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Medizinische Fakultät

Transcending Amino Acid Limitations: Enzymatic Halogenation for Phage Display Diversification

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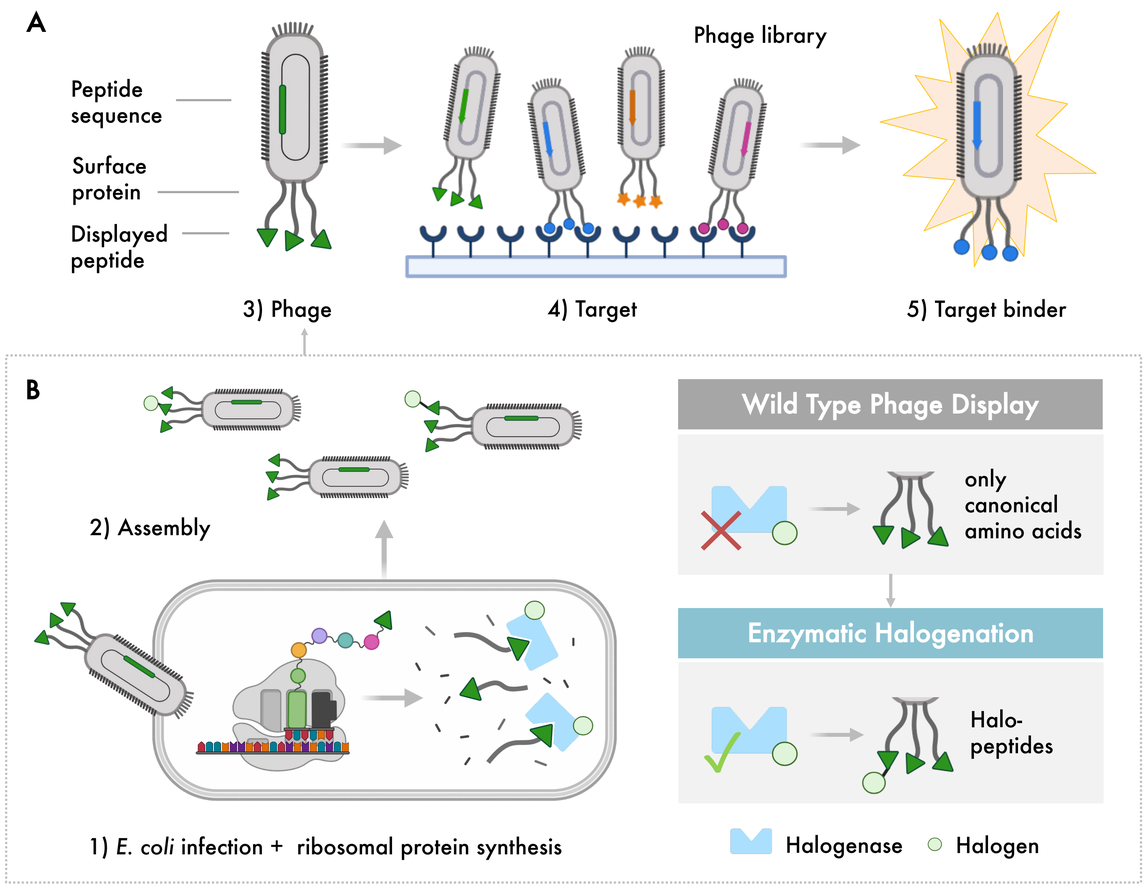
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Towards more drug-like peptides via enzymatic halogenation

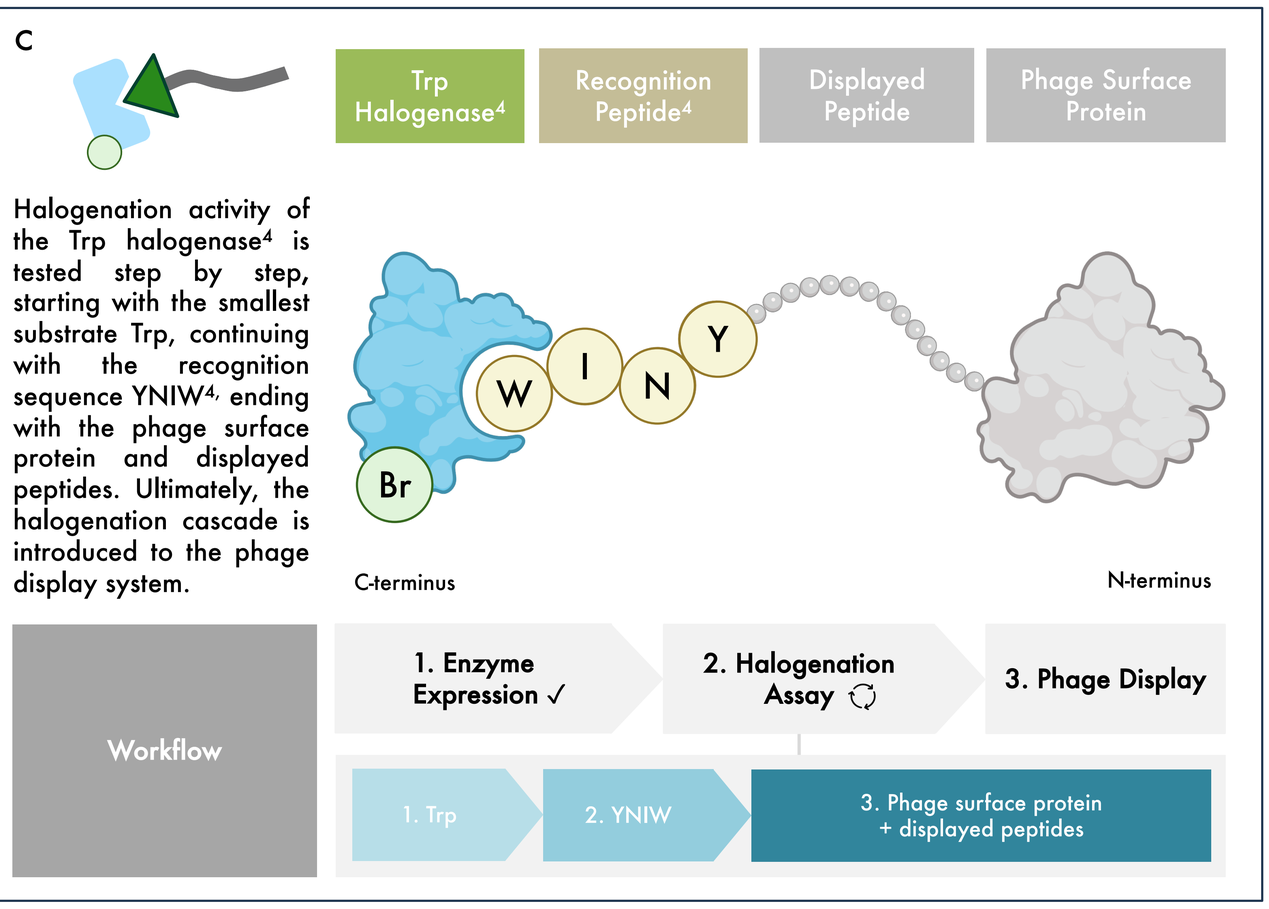
Over the past decades, peptides have gained significant attention in drug discovery due to their favorable pharmacological properties, including high potency, target selectivity, and low toxicity.[1] In this context, phage display has become a pivotal technology, capable of screening huge peptide libraries for target binders.[2] However, phage libraries are restricted to the 20 canonical amino acids, as displayed peptides are synthesized ribosomally.[3] Our study aims to address this challenge via enzymatic halogenation: as recent publications have demonstrated that several halogenases are capable of peptide or protein halogenation,[4] we hypothesize that selective halogenation of peptides displayed on phages is achievable.

Once established, this method could offer significant advantages over existing approaches: (1) site-selective halogenation without altering phage particles and (2) halogenated peptides as valuable intermediates for further modifications of phage display.

Ribosomal synthesis limits phage libraries to canonical amino acids

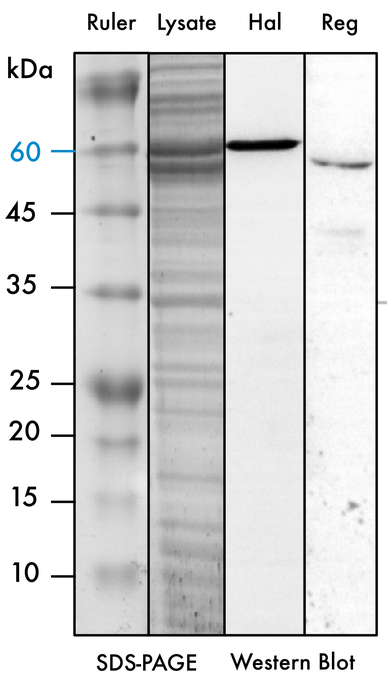


Enzymatic halogenation is assumed to make bromopeptides available

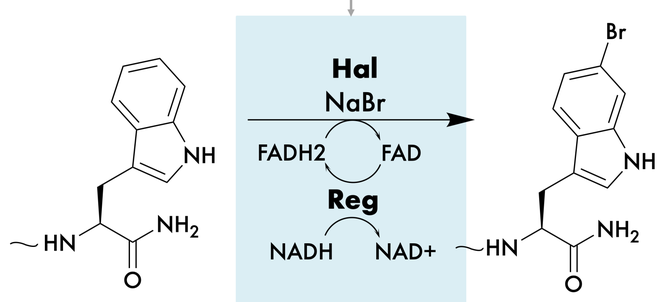


Halogenation assays show halogenation of Trp & recognition peptide

1. Enzyme Expression ✓ → 2. Halogenation Assay



Coexpression Lysate[5]
No purified proteins, contains proteases



Hal	Halogenation[4]	62 kDa
Reg	Cofactor Regeneration[4]	59 kDa

Halogenase & cofactor regeneration enzymes are co-expressed in *E. coli*. The cell lysate is directly used for the halogenation assay, therefore containing e.g. proteases as byproduct.[5]

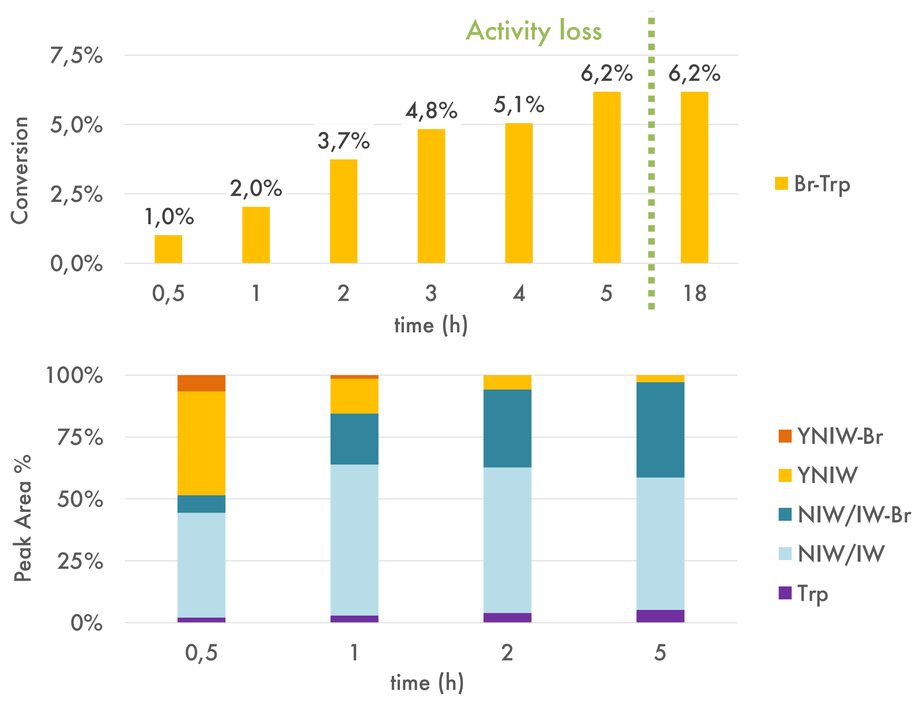
1. Trp

Halogenation ✓
Low conversion
Activity loss after 5h

2. YNIW

Halogenation ✓
Lysate proteases degrade peptides

Phage surface protein



Trp and YNIW are succesfully halogenated. Activity loss after 5h is in line with reported low total turnover number of halogenase family. WT Trp Halogenase was mutated in favor of YNIW, explaining low Trp halogenation. Protease degradation is assumed to not be a limitation in phage surface protein halogenation assays.

References
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[5] Montua & Sewald, ChemBioChem 2023, 24, e202300478.

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