Supplementary information

Concentration effects in the interaction of monoclonal antibodies (mAbs) with their immediate environment characterized by EPR spectroscopy

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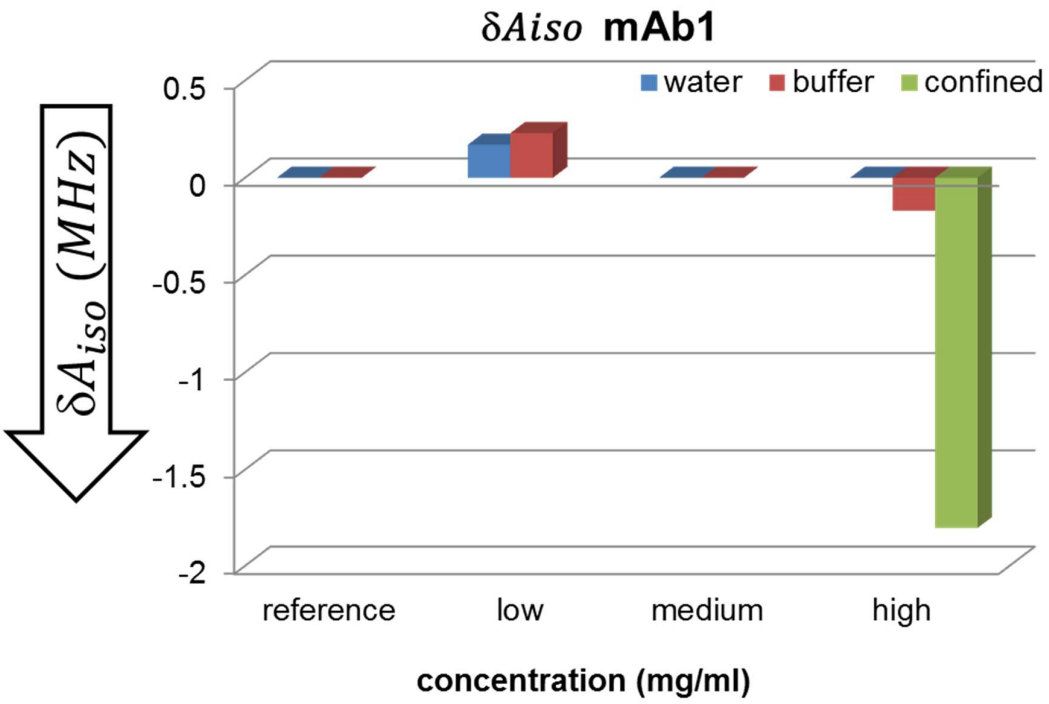


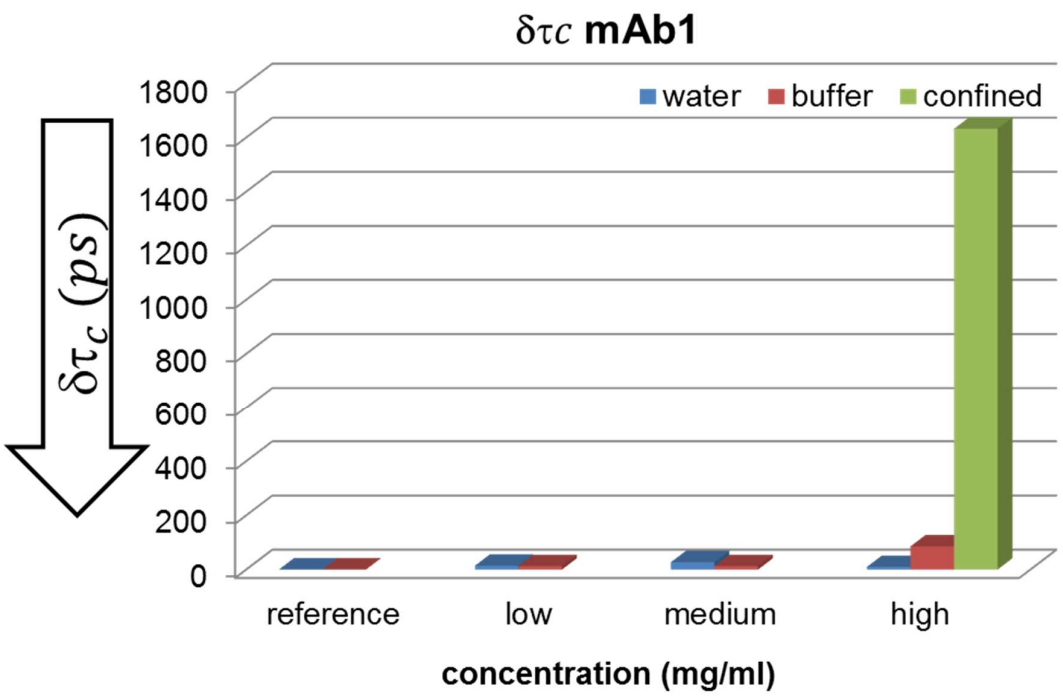
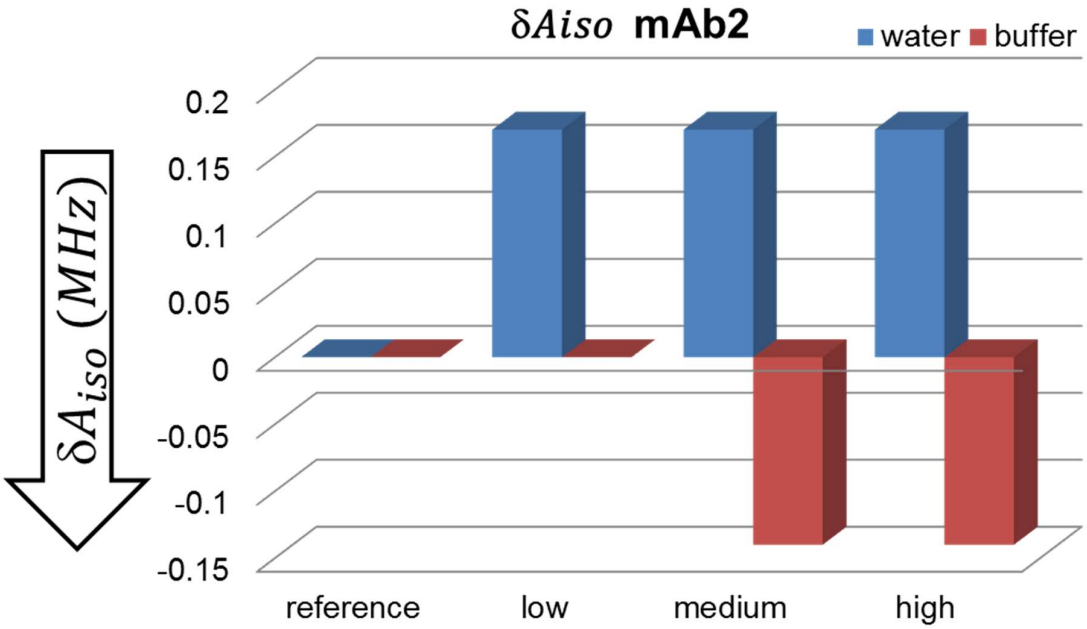


Fig.S2.mAb2-TEMPO



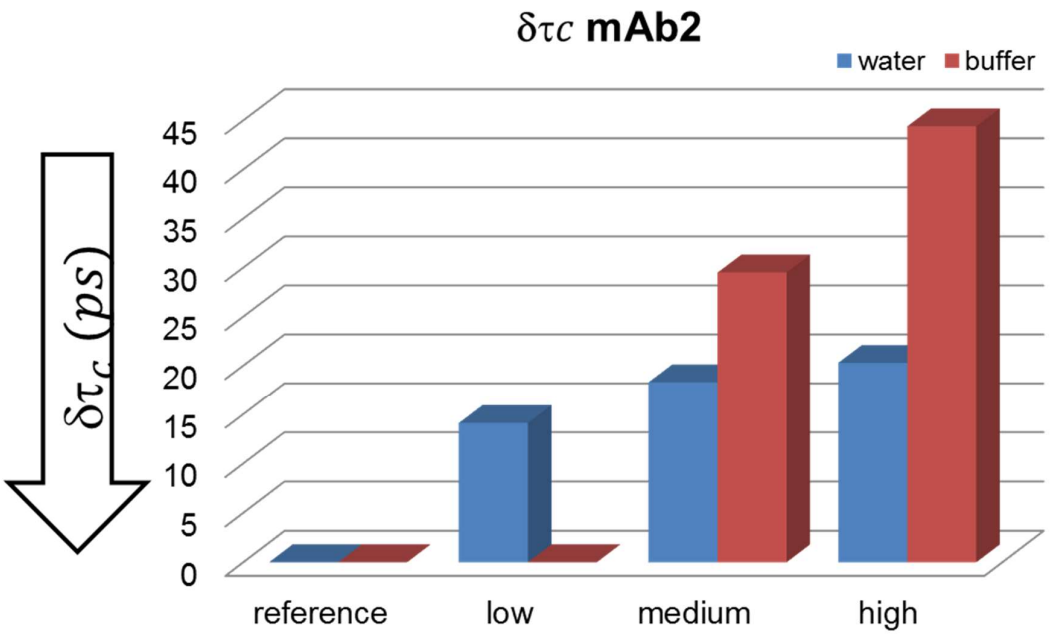


Fig.S3. mAb1-TEMPO-sim

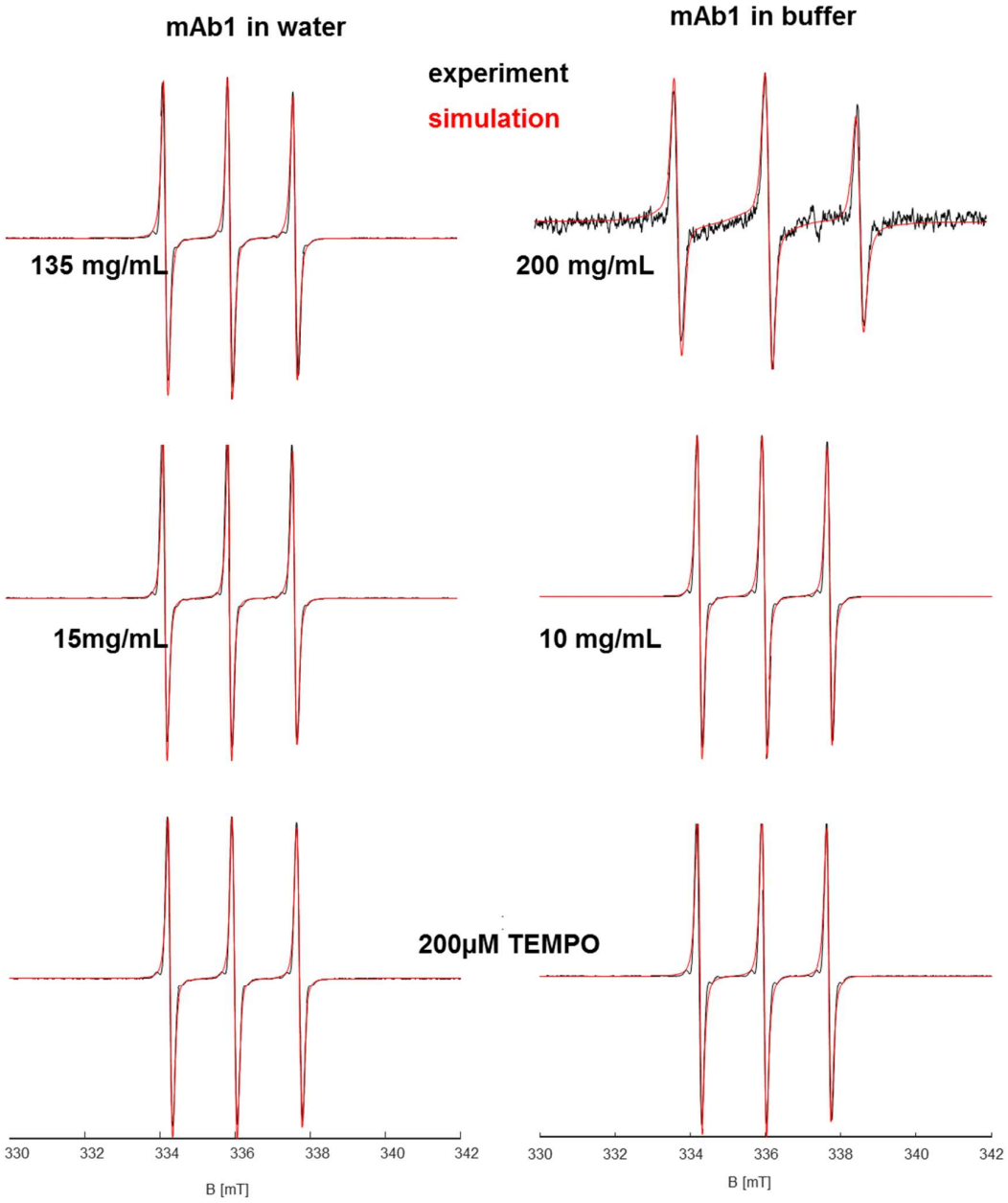


Fig.S3-1. mAb2-TEMPO-sim

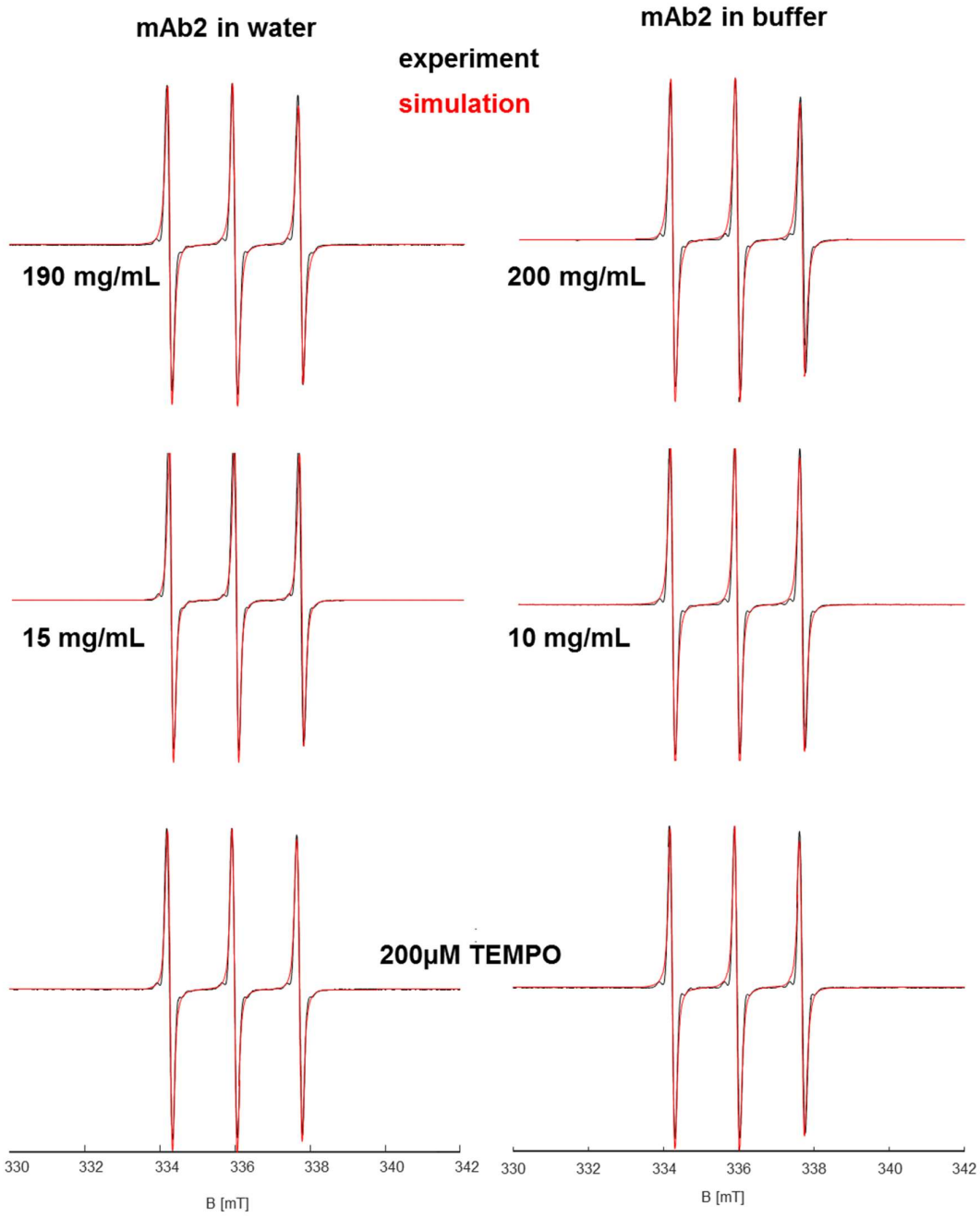


Fig.S4. mAb-TEMPO dynamics in buffer

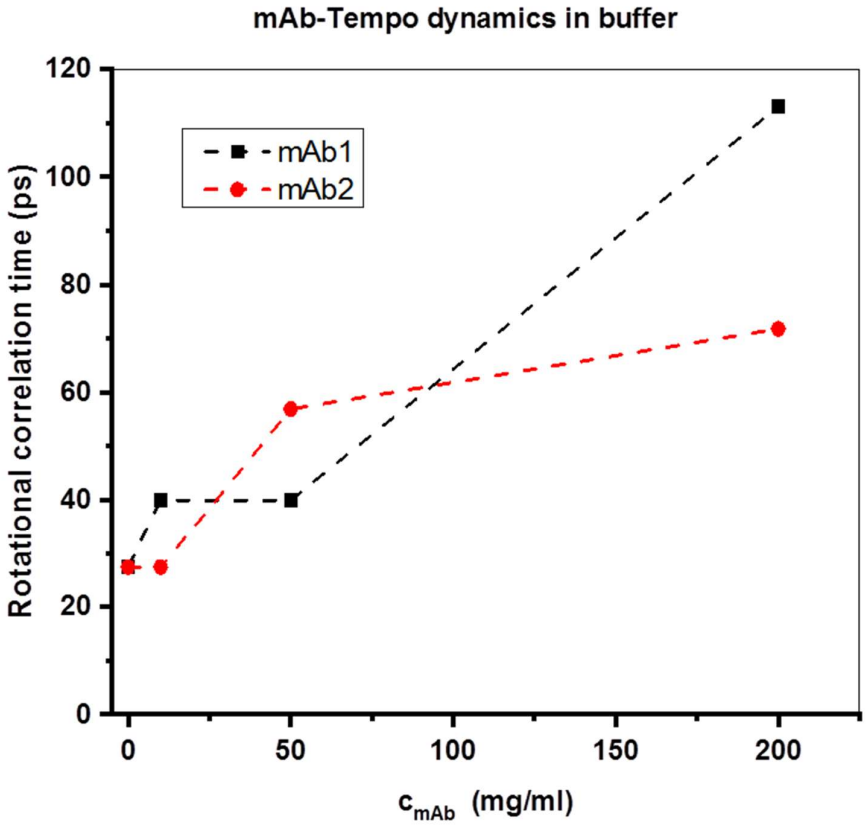
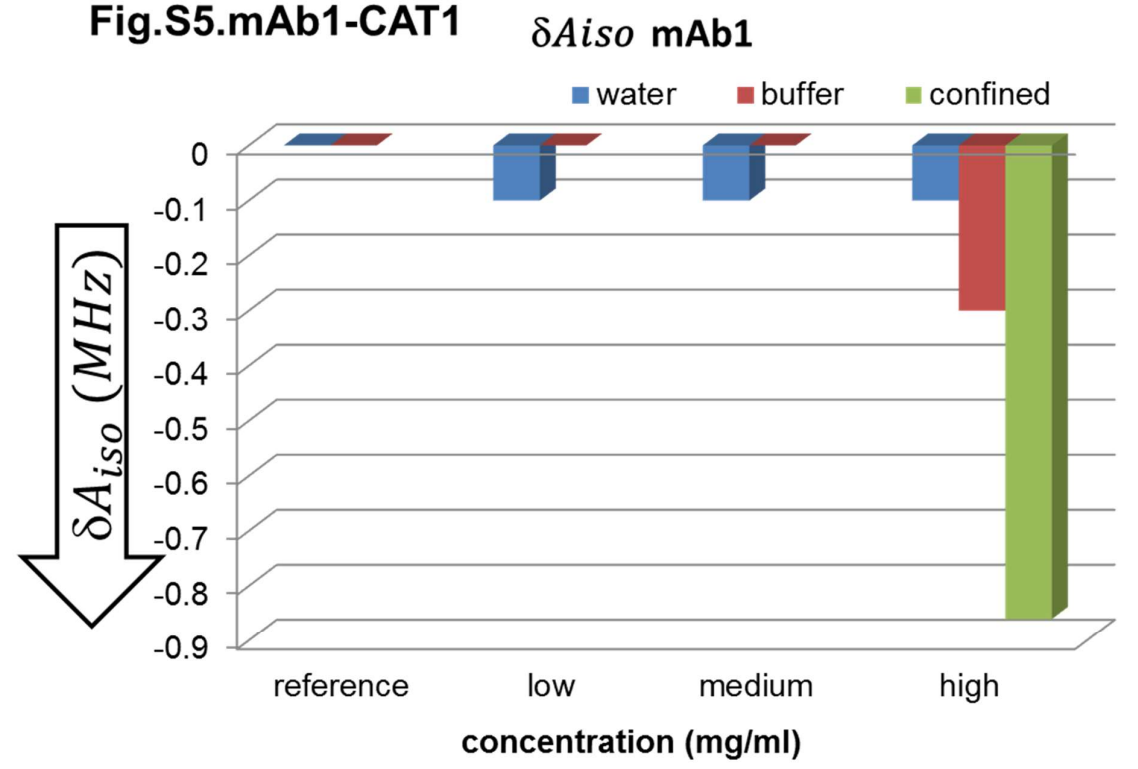


Fig.S5.mAb1-CAT1



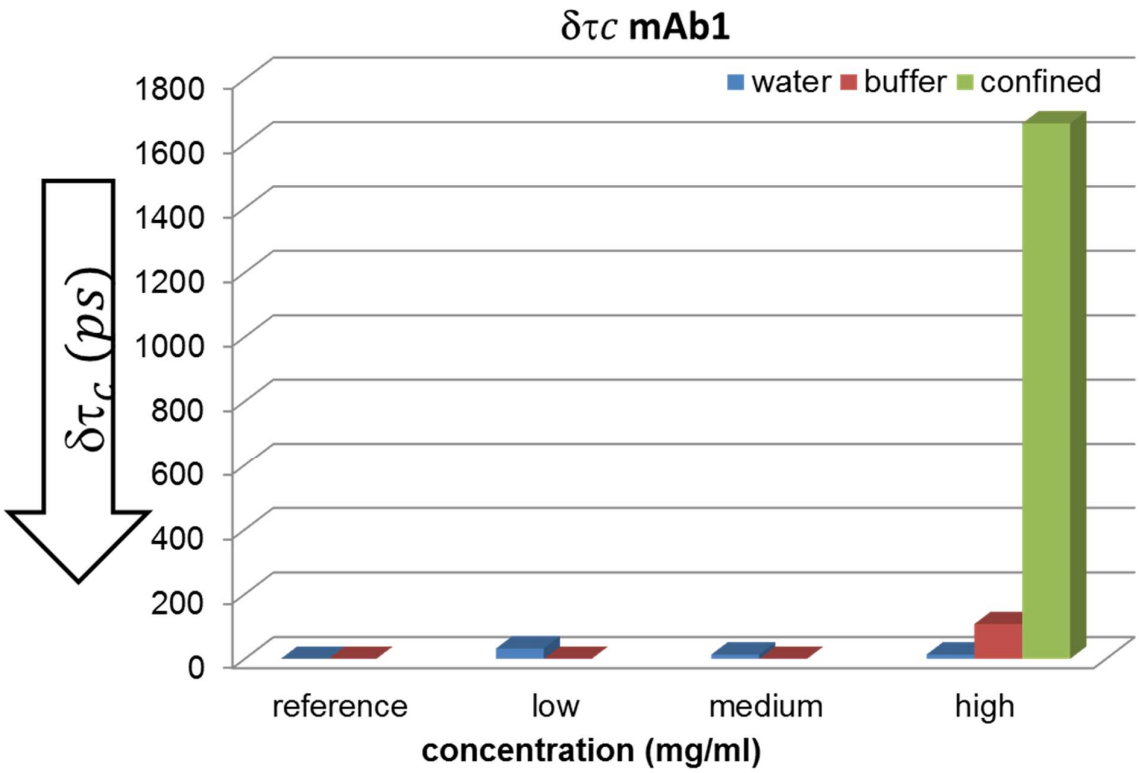
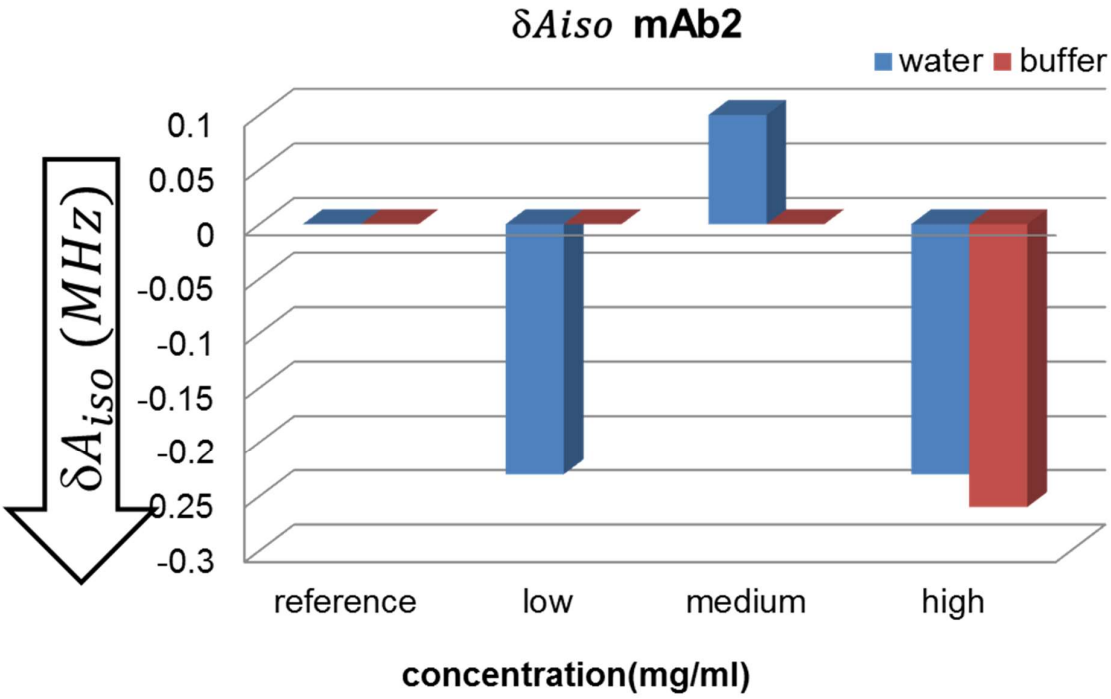


Fig.S6.mAb2-CAT1



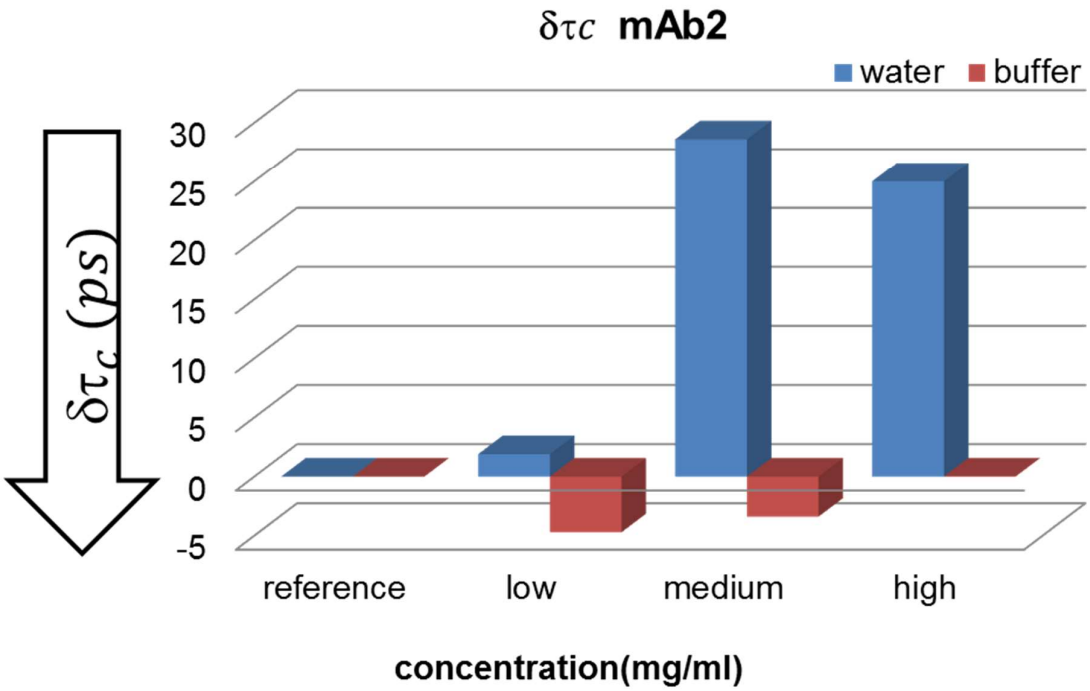


Fig.S7. mAb1-CAT1-sim

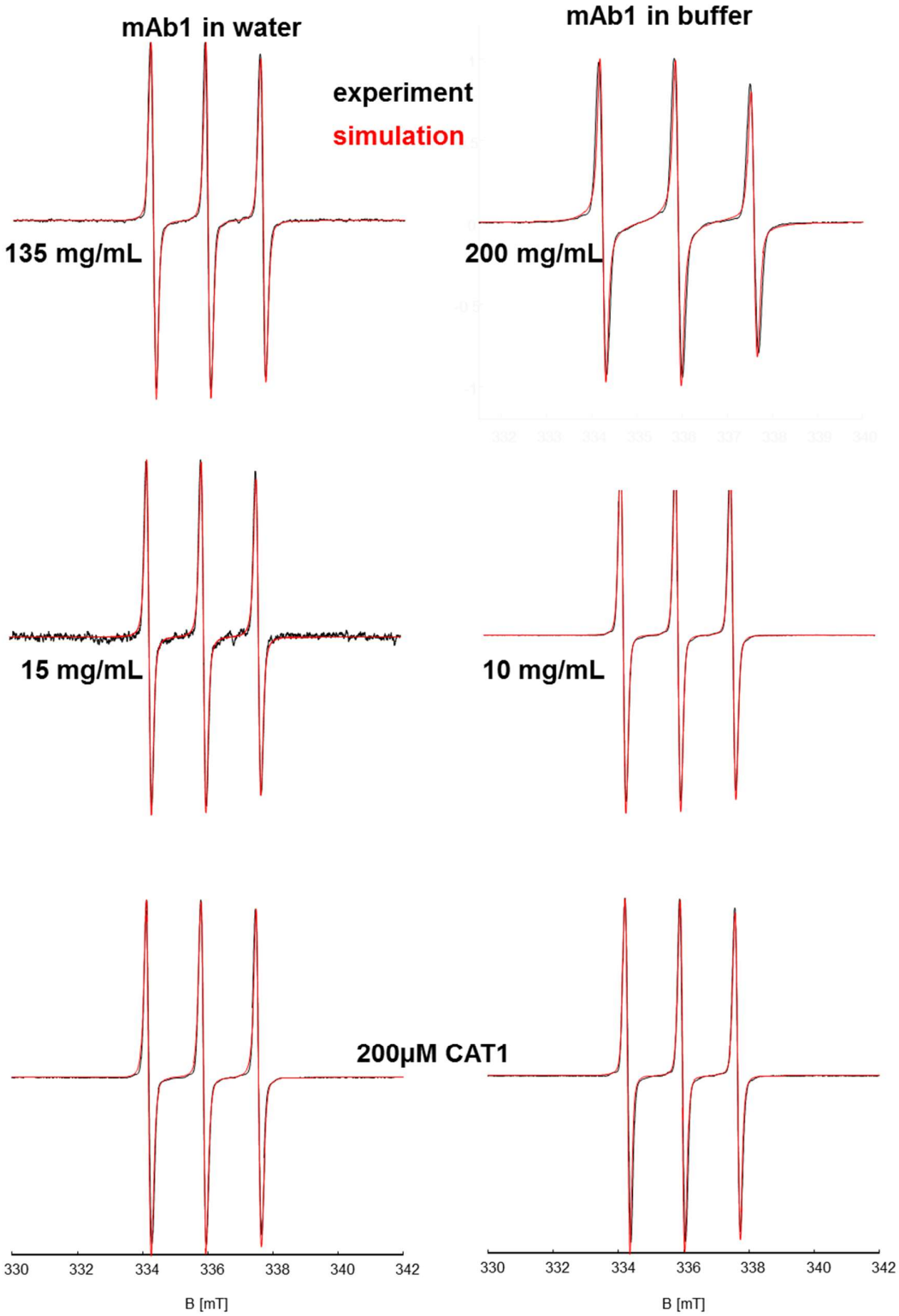


Fig.S7-1. mAb2-CAT1-sim

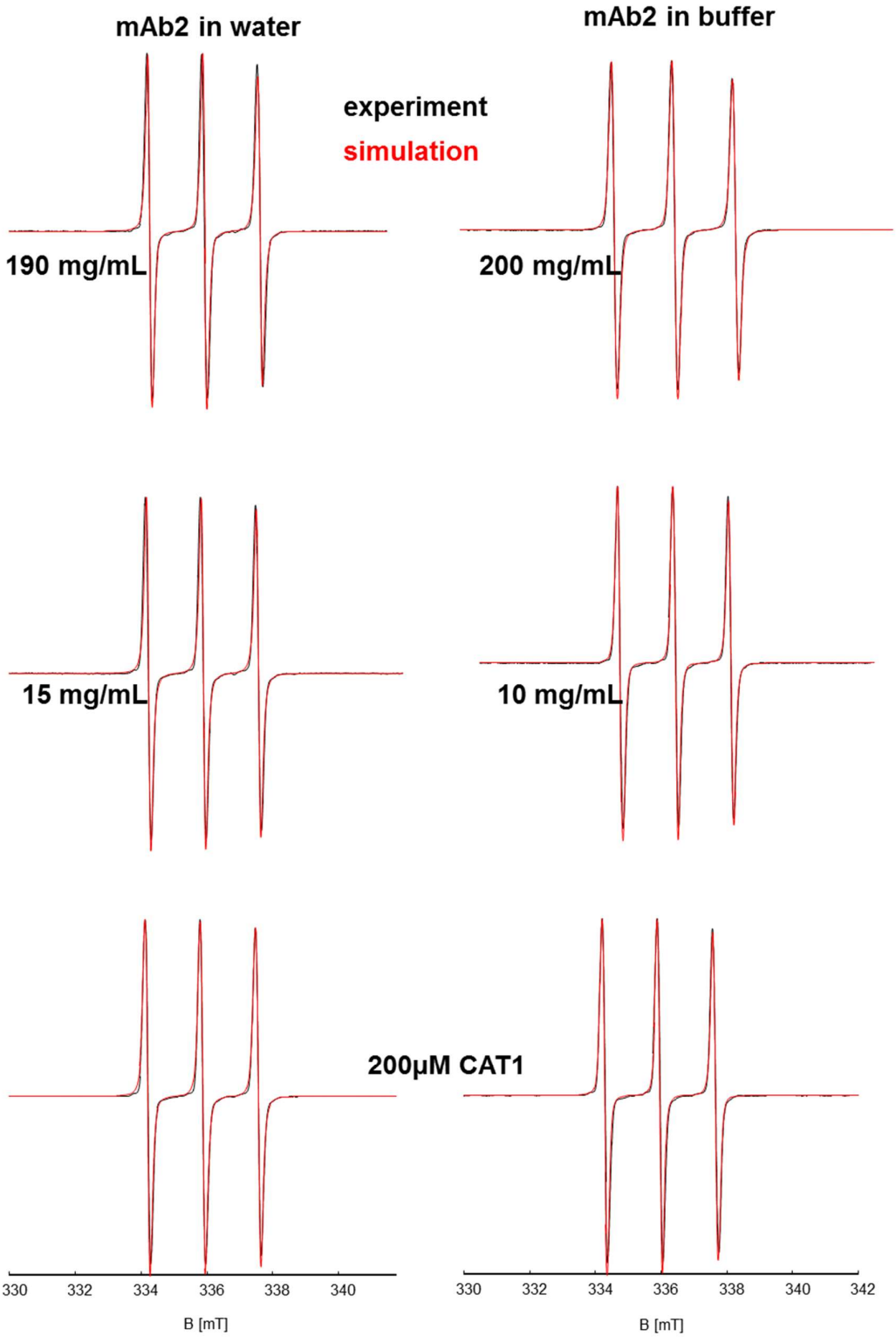


Fig.S8. mAb-CAT1 dynamics in buffer

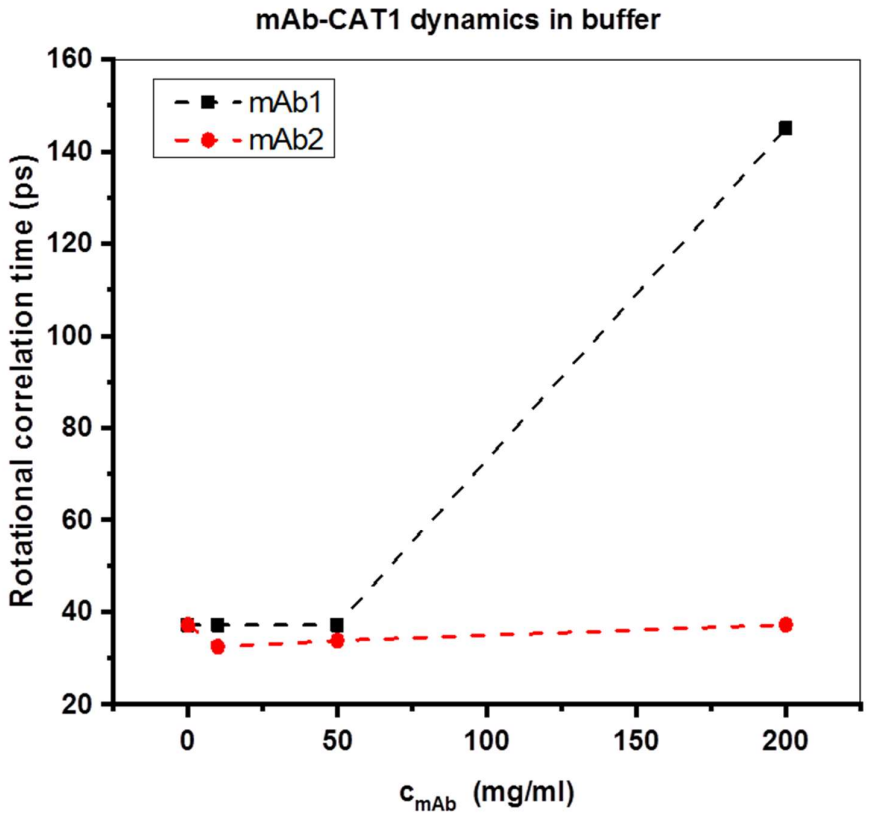


Fig.S9. mAb1-CITPRO-sim

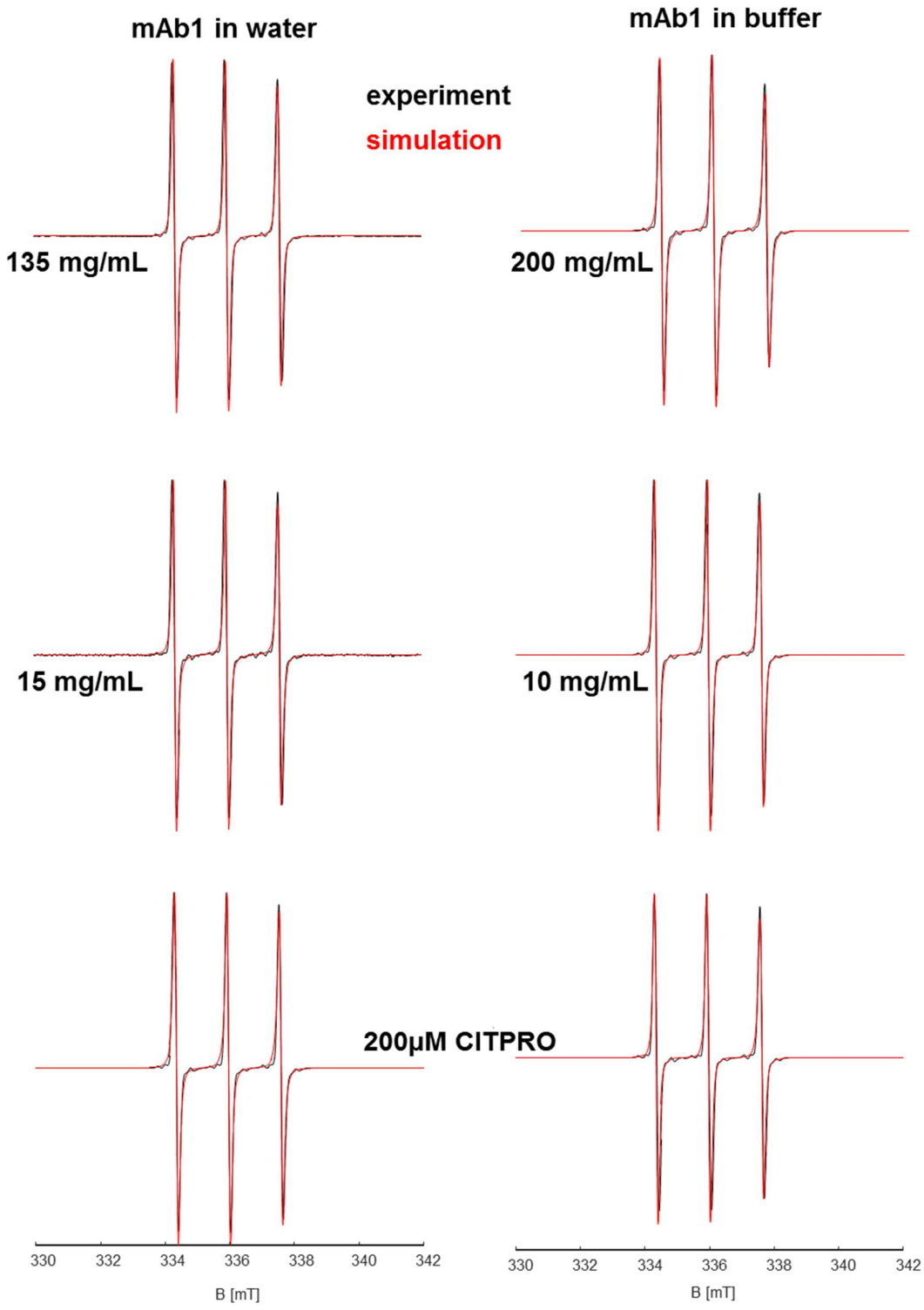


Fig.S9-1. mAb2-CITPRO-sim

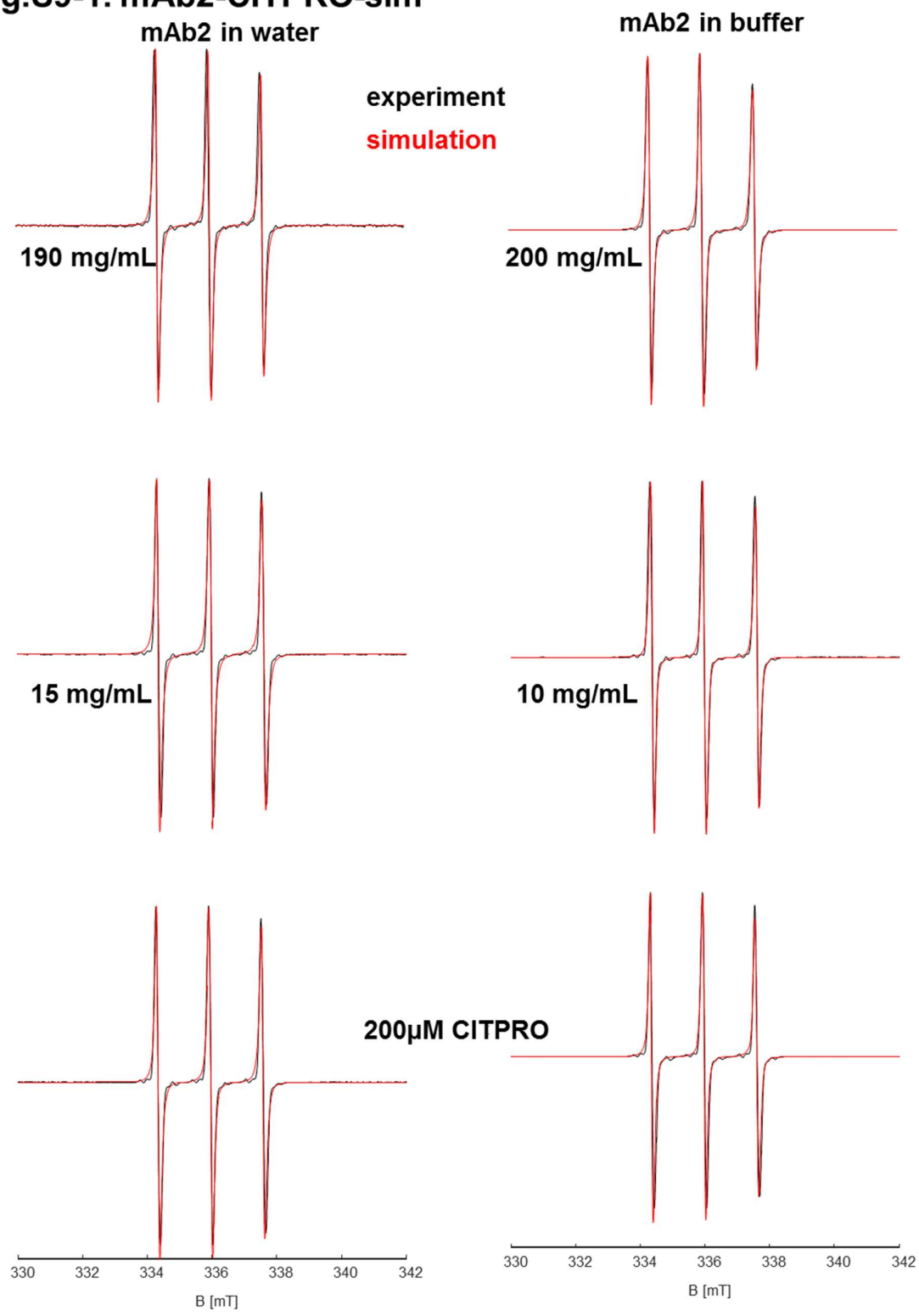
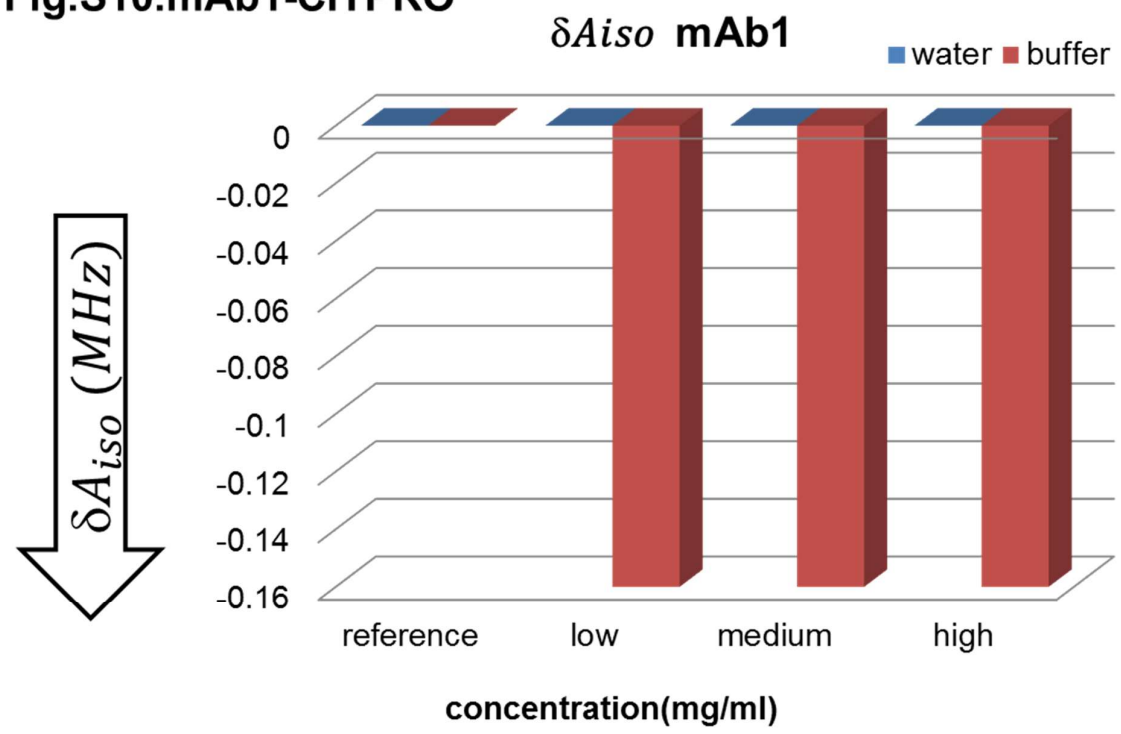
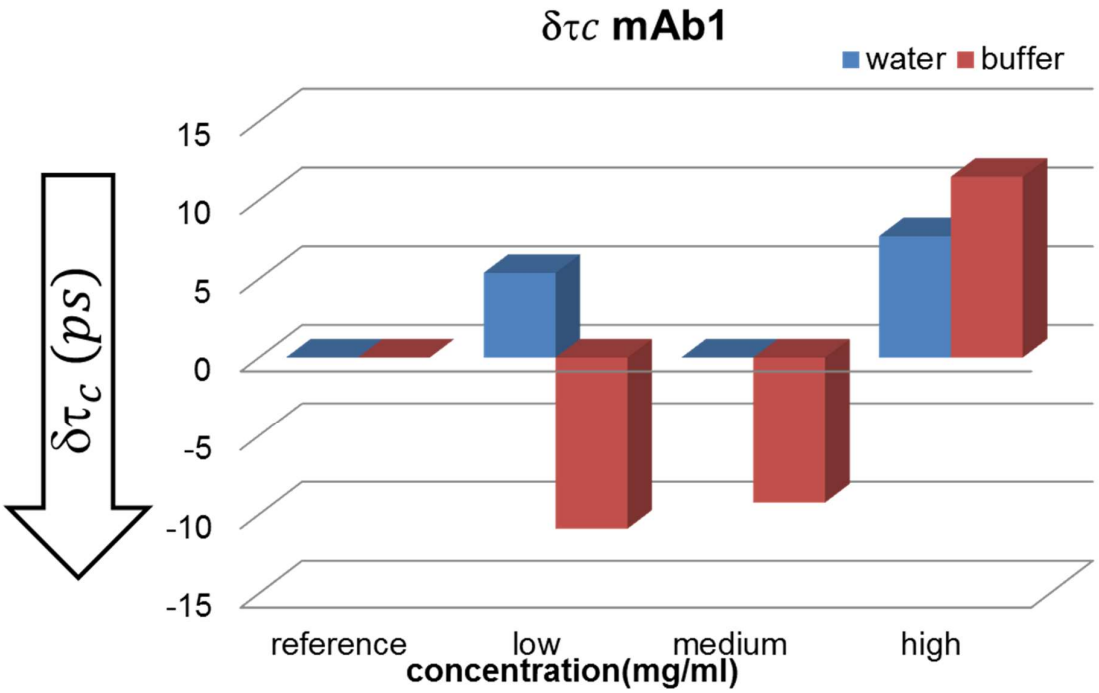


Fig.S10.mAb1-CITPRO





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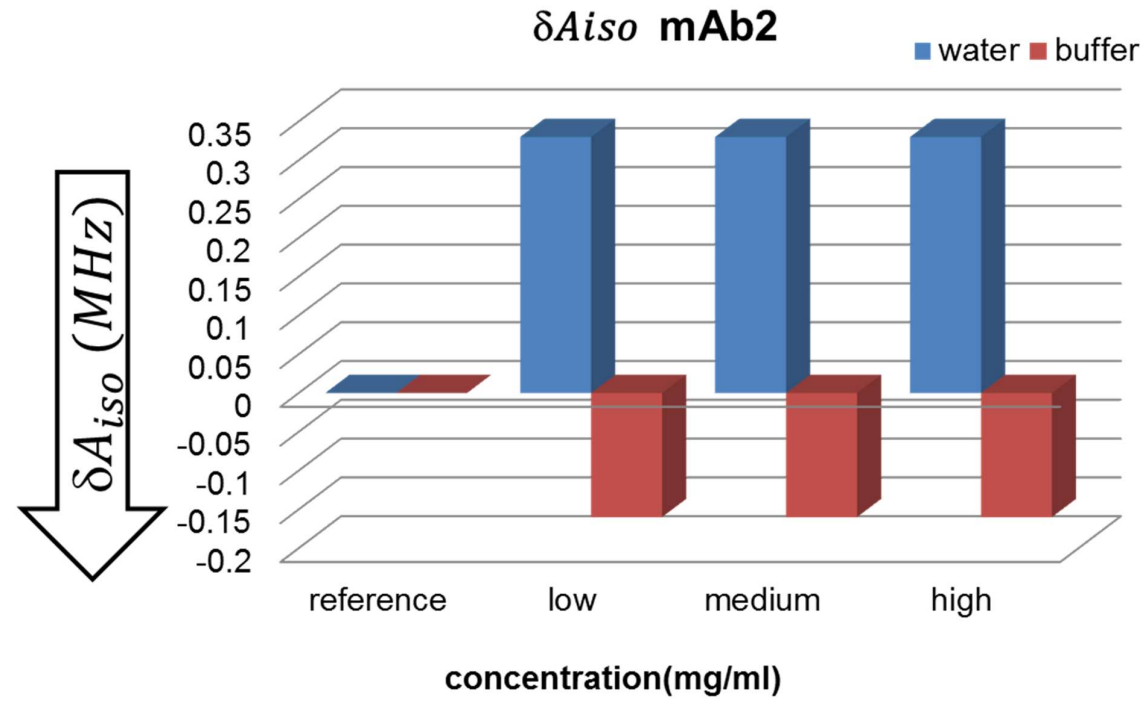

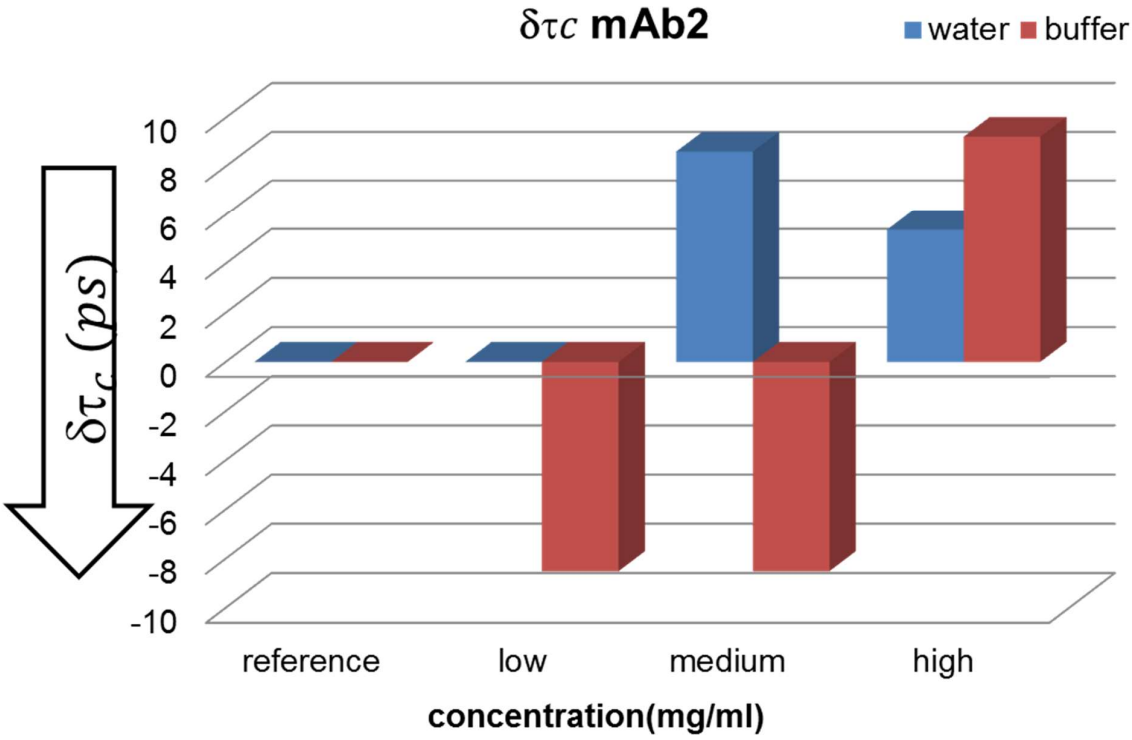

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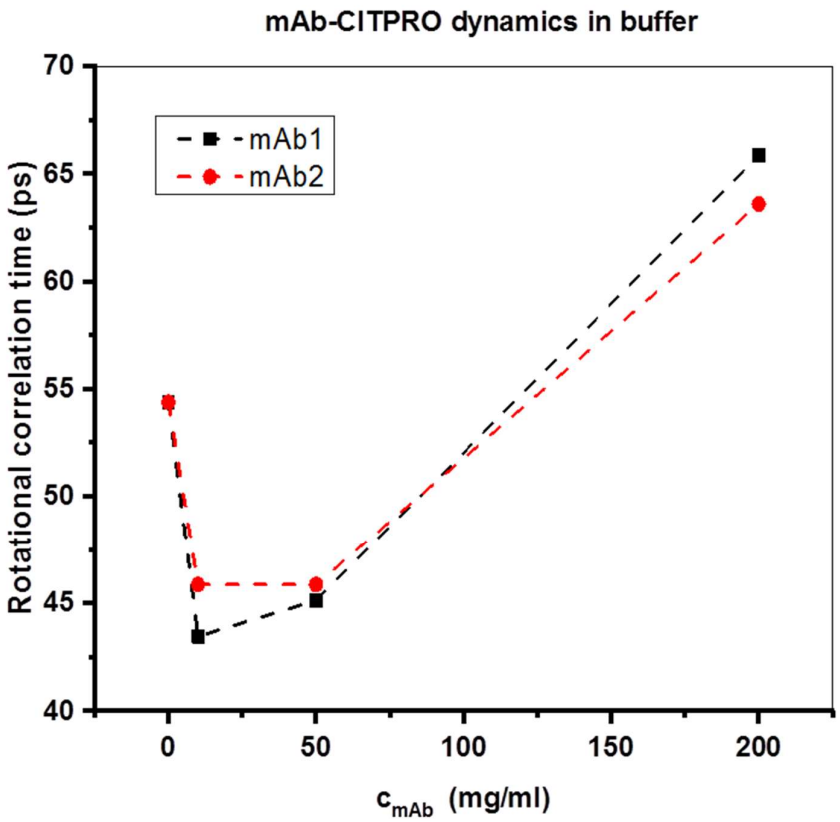


Fig.S13. Viscosity data for Glycerol as reference at different concentrations

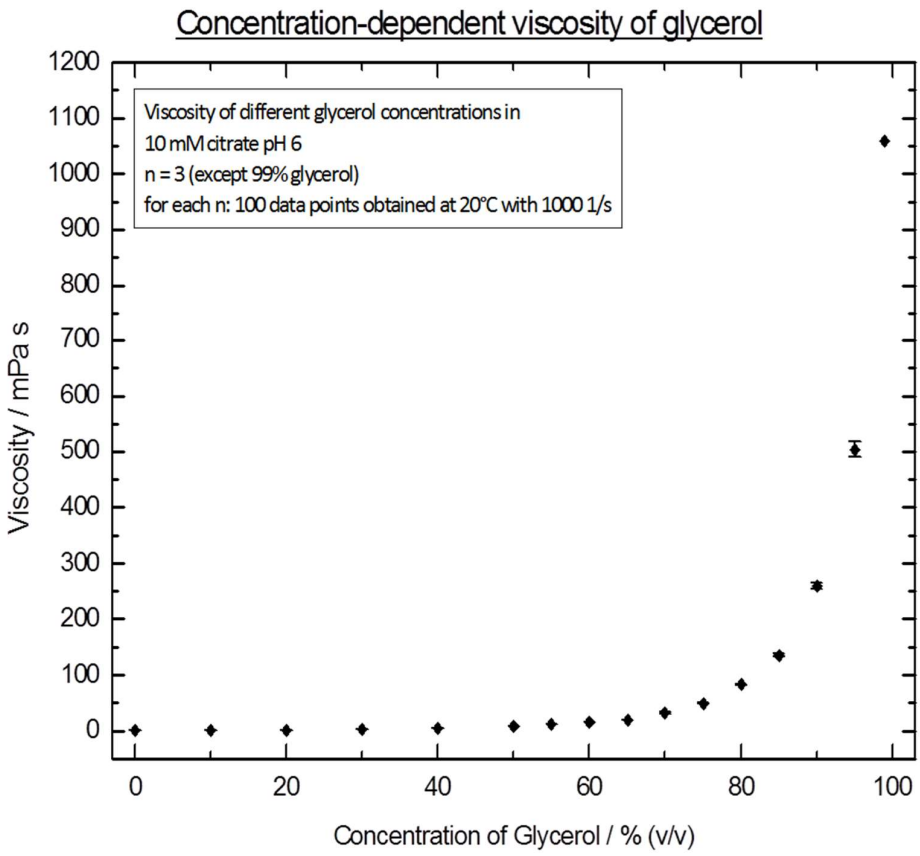


Fig.S14. EPR Spectra of CAT1-buffer mixed with Glycerol at different concentrations

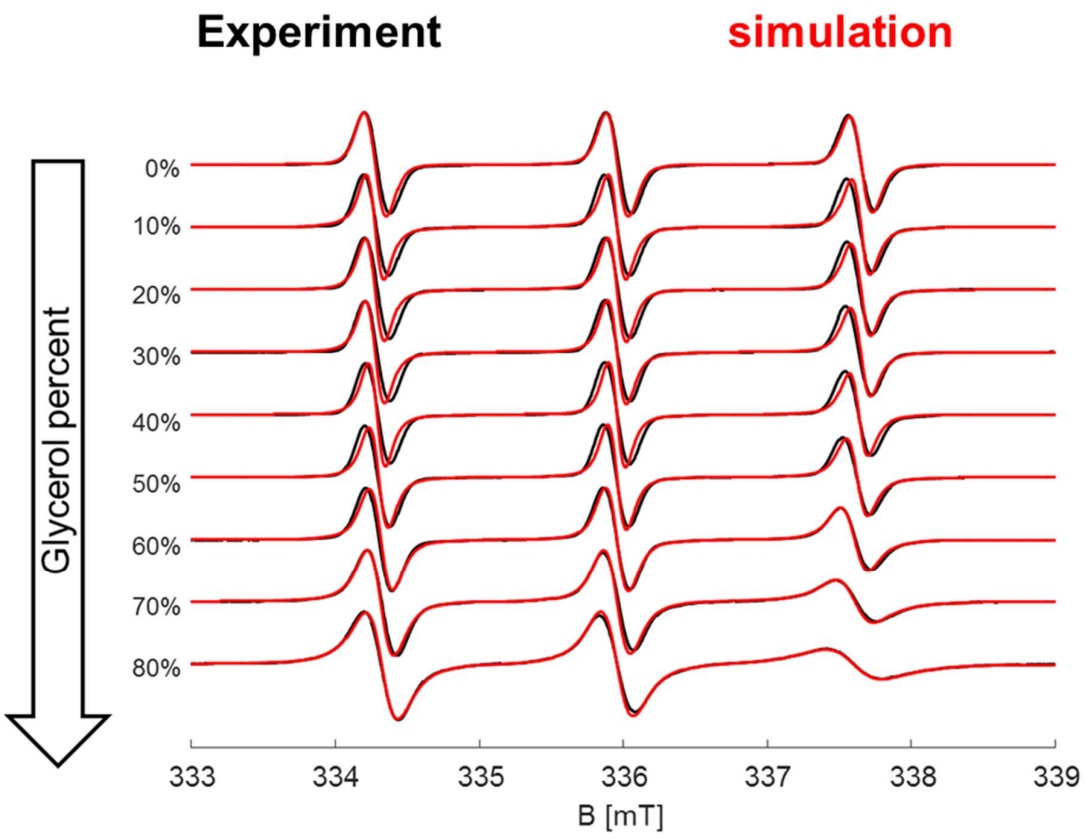


Fig.S15. EPR Spectra of CITPRO-buffer mixed with Glycerol at different concentrations

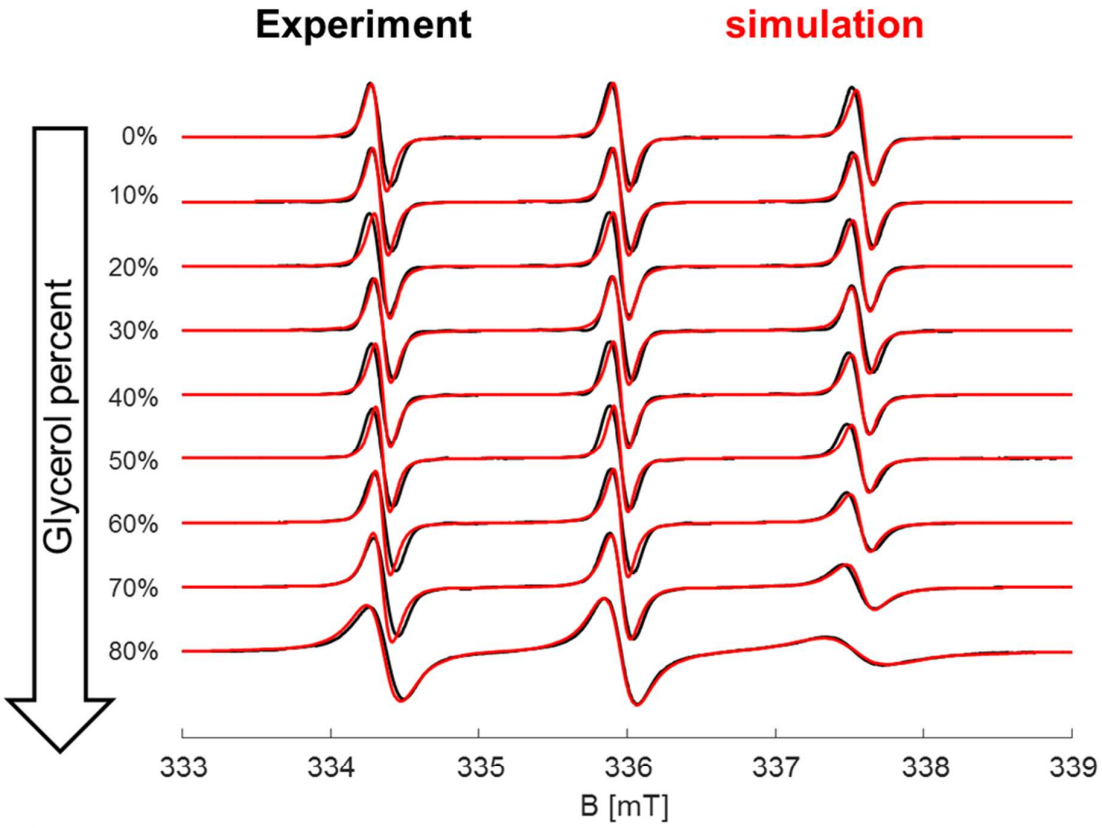


Fig.S16. Rheology results for (a)mAb1 and (b)mAb2 per concentration at different pH values.

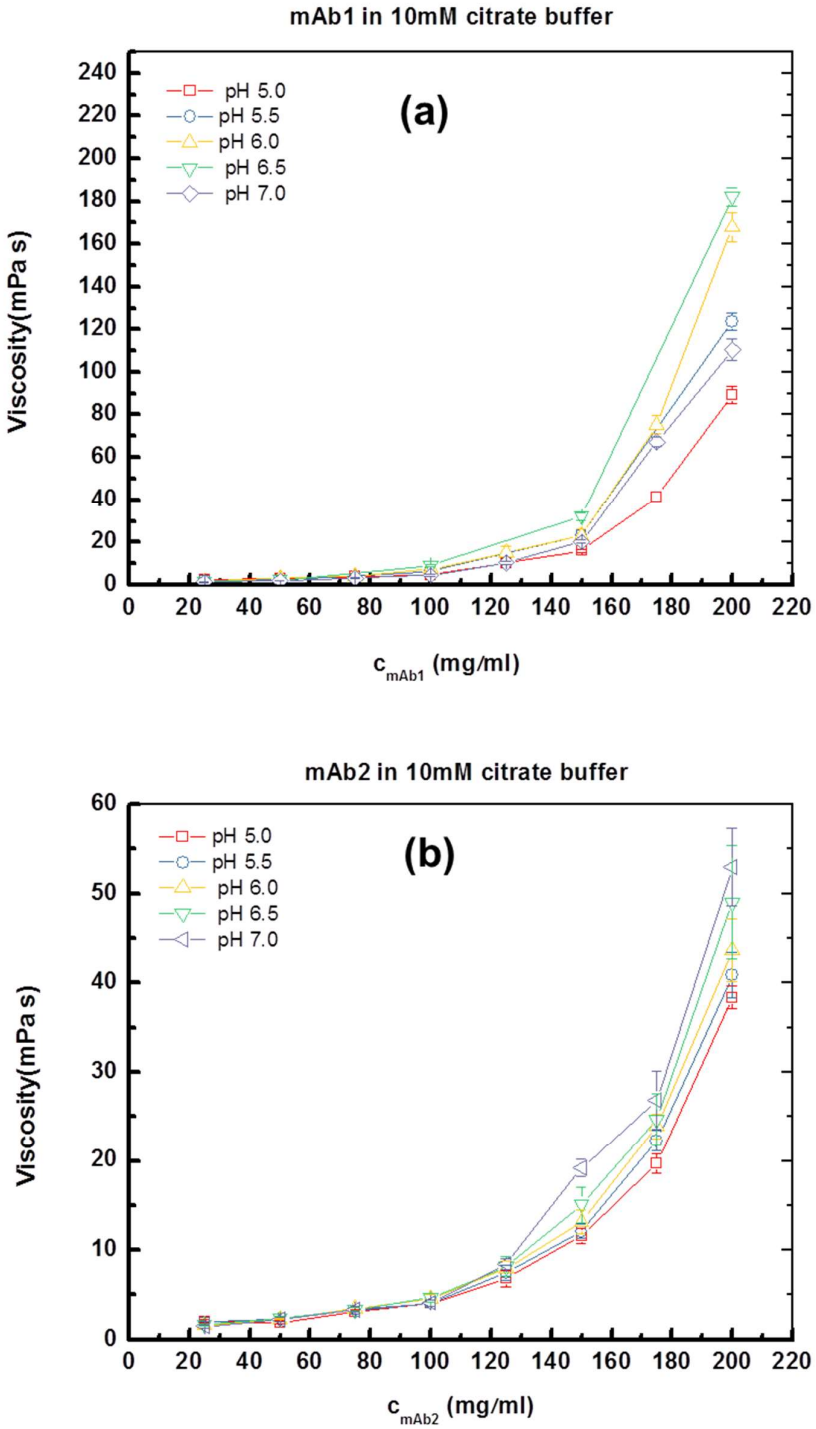
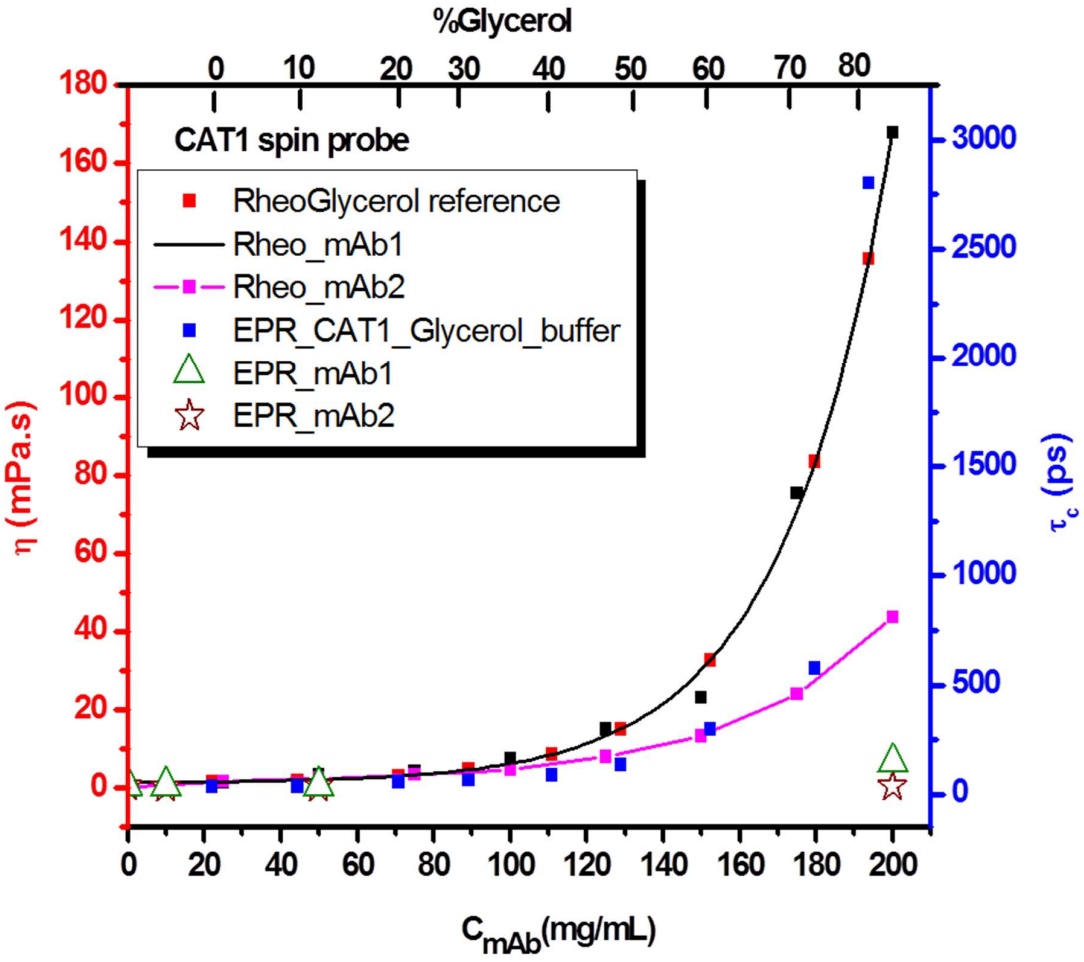


Fig.S17. Correlation diagram between viscosity and rotational correlation time for CAT1-containing systems



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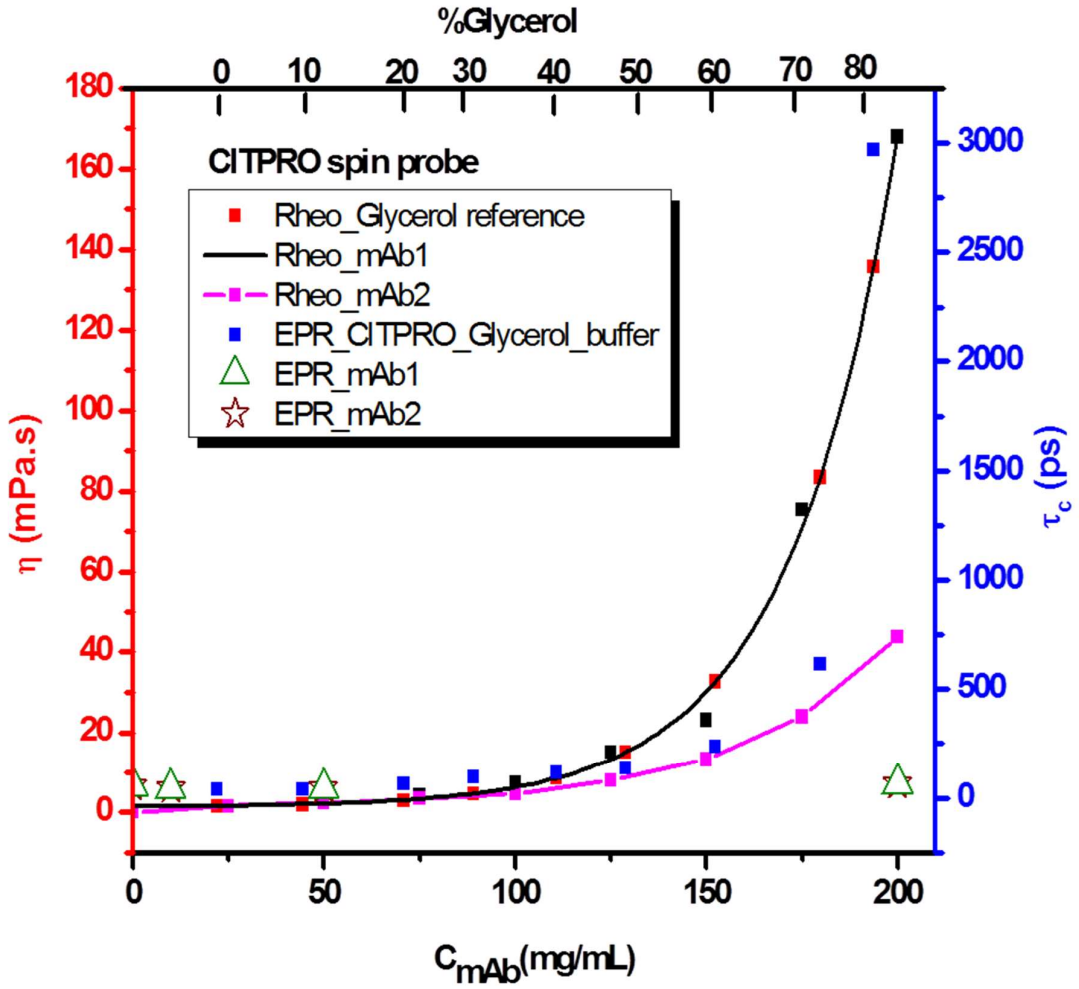
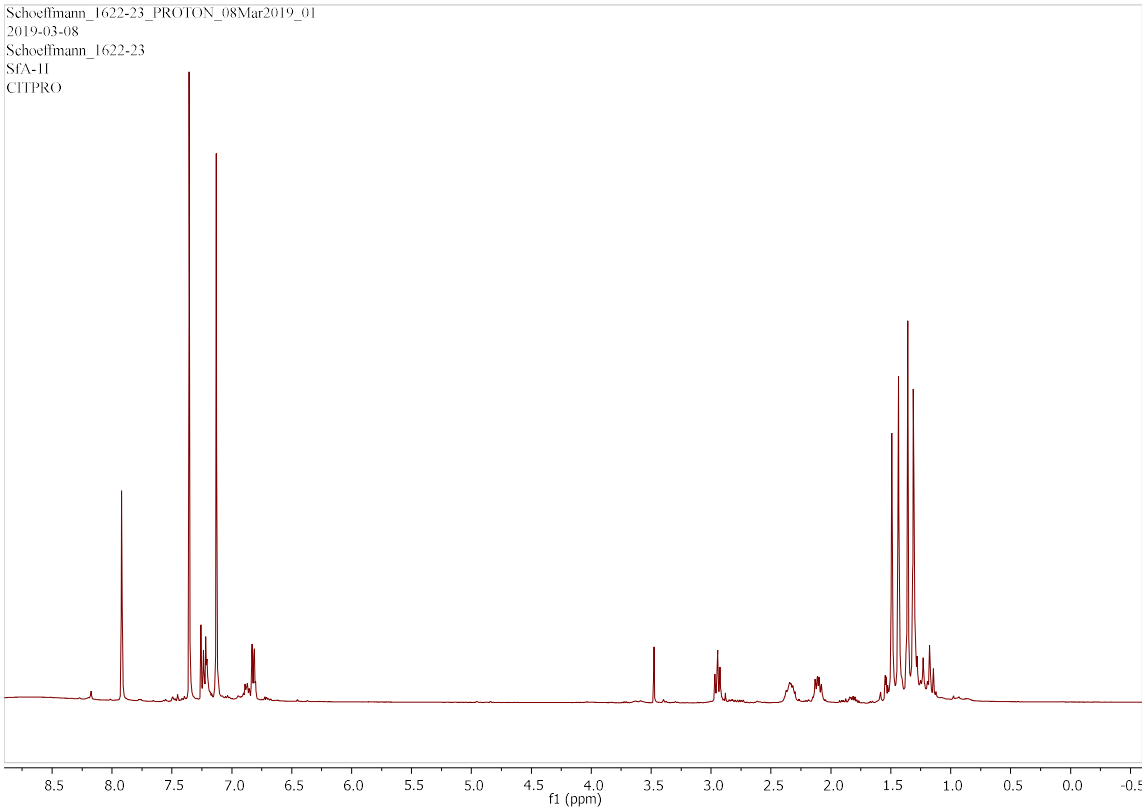
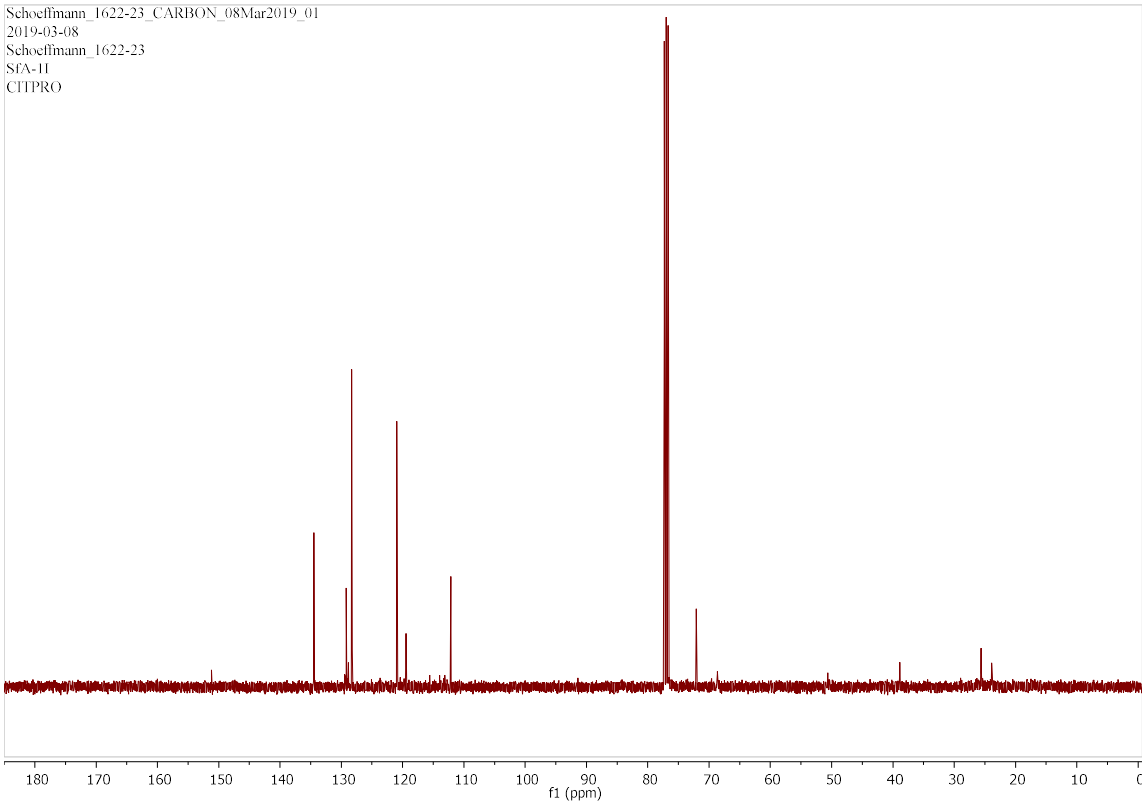


Fig.S19(a). ^{1}H-NMR CITPRO



^{1}H-NMR: 400 MHz, $CDCl_3$; δ = 1.10 – 1.75 (m, 12 H, -C**H**$_3$); 2.05 – 2.15 (m, 2 H, -C**H**$_2$-); 2.25 – 2.40 (m 1H, -C**H**COOC); 2.90 – 3.00 (t, 4 H, -C**H**$_2$-COOH); 3.48 (s, 1 H, -CN**H**C-);

Fig.S19(b). ^{13}C-NMR CITPRO



^{13}C-NMR: 500 MHz, $CDCl_3$; δ = 23.63 – 25.88 (m, 4 C, -**C**H_3); 38.55 – 39.24 (m, 2 C, -CH_2-COOH); 72.08 (s 1C, -**C**-CH_3); 76.26 – 77.51 (m, 1 C, HOOC-**C**-CH_2-COOH);

^{1}H and ^{13}C NMR spectra were recorded on an Agilent Technologies 400 MHz VNMRS spectrometer (400/100 MHz) or an Agilent Technologies 500 MHz DD2 spectrometer (500/125 MHz) with the use of CDCl3 or CD3OD as the internal standard. Chemical shifts (δ) are reported in ppm unit.

IR measurements were performed on a Tensor 27 FT-IT Spectrometer equipped with a BioATRCell II and a Nitrogen-cooled photovoltaic MCT Detector (all devices from Bruker Optics GmbH, Karlsruhe, Germany).

IR: OH: 3120 cm^{-1}; .CH_3 2973 cm^{-1}; CH_2 2932 cm^{-1}; -COOH 1718 cm^{-1}; -NO° 1362 cm^{-1}, 1573 cm^{-1}; -CH_2-1462 cm^{-1}; -COO^- 1323-1320 cm^{-1}

TableS1. mAb-spin probes simulation data in water and buffer. A_{iso} and τ_c are given in MHz and ps (* denotes confined structures).

	water	citrate buffer

		mAb [mg/ml]	(A_{iso}, $\varnothing_c$)	mAb [mg/ml]	(A_{iso}, $\varnothing_c$)
TEMPO	references	0	(48.33, 19.70)	0	(48.30, 27.40)
	mAb1	15	(48.50, 33.80)	10	(48.53, 40.00)
		75	(48.33, 46.80)	50	(48.30, 40.00)
		135	(48.33, 29.50)	200	(48.13, 113.00)
				200*	(46.50, 1660.00)
	mAb2	15	(48.50, 33.80)	10	(48.30, 33.80)
		75	(48.50, 38.00)	50	(48.16, 33.80)
		190	(48.50, 40.00)	200	(48.16, 40.00)
CAT1	references	0	(47.26, 16.60)	0	(47.36, 37.19)
	mAb1	15	(47.16, 48.70)	10	(47.36, 37.19)
		75	(47.16, 30.00)	50	(47.36, 37.19)
		135	(47.16, 30.00)	200	(47.06, 145.00)
				200*	(46.50, 1660.00)
	mAb2	15	(47.03, 18.50)	10	(47.36, 32.48)
		75	(47.36, 45.20)	50	(47.36, 33.80)
		190	(47.30, 41.60)	200	(47.10, 37.20)
CITPRO	references	0	(45.43, 37.19)	0	(45.76, 54.37)
	mAb1	15	(45.43, 42.57)	10	(45.60, 43.46)
		75	(45.43, 37.19)	50	(45.60, 45.12)
		135	(45.43, 44.90)	200	(45.60, 65.86)
	mAb2	15	(45.76, 37.19)	10	(45.60, 45.86)
		75	(45.76, 45.80)	50	(45.60, 45.86)
					(45.60,63.59)
		190	(45.76, 42.57)	200	

Table S2. Glycerol concentration based on its viscosity

%Glycerol	Viscosity (mPa.s)	Glycerol(mg/ml)
0	0	0

10	1.5	22.0
20	2.0	44.0
30	3.0	71.0
40	4.7	89.0
50	8.5	111.0
60	15.0	129.0
70	33.0	152.0
80	83.0	180.0