Supporting Materials

# Tau binds to lipid membrane surfaces via short amphipathic helices located in its microtubule-binding repeats

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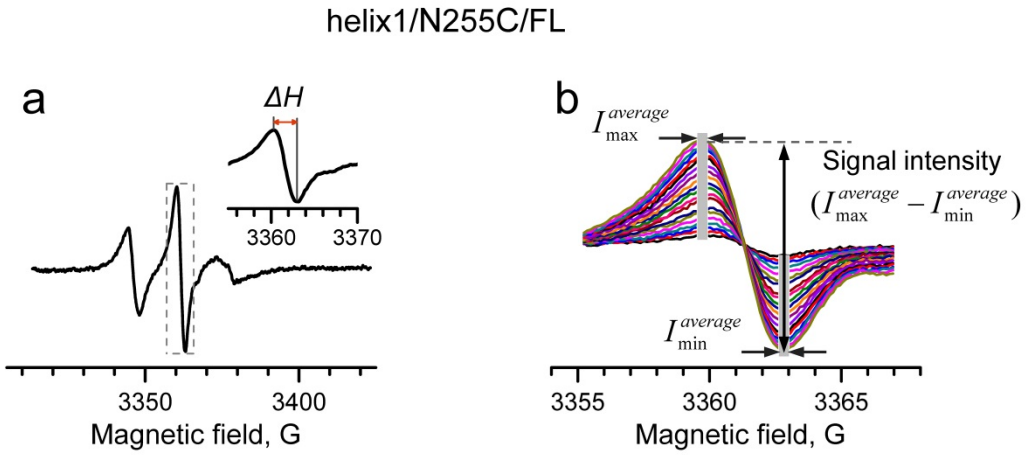


**Figure S1.** (***a***) The cw ESR spectrum of spin-labeled residue N255C in MBD repeat 1 (R1)/helix-1 of full length (FL) tau352 bound to liposomes of POPC/POPS is shown. The spectrum was recorded in air ($O_2$). The experimental parameters used were: 1.26 mW incident microwave power and 2.3 G field modulation amplitude. The central spectral line and its width, $\Delta H$, used in Eq.2 to estimate the nitroxide spin-label accessibilities to $O_2$ and NiEEDA are shown in the inset. (***b***) Central line of the nitroxide cw ESR spectrum recorded at incident microwave power over the range of 0.5 mW to 200 mW in air. The central line intensity was calculated as the difference between the maximal, $I_{max}$, and minimal, $I_{min}$, intensity value for each microwave power applied. $I_{max}$ and $I_{min}$ were averaged over a 1G interval (seven data-points).

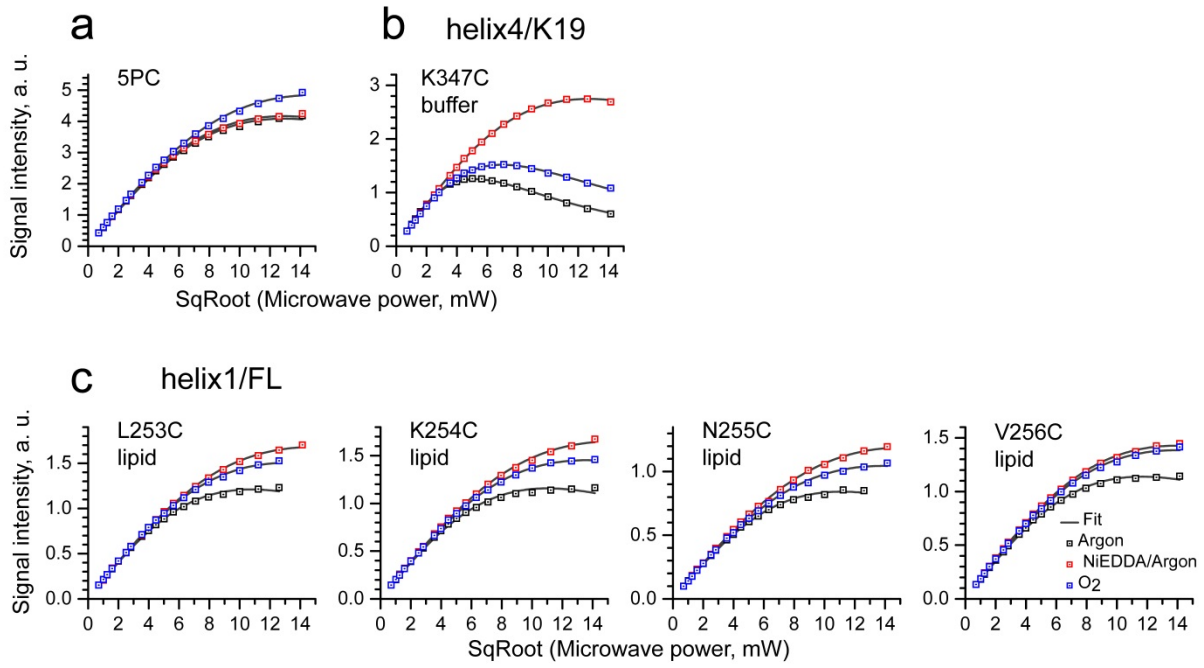


**Figure S2.** (***a***) Microwave power saturation data for the spin-labeled lipid 1-palmitoyl-2-stearoyl-(5-doxyl)-*sn*-glycero-3-phosphocholine (16:0-5 Doxyl PC, 5PC) in POPC/POPS liposomes, at a non-labeled:labeled lipid molar ratio of 1:690. (***b***) Microwave power saturation data for spin-labeled residue K347C in R4/helix-4 of tau K19 in buffer. Data for deoxygenated K347C in buffer were used as a reference to calculate the accessibilities to $O_2$ and NiEEDA of nitroxide spin-labels in membrane-bound tau constructs. (***c***) Representative microwave power saturation data for four spin-labeled residues, L253C, K254C, N255C and V256C in MBD R1/helix-1 of full length (FL) membrane-bound tau352. Data were normalized to a common value at 0.5 mW microwave power (first point). Data obtained in deoxygenated samples, in air ($O_2$) and in 5 mM NiEDDA are in black, blue and red, respectively. The curves fitted to Eq. 1 for each data-set are in gray. In the case of tau mutants, saturation of the nitroxide spectrum is affected by both $O_2$ and 5 mM NiEDDA. However, NiEDDA has a more pronounced effect at positions of charged/polar residues, i.e. K254C and N255C, and a lesser effect at positions of more hydrophobic, i.e. L253C and V256C. In the case of spin-labeled lipid, 5PC, the effect of 5 mM NiEDDA on the nitroxide spectrum power saturation is marginal.

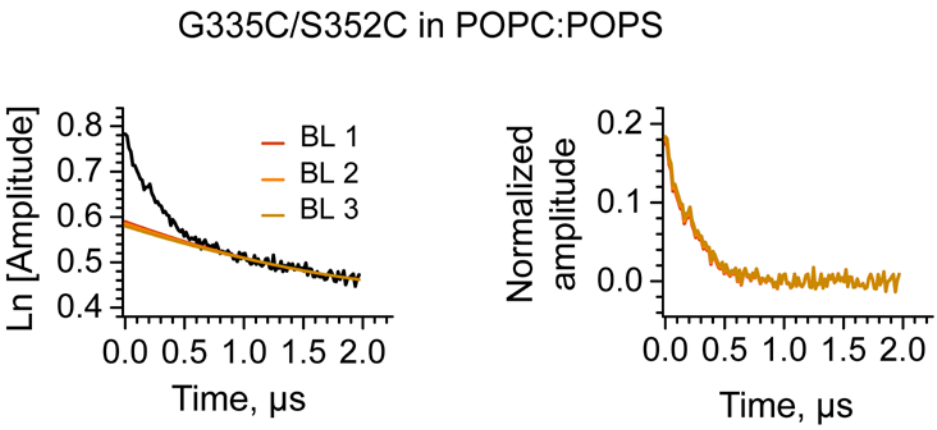


**Figure S3.** Effect of uncertainty in the background on the DEER signal: In the case of lipid samples the background was approximated by a second order polynomial function, which was manually optimized (three examples, BL1, BL2 and Bl3, shown in different shadows of orange-brown) and subtracted from the raw DEER signal plotted on a semi-logarithm scale (left) to produce the final DEER signals (right). The corrected DEER signals on the right are colored according to the base-lines in the left panel, but are essentially indistinguishable. In all other cases, a homogeneous (first order polynomial) background was subtracted from the raw DEER signals. The error produced by base-line uncertainty was negligible.

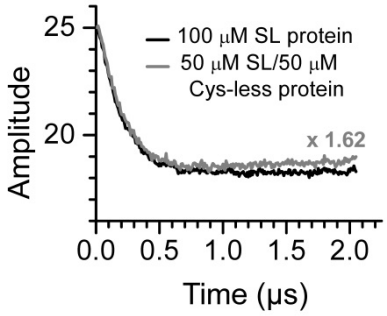


**Figure S4.** Raw time-domain DEER signals from samples of spin-labeled double cysteine mutant G273C/S320C tau K19: Data at 100 µM protein concentration is in black, and data for a magnetically diluted sample at 50 µM spin-labeled protein/50 µM cysteine-free protein is in gray. The buffer used contained 40% (w/v) Gly-d8. The signals from both samples are similar, ruling out any significant presence of protein aggregates in the samples.

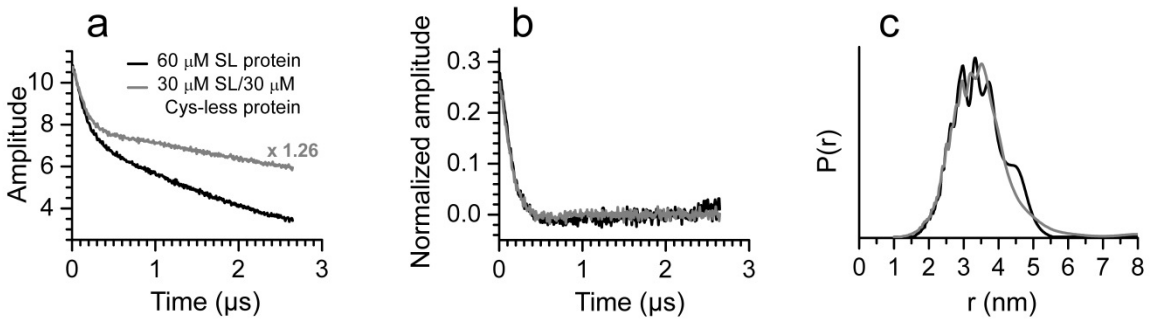


**Figure S5.** Data for spin-labeled double cysteine mutant G273C/S320C in tau K19 in the presence of 40 mM SDS: (***a***) raw experimental time-domain DEER signals for 60 μM spin-labeled protein (black) and magnetically diluted 30 μM spin-labeled/30 μM cysteine-free proteins (gray); (***b***) baseline corrected and normalized time-domain DEER signals for data in (***a***) plotted using the same colors; (***c***) distance distributions reconstructed from data in panel (***b***). Virtually no difference was observed between the baseline-corrected and normalized DEER signals from magnetically-diluted and non-diluted samples and the reconstructed distance distributions are also very similar. Small deviations in the distance distributions are most likely a result of different signal-to-noise ratios in the time-domain signals. Similar experiments were conducted in 450 mM SDS, and again no effect of magnetic dilution was observed.