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Table I. Data collection and refinement statistics.

	V21-BTN	V21-BTNtail	V21-IMNtail	V212-IMNtail
Data collection				
Space group	$P2_12_12$	$P2_12_12_1$	$P2_1$	$C222_1$
Unit-cell parameters (Å, □)	a = 67.68, b = 54.34, c = 60.06	a = 55.24, b = 85.46, c = 86.12	a = 59.97, b = 57.50, c = 71.18, β = 103.34	a = 77.42, b = 77.41, c = 172.83
Wavelength	0.98000	0.98000	0.90000	0.90000
Resolution (Å)	50–1.30 (1.35–1.30)[a]	50–1.50 (1.55–1.50)	50–1.20 (1.24–1.20)	50–1.70 (1.76–1.70)
R_{sym} (%)[b]	7.2 (40.6)	3.5 (23.2)	6.8 (30.9)	5.4 (35.3)
Total reflections	411,780	405,051	497,753	326,970
Unique reflections	54,829 (5,393)	64,021 (6,294)	143,314 (13,724)	55,926 (5,415)
$I/\sigma(I)$	26.8 (4.0)	17.6 (3.1)	18.3 (2.8)	26.4 (3.0)
Completeness (%)	99.1 (99.2)	97.4 (97.2)	96.6 (93.0)	97.0 (94.8)
Redundancy	7.5 (5.5)	6.3 (4.4)	3.5 (2.6)	5.8 (3.6)
Refinement				
Resolution	1.30	1.50	1.20	1.70
No. of reflections	51,662	60,475	135,778	52,687
R_{work} (%)[c]/R_{free} (%)[d]	16.0/20.1	17.3/23.6	13.4/16.1	19.7/24.4
No. of atoms				
Protein	1,840	3,734	3,656	3,713
Ligand/ion	37	159	126	128
Water	222	233	441	287
B factors				
Protein	14.8	14.7	10.0	18.6
Ligand/ion	14.6	33.6	14.4	35.2
Water	29.8	34.7	24.9	29.0
R.m.s deviations				
Bond length (Å)	0.026	0.018	0.026	0.025
Bond angles (□)	2.33	1.91	2.27	2.12
Ramachandran plot				
Favored (%)	96.15	95.39	97.01	94.56
Allowed (%)	3.42	3.56	2.99	5.44
Outliers (%)	0.43	1.05	0.00	0.00
PDB IDs	3WZN	3WZO	3WZP	3WZQ

[a]Values in parentheses are for the highest-resolution shell.

[b] R_{sym} is calculated as $\Sigma_{hkl}\Sigma_i|I_i(\mathrm{hkl}) - \langle I(hkl)\rangle|/\Sigma_{hkl}\Sigma_i I(hkl)$, where $I_i(hkl)$ is the intensity of an individual measurement of the reflection with Miller indices *hkl* and $\langle I(hkl)\rangle$ is the average intensity from multiple observations.

[c] $R_{work} = \Sigma_{hkl}||F_{obs}| - |F_{calc}||/\Sigma_{hkl}|F_{obs}|$, where F_{obs} and F_{calc} are the observed and calculated structure-factor amplitudes, respectively.

[d] R_{free} is computed in the same manner as R_{work} but using only a small set (5%) of randomly chosen intensities that were not used in the refinement of the model.

Table II. SA muteins, substituted residues and results of SPR measurements

Muteins	Substituted residues	K_d (M)	
		Biocytin	IMNtail
LISA-314		8.3×10^{-11}	not detected
V21	N23D, S27D	3.5×10^{-7} (1.0×10^{-7} [a])	1.5×10^{-7}
V212	N23D, S27D, S45N	not detected	5.9×10^{-7}
	N23D, S27D, S45H	not detected	5.2×10^{-7}
	N23D, S27D, S45Q	not detected	not detected
	N23D, S27D, S45A	2.4×10^{-5}	3.2×10^{-7}
	N23D, S27D, S45T	7.6×10^{-6}	7.6×10^{-7}

[a] The K_d value was measured with biotin.

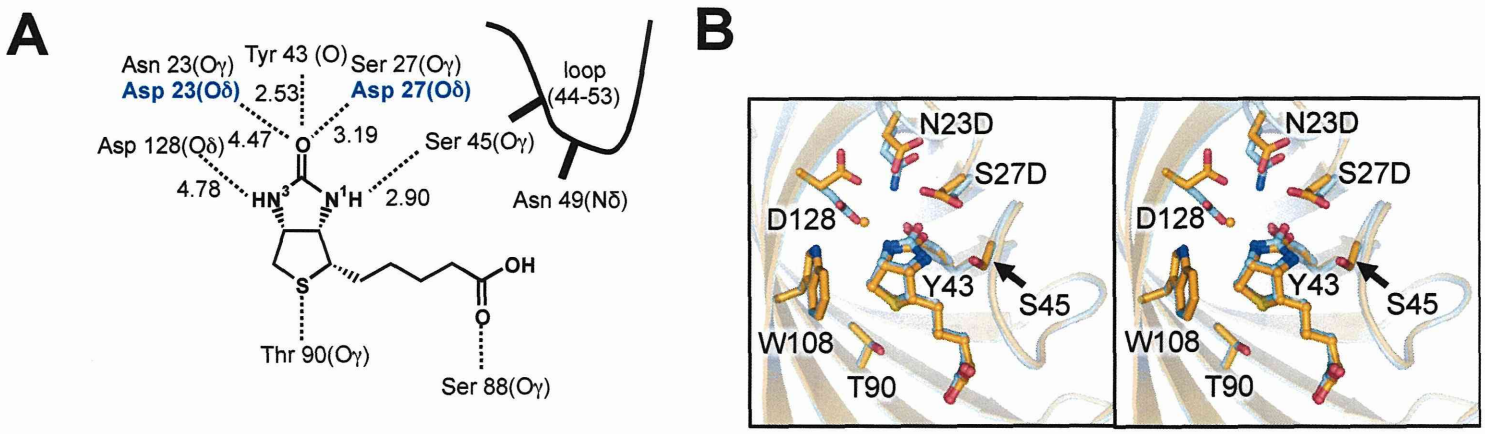
Figure Legends

Fig. 1. Binding mode of BTN in V21. (A) The distance between BTN and V21 are shown by the black dotted lines. The position numbers of nitrogen atoms are shown as superscripts. Residues in LISA-314 (*38*) are indicated in black and substituted residues in V21 are in blue. The distances (Å) between residues of V21 and BTN are represented schematically. (B) Superposition of the BTN bound to V21 (orange) was performed with the BTN bound to LISA-314 (PDB ID: 3WYQ (*38*); cyan). By introducing N23D/S27D substitutions, two hydrogen bonds, which were formed around the ureido oxygen of BTN in LISA-314, were lost in V21. The ureido oxygen of BTN in V21 moved away from Asp23 and Asp27.

Fig. 2. Binding mode of IMNtail in V21. (A) Structural formula of IMNtail. The position numbers of nitrogen atoms are shown in superscripts. (B) Structure of V21 tetramer (ribbon model) in complex with IMNtail (stick model). (C) Superposition of BTNtail-bound V21 (green) and IMNtail-bound V21 (magenta) structures. Unlike the ureido oxygen of BTNtail, the N^2 nitrogen of IMNtail interacts with Asp27. Ser45 forms hydrogen bond to IMNtail *via* a water molecule, though forming direct hydrogen bonds toward BTNtail.

Fig. 3 Binding mode of IMNtail in V212. (A) Superposition of IMNtail-bound V212 (yellow) and IMNtail-bound V21 (magenta) structures. The binding loop composed of 45–52 in V212 was formed differently. (B) Structural superposition of the V212-IMNtail protomer (yellow) with the V21-IMNtail (magenta), SA-WT-BTN (green) and SA-WT-apo (blue) protomers. For clarification, the ligand is only depicted for IMNtail bound in V212 as a stick model. The loop composed of residues 45–52 is indicated with a circle of red dots. The binding loop in V212 superposes well with the ligand free-SA-WT. (C) Schematic representations of the interactions between IMNtail and (i) V21 or (ii) V212 are shown. The position numbers of nitrogen atoms are shown in superscripts. The loop in V212 is presented with the dash-dot lines, because it forms an open conformation and does not interact with the ligand. The distances (Å) between amino-acid residues and IMNtail are represented by dotted lines. The distance between the carboxyl oxygen of Asp23 and the N^2 nitrogen of IMNtail in V212 is shorter than that in V21 by 0.86 Å. This indicates that the weak hydrogen bond in V21 is strengthened in V212.

Fig. 1


A
Asn 23(Oγ)
Asp 23(Oδ)
Tyr 43 (O)
2.53
Ser 27(Oγ)
Asp 27(Oδ)
loop
(44-53)
Asp 128(Oδ)
4.47
3.19
Ser 45(Oγ)
4.78
HN3
N1H
2.90
Asn 49(Nδ)
OH
O
S
Thr 90(Oγ)
Ser 88(Oγ)
B
N23D
S27D
D128
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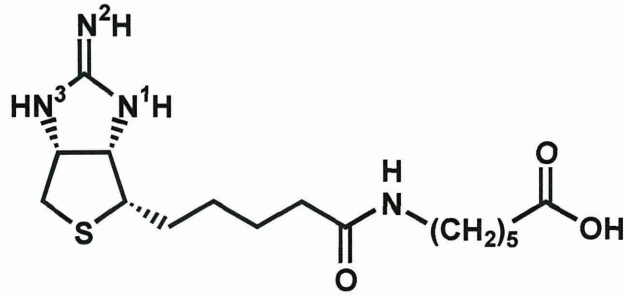
S45
W108
T90
N23D
S27D
D128
Y43
S45
W108
T90

Fig. 2

A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S
H
N
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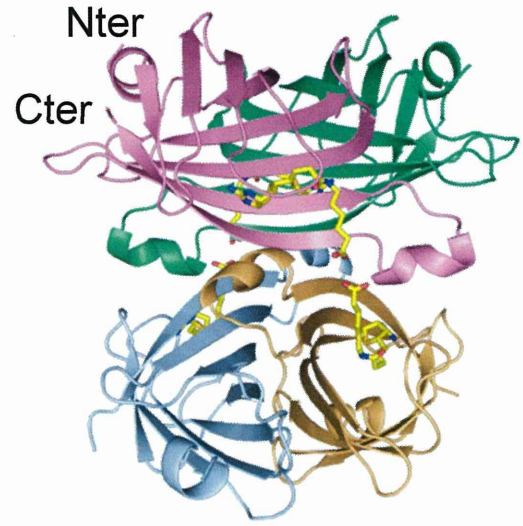
O
OH
O

B


Nter
Cter

C

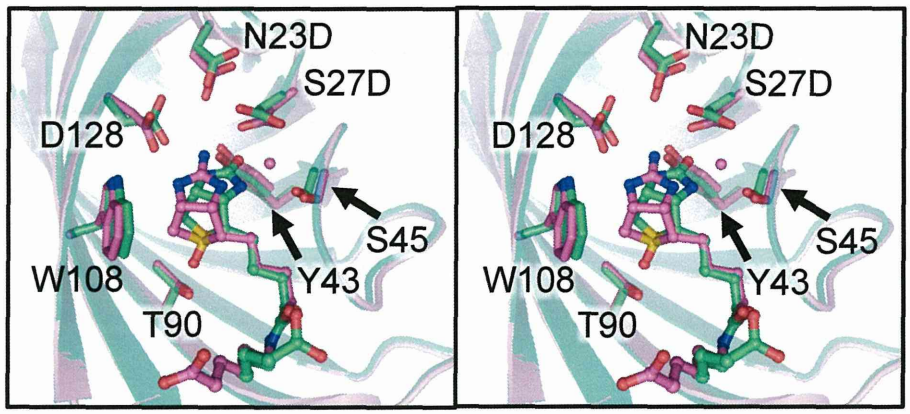
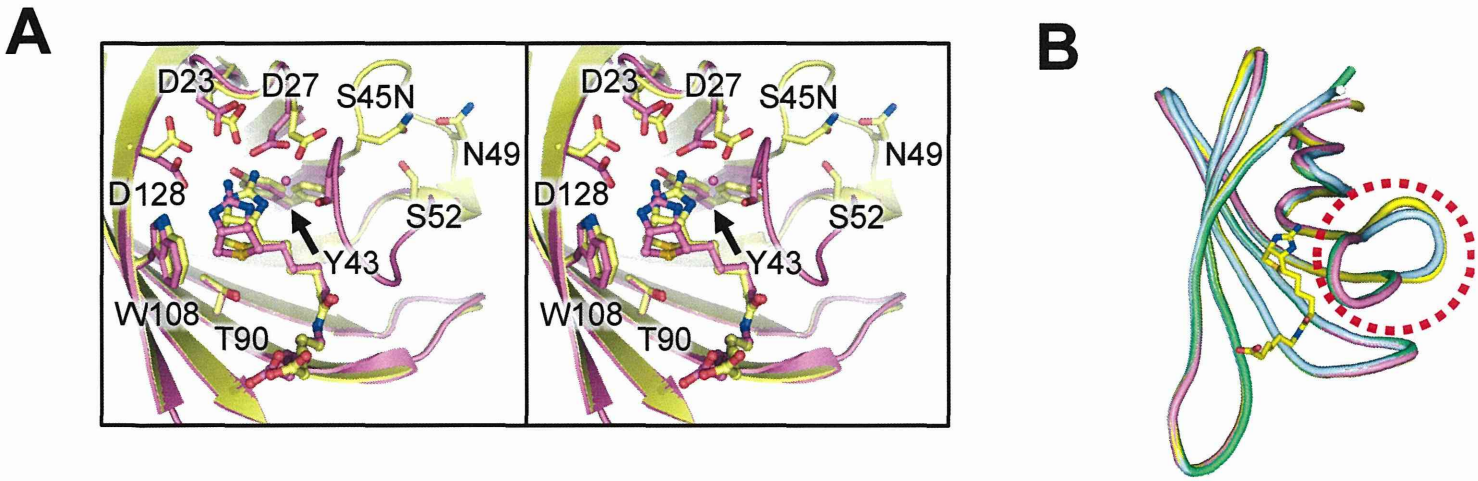

N23D
S27D
D128
S45
W108
Y43
T90
N23D
S27D
D128
S45
W108
Y43
T90

Fig. 3


A
D23
D27
S45N
N49
D128
S52
Y43
W108
T90
B
C
(i)
Asp 23(Oδ)
3.61
loop
(45-52)
Asp 27(Oδ)
Asp 128(Oδ)
2.73
Ser 45(Oγ)
3.06
2.85
2.46
2.76
Asn 49(Nδ)
2.71
3.26
3.00
Thr 90(Oγ)
Ser 88(Oγ)
(ii)
loop
(45-52)
Asp 128(Oδ)
Asp 23(Oδ)
2.52
2.75
Asp 27(Oδ)
2.90
2.71
2.77
2.87
3.31
2.88
Thr 90(Oγ)
Ser 88(Oγ)