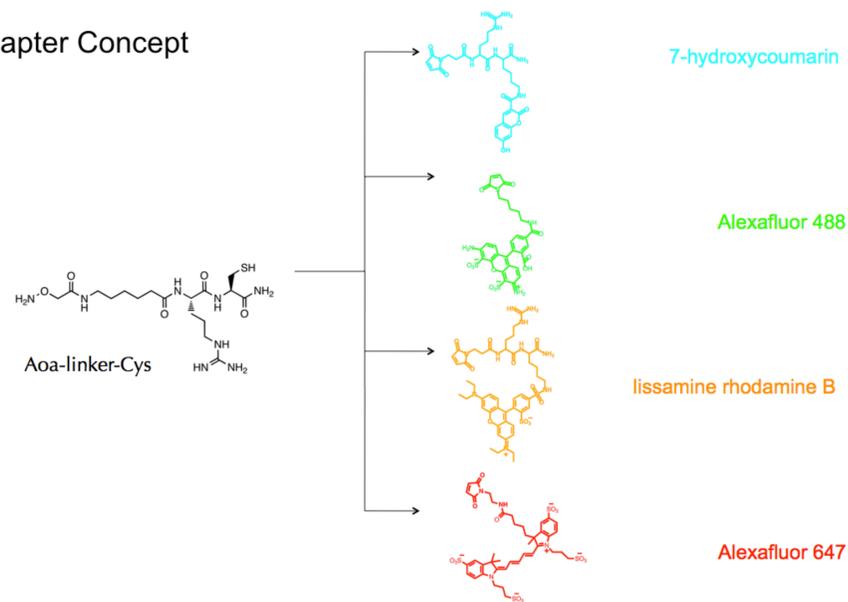


ADAPTER REAGENTS FOR PROTEIN SITE SPECIFIC DYE LABELING

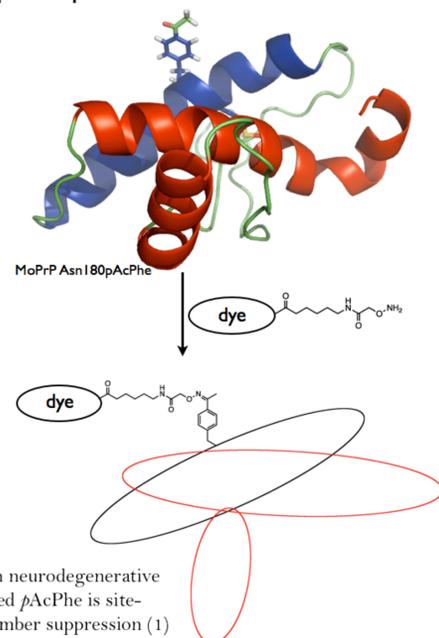
Darren A. Thompson, Eric G.B. Evans, Tomas Kasza, Glenn L. Millhauser, and Philip E. Dawson

There are many ways to site specifically label proteins, yet the vast majority of dyes are either maleimides or succinimides. If you want a different ligation chemistry than these two, you can put a graduate student on it and waste years of his/her life, pay through the nose, or settle on these conjugation strategies. We have developed a general approach to make conjugation ready reagents; many thousands of reporter molecules are conceivably adapted to the protein ligation chemistry of choice from a few simple reagents.

1. Adapter Concept



2. We label mouse prion protein



The prion protein is involved in neurodegenerative disease and ketone functionalized pAcPhe is site-specifically incorporated with amber suppression (1)

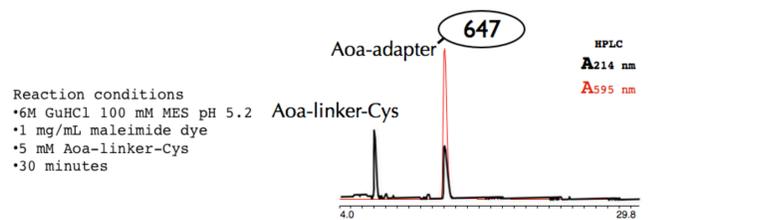
Selected References

- Noren, C. J., Anthony-Cahill, S. J., Griffith, M. C., and Schultz, P. G. (1989) A general method for site-specific incorporation of unnatural amino acids into proteins, *Science* 244, 182-188.
- Brustad, E. M., Lemke, E. A., Schultz, P. G., and Deniz, A. A. (2008) A general and efficient method for the site-specific dual-labeling of proteins for single molecule fluorescence resonance energy transfer, *J Am Chem Soc* 130, 17664-17665.
- Dirksen, A., Hackeng, T. M., and Dawson, P. E. (2006) Nucleophilic catalysis of oxime ligation, *Angew Chem Int Ed Engl* 45, 7581-7584.



3. Creation of Aoa-adapter-dye

The maleimide reaction modifies only the thiol of cysteine of adapters



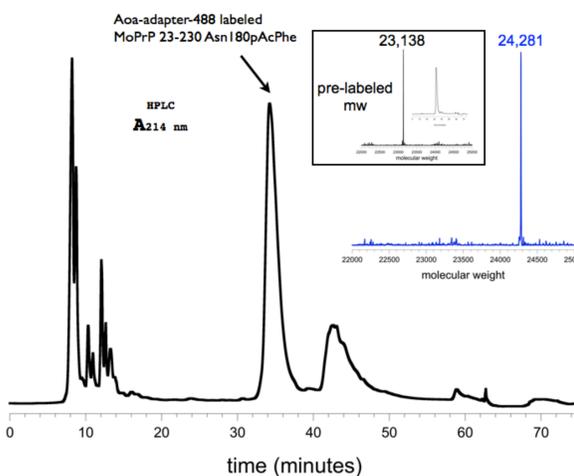
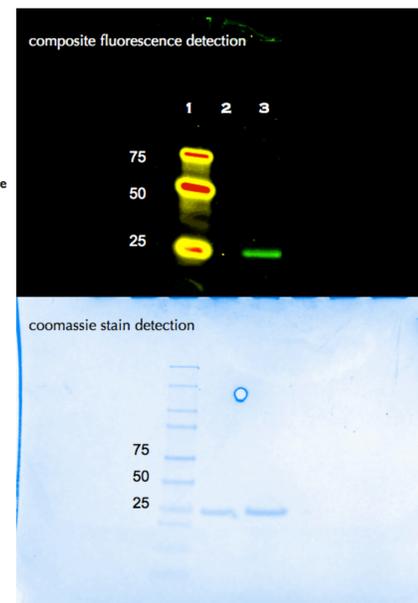
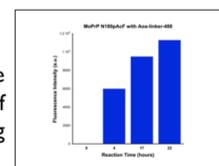
4. Protein Dye oxime Labeling

- west C precision plus (biorad) ladder
- wild type MoPrP 23-230
- Aoa-adapter-488 labeled MoPrP 23-230 Asn180pAcPhe

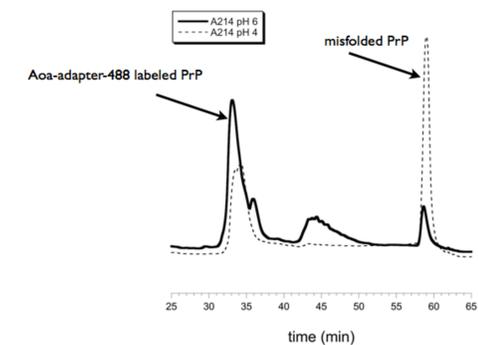
The aminoxy group site specifically labels only p-Acetylphenylalanine (2)

Reaction conditions
•25 mM MES pH 6
•100 μM pAcPhe prion protein
•1 mM Aoa-adapter-488
•10 mM anisidine (3)
•22 hours

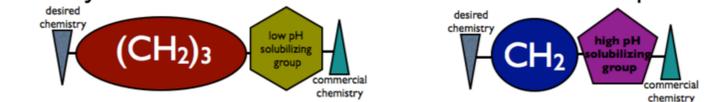
timecourse of labeling



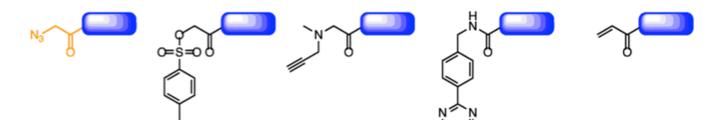
5. It is essential to avoid labeling Prion protein at acidic pH



6. Flexibility of SPSS makes linker modifications simple



7. Alternative protein conjugation chemistries easily explored

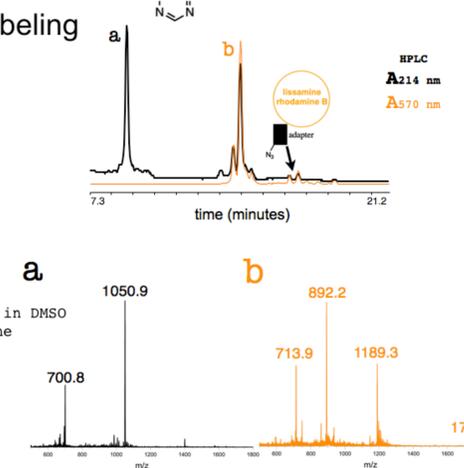


8. Peptide Dye "click" Labeling

The azide group site specifically labels only alkynes

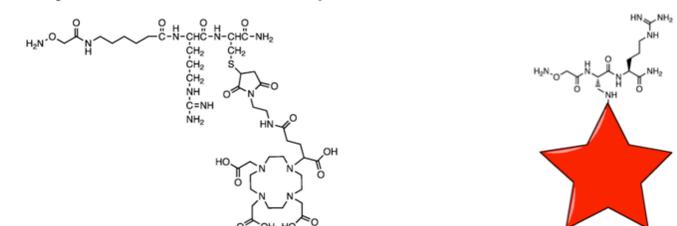
Reaction conditions
•10 mM peptide alkyne in DMSO
•5 mM N3-adapter-lissamine rhodamine in DMSO
•3 mM Cu(I)Br/ascorbate/aminoguanidine
•1.5% DIEA/.75% Lutidine
•30 minutes

a alkyne peptide
b clicked with azide-adapter-dye



9. Future directions

- Aoa-adapter-DOTA
- Dye for fluorescence polarization



Conclusions

- ✓ Reagent has "tune"-able properties rapidly accessed with solid phase synthesis
- ✓ Commercially available reporter molecules coupled in solution saving time and money
- ✓ Adapter soluble at 1 mM pH 6 > 22 hrs