Electronic Supplementary Material (ESI) for Molecular Systems Design & Engineering.

Evaluation of affibody charge modification identified by synthetic consensus design in molecular PET imaging of epidermal growth factor receptor

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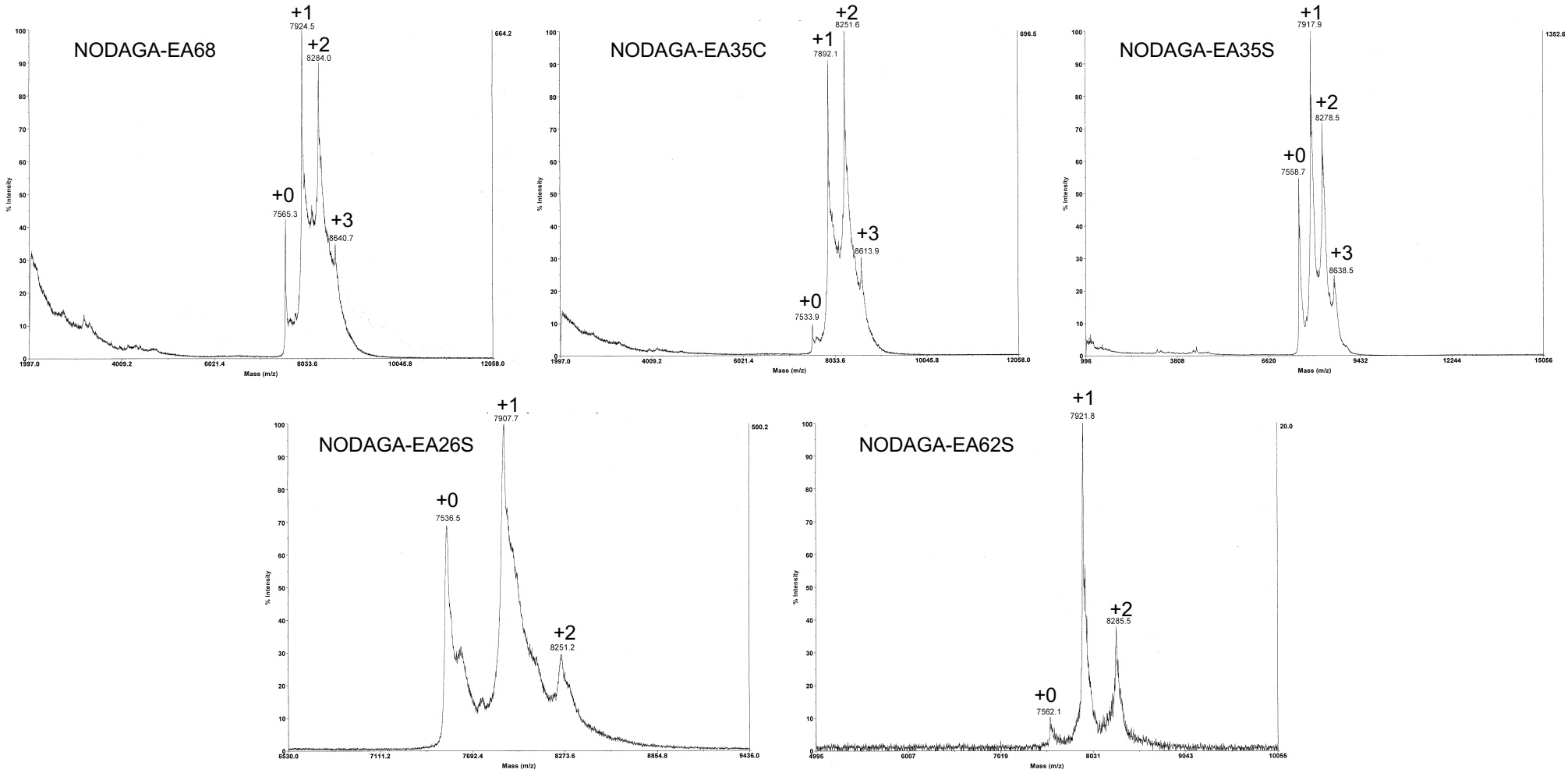
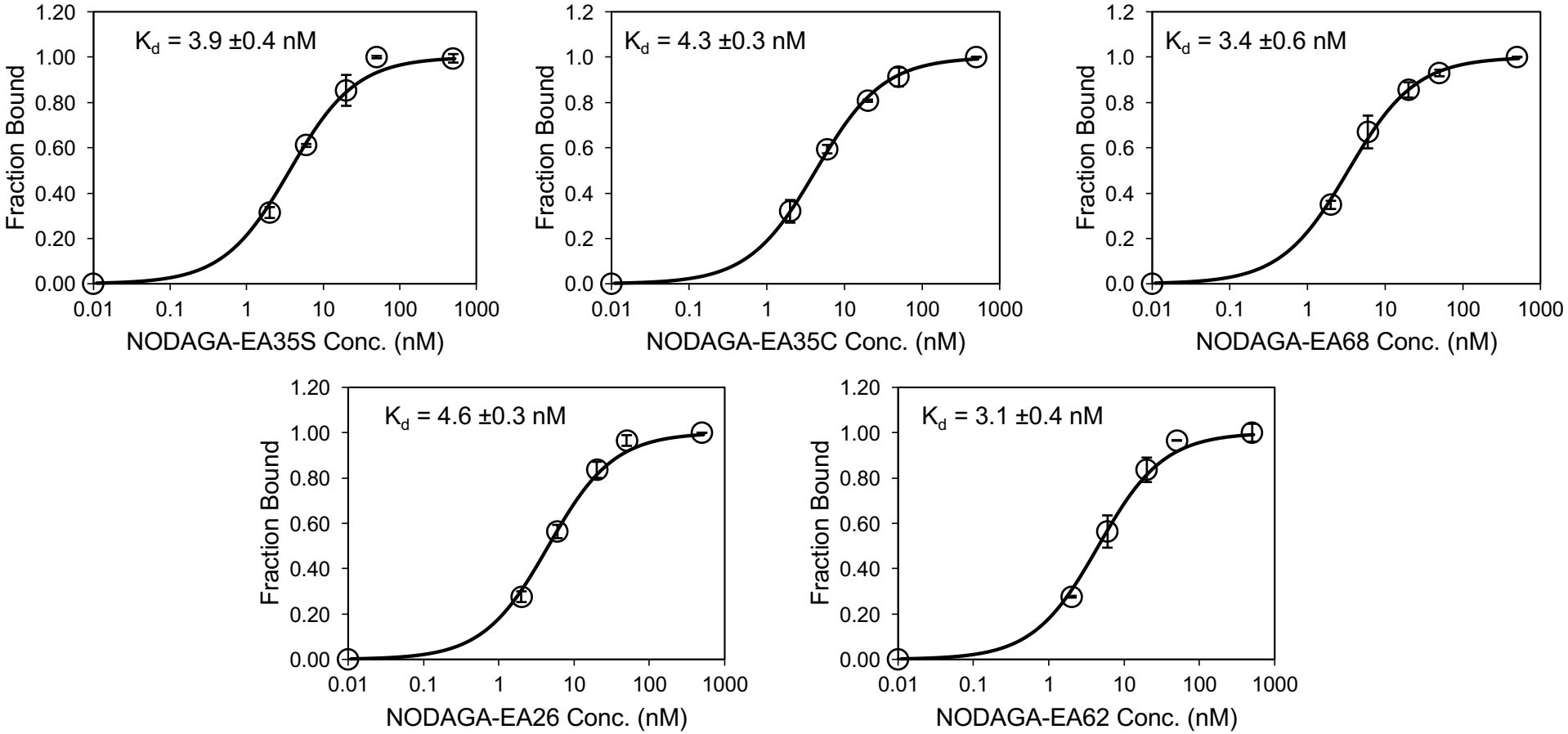
Supplementary Figures and Tables

Variant	Yield (mg/L)	K_d (nM)	T_m (°C)	2° Structure	Specificity (A431:MCF7 Binding Signals)
EA68	3.5 ± 0.3	5.3 ± 1.7	71	α-helical	109 ± 63
EA26S	0.62 ± 0.02	1.6 ± 1.2	68	α-helical	214 ± 134
EA62S	1.9 ± 0.3	2.5 ± 0.7	59	α-helical	342 ± 209
EA35S	7.0 ± 0.5	1.7 ± 0.5	68	α-helical	529 ± 297
EA35C	12.7 ± 0.9	6.9 ± 1.4	71	α-helical	74 ± 29

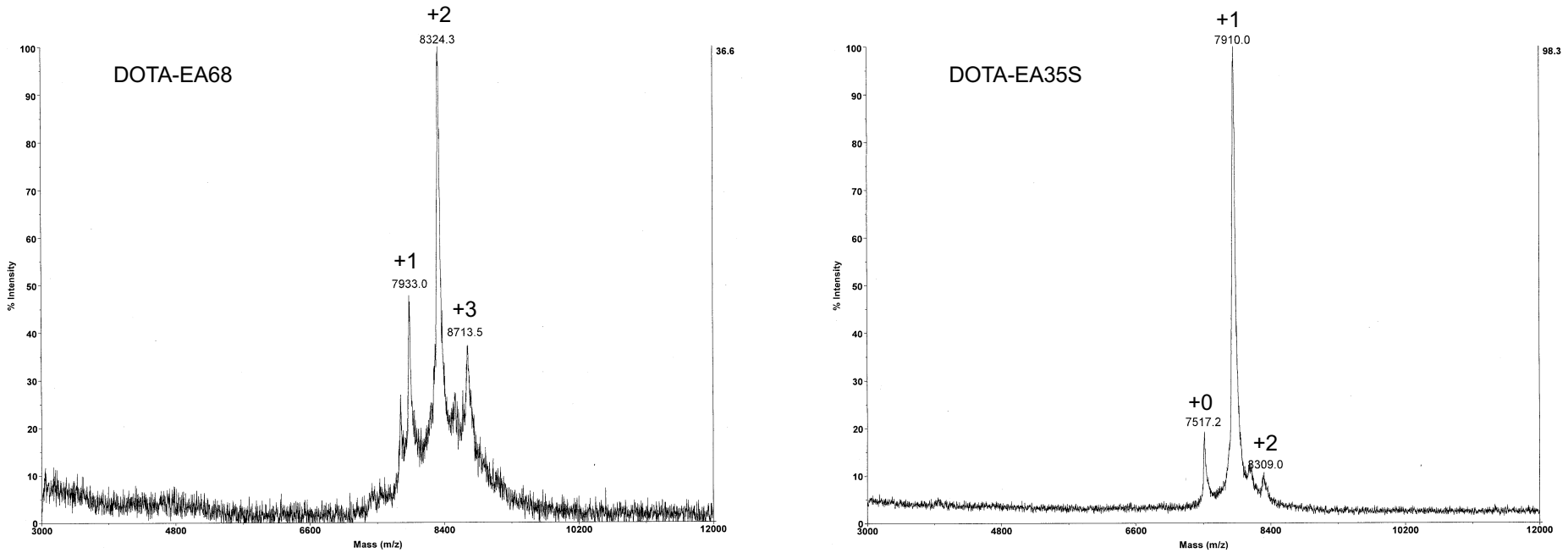
Supplemental Table 1. Biophysical properties of affibody clones used for PET/CT imaging.



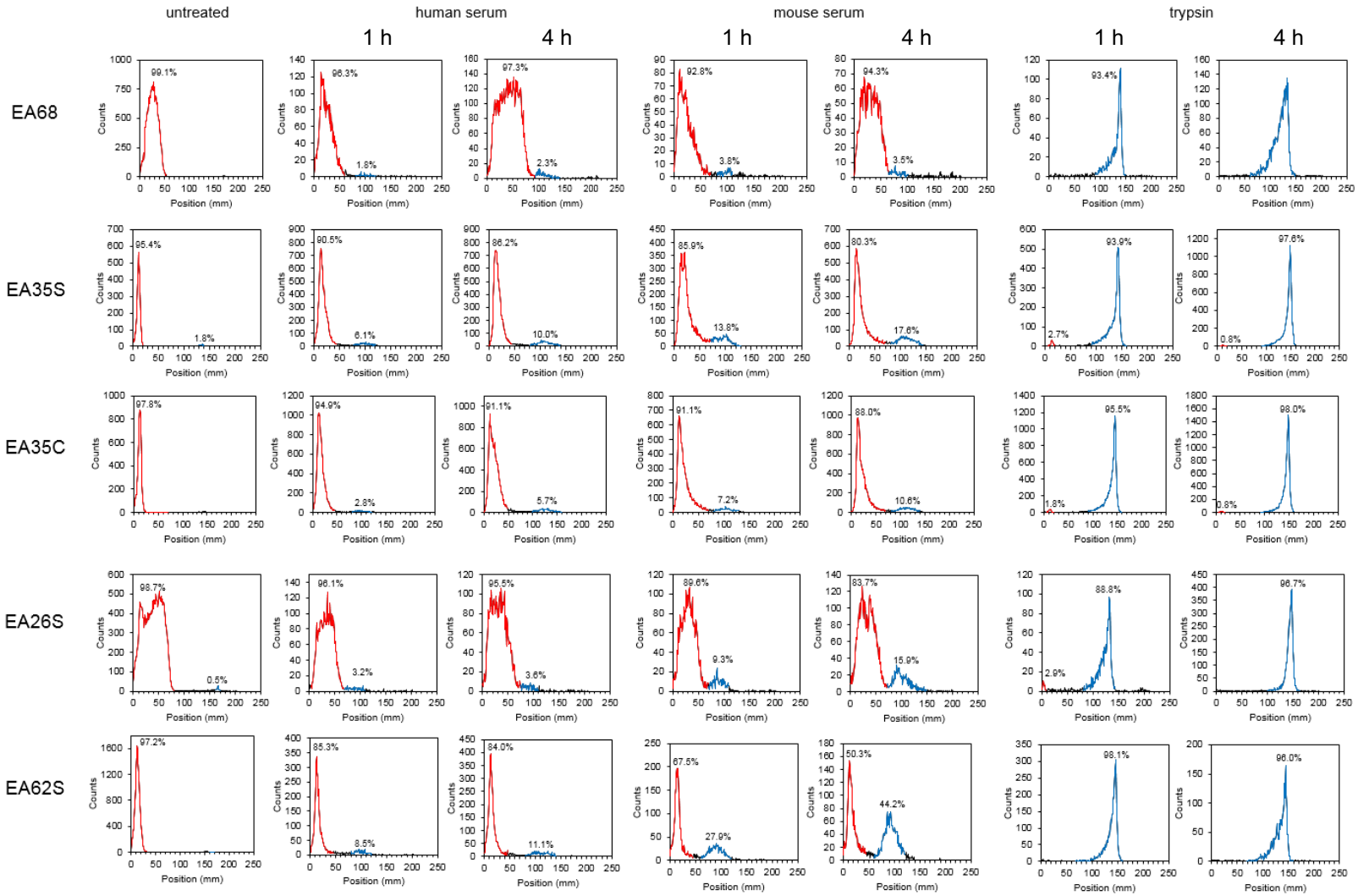
Supplemental Figure 1. Matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) of NODAGA-Affibody charge variants with 0, 1, 2, or 3 degrees of conjugation



Supplemental Figure 2. *NODAGA-Affibody target-affinity characterization.* EGFR-overexpressing A431 cells were mixed with the indicated concentration of NODAGA-conjugated affibody. Binding was detected with fluorophore-conjugated anti-His6 antibody via flow cytometry. Equilibrium dissociation constants were calculated assuming a 1:1 binding model. n = 3 measurements per variant. NODAGA-conjugated versions of EA35S and EA26 were nominally weaker binders to EGFR than their unconjugated counterparts (K_d = 3.9 ± 0.4 nM vs. 1.7 ± 0.5 nM and 4.6 ± 0.3 vs. 1.6 ± 1.2 nM respectively). However, these variances are slight and should not meaningfully impact *in vivo* biomarker localization. EA68 and EA62 showed no difference in binding affinity upon NODAGA conjugation and EA35C presented a slight improvement (4.3 ± 0.3 nM vs. 6.9 ± 1.4 nM).



Supplemental Figure 3. Matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) of DOTA-Affibody charge variants with 0, 1, 2, or 3 degrees of conjugation



Supplemental Figure 4. Radio chromatography of affibody clones left untreated, in serum, or in trypsin.