LABORATORI NAZIONALI DI FRASCATI
SIS – Pubblicazioni

LNF–03/004 (NT)
25 Marzo 2003

Biological Applications of Synchrotron Infrared spectroscopy in Europe
BASIE

Type of Instruments: Network of Excellence
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LSH-2002-1.1.3-2 Development of in-vivo imaging technologies for phenotyping and functional analysis in cells and animal models

LSH-2002-1.1.2-1 The 3D-structure determination of membrane proteins

With the sequencing of the human genome completed, life science is entering the new era of structural and functional genomics and proteomics, in which the rapid identification and instantaneous sequencing of genes central to cellular processes and diseases leads to questions concerning the structure and function of the gene products. These new challenges require new methods of analysis and interdisciplinary efforts. In structural genomics, great progress has been made at so-called “structure factories”. But there remains a deficit in methods for studying the relationship between structure and function in proteins. Recently, FTIR spectroscopy has emerged as the most powerful tool for structural/functional investigations in biology because of its ability to provide information on processes at multiple levels of organization. It has been used to investigate structural-functional relationships and molecular mechanisms at the atomic level in purified proteins, but also for large protein complexes; for investigations of environmental toxin pathways and programmed cell death in cells; and for tissue classification and disease diagnosis in tissue. Although, in all of these studies, SR IR light sources bring FTIR spectroscopy to the cutting edge in terms of the spectral range and spatial resolution, it remains true that it is used by a few specialized research groups. The new era of life science research coincides with an unprecedented development in European capabilities: we have two well established IRSR beamlines at Daresbury and Orsay, joined last year by three new ones and more are under construction or are planned. BASIE will bring together interdisciplinary scientists from 10 EU countries, ready to develop together specialized equipments, techniques and software optimized for a range of life science studies covering all levels of organization from molecules to tissues. In this way, BASIE may be the leader in Europe for these strategic researches.

B.1 Objectives of the network

With the sequencing of the human genome completed, life science is entering the new era of structural and functional genomics and proteomics, in which the rapid identification and instantaneous sequencing of genes central to cellular processes and diseases leads invariably to questions concerning the structure and function of the gene products. These new challenges require new methodological innovations. In structural genomics, great progress has been made with array-based crystallization technologies and high throughput crystallography at so-called "structure factories". But there remains a deficit to be made up in methods for studying the relationship between structure and function in proteins.

In the last decade, FTIR spectroscopy has emerged as one of the most powerful tools for structural/functional investigations in biological systems. The power of FTIR spectroscopy lies in its ability to provide information on biological processes at multiple levels of organization. It has been used to investigate structural-functional relationships and molecular mechanisms at the atomic level in purified proteins in vitro, but also for large protein complexes in natural and reconstituted membrane; for investigations of environmental toxin pathways and programmed cell death in cultured cells; and for tissue classification and disease diagnosis in tissue samples. In all of these studies, synchrotron infrared light sources bring FTIR spectroscopy to the cutting edge in terms of the extreme spectral range and spatial resolution made available. However, it remains true to say that biological FTIR spectroscopy is used by a relative limited number of specialized research groups and has not yet become a universally acceptable tool of biological and biomedical research comparable to, for example, X-ray crystallography or 2D electrophoresis.

The new era of life science research referred to above coincides with an unprecedented development in European synchrotron infrared capabilities. Currently, Europe has two[*] established synchrotron infrared beamlines at Daresbury and Orsay. These were joined last year by the newly commissioned beamlines in Frascati, Karlsruhe and Berlin. Further beamlines are now under construction at Trieste, Zurich and Grenoble, and two more are planned at Saclay and Culham. Thus the period of this proposal will see the number of infrared synchrotron beamlines in Europe increase from 2 to 10, giving a unique opportunity for Europe to assume the worldwide leadership in this field.

The BASIE Network of Excellence brings together the infrared beamline scientists at all the above-named synchrotron facilities with biophysicists, biochemists, cell biologists and clinicians from 10 European countries. The group will collaborate to develop specialized equipment, techniques and software at the European synchrotron facilities that is optimally adapted to the requirements of the biological research community, and will employ these for a range of biophysical, biochemical and biomedical studies covering all levels of organization from molecules to tissues. In this way, BASIE will lead the way to exploitation of FTIR spectroscopy as a universal tool for life science research, and at the same time will ensure European leadership in this field.

The Network is engaged to achieve in 60 months the following objectives, which may be grouped in five main categories. The order below does not reflect any priority or temporal hierarchy. All tasks will be pursued "in parallel" during the BASIE lifetime and are organized in workpackages, as described in detail in Sec. B.8:

- *Development of Improved Instrumentation for Infrared Synchrotron Radiation*

Even if IR Synchrotron Radiation (IRSR) is nowadays a well established technique, with about 25 beamlines operating in the world, no dedicated instrumentation has been developed up to now for

[*] The infrared beamline at MAXLAB in Sweden is excluded from this analysis, since it is used exclusively for high resolution spectroscopy in the gas phase.

this powerful source. Interferometers, microscopes and detectors that are mounted on the beamlines are the same as normally used with conventional sources. The severe experimental conditions often associated with measurements on biological samples stress the importance of this aspect, which will be addressed by BASIE. Moreover, the Network will use all its potential in terms of experience and know-how to support the construction and calibration of new European beamlines.
Therefore BASIE will perform the following verifiable objectives:
- Construction of new beamlines in Trieste, Villigen, and Grenoble. Design of the new beamlines for Diamond and Soleil;
- Study and exploitation of unconventional synchrotron radiation, like Edge Radiation;
- Development of new software for data acquisition, specific for the synchrotron source;
- Development of improved detectors for infrared microscopy;
- Identification of a system of standards for evaluating the performances of the IRSR source and instrumentation;
- Establishment of biological laboratories at the synchrotron facilities, where not already present.

• *Advanced Infrared Spectroscopy of Proteins in Vitro*

The high brilliance of Infrared Synchrotron Radiation, its intrinsic polarization properties, and its time structure, may provide an excellent signal-to-noise ratio when penetrating in the far infrared the small windows of high-pressure cells, when illuminating small areas at high angles of incidence, or when performing time-resolved experiments. The network is engaged to achieve the following verifiable tasks:

- Extensive use of ellipsometry for studies of protein crystals and oriented films;
- Realization of optical cells for infrared studies on proteins up to 0.7 GPa and use of these cells to study the pressure-induced folding and unfolding of proteins;
- Implementation of time-resolved and spatially-resolved techniques to study the temperature-induced protein aggregation and the protein conformational transitions linked to aggregation.
- Studies of protein absorption in the extreme far infrared (the region of the so-called “boson peak”) to detect collective modes of the protein/matrix system.
- Use of Infrared spectroscopy to determine the secondary structure of proteins in connection with computer simulation, X-ray scattering and neutron scattering at low angles;
- Use of the high brilliance of IRSR to study proteins at high dilution and proteins in microstructured flow cells.

• *Advanced Infrared Spectroscopy of Biomembranes and Membrane Proteins*

The structure and reactivity of membrane associated proteins is at the forefront of contemporary biochemical research. The experimental complexity of the field is due to the limitations imposed by the interfacial environment under study on existing techniques for spectroscopic and structural characterization. This project is aimed at achieving an understanding of membrane proteins utilizing vibrational spectroscopic techniques. The research involves both development of new techniques and application of existing techniques to the resolution of specific problems in membrane biochemistry and enzymology. In this framework, the network is engaged to achieve the following verifiable tasks:

- Development of technical expertise and methodologies for studying the interaction of biologically active peptides with lipid membranes at the air water interface by InfraRed Reflection and Absorption Spectroscopy (IRRAS) and Attenuated Total Reflection (ATR).
- Study of two mitochondrial respiratory enzymes, complex I and complex III, by use of electrochemically-induced ATR-FTIR redox difference spectroscopy

- IRSR determination of the extreme far infrared spectrum of the ATPase to detect the displacement of the nucleotide binding domain relative to the active site domain.

- *InfraredMicrospectroscopy of Single Cells*

The spectrum collected at an infrared microscope with the stroing contrast made possible by IRSR is the "fingerprint" of a single cell. The direction appropriate to BASIE is to develop an understanding of the nature of changes in cell chemistry, which correlate to cell abnormality. The program will involve work on cultured cell lines as well as cellular material derived from tissue and exfoliated samples from human subjects. We aim to study the changes in 'precancer' and inflammatory change to gain a complete understanding of the IR spectrum in human disease. The verifiable objectives we fix for the network are as follows:

- Development of procedures for culturing and maintaining cells on IR transparent supports, and for incorporating such cultures into liquid flow cells;
- Monitoring of cell processes such as differentiation, migration and the effects of drugs, approach to multi-drug resistance in cells, and to the relationship between morphological alterations and transcriptional activity;
- Fast identification of bacterial cells, at least at the young microcolony level;

- *Infrared Microspectroscopy of Tissues for Clinical Diagnosis and Deveopmental Analysis*

Spatially resolved IR measurements on human tissues help to understand the spectroscopic changes observed in biomedical infrared spectroscopy, for example in carcinogenesis or in arterial pathologies. We identified the following verifiable objectives:

- Use of IRSR microscopy in cancer diagnostics: colorectal adenocarcinoma, oral and cervical cancer
- Infrared mapping of aggregates of the pathological prion protein (PrPsc) and of other spectral markers in the central nervous system of TSE-infected Syrian hamsters;
- Identification of abnormalities in aneurismal aortas with respect to normal aortas;
- Spectral imprints of the degeneration and atrophy of neurons in the presence of oxidative stress, excitotoxicity, protein aggregation, and mitochondrial dysfunction.
- Mapping of the collagen structure in human bones, in the presence of aging and of different factors, which affect the bone evolution;
- Identification in plants, through IRSR microspectroscopy, of flowering signals (*florigen molecules*) travelling from the leaves to the Shoot Apical meristem (SAM), which trigger its conversion into floral meristem.

A provisional list of full names and employing organisation of the researchers who will participate in the BASIE Network of Excellence and have been counted on the form A3 in Part A of the proposal is the following.

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B.2 Relevance to the objectives of the LifeSciHealth Priority

The proposed network addresses to the development of high-throughput proteomics technologies for the analysis of the 3-D protein structural and conformational properties and for the identification of protein-protein interactions in complex biological samples and/or in the cell.
In particular, it has been widely recognized that the outcomes of genomics projects will have a major impact on technology when the molecular/atomic structures of the genic products (i.e. proteins) will be unravelled. This general statement is currently translated into several "structural proteomics" projects active worldwide. Such projects focus on the study of protein three-dimensional structure to gain deeper understanding of their basic functions, to control their mechanism of action *in vivo* and in technological applications, to scout novel drug-design principles. Structural proteomics has its roots in the application of synchrotron radiation in the hard X-ray band, through high-resolution diffraction, in the application of NMR techniques and in the simulative/bioinformatics approaches. However, X-ray crystallography requires crystals of good diffraction quality, whereas the application of NMR to structure determination is limited to small proteins. It is therefore clear that a significant fraction of proteins that will be expressed in structural proteomics projects now active will not be analyzable using the two methods.
It should be also observed that according to recent estimates, it might be that as much as 50 % of the proteins present in eucaryotes are disordered in their native state, and may get some structure when interacting with a ligand. This large pool of unstructured proteins is almost out of reach for crystallographs, as well as membrane proteins (ca. 30 % of the entire genome), that largely remain out from standard structural analysis.
Moreover, even when the crystal structure of a protein is known, it is more and more evident that the study of its structural and dynamical properties in solution and the analysis of its interactions with other cell constituents are the unique key to understand its biological activity. For elucidating protein function are then of special interest the structural changes occurring during aggregation and folding-unfolding processes, the stability of the protein structure against thermal, pressure and pH changes, the conformational changes associated to interactions with other proteins or determined by specific effectors, the protein-protein interaction mechanisms, the solvent effects as well as the dynamics of all the above indicates processes.
Several other physical techniques can be used to assess protein structure and to derive protein structure and dynamics, like in-solution small-angle X-ray or neutron scattering, neutron anelastic or quasi-elastic scattering, electron microscopy, CD UV/fluorescence, Raman and FT-IR spectroscopies, but detailed information can be derived only when the complementarity of these techniques is fully exploited.
Infrared (IR) spectroscopy has the ability to identify molecular constituents from their vibrational spectra. The molecular finger-print is found in the so-called mid-IR region (500 - 5000 cm^{-1}), which has been investigated in research laboratories and in industry for decades: FT-IR spectroscopy using conventional sources has been used to quantify protein secondary structure and to characterize changes in the secondary structure during folding/unfolding or activation/inhibition processes by effectors. IR microscopes equipped with conventional IR sources have been also used to examine biological samples in order to spatially resolve inhomogeneous samples and small particles: variations in nucleic acid, protein, and lipid content or structure have been observed to provide important details about the biochemistry of diseased states. However, unlike many X-ray based spectroscopies, which were made possible by the

advent of synchrotron radiation, IR spectroscopy has been used for a long time without the benefits of a synchrotron source. On the contrary, it has been demonstrated that owing to the broadband nature of the synchrotron beam with brightness 1000 times that of conventional sources, Fourier transform IR spectroscopy experiments are feasible on very diluted samples, on diffraction-limited sample areas at high signal-to-noise ratios and with relatively short data-acquisition times. A number of synchrotron IR microscopy experiments relevant to both structural and functional genomics can be then performed in the mid-IR spectral range, including time-resolved secondary structure analysis of protein in very diluted concentrations, time-resolved protein-folding studies in the microsecond time regime, IR imaging of neurons, bone and other biological tissues. Owing to the high flux output of synchrotron beam in the far-IR region (50 - 500 cm^{-1}), investigations of hydrogen bonding, of the dynamic molecular motions of biomolecules and of metal-ligand vibrational modes will be also possible.

The programme objectives of the LifeSciHealth Priority in the Thematic Area on Advanced genomics concerns the fundamental knowledge and basic tools for functional genomics in all organisms and includes both proteomics and structural genomics. Recent progress in sequencing of various genomes has uncovered thousands of proteins with little homology to characterized proteins, and hence, proteins with unknown function. The prospect of uncovering function through the acquisition of structural information on a genome wide scale (structural genomics) is appealing. According to the programme objectives, synchrotron IR spectroscopy should have a role to play in massive structure determination efforts, both as a structure determination tool for non-crystallizable proteins (in combination with other established experimental methods and computational predictive techniques), and as an experimental tool for monitoring protein conformation dynamics, protein-protein interactions and changes in secondary structure during protein folding/unfolding processes.

A network of researchers from four different areas (infrared synchrotron radiation specialists, biophysics, biology and biomedical) is then proposed. The network will operate at European level, promoting the integration of the infrared beamline scientists at all the European synchrotron facilities with biophysicists, biochemists, cell biologists and clinicians from 10 European countries. In particular, the group will collaborate to develop specialized high-throughput infrared equipment, techniques and software at the European synchrotron facilities optimally adapted to the development of researches in the fields of structural genomics and proteomics, which are important for elucidating protein function and are essential for drug design. It is then evident that the proposed network addresses to the objectives of the LifeSciHealth Priority in the areas 1.1.1 “Gene expression and proteomics” and 1.1.2 “Structural genomics”, referring in particular to the possibility:

- to enable researchers to better decipher the gene products and to define the complex regulatory networks that control fundamental biological processes in the cell (topic 1.1.1-2: “Development and application of high-throughput proteomics technologies for the generation of a large data set of protein-protein interactions”);
- to solve the bottleneck that preclude the determination at high-throughput of the structure of membrane proteins and membrane protein complexes by using optimized and integrated technologies (topic 1.1.2-1: “The 3-D structure determination of membrane proteins”).

Moreover, the network researches will also focus on developing, assembling, standardising and providing highly integrated and automated technological platforms and software at European synchrotron research centers for high-throughput infrared based structural genomics (topic 1.1.2-3: “Development of new hardware and software for the implementation of innovative automated technologies at synchrotron sites”).

One last point should be considered: the network researches will also point to validate and develop strategies for a optimized and diffuse application of synchrotron IR spectroscopy in medical diagnosis, for mapping cells and tissues, but also for allowing the chemical imaging within single cells as well as for permitting the detection of chemical variations at sub-cellular

levels. This objective addresses to several LifeSciHealth Priority topics, like the LSH-2002-2.2.0-5, in which multidisciplinary researches are considered to exploit molecular imaging for early detection of tumours and monitoring of treatments, in the pre-clinical and clinical setting, with a view to speed up their application in early stage diagnosis and therapeutic and prognosis assessment. The socio-economic benefits of such objectives are straightforward.

B.3 Potential Impact

European need for a Network of Excellence like BASIE.

Recent years have seen a sharp increase in the application of advanced techniques of vibrational spectroscopy to biophysical/biomedical studies. In part B1 we have stressed how a systematic use of InfraRed Synchrotron Radiation (IRSR), instead of conventional sources, may represent a breakthrough for those experiments. However, this opportunity is not being fully exploited by the biomedical/biophysical research community. This is in part due to a lack of awareness about the potential of the facilities, limited communication and cultural differences between the communities of potential users and suppliers.

A comparison with the situation in the USA, where the use of IRSR in biomedical applications is a current practice and gives excellent results, may be useful. Therein, the IRSR resources are much more numerous but also concentrated in order to achieve all possible synergies. At the NSLS facility in Brookhaven (New York) 6 infrared beamlines are working night and day for research institutions and industrial users: the same number as in whole Europe, where moreover they are spread from Daresbury to Berlin, from Lund to Frascati, with typically a single beamline for each facility. The new beamlines under construction in Europe, being the result of national programmes not coordinated with each other, will not considerably increase the efficiency of the system. This will be further affected by the transition of the British and French synchrotron radiation communities to the new rings Diamond ans Soleil, respectively.

It is clear that such a rapidly evolving situation should be governed in some way in order to achieve efficiency in a field that is important for Priority 1. The random geographical distribution of the European facilities for advanced infrared spectroscopy cannot be changed in the near future. However, this situation may become advantageous for the European users in terms of costs and comfort, provided that the synergies usually obtained by concentrating research in one place are instead achieved through coordination. In all domains of synchrotron radiation, coordination requires the joint efforts of the groups in charge of the beamlines, and those of users. This is even more true for IRSR, which has a shorter history than other synchrotron-based techniques, and for its applications to biomedical spectroscopy, that are still less explored than, for example, those to materials science.

If the present proposal will be accepted, money invested in coordination will also have the effect to divert national resources towards the objectives fixed by the EU Commission in the Sixth Framework Programme. This leverage effect will be a consequence of the particular structure of BASIE, which puts together beamtime providers and users. The European IRSR beamlines have been conceived to be “general purpose”. Therefore they are usually engaged in a variety of experiments proposed by the users and selected each time. The European Commission, when writing down the Sixth Framework Programme for 2002-2006, invited the European scientific community to focus on selected priorities, of which one is Life sciences. Thanks to the engagement in BASIE of all scientists in charge of the IRSR beamlines, a major percentage of the beamtime available in the European facilities will be devoted to the experiments of Priority 1. Moreover, investment on the experimental stations will be needed to host biological samples, where such facilities are not already present. In this way BASIE, by its simple existence, and at

the cost of coordination, will displace resources in beamtime, personnel, and national budget to one of the main objectives of the VI Programme of the EU.

Finally, the impact of a proposal like BASIE on the European panorama of Priority 1 will show up in a real change in culture and attitude. The groups of BASIE are not asking for financial support in order to continue to do the same research as before. As one easily understands by reading their curricula and lists of publications, most researchers involved will have to change habits and procedures in order to fulfil the tasks of BASIE. On one side, many biomedical spectroscopists will be induced to first enter a synchrotron facility, thus experiencing a major change of mind and updating their experimental ability. On the other side, the suppliers of infrared synchrotron radiation, who mostly have a background in optics and materials science, will learn to understand the needs of biomedical research, manage new samples, upgrade and modify the experimental stations on the beamlines.

BASIE, a virtual, unified research facility realized through coordination

The task of BASIE is ambitious. We wish to create a single "Virtual European Facility" devoted to the biomedical applications of Infrared Synchrotron Radiation. The disadvantages related to the geographical spread and to the individual management of the IRSR facilities will be overcome by means of coordination. To this purpose, the following actions will be undertaken:

- Establishing a set of collaborations in a few selected research topics in biomedical/biophysical research, that are envisioned to benefit from an increased application of vibrational spectroscopy/imaging. These collaborations will be aimed at producing a set of High-profile Experiments that will prove/establish/confirm the potential of IRSR vibrational spectroscopy and imaging for biophysical/biomedical research.

- Spreading scientific results within the network by organizing periodic Meetings, sending Circulars, creating a network Web site and a Database on-line on Infrared and Raman biomedical spectroscopies.

- Promoting and controlling the assignment of beamtime on the IRSR lines to the groups of BASIE: when needed, also adapting Procedures for access to IRSR to the specific needs of users.

- Providing organization for Joint Training, Lectures and Seminars at Ph. D. level, and Student/Staff Exchange

- Promoting the integration of External Instrumentation into the beamlines, programming instrumentation transfer or exchange.

- Defining and applying throughout the network suitable protocols for ensuring Biosafety at IRSR Beamlines.

- Promoting the participation of women to the experimental activities by taking into account specific needs in the allocation and choice of beamtime.

How BASIE will spread excellence and disseminate knowledge

Successful application of spectroscopic studies in the selected topics will provide the exposure required to increase awareness with other research groups outside of the network. This will obviously be achieved via publication of results in thematic journals and international

conferences covering a range of different research fields (e.g. microbiology, medicine, biophysics, biochemistry, medical engineering…).

However, specific action will be undertaken toward the rest of the scientific community, like:

- training of young researchers through Ph. D. and post-doctoral fellowships;
- training of students from European universities through short stages at the beamlines (at level of DEA, master, or laurea thesis);
- organization of visits and seminars for new potential users;
- maintaining and updating of the network Website and of the on-line database on biomedical applications of infrared spectroscopy.

Durable structuring impact on the European research in the field

We can easily foresee a durable impact for BASIE on the European research in the field, even after the end of funding. This prediction is not a generic auspice but is based on two sound arguments.

First of all, the core of BASIE is firmly anchored to the network of the European synchrotron facilities, all of which will host at least one infrared beamline at the expiration date for BASIE. The synchrotron facilities are permanent institutions, that will continue for a long time to be supported by the national governments or, in case of ESRF, by European funds. Individual researchers may turn over, but the infrared beamlines as well as the need of infrared radiation for the European community of Life sciences will be permanent data of the problem. It will then be easy to keep the groups of BASIE in close collaboration with each other at the end of the Network, maintaining the structure and the procedures outlined in the present proposal, even if the funds of the European community will be replaced each time by other sources.

The above considerations are confirmed by the experience with the HC&M network on Infrared Synchrotron Radiation that is recalled below (point 5). On that experience our second argument is based. Indeed, after the expiration date of that smaller network, the groups involved have continued to exchange information, to propose training fellowships to students of the other groups, to publish joint papers. Two years after the expiration of the network funds, the first book devoted to IRSR appeared [Infrared Synchrotron Radiation, ed. by P. Calvani (La Sapienza) and P. Roy (LURE), Editori Compositori, Bologna, 1998] with updated contributions from all former members of the network, in addition to papers from USA and Japan. Five years later, those members are still forming a scientific community to such extent, as they have been the "seed" for the present proposal.

Chances of achieving the BASIE objectives: two arguments

Finally, we would like to mention two factors that open a successful perspective to BASIE. The first one consists in the extremely short time needed for BASIE to grow up from a “seed” represented by the small number of groups already interacting with each other. Indeed, as one can easily check, BASIE (or any other similar project) was not among the Expressions of Interest sent to the European Commission in 2002. The idea for BASIE came out in January 2003 from informal talks among a few friends and colleagues of different countries. In February a meeting was organized in Frascati (Italy), and several groups were already represented. Most of the participants were biophysicists and biochemists, strongly interested to IRSR, but who never had the opportunity to use such advanced instrumentation. In the following weeks, as the proposal was more and more defined, many other groups which heard about BASIE joined the network. The final result is a Network of Excellence, which gathers many of the best European groups which apply Infrared and Raman spectroscopy to biological materials on one side, and most of the present and future European facilities for Infrared Synchrotron Radiation on the other side.

The second favourable factor is a previous experience in coordinating European groups. The "seed" from which BASIE was born formed in the years from 1993 through 1996. At that time, most of the European groups involved in the construction of the first IRSR beamlines gathered in the Human Capital & Mobility Network "Development of Synchrotron Radiation and Applications to Condensed Matter Studies". The network, funded by 250,000 Ecu and coordinated by P. Calvani of University *La Sapienza*, successfully reached its main objectives:
- construction of a new beamline in Frascati, by using the "know how" of all partners;
- training of young researchers;
- creation of a European community of IRSR spectroscopists through meetings, visits, exchange of information.

The success of that HC&M network is confirmed by considering the new IRSR facilities that have been built or are under construction in Europe [(Karlsruhe (D), Villigen (CH) and Trieste (I)]. All of them have been designed by young researchers trained within the HC&M network "Development of Infrared synchrotron radiation and applications to condensed matter studies" (Y-L. Mathis, A. Nucara, and S. Lupi, respectively). In the present proposal, the groups from Frascati (I), Roma (I), Orsay (F), and Daresbury (UK) that were leading the 1993-97 network, have been joined by the above three groups as well as by the group in charge for the beamlines in Berlin (D) and Grenoble (F). Actually, the whole European IRSR community joins BASIE, together with more than twenty groups of scientists, which will have a coordinated access to those beamlines.

B.3.1 Contribution to standards

BASIE may give major contributions to the definition of standards for Infrared Synchrotron Spectroscopy applied to biomedical research. The unprecedented possibility to systematically compare the performances of a large number of beamlines, interferometers, and detectors, with different design and operating procedures, will allow us to:

- define a standard protocol to measure the brilliance ratio between the synchrotron radiation and the conventional source (globar, Hg lamp). On this issue, figures published in the literature vary by one or two orders of magnitude, due to the absence of a widely accepted procedure.

- define a standard protocol to measure the signal-to-noise ratio for the synchrotron radiation and the conventional source on a 100% line in different spectral domains after given accumulation procedures.

- define standards for diffraction-limited and noise-limited spatial resolution in infrared microscopy, both with and without the use of the synchrotron. This is also an important issue for evaluating the realiability of infrared images of biological materials.

- define standards of statistical certainty for the presence or the absence of given spectral lines, a basic issue to determine the presence or the absence of a given component in a cell or a protein. Such standards will also provide criteria for the transferability of reference spectra between different spectrophotometers and facilities.

B.4 Degree of integration and the joint programme of activities

The BASIE Network of Excellence brings biophysicists, biochemists, cell biologists and clinicians from 10 European countries together with infrared beamline scientists at existing and planned European synchrotron facilities. The group will collaborate to develop specialized techniques for the life sciences at the European synchrotron facilities, and will employ these for a range of biophysical, biochemical and biomedical studies covering all levels of organization from molecules to tissues. In this way, BASIE achieves not only the critical mass but also the critical breadth to establish synchrotron FTIR spectroscopy as a key tool for life science research, and at the same time will ensure European leadership in this field.

The expected degree of integration will be brought about by activities in the areas of joint research, integration, management and spreading of excellence:

- Joint research activities

The key component of the joint programme of activities is the joint research programme. This is broken down into five groups of workpackages:

GROUP 1 SOURCES/EQUIPMENT/TECHNIQUES
GROUP 2 STUDIES OF PROTEINS IN VITRO
GROUP 3 STUDIES OF BIOMEMBRANES AND MEMBRANE PROTEINS
GROUP 4 STUDIES OF CELLS
GROUP 5 STUDIES OF TISSUES / CLINICAL DIAGNOSIS

Each of the workpackage groups involves the participation of between one third and one half of the NoE participants, with broad coverage across the disciplines represented. This will provide an interdisciplinary integration of expertise across all aspects of the project, in particular to ensure that the development of synchrotron infrared based techniques directly and completely addresses the requirements of life science research, and that the full potential of synchrotron infrared radiation is exploited in the experimental design and planning of the life science researchers.

There is a clear hierarchical organization of the workpackage groups, beginning with enabling technologies at the instrumentation level and then proceeding up the hierarchy of complexity of biological organization. This hierarchy also reflects the primary direction of information and expertise flow, with technical innovations from Group 1 flowing to the biological/biomedical workpackage groups, experimental and research insights from *in vitro* molecular studies flowing to more complex living systems, and fundamental discoveries concerning proteins, biomembranes and cells flowing toward clinical application in tissue studies and diagnostics.

Each workpackage group is comprised of a number of workpackages, with 27 workpackages in all. There are lateral connectivities between workpackages within each group, and vertical connectivities between individual workpackages in different groups. However, no interdependencies exist in the sense of commencement of work in one workpackage being dependent on completion or progress in another workpackage. The planning of the joint research acivities has been designed in such a way that all such interdependencies are handled internally within of individual workpackages, so that all can begin simultaneously in Month 0 of the programme.

The individual workpackages and their participants represent blocks of research activities in which multiple participants in the NoE will work jointly on a particular research topic or on closely related research topics. Integration between network participants is thus achieved in the first instance at the level of collaborative research work between the participants of individual workpackages. Integration between workpackages is achieved through the interconnectivies described above, as well as through the fact that most of the NoE members are participants in a number of individual workpackages.

- Integrating activities

The integrating activities with the BASIE NoE are defined so as to ensure the smooth functioning of the joint research programme participants as a coherent network. This begins with planning of the joint research at the network, workpackage group and workpackage levels, continues through the mobility of junior and senior researchers during execution of the program, and leads finally to the dissemination of insights and know-how by the instruments described below in Section B.4.1.

- Management activities

Efficient management activities are essential in a NoE of the scale and breadth of BASIE. As detailed in Section B.4.4, the initial composition of Executive Board and the Coordinator have already been appointed. Individual Board members will assume managerial responsibility for each of the five workpackage groups and for other activity areas. In addition, one NoE participant has been selected as Lead Participant for each of the 27 workpackages and will assume managerial responsibilities for the work in this workpackage. These nominations have been arranged in such a way that each NoE participant is Lead Participant of no more than one workpackage. Finally, all NoE participants will participate in the selection of a new Executive Board at regular intervals. We are confident that the planned management structures will ensure high efficiency as well as high acceptance of managerial decisions amongst the NoE participants.

- Spreading of Excellence activities

Since a primary goal of the BASIE NoE is to promote awareness of synchrotron infrared techniques amongst the biological and biomedical research communities in general, spreading of excellence activities play a central role. There will be an integrated and coordinated effort involving all NoE participants to disseminate technological expertise and research insights beyond the Network, as detailed in Section B.4.3.

NoE List of activities
Full duration of project

Project acronym – BASIE 1/5

	Partner 1 INFN-LNF	Partner 2 Sapienza	Partner 3 CNRS	Partner 4 FZK	Partner 5 SLS/PSI	Partner 6 CCLRC	Partner 7 BESSY
Integrating activities							
EXEC. BOARD	X	X	X	X			X
MEETINGS (ATTENDANCE)	X	X	X	X	X	X	X
MEETINGS (ORGANIZATION)	X	X	X	X			X
WORKSHOPS ON INSTRUM.	X	X	X	X	X	X	X
WORKSHOPS ON PROTEINS	X	X	X	X			X
WORKSHOPS ON MEMBRANES		X	X	X			
WORKSHOPS ON CELLS		X		X			
WORKSHOPS ON TISSUES	X	X	X	X			X
TRAINING (INSTRUMENTATION)	X	X	X	X	X	X	X
TRAINING (BIOMEDICALS)		X					
Joint research programme							
SOURCES/EQUIPMENT	X	X	X	X	X	X	X
STUDIES OF PROTEINS	X	X	X	X			X
STUDIES OF BIOMEMBRANES		X	X	X			
STUDIES OF CELLS		X		X			X
STUDIES OF TISSUES	X	X	X	X		X	X
Spreading of excellence activities							
PUBLICATIONS	X	X	X	X	X	X	X
ACCESS TO SYNCHROTRONS	X		X	X	X	X	X
BASIE NEWSLETTER/WEBSITE	X	X	X	X			X
COURSES	X	X	X	X	X	X	X
CONFERENCES (ORGANIZERS)	X	X	X	X			X
STUDENT TRAINING	X	X	X	X	X	X	X
Management activities							
COMMITTEE MEETINGS	X	X	X	X			X
ELECTION OF COMMITTEE	X	X	X	X	X	X	X

NoE List of activities
Full duration of project

Project acronym – BASIE 2/5

	Partner 8 ISAS	Partner 9 BOPT-IT	Partner 10 Elettra	Partner 11 DSFA	Partner 12 UNIPG	Partner 13 UNIVR	Partner 14 Nottingham
Integrating activities							
EXEC. BOARD							X
MEETINGS (ATTENDANCE)	X	X	X	X	X	X	X
MEETINGS (ORGANIZATION)							X
WORKSHOPS ON INSTRUM.		X	X				
WORKSHOPS ON PROTEINS	X			X	X		
WORKSHOPS ON MEMBRANES			X				
WORKSHOPS ON CELLS							X
WORKSHOPS ON TISSUES			X				
TRAINING (INSTRUMENTATION)		X	X				
TRAINING (BIOMEDICALS)	X			X	X	X	X
Joint research programme							
SOURCES/EQUIPMENT	X	X	X				
STUDIES OF PROTEINS	X	X	X	X	X		
STUDIES OF BIOMEMBRANES			X				X
STUDIES OF CELLS							X
STUDIES OF TISSUES			X			X	X
Spreading of excellence activities							
PUBLICATIONS	X	X	X	X	X	X	X
ACCESS TO SYNCHROTRONS			X				
BASIE NEWSLETTER/WEBSITE							X
COURSES	X	X	X	X	X	X	X
CONFERENCES (ORGANIZERS)							X
STUDENT TRAINING	X		X	X	X	X	X
Management activities							
COMMITTEE MEETINGS							X
ELECTION OF COMMITTEE	X	X	X	X	X	X	X

NoE List of activities
Full duration of project

Project acronym – BASIE 3/5

	Partner 15 ITC-CNR IBF TN	Partner 16 ULB	Partner 17 UPM	Partner 18 CEA	Partner 19 RKI	Partner 20 URCA	Partner 21 ERSF/Grenoble
Integrating activities							
EXEC. BOARD		X	X	X			
MEETINGS (ATTENDANCE)	X	X	X	X	X	X	X
MEETINGS (ORGANIZATION)		X	X	X			
WORKSHOPS ON INSTRUM.							X
WORKSHOPS ON PROTEINS	X		X				
WORKSHOPS ON MEMBRANES	X	X		X			
WORKSHOPS ON CELLS					X	X	
WORKSHOPS ON TISSUES					X	X	X
TRAINING (INSTRUMENTATION)							X
TRAINING (BIOMEDICALS)	X	X	X	X	X	X	
Joint research programme							
SOURCES/EQUIPMENT							X
STUDIES OF PROTEINS	X		X		X		
STUDIES OF BIOMEMBRANES	X	X	X	X			
STUDIES OF CELLS	X				X	X	
STUDIES OF TISSUES					X	X	X
Spreading of excellence activities							
PUBLICATIONS	X	X	X	X	X	X	X
ACCESS TO SYNCHROTRONS							X
BASIE NEWSLETTER/WEBSITE		X	X	X			
COURSES	X	X	X	X	X	X	X
CONFERENCES (ORGANIZERS)		X	X	X			
STUDENT TRAINING	X	X	X	X	X	X	X
Management activities							
COMMITTEE MEETINGS		X	X	X			
ELECTION OF COMMITTEE	X	X	X	X	X	X	X

NoE List of activities
Full duration of project

Project acronym – BASIE 4/5

	Partner 22 Strathclyde	Partner 23 Unimib	Partner 24 UNIVR/DP	Partner 25 TU Wien	Partner 26 SU	Partner 27 NTUA	Partner 28 FPNT, UMM
Integrating activities							
EXEC. BOARD							
MEETINGS (ATTENDANCE)	X	X	X	X	X	X	X
MEETINGS (ORGANIZATION)							
WORKSHOPS ON INSTRUM.							
WORKSHOPS ON PROTEINS		X		X	X		
WORKSHOPS ON MEMBRANES					X		
WORKSHOPS ON CELLS							
WORKSHOPS ON TISSUES						X	X
TRAINING (INSTRUMENTATION)							
TRAINING (BIOMEDICALS)	X	X	X	X	X	X	X
Joint research programme							
SOURCES/EQUIPMENT	X						
STUDIES OF PROTEINS	X	X		X	X		
STUDIES OF BIOMEMBRANES	X				X		
STUDIES OF CELLS			X	X			
STUDIES OF TISSUES						X	X
Spreading of excellence activities							
PUBLICATIONS	X	X	X	X	X	X	X
ACCESS TO SYNCHROTRONS							
BASIE NEWSLETTER/WEBSITE							
COURSES	X	X	X	X	X	X	X
CONFERENCES (ORGANIZERS)							
STUDENT TRAINING	X	X	X	X	X	X	X
Management activities							
COMMITTEE MEETINGS							
ELECTION OF COMMITTEE	X	X	X	X	X	X	X

NoE List of activities
Full duration of project

Project acronym – BASIE 5/5

	Partner 29 UCL	Partner 30 Leeds	TOTAL PARTNERS
Integrating activities			
EXEC. BOARD			9
MEETINGS (ATTENDANCE)	X	X	30
MEETINGS (ORGANIZATION)			9
WORKSHOPS ON INSTRUM.			10
WORKSHOPS ON PROTEINS			13
WORKSHOPS ON MEMBRANES	X		9
WORKSHOPS ON CELLS		X	6
WORKSHOPS ON TISSUES			11
TRAINING (INSTRUMENTATION)			10
TRAINING (BIOMEDICALS)	X	X	21
Joint research programme			
SOURCES/EQUIPMENT			12
STUDIES OF PROTEINS	X		18
STUDIES OF BIOMEMBRANES	X		11
STUDIES OF CELLS		X	10
STUDIES OF TISSUES		X	15
Spreading of excellence activities			
PUBLICATIONS	X	X	30
ACCESS TO SYNCHROTRONS			8
BASIE NEWSLETTER/WEBSITE			9
COURSES	X	X	30
CONFERENCES (ORGANIZERS)			9
STUDENT TRAINING	X	X	29
Management activities			
COMMITTEE MEETINGS			9
ELECTION OF COMMITTEE	X	X	30

B.4.1 Integrating activities

The integrating activities promoted by BASIE will include:

- Planning the joint research.

- Organizing and supporting the mobility of researchers.

Part of the funds will be employed to financially support the exchange of young researchers among different groups and their training. Shorter leaves will be organized for senior researchers. This program implies an action of advertisement, selection, and mobility planning that will be managed by the Coordinator in close collaboration with the Board and the groups involved.

- Promoting the flow of know-how and the dissemination of results within the network.

This action will develop through:
- maintainance and updating of the BASIE Website with its scientific database;
- organization of seminars held by senior researchers of the Network in the other Institutions involved;
- organization of a general meeting of the network every 18 months;
- organization of specialized workshops, open also to external groups, and focused on the workpackages of Section B.4.2.
- periodic diffusion of newsletters and circulars.

- Training European students.

This activity will include the organization of:
- at least two summer schools during the life of the Network;
- short courses at Ph. D. level in some of the universities involved in the project;
- stages for university students at the level of DEA, laurea, or master.

B.4.2 Programme for jointly executed research activities

WORKPACKAGES

GROUP 1 SOURCES/EQUIPMENT/TECHNIQUES

1.1 Novel synchrotron sources [WP 1]

Development and investigation of new approaches to the production of synchrotron IR radiation that provide benefits for biological users, e.g. edge radiation (for enhanced intensity at high spatial resolution), coherent emission (for enhanced intensity in the far infrared). This will include design of a novel insertion device for amplification of edge radiation in the IR range.

[1] “Magnetic field discontinuity as a new brighter source of infrared synchrotron radiation", Y-L. Mathis, P. Roy, B. Tremblay, A. Nucara, S. Lupi, P. Calvani, and A. Gerschel, Phys. Rev. Lett. 80, 1220 (1998).

[2] "Spectral distribution of Infrared synchrotron radiation by an insertion device and its edges", P. Roy, M. Cestelli Guidi, A. Nucara, O. Marcouille, P. Calvani, P. Giura, A. Paolone, and Y-L. Mathis, Phys. Rev. Lett. 84, 483 (2000).
[3] "Powerful, Steady State, Coherent Synchrotron Radiation at BESSY II", M. Abo-Bakr, J. Feikes, K. Holldack, H.-W. Hübers, U. Schade, G. Wüstefeld, European Particle Accelerator Conference (EPAC), 3.-7. June 2002, Proceedings, 778-780 (2002).
[4] "New higher brilliance sources of infrared synchrotron radiation: toward an edge radiation undulator", P. Roy, O. Marcouille, A. Paolone, P. Giura and A. Gerschel, Proceedings of the 12th International Conference on Fourier Transform Spectroscopy, ", Waseda University Enterprise Press (WUP), (2000)

Participants: 4 FZK/Karlsruhe, 3 CNRS, 7 BESSY/Berlin, 5 SLS-PSI, 10 Elettra

1.2 Construction of new IR beamlines [WP 2]

Construction of new beamlines at Elettra, SLS and at ESRF for infrared spectroscopy and microscopy. Infrared beamlines are planned at SOLEIL and DIAMOND. All of these IRSR beamlines will be mounted on third-generation storage rings with beams of high or very high energy. This will require an improved design of the extraction mirrors and of the focusing optics. The beamline at ESRF will offer a unique combination of IR and X-ray microscopy. SOLEIL is planning to have two infrared ports: one optimized in the mid-IR for microscopic studies, and one optimized in the far-IR domain. In addition, a second infrared beamline for biological applications is envisaged at ANKA.

[1] "First observation of Infrared Synchrotron Radiation at Elettra", S. Lupi, A. Nucara, P. Calvani, and M. Ferianis, Sync. Rad. News 14, 22 (2001).

Participants: 10 Elettra, 5 SLS-PSI, 21 ESRF, 4 FZK/Karlsruhe, 3 CNRS (SOLEIL), 6 CCLRC/Daresbury (DIAMOND)

1.3 Instrumentation [WP 3]

Development of specialized spectroscopy equipment and software, in place of the current practice of using standard commercial equipment that was not designed for synchrotron IR radiation. Some specific issues: avoiding optical axis obstruction in beamsplitters and objectives, coping with wavelength-dependent divergence, smaller detectors for lower noise, broadband interferometers and detection, mechanical tolerances and precision required for diffraction-limited spot sizes, software solutions for compensation of current decay, injection and other source intensity fluctuations.

The optimisation of commercial FT-IR optical benches for synchrotron light would be achieved by modifying the traditional optical elements for the synchrotron light with the purpose of managing the small dimensions of the beam and in particular the possibility to maintain the polarisation of the light by optimising the signal in plane of polarisation. Amongst the aspects under evaluation are:

- radius of curvature and the geometry of the mirrors
- porosity of the glass surfaces
- properties of the coating
- position and the dimension of the detectors
- optimisation of the laser alignment

A further specific goal is the implementation of fast response detectors for the FIR region. The first step would be reached by looking at the different technologies already available on the market in terms of sensible elements in the FIR region, then the development of a specific preamplifier for signal processing. The ideal goal is to reach TRS measurements able to discriminate signals in the range of μs in the spectral range of 600-5 cm^{-1}.

This workpackage will also cover the development of equipment and software for ellipsometry at BESSY. In this project it is planned to extend the spectroscopic ellipsometer set up by ISAS at the IR beamline of BESSY II for investigations of aligned biological films and biomolecular crystals. (see 2.2, WP 7).

A further goal within this workpackage is the construction of a sample holder that allows same-sample analysis using different techniques of microscopy and microspectroscopy. The holder will be designed to be compatible with both the IR microscope and the X-ray microscope currently under construction at ELETTRA. It will allow the precise reconstruction of alignment and positioning within the two machines, so that parallel microspectroscopy or imaging measurements can be executed on the same sample position. Expected samples are primarily tissues and cells.

Participants: 4 FZK/Karlsruhe, 1 INFN-LNF, 2 Sapienza/Roma, 3 CNRS, 9 BOPT-IT, 7 BESSY/Berlin, 8 ISAS-Dortmund, 22 Strathclyde

1.4 Standardization [WP 4]

Establishment of standardized tests and criteria for assessing the performance of synchrotron IR beamlines. They will imply the definition of protocols for the evaluation of brilliance ratio between the synchrotron source and conventional sources, signal-to-noise ratio, beam stability, data reproducibility, statistical certitude.

Participants: 4 FZK/Karlsruhe, 7 BESSY/Berlin, 3 CNRS, 10 Elettra, 1 INFN-LNF, 5 SLS-PSI, 6 CCLRC/Daresbury, 21 ESRF

1.5 Infrastructure for biologists at SR facilities [WP 5]

The existing laboratory support facilities associated with the IR beamline at synchrotron radiation facilities are in general not suitable for the most efficient use of synchrotron beamtime by biomedical infrared users. IR users often have access to only a generalized preparative laboratory, which is unsuitable for certain kinds of biological preparative work either because of lack of specialized equipment and/or because of laboratory safety regulations. At most, IR users may have limited access to a biological containment laboratory shared with users from other stations or located in other institutes on the campus. Both solutions are unsatisfactory in practice. Ideally users requiring to prepare clinical samples and tissue culture sample for IR analysis should have their own facility.

In the first instance, the Network proposes to support the establishment of a biological containment laboratory at one European synchrotron facility. An off-line laboratory is due to be provided at SRS Daresbury for IR users, and here we propose installing equipment suitable for preparing biomedical samples under class II biological safety conditions, including a class II microbiological safety cabinet, tissue culture incubator, inverted microscope and upright pathologists' microscope. The total planned investment for this first trial is modest (20 k€). Users

from other European countries requiring these facilities can be directed to the Daresbury laboratory, and the experience gained will be used to assess the requirement for similar facilites at other European synchrotrons.

Participants: 6 CCLRC/Daresbury, 1 INFN -LNF

GROUP 2 STUDIES OF PROTEINS IN VITRO

2.1 Protein folding/unfolding under high pressure [WP 6]

High pressure cells will be used to obtain infrared spectra of proteins up to 0.7 GPa in order to study their folding and unfolding in the far-infrared range. It should be in fact observed that while temperature produces simultaneous changes in both volume and thermal energy, high pressure applied on protein solutions perturbs the environment in a continuous, controlled way by changing only intermolecular protein distances [1,2]. Thus, combining the effects of pressure and temperature, a detailed description of the protein stability and protein-protein interactions in solution will be obtained, leading to new insights about the protein unfolding mechanism.

[1] “The effect of high pressure upon proteins and other biomolecules”, Weber, G., and H. G. Drickamer, Q. Rev. Biophys. 16:89-112 (1983)
[2] “Nuclear magnetic resonance at high pressure”, Jonas, J., Science. 216:1179-1184 (1982)

Participants: 2 Sapienza/Roma, 1 INFN-LNF, 3 CNRS, 17 UPM/Ancona

2.2 Polarization spectroscopy and ellipsometry of protein crystals and oriented films [WP 7]

ISAS set up a spectroscopic ellipsometer for the mid infrared spectral range at the IR beamline at BESSY II taking advantage of the high brilliance of the infrared synchrotron source. The gain in signal, which can be achieved with FT-IR ellipsometry at the IRIS beamline has been shown exemplarily for samples in the submillimeter range. In this project it is planned to extend the method for investigations of aligned biological films (e.g. protein membrane) and crystals (e.g. protein crystal). For this purpose the existing ellipsometer will be adapted for the measurements of biological samples. The necessary measurement strategies and data evolution will be developed. As scientific outcome of the project the optical constants and the molecular structure of membrane proteins will be determined.
Polarized IR spectroscopy has also been used by the ANKA group to determine the dipole orientations of individual absorbance difference bands in single protein crystals. [1] The maximum possible crystal thickness to avoid total absorbance in the amide I region is in the region of 10 μm. The high brilliance of the synchrotron IR source allows measurements of spot sizes of the same order of magnitude, so that any crystal habit can be studied. With the much larger measuring spot of conventional IR instrumentation, only platelets can be measured.

[1] "Oxidized-minus-reduced FTIR difference spectroscopy of cytochrome c crystals", D.A. Moss and J.T. Sage (1999), 8th European Conference on the Spectroscopy of Biological Molecules, Enschede, The Netherlands, August 29 - September 2, 1999

Participants: 8 ISAS/Dortmund, 7 BESSY/Berlin, 4 FZK/Karlsruhe

2.3 Protein misfolding and aggregation [WP 8]

The high brilliance of synchrotron IR sources will be exploited in order to perform time-resolved and spatially resolved studies of the temperature induced protein aggregation and of the conformational transitions linked to aggregation. We plan to investigate the aggregation behavior of proteins with different fractions of alpha/beta secondary structures and in different conditions (pH, temperature); bovine serum albumin (BSA), human serum albumin (HSA), lysozyme and apomyoglobin will be investigated initially, followed by extension to membrane proteins. The studies will include investigations of the role of trace metals (Cu, Zn, Al, Fe) in the process of formation and growth of aggregates by analysis of the small signals pertaining to the side chains of the residues that are involved in their binding, e.g. tyrosines, aspartates and glutamates. This will be complemented by results obtained with other techniques providing local structures such as EXAFS and XANES. The thermal unfolding of the lipocalins class of proteins and their temperature induced aggregation will be also investigated. Morever, processes leading to misfolding of proteins involved in human amyloid diseases, such as β_2 microglobulin (a major component in dialysis-related amyloidosis) and prion proteins (causative agents of transmissible spongiform encephalopathies) will be studied using static and time-resolved IR spectroscopy. These studies aimed at understanding early events of protein misfolding will also include proteins not involved in amyloid diseases but capable to assemble into amyloid-like structures under certain conditions (e.g., phosphoglycerate kinase).

[1] “Conversion of yeast phosphoglycerate kinase into amyloid-like structures”, Damaschun, G., Damaschun, H., Fabian, H., Gast, K., Kröber, R., Wieske, M., Zirwer, D., PROTEINS: Struct. Funct. Genet., 39, 204-211, (2000).

Participants: 11 DSFA/Palermo, 15 ITC-CNR IBF/Trento, 2 Sapienza/Roma, 17 UPM/Ancona, 23 Unimib/Milano, 19 RKI/Berlin

2.4 Extreme far IR spectroscopy of proteins [WP 9]

Exploitation of the far infrared region available from synchrotron IR sources to investigate the FIR absorption of proteins in the region 10 – 100 cm^{-1} (the region of the so-called “boson peak”), which is dominated by collective modes of the protein/matrix system. We propose to investigate the coupling between the protein and the environment around its surface by studying how the “boson peak” features (intensity, position and shape) change as a function of the kind of the solvent and of the hydration degree. In addition, we plan to investigate myoglobin and hemoglobin in different quaternary conformations; experiments will be performed in the temperature interval 5 – 320 K. Furthermore the infrared response of amino acids (aspartic and glutammic acids) within proteins in water solution in their native and unfolded states will be investigated in the region 50-400 cm^{-1}.

Participants: 11 DSFA/Palermo, 2 Sapienza/Roma, 12 UNIPG/Perugia, 7 BESSY/Berlin, 23 Unimib/Milano

2.5 Structure and dynamics of biomolecules in non-aqueous solvents and matrices [WP 10]

Enzymes can vigorously act as catalysts in anhydrous organic solvents. When placed in this unnatural milieu, enzymes acquire some remarkable novel properties, such as greatly enhanced thermostability and strikingly different specificity, including stereoselectivity. Non-aqueous

solvents also are used as novel processing media for purification of hydrophobic (e.g., membrane) proteins. Furthermore, the knowledge gained in these studies has turned out to be very useful in designing new strategies for transporting pharmaceutical proteins through biological barriers, such as the skin. We propose to use IR synchrotron radiation for the characterisation of the picosecond dynamics of proteins in organic solvents, which is supposed to be crucial for the onset of biological functionality, and of their secondary structure as affected by the environment. Typical systems we will investigate are proteins (lysozyme, cytochrome-c, _-lactoglobulin, myoglobin) embedded or solved in organic solvents as a function of the water content (glycerol, glucose, dimethylsulfoxide, ethylene-glycol, chloro-ethanol, trifluoro-ethanol, monohydric alcohols). The work will be extended to cover proteins enclosed in reverse micelles embedded in different matrices (silica hydrogels, trehalose glasses) in order to gain information on protein-protein and on protein-matrix interactions.

Participants: 11 DSFA/Palermo, 12 UNIPG/Perugia, 2 Sapienza/Roma, 3 CNRS, 17 UPM/Ancona

2.6 Exploitation of IR data for structure determination [WP 11]

This study will focus on a program in the field of the structural genomics, which is related to the structure characterisation of non-crystallizable proteins [1,2]. The work will start from the use of model protein systems (like cytochrome C, myoglobin, lysozyme and so on), to derive a Reverse Monte Carlo method to fit the secondary structure (or the preserved secondary structure, detected by FT-IR synchrotron spectroscopy) into protein shapes reconstructed by small angle X-ray and neutron scattering data during thermal or pressure induced folding/unfolding processes or in different experimental conditions (pH, non-aqueous solvents, ionic strength, proteins dissolved in lipid micelles) [3]. This will include studies of time-dependent conformational states of model protein systems, facilitated by the high intensity and high brightness which can be obtained in synchrotron sources. The secondary, tertiary and quaternary structure of the model proteins will be determined by the complementary use of time-resolved FT-IR synchrotron spectroscopy and protein shape reconstruction methods from time-resolved small angle X-ray scattering data. The final goal is the application of the techniques to non-crystallizable proteins (both soluble or from membranes), in order to establish a protocol to determine their structure in solution using an approach based on the combined use of computational prediction techniques, FT-IR synchrotron spectroscopy (to determine the secondary structure) and small angle X-ray and neutron scattering shape analysis.

An input to this workpackage can also be expected from polarization spectroscopy and ellipsometry of protein crystals (2.2).

[1] “Insights into biomolecular function from small-angle scattering”, Trewhella, J.,. Curr. Opin. Struct. Biol. 7:702–708 (1997)
[2] “Ligand-induced conformational changes in tissue transglutaminase: Monte Carlo analysis of small angle scattering data, ”Mariani P, Carsughi F, Spinozzi F, Romanzetti S, Meier G, Casadio R and Bergamini CM., Biophys. J. 78:3240-3251 (2000)
[3] “Structural Characterisation of the pH-Denatured States of Ferricytochrome-c by Synchrotron Small Angle X-Ray Scattering”, S. Cinelli, F. Spinozzi, R. Itri, S. Finet, F. Carsughi, G. Onori, P. Mariani, Biophys. Journal, 81:3522-3533 (2001)

Participants: 17 UPM/Ancona, 12 UNIPG/Perugia, 23 Unimib/Milano, 2 Sapienza/Roma, 3 CNRS, 8 ISAS/Dortmund

2.7 Ligand binding in proteins [WP 12]

This project is part of our ongoing effort to develop, use and validate vibrational spectroscopy as a new drug and herbicide development technique.

Infrared spectroscopy is a promising screening technology for drug development: it provides a wealth of direct label-free information on ligand-protein interactions and can be universally applied, also to membrane proteins, which are difficult to study with some other techniques. Infrared spectroscopy is fast, inexpensive and requires small amounts of material. We have started to map individual interactions between Ca^{2+}-ATPase and nucleotide with mid-infrared spectroscopy using ATP analogues in each of which a specific functional group is modified. This has enabled us to identify several functional groups that undergo key interactions with the ATPase.

However, a major obstacle for the general applicability of infrared spectroscopy to drug development is the high protein concentration needed presently (~mM) to record the small absorbance changes upon ligand binding. We will take advantage of the high brilliance of the synchrotron source to significantly reduce the spot size for measurement. This fact will allow us to dramatically reduce the amount of sample required for analysis and to carry out new types of experiments (c.f. section 2.8.).

We will extend present mapping studies focusing on the adenine moiety of ATP. The possibility of using a microstructured mixing cuvette relaxes the present requirement to photolyse a caged ATP analogue to initiate binding and therefore extends the number of analogues that can be investigated. Binding-induced infrared difference spectra are analysed mainly in the region near 1650 cm^{-1} where protein conformational changes are monitored. As the protein concentration needed is an important characteristic of a future infrared screening technology we will set a reference value and determine the lowest possible concentration that still enables reliable detection of binding.

A further topic within this workpackage is a study of NAD(P)H binding in transhydrogenase.

Participants: 26 SU/Stockholm, 4 FZK/Karlsruhe, 25 TU/Wien, 29 UCL/London

2.8 Micro flow cells and lab-on-a-chip devices for kinetic studies of protein folding and interactions [WP 13]

The high brilliance of synchrotron IR radiation will be exploited to enable rapid mixing experiments with protein solutions at dimensions close to the diffraction limits. This will yield benefits in terms of the extremely low sample volumes required as well as in the high time resolution achievable by diffusional mixing over micrometer distances. Microstructured flow cells will be developed for flow injection. [1,2] The Strathclyde group will contribute their experience with similar microfluidic systems for Raman spectroscopy [3]. Such system allows the shorter possible dead-time (less than 0.2 ms). Time resolution is limited by the space resolution in the detecting system and by the fluid speed (which should be pressurised). The small size of the exciting beam is paramount for optimal lateral resolution (and the corresponding time resolution). We will use automatic syringe driven injection system. The measuring cell will be microfabricated in Si and the typical diameter of the channels will be around 10 μm. In the first application the cell will be moved by a stepped motor in front of a narrowly focused beam taking data at one position at a time. For example 20 points for a 2 cm pathway will allow submillisecond resolution at a standard flux of 20 ml/min. Slower time courses will be followed by correspondingly slowing

down the flux. This technique will be make available to all partners. A possible future development will be the use of a diode array detector (64 x 64 matrix) for simultaneous recording from each position, allowing better resolution and faster accumulation of data with less sample consumption.

A new quality in experimental protocols and techniques will be gained by designing lab-on-a-chip devices to match the small diameter of the focused synchrotron beam. In a first study, microstructured devices will be developed for fast continued mixing of two micro-fluids (e.g. protein and ligand) to monitor bio-ligand interactions as a function of time. Both pressure driven and electro-osmotically driven techniques of fluid flow will be employed. The whole devices will be fabricated using modern micro-structuring techniques and employ layers of different functionality. Later, we plan to develop lab-on-a-chip devices capable of performing single and multiple mixing steps for quench flow experiments as well as electrophoretic separation steps on-chip. These devices will act as enabeling technology for a broad range of experiments planned by the bio-chemical and bio-medical groups of the BASIE network.

[1] "Stopped flow system for FTIR difference spectroscopy of biological macromolecules", R. Masuch and D.A. Moss (1999) 8th European Conference on the Spectroscopy of Biological Molecules, Enschede, The Netherlands, August 29 - September 2, 1999
[2] "Time-resolved FT-IR spectroscopy of chemical reactions in solution by fast diffusion-based mixing in a micromachined flow cell", P. Hinsmann, M. Haberkorn, J. Frank, P. Svasek, M. Harasek and B. Lendl Appl. Spectroc. 55, 241-251 (2001)
[3] "On chip SERRS - in situ substrate formation and improved detection using microfluidics", R. Keir, E. Igata, M. Arundell, W.E. Smith, D. Graham, C. McHugh and J.M. (2002) Anal. Chem. 74, 1503-1508

Participants: 4 FZK/Karlsruhe, 25 TU/Wien, 15 ITC-CNR IBF/Trento, 10 Elettra, 9 BOPT-IT, 17 UPM/Ancona, 22 Strathclyde

GROUP 3 STUDIES OF BIOMEMBRANES AND MEMBRANE PROTEINS

3.1 Peptide/lipid interactions [WP 14]

We plan to develop configurations, technical expertise, and methodologies for studying the interaction of biologically active peptides with lipid membranes at the air water interface. High resolution IRRAS of lipid-protein monolayers will be collected on an already available system, which can easily be moved to any of the SR IR beam line. The technique will also be used in connection with measurements of Surface Enhancement of Raman Signals (SERS), which be developed at Roma La Sapienza. This is a good model for studying peptide-lipid binding, e.g. in the case of amyloid peptides, but also for toxins, or antibiotics. As an even more interesting alternative, we will use single reflection crystals for total internal reflection of supported bilayers (or multilayers) at the water-crystal interface. This method is a much better mimic for all peptides that do, or can, cross the bilayer. Custom made Ge crystals are already available. We will use our technology and expertise for controlling and monitoring the lateral surface pressure during the experiment and during the preparation of the adhered layers using an available automated Langmuir Blodgett deposition system.

We will extend the above approach to a multiple reflections ATR configuration to develop new methods for High Throughput experiments using a multi-channeled configuration. We will prepare a sandwich of an IRE (e.g. a Ge or ZnSe or Si crystal) on top of a multi-channeled teflon

trough. By means of a stepped motor the crystal will be moved in front of a narrowly focused beam so that one channel is illuminated at a time. For example 16 channels will be carved for a 2 cm crystal. The crystal will carry a deposited (and chemically attached) lipid bilayer and will come in contact with a different peptide (or ligand) in each channel. Real time perfusion will be possible. HT experiments of cys- or ala-scanning of peptides with 20 to 40 residues will become easy. Isotope-editing, with the introduction of 13C labelled residues at fixed positions scanning the chain will also be possible. We will exploit our new peptide synthesizer to prepare the material to test by these procedures. Taking advantage of the highly polarized nature of the synchrotron IR beam, it will be possible to investigate the relative orientation of all components with respect to the IRE surface and to each other. For example to study in detail the mechanism of growth of the fibrils in solution and on a lipid surface, by deriving order parameters of the lipid and peptide part and correlating the differences. We will make available this to all other partners. As a possible future development we will pursue the use of a diode array detector for simultaneous recording from each channel.

A further development of this approach will be the construction of a spectroelectrochemical cell for IRRAS to study electrostatic effects on the structure of membranes and membrane proteins. The holder will be constructed to be interfaced to the Bruker IFS 66v interferometer on the beamline at Elettra. It will be designed to display the following features: a) reduce the thickness of the water layer in the optical path to μm size; b) allow potential control across the membrane over a range of a few 100's mV. The system will allow the measurement of electric field induced reorientation of membrane components with monolayer sensitivity. Samples will be monolayers and bilayers supported on a plane gold electrode. The cell will be tested using the following systems: alkanethiol monolayers, phospholipid monolayers and bilayers, polypeptides in membranes, proteins.

[1] "Secondary structure of sea anemone cytolysins in soluble and membrane bound form by infrared spectroscopy", Menestrina G., Cabiaux V. e Tejuca M., Biochem. Biophys. Res. Commun. 254,174 (1999)
[2] "Use of Fourier-transformed infrared spectroscopy (FTIR) for secondary structure determination of staphylococcal pore-forming toxins", Menestrina G., in: Bacterial toxins, methods and protocols, O. Holst ed., Humana Press, (Totowa, New Jersey), 115 (2000)

Participants: 15 ITC-CNR IBF/Trento, 16 ULB/Brussels, 2 Sapienza/Roma, 10 Elettra, 17 UPM/Ancona, 22 Strathclyde

3.2 Studies of membrane electron transfer complexes [WP 15]

The aim is to study aspects of structure and function of two key mitochondrial respiratory enzymes of biomedical interest, complex I and complex III, by use of electrochemically-induced ATR-FTIR redox difference spectroscopy and, through collaboration with Breton (Node #18), to extend the IR measurements into the low frequency region where metal-ligand vibrations are expected. Bovine forms of complexes I and III will be used and simpler model proteins will also be used for initial tests of technology and for aiding band assignments of the more complex proteins. The aim of the work is to understand the coupling between redox changes at the metallic cofactors sites and protonation and other structural changes in the proteins, and to probe the nature of the ubiquinone-binding sites in the proteins. Such work will shed light on the structure/function of these enzymes and might aid understanding of how they may misfunction in human disease states which have been associated with mutations in key subunits. The work will include the development of an electrochemical ATR-FTIR cell compatible with synchrotron IR facilities, and

test of this cell initially with small, soluble redox proteins (ferredoxin, cytochrome c) in the mid-IR range, later extending to the far-IR range and to membrane protein complexes.

Participants: 29 UCL/London, 18 CEA/Saclay, 4 FZK/Karlsruhe, 3 CNRS, 10 Elettra

3.3 Inter-domain movements in membrane transport proteins [WP 16]

The sarcoplasmic reticulum Ca^{2+}-ATPase belongs to the important P-type ATPase family, which is essential in maintaining ion gradients across biological membranes. The cytoplasmic portion of this membrane protein is divided into three domains, which form compact and loose structures depending on the enzyme state. The current understanding is that inter-domain movements are essential for catalysis. For example, vibrations involving the nucleotide binding domain are thought to bring the substrate ATP close to the active site.

In an ongoing study we have discovered that the conformational change of the ATPase upon nucleotide binding depends dramatically on the presence of individual interactions between nucleotide and ATPase. Mainly the extent of conformational change is affected, less its character. An attractive hypothesis is that in the complex with ATP, the nucleotide is tightly sandwiched between nucleotide binding domain and active site domain, closing the cleft between them; whereas the cleft is more open and the structure more loose with other analogues. Different nucleotides should then allow inter-domain vibrations to different extents.

To test this hypothesis, the absorbance spectrum of the ATPase will be investigated using synchrotron radiation in the far infrared region (several cm^{-1}). The lowest frequency modes of the protein are calculated to be those involving movement of the nucleotide binding domain relative to the active site domain. These modes will be studied for the nucleotide-free ATPase and its complexes with non- or slowly hydrolysable ATP analogues. The results will be correlated with results in the mid-infrared region.

A further topic to be addressed within this workpackage is the use of FTIR spectroscopy for an experimental test of the hypothesis of a mobile Rieske domain in complex III of the respiratory chain. This hypothesis has emerged as a result of differences structures observed when the complex is crystallized under different conditions, and has yet to be tested experimentally. The work would then be extended to complex I, where domain mobility hypotheses are emerging presently as a serious possibility.

Participants: 26 SU/Stockholm, 16 ULB/Brussels, 4 FZK/Karlsruhe, 29 UCL/London

GROUP 4 STUDIES OF CELLS

4.1 Culturing techniques on IR transparent substrates [WP 17]

As an enabling technology for further studies, methods will be developed for culturing and maintaining cells on IR transparent supports, and for incorporating such cultures into liquid flow cells. This will facilitate real-time IR spectroscopy studies of single living cells during treatment with various growth factors, therapeutic and other agents.

In advanced experiments lab-on-a-chip devices will be designed that are capable to trap cells temporarily. Once trapped the cells can be flushed with various agents in a highly controlled

fashion. After one experiment the cells will be released and new ones trapped for the subsequent experiment.

Participants: 4 FZK/Karlsruhe, 20 URCA/Reims, 14 Nottingham, 7 BESSY/Berlin, 30 Leeds, 25 TU/Wien

4.2 Tracking biochemical changes in single cells [WP 18]

Part of this activity follows from our current cancer diagnosis programme funded by the "Physics for Healthcare" initiative of the EPSRC. The original programme has concentrated on using the IR spectrum as a "fingerprint" of a single cell. The direction appropriate to this network is to develop an understanding of the nature of changes in cell chemistry, which correlate to cell abnormality. The programme will involve work on cultured cell lines as well as cellular material derived from tissue and exfoliated samples from human subjects. We aim to study the changes in 'precancer' and inflammatory change to gain a complete understanding of the IR spectrum in human disease.

Following on from this, we plan the application of oncology related growth factors to cells and to map their effect by detailed IR spectra. New treatments can be designed to act in a specific fashion e.g. drugs acting on individual growth factors. The effect of such treatments on prepared cells (and where available cells taken from patients who have, or have not responded to a particular modality) will be used to gain understanding of the biochemistry of the cell response to therapeutic agents. Cellular material from smokers and non-smokers will help us build insight into the basis of smoking related cellular change and its relationship to precancer and cancer.

Cell-level studies will also include the monitoring of cell processes such as differentiation, migration and the effects of drugs, approach to the phenomenon of multi-drug resistance in cells, and to study the relationship between morphological alterations and transcriptional activity during processes such as acetylation/deacetylation of histones. Similar studies will be employed to characterize the sequence of biochemical events arising from the exposure of cells to environmental toxins.

A further aspect of this workpackage involves the characterization of redox systems in RIPs-II dependent cell intoxication mechanisms. Ribosome Inhibiting Proteins type II (RIPs-II; e.g. ricin, abrin, volkensin) are heterodimeric proteins of molecular mass of approximately 60-65 kDa, consisting of an enzymatically active A-chain (A = active) connected by a disulfide bond to a B-chain (B = binding). To intoxicate cells RIPs-II must be endocytosed, transported to intracellular organelles, the inter-chain disulfide bridge must be reduced and the enzymatic subunit must translocate to the cytosol. The properties of RIPs-II can thus be exploited to investigate fundamental aspects of cell physiology, in particular the mechanisms involved in the reduction of disulfides and the role of disulfides in protein unfolding and in the retrograde passage of proteins to the cytosol across the membranes of the endoplasmic reticulum or other organelles. We plan to apply high resolution Infrared Synchrotron Radiation (IRSR) to collect spectra of individual proteins and of protein complexes in solution with particular interest on TrX, TrXR, PDI, ricin and RTA-ITs. We will extend this approach by applying IRSR to advanced Infrared Microspectroscopy to identify spectra of above mentioned molecules and protein aggregates by mapping them in living cells and to compare their variations in different conditions of cell stimulation. We will also apply IRSR to map these proteins in 3D cell culture models called Multicellular Tumor Spheroids (MTS).

Participants: 14 Nottingham, 20 URCA/Reims, 4 FZK/Karlsruhe, 2 Sapienza/Roma, 30 Leeds, 7 BESSY/Berlin, 24 UNIVR-DP/Verona, 15 ITC-CNR IBF/Trento

4.3 Fast identification and metabolic studies of bacteria [WP 19]

Studies of bacterial cells are envisaged, probably not at single cell level but at the young microcolony level. By using single detector mid-infrared instrumentation it has been shown that microspectroscopic information can be obtained quite early (after a few hours growth, colony size of 50-100 μm diameter), thus reducing the time needed, usually 1-2 days, to identify the microbe [1]. The use of an IR synchrotron source would permit to obtain IR spectra from colonies as small as 10 μm. Thus, this approach would allow to further reduce the time needed for microbial identification. Furthermore, it would be interesting, with such highly resolved approach, to study the heterogeneity of micro-colonies and the effects of antibacterial/antifungal agents on some important micro-organisms. This information would be very important for early identification and characterisation.

A further goal within this work package is an in-situ study of the interactions between microorganisms and the soil environment. This will lead to an understanding of how bacteria affect the chemistry of their surroundings and to proposed remediation strategies for neutralization of sulfate-containing acidic mining lakes. Bacteria can dramatically modify the rates and products of mineral dissolution reactions by controlling mineral solubility and surface reactivity (e.g. dissolution rates) [2]. Reaction products of interactions between sulfate reducing bacteria and surfaces of sulfate minerals will be determined by using synchrotron based IR microspectroscopy.

[1] Choo et al., Appl. Environ. Microbiol. **67**, 1461-1469 (2001)
[2] Göttlicher J., Gasharova B. (2000) in Applied Minerology in Research, Economy, Technology, Ecology and Culture, Rammlmair et al. (eds.), 2000, Balkema, Rotterdam, Volume 2, 557-560

Participants: 20 URCA/Reims, 19 RKI/Berlin, 4 FZK/Karlsruhe

GROUP 5 STUDIES OF TISSUES / CLINICAL DIAGNOSIS

5.1 Cancer diagnostics, colorectal adenocarcinoma [WP 20]

In an effort to understand and interpret IR spectroscopic changes observed in biomedical IR spectroscopy the group of Dieter Naumann at the Robert Koch Institute has undertaken spatially resolved IR measurements to study distinct human tissues. To give an example, the overall biochemical and tissue architecture of colorectal adenocarcinomas was studied by IR-microspectroscopy using single detector instrumentation and a thermal source (globar), or alternatively, applying the focal plane array (FPA) detector technique (step-scan technique, globar) [1]. The spatial resolution of these experiments was ca. 50 and 10 μm, respectively. The experiments revealed that defined tissue structures can be reproducible differentiated, identified, and classified by spatially resolved infrared microspectroscopy. Artificial Neural Network analysis of the infrared microspectra revealed an overall sensitivity of about 90% and a specificity of 98%. Furthermore, studies at high spatial resolution (FPA technique, 10 μm resolution) on colorectal adenocarcinomas revealed minor spectral changes between the carcinoma and epithelial cells of the crypts (these are the precursor cells of the carcinoma). For a better understanding of these carcinogenesis induced spectral changes it is of utmost importance to establish a correlation between those spectral alterations and well-known tissue parameters such as mitotic index, nuclear-cytosplasmic-ratio, or pathohistological grading of malignancy.

The interpretation of the results of the focal plane array detector technique is however, complicated by a poor spectral signal to noise ratio (S/N). The desired S/N values of > 200 at a spatial resolution of about 10 μm can be reached only in the continuous mode of the FT-IR spectrometer using an alternative ultra-bright light source.

The use of a synchrotron will considerably increase the throughput through small apertures and will therefore permit measurements at a high S/N at apertures close to the diffraction limit. We expect from the synchrotron experiments high quality infrared spectra (S/N > 200) at a spatial resolution of 8 μm. The planned measurements will be performed on sections of colorectal adenocarcinomas, which were already spectroscopically characterized at lower spatial resolution in our laboratory.

Tissue specimens are obtained from the Department of Pathology, Charité Medical Faculty of the Humboldt University, Robert-Rössle-Clinic at the Max-Delbrück-Centrum for Molecular Medicine in Berlin. The tissue specimens will be prepared at Robert Koch-Institut, Berlin and measured at BESSY and/or at ANKA.

[1] Lasch et al, Appl. Spectrosc. 56 (1): 1-9 (2002)

Participants: 19 RKI/Berlin, 7 BESSY/Berlin, 4 FZK/Karlsruhe

5.2 Cancer diagnostics, cervical, oral and laryngeal cancer [WP 21]

The group of Daresbury, Nottingham and Leeds has undertaken a project to investigate the use of infrared microspectroscopy and multivariate data analysis in enhancing the clinical screening programme for cervical and oral cancer [1,2]. Two areas where we wish to extend this research are as follows:

a) Liquid-based methods of cervical smear preparation have recently been adapted for use in preparation of samples for IR analysis. Only a small number of samples prepared in this way have so far been examined, and we would intend that a postdoctoral fellow spend a period of time collecting and analysing data on a wide range of cervical samples, including smears. Work has been done on exfoliated cells and brush biopsies in oral cancer and this should be extended to other sites, such as larynx, to obviate the morbidity resulting from serial biopsies, and exploring the use of liquid based preparations in a wider range of cancer and precancerous conditions.

b) A number of cervical carcinoma-derived and head and neck cell lines are available to the group. These represent a valuable resource, and no attempt has so far been made to characterise these spectroscopically, and to compare them with the cells observed in cervical smears and tissue sections of cervical cancer and pre-cancer.

c) New cell lines can be developed from patients who respond or fail to respond to particular therapies. This initiative will aid the understanding of cancer therapy and may reveal target areas for future initiatives.

[1] Chesters M.A., Tobin M.J., Griffin N.R., Fisher S.E. Oral Oncology VI, 193-194 (1999).
[2] M.J. Tobin, F. Rutten, M. Chesters, J. Chalmers, I. Symonds, S. Fisher, R. Allibone, A. Hitchcock, Investigating the potential for infrared microanalysis in cancer screening, European Clinical Laboratory 21 (4) 20-22 (2002).

Participants: 14 Nottingham, 6 CCLRC/Daresbury, 30 Leeds

5.3 Cancerogenesis and diagnostics of Hodgkin's lymphoma [WP 22]

Intense investigation is currently devoted to precisely define molecular signatures characterizing clinically relevant subtypes of human malignancies, aimed to better define the molecular pathogenesis of neoplasms, to find molecular predictors of survival, and also to search for new therapeutic strategies. The expression profile of neoplastic cells can be thoroughly investigated using different methodological approaches including DNA-microarray technology, 2D-proteomic and in situ analysis of tissue samples. Recently, the exciting possibility of investigating cellular complexity using a microscopic Fourier transform IR (micro-FTIR) technique has been provided by new technological developments of high-brilliance synchrotron radiation sources. An integrated investigation will be carried out on tissue samples obtained for diagnostic purposes (available in a large tissue-bank) using different methodological approaches. Selected types of human B-cell, T-cell and Hodgkin's lymphoma samples, will be investigated and characterised using molecular and immunophenotypic techniques using different probes and antibodies recognizing relevant gene products (especially those involved in the regulation of proliferation, apoptosis, cell-adhesion, as well as products of relevant oncogenes and tumour-suppressor genes). The multi-approach analysis will be carried out on the same tissue samples, and also at the single cell analysis level. FT-IR microspectroscopy analysis will be performed on different cell and tissue compartments, characterised by specific molecular abnormalities previously detected by molecular morphology.

Participants: 13 UNIVR/Verona, 1 INFN-LNF

5.4 Pathogenesis/diagnostics of transmissible spongiform encephalopathies [WP 23]

In this project the distribution of aggregates of the pathological prion protein (PrPsc) and the spatial distribution of other spectral markers in the central nervous system (CNS, e.g. dorsal root ganglia, cerebellum, medulla) of TSE-infected Syrian hamsters shall be investigated by synchrotron IR microspectroscopy. It is well known, that in the course of scrapie or BSE aggregates of PrPsc accumulate in defined anatomical structures of the CNS. In previous studies carried out by the group of Prof. Naumann at the Robert Koch-Institute, Berlin [1] it was shown that distinct histological CNS structures of scrapie-infected hamsters exhibit disease-specific spectral features. Also, in the course of the studies it could be demonstrated that these spectral features were not related to PrP^{sc}, but to certain independent 'surrogate markers', which supports the spread of scrapie pathology in the brain. The IR spectroscopic measurements were performed by using the '*conventional*' IR technique, i.e. with a limited spatial resolution (50 μm). It turned out that a resolution of 50 μm is insufficient to detect microdisperse accumulations of the pathological prion protein PrPsc in or around single neurons. Direct IR spectroscopic analysis of PrPsc (in situ) can be achieved only by increasing the spatial resolution by means of IR synchrotron radiation. The IRIS beamline at BESSY II would provide excellent conditions for carrying out such a study. Furthermore, it is planned to test whether PrPsc from different strains of Scrapie or BSE (Scrapie 263K and Me-7, or BSE-H) can be spectroscopically differentiated. The measurements will be performed on tissue from the CNS (dorsal root ganglia, cerebellum, medulla) from Syrian hamsters. These tissue samples will be prepared at Robert Koch-Institut, Berlin and measured at BESSY and/or at ANKA.

[1] Kneipp et al, J. Neuroscience. 22(8). pp. 2989-2997

Participants: 19 RKI/Berlin, 7 BESSY/Berlin, 4 FZK/Karlsruhe

5.5 Characterization of pathological tissue samples [WP 24]

Infrared and Raman microscopies are vibrational techniques that have proven to be very valuable for acquiring information at the composition, molecular, and environmental levels since they offer a "molecular finger-print" of the studied samples. The high-brightness and high spatial resolution offered by synchrotron sources improve signal-to-noise ratio and consequently reduce data acquisition times. IRSR is thus well suited for infrared microscopy which, coupled to complementary microRaman analysis, will provide high-quality spectral imaging in the biological and bio-medical fields. Pathologies that will be investigated include analysis of normal and aneurismal aortas, identification of abnormalities in thyroid samples, and characterization of gliomas.

Participants: 20 URCA/Reims, 2 Sapienza/Roma, 10 Elettra, 14 Nottingham, 30 Leeds, 13 UNIVR/Verona

5.6 Studies of CNS tissues in neurodegenerative disorders [WP 25]

These researches are conducted in co-operation with Institute of Neurology, Collegium Medicum, Jagiellonian University in Krakow, which studies grounds of pathogenesis of two neurological diseases, i.e. Parkinson's disease (PD) [1] and Amyotrophic Lateral Sclerosis (ALS) [2]. A common pathological hallmark of these diseases is the lost of neurons in selected parts of brain or spinal cord. The pathogenesis of PD and ALS is still not known. There are some putative biochemical mechanisms that may lead to degeneration and atrophy of nerve calls. The most frequently cited theories of degeneration and atrophy of neurons are oxidative stress, excitotoxicity, protein aggregation, and mitochondrial dysfunction. These processes lead to changes in main bio-organic components such as nucleic acids, lipids or proteins, etc. Moreover, the disruption of intracellular homeostasis is reflected by the products of these abnormal reactions or by intensify production of the defence system factors (such us antioxidants). The investigation with the use of SRIR spectroscopy will be applied for topographic and qualitative identification of compounds in thin tissue slices. The major benefits of the experiments will be: 1) Observations of local distribution of biochemical components in affected parts of human brain and spinal cord; 2) Comparison of chemical compounds accumulated in neurones and outside the nerve cells; 3) Recognition, if PD and ALS tissues reveal topographic and quantitative modification of biochemical compounds in comparison with the control group tissues; 4) Analysis of two-dimensional distribution of β-amyloid in structures of the pathological and control tissues; 5) Analysis of correlation between distribution of selected elements and biochemical components accumulated in different part of central nervous system tissue.

References:
[1] Krygowska-Wajs A, Lorens K, Thor P, Szczudlik A, Konturek SJ, Functional Neurology 2000, 15(1): 41-47.
[2] Adamek D, Tomik B, Pichor A, Ka_uza J, Szczudlik A., Folia Neuropathol. 2002; 40(3): 119-124.

Participants: 28 FPNT-UMM/Kracow, 3 CNRS, 21 ESRF

5.7 Studies of human bone [WP 26]

It is widely known that infrared spectroscopy is the technique, which has the ability to characterize and identify molecules and molecular groups or constituents from vibrational spectra.

The infrared spectrum is the molecular fingerprint of biomolecules. Our bio-material is the human bone and the osteopblastic cells. It is known that through out the life osteoclasts resorb bone tissue and osteoblasts replace the bones and regulate the total mass tissue. A number of factors, such as heredity, nutrition, hormones and exposure to sun light influence bone development, growth and repair. Our biomedical sample, which includes cells, proteins and tissues, contains an organic part which absorb in the region 2800-3000 cm^{-1} and the bands are assigned to anti-symmetric and symmetric stretching vibrations of CH_3 (2950 and 2874 cm^{-1}) and CH_2 (2922 and 2854 cm^{-1}) groups of the protein acyl chains. Also there is the amide I band at 1600-1700 cm^{-1} and amide II band at 1500-1560 cm^{-1}. In addition, there is the inorganic part of the bone, hydroxyapatite, which includes the phosphate (PO_4^{3-}) and carbonate (CO_3^{2-}) vibrations, which absorb in the region 900-1250 cm^{-1} (ν_1 and ν_3 phosphate vibrations) and in the 850-900 cm^{-1} region ($\nu_2 CO_3^{2-}$). In addition, there are also the bands at 603 $cm^{-1}$and 560 cm^{-1} (ν_4 of PO_4^{3-}). Therefore, the region of mid-infrared spectrum of 4000-400 cm^{-1} will be examined in our experiments. The far infrared region 400-40 cm^{-1} is also important and will be studied, since our samples contain a lot of water molecules.

The synchrotron infrared (IR) or near infrared beamline is important for our experiments. We need a high signal-to-noise ratio with relatively short data acquisition time. The proposed experiments are fine sliced bone samples and osteoblasts and the IR mapping of the bones could give important information concerning the analysis of the bone along the length of the bone and from the centre to the periphery of the bone. It is important to study the ratio of intensities phosphate/amide I and phosphate/carbonate. The most important information is the collagen structure, which depends on the age as well as of osteoporosis, ageing, etc.

We expect to obtain good quality FT-IR spectra of our samples in the region 2000-400 cm^{-1} in order to observe the protein-mineral part of the spectrum Amide I, Amide II, may be also Amide III (1300 cm^{-1}) and the CO_3^{2-} and PO_4^{3-} mineral bands and see how do change the phosphate/protein ratio as well as the PO_4^{3-}/CO_3^{2-} ratio of intensities in the spectra along the length of the bone and osteoblastic cells.

References:
[1] J. Anastassopoulou, Metal–DNA interactions**,** J. Mol. Structure, in press, 2003
[2] M. Petra, J. Anastassopoulou, A. Dovas, D. Yfantis and T. Theophanides, “Aging of human bones. An infrared study”, Metal Ion Biol. Med. 6, 736 (2000).
[3] T. Theophanides and J. Anastassopoulou, Copper and carcinogenesis, Revews Oncology and Heamatology, 42, 57-64, 2002
[4] J. Anastassopoulou and T. Theophanides, Magnesium-DNA interactions and the possible relation of magnesium to carcinogenesis. Irradiation and free radicals Oncology and Heamatology, 42, 79-91, 2002
[5] T. Theophanides & J. Anastassopoulou, “The use of Fourier transform infrared (FT-IR) spectroscopy in the structural analysis of nucleic acids”, in Biomolecular Structure and Dynamics: Recent Experimental and Theoretical Advances, Eds. G.Vergoten & T Theophanides, Kluwer Academic Publishers, The Netherland, 1997, pp. 273-284.
[6] M. Petra, J. Anastassopoulou & T. Theophanides, Synchrotron Infrared microscopy and imaging studies of paediatric osteonal human bones, submitted

Participants: 27 NTUA/Athens, 21 ESRF, 13 UNIVR/Verona

5.8 Distribution and movement of florigen in plant tissues [WP 27]

We aim to identify the chemical nature and mechanisms of the signals controlling flowering in response to light stimuli known as "florigen". Classic grafting studies demonstrate that "florigen" migrate from the leaves passing through the plant vascular system and is/are transmitted to the shoot apical meristem (SAM) to start its conversion into floral meristem.

This research proposes to combine a genomic and proteomic approach to isolate genes and identify their corresponding molecules controlling flowering in response to light signalling. A popular new genomic technology is the use of high-density DNA microarrays to isolate genes active in specific pathway. The analysis of distinct microarrays will produce a selection of candidate genes to be screened for a role in flower induction.

The c-DNA of these genes will be expressed in bacteria (E. coli) to produce proteins. Other candidate genes may be in-vitro transcribed into RNA. The spectral identity of these molecules (proteins, RNA) will be determined by SR-FTIR. Spectromicroscopy will in addition characterize the localization and movement of these flowering signals in the vascular and/or intercellular spaces. This technique may revel if any of these molecules is a "florigen" molecule travelling from the leaves to the SAM, monitoring molecular responses over time in vivo.

Participants: 1 INFN-LNF, 23 Unimib/Milano

B.4.3 Activities to spread excellence

Spreading of excellence plays a central role in the intentions of the BASIE Network. As already stated in Section B.3, synchrotron infrared light is not being fully exploited by the European biomedical/biophysical research community, and this is at least in part due to lack of awareness of the potential of the techniques described in the present proposal. The BASIE Network brings together not only a critical mass but also a critical breadth of expertise across life science research areas, who, in collaboration with the participating synchrotron physics and optics specialists, will develop and refine synchrotron-based techniques of relevance to life sciences and employ these in cutting edge research. This advertisement by example can be expected to provide the essential stimulus for widespread exploitation of synchrotron infrared light in European life science research.

- Publication of results

The primary activity to spread excellence will obviously be the publication of life science research results in the relevant scientific journals, an activity that will involve all participants of the NoE. The publication of decisive new research insights obtained with synchrotron infrared radiation will disseminate awareness of the techniques and their potential.

- Access to synchrotron facilities

All participating synchrotron facilities provide access to the scientific community through a process of peer review. Thus the specialized equipment for life science research to be developed at synchrotron infrared beamlines within the framework of the present proposal will automatically be available to scientists from outside the NoE for their own research activities.

- Network Publications

The NoE will use both paper-based media and a dedicated web site to disseminate knowledge and provide information on synchrotron-based infrared techniques and their application to biological research questions.

- Workshops/Courses

In addition to the internal NoE workshops described in Section B.4.1, regular workshops and courses open to participants from outside the NoE will be offered. Here, the NoE members will offer the necessary training to other life scientists interested in applying similar techniques in their own research.

- Conferences

The NoE will initiate a series of dedicated conferences on Biological Applications of Synchrotron Infrared in Europe, at which participants from inside and outside the NoE can present their results and engage in discussions with others in the field.

- Student training

Most NoE participants offer student research training at the undergraduate and postgraduate levels. The NoE offers a unique opportunity for the planning of student research projects in the life sciences that include short and long term stays at synchrotron facilities and training in synchrotron-based techniques. This is a dual-function activity, with a promotion of integration through exchange of students for the duration of the student research program, and a spreading of excellence effect as these students apply the knowledge gained in their subsequent careers.

B.4.4 Management activities

Given the dimension of BASIE, with 30 groups and more than 230 researchers participating, it is clear that not all of them can be involved in the management and direction of the network. In order to increase efficiency and speed in taking decisions, we chose an organization scheme as simple as possible.

At the top of BASIE there is a single committee, which unifies in itself all scientific and organizing activities. This is the Executive Board of 10 members, among which the Coordinator has already been selected. The present Board, that has prepared the proposal, will be integrated or partially replaced after 18 months since the start of the project.

The Board is expression of several countries and of all of the competences that are needed to manage the 5 *Workpackage* groups, as listed above in this Section. The Board members will take responsibility for the management activities in the following way:

- 5 members, selected by the Coordinator on the basis of their specific competences, will be in charge for the 5 *Workpackage* groups, one each. They will periodically report to the Coordinator and to the Board on the advancements of the *Workpackage* of competence. They will also advice the Board about the distribution of funds among the different activities of the corresponding *Workpackage*.

- Other 4 members of the Board, again selected by the Coordinator, will take in charge the following activities of the whole BASIE Network of Excellence:

- training and mobility;
- meeting and workshop organization;
- external relationships (including the website and database maintenance);
- financial control.

The 10th member, the Coordinator, will chair the Board. He will promote, control and coordinate all the activities of BASIE in close collaboration with the Board members. He will also fix a procedure to replace integrally or partially, or to integrate if needed, the Board after the first 18 months. The Coordinator will be helped by an Assistant who will be paid for this work on the funds of the network.

B.5 Description of the consortium and of the excellence of the participants

B.5 Description of the consortium and of the excellence of the participants

All participating groups of the BASIE Network of Excellence have shown their excellence in the respective fields and regularly publish in prestigious journals. Some of these groups are leaders of the European science and are renowned worldwide in Life Science researches. In the last years a new era of life science emerged and it may be associated with the availability of large synchrotron radiation facilities. In particular, for life science researches, the existing and the planned European synchrotron radiation infrared capabilities may offer a unique opportunity for Europe to assume the worldwide leadership in this field. This network includes almost all existing and planned IRSR facilities of FT IR spectroscopy and IR microscopy in Europe representing a real link to optimize resources and spread knowledge among countries and Institutions.
The participating enterprise is a large company, which is a world leader in the field of Infrared Spectroscopy and Imaging with FT techniques, and has an eminent position in the market of IR instrumentation in Life Science. Actually, Bruker Optics is the largest European Company producing and supplying FT-IR systems and most of its excellence has been generated by a strong cooperation with researcher based inside the European Community. The European nature of the company is stressed by the presence in Europe of both the R&D department and the production site for the entire world (The facilities are based in Ettlingen - Germany). The major achievements of the Bruker Optics in this field are:

- Entered the field of FTIR spectroscopy in 1974
- First infrared transmission/reflectance microscope in 1982
- Introduction of the IFS 120HR world's highest resolution instrument in 1986
- Introduction of Step-Scan Technology to the Research Community 1988
- Introduction of FT-Raman technology in 1989
- Introduction of dedicated FT-NIR product line in 1993
- Introduction of infrared microbial identification in 1996
- Introduction of MATRIX FT-NIR in 1999

The applicative effort of BRUKER in bioscience has always been testified by a long series of products dedicated to the study of protein secondary structure and the interaction ligand/protein.

Quality of the infrastructure

Participant #1 (Istituto Nazionale di Fisica Nucleare - Laboratori Nazionali di Frascati)
The Laboratori Nazionali di Frascati (LNF) are by far the largest laboratories of the Istituto Nazionale di Fisica Nucleare (INFN), the national agency for nuclear and particle physics research. The construction of LNF, which is also the eldest INFN laboratory, dates back to 1955. Since then, it has become a major focal point for both fundamental and applied particle and nuclear physics - not only in Italy - being a world pioneer in colliding beams research and one of the first synchrotron radiation laboratory in the world with its first experiments in the 60's on the electrosynchrotron. AdA (Anello di Accumulazione), the first electron/positron collider in the world, and ADONE were designed and realised entirely in Frascati. Moreover ADONE, before the shut-down in 1993 was the only synchrotron radiation source in Italy, it has been operational for 24 years, giving access to hundreds of Italian and foreign users. Due to its history, the laboratory hosts a number of facilities and a strong component of applied research is nowadays present thanks to the new synchrotron radiation laboratory that utilizes the radiation emitted by the storage ring DAΦNE.
The total investment cost for the facilities is about 140,000 kEuro, while the annual operating cost amounts to 21,000 kEuro. The tenured staff includes 129 researchers & engineers and 186 technicians & administrative personnel. All personnel is mainly involved in operating the facilities and supporting their users. Personnel annual cost is 8,200 kEuro/year. About 300 researchers from 50 institutions asked to use LNF's facilities during 2002.
The DAΦNE storage ring is used as an intense source of Synchrotron Radiation covering the range from the Infrared up to the soft X-rays. The Synchrotron Radiation facility (DAΦNE-Light) offers access to several experimental stations at three beamlines. One of these is SINBAD (Synchrotron INfrared Beamline At DAΦNE) that takes advantage of the brilliant Infrared photon emission from a bending magnet. Covering the whole IR domain from 10 to 10000 cm^{-1}, SINBAD is equipped with a Michelson interferometer (Bruker IFS55 with a maximum energy resolution of 0.5 cm^{-1}), modified to work under vacuum. These instruments operate in connection with Department of Physics of University La Sapienza (node n.2). An IR microscope is also available using an exit port of the interferometer system while another interferometer station will be installed during 2003. Due to the high current (typically above 1.5 A) at 0.51 GeV of energy the electron ring has unique characteristics as a brilliant SR source for applications in the IR photon range. Marcelli Augusto, its principal investigator, born in June, the 16th 1959 in Roma. He got his degree in Physics at Rome University La Sapienza in 1984. Since the December of 1985 is scientist at the INFN Frascati National Laboratory where he is involved in synchrotron radiation researches. From 1990 to 1996 he was a Contract Professor of Physics at Camerino University, but lectured also in the Universities of Roma I, Roma III and Salerno. He is member of the 'International Scientific Committee of the X-Ray and Inner-Shell Conference Series' (1997-2005) and in 2002 was Chairman with A. Bianconi of the 19th International Conference X-ray and Inner shell Process.
He has been the principal investigator for the INFN-LNF of two network CEE HC&M (CHRX-CT92-0034 and CHRX-CT94-0551). He is also experienced in training young scientist and was sponsor of many fellowships, including one for the Minister of Science and Education of Spain, and several university students.
For two years (1999-2000) he was responsible of the Computing and Natework Data System of the LNF laboratory of the INFN and Member of the INFN Board for the New Data Technologies. He was National Responsible of one experiment in the INFN National Committee V and Coordinator of a project concerning synchrotron radiation applications within the framework of the X Protocol of Scientific and Technological Cooperation between Italy and China. Since 2001 is Consultant of the IHEP (Institute of High Energy Physics) of the Chinese Academy of Science, for synchrotron radiation researches. Now is responsible of the Infrared SINBAD beamline

operational in the new DAFNE-Light laboratory. He is author of more than 160 scientific papers published in international journals.
The genomic and proteomic approach using the Dafne SR-FTIR spectromycroscopy proposed by G. Murtas will likely shed light on the "florigen" hypothesis. This was formulated by Chailakhyan in 1936 and predicted that inductive signal/s travel over a long distance from the leaves to the growing tip of the plant to induce flowering in response to photoperiod. The identification of these molecular flowering signal/s and movement in the plant in-vivo will be a pioneer experiment to study other inductive processes with an important role in the development of multicellular organisms.

Recent relevant publications
- A. Marcelli, E. Burattini, A. Nucara, P. Calvani, G. Cinque, C. Mencuccini, S. Lupi, F. Monti and M. Sanchez del Rio, The beamline SINBAD at DAFNE, Nuovo Cimento D, 20, 463 (1998)
- A. Marcelli, A. Nucara, D. Cannavo', E. Burattini, P. Calvani, G. Cinque, C. Mencuccini, S. Lupi and F. Monti, Infrared beamline SINBAD at DAFNE: expected performances at the sample site, Proceedings of the SPIE 99 Meeting Vol. 3775, 7 (Denver, 1999)
- Giovanni Murtas and Andrew J Millar, How plants tell the time, Current Opinion in Plant Biology 2000, 3:43-46 Current Biology Publications,
- A. Marcelli and C. Iliescu, Infrared synchrotron radiation: from condensed matter to biology researches, Acta Physica Polon. A, 100, 647 (2001)
- Giovanni Murtas, Paul H. Reeves., Shadansu Dash and Coupland, G., Early in short days 4, a mutation in Arabidopsis that causes early flowering and affects the expression of the flowering time gene FLC, Development 2002 , 129 (23): 5349.

Participant #2 (Università La Sapienza – Dipartimento di Fisica)
The group at Università La Sapienza has a long experience in the use and construction of Infrared Synchrotron Radiation beamlines. The principal investigator, P. Calvani, has been Coordinator of the EU HC&M network "Development of Infrared synchtron Radiation and applications to condensed matter studies", funded with 250,000 Ecu in 1994-97. A. Nucara and S. Lupi have played a leading role in the design of the Infrared beamlines of DAFNE (Frascati), ELETTRA (Trieste), and Villigen (the latter one by appointment of the ETH Zurich).
The group is responsible for the experimental station on the IRSR beamline of DAFNE and for the whole IRSR beamline under construction at ELETTRA. Moreover, it occupies 250 mq of laboratories at the Physics Dept. of University La Sapienza. They host 4 interferometers for the infrared, 1 infrared microscope, 4 monochromators for visible and UV rdiation, 1 micro-Raman spectrometer, 1 Energy dispersive X-Ray diffractometer, 1 Atomic Force Microscope, 2 Apparata for microwave spectroscopy, 2 fully equipped laboratories for the treatment of biological samples. The Raman group is led by P. Postorino, who is also an expert of high-pressure spectroscopy. Paola Maselli is responsible for the computing facilities.
Studies on biological materials are directed by A. Bonincontro, C. Cametti, and Agostina Congiu, who has been Coordinator of the Italian National Network "Biomolecules and Synchrotron Radiation" from 1998 to 2001. Focus is on the ionic transport in the membranes, on the interactions between the cell and heavy metal ions, and between amphiphylic molecules and DNA.
In the last five years, the group of La Sapienza has published 115 papers on international journals in Infrared, Raman and Microwave Spectroscopy of condensed matter and biological materials, of which 10 on top journals.

Recent relevant publications

- P. Calvani, G. De Marzi, P. Dore, S. Lupi, P. Maselli, F. D'Amore, S. Gagliardi, and S-W. Cheong, Infrared absorption from Charge Density Waves in magnetic manganites, Physical Review Letters 81, 4504 (1998).
- S. Lupi, P. Maselli, M. Capizzi, P. Calvani, P. Giura, and P. Roy, Evolution of an infrared polaron band through the phase diagram of NCCO, Physical Review Letters 83, 4852 (1999).
- A. Lucarelli, S. Lupi, M. Ortolani, P. Calvani, et al.,, The phase diagram of LSCO probed in the infrared, Physical Review Letters 90, 037002 (2003).
- M. Girasole, A. Cricenti, R. Generosi, A. Congiu Castellano, F. Boffi, A. Arcovito, G. Boumis , and G. Amiconi, Atomic force microscopy study of erythrocyte shape and membrane structure after treatment with a dihydro-pyridine drug, Applied Physics Letters 76, 3650 (2000).
- L.G. Quagliano, A. Congiu Castellano and E. El Gawhary, Effects of Cd^{2+} ions on living pancreatic cells: a Raman microspectroscopic study, in: Spectroscopy of biological molecules: new directions, ed. by J. Greve, G.J. Puppels and C. Otto, Kluwer Acad., 529 (1999)

Participant #3 (LURE - Centre Universitaire Paris Sud)

LURE is one of the pioneer laboratories in the field of synchrotron radiation. The emitted light, from the infrared to hard x-rays is used to study the structural as well as the optical, electronic and magnetic properties of chemical, physical and biological matter. 42 beamlines with different dedications, spread over two storage rings (DCI and SUPERACO) are open to a wide community of users. The techniques employed at the various beamlines concern: surface science, physical science, chemistry and biology. Surfaces, *i.e*. the last few atomic layers of a solid or liquid, are usually characterised by quite different features from those of bulk material. Understanding these properties is important for technology since a large variety of processes (catalysis, electrochemistry, lithography, self-organisation, diffusion, electronic transport, magnetism,…) are linked to surface phenomena. All these phenomena are investigated at various experimental stations in great breadth. Investigations in surface science presently make use of tunable light of high-energy resolution (high energy photoemission for the study of the electronic properties) and production of polarised light (magnetic dichroism). Investigations to combine circular dichroism with electron spin photoemission are under preparation. The host team concerned by the project is in charge of a far Infrared beamline dedicated to spectroscopy. The team has been at the origin of the discovery of a new source of infrared emission, namely the Edge radiation (see list of publication). This emission mechanism is now exploited in third generation light sources. Part of the LURE beamline will be transferred on the future third generation synchrotron radiation ring SOLEIL. The beamline AILES (Advanced Infrared Line from Edge for Spectroscopic studies) has been validated for construction in the first phase of development and its development led by the group leader will be completed in year 2007. The effort of the team is concentrated on the role of water in various systems including biological objects. Our team is known for its expertise in far infrared spectroscopy of various systems (see list of publications) The team leader, Dr. P. Roy is responsible for the Chemical science department at LURE and has a good experience in conducting projects. She has been the local coordinator for two EEC Networks. She is also experienced in training young scientist as she conducted 5 PhD students, 6 post-docs and dozen of MSc university students.

Recent relevant publications

- Boissière, J. B. Brubach, A. Mermet, G. de Marzi, E. Prouzet, P. Roy, Water confined between lamellar structures of AOT surfactants, An infrared investigation, J. Phys. Chem. B, 106 (5), 1032-1035, (2002).
- J.B. Brubach, A. Gerschel, V. Stradler, M.P. Kraft and P. Roy, Dependence of Water Dynamics upon Confinement Size, J. Phys. Chem. B, 105, 430 (2001).

- P. Roy, M. Cestelli, O. Marcouillé, A. Paolone, P. Giura, Y.-L. Mathis and A. Gerschel, Spectral distribution of infrared synchrotron radiation by an insertion device and its edges: A comparison between experimental and simulated specta, Phys. Rev. Lett. 84 (2000), 483.
- J. Orphal, Fourier Transform IR, invited review article, in Encyclopedia of Life Sciences, Nature Publishing Group, Macmillan Ltd., London, www.els.net, 2002.
- Y.-L. Mathis, P. Roy, B. Tremblay, A. Nucara, S. Lupi, P. Calvani, and A. Gerschel, Magnetic field discontinuity as a new brighter source of infrared synchrotron radiation, Phys. Rev. Lett. (1998), 80(3).

Participant #4 (Forschungszentrum Karlsruhe GmbH)

The new synchrotron ANKA (ANgstrøm source KArlsruhe, www.fzk.de/anka) is a 2.5 GeV electron facility located in the South West of Germany, on the campus of the Forschungszentrum Karlsruhe (FZK). The FZK with some 3,500 employees and 22 institutes is one of the biggest research centers within the Hermann von Helmholtz Association of National Research Centers (HGF). The Federal Republic of Germany and the State of Baden-Württemberg fund the Forschungszentrum Karlsruhe jointly. At the FZK, the Institute for Synchrotron Radiation (ISS) provides operation of the synchrotron facility and support for the users. ANKA is fully operational since the beginning of 2002. As a research infrastructure within the HGF, ANKA provides beamtime for fundamental and application-oriented research, to users from Germany and from abroad. Beamtime is available upon submission of proposals that are evaluated by external experts in a peer review process. A significant share of the beamtime is provided to industrial customers through our marketing subsidiary ANKA GmbH.

Eight beamlines at ANKA with 10 experimental stations are operational. The analytical beamlines cover techniques from spectroscopy to diffraction and are taking advantage of the large spectral range from far infrared to hard X-rays emitted by the bending magnets. Three more beamlines are devoted to lithography and are installed in a clean room area. One further X-ray beamline is operated by the Max-Planck society. Three beamlines are in the construction phase: - WERA, a soft X-ray beamline, - INE a beamline for actinide research, - SUL-X, an X-ray beamline for environmental studies, based on our first insertion device.

The dipole edge radiation based infrared beamline (ANKA-IR) is equipped with two main experimental set-ups. Each set-up is centered around one Fourier transform infrared (FTIR) interferometer, a Bruker IFS 66v/S. Both interferometers cover the entire spectral range from far- to near-infrared (4-10000 cm^{-1}) with a resolution down to 0.1 cm^{-1}. To avoid water and CO_2 absorption, these spectrometers are evacuated. One spectrometer is connected to an ellipsometer equipped with a cryostat for measurements at temperatures ranging from 4 to 475 K. Further set-ups for studies of electrochemical interfaces and studies at high-pressure will be installed on the same spectrometer in 2003. The second spectrometer is connected to an infrared microscope covering the mid- and near-infrared domain. A bolometer is being adapted to the microscope to extend the spectral range towards lower frequencies. A near UV fluorescence system will be installed in spring 2003 to increase sample contrast. To avoid water and CO_2 absorption, the microscope is N_2 purged. Different objectives are available for transmission and reflection measurements. For sample positioning, a motorized stage with 1 μm resolution is used.

The construction of two further dipole edge radiation based IR beamlines is considered at ANKA to answer the strong request of users for additional beamtime. Two more dipole vacuum chambers are already equipped with the necessary large output port and it is foreseen to install front-end components during next winter shutdown.

The host team detailed below consists of three beamline scientists running the existing ANKA-IR beamline and one scientist specialist of insertion devices.

Dr. Mathis has 12 years of experience in the field of infrared synchrotron radiation beamline development. His main achievements were the characterization of alternate sources like undulator

and bending magnet edges for IR light production at LURE and ANKA [Boris, 2002] synchrotrons, and their utilization for spectroscopy at electrochemical interfaces. He contributed to make the edge radiation sources popular amongst the synchrotron community by building and operating the ANKA-IR beamline, the first dipole edge radiation based beamline covering the entire spectral range from far- to near-IR. Since 1997 he has been the beamline scientist at the ANKA-IR facility.
Dr. Moss has 16 years of experience in the field on biomolecular infrared spectroscopy. His main achievements were the introduction of electrochemical techniques and microstructured rapid-mixing cells [Masuch, 2003] for FTIR difference spectroscopy of proteins. These tools have been widely adopted within the biomolecular FTIR community for studies of dynamic processes and molecular mechanisms in proteins. His own studies have mostly been devoted to the redox-active proteins and protein complexes of the photosynthetic and respiratory electron transport chains. Since 2001 he has been responsible for biological applications at the IR beamline [Moss, 2002].
Dr. Gasharova has 11 years of experience in mineralogy and crystallography. Her scientific background is mainly related to structural study of minerals by vibrational spectroscopy and X-ray diffraction combined with characterization of crystal surfaces and investigation of mineral surface reactions by Atomic Force Microscopy [Becker, 2001]. Her main achievements were in interdisciplinary research in the field of environmental mineralogy. Since 2002 she has been responsible for environmental applications at the ANKA-IR beamline.
Dr. Rossmanith has more than 30 years of experience in the field of particle accelerator physics. His main recent achievement was the invention of a revolutionary superconductive in-vacuo mini-undulator for light production at synchrotrons [Moser, 2002]. It has been successfully tested at the MAMI linear accelerator in Mainz, Germany. A version adapted for storage rings will be installed at ANKA in 2003 for use in the X-ray spectral range. Several synchrotron and free electron laser facilities world wide expressed their will to install duplicates of the devices. Singapore Synchrotron Light Source received already one. Since 1997 Dr. Rossmanith has been responsible for insertion devices development at the ANKA facility.

Recent relevant publications
- A.V. Boris, D. Munzar, N. N. Kovaleva, B. Liang, C.T. Lin, A. Dubroka, A.V. Pimenov, T. Holden, B. Keimer, Y.-L. Mathis and C. Bernhard, Josephson plasma resonance and phonon anomalies in trilayer $Bi_2Sr_2Ca_2Cu_3O_{10}$, Phys. Rev. Lett. 89, 277001 (2002)
- Masuch, R. and Moss, D. A. (2003) Stopped flow apparatus for time-resolved FT-IR difference spectroscopy of biological molecules in ^1H_2O. Appl. Spectrosc. (submitted)
- Moss, D.A. and Mathis, Y.-L. (2002) Infrared imaging at diffraction-limited spatial resolution with ANKA, the new synchrotron light source in Kalrsruhe. Shedding New Light On Disease, Reims, France, June 23-27, 2002
- U. Becker and B. Gasharova, AFM observations and simulations of jarosite growth at the molecular scale: probing the basis for the incorporation of foreign ions into jarosite as a storage mineral, Phys. Chem. Minerals 28, 545-556 (2001)
- H. O. Moser and R. Rossmanith, Magnetic field of superconductive in-vacuo undulators in comparison with permanent magnet undulators, Nucl. Instr. Meth. A490 (2002) 403

Participant #5 (Swiss Light Source (SLS) and Paul Scherrer Institute)
The Swiss Light Source (SLS) at the Paul Scherrer Institut is a third-generation synchrotron light source. With an energy of 2.4 GeV, it provides photon beams of high brightness for research in materials science, biology and chemistry. SLS should make possible state-of-the-art optical investigations. This team proposed the realization at SLS of an IR beamline where a wealth of optical techniques (far-infrared (FIR) Fourier spectrometry,IR-microscopy, IR-ellipsometry, tunable IR-ultraviolet (UV) source for Raman spectroscopy etc.) will be possible. The facility should be exploited for a variety of projects in biophysics or in order to address topics of the area

of interest for biology. A first technical project, summarizing this feasibility study, has been prepared and it can be consulted by the link "http://www.solidphys.ethz.ch/spectro/new.htm". Presently, the beamline is in the construction phase and the goal is to have an operative facility by the end of 2004.
The Zurich-PSI node has a long and well-recognized experience in the application of optical spectroscopic methods. Leonardo Degiorgi, its principal investigator, has been elected Assistant Professor at the Laboratorium für Festkörperphysik of ETH Zurich, as from August 1, 2000. Leonardo Degiorgi was born on December 18th 1960 in Lugano. He studied Physics at ETH Zurich from 1980 to 1985 and got his PhD. degree in 1990 under the supervision of Prof. Dr. P. Wachter. After a two years (1990-1992) Postdoc stage at the University of California in Los Angeles, he took over the leadership of the optical spectroscopic group at ETH Zurich, as associate researcher (Oberassistent). In 1996 he got the degree of lecturer in physics (Privatdozent). In November 1999 he was promoted to the Assistant Professor position, after he was appointed in 1998 as Associate Professor at the High Magnetic Field National Laboratory in Tallahassee, Florida. Magneto-optical investigation of strongly correlated systems and of novel materials with peculiar ground states is the main topic of research. In 1990 he was awarded with the ETH-prize for his PhD. thesis, in 1992 with the ETH-Tokyo Institute of Technology prize and in 1996 with the University Latsis prize. From 1994 to 2000 he was also holder of the Profil 2 scholarship of the Swiss National Science Foundation.

Recent relevant publications
- *Ch. Bosshard, R. Spreiter, L. Degiorgi and P. Günter, Infrared and Raman spectroscopy of the organic crystal DAST: Polarization dependence and contribution of molecular vibrations to the linear electro-optic effect, Phys. Rev. B 66, 205107 (2002)*
- R. Gaal, J.-P. Salvetat, J.-M. Bonard, L. Thien-Nga, S. Garaj, L. Forro', B. Ruzicka and L. Degiorgi, Pressure and doping dependence of electronic properties of carbon nanotube ropes, Electronic properties of novel materials - molecular nanostructures, Eds. H. Kuzmany et al., AIP Vol. 544 (2000), p. 404
- L. Forro', J.-P. Salvetat, J.-M. Bonard, R. Basca, N.H. Thomson, N.H., S. Garaj, L. Thien-Nga, R. Gaal, A. Kulik, B. Ruzicka, L. Degiorgi, A. Bachtold, C. Schönenberger, S. Pekker, K. Hernadi, Electronic and mechanical properties of carbon nanotubes, Science and Application of Nanotubes, Tomanek and Enbody (Eds.), Plenum Publishers, New York 2000
- B. Ruzicka, L. Degiorgi, R. Gaal, L. Thien-Nga, R. Bacsa, J.-P. Salvetat and L. Forro, Optical and dc conductivity study of potassium-doped single-walled carbon nanotube films, Phys. Rev. B61, R2468 (2000)

Participant #6 (Council for the Central Laboratory of the Research Councils)
Daresbury Laboratory is the site of the UK's synchrotron radiation source, the SRS. This facility provides access to a diverse portfolio of scientific instruments including beamlines for protein crystallography, non-crystalline diffraction, infrared spectroscopy, and UV circular dichroism. In addition to the SRS, Daresbury Laboratory is also host to other research facilities. These including a time resolved fluorescence imaging laboratory, a powerful scanning transmission electron microscope research unit "SuperSTEM" and the recently installed HPCx super computer. The laboratory is also noted for its achievements in detector development, and is host to ASTec, the UK centre for accelerator physics.
The SRS, the UK's only synchrotron light source, supports the needs of a wide variety of researchers. Two synchrotron infrared facilities are currently installed on the SRS, the first of which was originally designed for far-IR surface science, but has been modified for infrared microspectroscopy. A new dedicated infrared microspectroscopy beamline is currently being commissioned, and is expected to be available for users in late 2003. The facility attracts infrared users from a diverse research community, including earth science, biomedicine, food science, and

solid state chemistry. In collaboration with Lancaster University, we are currently exploring the use of near-field spectroscopic methods with synchrotron radiation.
The principal investigator from Daresbury, Mark Tobin, studied Genetics and Cell biology at Manchester University, and gained a PhD with the Medical Research Council in 1994. He currently has research collaborations in the clinical application of vibrational spectroscopy, and in the use of time resolved fluorescence single molecule imaging in biology. At Daresbury Laboratory he has been responsible for the development of synchrotron infrared microspectroscopy.

Recent relevant publications
- M.J. Tobin, F. Rutten, M. Chesters, J. Chalmers, I. Symonds, S. Fisher, R. Allibone, A. Hitchcock. Investigating the potential for infrared microanalysis in cancer screening, European Clinical Laboratory 21 (4) 20-22 (2002).
- L. Bozec, A. Hammiche, M. J. Tobin, J. M. Chalmers, N. J. Everall and H. M. Pollock Near-field photothermal Fourier transform infrared spectroscopy using synchrotron radiation, Meas. Sci. Technol. 13 1217-1222 (2002).
- L.G. Benning, V. Phoenix, N. Yee, M. Tobin, K.O. Konhauser, B.W. Mountain, Molecular Characterisation of cyanobacterial cells during silicification: a synchrotron-based infrared study. In: Geochemistry of the Earth's Surface, 6 259-263 (2002).
- P. Bouchon, P. Hollins, M. Pearson, D.L. Pyle, M.J. Tobin, Oil distribution in Fried Potatoes Monitored by Infrared Microspectroscopy. Journal of Food Science 66 (7) 918-923 (2001)
- M. A. Chesters, M.J. Tobin, N.R. Griffin, S.E. Fisher. Infrared spectroscopy – may it have a role in the diagnosis of oral cancer?, Oral Oncology VI, 193-194 (1999).

Participant #7 (Berliner Elektronenspeicherring-Gesellschaft für Synchrotronstrahlung m.b.H.)
The IR facility at BESSY was commissioned in 2002. The large acceptance beamline IRIS provides high brilliant infrared synchrotron radiation from the near to the far infrared wavelength region. Running the storage ring in a detected "low alpha" mode, an unique feature of BESSY, the beamline also provides coherent far infrared (THz and sub-THz) radiation, which is 10.000 times brighter than conventional synchrotron radiation. This opens a new region in the electromagnetic spectrum to explore the properties of biological relevant systems by means of FT IR spectroscopy. The beamline IRIS is equipped with two infrared Fourier transform spectrometers, an infrared microscope as well as an ellipsometer (the later is run by ISAS, Berlin) for life and material science investigations. Among the planned scientific activities are structural-biological researches by means of Fourier transform spectroscopy and time resolved investigations of biological systems as well as new developments in spectroscopic methods. It is planned to perform investigations on proteins, on biological tissues down to single cells, and to investigate protein crystals and thin biological films applying infrared ellipsometry with a high lateral resolution.
The coordinator of node 7 is Dr. Ulrich Schade, also coordinating the infrared activities at the beamline IRIS at BESSY. His expertise covers FT-IR spectroscopy, spectral IR ellipsometry and THz spectroscopy. Recent activities included the development of the large acceptance infrared beamline.

Recent relevant publications
- Peatman, W.B., and Schade U., A Brilliant Infrared Light Source at BESSY, Rev. Sci. Instr., 72, 1620-1624 (2001).
- Schade, U., Röseler, A., Korte E.H., Bartl, F., Hofmann K.P., Noll, T., and Peatman W.B., New infrared spectroscopic beamline at BESSY II, Rev. Sci. Instr. 73, 1568-1570 (2002).

- K.H. Korte, U. Schade, W.B. Peatman, A. Röseler, D. Tsankov, K. Hinrichs, Infrared ellipsometric view on monolayers: towards resolving structural details, Anal. Bioanal. Chem. 374, 665-671 (2002).
- K. Hinrichs, M. Gensch, A. Röseler, E.H. Korte, K. Sahre, K.-J. Eichhorn, N. Esser, and U. Schade, FT-IR synchrotron ellipsometry for studying the anisotropy of small organic samples, submit. to Appl. Spec. (2002).
- M. Abo-Bakr, J. Feikes, K. Holldack, P. Kuske, W. B. Peatman, U. Schade, and G. Wüstefeld Brillian, Coherent Far-Infrared (THz) Synchrotron Radiation, Phys. Rev. Lett. 90(9), 0948011-0948014 (2003).

Participant #8 (Gesellschaft zur Förderung der Spektrochemie und angewandten Spektroskopie e.V., Dortmund)

The Institute of Spectrochemistry and Applied Spectroscopy, with sections in Dortmund and Berlin, is a scientific research establishment of the Wissenschaftsgemeinschaft Gottfried Wilhelm Leibniz. ISAS works on solving analytical problems. For this it develops new or improves existing measurement principles, measurement techniques, analytical techniques, methods and instruments. Spectroscopical methods and the analytical contribution for solving problems in the areas of material and life sciences are the priority of the research and development at ISAS. The Infrared group of the ISAS Berlin works on development of infrared spectroscopic methods for the characterization of anisotropic layers. Organic and inorganic films down to a single atomic layer are accessible and assertions regarding crystalline order and molecular orientation are available. The laboratory equipment includes a FT-IR spectrometer (Bruker IFS 55) with the necessary accessories for polarization dependent FT-IR spectroscopy and among those a variety of different in-house-designed ellipsometers. ISAS Berlin, and its predecessor in the GDR the "Zentralinstitut für Optik und Spektroskopie", has made seminal contributions to the development of FT-IR ellipsometry into a ready-to-use technique and by that gained unique experience in modelling and evaluating the infrared ellipsometric measurements for the purpose of revealing structural details. In recent years BESSY cooperated with ISAS and the Charité Berlin in a joint effort to set up the infrared beamline IRIS. In particular, ISAS set up a specially designed spectroscopic infrared ellipsometer for the purpose of ellipsometric measurements of samples in the submillimeter range. ISAS is using its expertise in the field of infrared ellipsometry to adapt the measurement strategies and data evaluation for investigations of aligned biological films and protein crystals. The coordinator of the node, Karsten Hinrichs, is born in 1964 and is project leader for the synchrotron activities at ISAS-Berlin. Its recent scientific activities are devoted to the analysis of structural properties in anisotropic organic samples with infrared spectroscopic techniques.

Recent relevant publications

- U. Schade, A. Röseler, E.H. Korte, M. Scheer, W.B. Peatman, Measured characteristics of infrared edge radiation from BESSY II, Nucl. Instr and Meth A 455, 476 (2000)
- U. Schade, A. Röseler, E.H. Korte, F. Bartl, K.P. Hofmann, T. Noll, W.B. Peatman, New infrared spectroscopic beamline at BESSY II, Rev. Sci. Instr. 73, 1568 (2002).
- K. Hinrichs, D.Tsankov, E.H. Korte, A. Röseler, K. Sahre, K.-J. Eichhorn, Comparative Study of an Anisotropic Polymer Layer by Infrared Spectroscopic Techniques, Appl. Spectrosc. 56, 737 (2002)
- E. H. Korte, , U. Schade, W.B. Peatman, A. Röseler, D. Tsankov, K. Hinrichs, Infrared ellipsometric view on monolayers: towards resolving structural details, Anal. Bioanal. Chem. 374, 665 (2002).
- D. Tsankov, K. Hinrichs, E.H. Korte, R. Dietel and A. Röseler, Infrared ellipsometry of Langmuir-Blodgett films on gold – towards interpreting the molecular orientation, Langmuir 18, 6559 (2002).

Participant #9 (Bruker Optics Srl)
Bruker Optics is the largest European Company producing and supplying FT-IR systems and the leadership has been generated by a strong cooperation with researcher based inside the European Community. Bruker Optics Srl is an instrumentation company bound to his headquarters Bruker Optik Gmbh and serves the research community in Italy providing spectroscopic solution based on FT-technology.
The implementation and the development of our instruments (already used in many synchrotron IR lines for the performances already available) within a network, would have the clear goal of producing instruments able to satify more and more specific requirements of the researchers who work with them. Specifically our engagement would be concentrated in two different directions:

- Implementation of fast response detectors in the FIR region
- Optimisation of an existing FT-IR optical bench for a synchrotron light

From previous cooperation with the nodes of Laboratori Nazionali di Frascati (LNF-INFN), University of Roma "La Sapienza", Elettra in Trieste, Bruker Optics has already developed special competence on FT-IR applied to synchrotron radiation improving his competence in the field of vacuum adaptation of optical elements for FT-IR benches and with the nodes of Università Milano Bicocca, ITC-CNR of Trento has already cooperated to develop special attachments for protein analysis and protein- and peptide-lipid interaction.

Technical relevant information
- http://www.bruker.de/optik/index.html
- http://www.bruker.it
- www.brukeroptics.com

Participant #10 (Elettra Sincrotrone Trieste)
Elettra is a large multidisciplinary Synchrotron Light Laboratory, open for service to researchers in diverse basic and applied fields such as materials and life sciences, physics, chemistry and geology. Elettra has been built and is managed by "Sincrotrone Trieste", a non-profit Share Company (Società Consortile per Azioni) recognized of national interest by a State Law. Shareholders are local Authorities and national Research Institutions and Industrial partners. The main local shareholder is the Research Area (AREA Science Park). The two main national Research Institutions of reference are the National Institute for the Physics of Matter (INFM) and the National Research Council (CNR). The laboratory offers an international and competitive environment to researchers from Universities, national Laboratories and enterprises from all over the world. Elettra operates a storage ring at 2.0 or 2.4 GeV and is currently equipped with ultra-bright light sources in the spectral range from UV to X-rays. As part of its ongoing expansion program, the laboratory has designated as its first priority the construction of a new source of radiation covering the spectral range from the far to the near IR. The project is driven by a collaboration between Elettra and Universita' di Roma "La Sapienza". The beamline will extract both constant field and edge radiation from a bending magnet source and will find its application as a source for both spectroscopy and microscopy in several disciplines. End instrumentation will include a Bruker Hyperion 2000 microscope interfaced to a Bruker IFS 66v/S interferometer. The design stage of the project has been completed and construction is due to start in summer 2003. Additional information can be found at the URL:
http://www.elettra.trieste.it/experiments/beamlines/irsr/index.html.
The principal investigator of node 10, Luca Quaroni, has been hired by Elettra in Autumn 2002 with the purpose of coordinating beamline applications in the fields of biology, chemistry and

biochemistry. His background and interests focus on the application of spectroscopic techniques to the study of biochemical problems, with particular reference to applications of vibrational spectroscopy to enzymology, protein interaction with surfaces and membranes, bioinorganic chemistry, and reaction mechanisms. In addition, the members of node 10 display a wide range of interdisciplinary expertise complementing the competences required for node research. This includes expertise in microlithography (Drs. Di Fabrizio e Tormen), X-ray microscopy (Drs. Kiskinova and Kaulich), laser spectroscopy (Dr. Danailov), surface science (Drs. Kiskinova and Casalis) and optics (Dr. Cocco). The node has access to all the facilities present at Elettra, including workshops, biochemical, chemical, crystallographic and microlithografic laboratories. Finally the node enjoys extra-network collaborations throughout the scientific community in Trieste, including access to instrumentation and expertise at the Tecnologie Avanzate Superfici e Catalisi (TASC) laboratory, International Center for Theoretical Physics (ICTP), International Centre for Genetic Engineering and Biotechnology (ICGEB), International School for Advanced Studies (ISAS,SISSA), and Università di Trieste.

Recent relevant publications

- S. Smoukov, L. Quaroni, X. Wang, P. Doan, B.M. Hoffman, L. Que, Jr. ENDOR Evidence for a Hydroxo-Bridge Nucleophile Involved in Catalysis by a Dinuclear Hydrolase. J. Am. Chem. Soc., 124 (11), 2595-2603 (2002).
- S. Lin; L. Quaroni; W.S. White; T.M. Cotton; G. Chumanov.Localization of Carotenoids in Plasma Low-Density Lipoproteins Studied by Surface-Enhanced Resonance Raman Spectroscopy. Biopolymers 57(4), 249-256 (2000).
- L. Quaroni; W.E. Smith; Nitration of an Internal Tyrosine of Cytochrome C Probed by Resonance Raman Scattering. Biospectroscopy 5 (5, Suppl.) S71-S76 (1999).
- L. Vaccari, M. Altissimo, E. Di Fabrizio, F. De Grandis, G. Manzoni, F. Santoni, F. Graziani, A. Gerardino, F. Perennes, P. Miotti, Design and prototyping of a micropropulsion system for microsatellites attitude control and orbit correction, J. Vac. Sci. Technol. B 20(6), Nov/Dec, 2793-2797 (2002).
- Gunther S, Kaulich B, Gregoratti L, Kiskinova M, Photoelectron microscopy and applications in surface and materials science. Progr. Surf. Sci. 70 (4-8): 187-260 (2002).

Participant #11 (University of Palermo - Department of Physical and Astronomical Sciences)

The Palermo research unit will operate at the Department of Physical and Astronomical Sciences (DSFA) of the University of Palermo. DSFA is a medium sized department (about 60 researchers, 10 technical and administrative staff units and 20 PhD students). Several research lines are operative at DSFA, ranging from astrophysics to solid state physics (both theoretical, simulative and experimental), to biophysics. Our Molecular Biophysics group has been operative in the field of the spectroscopy of hemeproteins and metalloproteins for more that 15 years. We have developed new spectroscopic methods to investigate the dynamic properties of the active site of proteins: this has qualified our group as one of the leading groups in the field of protein dynamics studied with temperature-dependent spectroscopic methods. Very recently, our group has been active also in the field of protein-matrix interactions, with special attention to trehalose coating and sol-gel trapping of proteins. Our laboratory is fully equipped for spectroscopic (optical and NIR absorption, FTIR, fluorescence, CD) measurements in the temperature interval 4 – 365 K.

Our group has a well established experience on investigations of the dynamic properties of proteins by means (among other experimental techniques) of FTIR spectroscopy in the temperature interval 5 – 350 K. In particular, the following research fields have been investigated in the recent past:

- Energy landscape and dynamic properties of myoglobin embedded in trehalose glasses and their relationship with the dynamics of the matrix.
- Active site dynamics of metalloproteins in solution and encapsulated in silica hydrogels.

- Structural and dynamic properties of water confined in silica hydrogels.
- Conformational changes and aggregation processes of native and pathological proteins; dependence upon protein-solvent interactions.

Recent relevant publications

- A. Cupane, M. Levantino and M.G. Santangelo, Near infrared spectra of water confined in silica hydrogels in the temperature interval 365-5 K, J. Phys. Chem. B 106, 11123, 2002;
- M.G. Santangelo, M. Levantino and A. Cupane, Ferricytochrome c encapsulated in silica hydrogels: correlation between active site dynamics and solvent structure, Biophys. Chem. 103, 67-75, 2003;
- R.J. Lippski, E. Unger, W. Dreybrodt, V. Militello, M. Leone and R. Schweitzer-Stenner: Vibrational analysis of Ni(II) and Cu(II)-octamethylchlorin by polarized resonance Raman and FTIR spectroscopy, J. Raman. Spectr. 32, 521-541, 2001;
- F. Librizzi, C. Viappiani, S. Abbruzzetti and L. Cordone, Residual water modulates the dynamics of the protein and of the external matrix in trehalose coated MbCO: an infrared and flash-photolysis study, J. Chem. Phys. 116, 1193-1200, 2002.
- F. Librizzi, E. Vitrano and L. Cordone, Dehydration and crystallization of trehalose and sucrose glasses containing carbonmonoxy myoglobin, Biophys. J. 76, 2722-2734, 1999.

Participant #12 (University of Perugia - Department of Physics)

This group is, since many years, involved in the study of biological macromolecules and their aggregates, in particular, in the characterization of the structure, dynamics and thermal stability of biomolecules in aqueous and non-aqueous solvents. The scientific facilities available at the University of Perugia for this research program are :

- Equipment for Raman spectroscopy
- FTIR-VIS-UV spectrophometer
- Spectropolarimeter Jasco mod.810.
- Differential scanning calorimetry. Calorimetric measurements will be performed with a high-sensitivity Differential Scanning Calorimeter (SETARAM Mod. G III) specific for measurements on biological systems.

At the moment our group is involved in several Neutron Scattering (NS) experiments on European large scale facilities, in particular at the Institut Laue-Langevin in Grenoble, the most powerful neutron source in the world, and at the Hans-Meitner Institut in Berlin. NS is a powerful non invasive technique that allows to get detailed information on the biomolecule dynamics on a frequency range from 10^{+8} Hz to 10^{+13} Hz, where thermal motions crucial for the biological functionality take place.

The coordinator of the group is Giuseppe Onori, Full professor in Physics at the University of Perugia. His main interests are in Biophysics and Physics of Complex Systems. His studies refer to the role of the environment on dynamics, function and stability of proteins and nucleic acids, hydrophobic hydration and hydrophobic interactions, protein folding and properties of water confined in reverse micelles.

Recent relevant publications

- F.Boffi, A.Bonincontro, S.Cinelli, A.Congiu Castellano, A.De Francesco, S.Della Longa, M.Girasole and G.Onori, pH dependent local structure of ferricytochrome-c studied by X-ray absorption spectroscopy, Biophysical Journal,80,1473-1479 (2001)
- S.Cinelli, F.Spinozzi, R.Itri, S.Finet, F.Carsughi, G.Onori, P.Mariani, Structural characterization of pH-denatured states of ferricytochrome-c by synchrotron small angle X-ray scattering, Biophysical Journal 81,3522-3533(2001)
- M.Freda, G.Onori, A. Paciaroni, A.Santucci, Hydration and dynamics of Aerosol OT riverse micelles, J. Molecular Liquids 101, 55-68 (2002)

- A.Paciaroni, S.Cinelli, G.Onori, Effect of environment on the protein dynamical transition: a neutron scattering study, Biophysical Journal 83,1157-1164 (2002)
- Orecchini A., Paciaroni A., Bizzarri A.R., S. Cannistraro. Dynamics of different hydrogen classes in beta-lactoglobulin: A quasielastic neutron scattering investigation, J. Phys. Chem. B 106 (29): 7348-7354 JUL 25 2002

Participant #13 (Universita' di Verona)
The Verona node include different expertises related to Synchrotron Radiation Infrared Microspectroscopy, Molecular Pathology, Histopathology and Imaging. The internationally recognized level of the structures and of the participants guarantees the required degree of excellence of the research in all the fields involved. Prof. Procacci, Prof. Bartolozzi and Prof Menestrina are all Full Professors at the Faculty of Medicine of the University of Verona. Prof. Procacci is also the Director of the Department of "Scienze Morfologiche e Biomediche"; Prof. P. Bortolazzi is the Director of the Department of "Scienze Anestesiologiche e Chirurgiche" and prof. F. Menestrina is the Director of the Section of "Anatomia Patologica" of the Department of "Patologia". The Faculty of Medicine of Verona and the related Hospitals (Borgo Roma and Borgo Trento) have recently been declared Center of Excellence for several innovative techniques in the field of surgery and in the treatment of tumors. The coordinator of the node, Prof. E. Burattini, is Full Professor in Physics at the Faculty of Science of the University of Verona. He is one of the pioneer in Synchrotron Radiation activity (more than 30 year experience in the field) and in particular one of the pioneer of Synchrotron Radiation research in biomedical fields. He was the organizer of the first meeting on Synchrotron Radiation Applications to Digital Subtraction Angiography (SYRDA), which was held in Frascati in 1987. He was also the Director of the Enrico Fermi International School on Physics on "Biomedical Applications of Synchrotron Radiation" (Varenna, 1994). He is actually the Director of the DAΦNE-Light Laboratory, where one the most intense IRSR source has recently come into operation.

Recent relevant publications
- Chilosi M, Doglioni C, Yan Z, Lestani M, Menestrina F, Sorio C, Benedetti, A Vinante F, Pizzolo G, Inghirami G., Diffential expression of cyclin-dependent kinase 6 in cortical thymocytes and T-cell lymphoblastic lymphoma/leukemia, Am. J Pathol. 1998 Jan; 152(1):209-17.
- Chilosi M, Doglioni C, Magalini A, Inghirami G, Krampera M, Nadali G, Rahal D, Pedron S, Benedetti A, Scardoni M, Macri E, Lestani M, Menestrina F, Pizzolo G, Scarpa A., P21/WAF1 cyclin-kinase inhibitor expression in non-Hodgkin's lymphomas: a potential marker of p53 tumor-suppress or gene function, Blood. 1996 Nov 15; 88(10):4012-4020
- S. Ricci, A. Antonuzzo, L. Galli. M. Ferdeghini, L. Bodei, C. Orlandini, G. Boni, P.F. ConteOctreotide acetate long-acting release in patients with metastatic neuroendocrine tumors pretreated with lanreoide, Ann Oncol 2000; 11(9):1127-30
- M. Ballardin, F. Gemignani, L. Bodei, G. Mariani, M. Ferdeghini, A.M., Rossi, L. Migliore, R. Barale, Formation of micronuclei and of clastogenic factor(s) in patients receiving therapeutic doses of iodine-131, Mutat Res 2002 Feb 15; 514(1-2):77-85
- E. Burattini, F. Monti, G. Cinque, A. Marcelli, The DAFNE-Light Laboratory in Frascati: a new brilliant infrared, Synchrotron Radiation source for microspectroscopy experiments, Spectroscopy Europe 11/6 (1999) 6

Participant #14 (Nottingham University)
The School of Chemistry at the University of Nottingham has a large and vigorous research school with 40 academic staff, 60 research assistants and 150 PhD students. Chemical spectroscopy is one of the strengths of the school, supported by an infrastructure including laser systems, FTIR and Raman spectrometers and microscopes, surface analysis including SIMS and XPS analysis

systems and surface IR analysis systems, liquid phase and solid state NMR instrumentation, circular dichroism spectrometers and a SQUID magnetometer.
Specifically addressing the spectroscopic infrastructure for this programme there is:

- A Nicolet Continuum IR microscope /Nexus 870 spectrometer combination for IMS
- A Perkin Elmer Spotlight Infrared Imager, which will be based at the Daresbury laboratory IMS beamline
- A Nicolet Nexus 870 IR spectrometer with PM IRRAS attachment for surface studies.

Recent relevant publications
- M.A. Chesters, E.C. Hargreaves, M. Pearson, P. Hollins, D.A. Slater, J.M. Chalmers, B. Ruzicka, M. Surman and M.J. Tobin, Il Nuovo Cimento, 1998, 20D, 439-448.
- M.A. Chesters, M.J. Tobin, N.R. Griffin and S.E. Fisher, Oral Oncology, (1999) VI, 193-194.
- M.J. Tobin, F. Rutten, M.A. Chesters, J.M. Chalmers, I. Symonds, S. Fisher, R. Allibone and A. Hitchcock, Investigating the potential for infrared microanalysis in cancer screening, European Clinical Laboratory, (2002) 21 (4), 20-22.
- J.M. Chalmers and P.R. Griffiths, Eds. Handbook of Vibrational Spectroscopy, Vol 5, Applications in Life, Pharmaceutical and Natural Sciences, John Wiley and Sons, 2002, Biomedical Applications, pg. 3227-3388.

Participant #15 (ITC-CNR Institute of Biophysics at Trento)
The Section at Trento of the CNR Institute of Biophysics, consist of a group of researchers that has developed an original biophysical and biochemical approach to investigate structural and functional aspects of protein-lipid and peptide-lipid interactions. In particular we are interested in the mechanisms underlying membrane-damage in model and biological membranes, and we have become leader in the determination of molecular action and structure-function relation of bacterial and animal toxins that attack cell membrane. More recently, our expertise has been applied also to small peptides that are involved in the generation of diseases such as amyloidoses. The contribution we will provide will cover: determination of the 3D and secondary structure of each system component, including lipids, peptides and proteins, by integrating information coming from FTIR, CD, XAS, QLS fluorescence and mass spectroscopy in membrane and in solution; implementation of fast kinetic set-ups based on stopped- or continuous-flow approach; preparation of samples deposited with LB troughs with controlled surface pressure and with particular attention to developing high throughput devices; construction of 3D structural models by integrating collected data, homology building and structural predictions.
Our equipment and expertise cover a number of techniques for synthesis, purification, functional and structural characterisation of membrane active polypeptides, which include: a fully automated Fourier Transform Infrared spectroscopy (FTIR) with MCT anf Far-IR detectors, ATR (Attenuated Total Reflectance) and VADR (Variable Angle Diffuse Reflectance) attachments that can be used for single- or multiple-reflections on deposited mono- or multi-layers, all equipped with grid polarizers; possibility of simultaneous measurement of monolayer surface pressure.
- Circular Dichroism (CD);
- Quasi-Elastic Laser Light Scattering (QLS) for size and charge of particles;
- Peptide Synthesizer for the simultaneous synthesis of up to 6 peptides (max- 72 residues, 25 μmol),
- HPLC system with Photo Diode Array detector and digital Integrator
- Ultra-centrifugation, analytical and preparative electrophoresis;
- Computer-controlled Langmuir balance for the deposition of mono- and multi-layers;
- cell culture facilities;

- fluorescence spectroscopy (Photon-Counting, stopped-flow and 96-well kinetic microplate reader); computer modelling.

Recent relevant publications

- Menestrina, G., V. Cabiaux, and M. Tejuca. 1999. Secondary structure of sea anemone cytolysins in soluble and membrane bound form by infrared spectroscopy, Biochem. Biophys. Res. Commun. 254:174-180.
- de Leeuw, E., K. te Kaat, C. Moser, G. Menestrina, R. A. Demel, B. de Kruijff, B. Oudega, J. Luirink, and I. Sinning. 2000, Anionic phospholipids are involved in membrane association of FtsY and stimulate its GTPase activity. EMBO J. 19:531-541.
- Menestrina, G. 2000, Use of Fourier-transformed infrared spectroscopy (FTIR) for secondary structure determination of staphylococcal pore-forming toxins. Methods Mol Biol. 145:115-132.
- Alvarez, C., M. Dalla Serra, C. Potrich, I. Bernhart, M. Tejuca, D. Martinez, I. F. Pazos, M. E. Lanio, and G. Menestrina. 2001, Effects of lipid composition on membrane permeabilization by Sticholysin I and II, two cytolysins of the sea anemone Stichodactyla helianthus. Biophys. J. 80:2761-2774.
- Nuzzo, S., C.Meneghini, S.Mobilio, H.Haas, P.Riccio, A.Fasano, P.Cavatorta, and S.Morante. 2002, An X-ray Absorption Spectroscopy study of the Zinc environment in Langmuir-Blodgett phospholipid multilayers. Biophys. J. 83: 3507-3512.

Participant #16 (Université Libre de Bruxelles)

The Center of Structural Biology and Bioinformatics and Structure and Function of Biological Membranes of the ULB is one of the major centers of research in Europe, that developed unique competences in the field of infrared spectroscopy of biological membranes and proteins. Nationally, both the university and the National Fund for Scientific Research have built an infrastructure that presently counts 5 FTIR spectrometers, all fully equipped with MCT detectors, ATR and accessories for kinetic measurements in protein, including the step scan option. Internationally, visitors are present all year round from Europe and farther.

The laboratory developed very specific skills aimed at determining conformational changes relevant to activity (re-orientation of secondary structures and tertiary structure changes). ATR-FTIR spectra of membrane multilayers in buffer flow and release by photolysis of a “caged” precursor are used to modulate the environment. Sensitivity reaches about one residue on a total of 1000. Side chain ionisation, secondary structure and orientation of secondary structure can be monitored. Alternatively, membranes are subject to trans-membrane potentials. The step scan approach allows the recording of any change on a micro-second scale. Finally, the laboratory has acquired special skill in the recording and analysis of H/D exchange kinetic in proteins. In the resulting 2-D spectroscopy, resolution in the frequencies as well as of the time constants for the exchange can be achieved at the level of sub-molecular regions. Recently, we started using FTIR to identify cancer cell lines in brain tumors and leukemia. Similarly, determination of Leishmania species was achieved.

The laboratory also includes other facilities, from cell culture rooms to protein purification and characterization: electrospray-MS-MS, circular dichroism, fluorescence, patch-clamp and BLM.

Recent relevant publications

- O. Radresa, K. Ogata, S. Wodak, J.M. Ruysschaert, and E. Goormaghtigh, Modeling the three-dimensional structure of H+-ATPase of Neurospora crassa - Proposal for a proton pathway from the analysis of internal cavities, European Journal of Biochemistry 269 (2002), 5246-5258
- A. Gaigneaux, J.M. Ruysschaert, and E. Goormaghtigh, Infrared spectroscopy as a tool for discrimination between sensitive and multiresistant K562 cells, European Journal of Biochemistry 269 (2002), 1968-1973

- V. Grimard, C. Vigano, A. Margolles, R. Wattiez, H. W. van Veen, W. N. Konings, J.M. Ruysschaert, and E. Goormaghtigh, Structure and Dynamics of the Membrane- Embedded Domain of LmrA Investigated by Coupling Polarized ATR-FTIR Spectroscopy and 1H/2H Exchange, Biochemistry 40, (2001) 11876-11886
- A. Le-Saux, J.M. Ruysschaert, and E. Goormaghtigh, Membrane molecule reorientation in an electric field recorded by attenuated total reflection Fourier-transform infrared spectroscopy, Biophys J. 80 (2001), 324-330.
- R.I. Saba, J.M. Ruysschaert, A. Herchuelz, and E. Goormaghtigh, Fourier transform infrared spectroscopy of the reconstituted Na/Ca exchanger 70 kDa polypeptide, J.Biol.Chem. (1999) 274, 15510-15518.

Participant #17 (Università Politecnica delle Marche)

The Node 17 is the Biophysics research unit working at the Istituto di Scienze Fisiche of the Università Politecnica delle Marche in Ancona. The unit has a proven experience in the use of in-solution small angle X-ray and neutron scattering (SAS) techniques to analyse the structural and conformational properties of proteins in solution. In the last years, the Biophysics research unit has spent a large effort to improve Monte Carlo and reverse Monte Carlo methods to derive from SAS data protein shape and interactions in solution. Moreover, original multipole expansion particle shape reconstruction methods from SAS curves, that have been proved to help in assessing ab-initio protein structure and conformation in combination with computational prediction techniques, have been also derived. The unit staff is composed by 3 Senior researchers (2 Biohysicists, 1 Biochemist), 1 Post Doc (Biophysicist) with more than four years of research experience, and 3 PhD students (Biologists). Moreover, 2 Senior researchers (both Biochemists) and 1 PhD student (Biologist) from the University of Ferrara (Italy) will actively collaborate in the project, and then will be considered as external collaborators in the unit staff.

The Biophysics research unit has reached a stage of scientific maturity and physical implementation for the analysis of protein biophysical properties and structure. Moreover, the lab is fully equipped with a complete SAXS facility, composed of a high-resolution SAS camera with a curved monochromator and linear detector installed on a Rigaku RV300 rotating anode source (18 kW), a pinhole SAS camera equipped with a linear detector installed on a Philips X-ray generator (3.5 kW), a Bonse-Hart ultra Small Angle camera installed on a Philips X-ray generator (3.5 kW). The lab is also equipped with biocomputing facilities composed of Alpha workstations with original (in-house implemented) software for SAS particle shape rendering techniques. Moreover, the staff of the Biophysics research unit at the Istituto di Scienze Fisiche has a very good and proved expertise on the use of SAS beamlines at synchrotron european facilities (ESRF and Elettra, in particular) and at Neutron Sources (ILL, LLB, FZJ, HMI).

The coordinator of the node, Paolo Mariani is born in 1956 and is Associate Professor in Physics at the Faculty of Science of the University of Ancona. Its scientific activity is mainly devoted on the analysis of structural properties and polymorphism of molecules and systems of biological interest (including proteins, lipids and nucleic acid derivatives) using X-ray and neutron scattering techniques. Since 1998, the research activity was focused on protein structure and aggregation in solution, on protein folding/unfolding processes and on protein-protein interaction by using small angle X-ray and neutron scattering. He is author of more than 100 scientific papers published in international journals.

Recent relevant publications

- F. Spinozzi, F. Carsughi, P. Mariani, Particle shape reconstruction by Small-Angle Scattering, Integration of Group Theory and Maxinmum Entropy to multipole expansion method, Journal of Chemical Physics, 109, 10148-10158 (1998).

- G. Baldini, S. Beretta, G. Chirico, H. Franz, E. Maccioni, P. Mariani, F. Spinozzi, Salt-induced association of b-lactoglobulin by light and X-ray scattering, Macromolecules, 32, 6128-6138 (1999).
- P. Mariani, F. Carsughi, F. Spinozzi, S. Romanzetti, G. Meier, R. Casadio, C.M. Bergamini, Ligand-induced conformational changes in tissue Transglutaminase: Monte Carlo analysis of small angle scattering data, Biophysical Journal, 78, 3240-3251 (2000).
- S. Cinelli, F. Spinozzi, R. Itri, S. Finet, F. Carsughi, G. Onori, P. Mariani, Structural Characterisation of the pH-Denatured States of Ferricytochrome-c by Synchrotron Small Angle X-Ray Scattering, Biophys. Journal, 81, 3522-3533 (2001).
- F. Spinozzi, D. Gazzillo, A. Giacometti, P. Mariani, F. Carsughi, Interaction of proteins in solution from small angle scattering: a perturbative approach, Biophys. Journal, 82, 2165-2175 (2002).

Participant #18 (Commissariat à l'Energie Atomique)

Service de Bioénergétique is one entity amongst six that constitute the Département de Biologie Joliot Curie, a government funded (CEA) research institute, involved in biological research that is mainly but not exclusively fundamental. Within the Service, a series of four research teams focus on biophysical studies of photosynthetic electron transfer and of other metalloproteins involved in bioenergetic processes. These groups together make the Institution a uniquely powerful center for research in this area that has established a wealth of international collaborations and which attract many foreign researchers. In Framework Program 5, the Institution has been selected as a Marie Curie Training Site specializing in spectroscopic studies on the structure-function relationships in metalloproteins involved in bioenergetic and electron transfer processes. The originality of the laboratory is the use of advanced spectroscopic methods, which in many cases have been developed in-house. In most cases the research teams have technological expertise that is at the forefront or ahead of the field. A number of young PhD students have greatly benefited from training periods in the host institution.

The coordinator of the CEA node is Jacques Breton a researcher mainly devoted to structural spectroscopy of membrane proteins involved in bioenergetic processes (photosynthesis and respiration) with emphasis on structure-function relationships using FTIR difference spectroscopy.

Recent relevant publications

- Iwaki, M., Breton, J., Rich, P.R., ATR-FTIR difference spectroscopy of the P_M intermediate of bovine cytochrome c oxidase. Biochim. Biophys. Acta (2002), 1555, 116-121.
- Breton, J., Boullais, C., Mioskowski, C., Sebban, P., Baciou, L., Nabedryk, E., Vibrational spectroscopy favors a unique QB binding site at the proximal position in wild-type reaction centers and in the Pro-L209 Tyr mutant from Rhodobacter sphaeroides, Biochemistry, (2002), 41, 12921-12927.
- Rich, P.R., Breton, J. ATR-FTIR studies of redox changes in bovine cytochrome c oxidase: Resolution of the redox FTIR difference spectrum of heme a_3, Biochemistry, (2002), 41, 967-973.
- Iwaki, M., Andrianambinintsoa, S., Rich, P.R., Breton, J. Attenuated total reflection Fourier Transform infrared spectroscopy of redox transitions in photosynthetic reaction centers: comparison of perfusion- and light-induced difference spectra, Spectrochimica Acta Part A, (2002), 58, 1523-1533.
- Breton, J., Nabedryk, E., Clerici, A., Light-induced FTIR difference spectroscopy of photosynthetic charge separation between 9000 and 250 cm^{-1}, Vibrational Spectroscopy, (1999) 19, 71-75.

Participant #19 (Robert Koch-Institut)

The Robert Koch-Institute (RKI) is the central institute for health protection in Germany. It advices the Federal Ministery of Health and Social Safety (Bundesministerium für Gesundheit und soziale Sicherung, BMGS) in the field of infectious diseases and biomedicine. With its work the RKI creates the scientific foundation for effective preventive and control measures in Germany.
The research group of Prof. Naumann (RKI/P13 "Biomedical Spectroscopy") has a well-known and recognized expertise in the field of biomedical applications of infrared spectroscopy and structural IR spectroscopy (protein folding). Dieter Naumann, as its principal investigator and coordinator of node #19 is an associated professor at the Dept. of Chemistry at the Free University of Berlin/Germany. His scientific activity is mainly devoted to the study of biological objects such as microorganisms, body fluids and tissues by infrared spectroscopy and microspectroscopy. The originality of Dieter Naumann's research group is the combination of expertise in infrared spectroscopy, medicine and chemometrics.
The unit staff of P13 is composed by 3 senior researchers (1 Chemist, 1 Physicist and 1 MD) and 4 PhD students. The team actively collaborates with a number of institutions in Berlin (e.g. Charite, Medical Faculty of the Humboldt University in Berlin, Dr. Schade, IRIS beamline at BESSY II), Germany (e.g. David Moss, ANKA beamline at Forschungszentrum Karlsruhe), and worldwide.
The scientific facilities available for this proposal cover a number of different spectroscopic techniques: six IR spectrometer, three IR microscopes, as well as a Raman spectrometer, MALDI-TOF, CD and UV-V is spectrophotometer.

Recent relevant publications
- D. Naumann, D. Helm, and H. Labischinski, Microbiological Characterizations by FTIR Spectroscopy, Nature 351, 81-82 (1991)
- Troullier, A.; Reinstädler, D.; Dupont, Y.; Naumann, D.; Forge, V., Direct Evidence for transient non-native Secondary Structures During the Refolding of a-Lactalbumin by Time-Resolved Fourier Transform Infrared Spectroscopy. Nature Structural Biology 7, 78-86 (2000)
- Lasch P.; Petras T.; Ullrich O.; Backmann, J.: Naumann D.; & Grune, T. Hydrogen Peroxide Induced Damage of RNase A is Followed by Degradation by Proteasome and FT-IR Spectroscopy. J. Biol. Chem. 276: pp. 9492-9502. (2001)
- Kneipp, J.; Beekes, M.; Lasch, P.; & Naumann, D., Infrared Spectroscopy Can Be Applied to Detect Molecular Changes in situ in Preclinical Scrapie. J. Neuroscience. 22(8). pp. 2989-2997 (2002)
- Lasch P.; Haensch W.; Kidder, L.; Lewis, E.N. & Naumann D., Colorectal Adenocarcinoma Characterization by Spatially Resolved FT-IR Microspectroscopy. Appl. Spectrosc. 56 (1): pp. 1-9. (2002).

Participant #20 (Université de Reims Champagne-Ardenne)
The group at the URCA has, from many years, developed spectroscopic applications at the level of single living cell. More particularly, optical microspectroscopies (Raman, SERS Raman, FT-IR, fluorescence) and multispectral imaging analysis are used to study intracellular pharmacokinetics of drug in relation to biological processes (cytotoxicity, differentiation, resistance phenotype, …). Very recently, in collaboration with clinicians, the research has been focused on the applications of Raman and IR spectroscopies in the field of health engineering (identification of tumor tissues, early diagnosis and prognostic, early identification and characterization of microorganisms). The staff is composed by 16 Senior researchers, 3 Post Doc and 12 PhD Students. 5 Senior researchers and 7 PhD Students will actively collaborate in the project. The coordinator of the URCA node is Michel Manfait, head of the CNRS UMR 6142, who has an expertise in biomedical spectroscopy.

Recent relevant publications

- Sandt C, Sockalingum GD, Aubert D, Lepan H, Lepouse C, Jaussaud M, Leon A, Pinon JM, Manfait M, Toubas D., Use of Fourier-transform infrared spectroscopy for typing of Candida albicans strains isolated in intensive care units, J Clin Microbiol. (2003); 41(3): 954-9
- G.D. Sockalingum, C. Sandt, D. Toubas, J. Gomez, P. Pina, I. Bequinot, F. Witthuhn, D. Aubert, P. Allouch, M. Pinon, M. Manfait, FTIR characterization of some reference and clinical *Candida species*. Vibrational Spectroscopy (2002) 28, 137-147.
- A. Galichet, G.D. Sockalingum, A. Belarbi, M. Manfait, FTIR spectroscopic analysis of *Saccharomyces cerevisiae* cell walls: Study of an anomalous strain exhibiting a pink-colored cell phenotype. FEMS Mic. Lett., (2001) 197, 179-186.
- C. Kirschner, N.A. Ngo Thi, D. Naumann K. Maquelin, L.-P. Choo-Smith, G.J. Puppels, P. Pina, P.Y. Allouch, C. Sandt, G.D. Sockalingum, M. Manfait, D. Ami, S.M. Doglia, Classification and Identification of Enterococci Species: A Comparative Phenotypic, Genotypic and Vibrational Spectroscopic Study. J. Clin. Microbiol,, (2001) 39, 1763-1770.
- A. Beljebbar, G.D. Sockalingum, H. Morjani, M. Manfait, Raman and SERS microspectroscopy on living cells: a promising tool towards cellular-drug response and medical diagnosis. Biomedical Applications of Raman Spectroscopy, (1999) Michael D Morris, Abraham Katzir, Editors, SPIE, 3608, 175-184.

Participant #21(European Synchrotron Radiation Facility)

The development of micro-analysis and micro-spectroscopy methods, combining spatial and spectral resolutions has already attracted several scientific communities at the ESRF, in various fields spanning from Life Sciences to Earth Sciences and Materials Science. The ESRF provides today the best synchrotron based microanalysis facility in the 2-30 keV energy range. The scientific programme relies on more than 60 different experiments carried out by international groups every year on three dedicated beamlines. The proposed program aims to build a state-of-art IR microscope, which will be used in conjunction with two X-ray microscopy beamlines. Indeed, combined use of infrared and X-rays microscopes would constitute a very potential micro-characterisation facility, which will be unique in the world. It should be noted that the different scientific communities that are interested in performing microanalysis, using either IR or X-rays photons, are essentially the same. Most of the scientific cases require a multi-technique approach, consisting of a coupling of techniques providing chemical as well as structural information. Most of our scientific activities are method-oriented. A successful in-house research program requires a close collaboration with external groups having expertise in sample preparation and characterisation. An in-house development of complementary techniques such as IR microscopy will boost several programs by enhancing our micro-characterisation capability. Several programs initiated on bone structure/chemistry, micro-irradiation at the cell level, metal biochemistry in neuronal cells will immediately benefit from this project. Finally, this project will benefit from the expertise of P. Dumas (LURE) who is acting as consulting scientist.

The principal investigator from the ESRF, J. Susini, is head of the X-ray Microscopy and Micro-analysis Group, which consists of three beamlines associating 7 scientists, 5 post-doc fellows and 2 PhD students. Furthermore, this project will benefit from the expertise of P. Dumas (LURE) who is acting as consulting scientist. P. Dumas is a beamline scientist at LURE, who has initiated synchrotron based infrared microscopy at LURE, the French Synchrotron facility. Since 1999, the MIRAGE beamline has been very successful and has hosted tens of scientists, from various communities: Geology, Material Science, Forensic Science, Biology and Bio-medicine. P. Dumas has been collaborating very closely with the group at the National Synchrotron light Source, at Brookhaven National Laboratories, initially on far infrared surface science studies, and furthermore, on the characterisation and validation of the three IR microscopic beamlines in this Synchrotron facility. He is presently in charge of designing an IR microscopic facility at the new French synchrotron facility SOLEIL.

Recent relevant publications

- J. Susini, M. Salomé, B. Fayard, R. Ortega and B. Kaulich, The scanning X-ray microscopy at the ESRF, Surface Review and Letters, 9(1), (2002).
- Y. Dauphin, J.P. Cuif, J. Doucet, M. Salomé, J. Susini, C.T. Williams, Chemical forms of sulfur in calcitic biominerals determined by in situ mapping at the sulfur K-edge using x-ray absorption near-edge spectroscopy (XANES), Journal of Structural Biology, 125 (2003).
- L.M. Miller, G.L. Carr, M. Jackson, P. Dumas and G.P. Williams The Impact of Infrared Synchrotron Radiation in Biology: Past, Present and Future Synchrotron Radiation News vol. 13 (5) (2000).
- P. Dumas and G.P. Williams in Chemical Applications of Synchrotron Radiation, Advanced Series in Physical Chemistry, World Scientific, 12 (2001)
- N. Jamin, P. Dumas, J. Moncuit, W.H. Fridman, J.L. Teillaud, G.L. Carr, and G.P. Williams Highly resolved chemical imaging of living cells by using synchrotron infrared microspectrometry, Proceedings of the National Academy of Sciences, 95 (9), (1998)

Participant #22 (University of Strathclyde)

The Sensitive Analytical Spectroscopy Group is one of the leading research teams within the University of Strathclyde with a world-class reputation. It is led by Professor Smith and Dr Graham and has a world lead in the development of SERRS technology and in particular in its use in DNA analysis. The group holds a number of key patents in the area and is currently well funded by BBSRC, EPSRC, MOD, The Home Office, Avecia and the Royal Society of Chemistry. They have published a number of seminal papers on SERRS/DNA analysis in the last two years and have also published key papers on the exploitation of SERRS as a routine analytical technique. The skills base includes synthetic chemistry, analytical chemistry and Raman spectroscopy. There is a state-of-the-art multi line Raman facility that has been purpose built for SERRS and will be used during this programme.

Recent relevant publications

- Fruk, L., Grondin, A., Smith, W. E., Graham, D., A New Approach to Oligonucleotide Labelling Using Diels Alder Cycloadditions and Detection by SERRS, Chem. Commun. 2002, 18, 2100-2101.
- McHugh, C. J., Keir, R., Graham, D., Smith, W. E., Selective Functionalisation of TNT for Sensitive Detection by SERRS, Chem. Commun., 2002, 6, 580-581.
- McLaughlin, C., Graham, D., Smith, W. E., A Comparison of Resonant and Non-Resonant Conditions on the Concentration Dependence of Surface Enahnced Raman Scattering from a Dye Adsorbed on Silver Colloid, J. Phys. Chem., 2002, 106, 21, 5408-5412.
- Graham, D., Mallinder, B. J., Whitcombe, D., Smith, W. E., Surface Enhanced Resonance Raman Scattering (SERRS) - A First Example of its Use in Multiplex Genotyping, A European J. Chem. Phys. and Phys. Chem., 2001, 2, 12, 746-748.
- Graham, D., Mallinder, B. J.and Smith, W. E., Surface Enhanced Resonance Raman Scattering as a Novel Method of DNA Discrimination, Angewandte Chemie Int. Ed. Engl. 2000, 6, 1061-1063; Angewandte Chemie 2000, 112, 1103-1105.

Participant #23 (Università degli Studi di Milano Bicocca)

The Department of Biotechnologies and Biosciences of the University of Milano Bicocca was founded in 1999. In the Department scientists in biotechnology, biochemistry, genetics, as well as in organics chemistry, physical chemistry and biophysics, are involved in research and teaching (University Program in Biotechnology and in Biological Sciences, PhD School in Biotechnology.) Our present research is addressed to the study of proteins dynamics and structure by the complementary approaches of optical spectroscopic techniques, molecular modeling and protein

engineering, where we have a well-recognized experience. In the last years, we have started a FT-IR study of large molecular weight proteins in solution for the understanding of their structure–function relationship. Particular interest is given to lipases and lipocalins, wild- type proteins and their mutants produced in recombinant form in yeasts.
Silvia Maria Doglia is Associate Professor of Experimental Physics (Faculty of Sciences, University of Milano Bicocca) since 1987. Her research experience is in biophysics and in optical spectroscopy of biological systems. She has taken part to EC funded research projects (Science and Biomed II) and to the Erasmus and Socrates Programs, for the training of university students.

Recent relevant publications
- Vergani B., Kintrup M., Hillen W., Lami H., Piemont. E., Bombarda E., Alberti P. Doglia S.M., and Chabbert M., Backbone dynamics of Tet Repressor alfa8 -alfa9 loop, Biochemistry 39, 2759-2768 (2000).
- F. Orsini, D. Ami, A.M. Villa, G. Sala, M.G. Bellotti, and S.M. Doglia, FT-IR Microspectroscopy for microbiological studies, J. Microbiological Methods 42, 17-27 (2000)
- Monzani E, Alzuet G, Casella L, Redaelli C, Bassani C, Sanangelantoni A, Gullotti M, De Gioia L, Santagostini L, Chillemi F., Properties and reactivity of myoglobin reconstituted with chemically modified protohemin complexes, (2000) Biochemistry 39:9571-9582
- L. Choo-Smith, K. Maquelin,T. Van Vreeswijk, H.A. Bruning, G.J. Puppels, N.A. Ngo Thi, C. Kirschner, D. Naumann, D. Ami, A.M. Villa, F. Orsini, S.M. Doglia, H. Lamfarraj, G.D. Sockalingum, M. Manfait, P-Allouch and H.PH Endtz, Investigating microbial microcolony heterogeneity by vibrational spectroscopy, Appl. Environ. Microbiol. 67(4), 1461-9 (2001)
- Alquati, C.; De Gioia, L.; Santarossa, G.; Alberghina, L., Fantucci, P., Lotti, M., The cold-active lipase of Pseudomonas fragi: Heterologous expression, biochemical characterization and molecular modeling, European Journal of Biochemistry 269 (13), 3321-3328 (2002).

Participant #24 (Università di Verona - Dipartimento di Patologia - Sezione di Immunologia)
The Verona group of Dipartimento di Patologia has developed competence on purification of toxins and synthesis of carrier-toxin heteroconjugates, cloning, expression and purification of toxins mutants. The contribution which will be given will consist mainly in: preparing purified in vitro systems for studies of interactions between proteins. Cell cultures in monolayers and in 3D aggregates (Multicellular Tumor Spheroids) for the mapping of single protein or protein complexes within the cell in different redox conditions.
The facilities available for this proposal and the expertise cover a number of techniques of purification, functional and structural characterisation of protein toxins and synthesis of carrier-toxin conjugates, which include: ultra-centrifugation, FPLC, preparative electrophoresis, western blotting, semiquantitative PCR. A bank of cell lines is available for adherent cell cultures in monolayer, separation of cell subpopulations (cell sorting) or phenotyping (citoflurometers) and for 3D cell cultures (MTS). Major equipment is available (i.e. containment units for manipulation of highly contaminating biologic materials, ultracentrifuges, cell culture laboratories). Other facilities available in the Department may allow a whole range of experimental procedures (biochemistry, cell biology, molecular biology, microbiology, manipulation of human specimens, tissue microarray) to be carried out.

Recent relevant publications
- R. Chignola, D. Liberati, E. Chiesa, R. Foroni, G.C. Andrighetto, G. Tridente and M.Colombatti, On the growth dynamics of multicellular tumor spheroids: a preliminary report. In: “Chaos, fractals, models”, F.M.Guindani and G.Salvatori, Eds., Italian University Press, pp.371-377, 1997.
- I. Lorenzetti, A. Meneguzzi, G. Fracasso, C. Potrich, L. Costantini, E. Chiesa, G. Legname, G. Menestrina, G. Tridente, and M. Colombatti, Genetic grafting of membraneacting peptides to the

cytotoxin dianthin augments its ability to destabilize lipid bilayers and enhances its cytotoxic potential as the component of transferrin-toxin conjugates. Int.J. Cancer, 2000, 86:582-589.
- G. Fracasso and M. Colombatti, Effect of therapeutic macromolecules in spheroids, Crit. Rev. Oncol. Hematol., 2000, 36:159-178.
- M. Tommasi, D. Castelletti, M. Pasti, G. Fracasso, I. Lorenzetti, S. Sartoris, C. Pera, G.B. Ferrara, G. Tridente, and M.Colombatti, Identification of Ricin A chain HLA class II-restricted epitopes by human T-cell clones. Clin. Exp. Immunol., 2001, 125:391-400.
- G. Fracasso, G. Bellisola, S. Cingarlini, S. Righetti, E. Chiesa, D. Castelletti, T. Prayer-Galetti, F. Pagano, G. Tridente, and M. Colombatti, Anti-tumor effects of toxins targeted to the prostate specific membrane antigen. Prostate, 2002, 53:9-23.

Participant #25 (Vienna University of Technology)
The research group of Dr. Lendl on chemical analysis and vibrational spectroscopy at VUT has profound experience in the development and application of analytical techniques based on flow analysis (FIA, SIA, LC) and vibrational (FTIR and Raman) spectroscopic detection. Examples extend from on-line fermentation monitoring, where FTIR spectra of the dissolved reactants and of the micro-organism were used for process monitoring and process control, to fundamental research such as the development of lab-on-a-chip devices for fast time resolved FTIR spectroscopy of chemical reactions in solution. These devices can be used for the study of short living reaction intermediates, bio-ligand interactions as well as protein folding. The contribution to the network will focus on production and further development of miniaturized lab-on-a-chip devices for performing chemical reactions (fast mixing) and/or separations (capillary electrophoresis on a chip) in a miniaturized format with IR synchrotron detection. Using miniaturized devices highly accurate liquid handling is possible requiring only minute amounts of samples, which is of crucial importance especially when only very small amounts of sample are available. Furthermore, the small dimensions of the planned lab-on-a-chip devices enable to take full advantage of the high brilliance of the synchrotron sources. Lab-on-a-chip devices with IR synchrotron detection will therefore be used as an enabling technology that provides the experimental platform for a broad variety of experiments to be carried out within this project. Examples of possible application areas:

Heparin-binding proteins and peptides
Amyloid-forming proteins
Bio-ligand interaction studies.

Dr. Lendl is currently running the EU Marie Curie Training Site on Advanced and Applied Vibrational Spectroscopy and serves on the Editorial Boards of Vibrational Spectroscopy (Elsevier) and Applied Spectroscopy (Society for Applied Spectroscopy). His research group is embedded in an extensive network of national and international co-operations. Facilities available in the group encompass 5 high-end FTIR and FT Raman spectrometers, QCL lasers, CE and HPLC units as well as a variety of supporting equipment. Through in house co-operation efficient design of lab-on-a-chip devices using computational fluid dynamic, CFD as well as production in well equipped clean room facilities can be carried out.

Recent relevant publications
- P. Hinsmann, J. Frank, P. Svasek, M. Harasek and B. Lendl, Time resolved FT-IR spectroscopy of chemical reactions in solution by fast diffusion based mixing in a micromachined flow-cell, Appl. Spectrosc. 55 (2001) 241-251
- P. Hinsmann, J. Frank, P. Svasek, M. Harasek and B. Lendl, Design, Simulation and Application of a new micromixing device for time resolved Infrared spectroscopy of chemical reactions in solution, Lab Chip 1 (2001) 16-21
- Edelmann and B. Lendl, Towards the Optical Tongue: Flow-through Sensing of Tannin-Protein Interactions based on FTIR-Spectroscopy, JACS 124 (2002) 14741-14747

- M. Kölhed, P. Hinsmann, J. Frank, P. Svasek, B. Karlberg and B. Lendl, On-line Fourier Transform Infrared Detection in Capillary Electrophoresis, Anal. Chem. 74 (2002) 3842-3848.
- M. Kölhed, P. Hinsmann, B. Lendl and B. Karlberg, Micellar Electrokinetic Chromatography with On-Line Fourier Transform Infrared Detection, Electrophoresis 24 (2003) 687–692

Participant #26 (Stockholm University)
Stockholm University is one of Sweden's largest educational establishments with 34000 students and 3570 permanent employees. At the Department of Biochemistry and Biophysics, more than 30 principal investigators are engaged in internationally highly recognized research covering a broad range of subjects. These include molecular studies on photosynthesis, protein structure & function, membrane transport, protein folding & trafficing, membrane protein topology, and bioinformatics. The Department is well equipped and with most major biophysical techniques.
Our group has 15 years of experience in infrared difference spectroscopy on the Ca^{2+}-ATPase. Recently we have introduced mapping of ligand binding sites with infrared spectroscopy to identify and characterise ligand protein interactions.

Recent relevant publications
- M. Liu, A. Barth (2003): Mapping interactions between the Ca^{2+}-ATPase and its substrate ATP with infrared spectroscopy, J. Biol. Chem., in press
- M. Liu, A. Barth (2002): Mapping the nucleotide binding site of Ca^{2+}-ATPase with infrared spectroscopy: the effects of γ-phosphate binding, Biospectroscopy 67, 267-270
- F. von Germar, A. Barth, W. Mäntele (2000): Structural changes of the sarcoplasmic reticulum Ca^{2+}- ATPase upon nucleotide binding studied by rapid scan Fourier transform infrared spectroscopy, Biophys. J. 78, 1531-1540
- A. Barth (1999): Phosphoenzyme conversion of the sarcoplasmic reticulum Ca^{2+}-ATPase: molecular interpretation of infrared difference spectra, J. Biol. Chem. 274, 22170-22175
- A. Barth, W. Kreutz, W. Mäntele (1997): Ca^{2+} release from the phosphorylated and unphosphorylated sarcoplasmic reticulum Ca^{2+} ATPase results in parallel structural changes. An infrared spectroscopic study, J. Biol. Chem. 272, 25507-25510

Participant #27 (National Technical University of Athens)
National Technical University of Athens (NTUA) is one of the largest education establishments in Greece with 15,000 students and about 1800 permanent employees. The leader of the group of radiation Chemistry and Biospectroscopy is Assoc. Professor J. Anastassopoulou. We have two FT-IR instruments one of those BOMEM and the other one Nicolet, with accessories and other supporting equipments.
The research is devoted to the structure dynamics of bones, osteoblastic cells, DNA, proteins and membranes. Dr. Anastassopoulou has over 100 papers in scientific journals. Our laboratory is involving in teaching with courses at undergraduate and graduate levels.

Recent relevant publications
- J. Anastassopoulou, Metal-DNA interactions, J. Mol. Structure, in press, (2003)
- J. Anastassopoulou & T. Theophanides, Magnesium-DNA interactions and the possible relation of magnesium to carcinogenesis, Irradiation and free radicals. Cr. Rev Oncol. Heam. 42, 79-91, (2002)
- T. Theophanides & J. Anastassopoulou, Copper and carcinogenesis, Cr. Rev Oncol. Heam. 42, 57-64, (2002)
- M. Petra, J. Anastassopoulou, A. Dovas, D. Yfantis & T. Theophanides, Ageing of human bones. An infrared study, Metal Ion Biol. Med. 6, 763 (2000)

- J Anastassopoulou, B. Anifantakis, Z-A Anifantakis, A. Dovas & T. Theophanides, Thalassaemia and the role of free radicals in our understanding of iron transport and storage in haemoglobin, Bioinorganic Chem., 79, 327 (2000).

Participant #28 (University of Mining and Metallurgy - Faculty of Physics and Nuclear Techniques)

The Section at Faculty of Physics and Nuclear Techniques UMM co-operates with Institute of Neurology Collegium Medicum Jagiellonian University, which studies grounds of pathogenesis of neurodegenerative disorders. The main goal of this co-operation is investigation of human central nervous system (CNS) tissue with respect to biochemical changes that may be involved in neuropathological mechanisms. Selected techniques of X-ray fluorescence (XRF, PIXE, and micro-SRIXE) have been previously applied for elemental macro- and microanalysis of the CNS tissue. As continuation, the researches planned in the project will be devoted to the application of synchrotron infrared microspectroscopy (SRIR) for investigation of distribution of chemical components in CNS tissues with respect to neurodegenerative disorders. This study will be performed in connection to CNRS Orsay. The planned investigation of biochemical changes that may participate in neurodegenerative disorders, comprise also analysis of elemental oxidation states. This part of work will be performed using micro-XANES spectroscopy with the use of microbeam of synchrotron radiation (at ESRF, Grenoble). The application of synchrotron radiation (synchrotron infrared microspectroscopy and micro-XANES spectroscopy) for two-dimensional analysis of distribution of biochemical components in CNS tissue may significantly support investigations on pathogenesis of neurodegenerative disorders.

The co-ordinator of the FPNT, UMM node is Professor Marek Lankosz. The group is composed by 4 Researchers (2 Physicists, 1 Chemist, 1 Neuropathologist) and 2 Doctoral Students (Medical Physicist). Moreover, two neurologists (experts in neurodegenerative diseases) from the Institute of Neurology, CM UJ will actively collaborate in the project.

Recent relevant publications

- Lankosz M, Szczerbowska-Boruchowska M, Ostachowicz J, Adamek D, Krygowska-Wajs A, Tomik B, Bohic S, Simionovici A. Topographic and quantitative elemental analysis of human central nervous system tissue. ESRF Highlights 2002, p. 87-88.
- Szczerbowska-Boruchowska M, Lankosz M, Ostachowicz J, Adamek D, Krygowska-Wajs A, Tomik B, Szczudlik A, Simionovici A, Bohic S. Topographic and Quantitative Microanalysis of Human Central Nervous System Tissue using Synchrotron Radiation, X-Ray Spectrometry, submitted for publication.
- M. Boruchowska, M. Lankosz, D. Adamek, A. Korman. PIXE analysis of human brain tissue. X-Ray Spectrometry 30; 2001: 174-179.
- Adamek D, Tomik B, Pichor A, Ka_uza J, Szczudlik A., Folia Neuropathol. 2002; 40(3): 119-124.
- Szczerbowska-Boruchowska M, Lankosz M, Ostachowicz J, Adamek D, Krygowska-Wajs A, Tomik B, Szczudlik A, Simionovici A, Bohic S. Application of Synchrotron Radiation for Elemental Microanalysis of Human Central Nervous System Tissue, J. de Physique, in press.

Participant #29 (University College London)

The group at UCL is led by Professor Rich who works in the field of Molecular Bioenergetics. He has more than 170 publications in the area of biological electron and proton transfer and his Glynn Laboratory is renowned worldwide. The laboratory specialises in the application of advanced spectroscopic methods to structure/function studies of membrane proteins, especially those of the mitochondrial respiratory chain. In recent years, Professor Rich has been concerned primarily with the development of ATR-FTIR spectroscopic methods so that they can be applied to the study of membrane redox proteins and a series of key publications in this area have been

produced. Professor Rich has received various awards, culminating most recently with the award of the prestigious Keilin Medal and is routinely invited to give major lectures at the majority of international meetings in molecular bioenergetics. He has also been on numerous Editorial and Award Panels and is currently an Executive Editor of the Elsevier Journal BBA Bioenergetics and Chair of the Bioenergetics and Metabolomics Theme Panel of The Biochemical Society.
The major equipment of the laboratory are two Bruker research FTIR spectrometers equipped with purpose-built transmission and ATR devices, and with rapid and step scan facilities, and three near UV/visible transient kinetic spectrometers. All spectrometers have means of light-activation with continuous, xenon or laser flashes. ATR facilities include automatic buffer perfusion, light activation, ability to record visible spectra synchronously with IR spectra and electrochemical cycling (the latter still under development). The laboratory has all facilities for membrane protein preparation and characterisation and access to molecular biology technology. Active projects involve a wide range of international collaborators who supply various well-defined proteins and mutants.

Recent relevant publications
- Rich, P.R. and Breton, J., FTIR studies of the cyanide and CO adducts of fully reduced bovine cytochrome c oxidase, Biochemistry, 40, 6441-6449 (2001)
- Rich, P.R. and Breton, J., ATR-FTIR Studies of Redox Changes in Bovine Cytochrome *c* Oxidase: Resolution of the Redox FTIR Difference Spectrum of Heme a_3., Biochemistry, 41, 967-973 (2002)
- Ingledew, W.J., Smith, S.M.E., Salerno, J.C. and Rich, P.R., Neuronal *Nitric Oxide Synthase* ligand and protein vibrations at the substrate binding site. A study by FTIR, Biochemistry, Biochemistry, 41, 8377-8384 (2002)
- Rich, P.R., Rigby, S. and Heathcote, P., Radicals associated with the catalytic intermediates of bovine cytochrome *c* oxidase, Biochim. Biophys. Acta, 1554, 137-146 (2002)
- Iwaki, M., Breton, J. and Rich, P.R., ATR-FTIR difference spectroscopy of the P_M intermediate of bovine cytochrome *c* oxidase, Biochim. Biophys. Acta, 1555, 116-121 (2002)

Participant #30 (University of Leeds)
The group brings together clinical academic, pathological, biochemical and oral biology expertise in a project looking at characterisation of cancer and non-cancer tissues in the head & neck and possible important links between micro-organisms and cancer by studying these at cellular and sub-cellular level.
The team has experience in the application of infrared microspectroscopy through an EPSRC funded project using the Synchrotron at Daresbury, which has confirmed promising spectral changes. This next phase in the work will allow further definition of those changes and understanding of their relevance in terms of biochemical change.
The principal investigator, Sheila Fisher, is Senior Lecturer in Oral & Maxillofacial Surgery at the University of Leeds. Through the multidisciplinary head & neck clinic, the oncology team sees 8% of the total UK head & neck cancer population. Mrs Fisher's major research interest is in the potential of new technology to aid in the diagnosis and treatment and prevention of head & neck cancer. She has a long standing interest in the management of premalignant disease, in laser technology and more recently photodynamic therapy and electroporation as potential modalities of treatment. She is a member of the team led by, Professor Mike Chesters, Professor of Physical Chemistry at the University of Nottingham, collaborating on an EPSRC funded programme of research using synchrotron IMS for cancer diagnosis at single cell level.

Recent relevant publications
- M A Chesters, M J Tobin, N R Griffin and S E Fisher, Oral Oncology, (1999) VI, 193-194.

- Tobin MJ, Rutten F, Chesters MA, Chalmers JM, Symonds I, Fisher SE, Allibone R and Hitchcock A. (2002), Investigating the potential for infrared microanalysis in cancer screening. European Clinical Laboratory, 21, 20-22.
- Chesters M.A., Tobin M.J., Griffin N.R. and Fisher S.E. (1999). Infrared spectroscopy – may it have a role in the diagnosis of oral cancer? Pp. 193-194. In: Oral Oncology VI. Proceedings of the VIth International Congress on Oral Cancer. Ed Varma A.K. MacMillan Press. SBN 0333 932714.
- Srinivasan D., Fisher S.E., Seth R., Jenkins D., Edwards R. and Griffin N.R. (1997), Apoptosis and cell proliferation assessment in oral tumours Pp. 511-513. In: Oral Oncology V. Proceedings of the Vth International Congress on Oral Cancer, Ed. Varma A.K. MacMillan Press, SBN 0333 930797.
- Fisher S.E., Edwards R., Jenkins D., Seth R., Bradley P.J. and Griffin N.R. (1997),
Changes in cell markers, in multiple tumours, in a patient with long standing oral lichen planus. Pp. 493-495. In: Oral Oncology V, Proceedings of the Vth International Congress on Oral Cancer. Ed. Varma A.K. MacMillan Press. SBN 0333 930797.

A plan to extend the participation of European groups and companies and the potential expansion of the BASIE NoE exists and it is devoted to spread excellence, outside the boundary of this already large scientific community. Many teams involved in researches related to those proposed by the BASIE NoE exists, and a reliable expansion can be considered at least for two groups:
1) Staffordshire Oncology Centre - The Staffordshire Oncology Centre, North Satffordhsire Hospital, Stoke on Trent, serves a population of 845.000. The department has around 2000 new patient referrals a year. The facilities within the department include a Radiotherapy Unit with 2 Varian Linear Accelerators, a "Gulmay" superficial and orthovoltage Unit and a "Nucletron" simulator with CT facility and a third Linear Accelerator is planned. The Centre for Science and Technology in Medicine is based at Keele University, Staffordshire, U. K. and was rated 5A in the latest (2001) UK Research Assessment Exercise (RAE). The centre's research encompasses a cross-disciplinary approach to bioengineering and biomedical sciences.

2) The Biological Research Centre of the Institute of Biophysics located at Szeged in Hungary. The group leaded by Dr. Balázs Szalontai have two FTIR instruments, one of these is Bruker with rapid scan, step scan and FT Raman equipments. The research is devoted to the structure and dynamics of mostly bacteriorhodopsin as a protein, different intermediers, and the role of amino acid side chains in the proton transfer. In addition, the structure and dynamics of biological membranes, lipidoprotein interactions, mostly in photosynthetic membranes, using genetically modified lipid compositions to reveal the role of certain fatty acids in membrane dynamics and temperature adaptation.

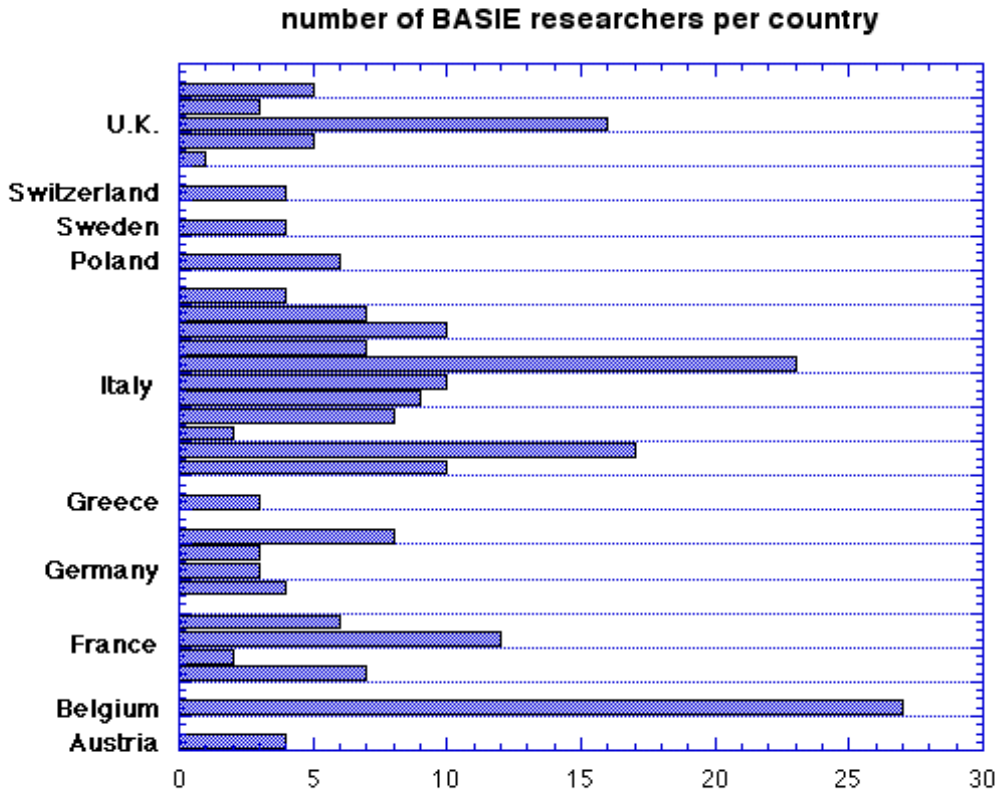
B.6 Quality of the integration

This section describes how the continuous evaluation aimed at examining and analysing well defined collaborative work will ensure the effectiveness for collaboration.

The European research in general, but also in life sciences suffers from insufficient resources and from fragmentation. National and regional research programmes are insufficiently geared up to each other and cross-border co-operation between universities and industry is not yet widespread. We need to strengthen competitiveness to permit growth and the creation of highly skilled jobs. The driving factor is primarily research, which expands the new knowledge base in life sciences.

Scientists working together (officially or not) is the rule more than the exception for successful research, as the level and diversity of knowledge can hardly be found in one individual or even in one team. Moreover some groups or individual may benefit from equipment or human resources that can be used effectively not only for their own thematic but also as a shared facility. For this reason, most of us tend to put aside our instinct of competition (temporarily) and we usually find that by working together we form stronger problem-solving collaborative relationships by bringing together human and/or technical resources.
Effective collaborations are able to generate positive outcomes such as quality research (amount, stature, creativity and visibility) of research and may also include financial savings and stronger international voice. However, these benefits need to be balanced against administration costs and staff time. In this network project, we are planning to influence the collaborative by strengthening: leadership, unity, communication, participation, organization, and successful accomplishments to which we could add goal setting.

A. Present situation
As for now, BASIE links 30 research teams with moderate levels of collaboration. These groups can be subdivided into three main types depending on their institution: 21 Biophysics/Biology and Medicine research laboratories, eight synchrotron radiation teams working in large research laboratories and one enterprise. The total number of researchers is close to 160, and ten European countries are represented. 24 out of the 30 laboratories are from four countries (Italy, France, Germany and the U.K.). These four countries represent more than 75 % of the total number of researchers and also include seven teams in charge of Infrared Synchrotron Radiation facilities. Most of the groups are relatively small (20 groups include not more than five people).



Two main areas to be coordinated by the Network proposal and their respective roles can be defined:

- The Synchrotron Radiation groups roles will be:
To develop and improve synchrotron IR sources and adapt (or develop) specific instrumentation for applying FT interferometry and IR microscopy with emphasis on far-IR detectors.

- The Biophysics Biology Biomedicine role will consist in:
using the advanced infrared spectroscopy/microscopy to study the protein structure, dynamics and functions and to provide the specific (clinically) relevant proteins and the research questions to be answered.

The large number of countries and the diversity of background as well as the geographical dispersion may limit the interactions and therefore the successful collaboration. For this reason, an Executive Board representative of countries, thematics and techniques will assist the coordinator in its leadership role. The composition of this Executive Board is discussed in section B.7.

B. The plan for coordinating research in collaboration
Using the structure as defined above, one coordinator assisted by a board chosen on the basis of their geographical as well as research theme complementarities, we have defined three actions which will guarantee a real and lasting collaboration effort:

Action 1 - Definition of *Workpackages* and identification of responsibility.
These 30 groups agreed on 27*Workpackages* defined as subjects where a collaboration would exist within the network. These relatively well defined projects are subdivided in five larger themes as shown in the detail in the section B.9. The basic idea of the planned network is to give resources on the basis of the collaborations rather than to a given group on the basis of its past accomplishments. For each work package a responsible will be identified and will take responsibility for communicating to the Executive Board the status and progress of the accomplishment through collaborative work. Monitoring the collaborations will include: number of visits, exchange of students and accomplishments (manuscripts, communications, thesis, etc…).

Action 2 - Responsibility taken by the Executive Board for the continuous evaluation.
The member of this board will not only represent the countries and techniques but will also be responsible for ensuring the effectiveness of the collaborations and will verify that each of the following factors are as well met as possible. For each factor, one (or more) member of the Executive Board will be explicitly responsible and will present the effectiveness of the collaboration as for this factor in the yearly meeting.

- Research and Evaluation - the collaboration for each *Workpackage* will conduct a needs assessment (resources and personnel) to establish its goals and the Executive Board will collect data to measure goal achievement;
- Sustainability - for each *Workpackage*, one team will present a plan for sustaining membership and resources. This will involve guidelines relating to terms and members;
- Communication - the collaboration will develop open and clear communication. There will be an established process for communication through meetings, web site, list of competence on subjects.
- Leadership - the adequacy and quality of representation of the Network by the Executive Board will be reconsidered every second year in the Network meeting

Action 3 - Distribution of resources based on the need of each node and the level of collaboration.

- Policies - At the start of the network, the distribution of resources will be decided both upon the size of each node and on the basis of the collaboration for accepted *Workpackages*; in subsequent meetings, it will be reconducted upon the meeting of the goals set.
- Resources - Every member of a collaboration for an accepted *Workpackage* will have access to needed resources. Resources refer to four types of capital: environmental, in-kind, financial, and human; the Executive Board will verify that more isolated communities receive opportunities for their work.
- Gender Issues - specific financing for women researchers will be guaranteed as described in the gender issues section.

Overall, each node will get involve in collaborative work and will provide elements for evaluation of the working packages. The Executive Board will provide guidance and assistance with working plan and will ensure that key components of partnerships are met. They will take responsibility for monitoring, evaluation and tracking.
However, we envisage the Network as a flexible unit, which is not rigidly delimited by its initial composition. Rather, within the duration of the project, we will continuosly stimulate each node to involve more groups into collaborating to the aims of the Network. Most naturally, but not exclusively, each node could involve other European teams with which is already collaborating. Such new collaborations should be separately funded (and administered), by applying to national or international granting agencies. This will achieve the purpose of conveying additional funds and manpower to the project, of increasing its impact on the scientific community and strengthening the quality and duration of its offsprings. The performance of each node will be evaluated also on these additional criteria.

B.7 Organisation and management

Our Network of Excellence involves 30 teams of scientists from Universities, Institutions and companies and accounts for 160 researchers and about 70 students.
The organizational, management and governance structure of the Network was defined as a joint effort in the following democratic way. The first teams of the proposal defined a date for a joint meeting of the proposal. The meeting was held the February 26 in Frascati. At the meeting attended 18 scientists representing the following nodes:

1) node #1 INFN/LNF Frascati,
2) node #2 Universita' La Sapienza (Roma)
3) node #3 CNRS Orsay
4) node #4 FZK Karlsruhe (delegated by the Institutions of the node 7-BESSY and node 19 Robert-Koch Inst. at Berlin)
5) node #5 PSI-ETH Zurich
6) node #9 Bruker (Milano)
7) node #10 Sincrotrone Trieste
8) node #11 Universita' Palermo
9) node #12 Universita' Perugia
10) node #17 Universita' Politecnico delle Marche (Ancona)

Scientists from other nodes: #21 - ESRF (Grenoble) and #23 - Universita' di Milano sent their suggestions, in a way that at least 14 groups were represented. At the meeting we first discussed our scientific researches and potential synergies outlining the benefit of a common large effort. All together agreed to present a proposal for a Network of Excellence and the selected title was:

Biological Applications of Synchrotron Infrared Spectroscopy in Europe

with the acronym BASIE. After, we discussed in detail the proposal and we defined several issues concerning the Network. The first of these issues was the composition of the Executive Board of the proposal. The Executive Board has been considered as a key element of the "organization and management" of the BASIE Network of Excellence. It is composed by 10 members first elected at the meeting for a period of 12-24 months. It is expected that, a first group of the members will be replaced during the 60 months proposed period of the Network life in order to have all groups represented within the Board during the entire life of the Network. The Executive Board Members represent the interests of the Institutions involved into the Network and their main role is to both address and facilitate the decision making within the Network on a rigorous democratic basis.
We plan that all the groups involved in the Network of Excellence will have an equal status. The practical main issues of co-ordination and functioning of the Network of Excellence via the Executive Board are:

a) information exchange (website, bulletin, etc.)
b) organization of meetings within NoE, workshops, etc.,
c) coordinate training activities,
d) distribution of resources with the budget,
e) gathering reports,
f) management of knowledge of intellectual property arising by the researches;
g) prepare official NoE documentations and report current activities when requested.

The Executive Board Committee should also provides a peer review of the research activity in the IR Synchrotron Radiation Facilities and, may help in allocation of beam time, on the different available facilities of the BASIE Network, of the experiments planned within the proposal considering the balance of allocation of resources across the different BASIE laboratories.

This scientific committee will meet on average two times a year as regular meetings, but also videoconference meetings will be considered to prompt react to urgent needs. It is assumed that this committee will prepare the annual report of the research program. However, a crucial management resource of the Network will be also the regular meetings, at workshops or schools, of the Executive Board members and the node coordinators.

The initial Executive Board Members of the BASIE NoE were:

1) Augusto Marcelli	#1 - LNF-INFN (Frascati)	marcelli@lnf.infn.it
2) Paolo Calvani	#2 - La Sapienza (Roma)	calvani@roma1.infn.it
3) Pascale Roy	#3 - CNRS/Paris	pascale.roy@lure.u-psud.fr
4) Paul Dumas	#3 - CNRS/Paris	paul.dumas@lure.u-psud.fr
5) David Moss	#4 - FZK/Karlsruhe	david.moss@anka.fzk.de
6) Ulrich Schade	#7 - BESSY/Berlin	schade@bessy.de
7) Michael Chesters	#14 - Univ. Nottingham	Michael.Chesters@Nottingham.AC.UK
8) Erik Goormaghtigh	#16 - ULB/Brussels	egoor@ulb.ac.be
9) Paolo Mariani	#17 - UPM (Ancona)	mariani@alisf1.unian.it
10) Jacques Breton	#18 - CEA/Saclay	cadara3@dsvidf.cea.fr

The Executive Board Members is formed by representatives of the Network nodes selected after discussion and following several independent criteria, which included of course the largest possible representativity of the nodes, but also the need to cover all the different scientific areas included in the BASIE proposal. The latter criteria were considered strategic in particular to trigger in the initial activity of the proposed Network.
The Executive Board will follow closely all aspects of the research initiatives: scientific value, organization, resources in terms of personnel and budget, progress and scientific results. The scientific background of the members of the Executive Board covers at the highest level the whole BASIE activity, that is divided into the 5 *Workpackage* groups listed in section B4. If BASIE will be approved, in its first meeting the Board will select 5 of its members. Each one of them will take in charge one *Workpackage*. They will periodically report to the Coordinator and to the Board on the advancements of the *Workpackage* of competence. They will also advice the Board about the distribution of funds to different activities.
The other 4 members of the Board, excluding the Coordinator, will take in charge an equal number of important activities of the whole network:
training, meeting organization, external relationships (including the website and the database), financial control.
During the meeting, after the nomination of the Executive Board Members, was also elected the Coordinator of the BASIE Network of Excellence: Augusto Marcelli (INFN-LNF, Frascati, node n.1) The network organizational structure is then adequate to the complexity of the scientific issues planned both on the short- and long-term period. The composition and the criteria considered for the Network Board match both the complexity of the network and the degree of integration required by the presence of groups with different expertise. However it is also limited in number so that any decision-making mechanisms can be adequate to respect the milestones.

After the meeting, other groups joined our proposal accounting for 10 EU and associated EU countries, joining the original the scientific project but adding relevant scientific contributions in different topics of the BASIE proposal. All these groups supported the name of the Executive Board members elected at the meeting.
For the future, another task of the Executive Board will be the discussion of the possible addition and integration of new participants (if any) during the period of Community funding. It will consider and decide the necessary changes to the NoE structure, define the new plan and add new milestones.

In order to deal with the actual complexity of this NoE, we intend to propose to the Community a Consortium composed by all groups without subcontractors.

All groups of the NoE agreed also, in case of success, to nominate a BASIE REVIEW COMMITTEE. This panel composed by at least three international (not European) independent experts in the appropriate fields covered by the BASIE proposal, will have the role to review during the life of the Network the activities of the cooperation and the quality of the results achieved. This review committee will be invited to analyze the structure and programs of the NoE on the basis of documentation collected time by time, with particular attention being devoted to the results obtained and the milestones reached. Actually this Committee will follow the progress of the Network and in particular of the workpackages, making the necessary recommendations to the Executive Board.

The Executive Board will be also constantly monitoring scientific advances and needs as well as the evolution of international and national legislation, regulations and ethical rules regarding the researches developed within the framework of the BASIE proposal. Executive Board members

according to their expertise have to dedicate time to these issues in order to identify potential problems. The role of the Executive Board may be also that to create specific panels of experts selected among the team members and/or experts of National Institutions to analyze the emerging issues related to the Network and prepare specific advices on emerging areas of Life Science (see Section B.9).

The management of the Network of Excellence and the co-operation between the participants will be carried out primarily by electronic means of communication, including the videoconferencing system, which is available now in almost all laboratories and major Institutions.
However, one of the most important aspects of this Network is the regular exchange of scientists and students between facilities and teams in order to accelerate the achievements of the scientific goals and to facilitate the development of the new facilities in commissioning or under construction and the associated technologies necessary to the advancement of the entire project.
Scientists and students from all teams will be invited to submit proposals to the operating facilities, to participate to the accelerator runs at the IRSR facilities, and to discuss the results to plan further achievements of the Network activities. In fact, because of many collaborations already exists among teams of the BASIE nodes, they work within the limit of the limited present resources and without coordination, sometimes duplicating efforts and resources. In view of the present existing competition in this strategic field with USA and Japan, this condition is really unfavourable for the European research and the goal to coordinate these efforts within a unique European framework, is one of the main motivation of the proposal.

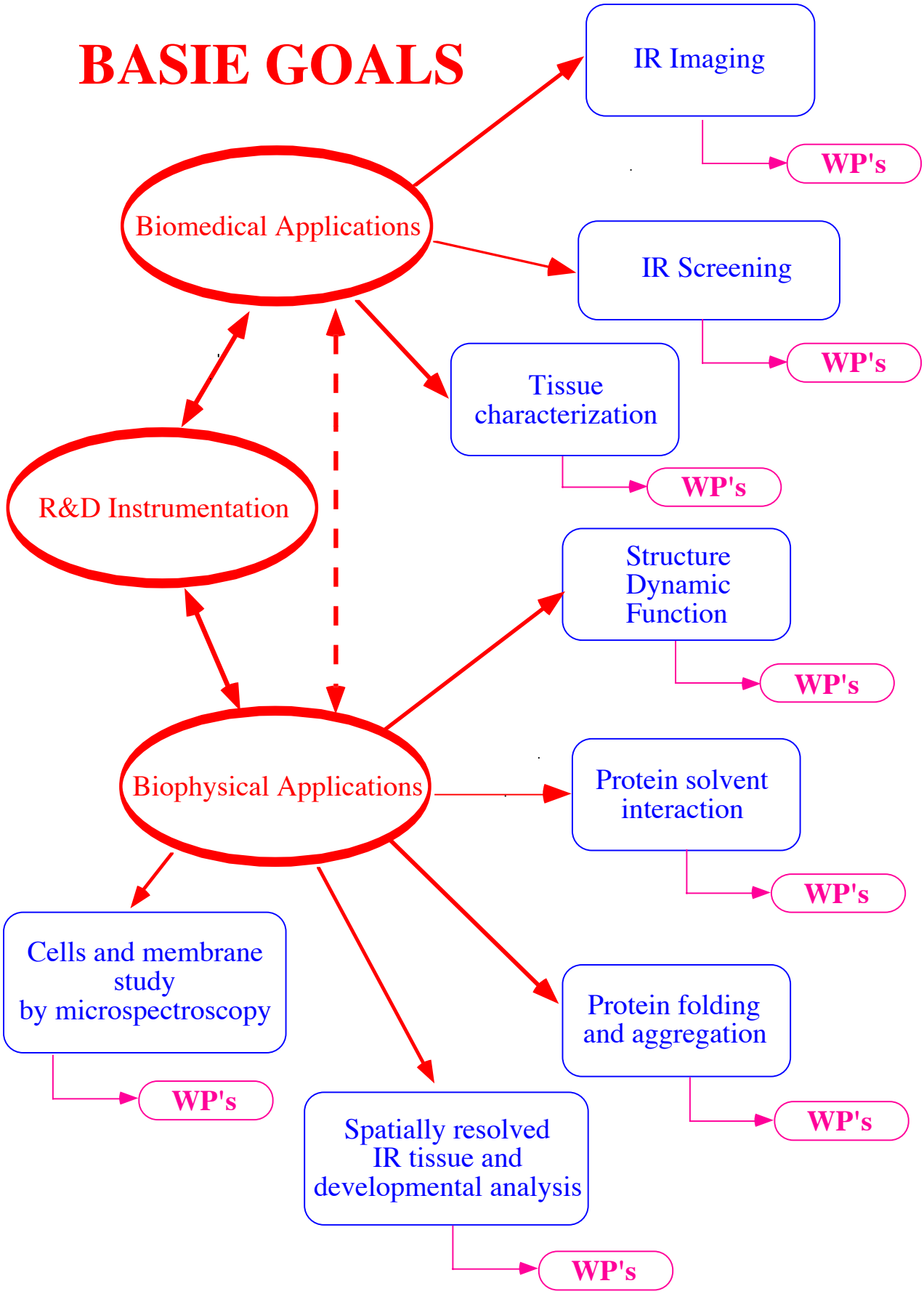
B.8 Joint Programme of Activities – first 18 months

The Joint Program of Activities that the BASIE NoE is engaged to perform in the first 18 months of its duration is reported in the following pages. This reduced plan is obviously based on the masterplan for the whole 60-month duration of BASIE, that has been described in detail in Section B.4. Therefore in the first 18 months there will be the 5 Main activities

GROUP 1 SOURCES/EQUIPMENT/TECHNIQUES
GROUP 2 STUDIES OF PROTEINS IN VITRO
GROUP 3 STUDIES OF BIOMEMBRANES AND MEMBRANE PROTEINS
GROUP 4 STUDIES OF CELLS
GROUP 5 STUDIES OF TISSUES / CLINICAL DIAGNOSIS

that in turn will be articulated in 27 *Workpackages (WP)*. However, a different description of the Network plan may provide a better insight into the Life science content of the proposed Network of Excellence.

As shown in Sec. B.4, some *Workpackages* concern the Research and Development of Infrared Synchrotron Instrumentation, an issue that is ancillary to both advanced Biophysical and Biomedical applications. Therefore the *Workpackages* can also be classified according to the following scheme which identifies three main areas (in red in the scheme), of which that concerning the instrumentation is ancillary to nine objectives, all of biophysical and biomedical interest (in blue in the scheme).

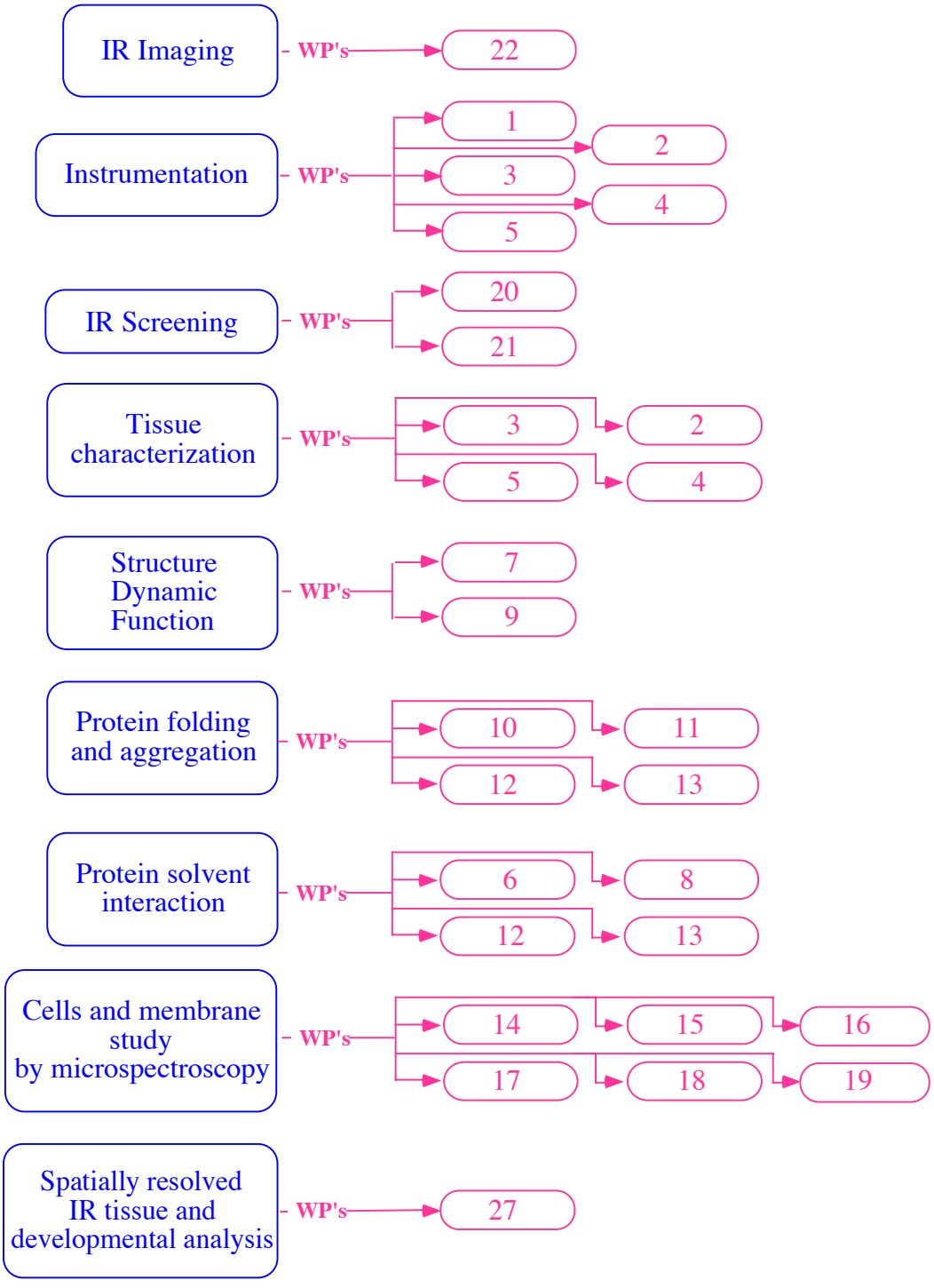

BASIE GOALS
Biomedical Applications
R&D Instrumentation
Biophysical Applications
IR Imaging
IR Screening
Tissue characterization
Structure Dynamic Function
Protein solvent interaction
Protein folding and aggregation
Spatially resolved IR tissue and developmental analysis
Cells and membrane study by microspectroscopy
WP's
WP's
WP's
WP's
WP's
WP's
WP's

The 6 objectives of biophysical interest for the first 18 months are:

- *Studies of single cells and model-membranes by microspectroscopy;*
- *Spatially resolved infrared analysis of tissues*
- *Studies of protein*
- *Folding and aggregation;*
- *Interaction with solvent;*
- *Functionality, through structure and dynamics;*

and the 3 objectives of biomedical interest are:

- *Imaging of biological materials by infrared microscopy;*
- *Early detection of cancer diseases aimed at screening risky groups of patients;*
- *Tissue characterization for further studies by different techniques.*

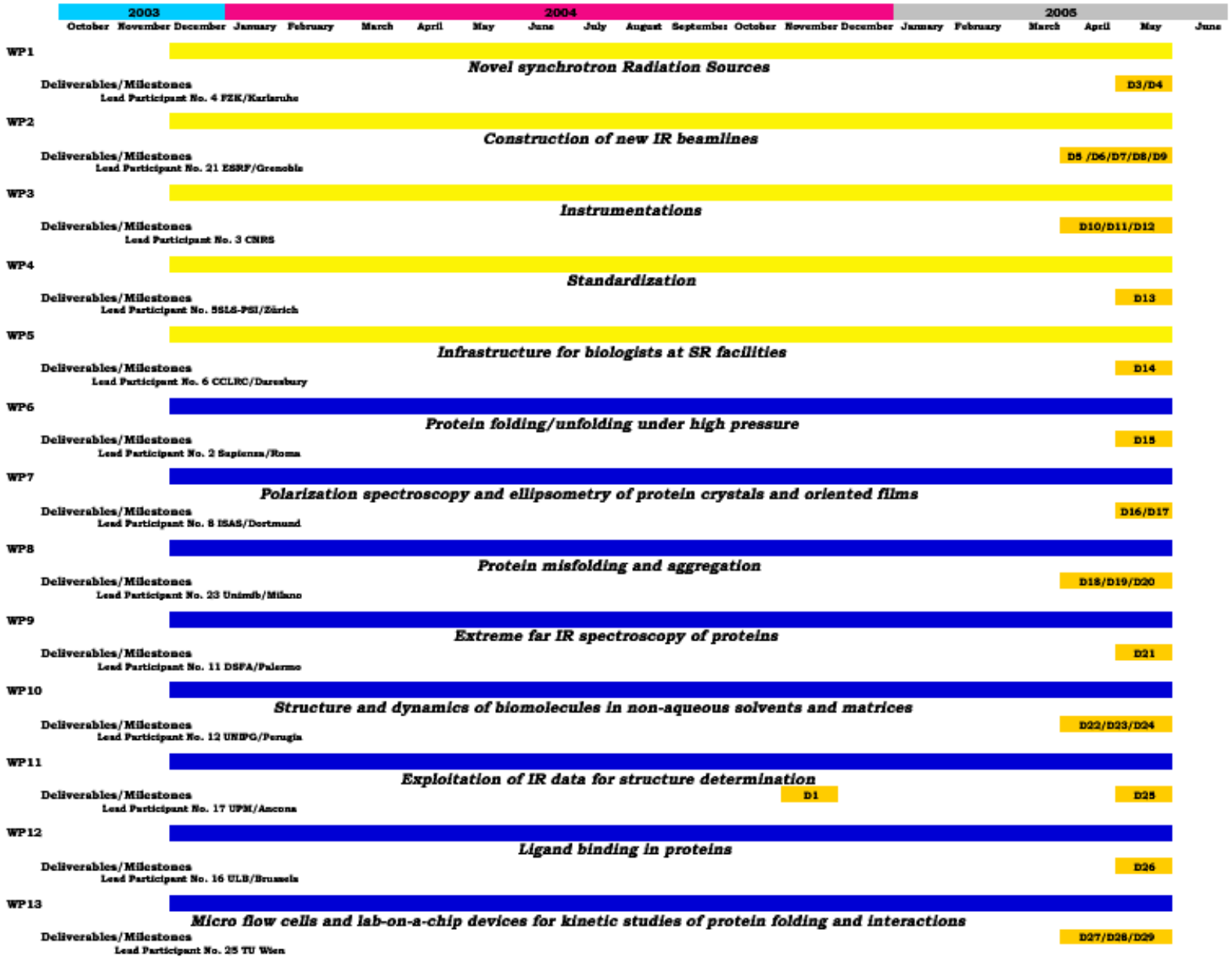


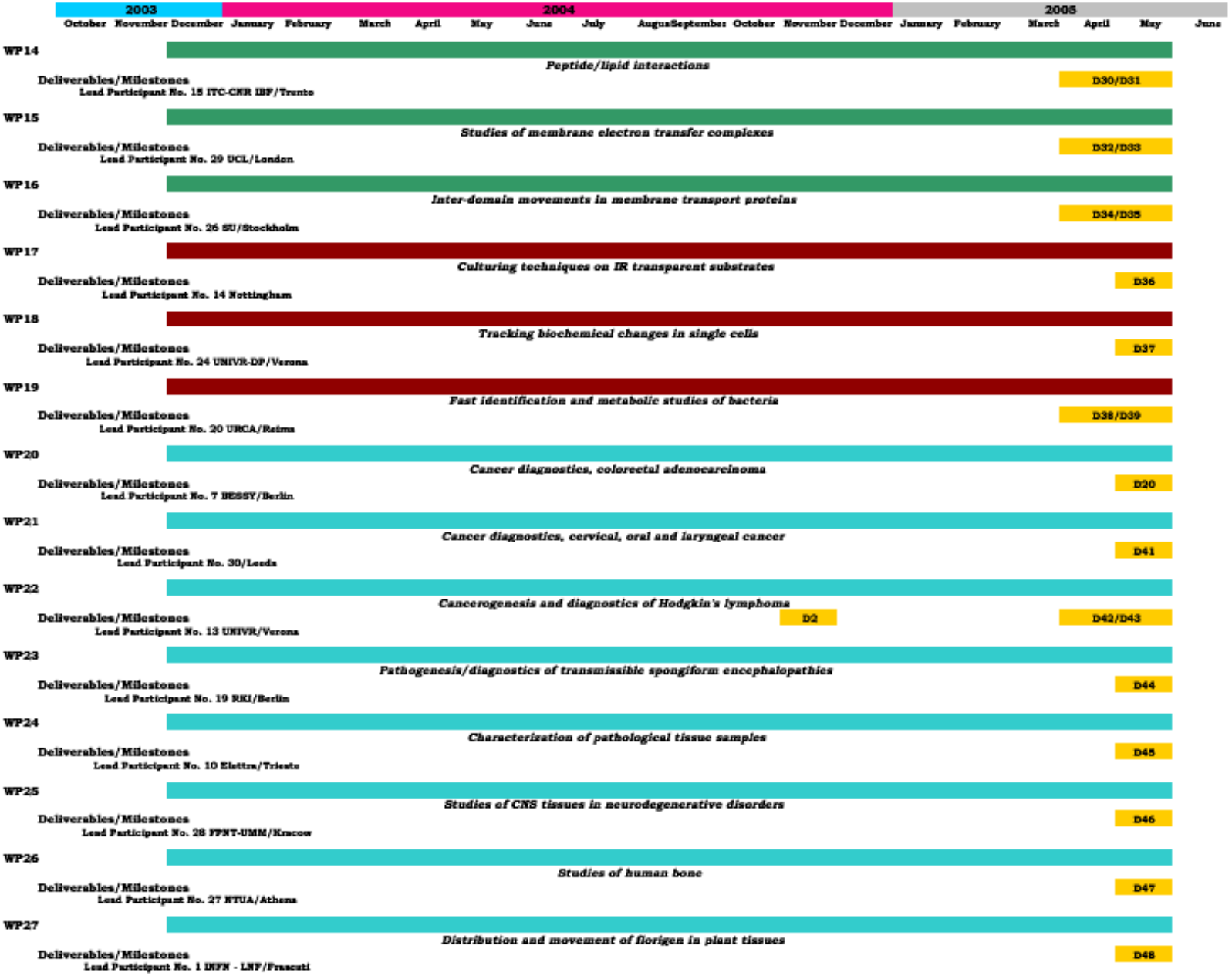
The first plot illustrates the primary flow of information and know-how, with technical innovations from the Instrumentation Area flowing to the biological/biomedical *Workpackage* groups but also receiving a feed-back from the users experience, with experimental insight from *in vitro* molecular studies flowing to more complex living systems, and fundamental discoveries concerning proteins, biomembranes and cells flowing toward clinical applications in tissue studies and diagnostics. The second plot describes the*Workpackages* as derived by the first scheme.

As far as the temporal scale of the first 18 months is concerned, we have managed the 27 WP's in such a way that all of them may proceed "in parallel", in spite of synergies and disciplinary interdipendency. This is allowed by the abundance of experimental laboratories and large facilities that are available to the present Network. As stressed in the Introduction to the proposal, the research field of BASIE suffers for lack of coordination, not for lack of experimental infrastructures. Therefore, for example, a wise use of the resources will provide enough beamtime on the existing infrared facilities for all needs, while the new ones are being built and commissioned.

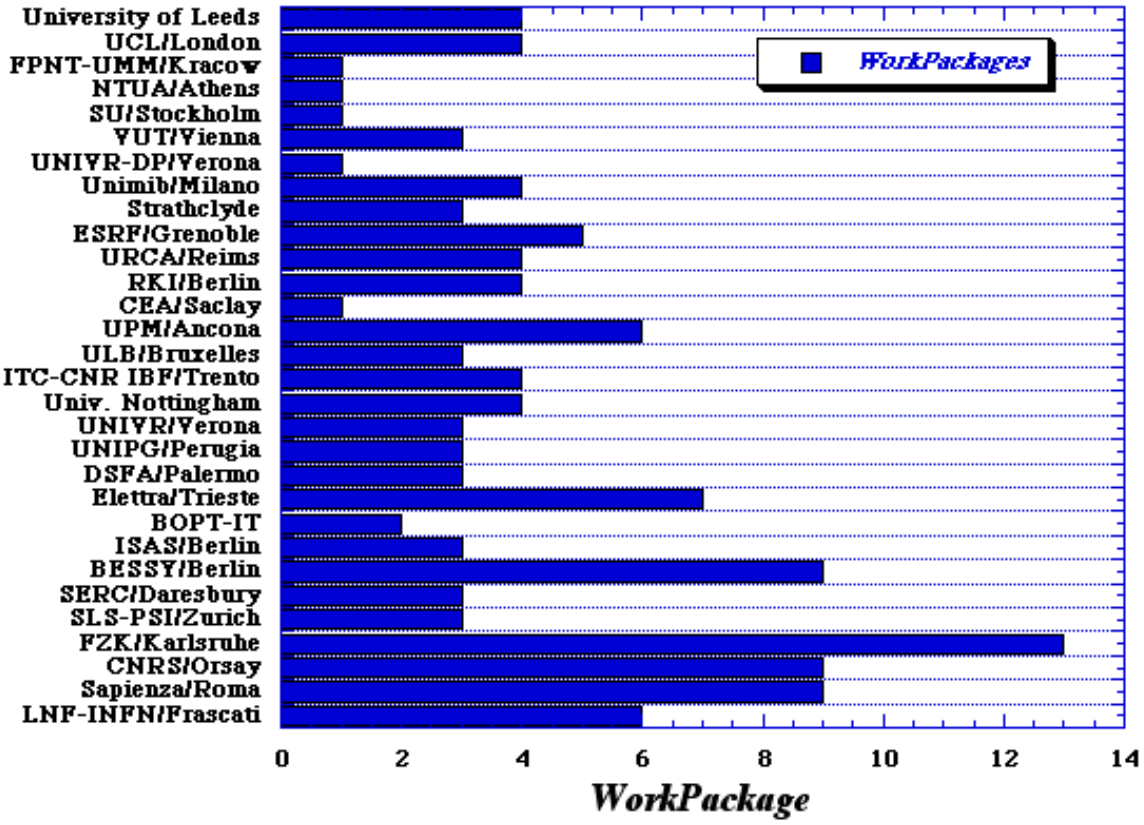
The following Ghant chart expresses this simple assumption. We believe that all the Network activities can start together in Month 0 of BASIE, which has been tentatively assumed to be November 2003.

BASIE WORKPACKAGE LIST (18 months)



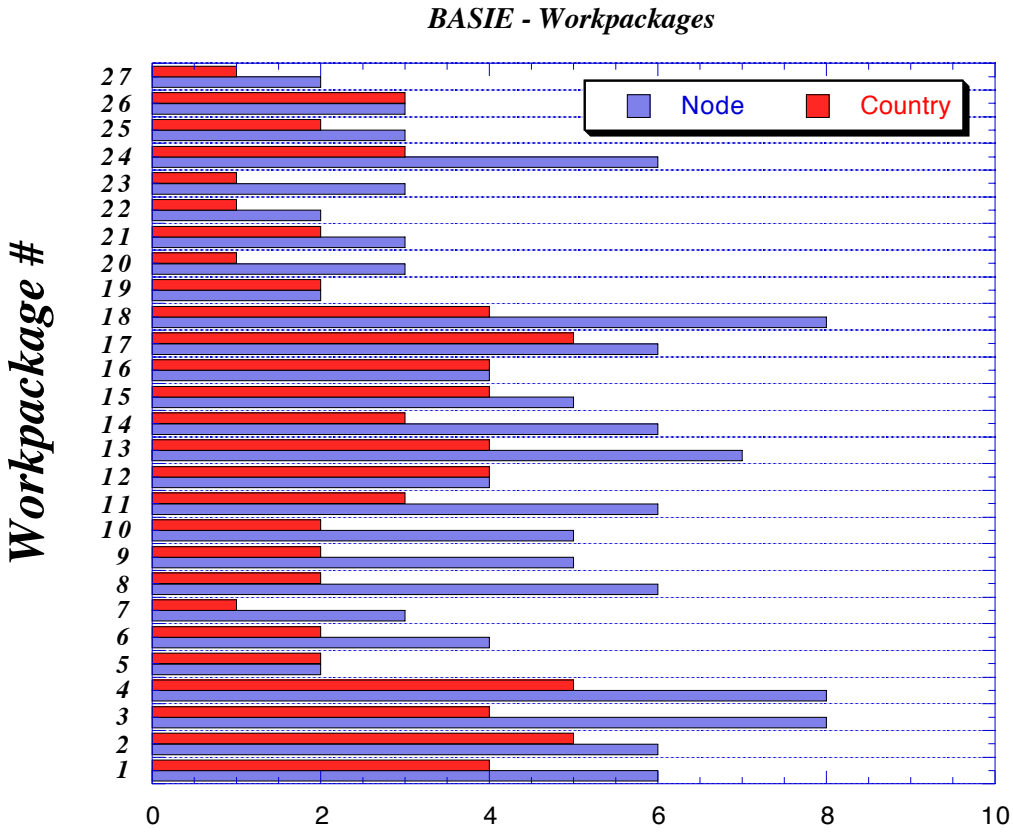

2003
2004
2005
October November December January February March April May June July AugusSeptembes October November December January February March April May June
WP14
Peptide/lipid interactions
Deliverables/Milestones
Lead Participant No. 15 ITC-CNR IBF/Trento
D30/D31
WP15
Studies of membrane electron transfer complexes
Deliverables/Milestones
Lead Participant No. 29 UCL/London
D32/D33
WP16
Inter-domain movements in membrane transport proteins
Deliverables/Milestones
Lead Participant No. 26 SU/Stockholm
D34/D35
WP17
Culturing techniques on IR transparent substrates
Deliverables/Milestones
Lead Participant No. 14 Nottingham
D36
WP18
Tracking biochemical changes in single cells
Deliverables/Milestones
Lead Participant No. 24 UNIVR-DP/Verona
D37
WP19
Fast identification and metabolic studies of bacteria
Deliverables/Milestones
Lead Participant No. 20 URCA/Reims
D38/D39
WP20
Cancer diagnostics, colorectal adenocarcinoma
Deliverables/Milestones
Lead Participant No. 7 BESSY/Berlin
D20
WP21
Cancer diagnostics, cervical, oral and laryngeal cancer
Deliverables/Milestones
Lead Participant No. 30/Leeds
D41
WP22
Cancerogenesis and diagnostics of Hodgkin's lymphoma
Deliverables/Milestones
Lead Participant No. 13 UNIVR/Verona
D2
D42/D43
WP23
Pathogenesis/diagnostics of transmissible spongiform encephalopathies
Deliverables/Milestones
Lead Participant No. 19 RKI/Berlin
D44
WP24
Characterization of pathological tissue samples
Deliverables/Milestones
Lead Participant No. 10 Elettra/Trieste
D45
WP25
Studies of CNS tissues in neurodegenerative disorders
Deliverables/Milestones
Lead Participant No. 28 FPNT-UMM/Krakow
D46
WP26
Studies of human bone
Deliverables/Milestones
Lead Participant No. 27 NTUA/Athens
D47
WP27
Distribution and movement of florigen in plant tissues
Deliverables/Milestones
Lead Participant No. 1 INFN - LNF/Frascati
D48

The distribution of the work among the 30 nodes of the Network in the first 18 months is instead shown in the following diagram. Integration, temporal "parallelism" and equilibrated distribution of the research activity may be appreciated by observing that the average number of *Workpackages* per node is more than 4, with much larger numbers only for those IRSR facilities that are already operational.



Each *Workpackage* involves the participation of a number of researchers between one third and one half of the NoE participants, with a broad coverage across the different scientific backgrounds.
The strong integration provided by the plan is illustrated in the next plot, where the number of different groups and countries participating to all the WP's is reported.

Potential risks for BASIE may basically come only by an abrupt shortening of the national funds to the synchrotron facilities, that are the heart of the proposal. Also considering present financial problems for R&D in some European countries, the plan of the first 18 months should not be affected. It has been done by taking into account prudential estimates based on the long-term plans of the research Institutions in the various countries. In any case, BASIE will be a flexible Network. At the end of the 18 months, also the financial situation will be examined and, in case of any fund shortening that is presently not predictable, appropriate measures will be undertaken by the Executive Board. These may include the involvement of other groups, possibly other industrial partners, which may provide the necessary support without radically changing our scientific plan or affect the excellence of the BASIE activities.



The next pages contain the forms with a short list of the Workpackage*s*, the Deliverables and the Milestones of the whole BASIE programme.

Finally we include the forms with the description of the 27 Workpackages for the first 18 months.

Workpackage list (18 months)

Work-package No*	Workpackage title	Lead participant No†	Start month‡	End month§	Deliv-erable No**
W1	Novel synchrotron sources	4	0	18	D3, D4

* Workpackage number: WP 1 – WP n.
† Number of the contractor leading the work in this workpackage.
‡ Relative start date for the work in the specific workpackages, month 0 marking the start of the project, and all other start dates being relative to this start date.
§ Relative end date, month 0 marking the start of the project, and all ends dates being relative to this start date.
** Deliverable number: Number for the deliverable(s)/result(s) mentioned in the workpackage: D1 - Dn

W2	Construction of new IR beamlines	21	0	18	D5, D6, D7, D8, D9
W3	Instrumentation	3	0	18	D10, D11, D12
W4	Standardization	5	0	18	D13
W5	Infrastructure for biologists at SR facilities	6	0	18	D14
W6	Protein folding/unfolding under high pressure	2	0	18	D15
W7	Polarization spectroscopy and ellipsometry of protein crystals and oriented films	8	0	18	D16, D17
W8	Protein misfolding and aggregation	23	0	18	D18, D19, D20
W9	Extreme far IR spectroscopy of proteins	11	0	18	D21
W10	Structure and dynamics of biomolecules in non-aqueous solvents and matrices	12	0	18	D22, D23, D24
W11	Exploitation of IR data for structure determination	17	0	18	D1, D25
W12	Ligand binding in proteins	16	0	18	D26
W13	Micro flow cells and lab-on-a-chip devices for kinetic studies of protein folding and interactions	25	0	18	D27, D28, D29
W14	Peptide/lipid interactions	15	0	18	D30, D31
W15	Studies of membrane electron transfer complexes	29	0	18	D32, D33
W16	Inter-domain movements in membrane transport proteins	26	0	18	D34, D35
W17	Culturing techniques on IR transparent substrates	14	0	18	D36
W18	Tracking biochemical changes in single cells	24	0	18	D37
W19	Fast identification and metabolic studies of bacteria	20	0	18	D38, D39

W20	Cancer diagnostics, colorectal adenocarcinoma	7	0	18	D40
W21	Cancer diagnostics, cervical, oral and laryngeal cancer	30	0	18	D41
W22	Cancerogenesis and diagnostics of Hodgkin's lymphoma	13	0	18	D42, D2, D43
W23	Pathogenesis/diagnostics of transmissible spongiform encephalopathies	19	0	18	D44
W24	Characterization of pathological tissue samples	10	0	18	D45
W25	Studies of CNS tissues in neurodegenerative disorders	28	0	18	D46
W26	Studies of human bone	27	0	18	D47
W27	Distribution and movement of florigen in plant tissues	1	0	18	D48
	TOTAL				

Deliverables/milestones list (18 months)

Deliverable/ Milestone no*	Deliverable/milestone title	Delivery/Achieve date †	Nature ‡	Dissemination level §
D1	Software for secondary structure fit on protein 3D shape reconstructed by SAS data	Month 12	M	CO

* Deliverable numbers in order of delivery dates: D1 – Dn.
† Month in which the deliverables will be available or milestone achieved. Month 0 marking the start of the project, and all dates being relative to this start date.
‡ Please indicate the nature of a deliverable using one of the following codes:
R = Report
P = Prototype
D = Demonstrator
O = Other
If milestone, indicate with **M**
§ Please indicate the dissemination level for deliverables using one of the following codes:
PU = Public
PP = Restricted to other programme participants (including the Commission Services)
RE = Restricted to a group specified by the consortium (including the Commission Services)
CO = Confidential, only for members of the consortium (including the Commission Services)

D2	First measurements of human B-cell T-cell and Hodgkin's lymphoma samples at the Frascati IR beamline	Month 12	M	CO
D3	Report on edge radiation	Month 18	R	CO
D4	Report on coherent emission	Month 18	R	CO
D5	Commissioned IR beamline at ELETTRA	Month 18	O	PU
D6	Commissioned IR beamline at SLS	Month 18	O	PU
D7	Commissioned IR beamline at ESRF	Month 18	O	RE
D8	Report on European IR beamtime demand/supply analysis in the biological/biomedical field, with recommendations for future action	Month 18	R	CO
D9	Interim report on progress with beamline construction at DIAMOND, SOLEIL and ANKA	Month 18	M	CO
D10	First-draft software for synchrotron-optimized data acquisition	Month 18	M	CO
D11	First tests of individual components for a synchrotron-optimized optical bench	Month 18	M	CO
D12	First tests of low area detector pre-prototypes	Month 18	M	CO
D13	Interim standard for performance tests and criteria based on experience at the synchrotron infrared beamlines in operation during the first 18 months of the program	Month 18	M	CO
D14	Commissioned laboratory for specialized biological sample handling	Month 18	O	PU
D15	Functional high pressure cell and preliminary results with at least one protein	Month 18	M	CO
D16	Commissioned biological IR ellipsometry station at BESSY	Month 18	O	PU
D17	First results on polarized IR difference spectroscopy of single crystals at ANKA-IR	Month 18	M	CO
D18	Interim report on aggregation studies with model proteins	Month 18	M	CO
D19	Interim report on unfolding/aggregation in lipocalins	Month 18	M	CO

D20	Interim report on misfolding in clinically relevant proteins	Month 18	M	CO
D21	Interim report on extreme far IR spectroscopy of proteins	Month 18	M	CO
D22	Interim report on proteins in organic solvents	Month 18	M	CO
D23	Interim report on proteins in reverse micelles	Month 18	M	CO
D24	Interim report on proteins in solid matrices	Month 18	M	CO
D25	Description of the conformational changes in few model proteins (cytochrome *c*, myoglobin, lysozyme) during pressure induced and thermal folding/unfolding processes	Month 18	M	CO
D26	Interim report on ligand binding studies	Month 18	M	CO
D27	Practical microstructured mixing/flow cells for studies of proteins	Month 18	**D**	CO
D28	Interim report on demonstration experiments with microstructed mixing/flow cells	Month 18	M	CO
D29	Interim report on design of lab-on-a-chip devices	Month 18	M	CO
D30	Practical apparatus for synchrotron-based infrared IRRAS and ATR spectroscopy of lipid monlolayers and bilayers	Month 18	D	CO
D31	Interim report on peptide-lipid interactions	Month 18	M	CO
D32	Practical electrochemical ATR-FTIR cell for mid- and far-IR redox difference spectra of proteins	Month 18	D	CO
D33	Interim report on redox difference spectra of mitochondrial complexes I and III	Month 18	M	CO
D34	Interim report on domain movements in Ca^{2+}-ATPase	Month 18	M	CO
D35	Interim report on domain movements in complex III	Month 18	M	CO
D36	Practical and validated experimental set-up for studies of single living cell using synchrotron FTIR spectroscopy	Month 18	D	CO
D37	Interim report on biochemical changes in single cells	Month 18	M	CO

D38	Interim report on microcolony identification	Month 18	M	CO
D39	Interim report on in situ sulfate bacteria metabolism	Month 18	M	CO
D40	Interim report on synchrotron-based tissue mapping and interpretation	Month 18	M	CO
D41	Interim report on infrared microspectroscopy-based cancer screening	Month 18	M	CO
D42	Optimized protocol for sample handling and data acquisition of human B-cell, T-cell and Hodgkin's lymphoma samples	Month 18	R	CO
D43	Interim report on multi–approach analysis of human B-cell T-cell and Hodgkin's lymphoma	Month 18	M	CO
D44	Interim report on TSE pathogenesis studies	Month 18	M	CO
D45	Interim report on diagnostically relevant infrared spectral signatures from human tissues	Month 18	M	CO
D46	Interim report on infrared microspectroscopic investigations of neurodegenerative tissue samples	Month 18	M	CO
D47	Interim report on synchrotron IR microspectroscopy of bone samples	Month 18	M	CO
D48	Interim report on flower induction signalling	Month 18	M	CO

Workpackage description (18 months)

Workpackage number	WP 1	**Start date or starting event:**				Month 0	
Participant id	4	3	7	5	10		

Objectives

- Improved understanding of the production and properties of edge radiation and coherent emission
- Development of a design for an insertion device dedicated to IR edge radiation
- Optimized exploitation of these sources for biophysical, biological and biomedical research

Description of work

- Measurements and modeling of the edge radiation properties at ANKA and LURE IR beamlines
- Measurements and modeling of the coherent emission at BESSY IR beamline
- Modeling of the magnetic field profile and calculation of the emission of the insertion device
- Reference design of the insertion device

Deliverables

D3 Report on edge radiation and insertion device design (**R**) – Month 18

D4 Report on coherent emission (**R**) – Month 18

Milestones

Workpackage description (18 months)

Workpackage number	WP 2	**Start date or starting event:**			Month 0		
Participant id	10	5	21	4	3	6	

Objectives

- Construction of new synchrotron infrared beamlines
- Elimination of demand/supply imbalance in respect of access to synchrotron infrared spectroscopy for European biological and biomedical scientists.

Description of work

- Completion of construction and commissioning of new infrared beamlines at ELETTRA, SLS and ESRF.
- Significant progress towards the planned new infrared beamlines at SOLEIL, DIAMOND and ANKA

Deliverables

D5 Commissioned IR beamline at ELETTRA (**O**) – Month 18

D6 Commissioned IR beamline at SLS (**O**) – Month 18

D7 Commissioned IR beamline at ESRF (**O**) – Month 18

D8 Report on European IR beamtime demand/supply analysis in the biological/biomedical field, with recommendations for future action (**R**) – Month 18

Milestones

D9 Interim report on progress with beamline construction at DIAMOND, SOLEIL and ANKA – Month 18

Workpackage description (18 months)

Workpackage number	WP 3	**Start date or starting event:**			Month 0		
Participant id	4	1	2	3	9	7	8
	22						

Objectives

- Optimization of the data quality obtainable at synchrotron IR light sources
- Full exploitation of the advantages of synchrotron IR light sources
- Optimized adaptation of experimental facilities at synchrotron IR beamlines to the special requirements of the biological/biomedical scientific communities

Description of work

- Development of new data acquisition software optimized for the typical characteristics of synchrotron infrared radiation (e.g. beam current decay compensation, injection artefact rejection)
- Optimal adaptation of existing optical benches to the unique characteristics of synchrotron radiation (low divergence, high brilliance) and to compensate for beam position variations
- Design of small area detectors to exploit the high brilliance of synchrotron sources, yielding higher acquisition speed and better signal-to-noise ratio
- Adaptation of IR ellipsometry equipment at for studies of biological samples
- Construction of sample holder for precisely aligned multiple techniques of microscopy and microspectroscopy with biological samples

Deliverables

Milestones

D10 First-draft software for synchrotron-optimized data acquisition – Month 18

D11 First tests of individual components for a synchrotron-optimized optical bench – Month 18

D12 First tests of low area detector pre-prototypes – Month 18

Workpackage description (18 months)

Workpackage number	WP 4	**Start date or starting event:**				Month 0	
Participant id	4	7	3	10	1	5	6
	21						

Objectives

- Establishment of standardized performance tests and criteria for synchrotron infrared beamlines
- Provision of objective criteria to assess the improvements achieved in WP 1 and WP 3

Description of work

- Round table discussions to evaluate individual performance tests and criteria currently in use and analyze obstacles to transferability between facilities
- Production of first-draft standardized performance tests and criteria
- Application of standardized tests and criteria at all operational facilities and comparative analysis of results
- Iterative rounds of application and improvement of standardized tests and criteria

Deliverables

Milestones

D13 Interim standard for performance tests and criteria based on experience at the synchrotron infrared beamlines in operation during the first 18 months of the program – Month 18

Workpackage description (18 months)

Workpackage number	WP 5	**Start date or starting event:**					
Participant id	6						

Objectives

- Provision of specialized biological handling capabilities at synchrotron radiation facilities, conforming to laboratory safety regulations with respect to biological materials

Description of work

- Equipment of the new IR users' preparative lab at SRS Daresbury with a class II microbiological safety cabinet, tissue culture incubator, inverted microscope and upright pathologists' microscope class II.

Deliverables

D14 Commissioned laboratory for specialized biological sample handling (**O**) – Month 18

Milestones

Workpackage description (18 months)

Workpackage number	WP 6	**Start date or starting event:**					
Participant id	2	1	3	17			

Objectives

- Contribution to general understanding of protein folding pathways and mechanisms

Description of work

- Construction of high pressure cells / adaptation of existing cells for studies of protein solutions in the far infrared range under temperature control
- Far infrared studies of protein folding/unfolding and dynamics as a function of pressure and temperature using various model proteins

Deliverables

Milestones

D15 Functional high pressure cell and preliminary results with at least one protein – Month 18

Workpackage description (18 months)

Workpackage number	WP 7	**Start date or starting event:**				Month 0	
Participant id	8	7	4				

Objectives

- Contribution to understanding of structure/function relationships and molecular mechanisms in proteins
- Sequence-specific band assignments by comparison of dipole orientations with available structural data
- Contribution to rapid structural determination applicable to proteins not accessible by X-ray crystallography or NMR spectroscopy

Description of work

- Adaptation of the ellipsometer at the BESSY IR beamline for biological samples
- Development of measurement strategies and data evaluation procedures for ellipsometry of protein crystals and oriented films
- Polarized IR difference spectroscopy of single protein crystals at ANKA

Deliverables

D16 Commissioned biological IR ellipsometry station at BESSY (**O**) – Month 18

Milestones

D17 First results on polarized IR difference spectroscopy of single crystals at ANKA-IR – Month 18

Workpackage description (18 months)

Workpackage number	WP 8	**Start date or starting event:**				Month 0	
Participant id	11	15	2	17	23	19	

Objectives

- Fundamental understanding of processes and mechanisms in protein misfolding and aggregation
- Fundamental understanding of molecular mechanisms in amyloid diseases and spongiform encephalopathies

Description of work

- IR spectroscopic studies of aggregation behaviour of model proteins under various conditions
- IR spectroscopic studies of the role of trace metals in aggregate formation and growth
- IR spectroscopic studies of unfolding and aggregation in lipocalins
- IR spectroscopic studies of misfolding in clinical relevant proteins (β_2 microglobulin, prions)

Deliverables

Milestones

D18 Interim report on aggregation studies with model proteins – Month 18

D19 Interim report on unfolding/aggregation in lipocalins – Month 18

D20 Interim report on misfolding in clinically relevant proteins – Month 18

Workpackage description (18 months)

Workpackage number	WP 9	**Start date or starting event:**				Month 0	
Participant id	11	2	12	7	23		

Objectives

- Advances in fundamental understanding of protein dynamics by synchrotron IR spectroscopy of proteins in the extreme far IR region 10 – 100 cm^{-1}, which is dominated by collective modes of the protein/matrix system.

Description of work

- Joint design discussions regarding the cryogenic apparatus needed for the experiments, simultaneous training of students at synchrotron IR sources (Months 0-6)
- Preliminary experiments at Palermo with conventional IR sources (Months 0-6)
- Synchrotron IR experiments with myoglobin and hemoglobin in different quaternary conformations, over the temperature range 0 – 320 K and in various matrices (Months 7-18)

Deliverables

Milestones

D21 Interim report on extreme far IR spectroscopy of proteins - Month 18

Workpackage description (18 months)

Workpackage number	WP 10	**Start date or starting event:**				Month 0	
Participant id	11	12	2	3	17		

Objectives

- Contribution to fundamental knowledge of the structure and dynamics of biomolecules in non-aqueous solvents and matrices
- Contribution to pharmaceutical issues of protein transport through biological barriers
- Contribution to fundamental knowledge in protein-matrix interactions

Description of work

- Use of synchrotron IR spectroscopy to characterize picosecond dynamics of model proteins (lysozyme, cytochrome *c*, β-lactoglobulin, myoglobin) in organic solvents as a function of water content
- Synchrotron IR studies of structure and dynamics of model proteins enclosed in reverse micelles
- Synchrotron IR studies of structure and dynamics of model proteins in solid matrices (silica hydrogels, trehalose glasses)

Deliverables

Milestones

D22 Interim report on proteins in organic solvents – Month 18

D23 Interim report on proteins in reverse micelles – Month 18

D24 Interim report on proteins in solid matrices – Month 18

Workpackage description (18 months)

Workpackage number	WP 11	**Start date or starting event:**				Month 0	
Participant id	17	12	23	2	3	8	

Objectives

- Development of alternative experimental strategies and approaches for determination of structural properties of soluble and membrane proteins

Description of work

- Derivation of a Reverse Monte Carlo method to fit the secondary structure of model proteins as determined by synchrotron FT-IR spectroscopy into protein shapes reconstructed by small angle X-ray and neutron scattering data.
- Application of above approach to time-resolved conformational states during thermal or pressure induced folding/unfolding processes or in different experimental conditions.
- Development of an experimental approach for structural determination of non-crystallizable proteins in solution based on the combined use of computational prediction techniques, FT-IR synchrotron spectroscopy (to determine the secondary structure) and small angle X-ray and neutron scattering shape analysis.

Deliverables

Milestones

D1 Software for secondary structure fit on protein 3D shape reconstructed by SAS data - Month 12

D25 Description of the conformational changes in few model proteins (cytochrome *c*, myoglobin, lysozyme) during pressure induced and thermal folding/unfolding processes - Month 18

Workpackage description (18 months)

Workpackage number	WP 12	**Start date or starting event:**			Month 0	
Participant id	26	4	25	29		

Objectives

- Establishment of FTIR spectroscopy as a general method for studying ligand binding in proteins
- Provision of a new technique for drug and herbicide development

Description of work

- Synchrotron IR spectroscopy of spectral changes occurring on ligand binding
- Use of ATP binding to Ca^{2+}-ATPase as a model system, involving ATP analogues with modified functional groups, in order to determine the role of each functional group in ligand binding

Deliverables

Milestones

D26 Interim report on ligand binding studies – Month 18

Workpackage description (18 months)

Workpackage number	WP 13	**Start date or starting event:**				Month 0	
Participant id	4	25	15	10	9	17	22

Objectives

- Development of novel methods for studies of protein folding and interactions

Description of work

- Development of micro mixing and flow cells for experiments with micro fluid volumes, matched to the spatial resolution of synchrotron IR spectroscopy
- Demonstration of the method with model proteins and protein-ligand systems in stopped flow, continuous flow and flow injection experiments
- Design steps towards microstructured lab-on-a-chip devices with added functionality (electro-osmotic fluid drive, multiple mixing steps, separations)

Deliverables

D27 Practical microstructured mixing/flow cells for studies of proteins (**D**) – Month 18

Milestones

D28 Interim report on demonstration experiments with microstructed mixing/flow cells – Month 18

D29 Interim report on design of lab-on-a-chip devices – Month 18

Workpackage description (18 months)

Workpackage number	WP 14	**Start date or starting event:**			Month 0		
Participant id	15	16	2	10	17	22	

Objectives

- Contribution to fundamental understanding of interactions between biomembranes and amyloid / toxin / antibiotic peptides

Description of work

- Adaptation of existing experimental apparatus for IRRAS of lipid monolayers at the air-water interface for use at a synchrotron IR beamline
- Development of experimental apparatus for ATR spectroscopy of supported bilayers and multilayers at the water-crystal interface
- Extension of above apparatus for high throughput measurements in a multi-channel configuration, using the high spatial resolution of the synchrotron IR to resolve individual microchannels
- Construction of a spectroelectrochemical cell for IRRAS to study electrostatic effects on the structure of membranes and membrane proteins.
- Experimental determination of structural changes and molecular mechanisms involved in peptide-lipid interactions

Deliverables

D30 Practical apparatus for synchrotron-based infrared IRRAS and ATR spectroscopy of lipid monlolayers and bilayers (**D**) – Month 18

Milestones

D31 Interim report on peptide-lipid interactions – Month 18

Workpackage description (18 months)

Workpackage number	WP 15	**Start date or starting event:**				Month 0	
Participant id	29	18	4	3	10		

Objectives

- Fundamental investigations of structure and function in mitochondrial respiratory enzymes of biomedical interest

Description of work

- Construction of an automated electrochemical ATR-FTIR cell to produce high quality mid-IR redox difference spectra of proteins. Test of the system to generate mid-IR redox difference spectra of the simple soluble model metalloproteins, ferredoxin, cytochrome **c** and plastocyanin (Months 0-6).
- Use of simple model proteins to test helium bolometer/ATR equipment with diamond and KRS-5 optics in the low frequency IR down to 300 cm^{-1} (Months 7-12)
- First electrochemically-induced ATR-FTIR redox difference spectra of of bovine mitochondrial forms of respiratory chain complexes I and III in the low frequency IR (Months 13-18)
- Development of an automated electrochemical cell for ATR-FTIR difference spectroscopy studies in a Bruker IFS 66v spectrometer functioning under vacuum (Months 13-18)

Deliverables

D32 Practical electrochemical ATR-FTIR cell for mid- and far-IR redox difference spectra of proteins (**D**) – Month 18

Milestones

D33 Interim report on redox difference spectra of mitochondrial complexes I and III – Month 18

Workpackage description (18 months)

Workpackage number	WP 16	**Start date or starting event:**			Month 0		
Participant id	26	16	4	29			

Objectives

- Fundamental investigations conformational molecular mechanisms in membrane transport proteins

Description of work

- Extreme far IR difference spectroscopy with synchotron light sources to study inter-domain movements in Ca^{2+}-ATPase resulting from binding of ATP and other nucleotides by caged precursor and/or rapid mixing techniques
- Synchrotron FTIR difference spectroscopy to study the hypothetical Rieske domain movement in complex III of the respiratory chain, using rapid mixing for ligand binding in combination with redox poising of the complex.

Deliverables

Milestones

D34 Interim report on domain movements in Ca^{2+}-ATPase – Month 18

D35 Interim report on domain movements in complex III – Month 18

Workpackage description (18 months)

Workpackage number	WP 17	**Start date or starting event:**				Month 0	
Participant id	4	20	14	7	30	25	

Objectives

- Development of enabling technologies for synchrotron IR studies of single living cells

Description of work

- Tests of cell survival and adhesion when grown in culture on various standard IR window materials, both untreated and with various physical and chemical surface treatments, using various standard cell lines
- Long-term observation of cell growth, development and reproduction in on candidate culture substrates using various cell lines
- Development of cell culture cuvettes with nutrient flow and temperature control for experiments involving chemical challenge of cultured cells during synchrotron FT-IR obervation

Deliverables

D36 Practical and validated experimental set-up for studies of single living cell using synchrotron FTIR spectroscopy (**D**) – Month 18

Milestones

Workpackage description (18 months)

Workpackage number	WP 18	**Start date or starting event:**			Month 0		
Participant id	14	20	4	2	30	7	24
	15						

Objectives

- Contributions to fundamental understanding of the nature of changes in cell chemistry which correlate to cell abnormality
- Contribution of fundamental insights relevant to cancer prevention and therapy
- Contribution of fundamental insights relevant to the mechanism of action of drugs at the cellular level
- Contribution of fundamental insights relevant to the mechanisms of cell damage through environmental toxins

Description of work

- Synchrotron IR studies of precancer- and inflammation-related changes in cultured cells, exfoliated cells and tissue samples from human subjects
- Synchrotron IR studies of the response of cells to oncology-related growth factors and to therapeutic agents
- Synchrotron IR studies of biochemical changes related to cell processes such as differentiation, migration and elevated transcriptional activity
- Synchrotron IR studies of cell responses to drugs and to environmental toxins
- Synchrotron IR studies of cell RIPs-II dependent cell intoxication mechanisms

Deliverables

Milestones

D37 Interim report on biochemical changes in single cells – Month 18

Workpackage description (18 months)

Workpackage number	WP 19	**Start date or starting event:**				Month 0	
Participant id	20	19	4				

Objectives

- Development of a novel technique for ultra-fast identification of bacteria
- Contribution to fundamental knowledge of interactions between bacteria and the soil environment, relevant to bioremediation

Description of work

- Transfer of existing data collection and analysis procedures for FTIR spectroscopic identification of bacteria to ultra-small microcolonies using synchrotron infrared microspectroscopy
- In situ determination of reaction products resulting from the interaction of sulfate-reducing bacteria and surfaces of sulfate minerals using synchrotron IR microspectrosopy

Deliverables

Milestones

D38 Interim report on microcolony identification – Month 18

D39 Interim report on in situ sulfate bacteria metabolism – Month 18

Workpackage description (18 months)

Workpackage number	WP 20	**Start date or starting event:**				Month 0	
Participant id	19	7	4				

Objectives

- Contribution to fundamental understanding of carcinogenesis-induced infrared spectral changes in human tissues
- Contribution to new methods of cancer diagnosis

Description of work

- Recording of high spatial resolution infrared spectral maps of colorectal adenocarcinoma tissue at using synchrotron infrared microspectroscopy
- Interpretation of data with multivariate data analysis
- Comparison of results with low resolution spectral maps recorded with standard laboratory instruments

Deliverables

Milestones

D40 Interim report on synchrotron-based tissue mapping and interpretation – Month 18

Workpackage description (18 months)

Workpackage number	WP 21	**Start date or starting event:**				Month 0	
Participant id	14	6	30				

Objectives

- Development of new techniques for clinical screening of cervical, oral and laryngeal cancer

Description of work

- Data collection from a statistically significant number of liquid-based cervical smear samples using synchrotron infrared microspectroscopy
- Extension of the work to cover exfoliated cells and brush biopsies for oral and laryngeal cancers
- Analysis of data with multivariate data analysis
- Comparison of data with relevant laboratory cell lines

Deliverables

Milestones

D41 Interim report on infrared microspectroscopy-based cancer screening– Month 18

Workpackage description (18 months)

Workpackage number	WP 22	**Start date or starting event:**				Month 0	
Participant id	13	1					

Objectives

- Contribution to fundamental understanding of the molecular basis of cancerogenesis
- Development of diagnostic molecular survival predictors in cancerogenesis
- Contribution of fundamental knowledge relevant to the development of new therapeutic strategies

Description of work

- Preparation of human B-cell, T-cell and Hodgkin's lymphoma samples as well as human bone tissues for FT-IR microspectroscopy at the synchrotron radiation IR beam in Frascati.
- Optimization of sample preparation techniques and data acquisition techniques
- Experimental determination of absorption bands of the molecular groups relevant for the spectroscopic analysis
- Preliminary comparison with results obtained by molecular and immunophenotypic techniques

Deliverables

D42 Optimized protocol for sample handling and data acquisition of human B-cell, T-cell and Hodgkin's lymphoma samples (**R**) – Month 18

Milestones

D2 First measurements of human B-cell T-cell and Hodgkin's lymphoma samples at the Frascati IR beamline – Month 12

D43 Interim report on multi–approach analysis of human B-cell T-cell and Hodgkin's lymphoma – Month 18

Workpackage description (18 months)

Workpackage number	WP 23	**Start date or starting event:**				Month 0	
Participant id	19	7	4				

Objectives

- Contribution to fundamental understanding of pathogenesis in transmissible spongiform encephalopathies
- Development of new techniques for diagnosis of transmissible spongiform encephalopathies

Description of work

- High-resolution synchrotron infrared microscopy of CNS tissues (dorsal root ganglia, cerebellum, medulla) from the Syrian hamster Scrapie model, allowing in situ analysis of microdisperse PrP^{sc} accumulations
- Investigation of possible spectroscopic differentiation of different Scrapie/BSE strains (Scrapie 263K, Scrapie Me-7, BSE-H)

Deliverables

Milestones

D44 Interim report on TSE pathogenesis studies – Month 18

Workpackage description (18 months)

Workpackage number	WP 24	**Start date or starting event:**				Month 0	
Participant id	20	2	10	14	30	13	

Objectives

- Contribution to fundamental knowledge of molecular fingerprints related to pathological conditions in human tissue samples
- Development of new diagnostic techniques

Description of work

- High resolution synchrotron infrared microspectroscopic mapping of pathological tissues including aneurismal aortas, thyroid abnormalities and gliomas
- Comparisons with spectral data from the relevant healthy tissues
- Multivariate analysis of data

Deliverables

Milestones

D45 Interim report on diagnostically relevant infrared spectral signatures from human tissues – Month 18

Workpackage description (18 months)

Workpackage number	WP 25	**Start date or starting event:**				Month 0	
Participant id	28	3	21				

Objectives

- Contribution to fundamental understanding of pathogenesis in Parkinson's disease and amyotrophic lateral sclerosis

Description of work

- High resolution synchrotron infrared microspectroscopic mapping of thin sections from pathological and control tissue samples
- Analysis of data in terms of local biochemical component distributions in affected human CNS tissues, comparison of compounds accumulating inside and outside nerve cells, analysis of β-amyloid distribution in pathological control tissues

Deliverables

Milestones

D46 Interim report on infrared microspectroscopic investigations of neurodegenerative tissue samples – Month 18

Workpackage description (18 months)

Workpackage number	WP 26	**Start date or starting event:**				Month 0	
Participant id	27	21	13				

Objectives

- Contribution to fundamental understanding of human bone structure, development, osteoporosis and ageing

Description of work

- High resolution synchrotron infrared microspectroscopic mapping of bone sample thin sections and osteoblasts
- Interpretation of data in terms of phosphate/protein and phosphate/carbonate ratios and collagen structure as a function of longitudinal and radial location within the bone

Deliverables

Milestones

D47 Interim report on synchrotron IR microspectroscopy of bone samples – Month 18

Workpackage description (18 months)

Workpackage number	WP 27	**Start date or starting event:**				Month 0	
Participant id	1	23					

Objectives

- Contribution to fundamental understanding of chemical nature and mechanisms of flower induction signalling in plant tissues

Description of work

- Recording of FTIR spectral signatures of molecules corresponding to candidate genes for a role in flower induction.
- High resolution synchrotron infrared microspectroscopy to characterize localization and movement of the candidate molecules in the vascular and/or intercellular spaces of plant tissues.

Deliverables

Milestones

D48 Interim report on flower induction signalling – Month 18

B.9 Ethical, safety and other EC-policy related issues

Recent spectacular advances in Life sciences have raised strong expectations about curing serious diseases and improving the quality of life. The new knowledge has enabled technical innovations such as genetic engineering, cellular cloning (although reproductive human cloning is prohibited by the Charter of Fundamental Rights of the European Union), biocatalysis, gene testing, gene therapy and other researches generally known under the name of biotechnologies. As there is an undisputed link between research and innovation, and the generation of health and welfare, such studies are strongly encouraged by the 6th Framework Programme. At the same time, they may imply sensitive issues, which raise concerns as to their ethical and social consequences.
The European Union is a community of law and of shared fundamental values and human rights, while respecting differences in cultural and ethical values and public morality. Therefore the European institutions have to take on board the concerns of the public opinion about the legal and ethical conditions under which research in Life science goes on.
In this context, the research activities proposed by the BASIE Network of Excellence take into account the ethical attitude of all EU Member States (and their constitutional and legal systems).
In particular, as far as human reproductive cloning and human embryonic stem cell research are concerned, we declare that the proposed NoE BASIE do not involve:

- any research activity aiming at human cloning for reproductive purposes;
- any research activity intended to modify the genetic heritage of human beings which could make such changes heritable*;
- any research activity intended to create human embryos solely for the purpose of research or for the purpose of stem cell procurement, including nuclear transfer by means of somatic cells;
- any research involving the use of human embryos or embryonic stem cells.

B.9.1 Ethical aspects

In carrying out the proposed project, all existing applicable safety provisions will be strictly respected. In addition, the Executive Board (see Section B.7) will be in charge to monitor the ethical, legal, social and safety issues that may eventually emerge during the life of the network.
At the stage of the proposal, 7 teams only intend to perform experiments that involve either human or animal biological specimens:

Node #13 (Universita' degli Studi di Verona)
The research project involves human tissues affected by the Hodgkin's lymphoma and human bone samples.

Node #14 (University of Nottingham)
The research programme involves:
- samples provided by human beings who are able to give consent;
- samples provided both by human beings in course of treatment and by healthy human volunteers;
- personal data.

Ethical permission will be obtained in advance for all the above aspects in compliance with the principles of the World Medical Association Declaration of Helsinki (October 2002). This work does **not** involve:
- Persons not able to give consent
- Children
- Human embryonic stem cells
- Human foetal tissue/human foetuses
- Genetic information
- Animals (any species)

Node #19 (Robert Koch - Institut, Berlin)
The research project involves human and animal tissues (tissue from human colorectal adenocarcinoma and CNS tissue from scrapie-infected Syrian hamsters). The proposed research is performed according to all safety provisions presently applicable in the countries where the research will be carried out.

Node #20 (Université de Reims Champagne-Ardenne)
The proposed research projects involve human tumour cells / tissues and animal tissues, and micro-organisms. The research programme involves two types of human biological samples:
- tissue sections taken from Cervical biopsies
Cervical biopsies are stored cryogenically. Tissue sections of 5 μm thickness are cryo-sectioned from biopsy material. Infrared analysis is carried out on unstained sections while parallel sections are stained and submitted to conventional cytological analysis. Infrared spectra from individual cells are measured from areas of the tissue section, which have been classified as normal or as characteristic of varying degrees of dysplasia. The objective of this part of the programme is to identify spectroscopic characteristics relating to abnormality.
- exfoliated material taken as part of the Pap cervical smear test procedure.
Cervical smear test material is worked up using the Thin Layer Cytology procedure, which produces a single layer of cells on a reflective slide for infrared analysis. The individual cells analysed are identified in a grid reference system and, after staining are subjected to conventional cytological assessment. This procedure allows any multivariate analysis of the cell IR spectra, which classifies the cells into sub-sets, to be tested against the conventional cytological analysis.
Ethical permission is obtained in advance for all the above aspects of this work in compliance with the principles of the World Medical Association Declaration of Helsinki (October 2002).

In implementing the proposed research the team will adhere strictly to all existing safety provisions applicable in the countries in which the research will be carried out.

Node #27 (National Technical University of Athens)
The research programme involves the use of human bones for structural and chemical composition studies only.

Node #28 (University of Mining and Metallurgy - Faculty of Physics and Nuclear Techniques - Krakov)
The research projects involve human central nervous system (CNS) tissue. The samples were taken during the autopsy from patients deceased with PD, ALS and from patient who died due to non-neurological conditions. These tissue samples were taken from selected parts of human brain and spinal cord. The specimens were frozen and cut into sections of 20 micrometers thick in a cryo-microtome. The slices were fixed onto thin film foils and freeze-dried. Infrared analysis is carried

out on unstained sections while parallel sections are stained and submitted to routine histopathological investigation. The researches will comprise two-dimensional microanalysis of biochemical components in thin tissue slices, using synchrotron IR microspectroscopy, (SRIR). In details, the planned experimental studies comprise:
1) Observations of local distribution of biochemical components in affected parts of human brain and spinal cord;
2) Comparison of chemical compounds accumulated in neurones and outside the nerve cells;
3) Recognition, if PD and ALS tissues reveal topographic and quantitative modification of biochemical compounds in comparison with the control group tissues;
4) Analysis of two-dimensional distribution of β-amyloid in structures of the pathological and control tissues;
5) Analysis of correlation between distribution of selected elements and biochemical components accumulated in different part of central nervous system tissue.
Moreover, evaluation of oxidation states of selected elements in these tissue samples will be performed using micro-XANES spectroscopy (also with respect to neurodegenerative disorders).

The researches were accepted by Jagiellonian University Bioethical Commission and are in accord with the policy related issue (Commision's opinion nr KBET/321/B/2002). In implementing the proposed research the team will adhere strictly to all existing safety provisions applicable in the countries in which the research will be carried out.

Node #30 (University of Leeds)
The research programme involves
- Samples provided by human beings who are able to give consent.
- Samples provided by human beings in the course of treatment and by healthy human volunteers.
- Personal data.
Ethical permission is obtained in advance for all the above aspects of this work in compliance with the principles of the World Medical Association Declaration of Helsinki (October 2002).

All of the other nodes of the Network of Excellence are **not** involved in investigations with human or animal beings and declared that
"Bioethical issues are not implicated in their proposed researches".

Declarations are included.

ISTITUTO NAZIONALE DI FISICA NUCLEARE

LABORATORI NAZIONALI DI FRASCATI

A. Marcelli

Frascati, March 12, 2003

To whom it may concern

As the coordinator of the node 1 of the BASIE NoE proposal, operative at INFN – Laboratori Nazionali di Frascati, I declare that: "Bioethical issues are not implicated in the proposed research program that will be performed by this node within the European Network of Excellence named *Biological Applications of Synchrotron Infrared Spectroscopy in Europe* (BASIE)".

In faith

(Dr. Augusto Marcelli)

P.O. Box 13, 00044 Frascati (Roma) Telefono 06/94032727 Fax 06/94033427

Prof. Paolo Calvani
DIPARTIMENTO DI FISICA
Università degli Studi di Roma "La Sapienza"
Piazzale Aldo Moro, 2
00185 Roma (Italy)

To whom it may concern

Roma, 20/3/2003

As the Coordinator of node 2 of the BASIE NoE proposal, operative at Dipartimento di Fisica, Universita' di Roma La Sapienza, I declare that:
"Bioethical issues are not implicated in the proposed research program that will be performed by this node within the European Network of Excellence named *Biological Applications of Synchrotron Infrared Spectroscopy in Europe* (BASIE)".

In faith,

Prof. Paolo Calvani

Fax +39 6 4463158 - Tel. Studio +39 6 49913493 - Laboratorio +39 6 49913507

L U R E

Laboratoire pour l'Utilisation du Rayonnement Électromagnétique

Bâtiment 209D - Centre Universitaire Paris-Sud - BP 34 - 91898 ORSAY Cedex
Tél. 01 64 46 80 00 - Télétax 01 64 46 41 48
http://www.lure.u-psud.fr

Orsay, 20 March 2003

To whom it may concern

As the local coordinator of the node 3 of the BASIE NoE proposal, at LURE, Orsay France, I declare that : "Bioethical issues are not involved in the proposed research program that will be performed by this node within the European Network of Excellence named *Biological Applications of Synchrotron Infrared Spectroscopy in Europe* (BASIE)".

faithfully yours,

Pascale Roy
Directrice de Recherche
CNRS

Forschungszentrum Karlsruhe
Technik und Umwelt

Institut für Synchrotronstrahlung
Synchrotron Light Source ANKA

Datum: 21. March 2003
Bearbeiter/-in: Dr. David Moss
Telefon: 07247/82-2689

To whom it may concern:

As the coordinator of the node 4 of the BASIE NoE proposal, operative at Forschungszentrum Karlsruhe, I declare that: "Bioethical issues are not implicated in the proposed research program that will be performed by this node within the European Network of Excellence named *Biological Applications of Synchrotron Infrared Spectroscopy in Europe* (BASIE)".

Dr. David Moss

Forschungszentrum Karlsruhe GmbH
Technik und Umwelt
Hermann-von-Helmholtz-Platz 1
76344 Eggenstein-Leopoldshafen

Telefon 07247 / 82-0, Telefax 07247 / 82 5070
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Eidgenössische Technische Hochschule Zürich
Ecole polytechnique fédérale de Zurich
Politecnico federale svizzero di Zurigo
Swiss Federal Institute of Technology Zurich

Solid State Physics Laboratory
Prof. Dr. L. Degiorgi

Phone ++41 1 633 22 41/22 68 (laboratory)
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Mailing address:
ETH-Zürich, HPF F16
CH-8093 Zürich, Switzerland

Dr. A.C. Marcelli
INFN
Frascati

Zurich, Monday, March 17, 2003

To whom it might concern

As principle investigator of Node #5 within the NoE named BASIE, I declare that bioethical issues are not implicated in the proposed research program.

Sincerely yours

L. Degiorgi

SYNCHROTRON RADIATION DEPARTMENT

Daresbury Laboratory
Daresbury Warrington
Cheshire
WA4 4AD

Telephone +44 (0) 1925 603584 (Direct)
Fax +44 (0) 1925 603124
Switchboard +44 (0) 1925 603000

21st March 2003

To whom it may concern

This is to confirm that work carried out at Daresbury Laboratory, as part of the proposed research programme, will only be carried out with prior ethical approval. All sample material will be provided either by the group of M. Chesters at Nottingham University (Participant 14), or by the group of S. Fisher at Leeds University (Participant 30). Each of these groups has provided a statement confirming that ethical approval that will be acquired for the use of human tissue and personal records.

Yours sincerely

Mark Tobin

Berliner Elektronenspeicherring-Gesellschaft
für Synchrotronstrahlung m.b.H.

BESSY

Albert-Einstein-Straße 15
D-12489 Berlin
Tel. 030/6392-2999
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Durchwahl/extension
Tel. 030/6392-3449
Fax 030/6392-2999

http://www.bessy.de
schade@bessy.de

Datum/date
2003-3-21

To whom it may concern

As the co-ordinator of the node 7 of the BASIE NoE proposal, operative at the Berliner Elektronenspeicherring-Gesellschaft für Synchrotronstrahlung m.b.H., I declare that bioethical issues are not implicated in the research program proposed by this node within the European Network of Excellence named Biological Applications of Synchrotron Infrared Spectroscopy in Europe (BASIE).

With best regards

Ulrich Schade

Dr. Ulrich Schade

ISAS

c/o Dr. K. Hinrichs
INSTITUT FÜR SPEKTROCHEMIE
UND ANGEWANDTE SPEKTROSKOPIE
INSTITUTSTEIL BERLIN

Leibniz Gemeinschaft

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e-mail hinrichs@isas-berlin.de
Internet http://www.isas-berlin.de

Ethical issues

20.03.2003

To whom it may concern

I hereby confirm that our research planned in the frame of the BASIE project does not involve ethical issues.

With best regards

Karsten Hinrichs

Dr. Karsten Hinrichs

Bankverbindung: Dresdner Bank AG Konto Institut für Spektrochemie (BLZ 440 800 50) 0 1 229 229 0

BRUKER OPTICS S.r.l. Unipersonale
Via G. Pascoli, 70/3
20133 MILANO
Tel. 02 70 63 63 70 r.a.
Fax 02 23 61 294
optics@bruker.it

Milan, March 24, 2003

To whom it may concern

As the coordinator of node 9 of the BASIE NoE proposal, operative at Bruker Optics S.r.l. in Milan,

I declare that

Bioethical issues are not implicated in the research program proposed by this node within the European Network of Excellence named BASIE (*Biological Applications on Synchrotron Infrared Spectroscopy in Europe*).

In faith

Bruker Optics S.r.l.
Dr. Alessandro Mondini

ALMON/lafer/022

ISO 9001:2000

TO WHOM IT MAY CONCERN

I hereby declare that research activity proposed to be carried out, as part of the joint research project presented by BASIE, at Elettra (BASIE Node #10) by research and technical personnel affiliated to Elettra and listed as components of Node #10 does not involve:

- human beings,
- human biological samples,
- personal data or genetic information, animals (any species),
- release into the environment of genetically modified micro-organisms or plants.

I also confirm that the proposed research does not involve:

- research activity aiming at human cloning for reproductive purposes,
- research activity intended to modify the genetic heritage of human beings which could make such changes heritable,
- research activities intended to create human embryos solely for the purpose of research or for the purpose of stem cell procurement, including by means of somatic cell nuclear transfer,
- research involving the use of human embryos or embryonic stem cells with the exception of banked or isolated human embryonic stem cells in culture.

Luca Quaroni, PhD

Trieste, March 14, 2003

Dipartimento di Scienze
Fisiche ed Astronomiche

DELL'UNIVERSITA' DI PALERMO
Via Archirafi, 36 - 90123 Palermo

Prof. Antonio Cupane
Tel. 091 6234 221
FAX 091 6162 461
Email: cupane@fisica.unipa.it

Palermo, March 13, 2003

To whom it may concern

In my quality of coordinator of the research unit acting at the Department of Physical and Astronomical Sciences of the University of Palermo, I declare that the research program that will be performed by the Palermo research unit within the European Network of Excellence "Biological Applications of Synchrotron Infrared Spectroscopy in Europe" (BASIE) does not involve ethical issues.

In faith

(Prof. Antonio Cupane)

UNIVERSITA' DEGLI STUDI DI PERUGIA
DIPARTIMENTO DI FISICA

PERUGIA, 21/05/03

I declare that the research of node 12 (Perugia University) does not involve ethical issues.

The coordinator of node 12

Giuseppe Onori

Giuseppe Onori

Università degli Studi di Verona
Dipartimento di Informatica

Ca' Vignal 2
Strada le Grazie 15
37134 Verona - Italia
Tel. +39 045 802 7069
Fax +39 045 802 7068

To whom it may concern

Ethical issues

I hereby declare that our proposed research project involves human tissues (Hodgkin's lymphoma) and human bone samples. Our research does not involve:

- any activities in human cloning for reproductive purposes
- human embryonic stem cells and human foetal tissue or human fetuses
- activties involving the use of human embryos or embryonic stem cells
- any activities intended to modify the genetic heritage of human beings which could make such changes heritable
- persons not able to give consent
- children

Ethical permission is obtained in advance for all the above aspects of this work in compliance with the principles of the World Medical Association Declaration of Helsinki (October 2002).

Safety provisions

I hereby declare that our proposed research project involves human tissues (Hodgkin's lymphoma) and human bone samples. In carrying out the proposed project I declare that all existing applicable safety provisions will be strictly respected.

Emilio Burattini

Prof. Emilio Burattini

Consiglio Nazionale delle Ricerche
Istituto di Biofisica
Sezione di Trento

Povo, 17.03.03

To whom it may concern

As the coordinator of the node 15 of the BASIE NoE proposal, operative at the Institute of Biophysics of the CNR, Section at Trento, I declare that bioethical issues are not implicated in the research program proposed by this node within the European Network of Excellence named Biological Applications of Synchrotron Infrared Spectroscopy in Europe (BASIE).
The studies, and expected results, do not have any known negative impact on humans, animals or environment. We will not use, or generate, modified organisms. We commit ourselves to respect all the relevant national and international safety regulations.

In faith

Gianfranco Menestrina

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Prof. E. Goormaghtigh

Phone: (32)-2-650.53.86
Fax: (32)-2-650.53.82
E-mail egoor@ulb.ac.be

March 13, 2003.

Professor Marcelli

Subject : Ethical issues

Dear Professor Marcelli,

I hereby confirm that that our research planned in the framework of the BASIE project does not involve ethical issues.

With my best regards

Erik Goormaghtigh

Prof. Paolo MARIANI

ISTITUTO DI SCIENZE FISICHE

Facoltà di Scienze Matematiche, Fisiche e Naturali -
Università Politecnica delle Marche
Via Ranieri, 65 - 60131 ANCONA (Italy)
tel 39.071.2204608 - fax 39.071.2204605
email: mariani@alisf1.unian.it

Ancona, March, 15, 2003

To whom it may concern

As Coordinator of the node 17 of the BASIE NoE proposal, operative at the Istituto di Scienze Fisiche - Università Politecnica delle Marche, I declare that "Bioethical issues are not implicated in the proposed research program that will be performed by this node within the European Network of Excellence named *Biological Applications of Synchrotron Infrared Spectroscopy in Europe* (BASIE)".

In faith

Paolo Mariani

COMMISSARIAT A L'ENERGIE ATOMIQUE - CENTRE D'ETUDES DE SACLAY - F 91191 GIF-SUR-YVETTE CEDEX

DIRECTION DES SCIENCES DU VIVANT

Service de Bioénergétique

Saclay, Mars 20, 2003

To Whom it may concern

As the coordinator of node 18 in the proposal for the European Network of Excellence named BASIE, I declare that ethical issues are not involved in the research program proposed by this node.

Jacques Breton

Directeur de Recherches au CEA

CORRESPONDANCE : SBE. Bât. 532, CEA SACLAY, 91191 GIF-SUR-YVETTE CEDEX, France
TELECOPIE / FACSIMILE : (33) 1.69.08.87.17. TELEPHONE : (33) 1.69.08.22.39
E-mail : cadara3@dsvidf.cea.fr

Prof. D. Naumann
Robert Koch-Institut, P13
Nordufer 20, 13353 Berlin
phone: +49-30-4547 2606
e-mail: NaumannD@rki.de

ROBERT KOCH INSTITUT

Robert Koch-Institut | Postfach 65 02 80 | 13302 Berlin

To whom it may concern,

Ethical issues

Unser Zeichen

Ihr Zeichen

Berlin, 18.03. 2003

Dr. Peter Lasch
Tel.: 030-45472405
Fax: 030-45472606

Besucheranschrift
Robert Koch-Institut
Nordufer 20
13353 Berlin

Tel. 01888.754-0
Fax 01888.754.2328
www.rki.de

I hereby declare that our proposed research projects involve human and animal tissues (tissue from human colorectal adenocarcinoma and CNS tissue from scrapie infected Syrian hamsters). Our spectroscopic studies do NOT involve:

- any activities in human cloning for reproductive purposes,
- human embryonic stem cells and human foetal tissue or human fetuses,
- activities intended to create human embryos solely for the purpose of research or for the purpose of stem cell procurement, including by means of somatic cell nuclear transfer,
- activities involving the use of human embryos or embryonic stem cells,
- genetic information,
- any activities intended to modify the genetic heritage of human beings which could make such changes heritable,
- persons not able to give consent,
- children.

Ethical permission is obtained in advance for all the above aspects of this work in compliance with the principles of the World Medical Association Declaration of Helsinki (October 2002).

Safety provisions

I hereby declare that our proposed research projects involve human and animal tissues (tissue from human colorectal adenocarcinoma and CNS tissue from scrapie infected Syrian hamsters). In implementing the proposed research I declare to adhere strictly to all existing safety provisions applicable in the countries in which the research is carried out.

Université de Reims Champagne-Ardenne

CNRS CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE

SPI

UNITE MEDIAN - CNRS UMR 6142

Médicament, Dynamique Intracellulaire et Architecture Nucléaire

UFR DE PHARMACIE - IFR53

Directeur : Michel MANFAIT

To whom it may concern,

Ethical issues

I hereby declare that our proposed research projects involve human tumour cells / tissues and animal tissues, and micro-organisms. Our spectroscopic studies do NOT involve :

. any activities in human cloning for reproductive purposes,

. human embryonic stem cells and human foetal tissue or human fetuses,

. activities intended to create human embryos solely for the purpose of research or for the purpose of stem cell procurement, including by means of somatic cell nuclear transfer,

. activities involving the use of human embryos or embryonic stem cells,

. genetic information,

. any activities intented to modify the genetic heritage of human being which could make such changes heritable,

. persons not able to give consent,

. children.

Ethical permission is obtained in advance for all the above aspects of this work in compliance with the principles of the World Medical Association Declaration of Helsinki (october 2002).

Safety provisions

I hereby declare that our proposed research projects involve human tumour cells / tissues and animal tissues. In implementing the proposed research I declare to adhere strictly to all existing safety provisions applicable in the countries in which the research will be carried out.

51 rue Cognacq-Jay, 51096 Reims Cedex, France
☎ : 0326913574 🖷 : 0326913550 International: (33) 3
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EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

J. SUSINI, Ph.D.
Head of the X-ray Microscopy Group
Experiment Division
Tel : (+33) (0)4 76 88 21 24
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E-mail : susini@esrf.fr

JS/JS 05-03
Grenoble – 20th March 2003

To whom it might concern,

As principal coordinator of the Node 21 within the *BASIE* NoE proposal, operative at the ESRF, I hereby confirm that bioethical issues are not implicated in the research program proposed by this node.

Yours truly,

J. Susini

Postal address: ESRF - BP 220 - F-38043 Grenoble Cedex - France
Location: Polygone Scientifique Louis Néel - 6, rue Jules Horowitz - Grenoble - France

UNIVERSITÀ DEGLI STUDI DI MILANO – BICOCCA
DIPARTIMENTO DI BIOTECNOLOGIE E BIOSCIENZE
Piazza della Scienza, 2 – 20126 MILANO
Prof.Silvia Maria Doglia
phone:+39 02 6448 3459; E-mail: silviamaria.doglia@unimib.it

To whom it may concern

As coordinator of the research node 23 of the BASIE NoE proposal, active at the Department of Biotechnologies and Biosciences of the University of Milano Bicocca, I declare that bioethical issues are not involved in the research program that will be performed by this node within the European Network of Excellence "Biological Applications of Synchrotron Infrared Spectroscopy in Europe" (BASIE) We are committed to respect all the national and international safety regulations.

In faith

Silvia Maria Doglia

DIPARTIMENTO DI PATOLOGIA
SEZIONE DI IMMUNOLOGIA
UNIVERSITÀ DEGLI STUDI DI VERONA – FACOLTA' di MEDICINA e CHIRURGIA

TO WHOM IT MAY CONCERN

I hereby declare that our proposed research does not involve:

- human beings,
- human biological samples,
- personal data or genetic information, animals (any species),
- release into the environment of genetically modified micro-organisms or plants.

I confirm that the proposed research does not involve:

- research activity aiming at human cloning for reproductive purposes,
- research activity intended to modify the genetic heritage of human beings which could make such changes heritable[1],
- research activities intended to create human embryos solely for the purpose of research or for the purpose of stem cell procurement, including by means of somatic cell nuclear transfer,
- research involving the use of human embryos or embryonic stem cells with the exception of banked or isolated human embryonic stem cells in culture[2].

Marco Colombatti, M.D.

Verona, March 13, 2003

[1] Research relating to cancer treatment of the gonads can be financed.

[2] Proposers should note that the Council and the Commission have agreed that detailed implementing provisions concerning research activities involving the use of human embryos and human embryonic stem cells which may be funded under the 6th Framework Programme shall be established by 31 December 2003. The Commission has stated that, during that period and pending establishment of the detailed implementing provisions, it will not propose to fund such research, with the exception of the study of banked or isolated human embryonic stem cells in culture.

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Ihr Zeichen	Ihre Nachricht vom	unser Zeichen	unsere Bearbeiterin	Telefon	Datum
				+43-1-58801 DW: 15140	20.05.03

To whom it may concern

Ref.: Network of Excellence, BASIE

As the coordinator of the node 25 of the BASIE NoE proposal, operative at the Institute of Chemical Technologies and Analytics of the Vienna University of Technology, I declare that bioethical issues are not implicated in the research program proposed by this node within the European Network of Excellence named Biological Applications of Synchrotron Infrared Spectroscopy in Europe (BASIE).
The studies , and expected results, do not have any known negative impact on humans, animals or environemnt. We will not use, or generate, modified organisms. We commit ourselves to respect all the relevant national and international safety regulations.

Sincerely,

Dr. Bernhard Lendl

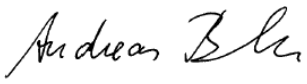
Department of Biochemistry and Biophysics

2003-03-24

To whom it may concern

As the coordinator of node 26 of the BASIE NoE proposal I declare that Bioethical issues are not implicated in the proposed research program that will be performed by this node within the European Network of Excellence named *Biological Applications of Synchrotron Infrared Spectroscopy in Europe* (BASIE).

Yours faithfully



Andreas Barth

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FAX: +30-210-7723184
E-MAIL: ianastas@central.ntua.gr

TO WHOM IT MAY CONCERN

- I hereby declare that our proposed research involves human bones for structural and chemical composition studies only and it does not involve any activity in human cloning for reproductive purposes.
- We do not create human embryos by means of somatic cell nuclear transfer

We are in accord with ethical EC-policy related issues.

Yours truly,

Ioanna Anastassopoulou
Assoc. Professor

Department of Radiometry
Faculty of Physics and Nuclear Techniques
University of Mining and Metallurgy
Al. Mickiewicza 30, 30-059 Kraków
Poland

Professor Marek Lankosz
tel. +48 12 617 41 66
fax +48 12 634 00 10
e-mail: lankosz@novell.ftj.agh.edu.pl

Kraków, March 17, 2003

TO WHOM IT MAY CONCERN

- I hereby declare that our proposed research involves human central nervous system tissue for biochemical studies and it does not involve any activity in human cloning for reproductive purposes.

- We do not create human embryos by means of somatic cell nuclear transfer.

We are in accord with Jagiellonian University Bioethical Commission-policy related issues; (Commission's opinion nr KBET/321/B/2002).

Prof. Marek Lankosz

Department of Biology
University College
Gower Street LONDON WC1E 6BT

Department Phone:- 020 7679 7098 Department FAX:- 020 7679 7096

Direct phone/fax:- 020 7679 7746 e-mail:- PRR@UCL.AC.UK

18th March 2003

To Whom It May Concern

As Principal Investigator of node #29 of the NoE of acronym BASIE, I declare that bioethical issues are not implicated in the proposed research programme and that there are no other no ethical or safety aspects.

Yours sincerely,

Professor Peter Rich

The Leeds Teaching Hospitals NHS
NHS Trust

March 2003

Ethical Declaration

All work, involving tissue or samples from patients, carried out under the auspices of the Network of excellence bid will be carried out according to the principles of the World Medical Association (Declaration of Helsinki, October 2000) and will be approved by the ethics committee of the Leeds Teaching Hospitals NHS Trust on behalf of the Trust and the University.

Sheila E. Fisher

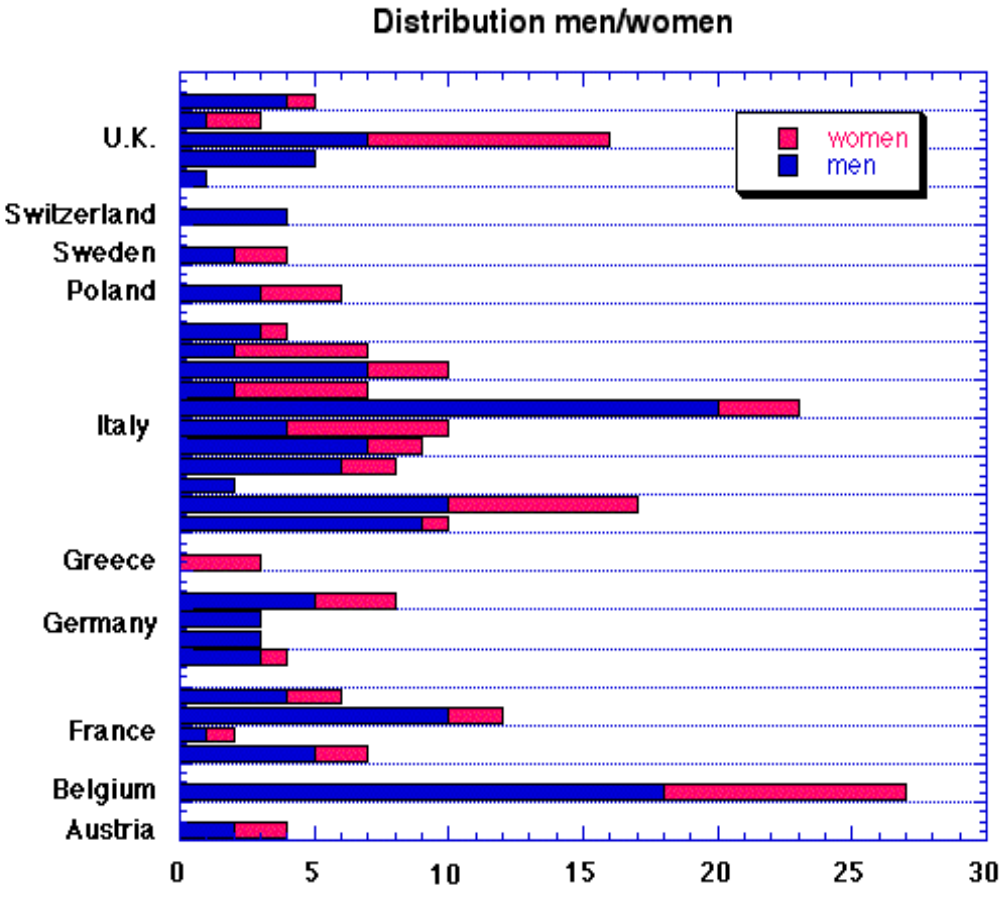
Sheila E. Fisher, MSc, FDS, FFD, FRCS.
Senior Lecturer/Hon Consultant Surgeon

B.10 Gender issues

In all research sectors, including the biological sciences, career progression for women in higher education is poorer than for men. At each successive career level, the proportion of women decreases significantly. In the present document, we will try to describe briefly the situation in our network. We will then briefly suggest some action that may help improve the situation at our (somehow limited) level.

The situation

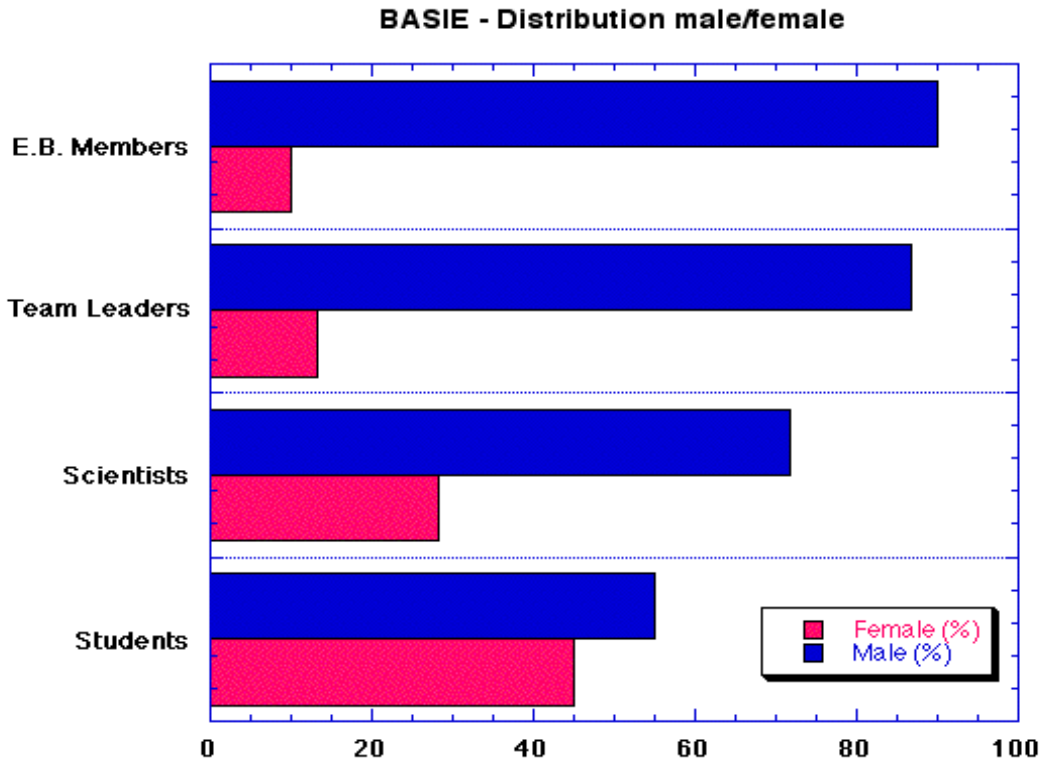
The present network is composed of 30 nodes with various backgrounds: 21 Biophysics/ Biology and Medicine research laboratories, 8 synchrotron radiation research laboratories and one enterprise. The total number of researchers is close to 160, and ten European countries are represented with four countries representing more than 75 % of the total number of researchers. In these nodes, the total number of women is important, with large discrepancies amongst nodes. The distribution of men/women in the various nodes grouped by countries are represented in the following chart.



Despite the fact that women have constituted over 50% of those graduating in the Bio-Science area for nearly 30 years, less than 10% of professors in the biosciences are women and this proportion does nor vary significantly throughout Europe. (HESA web site).

B.10.1 Participation of women and gender action plan

The distribution of male/female in our Network of Excellence composition is much the same: the proportion of female students within BASIE is 45.7 % (32/71), the ratio of female scientists drops to 28.1 % (45/160), the proportion of females in the team leader reaches a poor 13 % (4 out of 30) and only one is a member of the Executive Board of the NoE.



The poor representation of women in science is caused by many interplaying factors. Most of them are so complex, that only timely changes may play a role to solve them. There are others where we may help correcting slightly the situation. We engage ourselves to discuss them and work on how to solve specific problems.

The actions

- Representativity
We suggest that the proportion of women in the executive group should be proportional to the total proportion of researchers in the network. If accepted, during the first NoE meeting we will include at least two more women in the Executive board.

- Flexibility
There is evidence that certain aspect of the research culture - long, inflexible hours, short-term contracts that require freedom to relocate - are particularly problematic for women (but not only). We suggest to secure a certain amount of money to reimburse for baby sitting (child care) to allow parents to attend meetings or to perform experiments in other laboratories.

- Grants
The system of evaluation through which the majority of grants and other resources are allocated - was recently shown to have serious shortcomings[*]. To counter this, we plan to reserve grants especially for women to attend meetings and to present scientific results.

In short, the Network wants to clearly commit itself to increase the representation of women in research.

B.10.2 Gender aspects in research

Although the researches proposed within this Network of Excellence involve human and/or animal tissues or specimens (see section B.9) in some of the *Workpackage* described in detail in section B.4 and B.8, from a careful analysis of the proposed researches does not emerge any gender issue.

[*] The system of evaluation through which the majority of grants and other resources are allocated - the peer review system - was recently shown to have serious shortcomings. Two Swedish women scientists, Wenneras and Wold, conducted a study on the Swedish Medical Research Council's evaluation process. They reported that the likelihood of obtaining a grant was dependent not only on a scientist's productivity, but also their sex (males being more likely to secure funding) and their affiliation to a member of the evaluation committee. Published in Nature in 1997 under the title *'Nepotism and sexism in peer review'*, the study attracted much attention in the scientific community.