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

kinase to bind to the N lobe of the other kinase and activate it allosterically (*Cell*, 2006 *125*, 1030). Recent biophysical experiments, however, suggest EGFR dimers exist in the *absence* of ligand: FCS cross correlation (*Biophys. J*. 2007 *93*, 684) and brightness analysis (*Biophys. J*. 2007 *93*, 1021) measurements support prior claims of preformed dimers (e.g., *J. Mol. Biol*. 2001 *311*, 1011). Why aren't these preformed dimers active? We proposed the JM region, net charge = +8, binds to acidic lipids on the inner leaflet of the plasma membrane, which could prevent contact between kinase domains in a preformed dimer (*J Gen Physiol* 2005, *126*, 41). This suggests the "Twist model for ligand-dependent activation of preformed EGFR dimers" of Gadella and Jovin (*J. Cell Biol*. 1995 *129*, 1543) could be compatible with the elegant allosteric activation model from the Kuriyan laboratory (*Cell*, 2006 *125*, 1137). A combination of NMR, CD, infrared and fluorescence measurements on reconstituted TM + JM EGFR(622–660) peptides led us to 3 conclusions: the TM helix breaks at the membrane-solution interface; the unfolded JM region binds electrostatically to the membrane; binding of Ca^{2+}/calmodulin to the positively charged JM region can reverse its charge from +8 to −8 and release it from the membrane (*Biochemistry* 2006, *45*, 12704). Any factor that reduces the electrostatic interaction of the JM region with the plasma membrane should activate EGFR in the absence of ligand (*J. Biol. Chem*. 2007 *282*, 8474).

Platform J: Self-Assembled-Session: The Hidden Photophysics of Autofluorescent Proteins

108-Plat From the knowledge of protonation ground states to the development of photochromic Green Fluorescent Proteins

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Autofluorescent proteins (FPs), and particularly Green Fluorescent Proteins mutants (GFPs), have revolutionized biophysical and molecular biology studies owing to the genetic encoding of strong visible fluorescence in vivo (1). The optical properties of FPs originate from an organic emitter molecule buried inside a higly-ordered b-can tertiary structure. The engineering of FPs' optical properties by means of sequence mutation allows to obtain probes tailored for high-resolution intracellular studies.

Recently, scientific attention has been attracted by "hidden" photophysical properties of FPs (2). In this field, we have been involved in the development and application of photochromic GFPs mutants since 2001 (3–5) and we have shown that pH-dependent optical properties of most GFP mutants can be described by a general two-site ionization model (6). This model helped us to develop new ratiometric pH biosensors for intracellular use (7). Here, we shall show how the knowledge of the protonation ground states led us to generate new photochromic GFP mutants. Quantitative details on the photophysical mechanism underlying the observed photochromic behavior will be reported.

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109-Plat Pa-GFP Photoactivation Kinetics In Model And Cell Systems

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We present data related to the possibility to induce the photoactivation process on the photo-activatable green fluorescent protein paGFP, T203 variant (1), at different wavelengths, specifically in the (270–488) nm spectral range under linear conditions and in the infrared range under multi-photon absorption (2). In particular, exploiting UV irradiation to bring the molecules in the activated state it is possible to evaluate the absorption properties of activated and non activated form of the fluorescent protein in solution, without additional artefacts due to photobleaching or environmental conditions. These results were employed to separate the contribution of the two forms of the molecules via a home made linear unmixing algorithm. This analysis allowed to identify the kinetic behaviour of activated and non activated paGFP populations in more complex systems, like proteins layers and fixed cells while utilizing confocal and two-photon microscopy. In particular, a different fraction of molecules undergoing photoactivation was found depending on the energy delivered during the activation step. We demonstrate that this phenomenon is driven by the competition of photoactivation and photobleaching of the pre- and post-activated form of paGFPs.

In conclusion, we showed unreported photoactivation pathways of the proteins in the UV range that were useful for the comprehension of the nature and kinetics of photobleaching and photoactivation processes. These results are in tune with the broadening of two photon activation spectra (2) and with blue-photoactivation pathways (3) early reported.

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