Biochemical and EPR-spectroscopic Investigation into Heterologously Expressed Vinyl Chloride Reductive Dehalogenase (VcrA) from *Dehalococcoides mccartyi* strain VS

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SUPPORTING INFORMATION

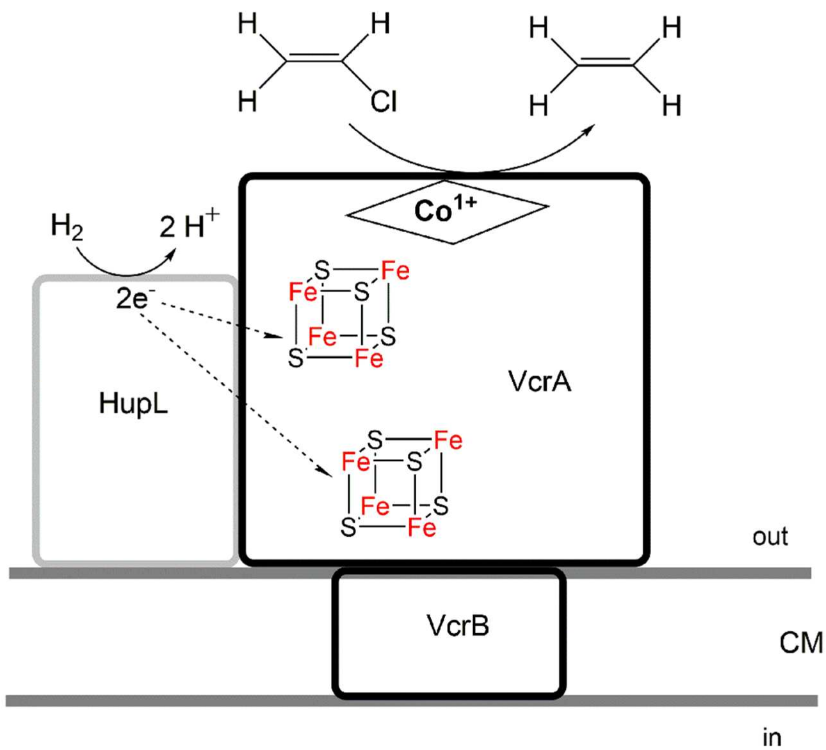

Fig S1. Model of vinyl chloride reduction in the *Dehalococcoides mccartyi* strain VS cell – VcrA (the catalytic vinyl chloride reductase subunit) is shown with two [4Fe-4S] clusters in red & black and a cartoon of a corrinoid in black. The entire complex of VcrA and hydrogenase (HupL), which presumably donates electrons to VcrA, as well as VcrB, which anchors VcrA to the membranes are oriented towards the periplasmic space. CM denotes the cytoplasmic membrane.

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                1                                                  50
                      ## # #                                    #
VcrA            .MSKFHKTIS RRDFMKGLGL AGAGIGAVAA SAPV...... .FHDIDELVS
DmccaTceA       MSEKYHSTVT RRDFMKRLGL AGAGAGALGA AVLAENNLPH EFKDVDDLLS
DEth195         MSEKYHSTVT RRDFMKRLGL AGAGAGALGA AVLAENNLPH EFKDVDDLLS
SmultivoPceA    MEKKKKPELS RRDFGK.LII GGGAAVTIAP FGVPGANAAE KEKNAAEIRQ
DesulfH         .....MGEIN RRNFLK.VSI LGAAAAAVAS ASAVKGMVSP LVADAADIVA
DesulfCprA      .......... .......... .......... .......... ..........
Consensus       ...k...... rrdf.k.l.. .ga.a.a.a. .......... ...d......

                51                                                100
                        1        #
VcrA            S.......EA NSTKDQPWYV KHREHFDPTI TVDWDIFDRY DGYQHKGVYE
DmccaTceA       AGKALEGDHA NKVNNEPWWV TTRDHEDPTC NIDWSLIKRY SGWNNQGAYF
DEth195         AGKALEGDHA NKVNNHPWWV TTRDHEDPTC NIDWSLIKRY SGWNNQGAYF
SmultivoPceA    QFAMTAGSPI IVNDKLERYA EVRTAFTH.. .......PTS FF........
DesulfH         PITETSEFPY KVDAKYQRYN SLKNFFEKTF DPEANKTPIK FHYDDVSKIT
DesulfCprA      .......... .......... .......... .......... ..........
Consensus       .......... ........y. ..r..f..t. .......... ..........

                101                                               150
                                               #
VcrA            GPPDAPFTSW GNR....... .LQVRMSGEE Q......KKR ILAAKKERFP
DmccaTceA       LPEDYLSPTY TGRRHTIVDA FREIKLQGKK YRDSAWIKSG IDWMKENIDP
DEth195         LPEDYLSPTY TGRRHTIVDS KLEIELQGKK YRDSAFIKSG IDWMKENIDP
SmultivoPceA    .KPNYKGEVK P......... .WFLSAYDEK VRQIENGENG PKMKAKNVGE
DesulfH         GKKDTGKDLP T......... .LNAERLGIK GRPATHTETS ILFHTQHLGA
DesulfCprA      .......... .......... .MENNEQRQQ TGMNRRSFLK VGAAATTMGV
Consensus       ...d...... .......... .......g.k .r........ i.......g.

                151                                               200
VcrA            GWDGGLHGRG DQRADALFYA VTQPFPGSGE EGHGLFQPYP .DQPGKFYAR
DmccaTceA       DYDPGELGYG DRREDALIYA ATNGSHNCWE ..NPLYGRYK GSRPYLSMRT
DEth195         DYDPGELGYG DRREDALIYA ATNGSHNCWE ..NPLYGRYE GSRPYLSMRT
SmultivoPceA    ARAGR..... ......ALEA AGWTLDINYG NIY....... ...PNRFFML
DesulfH         MLTQRHNETG WTGLDEALNA GAWAVEFDYS GFNAT..... GGGPGSVIPL
DesulfCprA      IGAIKAPAKV ANAAETMNYV PGPTNARSKL RPVHDFAGAK VRFVENNDEW
Consensus       .........g ....d...ya .......... .......... ...p......

                201                                               250
                                      #
VcrA            WGLYGPPHDS APPD...GSV PKWEGTPEDN FLMLRAAAKY FGAGGVGALN
DmccaTceA       MNGINGLHEF GHADIKTTNY PKWEGTPEEN LLIMRTAARY FGASSVGAIK
DEth195         MNGINGLHEF GHADIKTTNY PKWEGTPEEN LLIMRTAARY FGASSVGAIK
SmultivoPceA    WSGETMTNTQ LWAPV...GL DRRPPDTTDP V......... ....ELTNYV
DesulfH         YPINPMTNEI ANEPVMVPGL YNWDNIDVES VRQQGQQWKF ESKEEASKIV
DesulfCprA      LGTTKIISKV KKTSEADAGF MQAVRGLYGP DPQRGFFQFI AKHPFGGTIS
Consensus       .......... ........g. ..w....... .......... ......g.i.

                251                                               300
                                        #
VcrA            LADPKCKKLI YKKAQPMTLG KGTYSEIGGP GMIDAKIYPK VPDHA...VP
DmccarTceA      ITD.NVKKIF YTKAQPFSLG PW.YTITNFA EYIEYPV..P VDNYA...IP
DEth195         ITD.NVKKIF YAKVQPFCLG PW.YTITNMA EYIEYPV..P VDNYA...IP
SmultivoPceA    KFAARMAGAD LVGVARLN.R NWVYS...EA VTIPADVPYE QSLHKEIEKP
DesulfH         KKATRLLGAD LVGIAPYD.E RWTYSTWGRK IYKPCKMPNG RTKYLPWDLP
DesulfCprA      WARNLIAAED VVDGDAEPTK TPIPDPEQMS QHIRDCCYFL RADEVGIGKM
Consensus       .........d .v...p.... .w.ys..... ..i....... .........p
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             301                                                  350
              #
        VcrA INFKE..ADY SYYN...... DAEWVIPTK. .CESIFTFTL PQPQELNKRT
  DmccarTceA IVFEDVPADQ GHYSYKRFGG DDKIVVPNA. .LENIFTYTI MLPQKRFKYA
     DEth195 IVFEDIPADQ GHYSYKRFGG DDKIAVPNA. .LDNIFTYTI MLPEKRFKYA
SmultivoPceA IVFKD..VPL PI......ET DDELIIPNT. .CENVIVAGI AMNREMMQTA
     DesulfH KMLSG..GGV EVFGHAKFEP DWEKYAGFK. .PKSVIVFVL EEDYEAIRTS
  DesulfCprA PEYGYYTHHV SDTVGIMSKP VEECVTPVTK IYPNVIVVMI DQGIETMWAS
   Consensus i.f....... .......... d.e.v.p... ...n!iv..i ....e.....

             351                                                  400
                        #   #              #       #
        VcrA GGI..A.GAG SYTVYKDFAR VGTLVQMFIK YLGYHALYWP I.GWGPGGCF
   DmccaTceA HSV..PMDPC SCIAYPLFSE VEARIQQFIA GLGYNSMGGG VEAWGPGSAF
     DEth195 HSI..PMDPC SCIAYPLFTE VEARIQQFIA GLGYNSMGGG VEAWGPGSAF
SmultivoPceA PNS..MACAT TAFCYSRMCM FDMWLCQFIR YMGYYAIPSC .NGVGQSVAF
     DesulfH PSV..ISSAT VGKSYSNMAE VAYKIAVFLR KLGYYAAPCG .NDTGISVPM
  DesulfCprA TGYDGISGAM SMQSYFTSGC IAVIMAKYIR TLGYNARXHH AKNYEAIMPV
   Consensus ........a. s...Y..... v......%ir .$GY.a.... ....g....f

             401                                                  450
                     #    #        #                        #  #    #
        VcrA TTFD.GQGEQ GRTG.AAIHW KFGSSQRGSE RVITDLPIAP TPPIDAGMFE
   DmccaTceA GNLS.GLGEQ SRVS.SIIEP RYGSNTKGSL RMLTDLPLAP TKPIDAGIRE
     DEth195 GNLS.GLGEQ SRVS.SIIEP RYGSNTKGSL RMLTDLPLAP TKPIDAGIRE
SmultivoPceA AVEA.GLGQA SRMG.ACITP EFGPNVRLT. KVFTNMPLVP DKPIDFGVTE
     DesulfH AVQA.GLGEA GRNG.LLITQ KFGPRHRIA. KVYTDLELAP DKPRKFGVRE
  DesulfCprA CIMAAGLGEL SRTGDCAIHP RLGYRHKVA. AVTTDLPLAP DKPIDFGLLD
   Consensus ...a.GlG#. sR.g...I.p .fG...r... .v.T#$plaP dkPidfG..#

             451                                                  500
             #*  *  * #  *        #         #             #  ## #*
        VcrA FCKTCYICRD VCVSGGVHQE DEPTW...DS GNWWNVQGYL GYRTDWSGCH
   DmccaTceA FCKTCGICAE HCPTQAISHE G.PRY...DS PYWDCVSGYE GWHLDYHKCI
     DEth195 FCKTCGICAE HCPTQAISHE G.PRY...DS PHWDCVSGYE GWHLDYHKCI
SmultivoPceA FCETCKKCAR ECPSKAIT.. EGPRTF..EG RSIHNQSGKL QWQNDYNKCL
     DesulfH FCRLCKKCAD ACPAQAISHE KDPKVLQPED CEVAENPYTE KWHLDSNRCG
  DesulfCprA FCRVCKKCAD NCPNDAITFD EDP....... ...IEYNGYL RWNSDFKKCT
   Consensus FC.tCkkCad .Cp..a!..e ..P....... .......gyl .w..D..kC.

             501                                                  550
                         *  *   *  #         # # $
        VcrA N......... QCGMCQSSCP FTYLGLENAS LVHKIVKGVV ANTTVFNSFF
   DmccaTceA N......... .CTICEAVCP F..FTMSNNS WVHNLVKSTV ATTPVFNGFF
     DEth195 N......... .CTICEAVCP F..FTMSNNS WVHNLVKSTV ATTPVFNGFF
SmultivoPceA GYWPES..GG YCGVCVAVCP F....TKGNI WIHDGVEWLI DNTRFLDPLM
     DesulfH SFWAYN..GS PCSNCVAVCS W....NKVET WNHD.VARVA TQIPLLQDAA
  DesulfCprA EFRTTNEEGS SCGTCLKVCP W...NSKEDS WFHKAGVWVG SKGEAASTFL
   Consensus ........g. .Cg.C.avCp f.....k..s w.H..v..v. ..t.....f.

             551                                                599
                #    #         #
        VcrA TNMEKALGYG D.LTMENSNW WKEEGPIYGF DPGT...... .........
   DmccaTceA KNMEEAFGYG PRYSPSRDEW WASENPIRGA SVDIF..... .........
     DEth195 KNMEGAFGYG PRYSPSRDEW WASENPIRGA SVDIF..... .........
SmultivoPceA LGMDDALGYG AKRNI..TEV WDGKINTYGL DADHFRDTVS FRKDRVKKS
     DesulfH RKFDEWFGYN GPVNP..DER LESGY.VQNM VKDFWNNPES IKQ......
  DesulfCprA KSIDDIFGYG TE.TIEKYKW WLEWPEKYPL KPM....... .........
   Consensus ..m#.afGYg ........ew w......yg. ..d....... .........
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Fig S2. Amino acid sequence alignments with the GenBank accession numbers in brackets; VcrA from *Dehalococcoides* VS (ACZ62391.1) compared with DmccaTceA = *Dehalococcoides mccartyi* TceA (AFX81898.1); Deth195 = *Dehalococcoides ethenogenes* 195 dehalogenase (AAW39060.1); SmultivoPceA = *Sulfospirillum multivorans* PceA (AHJ12791.1); DesulfH = *Desulfitobacterium hafniense* Y51 dehalogenase (BAC00915.1) and DesulfCprA = *Desulfitobacterium dehalogenans* CprA (AAD44542.1). The * mark and blue shading refer to the conserved Cys residues for binding the FeS clusters; # suggests a possible catalytic role and $ refers to the conserved His. Yellow = single amino acid conserved in $\geq$5 of the 6 sequences. Grey = conservation in $\leq$4 out of 6 sequences. Green = multiple amino acid region with conservation in $\geq$5 of the 6 sequences. All markings are made with like-for-like amino acid substitution criteria (eg. E is considered conserved if E is changed into D in some entries). The portion of the sequence where the heading is underlined is a predicted TAT signal according to an earlier study (Mũller et al, 2004). After post-translational processing, the first amino acid of VcrA is a glutamate represented by the symbol 1 (E59 in this alignment).

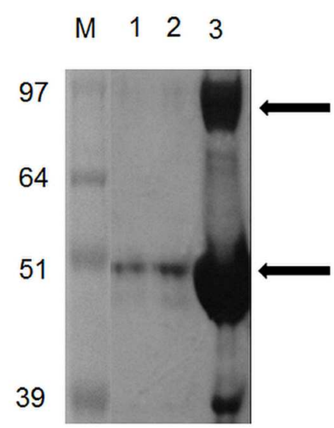

Fig S3. SDS-PAGE analysis of TEV digestion of VcrA-MBP. On the left of the gel, the sizes of the bands in the molecular mass marker are shown in kDa. The position of the arrows indicates the bands of interest, the upper shows VcrA-MBP and the lower VcrA without MBP. Lanes M = molecular mass marker, 1 & 2 = flow through from the Ni-IDA column after digestion; 3 = TEV protease digest of VcrA-MBP.

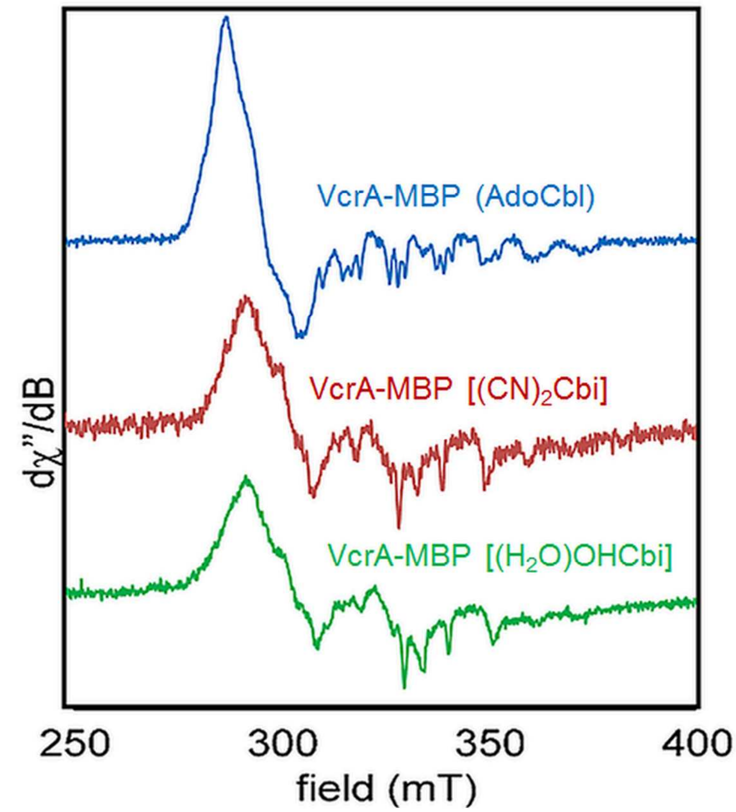

Fig S4. CW EPR spectra of VcrA-MBP reconstituted with different corrinoids and reduced with 2-mercaptoethanol. AdoCbl = adenosylcobalamin (blue); $(CN)_2Cbi$ = dicyanocobinamide (brown) and $(H_2O)OHCbi$ = aquahydroxocobinamide (green). The hyperfine structure due to axial ^{14}N coordination seen in the blue trace is absent in the other two samples (which lack the lower nitrogenous ligand), showing that the ^{14}N signal arises from the corrinoid cofactor and not from a proteinaceous ligand like a His residue.

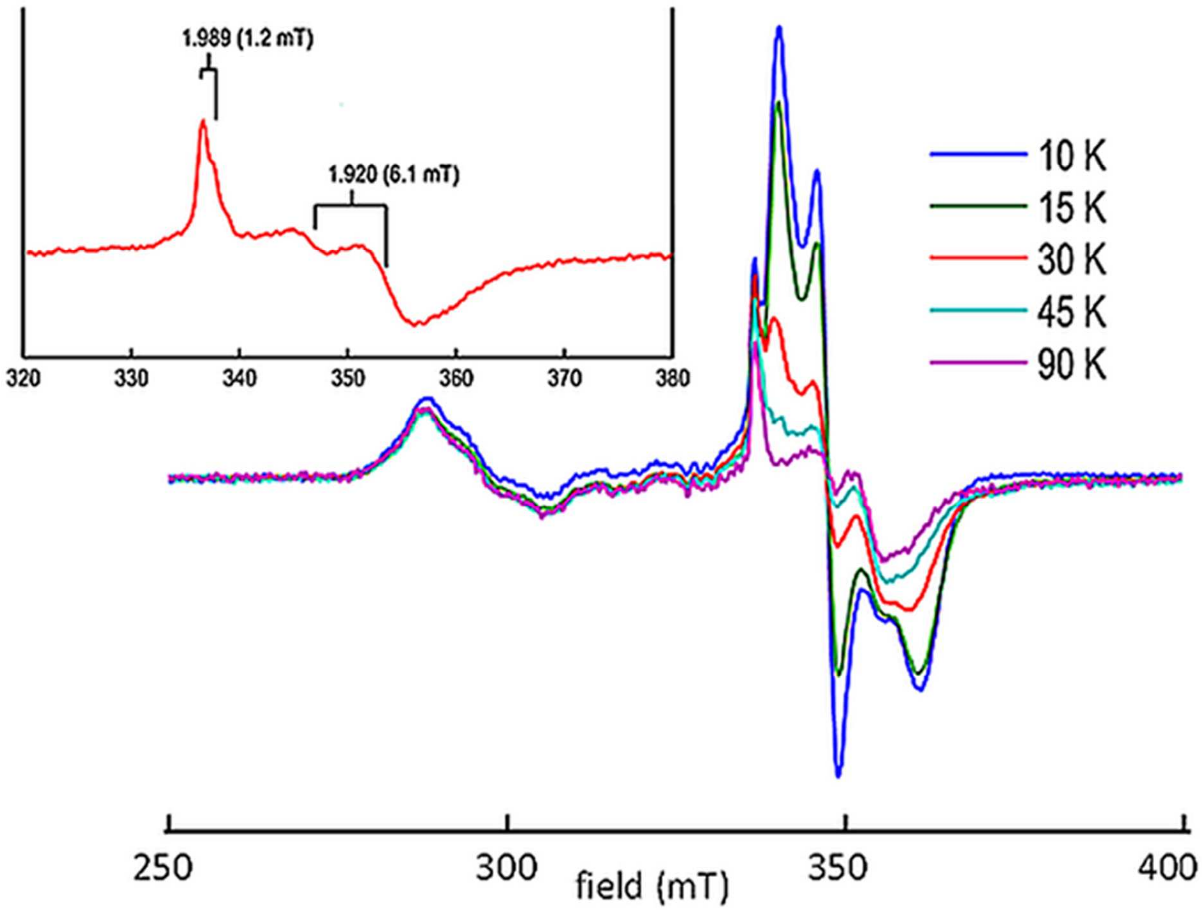

Fig S5. X band EPR spectra showing the temperature response of Ti(III)-reduced VcrA-MBP at 10, 15, 30, 45 and 90 K as indicated [inset shows the signals arising from Ti (III)]. The signals at *g*-values of 1.989 and 1.920 do not decay considerably with the rise of temperature, while the ones at *g*-values of 1.969 and 1.928 decay much faster, suggesting [4Fe-4S] cluster(s) in the reduced +1 state.

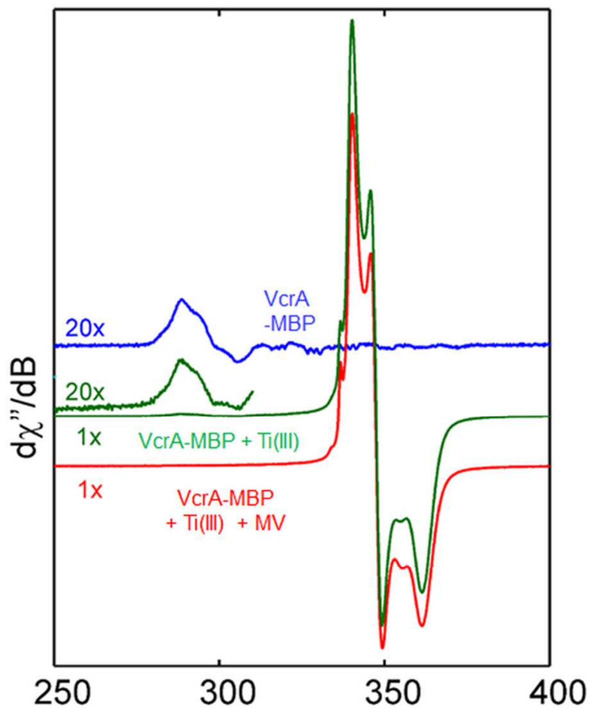

Fig S6. X-band spectra of VcrA-MBP following rapid desalting (which resulted in the loss of some bound cobalamin) after the reduction of each sample. The blue trace was measured after reduction with 2-mercaptoethanol, the green trace after Ti(III) reduction, and the red trace after reduction with Ti(III) and the electron carrier dye methyl viologen. The features attributed to S = 1/2 Ti(III) species are greatly diminished, whereas the $[4Fe\text{-}4S]^{1+}$ signal remains unchanged in the latter two samples. The Cob(II)alamin signals are abolished upon the reduction via methyl viologen, suggesting reduction to the Co(I) state.

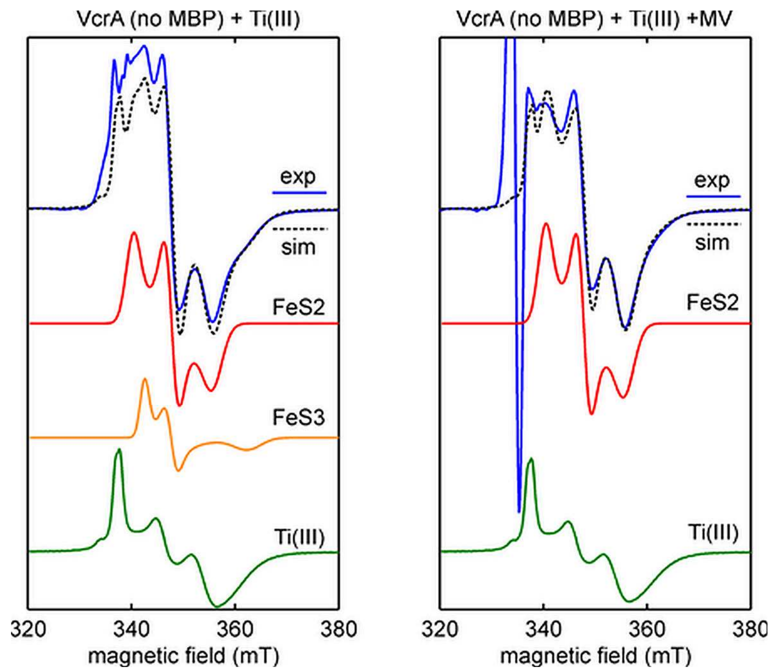

Figure S7. X-band (9.38 GHz) CW EPR spectra (blue traces) for reconstituted VrcA lacking the MBP tag and treated with 1.0 mM Ti(III)citrate (left panel) and 1.0 mM Ti(III)citrate + 0.5 mM methylviologen (right panel). Spectrometer settings are same as given in earlier CW EPR figures. Red trace is a simulation of an FeS cluster signal obtained using the parameters *g* = [1.957, 1.928, 1.849]; *g*-Strain = [0.01, 0.0125, 0.03] that was fit based on the difference spectrum of 15 K and 90 K spectra of VcrA (no MBP) +Ti(III) + MV. The orange trace is a simulation of an FeS cluster signal obtained using the parameters *g* = [1.970, 1.928, 1.885]; *g*-Strain = [0.018, 0.014, 0.023] that was fit based on the difference spectrum of the 15 K data of VcrA (no MBP) +Ti(III) *minus* VcrA (no MBP) +Ti(III) + MV. The green trace is a spectrum of the VcrA (no MBP) +Ti(III) sample collected at 90 K. All FeS cluster signals relax too quickly at this temperature and only the signals corresponding to Ti(III)-containing species remain. The dotted black trace is a summation of the FeS2, FeS3, and Ti(III) signals weighted by the concentrations given in Table S1. The MV sample underwent accidental oxidation during freezing and the [4Fe-4S] cluster corresponding to the FeS2 signal was converted into a [3Fe-4S] cluster.

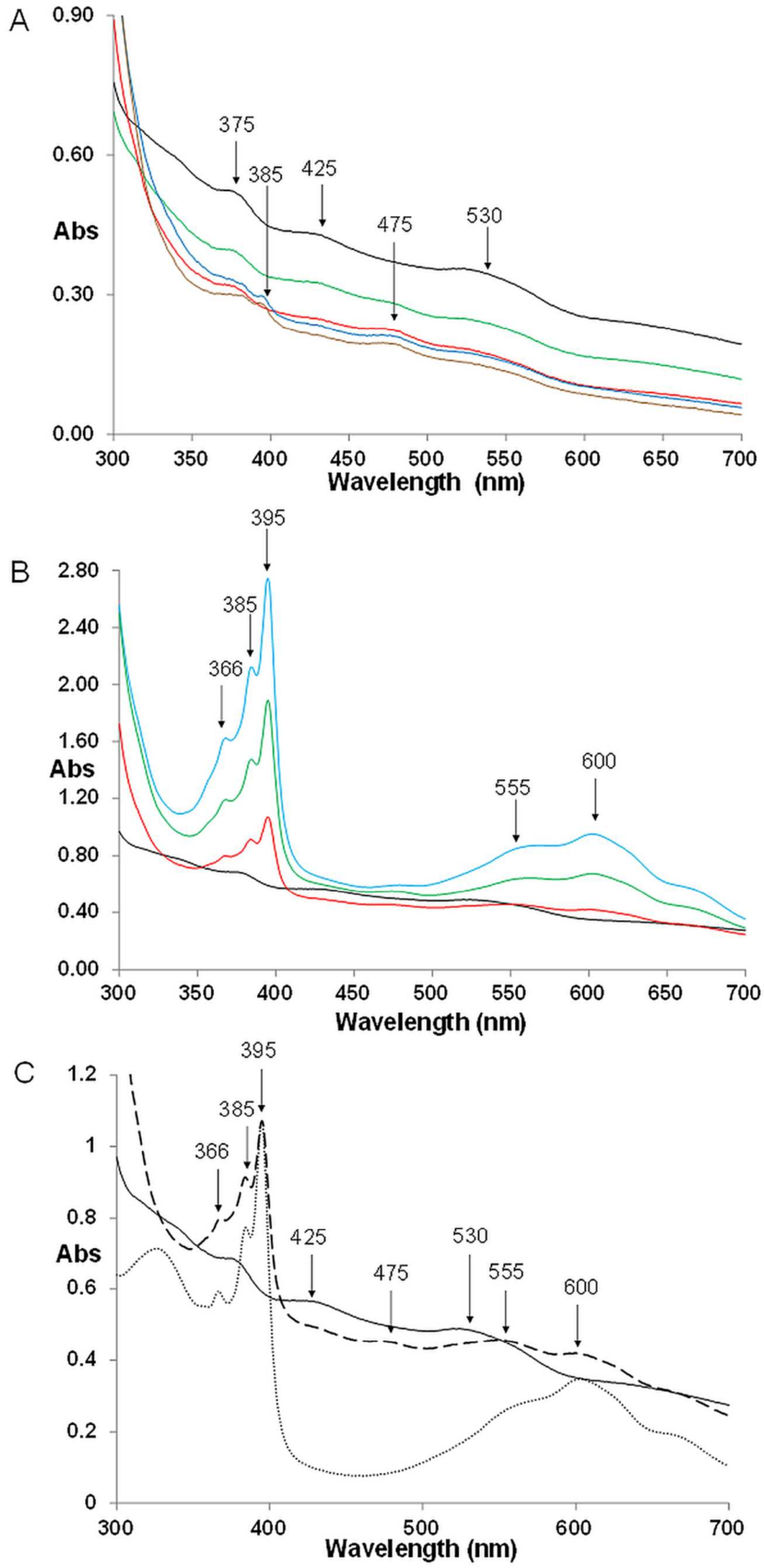
A
0.90
0.60
0.30
0.00
Abs
375
385
425
475
530
300
350
400
450
500
550
600
650
700
Wavelength (nm)
B
2.80
2.40
2.00
1.60
1.20
0.80
0.40
0.00
Abs
395
385
366
555
600
Wavelength (nm)
C
1.2
1
0.8
0.6
0.4
0.2
0
Abs
395
385
366
425
475
530
555
600
Wavelength (nm)

Fig S8. Electronic absorption spectra of VcrA-MBP

Panel A: 80 µM VcrA-MBP reduced with different concentrations of Ti(III)-citrate: black = 0 mM, unreduced (note the iron-sulfur peak at 420 nm & the Cob(II)alamin peaks at 375 and 530 nm); green = 0.2 mM; blue = 0.4 mM; red = 0.8 mM and brown = 1.6 mM. All the spectral features between 300 and 400 nm disappeared at 2.5 mM of Ti(III)-citrate (data not shown). The peak Co(I) is at 385 nm. **Panel B:** 100 µM VcrA-MBP reduced with 0.4 mM methyl viologen; sequential additions of Ti(III)-citrate induced the generation of peaks at 366, 385 and 395 nm, and two more broad peaks around 555 and 600 nm (black = 0 mM, unreduced; red = 0.3 mM; green = 0.6 mM and blue = 1 mM). The 600 nm peak belongs to reduced methyl viologen. **Panel C:** 100 µM unreduced VcrA-MBP (solid line), 100 µM of VcrA-MBP reduced with 0.4 mM methyl viologen and 0.3 mM Ti(III)-citrate (dashed line) and 0.4 mM free methyl viologen reduced with 0.3 mM Ti(III)-citrate (dotted line). Although the peaks at 366, 385, 395 and 600 nm could arise from free methyl viologen and thus mask the 385-390 nm peak of Co(I), the peak at 555 nm (shifting from 530 nm for unreduced VcrA-MBP) suggests a reduction of the Co(II) to the Co(I) state.

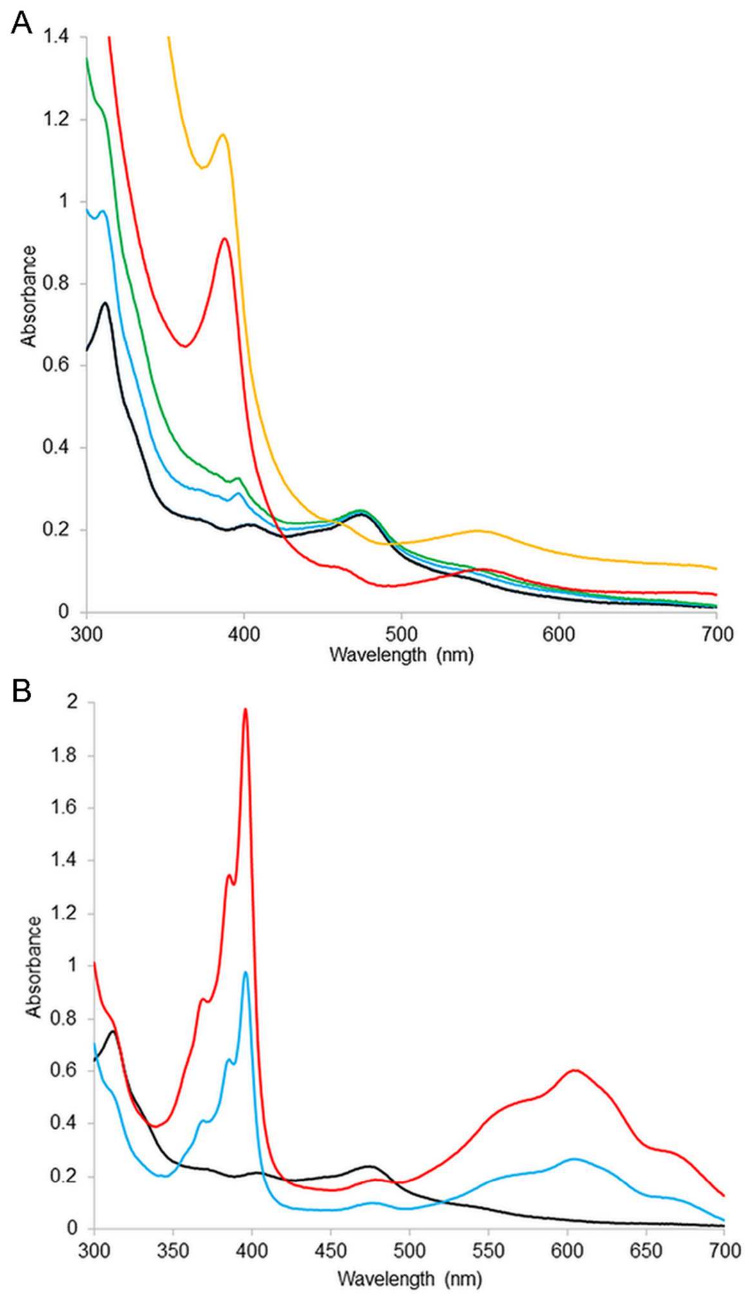

Fig S9. Electronic absorption spectra of TEV-protease digested and purified VcrA

Panel A: 50 µM, reconstituted with hydroxocobalamin & FeS clusters. Recorded upon sequential reduction by Ti(III)citrate – black (unreduced, 0 mM), sky blue (0.2 mM), green (0.4 mM), red (1 mM) and golden (1.5 mM); showing the emergence of a clear Cob(I)alamin peak at 390 nm at higher Ti(III) concentrations. Concomitant with the appearance of the Cob(I)alamin signal, the 475 nm Cob(II)alamin maximum is replaced by another at 465 nm (which shows that cobalamin is still bound to VcrA), while a new peak is also generated at 550 nm for Cob(I)alamin. The Cob(I)alamin signals are not observed above [Ti(III)citrate] = 2 mM since all maxima are masked by the Ti(III) absorption [data not shown]. Our spectra are strikingly similar to those published for the corrinoid iron sulfur protein (Kung et al, 2012). **Panel B:** 48 µM, reconstituted with hydroxocobalamin & FeS clusters] treated with 0.4 mM reduced methyl-viologen. The traces show the sequential reduction of VcrA by Ti(III)citrate – black (unreduced, 0 mM), sky blue (0.9 mM) and red (1.8 mM).

Table S1. Concentration (µM) of paramagnetic species in VcrA samples lacking the MBP Tag.

Sample	FeS2	FeS3	Ti(III)
VrcA(no MBP) + Ti(III)	23	60	114
VrcA(no MBP) + Ti(III) + MV	--	66	104