

# SUPPLEMENTARY INFORMATION

## **Ligand entry in human ileal bile acid-binding protein is mediated by histidine protonation**

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**Table S1** Exchange parameters derived from individual fit of  $^{15}\text{N}$  relaxation dispersion curves obtained for [80%  $^2\text{H}$ , 99%  $^{15}\text{N}$ -labeled] *apo* human I-BABP in 20 mM K-phosphate, 50 mM KCl, 0.05%  $\text{NaN}_3$ , 10%  $\text{D}_2\text{O}$ , pH=5.4 at 283 K.

Residue	$k_{\text{ex}}$ ( $\text{s}^{-1}$ )	$p_b$ (%)	$ \Delta\omega $ (Hz)	$R_{\text{ex}}$ (Hz)	$\Phi$ ( $\text{Hz}^2$ )
T3	54 $\pm$ 22	8 $\pm$ 5	73 $\pm$ 14	4.4 $\pm$ 0.4	405 $\pm$ 277
T73	10 $\pm$ 44	19 $\pm$ 6	232 $\pm$ 48	2.9 $\pm$ 0.8	8284 $\pm$ 3619
G76	1170 $\pm$ 322	21 $\pm$ 8	25 $\pm$ 17	3.4 $\pm$ 0.6	106 $\pm$ 110
V91	1852 $\pm$ 216	24 $\pm$ 3	44 $\pm$ 5	7.4 $\pm$ 0.5	358 $\pm$ 74
Y97	1057 $\pm$ 170	0.3 $\pm$ 0.4	195 $\pm$ 29	2.0 $\pm$ 0.6	127 $\pm$ 99
T100	479 $\pm$ 63	0.7 $\pm$ 0.2	159 $\pm$ 19	2.6 $\pm$ 0.3	172 $\pm$ 57
S101	651 $\pm$ 104	23 $\pm$ 5	18 $\pm$ 5	3.2 $\pm$ 0.3	57 $\pm$ 26
E110	480 $\pm$ 94	0.4 $\pm$ 0.3	171 $\pm$ 21	1.5 $\pm$ 0.3	113 $\pm$ 87

**Table S2** Exchange parameters derived from global fit of  $^{15}\text{N}$  relaxation dispersion curves obtained for [80%  $^2\text{H}$ , 99%  $^{15}\text{N}$ -labeled] *apo* human I-BABP in 20 mM K-phosphate, 50 mM KCl, 0.05%  $\text{NaN}_3$ , 10%  $\text{D}_2\text{O}$ , pH=6.3 at 283 K.

Residue	$ \Delta\omega $ (Hz)	$R_{\text{ex}}$ (Hz)	$\Phi$ ( $\text{Hz}^2$ )
	<b>'slow' cluster</b> $k_{\text{ex}} = 294 \pm 40 \text{ s}^{-1}$ $p_b = 1.8 \pm 0.2 \%$		
E11	47 $\pm$ 4	2.7 $\pm$ 0.2	42 $\pm$ 7
N13	34 $\pm$ 4	1.9 $\pm$ 0.3	23 $\pm$ 5
K35	55 $\pm$ 4	3.1 $\pm$ 0.2	60 $\pm$ 9
T38	40 $\pm$ 4	2.2 $\pm$ 0.2	30 $\pm$ 5
E39	45 $\pm$ 4	2.6 $\pm$ 0.2	39 $\pm$ 7
V40	43 $\pm$ 4	2.5 $\pm$ 0.2	36 $\pm$ 6
H57	60 $\pm$ 5	3.3 $\pm$ 0.2	70 $\pm$ 11
T58	97 $\pm$ 9	4.2 $\pm$ 0.2	183 $\pm$ 31
T60	89 $\pm$ 8	4.1 $\pm$ 0.2	154 $\pm$ 26
N61	38 $\pm$ 4	2.2 $\pm$ 0.2	29 $\pm$ 5
K62	44 $\pm$ 4	2.5 $\pm$ 0.2	38 $\pm$ 6
F63	39 $\pm$ 4	2.2 $\pm$ 0.3	29 $\pm$ 5
	<b>'fast' cluster</b> $k_{\text{ex}} = 836 \pm 59 \text{ s}^{-1}$ $p_b = 3.1 \pm 0.2 \%$		
E7	47 $\pm$ 2	2.6 $\pm$ 0.2	65 $\pm$ 6
I71	39 $\pm$ 3	1.8 $\pm$ 0.1	45 $\pm$ 6
Q72	42 $\pm$ 2	2.1 $\pm$ 0.2	52 $\pm$ 5
T73	170 $\pm$ 5	15.8 $\pm$ 0.1	857 $\pm$ 66
M74	65 $\pm$ 4	4.7 $\pm$ 0.2	126 $\pm$ 14
T78	59 $\pm$ 3	4.0 $\pm$ 0.1	105 $\pm$ 10
A81	49 $\pm$ 4	2.9 $\pm$ 0.2	72 $\pm$ 10
T82	56 $\pm$ 3	3.7 $\pm$ 0.2	94 $\pm$ 9
G88	67 $\pm$ 5	4.9 $\pm$ 0.1	133 $\pm$ 16
L90	43 $\pm$ 4	2.2 $\pm$ 0.2	54 $\pm$ 8
V91	210 $\pm$ 10	18.2 $\pm$ 0.1	1302 $\pm$ 121
N96	86 $\pm$ 6	7.2 $\pm$ 0.1	219 $\pm$ 26
Y97	67 $\pm$ 4	4.9 $\pm$ 0.1	133 $\pm$ 14
H98	84 $\pm$ 7	7.0 $\pm$ 0.1	211 $\pm$ 28
Q99	63 $\pm$ 5	4.5 $\pm$ 0.2	119 $\pm$ 15
T100	60 $\pm$ 6	4.1 $\pm$ 0.1	108 $\pm$ 17
S101	49 $\pm$ 5	2.9 $\pm$ 0.2	73 $\pm$ 12
E102	47 $\pm$ 4	2.6 $\pm$ 0.2	66 $\pm$ 9
V109	53 $\pm$ 7	3.3 $\pm$ 0.2	83 $\pm$ 16
E110	42 $\pm$ 2	2.1 $\pm$ 0.2	51 $\pm$ 5
T113	34 $\pm$ 2	1.5 $\pm$ 0.1	35 $\pm$ 4
G115	44 $\pm$ 2	2.4 $\pm$ 0.2	58 $\pm$ 5
Y119	45 $\pm$ 2	2.4 $\pm$ 0.1	60 $\pm$ 5
R121	50 $\pm$ 2	2.9 $\pm$ 0.2	74 $\pm$ 6

**Table S3** Exchange parameters derived from global fit of  $^{15}\text{N}$  relaxation dispersion curves obtained for [80%  $^2\text{H}$ , 99%  $^{15}\text{N}$ -labeled] *apo* human I-BABP in 20 mM K-phosphate, 50 mM KCl, 0.05%  $\text{NaN}_3$ , 10%  $\text{D}_2\text{O}$ , pH=6.8 at 283 K.

Residue	$ \Delta\omega $ (Hz)	$R_{\text{ex}}$ (Hz)	$\Phi$ (Hz <sup>2</sup> )
	<b><math>k_{\text{ex}} = 1467 \pm 161 \text{ s}^{-1}</math> <math>p_b = 4.4 \pm 0.3 \%</math></b>		
G4	45 $\pm$ 2	2.4 $\pm$ 0.2	95 $\pm$ 9
E7	51 $\pm$ 3	3.0 $\pm$ 0.1	119 $\pm$ 13
S10	51 $\pm$ 2	3.0 $\pm$ 0.1	118 $\pm$ 10
E11	62 $\pm$ 4	4.3 $\pm$ 0.2	175 $\pm$ 20
E16	50 $\pm$ 3	2.9 $\pm$ 0.2	115 $\pm$ 13
S25	58 $\pm$ 6	3.9 $\pm$ 0.2	156 $\pm$ 25
K35	47 $\pm$ 7	2.5 $\pm$ 0.3	100 $\pm$ 22
I36	59 $\pm$ 8	4.0 $\pm$ 0.3	160 $\pm$ 33
Q45	64 $\pm$ 4	4.7 $\pm$ 0.1	192 $\pm$ 21
F47	47 $\pm$ 5	2.6 $\pm$ 0.2	104 $\pm$ 17
H52	50 $\pm$ 4	2.9 $\pm$ 0.2	116 $\pm$ 15
H57	48 $\pm$ 6	2.7 $\pm$ 0.1	107 $\pm$ 20
T60	46 $\pm$ 5	2.5 $\pm$ 0.1	99 $\pm$ 17
N61	45 $\pm$ 7	2.4 $\pm$ 0.3	95 $\pm$ 22
F63	43 $\pm$ 5	2.2 $\pm$ 0.2	86 $\pm$ 15
T73	102 $\pm$ 9	10.9 $\pm$ 0.4	483 $\pm$ 69
G88	71 $\pm$ 8	5.6 $\pm$ 0.3	231 $\pm$ 40
V91	87 $\pm$ 6	8.3 $\pm$ 0.3	353 $\pm$ 42
N96	85 $\pm$ 8	8.0 $\pm$ 0.4	337 $\pm$ 50
Y97	96 $\pm$ 10	9.8 $\pm$ 0.3	424 $\pm$ 69
H98	71 $\pm$ 8	5.7 $\pm$ 0.2	233 $\pm$ 40
Q99	68 $\pm$ 8	5.1 $\pm$ 0.2	211 $\pm$ 38
T100	81 $\pm$ 10	7.3 $\pm$ 0.2	306 $\pm$ 57
S101	66 $\pm$ 9	4.9 $\pm$ 0.3	202 $\pm$ 41
E102	49 $\pm$ 7	2.8 $\pm$ 0.2	111 $\pm$ 24
I103	47 $\pm$ 7	2.5 $\pm$ 0.2	100 $\pm$ 22
T118	56 $\pm$ 6	3.6 $\pm$ 0.2	144 $\pm$ 24
Y119	48 $\pm$ 6	2.7 $\pm$ 0.3	107 $\pm$ 20

**Table S4** Exchange parameters derived from global fit of  $^{15}\text{N}$  relaxation dispersion curves obtained for [80%  $^2\text{H}$ , 99%  $^{15}\text{N}$ -labeled] *apo* human I-BABP in 20 mM K-phosphate, 50 mM KCl, 0.05%  $\text{NaN}_3$ , 10%  $\text{D}_2\text{O}$ , pH=8.0 at 283 K.

Residue	$ \Delta\omega $ (Hz)	$R_{\text{ex}}$ (Hz)	$\Phi$ (Hz <sup>2</sup> )
	<b><math>k_{\text{ex}} = 1453 \pm 194 \text{ s}^{-1}</math> <math>p_b = 5.7 \pm 0.4 \%</math></b>		
Y14	42±7	2.5±0.3	96±24
E16	37±5	1.9±0.3	73±15
K30	49±8	3.2±0.3	128±31
F63	30±6	1.3±0.2	49±14
I71	36±7	1.8±0.2	69±20
T73	111±12	14.9±0.2	665±112
A81	53±4	3.8±0.2	152±19
T82	66±5	5.8±0.3	236±30
L90	41±5	2.3±0.2	90±17
V91	129±15	19.3±0.2	906±162
N93	67±5	6.0±0.2	244±31
Y97	52±7	3.6±0.3	144±29
H98	82±9	8.8±0.2	367±63
Q99	55±5	4.1±0.3	164±24
T100	75±7	7.3±0.2	300±45
S101	46±6	3.0±0.2	117±23
T113	56±7	4.3±0.2	169±32
Y119	68±7	6.2±0.3	251±41