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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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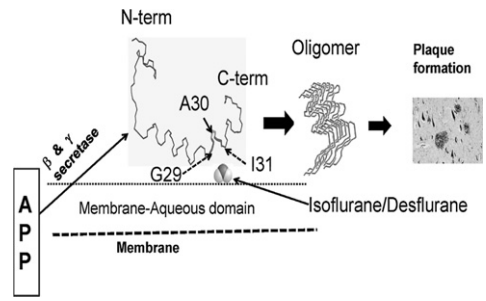

Figure 1 A plausible schematic diagram for the Aβ interactions with isoflurane and/or desflurane at a clinically relevant concentration that leads to oligomer formation. Aβ peptide is generated by the amyloid precursor protein (APP), by the action of β and γ secretase by the natural process, and the 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,