Literature report

Genetically Encoded Cleavable Protein Photocrosslinker

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Communication

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Protein-protein interactions



•Co-IP



Protein-protein interactions

 Protein photocrosslinking weak and transient interactions



photo affinity moieties

benzophenones aryl azide diazirine

The CAPP (Cleavage-and-capture After Protein Photocrosslinking) strategy



A general procedure for protein photocrosslinking using a cleavable photocrosslinker.



DiZSeK: N^ε-3-(3-methyl-3H-diazirine-3-yl)-propaminocarbonyl-γ-seleno-L-lysine

Chen, P. R. *Nat. Chem. Biol.* **2011, 7, 671-677.** Chen, P. R. *J. Am. Chem.Soc.* **2011, 133, 20581-20587.** 6

Why?

- 1. Unnatural amino acid
- 2. DiZSeK



Test and Verify

- the Pyrrolysine tRNA-synthetase
 (PyIRS)- tRNA Pyl CUA pair
- ESI-MS



1. The DiZSeK is incorporated into GFP-N149DiZSeK specifically and accurately.

• The H₂O₂-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 H_2O_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



• Quantification by azido-Fluor 488



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.

Number	Protein name	MASCOT score	Molecular weight (Da)	Number of matched peptides	Protein description
Spot 1					
1	UshA	924	60900	13	External UDP-glucose degradase
2	OppA	370	60975	6	Periplasmic oligopeptide-binding protein
3	DppA	132	60483	1	Periplasmic dipeptide transport protein
4	MasY	1022	60521	13	Malate synthase A
5	Syk1	931	57624	17	Lysyl-tRNA synthetase
6	GlpK	404	56480	6	Glycerol kinase
7	Syk2	248	57847	8	Lysyl-tRNA synthetase, heat inducible
8	PyrG	151	60792	5	CTP synthase
9	IlvB	114	60915	2	Acetolactate synthase isozyme 1 large subunit
10	TreC	208	64082	4	Trehalose-6-phosphate hydrolase

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 !