Supplementary Material

Influence of Molecular Design on the Targeting Properties of ABD-Fused Mono- and Bi-Valent Anti-HER3 Affibody Therapeutic Constructs

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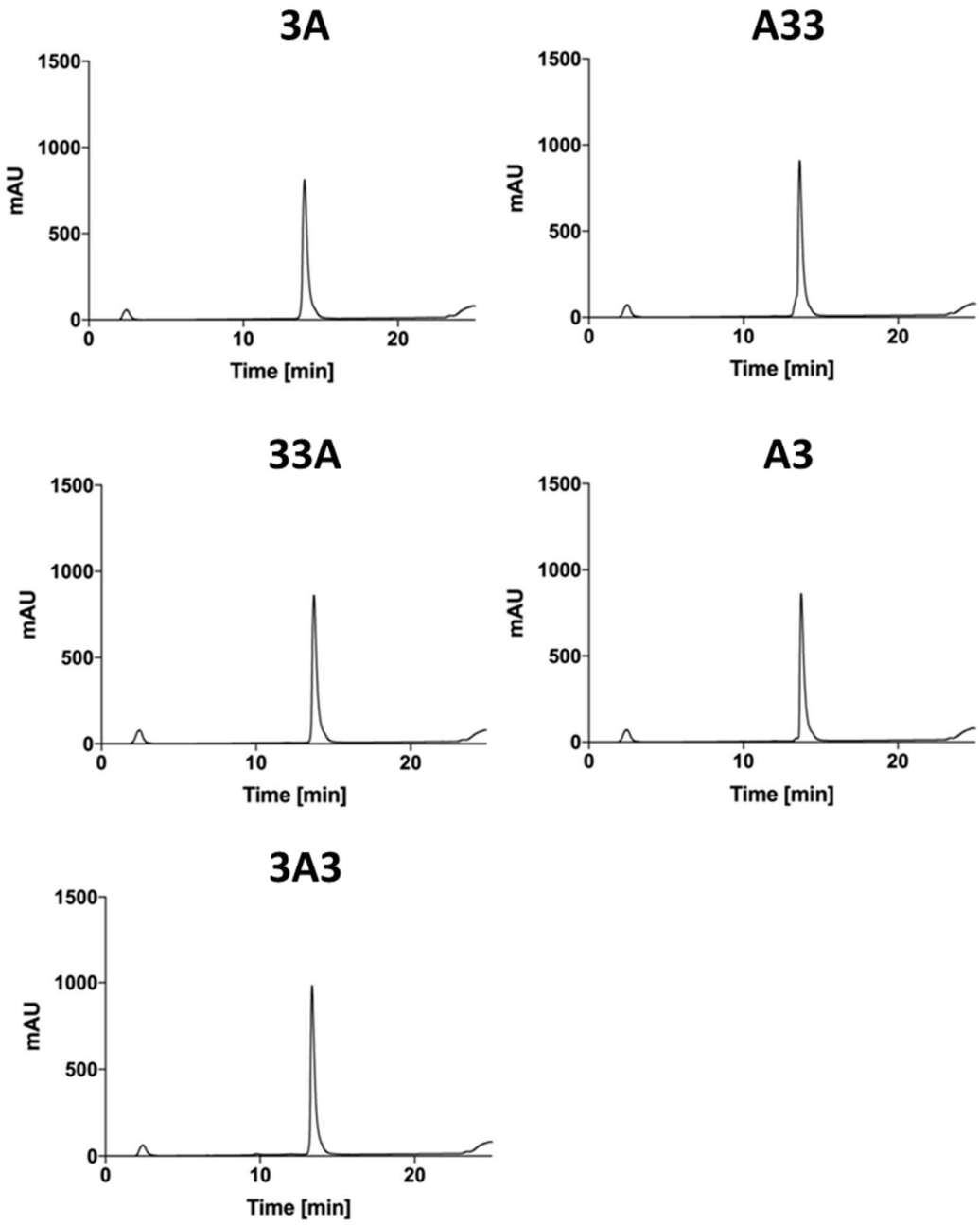

Figure S1: Purity determination. The purity of the five constructs was evaluated by absorbance measurement at 220 nm using RP-HPLC.

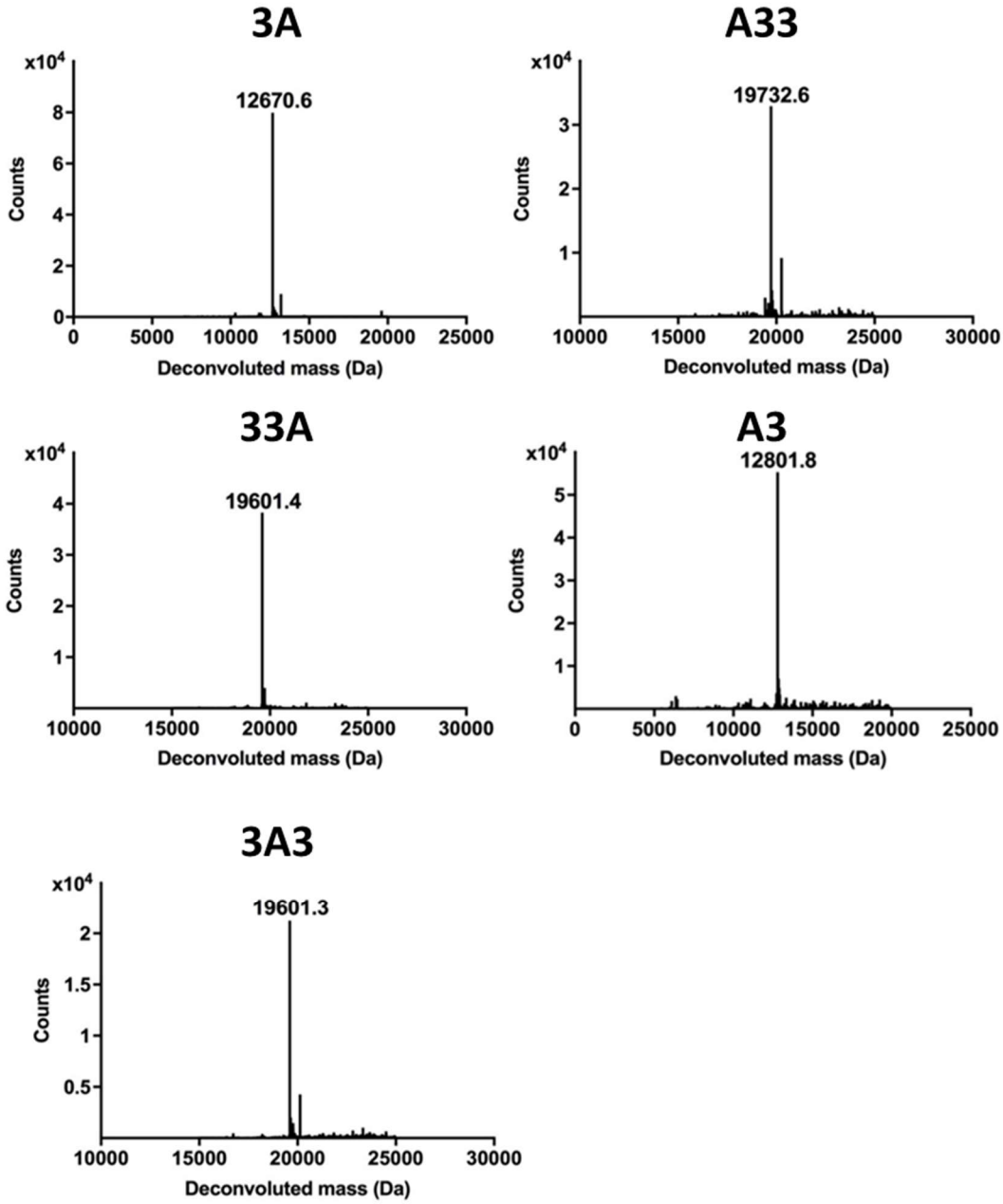

Figure S2: Mass determination. Results from ESI-MS confirming the identity of the constructs. The experimental molecular masses of the observed peaks are in accordance with the theoretical masses shown in Table 1.

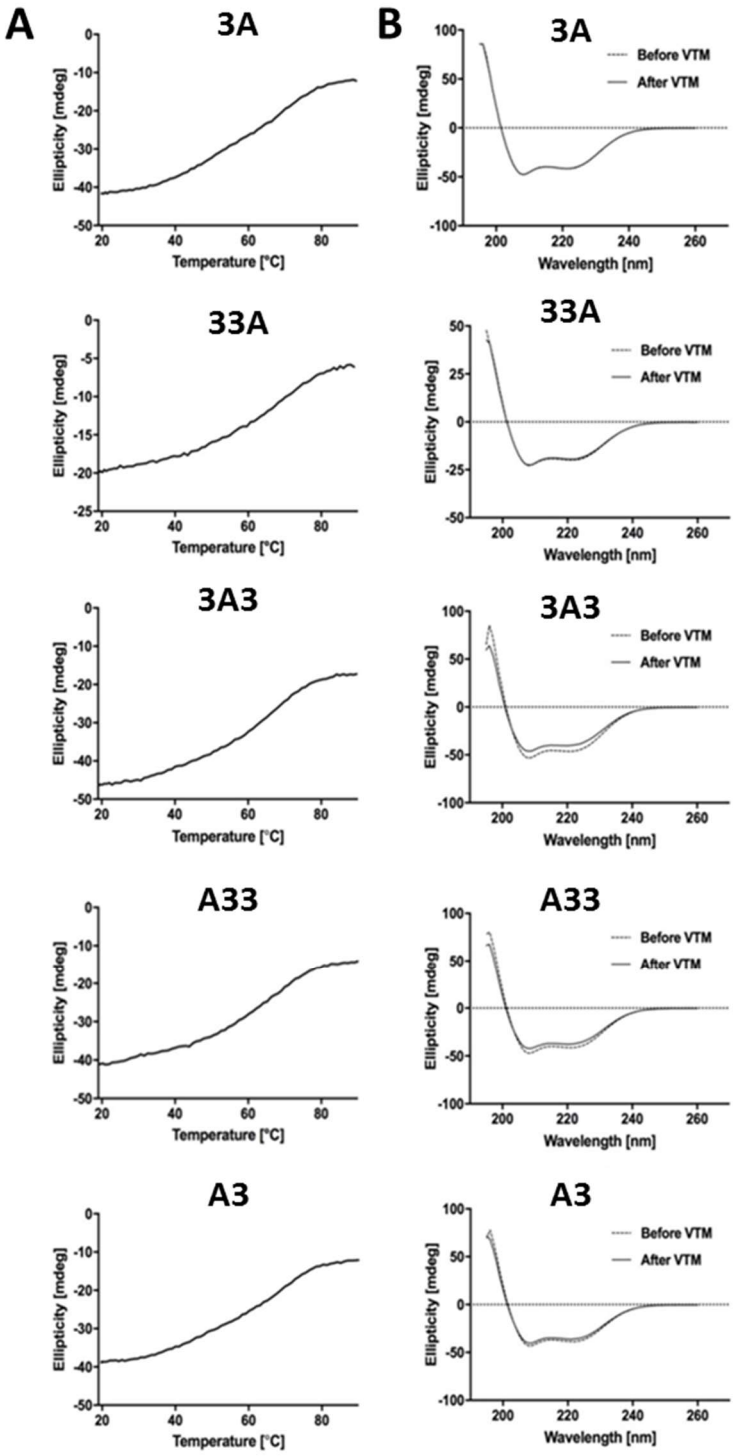

Figure S3: Analysis of thermal stability and refolding capacity of DOTA-conjugated constructs. A) overlay of circular dichroism spectra (195-260 nm) before and after thermal denaturation. B) Variable temperature measurement (VTM) spectra obtained at 221 nm while heating the sample from 20 °C to 90 °C. Melting temperatures (T_m) were determined by fitting the curves using a Boltzmann Sigmoidal model. The determined T_m values for the five constructs are presented in Table 1.

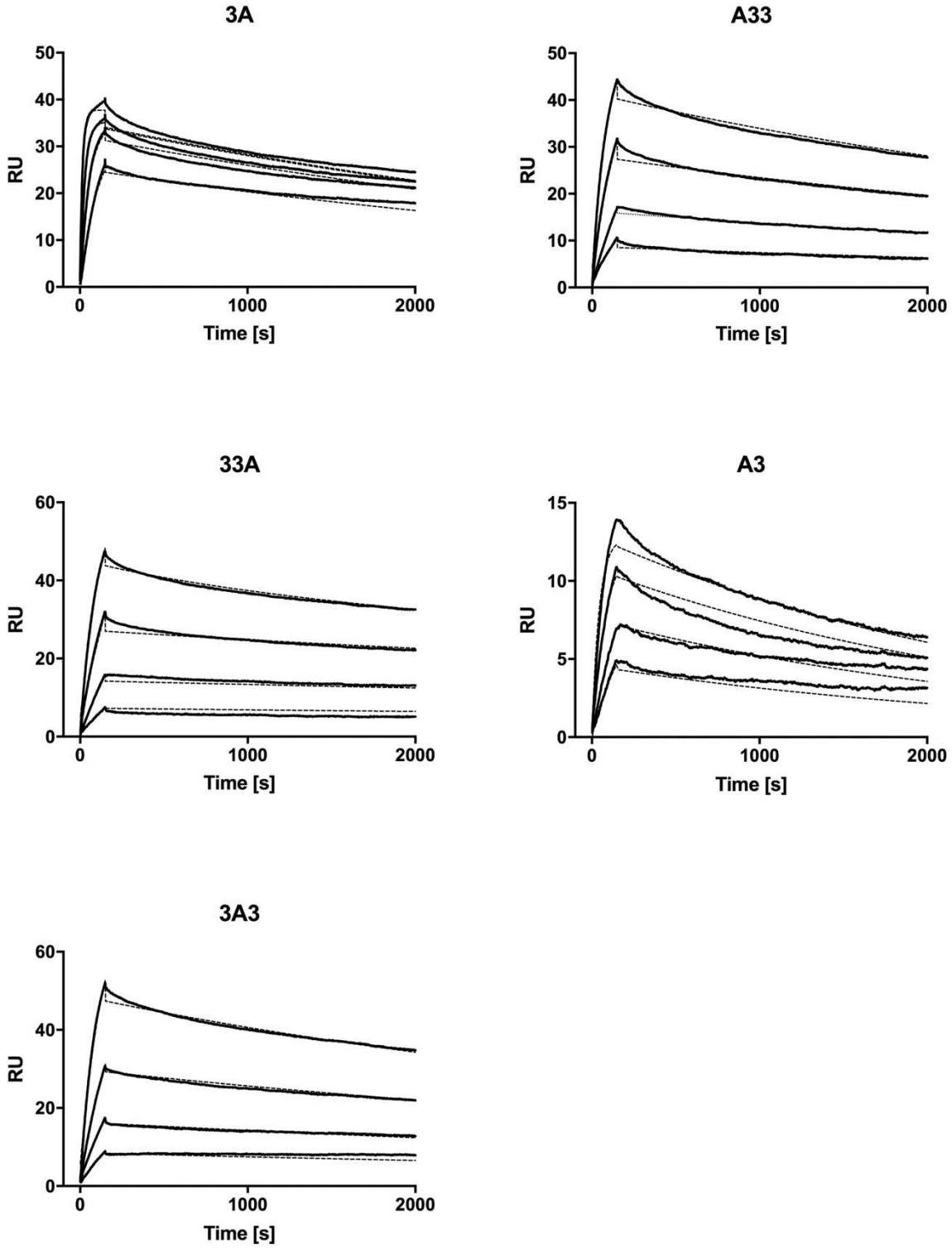

Figure S4: Analysis of binding affinity to HSA. Representative experimental sensorgrams (solid) with fitted curves (dashed) from SPR analysis for the five DOTA-conjugated ABD-fused anti-HER3 affibody constructs. Immobilized HSA was subjected to four concentrations of each construct (1.5625, 3.125, 6.25 and 12.5 nM). Monovalent affinities to HSA, based on a Langmuir 1:1 model, are presented in Table 1.

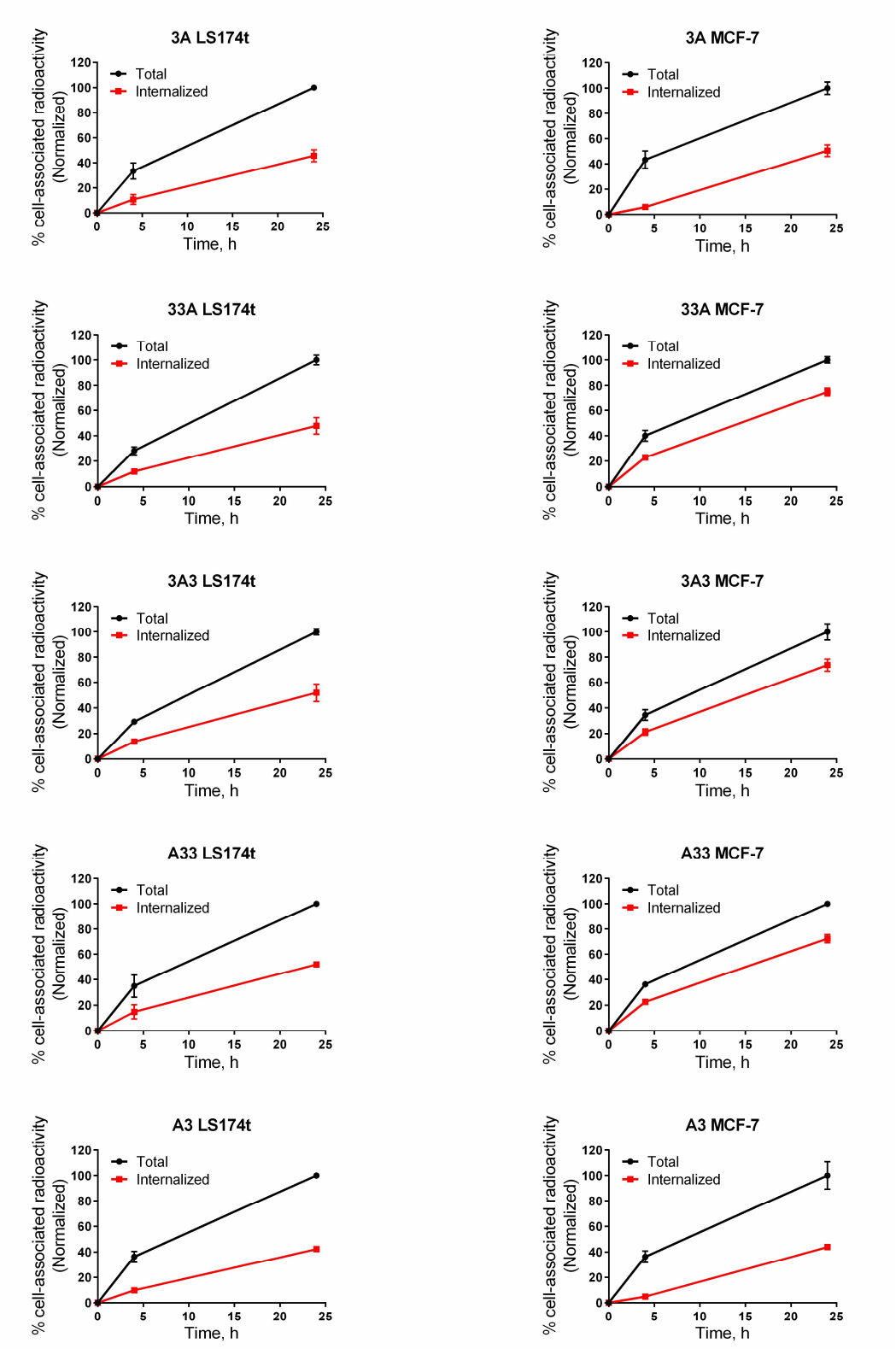

Figure S5: Cellular processing of different ^{111}In-labeled ABD-fused anti-HER3 affibody molecules by LS174T (left) and MCF-7 (right). HER3-expressing cell lines up to 24 h after continuous incubation. Data were normalized to the maximum uptake.