Membrane Receptors & Signal Transduction I

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Protein Flexibility Is One Type Of Biosignal
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According to protein model of biosignal, the protein flexibility is one type of biosignal. In view of protein thermodynamics structure theory, the protein flexibility can influence signal transduction activity by several ways. Firstly, it can modulate the rate of protein conformational change and then influence signal transduction activity (signal activity). Second, it can regulate protein conformational coupling and then regulate activity of other effectors (or signal transduction pathway). Third, it can influence the equilibrium between different protein conformational states and influence the initiation of signal transduction activity. Experimental test indicates that the urea shows anesthetics potency and thus the protein flexibility is related to action mechanism of general anesthetics. The protein flexibility has profound impact on all pathways of signal transduction.

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Membrane Proteins-bilayer Interplay: Insights From Coarse-grained Self-assembly And Potential Of Mean Force Simulations Of Rhodopsin In Model Bilayers
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Due to the transient nature of typical protein-protein interactions in biological membranes and other technical limitations, it is extremely difficult to devise robust experimental approaches to elucidate atomic details of complex membrane systems. By offering alternative and complementary information, theoretical and computational approaches have become extremely useful and valuable tools. Here we will present our recent development of new molecular models, which, while conserving the physicochemical properties of the different components in a system, reduce the complexity of the description of a system down to chemical entities (3 to 6 heavy atoms). These coarse-grained models allow simulating systems of size and for time scales relevant to their biological function. The results obtained from self-assembly simulations of visual pigment rhodopsin in model membranes [1] and potentials of mean force (PMF) of rhodopsins association will be presented. The data show that the membrane bilayer responds to the presence of a protein by inhomogeneous and localized deformations at the surface of the protein and that, whereas the amount of deformation (hydrophobic mismatch) strongly affects the propensity of the proteins to aggregate, the location of the deformation correlates with protein-protein interfaces. The PMFs further demonstrate that protein binding energies are strongly correlated to the interfaces involved and that the membrane bilayer has a significant contribution to the energy barriers encountered during association. This work brings new insights to our understanding of the forces driving protein self-organization in membrane bilayers. [1] X. Periole, T. Huber, S.-J. Marrink, and T.P. Sakmar. 2007. G Protein-Coupled Receptors Self-Assemble in Dynamics Simulations of Model Bilayers. J. Am. Chem. Soc. 129, 10126-10132.

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Docking of Insulin to its Receptor
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The human insulin receptor (IR) is a homodimeric membrane-spanning ligand-activated tyrosine kinase. Although a detailed theory describing how binding of the hormone insulin triggers kinase activity of IR remains elusive, the recently published crystal structure of the IR ectodomain offers the unique opportunity to use physical modeling to begin to construct this theory. We present here the results of ~100 ns of explicit-solvent all-atom molecular dynamics (MD) simulations of IR in both the apo and putative T and R-state insulin docked states. Our simulations confirm the large interdomain flexibility of IR and the stability of its dimeric interfaces. More importantly, our simulations demonstrate the evolution of large-scale asymmetry in IR relative to the crystal structure, a result that reflects the structural requirements of a "see-saw" mechanism that guarantees negative homeotropic allostery in insulin binding. This asymmetry also manifests itself in the opening of one of the two equivalent insulin binding pockets and closing of its partner. This result is significant because it allows for the first time computational docking of an intact molecule of insulin into its binding pocket on an intact IR ectodomain. We use a Monte-Carlo docking algorithm followed by MD equilibration to predict bound states of insulin on IR. These simulations allow us to identify unambiguously the residues on IR that form the "site-2" binding epitope which recognizes residues on insulin responsible for its hexamerization.

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Coupling Atomistic Simulation to a Continuum Based Model to Compute the Mechanical Properties of Focal Adhesions
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Mechanical Ventilation is causing inflammation in the lung either by rupture of tissue or by overstretching cells and starting signalling cascades. To prevent starting these, it is of great interest to connect ventilation parameters to stresses and strains in the lung on a cellular level.
In order to quantify forces transferred from the tissue to the cell, the mechanical properties of integrins play an important role. Large time and length scale differences between the cell and the integrin molecules make such computations difficult. Given the utility of continuum models based on Finite Element Method, it would be useful to couple the continuum methods to molecular dynamics techniques in order to compute forces between cells and tissue.
For coupling molecular information to the continuum level, we present a technique based on energy transition. Hereby the mechanical properties of on integrin bonds are computed with help of molecular dynamics. In order to model the complete focal adhesion, a spring recruitment model is included on the continuum side, representing more than one molecular bond. The approach includes dynamic effects from both scales. The focal adhesion is represented by a layer of finite elements with the intergin molecule bound to collagen. The deformation gradient of the element is scaled to the size of the protein. The actual gradient and the one from one time step earlier are transferred to the molecular scale. In the molecular dynamics simulations, the energy difference between both deformations is simulated first by slowly deforming and secondly equilibrating the protein. This information is transferred back to the continuum level and, under the assumption that focal adhesions remain connected, the number of bonds are calculated and modelled as multiple non-linear neo-Hookean material, representing parallel springs.

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In VSMCs, Beta-1 Integrin but not Syndecan-4 Gene Expression is Dependent on Matrix Stiffness
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Mechanotransduction is the process by which cells sense and convert mechanical stimuli into biochemical signals, and has been implicated in the development of certain pathologies. During atherosclerosis, the vessel wall experiences progressive stiffening, particularly at the site of developing plaques, and vascular smooth muscle cells are intimately involved in the development and maintenance of these plaques. In vitro, VSMCs have also been shown to be mechanosensing: cell spreading area, migration speed, and expression of characteristic cytoskeletal components is mediated by substrate stiffness. It is likely, then, that changes in arterial mechanics during atherosclerosis contribute to the pathological behavior of VSMCs, but our understanding of this process remains inadequate.
The beta-1 integrin subunit has been implicated in mechanosensing, but its uniqueness as a mechanotransducer has not been established. Syndecan-4 acts in synergy with the alpha5beta1 integrin, though its role in mechanotransduction is unknown.
We hypothesized that beta-1, but not syndecan-4, gene expression is regulated by substrate stiffness. We used polyacrylamide gels functionalized with fibronectin which mimic the mechanical properties of healthy (~30kPa) and diseased (~80kPa) arterial walls. Primary rat VSMCs were grown to 60% confluence on 18kPa, 35kPa, and 80kPa gels with uniform FN surface content, and collected for quantitative PCR analysis. Gene expression of beta-1 increased 1.5x with each increment in stiffness, while syndecan-4 levels were not affected. These results suggest that beta-1 integrin gene expression is sensitive to substrate mechanics, while syndecan-4 expression is not.
Because cell-cell contacts are also capable of mechanotransduction, we also looked at the effects of cell density on beta-1 integrin expression. Preliminary results suggest that increasing cell density eliminates the substrate-mediated increase in beta-1 expression, which suggests that VSMCs integrate mechanical stimuli from various sources to achieve physical homeostasis.

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Investigation of Signal Transduction through the HAMP Domain from Molecular Dynamics Simulations
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The HAMP domain connects the extracellular sensory with intracellular signaling domains in over 7,500 proteins including histidine kinases, adenylyl cyclases, chemotaxis receptors, and phosphatases, and thus plays an essential