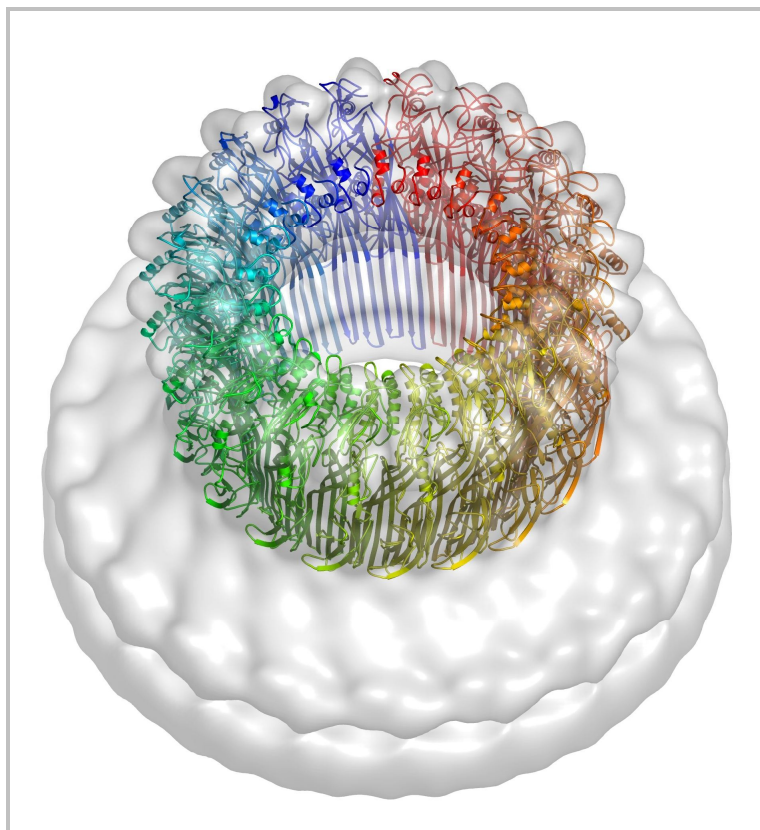


ISMB NEWS is a news bulletin published twice a year in order to share information between the various departments involved in the Institute of Structural and Molecular Biology. This includes information such as events, research highlights, new staff appointments, awards and grants.

Your comments, suggestions and contributions are welcome and will help us put together a newsletter which meets your expectations. Please email the ISMB administrator at ismb-admin@ismb.lon.ac.uk.

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The perforin pore delivers the 'kiss of death' to virus-infected cells and cancer cells in the body (see p.4)

Helen Saibil, ISMB

WELCOME TO... FAREWELL TO...

After spending five years as a Graduate administrator at UCL Research Department of Structural and Molecular Biology, Michael Wright left his position to move to the USA. We wish him all the best for the future. Welcome to Michael's replacement, Manu Davies, who started on Tuesday 11 January 2011.

On the academic side of things, Xuemei Yuan (UCL SMB) and Adam McKay (UCL Chemistry) both left UCL in September 2010. We wish them all success in the future.



Dr D. Flemming Hansen, Lecturer in Biomolecular NMR



"Motions and dynamics of macromolecules have fascinated me since the onset of my scientific career. In particular, I am interested in understanding how the molecular dance of a protein relate to biological function and also how the brief excursions that an enzyme makes from its ground state to minor populated excited states influence molecular function. After completing my PhD in biophysical protein chemistry at the University of Copenhagen (2005) I joined the NMR group of prof. Lewis E. Kay at the University of Toronto for postdoctoral training. The main focus of my postdoctoral research was development of NMR methodology to study protein dynamics and develop methods to characterise thermally excited and low populated states of proteins. These excited states often play important roles in ligand binding, molecular recognition, enzyme catalysis, and protein folding, however, they are 'invisible' to most of the traditional tools of structural biology. With our new methods we can now determine excited state structures by NMR relaxation dispersion experiment; so long as their lifetimes are between 0.5 and 10 ms and their population exceeds 0.5%. We applied our new methods to determine the structure and dynamics of protein folding intermediates, etc. In October 2010 I joined the ISMB with a BBSRC David Phillips fellowship. My main research areas are (1) Characterise the dynamics and mechanisms of interactions between histone deacetylases (HDACs) and their substrates (2) Develop new NMR methodology to characterise macromolecular motions, interactions, and dynamics, using HDACs as model systems."

[Details of work and papers at <http://www.smb.ucl.ac.uk/hansen>]

[d.hansen@ucl.ac.uk]

Flemming Hansen

Dr Andrew Osborne, Lecturer in Microbial Macromolecular Systems



"I am interested in understanding how proteins are translocated across cellular membranes. My previous work, in the laboratory of Tom Rapoport at Harvard Medical School, focused on trying to understand how polypeptides are translocated across the plasma membrane of bacteria. I subsequently spent some time in the laboratory of Kasturi Haldar at Notre Dame University, trying to understand a similar problem in the malaria parasite. My current focus remains trying to understand this process.

In humans, symptoms of malaria occur when the *Plasmodium* parasite enters the bloodstream, where it invades and replicates inside erythrocytes. Remarkably the parasite exports many proteins into the cytoplasm of the infected erythrocyte. This process is complex, as proteins have to be translocated across two membranes.

Current evidence suggests that protein export occurs via a novel pathway; the malaria parasite is a eukaryote and therefore its genome does not encode classical secretion systems found in bacteria. We will focus on identifying components of the export machinery, and understanding how proteins are recognized by the export machinery, subsequently unfolded, and translocated into the host cell. Another goal of my research is to establish what exported proteins do in infected red blood cells and why the parasite goes to such lengths to modify its host cell."

[Details of work and papers at http://www.ismb.lon.ac.uk/andrew_osborne.html]

[a.osborne@ucl.ac.uk]

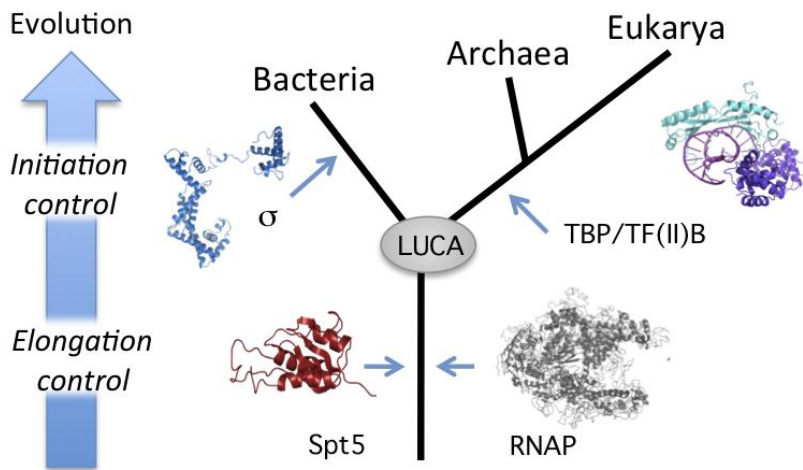
Andrew Osborne



RESEARCH HIGHLIGHTS

RNAP laboratory develops novel theory on evolution of transcription

Finn Werner and Dina Grohmann from the RNAP laboratory at UCL SMB have developed a novel theory on the evolution of transcription regulation – the ‘*elongation first hypothesis*’¹. By studying the structure and function of the engine of transcription, RNA polymerase (RNAP), and the factors that orchestrate its activities they conclude that the ancestral RNAP of the last universal common ancestor (LUCA) of all extant life was regulated during the *elongation phase* of the transcription cycle. This theory represents a paradigm shift in the field since according to textbook dogma RNAP - and thereby gene expression - is principally controlled at the level of transcription initiation.



All organisms alive today belong to one of the three ‘domains of life’ Bacteria, Archaea and Eukarya, and all are descendants from a hypothetical common ancestral life form, the LUCA. This kinship is reflected in the molecular architecture of RNAPs, which read the genetic information by transcribing DNA into RNA, and thereby act as ‘gatekeepers of the genome’². The activity of RNAP is controlled by transcription factors that are specific for the three phases of the transcription cycle: initiation, elongation and termination. The RNAP laboratory has recently carried out a functional and crystallographic analysis of the universally conserved elongation factor Spt4/5³. A careful scrutiny of its properties revealed an astonishing degree of similarity between its homologues across all three domains. This is in stark contrast to the factors that facilitate transcription initiation, which are not evolutionary conserved. In bacteria a family of proteins called σ factors is strictly required for initiation, whereas in Archaea and Eukarya the evolutionary unrelated TBP and TFIIB factors facilitate initiation. Werner and Grohmann were puzzled by the contrasting phylogeny of initiation and elongation factors, since the RNAP itself is highly conserved in the three domains. According to the law of parsimony (‘Occam’s razor’) it is most likely that the initiation factors emerged independently in the bacterial and archaeo-eukaryotic lineages of life - after LUCA and *not before* the split into separate domains. In summary, the ‘*elongation first hypothesis*’ states that (i) LUCA utilised neither σ nor TBP/TFIIB-like factors and initiated transcription in a factor-independent fashion, and (ii) LUCA’s RNAP was regulated by an ancestral form of Spt5 during the elongation phase of transcription. This implies that the regulation of elongation predates the regulation of initiation in evolution. Their findings will be published in the journal *Nature Reviews Microbiology* in February 2011.

¹ ‘Evolution of RNA polymerase in the three domains of life’, Finn Werner and Dina Grohmann, *Nature Reviews Microbiology* 2010 Feb; ² ‘Structure, function and evolution of RNAP – gatekeeper of the genome’, Dina Grohmann and Finn Werner, *Molecular Machines in Biology* (Cambridge University Press); ³ ‘Spt4/5 stimulates transcription elongation through the RNA polymerase clamp coiled-coil motif’. Hirtreiter A, Damsma GE, Cheung AC, Klose D, Grohmann D, Vojnic E, Martin AC, Cramer P, Werner F. *Nucleic Acids Research*, 2010 Jul 1;38(12):4040-51

Finn Werner



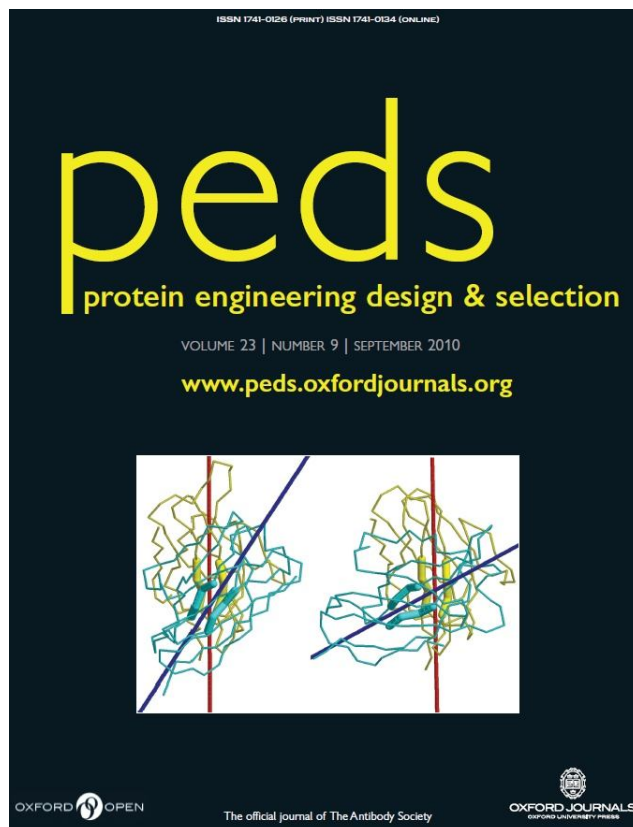
Analysis and Prediction of V_H/V_L Packing in Antibodies

The packing of V_H and V_L domains in antibodies can vary, influencing the topography of the antigen combining site. However, until recently, this has largely been ignored in modelling antibody structure. We performed an extensive analysis of the degree of variability observed in 567 known structures and found an approximately normal distribution with a mean of -45.6° and a standard deviation of 3.36° . The figure, used by PEDS as their front cover in September 2010, shows the two extremes of -31.0° and -60.8° . Lines in blue and red are drawn through conserved beta-strands (shown as thicker lines) at the interface between the heavy and light chain domains respectively. The light chain (in yellow) is in approximately the same orientation in both views.

In addition we developed a machine-learning approach to predict the packing angle. A neural network was trained on sets of interface residues and a genetic algorithm was designed to perform 'feature selection' to define which sets of interface residues could be used most successfully to perform the prediction. The training procedure was very computationally intensive, exploiting the central (pre-Legion) compute farms provided by UCL Research Computing. However, prediction is performed in a matter of seconds and has been made available as a web server (<http://www.bioinf.org.uk/abs/paps/>). The results of our machine learning not only provide a rapid method for predicting the packing angle, but also define a set of residues that influence V_H/V_L packing and therefore may be important in antibody humanization in order to obtain the correct binding site topography.

Abhinandan, K.R. and Martin, A.C.R. (2010). Analysis and Prediction of V_H/V_L Packing in Antibodies *Protein Engineering Design and Selection*, **23(9)**:689-697

Andrew C.R. Martin



New ISMB Featured articles and commentaries

Commentaries of the following articles are available at <http://www.ismb.lon.ac.uk/news.html>:

Template-free 13-protofilament microtubule- MAP assembly visualised at 8 Å resolution (22 November 2010)

Fourniol F, Sindelar CV, Amigues B, Clare DK, Thomas G, Perderiset M, Francis F, Houdusse A & Moores CA (2010). Template-free 13-protofilament microtubule-MAP assembly visualised at 8 Å resolution. *J Cell Biol.* 191:463-70. (DOI: 10.1083/jcb.201007081)

An ISMB commentary by Dr Clare Sansom and Dr Carolyn Moores is available. The original article was published in the November 1st 2010 issue of *The Journal of Cell Biology*.

Assassin's tricks revealed in nature (1 November 2010)

A team of researchers from the ISMB and Melbourne, Australia have shown how a protein called perforin punches holes in, and kills, rogue cells in our bodies. The discovery of the mechanism of this assassin has been published in the science journal *Nature*. An ISMB featured article and commentary by Michael Gross is available.



Membrane-receptor clustering and ligand binding: a special relationship

Many cellular processes, including neurotransmission and immunological response, require the clustering of membrane embedded receptors. The clustering can be modulated by a number of concomitant molecular recognition events, such as formation of lipid rafts, interaction with the cytoskeleton or the presence of antigens. It is therefore clear that molecular recognition in and near lipid bilayers, and in particular the interplay between the distribution of membrane-embedded receptors and in-solution ligands, is at the heart of cellular function.

Studies using simplified synthetic models, composed of a lipid membrane, a model receptor and model ligands have shown that multivalent ligands can induce clustering of the receptor, attributable to the chelate effect. Here, we have used a chemical model to show that the binding of a monovalent ligand and the clustering of a membrane-embedded receptor are closely related processes that modulate each other without the contribution of any apparent multivalence effect. Clearly, the confinement of the receptor within the surface reveals cooperative effects between clustering and binding that are too weak to detect in bulk solution systems. This work shows that for membrane-embedded receptors that undergo some degree of spontaneous clustering, analysis based on multivalence-mediated cooperativity are insufficient to fully describe the molecular recognition events induced by ligands in solution. Instead, a binding-clustering thermodynamic cycle is proposed for the analysis of the interaction of any kind of ligand with membrane-embedded receptors.

Tomas, S.; Milanesi L. (2010) Mutual modulation between membrane-embedded receptor clustering and ligand binding in lipid membranes. *Nature Chemistry*, 2, 1077-1083.

Salvador Tomas

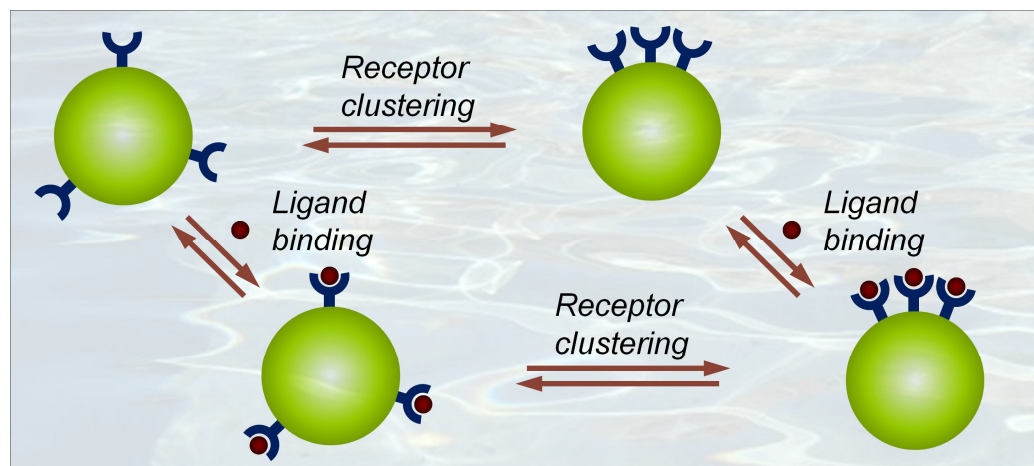


Figure 1: Thermodynamic cycle that relates the clustering of a membrane-embedded receptor and the binding of a monovalent ligand

STUDENT NEWS

Young Modeller's forum

Paul Ashford, a third-year PhD student at Birkbeck Biological Sciences, has won an award for his oral presentation at the Young Modellers' Forum of the Molecular Modelling and Graphics Society, which took place at SOAS on the 10th of December 2011. Paul, better known in the lab as Ash, presented at the forum a new computational method for visualising variable pockets on protein surfaces. Ash is supervised by Drs Irilenia Nobeli and Mark Williams and Professor David Moss, and his PhD work is supported by a BBSRC CASE studentship, co-funded by Pfizer.

World champion

Congratulations to Rob Williams, a fourth-year PhD student at Birkbeck Biological Sciences. Rob won the men's four world championship in rowing, thus becoming a world champion. A massive round of applause for him!



UPDATE ON CENTRES & LABORATORIES

News from the Peter Rich Glynn Laboratory of Bioenergetics

Establishment of a mid- and far-infrared spectroscopy platform facility with CIF funding.

The ISMB has gained another vital biophysical technique that is now available for all members and external users. With acquisition of a new CIF funded instrument, we are now able to offer both mid-IR (4000-800 cm^{-1}) and far-IR (800-200 cm^{-1}) spectroscopic analytical facilities (Bruker Vertex 80v and IFS/66s FTIR spectrometers), together with expert technical assistance if required for running samples and analysing data. These machines can be operated in transmission or 'ATR' mode and have DTGS, MCT and helium bolometer detectors. We also have technology to combine IR measurements with electrochemistry, photochemistry and cyclic addition/removal of ligands.

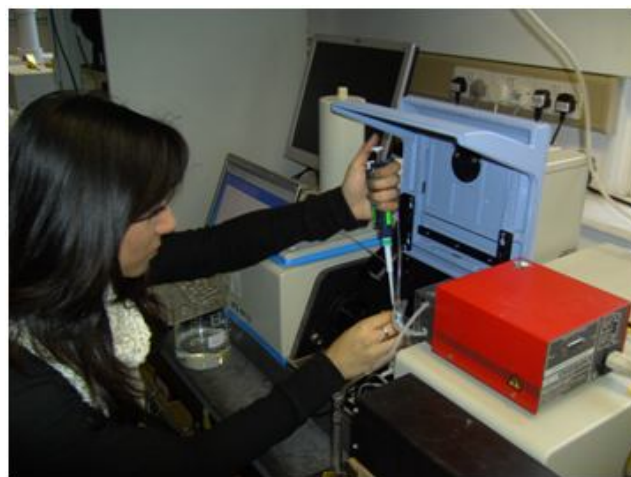
Vibrational infrared (IR) spectroscopy covers the 4000 – 200 cm^{-1} (2500 – 50000 nm) spectral range. It is a powerful chemical analytical tool that can be used to detect and analyse many types of chemicals and materials from their absorptions in this region due to their molecular vibrational properties. This frequency range provides spectroscopic information that is complementary to, and is generally more informative than, UV/visible/near-IR electronic spectra and almost all molecules have characteristic IR spectra. The technology is advancing rapidly, in large part driven by forensic, WMD detection, biomedical and other analytical interests. Its biological applications continue to increase and range widely from structural biology and analytical biochemistry to medical diagnostics.

Since sample requirements are very different for different types of applications, potential users should firstly contact Prof. Peter Rich (pr@ucl.ac.uk) to discuss project feasibility and sample requirements, together with likely machine requirements and access charges. Further information and booking will be available via the Biophysics Centre website: <http://biophysics.ismb.lon.ac.uk>.

Peter Rich

New Staff

Several new people have joined our laboratory recently. Raksha Dodia is a Ph.D. student and a former UCL undergraduate who is working on infrared characterisation of protein radicals, in collaboration with electrochemist Dr. Katherine Holt (UCL Chemistry). Talha Arooz is our new research technician who will be supervising users of the FTIR facility. Simona Bettini will join us for 6 months from 2nd February for part of her Ph.D. programme; this is part of a long-standing collaboration between our lab and the Physical Chemistry Department of Lecce University (Puglia, Italy). Dr. Lionel Truflandier is a postdoc working on a BBSRC-funded joint project with Dr. David Bowler (UCL Physics) on the development of computational methods for calculations of physical properties of very large molecules, in this case using cytochrome c oxidase as the test system. Dr. Amandine Maréchal continues work on our BBSRC-funded project on structural and mechanistic studies of cytochrome c oxidase (CcO). This programme has been greatly enhanced by the development of a system to prepare purified forms of his-tagged yeast CcO in which point mutations are being introduced into key function regions, a project in collaboration with yeast geneticist, Dr. Brigitte Meunier (Gif, France).



PhD student Raksha Dodia using FTIR in combination with electrochemistry to measure protein and cofactor structural changes in peroxidase intermediates



AWARDS, PRIZES & GRANTS

Grants applications - Deadlines

Funding body	Funding opportunities	Deadlines
BBSRC	Responsive Research Grants	25 May 2011
	Industry interchange programme	1 February 2011, 4pm
	The Genome Analysis Centre Capacity and Capability Challenge	18 March 2011
Wellcome Trust		Applications invited at any time
MRC Research board	Molecular and Cellular Medicine	4 pm on 18 May 2011
	Infections and Immunity	4 pm on 1 June 2011
MRC	Career development award	27 January 2011
	Methodology Research Programme	2 February 2011
EPSRC	Responsive Research grants	Applications invited at any time
Royal Society	Wolfson Research Merit Awards	10 March 2011
Royal Society of Chemistry	Prizes & Awards 2011	31 January 2011
Leverhulme Trust	Emeritus Fellowships	3 February 2011
European Molecular Biology Organisation (EMBO)	Long Term Fellowships	15 February 2011

VACANCIES AT THE ISMB

Lectureship / Senior Lectureship in 'Single Molecule Biophysics'

The Institute of Structural and Molecular Biology (ISMB) at UCL and Birkbeck and the London Centre for Nanotechnology (LCN) at UCL are seeking applications for a lectureship or a senior lectureship in the field of Single Molecule Biophysics, a position that will be held jointly between the two organisations.

Applications closing date: **15th March 2011**

For more details please visit the ISMB vacancies webpage at:
<http://www.ismb.lon.ac.uk/vacancies.html>

UPCOMING EVENTS

LSBC meeting	19 April 2011
ISMB Retreat	21st & 22nd June 2011
Venue: Robinson College, Cambridge	

More ISMB news on <http://www.ismb.lon.ac.uk/news.html>

To contribute to the next newsletter, please email Anne-Cécile Maffat at ismb-admin@ismb.lon.ac.uk.
Previous issues are available at <http://www.ismb.lon.ac.uk/newsletter.html>

