# Exploring the Protein-Like Folding Capabilities of Biomimetic Polymers

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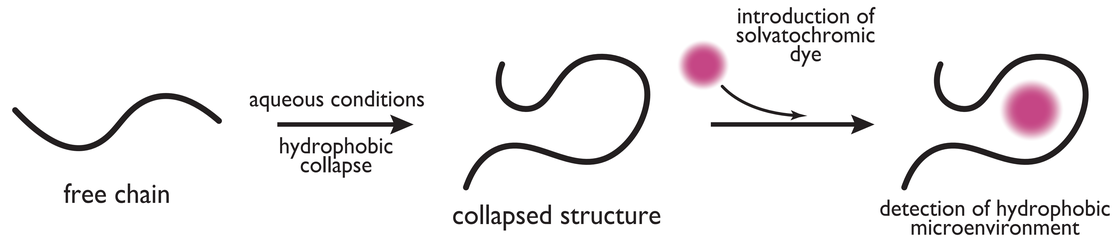
## Background

Peptoids are promising candidates for synthetic replication of the tightly-folded proteins that allow biological systems to function efficiently. By incubating sequences 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.

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

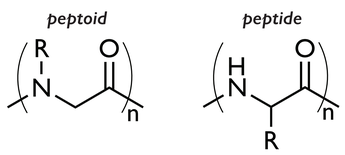



Peptoid scaffolds are hypothesized to form unstructured chains in hydrophobic environments and to collapse in aqueous solutions, and differences in sequence will cause different scaffolds to undergo varying amounts of collapse.

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-collapsed structures in an aqueous environment.

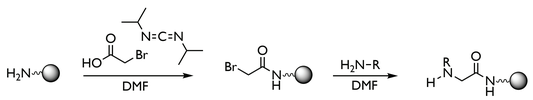
## Why Peptoids?




### Key Advantages of Peptoids

- Achiral
- Lack H-bonding
- Flexible
- Larger monomer scope
- Resistant to proteolysis[1]
- More scalable and stable than peptides[2,3]

## Synthesis and Current Scope

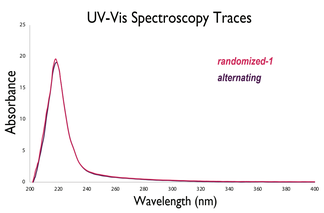


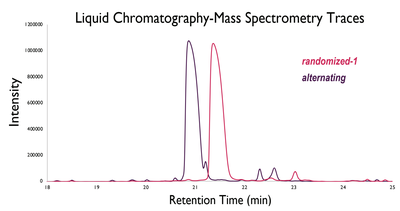

The solid-phase synthesis mechanism proceeds through two steps. The first step uses a haloacetic acid to acylate a resin-bound secondary amine after activation with N,N'-diisopropylcarbodiimide (DIC).[4] Then, a residue of choice is incorporated into the chain through nucleophilic displacement of the halogen. Through this method, residues are added stepwise, offering very precise synthesis.[5]


For preliminary assays, three 20-mer peptoid sequences were developed to hold the percent hydrophobicity of each chain at 50% and to span the space of sequence patterning.

## Standardizing Peptoid Quantities

On-bead assays of the three control sequences depicted above indicated that only the randomized-1 and alternating sequences had significant uptake of dye. Additionally, the diblock sequence was expected to undergo multichain assembly in solution phase, hindering the study of single-chain conformations. Therefore, the randomized-1 and alternating sequences were chosen for in-solution assays. Ultraviolet-visible light (UV-Vis) spectrophotometry was used to estimate the low concentrations of peptoid required for these assays. From literature precedent, the extinction coefficient was determined to be the same for both sequences.[6]

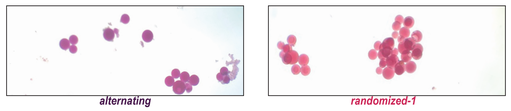



Absorbance values were used to standardize the concentrations of the randomized-1 and alternating sequences. As seen in the UV-Vis spectroscopy trace, the absorbance curves for the two peptoids are nearly identical in both peak intensity and in wavelength range. These results were confirmed using liquid chromatography-mass spectrometry (LCMS), 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

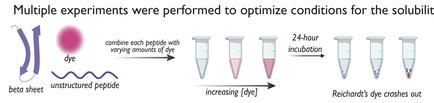
Moving from an on-bead assay to an off-bead assay, the same solvatochromic dye was initially used. This dye was Reichardt's dye, which appears bluer as more dye is solvated in a hydrophobic environment.[7] When incubated with the alternating and randomized-1 control peptoids on-bead, a difference in both shade and intensity was observed, indicating different degrees of collapse in the aqueous environment.




To retain as much biological relevancy as possible, a 95:5 ratio of aqueous:organic solvent was used. So as not to waste material, initial off-bead assays were developed by screening two peptide sequences with well-characterized secondary structure – one unstructured and the other forming a single-turn beta-sheet.

### Reichardt's Dye Crashing Out in Initial Screens with Peptide Sequences

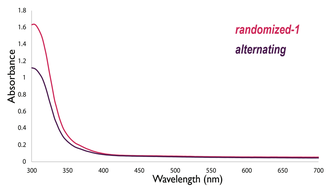
Multiple experiments were performed to optimize conditions for the solubility of Reichardt's dye, including:




- 95% HEPES (pH 7.6) and 5% DMSO
- 95% HEPES (pH 7.6) and 5% acetone
- Incubation at 37°C and 25°C

Optimized conditions for assay: 95% $H_2O$ and 5% acetone with incubation at 25°C.

### Lack of Absorbance in Visible Spectrum

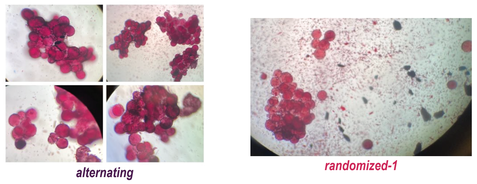



Using the optimized parameters above, peptoid was incubated with Reichardt's dye and the absorbance of samples was read using a UV-Vis spectrophotometer.

Absorbance was not observed in the region of visible light
This prohibited any comparison of $\lambda_{max}$ for the two peptoid sequences
After further on-bead assays with Reichardt's dye investigating pH effects on visualization, the unbuffered solution was likely too acidic to result in a colorful solution
This was unknown at the time, therefore other solvatochromic dyes were considered for these assays

## Off-Bead Nile Red Assay Design

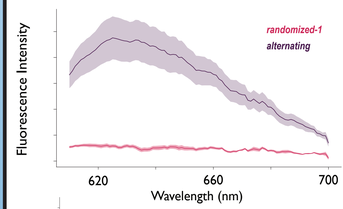
In prior on-bead assays, Nile Red was also explored as a potential solvatochromic dye, where it was more prone to crashing out of solution and there was minimal difference in bead colors under a microscope. However, these on-bead assays were unable to utilize the fluorescence of Nile Red due to sequences being immobilized on resin.




In a solution-phase assay, fluorescence could be used to detect the amount of dye present in the hydrophobic pocket of collapsed peptoid sequences. Like Reichardt's dye, Nile Red is more soluble in a hydrophobic environment. This led to the design of an in-solution dye assay following these optimized parameters:

| Sequences | [Peptoid] (mM) | Dye | [Dye] (mM) | Temperature | Aqueous : Organic Components |
|---|---|---|---|---|---|
| Randomized Alternating | 0.03 | Nile Red | 0.02 | 25°C | 95% $H_2O$ : 5% acetone |

## Off-Bead Fluorescence Assay and SEC Results

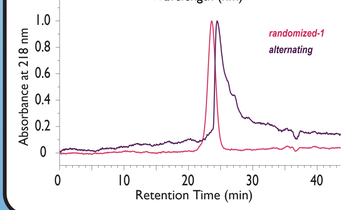



### *Fluorescence Data:*

Excitation of Nile Red dye at 586 nm, emission from 610-700 nm

Showed a significant difference between the fluorescence signal of the alternating and randomized-1 sequences over five replicate wells, the range of which is shown in the shading of signal curves

Introducing the alternating sequence to aqueous conditions resulted in a more hydrophobic microenvironment, implying a more collapsed structure




### *Aqueous Size-Exclusion Chromatography (SEC):*

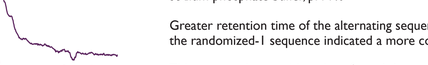
Used a low molecular weight column with a mobile phase of sodium phosphate buffer, pH 7.0

Greater retention time of the alternating sequence compared to the randomized-1 sequence indicated a more collapsed structure

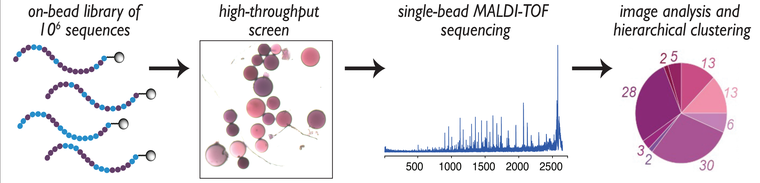
This was consistent with and confirmed the results from the fluorescence assay

## Current State of the Project

***Randomized-2***



Another randomized sequence has been designed to further span the patterning space of 50% 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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Additional thanks to alum ***Delaney Davis***