Figure 1S. The structure of biradical **BR**, used for calibration experiments.
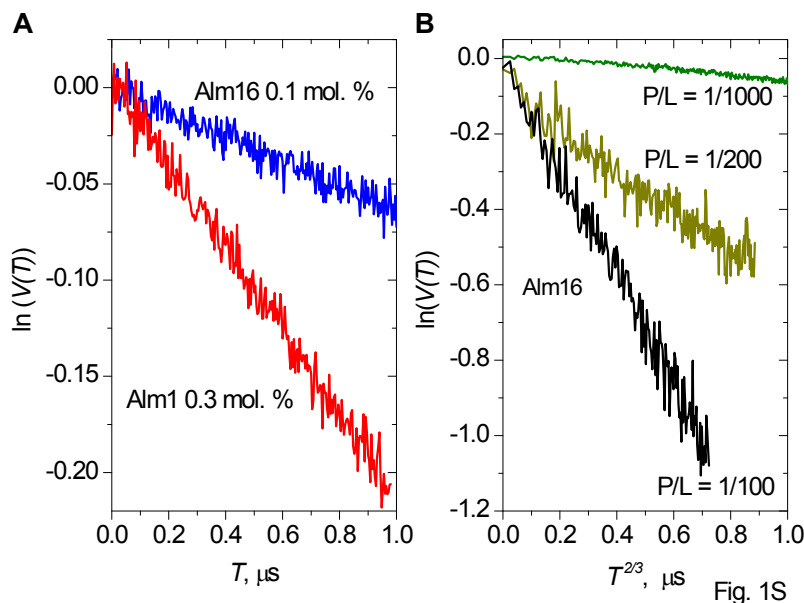

Fig. 1S

Fig.2S. (A) Semilogarithmic plot of 3-pulse ELDOR time traces for 0.1 mol. % Alm16 and 0.3 mol. % Alm1 in a glassy solvent (methanol+ethanol). The slope of linear approximations is expected to obey the theoretical relation [A. D. Milov, A. G. Maryasov, Yu. D. Tsvetkov, *Appl. Magn. Reson.*, *1998*, **15**, 107-143]:

$$\ln(E(T)/E(0)) = -\frac{8\pi^2}{9\sqrt{3}}\frac{g^2\mu_B^2}{h}Cp_BT,$$

where C is the volume spin label concentration taken in cm^{-3} units. The p_B value was 0.22 (calculated from Eq. (4) (main text)). The derived C values turned out to be $(1\pm0.15)*10^{17}$ cm^{-3} for Alm16 and $(3.03\pm0.05)*10^{17}$ cm^{-3} for Alm1, that corresponds to ~0.095 mol. % and ~0.285 mol. %, respectively. (B) 3-pulse ELDOR time traces for Alm16 at different peptide/lipid ratio in dry lipid film before hydration.
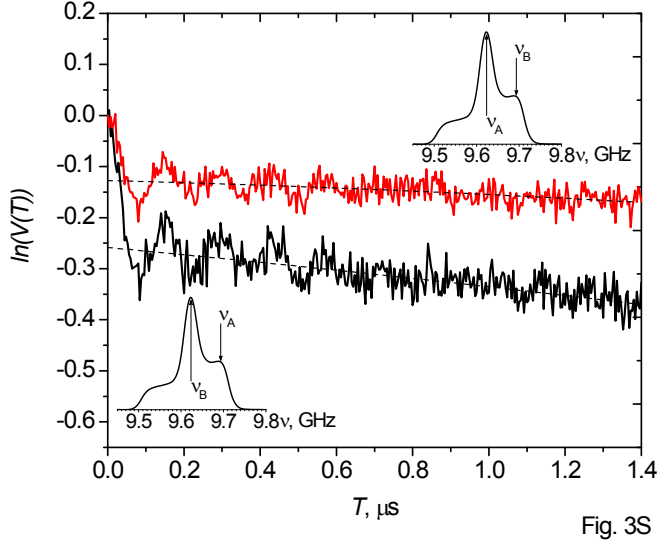
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Fig. 3S

Fig. 3S. 3-pulse ELDOR time traces in semilogarithmic plot for biradical BR for offset ν_A-ν_B=+70 MHz (lower curve) and ν_A-ν_B= - 70 MHz (upper curve), for positions of pumping and observation pulses in the EPR spectrum shown in the inserts. $V_{INTER}(T)$ decays in both cases are shown by dashed lines. The calculated by Eq. (4) (main text) the $p^0_{B(A)}$ value for the excitation in the spectral right position is 0.085 and at the spectral maximum is 0.21. Then, according to the

relation $p_B = \dfrac{p^0_B}{1 - p^0_A}$, the calculated values are $p_B(+70\,\text{MHz}) = 0.23$ and $p_B(-70\,\text{MHz}) = 0.11$, which are to be compared with the obtained from $V_{INTER}(\infty)$ values the experimental ones, $p_B(+70\,\text{MHz}) = 0.227$ and $p_B(-70\,\text{MHz}) = 0.12$.
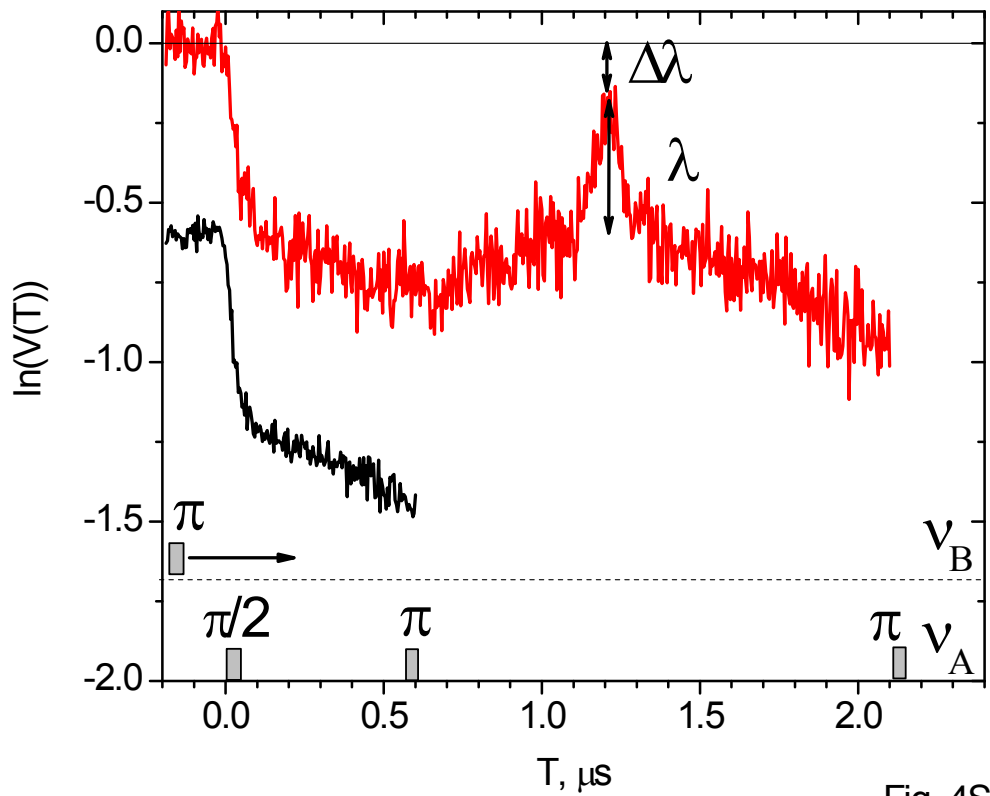
Fig. 4S. Comparison of 3- and 4-pulse ELDOR experiments for Alm16 at P/L =1/100. The pulses are depicted by rectangles. The depth of intra-cluster modulation in 4-pulse ELDOR is shown by λ, the underestimation in 4-pulse ELDOR experiment in compare with 3-pulse experiment is shown by $\Delta\lambda$. The number of scans and number of shots per point are the same for both experiments.
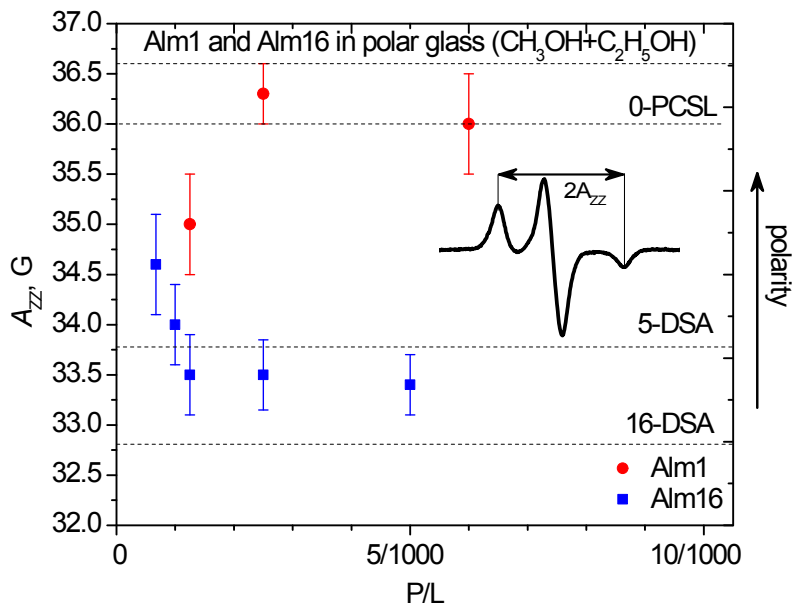
Fig. 5S. The A_{ZZ} splitting for Alm1 and Alm16 in POPC membrane at different P/L obtained from CW EPR spectra (see insert), taken at 100 K. For comparison, the A_{ZZ} values are shown by dotted lines for Alm1 and Alm16 in methanol/ethanol glass, for lipids spin-labeled at the polar head (0-PCSL), and for stearic acids labeled at the 5th or 16th acyl carbon positions (5-DSA and 16-DSA, respectively) in POPC membrane.
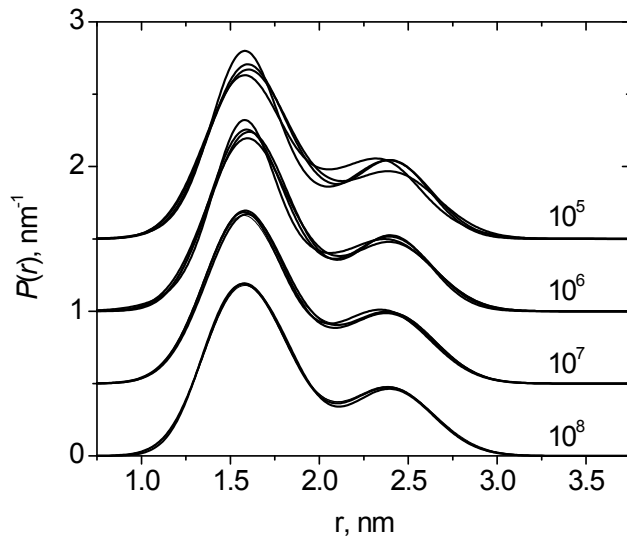
Fig. 6S

Figure 6S. Illustration of convergence of the three-Gaussian fit of PELDOR data in frequency domain employing a fully random Monte Carlo process: for different number of trials four independent realizations are shown. Data are shifted upwards for convenience. The sample is Alm16 in POPC membrane, P/L = 1/200.
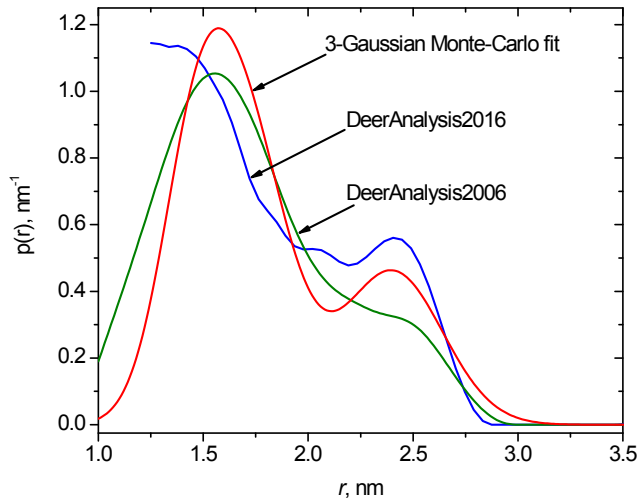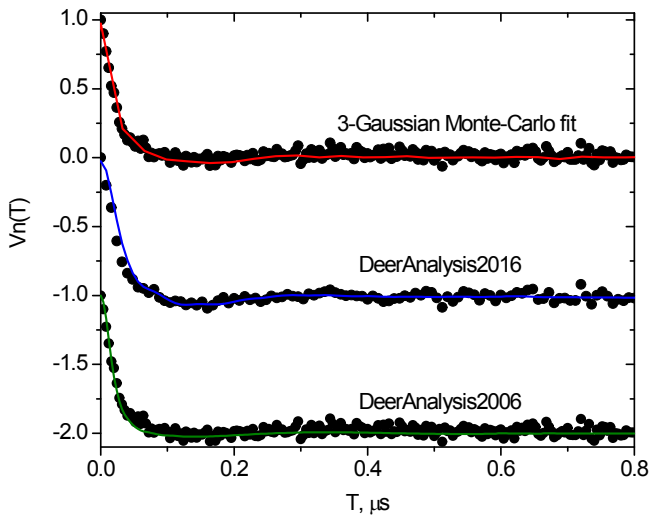
Fig. 7S

Fig. 8S

Figure 7S. The comparison of *P*(*r*) functions for Alm16, P/L= 1/200, by 3-Gaussian Monte-Carlo simulation (red curve) and Tikhonov regularization (simulation performed in DeerAnalysis software, version 2016 (blue curve) and version 2006 (green curve)).

Figure 8S. The time-domain experimental (circles) and simulated $V_n(T)$ curves for *P*(*r*) functions, calculated by different methods (see Fig. 7S).