Supporting Information

Single cell 'Glucose Nanosensor' verifies elevated glucose levels in individual cancer cells

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Materials and Methods

Chemicals:

Glucose Oxidase (GOx) from *Aspergillus Niger* ≥100,000 units/g (Type VII, lyophilized powder, EC Number 232-601-0), ferrocene (98 %), poly-l-lysine (PLL) (0.1 % solution in water), glutaraldehyde (GA) (25 % in water), L-ascorbate, zinc sulfate, copper sulfate, magnesium sulfate, sulforhodamine B and D-glucose were purchased from Sigma Aldrich. Silver wires were supplied from A-M Systems while uric acid (99 %) from Acros Organics. Dulbecco's Modified Eagle Medium (DMEM), trypsin (0.25 %, phenol red), penicillin-streptomycin and sterile phosphate buffered saline (PBS, pH 7.4) were bought from Gibco. 500 μm gridded plates were obtained from Ibidi.

Methods:

Nanopipettes were fabricated from quartz capillaries (with outside diameter of 1.00 mm and inside diameter of 0.70 mm bought (Sutter Instrument, Novato, CA)) using a P-2000 laser puller (Sutter Instrument, Novato, CA) as previously described.[1] A single pull cycle was used to fabricate nanopipettes. Pull parameters were heat=650, fil=3, vel=40, del=190 and pul=180. The mean diameter of the pore of the nanopipette is 106 $\pm$ 16 nm. Quartz nanopipettes were backfilled with a 10 μl solution containing 10 mM Ferrocene prepared in 100 mM PBS (pH 7, supplemented with 0.1 M KCl). An Ag/AgCl electrode was placed into the nanopipette as working electrode while another Ag/AgCl electrode immersed in the bath solution acting as a reference/counter electrode.

To use the nanopipette as a glucose nanosensor, we functionalized the nanopipette tip with GOx to the tip's inner surface using an Eppendorf Microloader. This was achieved by casting PLL to the inner walls of the nanopipette by backfilling the nanopipette with 0.01 % PLL solution (100mM phosphate buffer supplemented with10mM KCl) and baking the

nanopipettes at 120^0C for 30 min. Then nanopipette surface was treated with a 10 % (v/v) solution of glutaraldehyde (GA) in MilliQ water for 30 min. GOx was then reacted with the activated nanopipette walls. For this, a 6 mg/ml solution of GOx in PBS solution pH 7 was backfilled in the nanopipette and allowed to react with the activated glass surface at room temperature for 1 hour. Nanopipettes were backfilled using a microloader to ensure that only the tip proximity is exposed and centrifuged with 4 μl of solution for each step to minimize use of reagents as well as to limit surface modifications with nanopipette tip.

Nanopipette measurement setup:

The nanopipette instrument consists of an inverted microscope Olympus IX 70 with an attached eye piece camera (AM4023X Dino-Eye) to image cells. The nanopipettes are fixed to a microscope by a holder (Axon Instruments, Union City, CA). The holder is connected to an Axopatch 700B low-noise amplifier (Molecular Devices, Sunnyvale, CA) for current measurement, a MP-285 micromanipulator (Sutter Instrument, Novato, CA) for coarse control of the nanopipette positioning in the *X*, *Y*, and *Z* directions, a Nanocube piezo actuator (Physik Instrument, Irvine, CA) for fine control in the *X*, *Y*, and *Z* directions, and a PCIe-7851R Field Programmable Gate Array (FPGA) (National Instruments) for hardware control of the system. The system is operated using custom coded software written in LabVIEW. The amplifier was set to current-clamp (clamped at 1nA) mode with signal filter at 1kHz bandwidth. The signal was further digitized by an Axon Instruments Digidata 1322A. The data were then recorded by a LabView 9.0 home-made software, as described previously.[2]

While in the medium, the nanopipette tip is polarized with a positive bias preventing medium from flowing into the barrel. This bias potential generates an ion current through the liquid-liquid interface that is used as the input into a feedback loop analyzed by the SICM. Custom-designed software directs the nanopipette to the cell until it detects a 5 to 10 % drop

in the ionic current. At that point, the software stops the approach and initiates the penetration mode by lowering the nanopipette 0.8 μm at 100 nm/s velocity to pierce the cell membrane, and inserting the tip into intracellular media. The nanopipette is maintained inside the target cell for a pre-defined time of 60 seconds and then withdrawn to the previous location of the nanopipette tip. The procedure can then be repeated multiple times in the same cell.

Cell Culture:

Three different cell lines were used: BJ Human Fibroblasts were purchased from Stemgent (Cambridge, MA) and the breast cancer cell lines MDA-MB-231 and MCF7 were obtained from the Princeton Physical Sciences Oncology Center tissue biorepository. All cells were cultivated and maintained with normal DMEM cell media in the presence of glucose (25mM), 10% Fetal Bovine Serum and antibiotics (Penicillin and streptomycin). The glucose free media corresponded to DMEM with 10% Fetal Bovine Serum and antibiotics.

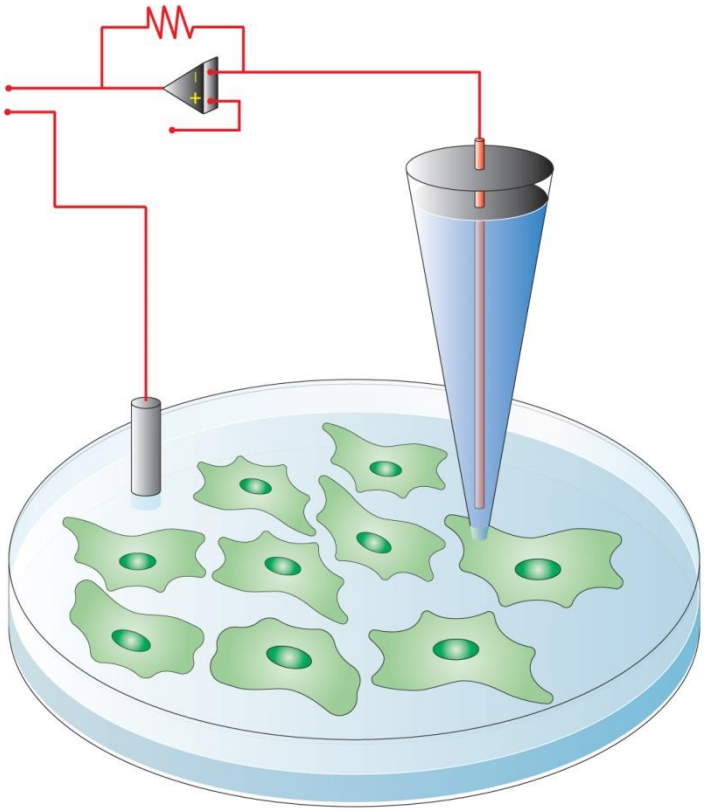

Figure S1 Schematic illustration of the single-cell glucose measurement with GOx-functionalized nanosensor. The nanosensor is backfilled with 10 mM PBS (pH 7.0) and a Ag/AgCl electrode is inserted, acting as working electrode. Another Ag/AgCl electrode is immersed into the bulk solution as auxiliary/reference electrode. All measurements are performed with an Axopatch 700B connected to a signal digitizer (DigiData 1322A) and the external resistor was 20 Ω.

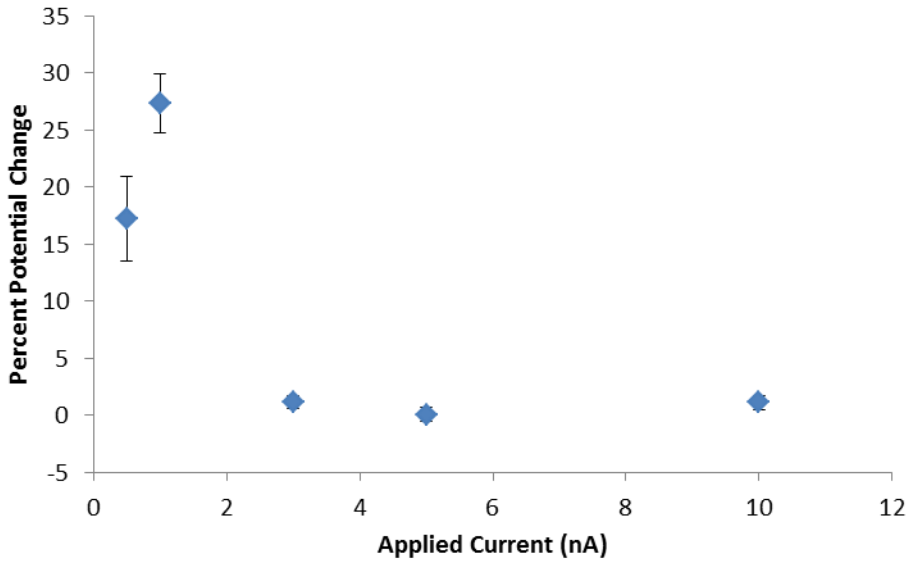

Figure S2 Optimization of applied current for glucose sensing. Percent potential change represents the response of glucose sensor to additions of 1mM glucose versus baseline signal. The error bars represent *n=3* replicate measurements.

Current-clamp is an electro-analytical technique in which a constant current is applied across working and counter electrodes. Applied current optimization is important for a sensitive glucose oxidase modified nanopipette. To optimize the applied current, we selected a range of current from 0.5 to 10nA, and evaluated the response of the sensors for 1mM glucose additions. The highest sensitivity was achieved at a current of 1 nA.

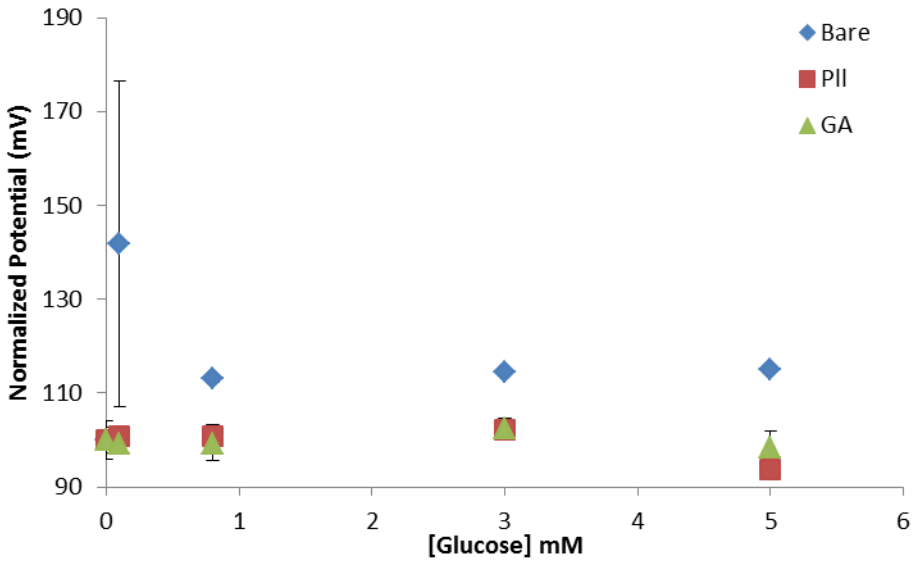

Figure S3 Potential response of quartz nanopipettes to glucose additions at different stages of surface modification. The error bars represent *n=3* replicate measurements. Supporting electrolyte was 10 mM PBS (pH 7.4).

To evaluate the effec of surface modifications of quartz nanopipettes, we measured the potential response as a function of glucose concentration. We spiked the bulk electrolyte with a glucose concentration range from 0.1 to 5 mM. The potential-response did not change, suggesting that the nanopipettes signal is only dependent to the presence of glucose oxidase at the tip but not the other surface finctionalities.

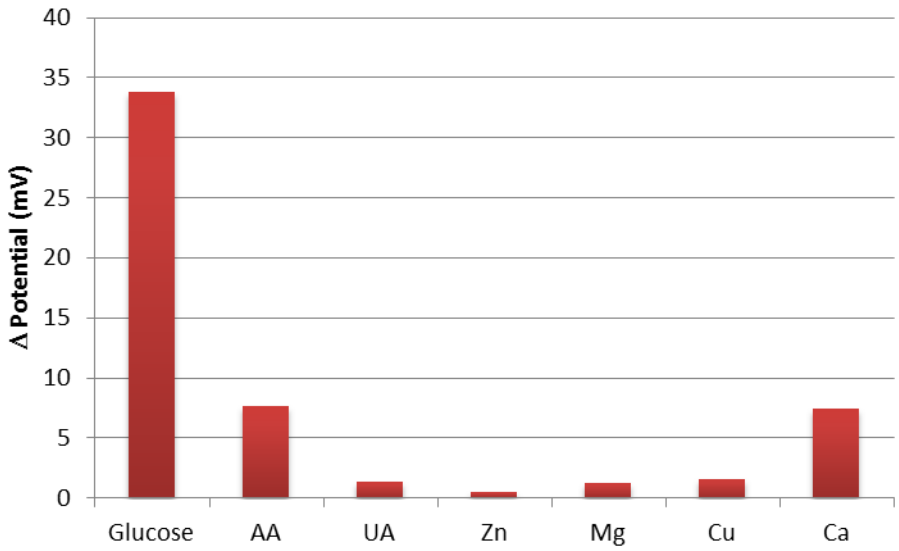

Figure S4 Detection selectivity and comparison of the responses of glucose *vs.* other common interferences present in physiological media at an applied current of 1nA. Supporting electrolyte was 10 mM PBS (pH 7.4).

The specificity of the glucose oxidase modified nanosensors was tested by the addition of biomolecules (100 μM ascorbic acid and uric acid) and ions (100 μM Zn^{2+}, Mg^{2+}, Cu^{2+} and Ca^{2+}) which are commonly present in cellular environment. Figure S4 shows the nanosensor selectivity of responses to these species versus the target analyte, glucose. For the concentration range, glucose oxidase modified nanosensor demonstrated high specificity for glucose compared to interferences. In other words the surface reaction taking place at nanopore was not significantly affected by the presence of other ionic and biochemical species; thus the potential-response of the nanopipette was mainly from the catalytic conversion of glucose by glucose oxidase.

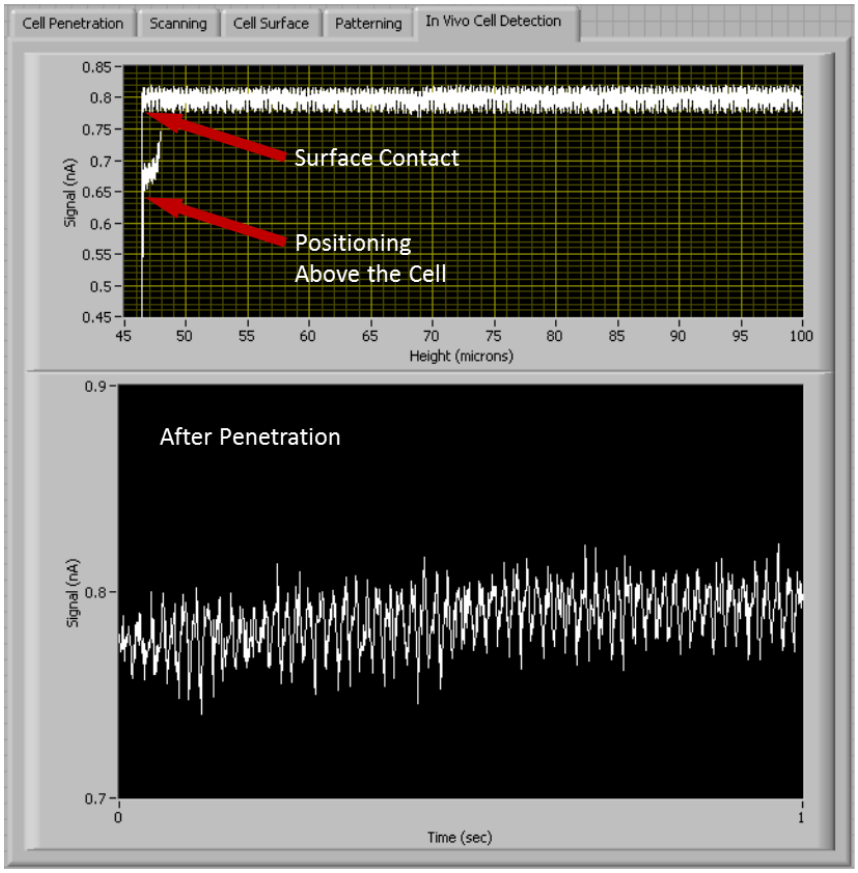

Figure S5 Live surface finding and penetration signals obtained from the customized SICM-based cell finding system.

We utilized our custom scanning ion conductance microscope (SICM) setup controlled with LabView software to perform cell finding and penetration with nanopipettes. Figure S5 displays real-time recordings of SICM during surface finding and after penetration. Top signal shows the change of ionic current *vs.* travel distance. A fixed potential of 500 mV is applied to perfrom surface finding and penetration steps. When the nanopipette is in close proximity to cell surface current dramatically drops due to a phenomenon called 'current squeeze', and nanopipette is positioned above the target cell. Then automatic penetration step takes place and current increases after the penetration (shown in bottom window).

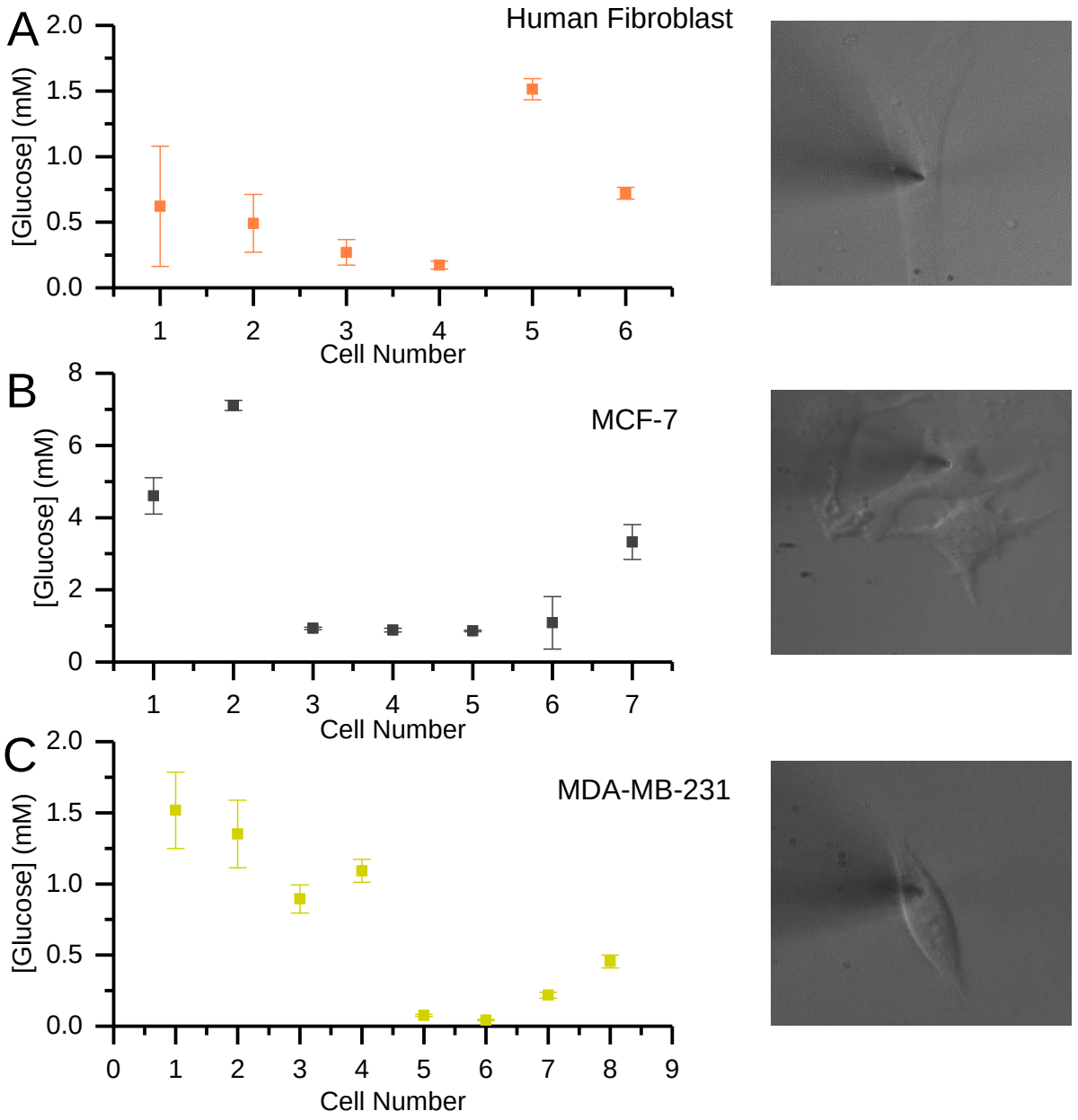

Figure S6 Intracellular glucose concentrations of individual cells measured by GOx-modified quartz nanopipette biosensor. Glucose levels recorded for **(A)** 6 human fibroblast, **(B)** 7 MCF-7 and **(C)** 8 MDA-MB-231 cells. Corresponding micrographs at the left panel demonstrate that intracellular measurements do not create any observable changes in cell morphology. Cells were incubated in 37 ^{o}C in 5% CO_2 atmosphere. Supporting cell media was 1X DMEM (supplemented with 10% FBS and 1% Pen/Strep) for all cell lines.

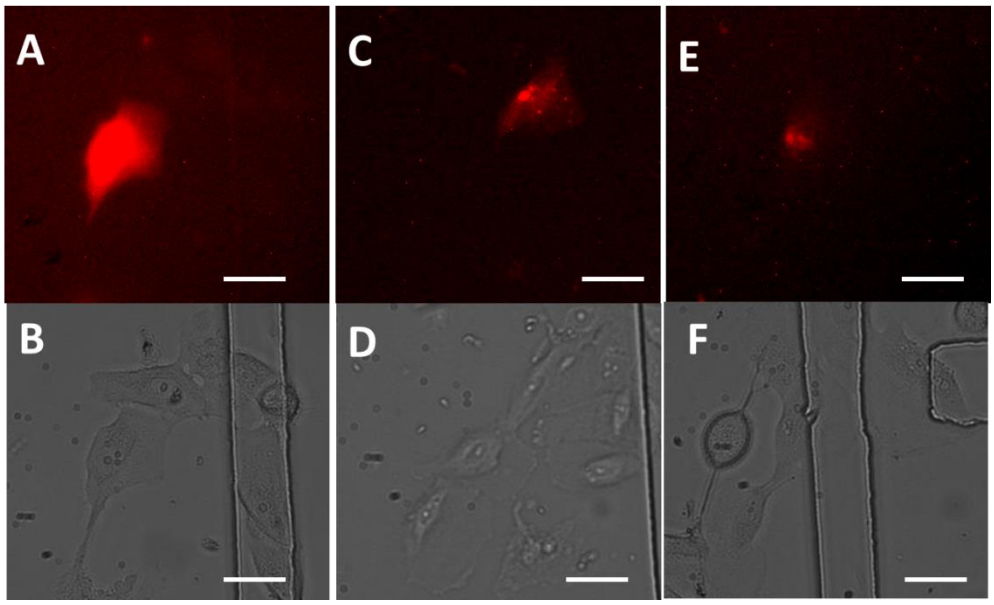

Figure S7 Investigation of viability of a cell after glucose measurement with a nanopipette. Rhodamine B is injected to the cell using a different nanopipette. Fluorescence pictures (top panel) are presented with respective bright field images at the bottom panel. The fluorescence micrographs are taken **(A)** 5 min, **(B)** 5 hrs and **(C)** 24 hrs post injection. This approach not only helps localizing the cell in later time even the shape and location is different but also demonstrates that the cell is alive after nanopipette manipulation was performed. Over time the fluorescence intensity decreases yet still significantly brighter at the emission wavelength.

References:

1. Ozel, R. E.; Lohith, A.; Mak, W. H.; Pourmand, N. *RSC Adv* **2015,** 5, 52436-52443.
2. Seger, R.; Actis, P.; Penfold, C.; Maalouf, M.; Vilozny, B.; Pourmand, N. *Nanoscale* **2012,** 4, 5843-5846.