Determination of the Oligomerization States of the Transmembrane Domains from SARS-CoV-2 Membrane Proteins

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Abstract

SARS-CoV-2 is the third highly infectious, highly deadly coronavirus to make the jump to humans in the last two decades. SARS-CoV-2, like other coronaviruses, is an enveloped virus with a small number of integral membrane proteins embedded in a lipid membrane. Each of these proteins has a transmembrane (TM) domain, a portion of the protein approximately 20 amino acids in length. Several proteins, including the Spike protein, are functional proteins only when trimeric–that is, three copies of the protein must self-assemble in order to carry out their function. Six proteins present in SARS-CoV-2 are known or hypothesized to have TM domains. These TM domains are highly conserved, and they are an underexplored region for potential drug discovery.

To examine the oligomerization state, three versions of each peptide of several of the TM proteins have been synthesized. One has a nitrobenzodiazole (NBD) fluorophore active in membranes, one has tetramethylrhodamine (TAMRA), a quencher of NBD, and one is unlabeled. These peptides are dissolved in a detergent above its CMC, which presents the peptides in micelles previously shown to be accurate mimics of membranes for the analysis of oligomerization states of TM domains. The three variants are used in a fluorescence-based assay which monitors the change in fluorescence as a function of added quencher peptide. The shape of the resulting fluorescence curve describes the oligomerization state. We describe the results of this experiment for two TM proteins, ORF 7a and ORF 3a.

Membrane Proteins in SARS-CoV-2

Protein	Function(s)	TM Domain Sequences	Literature Oligomerization State of Full Protein
Spike	Cell entry	1	Trimer
Envelope	Viral assembly, budding	1	Pentamer
Membrane	Viral assembly	3	Dimer
ORF 3a	Ion channel	3	Dimer or tetramer
ORF 7a	Immunomodulation, possibly structural	1	Monomer
ORF 7b	Structural	1	Dimer or tetramer

TM Domains Are Highly Conserved

A LogoPlot of the TM domain of the Spike protein of all β-coronaviruses ever sequenced from human hosts.

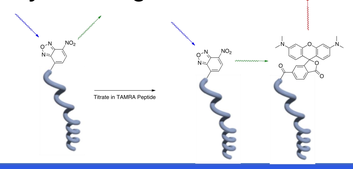
Peptides Synthesized

ORF 3a helix 1	IPIQASLPFGWLIVGVALLAV-KKKK
ORF 7a	LYSPIFLIVAAIVFITLCFTL-KKKK

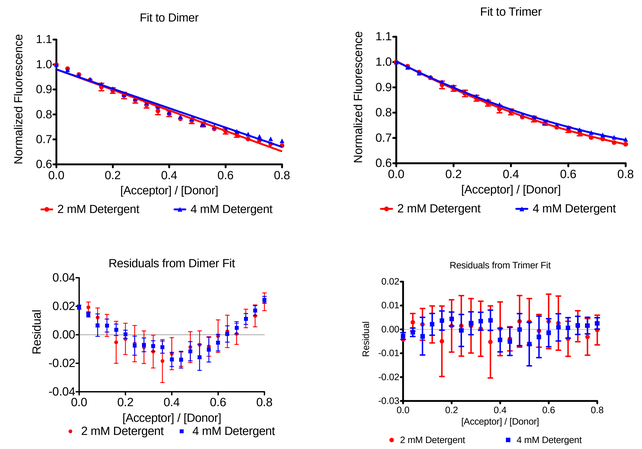
- C-terminal lysines added for solubility and handling
- Synthesized by undergraduates using manual Fmoc SPPS
- Synthesized 3 variants of each: NBD-labeled, TAMRA labeled, and unlabeled
- Purified by HPLC and analyzed by LCMS

Determining the Peptide Oligomerization State

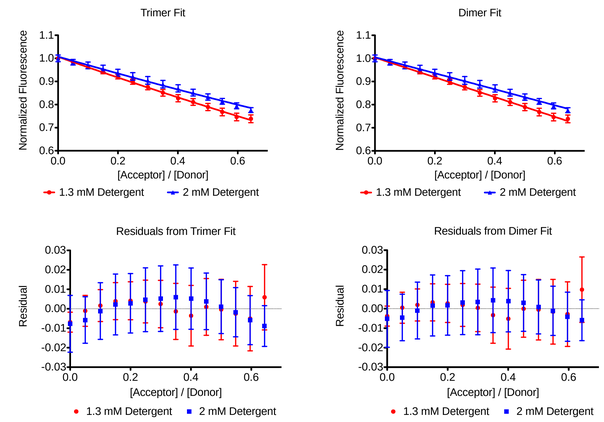
- Peptide dissolved in C14 betaine previously showed to be a good mimic for membrane bilayers in analyzing TM peptide biophysics
- Constant concentration of peptide and of NBD
- Titrate in solution of NBD/TAMRA labeled
- Shape of curves yields oligomerization state



ORF3a Data Fits Trimer



ORF7a Data Fits Dimer



Conclusions

- Undergraduates successfully synthesized and purified 25 amino-acid peptides
- Used fluorescence-based assay to determine peptide oligomerization state in a model membrane
- ORF3a TM domain a trimer
- ORF7a TM domain a dimer

Future Directions

- Synthesize other TM domains and determine their oligomerization states
- Analyze intermolecular determinants of oligomerization
- Use variant of fluorescence assay to determine intermolecular TM domain interactions

Acknowledgments

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