Supporting information for

One-pot construction of Quenchbodies using antibody binding proteins

Hee-Jin Jeong[a†], Tomoki Kojima[b], Jinhua Dong[a], Hiroyuki Ohashi[b,c] and Hiroshi Ueda [a]

[a.] Laboratory for Chemistry and Life Science, Institute for Innovative Research, Tokyo Institute of Technology, Japan.

e-mail: ueda@res.titech.ac.jp.

[b.] Department of Chemistry and Biotechnology, School of Engineering, The University of Tokyo, Japan.

[c.] Ushio Inc., Japan

[†] Current address: Dana-Farber Cancer Institute, USA.

SI EXPERIMENTAL PROCEDURES

Construction of murine IgG_1 anti-BGP Fab fragment

Since the pUQ1H vector for expressing anti-BGP Fab uses human antibody sequences for the constant region, we changed the C_{H1} and C_k domains to those of mouse antibody, and prepared mouse anti-BGP Fab. Mouse C_{H1} gene was amplified using primers mIgG1CH1XhoBack and mIgG1CH1_EagFor, pROX H chain[1] as a template, and KOD-plus DNA polymerase. Similarly, mouse Vk gene was amplified using primers Histagback and VkHind+D, and pUQ1H as a template. Mouse Ck gene was amplified using primers mCkBack and mCkBamfor, and pROX L chain[1] as a template. These three amplified fragments were connected by SOE PCR using mIgG1CH1XhoBack and mCKBamfor as primers, followed by ligation to *Xho*I- and *Bam*HI-digested pUQ1H(KTM219), resulting in pmUQ1H. The obtained plasmids were prepared using PureYield plasmid miniprep system, and the entire coding region sequences were confirmed.

Biolayer Interferometry analysis

To investigate the interaction between Z_{mab22_25} and Fab fragments, a biolayer interferometry (BLI)-based biosensor BLItz system (FortéBio, Pall, Menlo Park, CA) was employed. BLI is a label-free technology for measuring biomolecular interactions within the interactome [2]. It analyzes the interference pattern of white light reflected from immobilized protein layer on the biosensor, and an internal reference layer. Binding of molecules to the biosensor causes a shift in the interference pattern that can be measured. For immobilization onto the biosensor, Z_{mab22_25} was biotinylated as specified by the manufacturer, diluted in kinetic buffer (10 mM sodium phosphate, 150 mM NaCl, 0.005% Tween 20, 0.1% bovine serum albumin, pH 7.4) and added to a streptavidin biosensor (FortéBio). Anti-BGP mouse-human chimeric Fab and human Fab at concentrations of 50~200 nM, 4 μL each in kinetic buffer were used as analytes. After immobilization of probe, we first ran the machine for 30 s to obtain the baseline, followed by the addition of antibody to obtain the association curve for

120 s. By soaking in 250 μL of sample diluent, the dissociation curve was obtained for 180 s. Afterwards, the biosensor was regenerated by soaking for 60 s in 0.1 M glycine, pH 2.5. The association rate (k_{on}) and dissociation rate (k_{off}) constants, as well as their standard errors were calculated by fitting the curves using BLItz software. The K_A value was calculated by dividing k_{on} by k_{off}. Standard error (SE) of the ratio was calculated according to

$$\frac{M_1 \pm \varepsilon_1}{M_2 \pm \varepsilon_2} = \frac{M_1}{M_2} \pm \sqrt{(\frac{1}{M_2} \cdot \varepsilon_1)^2 + (\frac{M_1}{{M_2}^2} \cdot \varepsilon_2)^2} \quad (1)$$

where M_1 (M_2) and ε_1 (ε_2) are the mean and SE of k_{on} (k_{off}), respectively.

Table S1. Nucleotide sequences of primers used in this study.

Primer name	Nucleotide sequence (5'-3')
GSx2PAback	gggcggcggcagcggtggaggttcaaccgcgcaacaaaataacttc
GS3x1Pxfor	cgctgccgccgccctcattagactagtttacttc
GSx3PAback	gggcggcggcagcggtggaggttcaggaggtggatcaaccgcgcaacaaaataacttc
GS3x3PXfor	cgctgccgccgcccgaaccgcctccgctaccaccaccctcattagactagtttacttc
ProCXback	cgaagtaaactgctctaatgag
pROX2FabQ-Bamfor	cgtcgtccttgtagtcggatccactagtaacggc
Zmab22back	gtggacaacaaattcaacaaagaagcatggcgcgcacacatggaaattattcg
Zmab25for	caggcttgcaataaatgcataggcctgaaatgcattcagattcggcagacgaataatttccatgt
ZmabNdeBack	ggaattcatatggctcaagtggacaacaaattcaacaaag
ZmabNotFor	ggaattcgcggccgctttcggtgcctgtgcatc
Zmab25-3back	gcatttattgcaagcctgattgatgatccgagccagagcgcaaatctgctggccgaagc
Zmab25-4for	tttcggtgcctgtgcatcattcagttttttgcttcggccagcag
mIgG1CH1XhoBack	cggtcaccgtctcgagcgctaaaacgacaccccc
mIgG1CH1_EagFor	atgatgtgcggccgacacaattttcttgtccacc
Histagback	tcggccgcacatcatcatcaccatcacgg
VkHind+D	cagcacgtttgatttcaagcttggtcccccc
mCkBack	gaaatcaaacgtgctgatgctgcaccaactg
mCkBamfor	tcgtccttgtagtcggatccgccccccctcattcctgttgaagctcttg
mIgG1CH1XhoBack	cggtcaccgtctcgagcgctaaaacgacaccccc

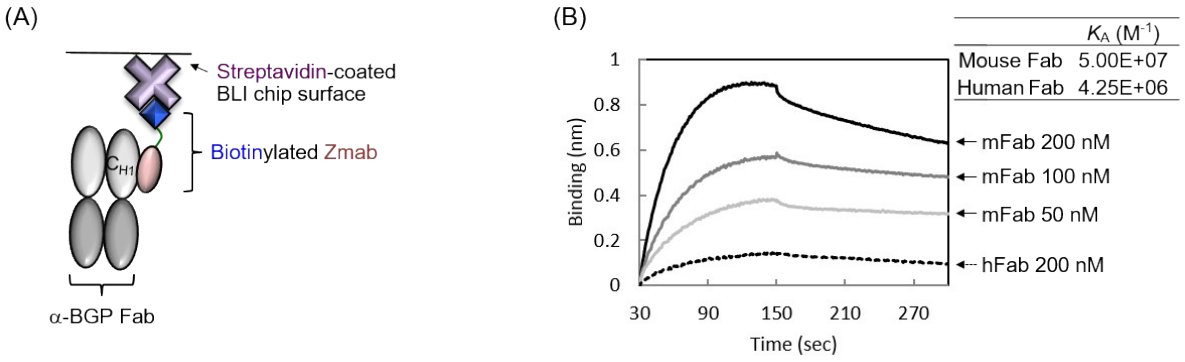

Figure S1. (A) Scheme of biolayer interferometry analysis. The C-terminally added Cys-tag was used for the biotinylation of $Zmab_{22\text{-}25}$. (B) Binding activity of $Zmab_{22\text{-}25}$ to mouse IgG_1 and mouse-human chimeric Fab fragments.

References

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2. R. L. Rich and D. G. Myszka, *Anal Biochem*, 2007, **361**, 1-6.