100-Plat Myosin II Plays a Specific Role in Driving Glioma Invasion of Normal Brain

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The ability of gliomas to invade normal brain enormously limits the efficacy of standard therapies. Targeting this invasive phenotype requires understanding how glioma cells move and what intracellular machinery they use to invade normal brain. In normal brain, glioma migration occurs in a two-step process that resembles how neural and glial progenitor cells migrate *in situ*. These steps consist of extension of a leading process, followed by forward propulsion of the cell body. We show that forward propulsion of the cell body requires the molecular motor myosin II when these cells have to crawl through the mechanically-constrained 3-dimensional matrix that characterizes the brain. By contrast, myosin II activity is unnecessary when these tumor cells migrate on a 2-dimensional surface in the absence of such mechanical constraints. Immunoblots and immunofluorescence reveal that tumors and their associated vasculature markedly up-regulate myosin IIA expression, and RNAi suppression of myosin IIA, but not myosin IIB, blocks tumor cell invasion. Our results thus demonstrate that myosin IIA has a specific and indispensable role in driving glioma invasion of normal brain, presumably by providing internal compressive forces need to propel the cell body and nucleus forward.

Symposium 5: Driving Forces in Macromolecular Binding

101-Symp Complexity in protein-protein interfaces

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Protein-protein interactions are characterized by a striking structural plasticity that allows contact points to adapt to conformational changes and multiple amino acid substitutions. As a result, the biophysics governing the steric and energetic properties of protein-protein interactions is extremely complex. The structure-function data base for human growth hormone (hGH) binding to its receptor (hGHR) is probably the most extensive available for any large protein-protein interface, but attempts to extract universal trends have been hindered by the incompleteness of the data set. We have developed combinatory techniques that have allowed us to access the structural and functional effects of all possible point mutations across the hGH-hGHR interface. This has allowed us to assess the binding effects of introducing all 20 amino acid types in the 35 positions comprising the hGH binding interface. This has produced the most comprehensive picture of adaptability in a large protein-protein interface that has ever been achieved and has led to some new and unexpected insights. We have determined that while there are a few hot-spot residues that are immutable, at least half of the 35 residues can be simultaneously mutated to alanine without affecting binding. Our data challenge the concept of conservative substitutions, especially in the case for hydrophilic residues. Sequence conservation across species is a poor predictor of functional importance. Hydrophobic residues in many instances can replace hydrophilic ones even if that residue is involved in a specific H-bonding or salt-bridge interaction. In a complementary phage display study, we show that high affinity interactions can be achieved using a simple binomial (Tyr, Ser) genetic code. This facilitates building high affinity and specific interactions with a highly restricted chemical diversity and demonstrates that conformational diversity trumps sequence diversity in protein-protein interactions.

102-Symp Biophysics of Interactions in Non-globular Proteins

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Protein-protein interactions mediated by non-globular proteins generally involve more than one folded protein domain in the interaction interface. Experimental results from two different interactions will be discussed; the LRP1/apoE interaction and the NF-kB/IkBa interaction. Isothermal titration calorimetry, SPR, NMR, and amide H/D exchange results taken together help to understand these interactions in more detail. In both of these cases, contributions to the energetics of the interaction come from more than one "hot spot" and are energetically cumulative. In addition, the overall favorable energy of interaction has a contribution from folding upon binding. The functional significance in terms of binding to multiple targets and binding kinetics will also be discussed.

103-Symp Understanding Protein-ligand Interactions: Correlating Structure and Thermodynamics

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Isothermal titration calorimetry (ITC) provides a direct method for determining thermodynamic parameters associated with biomolecular interactions. The understanding of the correlation between the change in thermodynamic parameters for a binding event and the perturbation in structure forms one cornerstone of biophysical science. Clearly, if correlations can be defined then predictive algorithms can be produced which will permit the calculation of compound affinities directly from high resolution structural data (and vice versa). This would have dramatic effects on the temporally and financially expensive processes associated with compound development in the pharmaceutical industry. Although significant effort has been made in this area, particularly in the last decade, the application of these principles to drug development is fraught with inconsistencies and inaccuracies. The SCORPIO database (www.biochem.ucl.ac.uk/scorpio/scorpio.html) provides a repository for thermodynamic data derived solely from ITC and thus is not prone to potential problems in derivation of the enthalpic (and hence entropic) contributions to binding inherent in spectroscopic methods. Data from SCORPIO has been used to assess potential correlations between thermodynamic parameters and structure. These

correlations, or lack thereof, will be discussed with respect to general protein ligand interactions and specifically in relation to drug development. The use of calorimetrically determined thermodynamic data input to decision making in drug development will be demonstrated.

104-Symp Computational Prediction of Ligand Binding Modes and Affinities

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Computer simulation has become an increasingly powerful tool for addressing problems in molecular recognition. We will discuss how the combination of efficient docking procedures with subsequent binding free energy calculations can be used to predict both binding modes and affinities with good accuracy and, in particular, recent results utilizing the linear interaction energy (LIE) method will be presented. We will further address the problems of sensitivity to the 3D receptor model, discrimination between right and wrong binding modes, the reliability of empirical scoring functions as well as mapping of key interactions for the recognition process. Examples will include both enzyme-inhibitor and ion channel-blocker complexes.

Symposium 6: EGF Receptor Signaling and Networks

105-Symp Extracellular control of EGF receptor

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It is well accepted that the first step in the activation of receptor tyrosine kinases (RTKs), such as those of the epidermal growth factor receptor (EGFR) family, involves ligand-induced receptor dimerization or alteration of a pre-existing dimers. X-ray crystal structures of the extracellular region of EGFR (sEGFR) reveal an unexpected mechanism for ligand-induced dimerization. All of the contacts across the dimer interface in the sEGFR dimer are mediated by the receptor, with the majority contributed by domain II. This contrasts with the case for most other RTKs where the ligand is the primary mediator of dimer contacts. In unliganded sEGFR the domain II dimerization interface is occluded in an intramolecular interaction with domain IV. Ligand binding promotes a dramatic domain rearrangement in sEGFR, exposing and stabilizing the domain II dimerization interface. This mechanism has substantial implication for extracellular control of EGFR activation.

The EGFR family is implicated in several disease states, perhaps most notably in many cancers. For example, EGFR activation in epithelial tumors has been linked with more aggressive disease and poorer outcomes. Drugs that inactivate EGFR through interaction with either the extracellular or intracellular regions of EGFR are under intense clinical investigation. The structures of the antigen binding fragments from several therapeutic antibodies have been determined in complex with the extracellular regions of EGFR family members. These structures indicate several possible mechanisms of inhibition. The structure-based models for EGFR activation suggest additional modes of binding that may also be effective. Finally impact of activating tumor mutations in evaluating the optimal mode of EGFR inhibition will be discussed.

106-Symp Structural and Mechanistic Studies of Cancer-causing Mutations in the EGFR Kinase Reveal A Novel Mechanism of Drug Resistance

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Mutations in the EGFR kinase are a cause of non-small cell lung cancer, and the presence of these mutations correlates with response to small-molecule tyrosine kinase inhibitors (TKIs). Interestingly, some of the mutant kinases are as much as ~100-fold more potently inhibited by gefitinib and erlotinib than the wild-type kinase, despite the fact that these TKIs were developed to target the WT enzyme. Structural studies reveal an altered binding mode of gefitinib in the L858R mutant, which may in part explain its enhanced sensitivity. Additionally, kinetic studies show that the L858R and other mutants have diminished affinity for ATP, rendering them more sensitive to these ATP-competitive TKIs. Although patients with tumors harboring a mutant EGFR initially respond to these drugs, longer-term efficacy has been limited by the emergence of drug resistance, often conferred by an additional mutation of Threonine 790 in the EGFR to Methionine (T790M). This "gatekeeper" mutation lies in the ATP binding pocket of the kinase, and has been thought to confer resistance by sterically interfering with drug binding. However, we show through binding studies, enzyme kinetics, and x-ray crystallography that the T790M mutant and L858R/T7890M double mutant retain low nanomolar affinity for gefitinib, and that the T790M mutation does not alter the binding mode of the inhibitors. Instead, clinically observed drug resistance is due to an increase in the ATP affinity conferred by the T790M substitution. Since TKIs must compete with ATP to achieve their intended effect, their effective potency is diminished by the enhanced ATP-affinity. Thus the T790M mutation is a "generic" resistance mutation that can be expected to diminish the potency of any ATP-competitive inhibitor. Irreversible inhibitors, as a class, overcome this effect through covalent binding.

107-Symp Role of the Intracellular Juxtamembrane Region in Activation of the EGFR

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The receptor tyrosine kinase EGFR has an extracellular ligand-binding domain, a helical transmembrane (TM) domain linked to a flexible, unfolded juxtamembrane (JM) region, and a kinase domain. A popular model of EGFR activation postulates ligand binding induces dimer formation, permitting the C lobe of one