Supporting Information

Broadening and Enhancing Functions of Antibody by Self-Assembling Multimerization at Cell Surface

Lian Li[1], Jiawei Wang[1], Yachao Li[1], D. Christopher Radford[2], Jiyuan Yang[1,*], Jindřich Kopeček[1,2,*]

[1]Department of Pharmaceutics and Pharmaceutical Chemistry/Center for Controlled Chemical Delivery, University of Utah, Salt Lake City, Utah 84112, USA.

[2]Department of Biomedical Engineering, University of Utah, Salt Lake City, Utah 84112, USA

Corresponding Authors: J. Kopeček, E-mail: jindrich.kopecek@utah.edu

J. Yang, E-mail: jiyuan.yang@utah.edu

1	Characterization of OBN-MORF1 conjugate	2
2	Characterization of HSA-$(MORF2)_{10}$ conjugate	3
3	SDS-PAGE gel electrophoresis analysis of conjugate hybridization	4
4	Comparison of OBN-MORF1 and OBN-$(MORF1)_3$ on apoptosis induction	5
5	Characterization of $Fab'_{(OBN)}$-MORF1 conjugate	6
6	Flow cytometry analysis of residual Raji cells (human CD10+CD19+) in the bone marrow	7

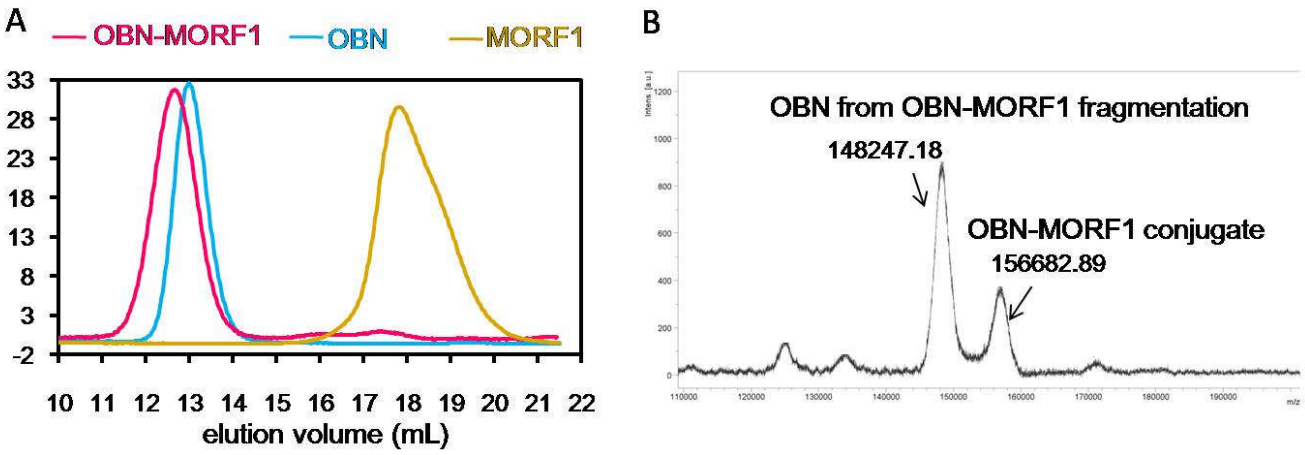


Figure S1. (A) Size-exclusion chromatography characterizations of OBN antibody, free MORF1, and OBN-MORF1 conjugate as determined on Superdex 200 10/300 GL column eluted with PBS (pH 7.2) at flow rate 0.4 mL/min and UV wavelength 280 nm. (B) MALDI-TOF mass spectrum for OBN-MORF1 conjugate. Results in (A) show that for the OBN-MORF1 conjugate, the peak (12.6 mL) shifts to smaller elution volume as compared with that of OBN antibody (13.1 mL) due to the molecular weight increase, and there is minimal absorbance at elution volume of 18.0 mL where the peak of free MORF1 appears, demonstrating the successful attachment of MORF1 to OBN and the minimal unconjugated MORF1 in OBN-MORF1 conjugate. According to the bicinchoninic acid protein assay for quantifying OBN concentration and UV–vis spectrophotometry for quantifying MORF1 concentration, the ratio of MORF1 to OBN in OBN-MORF1 conjugate is 1.2:1 (Table 1), indicating no free antibody in OBN-MORF1 conjugate as well. Results in (B) show the molecular weights of OBN and OBN-MORF1 conjugate are approximately 148.2 kDa and 156.7 kDa, respectively. Since the molecular weight of free MORF1 is about 8.6 kDa, this result demonstrates only one strand of MORF1 has been conjugated onto OBN antibody, which is consistent with the characterization result in Table 1. The OBN peak indicates fragmentation of OBN-MORF1 into OBN and MORF1 during analysis (similarly as shown in ChemBioChem 20, 1599-1605, 2019).

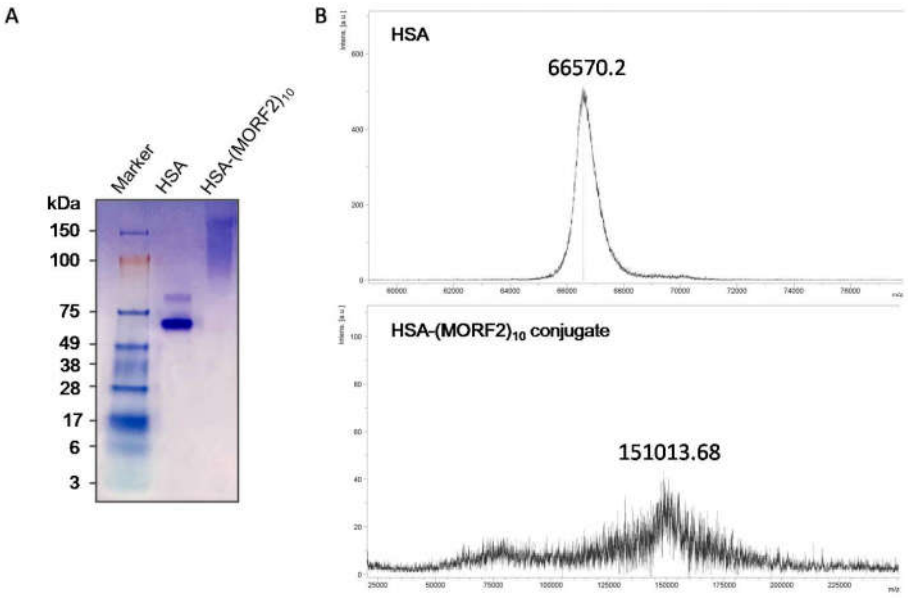


Figure S2. (A) SDS-PAGE gel electrophoresis analysis and (B) MALDI-TOF mass spectroscopy characterization of HSA and HSA-$(MORF2)_{10}$ conjugate. Results show that the molecular weight for HSA and HSA-$(MORF2)_{10}$ conjugate is approximately 67 kDa and 151 kDa, respectively. Since the molecular weight of MORF2 is 8.6 kDa, the MORF valence of HSA-$(MORF2)_{10}$ conjugate is calculated to be 9.8, which is consistent with the characterization in Table 1 (MORF2 valence=9.4) as determined by bicinchoninic acid protein assay for HSA concentration and UV–visspectrophotometry for MORF2 concentration.

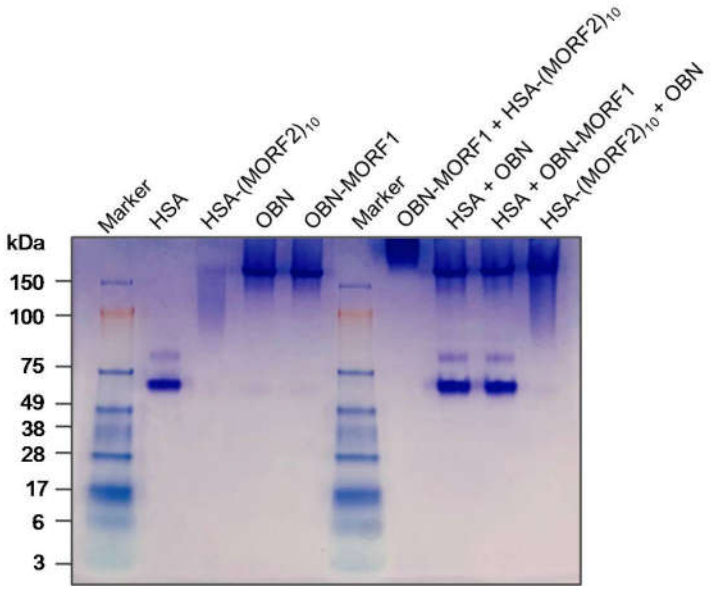
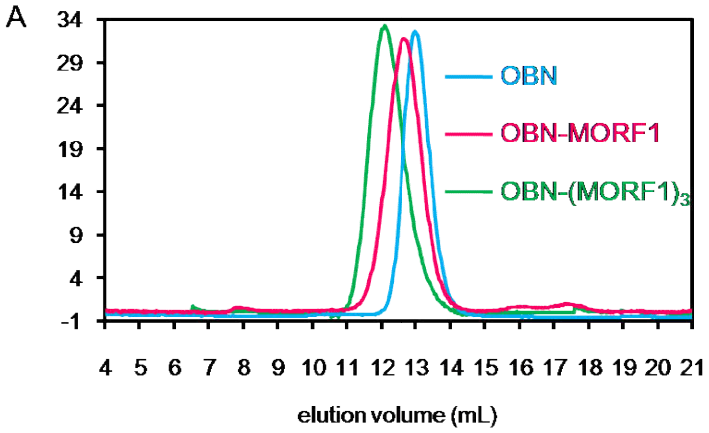


Figure S3. SDS-PAGE analysis of two conjugates, OBN-MORF1 and HSA-$(MORF2)_{10}$, and their hybridization. Samples (> 3 μg protein) were incubated with 1× Laemmli buffer without reducing reagents at 37 °C for 10 min prior to loading on 8-16% polyacrylamide gel. The mixtures of indicated two components were incubated at room temperature for 15 min. A significant change in the band position occurs between HSA and HSA-$(MORF2)_{10}$, because the MORF conjugation results in significant increase in molecular weight of HSA (from 67 kDa to ~ 150 kDa). However, the gel is insensitive to the change between OBN and OBN-MORF1, because there is only 5 % increase in molecular weight after MORF conjugation to OBN. When OBN-MORF1 and HSA-$(MORF2)_{10}$ are mixed, both bands of original conjugates disappear from the gel and a larger product is generated, indicating an efficient hybridization between these two conjugates. This is in contrast to the mixtures of HSA+OBN, HSA+OBN-MORF1 and HSA-$(MORF2)_{10}$ +OBN, which have obviously two separated bands of original conjugates, indicating the hybridization between OBN-MORF1 and HSA-$(MORF2)_{10}$ is mediated by the specific biorecognition between MORF1 and MORF2.

A



B

Antibody-MORF1 conjugate	MORF1 valence
OBN	0
OBN-MORF1	1.21
OBN-(MORF1)$_3$	3.39

C

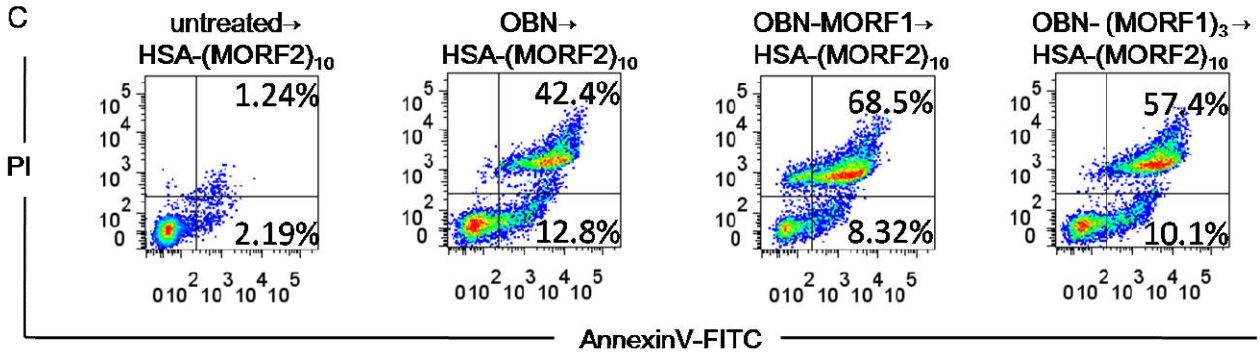


Figure S4. (A) Size-exclusion chromatography characterizations of OBN, OBN-MORF1 and OBN-(MORF1)$_3$ conjugates as determined on Superdex 200 10/300 GL column eluted with PBS (pH 7.2) at flow rate 0.4 mL/min and UV wavelength 280 nm. OBN-(MORF1)$_3$ conjugate was synthesized *via* the thiol-ene reaction between thiolated OBN and maleimide functionalized MORF1 with the feed molar ratio [MORF1] :[OBN] of 4.5:1. (B) MORF1 valence characterization of OBN, OBN-MORF1 and OBN-(MORF1)$_3$ conjugates as determined by bicinchoninic acid protein assay (for OBN concentration) and UV–vis spectrophotometry (for MORF concentration). (C) Apoptosis induction after Raji cells were consecutively exposed to OBN, OBN-MORF1 or OBN-(MORF1)$_3$ with 0.5 μM OBN equivalence for 1 h, and then HSA-(MORF2)$_{10}$ with 0.5 μM MORF equivalence for another 24 h.

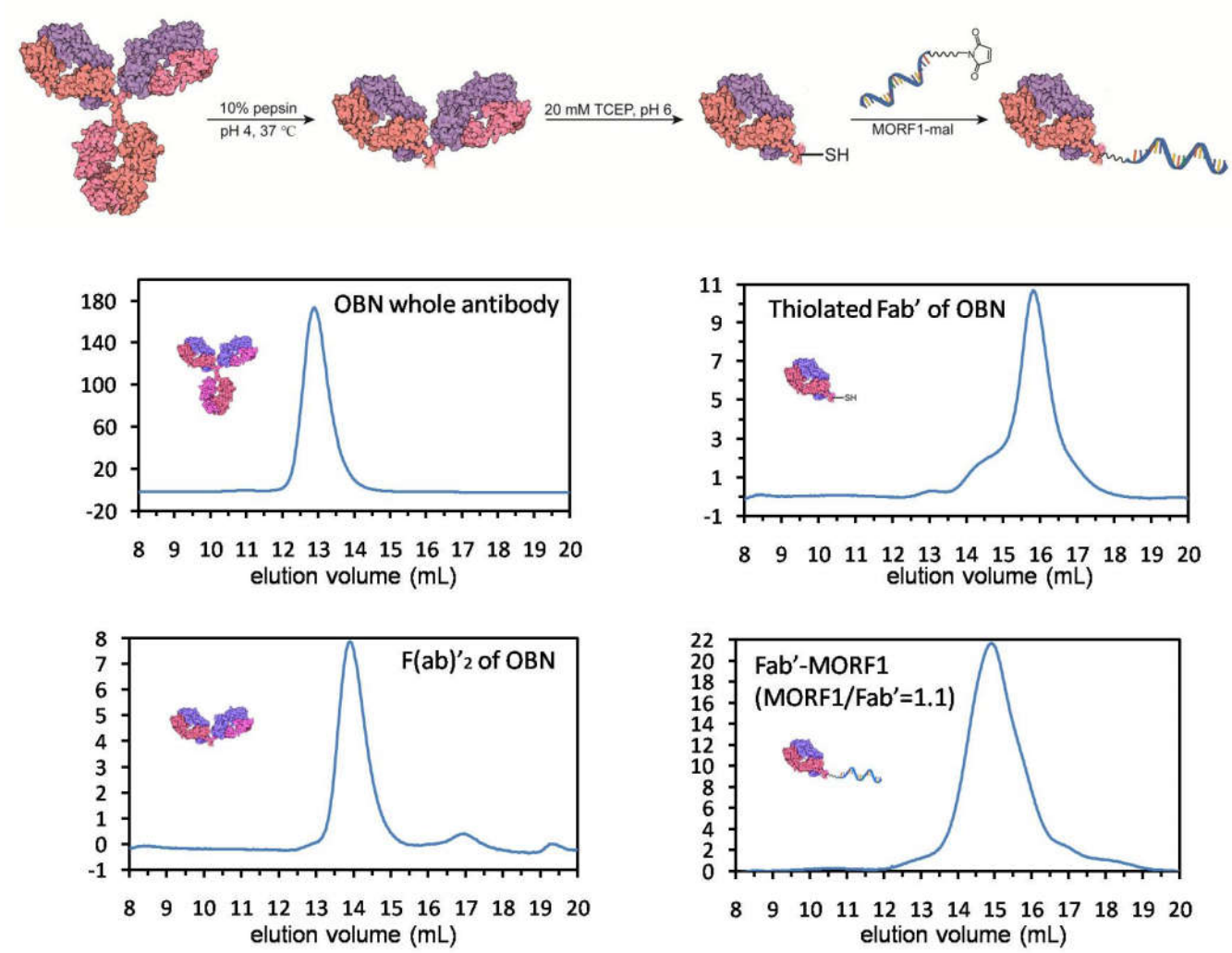


Figure S5. Size-exclusion chromatography characterization of OBN antibody, digested F(ab′)$_2$, reduced Fab′, and the final conjugate Fab′-MORF1 as determined on Superdex 200 10/300 GL column eluted with PBS (pH 7.2) at flow rate 0.4 mL/min and UV wavelength 280 nm.

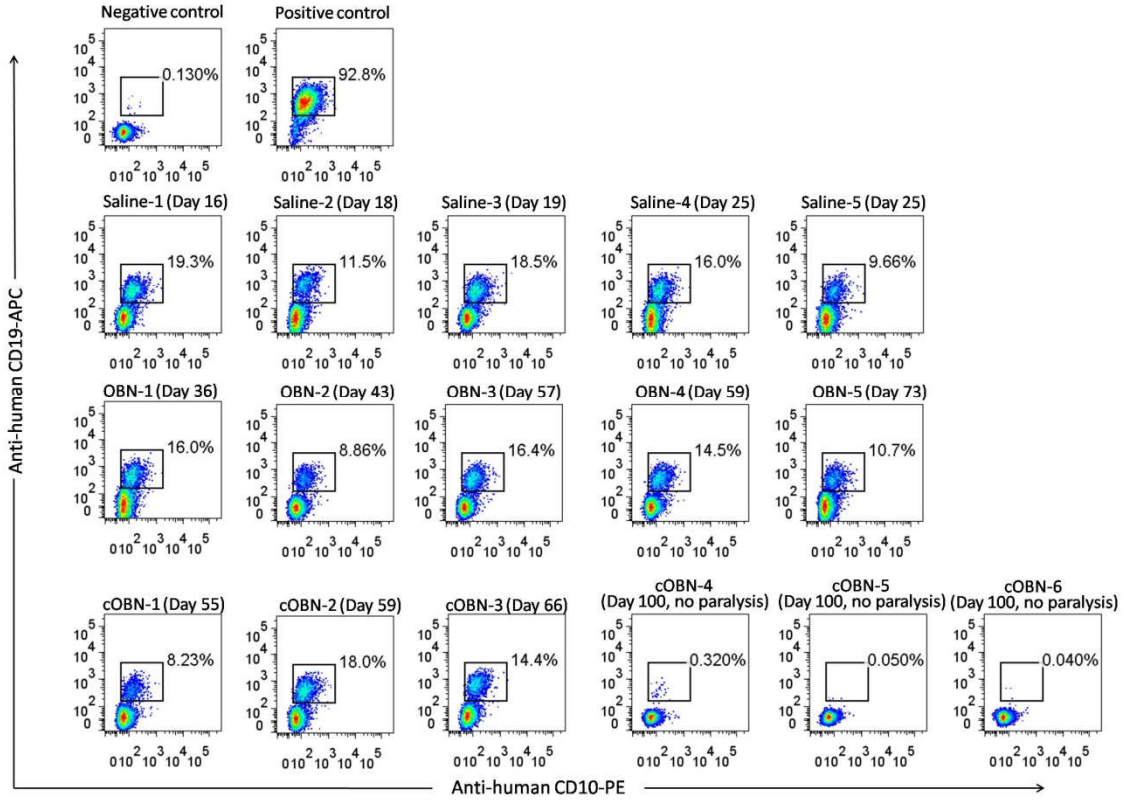


Figure S6. Flow cytometry analysis of residual Raji cells (human CD10+CD19+) in the bone marrow (BM). BM cells were isolated from the femur of mice at endpoint (onset of paralysis or at 100 days), and Raji cells were dual stained with PE-labeled mouse anti-human CD10 and APC-labeled mouse anti-human CD19 antibodies. BM cells isolated from native NRG mouse served as the negative control, and Raji cells served as the positive control.