Developing a nanobiosensor for non-invasive visualization of the AKT signaling pathways

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Target Audience: Researchers and clinicians who are interested in molecular and cellular MRI, particularly in non-invasive monitoring of gene expression, cell therapy, and transplantation, as well as pre-clinical drug screening with advanced MRI-based techniques.

Purpose: Elucidating signaling pathways in cancer is critical for developing new therapeutic targets. AKT (also known as Protein Kinase B (PKB)) is a serine/threonine-specific protein kinase, which is central to various cellular processes, including metabolism, cell proliferation, and cell death. Therefore, it is not surprising that it plays a critical role in cancer progression in terms of cell growth and response to chemotherapy[1]. Thus, developing an MRI-based biosensor specifically for the detection of AKT activity could be a game changer in the evaluation of new therapeutics in a pre-clinical setting, which may be translated directly to the clinic. Our overall goal is to develop a non-invasive platform to visualize the response to protein kinase inhibitors in brain cancer. For that end, we started with a screening for an ideal peptide that provides high CEST contrast, which is changed only upon phosphorylation by AKT.

Methods: Peptide synthesis was performed on a microwave-assisted peptide synthesizer Liberty (CEM, USA) as described before[2] using N-fluorenylmethyloxycarbonyl (Fmoc) chemistry with O-benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU) as activator. The crude peptide was purified using HPLC on a C12 reverse-phase column (>90% pure), and the resulted peptide mass was validated using MALDI-TOF mass-spectrometer. CEST experiments were performed on an 11.7T Bruker Avance system as previously described[3]. A modified RARE (TR/TE=6000/9.4 ms), including a magnetization transfer module (B_1=2.4-4.7 T/4000 ms), was used to acquire CEST-weighted images. The absolute water resonance frequency shift was measured using a modified WASSR method, with the same parameters as in CEST imaging, except for TR=1.5 sec and a saturation pulse of B_1=0.5 T/500 ms, which was used for B_0 correction for each voxel using MatLab. MTR asymmetry (MTR_{asym})= (S_- – S_+)/S_0 was computed at different offsets,   .

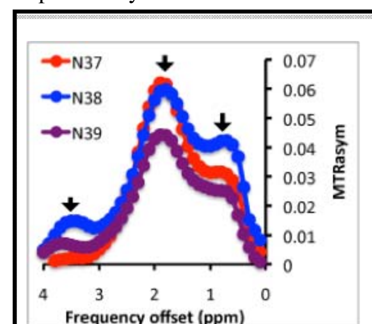

Fig. 1 MTR_{asym} of three different peptides. Arrows point to the peaks corresponding to hydroxyl (0.9 ppm), guanidyl (1.8 ppm), and amide (3.6 ppm) protons.

Results:
Initially we synthesized a set of 3 peptides (Table 1) that are corresponding to known AKT phosphorylation sequences. All three peptides have the conserved phosphorylation sequence RXRXX(S/T), whereas R is arginine, S is serine, T is threonine, and X is any amino acid. As can be seen in **Fig. 1**, each peptide displayed a specific MTR_{asym} profile with different contrast at the hydroxyl (0.9 ppm), guanidyl (1.8 ppm) and amide (3.6 ppm) protons.

To investigate how phosphorylation affects the MTR_{asym} of the biosensor (i.e., CEST contrast), we chose peptide N37 for further studies. We synthesized an identical peptide with the exception that the threonine residue was chemically phosphorylated. **Fig. 2.** demonstrates the feasibility of using CEST MRI to distinguish between a peptide known to be a specific target of AKT (RPRAATF), from a chemically phosphorylated peptide (RPRAApTF where pT=phophso-Threonine). These findings support the feasibility of constructing a biosensor for AKT based on chemical exchange.

Table 1.

N37	***RPRAATF****	***Arginine-Proline-Arginine-Alanine-Alanine-Threonine-Phenylalanine***
N38	***RPRTSSF***	***Arginine-Proline-Argginge-Threonine-Serine-Serine-Phenylalanine***
N39	***RSRKESY***	***Arginine-Serine-Arginine-Lysine-Glutamate-Serine-Tyrosine***

****Conserved amino acids are in blue. Phosphorylation site is underlined.***

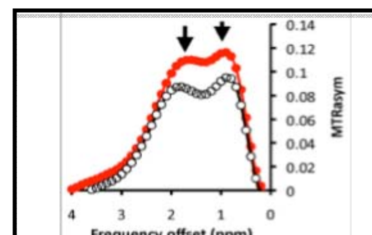

Fig. 2: CEST contrast of 2 mM RPRAATF (●) and RPRAApTF (○); arrows point to the reduction in the contrast at 1.8 ppm and 0.9 ppm due to the presence of a phosphorus group.

Discussion: In this study we investigated three peptides known to be specific substrates for the enzyme AKT. All three peptides gave high CEST contrast at 1.8 ppm, corresponding to the exchangable guanidyl proton (on the arginine side chain). Only peptide N39 provided contrast at 3.6 ppm, most likely due to the amide proton between the arginine and lysine.

The feasiblity of a genetically encoded biosensor for protein kinase A (PKA) has previously been demonstrated[4]. Such biosensors and reporters, using synthetic biology tools, can be integrated into the genome of bacteria as well as mammalian cells and subsequently be detected using CEST-MRI[2]. In this study we have chosen to investigate AKT due to its key role in cancer development. By engineering a genetically encoded biosensor, we aim to develop a new platform for pre-clinical sceening of anti-cancer drugs.

***Conclusion*:** We have tested three peptides as substrates of the enzyme AKT. All peptides generated CEST contrast. Adding phosphorus to the threonine residue resulted in a reduction of the contrast. These finidngs are the first step in optimizing a peptide that will be the most senstive and specific substrate for AKT.

***References*:**

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