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When studying the binding of ligands to macromolecules immobilized at a surface, the question arises whether the surface binding sites are heterogeneous, as one might expect considering many factors such as intrinsic surface roughness, nonuniform density distribution of polymeric linkers and nonuniform chemical attachment producing different protein orientations and conformations. We previously developed a computational tool to determine the distribution of affinity and kinetic rate constants from the analysis of experimental surface binding data. In order to avoid an ill-posed computational analysis, the previous approach used a regularization strategy assuming a priori an equal probability for all binding constants, which results in the broadest possible distribution of all that are consistent with the data. In the present work, we implemented a Bayesian approach of regularization to incorporate the opposite assumption, i.e. that the surface sites a priori are expected to be uniform (as expected in free solution). The data analysis with this new approach results in the narrowest distribution given the experimental data. We applied this method to several protein systems immobilized on a carboxymethyl dextran surface and with protein interactions measured by surface plasmon resonance. The obtained distributions are highly reproducible. The results demonstrate microheterogeneity of the binding sites on the surface, in addition to broad populations of significantly altered affinity. The variation of immobilization conditions and the total surface density of immobilized sites indicates a considerable impact of these parameters on the distribution of the surface binding sites.

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Proteomic Analysis of KvLQT1 and HERG-associated Proteins

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Background: We have generated LQT1 and LQT2 rabbit models by overexpressing the Flag-tagged pore mutant KvLQT1-Y315S or HERG-G628S, in the heart of transgenic rabbits. We undertook a proteomics approach in order to identify KvLQT1 as well as HERG-interacting proteins and differences in protein expression in the hearts of these transgenic rabbits.
Methods: Triton X-100 solubilized heart membranes were subjected to immuno-precipitation using anti-FLAG antibody. The FLAG-immunoprecipitated KvLQT1 and HERG-bound complexes were resolved by SDS-PAGE and stained with Commassie blue. Upon resolution, 15 protein bands each corresponding to KvLQT1 and HERG-interacting proteins were obtained. Individual protein bands were destained, excised and digested with trypsin. The resulting peptide mixtures were analyzed by high pressure liquid chromatography coupled with electrospray ionization tandem mass spectrometry. The results from mass spectra were searched against the mouse, rat, and human genomic data base using the SEQUEST software. 2-D DIGE technique in conjunction with proteomic studies was employed to investigate the differences in protein expression in the hearts of transgenic rabbits.
Results: Our results revealed that both channels were precipitated. In addition, several unique potential KvLQTI and HERG-interacting proteins along with the previously known KvLQT1-interacting protein such as calmodulin, and HERG-interacting proteins, such as Hsc70 and Hsp90 were detected. Our results also showed that there were four protein bands with varying molecular weights which were common for KvLQT1 and HERG-bound complexes, suggesting potential common KvLQT1 and HERG-interacting proteins. Furthermore, the results of 2D-DIGE have revealed the upregulation of several proteins in KvLQT1 transgenic rabbit heart.
Conclusions: We have identified potential unique proteins that interact with either KvLQT1 or HERG, as well as the several potential proteins that interact with both of these channels. These interacting proteins might play an important role in regulating the trafficking and localization of these channels.

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Computer Simulation of Protein-Protein Association Processes

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Protein-protein interactions are key components of most biological processes. About half of all cellular proteins appear to be parts of larger stable protein complexes whereas transient, pairwise protein-proteins interactions are crucial parts of bioenergetic and signal transduction pathways. Here, we will concentrate on fast assembling protein pairs where complementary electrostatic interactions accelerate the association processes by several orders of magnitude. Brownian and molecular dynamics simulations will be used to identify the energetic principles for these binding phenomena. First, we have studied the association free energy landscape for the barnase:barstar complex by Brownian Dynamics simulations [1]. We will use this system to introduce the concept of diffusional protein protein association on conformational energy landscapes. Interestingly, we found that single protein mutations can drastically alter the shape of the energy landscape and the location of the encounter complex. Secondly, unbiased molecular dynamics simulations were used to study the binding process of a proline-rich peptide to an SH3 domain [2]. In this case, stable complexes were formed within 20 - 130 ns of simulation. Depending on the orientation of the first contacts made, the peptide adopted one of three experimentally known binding modes on the adaptor domain. Association was found to be governed by the synergistic interplay of two types of driving forces for binding. The long-range electrostatic effects play the main role during diffusion and stabilize the transient complexes formed by the electrostatic parts in the interface. At short distances, this then enables partial dewetting at the interfaces to increase the probability for the collapse of the hydrophobic part of the interface and the convergence to the final specific complex.
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At Clinically Relevant Concentration Isoflurane and Desflurane Induce Abeta Oligomerization. Molecular Details from NMR Spectroscopy

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Anesthetics could be a risk factor for Alzheimer disease (AD). Findings from other laboratories confirm that several commonly used inhaled anesthetics may cause brain damage that accelerates the onset of AD . Using nuclear magnetic resonance spectroscopy, we previously evidenced that at high concentration (higher than clinically relevant concentration), halothane and isoflurane interact with specific amino acid residues (G29, A30 and I31) and induce Aβ oligomerization. Our present study, performed at clinically relevant anesthetic concentration indicates that two popularly used inhaled anesthetics, isoflurane and desflurane, induce Aβ oligomerization by inducing chemical shift changes of the amino acid residues (G29, A30 and I31). Experimental data at clinically relevant concentration reinforce that perturbation of these three crucial residues indeed play important role for the induction of Aβ oligomerization. A working model for Aβ olifomerization due to isoflurane and desflurane is presented in Figure 1.
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Mandal PK, Pettegrew JW, Abeta peptide interactions with isoflurane, propofol, thiopental and combined thiopental with halothane: A NMR study. Biochim Biophys Acta. 2008, Jul 14.
Correspondence Dr. Pravat Mandal

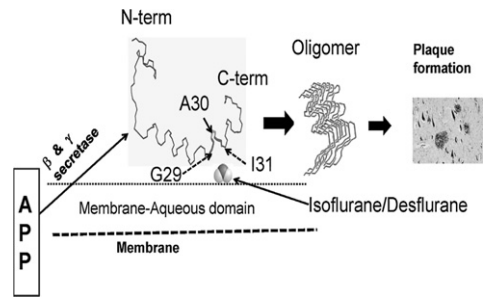

Figure 1 A plausible schematic diagram for the Aβ interactions with isoflurane and/or desflurane at a clinically relevant concentration that leads to oligomer formation. Aβ peptide is generated by the amyloid precursor protein (APP), by the action of β and γ secretase by the natural process, and the inhaled anesthetic interacts with three specific residues (G29, A30 and I31) and modulates Aβ oligomer formation, which are neurotoxic and produce plaque as seen in AD patients, on biopsy.

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Molecular Dynamics Simulations of a Single 11-Residue Beta-Sheet Adhesive and its Assembly

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Thirty synthetic peptides with varying degrees of adhesion strength toward wood strips are prepared and tested as glues by checking adhesive strength at different pHs and curing temperatures. Lysine-Lysine-Lysine repeats on both the N- and C-termini of the hydrophobic core give the highest adhesion strength. In the absence of water, the peptide retains a β-sheet structure. Experimental results show that these peptides form a nano fiber-like structure in the absence of water. Simulations of three of the adhesive sequences, KKKFLIVIKKK, KKKIGSIIKKK,

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.

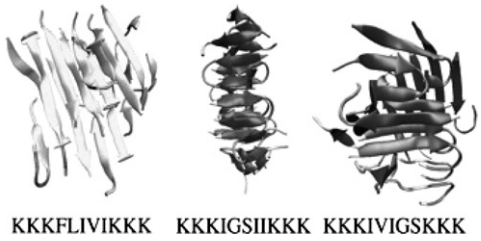

Figure 1: Assembly of Kh9K achieved using DMD

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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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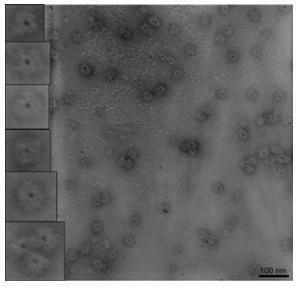
Inorganic nanoparticles such as colloidal gold, quantum dots or superparamagnetic iron oxide nanoparticles have unique optical and magnetic properties for a wide variety of biomedical applications. Here we present the design and biophysical analysis of a novel type of self-assembling polypeptide nanocapsule (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.

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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