conformations. Caspase-6 transitions exclusively to the canonical strand conformation only upon substrate binding. Glu-135, which showed noticeably different calculated pK_as in the helix and strand conformations, appears to play a key role in the interconversion between the helix and strand conformations. We have also mapped the local changes in the conformational flexibility of procaspase-6 at the discrete states that reflect series of cleavage events that ultimately lead to the fully active, substrate-bound state. The prodomain region was found to be intrinsically disordered, independent of the activation step of caspase-6; however, its complete removal resulted in the protection of the adjacent 26-32 region, suggesting a regulatory role. The molecular details of caspase-6 dynamics in solution provide a comprehensive scaffold for strategic design of therapeutics for neurodegenerative disorders. We have used this information to make the most potent caspase-6 inhibitor to date.

Platform: Membrane Receptors and Signal Transduction

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Rationalizing the Transport of Trojan Horse Compounds for Crossing the Outer Membrane of Gram- Bacteria

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One of the challenges of modern medicine is to find efficient antibiotics to counteract infections. The main concern is represented by Gram negative species, where antibiotics have to cross the outer membrane for reaching their targets. Among the possible strategy for resolving the permeation of antibiotics is the use of TonB dependent transporters, such as those expressed to capture iron from the environment. Existing siderophore molecules enriched by antiinfective properties (Trojan-Horse candidates) can be transported efficiently inside the cell. The high-resolution structures obtained recently using X-ray crystallography have open the way to a more detailed knowledge of transport mechanism. Thanks to the new structures, we have applied molecular simulations in combination with NMR spectroscopy to investigate at molecular level the formation of the molecule-ion complex in solution, the binding of the complex to the transporter and finally its diffusion along the interior of the transporter. In general the ion-siderophore complex in solution is the one recognized by the transporter, in a precise recognition pocket. Upon the recognition, the binding can provide a unique allosteric signal appearing to activate other regions of the transporters in order to control the self-transport of the ligand. This effect upon ligand binding promotes a novel idea that the internal diffusion does not require a large conformational change of the transporter, as suggested earlier. We obtained detailed molecular data to understand how to rationalize siderophores able to use transporters to cross the outer membrane. The three fundamental steps are: formation of the complex in solution, binding and recognition on the transporter, and internal diffusion. Only the first two steps seem to play a key role in the transport and both depend on subtle interactions ion-siderophore-transporter, modulated by selecting precise chemical groups.

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Atomistic Modeling of Neuro-cardiovascular Coupling Modulation

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Cardiac heart rhythm and vascular tone are tightly controlled by sympathetic and parasympathetic autonomic nervous systems and their dysregulation is associated with multiple cardiovascular disorders such as arrythmias and hypertension. At the molecular level such control is established through a complex set of events including synaptic neurotransmitter release, binding to myocyte adrenergic or cholinergic G-protein coupled receptors (GPCRs), subcellular signal transduction through G-proteins, neurotransmitter - GPCR unbinding, reuptake or degradation. In this study, funded by NIH Common Fund program "Stimulating Peripheral Activity to Relieve Conditions" (SPARC), we used atomistic modeling to identify structural, energetic and kinetic determinants of several molecular processes crucial for cardiovascular neuromodulation such as neurotransmitter interactions with GPCR / G-protein complex at neuroeffector junction as well as G-protein interaction with adenylyl cyclase (AC), a key enzyme for downstream subcellular signaling. To this end, we developed atomistic models of human β-adrenergic receptor (βAR) / G_s and muscarinic receptor (MR) / G_i GPCR / G-protein complexes in different conformational states using available structures and Rosetta structural modeling. We tested these models via molecular docking of βAR agonists norepinephrine and isoproterenol and MR agonists acetylcholine and carbachol. Moreover, receptor model structural stabilities were assessed via microsecond-long all-atom molecular dynamics (MD) simulations with and without ligand bound. Enhanced sampling atomistic MD runs were used to estimate receptor - ligand binding affinities and association / dissociation rates. Martini coarse-grained and Brownian dynamics implicit-solvent techniques were utilized to assess G-protein interactions with AC and GPCR as well. This information will be used to inform functional kinetic models of autonomic control of myocyte subcellular signaling, a crucial component of our predictive multi-scale neurocardiovascular simulator.

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Large Conductance Ca^{2+}-activated K^+ Channels Regulate LPS-induced Cytokine Secretion from Alveolar Epithelial and Endothelial Cells

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We previously established the role of stretch-activated TREK K^+ channels in inflammatory cytokine secretion form alveolar epithelial cells using in vitro and in vivo models of hyperoxia- and TNF-induced acute lung injury. Since the K^+ currents mediated by TREK channels are relatively small, we now investigated the role of large conductance Ca^{2+}-activated K^+ channels (BK channels) on inflammatory cytokine secretion in a lipopolysaccharide (LPS)-induced lung injury model. Our results show for the first time expression of BK channels in primary human alveolar epithelial cells (HPAEC) and in human pulmonary microvascular endothelial cells (HULEC) using semi-quantitative real-time PCR. We found that LPS induced a time- and dose-dependent increase in secretion of the proinflammatory cytokines IL-6 and CCL-2. Interestingly, inhibition of BK with Paxilline inhibited LPS-induced IL-6 secretion from epithelial and endothelial cells, whereas BK activation with NS1619 increased cytokine secretion. In contrast, LPS-induced CCL-2 secretion was increased after BK inhibition with Paxilline and decreased after BK activation with NS1619. Using fluometric assays, neither the resting membrane potential nor intracellular calcium concentrations were altered by LPS. Therefore, we report for the first time differential regulation of proinflammatory cytokine secretion from human alveolar epithelial and endothelial cells in a clinically relevant model of LPS-induced lung injury. We are currently investigating the mechanisms underlying BK regulation of cytokine secretion from these cells.

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Affinity and Stoichiometry of E-cadherin/EGFR Complexes-relevance to Proliferation and Force Transduction

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This study investigated the molecular mechanism of E-cadherin association with the epidermal growth factor receptor (EGFR) at cell surfaces. Cross talk between EGFR and the adhesion protein, epithelial E-cadherin is well known to regulate contact inhibited proliferation in epithelia. In addition, E-cadherin-mediated force transduction signaling involves EGF-dependent, EGFR activation. Despite biochemical evidence that E-cadherin and EGFR form hetero complexes, we do not know which protein domains mediate the association, the receptor binding affinity, or the complex stoichiometry. Moreover, the mechanism by which E-cadherin suppresses EGFR signaling is also unresolved. Here we used Full-Spectral-Imaging FRET (FSI-FRET) measurements to quantify the hetero complex stoichiometry and the binding affinity between E-cadherin and EGFR, at the plasma membranes of live cells. Domain deletions mapped the cadherin regions that mediate receptor binding. Co-immunoprecipitation studies qualitatively support the FSI-FRET results. FSI-FRET and co-IP measurements show that soluble EGF regulates the hetero complex formation. Additional measurements show that mechanically