Faculty of Chemistry - Organic and Bioorganic Chemistry - OCIII

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Enzymatic Peptide and Protein Bromination: The BromoTrp Tag

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Introduction: Flavin-Dependent Halogenases

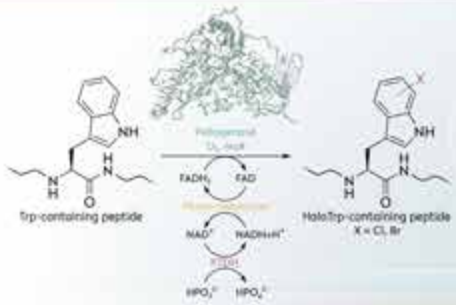
- Flavin-dependent tryptophan halogenases (FDHs) catalyze the chlorination, bromination or iodination of the Trp-sidechain indole at C5, C6 or C7.[1,2]
  - Environmentally-friendly conditions: aqueous buffer, neutral pH, halide-salt, ambient temp.
  - Remarkable regioselectivity, no oversubstitution
- Poor catalytic parameters, narrow substrate scope, stability and process scalability[3-5]
- FDHs require $FADH_2$ in stochiometric 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

Fig. 1. Mechanism of FDH-catalyzed halogenation. $FADH_2$ reacts with molecular oxygen, forming a C4a-hydroperoxyflavin intermediate. This is attacked by a halide ion ($X^- = Cl^-$, Br^-, I^-) to generate hypohalous acid as an activated halogen transfer reagent. After diffusion through an approx. 10 Å-long tunnel, catalytically essential Lys and Glu residues further activate HOX to generate the active halogenation species. Aryl halogenation proceeds via electrophilic aromatic substitution.

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 (^{75}Br, ^{76}Br), Auger emitters (^{77}Br, ^{80m}Br)

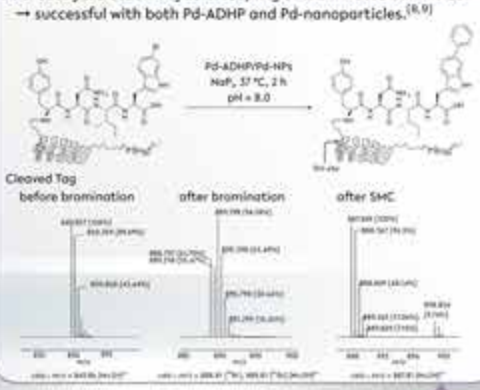
Late-Stage Enzymatic Halogenation



- We previously described effective peptide halogenation of N-terminal Trp-residues.[9]
- Halogenation of internal or C-terminal Trp → poor conversion

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 Thal:
Peptide Bromination? ☑
Retented Trp? ☑
tagged Proteins? ☑

Enzyme Engineering Campaign

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

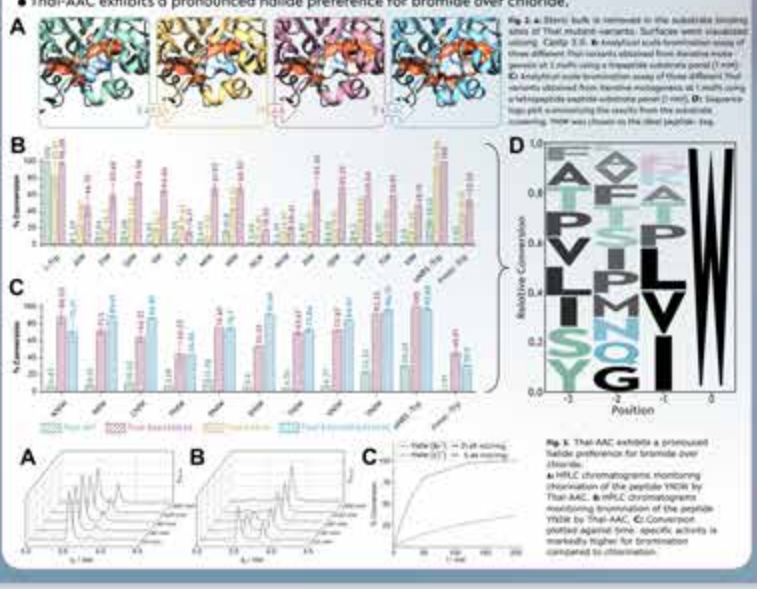


Fig. 3. Thal-AAC exhibits a pronounced halide preference for bromide over chloride. **a** HPLC chromatograms monitoring chlorination of the peptide YNIW by Thal-AAC. **b** HPLC chromatograms monitoring bromination of the peptide YNIW by Thal-AAC. **C** Conversion plotted against time. Specific activity is markedly higher for bromination compared to chlorination.

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

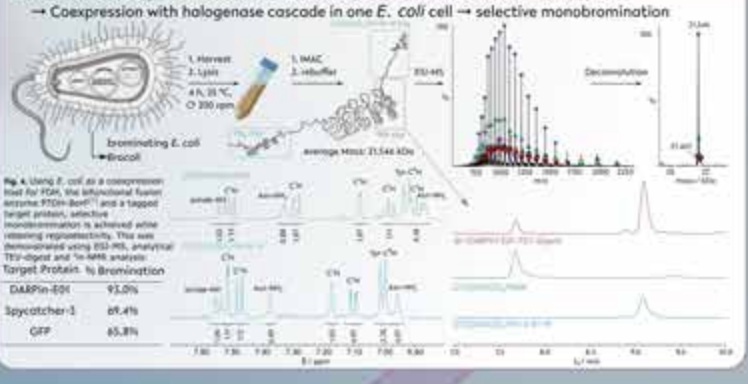


Fig. 4. Using *E. coli* as a coexpression host for FDH, the bifunctional fusion enzyme PltDH-BorH[11] and a tagged target protein, selective monobromination is achieved while retaining regioselectivity. This was demonstrated using ESI-MS, analytical TEV-digest and ^{1}H-NMR analysis.

Target Protein	% Bromination
DARPin-E01	93.0%
Spycatcher-1	69.4%
GFP	65.8%

References & Acknowledgements

Scan to read more!

https://sewaldlab.de