Antibody targeted PET Imaging of ^{64}Cu-DOTA-Anti-CEA PEGylated Lipid Nanodiscs in CEA positive tumors

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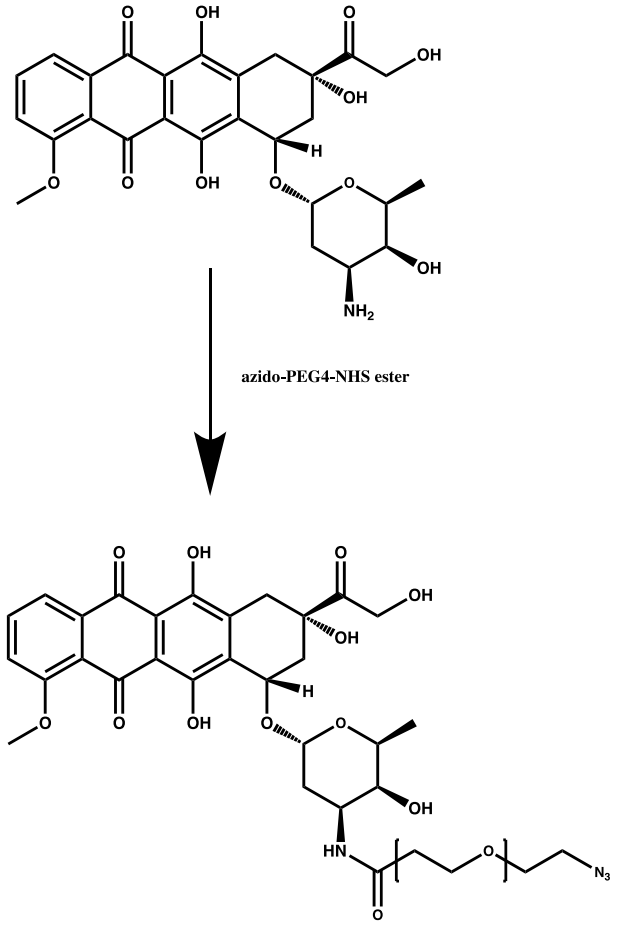
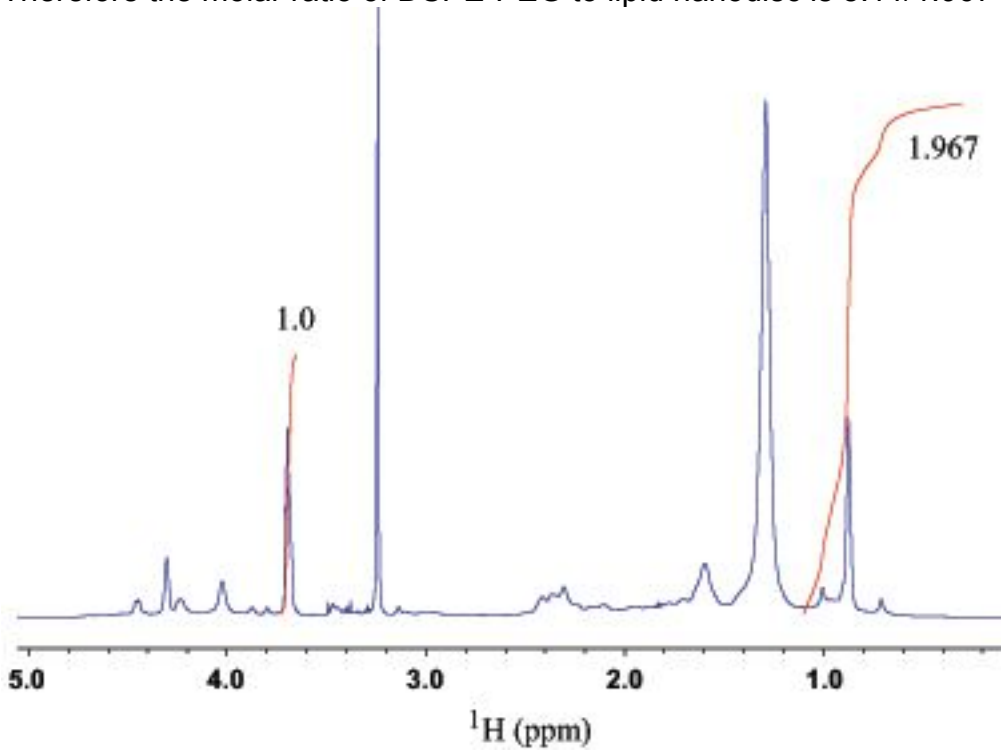
Supplementary Figures

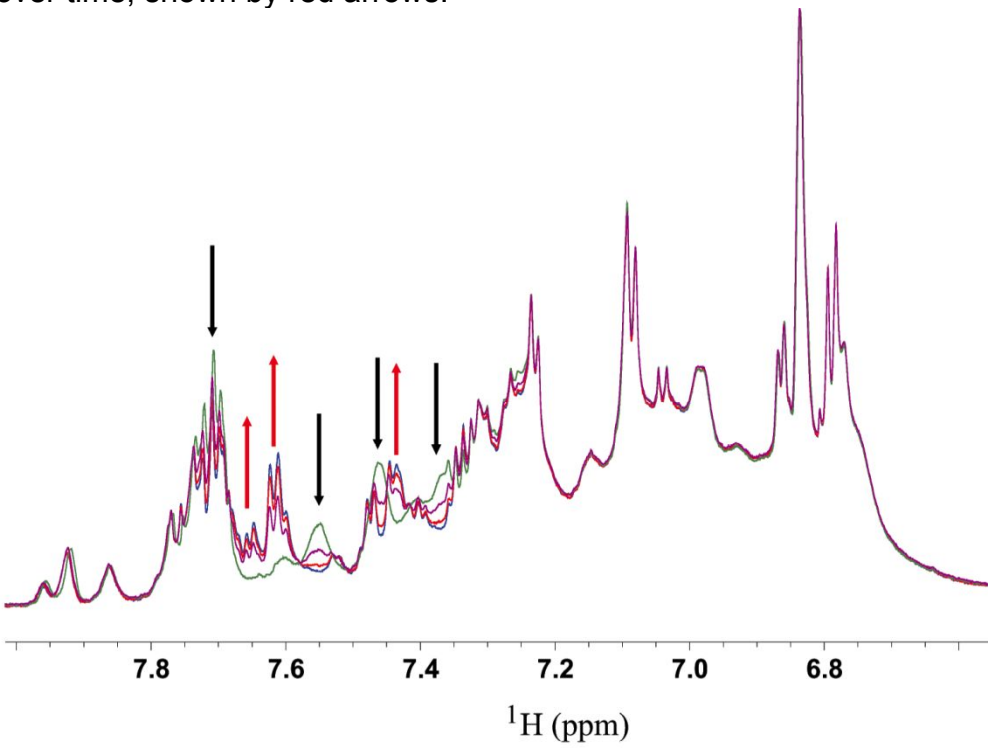
Supplementary scheme S1. Synthesis of azido-dPEG$_4$-dox. Dox-HCl in DMSO was reacted with a two-fold molar excess of azido-PEG$_4$-NHS in DMSO.



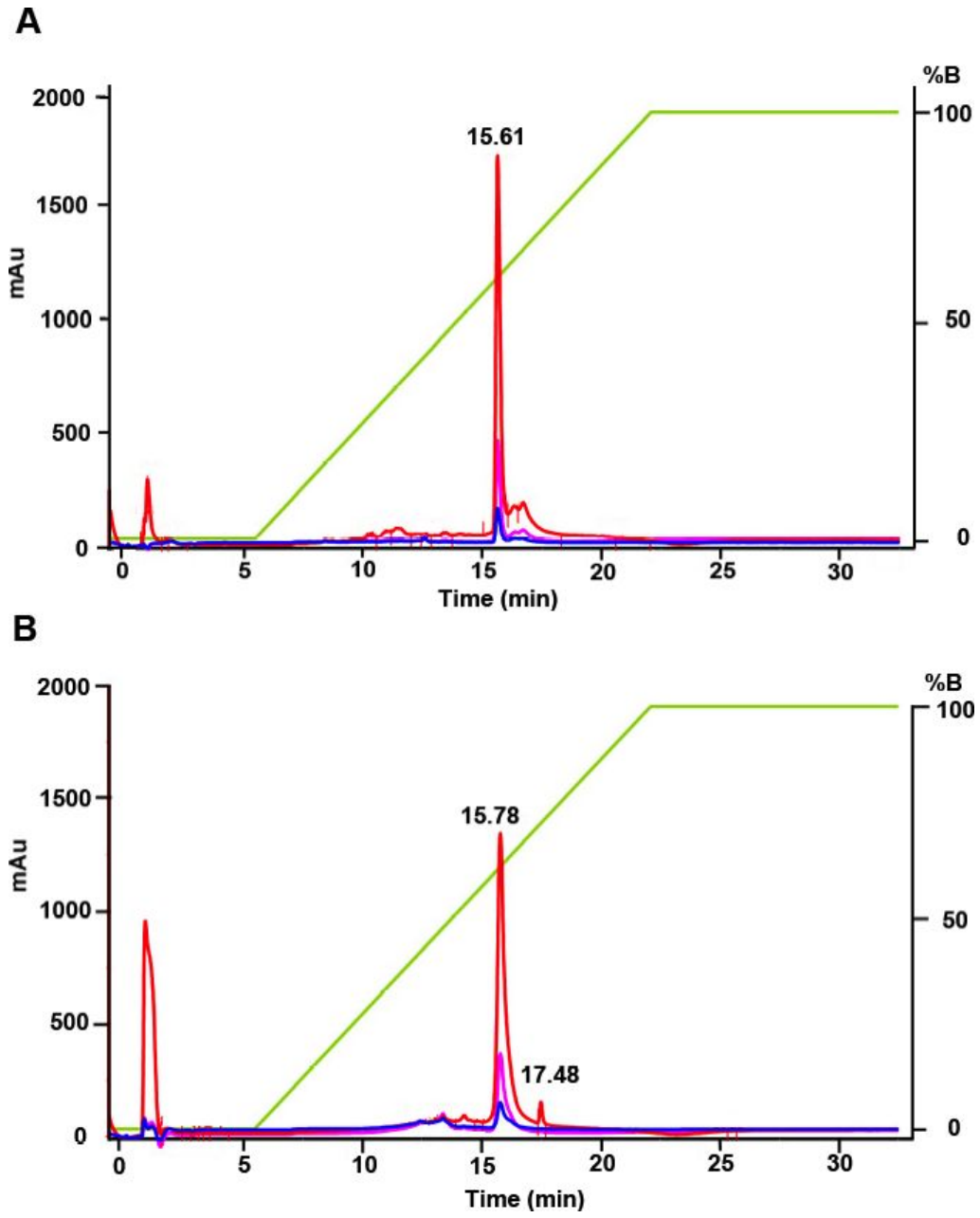
Supplementary Fig S1. Estimation of molar ratio of DSPE-PEG_{2000}-DBCO to lipid nanodisc by 1D ^{1}H NMR. Two regions of the spectrum were integrated to estimate the molar ratio: the PEG ethylene group from 3.73 to 3.65 ppm and the methyl region from 1.1 to 0.3 ppm for DMPC (plus Val, Ile and Leu in MSP1D1 and neglecting DSPE). There are 160 DMPC per nanodisc with a total of 960 methyl protons and 468 methyl protons (Val, Ile and Leu) from 2 MSP1D1 for a total of 1428 methyl protons. Since there are an average of 44 ethylene groups in PEG_{2000}, the total number of ethylene protons is 176. If the molar ratio expected is 1:1, then the integration of 1428 (methyl proton) vs integration of 176 (ethylene proton) should be 8.11. However, the actual ratio is 1.967/1.0= 1.967. Therefore the molar ratio of DSPE-PEG to lipid nanodisc is 8.11/1.967 = 4.12.



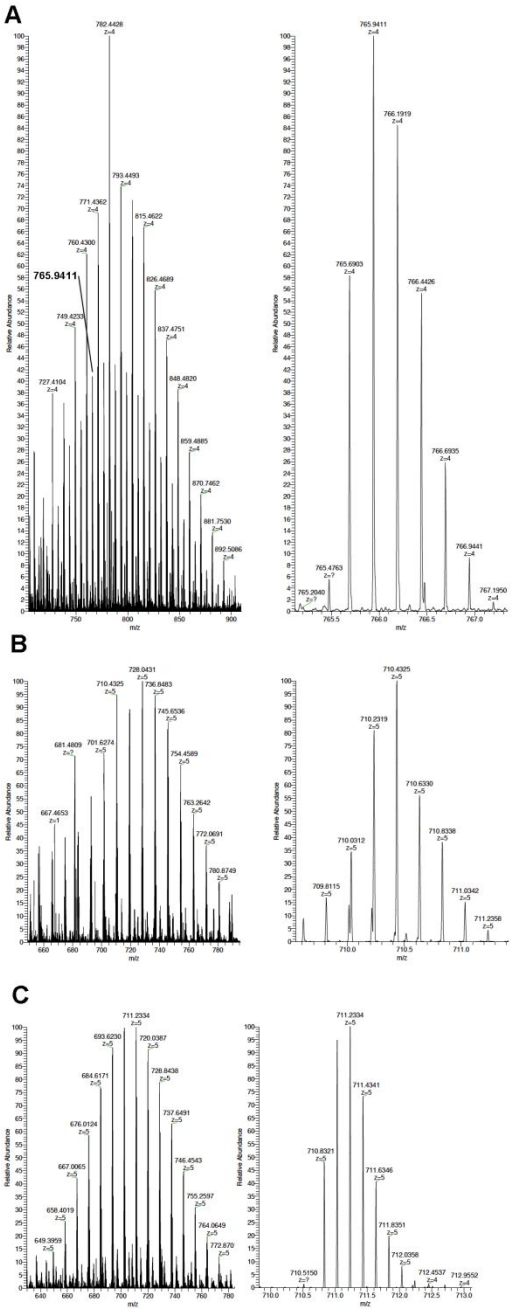
Supplementary Fig S2. Monitoring the reaction between azido-monoamide DOTA and DSPE-PEG$_{2000}$-DBCO on a lipid nanodisc by 1D ^{1}H NMR. The 1D ^{1}H NMR spectrum in green is from nanodisc-DBCO aromatic region without addition of azido-monoamide DOTA. The spectra in purple, red and blue are from the same sample with different reaction times after addition of azido-monoamide DOTA to nanodisc-DBCO at a molar ratio of 2.4:1 (azide:DBCO). The reaction times are 1 hour, 2 hour and 3.5 hour, respectively for the spectrum in color of purple, red and blue. The click reaction changed the aromatic proton local environment of DBCO due to structural changes, and which caused the chemical shift changes. Referring to the green spectrum for free DBCO, black arrows show progressive decrease in aromatic signals over time, while new aromatic signals grow in over time, shown by red arrows.



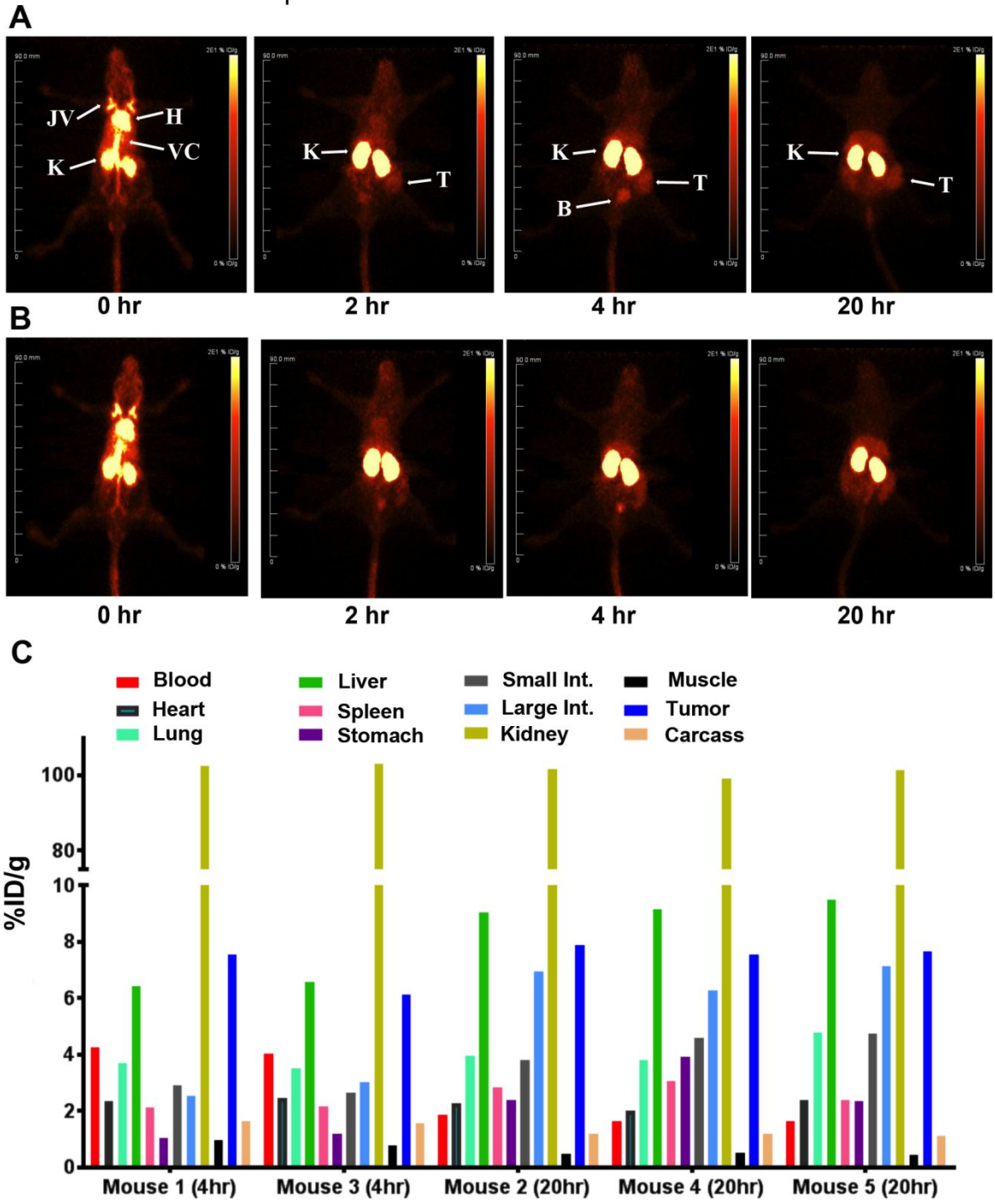
Supplementary Fig S3. RP-HPLC purification of Clicked products of azido-monoamide-DOTA and azido-PEG_4-Dox with DSPE-PEG_{2000}-DBCO. A. Azido-monoamide-DOTA Clicked to DSPE-PEG_{2000}-DBCO. **B.** Azido-PEG_4-Dox Clicked to DSPE-PEG_{2000}-DBCO.



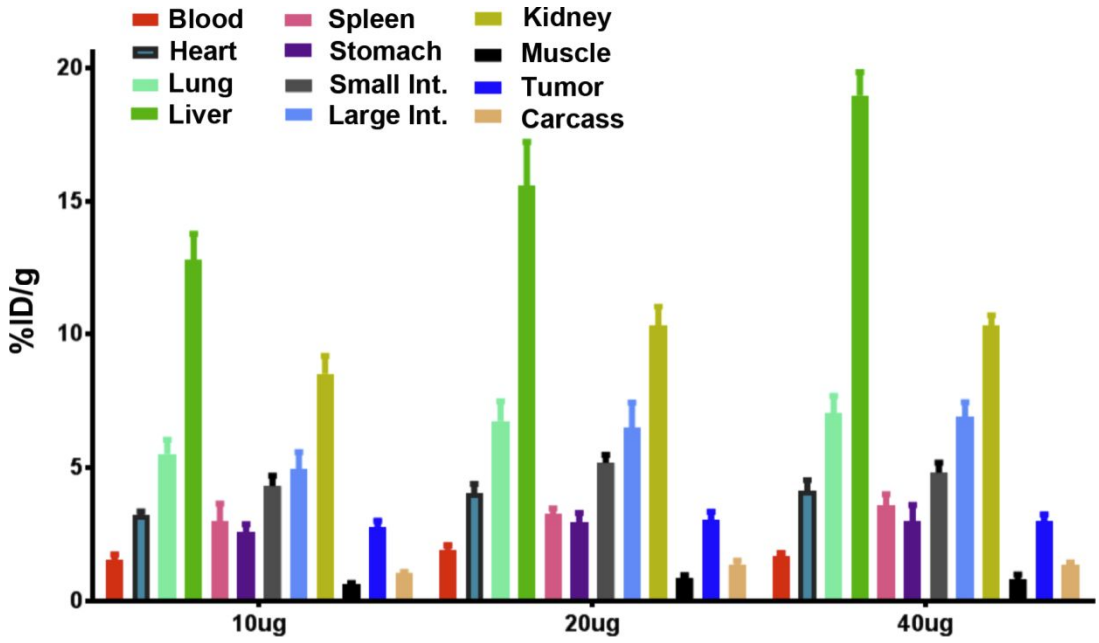
Supplementary Fig S4. High resolution mass spectrometry of DSPE-PEG_{2000}-DBCO and Clicked products of azido-monoamide-DOTA and azido-PEG_4-Dox with DSPE-PEG_{2000}-DBCO. A. DSPE-PEG_{2000}-DBCO (inset: blow up of the PEGn= 45 peak, no sodium; 771.4362 plus sodium). **B.** Clicked azido-monoamide-DOTA with DSPE-PEG_{2000}-DBCO (inset: blow up of PEGn=45 peak). **C.** Clicked azido-PEG_4-Dox with DSPE-PEG_{2000}-DBCO (inset: blow up of PEGn= 38 peak).



Supplementary Fig S5. PET imaging of CEA positive tumors in CEA transgenic mice with ^{64}Cu-DOTA-anti-CEA-Fab'. CEA Tg mice bearing CEA transfected E0771 cells in their right mammary fat pads were injected with 91.0 uCi (**A**, mouse 1) or 91.6 uCi (**B**, mouse 2) of DOTA-anti-CEA-Fab' and imaged at 0, 2, 4 and 20h. Major organs are indicated: JV- jugular veins, H =heart, K= kidney, T= tumor, L= liver. **C**. At 4 and 20 hr tissues were analyzed for ^{64}Cu-DOTA-anti-CEA-Fab' uptake.



Supplementary Fig S6. Biodistributions of CEA positive tumors in CEA transgenic mice with increasing amounts of ^{64}Cu-DOTA-anti-CEA-Fab' LNDs. The mass amount in micrograms is shown on the ordinate. Each preparation was labeled with 50 µCi of ^{64}Cu, injected into E0771/CEA tumor bearing CEA transgenic animals and biodistribution performed at 21 hrs post injection.



Supplementary Fig S7. MS analysis of DOTA-PEG_5-azide M5A. M5A was lightly reduced with TCEP, alkylated with bromo-PEG_5-azide on the reduced hinge thiols, and derivatized with NHS-DOTA. The product was analyzed by mass spectrometry on an Agilent Q-TOF. **A**: The light chain (LC) shows major peaks for the underivatized LC (23,894) and derivatives with one PEG_5-azide, one PEG_5-azide plus one DOTA. **B**: The heavy chain (HC) shows major peaks of HC plus DOTA plus 2 PEG_5-azide, HC plus 2 DOTA and 2 PEG_5-azide and HC plus 3 DOTA and 3 PEG_5-azide. The shape of the peaks and appearance of satellite peaks is due to the presence of carbohydrate on the HCs.