

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, KKKIGSIKKK a stacked β hairpin, and KKKIVIGSKKK 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

388-Pos Board B267

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.

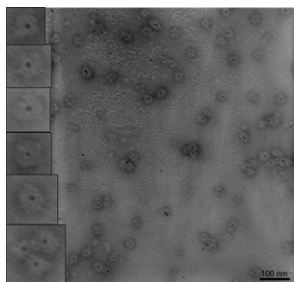
389-Pos Board B268

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 SAPNs 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.

390-Pos Board B269

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 bilayer¹. 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. Burkhartsmeier, J. Boehm, H. Bingen, P. Wang, G. Steinmetz, N.F. Evans, D.J. Spatz, J.P. *JACS* **2007**, *127*, 5306-5307.

391-Pos Board B270

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.

392-Pos Board B271

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 (Δ PRD) has been solved using cryo-electron microscopy (cryo-EM) and 3D