A fluorescent method for detecting ligand availability on the surface of self-assembled peptide nanofibers

Emily A. Verbus[1], Jai S. Rudra[1], Adam R. Urbach,[2] and Joel H. Collier[1]
[1]University of Chicago, Department of Surgery, Chicago, IL.
[2]Trinity University, Department of Chemistry, San Antonio, TX

Statement of Purpose: Self-assembling peptides with attached peptide ligands provide promising scaffold materials for regenerative medicine.[1] However, determining the amount of available ligand on the surface of fibrils has been challenging. We have thus sought a method for determining the amout of solvent-accessible ligand on the surface of self-assembled peptide fibrils using a novel macro-cyclic host, cucurbit[8]uril (Q8).[2] Q8 is a doughnut-shaped macrocycle composed of 8 methylene-bridged glycoluril units surrounding a hydrophobic cavity. It is able to bind to one equivalent of methyl viologen (MV), plus one other guest, in this case an N-terminal tryptophan residue (Trp).[2] When this binding occurs, Trp fluorescence is quenched.[3] By progressively quenching Trp fluorescence with increasing amounts of Q8-MV, the binding affinity of the host-guest complex can be calculated, as can the concentration of the available Trp in the system, in effect measuring the availability of the Trp residue on the surface of the fibrils (Figure 1a-c). By investigating different formulations of fibrils, as well as density of the Trp label on its surface, it can be determined if different processsing procedures influence the availability of the ligand.

Methods: *Peptide synthesis:* Q11 (QQKFQFQFEQQ) and W-Q11 (WSGSGQQKFQFQFEQQ) were both synthesized using standard Fmoc protocols. *Macro-cyclic host preparation:* The Q8-MV complex was prepared by as previously reported.[2] The complex was solubilized in PBS and filtered before the concentration was checked. *Fibril preparation:* Q11 and W-Q11 were mixed as dry powders in a 9:1 ratio, dissolved with Milli-Q water, and diluted with PBS to a 100 µM stock solution in order to initiate self-assembly.[1] The solution was then incubated for three days to allow for equilibration. Q8-MV was then titrated into peptide fibril solutions at variable concentrations and Trp fluorescence monitored from 300-500 nm with an excitation of 280 nm. 20 different concentrations were used ranging from 1-100 µM.

Results: Fluorescence of the Q11+W-Q11 solution behaved as expected. Upon introducing Q8-MV into the solution, the fluorescence intensity began to decrease owing to the interactions between the host complex and the terminal tryptophan (Figure 1d). The quenching of the fluorescence continued as the concentration of Q8-MV was increased and more host complexes bound to the free W in solution. This trend continued until the concentration reached 100 µM and the quenching ceased (Fig 1d). This is the point at which we believe all free W was bound to the Q8-MV host. The data obtained was then processed using a derived equilibrium fit equation, which takes into account the constant guest concentration of Q11+W-Q11 and variable host concentrations. A plot of the fraction of bound guest vs. the host concentration was created to judge the behavior of the newly formed

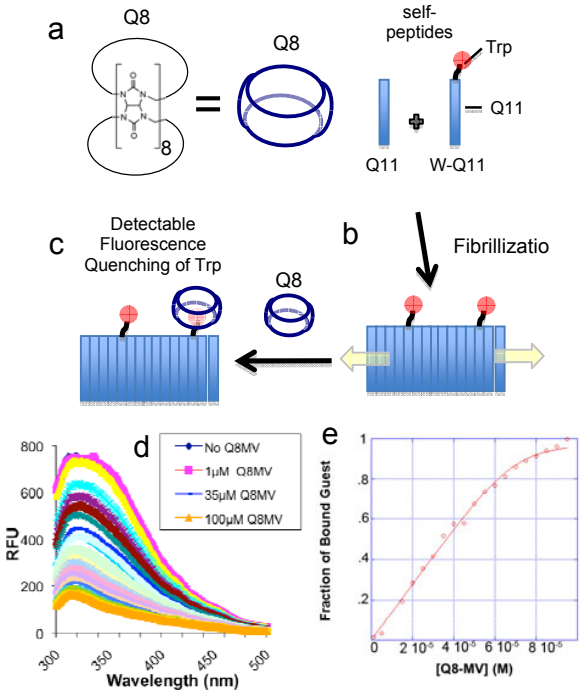

Figure 1. Schematic for measuring the availability of N-terminal Trp residues on the surface of self-assembled peptide fibrils using a cucurbit[8]uril host (a). Trp fluorescence readings obtained showing the resulting quenching while adding variable concentrations of Q8-MV to Q11+W-Q11 (b). Plot of processed data (c).

complex (Fig 1e). The results of this plot produced a K value of 7.55×10^5 M^{-1}, which is close to the accepted value of 1.3×10^5 M^{-1} for Q8MV-Trp interactions.[2] This methodology can then be utilized to calculate the amount of Trp available on the surface of the fibrils. The fit data has a high R^2 value of .997 and goes through the origin, indicating a well-behaved system.

Conclusions: By the fluorescence quenching behavior of this system, it can be concluded that Q8-MV interacts with the N-terminal Trp residue W-Q11 in a predictable manner, allowing the calculation of dissociation constants as well as the concentration of Trp available to the soluble host. In the time between abstract submission and the meeting, this method will be utilized to measure Trp availability on peptide fibrils in various contexts, including different ratios of peptides, mixing procedures, and over time.

References: 1. Collier JH. et al. Chem Soc Rev 2010; 39:3413-3424. 2. Reczek JJ. et al. JACS 2009; 131:2408-2415. 3. Bush ME et al., JACS 2005; 127, 14511-14517.