calculated from kinetic data. The differences in thermodynamic parameters measured for the cyclic and reduced peptide explain epitope mapping data obtained by NMR.

1270-Pos

Creating Steroidal Ligand-Receptor Pairs for Behavioral Studies of Androgen Receptor

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Heterologous agonist-receptor pairs designed after high resolution structural studies have yielded insights into specificity of signaling by protein kinases and G-protein coupled receptors. We are extending this concept to the realm of steroid nuclear receptors with a goal to learning the behavioral effects of steroid activation of the androgen receptor in the brain. We are designing a steroidal ligand-hormone receptor pair such that the novel ligand does not bind to endogenous androgen receptors (AR), and the designer receptor does not bind to endogenous androgens. We have synthesized novel steroids that do not bind endogenous AR. Since steroid binding affects the folding of the receptor, prediction of amino acid mutations in the androgen receptor that are needed to accommodate the steroidal ligand is difficult. To combat this issue, we are using a genetic selection in S. cerevisiae to reveal gain of function activity from a library of about 10 ⊥ 9 randomly mutated AR ligand binding domains. In this experiment, yeast strains containing a hormone-inducible HIS3 gene select for full length AR mutants that are active in the presence of our novel steroids.

1271-Pos

Molecular Dynamics Study of 2-Arachidonoyl-Sn-Glycero-3-Phosphoinositol (2-AGPI) and Lysophosphatidylinositol (LPI) in a POPC Bilayer and their GPR55 Docking Sites

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GPR55 is a Class A G protein-coupled receptor that has been shown to be activated by cannabinoids (Henstridge et al. FASEB J, 2008). The receptor is expressed in several mammalian tissues including several brain regions. It has been reported that GPR55 is also activated by LPI found in rat brain (Oka et al. BBRC, 2007), with 2-AGPI having the highest activity (Oka et al. J Biochem, 2009). Since 2-AGPI and LPI are lipids and are shown to interact with the membrane-embedded GPR55 receptor, we undertook a study of the location and conformations they can adopt in a phospholipid bilayer, as well as, of their interaction modes with GPR55. To this end, 2-AGPI and LPI were added to a fully hydrated, pre-equilibrated POPC bilayer (28 waters/lipid; 72 lipid molecules with 36 in each leaflet) and their behavior in POPC was studied using the NAMD2 molecular dynamics software package (NPAT ensemble; P 1atm, T 310K) with the CHARMM27 parameter set including data for polyunsaturated lipids, and the TIP3P water model. The MD studies place the 2-AGPI and LPI headgroups in the water-lipid interface with the inositol moiety either upright and solvated in water or bent and buried in the POPC headgroups. Extensive ligand inter- and intramolecular hydrogen bonding contributes to the inositol location and conformation. 2-AGPI's acyl tail is extremely flexible and prefers compact to moderately extended conformations, whereas LPI's tail prefers more extended ones. Following these MD studies, the ligands were docked in a GPR55 model in the TMHs 1,2,3, 6 and7 region using K2.60 as the primary interaction site. [Support: NIH RO1 DA023204 (MEA) and KO5 DA021358 (PHR)]

1272-Pos

Diminished Cooperativity: Comparing Linker Lengths in Synaptotagmin I C2A Domain

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Exocytosis of neurotransmitters is triggered by the initial influx of Ca2+. Synaptotagmin I is known to bind Ca2+ and the phospholipid membrane to modulate this process. The exact mechanism for this information transduction, however, is not well known. Synaptotagmin I contains two binding domains, C2A and C2B, that are tethered to a neurotransmitter containing, lipid vesicle with a flexible linker region. The wild type C2A domain acts as a Ca2+ dependent trigger by binding the calcium ions in a cooperative manner. We seek to understand the role that the linker region has on the binding properties of the protein. To do this, a shortened construct (amino acids 141-267 verses amino acids 97-265 of the previously studied long construct) has been utilized as a probe to examine the effects of the truncated linker region. Ca2+ and phospholipid binding assays have been carried out and monitored via steady state fluorescence to make a thermodynamic comparison between the two constructs. Binding partition functions have been derived for this purpose and clearly show the diminished linkage relationship between the binding sites of the shortened construct. This material is based in part upon work supported by the National Science Foundation under CAREER-MCB 0747339

1273-Pos

Selectin Mechanokinetics and Two-Dimensional Bond Formation Determine and are Reported by Nano-Motion Dynamic Patterns

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Binding between surface-tethered proteins at cellular interfaces has been considered two-dimensional because of the restricted motion of the two binding partners. Two-dimensional protein interactions between cells are critical for many biological processes, such as leukocyte vascular adhesion via selectins. Experimental measurements have yielded data on the kinetics of selectin bond formation and dissociation. Additionally, computational methods have been employed to integrate molecular and cellular properties to elucidate the factors that influence the dynamics of selectin-mediated rolling. Simulation methods focused on biomolecular properties promise to yield additional novel insights into the molecular component of adhesion with the assistance of measurements from improved assays. We performed an in silico investigation on the effects of the kinetic force dependence, molecular deformation, grouping adhesion receptors into clusters, two-dimensional bond formation, and nanoscale vertical transport on outputs that directly map to observable motions. Statistics describing the motion patterns tied simulated motions to experimentally reported quantities. Distributing adhesive forces among P-selectin/PSGL-1 molecules closely grouped in clusters was necessary to achieve pause times observed in microbead assays. Notably, rebinding events were enhanced by the reduced separation distance following initial sphere capture. The result demonstrates vertical transport can contribute to an enhancement in the apparent bond formation rate. The result also suggests a new mechanism that may be important for the rebinding events characteristic of stable leukocyte rolling. When selectin receptor and ligand are restricted to small, two-dimensional interaction zones during rolling, the resultant wobble was found to be dependent on the confinement model used. Insight into two-dimensional bond formation gained from flow cell assays might also therefore be important to understand processes involving extended cellular interactions, such as immunological synapse formation.

1274-Pos

Single-Molecule Force Spectroscopy of the Interactions Between Platelet Integrin αIIbβ3 and Monomeric Fibrin

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Platelet-fibrin interactions under hydrodynamic blood shear are mediated by the integrin αIIbβ3, but the mechanism of αIIbβ3 binding to fibrin is largely unknown, although interactions with fibrinogen have been extensively studied. We used the optical trap to measure forces required to separate a laser-trapped-bead coated with monomeric fibrin from a pedestal coatedwith purified αIIbβ3. Experiments were performed with recombinant fibrin obtained from thrombin-treated fibrinogen, either wild-type or variants lacking putative integrin-binding sites. Integrin-fibrin interactions manifested as a bimodal force histogram with rupture forces from 20 pN to 140 pN, similar to αIIbβ3-fibrinogen but with somewhat higher binding probability. To test a role of the γ-chain C-terminal 400-411 dodecapeptide, the major αIIbβ3-binding site in fibrinogen, the most abundant fibrin (γA/γA) was replaced with a splicing variant (γ'/γ'), in which the γC-terminus has new amino acids from 408 to 427. Unexpectedly, the lack of the γC400-411 motif did not affect the ability of fibrin to interact with αIIbβ3, suggesting that this structure may not be a major integrin-binding site in fibrin. At the same time, fibrin-integrin interactions were partially inhibited by the γC-dodecapeptide, indicating that the γC400-411 motif still may be involved, perhaps indirectly. Two RGD motifs, one located in the αC region and the other in the coiled-coil connector, were tested as the potential binding sites by using fibrin(ogen) variants αD574E and αD97E. Both of them had a reduced integrin-binding strength and displayed the cumulative binding probability about 2/3 of that of the wild-type fibrin, suggesting that the RGD motifs play a role in the αIIbβ3-fibrin interactions. Free γC-dodecapeptide did not affect the reactivity of the D574E and D97E mutants. The results suggest that the αIIbβ3-fibrin interactions involve the RGD sites rather than the γC400-411 motif.