Electronic Supplementary Material (ESI) for Nanoscale Advances.

Dark peptide discs for the investigation of membrane proteins in supported lipid bilayers: the case of synaptobrevin 2 (VAMP2)

Supporting Information

Alessandra Luchini, Frederik Grønbæk Tidemand, Nicolai Tidemand Johansen, Federica Sebastiani, Giacomo Corucci, Giovanna Fragneto, Marité Cárdenas, Lise Arleth

0.1 Protein sequences

VAMP2
MGSATAATAP PAAPAGEGGP PAPPPNLTSN RRLQQTQAQV DEVVDIMRVN VDKVLERDQK LSELDDRADA LQAGASQFET SAAKLKRKYW WKNLKMMIIL GVICAIILII IIVYFSTHHH HHH

α-synuclein
MGHHHHHHDY DIPTTENLYF QGMDVFMKGL SKAKEGVVAA AEKTKQGVAE AAGK-TKEGVL YVGSKTKEGV VHGVATVAEK TKEQVTNVGG AVVTGVTAVA QKTVEGAGSI AAATGFVKKD QLGKNEEGAP QEGILEDMPV DPDNEAYEMP SEEGYQDYEP EA

α-synuclein (TEV-cleaved)
GMDVFMKGL SKAKEGVVAA AEKTKQGVAE AAGKTKEGVL YVGSKTKEGV VHGVAT-VAEK TKEQVTNVGG AVVTGVTAVA QKTVEGAGSI AAATGFVKKD QLGKNEEGAP QEGILEDMPV DPDNEAYEMP SEEGYQDYEP EA

1 Protein production

1.0.1 α-synuclein

Protein expression was induced by addition of IPTG to a concentration of 1 mM. After 3 hrs, cells were harvested by centrifugation. The harvested cells were resuspended in 50 mM TrisHCl pH 8, 1 mM EDTA, 1 mM PMSF and added lysozyme to a concentration of 0.5 mg/ml and stored at -20 °C. The next day, the sample was thawed and added MgCl2 to a concentration of 5 mM, along with benzonase (MerckMillipore), and incubated until homogenous. The sample was further sonicated to ensure complete cell lysis. Cell debris was pelleted by centrifugation, and the protein

was purified from the supernatant on NiNTA resin. The resin was washed in 50 mM TrisHCl pH 8, 300 mM NaCl, 40 mM imidazole and the protein was eluted in 50 mM TrisHCl pH 8, 300 mM NaCl, 250 mM imidazole. The eluted protein was added TEV protease (1:100 w/w) and dialysed against 20 mM TrisHCl pH 8, 100 mM NaCl, 0.5 mM EDTA, 1 mM DTT at 4 °C over night. TEV protease and uncleaved α-synuclein was removed by NiNTA resin, and the sample was precipitated by adding ammonium sulfate to a concentration of 60 % w/v followed by incubation at 4 °C. The precipitated protein was pelleted by centrifugation (15000 g, 30 min, 4 °C) and resuspended in the smallest volume possible without visible precipitate. This sample was dialysed against a 2000 fold volume of 20 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM EDTA at 4 °C over night. Next day, the sample was aliquoted and frozen at -20 °C.

1.0.2 VAMP2

The temperature was lowered to 20 °C, and after 1 hr, protein expression was induced by addition of IPTG to a concentration of 0.5 mM. The cells were harvested after approx. 20 hrs. The harvested cells were resuspended and lysed as described above for αsynuclein. After sonication, the sample was added 1 % SDS to solubilize the protein. Cell debris was pelleted by centrifugation, and the supernatant was diluted to 3.3 fold before purification on NiNTA resin. The wash and elution buffers contained 1.1 mM 12:0 lysoPC. The eluted protein was concentrated using 3 kDa MWCO centrifugation filters (Satorius) and further purified by SEC, using a superdex 75 10/300 GL column equilibrated in 20 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 1.1 mM 12:0 lysoPC. A sample was collected from the main peak, aliquoted and stored at -20 °C.

2 Dark peptide disc preparation

The dark peptide discs were prepared according to the same protocol previously reported for the 18A peptide discs [1, 2]. Briefly, the lipids (1 mg, of either POPC or POPC/POPS 70/30 mol/mol) and the d18A peptide (1 mg) were dissolved in methanol, and subsequently, the organic solvent was removed under a nitrogen flow to produce a dried film with the mixed lipids and the d18A peptide. The film was dissolved with 0.5 ml of buffer with a composition of 20 mM Tris, 100 mM NaCl, 10 mM $CaCl_2$, $pH = 7.4$. The solution, containing 1.3 mM lipids and 0.3 mM 18A peptide, was purified by size exclusion chromatography (SEC) using a Superdex 200 10/300 increase column (GE Healthcare).

2.1 Neutron Reflectometry (NR)

NR data were analysed with an in-house developed code that is based on the Parratt formalism to calculate the reflectivity profile corresponding to a given structural model [3]. A standard approach in NR data analysis is to consider the sample as composed by a stack of layers, each of them characterized by a different thickness (t), scattering length density (ρ), solvent volume fraction (ϕ_s) and surface roughness (σ).The scattering length density is defined as

$$\rho = \sum_i \frac{n_i b_i}{V_m} \tag{1}$$

where n_i is the number of i-type nuclei and b_i is the corresponding coherent scattering length, while V_m is the molecular volume. Further details about the NR data analysis procedure are reported in supplementary materials.

The investigated SLBs were modelled as a stack of four layers: 1) silicon oxide, which spontaneously forms on the surface of the silicon support; 2) inner lipid headgroups in the proximity of the support surface; 3) lipid acyl chains belonging to the two bilayer leaflets; 4) outer lipid headgroups in contact with the bulk solvent. The structural parameters associated with layer 1 were obtained from the analysis of independent datasets collected for the silicon support in contact with D_2O and H_2O, respectively. The silicon oxide might have a different thickness from one substrate to another. In the specific case of the data shown in the main text, the silicon oxide thickness for the substrate used to characterise the POPC bilayer was 17Åwhereas that of the substrate used for the characterisation of the SLB with VAMP2 was 10 Å. All the parameters of layer 1 expect the solvent volume fraction were kept fixed during the sample data analysis. Preliminary data analysis showed that all the investigated samples are characterized by the same composition of the inner and outer leaflet and a symmetric structure. For this reason, in the final data analysis layer 2 and 4 were constrained to have the same parameter values.

Data collected for the SLBs with incorporated VAMP2 molecules were analysed according to the model previously reported for SLBs with membrane proteins [1]. In particular five layers were used to describe the samples: 1) silicon oxide, which spontaneously forms on the surface of the silicon support; 2) inner lipid headgroups in the proximity of the support surface with part of VAMP2; 3) lipid acyl chains belonging to the two bilayer leaflets with the VAMP2 transmembrane domain (TMD); 4) outer lipid headgroups in contact with the bulk solvent with part of VAMP2; 5) VAMP2 extramembrane domain. Also in this case, preliminary data analysis showed similar structural parameters for layer 2 and 4, which, in the final data analysis, were constrained to have the same parameter values. Because of the similar ρ values associated to the lipid headgroups including hydration (i.e. $\rho_{D_2O-buffer} = 3.66 \cdot 10^{-6}, \rho_{SMW-buffer} = 1.96 \cdot 10^{-6}, \rho_{H_2O-buffer} = 1.65 \cdot 10^{-6}$) and VAMP2 ($\rho_{D_2O-buffer} = 3.057 \cdot 10^{-6}, \rho_{SMW-buffer} = 2.284 \cdot 10^{-6}, \rho_{H_2O-buffer} = 1.81 \cdot 10^{-6}$), VAMP2 contribution were explicitly taken into account only in layer 3 and 5. In order to constrain layer 3 and 5 to reflect the same number of protein molecules, the scattering length density of layer 3 was expressed as

$$\rho_{layer3} = \phi_{acylchain}\rho_{acylchain} + \phi_{VAMP2TMD}\rho_{VAMP2TMD} \tag{2}$$

where $\rho_{acylchain}$ was calculated from the acyl chain chemical composition and molecular volume (921Å^3) and $\rho_{VAMP2TMD}$ was calculated from the protein sequence as $1.86 \cdot 10^{-6}$Å^{-2}. Finally, $\phi_{VAMP2TMD}$ was calculated as

$$\phi_{TMD} = \phi_{extramembrane}\frac{t_5 V_{TMD}}{t_3 V_{extramembrane}} \tag{3}$$

where t_3 and t_5 are thickness of layer 3 and 5 respectively and V_{TMD} is the volume of the transmembrane domain calculated as 2951 Å^3 and $V_{extramembrane}$ is the volume of the extramembrane domain calculated as 13351Å^3.

3 POPC/POPS supported lipid bilayer

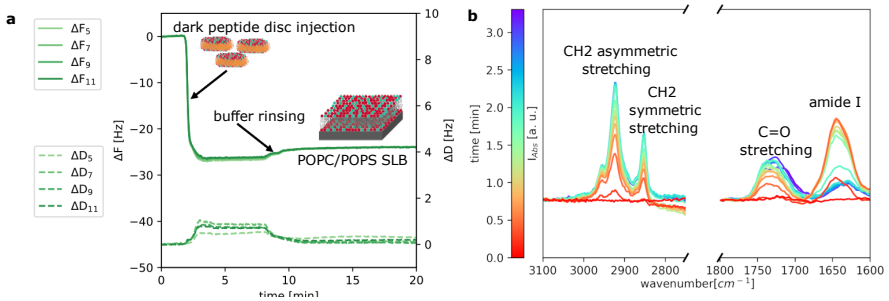

Figure 1: QCM-D data showing the formation of the POPC/POPS SLB by deposition of the dark peptidediscs and peptide removal (a). Time resolved ATR-FTIR spectra showing the formation of the POPC/POPS SLB by the dark peptide discs (b).

Figure 1 shows the data collected on the formation of a POPC/POPS supported lipid bilayer (SLB) by dark peptide discs. Figure SM1a shows the quartz crystal microbalance with dissipation monitoring (QCM-D) data with a trend similar to Figure 1b of the main text. Figure SM1b shows attuated total reflectance-FTIR (ATR-FTIR) spectra collected during the SLB formation. The amide I band increased in intensity during the dark peptide deposition. As observed in Figure 1e of the main text, a decreased in intensity was observed during buffer rinsing as a consequence of the d18A peptide removal. Upon peptide removal a residual signal was still detected at $1635 cm^{-1}$, which is associated to the PS headgroup absorption [4].

4 POPC/POPS SLB with VAMP2

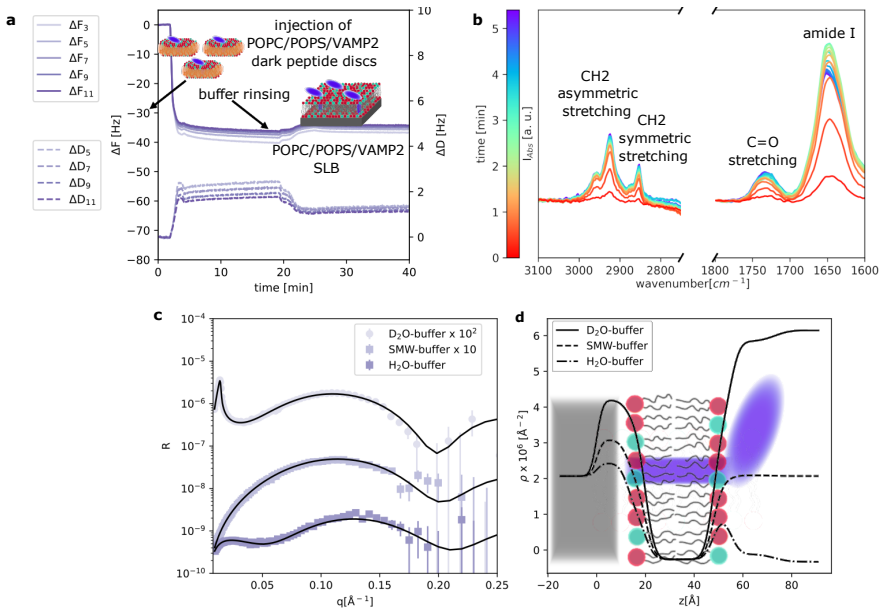

Figure 2: QCM-D data showing the formation of the SLB by deposition of the dark peptide discs and peptide removal (a). Time resolved ATR-FTIR spectra showing the formation of the SLB by the dark peptide discs (b). NR experimental data together with the fitting curves (c). Data and fits are offset for clarity. Scattering length density profiles calculated from NR data analysis (d).

5 Additional data on α-synuclein interaction with SLBs and SLBs with VAMP2

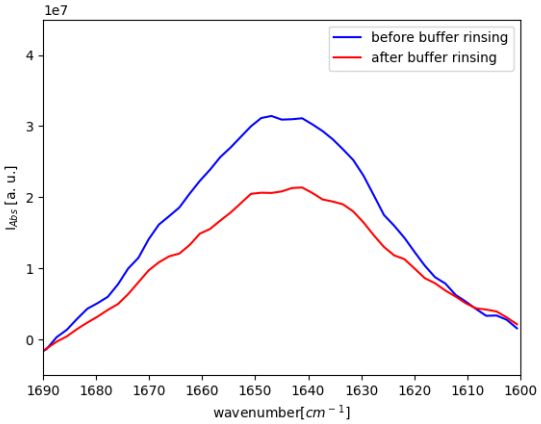

Figure 3: Amide I band measured for the POPC/POPS/VAMP2 + α-syn before (blue) and after (red) rinsing with buffer (0.5 mM Tris, pH=7.5). Similar results were obtained for POPC/VAMP2

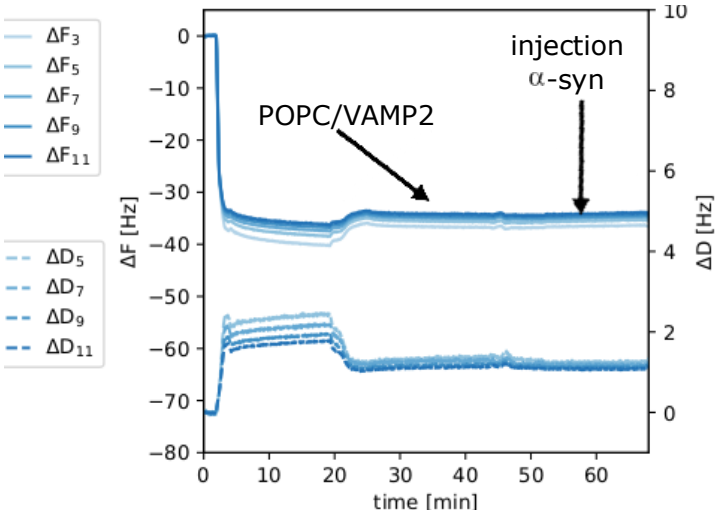

Figure 4: QCM-D data showing the formation of the POPC/VAMP2 SLB and the lack of additional signal variation upon injection of α-syn (10μM). The experiment was performed with the buffer with composition 20mM Tris, 100 mM NaCl, pH=7.5. Similar results were obtained for POPC/POPS/VAMP2 as well as for the pure lipid SLBs, i.e. POPC and POPC/POPS

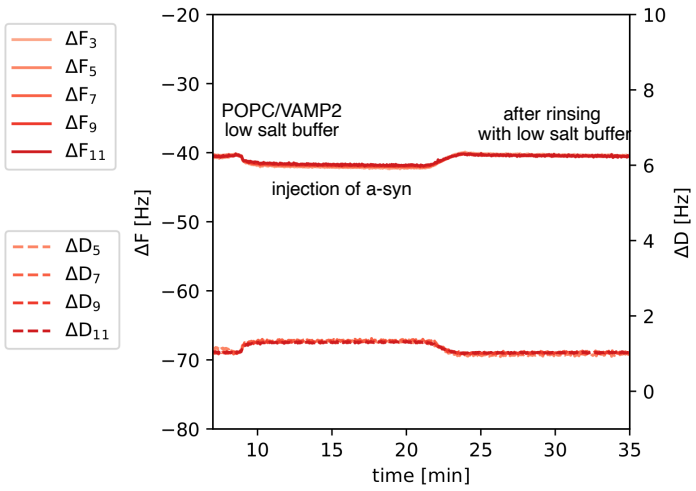

Figure 5: QCM-D data showing the formation of the POPC/VAMP2 SLB at the sensor in low salt buffer (0.5mM TRIS, pH=7.5). Injection of α-syn (10μM) produced a small decrease in the frequency shift, however as soon as fresh buffer solution was introduced in the QCM-D cell the initial ΔF value was restored. This suggests that buffer rising removed all the α-syn molecules initially injected.

References

[1] A. Luchini, F. G. Tidemand, N. T. Johansen, M. Campana, J. Sotres, M. Ploug, M. Cárdenas, and L. Arleth, "Peptide disc mediated control of membrane protein orientation in supported lipid bilayers for surface-sensitive investigations," *Analytical Chemistry*, vol. 92, no. 1, pp. 1081–1088, 2020.

[2] A. Luchini, F. Sebastiani, F. G. Tidemand, K. C. Batchu, M. Campana, G. Fragneto, M. Cárdenas, and L. Arleth, "Peptide discs as precursors of biologically relevant supported lipid bilayers," *Journal of Colloid and Interface Science*, vol. 585, pp. 376–385, 2021.

[3] L. G. Parratt, "Surface studies of solids by total reflection of x-rays," *Phys. Rev.*, vol. 95, pp. 359–369, 1954.

[4] D. Bach and I. Miller, "Attenuated total reflection (atr) fourier transform infrared spectroscopy of dimyristoyl phosphatidylserine–cholesterol mixtures," *Biochimica et Biophysica Acta (BBA) - Biomembranes*, vol. 1514, no. 2, pp. 318–326, 2001.