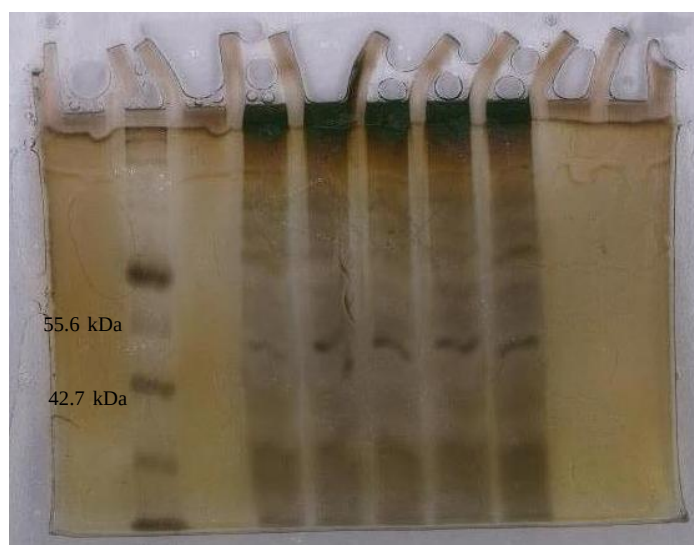


R161, K452 and R460 residues are vital for metal-citrate complex transport in the CitMHS secondary transport protein Cit_{Sc} from *Streptomyces coelicolor*

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S-1. Acrylamide gels of the JW *E. coli* cells expressing Cit_{Sc}, R161A, K452C, and R460C. Gel is a 10% polyacrylamide gel. Expression conditions are as describe din the text Markers are NEB Protein Marker, Broad Range (2-212 kDa). Cit_{Sc} is predicted to weigh ~50 kDa.

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CitSc      --MLTILGFAMIATFLVLIMMKKMSPIAALVLIPALFCVLVGKG-----
CitBs1    --MLAILGFLMLLVFMALIMTKRLSVLTALVLTPIVFALVAGFG-----
CitBs2    --MLAILGFVMMIVFMYLIMSNRLSALIALIVVPIVFALISGFG-----
CitEf     -MLLTI LAYGMIIIFMYVIMKKKLSPTSLVVIPLIFTLIAIATGVASPP
CitSm     MMLLTMLAYAMIIVFMYVVMKKKMTPTALTVMIPILMTIAVILTGSADFN

CitSc      -----AHLGDYVIDGVSSLAPTAAMLMFAIVYFGVMI
CitBs1    -----FTEVGDMMISGIQQVAPTAVMIMFAILYFGIMI
CitBs2    -----KDLGEMMIQGVTDLAPTGIMLLFAILYFGIMI
CitEf     P-----GADGVVPEVN-IGNFVLDGISTTSKTGIMLLFAILYFSIML
CitSm     ADAKFVAFVGDGGIAKDLTAIGPMVMYGINNTAKTGIMLLFAILYFSVML

CitSc      DVGLFDPVIRAILKFKADPMRIVVGTALLAAIVSLDGDGSTTFMITVSA
CitBs1    DTGLFDPVVGKILSMVKGDPKIVVGTAVLTMLVALDGDGSTTYMITTSA
CitBs2    DSGLFDPVIAKILSFVKGDPFKIAVGTAVLTMTISLDGDGSTTYMITIRA
CitEf     DAGLFDPIITKKMIYFAKGDPMKVLMATAVVAAVSLNGDGTITTLICCSA
CitSm     DAGLFDPIITEKMIRFAKGDPMKVLIATAVVAAVSLNGDGTITTLICCSA

CitSc      MYPLYKRLKMSLVVMTGVAAMANGVMNTLPWGGPTARAATALKVDATDIF
CitBs1    MLPLYLLLGIRPIILAGIAGVGMIMNTIPWGGATPRAASALGVDPAELT
CitBs2    MLPLYKRLGMNRLVLAGIAMLGSGVMNIIPWGEPTARVLASLKLDTSEVF
CitEf     FIPIYKKLNMKLMNLGVLVILQNTIMNLLPWGGPTARAMAVLEVD-ADIL
CitSm     FLPIYKKLDMKIMNLGVLIIQLQNTIMNLLPWGGPTARAMSVLGVG-PEIL

CitSc      VPMIPALAVGLVAVVV-LAYVLGLRERRRLGTLSLDGAPEREPETETVLV
CitBs1    GPMIPVIAAGMLCMVA-VAYVLGKAERKRLGVIELKQPANANE-----
CitBs2    TPLIPAMIAAGILWVIA-VAYILGKKERKRLGVISIDHAPSSDP-----
CitEf     SYLAPGMILSVLYVIFVAYRMGKKERARLGVTTLTDKLEEMT-----
CitSm     GYLAPGMILSLLYVICWVAPSMGRKERAKLGVIDLSEEDMRQLT-----

CitSc      GAGAGTGAAGLSGAGAGAGAGAARGAAGGPGAAGDRDTGAGGESDDDFKG
CitBs1    -----PAAAVE-----DEWK-
CitBs2    -----EAAP-----LK-
CitEf     -----IVNDPE-----LLEIR-
CitSm     -----DITDPD-----TLFIR-

CitSc      LDPNRPTLRRLYWFNALLTALLTAMIME-----LLPIPVLV
CitBs1    -----RPKLWWFNLLTSLIGCLVSG-----KVSILTIVLF
CitBs2    -----RPALQWFNLLTVALMAALITS-----LLPLPVLV
CitEf     -----RPKNFAFNGLTIVLIGWLVLSSFVGQLGLPSWIEPPLLLF
CitSm     -----RPKNFVFNAILTIGLITWLVAAGSFN-----KSIAMAPLLLF

CitSc      LIGAAALALTVNFPHIPDQKARIAAHADNVLVNVSVMVFAAAVFTGVLGTG
CitBs1    VIAFCIALIVNYPNLEHQRIAAHSSNVLAIGSMIFAAGVFTGILTGTG
CitBs2    MTAFVALMVNYPNVKEQQKRISAHAGNALNVVSMVFAAGIFTGILSGTK
CitEf     LVGTICIALMINYPKLDQSSRISANAGDAVQVILVFAAGVFMGLFQGS
CitSm     AVGTICIALMVNYPVLKDQSKRIGDNAGDAVQVILVFAAGIFMGLFQGS

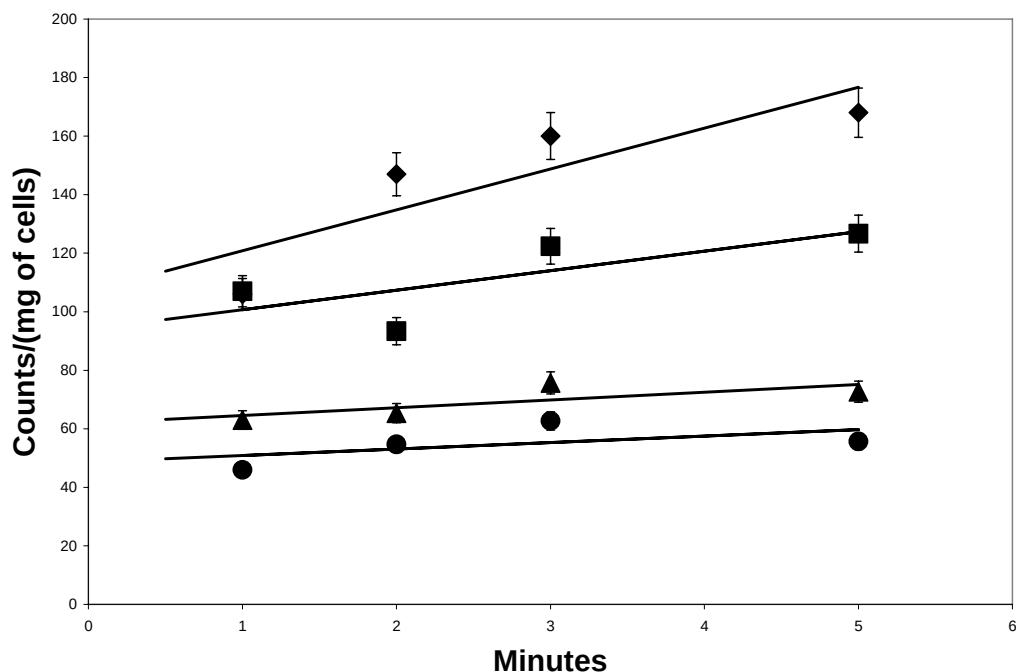
CitSc      MVDHMANWLVDITPDGMGPQMGVLVTGLLSLPLTYFMSNDGFYFGLPVLA
CitBs1    MVDEMAISLVSMIPEQMGGIIPAIVALTSGIFTFLMPNDAYFYFGLPILS
CitBs2    MVDAMAHSTRSLIPDAMGPHLPLITAIVSMPFTFFMSNDAFYFGLPIIA
CitEf     MANALANSFVNIIPKQLAGAWGLVIAFISAPGTFISNDGFYFGLPVLA
CitSm     MASALAQSFIATIPKQLAGFWGLVIALVSAPGTFISNDGFYFGLPVLA

CitSc      EAGQAHGVSTLEIARASIVGQPLHMSSPLVPVAVYVLVGMKVEFGDHTRF
CitBs1    ETAVAYGVDKVEIARASIIQQPIHMLSPLVPSTHLLVGLVGVSIDDHQKF
CitBs2    EAASAYGIDAAEIGRASLLGQPVHLLSPLVPSTYLLVGMAGVSFSDGHQKF
CitEf     EAGRSYGFDMNSMALASLMGQAFHLLSPLVAFIYLLRLTGLDMGQWQKE
CitSm     EAGAEGFNSMAMALASLMGQAFHLLSPLVAFIYLLRLTGLDMGEWQKE

CitSc      VVKWAVLTSLVILAAGILFGII-----
CitBs1    ALKWAVLAVIVMTAIALLIGASISV---
CitBs2    TIKWAVGTTIVMTAIALLIGIISF----
CitEf     SAKYALGIFVIFVVTIVALGHMPLFIPQN
CitSm     AAKYALIIIFVIFVVTIIMGQMPLYIPQ-

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S-2. CLUSTAWL Multiple Sequence Alignment of the five functionally characterized CitMHS members. Conserved and similar amino acids are in blue and green.



S-3. Ca^{2+} -citrate uptake by K452C. Solid diamonds represent Ca^{2+} -citrate uptake by K452C, solid squares represent uptake with non-induced wild type, closed circles represent free citrate control in wild type, and closed triangles represent Mg^{2+} -citrate transport. Experiments were performed at least in triplicate.