Heteroditopic binding of MR contrast agents for increased relaxivity

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Introduction: Fibrin-specific peptides conjugated to multiple Gd-chelates, e.g. EP-2104R and EP-1242, have shown efficacy in MR detection of thrombus.[1,2] Performance of these probes could be improved further if the fibrin-bound relaxivity (r_1) of the probe was increased while minimizing the relaxivity of the unbound probe. This would enable higher thrombus:background contrast at lower chemical doses. The relaxivity of these compounds is already fairly high because of the 4 Gd-chelates per molecule and an increase in the molecular tumbling rate upon binding to fibrin.[3,4] However the gain in relaxivity upon binding is limited by internal motion along the chemical bonds between the rigidly bound peptide pharmacophore and the gadolinium chelate, resulting in increased flexibility at the gadolinium ion.[4] For agents with multiple chelates, it is a challenge to conjugate the chelates in a way that reduces internal motion and yet does not deleteriously impact affinity. We hypothesized that a second small binding group at the N-terminus may provide additional fibrin affinity, while at the same time would restrict internal motion at the N-terminus upon fibrin binding and this would result in higher relaxivity. We evaluated the effect of peptide nucleic acids (PNA) at the N-terminus of a known fibrin contrast agent.

Methods: Standard Fmoc chemistry was used to elongate the peptide on 1,3-bis(aminomethyl)benzene trityl NovaSyn TGT resin (0.63 mmol/g). The PNA monomer was introduced at the N-terminus by using Fmoc-PNA(Bhoc)aeg-OH. Peptide conjugate binding to fibrin fragment DD(E) was measured by a fluorescence polarization (FP) assay.[4] Relaxivities were determined on a field cycling relaxometer at NY Medical College over the frequency range 0.01 to 50 MHz at 35 °C.[3,4]

Results & Discussion: We first screened a series of fibrin-specific peptide derivatives modified with a PNA monomer at the N-terminus and identified modifications that improved affinity. The peptide showing the highest fibrin affinity was then conjugated with four gadolinium DTPA chelates and the effects on the fibrin affinity and relaxivity were studied. Figure 1 shows that relaxivities for the 3 compounds are similar in buffer and plasma indicating similar rotational dynamics. Plasma data suggest that the PNA introduces little/no additional non-specific binding, otherwise we would expect increased plasma relaxivity. In fibrin all relaxivities are increased due to reduced rotation rates, but the Thymine-PNA modification for **Gd2-(T-PNA)-Pep-Gd2** dramatically improves target-bound relaxivity (more than 50% compared to the parent compound **Gd2-Pep-Gd2** and the control compound **Gd2-GlyGly-Pep-Gd2**).

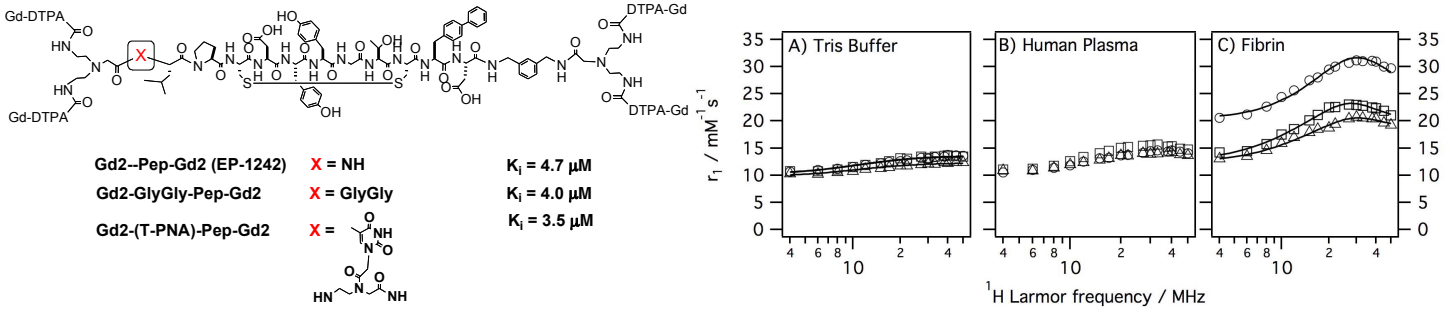

Figure 1. Left: Contrast agents. Right: ^{1}H NMRD relaxivity profiles of **Gd2-(T-PNA)-Pep-Gd2** ○, **Gd2-GlyGly-Pep-Gd2** △ and **Gd2-Pep-Gd2** □ in a) Tris buffer, b) human plasma, or c) human fibrin.

Figure 2 shows the mechanism of increased relaxivity by heteroditopic protein binding. In the absence of target protein fibrin, the molecule is flexible, undergoes fast tumbling, relaxivity is low. For EP-1242, fibrin binding reduces rotational motion and relaxivity is increased, but local motion limits relaxivity. Addition of second binding group (PNA) limits internal motion upon fibrin binding and boosts relaxivity.

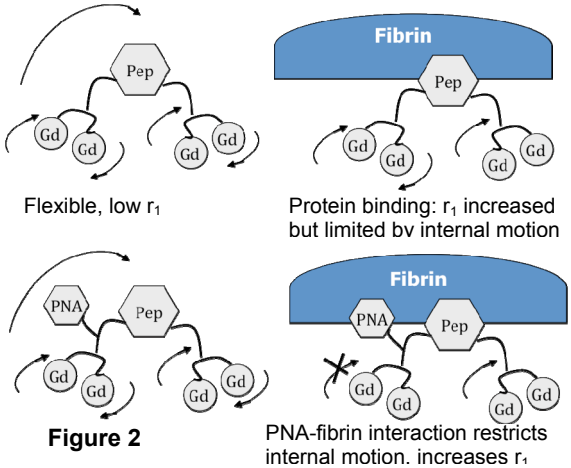

Figure 2

Conclusions: We demonstrate that a minor perturbation in probe structure can result in a 50% increase in relaxivity at the target, but does not increase off-target relaxivity. This heteroditopic binding approach is generalizable to other protein-targeted probes.

References: [1] Sirol et al., *Atherosclerosis,* 2005; 182:79. [2]Vymazal et al. *Invest Radiol*. 2009, 44:697. [3]Overoye-Chan et al. *J Am Chem Soc* 2008, 108:6025. [4]Zhang et al., *New J Chem*. 2010, 34:611.