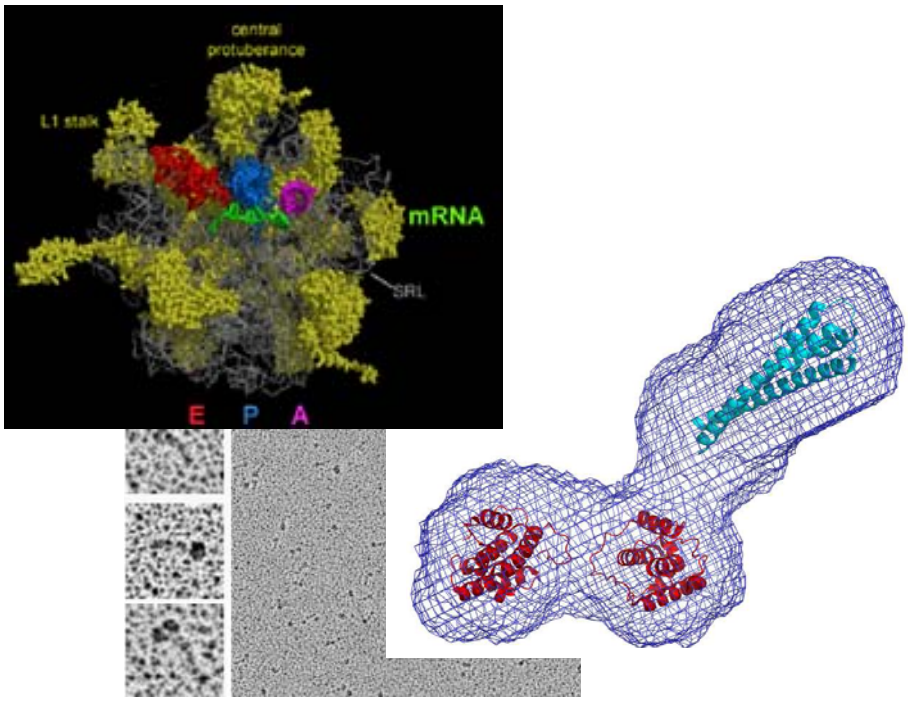


SLS Symposium

Physics Meets Biology

Tuesday, February 2, 2010

10:00 to 12:15, WBGB/019

Program

10:00 The Nobel Prize in Chemistry for 2009 - The cellular translation machinery at the atomic level
Rouven Bingel-Erlenmeyer

10:30 Solution structure of End Binding proteins (EB) determined by Small Angle X-ray Scattering
Ruben Martinez-Buey

11:00 Coffee

11:15 Structural biology studies of SAS6, an essential centrosome duplication protein
Ioannis Vakonakis

11:45 Ligand binding studies of the P_{II} protein GlnZ from *Azospirillum brasilense*
Daphné Truan

The Nobel Prize in Chemistry for 2009 - The cellular translation machinery at the atomic level

R. Bingel-Erlenmeyer, Swiss Light Source, Paul Scherrer Institut, CH-5232 Villigen; rouven.bingel-erlenmeyer@psi.ch

Genetic cellular information is stored in the genome sequence of the DNA (deoxyribonucleic acids). A large number of these sequences encode for proteins which carry out a vast variety of crucial functions in the living cell. The genetic information required at a given point of time is made available by transcription where the corresponding gene sequence of the stable DNA is transcribed into messenger RNA (messenger ribonucleic acids). The short-lived mRNA is subsequently translated into the respective amino acid sequence of the encoded protein. This highly complex and regulated process is accomplished by a huge macromolecular cellular assembly called the ribosome.

The Nobel Prize in chemistry for 2009 was awarded to Ada E. Yonath, Thomas A. Steitz and Venkatraman Ramakrishnan for their structural work on the ribosome. Their pioneering results elucidated the mechanism of this highly complex machinery at the atomic level. Nearly 30 years ago Yonath and co-workers started to crystallise ribosomes from extremophilic organisms in order to solve the structure by X-ray crystallography. At that time this endeavour was considered impossible, nevertheless the group of Ada Yonath was able to generate 3 dimensional ribosome crystals that allowed native data collection at the synchrotron. Unfortunately, the phase problem remained unsolved for a long time. It was the work of Thomas Steitz' group that yielded in the breakthrough of solving the phase problem. Finally in the year 2000, all three research groups published ribosome structures at atomic resolution. Since then especially the work of Venkatraman Ramakrishnan, a regular user of beam-line X06SA at the SLS, has largely contributed to the understanding of the accuracy and control of the translational process. Not only has the work of the Nobel Laureates enhanced our knowledge about this complex and fundamental cellular machine tremendously, but also initiated some structure based design on novel antibiotics.

In this presentation I will give an overview on the functionality of the ribosome and discuss the scientific achievements of the three Nobel Laureates. A focus will also be on the synchrotron beam-lines which are necessary to accomplish such challenging projects. Finally an outlook on the future of the ribosome research field will be given.

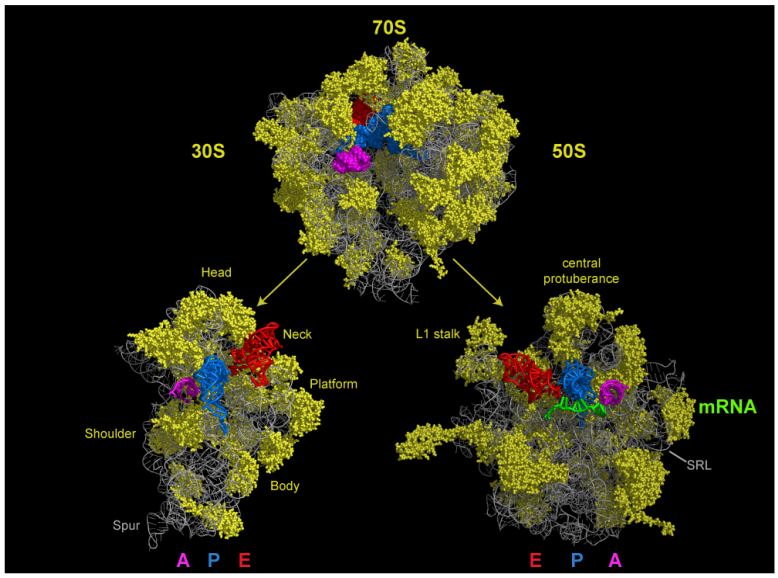


Figure 1: The bacterial ribosome. The bacterial ribosome is composed of a 30S (PDB-entry 2J00) and a 50S subunit (PDB-entry 2J01), which form together the translational active 70S ribosome. The tRNA molecules - which deliver the amino acids for the protein synthesis to the ribosome – are shown in the aminoacyl- (A, mangenta), peptidyl- (P, blue), and exit- (E, red) site and mark their respective binding sites on the ribosome. The mRNA molecule is highlighted in green.

References: http://nobelprize.org/nobel_prizes/chemistry/laureates/2009/cheadv09.pdf

Solution structure of End Binding proteins (EB) determined by Small Angle X-ray Scattering

Ruben Martinez-Buey & Michel O Steinmetz

Dept. Biology and Chemistry. Paul Scherrer Institut, 5232 Villigen PSI, Switzerland

ruben.martinez-buey@psi.ch

End binding (EB) proteins are highly conserved core components of microtubule (MT) plus-end tracking protein (+TIP) networks. EBs are 60 kDa dimeric proteins composed of two distinct, highly conserved functional modules connected by a long, poorly conserved linker: a calponin homology domain (CH), responsible for MT binding, and a coiled-coil structural motif (CC), responsible for dimerization and partner binding [1]. At present, no structural data is available for a functional full-length dimeric EB protein. We have performed SAXS experiments to determine the low-resolution structure of full-length EB proteins.

Our SAXS data on EB1 is compatible with a compact, slightly elongated molecule with a radius of gyration of 45Å and a maximum distance of 140Å (figure 1). The experimental sedimentation coefficient of EB1 (sw,20 = 3.5 S) agrees well with the one calculated from the *ab initio* SAXS model (sw,20 = 3.3 S). Model's overall excluded volume corresponds to a protein of around 60 kDa, further supporting our model. Similar structural parameters were obtained for the other human EB isoforms, EB2 and EB3.

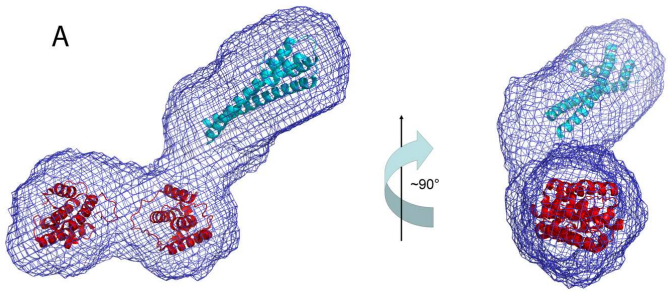


Figure 1.SAXS-derived low-resolution envelope (blue mesh) for EB1 as determined by DALAI-GA. X-ray crystal structures for the CH (red ribbons) and CC (cyan ribbons) domains (PDB codes 1PA7 and 1WU9, respectively) are docked inside.

Our model implies that the linker must be folded to some extent, with the two MT-binding domains in close proximity. This result readily explain why a dimeric molecule is needed to support microtubule processive growth in cells and anti-catastrophe activity, despite the fact that a single CH domain is the primary determinant of MT plus-end recognition [2]. Interestingly, two interacting CH domains also conform the MT-binding interface in the Ndc80 complex [3], another MT-biding protein complex. Furthermore, two CH domains in tandem form the F-actin binding region in most actin-binding proteins [4]. Together, these observations suggest that CH domains act in pairs to bind to cytoskeletal filaments.

References:

[1] Akhmanova, A., and Steinmetz, M.O. (2008). Tracking the ends: a dynamic protein network controls the fate of microtubule tips. Nat Rev Mol Cell Biol 9, 309-322.

[2] Komarova, Y., De Groot, C.O., Grigoriev, I., Gouveia, S.M., Munteanu, E.L., Schober, J.M., Honnappa, S., Buey, R.M., Hoogenraad, C.C., Dogterom, M., Borisy, G.G., Steinmetz, M.O., and Akhmanova, A. (2009). Mammalian end binding proteins control persistent microtubule growth. J Cell Biol 184, 691-706.

[3] Ciferri, C., Pasqualato, S., Screpanti, E., Varetti, G., Santaguida, S., Dos Reis, G., Maiolica, A., Polka, J., De Luca, J.G., De Wulf, P., Salek, M., Rappsilber, J., Moores, C.A., Salmon, E.D., and Musacchio, A. (2008). Implications for kinetochore-microtubule attachment from the structure of an engineered Ndc80 complex. Cell 133, 427-439.

[4] Gimona, M., Djinovic-Carugo, K., Kranewitter, W.J., and Winder, S.J. (2002). Functional plasticity of CH domains. FEBS Lett 513, 98-106.

Structural biology studies of SAS6, an essential centrosome duplication protein

***I. Vakonakis*[1], *M. Bortfeld*[1], *P. Gönczy*[2], *C. Schulze-Briese*[1], *M. Steinmetz*[1]**

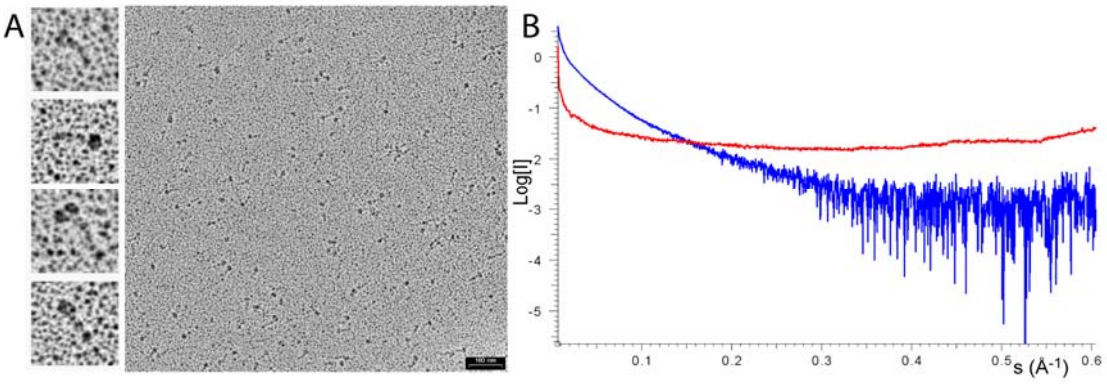
[1] *Swiss Light Source, Paul Scherrer Institute, CH 5232 Villigen, Switzerland*

[2] *Swiss Institute for Experimental Cancer Research, Swiss Federal Institute of Technology, School of Life Sciences, CH-1066 Lausanne, Switzerland*

ioannis.vakonakis@psi.ch

Centrosomes are primary cellular organization centres of microtubules, the molecular highways connecting cellular compartments, directing molecular motions and responsible for symmetric cell division. Centrosomal defects give rise to embryonic abnormalities, tunorigenesis and limit stem cell potential. In the mature cell they are composed of two centrioles, 9-fold symmetric structures that duplicate along with the cells [1]. Centriole formation is a process understood at ultrastructural terms through transmission electron microscopy studies; however, a high-resolution view of the underlying components and their structural interactions is lacking.

Here, we report the first structural / biophysical studies of *C. elegans* SAS6, a protein essential for the establishment of the centriole 9-fold symmetry [2] and conserved through the animal kingdom. A combination of biophysical methods allowed us to define domain boundaries in SAS6 that are suitable for further structural analysis. SAS6 is comprised of an independently folded, globular amino-terminal domain, an extended coiled-coil span and a flexible carboxy-terminal region. We show that the globular and coiled-coil domains harbour important protein interaction and chemical modification sites for centriole biogenesis. Electron microscopy and SAXS analysis of SAS6 constructs suggest a parallel dimeric configuration in solution with an elongated shape of over 35 nm in length. X-ray crystallographic studies of the SAS6 globular domain, now underway, will lead the way towards *in vivo* functional studies.



(A) Rotary metal shadowing electron micrographs of a SAS6 construct containing the globular and coiled-coil domains. The elongated shape and dimeric arrangement of this molecule can be seen in the inset magnifications. (B) SAXS data obtained from the same SAS6 construct. These data can be used to validate a model obtained by a combination of x-ray crystallography, electron microscopy and structure prediction methods.

1) Strnad, P. and Gönczy, P. *Trends in Cell Biology*, **18**, 389-396
2) Nakazawa, Y., Hiraki, M., *et al. Current Biology*, **17**, 2169-2174

Ligand binding studies of the P_{II} protein GlnZ from *Azospirillum brasilense*

Daphné Truan

Macromolecular Group, Swiss Light Source, Paul Scherrer Institute, daphne.truan[a]psi.ch

P_{II} proteins are widely spread in bacteria and plants. They play an important role by regulating the activities of many enzymes and other proteins involved in nitrogen assimilation in the cell. They are sensitive to a number of small so-called effector molecules, in particular to the intracellular ADP:ATP ratio as well as to other key metabolites signalling the nitrogen or carbon status of the cell. That is why they are often compared to CPUs.

In this talk, I will present structures that show how these ligands bind to GlnZ and I will address some technical crystallographic problems like for instance a rare case of twinning.