CYSTEINE–SPECIFIC DUAL LABELLING OF PROTEINS USING STRAINED [2.2.1]AZABICYCLIC VINYL SULFONES

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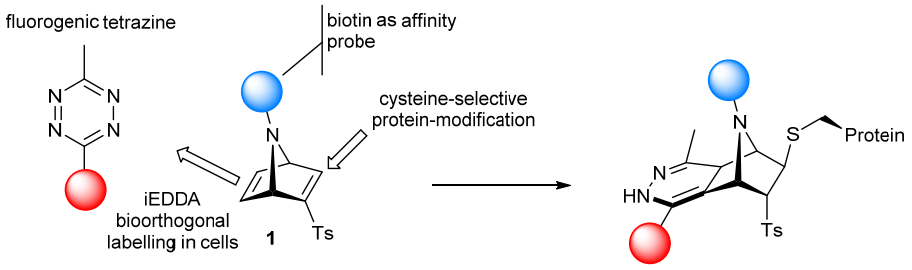
The development of simple and robust methodologies that enable the installation of two or more distinct synthetic modifications into a protein is an area of prominent interest.[1] For this purpose, the use of multifunctional scaffolds that simultaneously display two different modifications and one handle for site-selective protein modification, is the most straightforward strategy.

This work shows the use of [2.2.1]azabicyclic vinyl sulfones that simultaneously enable cysteine-selective protein modification and introduce a handle for further bioorthogonal ligation.[2] In particular, model biotinylated reagent **1** has been employed for the modification of two cysteine-tagged proteins, Ubiquitin and C2Am, under mild conditions. The dienophile present in the mono-labelled azabicycle-C2Am bioconjugate could react with a fluorogenic tetrazine in cells *via* inverse electron demand Diels-Alder (iEDDA) reaction to afford a dual-labelled bioconjugate. This new bioconjugate allowed selective apoptotic cells imaging.



[1] a) A. Maruani, M. E. B. Smith, E. Miranda, K. A. Chester, V. Chudasama, S. Caddick, *Nat. Commun.*, **2015**, *6*, 6645; b) M. R. Levengood, X. Zhang, J. H. Hunter, K. K. Emmerton, J. B. Miyamoto, T. S. Lewis, P. D. Senter, *Angew. Chem., Int. Ed.*, **2016**, *56*, 733.

[2] E. Gil de Montes, E. Jiménez-Moreno, B. L. Oliveira, C. D. Navo, P. M. S. D. Cal, G. Jiménez-Osés, I. Robina, A. J. Moreno-Vargas, G. J. L. Bernardes, *Chem. Sci.* **2019**, DOI: 10.1039/C9SC00125E.