Supporting Information.

Membrane Bound Alpha-Synuclein Forms an Extended Helix: Long-Distance Pulsed ESR Measurements Using Vesicles, Bicelles, and Rod-Like Micelles

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Protein expression, purification, and labelling.

Alpha-Synuclein (αS) mutants were expressed, purified, and spin-labeled with MTSSL as described in the literature [S1-S3]. For this work, eight double mutants were made – Q24C/E61C, Q24C/T72C, V3C/H50C, E13C/H50C, H50C/T72C, E20C/S42C, S42C/E61C, and E35C/H50C.

Preparation of liposome and bicelle bound protein samples

1-Palmitoyl-2-Olenoyl-*sn*-Glycero-3-Phosphocholine (POPC) and 1-Palmitoil-2-Olinoil-*sn*-Glycero-3-Phosphate (Monosodium Salt) (POPA) came in chloroform (25mg/ml), whereas 1,2-Dimyristoil-*sn*-Glycero-3-[Phospho-*rac*-(1-glycerol] (Sodium Salt) (DMPG), 1,2-Dimyritroil-*sn*-Glycero-3-Phospholine (DMPC) and 1,2-Dihexanoil-*sn*-Glycero-3-Phosphochlorine ([DHPC) were purchased from Avanti Polar Lipids and used as received. Perdeuterated phospholipids DMPC-d67 and DMPG-d54 were also purchased from this company and were used in some long-distance measurement (mutants Q24C/E61C, Q24C/T72C, V3C/H50C, E13C/H50C).

Chloroform or chloroform/methanol/water stock solutions were mixed to achieve the desired molar ratios of phospholipids and the solvent was removed with nitrogen gas flow. The resulting phospholipids films were dried overnight under vacuum to completely remove organic solvents. The dry lipid films were then rehydrated in 10 mM phosphate D_2O buffer, pH 7.4. In both lipid mixtures used in this work, namely, POPC/POPA and DMPC/DMPG (or DMPC-d67/DMPG-d54), the charged and uncharged phospholipids were in the ratio of 1:1. The total phospholipid concentration was 60 mM before spinning down (see below).

Small unilamellar vesicles (SUV's) were prepared following the procedure described in [S4] used with minor modifications. POPC/POPA vesicles were made by sonicating liposome solutions 3 times for 2 min at room temperature (RT) under anaerobic conditions. For multi-lamellar vesicles and titanium particles removal ready liposome solutions were centrifuged at

3000 x g for 45 min at RT. The supernatant, containing small liposomes, was collected. During the process the temperature of the liposome solution was maintained above 25°C [S5]. SUVs' of DMPC/DMPG were prepared following the same procedure, but in air. Both types of liposome solutions were stored in a water bath at 28 – 30 °C. Proteoliposomes were prepared by adding the protein stock solutions followed by incubation for 10 min at the same temperature. Finally, proteoliposome samples were loaded into ESR sample tubes and plunge-frozen in liquid nitrogen. The concentration of spin-labeled protein in the samples was in the range of 18 to 50.5 μM. Throughout all experiments only freshly made proteoliposome samples were used.

It is known that αS binds to both DMPC/DMPG and POPC/POPA liposomes and folds into a helical structure [S6, S7]. The sizes of vesicles were measured by transmission electron microscopy using negative staining, and they were in the range of 40 – 80 nm in diameter.

Isotropic bicelle protein samples were prepared using the following procedure. A chloroform solution of 13:25:6 mixture of DHPC, DMPC, and DMPG (using DMPC-d67 and DMPG-d54 when needed) was lyophilised as described above, and then redissolved to the final concentration of 16.7% (w/v) total phospholipid in the 10 mM phosphate D_2O buffer, pH 7.4. The total lipid concentration was 270 mM, with a composition corresponding to q of 2.6 (q = [DMPC + DMPG]/[DHPC]). After vortexing for ~10 min the solution was left for 30 min at RT, followed by cooling down and keeping at 4°C for the same period of time. Then it was vortexed again for 15 min at RT. The solution was placed for 30 min on a water bath at 38°C; kept for 15 min at 4°C; and then vortexed several times, cycling temperature between 4°C and RT. Prepared in this way, the bicellar stock solution was stored at -30°C. The desired lipid concentration was produced by diluting the stock solution. The solution was placed on a water bath for incubation at 20 °C, mixed with α-Synuclein for a total concentration of 162 mM phospholipid and 100 - 120 μM protein, and finally incubated for at least 20 min at the same temperature. 15 % (w/w) ethylene glycol-d4 was added to each sample and carefully mixed. Finally ca. 15 μL of the solutions was transferred into the sample tube and plunge-frozen in liquid nitrogen for pulsed ESR measurements.

The protein binding to bicelles and its folding was verified by CD spectroscopy. The bicelle radius was estimated by dynamic light scattering to be 10 nm.

Preparation of rod-like micelle bound protein samples

Rod-like micelle-bound protein samples were prepared by reconstituting spin-labeled protein at RT into a solution of 450 mM SDS-d25 in deuterium buffer – 10 mM Na_2HPO_4, 100 mM NaCl, pH 7.4. Samples were adjusted to a final protein concentration in the range of 70 to 100 μM. Solutions were gently mixed and 30% (w/w) glycerol-d8 was added and thoroughly mixed by vortexing. Freshly made solutions were then immediately transferred into the sample tubes and plunge frozen in liquid nitrogen.

DEER measurements and data analysis.

Four-pulse DEER experiments were conducted at 17.3 GHz and 60 K as described in [S8]. Long distances benefited from using bicelles, particularly due to increased phase relaxation times, when bicelles were prepared using deuterated lipids. Additional benefit was due to the uniform distribution of spins through the sample as opposed to the case of vesicles. This greatly simplified data analysis based on the L-curve Tikhonov regularization method [S8, S9] to reconstruct distances.

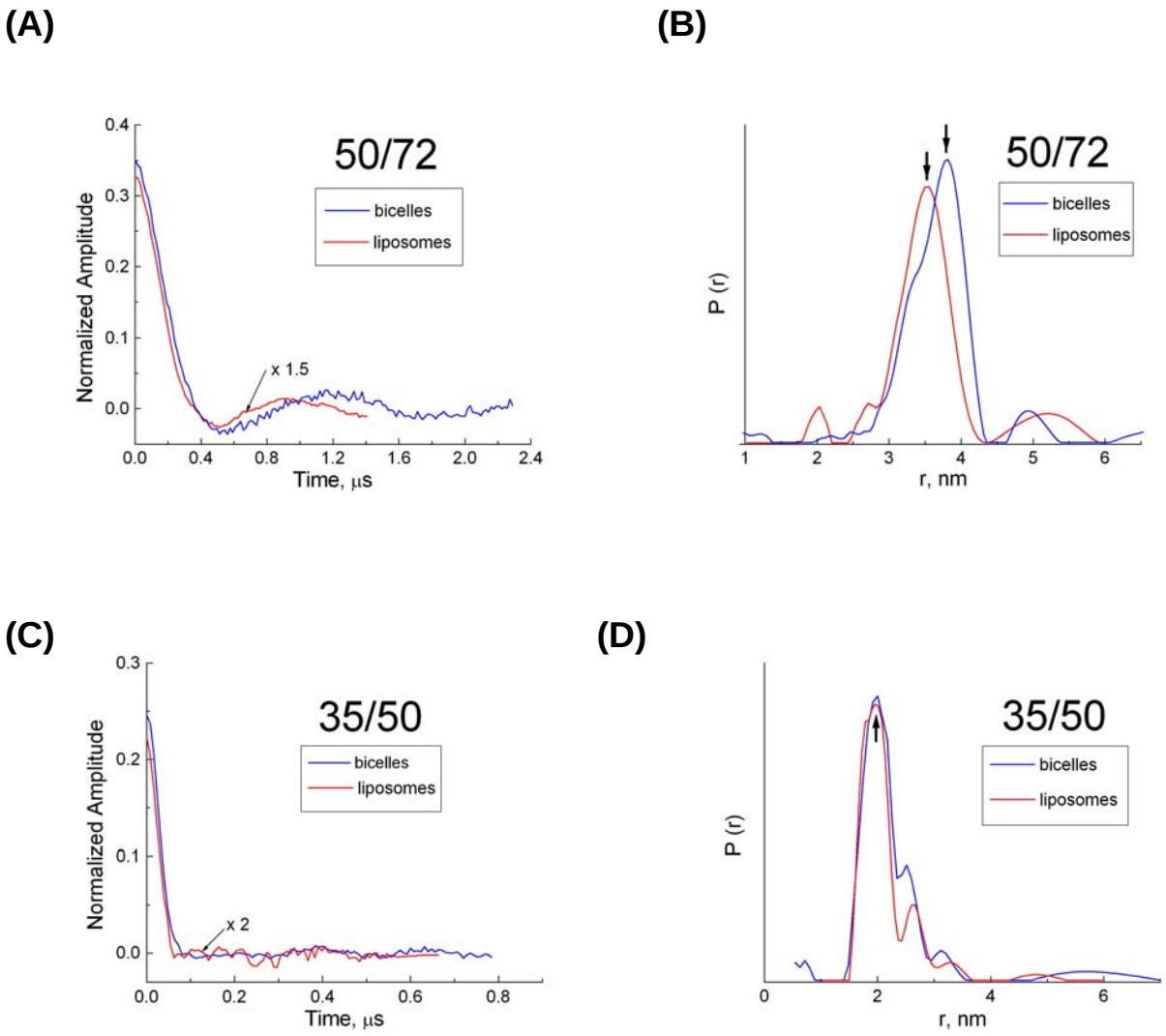


Figure S1. (A, C) – The time-domain DEER signals recorded for α-synuclein (αS) double mutants H50C/T72C and E35C/H50C in bicelles (blue) and SUV's of 1:1 DMPC:DMPG (red). Signal backgrounds were removed by subtracting out the linear (bicelles) or 2nd-degree polynomial (SUV's) fits using ca 60% of the latter data points in a logarithmic scale. (B, D) – The respective distance distributions obtained by the L-curve Tikhonov regularization method. Arrows indicate distances shown in the main text.

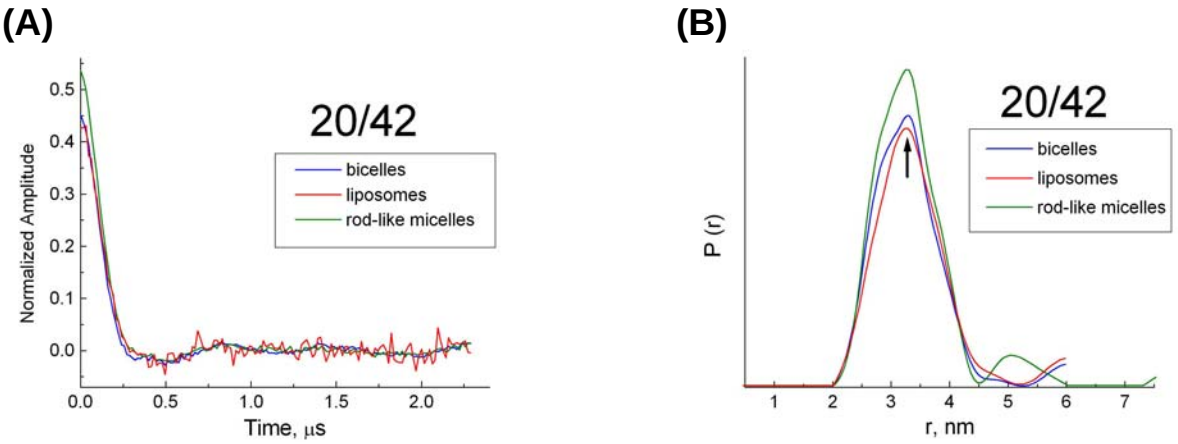


Figure S2. (A) – The time-domain DEER signals recorded for αS double mutant E20C/S42C in bicelles (blue), rod-like SDS micelles (green), and SUV's of 1:1 DMPC:DMPG (red). Signals backgrounds were removed by subtracting out the linear (bicelles and micelles) or 2nd-degree polynomial (SUV's) fits to ca 60% of the latter data points in log plots. (B) – The respective distance distributions yielded by the L-curve Tikhonov regularization method. Arrow indicates distances shown in the main text.

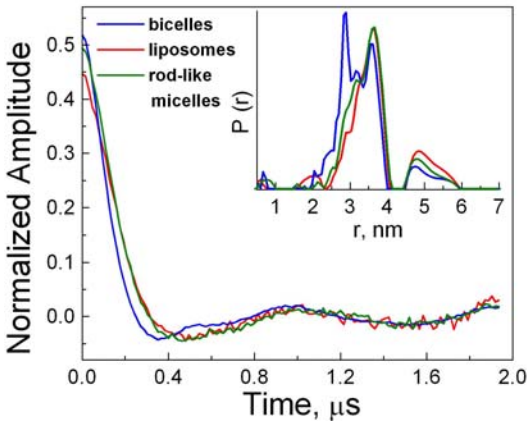
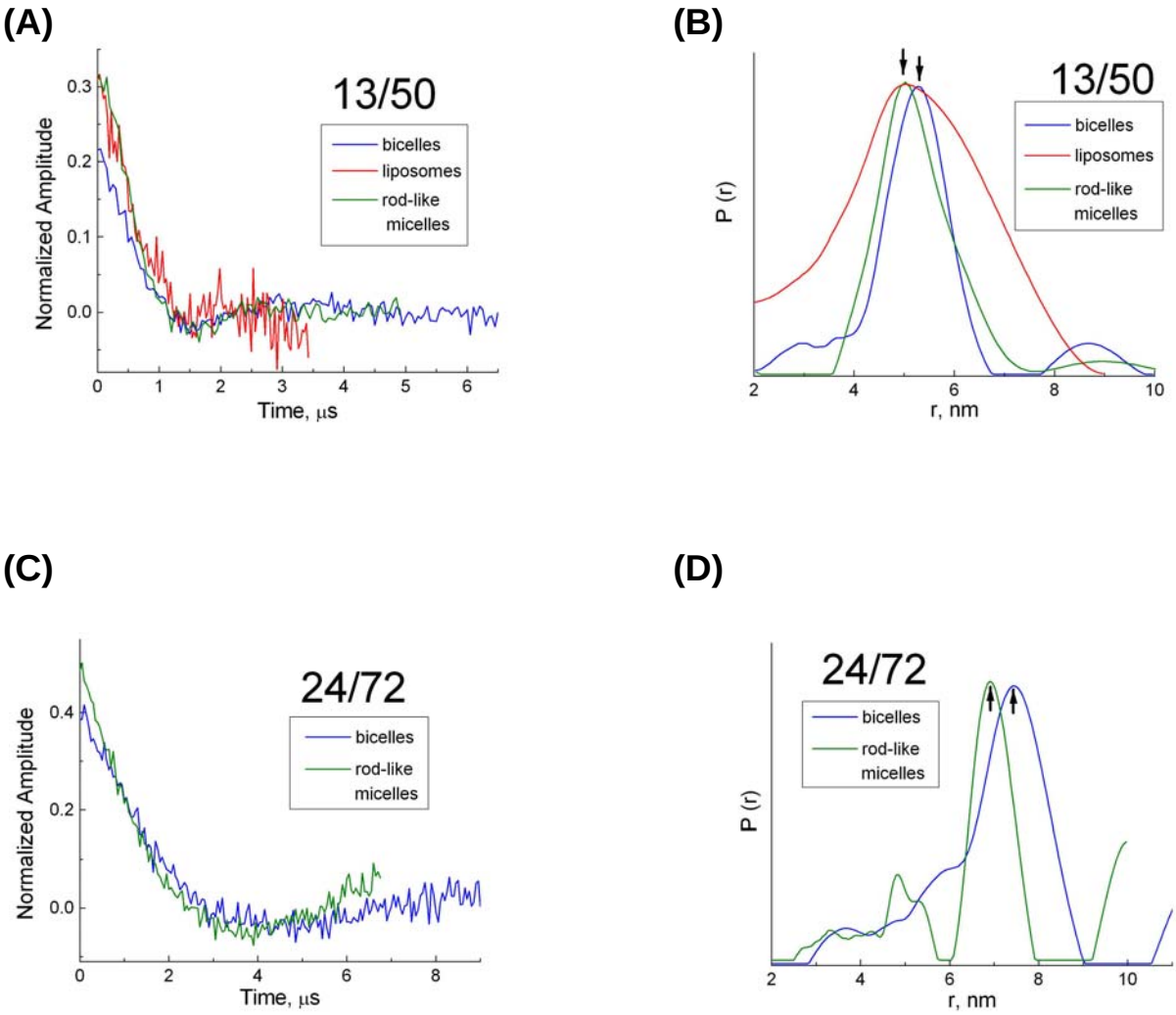


Figure S3. Time-domain DEER signals obtained for the double mutant S42C/E61C, reconstituted into 450 mM SDS (green), DMPC:DMPG 1:1 SUVs (red), and bicelles (blue). The backgrounds were removed in the log plot, as described above. The respective distance distributions, shown in the insert, were generated using L-curve Tikhonov regularization. Note, that for bicelles a sharp peak at ~2.8 nm points to the possibility of a (minor) conformation with a shorter distance.



Figures S4. (A, C) - Time-domain DEER signals for the labels on different sides of the "helix break", E13C/H50C and Q24C/T72C. The data were obtained in rod-like micelles of SDS (green), SUV's (red), and bicelles (blue). The backgrounds were removed as described above; Note that the evolution time has nearly doubled in the case of bicelles as compared to liposomes, with a concomitant increase in SNR and resolution in distance. (B, D) - Respective distance distributions produced by L-curve Tikhonov regularization.

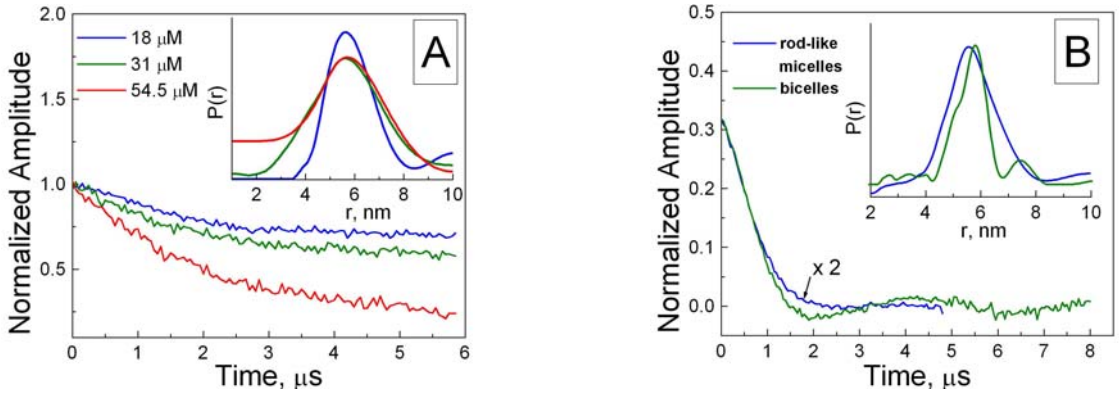


Figure S5. Time-domain Ku-band DEER signals recorded for αS double mutants Q24C/E61C. (A) - Raw data shown used three concentrations of αS bound to POPC:POPA (1:1) SUVs. The signal for the lowest concentration indicates that its profile is for the most part due to the intra-molecular dipolar signal, with the slope of the background signal being relatively small at 18 μM protein concentration. (B) – The DEER data shown for the same mutant bound to DMPC:DMPG bicelles and rod-like SDS micelles. The background was removed by subtracting out the linear part of signal decay in the log plot. In all cases the respective distance distributions, shown in inserts, were produced by the L-curve Tikhonov regularization method.

(A) **(B)**

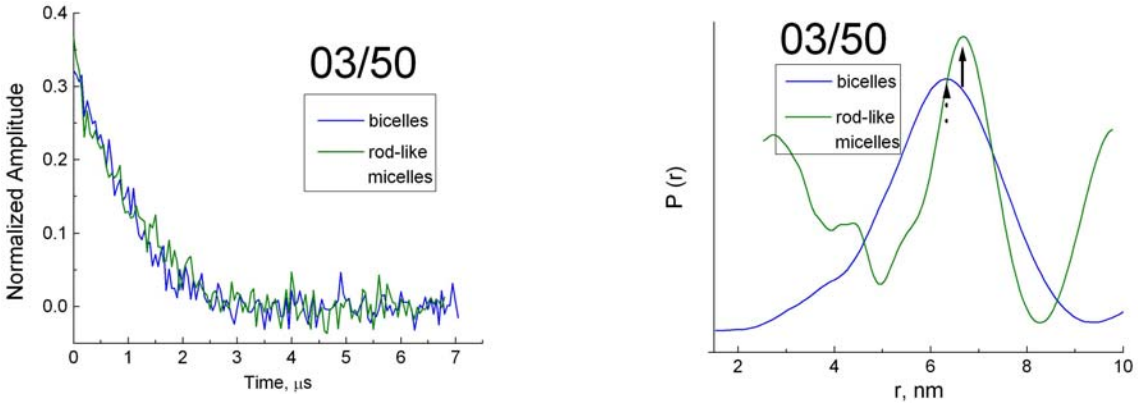


Figure S6. (A) - Time-domain Ku-band DEER signals recorded for αS double mutant V3C/H50C In bicelles (blue) and rod-like SDS micelles (green). (B) - The respective distance distributions, shown in inserts, were generated using the L-curve Tikhonov regularization method.

(A)

1 9 – I 20 – II 31 - III
MDVFMKGL SKAKEGVVAAA EKTKQGVAEAA GKTKEGVLYVG

42 – IV 53 57 – V 68 – VI 79 – VII
SKTKEGVVHGV ATVA EKTKEQVTNVG GAVVTGVTAVA QKTVEGAGSIA

90 100 140
AATGFVKKDQ LGKNEEGAPQEGILEDMPVDPDNEAYEMPSEEGYQDYEPEA

(B)

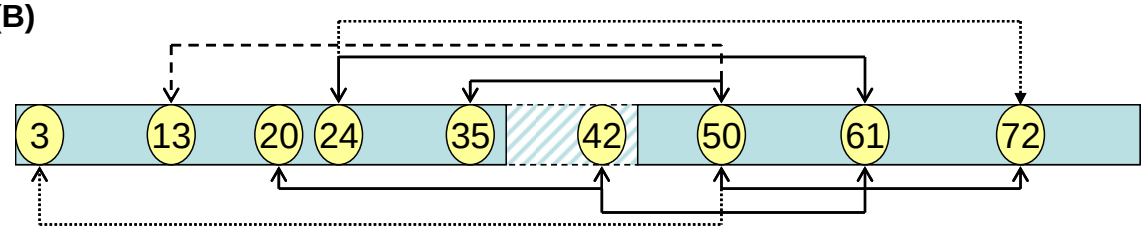


Figure S7. (A) – The amino-acid sequence of αS. Seven imperfect repeats in the N-terminal part of the protein are labeled with Roman numbers; (B) – A cartoon illustrating the selection of spin-labeled sites for the distance measurements.

References

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