and that antibody crosslinking either in *cis* or in *trans* can contribute to the inhibition potency.

1456-Plat
Molecular characterisation of the transglycosylases involved in lipopolysaccharide maturation and peptidoglycan biogenesis
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Lipopolysaccharide (LPS) O-antigens and the Peptidoglycan (PG) cell wall are core components of the cell envelope of Gram-negative bacteria. The assembly of both polysaccharides requires glycosyltransferases (GT) to generate the glycan polymer. In both cases this requires a homologous TM-embedded GT enzyme, with PG biosynthesis catalysed by RodA, a member of the Shape, Elongation, Division and Sporulation (SEDS) GT family, and the final stage of O-antigen maturation of LPS being controlled by WaaL. Structures of both *C. metallidurans* WaaL and *E. coli* RodA have been solved by using single particle cryo-electron microscopy, the latter in complex with the transpeptidase PBP2. We have used molecular modelling and dynamics simulations to understand how both enzymes engage with their substrates. In doing so we have identified key residues involved in their coordination and catalysis. For RodA, we identify two binding sites for Lipid II and propose a mechanism for Lipid II polymerization into peptidoglycan. We also identify two equivalent sites in WaaL for the binding of undecaprenyl-linked O-antigen and LPS. The active sites for both enzymes sit on the periplasmic face of the transmembrane bundle, with the lipidic substrates anchored within the cell membrane to allow catalysis to proceed. As part of this study we compare and contrast the two critical enzymes.

Platform: Modeling of Biological Systems

1457-Plat
Dynamical systems theory analyses reveal cell cycle-coupled paths of epithelial-to-mesenchymal transition
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Epithelial to mesenchymal transition, EMT, is involved in numerous biological processes such as wound healing, tissue fibrosis, and cancer metastasis. Existing literatures have been debated on whether the transition proceeds through a single or multiple paths, and how cell cycle couples to EMT. To address the above questions, we first generated scRNA-seq dataset, where mammary epithelial MCF10A cells were treated with different doses of TGFβ, an EMT inducer. Then we analyzed the data with dynamo, a machine-learning based analytical framework we developed to reconstruct single cell dynamical equations (Qiu et al. Cell, 2022, 185: 690-711). From the obtained vector fields, we applied the transition path analyses, which are originally developed in studying chemical reactions, on simulated single cell trajectories. The analyses reveal two unique types of transition paths, corresponding to either an arrest in the G1/S or G2/M phase, when cells undergo EMT. The existence of two paths agrees with our previous live cell imaging studies (Wang et al., Sci. Adv. 2020, 6:eaba9309; eLife 2022, 11:e74866), but not pseudotime analyses reported in the literature. Our analyses also reveal a surprising backward cell cycle propagation of cells arrested in G2/M to a G1/S attractor through mitotic skipping. We obtained similar results with a number of other EMT scRNA-seq data sets, then confirmed with live cell imaging using a A549-Vim/RFP-PCNA-EGFP cell line. Our results provide mechanistic understanding of how a cell makes the decision between EMT and cell cycle progression, and demonstrate the importance of analyzing single cell data in the formalism of dynamical systems (Xing, Phys. Biol. 2022, 19: 061001).

1458-Plat
Translation kinetics and diffusive timescales regulate mitochondrial localization of mRNAs in yeast and mammalian cells
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Eukaryotes employ various regulatory mechanisms to maintain subcellular organization in dynamic environments. Localization of nuclear-encoded mitochondrial mRNAs attunes the nuclear genome to mitochondrial protein levels and metabolic needs. Targeting nuclear-encoded mitochondrial mRNAs to the mitochondrial surface is crucial for cotranslational import and proper folding of mitochondrial membrane proteins. Hundreds of mitochondrial mRNAs contain a Mitochondria Targeting Sequence (“MTS”) and preferentially localize to mitochondria using nascent peptide-mediated interactions with mitochondrial import machinery. Recent studies in brewer’s yeast have illuminated MTS-mediated localization as a post-transcriptional mechanism of protein production, identifying two broad classes of mRNA localization patterns–constitutively localized or condition dependent. Our stochastic simulation incorporates gene-specific translation kinetics, MTS exposure and maturation, and diffusive search for mitochondria to explore and predict the localization patterns of mRNAs in brewer’s yeast without the need for mRNA-specific binding partners. Our results point to the interplay of global and gene-specific mechanisms for tuning mRNA localization in response to changing metabolic conditions. Interestingly, conditional and constitutive localization patterns are largely conserved for homologous mRNAs from brewer’s yeast to mammalian cells despite differences in cell size and gene-specific translation parameters. Notably, the conditional class is enriched for crucial TCA cycle genes, making these metabolic mRNAs sensitive to metabolic conditions in yeast and mammalian cells. Mammalian cell types also exhibit similar patterns in gene-specific kinetics for conditional and constitutive mRNAs. Both classes have similar ribosome densities whereas conditional mRNAs have significantly faster initiation and elongation rates. Given that patterns in mRNA localization and translation kinetics are conserved from brewer’s yeast to mammals, we posit that the physical mechanism of mRNA localization is conserved as well.

1459-Plat
High-speed imaging of giant unilamellar vesicle production in cDICE
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Giant unilamellar vesicles (GUVs) are cell-sized containers that are commonly used as three-dimensional model membranes in biophysics, as *in vitro* model systems in synthetic biology, and even as cargo carriers in various other research fields. Despite their ubiquitous use, there is still no one-size-fits-all GUV production method. Over the years, numerous methods have been developed, attempting to meet the demanding requirements of robustness, reliability, and high yield while simultaneously achieving robust encapsulation. Double emulsion-based methods are often praised for their apparent simplicity and good yields; hence, methods like continuous droplet interface crossing encapsulation (cDICE) that make use of this principle, have gained popularity in recent years. In cDICE, aqueous droplets that originate from a capillary orifice are continuously forced through an oil-water interface by centrifugal force, thereby forming a lipid bilayer and thus GUVs. Although cDICE and related methods are frequently used in the field, the complexity of the underlying principles and fluid dynamics has not been considered previously, and how exactly the GUVs are being formed remains unknown. To elucidate the process of GUV formation in cDICE, we have developed a high-speed microscopy setup that allows us to visualize GUV formation in real time. We focused on the capillary orifice, where initial droplet formation occurs, and on the oil-water interface, where droplets are converted into GUVs. Our experiments reveal a complex droplet formation process at the capillary orifice and suboptimal droplet transfer through the water-oil interface, which we explain using fluid dynamics and theoretical modeling. Our results are a first step towards explaining the widely observed variation in encapsulation efficiency and size polydispersity in cDICE. Ultimately, these results will contribute to a better understanding of GUV

formation processes in cDICE and in extension, in double emulsion-based methods in general.

1460-Plat
4D dynamical whole-cell simulations of a growing minimal cell
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JCVI-syn3A is a genetically minimal bacterial cell with a single chromosome consisting of 493 genes that has retained few regulatory proteins or small RNAs. We recently developed a 3D fully dynamical whole-cell model that simulates the first 20 minutes of the cell cycle for this minimal cell before replication and growth. This model, based on experimental characterization of Syn3A and measurements from numerous biochemical experiments in related organisms, consisted of complete reaction networks for genetic information processing and metabolism totaling over 7200 chemical reactions. We now extend this 4D model to simulate the trajectory of a complete cell cycle while we dynamically update the shape of the membrane, chromosome configuration (including replication state), and polysome structures. To capture the broad range of length- and time-scales of the various dynamic processes, we periodically communicate cell state information among several coupled simulation techniques, including: reaction-diffusion and chemical master equation simulations for stochastic reactions, numerical integration of ODE's for metabolic reactions, and coarse-grained Brownian dynamics for the chromosome and membrane. Membrane shapes during division are inferred from experimental fluorescent images of dividing cells. The chromosome configurations are placed using physically realistic energy functions. From the model, we determine time-dependent concentrations, spatial distributions, and reaction fluxes that offer insight into the principles of life for this minimal cell, for example: prediction of doubling time matching the experimental value, realistic distributions of mRNA half-lives, and time-dependent balance of ATP costs and production for all reactions in the cell.

1461-Plat
Utilizing micro-CT imaging to define collagen modeling in murine aorta
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We aim to demonstrate local aortic tissue biodynamics via collagen and elastin fiber remodeling in murine aorta at varying distension using microcomputed tomography (microCT). Ten week-old C57BL/6 mice were injected via tail vein with in-vivo collagen hybridizing peptide, which reforms the triple-helix structure of denatured collagen, and is visualized via fluorescein tag. Careful dissection and removal of the thoracoabdominal aorta was performed, which was pressurized with formalin at varying distensions. Samples were then prepared via heavy-metal staining, and fixed in epoxy resin. These were subsequently imaged at the microCT scanner at University of Chicago, as well as at the 2-BM-B beamline using the Advanced Photon Source at Argonne National Laboratory to obtain micron and sub-micron resolution images and thus provide multi-level views of the tissue. Clear visualization of distinct collagen and elastin layers was observed with microCT. The images also clearly illustrate the effect on individual collagen and elastin fiber organization created by overdistension and shear-stress on the aorta. Clear demonstration of the geometric structure of aortic elastin fibers was seen upon initial segmentation. Preliminary experiments demonstrate that microCT provides an excellent visualization of murine aorta at micron and submicron levels. Next steps will be to segment and mesh this data in order to simulate local tissue responses to stress via finite element analysis. This method will produce a multi-scale imaging model of aortic failure.

1462-Plat
Effect of physical and geometrical stimuli on microvascular dynamics
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The microvasculature is not only a passive organ composed of pipes responsible for the delivery of blood to tissues. It is a highly dynamic organ that actively regulates the passage of nutrients and immune cells to the tissue. More specific, this regulation is bi-directional, where immune cells signal with the blood vessel cells to regulate the recruitment of immune cells to the vasculature, and the subsequent passage of immune cells through the vasculature into the tissue. This regulation of cell trafficking through the vasculature is deregulated in diseases such as atherosclerosis or in cancer, where cancer cells are trafficking through the vasculature in similar manners to immune cells. Besides cell-cell interactions, the dynamics of the vasculature can also be regulated by physical stimuli, such as the properties of the underlying extracellular matrix, the forces the vascular cells exert on each other through cytoskeletal activity, or the geometrical properties of the vascular network. We present mathematical models that elucidate how physical, together with molecular or cellular, stimuli can regulate the dynamic movements of the vasculature, the formations of gaps between vascular cells, and the resulting trafficking of immune or cancer cells through the vasculature. With this model, validated by experiments, we demonstrate that gaps in the vasculature preferentially occur at tricellular junctions, and that cancer cells consequently transmigrate at these locations. We also demonstrate how force distributions change in realistic 3D microvasculature, compared to 2D monolayers, and how this changes gap formation, leakage, and transmigration in 2 versus 3D.

1463-Plat
Vast heterogeneity in cytoplasmic diffusion rates revealed by nanorheology and Doppelgänger simulations
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The cytoplasm is a complex, crowded, actively-driven environment whose biophysical characteristics modulate critical cellular processes such as cytoskeletal dynamics, phase separation, and stem-cell fate. Little is known about the variance in these cytoplasmic properties. We employed particle-tracking nano-rheology on genetically-encoded multimeric 40-nm nanoparticles (GEMs) to measure diffusion within the cytoplasm of the fission yeast *Schizosaccharomyces pombe*. We found that the apparent diffusion coefficients of individual GEM particles varied over a 400-fold range, while the average particle diffusivity for each individual cell spanned a 10-fold range. To determine the origin of this heterogeneity, we developed a Doppelgänger Simulation approach that uses stochastic simulations of GEM diffusion that replicate the experimental statistics on a particle-by-particle basis, such that each experimental track and cell had a one-to-one correspondence with their simulated counterpart. These simulations showed that the large intra- and inter-cellular variations in diffusivity could not be explained by experimental variability but could only be reproduced with stochastic models that assume an equally wide intra- and inter-cellular variation in cytoplasmic viscosity. Through experimental perturbations, we found that the variance in diffusivity was largely independent of factors such as temperature, cytoskeleton, cell cycle stage, and spatial locations, but was magnified by hyperosmotic shocks. The model additionally predicts diffusion through a heterogeneous cytoplasm should be weakly non-ergodic at short time scales, consistent with the experimental data. Taken together, our results provide a striking demonstration that the cytoplasm is not ''well-mixed'' but represents a highly heterogeneous environment in which subcellular components at the 40-nm size-scale experience dramatically different effective viscosities within an individual cell, as well as in different cells in the population. These findings carry significant implications for the origins and regulation of biological noise at cellular and subcellular levels.

1464-Plat
Towards cellular digital twins of in vivo tumors
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To this day, cancer remains an insufficiently understood disease plaguing humanity. In particular, the mechanisms driving tumor invasion still require extensive study. Current investigations address collective cellular behavior within tumors, which leads to solid or fluid tissue dynamics. Furthermore, the extracellular matrix (ECM) has come into focus as a driving force facilitating invasion. Large scale tumor simulations at subcellular resolution represent a promising computational tool to complement the experimental studies, and advances in computational power within HPC systems have enabled the simulation of such macroscopic tissue arrangements. We hereby present our work using Cells in Silico (CiS), a high performance framework for