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Fabrication of electron tunnelling probes for measuring single protein conductance

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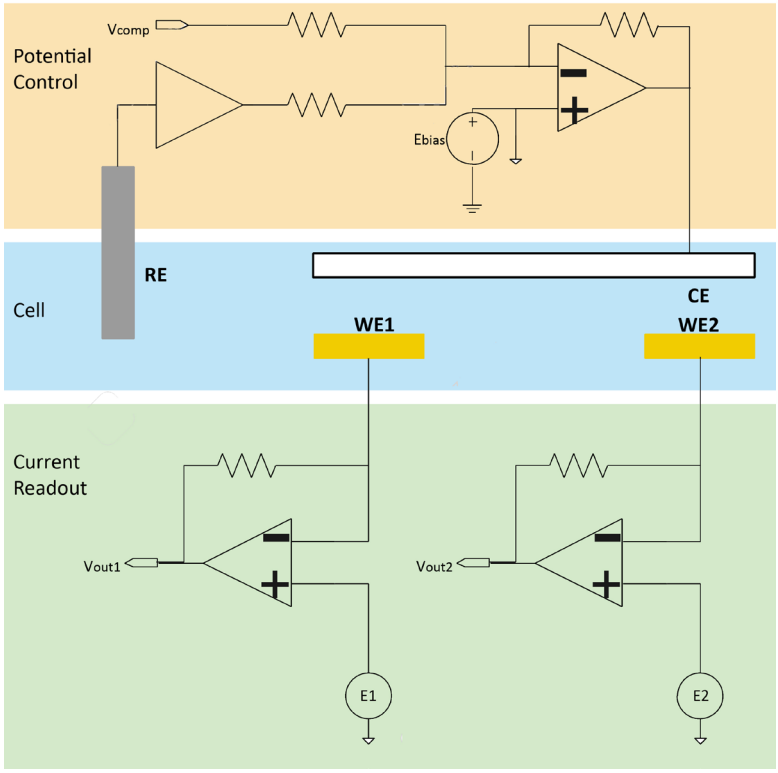
Methods for protein junctions	**Electrode configurations**	**Feature gap size**	**Number of molecules**	**Scalability**	**Reproducibility**	**Ref.**
Single-protein-coupled quantum mechanical tunneling probes	Symmetric	Adjustable	Single	High	Low	This work
Scanning tunnelling microscopy break junction	Asymmetric	Adjustable	Single	Low	High	1
Mechanically controlled break junctions	Symmetric	Adjustable	Single	Low	High	2
Crack-defined break junction	Symmetric	Sub-3 nm	Single	High	Low	3
Lithography-defined cutting protein junction based on single-walled carbon nanotubes	Symmetric	Less than 10 nm	Single	Middle	Middle	4
Nanowire cross-protein junction	Asymmetric	Sub-5 nm	Ensemble	Middle	Low	5
Liquid metal contact junctions	Asymmetric	Sub-5 nm	Ensemble	Low	Low	6

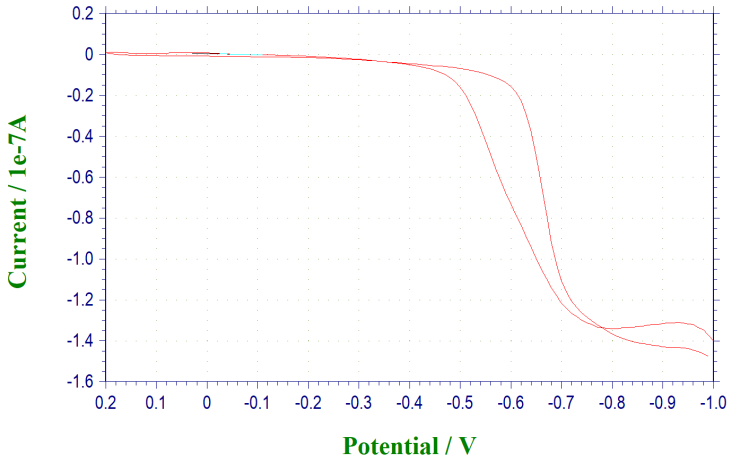
Supplementary Table 1 Comparison of protein junctions fabricated by different methods.

concept	explanation
Working electrode	An electrode where the target substance undergoes an electrochemical reaction.
Reference electrode	Used as a potential reference for the working electrode.
Counter electrode	The electrode that is opposite to the working electrode is also called an auxiliary electrode.
quasi-reference electrode	When there is no reasonable matching reference electrode to measure the electrochemical reaction, the selected one has the properties of a conductor, and the electrode surface does not have a clear electrochemically hardened electrode material to act as a reference electrode.
Potentiostat	A device that keeps the potential of the working electrode constant relative to the reference electrode. Its working principle is to accurately measure the current between the working electrode and the counter electrode.

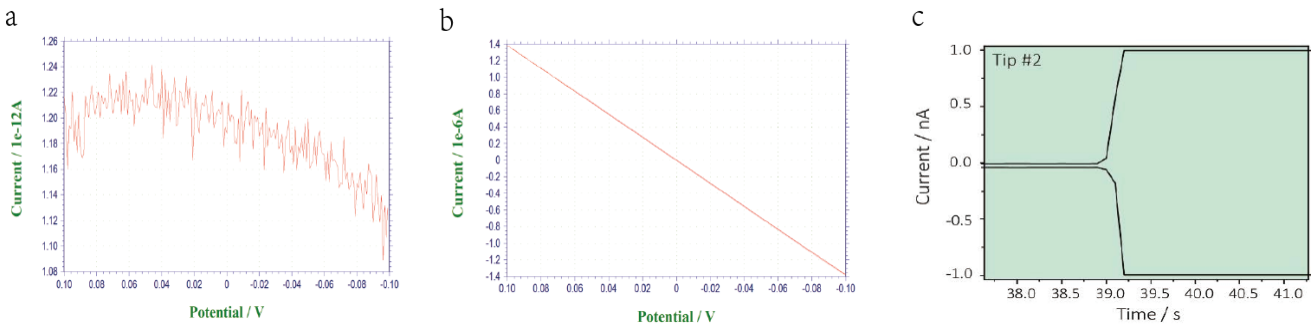
Supplementary Table 2 Explanations of electrochemical concepts.



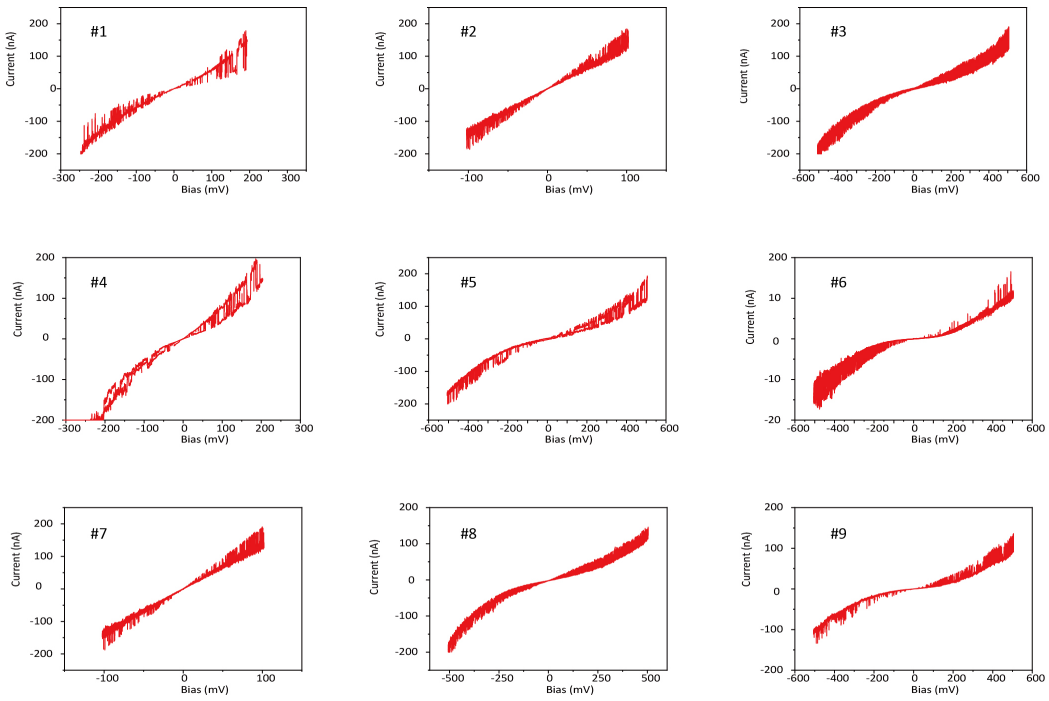
Supplementary Figure 1 Basic circuit diagrams of the bipotentiostat used for nanoelectrode fabrication.[7]



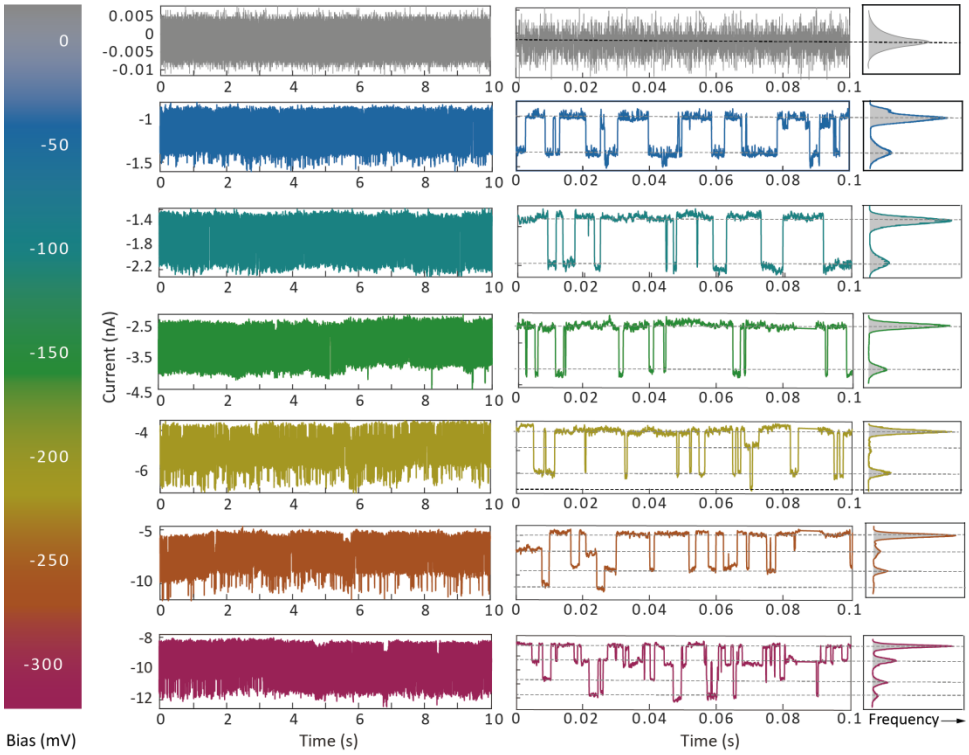
Supplementary Figure 2 CV measurement of the gold plating solution (Initial: -1 V, Final: 0.2 V, Sample rate: 0.01 V/s).



Supplementary Figure 3 (a-b) I-V between the two electrodes (a) before and (b) after electrical contact. (c) Electrochemical pre-deposition of gold on the carbon nanoelectrodes. An abrupt increase in the current was observed during the deposition due to the generation of a tunnelling current.



Supplementary Figure 4 Current-bias plots of different streptavidin-coupled QMT probes measured in 1 mM phosphate buffer solution (PBS) at room temperature (25 °C).



Supplementary Figure 5 Chronoamperometric traces of single streptavidin-bridged QMT probes at different bias using 1 mM PBS (pH 7.4) at 25 °C. Representative 10-s *I-t* traces are shown on the left and zoomed-in 0.1s traces are shown in the middle. The emergence of multiple conductance states becomes visible with increasing applied bias. The corresponding all-points current histograms are plotted on the right.

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