

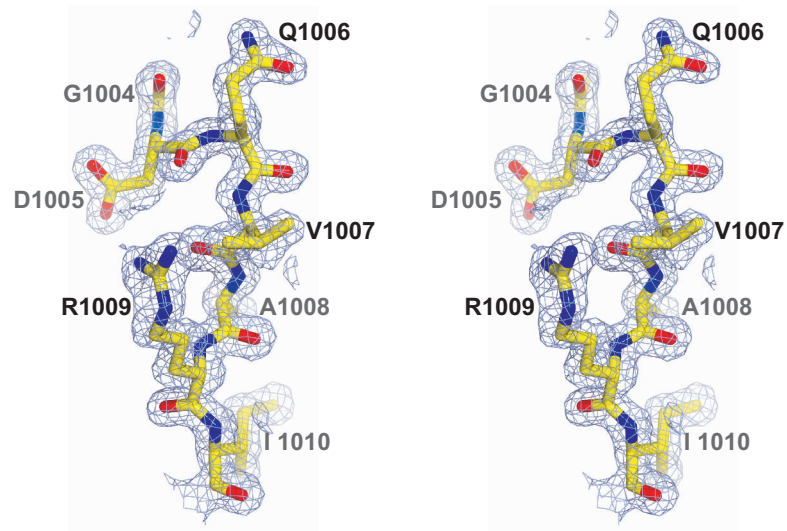
# **Structure of the Calx- $\beta$ domain of the integrin $\beta$ 4: insights into function and cation-independent stability.**

## **Supplementary Material**

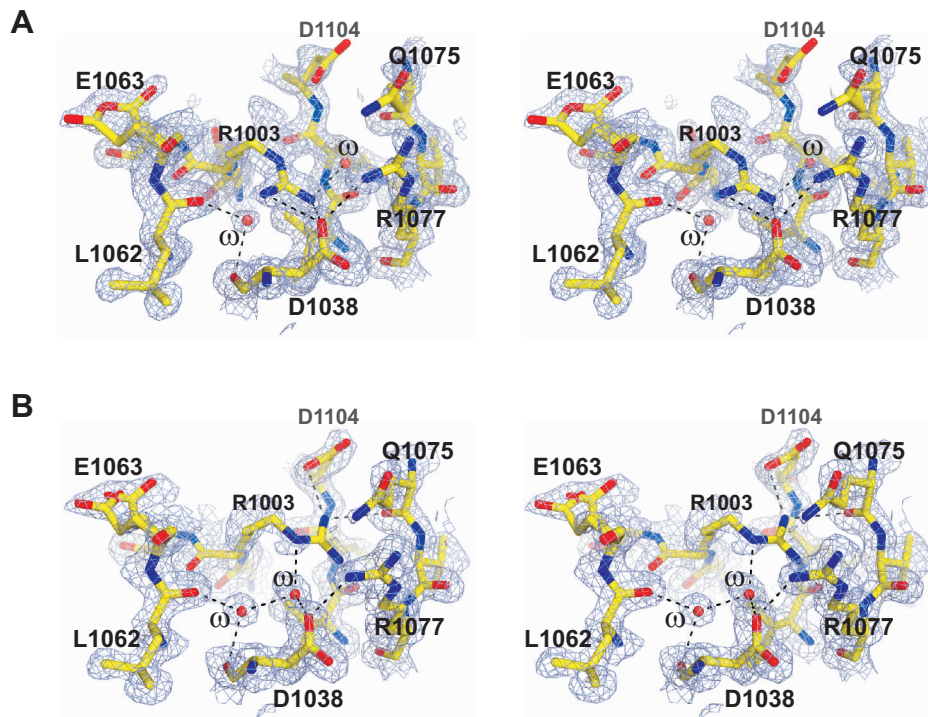
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**Supplementary figure 1.** Stereo representation of a simulated annealing omit map ( $2mF_o - DF_c$ , contoured at  $1.2 \sigma$ ) superimposed on the refined structure of the region (A'B loop and strand B) that was omitted for the calculation of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



**Supplementary figure 2.** Stereo representation of simulated annealing omit maps ( $2mF_o - DFC$ , contoured at  $1 \sigma$ ) 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

	 <b>F</b> 1077	  <b>G'</b> 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	-DEHFFVRLNLRVG--DAQGMFEPDGGGRP--K-----	-----GRLVAPLLATVTILDDDH	521
O02196_LOLOP	-DEHFYIRLSNLRVG--DSNGLFESGQAEAK-----	-----AQLANPFLATVMILDDDH	503
O18367_DROME	-DECFYIRLFPSEG-----	-----VKLAVPMIATVMILDDDH	553
O45630_CAEEL	-DEHFYLRCLNLRVR--TKDGI IIDPTRIGGLPV-----	-----AQLEMPNTATIMILDDDH	496
Q21609_CAEEL	-DEQFMVRLSQVRAF-----RSEHFSSVP-----	-----ARLGLAATATVIIVDDDH	510
P74440_SYNY3	-DKQFGLLVSLLEEGLPQNGEQAFFLAADANGS-----	-----QIRNWN-----YLPGESANSLTGGVINFAST-	3781
P73139_SYNY3	-DRQFSLLVSLNDGLEAGDWGDAFALAGDANGA-----	-----QIRRWN-----YLAGNWDNSVMGGLIDFST-	3554
NAC1_BOVIN	-NKTFFLEIGEPRLVEMSEKKALLLNELGGFTITGK-----	-----YLYGGQPVFR-----KVHAREHPLPSTIITIADEYDDKQ-	683
Q9PT19_ONCMY	-NKNFFLEMGE PQLLEMSEKAVLLQEIGGFVKT-----	-----GRDVYR-----KVQGRDNPVPATIIISLAEEGDEEAL	684
NAC2_RAT	-KDNFFIELGQPQWLKRGISALLLNQGDGDRKLT-----	-----AEEEEAQ-----RIAEMGKPV LGENCRLEVIIEESYD	668
NAC3_RAT	-QENFFIALGEPKWMERGISALLLSPEVTDKRLT-----	-----MEEEEAK-----RIAEMGKPV LGHEHPKLEVIIEESYE	675
O02196_LOLOP	-NETFFIWLDEPYLVKKPTG-----SSGSVVE-----	-----DDDP-----VLAE LGKPRRGENIKITVHIIESTE	640
O45630_CAEEL	-SDYFYIELSPPIWAKKMNDSLRIQERFQRMERKRGSSVASESKDSNTENGDDVETSLTPSQLEIAEMGKPRLGFTKQITITRESKE		666
Q21609_CAEEL	-HEDFYIELGEP IWHRELAD-----DEEG-----	-----IE---GKPI LG-FSRCKV VITEDRE	635
O18367_DROME	-DVSFKVHIGEPRLAPDELAAKI KEVEKK-----	-----PVQDLTELD-----RILLLSKPRNGELTTAYVRIRESQE	697
Q21895_CAEEL	-DEAFI IELLKVD-----EPGVSIGTR-----	-----RKATITIIISDDN	560
P73590_SYNY3	RAEKFRVVITSDHEG-----AHLRLDGLRLG-----	-----NTRLELVATVTTIEDEP	971
Q21895_CAEEL	-NDVFYVHLKIQDVD-----EDS-----		444
O16856_MICPR	-EEFFLLLA EILDP-----DDPRVNL TDP-----	-----ETMVII LDDD-	236
O16857_MICPR	-NETFPANL TLISDN-----DR--VTIDP-----	-----PMATVLI IDND-	1048
O16858_MICPR	-TEDLLAILSFPSGN-----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-----DDPMVVTEP-----	-----ELSEVI ISDTN-	348
O16857_MICPR	-PEDFFVT LST-----TDPSAEVDPD-----	-----RDVATATINDLD-	466
O16857_MICPR	-PEIFFADLMT-----DEERVTLDP-----	-----NRTTIVIIDAD-	585
O16857_MICPR	-RGDFFVNLT T-----SEPLVTLMP-----	-----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- $\beta$  domains. The 37 sequences that constitute the seed of the Calx- $\beta$  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  $\beta$ -strand of the Calx- $\beta$  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  $\beta$ 4 (ITB4\_HUMAN), the alignment includes sequences of Na<sup>+</sup>/Ca<sup>2+</sup>-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 *Microcystis proliferans* (O16856\_MICPR, O16857\_MICPR, O16858\_MICPR), and three proteins from *Synechocystis* sp. PCC6803 (P73139\_SYNY3, P73590\_SYNY3, P74440\_SYNY3). The  $\beta$ -strands of the Calx- $\beta$  domain, as observed in the  $\beta$ 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 Ca<sup>2+</sup> are shown in red boxes and the number of the residues of  $\beta$ 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., Cogill, 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.