Exploring the Protein-Like Folding Capabilities of Biomimetic Polymers
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Background
Peptoids are promising candidates for synthetic replication of the tight-folded proteins that allow biological systems to function efficiently. By incubating solutions with solvatochromic dyes following solid-phase synthesis, we have developed a novel way to probe what kinds of sequences are best-suited for collapse in aqueous environments. The uptake of these dyes by a peptoid indicates the extent to which that particular sequence can mimic protein-folding. Through our project, we have already shown that large libraries of peptoid sequences show a wide range of dye color and intensity in these on-bead dye assays, with bluer shades indicating a greater degree of hydrophobic collapse. However, the image-based data obtained from assays were difficult to quantitatively compare. This motivated a project which developed a novel, in-solution assay to reconstitute the results of on-bead assays and confirm hit sequences.

Why Peptoids?

![Peptoid vs Peptide](image1)

**Peptoid**
- Achiral
- Lack N-bonding
- Flexible
- Larger monomer scope
- Resistant to proteolysis
- More scalable and stable than peptides

**Peptide**
- Chiral
- Strong hydrogen bonding
- Stiff
- Smaller monomer scope
- Susceptible to proteolysis

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**Key Advantages of Peptoids**
- Achiral
- Lack N-bonding
- Flexible
- Larger monomer scope
- Resistant to proteolysis
- More scalable and stable than peptides

**Synthesis and Current Scope**
The solid-phase synthesis methodology proceeds through two steps. The first step uses a functionalized acid to achieve a resin-bound secondary amine via activation with N,N'-diisopropylcarbodiimide (DIC). Then, a residue of choice is incorporated into the chain through incubation with solvatochromic dyes following solid-phase synthesis.

**Current State of the Project**
Another randomized sequence has been designed to further explore the patterning space of 15% hydrophobic 20mers. Libraries containing over one million unique 20mer peptoid sequences have been synthesized, screened on-bead, and are being sequenced to determine trends that can be analyzed prior to subsequent off-bead assays.

**References and Acknowledgements**

**Experimental Results**

**How can we measure the compactness of peptoid sequences relative to others?**

- Absorbance values were used to standardize the concentrations of the randomized-1 and alternating sequences. As seen in the UV-Vis spectroscopy trace, the absorbance curves for the two peptoids are nearly identical in both peak intensity and wavelength range. These results were confirmed using liquid chromatography / mass spectrometry (LC/MS), where the consistency in intensity of the main peaks indicates comparable quantities of peptoids and confirms the use of UV-Vis for the standardization of peptoid quantities.

**Transitioning from an On-Bead to an Off-Bead Assay**

Moving from an on-bead assay to an off-bead assay, the same solvatochromic dye was initially used. This dye was Reichardt’s dye, which appears blue in a more hydrophilic environment. However, the image-based data obtained from assays were difficult to quantitatively compare. This motivated a project which developed a novel, in-solution assay to reconstitute the results of on-bead assays and confirm hit sequences.

**Off-Bead Nile Red Assay Design**

In prior on-bead assays, Nile Red was also explored as a potential solvatochromic dye, where it was more prone to crushing out of solution and thus was more sensitive in detecting collapse under a microscope. However, these on-bead assays were unable to detect the fluorescence of Nile Red due to sequences being immobilized in resin.

**Peptoid scaffolds are hypothesized to form unstructured chains in hydrophobic environments and to collapse in aqueous solutions, and differences in sequence will cause different scaffolds to undergo varying amounts of collapse.** Solvatochromic dyes that are more soluble in hydrophobic environments can be used to probe the degree to which a particular sequence has collapsed.

**Large libraries of sequences can therefore be screened to understand what sequence design parameters lead to more- or less-collapse structures in an aqueous environment.**