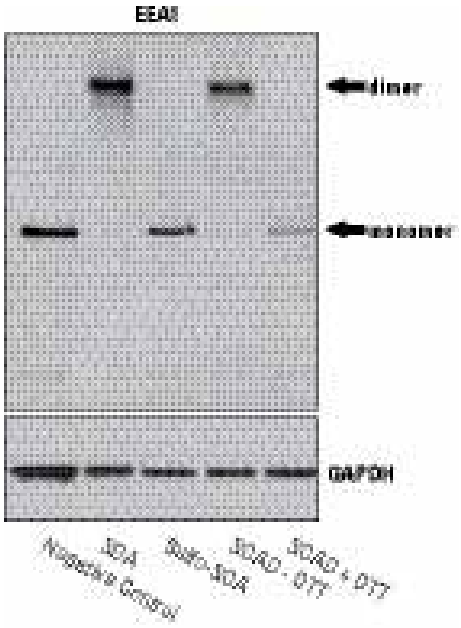
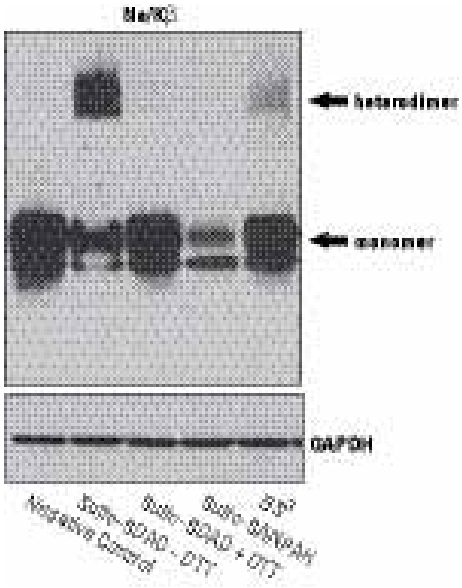


Intracellular crosslinking of EEA1 protein complex using Thermo Scientific Pierce NHS-Ester Diazirine (SDA) crosslinkers. HeLa cells (2 x 10^6) were labelled for 10min with 1mM of SDA, Sulfo-SDA, LC-SDA orSDAD in PBS. Non-reacted NHS esters were quenched with Tris•HCl, pH8.0 at a final concentration of 100mM for 5min and then rinsed with PBS. NHS-diazirine labelled cells and a mock-treated control were UV irradiated using a Stratalinker 2400 at 365nm for 15min at a distance of 40mm in PBS. After UV treatment, cells were lysed with Thermo Scientific Pierce M-PER mammalian protein extraction reagent (**PN78501**) and analysed for total protein concentration using the Pierce BCA protein assay (**PN23225**). Reducing sample buffer was added to 10µg of each sample with the exception of a duplicate SDAD-treated sample (-DTT) and separated by SDS-PAGE. Results were analysed by Western blot using anti-EEA1 antibodies.



Comparison of extracellular crosslinking of the Na/K ATPase complex sulfonated NHS ester crosslinkers. HeLa cells (2 x 10^6) were labelled for 10min with 1mM Sulfo-SDAD, BS3 or Sulfo-SANPAH in PBS. All sulfo-samples were quenched, irradiated and lysed as described in figure above before SDS-PAGE analysis and Western blotting with antibodies against the Na/Kβ subunit and GAPDH.

NHS-Diazirine crosslinkers, Thermo Scientific Pierce

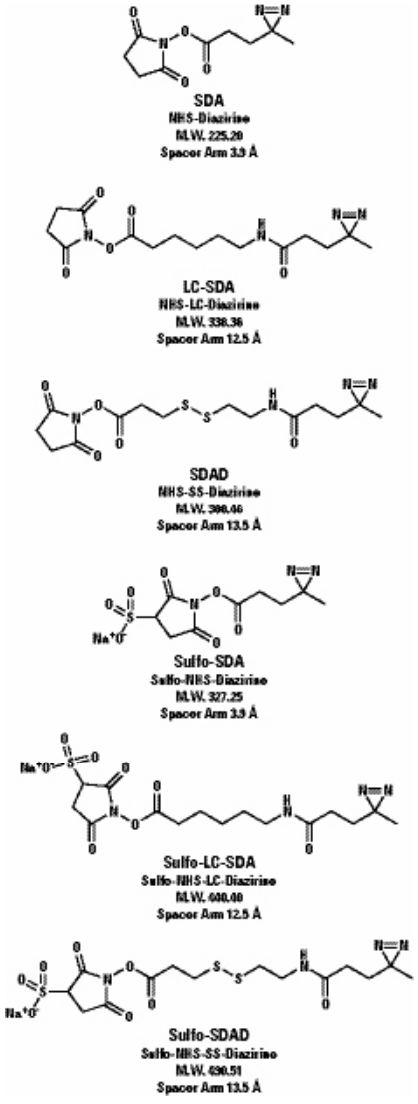
PN

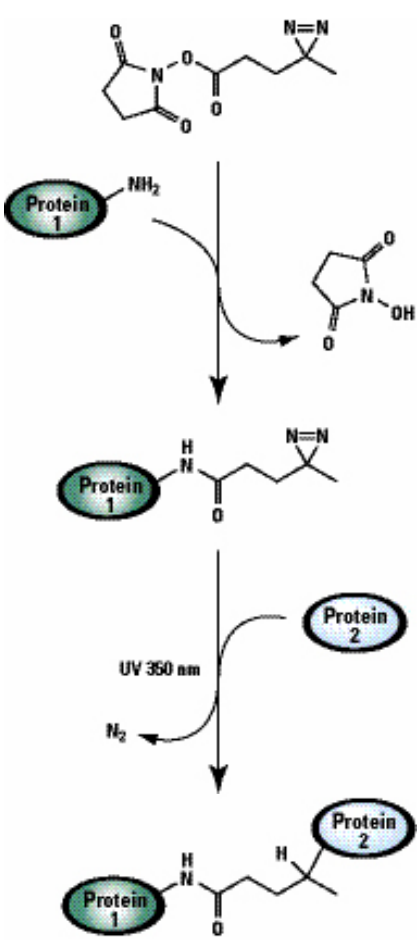
Heterobifunctional, amine-reactive photo-crosslinkers for studying protein interactions.

- Controllable – two step chemical crosslinking is activated using common laboratory UV lamps (330nm to 370nm)
- Reversible – disulfide containing spacers enable cleavage using reducing agents

The succinimidyl-ester diazirine (SDA) reagents are a new class of crosslinkers that combine proven amine-reactive chemistry with an innovative and efficient diazirine based photochemistry for conjugating amine-containing molecules to nearly any other functional group. The SDA crosslinkers include six compounds differing in spacer arm lengths, ability to cleave the crosslinked proteins, and presence or absence of a charged group for membrane permeability.
The NHS-ester diazirine derivatives (SDA, LC-SDA and SDAD) lack a charged group and are membrane-permeable. This property makes them ideal for intracellular and intramembrane conjugations. By contrast, Sulfo-SDA, Sulfo-LC-SDA and Sulfo-SDAD contain negatively charged sulfate groups that improve their water solubility and reduce cell membrane permeability, enabling their use for extracellular protein crosslinking. SDAD and Sulfo-SDAD also have a disulfide bond within the spacer that can be cleaved with reducing agents.

Catalogue No	Description	Quantity, mg
PN26167	**SDA** Succinimidyl 4,4-azipentanoate	50
PN26168	**LC-SDA** Succinimidyl 6-(4,4′-azipentanamido) hexanoate	50
PN26169	**SDAD** Succinimidyl 2-([4,4′-azipentanamido] ethyl)-1,3′-dithiopropionate	50
PN26173	**Sulfo-SDA** Sulfosuccinimidyl 4,4′-azipentanoate	50
PN26174	**Sulfo-LC-SDA** Sulfosuccinimidyl 6-(4,4′-azipentanamido)hexanoate	50
PN26175	**Sulfo-SDAD** Sulfosuccinimidyl 2-([4,4′-azipentanamido] ethyl)-1,3′-dithiopropionate	50





Mechanism of NHS-ester diazirine crosslinking.
N-hydroxysuccinimide (NHS) esters react efficiently with primary amine groups (–NH_2) in pH7-9 buffers to form stable amide bonds upon release of NHS. Photoactivation of diazirines with longwave UV light (330 to 370) creates reactive carbene intermediates. Such intermediates can form covalent bonds through addition reactions with any amino acid side chain or peptide backbone at distances corresponding to the spacer-arm lengths.