A fluorescent probe which allows highly specific thiol labeling at low pH

Determination of the thiol-disulfide status in biological systems is challenging as redox pools are easily perturbed during sample preparation. This is particularly pertinent under neutral to mildly alkaline conditions typically required for alkylation of thiols. Here we describe the synthesis and properties of a thiol-specific reagent, fluorescent cyclic activated disulfide (FCAD), which includes the fluorescein moiety as fluorophore and utilizes a variation of thiol-disulfide exchange chemistry. The leaving-group character of FCAD makes it reactive at pH 3, allowing modification at low pH, limiting thiol-disulfide exchange. Different applications are demonstrated including picomolar thiol detection, determination of redox potentials, and in-gel detection of labeled proteins.

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