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Supplementary Materials for

300-fold conductivity increase in microbial cytochrome nanowires due to temperature-induced restructuring of hydrogen bonding networks

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This PDF file includes:

Figs. S1 to S7
Tables S1 to S12

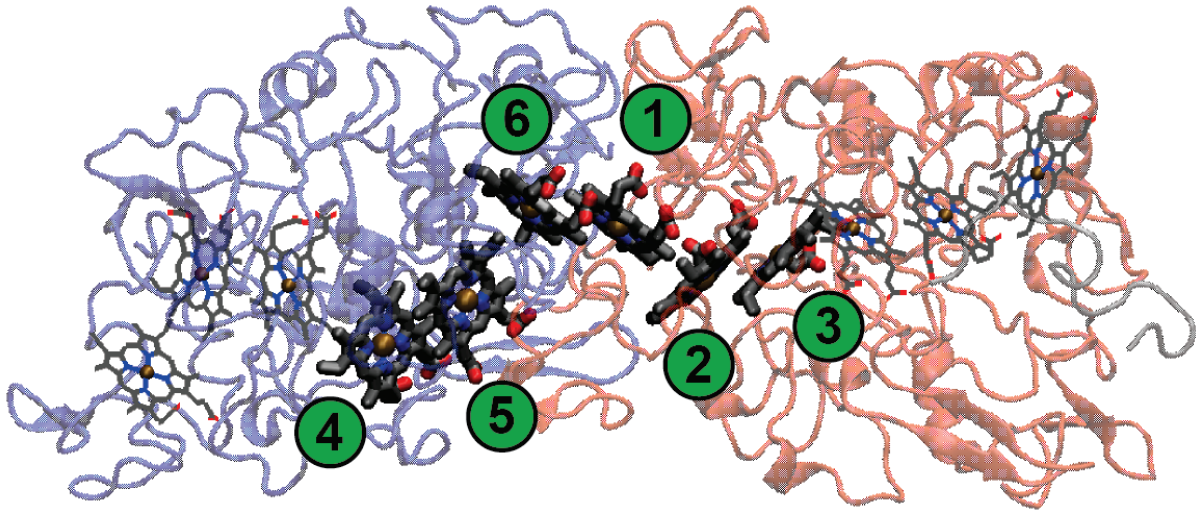

Fig. S1. Computational model of an OmcS dimer. Model system used for all computational characterization. Two full protein chains as well as the first 20 residues from a third chain are included in the model. The additional 20 residues from the third chain are included to satisfy coordination of heme 5 with Histidine 16 of the next protein chain. Our electronic structure calculations, electronic coupling calculations, distance analysis, and essential dynamics analysis were performed on the central six hemes highlighted here. The hemes closest to the edges of the system were excluded from our analysis to limit the potential influence of heme solvation.

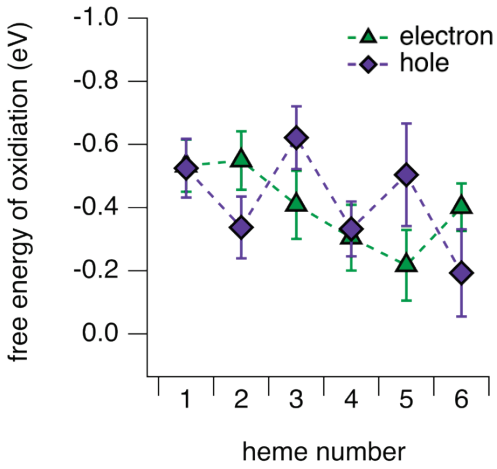

Fig. S2. Free energy landscape in a uniform dielectric environment. Redox potentials computed using the same ensemble of heme configurations that were used to generate Fig. 3A. For this calculation, the protein electrostatics were replaced with a dielectric continuum model. We used a dielectric constant of 4.7113 corresponding to chloroform to model a protein environment.

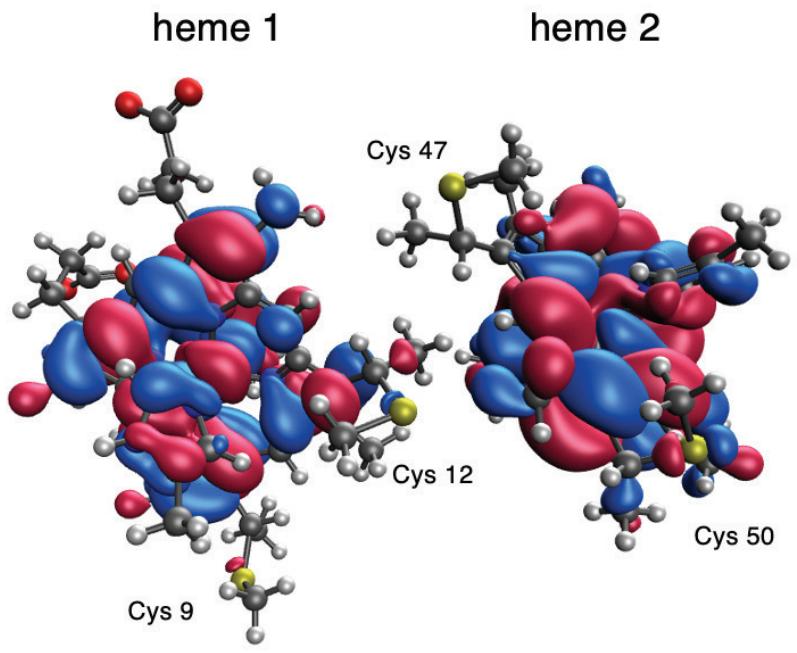

Fig. S3. Proximity of Cysteine 12 to heme 2 contributes to increased coupling between hemes 1 and 2. HOMO orbitals of reduced heme 1 and oxidized heme 2. This is a representative configuration. Orbitals are displayed using an isovalue of 0.01.

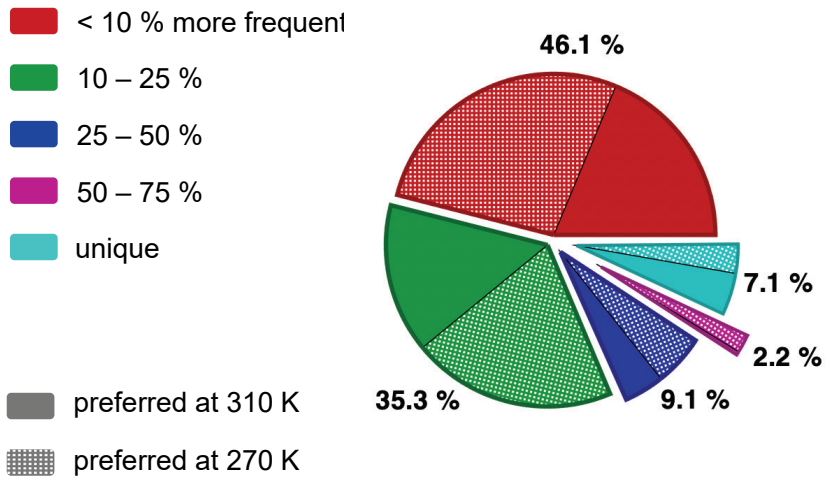

Fig. S4. The hydrogen bonding network changes significantly upon cooling. (**A**) Average number of hydrogen bonds realized in a selection of 200 snapshots from our fully oxidized OmcS model at 310 K and 270 K. (**B**) Analysis of the preference of the hydrogen bonding interactions at 310 K and 270 K.

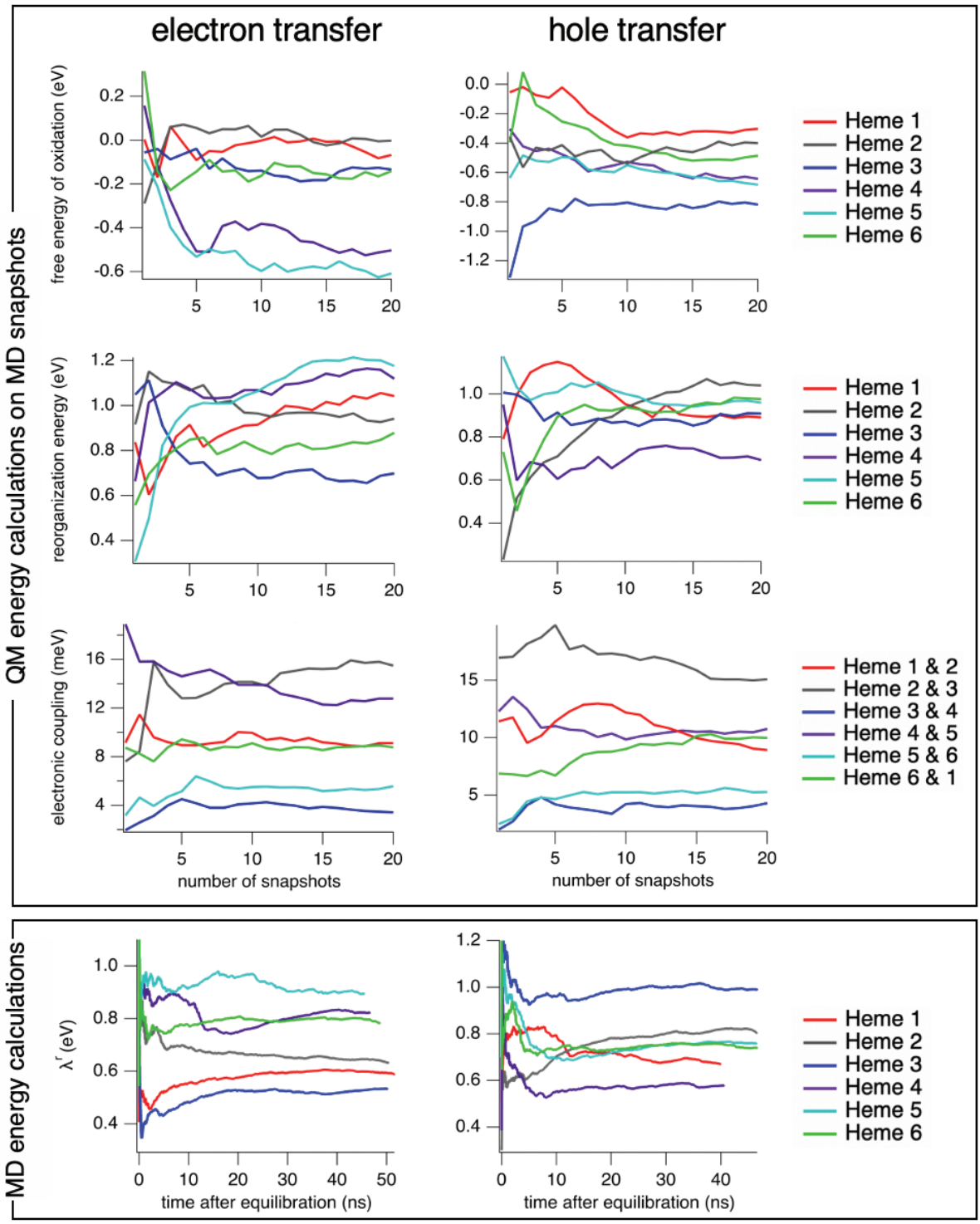

Fig. S5. Convergence of the free energy of oxidation, reorganization energy, and electronic coupling. The free energy of oxidation, reorganization energy, and electronic coupling plotted as a function of the number of snapshots included in the calculation. Left column: Oxidized hemes Right Column: reduced hemes.

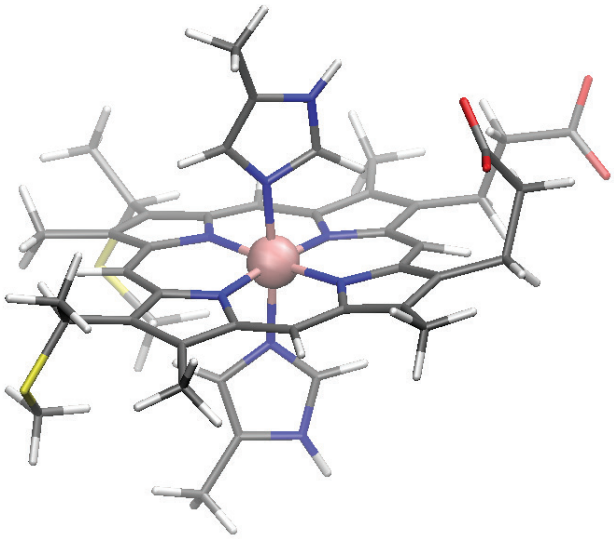

Fig. S6. QM region for electronic structure calculations. The QM region for the electronic structure calculations included the heme (porphyrin ring, iron center, propionate substituents) as well as the covalently bound cysteine and histidine amino acids. The amino acids were truncated at their C_β atom and a hydrogen was inserted to satisfy the C_β valency.

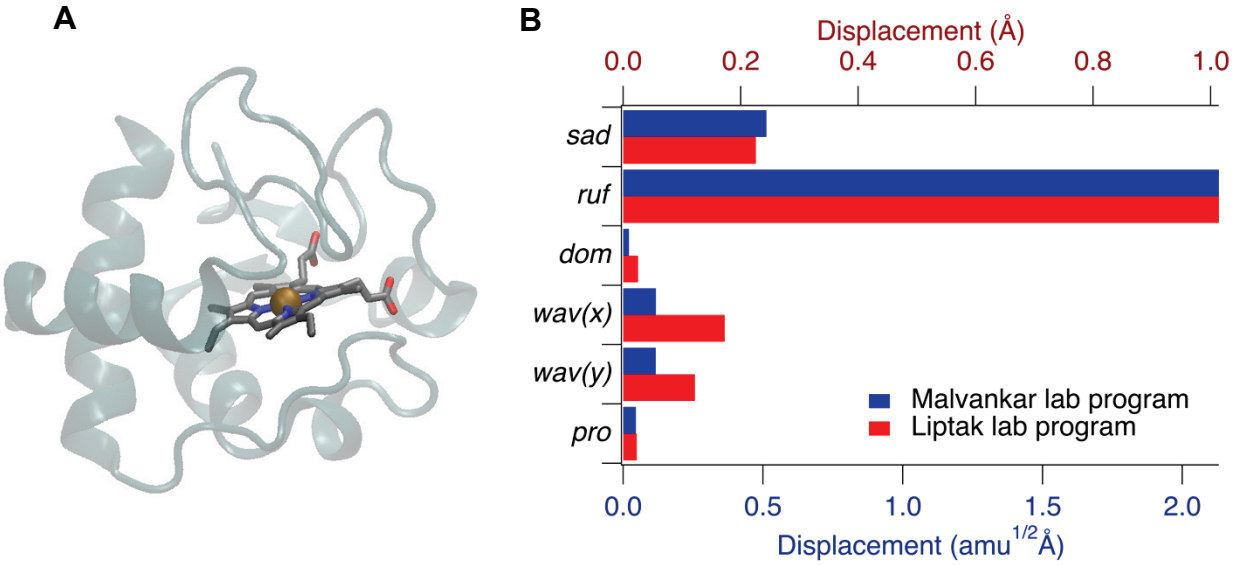

Fig. S7. Benchmarking NSD program with analysis of heme distortion in horse heart cytochrome c. (**A**) Structure of horse heart cytochrome c (PDB ID: 1HRC) showing the distorted heme geometry. (**B**) NSD analysis of the heme distortions in horse heart cytochrome c using our program (blue) and the program described in ref 38 of the Methods.

Table S1. Free energies of oxidation and reorganization energies for electron transfer half reaction at 310 K

Heme Number	Free energy of oxidation (eV)	Reorganization energy (eV) [LRA]	Reorganization energy (eV) [MD]
1	-0.191 ± 0.05	0.888 ± 0.05	0.586
2	-0.108 ± 0.04	0.949 ± 0.04	0.624
3	-0.126 ± 0.05	0.657 ± 0.05	0.532
4	-0.334 ± 0.04	0.881 ± 0.04	0.783
5	-0.521 ± 0.05	1.094 ± 0.05	0.874
6	-0.080 ± 0.06	0.781 ± 0.06	0.763

Table S2. Free energies of oxidation and reorganization energies for hole transfer half reaction at 310 K

Heme Number	Free energy of oxidation (eV)	Reorganization energy (eV) [LRA]	Reorganization energy (eV) [MD]
1	-0.491 ± 0.05	0.849 ± 0.05	0.667
2	-0.421 ± 0.05	1.054 ± 0.05	0.804
3	-0.727 ± 0.05	0.881 ± 0.05	1.004
4	-0.647 ± 0.05	0.697 ± 0.05	0.587
5	-0.826 ± 0.05	0.890 ± 0.05	0.758
6	-0.431 ± 0.06	0.912 ± 0.06	0.737

Table S3. Gibbs free energy, reorganization energy, and electronic coupling for heme-to-heme electron transfer at 310 K

Heme Pair	Gibbs Free Energy (eV)	Reorganization energy (eV)		Electronic coupling (meV) Mean ± s.e.m.
		LRA	MD	
1 – 2	-0.083	0.918	0.605	8.93 ± 0.8
2 – 3	0.018	0.803	0.578	15.9 ± 1.5
3 – 4	0.209	0.769	0.658	3.48 ± 0.4
4 – 5	0.186	0.987	0.829	12.4 ± 0.9
5 – 6	-0.440	0.938	0.819	5.68 ± 0.6
6 – 1	0.111	0.835	0.675	8.76 ± 0.6

Table S4. Gibbs free energy, reorganization energy, and electronic coupling for heme-to-heme hole transfer at 310 K

Heme Pair	Gibbs Free Energy (eV)	Reorganization energy (eV)		Electronic coupling (meV) Mean ± s.e.m.
		LRA	MD	
1 – 2	0.070	0.952	0.736	8.93 ± 1.1
2 – 3	-0.305	0.968	0.904	15.1 ± 1.1
3 – 4	0.080	0.789	0.795	4.31 ± 0.7
4 – 5	-0.179	0.793	0.672	10.8 ± 0.7
5 – 6	0.395	0.901	0.748	5.28 ± 0.5
6 – 1	-0.061	0.880	0.702	9.99 ± 0.9

Table S5. Electron transfer rates at 310 K

Heme numbers	LRA reorganization energy		MD reorganization energy	
	forward rate (s^{-1})	reverse rate (s^{-1})	forward rate (s^{-1})	reverse rate (s^{-1})
1 – 2	1.12×10^{9}	4.98×10^{7}	2.50×10^{10}	1.11×10^{9}
2 – 3	1.81×10^{9}	3.50×10^{9}	1.76×10^{10}	3.39×10^{10}
3 – 4	2.02×10^{6}	5.02×10^{9}	5.64×10^{6}	1.40×10^{10}
4 – 5	5.46×10^{6}	5.83×10^{9}	2.46×10^{7}	2.63×10^{10}
5 – 6	4.65×10^{10}	3.22×10^{3}	1.14×10^{11}	7.91×10^{3}
6 – 1	6.13×10^{7}	3.89×10^{9}	2.94×10^{8}	1.87×10^{10}

Table S6. Hole transfer rates at 310 K

Heme numbers	LRA reorganization energy		MD reorganization energy	
	forward rate (s^{-1})	reverse rate (s^{-1})	forward rate (s^{-1})	reverse rate (s^{-1})
1 – 2	4.71×10^{7}	6.42×10^{8}	3.98×10^{8}	5.42×10^{9}
2 – 3	5.49×10^{10}	5.97×10^{5}	9.66×10^{10}	1.05×10^{6}
3 – 4	4.45×10^{7}	8.88×10^{8}	4.18×10^{7}	8.36×10^{8}
4 – 5	2.50×10^{10}	3.12×10^{7}	7.90×10^{10}	9.86×10^{7}
5 – 6	1.20×10^{4}	3.48×10^{10}	3.95×10^{4}	1.14×10^{11}
6 – 1	1.43×10^{8}	1.38×10^{9}	8.43×10^{8}	8.15×10^{9}

Table S7. Free energies of oxidation and reorganization energies for electron transfer half reaction at 270 K

Heme Number	Free energy of oxidation (eV)	Reorganization energy (eV) [LRA]	Reorganization energy (eV) [MD]
1	-0.227 ± 0.06	1.016 ± 0.06	0.695
2	-0.200 ± 0.05	1.012 ± 0.05	0.712
3	-0.315 ± 0.04	0.962 ± 0.04	0.408
4	-0.221 ± 0.06	0.882 ± 0.06	0.686
5	-0.477 ± 0.04	0.988 ± 0.04	0.882
6	-0.107 ± 0.04	0.954 ± 0.04	0.878

Table S8. Gibbs free energy, reorganization energy, and electronic coupling for heme-to-heme electron transfer at 270 K

Heme Pair	Gibbs Free Energy (eV)	Reorganization energy (eV)		Electronic coupling (meV) Mean ± s.e.m.
		LRA	MD	
1 – 2	-0.027	1.014	0.704	9.07 ± 0.9
2 – 3	0.115	0.987	0.560	14.9 ± 1.6
3 – 4	-0.092	0.922	0.547	3.37 ± 0.5
4 – 5	0.254	0.935	0.784	12.7 ± 1.0
5 – 6	-0.370	0.971	0.880	5.56 ± 0.6
6 – 1	0.120	0.954	0.787	8.77 ± 0.6

Table S9. Electron transfer rates at 270 K

Heme numbers	LRA reorganization energy		MD reorganization energy	
	forward rate (s^{-1})	reverse rate (s^{-1})	forward rate (s^{-1})	reverse rate (s^{-1})
1 – 2	4.74×10^{7}	1.49×10^{7}	1.58×10^{9}	4.98×10^{8}
2 – 3	7.12×10^{6}	1.00×10^{9}	8.34×10^{8}	1.17×10^{11}
3 – 4	6.80×10^{7}	1.30×10^{6}	4.64×10^{9}	8.90×10^{7}
4 – 5	2.57×10^{5}	1.43×10^{10}	1.24×10^{6}	6.85×10^{10}
5 – 6	8.50×10^{9}	1.87×10^{3}	2.05×10^{10}	4.51×10^{3}
6 – 1	2.25×10^{6}	3.92×10^{8}	2.02×10^{7}	3.53×10^{9}

Table S10. MM reorganization energy parameters for electron transfer at 310 K

Heme number	λ^{St} (eV)	λ (eV)	κ_G (eV)
1	0.782	1.045	1.34
2	0.811	1.055	1.30
3	0.587	0.648	1.10
4	0.840	0.900	1.07
5	1.021	1.192	1.17
6	0.857	0.962	1.12

Table S11. MM reorganization energy parameters for hole transfer at 310 K

Heme number	λ^{St} (eV)	λ (eV)	κ_G (eV)
1	0.880	1.162	1.32
2	0.932	1.082	1.16
3	0.806	0.648	0.80
4	0.738	0.929	1.26
5	0.852	0.957	1.12
6	0.887	1.067	1.20

Table S12. MM reorganization energy parameters for electron transfer at 270 K

Heme number	λ^{St} (eV)	λ (eV)	κ_G (eV)
1	0.825	0.980	1.19
2	0.789	0.876	1.11
3	0.532	0.693	1.30
4	0.808	0.952	1.18
5	0.933	0.987	1.06
6	0.917	0.958	1.04