HYPER-CEST SIGNATURES OF FUNCTIONALIZED ^{129}XE FOR SENSING BIOMEMBRANE COMPOSITION

Leif Schröder[1], Jagoda Sloniec[2], Christopher Witte[1], Ute Resch-Genger[2], and Andreas Hennig[2]

[1]*Leibniz-Institut für Molekulare Pharmakologie (FMP), Berlin, Germany,* [2]*Bundesanstalt für Materialforschung und -prüfung (BAM), Berlin, Germany*

Introduction: Hyperpolarized xenon is a powerful NMR/MRI probe because it provides both high sensitivity and specificity. Its solubility in water and its huge chemical shift range motivated the design of xenon biosensors [1] and the Hyper-CEST technique [2]. Such sensors comprise a molecular cage (often a cryptophane derivative) to reversibly trap the hyperpolarized atoms and confer a chemical shift signature onto the noble gas and a targeting unit linked to the cage. They are often characterized by a certain hydrophobicity and show pronounced interactions with lipid membranes [3,4]. Although this can cause unwanted unspecific interaction with cellular membranes when the design aims for a targeted binding [4], a systematic approach may provide a method to decipher the membrane composition. Previous studies demonstrated a significant change in chemical shift for caged xenon upon interactions with bilayers of lipid emulsions, an effect much less pronounced for 'naked' xenon [3]. Hence, cryptophane cages appear to enhance the sensitivity of Xe to its molecular environment. Here, we present results of a systematic study with a functionalized cryptophane-A derivative that has been exposed to different well defined biomembrane models and that shows significantly different NMR signatures depending on the membrane composition. Fluorescence resonance energy transfer (FRET) from a suitable membrane-bound donor to a dye attached to the molecular cage was used as an independent method to verify partitioning of the cage construct into the membrane rather than translocation into the liposomal inner volume. A combination of optical and NMR data therefore provides access to interactions of xenon biosensors with biological membranes.

Methods: Cryptophane-A monoacid was coupled with commercially available activated esters of fluorescent dyes. DY682 was chosen because of its water solubility and high quantum yield (conjugate CA-DY682, Fig. 1). This was paired with nile red as a FRET donor, an established hydrophobic probe for lipid membranes [5]. It efficiently partitions into the membrane, thereby showing a high fluorescence in the spectral region of the CA-DY682 absorption. This enabled FRET to determine the partitioning coefficient of CA-DY682 into three different model membranes: uniformly sized, large unilamellar vesicles composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), and egg yolk phosphatidylcholine (EYPC) The vesicles were prepared by the extrusion method; their average diameter of 125-145 nm was determined by dynamic light scattering. NMR studies were performed at 9.4 T with hyperpolarized ^{129}Xe (ca. 15% spin polarization), bubbled into aqueous solutions containing HEPES buffer, ca. 1.6 mM lipids and 4.4 µM CA-DY682. The chemical shift of Xe bound to the cage was identified using the Hyper-CEST method [2], sweeping a 8 s continuous wave pulse ($B_1 \approx 1.2$ µT) over the spectral range ~52-82 ppm relative to the NMR signal of Xe in gas phase on top of the solution and detecting the xenon solution peak at ca. 190 ppm. This signal intensity is then plotted vs. saturation frequency as a z-spectrum.

Results and discussion: Exposing the cryptophane conjugate to nile red-labelled vesicles, FRET revealed significant accumulation of CA-DY682 in the biomembranes which was characterized by similar partitioning coefficients of $K_p = 5\text{-}7 \cdot 10^3$ (SD: $\pm 3 \cdot 10^3$) for the different phospholipids (K_p defined as the ratio of the dye concentration in the lipid phase, n_{lipid}/V_{lipid}, vs. concentration in the aqueous phase, n_{aq}/V_{aq}). However, Hyper-CEST signatures of CA-DY682 differ significantly, thus allowing differentiating between the membrane compositions. Fig. 2 shows comparison of z-spectra from free CA-DY682, POPC, and DPPC as an example. The differences in CEST response intensity can be assigned to different xenon exchange dynamics which might be a consequence of different membrane fluidity. DPPC yields a much less intense CEST peak for membrane-bound CA-DY682. This is presumably due to slower Xe exchange dynamics in the gel phase conditions of DPPC at 295 K compared to the more fluid POPC. Hence, Hyper-CEST yields complementary information to results reported in a recent study where Xe NMR signals were used to characterize different cell lines [6]: whereas the cell-related resonance of uncaged Xe was interpreted as exclusive intracellular signal after membrane translocation, the cryptophane conjugate could reveal cell-identity based on the membrane properties. Altogether, this could find applications, e.g., in discriminating cancer cells from normal cells since membrane fluidity appears to be significantly correlated with malignancy [7].

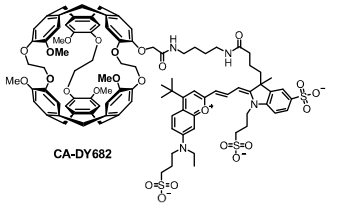

Fig. 1: Cryptophane A-dye conjugate for NMR of functionalized ^{129}Xe

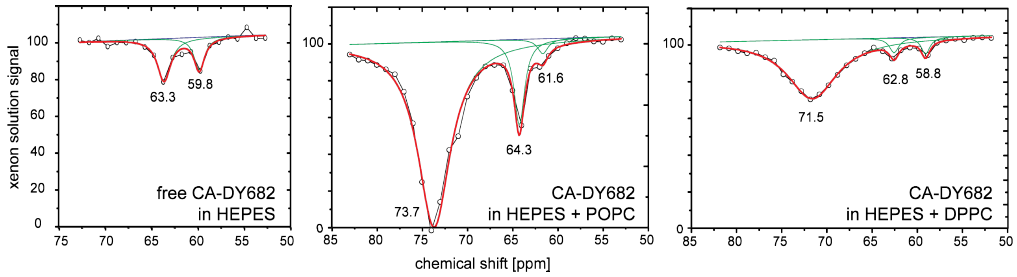

Fig. 2: CA-DY682 in HEPES buffer shows two CEST peaks in a z-spectrum (presumably due to the presence of monomers and dimers). Addition of POPC shifts peaks and yields an additional strong CEST response. DPPC causes a much weaker CEST response. (Raw data points (○) fitted to combination of 2-3 lorentzian saturation profiles, cumulative fit in red; all data recorded at 295 K.)

References

[1] Spence et al., *Proc. Natl. Acad. Sci. USA*. (2001) **98**: 10654-7
[2] Schröder et al., *Science* (2006) **314**: 446-9
[3] Meldrum et al., *J. Magn. Reson.* (2010) **205**: 242-6
[4] Boutin et al., *Bioorg. Med. Chem.* (2011) **19**:4135-43
[5] Kucherak et al., *J. Am. Chem. Soc.* (2010) **132**: 4907-16
[6] Boutin et al. *NMR Biomed.* (2011) DOI: 10.1002/nbm.1686
[7] Shinitzky, *Biochim. Biophys. Acta* (1984) **738**:251-61