Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

Kinetics and Mechanism of Mineral Respiration: How Iron Hemes Synchronize Electron Transfer Rates

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Supporting Information

I) NMR measurements

Experiments were carried out with samples of lyophilized OmcF protein from *Geobacter sulfurreducens* (Uniprot-Q74AE4) expressed as reported previously.[1] Buffer exchange was performed to remove Cl^- and PO_4^{3-} ions from the sample using the following procedure: OmcF samples were diluted into 8 mL of Milli-Q water and transferred into Amicon Ultra 15 Centrifugal filters Ultracel 3KDa. The samples were subsequently centrifuged at 10'000 rpm for 20 min. The remaining volume of each sample (ca. 500 µL) has been diluted in 8 mL of Milli-Q water and centrifuged in the above described conditions. This procedure was repeated 15 times and the samples were lyophilised with a FreeZone 4.5 Liter Cascade Benchtop Freeze Dry System (Labconco). The protein was further characterized by mass spectrometry (ESI-MS Bruker Esquire HCT) in H_2O/MeOH solution (1:1): 1035.5 $[OmcF+4H+2Na]^{9+}$, 932.0 $[OmcF+5H+2Na]^{10+}$, 847.4 $[OmcF+6H+2Na]^{11+}$, 776.7 $[OmcF+7H+2Na]^{12+}$, 716.8 $[OmcF+8H+2Na]^{13+}$, 665.9 $[OmcF+9H+2Na]^{14+}$, 621.4 $[OmcF+10H+2Na]^{15+}$. For titration with $AgNO_3$ (0.0-4.0 molar ratios) in HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), lyophilized protein was dissolved in 500 µL of D_2O + 10 mM HEPES buffer pD 7.8 to a concentration of 1.1 *mM* or in 500 µL of D_2O + 4 *mM* HEPES buffer pD 7.8 to a concentration of 0.2 *mM*. For $AgNO_3$ (0.0-7.2 *mM*) titration in competition with imidazole-d4, lyophilized protein was dissolved in 500 uL of D_2O + 20 *mM* imidazole-d4 pD 7.8 to a concentration of 1.0 *mM*. The exact concentration of OmcF was determined by measuring UV-Vis spectra of the mother solution diluted in H_2O to 10-20 μM OmcF concentration, and reduction of the Fe^{3+}/heme to the Fe^{2+}/heme with an excess of sodium dithionite as described by Lukat *et al*. ($\varepsilon_{552\ nm}$ = 23800 $M^{-1}.cm^{-1}$).[2] ^1H and ^{13}C-HSQC spectra (hsqcetgpsisp2) were recorded on Bruker Ascend 400 MHz NMR spectrometer upon adding increasing amounts of $AgNO_3$ deuterated solution of which the concentration (27 *mM* for direct titration and 114 *mM* for competition titration) has been previously determined by ICP analysis. The titration curves were fitted based on chemical shifts from methionine Met^{86} (taken from ^{13}C-HSQC) and from histidine His^{34} (two signals, ^1H spectrum) using the software DynaFit,[3] with the binding constants of $[Ag(imidazole)_n]^+$ (n = 1, 2) complexes (log K_{ass} = 2.96 and 3.75 for the 1:1 and 1:2 complexes respectively) determined by Czoik et al.[4] We set a binding constant K_{exch} = [OmcF][Im]/[OmcF-Im]= 4.10^{-3} for the exchange of the histidine residue by the imidazole from value reported in the literature.[5] Different sets of chemical shifts from the protein and from the protein-imidazole complex were used for the fitting so the binding constant reported is the average binding constant obtained for the difference sets, with each set containing one peak from His34-ε1, one peak from His34-δ2 and one peak from Met86-ε for each protein and protein-imidazole (6 experimental chemical shift variations for each set).

Binding constants were considered identical for the complexation of both OmcF and OmcF-imidazole complex.

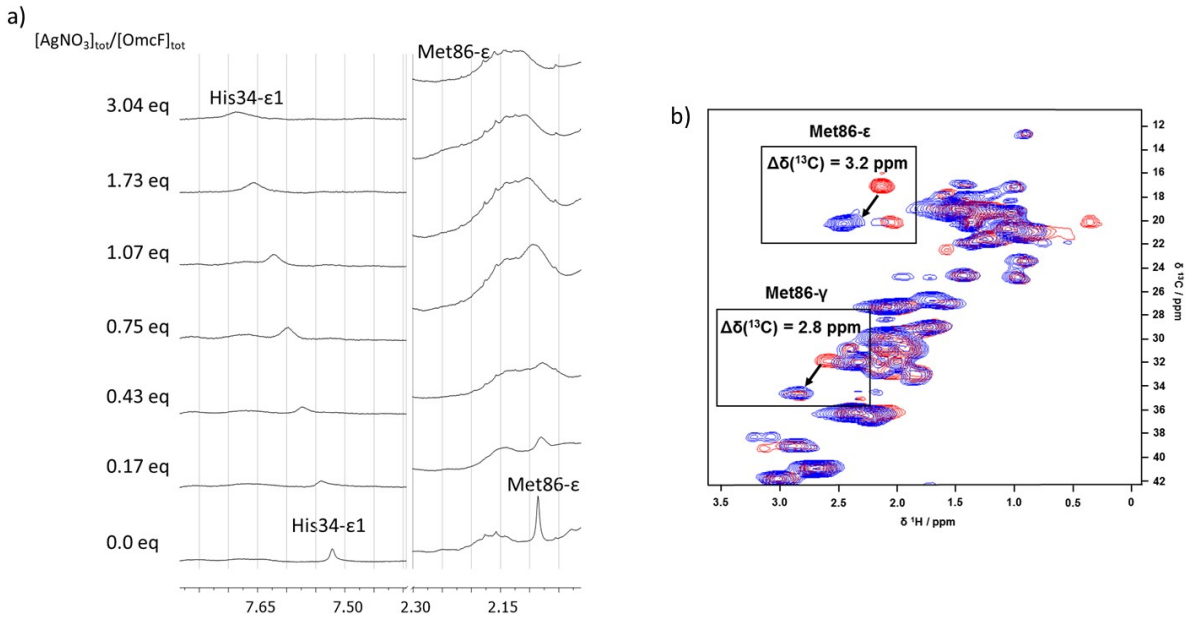


Figure S1. a) ^{1}H NMR titration of OmcF (1.13 mM) with $AgNO_3$ in D_2O + 10 *mM* HEPES buffer at 298K. b) ^{13}C-HSQC superimposition showing the chemical shift of the methionine residue.

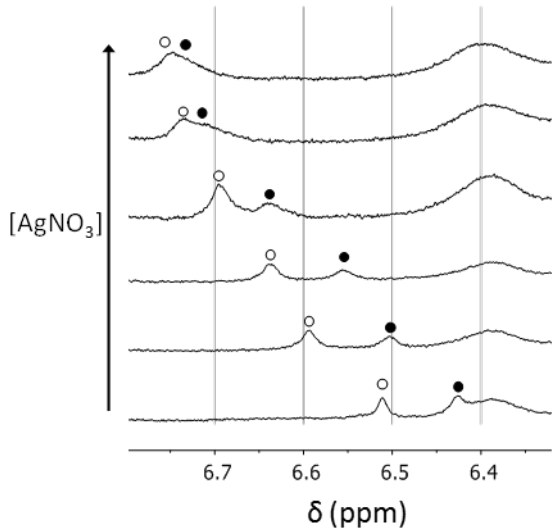


Figure S2. Proton chemical shift of $H^{\delta 2}$ of His^{34} upon addition of a 114 *mM* $AgNO_3$ solution in D_2O (0 to 7 *mM*) to a solution of OmcF protein (1.0 *mM*) in D_2O + 19.9 *mM* imidazole (pD 7.8).

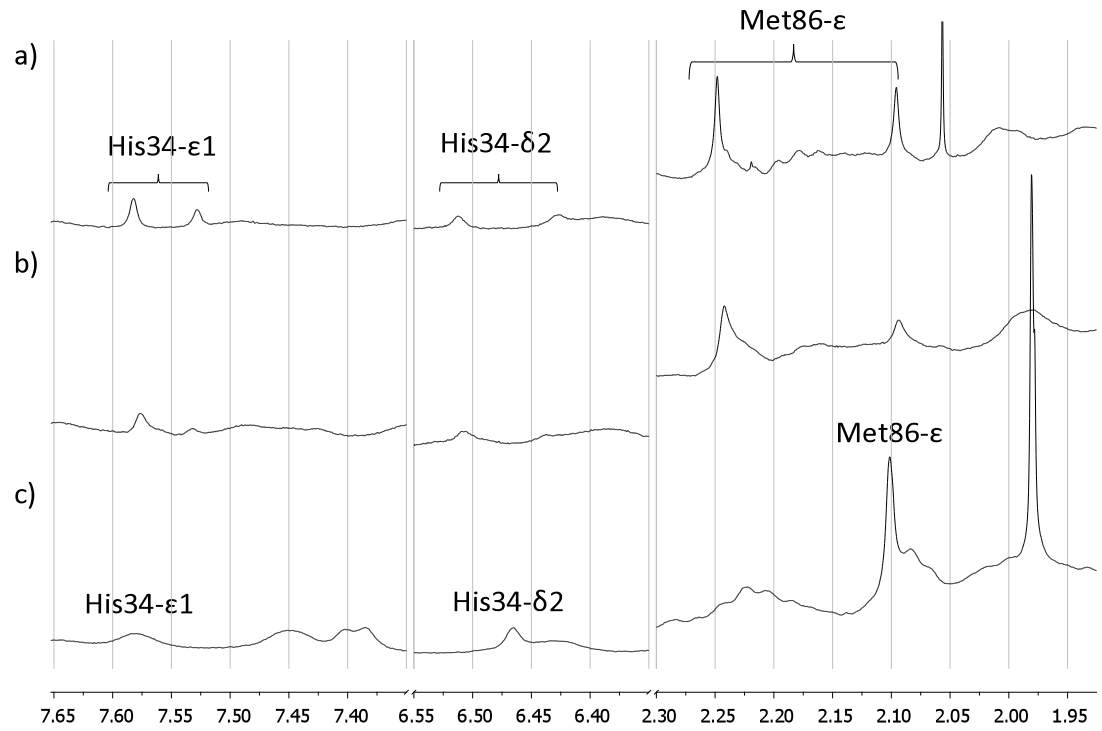


Figure S3. a) ^{1}H NMR spectra of OmcF (1.00 *mM*) with imidazole-d4 in D_2O at the beginning of the competition titration. b) and c) ^{1}H NMR spectra of OmcF (0.50 *mM*) in D_2O (c) with addition of a large excess of imidazole (b).

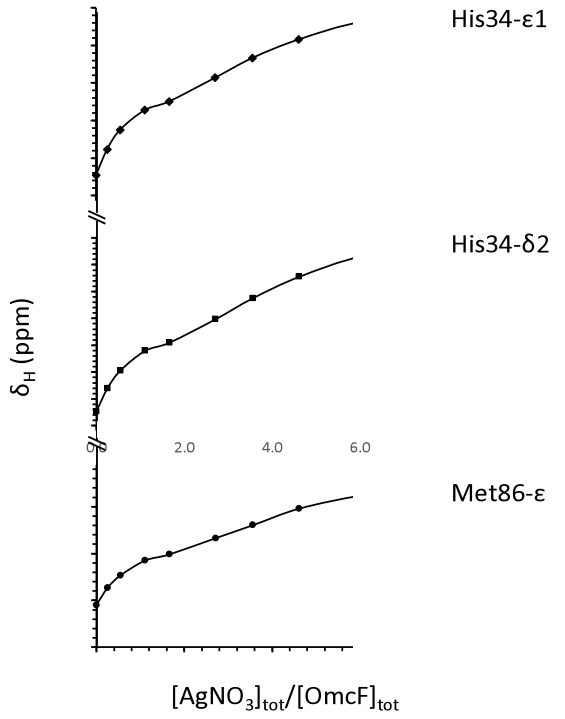


Figure S4. Variation of the proton chemical shifts of $H^{\varepsilon 1}$ and $H^{\delta 2}$ of His^{34} and H^{ε} of Met^{86} (plain shapes) upon addition of a 114 *mM* $AgNO_3$ solution in D_2O (0 to 7.18 *mM*) to a solution of OmcF protein (1.00 *mM*) and imidazole-d4 (19.9 *mM*) in D_2O.

[1] S. I. Vasylevskyi, S. Kracht, P. Corcosa, K. M. Fromm, B. Giese, M. Füegg, *Angew. Chem. Int. Ed.* **2017**, *56*, 5926-5930; *Angew. Chem*. **2017**, *129*,
[2] P. Lukat, M. Hoffmann, O. Einsle, *Acta Cryst.* **2008**, *D64*, 919–926.
[3] Copyright (c) by Petr Kuzmic (BioKin Ltd) 1996-2019 download on www.biokin.com/dynafit/
[4] R. Czoik, A. Heintz, E. John, W. Marczak, *Acta Phys. Pol. A* **2008**, *114*, A51-56.
[5] J. Hirst, S. K. Wilcox, J. Ai, P. Moënne-Loccoz, T. M. Loehr, D. B. Goodin, *Biochem.* **2001**, *40*, 1274-1283

II) *Geobacter sulfurreducens* growth medium

disodium fumarate	40 *mM*	Na_2MoO_4	1 *μM*
$NaHCO_3$	29.8 *mM*	$Ni(NOs)_2$	1 *μM*
$NaCH_3COO$	19.5 mM	Na_2WO_4	0.75 *μM*
NH_4CH_3COO	9.3 *mM*	pyridoxine	0.6 *μM*
NaH_2PO_4	3.0 *mM*	$CuSO_4$	0.4 *μM*
KNO_3	1.3 *mM*	nicotinic acid	0.4 *μM*
$MgSO_4$	122 *μM*	p-aminobenzoic acid	0.36 *μM*
nitrilotriacetic acid	78 *μM*	thioctic acid	0.24 *μM*
$MnSO_4$	30 *μM*	pantothenic acid	0.23 *μM*
$FeSO_4$	25 *μM*	$AlK(SO_4)_2$	0.2 *μM*
$ZnSO_4$	17 *μM*	thiamine	0.2 *μM*
$CaSO_4$	7 *μM*	riboflavin	0.13 *μM*
$CoSO_4$	4 *μM*	biotin	82 *nM*
KCl	2 *μM*	folic acid	45 *nM*
H_3BO_3	1.6 *μM*	cyanocobalamin	0.75 *nM*

Figure S5: A *Geobacter sulfurreducens* (DSM-12127) suspension has been obtained from the Leibniz Institute DSMZ GmbH, Germany. The bacteria were transferred into an anaerobic 100 mL glass bottle and 50 mL of a homemade growth medium was added under strictly anaerobic conditions. In the first *Geobacter sulfurreducens* culture, KCl (10 *mM*) and $FeSO_4$ (100 *μM*) were added to the medium. In the next cultures, Cl^- and Fe^{2+} concentrations were respectively decreased to 2 *μM* and 25 *μM* by dilution with the growth medium described above. The concentrations in Fe^{2+} ions were determined by ICP-OES analysis.

III) Plasmon resonance

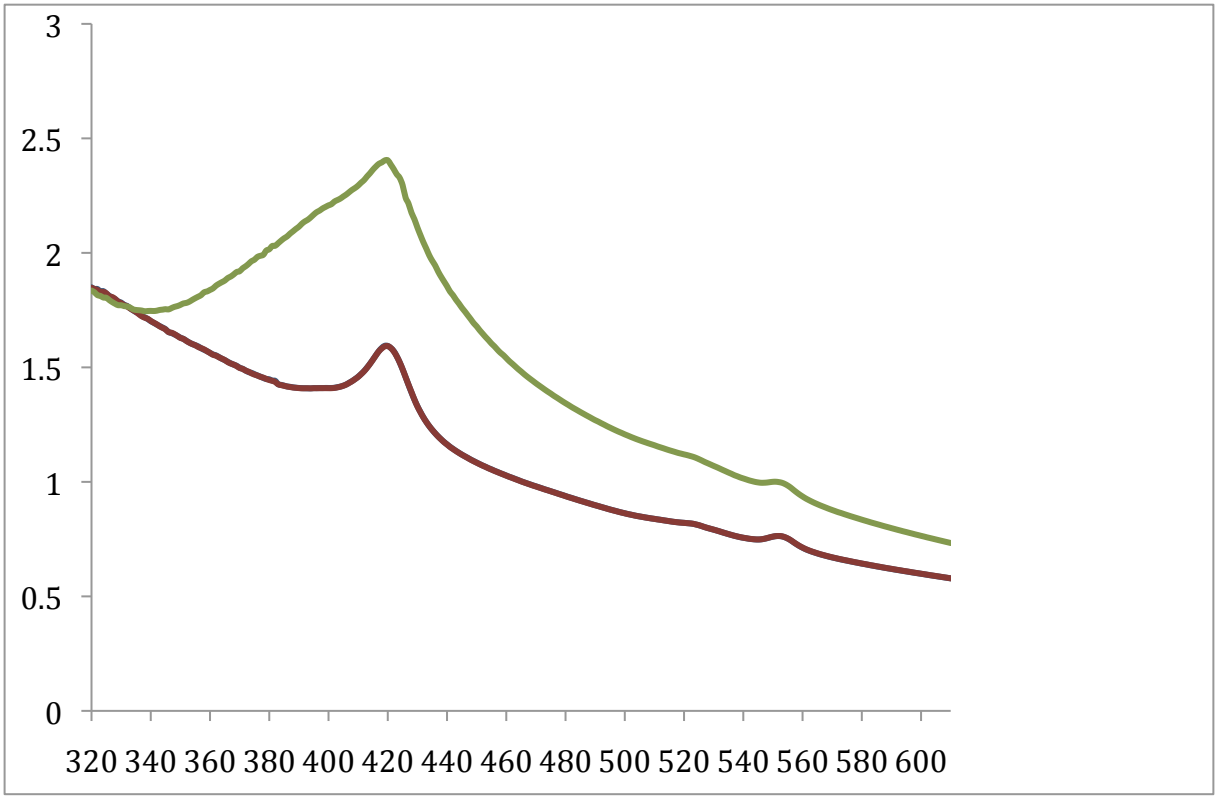


Figure S6: UV/Vis spectra of Geobacter sulfurreducens before (lower trace) and after reaction with 0.05 *mM* Ag^{+} ions (upper trace). The broadening of the Soret band at 400 nm shows the plasmon resonance of the synthesized AgNPs.

IV) Q-bands

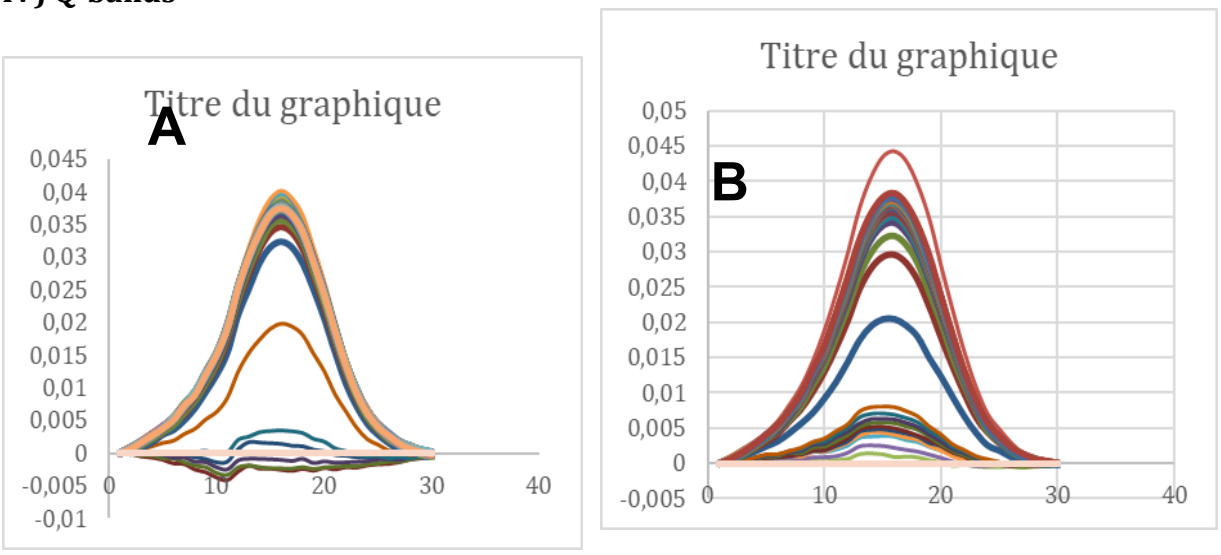
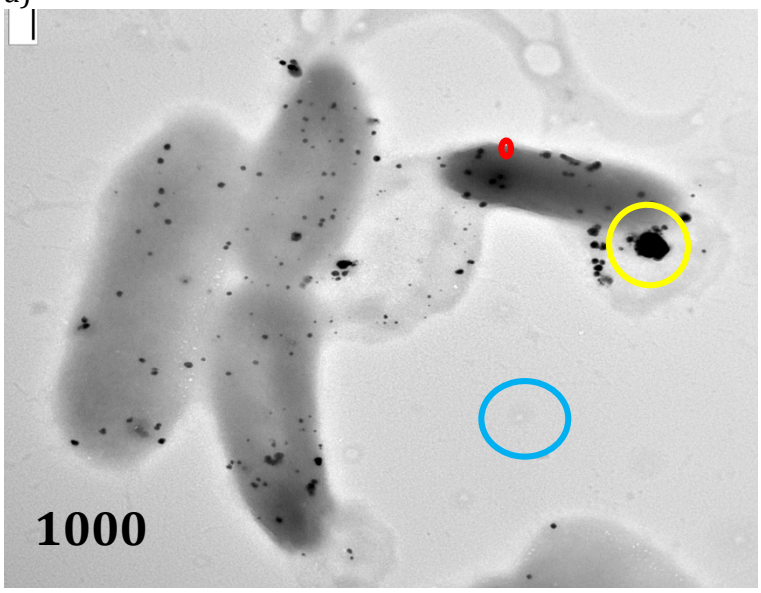


Figure S7: Increase of the Q-bands (Fe^{2+}/hemes) with increasing time during $AgNO_3$ addition (A), and during Na_2CrO_4 addition (B). The upper curve shows the intensity before addition of the oxidant. After addition of the oxidant the lowest curve appears, which increases with time.

V) TEM/EDS spectra of AgNPs at *Geobacter sulfurreducens*

a)



b)

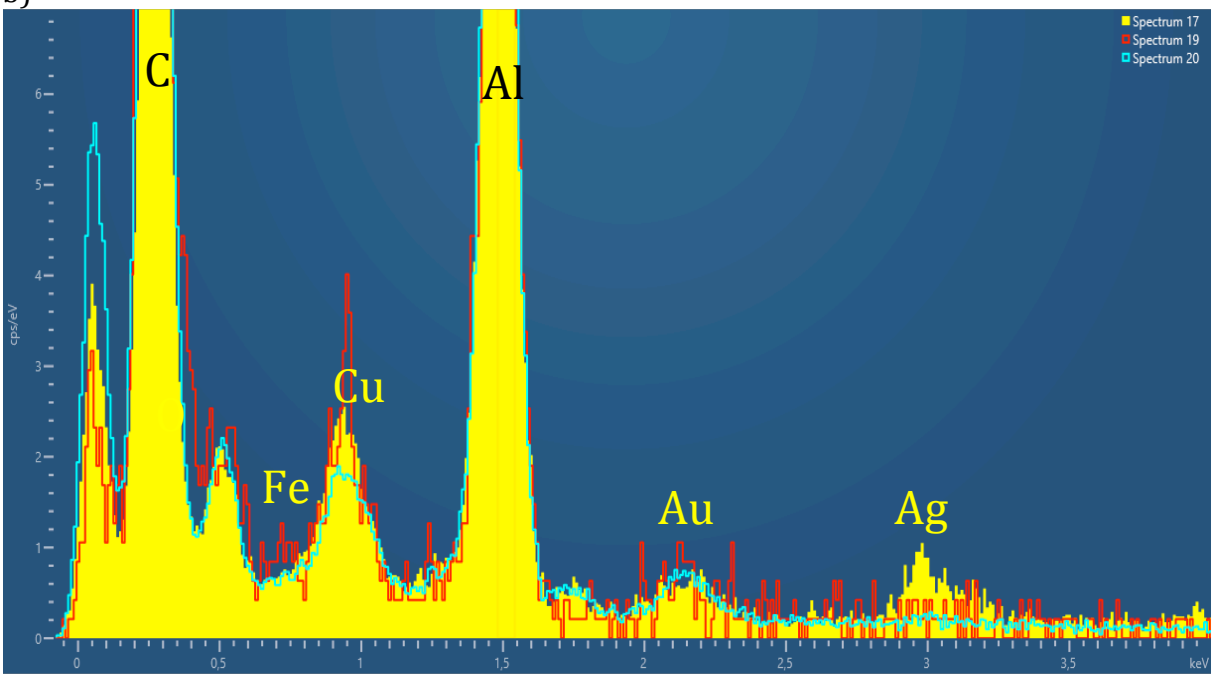
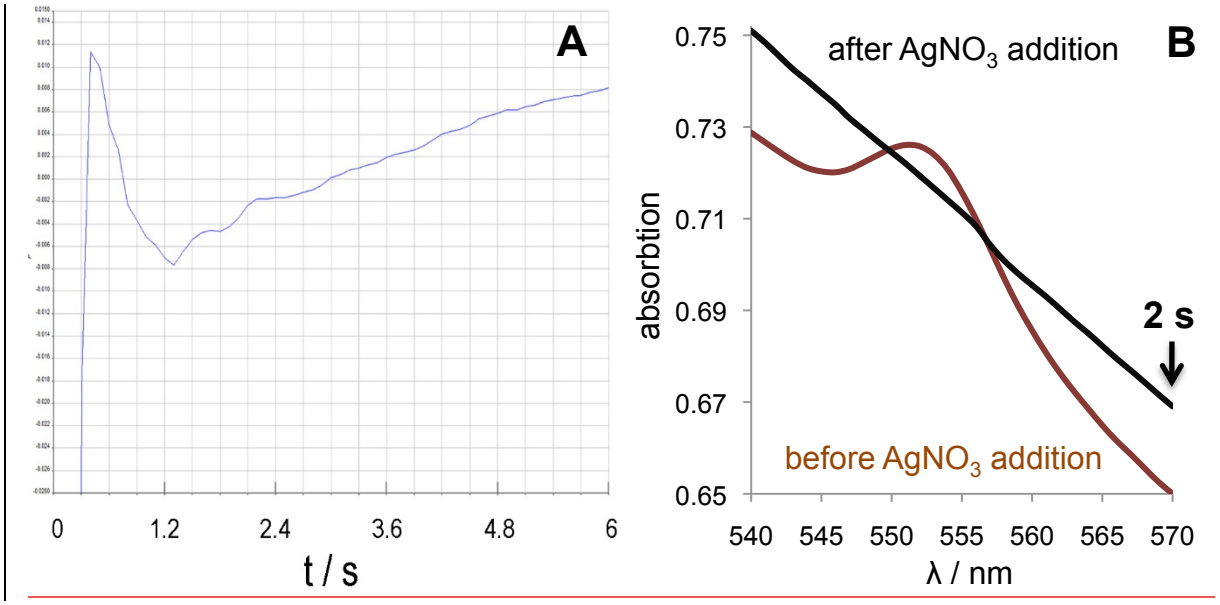


Figure S8: a) Electron microscopic image (TEM) of *G. sulfurreducens* incubated with 0.05 *mM* $AgNO_3$; b) EDS spectra in yellow, red and blue corresponding to the encircled areas shown on the TEM image. The yellow spectrum shows the presence of Ag (AgNPs). The red spectrum acquired close to the membrane shows Fe of the iron hemes. The blue field is outside (only the grid materials are present). We tested also for presence of chloride and phosphorous but these were not detectable by EDS in the yellow area. Hence formation of AgCl and Ag_3PO_4 can be excluded.

VI) Oxidation time of the Fe^{2+}/hemes by $AgNO_3$



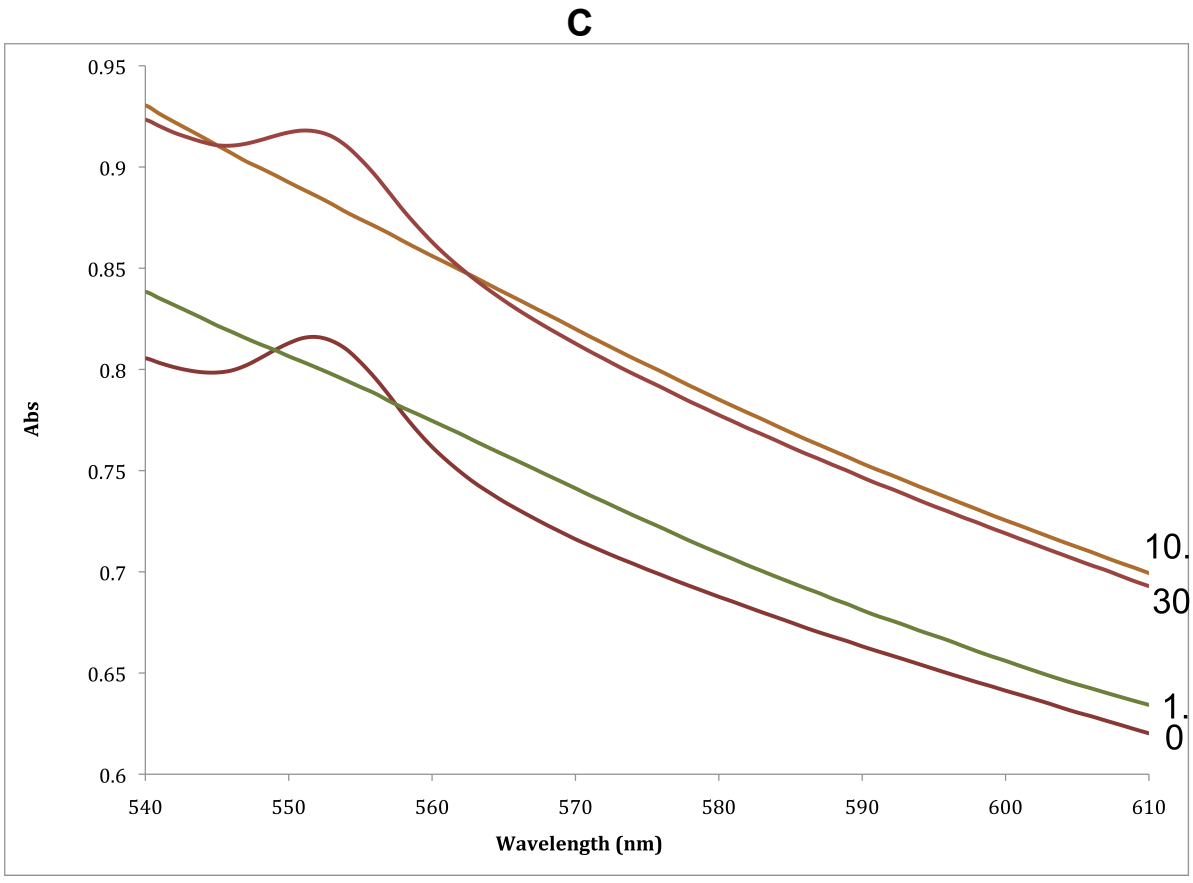


Figure S9: A) Time dependence of the UV/Vis spectrum at 550 nm (Q-band) after addition of $AgNO_3$ (0.05 *mM*) to *Geobacter sulfurreducens* (0.7 *pM*). The mixing took about 0.5 s (maximum of the curve). The minimum was reached 1.3 s after the injection of the $AgNO_3$ solution. The curve measures the decrease of the Fe^{2+}/hemes (Q-band) and the increase of the AgNPs (scattering). The UV/Vis absorption change for the Fe^{2+}/heme decrease is two times larger than for the AgNP increase during the first 2 s. From this we concluded that the minimum of the UV/Vis absorption is reached, when nearly all of the

Fe^{2+}/hemes were oxidized. B) Q- bands before and 2 s after $AgNO_3$ addition to *Geobacter sulfurreducens* (concentrations as above). The Fe^{2+}/hemes had been oxidized 2 s after $AgNO_3$ addition. The increase of the absorption is due to the increase of the scattering due to the AgNP formation. C) Line 0 is before $AgNO_3$ addition and line 2. is 2 s after $AgNO_3$ addition (like Figure S9B). Line 10. is at the end of the scattering increase and line 30. is at the end of the reaction (the Fe^{2+}/hemes had been regenerated by respiration). From the difference of lines 30. - 0 compared to 10. – 1. at 610 nm we concluded that in the first 2 s about 10% of the Ag+ ions had been reduced by the Fe^{2+}/hemes to AgNPs.

VII) Formation of AgCl nanocrystals

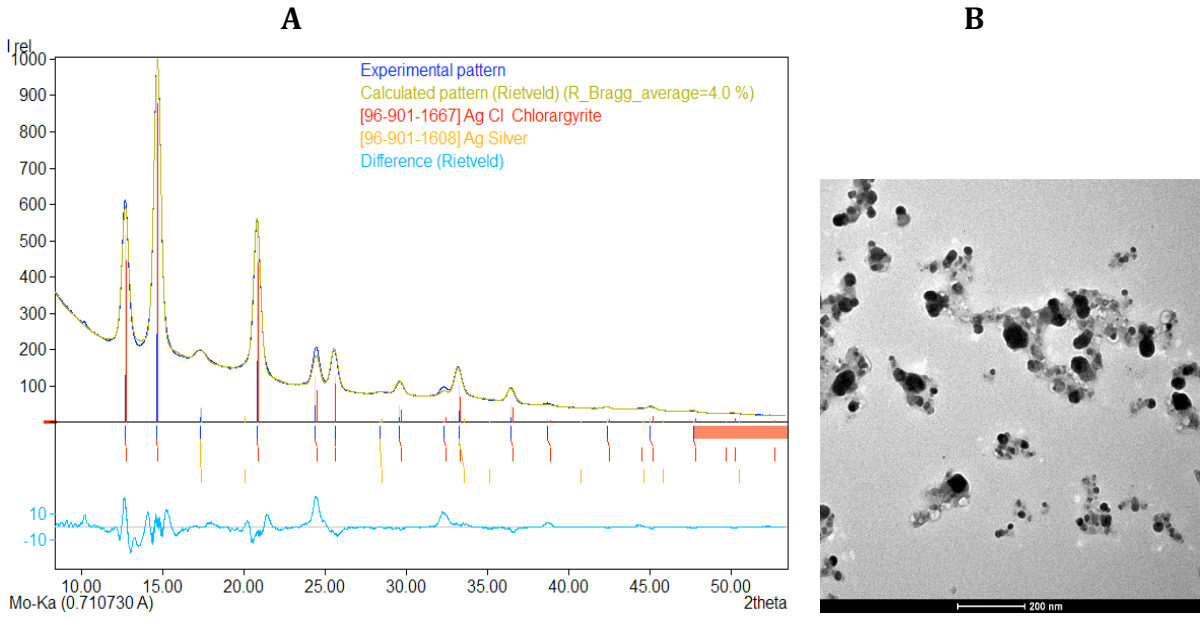


Figure S10: Powder X-ray (A) and TEM (B) of AgCl nanocrystals. Under strictly anaerobic conditions 0.05 mL of a 0.3 *mM* KCl solution was combined with 1.35 mL of the growth medium in the absence of *Geobacter sulfurreducens*. Injection of 0.1 mL of a 0.3 *mM* $AgNO_3$ solution generated AgCl nanocrystals.