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Nanodisc-T_m: Rapid functional assessment of nanodisc reconstituted membrane proteins by CPM assay

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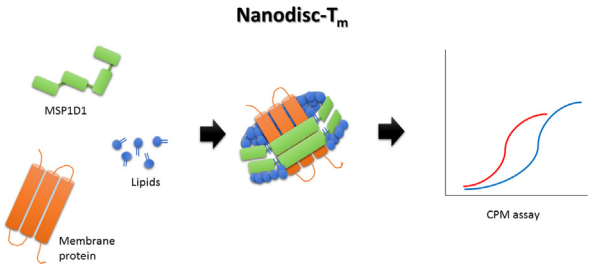
GRAPHICAL ABSTRACT



ABSTRACT

Membrane proteins are generally unstable in detergents. Therefore, biochemical and biophysical studies of membrane proteins in lipidic environments provides a near native-like environment suitable for membrane proteins. However, manipulation of proteins embedded in lipid bilayer has remained difficult. Methods such as nanodiscs and lipid cubic phase have been developed for easy manipulation of membrane proteins and have yielded significant insights into membrane proteins. Traditionally functional reconstitution of receptors in nanodiscs has been studied with radioligands. We present a simple and faster method for studying the functionality of reconstituted membrane proteins for routine characterization of protein batches after initial optimization of suitable conditions using radioligands. The benefits of the method are

- Faster and generic method to assess functional reconstitution of membrane proteins.
- Adaptable in high throughput format (≥96 well format).
- Stability measurement in near-native lipid environment and lipid dependent melting temperatures.

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Method details

Adenosine A2A receptor purification

Prepare 2% (w/v) cholesteryl hemi succinate (CHS) dissolved in 10% (w/v) *n*-dodecyl β-D-maltoside (DDM). Expression cassette for human adenosine A_{2A} receptor ($A_{2A}R$) consisted of hemagglutinin signal sequence, FLAG epitope, 10X histidine (His) tag, T4 lysozyme and amino acids encoding the receptor from 1 to 317 residues in pFastBac1 vector using *Spodoptera frugiperda* (Sf9) insect cells. Cell membranes were expressed and washed as described previously [3,9]. Prior to solubilisation thaw the membranes on ice with 4 mM theophylline.

1. Add iodoacetamide to a concentration of 2 mg/ml and incubate at 4 °C for 30 min. Membranes were solubilized by adding 2× solubilization buffer (50 mM Hepes pH 7.5, 1600 mM NaCl, 20% glycerol, 2% DDM-CHS mixture and 4 mM theophylline and incubated at 4 °C for 2.5 h. Unsolubilized membranes were centrifuged at 150,000*g* for 45 min.
2. For 1 l of biomass 1 ml of TALON (Clontech) immobilized metal affinity chromatography (IMAC) resin was used. The resin was pre-equilibrated with solubilization buffer. The supernatant was incubated with the resin overnight at 4 °C with 20 mM imidazole.
3. After overnight binding, the resin was washed with 10 column volume (10 ml) of wash buffer I (250 mM Hepes pH 7.4, 800 mM NaCl, 10 mM $MgCl_2$, 0.1% DDM:CHS, glycerol 10%, 25 mM imidazole, 8 mM adenosine triphosphate (ATP) and 100 μM ZM341285. This was followed by wash buffer II (50 mM Hepes pH 7.4, 800 mM NaCl, and 0.05% DDM-CHS, 10% glycerol, 50 mM imidazole and 100 μM ZM341285). The receptor was eluted in 25 mM Hepes pH 7.4, 800 mM NaCl, and 0.01% DDM: CHS, 10% glycerol, 220 mM imidazole and 100 μM ZM341285.
4. Concentrate the eluted receptors in a 100 kDa centricon centrifugal filter. Assess purity and monodispersity by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and analytical size-exclusion chromatography (aSEC).

Assembly of nanodiscs

1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine (POPS) were purchased from Avanti Polar Lipids. We have used Bio-beads, Biorad and Amberlite XAD-2, Sigma–Aldrich with equal success.

1. Solubilize lipids using sodium cholate detergent. Cholate concentration must be twice as lipid concentration. For example, solubilize 100 mM lipids in 200 mM cholate.
2. Using PD-10 column buffer exchange the receptor to 50 mM Hepes pH 7.4, 800 mM NaCl and 0.01% DDM-CHS.
3. Mix $A_{2A}R$: Membrane Scaffold Protein 1D1 (MSP1D1): lipid in a ratio of 1:10:700. POPC:POPS (7:3) was used in reconstitution. POPS is a negatively charged lipid. This is added to mimic the negative charge of the inner leaflet of the plasma membrane. The above mentioned ratio has been optimized to yield monomeric adenosine A_{2A} receptor in nanodiscs. Use His tag cleaved MSP1D1 protein.