

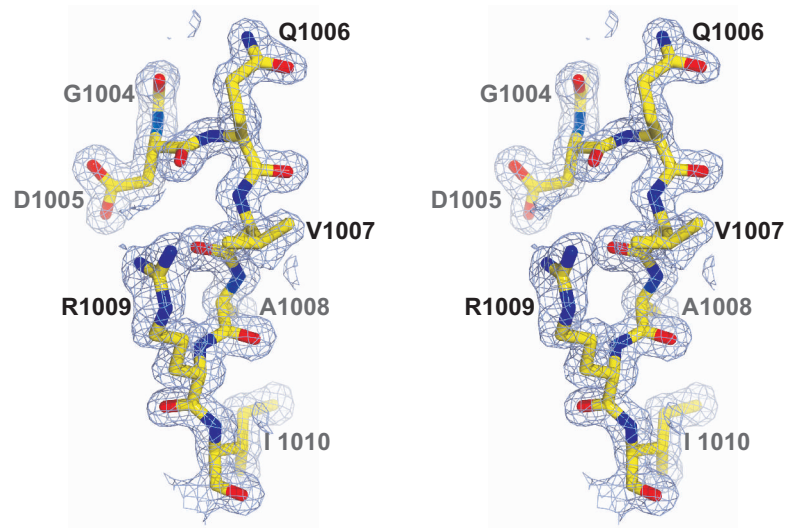
Structure of the Calx- β domain of the integrin β 4 subunit: insights into function and cation-independent stability.

Supplementary Material

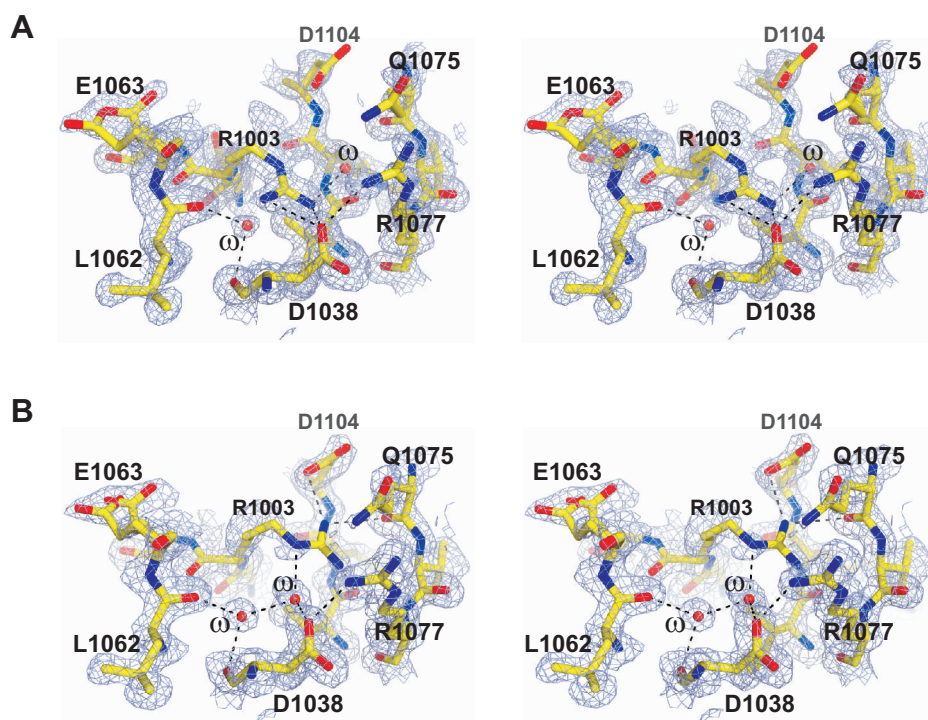
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Supplementary figure 1. Stereo representation of a simulated annealing omit map ($2mF_o - DF_c$, contoured at 1.2σ) superimposed on the refined structure of the region (A'B loop and strand B) that was omitted for the calculation of the map.



Supplementary figure 2. Stereo representation of simulated annealing omit maps ($2mFo-DFc$, contoured at 1σ) superimposed on the structures of the pseudo Ca^{2+} -binding sites of $\beta 4$ of the crystals soaked in 10 mM $CaCl_2$. (A) Molecule A and (B) Molecule B of the asymmetric unit. Maps were calculated after refinement using simulated annealing (initial temperature 5000 K) of models from which the regions shown were omitted.



Supplementary Figure 3

	 F 1077	  G' 1104/1105/1106	
ITB4_HUMAN	QVRRFHVQLSNPKFG-----	-----AHLGQPHSTTIIIRDPDE	1107
NAC1_BOVIN	-DENFLVHLSNVKVSLEASDGI LEASHVSTL-----	-----ACLGSPSTATVTIFDDDH	533
Q9PT19_ONCMY	-DEHFLVHLSNVKVI SEGTGYVQPRANHLDTL-----	-----AGLGLPCSATVTIFDDDH	538
NAC3_RAT	-DEHFFVRLSNVRVEEQLEEGMTPA-ILNSLPLPR-----	-----AVLASPCVATVTILDDDH	528
NAC2_RAT	-DEHFFVRLNLNRVG--DAQGMFEPDGGGRP--K-----	-----GRLVAPLLATVTILDDDH	521
O02196_LOLOP	-DEHFYIRLSNLRVG--DSNGLFESGQAEAK-----	-----AQLANPFLATVMILDDDH	503
O18367_DROME	-DECFYIRLFPNPEG-----	-----VKLAVPMIATVMILDDDH	553
O45630_CAEEL	-DEHFYLRCLNLRVR--TKDGI IIDPTRIGGLPV-----	-----AQLEMPNTATIMILDDDH	496
Q21609_CAEEL	-DEQFMVRLSQVRAF-----RSEHFSSVP-----	-----ARLGLAATATVIIVDDDH	510
P74440_SYNY3	-DKQFGLLVSLLEEGLPQNGEQAFFLAADANGS-----	-----QIRNWN-----YLPGESANSLTGGVINFS-	3781
P73139_SYNY3	-DRQFSLLVSLNDGLEAGDWGDAFALAGDANGA-----	-----QIRRW-----YLAGNWDNSVMGGLIDFST-	3554
NAC1_BOVIN	-NKTFFLEIGEPRLVEMSEKKALLNELGGFTITGK-----	-----YLYGGQPVFR-----KVHAREHPLPSTIITIADEYDDKQ-	683
Q9PT19_ONCMY	-NKNFFLEMGE PQLLEMSEKAVLLQEIGGFVKT-----	-----GRDVYR-----KVQGRDNPVPATIIISLAEEGDEEAL	684
NAC2_RAT	-KNFFIELGQPQWLKRGISALLNQGDGDRKLT-----	-----AEEEEAQ-----RIAEMGKPV LGENCRLEVIIEESYD	668
NAC3_RAT	-QENFFIALGEPKWMERGISALLSPEVTDKRLT-----	-----MEEEEAK-----RIAEMGKPV LGHEHPKLEVIIEESYE	675
O02196_LOLOP	-NETFFIWLDEPYLVKKPTG-----SSGSVVE-----	-----DDD-----VLAE LGKPRRGENIKITVHIIESTE	640
O45630_CAEEL	-SDYFYIELSPPIWAKKMNDSRIQERFQRMERKRGSVASESKDSNTENGDDVETSLTPSQLEIAEMGKPRLGFTKQITITRESKE		666
Q21609_CAEEL	-HEDFYIELGEP IWHRELAD-----DEEG-----	-----IE---GKPI LG-FSRCKV VITEDRE	635
O18367_DROME	-DVSFKVHIGEPRLAPDELAAKI KEVEKK-----	-----PVQDLTELD-----RILL SKPRNGELTTAYVRIRESQE	697
Q21895_CAEEL	-DEAFI IELLKVD-----EPGVSIGTR-----	-----RKATITIIISDDN	560
P73590_SYNY3	RAEKFRVVITSDHEG-----AHLRLDGLRLG-----	-----NTRLELVATVTTIEDEP	971
Q21895_CAEEL	-NDVFYVHLKIQDVD-----EDS-----		444
O16856_MICPR	-EEFFLLLA EILD P-----DDPRVNL TDP-----	-----ETMVII LDDD-	236
O16857_MICPR	-NETFPANL TLISDN-----DR--VTIDP-----	-----PMATVLI IDND-	1048
O16858_MICPR	-TEDLLAIL SFPSGN-----GPPLVLLDP-----	-----GMANITIFDDDD	682
O16858_MICPR	-IEMFSANLRLPAGS-----TD-IVFLDP-----	-----DRANATILDN DV	330
O16857_MICPR	-TENFTLVLDS-----DDN-VLLLP-----	-----EEAEVQIMDLGS	1163
O16858_MICPR	-VENFTLVLAS-----DDD-VLLLP-----	-----EEAEVLIDTTE	443
O16857_MICPR	-MENFTLALAS-----DNNDVILMP-----	-----EQADVEILD SGM	1515
O16857_MICPR	-EENVSLILMS-----DDPMVVT E P-----	-----ELSEVI ISDTN-	348
O16857_MICPR	-PEDFFVT LST-----TDPSAEVDPD-----	-----RDVATATINDLD-	466
O16857_MICPR	-PEIFFADLMT-----DEERVTLDP-----	-----NRTTIVIIDAD-	585
O16857_MICPR	-RGDFFVNLT T-----SEPLVT LMP-----	-----TTTVVMIDDEDE	816
O16857_MICPR	-NETFVGLLDA-----QGQPVIVDPP-----	-----REQAMVLITEDPA	235
O16857_MICPR	-DEDFMGSLEILT-----TGTNAQLVPG-----	-----TETAIVTILND-	702
O16856_MICPR	-TENFVVS LAELV-----ADEANVLVGV-----	-----QNVTVNIV---	354
O16858_MICPR	-SEDFSVVLAASP-----ASNQMVLSGD-----	-----LAMATVNIIDPN	1265

Supplementary Figure 3 (continuation)

Supplementary figure 3. Multiple sequence alignment of Calx- β domains. The 37 sequences that constitute the seed of the Calx- β family (PF03160) in the PFAM database (Finn *et al.*, 2008) were simultaneously aligned with the program CLUSTALW (Chenna *et al.*, 2003). The sequences were extended to include the C-terminal β -strand of the Calx- β domain, not present in the definition of the family in PFAM. Minor modifications in the alignment were introduced to reduce the presence of gaps inside secondary structure elements, when a reasonable sequence conservation pattern was observed. In addition to the human integrin β 4 (ITB4_HUMAN), the alignment includes sequences of Na⁺/Ca²⁺-exchangers from *Rattus norvegicus* (NAC2_RAT, NAC3_RAT), *Bos taurus* (NAC1_BOVIN), *Oncorhynchus mykiss* (Q9PT19_ONCMY), *Drosophila melanogaster* (O18367_DROME), *Caenorhabditis elegans* (Q21609_CAEEL, Q21895_CAEEL, O45630_CAEEL), *Loligo opalescens* (O02196_LOLOP), three isoforms of the MAFp3 aggregation factor from *Microciconia prolifera* (O16856_MICPR, O16857_MICPR, O16858_MICPR), and three proteins from *Synechocystis* sp. PCC6803 (P73139_SYNY3, P73590_SYNY3, P74440_SYNY3). The β -strands of the Calx- β domain, as observed in the β 4 structure, are shown on top of the alignment. Acidic residues that occupy positions equivalent to residues that in the CBDs of NCX1 participate in the direct coordination of Ca²⁺ are shown in red boxes and the number of the residues of β 4 in those positions are shown on top of the sequence alignment.

References

- Chenna, R., Sugawara, H., Koike, T., Lopez, R., Gibson, T. J., Higgins, D. G. & Thompson, J. D. (2003). *Nucleic Acids Res* 31, 3497-3500.
- Finn, R. D., Tate, J., Mistry, J., Coghill, P. C., Sammut, S. J., Hotz, H. R., Ceric, G., Forslund, K., Eddy, S. R., Sonnhammer, E. L. & Bateman, A. (2008). *Nucleic Acids Res* 36, D281-288.