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Supporting Material

Protein stabilization and the Hofmeister effect. The role of hydrophobic salvation

Xavier Tadeo, Blanca López-Méndez, David Castaño, Tamara Trigueros, and Oscar Millet

SUPPLEMENTARY MATERIAL

Table S1: *Legend for the multiple K to Q replacements.*

Name	*Legend*
Kx2Q	K42Q + K54Q
Kx3Q	K28Q + K42Q + K54Q
Kx4Q	K28Q + K42Q + K54Q + K61Q
Kx5Q	K23Q + K28Q + K42Q + K54Q + K61Q
Kx6Q	K23Q + K28Q + K41Q + K42Q + K54Q + K61Q

Table S2. *Summary of experimental restraints and statistics of the structure determination of the Kx5Q mutant. Average values over the 20 energy-refined conformers.*

NOE upper distance limits:	*1335*
Short-range, $\lvert i-j\rvert \le 1$	*631*
Medium-range, $1 < \lvert i-j\rvert < 5$	*226*
Long-range, $\lvert i-j\rvert \ge 5$	*498*
ϕ/ψ ***dihedral angle restraints from TALOS***	*76*
Maximal violation (Å)	*0.14*
Violations > 0.2 Å	*0*
CYANA target function (Å^2***)***	*0.54 ±0.11*
AMBER energy (kcal/mol)	*-3051.70 ±55.66*
Ramachandran plot statistics (%)	
residues in:	
most favoured regions	*88.4*
additionally allowed regions	*11.1*
generously allowed regions	*0.6*
disallowed regions	*0*
RMSD to mean coordinates (Å)	
backbone/heavy atoms (from 4 to 64)	*0.34 / 0.70*
RMSD to wild type ProtL, PDB ID 1HZ6 (Å)	
backbone (from 4 to 64)	*0.77*
backbone (secondary structure)	*0.58*

Table S3: *Experimental data for the single point mutants considered in the present study (in the absence of cosolute).*

Mutant	**Average T_m / ºC**	**Number of independent measurements (CD / Fluorescence)**
K7Q	64.6 ± 2.1	3 / 3
K23Q	66.1 ± 0.5	3 / 3
K28Q	71.3 ± 0.9	3 / 3
K41Q	69.4 ± 0.9	3 / 3
K42Q	63.8 ± 1.4	3 / 3
K54Q	67.1 ± 0.5	3 / 3
K61Q	65.5 ± 1.4	3 / 3
E2D	70.0 ± 0.8	4 / 4
E3D	68.0 ± 0.4	2 / 1
E21D	63.9 ± 1.4	2 / 3
E32D	70.9 ± 0.6	4 / 4
E46D	70.3 ± 0.7	4 / 4
K23A	63.1 ± 0.6	3 / 2
Kx2Q[(a)]	66.1 ± 0.5	3 / 3
Kx3Q[(a)]	69.4 ± 0.8	3 / 3
Kx4Q[(a)]	62.3 ± 0.6	3 / 3
Kx5Q[(a)]	61.9 ± 0.9	3 / 3
Kx6Q[(a)]	60.2 ± 0.4	3 / 3

(a) Legend: Kx2Q = K42Q,K54Q; Kx3Q = K28Q, K42Q, K54Q; Kx4Q = K28Q, K42Q, K54Q, K61Q; Kx5Q = K23Q, K28Q, K42Q, K54Q, K61Q; Kx6Q = K23Q, K28Q, K41Q, K42Q, K54Q, K61Q.

Table S4: *Solvent accessibilities and residues non-polar areas employed in equation 2.*

Mutant	$\chi^{F}_{np,Mut}$ [(a)]	$\chi^{F}_{np,WT}$ [(a)]
K7Q	0.867 ± 0.06	**0.738 ± 0.17**
K23Q	**0.751 ± 0.03**	**0.660 ± 0.18**
K28Q	**0.665 ± 0.10**	**0.363 ± 0.06**
K41Q	0.852 ± 0.08	**0.492 ± 0.20**
K42Q	**0.249 ± 0.07**	**0.375 ± 0.09**
K54Q	**0.313 ± 0.05**	**0.328 ± 0.07**
K61Q	**0.709 ± 0.14**	**0.623 ± 0.14**
E2D	0.448 ± 0.06	**0.307 ± 0.15**
E3D	0.384 ± 0.04	**0.416 ± 0.23**
E21D	0.682 ± 0.07	**0.698 ± 0.22**
E32D	0.757 ± 0.10	**0.522 ± 0.27**
E46D	0.539 ± 0.04	**0.504 ± 0.19**
K23A	0.696 ± 0.05	**0.660 ± 0.18**

$\chi^{U}_{np,K}$	$\chi^{U}_{np,Q}$	$\chi^{U}_{np,E}$	$\chi^{U}_{np,D}$	$\chi^{U}_{np,A}$	A^{K}_{np} /Å^2	A^{Q}_{np} /Å^2	A^{E}_{np} /Å^2	A^{D}_{np} /Å^2	A^{A}_{np} /Å^2
0.248[(b)]	0.295[(b)]	0.277[(b)]	0.222[(b)]	0.360[(b)]	122[(c)]	66[(c)]	69[(c)]	45[(c)]	86[(c)]

(a) Bold values are calculated from high resolution NMR structures (2PTL{Wikstrom, 1994 #142} or 2JZP). The 12 conformations of minimal energy have been used to estimate the error. Plain values have been obtained from homology models (using the servers' phyre {Bennett-Lovsey, 2007 #139}and swiss model workspace {Arnold, 2006 #125}). The error bars reflect the discrepancies between the models.

(b) Calculated from the expression: $\chi^{U}_{np} = A^{U}_{np} / A_{np}$. Values for A_{np} are taken from ref. {Bernado, 2006 #140}.

(c) Obtained from {Karplus, 1997 #134}.

Table S5: *Experimental T_m values for sodium sulfate.*

Sodium sulfate					
Mutant	***Conc. / mM***	***T_m / ºC***	***Mutant***	***Conc. / mM***	***T_m / ºC***
K7Q	250	72.6 ± 0.6	*K61Q*	500	78.5 ± 0.6
	500	78.2 ± 0.5		750	84.0 ± 0.7
	750	83.7 ± 0.4		1000	87.7 ± 0.4
	1000	88.2 ± 0.9	*Kx2Q*[(a)]	100	69.5 ± 0.3
K23Q	250	71.6 ± 1.4		250	71.9 ± 1.0
	500	77.0 ± 1.1		330	72.3 ± 0.3
	750	82.3 ± 0.1		450	76.3 ± 0.3
K28Q	200	74.0 ± 0.8		600	79.7 ± 0.4
	400	77.1 ± 0.7	*Kx3Q*[(a)]	50	70.3 ± 0.1
	800	82.5 ± 0.3		100	71.5 ± 0.1
K41Q	200	71.8 ± 1.3		200	74.2 ± 0.6
	400	77.6 ± 1.0		400	78.1 ± 1.4
	600	81.1 ± 1.0		750	82.9 ± 1.8
K42Q	200	65.9 ± 1.5	*Kx4Q*[(a)]	250	70.1 ± 0.2
	250	67.4 ± 0.4		400	73.1 ± 0.2
	400	70.1 ± 1.4		750	79.7 ± 1.3
	500	73.2 ± 1.1		850	82.0 ± 0.3
	600	75.4 ± 1.3	*Kx5Q*[(a)]	250	70.1 ± 0.9
	750	76.5 ± 0.5		500	76.7 ± 0.2
K54Q	200	73.2 ± 0.7		750	82.2 ± 1.6
	400	76.3 ± 0.6	*Kx6Q*[(a)]	250	67.2 ± 0.2
	600	79.4 ± 0.9		450	72.8 ± 0.3
	800	84.5 ± 0.7		660	79.9 ± 0.3
K61Q	250	72.0 ± 0.5			

(a) Legend: Kx2Q = K42Q, K54Q; Kx3Q = K28Q, K42Q, K54Q; Kx4Q = K28Q, K42Q, K54Q, K61Q; Kx5Q = K23Q, K28Q, K42Q, K54Q, K61Q; Kx6Q = K23Q, K28Q, K41Q, K42Q, K54Q, K61Q.

Table S6: *Experimental T_m values for sodium phosphate.*

Sodium phosphate					
Mutant	***Conc. / mM***	***T_m / ºC***	***Mutant***	***Conc. / mM***	***T_m / ºC***
K7Q	200	67.4 ± 0.3	*K61Q*	400	71.2 ± 0.9
	400	70.9 ± 0.5		600	75.2 ± 0.8
	600	74.3 ± 0.3		800	77.0 ± 1.0
	800	78.4 ± 0.6	*Kx2Q*[(a)]	200	70.1 ± 0.4
K23Q	200	69.1 ± 0.3		400	71.2 ± 1.6
	400	71.7 ± 0.2		600	75.9 ± 0.4
	600	75.7 ± 0.3		800	77.0 ± 1.0
	800	77.2 ± 0.4	*Kx3Q*[(a)]	200	73.3 ± 0.8
K28Q	200	72.9 ± 0.8		400	75.7 ± 0.9
	400	75.0 ± 0.6		600	79.3 ± 0.9
	800	78.6 ± 0.4		800	81.7 ± 0.7
K41Q	200	70.6 ± 0.8	*Kx4Q*[(a)]	200	66.7 ± 0.2
	400	75.8 ± 0.5		400	68.8 ± 0.1
	600	76.6 ± 1.5		600	72.0 ± 0.1
	800	75.4 ± 3.9		800	74.9 ± 0.5
K42Q	200	64.7 ± 0.9		900	77.2 ± 0.4
	400	69.7 ± 1.6	*Kx5Q*[(a)]	200	66.9 ± 0.3
	600	72.2 ± 1.1		400	70.7 ± 0.5
	800	75.2 ± 0.7		600	76.1 ± 0.8
K54Q	200	70.8 ± 0.7		800	77.9 ± 0.1
	400	72.7 ± 0.6	*Kx6Q*[(a)]	200	64.5 ± 0.4
	600	75.5 ± 0.7		400	68.9 ± 1.3
	800	77.4 ± 0.9		600	74.5 ± 0.5
K61Q	200	69.1 ± 0.7		800	80.5 ± 0.5

(a) Legend: Kx2Q = K42Q, K54Q; Kx3Q = K28Q, K42Q, K54Q; Kx4Q = K28Q, K42Q, K54Q, K61Q; Kx5Q = K23Q, K28Q, K42Q, K54Q, K61Q; Kx6Q = K23Q, K28Q, K41Q, K42Q, K54Q, K61Q.

Table S7: *Experimental T_m values for sodium fluoride.*

Sodium fluoride					
Mutant	***Conc. / mM***	***T_m / ºC***	***Mutant***	***Conc. / mM***	***T_m / ºC***
K7Q	200	68.0 ± 0.8	*K61Q*	200	68.5 ± 1.1
	400	71.1 ± 0.6		400	70.5 ± 1.0
	600	73.4 ± 1.5		600	74.3 ± 0.8
	800	75.6 ± 1.4		800	76.0 ± 0.8
K23Q	200	68.7 ± 1.1	*Kx2Q*[(a)]	200	69.3 ± 0.9
	400	72.1 ± 0.3		300	70.1 ± 0.4
	600	74.1 ± 0.3		400	71.3 ± 0.1
	800	76.1 ± 0.2		600	74.8 ± 0.4
K28Q	200	73.8 ± 1.7	*Kx3Q*[(a)]	200	72.1 ± 0.1
	400	76.1 ± 1.3		400	73.8 ± 0.6
	600	78.5 ± 1.6		600	77.1 ± 1.7
K41Q	200	72.2 ± 0.4		760	79.5 ± 3.3
	400	74.7 ± 1.3	*Kx4Q*[(a)]	200	66.0 ± 0.7
	600	79.2 ± 1.2		400	69.1 ± 0.5
	760	79.7 ± 0.9		600	71.6 ± 1.2
K42Q	200	64.2 ± 2.7		760	74.9 ± 0.2
	400	67.6 ± 0.5	*Kx5Q*[(a)]	200	66.9 ± 0.6
	600	69.2 ± 1.3		400	71.0 ± 0.4
	760	70.5 ± 0.6		640	74.3 ± 0.6
K54Q	200	71.1 ± 1.0	*Kx6Q*[(a)]	100	62.6 ± 2.1
	400	72.1 ± 1.0		250	65.0 ± 0.2
	600	75.3 ± 0.7		300	65.6 ± 0.7
	800	77.8 ± 1.3		500	70.4 ± 0.5

(a) Legend: Kx2Q = K42Q, K54Q; Kx3Q = K28Q, K42Q, K54Q; Kx4Q = K28Q, K42Q, K54Q, K61Q; Kx5Q = K23Q, K28Q, K42Q, K54Q, K61Q; Kx6Q = K23Q, K28Q, K41Q, K42Q, K54Q, K61Q.

Table S8: *Experimental T_m values for sodium nitrate.*

Sodium nitrate					
Mutant	***Conc. / mM***	***T_m / ºC***	***Mutant***	***Conc. / mM***	***T_m / ºC***
K7Q	200	63.6 ± 0.1	*K54Q*	750	63.5 ± 1.3
	400	62.3 ± 0.4		1000	62.4 ± 1.3
	600	61.8 ± 0.3	*K61Q*	250	65.2 ± 1.1
	800	60.0 ± 0.3		500	63.5 ± 1.0
K23Q	200	64.2 ± 0.5		1000	60.3 ± 0.8
	400	63.5 ± 0.7	*Kx2Q*[(a)]	500	63.3 ± 0.2
	600	62.3 ± 0.5		750	61.8 ± 0.5
	800	60.6 ± 0.3		1000	61.4 ± 0.3
K28Q	200	69.5 ± 0.8	*Kx3Q*[(a)]	250	67.8 ± 0.01
	400	68.7 ± 1.2		500	65.6 ± 0.4
	600	66.8 ± 0.3		750	63.2 ± 0.3
	800	65.6 ± 0.3	*Kx4Q*[(a)]	100	61.5 ± 0.1
K41Q	250	67.3 ± 2.7		250	61.1 ± 0.4
	500	65.5 ± 0.5		350	60.9 ± 0.01
	1000	63.1 ± 0.6		500	59.4 ± 0.9
K42Q	500	61.6 ± 1.3	*Kx5Q*[(a)]	250	60.2 ± 0.4
	1000	59.1 ± 0.9		500	58.1 ± 0.5
K54Q	250	66.2 ± 1.0		750	57.3 ± 0.2
	500	65.8 ± 0.7		1000	55.3 ± 0.7

(a) Legend: Kx2Q = K42Q, K54Q; Kx3Q = K28Q, K42Q, K54Q; Kx4Q = K28Q, K42Q, K54Q, K61Q; Kx5Q = K23Q, K28Q, K42Q, K54Q, K61Q.

Table S9: *Experimental T_m values for sodium perchlorate.*

Sodium perchlorate

Mutant	***Conc. / mM***	***T_m / ºC***	***Mutant***	***Conc. / mM***	***T_m / ºC***
K7Q	200	63.8 ± 0.8		800	56.8 ± 1.0
	400	61.8 ± 1.0	*K61Q*	200	63.4 ± 0.8
	600	58.8 ± 0.3		400	61.6 ± 0.8
	800	58.0 ± 0.9		600	58.7 ± 0.3
K23Q	200	63.4 ± 0.5		800	57.7 ± 0.5
	400	61.7 ± 0.4	*Kx2Q*[(a)]	250	61.9 ± 0.04
	600	60.2 ± 0.5		500	57.8 ± 0.2
	800	58.2 ± 0.4		1000	52.8 ± 1.5
K28Q	200	69.2 ± 0.3	*Kx3Q*[(a)]	100	67.7 ± 0.2
	400	66.7 ± 0.5		250	65.9 ± 0.2
	600	64.6 ± 0.4		750	60.8 ± 0.2
	800	61.8 ± 0.8	*Kx4Q*[(a)]	200	58.9 ± 0.9
K41Q	200	65.1 ± 0.6		600	56.5 ± 0.3
	400	59.6 ± 1.6		700	53.0 ± 0.9
	800	58.6 ± 1.5	*Kx5Q*[(a)]	250	59.0 ± 0.3
K42Q	200	59.9 ± 0.7		500	57.4 ± 0.7
	400	55.2 ± 0.6		750	54.5 ± 0.4
	600	55.1 ± 1.0		1000	53.3 ± 0.9
	800	53.9 ± 0.8	*Kx6Q*[(a)]	500	56.0 ± 0.7
K54Q	200	62.6 ± 0.8		750	52.0 ± 0.5
	400	61.0 ± 0.2		1000	49.5 ± 0.6
	600	57.6 ± 0.7			

(a) Legend: Kx2Q = K42Q, K54Q; Kx3Q = K28Q, K42Q, K54Q; Kx4Q = K28Q, K42Q, K54Q, K61Q; Kx5Q = K23Q, K28Q, K42Q, K54Q, K61Q; Kx6Q = K23Q, K28Q, K41Q, K42Q, K54Q, K61Q.

Table S10: *Experimental T_m values for sodium thiocyanate.*

		Sodium thiocyanate			
Mutant	***Conc. / mM***	***T_m / ºC***	***Mutant***	***Conc. / mM***	***T_m / ºC***
K7Q	200	63.0 ± 0.3	*K61Q*	600	53.1 ± 0.5
	400	57.7 ± 1.3		800	49.5 ± 0.8
	600	53.8 ± 0.8	*Kx2Q*[(a)]	250	58.9 ± 0.3
	800	49.9 ± 0.5		500	54.4 ± 0.8
K23Q	200	61.3 ± 0.7		750	50.7 ± 0.3
	400	57.9 ± 0.3		1000	47.1 ± 0.4
	600	52.2 ± 0.8	*Kx3Q*[(a)]	100	67.6 ± 0.2
K28Q	200	65.9 ± 0.5		250	64.9 ± 0.9
	400	60.6 ± 1.3		300	64.7 ± 0.5
	800	54.2 ± 0.6		375	63.4 ± 0.3
K41Q	200	64.4 ± 1.2		500	60.7 ± 0.5
	400	62.1 ± 0.8	*Kx4Q*[(a)]	100	60.1± 0.5
	600	58.7 ± 1.3		300	56.3 ± 0.3
	800	56.2 ± 0.6		400	54.0 ± 0.9
K42Q	200	59.5 ± 0.8	*Kx5Q*[(a)]	100	60.9 ± 0.4
	400	56.4 ± 0.6		200	58.0 ± 1.3
	600	53.3 ± 0.2		450	55.4 ± 0.8
	800	51.0 ± 0.7		600	52.2 ± 1.3
K54Q	250	59.4 ± 0.8		750	48.0 ± 0.6
	500	55.0 ± 0.1	*Kx6Q*[(a)]	200	55.7 ± 0.5
	750	50.7 ± 0.5		400	53.0 ± 0.8
	1000	46.5 ± 0.7		500	50.9 ± 0.3
K61Q	200	62.4 ± 0.8		650	47.0 ± 0.8
	400	57.3 ± 0.5		750	43.8 ± 0.4

(a) Legend: Kx2Q = K42Q, K54Q; Kx3Q = K28Q, K42Q, K54Q; Kx4Q = K28Q, K42Q, K54Q, K61Q; Kx5Q = K23Q, K28Q, K42Q, K54Q, K61Q; Kx6Q = K23Q, K28Q, K41Q, K42Q, K54Q, K61Q.

Table S11: *Comparison of the T_m variation produced by the cosolute ($\partial T_m / \partial C_3$) and the salting in coefficient for K7Q.*

Anion	$\partial T_m / \partial C_3$ *ºC·M^{-1}*	*Salting-in canstant(a) M^{-1}*
Sulfate	2.33	0.013
Phosphate	1.76	n.a.
Fluoride	1.43	0.027 (KF)
Nitrate	-5.5	n.a.
Perchlorate	-9.1	0.097
Thiocyanate	-19.3	0.077

(a) Data taken from ref. 4.