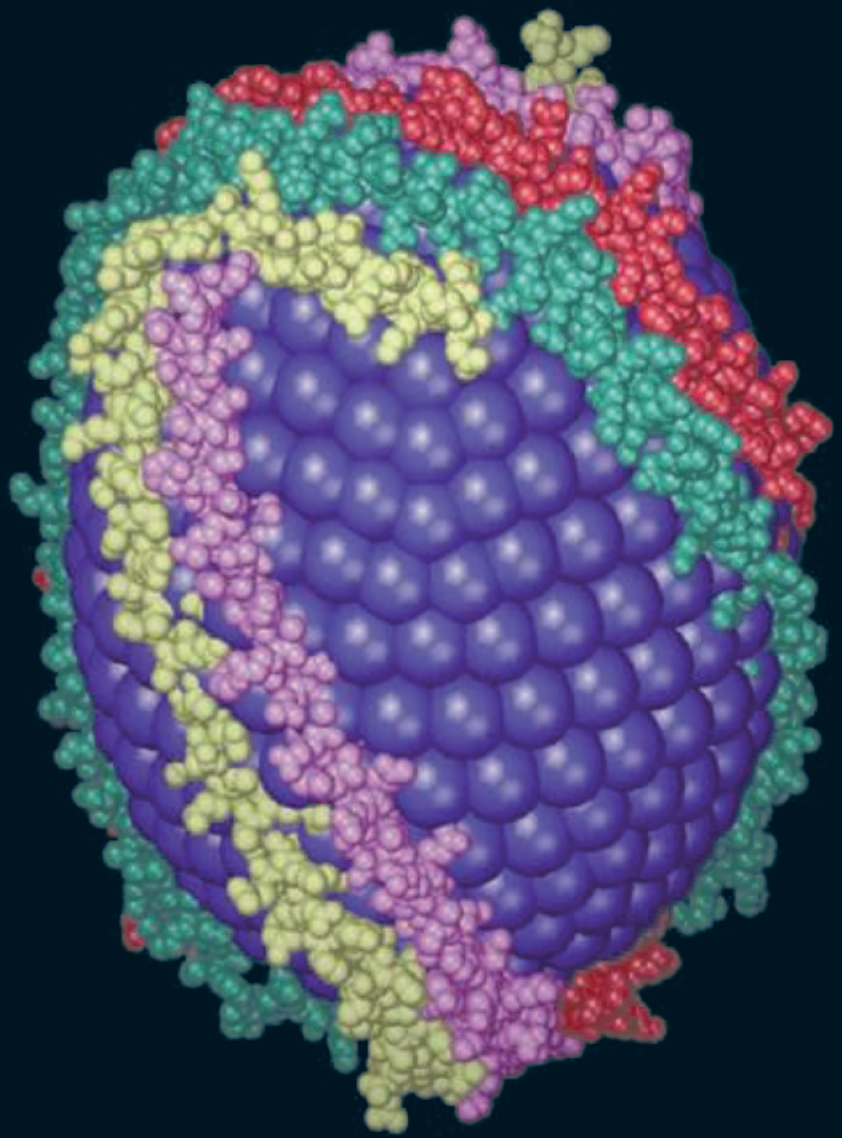
EUROPEAN SCIENCE FOUNDATION

SETTING SCIENCE AGENDAS FOR EUROPE

RESEARCH NETWORKING PROGRAMME

MAPPING THE DETAILED COMPOSITION (EPITOPE EXPOSURE) OF SURFACE-ADSORBED PROTEIN LAYERS ON BIOMATERIALS AND NANOPARTICLES – AN ALTERNATIVE APPROACH TO BIOCOMPATIBILITY AND NANOTOXICITY (EpitopeMap)

Standing Committee for Physical and Engineering Sciences (PESC)



www.esf.org

The European Science Foundation (ESF) is an independent, non-governmental organisation of national research organisations.
Our strength lies in the membership and in our ability to bring together the different domains of European science in order to meet the scientific challenges of the future. ESF's membership currently includes 80 influential national funding agencies, research-performing agencies and academies from 30 nations as its contributing members.
Since its establishment in 1974, ESF, which has its headquarters in Strasbourg with offices in Brussels and Ostend, has assembled a host of research organisations that span all disciplines of science in Europe, to create a common platform for cross-border cooperation.
We are dedicated to supporting our members in promoting science, scientific research and science policy across Europe. Through its activities and instruments ESF has made major contributions to science in a global context. The ESF covers the following scientific domains:

- Humanities
- Life, Earth and Environmental Sciences
- Medical Sciences
- Physical and Engineering Sciences
- Social Sciences
- Marine Sciences
- Nuclear Physics
- Polar Sciences
- Radio Astronomy Frequencies
- Space Sciences

Cover image:
Illustration of the concept of the nanoparticle-protein corona

Introduction

It has recently been argued that the effective unit of interest in the cell-nanomaterial interaction is not the nanoparticle per se, but the particle and its 'corona' of more or less strongly associated proteins from serum or other body fluids.[1, 2] Ultimately it is this corona of more or less disrupted proteins, 'expressed' at the surface of the particle that is 'read' by living cells. Given the enormous scientific, technological, and economic potential of nanoscience, e.g. nanomedicine, combined with the growing awareness of potential risks (nanotoxicology) it is surprising that the particle-protein complex is so poorly understood.

If our understanding of protein-nanoparticle interactions and their biological consequences is to be advanced we must identify the proteins that associate with particles in the complex multicomponent mixtures that are biological fluids. We require information on the binding affinities and stoichiometries for different combinations of proteins and nanoparticles, and ranking of the affinities of proteins that coexist in specific bodily fluids or cellular compartments. In time we believe it will become apparent that a whole conceptual framework, analogous to that pioneered by Langmuir for surfaces, will be required to fully address the challenges of nanoparticles interacting with biological systems.

Beyond that, and presenting unique challenges never yet faced by physical scientists, we will need to know the groups of proximate amino acid residues that are expressed at the outer surface of the adsorbed protein layer, for it is this collection of 'epitopes' (the epitope map) that ultimately gives the particle-protein complex its biological identity, not the particle itself. Advancing our knowledge in this arena is the goal of EpitopeMap, and it will be addressed by bringing together the leading scientists in Europe and beyond, to undertake a coordinated and collaborative research programme, involving exchanges of personnel and expertise, as well as training of our young people in the new cross-disciplinary approaches needed to address these broad and far-reaching questions.

Specific objectives of the EpitopeMap programme include:

- To understand how relevant (serum) proteins associate with and subsequently organise on biomaterial and nanoparticle surfaces;
- To acquire a deep understanding of the role of nanoparticles in fibrillation of amyloidogenic proteins *in situ*, and the role of stabilisation of protein conformation changes in the fibrillation process.

The running period of the ESF EpitopeMap Research Networking Programme is for five years from May 2007 to May 2012.

Framework of the Scientific Programme

Interactions between cells and biomaterials determine the level of success of medical implants and are crucial in determining the fate, transport and impacts in nanomedicine, nanodiagnostics and nano-Environmental Health and Safety (nano-EHS). A new paradigm for thinking about cell-biomaterial interactions is emerging, where it is the effect that the biomaterial has on the proteins that adsorb to the material upon contact with physiological solution that is important, rather than the actual nature of the biomaterial surface itself. The important parameter is thus the conformation and structure of the adsorbed protein layer, and in particular, the very outer protein layer, as this is what the cells actually see. This concept is illustrated in Figure 1.

In this programme we bring together scientists working in the traditionally separate areas of biomaterials, biophysics and nanoscience, in order to develop and apply the most cutting-edge characterisation techniques to understanding the nature of the surface-adsorbed protein layer on biomaterials and nanoparticles, and the effect of this on biocompatibility and nanoparticle toxicity.

Envisaged highlights of the programme include the exchange of ideas between the traditionally distinct research areas and the bringing together of a range of physical (characterisation and visualisation) techniques with biological and medical approaches to addressing the common goals, which will result in a great increase in the pace of understanding, a rational basis for risk assessment, and a reduction in the barriers to developing commercial applications of biomaterials and nanoparticles.

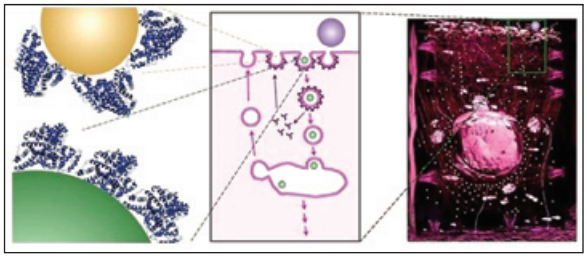


Figure 1. Because of their small size, nanoparticles can enter cells, and even the individual organelles in cells, such as the nucleus, the mitochondria and others. Here we show an image of the cell and its entry portals (receptors), showing that as the size of the particles decreases they become small enough to interact with these entry portals and can enter the cell. The interaction with the portal, and the subsequent pathway and fate of the nanoparticle following uptake is determined by the nature of the proteins adsorbed to the nanoparticle surface.

Nanoparticle-protein complexes

It is a (near) universal rule of materials in biology that the material is always covered by proteins immediately upon contact with a physiological environment, and we believe that this phenomenon will also be the key to understanding much of the bionanoscience world. For surfaces, this has long been known by scientists and industry involved in the development of biomaterials for use as medical implants, and it is understood that many of the early stage biological responses are determined by the nature of the deposited protein layer.[1, 3-6] Indeed, even much later stage responses are determined by the subsequent development of a biopolymer interface between the foreign material and tissue.[7] Strategies in medical-device research to minimise protein deposition, such as PEGylation of the surface,[8] are well known. The whole arena is, however, complex, and one should not automatically assume that a reduced protein load on the interface is beneficial, and surfaces prepared to achieve this may not be superior in terms of their long-term biocompatibility than those with protein adsorbed in a benign manner.

In the case of nanoparticles we believe that this paradigm will continue to be a key element of the story. In particular, we emphasise that most biology goes on at the surface of foreign materials, and the high surface to volume ratio of nanoparticles means that one is dealing with a very important issue (for example, there are 800m^2 surface area per litre solution at 1% concentration of 70nm particles). We are therefore potentially facing an issue that is similar to that of medical devices, but hugely amplified by the amount of surface exposed to living tissue, and in a complex mixture of proteins.

We conceive of the proteins associated with the particle as possessing a very wide range of affinities for the particle surface. In essence we expect a huge range of equilibrium constants (one for each protein) representing the quite different (and competitive) binding mechanisms present, as shown in Figure 2. This means that we see the proteins associated with the particle as a dynamic ‘corona’, rather than a solid fixed layer. The composition of the protein corona at any given time will be determined by the concentrations of the over 3 700 proteins in plasma, and the kinetic on and off rates (or equilibrium binding constants) of each protein for the particular nanoparticle. This corona may not immediately reach equilibrium when exposed to a biological fluid. Proteins with high concentrations and high association-rate constants will initially occupy the nanoparticle surface but may also dissociate quickly to be replaced by proteins of lower concentration, slower exchange and higher affinity. These relaxation processes may also be important when particles redistribute from one compartment or organ to another, such as

upon receptor-mediated endocytosis from the extracellular environment into the primary endosomal cavity, or from the cytosol to the nucleus. For example, a tightly associated protein that exchanges slowly may follow the nanoparticle as it endocytoses from the extracellular fluid into an intracellular location, while a protein with fast exchange will be replaced by an intracellular protein during or after such transfer. The biological outcome may also differ depending on the relative protein exchange between nanoparticles and cellular receptors.

It is clear that, in *understanding* how particles will interact with cells, these issues which are currently almost unstudied, are amongst the most fundamental. Many of these issues have never before been addressed systematically. No single case of a particle whose outer exposed surface is characterised in biologically relevant conditions exists yet in the literature, and rational attempts to relate nanoparticle characteristics to biological response (except in simple cases where the chemical substance is patently toxic) have not yet been successfully attempted. Success in this element, of fully characterising the particles in biological context, will require the most advanced physical, chemical, and biochemical approaches, as well as refinement of existing techniques to take into account the complex and dynamic nature of this new biological entity – the nanoparticle-protein complex.

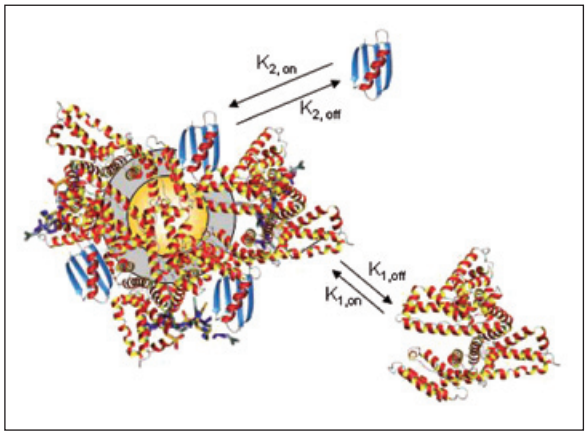


Figure 2. The proteins associated with the nanoparticle surface possess a very wide range of affinities for the particle surface. In essence we expect a huge range of equilibrium constants (one for each protein) representing the quite different (and competitive) binding mechanisms present. Identification and quantification of the proteins in the corona, their residence times and the evolution of the protein corona as the nanoparticle enters the cell and transports intra-cellularly to reach its final location are key goals of EpitopeMap.

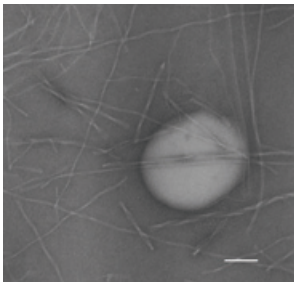


Figure 3. TEM image of protein fibrils formed in the presence of polymeric nanoparticles. Scale bar represents 100nm. Note that the fibrils do not grow out of the nanoparticle, suggesting that the fibrillation-competent oligomer dissociated from the nanoparticle surface at some point during the fibrillation process.

Protein fibrillation

Protein fibrillation is implicated in more than 20 different human disorders including Alzheimer's, Parkinson's and Creutzfeld-Jacob's diseases, sometimes referred to as amyolidosis diseases. In these diseases, the proteins take on an alternative structure that is largely independent of the protein's amino acid sequence and causes the proteins to form extended fibres of β-sheet structures. As a pre-state to forming the fibres, proteins arrange themselves into small oligomeric clusters. In many cases, including Alzheimer's disease, the toxic species is not the fibril per se but these small oligomeric species that form on the way to the fibril, although these oligomers have not been isolated in sufficient quantity for identification and physical characterisation, and thus, the exact nature of the oligomeric species, the number of protein monomers involved, and the mechanism of its formation are all unknown at this point.

We have recently published the first evidence that nanoparticles can play a significant role in altering the rate of protein fibrillation, in some cases shortening the lag-time before the onset of protein fibrillation,[9] and in other cases apparently disrupting the process even once a fibrillation-competent nucleus has been formed.[10] The ability to modulate the rate of protein fibrillation provides us with a powerful new tool to enrich and isolate the critical oligomers, study their molecular properties and structure, and unravel the molecular mechanisms behind oligomerisation and fibrillation, as well as offering an opening for development of diagnostics and treatment. Combining the ability to modulate the fibrillation rate with the ability to produce larger quantities of the fibrillation-competent species such as are needed for many physiochemical techniques will enable us to make real advances into the mechanistic aspects of protein fibrillation. Many different approaches will be combined from the partner laboratories, such as solid state NMR, proteomics, AFM, and Fluorescence Correlation Spectroscopy to understand the structures and epitopes involved in nanoparticle-induced protein fibrillation, and whether the resultant fibrils have similar morphologies as the fibrils formed under identical conditions in the absence of nanoparticles.

The need for detailed maps of the outer (exposed) surface of the nanoparticle-protein complex

There is considerable evidence in the scientific literature that adsorption of proteins to surfaces and to nanoparticles can alter the conformation of the proteins, as a result of electrostatic, hydrophobic or other interactions between the protein and the surface which drive the binding. Adsorption could therefore result in a variety of peptide units being expressed at the outer surface, in arrangements (here termed 'epitopes') that are not normally found in the native protein conformation, nor in the linear amino acid sequence of the protein, as illustrated in Figure 4.[11] Given the use of proteins in the regulation of cellular processes, it is conceivable that intra- and inter-cellular signalling events may be stimulated by these new epitopes present in the adsorbed protein layer.

The prediction of non-native peptide sequences in general is a matter of sophisticated calculation, and is a research topic for the EpitopeMap research network. A very primitive outline of the elements that would need to be considered in such predictions is given here. The central idea is that one should seek an understanding of the candidate epitopes expressed at the surface as a consequence of the adsorption of proteins to the surface. It is these groups of amino acids, often short sequences of three to ten residues, which will determine much of the biological response to materials and nanoparticles. Considering only the non-specific interactions that occur between proteins and nanoparticle surfaces, there are many parameters that need to be considered in order to predict the occurrence of new or cryptic epitopes. A non-exhaustive list of considerations includes the linear amino acid sequences, the solvent accessibility of the different amino acids, the protein-surface area ratio and the number of proteins that adsorb, how close the individual amino acids of the epitope would need to be to each other in order to function as an epitope, and the spatial arrangement of the amino acids such that they fit to the receptor (i.e. whether the sequence is spatially specific).[11]

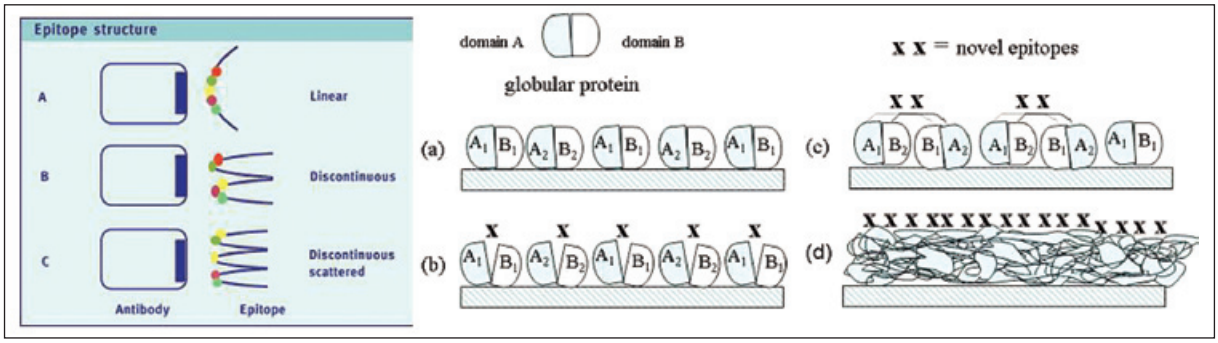


Figure 4. (a) Within a protein a sequence of amino acids that has a specific recognition function is called an epitope. A linear epitope is a straight sequence of covalently linked amino acids; a discontinuous epitope is composed of components who function together in the folded form of the protein but which are not adjacent in the liner sequence of the protein – these are also referred to as conformational epitopes. (b) When a protein interacts with a nanoparticle surface, various degrees of unfolding may occur, resulting in the loss of some conformational epitopes and/or formation of new 'cryptic' epitopes which do not occur in the native protein. These cryptic epitopes may induce new biological responses which cannot be predicted at present, and which could have potentially significant consequences for bicompatibility and safety of the nanoparticles.

Scientific Activities in the EpitopeMap Programme

The aim of the EpitopeMap network is to promote European collaboration in the arena of nanoparticle-protein interactions, and the manifestation of these interactions as altered protein conformation, altered protein surface expression and altered protein aggregation and fibrillation behaviours.

EpitopeMap comprises a cross-disciplinary alliance of leading research groups from Europe working on an ambitious long-term programme that aims to develop a detailed understanding of the nature of the exposed surface of the adsorbed protein layers on biomaterials and nanoparticles, i.e. the surfaces that cells actually come into contact with. The network encompasses competences, skills and knowledge from physical and biophysical chemistry, through sophisticated experimental techniques, to biological function, drawing expertise from both the biomaterials and nanoscience communities. The cross-disciplinary nature of the network is central for the programmes' success, and offers excellent opportunities for training of young scientists in conducting research at the interface between the physical sciences and the life sciences.

Because the area of nanoparticle-cell interactions is a newly emerging discipline, the focus of the EpitopeMap programme on researcher mobility and training via the longer term research visits (up to 6 months) will result in a concerted effort to train PhD students and post-docs in a cross-disciplinary manner by exposing them to areas not directly related to their PhD field (i.e. sending physical chemistry students to biology labs and vice versa, and having biomaterials people work with nanoparticles and vice versa), thus widening their knowledge-base and providing access to new ideas, methodologies and applications. EpitopeMap, with its fundamental and challenging approach is expected to attract top students in Europe, who will work in an environment that supports experimentation and stimulates the generation of new ideas. The availability of staff trained in these areas will represent a key asset for Europe in terms of competitiveness, the development of a knowledge-based economy, and in developing a dynamic attractive European research platform to help keep European researchers in Europe.

Workshops and conferences

The Steering Committee will organise 2-3 international workshops with around 40 participants, an International school in Year 4 which will be open to all ESF member country students, and a Programme Conference in Year 5 to showcase the outputs from the network (100 people).

- Workshops for programme collaborators will be organised on specific aspects of the programme. Possible topics for discussion at workshops include "What can biomaterials research teach nanoscientists regarding biocompatibility?", "State-of-the-art techniques and their application to determination of the conformational state of surface-adsorbed proteins" or "Development of novel approaches to understanding the conformation of surface-adsorbed proteins".
- An International School will be organised on the topic "Proteins as the drivers of biocompatibility – methods for determining the explicit nature of the adsorbed protein layer". Key scientists from each of the collaborating groups will give lectures on their area of expertise, with particular emphasis on the novel techniques used to determine protein conformation upon adsorption to materials, including instrumental techniques such as neutron reflectivity, solid state NMR and plasmon spectroscopy, and biological techniques such as limited proteolysis, proteomics, mass spectroscopy, and radiolabelling. This school will be the first in the nanotechnology area on the topic of interfacing physical chemistry approaches with classical protein science and newer emerging technologies such as proteomics. The course will provide the necessary physical chemistry background for biologists, and the necessary protein and basic biology background for physical chemists, resulting in a truly inter-disciplinary school that will prepare young scientists for the challenges of research at the interface between chemistry, physics, biology and technology which is becoming increasingly necessary in today's research environment.
- A Programme Conference will be organised in Year 5, which will bring together all of the participants, as well as other international researchers to whom the topic is of interest. The proceedings of the workshop will be published as a book on this topic, as to date there are no books dealing with this topic.

Interested parties who wish to organise workshops/events within the EpitopeMap programme are invited to submit their suggestions via the EpitopeMap website: **www.esf.org/epitopemap** ("science meetings" section).

Exchange grants

These are mainly for young scientists, who need further training and expertise in new experimental methods, which will broaden their skills-base, introduce them to new research environments and expose them to top-class European scientists. An additional aspect will be that these collaborations will strengthen the integration of European research. These grants are intended to facilitate the transfer of knowledge and techniques relevant to research from one laboratory to another within Europe (at least one contributing country should be involved). The grants are for a period of 15 days to 3 months.

Applications are encouraged from researchers from all European countries, including partner laboratories, who would like to undertake a scientific research project related to the topics of the EpitopeMap network at one of the partner laboratories. Application form available at: **http://www.esf.org/epitopemap**

Short visit grants

These cover the costs of short visits of senior researchers working in the area of the programme, in order to carry out joint work primarily in one of the EpitopeMap participating laboratories. These visits are typically 3-15 days in duration.
Applications are encouraged from researchers from all European countries, including partner laboratories, who would like to undertake a short scientific visit to one of the partner laboratories. Application form available at: **http://www.esf.org/epitopemap**

Bibliography

1. Lynch, I., Dawson, K.A., Linse, S., *Detecting crytpic epitopes in proteins adsorbed onto nanoparticles.* Science STKE, 2006. 327: p. pp pe 14.
2. Cedervall, T., Lynch, I., Lindman, S., Nilsson, H., Thulin, E., Linse, S., Dawson K.A., *Novel methods to quantify binding rates and affinities of plasma proteins to nanoparticles: Effects of nanoparticle composition and size.* In press PNAS, 2006.
3. Gray, J.J., *The interaction of proteins with solid surfaces.* Curr. Opin. Struct. Biol., 2004. 14: p. 110-115.
4. Anderson, J.M., *Annu. Rev. Mater. Res.*, 2001. *31*: p. 81-110.
5. Shen, M., Garcia, I., Maier, R.V., Horbett, T.A., *Effects of adsorbed proteins and surface chemistry on foreign body giant cell formation, tumor necrosis factor alpha release and procoagulant activity of monocytes.* J Biomed Mater Res A., 2004. 70: p. 533-541.
6. Norde, W., Lyklema, J., *Why proteins prefer interfaces.* J. Biomater. Sci. Polym. Ed., 1991. 2: p. 183-202.
7. Welt.G.P., R., C., *Inflammation and Restenosis in the Stent Era.* Arteriosclerosis, Thrombosis, and Vascular Biology., 2002. 22: p. 1769-1776.
8. Norde, W., Gage, D., *Interaction of bovine serum albumin and human blood plasma with PEO-tethered surfaces: influence of PEO chain length, grafting density, and temperature.* Langmuir, 2004. 20: p. 4162-4167.
9. Linse, S., Cabaleiro-Lago, C., Xue, W.-F., Lynch, I., Lindman, S., Thulin, E., Radford, S., Dawson, K.A., *Nucleation of protein fibrillation by nanoparticles.* PNAS, 2007. 104: p. 8691-8696.
10. Cabaleiro-Lago, C., Quinlan-Pluck, F., Linse, S., Lynch, I. Dawson, K.A., *Disruption of fibrillation-competent protein oligomers by nanoparticles.* JACS, 2008, ASAP Published.
11. Lynch, I., *Are there Generic Mechanisms Governing Interactions between Nanoparticles and Cells? Epitope Mapping For the outer layer of the Protein-material Interface.* Physica A, 2007. 373: p. 511-520.

Funding

ESF Research Networking Programmes are principally funded by the Foundation's Member Organisations on an *à la carte* basis. EpitopeMap is supported by:

- **Fonds zur Förderung der wissenschaftlichen Forschung in Österreich (FWF)**
  Austrian Science Research Fund, Austria
- **Fonds voor Wetenschappelijk Onderzoek-Vlaanderen (FWO)**
  Research Foundation Flanders, Belgium
- **Ίδρυμα Προώθησης Έρευνας (RPF)**
  Cyprus Research Promotion Foundation, Cyprus
- **Eesti Teadusfond (ETF)**
  Estonian Science Foundation, Estonia
- **Suomen Akatemia/Finlands Akademi**
  Academy of Finland, Finland
- **Deutsche Forschungsgemeinschaft (DFG)**
  German Research Foundation, Germany
- **Irish Research Council for Sciences, Engineering and Technology (IRCSET),** Ireland
- **Fonds National de la Recherche (FNR)**
  National Research Fund, Luxembourg
- **Vetenskapsrådet (VR)**
  Swedish Research Council, Sweden
- **Schweizerischer Nationalfonds (SNF)**
  Swiss National Science Foundation, Switzerland

EpitopeMap Steering Committee

Professor Kenneth Dawson (Chair)
Department of Chemistry
School of Chemistry and Chemical Biology
University College Dublin
Belfield • Dublin 4 • Ireland
Tel: +353 1 716 2300
Fax: +353 1 716 2127
Email: Kenneth@fiachra.ucd.ie

Dr. Jean-Nicolas Audinot
Dept. Science et Analyse des Matériaux (SAM)
Centre de Recherche Public-Gabriel Lippmann
41, rue du Brill • 4422 Belvaux • Luxembourg
Tel: +352 47 02 61 521
Email: audinot@lippmann.lu

Dr. Theodora Krasia-Christoforou
Department of Mechanical and Manufacturing
Engineering • University of Cyprus
PO Box 20537 • 1678 Nicosia • Cyprus
Tel: + 357 22 892288
Fax: +357 22 892254
Email: krasia@ucy.ac.cy

Dr. Tonu Kesvatera
The National Institute of Chemical Physics
and Biophysics
Akadeemia tee 23 • 12618 Tallinn • Estonia
Tel: +372 6 398 378
Email: kesvater@kbfi.ee

Professor Guido Maes
Department of Chemistry, Physical and Analytical
Chemistry • University of Leuven
Celestijnenlaan 200 F • 3001 Heverlee • Belgium
Tel: +32 16 32 74 50
Email: Guido.maes@chem.kuleuven.ac.be

Dr. Beat Meier
Laboratory for Physical Chemistry
ETH Hönggerberg
Faculty of Physical Chemistry
ETH Zurich
Wolfgang Pauli str. 10 • 8093 Zurich • Switzerland
Tel: +41 446 324 401
Fax: +41 163 216 21
Email: beat.meier@nmr.phys.chem.ethz.ch

Professor Markus Linder
VTT Biotechnology
Technical Research Centre of Finland
Tietotie 32 (TT2) • Espoo • Finland
Tel: +358 9 456 51 36
Fax: +358 9 455 21 03
Email: markus.linder@vtt.fi

Professor Joachim Rädler
Institut für Experimentalphysik
Ludwig-Maximilians-Universität München
Geschwister Scholl Platz 1 • 80539 Munich • Germany
Tel: +49 89 2180 2437
Fax: +49 89 2180 3182
Email: joachim.raedler@lmu.de

Professor Manfred Sippl
Center for Applied Molecular Engineering
Institut ür Chemie und Biochemie
University of Salzburg
Jakob Haringer str. 1 • 5020 Salzburg • Austria
Tel: +43 662 8044 5797
Fax: +43 662 454 889
Email: sippl@came.sbg.ac.at

Professor Sara Linse
Department of Biophysical Chemistry
Technical Faculty • Lund University
Chemical Center
PO Box 124 • 221 00 Lund • Sweden
Tel: +46 462 228 246
Fax: +46 462 224 543
Email: sara.linse@bpc.lu.se

Programme Coordinator:
Dr. Iseult Lynch
Irish Centre for Colloid Science & Biomaterials
School of Chemistry & Chemical Biology
University College Dublin
Belfield • Dublin 4 • Ireland
Tel: +353 1 7162418
Fax: +353 1 716212
Email: iseult@fiachra.ucd.ie

ESF Liaison

Dr. Patrick Bressler
Science

Ms. Chantal Durant
Administration

Physical and Engineering Sciences Unit (PESC)
European Science Foundation
1 quai Lezay-Marnésia
BP 90015 • 67080 Strasbourg cedex • France
Tel: +33 3 88 76 71 27
Fax: +33 3 88 37 05 32
Email: cdurant@esf.org

For the latest information on this Research Networking Programme consult the EpitopeMap website:
www.esf.org/epitopemap

EUROPEAN SCIENCE FOUNDATION
SETTING SCIENCE AGENDAS FOR EUROPE

1 quai Lezay-Marnésia | BP 90015
67080 Strasbourg cedex | France
Tel: +33 (0)3 88 76 71 00 | Fax: +33 (0)3 88 37 05 32
www.esf.org

Print run: 1500 – February 2009