799-Plat
Deep-learning-based analysis of combinatorial synthetic T-cell receptor libraries
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Current CAR T cells can show poor proliferation, susceptibility to exhaustion, and low persistence. How we currently engineer such chimeric receptors remains ad hoc and relatively limited in scope. It prevents a wide use of CART therapy and hinders the discovery of new CAR T cells. In this work, we innovatively construct combinatorial synthetic T cell receptor libraries to create synthetic T cell receptors, which cannot be found in nature, using functional linear motifs. However, this approach is still limited due to the expense of experiments. It is also difficult to directly analyze, model, and interpret these experimental data due to the noise and incompleteness. The Deep-learning-assisted analysis that we developed removes these bottlenecks and helps researchers to have significant findings based on the libraries. It can capture relationships between the sequence of chimeric receptors and its functional performance and compensate for the missing information from the experiments to help the researchers have a mechanistic understanding of the relationship between CAR signaling motifs and cell phenotype and massive acceleration of designing novel CAR T cells. Our approach enables the rational engineering of CAR T cells.

800-Plat
Accelerating single particle discoveries using machine learning
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Nanoscale biomolecular recognitions are a crucial component of life manifesting themselves as interactionsbetween; enzymes and substrate, viruses and cells, mucus and drug-loaded nanocarriers. Our currentunderstanding of these nanoscale interactions primarily relies on ensemble techniques reporting the behavior ofa large ensemble of biomolecules, which masks heterogeneous behavior and thus the actual underlying biology [1]. Single particle methods enable direct observation of heterogeneous behavior of thousands of particles inparallel enabling observation of internalization pathways etc. However, the vast information content in singleparticle methods poses a significant impediment in the analysis as manual annotation is time consuming and maybe subjected to unconscious biases.Herein, we developed a machine learning framework for single particle tracking data analysis, processing, andclassification. This method allows for dissecting the features that underlie diffusional behavior and establishingmolecular identity, regardless of the underlying diffusion type. Combined with our single-molecule fluorescencemicroscopy assay to track thousands of individual biomolecules on cells allows us to directly classify distinctpatterns of diffusional properties and link these to mechanistic insights of various biological systems such asenzymes, insulin, and drug-loaded nano-carriers [2]. [1] S. SR. Bohr et al, Sci Rep 9, 16169 (2019). [2] H. D. Pinholt et al, Proceedings of the National Academy of Sciences (PNAS) 2021, 118 (31).

801-Plat
Large-scale simulation of tumor spheroid invasion dynamics
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Cancer remains an insufficiently understood and often deadly disease to this day. In particular, the mechanisms driving tumor invasion and metastasis are a topic of interest. Since cancer is a tissue-level disease that may involve billions of cells, theoretical models need to be sufficiently flexible in order to accurately study the disease in silico across the scales. Here, we present our work using Cells in Silico (CiS), a high performance framework for large-scale tissue modeling previously developed by us. Based on both a cellular potts model and an agent-based layer, CiS is capable of accurately representing physical and biological properties at subcellular resolution, while being able to simulate hundreds of millions of cells at a time. A major challenge of simulating single cell tissue dynamics is the approximation of model parameters. Here, we investigate the invasion dynamics of tumor spheroids into a collagen matrix. Recent developments in microscopy have enabled the analysis of tumor spheroids at single-cell resolution, and therefore permit the direct comparison of experiments and simulations. We find that the invasive behavior depends strongly on ECM density, motility and cell stiffness, and we specify parameter regimes for different invasion modes, thereby enlarging the parameter space that is experimentally accessible. Specifically, we investigate the influence of the ECM density on collective invasion. We further apply our model to data on spheroid fusions to investigate collective flow within the tissue.

802-Plat
Optimal checkpoint strategies balancing risk and speed
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Why biological quality-control systems fail is often mysterious. Checkpoints in yeast and animals are overridden (adapt) after prolonged arrests allowing self-replication to proceed despite the continued presence of errors. Although critical for biological systems, checkpoint adaptation is not understood quantitatively or at the system level by experiment or theory. To uncover potential patterns in checkpoint adaptation, we derived the mathematically optimal checkpoint strategy, balancing the trade-off between risk and opportunities for growth. We demonstrate that the mathematical problem of finding the optimal strategy maps onto the question of calculating the optimal absorbing boundary for a random walk, which we show can be solved efficiently recursively. The theory predicts the optimal override time based on two inputs, the statistics i) of error correction and ii) of survival. We applied the theory experimentally to the DNA damage checkpoint in budding yeast whose override is not understood quantitatively, functionally, or at the system level. Using a fluorescent construct which allowed cells with DNA breaks to be isolated by flow cytometry, we quantified i) the probability distribution of repair for a double-strand DNA break (DSB), including for rare events deep in the tail of the distribution, as well as ii) the survival probability after override. Based on these two measurements, the optimal checkpoint theory predicted remarkably accurately the DNA damage checkpoint override times as a function of DSB numbers, which we also measured for the first time precisely. Thus, a first-principles calculation uncovered hitherto hidden patterns underlying the highly noisy checkpoint override process. Our multi-DSB results revise well-known bulk culture measurements and show that override is a more general phenomenon than previously thought. Further, we show that override is an advantageous strategy in cells with wild-type DNA repair genes.

803-Plat
Genomic analysis of AlphaFold2-predicted structures identifies maps of 3D essential sites in 243 neurodevelopmental disorder-associated proteins
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Neurodevelopmental disorders (NDDs) are heterogeneous, congenital conditions, often caused by missense variants with varying, hard-to-elucidate molecular effects (e.g., gain- or loss-of-function). Functionally indispensable regions in three-dimensional (3D) structures of proteins encoded by associated genes can provide insights into the molecular mechanisms of disease variants. The recent success of AlphaFold2 in predicting protein structures at an accuracy matching experimental methods opened an avenue to computationally identify 3D essential sites for disease genes with no available experimental structure (67%). Here, we propose a new methodology to identify maps of essential sites (i.e., amino acid residues) in 3D, called Essential3D sites. The maps are created for 243 NDD genes/proteins by taking a consensus of three lines of evidence: 3D sites that are conserved across human gene paralogs and intolerant of missense variants (determined by 3D transformation of sequence-based scores), and that are enriched (burden analysis) for pathogenic variations (N=9,433; ClinVar and HGMD) compared to reference population variations (N=89,401; gnomAD database). By association analysis of variants from three different exome sequencing studies of autism, developmental disorders, and epilepsies (N=1,951) and, those from the UK-Biobank

(N=52,042), we found that Essential3D sites are enriched in NDD variants (8-fold, *P*=8.1e-159; Fisher's Exact test). A similar burden of Essential3D sites was observed in *de novo* variants (denovo-db), compared to DiscovEHR-variants (6-fold, *P*=1.1e-11). We further show that Essential3D sites present mutation-sensitive locations for yet unseen variants (average ΔE of substitutions by EVmutation=-6.6). Finally, we identified hotspots of Essential3D sites across AlphaFold2-predicted structures, revealing intramembrane (*P*=1.6e-231), transmembrane (*P*=1.0e-300), and nucleotide phosphate-binding regions (*P*=9.0e-118) as key features of Essential3D sites. Our structure-guided method to prioritize essential sites in proteins is complementary to sequence-based methods, which we validated for NDD genes, and aim to apply proteome-wide.

Symposium: Membrane Tension

804-Symp
Mechanical signaling at the cell membrane
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Chemistry and Chemical Biology, Rutgers University, Piscataway, NJ, USA.
Cell membranes are highly dynamic and experience constant mechanical perturbations. The ensuing change in membrane tension is a fundamental regulator of many membrane signaling and cellular transport processes. In most non-motile cells, local perturbations to membrane tension remain localized, leading to subcellular Ca^{2+} influx and vesicle fusion events. Exception to this rule can be found in the axon of neurons, where a rapid propagation of membrane tension coordinates the growth and branching of the axon across hundreds of microns. However, it has been unclear how mechanosensitive molecules in the cell membrane would move in respond to localized mechanical and geometrical cues. In this talk, I will discuss the implications of membrane tension propagation, with a focus on the distribution and function of mechanosensitive proteins, such as PIEZO and GPI-anchored proteins, in respond to local perturbations to the cell membrane.

805-Symp
***In vitro* morphogenesis by cellular tornadoes**
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Tissues acquire function and shape via differentiation and morphogenesis. Both processes are driven by coordinating cellular forces and shapes at the tissue scale, but general principles governing this interplay remain to be discovered. Here, we report that self-organization of myoblasts around integer topological defects, namely spirals and asters, suffices to establish complex multicellular architectures. In particular, these arrangements can trigger localized cell differentiation or, alternatively, when differentiation is inhibited, they can drive the growth of swirling protrusions. Both localized differentiation and growth of cellular vortices require specific stress patterns. By analyzing the experimental velocity and orientational fields through active gel theory, we show that integer topological defects can generate force gradients that concentrate compressive stresses. We reveal these gradients by assessing spatial changes in nuclear volume and deformations of elastic pillars. Altogether, we propose integer topological defects as mechanical organizing centers controlling differentiation and morphogenesis.

806-Symp
Elucidating the role of membrane tension in cellular processes using continuum modeling
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Membrane tension plays a critical role in many cellular processes. Experiments using both cellular and reconstituted systems have shown that tension plays a critical role in membrane-protein interactions for curvature generation. Cellular membranes can be thought of as elastic lipid bilayers that contain a variety of proteins, including ion channels, receptors and scaffolding proteins. These proteins are known to diffuse and aggregate in the plane of the membrane and to influence the bending of the membrane. Experiments have shown that lipid flow in the plane of the membrane is closely coupled with the diffusion and aggregation of proteins. Thus, there is a need for a comprehensive framework that accounts for the interplay between these processes. In this talk, I will discuss some recent theoretical and computational developments from my group using continuum modeling that will allow for a better comparison of membrane deformations with experiments. Our primary focus will be membrane trafficking, particularly endocytosis but the theoretical developments are broadly applicable to many membrane curvature generating processes.

Symposium: Structure-Function of Long Non-Coding RNAs

807-Symp
G quadruplex structures in NEAT1: potential functions in regulating its stability and its interactions with the fused in sarcoma protein
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Paraspeckles are membraneless organelles found within mammalian nuclei comprised of RNA and proteins. The long non-coding RNA, nuclear enriched abundant transcript 1 (NEAT1) has been shown to be essential for paraspeckles formation. NEAT1 has two isoforms, a short one of ∼3.7 kB (NEAT1_1) and a long one of ∼22.7 kB (NEAT1_2), and each paraspeckle may contain up to fifty NEAT1 RNAs. We and others identified five regions within NEAT1 that have the potential to form G-quadruplex structures (GQs), four of which are common to both isoforms and one found only in the long isoform NEAT1_2. We show that the GQ structure unique to the longer NEAT1_2 is essential for the formation of the triple helix (TH) structure, which has been previously shown to control the stability of this NEAT1 isoform. Thus, the presence of this GQ structure confers an additional layer of regulation of the stability of NEAT1_2.Interestingly, it has been shown that there is an increased abundance of paraspeckles in the early stages of amyotrophic lateral sclerosis (ALS). ALS patients also have upregulated levels of NEAT1 and an essential paraspeckle protein, ribonuclear protein fused in sarcoma (FUS), within their motor neurons. In this work we also show that FUS binds to NEAT1 GQs through its C-terminal arginine-glycine-glycine RGG domain (RGG3). This raises the possibility that the NEAT1-FUS interactions are altered in ALS and they might be a target for therapeutic intervention.

808-Symp
Dynamic intramolecular lncRNA triplexes: transcript stability elements and small molecule targets
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An intriguing new class of intramolecular RNA triple helices protects the 3′ end of several lncRNA transcripts from degradation. Transcript abundance is dependent on an element for nuclear expression (ENE), which contains a U-rich internal loop that sequesters A-rich sequences at the lncRNA 3↑ terminus to form a triple helix. We investigated two ENE triplexes of varying lengths and with distinct peripherally stacked duplexes, both of which sufficiently evade degradation machinery in disease causing lncRNAs. Through a combination of mutational analysis and biophysical experimentation complemented with computational simulations our results demonstrate that finely tuned structural dynamics regulate transcript protection. In an effort to explore exogenous control of these ENE triplexes, we employed small molecule microarrays to discover three unique binders. These novel chemotypes exhibit triplex specificity with moderate affinity. Small molecule recognition is coupled to triplex structural rearrangements, which in turn inhibit triplex function and result in the reduction of lncRNA transcript levels. In summary, triplex dynamics mediate small molecule recognition and the ENE protective function.

809-Symp
The multiple dimensions of lncRNAS: how 3D structure determines meg3 function
Marco Marcia.
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Long non-coding RNAs (lncRNAs) are key regulators of gene expression, playing active roles in epigenetics, transcriptional and translational regulation, and chromatin scaffolding. However, because of their recent discovery and molecular complexity, lncRNAs are still very poorly characterized from a mechanistic perspective raising outstanding biological questions on how they selectively and efficiently tune gene expression. In my lab, we are interested