phase separation due to differentiated cell-cell adhesion. Various nontrivial emerging patterns resembling observed tumor morphology were identified over the parameter space of our model. However, the previous development and analysis of our model have been implicitly based on the assumption of a homogeneous microenvironmental background and unrestricted boundaries. Most clinically relevant tumors are constrained by particular organ tissue structures that may co-evolve with the progressing tumors and have profound impact on tumor-microenvironment interactions. Here we adopt a recently developed diffuse-domain approach, utilizing the Cahn-Hilliard equation framework we have previously established, to adapt partial-differential-equation models of tumor growth to a co-evolving tissue geometry. We will demonstrate this approach by modeling the growth of lymphoma within a lymph node and ductal carcinoma in situ within mammary ducts.

2540-Pos Board B559

Biochemical Response and the Effects of Bariatric Surgeries on Type 2 Diabetes

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A general method is introduced for calculating the biochemical response to pharmaceuticals, surgeries, or other medical interventions. This method is then applied in a simple model of the response to Roux-en-Y-gastric bypass (RYGB) surgery and related procedures. We specifically address the amazing fact that glucose homeostasis is usually achieved immediately after RYGB surgery, long before there is any appreciable weight loss. This result is usually attributed to a dramatic increase in an incretin, glucagon-like peptide 1 (GLP-1), but our model indicates that this mechanism alone is not sufficient to explain the largest declines in glucose levels or measured values of the homeostatic model assessment insulin resistance (HOMA-IR). The most robust additional mechanism would be production of a substance which opens an insulin-independent pathway for glucose transport into cells, analogous to the established insulin-independent pathway associated with exercise.

2541-Pos Board B560

Modeling Cluster Formation by Multivalent Interacting Proteins: Nephrin-Nck-NWasp Signaling in the Foot Process of Kidney Podocytes

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Intracellular function is often defined by clusters consisting of multiple multivalent molecules. Studying these clusters represents a significant challenge because of the potentially infinite number of cluster compositions and the intermediate complexes that are formed while clusters are formed. To make the matter worse, many clusters are very liquid, the affinities of the many bimolecular site interactions are quite modest, implying that off-rates are relatively rapid. Thus, we need efficient methods to predict the average composition of these ensembles, characterizing number of molecules of different types, number of bonds per different molecule types, and other parameters defining the size and structure of the cluster. Here we present a stochastic steady state algorithm for multivalent interacting molecules to determine cluster compositions and sizes based on probability that each type of binding site is bound. The advantage of the method is in its efficiency: tracking the formation of the cluster over time would require computation of binding and unbinding steps; instead, we identify a distribution of cluster compositions at the time point of interest based on the pairwise binding probability among multiple sites within interacting molecules. The method is applied to the system Nephrin-Nck-NWasp. The interaction between these three multi-domain molecules is required for maintenance of the podocyte foot processes cytoskeleton, the key cellular structure in the kidney slit diaphragm filtration system. The weak individual site pair affinities and estimated nephrin concentrations at the slit diaphragm by themselves would be insufficient to promote actin polymerization. We use our method to address how the multi domain and cooperative mechanisms could provide such function. Supported by NIH grants TR01DK087650 and P41GM103313.

Synaptic Transmission

2542-Pos Board B561

Synaptic Vesicle Capture by Intact CaV2.2 Channels

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The fusion of synaptic vesicles (SVs) at the presynaptic transmitter release face is gated by Ca^{2+} influx from nearby voltage gated calcium channels (CaV). Our early functional studies argued that the CaV and SV are linked by a molecular anchor or 'tether' and recent studies have proposed a direct cytoplasmic link to the channel distal C terminal. In order to explore CaV-SV binding we developed an in vitro assay, termed SV-PD, to test for capture of purified, intact SVs. Antibody-immobilized presynaptic or expressed CaV2.2 channels but not plain beads, IgG or pre-blocked antibody successfully captured SVs, as assessed by Western blot for a variety of protein markers. SV-PD was also observed with a distal C terminal fusion protein, C3strep, supporting involvement of this CaV region. Our results favor the model where presynaptic CaV can tether SVs directly, independently of the surface membrane.

2543-Pos Board B562

Optical Modulation of Neurotransmission

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The computational properties of an isolated neuron have been analyzed in detail by postsynaptic activation with caged compounds. However, new tools are needed to manipulate neurotransmission at individual synapses in order to understand how a neuron integrates physiological stimuli received from presynaptic neurons within a circuit. Here we describe a method to control neurotransmitter exocytosis at the presynaptic compartment by using a light-gated glutamate receptor (LiGluR). In chromaffin cells, LiGluR supports exocytosis by means of a calcium influx that is comparable to voltage-gated calcium channels. Presynaptic expression of LiGluR in hippocampal neurons enables direct and reversible control of neurotransmission with light, and allows modulating the firing rate of the postsynaptic neuron with the wavelength of illumination. This method constitutes an important step toward the determination of the complex transfer function of individual synapses.

2544-Pos Board B563

Molecular Mechanisms of Synchronous Synaptic Transmission

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Complexin and Synaptotagmin work in concert to couple the SNARE-mediated membrane fusion machinery to the triggering signal i.e. influx of Ca^{2+} ions. The SNARE assembly process is arrested at a very late stage by Complexin, to prevent spontaneous fusion events and the Complexin clamp is reversed by Synaptotagmin in a Ca^{2+}-dependent manner to synchronize the neurotransmitter release. To understand the mechanistic details of this process, we developed a Nanodisc based system, which allows us to generate trans-SNARE complexes under soluble conditions. SNAREs on Nanodisc assemble but do not fuse due to the topological constraints placed by the Nanodiscs. We employed a VAMP construct (VAMP-4X) which carries mutations in the C-terminal hydrophobic layers that prevent assembly of this region with the t-SNARE to accurately recreate the pre-fusion SNARE complex between two bilayers that mimic the vesicle-bilayer junction in docked vesicles. Fluorescence analysis show that the Synaptotagmin binds to the pre-fusion SNAREpin between the two bilayer under Ca^{2+} free conditions and the primary interaction is such that SNARE assembly or Complexin binding does not affect it. Calcium- additions triggers a very rapid co-penetration of the Ca^{2+}-binding loops of both C2 domains into the bilayer, with somewhat higher preference to the bilayer containing t-SNAREs and this partitioning could be further augmented by the addition of PIP2. However, Synaptotagmin maintains its position on the SNARE complex during the whole process. Recent data has shown that Complexin arrests fusion by blocking the complete assembly of SNAREpins in adjacent complexes i.e. a trans-clamping interaction. Our results suggest a simple, one-step physical mechanism by which Synaptotagmin could trigger the reversal of the trans-Complexin clamp and activate fusion, in response to Calcium.

2545-Pos Board B564

Calcium Triggered Membrane Penetration of Synaptotagmin may Provide the Driving Force to Reverse the Complexin Clamp

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Synaptic proteins, Complexin and Synaptotagmin act in sync to achieve the speed and the accuracy of the synaptic transmission. Complexin (CPX) arrests the SNARE assembly in the late stages promoting the docking of the synaptic vesicles at the active zone and along with Synaptotagmin, the calcium sensor, synchronizes the fusion of these vesicles with the influx of calcium ions following the nerve impulse. Recently, we obtained the first X-ray crystal structure representing the clamped state of SNAREpin at the docked vesicles, which shows that Complexin arrests fusion by blocking the complete assembly of the v-SNAREs in the adjacent SNARE complex. This trans- interaction generates an unusual zigzag array of half-zippered (pre-fusion) SNARE complexes between the two bilayers. Here we present experiments using a novel Nanodisc-based system that mimics a vesicle-bilayer junction that suggests

a simple model for the reversal of the CPX clamp by Synaptotagmin and calcium. In this model, Synaptotagmin binds the stalled CPX-SNARE complex via its interactions with the t-SNAREs and upon binding Calcium, the membrane loops inserts rapidly into the bilayers, while Synaptotagmin stays bound in place on the SNAREpins. This is expected to perturb the attached SNAREpin out of the planarity of the zigzag array and thus, trigger the rapid disassembly of the stalled zigzag structure allowing the SNAREs to complete zippering and open a fusion pore.

2546-Pos Board B565

The Dorsal Root Ganglion Sandwich Synapse: Novel Transglial Signaling between Neuronal Somata

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The dorsal root ganglion (DRG) contains a subset of closely-apposed neuronal somata (*NS*) that are separated solely by a thin satellite glial cell (*SGC*) membrane septum to form a *NS-glial cell-NS* (*NGlN*) cell trimer. We recently reported that stimulation of one *NS* evokes a delayed, noisy and long-duration inward current in both itself and its passive partner that was blocked by suramin, a general purinergic antagonist. Here we test the hypothesis that *NGlN* transmission involves purinergic activation of the *SGC* and its release of an excitatory transmitter. Stimulation of the NS triggered a sustained current noise in the *SGC*. Block of transmission through the *NGlN* by reactive blue 2 or thapsigargin, aCa^{2+} store-depletion agent, implicated a Ca^{2+} store discharge-linked P2Y receptor. P2Y2 was identified by simulation of the *NGlN*-like transmission by puff of UTP onto the *SGC*. Block of the UTP effect by BAPTA, an intracellular Ca^{2+} scavenger, supported the involvement of *SGC* Ca^{2+} stores in the signaling pathway. The response to UTP was also blocked by AP5, which, along with the N2B subunit-specific antagonist ifenprodil, inhibited *NGlN* transmission, implicating a glutamatergic pathway via postsynaptic NMDA receptors. Puff of glutamate could evoke transmission-like current in the *NS*. Immunocytochemistry localized the NMDA receptor subunit NR2B to the *NS* membrane, abutting staining for P2Y2 on the *SGC* septum. We infer that *NGlN* transmission involves secretion of ATP from the *NS*, *SGC* Ca^{2+} store discharge via P2Y2 receptors and release of glutamate to activate *NS* postsynaptic NMDA receptors. Thus, the *NS* of the *NGlN* trimer communicate via a ''Sandwich Synapse'' transglial pathway, a novel signaling mechanism that may contribute to information transfer in other regions of the nervous system.

2547-Pos Board B566

The Interactions of Dopamine and L-Dopa with Lipid Headgroup and its Implication for Neurotransmitters Metabolism

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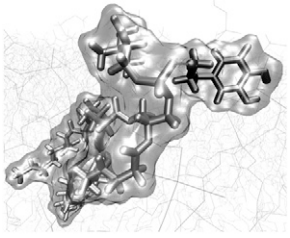
Atomistic molecular dynamics simulations were used to investigate the interactions between the neurotransmitter dopamine and its precursor L-dopa with membrane lipids. The results indicate that these molecules strongly interact with the lipid head groups e.g. via hydrogen bonds. These interactions anchor the dopamine and L-dopa to the membrane interfacial region. The strength of this bonding is dependent on lipids present in membrane. Presence of phosphatidylserine resulted in increased level of bonding strength with a lifetime longer than the timescale of our simulations.

The high membrane association of dopamine and L-dopa both, extracellularly, might favour the availability of these compounds for cell membrane uptake processes and, intracellularly, can accentuate the importance of membrane-bound metabolizing enzymes over their soluble counterparts.



We can also hypothesize that excessive association of dopamine and its precursor L-dopa with the membranes in the situation, when the concentration of phosphatidylserine is increased, may eventually limit the free use of dopamine as a synaptic transmitter what could possibly be a molecular level mechanism responsible for some of neurodegenerative disorders.

2548-Pos Board B567

Mapping the Energy Landscapes of the Glycine Receptor in the Post-Synaptic Neuronal Membrane

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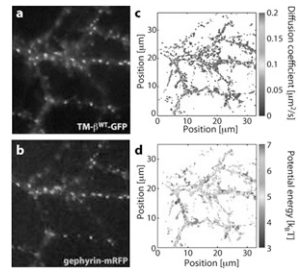
The movement of proteins in the cell membrane is governed by the local friction and their interactions with molecular partners. Yet, most experimental descriptions fail to unequivocally distinguish these effects; instead, they combine the diffusive and energetic contributions into an effective diffusion coefficient or anomalous exponent. Here, we show how the diffusion and energy landscapes of membrane proteins can be mapped separately over the entire cell surface using high-density single-molecule imaging and statistical inference [1]. In the case of glycine neuroreceptors, we demonstrate that scaffolds at inhibitory synapses act as energy traps with a depth modulated by the properties of the intracellular loop that mediates the receptor-scaffold interactions. Furthermore, we bridge the gap between local properties of the membrane environment and characteristics of the mobility at the cellular scale by simulating random walks in the inferred maps and computing estimators such as the propagator, mean square displacement, and first-passage time. Results are used to investigate the relation between numbers of receptors and synaptic plasticity. Overall, our approach provides a versatile framework with which to analyze biochemical interactions in situ.



[1] J.-B Masson et al, Nat. Chem. (submitted)

2549-Pos Board B568

High Precision Release of Neurotransmitter - A New Tool

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Modern imaging techniques have become a powerful tool for investigating excitation patterns and signaling pathways in the brain. However, the high complexity of brain topology and the resolution limit of in vivo techniques make it difficult to study isolated small neuron circuits. Micro-electrode arrays (MEAs) are able to record activity of in vitro neuron networks and to stimulate locally, but such electrical approach cannot easily be applied to stimulate single cells. Large stimulus artifacts and poor control over the spread of electrical stimuli in medium create the main disadvantages when studying the role of single cells in small networks. One promising solution is to interact with individual neural cells by mimicking chemical signaling. Recently developed systems for precise neurotransmitter release include micropipettes, microfluidic substrates and caged compounds.

In this project we propose to combine MEA technology (Multi Channel Systems GmbH, Switzerland) with novel FluidFM technology. The FluidFM (Cytosurge AG, Switzerland) has hollow atomic-force microscopy (AFM) cantilevers acting as force-controlled nanopipettes. We present the ability to locally release neurotransmitters on the cell membrane with precise control over applied force (sub-nN) and spatial position (µm). For those experiments we used FluidFM cantilevers with 2 µm openings, where the microfluidic channel was filled with physiological solution containing 200 µM glutamate. In order to chemically induce a local electrical response in a culture of dissociated hippocampal neurons from E18 rat, we first brought the FluidFM cantilever in contact with the cell membrane. We then applied pressure-pulses between 50 mbar and 300 mbar with 300 ms duration to locally eject sub picoliter volumes of the neurotransmitter.

We are now working towards applying single cell stimulations to well defined networks.

2550-Pos Board B569

Cocaine Preferentially Potentiates Fast Releasable Vesicle Pool in Mouse Dopaminergic Striatum *In Vivo*

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