

Supporting Information

An Albumin-Holliday Junction Biomolecular Modular Design for Programmable Multifunctionality and Prolonged Circulation

Anders Dinesen [†], Veronica L. Andersen [†], Marwa Elkhatab [†], Diego Pilati [†], Pernille Bech [†], Elisabeth Fuchs [†], Torbjørn R. Samuelsen [†], Alexander Winther [†], Yunpeng Cai [†], Anders Märcher [‡], Archie Wall [§], Marjan Omer [†], Jesper S. Nielsen [†], Vijay Chudasama [§], James R. Baker [§], Kurt V. Gothelf [‡], Jesper Wengel [¶], Jørgen Kjems [†], and Kenneth A. Howard ^{†,*}

[†] Interdisciplinary Nanoscience Center (iNANO) and Department of Molecular Biology and Genetics, Aarhus University, DK-8000 Aarhus C, Denmark

[‡] Interdisciplinary Nanoscience Center (iNANO) and Department of Chemistry, Aarhus University, DK-8000 Aarhus C, Denmark

[§] Department of Chemistry, University College London, London, WC1H 0AJ, UK

[¶] Nucleic Acid Center, Department of Physics, Chemistry, and Pharmacy, University of Southern Denmark, DK-5230 Odense M, Denmark

* Correspondence: kenh@inano.au.dk

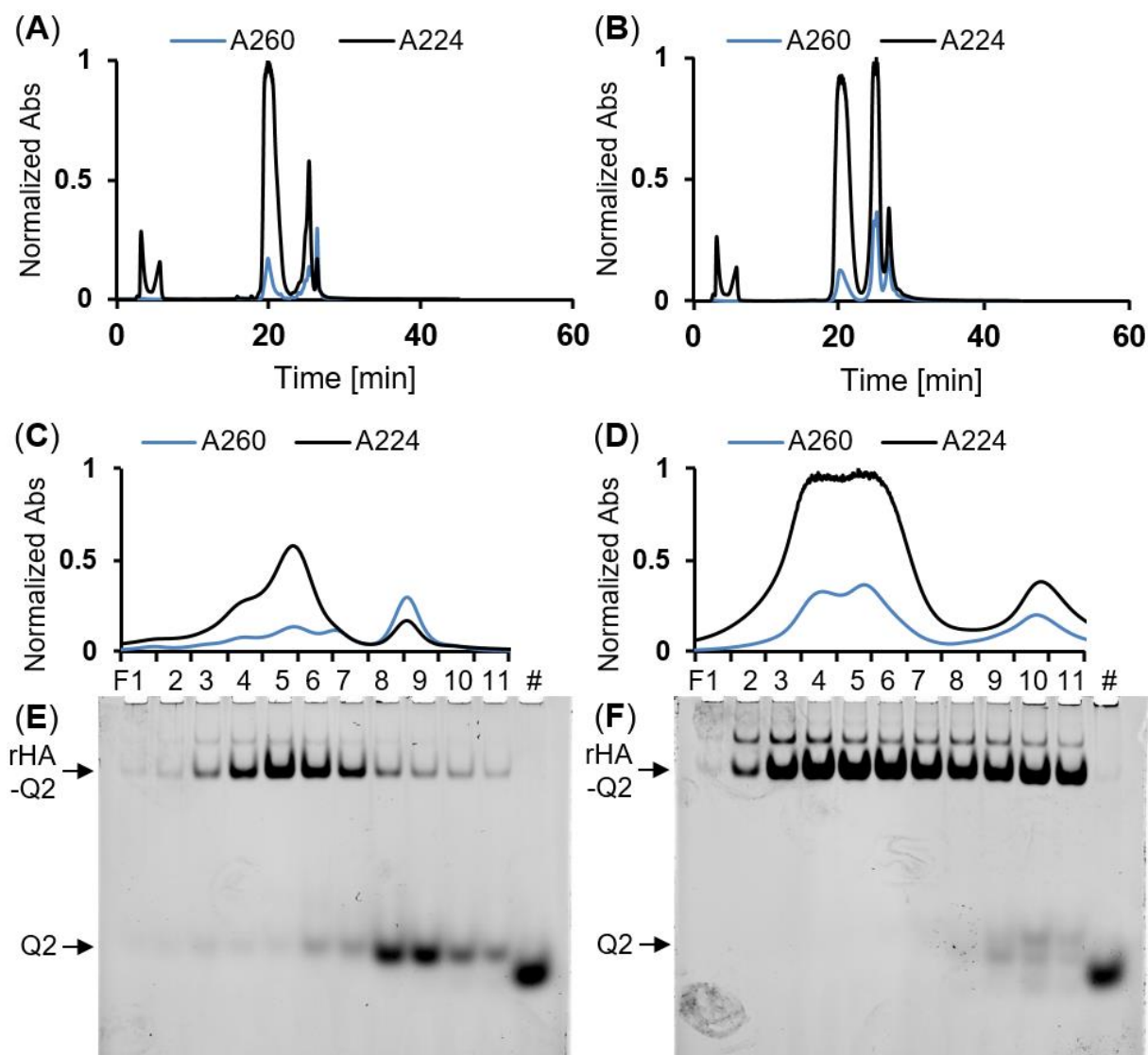


Figure S1. Optimization of the conjugation of Q2 to rHA and purification. (A, C, E) conjugation using SM(PEG)₈ linker, (B, D, F) conjugation using MBM-BCN linker. (A, B) full IEX-HPLC chromatogram showing absorbance at 224 nm indicating protein and 260 nm indicating oligonucleotide. The peak around 20 min corresponds to unconjugated excess rHA (C, D) zoomed in chromatogram corresponding to 24-27 min in A and B. Collected fraction numbers are shown below the chromatogram. (E, F) SYBR gold stained native PAGE gel of fraction 1-11 of the corresponding IEX-HPLC purification as shown above. # indicates a Q2 control lane.

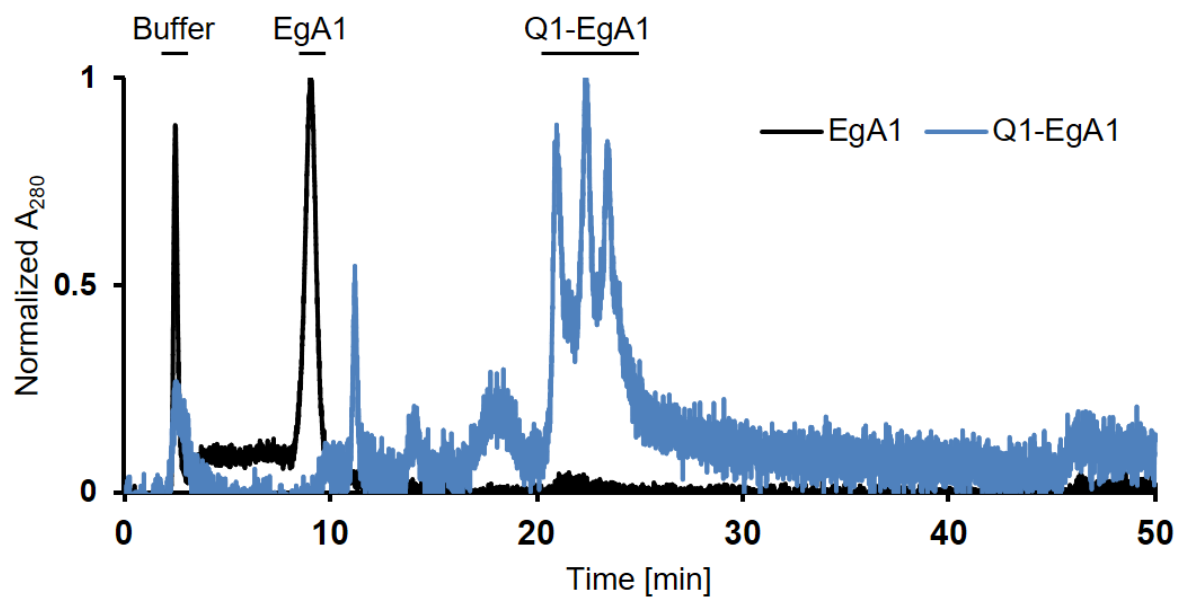
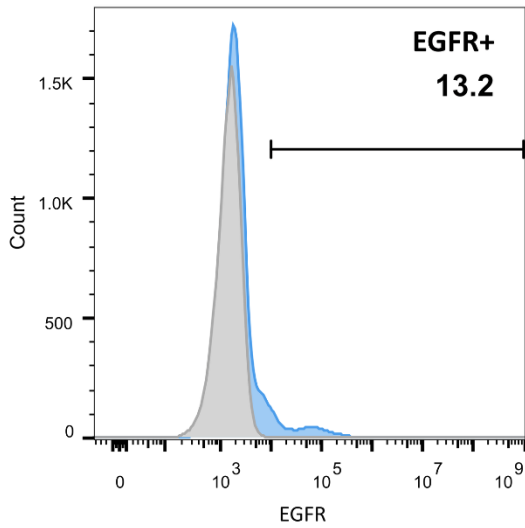
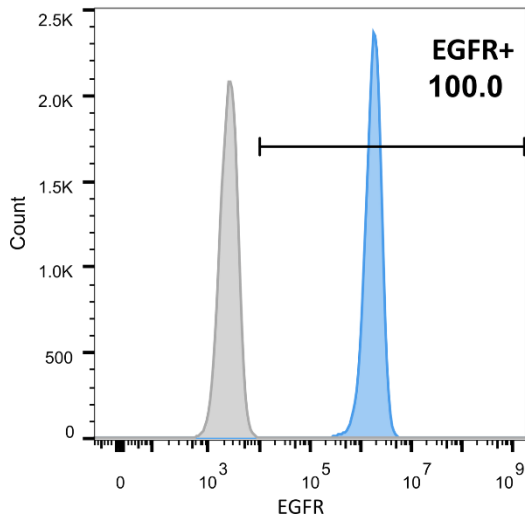


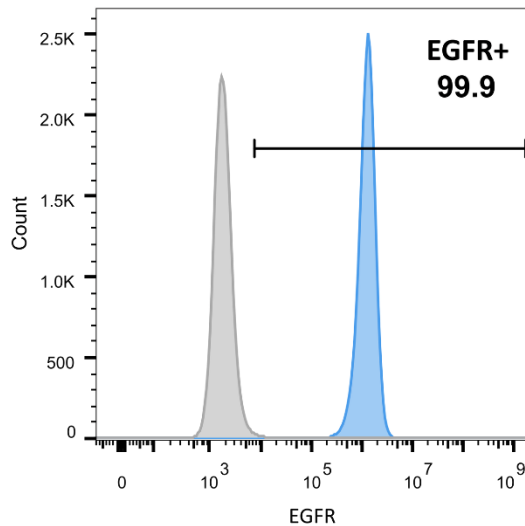
Figure S2. Analytical IEX-HPLC of Q1-EgA1 showing no excess EgA1 in the conjugated and purified Q1-EgA1 sample.



3T3



3T3 EGFR



A431

Figure S3. Flow cytometry confirmation of EGFR expression levels of the investigated cell lines by cetuximab. Blue indicates cells stained via cetuximab and anti-human IgG Alexa488, and grey indicates an unstained control. The inserted number in the histogram indicates the percentage of EGFR-positive cells.

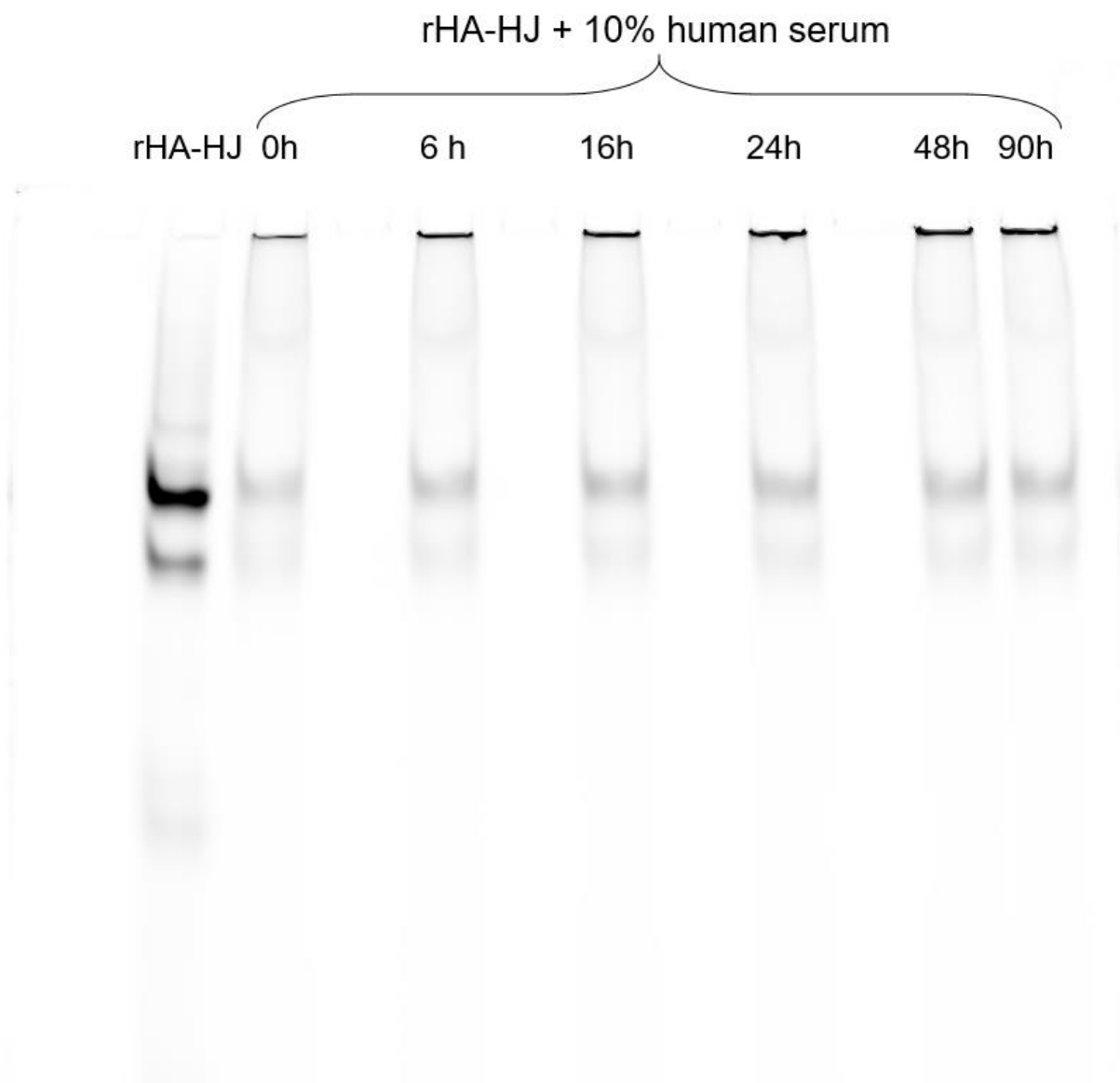


Figure S4. Native PAGE showing rHA-HJ assembly stability following incubation in 10% human serum at 37°C over time.

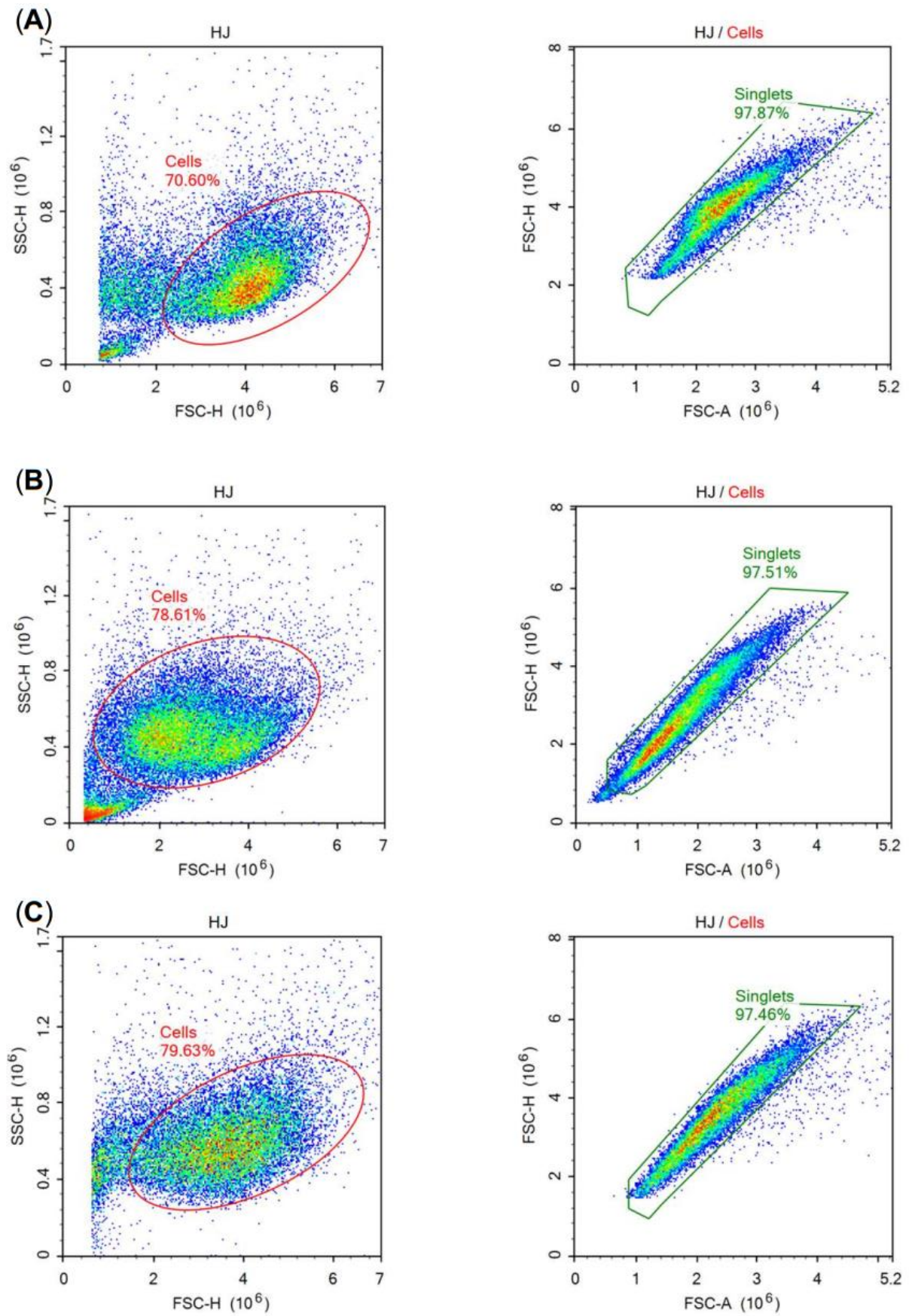


Figure S5. Flow cytometry gating strategy for the cell lines (A) 3T3, (B) 3T3-EGFR, and (C) A431.