# Supplementary Information

for

# Time-resolved Monitoring of Enzyme Activity with Ultrafast Hyper-CEST Spectroscopy

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Content

## 1. Estimating the specific activity

The variation of the enzyme concentration allows deriving a specific activity per mass of used enzyme. The obtained reaction rates, $v_0$, from the CEST analysis were plotted vs. used enzyme concentration and a linear fit with offset 0 was performed using the Origin Pro© fitting routine. Data is shown in Fig. S-1 and compared with results from $T_2$ analysis (explained in S2). The values of (75±3) µmol/g/min and (75±2) µmol/g/min, respectively, agree perfectly with data provided in the supplementary information in ref. [1].

Correlation between the reaction velocities from CEST analysis and from the relaxation analysis is shown in Fig. S-1(b) and confirms the high agreement of both evaluations.

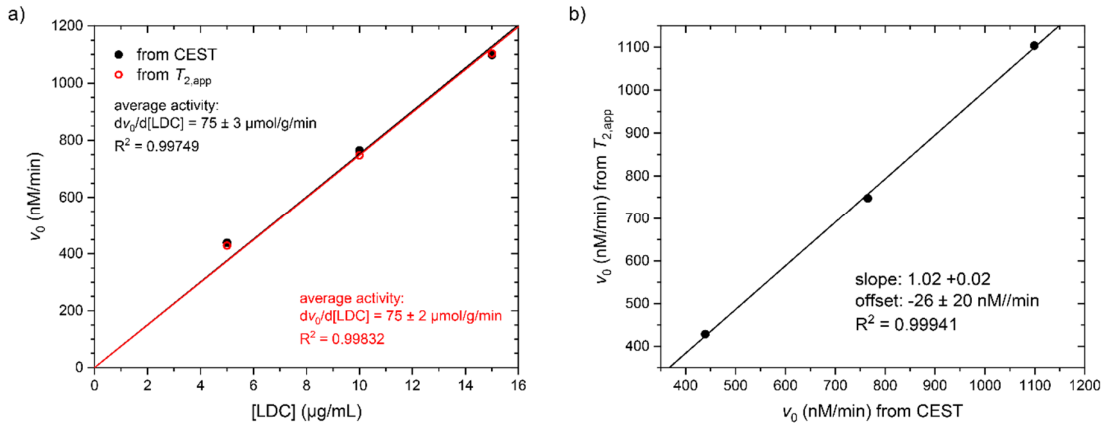


**Figure S-1:** Average activities derived from reaction rates at different enzyme concentrations. **(a)** Comparison of results from CEST analysis and from $T_2$-derived data. Reaction velocities from CEST are consistently higher and yield an activity of (75±3) µmol/g/min. However, $T_2$ analysis yields practically the same slope with (75±2) µmol/g/min. **(b)** The individual reaction rate values from both methods clearly correlate as any change in $v_0$ from CEST reflects in a change in $v_0$ from $T_2$ with a scaling factor of 102±2 %. The offset of (-26±20) nM/min is rather close to 0.

## 2. Estimating the apparent $T_2$ from UCS measurements

$T_2$ relaxation rates can be conveniently estimated with turbo spin-echo (TSE) sequences.[2] Since we used such a TSE sequence[a] to acquire the ultrafast Hyper-CEST spectra in our experiments, we readily have access to apparent $T_2$ information without conducting further experiments. The decaying spin-echo train of a single UCS reference scan is shown in Fig. S-2. We estimated the apparent $T_2$ decay time by fitting the envelope of this decay with a mono-exponential function. The resulting decay times for each time point and for all three LDC concentrations are plotted in Fig. 4 in the main article. Note from all UCS scans, we used only the reference scans without saturation pulse for the $T_2$ estimation, as they had a much higher signal-to-noise ratio. Note that the apparent $T_2$ estimation we performed might be biased by factors such as pulse imperfections of the refocusing pulses and by diffusion effects. For a more accurate $T_2$ measurement, repeated single spin-echo experiments with varying echo time are conventionally used.

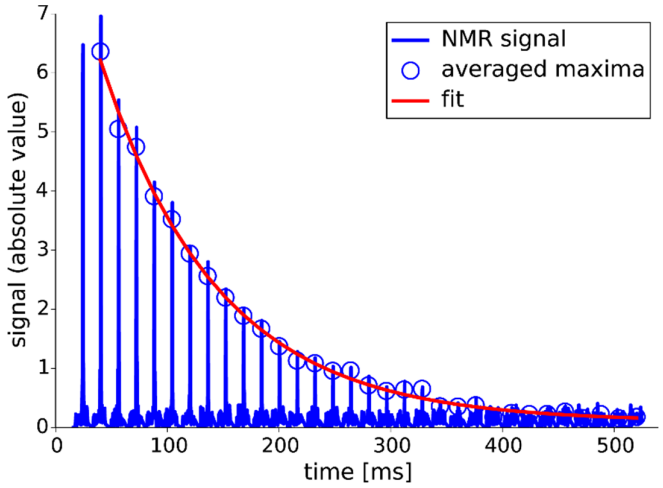


**Figure S-2:** Turbo spin-echo train of a UCS scan without saturation pulse, showing the apparent $T_2$ decay. The apparent $T_2$ decay time was extracted from the data by means of a mono-exponential fit (red line). The data points for the fit (circles) were obtained by averaging the two points with maximum signal for each echo.

[a] Note that our sequence also included gradients to encode spatial information. Each echo is hence actually a combination of a gradient-echo and a spin-echo. However, at the time of the echo formation, the gradients are balanced, such that the maximum echo intensity should be equal to the pure spin-echo intensity.

The extracted relaxation times were plotted against time and the second derivative of the smoothed curve was used to determine the kink representing the cut-off time. Derivatives for the three different enzyme concentrations are shown in Fig. S-3. To avoid excessive smoothing of the kink behavior, a smoothing window size of 3 was used. Remaining noise still shows up in the second derivatives but the characteristic transition into the plateau of the relaxation time can be identified by the largest oscillation between positive and negative values in Fig. S-3.

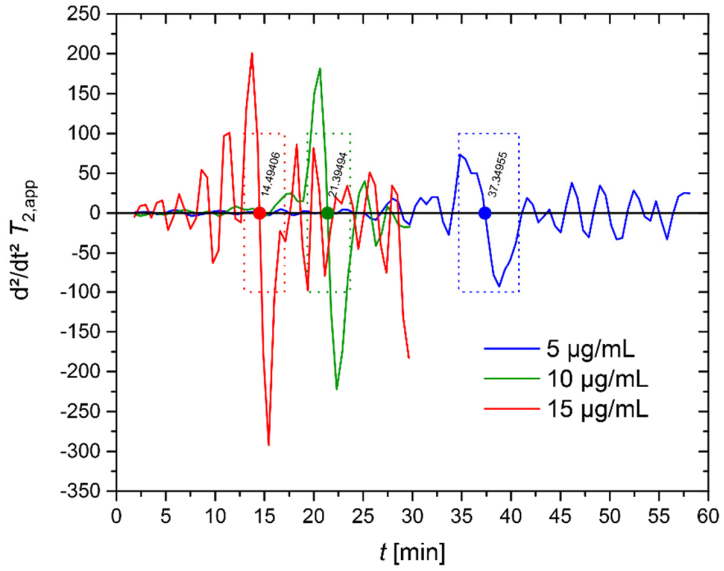


**Figure S-3:** Second derivative plots of the second derivation $d^2/dt^2\ T_{2,app}(t)$. The extracted cut-off times for the three enzyme concentrations are 37.35, 21.39, and 14.49 min.

## References


(1) Schnurr, M.; Sloniec-Myszk, J.; Döpfert, J.; Schröder, L.; Hennig, A. *Angew. Chem. Int. Ed.* **2015**, *54* (45), 13444.

(2) Neumann, D.; Blaimer, M.; Jakob, P. M.; Breuer, F. A. *Magn. Reson. Mater. Phys. Biol. Med.* **2014**, *27* (6), 567.