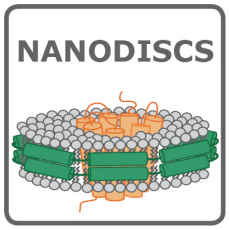

Nanodisc products and services

Cube Biotech offers an entire portfolio of nanodisc products and services. These include:

- Variety of lyophilized MSP proteins for reconstitution of solubilized proteins
- Nanodisc assembly kits for use with cell-free expression systems
- Empty nanodiscs for use with cell-free expression systems
- Reconstitution of already isolated proteins
- Full service from membrane protein expression to reconstitution into nanodiscs

Fig. 1 Cube Biotech assembly kit
Nanodisc assembly kits for use with cell-free expression systems, consisting of MSP protein, phospholipid, and sodium cholate in precisely aliquoted amounts.

What are nanodiscs?

Nanodiscs were first described by Sligar and coworkers (1,2). Nanodiscs provide a phospholipid bilayer system held together by membrane scaffold proteins (MSPs). MSPs are truncated forms of apolipoprotein (apo) A-I which wrap around a patch of a lipid bilayer to form a disc-like particle or nanodisc (3).
MSPs provide a hydrophobic surface facing the lipids, and a hydrophilic surface at the outside. This setup makes nanodiscs highly soluble in aqueous solutions and allows for the solubilization of membrane proteins in the absence of detergents.
These nanobilayer particles are about 7-13 nm in diameter, depending on the mutation variant of MSP used. Most widely employed are MSP1D1 and MSP1D1-deltaH5, but also other deletion mutants of MSP1D1 are suitable for the generation of nanodiscs (3). Most commonly used phospholipids are dimyristoyl-glycero-phosphocholine (DMPC) or palmitoyl-oleoyl-phosphatidylcholine (POPC) in combination with the detergent sodium cholate.

Why nanodiscs?

Nanodiscs have a number of advantages compared to other systems for membrane protein solubilization and reconstitution, in particular for ligand binding studies, analysis of conformational dynamics, and protein interaction studies (4). Nanodiscs can be used to reconstitute membrane proteins such as GPCRs or transporters in an artificial environment resembling the native membrane. These nanodisc-solubilized proteins can be directly purified by normal chromatographic procedures without the need for detergents. The resulting assembly allows for access to the physiologically intracellular and extracellular faces of the protein and thus allows unrestricted access of antagonists, agonists, G proteins and other interaction partners (5).

Reconstitution of proteins into nanodiscs

Reconstitution of detergent-solubilized membrane proteins

There are two ways by which membrane proteins can be reconstituted into nanodiscs. One is to solubilize and purify the membrane protein in the presence of a suitable detergent, and then add membrane scaffold proteins and phospholipids to the mix. Nanodiscs containing the membrane protein will form spontaneously, and can be purified e.g. using size exclusion chromatography after detergent removal. See Fig. 2, left side, (4,5)

Combining nanodiscs and cell-free expression systems

As an alternative method, membrane proteins can be expressed in cell-free systems with added, pre-assembled nanodiscs that integrate the nascent membrane protein (6). In this method, detergents are not required, minimizing possible artifacts. Yields obtained in cell-free expression systems are usually limited to a few micrograms of protein, but offer the possibility to include modifications such as biotinylation or isotope labelling. See Fig. 2, right side.

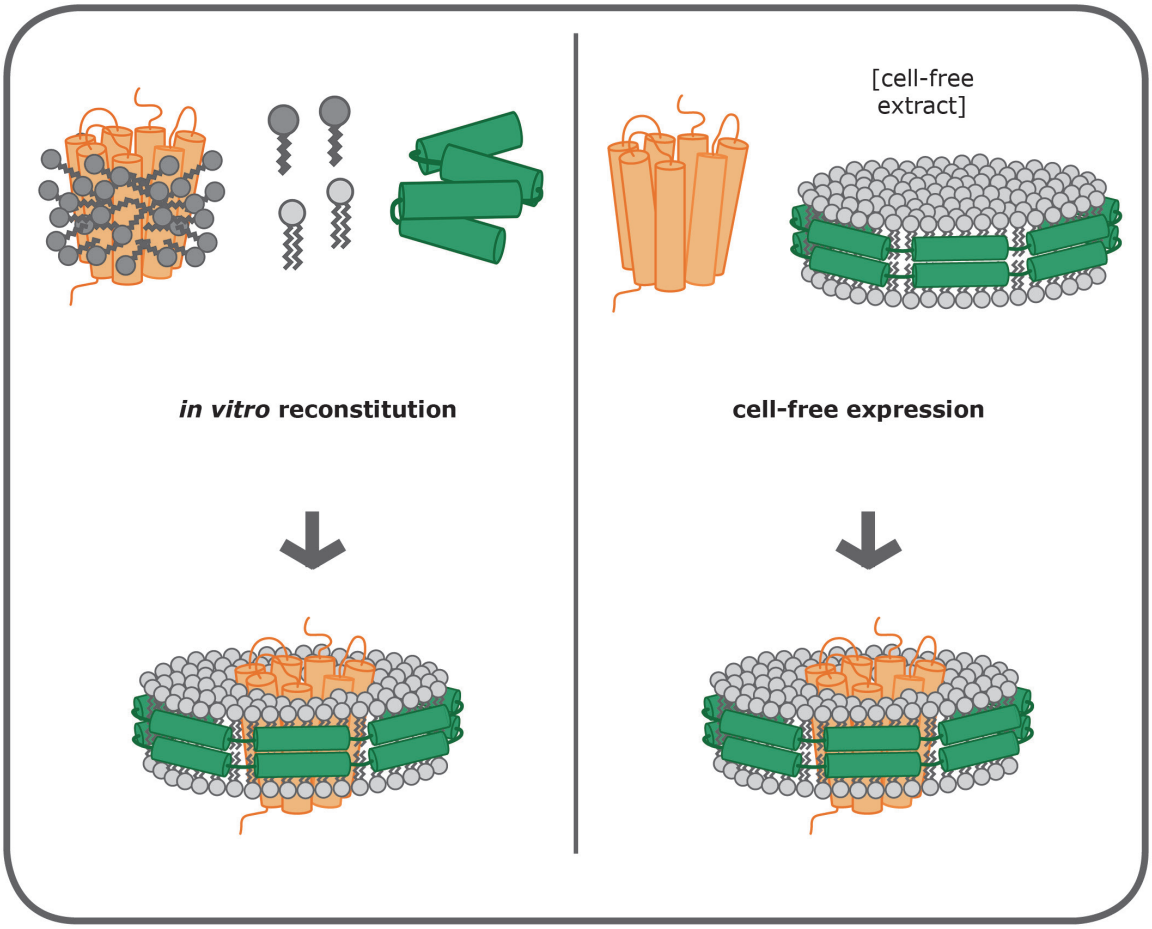

Fig. 2: Schematic view of two methods of incorporating membrane proteins into nanodiscs.

Left: Target protein (orange) is solubilized in detergent (dark grey) and mixed with lyophilized membrane scaffold protein (MSP, green), phospholipids (light grey), and detergents.

Right: Already assembled nanodiscs consisting of MSP and phospholipids are added to a cell-free expression reaction, and the nascent target protein inserts into the nanodisc.

Nanodisc applications

Nanodiscs provide the perfect environment to stabilize membrane proteins and allow to study binding of ligands, agonists or antagonists by methods such as NMR and SPR (7,8). Membrane scaffold proteins can be tagged with poly-His or other affinity tags to facilitate purification, detection, and immobilization.

Other applications performed with nanodiscs include resonance Raman (9), Cryo-EM (10), MALDI (11), protein activation studies (12) and time-resolved fluorescence spectroscopy (13). Antigens reconstituted into nanodiscs have even been used to raise immunogenic response in mice, showing their potential to be used as vaccines (14). Recently, the entire *E.coli* membrane proteome was reconstituted into nanodiscs, thereby creating a solubilized membrane protein library for further studies (15). Table 1 shows a number of examples for nanodisc applications.

The number of publications has grown significantly in the past few years, and more applications will surely come up as more researchers begin to use this exciting new technology.

Application	Protein studied	Origin	Reference
Binding studies of agonist, antagonist and radiolabel exchange in G proteins	beta andrenergic receptor 2	human	(5)
Nuclear magnetic resonance (NMR)	OmpX, CD4 mutant	bacterial, human	(3,7)
Surface plasmon resonance (SPR)	CD4 mutant	human	(8)
Resonance Raman spectroscopy	Cytochrome P450	mammalian	(9)
Cryo electron microscopy	SecYEG complex	*E.coli*	(10)
Matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS)	Various	Various	(11,15)
Protein phosphorylation / activation studies	Epidermal growth factor receptor (EGFR)	human	(12)
Electron microscopy (EM)	Light harvesting complex II (LHCII)	spinach	(13)
Time-resolved fluorescence spectroscopy	Light harvesting complex II (LHCII)	spinach	(13)
Vaccination of mice	Hemagglutinin A (HA)	influenza virus	(14)

Table 1. Examples for applications published for membrane proteins reconstituted into nanodiscs.

Ordering Information

Catalog Number	Product Description
26112 / 26116	MSP1D1 protein, his-tagged (2 mg) / (10 mg) 2 mg / 5 x 2 mg lyophilized protein
26132 / 26136	MSP1D1 protein (2 mg) / (10 mg) 2 mg / 5 x 2 mg lyophilized protein, His-tag removed by protease digest
26122 / 26126	MSP1D1 delta H5 protein, his-tagged (2 mg) / (10 mg) 2 mg / 5 x 2 mg lyophilized protein
26142 / 26146	MSP1D1 delta H5 protein (2 mg) / (10 mg) 2 mg lyophilized protein, His-tag removed by protease digest
26211	Nanodisc Assembly Kit MSP1D1-His_DMPC Contains 2 mg MSP1D1-His protein, pre-aliquoted DMPC and sodium cholate
26221	Nanodisc Assembly Kit MSP1D1_DMPC Contains 2 mg MSP1D1 protein, pre-aliquoted DMPC and sodium cholate
26231	Nanodisc Assembly Kit MSP1D1 delta H5-His_DMPC Contains 2 mg MSP1D1 delta H5-His protein, pre-aliquoted DMPC and sodium cholate
26241	Nanodisc Assembly Kit MSP1D1 delta H5_DMPC Contains 2 mg MSP1D1 delta H5 protein, pre-aliquoted DMPC and sodium cholate
Inquire	Pre-assembled nanodiscs For cell-free expression assays
Inquire	Protein expression and Nanodisc assembly services Project-dependent

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