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1130-Pos

Insight into Bcl-2 proteins' functioning at mitochondrial membrane level

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Programmed cell death (apoptosis) is essential in life. In its intrinsic apoptotic pathway opposing members of the B-cell lymphoma 2 (Bcl-2) protein family control the permeability of the mitochondrial outer membrane (MOM) and the release of apoptotic factors such as cytochrome c. Any misregulation of this process can cause disorders most prominently cancer, where often upregulation of cell protecting (anti-apoptotic) Bcl-2 members such as the Bcl-2 membrane protein itself plays a notorious role by blocking MOM perforation by - often drug induced - apoptotic proteins such as Bax which would cause cancer cell death normally. Here, we apply neutron reflectometry (NR) on supported lipid bilayers which mimic MOM environment and solid state/liquid state NMR spectroscopy to unravel the molecular basis driving opposing proteins to interact with each other at the MOM; a mechanism which is not really understood yet due to lack of high-resolution structural insight. Based on our central hypothesis that Bcl-2 drives its cell-protecting function at a membrane-embedded location as revealed by NR (1), we focus i) to determine the structure of human Bcl-2 protein in its membrane setting by combining solution and solid-state NMR; ii) use NR to study the kinetics and lipid/protein pore assemblied upon binding of Bax to mitochondrial membranes and its membrane destroying activities there; and iii) unravel the nature of direct interaction between Bcl-2 and Bax to neutralize each other. Knowledge generated here, will be indispensable in understanding the regulative function of the Bcl-2 family at mitochondrial membranes. 1. A. Ul Mushtaq, J. Åden, L.A. Clifton, H. Wacklin-Knecht, M. Campana, A.P.G. Dingeldein, C. Persson, T. Sparrman, G. Gröbner. Neutron reflectometry and NMR spectroscopy of full length Bcl-2 protein reveal its membrane localization and conformation. Communications Biology 4 (2021) 507.

1131-Pos

Nano and micro-vesicle diffusion through proteins, electrostatics mediate its interactions

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The primary biochemical communication in the body is mediated by vesicles, from energy transport to neural signals. Understanding the driving forces of its diffusion will proportionate light to comprehend the nature of some diseases. In this work, we investigate the diffusion of nano and microvesicles in a bovine serum albumin solution, as we alter their temperature and electrostatic charge. Their molecular interactions were studied by differential scanning calorimetry and its short-long range interactions by dynamic light scattering and video tracking. Results showed that the phase transition of the lipids alters its diffusion drastically and that the nano-size vesicles with net negative electrostatic charge are the most reactive to the protein.

1132-Pos

Nanodiscs as a novel approach to resolve inter-protein energy transfer within the photosynthetic membrane of purple bacteria

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Photosynthetic purple bacteria capture sunlight and convert it to chemical energy with almost 100% quantum efficiency: almost every photon absorbed leads to charge separation. Such high efficiency is achieved through a series of ultrafast inter-protein energy transfer events within an antenna network of light-harvesting proteins located in the photosynthetic membrane. Understanding how the organization of proteins within the membrane leads to efficient energy transfer is imperative to designing efficient artificial solar harvesting techniques. Determining inter-protein energy transfer between light-harvesting complex 2 (LH2) proteins, the most common light-harvesting protein *in vivo*, has proven challenging due to the heterogeneous organization of proteins within the membrane environment. In this work, we introduce model membrane nanodiscs as a novel technique to reconstruct the complex membrane environment in a controlled manner. By forming nanodiscs large enough to incorporate two variants of LH2, we can directly resolve inter-protein energy transfer. By controlling nanodisc size, we can change the inter-protein distance and resolve the effect of membrane organization on energy transfer rate. Using a combination of ultrafast transient absorption spectroscopy, cryogenic electron microscopy, and quantum chemical calculations, we find that LH2 complexes prefer to associate closely in the membrane (25 Å), with an energy transfer rate of 5.7 ps. The results suggest that these tightly-packed LH2s are important for long-distance energy transfer, as the 25 Å distance is similar to the most common inter-protein distance *in vivo*. Overall, our work introduces nanodiscs as a platform to study complex energy transfer events and the effect of membrane organization on critical biological processes.

1133-Pos

Achieving anticancer peptides: Protein-lipid interactions in breast cancer models

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Within the last decade, bioactive peptides with anti-cancer properties have become part of the therapeutic arsenal. While being less hazardous to normal cells, membrane-lytic peptides function as possible anti-cancer drugs that specifically target tumour cells leading to necrotic cell death. Anti-cancer peptides (ACP) can be easily restructured, have great selectivity and penetration efficiency, and are obtained from a variety of natural sources. However, the malignant cell selectivity of ACPs remains elusive. Human triple-negative breast cancer (TNBC) tumours are known for having a poor prognosis and limited treatment options. By understanding the lipidome of TNBC cells in comparison with their healthy counterparts, the development of targeted treatments can be better realised. This work builds on our understanding of TNBC tumours and explores protein-lipid interactions using lipidomic and mass spectrometry approaches to better understand the molecular determinants of ACP selectivity.

1134-Pos

Novel approaches to understand function and explore inhibition within protein-membrane interfaces

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Despite their importance in biology and disease, peripheral membrane proteins (PMPs) have presented a significant challenge. This class of proteins reversibly binds to membranes to perform function. High-resolution functional and structural studies of PMPs are often limited to their water-solubilized state due to technical limitations. Large blind spots in functional interactions within membranes are therefore common and require methodological and technological advancement to overcome. Additionally, inhibitor development for PMPs in their functionally relevant, membrane embedded state is difficult, often impossible, using current methods. We address these barriers by utilizing newly developed membrane mimicking reverse micelles (mmRMs), which house PMPs within a spherical, nanoscale assembly of lipids. Studies using mmRMs have been applied to several PMPs, including glutathione peroxidase 4 (GPx4). Use of mmRMs to investigate PMPs has proven advantageous over other membrane models for various NMR-based experimental approaches. Observations of protein-lipid interactions and lipid specificity determinations are greatly enhanced. Applying mmRMs to PMP structural analysis promises to reveal greater detail of the effects of membrane binding. Finally, mmRMs are helping to overcome challenges associated with inhibitor discovery and design for membrane embedded PMPs. mmRM-based screening for fragments in the protein-membrane interface reveals building blocks that may be advanced to inhibitors. Furthermore, discovery of small-molecule binders within membrane interfaces promises to reveal fundamental properties of this largely unexplored chemical space. Together, these approaches promise to advance our