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Highlights

- Single-molecule electrochemistry of duplex and G-quadruplex oligonucleotides (OGNs)
- Electrochemical *in situ* STM of pure and redox marked duplex and G-quadruplex OGNs
- Iron protoporphyrin IX labeled 15-base 12G-quadruplex (DNAzyme) at single-crystal electrodes
- Long-range electrochemical electron transfer of redox labelled duplex OGNs and DNAzyme

ACCEPTED MANUSCRIPT

Electrochemical Single-molecule Conductivity of Duplex and Quadruplex DNA

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Photoinduced and electrochemical charge transport in DNA (oligonucleotides, OGNs) and the notions “hopping”, superexchange, polaron, and vibrationally gated charge transport have been in focus over more than two decades. In recent years mapping of electrochemical charge transport of pure and redox marked single- and double-strand OGNs has reached the single-molecule level based *i.a.* on electrochemical *in situ* scanning tunnelling microscopy (STM) and break-junction (B-J) STM. There are much fewer such reports on “non-canonical” OGN structures such as G-quadruplexes. We discuss first single-molecule electrochemical conductivity of pure and redox marked duplex OGNs, and address next electrochemistry and electrochemical conductivity in the few reported monolayer and single-molecule G-quadruplex studies. Facile electrochemical electron transfer of iron protoporphyrin IX stacked onto three-quarter 12-guanine quadruplex (“DNAzyme”) and *in situ* high-resolution molecular structures are particularly noted.

1. Introduction

Experimental and theoretical studies of electron or hole transfer, ET/HT of single (bio)molecules such as proteins¹⁻⁹ and DNA¹⁰⁻¹⁶ have reached high levels of sophistication. The ways single redox metalloprotein molecules operate are increasingly well understood, e.g. from bioelectrochemistry and electrochemical scanning tunnelling microscopy/spectroscopy (*in situ* STM/STS) directly in aqueous biological media. DNA (oligonucleotides, OGN) conductivity offers other perspectives for oxidative damage repair¹⁷⁻¹⁹ and molecular “devices”^{16,20-24}. Binding of organic redox molecules or transition metal complexes are core notions in long-range ET through the double strand^{17-19,25-28}, extended now to PNA, treated elsewhere in this Current Opinion issue, and G-quadruplexes addressed presently.

DNA conductivity rests broadly on: (1) photo-excitation, followed by hole transport along the OGN strand^{17-19,21-24}; (2) electrochemical ET of a chelator “through” surface bound OGN²⁶⁻²⁸; (3) single-molecule imaging and conductivity by STM and B-J STM^{1,10,11,15}. “Hopping” and “superexchange” (tunnelling) are two limiting mechanisms^{20,21}, with polaron conductivity²⁹ and ET/HT via dynamically populated intermediate states³⁰⁻³⁴ as other notions. Hopping involves sequential, vibrationally relaxed ET between base pairs or base pair clusters, Fig.1. The generic rate constants are^{20,33}

$$W_{hop} = \kappa \frac{\omega_{eff}}{2\pi} \exp\left(-\frac{[\lambda + \Delta G^0]^2}{4\lambda k_B T}\right); \kappa = (T_{DA})^2 \sqrt{\frac{4\pi^3}{\lambda \hbar^2 \omega_{eff}^2 k_B T}}, \text{ if } \kappa < 1, \text{ otherwise } \kappa = 1 \quad (1)$$

λ is the reorganization free energy, ΔG^0 the reaction free energy, ω_{eff} the effective vibrational frequency of the ET hop, k_B Boltzmann’s constant and T the temperature. T_{DA} is the electronic transmission coefficient κ couples the donor (D) and acceptor (A) states, commonly dominated by guanine-cytosine (GC) pairs, via intermediate adenine-thymine (AT) tunnelling “matter”^{20,21}. A coarse-grained distance (R) dependent T_{DA} form for ET/HT through M intermediate states is

$$T_{DA} \approx \frac{\beta_D \beta_A}{\gamma} \left(\frac{\gamma}{\Delta \varepsilon}\right)^M \rightarrow T_{DA} = (T_{DA}^0) \exp(-\beta_{eff} R); \beta_{eff} = \frac{1}{a} \ln \frac{\Delta \varepsilon}{\gamma} \quad (2)$$

where $\Delta \varepsilon$ is the energy gap between the donor and nearest bridge group levels, β_D and β_A coupling of the donor and acceptor to their nearest bridge groups, and γ the electronic coupling between neighbouring bridge groups of structural extension a . Large $\Delta \varepsilon$ and small γ thus give fast distance fall-off. “Superexchange”, eq.(2), means that ET/HT is transmitted via high-energy AT base pairs, without physical charge localization but by efficient off-resonance nearest neighbour coupling. Extensions of eqs.(1) and (2) have become a frame for multiphonon *in situ* STM processes and current/overpotential/bias voltage STS of single redox (bio)molecules^{2,31,32,-35}.

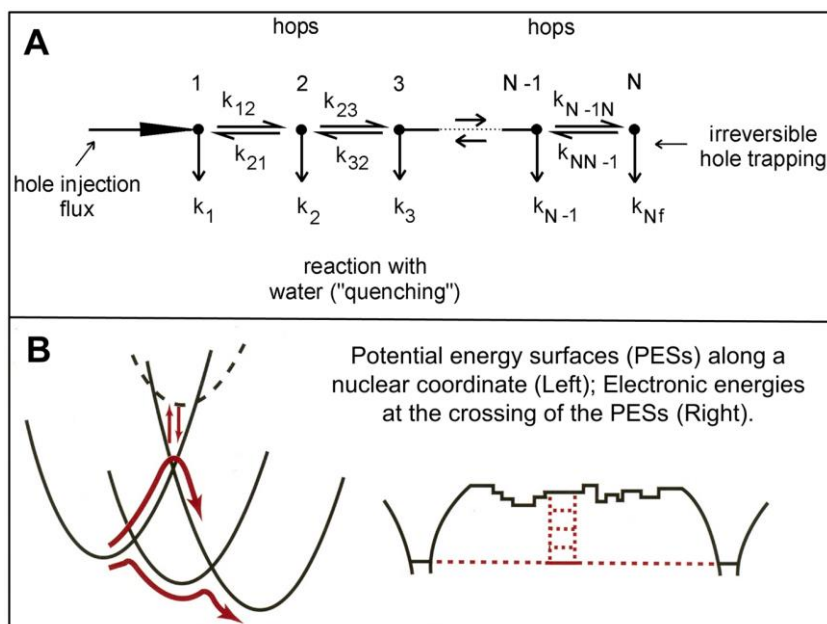


Fig. 1 Two schematic views of long-range ET/HT through OGN molecular strands. Details in text.

The two limiting mechanisms are illustrated in Fig.1. Fig.1A illustrates photo-induced ET/HT and random “hopping” of the excess charge along the strand, interrupted by “quenching” ($k_1 \dots k_{Nf}$) and eventual trapping. Fig.1B, left shows corresponding potential (free) energy surfaces spanned by a nuclear coordinate to show differences between “hopping” and superexchange via a single intermediate electronic state. “Hopping” via a temporarily populated low-lying intermediate state is represented by the fully drawn intermediate state potential surface. This state may or may not relax vibrationally prior to the subsequent ET/HT step. In “superexchange” a high-energy intermediate state (drawn by the dashed line) mediates ET/HT purely electronically, without physical electronic population. Fig.1B, right shows a corresponding electronic energy diagram spanned by the spatial coordinate(s) of the transferring electron (hole). The Coulomb-like potential wells are at resonance between the donor and acceptor states at the crossing of the initial and final potential surfaces. The potential wells drawn in dashed burgundy lines illustrate intermediate electronic energies from superexchange (high energy) to “hopping” with resonance between the three states.

Hopping has emerged as a consistent mechanism for photo-induced long-range, weakly distance dependent ET/HT (> 200 nm)¹⁷⁻²⁴ with $\tau_{cs}^N \approx (W_{hop})^{-1} N^\eta$ where τ_{cs}^N is the charge separation time, N the number of hops, and $\eta \approx 1-2$ a numerical factor^{20,21}. Electrochemical “hopping” is less obvious, as this involves electrochemical oxidation of DNA bases. Such signals have eluded identification at gold substrate electrodes most commonly used in electrochemical studies of long-range OGN ET/HT, and would be disturbed by electrode or solvent oxidation. Complex molecules are also subject to potential dependent surface reorganization reflected in capacitive signals that can resemble Faradaic signals^{36,37}. Such signals can also show weak distance dependence and high sensitivity to base pair mismatch³⁷. Polaron conductivity²⁹ is an attractive notion, as much fewer “hops” are needed, but electrochemical polaron signals have not been identified either. In the following we consider first recent single-molecule electrochemical *in situ* studies of duplex OGNs in Section 2, followed by a discussion of monolayer and single-molecule electrochemical behaviour of simple representatives of non-canonical G-quadruplexes in Section 3.

2. *In situ* STM imaging and conductivity of pure and redox marked duplex OGNs

2.1 *In situ* STM imaging

We consider first single-molecule *in situ* STM of single- and duplex-strand (ss and ds) OGNs. As for

single-molecule protein studies, *in situ* STM of DNA/OGNs holds other challenges than imaging in air/UHV^{2,15,25,27}. Solvation favours upright orientation, at potentials negative of the potential of zero charge, while target OGN molecules in air/UHV virtually always lie down on the substrate surfaces. *In situ* STM resolution of OGN in aqueous media is, however, lower than in UHV and has only recently reached the single-molecule level^{12,13,27}. Oshiro and Maeda recorded single-molecule *in situ* STM of 11 bp OGNs that testify to upright OGN orientation and notably, to different STM contrasts for ss and ds OGNs, Fig.2A.¹² Mechanistic implications were not, however, elaborated. Methylation of single-strand DNA on the Au electrode caused the conductivity of an 8 base pair OGN to decrease, enabling again ss and ds molecular scale distinction, Fig. 2B (step 3).¹³ Hansen, Salvatore, and associates used a different approach. They reported *in situ* STM of 13-20 bp duplex OGNs attached to Au(111)-surfaces by a thiol linker.^{25,27} Targets were normal and “unlocked” DNA (UNA), functionalized by terpyridine linked $\text{Ru}^{3+/2+}$, $\text{Os}^{3+/2+}$ and $\text{Fe}^{3+/2+}$ (Fig. 2C-D).²⁵ Single-molecule resolution was achieved, with significantly stronger contrasts of redox marked than of unmarked OGNs, Fig.2E reflecting different single-molecule ET/HT mechanisms, cf. below.^{25,27}

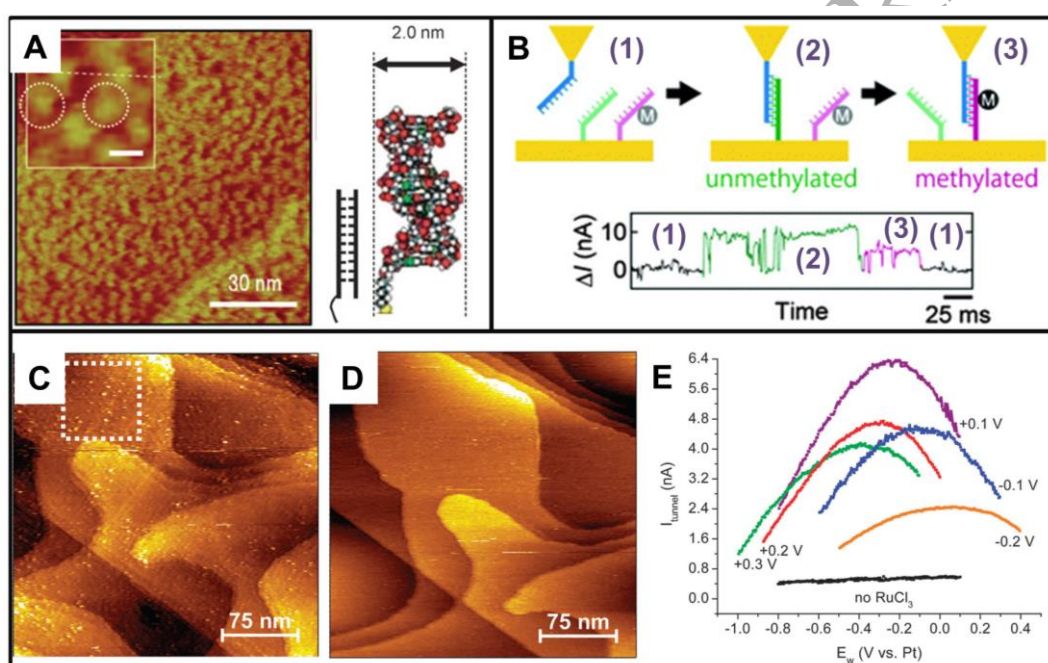


Fig. 2 (A) *In situ* STM image of 11-base ds OGN on Au-electrode surface (10 mM phosphate, pH 7.3). $E_{\text{sample}} = -0.2\text{V}$, $E_{\text{bias}} = 0.2\text{ V}$ (sample negative), $I_{\text{tunneling}} = 200\text{ pA}$. (B) Tunnelling current change of double-strand DNA by methylation in 0.1 M NaClO_4 . (C and D) *In situ* STM images of 15-base terpy-derivatized ds-UNA exposed to Ru(III) . $E_{\text{bias}} = 0.2\text{ V}$. $E_{\text{sample}} = -0.2\text{V}$ (C) and -0.6 V (D). (E) $I_{\text{tunneling}}$ vs. E_{sample} at fixed E_{bias} . 10 mM phosphate, pH 7.0). From Refs.^{12,13,27} with permission.

2.2 *In situ* electrochemical single-molecule conductivity of pure and redox marked duplex OGNs

In situ single-molecule DNA/OGN studies have focused on conductivity rather than imaging. Single-molecule visualization is appealing, but conductivity a more direct representation of biological single-molecule action, even though conductivity and STM/cAFM imaging are physically closely related. Tao pioneered *in situ* STM of redox molecules³⁸. In many recent reports, using STM (B-J) techniques supported by quantum chemical computations and transport calculations³⁹, Tao and associates have mapped electronic conductivity of ss and ds OGNs of variable length and composition. These studies, followed by studies by Diéz-Pérez, Hihath, and associates^{10,11,15,40,41} offer recent examples. The high data accuracy offers insight at several levels. Exponential distance decay was, for example observed but the decay factors, β_{eff} assume values ranging from 0.6-0.7 \AA^{-1} characteristic of tunnelling (superexchange) to much smaller values, characteristic of hopping or gating, cf. discussion in Section 1. The distance variation thus depends sensitively on base

composition, stacking, and other structural details. Periodic oscillations in the conductivity/distance correlation¹¹ were other observations. The parameters $\Delta\varepsilon$ and γ in the coarse-grained distance relation eq.(2) in fact already depend on the distance and base composition, itself a source of oscillatory behaviour, particularly for small values of the $\gamma/\Delta\varepsilon$ ratio. The oscillatory behaviour was framed by the Büttiker theory of “partially coherent” charge transfer with “incoherent corrections”³⁹. In other terms such notions relate to vibrationally activated multi-electron transfer in molecular junctions that prevails for strong electronic coupling of the target molecule to the enclosing electrodes^{2,8,34,35}. The multifarious single-molecule electrochemical OGN conductivity behaviour thus supports base pair specific behaviour further sensitive to temperature and electrolyte composition.

In several studies, Kawai, Ohshiro and associates, Nishino and associates, and Majima and associates reported single-molecule *in situ* conductivity of pure and chemically modified OGNs²²⁻²⁴. Core notions were functionalized probing tips, and tunnelling current-distance and current-time recording as introduced by Nichols, Haiss, and associates⁴². The data included single-molecule mapping of ss/ds hybridization, and mismatch (polymorphism) detection. Together with the comprehensive studies of Tao and associates, these recent results have further lifted single-molecule DNA science. Transition metal binding to immobilized OGNs offers other current/overpotential/bias voltage STS, with strong signals particularly from Ru-bound OGNs²⁷. The STS correlations showed systematically the multiphonon two-step hopping STS characteristics, Fig.2E, based on the views represented by eqs.(1) and (2) and by Fig.1B, lower left, with spectral features around the equilibrium potential but broadened by Ru-binding to multiple binding sites. Additional “hopping” conductivity channels along the bound Ru-sites are therefore envisaged.

3. Electrochemistry, *in situ* STM imaging and conductivity of DNA G-quadruplexes

3.1 Why electrochemical single-molecule approaches? G-quadruplex structure and the DNAzyme

DNA strands assemble into triplex and quadruplex structures. Interest in the latter started with the discovery of hundreds of six-nucleotide single strand G-rich tandem repeats [(TTAGGG)_n] forming “non-canonical” Hoogsteen quartets terminating the duplex DNA and protecting eukaryotic chromosome maintenance (Fig.3A and B).⁴³⁻⁴⁶ The quadruplexes, are denoted “telomeres” (Gr.: telos = end; meros = part). They are conjectured to hold keys to age and cancer⁴⁷⁻⁵⁴ and stabilized by alkali metal ions and divalent cations (Pb²⁺, Sr²⁺).⁵⁵⁻⁵⁷ Porphyrins can stack onto the terminal G-quartet planes by π - π interactions as a telomerase inhibitor.^{58,59} G-quartet bound porphyrins also exhibit peroxidase activity and ultra-sensitivity to K⁺, Na⁺ and Pb²⁺. All this has prompted the notion “DNAzyme” as a biomimetic enzyme (Fig. 3C).⁶⁰⁻⁶¹ Novel electrochemical approaches to DNAzyme structure and enzyme dynamics based on single-molecule conductivity are therefore warranted, as for duplex OGNs and recently for the copper enzymes nitrite reductase⁶² and laccase⁶³.

3.2 A G-quadruplexes as molecular electronic conductors

As for duplex DNA, the attractive looking three-dimensional (3D) variable-length G-quadruplexes have prompted views on G-quadruplexes in molecular electronics⁶⁵. Similar to “smart” transition metal complexes^{1,2,14,66}, metalloproteins^{2,6-8}, and redox marked duplex OGNs^{25,27}, single-molecule rectification, amplification etc. can be expected, provided that G-quadruplexes are good single-molecule conductors, as expected by the electronically delocalized 4G-base quartets and strong π - π electronic interactions among the G-quartets.

3.2.1 Electrochemistry of G-quadruplex structures

Photo-induced G-quadruplex conductivity in aqueous media is established⁶⁸⁻⁷⁰, but electrochemical studies of G-quadruplexes are very few. Doneaux and associates reported voltammetric and spectroscopic studies of [Ru(NH₃)₆]³⁺ on polycrystalline gold surfaces modified by G-quadruplexes and G-duplex monolayers.⁶⁷ [Ru(NH₃)₆]³⁺ binds selectively to G-quadruplex in the adlayers by nonelectrostatic interactions giving a new reduction peak at -0.14 V (vs. SCE) in successive ac voltammograms.⁶⁷ Apart from electroanalytical perspectives, this could point to G-quadruplex

hopping via bound $[\text{Ru}(\text{NH}_3)_6]^{3+}$, as for Ru-complex binding to ds-OGNs, Section 2.

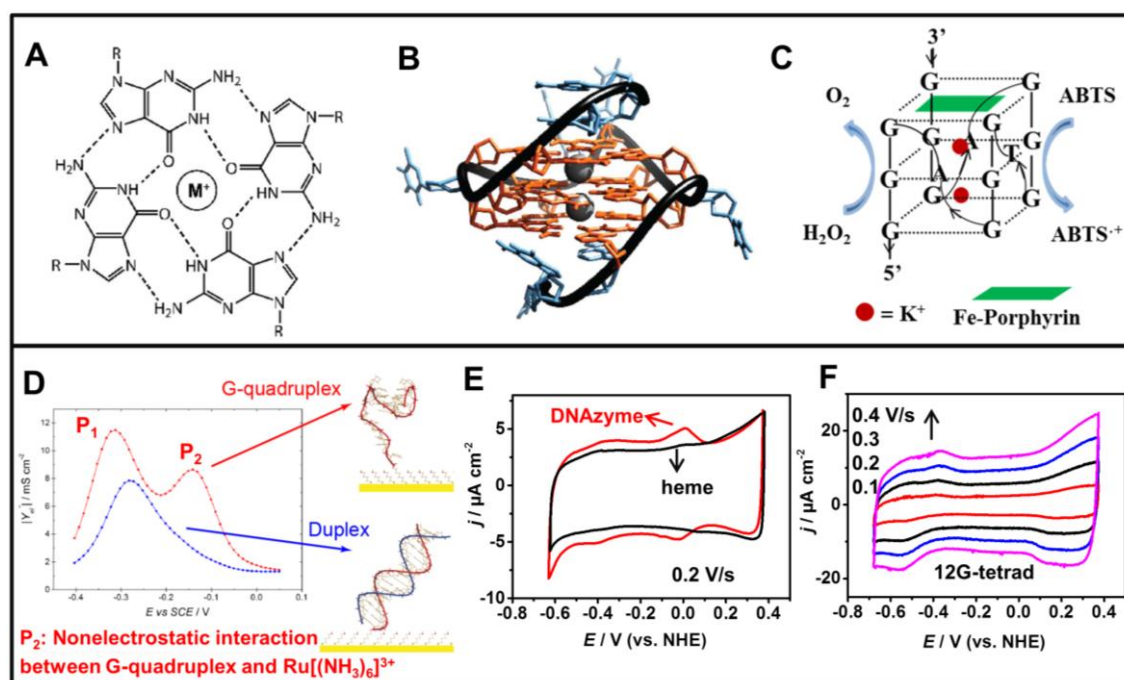


Fig. 3 (A) G-quartet structure coordinated with metal cations. (B) Solution NMR structure of c-myc Pu22 sequence 5'-TGAGGGTGGGTAGGGTGGGTAA-3' (PDB ID: 1XAV). From Ref.⁴⁴ with permission. (C) DNAzyme of G-quadruplex with iron porphyrin IX (FePPIX, or heme). From Ref.⁶⁴ with permission. (D) Successive ac voltammograms at G-quadruplex and duplex DNA modified electrodes. 10 mM Tris buffer (pH 7.4) + 100 μM $[\text{Ru}(\text{NH}_3)_6]^{3+}$. One voltammogram plotted every two measurements. $f = 37$ Hz, $T = 20$ °C. From Ref.⁶⁷ with permission. (E and F) CVs of 12G-quadruplex based DNAzyme and free heme (E) and of FePPIX free 12G-quadruplex (F) on Au(111)-electrode in 5 mM sodium acetate, pH 7.2. From Ref.⁶⁴ with permission.

Carbon materials enable following electrochemical guanine hole generation. Olivera-Brett and associates reported recently a voltammetric and AFM study of the time evolution of G₁₀, TG₉ and TG₈T on glassy carbon (voltammetry) and highly oriented pyrolytic graphite (AFM)⁷¹ from single strands to folded G-quadruplexes. AFM (in air) disclosed morphologically different aggregates in the adlayers. Molecular resolution was not achieved, and the AFM air environment poses recognized limitations, but use of carbon might offer promise for high-resolution mapping of electron or hole creation, once binding in well-defined orientations on the electrode surfaces can be controlled.

Zhang and associates recently reported a combined electrochemical and *in situ* STM study of the smallest, 12G-containing (5'-SH-(CH₂)₆-GGG A GGGT GGGA GGG) G-quadruplex FePPIX DNAzyme, on Au(111)-electrode surfaces, Fig. 3C⁶⁴. Fig. 3E shows cyclic voltammograms (CVs) of DNAzyme with pure FePPIX (heme) modified Au(111)-electrode as a reference. A pair of voltammetric peaks at 0.0 V (NHE) correspond to a dense monolayer, notably with a large electrochemical rate constant (6.0 ± 0.4) s⁻¹. The peak potential is significantly higher than for FePPIX on graphite (≈ -0.2 V vs NHE) due to specific FePPIX/G-quartet interactions. The large rate constant suggests facile ET/HT through the G-quadruplex, but this is not conclusive, as the FePPIX binding end of the G-quadruplex is presently unknown, cf. further Section 3.2.2. Another pair of peaks around -0.4 V for both DNAzyme and FePPIX free G-quadruplex was assigned a capacitive origin as for the reorientational capacitive peak reported for duplex OGNs, Fig. 3F.³⁷

3.2.2 Hopping and G-quadruplex conductivity

Variable-length G-quadruplexes form differently stacked conformations, with strong π - π couplings⁶⁸. In comparison to duplex OGNs photo-induced ET/HT in solution, there are much fewer reports of G-quadruplex conductivity^{24,65,69,70}. These studies point to higher conductivity due to the larger number of π -contacts and more delocalized electronic states, but the same issues are left as for G-duplex electrochemical conductivity, i.e. how the excess charges are created electrochemically in the first place, and why voltammetric ET/HT signals are not apparent.

Theoretical studies have addressed the coupling between differently stacked G-bases^{68,71}. The couplings are strong, with $T_{DA} \approx 0.03$ - 0.3 eV. Such values imply that individual hops would be in the adiabatic limit, $\kappa \rightarrow 1$, eq.(1)³³ and tunnelling only reflected in lowered activation free energy, $\Delta G^\ddagger \rightarrow \Delta G^\ddagger - \frac{1}{2}T_{DA}$. With reorganization free energies of 0.5-1 eV the activation free energy can therefore almost vanish for favourably stacked G-quartets and hopping be an efficient photo-conductivity hopping mechanism, eqs.(1) and (2), once the excess electrons or holes have been created.

3.2.3 Single-molecule G-quadruplex conductivity – STM, cAFM and *in situ* STM

Following comprehensive studies of duplex OGNs, Porath and associates reported single-molecule mapping of long G-quadruplexes immobilized laterally on mica surfaces, using STM and cAFM^{65,72}. Sub-molecular image resolution was achieved and single-molecule conductivity mapped, albeit in non-biological (vacuum) environments. Weak conductivity distance dependence over > 100 nm could be mapped by positioning the cAFM tip along the strands, with current observed at several volts threshold voltages, strongly indicative of “hopping” conductivity. These discoveries are important, but air/vacuum and high voltages leave issues regarding solvation, counter ions, biomolecules under external stress etc. compared with natural biological environments. Such questions can be approached differently, by electrochemical STM (or cAFM) and have been addressed recently⁶⁴. *In situ* STM to molecular resolution of the 5'GGG A GGGT GGG A GGG 3' G4-quadruplex DNzyme in biological buffer under electrochemical control was achieved, cf. Section 3.2.1, disclosing different surface structures. Domain 1, Fig.4 shows both quadratic molecular scale and larger octagonal entities (Fig.4C). The former resemble the 12G-quadruplex box. The larger octagonal units could then be water networks stretching out from the quartets, Fig. 4D. Similar quartet structures appear in UHV G-assemblies on Au(111) surfaces.⁷³ The larger side length, 1.6 ± 0.2 nm of the *in situ* 12G-quadruplex than for the UHV G-quartet (0.67 nm) again most likely discloses water networks. Domain 2 shows linear arrays, perhaps unfolded single strands. Even though voltammetrically robust, surface bound G-quadruplexes thus appear to be mobile molecular units. In this respect they follow duplex OGNs.

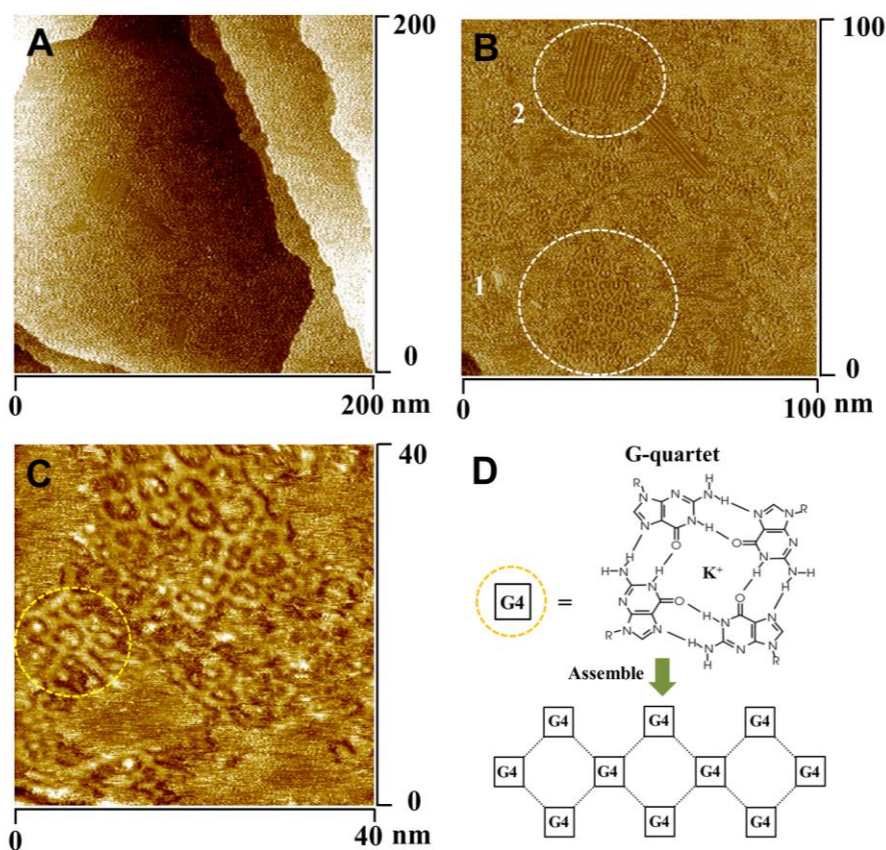


Fig. 4 (A–C) *In situ* STM images of DNAzyme on Au(111)-electrode surface. 5 mM sodium acetate, pH 7.0. $E_{sample} = 0.49$ V (vs. NHE), $E_{bias} = -0.48$ V. $I_{tunneling} = 0.09$ nA. (D) Schematic structure of the G- and octagonal G-tetrad superstructures. From Ref.⁶⁴ with permission.

4. Concluding remarks

We have provided a discussion of single-molecule *in situ* imaging and conductivity of duplex and quadruplex DNA/OGNs with emphasis on electrochemistry on single-crystal Au(111)-electrode surfaces and *in situ* STM. The mechanistic challenges regarding OGN conduction in air/UHV and aqueous media noted for duplex OGNs apply as well to quadruplex OGNs, for which, however, only few electrochemical and *in situ* single-molecule studies are reported. With a view on the success of B-J techniques for single-molecule *in situ* duplex OGN conductivity, this technology is likely to hold promise also for systematic single-molecule, variable-length G-quadruplex conductivity. In view of the strong π - π interactions between the G-quartets such studies could also open paths to the subtle region between strongly adiabatic hopping and superexchange/tunnelling in OGN charge transport. The outcomes of such studies could also offer guidance to new types of G-quadruplex sensors and possible forthcoming molecular scale electronics.

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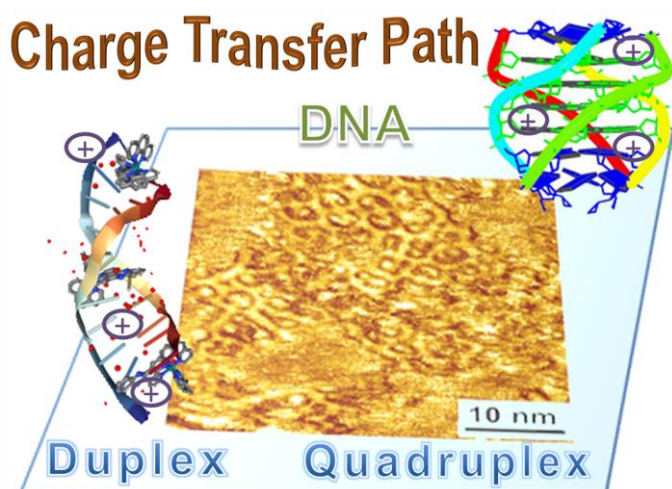
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