# **Supporting Information**

# An in-situ Surface Electrochemistry Approach toward Whole-cell Studies: Structure and Reactivity of a *Geobacter sulfurreducens* Submonolayers on Electrified Metal/Electrolyte Interfaces\*\*

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## References

### SI 1. Immersion experiment

Immersion experiments of the bare Au and Ag electrodes in 50 mM bicarbonate buffer + 20 mM sodium acetate solution enabled an estimation of the potential of zero charge as  $E_{pzc} \approx 0.0$  V (Au) and -0.1 V (Ag) vs. Ag/AgCl, respectively, by following the procedure previously described in ref. [S1, S2].



**Figure S1:** Immersion charge upon contact of an Au (full square) or Ag (empty triangle) electrode at various potentials in a 50 mM bicarbonate buffer solution containing 20 mM sodium acetate in a hanging meniscus configuration. The charge was obtained by the integration of the measured current transients. The zero crossing represents an estimation of the potential of zero charge.

### SI 2. Biofilm growth chronoamperometry and turnover cyclic voltammetry



**Figure S2**: (a) Chronoamperometric curve of the *Gs* biofilm formation using an Au electrode polarised at 0.40 V in a bicarbonate buffered growing media saturated with a mixture of  $N_2$ +CO<sub>2</sub> (4:1). (b) Cyclic voltammograms of the active *Gs* biofilm under turnover conditions in the presence of acetate after 7 and 11 days at a scan rate of 0.01Vs<sup>-1</sup>.

SI 3. Microscopic studies of a Geobacter sulfurreducens submonolayers on an Au surface



**Figure S3**: (a) Atomic force microscopy image of a *Gs* submonolayer on an Au electrode after 12 h of continuous polarisation at 0.40 V(Ag/AgCl) in 50 mM bicarbonate buffer solution containing 20 mM acetate in N<sub>2</sub> + CO<sub>2</sub> (4:1). The average coverage of *Gs* cells was  $10 \pm 2$  cells per 100  $\mu$ m<sup>2</sup>. (b) Size distribution histograms of the cell width with a most probable value of 0.6  $\pm$  0.1  $\mu$ m and (c) size distribution of the cell length with a most probable value of 1.6  $\pm$  0.3  $\mu$ m, as obtained from the analysis of several SEM and AFM images. (d) Atomic force microscopy image of a single *Gs* cell on an Au surface (1.5 × 1.5  $\mu$ m<sup>2</sup>): The inset shows a typical cross section.

SI 4. Steady state cyclic voltammetry of a Geobacter sulfurreducens submonolayer



**Figure S4**: (a) Cyclic voltammograms of a Gs submonolayer at steady state as recorded with different scan rates (1, 2, 5 and 10 mVs<sup>-1</sup>). (b) Integrated cathodic charges (between 0.40 V and -0.60 V) in dependence on the scan rate indicating that the electrochemical response of a Gs submonolayer can be attributed to a surface-confined redox process.

#### SI 5. Steady state EC-ATR-SEIRAS of a Geobacter sulfurreducens submonolayer

The ATR-SEIRA spectra were recorded during a simultaneous sweep at 5 mV/s. The SEIRA spectra reported in ref [S3] were acquired by SNIFTIRS. In this analysis, interferograms are collected at two different potentials (reference and sample potentials) during the rapid potential steps between two potentials, and the potential step was repeated until a desired signal-to-noise ratio is reached. But it is crucial in the SNIFTIRS experiment that the electrochemical process of the sample must be fast and reversible between two potentials. However, steady state SEIRA spectra showed that the redox process of the outermost cytochromes accompanied with the conformational change. Although the redox process is reversible, it is rather slow process compared to the acquisition time for SNIFTIRS. For this reason, SEIRA spectra was recorded with slow potential sweeping at 5 mV/s, avoiding a rapid potential steps during the measurements. In this way, the spectra shown in our study and in ref [S3] are different.



**Figure S5**: (a) Series of potential-dependent SEIRA spectra of a *Gs* submonolayer under steady state conditions as recorded in 50 mM bicarbonate buffer + 20 mM acetate solution. The spectra were acquired during a potential sweep (0.005 Vs<sup>-1</sup>) from 0.20 V to -0.60 V (Ag/AgCl). The reference potential was 0.20 V. (b) Spectrum of the reduced form of *Gs* under steady state conditions as acquired at -0.60 V (reference spectrum taken at 0.20 V, where the oxidized form of *Gs* is dominant). The absorbance *A* is defined as  $A = -\log(R_s/R_{ref})$ , where  $R_s$  and  $R_{ref}$  are the reflectance values of the single beam spectra at the sample and at the reference potential, respectively. Inset: an enlarged part of the spectrum in 1500 < v < 1800 cm<sup>-1</sup>.





**Figure S6**: (a) Scanning electron microscopy image of a *Gs* submonolayer mixed with Ag NPs. A controlled amount of *Gs* cells mixed with Ag NPs was casted-and-dried in an Ar atmosphere on an Au electrode surface. The average coverage of the *Gs* cells was 9.4 cells per 100  $\mu$ m<sup>2</sup>, similar to that prepared by continuous polarisation. Several white particles, aggregated together with bacterial cells, were observed in the SEM images. The EDX analysis demonstrated that these are Ag particles, suggesting that the Ag nanoparticles aggregate with the microbial cell surface on Au electrodes. (b) UV-Vis absorption spectrum of a solution of citrate-stabilised Ag NPs as recorded with an UV-vis-NIR spectrophotometer (CARY 5E, Varian) with a resolution of 2 nm.

SI 7. Electrochemical response of a Geobacter sulfurreducens submonolayer deposited on an Ag surface



**Figure S7**: Cyclic voltammograms of a *Gs* submonolayer on an Ag electrode recorded with 0.01 V s<sup>-1</sup> of 8 subsequent cycles in the absence (a) and in the presence (b) of Ag NPs after 12 h of continuous polarisation at 0.00 V vs. Ag/AgCl in a 50 mM bicarbonate buffer solution containing 20 mM acetate bubbled with N<sub>2</sub>: CO<sub>2</sub> (4:1).

## SI 8. EC-GM-SERS of a Geobacter sulfurreducens submonolayer



**Figure S8**: (a) Normal Raman spectrum of solid *Gs* on a glass slide in an Ar atmosphere with 532 nm excitation. The band assignments are summarised in Table S2. The peak observed at 748 cm<sup>-1</sup> ( $v_{15}$ ) is taken as a marker to monitor the redox state of the bacteria. The peaks at 1127 ( $v_{22}$ ), 1312 ( $v_{21}$ ) and 1582 cm<sup>-1</sup> ( $v_2$ ) are enhanced significantly in the normal Raman spectrum. In case of heme and metalloporphyrins, one can find two strong visible and near-ultraviolet absorption bands, which represent two  $\pi$ - $\pi$ \* electronic transitions. The Soret absorption band (410 - 420 nm) enhances the totally symmetric Raman modes ( $v_4$ ,  $v_3$  and  $v_2$  mode), while excitation in the Q-band region (520-550 nm) leads to an enhancement of the depolarised modes. The anomalously polarized modes are also enhanced due to the vibronic coupling between the Soret and the Q-band transition <sup>[S4]</sup>. (b) blank GM-SER spectrum of Ag NPs on an Ag surface in buffer solution containing acetate (1) as well as normal Raman spectra of the buffer solution (2) and of the acetate solution (3), respectively. The spectra were acquired with 532 nm excitation.

#### SI S9. Redox titration and data fitting

The potential-dependent molar fractions ( $X_a$ ) of the oxidized and of the reduced form of *Gs* were evaluated by a component analysis of the  $v_{15}$  band in the experimental GM-SER spectra. Each spectrum in the wavenumber range between 745-748 cm<sup>-1</sup> was deconvoluted in two superimposed bands, one representing the oxidized and the other the reduced form as derived from the Raman spectra at 0.00 and -0.60 V, respectively. Then, the molar fractions ( $X_a$ ) at the different applied potentials were obtained from the integrated intensities of the two components.

The Nernst equation was applied to simulate the potential-induced transition between an "effective" oxidized  $(X_{Ox})$  and a reduced  $(X_{Re})$  form of *Gs*:

$$X_{\text{Ox}} = \frac{\exp\left[\frac{n_{\text{eff}} F}{RT} (E - E_{\text{eff}})\right]}{1 + \exp\left[\frac{nF}{RT} (E - E_{\text{eff}})\right]}$$
$$X_{\text{Re}} = \frac{1}{1 + \exp\left[\frac{n_{\text{eff}} F}{RT} (E - E_{\text{eff}})\right]}$$

where  $E_{\text{eff}}$  and  $n_{\text{eff}}$  are the effective redox potential and the effective number of exchanged electrons. The experimental data could be represented with an accuracy  $R^2 > 0.99$ . The fit parameter  $E_{\text{eff}} = (-0.32 \pm 0.01) \text{ V}$  and  $n_{\text{eff}} = (0.8 \pm 0.2)$  were obtained. For a discussion of these parameters, we refer to the main text.

| Assignment <sup>[a]</sup>              | Accumulation | Potential cycling | Steady state |          | Ref     |
|--|--------------|-------------------|--------------|----------|---------|
|  |              |                   | Positive     | Negative |         |
| δ(H <sub>2</sub> O)                    | 1680         | 1678              | 1629         |          |         |
| Amide I $\beta$ -turn type III         |              |                   | 1686         |          | [S3,S5] |
| Amide I $\beta$ -turn type III         |              |                   |              | 1668     | [S3,S5] |
| Amide I α-helix                        | 1659         |                   |              |          | [S5,S6] |
| v37: $v(C_aC_m)_{asym}$                | 1602         | 1601              |              |          | [S3,S5] |
| Amide II β-turn type III               | 1565         | 1569              |              | 1564     | [85]    |
| v38: v( $C_bC_b$ )                     | 1531         |                   |              |          | [S7]    |
| C-H bending                            | 1416         | 1415              |              |          | [85]    |
| v41: $\nu(pyr \frac{1}{2} ring)_{sym}$ | 1387         | 1387              |              |          | [S7]    |
| Amine III $\beta$ -turn type III       | 1309         | 1310              |              |          | [85]    |
| v42: $\delta(C_mH)$                    | 1254         | 1254              |              |          | [S7]    |
| PLS                                    | 1069         | 1074              |              |          | [S6]    |

# Table S1: Assignation of bands in the ATR-SEIRAS of Geobacter sulfurreducens.

[a] Assignations are based on those previously indicated for proteins, cytochrome C [S3, S5, S6]

|                 | GM-SERS          |                 |                 | Normal<br>Raman |                                |              |
|-----------------|------------------|-----------------|-----------------|-----------------|--------------------------------|--------------|
| Symmetry        | Oxidised<br>(LS) | Reduced<br>(LS) | Reduced<br>(HS) |                 | Vibrational mode               | Ref          |
| V <sub>15</sub> | 748              | 74              | 45              | 748             | Pyr breath                     | [S8]         |
| V <sub>22</sub> | 1127             | 11              | 25              | 1127            | v(pyr 1/2ring) <sub>asym</sub> | [S8]         |
| V <sub>30</sub> | 1168             | 11              | 67              | 1170            | v(pyr 1/2ring) <sub>asym</sub> | [S8]         |
| v <sub>13</sub> | 1236             | 12              | 230             | 1245            | δ(C <sub>m</sub> H)            | [S8]         |
| $v_{21}$        | 1316             | 13              | 16              | 1312            | $\delta(C_mH)$                 | [\$8,\$9]    |
| $v_4$           | 1371             | 13              | 59              | 1365            | v(pyr 1/2ring) <sub>sym</sub>  | [S4,S8, S9]  |
| V <sub>29</sub> | 1404             | 13              | 98              | 1395            | v(pyr 1/4ring)                 | [S8-S10]     |
| $v_3$           | -                | 1494            | 1470            | 1497            | $\nu(C_aC_m)$                  | [S4 S8-S10]  |
| $v_{11}$        | 1565             | 15              | 537             | -               | $\nu(C_bC_b)$                  | [S4, S8, S9] |
| $v_2$           | 1583             | 1562            | 1592            | 1582            | $\nu(C_aC_m)_{asym}$           | [S4, S8-S10] |
| $\nu_{10}$      | 1636             | 1627            | 1607            | 1635            | $\nu(C_aC_m)_{asym}$           | [S4, S8-S10] |

**Table S2**. Assignment of the heme-related bands of a *Geobacter sulfurreducens* submonolayer in GM-SERS and normal Raman spectroscopy

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