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Electrochemistry of complex molecular and biomolecular scale entities

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Structural mapping of intermediate size and large molecules and biomolecules at ultra-high resolution using single-crystal electrodes and *in situ* scanning tunnelling microscopy continues to disclose surprising findings. *In situ* scanning tunnelling spectroscopy (STS) has also recently disclosed new electrochemical conductivity features at the level of the single molecule. We overview briefly elements of this development over the last few years, with focus on three recent discoveries: (1) a new packing mode of a core monolayer target thiol, the amino acid cysteine (Cys) on Au(100)-electrodes, quite different from Cys packing on Au(111)- and Au(110)-electrodes; (2) transition of a core ferrocene *in situ* STS probe from stochastic single-molecule to macroscopic behaviour, a concept at the heart of nanoscience; (3) unexpected behaviour of the large molybdenum enzyme sulfite oxidase, when going from macroscopic to single-molecule electrochemistry. We compare these studies with other recent discoveries of single-molecule protein conductivity and molecular scale inorganic nanostructures.

Keywords: Single-entity electrochemistry; Scanning tunnelling spectroscopy; Cysteine on Au(100); Stochastic conductivity of redox molecules; Sulfite oxidase.

1 An Electrochemical and Bioelectrochemical Renaissance

A new era transforming electrochemistry into highly sophisticated condensed matter physical science began in the late 1970's, [1-3]. Core notions were "clean", atomically planar electrode surfaces and a range of spectroscopies, supported by statistical physics and electronic structure theories [4-8]. Well-defined microenvironments [9,10] paved the way for electrochemical

scanning tunnelling and atomic force microscopy (*in situ* STM and AFM), [11-14] bringing electrochemical surface mapping to atomic and sub-molecular resolution [3,13-19]. Similar boundary-traversing efforts were introduced in bioelectrochemistry where large and fragile redox metalloproteins directly in aqueous biological media can now be mapped to single-molecule resolution [3,17]. Single-molecule electrochemistry has prompted new concepts of electron transfer (ET) relating to the finite-size system nature (stochastic vs. statistical) [18], and to new single-molecule ET ***phenomena*** [17,19-21].

2. Molecular Scale Electrochemical Science

Nanoscale objects display novel size-dependent properties, with quantum mechanical phenomena such as electron tunnelling or interference, and physical laws such as the renowned Ohm's law taking new forms. Metal and semiconductor nanoparticles (NPs), and 2D and 3D graphene are non-traditional materials in electrocatalysis, new battery types, and (bio)fuel cells [22]. New metalloenzyme and DNA electrochemistry also qualify as nanoscale (bio)electrochemistry [23-29]. Single-molecule optics monitor truly single-molecule signals [28], but single-molecule electrochemistry such as *in situ* STM strictly speaking monitors successive ET events. Single-ET events may, however, be observed in collision electrochemistry, where signals from individual colliding molecular scale entities exchange electrons [30]. Single-molecule electrochemistry combined with optical processes has led to other single-event electrochemistry [28,31-34].

We address here three chosen cases of single-molecule electrochemistry, developed over the last few years: ***(1)*** Single-molecule structure and reactivity of thiol-based SAMs, with novel focus on the amino acid cysteine. ***(2)*** Single-molecule structure and function of "smarter", redox molecules that can be brought to "do something" under electrochemical control. ***(3)*** Extension of ***(2)*** to redox metalloproteins, with new focus on the intriguing molybdenum enzyme human sulfite oxidase (*h*SO). In the Conclusion Section we note some other systems that qualify as "electrochemistry of molecular scale single-entities".

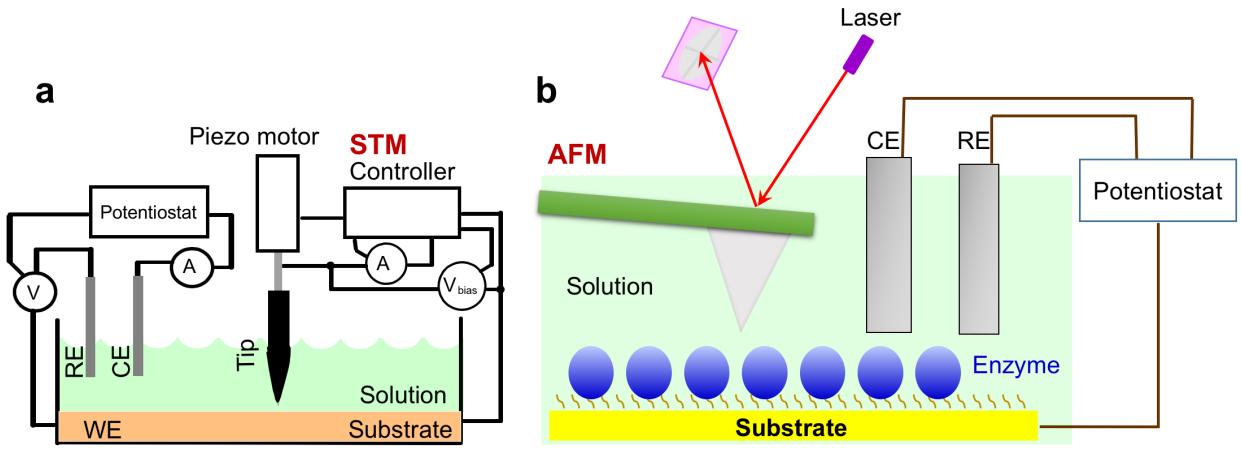

Figure 1. (a) Principal scheme of *in situ* (*in operando*) STM. The electrode/solution and the tip/electrode potentials are controlled independently relative to a common reference electrode, with the tip coated with an insulating material except at the very end. (b) Analogous electrochemical *in situ* AFM. Components not drawn to scale.

2.1 Electrochemical In Situ/Operando STM and AFM

In situ ("in operando") electrochemical STM and AFM [17,21], Figure 1, offer tunnelling current/overpotential and tunnelling current/bias voltage correlations, and are now powerful tools for mapping single-molecule structure and function of organic and inorganic molecules [2,15-17,32,35,36], and biomolecules such as redox (metallo)proteins [17,23-27]. Methodology [18,23-25,27,35] and phenomenological theory [19,21,37] of *in situ* STM of complex redox molecules are described in detail and overviewed elsewhere [17,26,32]. NP single-ET charging [33,34] and "nanoimpacts" [30] are other new electrochemical single-entity phenomena.

Following Tao's early work (1996) [35], a number of redox molecules in aqueous and ionic liquid media have been mapped to single-molecule resolution [2,14-17,23-29,32]. In redox molecules, an electronic level is electrochemically accessible, potentially imposing "hopping" character on the *in situ* STM process. Two-step "hopping", with successive ET between tip and molecule (rate constants $k_{tip,mol}$, $k_{mol,tip}$) and between molecule and electrode ($k_{electr,mol}$) [17,19-21], is represented by the current density, $i[(E-E_0);\ V_{vias}]$, or conductivity $<G> = dI[(E-E_0)]/dV_{bias}$, form

$$i\left[\left(E-E_0\right);V_{bias}\right]=en\frac{k_{tip,mol}k_{electr,mol}}{k_{mol,tip}+k_{electr,mol}} \qquad (1)$$

E is the substrate potential, E_0 the equilibrium potential, V_{bias} the bias voltage, e the electronic charge, and n a coarse-grained measure of the number of electrons transferred in a single two-step

in situ STM event [17,21]. This form gives a peak in the *i[(E-E$_0$)]* correlation, observed by a variety of complex molecules, but with noted challenges. Transition metal complexes and metalloproteins show for example significantly higher single-molecule conductivity compared to what is expected from the macroscopic electrochemical rate constant [3,17,26]. Solvent "freezing", resonance effects in transitions between superexchange and "hopping", and strong, adiabatic contact between target molecule and enclosing electrodes are possible clues [37,38]. The latter would be represented by large values of the parameter n ($n >> 1$), also recast as the broadening factor of Anderson-Newns theory, Δ [17, 37]. n is determined by the electronic coupling between the target redox molecule and the enclosing electrodes [17,21,38]. Notably therefore, the electronic coupling factor appears explicitly in the *in situ* STM tunneling current form, also in the adiabatic limit of strong electronic coupling.

A different, sigmoidal Nernstian-like correlation has also been observed [18],

$$< G >= G_{red} + \frac{1}{1+\exp\left[\frac{e}{k_B T}\left(E - E_0\right)\right]}\left(G_{ox} - G_{red}\right) \qquad (2)$$

where G_{ox} and G_{red} are the conductivities of the oxidized and reduced form respectively, k_B Boltzmann's constant, and T the temperature. This form will be in our focus below.

3 Imaging and Packing of Non-redox Molecules – Thiol-based SAMs

3.1 The Au-S Bond in Electrochemical thiol-based SAMs

Au-surfaces and AuNPs are widely used in heterogeneous catalysis and electrocatalysis, and in medical diagnostics [39, 40]. AuNPs must be protected by SAMs to prevent clogging, and SAMs can tailor Au-surfaces for catalytic and other purposes. Functionalized thiols, R-SH have come to an almost "iconic" status as Au-protecting/functionalizing SAMs. The electronically "soft" -S• ligand binds well to the "soft" Au-surface in a strong Au(0)-S(0) van der Waals unit [41-43]

$$Au(0) + HS(-I)\text{-}R \rightleftarrows Au(0)\text{-}\bullet S(0)\text{-}R + \tfrac{1}{2}H_2 \qquad (3)$$

The reverse process is reductive desorption, associated with sharp voltammetric peak(s). The R-residues can be hydrophobic, hydrophilic, electrostatically charged or neutral, and structurally

large or small. Au-thiyl SAM structures are determined not only by the Au-S bond, but also by lateral interactions, the solvent and electrolytes, and by the atomic structure of the metallic (gold) substrate itself [44,45]. Single-crystal electrochemistry, surface spectroscopies, and *in situ* STM, with strong computational support, have led to high-resolution mapping ranging from the single, chiral or achiral molecule to whole, also chiral or achiral thiol SAM domains, and the whole SAM formation process [43]. We discuss here a particular thiol target, cysteine, the only natural amino acid with a free thiol side group. Recent data have added novel insight in the multifarious ways this metabolically important biomolecule binds to Au-electrode surfaces, Figure 2 [45].

3.2 Cysteine Packing on Au(111)-, Au(110)- and Au(100)-electrode Surfaces

Cys on Au(111) forms highly ordered monolayers with (√3 × √3)R30°, (3√3 × 6)R30°, or (4 × √7)R19° cluster-like networks depending on electrolyte and pH, but a c(2×2) SAM on Au(110)-surfaces, Figure 2 [44,45]. Cys on Au(100)-electrode surfaces shows a SAM pattern further quite different from Cys on Au(111) and Au(110), Figure 2. Novel observations were:

- Two voltammetric reductive desorption peaks testify to ***two*** Cys Au-S binding modes. This is contrary to Cys on Au(111)- and Au(110)- surfaces, for which only a single peak is apparent, but accords with Surface Plasmon (SP) data [46] and DFT computations [45,47,48].
- *In situ* STM discloses "stripe-like" packing, as opposed to two-dimensional Cys networks on Au(111) and Au(110) [44,45]. This is intriguing in view of the feature-less Au(100) structure compared to the Au(110) missing row structure. The SAM matches two unit cells, (11 × 2)-2Cys and (7 × 2)-2Cys, according with two different Cys orientations or a double-SAM, as suggested by SPS.
- As opposed to all previous *in situ* STM studies of Cys on Au-electrode surfaces, both L- and D-enantiomers as well as racemic L/D were mapped. Separate L- and D-enantiomer voltammetry and *in situ* STM were virtually indistinguishable, whereas racemic L/D showed a number of identified irregularities. Similar differences were reported for the chiral R- and S-2-butane thiols [49].
- The time evolution of the striped layer formation was followed by a novel Monte Carlo approach [45], and the controlling physical forces identified, bearing lines to earlier studies of the dynamics of cysteamine [50] and homocysteine SAMs [51] on Au(111).

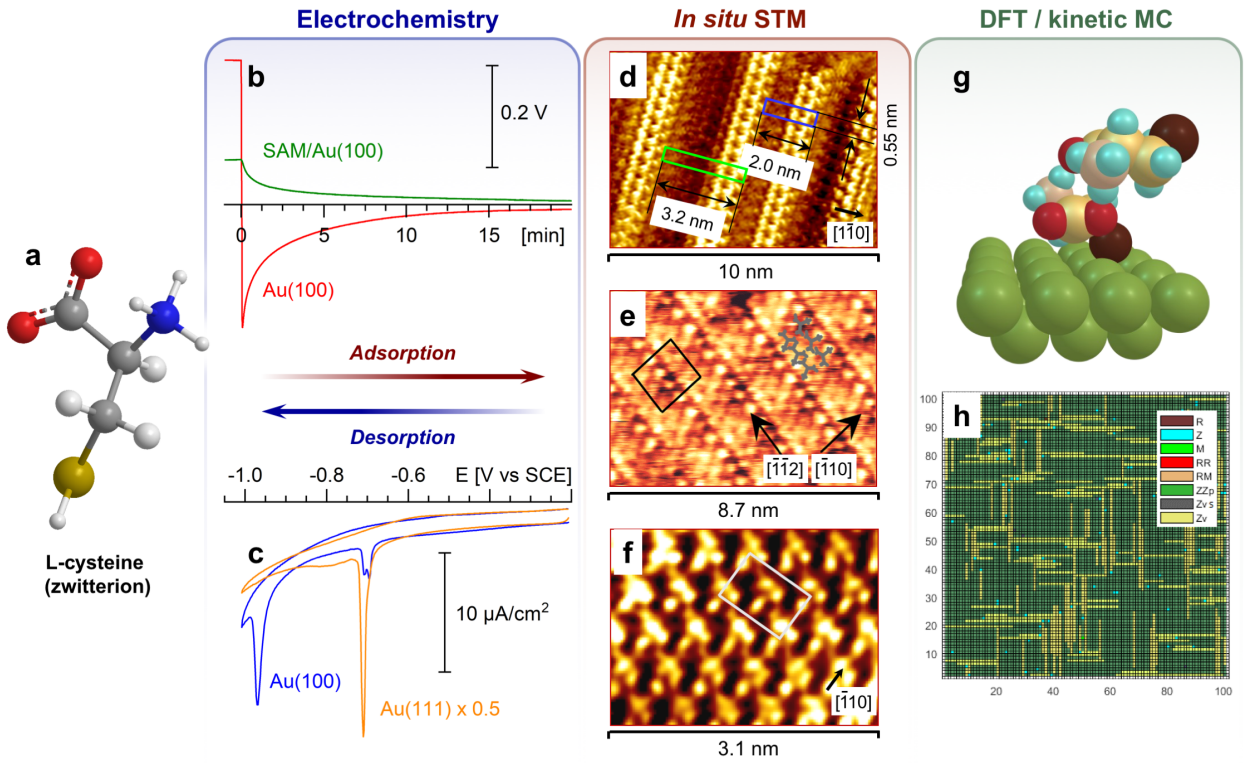

Figure 2. Overview of Cys SAM patterns on electrochemical Au(100)-, Au(111)- and Au(110)- electrode surfaces. (a) Molecular structure of the dominating zwitterionic Cys form. (b) Chronoamperometric traces following Cys adsorption on Au(100)-electrode surfaces; (c) Reductive Cys desorption from Au(100) with ***two*** peaks and from Au(111) with ***a single peak***. High-resolution Cys *in situ* STM images on Au(100)- (d), Au(111)- (e), and Au(110)-electrode surface (f). Single-molecule and even sub-molecular resolution ((Au(110)) is apparent. (g) Optimized DFT structure of zwitterionic Cys on Au(100)-electrochemical electrode and (h) Monte Carlo illustration of the time evolution of Cys adsorption on the electrochemical Au(100) surface. Data from Refs.[44,45,47,48].

Altogether the Cys SAM structure and formation dynamics on Au(100) thus testify to continued prodigious importance and new discoveries in Au-S based single-molecule electrochemistry.

4 Single-molecule Electrochemical Function of "Smart" Non-biological Molecules

Tao's study of Fe-protoporphyrin IX reported the first *in situ* STS $j_{tunn}/(E\text{-}E_0)$ correlation with a maximum around the equilibrium potential [35]. Single-molecule reports of other organic and inorganic redox molecules, with up to two orders of magnitude on-off current ratios have followed as noted [17,32,36]. Other single-molecule issues are reported over the last few years, for example: Mechanical effects on the conductivity [52]; the solvent and the electrochemical double layer in non-traditional micro-environments [53]; comparison between aqueous and ionic liquid media [32,53]; quantum interference [54,55]; resonance effects [56] (cf. early report [57]); porphyrin metal complexes in catalytic action [58,59]; and stochastic effects [18]. Recent reports on multi-electronic level polyoxometalates [60] add other single-molecule mechanistic aspects.

In a quite recent study, Li, Tao, and associates introduced a novel approach to stochastic vs. bulk molecular conductivity [18]. The target system was ferrocene in acetonitrile solution covalently linked to Au(111) and a Au-tip by variable-length alkanethiols, Figure 3. The study reports single-molecule *in situ* STS, the average of which displays a sigmoidal pattern as for viologen in aqueous solution [32], and the polyoxometalate in ionic liquid [60]. Nernstian-like equilibrium prevails for the averaged conductivity of 3C-Fc and 8C-Fc traces, but the single-molecule traces showed subtle tunnelling current fluctuations between low and high single-molecule conductivity ("telegraphic noise") beginning close to the equilibrium potential. The slower the interfacial rate process, the stronger the fluctuations. The study thus illuminates strikingly transition from stochastic single-molecule to macroscopic behaviour, and may offer clues to bridging the limits represented by eq.(1) and eq.(2).

5 Redox metalloproteins and metalloenzymes

5.1 Single-molecule Bioelectrochemistry of Simple Redox Metalloproteins

Single-molecule *in situ* STM was early brought to include large biomolecules, with heme-containing horse heart cytochrome *c* [61] and horseradish peroxidase [62] in focus. What has later become a "paradigmatic" *in situ* STM metalloprotein, the blue copper protein azurin (*Pseudomonas aeruginosa*) was introduced slightly later [23,63,64], followed by studies of protein immobilization, single-crystal electrochemistry, and *in situ* STM/STS [23,24,64]. Bacterial cytochrome (cyt) b_{562} is another well-characterized single-molecule *in situ* STM/STS electrochemical redox metalloprotein target [25].

5.2 Electrochemical STM and AFM of Metalloenzymes, and Some "Puzzles"

Other single-molecule proteins and protein/DNA complexes have been studied and reviewed [26,27,65-67]. The blue copper ***enzymes***, laccase (Lc) and nitrite reductase (CuNir) have been focus enzymes. A common observation is that metalloenzymes show no voltammetry of their own, but once substrate, say dioxygen or nitrite is let in, strong catalytic signals appear. In CuNiR and Lc electrons are let in at the type 1 Cu centre, and out towards dioxygen or nitrite reduction at the Type 2/3 or Type 2 centre. This requires a conductivity channel between the two centres, where CuNiR and Lc offer a single-molecule clue. A direct link between the Cu-ligands connect the Type 1 and Type 2 or type 2/3 centres. *In situ* STM of CuNiR and Lc both give single-molecule resolution, but only when NO_2^- (O_2) is present, as if NO_2^- (O_2) indeed triggers single-molecule

conductivity from a resting to an active state. A related intramolecular ET pattern is displayed by the "asymmetric" voltammetry of the two-centre heme protein cytochrome c_4 [68]. We refer to recent reviews [17,26,64,67] and address instead a different class of metalloenzyme targets, the sulfite oxidases (SOs) [69], which are novel in single-molecule enzyme electrochemistry.

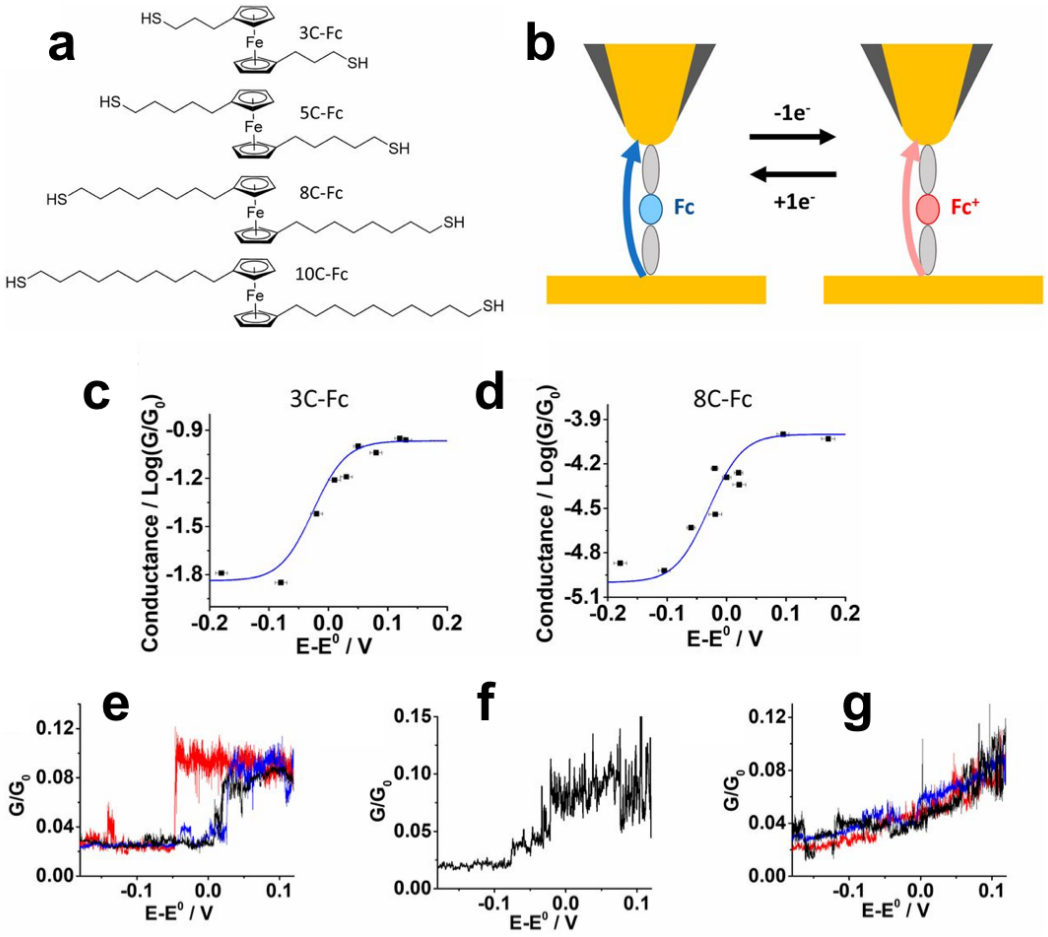

Figure 3. (a) Ferrocene target compounds; (b) Scheme of charge transport through a ferrocene molecule. (c) Averaged conductance vs. overpotential, 3-Carbon and (d) 8-Carbon linker. The blue curves are Nernstian fittings. (e-g) Single-molecule conductance vs. potential for 3-Carbon-Fc. Different fluctuation patterns around the equilibrium potential: (e) Discrete two level, (f) intermediate, and (g) continuous variations of conductance with potential. Red, blue and black colors represent different potential sweeps. Adapted from [18].

5.3 A Novel Bioelectrochemical Single-molecule Enzyme Player –Human Sulfite Oxidase

The SOs are dimeric metalloenzymes with a catalytic molybdenum cofactor (Moco) and a cytochrome b_5 domain, separated by a flexible ten-residue polypeptide, Figure 4. Electrons liberated by sulphite oxidation at the Moco centre are relayed by heme b_5 to an external electron acceptor or a solid electrode [70-72]. Conformational gating between an open and a closed

conformation is a core step (Figure 4b). Electrochemistry of SO from chicken (*c*SO) and humans (*h*SO) have been extensively reported and the mechanism mapped in detail.

*h*SO on Au(111)-electrode surfaces modified by an ω-amino octanethiol (AOT) SAM gives attractive electrocatalytic voltammograms [69]. *h*SO also offers single-molecule *in situ* STM challenges: ***(a)*** Moco reflects the complex chemical properties of molybdenum with several oxidation states, and high electronic density expectedly suitable for *in situ* STM; ***(b)*** as for the blue copper enzymes, the substrate is a small molecule, sulfite. Binding is not structurally detectable by STM, but can trigger electronic changes in the enzyme reflected in the *in situ* STM/STS patterns; ***(c)*** The conformational change between a "closed" and an "open" state, triggered by sulfite offers new *in situ* STM/STS challenges.

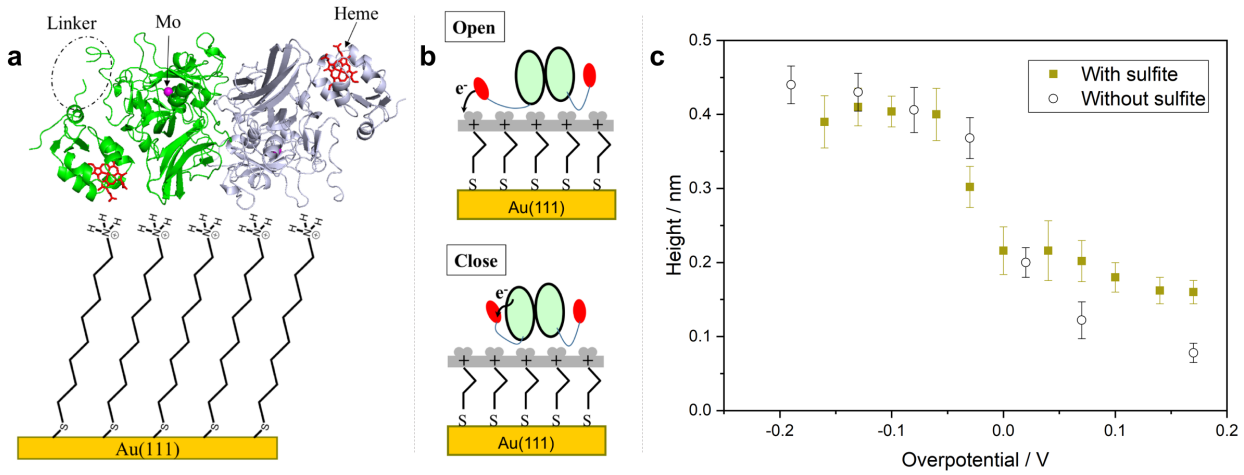

Figure 4 (a) Schematic view of *h*SO binding on electrode surface modified by a positively charged SAM (PDB: 1SOX). (b) Schematic view of switch between open and closed *h*SO conformations of electrochemical surface bound *h*SO. (c) Apparent height variation with the overpotential of *h*SO on AOT SAM modified Au(111)-electrode surfaces in the absence and presence of 5 mM sulfite. Details in [69].

In situ STM discloses a 2-5 % coverage of potential dependent molecular scale strong contrasts on the AOT SAM, with a clear transition from a low-conduction state at high potentials to a high-conduction state at low potentials. *In situ* STM/STS of *h*SO differs in two major respects from the Cu-enzymes [65-67] and the simpler ET copper protein azurin [17,26,67] by sigmoidal *in situ* STS, indicative of either Nernstian equilibration [18] or "gated" tunnelling [73]. The "flip-flop" mechanism could favour the latter. A third rationale could be the large bias voltage needed (-0.4 V). A mechanism of coherent, multi-ET would then prevail in an intermediate overpotential range, before tunnelling current decline sets in at high negative overpotentials [74]. A second observation is that *in situ* STM/STS in the absence and presence of sulfite is almost indistinguishable. This

suggests that single-molecule enzyme activity is not directly monitored as for the Cu-enzymes, but a tunnelling process through one or both of the enzyme domains, independently of sulfite.
The electronic origin of the *in situ* STM/STS contrasts remains an issue. The contrast resolution is not presently at a level, where the two domains can be distinguished, and the midpoint potentials of the two centres are too close, -173 and -193 mV, vs. SCE, respectively [69] for *in situ* STS to enable resolution into separate heme b_5 and Moco contributions. The *in situ* STS profile therefore reflects a composite process, in which both Moco and heme b_5 may be involved. Tunnelling current/bias voltage correlations and recording of current rectification [75] may here offer clues.

6 Some Concluding Notes and Perspectives

New conceptual, theoretical, and experimental notions in high-resolution electrochemistry of complex molecules seem to be underway:
Conceptual and theoretical challenges. Issues relating to the "elementary" *in operando* STM process presently are: coupling of the target molecule to the electrodes; the stochastic nature of small molecular assemblies; and, the molecular scale solvent environment. Challenges relating to strong-coupling adiabatic electrochemical ET are conveniently addressed by Anderson-Newns formalism [17,37]. Strong electronic overlap appears at first solely as a lowered activation free energy, but adiabatic ET extends to other properties including long-range ET across SAMs, and further to magnetic properties, say in dioxygen reduction, or transition metal complexes involving spin changes. To this adds the need for large-scale theoretical efforts in solvation dynamics.
Non-traditional molecular scale chemical target systems. Au-, Pt- and core-shell NPs, used biomedically, in catalysis, electrochemical sensing, and in fuel cells are prospective novel molecular scale single-entity targets [39,40]. When catalytic reactant, product, and reaction intermediates are adsorbed on the NPs, it is expectable that surface electronic structures can invoke catalytic effects, but PdNPs, PtNPs and AuNPs also catalyse simple (bio)electrochemical ET processes, presently in strong focus [40,59,76,77]. Along this line, nanoporous metals, say nanoporous gold (NPG) are in focus as new electrode materials with interesting properties [78].
Overviews of carbon materials are prodigiously available [22,79,80]. Even pristine graphene is electrochemically attractive due to unique electronic properties (the Dirac point, near linear ascending and descending DOS around the Fermi level). Complex ("crumbled") 2D and 3D graphene, and graphene doping offer theoretical and *in situ* STM challenges with catalytic properties attributed to defects, the catalytic role of which is, however, presently poorly understood

[79,81]. Entirely new electrochemical materials, e.g. layered perovskites [82], metal-organic-frameworks ("MOFs") [83], and molecular magnetic materials [84], "smarter" than graphene are, finally underway and could be probed, using spintronics notions and magnetic STM/AFM.

Prospective challenges and single-biomolecule systems. The confined *in situ* STM space poses recognized challenges. The molecular target and electrolyte ionic dimensions are comparable to the tunnelling gap width, and common notions such as Gouy-Chapman length need new approaches [85], and confinement can impose "freezing" of solvent configurational fluctuations, viewed as tunnelling barrier indentations or temporary sites for electron hopping.

In new single-molecule conductivity studies of redox and non-redox proteins, Lindsay and associates [86,87], and Gorostizas and associates [88] offered evidence that single-molecule and inter-protein *in situ* STM based ET can extend to longer distances with slower distance fall-off than according with common views of "long-range" ET. The actual distances in question, up to 10 nm are, however, in fact comparable to the dimensions of, say two contacting protein molecules or a single large protein complex including hydration layers. Transferred to the electrochemical interface, use of the powerful quantum molecular dynamics methodology successful in the mapping long-range proton-coupled ET in other proteins might therefore be warranted [89].

The electrochemical interface is complex, non-uniform, and anisotropic. Comparison between the reviews of Schmickler [90] (1996) and of Magnussen and Gross [91] (2019) illuminates development in this understanding. With this background, we discussed three single-molecule systems relating to different aspects of recent molecular and biomolecular single-molecule electrochemistry: ***(1)*** Cysteine adsorption on the three low-index Au-electrode surfaces, showing an unusual Cys pattern on Au(100), rationalized by DFT and Monte Carlo computations; ***(2)*** stochastic *in situ* STS, disclosing a bridge between single-molecule and macroscopic behaviour as a core nanoscience concept; ***(3)*** the first *in situ* STM/STS of a large molybdenum enzyme, *h*SO. The three systems each offer entirely new electrochemical molecular scale insight, perhaps extendable to molecular electronics concepts, and warranting new theoretical efforts.

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References

- Paper of particular interest

• • Paper of outstanding interest

[1] Furtak TE, Kliewer KL, Lynch DW, Eds.: **Non-traditional Approaches to the Study of the Solid-electrolyte Interface**. *Surf Sci* 1980, **101**:1-681.

[2] Kolb DM: **Electrochemical Surface Science**. *Angew Chem Int Ed* 2001, **40**:1162-1181.

• Although now of some age, this paper has maintained a status as a pioneering overview of the new electrochemistry.

[3] Zhang J, Albrecht T, Chi Q, Kuznetsov AM, Ulstrup J: *Charge Transfer and Interfacial Bioelectrochemistry at the Nanoscale and Single-molecule Levels*. Bioinorganic Electrochemistry, Dordrecht: Springer; 2008:249-302.

[4] Carnie SL, Torrie GM: **The Statistical Mechanics of the Electrical Double-Layer**. *Adv Chem Phys* 1984, **56**:141-253.

[5] Henderson D, Blum L: **A Simple Theory of the Electric Double-layer Including Solvent Effects**. *J Electroanal Chem* 1982, **132**:1-13.

[6] Schmickler W, Henderson D: **New Models for the Structure of the Electrochemical Interface**. *Progr Surf Sci* 1986, **22**:323-419.

[7] Kornyshev AA: *Solvation of a Metal Surface*. In: The Chemical Physics of Solvation. Part C. Solvation Phenomena in Specific Physical, Chemical and Biological Systems, Dogonadze RR, Kálmán E, Kornyshev AA, Ulstrup J: Eds. Amsterdam: Elsevier, Inc; 1988:355-400.

[8] Schmickler W, Guidelli R: **The Partial Charge Transfer**. *Electrochim Acta* 2014, **129**:489-505.

[9] Hubbard AT: **Electrochemistry of Well-defined Surfaces**. *Acc Chem Res* 1980, **13**:177-184.

[10] Hamelin A: **Cyclic Voltammetry at Gold Single-crystal Surfaces. Part 1. Behaviour at Low-index Faces**. *J Electroanal Chem* 1996, **407**:1-11.

[11] Wiechers J, Twomey T, Kolb DM, Behm RJ: **An In Situ Scanning Tunneling Microscopy Study of Au(111) with Atomic Scale Resolution**. *J Electroanal Chem* 1988, **248**:451-460.

[12] Kolb DM, Nichols RJ, Behm RJ: *The Application of Scanning Tunnelling Microscopy to Electrochemistry*. In: Guidelli R: Ed. Electrified Interfaces in Physics, Chemistry and Biology, NATO Adv. Sci. Inst. Series C, Mathematical and Physical Sciences; 1992, 355:275-292.

[13] Gewirth AA, Niece BK, **Electrochemical Applications of In Situ Scanning Probe Microscopy**. *Chem Rev* 1997, **97**:1129-1162.

[14] Chen CJ: *Introduction to Scanning Tunneling Microscopy*, 2nd Ed. Oxford: Oxford Science Publications; 2007.

[15] Yoshimoto S, Itaya K: **Adsorption and Assembly of Ions and Organic Molecules at Electrochemical Interfaces: Nanoscale Aspects**. *Ann Rev Anal Chem* 2013, **6**:213-235.

[16] Dretschkow T, Dakkouri AS, Wandlowski T: **In-Situ Scanning Tunneling Microscopy Study of Uracil on Au(111) and Au(100)**. *Langmuir* 1997, **13**:2843-2856.

[17] Zhang J, Kuznetsov AM, Medvedev IG, Chi Q, Albrecht T, Jensen PS, Ulstrup J: **Single-molecule Electron Transfer in Electrochemical Environments**. *Chem Rev* 2008, **108**:2737-2791.

• Detailed overview of electrochemical electron transfer and *in situ* STM/STS of single molecules and biomolecules

[18] Li Y, Wang H, Wang Z, Qiao Y, Ulstrup J, Chen H-Y, Zhou G, Tao N: **Transition from Stochastic Events to Deterministic Ensemble Average in Electron Transfer Reactions Revealed by Single-molecule conductance measurement**. *Proc Nat Acad Sci USA* 2019, **116**:3407-3412.

• • The first quantitative study of the transition from single-molecule stochastic to macroscopic behaviour of a redox molecule in scanning tunneling spectroscopy.

[19] Kuznetsov AM, Ulstrup J: **Dissipative Relaxation of a Low-energy Intermediate Electronic State in Three-Level Electron Transfer**. *Chem Phys* 1991, **157**:25-33.

[20] Kuznetsov AM, Sommer-Larsen P, Ulstrup J: **Resonance and Environmental Fluctuation Effects in STM Currents through Large Adsorbed Molecules**. *Surf Sci* 1992, **275**:52-64.

[21] Kuznetsov AM, Ulstrup J: **Mechanisms of In Situ Scanning Tunnelling Microscopy of Organized Redox Molecular Assemblies**. *J. Phys. Chem. A* 2000, **104**: 11531-11540. Errata: *J Phys Chem A* 2001, **105**:7494-7494.

[22] Tang J, Yan X, Engelbrekt C, Ulstrup J, Magner E, Xiao X, Zhang J: **Development of Graphene-based**

Enzymatic Biofuel Cells: A Minireview. *Bioelectrochemistry* 2020, **134**:107537.

[23] Chi Q, Zhang J, Nielsen JU, Friis EP, Chorkendorff I, Canters GW, Andersen JET, Ulstrup J: **Molecular Monolayers and Interfacial Electron Transfer of Pseudomonas Aeruginosa Azurin on Au(111)**. *J Am Chem Soc* 2000, **122**:4047-4055.

[24] Alessandrini A, Gerunda M, Canters GW, Verbeet MP, Facci P: **Electron Tunnelling through Azurin is Mediated by the Active Site Cu ion**. *Chem Phys Lett* 2003, **376**:625-630.

[25] Della Pia EA, Chi Q, Macdonald JE, Ulstrup J, Jones DD, Elliott M: **Fast Electron Transfer through a Single Molecule Natively Structured Redox Protein**. *Nanoscale* 2012, **4**:7106-7113.

[26] Zhang J, Chi Q, Hansen AG, Jensen PS, Salvatore P, Ulstrup J: **Interfacial Electrochemical Electron Transfer in Biology-towards the Level of the Single Molecule**. *FEBS Letters* 2012, **586**:526-535.

[27] Lopez-Martinez M, Artés JM, Sarasso V, Carminati M, Diez-Perez I, Sanz F, Gorostiza P: **Differential Electrochemical Conductance Imaging at the Nanoscale**. *Small* 2017, **13**:1700958.

[28] Mathwig K, Aartsma TJ, Canters GW, Lemay SG: **Nanoscale Methods for Single-molecule Electrochemistry**. *Ann Rev Anal Chem* 2014, **7**:383-404.

[29] Albrecht T, Horswell S, Allerston LK, Rees NV, Rodroguez P: **Electrochemical Processes at the Nanoscale**. *Curr Opin Electrochem* 2018, **7**:138-145.

[30] Sokolov SV, Eloul S, Kätelhön E, Batchelor-McAuley C, Compton RG: **Electrode-Particle Impacts: A Users Guide**. *Phys Chem Chem Phys* 2017, **19**:28-43.

- A vivid overview of the new electrochemical single-electron technique based on nanoscale entity productive collisions with electrochemical electrode surfaces.

[31] Fan Y, Anderson TJ, Zhang B: **Single-molecule Electrochemistry: From Redox Cycling to Single Redox Events**. *Curr Opin Electrochem* 2018, **7**:81-86.

[32] Nichols R J, Higgins SJ: **Single Molecule Nanoelectrochemistry in Electrical Junctions**. *Acc Chem Res* 2016, **49**:2640-2648.

- Detailed overview of electrochemistry and single-molecule *in situ* STM/STS of redox molecules.

[33] Chen S, Murray RW Feldberg SW, **Quantized Capacitance Charging of Monolayer-protected Gold Clusters**. *J Phys Chem B* 1998, **102**:9898-9907.

[34] Albrecht T, Mertens SFL, Ulstrup J: **Intrinsic Multi-state Switching of Gold Clusters through Electrochemical Gating**. *J Am Chem Soc* 2007, **129**:9162-9167.

[35] Tao N: **Probing Potential-tuned Resonant Tunneling through Redox Molecules with Scanning Tunneling Microscopy**. *Phys Rev Lett* 1996, **76**:4066-4069.

- Still of pioneering status as the first report of electrochemical single-molecule *in situ* STS.

[36] Petrangolini P, Alessandrini A, Berti L, Facci P: **An Electrochemical Tunneling Microscopy Study of 2-(6-Mercaptoalkyl)hydroquinone Molecules on Au(111)**. *J Am Chem Soc* 2010, **132**:7445-7453.

[37] Medvedev IG: **Analytical Expression for the Tunnel Current through the Redox-mediated Tunnel Contact in the Case of the Adiabatic Electron Transfer at One of the Working Electrodes and Any Possible Type of the Electron Transfer at the Other Electrode**. *J Chem Phys* 2017, **124**:194108.

[38] Nazmutdinov RR, Ulstrup J: *Retrospective and Prospective Views of Electrochemical Electron Transfer Processes: Theory and Computations*. Book chapter in: Atomic-Scale Modelling of Electrochemical Systems. Laasonen K, Tomi Laurila T, Melander: Eds, John Wiley & Sons, Inc; 2020.

[39] Louis C, Pluchery O: *Gold Nanoparticles for Physics, Chemistry and Biology*. London: World Scientific Publishing Europe, Inc; 2012.

[40] Shermukhamedov S, Nazmutdinov RR, Zinkicheva TT, Bronshtein MD, Zhang J, Mao B, Tian ZQ, Yan J, Wu DY, Ulstrup J: **Electronic Spillover from a Metallic Nanoparticle: Can Simple Electrochemical Electron Transfer processes be Catalyzed by Electronic Coupling of a Molecular Scale Gold Nanoparticle Simultaneously to the Redox Molecule and the Electrode**. *J Am Chem Soc* 2020, **142**:10646-10658.

- A new theoretical and computational approach to the possible role of single molecular scale gold nanoparticles in the catalysis of simple electrochemical electron transfer processes.

[41] Chi Q, Ford MJ, Halder A, Hush NS, Reimers JR, Ulstrup J: **Sulfur Ligand Mediated Electrochemistry of Gold surfaces and Nanoparticles: What, How, and Why?**. *Curr Opin Electrochem* 2017, **1**:7-15.

[42] Reimers JR, Ford MJ, Halder A, Ulstrup J, Hush NS: **Gold Surfaces and Nanoparticles are Protected by Au(0)-thiyl Species and Are Destroyed when Au(I)-thiolates Form**. *Proc Nat Acad Sci USA* 2016, **113**:E1424-E1433.

- Paper unique in describing in great theoretical detail the Au-S bonding and electronic structure.

[43] Inkpen MS, Liu Z, Li H, Campos LM, Neaton JB, Venkataraman L: **Non-chemisorbed Gold-sulfur Binding Prevails in Self-assembled Monolayers**. *Nat Chem* 2019, **11**:351-358.

- Detailed experimental documentation of non-covalent binding in thiol adsorption on Au-electrode surfaces.

[44] Zhang J, Welinder AC, Chi Q, Ulstrup J: **Electrochemically Controlled Self-assembled Monolayers Characterized with Molecular and Sub-molecular Resolution**. *Phys Chem Chem Phys* 2011, **12**:9999-10011.

[45] Engelbrekt C, Nazmutdinov RR, Zinkicheva TT, Glukhov DV, Yan J, Mao B, Ulstrup J, Zhang J: **Chemistry of Cysteine Assembly on Au(100): Electrochemistry, In Situ STM and Molecular Modeling**. *Nanoscale* 2019, **11**:17235-17251.

- The first comprehensive study of the adsorption of the amino acid cysteine on Au(100)-electrode surfaces; an entirely novel adsorption pattern disclosed, quite different from Cys adsorption on Au(111) and Au(110).

[46] Tawil N, Hatef A, Sacher E, Maisonneuve M, Gervais T, Mandeville R, Meunier M: **Surface Plasmon Resonance Determination of the Binding Mechanisms of L-Cysteine and Mercaptoundecanoic Acid on Gold**. *J Phys Chem C* 2013, **117**:6712-6718.

[47] Nazmutdinov RR, Zhang J, Zinkicheva TT, Manyurov IR, Ulstrup J: **Adsorption and In Situ Scanning Tunneling Microscopy of Cysteine on Au(111): Structure, Energy and Tunneling Contrasts**. *Langmuir* 2006, **22**:7556-7567.

[48] Zhang J, Chi Q, Nazmutdinov RR, Zinkicheva TT, Bronshtein MD: **Submolecular Electronic Mapping of Single Cysteine Molecules by In Situ Scanning Tunneling Microscopy**. *Langmuir* 2009, **25**:2232-2240.

[49] Ouyang R, Yan J, Jensen PS, Ascic E, Gan S, Tanner D, Mao B, Niu L, Zhang J, Tang C, Hush NS, Reimers JR, Ulstrup J: **Intermixed Adatom and Surface Bound Adsorbates in Regular Self-assembled Monolayers of Racemic 2-Butanethiol on Au(111)**. *ChemPhysChem* 2015, **16**:928-932.

[50] Zhang J, Bilič A, Reimers JR, Hush NS, Ulstrup J: **Co-existence of multiple conformations in Cysteamine Monolayers on Au(111)**. *J Phys Chem B* 2005, **109**:15355-15367.

[51] Zhang J, Demetriou A, Welinder AC, Albrecht T, Nichols RJ, Ulstrup J: **Potential-Induced Structural Transitions of Homocysteine Monolayers on Au(111) electrode surfaces**. *Chem Phys* 2005, **319**:210-221.

[52] Wang H, Wang ZX, Wang Y, Hihath J, Chen HI, Li YQ, Tao NJ: **Potential Dependence of Mechanical Stability and Electronic Coupling of Single S-Au Bonds**. *J Am Chem Soc* 2019, **140**:18074-18081.

[53] Liu S, Peng J, Chen L, Sebastián P, Feliu JM, Yan J, Mao B: **In-situ STM and AFM Studies of Electrochemical Interfaces in Imidazolium-based Ionic Liquids**. *Electrochim Acta* 2019, **309**:11-17.

[54] Li Y, Buerkle M, Li G, Rostamian A, Wang H, Wang Z, Bowler DR, Miyazaki T, Xiang L, Asai Y, Zhou G, Tao N: **Gate Controlling of Quantum Interference and Direct Observation of Anti-resonances in Single Molecule Charge Transport**. *Nat Mater* 2020, **19**:127-127.

[55] Naghibi S, Ismael AK, Vezzoli A, Al-Khaykanee MK, Zheng X, Grace IM, Bethell D, Higgins SJ, Lambert CJ, Nichols RJ: **Synthetic Control of Quantum Interference by Regulating Charge on a Single Atom in Heteroaromatic Molecular Junctions**. *J Phys Chem Lett* 2019, **10**:6419-6424.

[56] Zang Y, Ray S, Fung ED, Borges A, Garner MH, Steigerwald ML, Solomon GC, Patil S, Venkataraman L: **Resonant Transport in Single Diketopyrrolopyrrol Junctions**. *J Am Chem Soc* 2018, **140**:13167-13170.

[57] Kharkats YuI, Ulstrup J: **Resonance Effects in 3-Center Long-range Electron Transfer**. *Chem Phys Lett* 1991, **182**:81-87.

[58] Wang X, Cai Z, Wang D, Wan L: **Molecular Evidence for the Molecular Process of Cobalt Porphyrin Catalyzed Oxygen Evolution in Alkaline Solution**. *J Am Chem Soc* 2019, **141**:7665-7669.

[59] Herrera SE, Davia FG, Williams FJ, Calvo EJ: **Metal Nanoparticle Enhancement of Electron Transfer to Tethered Redox Centers through Self-assembled Molecular Films**. *Langmuir* 2019, **35**:6297-6303.

[60] Wu C, Qiao X, Robertson CM, Higgins SJ, Cai C, Nichols RJ, Vezzoli AA: **Chemically Soldered Polyoxometallate Single-Molecule Transistor**. *Angew Chem Int Ed* 2020, **59**:12029-12034.

- • A uniquely comprehensive experimental study of the single-molecule behaviour of a polyoxometallate in an ionic liquid based on *in situ* STS and a range of other techniques.

[61] Andersen JET, Moller P, Pedersen MV, Ulstrup J: **Cytochrome c Dynamics at Gold and Glassy Carbon Surfaces Monitored by In Situ Scanning Tunnel Microscopy**. *Surf Sci* 1995, **325**:193-205.

[62] Zhang J, Chi Q, Dong SJ, Wang E: **In situ Electrochemical Scanning Tunnelling Microscopy Investigation of Structure for Horseradish Peroxidase and its Electrocatalytic Property**. *Bioeletrochem Bioenerg* 1996, **39**:267-274.

[63] Friis EP, Andersen JET, Madsen LL, Moller P, Ulstrup J: **In Situ STM and AFM of the Copper Protein Pseudonomans Aeruginosa Azurin**. *J Electroanal Chem* 1997, **431**:35-38.

- First introduction of the electron transfer protein azurin as a "paradigmatic" *in situ* STM probe, picked up and followed by other groups.

[64] Alessandrini A, Facci P: **Electron Transfer in Nanobiodevices**. *Eur Polym J* 2016, **83**:450-466.

[65] Zhang J, Welinder AC, Hansen AG, Christensen HEM, Ulstrup J: **Catalytic Monolayer Voltammetry and In Situ Scanning Tunnelling Microscopy of Copper Nitrite Reductase on Cysteamine-modified Au(111)-Electrodes**. *J Phys Chem B* 2003, **104**:12480-12484.

[66] Climent V, Zhang J, Friis EP, Oestergaard LH, Ulstrup J: **Voltammetry and In situ Scanning Tunnelling Microscopy of Laccases and Bilirubin Oxidase in Electrocatalytic Dioxygen Reduction on Au(111) Single-crystal Electrodes**. *J Phys Chem C* 2012, **116**:1232-1243.

[67] Salvatore P, Zeng D, Karlsen KK, Chi Q, Wengel J, Ulstrup J: **Electrochemistry of Single Metalloprotein and DNA-based Molecules at Au(111)-electrode Surfaces**. *ChemPhysChem* 2013, **14**:2101-2111.

[68] Nazmutdinov RR, Bronshtein MD, Zinkicheva TT, Chi Q, Zhang J, Ulstrup J: **Modeling and Computations of the Intramolecular Electron Transfer in the Two-heme Protein Cytochrome c_4. Phys. Chem. Chem. Phys.** 2012,**14**: 5953-5965.

[69] Yan J, Froekjaer EE, Engelbrekt C, Leimkühler S, Ulstrup J, Wollenberger U, Zhang J: **Voltammetry and Single-molecule In Situ Scanning Tunnelling Microscopy of the Redox Metalloenzyme Human Sulfite Oxidase**. *ChemElectroChem* 2020, Submitted.

• The first electrochemical single-molecule approach to the intriguing class of molybdenum redox enzymes.

[70] Feng C, Tollin G, Enemark JH: **Sulfite oxidizing enzymes**. *Biochim Biophys Acta* 2007, **1774**:527-539.

[71] Frasca S, Rojas O, Salewski J, Neumann B, Stiba K, Weidinger IM, Tiersch B, Leimkuehler S, Koetz J, Wollenberger U: **Human sulfite oxidase electrochemistry on gold nanoparticles modified electrode**. *Bioelectrochemistry* 2012, **87**:33–41.

[72] Zeng T, Leimkuehler S, Wollenberger U, Fourmond V: **Transient Catalytic Voltammetry of Sulfite Oxidase Reveals Rate Limiting Conformational Changes**. *J Am Chem Soc* 2017, **139**:11559-11567.

[73] Haiss W, Albrecht T, van Zalinge H, Higgins SJ, Bethell D, Höhenreich H, Schiffrin DJ, Nichols RJ, Kuznetsov AM, Zhang J, Chi Q, Ulstrup J: **Single-molecule conductance of redox molecules in electrochemical scanning tunnelling microscopy**. *J Phys Chem B* 2007, **111**:6703-6712.

[74] Zhang J, Kuznetsov AM, Ulstrup J: **In situ scanning tunnelling microscopy of redox molecules. Coherent electron transfer at large bias voltages**. *J Electroanal Chem* 2003, **541**:133-146.

[75] Kuznetsov AM, Ulstrup J: **Mechanisms of molecular electronic rectification through electronic levels with strong vibrational coupling**. *J Chem Phys* 2002, **116**:2149-2165.

[76] Chazalviel J-N, Allongue P: **On the Origin of Efficient Nanoparticle Mediated Electron Transfer across a Self-assembled Monolayer**. *J Am Chem Soc* 2011, **133**:762-764.

[77] Kleijn SE, Lai SCS, Koper MTM, Unwin P: **Electrochemistry of Nanoparticles**. *Angew Chem Int Ed* 2014, **53**:3558-3586.

[78] Xiao X, Si P, Magner E: **An Overview of Dealloyed Nanoporous Gold in Bioelectrochemistry**. *Bioelectrochemistry* 2016, **109**:117-226.

[79] Ambrosi A, Chua CK, Bonanni A, Pumera M: **Electrochemistry of Graphene and Related Materials**. *Chem Rev* 2014, **114**:7150-7188.

[80] McCreery RL: **Advanced Carbon Materials for Molecular Electrochemistry**. *Chem Rev* 2008, **108**:2646-2687.

[81] Nazmutdinov RR, Zinkicheva TT, Shermukhamedov SA, Zhang J, Ulstrup J: **Electrochemistry of single molecules and biomolecules, molecular scale nanostructures, and low-dimensional systems-An integrated experimental, theoretical, and computational approach**. *Curr Opin Electrochem* 2018, **7**:179-187.

[82] Kamat PV, Pradhan N, Schanze K, Weiss PS, Buriak J, Stang P, Odom TW, Hartland G: **Challenges and Opportunities in Designing Perovskite Nanocrystal Heterostructures**. *ACS Energy Lett* 2020, **5**:2253-2255.

[83] Huang W, Tang J, Diao F, Engelbrekt C, Ulstrup J, Xiao X, Moelhave K: **Recent progress of 2D metal-organic frameworks and their derivatives for oxygen revolution electrocatalysis**. *ChemElectroChem* 2020, in press.

[84] Pedersen KS, Bendix J, Clerac R: **Single-molecule Magnet Engineering: Building-block Approaches**. *Chem Comm* 2014, **50**:4396-4415.

[85] Kornyshev AA,Kuznetsov AM, Ulstrup J: **In Situ Superexchange Electron Transfer through. a Single Molecule: A Rectifying Effect**. *Proc Nat Acad Sci USA* 2006, **103**:6799-6804.

[86] Zhang B, Lindsay S: **Electronic Decay Length in a Protein Molecule**. *Nano Lett* 2019, **19**:4017-4022.

[87] Zhang B, Song W, Pang P, Lai H, Chen Q, Zhang P, Lindsay S: **Role of contacts in long-range protein conductance**. *Proc Nat Acad Sci USA* 2019, **116**:5886-5891.

•• A detailed study documenting the crucial molecule/electrode importance in long-range electronic conductivity through redox and non-redox protein molecules.

[88] Lagunas A, Guerra-Castellano A, Nin-Hill A, Diaz-Moreno I, De la Rosa MA, Samitier J, Rovira C, Gorostiza P: **Long Distance Electron Transfer through the Aqueous Solution between Redox Partner Proteins**. *Nat Commun* 2018, **9**:5157.

- A single-molecule *in situ* STS study of the interaction between two protein complexes involving electronic contact through the protein hydration layers.

[89] Xu JC, Sharpe MA, Qin L, Ferguson-Miller S, Voth GA: **Storage of an Excess Proton in the Hydrogen-bonded Network of the D-pathway of Cytochrome c Oxidase: Identification of a Protonated Water Cluster**. *J Am Chem Soc* 2007, **129**:2910-2913.

[90] Schmickler W: **Electronic Effects in the Electric Double Layer**. *Chem Rev* 1996, **96**:3177-3200.

[91] Magnussen OM, Gross A: **Toward an Atomic-Scale Understanding of Electrochemical Interface Structure and Dynamics**. *J Am Chem Soc* 2019, **141**:4777-4790.