Incorporation of a Disulfide Mimic into an Fc Affinity Peptide: Synthesis and Binding

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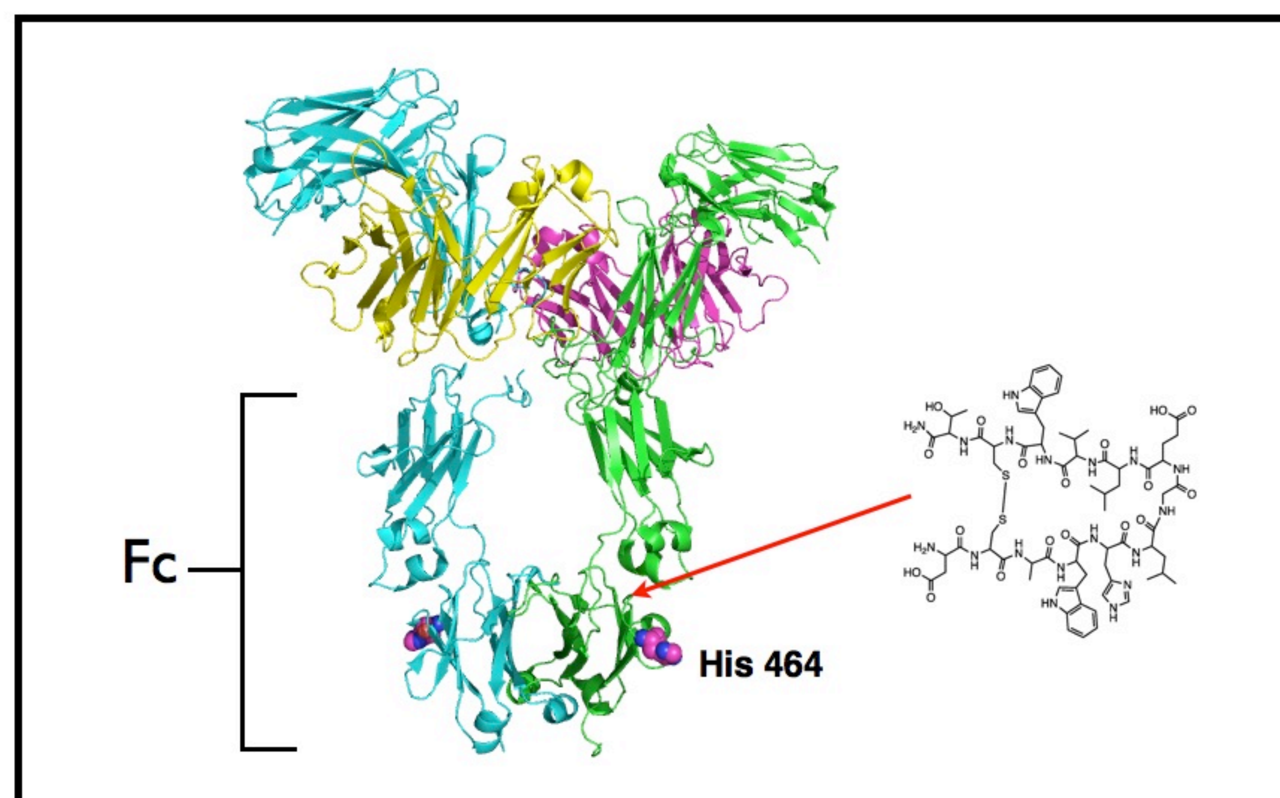
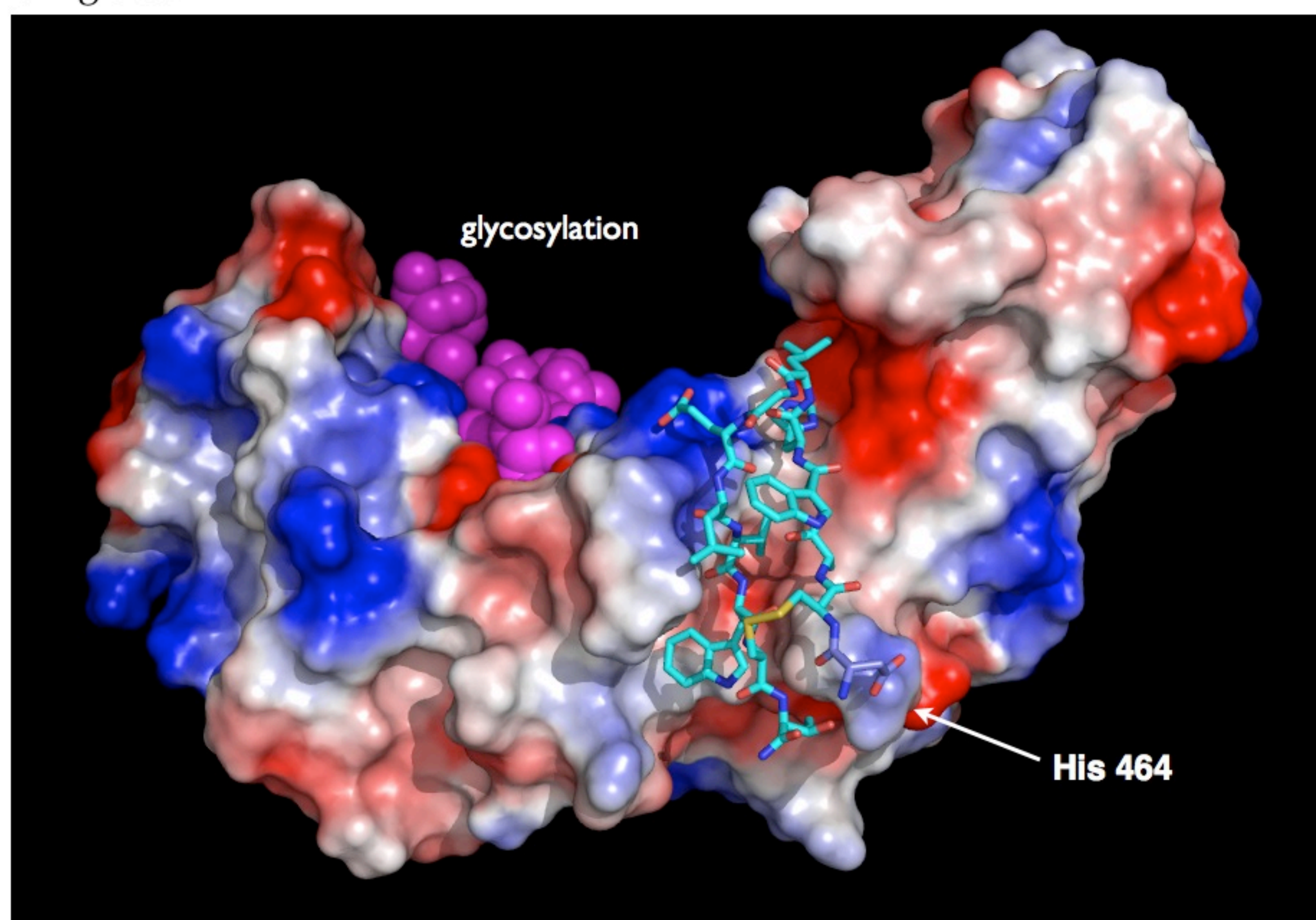
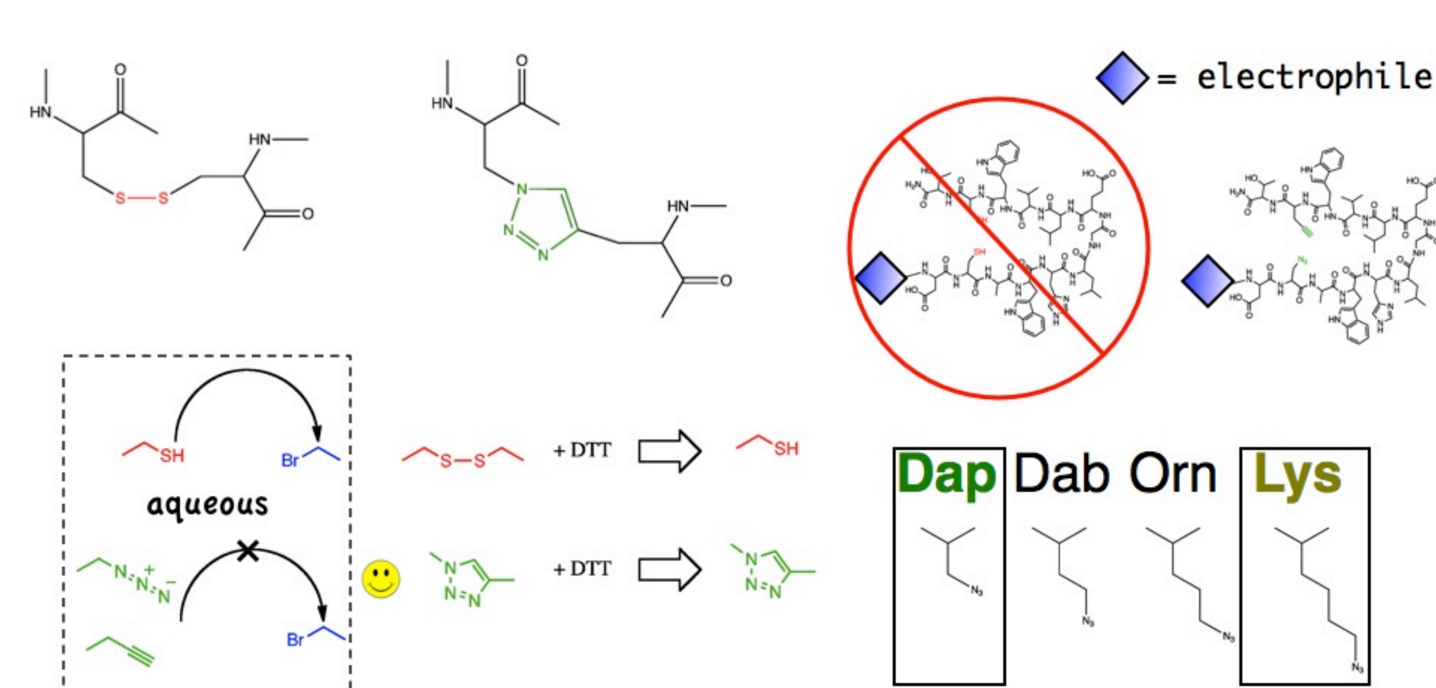
The copper catalyzed azide-alkyne cycloaddition or "Click" reaction provides a unique and attractive alternative to disulfide formation (1-3). The short peptide Fc-III is an affinity ligand for the Fc constant region of the human IgG antibody having a disulfide bond (4). Although they differ fundamentally, in that the disulfide bond forms through an oxidation of nucleophiles easily disrupted by reducing agents commonly utilized in protein chemistry while the Click mechanism involves no net change in electron number resulting in a stable triazole ring, sterically the two types of closures should be quite similar. In the modified Fc-III peptide the amino acid propargylglycine is substituted for one of the cysteines and serves as the alkyne source while the side chain azide-protected diaminopropionic acid replaces the other cysteine. The conformational constraint introduced by a disulfide bond has been reproduced by other bridging chemistries (e.g. Grubbs, cyclic lactams, thioethers, etc.) with a variable degree of success. The orthogonal nature and simplicity of this process produces a molecule in high yield employing universal SPPS methods free from the pitfalls normally associated with cysteine.



Human IgG b12 binding site (red arrow) for Fc-III peptide (1HZH.pdb). The Fc fragment sequence is constant in human IgG, and nucleophilic amino acids such as His 464 (1HZH numbering) are located near the Fc-III binding site. Our long term goal is to selectively modify Fc residues near to, but not occluding the Fc-III binding site.



Human Fc in complex with Fc-III peptide (1DN2.pdb, electrostatic surface of monomer). The peptide binds with low nM affinity to the hinge region and the N-and C-termini protrude toward the exterior of the Fc fragment, (N-terminus highlighted in blue).

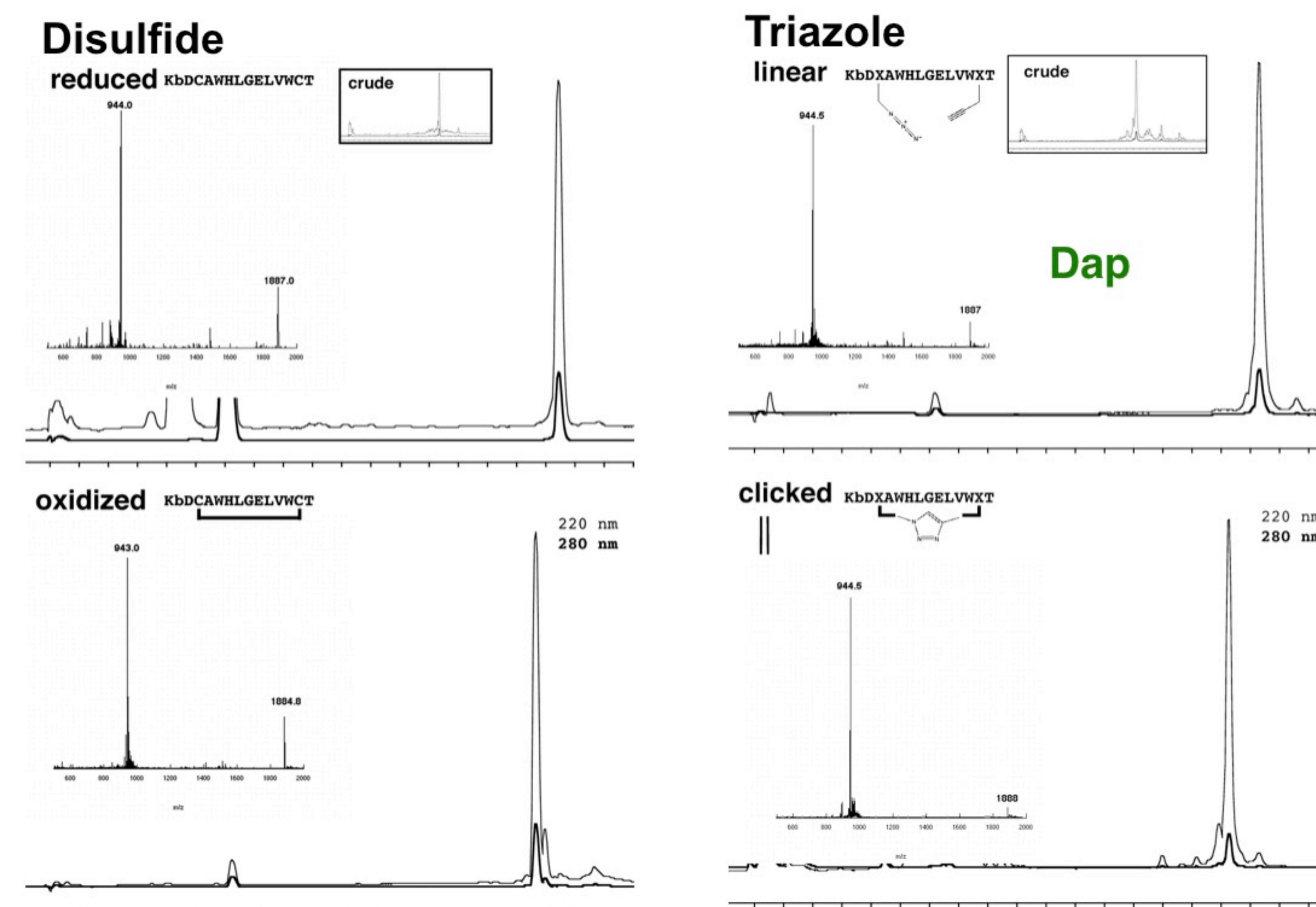


Bioconjugation reagents are typically electrophiles. We replace the disulfide bridge with a triazole, dramatically simplifying synthesis.

Cyclic disulfide and triazole Fc-III variants Cystine/triazole MW and retention times.	t_r (min)	mH^+ calc. (g/mol)	mH^+ obs. (amu)
D**C**AWHLGELVW**C**T	20.9	1529	1529
K(ε-Biotin)D**C**AWHLGELVW**C**T	18.7	1885	1885
K(ε-Biotin)D**B**AWHLGELVW**X**T	18.5	1887	1888
K(ε-Biotin)D**J**AWHLGELVW**X**T	21.5	1929	1930
D**J**AWHLGELVW**X**T	22.2	1575	1576

B=Dap(N3), J=Lys(N3), X=propargylglycine. HPLC: Jupiter Proteo 4.6 x 150 mm 90 Å, 0 to 70% B / 30 min A: H_2O ,.05% TFA, B: 90% ACN/H_2O .045% TFA.

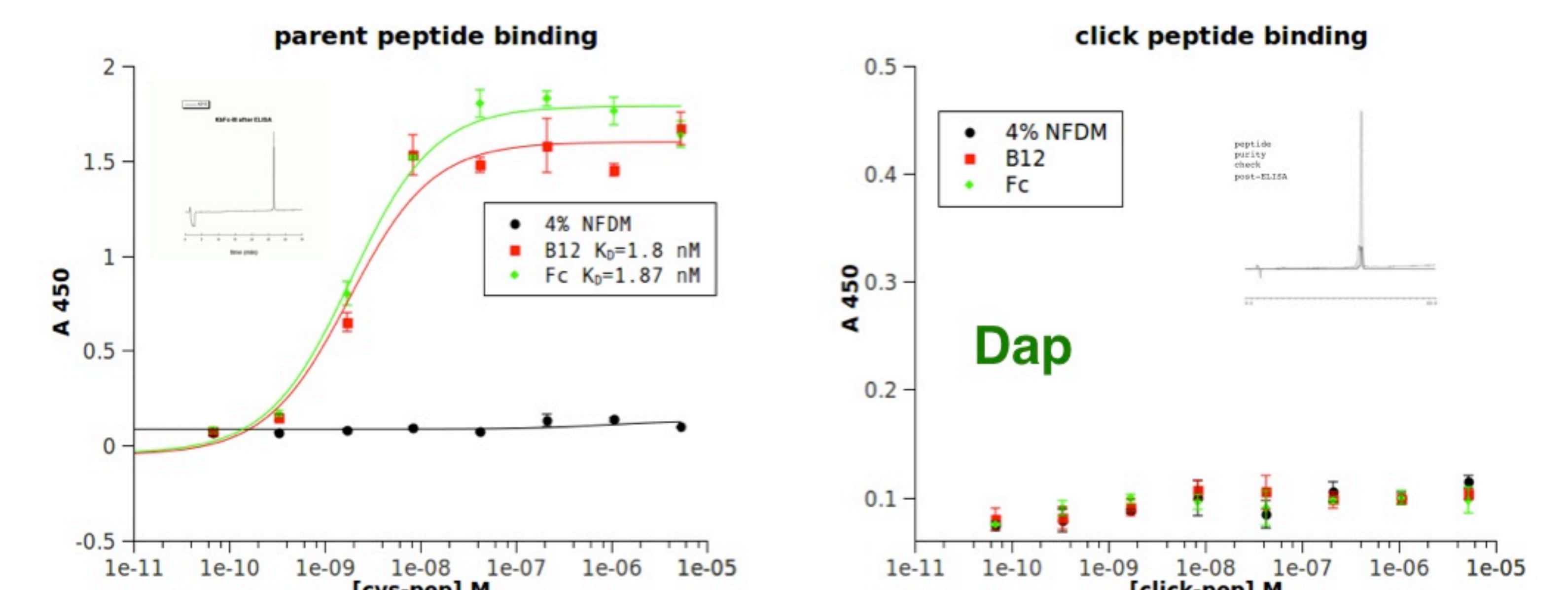
Aqueous Cyclization of Fc-III and Dap / triazole isostere



Oxidation/Click Conditions

- **oxidation** initiated by dissolving prep HPLC purified peptide (1 mg/mL) 6 M GuHCl .1 M Tris pH 8, diluting 10 fold with .1 M Tris pH 8, and stirring ON
- **CuAAC ligation** conditions were adopted from reference 5.
  final reaction concentration were: 0.1 mM peptide, 0.3 M Na_2HPO_4 pH 7, 5 mM Na Ascorbate, 5 mM aminoguanidine, 0.1 mM $CuSO_4$, 0.5 mM Tris(3-hydroxypropyltriazolylmethyl)amine (THPTA) ligand.
- **CuAAC Protocol:**
  add 3 mL peptide (3 mg/mL 6M GuHCl 0.05% TFA), 30 mL Na_2HPO_4 buffer, 1.925 mL fresh 100 mM Na Ascorbate, 1.925 mL 100 mM aminoguanidine, 577.5 μL premixed 20 mM $CuSO_4$ (192.5 μL) and 50 mM THPTA (385 μL) in 50 mL tube, 1 hour, lower pH 4 mL with AcOH, filter, purify.

Binding of biotin-Fc-III analogs by ELISA



Dap triazole isostere does not bind to IgG or Fc fragments suggesting that the triazole is unable to adopt the required disulfide geometry.

Expanded Fc-III analogs: The triazole link can be tuned by varying the side chain length with Dab, Orn and Lys as shown by Meldal (1). The Lysine analog (6) in this series was synthesized under the previously described conditions with excellent results.

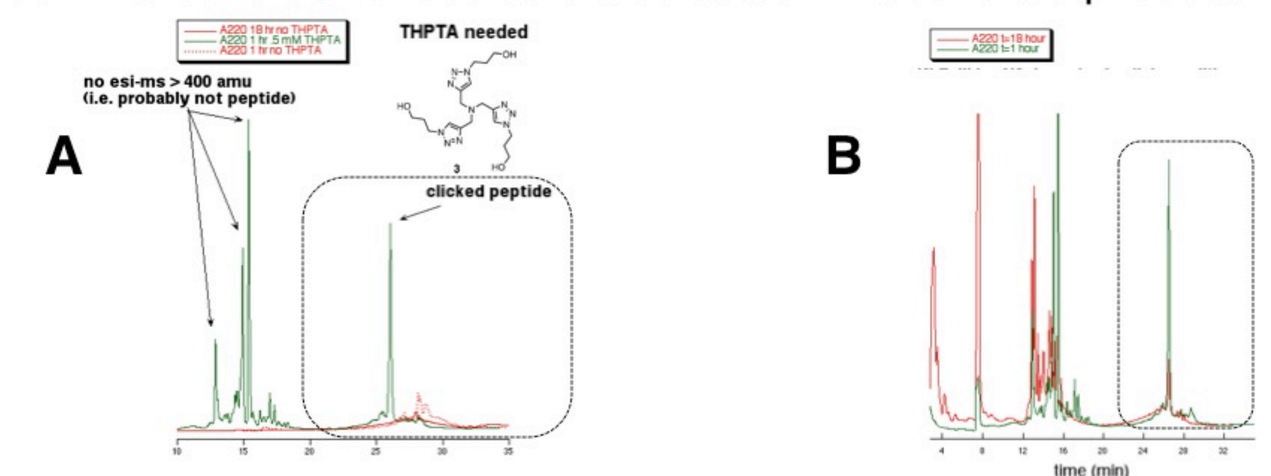
clicked KbDXAWHLGELVWXT

Lys

Observations on using CuAAC with unprotected peptides.

A) Reactions performed in aqueous buffer without THPTA ligand and aminoguanidine did not yield detectable cyclic products.

B) Prolonged exposure of the Fc-III peptide to optimized aqueous CuAAC conditions lead to a decrease in recovered product.



We have demonstrated a robust method for aqueous peptide cyclization by "click" chemistry, including an example of a 4 bond intrachain bridge (7). A more thorough sampling of bridge lengths and switching positions of the azide and alkyne amino acids may yield a better biological mimic. This method is compatible with proteins or peptides containing two or more disulfides, conceivably we could replace one disulfide with a cyclic triazole, isolating that bond (i.e. locking it down), and testing the contribution of the others to binding, folding, and stability,

1. Holland-Nell K, Meldal M. Maintaining Biological Activity by Using Triazoles as Disufide Bond Mimetics. *Angew Chem Int Edit*. **2011**;50(22):5204-6.
2. Rostovtsev VV, Green LG, Fokin VV, Sharpless KB. A stepwise Huisgen cycloaddition process: Copper(I)-catalyzed regioselective "ligation" of azides and terminal alkynes. *Angew Chem Int Edit*. **2002**;41(14):2596-9.
3. Tornøe CW, Christensen C, Meldal M. Peptidotriazoles on Solid Phase: [1,2,3]-Triazoles by Regiospecific Copper(I)-Catalyzed 1,3-Dipolar Cycloadditions of Terminal Alkynes to Azides. *J Org Chem.* **2002**;67(9):3057-64.
4. DeLano WL, Ultsch MH, de Vos AM, Wells JA. Convergent solutions to binding at a protein-protein interface. *Science*. **2000**;287(5456):1279-83.
5. Hong V, Presolski SI, Ma C, Finn MG. Analysis and optimization of copper-catalyzed azide alkyne cycloaddition for bioconjugation. *Angew Chem Int Ed Engl.* **2009**;48(52):9879-83.
6. Isaad AL, Papini AM, Chorev M, Rovero P. Side chain-to-side chain cyclization by click reaction. *J Pept Sci.* **2009**;15(7):451-4.
7. Roice M, Johannsen I, Meldal M. High capacity poly(ethylene glycol) based amino polymers for peptide and organic synthesis. Qsar Comb Sci. 2004;23(8):662-73.

Acknowledgements

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