Supporting Information for

Localization of Therapeutic Fab-CHP Conjugates to Sites of Denatured Collagen for the Treatment of Rheumatoid Arthritis

Keith J. Arlotta[‡], Boi Hoa San[‡,†], Hong-Hua Mu[#], S. Michael Yu[‡,§,*], Shawn C. Owen[‡,§,*]

‡ Department of Biomedical Engineering, University of Utah, SLC, UT 84112

Department of Internal Medicine, University of Utah, SLC, UT 84132

§ Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, SLC, UT 84112

Present Address:

† Beckman Coulter Life Sciences, Miami, FL

* Corresponding authors: SMY, michael.yu@utah.edu ; SCO, Shawn.Owen@hsc.utah.edu

Supporting Information

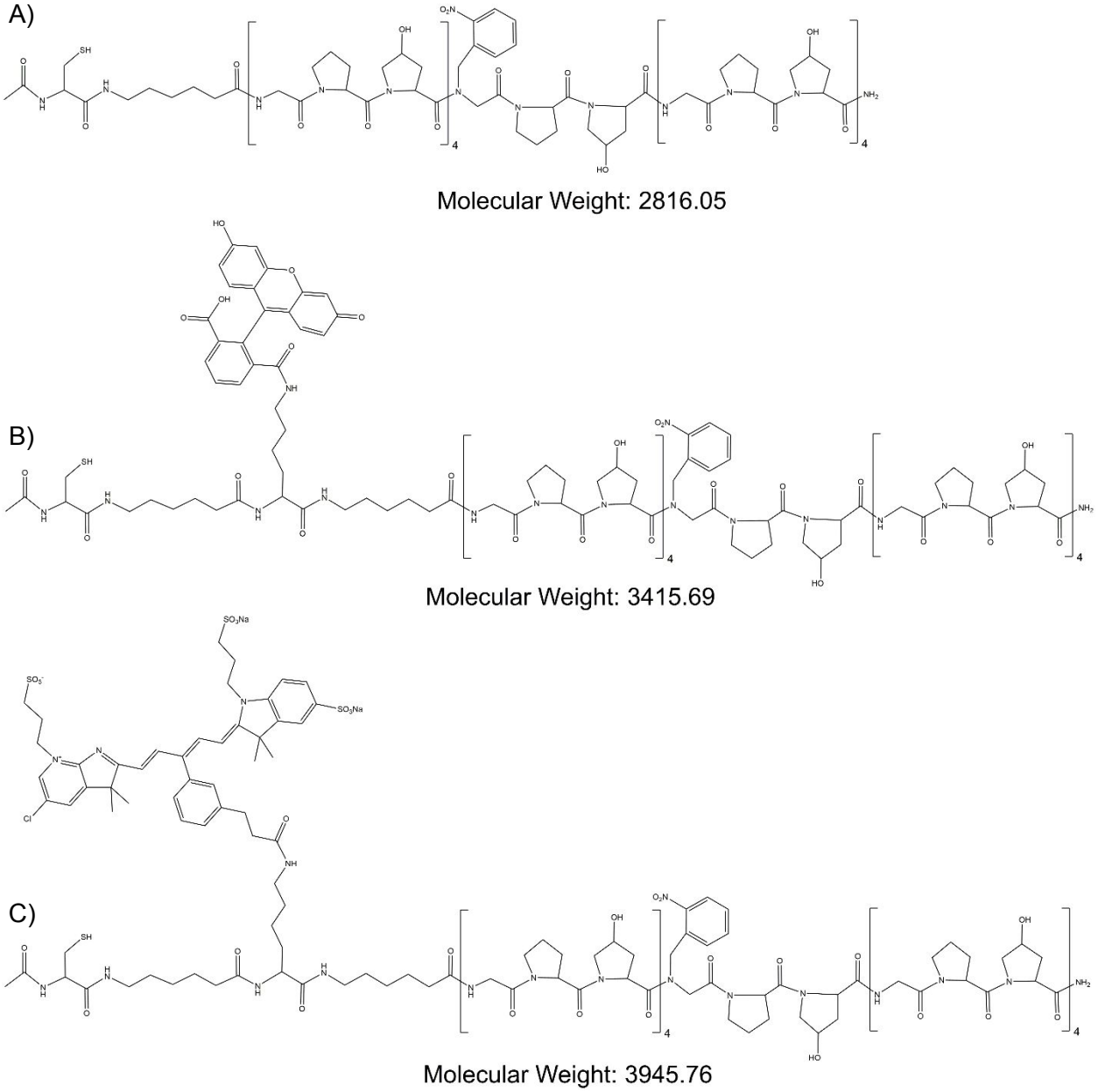

Figure S1. Chemical structures of A) Ac-C-Ahx-NB(GPO)$_9$, B) Ac-C-Ahx-K(CF)-Ahx-NB(GPO)$_9$, and C) Ac-C-Ahx-K(IR680)-Ahx-NB(GPO)$_9$ with corresponding molecular weights.

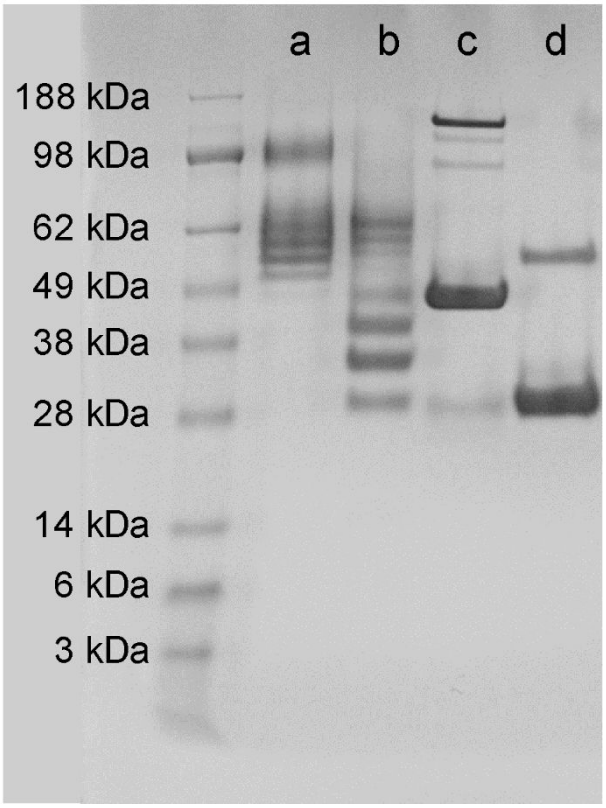

Figure S2. SDS-PAGE analysis of papain-digested infliximab and conjugated iFab-CHP. Lanes are as follows: a) iFab-CHP denatured, non-reduced b) iFab-CHP denatured, reduced c) iFab denatured, non-reduced d) iFab denatured, reduced.

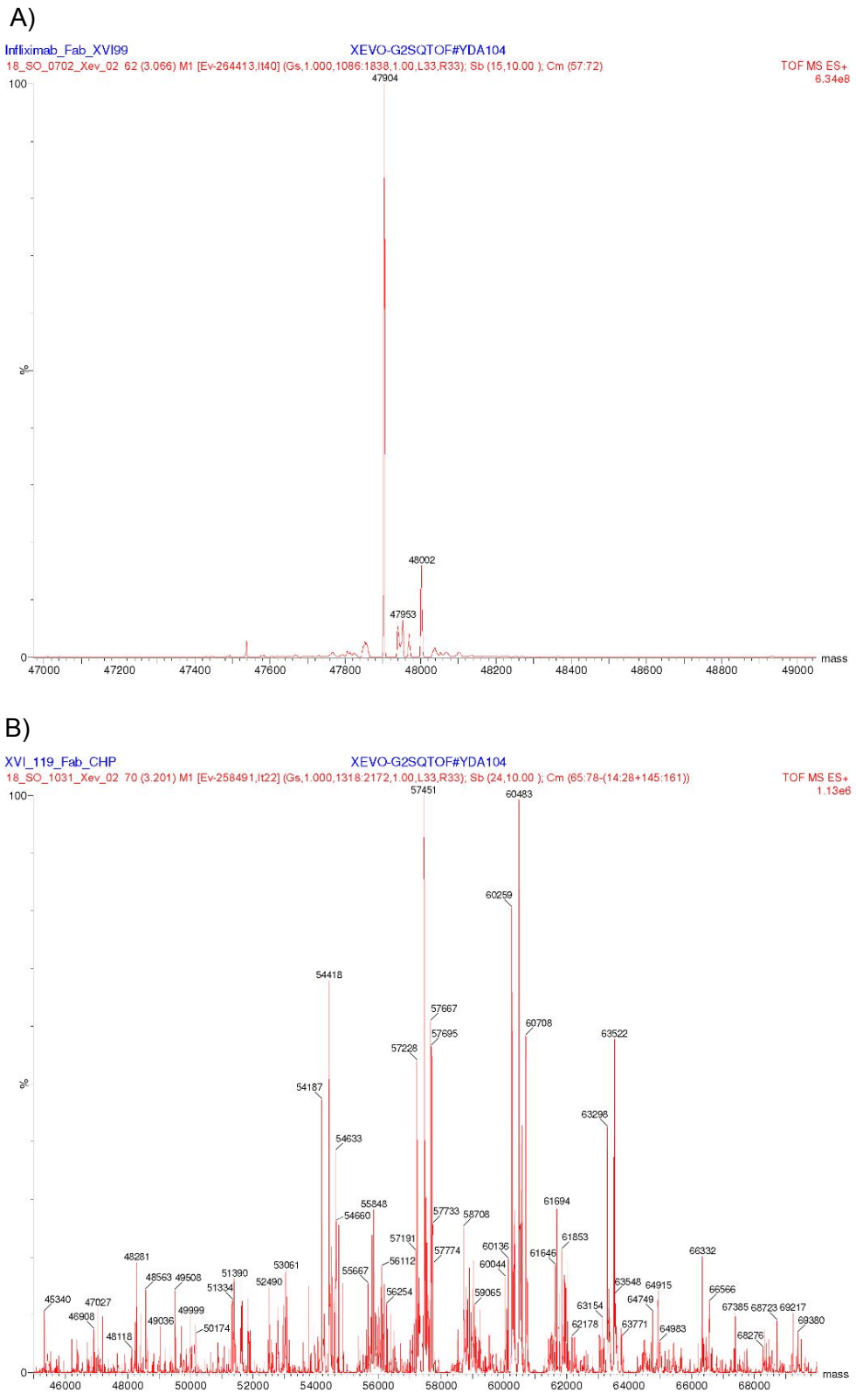

Figure S3. Deconvoluted, intact LC-MS spectra of A) iFab and B) iFab-CHP. These spectra correspond with the simplified overlay presented in Figure 1.

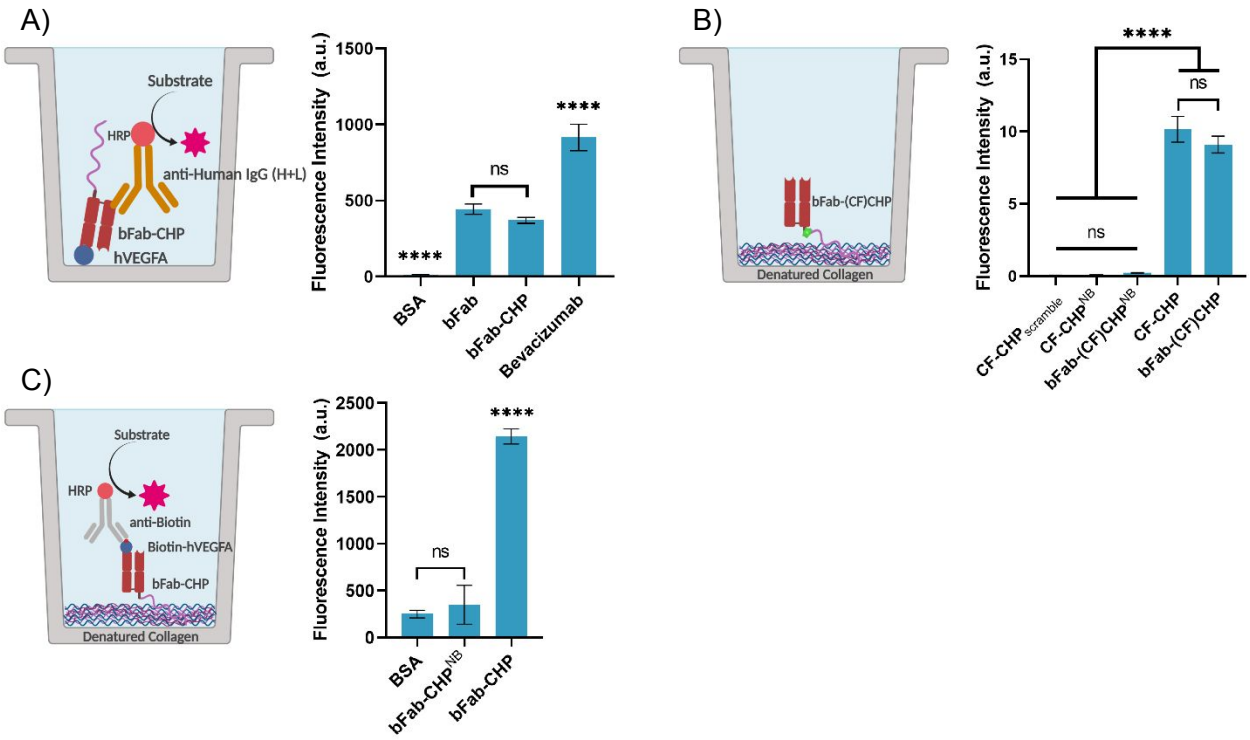

Figure S4. ELISA-like binding assays were used to confirm the ability of bFab-CHP to bind A) immobilized hVEGFA, B) dCol, and C) dcol and hVEGFA simultaneously. A) No statistically significant difference in hVEGFA binding was observed for bFab-CHP relative to bFab. B) No statistically significant difference in dCol binding was observed for bFab-(CF)CHP relative to CF-CHP, while caged CF-CHP^{NB} and bFab-(CF)CHP^{NB} binding of dCol was negligible. C) bFab-CHP showed significantly greater simultaneous binding of dCol and hVEGFA relative to both caged bFab-CHP^{NB} and BSA. Values are shown as a mean ± standard deviation (*n*=3); Statistical significance is defined as follows: ns = P>0.05; *P≤0.05; **P<0.01; ***P<0.001; ****P<0.0001.

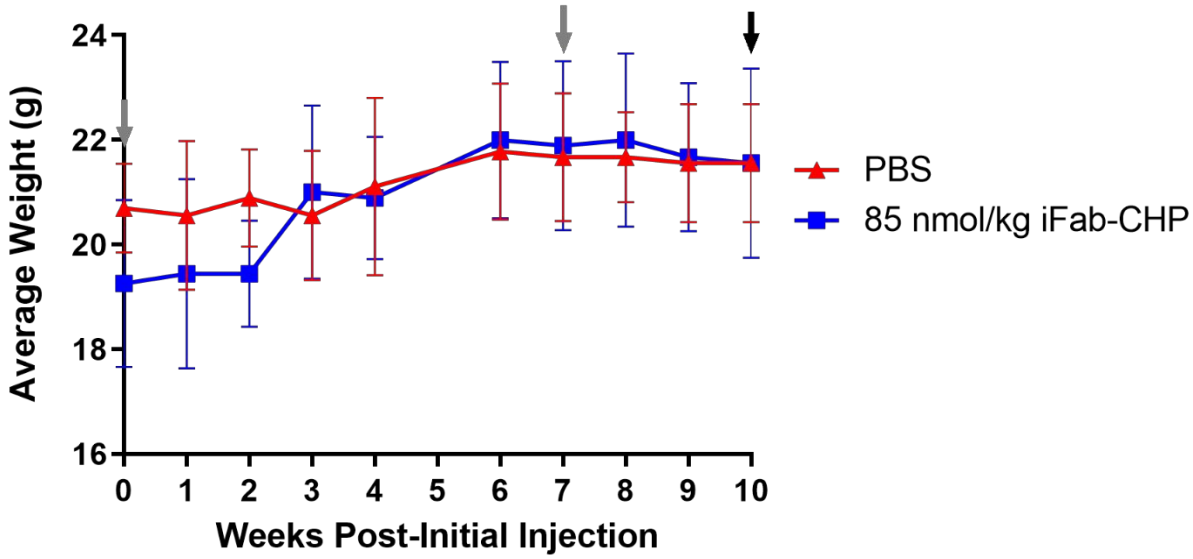

Figure S5. Weights of PBS or iFab-CHP treated arthritic female mice (*n*=3) averaged over each week. Grey arrows indicate the weeks mice were injected and the black arrow indicates the week mice were sacrificed. Weights are presented as a mean ± standard deviation. No significant differences were observed at any time point (P>0.05).

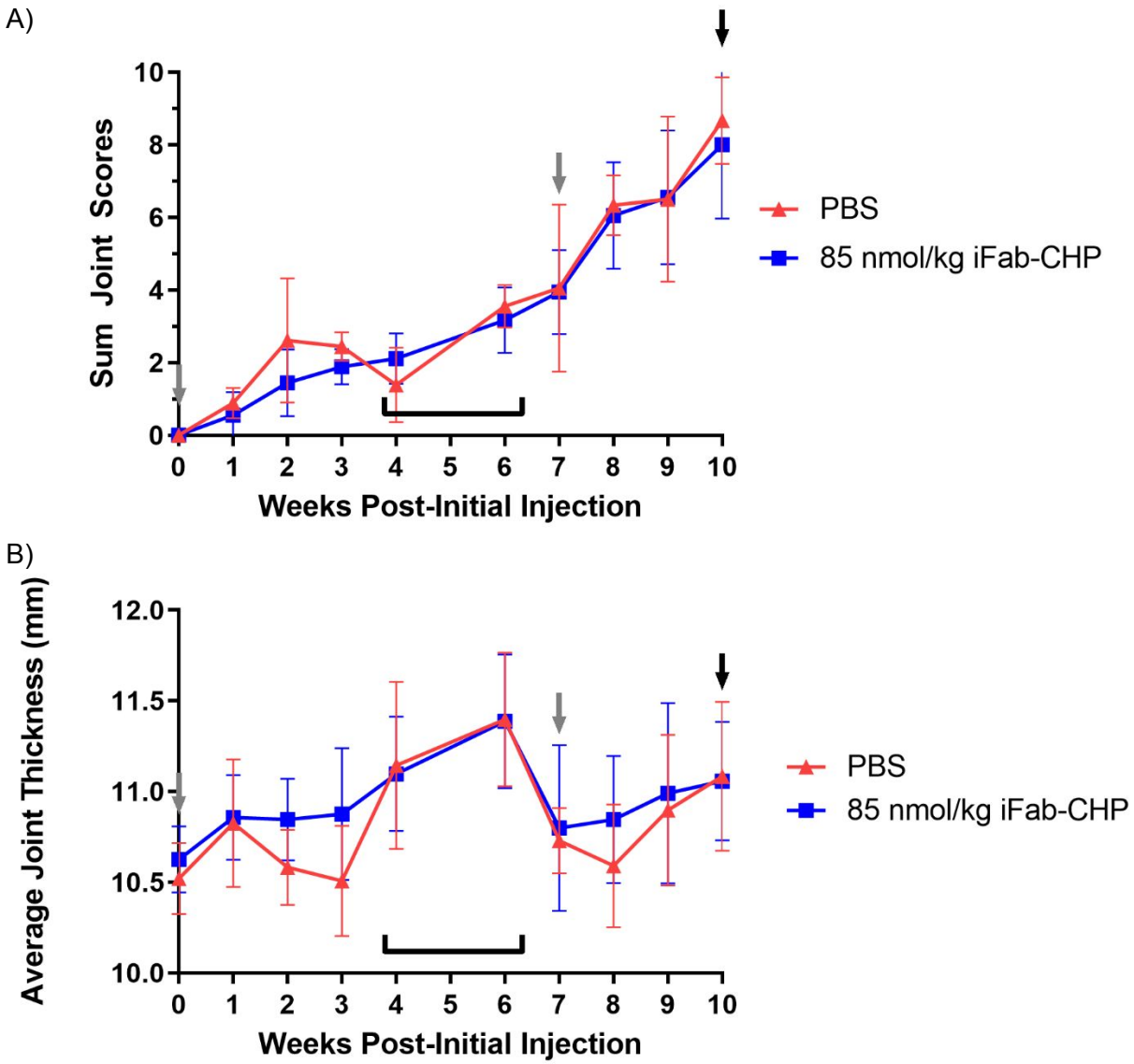

Figure S6. A) Summed joint scoring data averaged from three measurements per week (maximum score 32). B) Summed hind paw width and thickness measurements averaged from three measurements per week. Scores and measurements were summed for each mouse and averaged among that treatment group for each week. All scores and measurements are presented as a mean ± standard deviation (n=3). Grey arrows indicate the weeks mice were injected and the black arrows indicates the week mice were sacrificed. Black brackets indicate time points at which mice were assessed by a different blinded observer due to scheduling conflicts. No statistically significant differences were observed at any time point ($P>0.05$).