

Supplemental Figures and Tables

Figure S1 AA sequences for #7TR and H34 light chains

- a) #7TR human catalytic antibody light chain
- b) H34 human catalytic antibody light chain

Figure S2 Chemical structure of FRET adducted peptide substrates

For each peptide, MCA (4-methyl-coumaryl-7-amide; Fluorescent reagent) was adducted at N-terminal and DNP (2,4-dinitrophenyl; Quenching reagent). Lysine was inserted in order to bind with DNP.

- a) FRET-A β substrate; FRET was adducted for the peptide from 26 to 33 of A β .
- b) FRET-PD1 substrate; FRET was adducted for the peptide from 123 to 140 of PD-1 (this region is the epitope of anti PD-1 mAb, Nivolumab).

Figure S3 Procedure, chromatography and SDS-PAGE analysis in 1st purification step (Ni-NTA column chromatography)

Amino acid sequences of #7TR and H34 human light chains

- a) **#7TR**: Subgroup II of human kappa light chain (Kabat's classification)
MDVVM TQSP LSLP VTPG EPAS ISC RSSQSLHSNTRNYLD WYLQKPGQSPQLLIY LGSN RAS
GVPDR FSGSGSGTDFTLKISRVEAEDVGVYYC MQALQTPRT FGQG TKVEIKRTVAAPSVFIFP
PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKLYACEVTHQGLSSPVTKSFNRGEC(LEHHHHHHH)-----MW=25170.8
- b) **H34**: Subgroup I of human kappa light chain (Kabat's classification)
MDIQMTQSPSTLSASVGDRVITTC RASQSISSWLA WYQQKPGKAPKVLIIY KASTLES GVPLR
FSGSGSGTEFTLTISSLQPDDFATYYC QQYSTYRT FGQG TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH
KVYACEVTHQGLSSPVTKSFNRGEC(LEHHHHHHH)-----MW=24575.1

Red: CDR-1

Blue: CDR-2

Green: CDR-3

Methionine at 0th position is adducted because of insertion of a restriction enzyme site (*NcoI*).

(LEHHHHHHH) is adducted because of insertion of His-tag.

Figure S1a and S1b

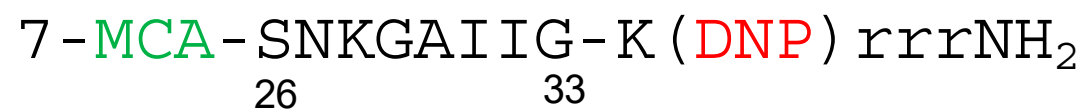
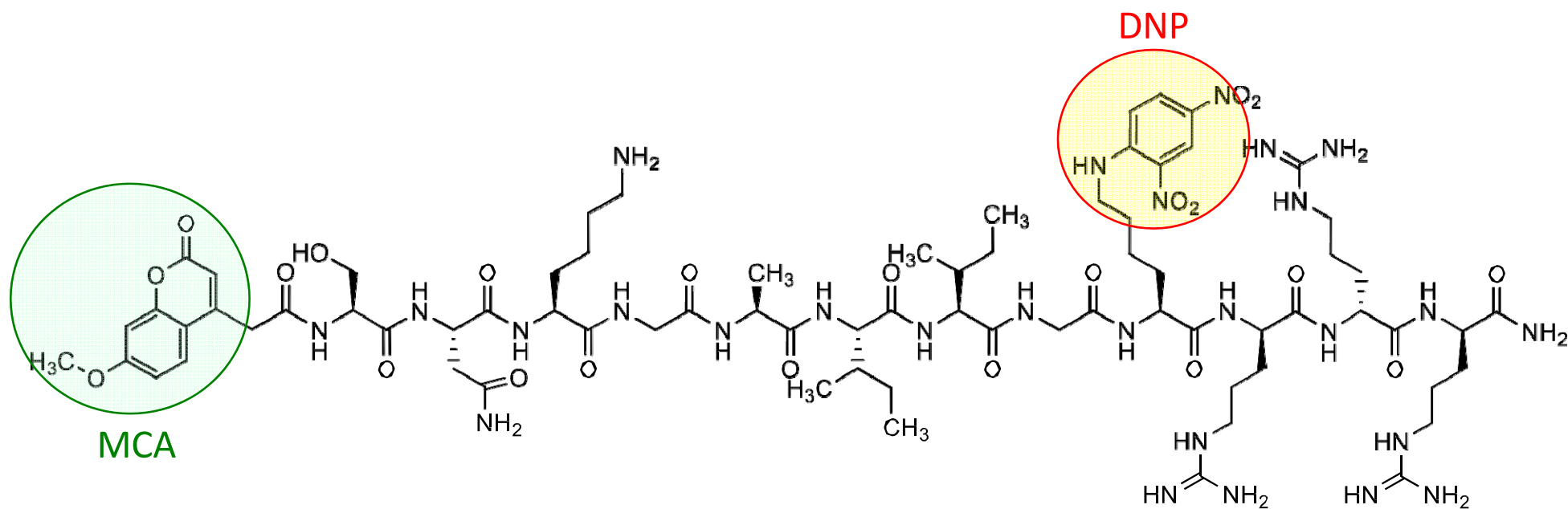
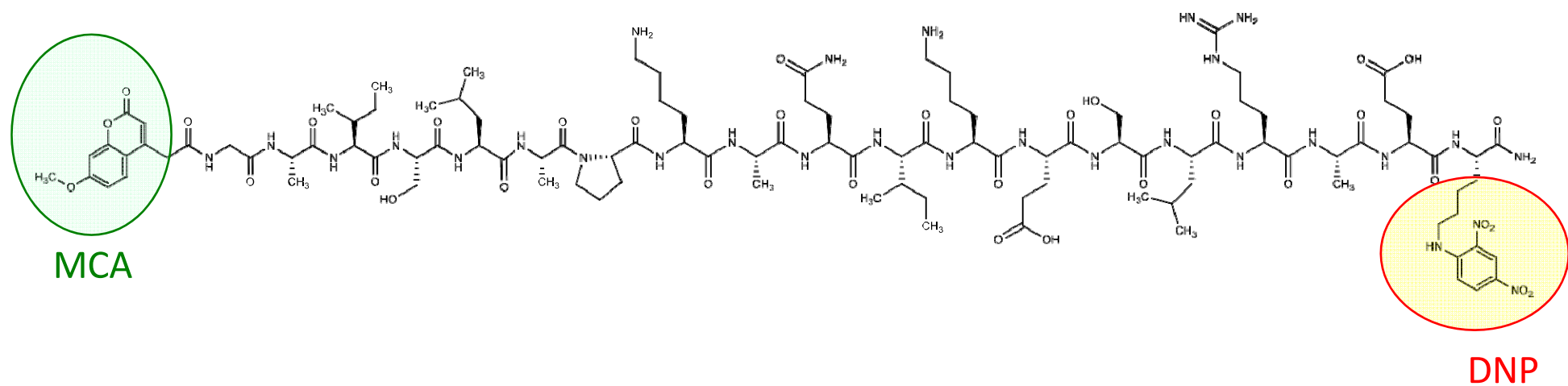


Figure S2a



7-MCA-GAISLAPKAQIKESLRAE-K (DNP) -NH₂

Figure S2b

First purification step (Ni-NTA Column chromatography)

【Procedure】

Ni-NTA Column chromatography

Column; Ni-NTA agarose gel(QIAGEN)

Buffer; 250 mM NaCl, 25 mM Tris-HCl,pH8.0

Imidazole conc.;30-300 mM

1.25 eq CuCl₂ addition.
Incubation overnight

Concentration

50 mM EDTA addition.
Incubation 1 h

Dialysis for PBS

Filtration (0.2μM). Stored at 4°C

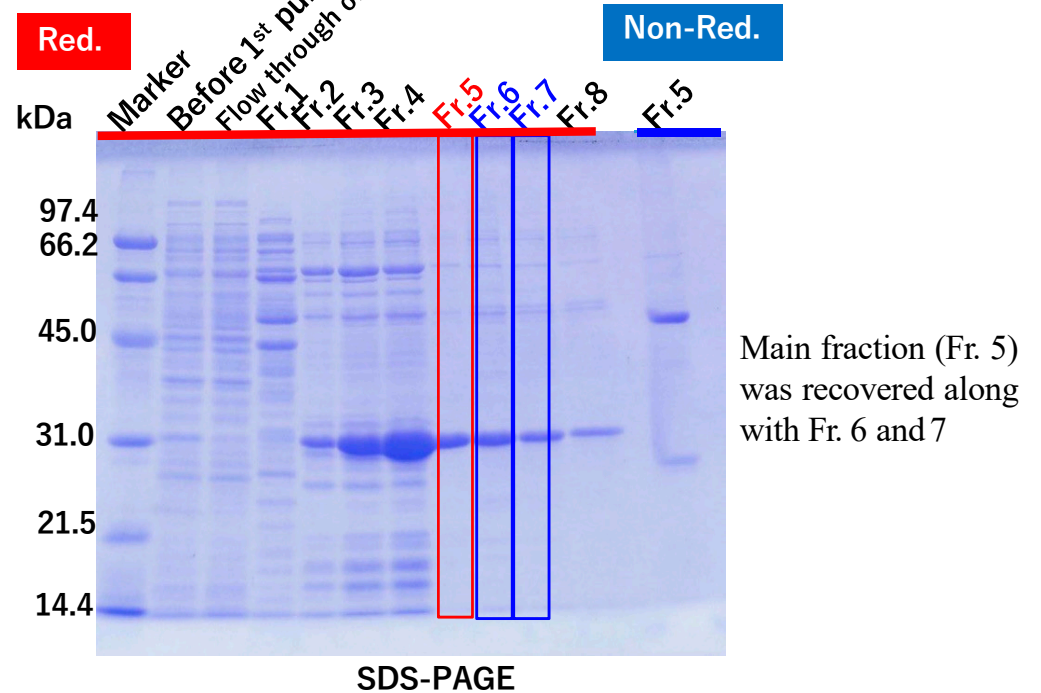
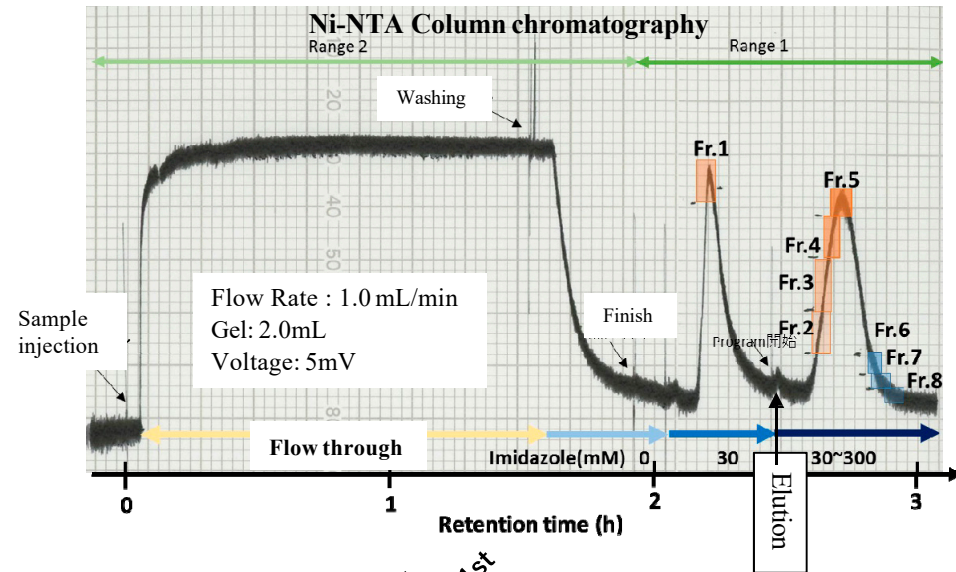


Figure S3

Fr.5: Main fraction (applied with 1/10 dilution)

Table S1. Summary for preparations for #7TR light chain

Route #	First purification Method	Second purification Method	pH	NaCl	Catalytic activity
"1"	Ni-NTA	-	8.0	250mM	moderate
"2"	Ni-NTA	cation-exchange	5.5	100-500mM	low
"3"	Ni-NTA	cation-exchange	8.0	0 mM	moderate
"4"	Ni-NTA	size-exclusion	5.5	137 mM	low
"5"	Ni-NTA	size-exclusion	7.4	137 mM	high
"6"	Ni-NTA	size-exclusion	8.0	137 mM	high
"7"	Ni-NTA	size-exclusion	5.5	0 mM	extremely low
"8"	Ni-NTA	size-exclusion	8.0	0 mM	low

Table S2. Kinetic values for H34 and other catalytic antibody light chains

Catalytic antibody		k _{cat} (min ⁻¹)	K _m (M)	k _{cat} /K _m (min ⁻¹ M ⁻¹)	substrate
#7TR (route “5” (pH7.4)		2.5 x 10 ⁻³	1.05x10 ⁻⁴	2.38 x 10	Arg-pNA
L12 light chain		1.6 x 10 ⁻³	5.3 x 10 ⁻⁵	3.0 × 10	Pro-Phe-Arg-MCA ¹⁶⁾
H34	Purification (I); Ni-NTA at pH8.0	5.5 × 10 ⁻²	3.2 × 10 ⁻⁶	1.7 × 10 ⁴	FRET-PD-1
	Purification (II); cation exchange at pH5.5	5.8 × 10 ⁻³	7.9 × 10 ⁻⁶	7.4 × 10 ²	FRET-PD-1
	Purification (III); size-exclusion at pH7.4	1.8 × 10 ⁻¹	3.1 × 10 ⁻⁶	5.9 × 10 ⁴	FRET-PD-1
aIgV		3.0 x 10 ⁻¹	8.0 x 10 ⁻⁵	3.8 × 10 ³	Aβ ³⁴⁾