Supporting Information

Surface Immobilizable Chelator for Label-free Electrical Detection of Pyrophosphate

David J. Liu,*[a] Grace M. Credo,[a] Xing Su, Kai Wu,[a] Hsiao C. Lim,[a] Oguz H. Elibol,[a,b] Rashid Bashir[c], and Madoo Varma[a,*]

[a] *Integrated Biosystems Lab, Intel Labs, Intel Corporation, 2200 Mission College Blvd., Santa Clara, CA 95054, USA*
[b] *Birck Nanotechnology Center, Purdue University, West Lafayette, IN, 47907, USA*
[c] *Departments of Electrical and Computer Engineering and Bioengineering, Micro and Nanotechnology Laboratory, University of Illinois at Urbana-Champaign, Urbana, IL, 61801, USA.*

Materials and methods: All materials were purchased from Sigma-Aldrich or BASF and were used without further purification unless otherwise noted in the text. NMR spectra were obtained with Varian 300 MHz from Varian, Inc. LC-MS spectra were obtained with Thermo Finnigan LCQ Advantage system from Thermo Fisher Scientific. UV-Vis and fluorescence spectra were obtained with a SpectraMax M2 from Molecular Devices. Time-of-Flight Secondary Ion Mass Spectrometry (TOF SIMS) was performed with an ION-TOF system (Germany) using a pulsed 25 kV Bi primary ion source. Ellipsometry was performed with a M2000FI Variable Angle Spectroscopic Ellipsometer from J. A. Woollam (Lincoln, NE) scanning 685 wavelengths between 240 nm and 1600 nm at 65º, 70º, and 75º for samples on silicon and 45º for samples on gold. AFM was performed using a Dimension V Atomic Force Microscope from Veeco (Santa Barbara, CA). FET measurements were performed with a Signatone Probe Station interfaced with Keithley Semiconductor Characterization System (SCS).

Synthesis of tosylate of 5-nitro-m-xylene-α,α'-diol (1): To a solution of 5-nitro-m-xylene-α,α'-diol (366 mg, 2.0 mmol) in 15 mL THF was added 15 mL of 0.5 N sodium hydroxide, followed by a solution of p-toluenesulfonyl chloride (2.3 g, 12.0 mmol) of in 15 mL of THF. The mixture was stirred at room temperature for 4 hours and was then partitioned between ethyl acetate and water. The organic phase was washed three times with water and dried over anhydrous sodium sulfate. The solvent was then removed under reduced pressure and the residue was purified by flash chromatograph using a 2:1 to 1:1 mixture of hexane and ethyl acetate affording colorless oil (641 mg, 95% yield). NMR ($CDCl_3$) 8.00 ppm (s, 2H), 7.79 ppm (d, 4H), 7.53 ppm (s, 1H), 7.36 ppm (d, 4H), 5.10 ppm (s, 4H), 2.47 ppm (s, 6H). MS (ESI +): *m/z* 508.9 (MW + 18) (calculated M.W. 491.53).

Synthesis of (2): To a solution of **1** (215 mg, 0.64 mmol) in 5 mL of acetonitrile, were added sodium carbonate (260 mg, 2.45 mmol) and potassium iodide (395 mg, 2.37 mmol), followed by di-(2-picolyl)amine (DPA) (240 µL, 1.3 mmol). The mixture was stirred at room temperature for 2 hours and the solvent was removed under reduced pressure. The residue was dispersed in ethyl acetate and water. The organic phase was washed with water three times and dried over sodium sulfate. The solvent was again removed under reduced pressure and the yellow residue was used in next step without further purification since NMR indicated sufficient purity. NMR ($CDCl_3$) 8.56 ppm (m, 4H), 8.15 ppm (s, 2H), 7.74 ppm (s, 1H), 7.62 ppm (m, 4H), 7.52 ppm (m, 4H), 7.13 ppm (m, 4H), 3.83 ppm (s, 8H), 3.79 ppm (s, 4H). MS (ESI+): *m/z* 546.72 (calculated M.W. 545.63).

Synthesis of chelator without zinc ions (3a): To a solution of **2** (54 mg, 0.1 mmol) in 5 mL of methanol was added 10% Pd on activated carbon (100 mg). The system was purged with a vacuum pump and back-filled with hydrogen using a hydrogen-filled balloon and T-adapter. This

was repeated three more times and the mixture was stirred under hydrogen (1 atm) for 4 hours. The reaction was monitored with thin layer chromatography on alumina plate. The carbon was then filtered and the solvent was removed under reduced pressure. The residue was flashed through an aluminum oxide column (activated, basic, ~150 mesh, 58 Å, Aldrich) using 5% methanol in dichloromethane. The fractions were pooled and the solvents were removed under reduced pressure affording colorless oil (50 mg, 97% yield). NMR (CD_3OD): 8.39 ppm (m, 4H), 7.64-7.75 ppm (m, 8H), 7.25 ppm (m, 4H), 6.81 ppm (s, 1H), 6.69 ppm (s, 2H), 3.74 ppm (s, 8H), 3.54 ppm (s, 4H). MS (ESI+): *m/z* 516.2 (calculated M.W. 515.38)

Formation of zinc-coordinated PPi chelator (**3**): A 2 mM solution of **3a** was made by dissolving 30 mg (0.058 mmol) of **3** in 2.9 mL of 50% methanol in water and a 4 mM solution of zinc nitrate hexahydrate was made by dissolving 19 mg of zinc nitrate hexahydrate in 16 mL of water. 1.91 mL of 2 mM solution of **3** and 0.955 mL of 4 mM solution of zinc nitrate hexahydrate were mixed together affording a turbid mixture, which became clear after 1 mL of acetonitrile was added. The solution was put on rotary evaporator and was rotated for 30 min before the solvent was removed under reduced pressure. The residue was mixed with 3.82 mL of 75% water in acetonitrile to make a 1 mM chelator solution.

Binding of a fluorescent dye to immobilizable PPi chelator[1]: A coumarin-based fluorescent indicator dye, (6,7-dihydroxy-2-oxo-2H-chromen-4-yl)methanesulfonate, (10 mM) was mixed with various concentrations of immobilizable chelator in 50 µL of HEPES buffer (10 mM, pH7.4). The fluorescence was monitored at 480 nm when excited at 347 nm. Data were obtained in triplicates.

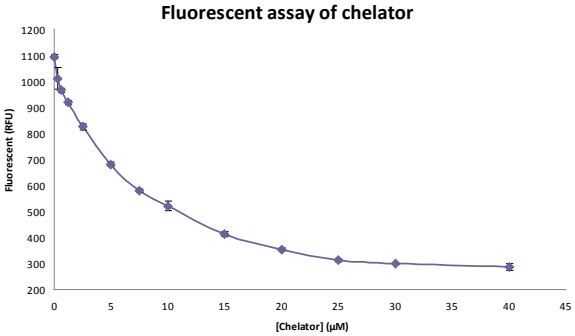

Figure S1. Binding of a fluorescent dye to immobilizable PPi chelator (**3**) in solution.

Colorimetric evaluation of PPi chelator[2]

This assay is very similar to fluorescent assay, except the fluorescent dye is replaced with pyrocatechol violet (PV). The absorption was monitored with UV-Vis spectroscopy at 444 nm. Data were obtained in triplicates.

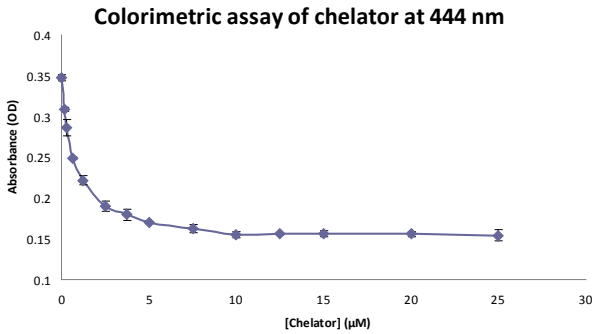

Figure S2. Binding of a fluorescent dye to immobilizable PPi chelator (**3**) in solution.

Pyrophosphate/Phosphate/dATP displacement with colorimetric dye

A complex mix of 50 µM of chelator, 50 µM of pyrocatechol violet dye, and 10 mM of HEPES buffer, pH7.4 (assay mix A) was prepared for the assay. The concentration of the samples, in this case, pyrophosphate, phosphate, and dATP was calculated to 0, 0.78, 1.56, 3.125, 6.25, 12.5, 25, and 50 µM when added to 50 µl of the assay mix A. Water was added to the reaction mixture for a total volume of 100 µl for each sample. The samples were incubated at room temperature for 10 minutes in a clear 96 well plate. After incubation, the samples' absorbance readings were read with SpectraMax at two wavelengths (444 nm and 624 nm). The absorbance readings were analyzed by calculating the mean and standard deviation. Line graphs of absorbance reading versus the concentration of the samples were plotted to determine the binding constant.

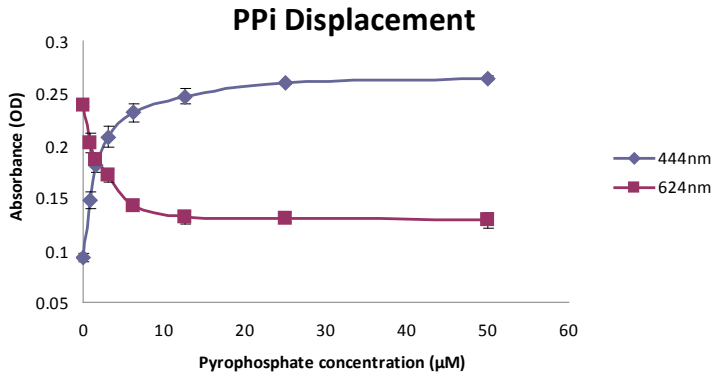

Figure S3. Colorimetric competitive displacement assay of immobilizable chelator with PPi when monitored at 444 nm and 624 nm.

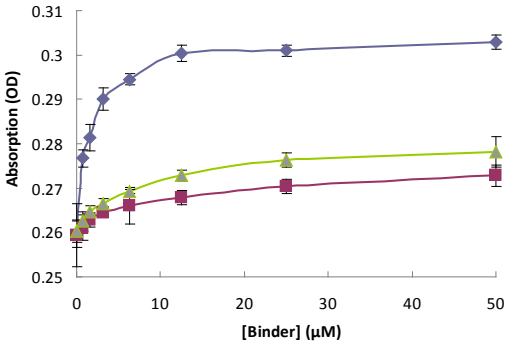

Figure S4. Colorimetric competitive displacement assay of immobilizable chelator with various binders PPi (♦), dATP (▲) and Pi (■) at 444 nm.

Pyrophosphate/Phosphate/dATP displacement with fluorescent dye

For the fluorescent assay, a mix of 10 µM chelator, 10 µM fluorescent dye, 10 mM of HEPES buffer, pH7.4, and 150 mM of NaCl were prepared. The corresponding amount of pyrophosphate, phosphate, and dATP solutions was added to 22.5 µl of fluorescent assay mix at 0, 0.625, 1.25, 2.5, 5, 7.5, 10, 15, 20, 25, 30, 40, and 50 µM concentrations. Water was used to fill the mixture to a final volume of 50 µl. The samples were incubated at room temperature for 10 minutes in a black 384 well micro-titer plate. The SpectraMax was set up with the excitation maximum of the fluorescent dye at 347 nm and maximum emission at 480 nm. The samples were read after

incubation and the standard deviation was computed. Line graphs of the relative fluorescent unit versus the concentration of the samples were plotted.

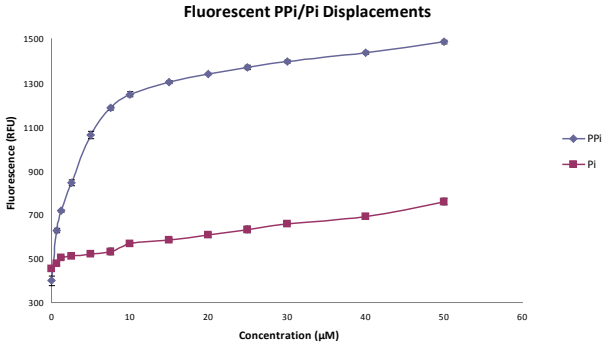

Figure S5. Fluorescent competitive displacement assay of immobilizable chelator with PPi & Pi.

Field-effect transistor device information

Previously reported work in Professor Rashid Bashir's group successfully demonstrated pH detection[3] and DNA match-mismatch detection[4] using nanoplate silicon field-effect sensor devices. Similar devices were used to demonstrate electrical pyrophosphate detection on FET devices. In brief, 8 inch bonded Silicon on Insulator (SOI) wafers (SOITEC), doped p-type at $10^{15}/cm^2$ with a buried oxide thickness of 1450 Å and superficial silicon layer thickness of 550 Å were laser cut into 4 inch wafers (Ultrasil Corp.) and used as a starting material. After fabrication, the wafer was diced into individual dies of size 4 mm by 7 mm. Each die contained multiple devices. Devices were designed to have an active area width of 2 μm and varying lengths of 5, 10 or 20 μm in length depending on the device location. The nanoplate sensor device reported in this work was 2 μm wide, 10 μm long and coated with a silicon dioxide dielectric approximately 27 nm thick. Other devices on the same chip and other chips exhibited behavior similar to that reported in this work.

Surface immobilization of chelator on FET devices

Substrates were cleaned in fresh, hot piranha (1:3 hydrogen peroxide:sulfuric acid) for at least 30 minutes. *Caution: Piranha solution is highly corrosive and should be used with extreme caution.* Afterward, samples were rinsed thoroughly in water.

Aldehyde functional groups were introduced to the device surface by silanization with 1%-2% triethoxysilylbutyraldehyde (TESBA, Gelest) in a mixture of anhydrous ethanol (Sigma) and DI water (1-2%) for at least 15 minutes at room temperature. Afterwards, the chips were rinsed with copious amounts of ethanol and water, dried under nitrogen, and annealed at 110 ˚C for 10 minutes.

To attach chelator molecules covalently on the FET device surface covered with aldehyde groups, 1 mM chelator solution was prepared in 100 mM sodium borate buffer (pH8) and 20 μL of this solution was deposited on the device surface placed in a humidity chamber. After 60 min, 20 μL of freshly prepared reducing agent, 0.2 M sodium triacetoxyborohydride (BASF), was mixed with the chelator solution and the reaction was allowed to proceed overnight. Then the device surface was washed four times with 10 mM borate buffer (pH8) and once with the control buffer (6.25 mM NaCl, 1.25 mM $MgCl_2$, 10 mM Tris pH8 and 1 mM DTT) and stored under the control buffer before measurements. For surface characterization with ellipsometry, AFM or TOF-SIMS, devices and silicon pieces were dried under nitrogen gas and stored in vacuum packed containers until analysis.

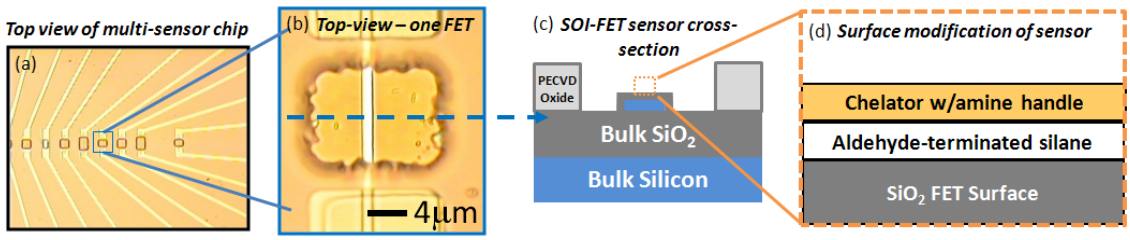

Figure S6. Schematic of modified device surfaces: a) optical microscopy image of several nanoscale thickness <30 nm SOI FET devices with 2 µm wide sensor areas – view from top, b) top view of individual device, c) cross-section of device – view from end, d) chemical modification layers of sensor surface, with chelator and silane on oxide or oxynitride

Surface characterization of immobilized chelator

Ellipsometry and atomic force microscopy (AFM): Spectroscopic ellipsometer and AFM measurements of chemical modifications of silicon substrates were consistent with monolayer formation of silane and subsequent chelator on silane on silicon, as shown in Figure S7 and Table 1. In Table 2, Spectroscopic ellipsometer measurements of chemical modifications of gold were consistent with submonolayer formation of chelator on gold.

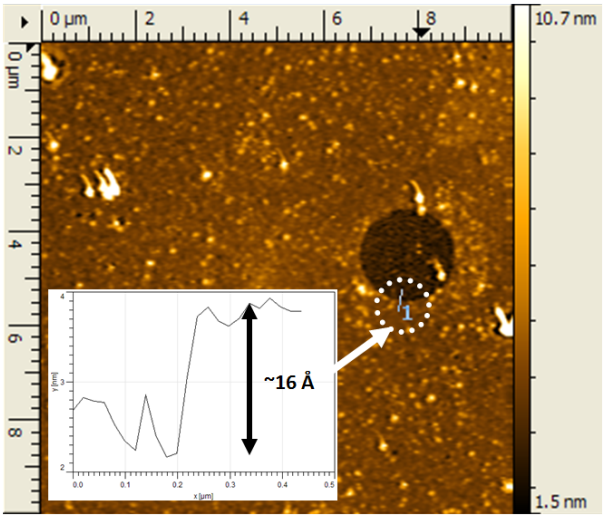

Figure S7. Atomic force microscopy (AFM) image of chemically modified SiO_2 on Si substrate. Void region is caused by bubble formation during STAB reduction step. Cross-section height corresponds to expected thickness of chelator modification on silane.

Table 1. Variable angle spectroscopic ellipsometry (VASE) of modified silicon surfaces

Modification step	Organic layer thickness (Å)	Observed change in thickness (Å)	Expected top monolayer thickness (Å)
Modified silane (TESBA)	9.370	9.370	~8
Immobilized chelator on silane	32.220	22.850	~16

Table 2. Variable angle spectroscopic ellipsometry (VASE) of modified gold surfaces

Modification step	Organic layer thickness (Å)	Observed change in thickness (Å)	Expected top monolayer thickness (Å)
Glutaraldehyde-modified aminoundecanethiol	16.476	16.476	~16
Immobilized chelator on glutaraldehyde-modified aminoundecanethiol	28.186	11.71	~16

TOF-SIMS: TOF SIMS spectra of chelator samples on gold and silicon confirm the presence of a 544 *m/z* ion consistent with the molecular mass of the positively charged chelator molecule attached to molecular fragment C_2H_4. In addition, other $C_xH_yN_z$ fragments are present. Reference samples, including an alternative procedure for negative control (chelator immobilization reaction, but no silane on surface), the linker silane only on silicon, unmodified silicon, and unmodified gold exhibited negligible levels of this peak.

FET measurements in solution

Each device used in pyrosphosphate detection measurements was characterized in a variety of control conditions, including dry, blank buffer (no pyrophosphate), and no chelator modification. For dry current-voltage (IV) measurements, the backgate voltage (V_{bg}) was swept from -20 V to 0 V to -20 V at drain-source voltage (V_{ds}) set at 0.03 or 0.1 V. For wet IV measurements, polydimethylsiloxane (PDMS) was used to create a large well around several many devices on one side of the device die. To this was added 3-6 µL of the indicated solutions, such as control buffer, control buffer with Zn^{2+}, PPi in buffer with Zn^{2+}, and control buffer with Zn^{2+}, after incubation/rinse in 0.1 M acetic acid. For wet measurements, V_{bg} was swept from -15 V to 0 V (or 2 V) to -15 V with V_{ds} = 0.03 V and 0.1 V. After each wet measurement, the device and PDMS well were removed from the probe station and flushed with water. After flushing with water and control buffer, the PDMS was assembled on the device surface again and buffer solution was added. Illumination lights on microscope were turned off during measurements.

The figure in the main paper shows I_{ds}-V_{bg} curves for a chelator–modified FET device in tris control buffer before and after exposure to PPi. The Tris buffer contains $MgCl_2$, NaCl, and $Zn(NO_3)_2$. The curve marked "Buffer – Before" represents the measurement obtained after exposing the chelator-coated FET device to a control Tris buffer at pH8 and scanning the backgate voltage from -15 V to 2 V to -15 V. The curve marked "Buffer – with PPi" was obtained after rinsing the device with water and then exposing the device to the same type of buffer mixture with 25 µM PPi. The curve marked "Buffer – After" represents the measurement obtained after the device was exposed to 0.1 M acetic acid at pH3 for 5 minutes, rinsed with water, and then exposed to the original control buffer at pH8.

Figure S8 in this section demonstrates the relatively limited response of a polyethylene glycol (PEG) modified FET device to several different solutions: pH8 Tris buffer before and after PPi exposure, 25 µM PPi and 0.1 M acetic acid (pH3).

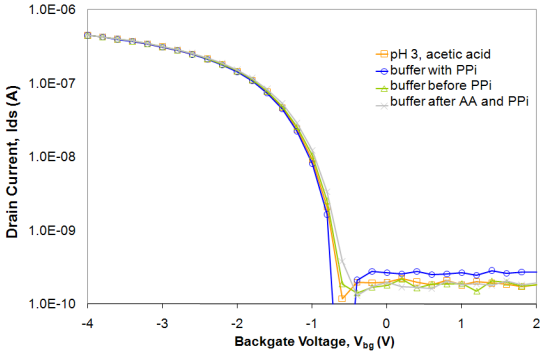

Figure S8. Relatively PPi-insensitive current-potential curve response of a HO-PEG_4-silane modified FET device to 25 μM PPi ('buffer with PPi'), 0.1 M acetic acid ('pH3, acetic acid'), Tris buffer before and after exposure to 25 μM PPi ('buffer before PPi' and 'buffer after AA and PPi')

References

1. R. G. M. Hanshaw, H. Jiang, B. D. Smith, *Tet. Lett.* 2004, **45**, 8721-4
2. M. S. Han, D.H. Kim, *Angew. Chem. Int. Ed. Engl.* 2002, **41**, (20), 3809-11.
3. Y.-S. Liu, P. P. Banada, S. Bhattacharya, A. K. Bhunia, and R. Bashir, *Appl. Phys. Lett.* 2008, **92**, 143902.
4. O. H. Elibol, B. Reddy, Jr., and R. Bashir, *Appl. Phys. Lett.* 2008, **93**, 131908.