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SUPPORTING INFORMATION

Single Stop Analysis of a Protein Surface using Molecular Probe Electrochemistry

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1. **Experimental Section**

2. **Spectra of Na[*o*-COSAN] and Na[*o*-COSAN] with amino acids.**

Fig S1. $^{1}H\{^{11}B\}$-NMR of Na[*o*-COSAN] 2mM in D_2O, with peak assignation.
Fig S2. $^{11}B\{^{1}H\}$-NMR of Na[*o*-COSAN] 2mM in D_2O, with peak assignation.

Fig S3. a) $^{1}H\{^{11}B\}$-NMR of Na[*o*-COSAN] 2mM (red), Na[*o*-COSAN] 2mM + L-arginine 2 mM (blue) and Na[*o*-COSAN] 2mM + L-arginine 60 mM (black) in D_2O. b) ^{1}H-NMR of L-arginine in D_2O for comparison.

Fig S4 $^{11}B\{^{1}H\}$-NMR of Na[*o*-COSAN] 2mM (red), Na[*o*-COSAN] 2mM + L-arginine 2 mM (blue) and Na[*o*-COSAN] 2mM + L-arginine 60 mM (black) in D_2O.

Fig S5. a)$^{1}H\{^{11}B\}$-NMR of Na[*o*-COSAN] 2mM (red), Na[*o*-COSAN] 2mM + L-histidine 2 mM (blue) and Na[*o*-COSAN] 2mM + L-histidine 60 mM (black) in D_2O. b) ^{1}H-NMR of L-histidine in D_2O for comparison.

Fig S6. $^{11}B\{^{1}H\}$-NMR of Na[*o*-COSAN] 2mM (red), Na[*o*-COSAN] 2mM + L-histidine 2 mM (blue) and Na[*o*-COSAN] 2mM + L-histidine 60 mM (black) in D_2O.

Fig S7. a) $^{1}H\{^{11}B\}$-NMR of Na[*o*-COSAN] 2mM (red), Na[*o*-COSAN] 2mM + L-lysine 2 mM (blue) and Na[*o*-COSAN] 2mM + L-lysine 60 mM (black) in D_2O. b) ^{1}H-NMR of L- lysine in D_2O for comparison.

Fig S8. $^{11}B\{^{1}H\}$-NMR of Na[*o*-COSAN] 2mM (red), Na[*o*-COSAN] 2mM + L-histidine 2 mM (blue) and Na[*o*-COSAN] 2mM + L-lysine 60 mM (black) in D_2O.

3. **Cyclic voltammogram and DPV measurement.**

Fig. S9. Representative electrochemical measurements for the study. **(a)** CV and **(b)** DPV curve with 1mM Na[*o*-COSAN] in 0.1M NaCl with increasing concentration of the protein BSA measured with a scan rate of 50mV/s.

Table S1. The current intensity values obtained from the DPV experiments for the various proteins with increasing concentration in presence of $[o\text{-COSAN}]^-$.

Fig. S10. Individual plots displaying the normalized current density calculated by considering the surface area of each of the protein *vs.* the protein to $[o\text{-COSAN}]^-$ fraction for different proteins.

Fig. S11. The normalized current density *vs.* the hydrodynamic radius at different protein/$[o\text{-COSAN}]^-$ ratio with the fitting parameters.

4. **Dynamic Light Scattering measurements:**

Fig. S12. Representative DLS measurements for BSA in **(a)** Water and **(b)** 0.1M NaCl (electrolyte).

Table S2. Hydrodynamic diameters of the different proteins in different conditions.

1. Materials and methods

Chemicals & proteins required: Cs[*o*-COSAN] was synthesized from 1,2-*closo*-$C_2B_{10}H_{12}$ from Katchem Spol.sr.o (Kralupy nad Vltavou, Czech Republic) as reported in the literature.[1] The sodium salts of [*o*-COSAN]$^-$ species was obtained by means of cationic exchange resin of the corresponding cesium salt.[2]

Hydrochloric acid (HCl), sodium chloride (NaCl), acetonitrile (ACN), serum albumin (bovine), carbonic anhydrase (bovine erythrocytes), catalase (bovine liver), haemoglobin (bovine blood), γ-globulin (bovine blood), peroxidase (horseradish), myoglobin (equine skeletal muscle), histone (calf thymus), lysozyme (chicken egg white), L-lysine, L-arginine, L-histidine and L-glutamine were all purchased from Sigma-Aldrich and used without any further purifications.

Dynamic Light Scattering (DLS) experiments: The DLS experiments were performed in a ZETASIZER NANO ZS (Malvern Instruments Ltd.) equipped with a He-Ne 633nm laser. 5 mL solutions having a protein concentration of 15μM were prepared in water as well as in 0.1 M NaCl for each of the 9 proteins under study, individually, and the different hydrodynamic radii were recorded.

Electrochemical measurements: The cyclic voltametric (CV) as well as the differential pulse voltametric (DPV) measurements were performed in an AutoLab PGSTAT302N potentiostat/galvanostat from Metrohm using its in-built software for analysis. The experiments were performed using a three-electrode system with glassy carbon (GC) as the working, Ag/AgCl (3M KCl) as the reference and Pt wire as the counter electrodes with 0.1M NaCl as the electrolyte. In a typical electrochemical experiment, 10 mL solution of 1mM Na[*o*-COSAN] (346.74 g/mol) in 0.1M NaCl (58.44 g/mol) was prepared in an electrochemical cell to which the protein was added in mg depending on the molecular weight of the different proteins to attain the required protein concentration, sequentially. The resulting solution was mixed vigorously to ensure homogenous distribution of the particles. All the measurements were performed in an inert N_2 atmosphere with 3 scans for each measurement and a scan rate of 50mV/s.

NMR experiments: The $^1H\{^{11}B\}$-NMR (400.13 MHz) and $^{11}B\{^1H\}$-NMR (128.37 MHz) spectra were recorded on a Bruker NEO 400 instrument equipped with appropriate decoupling accessories. 16 scans were recorded for $^1H\{^{11}B\}$ NMR spectra and 256 for $^{11}B\{^1H\}$-NMR. All NMR spectra were recorded using performed in D_2O 22 °C. The $^{11}B\{^1H\}$ NMR chemical shift values were referenced to external $BF_3{\cdot}OEt_2$, while the $^1H\{^{11}B\}$ NMR chemical shift values were referenced to $SiMe_4$. Chemical shifts are reported in units of parts per million downfield from reference. The experiments were performed at a fixed [*o*-COSAN]$^-$ concentration of 2mM in D_2O with increasing amounts of the amino acid to be studied, which resulted in final concentrations of 2mM and 60mM amino acid.

To further reinforce these data, the same experiments were carried out with compound [8,8'-I_2- $Co(C_2B_9H_{10})_2$]$^-$ which, unlike [*o*-COSAN]$^-$, a cisoid rotamer, is a transoid. Despite these differences, the same results were obtained as with [*o*-COSAN]$^-$ proving that these experiments are reproducible even with variations of [*o*-COSAN]$^-$.

2. Spectra of Na[*o*-COSAN] with amino-acids

Fig. S1. $^{1}H\{^{11}B\}$-NMR of Na[*o*-COSAN] 2mM in D_2O, with peak assignation.

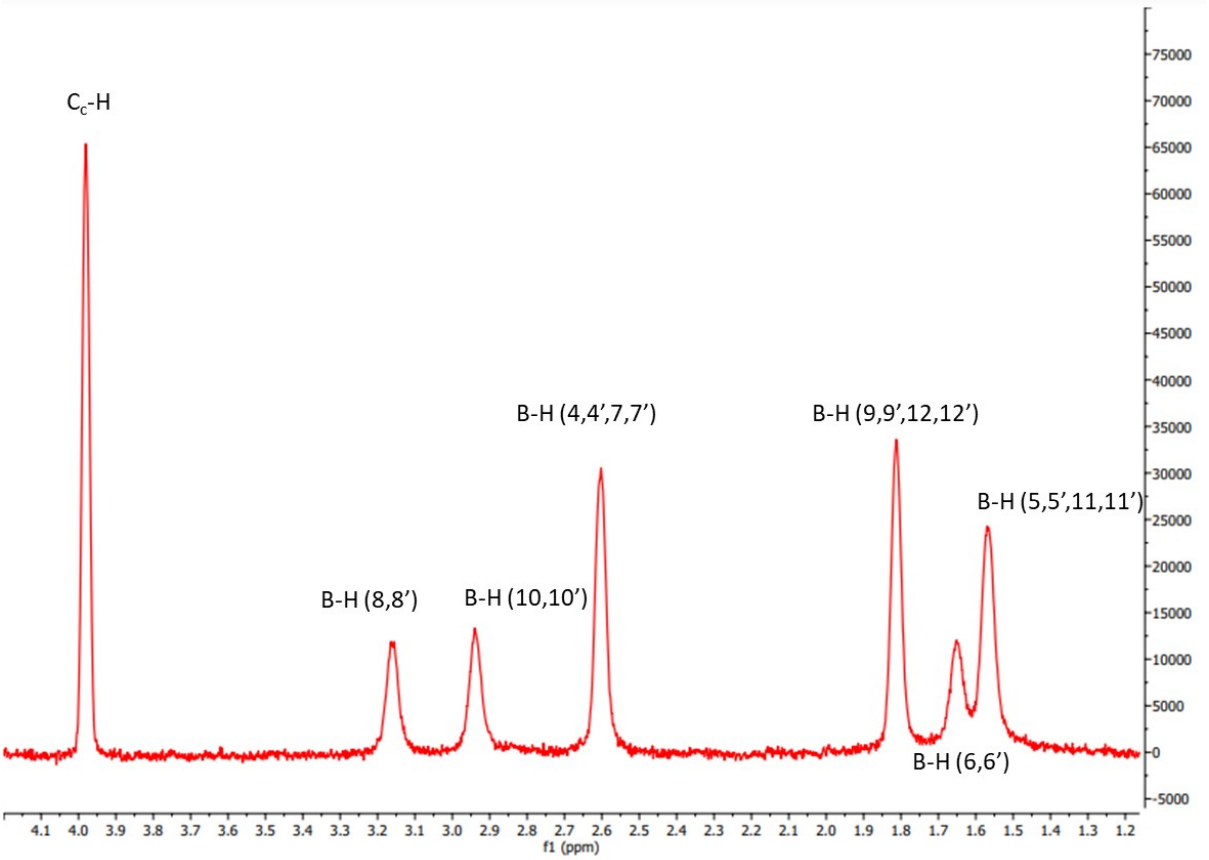


Fig. S2. $^{11}B\{^{1}H\}$-NMR of Na[*o*-COSAN] 2mM in D_2O, with peak assignation.

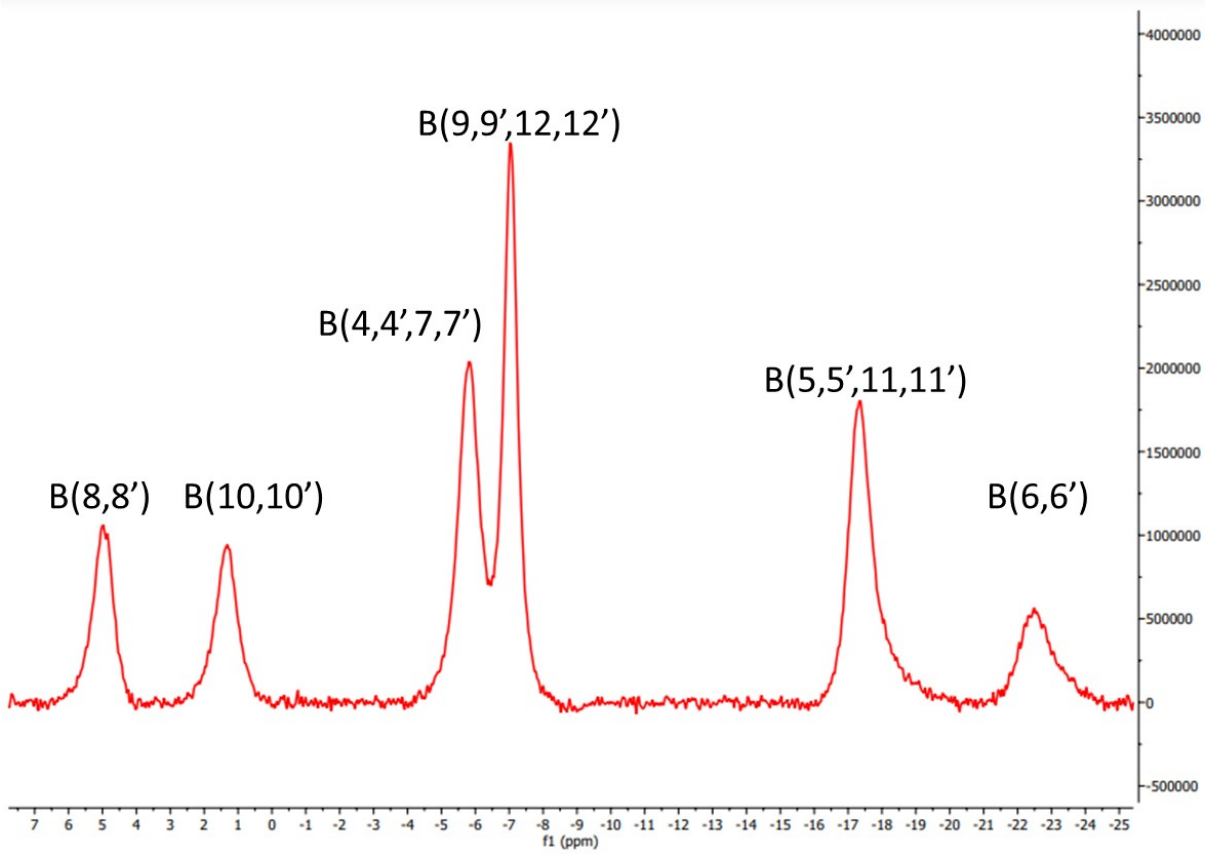
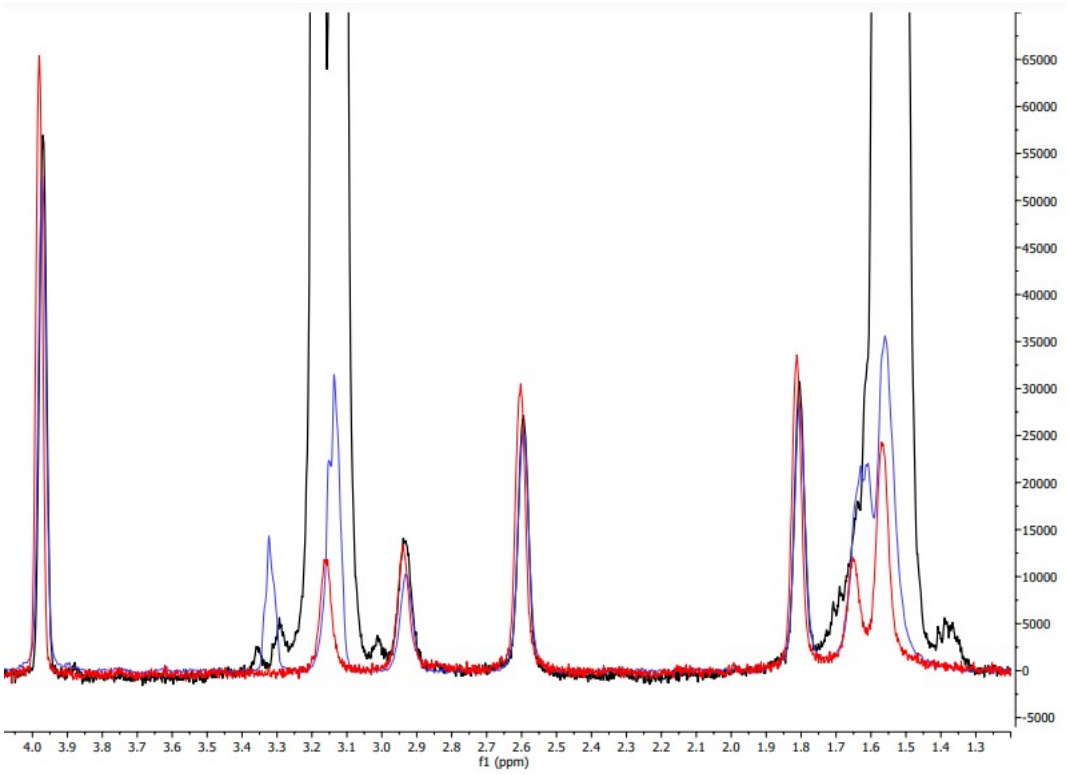


Fig. S3. a) $^{1}H\{^{11}B\}$-NMR of Na[*o*-COSAN] 2mM (red), Na[*o*-COSAN] 2mM + L-arginine 2 mM (blue) and Na[*o*-COSAN] 2mM + L-arginine 60 mM (black) in D_2O. b) ^{1}H-NMR of L-arginine in D_2O for comparison.

a)



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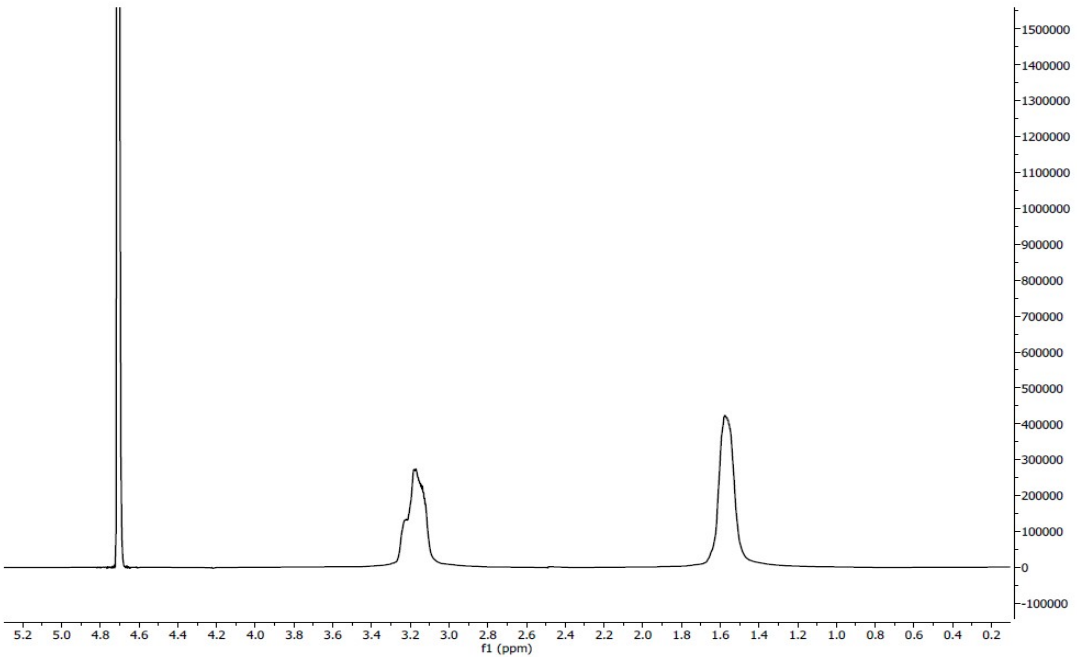

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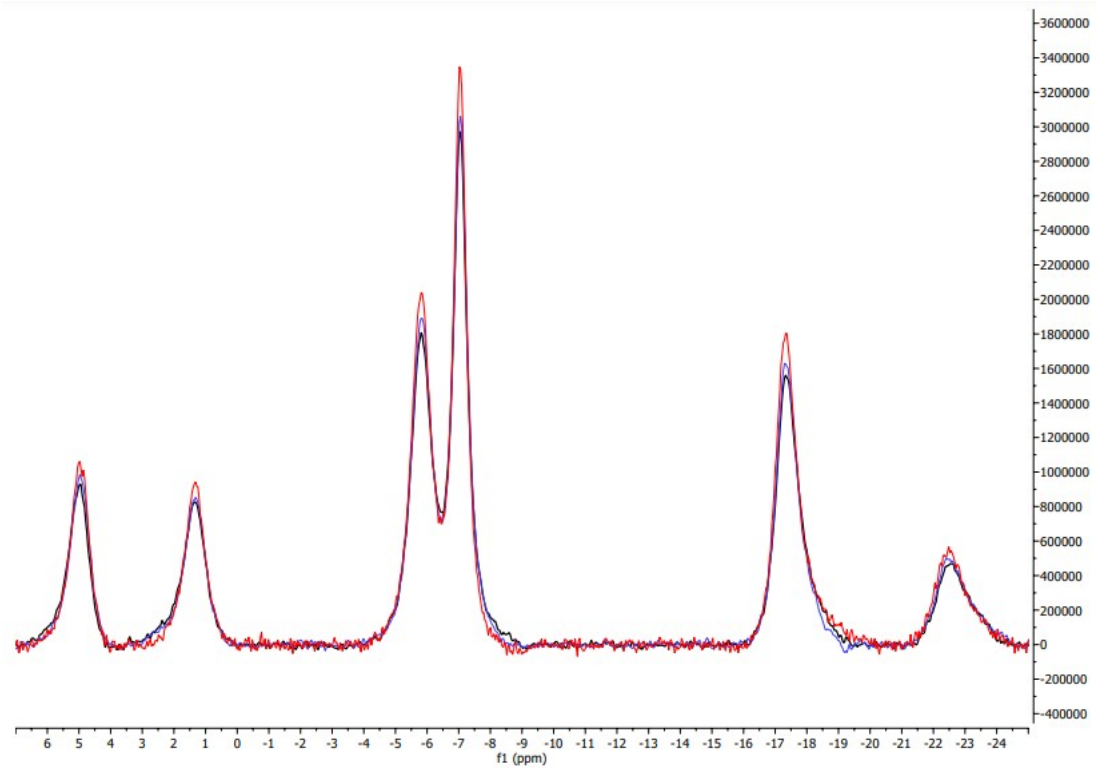

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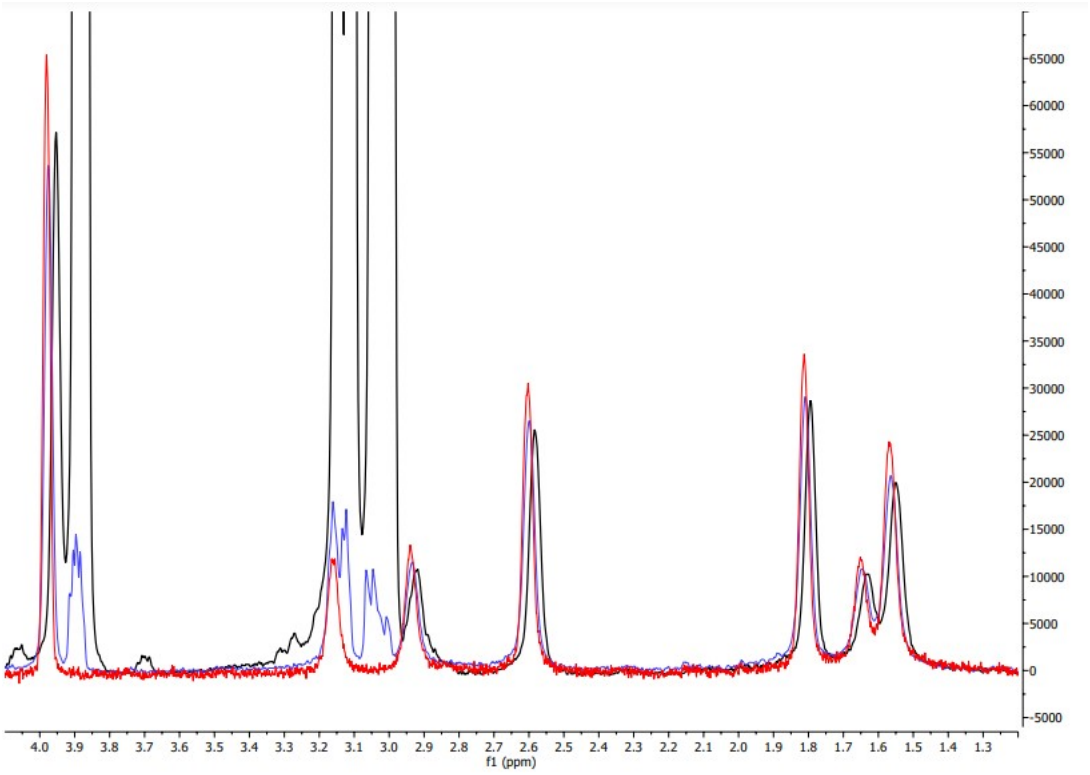

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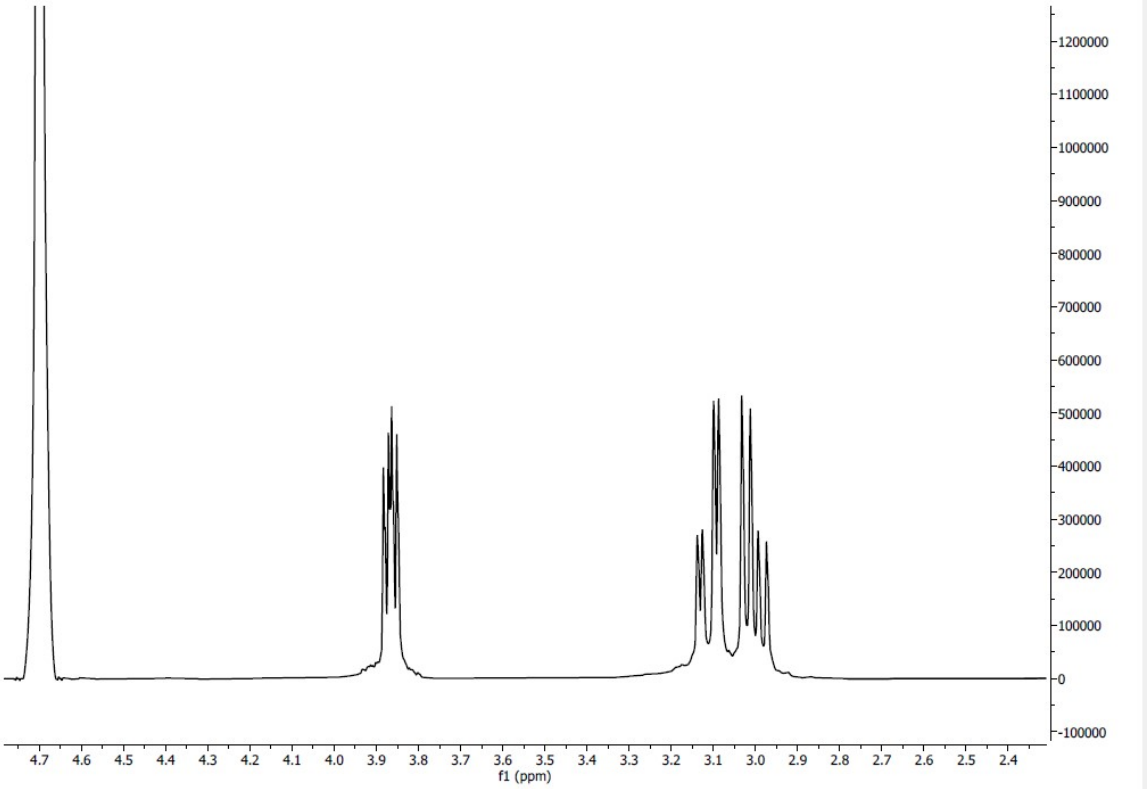

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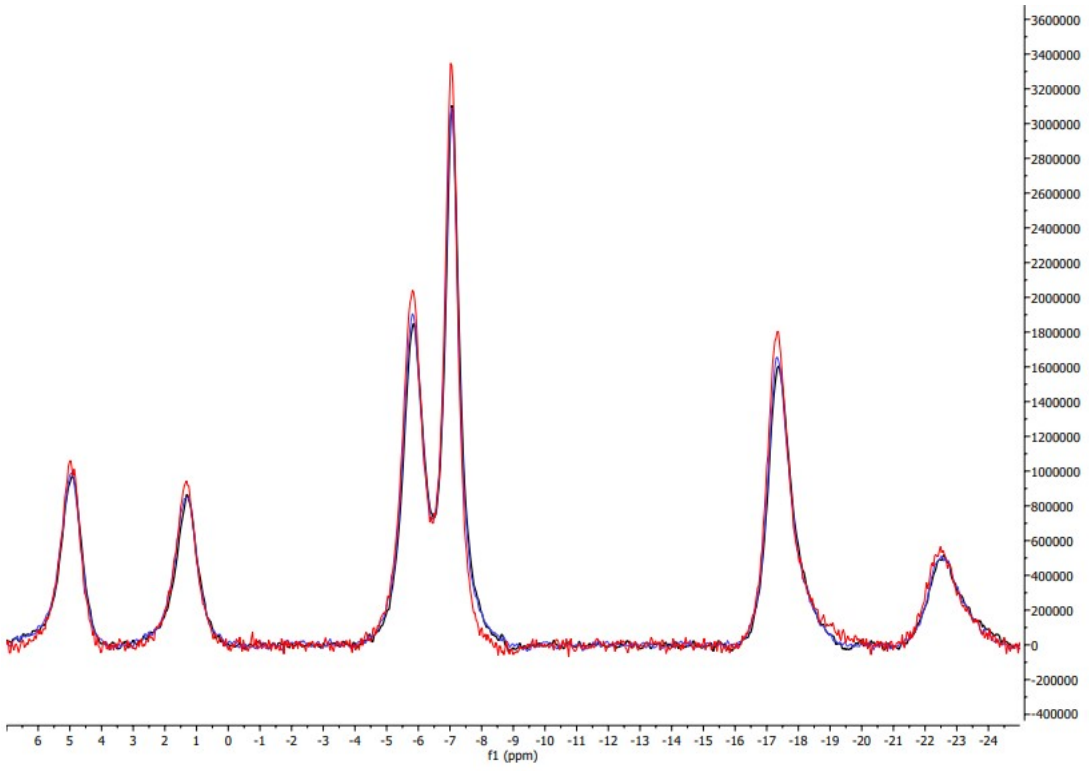

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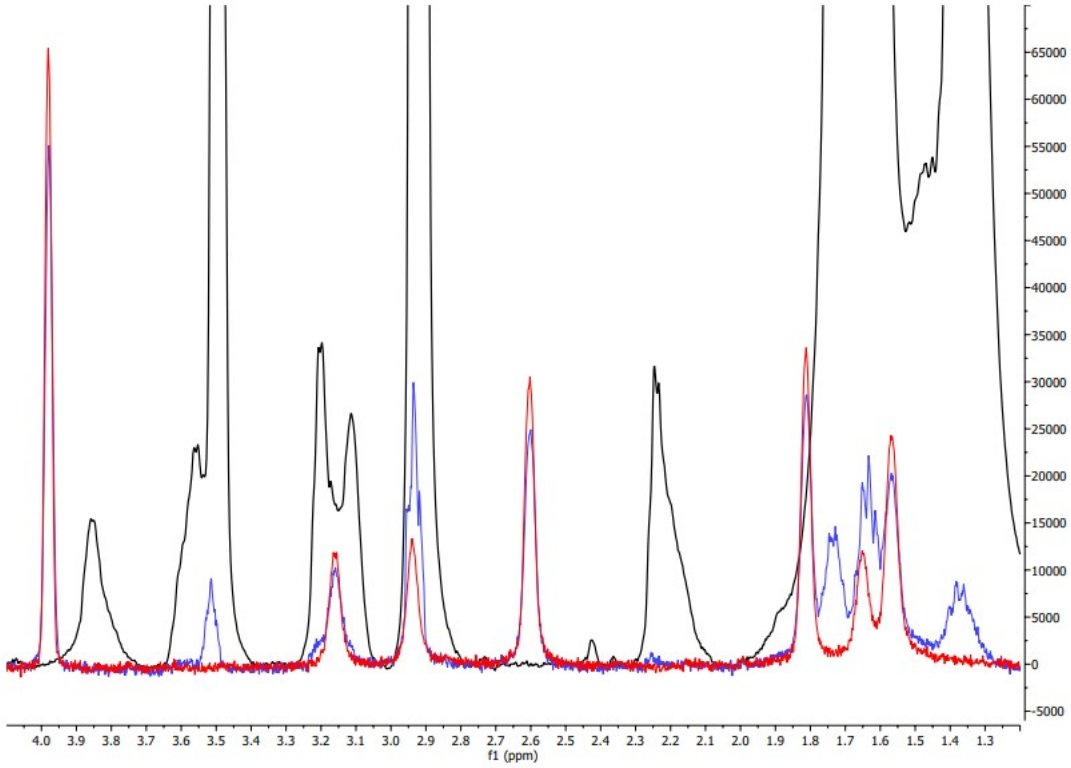

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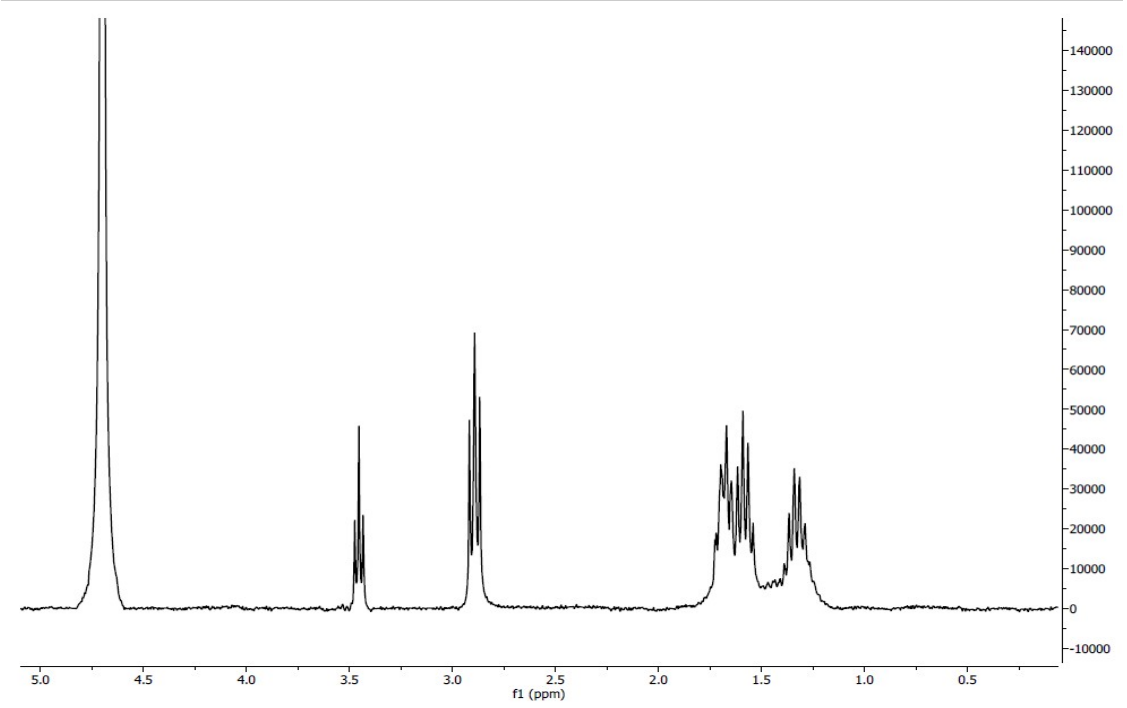

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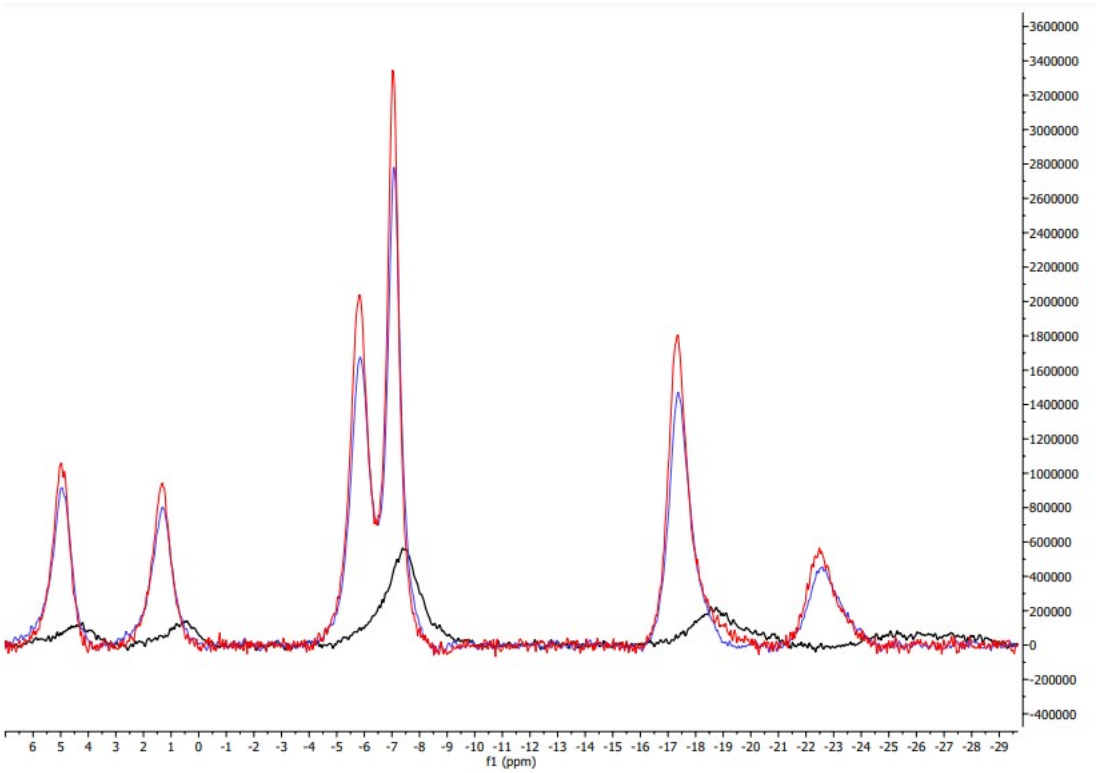

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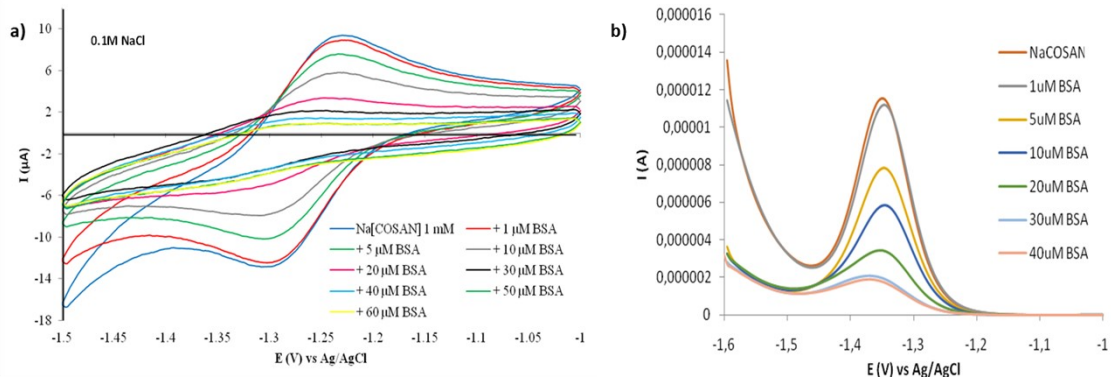


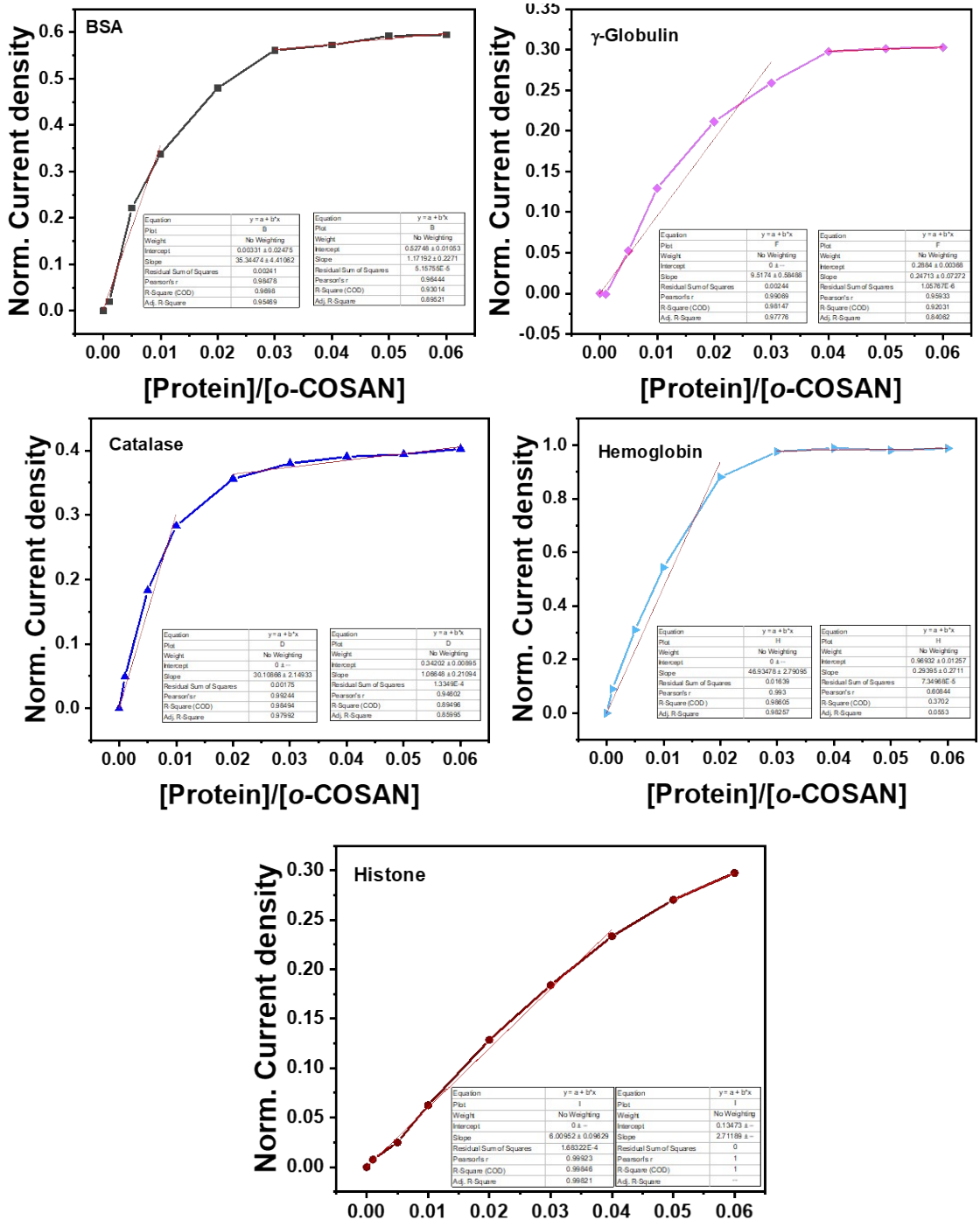
Table S1. The current intensity values obtained from the DPV experiments for the various proteins with increasing concentration in presence of [*o*-COSAN]$^-$ where X denotes the protein.

[X]/[*o*-COSAN]$^-$	Current, I (μA)								
	BSA	γ-Globulin	Catalase	Hemo-globin	Histone	Lysozyme	HRP	CA	Myo-globin
0	11.52	11.30	11.52	11.25	11.20	11.56	10.56	11.3	10.84
0.001	11.20	11.33	10.18	10.34	10.95	11.25	10.08	11.26	10.50
0.005	7.81	9.65	6.54	8.07	10.37	10.71	9.08	10.55	9.47
0.01	5.84	7.24	3.81	5.69	9.12	9.82	8.03	9.93	8.64
0.02	3.46	4.66	1.82	2.24	6.91	8.27	6.87	8.86	7.33
0.03	2.08	3.16	1.17	1.27	5.06	6.43	5.97	7.92	6.21
0.04	1.90	1.96	0.89	1.14	3.41	5.01	5.33	6.95	5.10
0.05	1.57	1.84	0.78	1.22	2.17	4.09	4.69	6.001	4.13
0.06	1.53	1.80	0.56	1.14	1.27	3.37	4.07	5.21	3.31

Current intensity of bound [*o*-COSAN]$^-$ is calculated as:

Current intensity of Bound [*o*-COSAN]$^-$ = Current intensity at 0 $_{[X]/[\ o\text{-COSAN}]^-}$ – Current intensity at [X]/[*o*-COSAN]$^-$

Fig. S10. Individual plots displaying the normalized current density calculated by considering the surface area of each of the protein *vs.* the protein to [*o*-COSAN]$^-$ fraction for different



proteins.

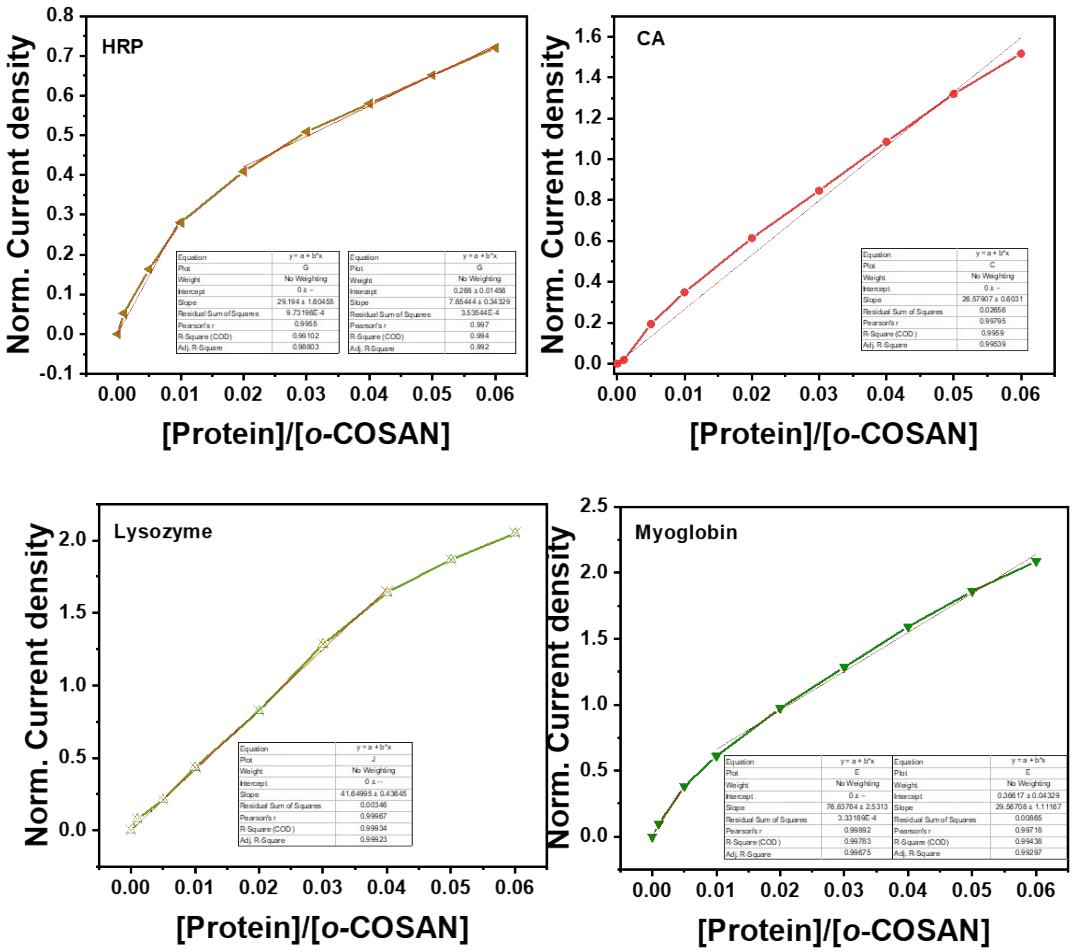

HRP
Norm. Current density
[Protein]/[o-COSAN]
CA
Norm. Current density
[Protein]/[o-COSAN]
Lysozyme
Norm. Current density
[Protein]/[o-COSAN]
Myoglobin
Norm. Current density
[Protein]/[o-COSAN]

Fig. S11. The normalized current density *vs.* the hydrodynamic radius at different protein/[*o*-COSAN]$^-$ ratio with the fitting parameters.

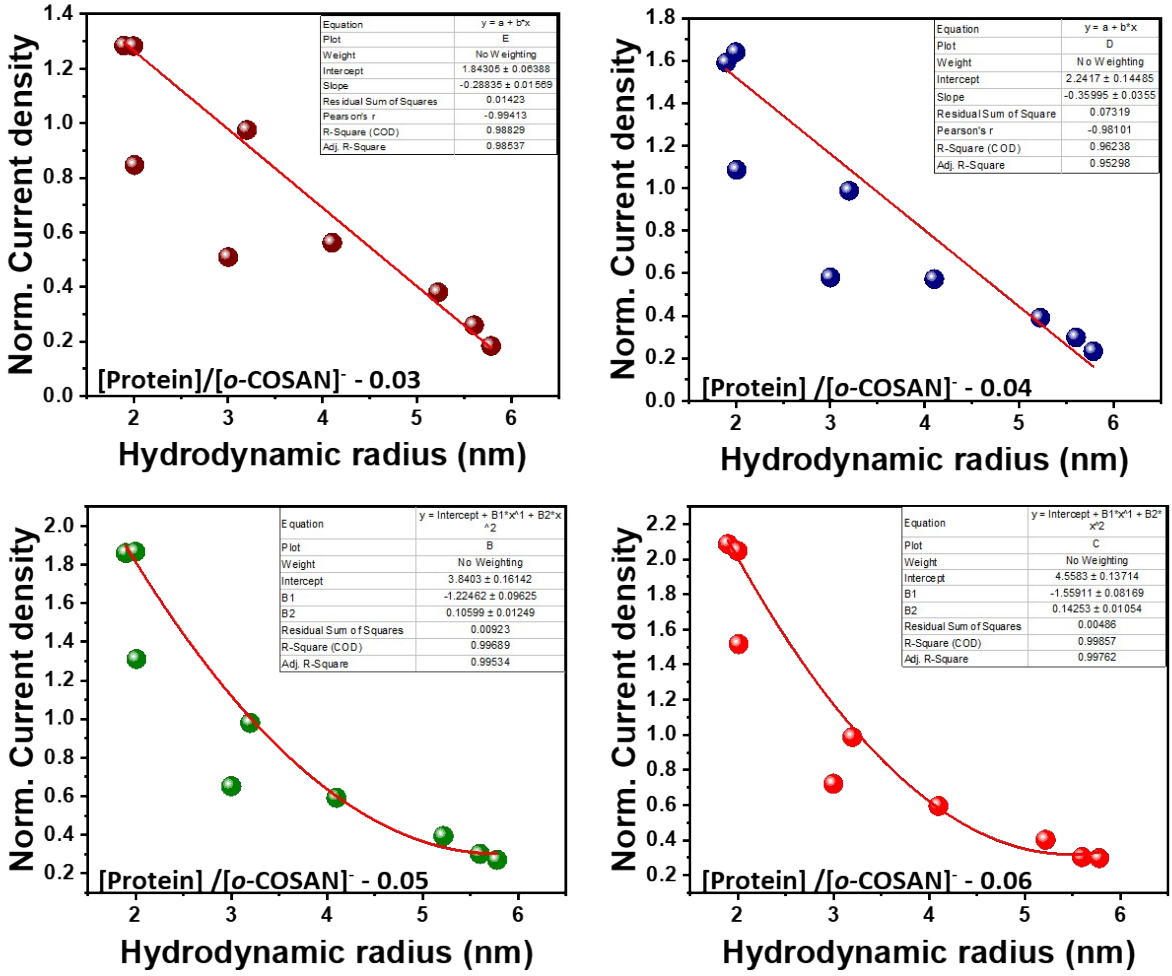


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Fig. S12. Representative DLS measurements for BSA in **(a)** Water and **(b)** 0.1M NaCl (electrolyte).

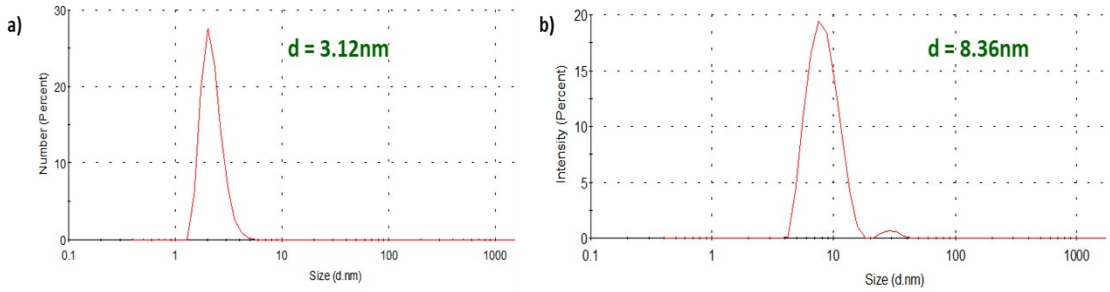

Table S2. Hydrodynamic diameters of the different proteins in different conditions.

Protein	Hydrodynamic Diameter, d_H (nm)	
	Water	**0.1M NaCl**
BSA	3.12	8.26
γ-Globulin	7.9	10.90
Hemoglobin	6.90	6.40
Histone	1.55	11.60
Lysozyme	1.42	4.01
HRP	5.27	6.08
CA	8.66	4.02
Myoglobin	4.09	3.80

References

1 M. F. Hawthorne and T. D. Andrews, Carborane analogues of cobalticinium ion, *Chem. Commun.*, 1965, **19**, 443–444.

2 A. Zaulet, F. Teixidor, P. Bauduin, O. Diat, P. Hirva, A. Ofori and C. Viñas, Deciphering the role of the cation in anionic cobaltabisdicarbollide clusters, *J. Organomet. Chem.*, 2018, **865**, 214–225.