

Centre for Self Organising Molecular Systems

Annual Report 2008

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Foreword

People often ask me what it's like working in a university; what do I do apart from lecturing; and that old, extremely irritating favourite: "but I thought you had three months holiday over the Summer"! My replies at the moment are: "I'm never bored" (I don't have time to be!), and "no two days are ever the same". That's certainly true where SOMS is concerned. We've all been going "full steam ahead" for the past couple of years, in pretty much every aspect of academic endeavour: research, teaching, income generation, course development, and spin-out company activity. As I write, SOMS is currently involved in research programmes totalling around £6.5 million, and is leading or participating in new research proposals worth £28 million. SOMS activities involve over 35 academic staff at Leeds, as well as partnerships and collaborations with Sheffield, York, Stuttgart, Ghent, Queensland and Fudan universities, and the Rudjer Boskovic Institute in Croatia. Our current industrial collaborators include Dow Chemical, Merck, Proctor & Gamble, GSK, UK government Health and Safety Laboratory, Interchem Hellas (Greece), Suprapolix (Netherlands), and Mare Engineering (Italy).

Within the SOMS Centre itself, we have been delighted to welcome two new members of staff: our new secretary Katie Moore, who joined SOMS in October 2007, and Dr. Stefan Auer, who arrived in August 2007 to take up an RCUK fellowship in Computational Biology. Expansion of our staff base is a key priority for SOMS over the next few years, with firm plans to appoint two new academic staff members in Autumn 2008, in the area of nanomedicine. This has been made possible by Leeds University's recent investment in interdisciplinary research, specifically in the form of the new Biomedical Health Research Centre (BHRC), a virtual centre which will support strong new partnerships between clinical research and practice, and the underpinning physical and life sciences.

The SOMS degree portfolio has expanded significantly, with the launch of two new MSc programmes: Nanomaterials for Nanoengineering in 2006, and Bionanotechnology in 2007. A particular highlight, last year, was the graduation of our first ever group of Nanotechnology BSc students. Indeed, as far as we are aware, these were the first students to graduate from an integrated nanotechnology BSc degree anywhere in the UK.

SOMS also gave birth, this year! - to a new company. "Organisense" is the brainchild of Dr. Andrew Nelson, and has been set up to commercialise his well-established research on membrane-based biosensors. Elsewhere on SOMS premises, we have set up a new biochemistry laboratory and a new analytical laboratory including fluorescence microscopy, Fourier Transform infra-red spectroscopy and high performance liquid chromatography, with circular dichroism spectroscopy also due for installation.

We hope you find this report useful and informative, and look forward to meeting you and working with you throughout the year.

*Robert W Kelsall,
Leeds, May 2008.*

1. Research Strategy

SOMS research in the long-standing themes of self-assembling peptides and supported biomembranes continues to flourish. In the biomembranes theme, as reported on page 4, we have extended the complexity of our model membrane systems; we have demonstrated the fabrication of more sophisticated membrane topologies on solid surfaces, and we have demonstrated robust and discriminative biosensor performance in supported membrane devices. Surface functionalisation has also been a feature of our self-assembling peptides research, with work on peptide tape formation on inorganic surfaces, as well as demonstrations of peptide aerogel formation for catalysis and biomineralisation using peptide networks in dental applications.

The discotic liquid crystal work in SOMS is now being scaled down from its heyday, but the last year has seen some important and long-sought-after achievements, including the synthesis of discotics which show both electron and hole conduction, and the demonstration of lateral alignment of discotic columns on a solid surface.

Directed assembly – the exercise of control over molecular self-assembly and self-organisation - is a theme that we wish to develop in SOMS, and is becoming increasingly apparent in across all our research areas. More details are given on page 9.

Our new theme area of nanotoxicity has been progressing well, as a result of various initiatives and consortium-building exercises (see page 11). In view of the prominence of medical applications across all the SOMS research areas, we are now planning to take forward our nanotoxicity research within development of the broader theme area of nanomedicine. This activity will be catalysed by the appointment into SOMS of two new academic staff members as part of the university's Biomedical Health Research Centre initiative. Both SOMS posts are in the nanomedicine area: one will be focussed on nanotoxicity, and the second, on molecular self assembly for targeted drug delivery. Both posts will involve identified partnerships with medical or clinical research groups at Leeds. The combined focus in SOMS on nanoscience and healthcare research correlates strongly with two of the four specific areas identified by the UK Engineering and Physical Sciences Research Council for priority support over the next four years. These two areas have also been allocated a combined budget of €9.6 million in the European Union Framework 7 research programme.

2. Research Reports

2.1 Supported & Suspended Biomembranes (Theme Leader: Dr Lars Jeuken)

Biological membranes perform a wide variety of essential tasks, ranging from cellular nutrition and drug uptake to neurotransmission. The membranes of cells are selective barriers and drugs and/or toxins will have no effect if they cannot pass through them. But it is not only organic chemicals that are required to cross the cell membrane. Signals must also pass through the membrane before they can affect the cell and, like nutrient uptake, signal transduction is regulated by membrane proteins.

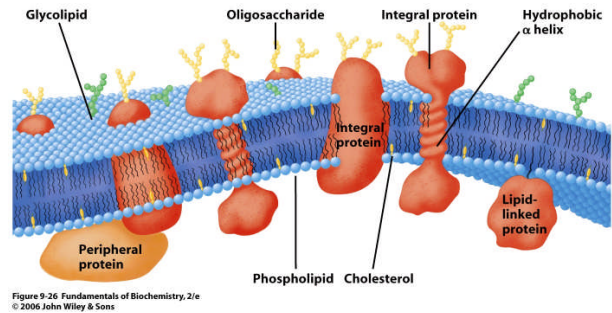


Figure 9-26 Fundamentals of Biochemistry, 2/e
© 2006 John Wiley & Sons

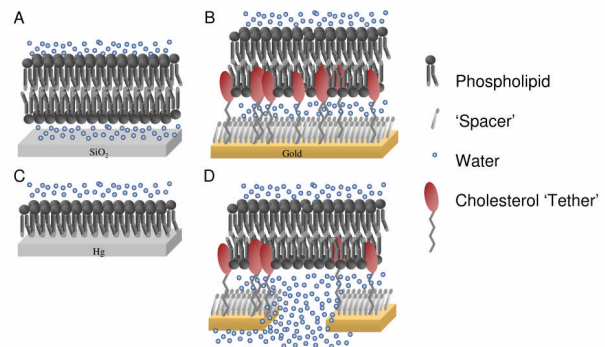
Diagram of a plasma membrane (Voet, Voet and Pratt in *Biochemistry*)

In the SOMS Centre, biological membranes form an important research theme which focuses on the coupling of biomembranes and solid surfaces (electrodes). This hybrid bio-electronic technology, *solid-supported membranes*, and enables biological responses to be converted in real-time into electronic information and allows detailed mechanistic studies of biological processes such as metabolism.

The adjacent figure shows four different classes of solid-supported membranes under investigation in the SOMS Centre, each of which has its own benefits and applications.

During 2007 we achieved several milestones in:

- 1) Phospholipid monolayers, fractal dimension and cholesterol
- 2) Peptide interactions with model membranes
- 3) Tethered bilayer lipid membranes and membrane protein incorporation
- 4) Cell walls and cytoskeletons
- 5) Biological electron transfer
- 6) Programmable dynamic surfaces



Schematic representation of the type of *solid-supported membranes* which are used at the SOMS centre.

- A. supported bilayer lipid membrane on silicon-oxide or mica
- B. tethered bilayer lipid membrane on gold
- C. supported monolayer lipid membrane on mercury
- D. suspended bilayer lipid membrane on silicon-oxide or gold

Phospholipid monolayers, fractal dimension and cholesterol*(Dr. A. Nelson)*

Phospholipid monolayers on mercury form a unique system of supported model membranes which are defect-free, self-sealing and impermeable to ions and thus ideal for sensitive measurement of a wide variety of molecular interactions. The atomically flat nature of the mercury substrate makes this system ideally suited to perform detailed impedance studies¹. The sensitivity of these measurements was utilised in the determination of the specific capacitance of membranes composed of varying fractions of cholesterol². This has important consequences as cell capacitance is used to estimate cell membrane area and membrane invaginations. By using these accurate capacitance measurements, the membrane fraction and protein contents of transverse (t-) tubules of cardiac ventricular myocytes were determined². The interaction of phospholipid monolayers with cholesterol and other additives of varying polarity was studied to determine the relationship between electrochemical fractal dimension measurements and impedance measurements³.

Peptide interactions with model membranes*(Dr. A. Aggeli and Dr. A. Nelson)*

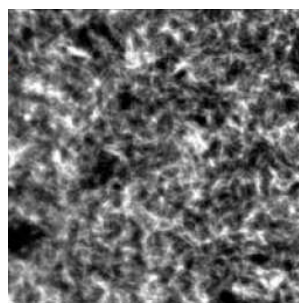
The extreme sensitivity of the phospholipid coated mercury surface also has pharmaceutical or biosensor applications and lipid interactions have been characterised with: (a) the complement-derived antimicrobial peptide CNY21⁴, (b) the human anti-microbial peptide LL-37⁵, the antibiotic compound gramicidin^{6,7}, and bispyridinium compounds⁸ which are used as treatment against nerve agents⁹.

Tethered bilayer lipid membranes and membrane protein incorporation*(Prof. R.J. Bushby Dr. L.J.C. Jeuken and Prof. S.D. Evans)*

We have made several recent technological advances in our supported and tethered membrane systems, including the use of ultra-flat gold, and surface characterisation using ATR-FTIR⁹, height-imaging AFM¹⁰ and colloidal probe AFM¹¹. In the past, some success has been obtained by incorporating membrane proteins in these and other supported and tethered lipid membranes¹². However, this is a technologically complicated process with variable results. The groups of Prof. S.D. Evans and L.J.C. Jeuken have developed new methods in which the whole bacterial membrane, which consists of up to 60% of protein, is used to prepare solid-supported planar membranes¹³. This novel type of system is very powerful for studying complex processes such as cell wall formation or electron transfer in the metabolic electron transport chain.

Cell walls and cytoskeletons*(Prof. R.J. Bushby, Dr. J. Colyer, Dr. L.J.C. Jeuken and Prof. S.D. Evans)*

Cell membranes in biology are complicated structures which extend beyond the central lipid bilayer with membrane proteins. In order to fabricate more accurate membrane models, bacterial cell walls and actin 'scaffolds' have been constructed on top of our hybrid membrane-



Fluorescent actin filaments bound to a hybrid membrane electrode surface

electrode systems. Bacterial cell walls have been formed on bacterial membrane surfaces by supplying the chemical building blocks of a cell wall and using the natural enzymes in the bacterial membrane to form the chemical bonds needed to form the cell wall polymer¹⁴. This system will have direct application in the study of cell wall formation and inhibition thereof by antibiotic reagents. Actin scaffolds have been formed by incorporating a small membrane protein, ponticulin, in the hybrid membrane-electrode system (see figure): the rôle of ponticulin in biological systems is to nucleate actin polymerisation and to serve as an anchor point for the actin polymer¹⁴.

Biological electron transfer

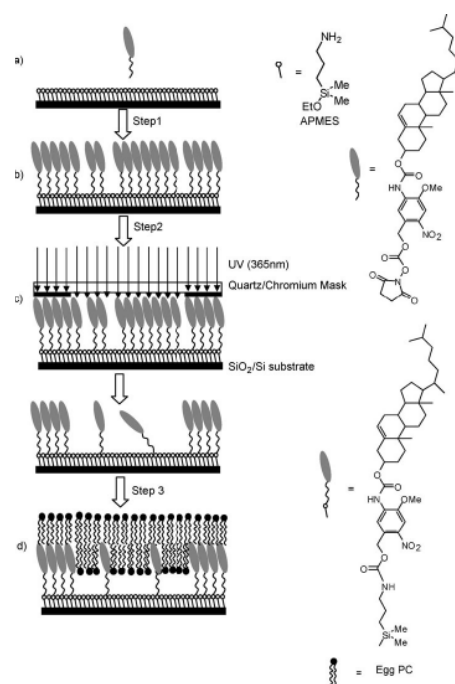
(Prof. R.J. Bushby, Prof. S.D. Evans, Prof. P.J.F. Henderson and Dr. L.J.C. Jeuken)

Electron and charge transport in and across the membrane is an important aspect of membrane biology. SOMS is using the hybrid membrane-electrode platform to study quinone enzymes. In 2007 the electrochemistry of ubiquinone in the membrane was characterised and the electrochemical data provided insight into the finite water layer that is located between the membrane and the solid substrate¹⁵. It was found that electrons can be efficiently transferred between the electrode surface and the enzymes via the naturally occurring quinone pool, which in nature is also used to pass electrons (and protons) between enzymes and across the membrane.

Programmable Dynamic Surfaces

(Prof. R.J. Bushby and Prof. S.D. Evans)

The majority of, if not all, surfaces employed for device fabrication and sensing applications are solid. They present an immobile hard support to which organic and inorganic materials can be attached and patterned. Generally the surface itself plays a passive role, and following deposition there is little one can do to change the location of the assembled elements. As part of a major Research Council 'Basic Technologies' grant we are involved in the creation of surfaces that are dynamic and on which the individual elements can be moved around. This exploits two underlying technologies which have been largely developed within the SOMS centre: firstly, the production of supported biomembranes (the requisite two-dimensional fluids), and secondly, the functional patterning of SAM surfaces using soft UV in a standard mask aligner (the tool used to pattern these membranes on to the surface) to irradiate photocleavable fluorocarbon and cholesterol derivatives as shown in the adjacent figure¹⁶⁻¹⁸. This work has been further extended to demonstrate re-configuration of molecules within a lipid bilayer using an electric field - an example of directed assembly, see section 2.2 for details.



Schematic diagram of the fabrication steps required to form a patterned lipid bilayer on silica surface using soft UV radiation.

Summary and future planning

The above results were published in 17 peer-reviewed publications in 2007/08 (with a further one in press) and were presented in various national and international conferences. The projects are currently continued by four PhD students and three postdoctoral researchers, funded by the EPSRC, BBSRC, Philips, Royal Society, MoD-DSTL, RSC, HSL and EU FW6. Plans for the next year include:

- transform the phospholipid coated mercury system to a robust sensing device. This involves using thin film mercury coated iridium electrodes arranged in a microfabricated array (with Dr. P. Steenson, School of Electronic & Electrical Engineering).
- prepare micro to nanoscale substrates to serve as array platforms for the supported bilayers. Part of this research is performed by an Industrial Case PhD student in collaboration with Philips.
- use the tethered membrane system to study the biochemistry of quinone enzymes.
- develop a system in which vesicles filled with pH sensitive dyes are adsorbed on the electrode surface to study proton-pumping enzymes using a combination of electrochemistry and fluorescence spectroscopy.
- continue the work on actin and cell-walls and perform microscopic and spectroscopic characterisation to increase our understanding of these platforms.

List of publications

- (1) Gasparovic, B., Risovic, D., Cosovic, B., and Nelson, A. (2007) The influence of frequency on fractal dimension of adsorbed layers. *Electrochim. Acta* **52**, 2527-2534.
- (2) Pásek, M., Brette, F., Nelson, A., Pearce, C., Qaiser, A., Christe, G., and Orchard, C. H. (2008) Quantification of t-tubule area and protein distribution in rat cardiac ventricular myocytes. *Prog. Biophys. Mol. Biol.* **96**, 258-80.
- (3) Gasparovic, B., Frka-Milosavljevic, S., Kozarac, Z and Nelson, A. (2008) *Anal. Chim. Acta. In press.*
- (4) Ringstad, L., Protopapa, E., Lindholm-Sethson, B., Schmidtchen, A., Nelson, A., and Malmsten, M. (2008) An electrochemical study into the interaction between complement-derived peptides and DOPC mono- and bilayers. *Langmuir* **24**, 208-216.
- (5) Neville, F., Gidalevitz, D., Kale, G., and Nelson, A. (2007) Electrochemical screening of anti-microbial peptide LL-37 interaction with phospholipids. *Bioelectrochemistry* **70**, 205-213.
- (6) Monné, J., Diez, Y., Puy, J., Galceran, J., and Nelson, A. (2007) Interpreting ion fluxes to channel arrays in monolayers. *Langmuir* **23**, 10581-10588.
- (7) Lindholm-Sethson, B., Geladi, P., Koeppe, R. E., Jonsson, O., Nilsson, D., and Nelson, A. (2007) Multivariate data analysis for enhanced interpretation of electrochemical impedance spectra of gramicidin-ion interactions in phospholipid monolayers. *Langmuir* **23**, 5029-5032.
- (8) Merrifield, J., Tattersall, J., Bird, M., and Nelson, A. (2007) Interaction of bispyridinium compounds with phospholipid layers in presence and absence of electric field. *Electroanalysis* **19**, 272-279.
- (9) Erbe, A., Bushby, R. J., Evans, S. D., and Jeuken, L. J. C. (2007) Tethered bilayer lipid membranes studied by simultaneous attenuated total reflectance infrared spectroscopy and electrochemical impedance spectroscopy. *J. Phys. Chem. B* **111**, 3515-3524.
- (10) Jeuken, L. J. C., Daskalakis, N. N., Han, X., Sheikh, K., Erbe, A., Bushby, R. J., and Evans, S. D. (2007) Phase separation in mixed self-assembled monolayers and its effect on biomimetic membranes. *Sens. Actuators B* **124**, 501-509.
- (11) Jeuken, L. J. C., Connell, S. D., Henderson, P. J. F., Gennis, R. B., Evans, S. D., and Bushby, R. J. (2006) Redox enzymes in tethered membranes. *J. Am. Chem. Soc.* **128**, 1711-1716.

- (12) Dodd, C., Johnson, B. R. G., Jeuken, L. J. C., and Evans, S. D. (2008) Native *E. coli* Inner Membrane Incorporation in Solid Supported Lipid Bilayer Membranes. *Biointerphases*, **3**, FA59-FA67.
- (13) Spencelayh, M. J., Cheng, Y. L., Bushby, R. J., Bugg, T. D. H., Li, J. J., Henderson, P. L. F., O'Reilly, J., and Evans, S. D. (2006) Antibiotic action and peptidoglycan formation on tethered lipid bilayer membranes. *Angew. Chem.-Int. Edit.* **45**, 2111-2116.
- (14) Barfoot, R. J., Sheikh, K., Johnson, B. R. G., Colyer, J., Miles, R. E., and Evans, S. D. (2008) Minimal F-Actin Cytoskeletal System for Planar Supported Phospholipid Bilayers. *Langmuir*, (in press).
- (15) Jeuken, L. J. C., Bushby, R. J., and Evans, S. D. (2007) Proton transport into a tethered bilayer lipid membrane. *Electrochem. Comm.* **9**, 610-614.
- (16) K. Critchley, R. Ducker, J. P. Bramble, L. Zhang, R. J. Bushby, G. J. Leggett and S. D. Evans, 'Photodeprotection patterning of self-assembled monolayers', *Journal of Experimental Nanoscience*, 2007, **2**, 278-290.
- (17) Han, X. J., Critchley, K., Zhang, L. X., Pradeep, S. N. D., Bushby, R. J., and Evans, S. D. (2007) A novel method to fabricate patterned bilayer lipid membranes. *Langmuir* **23**, 1354-1358.
- (18) Han, X. J., Pradeep, S. N. D., Critchley, K., Sheikh, K., Bushby, R. J., and Evans, S. D. (2007) Supported bilayer lipid membrane arrays on photopatterned self-assembled monolayers. *Chem.-Eur. J.* **13**, 7957-7964.

2.2 Directed Assembly

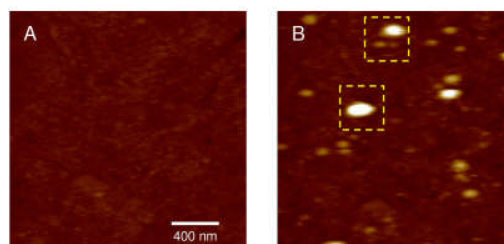
Following studies of the underlying science of spontaneous self-assembly and self-organisation processes in various molecular systems, it is a logical next step to investigate means of controlling such processes, in order to tailor the end results to specific applications, or to develop 'smart' dynamic molecular systems with customised morphological responses. Consequently, investigation of 'directed assembly' is a strategic aim for virtually all our scientific themes, and some current examples are given below.

Effects of electric fields on the membrane structure

(Dr. A. Nelson and Dr. L.J.C. Jeuken)

Investigating the effects of electric fields on membranes provide a fundamental understanding of the physical mechanisms which underlie electroporation in bilayers and cell membranes. Phospholipid monolayers on mercury in the presence of electric fields show three phase transitions at potentials -0.94 V, -1.0 V and -1.25 V versus Ag/AgCl respectively. Impedance data of the phase transition at a potential of -1.0 V was found to be consistent with a model whereby a structured electrolyte-phospholipid emulsion breaks up into two phases: electrolyte and phospholipid¹.

Electric-field effects were also studied on tethered bilayer lipid membranes using atomic-force microscopy and impedance spectroscopy². It was found that at electric fields of less than 0.45 V across the membrane the conductance of the SLM increases and membrane areas of less than 150 nm in size are found to elevate from the surface up to 15 nm in height. This structural change could either be due to (a) an increase in the lipid area due to changes in the lateral pressure or (b) ion transfer through the membrane, creating pockets of electrolyte between the membrane and the solid-surface.



AFM images of the same area of a SLM while applying electric fields of either (A) ~ 0.2 V and (B) ~ 0.55 V across the tethered membranes.

Electroinsertion of peptides or pharmaceuticals into phospholipid monolayers

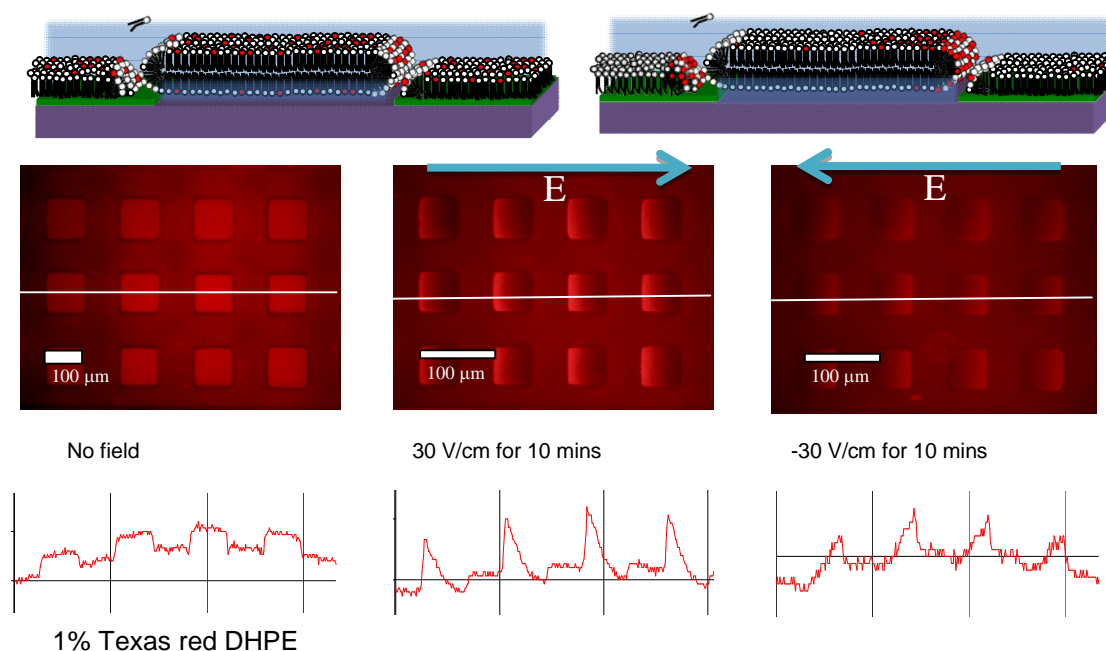
(Dr. L. A. Nelson and Dr. A. Aggeli).

A comprehensive series of experiments has shown that the application of electric fields across phospholipid monolayers can facilitate the penetration and insertion of positively charged peptide molecules into the layers. Similar experiments with doubly charged bispyridinium compounds have shown that this insertion can be reversed by a reversal of the electric field direction³. The reversibility of the insertion depends very much on the structure of the compound. These experiments show that small electric fields could be used to insert therapeutic compounds into cell membranes.

Electric field induced reconfiguration of lipid bilayers

(Prof R J Bushby and Prof S D Evans)

Section 2.1 described the formation of patterned bilayer structures on solid surfaces, using a combination of conventional optical lithography and photocleaving techniques^{4,5}. This work has been extended to form regular bilayer patches which can be electrically contacted. The figure below shows a patterned surface in which the square patches contain a fluid supported bilayer. As can be seen, when a charged fluorescent solute is incorporated, with no applied bias, the solute is randomly distributed throughout the patch, but when a field is applied, the charged solute molecules are re-ordered, and can be moved from side to side by switching the polarity of the field. We are currently demonstrating electrically induced movement of proteins, vesicles, and nanoparticles, and systems in which individual components are moved and even separated within photopatterned channels of lipid.



Top: schematic diagram showing charged lipid molecules in a bilayer before (left) and after (right) application of an electric field. Middle: fluorescence microscopy of an array of electrically contacted lipid bilayer patches, with no field, and with positive and negative fields. Bottom: corresponding concentration profiles of fluorescently tagged lipid molecules.

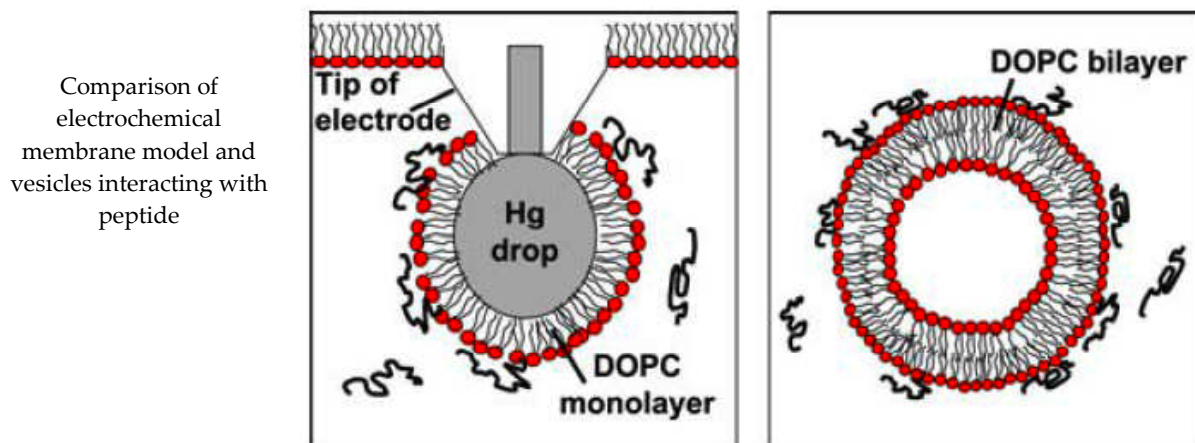
List of publications

- (1) Nelson, A. (2007) Electrochemical analysis of a phospholipid phase transition. *J. Electroanal. Chem.* **601**, 83-93.
- (2) Jeuken, L. J. C. (2008) AFM study on the electric-field effects on supported bilayer lipid membranes. *Biophys. J.* **94**, 4711-4717
- (3) Merrifield, J., Tattersall, J., Bird, M., and Nelson, A. (2007) Interaction of bispyridinium compounds with phospholipid layers in presence and absence of electric field. *Electroanalysis* **19**, 272-279.
- (4) Han, X. J., Critchley, K., Zhang, L. X., Pradeep, S. N. D., Bushby, R. J., and Evans, S. D. (2007) A novel method to fabricate patterned bilayer lipid membranes. *Langmuir* **23**, 1354-1358.
- (5) Han, X. J., Pradeep, S. N. D., Critchley, K., Sheikh, K., Bushby, R. J., and Evans, S. D. (2007) Supported bilayer lipid membrane arrays on photopatterned self-assembled monolayers. *Chem.-Eur. J.* **13**, 7957-7964.

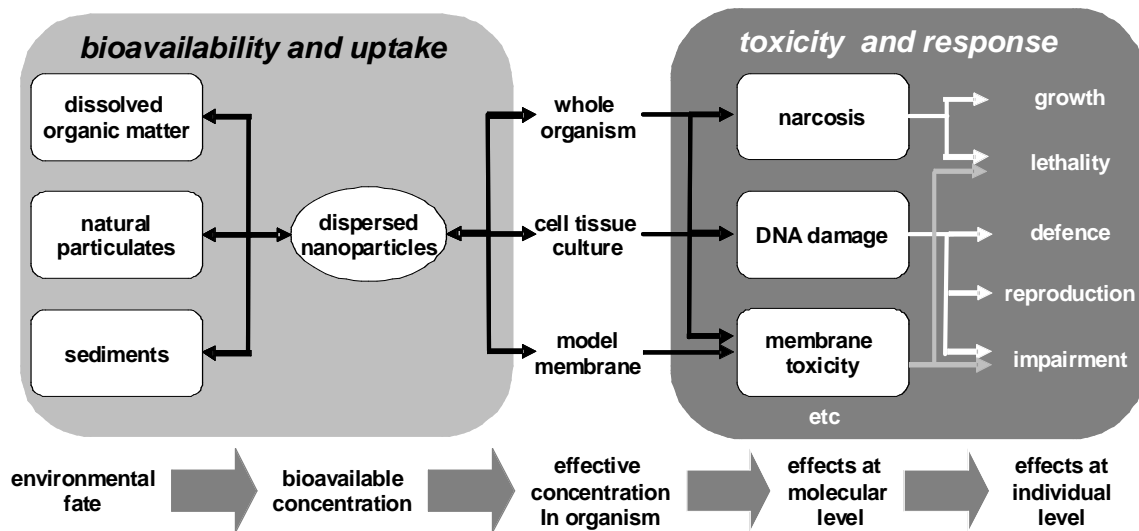
2.3 Nanotoxicology (Theme Leader: Dr L. A. Nelson)

In the last decade there has been an exponential increase in the use of nanoparticles and nanomaterials for a multitude of applications ranging from pharmaceuticals and personal care products to industrial uses. At the same time very little is known of their activity on human health and their impact on environmental organisms. There is therefore an urgent need to address this crucial uncertainty. Furthermore, the topic offers considerable scientific novelty and scope for research, since the toxicology of engineered nanomaterials and nanoparticles involves many interesting mechanisms which are specific to the structure and functionality of the materials involved.

The SOMS Centre is establishing an interdisciplinary activity with the aim of understanding the toxicological impact of nanomaterials on human health and the environment. Our particular contribution is in expertise in the characterisation of nanoparticles using membrane models specifically designed for toxicity sensing¹, combined with expertise in environmental toxicology. In the SOMS Centre we have been working for several years on the activity of peptides at the biological membrane level using simple model membrane systems. We have shown that when a model membrane system is exposed to a self assembled peptide, it is the peptide monomer in equilibrium with the peptidic aggregate which is biomembrane active. We have also demonstrated the implementation of electrochemical model membrane systems as toxicity sensors. These systems show the same activity to anti-microbial peptides as observed in bilayer vesicles². The figure below shows a comparison of the two systems in their sensing of peptides.



SOMS has been developing a growing nanotoxicology community within Leeds, and with partner organisations across the UK and Europe. Funding has been obtained by L A Nelson from the NERC-DEFRA environmental nanotoxicological initiative (ENI) for a pilot project. Our strategy is to develop a multidisciplinary holistic approach to the relationship between the structure and functionality of engineered nanoparticles and their behaviour, fate and impact in the aquatic environment, as shown in the figure below. A trans-European consortium, led by SOMS, and including academic groups from the Netherlands, Belgium and Italy, and a large UK toxicity testing company, is in the process of acquiring funding for this work.



Effects of bioavailability and uptake on toxicity and response

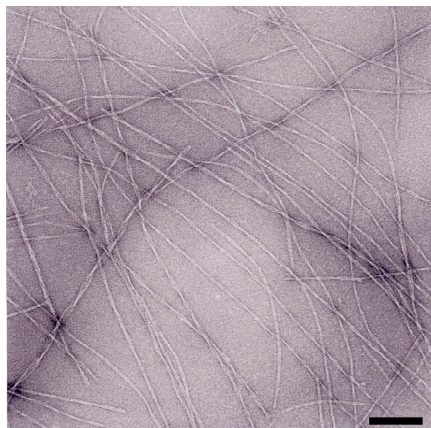
SOMS has also been active nanotoxicology policy development at the national level, with Nelson recently contributing to a UK Government commissioned report on nanotoxicology in the UK³.

List of publications

- (1) Nelson, LA. and Coldrick, Z. UK Patent No 0714866.1, filed 31/07/07.
- (2) Ringstad, L., Protopapa, E., Lindholm-Sethson, B., Schmidtchen, A., Nelson, A. & Malmsten, M. An Electrochemical study into the interaction between complement-derived peptides and DOPC mono- and bilayers. *Langmuir*. 2007, **24**, 208-216.
- (3) An assessment of regulatory testing strategies and methods for characterising the ecotoxicological hazards of nanomaterials Draft Report, Watts and Crane Associates, May 2007.

2.4 Self-assembling peptides (Theme Leader: Dr A. Aggeli)

The main objectives of our research are: (a) to understand quantitatively the fundamental mechanisms and principles that drive biological-like β -sheet peptide self-assembly in solution and at interfaces¹ and (b) to harness this insight in order to produce designed peptidic nanostructured materials and devices with a combination of desirable properties appropriate for the chemical and pharmaceutical industries².

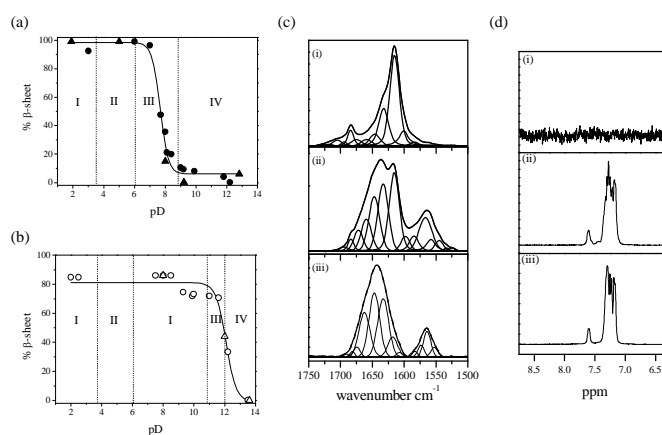


Peptide fibrils (scale bar: 200nm)

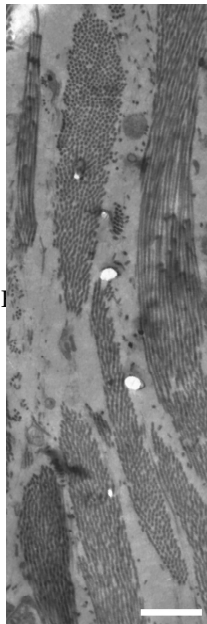
pH-responsive peptides were shown to undergo a transition from gel-forming β -sheets to monomeric random coil fluids at a characteristic pH controlled by peptide design. By screening the electrostatic repulsions between peptides with the addition of salt, the conformational transition was seen to shift by several pH units, due to changes in the magnitudes of the thermodynamic parameters that govern self assembly.

These basic studies provided the inspiration for the application of peptide nanocoatings into externally responsive self-assembling mineral particles (Dr. D. Bryant and Dr R. Moreno, in collaboration with Prof. R. Williams). The basic understanding also enabled us to design peptides that self-assemble into nanofibrillar gel networks not only in pure solvents but also in the complex biological medium. It was shown directly for the first time that the long semirigid peptide nanofibrils further organise spontaneously in solution to form aligned microdomains, giving rise to bundles, responsible for the observed liquid crystallinity of fibrillar solutions and gels (see figure overleaf). Peptides were also designed to have specific surface properties that enabled them to act as efficient templates for the nucleation of hydroxyapatite (HAP) crystals, the mineral in teeth and bones (A. Firth in collaboration with Prof. J. Kirkham, Dental Inst.). In a project "Filling without drilling", we

A fundamental study that we published last year was the investigation of the effect of solution ionic strength on the self-assembly of pH-responsive fibril-forming peptides (see adjacent figure). The acquisition of quantitative and reproducible self-assembly data is a well-recognised challenge. We were able to use successfully a range of techniques to achieve this, such as ¹H NMR, FTIR and, for the first time, mass spectroscopy, which due to its sensitivity enabled us to study peptides with very low self-assembly concentration (T. Knapman in collaboration with Dr A. Ashcroft, Biochemistry).



pH-induced peptide assembly and dis-assembly

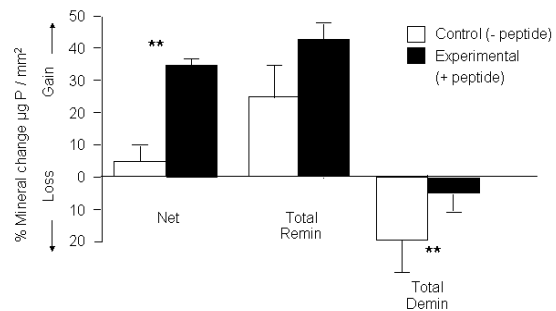


Peptide gel.
Scale bar: 1 μm

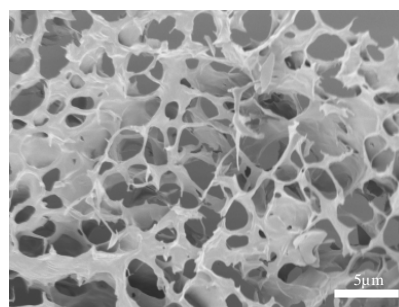
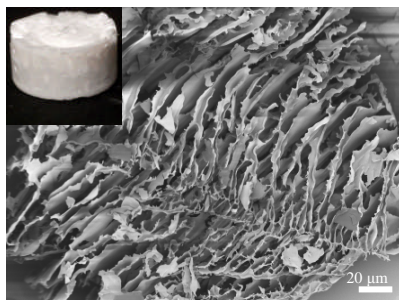
applied a fluid solution of monomeric peptide in dental caries-like lesions, followed by *in situ* triggered peptide self-assembly within the dental microcavities.

It was demonstrated that a single application of the peptide solution increased the total mineral gain of the tooth by 30% in the course of 5 days, this phenomenon being attributed to increased remineralisation and decreased demineralisation³ (see figure below).

Impact of peptide gel on dental remineralisation



From a material point of view, there is a lot of interest not only in wet gels, but also in dry aerogels, where all the solvent is carefully removed and replaced by air. In an industrially-funded study, we explored supercritical fluid drying (SCF) and freeze-drying (FD) as two separate methods for aerogel formation⁴. SCF caused collapse of the gel network but FD proved successful in creating self-supporting aerogels characterised by controllable internal open porous architecture, potentially useful for catalysis and filtration applications (see figures below).



Peptide aerogels

The results were published in five peer-reviewed publications and a book chapter and presented in national and international meetings, e.g. in Australia, America, Netherlands, Greece, London and York, whilst we also organised a successful three-day international consortium meeting at Weetwood Hall in Leeds, 19-21/9/2007. The projects were carried out by six postdoctoral researchers, nine PhD and two project students, funded by the European Union, EPSRC, the Royal Society, the Dutch Polymer Institute and the chemical and pharmaceutical industry, with a total of *ca* £2M. The work was facilitated by the set up of a new peptide analytical laboratory in the SOMS centre last year, with state-of-the-art spectroscopic and microscopic facilities for investigation of self-assembling systems.

Ongoing projects include:

- fundamental, quantitative studies on molecular engineering with self-assembling peptides (Dr S. Scanlon, Dr B. Liu in collaboration with Dr S. Harris and Prof. T. C. B. Mcleish, IRC in polymer science);
- functional peptide coatings (Dr C. Whitehouse, R. Guilliat, M. d'Souza);
- peptide – lipid interactions (E. Protopapa in collaboration with Dr A. Nelson, SOMS);
- tissue engineering studies (S. Maude, S. Kyle; L.R. Tai in collaboration with Prof. P. Kocienski, Chemistry),
- bone tissue engineering (J. Burke in collaboration with Prof. J. Kirkham and Dr X. Yang, Dental Inst.),
- cartilage tissue engineering (Dr S. Wilshaw, Dr J. Katta, in collaboration with Profs. E. Ingham and J. Fisher iMBE, and Dr E. Kainmueller in collaboration with Dr S. Warriner, Chemistry).

List of publications

- (1) Davies R.P.W. and Aggeli A., Mechanisms and principles of peptide self-assembly *Advances in Chemical Engineering* in press
- (2) Scanlon S. and Aggeli A., Self-assembling peptide nanotubes *Nano Today* in press
- (3) Kirkham J., Firth A., Vernals D., Boden N., Robinson C., Shore R.C., Brookes S.J., Aggeli A., Self-assembling peptide scaffolds promote enamel remineralisation *Journal of Dental Research*, 2007, **86**, 426-430,
- (4) Scanlon S., Aggeli A., Boden N., Koopmans R.J., Brydson R. and Rayner C.M., Peptide aerogels comprising self-assembling nanofibrils *Micro & Nano Letters*, 2007, **2**, 24-29,
- (5) Carrick L.M., Aggeli A., Boden N., Fisher J., Ingham E. & Waigh T.A., Effect of ionic strength on the self-assembly, morphology and gelation of pH responsive β -sheet tape-forming peptides *Tetrahedron*, 2007, **63**, 7457-746
- (6) Knapman T.W., Aggeli A. and Ashcroft A.E., Critical concentrations of β -sheet peptide self-assembly quantified directly by nanoelectrospray ionization mass spectrometry *Rapid Communications in Mass Spectrometry* in press

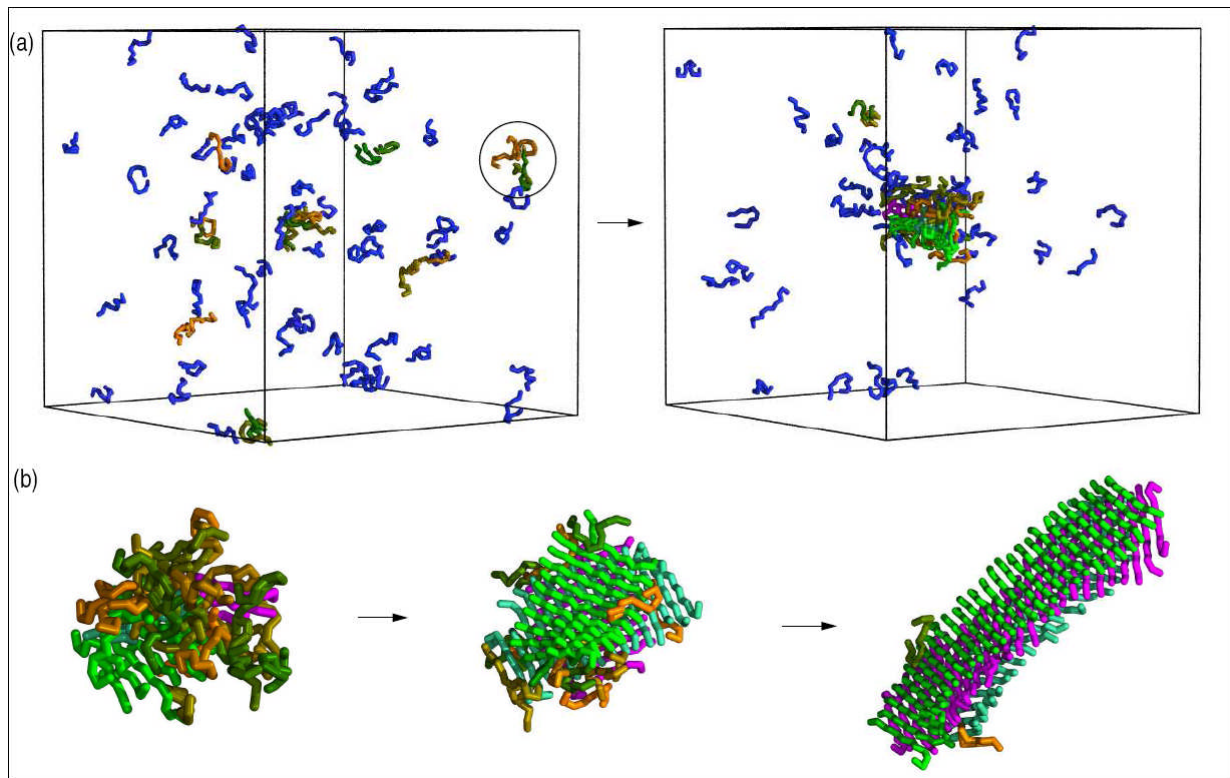
2.5 Simulating the self-assembly of bio-molecules (Dr Stefan Auer)

Our research is focused on the application of theoretical computational tools developed in soft condensed matter physics to investigate the phase behaviour and transitions of complex systems of biomolecules. From a purely statistical mechanical point of view an ensemble of many peptides and proteins represents a new and important system which should help to connect our understanding of colloidal systems, polymers, and proteins. More specifically we are studying a transition that is of particular interest in molecular biology: the aggregation of proteins into amyloid-like structures – structures that are associated with neurological and systemic diseases such as Alzheimer's disease or type II diabetes. Experimental investigations of the early stages of the formation of fibrillar aggregates are notoriously difficult, mainly due to the transient nature of the protein aggregates and their structural heterogeneity.

We approach this problem from a theoretical angle and simulate protein aggregation. The challenges of computational studies in biology are two fold. On the one hand the accessible timescale in molecular simulations is at present orders of magnitude shorter than the typical time-scales of molecular processes in the cell, which prohibits simulation even the simplest biological process: protein folding. On the other hand, if unlimited computer power were available, the biological processes could be simulated perfectly but this still would not guarantee our full understanding of the phenomena. We are approaching this problem by developing and applying a coarse grained physical representation for proteins that requires much less computational time than all-atom simulations, and enables us to address the physical aspects of protein assembly. The basic idea of the model is to incorporate the minimum number of ingredients that are common to all proteins, such as steric constraints, hydrogen bonding and hydrophobicity, and which are necessary for the existence of a marginally compact phase for polymers¹.

We have recently shown that by using a generic representation of the proteins, in combination with state-of-the-art simulation techniques, it is possible to simulate the aggregation of peptides into amyloid-like structures². The simulations reveal a two-step nucleation mechanism in which the peptide molecules initially collapse into oligomers that subsequently convert into β -rich structures (see figure below). A direct calculation of the nucleation barriers involved in this condensation-ordering mechanism provides a theoretical framework that unifies various mechanisms proposed by experimental studies. In turn, this simulation work has inspired experiments that quantitatively measure the kinetics and thermodynamics of amyloid formation³.

The work has been performed in collaboration with Dr M. Vendruscolo and Prof. C. Dobson (Cambridge University) who are both world leaders in the field of protein folding and aggregation. In future we aim to strengthen our links with the groups of Dr A. Trovato, Prof F. Seno and Prof. A. Maritan (Padova University), who are experts in theoretical biophysics, and who developed the protein model.



Simulation of peptide aggregation

List of recent publications

- (1) S. Auer, M. A. Miller, S.V. Krivov, C.M Dobson, M. Karplus, and M. Vendruscolo, *Phys. Rev. Lett.* **99**, 178104 (2007)
- (2) S. Auer, C.M. Dobson, and M. Vendruscolo, Characterization of the nucleation barriers for protein aggregation and amyloid formation, *HFSP Journal*, **1**, 137 (2007)
- (3) T. P. J. Knowles, W. Shu, G. L. Devlin, S. Meehan, S. Auer, C.M. Dobson, and M. E. Welland, Kinetics and thermodynamics of amyloid formation from direct measurement of fluctuations in fibril mass, *Proc. Natl. Acad. Sci. USA* **104**, 10016 (2007)

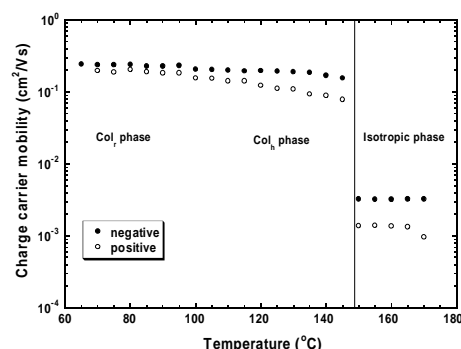
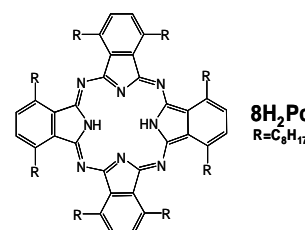
2.6 Discotic Liquid Crystals (Theme Leader: Professor Richard Bushby)

Discotic liquid crystals (DLCs) have only been known for about thirty years and applications are still modest in comparison to the much longer established calamitic liquid crystals. Sales of calamitic liquid crystal displays are now approximately \$40 billion per annum, but sales of discotics are also growing. Their use as optical compensating films (to improve the angle of view of displays) is now well established and recently Fuji launched a new range of specialist discotic liquid crystal-based lubricants.

From the beginning SOMS played a major role in the development of DLC science. Our main interest in them has been as self-organising/self-repairing organic semiconductors. It is a field of research which we began at Leeds and which is continuing to grow world-wide. We are interested in all aspects of the problem: synthesis of the liquid crystals, characterisation of their conductive properties, and the theory of conduction.

Highlights of our recently published and current work are:

1. The discovery of the best (highest mobility) liquid crystal so far: the phthalocyanine derivative shown in the adjacent figure. It not only has exceptionally high mobility but is a carrier for both holes (positive charges) and electrons (negative charges). Organic conductors such as this are of increasing importance commercially. They cannot rival silicon for some 'high-end' applications but in other areas (for example the printing of electronic tags which will eventually replace bar codes) they win out over conventional materials because they are so much cheaper to process.



2. Extensions to our ferric chloride/methanol method of synthesising triphenylene-based discotics. This is the route used for the synthesis of all commercial discotic liquid crystals and – on the basis that our first paper on this is one of their most cited papers ever – we published an invited commentary on the reaction to a special anniversary issue of the journal *Liquid Crystals* which appeared at the beginning of 2007.
3. The solution of the long-standing problem of how to align DLCs in a planar fashion with control of the azimuthal angle – something that is required for the development of the DLC field effect transistor (with Prof. S. D. Evans, School of Physics & Astronomy).

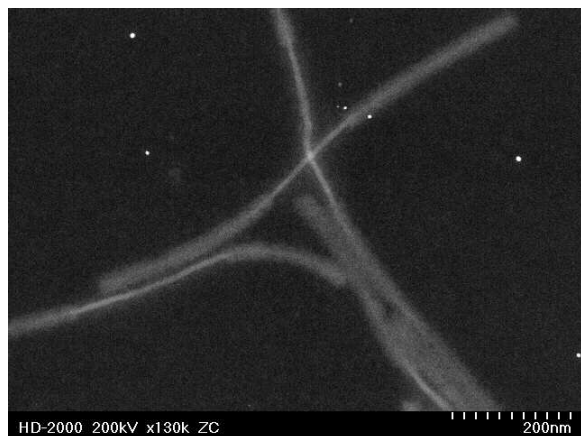
List of recent publications

- (1) H. Iino, Y Takayashi, J.Hanna, R.J.Bushby and D. Haarer, 'High electron mobility of $0.1 \text{ cm}^2 \text{ V}^{-1}\text{s}^{-1}$ in the highly ordered columnar phase of hexahexylthiotriphenylene' *Applied Physics Letters*, 2005, **87**, 192105.
- (2) H. Iino, J. Hanna, D. Haarer and R.J. Bushby, 'Fast electronic transport in discotic columnar phases of triphenylene derivatives' *Japanese Journal of Applied Physics Part1*, 2006, **45**, 430-433.
- (3) H. Iino, J. Hanna, R.J. Bushby and B.J.Whittaker, Conductivity in a discotic liquid crystal with a strong lateral dipole moment, *Journal of Applied Physics*, 2006, **110**, 043716.
- (4) R. C. Borner, R. J. Bushby, and A. N. Cammidge, 'Ferric chloride/methanol in the preparation of triphenylene-based discotic liquid crystals', *Liquid Crystals*, 2006, **33**, 1439-1442.
- (5) N. Boden, R. C. Borner, R. J. Bushby, A. N. Cammidge, and M. V. Jesudason, 'The synthesis of triphenylene-based discotic mesogens – New and improved routes' *Liquid Crystals*, 2006, **33**, 1443-1448.

2.7 Supramolecular Assembly (Dr. Xiaosong Wang)

My research focuses on the development of supramolecular functional nanomaterials through the synthesis and self-assembly of macromolecules. I have reported this year in *Science* that we are able to synthesize well-defined polymer supramolecular nanostructures through the self-assembly of organometallic polymers¹, just as we are able to design macromolecular architectures using living polymerization. This pioneering work is opening up a new area of research: 'living supramolecular chemistry'.

Our current research starts from the synthesis of well-defined polymers using living polymerization techniques including Atom Transfer Radical Polymerization (ATRP), Radical Addition Fragmentation Chain Transfer Polymerization (RAFT) and anionic polymerization. A range of macromolecules with well-controlled structures in terms of their chemical compositions, architectures, molecular weight and molecular weight distribution are targeted. Through investigation of the self-assembly behaviour of these novel polymers, we intend to develop our understanding of controlled aggregation processes for well-defined nanostructures. In addition to this fundamental research into supramolecular chemistry, self-assembly of these polymers also leads to a range of functional materials which have the potential for use in various applications including nanoelectronics and biomedicine.



Block co-micelles synthesized using living supramolecular polymerization

References

- (1) X. S. Wang, G. Guerin, H. Wang, Y. S. Wang, I. Manners, M. A. Winnik, *Science*, **317**, 644-647. (2007)

3. Education and Training

The *nanofolio* nanotechnology Masters portfolio, based in the SOMS Centre at Leeds and delivered in partnership with Sheffield University, is one of the largest and longest established nanotechnology education activities in Europe. Nanofolio currently includes four interrelated MSc programmes: Nanoscale Science and Technology, Nanoelectronics and Nanomechanics, Nanomaterials for Nanoengineering, and Bionanotechnology. Our most recent programme – Bionanotechnology – has enabled us, for the first time, to admit biology and biochemistry graduates onto a nanotechnology Masters degree, and features new modules in Biophotonics and Bioimaging, Biosensors, and Nanotoxicology. In 2007/8, *nanofolio* attracted sponsorship from Intel, who are providing financial support to a student on the Nanoelectronics and Nanomechanics programme, combined with a 3-month paid project placement.

The *nanofolio* website, www.nanofolio.org, has been expanded into a significant on-line nanotechnology resource, featuring a non-specialist's "What is Nanotechnology" site, nanotechnology research highlights, as well as hosting the EPSRC Nanotechnology Image Library. *Nanofolio* educational material also includes the course textbook, "Nanoscale Science and Technology", published by Wiley, which is a useful resource for graduate research scientists as well as for our Masters students.

The next programme in the *nanofolio* series will be the MSc in Nanomanufacturing. In contrast with our other programmes, this course is being designed primarily for participation by industrial employees for 'continuing professional development' purposes, either in part-time or 'day-release' study modes. New modules include Nano-business, innovation and ethics, Nanometrology, and Industrial Scale Nanomanufacturing.

July 2007 saw the first ever graduation of students from our Nanotechnology BSc course. These students are, we believe, the UK's first nanotechnology graduates at Bachelors level. The Nanotechnology BSc is a highly multidisciplinary course which combines education in the fundamental physical and life sciences with study of state-of-art topics in nanotechnology. Students are provided with unique opportunities for hands-on experience from an early stage, including clean room semiconductor fabrication work in year 1, and atomic-resolution scanning probe microscopy in year 2. A particularly successful feature of the course has been the final year nanotechnology projects. Projects were made available in a wide range of nanotechnology-based laboratories across the campus, and students were able to devote one third of their final year's effort to this work. One project was so successful that it has led to a patent application in conjunction with a major multinational company. In 2007, the course recruited its largest ever cohort of 17 students.

SOMS is also a participant in the new Doctoral Training Centre for Tissue Engineering and Regenerative Medicine. The Centre, which involves staff from Leeds, Sheffield and York universities, is supported by a £6 million EPSRC grant to provide a four year integrated research training programme for PhD students, including specialist taught modules and industrial placements alongside their research projects.

4. Commercial activities

SOMS currently hosts two university spin-out companies on its premises. *Scensive technologies Ltd* specialises in multi-functional chemical sensors and associated detection instrumentation, including electronic noses. These devices were originally based on liquid crystals developed in the SOMS centre, but now use semiconducting polymeric materials. Scensive's chemistry and sensor testing operation is currently based in the SOMS centre. *Organisense* is a new spin-out company, established in April 2008 with pump-priming funding from Techtran PLC to commercialise the mercury/phospholipid biosensor technology developed by Dr. Andrew Nelson. Much of the recent proof of concept work has been carried out by PhD student Zachary Coldrick with device fabrication expertise provided by Dr. Paul Steenson. This stable, mature, sensing technology has potential applications in environmental monitoring and toxicity screening, water treatment, healthcare, and homeland security.

5. SOMS Seminar Series

Semester 2 – 2006/2007

Dr Ben Murray, (School of Chemistry, University of Leeds), *Phase changes of atmospheric aerosols*

Dr Brent Murray, (Food Science, University of Leeds), *Nanoparticles and foam stabilization*

Professor J. H. Lakey, (Institute for Cell and Molecular Biosciences, University of Newcastle), *Self assembled membrane protein monolayers on gold*

Dr Xiaosong Wang, (Department of Colour Science, University of Leeds), *Functional nanomaterials via the self-assembly of block copolymers*

Dr Michael Butler, (Unilever, Colworth), *Self and directed-assembly for consumer applications: new organic and inorganic materials for controlled delivery and optical appearance*

Semester 1 - 2007/2008

Dr Stefan Auer (SOMS, University of Leeds), *Simulations of Phase Transitions from Colloids to Proteins*

Dr Toby Jenkins (Department of Chemistry, University of Bath), *Toxin-membrane Interactions on Bilayer Systems*

Dr David Adams (Faculty of Biological Sciences, University of Leeds), *Nanoscale Analysis of Cyanobacterial Ultrastructure and its Implications for the Mechanism of Gliding Motility*

Dr Robert Dryfe (University of Manchester), *Nucleation and Growth at the Liquid-Liquid Interface*

Professor Peter Meyer (Centre for Plant Sciences, University of Leeds) *Natural Antisense Transcripts in Plants*

Professor Derek Woolfson (Engineering Materials, University of Bristol) *Towards Synthetic Self-Organising Biomolecular Systems from the Bottom Up*

Dr Andrew Mount (School of Chemistry, University of Edinburgh) *Genomic Nanoprocessing; Towards Electrochemically Controllable DNA Switches*

Semester 2 - 2007/2008

Professor J.H. Lakey (University of Newcastle, Chemistry) *A couple of new ways to look at supported model membranes*

Dr Tim Gibson, Scensive (University of Leeds) *Smells and Nano-dogs: The Continuing Story of the Bloodhound*

Professor Suzy Jarvis (Trinity College, Dublin) *Interactions of amyloid peptides with membranes*

Professor Peter Henderson (University of Leeds) *Membrane transport proteins – the interface between biology, chemistry and physics*

Dr David Middleton (Chemistry, Liverpool) *Analysing the molecular architecture of self-organising biomaterials with solid-state NMR*

Dr. Andy Brown (SPEME, Leeds) *EM nanoparticle characterisation for chemical biological interactions*

Professor Philip Moriarty (University of Nottingham) *Self-organisation in Dewetting Nanofluids*

6. SOMS Events

October 2006 – January 2008

- October 2006** *Nanocharacterisation Short Course*
 A one week short course describing a comprehensive range of methods and tools for characterising nanostructures. The workshop was divided into five, individually themed days: “general characterisation techniques and photon based spectroscopies”, “x-ray and neutron diffraction”, “electron microscopy”, “surface analytical techniques” and “scanning probe microscopy”, and combined lectures by leading nanotechnology researchers with laboratory visits, demonstrations and practical work.
- November 2006** *Poster Afternoon*
 A display of SOMS recent research posters organised by Elisabeth Protopapa.
- December 2006** *Nanotoxicity Workshop*
 A specialist one day workshop to discuss UK capabilities and funding prospects in nanotoxicity.
- June 2007** *SOMS AGM*
- September 2007** *“Bio-based Functional Materials from Engineered Self-Assembling Peptides”*
 European Consortium Meeting, Weetwood Hall, Leeds.
- November 2007** *Poster Afternoon*
 A display of SOMS recent research posters organised by Zachary Coldrick and Nikolaos Daskalakis.
- December 2007** *EU Nanotoxicity Consortium Workshop*
 A closed workshop involving partners in the European Union ‘ENNSATOX’ Framework 7 research proposal on environmental nanotoxicology.
- January 2008** *SOMS Industrial Workshop*
 This one day workshop featured presentations of research from SOMS and partner research groups at Leeds with activities in molecular self-assembly, with an emphasis on actual or potential industrial relevance and applications. Delegates included representatives from the defence, pharmaceutical, personal care and food industries.
- January 2008** *Food Science Workshop*
 A ½ day University of Leeds workshop to explore prospects for collaboration between SOMS and Food Colloids researchers.

7. SOMS Membership List

Dr David Adams	(IICB)	Dr Jim Henderson	(PaA)
Dr Amalia Aggeli	(SoC/SOMS)	Dr Malcolm Hunter	(IMSB)
Dr Richard Ansell	(SoC)	Professor Eileen Ingham	(IMCB)
Dr Stefan Auer	(SOMS)	Dr Lars Jeuken	(IMSB)
Professor Godfrey Beddard	(SoC)	Dr Robert W Kelsall	(SOMS/EEE)
Professor Neville Boden		Professor Jennifer Kirkham	(OB)
Dr Andrew Brown	(IMR)	Professor Tom McLeish	(PaA)
Professor Rik Brydson	(IMR)	Professor Bob Miles	(EEE)
Professor Richard J Bushby	(SOMS)	Dr Paul Millner	(IMSB)
Prof John Colyer	(IMSB)	Dr Steve Milne	(IMR)
Dr Simon Connell	(PaA)	Dr Andrew Nelson	(SOMS)
Professor Steve Evans	(PaA)	Professor Peter Olmsted	(PaA)
Professor John Findlay	(IMSB)	Professor Chris Rayner	(SoC)
Dr Colin Fishwick	(SoC)	Dr Mike Routledge	(LIGHT)
Dr Tim Gibson	(Scensive)	Dr Paul Steenson	(EEE)
Professor John Griffiths		Dr. X Wang	(DCS)
Professor Jim Guthrie	(DCS)	Professor Ben Whitaker	(SoC)
Dr. Sarah Harris	(PaA)	Dr Xuebin Yang	(OB)
Professor Alastair Hay	(LIGHT)		

SOMS Research Fellows

Dr Achalkumar A. Sudhakar	Dr Shane Scanlon
Dr David Bryant	Dr Sophie Weiss
Dr. Eva Kainmueller	Dr Conor Whitehouse
Dr Jonathan Merrifield	

SOMS Research Students

Zachary Coldrick	James Kendall
Faye Bygrave	Sholto McLaren
Nikolaos Daskalakis	Steven Maude
Robert Philip Wynn Davies	Sunwat Nanan
Ashley Firth	Elisabeth Protopapa
Robert Guilliat	Lik Ren Tai
Xinjun Han	Dan Tate

Affiliations:

DCS – Dept. of Colour Science; EEE – School of Electronic & Electrical Eng.; IMR – Institute of Materials Research, School of Process, Environmental & Materials Eng.; IICB – Institute of Integrative and Comparative Biology; IMCB – Institute of Molecular and Cellular Biology; IMSB – Institute of Membrane and Systems Biology; LIGHT - Leeds Institute of Genetics, Health and Therapeutics; OB – Dept. of Oral Biology, Leeds Dental Institute; PaA – School of Physics & Astronomy; SoC – School of Chemistry