Tracking Antibody Distribution with Near-Infrared Fluorescent Dyes: Impact of Dye Structure and Degree of Labeling on Plasma Clearance

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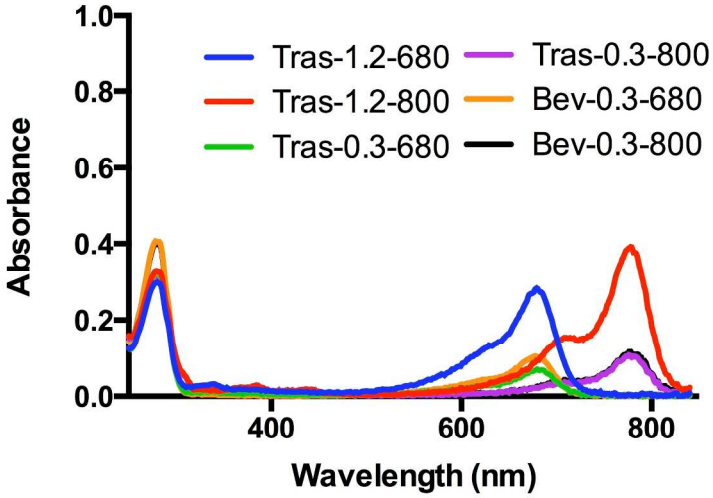
Supplementary Data

Supplemental Figures – Figures S1 to S5

Supplemental Table – Table S1

Supplemental References

Supplemental Figures



Antibody-Dye Conjugate	280nm Absorbance	Dye Max Absorbance	Protein Conc. (μM)	Dye Conc. (μM)	DoL
Bev-0.3-800	0.399	0.115	17.5	4.8	0.3
Tras-0.3-800	0.314	0.108	13.7	4.5	0.3
Tras-1.2-800	0.326	0.392	13.6	16.3	1.2
Bev-0.3-680	0.405	0.103	17.8	5.7	0.3
Tras-0.3-680	0.323	0.070	14.2	3.9	0.3
Tras-1.2-680	0.300	0.283	12.7	15.7	1.2

Figure S1 – Antibody-dye absorbance spectra. After reaction and purification, the absorbance spectrum for each antibody-dye conjugate was used to determine the DoL as described in the methods section. DoL was determined by dividing bulk fluorophore concentration by antibody concentration. Tras, trastuzumab; Bev, bevacizumab.

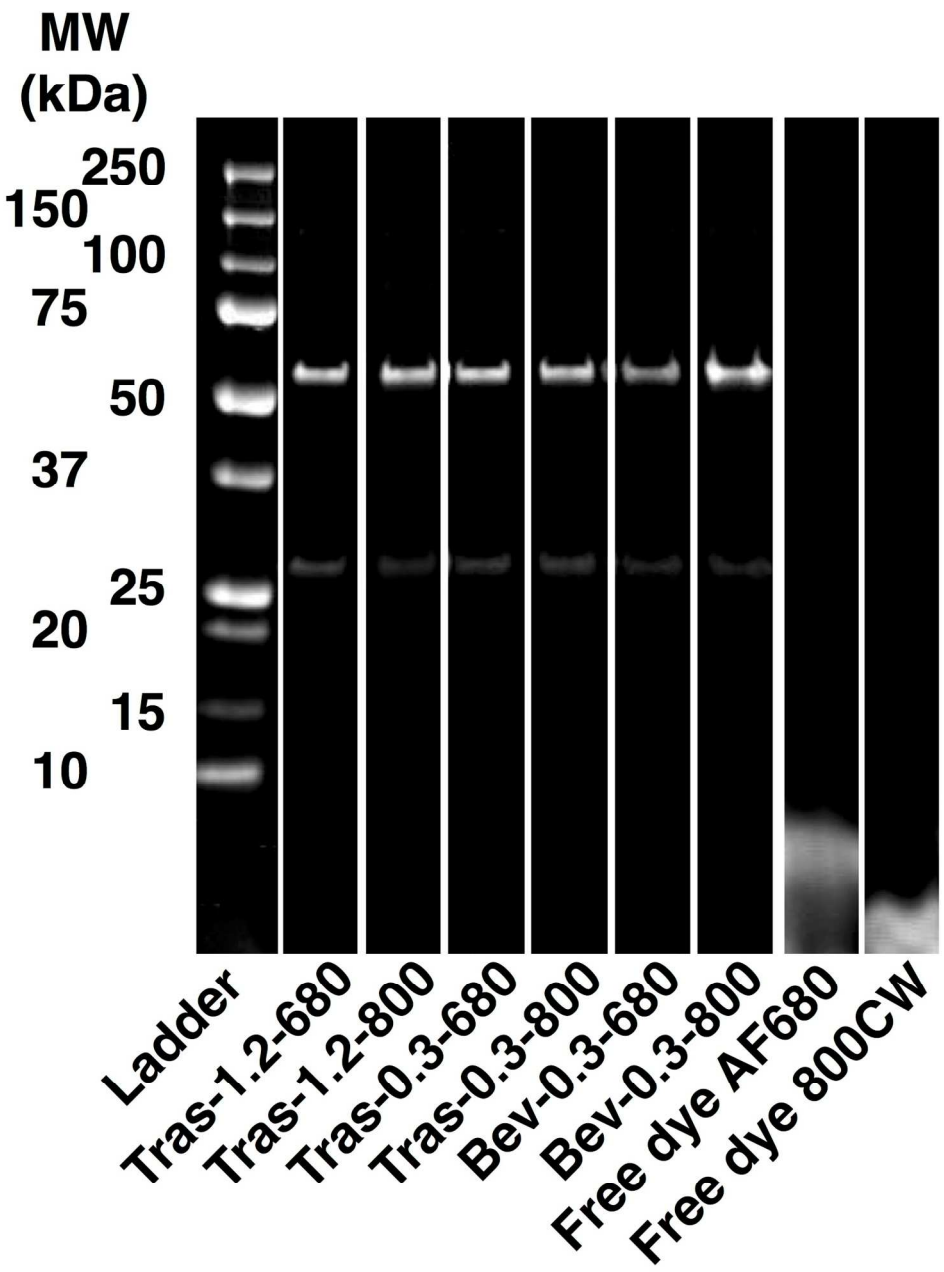


Figure S2 - SDS-PAGE of antibody-dye conjugates and free dye. After reaction and purification all conjugates were run on SDS-PAGE and scanned on the Odyssey CLx NIR scanner to ensure free dye was removed. Window leveling adjusted for similar brightness from heavy chain. Tras, trastuzumab; Bev, bevacizumab.

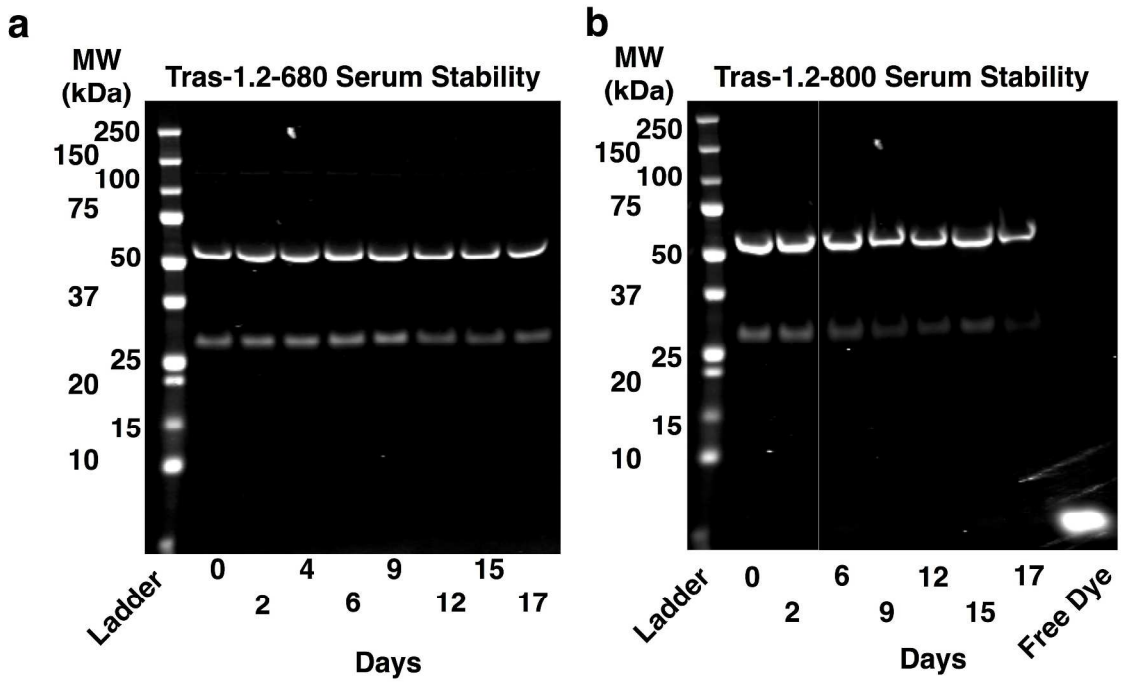


Figure S3 – SDS-PAGE of serum stability samples. Serum stability samples show intact antibody out to 17 days and no detectable formation of free dye.

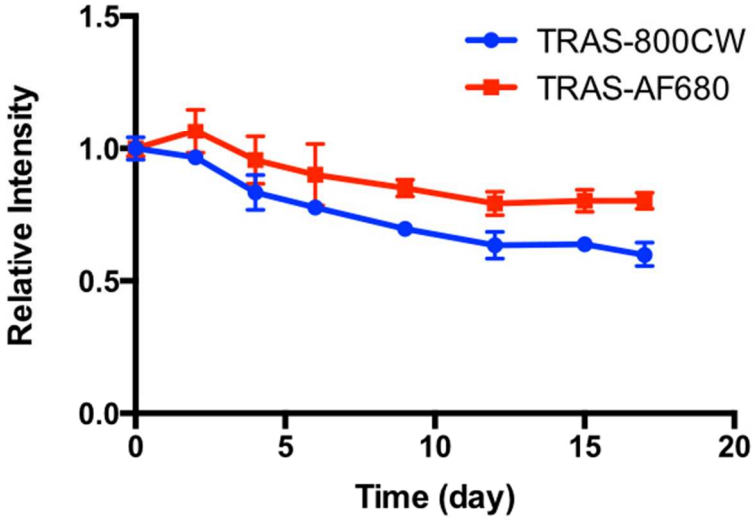


Figure S4 – Antibody-dye conjugate serum stability. Both Tras-AF680 and Tras-800CW show slight loss in fluorescence over 17 days (80% and 60% of initial, respectively); however, the loss in fluorescence does not account for the rapid drop in fluorescent signal seen with 800CW in Fig. 2. Therefore, the loss of fluorescent signal in Fig. 2 is due to clearance of the antibody-dye conjugate, not loss of fluorescence from the dye being in serum. Tras-AF680 and Tras-800CW, both with a DoL of 1.2, were mixed with fetal bovine serum (FBS) at 30nM. The serum/antibody-dye aliquots were thawed rapidly in a 37°C water bath and incubated at 37°C for the number of days shown. The samples were thawed and placed at 37°C in reverse order (17 day time point first) so the samples could all be measured on the same day. 15 µL of each sample was scanned in a 384 well plate on the Odyssey CLx, and the experiment was performed in triplicate.

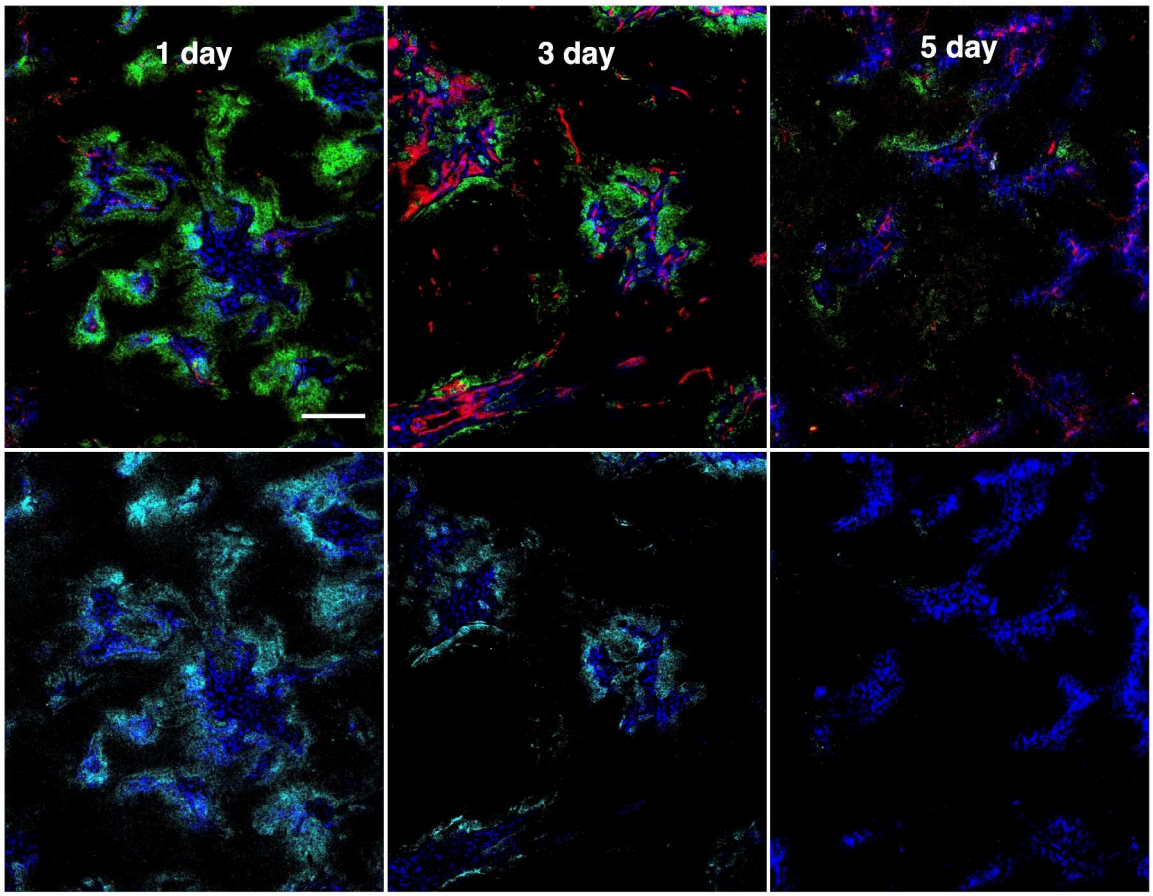


Figure S5 – Comparison of fluorescence detection of directly labeled antibody with a residualizing probe versus immunofluorescence detection of intact antibody over 5 days following administration of 3.6 mg/kg T-DM1-AF680 (green). Hoechst 33342 (blue) was administered 30min prior to sacrifice. Anti-mouse CD31-AF555 (red) and anti-human IgG Fc–AF488(cyan) were labeled *ex vivo*. Scale bar is 200μm.

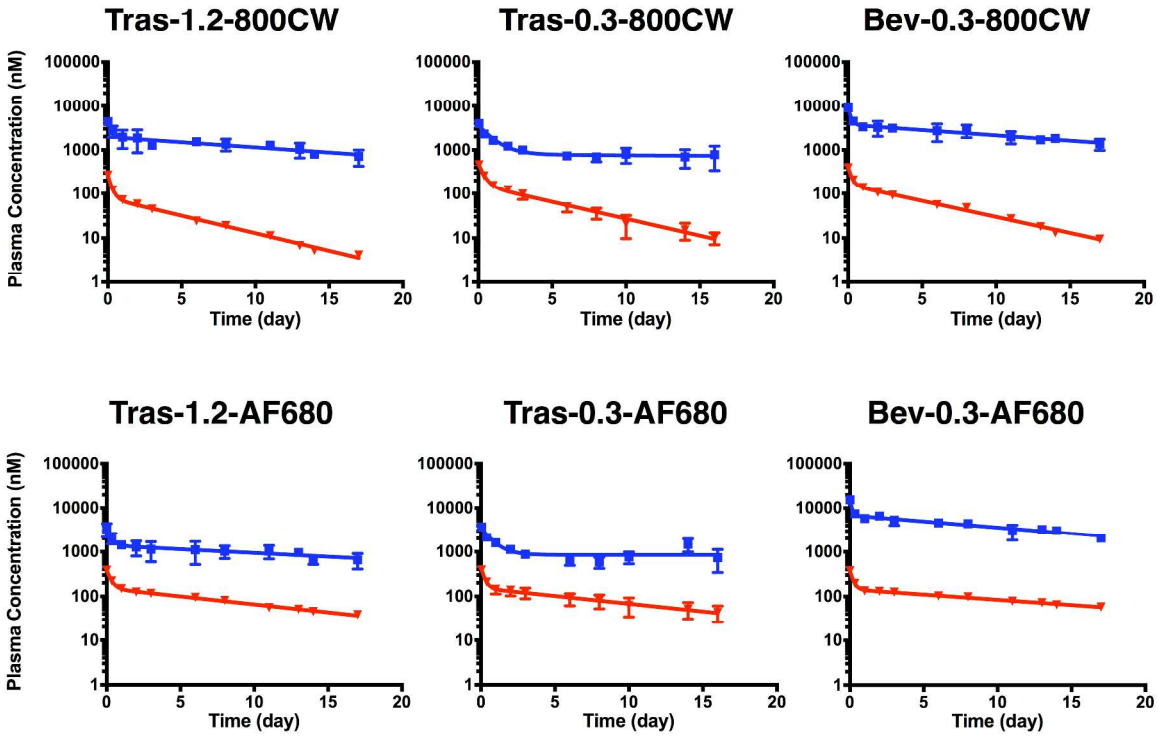


Figure S6 – Absolute concentrations of antibody-dye conjugate plasma clearance. The plasma concentration as measured by fluorescence (red) and ELISA (blue) for trastuzumab-dye conjugates with a low (1.2) and tracer (0.3) DOL (left and middle, respectively), and bevacizumab-dye conjugates with a tracer DOL (right). ELISA concentrations are higher due to the 550μg of total antibody versus 50μg of labeled antibody. Figures 2 and 3 were generated by normalized each data set to the initial concentration. Tras, trastuzumab; Bev, bevacizumab.

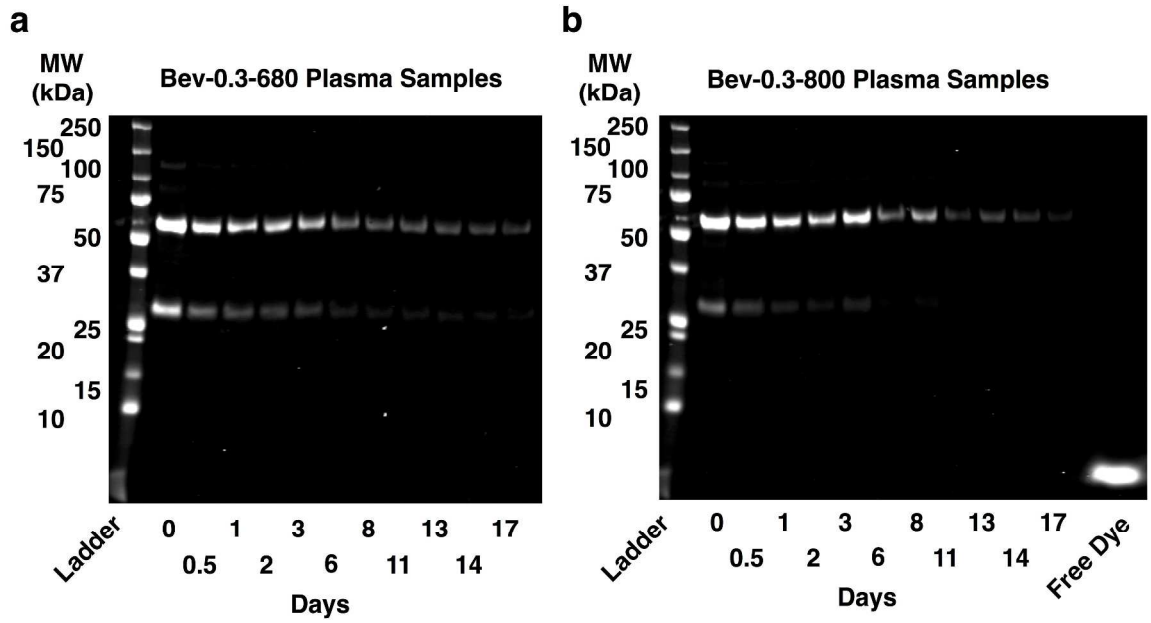


Figure S7 – SDS-PAGE of bevacizumab-AF680 (A) and bevacizumab-800CW (B) plasma clearance samples. Over the course of the experiment bevacizumab-dye conjugates remain intact and there is no detectable formation of free dye.

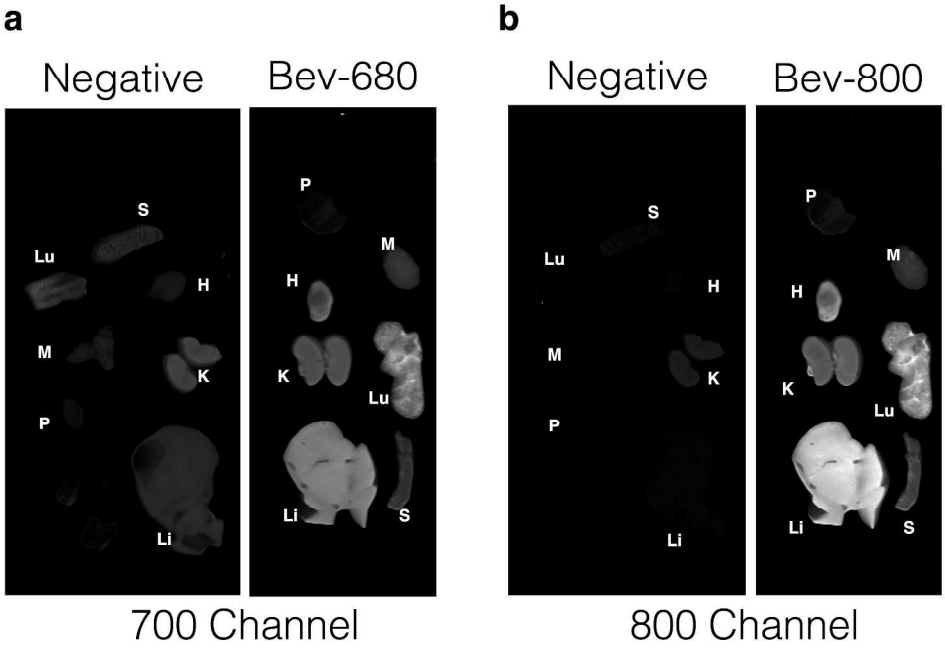


Figure S8 – Fluorescence scan of whole organs. (A) 700-channel scan of negative (uninjected) organs and bevacizumab-AF680 48 hours after injection. (B) 800-channel scan of negative (uninjected) organs and bevacizumab-AF680 48 hours after injection. Before further processing for biodistribution, whole organs were scanned on the Odyssey Clx. Qualitatively the signal intensity from organ scans matches the quantitative uptake from biodistributions (Fig. 5). Window leveling is the same for each channel. Li, liver; Lu, lung; K, kidneys; S, spleen; H, heart; M, muscle; P, pancreas.

Table S1 – Plasma clearance fitted biexponential parameters from PRISM.

Conjugate	% alpha	alpha (day^{-1})	beta (day^{-1})	AUC	95% C.I. % alpha	95% C.I. alpha (day^{-1})	95% C.I. beta (day^{-1})
Bev-0.3-680-F	61.6	4.410	0.054	7.27	59.89 to 63.22	3.617 to 5.203	0.04963 to 0.05818
Bev-0.3-680-E	55.3	7.741	0.065	6.94	48.90 to 61.74	-1.262 to 16.74	0.05066 to 0.07952
Bev-0.3-800-F	60.5	4.123	0.166	2.53	55.28 to 65.72	1.917 to 6.330	0.1530 to 0.1790
Bev-0.3-800-E	58.9	5.817	0.056	7.41	54.69 to 63.12	2.694 to 8.940	0.04597 to 0.06646
Tras-1.2-680-F*	60.6	3.316	0.083	4.92	58.38 to 62.83	2.594 to 4.039	0.07771 to 0.08864
Tras-1.2-680-E*	58.4	3.063	0.038	11.10	4.022 to 57.25	-3435 to 3489	0.03920 to 0.1205
Tras-1.2-800-F	70.8	3.917	0.181	1.79	66.39 to 75.28	2.220 to 5.615	0.1663 to 0.1961
Tras-1.2-800-E	55.3	3.505	0.054	8.38	46.11 to 64.54	0.1096 to 6.900	0.03424 to 0.07439
Tras-0.3-680-F	61.6	3.403	0.080	5.01	57.86 to 65.35	2.204 to 4.603	0.06927 to 0.08975
Tras-0.3-680-E	80.9	1.083	0.011	18.01	69.95 to 91.94	0.4738 to 1.692	0.0 to 0.06237
Tras-0.3-800-F	66.6	2.383	0.174	2.20	58.86 to 74.28	1.038 to 3.727	0.1505 to 0.1968
Tras-0.3-800-E	78.5	1.234	0.011	21.05	71.90 to 85.14	0.7783 to 1.690	0.0 to 0.03854

Tras, trastuzumab; Bev, bevacizumab; F, fluorescence; E, ELISA.
Note: These data (*) were published in the supplementary data section (Fig. S7) of [1]

References

1. Cilliers, C.; Guo, H.; Liao, J.; Christodolu, N.; Thurber, G. M. Multiscale Modeling of Antibody-Drug Conjugates: Connecting Tissue and Cellular Distribution to Whole Animal Pharmacokinetics and Potential Implications for Efficacy. *The AAPS journal* **2016**.