Investigating Stereochemically-Driven Peptide Assembly via Molecular Dynamics Simulations

Clare Cocker[1] & Liza Harold[2], Rachel Letteri,[1] and Kyle Lampe[1,2] .
[1]Department of Chemical Engineering & [2]Department of Biomedical Engineering, University of Virginia

Statement of Purpose: Injectable hydrogels can promote tissue repair following damage due to injury, stroke, or neurodegenerative disease. Here we describe hydrogels formed from short, self-assembling peptides that are injectable, mimic neural physiology, and provide a delivery mechanism for neural stem cells to the brain. We investigated the amino acid sequence lysine (K), tyrosine (Y), phenylalanine (F), isoleucine (I), and leucine (L), or KYFIL. This peptide is one of a class of rapidly assembling pentapeptides for injectable delivery (RAPID) hydrogels developed by the Lampe group, which are capable of self-healing and protecting cells during injection (Tang et al, 2019). We observed that mixtures of L-KYFIL and D-KYFIL exhibit distinct morphology compared to the enantiomerically pure peptide hydrogels. TEM showed enantiomerically pure peptide hydrogels to assemble into twisted fibers (Figure 1a). However, in hydrogels from racemic mixtures, peptides assemble into plate-like structures (Figure 1b). Through molecular dynamic simulations, we investigated the role of stereochemistry in driving assembly of KYFIL peptides, with the goal of employing stereochemistry in tuning KYFIL hydrogel morphology and mechanics.

Methods: We conducted atomistic molecular dynamics simulations through Nanoscale Molecular Dynamics (NAMD) and performed analysis through Visual Molecular Dynamics (VMD). We placed 64 amidated KYFIL peptides, varying the ratio of L:D-form peptides, in a 4x4x4 grid in random orientations. We solvated the peptides with explicit water and added Na^+ and Cl^- ions to reach physiological ion concentrations. Under constant pressure and temperature to mimic benchtop conditions, we followed the trajectory of KYFIL in NAMD for 200 ns. With the output of the simulation we investigated the composition of "clusters" of peptides, or groups of peptides within ~25 Å of each other. We also used in-house scripts to quantify the relative solvent accessible surface area (RelSASA) of each amino acid in KYFIL, measure the number of hydrogen bonds over time between amino acids, and generate Ramachandran plots to probe the secondary structure of the peptides.

Results: Hydrogen bond data suggest that the rate of bond formation varies with enantiomeric ratio. RelSASA analysis confirmed burying of hydrophobic amino acids within clusters.Secondary structure classification demonstrated beta sheet formation in L-KYFIL while revealing possible software limits of D-KYFIL analysis. The trajectory of the peptides during simulation

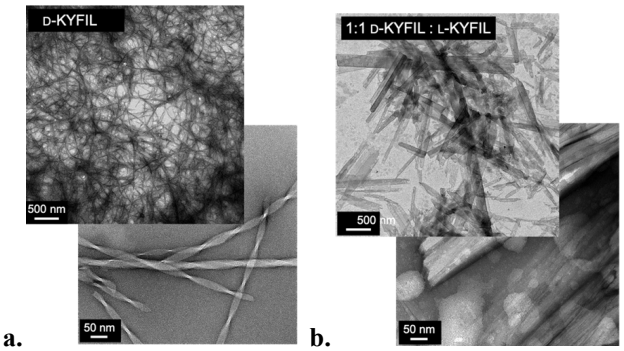

Figure 1: a. TEM images of pure D-KYFIL fibers; b. 1L:1D KYFIL plates (unpublished).

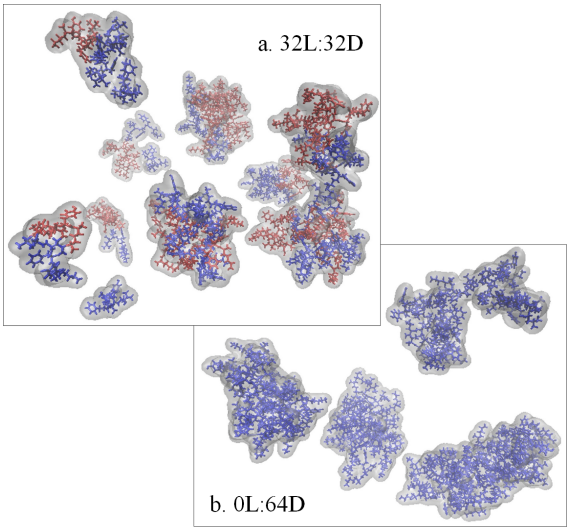

Figure 2: a. 32L:32D KYFIL clusters made of L (in red) and D (in blue); b. Pure 64D-KYFIL clusters at 200 ns.

revealed clusters smaller in size (~6 peptides per a cluster) but larger in quantity in racemic mixtures (Figure 2a) compared to simulations with only pure L or D peptides. In contrast, enantiomerically pure L and D peptides formed fewer, but larger clusters, with ~15 peptides per a cluster (Figure 2b). We observed that L-KYFIL and D-KYFIL cluster together, rather than self-sorting into enantiomerically pure clusters (Figure 2a). Together, these results highlight stereochemistry's role as a tunable driving force in peptide assembly. In summary, we established methods for simulating stereochemistry-driven assembly of peptides. Going forward, we will employ these methods to understand and inform the design of enantiomeric peptide mixtures for tissue engineering and regenerative medicine.

References:

Tang, JD. *J Am Chem Soc*. 2019; *141*(12), 4886–4899.