Supporting Information for:

Probing the dynamic properties of two sites simultaneously in a protein-protein interaction process: a SDSL-EPR study

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EPR spectral shape simulations –

All EPR spectra were simulated using an extended version of the ROKI software[1].

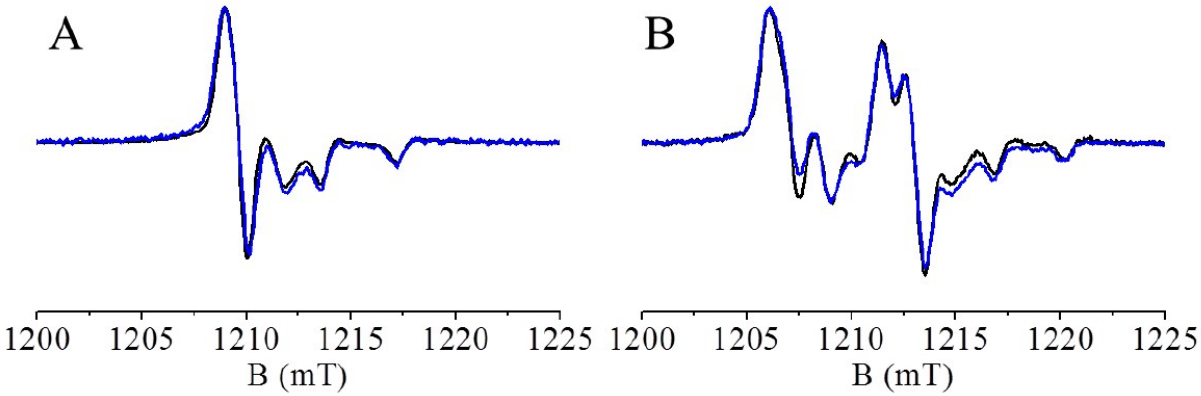

Figure S1. Experimental (black line) and simulated (blue line) Q-band EPR spectra of S407C N_{TAIL}^{P} (spectrum A) and S407C N_{TAIL}^{PP} (spectrum B), recorded at 100K using a 100 µM solution in 10 mM sodium phosphate buffer at pH 7 in the presence of 30% (v/v) glycerol used as cryoprotectant. The microwave power was 1 µW and the magnetic field modulation amplitude was 0.25 mT. Parameters used for the simulation:
spectrum A: g_{xx} = 2.0066, g_{yy} = 2.0066, g_{zz} = 2.0017, A_{Nxx} = 0.8 mT, A_{Nyy} = 0.8 mT, A_{Nzz} = 3.6 mT.
spectrum B: g_{xx} = 2.0088, g_{yy} = 2.0059, g_{zz} = 2.0020, A_{Pxx} = 5.2 mT, A_{Pyy} = 5.7 mT, A_{Pzz} = 6.3 mT, A_{Nxx} = 0.4 mT, A_{Nyy} = 0.6 mT, A_{Nzz} = 3.3 mT.

For all EPR spectra recorded at room temperature, simulations were performed considering axial g- and A-tensors. Their mean values and the range of allowed variations are given in the table below.

	$g_{\perp}$	$g_{//}$	$A_{N\perp}$	$A_{N//}$	$A_{P\perp}$	$A_{P//}$
P	2.0068 (5)	2.0018 (5)	0.70 (6) mT	2.7 (1) mT		
PP	2.0070 (5)	2.0022 (5)	0.7 (1) mT	3.3(2) mT	4.7(2) mT	5.5(4) mT

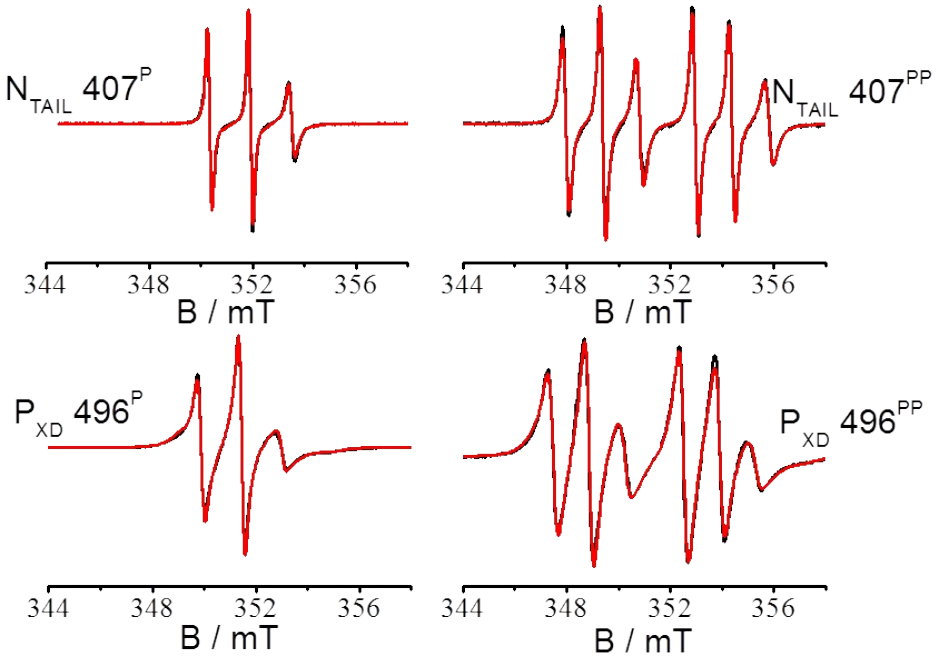

Figure S2. Amplitude normalized RT EPR spectra of N_{TAIL} S407C$^{P/PP}$ and P_{XD} K496C$^{P/PP}$ (black) superimposed with the simulated spectra using ROKI software (red). [2]

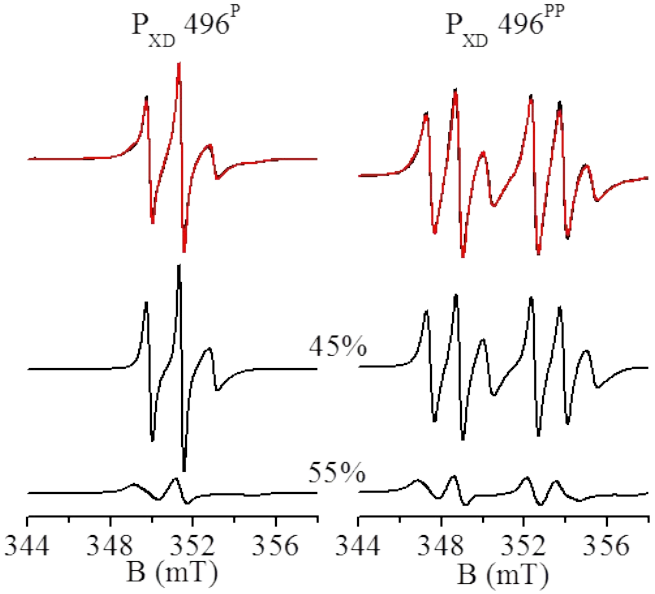

Figure S3. Amplitude normalized RT EPR spectra of P_{XD} K496C^{P} and P_{XD} K496C^{PP} along with their simulated spectra (red) as obtained using the ROKI software.[1] The simulation required to introduce two components accounting for 45 (narrow component) and 55 % (broad component).

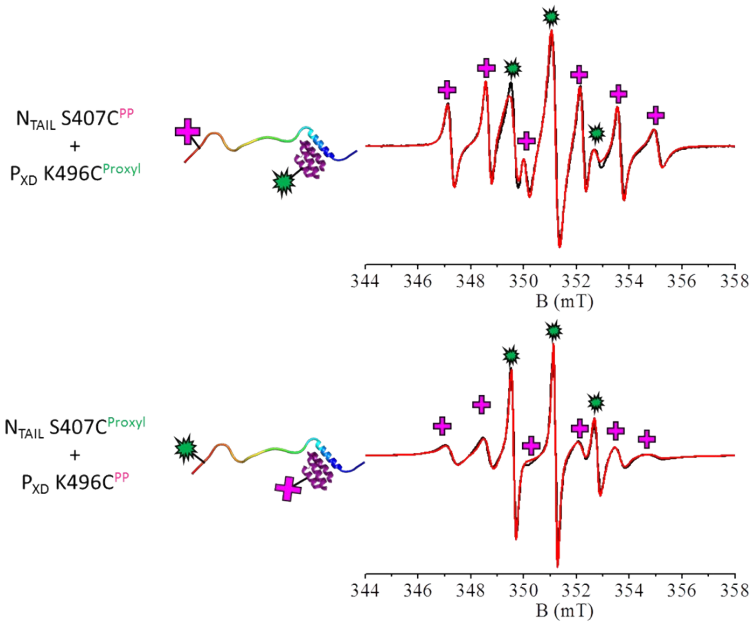

Figure S4. RT EPR spectra of equimolar mixtures: N_{TAIL} S407C^{PP} and P_{XD} K496C^{P} and N_{TAIL} S407C^{P} and P_{XD} K496C^{PP}. Simulated spectra in red. P and PP are illustrated by a green star and a pink cross respectively. EPR lines corresponding to each label are indicated on the EPR spectra. The overlapping between the two signals is only partial.

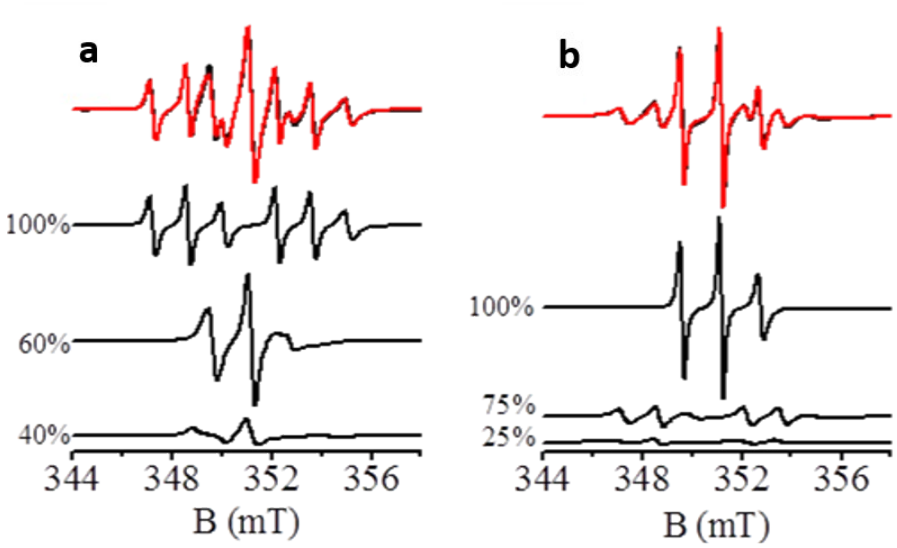

Figure S5. RT EPR spectra of equimolar mixtures of (a) N_{TAIL} S407C^{PP} and P_{XD} K496C^{P} and N_{TAIL} S407C^{P} and P_{XD} K496C^{PP}. Spectra were simulated using the ROKI software (red). Decomposition of spectra shows the components used to obtain the best simulations.

1. A. Rockenbauer; L. Korecz, *App. Magn. Reson.,* 1996, **10** (1-3), 29-43.