Supporting Information: Elliptical Structure of Phospholipid Bilayer Nanodiscs Encapsulated by Scaffold Proteins: Casting the Roles of the Lipids and the Protein

Nicholas Skar-Gislinge,[†] Jens Bæk Simonsen,[†] Kell Mortensen,[†] Robert Feidenhans'l,[‡] Stephen G. Sligar,[¶] Birger Lindberg Møller,[§] Thomas Bjørnholm,[‡] and Lise Arleth[*,†]

Biophysics, Faculty of Life Sciences, University of Copenhagen, Denmark, Nano Science Center, Faculty of Sciences, University of Copenhagen, Denmark, Department of Biochemistry, University of Illinois at Urbana-Champaign, Illinois USA, and Plant Biology and Biotechnology, Faculty of Life Sciences, University of Copenhagen, Denmark

E-mail: lia@life.ku.dk

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[*]To whom correspondence should be addressed
[†]Biophysics, Faculty of Life Sciences, University of Copenhagen, Denmark
[‡]Nano Science Center, Faculty of Sciences, University of Copenhagen, Denmark
[¶]Department of Biochemistry, University of Illinois at Urbana-Champaign, Illinois USA
[§]Plant Biology and Biotechnology, Faculty of Life Sciences, University of Copenhagen, Denmark

Experimental

Preparation of Nanodiscs:

Nanodiscs were prepared with the his-tagged membrane scaffolding protein MSP1D1 and, respectively, POPC (palmityl-oleoyl-phosphatidyl choline) and DLPC (Di-lauryl-phosphatidyl choline). The DLPC and POPC were acquired from Avanti Polar Lipids (AL, USA), whereas the MSP1D1 was provided by the Sligar lab.[1] The nanodiscs were prepared as previously described by Sligar et al:[2] An amount of phospholipid was dried and then quantified gravimetrically and finally resuspended in a 20mM Tris buffer at $pH = 7.4$ containing 100mM sodium cholate and 100mM NaCl to a final concentration of 50mM. The phospholipid stock solution was mixed with a MSP stock solution to reach the relevant lipid to MSP molar ratios. For DLPC this is 80:1 and for POPC it is 65:1 plus 5 % excess. Finally, a buffer with 100mM NaCl and 20mM Tris was added in order to achieve a final phospholipid concentration of $\sim$10mM. The final mixture was left to incubate for 1h at 4°C (just above t_m in both cases). Cholate was removed by addition 1g bio-beads (Bio-Rad) per ml of mixture and shaking gently for 3-8 h. The nanodiscs were purified using a Superdex-200 300/10 SEC column on an Äkta purifier. The chromatogram showed a peak at 12.5 *ml* characteristic for nanodiscs.[2] The peak was collected in 8 fractions. It was tested by SAXS that the 4 most concentrated fractions were similar and they were combined to maximize the yield. When needed, the samples were concentrated using spin-filters with a 100kDa cutoff.

Densitometry:

The partial specific molar volume of the phospholipids was measured using a vibrating tube densitometer (DMA 5000 from Anton Paar, Graz Austria). A stock of each phospholipid in milliQ water at 1.5% mass percent lipid was prepared. An amount of phospholipid was quantified gravimetrically (drying and weighing) and a small amount of radioactivity in the form of tritium labeled DMPC (DLPC samples) or POPC (POPC samples) was added. The initial ratio between lipid and β-radioactivity was then used to quantify the exact concentration of the further dilutions. The

stocks were then resuspended in milliQ water and heated and cooled between 30°C to 4°C ten times over night while gently shaken. To dissolve the last lipids, the solution was placed in an ultra sonic bath until the mixture was clear. The stock was then used to prepare three samples of each lipid at concentrations: 0.5%, 1% and 1.5% (mass). The density of the samples was measured in a temperature range from 20°C to 0°C with a two degree step. Before each measurement the sample was sonicated for additional 2 min. The measurements were repeated three times.
The three measurements together with a blank pure water sample were then used to extrapolate the density at a 0% lipid concentration and the partial specific molar volumes of DLPC and POPC were calculated. The result is shown as open diamonds in Figure 6 (A).

SAXS and SANS measurements:

SAXS measurements for the POPC and DLPC nanodisc temperature scans (Figure 3 and Figure 4 (B)) were performed at the Synchotron Soléil (Paris, France) at the SWING beamline. Each nanodisc sample was measured at temperatures between 20°C and 1°C and each measurement was performed by exposing the sample to 10 $\times$ 500 ms of radiation while flowing in order to minimize radiation damage. The samples were delivered to the measuring point using an adapted HPLC setup, enabling the samples to be stored at the measurement temperature. A combination of the wavelength λ of the incoming X-ray beam and the sample detector distance was chosen such that a q-range from 0.007 1/Å to 0.5 1/Å was covered. The scattering vector q is defined by $q = 4\pi \sin\theta/\lambda$, where θ is the half scattering angle and λ is the wavelength of the incoming X-ray beam.

The measured 10 $\times$ 500 ms blocks data were dezingered, averaged and background subtracted with the appropriate buffer backgrounds using the program BioXTAS RAW.[3] The absolute scale calibration was performed using H_2O and double checked against a lysozyme sample with a well-known concentration. This allows for absolute scale calibrating the data to an accuracy better than 10%. A dilution series of the two kinds of nanodisc was measured to check that concentration dependent effects could be neglected.

SANS and SAXS data sets for the simultaneous analysis (Figure 4) were acquired at, respectively, the SANS instrument D11 at Institut Laue Langevin (ILL) and at the Bio-SAXS instrument at beamline ID14-3 at European Synchrotron Radiation Facility (ESRF). Both facilities are located in Grenoble, France. For the SANS measurements a combination of two instrumental settings were used to obtain a sufficiently wide *q*-range. Absolute scale calibration was performed using H_2O according to the standard procedures at the facility. The samples were placed in cylindrical Hellma quartz cells during the measurements. For the SAXS measurements we used the fixed instrument setup including the HPLC-based automatic sample loading robot standard to the beamline ID14-3. Absolute scale calibration was performed using Bovine Serum Albumin as the external reference.

The SANS measurements were performed two days before the SAXS measurements. Ahead of the SANS measurements the usual H_2O-based buffer used in the nanodisc preparation, had been substituted for corresponding buffers with, respectively 100% D_2O and 42% D_2O/58% H_2O. In the latter contrast, the membrane scaffolding protein is contrast matched and in practice invisible in the experiments. The isotope substitution of the buffers was performed using centrifugal spin-filters with a cut-off of 100 kDa.

Notes and References

(1) http://sligarlab.life.uiuc.edu/nanodisc.html, **2010**.

(2) Bayburt, T. H.; Grinkova, Y. V.; Sligar, S. G. *Nano Lett.* **2002**, *2*, 853–856.

(3) Nielsen, S. S.; Toft, K. N.; Snakenborg, D.; Jeppesen, M. G.; Jacobsen, J. K.; Vestergaard, B.; Kutter, J. P.; Arleth, L. *J Appl Crystallogr* **2009**, *42*, 959–964.