

# Supplementary Data for Non-Native $\alpha$ -Helices in the Initial Folding Intermediate Facilitate the Ordered Assembly of the $\beta$ -Barrel in $\beta$ -Lactoglobulin

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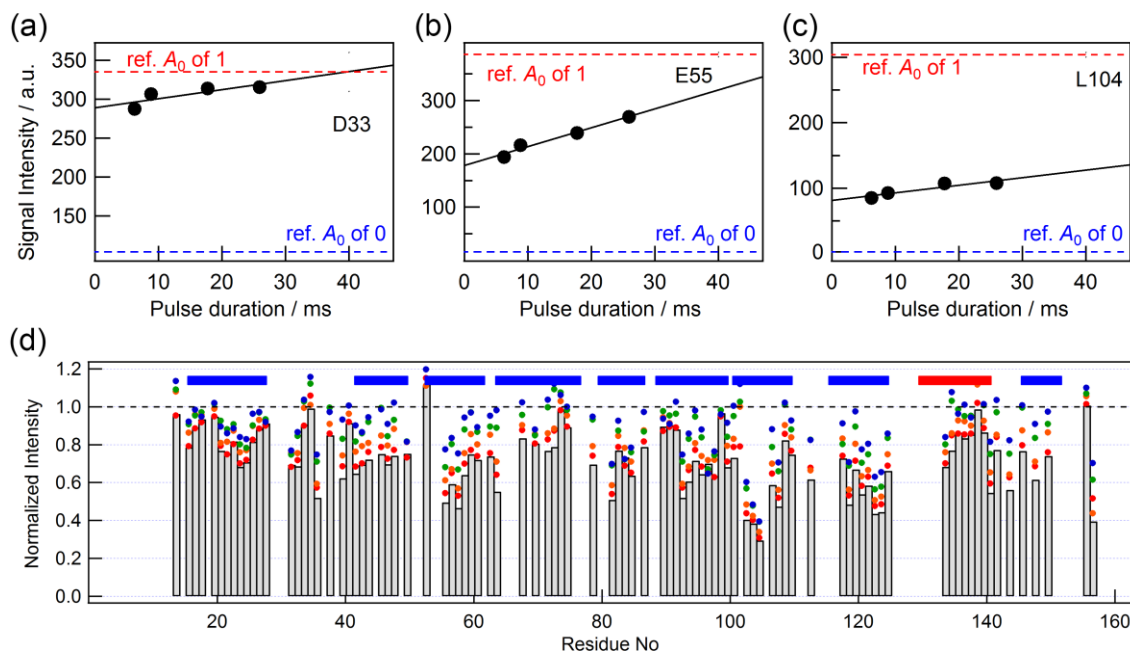
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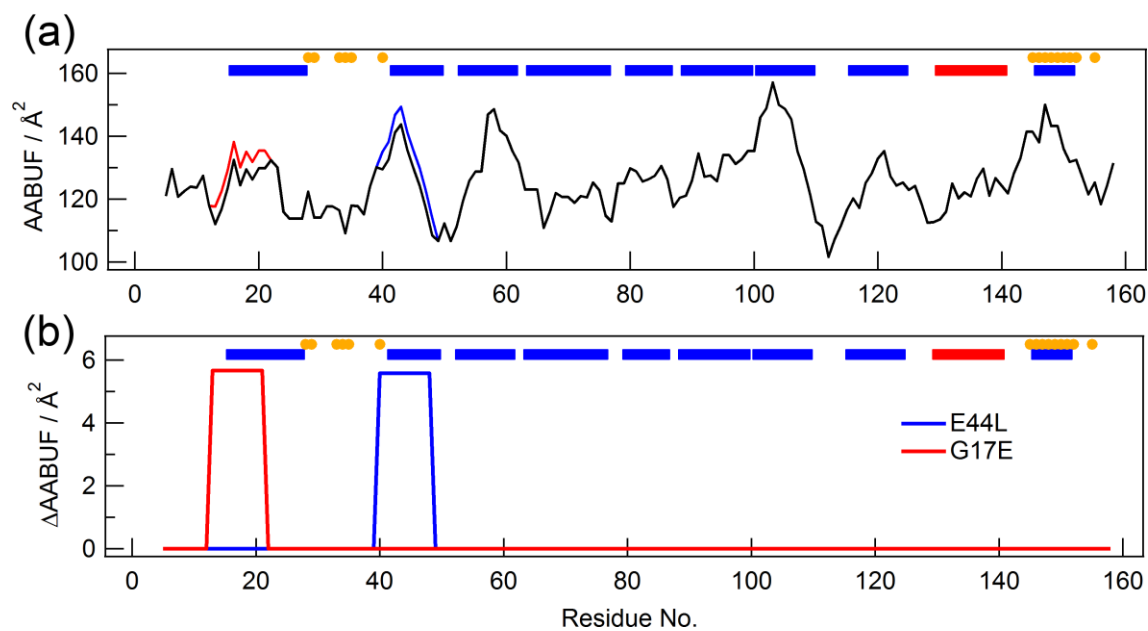
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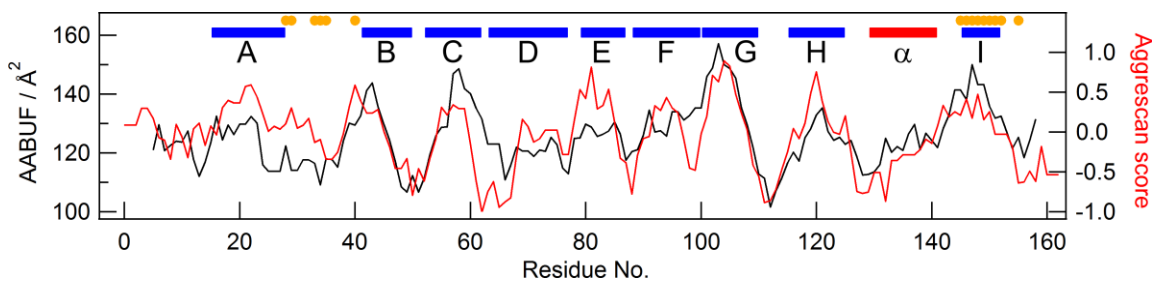
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**Figure S1.** Data analysis procedures of the quenched-flow pH-pulse D/H exchange experiments for the estimation of the zero-extrapolated intensities ( $A_0$ ) for WT\*  $\beta$ LG. (a-c) Representative data of signal intensities for D33 (a), E55 (b), and L104 (c) at different durations of pH pulse. The solid line is the extrapolation line, where the y-axis section indicates the pre-normalized  $A_0$  value. The red and blue broken lines indicate reference intensities for the initial (no exchange) and final (fully exchanged) states, respectively. (d) Normalized  $A_0$  values for each residue (indicated as bars). The normalized signal intensities at different pulse durations for each residue are also plotted, where the duration times are 6.2 (red), 8.8 (orange), 17.7 (green), and 25.9 (blue) ms, respectively.



**Figure S2.** (a) Comparison of the AABUF (Average Area Buried Upon Folding) patterns of WT\* (black), E44L (Blue) and G17E (red). (b) Difference of AABUF scores between E44L and WT\* (blue) and difference between G17E and WT\* (red), respectively. The per-residue AABUF was calculated using the method described by Rose *et al.*<sup>1</sup>, utilizing the ExpASy website, with a nine-residue moving-average window.



**Figure S3.** Plots of AABUF (black) and Aggrescan scores (red) from the amino acid sequence of  $\beta$ LG. The Aggrescan score was a4v (window average of the amino-acid aggregation-propensity value) using the method by Ventura and co-workers.<sup>2, 3</sup> The a4v values calculated for each residue were obtained utilizing the Aggrescan website.

**Table S1.** HSQC signal assignment of the  $\beta$ LG mutants in the native conditions

	WT*		E44L		G17E	
	$\delta_N$ /ppm	$\delta_H$ /ppm	$\delta_N$ /ppm	$\delta_H$ /ppm	$\delta_N$ /ppm	$\delta_H$ /ppm
A1	-	-	-	-	-	-
I2	-	-	-	-	-	-
V3	-	-	-	-	-	-
T4	-	-	-	-	-	-
Q5	-	-	-	-	-	-
T6	111.5	7.77	111.4	7.78	-	-
M7	120.4	7.77	-	-	120.3	7.83
K8	127.9	8.71	128.0	8.71	128.8	8.70
G9	109.8	8.59	109.9	8.60	109.8	8.67
L10	120.3	7.58	120.3	7.58	-	-
D11	127.1	8.63	127.1	8.63	127.0	8.65
I12	122.9	8.87	123.3	8.90	122.7	8.73
Q13	117.1	8.00	117.1	8.00	117.5	8.01
K14	113.9	7.23	113.9	7.23	113.5	7.15
V15	110.3	7.38	110.4	7.38	108.6	7.40
A16	120.9	6.80	120.9	6.79	-	-
G/E17	110.3	9.20	110.3	9.21	-	-
T18	118.4	8.26	118.4	8.26	118.4	8.27
W19	124.3	7.45	124.7	7.48	-	-
Y20	112.1	9.06	111.6	9.02	112.6	8.90
S21	119.4	9.87	119.4	9.94	119.3	9.78
L22	131.6	9.27	132.0	9.29	130.8	9.20
A23	115.8	7.74	115.9	7.74	-	-
M24	114.6	9.03	114.5	9.06	114.6	9.07
A25	123.4	9.30	123.5	9.31	123.5	9.31
A26	119.7	8.25	119.7	8.26	119.7	8.27
S27	116.4	8.32	116.3	8.33	116.3	8.36
D28	-	-	120.4	7.78	-	-
I29	124.2	8.78	124.2	8.79	124.2	8.79
S30	114.3	7.99	114.3	7.99	114.4	8.00
L31	117.2	7.61	117.3	7.61	117.3	7.60
L32	111.3	7.05	111.3	7.05	111.2	7.05
D33	118.7	7.76	118.6	7.75	118.7	7.76
A34	120.8	7.64	120.8	7.66	120.8	7.64
Q35	121.5	9.03	121.6	9.05	-	-
S36	107.6	7.72	107.6	7.73	107.7	7.73
A37	126.3	7.54	126.3	7.54	126.3	7.53
P38	-	-	-	-	-	-
L39	114.2	7.78	114.3	7.77	114.1	7.79
R40	122.3	7.41	122.3	7.38	122.2	7.40
V41	117.0	6.96	117.1	6.95	117.2	6.99
Y42	120.6	8.62	120.5	8.62	120.8	8.66
V43	125.3	8.73	124.8	8.74	125.2	8.64
E/L44	122.0	9.00	-	-	122.0	8.99
E45	116.4	7.62	115.2	7.49	115.7	7.70
L46	122.0	8.67	121.9	8.63	122.2	8.58
K47	122.6	9.17	122.6	9.15	-	-
P48	-	-	-	-	-	-
T49	114.4	8.61	114.5	8.62	114.2	8.59
P50	-	-	-	-	-	-

E51	112.3	7.54	112.3	7.53	112.3	7.54
G52	107.3	8.29	107.3	8.29	107.2	8.31
D53	115.5	7.18	115.5	7.18	115.8	7.20
L54	119.4	8.83	119.4	8.83	119.5	8.81
E55	126.1	8.56	126.0	8.59	126.7	8.60
I56	126.5	9.26	126.5	9.23	126.0	9.19
L57	128.6	8.49	128.7	8.45	128.5	8.64
L58	123.3	9.04	123.6	9.11	123.7	9.09
Q59	117.7	9.25	117.7	9.37	117.8	9.27
K60	121.5	8.96	120.9	8.99	121.5	8.94
W61	129.9	9.77	129.3	9.77	129.7	9.74
E62	127.4	8.76	127.3	8.77	127.4	8.76
N63	121.9	8.97	122.0	9.02	122.4	8.96
D64	110.9	8.15	110.9	8.19	110.9	8.12
E65	114.5	6.73	114.5	6.74	114.5	6.75
C66	120.2	8.68	120.4	8.68	120.2	8.68
A67	133.6	8.98	133.7	8.92	133.3	8.97
Q68	118.5	8.42	118.4	8.41	118.7	8.42
K69	123.5	9.17	123.8	9.13	123.7	9.18
K70	123.6	8.54	123.2	8.50	124.4	8.55
I71	124.3	8.97	123.9	8.95	125.0	9.08
I72	125.6	8.50	125.2	8.48	125.3	8.47
A73	133.5	9.26	133.4	9.27	133.5	9.33
E74	124.5	9.42	124.5	9.44	124.5	9.42
K75	123.3	8.62	123.2	8.61	123.1	8.57
T76	112.7	8.45	112.7	8.45	112.7	8.47
K77	116.5	8.24	116.5	8.24	116.5	8.23
I78	122.4	7.98	122.4	7.98	122.3	7.98

P79	-	-	-	-	-	-
A80	115.4	7.91	115.4	7.90	115.5	7.97
V81	117.4	7.28	117.4	7.29	117.4	7.30
F82	126.4	9.56	126.4	9.56	126.4	9.56
K83	123.5	9.50	123.4	9.51	123.5	9.54
I84	116.2	7.69	116.3	7.69	116.2	7.70
D85	119.6	8.77	119.6	8.80	119.9	8.76
A86	121.9	7.20	121.9	7.19	121.9	7.21
L87	114.5	9.00	114.5	9.00	114.7	9.01
N88	111.3	8.71	111.4	8.71	111.4	8.71
E89	117.4	8.26	117.4	8.26	117.4	8.27
N90	115.7	9.11	115.6	9.10	115.7	9.11
K91	121.7	8.58	121.7	8.57	121.7	8.58
V92	121.5	9.03	121.4	9.01	-	-
L93	126.1	9.44	126.1	9.45	126.0	9.42
V94	123.8	9.32	123.9	9.33	123.8	9.33
L95	128.2	8.50	128.2	8.50	127.9	8.52
D96	110.3	7.19	110.3	7.19	110.5	7.18
T97	119.3	7.75	119.3	7.75	119.2	7.75
D98	123.5	6.81	123.5	6.82	123.6	6.80
Y99	117.6	9.01	117.6	9.01	116.7	9.01
K100	119.9	8.96	119.9	8.96	120.3	8.89
K101	119.5	9.56	119.5	9.56	-	-
Y102	116.8	9.14	116.8	9.14	116.7	9.14
L103	124.8	8.94	124.8	8.94	124.8	8.89
L104	125.1	9.40	125.1	9.40	125.1	9.34
F105	121.6	9.05	121.4	9.01	121.6	9.05
C106	116.8	9.63	116.8	9.64	117.0	9.64

M107	120.7	9.94	120.7	9.94	120.5	9.90
E108	113.7	8.38	113.6	8.38	113.7	8.39
N109	120.2	9.41	120.3	9.41	120.2	9.40
S110	122.1	9.79	122.1	9.79	122.1	9.80
A111	123.5	8.33	123.5	8.33	123.5	8.33
E112	113.6	7.73	113.6	7.74	113.5	7.73
P113	-	-	-	-	-	-
E114	115.1	8.32	115.1	8.32	115.0	8.33
Q115	116.1	7.84	116.1	7.84	116.0	7.85
S116	109.6	7.55	109.6	7.55	109.7	7.55
L117	126.2	7.22	126.3	7.21	126.4	7.24
V118	126.3	8.81	126.3	8.81	126.2	8.82
C119	121.9	9.35	121.9	9.35	122.1	9.38
Q120	118.5	9.29	118.5	9.28	118.6	9.35
A121	120.6	7.74	120.5	7.74	121.0	7.76
L122	122.5	9.69	122.5	9.69	122.7	9.52
V123	110.9	9.36	110.8	9.34	111.3	9.31
R124	119.8	7.94	119.8	7.95	119.9	7.92
T125	109.0	7.55	109.3	7.55	109.4	7.49
P126	-	-	-	-	-	-
E127	118.9	8.26	118.6	8.24	119.0	8.25
V128	117.6	8.14	117.7	8.14	117.8	8.12
D129	-	-	-	-	-	-
D130	122.9	8.99	122.8	8.87	122.7	8.98
E131	121.1	8.40	121.1	8.41	121.3	8.34
A132	120.5	7.82	120.5	7.84	120.3	7.90
L133	115.8	7.73	115.9	7.79	116.1	7.75
E134	119.9	8.08	119.9	8.07	119.9	8.07

K135	119.2	8.14	119.2	8.13	119.3	8.11
F136	122.1	8.52	122.0	8.53	122.0	8.48
D137	117.0	8.62	117.0	8.62	117.1	8.67
K138	119.2	7.78	119.2	7.77	119.3	7.79
A139	121.8	7.85	121.8	7.86	121.8	7.86
L140	114.6	7.54	114.5	7.54	114.6	7.54
K141	119.6	7.12	119.6	7.12	119.7	7.13
A142	118.4	7.85	118.4	7.83	118.4	7.86
L143	119.7	7.69	119.8	7.68	119.8	7.69
P144	-	-	-	-	-	-
M145	117.9	7.67	118.0	7.67	118.1	7.68
H146	117.9	8.80	117.9	8.80	118.0	8.79
I147	117.5	7.71	117.4	7.70	117.5	7.71
R148	126.6	8.35	126.6	8.35	126.6	8.35
L149	124.2	9.21	124.1	9.22	124.1	9.19
S150	116.5	8.24	116.5	8.24	116.5	8.23
F151	119.9	8.02	119.7	8.03	119.5	8.07
N152	119.6	8.78	119.6	8.80	119.3	8.78
P153	-	-	-	-	-	-
T154	111.9	7.82	111.9	7.82	111.7	7.80
Q155	119.3	7.82	119.4	7.83	119.5	7.85
L156	116.2	7.55	115.8	7.46	-	-
E157	112.4	7.06	112.2	7.04	-	-
E158	118.8	7.18	118.9	7.10	-	-
Q159	-	-	-	-	-	-
C160	-	-	-	-	-	-
H161	-	-	-	-	-	-
I162	-	-	-	-	-	-

**Table S2.** HSQC signal assignment of the  $\beta$ LG mutants in the unfolding conditions in 8 M urea

	WT*		E44L		G17E								
	$\delta_N$ /ppm	$\delta_H$ /ppm	$\delta_N$ /ppm	$\delta_H$ /ppm	$\delta_N$ /ppm	$\delta_H$ /ppm							
A1	-	-	-	-	-	-	A25	124.8	8.00	124.6	8.05	124.7	7.96
I2	120.9	8.44	120.9	8.51	121.0	8.42	A26	123.0	8.03	122.9	8.09	123.0	7.99
V3	123.4	8.04	123.3	8.10	123.4	8.02	S27	114.0	7.99	113.9	8.06	114.0	7.97
T4	118.3	8.01	118.2	8.07	118.3	7.99	D28	120.8	8.21	120.7	8.27	120.9	8.19
Q5	-	-	-	-	-	-	I29	120.3	7.78	120.2	7.84	120.3	7.75
T6	115.5	8.06	115.4	8.12	115.5	8.04	S30	118.8	8.09	118.7	8.14	118.8	8.06
M7	122.8	8.23	122.7	8.30	122.8	8.21	L31	124.1	7.98	124.1	8.04	124.2	7.96
K8	122.8	8.20	122.6	8.25	122.8	8.17	L32	121.5	7.89	121.5	7.97	121.6	7.89
G9	109.8	8.19	109.7	8.25	109.8	8.17	D33	119.1	8.14	118.9	8.23	119.0	8.15
L10	121.1	7.88	121.0	7.94	121.2	7.86	A34	123.9	7.95	123.9	8.02	124.0	7.93
D11	120.4	8.39	120.1	8.47	120.3	8.39	Q35	118.4	8.05	118.4	8.11	118.5	8.03
I12	121.2	7.87	121.2	7.94	121.4	7.86	S36	116.4	7.99	116.3	8.06	116.5	7.98
Q13	124.0	8.22	124.0	8.28	124.2	8.20	A37	126.3	8.03	126.3	8.10	126.4	8.02
K14	123.1	8.15	123.1	8.22	123.2	8.13	P38	-	-	-	-	-	-
V15	121.3	7.96	121.3	8.04	121.4	7.94	L39	122.5	8.09	122.4	8.16	122.6	8.07
A16	127.8	8.22	127.8	8.29	127.6	8.14	R40	122.8	8.25	122.7	8.32	122.8	8.23
G/E17	107.9	8.05	107.8	8.12	-	-	V41	121.5	7.94	121.3	8.01	121.5	7.93
T18	113.2	7.77	113.2	7.84	114.8	7.89	Y42	124.8	8.20	124.5	8.24	124.8	8.18
W19	123.2	7.98	123.1	8.05	122.9	7.90	V43	123.0	7.95	-	-	123.0	7.94
Y20	121.4	7.63	121.3	7.70	121.2	7.62	E/L44	124.2	8.18	126.1	8.14	124.2	8.15
S21	116.8	7.92	116.7	7.99	116.8	7.91	E45	122.2	8.22	122.1	8.29	122.2	8.20
L22	124.4	8.00	124.4	8.07	124.3	7.97	L46	124.0	8.12	124.2	8.17	124.1	8.10
A23	123.9	7.98	123.8	8.05	123.8	7.96	K47	123.5	8.30	123.4	8.37	123.6	8.28
M24	119.1	7.96	119.0	8.02	119.1	7.93	P48	-	-	-	-	-	-
							T49	116.9	8.13	116.8	8.18	117.0	8.11
							P50	-	-	-	-	-	-



E51	120.2	8.20	120.0	8.25	120.3	8.18
G52	109.3	8.17	109.2	8.23	109.3	8.15
D53	118.6	8.08	118.4	8.16	118.5	8.07
L54	-	-	-	-	-	-
E55	120.8	8.09	120.8	8.16	120.9	8.08
I56	121.5	7.84	121.5	7.92	121.6	7.83
L57	125.5	8.00	125.5	8.07	125.6	7.99
L58	-	-	-	-	-	-
Q59	121.5	8.27	121.4	8.34	121.6	8.25
K60	122.1	8.06	122.0	8.13	122.1	8.05
W61	-	-	-	-	-	-
E62	-	-	-	-	-	-
N63	119.3	8.24	119.2	8.30	119.3	8.22
D64	119.5	8.18	119.1	8.26	119.3	8.18
E65	120.2	8.12	120.0	8.17	120.1	8.08
C66	-	-	-	-	-	-
A67	-	-	-	-	-	-
Q68	-	-	-	-	-	-
K69	122.9	8.17	123.0	8.24	123.1	8.16
K70	123.4	8.22	123.4	8.30	123.6	8.21
I71	123.7	8.15	123.7	8.22	123.8	8.14
I72	126.2	8.14	126.2	8.21	126.4	8.13
A73	128.6	8.17	128.5	8.24	128.7	8.15
E74	120.4	8.13	120.2	8.19	120.4	8.10
K75	122.8	8.27	122.8	8.34	123.0	8.25
T76	115.7	7.98	115.6	8.06	115.7	7.97
K77	124.0	8.23	124.0	8.29	124.2	8.20
I78	-	-	-	-	-	-

P79	-	-	-	-	-	-
A80	124.5	8.09	124.4	8.16	124.5	8.08
V81	118.6	7.76	118.5	7.82	118.6	7.74
F82	124.0	8.08	123.9	8.14	124.0	8.06
K83	123.9	8.12	123.7	8.18	123.9	8.10
I84	122.5	8.05	122.7	8.16	122.9	8.07
D85	123.5	8.39	123.1	8.48	123.3	8.40
A86	124.4	8.01	124.4	8.08	124.6	7.99
L87	120.3	7.91	120.4	7.97	120.5	7.88
N88	118.8	8.17	118.8	8.25	118.9	8.16
E89	120.4	8.08	120.3	8.15	120.4	8.06
N90	119.2	8.08	119.0	8.14	119.1	8.06
K91	121.4	8.01	121.3	8.08	121.5	8.00
V92	121.5	7.95	121.3	8.01	121.5	7.93
L93	-	-	-	-	-	-
V94	122.7	8.06	122.6	8.12	122.8	8.04
L95	126.2	8.16	126.1	8.21	126.2	8.12
D96	120.3	8.34	120.0	8.44	120.1	8.35
T97	113.9	7.92	113.8	7.99	114.0	7.90
D98	120.8	8.14	120.4	8.21	120.5	8.12
Y99	120.7	7.81	120.6	7.88	120.8	7.79
K100	122.1	7.97	122.2	8.05	122.4	7.97
K101	121.4	7.90	121.5	7.97	121.6	7.89
Y102	123.8	8.07	123.7	8.13	123.8	8.05
L103	-	-	-	-	-	-
L104	-	-	-	-	-	-
F105	120.5	8.12	120.5	8.18	120.7	8.11
C106	-	-	-	-	-	-

M107	-	-	-	-	-	-
E108	121.0	8.18	120.9	8.25	121.1	8.17
N109	119.7	8.28	119.6	8.36	119.7	8.27
S110	116.4	8.10	116.4	8.17	116.5	8.09
A111	124.3	8.08	124.2	8.15	124.3	8.07
E112	119.7	8.07	119.7	8.15	119.8	8.05
P113	-	-	-	-	-	-
E114	118.0	8.11	117.9	8.16	118.1	8.07
Q115	119.9	8.08	119.8	8.15	119.9	8.07
S116	115.7	7.99	115.6	8.06	115.7	7.97
L117	123.7	8.04	123.7	8.12	123.8	8.03
V118	119.4	7.88	119.4	7.95	119.5	7.87
C119	121.3	8.09	121.2	8.16	121.4	8.07
Q120	-	-	-	-	-	-
A121	-	-	-	-	-	-
L122	-	-	-	-	-	-
V123	121.0	7.95	121.0	8.02	121.1	7.93
R124	124.8	8.21	124.9	8.28	125.1	8.20
T125	117.7	8.05	117.6	8.11	117.8	8.03
P126	-	-	-	-	-	-
E127	120.7	8.18	120.6	8.24	120.8	8.16
V128	120.7	7.96	120.5	8.01	120.6	7.93
D129	126.5	8.12	126.4	8.18	126.6	8.10
D130	-	-	-	-	-	-
E131	-	-	-	-	-	-
A132	123.4	7.91	123.5	7.97	123.6	7.89
L133	120.0	7.75	120.1	7.83	120.2	7.75
E134	120.4	7.98	120.4	8.07	120.6	7.99

K135	121.6	7.98	121.6	8.06	121.8	7.98
F136	120.4	7.98	120.3	8.05	120.5	7.97
D137	120.9	8.22	120.5	8.29	120.6	8.20
K138	122.1	8.05	122.2	8.12	122.3	8.04
A139	-	-	-	-	-	-
L140	121.4	7.85	121.4	7.93	121.5	7.84
K141	122.3	8.13	122.3	8.20	122.5	8.12
A142	125.3	8.06	125.3	8.13	125.5	8.05
L143	123.1	8.10	123.1	8.17	123.2	8.09
P144	-	-	-	-	-	-
M145	120.7	8.23	120.6	8.31	120.8	8.23
H146	120.0	8.47	119.9	8.53	120.1	8.45
I147	122.7	8.06	122.6	8.12	122.8	8.04
R148	125.8	8.32	125.8	8.39	125.9	8.30
L149	125.0	8.23	125.0	8.30	125.1	8.21
S150	116.7	8.16	116.6	8.22	116.7	8.14
F151	122.1	8.09	122.1	8.16	122.2	8.08
N152	122.1	8.28	122.0	8.35	122.1	8.26
P153	-	-	-	-	-	-
T154	113.3	7.90	113.2	7.96	113.3	7.88
Q155	121.9	7.97	121.8	8.03	122.0	7.95
L156	122.7	7.97	122.5	8.03	122.7	7.94
E157	120.5	8.10	120.4	8.16	120.6	8.08
E158	121.1	8.13	120.9	8.18	121.0	8.09
Q159	-	-	-	-	-	-
C160	-	-	-	-	-	-
H161	-	-	-	-	-	-
I162	-	-	-	-	-	-

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