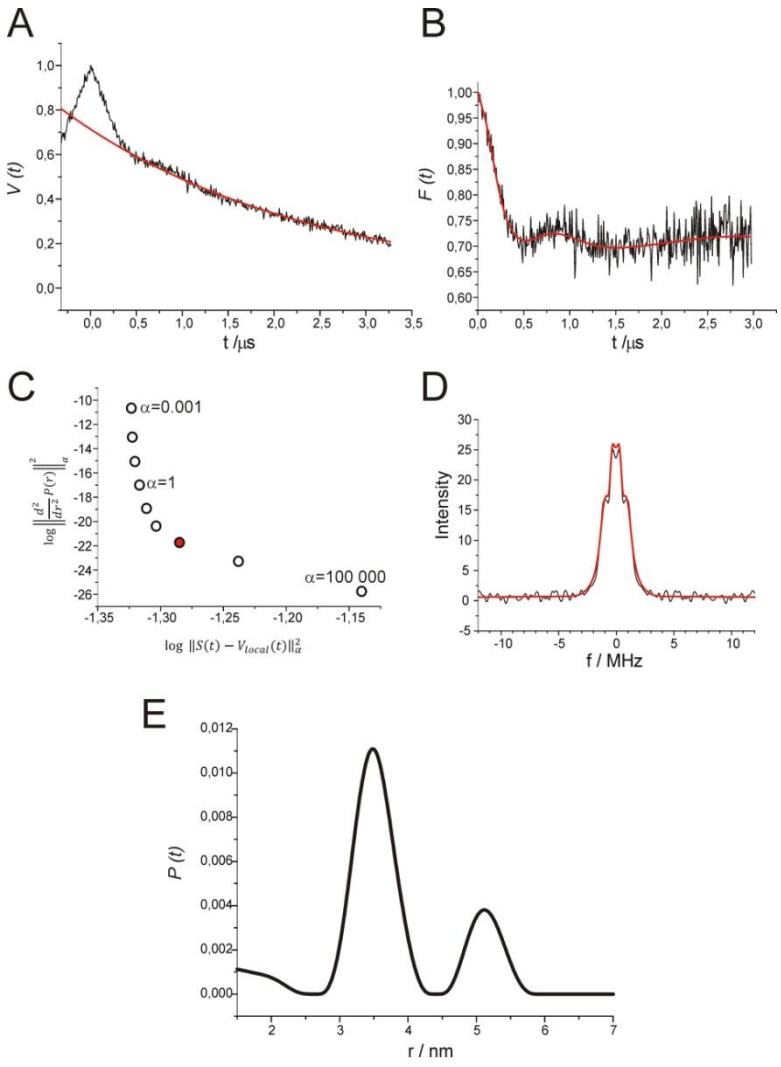
Supplementary Information

DEER data analysis with DeerAnalysis.



Supplementary Figure 1. The course of DEER data analysis by this method exemplified for the data for MnmE-E287R1 + GidA with GppNHp in the presence of K^+.

A. raw DEER signal *V(t)* (black) and background (red line) obtained from the 3D distribution model for the background implemented in the software. The background fit is obtained by fitting of the background function to the last third of *V(t)*.

B. Dipolar evolution data *F(t)* (black) obtained by dividing *V(t)* by the background function. The quality of background subtraction can be assessed by inspection of the Pake Pattern (D, black), which is the fourier transform of *F(t)*.

The Fit to this data (red line) is obtained by Tikhonov regularization. Basis of this approach is the computation of a simulated time domain signal *S*(*t*) from a given distance distribution *P*(*r*) by means of a kernel function *K* (*t*, *r*):

$$S(t) = K(t,r) \cdot P(r) \qquad (1)$$

For ideal microwave pulses, the kernel function for the DEER experiment is given by

$$K(t,r) = \int_0^1 \cos[(3x^2 - 1) \cdot \omega_{DD} \cdot t]dx \quad (2)$$

with

$$\omega_{DD}(r) = \frac{2\pi \cdot 52.04\ MHz\ nm^{-3}}{r^3} \quad (3)$$

for nitroxide spin labels.

In Tikhonov regularization the optimum distance distribution (E) is then found by minimizing the function

$$G_\alpha(P) = \|S(t) - V_{local}(t)\|^2 + \alpha \cdot \left\|\frac{d^2}{dr^2}P(r)\right\|^2, \quad (4)$$

where α is the so called regularization parameter. The first term on the right hand side of Equ. (4) is the mean square deviation between the simulated and the experimental DEER time domain data and the second term is the square norm of the second derivative of the distance distribution *P* (*r*), weighted by α. Variation of the regularization parameter is used to find the best compromise between smoothness, i.e. suppression of artifacts introduced by noise (high values of α), and resolution of *P* (*r*), higher resolution (low values of α). Determination of the optimum regularization parameter is performed by the *L curve criterion*, where the logarithm of the smoothness of *P* (*r*) is plotted against the logarithm of the mean square deviation (C), which allows to choose the value of α that gives the distance distribution with maximum smoothness representing a good fit to the experimental data (B, red line). The red line in D is the fourier transform (Pake Pattern) of the fit.

Table S1:

Dissociation constants (K_D) of MTSL-labeled mutant MnmE proteins and GidA, determined for the GDP-bound state in presence of 100 mM NaCl by fluorescence equilibrium titration.

Proteins	K_D in µM	% MnmE-GidA-complex at 200 µM MnmE, 100 µM GidA [2]
MnmE – wildtype + GidA	3,0 ± 0.16 [1]	88
MnmE – K95R1 + GidA	1.3 ± 0.1	92
MnmE – I105R1 + GidA	4.2 ± 1.7	87
MnmE – S278R1 + GidA	1.8 ± 0.5	91
MnmE – E287R1 + GidA	1.3 ± 0.3	92
MnmE – D366R1 + GidA	1.3 ± 0.3	92

[1] Taken from Meyer et al., 2009 (1)

[2] Fraction of complex to total MnmE-protein, calculated from a bimolecular mass law equation using the respective value of K_D and the minimum concentrations of MnmE and GidA (200 µM, respectively) applied in pulse EPR-measurements.

References

1. Meyer, S., Wittinghofer, A., and Versees, W. (2009) *J. Mol. Biol.* **392,** 910-922