Tuning histidine proton exchange rates for CEST contrast through peptide design

Nikita Oskolkov[1], Xiaolei Song[1], Kannie W.Y. Chan[1], Michael Giano[2], Jeff W.M. Bulte[1,3], Peter C.M. van Zijl[1,3], Joel Schneider[2], and Michael T. McMahon[1,3]

[1]*Russell H. Morgan Department of Radiology and Radiological Science, The Johns Hopkins University School of Medicine, Baltimore, MD, United States,* [2]*National Cancer Institute, National Cancer Institute, Frederick, MD, United States,* [3]*F.M. Kirby Research Center for Functional Brain Imaging, Kennedy Krieger Institute, Baltimore, MD, United States*

Target audience: Researchers who are interested in tissue engineering and molecular imaging using diamagnetic CEST contrast agents.

Purpose: One of the main problems facing CEST contrast agents is their relative low sensitivity, with reductions resulting from direct saturation and tissue magnetization transfer contrast (MTC). Previous studies have shown that increasing the chemical shift provided the exchange rate is properly tuned, can enhance this sensitivity. So far, the chemical shifts of exchangeable imino protons have been the largest (6 ppm with respect to water or higher) reported for diamagnetic CEST agents, such as thymidine, uridine, and polyuridilic acid [1, 2]. A recent study demonstrated that H**δ**1 and H**ε**2 protons of His57 in the catalytic triad of bovine chymotrypsinogen-A (bCT-A) **(Fig.1)** demonstrated strong CEST contrast with chemical shifts between 5-15 ppm downfield from water [3].

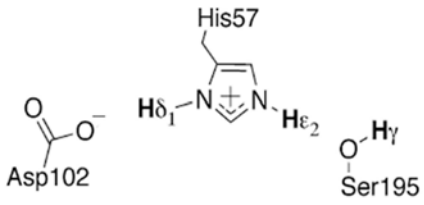

Fig.1 Catalytic triad of bCT-A

In this study we describe a new strategy for designing peptide CEST contrast agents based around histidine protons, which have strong electrostatic interactions-hydrogen bonding with hydroxyl or carboxyl functional groups of surrounding amino acids in order to form a β-sheets secondary structure with the tendency to self-assemble into hydrogels. These designed peptides can produce MRI contrast and could be used as a hydrogel scaffolds for cell therapy because of their unique properties, swelling, mechanical properties, biodegradation, and diffusion.

Methods: Peptide synthesis was performed in a microwave-assisted peptide synthesizer Liberty1 (CEM, USA) using N-fluorenylmethyloxycarbonyl (Fmoc) chemistry with O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU) as activator. The crude peptides **(Table 1)** were purified using HPLC on C12 reverse-phase column. The molecular weight was confirmed by MALDI-TOF mass-spectrometry. CEST images were acquired from -12 to 12 ppm (step=0.2ppm) for the synthesized peptide hydrogels in saline buffer, pH=7.2-7.4, at a concentration of 20 mg/ml (2% wt/vol). CEST experiments were performed at 37 C and 11.7T Bruker horizontal bore scanner using and a saturation pulse with B1=3.6 and 14.7 μT, and T_{sat}=3 s followed by a RARE readout (TR/TE=6000/19 ms). Data processing was performed using custom-written scripts in Matlab. Z-spectra were calculated from the mean of ROI for each sample after B_0 correction. $MTR_{asym}=(S_{-\Delta\omega}-S_{+\Delta\omega})/S_{-\Delta\omega}$ was computed at different offsets Δω. Transmission Electron Microscopy (TEM) was performed with negatively stained samples by 2% (wt/vol) aqueous uranyl acetate.

Table 1. The list of compounds used in this work.

Name	Sequence
N23	VDVSVDVSVDVSVpPTVHVHVHVHV-NH_2
N24	VDSVDSVDSVpPTVHVHVHV-NH_2
N25	VDDVDDVDDVpPTVHVHVHV-NH_2
N26	VSSVSSVSSVpPTVHVHVHV-NH_2
N27	VDVDVDVpPTVHVHVHV-NH_2
N28	VDVDVDVpPTHVHVHV-NH_2

Results and Discussion: Our approach involves incorporating multiple residues within the triad (histidine, serine, and aspartate), a "Val-Pro-Pro-Thr" tetrapeptide in the middle, and using valine every second residue to create a sequence with a tendency to self-assemble into hydrogels similar to the MAX1 and MAX8 peptides [4, 5]. Based on these elements, it should be feasible to create short peptides with strong hydrogen bonding inside this triad to influence the chemical shift and exchange rates of the protons based on the formation of two β-sheets connected by a type II' turn. This structure results in salt- and pH-dependent folding.

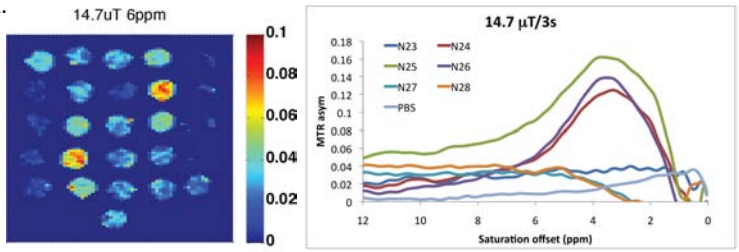

Fig. 2 MTR asymmetry of six designed peptides at pH7.3 at 14.7 μT

MR images of prepared hydrogels showed that the chemical shift difference between exchangeable protons on the designed peptides and bulk water is in the range of 3-6 ppm with a broad shoulder toward +12 ppm under basic conditions **(Fig.2)**, which switches at acidic pH to no contrast. This shoulder presumably is associated with the heterocyclic histidine protons, with the hydrogen bonding adjusted through pH. The CEST contrast is shifted further than 5 ppm and dependent on the peptide structure. Based on the power dependence of this contrast, the exchange rate of these protons is still too fast for proper detection for the peptides synthesized. Detailed structural analysis demonstrates the supermolecular structure of hydrogels composed of non-covalent cross-linked fibrils that are monodisperse in diameter with size ~6 nm for N24 and ~9 nm for N28 and have the tendency to form polydisperse network demonstrated by TEM analysis **(Fig.3)**

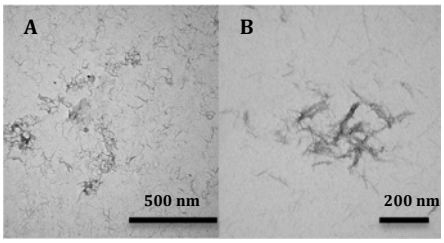

Fig.3. TEM micrographs of N24 **(A)** and N28 **(B)** hydrogels showing the fibrils formation

Conclusion: The peptides prepared form unique three-dimensional structures, which should be suitable for immunoprotecting cells. In addition, they generate sufficient CEST contrast for detection, making them a promising biodegradable contrast material for monitoring cell therapy.

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References: **1.** Ward, K.M., A.H. Aletras, and R.S. Balaban, *A new class of contrast agents for MRI based on proton chemical exchange dependent saturation transfer (CEST).* J Magn Reson, 2000. 143(1): p. 79-87. **2.** Snoussi, K., et al., *Sensitive CEST agents based on nucleic acid imino proton exchange: detection of poly(rU) and of a dendrimer-poly(rU) model for nucleic acid delivery and pharmacology.* Magn Reson Med, 2003. 49(6): p. 998-1005. **3.** Lauzon, C.B., P. van Zijl, and J.T. Stivers, *Using the water signal to detect invisible exchanging protons in the catalytic triad of a serine protease.* J Biomol NMR, 2011. 50(4): p. 299-314. **4.** Schneider, J.P., et al., *Responsive hydrogels from the intramolecular folding and self-assembly of a designed peptide.* J Am Chem Soc, 2002. 124(50): p. 15030-7. **5.** Haines-Butterick, L., et al., *Controlling hydrogelation kinetics by peptide design for three-dimensional encapsulation and injectable delivery of cells.* Proc Natl Acad Sci U S A, 2007. 104(19): p. 7791-6.