Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information

DEER distance measurements on trityl/trityl and Gd(III)/trityl labelled proteins

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Contents

1. Characterization of CT02MA

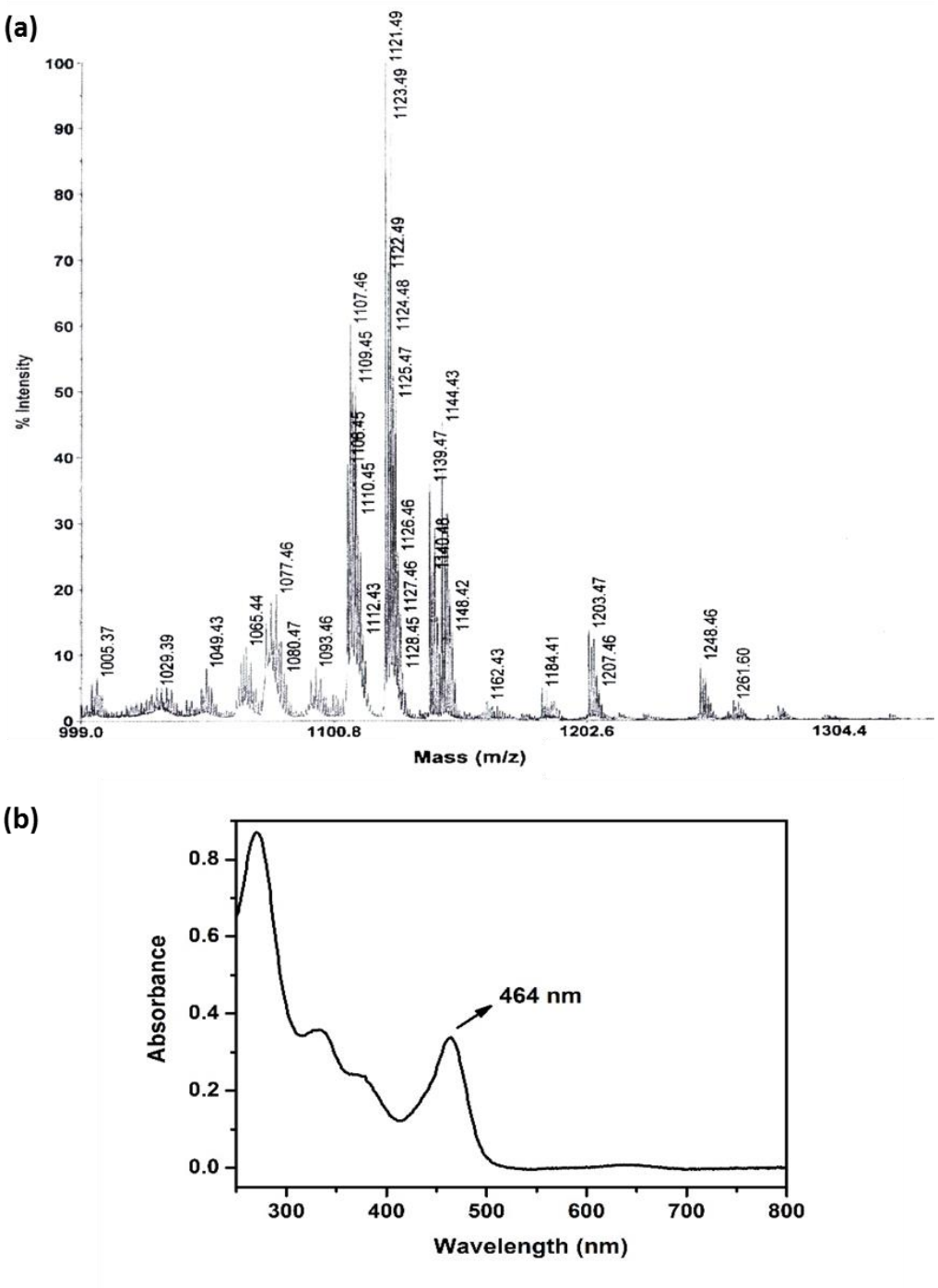

Figure S1. (a) MALDI-TOF spectrum in DHB matrix. The spectrum was acquired on a ABSciex TOF/TOF 5800 system (Applied biosystems intl., inc, CA, USA) equipped with a 355 nm laser and the spectrum was obtained in reflector positive mode with parameters: detector voltage 12.0 kV, ion source 1.59 kV, lens 1 4.0 kV and the laser pulse was set to 200 Hz. (b) UV-Vis spectrum of 20 µM CT02MA in PBS buffer (20 mM, pH 7.4).

2. Estimation of labelling efficiency

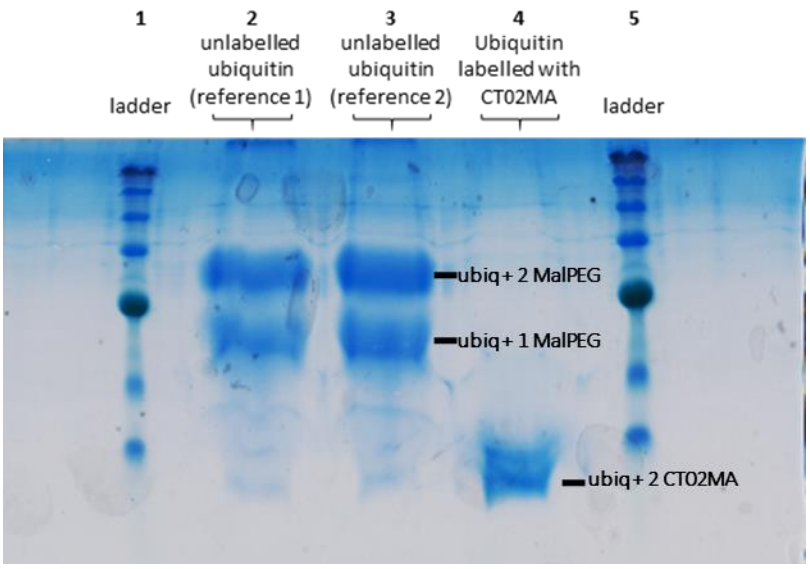

Figure S2. SDS-page of ubiquitin after binding to MalPEG for testing labelling efficiency. Conditions: 18% acrylamide gel, 200 V. As reference we used unlabelled ubiquitin and observed the appearance of the mono- and doubly labelled MalPEG-labelled ubiquitin (lanes 2 and 3).

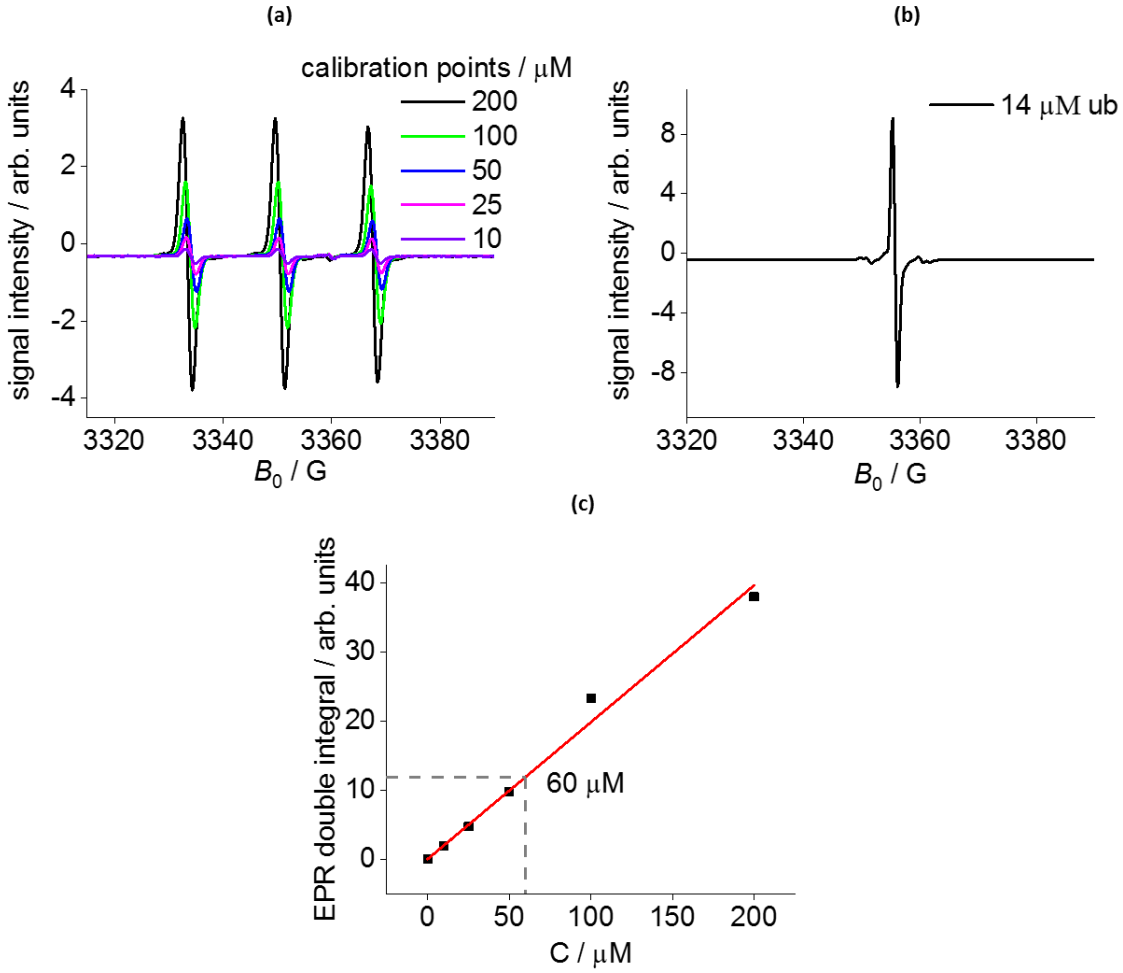

Figure S3. CW-EPR spectrum of (a) different MTSL concentrations (200, 100, 50, 25 and 10 μM) used for the calibration curve and of (b) ubiquitin-trityl. (c) Plot of the double integral of the EPR spectra of the calibration points. The double integral value of the trityl-labelled ubiquitin (unknown spin concentration, 14 μM labelled ubiquitin as estimated from Western blot, see Figure S4) yielded 60 μM spin concentration and is shown in dashed grey lines. Parameters: 9.419 GHz, 34 dB attenuation, 16 scans, 1.0 G modulation amplitude.

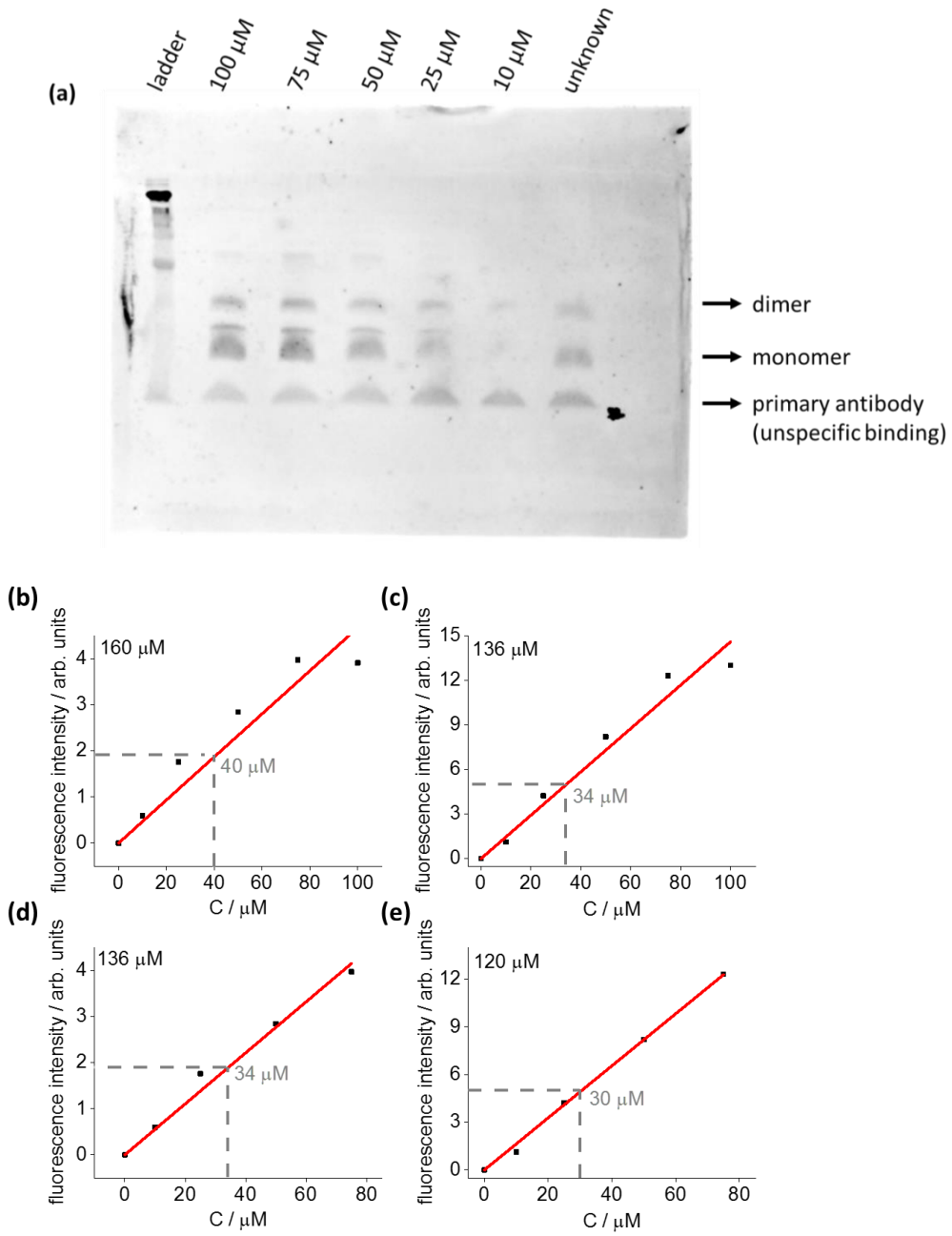

Figure S4. (a) Western blot of ubiquitin with the calibration points (100, 75, 50, 25 and 10 µM) and the unknown sample (4 × diluted CT02MA-labelled ubiquitin) and plot of fluorescence intensity *vs* concentration for dimer (b, d) and monomer (c, e) bands using all data points (b, c) and excluding the 100 µM data point (d, e). From these plots and from the intensity of the unknown sample we estimated the concentration of the diluted unknown (values given in grey) which we then multiplied by four (values given in black). Given that monomer and dimer bands showed good agreement in their fluorescence signals we took their mean value for quantifying the concentration of ubiquitin which was found to be 138 µM.

3. PD-EPR sequences and conditions

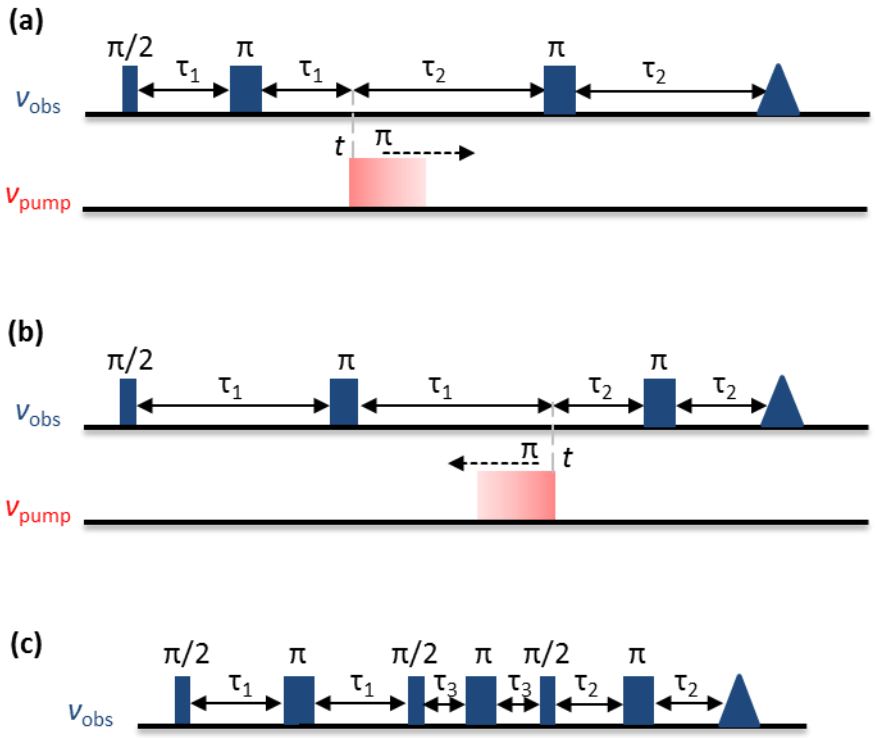

Figure S5. (a) Standard, (b) 'reversed' DEER[1-3] and (c) DQC[4] sequences for measuring spin-spin distances.

Table S1. Parameters of the DEER measurements on GB1-trityl.

protein	GB1						
label	CT02MA						
experiment	'reversed' DEER						
set-up	**1**	**2**	**3**	**4**	**5**	**6**	**7**
π/2-π pulse length / ns (ν_{obs} / GHz)	50-100 (94.91)	50-100 (94.91)	50-100 (94.91)	50-100 (94.885)	50-100 (94.91)	50-100 (94.89)	50-100 (94.90)
pump pulse length / ns (ν_{pump} / GHz)	50 (94.89)	30 (94.88)	30 (94.885)	30 (94.91)	128 (94.86-94.89)*	128 (94.91-94.94)*	128, 128 (94.86-94.88, 94.92-94.94)*
τ_1 / µs	5.2	5.2	5.2	5.2	5.2	5.2	5.2
τ_2 / µs	3	3	3	3	3	3	3
starting *t* / ns	-200	-200	-200	-200	-200	-200	-200
Δ*t* / ns	25	25	25	25	25	25	25
repetition time / ms	10	10	10	10	10	10	10
SNR /min^{-1}	0.094	0.059	0.111	0.055	0.079	0.075	0.055

*in all Tables it refers to chirp pulses covering the stated range of frequencies.

Table S2. Parameters of the DQC measurements on GB1-trityl and ubiquitin-trityl.

protein	GB1	ubiquitin
label	CT02MA	CT02MA
experiment	DQC	DQC
π/2-π pulse length / ns (ν_{obs} / GHz)	10-20 (9.80)	10-20 (9.80)
τ_1 / µs	2.1	1.6
τ_2 / µs	2.4	1.9
τ_3 / ns	30	30
starting *t* / ns	-300	-300
Δ*t* / ns	20	20
repetition time / ms	1	3

Deuterium modulation was suppressed by incrementing τ_1 and τ_2 three times by 108 ns and summing the obtained spectra.[5]

Table S3. Parameters of the DEER measurements on GB1-Gd(III) and ubiquitin-Gd(III).

protein	GB1	ubiquitin
label	BrPy-DO3MA-Gd(III)	BrPy-DO3MA-Gd(III)
experiment	standard DEER	standard DEER
π/2-π pulse length / ns (ν_{obs} / GHz)	15-30 (94.95)	15-30 (94.95)
pump pulse length / ns (ν_{pump} / GHz)	15 (94.85)	15 (94.85)
τ_1 / µs	0.35	0.35
τ_2 / µs	6.2	6.0
starting *t* / ns	-200	-200
Δ*t* / ns	30	30
repetition time / ms	0.8	0.8

Table S4. Parameters of the DEER measurements on ubiquitin-trityl.

protein	ubiquitin	
label	CT02MA	
experiment	'reversed' DEER	
set-up	**3**	**7**
π/2-π pulse length / ns (ν_{obs} / GHz)	50-100 (94.91)	50-100 (94.90)
pump pulse length / ns (ν_{pump} / GHz)	30 (94.885)	128, 128 (94.86-94.88, 94.92-94.94)*
τ_1 / µs	4.5	4.7
τ_2 / µs	3	3
starting *t* / ns	-200	-200
Δ*t* / ns	25	20
repetition time / ms	10	10
SNR / min^{-1}	8.06E-02	4.87E-02

Table S5. Parameters of the DEER measurements on ubiquitin-trityl/Gd(III).

set-up	**1**	**2**	**3**	**4**	**5**
observe	Gd(III)	Gd(III)	Gd(III)	Gd(III)	Gd(III)
pump	trityl	trityl	trityl	trityl	Gd(III)
cavity	single	single	dual	dual	single
DEER set-up	standard	'reversed'	standard	'reversed'	'reversed'
π/2-π pulse length / ns (ν_{obs} / GHz)	15-30 (94.8)	15-30 (94.76)	40-80 (94.48)	40-80 (94.48)	15-30 (94.95)
pump pulse length / ns (ν_{pump} / GHz)	25 (94.9)	96 (94.85-94.95)*	75 (95.0)	200 (94.97-95.03)*	96 (94.55-94.85)*
τ_1 / µs	0.375	4.0	0.375	3.2	4.2
τ_2 / µs	4.0	3.0	3.3	1.0	3.0
starting t / ns	-210	-200	-210	-200	-200
Δt / ns	30	25	30	25	25
repetition time / ms	1	0.8	0.8	0.8	0.8
SNR /min^{-1}	0.038	0.052	0.088	0.157	0.108

4. Additional EPR data on GB1-CT02MA

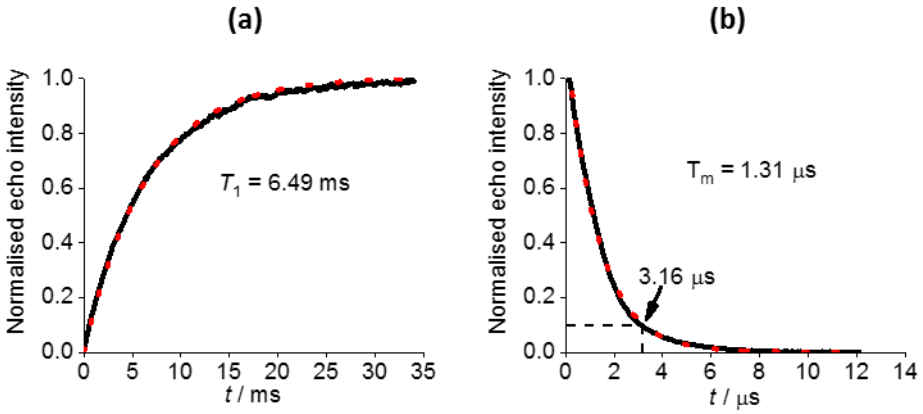

Figure S6. (a) Saturation recovery measurement and (b) two pulse echo decay traces of GB1-trityl. The longitudinal relaxation T_1 after fitting a mono-exponential fit function (red dotted line) is given in (a) and the value at which the echo decays to 10% of its initial value and the phase memory time T_m of the trityl spins after fitting a mono-exponential decay function (red dotted line) are given on (b).

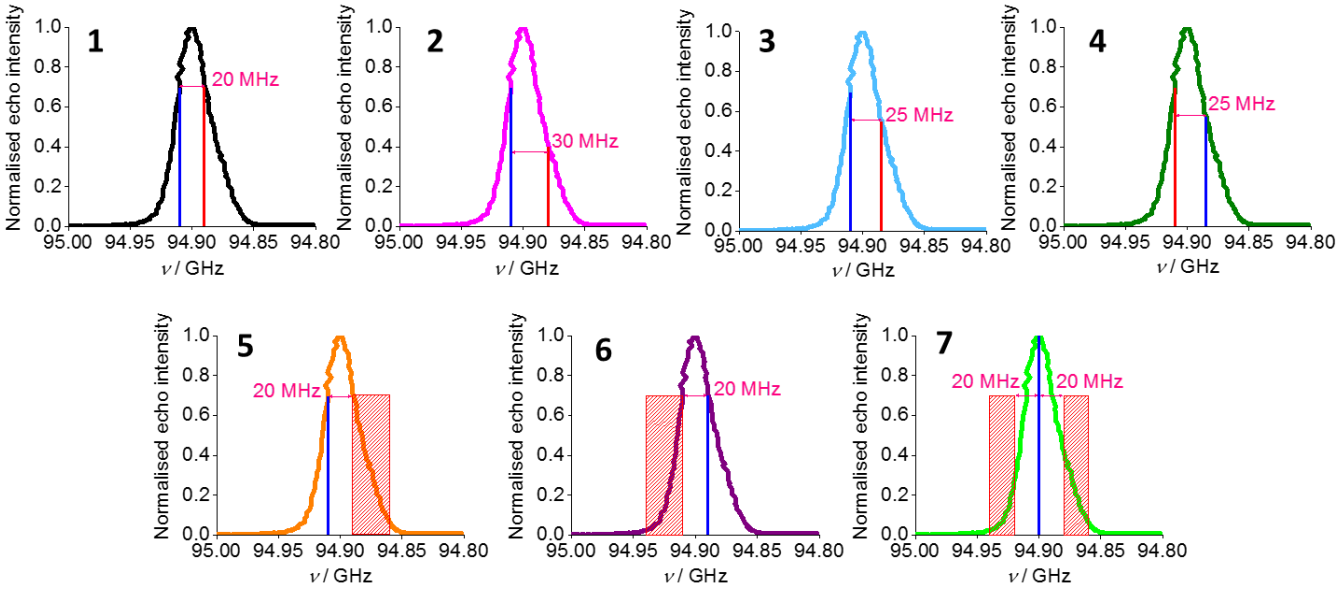

Figure S7. The different DEER experimental set-ups using rectangular (set-ups **1**-**4**) and chirp (set-ups **5**-**7**) pump pulse(s) on GB1-trityl, with the experimental set-up details given in Table S1. The red lines denote the pumped spins and the blue lines denote the observed spins.

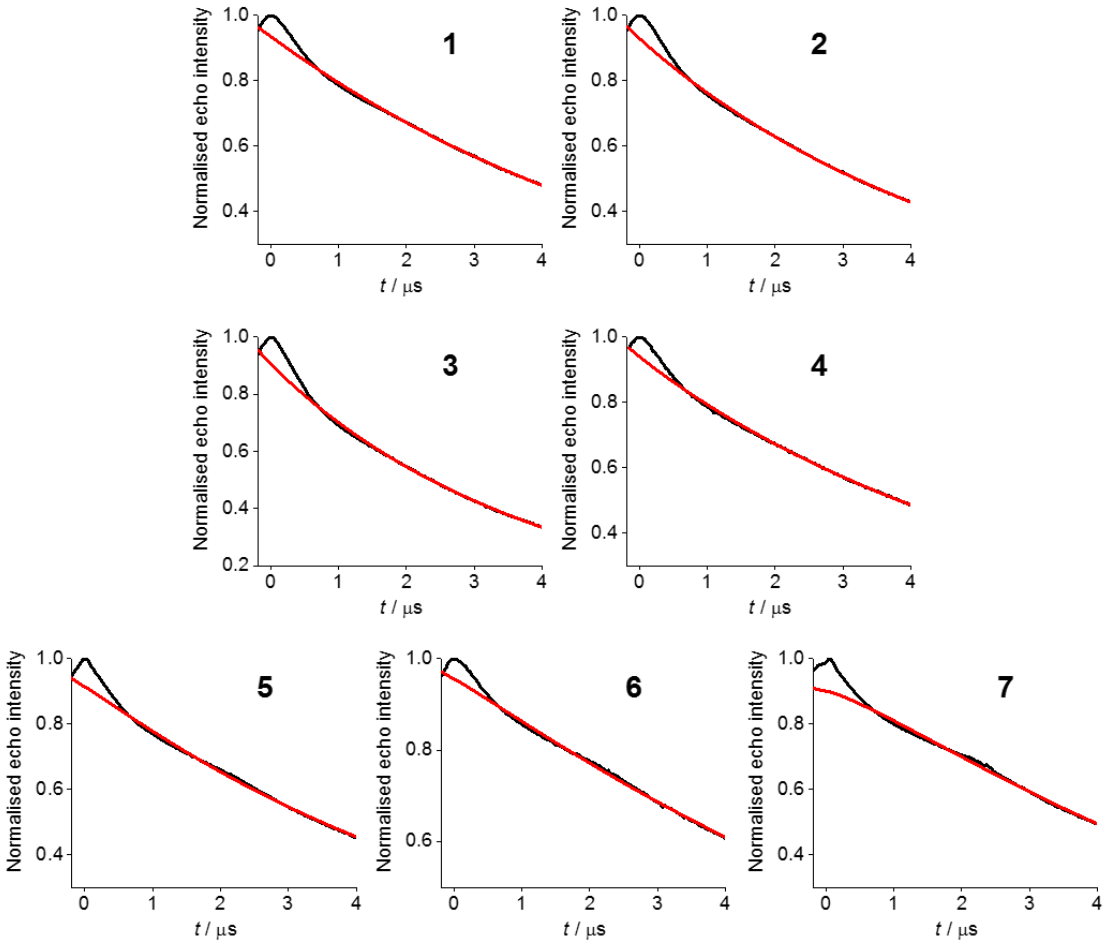

Figure S8. DEER primary data of GB1-trityl with experimental set-ups shown in Figure S7 and detailed in Table S1. The red line indicates the background decay function.

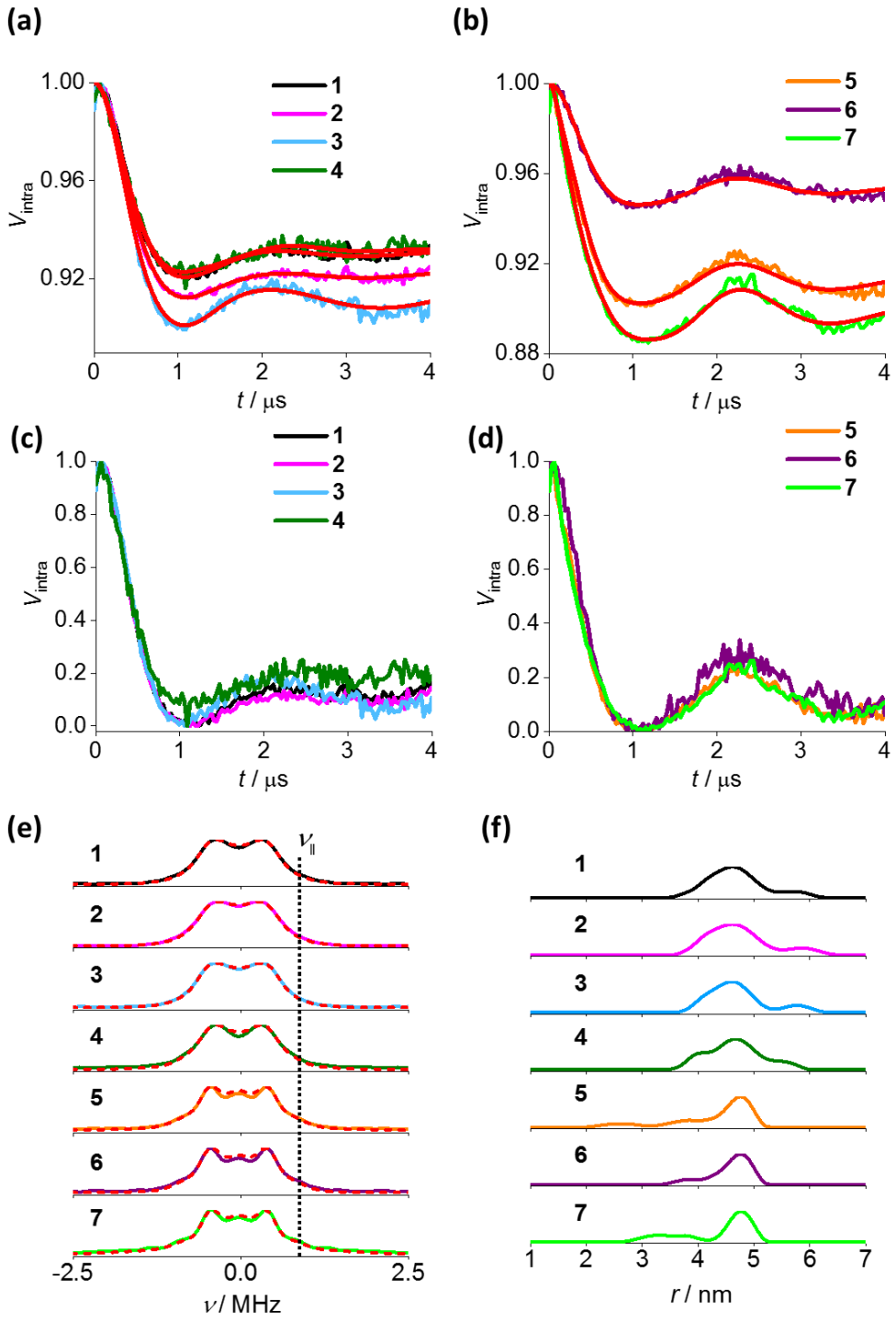

Figure S9. DEER results of GB1-trityl measured with set-ups given in Figure S7 and detailed in Table S1. Background corrected DEER traces with (a) rectangular and (b) chirp pump pulse(s), (c) and (d) as in (a) and (b) but the data is normalized from 0 to 1.0 and (e) respective frequency spectra and (f) distance distributions. The red solid lines in (a) and (b) and dashed lines in (c) are the fit to the data after Tikhonov regularization. The measurements were run 9.7, 9.2, 12.2, 8.7, 14.3, 8.2 and 12.8 hrs for set-ups **1** to **7**, respectively. The black dotted line denotes the frequency of the parallel component ($\nu_{\parallel}$) of the Pake pattern. Primary DEER traces are given in Figure S8.

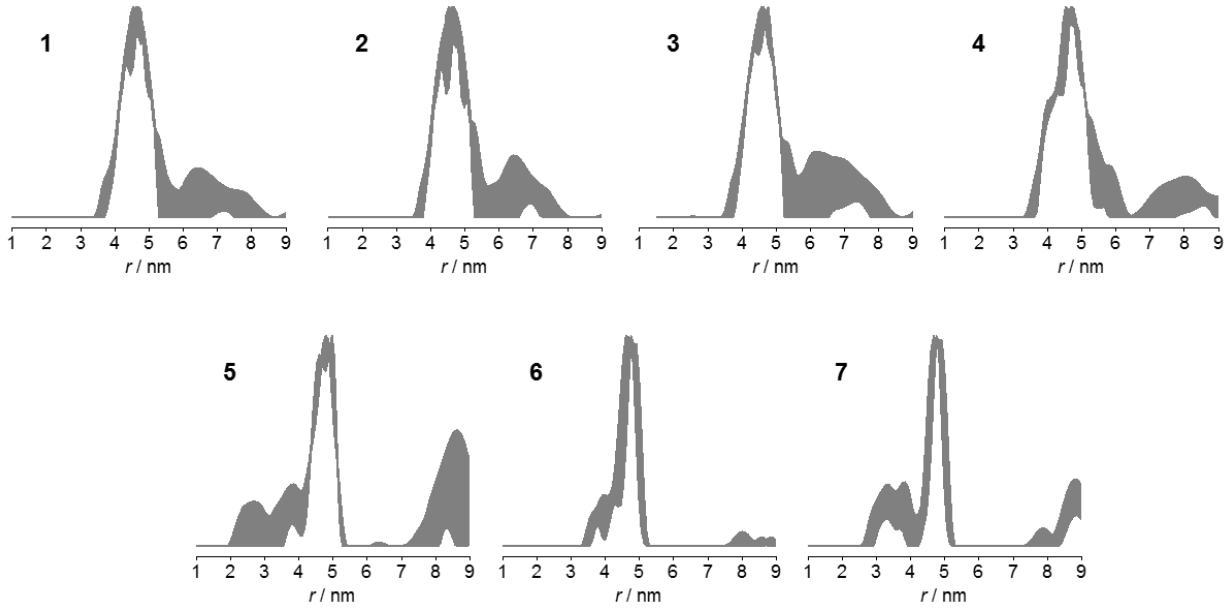

Figure S10. Validated distance distributions of the DEER data on the GB1-trityl with experimental set-ups given in the Figure S7 and detailed in Table S1.

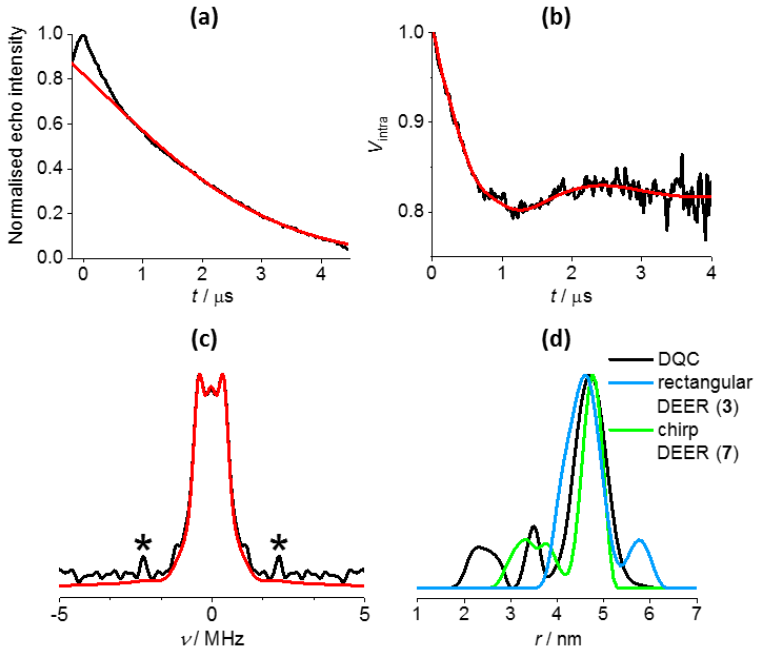

Figure S11. DQC results of GB1-trityl measured at 50 K at X-band. (a) Primary DQC data with the red line indicating the background decay function, (b) background-corrected DQC data with the red line indicating the fit to the data, (c) frequency spectrum with the red line indicating the fit to the data (* corresponds the signals due to ^{2}H modulation) and (d) distance distribution and comparison with the DEER distance distributions (set-up in parenthesis). The measurement was run 48 hrs (12 hrs for each set of τ_1 and τ_2 values).

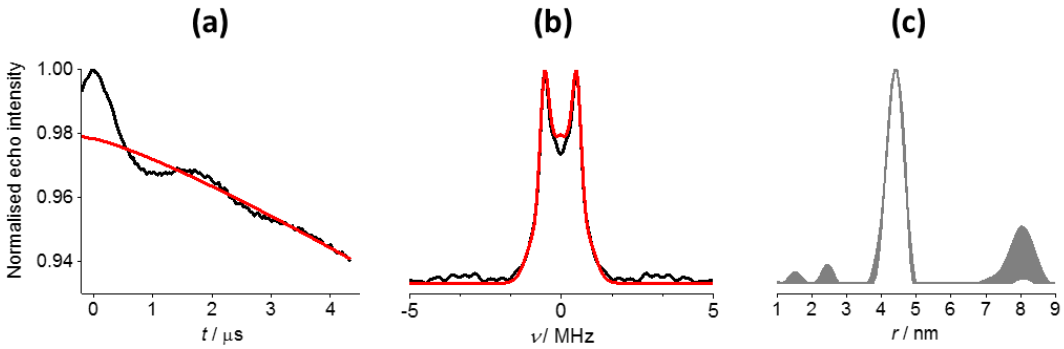

Figure S12. GB1-Gd(III) with rectangular DEER. (a) Primary DEER data with the red line indicating the background decay function, (b) frequency spectrum with the red line indicating the fit to the data and (c) validated distance distribution. The measurement was run 12.3 hrs.

5. Additional EPR data on ubiquitin-CT02MA

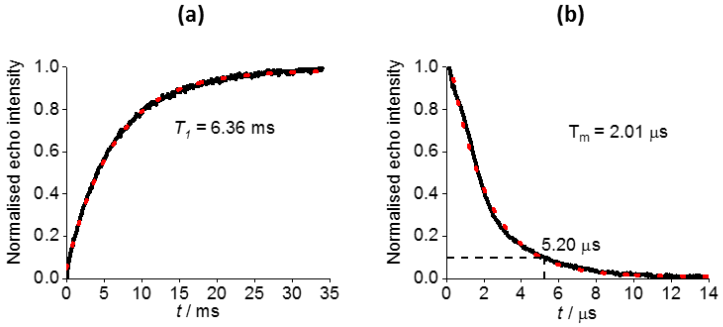

Figure S13. (a) Saturation recovery measurement and (b) two pulse echo decay traces of ubiquitin-trityl. The longitudinal relaxation T_1 after fitting a mono-exponential fit function (red dotted line) is given in (a) and the value at which the echo decays to 10% of its initial value and the T_m of the trityl spins after fitting a mono-exponential decay function (red dotted line) are given on (b).

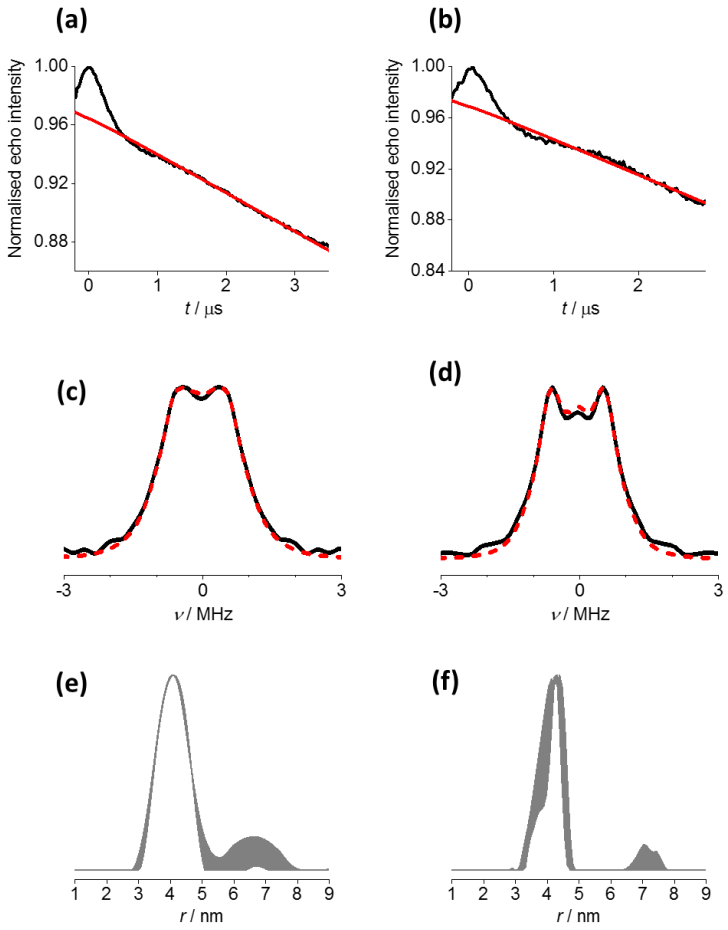

Figure S14. Ubiquitin-trityl measured with rectangular (a, c, e) and chirp (b, d, f) DEER. (a, b) Primary DEER data with the red solid line indicating the background decay function, (c, d) frequency spectra with the red dotted line indicating the fit to the data and (e, f) validated distance distributions. The measurements were run 11.5 and 9.8 hrs for rectangular and chirp DEER, respectively.

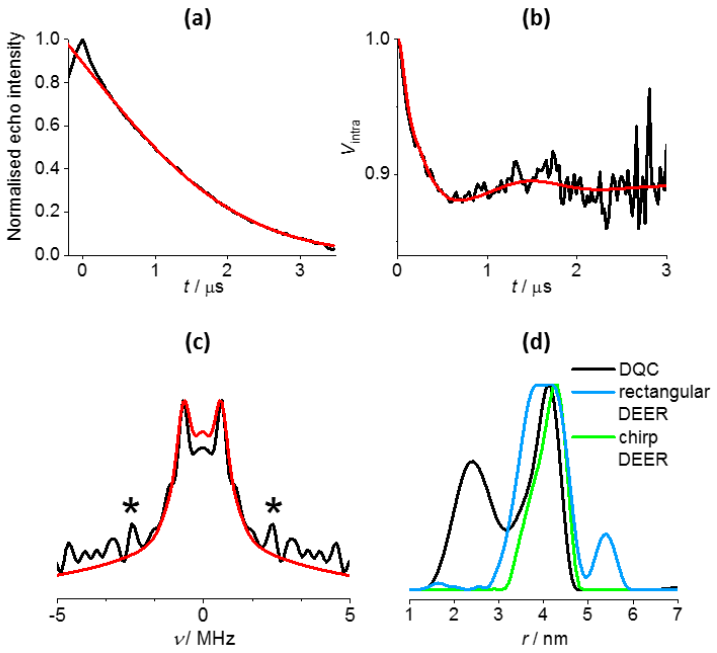

Figure S15. DQC results of ubiquitin-trityl measured at 50 K at X-band. (a) Primary DQC data with the red line indicating the background decay function, (b) background-corrected DQC data with the red line indicating the fit to the data, (c) frequency spectrum with the red line indicating the fit to the data data (* corresponds the signals due to ^{2}H modulation) and (d) distance distribution and comparison with the DEER distance distributions (set-up in parenthesis). The measurement was run 48 hrs (12 hrs for each set of τ_1 and τ_2 values).

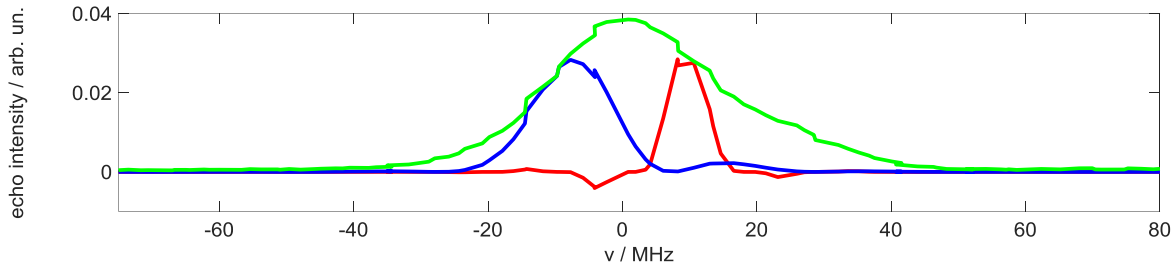

Figure S16. Ubiquitin-trityl ED-EPR spectrum (green) and excitation profile of rectangular observe echo sequence (blue) and pump (red) pulse for set-up **1**. The expected modulation depth is 28%.

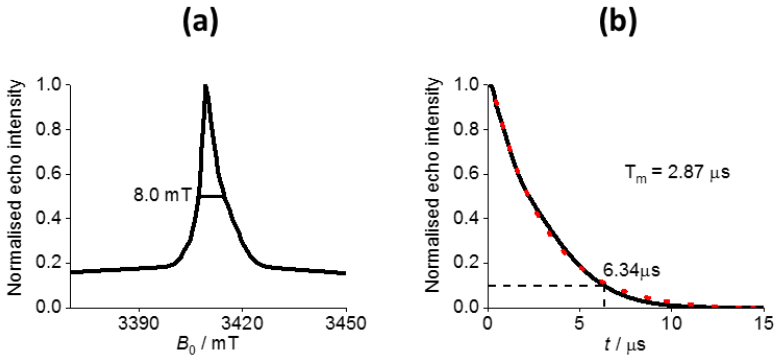

Figure S17. (a) ED-EPR spectrum and (b) two pulse echo decay trace of ubiquitin-Gd(III). The value at which the echo decays to 10% of its initial value and the T_m of the trityl spins after fitting a mono-exponential decay function (red dotted lines) are given on (b).

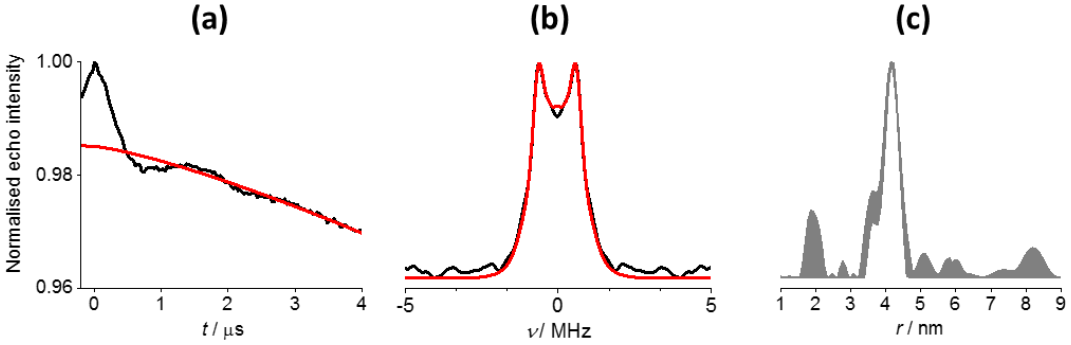

Figure S18. Ubiquitin-Gd(III) measured with rectangular DEER. (a) Primary DEER data with the red line indicating the background decay function, (b) frequency spectrum with the red line indicating the fit to the data and (c) validated distance distribution. The measurement was run 7.4 hrs.

6. Additional EPR data on ubiquitin-CT02MA/BrPy-DO3MA-Gd(III)

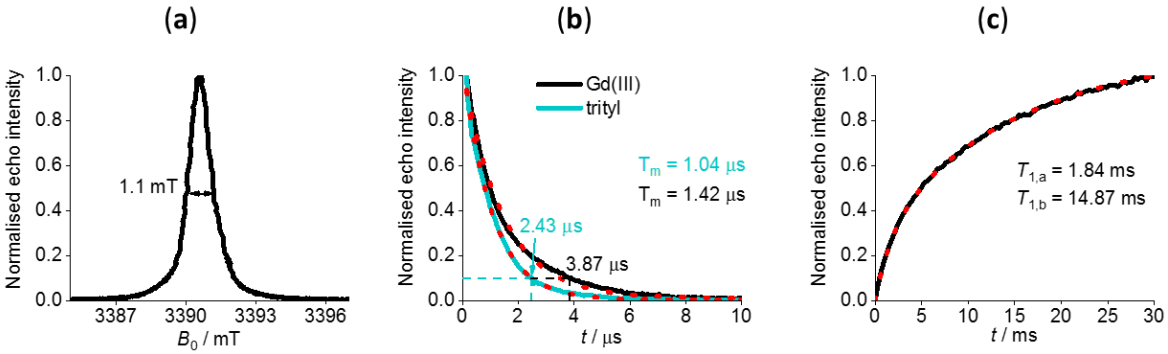

Figure S19. (a) ED-EPR spectrum of the trityl spins only (optimized for the trityl), (b) two pulse echo decay traces and (c) saturation recovery measurement measured at the maxima of the trityl and Gd(III) spectra at 10 K (a, b) and at 20 K (c). A fast and a slow component of longitudinal relaxation T_1 after fitting a bi-exponential fit function (red dotted line) is given in (c) and the values at which the echo decays to 10% of its initial value and the T_m of the trityl and Gd(III) spins after fitting a mono-exponential decay function (red dotted lines) are given in (b).

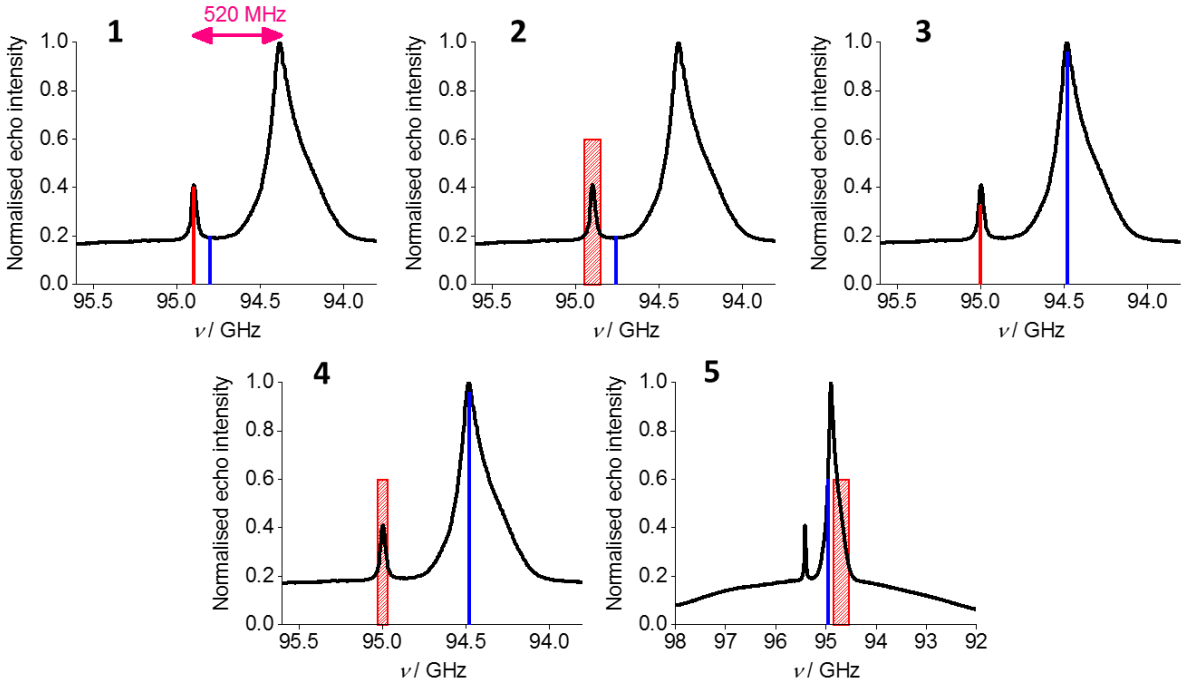

Figure S20. The different DEER experimental set-ups using rectangular (set-ups **1**, **3**) and chirp (set-ups **2**, **4**, **5**) pump pulse(s) on ubiquitin-trityl/Gd(III), with the experimental set-up details given in Table S5. Set-up **5** is designed for Gd(III)-Gd(III) distance measurements while all the others for Gd(III)-trityl distance measurements. The red lines denote the pumped spins and the blue lines denote the observed spins.

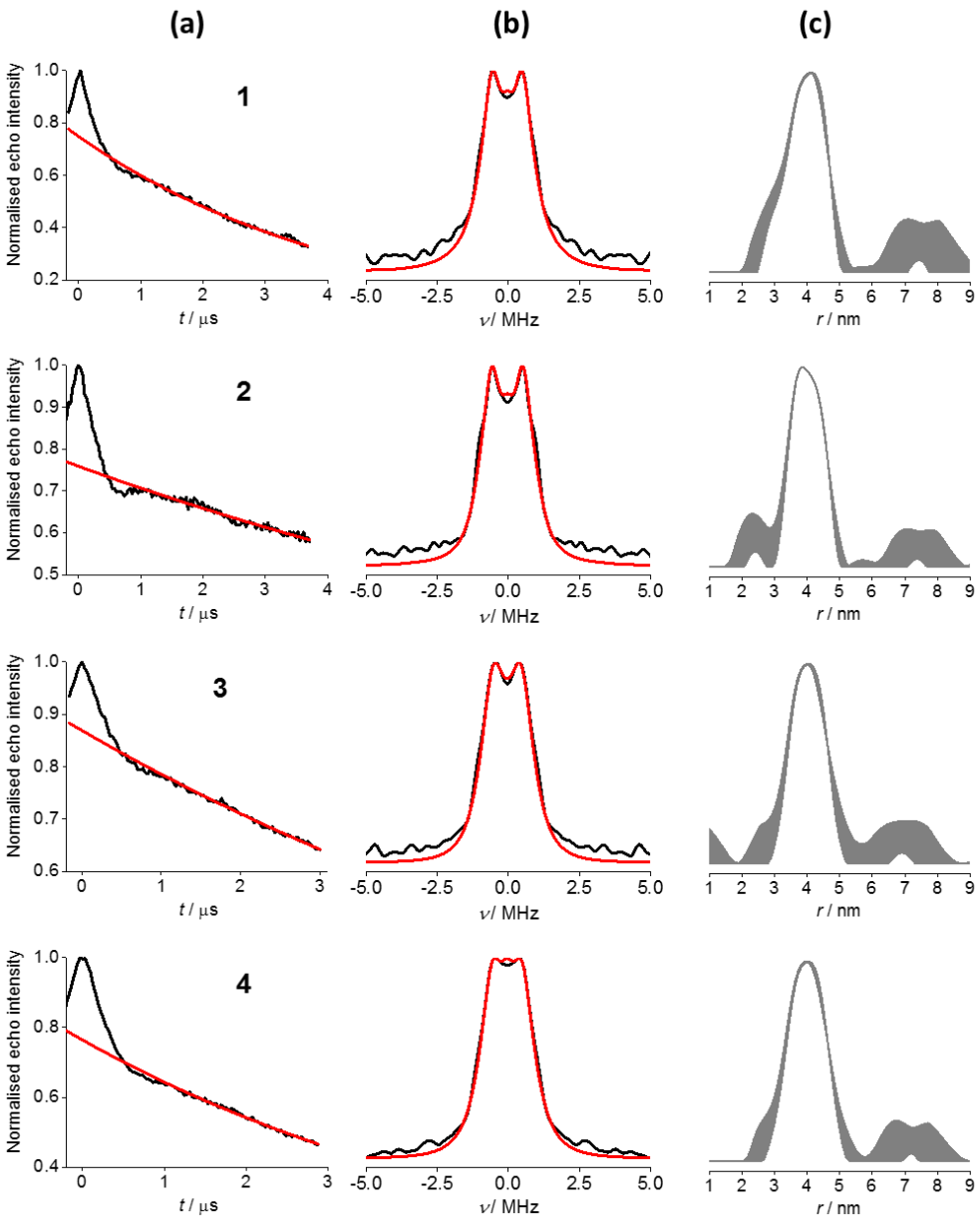

Figure S21. DEER results on ubiquitin-trityl/Gd(III) using the single or dual mode cavity (the set-up is given in parenthesis). (a) Primary DEER data with the red line indicating the background decay function, (b) respective frequency spectra with the red line indicating the fit to the data after Tikhonov regularization and (c) validated distance distributions.

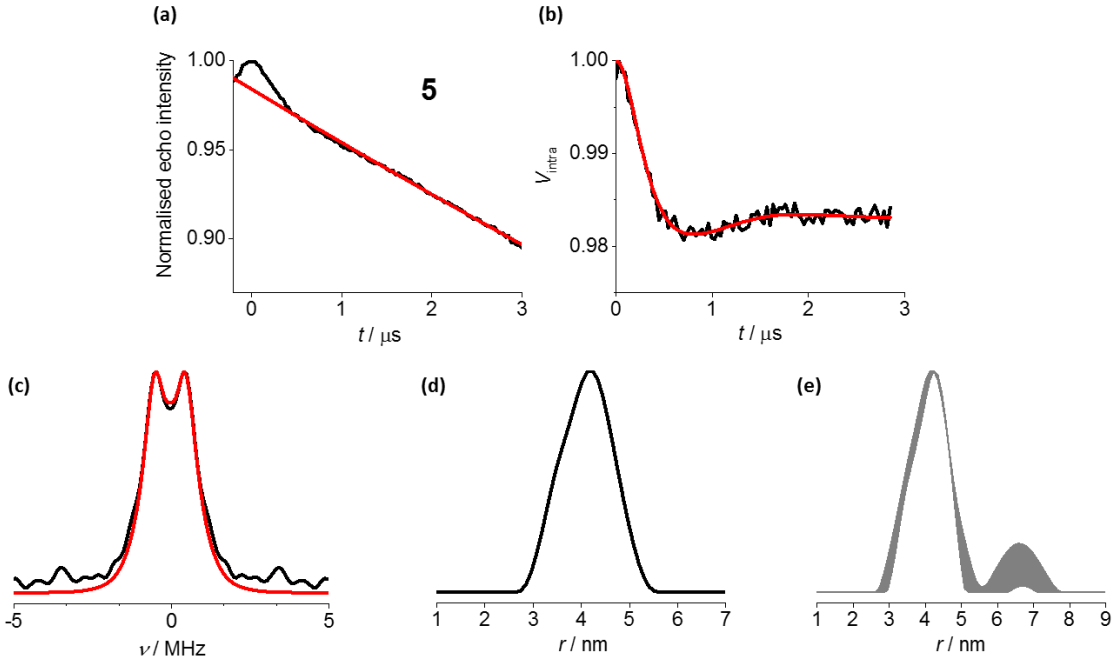

Figure S22. Ubiquitin-trityl/Gd(III) measured with chirp DEER with the single mode cavity (set-up **5**, in Figure S20) to observe Gd(III)-Gd(III) distances. (a) Primary DEER data with the red line indicating the background decay function, (b) background corrected DEER data with the red line indicating the fit to the data and (c) frequency spectrum with the red line indicating the fit to the data, (d) corresponding distance distribution and (e) validated distance distribution. The experimental set-up details given in Table S5. The measurement was run 3.9 hrs.

References

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5. G. Y. Shevelev, O. A. Krumkacheva, A. A. Lomzov, A. A. Kuzhelev, O. Y. Rogozhnikova, D. V. Trukhin, T. I. Troitskaya, V. M. Tormyshev, M. V. Fedin, D. V. Pyshnyi and E. G. Bagryanskaya, *J. Am. Chem. Soc.*, 2014, **136**, 9874-9877.