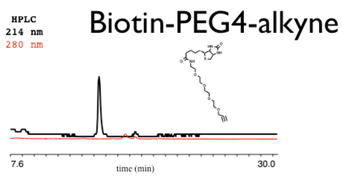
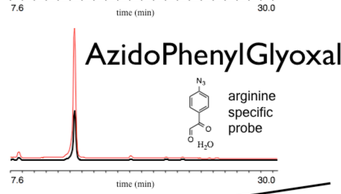
Biotin-PEG4-TPG hydrate: a novel reagent for the bioconjugation of arginine residues in peptides and proteins

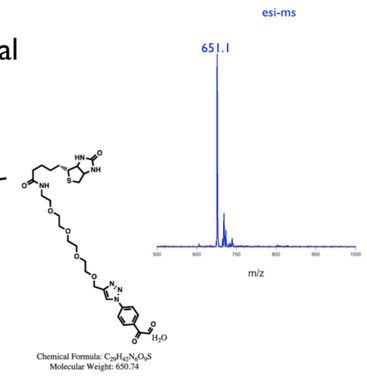
Darren A. Thompson and Philip E. Dawson

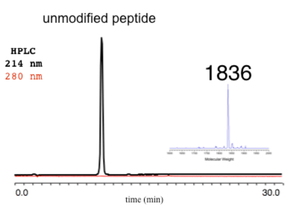
Biotin is an extremely useful molecule in basic biochemistry research. Reagents for intrinsic cysteine and lysine modification exist and represent a multi-million dollar market. The side chain of arginine is typically found on the protein surface and its directed conjugation is an attractive alternative site for derivatization. Using a high yielding click / CuAAC approach, biotin-PEG4-alkyne and azidophenylglyoxal were combined to create a novel biotinylation reagent. Examples of the ease of use and broad utility of this approach in biotechnology are given with enzyme, antibody and peptide ligations.

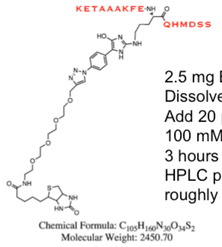


Biotin-PEG4-alkyne

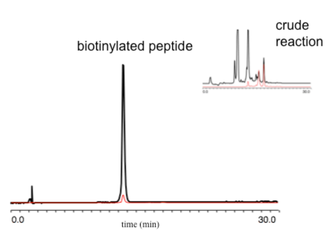


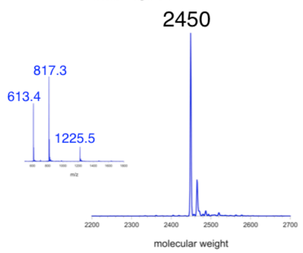


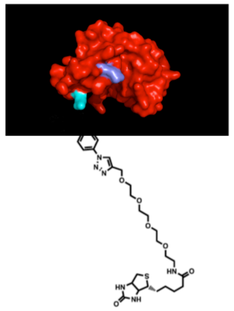




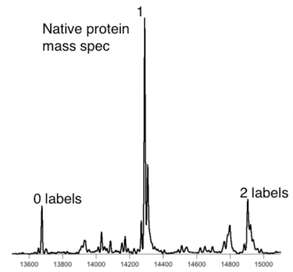
2.5 mg Biotin-PEG4-TPG reagent
Dissolved 120 µL ACN:H_2O
Add 20 µL to 250 µL 1 mM 16-mer
100 mM $KHCO_3$ pH 8.5
3 hours 37 ºC
HPLC purify
roughly stoichiometric reagent

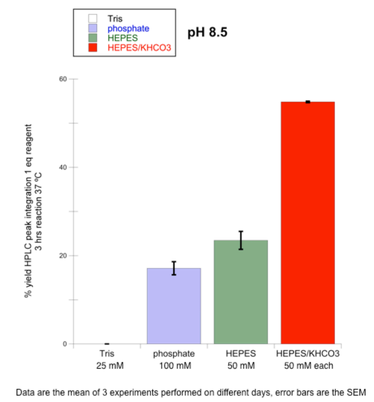






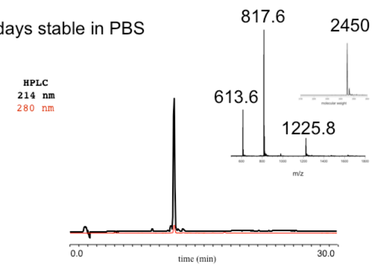
2.5 mg Biotin-PEG4-TPG reagent
Dissolved 120 µL 1:1 ACN:H_2O
Add 20 µL to 200 µL 100 µM RNAse A
100 mM $KHCO_3$ pH 8.5
3 hours 37 ºC
10 kDa cutoff amicon filter 2X
roughly 30 equivalents reagent





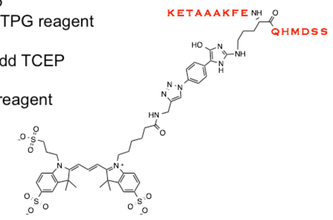
Data are the mean of 3 experiments performed on different days, error bars are the SEM

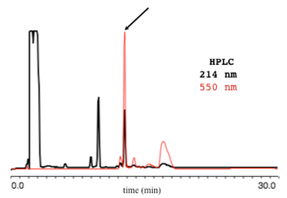
31 days stable in PBS

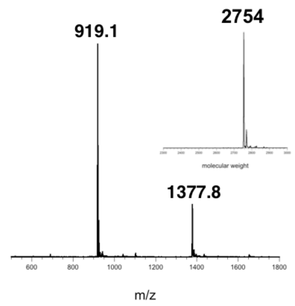


Coming soon Cy3-TPG

200 µL 1 mM 16-mer in
100 mM $KHCO_3$ pH 8.5
add to dry 200 µg Cy3-TPG reagent
3 hours 37 ºC
following conjugation add TCEP
HPLC purify
roughly .9 equivalents reagent

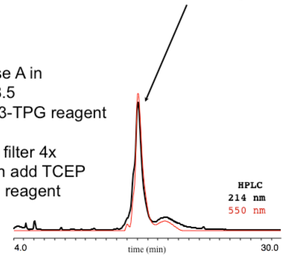


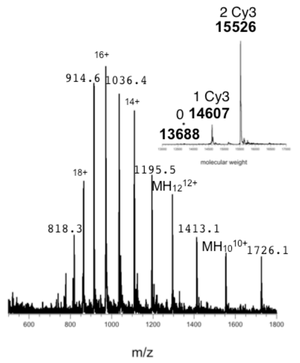


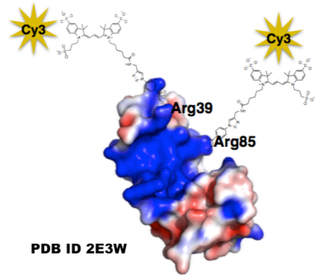


RNAse A + 2 Cy3 labels

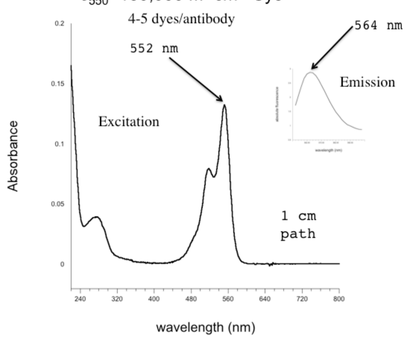
200 µL 100 µM RNAse A in
100 mM $KHCO_3$ pH 8.5
add to dry 200 µg Cy3-TPG reagent
3 hours 37 ºC
10 kDa cutoff amicon filter 4x
following spin filtration add TCEP
roughly 9 equivalents reagent

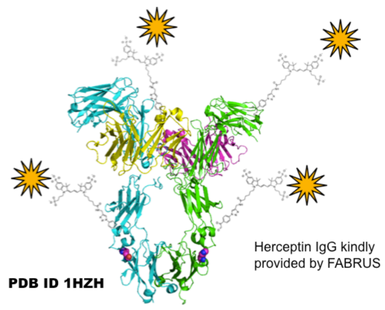






ε_{280}=208,380 $M^{-1}cm^{-1}$ Herceptin
ε_{550}=150,000 $M^{-1}cm^{-1}$ Cy3
4-5 dyes/antibody





Herceptin IgG kindly provided by FABRUS

Newly available from sigma-aldrich ALD00031

(a) Diels, O.; Schleich, K., The formation and properties of compounds originating from 1 2-diketones and benzamidine. I Diacteyl and benzamidine. *Ber Dtsch Chem Ges* **1916,** *49*, 1711-1721; (b) Takahashi, K., Reaction of Phenylglyoxal with Arginine Residues in Proteins. *J Biol Chem* **1968,** *243* (23), 6171-6179; (c) Politz, S. M.; Noller, H. F.; Mcwhirter, P. D., Ribonucleic Acid-Protein Cross-Linking in Escherichia-Coli Ribosomes - (4-Azidophenyl)Glyoxal, a Novel Heterobifunctional Reagent. *Biochemistry-Us* **1981,** *20* (2), 372-378; (d) Kolb, H. C.; Finn, M. G.; Sharpless, K. B., Click chemistry: Diverse chemical function from a few good reactions. *Angew Chem Int Edit* **2001,** *40* (11), 2004-2021; (e) Bicker, K. L.; Subramanian, V.; Chumanevich, A. A.; Hofseth, L. J.; Thompson, P. R., Seeing Citrulline: Development of a Phenylglyoxal-Based Probe To Visualize Protein Citrullination. *J Am Chem Soc* **2012,** *134* (41), 17015-17018.

HPLC conditions: 0-70% B 30 min, 1 mL/min, Phenomenex Jupiter Proteo 4.6 x 150 mm 4 µm 90 Å, HP1050

ESI-MS conditions: ABSCIEX API-2000

The Scripps Research Institute®