Electric Supplementary Information for

Enhanced β2-microglobulin binding of a "navigator" molecule bearing a single-chain variable fragment antibody for artificial switching of metabolic processing pathways

Yusuke Kambe,[a] Ken Kuwahara,[a,b] Mitsuru Sato,[c] Takahiko Nakaoki [b] and Tetsuji Yamaoka*[a]

[a] Department of Biomedical Engineering, National Cerebral and Cardiovascular Center (NCVC) Research Institute, 6-1 Kishibe-Shimmachi, Suita, Osaka 564-8565, Japan
[b] Department of Materials Chemistry, Ryukoku University, Seta, Otsu 520-2194, Japan
[c] Animal Bioregulation Unit, Division of Animal Sciences, Institute of Agrobiological Sciences, National Agriculture and Food Research Organization (NARO), 1-2 Owashi, Tsukuba, Ibaraki, 305-8634, Japan

*Correspondence to:
Tetsuji Yamaoka
Department of Biomedical Engineering, NCVC Research Institute, 6-1 Kishibe-Shimmachi, Suita, Osaka 564-8565, Japan
Tel: +81-6-6170-1070 (ext. 31009); Fax: +81-6-6170-1702; E-mail: yamtet@ncvc.go.jp

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Table S1. Primer sequences for the construction of the plasmid pCAG[V_H–linker–V_L].

Primer name	Sequence (5′–3′)
VH-IgG1-5	AAGCAGTGGTATCAACGCAGAGTACGCG
VH-IgG1-3	CCCTGGGCACAATTTTCTTGTCCACC
VL-kappa-5	AAGCAGTGGTATCAACGCAGAGTACGCG
VL-kappa-3	CCTGTTGAAGCTCTTGACAATGGGTG
#1	CGAATGCGGCCGCGCCACCATGGACGTTCAGCTGCAGCAG TC
#2	CAGAACCACCACCCCCGGCTGAGGAGACGGTGACTGAGG
#3	GGTGGAGGAGGTTCTGACATTCAGATGACCCAGTC
#4	CTAGTCTAGACCGTTTGATTTCCAGCTTGGTGCC
#5	TCTGAATGTCAGAACCTCCTCCACCTGATCCTCCACCTCCA GAACCACCACCCCC
#6	CTCCTCAGCCGGGGGTGGTGGTTCTGGAGGTGGAGGATCA GGTGGAGGAGGTTCT

NotI (GCGGCCGC) and XbaI (TCTAGA) recognition sites are underlined; sequences matching the cDNA of V_H or V_L are shown in red.

Table S2. Primer sequences for the construction of the plasmid pET[ApoE NTD–$(GGGGS)_3$–scFv].

Primer name	Sequence (5′–3′)
GGGGSx1-26VH-5	GGGATCCGGTGGAGGTGGTAGCGAGGTTCAGCTGC
26VL-3	GAAGC*TTATTA*CCGTTTGATTTCC

BamHI (GGATCC) and HindIII (AAGCTT) recognition sites are underlined; sequences matching the cDNA of scFv are in red; stop codon is italicized.

Table S3. Amino acid sequences of the navigator proteins.

Navigator protein name	Sequence (N–C)
ApoE NTD–scFv	MHHHHHHSSGLVPRGSGMKETAAAKFERQHMDSPDLGTD DDDKAMEGEPEVTDQLEWQSNQPWEQALNRFWDYLRWV QTLSDQVQEELQSSQVTQELTALMEDTMTEVKAYKKELEE QLGPVAEETRARLGKEVQAAQARLGADMEDLRNRLGQYR NEVHTMLGQSTEEIRARLSTHLRKMRKRLMRDAEDLQKRL AVYKAGAREGAERGVSAIRERLGPLVEQGRQRGGGGSGG GGSGGGGS**EVQLQQSGAELVKPGASVKLSCTAS<u>AFNIKN TY</u>IHWVKQRPEQGLEWIGR<u>IDFANGNT</u>KYDPKFQGKAT LTADTSSNTAYLQLSSLTSEDTAVYYC<u>ARAAYYKYDGA MDY</u>WGQGTSVTVSSA**GGGGSGGGGSGGGGS*DIQMTQSPA SQSASLGESVTITCLAS<u>QTIGTW</u>LAWYQQKPGKSPQLLIY<u>AAT</u>SL ADGVPSRFSGSGSGTKFSFKISSLQAEDFVSYYC<u>QQLYSSPWT</u>F GGGTKLEIKR*
ApoE NTD–MHC α3	MHHHHHHSSGLVPRGSGMKETAAAKFERQHMDSPDLGTD DDDKAMEGEPEVTDQLEWQSNQPWEQALNRFWDYLRWV QTLSDQVQEELQSSQVTQELTALMEDTMTEVKAYKKELEE QLGPVAEETRARLGKEVQAAQARLGADMEDLRNRLGQYR NEVHTMLGQSTEEIRARLSTHLRKMRKRLMRDAEDLQKRL AVYKAGAREGAERGVSAIRERLGPLVEQGRQRGGGGSGG GGSGGGGSRTDSPKAHVTHHPRSKGEVTLRCWALGFYPAD ITLTWQLNGEELTQKMELVETRPAGDGTFQKWASVVVPLG KEQNYTCRVYHEGLPEPLTLRW

Sequences of ApoE NTD are denoted in blue; sequences of scFv and MHC α3 are denoted in red; sequences of V_H and V_L are in bold and italicized, respectively; sequences of complementary determining regions in scFv are underlined.

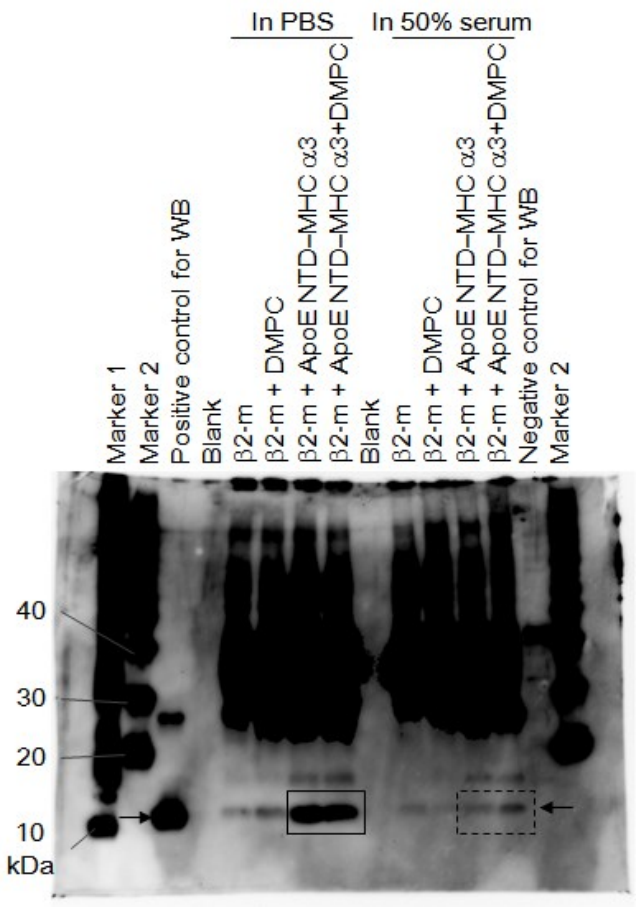

Fig. S1. IP assay for characterizing the ApoE NTD–MHC α3 binding to β2-m in PBS and 50% (v/v) serum. ApoE NTD–MHC α3 or ApoE NTD–MHC α3+DMPC (ApoE NTD–MHC α3: 8 μM, DMPC: 0 or 0.04 mg/mL) and human β2-m (5 μM) were mixed in PBS or PBS containing 50% (v/v) murine serum and incubated at RT o/n. Magnetic beads conjugated with mouse anti-MHC class I H2-D^b MAb (ab25228; Abcam, UK) were added to the solution of ApoE NTD–MHC α3 and β2-m. Then, an IP assay was performed as recommended by the manufacturer (Tamagawa Seiki). The resultant immunocomplexes were separated by SDS-PAGE under reducing conditions and were transferred to a polyvinylidene fluoride membrane. Then, the membrane was blocked with EzBlock Chem and was probed with the rabbit anti-β2-m PAb, followed by incubation with the HRP-conjugated goat anti-rabbit IgG PAb. Immunoactive proteins were detected using a Chemi-Lumi one super kit (Nacalai Tesque) and a chemiluminescence imaging system. As the positive and negative controls for WB, β2-m and ApoE NTD–MHC α3 were run on the gel, respectively. Novex® sharp protein standard and MagicMark™ XP Western protein standard (Invitrogen) were used as markers 1 and 2, respectively. Arrows indicate the positions of bands derived from β2-m.

Compared to PBS (solid box), the band derived from β2-m captured by ApoE NTD–MHC α3 or ApoE NTD–MHC α3+DMPC in 50% (v/v) serum was thinner and brighter (dashed box), indicating that the ApoE NTD–MHC α3 binding to β2-m was reduced by serum components.

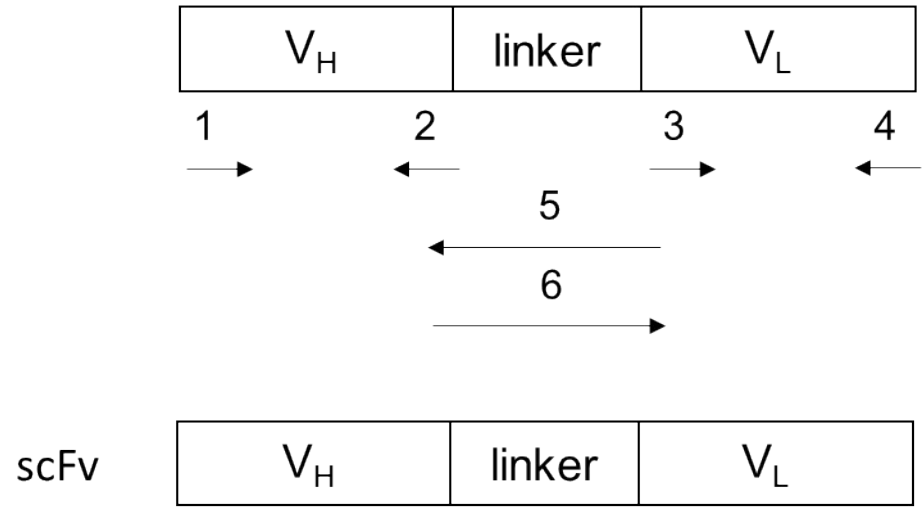

Fig. S2. Construction of cDNA encoding anti-β2-m scFv by cloning the V_H and V_L from hybridoma cells that produce anti-β2-m MAb. The arrows represent the primers used to amplify the antibody fragments.

A four-step PCR was performed for generating appropriate cDNA fragments encoding the V_H and V_L regions. Primer sequences (Sigma-Aldrich, MO) are listed in Table S1. Total RNA from hybridoma cells was reverse-transcribed using a SMART^{TM} RACE cDNA amplification kit (Clontech, CA). The cDNA fragments for the V_H and V_L regions were generated by PCR using isotype-specific primers (V_H, sense primer VH-IgG1-5 and reverse primer VH-IgG1-3; V_L, sense primer VL-kappa-5 and reverse primer VL-kappa-3). The second PCR amplification was performed using the following primer sets: V_H, sense primer #1 and reverse primer #2; V_L, sense primer #3 and reverse primer #4. The third PCR products were amplified using the following primer sets: V_H-linker, sense primer #1 and reverse primer #5; linker-V_L, sense primer #6 and reverse primer #4. The third PCR products, V_H-linker and linker-V_L, were mixed and then the fourth PCR amplification was performed with the following primer sets: sense primer #1 and reverse primer #4. The fourth PCR product was treated with NotI and XbaI and cloned into the pCAGGS-MCS vector, which was designated as pCAG[V_H–linker–V_L].

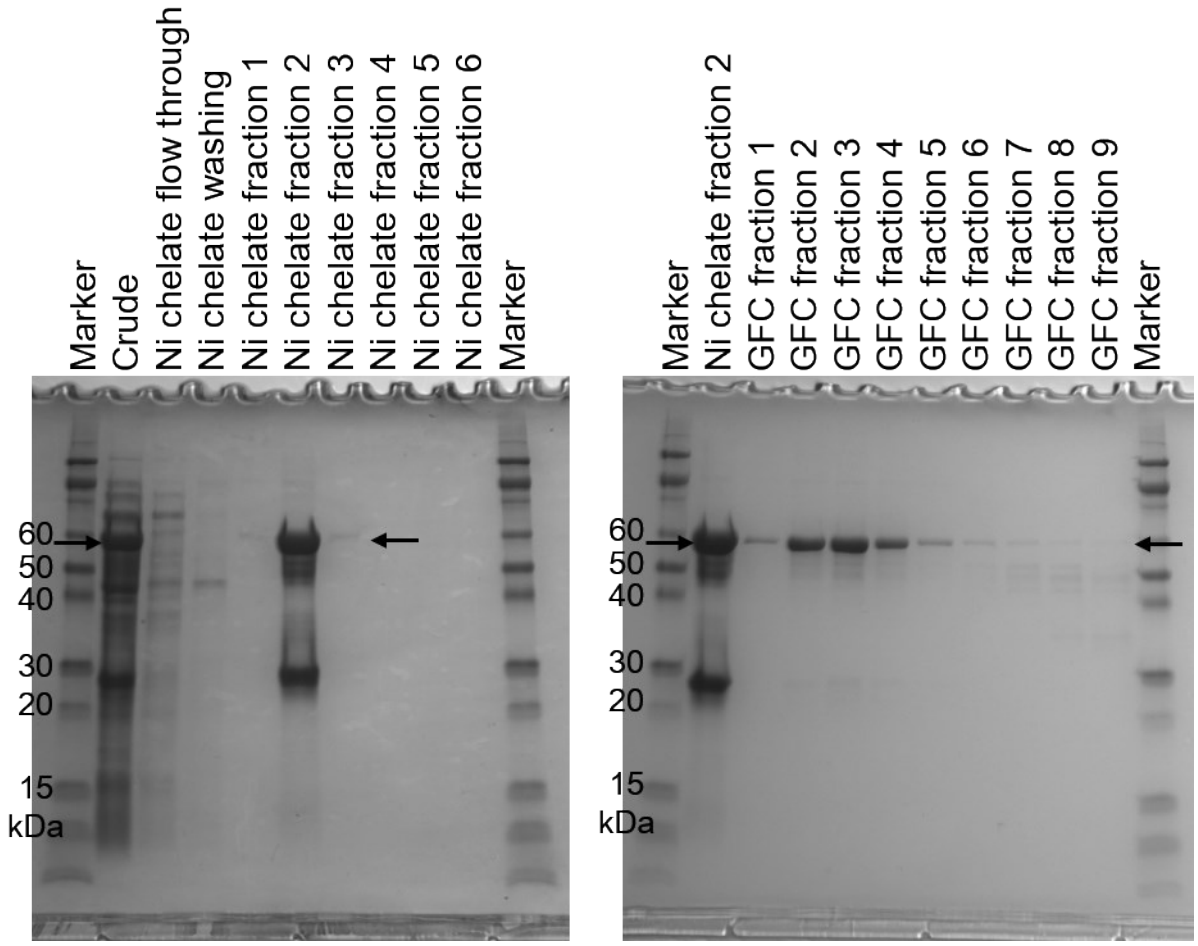

Fig. S3. Purification of ApoE NTD–scFv. *E. coli* cells transformed with pET[ApoE NTD–$(GGGGS)_3$–scFv] were resuspended and lysed in 100 mM NaH_2PO_4, 10 mM Tris, 8 M urea, and 5 mM imidazole (pH 8.0). After centrifugation and filtration, ApoE NTD–scFv with an N-terminal His tag was first purified on the Ni-chelate column. The fusion protein was further purified on the gel filtration column (GFC). Proteins in solutions obtained in the purification process were analyzed by SDS-PAGE on Mini-PROTEAN® TGX™ gels under reducing conditions. Separated proteins were fixed and visualized using the EzStain aqua solution (Atto, Japan). Novex® sharp protein standard was used as the marker. Arrows indicate the position of bands derived from ApoE NTD–scFv. The intensity of protein bands was determined using the ImageJ software, which revealed that the purity of ApoE NTD–scFv was 90%.

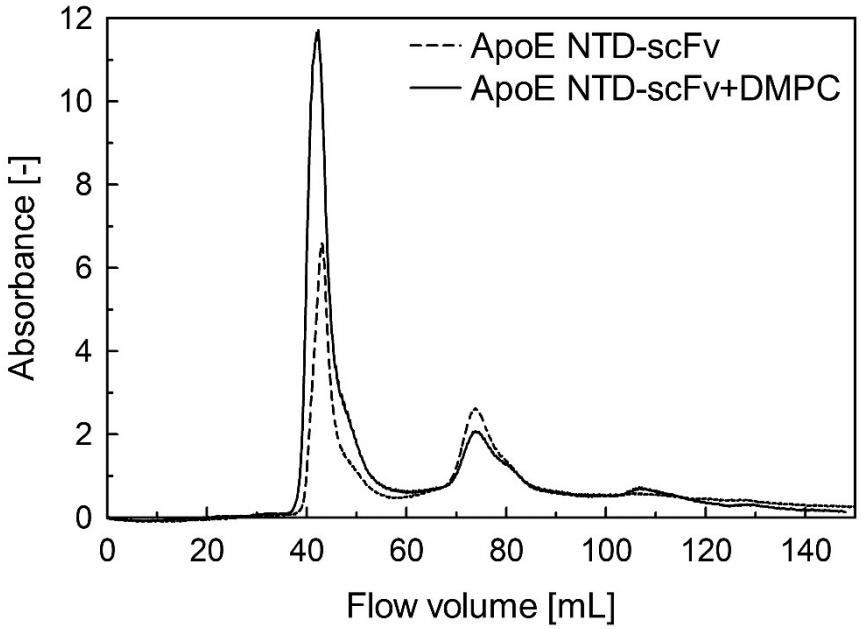

Fig. S4. Gel filtration chromatographs of ApoE NTD–scFv and ApoE NTD–scFv+DMPC. ApoE NTD–scFv (37.5 μM) or ApoE NTD–scFv+DMPC (ApoE NTD–scFv: 37.5 μM; DMPC: 0.8 mg/mL) was loaded onto a GFC (HiLoad 16/600 Superdex 200 pg; GE Healthcare) at a volume of 600 μL and run at a flow speed of 1 mL/min, while absorbance at 280 nm was detected.

Both ApoE NTD–scFv and ApoE NTD–scFv+DMPC showed two peaks. The peaks at 42 mL and 74 mL in the ApoE NTD–scFv+DMPC group were higher and lower, respectively, than those in the ApoE NTD–scFv group. These results suggested that the apparent MW of ApoE NTD–scFv+DMPC was larger than that of ApoE NTD–scFv due to the formation of the complex of ApoE NTD–scFv and DMPC.

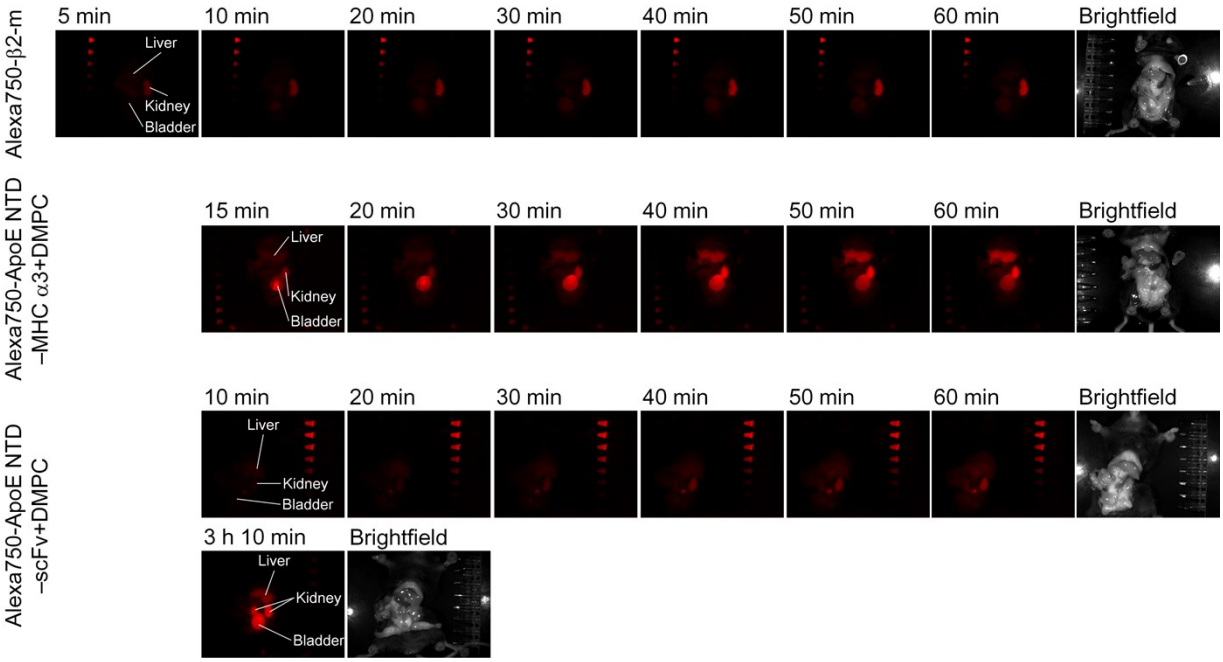

Fig. S5. Time-dependent changes in the body distribution of Alexa750-β2-m, Alexa750-ApoE NTD–MHC α3+DMPC, and Alexa750-ApoE NTD–scFv+DMPC after intravenous injection. Alexa750-β2-m (12.5 μM), Alexa750-ApoE NTD–MHC α3+DMPC (ApoE NTD–MHC α3: 12.5 μM; DMPC: 0.8 mg/mL), or Alexa750-ApoE NTD–scFv+DMPC (ApoE NTD–scFv: 12.5 μM; DMPC: 0.8 mg/mL) were injected into anesthetized mice (C57Bl/6N; male; 9–16 weeks old; 24.7–28.1 g) intravenously (for the labeled protein concentration of approximately 1 μM in the blood). After injection, the mice were laparotomized and observed until 60 min, except for a mouse with the Alexa750-ApoE NTD–scFv+DMPC injection (3 h and 10 min), using the Maestro *in vivo* imaging system (Cambridge Research & Instrumentation, MA).

The accumulation of Alexa750-β2-m in the kidney was observed at 5 min post-injection. In contrast, that of Alexa750-ApoE NTD–MHC α3+DMPC in the liver was obvious at 40 min. Alexa750-ApoE NTD–scFv+DMPC accumulated in the liver by 3 h and 10 min post-injection.