EMBO Practical Course on the Structural Characterization of Macromolecular Complexes
2-7 June 2008, Grenoble France

The aim of this course was to teach Ph.D. students and post-docs how to exploit diverse techniques and strategies to expedite difficult structural biology projects involving the study of macromolecular complexes. More specifically, the course concerned the techniques used to produce, purify, reconstitute and characterize multi-subunit protein and protein/nucleic acid complexes.

The course, which ran from Monday June 2 till Saturday June 6, was structured into a series of morning seminars and afternoon practicals. The topics of the seminars included: expression in bacteria, insect and mammalian cells; affinity methods to purify protein components or entire complexes; bioinformatic analysis of interaction networks; biochemical and biophysical characterization of complexes; and structural analysis by crystallography, cryo-electron microscopy, NMR, and small angle X-ray/neutron scattering.

There were five different practicals. Each lasting approximately four hours and each was run by 3-5 tutors. The 20 course participants were divided into five groups of four, which rotated through the various practicals throughout the week, such that the tutor/participant ratio was approximately 1:1. The five practicals concerned:

(1) Crystallization, crystallography and data collection at a synchrotron beamline;

(2) Protein-nucleic acid interactions (including RNA transcription reactions and a gel shift assay) as well as limited proteolysis to probe structural features of a multi-protein complex;

(3) Electron microscopy: sample preparation, data analysis and fitting of atomic structures into EM maps;

(4) Biophysical/biochemical characterization of samples: circular dichroism, multi-angle laser light scattering; and cross-linking experiments;

(5) Thermofluor stability assay to survey the effect of different buffer conditions on protein complex stability, and the use of isothermal calorimetry to measure protein-ligand interactions.

In particular, participants were invited to bring their own samples (which several of them did) to use in this last practical.

At the end of the course, a feedback session was held in which participants were asked to score on a scale from 1(very poor) to 5(very good) all the seminars, practicals and evening events. Notably, no participant gave any score lower than 3 (satisfactory), and most scores were either 4 or 5. The average for all seminars was 4.4, for practicals was 4.5 and for evening events 4.7. In general, everyone involved in the course - organizers, speakers, tutors and course participants – found the course highly rewarding. This is reflected in the general comments that 17 of the 20 course participants included in their evaluations, which are given below.

General Comments from Course Participants

(1) In general everything was very good, it was specially good that the speakers stayed here most of the week, giving the possibility to discuss. Very professional organization.

(2) Poster printouts for the other participants would be helpful to remember projects of other participants
Improvement: greater focus on result discussion of the experiments done in the labs than actually calculating reaction mixes (protein-nucleic acid course). Great organization, excellent speakers, very helpful tips from speakers, tutors, etc; keep on going like that! I really enjoyed this course! Than you very much!

(3) Very good practical very good tutors; I learnt a lot of things; I am impatient to make such experiment in my lab.

(4) All course was very good

(5) Put the project summary of all the participant in the notebook in addition to poster clip session.
Good timing for me. Change nothing. Maybe each speaker talk and practicals would give a reference publication - like this we could read it during the workhop and increase/improve our discussion. The invited spekaers were really accessible and free to speak with.
What I prefer during the course=the practicals (really well done)
What I liked least = The weather. Please invite the sun next time and it will be perfect.
The best workshop I participated in - thanks

(6) More poster time needed
To keep: morning lectures and afternoon practicals, highly skilled tutors, excellent spekaers a lot of experience;
social events - time to mingle, talk and discuss our research; early recognition of participants, speakers and tutors
To change: some of the lectures gave much information of the results part, it would be better to emphasize more about the methods and the practical notes/examples the spekaers has to share
Overall: an excellent course!

(7) Eventually include Biacore/SPR and native gels; Great course!

(8) Fantastic course, I really feel I got a lot from it. Maybe some more information about dealing with membrane proteins?

(9) Missing techniques: AFM, biacore, protein-protein gel shifts?
I liked all and everything, the least maybe the Wednesday guest lecture (too detailed, not as relevant to us as the other lectures), also quite late so we were tired. Thank you again! It was very helpful, stimulating. Speakers were giving a lot of advice in spare time, participants were all also interested and motivated.

(10) It was a real delight for me to be here. I have met so many other scientists and had a lot of scientific ineteractions. Also the practical courses were very interesting. It was really great!

(11) In some practicals, I think if only show a example and discuss a result would be better even if the people know the technique. I like too much the proximity with the speakers

(12) I think that at the first day of the course, more proper introduce should be done
The course was extremly good. I learn a lot and met other people. My English is not so good for telling how good I feel regarding the course. Thank you all very much

(13) Big thanks! To all organizers, speakers and tutors

(14) In general I liked the course a lot. Perhaps there could have been a bit more crystallography
The protein exrpession lectures were excellent.

(15) I would like more seminars about X-ray, about the fundamental of the technique; the course was excellent, a very very nice experience. Thank You!

(16) Very nice atmosphere: encouraging to ask/discuss broad choice of topics/spekaers was very good!
Maybe shorter talks/more discussion time.

(17) A rewarding and worthwhile course. A good balance of lectures and practicals and a healthy number of social/evening events. My only reservation is that the expression strategies lectures jumped into optimizing expression rather than giving a

workplan of expression in that system (especially baculovirus and mammalian cell talks). One or two of the late talks were a little theory heavy rather than focussing on what can and can't be done (though I accept that you need to understand the theory to know this). These are picky criticisms though and all talks and practicals were excellent and it was interesting to see talks that I perhaps wansn't initially interested in (e.g. the protein-protein in vivo interaction studies) I believe that the attitude of speakers was correct - complexes are hard but they can be managed - and wasn't falsely negative / opitimistic; and I was particularly impressed on how they treated participants as equals. Finally, unlike many conferences I've been to, I had no issues with organization / prior information. Basically, please keep running this course!

Programme at a Glance

	Mon Jun 2	Tues Jun 3	Wed Jun 4	Thur Jun 5	Fri Jun 6	Sat Jun 7
Chair:	***D. Hart***	***E. Gordon***	***W.Weissenhorn***	***G. Schoehn***	***S. Cusack***	***C. Petosa***
9:00–9:45	*Bacterial Expression* **Barbara Morris**	*Co-expression in E.coli* **Christophe Romier**	*SAXS & SANS* **Dmitri Svergun**	*Imaging by EM* **Patrick Schultz**	*Crystallizat^{n} strategies* **Song Tan**	*Synchrotrons Beamlines* **Martin Walsh**
9:45–10:30	*Baculovirus expression* **Imre Berger**	*Strategies re: complexes* **Bertrand Séraphin**	*AUC & ITC* **Vladimir Rybin**	*single particle cryo-EM* **Holger Stark**	*Molecular Imaging* **Jan Ellenberg**	*Mass spec of assemblies* **Frank Sobott**
Coffee						
11:00–11:45	*Mammalian cell expression* **Radu Aricescu**	*Producing Complexes* **Song Tan**	*TAP analysis of complexes* **Anne-Claude Gavin**	*EM Docking* **Willy Wriggers**	*cryo-EM of SRP/ribosome* **Christiane Schaffitzel**	*enhanceo-some* **Daniel Panne**
11:45–12:30	*HTP methods* **Christian Kambach**	*Preparing multi-subunit complexes* **Chris Oubridge**	*MALS* **Marc Jamin** --- *H/D-MS* **Ricardo Pires**	*NMR of complexes* **Michael Sattler**	*yeast transcription system III* **Carlos F.-Tornero**	*Final remarks* **Carlo Petosa**
Lunch						
14:00–18:00	Practicals	Practicals	Practicals	Practicals	Practicals	
18:00–19:00	Poster clip presentations	Poster Session 1	**Guest lecture: Titia Sixma**	Poster Session 2	Feedback Session	
Dinner			**Novagen Buffet**		**Banquet at Le Sappey**	

Afternoon Practicals:

1. Macromolecular Crystallography
2. Protein-Nucleic Acid interactions/Limited Proteolysis
3. Electron Microscopy, Analysis and Fitting
4. Biophysical & Biochemical Characterization of Complexes
5. Thermofluor Stability Assay

Detailed Programme

Monday 2 June

Expression Strategies Chair: Darren Hart Chadwick Amphitheatre		
9:00 - 9.10	Welcome	**Carlo Petosa**
9:10 - 9.55	Expression strategies using bacterial systems	**Barbara Morris** Novagen / EMD Chemicals Madison WI, USA
9:55 - 10.40	Baculovirus expression strategies for multiprotein complexes	**Imre Berger** EMBL Grenoble
	Coffee	
11:00 - 11.45	Large-scale expression of proteins (and complexes) in mammalian cells	**Radu Aricescu** University of Oxford, UK
11:45 - 12.30	HTP Methods Great and Small : high throughput at the brownie scale and beyond	**Christian Kambach** Paul Scherrer Institute Villigen, Switzerland
	Lunch	
14:00 - 18:00	Practicals (Meet in ILL 19/20 hall)	
18:00 - 19:00	Poster clip presentations (EMBL Seminar Room)	
19:00 - 20:00	**Dinner (canteen)**	

Tuesday 3 June

Isolation of Functional Complexes Chair: Elspeth Gordon Chadwick Amphitheatre		
9:00 - 9:45	Strategies for the co-expression of protein complexes in Escherichia coli	**Christophe Romier** IGBMC Illkirch, France
9:45 - 10:30	Strategies for identifying, overproducing and characterizing protein complexes	**Bertrand Séraphin** CGM-CNRS Gif-sur-Yvette, France
	Coffee	
11:00 - 11:45	Production of protein complexes	**Song Tan** Penn State University, University Park, PA, USA
11:45 - 12:30	Preparation of recombinant multi-subunit complexes for crystallization	**Chris Oubridge** MRC-LMB Cambridge, UK
	Lunch	
14:00 - 18:00	Practicals	
18:00 - 19:00	Poster Session 1	
19:00 - 20:00	**Dinner (canteen)**	

Wednesday 4 June

Characterization of macromolecular complexes Chair: Winfried Weissenhorn Chadwick Amphitheatre		
9:00 - 9:45	Structure analysis of biomacromolecular solutions by small-angle X-ray and neutron scattering	**Dmitri Svergun** EMBL Hamburg, Germany
9:45 - 10:30	Analytical ultracentrifugation and isothermal calorimetry in the study of protein complexes	**Vladimir Rybin** EMBL Heidelberg, Germany
	Coffee	
11:00 - 11:45	Tandem Affinity Purification of protein complexes: lessons from a genome-wide screen	**Anne-Claude Gavin** EMBL Heidelberg, Germany
11:45 - 12:10	The use of size exclusion chromatography and multi-angle laser light scattering (MALS) to characterize macromolecular complexes	**Marc Jamin** UVHCI Grenoble
12:10 - 12:35	Analysis of the conformational dynamics of a protein involved in retroviral budding using hydrogen/deuterium exchange and mass spectrometry	**Ricardo Pires** EMBL Grenoble
	Lunch	
14:00 - 18:00	Practicals	
18:00 - 19:00	**Guest Lecture (Chadwick Amphitheatre):** Protein-protein interaction modulating ubiquitin and SUMO conjugation	**Titia Sixma** NKI Amsterdam, The Netherlands
19:00 - 20:00	**Novagen Dinner (ILL Hall 19/20)**	

Thursday 5 June

Electron microscopy & NMR Chair: Guy Schoehn, Chadwick Amphitheatre		
9:00 - 9:45	Imaging biological macromolecules with electron microscopes	**Patrick Schultz** IGBMC Illkirch, France
9:45 - 10:30	Structure determination of dynamic macromolecular complexes by single particle cryo-EM	**Holger Stark** MPI Göttingen, Germany
	Coffee	
11:00 - 11:45	EM interpretation with Situs and Sculptor	**Willy Wriggers,** D.E. Shaw Research, New York, USA
11:45 - 12:30	Studying macromolecular complexes by NMR	**Michael Sattler** Technische Universität München, Germany
	Lunch	
14:00 - 18:00	Practicals	
18:00 - 19:00	Poster Session II	
19:00 - 20:00	**Dinner (canteen)**	

Friday 6 June

Strategies in structural and functional analysis Chair: Stephen Cusack, Chadwick Amphitheatre		
9:00 - 9:45	Crystallization strategies	**Song Tan** Penn State University, University Park, PA, USA
9:45 - 10:30	Fluorescence microscopy-based methods to study macromolecular complexes in live cells	**Jan Ellenberg** EMBL Heidelberg
	Coffee	
11:00 - 11:45	Cryo-EM studies of co-translational targeting and translocation	**Christiane Schaffitzel** EMBL Grenoble
11:45 - 12:30	Structural studies on yeast transcription system III	**Carlos Fernández-Tornero** EMBL Heidelberg
	Lunch	
14:00 - 18:00	Practicals	
18:00 - 18:20	Feedback session / EMBO Questionnaire	
approx. 18:30	**Bus to LeSappey & Banquet**	

Saturday 7 June

More tips/techniques Chair: Carlo Petosa, EMBL Seminar Room		
9:00 - 9:45	Synchrotrons, beamlines, and all that jazz	**Martin Walsh** MRC France, BM-14
9:45 - 10:30	Mass spectrometry of noncovalent assemblies	**Frank Sobott** University of Oxford, UK
10:30 - 11:15	Crystallizing the enhanceosome	**Daniel Panne** EMBL Grenoble
11:15 - 11:25	Final remarks	**Carlo Petosa** EMBL Grenoble