

Ultrafast ^{129}Xe Hyper-CEST

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Target Audience: Researchers interested in CEST, HyperCEST, and in hyperpolarized contrast agents and biomarkers.

Overview: ^{129}Xe biosensors have been demonstrated to provide sensitive probes of biological events. Very sensitive detection thresholds can be reached with the HyperCEST approach [1]. With hyperpolarized species it is often difficult to maintain a stable level of magnetization over consecutive experiments, which renders their detection at the trace level cumbersome, even when combined with chemical exchange saturation transfer (CEST). We report herein the use of ultra-fast Z-spectroscopy (UFZ) [2] as a powerful means to detect low concentrations of ^{129}Xe NMR-based sensors in a single shot. This experiment enables a multiplexed detection of several sensors, as well as the extraction of the exchange buildup rate constant in a single-shot fashion. Further extensions involve the one-shot acquisition of Z-spectra as a function of several different saturation times. The ultrafast method makes it possible to provide ultra-fast and high-throughput sampling of biomarker mixtures.

Method: Fig. 1 shows the pulse sequence and the Z-spectra obtained from a single shot (+ one reference scan) of a mixture of two water-soluble cryptophanes in H_2O : cryptophane-222-hexacarboxylate (compound **1**) and cryptophane-233-hexacarboxylate (compound **2**). Their xenon binding constants and in-out exchange rates at room temperature are significantly different ($K \sim 6800 \text{ M}^{-1}$, $k_{\text{ex}} \sim 3.2 \text{ s}^{-1}$ for **1**, $K \sim 2000 \text{ M}^{-1}$, $k_{\text{ex}} \sim 37 \text{ s}^{-1}$ for **2**). Concentrations are 30 and 90 μM , respectively. Both gradients are applied along Z. At the end of the saturation, a gradient crusher is applied along X. CW saturation at 7.1 μT during 2.4 seconds, in the presence of a $24 \text{ G}\cdot\text{cm}^{-1}$ gradient along Z ($S_{\text{on}}(z)$). For both spectra, the acquisition gradient G_{acq} was also $24 \text{ G}\cdot\text{cm}^{-1}$. Fig. 1c shows a normalized ^{129}Xe UFZ spectrum obtained by computing $(S_{\text{off}}(z) - S_{\text{on}}(z))/S_{\text{off}}(z)$. For Fig. 2 a mixture of 4 hexacarboxylated cryptophanes at 6 μM each is used.

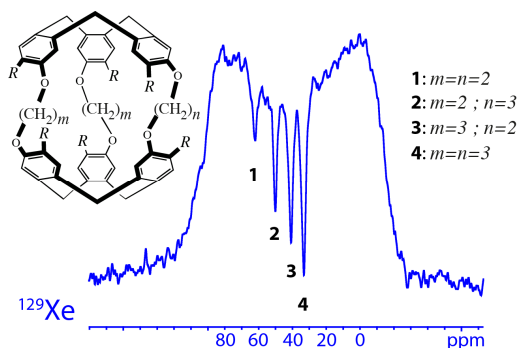


Fig. 2: ^{129}Xe single-shot UFZ spectrum recorded on a mixture of four hexacarboxylated cryptophanes. Conc: 6 μM each. Sat.: 7.5 s CW @ 2.4 μT .

short. One can therefore read out multiple echoes and co-add the results.

The normalized HyperCEST UFZ spectrum $((S_{\text{on}}(z) - S_{\text{off}}(z))/S_{\text{off}}(z))$, shown in Figure 1c, enables a clear separation of the signals of xenon caged in **1** and in **2**. From this spectrum, and from the experiments performed on other cryptophane mixtures (Fig. 2), we can deduce that ^{129}Xe signals with a chemical shift splitting of ~ 4 ppm (550 Hz) can be separated, a value comparable to the results of other groups working with conventional HyperCEST.

Discussion: Xenon in several cryptophanes can be simultaneously detected with UFZ, provided that the resonance frequencies differ by more than ca. 500 Hz. Further improvements allow one to acquire full UFZ spectra at different saturation times in one shot (Fig. 3).

Conclusion: We show here that the recently published UFZ-spectroscopy method [2] can be of high value for hyperpolarized samples, as it enables one to avoid complications from fluctuations in the magnetization, and to detect low amounts of xenon in exchange in HyperCEST experiments. Further speedup of such methodology could be achieved by combining UFZ with the parallel measurement of several samples. These methods could become very useful for high throughput measurements of samples.

References: 1. Schröder, L.; Lowery, T. J.; Hilty, C.; Wemmer, D. E.; Pines, A. *Science* **2006**, *314*, 446-449.
2. Xu, X.; Lee, J.-S.; Jerschow, A. *Angew. Chem. Int. Ed.* **2013**, *52*, 8281-8284.

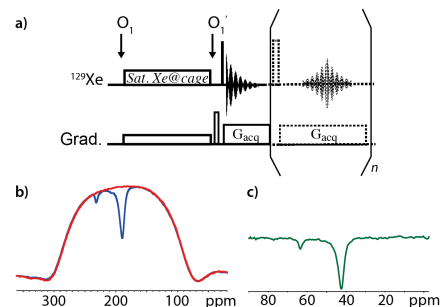


Fig. 1: (a) ^{129}Xe UFZ pulse sequence. (b) Raw data, (c) HyperCEST asymmetry curve from one-shot measurement of a mixture of **1** and **2**.

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Results: The original sequence is applied by first placing the saturation offset frequency in the middle of the high field region corresponding to caged xenon (around 50 ppm), while for detection, the offset is placed at the resonance frequency of dissolved xenon (200 ppm). This enables the use of a much lower saturation gradient value G_{sat} , as one needs now to cover a spectral window of only approximately 30 ppm (instead of 200 ppm). Also, since the signal is recorded in the presence of a gradient, the signal lifetime is

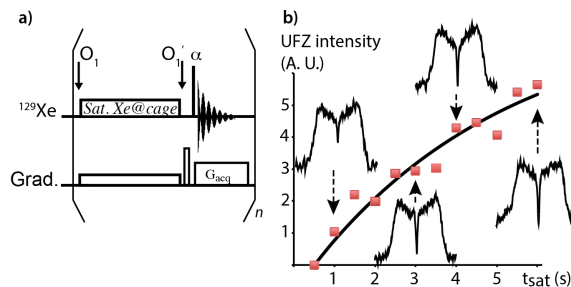


Fig. 3: One-shot UFZ spectroscopy with different saturation times.