

ISMB NEWS is a news bulletin that will be published twice a year. The aim is to share information between the various departments involved in the Institute of Structural 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 putting together a newsletter meeting your expectations. Please email ismb-admin@ismb.lon.ac.uk.

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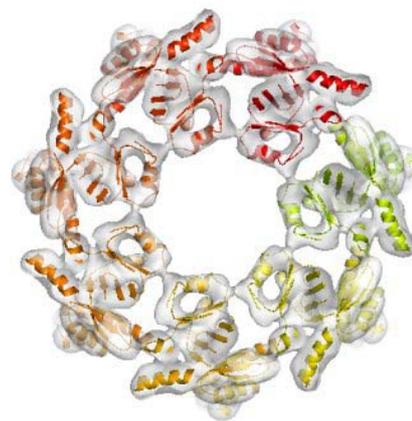
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Slice through a cryo EM map of GroEL, shown as a semi-transparent surface, with the domain atomic structures (colours) docked into the density.

Professor Helen Saibil, ISMB

UPCOMING EVENTS

ISMB Friday Wraps 25 January 2008
 29 February 2008
 25 April 2008
 30 May 2008

ISMB Symposium 19-20 June 2008

(Programme included at the end of this issue)

WELCOME TO...

The ISMB has recently appointed three lecturers in Structural Biology. **Dr Cara Vaughan** took up an X-ray lectureship in the School of Crystallography at Birkbeck whereas **Dr John Christodoulou** and **Dr Dmitri Ivanov** have joined the NMR group in the department of Biochemistry and Molecular Biology at UCL.

We are also pleased to welcome two new ISMB core members, **Professor Steve Wood** from UCL Department of Medicine and **Dr Adam McKay** from UCL Department of Chemistry.

IN FOCUS: ISMB PROFILES

Dr Cara Vaughan

"I started my scientific career in the laboratory of Prof Sir Alan Fersht, using protein crystallography as part of a multi-disciplinary approach to understand how interactions at the level of individual amino-acids contribute energetically to global protein-protein interactions. During this time I became fascinated by how highly specific protein-protein interactions within the crowded cellular milieu control cell fate and my research since then has focused on the structural biology of protein complexes in the context of disease. After studying the role of Hsp90 in facilitating oncogenic transformation via its interaction with kinases in the laboratory of Prof Laurence Pearl at the ICR, I am now interested in the role of Hsp90 in mitosis, with a particular emphasis on the kinetochore. Proper functioning of the kinetochore is crucial for accurate segregation of chromosomal DNA during cell division and misregulation leads to aneuploidy, a characteristic of many cancers."



Cara Vaughan

Dr John Christodoulou



"I'm returning to London as an ISMB lecturer (I undertook my PhD at Birkbeck) after periods at Cambridge University and at The Scripps Research Institute in California. My work over recent years with Chris Dobson and colleagues has focused on using solution state Nuclear Magnetic Resonance (NMR) methods in the study of protein folding and misfolding. My group at the ISMB is focused on using the latest NMR methods to understand protein folding of newly synthesized polypeptide chains e.g Structure and Dynamics of a ribosome-bound nascent chain by NMR spectroscopy Hsu, S-T. D., Fucini, P., Cabrita. L.D., Launay, H., Dobson, C.M., Christodoulou, J., P.N.A.S. (2007) 104, 16516. The aim is to structurally analyze protein chains while they are being created by taking structural snapshots of the functioning ribosomes. For this work, I was recently awarded a HFSP Young Investigators Award in collaboration with two groups from Frankfurt and Riken."

John Christodoulou

RESEARCH HIGHLIGHTS

Dichroweb

Dichroweb, a circular dichroism analysis webserver created in the School of Crystallography, Birkbeck, was highlighted as a case study of special merit in the recent 2007 RCUK "Study on the economic impact of research councils". It was also cited as paralleling for both academic and industrial spectroscopy the impact of the CCP4 programmes for crystallography. It is a resource that now has more than 1500 registered users worldwide.

(Whitmore, L. and Wallace, B.A. (2004) Nucleic Acids Research 32:W668-673.; Whitmore and Wallace (2007) Biopolymers doi 10.1002/bip.20853)

More information:

www.cryst.bbk.ac.uk/cdweb/html/home.html

Bonnie Wallace



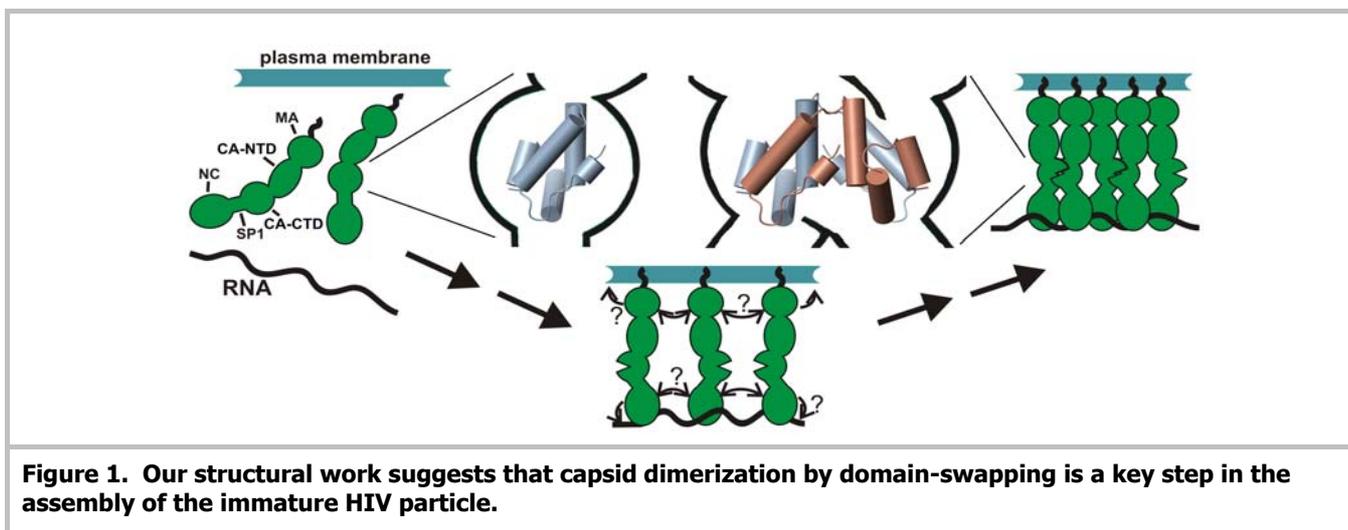
RESEARCH HIGHLIGHTS

Dr Dmitri Ivanov's research interests

The primary objective of my research is to understand fundamental structural principles governing formation and function of macromolecular assemblies. To address these questions we use a combination of biophysical techniques: X-ray crystallography, for example, is remarkably robust in producing high resolution structures of macromolecules once crystals are available, while NMR provides unique information on protein dynamics in solution and may identify protein segments problematic for crystallization efforts. Using NMR, X-ray and other techniques we were able to shed light on the elusive function of a conserved sequence element within the HIV retrovirus, known as the major homology region. Our findings indicate that this protein segment may participate in an

unusual assembly mechanism known as three-dimensional domain swapping, explaining its functional significance in retroviral assembly (*Mol. Cell* (2005) **17**:137; *PNAS USA* (2007) **104**:435). In another example of such multi-method approach we elucidated the mechanism of recruitment of ERCC1/XPF nuclease to sites of DNA damage by the damage-sensing protein XPA, one of the key assembly events in Nucleotide Excision Repair (NER) (*EMBO J.* (2007) Epub Oct 18). Our work provides another example that X-ray crystallography and NMR are complementary rather than rival techniques of modern structural biology.

Dmitri Ivanov



The Pace of Protein Buffer Screening is Hotting Up

In order to obtain high resolution protein crystals for X-ray crystallography or useful NMR spectra of a protein it is important to have a stable construct that is correctly folded. Changes in pH, ionic strength and additive conditions can affect the thermal and dynamic stability of a protein. To give the best conditions for protein crystallisation or NMR it is sensible to seek the optimal sample conditions. In general this objective can represent a laborious, and certainly tedious exercise.

Paul Leonard in Paul Driscoll's group has been putting into practice previously reported

methods for high throughput screening of buffer conditions, additives and drug-like compounds to search for conditions that improve protein stability. We have been using the differential scanning fluorimetry (DFS or "Thermofluor") method developed by researchers in industry (Pantoliano et al., *J. Biomol. Screen.* (2001) **6**:429-440,) and popularised by the Structure Genomics Consortium (Vedadi et al., *PNAS* (2006) **103**: 15835-40) and others (Ericsson et al. *Anal. Biochem.* (2006) **357**:289-298). The experiment uses an extrinsic fluorophore (e.g. SYPRO orange) that is sensitive to hydrophobic surfaces to monitor the temperature-dependent

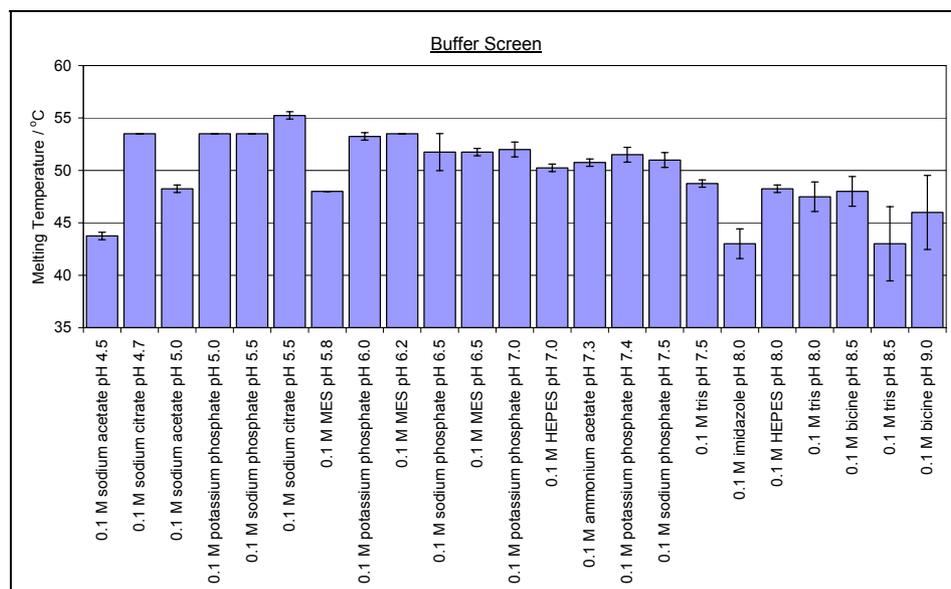
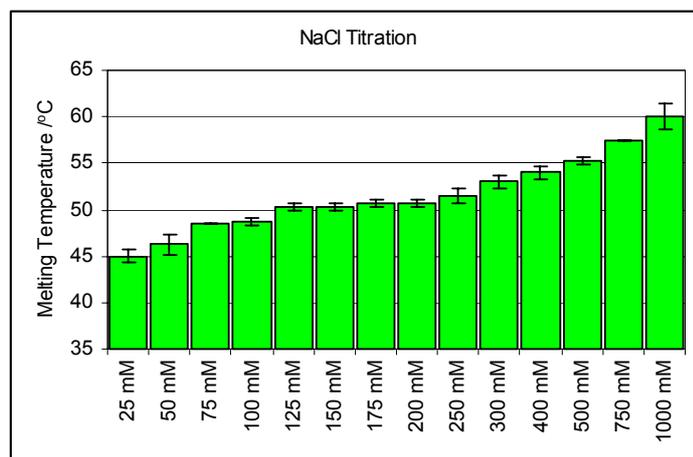
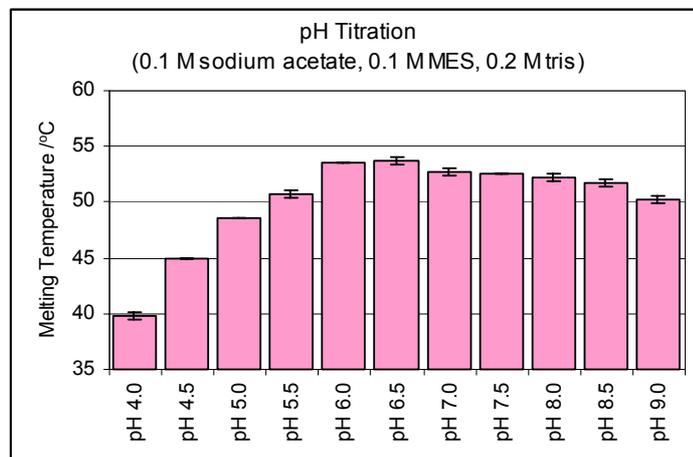
unfolding of a protein using a quantitative RT-PCR machine. In principle, compared to other methods, this type of screen is very efficient in terms of the amount of protein required, and thereby the number of conditions/additives that can be tested. Whilst this approach is not new, Paul Leonard has developed several spreadsheet templates to rapidly interpret the DFS output from our 96-well plate Bio-Rad MyIQ instrument, so the optimum buffer conditions for a protein can be determined almost immediately after the experiment is complete, allowing the testing and results analysis of 80 different buffer conditions, in duplicate, in just three hours.

A subset of results for a simple additive screen applied to a well-behaved protein under investigation in our laboratory are shown in the these diagrams. In this case we witness a strong pH-, salt-, and buffer-dependent changes in the apparent melting temperature of the protein. In other experiments we have been screening the interactions with a number of proteins of a commercially available chemical library of low molecular weight fragments, in some cases obtaining an increase in T_m of more than 3°C, suggesting a productive interaction between the protein and the library compound. Others using this technique report that crystallisability and crystal order (resolution) can be improved in the presence of stabilizing additives found by such methods.

More generally, DFS can be used to test the 'foldedness' of a given expressed polypeptide, and our MyIQ is also being used by Renos Savva and colleagues in their 'domain hunting' work at

Birkbeck. If anyone is interested in using these screening methods on their protein then they should contact either of the Pauls for more information (p.leonard@ucl.ac.uk or p.driscoll@ucl.ac.uk)

Paul Leonard and Paul Driscoll



AWARDS AND GRANTS

Wellcome Trust funds 4-year PhD programme in Structural, Computational and Chemical Biology

The ISMB, together with the Bloomsbury Centre for Bioinformatics and MRC-NIMR, has been awarded funding for a 4-year PhD programme comprising 30 studentships over a 6 year period. The programme is one of twelve new PhD opportunities supported by the Wellcome Trust. Funding is matched by UCL, Birkbeck College and NIMR who will provide a further four studentships in the programme. The first intake will be in September 2008 and the students will follow a series of tailored courses in structural biology, bioinformatics and chemical biology. The students will also have three research project rotations in the first year, at the end of which they will choose their main PhD topic for years two to four. There are 60 supervisors involved in the programme, covering a wide range of disciplines.

The Director, Prof. Gabriel Waksman said "This is a very exciting opportunity to promote interdisciplinary science within UCL, Birkbeck and NIMR. We are very pleased that the Wellcome Trust has chosen to recognise the international research excellence at all three institutions in the fields of structural, computational and chemical biology."

The SCCB programme joins two further Wellcome Trust-funded programmes, the new Developmental and Stem Cell Biology (Director Prof. Claudio Stern) and the long-running Neuroscience programme led by Prof. Dave Attwell.

Further details of the programme can be found at www.ismb.lon.ac.uk/wellcome_studentships.html

Jacky Pallas

Gabriel Waksman elected EMBO member

We are glad to announce Gabriel Waksman's election as a member of the European Molecular Biology Organization (EMBO). The organization announced the election of 50 leading scientists to its membership on the 14th of November 2007. These new EMBO Members join a community of over 1,300 members in Europe and more than 80 associate members worldwide.

Election as an EMBO Member is a tribute to the significant contribution to the advancement of science made by each of these researchers. EMBO elects new members annually on the basis of scientific excellence.



Structure of the HP0525 ATPase, a molecular motor powering the type IV secretion machinery in *Helicobacter pylori*.

Professor Gabriel waksman, ISMB

Grants applications - Deadlines

Funding body	Funding opportunities	Deadlines
BBSRC	Tools & Resources development Fund	4 pm on 10 January 2008
	Responsive Research Grants	22 January 2008, 23 April 2008
	Longer Larger Grant scheme (LoLa)	12 March 2008
Wellcome Trust		Applications invited at any time
MRC Research board	Infections and Immunity	4 pm on 16 January 2008, 7 May 2008
	Molecular and Cellular Medicine	4 pm on 9 January 2008, 30 April 2008
EPSRC	Responsive Research grants	Applications invited at any time
	BBSRC-EPSRC Funding Initiative for Bioprocessing of Biopharmaceuticals	23 January 2008

CENTRES & LABORATORIES UPDATES

Power to the people: new computing resources at BCB

November sees the delivery and installation of £200,000 of new high performance computing at three sites in the Bloomsbury Centre for Bioinformatics. The BCB received funding for the three new computer clusters in 2007 after a successful submission to the BBSRC Joint Research Equipment Initiative. The three clusters will be sited in the Departments of Computer Science and Biochemistry and Molecular Biology, UCL, and the School of Crystallography, Birkbeck. These clusters were purchased from IBM who also donated some additional nodes. The equipment was delivered at the end of October and is now being installed. Each cluster will provide at least 24 Dual socket quad core compute nodes giving a total of 192 cores. The new clusters will extend the computing infrastructure in each department and allow for more intensive applications. As part of the project, the three clusters will be joined using GRID technology and used to structurally annotate the human proteome. The new equipment will come online in early 2008.



Jacky Pallas

A new structural biology laboratory at UCL Department of Medicine

On November 1st 2007 a new structural biology laboratory was born in the UCL Department of Medicine. It is currently located on the top layer of the Medical School Building on the Royal Free Campus, just up the road from Belsize Park underground station. In the summer of 2008 the lab will move into refurbished space on the second floor. Moving down the building is entirely in keeping with the subterranean origins of many of the research group. Steve Wood, Jon Cooper, Alun Coker and Raj Gill all spent their early years in the basement at Birkbeck College while Simon Kolstoe, Halina Mikolajek and Mohinder Pal are younger creatures of the light. So some old faces and some new will join the London structural biology scene. The new laboratory will operate as part of the Centre for Amyloidosis and Acute Phase Proteins, directed by Mark Pepys, and translational research in drug discovery, targeting amyloidosis and inflammation will be one of the major foci. We are also interested in enzymes of haem biosynthesis, aspartic proteinases, type III secretion/injectisome components, caexctin in invertebrate neuron signal transduction and hope to establish collaborative research ventures across UCL and beyond.



Steve Wood

The analytical ultracentrifugation laboratory at UCL

Analytical ultracentrifugation (AUC) methods are enjoying a resurgence of interest in recent years for reason of new sample detection methods and much improved data handling and structural analyses. Major grant funding from the BBSRC and two Charities to Steve Perkins and others earlier this year in 2007 resulted in the purchase of a second AUC instrument for the Department of Biochemistry and Molecular Biology at UCL. Amongst other things, the commissioning of the second instrument now makes possible simultaneous velocity and equilibrium experiments so that protein associative behaviour in solution can be explored in detail.



Figure 1. Equipment. The AUC laboratory is pictured with both instruments side by side. For these, we possess four-hole and eight-hole rotors and sets of six-sector equilibrium cells and two-sector velocity cells. AUC data are saved onto a networked server and permanently archived. Data processing is performed in a computing laboratory supported by the latest DCDT+, SEDFIT and SEDPHAT software packages in which we are expert, helped by our home-written "easy-to-read" manuals. If required, sample and buffer densities can be measured on an Anton-Paar DMA 5000 densitometer.

Research with the analytical ultracentrifuge enables medium resolution structure determinations of large proteins using constrained modelling, complements new NMR and crystal structure determinations, and identifies protein solution properties. AUC methods can be applied to a wide range of biological systems with many useful outcomes. Studies of protein-protein interactions are facilitated by working with the protein in free solution, i.e. there are no potential artefacts that may result as with chip-based methods.

There are two types of experiments: (1) In **sedimentation equilibrium** experiments at low rotor speeds, diffusion opposes the process of sedimentation. When the two opposing forces reach equilibrium, the sample distribution is exponential in appearance. Molecular weights are obtained and equilibria can be studied to yield dissociation constants. (2) In **sedimentation velocity** experiments, if the sample is subjected to a high speed centrifugal field, the rapid sedimentation of protein towards the bottom of the cell occurs. The sedimentation coefficient provides structural data for comparison with crystallography, scattering and NMR, and sample polydispersity is readily analysed using size distribution $c(s)$ plots.

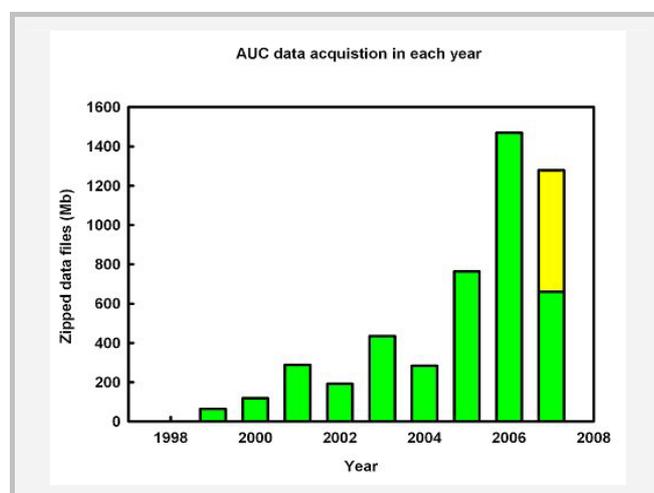


Figure 2. User statistics. Since the installation of our first machine in 1998, our data acquisition rate has grown steadily. In 2006, we obtained over 1 Gb of data (after compression) (green bars). With the second machine installed (yellow bar), we already matched our 2006 data accumulation after 10 months in 2007, and look forward to a very interesting year in 2008. Our results are published in mainstream journals such as J. Mol. Biol. and others.

We organised the well-attended 15th International AUC Conference at UCL in April 2006. Our track record with the AUC enables us to claim that we are the most successful AUC laboratory in the London area. We are always interested in new AUC collaborations with colleagues. For this, please contact either of us by email:

jayesh@biochemistry.ucl.ac.uk
or *s.perkins@medsch.ucl.ac.uk*

Steve Perkins and Jayesh Gor



ISMB Symposium 2008

19/20 June 2008

Structural biology programme

Steve Harrison (Harvard University)

Neil McDonald (ISMB)

Marc Baldus (MPI, Goettingen)

Chemical biology programme

Tom Muir (Rockefeller University)

Stefan Howorka (ISMB)

Peter Seeberger (ETH Zurich)

Biophysics/Proteomics programmes

Carlos Bustamante (UC Berkeley)

Peter Rich (ISMB)

Ruedi Aebersold (Institute of Molecular Systems Biology, Zurich)

Bioinformatics programme

Peer Bork (EMBL)

Irilenia Nobeli (ISMB)

Michael Sternberg (Imperial College)