EUROPEAN SCIENCE FOUNDATION
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Exploratory Workshops Scheme

Standing Committees for:

- **Life, Earth and Environmental Sciences (LESC)**
- **Physical and Engineering Sciences (PESC)**

ESF Exploratory Workshop on

Biosupramolecular Chemistry

Coombe Lodge - Blagdon
Bristol, United Kingdom, 4 - 8 September 2007

Convened by:
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Final Report

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Executive Summary:

This 3-day workshop was designed to explore science at the "biosupramolecular interface", i.e. the boundary between supramolecular chemistry and biology.

The background and our motivation for establishing the meeting were as follows. Scientific progress is often most rapid and significant at the interface between disciplines, and this particular boundary could be among the most fertile in modern science. From the chemical side, supramolecular chemists look across to biology as a major source of inspiration. If their subject is defined as "the designed chemistry of the intermolecular bond" (J.-M. Lehn), it is clear that Nature is a far better supramolecular chemist than any human. Biology shows us that remarkable feats may be achieved by carefully structured molecules operating through non-covalent interactions and encourages us to develop our own capabilities. It also provides opportunities: supramolecular systems can potentially be used to influence biology, and also to elucidate basic principles through biomimicry. On the biological side, workers are equally concerned with the fundamentals of non-covalent interactions, and some have recognised that biomolecules can themselves be used in the same way that supramolecular chemists use synthetic systems. By re-engineering the natural systems, new functions and

effects can be obtained. The two communities have much in common and, potentially, could achieve a great deal by working together. The supramolecular chemist can contribute synthetic skills, design strategies based on unnatural components, the measurement techniques of physical organic chemistry and a theoretical approach rooted in the physical sciences. The biologist brings the techniques of molecular biology, designs based on natural systems (especially biopolymers) and an appreciation of the biological questions that could be addressed by collaborative work

As planned, the meeting brought together researchers from both sides of the biosupramolecular interface, to discuss current research, future directions and potential collaborations. The first aim was to present a broad picture of biosupramolecular chemistry, viewing the areas of mutual interest from both sides of the boundary. We did this through a series of individual 20 – 25 minute presentations, in the following areas: (a) model studies and principles of molecular recognition, (b) peptides and proteins (structure and recognition), (c) carbohydrates & (d) nucleic acids, (e) enzymes & mimics, and (f) membranes. A key concern was to encourage open communication, and, so, we adopted the following policy on confidentiality, used by the Gordon Research Conferences:
"Each participant agrees that any information presented at the workshop, whether in a formal talk or discussion, is a private communication from the individual making the contribution and is presented with the restriction that such information is not for public use. ..... Each participant assumes sole responsibility for the protection and preservation of any intellectual property rights in such member's contributions to the workshop"

This worked extremely well and contributions were deep, informative and thought-provoking. This stimulated post-talk and post-session discussions extremely well. Discussion sessions included formal 10 – 15 minutes slots after each talk, less-formal debates over meals and during the small social programme (walks in the Avon countryside), and the formal meeting wrap-up. Discussions were always lively, vibrant and well-informed. They included all participants, drawing on their considerable collective and broad expertise and experience.

Having reviewed the core areas through individual contributions and discussions, the participants considered future trends, and especially those directions where collaboration could open new doors. Possible proposals for the EU 7th Framework Programme were discussed, and at least one is being actively considered. A number of 2- and 3-centre collaborations were also initiated. In addition, another clear and positive outcome was that a kernal for the development of a community in biosupramolecular chemistry has been established, and that a number of the participants expressed an interest in taking this forward, possibly through an application to the ESF for a larger meeting in 2009.

Scientific Report

The meeting was divided into 7 sessions focussing on different themes within biosupramolecular chemistry. Most sessions included presenters from both the supramolecular chemical and biological sides of the interface.

Model systems and principles of molecular recognition

To begin the meeting, we planned a session which would illustrate the use of chemistry to explain biology. The lectures described studies aimed at understanding the fundamentals of non-covalent bonding, and also attempts to demonstrate understanding through biomimicry.

Christopher Hunter (Sheffield, UK) focussed on the importance of solvent-solute interactions in self-assembling systems. The presentation concluded that the free energy of inter-molecular interaction and recognition is expressible as changes in the enthalpic component of free energy due to differences in solvent and solute polarity, approximating entropic contributions as a constant value.

Thomas Schrader (Duisburg, DE) discussed interference with, and mimicry of, biological processes via artificial receptors. Amplification of recognition effects by inclusion of receptors in lipid membranes was also examined. Finally, the idea of multi-valency in protein surface recognition, specifically in the Tat-TAR complex, was also presented.

Francesco Sansone (Parma, IT) detailed the benefits afforded by ligand multi-valency in molecular recognition. Benefits were explored in a variety of calixarenes. Glyco-calixarenes were used as a tetravalent mimic of the GM1 cell-surface glyco-lipid receptor to inhibit the action of the cholera toxin. A guanidino-calixarene system was also presented, which was able to induce DNA transfection by condensation of DNA.

Ulrich Koert (Marburg, DE) described molecular recognition systems based on both organic molecules and biologically-inspired systems. Intra-molecular conformational change in a triple-ring structure was induced at one end of the molecule and manifested at the opposing end. Hybrid ion-channels obtained

by modification of natural channels (gramicidin A) were also presented. Channel selectivity and activity were modified by including non-natural amino acids in central positions and a covalently-attached crown ether at the entrance.

Janusz Jurczak (Warsaw, PL) presented the use of high-pressure techniques to cross energy barriers in macro-cyclisation reactions. The high-pressure approach was combined with a templated dynamic combinatorial approach to control the proportions of racemates and achieve the desired compound chiral character.

Peptides and proteins: structure and recognition

Peptides and proteins are the key materials used by biology. We can see them as targets, or as tools to be used for our own ends. In either case their recognition and structural properties are central to their importance. These aspects were explored in this session.

Jeremy Kilburn (Southampton, UK) showed a combinatorial approach to the formation of a peptide-based molecular tweezer design. A scheme was shown to use a guanidinium derivative of one peptide scaffold as a tweezer for a carboxylate moiety. A similar technique was also shown that has potentially produced an effective β-sheet binder.

Helma Wennemers (Basel, CH) presented peptide-tweezer-based receptor designs. These designs were based on a diketopiperazine scaffold and were shown to bind specific short peptides. These systems could find uses in combination with vesicles for diagnostic/synthetic antibodies, sensors and liquid crystals. Description was also given of the formation of novel poly(azido-proline) helices as functionalisable nano-scaffolds.

Javier de Mendoza (Tarragona, ES) spoke of protein surface recognition by an artificial guanidinium receptor. A useful reminder that a good receptor is characterised by not only good steric complementarity, but also preorganisation and flexibility-induced fit was also given. A novel oligomeric bicyclic guanidinium system was presented for the binding of oxoanions. Emphasis was also placed on non-covalent protein-protein

interaction sites as potential drug targets. HIV-protease was given as a graphic example and a modified short peptide that was able to target this interaction was presented.

Dek Woolfson (Bristol, UK) presented a *de novo* designed system of peptides. Through non-covalent interactions, the peptides are able to self-assemble into a "sticky-ended", hetero-dimeric coiled-coil motif. The sticky- ended dimer permits further assembly in a hierarchical fashion to form a highly-ordered nano-fibril. A number of derivatives were also presented that produce kinked, branched and functionalised fibres. More ambitious potential design targets were also presented—for example, for a peptide-based molecular motor—to stimulate discussion.

Thomas Scheibel (Munich, DE) described a novel method for the production of artificial spider silks through modularly engineered bacterial expression of silk domains. Protein expression was combined with microfluidics to produce artificial silks of comparable mechanical properties to natural products. Designs were further augmented with amino acid substitutions to allow for covalent functionalisation of silk fibres.

Carbohydrates and nucleic acids

Carbohydrates and nucleic acids are the other major biopolymers. Both are used by biology to carry information, stored as structural variation and read through molecular recognition. These lectures addressed the understanding and manipulation of carbohydrate/nucleic acid recognition.

Anthony Davis (Bristol, UK) explored a critical gap that exists between the varied use of carbohydrates as recognition motifs in natural systems and our current understanding of such recognition processes. A novel synthetic system was presented that is able to recognise carbohydrates with a good degree of selectivity and the possibility of the use of the system in sensing applications was discussed.

Anna Bernardi (Milan, IT) spoke on the subject of biomolecular recognition of carbohydrates, specifically in the context of recognition of GP120 of HIV. By using a designed hydrophobic motif in combination with mimicking

moieties presented by the sugar, it was possible to design a ligand that out-competed the natural ligand.

Hans-Joachim Gabius (Munich, DE) discussed the role of glycans in malignancy. Discussion was given of the intricate role of ganglioside GM1 in several forms of cancer, such as neuroblastoma and pancreatic carcinoma. The speaker also gave details of how human galectin 1 can be used in the treatment of these pathologies due to its affinity of binding to ganglioside GM1.

Peter Nielsen (Copenhagen, DN) described the development and uses of peptide nucleic acid (PNA) as antisense gene targeting. Details were also given of how the extremely tight binding of PNA to DNA can be used to form higher-order DNA assemblies using PNA clamps.

Christian Ochsenfeld (Tübingen, DE) presented a novel method for performing quantum chemical calculations on molecular systems with large numbers of atoms with O(N) scaling. The methods outlined have been used to calculate the effects of solvent-solute interactions on the NMR spectra of a molecular tweezer.

Enzymes and proteins: structure, selection and mimetics

This session addressed the second role of proteins, that of catalysis. Enzymes serve as targets for medicinal chemistry, tools for chemical processing and inspiration for the design of non-biological catalysts. The lectures focussed mainly on the last two aspects, although all the work in this and other sessions serves as useful background for drug development.

Mikael Bols (Aarhus, DN) presented synthetic organic catalyst systems based on a cyclodextrin scaffold. Using a novel selective deprotection chemistry, the reactive site of the catalyst was tailored to perform as a glycosidase. Development of the system continues with attempts to conformationally-restrict the reactive site so that undesired intra-catalyst reactions are avoided.

Paolo Scrimin (Padova, IT) spoke about using dendritic scaffolds to enforce cooperativity in the action of catalytic molecules. Similar enhancement effects were seen with the use of catalytic peptides tethered to nanoparticles. Functionalised micellar systems were also presented, but did not appear to operating cooperatively.

Andrei Lupas (MPI Tübingen, DE) discussed how one can dissect the apparent complexity of protein tertiary structure from the perspective of its evolutionary development and find a small number of discrete motifs from which all proteins are constructed. A "dictionary" of such motifs was presented and it was shown how this technique can be used to decipher evolutionary relationships between proteins.

Philippe Minard (Paris, FR) described the *in vitro* evolution of new binding and functionality into an existing protein scaffold. Protein libraries were screened using phage display and several novel and unexpected features were found in the new proteins, such as dimerisation on binding and co-operative binding of multiple ligands.

Andrew Griffiths (Strasbourg, FR) described a novel, discretised microfluidic apparatus based on emulsions formed by a water/perfluorinated hydrocarbon system. Example of how the small volume (pl – nl) and low poly-dispersity (< 3%) of the water-phase droplets can be advantageous in the context of performing massively-parallel PCR and bacterial cell culture were also given.

At the membrane: natural and synthetic systems

The biological membrane is a major frontier for biosciences. This session described contributions to the understanding and exploitation of membrane-bound systems.

Simon Scheuring (Paris, FR) presented detailed probe microscopy studies of *ex vivo* bacterial light-harvesting complexes. This unique study showed how stoichiometric changes in LH1 & LH2 populations allow adaptations across a range of light conditions. Also discussed was a novel two-chamber technique that allows the measurement of physiochemical effects across membranes, such as ion pumping etc.

Stefan Matile (Geneva, CH) spoke about the design and production of synthetic ion channels and photo-activated proton pumps. The systems presented were based upon an oligophenyl backbone, from which a variety of pendant groups can be attached. Using either short, β-sheet-forming peptides or planar, aromatic molecules as pendant groups, a variety of static and ligand-gated ion conduction channels were formed in lipid bilayers. Also presented was a further modification of the system that transformed it into a proton pump in the presence of a light source.

Hagan Bayley (Oxford, UK) discussed a methodology for the engineering of protein pores for uses in nanotechnology applications. The method is based on the use of lipid-stabilised water/hexadecane emulsions whereby a target membrane protein is encapsulated in the water phase. Two regions of water phase can be brought into contact with micromanipulators and the properties of a resulting ion channel (formed by the protein insertion into the newly-created bilayer) can be characterised. Ideas for how this technique might be scaled for high-throughput applications were also presented.

The industrial viewpoint

Input from industry will be important if biosupramolecular chemistry is to become established as an applied research theme. Unfortunately, the industrial representation was less than expected, as two participants were forced to withdraw at the last minute. However, we appreciated the fascinating contribution from *Benedikt Sas* (Kemin Pharma, BE), who described the development and manufacture of a novel class of therapeutics,

based on the use of whole bacterial organisms. Data from pre-market trials was presented and showed the treatment to be effective against a wide range of gastrointestinal maladies.

The final session - general discussion

The meeting concluded with a discussion aimed at refining the concept of biosupramolecular chemistry, and highlighting areas with special potential for future progress. The conclusions are discussed in the following section.

Assessment of the Results

The aims of this workshop were (i) to bring together scientists from either side of the biosupramolecular interface, (ii) to expose them to each others' reearch programmes, (iii) to stimulate and facilitate future collaborations. The first two were certainly achieved. The third is a long-term goal and must be considered "work in progress", but a number of important steps have been taken.

Firstly there was general agreement that the border between supramolecular chemistry and biology, as represented at this meeting, is a viable and potentially fruitful research area. The term "Biosupramolecular Chemistry", as used for the workshop, was thought to be appropriate.

Secondly, to encourage the development of this area, it was felt strongly that a series of further meetings would be desirable. Several participants indicated their willingness to organise and host these meetings.

Thirdly, to stimulate further action (especially the development of joint research proposals), the participants were asked to define priorities and suggest directions for future research in the area. The results were grouped into "Fundamental" and "Applied topics as follows":

- **Fundamental**
  - *Non-covalent interactions between polar units in water*. This aspect of molecular recognition is still poorly understood, and a concerted effort to solve the problems would be very timely.
  - *Learning from biology and feeding back into predictive biology/supramolecular design.* This theme unites many of the participants, and represents a good area for collaboration.
  - *Improved methodologies for de novo design.* The de novo design of functional molecules is a major goal, but methodologies are not yet reliable.
  - *Selection (dynamic cominatorial chemistry & directed evolution).* As an alternative design strategy, selection has great potential.
  - *Beyond recognition into catalysis.* In principle catalysis can be considered an extension of molecular recognition, but putting this concept into practice requires further effort.
  - *Multivalency.* Multivalency is widely exploited in biomolecular recognition and is a timely topic for study.

- *Complexity science and multi-component systems*. As a future direction, the group recognized the importance of developing system of increasing complexity, and possibly those with "emergent properties": the biosupramolecular systems currently being designed and developed usually have one or a small number of molecular components; future challenges in the area will undoubtedly include the design of systems with multiple and interacting components. The overlap between this area and the emerging fields of synthetic biology and possibly systems biology was recognized and considered.

- **Applied**
  - *Sensors (including membrane engineering).* The development of sensors for use in biological contexts is a major potential application of biosupramolecular chemistry. The engineering of selective channels in membranes was seen as a powerful approach.
  - *"Intelligent drugs".* A variety of ideas emerged here, including: targeting drug molecules by tagging them with "biological postal-code"; the development of "first-in-class" drugs for unexplored targets; new strategies in drug design based on molecular recognition/supramolecular principles; and targeting protein surfaces and protein-protein interactions.
  - *Hybrid systems/materials for advanced performace (eg bringing biomolecular recognition and synthetic components together).* This is related to the complexity issue raised above: in biology most materials are hybrids, which brings added strength and function. Materials design based on biosupramolecular systems will have to consider this.
  - *High-throughput technology.* Industry has benefited substantially from high-throughput assays. Biosupramolecular chemistry can make a substantial input to this type of technology.

FINAL PROGRAMME

Presentations should be 25 minutes in length, to allow discussion time of 10 minutes

Tuesday 4 September 2007

Arrival

Wednesday 5 September 2007

Morning Session: Model systems and principles of molecular recognition
Chair Tony Davis

08:30-09:00	**Welcome** **Tony Davis, Dek Woolfson,** (Bristol, UK)
09:00-09:35	**Chris Hunter**, Sheffield, UK
09:35-10:10	**Thomas Schrader**, Marburg, Germany
10:10-10:45	**Francesco Sansone**, Parma, Italy
10:45-11:15	*Coffea & Tea*
11:15-11:50	**Ulrich Koert**, Marburg, Germany
11:50-12:25	**Janusz Jurczak**, Warsaw, Poland
12:30-14:00	*Lunch*

Afternoon Session: Peptides and proteins: structure and recognition
Chair Chris Hunter

14:00-14:35	**Jeremy Kilburn**, Southampton, UK
14:35-15:10	**Helma Wennemers**, Basel, Switzerland
15:10-15:45	**Javier de Mendoza**, Tarragona, Spain
15:45-16:15	*Coffea & Tea*
16:15-16:50	**Dek Woolfson**, Bristol, UK
16:50-17:25	**Thomas Scheibel,** Munich, Germany
17:25	**Free time for Discussion**
19:00	*Dinner*

Thursday 6 September 2007

Morning Session: Carbohydrates and nucleic acids
Chair Javier de Mendoza

09:00-09:35	**Tony Davis**, Bristol, UK
09:35-10:10	**Anna Bernadi**, Milan, Italy
10:10-10:45	**Hans-Joachim Gabius**, Munich, Germany
10:45-11:15	*Coffea & Tea*
11:15-11:50	**Peter Nielsen**, Copenhagen, Denmark
11:50-12:25	**Christian Ochsenfeld**, Tübingen, Germany
12:30-14:00	*Lunch*

Afternoon Session: Enzymes and proteins: structure, selection and mimetics
Chair Thomas Scheibel

14:00-14:35	**Mikael Bols**, Aarhus, Denmark
14:35-15:10	**Paolo Scrimin**, Padova, Italy
15:10-15:45	*Coffea & Tea*
15:45-16:15	**Andrei Lupas**, MPI, Tübingen, Germany
16:15-16:50	**Philippe Minard,** Paris, France
16:50-17:25	**Andrew Griffiths,** Strasbourg, France
17:25	**Free time for Discussion**
19:00	*Dinner*

Friday 7 September 2007

Morning Session 1: At the membrane: natural and synthetic systems
Chair Andrei Lupas

09:00-09:35	**Simon Scheuring,** Institut Curie, Paris, France
09:35-10:10	**Stefan Matile**, Geneva, Switzerland
10:10-10:45	*Coffea & Tea*

10:45-11:15 **Hagan Bayley,** Oxford, UK

Morning Session 2: The industrial viewpoint

11:15-11:50 **Benedikt Sas**, Kemin Pharma, Belgium

12:00-14:00 *Lunch*

Afternoon Session

14:00-16:30 Discussion session - summary, feedback, agreed conclusions and future directions *with Coffea & Tea*

16:30 *Departures / Free time / Further informal discussions*

19:00 *Dinner*

Statistical Information

Age structure

35 – 45: 13 participants

45 – 55: 7 participants

55 +: 4 participants

Gender

Male: 22 participants

Female: 2 participants

Country of Origin

Belgium: 1 participant

Denmark: 2 participants

France: 3 participants

Germany: 6 participants

Italy: 3 participants

Poland: 1 participant

Spain: 2 participants

Switzerland: 2 participants

UK: 5 participants

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