headgroups, but without the G-protein. Consistent with our hypothesis, the TM6 – TM7 interactions are similar to the G-protein coupled state in the presence of PS, but similar to the inactive state in the absence of PS. Moreover, the interaction of a PS headgroup with a key residue on TM6 recapitulates the same interaction with the glutamic acid, but only when the receptor is in the active state.

2483-Pos

Molecular determinants of distinctive opioid receptor subtype affinities

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Despite being targeted by over 30% of the drugs available on the market, G-Protein Coupled Receptors (GPCR) have proven to be notoriously difficult to characterize on an atomistic level. While it is known that helix 6 (TM6) movement is necessary for signal transduction, structural details of activation remain elusive and each receptor is generally studied individually, making comparisons between homologous proteins challenging. Given their homology, any newly discovered drug targeting a specific GPCR may be promiscuous, adding work- and cost-intensive dimensions to the drug discovery process. Some of the GPCRs that seem the most appealing as drug targets are opioid receptors. As opioids are potent pain relievers with widespread use and addictive properties, understanding how drugs bind particular opioid receptor subtypes would allow the design of non-addictive derivatives and thus help alleviate the ongoing opioid crisis - in the United States only, it is estimated that 4 million people use opioids recreationally or are dependent on them, with about half overdose-related deaths being related to synthetic opioids. In this work, we have used enhanced-sampling molecular dynamics simulations to explore the conformational dynamics of different subtypes of opioid receptors and uncovered the molecular determinants of their affinity to various endogenous peptides and synthetic opioids. Using enhanced sampling techniques, we have obtained free energy surfaces (FES) of activation of the μ-, κ-, and δ-subtypes. By comparing the metastable states obtained from the FES, we have pinpointed key structural differences relevant to the activation process. These findings will allow the development of simple and accurate structure-activity relationship, which in turn will guide the design of new, less addictive opioids.

2484-Pos

AI-assisted de novo design of selective κ-opioid receptor antagonists for the treatment of opioid addiction

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Daunting estimates of overdose fatalities, costs of healthcare, lost productivity, and criminal justice involvement for the misuse of prescription opioids have converted opioid use disorders into a major national crisis in the United States. Among likely efficacious pharmacological interventions for the treatment of opioid dependence are those that can attenuate brain reward deficits experienced during periods of abstinence. Pharmacological blockade of κ-opioid receptors (KOR) has been shown to abolish brain reward deficits detected in rodents during withdrawal from opioids, as well as to reduce the escalation of opioid use in rats with extended access to opioids. Although KOR antagonists represent promising candidates for the treatment of opioid addiction, very few potent selective KOR antagonists are known to date and most of them exhibit significant safety concerns. Here, we used a deep learning framework for the *de novo* design of ligands with predefined molecular properties to predict new chemotypes with KOR antagonistic activity. Specifically, we pre-trained a deep generative tensorial model to learn a mapping of the chemical space from a ZINC subset of purchasable drug-like molecules, as well as sets of effective ($IC_{50} < 1$ μM) or less effective ($IC_{50} \geq 1$ μM) known KOR inhibitors from the ChEMBL database, while simultaneously encoding the relationship between the molecules and their properties. Subsequently, we biased the resulting generative model towards the development of putative KOR antagonists with a reinforcement learning algorithm that rewarded similarity to the antagonist binding mode revealed by the JDTic-bound KOR crystal structure, as well as novelty, synthetic accessibility, absence of unstable and reactive moieties, drug-like solubility, and blood-brain barrier permeability. The generated molecules were prioritized for chemical synthesis and functional evaluation based on their predicted optimal interactions with the receptor.

2485-Pos

Dissecting diffusion and oligomerization dynamics of GPCRs in live cells using single-particle tracking

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Crucial insights about the signaling and trafficking mechanisms of G Protein Coupled Receptors (GPCRs) can be derived from their membrane diffusion properties. Previous studies in our lab (Shivnaraine et al., *JACS* 2016, Li at al., *Biophys. J.* 2018) have shown that the M2 muscarinic receptor (M_2R) can be purified as oligomers, yet the oligomerization dynamics and the implications for signalling in live cells remain elusive. Here we used single-molecule fluorescence techniques, such as single-particle tracking (SPT) and single-molecule photobleaching (smPB), to characterize the dynamic distribution of M_2R in HEK293 cells. To that end, receptors have been co-expressed at their N-terminus either with a green fluorescent protein (eGFP), or with a HaloTag for labelling with HaloTag ligand (HTL) dyes, such as JF549 HTL. For comparison, we applied the same techniques to μ-opioid receptors (MORs) in live U2OS cells. For both cases, measured diffusion maps are spatially and temporally heterogeneous, with receptors transitioning between normal and anomalous diffusion regimes. The analysis of experimental data was reinforced by simulations and by control measurements on monomeric (CD86) and dimeric (CD28) membrane proteins. Intensity traces of immobile, single receptor complexes and of monomeric/dimeric controls in the membrane of fixed cells were analyzed using an in-house smPB code based on a Bayesian algorithm (Garry et al., *J. Chem. Phys.* 2020). The results confirm the presence, correlated with expression levels, of higher order M_2R oligomers in cells, and lay out the foundation for subsequent studies of their functional role.

2486-Pos

Comprehensive elucidation of developmental functions of arachidonic acid-derived lipid mediators in zebrafish

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Prostaglandins (PGs) and leukotrienes (LTs) are major physiologically active lipid, which are biosynthesized from arachidonic acid (AA). AA is released from membrane phospholipids by phospholipase A_2 (PLA_2), and thus PGs and LTs are derived from membrane phospholipids. Zebrafish is a vertebrate model organism that has been widely used in the studies on development and drug discovery for the reasons as follows: (i) embryo development occurs outside the maternal body, (ii) embryo is transparent, (iii) high conservation between human and zebrafish genes, etc. Nevertheless, the systematic identification and characterization of zebrafish PG and LT receptors have not been performed. Therefore, we identified twelve zebrafish PG receptors and characterized their pharmacological properties. Furthermore, to identify the physiological role of zebrafish PG receptors, we generated each receptor-deficient zebrafish, and analyzed developmental processes. Here, we report that PGE_2 plays essential roles in the embryonic lymphatic development through the EP3 receptor, one of the PGE_2 receptors. Using pharmacological and genetic approaches, we demonstrated that the COX1-derived PGE_2-EP3 pathway is required for embryonic lymphatic development by upregulating the expression of critical factors for the lymphatic specification. We recently focused the zebrafish cysteinyl LT (cysLT) receptors, cloned four zebrafish cysLT receptors, and analyzed the expression profile of these receptors in developmental stages. As results, we revealed that the four zebrafish cysLT receptors show unique tissue distribution patterns. Each receptor may hence be involved in unique physiological functions. This work provides further insights into the diverse functions of arachidonate metabolites in zebrafish.

2487-Pos

The role of cardiac capillary endothelial cells in electro-metabolic signaling and blood flow regulation

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The *''Electro-metabolic signaling (EMS)''* hypothesis of blood flow control postulates that ventricular myocytes themselves are simultaneously the end-stage metabolic consumers, sensors and master controllers of their own blood