Maintaining Hyper-CEST performance of a dye-labelled cryptophane cage

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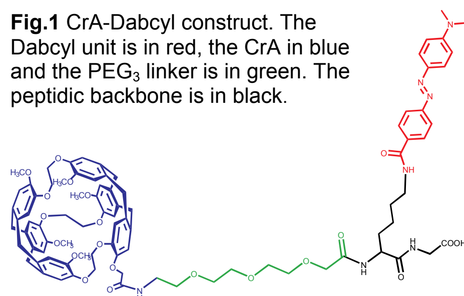
Introduction: The xenon biosensor approach aims to establish a new type of functionalized contrast agent that combines high specificity of the ^{129}Xe chemical shift range with high sensitivity of hyperpolarized nuclei [1]. An essential part of such sensors is a host structure for the noble gas. Various cryptophane cages have been studied in this context since they exhibit a high binding constant for xenon [2]. This is a rather new field of research and little is known so far about the behaviour of these hydrophobic cages in biologic environment, especially their interactions with cells. Fluorophore-labelled cryptophanes have been proposed to study cell uptake and accumulation on a microscopic scale [3]. Such conjugates offer the ability to understand biochemical behaviour of cryptophanes and could be used as bi-functional contrast agent for MRI and fluorescence microscopy. Though hyperpolarized nuclei are part of the biosensor concept additional sensitivity enhancement techniques, such as Hyper-CEST, were developed to extend the method to nanomolar detection limits [4,5]. Based on chemical exchange of the temporarily caged xenon, this method is sensitive to the immediate environment of the cage cavity and coupling of additional functional units could influence the signal amplification. Systematic understanding of the cryptophane behaviour by pairing fluorescence data with NMR studies on cell cultures should therefore use conjugates that maintain as much of the signal amplification as possible. So far, no comparison of the Hyper-CEST performance of an unmodified and a functionalized cage has addressed this aspect. Here, we investigate the possibility to maintain full efficiency of CEST effect of a cryptophane-A cage after coupling to a fluorescent dye.

Methods: A PEGylated fluorigenic building block was synthesized via Fmoc-solid phase peptide synthesis. Lysine bearing a Dabcyl-group on the ε-amino terminus was coupled on resin with NH-PEG$_3$-COOH, using COMU as a coupling reagent [6]. The building block was then cleaved from the resin and purified via HPLC-UV. Cryptophane-A monoacid (CrA) was coupled in solution with the PEGylated fluorogenic building block using HATU (1 eq.) and DIEA (2 eq.). CrA-Dabcyl (Fig.1), was then purified via HPLC-UV and the mass was confirmed using MALDI-TOF mass spectrometry. The linker length was chosen to leave enough flexibility between the xenon host and the fluorophore. CrA is highly hydrophobic and requires organic solvents. Although this problem is addressed for CrA-Dabcyl by introducing the PEG$_3$ group, comparison of the Hyper-CEST performance should be performed in the same solvent. Hence, solutions with 90% DMSO and 10% H_2O were prepared to study CrA and CrA-Dabcyl (both at ca. 336 μM concentration). H_2O was added to have line widths that are somewhat more similar to future applications in aqueous solutions. ^{129}Xe NMR spectra were recorded on a 9.4 T spectrometer. Hyperpolarized Xe was produced with a custom designed hyperpolarizer yielding ca. 16% spin-polarization for a mixture of 5% Xe, 10% N_2 and 85 % He. The gas was bubbled into a 10 mm test tube for 20 s followed by a 8 s wait time for bubbles to disappear. For characterizing the saturation frequency response of both constructs, z-spectra were recorded with a 3 s saturation pulse (B_1 = 3.8 μT) that was swept ~159-174 ppm upfield from the detected Xe solution peak. Dynamics of the saturation transfer were studied in an experiment with increasing saturation delay (0-3 s, B_1 = 3.8 μT; using a variable delay before saturation to compensate for relaxation effects). All spectra were integrated around the solution peak and the resulting signal intensity normalized to an average signal with no saturation. Z-spectra were fit to a Lorentzian profile (assuming the weak saturation approximation [7]) and Hyper-CEST dynamics to an exponential decay.

Results and discussion: Fig. 2 a) shows the z-spectra of both constructs. Both profiles follow a Lorentzian shape with only minor differences in the width of the CEST responses: 4.5±0.2 ppm for CrA-Dabcyl and 5.3±0.3ppm for CrA. Although this might indicate that Xe exchange is slightly faster for the unmodified cage, the exponential signal decay upon increasing CEST saturation time, Fig. 2 b), does not show systematic differences. The time constants for the decay under these specific saturation parameters are 425±19 ms and 451±19 ms for CrA-Dabcyl and CrA, respectively. Altogether, these results show that fluorophore-labelling of CrA did not hamper Hyper-CEST performance in this case. Although the mass of CrA-Dabcyl (MW = 1563.67) is ca. 67% higher than CrA (MW = 938.35), molecular dynamics and Xe exchange parameters do not change enough to decrease the signal amplification. The PEG linker obviously also provides enough flexibility to avoid potential blocking of the cavity entrances due to the fluorophore. Hence, fluorescence studies on biosensor accumulation in cells can be paired with highly sensitive NMR experiments to detect such constructs at low concentrations.

Fig.1 CrA-Dabcyl construct. The Dabcyl unit is in red, the CrA in blue and the PEG$_3$ linker is in green. The peptidic backbone is in black.



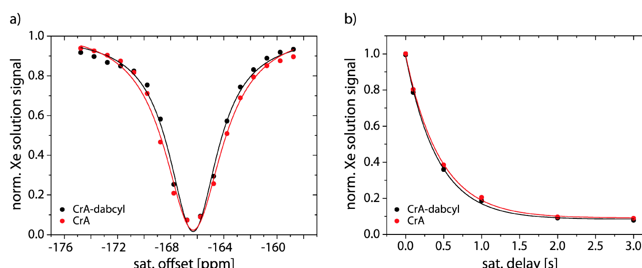


Fig.2 a) The Xe solution peak was integrated after saturating at a range of different frequencies around the Xe in CrA and CrA-Dabcyl resonance.
b) The Xe solution peak was monitored while varying the saturation pulse length from 0.1 to 3 seconds for both constructs.

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