# Biomacromolecular interactions, assemblies and machines: A structural view

Report on the Murnau Conference 2005 on *Structural Biology of Molecular Recognition*

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Molecular interactions are integral to all processes vital to life. Structural biology in its numerous facets has delivered ever more detailed three-dimensional descriptions of such interactions, ranging from complexes between proteins (or nucleic acids) and low molecular weight ligands, protein-protein and protein-nucleic acid complexes to large macromolecular assemblies and machines. Dramatic technical improvements in X-ray crystallography, biomolecular NMR spectroscopy and electron microscopy in the recent years led to unprecendently detailed pictures on biomolecular dynamics, supramolecular assemblies and interaction networks. In the future a detailed understanding of the complex macromolecular interplay within the cellular context will require a much tighter integration of biocomputational approaches with the diverse experimental techniques.

In September 2005 nearly two hundred structural biologists, biochemists and biophysicists met in Murnau, a picturesque town in the Bavarian alpine upland, at the occasion of the Murnau conference titled “Structural Biology of Molecular Recognition” (*http://www.murnauconference.de)* to discuss the status of the field. The meeting was structured in five scientific sessions focussing on protein-protein interactions (chaired by W. Kuehlbrandt, Frankfurt), protein-nucleic acid interactions (chaired by P. Cramer, Munich), prediction and design of molecular interactions (chaired by Y. Muller, Erlangen), dynamics and transient interactions (chaired by M. Sattler, Heidelberg) and large macromolecular assemblies (chaired by. M. Gruetter, Zurich).

**Protein-protein interactions** are critical for essentially all cellular processes. Matthias Wilmanns (EMBL Hamburg) and James Hurley (NIH Bethesda) presented new crystal structures on multisubunit complexes involved in protein translocation into peroxisomes (Wilmanns) and protein sorting and virus budding (Hurley). Protein translocation into peroxisomes is mediated by a signal-assembled translocon comprising about 30 different proteins. The Pex receptors as part of the translocon recognize import cargo

such as sterol carrier protein 2 (SCP-2).[1,2] The structure of the Pex-5 receptor-SCP-2 complex shows two interactions sites, with the signal peptide binding site forming a tunnel in the receptor. Comparison of the receptor structure with and without cargo indicates major conformational changes, including the unfolding of an entire loop. The system shows striking similarities to the karyopherins involved in nuclear transport.

The multivesicular-body (MVB) pathway involved in endosomal protein sorting and trafficking and HIV budding is highly conserved from yeast to man and involves three higher order protein complexes, termed ESCRT-I, -II and –III (Figure 1). The recognition signal for cargo protein sorting is ubiquitination. Numerous structures of proteins belonging to the ESCRT protein complexes have been recently determined, including the ESCRT-II core heterotrimeric complex.[3] The Y-shaped ESCRT-II core contains three different $\alpha$-helical proteins, one of which is present in two copies. The main interactions occur through coiled-coil helices leading to a rigid scaffold with protruding ends that allow for interactions with other ESCRT complexes and ubiquitin binding and thereby enable swift transfer of ubiquitinylated proteins from one ESCRT complex to the next. The core of the ESCRT-III targeting domain Bro1 shows a boomerang shaped structure made of three tetratricopeptide repeats.[4] The conspicuous shape of the structure suggests it to function as a negative membrane curvature sensor during the budding process.

**Figure 1**

Following on the astonishing structures of these multimodular protein-protein complexes, Eric Sundberg (Boston) and Erwin de Genst (Brussels) presented detailed studies on the structural basis of affinity maturation in protein-protein interfaces. Sundberg analyzed structural, enthalpic and entropic contributions of five mutations in

an artificially affinity matured T-cell receptor, which result in a cumulative 1500 fold increase in affinity.[5] Since changes of the complexity observed here cannot be predicted *a priori* at present, rational and evolutionary (randomized) approaches must be combined to enable substantial redesigns of molecular interactions amongst biomacromolecules (see below; Andreas Plueckthun). De Genst (Brussels) presented structural and functional data on camelid antibodies that are entirely lacking the light chains, showing that affinity maturation of camelid single domain antibodies is mainly driven by enthalpy.[6]

Stephan Grzesiek (Basel) presented studies on protein folding and ligand binding using advanced NMR methods based on measurements of residual dipolar couplings and scalar coupling across hydrogen bonds.[7] Using these methods detailed insight is achieved into the conformation and dynamics of macromolecules under different physicochemical conditions. As an example, Grzesiek presented the structure of the antibiotic binding protein TipAS, which plays an important role in bacterial multidrug resistance. In the absence of the antibiotic TipAS is partially unfolded. The protein folds completely upon ligand binding This system might lend some support to the proposal that a fly-casting mechanism may speed up molecular recogntion.[7] The session was the concluded with the crystal structure of the membrane-bound respiratory complex NarGHI from *E. coli,* which was presented by Michaela Bertero (Vancouver) and which provides new insights into the proton-motive force generation by nitrate respiration.[8]

In recent years immense progress has been achieved towards a structural understanding of the complex machineries controlling the central processes of transcription, splicing, translation and DNA repair. Opening the session on **Protein-Nucleic Acid Interactions**, Alfred Pingoud (Gießen) reported how the covalent linkage of a triple-helix forming oligonucleotide to a restriction endonuclease can specifically target the

enzyme to a restriction site.[9] The potential of such engineered nucleases for their use in gene therapy was discussed.

RNA-polymerase II is responsible for the synthesis of eukaryotic mRNA during transcription. Interestingly mRNA processing is directly coupled to transcription via the flexible C-terminal domain (CTD), which consists of heptapeptide repeats. Anton Meinhart (Heidelberg/Munich) presented the structure of the complex between the CTD-binding domain of 3'-RNA-processing factor Pcf11 and a phosphorylated CTD heptapeptide which suggests that CTD might form a compact β-spiral that gets partially unwound in a phosphorylation dependent manner during RNA processing.[10] Karim Armache (Munich) summarized the current understanding of the mRNA transcription mechanism based on the refined crystal structures of the complete 12-subunit RNA polymerase II in free form, an elongation complex with a DNA bubble and RNA, and an elongation complex that contained the elongation factor TFIIS (Figure 2). The studies revealed the nature of the “tunable” active site of the enzyme that carries out both mRNA synthesis and cleavage.[11,12]

**Figure 2**

Tom Steitz (Yale) completed the current view on the transcription process by describing his data on the T7-phage RNA polymerase, which undergoes dramatic refolding upon its transition from an initiation to an elongation complex. During this process, the upstream promoter-binding site is destroyed and the RNA exit site is created.[13] In a second part of his presentation Steitz showed impressive movies that summarize the progress in understanding protein synthesis during mRNA translation at the ribosome active site, based on structures of the large ribosomal subunit in complex with various substrate analogues.[14]

Alexandra Deaconescu from the Darst laboratory (New York) presented the structure of the bacterial transcription-repair coupling factor Mfd, providing insight into Mfd action at a stage when the RNA polymerase is stalled at a DNA lesion. The structure contains eight different domains, including an ATPase domain that can generate force by translocating along the DNA upstream of a lesion, thereby disrupting the elongation complex and freeing the lesion for access to the repair machinery. Lars-Oliver Essen (Marburg) presented the molecular mechanism for the repair of cis-*syn* cyclobutane pyrimidine dimer (CPD) induced DNA damage by the enzyme photolyase. These lesions are introduced into the DNA by UV light. The structure of photolyase in complex with a CPD lesion-containing DNA revealed the partial unwinding of DNA and the relative position of the lesion with respect to the flavin cofactor. Strikingly, X-rays could induce repair of the lesion within the crystal, as demonstrated by difference Fourier maps.[15] Esben Lorentzen from the group of Elena Conti (EMBL Heidelberg) reported new structures of the archaeal exosome RNase PH core liganded with RNA, providing insights into how the 3'-end of the RNA is placed into the active site and how the enzyme is rendered sequence-unspecific.[16] The session was concluded by Karl-Peter Hopfner (Munich), who reported the first structure of an enzyme of the Swi/Snf family of ATPases, in free form and bound to a DNA duplex. Current models for how the enzyme can translocate along DNA by tracking the minor groove were discussed.[17]

The session on **Prediction and Design of Molecular Interactions** was primarily focussed on the design of new molecular interactions combining both structural and functional approaches as well as on the prediction of so far undiscovered interactions by means of bioinformatics. The talk of Andreas Plückthun (Zürich) superbly revealed how stable non-antibody protein binders were discovered by combining rational design and affinity selection.[18] In a first step, a highly stable version of an ankyrin repeat protein was generated by consensus design.[19] Subsequently, ribosome display (a method de-

veloped in the Plückthun lab) against a variety of baits resulted in affinity-selected Designed Ankyrin Repeat Proteins (DARPins; Figure 3). It was proposed that DARPins may be utilized in a similar fashion as monoclonal antibodies in both basic research and therapeutic applications. With respect to the latter, interesting data were shown for DARPins, that bind very tightly to HER-2.

**Figure 3**

Thomas Wollert (Braunschweig) re-engineered the interface of internalin A from *Listeria monocytogenes* and its receptor human E-cadherin[20] by introducing three rationally designed mutants into internalin A. This resulted in an affinity increase of three orders of magnitude. Structural analysis and isothermal titration calorimetry showed that the increase in affinity was gained predominantly through the removal of steric strain and the exclusion of water molecules from the protein-protein interface. Rob van Montfort (Astex Technologies) moved the focus from protein design to drug design and presented examples on the fragment based discovery of lead structures for protein tyrosine phosphatase 1B (PTP-1B) and a protein kinase.[21] Marie-Helene Le Du (Gif-sur-Yvette) described crystal structures of the tumor marker proteins placental alkaline phosphatase (PLAP)[22] and urokinase plasminogen activator receptor (uPAR)[23] in complex with a peptide that acts as a competitive inhibitor of the uPAR:uPA interaction. She then discussed how structure-function studies can evolve such biomarker proteins to therapeutic targets.

Rob Russel (Heidelberg) and Tanja Kortemme (San Francisco) presented computational approaches for the identification and redesign of protein interaction pairs. Rob Russell (EMBL Heidelberg) discussed how protein-protein interactions can be iden-

tified from genomics and proteomics data and how known structural data can be applied to refine such searches and what the limitations are.[24]

Tanja Kortemme and her colleagues used computer algorithms to reengineer the interface of protein complexes with known 3D structure to create novel amino acid interaction pairs. They demonstrate, that computational repacking of side chain rotamers is achieved best when using an empiric orientation dependent description for the quantification of hydrogen-bonding energies.[25] Closing the session Dirk Röser (Göttingen) presented the structure and catalytic mechanism of the human formylglycine generating enzyme (FGE).[26] Formylglycine is a key catalytic component for sulfatases so that mutations in FGE cause multiple sulfatase deficiency, a fatal autosomal recessive syndrome.

**Dynamic properties and transient interactions** of biomacromolecules are receiving increasing attention for the analysis of cellular processes as well as for the development of more reliable computational procedures in protein and drug design. Opening the session, John Ellis (Warwick) advocated macromolecular crowding as an important variable in the design of biochemical experiments. The high protein concentration in the cell (80-400 g/l) results in reduced diffusion rates and increased aggregation, which has profound effects on the biochemical and biophysical properties of proteins. Andrea Schmidt (Hamburg) proposed to extract information about functionally relevant motions in proteins from anisotropic atomic displacement parameters in high-resolution crystal structures.[27] Frederic Allain (Zürich) presented structural insight into the regulation of alternative splicing. NMR structures of the polypyrimidine tract binding protein (PTB) and Fox proteins were determined in complex with single stranded RNA-oligonucleotides. It was found that two of the four RNA binding domains of PTB interact extensively in a manner that leads to the positioning of the bound oligonucleotides in an antiparallel orientation. This finding suggests that PTB induces RNA looping upon bind-

ing to two pyrimidine tracts in the same pre-mRNA. Consequently, structural models explaining how PTB acts as repressor of alternative splicing can be derived.[28] Matthias Görlach (Jena) presented structural and functional studies of the stemloop D from the 5'-terminal cloverleaf-RNA. In coxsackieviruses and other entero- and rhinoviruses viral and cellular proteins bind to the 5'-cloverleaf to form a ribonucleoprotein complex, which is essential for the initiation of viral RNA replication. Within this complex the tetraloop that caps stemloop D is the cognate RNA ligand for the viral proteinase 3C. Solution structures and NMR binding data for the apical tetraloops in stemloop D suggest that the viral proteinase 3C recognizes the conserved loop structure, rather than a specific RNA sequence.[29]

Harald Schwalbe (Frankfurt) investigated dynamics features of ligand mediated rearrangements in protein kinases and riboswitches. For protein kinase p38 he demonstrated that a key active site motif, the so called DFG loop, is in slow conformational exchange in the non-liganded enzyme. Some highly specific p38 inhibitors[30] "freeze" this equilibrium in a specific conformation, referred to as "DFGout".[31] The spectral changes associated with the reduced conformational freedom provide an analytical tool to rapidly detect such inhibitors for p38 kinase. In a second part of the presentation time-resolved NMR studies of riboswitches were shown to provide insight into mechanisms and timescales of ligand induced rearrangements.[32] Hans Bartunik (Hamburg) showed detailed structural analysis of the protein-ligand complexes of all reaction intermediates of 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase from *M. tuberculosis*, a potential target for new antimycobacterial drugs.

The Murnau Conference concluded with a session focussed on **macromolecular assemblies** that illustrated how supramolecular complexes and molecular machines such as the spliceosome, the ribosome, fatty acid synthase and even entire bacteriophages can be studied at the molecular/atomic level by hybrid approaches involving

structural information on individual proteins and complexes revealed by X-ray crystallographic and high resolution cryo-electron microscopy. The contributions of Holger Stark (Göttingen) on spliceosomes,[33] of Kirsten Model (Cambridge) on the architecture of mitochondrial membrane translocators TOM and TIM in the outer and inner membranes of mitochondria[34] and of Beate Rockel (Martinsried)[35] on the architecture and assembly mechanism of tripeptidyl peptidase II represented highlights on the progress made in single particle cryo-electron microscopy and its growing importance for elucidating the function of large biological assemblies at increasing resolution.

Markus Wahl (Göttingen) presented crystal structures of *T. maritima* ribosomal subunits L10-L7/L12 that constitute the so-called ribosomal stalk and refined features of the structure L10 subunit on the 50S subunit (Figure 4). Identification of these elements in cryo-EM reconstitutions of the *E. coli* ribosome provides a detailed structural understanding of the ribosomal stalk.[36] It also suggests how the mobile L7/L12 subunits of the stalk can “catch” translation factors from the surrounding medium and can transfer them to the ribosomal factor binding site, where the L12 stabilizes the positioning and active conformation of the factor.

**Figure 4**

Using a similar approach, Michael Rossmann (Purdue) combined crystallographic and cryo-electron microscopic investigations of the *T4*-bacteriophage tail to an astonishing view on the molecular warfare between *Escherichia coli* and bacteriophage *T4*.[37] Comparisons of multiple structures allowed to describe the significant conformational changes that occur upon puncturing of the bacterial membranes and the cell wall as well as during genome injection. The findings open intriguing questions on how the en-

ergy for the mechanical infection processes is stored in the supramolecular assembly of the phage.

**Figure 5**

X-ray crystallography by itself allows to address larger and larger assemblies, as it was impressively demonstrated in the contributions by Erez Pyetan (Rehovot) and Nenad Ban (Zurich). Pyetan discussed a structure and dynamics of trigger factor in complex with a eubacterial ribosome,[38] while Ban reported on the recently determined structure of the fatty acid synthase megadalton assembly. In the latter case a 4 Å electron density map was utilized to assemble the known crystal structures of individual catalytic subunits to a stunningly detailed picture of the supramolecular architecture of this multimodular enzyme complex. Mechanistic details on the sequential steps of catalysis were discussed in the light of this new structure.

In summary, the Murnau Conference impressively demonstrated how structural biology has matured to a multidisciplinary effort. Biological systems of increasing complexity are now addressed at an overwhelming rate by combining a variety of experimental approaches. Combined application of protein crystallography and NMR allows the assignment of dynamic properties to high resolution structures of biomolecules. The modelling of high resolution structures derived from crystallography into lower resolution envelopes from electron microscopy or crystallography now provides atomic resolution views on supramolecular assemblies of unprecedented complexity. The ultimate goal of biostructural studies – an atomic resolution view of the cell – may soon come within reach.[39]

After an exiting start in 2005 there is reason to believe that the Murnau Conference will evolve to a first-class meeting in the middle of Europe for the topical discus-

sion of recent and exciting findings in biostructural research. The next Murnau Conference is planned for September 2007.

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## Figure Legends

**Figure 1:** Trafficking of ubiquitinated membrane proteins by ESCRTs. ESCRT-I binds Ub-cargo through the UEV domain of Vps23. The second NZF domain of Vps36 binds the Ub-cargo. Reprinted with permission.[3]

**Figure 2:** Front view of a ribbon model of the protein subunits and nucleic acids of RNA polymerase II. Subunits Rpb4 and Rpb7 are shown in red and blue respectively. Transcription elongation factor TFIIS is shown in orange and nucleic acids in blue, cyan and red for template DNA, nontemplate DNA and product RNA respectively.

**Figure 3:** Crystal structure of an affinity-selected Designed Ankyrin Repeat Protein (DARPin) interacting with its target aminoglycoside phosphotransferase. Interaction residues are colored according to the the percentage of change of the solvent accessible surface area upon complex formation (yellow < 20 % cange; red > 80 % change). The figure is adapted from Binz *et al.*[18b]

**Figure 4:** Surface representation of a 50S ribosomal subunit (proteins - black, rRNA - gray) including a full size L12 stalk (color code as depicted) with six copies of protein L12 and a superimposed outline of the 30S subunit (semi-transparent). Proposed functional motions are indicated. The figure is adapted from Diaconu et al.[37]

**Figure 5:** Structure of the tail of bacteriophage *T4*. The left part of the figure shows the tail tube displaying one of the strands of the six start helix representing the contractile sheath around the tail tube. In green is shown the single turn of the extended sheath, in

brown the double turn of the contracted sheath. Thus, the tail tube and associated pin at its end turns through one revolution as the tail sheath contracts during the infection of an *E. coli* cell. The right part of the figure shows the contracted sheath (green) and the star shaped base-plate surrounding the tail tube. The various identified proteins in the baseplate are shown in different colors. The figure was adapted from Figure 5 of [37a] and Figure 1 of [37b]. Reprinted with permission.