Credo et al., Label-free PPi detection from DNA polymerase reactions on FETs, Dec 2011 (revised paper submitted to Analyst)

Supplemental Information

Label-free electrical detection of pyrophosphate generated from DNA polymerase reactions on field-effect devices

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Concentration Dependent PPi Response on Chelator-modified SOI-FET devices

In Figure S1a, the magnitude of the PPi-dependent response on a chelator-modified SOI-FET device (with no DNA colonies) was measured as a function of different PPi concentration (0, 0.3, 0.6, 1.3, 2.5, 5 and 25 μM). We observed saturation of the chelator response at ~2 μM PPi as shown in Figure S1b. Compared to buffer without PPi, the threshold voltage of the chelator-modified device shifted by -0.5 V at 1 nA upon exposure to PPi indicating decreased current, contrary to what would be expected from simple net-charge based models.[1] In comparison, control devices with aldehyde modification did not respond reversibly to PPi and control devices with polyethylene glycol alcohol (-PEG$_4$-OH) termination instead of chelator functional groups exhibited no response to PPi in solution or changes in solution pH.[2] These results were duplicated with several other devices during the same set of experiments and the collected results indicate that the response of chelator-modified field-effect sensors is a function of all related surface and device phenomena, not simply due to the charge of the target species.[3, 4] We also observe results contrary to the expected charge-based response when we expose the SOI-FET devices to positively charged homotetramer protein avidin (pI = 10.5; see main paper) in borate buffer at pH 8.0.
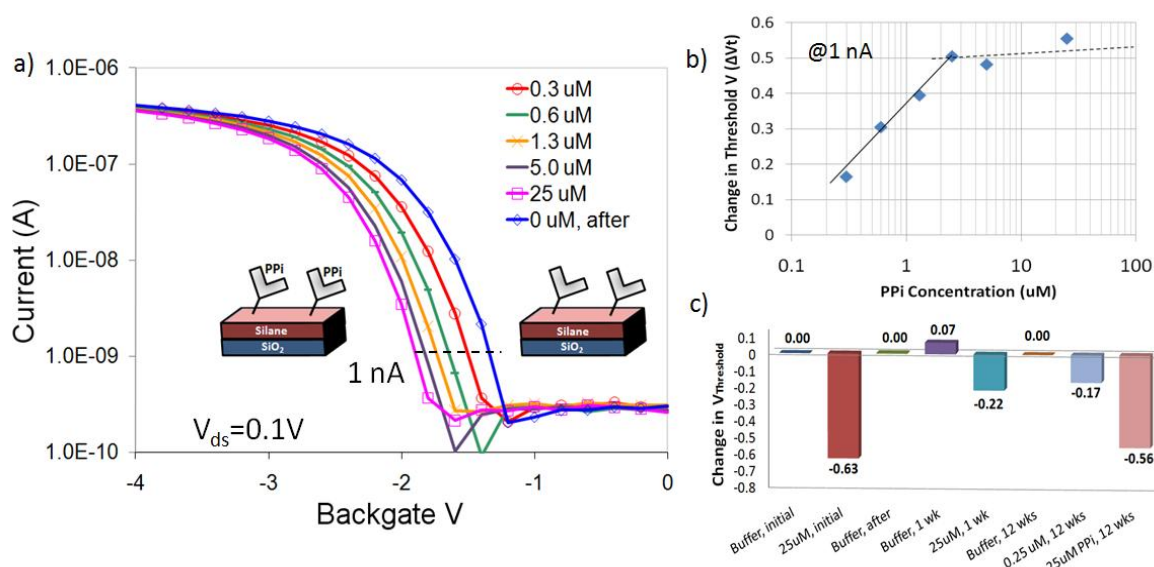

Figure S1 Concentration dependent response for a chelator modified FET device: a) I-V characteristics of a chelator-modified nanoplate exposed to different concentrations of PPi and treated with dilute acid to remove PPi, b) shift in threshold voltage of I-V curve at 1nA as function of PPi concentration, c) shift at 1nA as function of surface modification and buffer composition on different days.

Reusing Devices for PPi sensing

The observed PPi sensing response is expected to be dependent on the coverage and quality of the chelator monolayer, which affects the areal density of PPi binding sites. We observed the attenuation of PPi response with aging of the original silane-based surface modification.[5] In Figure S1c, a specific device was modified with chelator, exposed to control buffers without and with

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25 μM PPi on Day 1, stored in buffer for 6 more days and then exposed to PPi again. The device response compared to control buffer was measurable and in the negative direction (-0.22 V), but less than the original shift in threshold voltage (-0.63 V). However, after piranha cleaning and fresh surface modification, the devices could be reused over the course of many months. For example, in Figure S1c, we measured similar response in PPi detection measurements on the same sensor after chelator modification, a shift in threshold voltage at 1nA of -0.63 V decreasing slightly to -0.56 V, within 10% of the original shift measured three months prior.
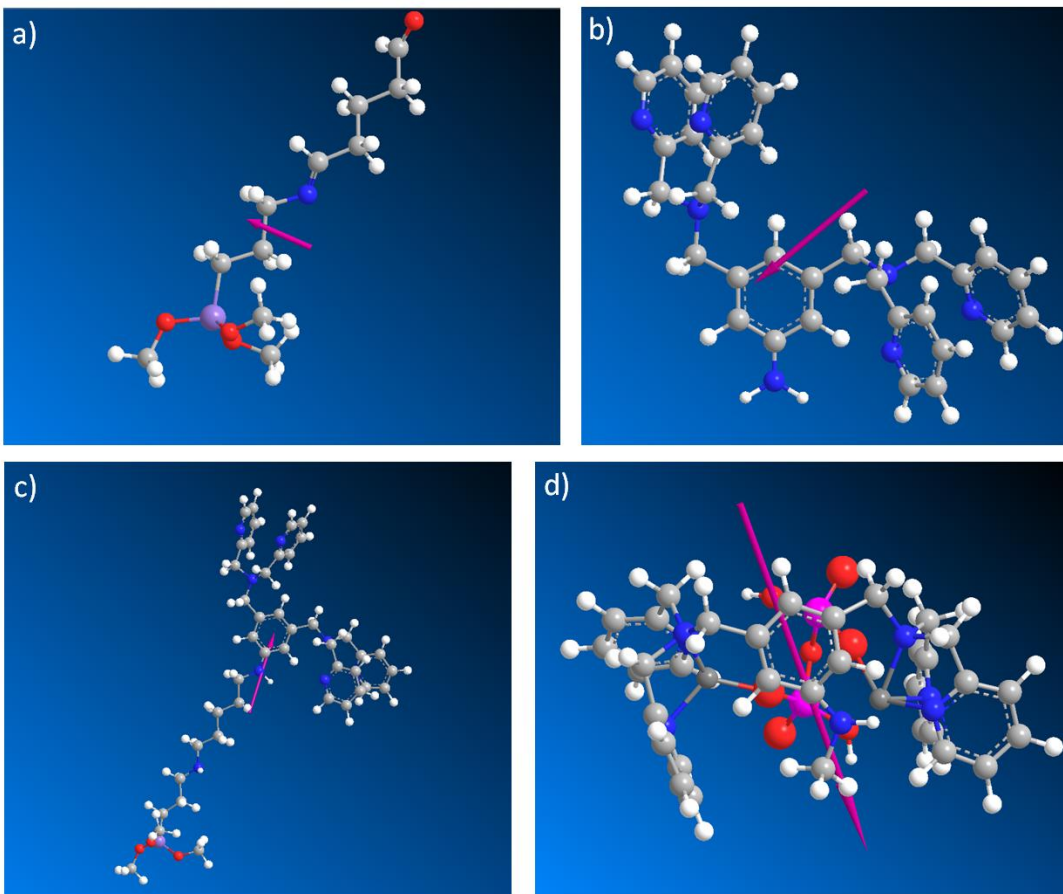
Molecular Dipole Moment Calculations

Figure S2 GAMESS calculated dipole moment of following molecules: a) glutaraldehyde modified aminopropyltriethoxysilane (APTES), b) one structure of the chelator molecule with one conformation of molecular 'arms', c) same version of the chelator attached to the glutaraldehyde-modified silane layer with different dipole moment compared to APTES-GA silane layer only, and d) the chelator complexed with Zn^{2+} and pyrophosphate. When bound to pyrophosphate, the chelator is limited to one conformation. In contrast, the two uncomplexed 'arms' of the chelator are free to rotate, affecting the dipole moment of the molecule and potentially affecting the threshold voltage of the SOI-FET sensor.

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Additional information

Table S1 Oligonucleotide sequences

Oligo Name	Sequence	Comments
PCR upstream primer	5'-pCTGCAATGATACCGCGAGACCCA-3'	For pUC19 plasmid
PCR downstream primer	5'-pCCTTGATCGTTGGGAACCGGAG-3'	For pUC19 plasmid
RC1	5'-pAGCTCGGCGGCCGCTTAAGT-3'	Used to form T-tailed RCA adaptor (1:1:1:1:1 mix)
RC2Tb	biotin-spacer-CTCCTATCACTTAAGCGGCCGCCGAGCTT-3'	Used to form T-tailed RCA adaptor (1:1:1:1:1 mix)
RC3T	5'-pACGTCCGTACGTTCGGAACCT–3'	Used to form T-tailed RCA adaptor (1:1:1:1:1 mix)
RC4	5'-pGGTTCCGAACGTACGGACGTCCAGCTGAG-3'	Used to form T-tailed RCA adaptor (1:1:1:1:1 mix); 3' locked nucleotide, resistant to 3'→5' exonuclease
RC5	5'-pGATAGGAGATCTCAGCTGG-3'	Used to form T-tailed RCA adaptor (1:1:1:1:1 mix)
DNA2	5'-/5amMC6/GTC GCG CAA AAA TAC CTA GTC G+AC GTG GTC CTT/iBiodT/TT GG+A CCA CGT CGA CT+A G-3'	Hairpin DNA used for polymerase reaction in solution

Table S2 pK$_a$ Information[6]

Multistep dissociation - Pyrophosphoric acid, $H_4P_2O_7$		
pyrophosphoric acid, step 1	$K_{a1} = 1.4 \times 10^{-1}$	$pK_{a1} = 0.85$[a]
pyrophosphoric acid, step 2	$K_{a2} = 3.2 \times 10^{-2}$	$pK_{a2} = 1.49$[a]
pyrophosphoric acid, step 3	$K_{a3} = 1.7 \times 10^{-6}$	$pK_{a3} = 5.77$[a]
pyrophosphoric acid, step 4	$K_{a4} = 6 \times 10^{-9}$	$pK_{a4} = 8.22$[a]

[a]Source: *Handbook of Chemistry and Physics*, 73rd edition (1992-1993), page 8-14 (Dissociation Constants of Inorganic Acids in Aqueous Solutions)