THE IMPORTANCE OF DETERGENT SELECTION: ARE YOU CHOOSING THE RIGHT DETERGENT FOR YOUR MEMBRANE PROTEIN?

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In the time that has elapsed since the deposition of the first membrane protein structure in 1985, we have witnessed many significant strides in the field. Despite the substantial growth and tremendous success, bottlenecks persist throughout the membrane protein workflow, including expression, solubilization, purification and crystallization. For just as long, Anatrace Products has been a trusted resource in membrane protein research, due to their large portfolio of detergents and lipids, and unsurpassable standards in quality and reproducibility.

We are excited to present an updated overview of detergent usage in membrane protein solubilization, purification, and crystallization which highlights the importance of detergent choice throughout each step of the membrane protein crystallization pipeline. Always committed to innovating, we have developed new tools to simplify the detergent selection process. Data will presented from our Analytic Extractor and Analytic Selector kits, designed to facilitate the choice of detergent for protein extraction (Extractor) and downstream applications including crystallization, NMR, EM, and binding studies (Selector). Lastly, we will introduce new detergent offerings, including novel trehalose-based detergents, and bicelle kits for crystallization.

Session II - Crystallization of Macromolecular Complexes
Sunday, July 3 - afternoon

GENERATION OF ANTIBODY-Fab REAGENTS TO CAPTURE AND STABILIZE FUNCTIONALLY IMPORTANT CONFORMATIONAL STATES OF PROTEINS TO FACILITATE THEIR STRUCTURE DETERMINATION BY CRYSTALLOGRAPHY AND SINGLE PARTICLE CRYO-EM

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A high throughput phage display pipeline has been established to generate Fab-based synthetic antibodies that have been successfully used as high performance crystallization chaperones. The phage selection strategies have been developed to target Fab binding to specific surface epitopes or to capture and stabilize functional conformational states to facilitate probing both the protein’s static and dynamic features, as well as the transitions between states. In most cases, the high throughput capability of the pipeline provides researchers with at least 10 unique Fabs that perform a desired function. A further enhancement for the generation of Fabs for membrane proteins is the use of lipid-filled nanodiscs to provide membrane-like environments for membrane proteins during phage display selections. Fabs generated from nanodisc formats provide guidelines for which detergents best (and worst) mimic the protein’s membrane-like lipid environment, which can guide choices for setting up crystallization and Cryo-EM experiments.
MACROMOLECULAR MACHINES IN GENOME MAINTENANCE
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UV-exposure of the skin result in the covalent cross-links of neighboring DNA nucleotides, introducing mutations in the genome if left unrepaired. Patients suffering from Xeroderma pigmentosum (XP) fail to effectively repair these DNA lesions, resulting in heightened propensity to develop skin cancers (melanomas, squamous cell, & basal cell carcinomas). My lab has solved the structures of these protein complexes and delineated their mode of action. We provided the mechanism by which this molecular sunscreen works, and how it is lost in XP cancer patients. In the presence of this DNA repair machine skin cancer rates are suppressed by more 1000-fold providing a major means of safeguarding the genome.

CRYSTALLIZATION OF CHROMATIN COMPLEXES
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Chromatin complexes of chromatin factors or enzymes bound to the nucleosome offer multiple challenges common to crystallizing multicomponent macromolecular complexes: producing milligram quantities of homogeneous sample, growing crystals of the desired complex and improving diffraction of the crystals to high resolution. I will describe our efforts to crystallize different chromatin complexes, including the RCC1/nucleosome complex and the Polycomb PRC1/nucleosome complex, and what we have learned from these efforts. For the 300 kDa RCC1/nucleosome complex, varying the RCC1 (Regulator of Chromosome Condesation) species improved initial imperfect crystals to block single crystals which diffracted anisotropically and finally to block single crystals which diffracted isotropically. Post-crystallization soak of crystals in dehydrating solutions made it possible to collect diffraction data to 2.9 Å. I will also describe procedures we use to optimize complex reconstitutions and to evaluate whether initial crystals contain the chromatin factor or enzyme.
Cell cycle gene expression is mediated by two transcription factor complexes known as DREAM and Myb-MuvB (MMB). DREAM represses cell cycle genes in quiescence and G1. Upon cell cycle entry, DREAM dissociates and MMB binds promoters to activate gene expression. While studies have identified the broad transcriptional roles of DREAM and MMB, the structure and biochemical function of these complexes have been poorly characterized. Both complexes share a core scaffold of five proteins known as MuvB, which binds DNA and histones. We are using X-ray crystallography to study the structure and function of MuvB and to understand how the association of MuvB with repressing and activating transcription factors is regulated. Our strategy relies on co-expression of multi-component complexes and subcomplexes using the baculovirus system. We have successfully designed subcomplexes suitable for crystallization using structure prediction, co-precipitation, and limited proteolysis. X-ray structures of these subcomplexes have revealed how MuvB interacts with DNA, how DREAM and MMB are assembled, and how the complexes bind histones. Our structures and complimentary data support a novel nucleosome-positioning model for MuvB function.

Figure 1. X-ray crystallography studies of the MMB complex. Schematic for the proposed domain organization is in the center. Crystals, diffraction data, and structures are shown for several subcomplexes.