1311-Pos
Specificity, cooperativity, synergy, and mechanisms of splice-modifying drugs
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Drugs that target pre-mRNA splicing hold great therapeutic potential, but the quantitative understanding of how these drugs work is limited. Here we introduce a biophysical modeling framework that quantitatively describes the genomic specificity, sequence specificity, and concentration-dependent behavior of splice-modifying drugs. Using massively parallel splicing assays, RNA-seq experiments, and precision dose-response curves, we apply this framework to drugs developed for treating spinal muscular atrophy and familial dysautonomia. The results quantitatively define the specificities of risdiplam and branaplam for 5' splice site sequences, strongly suggest that branaplam recognizes 5' splice sites in two distinct molecular conformations, and disprove the prevailing two-site hypothesis for risdiplam activity at *SMN2* exon 7. Remarkably, the results also show that single-drug cooperativity and multi-drug synergy are widespread among splice-modifying drugs more generally. Our biophysical modeling approach thus clarifies the mechanisms of existing splice-modifying treatments and provides a quantitative basis for the rational development of new therapies.

1312-Pos
Unraveling context-specific mechanisms governing calcium-YAP/TAZ distinct relationships through computational modeling
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Yes-associated protein (YAP) and its homolog TAZ are transducers of several biochemical and biomechanical signals, serving to integrate multiplexed inputs from the microenvironment into higher level cellular functions such as proliferation, differentiation, apoptosis, migration, and hemostasis. Emerging evidence suggests that calcium is a key second messenger that closely connects microenvironmental input signals and YAP/TAZ regulation. However, studies that directly modulate Ca^{2+} have reported contradictory YAP/TAZ responses: In some studies, a reduction in Ca^{2+} influx increases the activity of YAP/TAZ, while in others an increase in Ca^{2+} influx activates YAP/TAZ. Importantly, Ca^{2+} and YAP/TAZ exhibit distinct spatiotemporal dynamics, making it difficult to unravel their connections from a purely experimental approach. In this study, we developed a network model of Ca^{2+}-mediated YAP/TAZ signaling to investigate how temporal dynamics and crosstalk of signaling pathways interacting with calcium can alter YAP/TAZ response. By including six signaling modules (e.g., GPCR, IP3- Ca^{2+}, Kinases, RhoA, F-actin, and Hippo-YAP/TAZ) that interact with calcium, we investigated both transient and steady-state cell response to Angiotensin II, Thapsigargin, and ECM stiffness stimuli. The model predicts a context-dependent relationship between calcium signaling and YAP/TAZ activation primarily mediated by PKC, DAG, CaMKII, and F-actin. Model results illustrate the role of calcium dynamics and CaMKII bistable response in switching the direction of changes in Ca^{2+}-induced YAP/TAZ activity. Frequency-dependent YAP/TAZ response revealed the competition between upstream regulators of LATS1/2, leading to the YAP/TAZ non-monotonic response to periodic GPCR stimulation. We also identified key roles of Ca^{2+} and CaMKII dynamics in shaping the nonlinear relationship between cell size and YAP/TAZ activity. The model predicts Ca^{2+}-YAP/TAZ distinct relationships in different settings consistent with experiments. The model predictions provide new insights into the underlying mechanisms responsible for the controversial Ca^{2+}-YAP/TAZ relationship observed in experiments.

1313-Pos
Kinetic modelling of ligand trapping inside acidic vesicles containing alpha-4 beta-2 nicotinic receptors as an explanation for long-term cessation effect of varenicline (Chantix)
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Govind's et. al. 2017 paper proposes that the smoking cessation effect of varenicline (or epibatidine) is caused by these molecules being trapped inside acidic vesicles containing $\alpha4\beta2$ nicotinic receptors. These ligands are weak bases with high affinity to these receptors. The trapping would lead to their slower release and longer desensitization of the receptors, thereby reducing nicotine upregulation. Meanwhile, nicotine, with lower pKa and lower binding affinity is not trapped within the vesicles. It is unclear whether the acidity of the vesicles and the presence of receptors is sufficient to explain the biphasic trapping behavior observed empirically. To better understand this phenomenon, we model flux of ligands between cellular compartments using Flick's first law of diffusion and the Nernst-Plank Equation. The ratio of neutral to charged ligands within each compartment is calculated via Henderson-Hasselbach equation. Main parameters in the model include the number of vesicles, receptors per vesicle, ratio of vesicles to non-vesicle receptors, pH, membrane permeability, pKa and dissociation constants of the ligand. The system is solved numerically using MATLAB and optimized against empirical data. The results show the experimentally observed biphasic release of epibatidine, affirming the hypothesis of trapping inside the vesicles. Our model also shows that the main trapping effect is due to the presence of receptors inside vesicles, not those located on the cell surface and the high affinity of these ligands to the receptors. The exact acidity level inside the vesicles is not as important. Our model is also able to predict the much faster nicotine outward flow compared with epibatidine. This model can be used to predict the kinetics of other weak basic ligands like those used in positron emission tomography.

1314-Pos
Maximizing biological discovery through global computational analyses of unbiased apex biotinylation data on chemokine receptors
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Chemokine receptors CCR2 and CCR5 shape inflammatory responses by driving monocyte recruitment to sites of injury. Unfortunately, through the same mechanism they contribute to the formation of onco-protective tumor microenvironments in cancer and tissue fibrosis in chronic inflammatory conditions. These associations motivated targeting CCR2 and CCR5 in nonalcoholic fatty liver disease and pancreatic cancer, among others.
CCR2 and CCR5 traffick through the cell's endosomal system constitutively and in an agonist-dependent manner; this process contributes to variations in chemokine pharmacology for CCR5 (e.g. existence of superagonists that act as functional antagonists) and to unexpected side effects of CCR2 antagonists (e.g. systemic elevation in chemokine levels). However, the trafficking patterns of these receptors are poorly characterized and specific molecular interactions that regulate them are unknown.
Ascorbic-acid peroxidase (APEX) proximity biotinylation, followed by mass spectrometry, allows for unbiased time-resolved assessment of receptor localization and interactome in live cells. Unfortunately, the resulting datasets are often incomplete and dominated by abundant proteins that mask the subtle effects of treatments. Additionally, massive experiments spanning multiple receptors are hard to design, and when the experiments are conducted separately, technical variation prevents adequate comparisons.
Here we present a novel approach for in-depth computational analyses of APEX data, and demonstrate its utility for CCR2 and CCR5. Through optimal use of linear modeling, we maximize the recovery of significant proteins and enable comparisons between datasets acquired separately, to delineate system-wide treatment effects and to reveal fundamental differences between the receptors. By calculating chemokine response profiles distances with subsequent hierarchical clustering, we discern trafficking patterns of both receptors with a high level of granularity. The findings help understand previously unknown molecular determinants of constitutive and ligand-induced trafficking of CCR2 and CCR5.

1315-Pos
Contact map dependence of a T-cell receptor binding repertoire
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The adaptive immune system protects the host from pathogens by discriminating between host and foreign signatures. T cells of the adaptive immune system undergo training to avoid recognition of self-antigenic signatures displayed

as peptides bound to the major histocompatibility complex (pMHC) by their T-cell receptors (TCRs). Similarly, T cell responses are directed toward TCRs recognizing non-self pMHC. Reliable prediction of relevant TCR-pMHC recognition is centrally important for understanding adaptive immunity predicting optimized infections and cancer, but currently prohibited by the theoretical diversity of antigens ($\sim 10^{20}$) and TCR signatures. We study an affinity-based model for TCR-pMHC binding that uses a crystal structure to inform a distance-based contact map that weights the pairwise amino acid interactions between TCR and pMHC. We find that the TCR-pMHC binding energy distribution depends on the number of contacts and the topology of the contact map of choice; this impacts T-cell negative selection outcome, with higher T-cell recognition probability at higher variances. In addition, we quantify the degree to which neoantigens with mutations in sites with higher contacts are recognized at a higher rate.

1316-Pos

Reaction-diffusion model of Rho-GTPase signaling quantitatively reproduces different types of complex actin dynamics

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In this work, we developed a computational model of Rho-GTPase activity and applied it to investigate actin wave dynamics in cell cortex. Here we focused on cell-level dynamics of cortical actin in oocytes of two organisms: Patiria miniata (starfish) and Xenopus laevis (frog). The model showed a defining role of the early low-activity phase of pattern formation in the development of long-term, high-activity wave dynamics. To our best knowledge, such low-activity dynamics was not described before, however different paths of its destabilization explain GTPase activity at the later experimentally observable phase. In starfish, this complex transient behavior leads to the formation of multiple distinct regions of coherent activity (that we termed 'wave domains'). In frog, spatiotemporal dynamics is different and does not exhibit wave domains. By accounting for the intrinsic noise, our model quantitatively reproduced experimentally observed dynamics in both starfish and frog. We determined parameters responsible for the transition from starfish to frog phenotype, which shed light on the difference in the regulatory pathways of the two organisms. For our quantitative analysis, we developed a novel approach to identification and characterization of wave domains allowing for direct comparison of simulated and experimental images. Overall, our findings provide an insight into the organization of the Rho signaling motif responsible for very complex but still computationally reproducible cell-level actin dynamics.

1317-Pos

Experimental methods and mathematical modeling quantify and predict DUSP1 RNA expression dynamics in response to dexamethasone

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Overactivation of mitogen-activated protein kinase (MAPK) signaling pathways is key to multiple inflammatory responses, and synthetic glucocorticoids (GC), such as Dexamethasone (Dex), have long been used to treat inflammatory pathologies. Upon cell entry, Dex binds to the glucocorticoid receptor (GR), which initiates translocation to the nucleus. Nuclear GR then interacts with Glucocorticoid Regulatory Elements (GRE's) to promote transcription of anti-inflammatory genes including dual-specificity phosphatase 1 (DUSP1). In turn, DUSP1 encodes for the protein mitogen-activated protein kinase phosphatase 1 (MKP-1), which regulates the cell's anti-inflammatory response through dephosphorylation of the P38 and JUN N-terminal kinase (JNK) MAPK pathways. In this presentation, we combine single-cell measurements and discrete stochastic models to elucidate the spatiotemporal mechanisms by which Dex modulates GR localization and DUSP1 transcription. Using single-molecule inexpensive fluorescent *in situ* hybridization, we quantify the nascent transcription, spatial localization, and cellular heterogeneity of DUSP1 mRNA for hundreds of individual HeLa cells at 20 different time points following application of continuous 100 nM Dex stimulation. We observe a rapid increase in DUSP1 transcription sites (TS's) as early as 10-20 minutes, followed by a four-fold increase in expression of mature DUSP1 mRNA that peaks at 75-90 minutes and a temporary decrease in DUSP1 expression at 120-150 minutes post stimulation. We found that expression levels stay elevated during longer time course experiments, and high cellular heterogeneity is observed throughout the post-stimuli response. We used these experimental data to infer parameters and mechanisms for a discrete stochastic model that quantitatively reproduces the time-varying probability distributions for single-cell DUSP1 expression at all time points following Dex stimulation. Finally, we use this model inferred from DUSP1 expression to predict GR translocation dynamics and compare to direct measurement of GR localization dynamic using single-cell immunocytochemistry.

1318-Pos

Analysis of robustness of oscillations in models of the mammalian circadian clock

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The mammalian circadian clock mechanism features robust generation of $\sim$24 h rhythmicity under varied levels of the key clock proteins. To identify such robustness, we use bifurcation analysis to examine the models developed by Kim & Forger. At the core of their original 'single negative feedback' (SNF) model is a negative feedback loop whereby PER binds to and inhibits its transcriptional activator, BMAL1. For robust oscillations to occur, the dissociation constant of PER:BMAL1 complex, K_d, must be several orders of magnitude smaller than a reasonable expectation of 1-10 nM for this protein complex. We relax this constraint by two modifications: first, by introducing a multistep reaction chain for posttranscriptional modifications of *Per* mRNA and post-translational phosphorylations of PER, and second, by replacing the first-order rate law for nuclear degradation of PER by a Michaelis-Menten rate law. These modifications lengthen time delays, thereby increasing the maximum allowable K_d for robust oscillations to $\sim$2 nM. In a third modification, we consider an alternative rate law for *Per* gene transcription to resolve an unrealistically large transcription rate at very low concentrations of BMAL1. Additionally, we studied extensions of the SNF model to include a second negative feedback loop (involving REV-ERB) or a supplementary positive feedback loop (involving ROR). Contrary to Kim & Forger's observations of these extended models, with our modifications, the supplementary positive feedback loop makes the oscillations more robust than observed in the models with one or two negative feedback loops. However, all three models are similarly robust when accounting for $\sim$24 h period with $K_d \geq 1$ nM. Our results suggest crucial roles of the biochemical details of *Per* gene expression and provide testable predictions for future studies.

1319-Pos

Thermoresponsive artificial cells

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We build thermoresponsive phospholipid-based artificial cell model compartments that respond to mild temperature changes in the environment by performing reversible shape deformations, enabling material transfer between entities, and migration. The temperature increase is in the laboratory applied by means of an IR-B-laser, and induces the self-organization of lipid compartments into dynamic clusters. The clusters are motile and can migrate along the temperature gradient. To increase the thermoresponsiveness, we encapsulate poly-N-isopropylacrylamide (PNIPAAm) in the lipid compartments. The polymer solution undergoes reversible gel formation when heated above its lower critical solution temperature (LCST) of 32 °C. We observe the formation of microaggregates inside the lipid compartments which can interact with the membrane and induce membrane deformations. Our experimental system comprises a contactless method to generate compartmentalized multicellular model structures with thermoresponsive properties.

1320-Pos

Bifurcation and multistability in three-gene-driven network models

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Control of transcription presides over a vast array of biological processes. This control typically manifests through a web of regulatory circuits with different genes interacting under a range of feedback architectures, often exhibiting multistability as a result. Our work uses a geometric approach grounded in bifurcation theory to study the stability profile of a mutually repressing three-gene network across different regions of an unconstrained parameter space. The symmetric network exhibits a distinct dynamic topology as the relative repressive strengths among the genes change, with greater complexity as the genes become more similar in their regulatory activity. We also observe transitions across topologies in the bifurcation plane, and the parameter thresholds under which they occur. These boundaries broaden in parameter space as coupling sensitivity rises via the Hill coefficient and as a higher growth rate implies