Illuminating the assembly and cellular interactions of a trimer derived from Aβ

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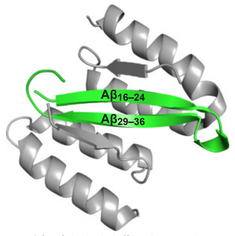
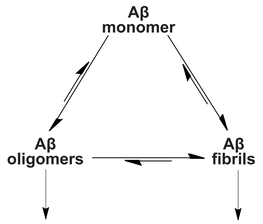
Introduction

Understanding the solution phase and biological behavior of the β-amyloid (Aβ) peptide is key to understanding Alzheimer's Disease (AD). Oligomeric assemblies of Aβ are suspected to be the leading cause of neurotoxicity, but their mechanism of action remains poorly understood. The Nowick lab has established a chemical model for Aβ oligomers by stabilizing fragments of Aβ in a macrocyclic β-hairpin structure and then covalently stabilizing these macrocycles into covalently-linked trimers. Solution phase studies of the covalently-linked trimers have thus far been limited to SEC and SDS-PAGE, but fluorescent labeling has emerged as a new tool to study the solution phase and biological behavior of the trimers. Fluorescence spectroscopy and fluorescence microscopy of the fluorescently-labeled trimers have provided insights into Aβ oligomers.

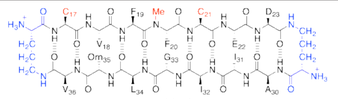


$Aβ_{40}$ β-hairpin/affibody complex (PDB 2OTK)

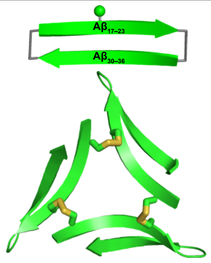


Hoyer, W. Grönwall, C.; Jonsson, A.; Ståhl, S.; Härd, T. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 5099–5104.

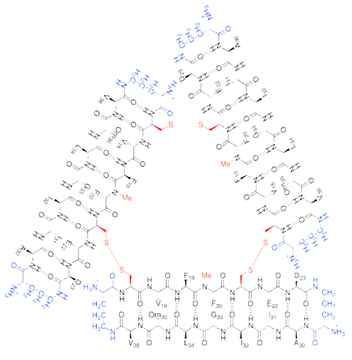
Macrocyclic β-Hairpin Peptides

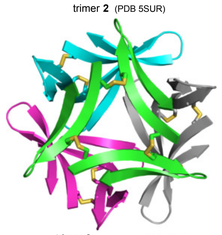


peptide **1**



trimer **2** (PDB 5SUR)

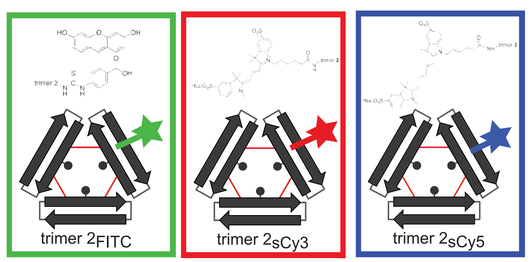




trimer **2** dodecamer (PDB 5SUR)

Spencer, R. K.; Li, H.; Nowick, J. S. *J. Am. Chem. Soc.* **2014**, *136*, 5595–5598.
Kreutzer, A. G.; Yoo, S.; Spencer, R. K.; Nowick, J. S. *J. Am. Chem. Soc.* **2017**, *139*, 966–975.

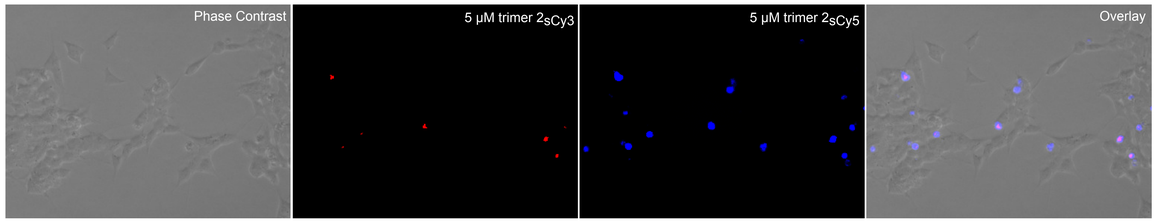
Fluorescent Labeling



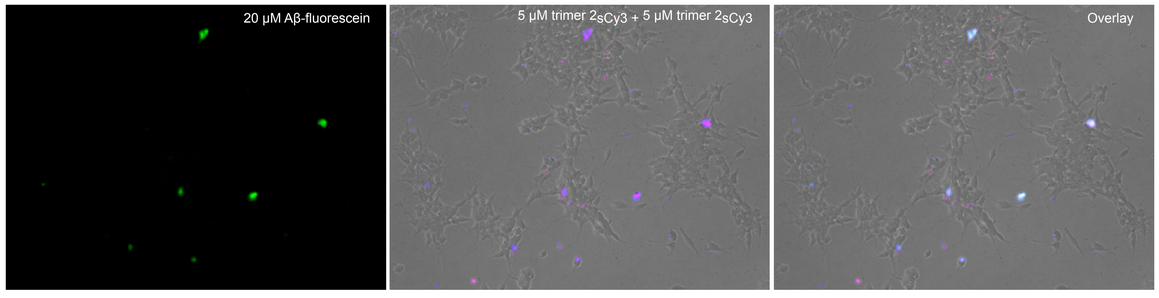
peptide	Fluorescent label	fluor. quantity	trimer quantity	reaction solvent	reaction volume	reaction time	C18 column purification temperature	average yield
trimer 2_{FITC}	FITC	880 μg	3 mg	DMSO/$NaHCO_3$	0.375 mL	24 h	80 °C	8%
trimer 2_{sCy3}	NHS-ester sulfo-cy3	20 μg	3 mg	$NaHCO_3$	1 mL	0.75 h	40 °C	56%
trimer 2_{sCy5}	NHS-ester sulfo-cy5	25 μg	3 mg	$NaHCO_3$	1 mL	0.75 h	40 °C	50%

Fluorescence Microscopy

Red = trimer 2_{sCy3} Blue = trimer 2_{sCy5} Green = Aβ-fluorescein



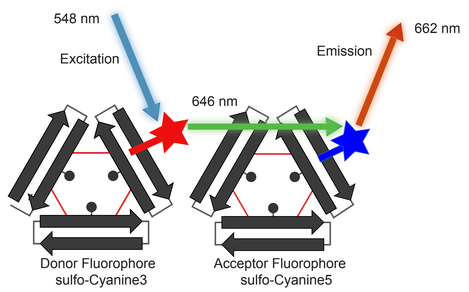
Fluorescence microscopy was used to visualize trimer 2_{sCy3} and trimer 2_{sCy5} in the presence of SH-SY5Y cells. After treating cells with 5 μM of each compound for 24 h, trimer 2_{sCy3} and trimer 2_{sCy5} colocalized on the cell membrane, which indicates that the trimers assemble on the SH-SY5Y cell membrane.

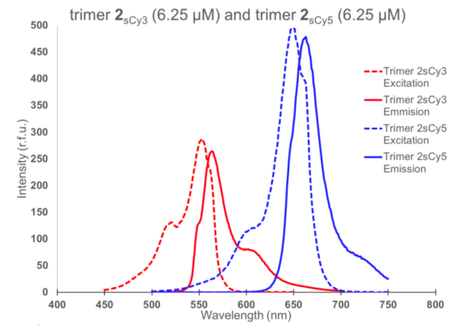


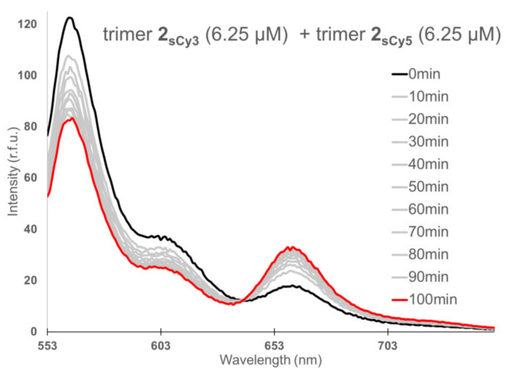
Fluoresecnce microscopy was then used to visualize Aβ-fluorescein in the presence of SH-SY5Y cells. In addition to treatment with 20 μM Aβ-fluorescein, cells were treated with 5 μM of trimer 2_{sCy3} and 5 μM trimer 2_{sCy5}. After 24 h, trimer 2_{sCy3} and trimer 2_{sCy5} colocalized with Aβ-fluorescein on the membrane of SH-SY5Y cells. This result suggests that the trimer model interacts with Aβ and supports the biological signifance of trimers.

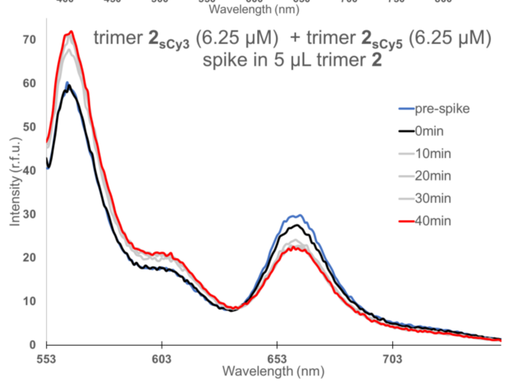
Fluorescence Spectroscopy

FRET model of fluorescently-labeled trimer



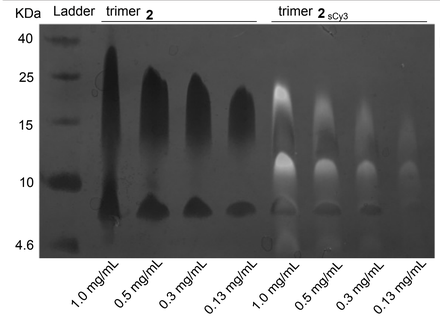


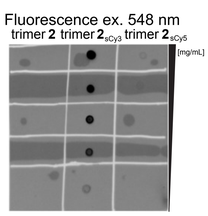


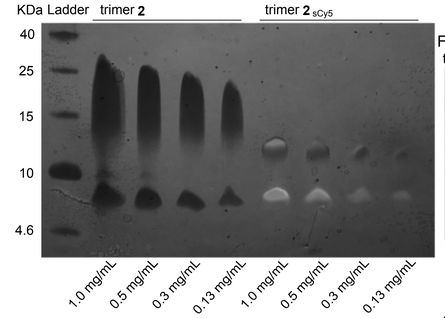


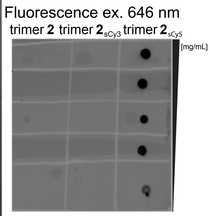
A mixture of trimers in solution was observed for 100 minutes. The decrease of emission at 563 nm and increase of emission at 662 nm indicates a FRET event and implies a dynamic nature of trimers in solution. After 100 minutes, additional trimer without a fluorescent label was added, and the FRET event appeared to diminish.

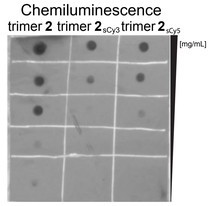
SDS-PAGE and Dot Blot











Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to study the assembly of sulfo-cyanine conjugated trimer. Fluorescent image (white) and silver stain (black) were used to visualize the peptide. Trimer 2_{sCy3} appears to assemble as a putative dodecamer. Trimer 2_{sCy5} appears to run consisent with the molecular weight of a hexamer. Dot blot analysis was used to determine if sulfo-cyanine conjugation disrupted recognition by an antibody raised against trimer 2. This experiment revealed that the sulfo-cyanine retained reactivity with the antibody.

Conclusions

Fluorescent labeling of trimer 2 and subsequent fluorescence spectroscopy and fluorescence microscopy has revealed insights into the biological and solution-phase assembly of trimers derived from Aβ. Fluorescence spectroscopy was used to demonstrate the presence of FRET events, suggesting close assembly of trimers in solution which increase over time. Fluorescence microscopy was used to visualize the trimers in the presence of cells, showing the colocalization of Aβ with the trimers. These findings provide evidence that support the biological significance of triangular trimers.

The Nowick Laboratory