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Supplemental Information

Real-Time Insights into Biological Events: In-Cell Processes and Protein-Ligand Interactions

Linda Cerofolini, Stefano Giuntini, Letizia Barbieri, Matteo Pennestri, Anna Codina, Marco Fragai, Lucia Banci, Enrico Luchinat, and Enrico Ravera

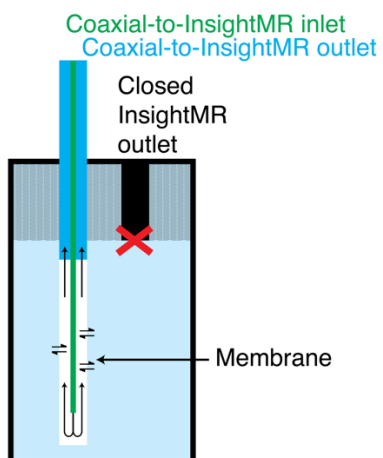


Figure S1. Scheme of the experimental setup in the case of using the InsightMR tool and photograph of the assembled tube.

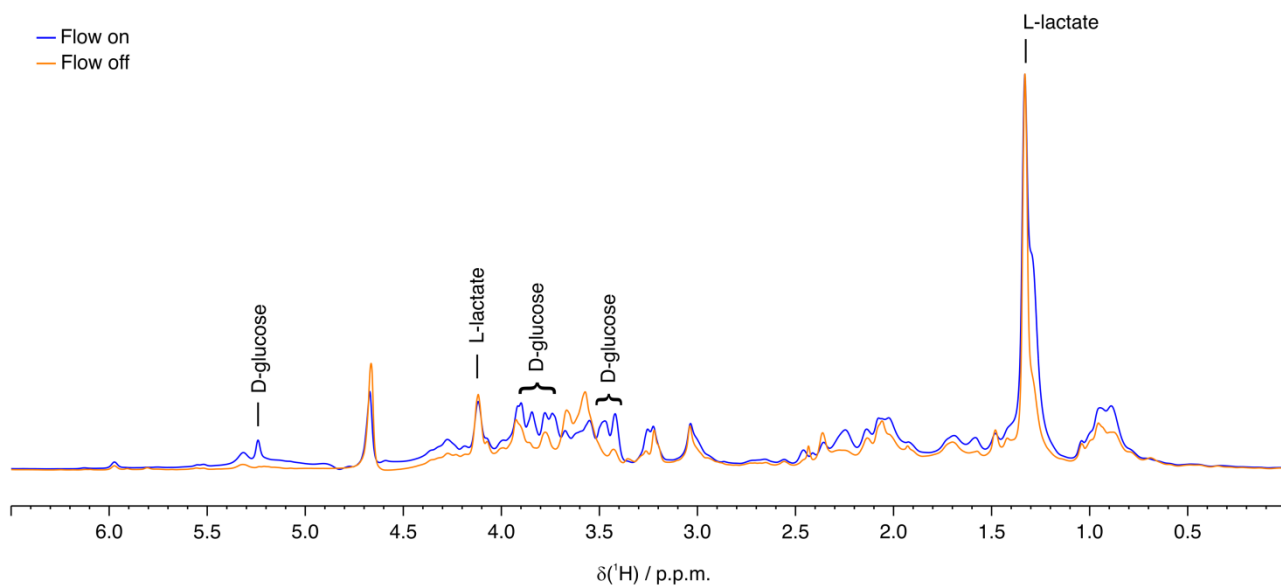


Figure S2. ¹H NMR spectra of cells in the bioreactor (blue) and in the control sample (orange) collected after 12 hours. Signals arising from D-glucose and L-lactate are labeled.

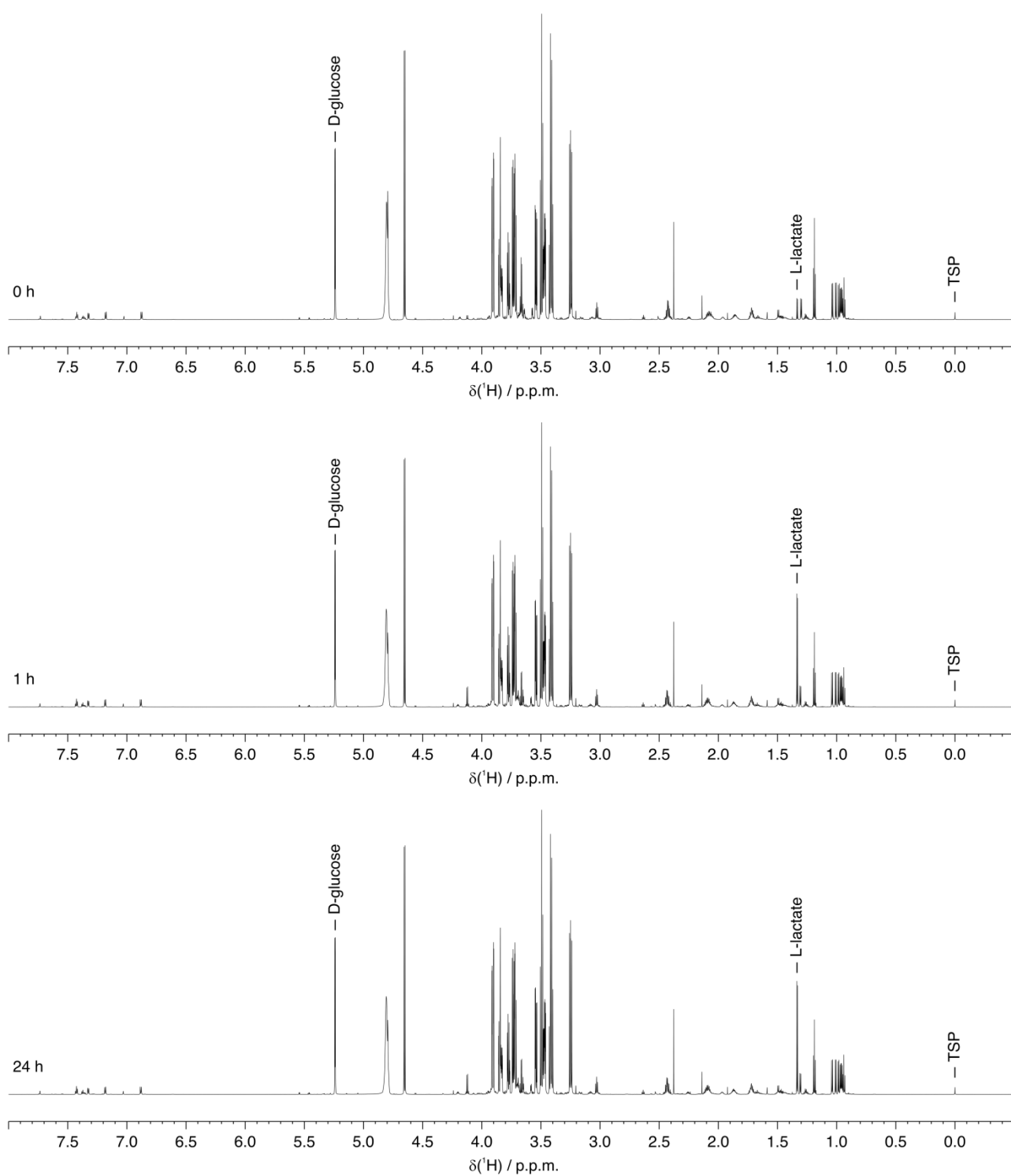


Figure S3. Representative ^1H NMR spectra acquired on the spent medium at collected at different times of nutrient flow. Top: 0 h, middle: 1 h, bottom: 24 h. For the quantification of D-glucose and L-lactate, the labeled signals were integrated and $34 \mu\text{M}$ 3-(trimethylsilyl)-2,2,3,3-tetradeuteropropionic acid (TSP) was used as internal standard.

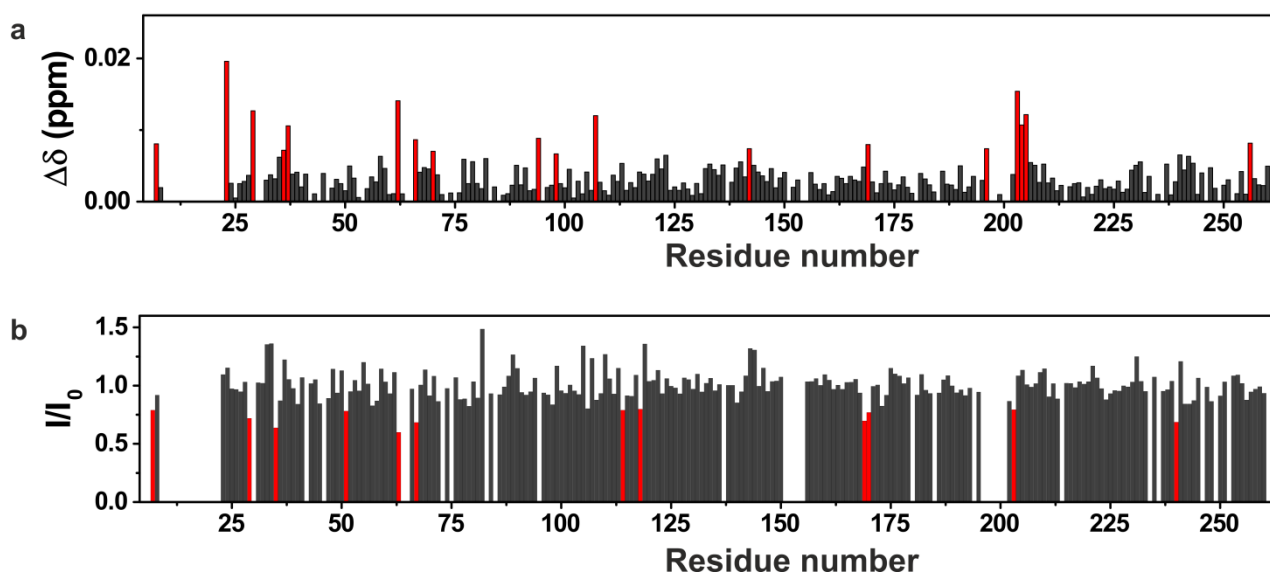


Figure S4. Chemical shift perturbation (CSP) (A) and intensity decreases between the uninhibited Zn(II)-hCAII and Zn(II)-hCAII in the presence of benzoate, evaluated according to the formula $\Delta\delta = 1/2 \sqrt{\Delta\delta_H^2 + (\Delta\delta_N/5)^2}$; the residues exhibiting the largest CSP are highlighted in red.

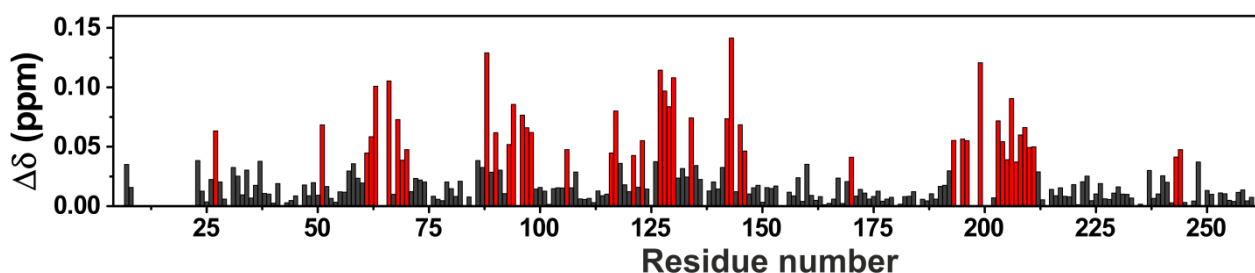


Figure S5. Chemical shift perturbation (CSP) between the uninhibited Zn(II)-hCAII and Zn(II)-hCAII in the presence of furosemide, evaluated according to the formula $\Delta\delta = 1/2 \sqrt{\Delta\delta_H^2 + (\Delta\delta_N/5)^2}$; the residues exhibiting the largest CSP are highlighted in red.

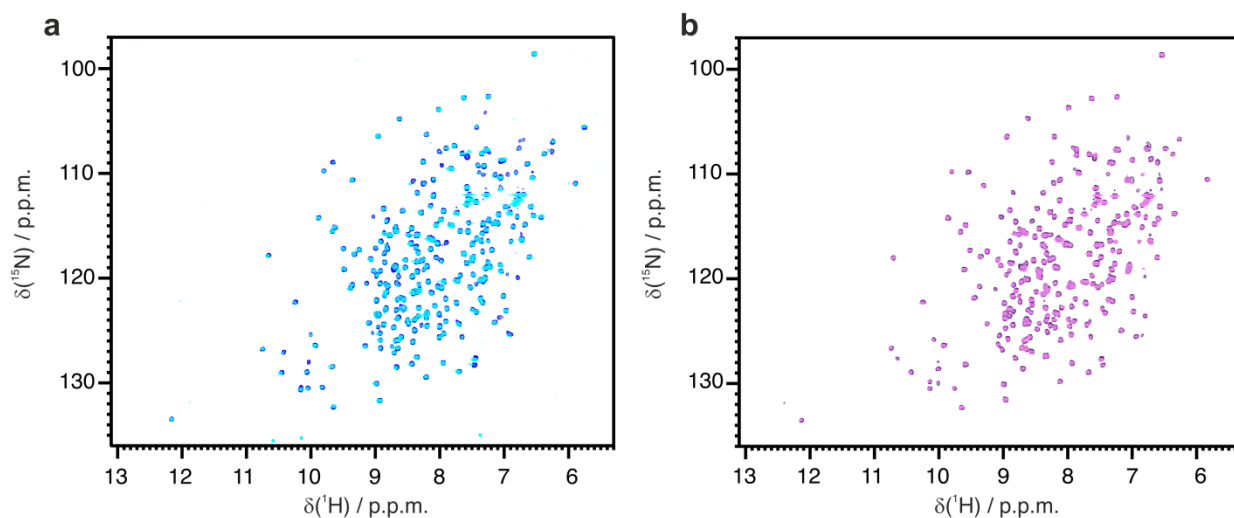


Figure S6. Overlay of the 2D ^1H - ^{15}N HSQC of hCAII. The effects of benzoate (a) and furosemide (b) in the in-flow based approach (blue and violet, respectively) are compared with those of a standard protein-ligand interactions experiment (cyan and pink, respectively) obtained at the same concentration of benzoate (5 mmol dm^{-3}) or furosemide (0.3 mmol dm^{-3}).

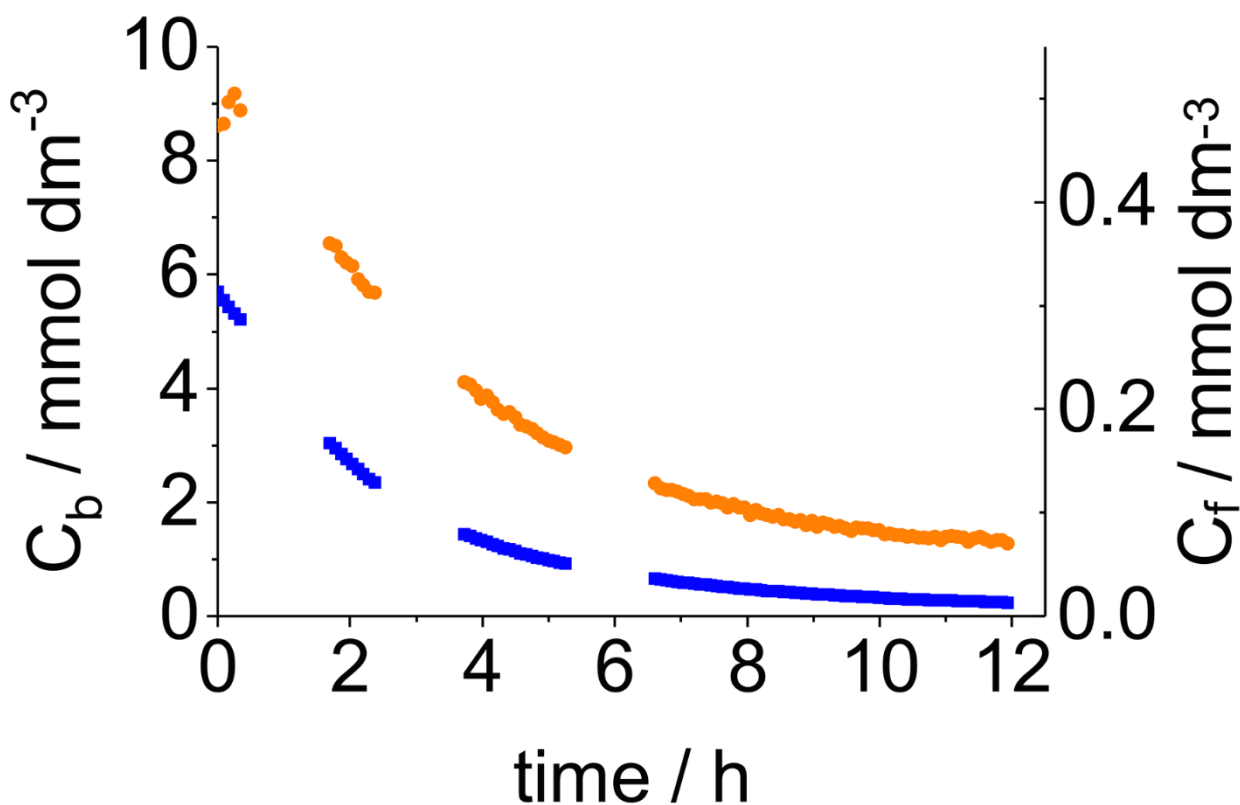


Figure S7. Concentration of benzoate and furosemide as a function of time while flowing a ligand-free buffer

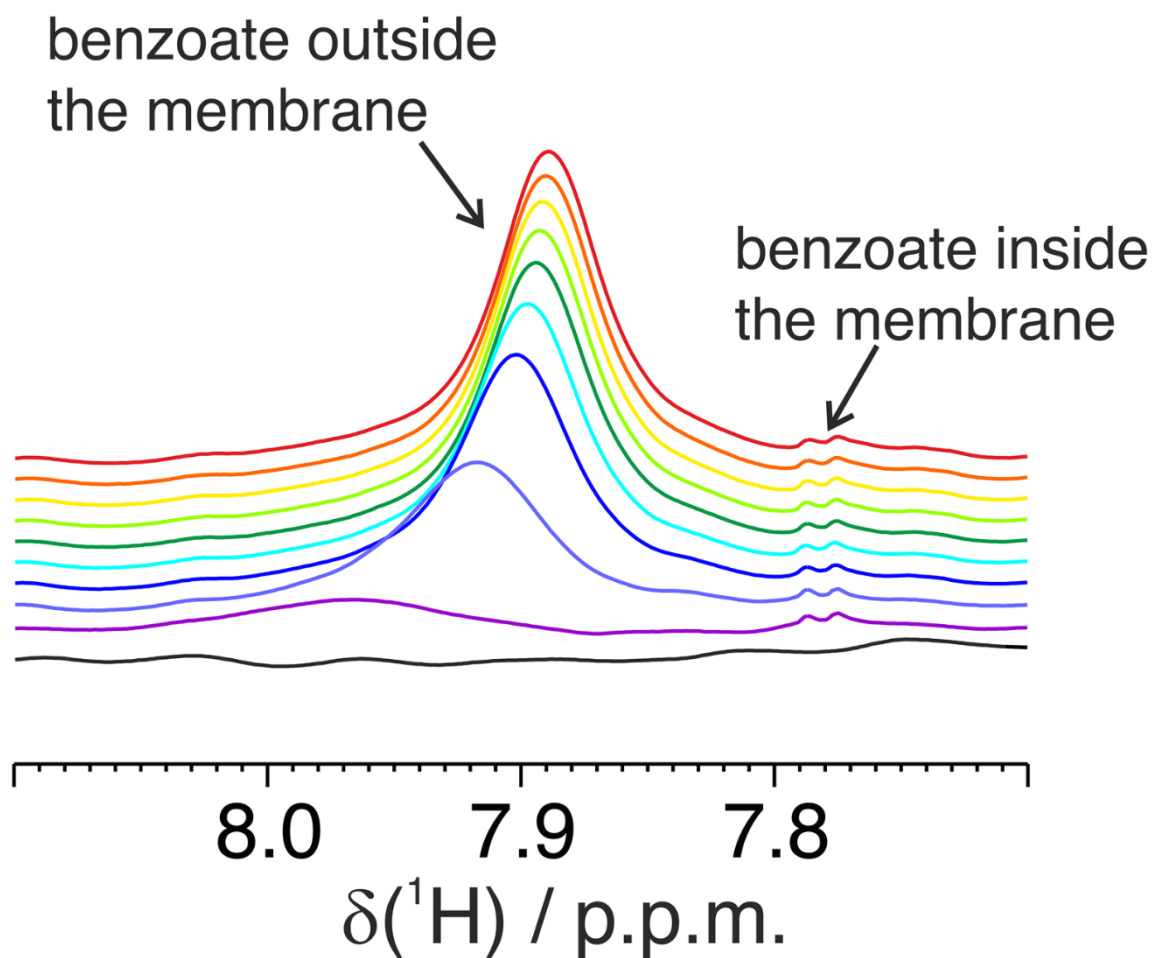


Figure S8. Stacked plot of the ortho hydrogen signal of benzoate in the ^1H -1D spectrum while flowing a benzoate solution at 20 mmol dm^{-3} : as the concentration increases the signal in fast exchange becomes narrower and less shifted with respect to the signal of free benzoate – bottom to top: 0, 1.36, 2.71, 4.07, 5.43, 6.79, 8.14, 9.50, 10.85, 12.21 hours

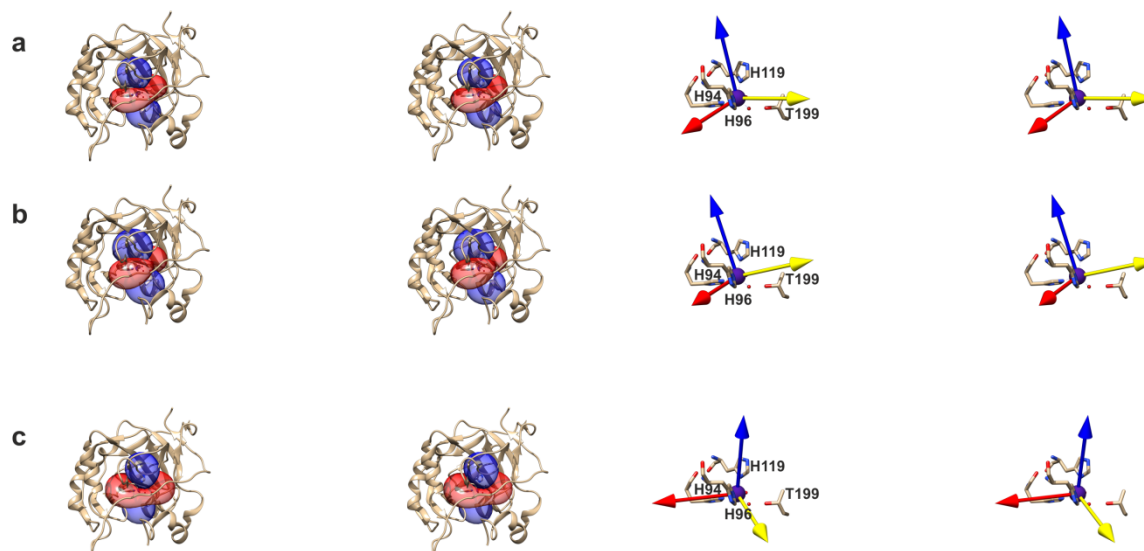


Figure S9. Stereo cross-eyed graphical representation of the magnetic susceptibility anisotropy tensor orientations and the PCS isosurfaces of 1 (blue) and -1 (red) ppm obtained for: uninhibited Co(II)-hCAII at pH 6.8 (A), cobalt(II)-hCAII bound to furosemide (B) (taken from reference (29)), Co(II)-hCAII bound to benzoate (C). The x, y and z axes (corresponding to the directions with the smallest, intermediate and largest magnetic susceptibility) are represented as red, yellow and blue arrows, respectively; the positive isosurfaces are in blue and the negative are in red.

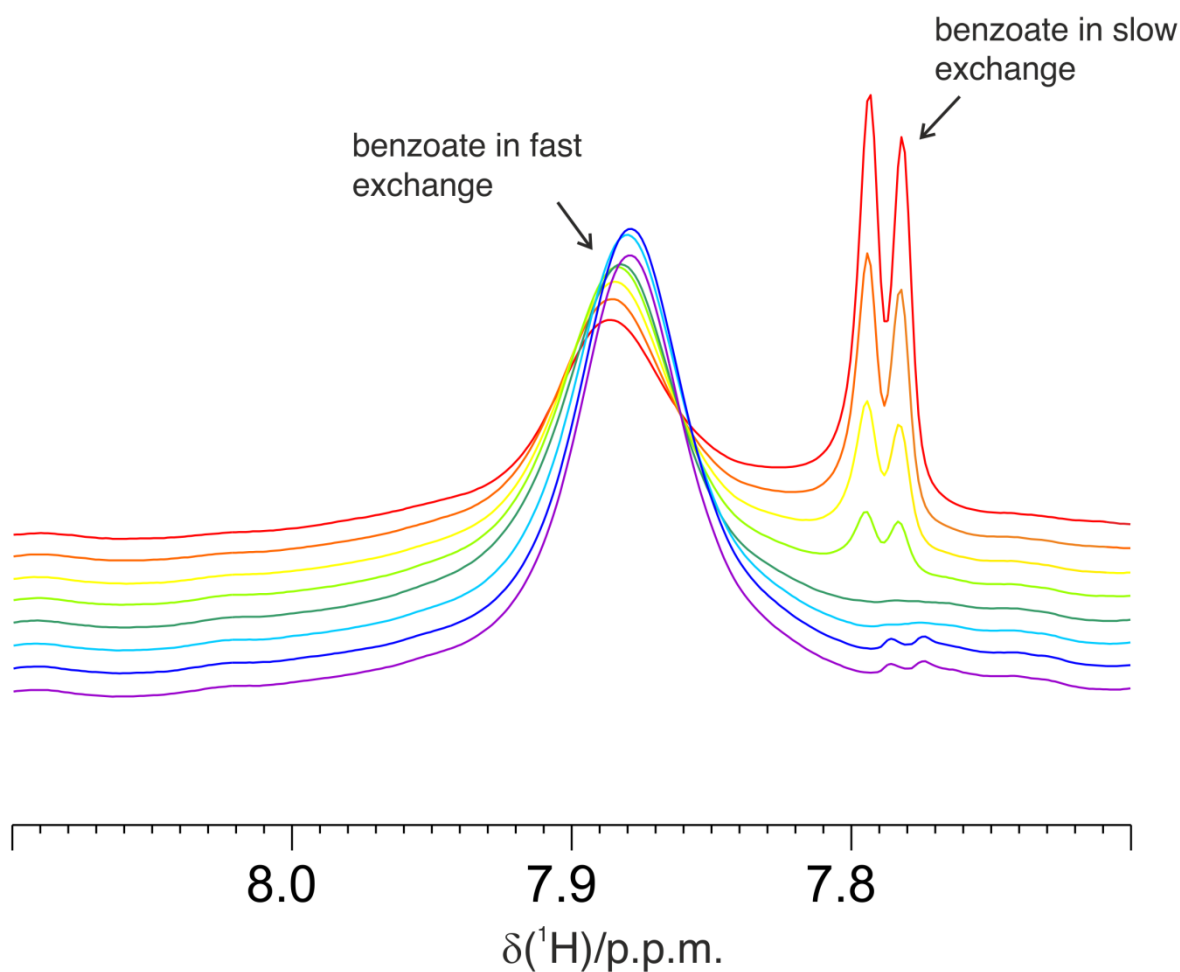


Figure S10. Stacked plot of the ortho hydrogen signal of benzoate in the ^1H -1D spectrum measured while flowing a furosemide solution at 2 mmol dm^{-3} when the InsightMR tube contains cobalt(II)-loaded hCAII. A diffusion front of furosemide is formed: the benzoate molecules that are behind the furosemide front and those that are beyond are in slow exchange with one another, so that those that are behind show the spectra typical of the free molecule, whereas those beyond still experience shift and broadening, as they remain in fast exchange with the paramagnetic enzyme molecules that are not yet reached by furosemide molecules.

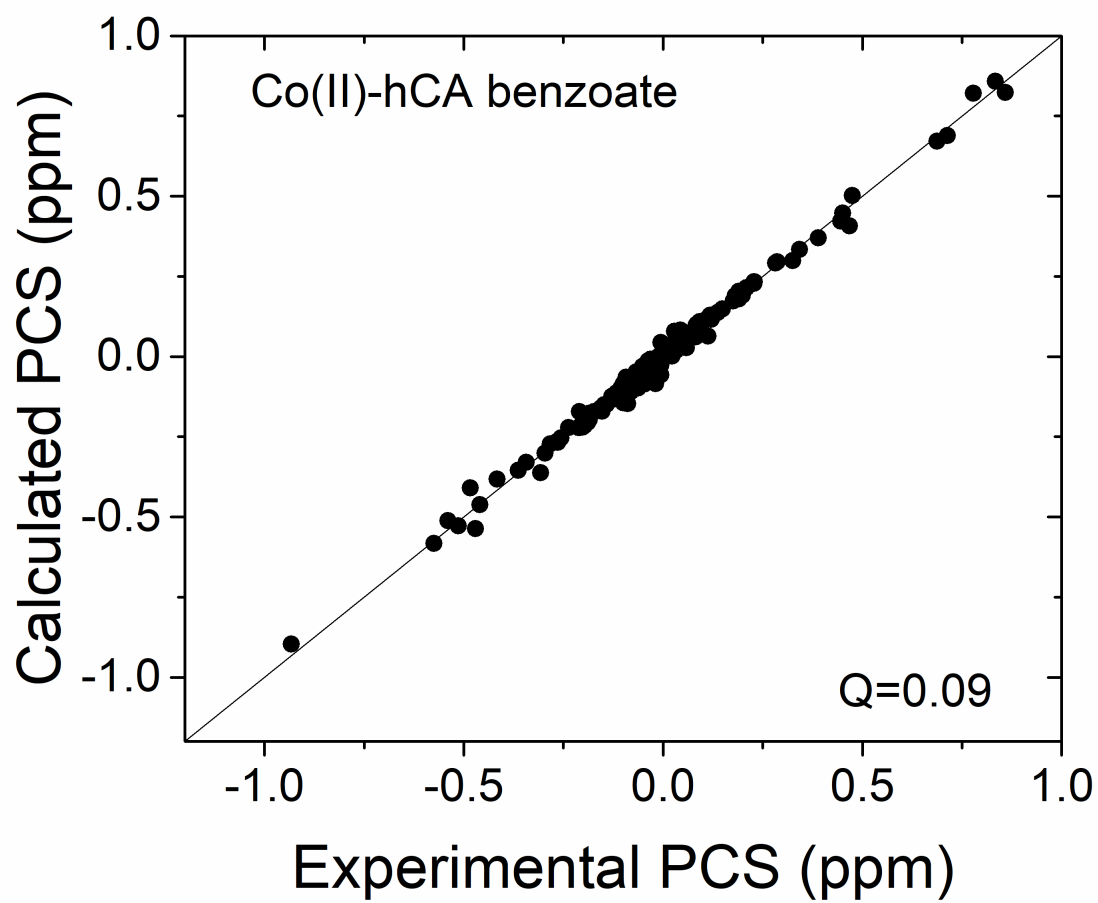


Figure S11. Correlation plot between experimental and calculated PCS collected for the Co(II)-substituted carbonic anhydrase in the presence of benzoate.

Table S1. Information on the custom CMA probes as provided by CMA microdialysis– a division of Harvard Bioscience, Solna, Sweden.

base model	CMA 20
outlet length	50 mm
inlet length	50 mm
shaft length	120 mm
membrane length	35 mm

Table S2. Tensor parameters calculated with the program FANTEN for the benzoate-hCAII adduct. The data have been compared with the parameters obtained in ref. (29) for hCo(II)-hCAII uninhibited and in the presence of furosemide. The tensor orientations are expressed as angles of the principal axes directions with the metal-ligand direction, defined as the direction from the metal to the coordinating atom in the ligand.

	Co(II)-hCAII pH 6.8	Co(II)-hCAII- furosemide pH 6.8	Co-hCAII benzoate
$\Delta\chi_{ax}$ (10^{-32} m^3)	2.81 ± 0.03	3.09 ± 0.02	3.25 ± 0.02
$\Delta\chi_{rh}$ (10^{-32} m^3)	-0.63 ± 0.02	-1.69 ± 0.02	-0.94 ± 0.03
<i>x</i> -His 96	$32 \pm 1^\circ$	$22 \pm 0.5^\circ$	$42 \pm 1.5^\circ$
<i>y</i> -His 94	$19 \pm 1^\circ$	$7 \pm 0.5^\circ$	$46 \pm 1.8^\circ$
<i>z</i> -His 119	$33 \pm 0.2^\circ$	$33 \pm 0.4^\circ$	$36 \pm 0.3^\circ$