Supporting Information

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SI Text

Background for Pulse Dipolar ESR Techniques Used. The signal $V(t)$ recorded by the 4-pulse DEER sequence (1–3) is typically a product, $V_{inter}(t)V_{intra}(t)$, with $V_{intra} = V_0\langle 1 - p(1 - \cos\omega(\mathbf{r})t)\rangle_{\mathbf{r}}$ containing the desired intramolecular dipolar signal, $V_{dip}(t) = pV_0\langle\cos\omega(\mathbf{r})t\rangle_{\mathbf{r}}$ originating from the dipole–dipole interaction of the electron spins residing on the same molecule. Here, the characteristic frequency $\omega(\mathbf{r}) = \gamma_e^2 \hbar(1 - 3\cos^2\Theta)/r^3$ is produced by the dipolar coupling between 2 electron spins separated by $r \equiv |\mathbf{r}|$, Θ is the angle between $\mathbf{r}$ and the static polarizing magnetic field, $\hbar$ is Planck's constant, and γ_e is the gyromagnetic ratio for an electron spin. Also, p is the fraction of spins flipped by the pumping pulse. The remainder of V_{intra}, that is, $V_0(1 - p)$, is a constant background from the fraction of spins that contribute to $V(t)$ but have their partners unaffected by the pump pulse and do not add to dipolar signal. Removing this background isolates the $V_{dip}(t)$. As noted above, $V(t)$, recorded in DEER, is modified by a monotonically-decaying V_{inter}, which is produced by dipole–dipole couplings with all other electron spins surrounding the molecule. This presents a task of extracting V_{intra} because the exact time dependence of V_{inter} is often unknown and has to be approximated e.g., by fitting it to a low-order polynomial, using a sufficient number of the latter data points, with subsequent extrapolation of the remaining points. The isolated dipolar signals were analyzed by the L-curve Tikhonov regularization method (4) and refined by MEM (5) to recover the distance distribution, $P(r)$. Typical dipolar signals and respective $P(r)$s are shown in Fig. S1.

VAMP2 Dimerization. In the case of an oligomer of order N, bearing nitroxide spins $k = 1, .., N$, the dipolar signal can be written in the spirit of ref. 6:

$$V_{intra}(t) = V(0)\frac{1}{N}\left\langle \sum_{i=1}^{N} \prod_{k \neq i} (1 - f_k p_{ik} v_{ik}(r_{ik}, t)) \right\rangle_{r_{ik}}. \quad [\text{s1}]$$

Here, $v_{ik}(\mathbf{r}_{ik}, t) \equiv \langle 1 - f_k p_{ik}\cos(\omega(\mathbf{r}_{ik})t)\rangle_{\mathbf{r}}$ is the intermolecular dipolar signal detected on spin i when pumping on spin k, p_{ik} is

the probability of flipping spin k by the pump pulse; $\mathbf{r}_{ik}$ is the vector connecting spins i and k. Also, f_k is the probability for the site k to be populated with spin label. Angular brackets denote averaging over all possible values of $\mathbf{r}_{ik}$. All $v_{ik}(t)$ are zero at $t = 0$ and unity at $t = \infty$. We first assume that all f_k are equal to unity. Because $p_{ik} \ll 1$ at sufficiently large t, and the p_{ik} normally exhibit only small variations, one can expand Eq. **s1** as $V_{intra}(t) \cong V(0)[1 - (N - 1)p]$. Thus the signal "modulation depth," $1 - V(\infty)/V(0)$, is $(N - 1)p$, i.e., this is a measure of the number of spins in the oligomer (7, 8). For just 2 spins $N - 1 = 1$, thus when one finds the modulation depth to be consistent with the calculated value of p, then there is a dimer. Certainly, care should be taken to make sure that $f \approx 1$ or its value is known. The signal should be sampled over a sufficiently long time interval, t_m, to ensure $v(t_m) \approx 1$, (t_m of 1–2 μs was sufficient for VAMP2). We show here a comprehensive example of the data obtained for VAMP2 singly labeled at position 109 reconstituted into α-dodecyl-maltoside (α-DDM) micelles and into the lipid environments, both with and without the addition of cholesterol. Because this mutant is >80% labeled, the dipolar amplitudes agree well with just a dimer in α-DDM and POPC/DOPS. Signals from α-DDM and POPC/DOPS/Chol demonstrate single narrow distributions with average distances of 2.85 and 2.95 nm, respectively. This is clear indication that there are similar dimeric structures in these 2 cases. The signal shows signs of aggregation of dimers, (seen as a broad background in Fig. S2d, red curve) in the latter case. The strongly oscillating signals in Fig. S2 a and c point to the nearly parallel "cones" sampled by MTSL side chains (9–11), leading to such a narrow distance distributions, thus giving a further support to the dimeric state. For the most of other mutants in detergents, the oscillations are less pronounced or absent, which can be attributed to the flexibility of MTSL side chains. The dimer in the absence of cholesterol shows no oscillations for all sites studied, indicating that the structure of the dimer is more conformationally heterogeneous than in the other 2 cases. The average distances however are in the range of 2.0–3.5 nm, with the larger values corresponding to the C terminus.

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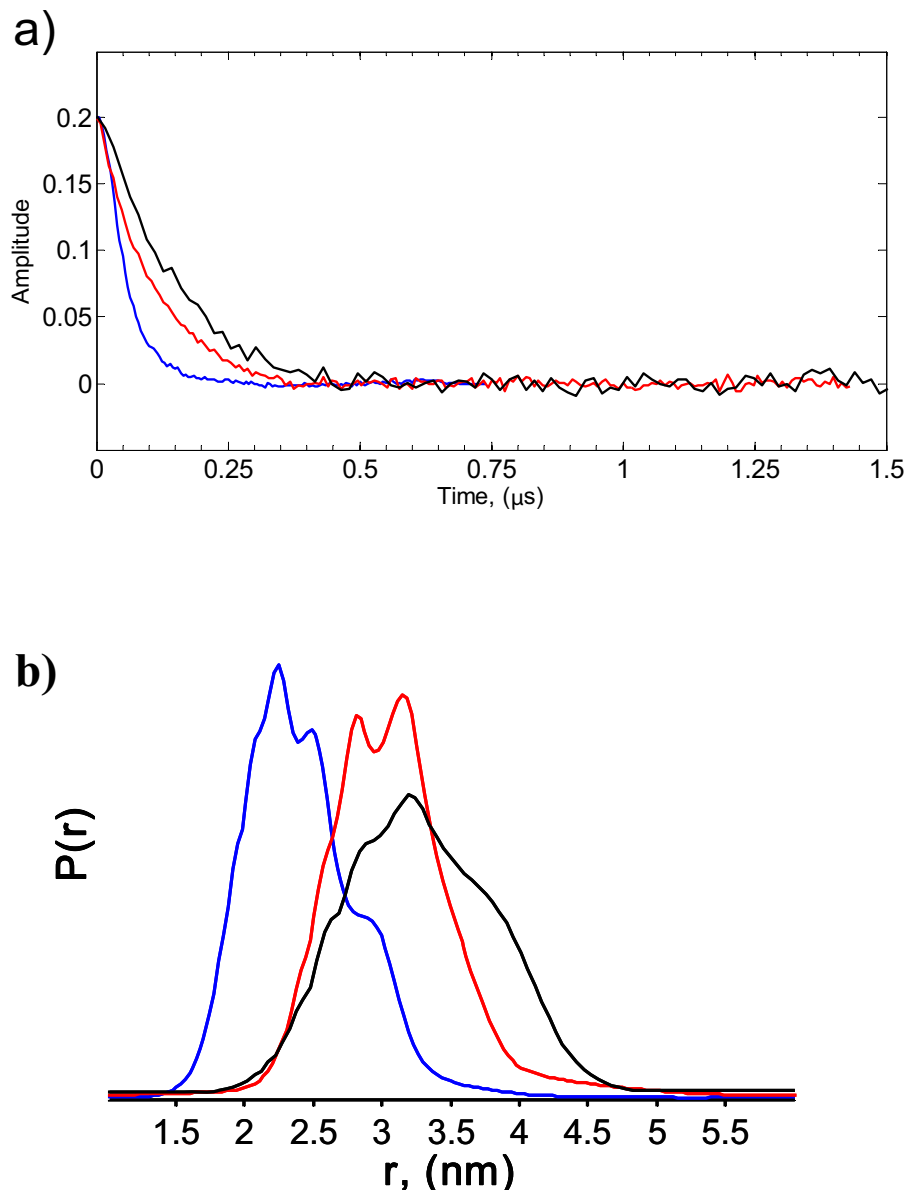
Fig. S1. Representative examples of DEER data for VAMP2 labeled at positions 100, 112, and 115. (*a*) Time-domain DEER signals. Signal backgrounds were removed by subtracting out second-degree polynomial fits using ≈60% of the latter data points in a logarithmic scale. Signals were normalized to a common value of 0.2 at zero time. (*b*) The respective distance distributions obtained by the L-curve Tikhonov regularization method with MEM refinement that included baseline fine adjustment into the fit.
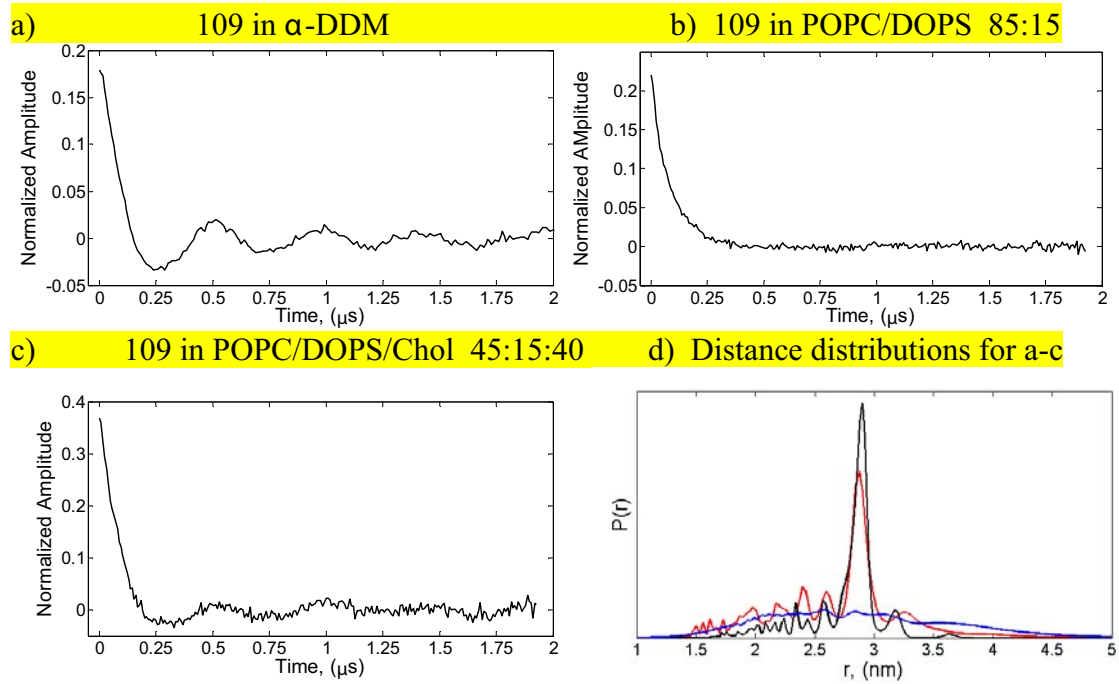
Fig. S2. VAMP2 dimer formation in detergent and vesicles. Normalized dipolar signals are shown after making standard correction for simple exponential background (in detergent) or slightly deviating from this (for liposomes). (*a*) The data were recorded for 109 mutants in α-DDM. (*b* and *c*) Shown are 109 in POPC/DOPS (85:15) and POPC/DOPS/cholesterol (45:15:40), respectively. (*d*) Normalized distance distributions for *a* (black), *b* (blue), and *c* (red).
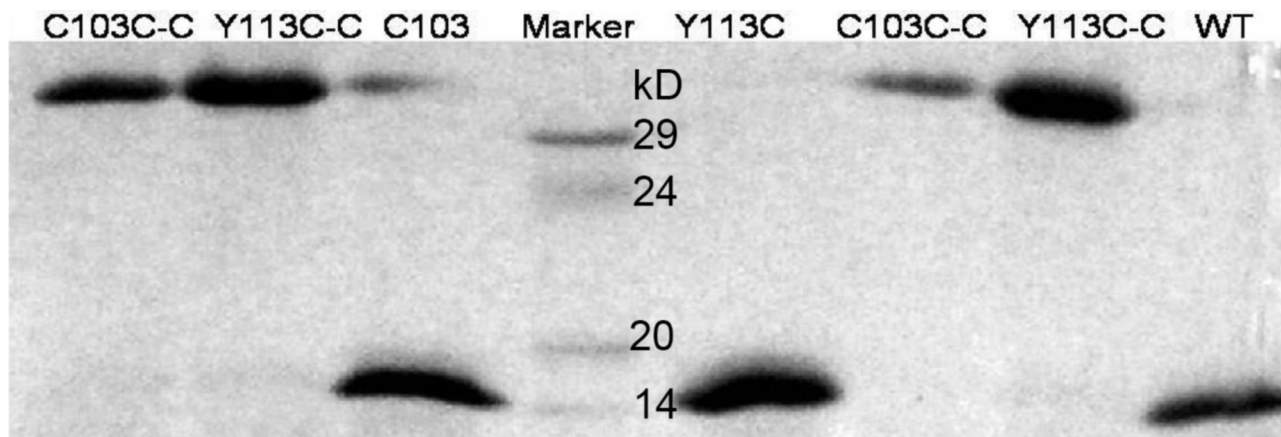
Fig. S3. SDS/PAGE gel analysis for cross-linked dimers. Y113C-C and C103C-C are the cross-linked dimers. The terms Y113C and C103 are for the noncross-linked dimers. Lanes 6 and 7 represent the proteins reconstituted into liposomes. Others represent the protein samples in detergent. The error bars were obtained from at least $n = 4$ independent measurements from four different preparations.