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A Renewable Nanosensor Based on a Glass Nanopipette

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The development of sensors capable of rapidly and sensitively probing analyte concentration on the nanoscale is required in both physical science and biology.[1] Electrodes, ranging in size from micrometers to a few nanometers, have been used, either with potentiometric or amperometric detection,[2] with a time resolution limit down to a few microseconds[3] and sensitivity to detect single molecules and even individual electron-transfer events.[4] Analyte fluxes from cells have also been mapped.[5] However, for mapping analyte concentration, robust control of the probe−sample distance can be an issue in liquid requiring feedback techniques such as force based distance control.[6] Chemiluminescence[7] or, more generally, fluorescence detection is an alternative, and sensing beyond the diffraction limit is usually based on fiber-optic tips.[1] These tips are either functionalized with biological recognition elements[8] or reporter dyes attached on membranes[9] or by co-photopolymerization.[10] Micropipettes with reporter dyes attached using a slow-setting sol−gel glass have also been developed.[11] However, all these techniques rely on near-field fluorescence and require the attachment of fluorescent dye. This dye has to be functionalized and is also prone to photobleaching without replenishment.[12] Here we present a general method to measure local concentration using a 100 nm sized probe with any unmodified reporter fluorophore. This has a time resolution of milliseconds with the option of probe−sample distance feedback control.

Our nanosensor is simple to fabricate and is based on a principle described previously[13−15] where a charged fluorescent molecule, in this case a reporter dye, is trapped and concentrated in solution at the nanopipette tip, as shown in Figure 1. The dye is excited by focusing a laser beam using far-field optics at the 100 nm inner diameter pipette tip, producing a local nanosensor where the fluorescence is then dependent on analyte concentration in the bath. This has the flexibility of trapping essentially any reporter dye molecule at the tip in solution without copolymerization or extensive preparation. The dye at the tip is renewed from well inside the nanopipette so that the reporter dye is replenished, providing continuous measurements.

Here we show SNARF-1-dextran, a negatively charged ratiometric pH-sensitive fluorophore, to demonstrate the concept of the nanopipette as a pH sensor.[16] The sensor measured bath pH from 4.0 to 9.2 and responded to the addition of acid in less than 30 ms (see Figure 2a and Supporting Information). However, the stability of the nanosensor's fluorescence signal also enabled an intensity-based sodium sensor to be developed, using the negatively charged CoroNa Green dye. CoroNa Green (50 nM) was trapped at +5 V pipette voltage, and the fluorescence intensity was found to increase as a function of bath NaCl concentration, as shown in Figure 2b. Using the standard binding equation,[17] the intensity-based response

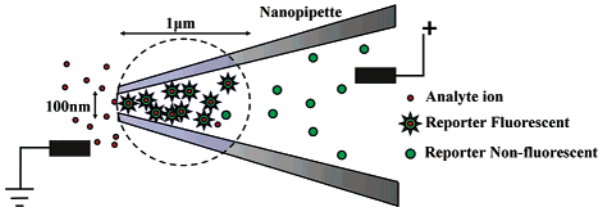

Figure 1. Schematic of the nanosensor: reporter dye molecules are trapped because of a balance of forces acting on the fluorophore in the nanopipette tip (see Supporting Information). Analyte ions diffuse into the tip and bind to the reporter molecules changing their fluorescence. This fluorescence is then collected by the confocal optics.

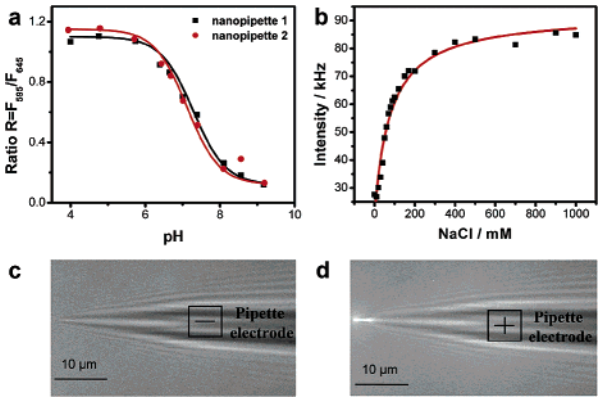

Figure 2. (a) Confocal ratiometric measurements of SNARF-1-dextran (excited at 488 nm) trapped at the nanopipette tip, with varying pH for two different pipettes. (b) Confocal fluorescent intensity measurements of CoroNa Green with varying NaCl concentration. (c) Global illumination of the nanopipette with CoroNa Green but at a negative pipette voltage. (d) Global illumination showing trapped CoroNa Green dye with a positive pipette voltage.

of CoroNa Green to physiological levels of sodium in bulk solution yielded the corresponding K_d of 79 ± 11 mM, close to the literature value of 80 mM.[18]

The ability to spatially and temporally map a nanopipette-based sodium source was first demonstrated with global laser illumination (see Supporting Information). The sodium source consisted of 5 M NaCl loaded into the "source" 100 nm diameter nanopipette and mounted on a separate micromanipulator. Application of a positive voltage to the source pipette electrode caused it to dose out Na^+ ions which were detected by the "sensor" nanopipette as an increase in fluorescence (see Figure 3 and Supporting Information videos). This was then repeated confocally for optimum sensitivity.

The concentration dependence $c(r)$ with radial distance r was fitted to steady-state diffusion from a hemispherical pore[19] (see Supporting Information and Figure 4a). Intensity changes near the tip could be mapped out down to the 200 nm steps of the

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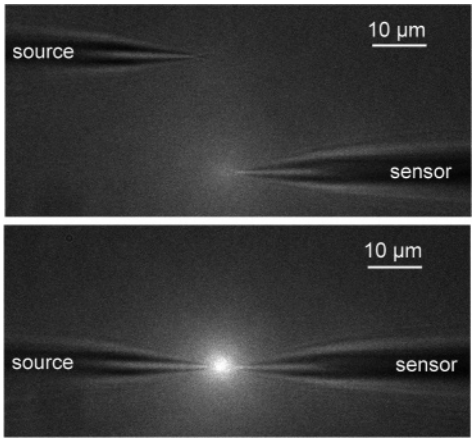

Figure 3. Mapping of the pipette nanosource: global images of the lateral mapping, showing the fluorescent response of the nanosensor to the proximity of the nanosource.

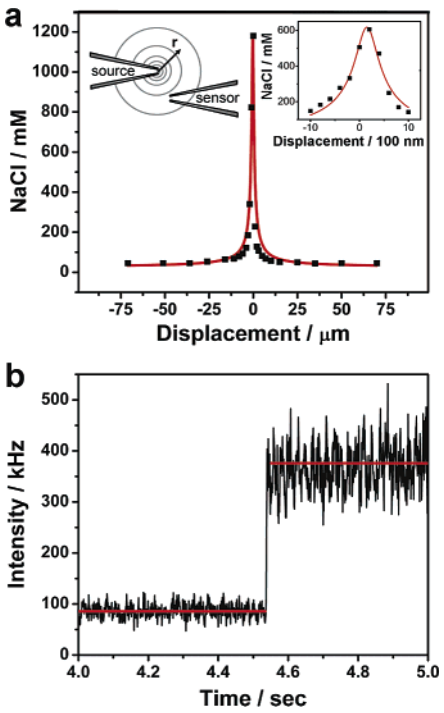

Figure 4. (a) Confocal fluorescent intensity measurement of the mapping fitted to the steady-state diffusion from a hemispherical electrode. Inset: (1) theoretical treatment of mapping the NaCl concentration using a hemispherical electrode source; (2) detailed mapping of the tip area with fwhm of 830 nm fitted to the source diffusion separation of 240 nm. (b) Nanosensor time response of 1.9 ± 1.4 ms due to the nanosource being triggered by application of a positive voltage of +10 V.

micromanipulator. The fwhm measured from mapping the nanosource at ~240 nm separation[20] was 830 nm. This corresponds to a sensor resolution of ~600 nm (see inset of Figure 4a and Supporting Information). When the source and sensor nanopipettes were aligned, a more accurate measure of the time resolution of the sensor was found to be 1.9 ± 1.4 ms (Figure 4b).

In summary, we have demonstrated the ability to detect both ratiometric and intensity-based fluorescent changes for physiological levels of pH and sodium, respectively, using a simply fabricated nanosensor. The method should be generally applicable to any fluorescence-based reporter dye and thus a wide range of analytes and concentrations. The time resolution has been measured to be ~2 ms, and the spatial resolution of 600 nm demonstrated here can be further improved by reducing the probe−sample separation. Simultaneous confocal microscopy and scanning ion conductance microscopy, where distance feedback control allows the probe−sample separation to be reduced to the pipette radius of 50 nm, have already been demonstrated on live cells.[21] This opens up the possibility for nanoscale mapping of analytes over living cells using this new sensor.

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Supporting Information Available: Experimental details, supporting figures and videos. This material is available free of charge via the Internet at http://pubs.acs.org.

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