Electronic Supporting Information (ESI)

Magnetic carbon allotrope catalytic micromotors for 'on-chip' molecular operations

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Supporting videos

SI Video 1. Navigation of MWCNTs-Ni-PtNPs micromotors in the different reservoirs of a PDMS chip

SI Video 2. *'Stop-turn'* motion of CB-Ni-PtNPs micromotors under a magnetic field

SI Video 3. *'On-chip'* dynamic loading and unloading of Rhodamine 6G using CB-Ni-PtNPs micromotors

SI Video 4. *'On-chip'* capture-transport of N-Acetylglucosamine modified particles with WGA modified MW-Ni-PtNPs micromotors containing a protective OPD layer

SI Video 5. Control experiments using WGA modified micromotors

SI Video 6. Control experiments using ConA modified micromotors

Figures

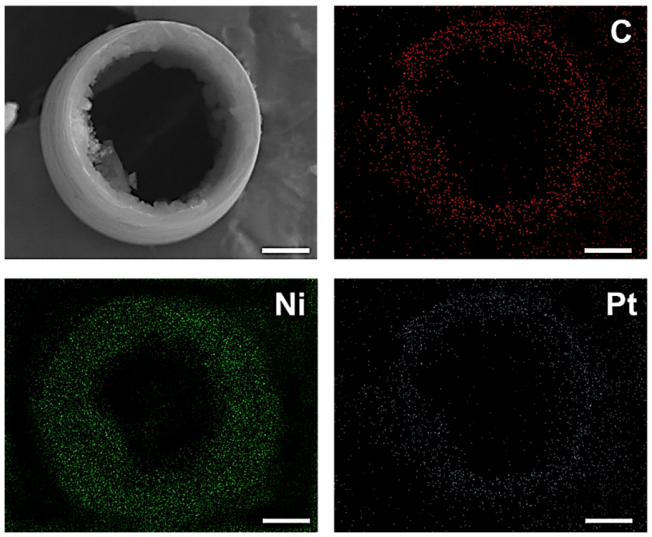


Fig. S1. EDX and SEM images showing the element distribution of a CB-Ni-PtNPs. Scale bar, 1 µm.

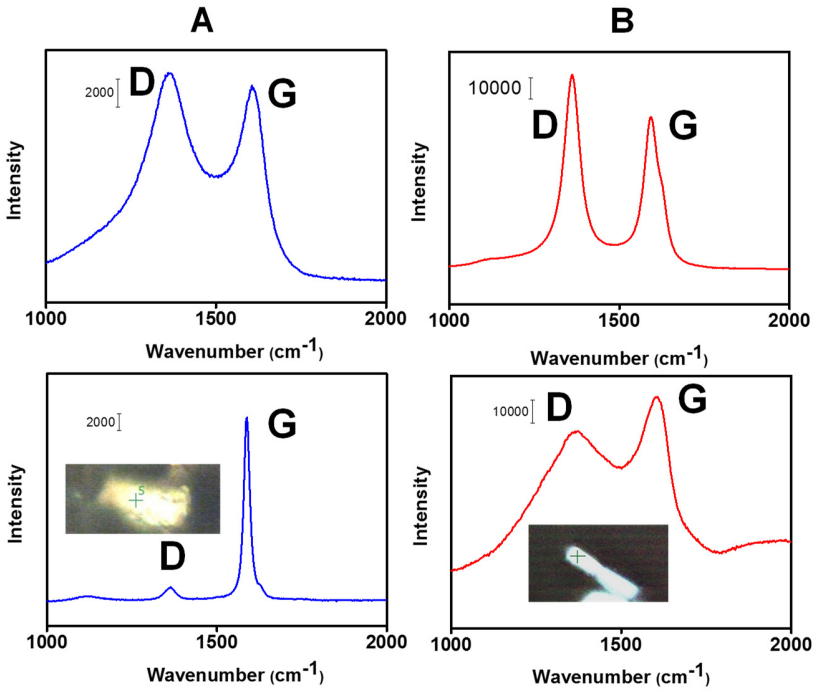


Fig. S2. Raman spectra of (A) Carbon black and (B) MW micromotors showing D ang G bands. For comparison, top part display the Raman spectra of the starting material used for electrodeposition.

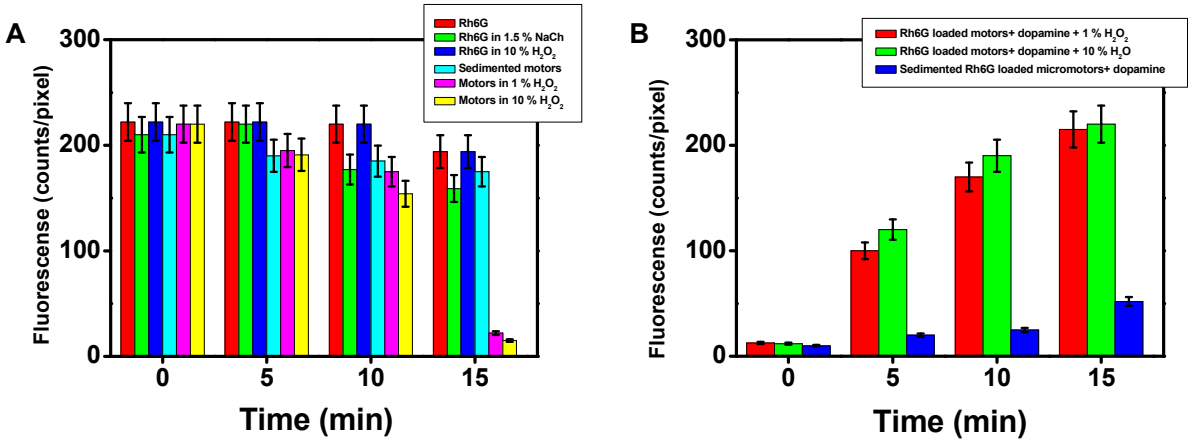


Fig. S3. (A) Influence of the reaction time and peroxide fuel upon the Rh6G loading-removal. (B) Dependence of the dopamine-triggered fluorescence recovery upon time and concentration of peroxide fuel (n=3).

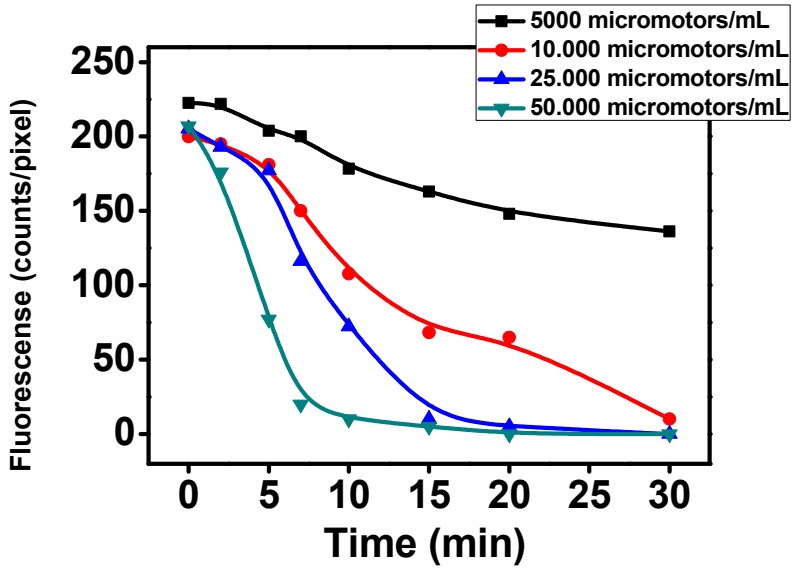


Fig. S4. Influence of the number of micromotors and time upon the Rh6G loading-removal. Experimental conditions: 1 % H_2O_2, surfactant, 1.5 % (w/v) sodium cholate.

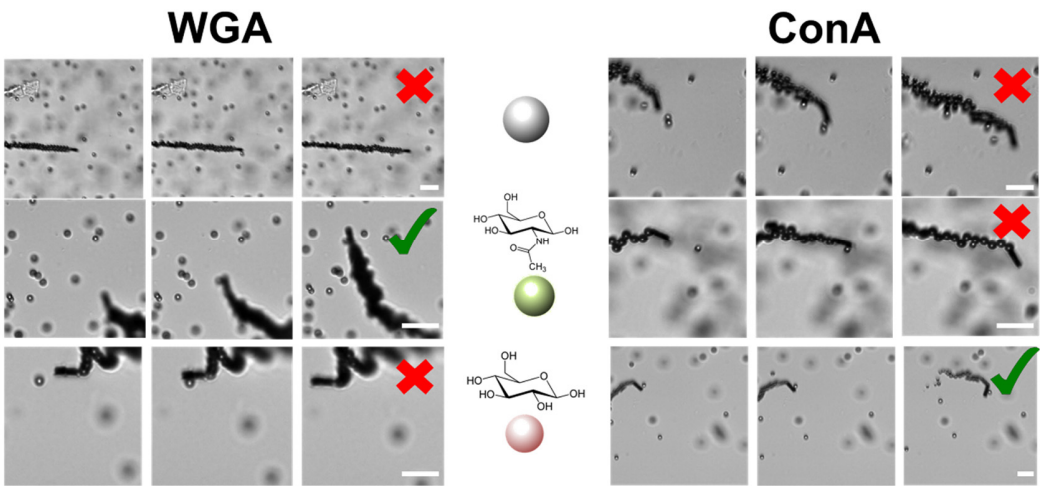


Fig. S5. Selectivity of the motor isolation protocol using MW-Ni-PtNPs micromotors modified with WGA and ConA: interaction with bare PS particles (white dots), N-Acetylglucosamine-incubated PS (green dots) and glucose-incubated PS (red dots). Scale bars, 2 µm. Experimental conditions: 5 % H_2O_2, surfactant, 1.5 % (w/v) sodium cholate.

Raman spectra of CB and MW micromotors. Fig. S2 illustrates the Raman spectra of the CB and MW materials in both pristine and on the board of micromotors. As expected, in all cases, the Raman spectra of CB and MW displayed well-defined D bands at 1360 cm^{-1} and a G bands at 1590 cm^{-1}. The D band arises from the out-of-plane vibrational modes and is indicative of the number of sp^3 carbon atoms present, whereas the G band arises from the presence of in-plane sp^2 vibrations.[1,2] The intensity ratio of the D and G lines (I_D/I_G ratio), therefore, provides important information about the composition and domains in-plane giving valuable information regarding the average size of the sp^2 carbon domains as well.[3]

The I_D/I_G ratio was calculated to be 1,05 and 1,25 for pristine CB and MW samples, and 0,08 and 0,91 for the micromotors, respectively. After CB deposition (Fig. S2A, bottom), "G" band predominates over "D" band and the broad band between them disappear when compared to the pristine material. This indicated a higher degree of organization of the CB after deposition. Indeed, similar spectra can be found in literature for graphite powder.[4] This can be attributed to the slow deposition of the CB in the pores of the membrane template, giving rise to the union of particles between them forming an ordered material more similar to graphite powder. For MW (see Fig. S2 B), Raman spectra before displays very well-defined peaks with the absence of a broad band between them, indicating thus a high degree of organization. Raman spectra after the electrodeposition shows a lower relationship between D/G band. This increment into the disorder could be attributed to decrease in the size of nanotubes to nanocrystals size.[5]

1. E. B. Barros, K. Sato, G. G. Samsonidze, A. G. Souza Filho, M. S. Dresselhaus, R. Saito, *Phys. Rev. B*, 2011, **83**, 245435.

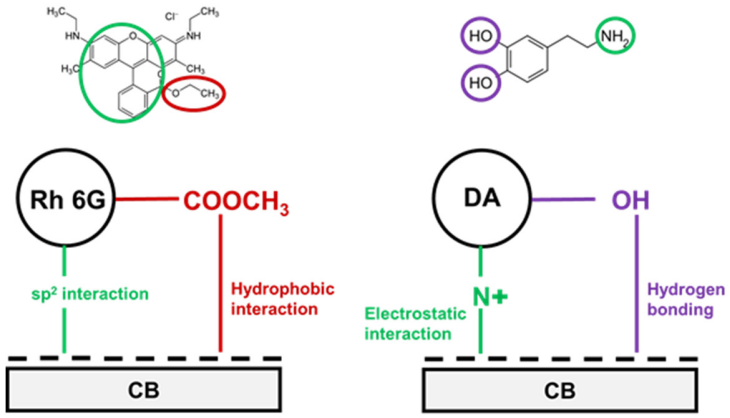
2. S. Stankovich, D. A. Dikin, R. D. Piner, K. A. Kohlhaas, A. Kleinhammes, Y. Jia, Y. Wu, S. T. Nguyen, R. S. Ruoff, *Carbon*, 2007, **45**, 1558-1565.

3. M. Cheng, R. Yang, L. Zhang, Z. Shi, W. Yang, D. Wang, G. Xie, D. Shi, G. Zhang, *Carbon*, 2012, **50**, 2581-2587.

4. S. Kurita, A. Yoshimura, H. Kawamoto, T. Uchida, K. Kojima, M. Tachibana, P. Molina-Morales, H. Nakai, *J. Appl. Phys*, 2005, **97**, 104320.

5. J. Zhou, C. Booker, R. Li, X. Zhou, T. K. Sham, X. Sun, Z. Ding. J. Am. Chem. Soc., 2007, **129**, 744-745.

Adsorption/desorption mechanism of Rhodamine 6G and dopamine on carbon black micromotors. Carbon black (CB) consists of aggregated spheroid particles, primarily of aggregated carbon combined into an extended aromatic network. Carbon black surfaces (such as that found in our micromotors) have a significant amount of oxides, which are favorable for adsorption of cationic species and sp^2 groups for interaction with aromatic compounds. Also, the adsorption capacity is highly dependent on the pH of the solution which affects the degree of ionization of adsorbate. At the pH of the assay, 7.3 , amine groups in Rhodamine 6G (Rh6G) are mainly non-protonated. Thus, the interaction mechanism with carbon black is mainly due to sp^2 interactions with aromatic groups. In addition, hydrophobic interaction between the micromotor surface and the ester group ($–COOCH_3$) in Rh6G molecules might also took place (see figure at the bottom). In the case of dopamine, a cationic specie, the amine sites are protonated at the pH of the assay and thus such compound have higher affinity for the negative oxygen groups present in the micromotor surface via electrostatic interactions (see also the figure at the bottom). Since electrostatic interactions are stronger than sp^2 bonds, dopamine preferentially adsorbs on the CB micromotors leading to the displacement of Rhodamine 6G. In addition, dopamine can further interact with the oxygen groups due to hydrogen bonding. The adsorption of dopamine can lead to desorption of Rh6G molecules from the CB micromotor surface owing to its weak adsorption on the surface with low free energy adsorption. In other words, dopamine predominates in competitive adsorption from mixed solution due to stronger affinity to the CB surface in the micromotor.



Scheme 1. Adsorption of Rhodamine 6G and dopamine on carbon black micromotors

Equipments and Reagents. All reagents, lectin from Triticum vulgaris (wheat, WGA), Concavalin A from Canavalia Ensiformis (jack bean, ConA), bovine serum albumin, carbon black, multiwalled carbon nanotubes and polystyrene microparticles were purchased from Sigma-Aldrich and were employed without further purification. Human plasma was obtained from healthy volunteers following standard protocols. Blood was collected in commercially available anticoagulant-treated tubes (EDTA) and centrifugation for 10 minutes at 1,500 x g. The resulting supernatant, designated plasma, was immediately transferred into a clean polypropylene tube and maintained at 2–8°C while handling. All potentials are referred to Ag/AgCl (3 M) reference electrode. Template electrochemical deposition of microtubes was carried out using an Autolab PGSTAT 12 (Eco Chemie, Utrecht, The Netherlands). Scanning electron microscopy (SEM) images were obtained with a NovaNano FE-SEM 230 FEI instrument, using an acceleration voltage of 10 kV. Energy-dispersive X-ray mapping analysis was performed using a Bruker X flash 4010 EDX

detector attached to SEM instrument, using an acceleration voltage of 22 kV. An inverted optical microscope (Nikon Eclipse Instrument Inc. Ti-S/L100), coupled with 4X, 10X, 20X and 40X objectives, and a Hamamatsu digital camera C11440 and NIS Elements AR 3.2 software, were used for capturing movies at a rate of 25 frames per second. The speed of the micromotors was tracked using a NIS Elements tracking module. The microscope includes an Epi-fluorescence attachment with a filter cube with a FTIC filter (530 ± 43 nm) use for fluorescence studies of Rhodamine 6G. Aqueous hydrogen peroxide solutions, with concentration of 0.5 to 5%, were used as the chemical fuel. Sodium cholate was used as surfactant in all propulsion experiments.

Chip design and fabrication. Chip experiments were carried out using polydimethylsiloxane (PDMS) chips. The structure compromise 2 µm hand-made reservoirs with a hexagon-shape middle reservoir and ~300 µm wide channels. The chips were rinsed with NaOH 0.1 M and ultrapure water before use. The chips are attached to a glass slide for seal the channels and filled with 1.5 % of sodium cholate solution. For Rhodamine 6G experiments, Rhodamine was added to a reservoir to obtain a final concentration 40 µg/L and finally micromotors and hydrogen peroxide fuel, 1 %, were added. After fluorescence quenching, dopamine solution was added to obtain 1 mM or 10 µM solutions to recover Rhodamine 6G fluorescence by competitive union of both analytes onto carbon black microtubes surface. For lectin recognition experiments the chip, filled with surfactant, then 1 µL of micromotors and 1 µL of PS-sugar suspension were added in one reservoir. Finally, hydrogen peroxide fuel was added to obtain a final concentration of 1 %.

Electrochemical synthesis of CB -Ni-PtNP, MWCNTs-Ni-PtNP and MWCNTS-Ni-PtNPs-OPD micromotors. Carbon black-Ni-PtNP (CB-Ni-PtNP) micromotors were prepared by direct deposition of the carbon nanomaterial. MWCNTs-Ni-PtNPs were prepared by direct electroreduction of the nanomaterial. The deposition or electrochemical reduction takes place into a 5 µm-diameter conical pores of a polycarbonate membrane (Catalog No. 7060–2513; Whatman, New Jersey, USA). A thin gold film was first sputtered on the branched side of the membrane to serve as a working electrode. The membrane was assembled in a Teflon plating cell with aluminum foil serving as an electrical contact for the subsequent deposition. CB (0.1 mg·mL^{-1}) or MWCNTs (0.1 mg·mL^{-1}) were dispersed in a solution containing 0.5 M Na_2SO_4 by ultrasonication. The simultaneous electrochemical reduction and deposition was carried out using cyclic voltammetry (CV, over +0.3 to –1.5 V vs Ag/AgCl (3 M), at 50 mV s^{-1} for ten cycles), using a Pt wire as counter electrode. Subsequently, a nickel tube layer was plated inside the reduced carbon layer by galvanostatic method using a solution containing a mixture of 20 g/L of $NiCl_2{\cdot}6H_2O$, 515 g/L of $Ni(H_2NSO_3)_2{\cdot}4H_2O$, and 20 g/L of H_3BO_3. First, 10 pulses of -20 mA were applied for 0.1s to generate nucleation spots. Then, a constant current of -6 mA was applied for 300 s to grow the nickel layer. After that an inner PtNP layer was amperometrically deposited at -0.4 V for 0.3 C from a solution containing 4 mM H_2PtCl_6 in 0.5 M boric acid. Poly-o-phenylenediamine layer (OPD) was deposited from a solution containing 5 mM of o-phenylenediamine in 0.054 M NaH_2PO_4-Na_2HPO_4 (pH 8) and 0.005 M NaCl by CV over 0-0.8 V (20 scans). The sputtered gold layer was gently removed by hand polishing with 0.05 µm alumina slurry. The membrane was then dissolved in methylene chloride for 30 min to completely release the microtubes. The micromotors were collected by centrifugation at 7000 rpm for 3 min and washed repeatedly with isopropanol, ethanol and three times with ultrapure water (18.2 Ω cm), with a 3 min centrifugation following each wash. All microtubes were stored in ultrapure water at room temperature when not in use. The template preparation method results in reproducible micromotors.

Propulsion of MW-Ni-PtNPs-OPD micromotors in BSA and human plasma. Micromotor propulsion was evaluated using solution containing bovine serum albumin (BSA) from 0% to 1% (0-150 µM) prepared in 0.01 M PBS buffer. 1 % H_2O_2 and 1.5 % sodium cholate were used as fuel and surfactant, respectively. Micromotor speed was compared with MW-Ni-PtNPs micromotors under

similar conditions. For selective isolation and transport experiments using WGA micromotors, raw human plasma was used without further dilution. For comparison, motion of micromotors not containing OPD layer was also tested in human serum and as expected, no efficient motion was noted (not shown).

Lectin functionalization. 300 µL of the MWCNTs-Ni-PtNP micromotors suspension was centrifugated to collect the micromotors in an eppendorf vial. 1 mL of 0.1 M MES buffer solution, at pH 5.0, containing 10 mM 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) and 20 mM of N-hydroxylsulfosuccinimide (NHS) was added to the micromotors and put under stirring for 30 min. After that, the micromotors were rinsed with acetate buffer (0.1 M, pH 5.0) containing 1 mM of Mn^{2+} and 1mM of Ca^{2+}, and incubated with 9 mg/mL solution of ConA or WGA in acetate buffer (0.1 M, pH 5.0) containing 1 mM of Mn^{2+} and 1 mM of Ca^{2+} for 2 hours. Finally, the surface was blocked with ethanolamine 1 M and the rockets were stored in acetate buffer (0.1 M pH 5.0) at 4 ºC in absence of light.

Polystyrene Particles-sugar preparation. 10 µL of a commercial suspension of polystyrene particles (PS) were transferred to an eppendorf vial and mixed with 190 µL of 70 mM sugar solutions (Glucose and N-Acetylglucosamine) in phosphate buffer 0.01 M pH 8.5. The suspension was incubated overnight at room temperature. The PS-modified particles were rinsed with phosphate buffer (0.01 M, pH 8.5) three times. Finally, the particles were re-suspended in the same buffer and stored at 4ºC.