Electrophilic affibodies forming covalent bonds to protein targets.

SUPPLEMENTAL DATA

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Fig. 1. Testing EBA coupling to Affibody D36C. We analyzed Affibody D36C modification by SDS-PAGE and Coomassie staining, with or without oxidation via CuCl₂. In lane 1 Affibody D36C was not modified and not oxidized. In all other lanes, samples were oxidized. In lane 2 Affibody D36C was unmodified, in lane 3 reacted with EBA followed by IAA, and in lane 4 with IAA alone or lane 5 with EBA alone. IAA is more reactive at cysteine modification than EBA and is a positive control. Bands corresponding to Affibody D36C monomer or an Affibody D36C disulfide-bonded homodimer are marked. Resistance to copper-induced disulfide formation indicates that the cysteine has been labeled by EBA or IAA.

Supplemental Fig. 2. Analysis of affibody modification and interaction with ZSPA by mass spectrometry. A. Spectrum of Affibody D36C after reaction with EBA. B. Spectrum of the negative control for covalent Affibody:ZSPA interaction, where Affibody D36C was labeled with IAA instead of EBA, before incubating with ZSPA N6C.
Supplemental Figure 1

Supplemental Figure 2

A

B

MS expected/observed:
Affibody-IAA 10,848/10,848
ZSPA N6C 9,125/9,125