Electronic Supplementary Material (ESI) for RSC Chemical Biology.

Supplementary Materials

Fig. S1 : Molecular structures

a) PD-1 molecule and the targeting peptide designing a FRET-PD-1 substrate (human type; 123-140)
b) The structure of FRET-PD-1 (mouse type; 123-140)
c) The structure of FRET-Amyloid beta (Aβ, human type; 26−33)
d) The structure of FRET-Tau (human type; 391−408)
e) Reaction profile for the cleavage of FRET-PD-1(mouse; 123-140) by H34

Fig. S2 : Mass spectroscopic analysis for the peaks detected in HPLC

The peak appearing at the retention time of 29.6 min was analyzed by mass spectroscopy.
a) Former peak; The divalent mass was detected at 619.81 *m/2z*, which was identified as a fragment of NH_2-I-K-E-S-L-R-A-E-K(DNP)-NH_2, from the FRET-PD1 substrate.
b) Latter peak; The divalent mass was detected at 586.28 *m/2z*, which was identified as a fragment of 7-MCA-G-A-I-S-L-A-P-K-A-Q-OH.

Fig. S3 :AA sequences for PD-1, rPD-1 and two fragments of PD-1

a) AA sequence of human PD-1
b) Recombinant of human PD-1 (by ENZO Ltd.; aa 25-167): Easily to be dimer or trimer
c) Large molecule of fragmented PD-1
d) Small molecule of fragmented PD-1

Fig. S4: Molecular modelling

a) **H34;** Asp^{1} in H34; ball & stick in pink, Arg^{96} in H34; ball & stick in light blue, CDR-3; wire in light blue

b) **H34-Pro^{95}(+);** Asp^{1} in H34-Pro^{95}(+); ball & stick in red, Arg^{96} in H34-Pro^{95}(+); ball & stick in blue, Pro^{95} in H34-Pro^{95}(+); ball & stick in cyan, CDR-3; wire in light blue

The residue Asp^{1} (ball & stick in pink) in H34 orients the structure more toward the inner side than in H34-Pro^{95} (+) mutant (ball & stick in red). The distance between the carboxyl oxygen of Asp^{1} and the guanidinium nitrogen of the Arg^{96} residue (ball & stick in light blue) in the CDR3 is shorter (9.65 Å) than in the mutant (13.54 Å) (ball & stick in blue) by a factor of ~4 Å.

c**) Location of Zn (II);** Arg^{24} in H34; ball & stick in light blue, Glu^{70} in H34; ball & stick in green, Asp^{1} and CDR-3 are the same colour as used in H34.

H34 has no histidine residues. Of the basic amino acids (Arg^{24} Arg ^{61}, Arg^{96} and Lys^{50}), Arg^{24} is located next to Glu^{70}, where the distance between Cα for Glu^{70} and for Arg^{24} is 5.286 Å and between Glu^{70} (O) - Arg^{24} (N)) is 2.88 Å. This may be the optimal position for Zn(II) to coordinate with the oxygen of Glu^{70} and the nitrogen of Arg^{24}, in analogy to the situation in carboxypeptidase.

d) Structure of carboxypeptidase (PDB ID: 1M4L)

Fig. S5

AA sequence for H34 antibody light chain (full size) and a zinc finger motif.

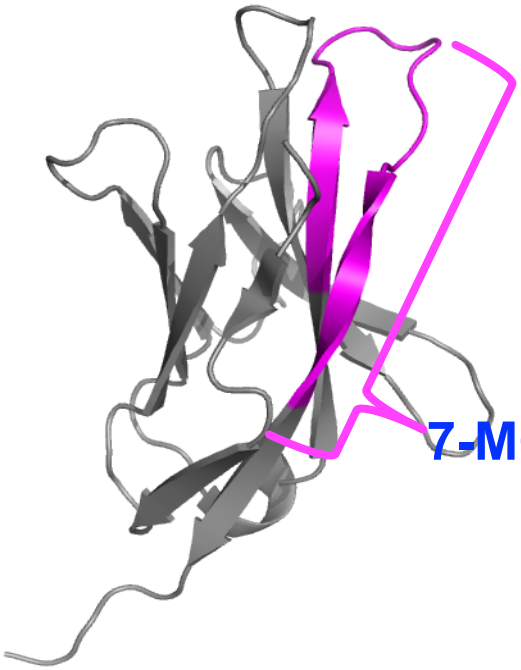
Fig. S6

Brief explanation for making a protein bank of human light chains

AA sequence and structure of human PD-1

(The sequence of 123-140 was selected for the targeting)

Structure of human PD-1
(PBD id: 2m2d)



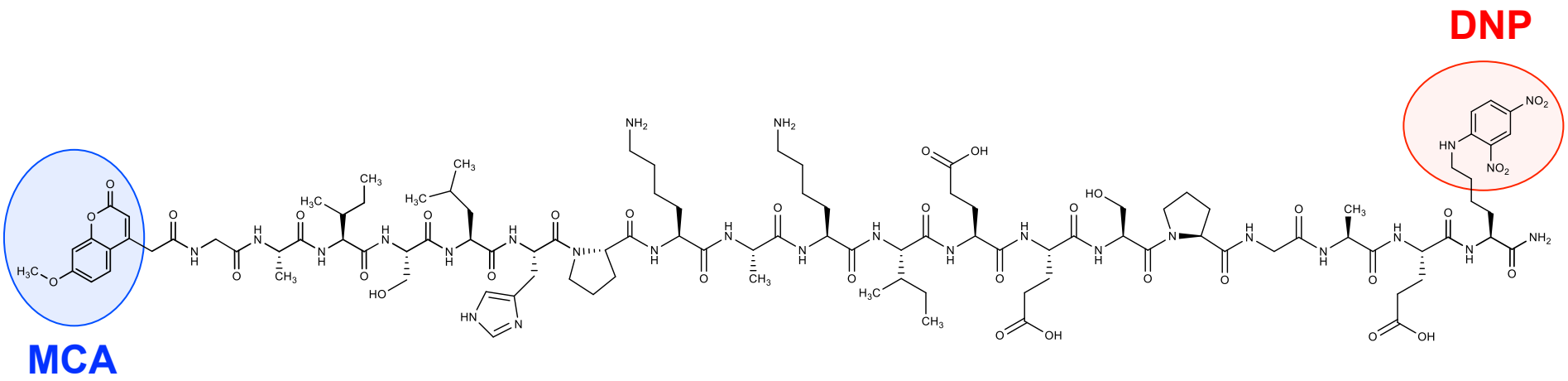
Amino acid sequence of human PD-1 (288mer)

MQIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPPTFSPALLVVTEGD
NATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFR
VTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRV
TERRAE VPTAHPSPSPRSAGQFQTLV VGVVGGLLGSLVLLVWVLAV
ICSRAARGTIGARRTGQPLKEDPSAVPVFSVDYGELDFQWREKTPEPP
VPCVPEQTEYAT IVFPSGMGTSSPARRGSADGPRSAQPLRPEDGHCS
WPL

7-MCA-GAISLAPKAQIKESLRAE-K(DNP)-NH_2
(123-140 ;18 mer)

Fig. S1a

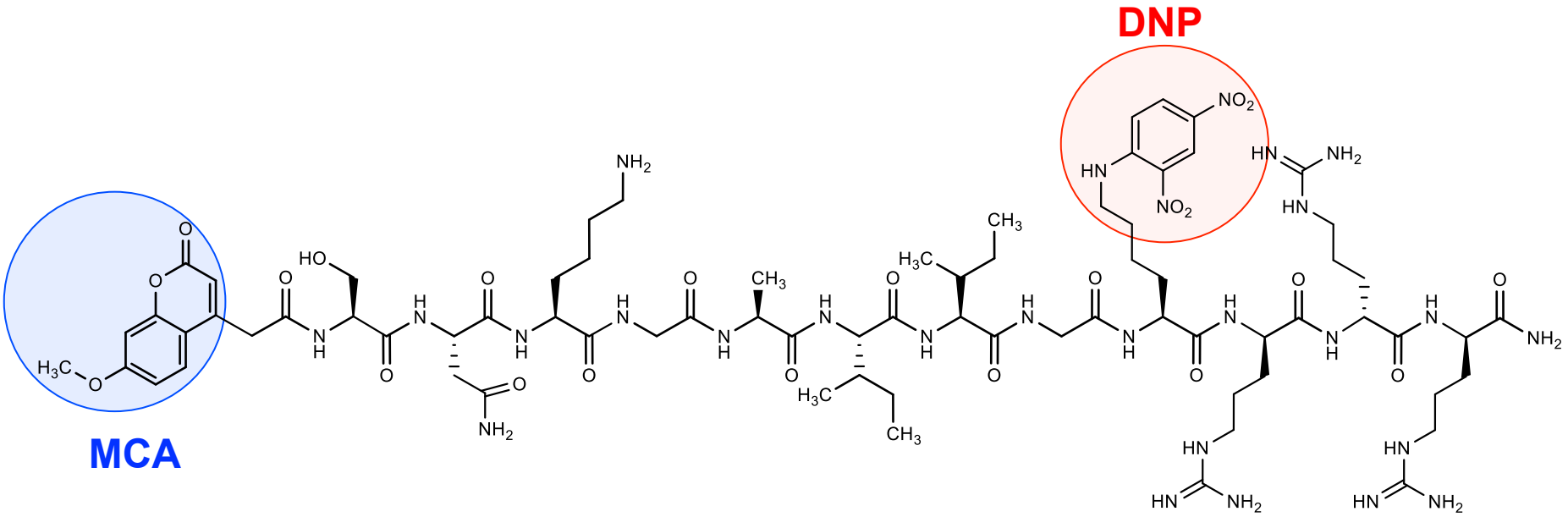
The structure of FRET-PD-1 (mouse type; 123-140)



$$\text{7-MCA-}\overset{123}{\text{G}}\text{AISLHPKAKIEESPGA}\overset{140}{\text{E}}\text{-K(DNP)-NH}_2$$

Fig. S1b

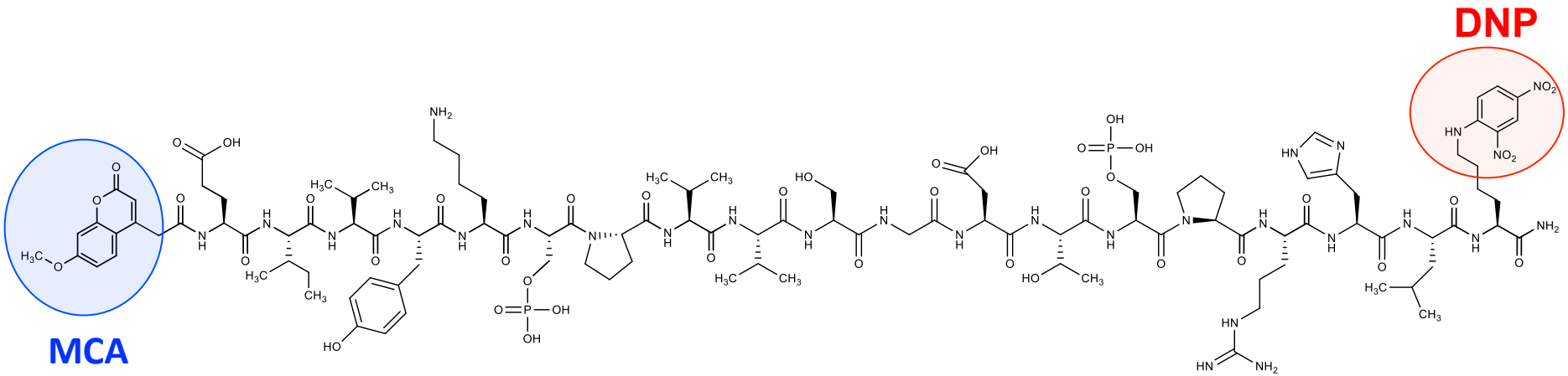
The structure of FRET-Amyloid beta (Aβ, human type; 26-33)



^{26} ^{33}
7-MCA-SNKGAIIG-K(DNP)-rrr-NH_2

Fig. S1c

The structure of FRET-Tau (human type; 391-408)



391 408

7-MCA-EIVYKpSPVVSGNVpSPRHL-K(DNP)

Fig. S1d

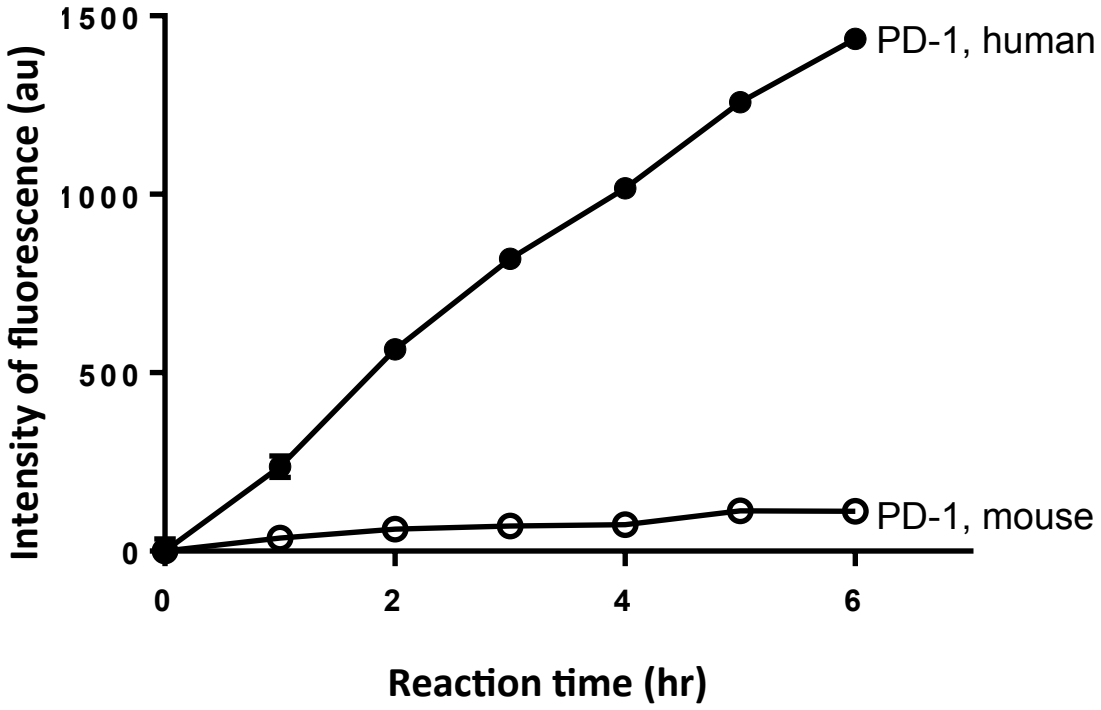

1500
1000
500
0
Intensity of fluorescence (au)
0
2
4
6
Reaction time (hr)
PD-1, human
PD-1, mouse

Fig. S1e

Mass spectroscopic analysis for the peaks detected in HPLC

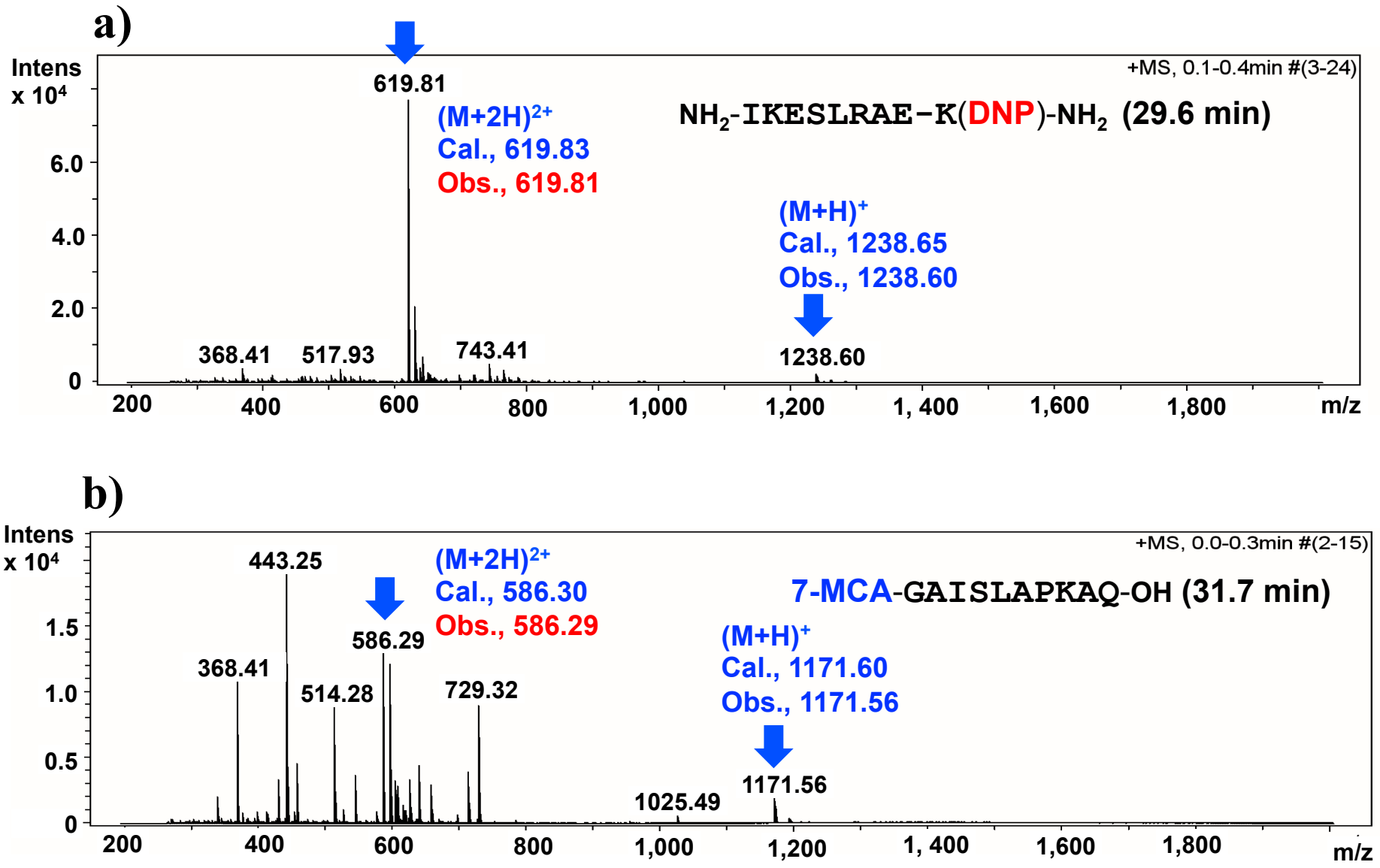


Fig. S2a & 2b

a) AA sequence of human PD-1 (full size):

MQIPQAPWPV VWAVLQLGWR PGWFLDSPDR PWNPPTFSPA LLVVTEGDNA TFTCSFSNTS
ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT
YLCGAISLAP KAQIKESLRA ELRVTERRAE VPTAHPSPSP RSAGQFQTLV VGVVGGLLGS
LVLLVWVLAV ICSRAARGTI GARRTGQPLK EDPSAVPVFS VDYGELDFQW REKTPEPPVP
CVPEQTEYAT IVFPSGMGTS SPARRGSADG PRSAQPLRPE DGHCSWPL

b) Recombinant of human PD-1 (by ENZO Ltd.; aa 25-167):

Easily to be dimerized or trimerized

MW; ~19.1kDa

His-tag V5 epitope-tag FLAG-tag

(HHHHHH-GKPIPNPLLGLDST-DYKDDDDK) -----DSPDR PWNPPTFSPA LLVVTEGDNA
TFTCSFSNTS ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV
VRARRNDSGT YLCGAISLAP KAQIKESLRA ELRVTERRAE VPTAHPSPSP RSAGQFQ

Cleaved bond

Fig. S3a and S3b

c) Fragmented PD-1 (detected as a dimer at~28kDa and a monomer at ~17kDa)

MW; ~15.3kDa

His-tag V5 epitope-tag FLAG-tag

(HHHHHH-GKPIPNPLLGLDST-DYKDDDDK) -----DSPDR PWNPPTFSPA LLVVTEGDNA TFTCSFSNTS ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT YLCGAISLAP KAQ

d) Small fragmented PD-1 (not detected) **MW; 3.8kDa**

IKESLRA ELRVTERRAE VPTAHPSPSP RSAGQFQ

Fig. S3c and 3d

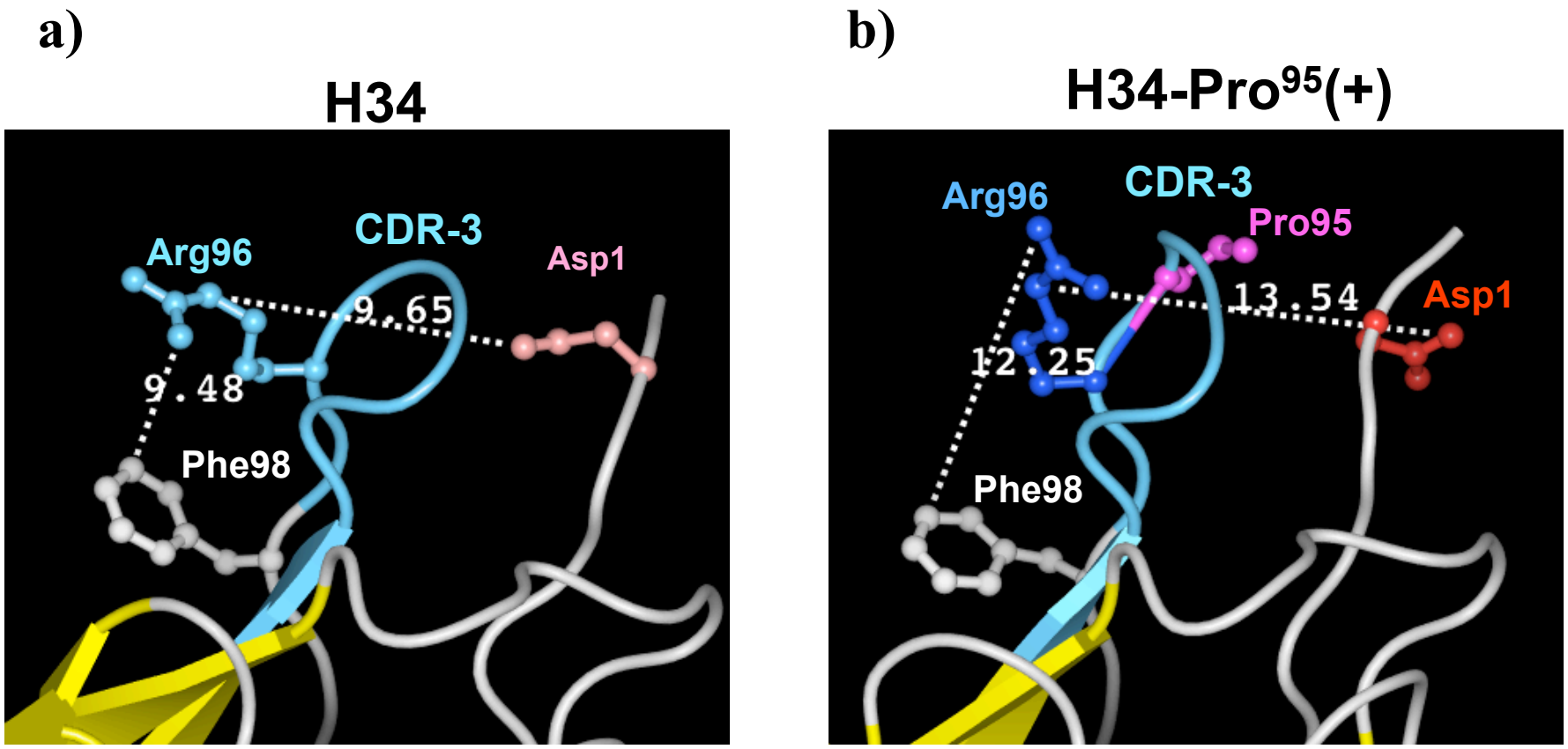

a)
H34
Arg96
CDR-3
Asp1
9.65
9.48
Phe98
b)
H34-Pro95(+)
Arg96
CDR-3
Pro95
13.54
Asp1
12.25
Phe98

Fig. S4a and b

c)

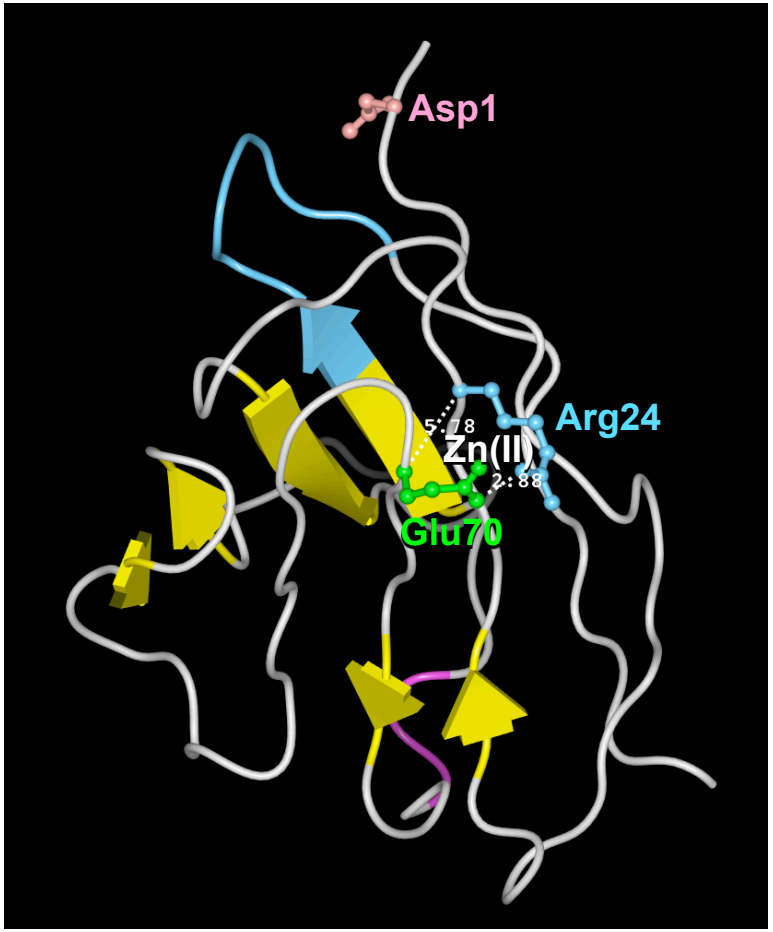


Fig. S4c

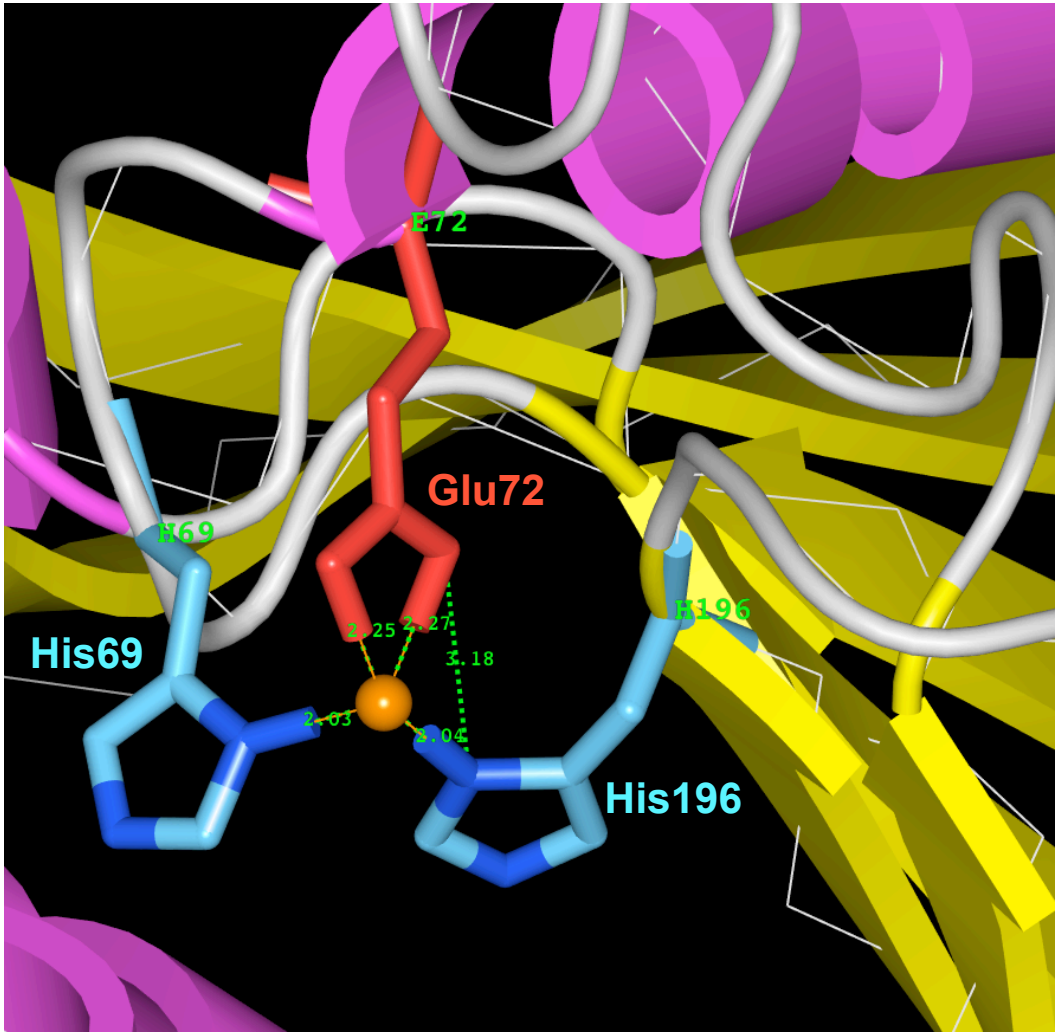


Fig. S4d

AA sequence for H34 antibody light chain (full size)

```
1           10          20          30          40          50          60          70
DIQMTQSPST LSASVGDRVT ITCRASQSIS SWLAWYQQKP GKAPKVLIYK ASTLESGVPL RFSGSGSGTE

71          80          90          100         110         120         130         140
FTLTISSLQP DDFATYYCQQ YSTYRTFGQG TKVEIKRTVA APSVFIFPPS DEQLKSGTAS VVCLLNNFYP

141         150         160         170         180         190         200         210
REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLTL SKADYEKHKV YACEVTHQGL SSPVTKSFNR

211
GEC(LEHHHHHH)
```

Double underline indicates the zinc finger motif.

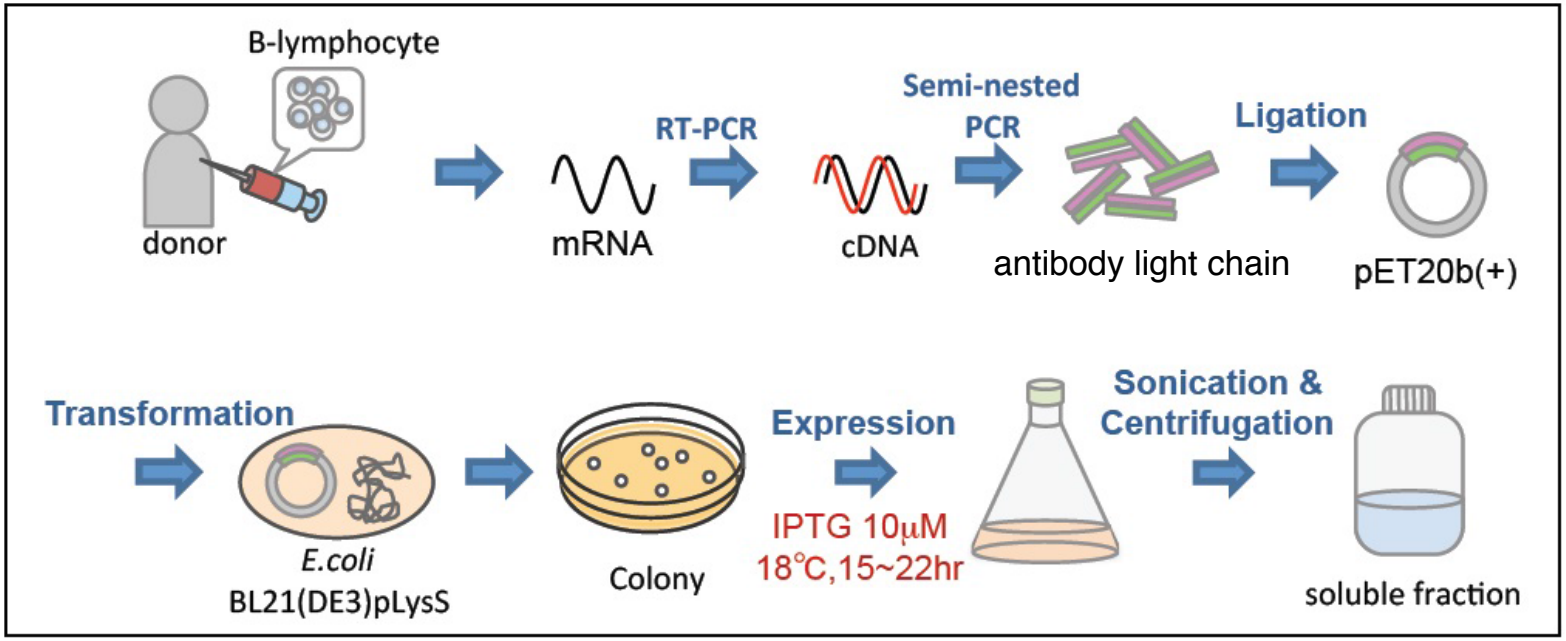
Red; CDR-1, **Blue; CDR-2**, **Green; CDR-3**

LE: aa sequence for *Xho* I

HHHHHH: aa sequence for His tag

In the expressed H34, Methionine residue exists at 0th position

Fig. S5



Regarding the generation of the protein bank, the detail explanation is described in several references (Hifumi *et al.*,Vol. 4, Chapter 2 (pp28-57), Frontiers in Clinical Drug Research-Anti Infectives, 2017 (Bentham Science Publishers)); Hifumi *et al.*, *FASEB J.*, **26**, 1607-1615(2012)). The above figure describes about the brief flow chart for obtaining the soluble fraction of *E.coli* expressing human light chain. Each gene of antibody light chain from donors was cloned in DH5α for sequencing. The DNA fragments were inserted in an expression vector pET20b(+). Then, each was transformed into each *E. coli (*BL21(DE3)pLysS). The cells were harvested and recovered by ultra-sonication. The soluble fraction was submitted to several kinds of purification columns such as Ni-NTA affinity column, cation exchange column, size exclusion column etc. After the purification, it was passed through 0.22um-filter for sterilize. After the determination of the protein concentration by a DC protein assay, it was stored at 4°C and/or frozen. Over hundreds of human antibody light chain have been stored for these ten years.

Fig. S6