Supplemental Information

1. Ruggedness and reproducibility of the integrated separation-based sensor.

The ruggedness of the system and its ability to perform while in motion, akin to that in a *freely roaming* animal, was evaluated. Two motion tests were performed. The first was a cart test in which the system was placed on a rolling cart in motion. The system was set up for the analysis of a 1 mM solution of nitrite and 1 mM peroxide in 10 mM borate with 2 mM TTAB at pH 9.2 as the perfusate. The perfusate was introduced into the microfluidic device using direct injection via a CMA 107 syringe pump at a flow rate of 1 $\mu L/min^{-1}$. The separation buffer was 10 mM borate with 2 mM TTAB at pH 9.2 and a voltage of –1600 V was applied at the buffer reservoir for the separation. The platinum working electrode was held at 1.1 V vs. a platinum counter electrode. The second motion test performed used a Stovall Belly Dancer™ Shaker, 220/240VAC (Greensboro, NC) with the system being shaken at various rates of rotation for a series of injections. System analysis was performed under the same conditions as stated previously for the cart test.

To evaluate how reproducible the response of the system was over an extended period of time, a 1 mM solution of nitrite in 50 mM phosphate buffer was used as the perfusate and was introduced into the microfluidic device using direct injection via a CMA 107 syringe pump at a flow rate of 1 $\mu L/min^{-1}$ over a 24-h period. A sample injection was performed every 2 min during the course of the experiment. The separation buffer was 50 mM phosphate at pH 7.4, and a voltage of –1600 V was applied at the buffer reservoir for the separation. The platinum working electrode was held at 1.1 V vs. a platinum counter electrode. The buffers in the buffer reservoir, buffer waste, and

sample waste were manually replenished during the experiment to prevent ion depletion from affecting the separation.

The device was tested for ruggedness in anticipation of the environment that the system would encounter when deployed on a *freely roaming* animal. One of the largest concerns for the system was its ability to perform continuously while the animal was in motion. A cart test was performed to evaluate the system under random and vigorous motion to ensure that movement did not interfere with the electrophoretic separation and detection (Figure S1). The cart was pushed at random speeds and decelerated, both over time and immediately, to assess how this type of movement would impact the function of the system. Excessive noise can be attributed to bumping the cart vigorously into hard surfaces and the rapid deceleration due to forced impact with vertical support structures.

A more rhythmic motion was also tested to simulate noise caused by locomotion. In an effort to simulate this, a Stovall Belly Dancer™ Shaker was used at various rates of rotation. A slight increase in baseline noise of approximately ±0.6 nA due to the simulation locomotion was seen (Figure S2). However, this increase did not disrupt the electrophoretic separation or the detection of target analytes.

2. *In vitro* evaluation of on-line microdialysis-microchip electrophoresis with electrochemical detection using DEA-NONOate

The production of nitrite from DEA-NONOate was performed using a BASi 1 cm loop microdialysis probe secured inside a 2 mL reaction vial containing 1 mL of 50 mM phosphate buffer at pH 10 along with 100 μL of 48 mM DEA-NONOate. The microdialysis perfusate consisted of 50 mM phosphate buffer at pH 7.4. The flow rate

was 1 μL/min. To initiate the reaction, the pH in the reaction well was carefully lowered to pH 7.4 using 0.05M HCl. The separation buffer was 50 mM phosphate at pH 7.4, and a voltage of –1600 volts was applied at the buffer reservoir for the separation. Finally, the platinum working electrode was held at 1.1 volt vs. a platinum counter electrode.

In an effort to test the ability of the system to monitor the generation of nitrite production *in vivo,* an *in vitro* experiment was performed. This was accomplished through monitoring nitrite production from the acid-catalyzed decomposition of DEA-NONOate. At pH values below 8, DEA-NONOate rapidly releases nitric oxide by spontaneous disassociation; this readily oxidizes to the more stable oxidation product, nitrite, in the presence of oxygen. Release was accomplished by lowering the pH of a vial containing DEA-nanoate and following the production of nitrite from 10 to 7.4 as a function of time (Figure S3). The series of electropherograms shown started at 4 min after the pH was lowered to 7.4. There was no visible peak for nitrite before or during the initiation of the reaction. The peak intensity then rapidly increased from a 0.4 nA peak for the first injection, as shown in Figure S3, to a 1.7 nA peak for the last injection at the 15-min mark. After this time, the concentration of nitrite within the sample stabilized and no increase in peak intensity was seen. Figure S3 shows the electropherograms obtained for the release of nitrite with the nitrite peak highlighted.

3. Optimization of buffer system for *in vivo* analysis

In vivo experiments must be conducted using a perfusate that is compatible with the tissue being sampled to maintain tissue integrity. Therefore, the perfusate was modified from that used for the *in vitro* experiments to minimize disturbance of the native

electrolyte levels for the subcutaneous sampling experiments. The subcutaneous sampling perfusate buffer or "pseudo saline" consisted of a 50 mM phosphate buffer with 119 mM NaCl, and the separation buffer consisted of 50 mM phosphate buffer at pH 7.4. The perfusate solution could not be used as the run buffer because the additional NaCl increased the conductivity of the buffer, leading to extremely high currents and Joule heating.

The double T design used for these studies allowed the use of contrasting conductivity buffers for the microdialysis perfusate and the electrophoresis separation. . In this application, we found that the addition of NaCl to the perfusate was actually beneficial and produced isotachophoretic stacking of nitrite, leading to an improvement in the LOD for this analyte. The chloride ions in the perfusate acted as the leading electrolyte while the phosphate ions in the run buffer acted as the terminating electrolyte.[Figure S4 shows the effects of the perfusate composition on the separation and the corresponding peak height for nitrite. The electropherogram for nitrite using a 50 mM phosphate buffer for the perfusate and 50 mM phosphate buffer for the separation is shown in Figure S4A. By adding 119 mM NaCl to the perfusate, to create a pseudo saline solution, and keeping the separation buffer the same, a distinctive dip in the baseline (Figure S4C) due to the NaCl can be seen. The addition of NaCl also caused an increase in signal intensity for nitrite as shown in Figure S4B.

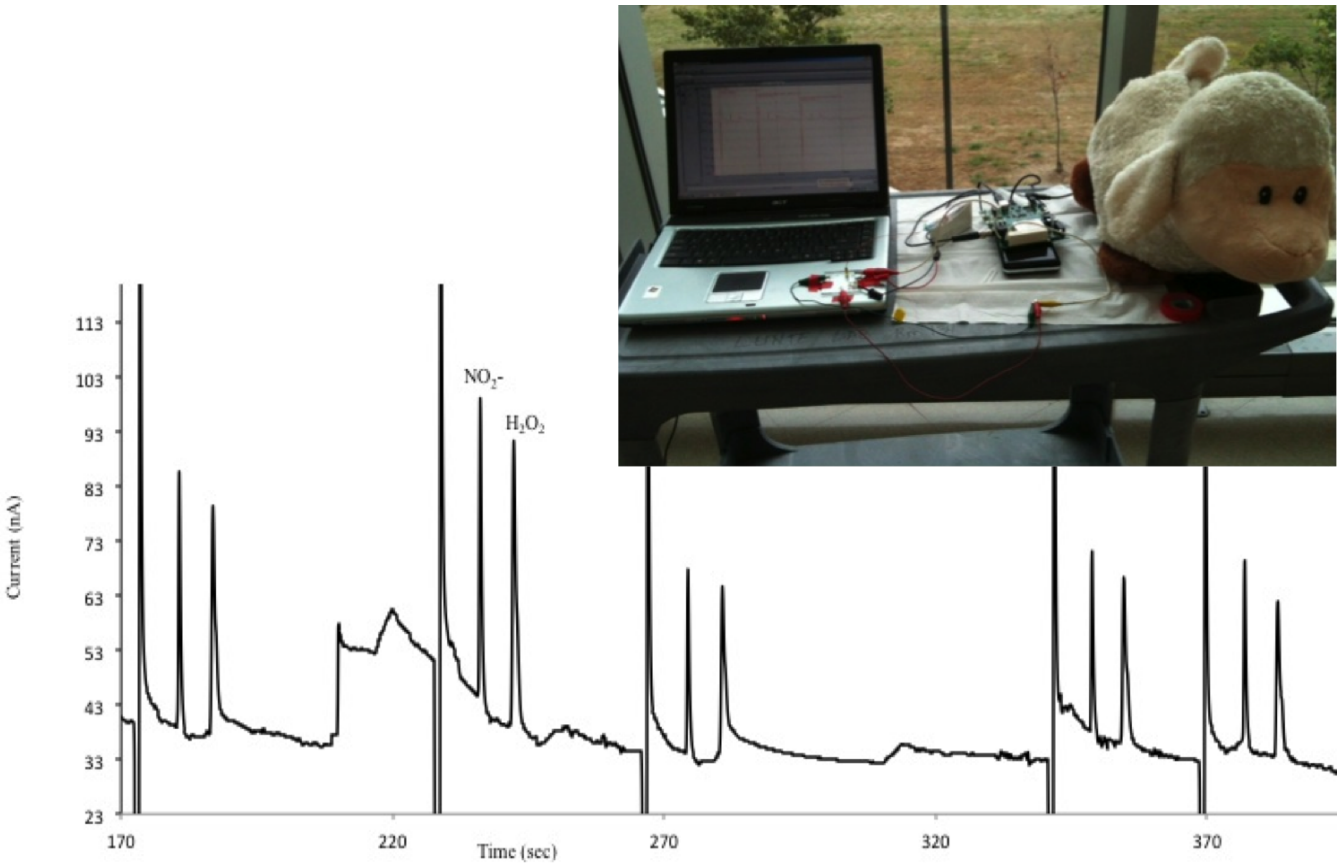

Figure S1. A "cart test" for the analysis on the effects of rapid and vigorous motion. Perfusate contained 1 mM nitrite with 1 mM peroxide in 10 mM boric acid buffer with 2 mM TTAB. Separation buffer: 10 mM boric acid with 2 mM TTAB. Separation voltage was –1600 V with a 1 s injection and the working electrode potential set to 1.1 V versus platinum pseudo reference electrode. Pump flow rate was set at 1 μL/min.

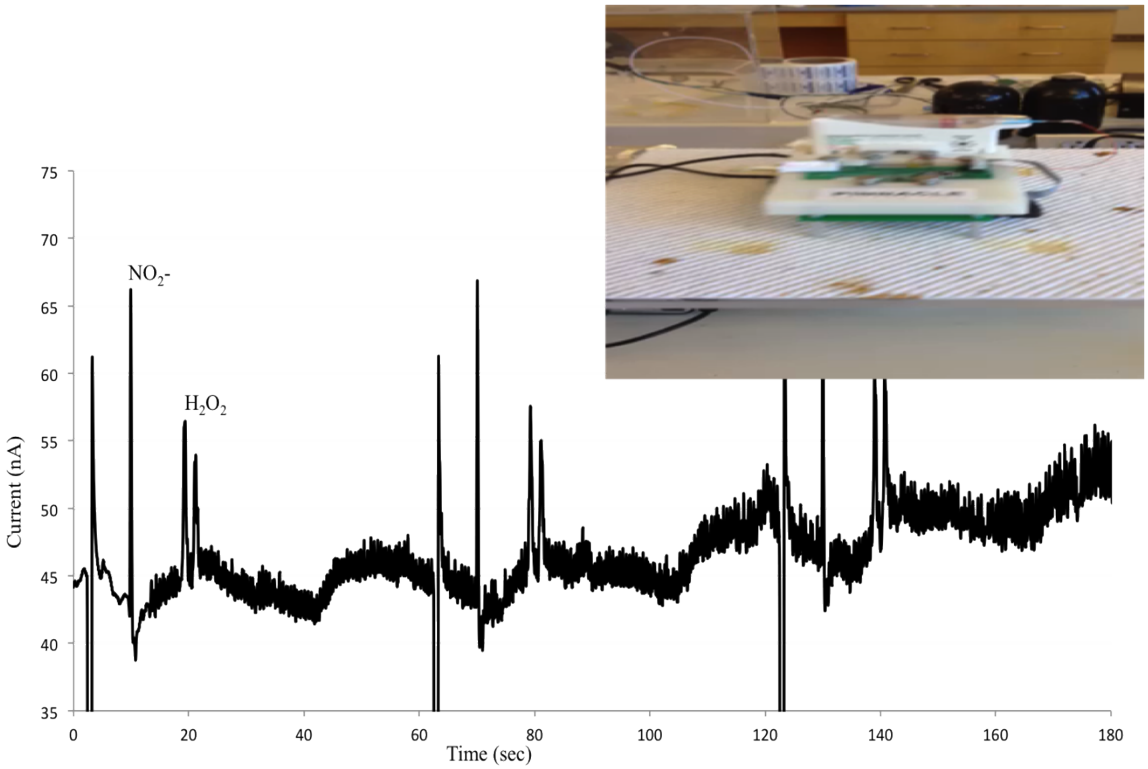

Figure S2. A Stovall Belly Dancer™ Shaker, 220/240VAC, with the system being shaken at various rates of rotation for analysis of the effects on system performance due to motion. Operating conditions are identical to those in Figure S1.

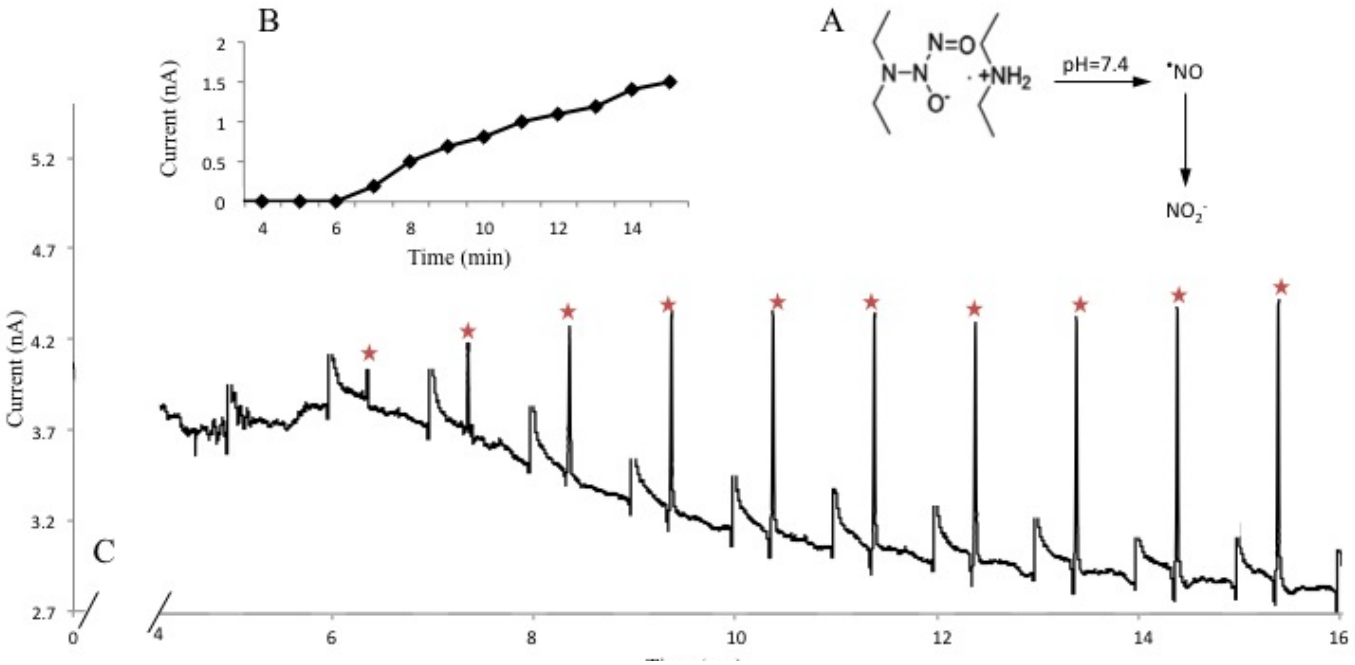

Figure S3. Monitoring nitrite production by DEA NONOate. A 2-mL reaction well holding the microdialysis probe contained 1 mL of 50 mM phosphate buffer at pH 10 and 100 uL of 48 mM DEA NONOate. HCl was added to the reaction well to reach pH 7.4 and stimulate nitrite production. Separation buffer and perfusate: 50 mM phosphate buffer at pH 7.4. Flow rate was 1 μL/min^{-1}. (A) DEA NONOate reaction. (B) Electropherogram of nitrite production over time t = 0 is 4 min after start of reaction. (C) Peak height vs. time for nitrite is shown using MD-MCE system.

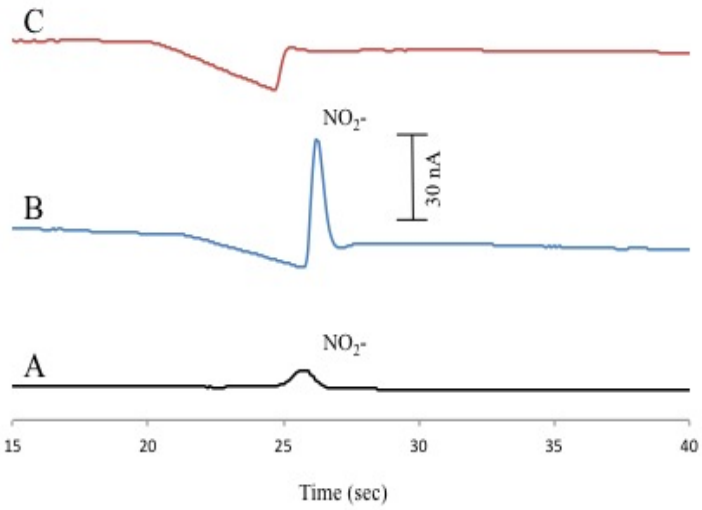

Figure S4. Effect of perfusate composition on response for 1 mM nitrite. (A) 50 mM phosphate buffer. (B) 50 mM phosphate buffer containing 119 mM NaCl. (C) Blank using a perfusate of 50 mM phosphate buffer and 119 mM NaCl. Separation buffer: 50 mM phosphate at pH 7.4. Separation voltage was –1600 V with a 1-s injection, and the electrode bias set to 1.1 V with platinum counter. Pump flow rate was set at 1 μL/min^{-1}.