Cysteine chemoselective ligation with carbonylacrylic reagents for the construction of functional and plasma stable conjugates

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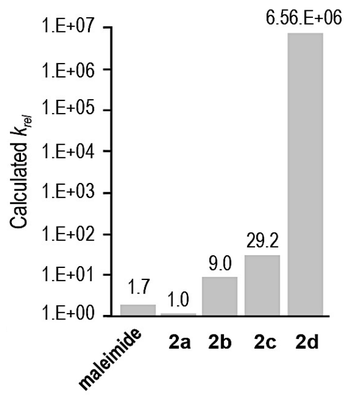
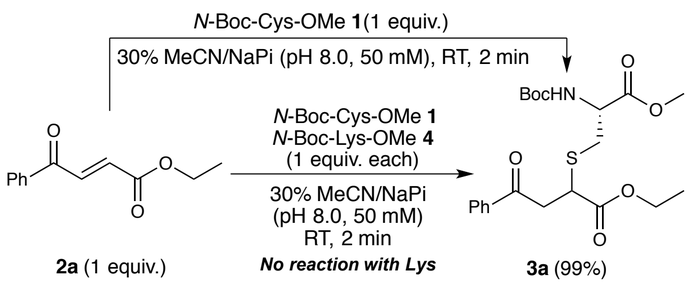
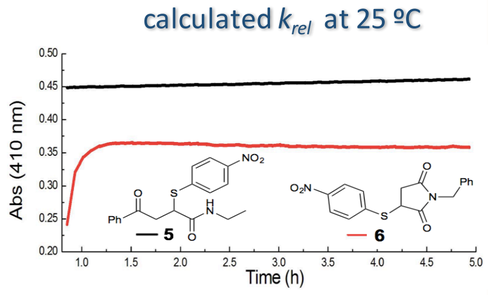
CYSTEINE SELECTIVE LABELING

Maleimides remain the reagents of choice for the preparation of therapeutic and imaging protein conjugates despite the known rapid retro-Michael addition *in vivo*. Here, we present the rational design of carbonylacrylic reagents for stable and irreversible chemoselective cysteine (Cys) bioconjugation.



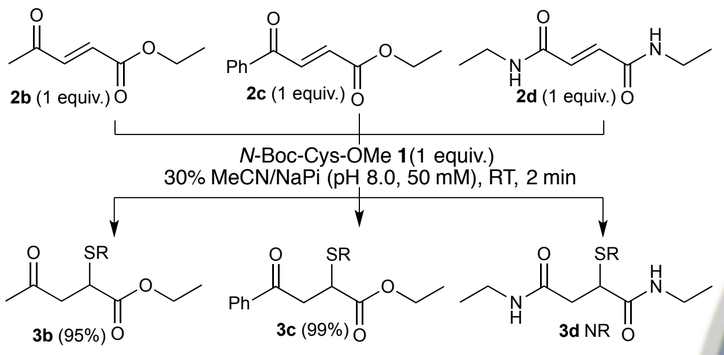
calculated k_{rel} at 25 ºC



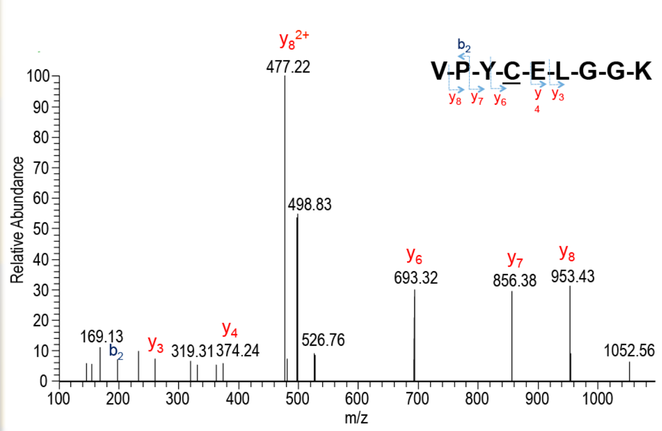


Kinetic assays

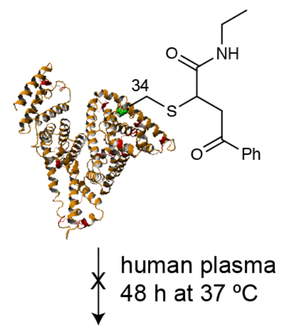
Complete Cys-chemoselectivity.



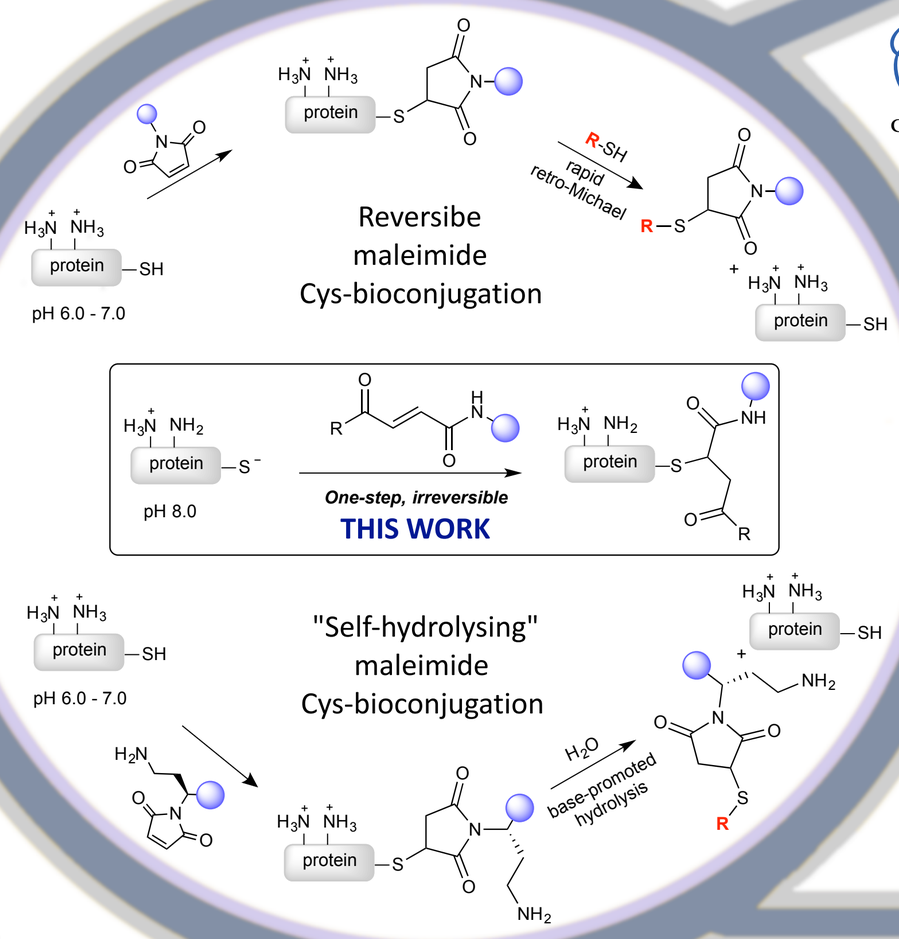
2c for optimal protein functionalization.

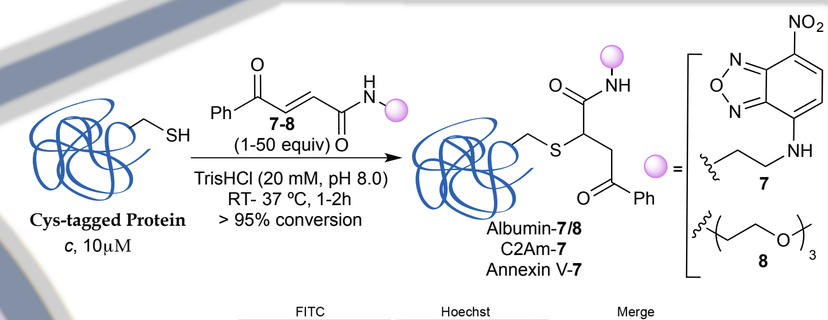


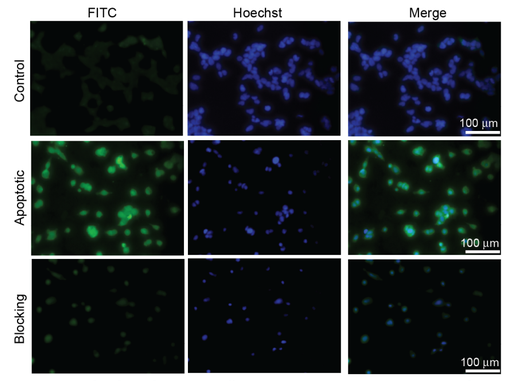
MS/MS spectrum of the *m/z* 526.75 doubly charged ion of the tryptic peptide YPYCELGGK, containing the modification at the Cys residue of C2Am–**2c**.



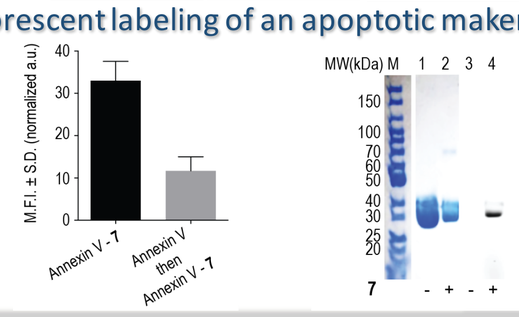
Stability of the albumin conjugate with **2c** in human plasma as well as in the presence of 10 mM GSH.





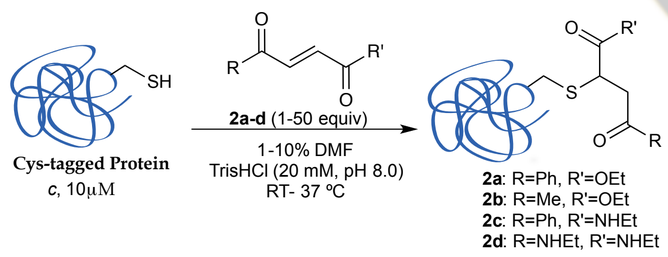


Fluorescent labeling of an apoptotic maker protein.

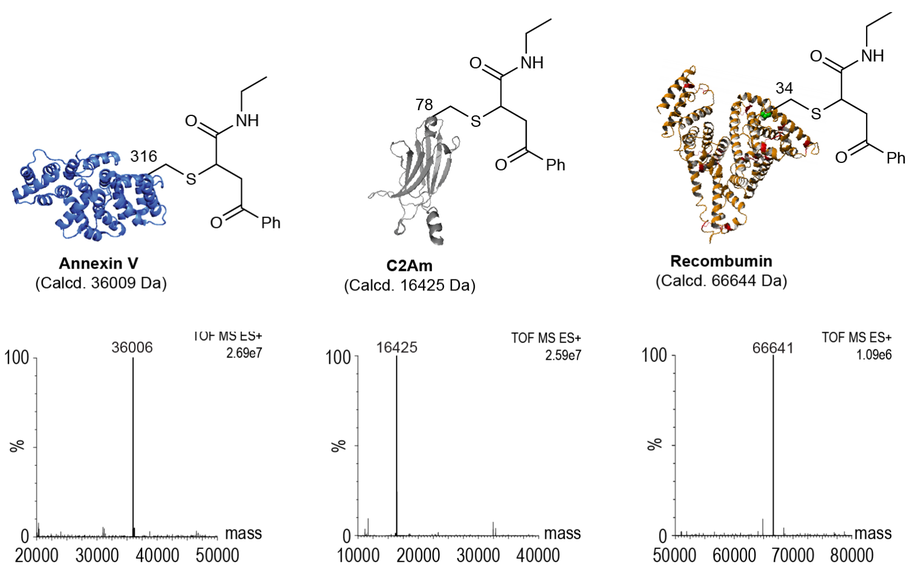


PROTEIN BIOCONJUGATION

The 3-carbonylacrylic derivatives **2a–d** were evaluated for the labeling of Annexin V, C2Am (C2A domain of synaptotagmin-I) and recombinant human albumin –Recombumin® (Albumedix Ltd),

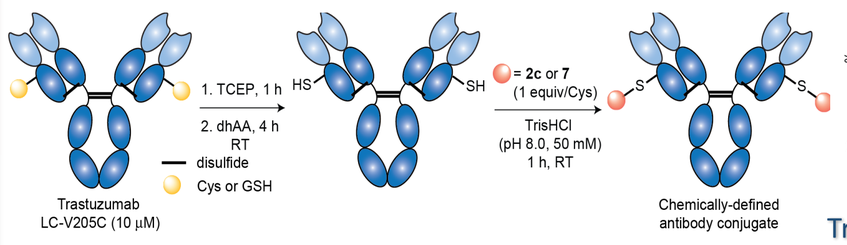


Protein Cys-bioconjugation with carbonylacrylic reagent **2c**.

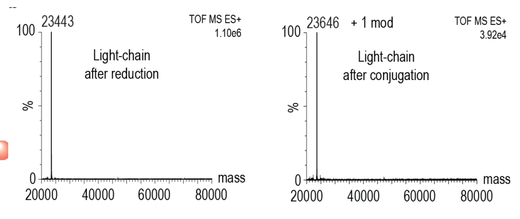


ESI–MS spectra of conjugates.

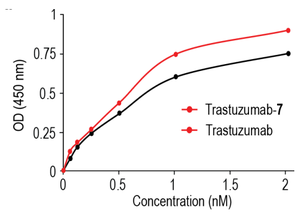
ANTIBODY BIOCONJUGATION



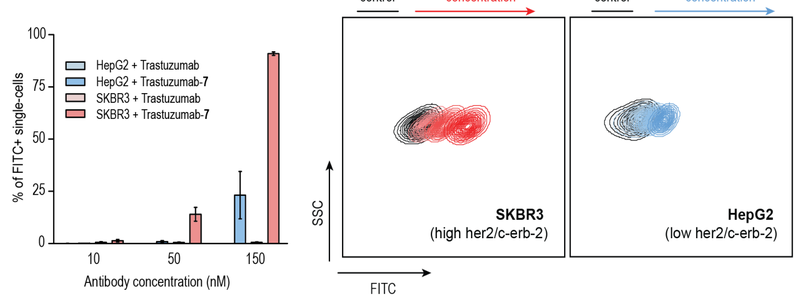
Chemoselective and equimolar bioconjugation of Trastuzumab using **2c** and **7**.



ESI–MS spectra of the light-chain of Trastuzumab before and after conjugation with **2c**.



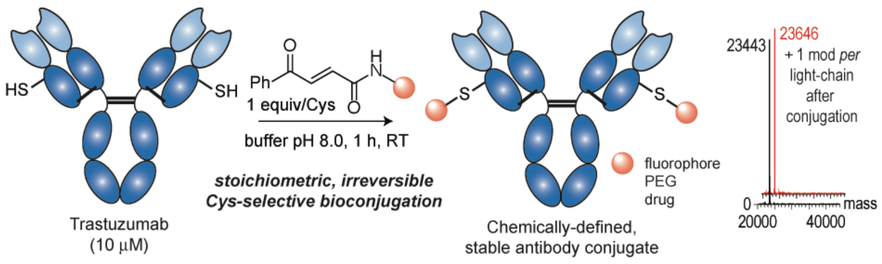
Binding activity data for native Trastuzumab and Trastuzumab-**7** obtained by ELISA.



Analysis of specificity of the Trastuzumab-**7** towards her2/c-erb-2 by flow-cytometry.

CONCLUSIONS

A direct and irreversible Cys-selective bioconjugation is reported using simple carbonylacrylic reagents. The conjugates formed are fully stable when exposed to GSH and in plasma, and retain their function, as evidenced by the selective imaging of apoptotic and high expression her2+ cells. The direct chemoselective and irreversible Cys-conjugation technology disclosed herein will find significant use for the preparation of imaging and therapeutic conjugates for *in vivo* purposes



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