Supplementary Information for

Development of bioluminescent Switchbody, antigen-triggered enzyme switch and elucidation of its principle

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Keywords: protein switch, homogeneous immunoassay, X-ray structural analysis, solution NMR, MD simulation

Supplementary Table 1. Characterization of Switchbodies.

Sample	Buffer	Response (-fold)	EC_{50} (nM)	LOD (nM)
Switchbody (L1)	PBST	3.5	3.3	0.32
	20% Serum	5.5	165	1.3
	20% Plasma	5.9	163	2.7
Switchbody (L2)	PBST	4.0	10	1.2
	20% Serum	4.8	107	4.6
	20% Plasma	5.4	80	1.6

Data are shown as mean of triplicates.

Supplementary Table 2. Crystallographic data collection and refinement statistics.

Data collection	
Space group	$P2_12_12$
Unit-cell parameters (Å)	a = 95.84 b = 65.91 c = 69.56
X-ray source	PF BL-5A
Wavelength (Å)	1.00
Resolution (Å)	47.92–1.95 (2.00–1.95)
Unique reflections	32857 (2290)
Average redundancy	13.1 (12.7)
Completeness (%)	100.0 (100.0)
I / σ(I)	23.9 (2.2)
R_{meas} (%)	7.6 (130.8)
$CC_{1/2}$	1.000 (0.850)
Refinement	
Resolution (Å)	47.92–1.95
No. of reflections	31276
No. of protein atoms	3274
No. of water atoms	273
No. of other atoms	6
R_{work} (%)	20.2
R_{free} (%)	23.8
RMSD bond length (Å)	0.012
RMSD bond angles (°)	1.47
Average B-factor (Å^2)	41.3
Ramachandran plot	
In preferred regions (%)	97.4
In allowed regions (%)	2.6
Outliers (%)	0
PDB ID	9LUK

All numbers in parentheses represent the last outer shell statistics.

R_{free} is calculated for 4.7% of randomly selected reflections excluded from refinement.

Ramachandran plot analyses of the models were performed using RAMPAGE.

Supplementary Table 3. Estimated the apparent transverse relaxation rate constant R_2.

Signal	R_2 (s^{-1})
a	20 ± 3.7
b	19 ± 5.1
c	17 ± 4.6
d	22 ± 2.8
e	22 ± 6.0
x	4.9 ± 0.63
y	20 ± 6.9

Data are shown as mean ± standard deviation of curve fitting using Monte Carlo method (N = 128).

Supplementary Table 4. Contact frequency of Switchbody (L1) without antigen.

Rank	Residue pair		Contact frequency (%)
	HiBiT	scFv	
1	R5	$E50_H$	20
2	K9	$D52_H$	19
3	K9	$D54_H$	19
4	I10	$F29_H$	18
5	R5	$Y96_L$	18
6	S11	$S97_H$	16
7	S11	$W33_H$	13
8	K9	$W33_H$	12
9	W4	$E50_H$	12
10	L6	$K73_H$	11

Supplementary Table 5. Contact frequency of Switchbody (L1) with antigen.

Rank	Residue pair		Contact frequency (%)
	HiBiT	scFv	
1	F7	$V98_H$	30
2	W4	$S97_H$	23
3	S11	$Y32_H$	19
4	I10	$T96_H$	19
5	I10	$V98_H$	19
6	W4	$D30_L$	18
7	F7	$T96_H$	17
8	S2	$D30_L$	17
9	L6	$V98_H$	16
10	R5	$D52_H$	15

Supplementary Table 6. Luminescence response of PM-HiBiT/IgG complexes using PM-HiBiT carrying different linker length.

IgG	Response (-fold)			
	L0	L1	L2	L3
KTM219	1.1	1.2	1.6	1.7
P20.1	1.4	1.3	1.4	1.4
ME.125	1.4	1.4	2.0	1.3

Data are shown as a single measurement only for screening purpose.

Supplementary Table 7. Characterization of PM-HiBiT/IgG complexes.

PM-HiBiT/IgG	Response (-fold)	EC_{50}	LOD
L3/KTM219	1.7	14 nM	1.9 nM
L3/P20.1	1.7	6.9 μM	0.33 μM
L2/ME.125	1.8	2.8 nM	1.5 nM

Data are shown as mean of triplicates.

Supplementary Table 8. Half-life of binding activity after 60°C.

Variant	$T_{1/2}$ (h)
WT	2.2
$Y27_HS$	3.8
$Y27_HT$	2.7
$F29_HS$	2.7
$L9_LS$	1.7
$L9_LT$	1.5

Supplementary Table 9. Amino acid sequences of scFvs and Switchbodies.

Name	Sequence
scFv	MQVKLQQSGAEFVKAGASVKLSCKTSGYTFNNYWIHWVKQSPGQGLEWIGEIDPSDGYS NYNQKFKGKATLTVDKSSSTAYMHLNSLTSEDSAVYYCTSSTSVGGSWGQGTTVTVSSG GGGSGGGGSGGGGSDIELTQSPLSLPVSLGDQASISCTSSQSLLHSNGDTYLHWYLQKP GQSPKLLIYTLSNRFSGVPDRFSGSGSGTDFTLKISRVEAADLGIYFCSQTTHVPYTFG GGTKLEIKRGGGSHHHHHHGGSDYKDDDDK*
Switchbody (L1)	MSVSGWRLFKKISGGGSTGQVKLQQSGAEFVKAGASVKLSCKTSGYTFNNYWIHWVKQS PGQGLEWIGEIDPSDGYSNYNQKFKGKATLTVDKSSSTAYMHLNSLTSEDSAVYYCTSS TSVGGSWGQGTTVTVSSGGGGSGGGGSGGGGSDIELTQSPLSLPVSLGDQASISCTSSQ SLLHSNGDTYLHWYLQKPGQSPKLLIYTLSNRFSGVPDRFSGSGSGTDFTLKISRVEAA DLGIYFCSQTTHVPYTFGGGTKLEIKRGGGSHHHHHHGGSDYKDDDDK*
Switchbody (L2)	MSVSGWRLFKKISGGGSGGGSTGQVKLQQSGAEFVKAGASVKLSCKTSGYTFNNYWIHW VKQSPGQGLEWIGEIDPSDGYSNYNQKFKGKATLTVDKSSSTAYMHLNSLTSEDSAVYY CTSSTSVGGSWGQGTTVTVSSGGGGSGGGGSGGGGSDIELTQSPLSLPVSLGDQASISC TSSQSLLHSNGDTYLHWYLQKPGQSPKLLIYTLSNRFSGVPDRFSGSGSGTDFTLKISR VEAADLGIYFCSQTTHVPYTFGGGTKLEIKRGGGSHHHHHHGGSDYKDDDDK*
scFv ($Y27_HS$) for NMR	MSKIKHHHHHHSSGENLYFQGGGGSTGQVKLQQSGAEFVKAGASVKLSCKTSGSTFNNY WIHWVKQSPGQGLEWIGEIDPSDGYSNYNQKFKGKATLTVDKSSSTAYMHLNSLTSEDS AVYYCTSSTSVGGSWGQGTTVTVSSGGGGSGGGGSGGGGSDIELTQSPLSLPVSLGDQA SISCTSSQSLLHSNGDTYLHWYLQKPGQSPKLLIYTLSNRFSGVPDRFSGSGSGTDFTL KISRVEAADLGIYFCSQTTHVPYTFGGGTKLEIKR*
Switchbody (L1, $Y27_HS$) for NMR	MSKIKHHHHHHSSGENLYFQGVSGWRLFKKISGGGSTGQVKLQQSGAEFVKAGASVKLS CKTSGSTFNNYWIHWVKQSPGQGLEWIGEIDPSDGYSNYNQKFKGKATLTVDKSSSTAY MHLNSLTSEDSAVYYCTSSTSVGGSWGQGTTVTVSSGGGGSGGGGSGGGGSDIELTQSP LSLPVSLGDQASISCTSSQSLLHSNGDTYLHWYLQKPGQSPKLLIYTLSNRFSGVPDRF SGSGSGTDFTLKISRVEAADLGIYFCSQTTHVPYTFGGGTKLEIKR*

VH and VL; HiBiT; TEV site; His-tag and FLAG-tag

Supplementary Table 10. Amino acid sequences of LgBiT.

Name	Sequence
LgBiT	MVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRIVRSGENALKIDIH VIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRPYEG IAVFDGKKITVTGTLWNGNKIIDERLITPDGSMLFRVTINSLEHHHHHH*

LgBiT; His-tag

Supplementary Table 11. Amino acid sequences of Protein M and PM-HiBiTs.

Name	Sequence
Protein M	MGNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGSDRPVPKTEISGLIKTGDN FITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYDDVFRQ VPSFSGWSNTKATTVSTSNNLTYDKWTYYAAKGSPLYDSYPNHSFEDVKTLAIDAKDIS ALKTTIDSEKPTYLIIRGLSGNGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVE LEFYSTSKANSFGFNPLVLGSKTNVIYDLFASKPFTHIDLTQVTLQNSDNSAIDANKLK QAVGDIYNYRRFERQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNLHLEEAY REGDNTYYRVNENYYPGASIYENERASRDSEFQNEILKR*
PM-HiBiT (L0)	MGNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGSDRPVPKTEISGLIKTGDN FITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYDDVFRQ VPSFSGWSNTKATTVSTSNNLTYDKWTYYAAKGSPLYDSYPNHSFEDVKTLAIDAKDIS ALKTTIDSEKPTYLIIRGLSGNGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVE LEFYSTSKANSFGFNPLVLGSKTNVIYDLFASKPFTHIDLTQVTLQNSDNSAIDANKLK QAVGDIYNYRRFERQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNLHLEEAY REGDNTYYRVNENYYPGASIYENERASRDSEFQNEILKRAASVSGWRLFKKIS*
PM-HiBiT (L1)	MGNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGSDRPVPKTEISGLIKTGDN FITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYDDVFRQ VPSFSGWSNTKATTVSTSNNLTYDKWTYYAAKGSPLYDSYPNHSFEDVKTLAIDAKDIS ALKTTIDSEKPTYLIIRGLSGNGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVE LEFYSTSKANSFGFNPLVLGSKTNVIYDLFASKPFTHIDLTQVTLQNSDNSAIDANKLK QAVGDIYNYRRFERQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNLHLEEAY REGDNTYYRVNENYYPGASIYENERASRDSEFQNEILKRAASGGGSVSGWRLFKKIS*
PM-HiBiT (L2)	MGNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGSDRPVPKTEISGLIKTGDN FITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYDDVFRQ VPSFSGWSNTKATTVSTSNNLTYDKWTYYAAKGSPLYDSYPNHSFEDVKTLAIDAKDIS ALKTTIDSEKPTYLIIRGLSGNGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVE LEFYSTSKANSFGFNPLVLGSKTNVIYDLFASKPFTHIDLTQVTLQNSDNSAIDANKLK QAVGDIYNYRRFERQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNLHLEEAY REGDNTYYRVNENYYPGASIYENERASRDSEFQNEILKRAASGGGSGGGSVSGWRLFKK IS*
PM-HiBiT (L3)	MGNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGSDRPVPKTEISGLIKTGDN FITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYDDVFRQ VPSFSGWSNTKATTVSTSNNLTYDKWTYYAAKGSPLYDSYPNHSFEDVKTLAIDAKDIS ALKTTIDSEKPTYLIIRGLSGNGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVE LEFYSTSKANSFGFNPLVLGSKTNVIYDLFASKPFTHIDLTQVTLQNSDNSAIDANKLK

QAVGDIYNYRRFERQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNLHLEEAY
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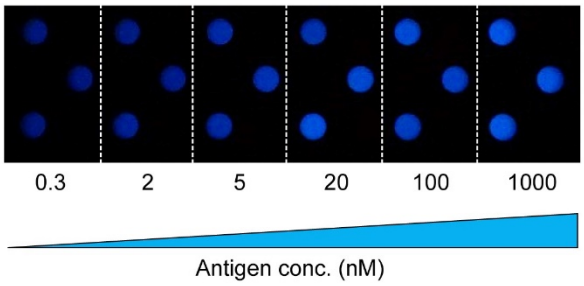
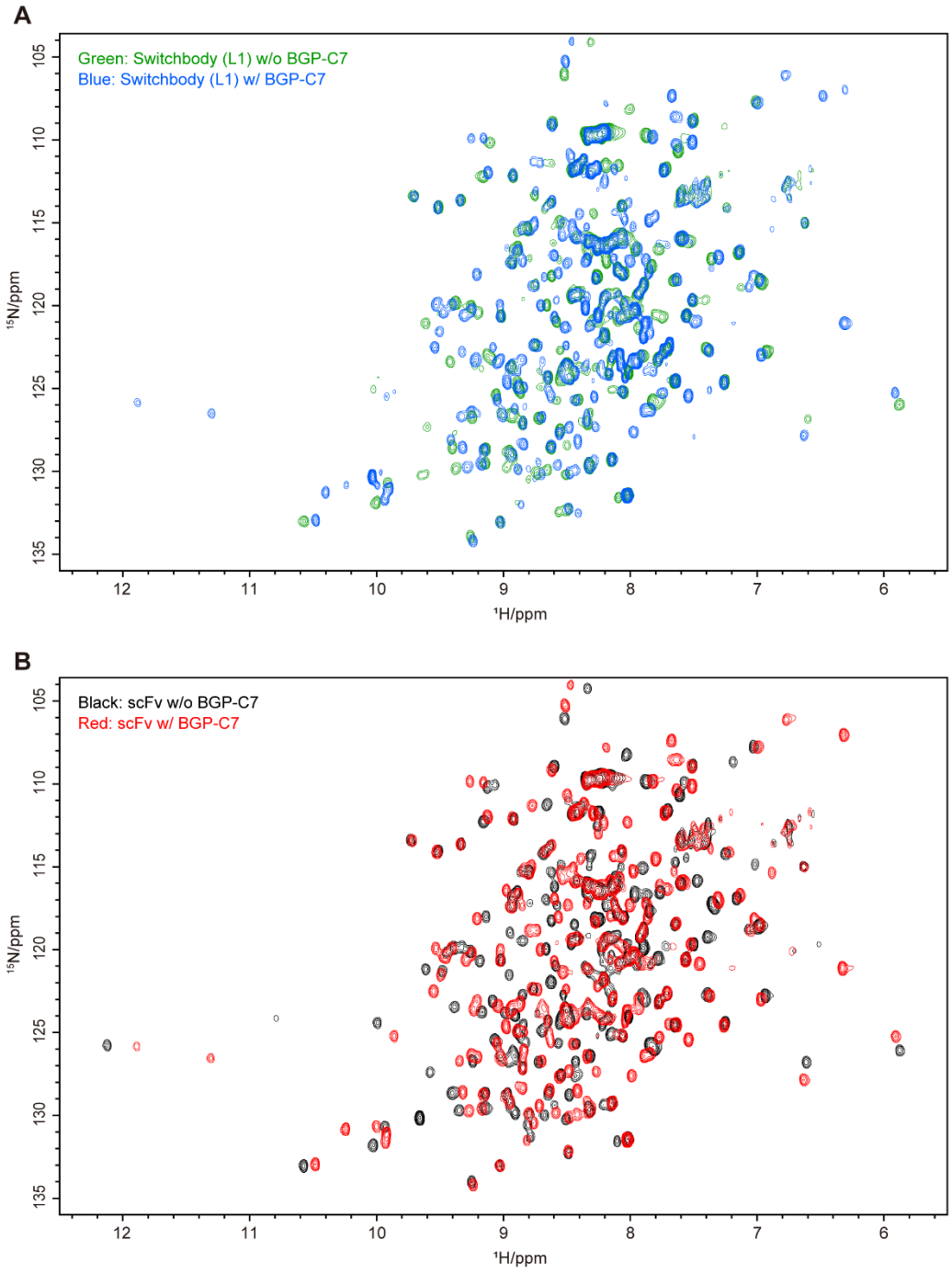
LFKKIS*

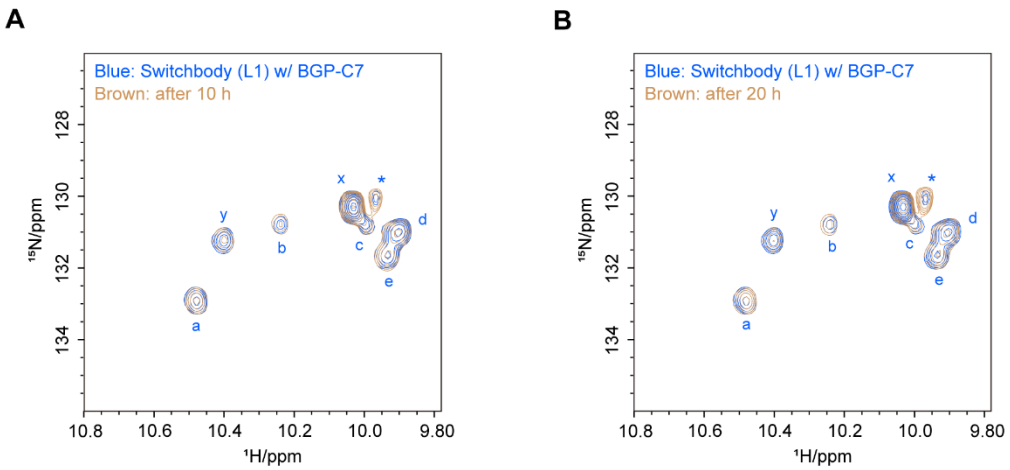
Protein M; HiBiT; His-tag; Linker



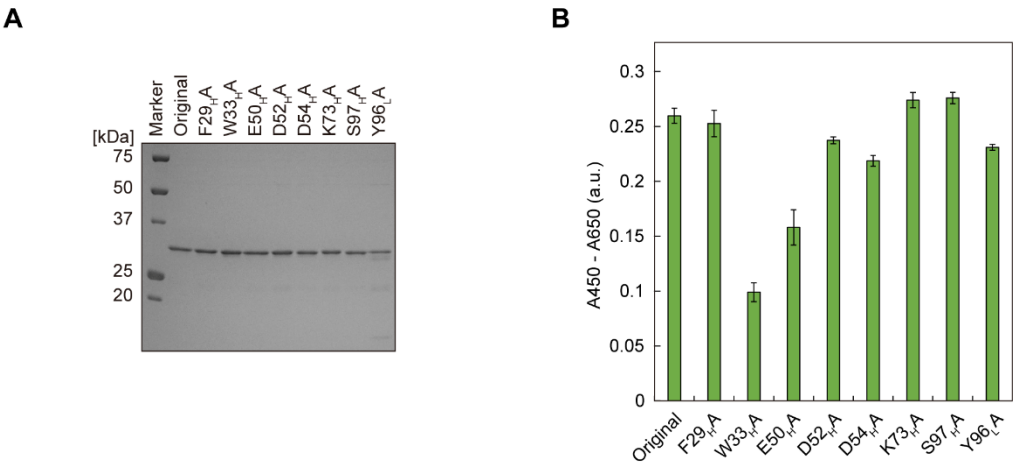
Supplementary Figure 1. Bioluminescence images of the 5 nM Switchbody (L1) with various concentrations of BGP-C7 captured using a digital camera.



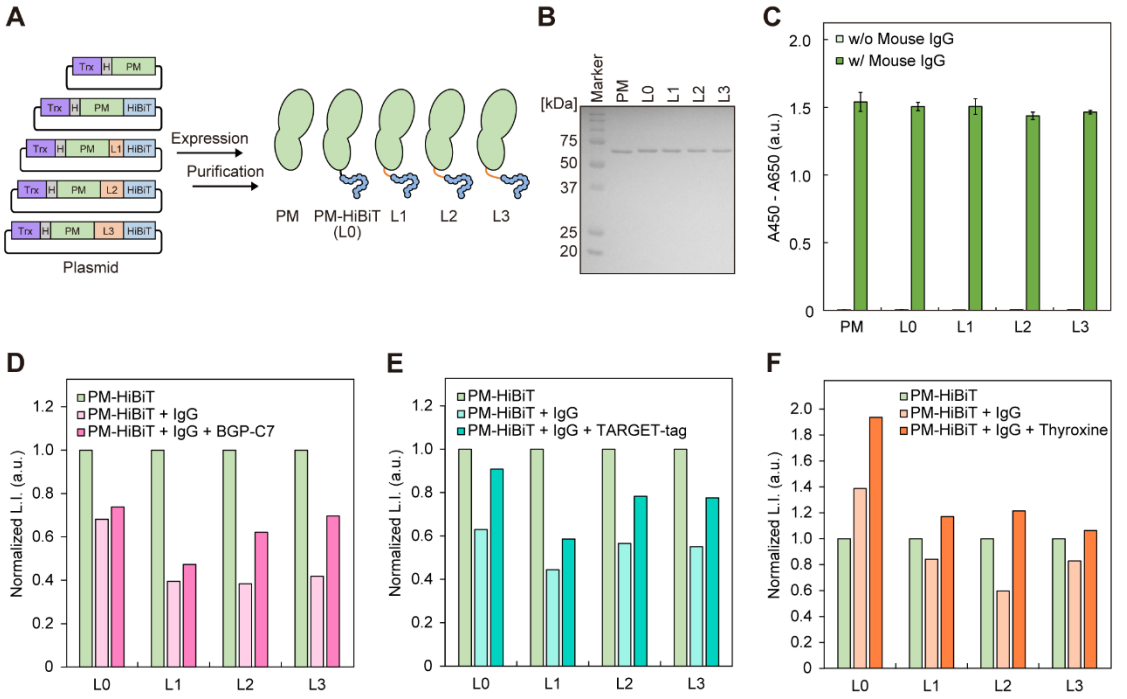
Supplementary Figure 2. Overlay of ^{1}H-^{15}N HSQC spectra. (**A**) Switchbody (L1) with and without antigen. (**B**) scFv with and without antigen.



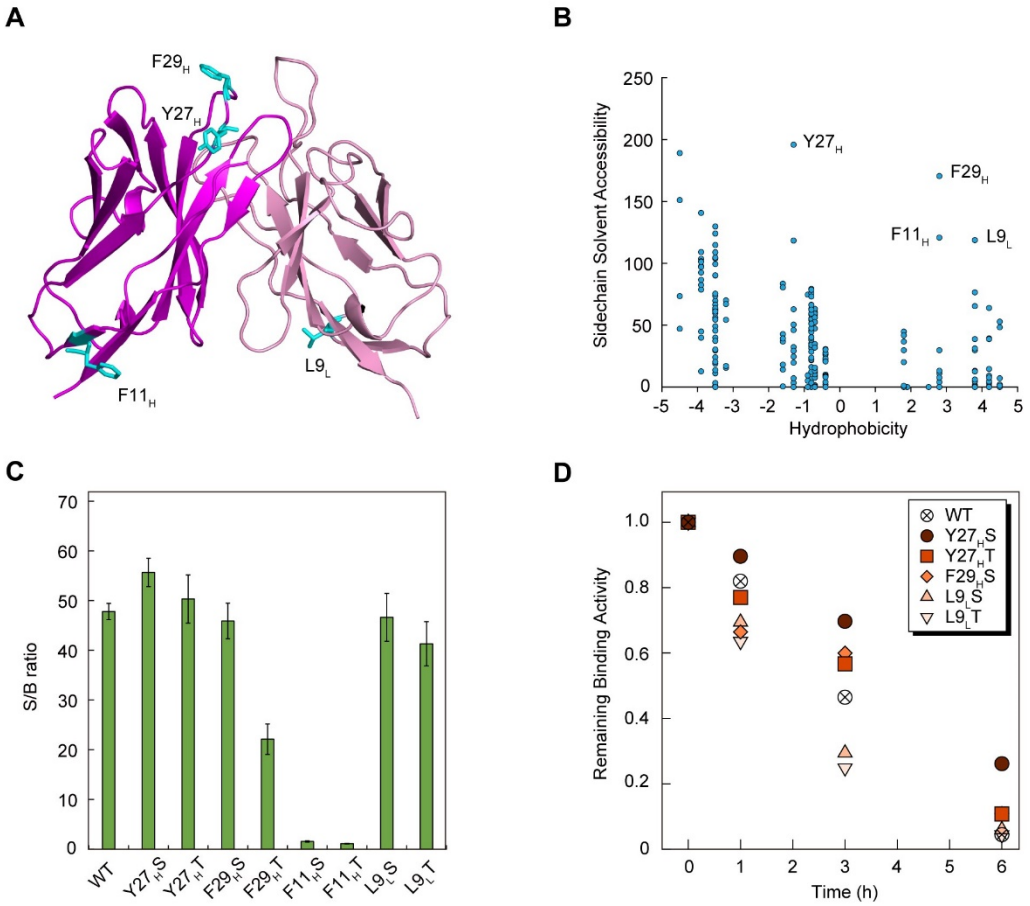
Supplementary Figure 3. Overlay of ^{1}H-^{15}N HSQC spectra. (**A**) Switchbody (L1) with antigen and after 10 hours. (**B**) Switchbody (L1) with antigen and after 20 hours.



Supplementary Figure 4. Preparation of Switchbody mutants. (**A**) SDS-PAGE analysis of purified Switchbody mutants. (**B**) Binding activity of Switchbody mutants to immobilized antigen, BGP-C11 examined by ELISA. Data are shown as mean ± standard deviation of triplicates. a.u.: arbitrary unit.



Supplementary Figure 5. Preparation and characterization of PM-HiBiT. (**A**) Schematic illustration of the preparation process for PM and PM-HiBiTs. L1: (G_3S) linker, L2: $(G_3S)_2$ linker, L3: $(G_3S)_3$ linker, H: 6×His-tag, Trx: thioredoxin. (**B**) SDS-PAGE analysis of purified PM and PM-HiBiTs. (**C**) Binding activity of PM and PM-HiBiTs to immobilized mouse IgG examined by ELISA. Data are shown as mean ± standard deviation of triplicates. (**D–F**) Luminescence intensity of complexes of 1 nM PM-HiBiT and 5 nM IgG. The data are presented as a single measurement value for screening purposes only. L.I.: luminescence intensity. a.u.: arbitrary unit.



Supplementary Figure 6. Preparation of thermostable scFv for NMR study. (**A**) Model structure of the scFv generated from the crystal structure (PDB ID: 5X5X) (VH in magenta, VL in pink, candidates for mutation in cyan). (**B**) Selection of hydrophobic amino acids with large solvent accessible surface area on the scFv. Selected amino acid was mutated to serine or threonine. (**C**) Binding activity of mutants to immobilized antigen, BGP-C11 examined by ELISA. S/B ratio were calculated from absorbance signal with antigen divided by without antigen. Data are shown as mean ± standard deviation of triplicates. (**D**) Remaining binding activity of mutants to immobilized antigen after 60°C with various incubation time. Data are shown as mean of triplicates.