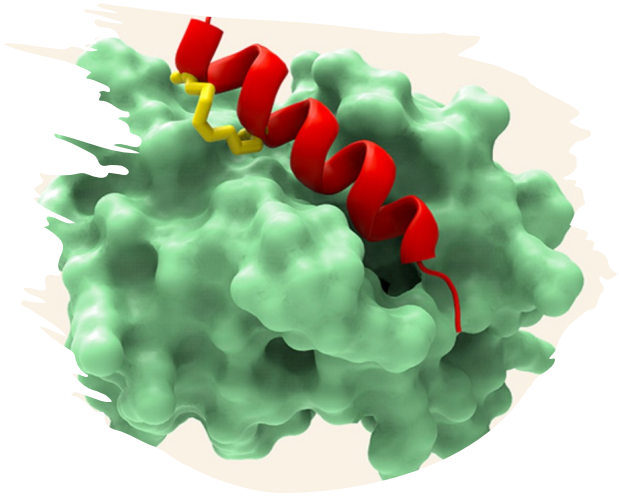
Photoaffinity Labeling Preferences of Diazirines

Literature Seminar
2021/12/03
Lin Yuanqi

Contents

Photoaffinity Labeling (PAL)



chemical probe

covalently bind to target by light activation

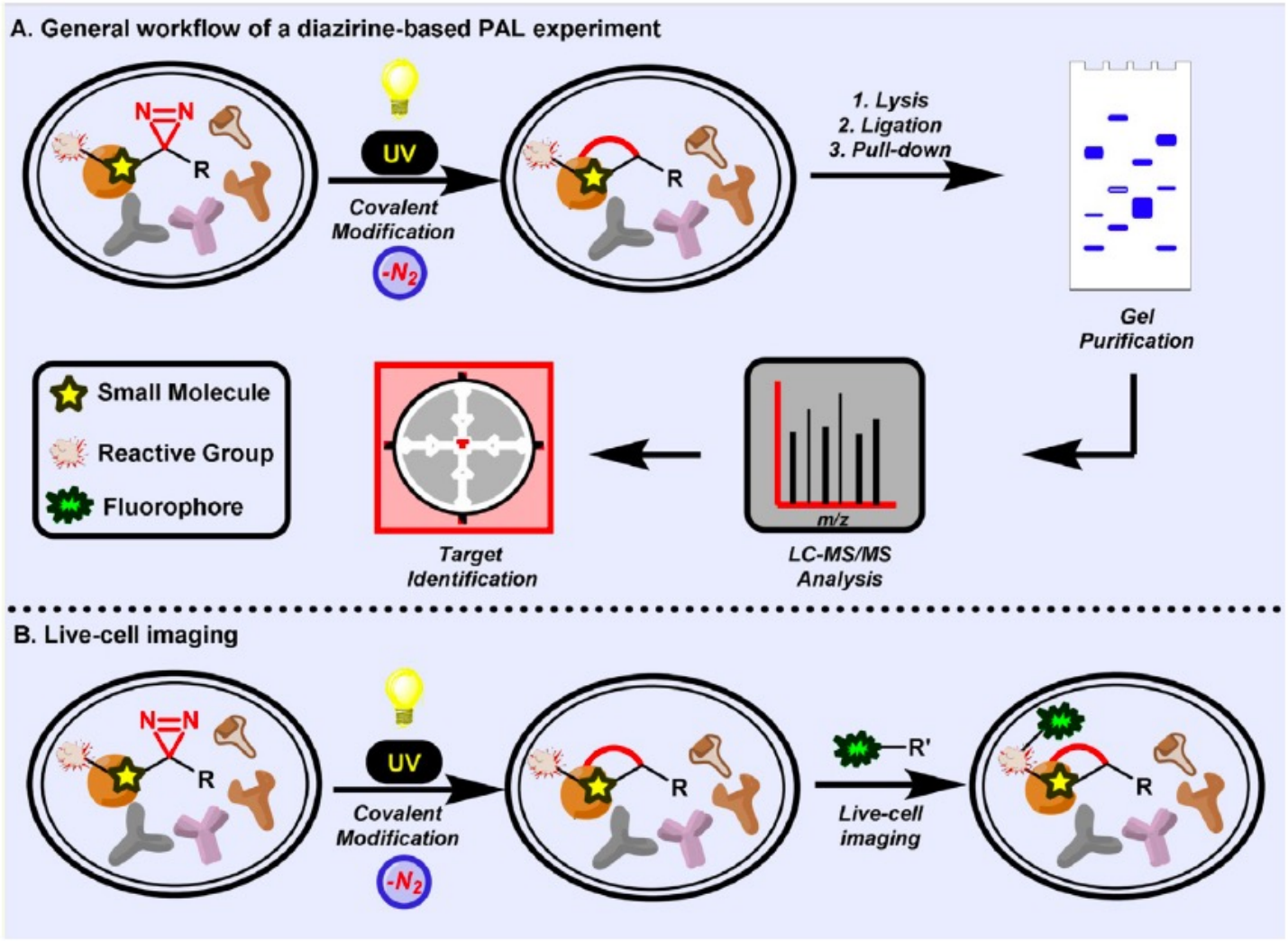
application

- identification of molecular interactions
- identification of new drug targets
- probing the location and structure of binding sites

ideal traits

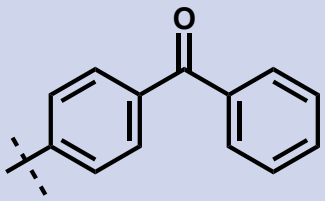
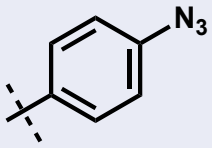
- ❖ stability in the dark at a range of pHs
- ❖ similarity to the parent compound with comparable activity and affinity levels
- ❖ little steric interference to binding
- ❖ minimal damage during activation
- ❖ generation of reactive intermediates to form stable adduct

Work flow



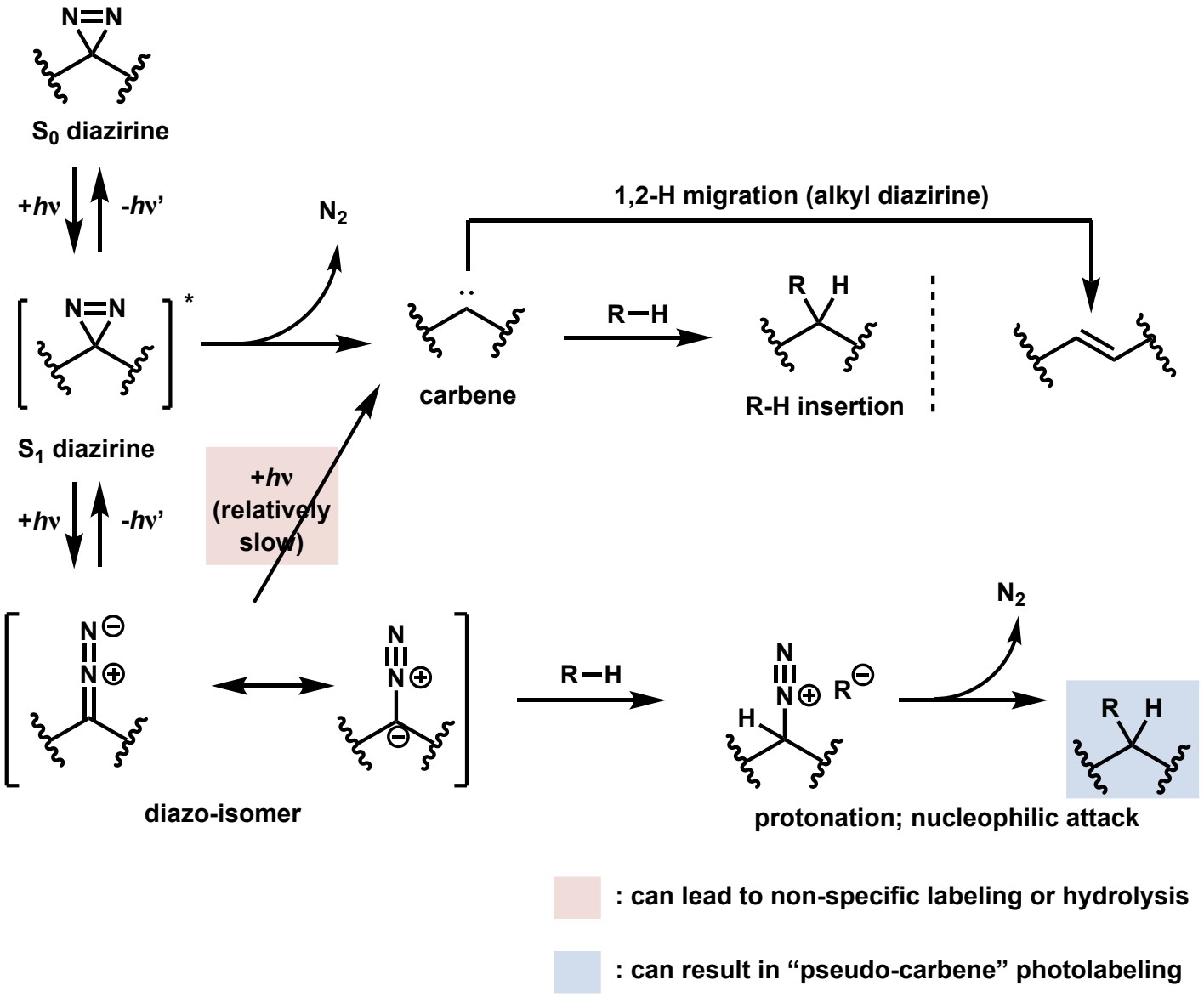
Halloran, M. W.; Lumb, J. P. *Chem. Eur. J.* **2019**, *25*, 4885.

Comparison of Commonly Used Photo-activatable Groups (PAG)

PAG	activation wavelength	advantages	disadvantages
 benzophenone	350 - 360 nm	-commercially available -non-damaging activation wavelengths	-bulky size -prolonged irradiation times
 arylazide	< 300 nm	-readily synthesized -small size	-low activation wavelengths -tendency to undergo undesired side reactions
 diazirine	350 - 380 nm	-fast kinetics -small size -non-damaging activation wavelengths	-tedious synthesis -formation of undesired diazo-isomer

Halloran, M. W.; Lumb, J. P. *Chem. Eur. J.* **2019**, *25*, 4885.

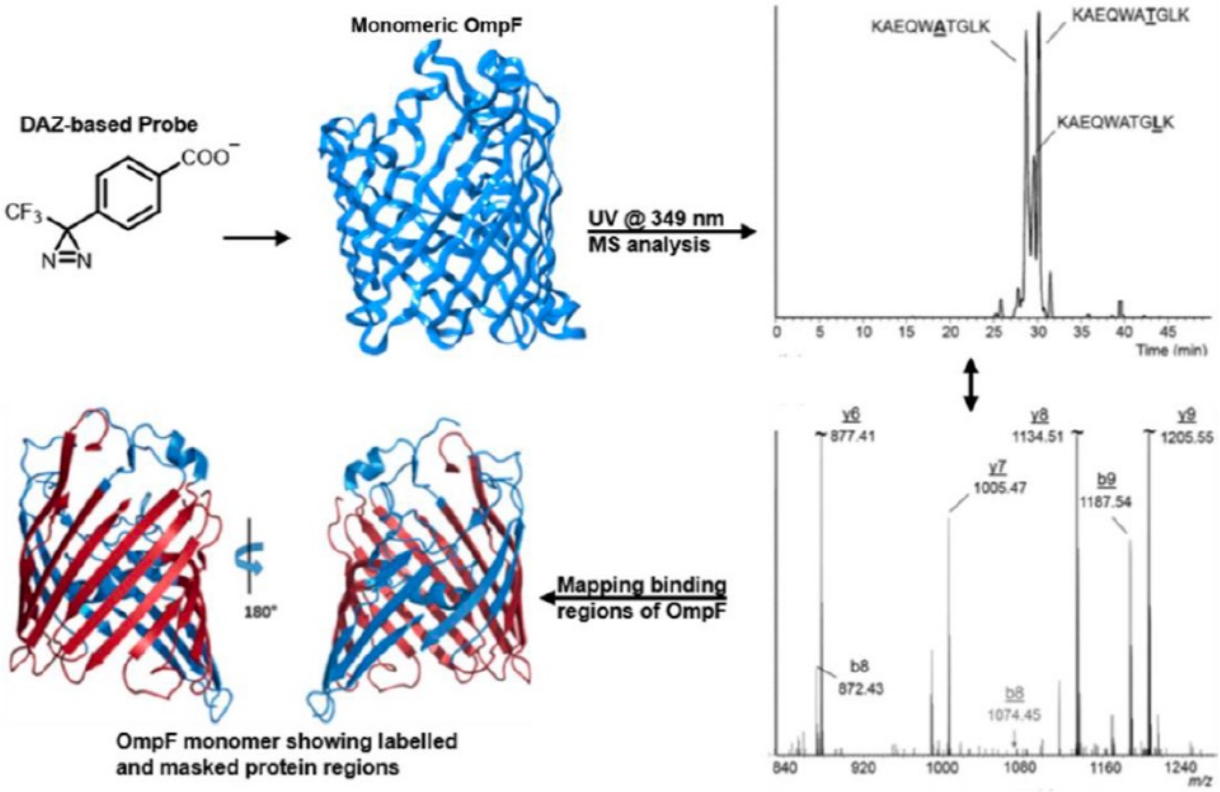
Photochemical Reactivity of Diazirines



Zhang, T.; Ondrus, A. E. *Synlett*. **2021**, *32*, 1053.

Application

1. Target engagement and discovery of novel bioactive compounds
2. Studying complex protein interactions
3. probing membrane proteins and other protein–biomolecule interactions
4. Molecular markers for imaging studies



protein in red: labelled
protein in blue: unlabeled

Diazirines-modified OmpF allows successful mapping of proteins' binding regions.

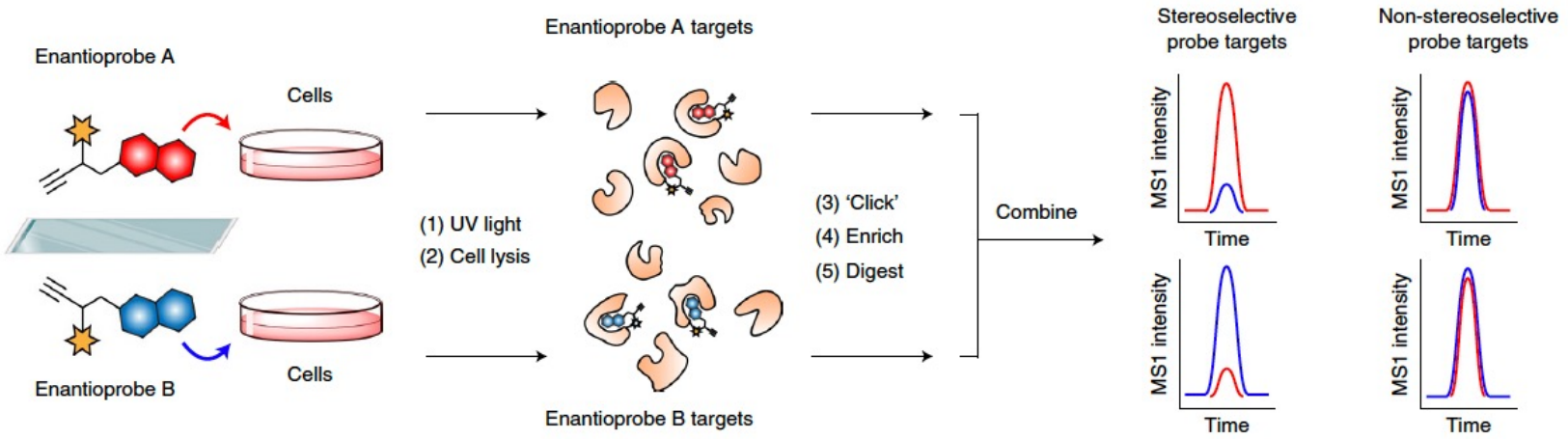
Hassan, M. M.; Olaoye, O. O. *Molecules.* ***2020****, 25*, 2285.

Remaining Problems and trials (1)

The incomplete understanding of the labeling preferences of the diazirine limits the design and interpretation of these experiments.

enantioprobes

Enantioprobes offer a highly efficient way to discover small-molecule/protein interactions in human cells.



1) Wang, Y.; Dix, M. M.; Bianco, G.; Remberg, J. R.;Lee, H. Y.; Kalocsay, M.; Gygi, S. P.; Vite, G.; Lawrence, R. M.; Parker, C. G.; Cravatt, B. F. *Nat. Chem*. **2019**, *11*, 1113. 2) 200530_LS_Koichi_Kamiya.

Remaining Problems and trials (2)

Evidence shows that alkyl diazirine preferentially react with acidic residues.

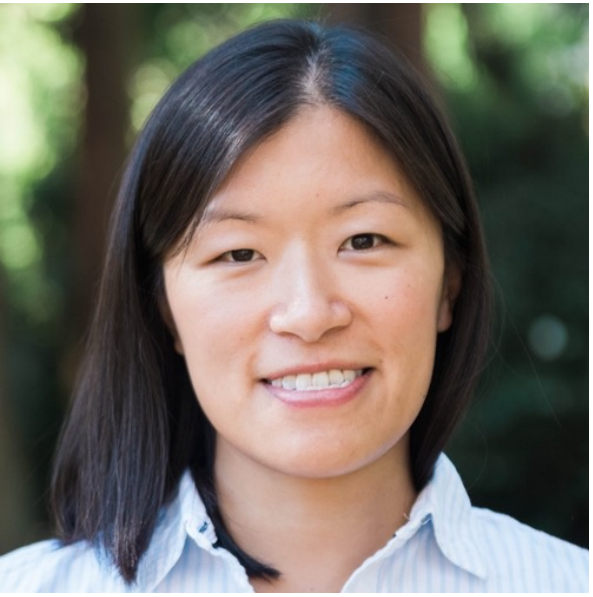
Systematic establishment of how alkyl diazirines exhibit differential reactivity preferences from aryl diazirines is needed.

facilitate interpretation of the measurements and conclusions from PAL experiments using diazirines.

West, A. V.; Muncipinto, G.; Wu, H. Y.; Huang, A. C.; Labenski, M. T.; Jones, L. H.; Woo, C. M. *J. Am. Chem. Soc*. **2021**, *143*, 6691.

Contents

Associate Professor Christina M. Woo

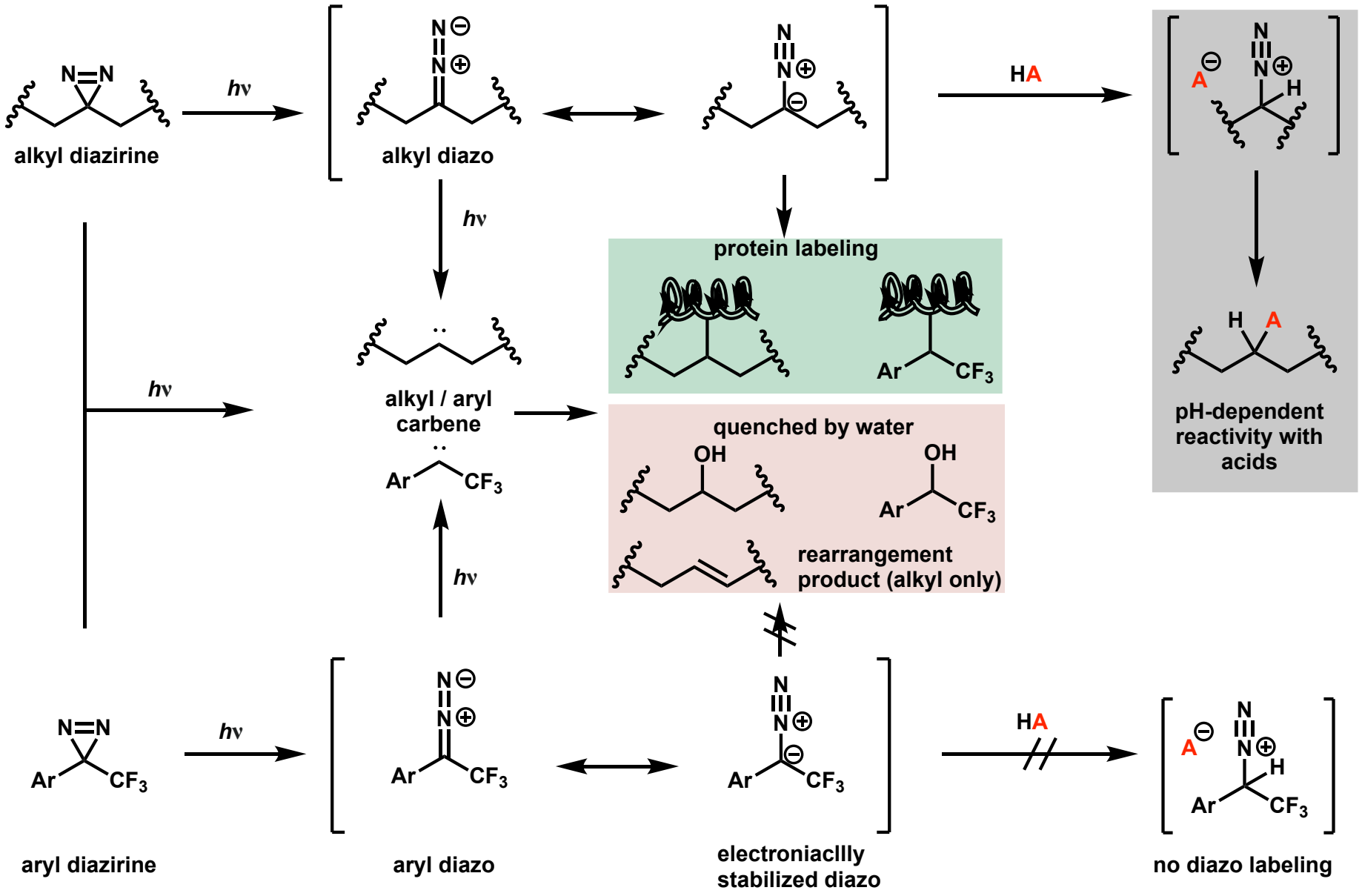


2004-2008	B.A. in Chemistry @ Wellesley College (Prof. Dora Carrico-Moniz)
2008-2013	Ph.D. in Chemistry @ Yale University (Prof. Seth B. Herzon)
2013-2016	Jane Coffin Childs and Burroughs Wellcome Fund Postdoctoral Fellow University of California (Prof. Carolyn R. Bertozzi)
2016-2020	Assistant Professor @ Harvard University
2020-	Associate Professor @ Harvard University

Research field:
Chemical control of cellular signaling

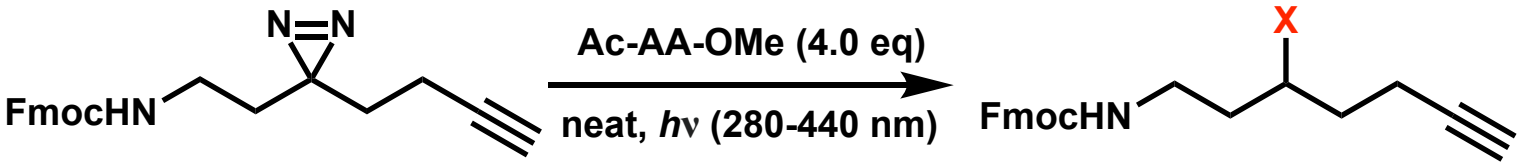
- **Discovery of small molecule binding site hotspots**
- **Chemical control of the O-GlcNAc proteome**
- **Chemical control of the ubiquitin proteasome system**

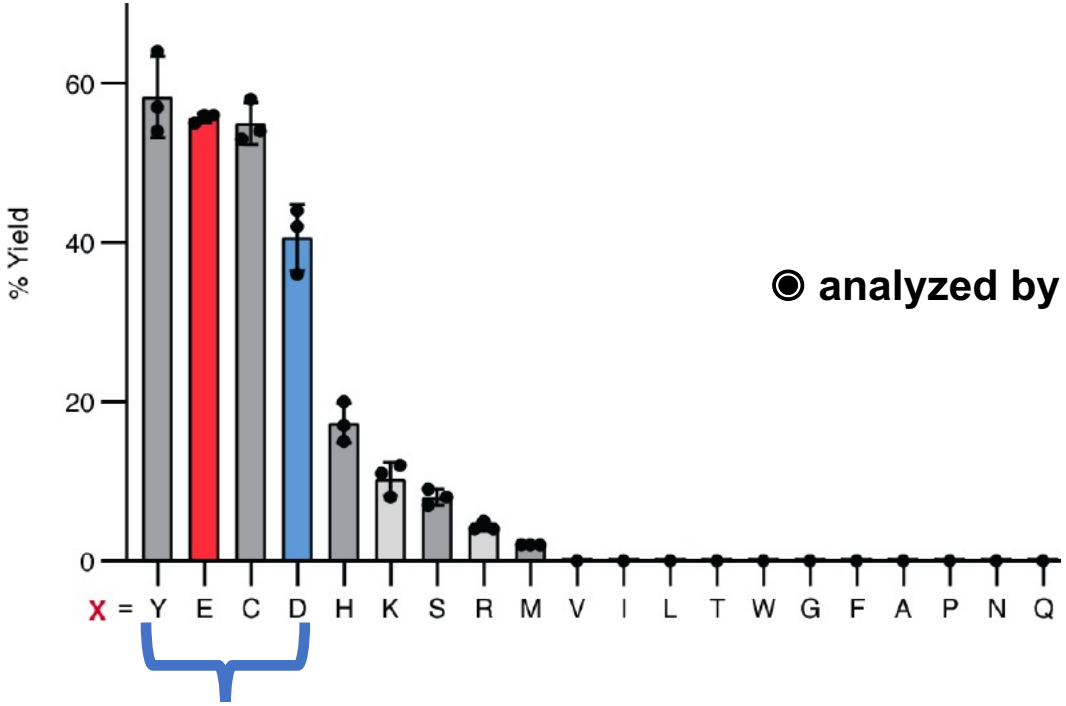
Overview of diazirine reactivity pathways



West, A. V.; Muncipinto, G.; Wu, H. Y.; Huang, A. C.; Labenski, M. T.; Jones, L. H.; Woo, C. M. *J. Am. Chem. Soc.* **2021**, *143*, 6691.

Reactivity of Alkyl Diazirine Under Neat Conditions



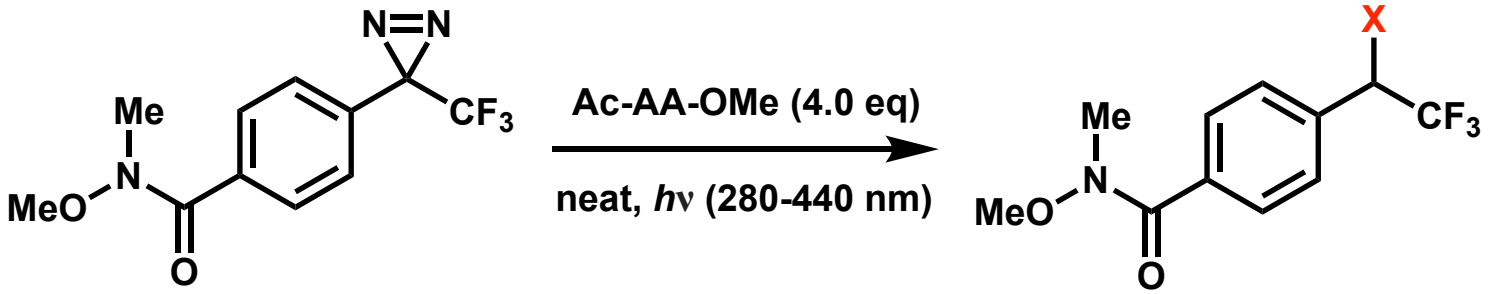


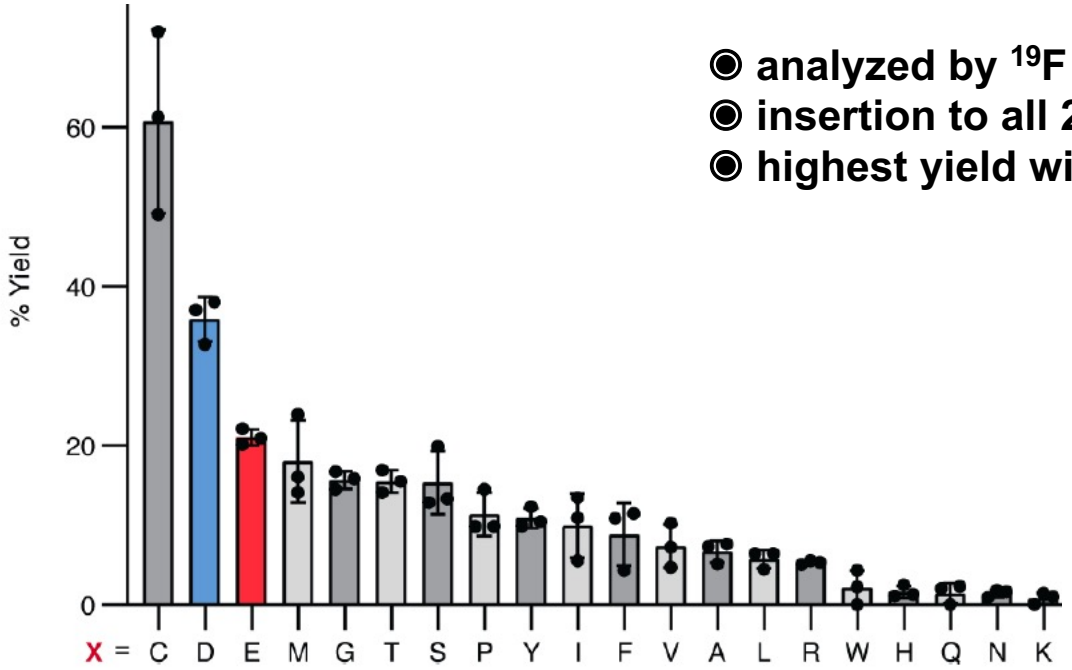
◉ analyzed by LC-MS

acidic polar amino acids (40-60%)

West, A. V.; Muncipinto, G.; Wu, H. Y.; Huang, A. C.; Labenski, M. T.; Jones, L. H.; Woo, C. M. *J. Am. Chem. Soc.* **2021**, *143*, 6691.

Reactivity of Aryl Diazirine Under Neat Conditions

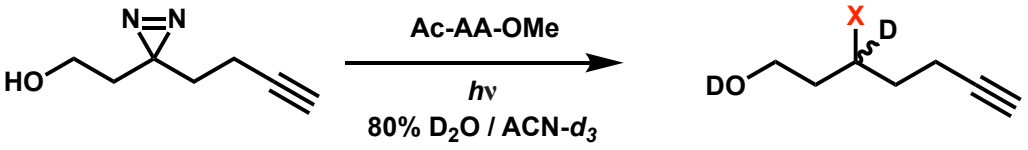


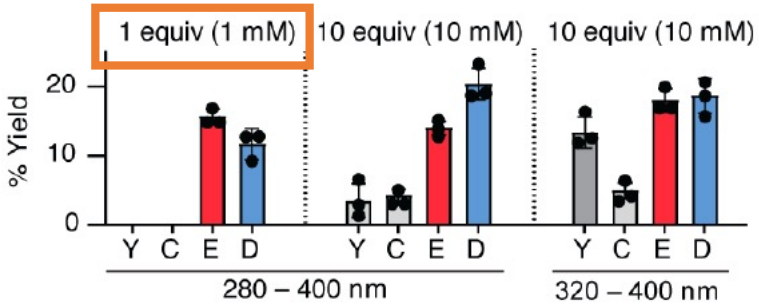


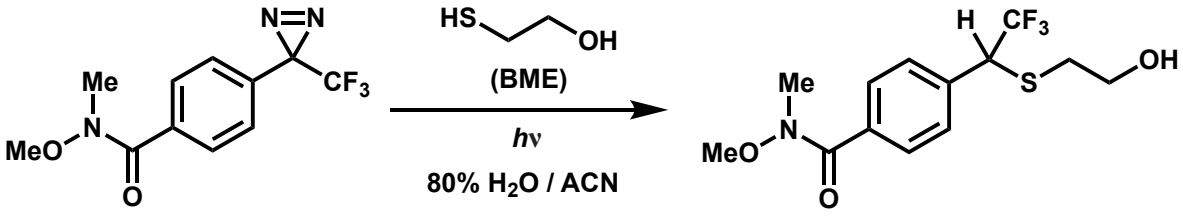
- analyzed by ^{19}F NMR
- insertion to all 20 amino acids
- highest yield with cysteine

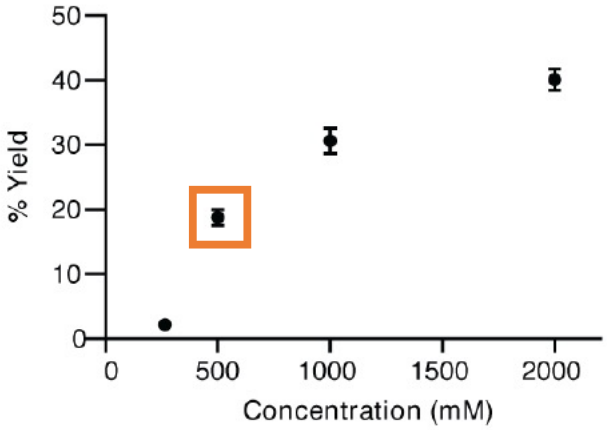
West, A. V.; Muncipinto, G.; Wu, H. Y.; Huang, A. C.; Labenski, M. T.; Jones, L. H.; Woo, C. M. *J. Am. Chem. Soc.* **2021**, *143*, 6691.

Reactivity of Alkyl and Aryl Diazirine in Aqueous Solution





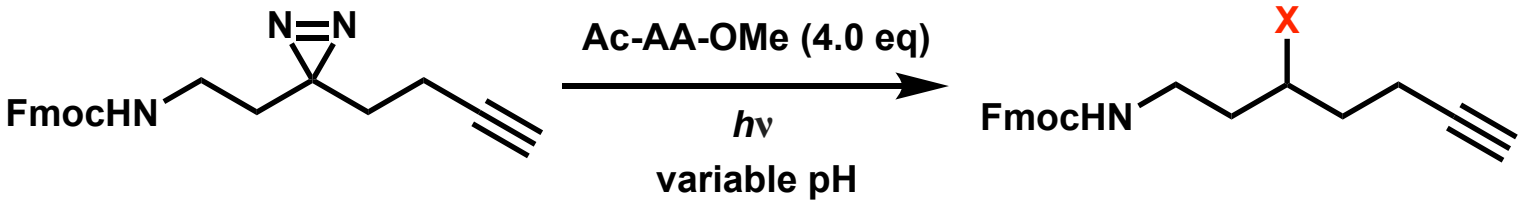


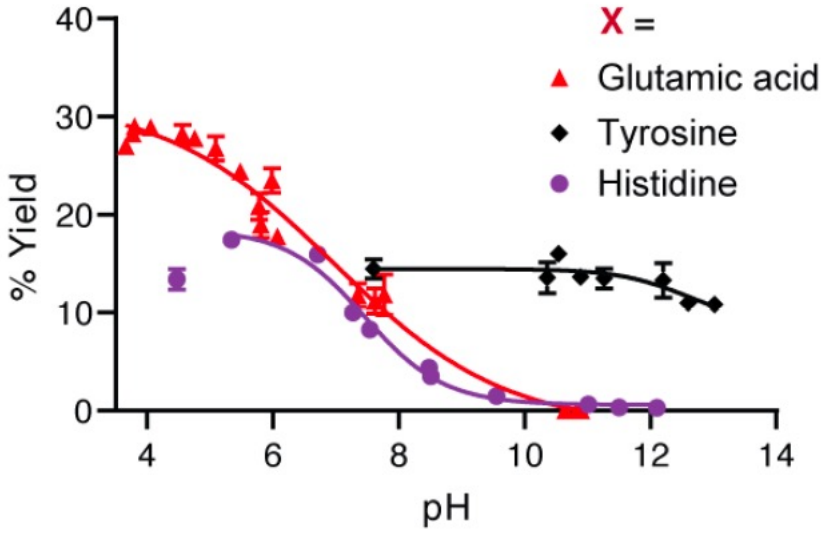


☆ Aryl diazirine reacts through a short-lived carbene.
☆ Aryl diazo intermediate is not driving alternate reaction.

West, A. V.; Muncipinto, G.; Wu, H. Y.; Huang, A. C.; Labenski, M. T.; Jones, L. H.; Woo, C. M. *J. Am. Chem. Soc.* **2021**, *143*, 6691.

Alkyl Diazirine Labeling of Amino Acids: pH-dependent

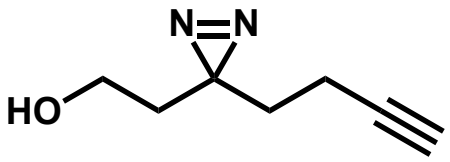




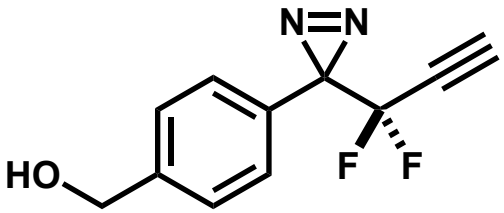
Alkyl diazirine generates a reactive intermediate that leads to preferential reactivity with protonated acidic residues in aqueous solution.

West, A. V.; Muncipinto, G.; Wu, H. Y.; Huang, A. C.; Labenski, M. T.; Jones, L. H.; Woo, C. M. *J. Am. Chem. Soc.* **2021**, *143*, 6691.

Alkyl Diazirine Labeling of Single Proteins: pH-dependent



or



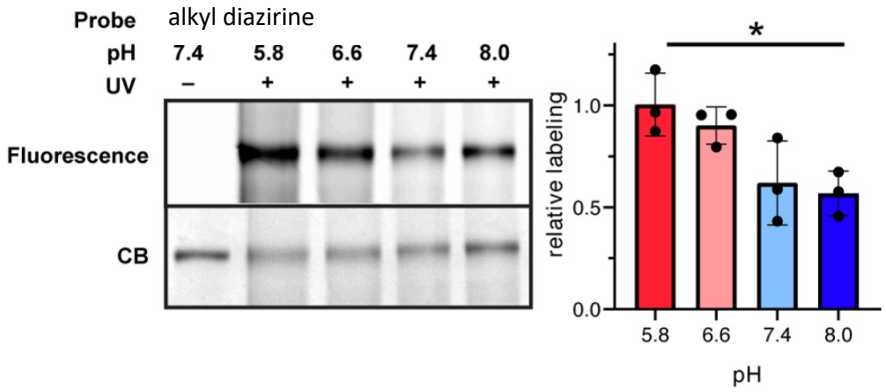
BSA

1. Tris buffer, *hν*

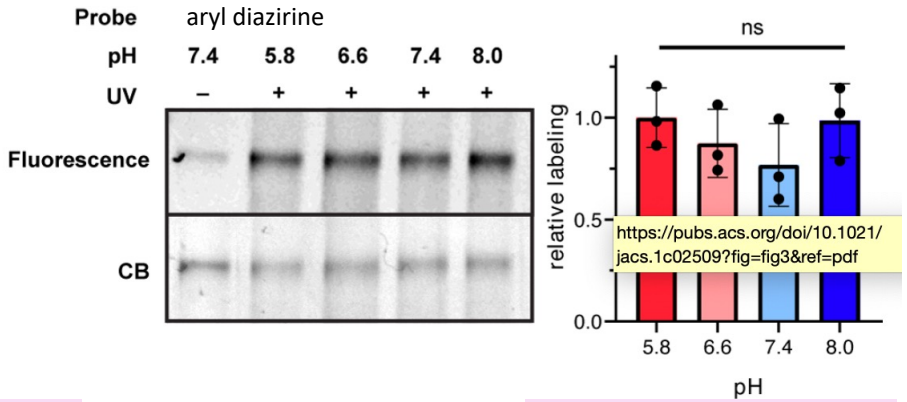
2. Azide-Flour 488, CuAAC

In-gel flurescence

BSA: bovine serum albumin, an individual protein



higher at lower pH



pH independent

The differential reactive intermediates from the alkyl diazirine and the aryl diazirine predictably impact observed labeling patterns on individual substrates.

West, A. V.; Muncipinto, G.; Wu, H. Y.; Huang, A. C.; Labenski, M. T.; Jones, L. H.; Woo, C. M. *J. Am. Chem. Soc.* **2021**, *143*, 6691.

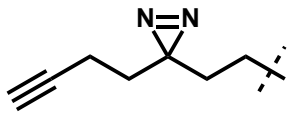
Photolabeling in Cells with PAL Probes

in vitro: pH dependence of alkyl diazirine

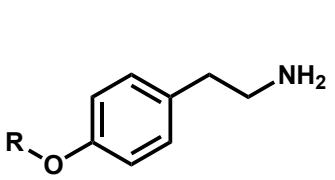
in vivo:

Representative probe structures

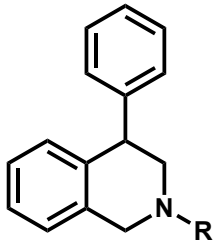
R =



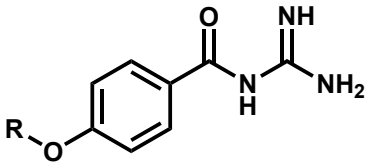
+1



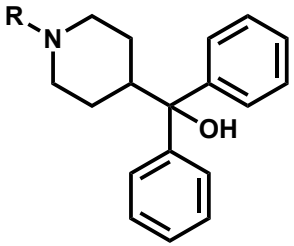
JN26



JN849

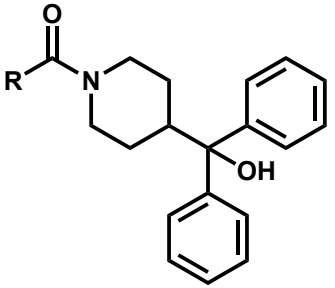


JN935

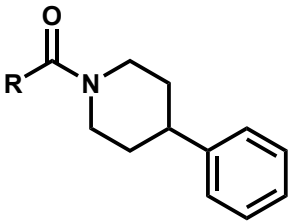


JN938

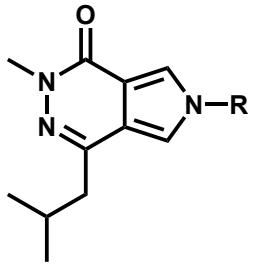
0



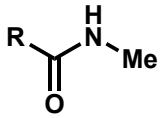
JN939



JN3

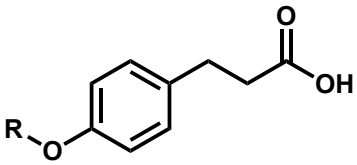


JN24

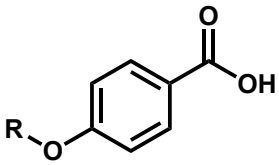


JN1

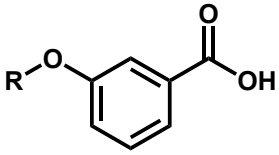
-1



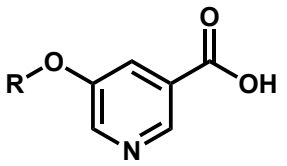
JN33



JN12



JN20



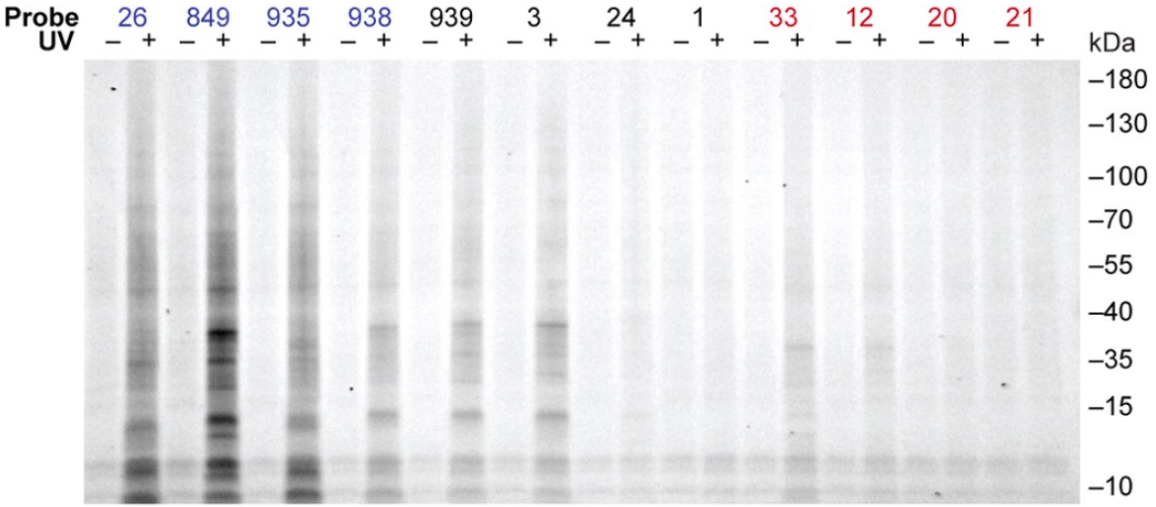
JN21

West, A. V.; Muncipinto, G.; Wu, H. Y.; Huang, A. C.; Labenski, M. T.; Jones, L. H.; Woo, C. M. *J. Am. Chem. Soc.* **2021**, *143*, 6691.

Photolabeling in cells with PAL probes (1)

varying PAL probes

gel visualization



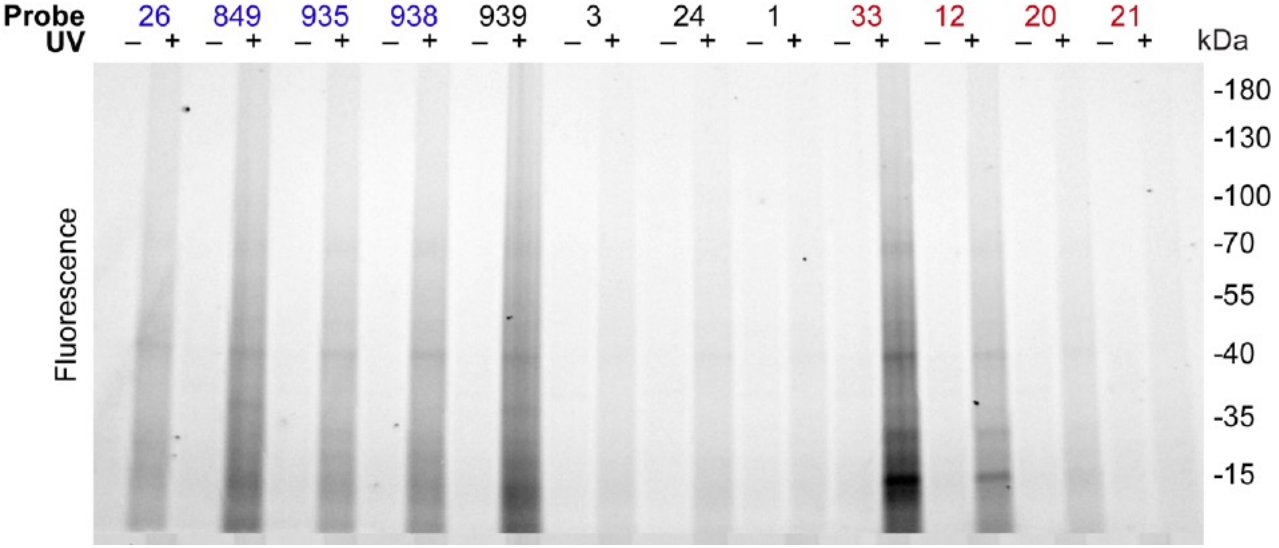
probes with a net positive charge: higher labeling

West, A. V.; Muncipinto, G.; Wu, H. Y.; Huang, A. C.; Labenski, M. T.; Jones, L. H.; Woo, C. M. *J. Am. Chem. Soc.* **2021**, *143*, 6691.

Photolabeling in cells with PAL probes (2)

varying PAL probes

gel visualization

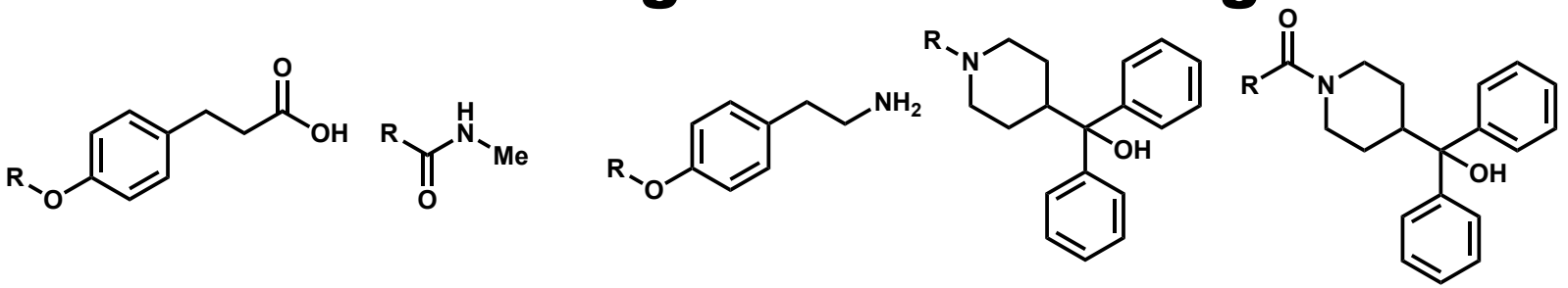


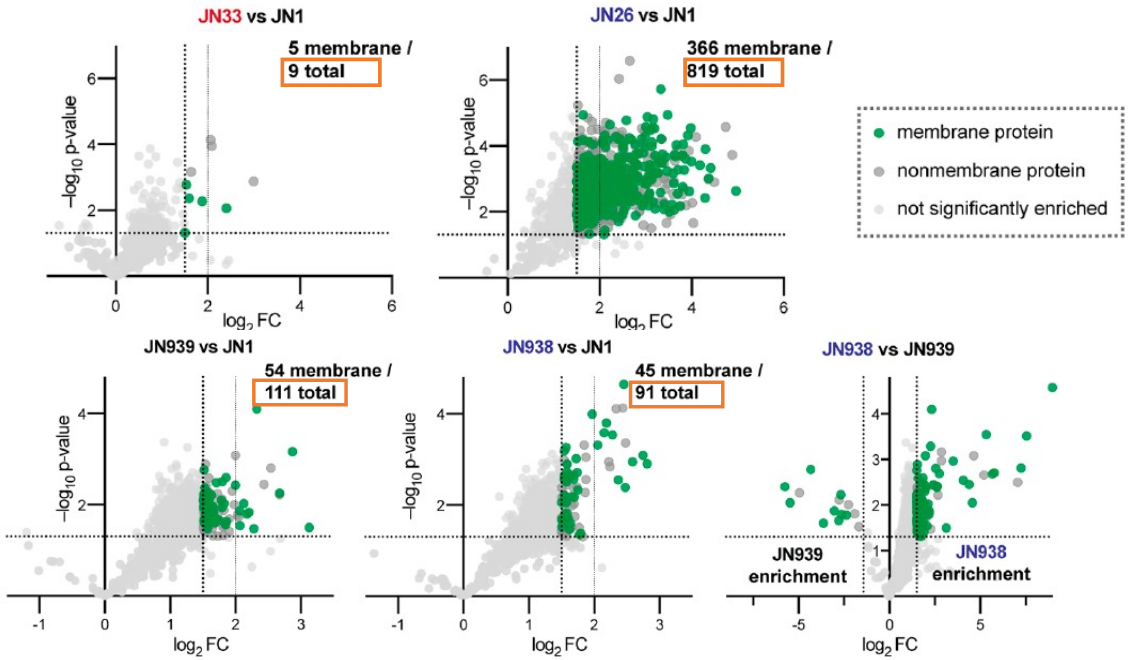
positively charged probes: higher labeling propensities (in general)

- In general, increased interaction between the probes and negatively charged protein surfaces composed of acidic residues was shown.
- Overall labeling yields from a particular probe reflect a composite of the diazirine chemistry and cellular permeability.

West, A. V.; Muncipinto, G.; Wu, H. Y.; Huang, A. C.; Labenski, M. T.; Jones, L. H.; Woo, C. M. *J. Am. Chem. Soc.* **2021**, *143*, 6691.

Contribution of Charge to the Labeling Efficiency





positively charged JN26: a much higher degree of labeling

charge preference of neutral versus positively charged probes with larger, more hydrophobic structures: diminished but still noticeable

increased localization to protein surfaces that are more readily cross-linked by alkyl diazirine chemistry

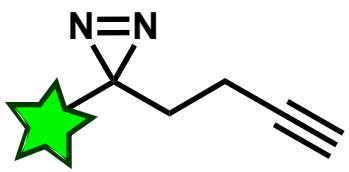
Alkyl diazirine probes with a net positive charge will yield elevated labeling of the proteome.

West, A. V.; Muncipinto, G.; Wu, H. Y.; Huang, A. C.; Labenski, M. T.; Jones, L. H.; Woo, C. M. *J. Am. Chem. Soc.* **2021**, *143*, 6691.

Discussion (1)

for design and interpretation of PAL experiments

By altering the net overall charge, design of the probe can be tuned to enhance or reduce protein capture.



Alkyl diazirine probe

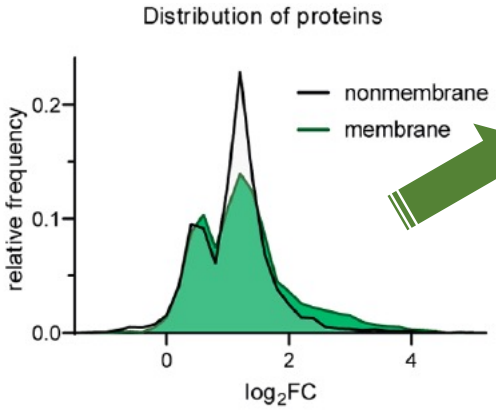
-1 / 0 → **selectively visualize stronger binding interactions**

+1 → **capture more transient interactions in the proteome**

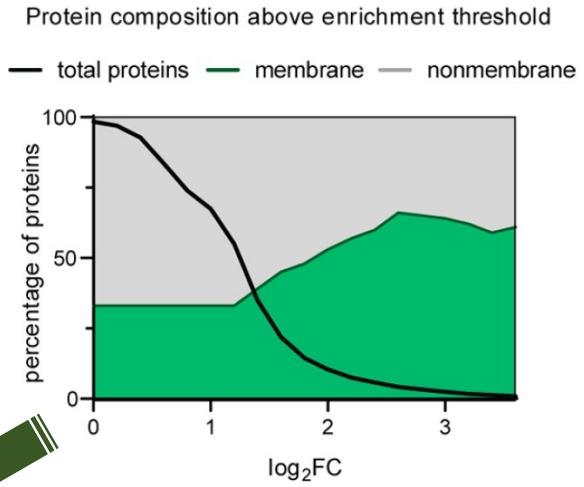
The charge state of the molecule is readily altered by the type of linker chemistry employed.

West, A. V.; Muncipinto, G.; Wu, H. Y.; Huang, A. C.; Labenski, M. T.; Jones, L. H.; Woo, C. M. *J. Am. Chem. Soc.* **2021**, *143*, 6691.

PAL Probes Disproportionately Label Membrane Proteins in Cells



Alkyl diazirine probes possess an enrichment bias for membrane proteins.



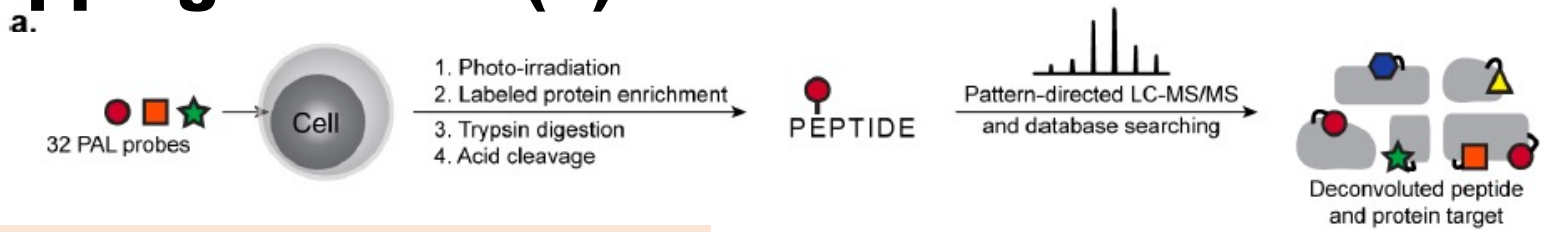
Membrane proteins were over-represented after filtering for protein enrichment.

Membrane proteins were disproportionately common.

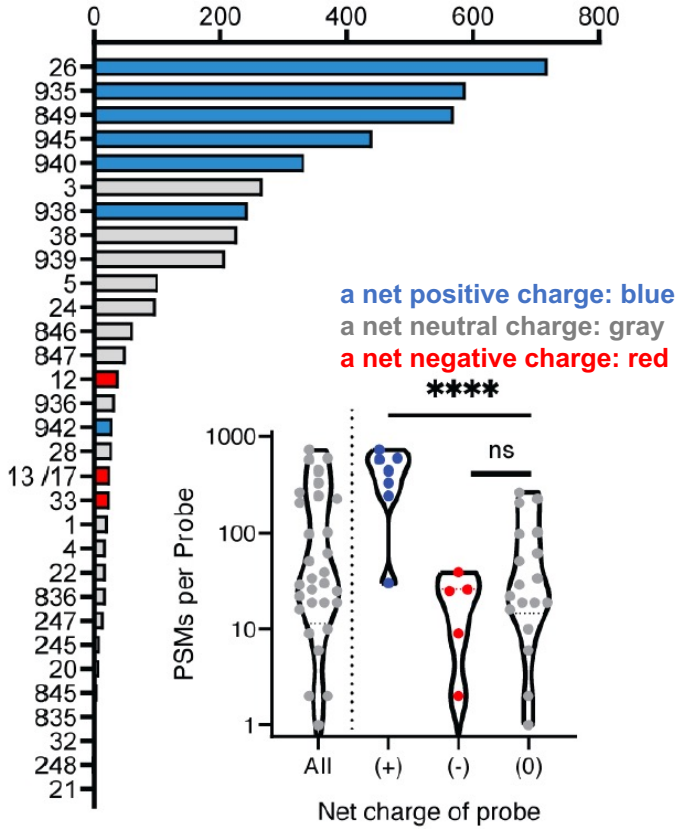
The preferential labeling of membrane proteins by alkyl diazirine probes may reflect the elevated pK_a of Glu and Asp residues in lipid bilayers.

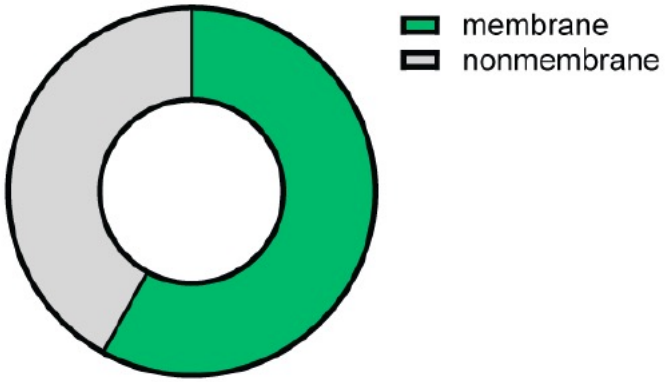
West, A. V.; Muncipinto, G.; Wu, H. Y.; Huang, A. C.; Labenski, M. T.; Jones, L. H.; Woo, C. M. *J. Am. Chem. Soc*. **2021**, *143*, 6691.

Improvement in Interpretation of Binding Site Mapping Results (1)



Count of peptide spectral matches (PSMs) assigned to each PAL probe





The increased labeling of membrane proteins is a result of the labeling propensity of alkyl diazirine chemistry.

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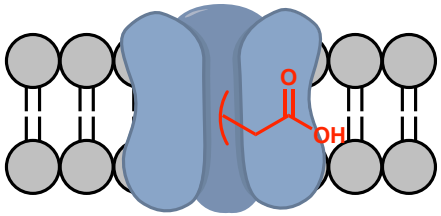
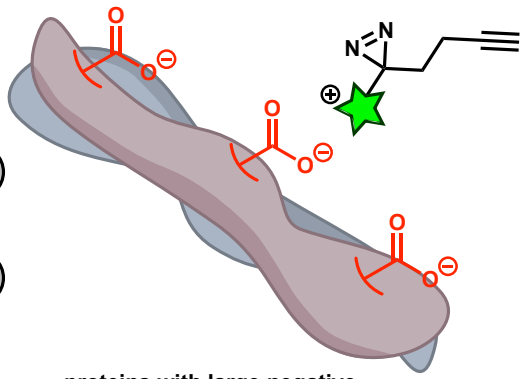
Discussion (2)



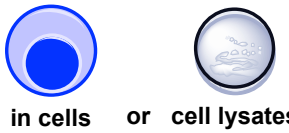
membrane proteins



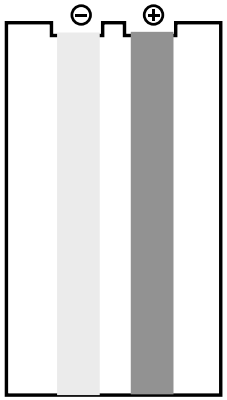
proteins with large negative electrostatic surfaces

pH-dependent labeling

enhance the utility for visualizing binding over a range of affinities with membrane proteins

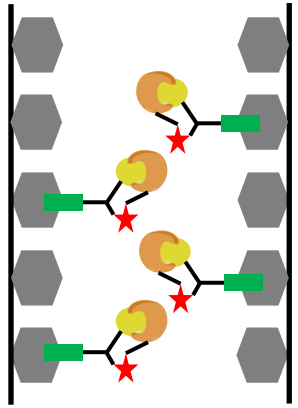


in cells or cell lysates



higher labeling yields from probes with a positive charge

increased membrane protein enrichment: independent of the charge of the probe



It is potential that the subcellular localization of positively charged probes to the membrane is synergistic with the pH dependent reactivity of alkyl diazirines.

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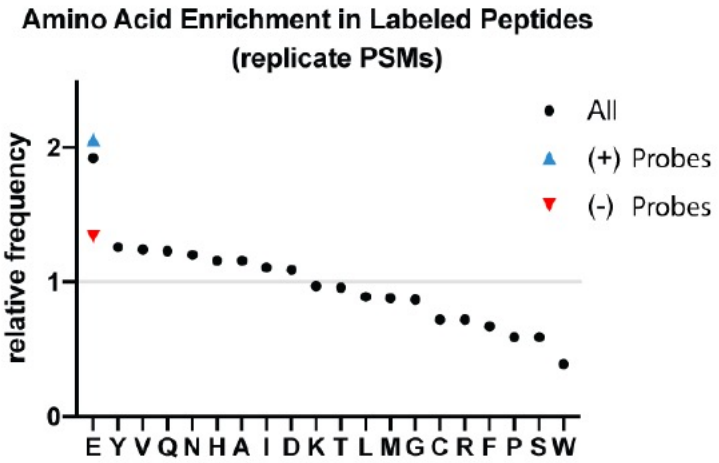
Improvement in Interpretation of Binding Site Mapping Results (2)



- Glutamic acid:
  most enriched
  increased specifically
- aspartic acid:
  limited enrichment

- differences in steric accessibility
- lower pK_a of Asp to Glu

- glutamic acid: highest propensity for labeling
- factor for labeling frequency: local pK_a environment, physical properties of the photoaffinity probe

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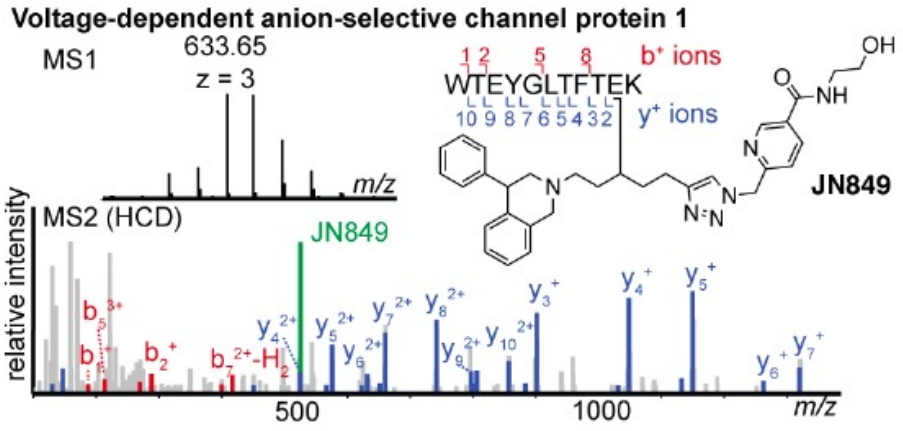
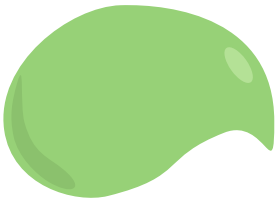
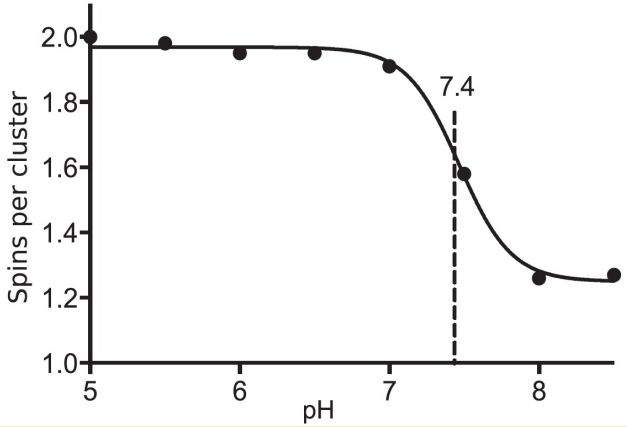
Improvement in Interpretation of Binding Site Mapping Results (3)



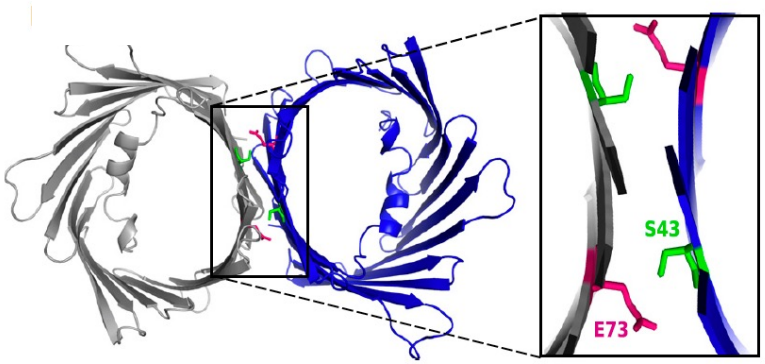


VDAC1 E73

- ✪ high membrane p*K*a
- ✪ ability to sensitively mediate dimerization of VDAC1



mVDAC1 forms a dimer at low pH.



- ◉ mVDAC1 dimer model
- ◉ form a function of pH with pK_a 7.4
- ◉ mitochondrial metabolic regulation

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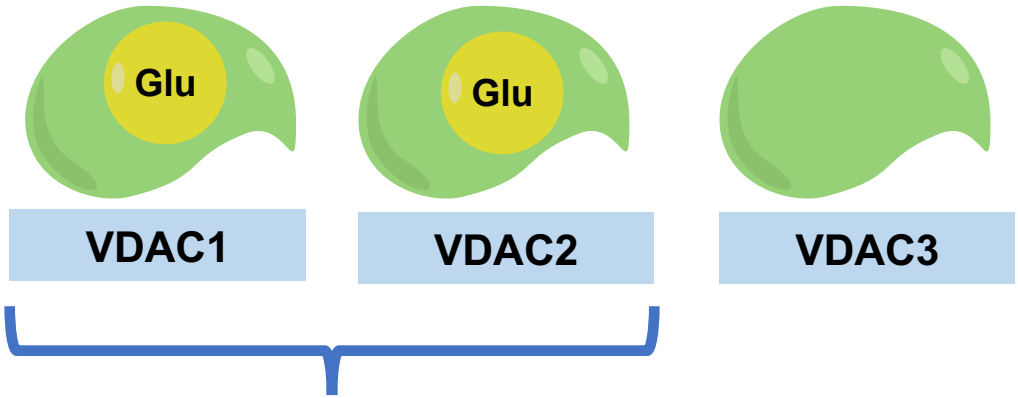
Improvement in Interpretation of Binding Site Mapping Results (4)



VDAC1 and VDAC2 are frequently enriched targets in alkyl diazirine studies.

VDAC-1

```
        10         20         30         40         50
MAVPPTYADL GKSARDVFTK GYGFGLIKLD LKTKSENGLE FTSSGSANTE
        60         70         80         90        100
TTKVTGSLET KYRWTEYGLT FTEKWNTDNT LGTEITVEDQ LARGLKLTFD
       110        120        130        140        150
SSFSPNTGKK NAKIKTGYKR EHINLGCDMD FDIAGPSIRG ALVLGYEGWL
       160        170        180        190        200
AGYQMNFETA KSRVTQSNFA VGYKTDEFQL HTNVNDGTEF GGSIYQKVNK
       210        220        230        240        250
KLETAVNLAW TAGNSNTRFG IAAKYQIDPD ACFSAKVNNS SLIGLGYTQT
       260        270        280
LKPGIKLTLS ALLDGKNVNA GGHKLGLGLE FQA
```

VDAC-2

```
        10         20         30         40         50
MATHGQTCAR PMCIPPSYAD LGKAARDIFN KGFGFGLVKL DVKTKSCSGV
        60         70         80         90        100
EFSTSGSSNT DTGKVTGTLE TKYKWCEYGL TFTEKWNTDN TLGTEIAIED
       110        120        130        140        150
QICQGLKLTF DTTFSPNTGK KSGKIKSSYK RECINLGCDV DFDFAGPAIH
       160        170        180        190        200
GSAVFGYEGW LAGYQMTFDS AKSKLTRNNF AVGYRTGDFQ LHTNVNDGTE
       210        220        230        240        250
FGGSIYQKVC EDLDTSVNLA WTSGTNCTRF GIAAKYQLDP TASISAKVNN
       260        270        280        290
SSLIGVGYTQ TLRPGVKLTL SALVDGKSIN AGGHKVGLAL ELEA
```

VDAC-3

```
        10         20         30         40         50
MCNTPTYCDL GKAAKDVFNK GYGFGMVKID LKTKSCSGVE FSTSGHAYTD
        60         70         80         90        100
TGKASGNLET KYKVCNYGLT FTQKWNTDNT LGTEISWENK LAEGLKLTLD
       110        120        130        140        150
TIFVPNTGKK SGKLKASYKR DCFSVGSNVD IDFSGPTIYG WAVLAFEGWL
       160        170        180        190        200
AGYQMSFDTA KSKLSQNNFA LGYKAADFQL HTHVNDGTEF GGSIYQKVNE
       210        220        230        240        250
KIETSINLAW TAGSNNTRFG IAAKYMLDCR TSLSAKVNNA SLIGLGYTQT
       260        270        280
LRPGVKLTLS ALIDGKNFSA GGHKVGLGFE LEA
```

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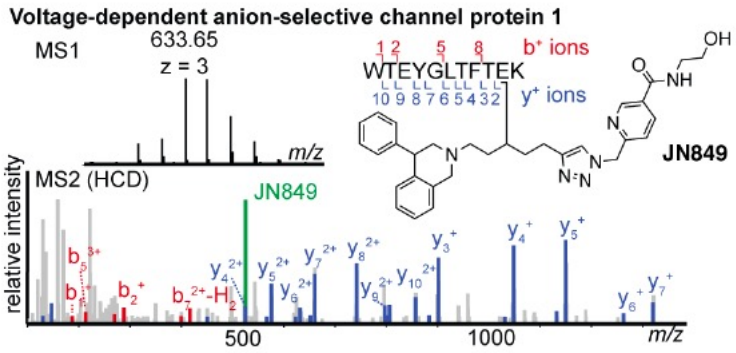
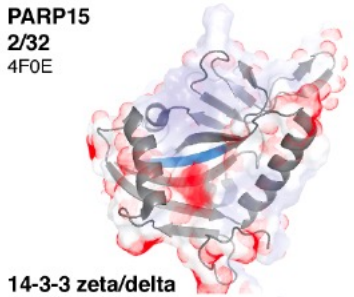
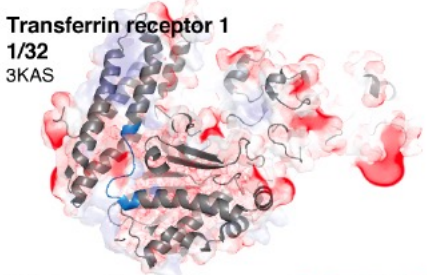
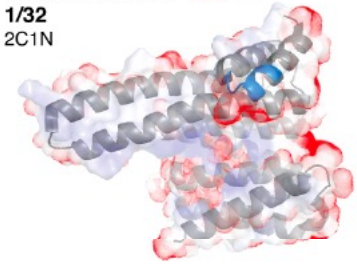
Improvement in Interpretation of Binding Site Mapping Results (5)

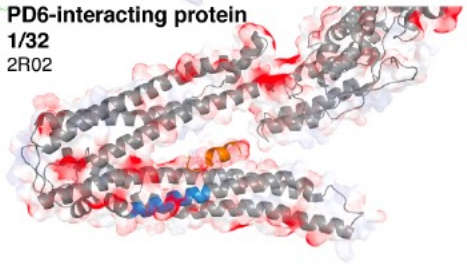
negative electrostatic maps (red)
binding site (blue)





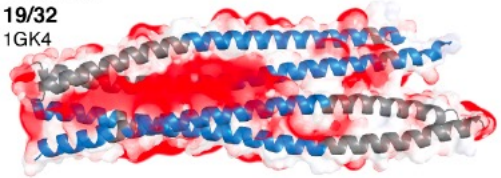


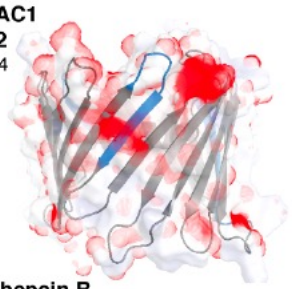


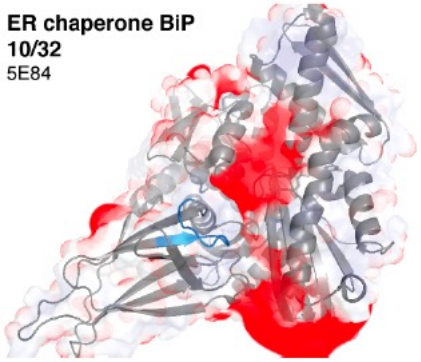


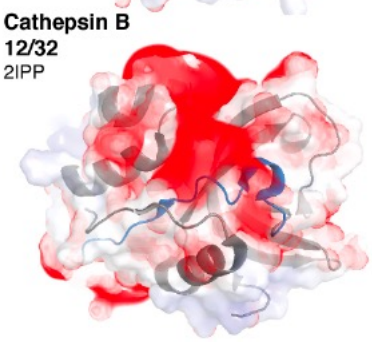
labeled by only one or two PAL probes

labeled by multiple PAL probes









Proteins that are more frequently labeled by alkyl diazirine chemistry typically have negative electrostatic regions that are characterized by a density of acidic residues or those with a relatively high localized pK_a.

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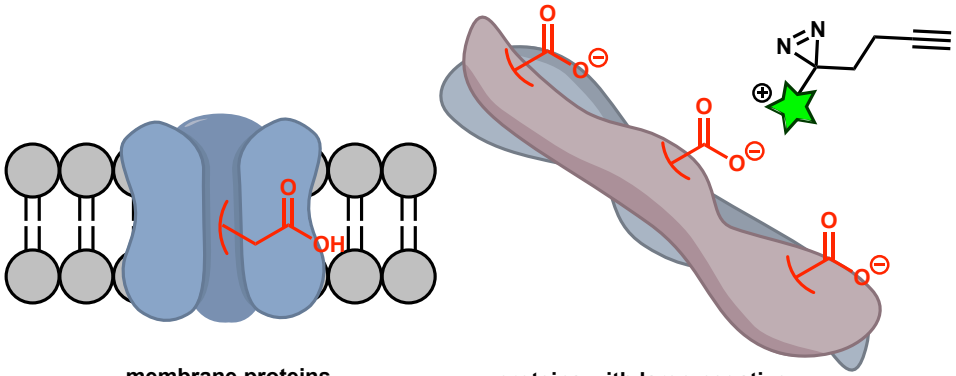
Conclusion and Perspectives

1. The alkyl diazirine preferentially reacts with acidic amino acid residues because of the major contribution of a reactive diazo intermediate.



2. Understanding the reactivity pattern of alkyl diazirines therefore facilitates design and interpretation of PAL experiments.

3. It is expected for generation of an "ideal" PAL functional group or development of methods to selectively map noncovalent interactions with certain regions of the cell or types or proteins.