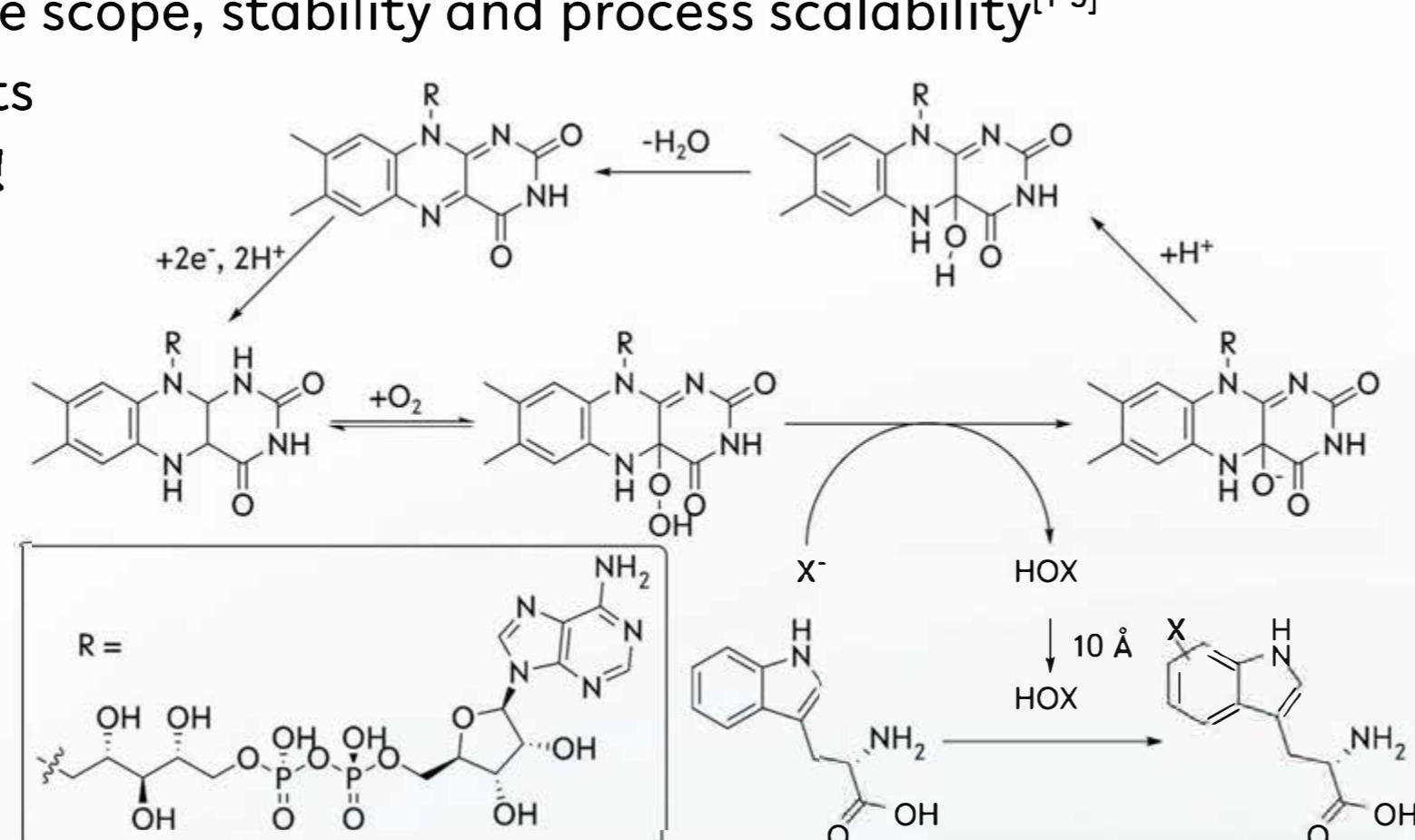


Enzymatic Peptide and Protein Bromination: The BromoTrp Tag

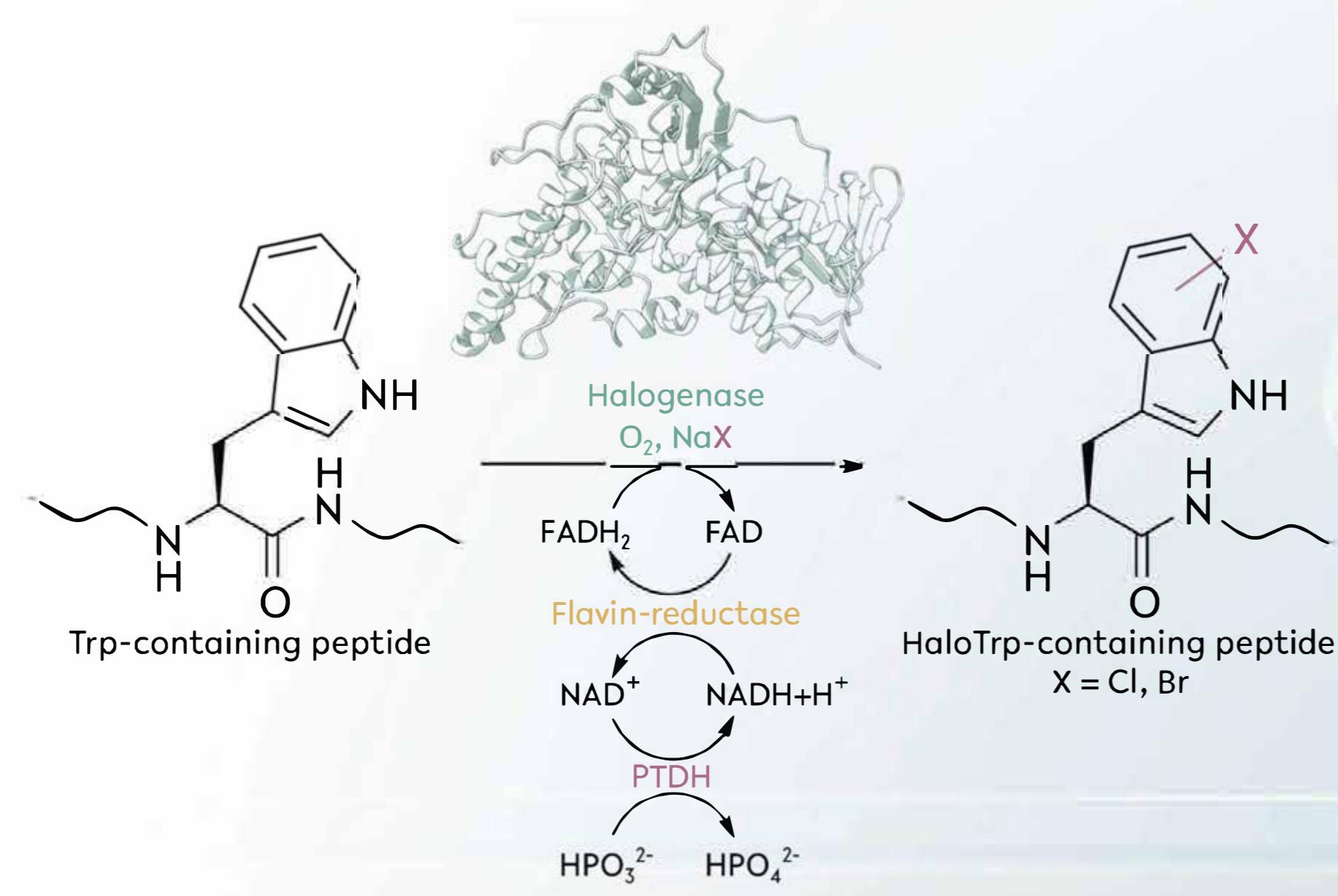
Nicolai Montua¹ and Norbert Sewald¹

Introduction: Flavin-Dependent Halogenases

- Flavin-dependent tryptophan halogenases (FDHs) catalyze the chlorination, bromination or iodination of the Trp-sidechain indole at C5, C6 or C7.^[1,2]
 - Environmentally-friendly conditions: aqueous buffer, neutral pH, halide-salt, ambient temp.
 - Remarkable regioselectivity, no oversubstitution
 - Poor catalytic parameters, narrow substrate scope, stability and process scalability^[1-3]
 - FDHs require FADH₂ in stoichiometric amounts
 - ↳ Cofactor regeneration strategy required!
 - Halotryptophan is part of many bioactive natural products & peptides.^[4,5]
 - Br-Trp: useful chemical handle for transition metal catalyzed cross-coupling reactions



Late-Stage Enzymatic Halogenation



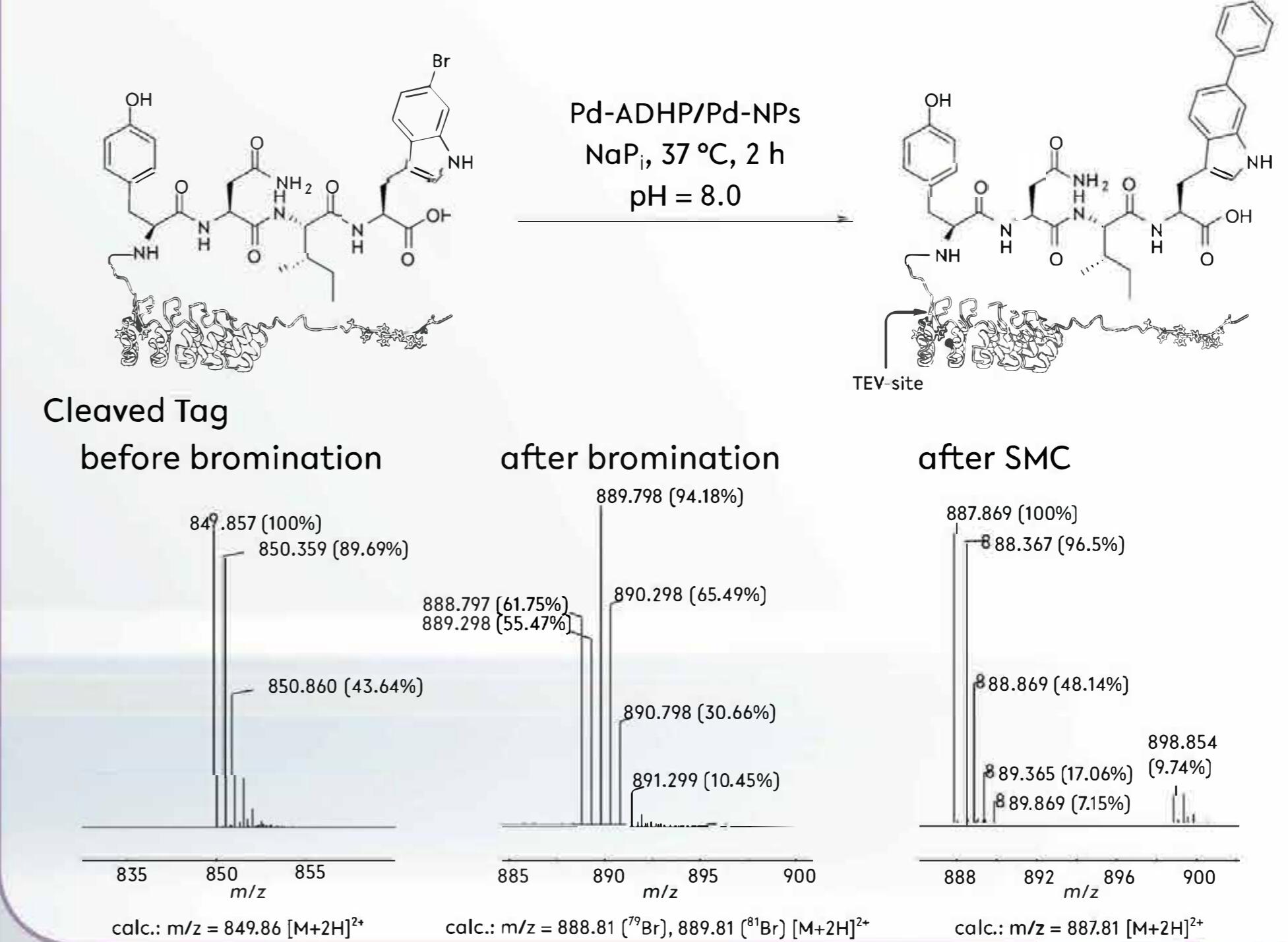
- We previously described effective peptide halogenation of N-terminal Trp-residues.^[6]
 - Halogenation of internal or C-terminal Trp → poor conversion

Summary & Outlook

- Enzyme engineering campaign over three rounds of mutagenesis improved conversion of peptides with C-terminal Trp by up to 25-fold.
 - Regioselectivity for indole-C6 fully retained
 - conversion of N-protected Trp-derivatives also greatly improved
 - triple mutant Thal-AAC → strong bromide preference vs. chloride
 - YNIW as a viable protein tag → "BromoTrp Tag"
 - Post-translational protein bromination
 - ↳ Successful for three model proteins (66-93% conversion)
 - Suzuki coupling of brominated DARPin-E01 with phenyl boronic acid
 - Potential applications:
 - Novel bioconjugation technique (e.g. for ADCs) → SMC yields stable C-C-Bond
 - Fluorogenic protein labelling^[10]
 - Protein macrocyclization,^[12] Palladium-catalyzed protein-protein cross-coupling^[13]
 - Radiobromine labelling^[14] → positron emitters (⁷⁵Br, ⁷⁶Br), Auger emitters (⁷⁷Br, ^{80m}Br)

Bioconjugation by Suzuki-coupling

- Pd-catalyzed Suzuki-Miyaura coupling of brominated DARPin-E01
→ successful with both Pd-ADHP and Pd-nanoparticles.^[8,9]



Protein Engineering of β -Thal:

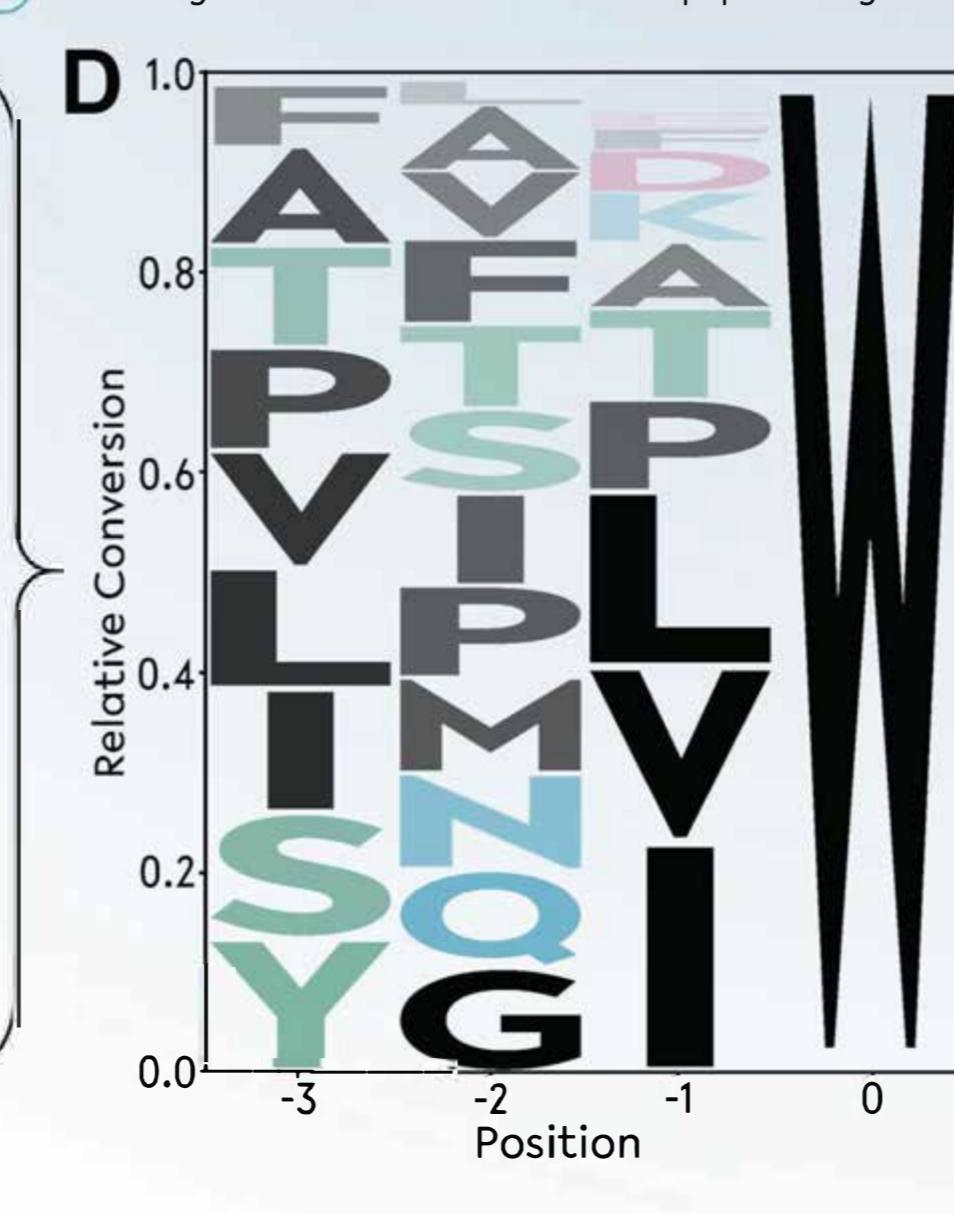
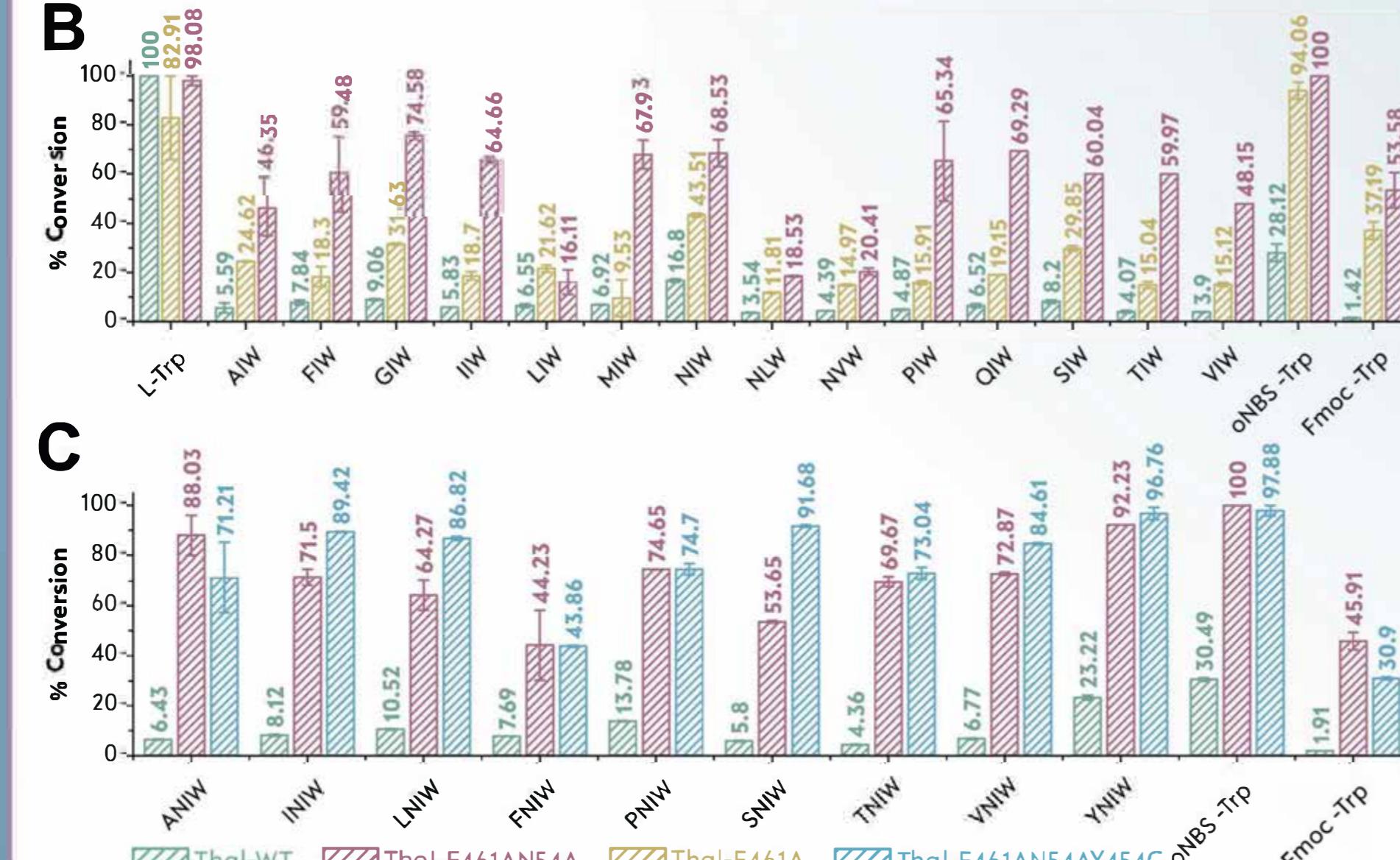
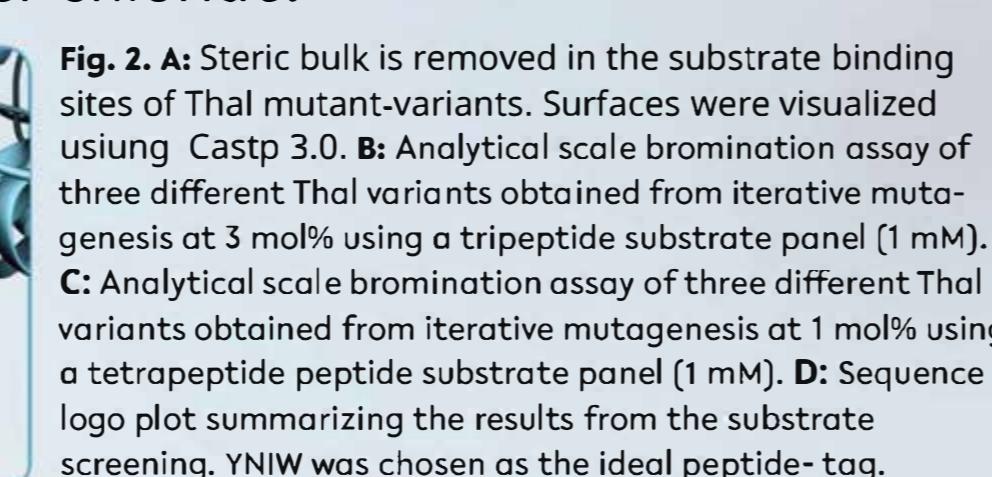
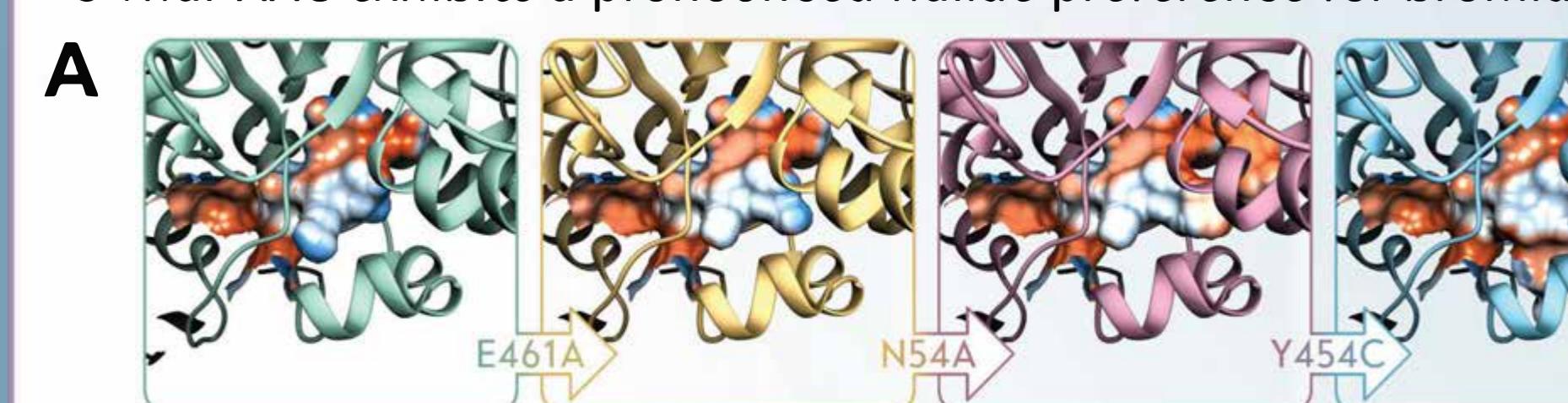
Peptide Bromination?

Protected Trp?

tagged Proteins?

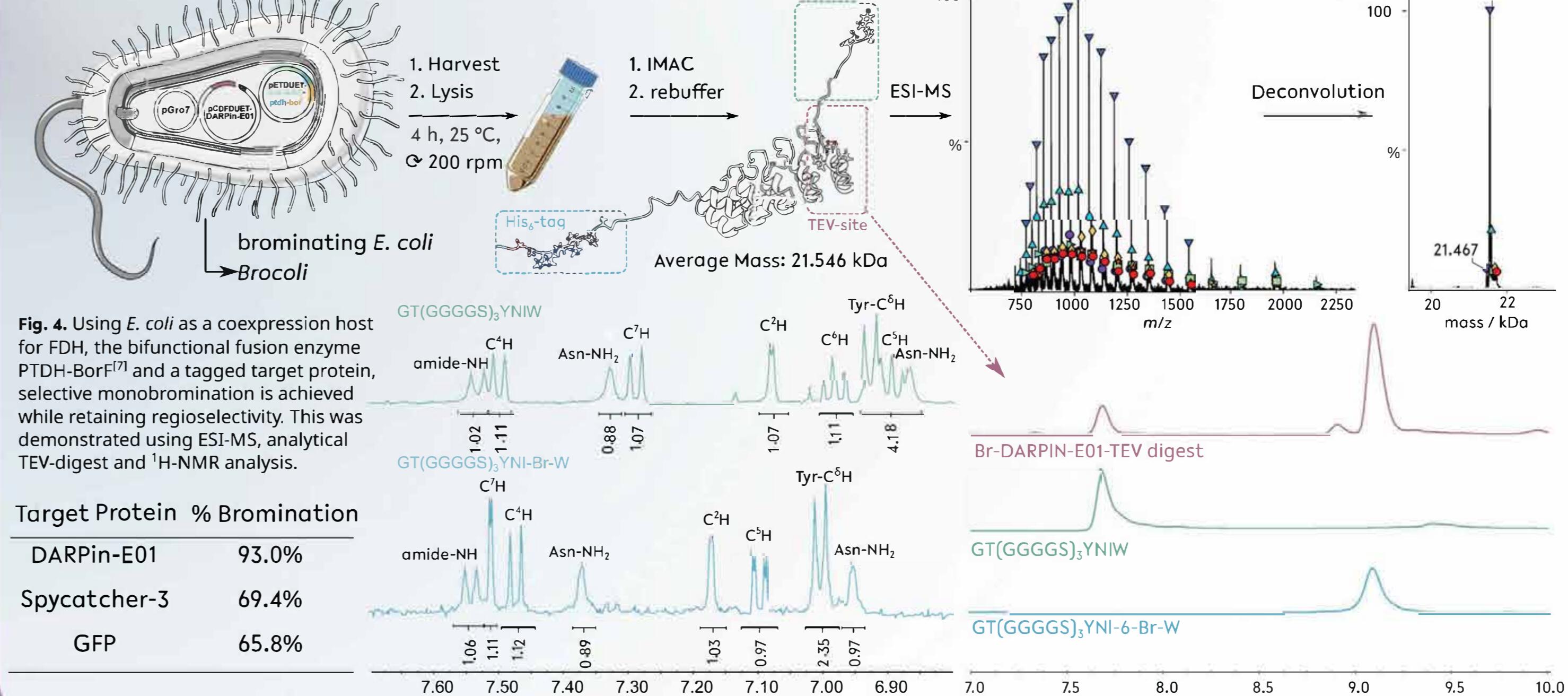
Enzyme Engineering Campaign

- Enzyme engineering extended the substrate scope of Thal to peptides with Trp at the C-terminus and N^{α} -protected Trp derivatives; Conversions improved up to 25 \times using a triple mutant variant of Thal.
 - Thql-AAC exhibits a pronounced halide preference for bromide over chloride.



The BromoTrp Tag: Selective Protein Bromination

- The BromoTrp tag YNIW was appended to model proteins via a flexible linker peptide
→ Coexpression with halogenase cascade in one *E. coli* cell → selective monobromination



References & Acknowledgements

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