

Fast electron transfer through a single molecule natively-structured redox protein. Supporting Information.

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1 Methods

1.1 Preparation of protein monolayer and electrochemical measurements

All proteins used in this study were prepared as described previously^{1,2}. Au(111) bead single crystals electrodes were electropolished in 1 M H₂SO₄ and washed with 1 M HCl and water, then annealed for 8 hours at 880°C. Prior to protein deposition the electrodes were H₂ flame annealed and the Au(111) single crystal surface was protected with hydrogen saturated water. The electrodes were subsequently incubated in 30-60 μM protein solution with 50 μM DTT (1,4-dithiothreitol) diluent at 4°C overnight. This same procedure was used for all proteins. Prior to undertaking the experiments the electrodes were rinsed with water to remove the non-chemically adsorbed molecules. The proteins were checked for stability by measuring the UV-Vis spectra before and after the incubation. The electrochemical analyses were performed using an Autolab potentiostat (Eco Chemie, Netherlands) controlled by a general purpose electrochemical software package (GPES). A homemade glass cell was used for the measurements; a platinum wire gauze and a freshly prepared reversible hydrogen electrode (RHE) were the counter and reference electrode respectively. At the end of each experiment the RHE potential was measured against SCE potential and the obtained value was used to correct the measured potentials. Purified argon gas was bubbled through the electrochemical cell solutions prior to each experiment for 10 minutes to generate an inert atmosphere. A constant gas stream was flowed through the electrochemical cell during the experiments. The sample was allowed to equilibrate for 5 minutes in the same buffer as the protein, 10 mM sodium phosphate buffer pH 6.2. Scanning rates were in the range 0.05 V/s - 30 V/s and the potential window was from -0.3 V to + 0.2 V vs SCE. All glassware was boiled in 20% nitric acid solution and washed with Millipore water prior to the experiments.

1.2 ECSTM imaging

Single crystal Au(111) electrodes were used to perform in situ ECSTM experiments with a PicoSPM system (Molecular Imaging Co., USA) connected to a bipotentiostat for controlling substrate and tip potentials. ECSTM investigations were performed in constant current mode (set point 30-40 pA). Electrochemically etched STM tips were made from 0.25 mm diameter platinum-iridium (90%-10%) wires and insulated with a layer of Apiezon wax. A homemade Teflon electrochemical cell was used for the ECSTM experiments. Counter and reference electrodes were both platinum wire gauze. The reference potential was measured versus SCE in the end of each experiment. All the experiments were carried out in a specially designed Argon chamber.

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1.3 $I_t - z$ Measurement and analysis

Before starting the $I_t - z$ experiments, the substrate was imaged and the tip checked for integrity. After achieving molecular resolution and when the system lateral drift was as low as a few pm/s, the STM tip was placed on top of a single molecule at a current set-point of 35 pA and a voltage bias of -100 mV. The STM feedback loop was then switched off and the tip was moved towards the molecule by 0.5 nm. The approach was stopped for 50 ms in order to help form a molecular junction between the tip and the protein and the tip was then moved away from the surface and the current measured as function of tip travelled distance (driving rate 40 nm/s). Two hundred $I_t - z$ traces were recorded at each overpotential and, before varying the working potential, the sample was imaged to check for protein stability and system lateral drift. At least 2000 traces were recorded for each family of protein and we observed three types of $I_t - z$ traces. The working electrode potential was changed so that the proteins were in the fully reduced, at the equilibrium redox potential and in the fully oxidised state.

2 DNA and protein sequences

2.1 The DNA sequence for wild-type cyt *b*₅₆₂:

GCAGATCTTGAAGACAATATGGAAACCCTCAACGACAATTTAAAAGTGATCGAAAAAGCG	60
GATAACGCGGCGCAAGTCAAAGACGCGTTAACGAAGATGCGCGCCGCAGCGCTGGATGCG	120
CAAAAAGCAACGCCGCCGAAGCTCGAAGATAAATCACCGGACAGCCCCGAAATGAAAGAT	180
TTCCGCCACGGTTTTCGACATTCTGGTCGGTCAGATTGACGACGCGCTGAAGCTGGCAAAT	240
GAAGGTAAAGTAAAAGAAGCGCAGGCTGCTGCAGAGCAACTGAAAACGACCCGCAACGCC	300
TATCACCAGAAGTATCGT	318

2.2 The protein sequence for wild-type cyt *b*₅₆₂:

1	5	10	15	20	25																			
A	D	L	E	D	N	M	E	T	L	N	D	N	L	K	V	I	E	K	A	D	N	A	A	Q
26	30	35	40	45	50																			
V	K	D	A	L	T	K	M	R	A	A	A	L	D	A	Q	K	A	T	P	P	K	L	E	D
51	55	60	65	70	75																			
K	S	P	D	S	P	E	M	K	D	F	R	H	G	F	D	I	L	V	G	Q	I	D	D	A
76	80	85	90	95	100																			
L	K	L	A	N	E	G	K	V	K	E	A	Q	A	A	A	E	Q	L	K	T	T	R	N	A
101	105																							
Y	H	Q	K	Y	R																			

2.3 The DNA sequence for D50C cyt *b*₅₆₂:

GCAGATCTTGAAGACAATATGGAAACCCTCAACGACAATTTAAAAGTGATCGAAAAAGCG	60
GATAACGCGGGCGCAAGTCAAAGACGCGTTAACGAAGATGCGCGCCGCAGCGCTGGATGCG	120
CAAAAAGCAACGCCGCCGAAGCTCGAA	180
TGTTAAATCACCGGACAGCCCGGAAATGAAAGAT	240
TTCCGCCACGGTTTTCGACATTCTGGTCGGTCAGATTGACGACGCGCTGAAGCTGGCAAAT	300
GAAGGTAAAGTAAAAGAAGCGCAGGCTGCTGCAGAGCAACTGAAAACGACCCGCAACGCC	318
TATCACCAGAAGTATCGT	

2.4 The protein sequence for D50C cyt *b*₅₆₂:

1	5	10	15	20	25
A	D	L	E	D	N
M	E	T	L	N	D
N	L	K	V	I	E
K	A	D	N	A	A
Q					
26	30	35	40	45	50
V	K	D	A	L	T
K	M	R	A	A	A
L	D	A	Q	K	A
T	P	P	K	L	E
C					
51	55	60	65	70	75
K	S	P	D	S	P
E	M	K	D	F	R
H	G	F	D	I	L
V	G	Q	I	D	D
A					
76	80	85	90	95	100
L	K	L	A	N	E
G	K	V	K	E	A
Q	A	A	A	E	Q
L	K	T	T	R	N
A					
101	105				
Y	H	Q	K	Y	R

2.5 The DNA sequence for SH-LA cyt *b*₅₆₂:

GCAGATCTTGAAGACAATATGGAAACCCTCAACGACAATTTAAAAGTGATCGAAAAAGCG	60
TGTAAACGCGGGCGCAAGTCAAAGACGCGTTAACGAAGATGCGCGCCGCAGCGCTGGATGCG	120
CAAAAAGCAACGCCGCCGAAGCTCGAA	180
TGTTAAATCACCGGACAGCCCGGAAATGAAAGAT	240
TTCCGCCACGGTTTTCGACATTCTGGTCGGTCAGATTGACGACGCGCTGAAGCTGGCAAAT	300
GAAGGTAAAGTAAAAGAAGCGCAGGCTGCTGCAGAGCAACTGAAAACGACCCGCAACGCC	318
TATCACCAGAAGTATCGT	

2.6 The protein sequence for SH-LA cyt *b*₅₆₂:

1 5 10 15 20 25
A D L E D N M E T L N D N L K V I E K A C N A A Q

26 30 35 40 45 50
V K D A L T K M R A A A L D A Q K A T P P K L E C

51 55 60 65 70 75
K S P D S P E M K D F R H G F D I L V G Q I D D A

76 80 85 90 95 100
L K L A N E G K V K E A Q A A A E Q L K T T R N A

101 105
Y H Q K Y R

2.7 The DNA sequence for SH-SA cyt *b*₅₆₂:

GCAGATCTTGAA TGT AATATGGAAACCCTCAACGACAATTTAAAAGTGATCGAAAAAGCG 60
GATAACGCGGCGCAAGTCAAAGACGCGTTAACGAAGATGCGCGCCGCAGCGCTGGATGCG 120
CAAAAAGCAACGCCGCGCGAAGCTCGAAGATAAATCACCGGACAGCCCGGAAATGAAAGAT 180
TTCCGCCACGGTTTCGACATTCTGGTCGGTCAGATTGACGACGCGCTGAAGCTGGCAAAT 240
GAAGGTAAAGTAAAAGAAGCGCAGGCTGCTGCAGAGCAACTGAAAACGACCCGCAACGCC 300
TATCACCAG TGT TATCGT 318

2.8 The protein sequence for SH-SA cyt *b*₅₆₂:

1 5 10 15 20 25
A D L E C N M E T L N D N L K V I E K A C N A A Q

26 30 35 40 45 50
V K D A L T K M R A A A L D A Q K A T P P K L E D

51 55 60 65 70 75
K S P D S P E M K D F R H G F D I L V G Q I D D A

76 80 85 90 95 100
L K L A N E G K V K E A Q A A A E Q L K T T R N A

101 105
Y H Q K C R

3 Statistical analysis of proteins dimensions

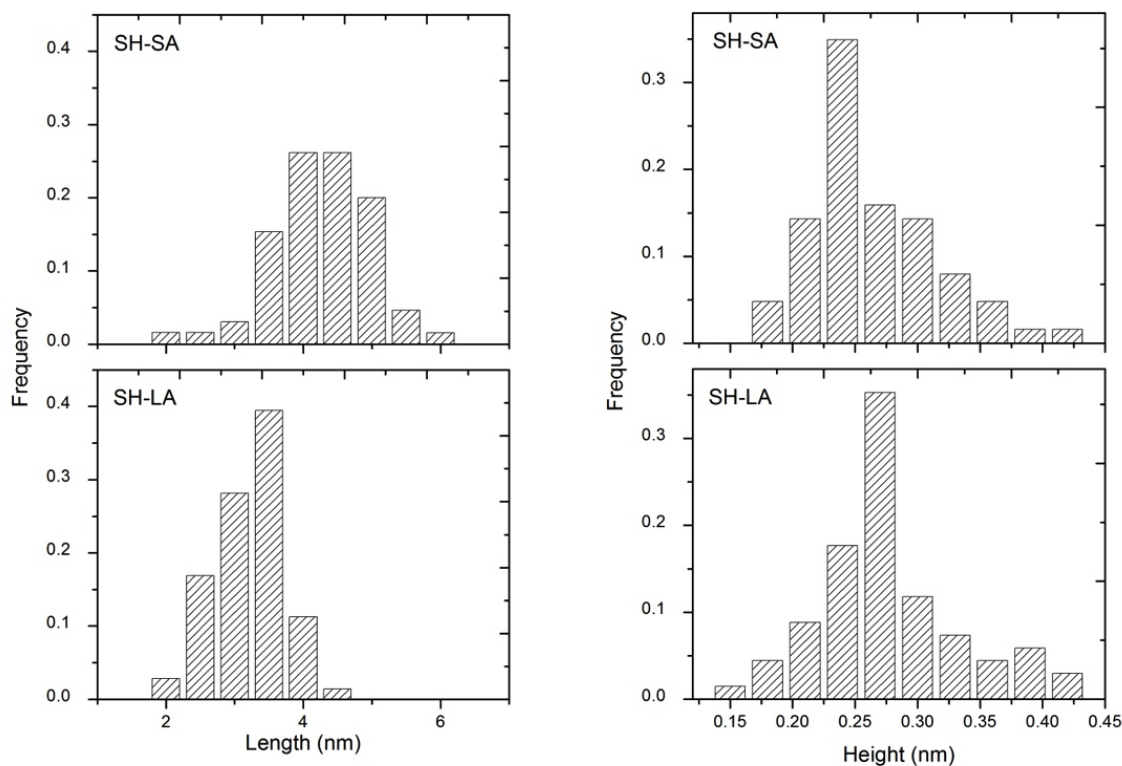


Fig. 1 Lateral (left) and vertical (right) dimensions of 230 different SH-SA and different 230 SH-LA molecules adsorbed on Au(111) surfaces. The data were obtained from images of 100×100 nm size, recorded in 10 mM phosphate buffer pH 6.2, $I_t = 35$ pA, $V_b = -0.40$ V, $E_s = -0.10$ V vs SCE.

4 STM images of apo-cyt b_{562}

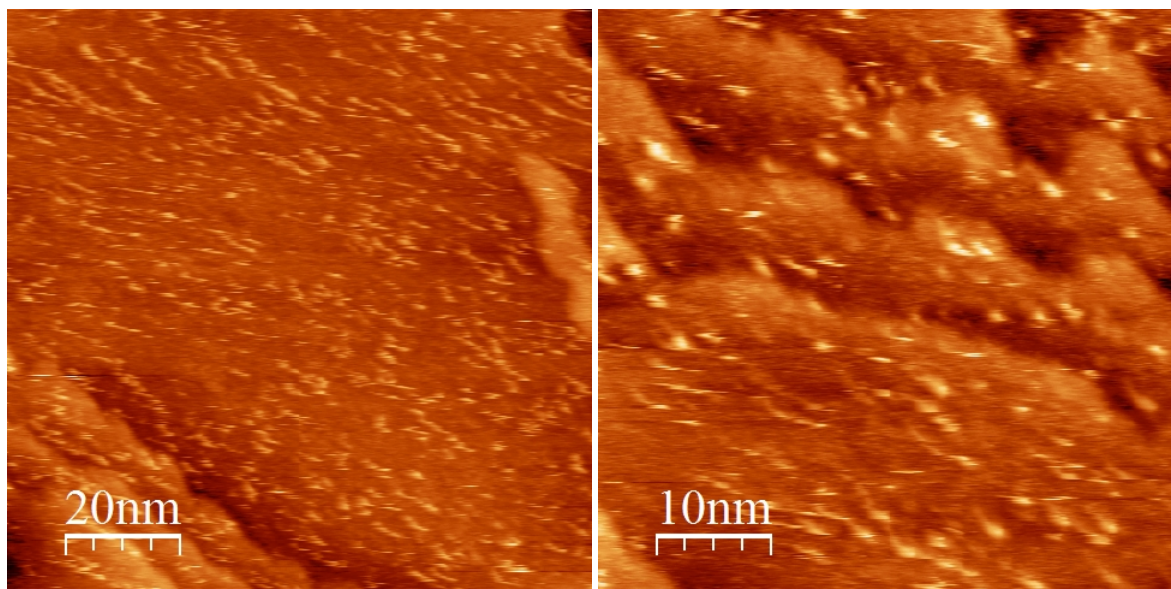


Fig. 2 EC-STM images of SH-LA apo-cyt b_{562} deposited on Au(111). $I_t = 50$ pA, $V_b = -0.2$ V, $E_s = -0.05$ V vs SCE, z -range = 1.8 nm.

5 Electrochemical data

5.1 A. Cyclic voltammograms

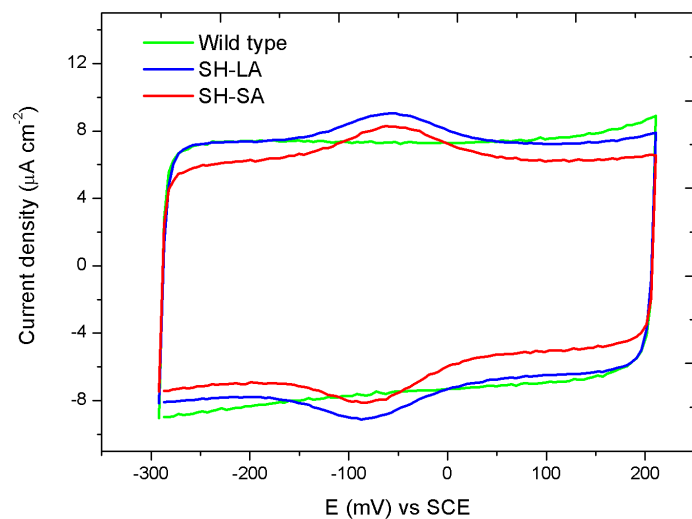


Fig. 3 Cyclic voltammograms recorded for holo-wild type, holo-SH-SA and holo-SH-LA cyt b_{562} adsorbed on Au(111) in a phosphate buffer (10 mM, pH 6.2). Scan rate 700 mV s^{-1} .

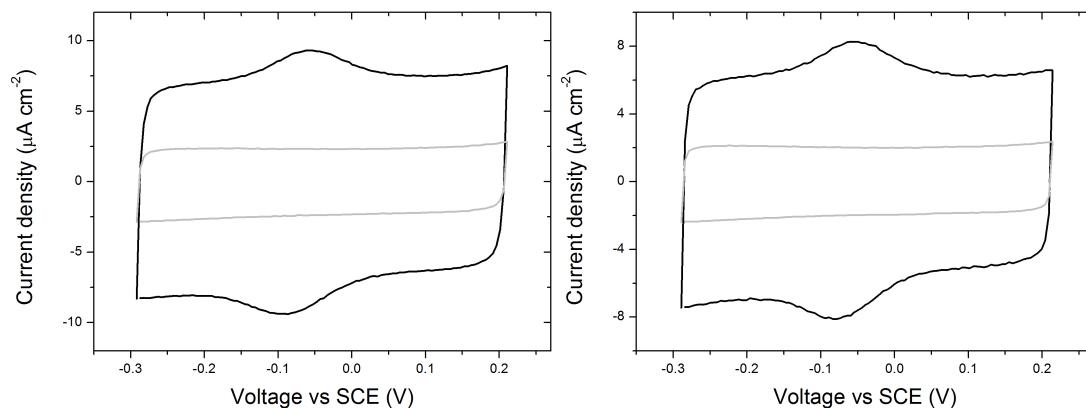


Fig. 4 Cyclic voltammograms recorded for apo- (grey line) and holo- (black line) cyt b_{562} SH-SA (left) and SH-LA (right) adsorbed on Au(111) in a phosphate buffer (10 mM, pH 6.2). Scan rate 700 mV s^{-1} .

5.2 B. Cyclic voltammograms at low scan rates

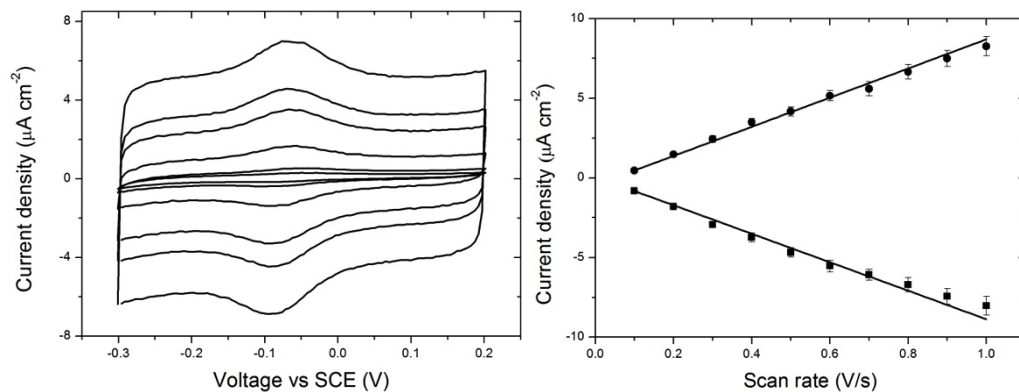


Fig. 5 Cyclic voltammograms of cytochrome *b*₅₆₂ SH-SA-Au(111) in 10 mM phosphate buffer (pH 6.2) at various scan rates (left) and relation of the peak currents versus scan rates (right). Scan rates in the left plot are 0.2, 0.3, 0.5, 0.7, 0.8 and 1.0 V s⁻¹.

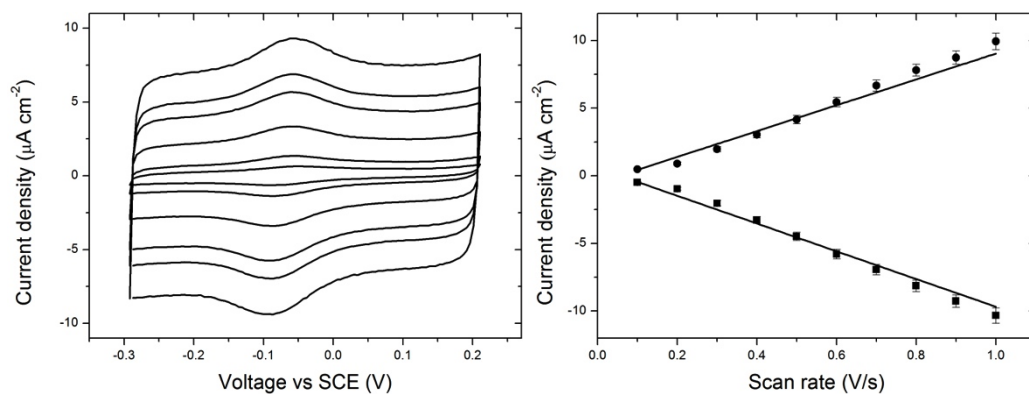


Fig. 6 Cyclic voltammograms of cytochrome *b*₅₆₂ SH-LA-Au(111) in 10 mM phosphate buffer (pH 6.2) at various scan rates (left) and relation of the peak currents versus scan rates (right). Scan rates in the left plot are 0.2, 0.3, 0.5, 0.7, 0.8 and 1.0 V s⁻¹.

6 In situ STM images

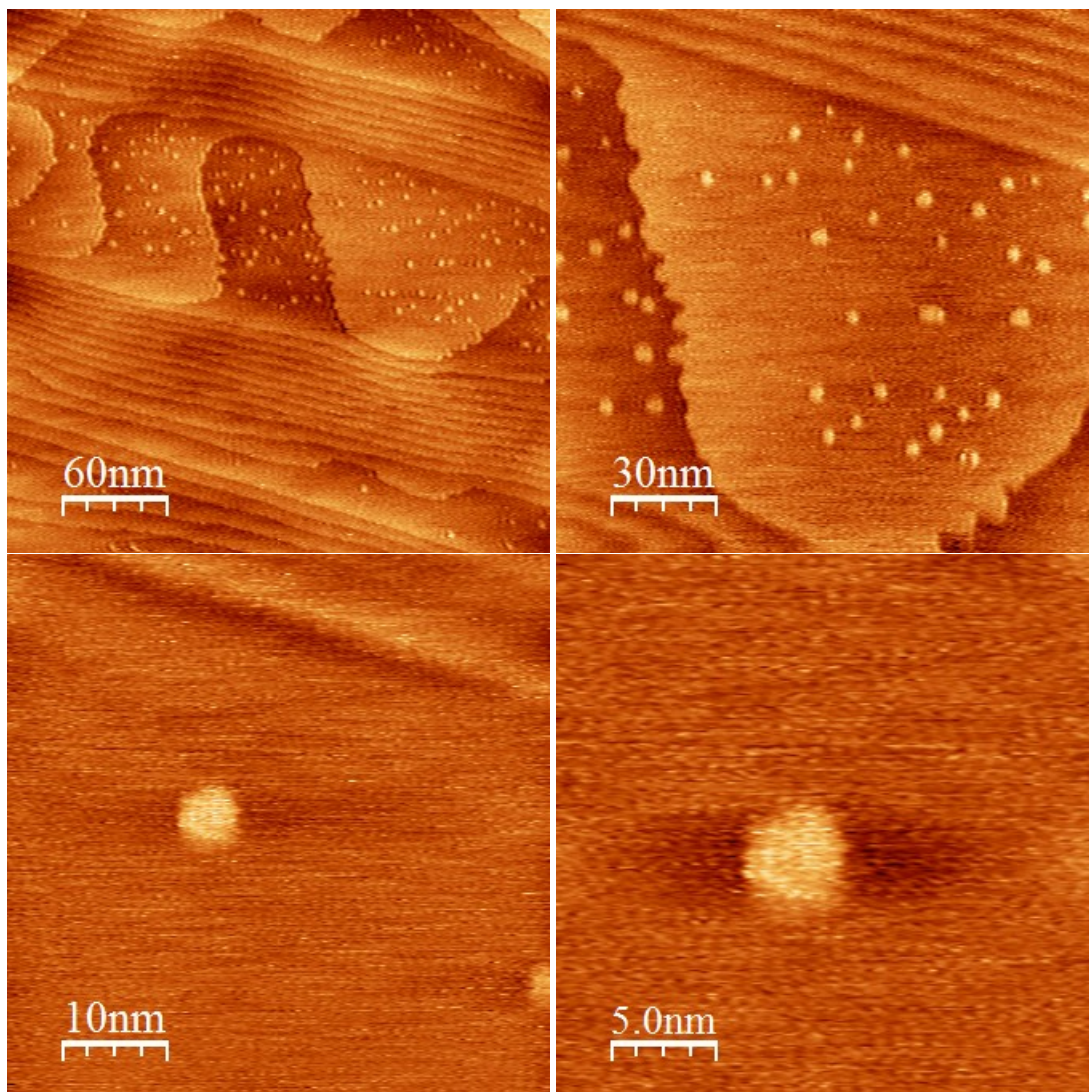


Fig. 7 Series of EC-STM images recorded before STS experiments ($I_t = 50$ pA, $V_b = -0.14$ V, $E_s = -0.05$ V vs SCE). Smaller areas are consecutively scanned and when the system drift is very low, the STS experiments are performed. The bottom images show the protein before (left) and after (right) the STS experiments.

7 $I_t - z$ histograms

The $I_t - z$ traces (each comprising 400 data points) presented in the main text were smoothed by convolution with a Gaussian kernel of 7-point width σ before binning into histograms (150 bins over the full conductance range of 50 nS). The smoothed example traces for the LA and SA molecules are shown here. This procedure yields less noisy histograms (presented in the main text) and sharpens the peak positions.

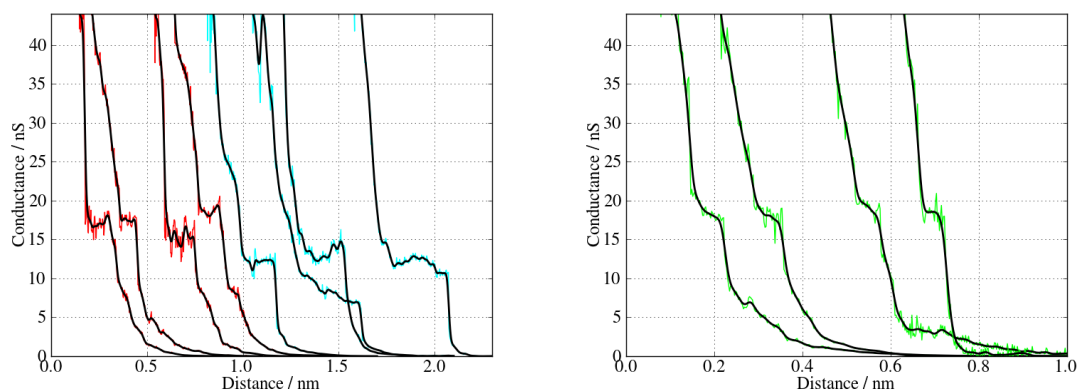


Fig. 8 Same representative $I_t - z$ traces for cyt b_{562} SH-LA (left), and cyt b_{562} SH-SA (right) showing the effect of the data smoothing procedure (black curves).

Histograms from the un-smoothed data are shown here for comparison.

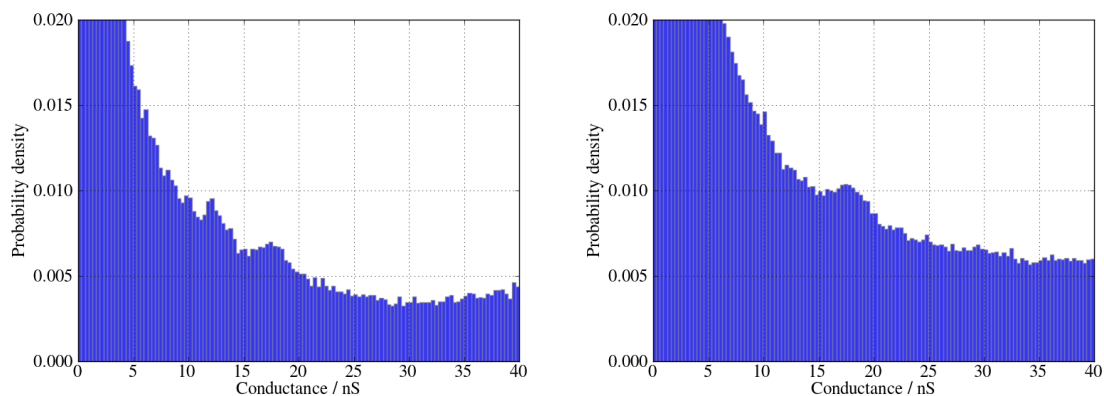


Fig. 9 Histograms obtained for all $I_t - z$ traces without selection. Left panel cyt b_{562} SH-LA (2400 traces), right panel cyt b_{562} SH-SA (2200 traces). The measurements were performed in 10 mM phosphate buffer (pH 6.2) at a fixed tip-substrate bias voltage of -0.1 V, and at the equilibrium redox potential of the protein molecules (i.e. $\eta = 0$ mV).

References

- 1 E. A. Della Pia, Q. Chi, D. D. Jones, J. E. Macdonald, J. Ulstrup and M. Elliott, *Nano Lett.*, 2011, **11**, 176–82.
- 2 E. A. Della Pia, M. Elliott, D. D. Jones and J. E. Macdonald, *ACS Nano*, 2012, **6**, 355–61.