



The Expression and Characterization of Disulfide-bond Stabilized Amyloid- β peptides

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Research gap: A β oligomers are challenging to study

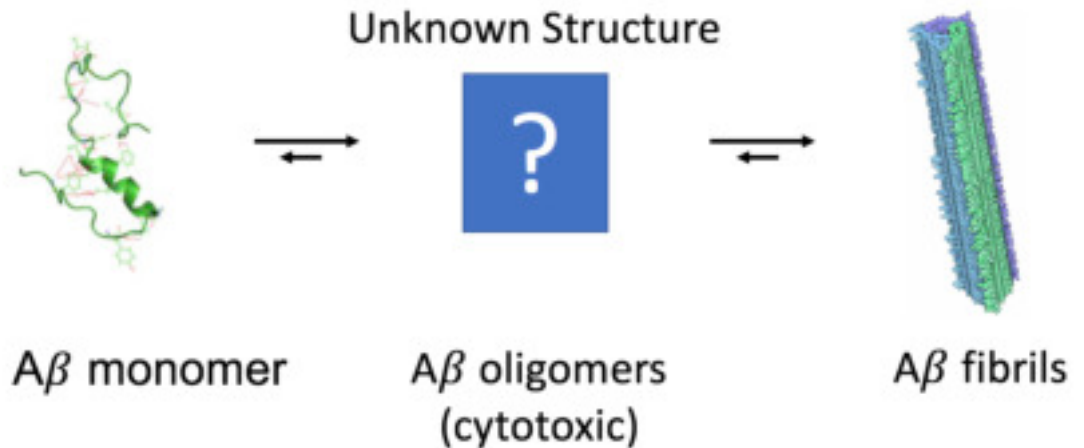
□ Amyloid- β (A β)

❖ Central to the pathogenesis of Alzheimer's disease

□ A β oligomers

❖ Toxic

❖ Aggregation-prone



Kreutzer, A. G. et al. *J. Am. Chem. Soc.* **2017**, 139, 966–975.

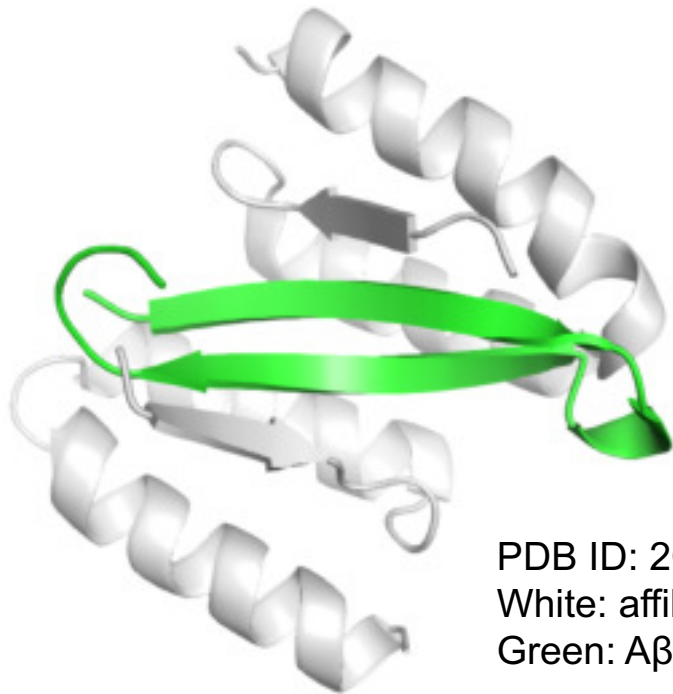
Knowles, T. P. J. et al. *Nat. Rev. Mol. Cell Biol.* **2014**, 15, 384–396.

Vivekanandan, S. et al. *Biochem. Biophys. Res. Commun.* **2011**, 411, 312.

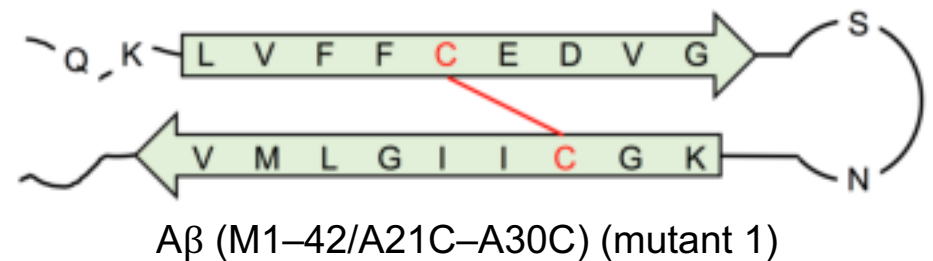
<http://pdb101.rcsb.org/motm/189>

Inspiration: β -hairpin is required for A β oligomerization

- β -hairpin \rightarrow A β oligomers
- Conformational change \rightarrow A β fibrils



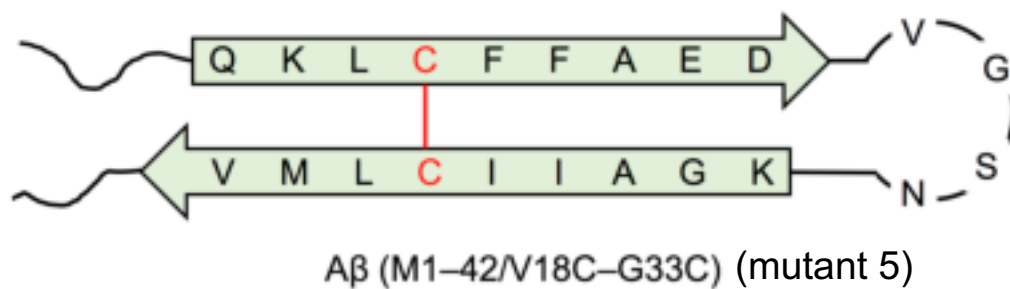
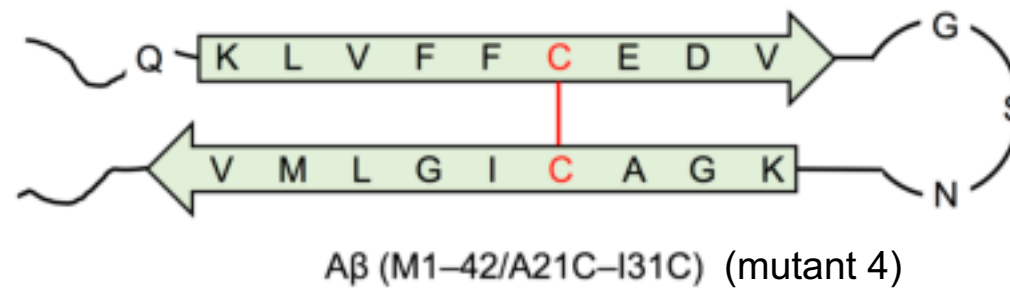
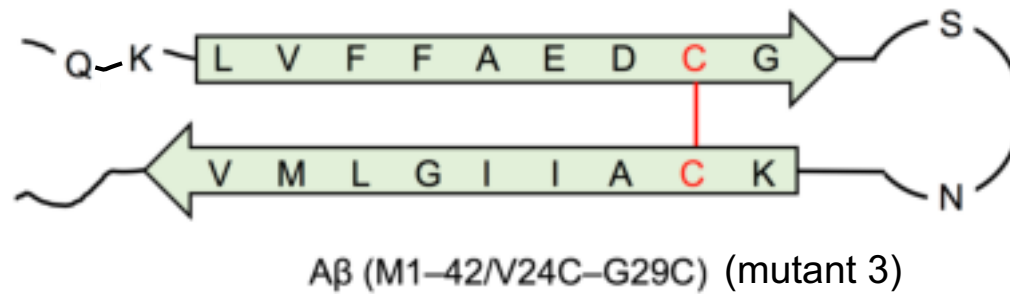
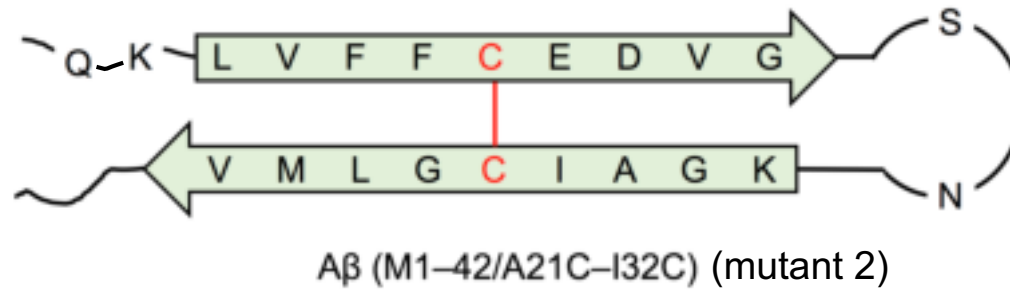
PDB ID: 2OTK
White: affibody
Green: A β β -hairpin



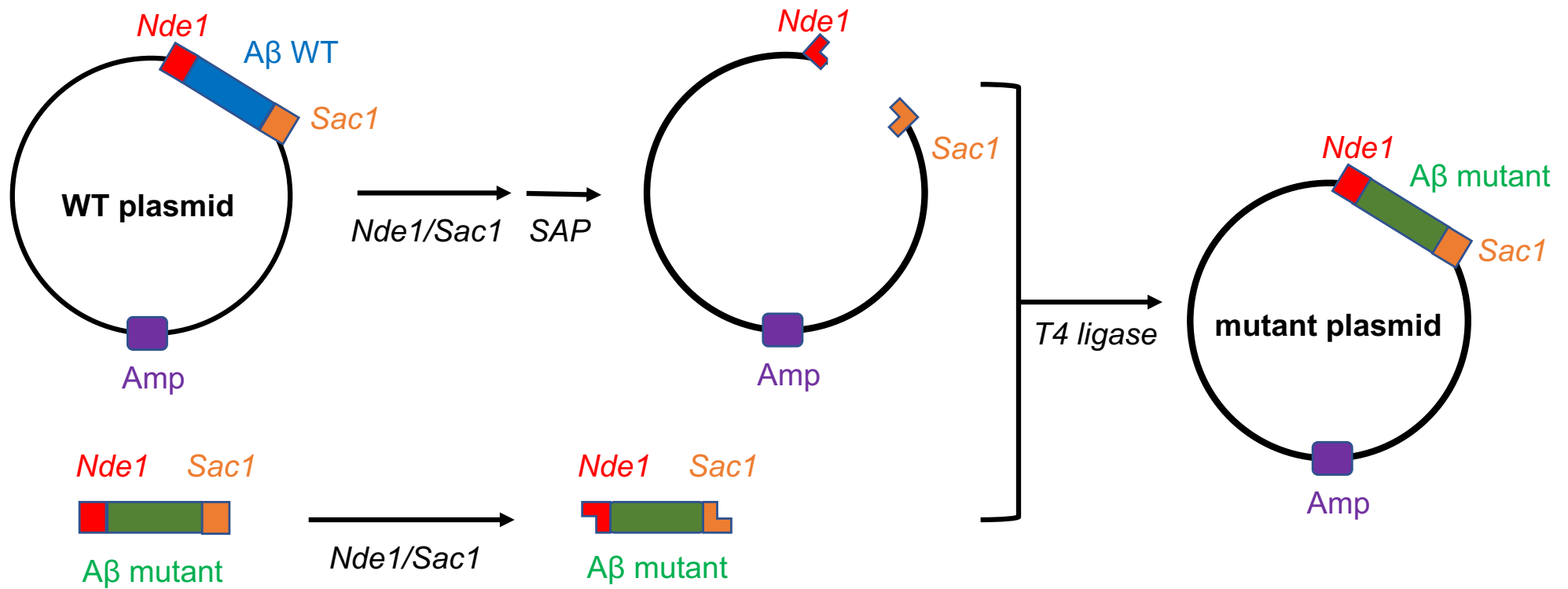
Hoyer, W. et al. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 5099–5104.

Sandberg, A. et al. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 15595–15600.

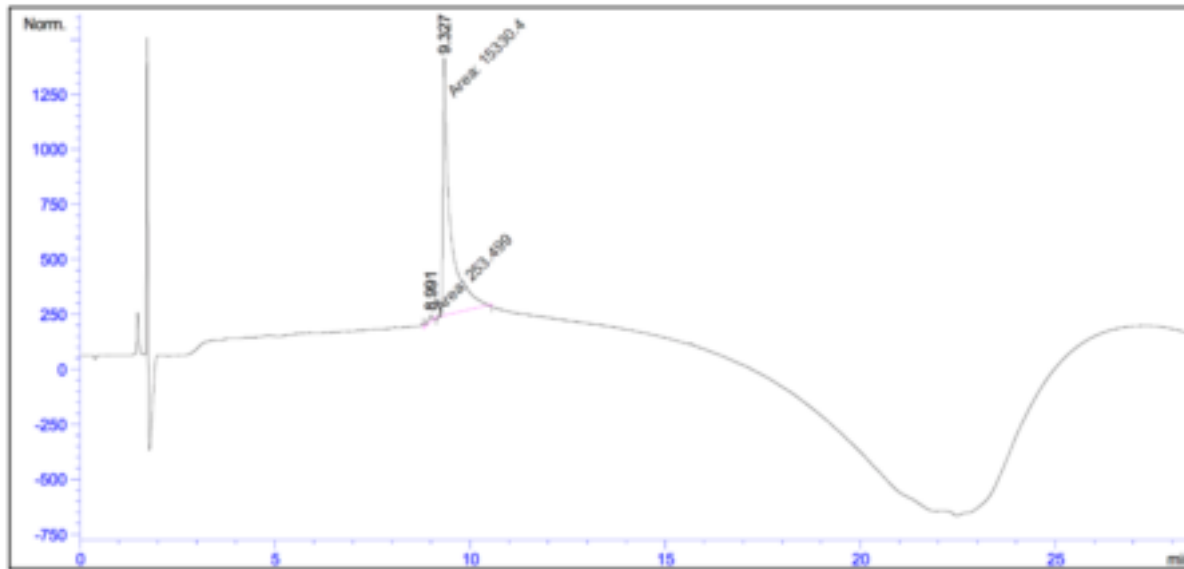
Research goal: study effects of β -hairpin alignment of A β



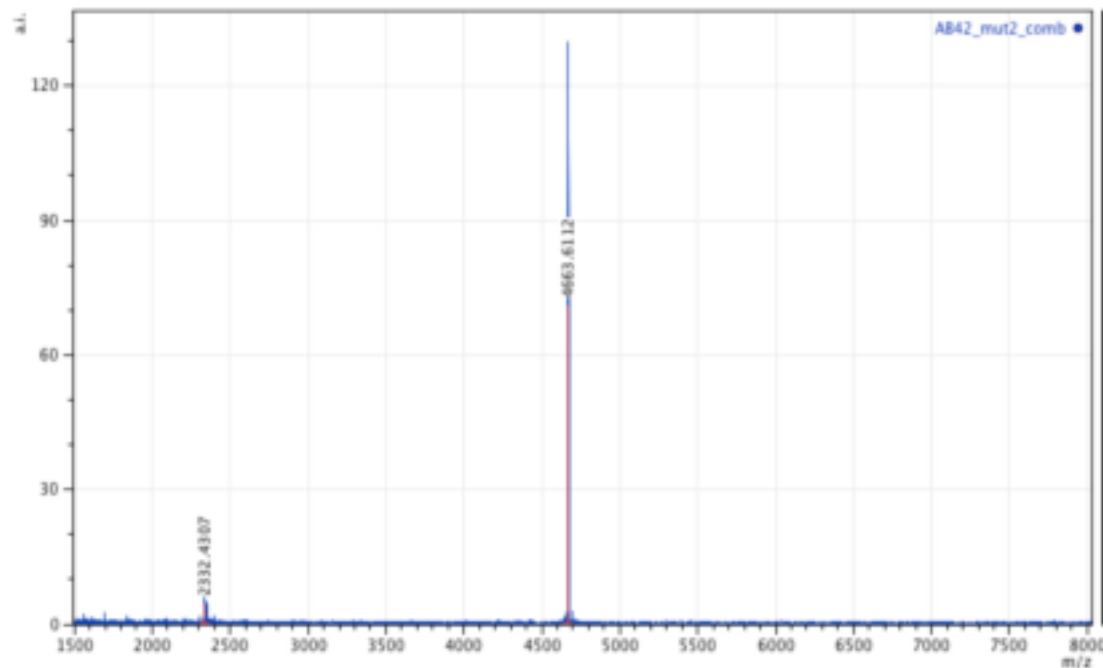
Mutant A β plasmids were generated through molecular cloning



Disulfide-bond stabilized A β were expressed and purified



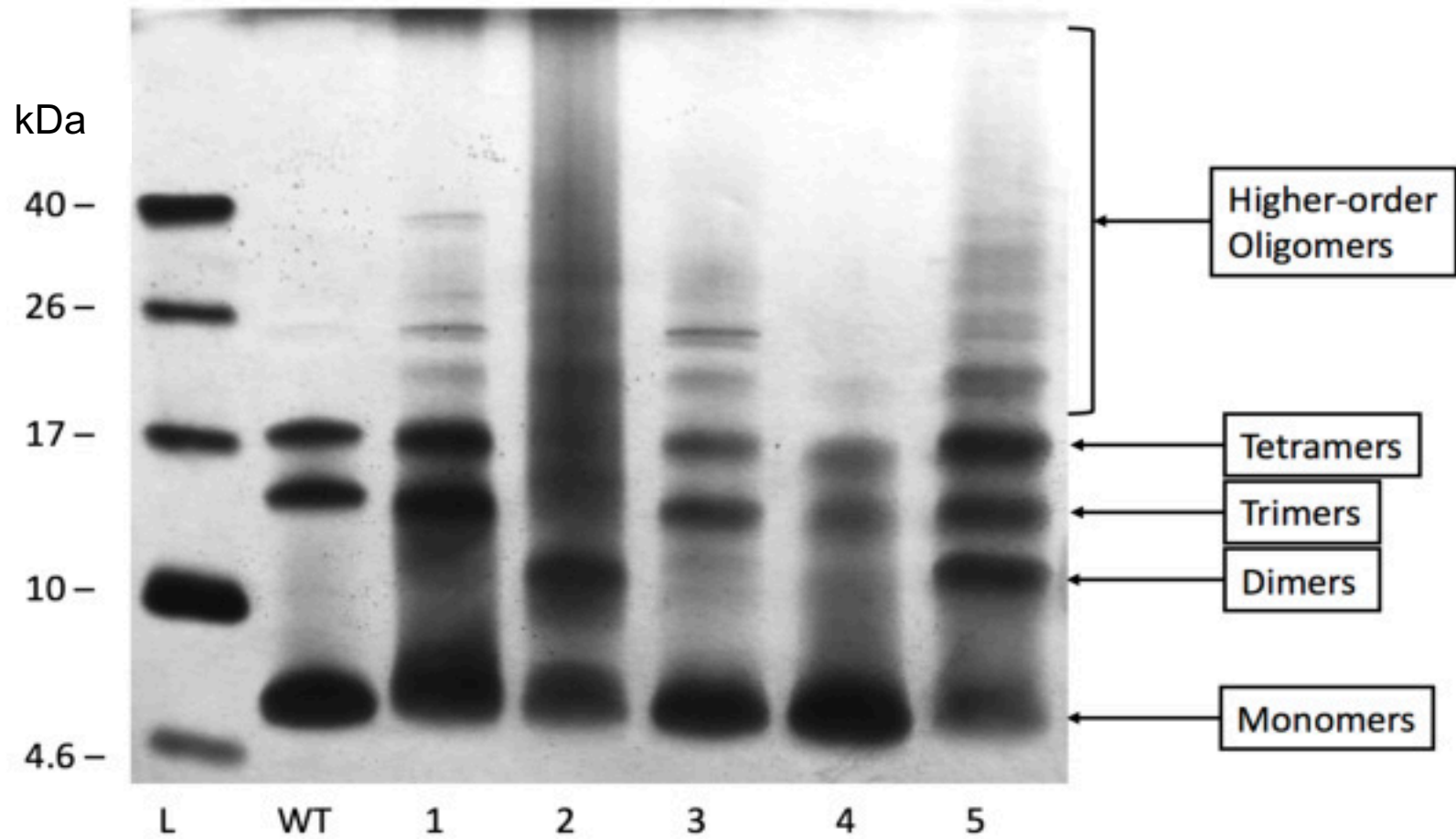
Representative HPLC:
A β (M1-42/A21C-I32C)
(mutant 2)



Representative mass spec:
A β (M1-42/A21C-I32C)
(mutant 2)

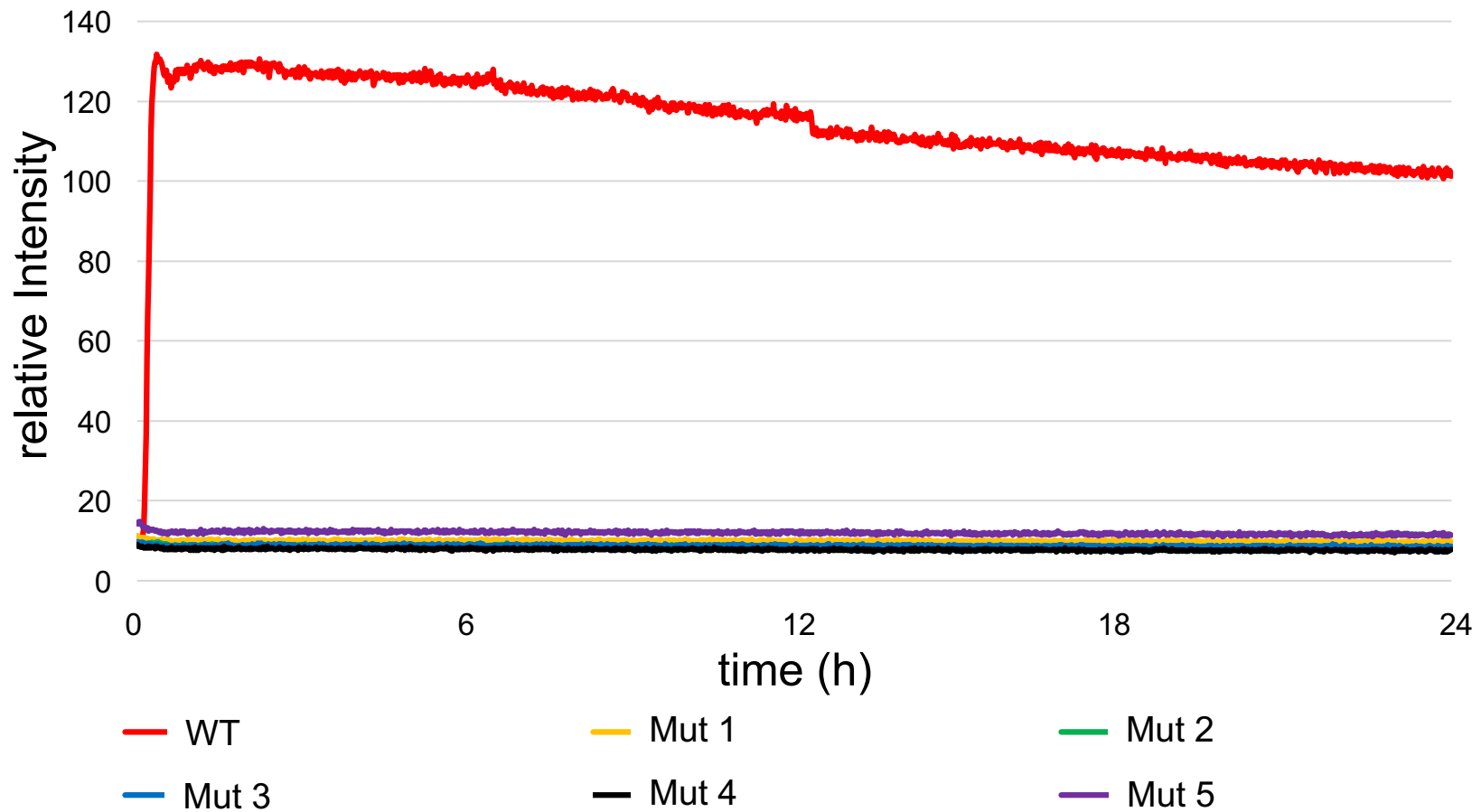
Disulfide-bond stabilized mutants like to form oligomers

SDS-PAGE results of A β (M1–42) wild-type and disulfide-stabilized peptides (at 31.25 μ M)



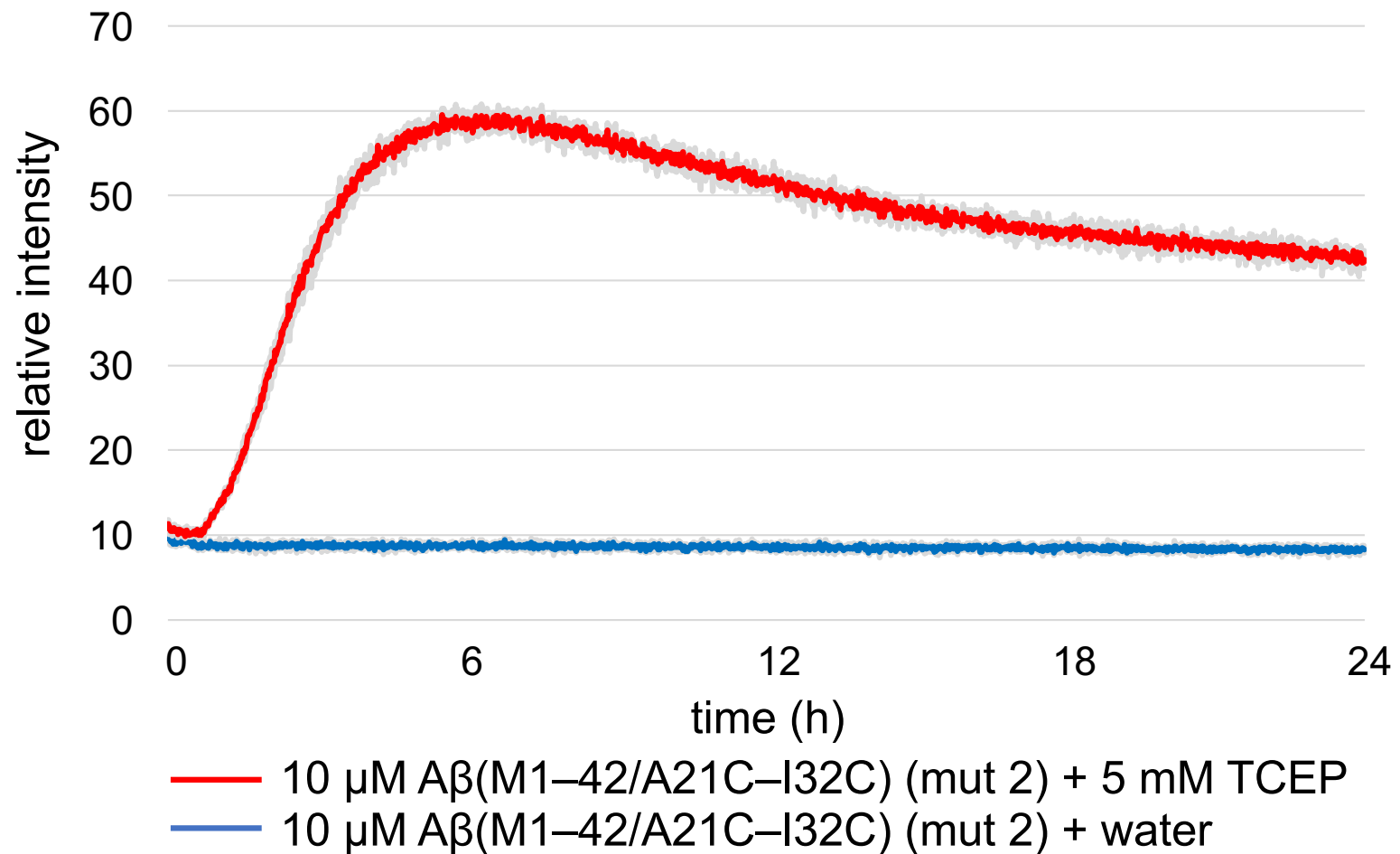
Disulfide-bond stabilized mutants do not form fibrils

ThT assay results of A β (M1–42) wild-type and disulfide-stabilized peptides (at 10 μ M)



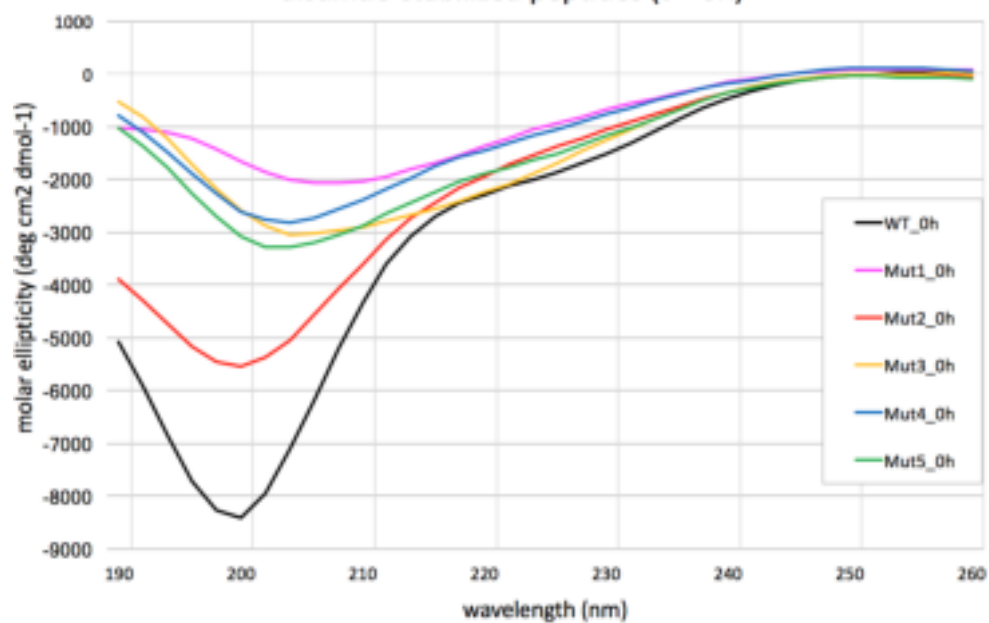
Reduction of the disulfide-bond induced the formation of the fibrils

ThT assay results of A β (M1–42/A21C–I32C) in the absence or presence of TCEP reducing agent (at 10 μ M)

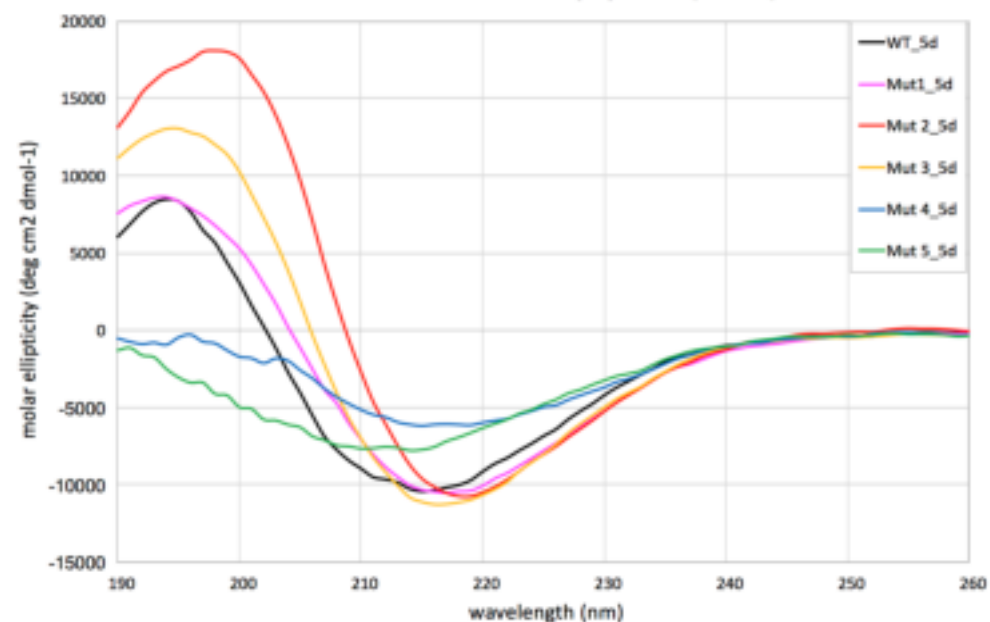


Disulfide-bond stabilized mutants – circular dichroism

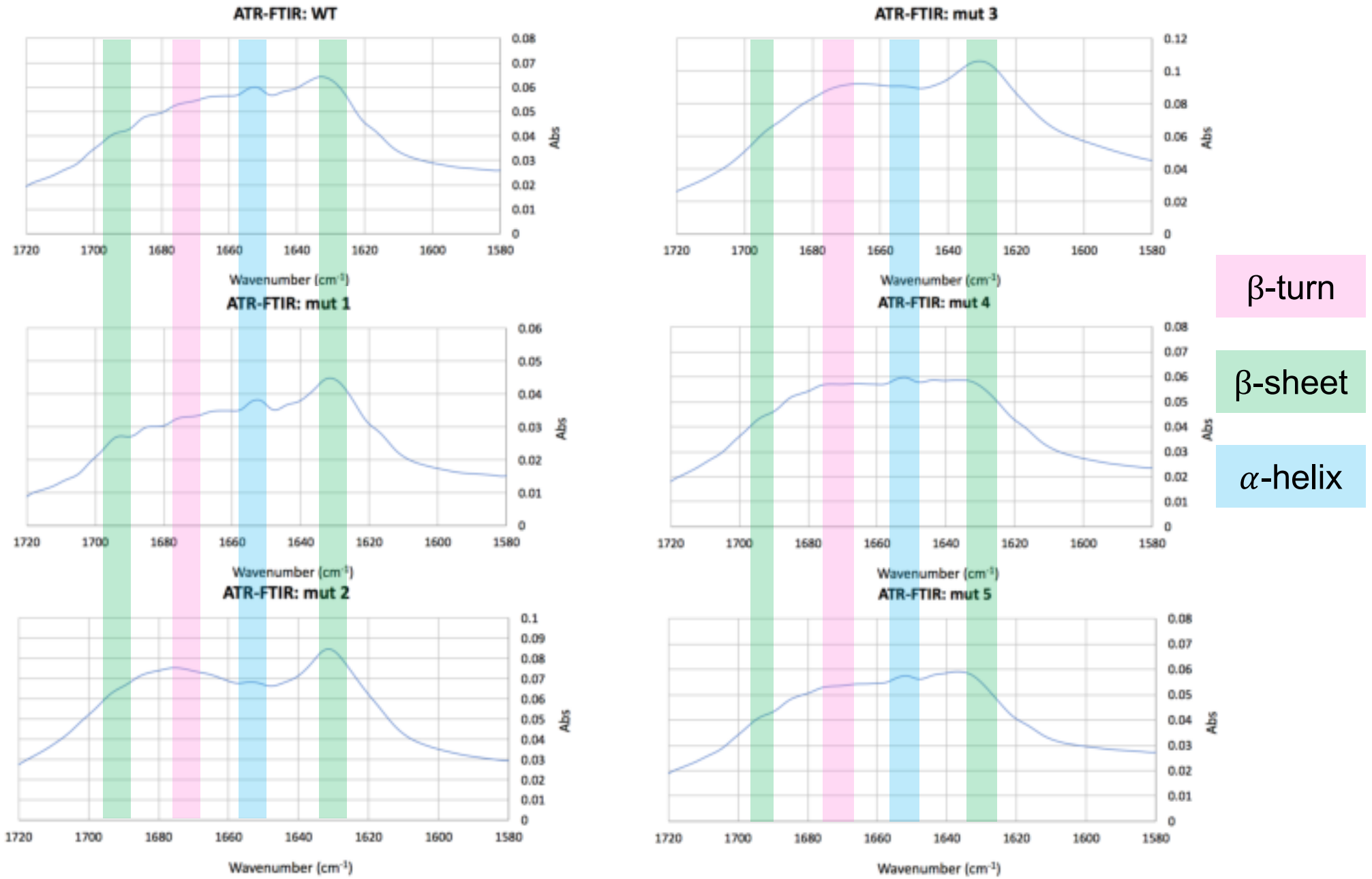
Circular dichroism of A β (M1–42) wild-type and disulfide-stabilized peptides (t = 0h)



Circular dichroism of A β (M1–42) wild-type and disulfide-stabilized peptides (t = 5d)

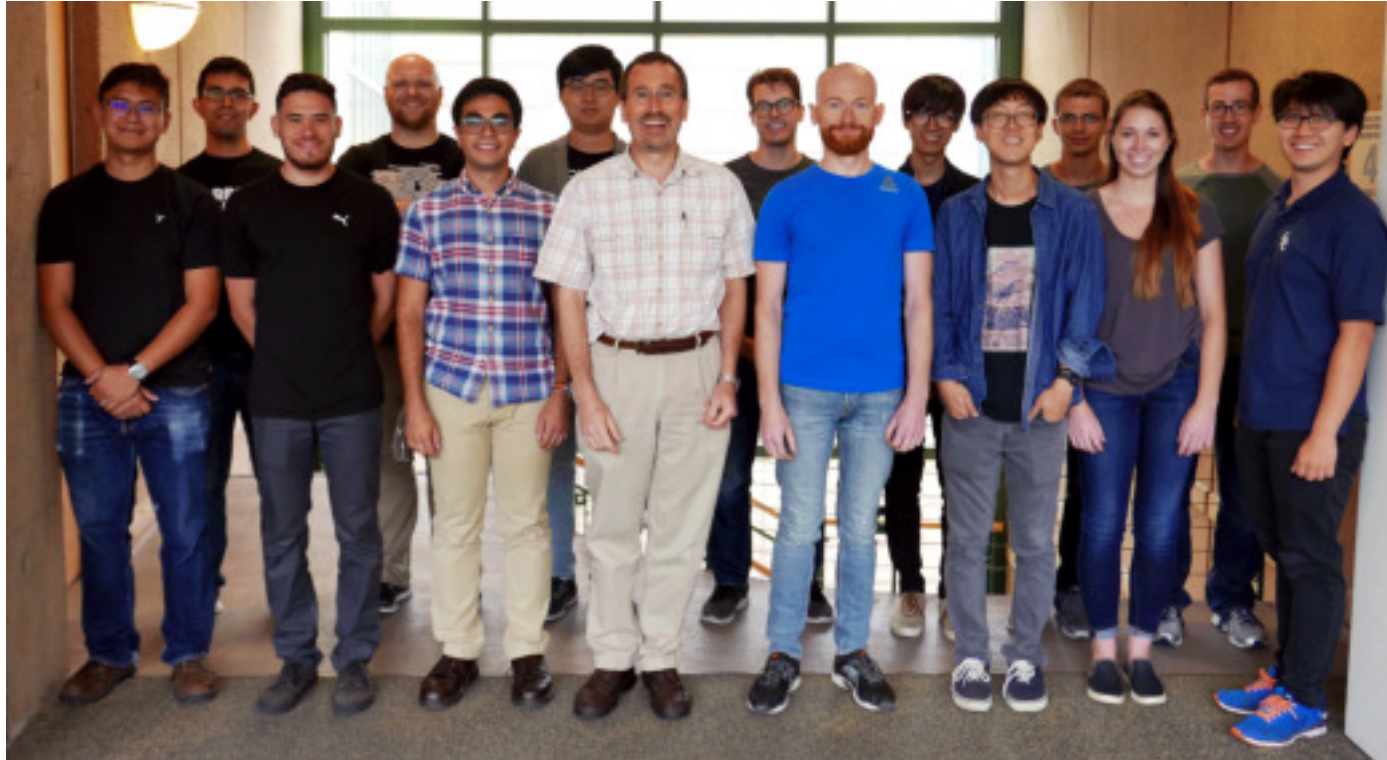


Disulfide-bond stabilized mutants – ATR-FTIR



Acknowledgments

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- UCI Laser Spectroscopy facility (Dmitry Fishman and Christian Baca)
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