

Electronic Supplementary information for

Efficient and rapid linker optimization with heterodimeric coiled coils improves the response of fluorescent biosensors comprising antibody and protein M

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Supplementary Table

Table S1. The DNA sequence of the primers used for the construction of PM-E4s.

Primer name	Nucleotide Sequence (5'-3')
Bsu_SMA_E4back	AAACGTGCGGCCTCAATGGCTGAAATCGCTGCAC
Bsu_SGGGSMA_E4back	AAACGTGCGGCCTCAGGTGGAGGGAGCATGGCTGAAATCGCTGCAC
Bsu_SGS_E4back	AAACGTGCGGCCTCAGGGTCTGAAATCGCTGCAC
Bsu_SGGSGG_E4back	AAACGTGCGGCCTCAGGTGGAGGGAGCGGGGTGAAATCGCTGCAC
E4-2_Bottom	ACTTTAGCGACGTGACCTCTTTCTTTAACGTCGGAATCTTTTCTTTATCG
E4-3_Top	CCTTAGAAAAAGAAATAGCAGCGTTGGAAAAGGAAATCGCAGCATTGGAG
Eag_E4For_v2	CCTTTAGCGTCGTAACCTCTCATTATTGCCGGCGTGAGCTCGT

Table S2. Amino acid sequences of peptides for antigen and K4s before dye modification.

Peptide name	Sequence	M.W.
BGP-C7	NH ₂ -RRFYGPV-COOH	894.0
C-K4	NH ₂ -C KIAALKEKIAALKEKIAALKEKIAALKE-COOH	3136.9
C-G3S-K4	NH ₂ -C GGGG KIAALKEKIAALKEKIAALKEKIAALKE-COOH	3395.1
C-G3SG3S-K4	NH ₂ -C GGGSGGGG KIAALKEKIAALKEKIAALKEKIAALKE-CONH ₂	3637.0

A Anti-BGP Fab

		T-K4 linker		
		(GGGS) ₀	(GGGS) ₁	(GGGS) ₂
PM-E4 linker	SMA	0.79 ± 0.017	0.81 ± 0.0087	0.62 ± 0.017
	SGS	0.72 ± 0.020	0.35 ± 0.018	0.38 ± 0.020
	SGGG SMA	0.43 ± 0.017	0.23 ± 0.0079	0.16 ± 0.0014
	SGGG SGG	0.84 ± 0.031	0.23 ± 0.0073	0.28 ± 0.027

B Anti-CS IgG

		T-K4 linker		
		(GGGS) ₀	(GGGS) ₁	(GGGS) ₂
PM-E4 linker	SMA	0.79 ± 0.0041	0.72 ± 0.027	0.58 ± 0.017
	SGS	0.48 ± 0.0093	0.54 ± 0.024	0.53 ± 0.023
	SGGG SMA	0.38 ± 0.012	0.49 ± 0.0091	0.50 ± 0.019
	SGGG SGG	0.41 ± 0.0079	0.44 ± 0.0044	0.45 ± 0.023

C Anti-TS IgG

		T-K4 linker		
		(GGGS) ₀	(GGGS) ₁	(GGGS) ₂
PM-E4 linker	SMA	0.79 ± 0.028	0.91 ± 0.038	0.81 ± 0.014
	SGS	0.58 ± 0.012	0.62 ± 0.0070	0.69 ± 0.023
	SGGG SMA	0.55 ± 0.031	0.54 ± 0.028	0.59 ± 0.017
	SGGG SGG	0.53 ± 0.016	0.55 ± 0.012	0.56 ± 0.013

D Anti-Digoxin IgG

		T-K4 linker		
		(GGGS) ₀	(GGGS) ₁	(GGGS) ₂
PM-E4 linker	SMA	1.2 ± 0.076	1.1 ± 0.074	1.0 ± 0.033
	SGS	0.94 ± 0.051	1.0 ± 0.022	1.0 ± 0.0058
	SGGG SMA	0.80 ± 0.012	0.82 ± 0.020	0.92 ± 0.015
	SGGG SGG	0.77 ± 0.070	0.97 ± 0.014	0.92 ± 0.027

Table S3. Quenching levels of 12 combinations of CQ-probe using TAMRA with different linkers mixed with anti-BGP Fab (50 nM), anti-CS IgG (5.0 nM), anti-TS IgG (5.0 nM), and anti-digoxin IgG (10 nM). CQ-probe concentration in all experiments was 1.0 nM. The data represent means ± standard deviation (n = 3).

A BGP-C7

PM-E4 linker / T-K4 linker	+ Anti-BGP Fab (30 nM)	+ BGP-C7 (3.0 μ M)	Response
SGGGSMA/ (GGGS) ₂	0.25 \pm 0.011	0.95 \pm 0.030	3.7
SGGGS GG/ (GGGS) ₁	0.20 \pm 0.017	0.88 \pm 0.033	4.3

B CS

PM-E4 linker / T-K4 linker	+ Anti-CS IgG (5.0 nM)	+ CS (1.0 mM)	Response
SGGGSMA/ (GGGS) ₀	0.41 \pm 0.021	1.1 \pm 0.062	2.6
SGGGS GG/ (GGGS) ₀	0.42 \pm 0.015	0.90 \pm 0.0059	2.1

C TS

PM-E4 linker / T-K4 linker	+ Anti-TS IgG (5.0 nM)	+ TS (1.0 μ M)	Response
SGGGSMA/ (GGGS) ₀	0.55 \pm 0.013	1.1 \pm 0.016	1.9
SGGGSMA/ (GGGS) ₁	0.53 \pm 0.0088	1.0 \pm 0.018	1.9
SGGGS GG/ (GGGS) ₀	0.55 \pm 0.013	0.99 \pm 0.065	1.8
SGGGS GG/ (GGGS) ₁	0.52 \pm 0.025	0.93 \pm 0.0035	1.8

D Digoxin

PM-E4 linker / T-K4 linker	+ Anti-digoxin IgG (10 nM)	+ Digoxin (1.0 μ M)	Response
SGGGSMA/ (GGGS) ₀	0.80 \pm 0.012	0.93 \pm 0.028	1.2
SGGGS GG/ (GGGS) ₀	0.77 \pm 0.043	1.0 \pm 0.045	1.3

Table S4. Fluorescence responses for selected T-K4 labeled CQ-probes/antibody complex against BGP-C7, cortisol, testosterone, and digoxin. The data represent the means \pm standard deviation (n = 3).

A BGP-C7

	Max response (-fold)	Δ F.I. (%)	$\frac{\Delta$ F.I.CQ-probe / Δ F.I.PM Q-probe	EC50 (nM)	LOD (nM)
CQ-probe (TAMRA)	4.6	360	2.6	140	18
PM Q-probe (TAMRA)	2.4	140		85	27
CQ-probe (R6G)	8.9	790	13	520	11
PM Q-probe (R6G)	1.6	61		260	15

B CS

	Max response (-fold)	Δ F.I. (%)	$\frac{\Delta$ F.I.CQ-probe / Δ F.I.PM Q-probe	EC50 (nM)	LOD (nM)
CQ-probe (TAMRA)	2.5	150	1.5	3.9	0.25
PM Q-probe (TAMRA)	2.0	100		7.1	0.34
CQ-probe (R6G)	4.7	370	9.7	210	1.6
PM Q-probe (R6G)	1.4	38		170	15

C TS

	Max response (-fold)	Δ F.I. (%)	$\frac{\Delta$ F.I.CQ-probe / Δ F.I.PM Q-probe	EC50 (nM)	LOD (nM)
CQ-probe (TAMRA)	2.1	110	1.2	8.2	2.0
PM Q-probe (TAMRA)	1.9	94		11	4.3
CQ-probe (R6G)	1.8	84	1.3	5.3	1.7
PM Q-probe (R6G)	1.4	40		4.4	1.2

D Digoxin

	Max response (-fold)	Δ F.I. (%)	$\frac{\Delta$ F.I.CQ-probe / Δ F.I.PM Q-probe	EC50 (nM)	LOD (nM)
CQ-probe (TAMRA)	*	*	*	*	*
PM Q-probe (TAMRA)	*	*		*	*
CQ-probe (R6G)	1.6	59	1.8	3.7	0.19
PM Q-probe (R6G)	1.3	32		5.4	0.43

* not calculated due to R2 values less than 0.96

Table S5. Characterization of CQ-probe and PM Q-probe complexes with antibodies against BGP-C7, cortisol, testosterone, and digoxin. An average of three independent measurements is shown.

A Anti-BGP Fab

		R-K4 linker		
		(GGGS) ₀	(GGGS) ₁	(GGGS) ₂
PM-E4 linker	SMA	0.28 ± 0.013	0.39 ± 0.017	0.19 ± 0.020
	SGS	0.21 ± 0.013	0.41 ± 0.0098	0.12 ± 0.0049
	SGGG SMA	0.13 ± 0.0087	0.14 ± 0.0065	0.14 ± 0.012
	SGGG SGG	0.12 ± 0.010	0.12 ± 0.0063	0.12 ± 0.0040

B Anti-CS IgG

		R-K4 linker		
		(GGGS) ₀	(GGGS) ₁	(GGGS) ₂
PM-E4 linker	SMA	0.32 ± 0.0037	0.44 ± 0.0062	0.24 ± 0.0097
	SGS	0.27 ± 0.0048	0.35 ± 0.0069	0.18 ± 0.0064
	SGGG SMA	0.30 ± 0.0065	0.25 ± 0.011	0.28 ± 0.019
	SGGG SGG	0.30 ± 0.0038	0.22 ± 0.0029	0.21 ± 0.0048

C Anti-TS IgG

		R-K4 linker		
		(GGGS) ₀	(GGGS) ₁	(GGGS) ₂
PM-E4 linker	SMA	0.86 ± 0.0046	0.94 ± 0.012	0.93 ± 0.0078
	SGS	0.75 ± 0.011	0.83 ± 0.019	0.74 ± 0.0068
	SGGG SMA	0.77 ± 0.0087	0.68 ± 0.024	0.85 ± 0.0047
	SGGG SGG	0.70 ± 0.0062	0.70 ± 0.013	0.77 ± 0.014

D Anti-Digoxin IgG

		R-K4 linker		
		(GGGS) ₀	(GGGS) ₁	(GGGS) ₂
PM-E4 linker	SMA	0.99 ± 0.021	1.0 ± 0.0085	0.88 ± 0.015
	SGS	0.91 ± 0.019	0.84 ± 0.015	0.79 ± 0.010
	SGGG SMA	0.77 ± 0.015	1.1 ± 0.0030	0.78 ± 0.022
	SGGG SGG	0.73 ± 0.0093	1.1 ± 0.0090	1.1 ± 0.032

Table S6. Quenching levels of 12 combinations of CQ-probe using R6G with different linkers mixed with anti-BGP Fab (50 nM), anti-CS IgG (5.0 nM), anti-TS IgG (5.0 nM), and anti-digoxin IgG (10 nM). CQ-probe concentration in all experiments was 1.0 nM. The data represent means ± standard deviation (n = 3).

A BGP-C7

PM-E4 linker / R-K4 linker	+ Anti-BGP Fab (30 nM)	+ BGP-C7 (3.0 μ M)	Response
SGS/(GGGS) ₂	0.13 \pm 0.016	1.2 \pm 0.011	9.0
SGGGSMA/(GGGS) ₀	0.16 \pm 0.049	1.3 \pm 0.013	8.2
SGGGSMA/(GGGS) ₁	0.18 \pm 0.020	1.2 \pm 0.018	6.6
SGGGSMA/(GGGS) ₂	0.13 \pm 0.019	1.2 \pm 0.0088	9.2
SGGSSGG/(GGGS) ₀	0.15 \pm 0.063	1.2 \pm 0.0055	7.9
SGGSSGG/(GGGS) ₁	0.16 \pm 0.065	1.2 \pm 0.011	7.3
SGGSSGG/(GGGS) ₂	0.13 \pm 0.031	1.2 \pm 0.0055	9.0

B CS

PM-E4 linker / R-K4 linker	+ Anti-CS IgG (5.0 nM)	+ CS (1.0 mM)	Response
SGS/(GGGS) ₂	0.24 \pm 0.0034	0.87 \pm 0.028	3.6
SGGSSGG/(GGGS) ₁	0.24 \pm 0.031	0.82 \pm 0.027	3.5
SGGSSGG/(GGGS) ₂	0.23 \pm 0.076	0.83 \pm 0.0051	3.6

C TS

PM-E4 linker / R-K4 linker	+ Anti-TS IgG (5.0 nM)	+ TS (1.0 μ M)	Response
SGGGSMA/(GGGS) ₁	0.57 \pm 0.012	1.1 \pm 0.0046	1.9
SGGSSGG/(GGGS) ₀	0.62 \pm 0.0098	1.1 \pm 0.020	1.8
SGGSSGG/(GGGS) ₁	0.60 \pm 0.021	1.1 \pm 0.025	1.8

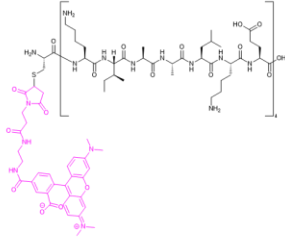
D Digoxin

PM-E4 linker / R-K4 linker	+ Anti-digoxin IgG (10 nM)	+ Digoxin (1.0 μ M)	Response
SGGGSMA/(GGGS) ₀	0.86 \pm 0.0020	1.2 \pm 0.017	1.4
SGGSSGG/(GGGS) ₀	0.83 \pm 0.011	1.2 \pm 0.015	1.5

Table S7. Fluorescence responses for selected R-K4 labeled CQ-probes/antibody complexes against BGP-C7, cortisol, testosterone, and digoxin. The data represent means \pm standard deviation (n = 3).

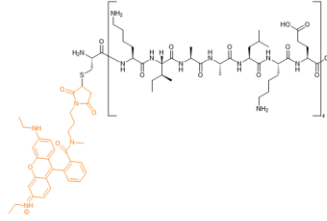
A

T-K4

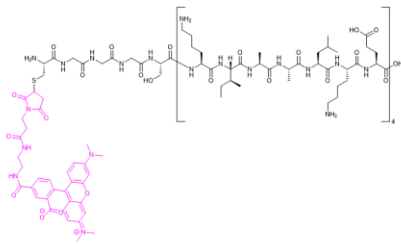


B

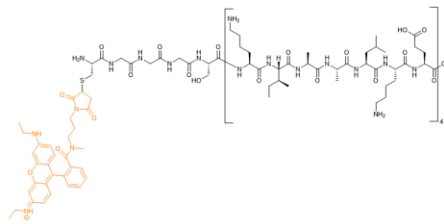
R-K4



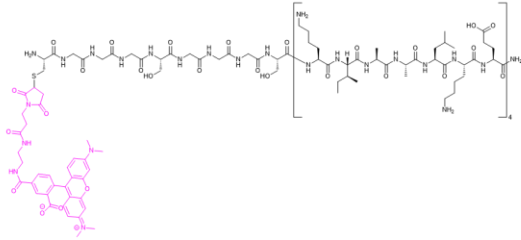
T-GGGS-K4



R-GGGS-K4



T-GGGSGGGS-K4



R-GGGSGGGS-K4

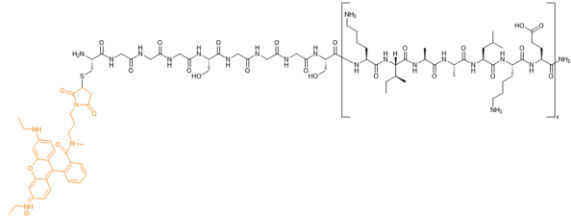


Figure S1. The molecular structure of dye-labeled K4 peptides.

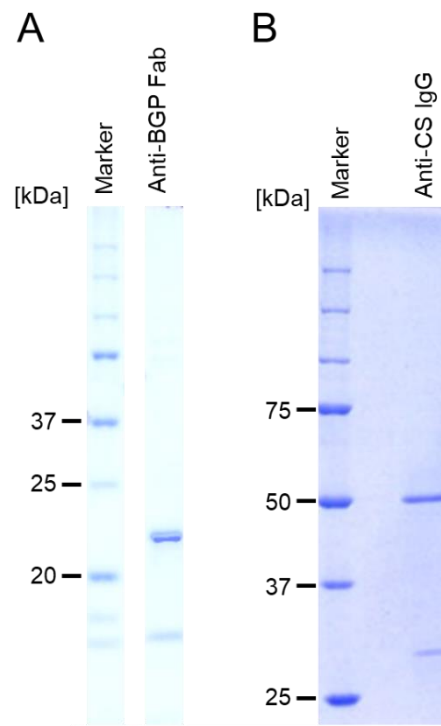


Figure S2. SDS-PAGE analysis of purified antibodies. (A) Anti-BGP Fab. (B) Anti-CS IgG.

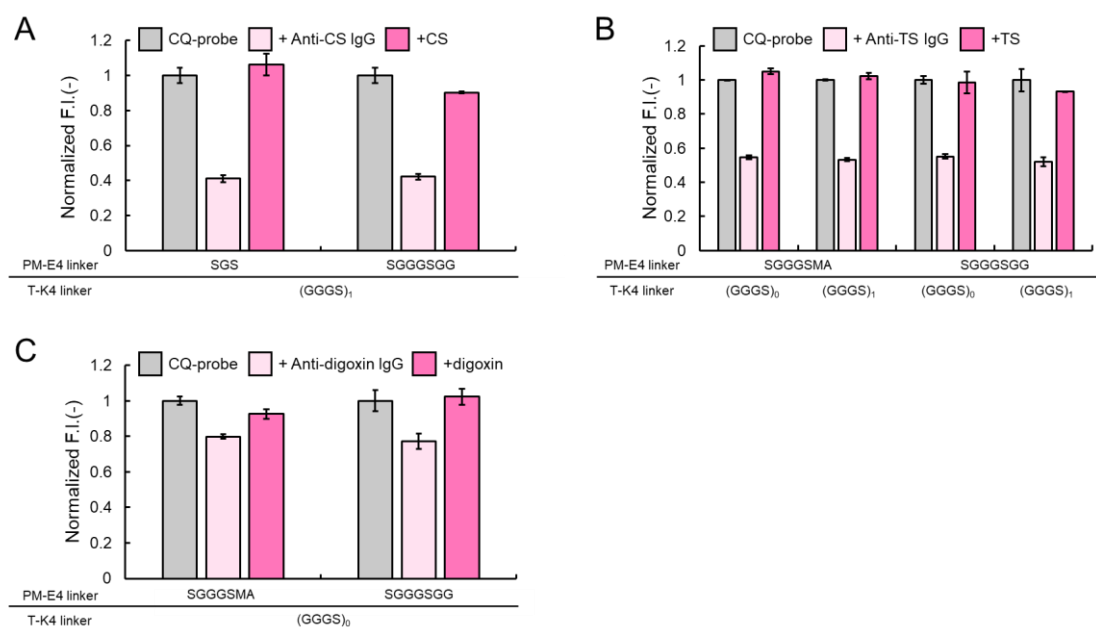


Figure S3. Fluorescence responses of selected T-K4 labeled CQ-probe/anti-hapten IgG complexes against antigens. (A) Complex with anti-cortisol IgG (5.0 nM) against cortisol (1.0 mM). (B) Complex with anti-testosterone IgG (5.0 nM) against testosterone (1.0 μ M). (C) Complex with anti-digoxin IgG (10 nM) against digoxin (1.0 μ M). CQ-probe concentration in all experiments was 1.0 nM. The data represent means \pm standard deviation ($n = 3$).

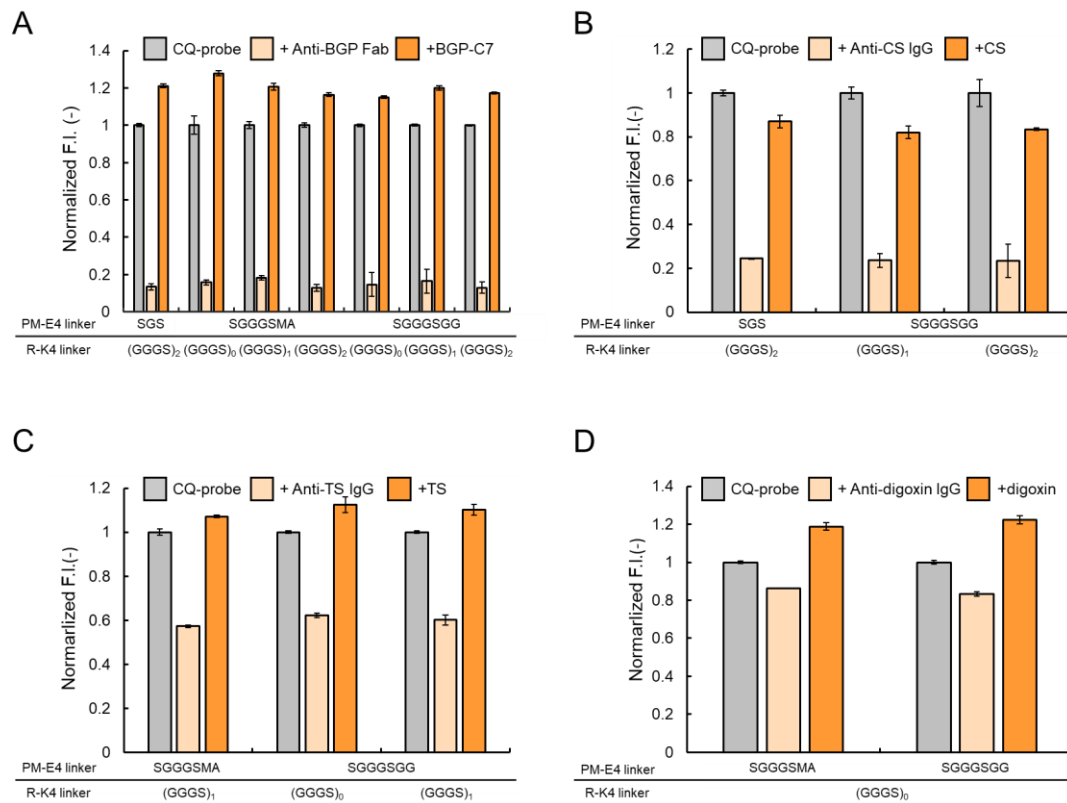


Figure S4. Fluorescence responses of selected R-K4 labeled CQ-probe/IgG complexes against antigens. (A) Complex with anti-BGP Fab (30 nM) against BGP-C7 (3.0 μ M). (B) Complex with anti-cortisol IgG (5.0 nM) against cortisol (1.0 mM). (C) Complex with anti-testosterone IgG (5.0 nM) against testosterone (1.0 μ M). (D) Complex with anti-digoxin IgG (10 nM) against digoxin (1.0 μ M). CQ-probe concentration in all experiments was 1.0 nM. The data represent means \pm standard deviation ($n = 3$).

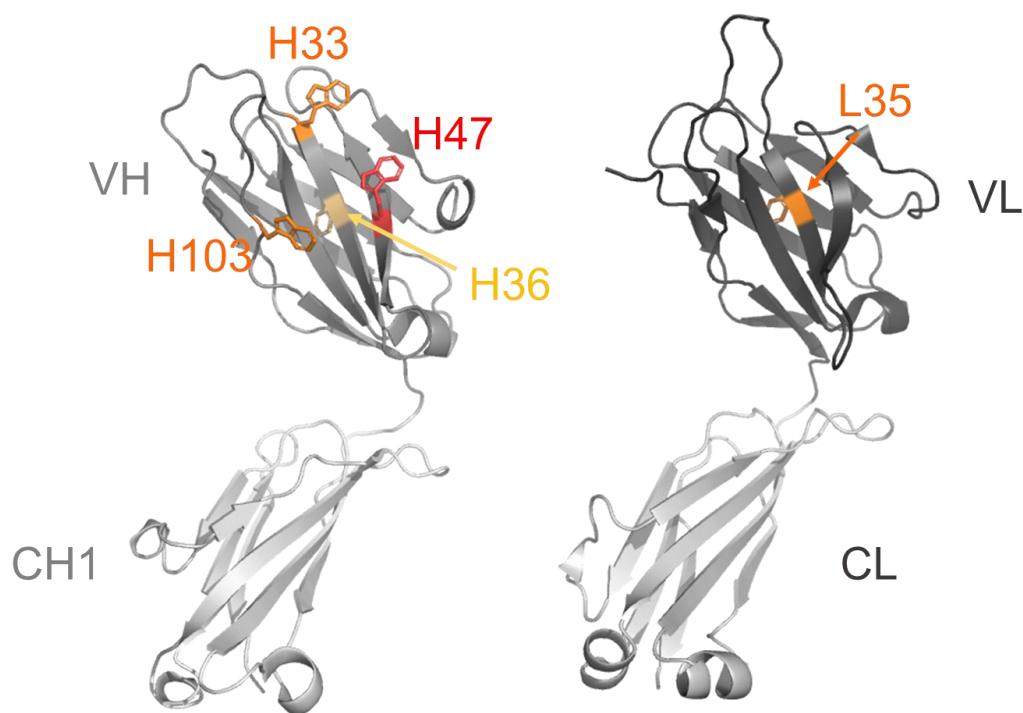


Figure S5. The 3D structure of the heavy chain (left) and light chain (right) of anti-BGP Fab, KTM219 (PDB: 5X5X). The sides facing each other in Fab are shown toward this side. The Trp residues were given in different colors depending on their contribution to the fluorescence response. The contribution was based on the percentages of fluorescence response after Phe mutation calculated from descriptions in the previous paper (45% for H47; 55-66% for H33, H103, L35; 84% for H36).⁴ The high contribution is red, the moderate is orange, and the low is yellow.

Reference

R. Abe, H. Ohashi, I. Iijima, M. Ihara, H. Takagi, T. Hohsaka and H. Ueda, *J. Am. Chem. Soc.*, 2011, **133**, 17386–17394.