Electrophilic affibodies forming covalent bonds to protein targets.

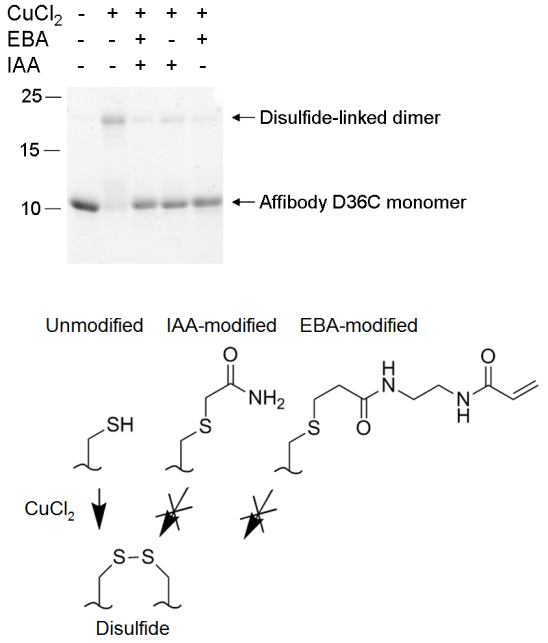
SUPPLEMENTAL DATA

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 $CuCl_2$. 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