SUPPORTING INFORMATION

```
Parachlamydia acanthamoebae ------------------------------------------------------------  0
Campylobacter jejuni        --------------------------------LDPNL-----------------------  5
Wolinella succinogenes      --------------------------------FERWT-----------------------  5
Marichromatium purpuratum   PACVRCHGPGGVGAGAVFPPLAGQPYSYLLAQLQAWGTGRRHGEPMALMGAVAGRLDADE  180
Thiomonas intermedia        ------------------------------------------------------------  0
Sideroxydans lithotrophicus ------------------------------------------------------------  0
Pseudomonas stutzeri        ------------------------------------------------------------  0
Allochromatium vinosum      ------------------------------------------------------------  0
Psychrobacter arcticus      -------------------------------------------------------SDNTE  5

Parachlamydia acanthamoebae ----------------------------------SSN-----------------------  3
Campylobacter jejuni        --------EK----------------------TKSATGIDLPTAKWNLPKALNED-----  30
Wolinella succinogenes      --------LE----------------------ARGKSGYDAPKLEWVEGKTINEIKNPTI  35
Marichromatium purpuratum   QRALAAYFATRPLAVADPADDLPDPSPPPATASVSTPAMTVAGANAVVPEHLGAV-----  235
Thiomonas intermedia        ------------------------------------------ADAPMAP-PKSEI-----  12
Sideroxydans lithotrophicus ---------------------------------------------------ASET-----  4
Pseudomonas stutzeri        ------------------------------------------AEIKMDDQSQLTQ-----  13
Allochromatium vinosum      ------------------------------------------------------------  0
Psychrobacter arcticus      TKAVERVEEAAALA---RVKDLEA------RAEALKS--NMPAANDMTA---TAG-----  46

Parachlamydia acanthamoebae --GMSSSNDTQEWRTTLLDPEMAPENIKPLVMQGFRILFDTKK--------HAPQYAGDE  53
Campylobacter jejuni        -------------GTIDETKMPKNSEYSKMVILGNKILNETSKYVGPQAKDPKKRFAGNN  77
Wolinella succinogenes      QYALPKFEGDVFWDTINAKPVVPDSIYGEFVQYGYELFVNTQAVIGPEVADPNMRYSGNN  95
Marichromatium purpuratum   --PAGRAEAAS-RFTPPSRDALPEGPLGEMVRLGARLFRHTNTD------PRSAPHVGND  286
Thiomonas intermedia        --NA-AVGTGA-KFTPPPESAIPDDDFGKMVKLGRDIMLDTPK--------YAKDYVGNT  60
Sideroxydans lithotrophicus --STPTAAKAL-VFTPPNDEEIPNNEFGKIVRQGKNIFEDTQH--------YAKQYVGNS  53
Pseudomonas stutzeri        --KAGKGAGES-YFQPPQEKDLPANAYGELVQQGRAIFVDTQK--------YAAEYVGNG  62
Allochromatium vinosum      -----EEPPTV-ALTVPAAALLPDGALGESIVRGRRYLSDTPA--------QLPDFVGNG  46
Psychrobacter arcticus      --GTDTASGKP-TIKMPDESTIPDDEFGAAVRRGLQISNHTYK--------ELPNNVGNQ  95

Parachlamydia acanthamoebae ISCTNCHFNCGNTFGGENNGISLVGVTKKYPRAILD-NPHYTLEERINACFTKSLNG---  109
Campylobacter jejuni        LSCSSCHANGG----SVQNQSGFVGIWARFPQYNARGDKVITLADRINGCFERSMNG---  130
Wolinella succinogenes      LSCNSCHLGAG----TAKYAAPLVDNHANFPQYRNRENSLGTMAARVNGCMQRSMNG---  148
Marichromatium purpuratum   QTCAGCHLDNG----RRADASPMWAAWVAYPAYRGKNQRVDTMAERIQGCFRYSMNAQDS  342
Thiomonas intermedia        LSCVNCHTDAG----RMAGSAPLWAAYVSYPAYRGKNKKVNTFEERLQGCFKFSQNG---  113
Sideroxydans lithotrophicus LNCVNCHLASG----RKENSSPLWAAYVRYPAYRAKNNKVNTYEERIQGCFKYSLNG---  106
Pseudomonas stutzeri        MNCTNCHLEQG----RKANSAPLWGAYPMYPAYRKKNDKVNSYAERVQGCFQFSMNG---  115
Allochromatium vinosum      LACRHCHPGRDGEVGTEANAAPFVGVVGRFPQYSARHGRLITLEQRIGDCFERSLNG---  103
Psychrobacter arcticus      LNCTSCHLGNG----SEAYAAPWNNTPSVYPNYSKRTGRINTIQERINGCFERSLNG---  148

Parachlamydia acanthamoebae ---KPVPLKSKEMKAMIAYLEWISKGVSNQA---PWLGLKKLRS----HAIPNAEKGNAL  159
Campylobacter jejuni        ---KRMPSDTPEMKAMLTYMQWLSQGVPVGA-KIEGQGLKKIDF---ISRAADPKKGKAI  183
Wolinella succinogenes      ---YPLPAEGKEMKAFLAYIHWLGQGIPVGA-KIEGRSLKTVDRKMVQQNAADVKNGAEV  204
Marichromatium purpuratum   VSGQVPETNGLVLDALQSYIFWLATGAPTGDTAMSGRGYPRLQP---PAEGFDRTRGAAL  399
Thiomonas intermedia        ---KAPPLGSKTLVALESYSYWLSKGLPVDE-KVAGRGYPNLPE---PQQAPDYVRGQKV  166
Sideroxydans lithotrophicus ---KAPAVDSPEMVALVTYSYWLATGAPVGA-KLKGAGYPEVPK---PGLIPDANRGKTV  159
Pseudomonas stutzeri        ---TPPAADSHVINALTAYSYWLSTGAPTGQ-ELPGRAYPEVPQ---PQGGFDIAKGKQI  168
Allochromatium vinosum      ---RALALDHPALIDMLAYMSWLSQGVPVGA-VVAGHGIPTLTL---E-REPDGVHGEAL  155
Psychrobacter arcticus      ---KALDLNSDDMNAMVSYMSWLSQDMPFGV-SPEGSGFVKVDK---T-LEPNTDNGKKL  200

Parachlamydia acanthamoebae YQQHCAACHGKDGEGQLRPD------NLKYPPLWGN-HSFNQAAGMNDLPTLAAFIYDNM  212
Campylobacter jejuni        YMDKCAVCHQENGLGLKNEDSA--GAYYLYPPLWGD-DSYNTGAGMYRLIKAASYIKENM  240
Wolinella succinogenes      YARDCASCHGAEGEGLRRESKDGKPAGYEFPPLWGSDDTYNTGAGMYRTLKAADFIKSTM  264
Marichromatium purpuratum   YAEHCALCHGAEGEGLL-VD-----GEVVFPPLWGP-RSYNWGAGMHRVDTAAAFIAANM  452
Thiomonas intermedia        YEAKCILCHAANGEGQY-VN-----GETVFPPLWGP-KSFNWGAGMGSYKNAAKFIYANM  219
Sideroxydans lithotrophicus FVENCQVCHGSNGEGKK-VD-----GKYIFPPLWGS-ESFNWGAGMHRINTAAAFIKANM  212
Pseudomonas stutzeri        YAEQCAVCHGDDGQGQK-AG-----GGYVFPPLWGK-DSFNWGAGMHRINTAAAFIKESM  221
Allochromatium vinosum      YQARCLACHGADGSGTLDAD-----GRYLFPPLWGP-RSFNTGAGMNRQATAAGFIKHKM  209
Psychrobacter arcticus      FAEKCSVCHGATGEGQYNDD-----GTYVYPAIAGD-KSFNDGAGMARTYTAASFIKGKM  254

Parachlamydia acanthamoebae PYQEP-RLTVEEVLDIAAYITSQ--PRPIEIRTP--------------------------  243
Campylobacter jejuni        PQGAP-DLSLEDAYDVAAYMNSQ--ARPIKANRDKDFPDRKVK---PLDMD---VGPYDD  291
Wolinella succinogenes      PKGAP-TLSDKDAYDVAAFINDYSHYRTVKLNRQNDFVDPKVR---VPDHDQ--PGPYGP  318
Marichromatium purpuratum   PLLDTVRLTPQEAWDVAAYINAH--ERPQDPRF----DG-SVERTAARFHASP-FDLYGE  504
Thiomonas intermedia        PYGMSYSLSPQEAWDVAYFMDAQ--ERPQDPRW----QG-SVAATRAKFHDSK-FSLYGT  271
Sideroxydans lithotrophicus PLSKGGTLTDQEAWDVATFVMSH--ERPQDPRF----KG-NVAQTKKEYHDE--NCRYGE  263
Pseudomonas stutzeri        PLGKGGSLSDADAWHVAAYMNSH--ERPQDPRL----IEGSVEKTRLKYHANDGVNLYGQ  275
Allochromatium vinosum      PLGADDSLSDEEAWDVAGFVLTH--PRPLFQEPTGD------------------------  243
Psychrobacter arcticus      PFGQGGSLSDQEAVDIASYFTHL--PRPIKANKDKDWPNGDAPKDVRR------------  300

Parachlamydia acanthamoebae ----------------------------------  243
Campylobacter jejuni        S---------FSTTQHRYGPYTNMIKK-------  309
Wolinella succinogenes      EGSYIFPDEGKTQMDYKVGPYKGIIKQKPAAK--  350
Marichromatium purpuratum   P----------------LGVDGAVLGQGVAKD--  520
Thiomonas intermedia        K------------------VNGKLLGDIGAPKPR  287
Sideroxydans lithotrophicus T------------------VNGKVLGGKHQTAAK  279
Pseudomonas stutzeri        Q------------------VDGALLGQGVK----  287
Allochromatium vinosum      ----------------------------------  243
Psychrobacter arcticus      ----------------------------------  300
```

Figure S1. Sequence alignment for TsdAs from *Parachlamydia acanthamoebae*, *Campylobacter jejuni*, *Wolinella succinogenes*, *Allochromatium vinosum*, *Marichromatium purpuratum*, *Thiomonas intermedia*, *Sideroxydans lithotrophicus* and *Pseudomonas stutzeri*. Heme binding motifs including proximal His ligand are indicated in blue with the distal Cys ligand to Heme 1 in red and M(K) ligand to Heme 2 in brown.

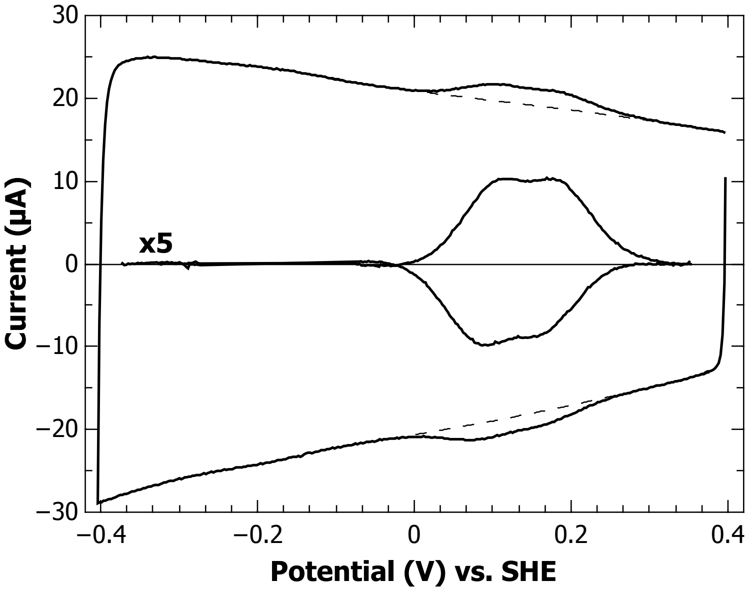

Figure S2. Representative cyclic voltammetry of a TsdA protein adsorbed on IO-ITO. Voltammogram (continuous line) is for *Cj*TsdA C138M in 50 mM HEPES, 50 mM NaCl, pH 7, 4 °C measured at 10 mV s^{-1}. Inset: the protein (Faradaic) response obtained by subtraction of a baseline (dashed line) from the measured current.

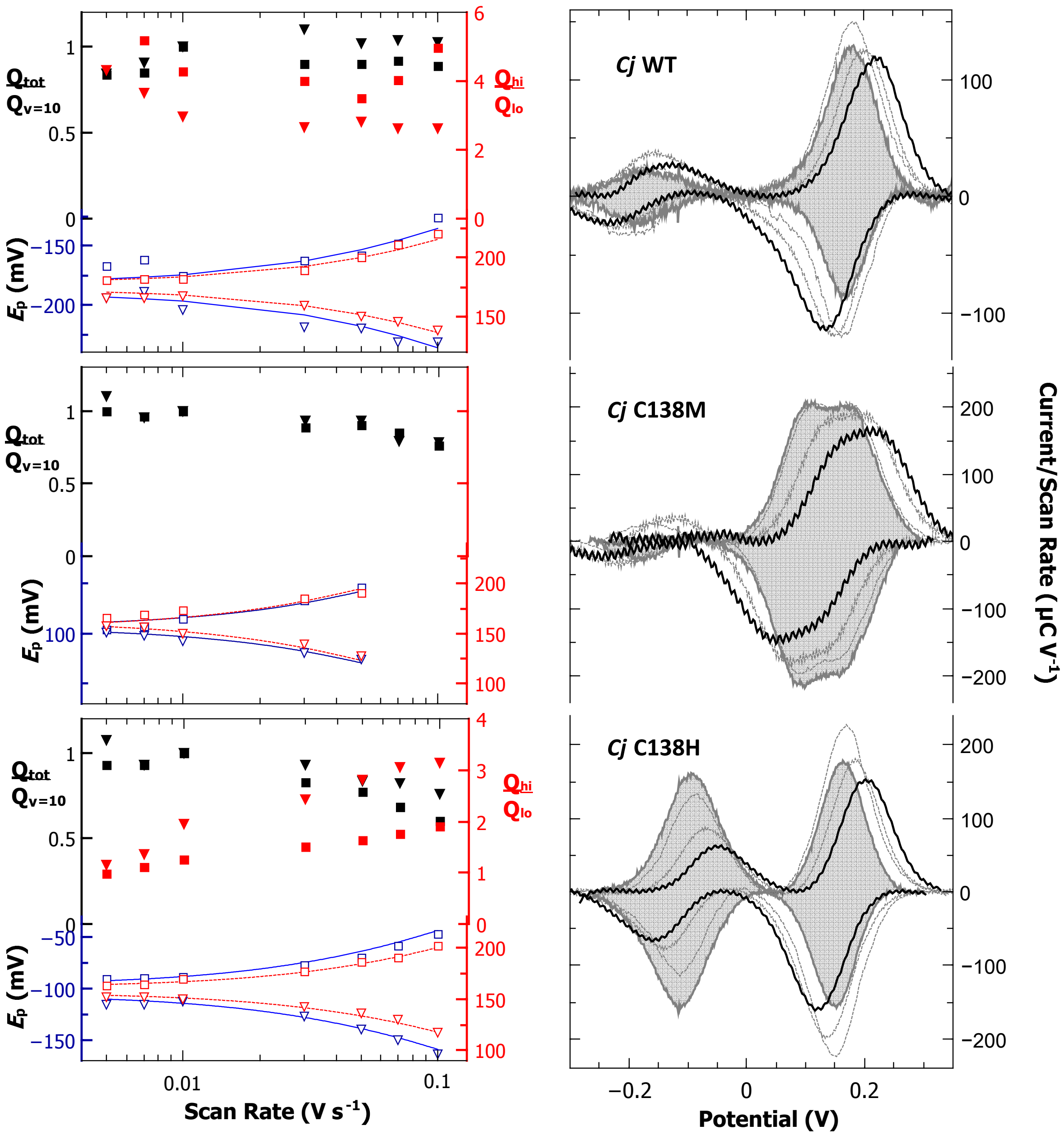

Figure S3. Scan-rate dependence of the cyclic voltammetry for *Cj*TsdA and Heme 1 variants adsorbed on IO-ITO electrodes.

(Right) Representative baseline subtracted cyclic voltammograms for the indicated proteins recorded at 5 (grey line, shaded area), 10, 50 and 100 (black line) mV s^{-1}. Data recorded in 50 mM HEPES, 50 mM NaCl, pH 7 at 4 °C.

(Left) Scan rate dependence of voltammetric features.

Upper part of panels. Black: total moles of electrons (Q_{tot}) giving rise to the oxidative (circles) and reductive (triangles) peaks normalized to those at 10 mV s^{-1} (black). Red: ratio of mol. electrons in the low potential peak (Q_{hi}) to mol. electrons in the high potential peak (Q_{lo}) for oxidative (circles) and reductive (triangles) peaks.

Lower part of panels. Red: peak potentials (E_p) for oxidative (circles) and reductive (triangles) peaks at low (blue) and high (red) potential. Heterogeneous rate constants (k_o / s^{-1}) were obtained from fits to the data using Jellyfit v2.1 available from (https://www.fbs.leeds.ac.uk/jeukengroup/freeware/Jellyfit.php). k_0 values used for low/high E_m peaks were 1.06/1.48 s^{-1} (*Cj* WT), 0.78/0.67 s^{-1} (*Cj* C138M) and

0.87/1.38 s^{-1} (*Cj* C138H). E_m was equal to the average of the corresponding E_p values. Examples of similar analyses can be found in Jeuken, L.J.C., McEvoy, J.P. & Armstrong, F.A. (2002) J. Phys. Chem. B 106 2304-2313.

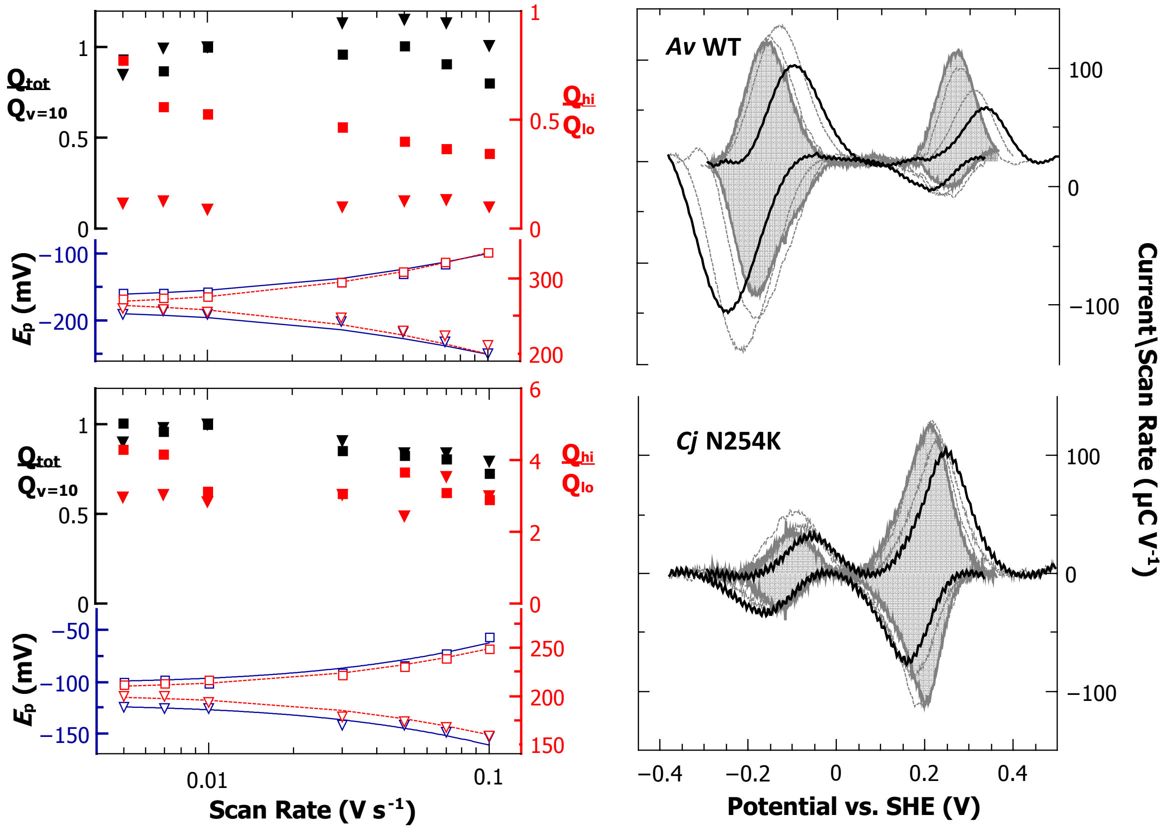

Figure S4. Scan-rate dependence of cyclic voltammetry for *Av*TsdA and *Cj*TsdA N254K adsorbed on IO-ITO electrodes.

(Right) Representative baseline subtracted cyclic voltammograms for the indicated proteins recorded at 5 (grey line, shaded area), 10, 50 and 100 (black line) mV s^{-1}. Data recorded in 50 mM HEPES, 50 mM NaCl, pH 7 at 4 °C.

(Left) Scan rate dependence of voltammetric features.

Upper part of panels. Black: total moles of electrons (Q_{tot}) giving rise to the oxidative (circles) and reductive (triangles) peaks normalized to those 10 mV s^{-1} (black). Red: ratio of mol. electrons in the high potential peak (Q_{hi}) to mol. electrons in the low potential peak (Q_{lo}) for oxidative (circles) and reductive (triangles) peaks.

Lower part of panels. Red: peak potentials (E_p) for oxidative (circles) and reductive (triangles) peaks at low (blue) and high (red) potential. Heterogeneous rate constants (k_o / s^{-1}) were obtained from fits to the data using Jellyfit v2.1 available from (https://www.fbs.leeds.ac.uk/jeukengroup/freeware/Jellyfit.php). k_0 values used for low/high E_m peaks were 0.67/0.72 s^{-1} (*Av* WT) and 1.32/1.22 s^{-1} (*Cj* N254K). E_m was equal to the average of the corresponding E_p values. Examples of similar analyses can be found in Jeuken, L.J.C., McEvoy, J.P. & Armstrong, F.A. (2002) J. Phys. Chem. B 106 2304-2313.

Table S1. Peak wavelengths (λ) and intensities ($\Delta\varepsilon$) for low-spin Fe(III) charge-transfer transitions in nIR MCD. ox = oxidized form, asc = ascorbate reduced form.

λ / nm ($\Delta\varepsilon$ / M^{-1} cm^{-1} T^{-1})		Heme 1			Heme 2	
		His/Cys$^-$	His/His	His/Met	His/Lys	His/Met
*Av*TsdA (407/208)*	ox				1505 (1.6)	
	asc	1240 (0.42)				
*Cj*TsdA (415/190)	ox	1215				1825 (0.74)
	asc	1225 (0.50)				
*Cj*TsdA C138M (413/210)	ox			1735† (0.51)		1825‡ (0.74)
	asc					
*Cj*TsdA C138H (411/230)	ox		1480			1820 (0.82)
	asc		1480 (1.03)			
*Cj*TsdA N254K (408/200)	ox	1250			1525 nm (0.75)	1825 (0.47)
	asc	1250 (0.41)				

* The Soret absorption peak wavelength and extinction coefficients (nm/mM^{-1}cm^{-1}) are shown in brackets for each species.

† by subtraction.

‡ assumed to be the same as *Cj*TsdA.

Table S2. E_m values derived from modelling of voltammograms, see Methods for details.

	Heme 1		Heme 2			
*Av*TsdA	His/Cys$^-$	−181 mV	His/Lys	−129 mV	His/Met	+266 mV
*Cj*TsdA	His/Cys$^-$	−186 mV			His/Met	+172 mV
*Cj*TsdA C138M	His/Met	+94 mV			His/Met	+174 mV
*Cj*TsdA C138H	His/His	−100 mV			His/Met	+160 mV
*Cj*TsdA N254K	His/Cys$^-$	−115 mV	His/Lys	−171 mV	His/Met	+203 mV

Table S3. Moles of electrons transferred (*Q*) in the baseline-subtracted and modelled peak areas for cyclic voltammetry of adsorbed TsdA proteins. Bolded values (± σ) are average of three scans performed at 10 mV s^{-1} in 50 mM HEPES, 50 mM NaCl, pH 7, 4 °C with the exception of *Cj*TsdA

N254K scan 1. Italicized values are for the modelled contributions shown in Fig 5 and 8 with grey values assigned to Heme 1 and blue values assigned to Heme 2. Ox = oxidized, red = reduced, lo = low potential, hi = high potential.

	Low Potential Peak (mol e^-)		**High Potential Peak (pmol e^-)**		**Total (pmol e^-)**	
Enzyme	$Q^{\mathrm{ox}}_{\mathrm{lo}}$	$Q^{\mathrm{red}}_{\mathrm{lo}}$	$Q^{\mathrm{ox}}_{\mathrm{hi}}$	$Q^{\mathrm{red}}_{\mathrm{hi}}$	$Q^{\mathrm{ox}}_{\mathrm{tot}}$	$Q^{\mathrm{red}}_{\mathrm{tot}}$
***Cj*TsdA**	**41 (± 10)**	**52 (± 10)**	**166 (± 10)**	**135 (± 21)**	**207 (± 10)**	**187 (± 21)**
	31	*41*	*145*	*124*	*176*	*165*
***Av*TsdA**	**176 (± 10)**	**269 (± 21)**	**93 (± 10)**	**21 (± 10)**	**269 (± 10)**	**300 (± 21)**
	114, *31*	*135*, *83*	*93*	*31*	*238*	*249*
***Cj*TsdA C138M**			**373 (± 21)**	**352 (± 10)**	**373 (± 21)**	**352 (± 10)**
			176, *176*	*166*, *145*	*352*	*311*
***Cj*TsdA C138H**	**155 (± 10)**	**124 (± 10)**	**207 (± 10)**	**238 (± 21)**	**373 (± 10)**	**352 (± 21)**
	135	*104*	*218*	*218*	*353*	*322*
***Cj*TsdA N254K scan 1**	**52**	**145**	**165**	**83**	**218**	**228**
	21, *41*	*62*, *73*	*124*	*52*	*187*	*187*
steady-state	**52 (± 10)**	**31 (± 10)**	**155 (± 10)**	**135 (± 21)**	**207 (± 10)**	**166 (± 10)**
	52	*41*	*124*	*104*	*176*	*145*