Ganglioside-embedding small bicelles for probing membrane-landing processes of intrinsically disordered proteins

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Supplementary Information

Gangliosides and phospholipids, phospholipids 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-dihexanoyl-sn-glycero-3-phosphocholine (DHPC), were purchased from Carbosynth Limited, Enzo Life Sciences, or Avanti Polar Lipids, Inc. and were used without further purifications. Expression of $^{15}$N-labeled $\alpha$SN was performed as previously described.$^{1,2}$ The isotopically labeled peptide corresponding to the N-terminal 30 amino acid residues of $\alpha$SN was expressed and purified as a recombinant ubiquitin extension according to the literature$^3$ with modifications. Cells were grown in M9 minimal media containing $[^{15}$N]NH$_4$Cl (1 g/L). After sonication, the centrifugation pellet was dissolved in 50 mM Tris-HCl buffer (pH 8.0) containing 150 mM NaCl and 8 M urea. Hexahistidine-tagged ubiquitin (His$_6$-Ub) fused with the peptide was purified by a Ni$^{2+}$-nitrilotriacetic acid affinity column (GE Healthcare). The peptide was enzymatically cleaved from His$_6$-Ub by incubation with recombinant glutathione S-transferase-tagged yeast ubiquitin hydrolase-1. The liberated peptide was purified by reverse-phase chromatography using an octylsilane column (Sunniest C8, ChromaNik) with a linear gradient of acetonitrile. The fraction containing the peptide was collected and lyophilized.

All spectroscopic measurements were performed in 20 mM sodium phosphate buffer (pH 7.0) containing 100 mM NaCl. $^{31}$P NMR and DOSY spectra of the bicelles ([ganglioside] = 5 mM, ganglioside:DMPC:DHPC = 1:1:4) were measured on JEOL LA500 and ECA-600 spectrometers at 283 K. Diffusion coefficients were analyzed using the Delta 4.3.6 program. DLS observations were performed with a Wyatt Technology DynaPro at 283 K and 10 scans were averaged for each sample. Observed hydrodynamic radii of DMPC/DHPC bicelle ([DMPC] = 10 mM, DMPC:DHPC = 2:4) as a control was 6.1±0.1 nm. The values are mean ±SD of three independent experiments.
CD spectra of αSN were measured at room temperature on Jasco J-725 apparatus using a 1.0 mm path length quartz cell. Four scans were averaged for each sample and the averaged blank spectra were subtracted. DMPC/DHPC bicelle ([DMPC] = 4 mM, DMPC:DHPC = 2:6) was used as a control.

NMR spectra of αSN and the peptide were acquired at 283 K with JEOL ECA-600, and ECA-920 spectrometers. The spectra were processed and analyzed with the nmrPipe/Sparky program. Isotopically labeled proteins and the bicelles were dissolved with 10% (v/v) D₂O. The NMR assignments of αSN have been previously described and the backbone assignments for the peptide were performed using a standard set of double- and triple-resonance experiments. For ¹H-¹⁵N HSQC measurements of αSN, the spectra were recorded at a ¹H observation frequency of 920.7 MHz with 256 (t₁) × 1024 (t₂) complex points. DMPC/DHPC bicelle ([DMPC] = 10 mM, DMPC:DHPC = 2:4) and the aggregates containing GM1 (5 mM) and DHPC (20 mM) were used as a control. ¹H-¹⁵N HSQC and ¹⁵N relaxation measurements of the peptide were recorded at 600.2 MHz of ¹H observation frequency with 128 (t₁) × 1024 (t₂) complex points. T₂ data sets were recorded in a randomized order regarding relaxation delays.

REFERENCES


Figure S1. $^1$H-$^{15}$N HSQC spectra of $^{15}$N-labeled αSN in the presence of (a) DMPC/DHPC bicelle, (b) GM1-, (c) GM2-, and (d) GM3-containing bicelles.