Literature report

Genetically Encoded Cleavable Protein Photocrosslinker

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Protein-protein interactions

- **FRET**
- **Pull-down**
- **Co-IP**
Protein-protein interactions

- Protein photocrosslinking weak and transient interactions

The CAPP (Cleavage-and-capture After Protein Photocrosslinking) strategy
A general procedure for protein photocrosslinking using a cleavable photocrosslinker.
DiZSeK: $\text{N}^\varepsilon$-3-(3-methyl-3H-diazirine-3-yl)-propaminocarbonyl-$\gamma$-seleno-L-lysine

Chen, P. R. Nat. Chem. Biol. **2011, 7, 671-677.**
Chen, P. R. J. Am. Chem. Soc. **2011, 133, 20581-20587.**
Why?

1. Unnatural amino acid
2. DiZSeK
Test and Verify

- the Pyrrolysine tRNA-synthetase (PylRS)- tRNA\textsubscript{CUA}\textsuperscript{Pyl} pair

- ESI-MS

1. The DiZSeK is incorporated into GFP-N149DiZSeK specifically and accurately.
• The H$_2$O$_2$-mediated oxidative cleavage

2. The GFP-N149DiZSeK can be converted to GFP-N149Dha
the photocrosslinking efficiency with DiZSeK or DiZPK incorporating in model HdeA protein.

3. The DiZSeK probe showed photocrosslinking efficiency similar to that of DiZPK photocrosslinker.
4. The $\text{H}_2\text{O}_2$-mediated cleavage reaction may be used on the Se handle of DiZSeK in order to yield an efficient separation of bait and prey proteins after photocrosslinking.
CAPP: proof-of-concept

- Design a covalently linked GFP-Biotin conjugated system as the “prey-bait” model
• LC-MS/MS to detect the desired cleaved products
The CAPP strategy exhibits high efficiency in capturing the in situ generated prey proteins after cleavage of the crosslinked prey-bait complexes.
Application

- direct profiling of in vivo binding proteins of HdeA under acid stress by using the DiZSeK probe and CAPP strategy in conjunction with 2D-PAGE
The DiZSeK cleavable photocrosslinker, CAPP strategy, and 2D-PAGE offer a powerful tool for the systematic profiling of the interaction protein targets of a given protein in living cells.

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Summary

• Developed a genetically encoded Se-containing cleavable protein photocrosslinker;

• Developed a cleavage-and-capturing of interaction proteins after the photocrosslinking (CAPP) strategy;

• This CAPP strategy, in conjunction with the 2D-PAGE proteomics and MS analysis, is a powerful tool for protein-protein interactions.
Thank you!