Developing Pan-cancer targeting MRI contrast agents that self-assemble in malignant tumors

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Target audience. The target audience for this presentation is molecular imaging specialists interested in contrast agents.

Purpose. Developing novel classes of MRI imaging agent that target cancer hallmarks such as the low extracellular pH rather than specific receptors could in principle target most cancers (e.g. those with positive FDG-PET scans) and *enable <1 mm resolution and higher clinical detection sensitivity.* To this end we are creating a new type of synthetically flexible peptide amphiphile that uses an entirely novel mechanism for delivering and trapping a high concentration of imaging agent in malignant tumors by spontaneously self-assembling itself into larger, more slowly diffusing objects in the acidic extracellular microenvironment (**Fig. 1**).

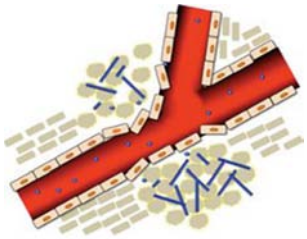

Fig 1. Blue vehicle self assembles to nanofibers in acidic tumor microenvironment

Methods. We have synthesized a prototype series of molecules called peptide amphiphiles (PA) that are comprised of a sequence of 8-10 biocompatible amino acids, a lipid tail, and an FDA-approved Gd^{3+} chelator, that transform from<< 100nm diameter spherical micelles (nanospheres) to micron-sized microfibers under the slightly more acidic pHe values of cancer (**Fig. 2**). Using a combination of critical micelle concentration and circular dichroism measurements, we have elucidated the transition pH and self-assembly phase diagram in simulated serum environments. Relaxivity measurements were performed on these agents. We have determined the influence of serum proteins on the transition pH by performing fluorescence anisotropy measurements in pure mouse serum with chromophore-labeled PAs. Finally, to determine the influence of self-assembly behavior (nanospheres, microfibers, and transition below $pH_e > 6.6$) on retention in tumor environments, biodistribution experiments using ^{177}Lu labeled PA were performed in mice.

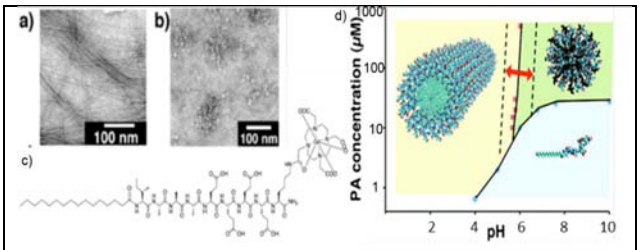

Fig 2. a, b) TEM images of 0.5 mM of PA, measured at pH **a)** 4.0 and **b)** 10.0. **(c)** Molecular structure of PA **(d)** Concentration–pH self-assembly phase diagram of PA. All samples were prepared in 150 mM NaCl and 2.2 mM $CaCl_2$.

Results . The biocompatible PAs undergo a self-assembly transition under physiological conditions from ~10 nm diameter spherical micelles ($pH \geq 6.6$ (at 10 μM to 1 mM) to >1000 nm microfibers, via random coil to beta sheet transitions (**Fig. 2**).[1] By balancing the supramolecular forces via peptide sequence, the transition can be systematically shifted by 1-2 pH units. The relaxivity values of the microfiber and spherical agents were 8.3 and 6.6mM^{-1} s^{-1}, respectively. The presence of serum albumin shifts the equilibrium from nanospheres to microfibers at significantly more basic pH values. The biodistribution measurements at 1 – 24 h post IV administration of 0.5 umol/kg revealed a clearance half-time of ~7 h vs >30 for transitioning vs fixed micelle state derivatives with even tissue vs mainly spleen/liver retention, respectively.

Discussion. We demonstrated through an array of molecules that we can control state transitions with pH and concentrations. In contrast to traditional surfactants that tend to bind serum albumin as isolated molecules, albumin promotes the self-assembly into nanofiber systems. Biodistribution results indicate that the spherical micelles have biodistribution profiles similar to conventional micelles, while transitioning systems have profiles more like smaller molecules.

Conclusion. We have developed a molecular design strategy for controlling the PA self-assembly transition behavior to tune the transition in serum. If we can harness the power of self-assembly to develop Gd-based MRI contrast agents that change shape and size sufficiently to trap Gd-PA in the acidic tumor environment, we should be able to image tumors at the spatial resolution of MRI using Warburg metabolism (like FDG PET) as the target.

References.

1. Ghosh A, Arijit, Haverick M, Stump, K, Yang, XY, Tweedle MF, Goldberger, JE. Fine-Tuning the pH Trigger of Self-Assembly. J Am Chem Soc 2012, 134: 3647-3650.