and KKKIVIGSKKK, obtained via Discrete Molecular Dynamics, show that they assemble to form organized associations. For each of these peptides, sixteen peptides are randomly positioned within a cubic simulation box, leading to several initial configurations in which peptides are spatially separated in nonnumeric, random, coil-like forms. For each system, eight starting configurations are used in the production runs. The figure shows the assembly of each, with KKKFLIVIKK exhibiting a flat planar structure, KKKIGSIIKKK a stacked β hairpin, and KKKI VIGSKKK being amorphous. Molecular dynamics simulations are performed to understand the dynamics and functionality of beta-sheet adhesives, using CHARMM and NAMD. A detailed analysis of the results is presented.

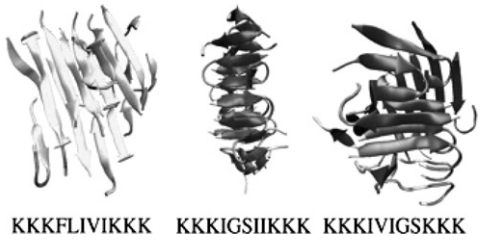

Figure 1: Assembly of Kh9K achieved using DMD

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Peptide Nanocapsules As Novel Immunogens:Design And Biophysical Analysis Of A Prototype SARS Vaccine

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Severe Acute Respiratory Syndrome (SARS) is an infectious disease caused by a novel coronavirus that cost nearly 800 lives. While there have been no recent outbreaks of the disease, the threat remains as SARS coronavirus (SARS-CoV) like strains are still existing in animal reservoirs. Therefore, the development of a vaccine is in grave need. We have designed and produced a prototype SARS vaccine: a self-assembling polypeptide nanocapsule that repetitively displays a SARS B-cell epitope from the C-terminal heptad repeat of the virus' spike protein. The peptide forming the nanocapsule consists of the pentameric coiled-coil domain of COMP at the N-terminus joined by a short linker segment to a *de novo* designed trimeric coiled-coil domain at the C-terminus. The SARS epitope is ideally suited to extend this trimeric coiled-coil as it is itself a trimeric coiled-coil. Circular dichroism of the refolded nanocapsules revealed a highly α-helical structure. Proper self-assembly of the peptide into nanocapsules was verified by TEM and DLS, both showing nanocapsules in the 25nm to 30nm size range. The number of peptide chains per nanocapsule was then determined by analytical ultracentrifugation and the average was 110 peptide chains per nanocapsule. Immunization experiments with these SARS-nanocapsules were performed with Balb/c mice. An investigation of the binding properties of the elicited antibodies showed that they were highly conformation specific for the coiled-coil epitope since they specifically recognized the native trimeric conformation of C-terminal heptad repeat region. The antisera also exhibited neutralization activity in an *in vitro* infection inhibition assay. We conclude that these peptide nanocapsules represent a promising platform for vaccine design, in particular for diseases that are characterized by neutralizing epitopes with coiled-coil conformation such as SARS-CoV or other enveloped viruses.

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Peptide Nanocapsules and Their Conjugation with Inorganic Nanoparticles

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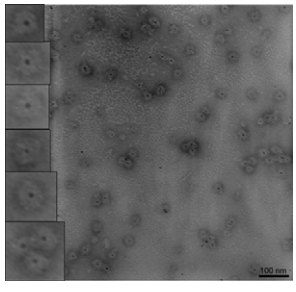
Inorganic nanoparticles such as colloidal gold, quantum dots or superparamagnetic iron oxide nanoparticles have unique optical and magnetic properties for a wide variety of biomedical applications. Here we present the design and biophysical analysis of a novel type of self-assembling polypeptide nanocapsule (SAPN), which can be used to encapsulate such inorganic nanoparticles. The peptide chain is composed of the pentameric coiled-coil domain at the N-terminus and a trimeric coiled-coil domain at the C-terminus. At either end a functional peptide sequence can be attached to provide useful biological functions for cell targeting or cell penetration. The SAPN are formed in a self-assembly process of the coiled-coil oligomerization domains. The central cavity of the SAPN can be modified with positively charged residues making it ideally suited for encapsulation of inorganic nanoparticles which are coated with negatively charged ligands. We have successfully encapsulated negatively charged gold nanoparticles and quantum dots into the SAPN. Such peptide-inorganic hybrid nanocomposites combine the optical properties of the inorganic nanoparticles and the biological functionality of the SAPN and hence may be useful for cell targeting and imaging applications.



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Properties of Glycan-Rich Pericellular Coats - A Study on a Well-Defined Model System

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The plasma membrane is commonly considered the boundary of the living cell, although peripheral polysaccharides and glycoproteins often self-organize into an additional coating layer on the cell surface. Chondrocytes and oocytes, for example, build strongly hydrated coats that are rich in the polysaccharide hyaluronan, and that can reach several micrometers in thickness. These pericellular coats play a crucial role in the general protection of the cell, and act as a mediator in the communication with its environment. The highly hydrated nature of these coats, and the complex structure and dynamics of the living cell make them difficult to probe in their native environment or to determine the coat's structure with high resolution methods. Therefore, to understand *structure/function inter-relationships* of these coats it is vital to move from living cells to simplified model systems.

We have recently developed a new method to create *in vitro* model systems of the pericellular coat that is based on the end-grafting of hyaluronan to a supported lipid bilayer[1]. The model systems are well-controlled and capture characteristic properties of the pericellular coat, including its dimensions and hydration. With these models, the dynamics of coat reorganization and relevant physico-chemical properties can be investigated in a quantitative manner, and related to polymer physics theory.

Here, we present data on the characterization of the properties inherent to films of end-grafted hyaluronan, including its permeability to solutes, its response to hyaluronan-binding proteins and its mechanical properties. Ultimately, we expect to gain novel information about the relationship between the pericellular coat's composition, supramolecular structure and biological function.

(1) Richter, R.P. Hock, K.K. Burkhartsmeyer, J. Boehm, H. Bingen, P. Wang, G. Steinmetz, N.F. Evans, D.J. Spatz, J.P. *JACS* **2007**, *127*, 5306-5307.

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Conformational Change of ClpP from *Bacillus subtilis* Characterized by Electron Microscopic study

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The ATP-dependent chaperone/protease complex ClpXP is the important molecule for protein degradation in most bacteria or in mitochondria and chloroplast of eukaryotes. ClpXP consists of two different proteins; ClpP is a proteolytic component that has 14 identical subunits organized in two stacked heptameric rings and ClpX is a hexameric AAA-ATPase that binds, denatures, and translocates protein substrates. We have obtained the images of ClpP from *Bacillus subtilis* (BsP) and from *E. coli* ClpP (EcP) using electron microscopy and checked its ring sizes against two peptide substrates. The model of ClpP from BsP shows the similarity with the previously solved structures of ClpP from another species, especially with *E. coli* ClpP (EcP). Although the structural and sequential resemblance between *E. coli* and *B. subtilis* species is significantly high, ClpX from *E. coli* is not able to stimulate the proteolytic activity of BsP and ClpX from *B. subtilis* also is not able to stimulate that of EcP. We believe that the difference in this function is also shown in the EM images of ClpP from BsP and EcP with different substrates by internal structure changes.

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The Role of the Proline Rich Domain in the Structural Organization of Dynamin

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Dynamin is a mechanochemical enzyme involved in numerous membrane vesiculation events including endocytosis. During these processes, dynamin self assembles into small spirals at the necks of budding pits and facilitates membrane fission following GTP hydrolysis. Dynamin consists of five distinct domains: a N- terminal GTPase domain, a middle domain, a pleckstrin homology (PH) domain, a GTPase effector domain (GED), and a C-terminal proline-rich domain (PRD). To date, the structure of a PRD deletion mutant of human dynamin 1 (ΔPRD) has been solved using cryo-electron microscopy (cryo-EM) and 3D

reconstruction methods (Zhang and Hinshaw, 2001, Chen et al., 2004). Crystal structures of the GTPase and PH domains from various species have been fitted to the structure of the ΔPRD dynamin phospholipid tube in its constricted and non constricted states (Mears et al., 2007). The PRD interacts with the SH3 domains of several proteins involved in signaling pathways. We are using cryo-EM and a single particle approach to solve the structures of the full length protein-lipid tubes in the constricted and non-constricted states. In both states, we have observed a decrease in the number of subunits per turn compared to previous structures of ΔPRD dynamin tubes. This suggests that the presence of the PRD changes the arrangement of the dynamin domains around the phospholipid tube. Further evidence of a direct interaction between the GTPase domain and the PRD is provided by simultaneous immunogold labeling of the two terminal domains.

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[1] Zhang, P. & Hinshaw, J.E. Nat Cell Biol. 3, 922-926 (2001).
[2] Chen, Y.J., Zhang, P., Egelman, E.H. & Hinshaw, J.E. Nat Struct Mol Biol 11, 574-575 (2004).
[3] Mears, J.A., Ray, P. & Hinshaw, J.E. Structure 15, 1190-1202 (2007).

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Structural Basis For HIV-1 DNA Integration in the Human Genome

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Integration of the human immunodeficiency virus type 1 (HIV-1) cDNA into the human genome is catalyzed by the viral integrase protein that requires the lens epithelium-derived growth factor (LEDGF), a cellular transcriptional coactivator. In the presence of LEDGF, integrase forms a stable complex *in vitro* and importantly becomes soluble by contrast with integrase alone which aggregates and precipitates. Using cryo-electron microscopy (EM) and single-particle reconstruction, we obtained three-dimensional structures of the wild type full length integrase-LEDGF complex with and without DNA. The stoichiometry of the complex was found to be $(integrase)_4$-$(LEDGF)_2$ and existing atomic structures were unambiguous positioned in the EM map. *In vitro* functional assays reveal that LEDGF increases integrase activity likely in maintaining a stable and functional integrase structure. Upon DNA binding, IN undergoes large conformational changes. Cryo-EM structure underlines the path of viral and target DNA and a model for DNA integration in human DNA is proposed.

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Structural Studies of a Phycobilisome

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Phycobilisomes are protein complexes present in cyanobacteria and red algae; they are involved in light harvesting and conduction of light and the aim of this study has been to understand the high efficiency observed in these processes The structure of the phycobilisome from an eukaryotic algae *Gracilaria chilensis* was studied by biochemical methods in order to obtain intact phycobilisomes and to obtain their phycobiliprotein components, phycoerythrin, phycocyanin and allophycocyanin. The structure of phycobilisomes has been studies by electron microscopy and electrophoresis and by theoretical methods; the structure of phycobiliproteins has been studied by protein crystallography and because they are chromophorylated, their properties also were studied by absorption and emission spectroscopy. We have also built a theoretical docking model for an antenna formed by two units of phycoerythrin and two units of phycocyanin. This model was used to obtain the k_T for the transfer in resonance of the light; the pathway of the light was calculated through the antenna. An evaluation of the model was performed by comparison with the electron microscopy images. The effect the protein environment on the spectroscopic properties of the chromophoric groups was also considered and analysed.
FONDECYT 108.0267.

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Quantification of the Exchange of Subunits from Membrane Protein Complexes Using Foerster Transfer Recovery (FTR)

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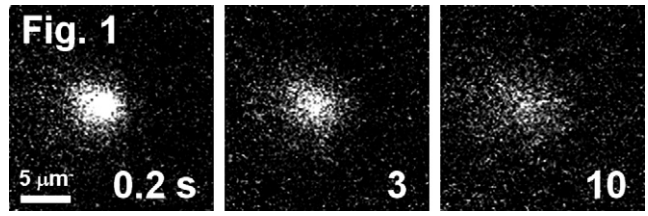
To quantify the exchange of subunits of membrane protein complexes cells expressing CFP/YFP-tagged phospholamban (PLB) were observed by total internal reflection fluorescence (TIRF) microscopy. We performed YFP-selective photobleaching of spots, lines, or larger regions of interest on the basal surface of the cells. This resulted in enhanced CFP fluorescence, indicating CFP-YFP fluorescence resonance energy transfer (FRET). Subsequent spatial broadening of this ''pseudo-photoactivated'' CFP fluorescence was analyzed as a measure of the lateral diffusion of PLB complexes away from the target region. In addition, exchange of bleached and unbleached YFP-PLB from complexes restored FRET over time. This process of Foerster transfer recovery (FTR) was taken to indicate the rate of exchange of fluorescently-labeled subunits of the membrane protein complex. Diffusion and exchange processes were quantified by image analysis using a custom MatLab application for 2-dimensional Gaussian fitting. In addition to its application to FTR, this approach may be useful for cytoplasmic proteins as a way of quantifying dynamic membrane recruitment and lateral diffusion on the plane of the bilayer. Fig. 1 shows the diffusion of acceptor-photobleached CFP/YFP-PLB complexes from a target region, followed by subunit exchange.



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Ligand Binding and Sickle Hemoglobin Polymerization Kinetics: Implication for Therapies

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Sickle Cell Disease results from a point mutation on the beta subgroups of hemoglobin. When hemoglobin releases its four ligands it changes from a relaxed (R) structure to a tense (T) structure and the mutation causes polymer chains to grow. Typical in vitro experiments measure this through complete photolysis of a COHbS sample with a laser and then quantify the scattered light from growing polymers. However, in vivo, many molecules are partially liganded due to the incomplete transfer of oxygen from red blood cells to the surrounding tissue. Liganded T state molecules could contribute to polymer growth, although until now the effect on the kinetics of fractional saturation was unknown. We examined the effects of introducing NO into COHbS samples. The strong binding of NO to HbS keeps its ligand distribution unchanged during the COHbS experiment. We found that the NOHbS caused the polymerization rate to decrease by 50% due to tertiary inhibition of the partially bound T state hemoglobin. We ruled out the possible effects of non-polymerizing R state NO Hb through a flash photolysis experiment, where photolysis curves were analyzed for an initial fast recombination of CO to R state Hb. Only an insignificant possible amount of R state was found ($<3\%$), and could not account for the effects recorded. The effect of partial ligation on polymerization is important in analyzing possible therapies for sickle cell disease. One possible therapy would be to alter the oxygen affinity of Hb, thereby decreasing the number of fractional intermediates and decreasing the number of T state HbS overall.

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Fiber Depolymerization: Fracture, Fragments, Vanishing Times and Stochastics in Sickle Hemoglobin

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Polymerization of sickle cell deoxyhemoglobin (HbS) into stiff fibers lies at the root pathology in sickle cell disease. It induces red cell rigidification, cell membrane damage with myriad pathophysiological consequences, and hemolysis and anemia. The well characterized polymerization kinetics bear intimate relation to pathogenesis, but the role of the less well characterized fiber depolymerization remains to be defined. Its rates may be important in at least 3 ways: i) they govern whether residual polymers fail to dissolve in the lungs and pass into the systemic circulation, facilitating repolymerization; ii) they may affect resolution of vaso-occlusion in sickle cell crises; iii) delayed dissolution might exacerbate cellular damage. Here we observe depolymerization experimentally and develop a theoretical model that encompasses fiber fracture, fragment formation, stochastics and the probabilistic distribution of fiber vanishing times. We use Monte Carlo simulations to show when dissolution is rapid and when slow. Experimentally, we demonstrate fracture in real time and show that dissolution of a fiber does not proceed uniformly in time and space and thus is stochastic. We derive an analytic equation for the distribution of