MEETING REPORT

Structures and diseases

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Structural biology continues to make significant contributions towards an atomic resolution understanding of molecular constituents and mechanisms underlying human diseases as recently discussed at the international *Murnau Conference on Structural Biology of Disease Mechanism* held in Murnau, Germany.

From its very beginning biostructural research has not only provided tremendous breakthroughs in basic biological processes but has also significantly contributed to the understanding of the molecular mechanisms underlying human disease. This was first exemplified when Max Perutz and colleagues elucidated the molecular pathology of human hemoglobin mutations[1] leading to sickle cell anemia. More recently, the three-dimensional structures of many human and pathogen derived proteins have served as templates for structure based drug design, a method which is now broadly applied in the discovery and optimization of small molecule therapeutics. Nowadays, many of the required target structures are determined at greatly increased rates in the context of structural genomics[2]. Beyond single protein approaches, structural biology is now *en route* towards a high resolution picture of the cell assessing permanent and transient protein-protein and protein-nucleic acid complexes at steadily increasing levels of complexity. One would assume, that an ever

more integrated understanding of the molecular mechanism of human disease will emerge on this path, while a formidable challenge to the field may arise in translating the emerging level of biomolecular complexity into concrete intervention strategies. In early September 2007 about 180 structural biologists and biochemists met in the picturesque town of Murnau, located at lake Staffelsee in the Bavarian alpine upland, to discuss and reflect on these questions, in view of recent biostructural data that relate to the molecular determinants of human diseases including microbial and viral infections, protein misfolding diseases, cancer and metabolic diseases. These topics were addressed during five scientific sessions accompanied by two poster sessions and ample time for discussions (Figure 1). The scientific part of the meeting was complemented by a popular traditional Bavarian style social programme.

The meeting started out with the "Murnau lecture" held by Wim Hol (University of Washington, Seattle), who has focussed his research for several decades on the structures of key proteins from often neglected tropical diseases. In his lecture Wim Hol gave an impressive overview on the molecular mechanism of maturation and activity of cholera toxin starting from its assembly in the periplasm via its translocation out of the cell by a type II secretion system up to its subsequent interactions with host cell components[3 4]. With the example of the Medical Structural Genomics of Pathogenic Protozoa (MSGPP) program, he also demonstrated the power of integrating structural biology and biophysical screening approaches to quickly obtain novel lead structures for potential drug targets[5].

The first session on infectious diseases was opened by Gabriel Waksman (Institute of Structural Molecular Biology, UCL and Birkbeck College, London), who investigates the assembly process of bacterial pili, which are multisubunit assemblies on the bacterial surface. In uropathogenic *Escherichia coli* they function as virulence determinants critical for host cell recognition. He highlighted the role of a specific chaperone that donates one of its β-strands to the incomplete Ig fold of the subunits while these are transported to an outer membrane platform called usher, where the pilus is assembled. Here, the chaperone strand is exchanged with the strand of the neighbouring subunit of the growing pilus in a process referred to as "donor-strand exchange"[6]. Waksman furthermore combined biostructural and biophysical methods to demonstrate that the exchange mechanism proceeds through a zip-in-zip-out process involving a transient intermediate complex that plays key roles in subunit ordering and pilus biogenesis termination[7 8]. This multidisciplinary approach also led to the characterization of small molecule inhibitors of pilus formation, bicyclic 2-pyridones termed pilicides, which suppress the assembly of this multimodular virulence factor by interrupting the protein-protein

interactions of the chaperone with the usher[9] This approach nicely demonstrates how the cooperative assembly of a fibrillar virulence factor on the cell surface can be disrupted by targeting non-cooperative events during the assembly process.

Similar to pili, type III secretion systems (T3SS) are important virulence elements of well-known Gram-negative bacterial pathogens like *Shigella flexneri* and *Yersinia pestis*. On the bacterial surface T3SS form needle-like devices which can penetrate the host cell membrane and deliver effectors into the host cell where they often cause a "reprogramming" of host cell processes for the pathogens' benefit. Steven Johnson (University of Oxford), who is studying the assembly of the complex T3SS apparatus, presented a hybrid approach utilizing electron microscopy, X-ray crystallography and molecular modelling. This has provided a first near atomic model of a T3SS needle[10] and atomic models of proteins associated with the tip of the needle[11]. Investigations are currently underway to probe the structural basis for regulation of assembly of this complex multi-protein structure. Erec Stebbins (Rockefeller University, New York) presented a wealth of structural data on the mechanisms of potentially druggable T3SS effectors of pathogenic bacteria. A striking example is the *Yersinia* protein kinase A (YpkA) which is able to disrupt the actin cytoskeleton of the host cell. The structure of YpkA in complex with the small GTPase Rac1 shows that YpkA acts as a mimic of guanine nucleotide dissociation inhibitors (GDIs) for Rac1 leading to the disruption of the actin cytoskeleton[12]. Deshmukh Gopaul (Institute Pasteur, Paris) presented data on the exchange of genetic information between bacteria, a process critical for bacterial adaptation to changing environmental conditions and resistance against antibiotics. This process relies on integron integrases, enzymes which mediate DNA recombination between short symmetric DNA sequences. The structure of the integron integrase from *Vibrio cholerae* bound to a DNA substrate shows that the enzyme recognizes the structure of the DNA rather than a specific sequence thus providing a structural explanation for its broad substrate specificity[13]. Gunther Kern and Gautam Sanyal (AstraZeneca, Boston) presented the industrial perspective on the search for new antibiotics by further exploiting the notoriously vulnerable cell wall biosynthesis. They showed that glutamate racemases, that exist in distinctly functional oligomeric states, are highly suitable target enzymes for narrow-spectrum antimicrobial agents that are badly needed e.g. against nosocomial, i.e. hospital-acquired infections[14]. Hartmut Niemann (Helmholtz Centre for Infection Research, Braunschweig/University of Bielefeld) showed how a bacterial invasion protein (InlB from *Listeria monocytogenes*) exploits the signalling pathway set off by the receptor tyrosine kinase and protooncogene Met to promote bacterial uptake by the host cell. The structure of the complex between InlB and human Met shows that InlB specifically interacts via its leucine-rich

repeat domain with the first immunoglobulin-like domain of the Met ectodomain stalk leading to a clamping of the receptor into a signalling competent state. InlB thus functionally mimics the natural ligand hepatocyte growth factor/scatter factor (HGF/SF) albeit binding Met at a different domain. The structure provides general insight into the activation mechanism of an important cancer drug target.[15]. Stephen Matthews (Imperial College London) presented the latest results on the host cell recognition by the protozoan parasite *Toxoplasma gondii*, where so-called microneme proteins (MICs) are secreted to allow for host cell attachment and penetration. The structure of a MIC reveals the presence of a novel microneme adhesive repeat (MAR)-domain which specifically interacts with sialyated host cell oligosaccharides[16] .

Rolf Hilgenfeld (University of Lübeck) started the session on viral diseases. He reviewed the work of his lab on proteases of RNA viruses, like SARS coronavirus, human coronavirus 229E, coxsackievirus B3 and highlighted recent structural data on the falcipain-2 (FP-2) from *P. falciparum* . Implications for the structure based design of active site directed and allosteric inhibitors for these cysteine proteases were discussed[17].

Young Do Kwon from Peter Kwong's group (NIH Bethesda) shifted the focus to HIV-1. One of the main obstacles for successful vaccination against human immunodeficienty virus type 1 (HIV-1) is its ability to evade the humoral immune defense by glycosylation and conformational masking. The conserved binding site for the human receptor CD4 on the HIV-1 gp120 envelope glycoprotein, however, is accessible and therefore in principle a prime site for attack. Quite unexpectedly, however, most CD4-binding site-directed antibodies are not able to neutralize primary HIV-1 isolates. To explore this mechanism of viral evasion Kwon investigated the structure of the complex of gp120 with the non-neutralizing antibody F105 and compared this structure to that of gp120 with the potently neutralizing antibody b12[18] (Figure 2). The analysis revealed that gp120 uncovers an immunofascile hydrophobic surface to which F105 binds. The novel F105-induced conformation of gp120 is poorly compatible with quaternary interactions in the viral spike. In this manner the shape-shifting gp120 is able to decoy the immune system into making non-neutralizing antibodies, which it easily evades. Dennis Bamford (University of Helsinki) explored the architectural principles of capsids belonging to viruses that infect a variety of different hosts from bacteria to humans to suggest a polyphyletic origin of viruses, i.e. that early cells were infected with many different viruses and that only a very limited number of folds have been selected to assemble viable virus coats[19]. Eloise Mastrangelo (University of Milano) from Martino Bolgnesi's group presented a structure-function study on the NS3 protease/helicase from Kunjin virus, a flavivirus affecting livestock and man. NS3 displays increased RNA-helicase activity, compared to its isolated helicase domain. Docking of the protease/helicase domains within a small angle X-

ray scattering (SAXS) derived envelope showed an elongated molecular assembly. Domain rearrangements at the protease/helicase interface, once RNA is bound, may explain the unwinding efficiency of NS3[20]. Winfried Weissenhorn (EMBL Grenoble) presented the three-dimensional structure of the rabies virus nucleoprotein/RNA-complex at 3.5 Å resolution. The complex consists of 99 nucleotides bound to 11 nucleoproteins arranged in an undecameric ring structure and revealing how non-segmented negative strand RNA viruses compact and protect their genome[21].

Various microbial and viral pathogens are efficiently cleared from the blood stream by the complement system which is an important constituent of the innate immunity. The complement protein C3 in its activated form (C3b), which has recently been a matter of hot debate in the protein crystallographic community, binds to the pathogens and tags them for phagocytosis by macrophages via the complement receptors (CRIg). Christian Wiesmann (Genentech, San Francisco) presented the structure of the complex between C3b and CRIg which sheds light on the dramatic structural rearrangements that take place during complement activation[22]. Wiesmann and colleagues furthermore combined structural and function data to demonstrate that CRIg inhbits alternative pathway convertases, thereby providing interesting hints for the development of therapeutics targeting the complement system, which is involved in various human diseases.

The session on protein misfolding diseases was started out by Roland Riek, a Swiss NMR-expert (Salk Institute, La Jolla), who very recently returned back to the ETH (Zurich). Riek combined solid-state NMR-methods, electron microscopy (EM) and H/D exchange methods to identify fibrillogenic sequences in various proteins, including the amyloid peptide Aβ(1-42), implicated in Alzheimer's disease. He demonstrated that the Aβ(1-42) fibril consists of two layers of parallel β-sheets and that multiple fibrils are twisted around each other in the amyloid plaque. Riek furthermore investigated structure toxicity relationships of Aβ(1-42) mutants, indicating that the toxicity of Aβ(1-42) relates to the morphology of the aggregate formed. These data may represent important progress towards identification of the disease relevant steps and intervention options early in the amyloid formation process[23 24]. In the discussion Riek suggested to consider amyloid fibrils not only as toxic species but also as macromolecular assemblies that may be used in nanotechnology as a deposit form for the triggered release of bioactive peptides.

Marcus Fändrich (Leibniz Institute, Jena) reported on the three-dimensional EM reconstruction of an Aβ(1-40) amyloid fibril obtained in collaboration with Niko Grigorieff (Brandeis University, Waltham). Already at 26 Å resolution, the presented EM-map deviates significantly from previous models of Aβ(1-40) fibrils and shows

an entirely different fibril cross section. Based on fibril classification results, however, Fändrich also demonstrated that Aβ(1-40) fibrils can be vastly heterogeneous and that different fibril structures can occur. This heterogeneity is of importance when considering the biological or structural properties of a given amyloid sample. Luigi Vitagliano (IBB, CNR, Naples) presented insights on fibril models from molecular dynamics calculations. Starting from the crystal structure of hexa- or heptapeptides from Eisenberg's lab (see below), it was convincingly demonstrated that all models acquire a twist after having gone through some simulation[25].

It was also shown that the minimally stable oligomer was a pentamer of hexapeptides, where one of the peptides was significantly shielded by the other four. Christian Betzel (University of Hamburg) presented work on aggregated prion proteins. They developed a cell-free assay that converts cellular PrP into an isoform similar to PrPSc by mimicking oxidative stress, which leads to Fenton-like oxidations. Two initial oligomers were obtained on the pathway of aggregation. One of them consisting of about 25 PrP molecules could be isolated and studied by small angle X-ray scattering (SAXS). This data provides structural insights into the mechanism of PrP oligomerization, which is important for the design of effective therapeutic strategies[26]. The session was concluded by a superb presentation by David Eisenberg (UCLA). He started with a brief historical overview of early fibril models that were derived from cross-β-diffraction images, before moving to recent work on amyloidogenic peptides. The first 40 amino acids of the yeast protein Sup35 are the amyloid part of the protein and it could be shown that peptides consisting of as few as four amino acids (e.g. NNQQ) are able to form fibrils[27]. Another fibril forming peptide is the heptapeptide GNNQQNY[28]. Crystals of GNNQQNY form almost immediately, but they are tiny in two dimensions, posing a great challenge for the collection of decent X-ray data. Since it could be observed that real fibrils form at the tip of the crystals, it can be speculated that the straight β-structure observed inside the crystals presents a very good model for the cross-β-structure found in fibrils. Based on the about 30 crystal structures of amyloid peptides, which have over the past few years been determined in the Eisenberg lab[29], computational approaches were developed, which are able to predict the amyloid propensity of peptide stretches in proteins. Using this method, LVEALYL was proposed as a potentially fibrillogenic sequence in human insulin. Interestingly in natively folded insulin, LVEALYL is part of an α-helix.

In summary, this session gave a very good overview on the achievements in this field that have been made over just the past few years. Even though it might be long ways until a drug against the disease discovered by and named after Aloys Alzheimer[30] will be developed and be available for patients, it is truly remarkable how

much nowadays is known of a process which a few years ago was the subject of wild speculations. The work of Eisenberg, Riek and many others have revealed the structural principles of fibril formation and recent studies are now starting to reveal structure fibril formation and structure toxicity relationships which may provide a basis for the design of agents that inhibit critical steps in the early stages of the fibril assembly.

Alan Fersht (University of Cambridge) opened the session on cancer and reported on the tumor suppressor protein p53. In about half of all human cancers, this protein is inactivated by mutations, many of which simply lower the thermal stability of the core domain below body temperature. Fersht's group has applied biophysical and biostructural studies to set a basis for the design of compounds that would rescue the function of p53 mutants as a potential cancer therapeutic strategy. He demonstrated how the combination of SAXS, electron microscopy, and NMR studies, together with previous crystallographic results, resulted in the complete architecture of the p53 tetramer, which includes large intrinsically unstructured regions. A stabilized mutant was engineered and high resolution crystal structures of oncogenic mutants were solved based on this framework, indicating that several cancer mutations cause surface cavities which appear as appropriate targets for drug design geared towards the a chemical rescue of p53 function[31]. Underscoring this approach, Fersht also showed how a short stabilizing peptide was able to rescue the function of a p53 variant that was inactive due to decreased stability[32]. Holger Rehmann (University Medical Centre Utrecht) reported on the structural basis of the regulation of the guanine nucleotide exchange factor (GEF) Epac by cyclic AMP (cAMP). Rehmann compared the crystal structures of cAMP free Epac and the trimeric complex of Epac, cAMP and the small G-protein Rap, which revealed a dramatic conformational change in a hairpin-like “lid” region of the protein that closes over cAMP upon its binding[33]. The closure of the cAMP-binding site apparently discriminates cAMP against cGMP. The function of this molecular switch is to activate Rap, which in turn regulates several downstream events. The structure of the Epac•cAMP•Rap complex showed how these proteins associated after cAMP binding. Alfred Wittinghofer (Max-Planck-Institute Dortmund) demonstrated efforts to use their detailed understanding of the GTPase reaction of Ras to devise potential strategies for cancer therapy. Ras mutations that decrease or abolish Ras function are found in about a quarter of all human cancers, and thus restoring Ras activity is expected to be a valuable therapeutic approach. So far the search for Ras activating small molecules has not been successful. In the crystal structure of the Rap-RapGAP complex, a novel “Asn thumb” in the GAP protein approaches the GTPase active site in Rap, and provides a catalytic Asn side chain in *trans*, instead of the normal Gln in *cis*.

Wittinghofer mentioned that following this recognition principle they now found a small molecule of undisclosed structure that could induce activity of an oncogenic inactive Ras variant *in vitro*[34]. Guillermo Montoya (Spanish National Cancer Center Madrid) reported on the molecular basis of substrate recognition by polo-like kinase 1 (Plk1) and its implications for centrosomal localization of this enzyme[35]. Claus Kuhn (Gene Center Munich) reported on the functional architecture of yeast RNA polymerase I, which could be unraveled by combining cryo electron-microscopy, X-ray crystallography and homology modeling. Regulation of RNA polymerase I is the end point of several growth signals for eukaryotic cells, exemplifying the importance of the enzyme carrying out synthesis of ribosomal RNA, which is the first step in ribosome biogenesis. Kuhn showed how biochemical and genetic experiments are nicely complementing the structural data, and how RNA polymerase I deviates in structure and function from RNA polymerase II [36].

Finally, Titia Sixma (Netherlands Cancer Institute Amsterdam) reported on the modulation of the enzymatic activity of E2 and E3 enzymes in ubiquitin conjugation by another type of protein modification, the so-called SUMOylation. The importance of these signalling pathways makes them prime drug targets. In close collaboration with Andrea Pichler (Vienna) and Frauke Melchior (Göttingen) Sixma demonstrated how SUMOylation can influence the ubiquitination pathway. In the E2 enzyme, a lysine residue gets SUMOylated, although it lies outside three consensus SUMOylation motifs. The crystal structure of SUMOylated E2 showed that the E2 is unchanged by sumoylation, and its effect on enzymatic activity is by modulating binding. Sixma further pointed out that recognition of the SUMOylation site depends on the surrounding structure of the site. Finally, she showed that SUMO can bind on different sites on Ubc9, dependenting on whether a covalent thioester-linked complex, a modified lysine or a non-covalent complex, forms[37].

The final session started with structures of extracellular receptors critical for the understanding of metabolic control and cardiometabolic disease. Michael Lawrence (Walter and Eliza Hall Institute Parkville, Australia) presented the recent 3.8 Å structure of the insulin receptor ectodomain that was crystallized in complex with four Fabs and in the presence of an insulin mimetic peptide[38 39]. Within this complex, the receptor assumes an antiparallel conformation, in which a bound insulin could crosslink the L1 domain of one receptor molecule with the fibronectin-III-1 domain of the second receptor molecule, thereby providing the first view of the spatial arrangement of low and high affinity insulin binding sites.

As a second long expected highlight Günter Fritz (University of Konstanz) presented the yet unpublished structure of the ligand-binding domain of the receptor of advanced glycation endproducts RAGE. RAGE is a multiligand receptor binding advanced glycation endproducts, S100 proteins, HMGB1, and amyloid-beta.

Activation of RAGE is key to a number of chronic diseases such as diabetes, inflammation, artheriosclerosis and neurodegeneration. The receptor is currently discussed as a new therapeutic target[40] [41]. Armin Ruf (Hoffmann-La Roche Basel) provided a view on the structure guided design of two novel classes of PPARα/γ dual agonists whose profile appears well suited for addressing both hyperglycemia as well as the enhanced cardiovascular risk of diabetic patients[42].

Annalisa Pastore (MRC London) shifted the focus of the session to rare diseases and presented her recent findings on the anomalous expansion of polyglutamine sequences as basis for neurodegenerative misfolding diseases[43]. Antti Haapalainen (University of Oulu, Finland) presented structural and enzymological studies of the human mitochondrial acetoacteyl-CoA thiolase T2, an enzyme for which loss-of-function mutations result in severe health problems due to ketoacidosis[44]. Markus Wahl (Max-Planck-Institute Göttingen) combined structural and functional studies in search of a molecular basis for RP13, a severe form of retinitis pigmentosa, which is an inherited disease leading to a progressive retina degeneration and blindness. The C-terminal fragment of the spliceosomal factor Prp8 carries several mutations linked to RP13. Combination of structural and biochemical data supports a model where (i) this fragment of Prp8 serves as a protein-protein interaction scaffold for the assembly of spliceosomal factors such as DEAD box protein Brr2 and GTPase Snu114 and where (ii) viable PR13 related mutations weaken but do not abolish these interactions[45]. While this model deepens the understanding of the molecular mechanism and the tissue specificity of the disease, it is not obvious at present how to translate the available data into intervention strategies for the disease. Ryota Kuroki (Japan Atomic Energy Agency, Ibaraki, Japan) presented recent structural studies on the 2:2 complex of human Granulocyte Colony-Stimulating Factor (GCSF), an important cytokine for the treatment of patients suffering from granulopenia with it’s receptor GCSF-R. While various stochiometries had been discussed for this assembly Kuroki's work undscores the relevance of the 2:2 complex in line with thermodynamic and mutational analyses[46].

Roger Williams (MRC Cambrige) closed the session with a comprehensive overview on recent structural results which continue to shape our understanding of the cellular ESCRT machinery[47]. The ESCRT complex mediates the trafficking of mono-ubiquitinylated proteins to lysosomes via the multivesicular budding (MVB) pathway. This principal cellular process is linked to cell surface receptor downregulation via the lysosomal degradation of membrane proteins. As an exit pathway of the budding HIV virus and other retroviruses, this pathway is furthermore critically involved in the lifecyle of such pathogens. Williams demonstrated that a molecular understanding has been developed for many individual players in the MVB pathway, for their

bimolecular assemblies and for their reaction to the sorting signal ubiquitin. Major challenges remain in understanding the higher level molecular organization of the ESCRT lattice together with a molecular mechanism for vesicle budding. Solution of these challenges will require the application of hybrid methods to close the resolution gap between X-ray structures and current EM reconstructions.

Studies of HIV budding indicate that some elements of the ESCRT pathway are required for viral budding. By understanding the higher-order assembly of ESCRTs on endosomes versus the plasma membrane, treatment options can focus on HIV-mediated assemblies at the plasma membrane.

In summary, the 2007 Murnau conference featured 31 presentations and 66 posters on many molecular aspects underlying human disease. Several of the presentations demonstrate how an advanced biomolecular understanding of disease relevant factors opens new strategies for the design of (i) interfering small molecules and (ii) of screening procedure in search of such compounds. In other cases, the complexity of multiprotein complexes and supramolecular assemblies that are now accessible for biostructural methods appears to overwhelm our current ability to translate the emerging higher order biomolecular view into concrete options for therapeutic intervention. As demonstrated by Hol, Waksman, Fersht, Wittinghofer, Riek and many others this step can only come in reach when biostructural research is tightly integrated with biophysical, biochemical and cellular studies. This strategy (paradigm) of integrating functional and structural studies of ever more complex cellular processes in search of new therapeutic entry points has been reflected in many presentations and discussions at Murnau. For the upcoming meeting in 2010 it may be of interest to focus on the the interface of structural and chemical biology where biostructural are utilized to reveal the action mechanism of novel bioactive compounds as an entry point for improved intervention strategies[48]

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

References:

1. Morimoto, H., Lehmann, H., Perutz, M.F. *Nature* **232**, 408-413 (1971).

2. Kuhn P., Wilson, K., Patch, M.G. & Stevens R.C. The genesis of high-throughput structure-based drug discovery using protein crystallography *Curr. Opin. Chem. Biol.* **6**, 704-710 (2002).

3. Abendroth, J., Murphy, P., Sandkvist, M., Bagdasarian, M. & Hol, W. G. The X-ray structure of the type II secretion system complex formed by the N-terminal domain of EpsE and the cytoplasmic domain of EpsL of Vibrio cholerae *J. Mol. Biol.* **348**, 845-855 (2005).

4. O'Neal C.J., Jobling, M.G., Holmes, R.K. & Hol, W.G. Biological and biochemical characterization of variant A subunits of cholera toxin constructed by site-directed mutagenesis *Science* **309**, 1093-1096 (2005).

5. Bosch, J. *et al*. Using fragment cocktail crystallography to assist inhibitor design of Trypanosoma brucei nucleoside 2-deoxyribosyltransferase *J. Med. Chem.* **49**, 5939-5946 (2006).

6. Sauer, F. *et al.* Structural basis of chaperone function and pilus biogenesis *Science* **285**, 1058-1061 (1999).

7. Remaut, H. *et al.* Donor-strand exchange in chaperone-assisted pilus assembly proceeds through a concerted beta-strand displacement mechanism *Mol. Cell* **22**, 831-842 (2006).

8. Verger, D. *et al.* Molecular mechanism of P pilus termination in uropathogenic E. coli. *EMBO reports* **7**, 1228-32 (2006).

9. Pinkner, J.S. *et al.* New class of rationally designed small compounds inhibit pilus biogenesis in uropathogenic bacteria *Proc. Natl. Acad. Sci. USA* **103**, 17897-902 (2006).

10. Deane, J.E. *et al*. Molecular model of a type III secretion system needle: Implications for host-cell sensing *Proc. Natl. Acad. Sci. USA*. **103**, 12529-33 (2006).

11. Johnson, S. *et al.* Self-chaperoning of the type III secretion system needle tip proteins IpaD and BipD *J. Biol. Chem*. **282**, 4035-4044 (2007).

12. Prehna, G., Ivanov, M.I., Bliska, J.B. & Stebbins, C.E. virulence depends on mimicry of host Rho-family nucleotide dissociation inhibitors *Cell* **126**, 869-880 (2006).

13. MacDonald, D., Demarre, G., Bouvier, M., Mazel, D. & Gopaul, D.N. Structural basis for broad DNA-specificity in integron recombination *Nature* **440**, 1157-1162 (2006).

14. Lundqvist, T. *et al.* Exploitation of structural and regulatory diversity in glutamate racemases *Nature* **447**, 817-822 (2007).

15. Niemann, H.H. *et al*. Structure of the human receptor tyrosine kinase met in complex with the Listeria invasion protein InlB *Cell* **130,** 235-246 (2007).

16. Blumenschein, T.M.A. *et al.* Atomic resolution insight into host cell recognition by Toxoplasma gondii *EMBO J*. **26**, 2808-2820 (2007).

17. Hogg, T. *et al*. Structural and functional characterization of falcipain-2, a hemoglobinase from the malarial parasite Plasmodium falciparum *J. Biol. Chem*. **281**, 25425-25437 (2006).

18. Zhou, T. *et al.* Structural definition of a conserved neutralization epitope on HIV-1 gp120 *Nature* **445**, 732-737 (2007).

19. Bamford, D.H., Grimes, J.M. & Stuart, D.I. What does structure tell us about virus evolution? *Curr. Opin. Struct. Biol.* **15**, 655-663 (2005).

20. Mastrangelo, E. *et al.* Crystal structure and activity of Kunjin virus NS3 helicase, protease and helicase domains assembly in the full length NS3 protein *J. Mol. Biol.* **372**, 444-455 (2007).

21. Albertini, A. *et al*. Crystal structure of the rabies virus nucleoprotein reveals complete RNA sequestering *Science* **313**, 306-303 (2006).

22. Wiesmann, C. *et al.* Structure of C3b in complex with CRIg gives insights into regulation of complement activation *Nature* **444**, 217-220 (2006).

23. Ritter, C. *et al.* Correlation of structural elements and infectivity of the HET-s prion *Nature* **435**, 844-848 (2005).

24. Lührs, T. *et al. Proc. Natl. Acad. Sci. U.S.A.* **102**, 17342–17347 (2005).

25. Esposito, L., Pedone, C. & Vitagliano, L. Molecular dynamics analyses of cross â-spine steric zipper models: â-sheet twisting and aggregation *Proc. Natl. Acad. Sci. U S A*. **103**, 11533-11538 (2006).

26. Redecke. L. *et al.* Structural characterization of β-sheeted oligomers formed on the pathway of oxidative prion protein aggregation in vitro. *J. Struct. Biol.*, **157** 308-320 (2007).

27. Balbirnie, M., Grothe, R. & Eisenberg, D. An amyloid-forming peptide from the yeast prion Sup35 reveals a dehydrated β-sheet structure for amyloid *Proc. Natl. Acad. Sci. U.S.A.* **98**, 2375–2380 (2001).

28. Nelson, R. *et al.* Structure of the cross-beta spine of amyloid-like fibrils *Nature* **435**, 773–778 (2005).

29. Sawaya, M. R. *et al*. Atomic structures of amyloid cross-β spines reveal varied steric zippers *Nature* **447**, 453-457 (2007).

30. Alzheimer, A. Über eine eigenartige Erkrankung der Hirnrinde. Vortrag in der Versammlung Südwestdeutscher Irrenärzte in Tübingen am 3. November 1906. Referiert als Eigenbericht in *Allgemeine Zeitschrift für Psychiatrie und psychisch-gerichtliche Medizin* **64,** 146 (1907).

31. Joerger, A.C. & Fersht, A.R. Structure-function-rescue: the diverse nature of common p53 cancer mutants *Oncogene* **26**, 2226-2242 (2007).

32. Tidow, H. *et al*. Quaternary structures of tumor suppressor p53 and a specific p53 DNA complex *Proc. Natl. Acad. Sci. USA*. **104**, 12324-12329 (2007).

33. Rehmann, H., Das, J., Knipscheer, P., Wittinghofer, A. & Bos, J. L. Structure of the cyclic-AMP-responsive exchange factor Epac2 in its auto-inhibited state *Nature* **439**, 625-628 (2006).

34. Bos, J.L., Rehmann, H. & Wittinghofer, A. GEFs and GAPs: critical elements in the control of small G proteins *Cell* **129,** 865-877 (2007).

35. Garcia-Alvarez, B., de Carcer, G., Ibanez, S., Bragado-Nilsson, E. & Montoya, G., Molecular and structural basis of polo-like kinase 1 substrate recognition: Implications in centrosomal localization. *Proc. Natl. Acad. Sci, USA* **104**, 3107-3112 (2007).

36. Kuhn, C. *et al.* Functional Architecture of RNA Polymerase I *Cell (in press).*

37. Knipscheer, P., van Dijk, W.J., Olsen, J.V., Mann, M. & Sixma, T.K. Noncovalent interaction between Ubc9 and SUMO promotes SUMO chain formation. *EMBO J*. **26,** 2797-2807 (2007).

38. McKern, N.M *et al.* Structure of the insulin receptor ectodomain reveals a folded-over conformation. *Nature* **443**, 218-221 (2006).

39. Ward, C.W., Lawrence, M.C., Streltsov, V.A., Adams, T.E. & McKern, N.M. The insulin and EGF receptor structures: new insights into ligand-induced receptor activation *Trends in Biochem. Sci*, **32**, 129-137 (2007).

40. Bierhaus, A., Stern, D.M. & Nawroth, R. RAGE in inflammation: a new therapeutic target? *Curr. Opin. Investig. Drugs* **11**, 985-991 (2006).

41. Ostendorp, T. *et al.* Structural and functional insights into RAGE activation by multimeric S100B *EMBO J.* **26**,3868-78 (2006).

42. Kuhn, B. *et al.* Structure-based design of indole propionic acids as novel PPARα/γ co-agonists *Bioorganic & Medicinal Chemistry Letters* **16,** 4016–4020 (2006).

43. Nicastro, G., Menon, R., Masino, L., McDonalds, N.O. & Pastore, A. The solution structure of the Josephin domain of ataxin-3: Structural determinants for molecular recognition *Proc. Natl. Acad. Sci. USA* **102**, 10493-10498 (2005).

44. Haapalainen, A.M.*et al.* Crystallographic and kinetic studies of human mitochondrial acetoacetyl-CoA thiolase (T2): the importance of potassium and chloride ions for its structure and function *Biochemistry* **46,** 4305-4321 (2007).

45. Pena, V., Liu, S., Bujnicki, J., Lührmann, R. & Wahl, M. C. Structure of a Multipartite Protein-Protein Interaction Domain in Splicing Factor Prp8 and its Link to *Retinitis Pigmentosa Mol. Cell* **25**, 615-624 (2007).

46. Tamada, T. *et al.* Homodimeric cross-over structure of the human GCSF-receptor signaling complex *Proc*. *Natl. Acad. Sci. USA* **103**, 3135-3140 (2006).

47. Obita T, *et al.* Structural basis for selective recognition of ESCRT-III by the AAA ATPase Vps4. *Nature* **449**, 735-739 (2007).

48. Schreiber, S.L. Small Molecules: The missing link in the central dogma *Nat. Chem. Biol.* **1**, 64-66 (2005).

Figure legends:

Figure 1: Murnau group photograph

Figure 2: The site on HIV-1 gp120 (grey) of interaction with the CD4 receptor (yellow) represents a conserved accessible surface on HIV-1. Indeed, many commonly elicited antibodies, called CD4-binding-site antibodies, compete with CD4 for binding to gp120. However, most of these are only weakly neutralizing, and relatively inpotent against primary isolates of HIV-1. One exception is the b12 antibody, a rare broadly neutralizing antibody. While all of the CD4-binding site ligands appear to have an extended loop tipped by a hydrophobic residue (red), it appears that b12 recognizes gp120 in a slightly different way than CD4. Structures of gp120 with non-potent CD4-binding-site antibodies should reveal how HIV-1 manages to avoid neutralization by these and also how an appropriate immunogen might be designed to elicit more b12-like antibodies.