Rapid kinetic evaluation of diverse chemical transformations on bacteriophage-displayed peptides

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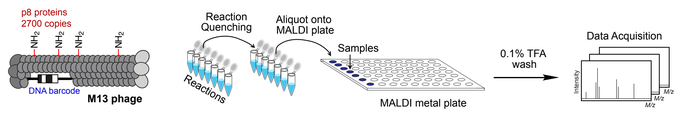
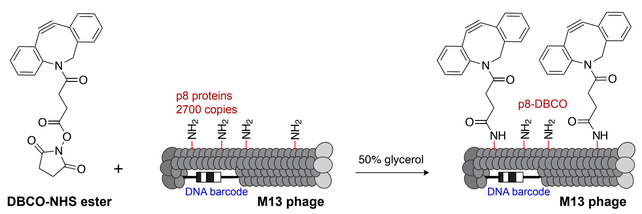
Introduction

Chemically modified genetically encoded peptide libraries encompass broader chemical space that enables rapid discovery of proteolytically resistant and potent drug leads. However, evaluating chemical modifications on phage-displayed peptide remains challenging due to their ultra-low concentrations. Despite extensive efforts to assess these modifications—such as using ESI-MS for identifying modifications of phage-displayed peptides[1] or using MALDI-TOF-MS to detect chemical transformations on p8-displayed peptides[2]—current methods are often time-consuming or impractical for certain applications, hindering the development of new modification strategies for generating chemically modified peptide libraries in drug discovery. Herein, we propose a strategy that enables rapid display of peptides regards of sequence and size on phage and offer convenient kinetic evaluation of chemical transformation on phage-displayed peptide. In this work, DBCO was installed on p8 proteins of M13 phage, followed by "click" chemistry attachment of chemically synthesized azido peptides. Subsequently, the kinetics of diverse chemical transformations on phage-displayed peptide were evaluated using MALDI-TOF-MS, highlighting the extreme convenience and rapidity of this method. All modification, detection, and purification (if needed) steps can be performed in parallel, with the potential for fully automation, significantly advancing the development of new chemical modification strategies of peptide libraries.

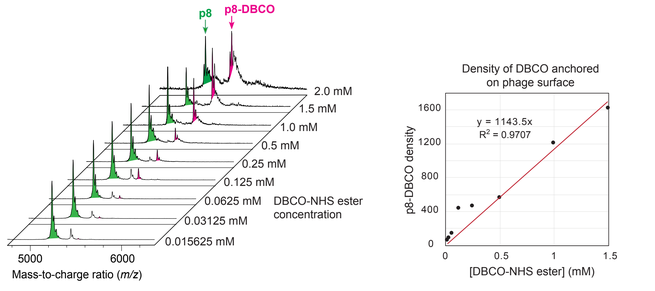


Grafting peptide onto p8 proteins

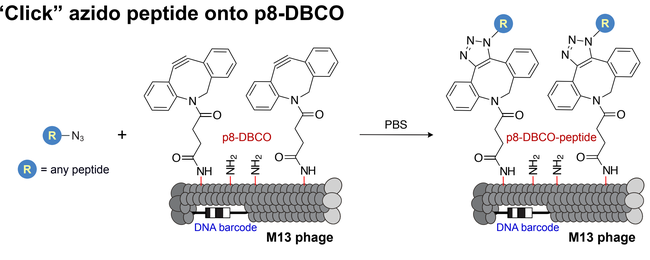
Installation of DBCO onto p8 protein of M13 phage

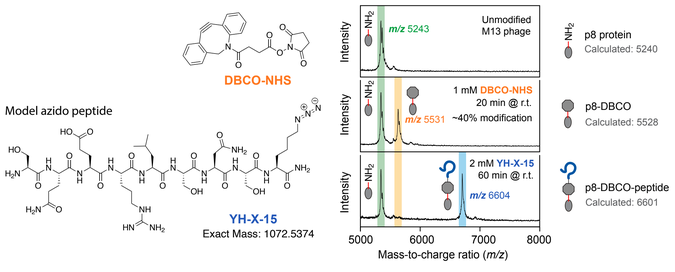


Fine-turning of DBCO density anchored on phage



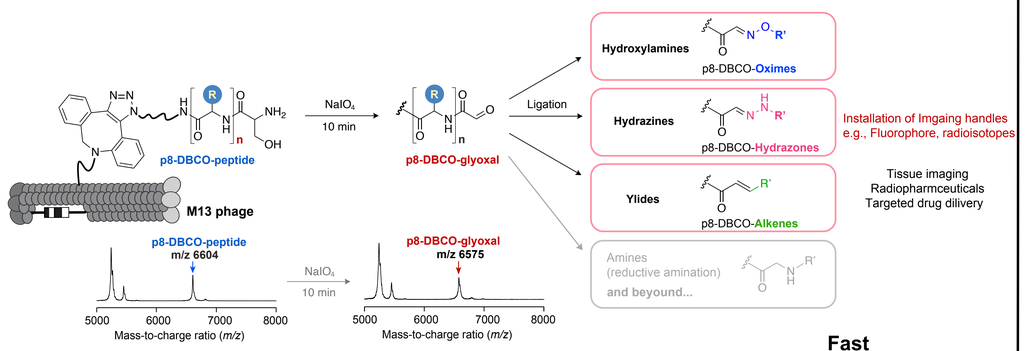
"Click" azido peptide onto p8-DBCO





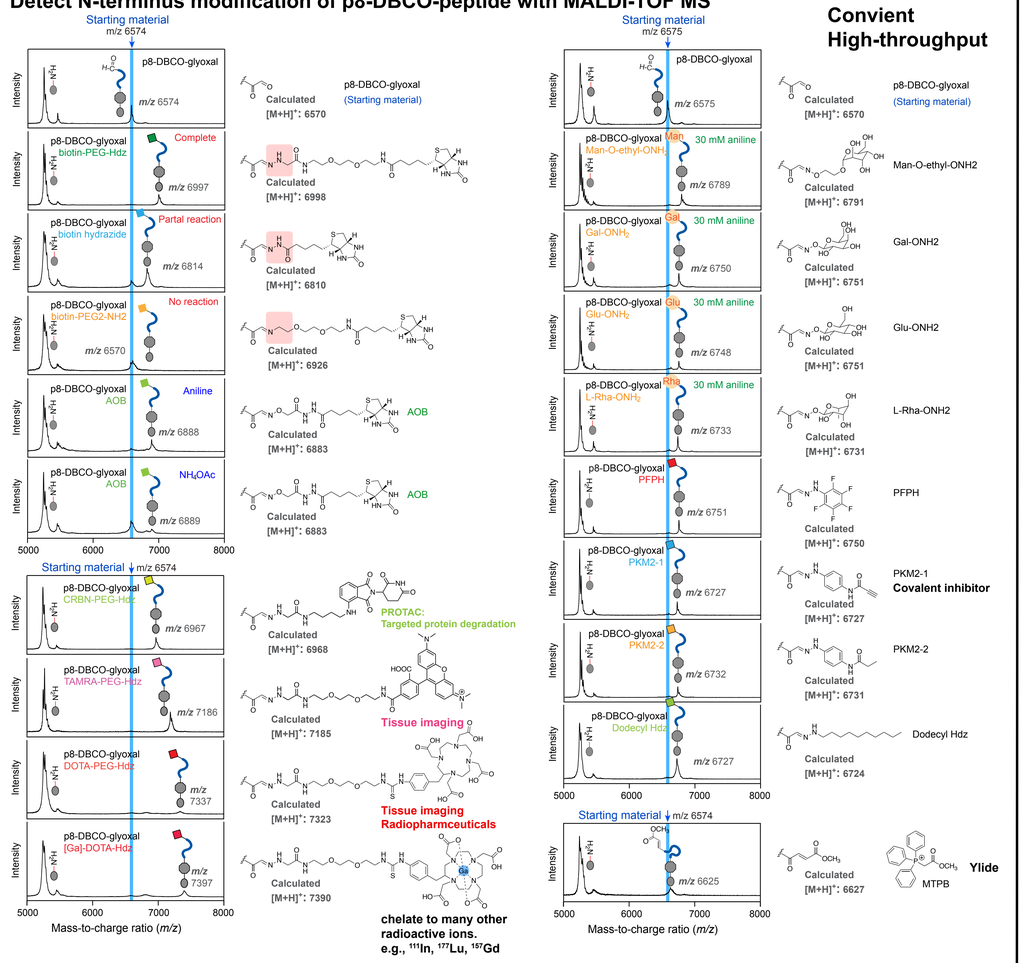
Diverse modification of chemically-displayed peptide

Diverse N-terminus modification on p8-DBCO-peptide (an easy-start)



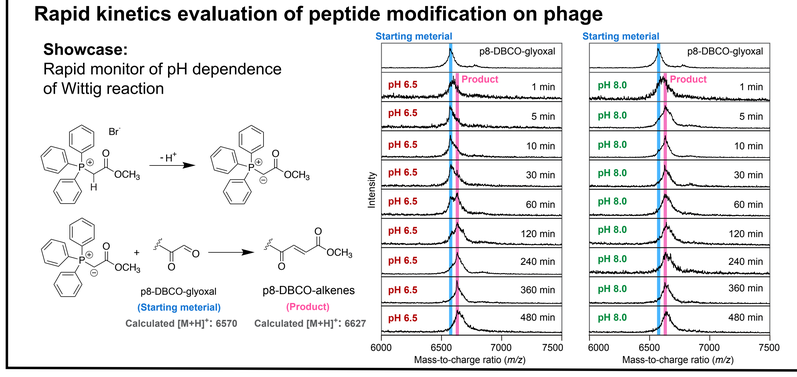
Detect N-terminus modification of p8-DBCO-peptide with MALDI-TOF MS

Fast
Low-cost
Convient
High-throughput



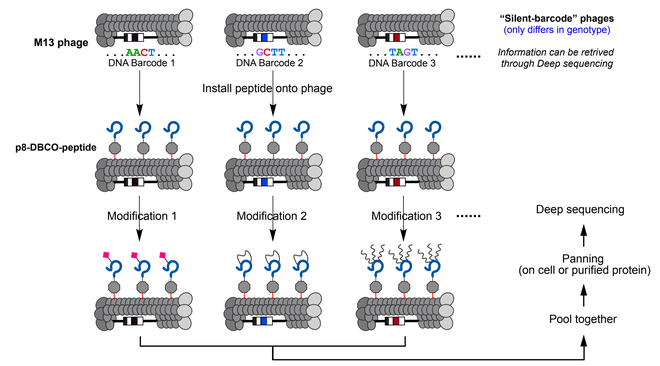
Rapid kinetics evaluation of peptide modification on phage

Showcase:
Rapid monitor of pH dependence of Wittig reaction

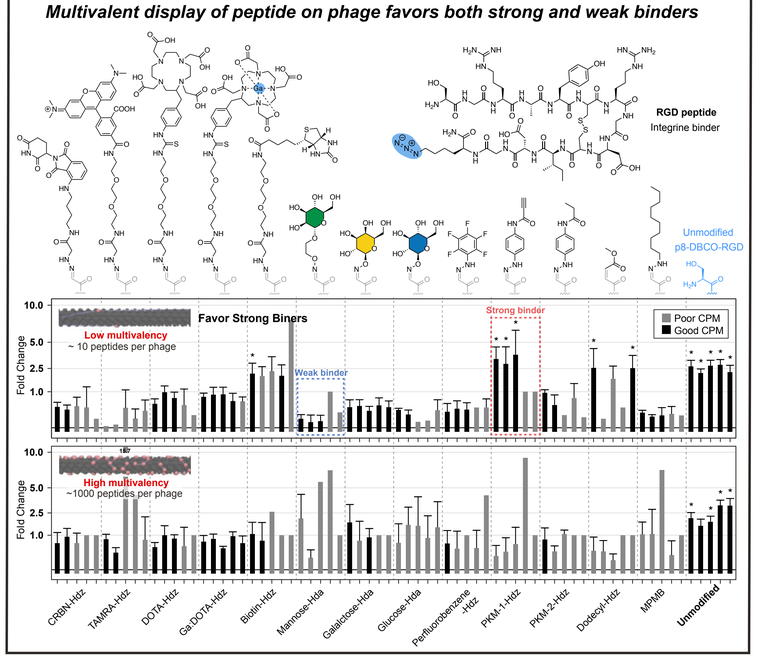


Effect of modifications on binding

Study the effect of different modifications to peptide-protein interactions



Multivalent display of peptide on phage favors both strong and weak binders



Conclusions

1) Chemically display peptides on p8 proteins provided a rapid and high-throughput way to study reaction kinetics on peptides.

2) Multivalency has significant influences on the interaction of integrine with p8-displayed RGD-peptides. Low multivalence tends to favoring strong binders and high multivalency favors both strong and weak binders.

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References

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3. Ng, S.; Tjhung, K. F.; Paschal, B. M.; Noren, C. J.; Derda, R. Chemical Posttranslational Modification of Phage-Displayed Peptides. Peptide Libraries: Methods and Protocols 2015, 155, 155–172.