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Supplement to: Pentameric Assembly of Potassium Channel Tetramerization Domain-Containing Protein 5 (KCTD5) by Irina S. Dementieva, Valentina Tereshko, Zoe A. McCrossan, Elena Solomaha, Daniel Araki, Chen Xu, Nikolaus Grigorieff and Steve A. N. Goldstein

Supplement Figure legends

Supplement Figure 1. The C-terminal KCTD5 module. (a-c) Conformational diversity of β5-β6 loops crowning the KCTD5 C-terminal module as determined by X-ray crystallography. (**a**) Ribbon representation of individual subunits superimposed on the β5-β6 hairpin: three loops in cyan, blue and yellow are from high-salt structure; the loop shown in red is from low-salt structure. Four other subunits in the pentameric assembly are included in pale colors. (**b-c**). Selected conformation of β5-β6 loop in high- (**b**) and low- (**c**) salt crystals shown in ball-and-stick presentation with the residues 189-200 (SSYNYGNEDQAE) are labeled. The acidic Glu196, Asp197 (β5-β6 loop) and Glu165-167 (β4-α6 turn) that are exposed to solvent are boxed. **(d)** The Cα-trace of the C-terminal KCTD5 domain, residues 155-210 (in stereo). **(e)** The SigmaA-weighted 2Fobs-Fcalc electron density map contoured at 1σ level around β5-β6 loop and b4-a6 turn residues in high-salt KCTD5 crystal structure.

Supplement Figure 2. The H-bond interactions in the N-terminal (a) and C-terminal (b) KCTD5 pentameric modules. The distances (averaged over five independent interfaces) from the high-salt KCTD5 and truncated N-terminal KCTD5 structures are in (**a**) on left in right, respectively; the distances from high-salt KCTD5 are in (**b**). (**c**) The comparative alignment of the residues found at the interface between the adjacent subunits in the KCTD5 and Kv T1-domain assemblies. The T1-domain H-bonded residues are indicated in black; those that are not involved in the H-bond formation are shown in gray. Four key aliphatic substitutions in KCTD5 are shown in red; other KCTD5 residues are colored to match the BTB coloring scheme (shown on left) with the secondary structure elements indicated in the middle. The H-bonded moieties judged crucial for circular arrangement in Kv channel T1 crystal structures and conserved in KCTD5 are highlighted in yelow in (**a**). KCTD5 and Kv4.2 have one extra common pair (boxed in yellow) due to the presence of Asp residue (analogous to Asp95 in KCTD5) in the β3-α3 loop. Kv T1-domain X-ray structures used: Kv1.1 (PDBid 1T1D), Kv3.1 (PDBid 3KVT), Kv4.2 (PDBid 1NN7).

Figure S1

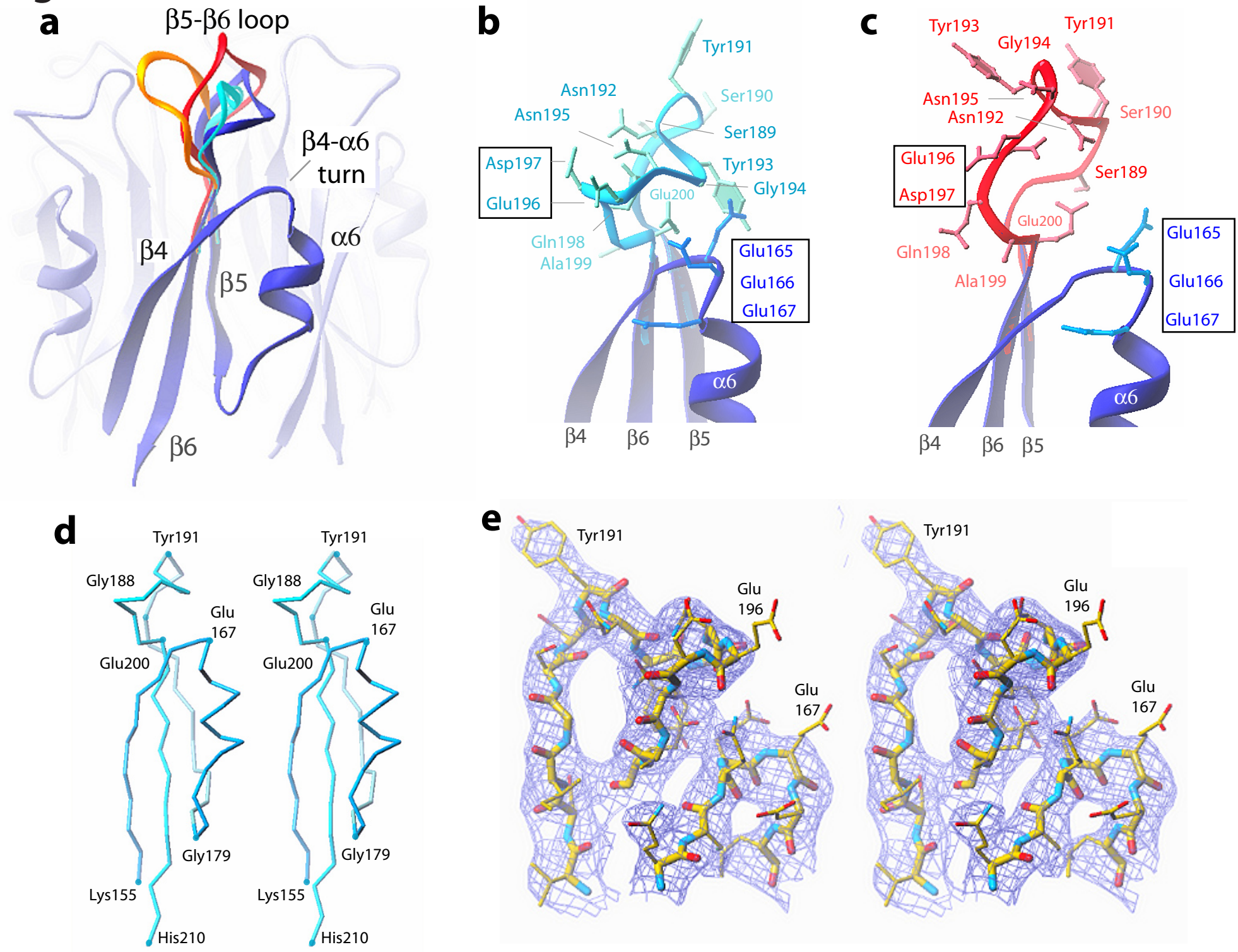
a
β5-β6 loop
β4-α6
turn
α6
β4
β5
β6
b
Tyr191
Asn192
Ser190
Asn195
Ser189
Asp197
Tyr193
Glu196
Gly194
Glu200
Gln198
Ala199
Glu165
Glu166
Glu167
α6
β4
β6
β5
c
Tyr193
Tyr191
Gly194
Asn195
Asn192
Ser190
Glu196
Ser189
Asp197
Glu200
Gln198
Ala199
Glu165
Glu166
Glu167
α6
β4
β6
β5
d
Tyr191
Tyr191
Gly188
Gly188
Glu
167
Glu
167
Glu200
Glu200
Gly179
Gly179
Lys155
Lys155
His210
His210
e
Tyr191
Tyr191
Glu
196
Glu
196
Glu
167
Glu
167

Figure S2

a

H-bond Distance, A		subunit 1 residue	atom		subunit2 atom	residue
2.8	3.2	Glu124	OE1	– – –	NZ	Lys110
3.3	3.2	Asp116	O	– – –	N	Lys115
3.2	3.0	Ala118	N	– – –	OD1	Asn114
2.9	2.5					
2.9	3.5	Tyr 98	OH	– – –	ND2	Asn114
2.6	3.5	Asp95	D2	– – –	ND2	Asn104
3.0	2.8	Asp93	OD1	– – –	NE	Arg107
3.1	3.1				NH1	Arg107
2.8	2.8					
3.5	3.3				OG1	Thr57
2.8	2.8		OD2	– – –	OG1	Thr58
2.7	2.6				OG1	Thr61
3.2	3.1	Gly51	O	– – –	N	Leu56
3.4	3.1	Asp83	OD1	– – –	NE1	Trp45

b

H-bond Distance, A	subunit 1 residue	atom		subunit2 atom	residue
2.9	Gln183	OE1	– – –	N	Leu184
2.9		NE2	– – –	O	Leu184
3.2	Tyr158	OH	– – –	N	Phe181

c

subunit 1						subunit 2			
Kv1.1	Kv3.1	Kv4.2	KCTD5	Interface		KCTD5	Kv4.2	Kv3.1	Kv1.1
Glu 144	Glu 87	Glu 117	Glu 124	α4-helix	α3-α4	Lys 110	Lys 103	Lys 73	Arg 130
Asn 136	Asp 79	His 109	Asp 116	α3-α4	α3-α4	Lys 115	Arg 108	Thr 78	Val 135
Asp 140				α3-α4 loop	α3-α4 loop	Val 112	His 105	His 75	Arg 132
Pro 138	Cys 81	Cys 111	Ala 118						
Ser 117	Val 61	91	Tyr 98	α3-helix		Asn 114	Pro 107	Pro 77	Pro 134
Arg 115	Pro 59	Pro 89		β3–α3 turn	α3-helix	Asn 104	Asn 97	Asn 67	Tyr 123
Asn 114	His 58	Asp 88	Asp 95			Gly 100	Arg 93	Gln 64	Asp 119
Asp 112	Asp 56	Asp 86	Asp 93	β3–α3 turn	α3-helix	Arg 107	Arg 100	Arg 70	Gln 126
					β2-α1 loop α1–helix	Thr 57,58 61	Thr 54 58	Thr 23 27	Thr 83 87
Ser 73	Gly 17	Ser 48	Gly 51	β1–β2 turn	β2-strand	Leu 56	Gln 53	Glu 22	Glu 78
Asn 71	Asn 15	Asn 46	Asn 49						
			Asp 83	α1–α2 loop	β1-strand	Trp 45			

L3 L2 L1

BTB segment