

Quantum Mechanical Tunnelling Probes with Redox Cycling for Ultra-Sensitive Detection of Biomolecules

Long Yi, ^{[a],[b],[c]} Tao Jiang, ^[a] Ren Ren, ^[c] Ji Cao, ^[d] Joshua B. Edel, * ^[c] Aleksandar P. Ivanov, * ^[c] Longhua Tang, * ^{[a],[b]}

[a] Long Yi, Tao Jiang, Prof. Longhua Tang

State Key Laboratory of Extreme Photonics and Instrumentation, College of Optical Science and Engineering
Zhejiang University, Hangzhou, 310027 China.
lhtang@zju.edu.cn

[b] Long Yi, Prof Longhua Tang

Nanhu Brain-Computer Interface Institute, Second Affiliated Hospital School of Medicine.
Hangzhou, Zhejiang, 311100, China

[c] Long Yi, Dr. Ren Ren, Prof. Joshua B. Edel, Prof. Aleksandar P. Ivanov

Department of Chemistry, Molecular Science Research Hub
Imperial College London, White City Campus
82 Wood Lane, London, W12 0BZ, UK
joshua.edel@imperial.ac.uk, alex.ivanov@imperial.ac.uk

[d] Prof. Ji Cao

Institute of Pharmacology and Toxicology, Zhejiang Province Key Laboratory of Anti-Cancer Drug Research
College of Pharmaceutical Sciences, Zhejiang University
Hangzhou, China.

Abstract: Quantum mechanical tunnelling sensors (QMTs) have emerged as a promising technology for next-generation single-molecule detection. When QMT sensors are combined with redox species, repeated oxidation and reduction (redox cycling) can also be observed. We developed robust QMT probes with electrode gap distances below 2 nm. Using the generator-collector (GC) mode, we verified that redox cycling of the ferrocyanide/ferricyanide ($\text{Fe}(\text{CN})_6^{3-/4-}$) couple occurs both in the tunnelling regime and on the electrode surface. Our findings indicated that the current enhancement is affected by both the gap distance and surface modifications of the probes. These QMT probes exhibited remarkable sensitivity, capable of detecting $\text{Fe}(\text{CN})_6^{3-/4-}$ concentrations as low as sub-picomolar levels. Utilising this ability to modulate redox reactions, we adapted the QMT probes to serve as electrochemical sensors for detecting viral proteins. By modifying the electrode surfaces, our functionalised QMT probes achieved sub-pM detection limits with high selectivity in biofluids such as nasopharyngeal secretions. These findings highlight the potential of QMT probes to develop into a new class of electrochemical tunnelling sensors, offering significant advancements in biomedical diagnostics.

Introduction

Single-entity electrochemistry has emerged as a powerful tool for probing fundamental biological and chemical processes at the molecular level.^[1] Within this broad field, redox cycling electrochemistry stands out for its ability to monitor electron transfer processes directly through a redox cycling reaction, which provides amplification of the current response.^[2] To achieve this redox cycling behaviour, two closely spaced parallel

electrodes, with separations ranging from micrometres to nanometres, create a confined space where redox reactions occur.^[3] The reactant is generated at a high potential at one electrode, while the reverse reaction occurs at the opposite electrode, indicating a charge transfer process.^[3b] Over the past few decades, advancements in this area have enabled redox cycling detection on various electrode architectures,^[4] including scanning electrochemical microscopy,^[5] nanopore electrode array,^[6] nanogap device.^[7] These innovations have significantly improved sensitivity compared to traditional electrochemical methods and reduced detection volumes to ~10 attoliters.^[8] Consequently, redox cycling has become a promising technique for studying reaction kinetics and provides novel opportunities for single-molecule detection.^[5b, 9] Currently, redox cycling studies typically employ electrode gaps ranging from tens of nanometres to micrometres. Therefore, investigating redox cycling within sub-2 nm nanogap devices is particularly valuable, as it could further enhance our understanding of these processes and push the boundaries of detection sensitivity.

When a voltage bias is applied across two electrodes separated by a gap usually smaller than 2 nm, electrons can traverse the gap through a phenomenon known as quantum tunnelling.^[10] This phenomenon can be detected and quantified by measuring the resulting current.^[11] The tunnelling current is influenced by several factors, including the applied voltage, the distance between the electrodes, and the properties of the medium within the gap.^[10a] Measuring the tunnelling current provides a unique opportunity to investigate the molecular species present in the gap.^[10b, 12] The current is highly sensitive to the molecular

RESEARCH ARTICLE

environment and responsive to dynamic changes such as molecular reorganisation or chemical modifications.^[13]

Despite its potential, fabricating stable sub-2 nm nanogaps in a controlled and straightforward manner remains a significant challenge. Various methods have been developed to address this, including break junction techniques,^[14] electromigration,^[15] electrodeposition,^[16] advanced lithographic techniques,^{[17],[18]} To overcome these challenges, our team has developed a robust and straightforward protocol for creating QMT probes, which we have successfully applied to the detection of single nucleotides and proteins.^[19] In addition to sensing single molecules, the structure of QMT probes meets the demand to detect redox cycling behaviours by direct tunnelling.

Our study observed redox cycling response using cyclic voltammetry and chronoamperometry measurements. We demonstrated that the presence of the redox-active $\text{Fe}(\text{CN})_6^{3-/4-}$ couple enhances the current through redox cycling between tunnelling junctions. Our findings revealed that the enhanced current density exhibits a linear relationship with the gap distance from sub-nanometer scales up to 2 nm. We also observed that

the extent of redox cycling enhancement depends significantly on the concentration of the $\text{Fe}(\text{CN})_6^{3-/4-}$ couple and the voltage bias applied to the tunnelling nanoelectrodes. Importantly, we identified a shielding effect on redox cycling influenced by surface modifications. Based on this phenomenon, we functionalised QMT probes with aptamers through surface modification, enabling the detection of target molecules and advancing their potential as single-molecule sensors.

Results and Discussion

Fabrication, Characterisation and Redox Cycling Observation of Au quantum mechanical tunnelling Probe

Figure 1(A, B) demonstrates a schematic of the structure of the QMT probe and the redox cycling pathway. Briefly, the QMT probe is fabricated using a laser-pulled dual-barrel quartz capillary filled with carbon through a pyrolysis process. Gold is then electrochemically deposited on the carbon nanoelectrodes using real-time feedback, forming an Au nanoelectrode pair within the tunnelling distance.^{[19],[20]}

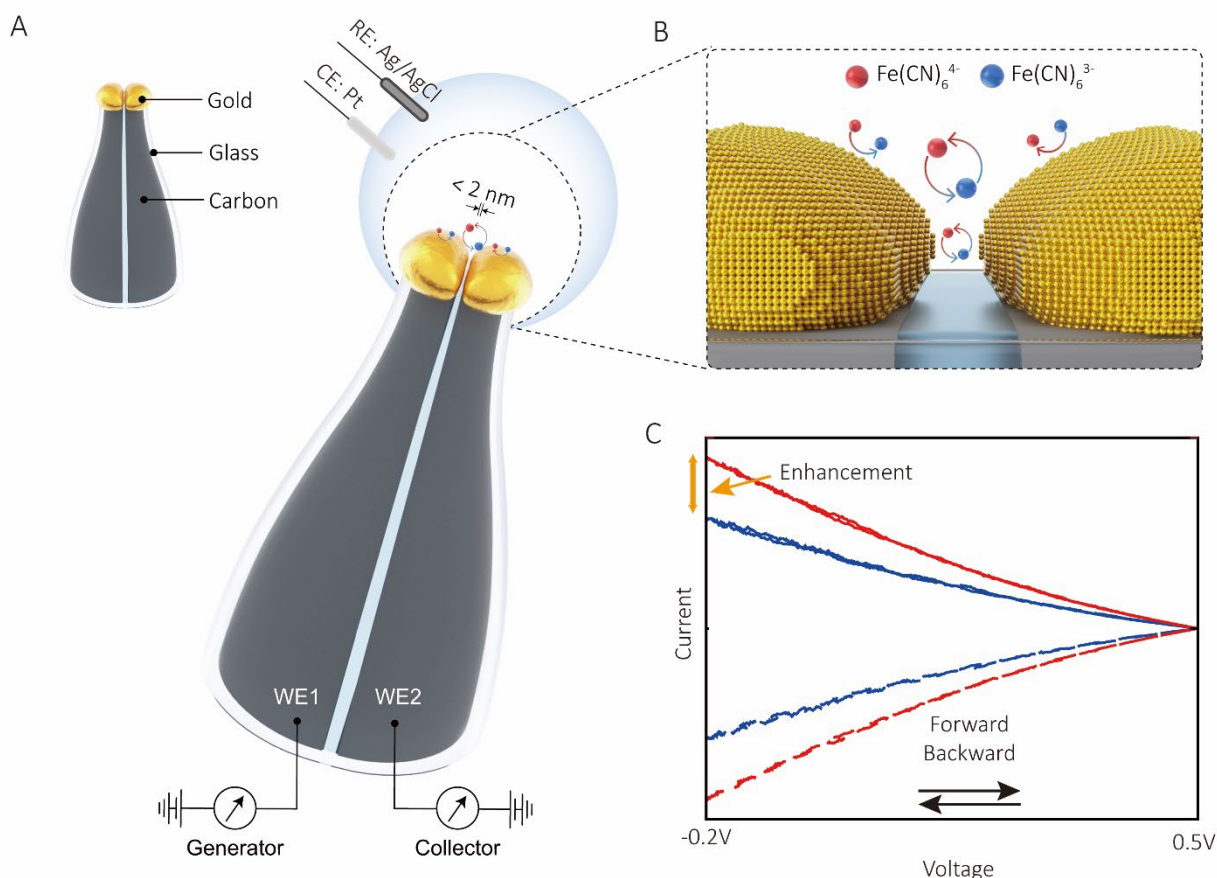


Figure 1. Schematic of redox cycling enhancement on QMT probe. The probe consists of an Au tunnelling junction deposited on dual-barrel carbon nanoelectrodes fabricated through carbon pyrolytic deposition. When redox-active reagents ($\text{Fe}(\text{CN})_6^{3-/4-}$ couple) are introduced, redox reactions occur separately on each side. A fixed positive bias is applied to one Au nanoelectrode (WE1) to generate the reduction product as a generator. Meanwhile, from negative to positive voltage, the other nanoelectrode (WE2) collects the reduction product as a collector. This process results in a significant current enhancement in the CV measurement (red curve) compared to the tunnelling background (blue curve) (reference electrode (RE): Ag/AgCl, counter electrode (CE): Pt, working electrode 1 (WE1): Au tunnelling junction, working electrode 2 (WE2): Au tunnelling junction).

To evaluate the properties of the QMT probes, current-voltage (I-V) measurements were conducted in deionised water to estimate the characteristics of the tunnelling junction. Figure S1(B) depicts a typical I-V response of the tunnelling junction (blue curve) compared to an open circuit (black curve). Upon adding a 1 nM $\text{Fe}(\text{CN})_6^{3-/4-}$ solution diluted in deionised water, the current increased (red curve) compared to a baseline measurement only in deionised water. This increase indicates that the presence of the $\text{Fe}(\text{CN})_6^{3-/4-}$ couple enhances the tunnelling conductivity. To confirm the stability of the gap and exclude interference from gap distance changes due to applied potential, we recorded the tunnelling background before and after measurements, demonstrating the stability of our device for redox cycling observations (Figure S3).

Beyond direct I-V characterisation, we employed the "Generator and Collector" mode, a model frequently used in single-molecule redox cycling electrochemistry, to investigate further redox cycling with the QMT probes.^[6] In this mode, one working electrode (WE1) is held at a constant voltage to drive one-half of the redox reaction, while the other working electrode (WE2) is scanned across a range of voltages using cyclic voltammetry (CV), covering the reduction-oxidation potential range.^[21] During the measurement, molecules are reduced at one electrode and oxidised at the other, resulting in enhanced current amplitude, as observed in Figure 1(C).^[22] To quantify the effect of $\text{Fe}(\text{CN})_6^{3-/4-}$ couple, relative limiting current enhancement is calculated as $\Delta I_{\text{relative}} = I_{\text{Fe}^{3+}/\text{Fe}^{2+}} - I_{\text{background}}$ at certain V_{bias} , as depicted in Figure 1(C).^[23]

Redox cycling enhancement

The QMT probe's geometry consists of gold deposited on coplanar carbon nanoelectrodes, which can also facilitate redox reactions.^[24] We performed in-situ redox cycling characterisation at each step of the fabrication process to identify the conditions necessary for observing redox cycling. Figure 2(A) and (B) show the in-situ fabrication of the tunnelling junction with real-time CV measurements to monitor redox cycling as the nanogap distance changes. To mitigate the hindered diffusion effect of redox reagents during measurements, 1 mM $\text{Fe}(\text{CN})_6^{3-/4-}$ in deionised water was applied.

Initially, the tunnelling probes comprised two coplanar carbon nanoelectrodes separated by a barrel, with a separation distance of 10–20 nm based on the barrel thickness from previous studies.^[20] In this configuration (step i), the CV response indicated leakage current without any observable redox cycling behaviour. Subsequently, Au was deposited onto the carbon electrodes using a potentiostatic method to form Au nanogap electrodes with gap distances ranging from 2 to 10 nm (step ii). At this stage, no tunnelling response was detected, and the CV of the $\text{Fe}(\text{CN})_6^{3-/4-}$ couple showed leakage current with a larger amplitude compared

to step i, confirming the presence of Au on the carbon but without redox cycling on the Au nanogap electrodes.

Finally, after feedback-controlled electrodeposition, the tunnelling junction was formed (step iii). The tunnelling junction was established with gap distances estimated to be between 0.3 nm and 2 nm.^[19] Meanwhile, a border sensing space is also formed, which surrounds the tunnelling junction. It extends beyond the actual junction but remains close enough to allow electron tunnelling through redox-active molecules, acting as a transitional interface that bridges direct electron transfer with broader electrochemical activity on the electrode surface.^[20] At this stage, a significant enhancement in the relative current was observed. Through in-situ monitoring of redox cycling at various stages of nanogap probe fabrication, we determined that redox cycling occurs at the tunnelling junction in our device configuration.

To identify where reactions occur on the QMT probes, we applied surface modifications using alkyl sulfide. We analysed the CV responses of a 1 nM $\text{Fe}(\text{CN})_6^{3-/4-}$ solution before and after functionalisation with 2-propyl disulfide, as shown in Figure S4 (A). Due to its small size, 2-propyl disulfide fits well within the sub-2 nm tunnelling gap, allowing optimal surface coverage on the tunnelling junctions.^[25] The CV analysis in Figure 2(C) reveals that, following surface modification, the current enhancement ratio was significantly lowered to 0.26 ± 0.21 . Given the variations in electrode structures between different devices, substantial differences in redox cycling behaviour are expected, influenced by the coverage of the functionalising molecules and the gap distance. This outcome demonstrates that surface modification effectively shields redox cycling, though redox processes within the tunnelling regime persist, enhancing the CV response.

In redox cycling electrochemistry, the concentration of redox reagents contributes to the current amplitude by ion migration and accumulation.^[26] To explore the effect of concentration on redox cycling enhancement in QMT probes, we performed real-time titration of $\text{Fe}(\text{CN})_6^{3-/4-}$, as depicted in Figure S6. At a bias of 500 mV, we observed that the current amplitude increased with rising $\text{Fe}(\text{CN})_6^{3-/4-}$ concentration. After accounting for the tunnelling background at each concentration, the relative current enhancement changes were plotted against concentration in Figure 2(D). The results demonstrated that in the absence of a supporting electrolyte, the relative current data fit the Hill equation, modelling Langmuir-Hill adsorption, indicating a half-maximal relative current response at around 0.11 pM concentration.^[27] The concentration change in the solution varies the number of redox-active molecules on the electrode surface of QMT probes, which interferes with the redox cycling enhancement as an electrochemical-related behaviour.^[28] This ultra-low concentration sensitivity demonstrates the high potential of QMT probes for detecting minute quantities of analytes and suggests optimal concentration settings for future biological applications.

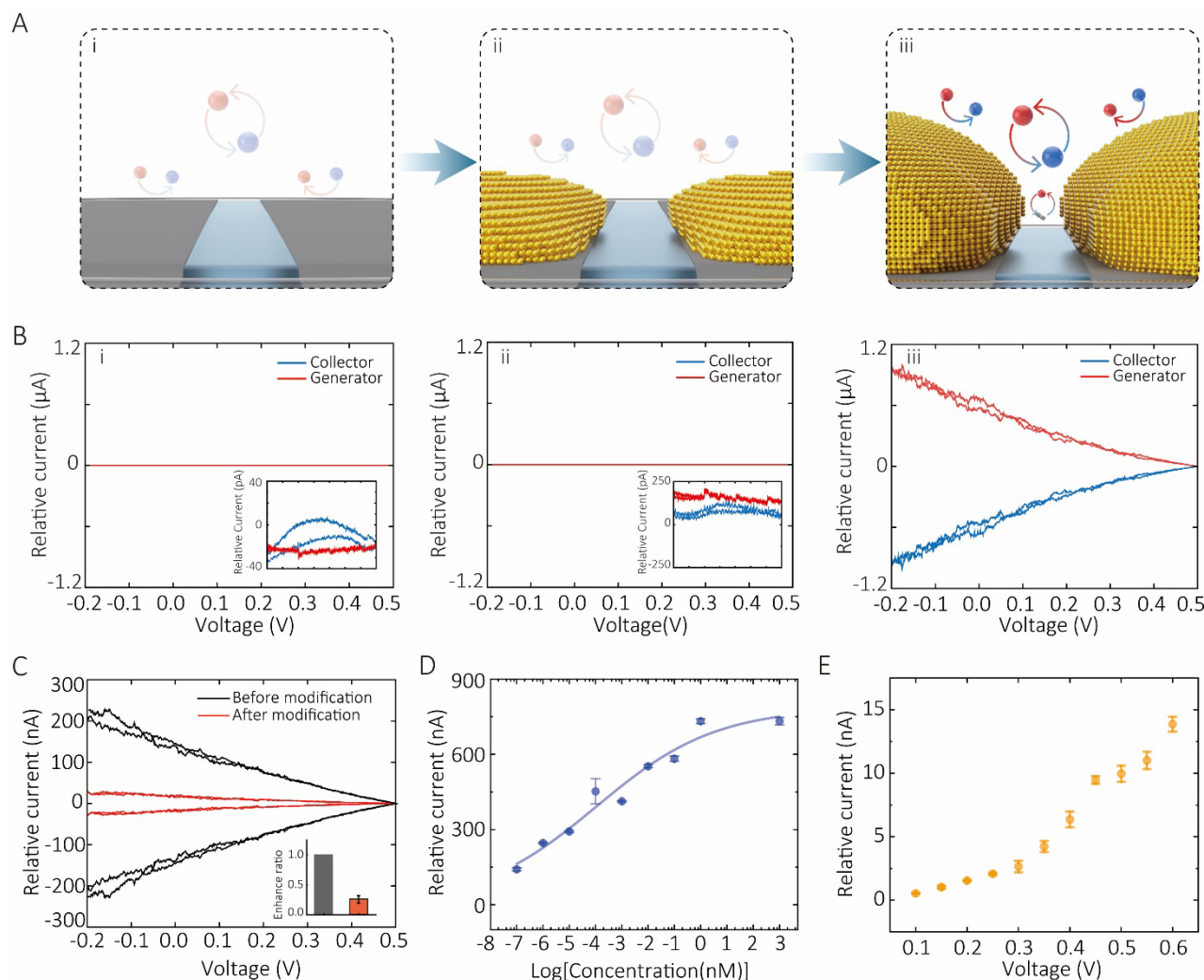


Figure 2. Characterisation of redox cycling on QMT. (A) Sequential fabrication stages of the QMT probes: (i) Dual-barrel carbon electrodes, (ii) Au nanoelectrodes with a wide nanogap (gap distance > 2 nm), and (iii) Au tunnelling junction with a narrow nanogap (gap distance < 2 nm). (B) CVs depicting the redox cycling measurements for each fabrication stage using 1 mM $\text{K}_3\text{Fe}(\text{CN})_6/\text{K}_2\text{Fe}(\text{CN})_6$ as the analyte. (C) Comparative CV measurements illustrating the relative changes before (black) and after (red) surface modification with 2-propyl disulfide in a 1 mM $\text{K}_3\text{Fe}(\text{CN})_6/\text{K}_2\text{Fe}(\text{CN})_6$ solution (diluted with deionised water). The scanning range of WE2 is from -200 mV to 500 mV at a scanning rate of 100 mV/s, while WE1 is held at 500 mV (Reference Electrode: Ag/AgCl, Counter Electrode: Pt, Working Electrode: Au). The error bars represent standard error calculated from measurements with separate devices ($N=4$). (D) Plot of relative current as a function of $\text{K}_3\text{Fe}(\text{CN})_6/\text{K}_2\text{Fe}(\text{CN})_6$ analyte concentration changes at 500 mV. (Reference Electrode: Ag/AgCl, Counter Electrode: Pt, Working Electrode 1: Au at 500 mV, Working Electrode 2: Au at 0 mV). The blue curve represents Hill fitting based on the Langmuir-Hill adsorption model ($R^2 = 0.96$). Error bars represent one standard deviation from the mean. (E) Plot of relative current as a function of potential change for 1 mM $\text{K}_3\text{Fe}(\text{CN})_6/\text{K}_2\text{Fe}(\text{CN})_6$ analyte (Reference/Counter Electrode: Ag/AgCl, Working Electrode 1: Au at 500 mV, Working Electrode 2: Au at 0 mV). Error bars represent one standard deviation from the mean.

To examine the response across different voltage biases, we performed chronoamperometry measurements on 1 nM $\text{Fe}(\text{CN})_6^{3-/4-}$ solutions, varying the bias from 100 mV to 600 mV. Figure 2(E) presents the relative current at each voltage. As the voltage increased from reduction to oxidation potentials, the relative conductance followed a sigmoidal-like trend, consistent with the two-state redox behaviour.^[29] Moreover, the increase in the generator potential changes the Fermi level of both electrodes. When the scanning range encompasses the redox potential, the

electrons can hop through the redox active molecule and produce a relatively limited current enhancement on the CV response shown in Figure S9. This phenomenon also highlights the engagement of tunnelling characters.^[30] Furthermore, The QMT probes demonstrated selectivity based on the reversibility of reactions, as illustrated in Figure S7.^[21] The corresponding I-t response further confirmed the selectivity of QMT probes for reversible reactions, highlighting their potential as promising electrochemical sensors.

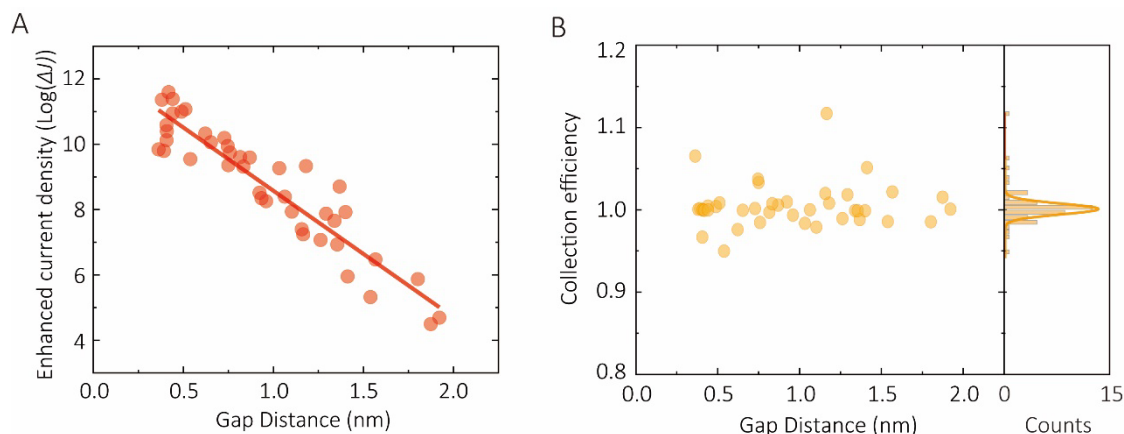


Figure 3. Redox cycling in the tunnelling regime. (A) The plot of mean enhanced current density ($\text{Log}(\Delta J)$) versus gap distance for 37 devices, measured at -200 mV in a $1 \mu\text{M}$ $\text{K}_3\text{Fe}(\text{CN})_6/\text{K}_2\text{Fe}(\text{CN})_6$ solution. The red line represents the linear fit of the data. ($R^2 = 0.87$) (B) Plot of collection efficiency versus gap distance for 37 devices, measured at -200 mV in a $1 \mu\text{M}$ $\text{K}_3\text{Fe}(\text{CN})_6/\text{K}_2\text{Fe}(\text{CN})_6$ analyte.

Redox cycling in the tunnelling regime

In traditional single-entity electrochemistry, the distance between the generator and the collector electrodes significantly impacts current amplitude enhancement.^[5b] To further investigate the relationship between the nanogap distance and redox cycling, we analysed QMT probes with various redox cycling characteristics. Figure S1(A) shows typical tunnelling I-V backgrounds (dotted line) and fitted data (solid line) for different QMT probes, using the Simmons tunnelling model to estimate gap distance, tunnelling area, and barrier height. Given that the CV measurement of redox cycling reagents includes background tunnelling current, to minimise variations in gap distance between devices, we introduced relative enhanced current density $\Delta J = \Delta I_{\text{relative}}/S$, to normalise the redox cycling enhancement rate on the tunnelling junction.^[29a] S represents the area of the tunnelling junction (based on simulation). To maximise redox cycling on QMT probes, we recorded CV measurements at -200 mV with a relatively high concentration of $1 \mu\text{M}$ $\text{Fe}(\text{CN})_6^{3-/4-}$.^[22] The red line represents the linear fit of the data plots based on the logarithmic form of the Simmons tunnelling model.^[10a] The experimental results followed a linear trend with a slope of -3.9 ± 0.24 ($R^2 = 0.87$), indicating that larger gap distances produced lower relative current density ratios. Figure 3(A) illustrates the redox cycling enhancement rate for 37 devices. In addition to changes in relative enhanced current density, we also analysed the collection efficiency to assess the redox cycling effect. Figure 3(B) shows the collection efficiency ϕ_r for 37 devices with different gap distances, calculated using

$$\phi_r = \frac{I_{\text{Generator}}}{I_{\text{Collector}}}$$

where $i_{\text{Generator}}$ and $i_{\text{Collector}}$ represented the relative current of the generator and collector, respectively.^[22] The results indicated

an average ϕ_r nearly 100%. This high collection efficiency suggests that once a molecule is oxidised at one electrode, reduction occurs almost instantaneously at the other electrode. This high conversion rate confirms that redox cycling occurs within the confined space of the QMT probes. Furthermore, the conversion of $\text{Fe}(\text{CN})_6^{3-}$ and $\text{Fe}(\text{CN})_6^{4-}$ is independent of the gap distance and the surface structure variations of the tunnelling junction. In addition, in Figure S8, both $1 \mu\text{M}$ to 10 M KNO_3 did not produce significant variation in relative current response, indicating that ion migration will not interfere with the redox cycling enhancement. Thus, the relationship between gap distance and collection efficiency provides evidence for redox cycling reactions occurring in the tunnelling regime.

Compared to models focusing on the conductance of a single molecule, the QMT probes provide expanded spaces for redox-active analytes to interact predominantly with the tunnelling junction via diffusion. To more accurately explain the behaviours observed with QMT probes, we proposed a possible mechanism that integrates electrochemical and tunnelling models. When the electrode potential aligns with the redox potential, redox reactions that occur in the tunnelling regime suggest rapid, continuous conductance switching between oxidation and reduction states. This behaviour implies a hopping-like mechanism.^[29a, 29c, 30a] Simultaneously, the electrode surface can initiate redox cycling reactions. During this electrochemical process, the tunnelling junction acts as a bridge for direct electron transport from one electrode to the other, supporting electron transfer through the redox-active molecules located within the junction.^[20, 30b, 31] This comprehensive approach helps elucidate the complex dynamics of redox cycling in QMT probes, highlighting their potential as sophisticated electrochemical sensors.

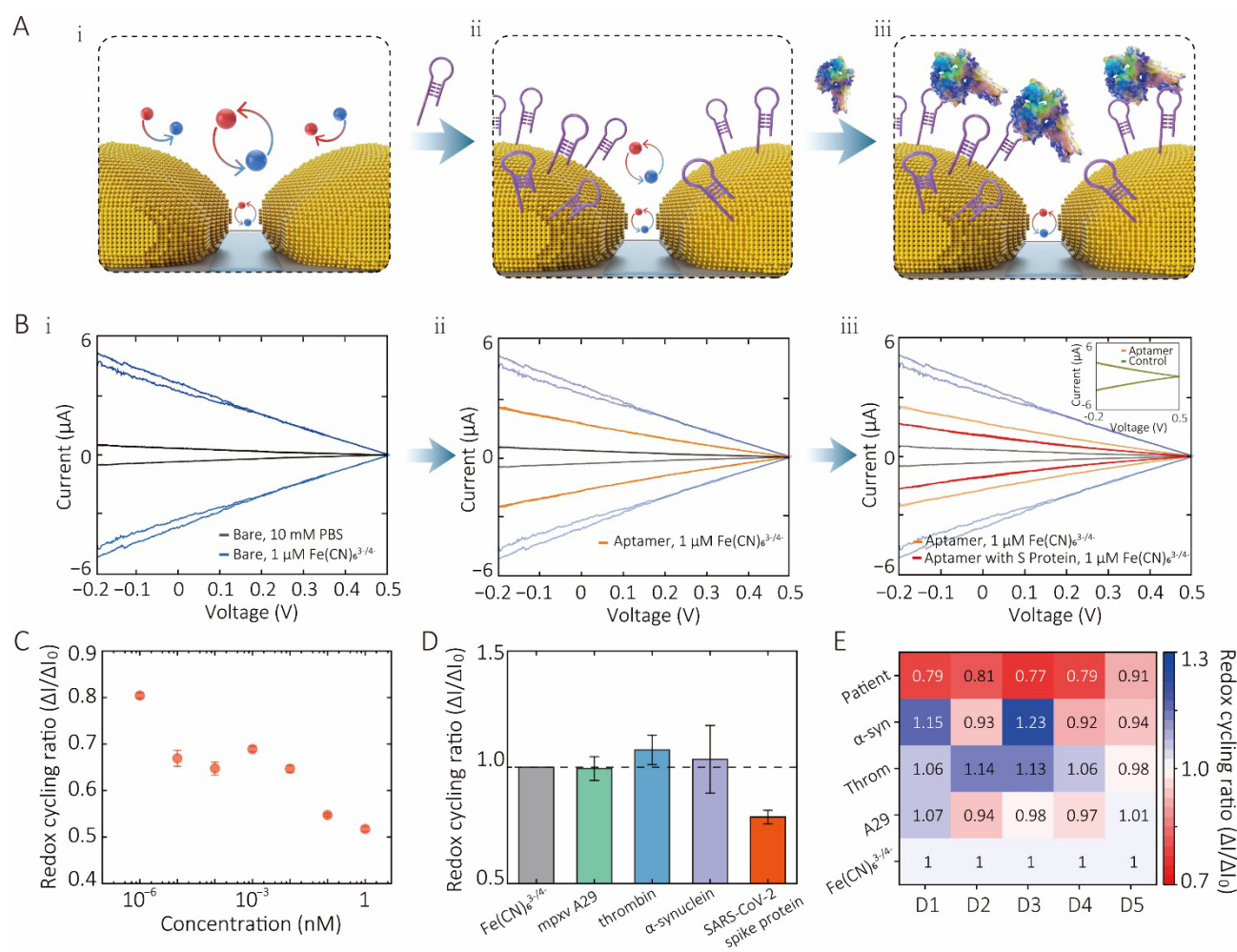


Figure 4. redox cycling-based tunnelling sensor for single molecule detection. (A) Schematic of the redox cycling-based tunnelling sensor and (B) representative CV responses: the background (black), redox cycling enhancement with $1 \mu\text{M } \text{Fe}(\text{CN})_6^{3-/4-}$ (blue), first shielding effect due to aptamer surface modification (orange), and second shielding by the target protein (red). (C) Plot of the redox cycling ratio versus target protein concentration change, with the RE: Ag/AgCl, CE: Pt, WE: Au. Error bars represent one standard deviation from the mean. (D) Bar chart showing the changes in redox cycling ratios for different proteins. The $1 \text{ nM } \text{K}_3\text{Fe}(\text{CN})_6/\text{K}_2\text{Fe}(\text{CN})_6$ background set the redox cycling ratio at 1. Non-specific proteins (mpxv A29, thrombin, and α -synuclein) show no significant difference in redox cycling ratio compared to the $\text{K}_3\text{Fe}(\text{CN})_6/\text{K}_2\text{Fe}(\text{CN})_6$ background. For the target protein (S1 unit of SARS-CoV-2 spike protein, red bar), binding with the aptamer leads to a noticeable redox shielding effect in the CV response compared to the background. The error bars represent standard error calculated from measurements with separate devices ($N=5$). (E) Heat map illustrating the detection of the target protein in clinical samples (dilutions ranging from 1 to 10,000, diluted in 10 mM PBS) across five different devices. The control protein concentration was 1 pM (diluted in a 10 mM PBS solution). The scanning range of WE2 is from -200 mV to 500 mV, with a scanning rate of 100 mV/s, while WE1 is held at 500 mV. Re: Ag/AgCl, CE: Pt, WE: Au.

Redox cycling-based QMT sensor for ultra-low concentration detection

The number of redox molecules involved in redox cycling measurement can interfere with the CV response.^[12a] Consequently, when small molecules are captured on the tunnelling junction, they will block the electrode surface from undergoing redox cycling and, thus, can be detected by observing the CV response. We applied this shielding principle to use redox cycling QMT probes for sensing the SARS-CoV-2 S protein by functionalising the Au tunnelling junction with SARS-AR10 aptamer that binds specifically to the S1 unit of SARS-CoV-2 spike protein (see SI).^[20, 32] Figure 4(A) illustrates the mechanism behind the redox cycling shielding-based sensor. Initially, the tunnelling background (black curve) and the redox cycling enhancement of $1 \mu\text{M } \text{Fe}^{3+}/\text{Fe}^{2+}$ (blue curve) were recorded in a

10 mM PBS solution. As the tunnelling current lacks selectivity to distinguish the molecule, thus aptamer SAM is introduced to bind with the target molecules and prevent the non-specific adsorption of non-target molecules on the electrode surface. The structure of the aptamer is detailed in Figure S9(A). After overnight modification with the aptamer on the bare tunnelling junction, the redox cycling of $\text{Fe}^{3+}/\text{Fe}^{2+}$ was shielded, as shown in the CV response (Figure 4(B), ii, orange curve). As both proteins and aptamers are larger than the gap distance, they can only be anchored to the electrode surface, occupying space for redox cycling, while the number of redox-active species in the confined tunnelling junction remains unchanged. Proteins were added to the solution for an incubation period of 30 minutes before the CV measurement. The addition of non-target proteins (e.g., thrombin: 36.05 kDa, α -synuclein monomer: 14.5 kDa, ^[33] mpxv A29: 14

kDa,^[34] SARS-CoV-2 S protein: 76.5 kDa) resulted in CV responses (Figure 4(B) iii, green curve) similar to the initial redox shielding response, indicating no significant binding to the aptamer. However, when the target S protein is bound to the aptamer, it is immobilised on the electrode surface and reduces the number of $\text{Fe}(\text{CN})_6^{3-/4-}$ molecules on the tunnelling electrode surface and in the tunnelling regime, leading to a second redox cycling shielding effect (Figure 4(B), iii, red curve) in the CV response.

To accurately quantify the shielding effect by aptamer modification and protein, we calculated the redox cycling ratio, $\Delta I/\Delta I_0$, where ΔI represents the relative current after the introduction of analytes, and ΔI_0 represents the relative current background, respectively. To ensure complete binding between the protein and the aptamer, proteins were added to the solution 30 minutes prior to the CV measurement. This incubation period was chosen based on the real-time monitoring of the I-t response illustrated in Figure S11. Figure 4(C) shows the changes in the redox cycling ratio with increasing target SARS-CoV-2 S protein concentration, from 1 fM to 1 nM, in 10-fold increments. The shielding ratio decreased from 0.80 ± 0.01 at 1 fM to 0.52 ± 0.01 at 1 nM, demonstrating high sensitivity for detecting target molecules at sub-pM concentrations.

The selectivity measurements of the modified QMT probes are shown in Figure 4(D). The aptamers, attached to the Au tunnelling junction via S-Au bonds, mitigate non-specific protein adsorption. For non-specific proteins, such as mpxv A29, thrombin, and α -synuclein, the redox cycling ratio remained close to the $\text{Fe}(\text{CN})_6^{3-/4-}$ background, with values of 0.99 ± 0.05 , 1.07 ± 0.06 , and 1.03 ± 0.15 , respectively. However, the introduction of the target SARS-CoV-2 S protein resulted in a significantly lower redox cycling ratio of 0.79 ± 0.03 , illustrating that the specific binding between the target protein and the aptamer occupied the rest of the space on the electrode surface, and therefore, reduces the number of redox-active species in these regions. Consequently, a distinctive secondary shielding effect on the current response was observed. This selectivity highlights the potential of these devices in distinguishing target proteins.

Given the high sensitivity and selectivity of the aptamer-modified QMT probes, we applied them to detect the wild-type S1 unit of SARS-CoV-2 spike protein in clinical samples to evaluate their diagnostic applicability. Further details regarding this process can be found in the 'Methods' section. It is important to note that these represent proof-of-principle measurements and validation with further samples is required.^[35] Figure 4(E) presents a heat map showing detection in a nasopharyngeal secretions sample on five different devices. Although device-to-device variations, such as differences in surface roughness and electrode geometry, can influence the absolute current responses, the observed response in the redox cycling ratios allowed us to detect the shielding effect of the S1 protein, as shown in Figure 4(D), indicating that the functionalised quantum mechanical tunnelling probe can identify target protein from mixtures in complex biofluids. This indicates that the functionalised quantum mechanical tunnelling probe can

effectively identify target proteins from complex mixtures. Compared with state-of-the-art methods, the functionalised redox cycling-based QMT probe provides the benefits of label-free detection and advantages in terms of detection limits and selectivity.^[36]

Conclusion

In conclusion, we successfully observed the redox cycling of redox-active species ($\text{Fe}(\text{CN})_6^{3-/4-}$) on QMT probes with gap distances of less than 2 nm. Our study reveals that redox cycling within the tunnelling regime significantly enhances the current response, with surface modifications at the tunnelling junction playing a crucial role in this enhancement. The QMT probes exhibited high sensitivity to $\text{Fe}(\text{CN})_6^{3-/4-}$ concentration changes at sub-picomolar levels, enabling their use as electrochemical sensors. By modulating the redox reaction at the tunnelling junction, these probes also respond to voltage variations, further enhancing their sensitivity. Functionalisation with aptamers enabled the detection of target proteins at sub-pM concentrations, with high selectivity for identifying specific proteins in complex samples. This work introduces a novel approach to activating redox-active reactions through precise control of surface potential, which could deepen our understanding of complex biological processes, such as redox-active enzymatic reactions at the single-molecule level. These capabilities open new pathways for redox studies in quantum tunnelling and hold great potential for bioanalytical applications.

Supporting Information

The authors have cited additional references within the Supporting Information.^[20, 35, 37]

Acknowledgement

This research was supported by the National Natural Science Foundation of China (grant nos. 21874119 and 62127818), the Natural Science Foundation of Zhejiang Province (grant no. LR22F050003) and the Fundamental Research Funds for the Central Universities. API and JBE acknowledge support from EPSRC grant EP/V049070/1 and Analytical Chemistry Trust Fund grant 600322/05. This project has also received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement nos. 724300 and 875525).

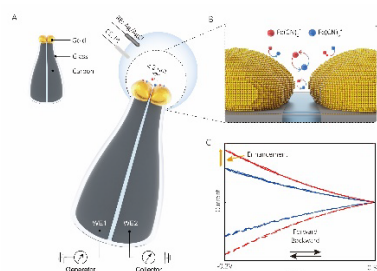
Keywords: Quantum Tunnelling Sensors • Redox Cycling Reaction • Single-Molecular Detection • Biosensor

- [1] Y.-L. Ying, J. Wang, A. R. Leach, Y. Jiang, R. Gao, C. Xu, M. A. Edwards, A. D. Pendergast, H. Ren, C. K. T. Weatherly, W. Wang, P. Actis, L. Mao, H. S. White, Y.-T. Long, *Science China Chemistry* **2020**, 63, 589-618.

- [2] S. G. Lemay, S. Kang, K. Mathwig, P. S. Singh, *Accounts of Chemical Research* **2013**, 46, 369-377.
[3] aB. McDuffie, L. B. Anderson, C. N. Reilly, *Analytical Chemistry* **1966**, 38, 883-890; bT. Kai, C. G. Zoski, A. J. Bard, *Chemical Communications* **2018**, 54, 1934-1947.

- [4] S. M. Oja, Y. Fan, C. M. Armstrong, P. Defnet, B. Zhang, *Analytical Chemistry* **2016**, *88*, 414-430.
- [5] aA. J. Bard, F.-R. F. Fan, *Accounts of Chemical Research* **1996**, *29*, 572-578; bT. Kai, M. Zhou, Z. Duan, G. A. Henkelman, A. J. Bard, *Journal of the American Chemical Society* **2017**, *139*, 18552-18557; cJ. C. Byers, B. Paulose Nadappuram, D. Perry, K. McKelvey, A. W. Colburn, P. R. Unwin, *Analytical Chemistry* **2015**, *87*, 10450-10456.
- [6] K. Fu, S.-R. Kwon, D. Han, P. W. Bohn, *Accounts of Chemical Research* **2020**, *53*, 719-728.
- [7] P. S. Singh, E. Kätelhön, K. Mathwig, B. Wolfrum, S. G. Lemay, *ACS Nano* **2012**, *6*, 9662-9671.
- [8] S.-R. Kwon, K. Fu, D. Han, P. W. Bohn, *ACS Nano* **2018**, *12*, 12923-12931.
- [9] H. Do, S.-R. Kwon, S. Baek, C. S. Madukoma, M. K. Smiley, L. E. Dietrich, J. D. Shrout, P. W. Bohn, *Analyst* **2021**, *146*, 1346-1354.
- [10] aJ. G. Simmons, *Journal of Applied Physics* **1963**, *34*, 1793-1803; bS.-M. Lu, H.-W. Wang, M. Chen, B.-K. Xie, Y.-T. Long, *Angewandte Chemie International Edition*, n/a, e202404170.
- [11] T. Albrecht, *Nature Communications* **2012**, *3*, 829.
- [12] aK. Mathwig, T. J. Aartsma, G. W. Canters, S. G. Lemay, *Annu Rev Anal Chem (Palo Alto Calif)* **2014**, *7*, 383-404; bS.-M. Lu, J.-F. Chen, Y.-Y. Peng, W. Ma, H. Ma, H.-F. Wang, P. Hu, Y.-T. Long, *Journal of the American Chemical Society* **2021**, *143*, 12428-12432.
- [13] aM. Di Ventra, M. Taniguchi, *Nature Nanotechnology* **2016**, *11*, 117-126; bY. Li, J. M. Artés, B. Demir, S. Gokce, H. M. Mohammad, M. Alangari, M. P. Anantram, E. E. Oren, J. Hihath, *Nature Nanotechnology* **2018**, *13*, 1167-1173; cY. Zhao, B. Ashcroft, P. Zhang, H. Liu, S. Sen, W. Song, J. Im, B. Gyrfas, S. Manna, S. Biswas, C. Borges, S. Lindsay, *Nature Nanotechnology* **2014**, *9*, 466-473; dT. Jiang, B.-F. Zeng, B. Zhang, L. Tang, *Chemical Society Reviews* **2023**, *52*, 5968-6002.
- [14] M. Tsutsui, M. Taniguchi, K. Yokota, T. Kawai, *Nature Nanotechnology* **2010**, *5*, 286-290.
- [15] H. Park, A. K. L. Lim, A. P. Alivisatos, J. Park, P. L. McEuen, *Applied Physics Letters* **1999**, *75*, 301-303.
- [16] C. Z. Li, H. X. He, N. J. Tao, *Applied Physics Letters* **2000**, *77*, 3995-3997.
- [17] A. P. Ivanov, E. Instuli, C. M. McGilvery, G. Baldwin, D. W. McComb, T. Albrecht, J. B. Edel, *Nano Letters* **2011**, *11*, 279-285; bA. P. Ivanov, K. J. Freedman, M. J. Kim, T. Albrecht, J. B. Edel, *ACS Nano* **2014**, *8*, 1940-1948.
- [18] Q. He, L. Tang, *Biosensors and Bioelectronics* **2022**, *213*, 114486.
- [19] L. Tang, B. P. Nadappuram, P. Cadinu, Z. Zhao, L. Xue, L. Yi, R. Ren, J. Wang, A. P. Ivanov, J. B. Edel, *Nature Communications* **2021**, *12*, 913.
- [20] L. Tang, L. Yi, T. Jiang, R. Ren, B. Paulose Nadappuram, B. Zhang, J. Wu, X. Liu, S. Lindsay, J. B. Edel, A. P. Ivanov, *Science Advances* **2022**, *8*, eabm8149.
- [21] K. Fu, D. Han, C. Ma, P. W. Bohn, *Nanoscale* **2017**, *9*, 5164-5171.
- [22] C. Ma, N. M. Contento, L. R. Gibson, P. W. Bohn, *ACS Nano* **2013**, *7*, 5483-5490.
- [23] C. H. Ryu, H. Ren, *Nano Letters* **2024**, *24*, 6112-6116.
- [24] K. Mathwig, T. J. Aartsma, G. W. Canters, S. G. Lemay, *Annual Review of Analytical Chemistry* **2014**, *7*, 383-404.
- [25] H. Hinterwirth, S. Kappel, T. Waitz, T. Prohaska, W. Lindner, M. Lämmerhofer, *ACS Nano* **2013**, *7*, 1129-1136.
- [26] C. Ma, W. Xu, W. R. A. Wichert, P. W. Bohn, *ACS Nano* **2016**, *10*, 3658-3664.
- [27] P. Pang, B. A. Ashcroft, W. Song, P. Zhang, S. Biswas, Q. Qing, J. Yang, R. J. Nemanich, J. Bai, J. T. Smith, K. Reuter, V. S. K. Balagurusamy, Y. Astier, G. Stolovitzky, S. Lindsay, *ACS Nano* **2014**, *8*, 11994-12003.
- [28] J. Reitemeier, S. Baek, P. W. Bohn, *ACS Applied Materials & Interfaces* **2023**, *15*, 39707-39715.
- [29] aY. Li, H. Wang, Z. Wang, Y. Qiao, J. Ulstrup, H.-Y. Chen, G. Zhou, N. Tao, *Proceedings of the National Academy of Sciences* **2019**, *116*, 3407-3412; bY. Li, N. L. Haworth, L. Xiang, S. Ciampi, M. L. Coote, N. Tao, *Journal of the American Chemical Society* **2017**, *139*, 14699-14706; cX. Xiao, D. Brune, J. He, S. Lindsay, C. B. Gorman, N. Tao, *Chemical Physics* **2006**, *326*, 138-143.
- [30] aR. R. Nazmutdinov, S. A. Shermokhamedov, T. T. Zinkicheva, J. Ulstrup, X. Xiao, *Chemical Society Reviews* **2023**, *52*, 6230-6253; bT. Albrecht, S. F. L. Mertens, J. Ulstrup, *Journal of the American Chemical Society* **2007**, *129*, 9162-9167; cT. Albrecht, A. Guckian, J. Ulstrup, J. G. Vos, *Nano Letters* **2005**, *5*, 1451-1455.
- [31] Y. Han, C. A. Nijhuis, *Chemistry – An Asian Journal* **2020**, *15*, 3752-3770.
- [32] A. S. Peinetti, R. J. Lake, W. Cong, L. Cooper, Y. Wu, Y. Ma, G. T. Pawel, M. E. Toimil-Molares, C. Trautmann, L. Rong, B. Mariñas, O. Azzaroni, Y. Lu, *Sci Adv* **2021**, *7*, eabh2848.
- [33] Y. Liu, X. Wang, G. Campolo, X. Teng, L. Ying, J. B. Edel, A. P. Ivanov, *ACS Nano* **2023**, *17*, 22999-23009.
- [34] S. Cai, R. Ren, J. He, X. Wang, Z. Zhang, Z. Luo, W. Tan, Y. Korchev, J. B. Edel, A. P. Ivanov, *Nano Letters* **2023**, *23*, 11438-11446.
- [35] R. Ren, S. Cai, X. Fang, X. Wang, Z. Zhang, M. Damiani, C. Hudlerova, A. Rosa, J. Hope, N. J. Cook, P. Gorelkin, A. Erofeev, P. Novak, A. Badhan, M. Crone, P. Freemont, G. P. Taylor, L. Tang, C. Edwards, A. Shevchuk, P. Cherepanov, Z. Luo, W. Tan, Y. Korchev, A. P. Ivanov, J. B. Edel, *Nature Communications* **2023**, *14*, 7362.
- [36] C. W. Fuller, P. S. Padayatti, H. Abderrahim, L. Adamiak, N. Alagar, N. Ananthapadmanabhan, J. Baek, S. Chinni, C. Choi, K. J. Delaney, R. Dubielzig, J. Frkanec, C. Garcia, C. Gardner, D. Gebhardt, T. Geiser, Z. Gutierrez, D. A. Hall, A. P. Hodges, G. Hou, S. Jain, T. Jones, R. Lobaton, Z. Majzik, A. Marte, P. Mohan, P. Mola, P. Mudondo, J. Mullinix, T. Nguyen, F. Ollinger, S. Orr, Y. Ouyang, P. Pan, N. Park, D. Porras, K. Prabhu, C. Reese, T. Ruel, T. Sauerbrey, J. R. Sawyer, P. Sinha, J. Tu, A. G. Venkatesh, S. Vijaykumar, L. Zheng, S. Jin, J. M. Tour, G. M. Church, P. W. Mola, B. Merriman, *Proceedings of the National Academy of Sciences* **2022**, *119*, e2112812119.
- [37] aM. Zuker, *Nucleic Acids Research* **2003**, *31*, 3406-3415; bA. S. Peinetti, R. J. Lake, W. Cong, L. Cooper, Y. Wu, Y. Ma, G. T. Pawel, M. E. Toimil-Molares, C. Trautmann, L. Rong, B. Mariñas, O. Azzaroni, Y. Lu, *Science Advances* **2021**, *7*, eabh2848; cT. Jiang, L. Yi, X. Liu, A. P. Ivanov, J. B. Edel, L. Tang, *Nature Protocols* **2023**, *18*, 2579-2599.

Entry for the Table of Contents



In this work, we fabricate quantum mechanical tunnelling probes with sub-2 nm gap distance. The generator-collector mode was applied to detect that the redox cycling of free diffused $\text{Fe}(\text{CN})_6^{3-/4-}$ produced a current enhancement with great sensitivity to analyte concentration variation and voltage bias set. Therefore, functionalised quantum mechanical tunnelling probes can be applied as electrochemical tunnelling biosensors to detect target proteins in mixed samples.

Institute and/or researcher Twitter usernames: @Longyi0803 @EdelResearch @IvanovGroup @LONGHUA18