repeats. Our crystal structures, simulations, and binding assays show how the tip of protocadherin-15 and some of its variants form a mechanically strong and calcium-dependent heterophilic complex with the cadherin-23 tip. In addition, structures and simulations of protocadherin-15 EC repeats show how non-canonical linker regions may alter protocadherin-15's tertiary structure and elasticity. Overall, our results provide a molecular view of tip link mechanics and identify the molecular determinants of tip link function in vertebrate mechanosensation.

58-Symp
Mechanical Forces in B cell Activation
Pavel Tolar.
MRC National Institute for Medical Research, London, United Kingdom.
The generation of protective antibodies depends on the selective expansion of B cells with the strongest binding of their B cell receptors (BCRs) to foreign antigens. This process starts by formation of B cell immune synapses with antigen-presenting cells. BCR signaling in immune synapses triggers extraction of the antigens, leading to B cell antigen processing and presentation to helper T cells - a step that ultimately controls the relative expansion of B cell clones. To internalize antigen from immune synapses, B cells generate tensile forces by activating the non-muscle myosin IIa. Myosin contractility invaginates synaptic antigen clusters and promotes antigen internalization by clathrin-mediated endocytosis. Forces generated by myosin IIa in B cell synapses rupture low avidity interactions between the BCR and antigens and provide thus a negative feedback to BCR antigen binding and signaling, which promotes B cell affinity discrimination. These results suggest that B cells use mechanical forces to test the strength of antigen binding to the BCR. The location, intensity and timing of the forces are distinctly regulated in B cell subsets. In naive B cells, antigen clusters form and grow in lamellipodia, move centripetally and are collected in the center of the synapse. Tensile forces are applied at the base of lamellipodia, creating a delay that allows cluster growth, increase in cluster avidity and greater sensitivity of endocytosis. In contrast, germinal centre B cells, which undergo affinity selection, apply strong forces on small antigen clusters in the periphery of the synapse. This synaptic architecture of germinal center B cells is associated with higher stringency of affinity discrimination. Thus, B cell selection is regulated by the architecture of immune synapses through the coordination of signaling, contractility and endocytosis.

59-Symp
Navigating a Maze - Sensing and Responding to Mechanical Obstacles during Cellular Invasive Growth
Carlos Agudelo[1], Amir Sanati Nezhad[1], Mahmood Ghanbari[1], Muthukumaran Packirisamy[1], **Anja Geitmann**[2].
[1]Concordia University, Montreal, QC, Canada, [2]University of Montreal, Montreal, QC, Canada.
The tool delivering the sperm cells in the flowering plants, the pollen tube, has to invade multiple tissues to reach its target within the pistil, the female gametophyte. To accomplish this task the rapidly growing tube must perceive the geometry and mechanical consistency of the growth substrate as well as directional cues guiding it towards the target. To penetrate the pistillar tissues the pollen tube also needs to produce invasive forces to overcome any mechanical impedance. To investigate the directional growth behavior, the role of chemical, mechanical and electrical guidance cues, and the ability to cope with mechanical obstacles we devised the TipChip, a microfluidic experimental platform that allows us to assess these parameters in quantitative manner. Pollen grains are trapped individually within the microfluidic network, and the germinating pollen tubes are guided into microchannels in which they are exposed to test assays such as calibrated micro-cantilevers, highly localized electrical fields, or micron-sharp chemical gradients. These approaches have enabled us to quantify the invasive and dilating force of growing pollen tubes, revealing that the tubes employ the internal turgor pressure to generate this force, but that they modulate it by regulating the cell wall mechanical properties. The use of precisely calibrated electrical fields has revealed that medium conductivity plays an important role in mediating the effect of the field on the cellular response. Finally, navigation in a chemical gradient seems to be governed by complex strategies that overlap and must be integrated depending on the nature of the cues provided. Our experiments illustrate that to fully understand the strategies governing pollen tube growth, in vitro studies must be rendered more relevant for the in vivo situation by simulating the micro-environment of the pistil.

Platform: Molecular Simulation: Structure and Interactions

60-Plat
Role of Desolvation in Thermodynamics and Kinetics of Ligand Binding to a Protein
Jagannath Mondal, Richard Friesner, Bruce J. Berne.
Chemistry, Columbia University, New York, NY, USA.
Computer simulations are used to determine the free energy landscape for the binding of the anti-cancer drug Dasatinib to its src kinase receptor and show that before settling into a free energy basin the ligand must surmount a free energy barrier. An analysis based on using both the ligand-pocket separation and the pocket-water occupancy as reaction coordinates shows that the free energy barrier is a result of the free energy cost for almost complete desolvation of the binding pocket. The simulations further show that the barrier is not a result of the reorganization free energy of the binding pocket. Although a continuum solvent model gives the location of free energy minima, it is not able to reproduce the intermediate free energy barrier. Finally, it is shown that a kinetic model for the on rate constant in which the ligand diffuses up to a doorway state and then surmounts the desolvation free energy barrier is consistent with published very long time simulations of the ligand binding kinetics for this system [D. E. Shaw et al., J. Am. Chem. Soc. 2011, 133, 9181-9183]

61-Plat
Insights into the Stabilizing role of Cholesterol for the Amyloid Precursor Protein
Martina Audagnotto[1], Matteo Dal Peraro[2].
[1]School of Life Sciences, Institute of Bioengineering, Ecole Polytechnique Fédérale de Lausanne (EPFL), CH1015 Lausanne, Switzerland, [2]School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne (EPFL), CH1015 Lausanne, Switzerland.
The Amyloid Precursor Protein (APP) is a type-I transmembrane glycoprotein that is present in the synaptic plasma membrane (SPM). Its cleavage by γ-secretase produces Aβ amyloids, which are major components of the plaques observed in patients affected by Alzheimer disease (AD).
In the last years, APP has been proposed to have both a monomeric and a homodimeric structures. In particular, two possible dimerization interfaces were proposed: namely, G(700)XXXG(704)XXXG(708) and G(709)XXXA(713) of which, as suggested by NMR and EPR experiments, the former can represent a cholesterol binding motif key for the stabilization of APP transmembrane domain. However, at today, there is no consensus on the role of these two motifs on the structural and dynamic properties of APP. In this study, we aim at dissecting, using atomistic molecular dynamics (MD) simulations and realistic models of the SPM the role of these binding motifs for the stability of APP transmembrane domain. Preliminary results show that cholesterol molecules have high affinity for both the G(700)XXXG(704) XXXG(708) and G(709)XXXA(713) motif, promoting the destabilization of the homodimeric APP structure. The molecular description of the APP dimerization dynamics at the SPM can have specific and important implications for γ-secretase cleavage and the following production of amyloidogenic peptides related with AD.

62-Plat
Pharmacophore Modeling using Site-Identification by Ligand Competitive Saturation (SILCS) Method with Multiple Probe Molecules
Wenbo Yu, E. Prabhu Raman, Sirish Kaushik Lakkaraju, Lei Fang, Alexander D. MacKerell, Jr.
Department of Pharmaceutical Sciences, University of Maryland, Baltimore, Baltimore, MD, USA.
Receptor-based pharmacophore modeling is a computer-aided drug design technique that uses the structure of the target protein to identify novel ligands that may bind[1]. Typical receptor-based pharmacophore modeling methods are limited by the neglect of protein flexibility and desolvation effects, since only a single or limited number of receptor conformations are considered in the modeling which is usually performed in vacuum or with a limited representation of the aqueous solvent environment. The SILCS assisted pharmacophore modeling protocol (SILCS-Pharm)[2] was introduced recently to address these issues since SILCS[3] naturally takes both protein flexibility and desolvation effects into account by using full MD simulations to determine 3D maps of the functional group-binding patterns on a target receptor. In the present work, SILCS-Pharm protocol is extended to use a wider range of fragments including benzene, propane, methanol, formamide, acetaldehyde,