

Supporting Information for

Transverse tunneling through DNA hydrogen bonded to an electrode

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1. Materials and methods

Aminopropyltrimethoxysilane (APDM) was purchased from Gelest, Inc. (Morrisville, PA). 3,3'-dithiobis(succinimidyl propionate) was from Pierce Biotechnology, Inc. (Rockford, IL). Maleimide-PEG-NHS (MW=5000Da) was from Nektar Therapeutics (San Carlos, CA). All other chemicals and anhydrous solvents are from Aldrich (Milwaukee, WI). DNA oligomers were acquired from Integrated DNA Technologies (Coralville, IA).

NMR spectra were recorded on the Varian Inova 500 MHz instrument in the Magnetic Resonance Research Center at ASU. Matrix assisted laser desorption ionization time-of-flight (MALDITOF) mass spectra were recorded on a VG ToFSpec spectrometer, Proteomics and Protein Chemistry Lab at ASU. The ozone cleaner is a UV Clean #135500 (Boekel Inc.).

Thickness measurements were performed on an LSE Stokes Ellipsometer (Gaertner Scientific Corp.) at a wavelength of 632.8 nm with an incident angle of 70 degrees. FTIR spectra were collected at a grazing angle of 80 ° on the Smart SAGA (Specular Apertured Grazing Angle) accessory coupled into a Thermo-Nicolet 6700 FTIR spectrometer equipped with a mercury cadmium tellurium (MCT) detector. A polarizer minimizes 'S' polarized light and increases sensitivity. The entire FTIR system is protected by nitrogen generated from a flow-controlled liquid nitrogen tank.

Surface Plasmon resonance (SPR) measurements were made on a BI-SPR1000 system (Biosensing Instruments, Tempe, AZ). The flow speed was 10 $\mu\text{L}/\text{min}$. Water or 0.5 mM Tris-HCl buffer were used as a carrier.

2. STM and AFM Imaging

STM and AFM imaging were carried out using both PicoSTM and PicoPlus systems from Agilent, Chandler. Pt-Ir probes, prepared as described previously,¹ were used for STM images. AFM images taken using tapping mode in air (MI-300 probes, resonant frequency 280 kHz). All STM data were collected in freshly prepared solutions at a sample bias of 0.5 V and tunneling currents of 10~30 pA. All image processing and analysis was carried out using Gwyddion (<http://gwyddion.net/>).

DNA adsorption onto the guanidinium monolayer was carried out by exposing the freshly-prepared guanidinium monolayer to a 50 μL drop of 5 $\mu\text{g}/\text{mL}$ DNA in 0.5 mM Tris buffer (pH 7.0) for approximately 2 hours and then rinsing before imaging under buffer. In-situ injection (Figures 2a and b in the main text) was carried out using a 0.1 $\mu\text{g}/\text{ml}$ solution of DNA introduced by a SP200 syringe pump (WPI, Florida).

3. DNA Minicircles

DNA minicircles were synthesized using oligomers of the following sequences

5'-cgatgaattcctgtacggaatggattgaccgatggaaaaacgggcaaaaaacggcaaaaaacgggcaaaaaacggcaaaaaacgggcaaaaaac
cctgctagacgcctagaggcatgccctagggcatgcccgttctacaac gtgatgcgat-3' and

5'-cgatcgcacacgtttagcaatcgggcatgccctagggcatgcccttaggcgtctagcagggtttttgcccgtttttgcccgtttttgcccgtttttgccc
gtttttgcccgtttttccatcggtcaatccattccgtacaggaattcat-3'.

To make the final 156 bp duplex with CG cohesive ends, equal molar concentrations of top and bottom strands were mixed and hybridized in 10 mM Tris-HCl, pH 8.0, 50 mM KCl and 0.1 mM EDTA and the sample was heated to 95 °C for 10 mins followed by slow cooling to room temperature. DNA duplex was confirmed and purified by 10% nondenaturing polyacrylamide gel. The DNA duplex was then phosphorylated at both 5'-ends by using T4-DNA polynucleotide kinase. Cyclization was carried out with 10 nM total DNA and 500 units/ml T4 ligase. The reaction was incubated at 21 °C for 5 hrs then heated to 65°C for 15 mins to terminate the ligation. Circular product was separated from linear molecules by using Bal 31 exonuclease and analysis on a 10% nondenaturing polyacrylamide gel. Bal 31 exonuclease preferentially degrades linear duplex DNA rather than circular DNA. The desired circular DNA band was excised and eluted in 10 mM Tris-HCl, pH 8.0.

4. Preparation and characterization of β -mercaptoethylguanidine monolayers.

Synthesis of β -mercaptoethylguanidine

Cystamine dihydrochloride (0.25 g, 1.1 mmol) was dissolved in anhydrous DMF (1.5 ml). To the solution, N,N-bis(tert-butoxycarbonyl)thiourea (729 mg, 2.2 mmol) was added, followed by addition of anhydrous triethylamine. (0.373 ml, 2.64 mmol). A suspension of Mukaiyanma's reagent (675 mg, 3 mmol) in anhydrous DMF (1.5 ml) was

added dropwise to the reaction mixture. The reaction was stirred at room temperature overnight, and then was diluted with water (2.2 ml) and extracted with diethyl ether (3 x 2.2 ml). The organic layer was dried over sodium sulfate and evaporated to dryness. The crude product was purified by column chromatography (ethyl acetate: hexane = 1:4). The yield was about 14%. The tert-butoxycarbonyl group was deprotected with 50% TFA in DCM for two hours. The excess TFA was evaporated under vacuum. The product was then dissolved in 2 ml water and washed with DCM twice to remove organic impurities. Pure Bis(2-guanidinoethyl) disulfide was obtained as a trifluoroacetate salt. $^1\text{H NMR}$ (D_2O) δ 3.338 (t, 4H), 2.727 (t, 4H); MALDI MS m/z : 237.098 (calcd 237.095 for $\text{C}_6\text{H}_{16}\text{N}_6\text{S}_2$). β -mercaptoethylguanidine was obtained by treating bis(2-guanidinoethyl) disulfide with immobilized TCEP (Tris[2-carboxyethyl] phosphine hydrochloride) disulfide reducing gel (Pierce) immediately prior to use.

Monolayer preparation and characterization

A freshly hydrogen-flame annealed gold substrate² (Agilent, Chandler, AZ) was immersed in a 0.5 mM aqueous solution of β -mercaptoethylguanidine for 15-30 minutes, and then rinsed with 1M NaCl solution and doubly distilled deionized water. The molecular resolution image shown in Figure 1b of the main text was taken by monitoring the formation of the monolayer in-situ after injection of the β -mercaptoethylguanidine solution into the liquid cell of the STM.

The thickness of the guanidinium SAM was determined by averaging ellipsometry readings at eight different locations on the sample. Optical constants of the bare gold substrate were measured before deposition of molecules, yielding $n=0.2$ and $k=-3.54$. The guanidinium SAM optical constants were chosen to be $n_f = 1.46$ and $k_f = 0$,

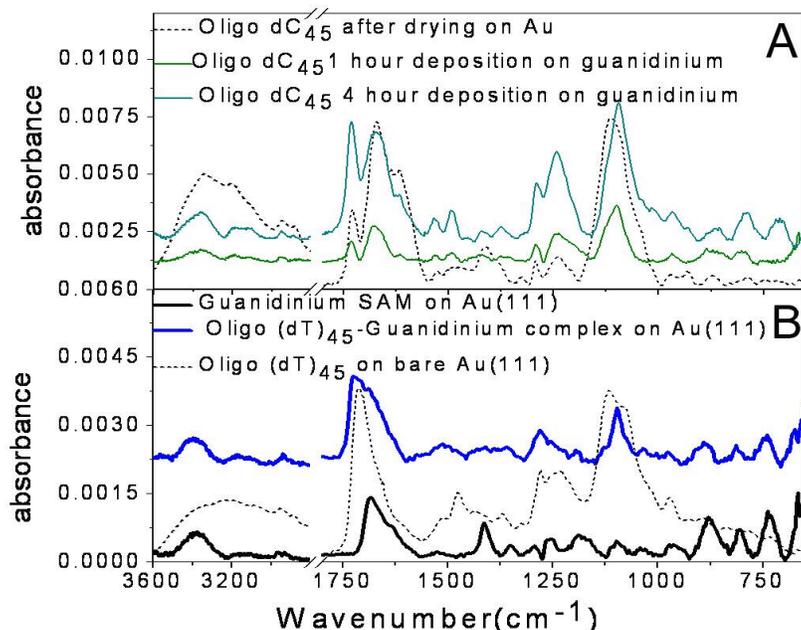


Figure S1. FTIR spectra of guanidinium and guanidinium-DNA complexes on Au(111). (A) Oligo (dC)₄₅-guanidinium spectrum at two immobilization times (solid curves) and the spectrum of oligo (dC)₄₅ dried onto bare gold (dashed line – intensity scaled by a factor 0.3). (B) FTIR spectrum of oligo (T)₄₅-guanidinium complex (solid blue line). The dried-on sample spectrum (0.15x) is shown by the dashed line. The solid black line is for the guanidinium adlayer alone, yielding a thickness of 0.99 ± 0.03 nm. While this is close to the expected height for an upright molecule of 1 nm (estimated by Chemdraw 3D) it is also possible that the adlayer is tilted, with the extra height coming from an adventitious adsorbate layer on this highly active surface (the surface clearly becoming highly contaminated after just a few hours of storage in buffer).

A FTIR spectra of a guanidinium monolayer on Au(111) surface is shown in Figure S1b (black line). The bands at 3356 cm^{-1} and 1683 cm^{-1} arise from NH_2 stretching and $\text{C}=\text{N}$ stretching of guanidine, respectively. The peak at 2948 cm^{-1} is attributed to C-H stretching and 1415 cm^{-1} is attributed to C-H bending.

The Ellipsometry and FTIR, together with the hydrophilic nature of the surface support the formation of a guanidinium monolayer on the gold surface.

5. Effect of phosphate adsorption on guanidinium monolayers.

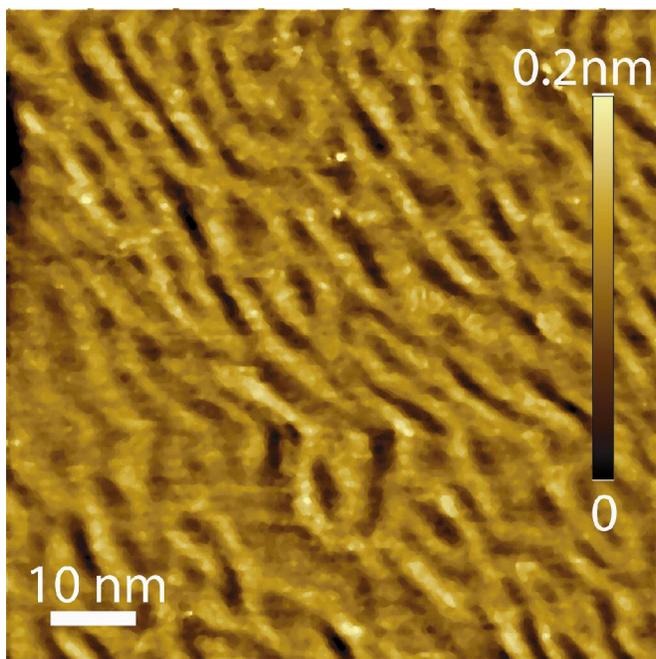


Figure S2: STM image of a guanidinium monolayer taken in phosphate buffer. Images obtained at 0.5V, 10 pA. Height scale is on the right.

An image of the guanidinium surface after exposure to phosphate buffer (pH7.0) is shown in Figure S2. The surface was first imaged in 0.5 mM Tris-HCl, yielding the characteristic ‘pitted’ surface shown in Figure 1a of the main text. After addition of 5 mM phosphate buffer into the liquid cell, the surface at first became unstable, settling into the striped arrangement shown in Figure S2 after a few hours

(this image is characteristic of the whole surface). Since the corrugations (of ca 1 Å) are

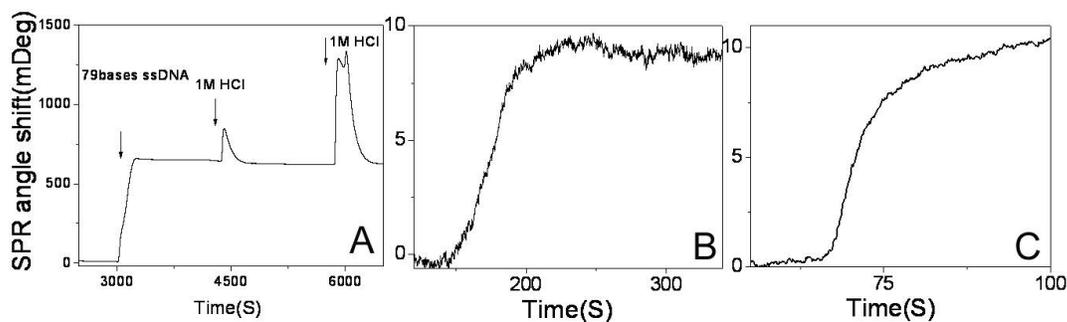


Figure S3: SPR of DNA adsorption onto guanidinium monolayers. (A) 0.8 $\mu\text{g/ml}$ of 79 base ssDNA in 0.5 mM Tris-HCl, pH 7.0; (B) 0.2 $\mu\text{g/ml}$ of Litmus 28i Vector (2.8kbp dsDNA from New England Biolabs) in 0.5mM Tris-HCl buffer, pH 7.0; (C) 0.78 $\mu\text{g/ml}$ of M13 (6.4kb ssDNA from New England Biolabs) in 0.5mM Tris-HCl buffer, pH 7.0. 20 μL aliquots were injected into the flowing buffer.

now significantly less deep than the height of a gold atomic terrace (2.5 Å) we conclude

that the surface layer has become re-ordered in such a way as to remove the “pits” and restore a flat gold surface.

6. Characterization of DNA adsorption from solution onto guanidinium monolayers.

Surface Plasmon Resonance Studies

SPR data for DNA adsorption is shown in Figure S3 as a function of time after the injection of a 30 μ L aliquot of DNA into 0.5mM TrisHCl buffer (pH 7.0) flowing through the cell at 10 μ L/minute. Panel A shows data for a 79 base ssDNA oligomer. The second and third arrows (left to right) point to features caused by the injection of 1M HCl. The acid treatment clearly fails to remove the adsorbed DNA. Data for the dsDNA imaged in Figure 2 of the main text (Litmus 28i vector) are shown in panel B. Data for the M13 ssDNA (Figure 2d of the main text) are shown in panel C.

Sample	Thickness (nm) 300 bp	Thickness (nm) 1800 bp	Thickness (nm) 2800 bp
Guanidinium (Gd) (nf=1.46)	0.62 \pm 0.07	1.01 \pm 0.03	0.96 \pm 0.02
DNA-Gd (nf=1.46)	0.95 \pm 0.07 Δ t = 0.32 nm	1.31 \pm 0.16 Δ t = 0.3 nm	1.09 \pm 0.05 Δ t = 0.13 nm
DNA-Gd (nf=1.42)	1.3 \pm 0.07 Δ t = 0.68 nm	1.48 \pm 0.16 Δ t = 0.47 nm	1.14 \pm 0.02 Δ t = 0.18 nm

Table S1: Thickness of guanidinium monolayers before and after dsDNA adsorption as a function of the length of the DNA.

Ellipsometry

Data for three different dsDNA adsorbates (300, 1,800 and 2,800 bp) are listed in Table S1. We have assumed that the refractive index of the guanidinium is 1.46 (a common

value for similar molecules). The refractive index of DNA ranges from 1.46 to 1.42, depending on the degree of hydration.³

Two striking features of these data are (a) that the estimated DNA heights are substantially less than the expected 2 nm, and (b) the apparent height decreases with increase in molecular weight. Since AFM images (see below) suggest full monolayer coverage, the anomalously low heights may reflect the displacement of an adventitious adlayer on the guanidinium by the (strongly adsorbed) DNA. The decrease of apparent coverage with increasing molecular weight is consistent with the observation that adlayers of lower molecular weight appear to be almost perfectly smooth (see below) whereas the higher molecular weight material adsorbs with many obvious defects (e.g. Figure 2d in the main text). In any event, these data are not consistent with multilayer adsorption.

FTIR Spectroscopy

FTIR spectra of guanidinium-DNA complexes on gold substrate are shown in Figure S1. The spectra shown in dashed lines are for bulk DNA obtained by drying DNA solutions onto the Au (111) substrate. The samples are oligo (dC)₄₅ and oligo (dT)₄₅. The similarity of the spectra of bulk DNA and of guanidinium-DNA complexes demonstrates DNA attachment to the guanidinium surface. The absorptions at 1730 cm⁻¹ (panel A) and 1724 cm⁻¹ (panel B) correspond to carbonyl group of the bases thymine and cytosine. The increase of the peak at 1730 cm⁻¹ (Figure S1A) with immobilization time is consistent with the observation of Petrovykh et al. on thiolated DNA.⁴ The bands at 1243 cm⁻¹ and 1095 cm⁻¹ (panel A) and 1253 cm⁻¹ and 1097 cm⁻¹ (panel B) are attributed to the

antisymmetric and symmetric stretching of the phosphate.^{5,6} In both Figures S1A and B the shift of phosphate peak from 1110 cm⁻¹ (bulk DNA) to 1095 cm⁻¹ (guanidinium-DNA

complex) may be due to the interaction between the guanidine and the phosphate.

7. STM images of linear single stranded DNA oligomers

Figure S4 shows STM images of adlayers of single stranded oligomers on the guanidinium monolayer. Representative scans of the substrates prior to incubation with DNA are shown in the left panels and images taken after incubation with DNA are shown in the right panels. Data are for a 12 base (A), 40 base (B) and 79 base oligomer, incubated at ca 1 µg/ml concentration with the guanidinium substrate for 2 hours. The surfaces change dramatically on exposure to DNA. The longer DNAs (B and C)

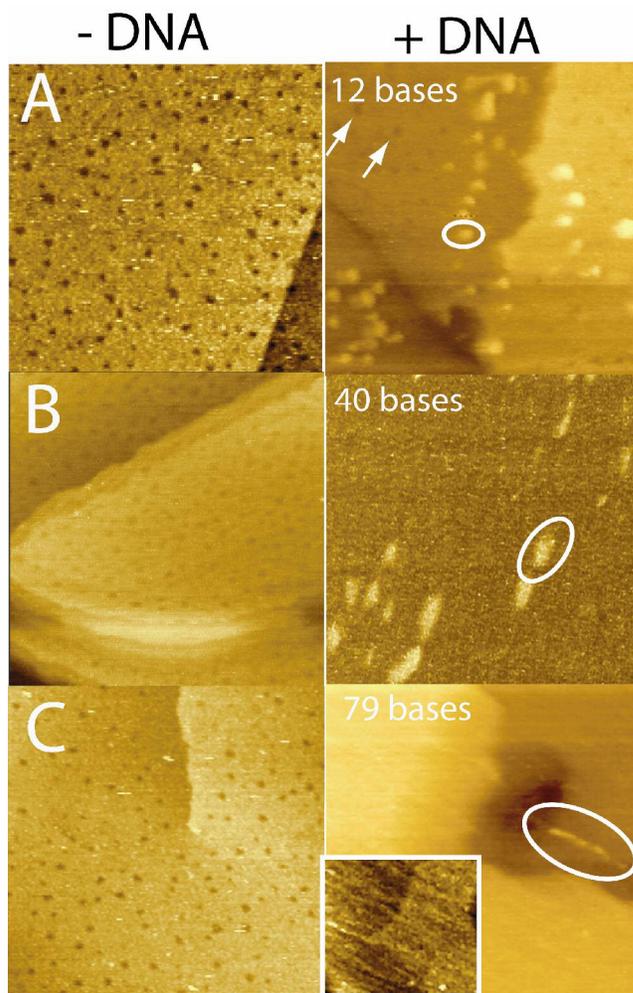


Figure S4: STM images (100 nm x 100 nm) before (left panels) and after (right panels) incubation with ssDNA of lengths 12 b (A) 40 b (B) and 79 b (C). The 40 (B) and 79 (C) base adlayers are smooth, lacking the characteristic pits seen in the pre-incubation images and the 12 b image (arrows, right panel in A). Characteristic molecular features (circled) stand out from the adlayers. Stretching the contrast of images (inset white box in C) shows that the 'smooth' surface is composed of densely-packed molecules.

causes the pits to disappear, and the surface looks smooth, except for the appearance of

rod-like ‘defects’. These appear to be isolated molecules that are not as well packed into the adlayer, because their dimensions scale with those of the added DNA (a summary of a number of images is given in Table S2). The 12 base oligomers does not appear to form a dense layer and the pits coexist with the DNA (arrows in right panel in A). Stretching the image contrast on a flat region reveals a fibrous texture (white box inset in C) suggesting that the ‘smooth’ regions’ are composed of densely packed DNA.

Sample	Approximate length of image features (nm)	Calculated length (0.34 nm/base)
12 bases	5±1	4.1
40 bases	9±3	13.6
79 bases	30±5	26.8

Table S2: Comparison of lengths of features in the STM images with calculated DNA lengths. Calculated length assumes B form DNA.

8. AFM measurements of the adhesion of single ssDNA molecules

Ultrasharp CSC11/AIBS cantilevers (MikroMash) were used in measuring the adhesion of ssDNA to guanidinium surfaces. In order to functionalize the probes they were first placed in the ozone cleaner for 10 minutes, immediately dipped into fresh piranha solution for no more than 30 seconds (to prevent damage to the metallization), rinsed with tris-buffer and put into a solution of APDM (200 μ l, 1.4 μ mol) in 95% ethanol (1 ml). After 5 minutes, the tips were rinsed with water, placed in a clean dessicator flushed with argon, and placed under vacuum (10 torr) for 1 hour. The tips were removed from the vacuum and placed in solution of 3,3'-dithiobis(succinimidyl propionate) (0.025 g, 0.062 mmol) in DMF (1 ml) for half an hour. The tips were then rinsed with water and then allowed to sit in 10 mM tris(2-carboxyethyl)phosphine hydrochloride in 5 mM PBS (pH 7.0) for 15 minutes. While waiting for the tips maleimide-PEG-NHS (0.1 mg, 22 nmol) was added to DNA (single stranded, 60 base

oligo with a 3' aminohexane modification from Integrated DNA Technologies (Coralville, IA) (10.5 nmol in 25µl nanopure water), the solution was diluted with 50 µl of 100 mM phosphate buffer (pH 6.5) with 5 mM EDTA and 1M NaCl . Before adding the tips to the DNA-PEG solution, the tips were rinsed in 100 mM phosphate buffer (pH 6.5) with 5mM EDTA and 1M NaCl. The tips were allowed to react for 2 hours in the DNA-PEG solution. The probes were then rinsed with water and used immediately.

The AFM was interfaced to a LabView control system via a custom modification of the head electronics. The AFM approach was controlled with custom software using the Measurement Studio (National Instruments) in Visual Basic (Microsoft). The guanidinium surface was placed into the liquid cell of the microscope and covered in 100 mM Tris-HCl (pH 7.5) buffer. A freshly-functionalized probe was inserted into the scanner and lowered onto a guanidinium functionalized gold surface using Measurement Studio until a deflection increase of ~100 pN was detected, held for 4-6 s to allow the DNA to make contacts with the guanidinium then retracted while force-distance curves were recorded.

9. Noise and read speed

The mean square Johnson voltage noise in a resistor of R Ohms at a temperature T is given by⁷

$$V^2 = 4k_B TRB \quad (S1)$$

The equivalent current noise is $i^2 = \frac{4k_B TB}{R}$.

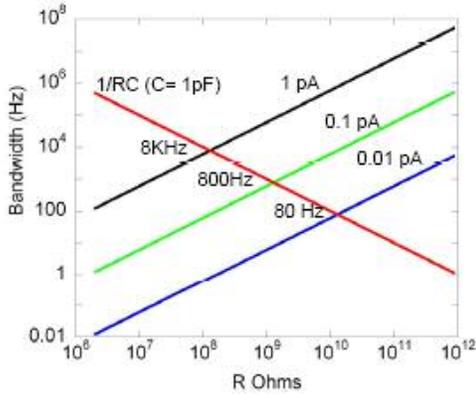


Figure S5: Maximum bandwidth owing to RC time constant (red line) and Johnson noise (black line: 1 pA signal, green line: 0.1 pA signal, blue line: 0.01 pA signal) as a function of the value of the current-conversion resistor. 1 pF input capacitance is assumed.

The very smallest signal that could be detected would be just equal to this noise. Calling this i_{\min} , the maximum achievable bandwidth is then

$$B = \frac{i_{\min}^2 R}{4k_B T} \quad \text{or} \quad B = 6.04 \times 10^{-5} R i_{\min}^2 \quad \text{at } 300 \text{ K with } i_{\min} \text{ in units of pA.} \quad (\text{S2})$$

The bandwidth increases linearly with the current-to-voltage conversion resistor for a given i_{\min} . However, the current to voltage conversion amplifier will have a finite input capacitance, C_{in} and this will limit the maximum possible bandwidth to

$$B_{\max} \approx \frac{1}{RC_{in}}. \quad (\text{S3})$$

Optimistically, C_{in} could be as small as 1 pF.

Equations S2 and S3 are plotted in Figure S5 as a function of R for $C=1\text{pF}$ and $i_{\min} = 1\text{pA}$, 0.1 pA and 0.01 pA. The intercept gives the largest possible bandwidth.

References for the supplementary information

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