OPTIMIZATION OF ANALYTICAL TOOLS FOR NANODISC CHARACTERIZATION

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ADIMAB

BACKGROUND

Membrane proteins are the most prominent drug targets, with G-Protein Coupled Receptors (GPCRs) comprising a key target class.[1] However, membrane proteins are difficult to study *in vitro* outside the cellular environment due to their low abundance and hydrophobicity. Detergents have traditionally been employed to stabilize the hydrophobic regions of membrane proteins; however, these can impact native conformations and disrupt weakly associated protein complexes. Since the cell membrane is required for proper folding and activity, various membrane mimetics have been employed to provide a native-like environment.

One type of membrane mimetic is a protein nanodisc. Nanodiscs are water-soluble molecules that contain a membrane protein surrounded by a lipid bilayer encircled by a scaffold that acts as a "belt." The scaffold can be two copies of a membrane scaffold protein (MSP) or a polymer. MSP-based nanodiscs first require detergent solubilization of the membrane protein, followed by dialysis for nanodisc formation and form large nanodiscs up to 50 nm.[2] In contrast, polymer-based nanodiscs can directly extract membrane proteins from a cell membrane, preserving its native lipid environment. However, the extraction process can be inefficient and have low yield.

Here, we show the efforts to generate and characterize in-house produced nanodiscs. The nanodiscs are homogeneous and functional, making them high quality reagents for our flow cytometry-based selections.

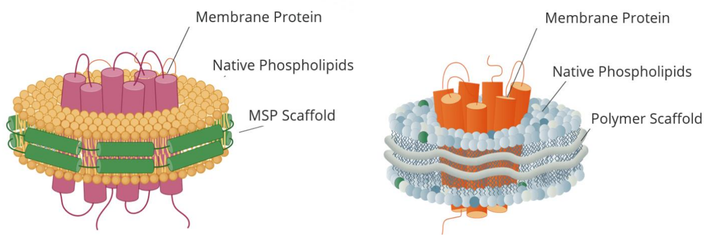

Figure 1. Types of nanodiscs, a membrane mimetic, featuring different scaffold types. Left, membrane scaffold protein (MSP) scaffold[3]. Right, polymer-based scaffold[4].

COMMERCIALLY-SOURCED NANODISCS HAVE VARYING QUALITY

Dynamic light scattering (DLS) is typically used to assess the size of nanodisc-stabilized membrane proteins. In DLS, intensity fluctuations of scattered light from Brownian motion is used to determine the size of particles in solution. From the diffusion coefficient, the average hydrodynamic radius of each population within the sample can be determined to evaluate its size and assess homogeneity.

Nanodiscs carrying the same GPCR were sourced from 3 different vendors using either a polymer or MSP scaffold. Their size and homogeneity were assessed using dynamic light scattering (DLS) and show varying size distributions.

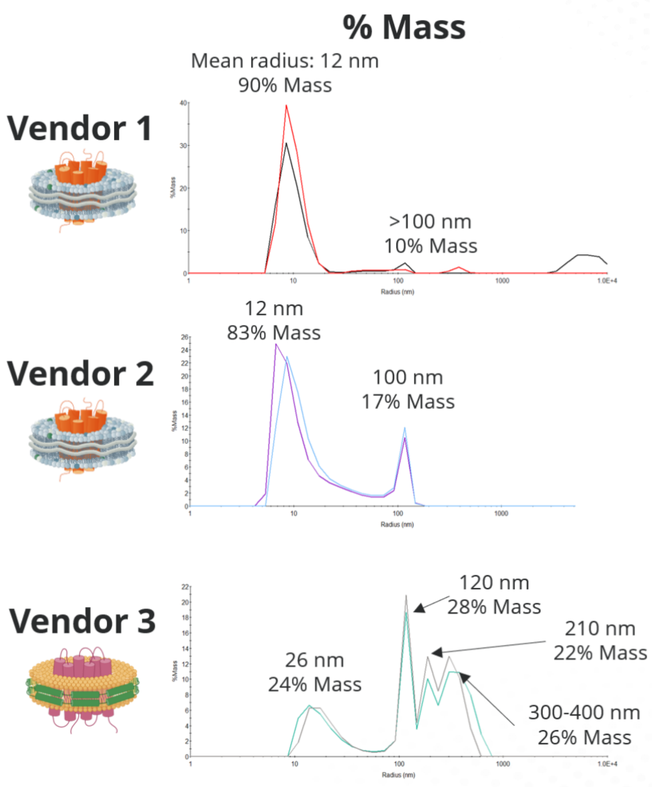

Figure 2. Dynamic light scattering % mass data showing the hydrodynamic radii for nanodiscs commercially sourced from 3 different vendors. Vendors 1 and 2 have similar profiles, while Vendor 3 is largely aggregated. Two replicates shown.

DLS TO INFORM IN-HOUSE NANODISC PURIFICATION STRATEGY

The varying homogeneity of commercially available nanodiscs is one of the motivations of our in-house efforts to produce nanodiscs. Here we use polymers to directly extract a GPCR from cell membranes for purification and characterization. DLS was used to assess the quality of purification elutions, to inform the purification strategy and assess the quality of the final purified material.

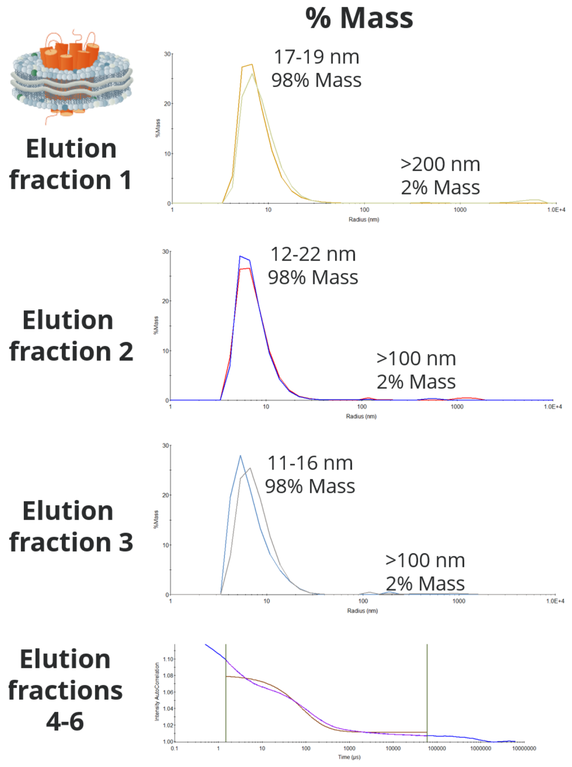

Figure 3. Dynamic light scattering % mass data showing the size distribution for purification elution fractions 1-3; and an example intensity autocorrelation plots for elution fractions 4-6. Elution fractions 1-3 have high homogeneity with most molecules ~20 nm in radius, while elution fractions 4-6 are too heterogeneous to analyze and were not pooled. Two replicates shown.

IN-HOUSE NANODISCS BIOTINYLATED *IN SITU* ARE FUNCTIONAL

A GPCR was biotinylated *in situ* upon expression in mammalian cells. The subsequent biotinylated nanodiscs were tested for activity using biolayer interferometry (BLI). The biotinylated nanodisc was loaded to streptavidin sensors against its antibody in solution.

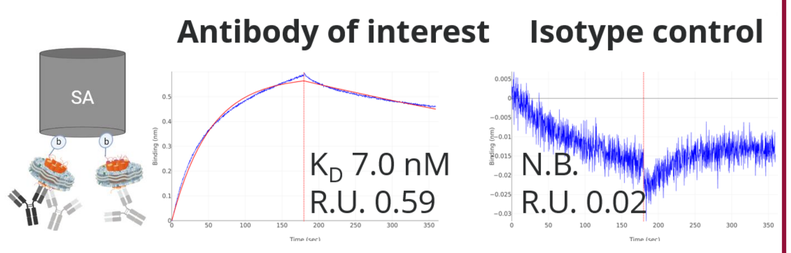

Figure 4. Biolayer interferometry (BLI) characterization indicates *in situ* biotinylated nanodiscs show activity towards their specific antibody. SA, streptavidin; R.U., response units; N.B., non-binder.

NANODISCS CAN BE CAPTURED VIA TAG FOR KINETICS CHARACTERIZATION

In-house nanodiscs can be captured via a twinstrep tag for kinetics characterization. An anti-Strep antibody can be used to capture the twinstrep-tagged GPCR containing nanodisc on a sensor with the subsequent functional antibody in solution. This highlights the ability to design the tag system for the intended target and use in characterization and in flow cytometry-based selections.

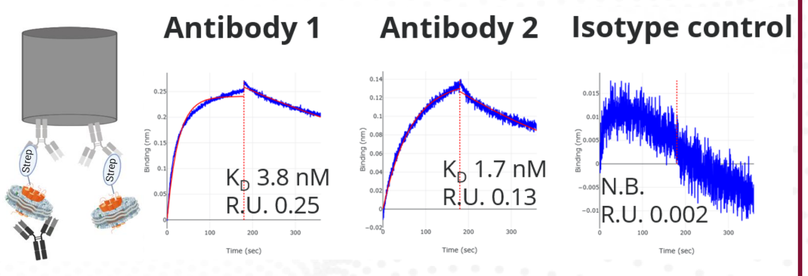

Figure 5. Biolayer interferometry (BLI) kinetics characterization using an anti-Strep antibody to capture the twinstrep-tagged nanodisc on sensor, with the functional antibodies in solution. Both antibodies bind with the expected affinity, while the isotype control does not bind. R.U., response units; N.B., non-binder.

POLYMER SCREEN TO GENERATE IN-HOUSE NANODISCS

Nanodiscs were generated using a polymer screen from Cube Biotech[3] to test membrane extraction efficiency, size and homogeneity for a GPCR of interest. A dot blot probing with an anti-strep antibody for the twinstrep-tagged nanodisc was used to assess the extraction efficiency with each polymer. Nanodiscs with Amphipol 18 had the highest amount of active protein and had high homogeneity for the target protein.

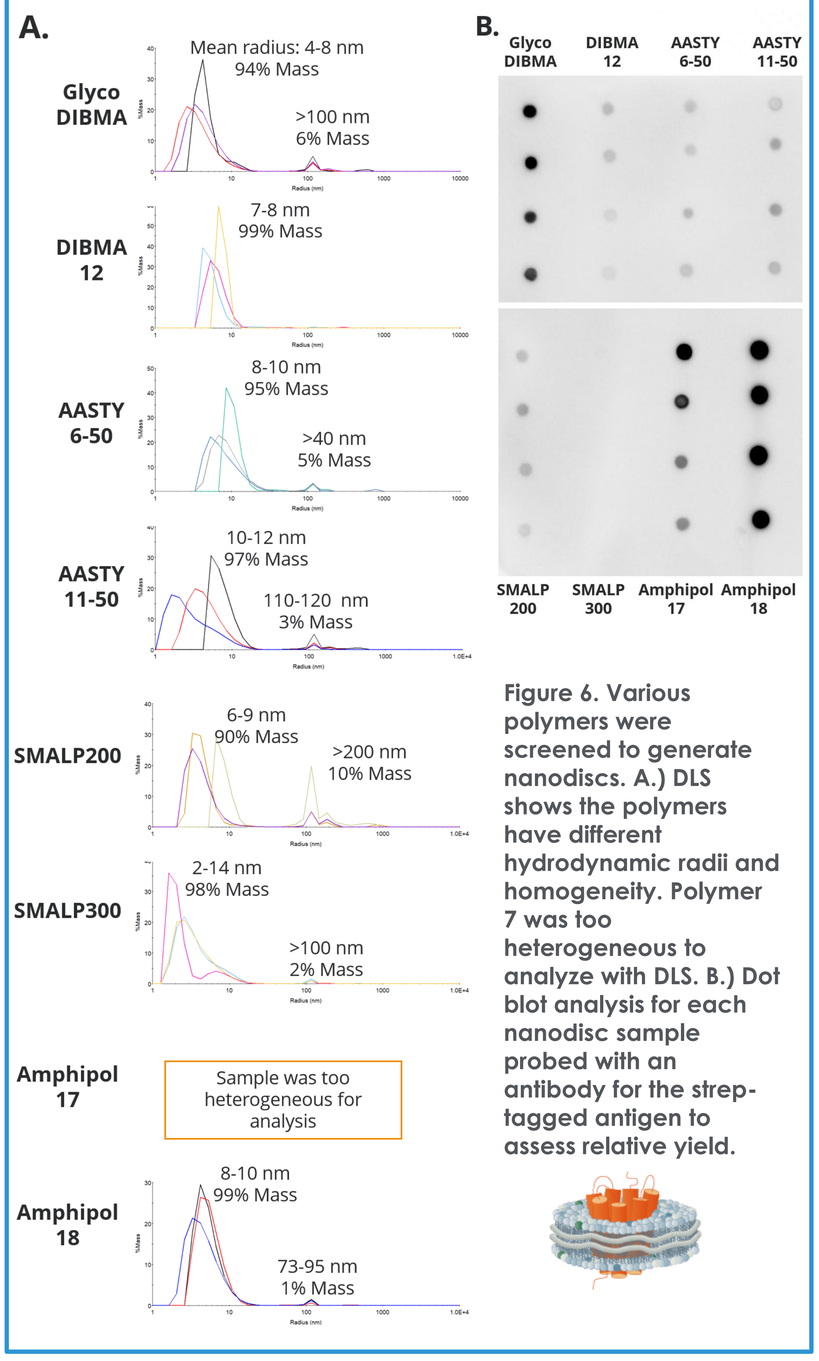

Figure 6. Various polymers were screened to generate nanodiscs. A.) DLS shows the polymers have different hydrodynamic radii and homogeneity. Polymer 7 was too heterogeneous to analyze with DLS. B.) Dot blot analysis for each nanodisc sample probed with an antibody for the strep-tagged antigen to assess relative yield.

SUMMARY

In-house produced nanodiscs offer promise in their homogeneity and functionality for use in both antibody selections and characterization. Here, we highlight a workflow to screen polymers to target various membrane proteins of interest and assess their quality, yield, and activity. We have applied this workflow to several membrane obligate targets and further validation and optimization are in progress.

ACKNOWLEDGMENTS

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REFERENCES

[1]Hauser, AS, et al. 2019. Trends in GPCR drug discovery: new agents, targets and indications. Nat Rev Drug Discov. 16(12):829-842. PMID: 29075003.

[2]Young, JW. 2023. Biochem Soc Trans. 51(3):1405-1416. PMID: 37345653.

[3]Biorender

[4]CUBE Biotech. What are Nanodiscs – Copolymers. https://cube-biotech.com/our-science/membrane-protein-stabilization/copolymer-nanodisc/