Supporting Information

Photo-controlled reversible binding between the protein A-derived Z domain and immunoglobulin G

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Peptide synthesis, purification and crosslinking

The molecular weights of the unconjugated and photoswitch-conjugated peptides were analyzed by MALDI-MS. Because of calibration issues with the instrument (MALDI TOF/TOF analyzer, Sciex), mass differences of +/- 25 Da were often seen, also with reference proteins, and therefore not considered significant.

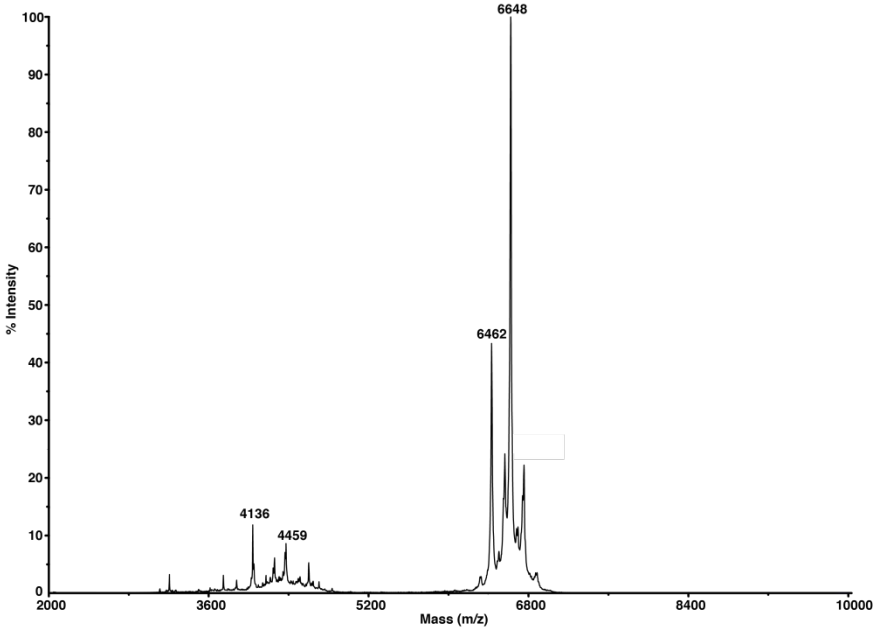

Figure S1. MALDI-MS spectrum of unconjugated, HPLC-purified Z_{C2} (theoretical MW: 6661 Da).

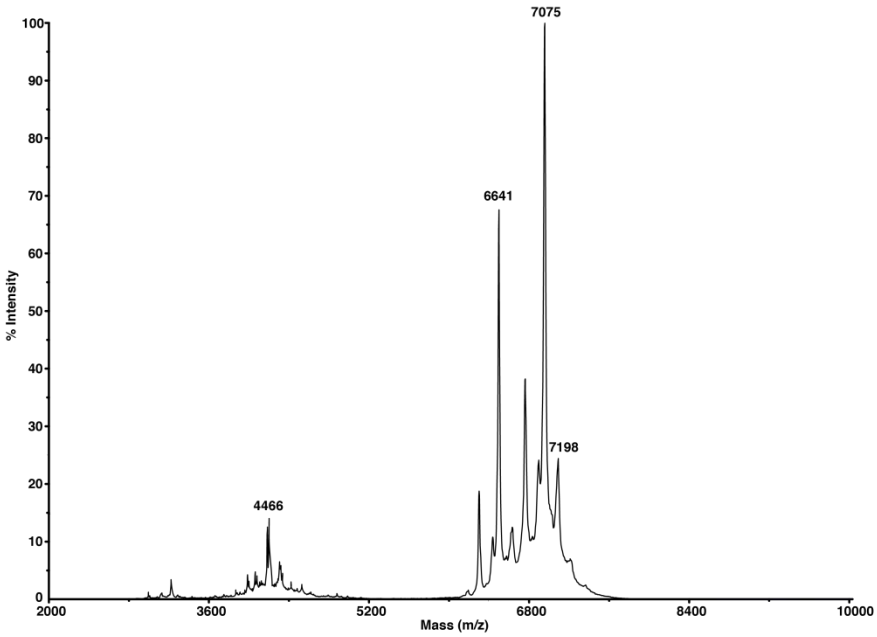

Figure S2. MALDI-MS spectrum of photoswitch-conjugated, HPLC-purified Z_{C2} (theoretical MW: 7092 Da).

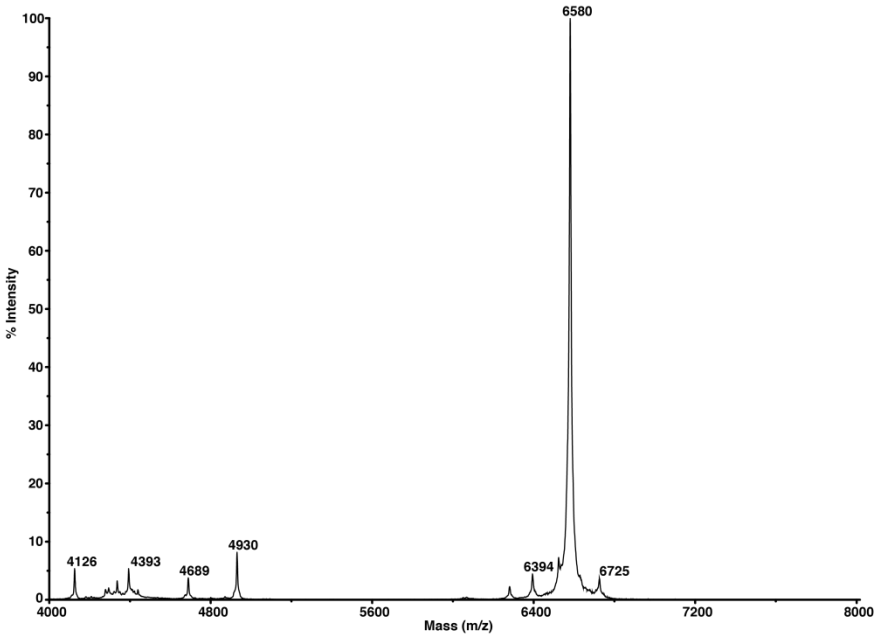

Figure S3. MALDI-MS spectrum of unconjugated, HPLC-purified Z_{C3} (theoretical MW: 6600 Da).

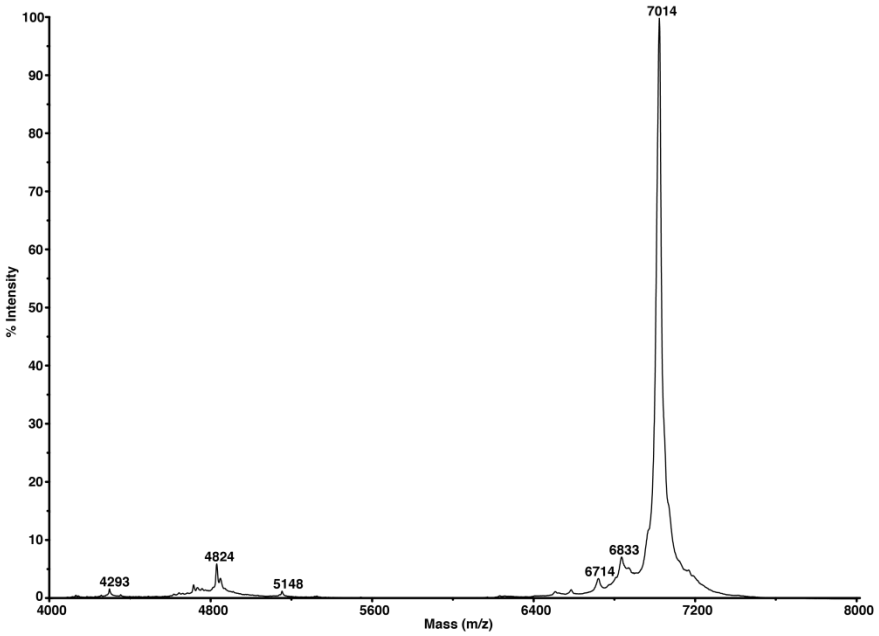

Figure S4. MALDI-MS spectrum of photoswitch-conjugated, HPLC-purified Z_{C3} (theoretical MW: 7031 Da).

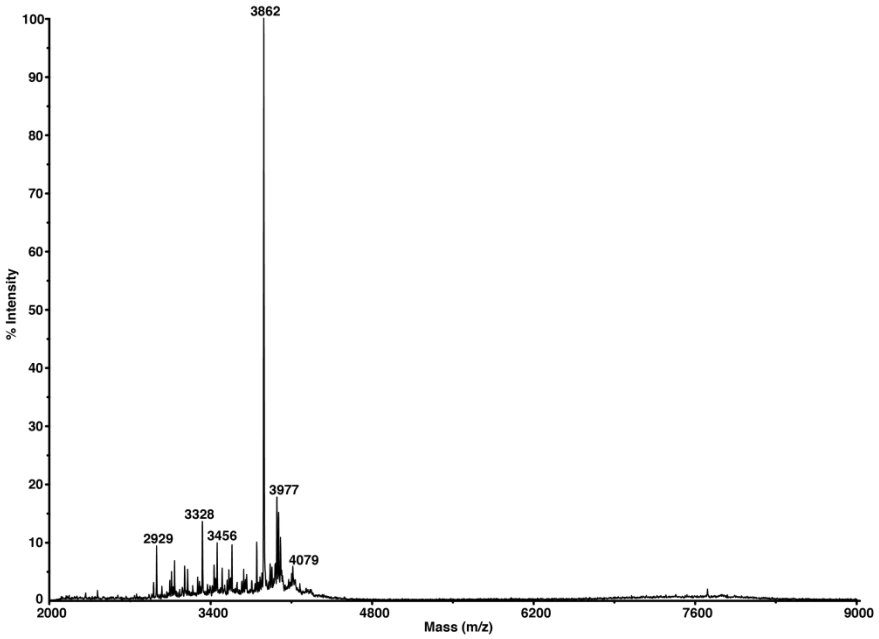

Figure S5. MALDI-MS spectrum of unconjugated, HPLC-purified Z_{C4} (theoretical MW: 3862 Da).

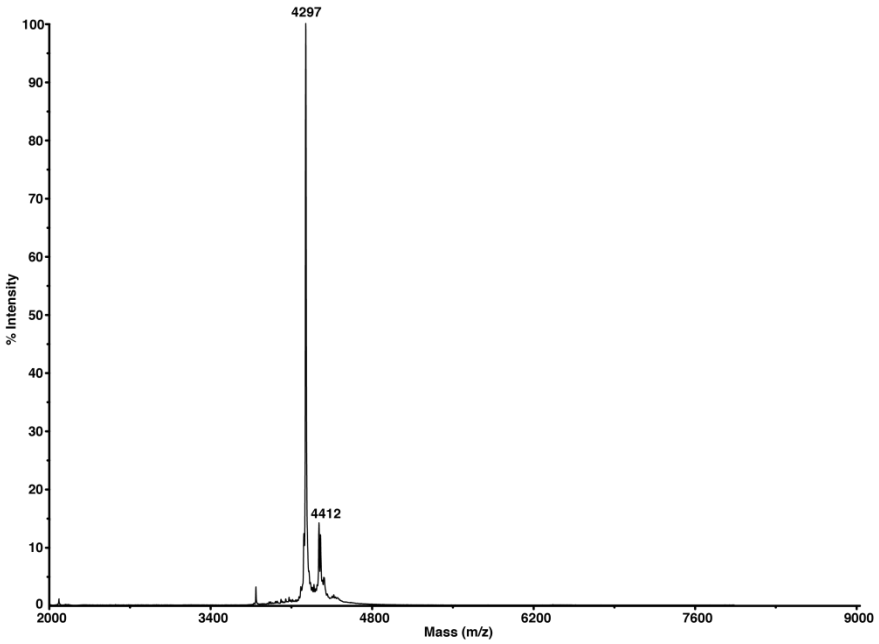

Figure S6. MALDI-MS spectrum of photoswitch-conjugated, HPLC-purified Z_{C4} (theoretical MW: 4293 Da).

The efficiency of the optimized conjugation of photoswitch to the ZC3 variant was analyzed by MALDI-MS before and after the conjugation reaction.

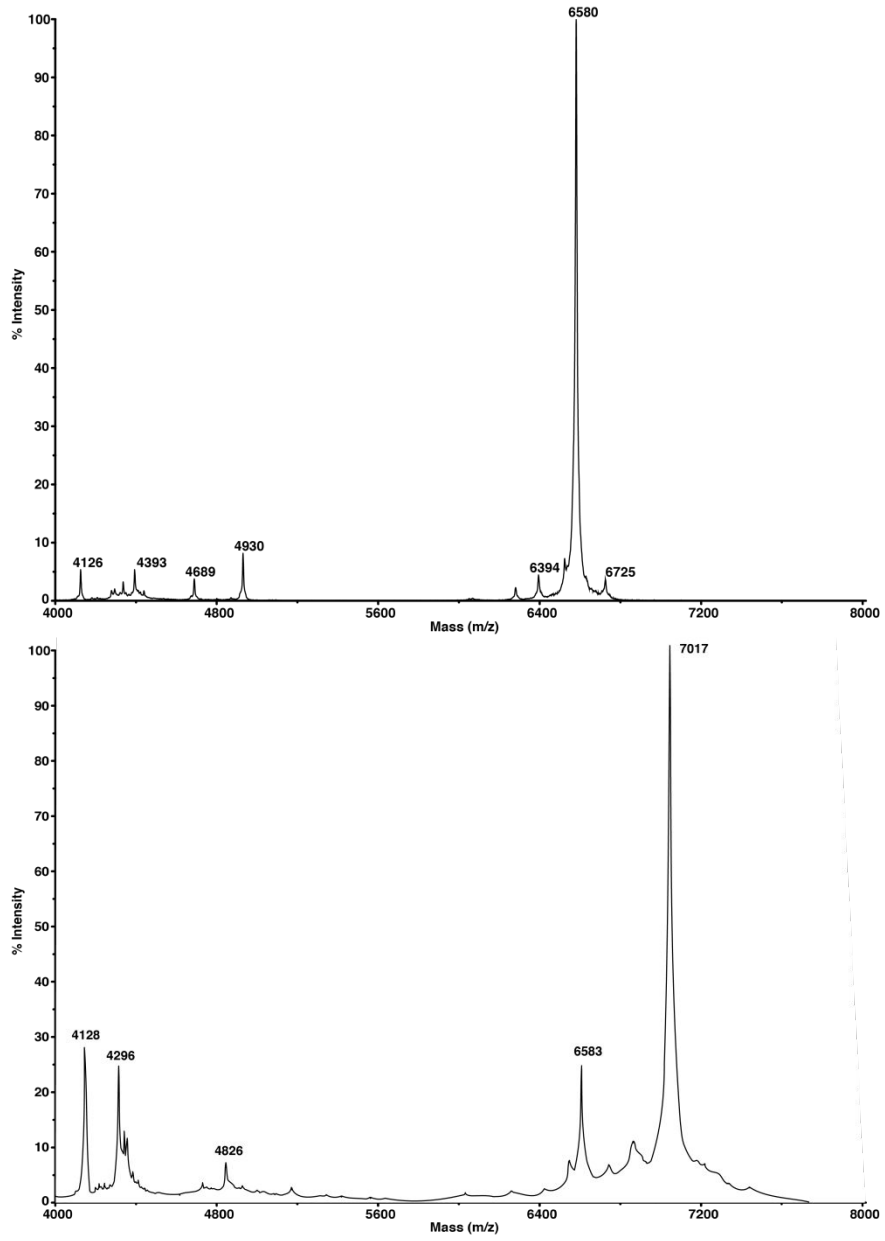

Figure S7. MALDI-MS spectrum of Z_{C3} before (above) and after (below) photoswitch conjugation (theoretical MW: 6600 Da and 7031 Da, respectively).

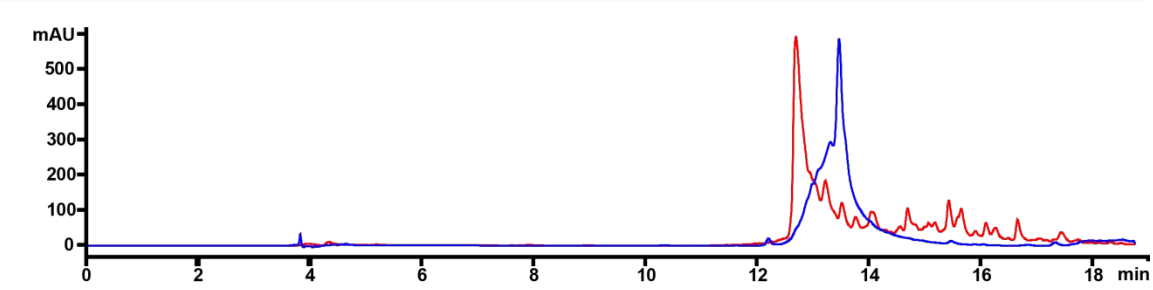

Figure S8. HPLC elution profiles for purification of crude, unconjugated Z_{C3} (red curve) and photoswitch-conjugated Z_{C3} (blue curve).

Photoswitch screening and elution assay

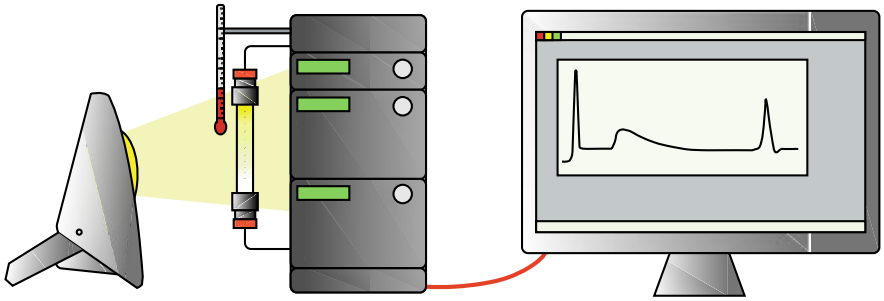

Figure S9. The setup of the light screening assay. The chromatography columns were coupled to an ÄKTA Explorer (GE Healthcare) and the temperature was kept constant with pressurized air flow while the light was turned on.

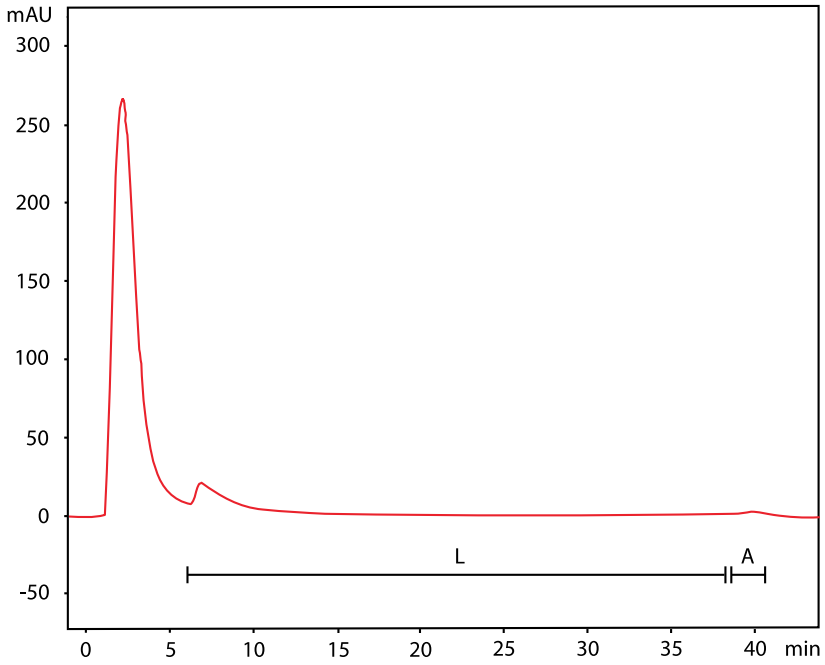

Figure S10. Elution profile of Z_{C4} (red curve) on an IgG-sepharose column. The column was exposed to light (λ= 400 nm) during the segment marked with (L), and exposed to acid (0.1 M acetic acid, pH 3.3) during the segment marked with (A).

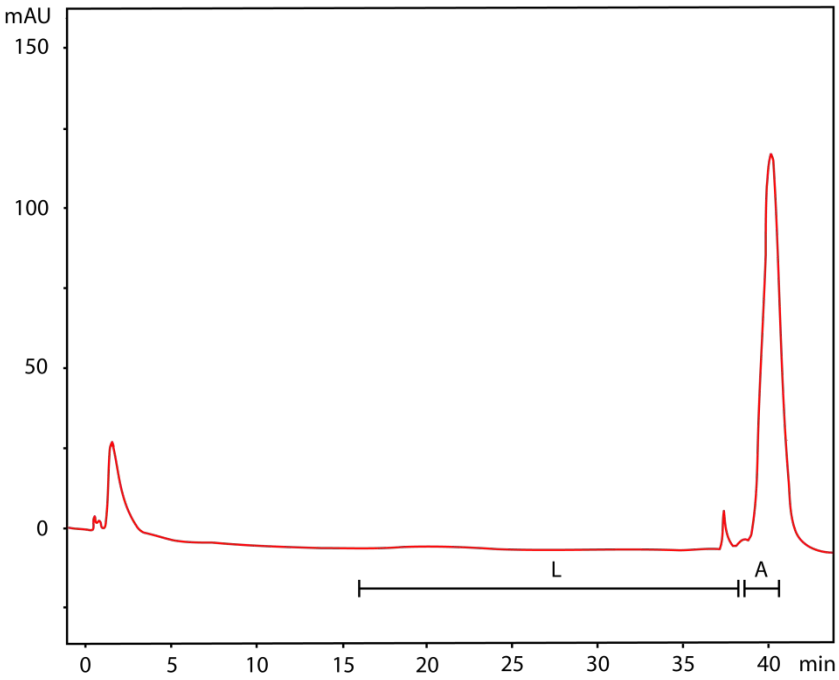

Figure S11. Elution profile of Z_{C2} (red curve) on an IgG-sepharose column. The column was exposed to light (λ= 400 nm) during the segment marked with (L), and exposed to acid (0.1 M acetic acid, pH 3.3) during the segment marked with (A).

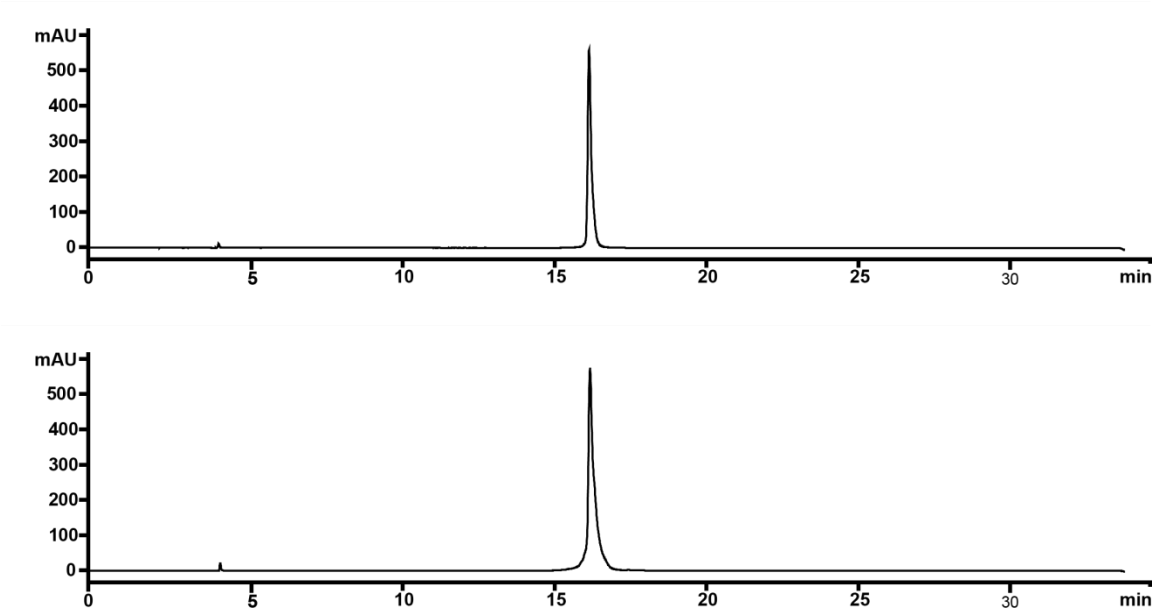

Figure S12. HPLC analysis of Z_{C3} before the light elution assay (above) and the light-eluted fraction from the light elution assay (below).

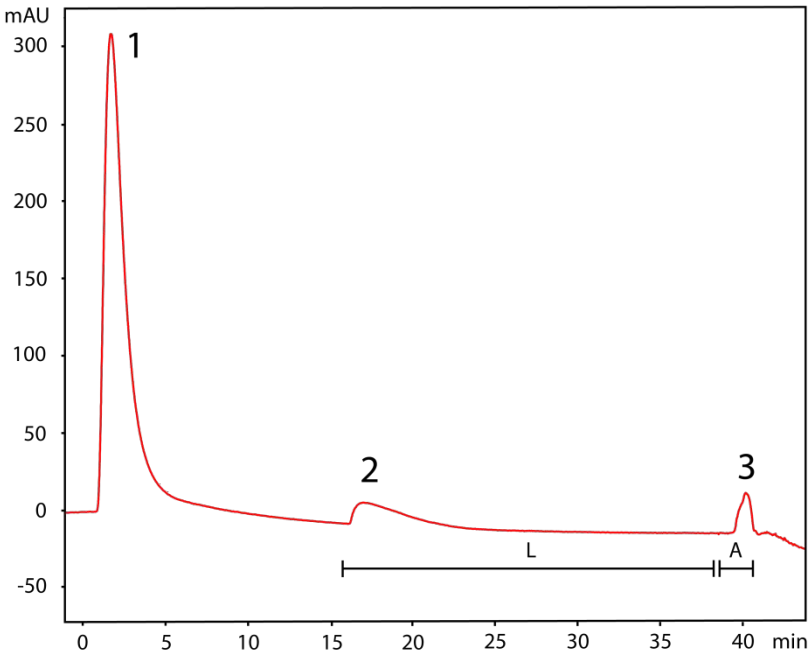

Figure S13. Elution profile of Z_{C3} (red curve) on an IgG-sepharose column. The column was exposed to light (λ= 400 nm) during the segment marked with (L), and exposed to acid (0.1 M acetic acid, pH 3.3) during the segment marked with (A). The three peaks marked 1, 2, and 3 were collected and analyzed by MALDI-MS.

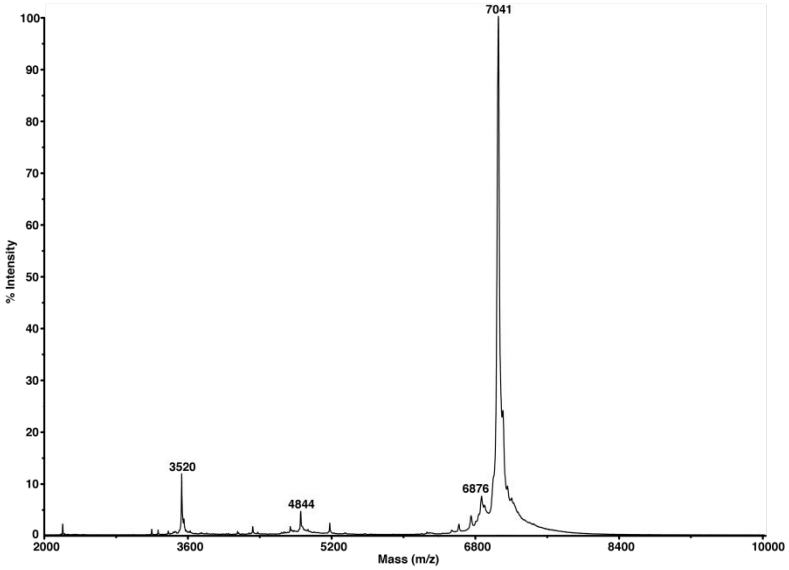

Figure S14. MALDI-MS spectrum of peak 1 from the photoelution of Z_{C3} (see **Fig. S13**) (theoretical MW: 7031 Da for photoconjugated Z_{C3} and 6600 Da for unconjugated Z_{C3}).

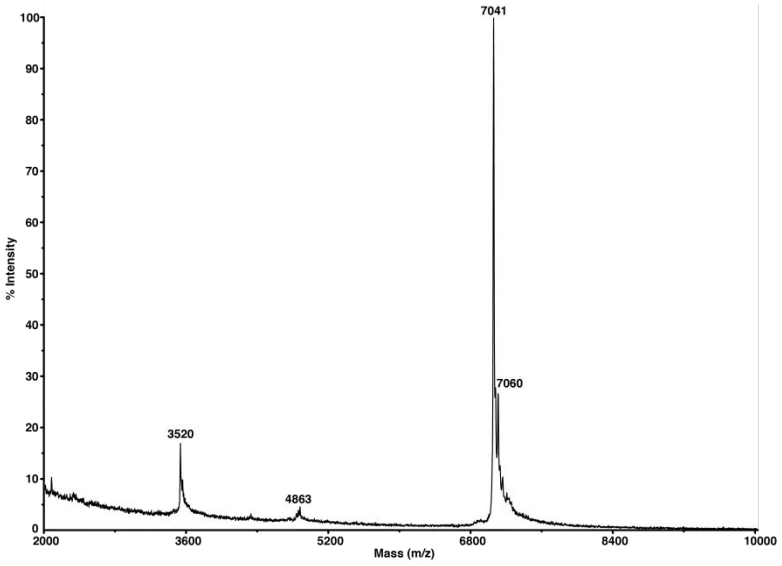

Figure S15. MALDI-MS spectrum of peak 2 from the photoelution of Z_{C3} (see **Fig. S13**) (theoretical MW: 7031 Da for photoconjugated Z_{C3} and 6600 Da for unconjugated Z_{C3}).

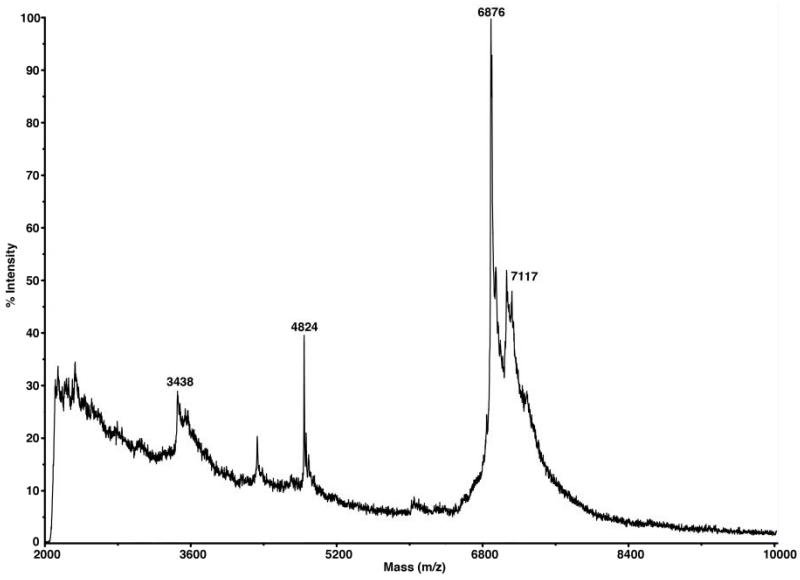

Figure S16. MALDI-MS spectrum of peak 3 from the photoelution of Z_{C3} (see **Fig. S13**) (theoretical MW: 7031 Da for photoconjugated Z_{C3} and 6600 Da for unconjugated Z_{C3}).

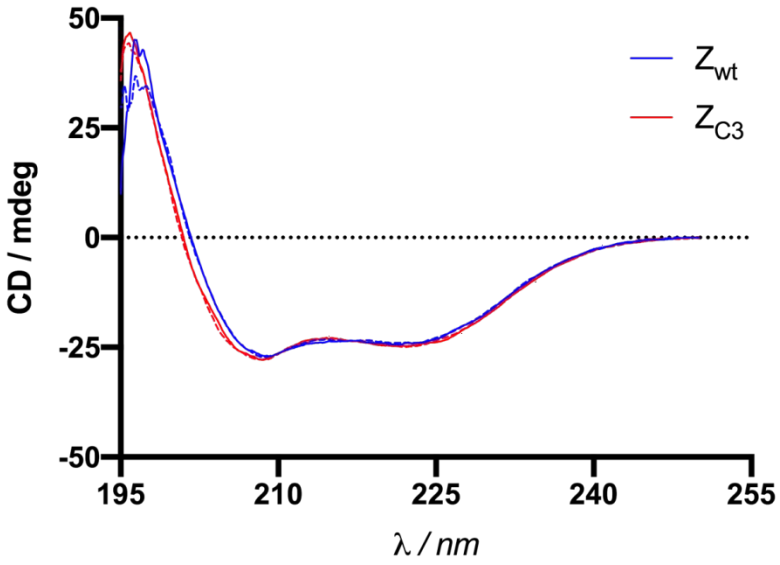

Figure S17. CD spectra of Z_{C3} (16 μM) and Z_{wt} (16 μM) before (solid line) and after (dotted line) exposure to the light source used for the light elution assay.