large-scale tissue simulation previously developed by us. Combining a cellular Potts model and an agent-based layer, CiS is capable of simulating tissues composed of tens of millions of cells, while accurately representing many physical and biological properties. However, in order to realistically represent tumor dynamics, CiS needs to be parameterized. We present strategies and pitfalls in investigating multiple experimental data sources for this task. In particular, we highlight recent success utilizing a feature-extraction based deviation score method for the comparison of simulated and experimental tumor spheroids.

Platform: Mitochondria, Endoplasmic Reticulum and Bioenergetics

1465-Plat

Living on hydrazine: Metabolic protein complexes from an anaerobic ammonium oxidizer

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The discovery of anammox bacteria in the 1990s has dramatically changed our understanding of the global nitrogen cycle. Anammox bacteria are now believed to be responsible for up to 30 to 70% of the nitrogen removal from the oceans. These organisms have the unique metabolic ability to combine ammonium and nitrite to form dinitrogen gas, a process that takes place in a special cellular compartment, the anammoxosome. To elucidate how bacteria perform such extraordinary chemistry, we have determined the structures of the key enzymes in this process. Central to harvesting the energy from hydrazine is the hydrazine dehydrogenase (HDH), which converts hydrazine into dinitrogen gas, liberating four extremely low-potential electrons (−750 mV). Our crystal and cryo-EM structures reveal that this 1.7-MDa complex contains an extended system of 192 heme groups spanning the entire complex, which is only accessible via narrow holes in the side of the complex. Moreover, we identified an unexpected assembly factor for this complex. In addition, anammox bacteria obtain additional reducing equivalents through the oxidation of nitrite to nitrate, which is catalyzed by a nitrite oxidoreductase (NXR) related to the NXR from nitrifying bacteria. Despite its importance in the biogeochemical nitrogen cycle, essential issues on NXR functions remain unanswered, particularly due to the lack of structural information. To meet this challenge, we used a multiscale approach combining cryo-electron tomography, crystallography, single-particle cryo-EM together with reconstitution studies and enzyme kinetics to characterize NXR. We show that, in contrast to what was shown for NXR in NOB, NXR of anammox bacteria forms tubule-like structures inside the anammoxosome held together by a novel subunit NXR-T. As with the hydrazine dehydrogenase structure, our multiscale structure of the anammox NXR tubules suggest how electrons are passed on to redox partners.

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Disordered regions of respiratory supercomplexes offer new pathways for substrate channeling in crowded membranes

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Are protein supercomplexes biological redundancies or do they have a functional role? This question has been perplexing biophysicists and bioenergeticists for decades, especially on the significances in crowded environments. Now, cryo-EM has brought forth remarkable insights into the structures of supercomplexes, but functional relevance of their intrinsically disordered regions remains a mystery. First, using a combination of maximum entropy (MaxEnT)-guided molecular simulations with low-resolution cryo-EM data, we have resolved the disordered Qcr6 subunit of respiratory complex III (CIII). Second, porting this whole-CIII model into the crowded yeast $CIII_2CIV_2$ supercomplex, long-timescale Brownian Dynamics computations were performed. We found that the negative charge on the disordered region surprisingly cooperates with that of the anionic lipids in the mitochondrial membrane to attract a pool of complementary protein substrates in its vicinity. Third, we discovered how the transient fold of Qcr6 expedites directional diffusion of the substrates from CIII to CIV that simultaneously leverage the supercomplex architecture as well as the electrostatic environment of the membrane. A re-classification of the EM images based on our computational model have brought to light new density features that indeed describe the 2-dimensional dynamic of the substrate in crowded supercomplexes. Finally, using multisequence alignment via Alphafold2, mutations are designed to control the diffusivity of substrates by tuning the disorder of the protein surface, for future biochemical validation.

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ATP synthase c-subunit leak channel as a novel therapeutic target

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Mitochondrial ATP synthase plays a key role in cell life and death by catalyzing the ATP synthesis and housing a leak channel of mitochondrial permeability transition. Various pharmacological and natural compounds that target mitochondria and ATP synthase were under clinical trials recently including bedaquiline (against mycobacterial tuberculosis) and resveratrol (for treating Alzheimer's disease). Here in this work, we are evaluating the effects of these compounds on the structure and leak channel activity of ATP synthase. Purified ATP synthase from porcine heart mitochondria was reconstituted in a planar lipid bilayer to study the single-channel activity of ATP synthase. Electrophysiological experiments revealed a profound inhibitory effect of bedaquiline on the single channel activity of ATP synthase. Additionally, we observed a concentration-dependent dual role of resveratrol on ATP synthase channel activity. Higher concentrations of resveratrol activated the channel while nanomolar concentrations inhibited the ATP synthase leak channel activity. Similar results were obtained with a less sensitive but more classical calcium retention capacity (CRC) assay for measuring the mitochondrial permeability transition pore (mPTP) opening, establishing the involvement of these compounds in modulating mPTP. Currently, we are investigating the ATP synthase structure bound to these compounds by cryo-electron microscopy (cryo-EM) to explore the channel gating mechanism. These findings will lead to the development of structure-based therapeutic drugs targeting the ATP synthase c-subunit leak channel for treating mPTP-related diseases.

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A novel therapeutic strategy against mitochondrial respiratory chain dysfunction-linked diseases

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Mitochondrial diseases result from a decreased oxidative phosphorylation (OXPHOS) that leads to a broad spectrum of incurable pathologies. Our goal was to understand whether membrane permeant small molecule(s) can be exploited to treat OXPHOS-related diseases as an alternative to gene therapy. Therefore, we selected some molecules for their ability to replace the redox functions of complex III and among them identified pyocyanin as a promising agent. Pyocyanin is a bacterial redox cycler that can shuttle electrons from reduced coenzyme Q to cytochrome c, acting as an electron shunt. Sub-μM dose of pyocyanin is harmless, restores respiration and increases ATP production in *Ttc19*$^{-/-}$ mouse embryonic fibroblasts as well as in fibroblasts from patients harboring pathogenic mutations in three different assembly/stabilization factors of complex III (namely, TTC19, BCS1L and LYRM7). The drug normalized the mitochondrial membrane potential, mildly increased ROS production, and triggered mitochondrial biogenesis. These in vitro effects were confirmed in both *Drosophila melanogaster*^{TTC19KO}, in *Danio rerio*^{TTC19KD}. Here we show that pyocyanin and its newly synthesized derivative with enhanced life-time and tissue distribution exhibited a benefit in Ttc19 KO mouse model as well. Indeed, in all these models, administration of low, non-toxic concentration of pyocyanin significantly ameliorated movement proficiency, without inducing toxicity. Likewise, pyocyanin, able to receive electrons from NADH, showed a beneficial effect also in the case of cells and mice with complex I disease. Our results point to exploitation of redox cyclers for therapy against diseases due to OXPHOS dysfunction.