Glucose driven catalytic nanomotor to create motion at micro scale

Arun Kumar[1, *], Hideyo Takatsuki[2], Chang K. Choi[3], Ayusman Sen[4], Eric Blough[2, 5]

[1]Nanomedicine Research Laboratory, Department of Medical Laboratory Science, College of Health Sciences, University of Delaware, Newark, DE 19716, USA; [2]Center for Diagnostic Nanosystems, Marshall University, Huntington, WV 25755,USA; [3]Mechanical Engineering-Engineering Mechanics, Michigan Technological University, Houghton, MI 49931, USA; [4]Department of Chemistry, Pennsylvania State University, University Park, PA 16802, USA; [5]Department of Pharmaceutical Sciences and Research, School of Pharmacy, Marshall University, Huntington, WV 25755, USA.

Received: January 23, 2013; accepted: October 2, 2013.

Catalytic bionanomotors were constructed by selectively immobilizing glucose oxidase onto the surface of platinum / gold bi-metallic rods. Comparison of non-functionalized and functionalized nanorods using Fourier transform infrared spectroscopy was consistent with the presence of protein adsorption onto the surface of the nanorods. Incubation of functionalized, but not non-functionalized, nanorods in glucose solution (1 – 5 M) resulted in nanorod translocation which was found to vary as a function of glucose and glucose oxidase concentration. Taken together these data suggest that the fabricated device can undergo glucose dependent movement and can be used as another technique for sensing glucose.

***Corresponding author:** Arun Kumar, College of Health Sciences, University of Delaware, 305 Willard Education Hall Building, Newark, DE 19716, USA. Phone: +1 302 831 4552. Fax: +1 302 831 4180. E-mail: arunk@udel.edu.

Introduction

The enzymatic conversion of chemical energy to produce motion is widespread in nature and is the basis of muscle contraction [1]. Other applications using this process include intracellular transport, e.g. vesicle transport by kinesin and the movement of ions up a concentration gradient. Although already exploited by the cell, the creation of man-made miniature "engines" that can convert stored chemical energy to motion remains a challenge. Recent work has demonstrated that the catalytic decomposition of hydrogen peroxide can be used to propel micro scale objects on a water surface [2-4]. So called "camphor motors", in which small particles of camphor move at the air water interface by slowly dissolving and spreading a hydrophobic "wake" behind them have also shown promise [5, 6]. Herein, we report our work regarding the development of a "glucose powered" bio-catalytic nanomotor fabricated from platinum/gold (Pt/Au) nanorods that have been selectively functionalized with the enzyme glucose oxidase. Our findings suggest that this approach may have applicability for the development of new types of micro scale transport [7].

Materials and Methods

1. Fabrication of Pt/Au nanorods

Bimetallic Pt/Au nanorods were synthesized electrochemically using alumina membranes as reported previously [8]. Nanorods were

characterized by transmission electron microscopy as outlined elsewhere [9].

2. Immobilization of glucose oxidase onto the surface of the Pt/Au nanorods

Nanorods were cleaned with absolute ethanol (3 times for 5 mins) and dried under nitrogen before immersion in 50 mM PBS (pH 7.5) containing glucose oxidase (GOx) (5-10 mg/ml). A deposition time of 90s at room temperature yielded maximum nanorod velocity at 5 mg/ml of GOx and was chosen to evaluate the effect of GOX concentration on nanorod movement (data not shown). Immediately after adsorption, samples were rinsed with PBS buffer (pH 7.2) and then rinsed with deionized water to remove any residual salt or weakly adsorbed enzyme. After washing, samples were dried with nitrogen for 2 min and stored at -20 °C.

3. FTIR (Fourier transform infrared spectroscopy) analysis

Nanorods were washed three times with distilled water and then rinsed with 50% ethanol two times. After drying, the FTIR spectra were collected at room temperature using a spectrophotometer (iS10 Thermo-Fisher Scientific).

4. Evaluation of nanorod movement

Nanorods were suspended in water (100 µL) containing different concentrations of glucose (0 - 5 M). Flow cells were constructed using double-sided tape (NW-10, Nichiban Co, Japan) to form a chamber between the coverslip and a glass slide. Movement of the nanorods was observed at room temperature (20 - 23°C) using a microscope (Olympus BH-2, Japan) with a 40x objective through a CCD camera (Hamamatsu Photonics, Japan) and digitally recorded onto a computer (Dell, Dimension 4300). Video clips were collected at 2 frames/sec over a 30 sec time period and analyzed using Image J (http://rsb.info.nih.gov/ij/). Motion analysis was performed by examining all of the nanorods in each of the image frames tracking and the leading end of each nanorod.

5. Statistical analysis

Results are presented as mean ± SEM (Standard Error Mean). Comparisons between groups were performed using the Students t-tests or two-way analysis of variance (ANOVA) and post-hoc testing as appropriate. The level of significance accepted a priori was $P \leq 0.05$.

Results

1. Characterization of nanorod size and protein adsorption

Scanning electron microscopy after carbon sputtering showed the Pt/Au nanorods to be ~370 nm in diameter and ~2 µm in length (Figure 1). FTIR spectroscopy was used to determine whether the adsorption procedure resulted in the deposition of protein onto the surface of the Pt/Au nanorods. Compared to that observed for the non-functionalized nanorods, the FTIR spectrum of the functionalized nanorods showed evidence consistent with the presence of protein (GOx) on the nanorods (Figure 2). Specifically, in the functionalized samples we noted changes in the amide I (1700 - 1600 cm^{-1}) and II (1600 - 1500 cm^{-1}) bands. It is thought that the amide I band is caused by C=O stretching vibrations of peptide linkages in the protein backbone while the amide II band results from a combination of N-H in plane bending and C-N stretching of the peptide groups.

2. Glucose driven movement

Motion analysis of nanorods in solutions with and without glucose suggested that translocation velocity increased with increasing glucose oxidase and glucose concentration (V_{avg}: un-functionalized nanorod (no glucose oxidase): 0.0 ± 0.0 µm/s; V_{Avg}: functionalized nanorod 0.1 (mg/ml) glucose oxidase: 0.4 ± 0.05, 0.9 ± 0.1, 1.3 ± 0.1, 1.4 ±0.2, and 1.4 ± 0.2 µm/s for 1, 2, 3, 4, and 5 M glucose respectively; V_{Avg} 0.5 (mg/ml) glucose oxidase: 1.2 ± 0.1, 1.4 ± 0.2, 1.6 ± 0.2, 1.8 ± 0.2, and 2.3 ± 0.3 µm/s for 1, 2, 3, 4, and 5 M glucose, respectively; V_{avg} 5.0

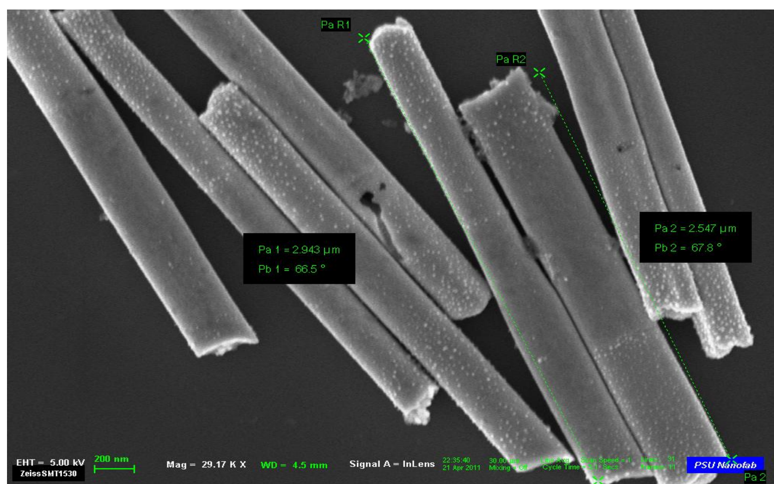


Figure 1. SEM analysis of nanorod dimensions.

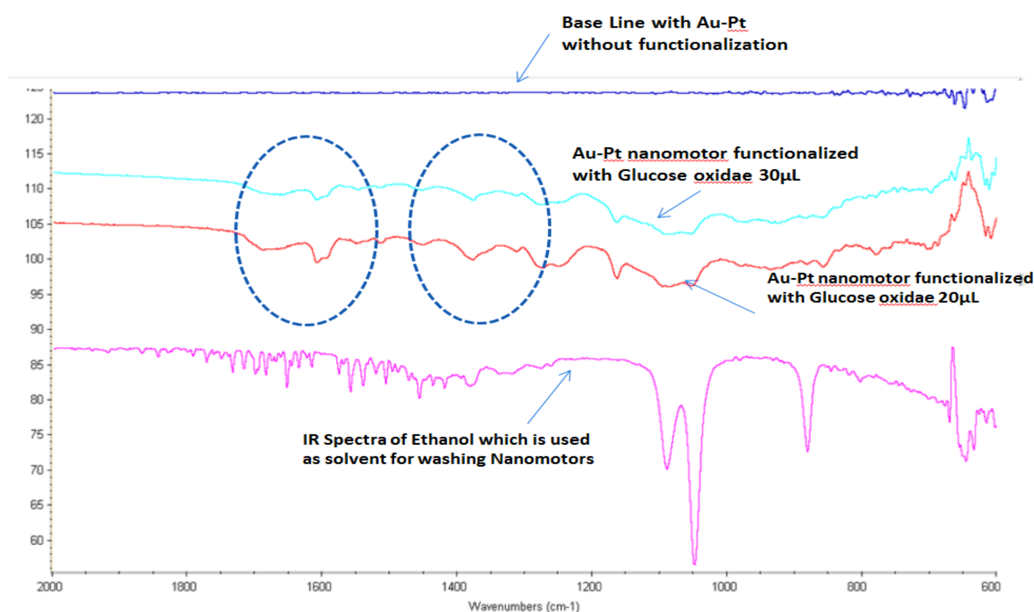


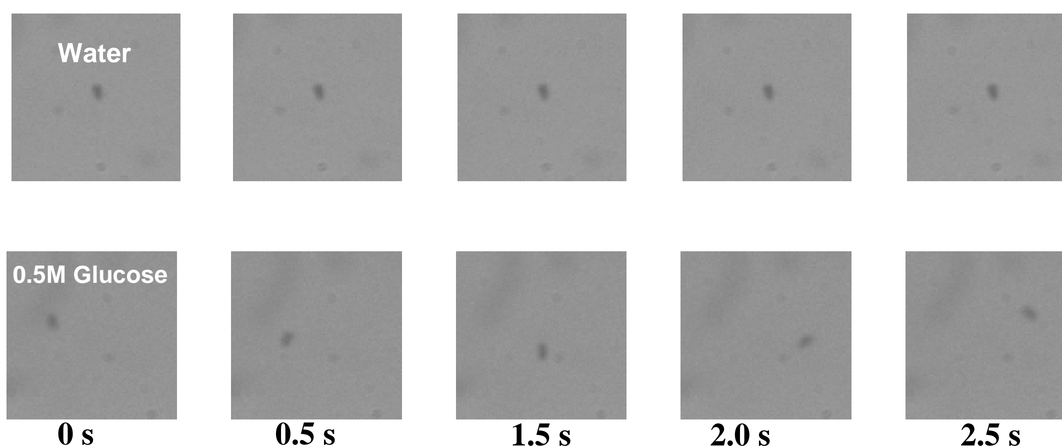
Figure 2. FTIR spectrum of immobilized GOx over the surface of the nanorods. Au-Pt nanorod after incubation with 30 µL of 10 mg/ml GOx (A) and 20 µL of 10 mg/ml GOx (B). FTIR spectrum of ethanol wash (C).

(mg/ml) glucose oxidase: 1.4 ± 0.2, 1.5 ± 0.2, 1.7 ± 0.3, 2.1± 0.2, and 1.8 ± 0.2 µm/s for 1, 2, 3, 4, and 5 M glucose, respectively (Figure 3).

Discussion

Previous work has demonstrated that bimetallic Pt-Au nanomotors can be powered by hydrogen peroxide (H_2O_2) fuels [3, 9]. Although not fully understood, it is thought that the motive force is derived from platinum catalyzing the decomposition of H_2O_2 to water and oxygen which gives rise to an interfacial tension gradient that is continuously re-established due to the catalytic nature of the platinum [10]. Although quite promising, the application of these types of Pt-Au nanomotors to biological

Panel A



Panel B

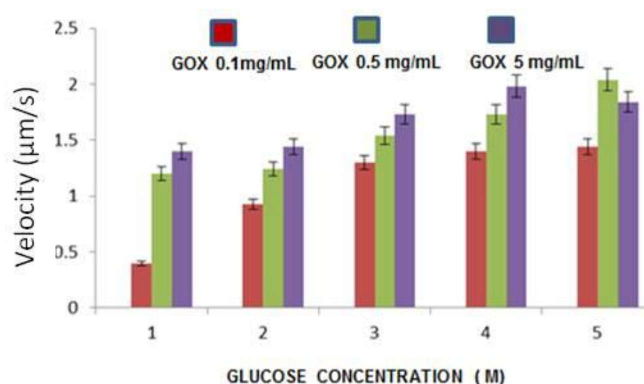


Figure 3. Image series showing the movement of functionalized (0.5 mg/ml GOx) nanorods in water in an in 0.5 M glucose (Panel A). Velocity of nanorods as a function of different glucose oxidase and glucose concentration (Panel B).

systems has been hampered by the requirement of having to use a potentially toxic fuel source. In an attempt to circumvent this limitation, we investigated whether the coupling of glucose oxidase to the surface of the Pt-Au nanorods allows these structures to utilize glucose as a fuel source.

Glucose oxidase (GOx) is an oxido-reductase that catalyzes the oxidation of glucose to hydrogen peroxide and D-glucono-δ-lactone [10]. Besides its role in the metabolism of sugars, it acts as a bactericide in many cells [11]. GOx is widely used in industry and is also routinely employed in enzyme electrode biosensors designed to detect glucose levels. In our approach we immobilized the GOx enzyme to the surface of the Pt-Au nanorods via physioadsorption. We hypothesized that the GOx, when exposed to glucose, would catalyze the production of H_2O_2 which then be broken down by the platinum portion of the nanorod to release electron per molecule and provide the motive force needed to move the nanorods (Figure 4). Binding of protein to the surface was characterized by FTIR (Figure 2). After confirming immobilization of GOx, we incubated functionalized nanorods in solutions of varying glucose concentration. The nanorods exhibited different behavior in the presence and absence of glucose. As expected, incubation of the functionalized nanorods in

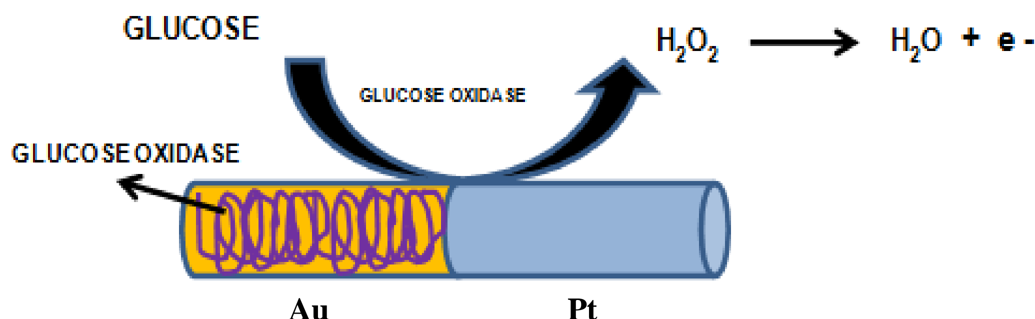


Figure 4. Schematic representation of proposed mechanism of nanorod movement.

water gave rise to Brownian motion; however, when placed in glucose containing solutions (1 - 5 M) nanorod translocation was clearly evident. In addition, the velocity of translocation appeared to vary in a glucose and glucose oxidase dependent fashion (Figure 3, Panel B).

Taken together, these data demonstrate that it is possible to power the motion of a nanoscale object by using glucose as a fuel source and surface enzymatic reactions. Although the glucose concentrations used in the present study were non-physiological it is possible that other immobilization strategies could give rise to great GOx density onto the surface of the nanorods thus allowing movement at lower glucose concentrations. Experiments toward this end are currently in progress.

Acknowledgement

Grant support for this project was funded by DOE funding #DE-SC0005162 to E.R.B. and startup funds to Arun Kumar at University of Delaware.

Reference

1. Pantaloni D, Clainche CL, Carlier MF. 2001. Mechanism of actin-based motility. Science **292**(5521): 1502-1506.
2. Kagan D, Laocharoensuk R, Zimmerman M, Clawson C, Balasubramanian S, Kang D, Bishop D, Sattayasamitsathit S, Zhang L, Wang J. 2010. Rapid delivery of drug carriers propelled and navigated by catalytic nanoshuttles. Small **6**(23): 2741-2747.
3. Laocharoensuk R, Burdick J, Wang J. 2008. Carbon-nanotube-induced acceleration of catalytic nanomotors. ACS nano **2**(5): 1069-1075.
4. Paxton WF, Sundararajan S, Mallouk TE, Sen A. 2006. Chemical locomotion. Angew Chem Int Ed Engl. **45**(33): 5420-5429.
5. Nagayama M, Yadome M, Murakami M, Kato N, Kirisaka J, Nakata S. 2009. Bifurcation of self-motion depending on the reaction order. Phys Chem Chem Phys **11**(7): 1085-1090.
6. Nakata S, Doi Y, Kitahata H. 2004. Synchronized motion of a mobile boundary driven by a camphor fragment. Journal of colloid and interface science **279**(2): 503-508.
7. Retterer ST, Siuti P, Choi CK, Thomas DK, Doktycz MJ. 2010. Development and fabrication of nanoporous silicon-based bioreactors within a microfluidic chip. Lab chip 10(9): 1174-1181.
8. Simmchen J, Baeza A, Ruiz D, Esplandiu MJ, Vallet-Regí M. 2012. Asymmetric Hybrid Silica Nanomotors for Capture and Cargo Transport: Towards a Novel Motion-Based DNA Sensor. Small 8(13):2053-2059.
9. Wang Y, Fei ST, Byun YM, Lammert PE, Crespi VH, Sen A, Mallouk TE. 2009. Dynamic interactions between fast microscale rotors. J Am Chem Soc **131**(29): 9926-9927.
10. Free AH. 1963. Enzymatic Determinations of Glucose. Advances in Clinical Chemistry **6**: 67-96.
11. Carroll SF and Martinez RJ. 1981. Antibacterial peptide from normal rabbit serum. 3. Inhibition of microbial electron transport. Biochemistry **20**(21): p. 5988-5994.