CPPC 2021
First Canadian Peptide and Protein Community Virtual Symposium
27–28 MAY 2021 | ONLINE
Canadian Peptide Protein Community
biomedicines
MDPI

MALDI/MS as a tool to rapidly screen peptide libraries for novel substrates of Farnesyltransferase

Garrett L. Schey[1*], Emily R. Hildebrandt[2], Sadie X. H. Novak[3], James L. Hougland[3], Walter K. Schmidt[2], Mark D. Distefano[1]

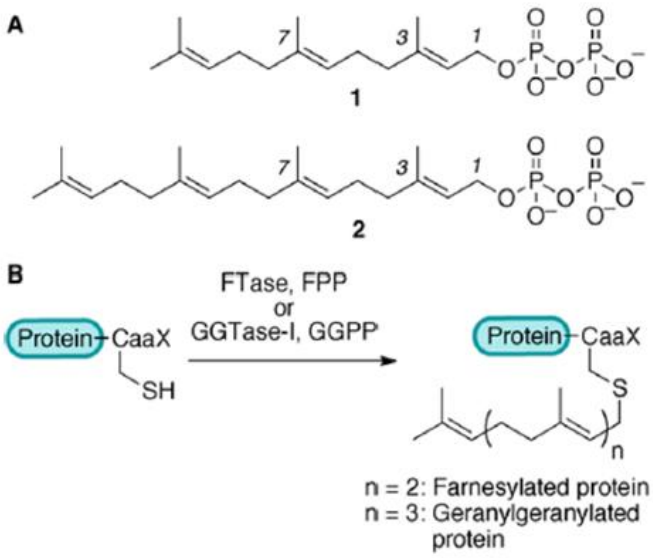
[1] Department of Medicinal Chemistry, University of Minnesota, Minneapolis, Minnesota USA
[2] Department of Biochemistry and Molecular Biology, University of Georgia, Athens, Georgia USA
[3] Department of Chemistry, Syracuse University, Syracuse, New York USA

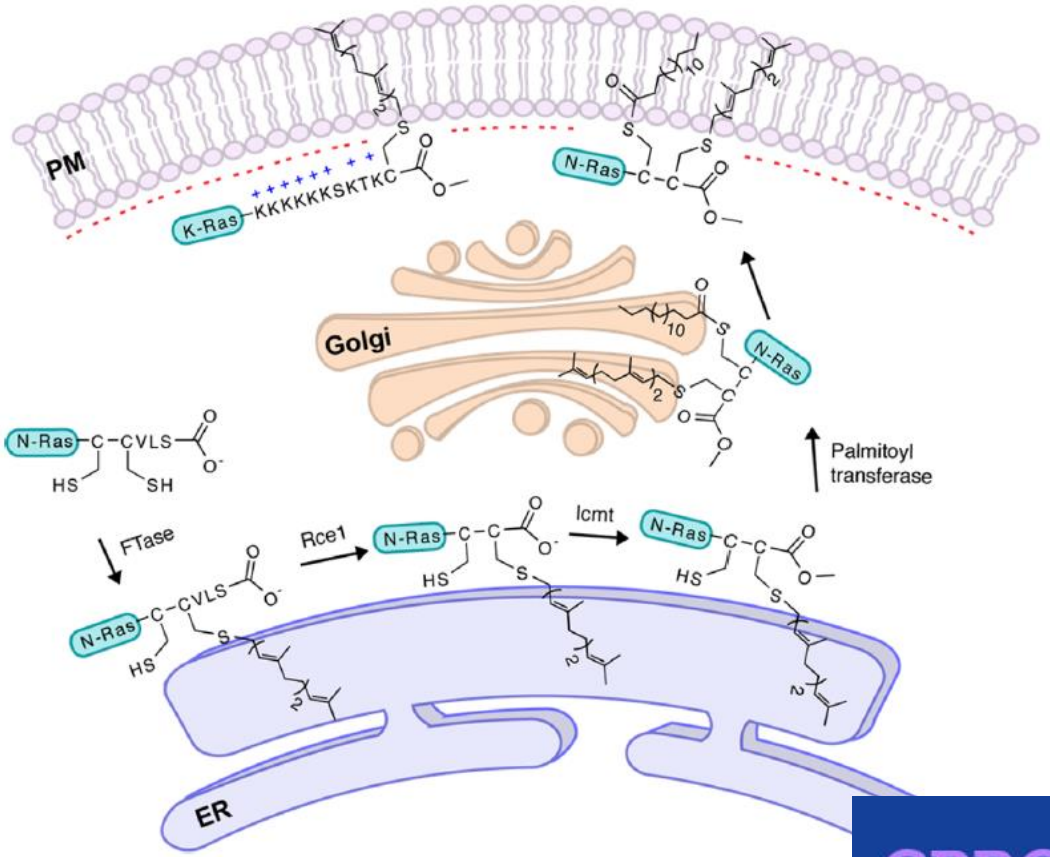
* Corresponding author: schey013@umn.edu

Abstract:
Protein farnesylation is a post-translational modification where a 15 carbon farnesyl isoprenoid is appended to the C-terminal end of a protein by Farnesyltransferase (FTase). In the canonical understanding of FTase, the isoprenoids are attached to the Cysteine residue of a four amino acid CaaX box sequence. However, recent work has shown that five amino acid sequences can be recognized, such as the pentapeptide CMIIM. This new discovery greatly increases the number of potential FTase substrates, as the FTase is already known to tolerate a wide variety of amino acids in the canonical CaaX sequence. With the goal of developing a more rapid and methodical method to evaluate potential substrates, we envisioned using MALDI to assay libraries of 10 peptides at a time, varying one amino acid in the CaaX box to all 20 canonical amino acids over two libraries. Through this method we observed 30 hits in the mass spectrum and chose eight for further evaluation. Seven of these sequences are novel substrates for FTase, with several meeting or surpassing the in vitro efficiency of the benchmark sequence CMIIM. Additionally, in vivo experiments in yeast demonstrate that proteins bearing these sequences can be efficiently prenylated in a biological context.

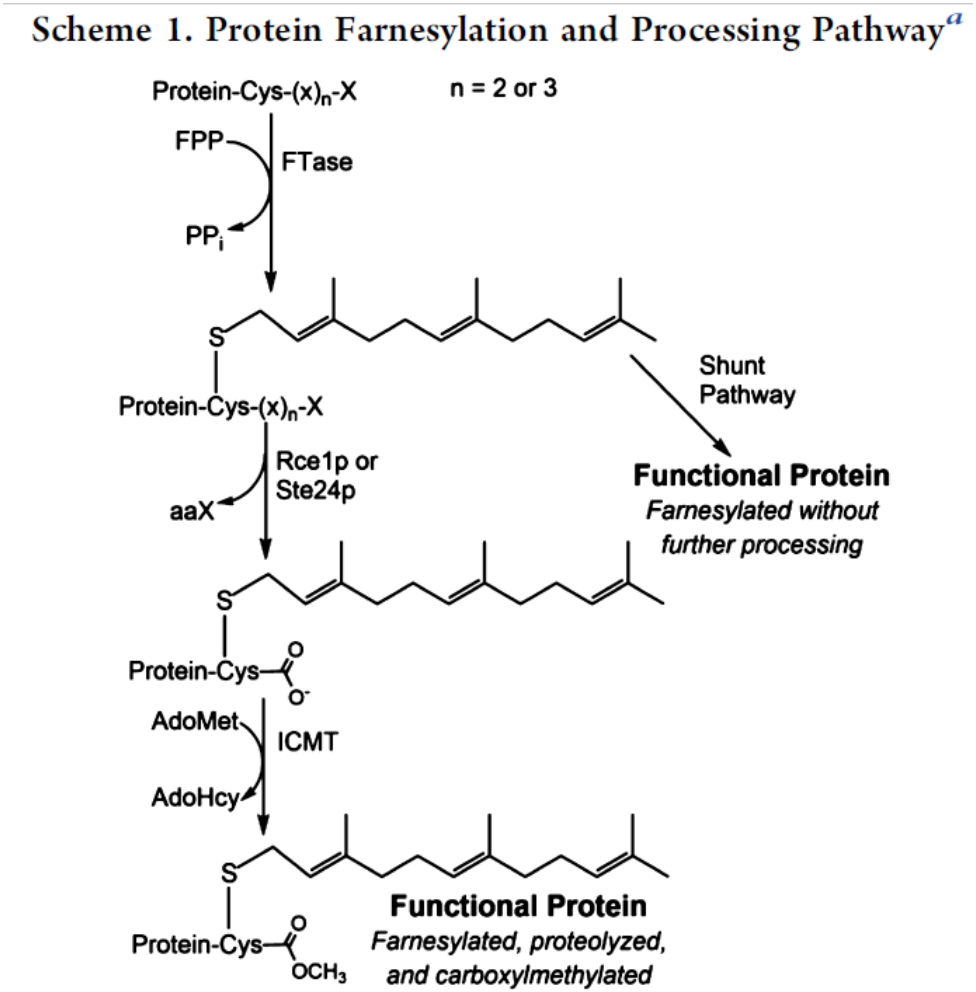
Keywords: Prenylation; peptide libraries; MALDI

Protein prenylation – the anchor of life



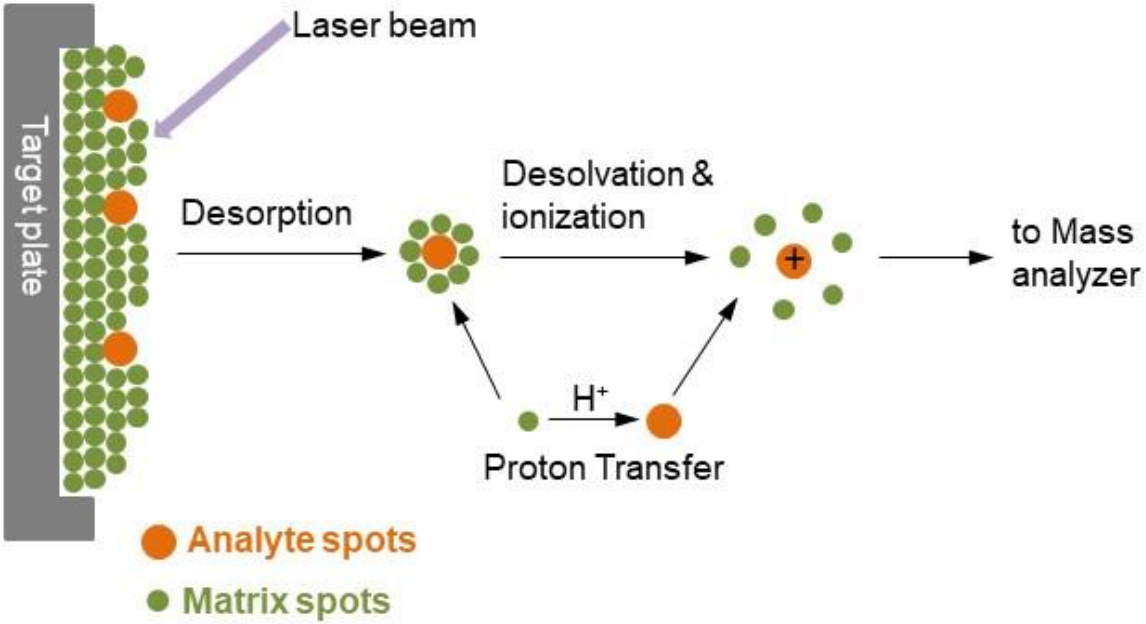


The canonical view of a CaaX box is expanding



Blanden, et al. J Biol Chem 2018

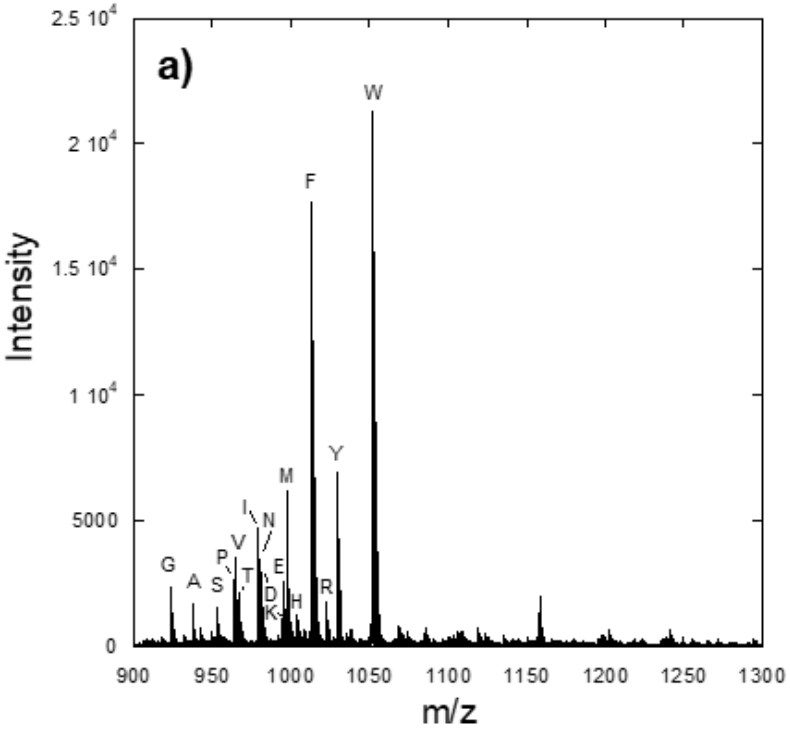
MALDI has several advantages as a library screening tool

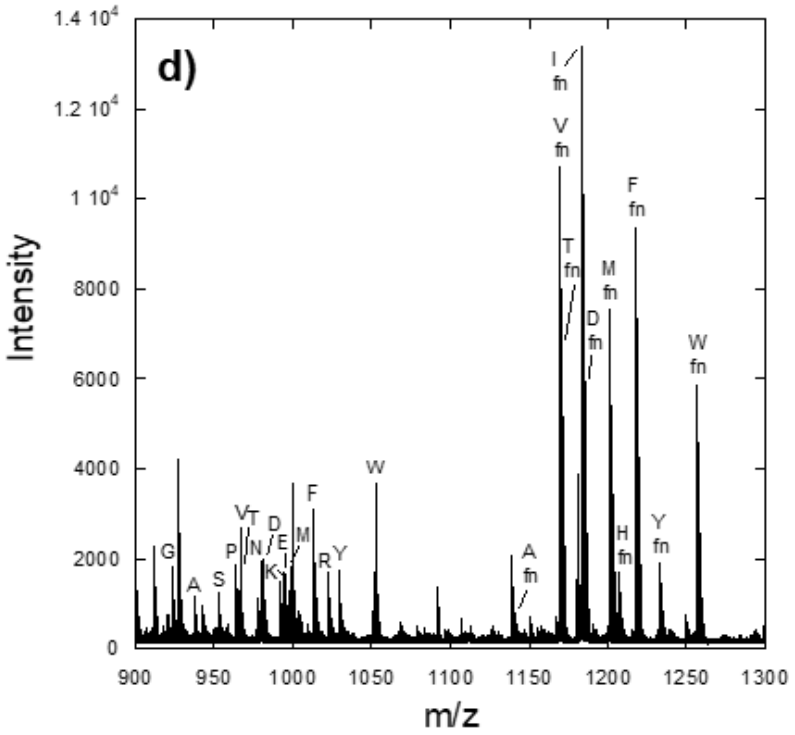


- Small sample volume
- High throughput
- Single charged species for simple analysis of complex mixtures
- Gentle ionization reduces fragmentation

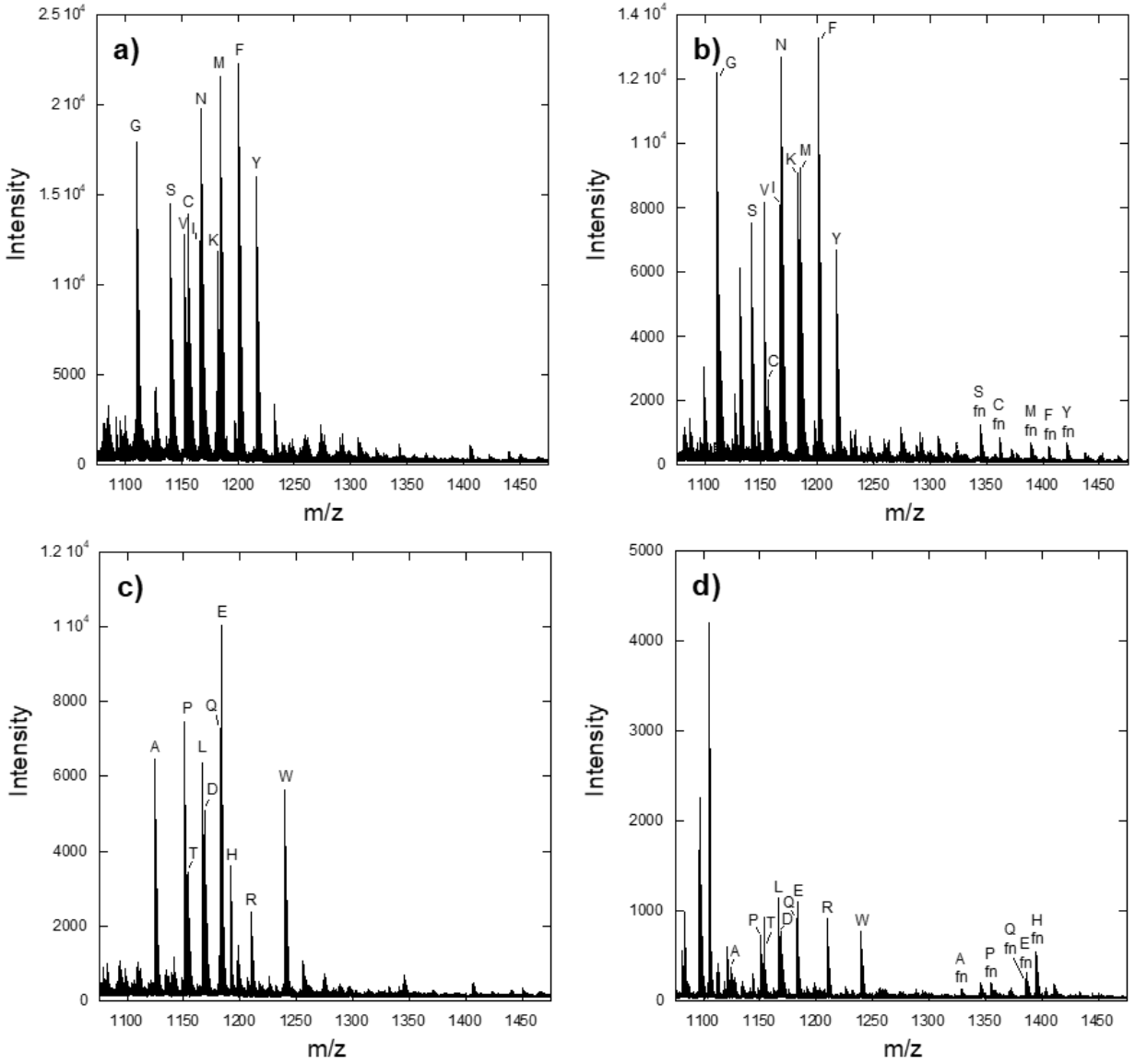
https://www.creative-proteomics.com/technology/maldi-tof-mass-spectrometry.htm

MALDI analysis of a previously studied CVIA library - RAGCVXA





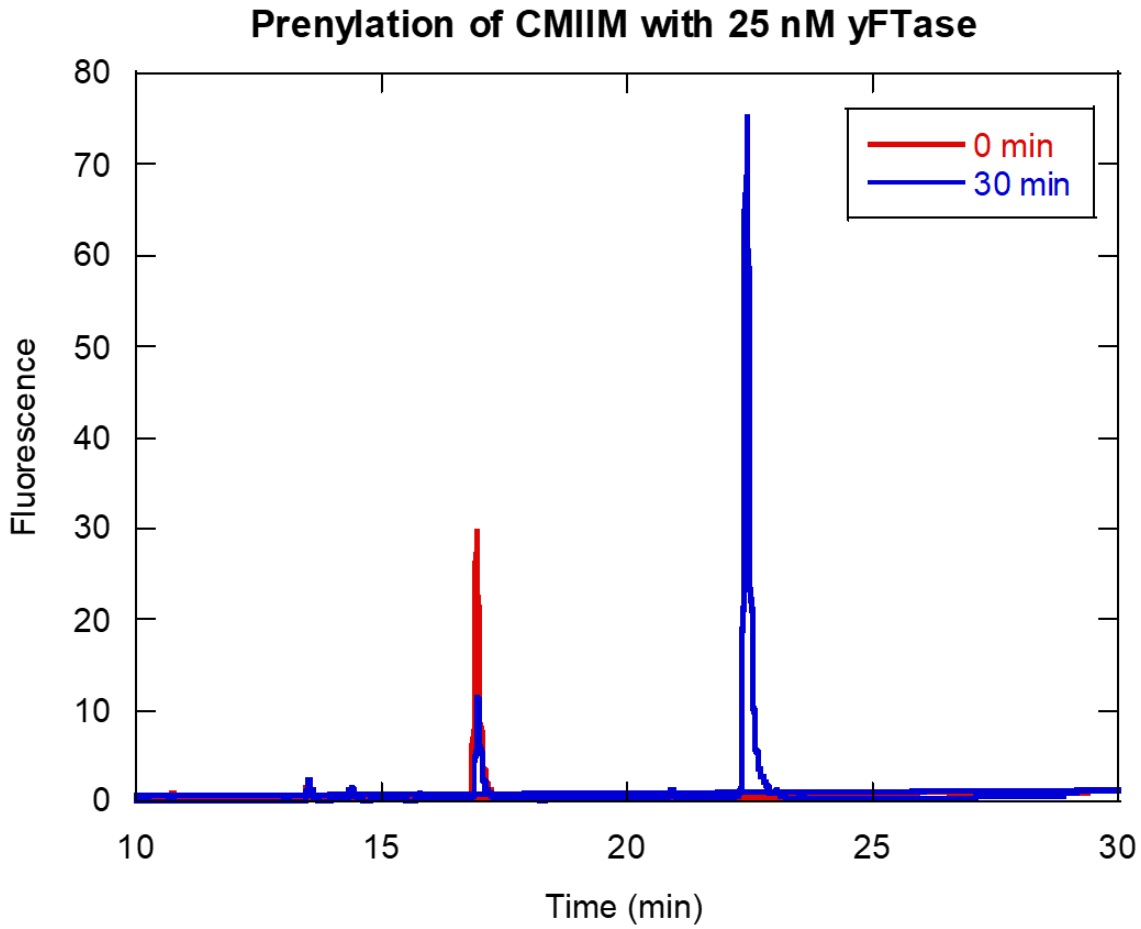
Use of MALDI to observe prenylation in peptide libraries based on CMIIM - CXIIM



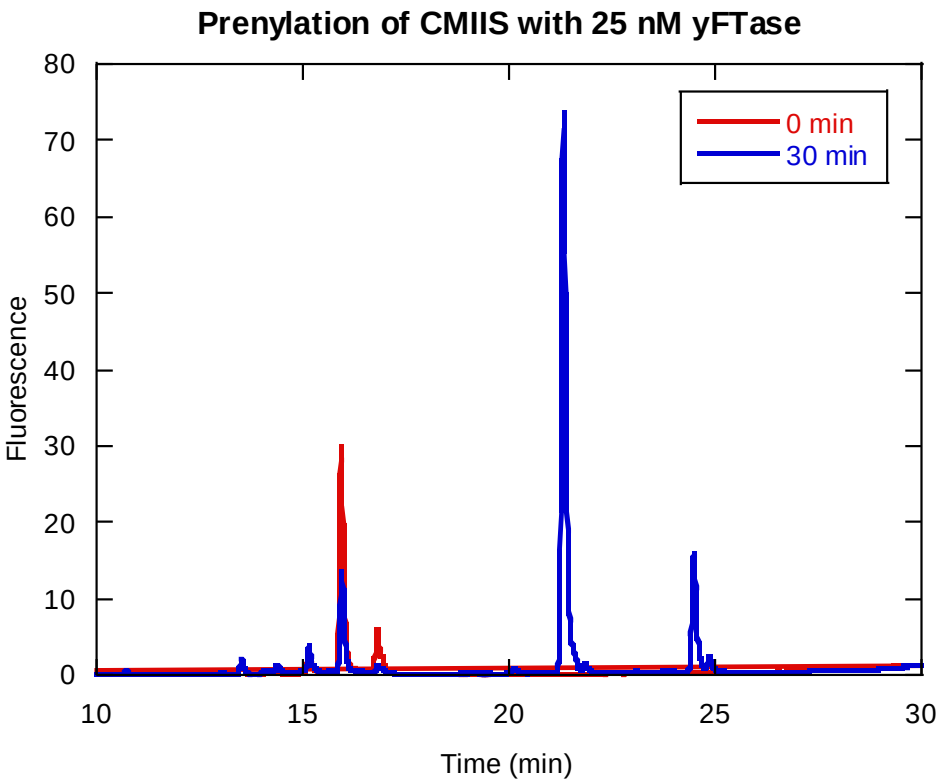
30 new potentially prenylated CaaaX sequences were discovered

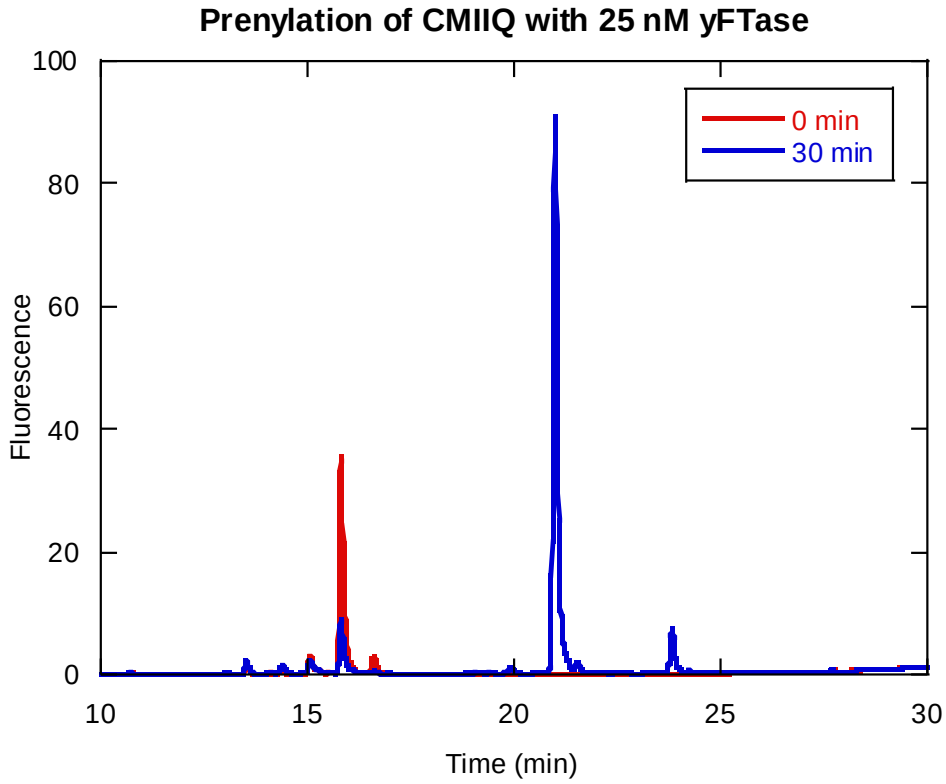
Library sequence	Observed amino acid hits
CXIIM	S, C, M, F, Y, A, P, Q, E, H
CMXIM	G, S, N, K, Q, E, H, R
CMIXM	G, N, M, A, T, L, Q, E, H
CMIIX	S, C, K, A, Q, M

Detecting HPLC retention time shifts of prenylated peptides by fluorescence

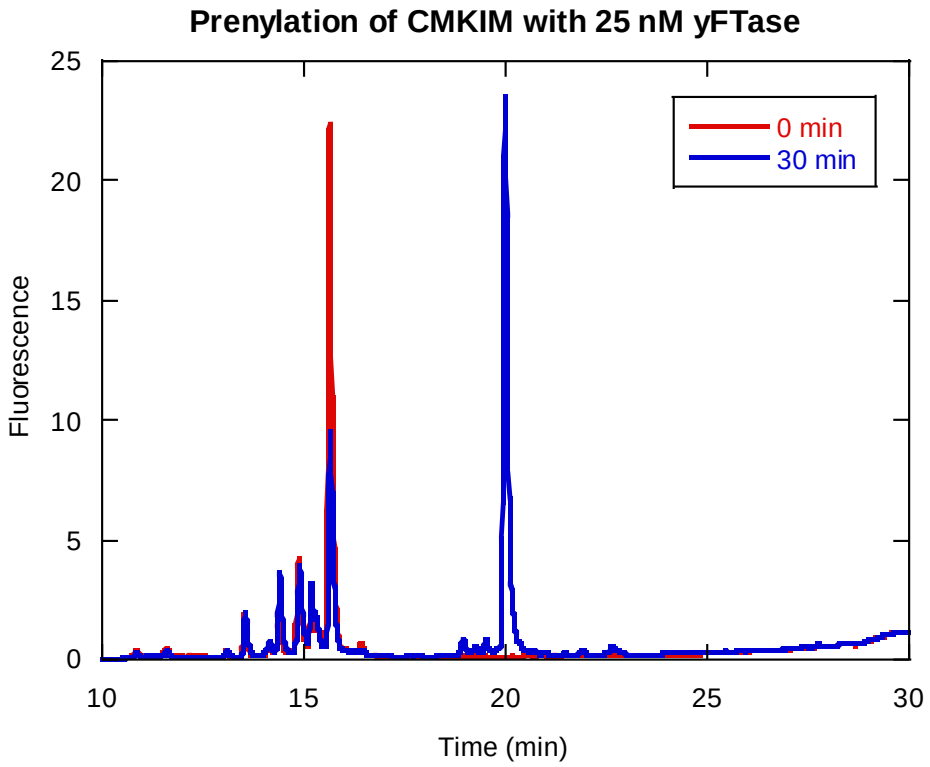


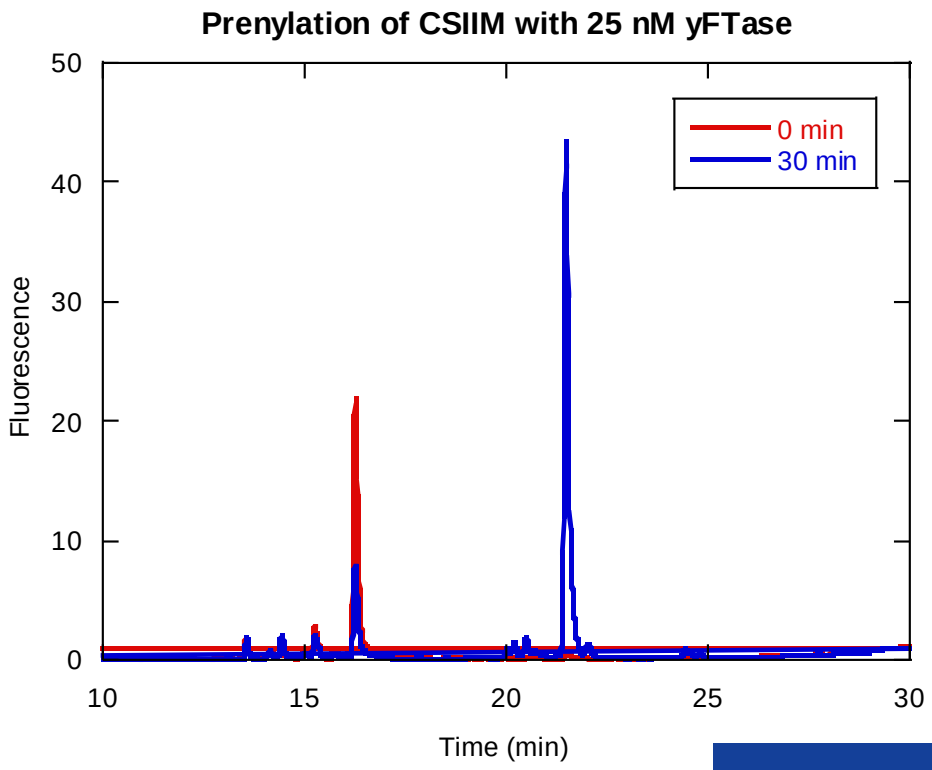
CMIIS and CMIIQ showed excellent conversion to prenylated product





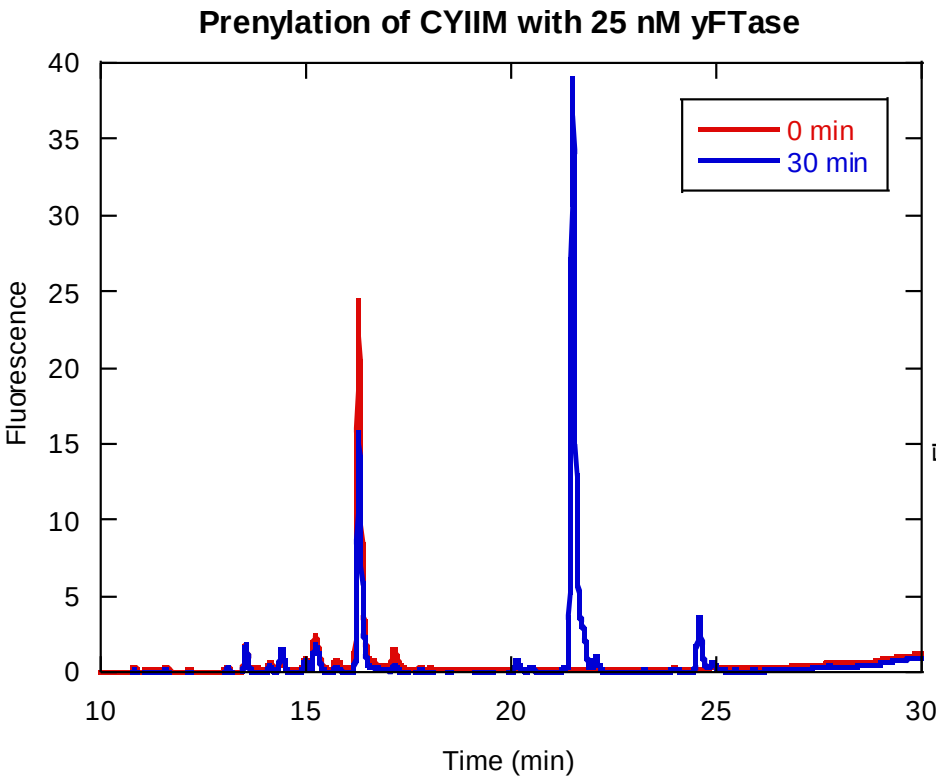
CMKIM and CSIIM showed excellent conversion to prenylated product

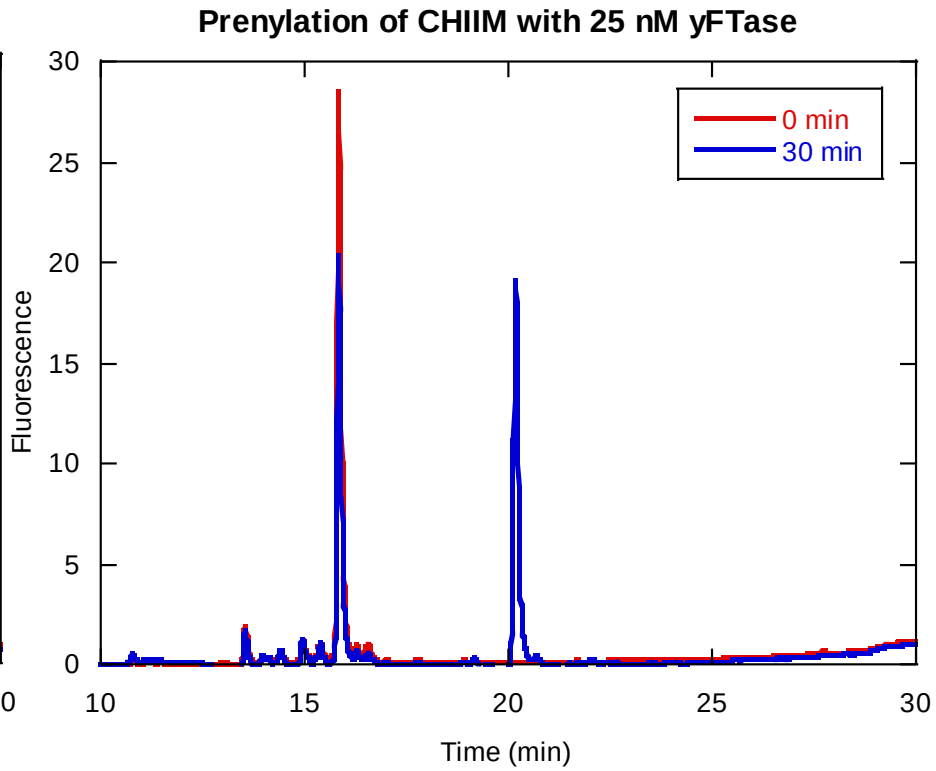




CPPC 2021

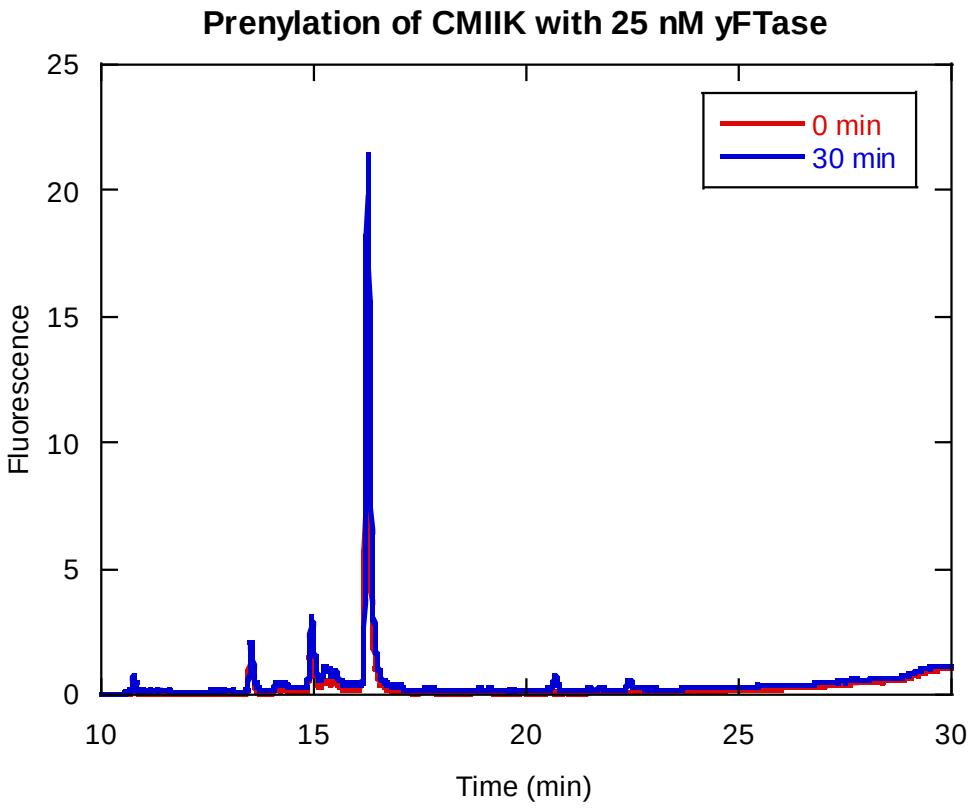
CYIIM and CHIIM showed some conversion to prenylated product

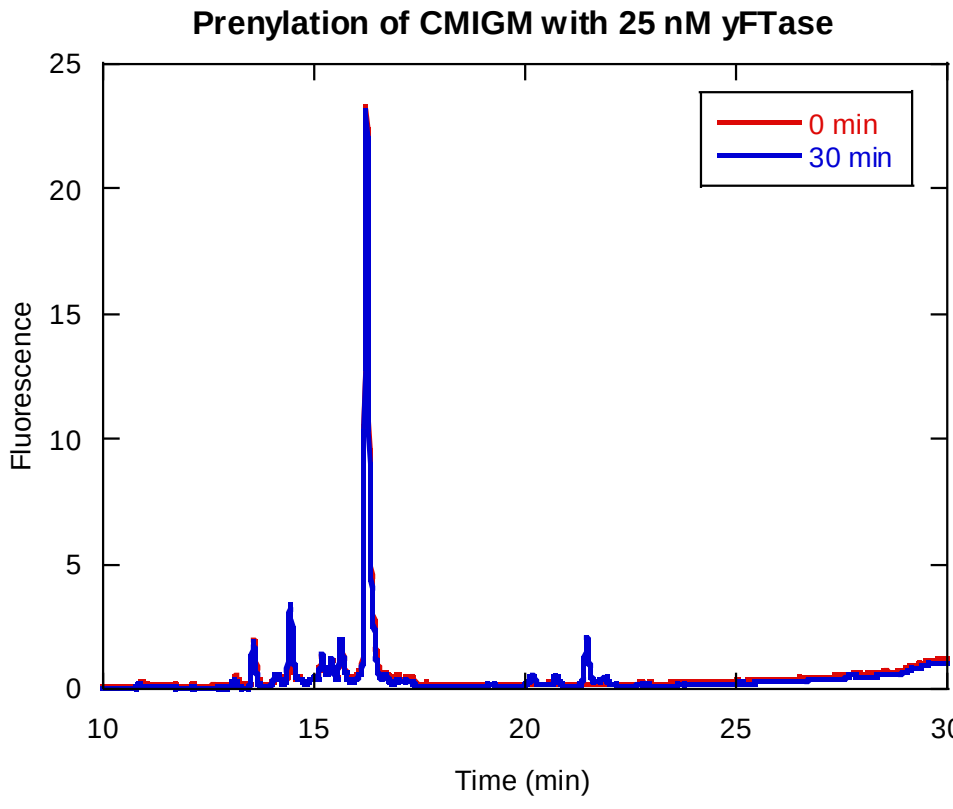




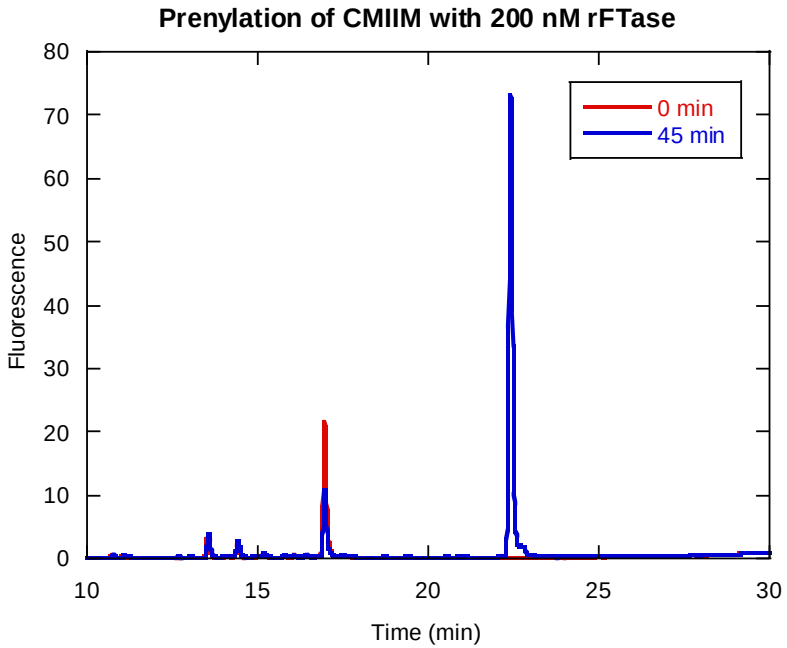
CPPC 2021

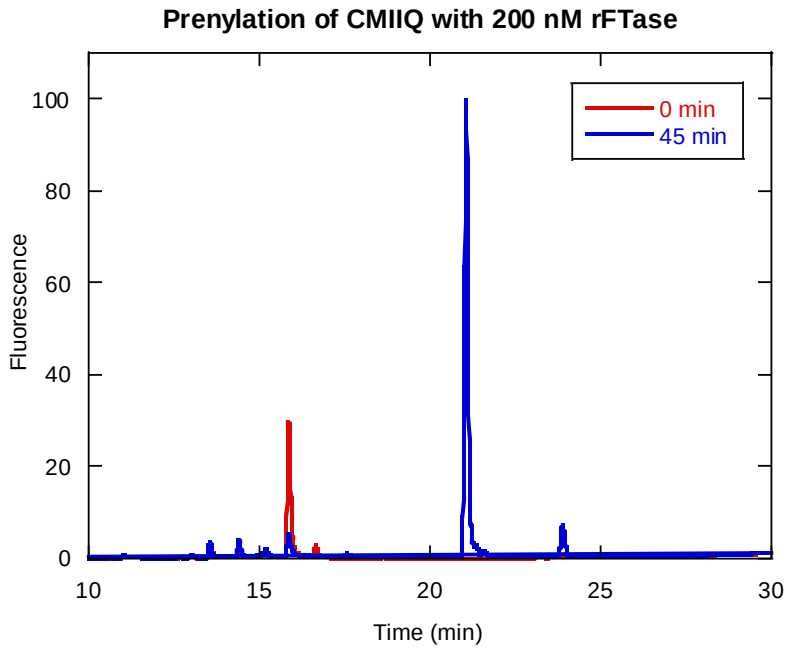
CMIIK and CMIGM did not show conversion to prenylated product





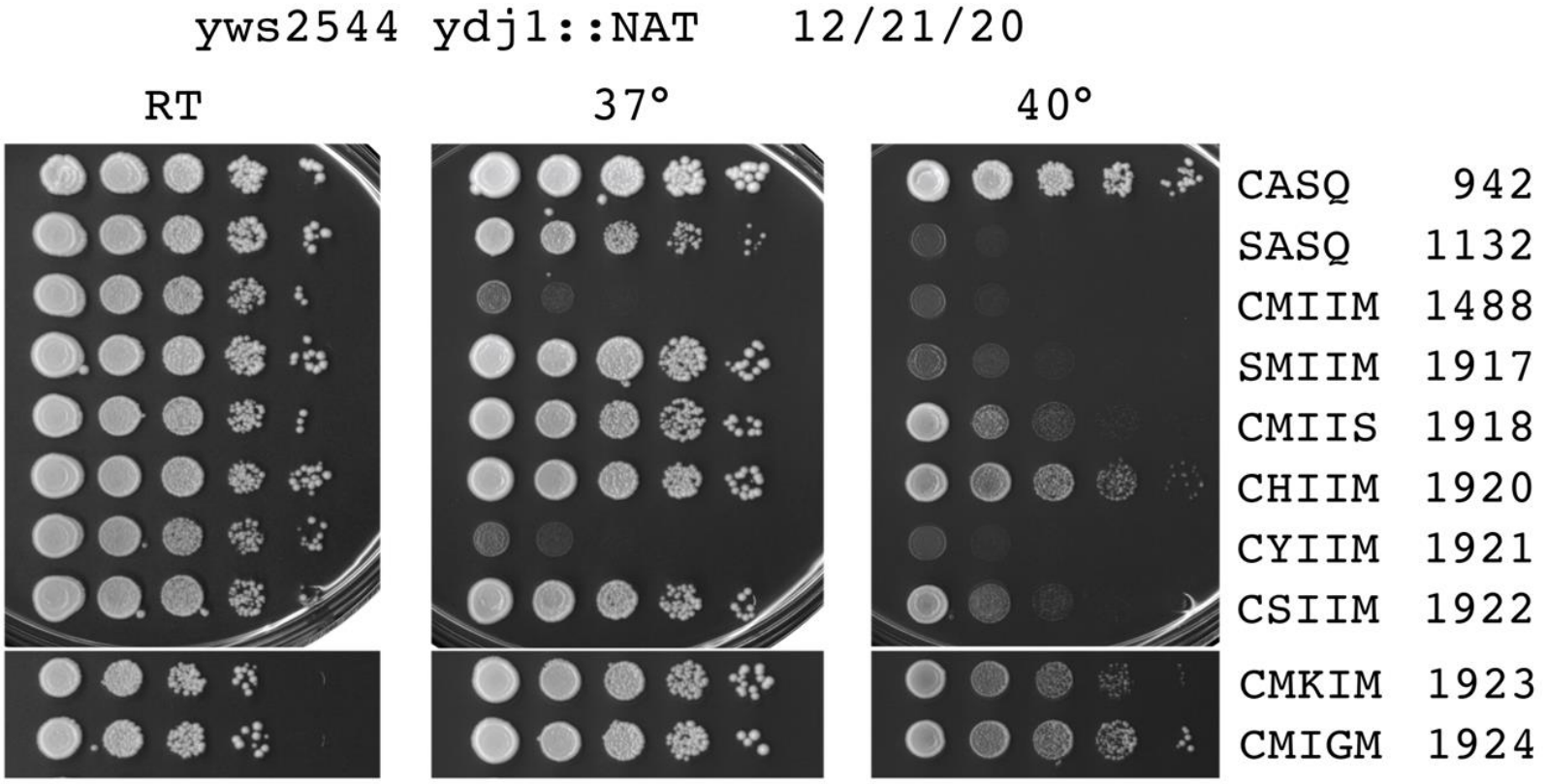
Only two of the CaaaX peptides were substrates of rat FTase





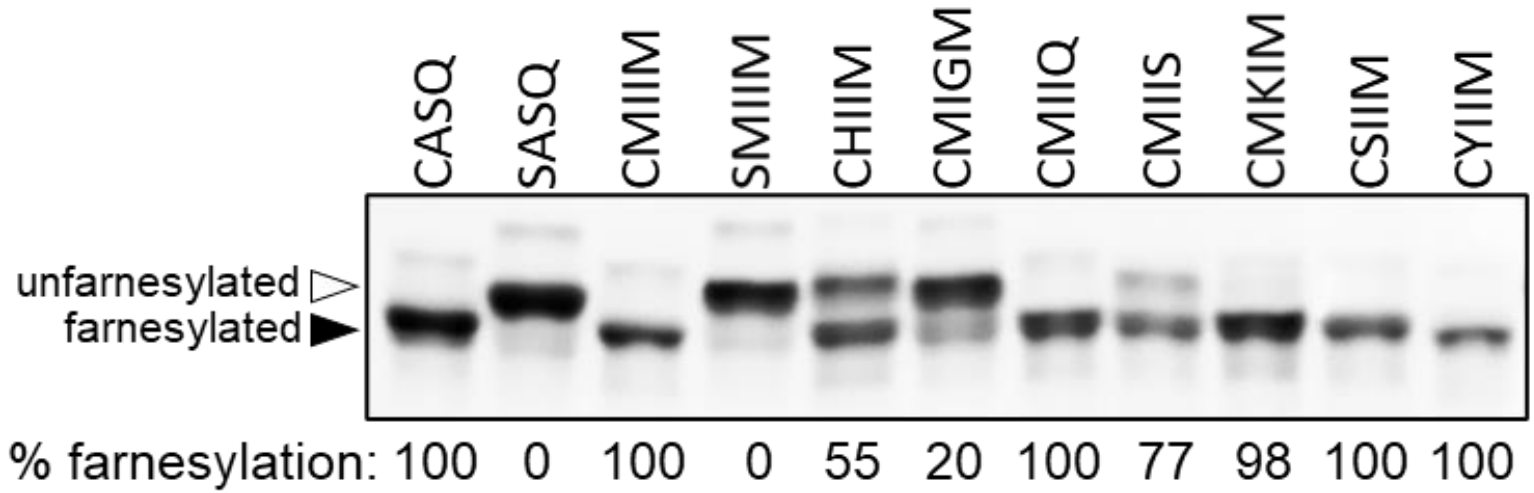
% conversion	CMIIM	CMIIS	CMIIQ	CMIIK	CHIIM	CYIIM	CSIIM	CMKIM	CMIGM
25 nM yFTase	56	61	76	N/A	8	31	64	54	N/A
200 nM rFTase	21	5	5	N/A	< 1	< 1	1	1	N/A
200 nM rFTase, 35 C	50	25	80	N/A	5	< 1	1	1	N/A

Thermotolerance of CaaaX hits is variable



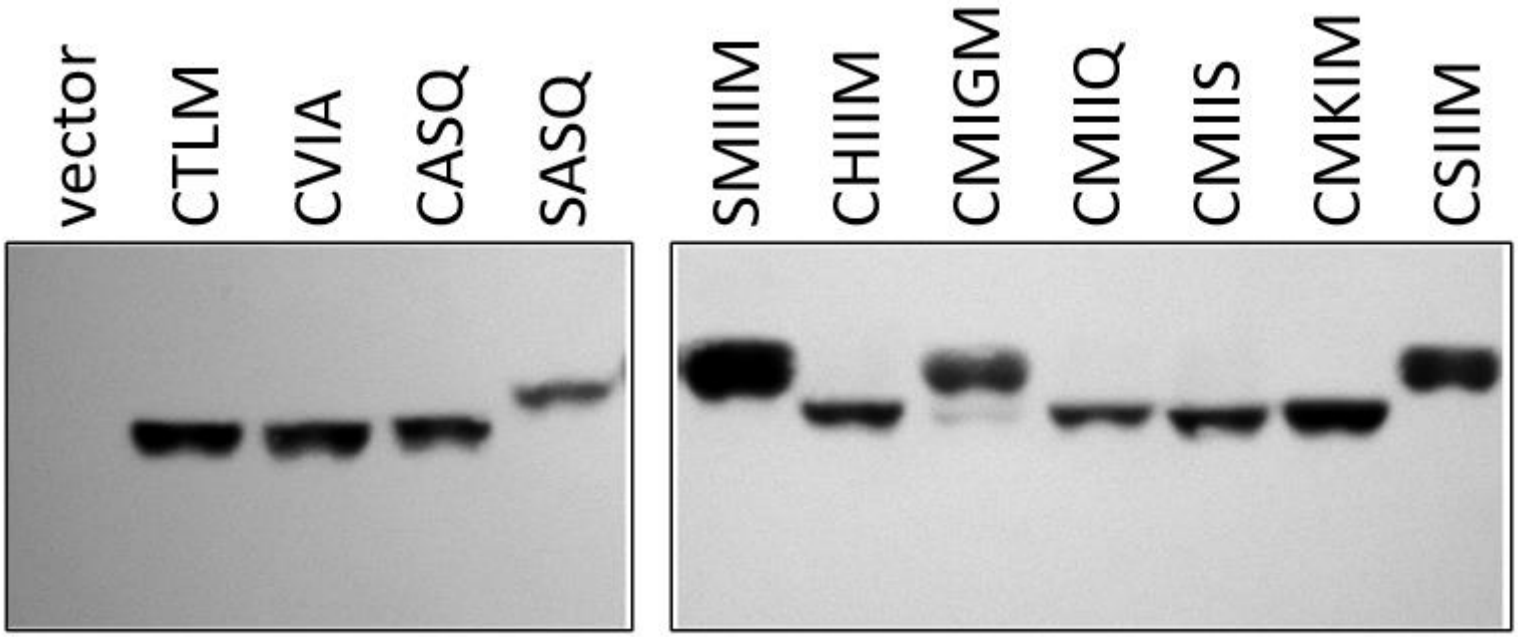
Courtesy of Walter Schmidt

Western Blot Gel shift assay of CaaaX hits in yeast shows prenylation in a biological context



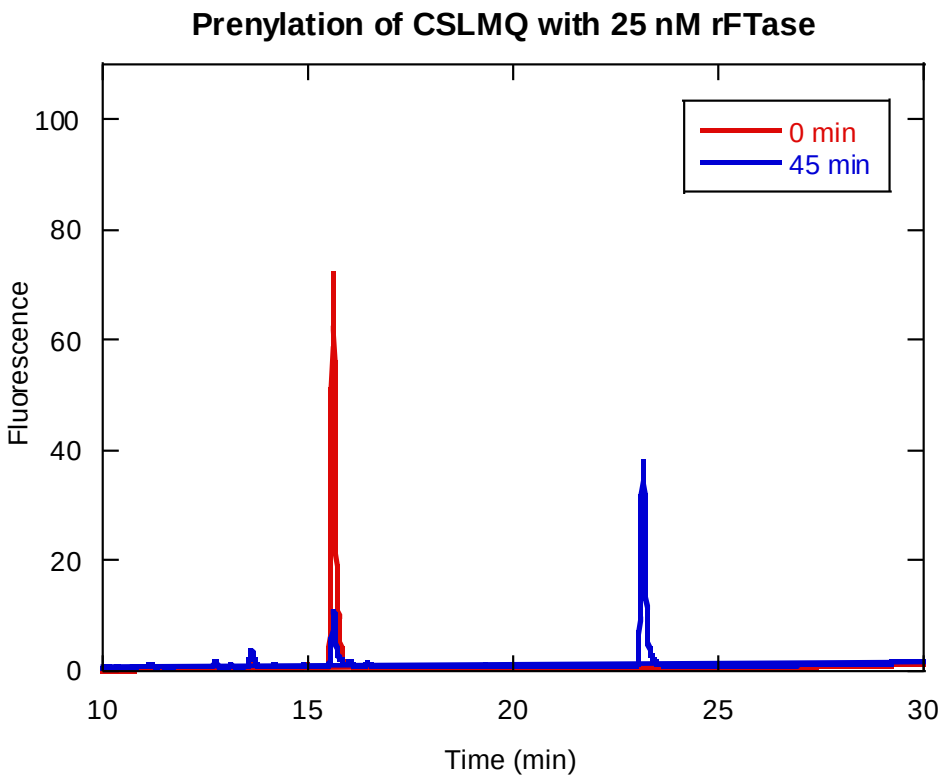
Courtesy of Walter Schmidt

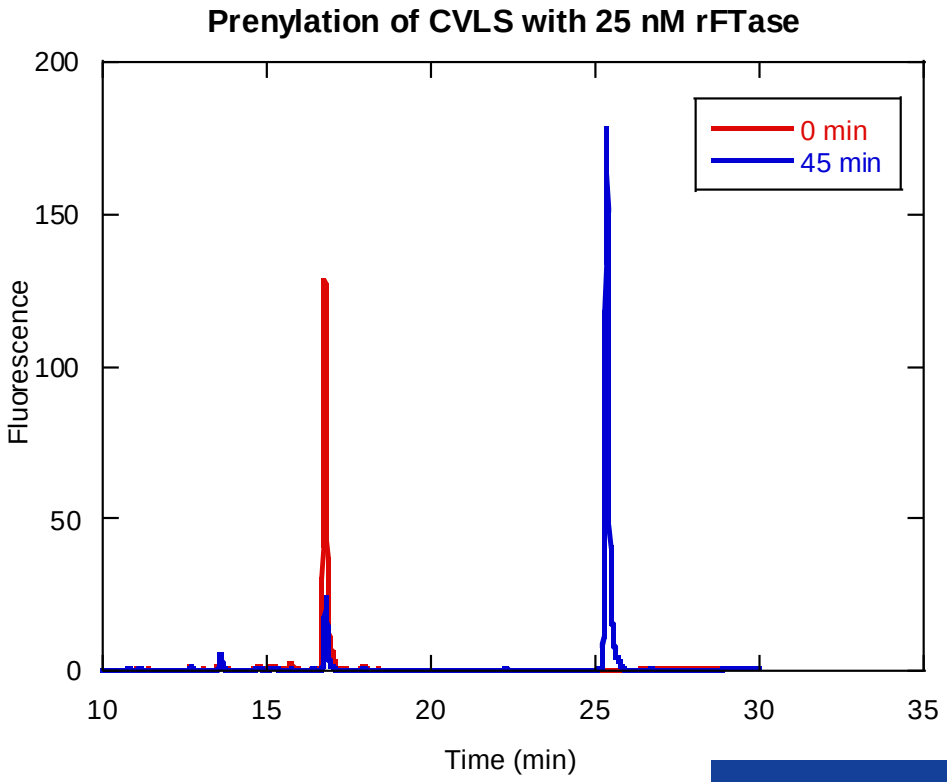
A strain of yeast expressing human FTase also shows excellent conversion of CaaaX hits



Courtesy of Walter Schmidt

The human genome contains several canonical CaaaX sequences which are potentially prenylated *in vivo*





Conclusions and future directions

- 30 potentially novel CaaaX sequences based on CMIIM were discovered using yFTase
- Of the eight hits chosen for follow up, six were found to qualitatively convert with a moderate to excellent efficency, including some with amino acids that are unusual in a CaaX sequence
- There are several potentially prenylated CaaaX sequences in the human genome
  - Next steps will be determining if these proteins are actually prenylated *in vivo*
- This methodology could be used to quickly screen mutant FTases to check for substrate specificity both for peptides and bioorthogonal isoprenoids.

Acknowledgments

- Distefano group
- Peter Buttery
- Schmidt Lab – Georgia
- Hougland Lab – Syracuse
- CPPC committee

CPPC 2021