Supporting Information

Enhancing Reactivity for Bioorthogonal Pretargeting by Unmasking Antibody Conjugated *trans*-Cyclooctenes

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Characterization of heterobifunctional DBCO-PEG$_4$-TCO

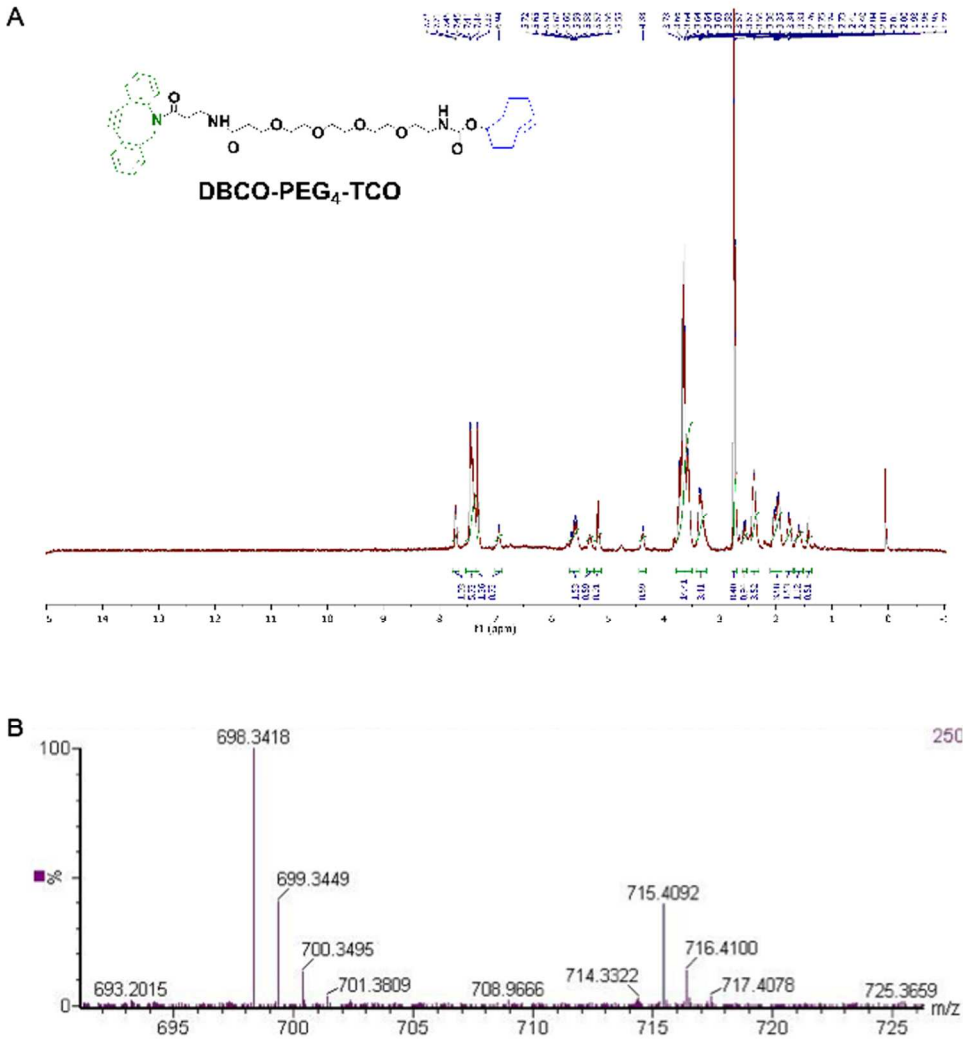

Figure S1. (A) proton NMR and (B) mass spectrometry.

MALDI-TOF analysis of all modified antibodies

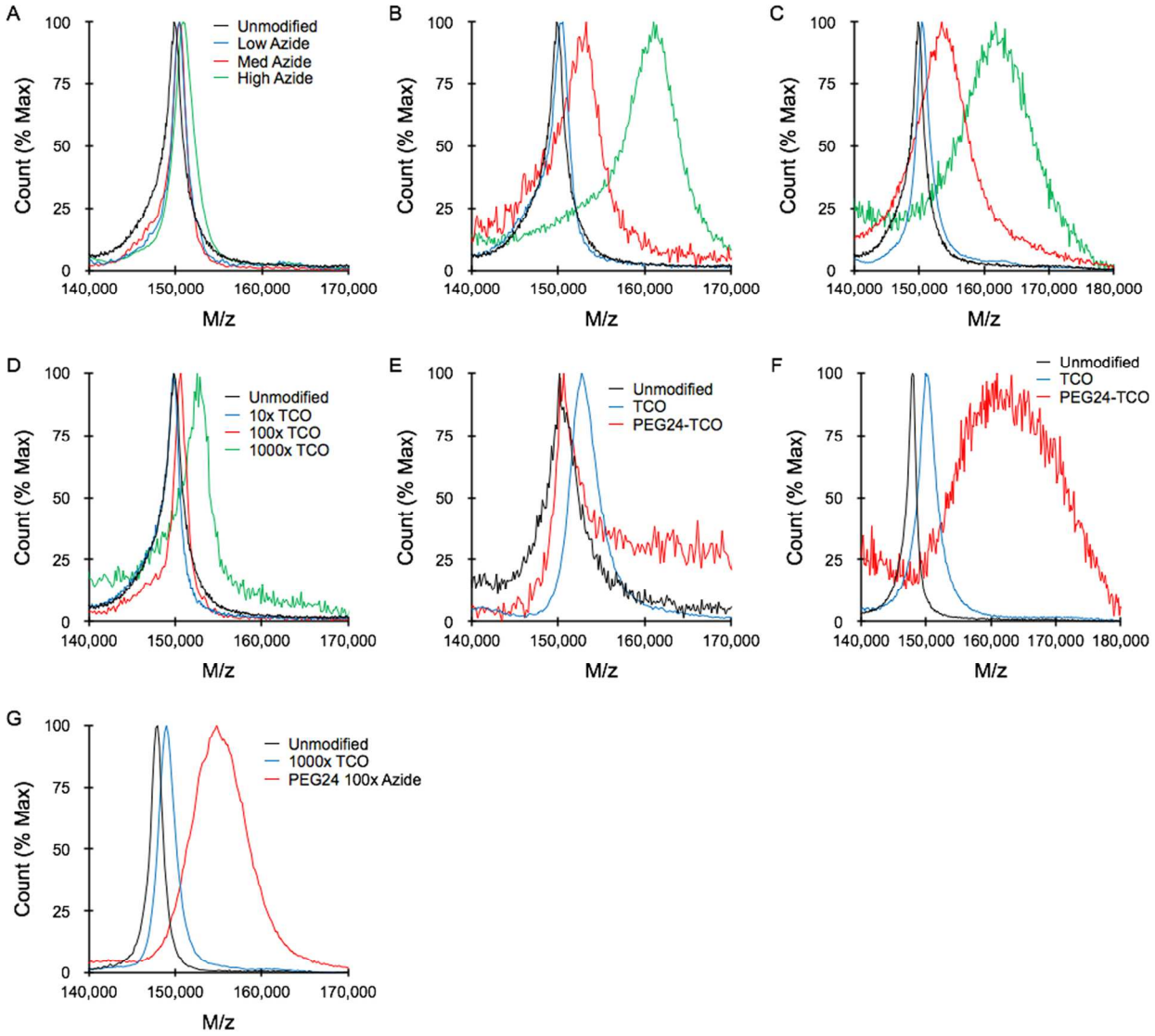

Figure S2. Representative MALDI-TOF results. (A-C) Anti-EGFR antibody conjugated with azide and measured (A) directly or after reaction with (B) PEG_4-TCO or (C) PEG_{24}-TCO. (D) Anti-EGFR antibody conjugated after conjugation with TCO. (E-G) Conjugation of TCO and PEG_{24}-TCO to (E) anti-EpCAM, (F) anti-TfR, and (G) non-binding control antibodies.

Varying solvent concentration to reveal masked TCOs

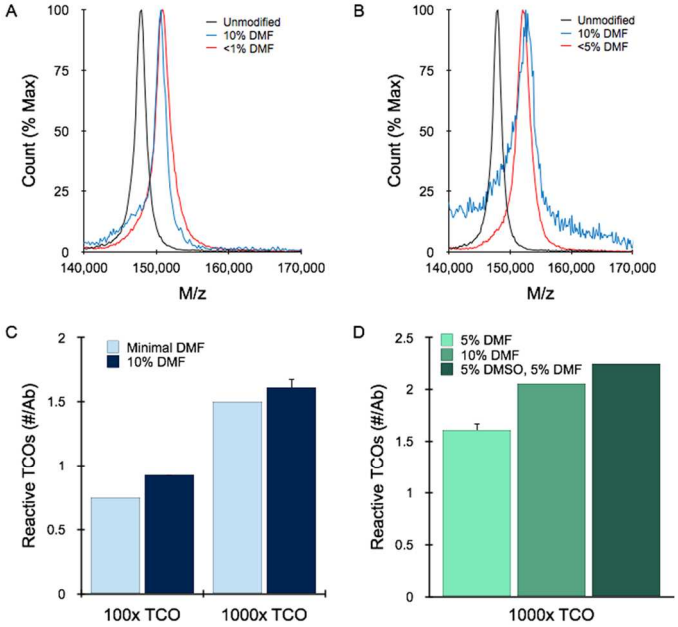

Figure S3. Effect of polar solvent concentration during antibody conjugation with TCO and tetrazine reaction. MALDI-TOF data for anti-EGFR antibodies reacted with (A) 100-fold or (B) 1000-fold molar equivalents of NHS-TCO in the presence of minimal or 10% DMF, showing no effect of DMF concentration on total TCO conjugation level. (C) Increasing DMF concentration did slightly enhance TCO reactivity, however. (D) Adding more DMF or DMSO during the reaction of the TCO-antibody with tetrazine-Oregon Green 488 revealed up to 30% more TCOs, but most modifications still remained non-functional. Error bars represent the standard error of at least three independent experiments.

Binding of anti-EGFR antibody modified with azide-, TCO, and TCO-PEG

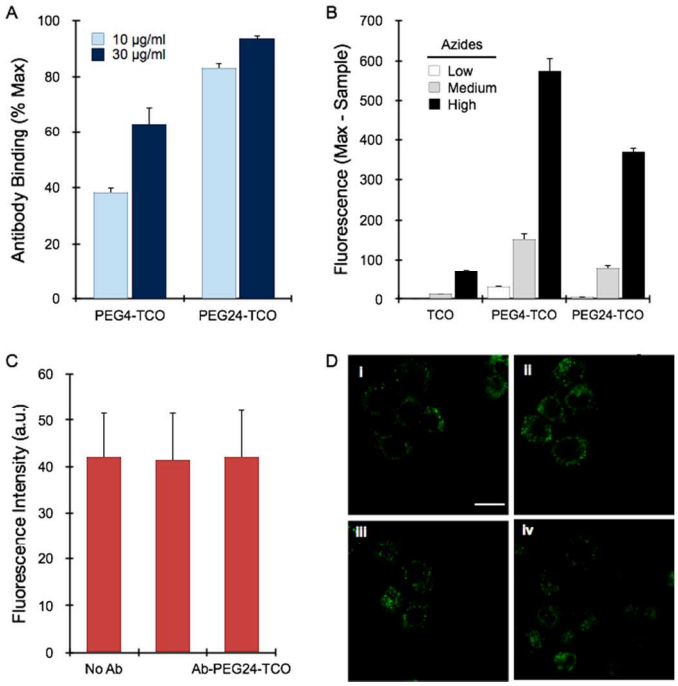

Figure S4. Binding and background levels for TCO-PEG-modified anti-EGFR antibody. (A) Increasing antibody concentration to 30 µg/mL helped recover reduced binding. (B) Raw fluorescence signals measured by flow cytometry after reacting tetrazine-Oregon Green 488 (background subtracted). (C) Background signal levels of tetrazine-Oregon Green 488 were identical using no antibody or a non-bonding control antibody modified with TCO and PEG_{24}-TCO. (D) Confocal images of live A431 cancer cells labeled with (i,ii) no antibody or non-bonding control antibody modified with (iii) TCO or (iv) PEG_{24}-TCO. Cells were then (i) left untreated to show autofluorescence or probed with (ii-iv) tetrazine-Oregon Green 488 at 50 nM for 30 min. Scale bar: 20 µm. Error bars represent the standard error of at least three independent experiments.

Background control signals from tetrazine-modified QDs

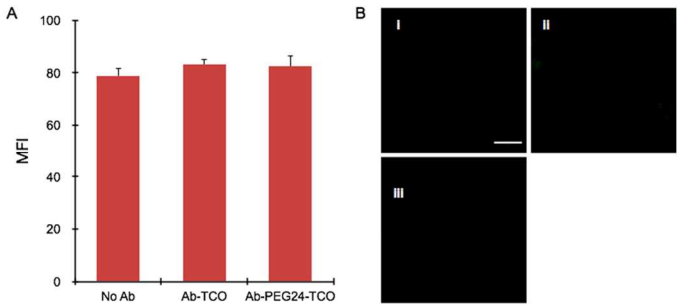

Figure S5. (A) Background levels for tetrazine-QD were identical after labeling A431 cells with no antibody or a non-bonding control antibody modified with TCO and PEG_{24}-TCO. Tetrazine-QD were incubated for 30 min at 50 nM. (B) Confocal images of A431 cancer cells treated with (i) no antibody or a non-bonding control antibody modified with (ii) TCO or (iii) PEG_{24}-TCO. Cells were then probed with tetrazine-QDs at 50 nM for 30 min. Scale bar: 20 μm. Error bars represent the standard error of at least three independent experiments.

Performance of TCO-PEG-modified anti-EpCAM and anti-TfR antibodies

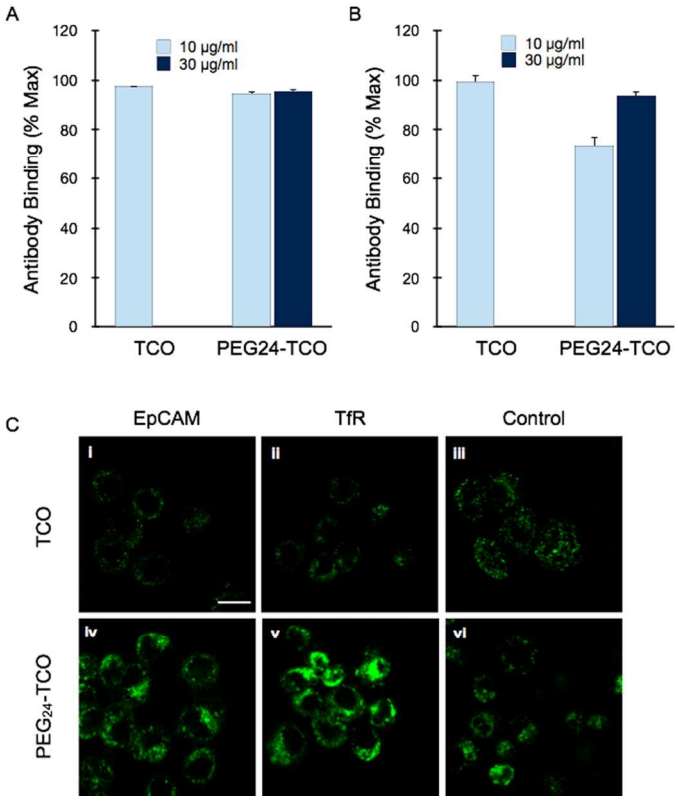

Figure S6. Binding and background levels for TCO-PEG-modified anti-EpCAM and TfR antibodies. (A,B) Relative antibody binding of TCO- and TCO-PEG_{24}-modified (a) anti-EpCAM and (b) anti-TfR antibodies at 10 or 30 µg/mL. (C) Confocal images of live A431 cells labeled with (i, iv) anti-EpCAM, (ii, v) anti-TfR, or (iii, vi) non-binding control antibodies. The antibodies were modified with (i-iii) TCO or (iv-vi) PEG_{24}-TCO at the highest densities, and reacted with tetrazine-Oregon Green 488 at 50 nM for 30 min. Scale bar: 20 µm. Error bars represent the standard error of at least three independent experiments.