"New" Uses for "Old" Arginine Specific Conjugation/Crosslinking Reagents

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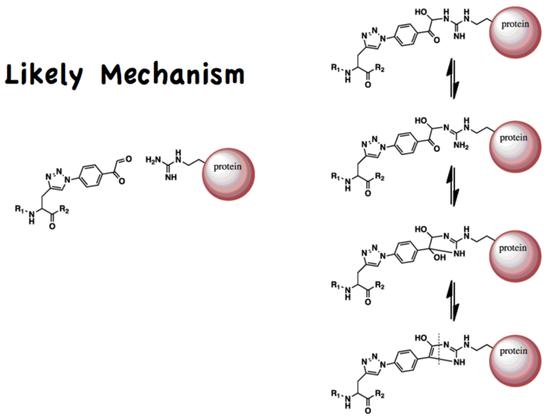
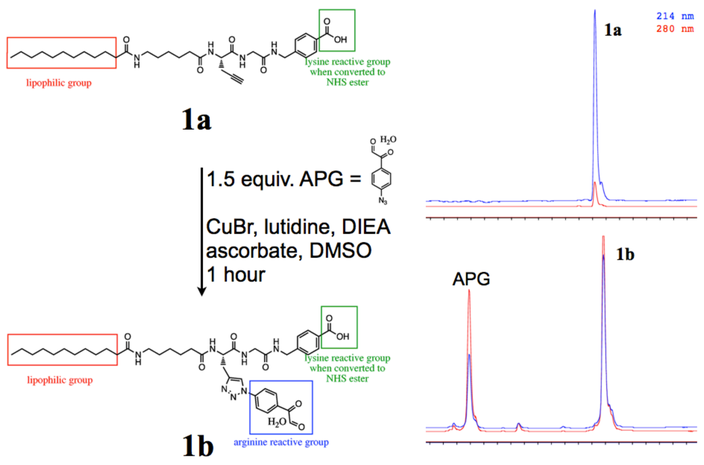
The remarkably specific, moderately reactive reagent phenylglyoxal covalently modifies guanidino groups forming a meta-stable, heterocyclic dihydroxydehydroimidazole linkage, which, when dehydrated, creates a durable moiety. Arginine represents just 4.2% of all amino acids in vertebrate proteins, but is highly abundant on the surface of proteins. In contrast to the widely popular lysine and cysteine directed crosslinkers, arginine specific reagents have traditionally been underutilized, due, in part, to the lack of readily available linkers. We have developed a straightforward approach for the introduction of the commercially available para-Azidophenylglyoxal group into peptides using CuAAC / click chemistry. Propargyl groups can be inserted either as an unnatural amino acid or through the reaction of propargyl bromide with a terminal/ side chain amine. para-Azidophenylglyoxal can be "clicked" onto the alkyne, generating a payload attached arginine targeted crosslinking agent. Applications of this technology through chemoselective conjugation to RNAse A 1-16, the RNAse A protein, and covalent trapping trimers of HIV Env are demonstrated.

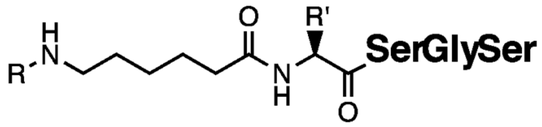
Likely Mechanism

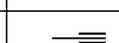
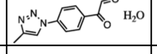
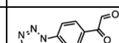
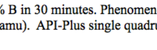


Incorporation of phenylglyoxal into complex probes



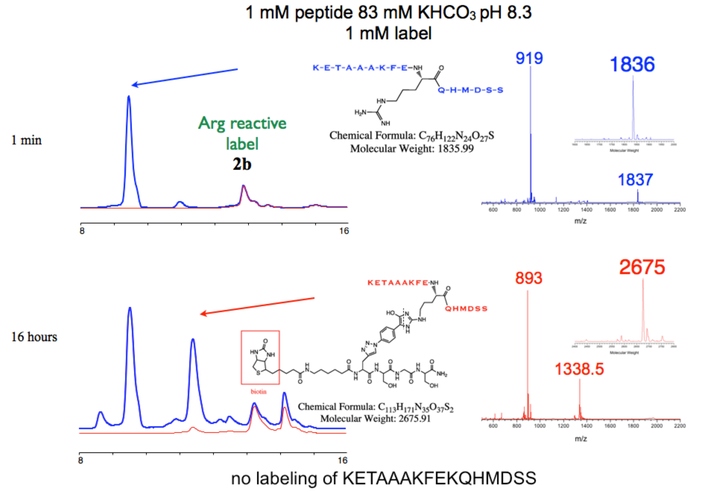
Modular approach to triazolylphenylglyoxal (TPG) probes



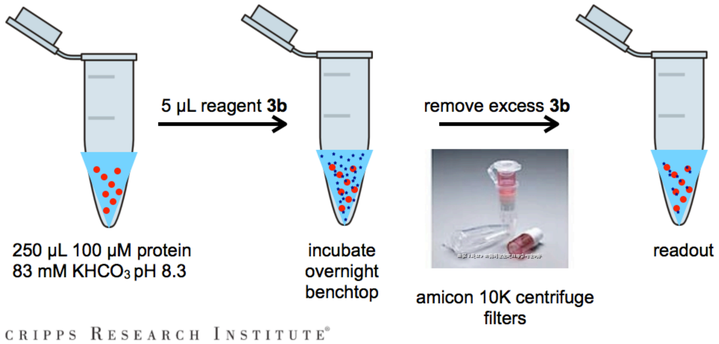
	R	R'	t_R[a]	esi-ms[b]
2a	biotin		11.95	684.0
2b	biotin		12.54	876.5
3a	coumarin		14.38	645.0
3b	coumarin		14.96	838.5

[a]rp-hplc retention time (min) 0-70% B in 30 minutes. Phenomenex Jupiter Proteo 4 µm 90 Å 4.6 x 150 mm
[b]experimentally determined mass (amu). API-Plus single quadrupole

TPG probes chemoselectively label peptides

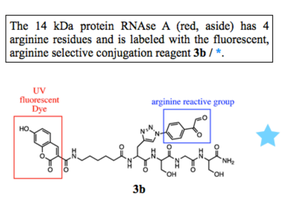


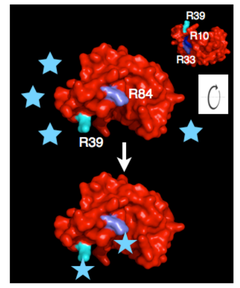
Easy protein labeling



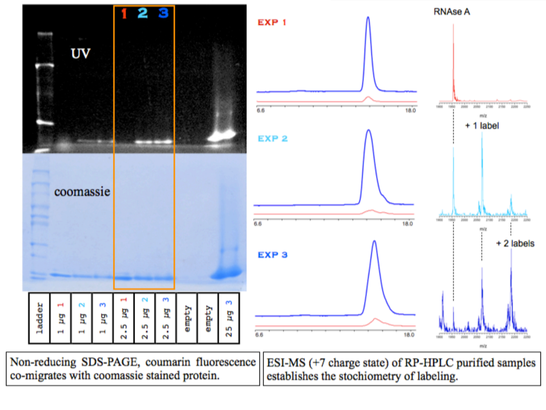
TPG probes chemoselectively label proteins

The 14 kDa protein RNAse A (red, aside) has 4 arginine residues and is labeled with the fluorescent, arginine selective conjugation reagent **3b** / *.





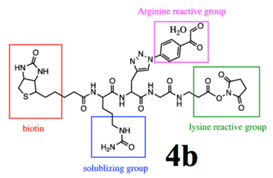
EXP1 RNAse A control
EXP2 RNAse A + 0.7 mM label **3b**
EXP3 RNAse A + 1.4 mM label **3b**

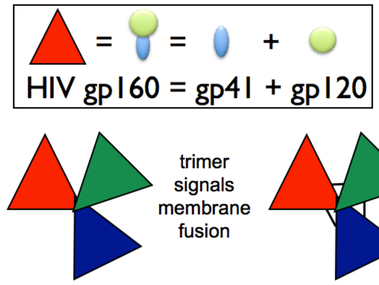


Non-reducing SDS-PAGE, coumarin fluorescence co-migrates with coomassie stained protein.

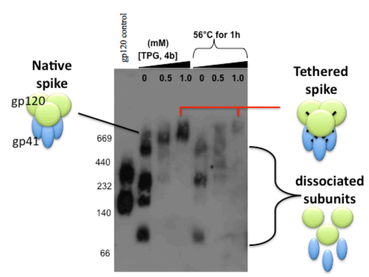
ESI-MS (+7 charge state) of RP-HPLC purified samples establishes the stochiometry of labeling.

TPG probes crosslink HIV spike proteins





Western Blot of Native PAGE



Crosslinking of trimers on surface of JRFL virus. Virons were incubated with 0, 0.5, or 1 mM **4b** at pH 7.5 for 30 min. Blue Native PAGE was developed with a cocktail of neutralizing mAbs to gp41 and gp120.

Conclusion

- Mild buffer conditions for labeling (pH 7-8).
- Compatible with lysine labeling / NHS esters.
- Ligation product stable over 5 days in PBS / Tris and lyophilization.
- Introduced quantitatively using commercially available reagents.

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