SUPPORTING INFORMATION

Functional Assays of Membrane-Bound Proteins by SAMDI-TOF Mass Spectrometry

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Nanodisc preparation

Materials - MSP1D1 was expressed and purified as described.[11] POPC was obtained from Avanti Polar Lipids (Alabaster, AL), octyl glucoside was obtained from Anatrace (Maumee, OH). Rod outer segments were isolated from frozen bovine retina (SPCI Retina Inc., spci_retina@msn.com) and rhodopsin purified on conA sepharose (GE Healthcare, Piscataway, NJ) using published procedures.[14,15] Transducin was purified as described[16] and quantitated by fluorescence.[12,13] GTPγS was from Sigma (St. Louis, MO). Standard buffer is 10 mM Tris pH 7.4, 0.1 M NaCl, 0.01% NaN_3 using MilliQ purified water (Millipore).

Nanodisc self-assembly and purification - All rhodopsin manipulations were performed under dim red light (Kodak #2 filter; long pass cut off ~650nm). Nanodiscs were self-assembled at a mole ratio of 700:10:1 POPC:MSP1D1:rhodopsin. Octyl glucoside (0.5 M stock in water) was added to conA purified rhodopsin for a final concentration of 90 mM to ensure monomerization. The rhodopsin stock (117 micromolar) was added to a mixture of MSP1E3D1 (390 micromolar) and POPC/cholate (0.2 M/0.4 M sodium cholate in buffer) at the correct ratio. A protease inhibitor cocktail (Roche, complete without EDTA) was added followed by a brief incubation on ice. An equal volume of moist Amberlite XAD-2 (Supelco), prepared by washing extensively with methanol followed by water and decantation of fine particles, was added to the nanodisc assembly mixture to remove detergent. Detergent removal was allowed to proceed overnight at 4 °C with gentle agitation. The Amberlite was removed by centrifugation. After buffer exchange on a Biogel P6 spin column (BioRad) the sample was purified on conA sepharose to remove bare disks. The sample was concentrated with a 10 kDa MW cutoff centrifugal device and injected onto a Superdex 200 prep grade column (1.6 x 30 cm) run at 0.5 mL/min. The nanodisc peak was pooled and concentrated as above.

Preparation of Actacn-presenting SAMs.

Self-assembled monolayers (SAMs) of alkanethiolates on gold were prepared as described previously.[19] Briefly, titanium (60 Å) and then gold (220 Å) were evaporated onto microscope coverslips (25 mm x 50 mm) using an electron beam evaporator (Thermionics VE-100) at a rate of 0.2-0.4 nm/s and at a pressure of 9 x 10^{-7} torr. Maleimide-presenting SAMs (at a density of 5%) were prepared by immersing the coverslips in an ethanolic

solution containing an asymmetric maleimide-terminated disulfide and a symmetric tri(ethylene glycol) disulfide in a ratio of 1:9 at a total disulfide concentration of 1 mM. The monolayers were allowed to form for 12 hours, rinsed with ethanol and dried under a stream of nitrogen. Solutions of thiol-terminated Actacn ligand (1 mM in phosphate buffered saline, pH = 7.2) were applied to the maleimide-terminated monolayers for one hour at 30 ^{o}C in a humidified chamber. Chips were stored in ethanol at -20^{o}C for up to one week.

Nanoassembly immobilization onto Actacn-presenting SAMs and functional assyas

Prior to immobilization of the nanodiscs, monolayers were rinsed with water and ethanol and dried under a stream of nitrogen. The Actacn-terminated monolayers were loaded with Ni^{2+} by incubating the chips with $NiSO_4$ (1 mM in water) for 5 minutes, followed by a rinse with water and ethanol. Solutions of nanodiscs with and without rhodopsin (7.5 μM, 3uL for 1 mm x 1 mm chips) in buffer (10mM Tris pH = 7.4, 0.1 M NaCl, 0.01% NaN_3) were applied and incubated at room temperature in a dark room with dim red light for 15-30 minutes. The monolayers were then washed with water. Mixtures of rhodopsin-loaded nanodiscs and transducin (10 μM) were exposed to ambient light, and the chips were incubated at room temperature for 15-30 minutes. For experiments with the small molecule inhibitor, an excess of an aqueous solution of GTPγS (1 mM) was applied to the monolayers after the rhodopsin-transducin complex was formed. The monolayers were then washed with water and dried under nitrogen. Matrix solution was applied in a thin layer (sinapinic acid 5 mg/mL in acetonitrile; 0.3 μL/chip) and mass spectra were acquired as described below.

MALDI-TOF analysis

Mass analysis was performed using a Voyager-DE™ PRO Biospectrometry™ mass spectrometer (Applied Biosystems, Framingham, MA) with a 337 nm nitrogen laser as a desorption/ionization source. Solutions of matrix (5 mg/mL in anhydrous acetonitrile) were prepared using either sinapinic acid (SA) for monitoring proteins in the high *m/z* range or 2,4,6-trihydroxyacetophenone (THAP) for characterizing the maleimide-terminated and Actacn-terminated monolayers. Matrix solutions (0.3 μL) were applied on monolayers measuring 1.0 mm^2 and dried rapidly under vacuum. For control experiments performed with soluble reagents, aqueous solutions of nanodisc (1 μM, 1 μL) were mixed with 10 μL of a matrix solution (10 mg/mL in a 50:50 acetonitrile:water solution containing 0.1% trifluoroacetic acid). Mass spectra of proteins were acquired using a 25 kV accelerating voltage, linear operating mode, and positive polarity. Mass spectra of small molecules were acquired using a 20 kV accelerating voltage, reflector operating mode, and positive polarity.

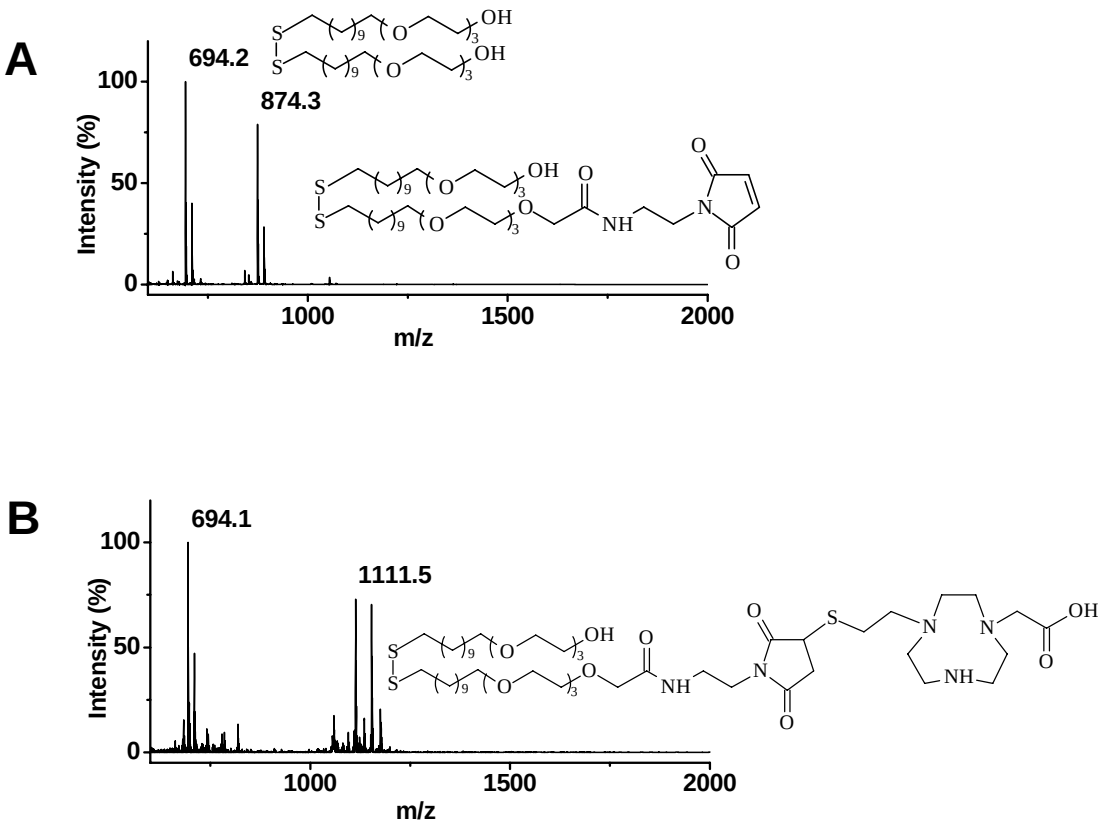

Figure S1. SAMDI characterization of maleimide-presenting monolayers before (A) and after (B) incubation with Actacn-thiol ligand. Peaks correspond to the disulfide reagents shown with each spectrum (usually observed as sodium and potassium adducts).

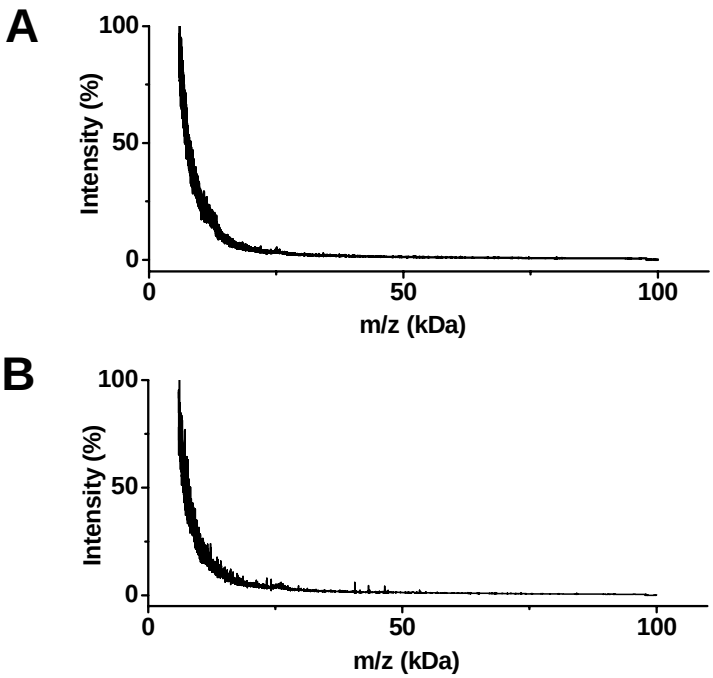

Figure S2. SAMDI characterization of nanodiscs immobilized onto maleimide-presenting SAMs (no ligand) (A) and onto Actacn-presenting SAMs after treatment with a solutio of imidazole (150 mM) that competes with the his-tag for binding to the Ni^{2+}-chelating ligand (B). Both experiments show the absence of the MSP1 protein peak at 25 kDa and therefore confirm the specificity of the immobilization reaction.