Supplementary Material for

GroEL Walks the Fine Line: The Subtle Balance of Substrate and Co-chaperonin Binding by GroEL

A Combinatorial Investigation by Design, Selection and Screening

Martin Kawe[1] and Andreas Plückthun[1*]

[1]Biochemisches Institut, Universität Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland.

*Corresponding author. Tel.: +41-44-635 5570; Fax: +41-44-635 5712; E-mail: plueckthun@bioc.unizh.ch

Table 1. Supplement

Amino acid residues encoded by NNK and NNB

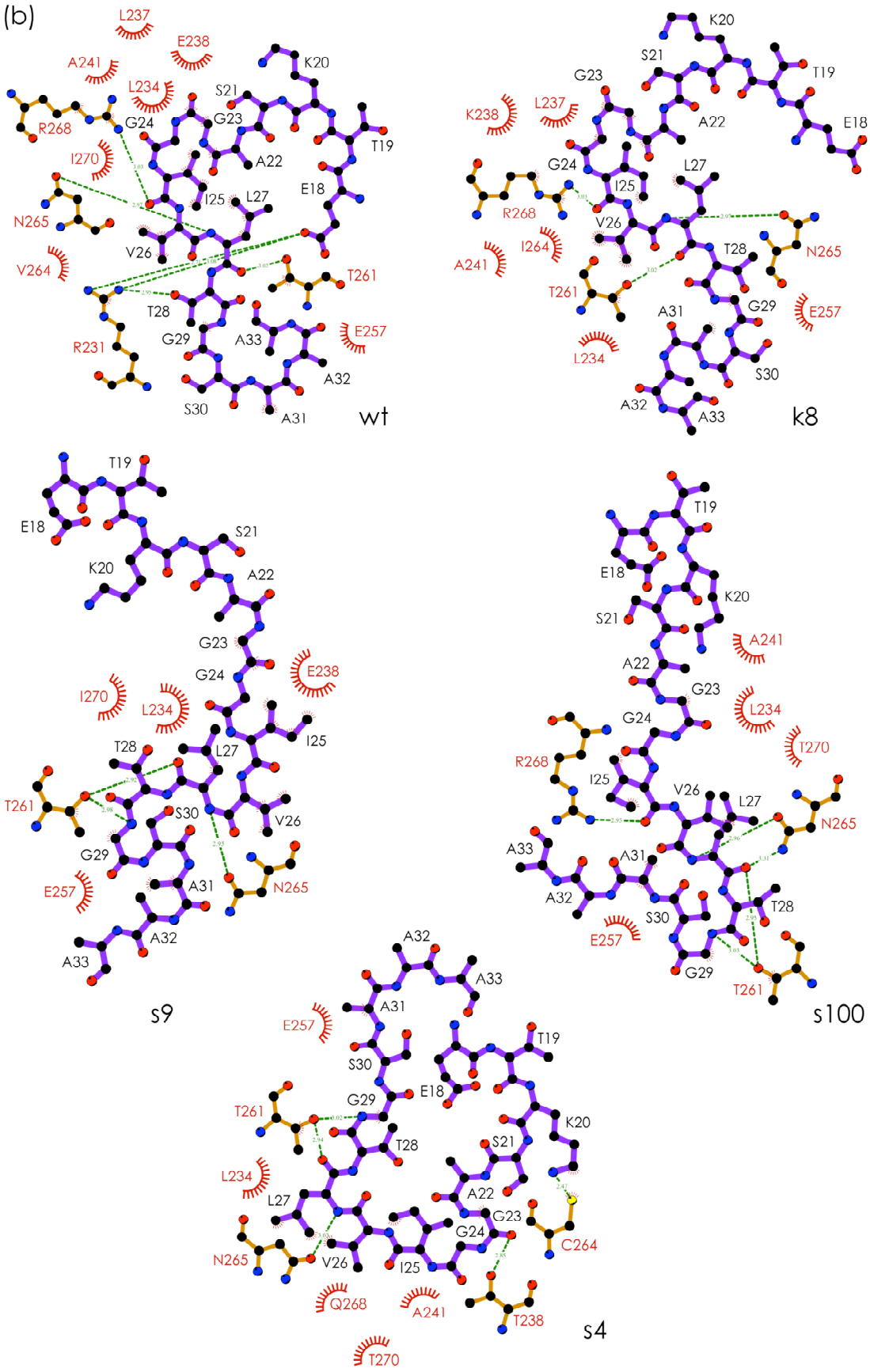
NNK				NBB			
amino acid	relative frequency	amino acid	relative frequency	Amino acid	relative frequency	amino acid	relative frequency
Gly	2	Ser	3	Gly	3	Ser	5
Ala	2	Thr	2	Ala	3	Thr	3
Val	2	Asn	1	Val	3	Asn	–
Leu	3	Gln	1	Leu	4	Gln	–
Ile	1	Asp	1	Ile	2	Asp	–
Cys	1	Glu	1	Cys	2	Glu	–
Met	1	His	1	Met	1	His	–
Phe	1	Lys	1	Phe	2	Lys	–
Tyr	1	Arg	3	Tyr	–	Arg	4
Trp	1	Stop	1	Trp	1	Stop	–
Pro	2			Pro	3		

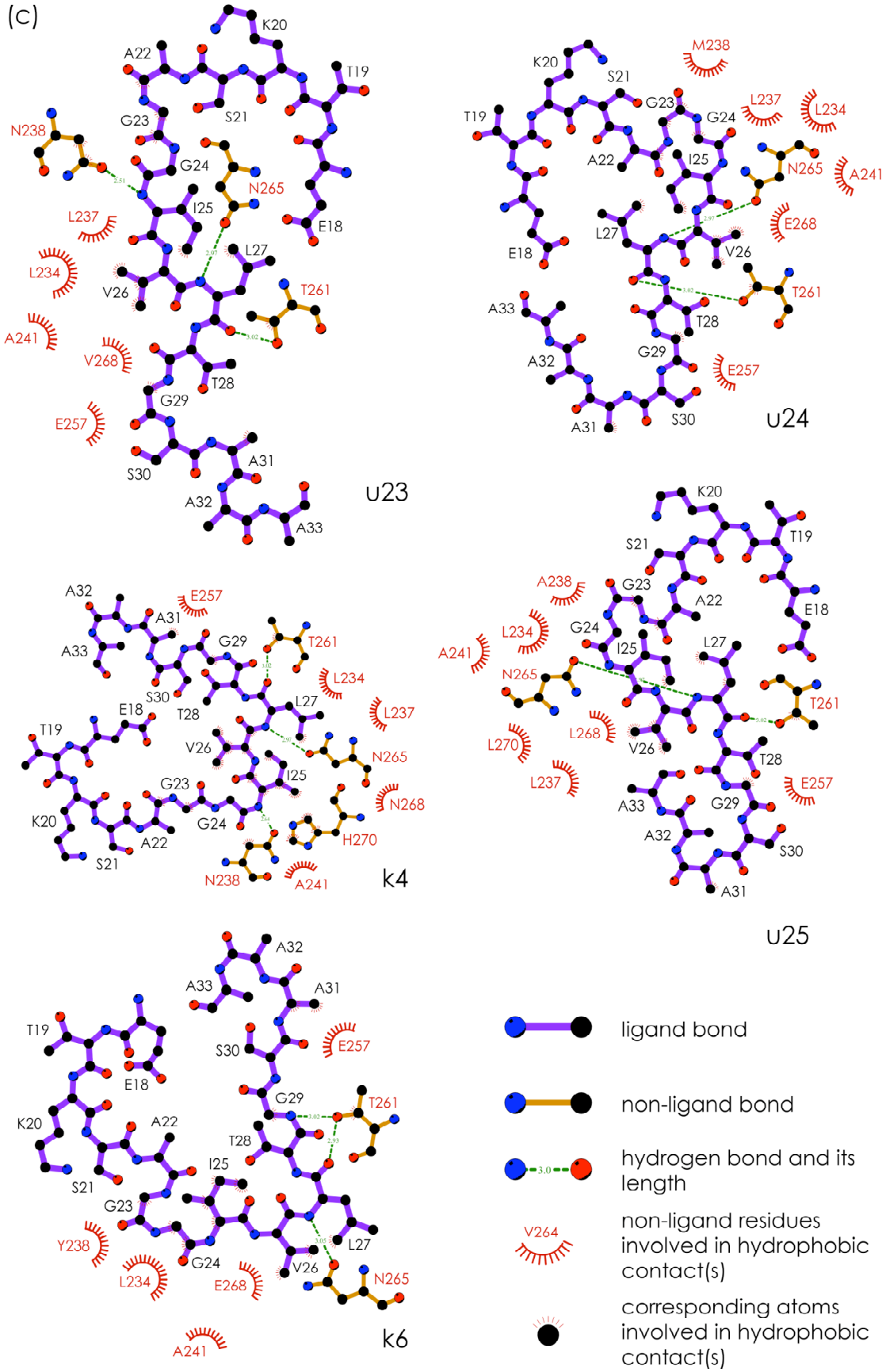
Figure 1. Supplement H-bonding network of residues in the GroEL-GroES interface. (a) Residues of the GroEL-GroES protein-protein interface involved in H-bonding are listed. Residues in bold specify positions randomized in the GroEL apical domain library. Positions shaded in grey are involved in H-bonding in $GroEL_{wt}$. The amino acid at these positions and the number of H-bonds they contribute is given for each GroEL variant. The H-bonding network was calculated using MOLMOL [1] after standard amino-acid side chain energy minimization performed with the Swiss PDB-viewer [2]; (b) and (c) Schematic diagram of the GroEL-GroES protein-protein interface of one chaperonin subunit for each of the analyzed GroEL variants generated by using the program LIGPLOT [3]. H-bonds between GroEL and GroES amino acid residues and their respective length are depicted in green.

Insert Figure

(a)

Residue_#	wt	wt	k8	k8	s4	s4	s9	s9	s100	s100	u23	u23	u24	u24	u25	u25	k4	k4	k6	k6
231	R	**2**	A		Y		T		V		G		G		P		L		G	
238	E		K		T	**1**	E		S		N	**1**	M		A		N	**1**	Y	
261	T	**1**	T	**1**	T	**2**		**2**	T	**2**	T	**1**	T	**1**	T	**1**	T	**1**	T	**2**
264	V		I		C	**1**	V		I		P		P		A		T		R	
265	N	**1**	N	**1**	N	**1**	N	**1**	N	**1**	N	**1**	N	**1**	N	**1**	N	**1**	N	**1**
268	R	**1**	R	**1**	Q		M		R	**1**	V		E		L		N		E	


(b)
L237
E238
A241
L234
S21
K20
R268
G24
G23
T19
I270
A22
E18
I25
L27
N265
V26
V264
T261
T28
E257
G29
A33
R231
A32
S30
A31
wt
K20
S21
G23
T19
K238
L237
A22
E18
G24
L27
I25
R268
V26
N265
I264
A241
T28
T261
E257
A31
G29
L234
S30
A32
A33
k8
T19
E18
S21
K20
A22
G23
E238
G24
I270
L234
I25
T28
L27
T261
S30
V26
G29
N265
E257
A31
A32
A33
s9
T19
E18
K20
S21
A241
A22
G23
L234
G24
I270
R268
I25
V26
L27
N265
A33
A31
A32
S30
T28
E257
G29
T261
s100
A32
A33
A31
E257
T19
S30
E18
G29
K20
T261
T28
S21
L234
A22
L27
G23
G24
C264
N265
V26
I25
Q268
A241
T238
T270
s4


(c)
K20
A22
T19
S21
G23
N238
G24
N265
I25
L237
E18
L234
L27
V26
T261
A241
V268
T28
G29
E257
A31
S30
A32
A33
u23
M238
K20
S21
T19
G23
L237
L234
G24
A22
I25
N265
A241
E268
L27
E18
V26
T261
A33
T28
G29
A32
E257
A31
S30
u24
A32
E257
A31
A33
G29
T261
L234
S30
E18
T28
L27
L237
T19
V26
N265
I25
G23
N268
K20
G24
H270
A22
S21
N238
A241
k4
K20
S21
T19
A238
G23
A22
E18
L234
G24
A241
I25
L27
N265
T261
L268
V26
L270
T28
L237
E257
A33
G29
A32
S30
A31
u25
A32
A33
A31
T19
S30
E257
E18
G29
T261
K20
A22
T28
I25
S21
G23
Y238
L27
G24
V26
N265
L234
E268
A241
k6
ligand bond
non-ligand bond
3.0
hydrogen bond and its length
V264
non-ligand residues involved in hydrophobic contact(s)
corresponding atoms involved in hydrophobic contact(s)

References

1. Koradi, R., Billeter, M. & Wüthrich, K. (1996). MOLMOL: a program for display and analysis of macromolecular structures. *J. Mol. Graph.* **14**, 51-55.

2. Guex, N. & Peitsch, M. C. (1997). SWISS-MODEL and the Swiss-PDPViewer: An environment for comparative protein modeling. *Electrophoresis* **18**, 2714-2723.

3. Wallace, A. C., Laskowski, R. A. & Thornton, J. M. (1995). LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. *Protein Eng.* **8**, 127-134.