Supporting Information for:

A Rapid Pathway Toward a Superb Gene Delivery System: Programming Structural and Functional Diversity into a Supramolecular Nanoparticle Library

Hao Wang[‡,2-5], Kan Liu[‡,1], Kuan-Ju Chen[2-5], Yujie Lu[6], Shutao Wang[2-5], Wei-Yu Lin[2-5], Feng Guo[2-5,7], Ken-ichiro Kamei[2-5], Yi-Chun Chen[2-5], Minori Ohashi[2-5], Mingwei Wang[2-5], Mitch André Garcia[2-5], Xing-Zhong Zhao[7], Clifton K.-F. Shen[2-4], Hsian-Rong Tseng[2-5]

[1]College of Electronics and Information Engineering, Wuhan Textile University, Wuhan, 430073, China.

[2]Crump Institute for Molecular Imaging, [3]California NanoSystems Institute, [4]Department of Molecular and Medical Pharmacology, [5]Institute for Molecular Medicine, University of California, Los Angeles, CA 90095, USA. Email: kshen@mednet.ucla.edu and hrtseng@mednet.ucla.edu

[6]Center for Molecular Imaging, Institute of Molecular Medicine, University of Texas Health Science Center at Houston, 1825 Pressler Street SRB 330A, Houston, TX 77030, USA.

[7]Department of Physics, School of Physics, Center of Nanoscience and Nanotechnology, Wuhan University, Wuhan, 430072, China.

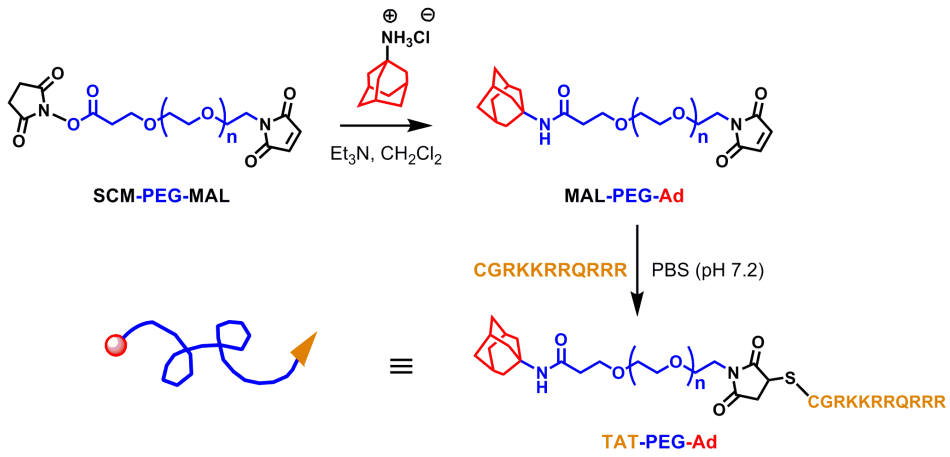
[‡]These authors contributed equally to the work.

RECEIVED DATE (to be automatically inserted after your manuscript is accepted if required according to the journal that you are submitting your paper to)

Table of Contents

1. Experimental Section

1.1. Synthesis of TAT-PEG-Ad



Scheme S1. Synthesis of TAT-PEG-Ad targeting ligand

To a solution of 1-adamantanamine hydrochloride (0.94 mg, 5.0 μmol, 5.0 equiv.) in CH_2Cl_2(1 mL), triethylamine (0.60 mg, 5.0 μmol, 5.0 equiv.) and SCM-PEG-MAL (5 mg, 1.0 mmol, 1.0 equiv.) were added in sequence. The reaction mixture was stirred at room temperature for 2 h. After the reaction, the solvent was subsequently removed *in vacuo*, and the PBS buffer solution (1 mL) containing CGRKKRRQRRR (7.50 mg, 5.0 μmol, 5.0 equiv.) was added to the reaction residue. The mixture was stirred for another 2 h at room temperature, followed by remove of insoluble 1-adamantanamine by filtration. The solution was then dialyzed with Slide-A-Lyzer® dialysis cassette (MWCO, 3.5 kD) against water overnight and lyophilized to give TAT-PEG-Ad (4.2 mg, 0.65 μmol), a white powder in 65% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.76-9.32 (br, protons on TAT), 3.41-3.50 (br, protons on PEG), 1.12-1.23 (br, protons on Ad). MS (MALDI-TOF, positive mode, DHB): the observed Mn for SCM-PEG-MAL was 5373.49; the Mn value of TAT-PEG-Ad based on the SCM-PEG-MAL was calculated as 6911.08 ($M+H^+$); found: 6913.72.

1.2. Quantification of functional ligand coverage on TAT/RGD-DNA⊂SNPs (1)

According to our previously reported method[1], a fluorophore (FITC) labeled molecule (FITC-PEG-Ad) as a ligand analogue was synthesized to quantify the functional ligand coverage on the DNA⊂SNPs (**1**). The FITC-PEG-Ad distribution both in free and bonded states are quantify using UV/Vis spectroscopy by monitor the absorption of FITC at 490 nm. Different mixing ratios of FITC-PEG-Ad (0, 1, 5, 10, 16, 20 and 100% based on

Ad-PEG) were added to the 40-nm DNA⊂SNPs. From the curved obtained in Fig. S1, it was determined that the optimal TAT/RGD-DNA⊂SNPs (**1**) with 5±1% and 9±1% (based on Ad-PEG) ligand surface coverages was observed.

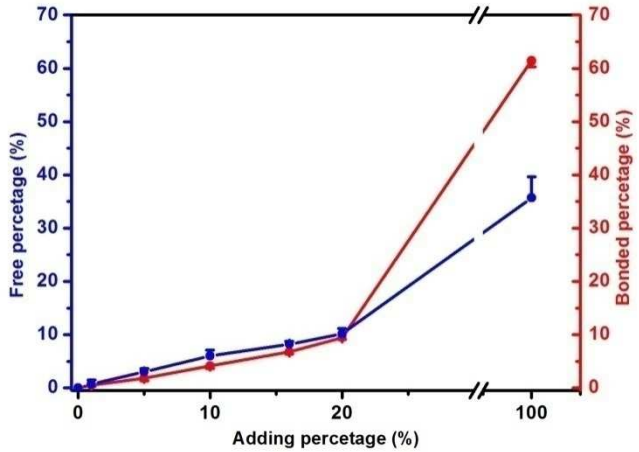


Figure S1 The amount of free and bonded **FITC-PEG-Ad** in the mixture was determined by UV/Vis spectroscopy. Blue and red curves represented the amount of free and bonded **FITC-PEG-Ad**.

2. DCM setup and operation

2.1. Fabrication of DCM

The polydimethylsiloxane (PDMS) microfluidic chips[2,3] were fabricated following the standard multi-layer soft lithography technology.[4-8] Two different sets of fluidic layer mold and control layer mold were separately fabricated by standard photolithographic process. The fluidic layer mold utilized for fabrication of the fluidic channels was made by patterning 45 mm thick positive photoresist (AZ 50XT) on the silicon wafer (channel width: 300 mm, channel height: 45 mm). The control layer mold utilized for the control channels was made by patterning negative photoresist (SU8-2050) on the silicon wafer (channel width: 100 mm, channel height: 40 μm). In order to achieve reliable performance of valve, the width of the control channel was set at 250 mm in sections where the valve modules are located.

Before fabricating the PDMS microfluidic chips, all molds were pretreated by exposure to trimethylsilyl chloride (TMSCl) vapor for 10 minutes. Well-mixed PDMS prepolymer (GE RTV615 A and B, total 36 g, mixing ratio A:B = 5:1) was poured onto the fluid layer molds to give 6 mm-thick fluidic layers. Another portion of PDMS prepolymer (GE RTV615, total 10 g, mixing ratio A:B = 20:1) was mixed and then spin-

coated on the control layer molds at 1500 RPM for 60 s. The fluidic and control layers were cured at 80°C oven for 15 minutes and 18 minutes, respectively. After baking, the fluidic layers were peeled off from the mold, aligned onto the corresponding control layers, and then baked at 80°C for at least 6 h to bond them. The assembled layers were peeled off from the control layer molds when the fluidic and control layers were bonded together. Holes were then punched to form ports connected to the fluidic layer channels for reagent inlets and outlets, and ports connected to the control layer channels for valve actuation with hydraulic fluid. Adhesion of the layers to a clean glass microscope slides to seal the control channels were achieved by oxygen plasma bonding. The microfluidic device was baked in an oven at 80°C for 72 h to restore the intrinsic hydrophobicity of PDMS surfaces needed to minimize the residue lost on channel walls when moving aqueous slugs.

2.2. Control interface

The pneumatic control system consists of 2 sets of 48-channel manifolds (electronic solenoid valves, SMC Series S070). On-chip microvalves were actuated by pressurizing the corresponding control channel, filled with water, to 60 psi. When the manifold was activated, pressure was transferred from the solenoids to the chip *via* PTFE microbore tubing (0.022" inner diameter, Cole Parmer) connected to 22-gauge stainless steel tubes and the valve in control layer would close the corresponding fluidic channel. All the valves were automatically controlled through a self-fabricated control system by a software program written in LabView (National Instruments).

2.3. CIM workflow

The schematic diagrams (Fig. S2) summarize the step by step operation of slug generation in CIM, which consisted of eight sequential steps for synchronously performing the DNA⊂SNPs synthesis by parallel eight units *via* the cooperation of valves. The device uses integrated microvalves to render slug formation relatively minimizing waste, permitting operation with tiny volumes, and enabling slug formation to be stopped and restarted on demand. At the same time, all the reagents inside the slugs could be mixed well along the tubing.[9,10]

The DNA⊂SNPs is produced by cycling through the sequence of valves states in Fig. S2 under the control of a digital program: (a) close all the microvalves: initialize microfluidic device (it takes *ca.* 50 ms); (b) open dividing valves and vacuum valve: apply vacuum (-22 inHg) to release the back pressure of fluidic channels to ensure that all the reagent filling chambers are on the same vacuum level (it takes *ca.* 150 ms); (c) close dividing valves: forming the desired volume chambers to be ready for filling the reagents (it takes *ca.* 50 ms); (d) open inlet valves: all the reagents (reagent **2**, **3**, **4**, **5**, **6**, **7** and two PBS) are synchronously filled into the chambers by using 30 psi positive pressure (it takes *ca.* 150 ms); (e) close inlet valves: re-close to seal the

reagent chambers (it takes *ca.* 50 ms); (f) open dividing valves and nitrogen valve: merge reagents to form eight individual parallel slugs (it takes *ca.* 50 ms); (g-h) sequentially open the trap valves at the end of each unit: sequentially drive the eight slugs out of the device within 1.2 s by 8-10 psi nitrogen. The total time (from step a to step h) to generate eight slugs is *ca.* 1.7 s.

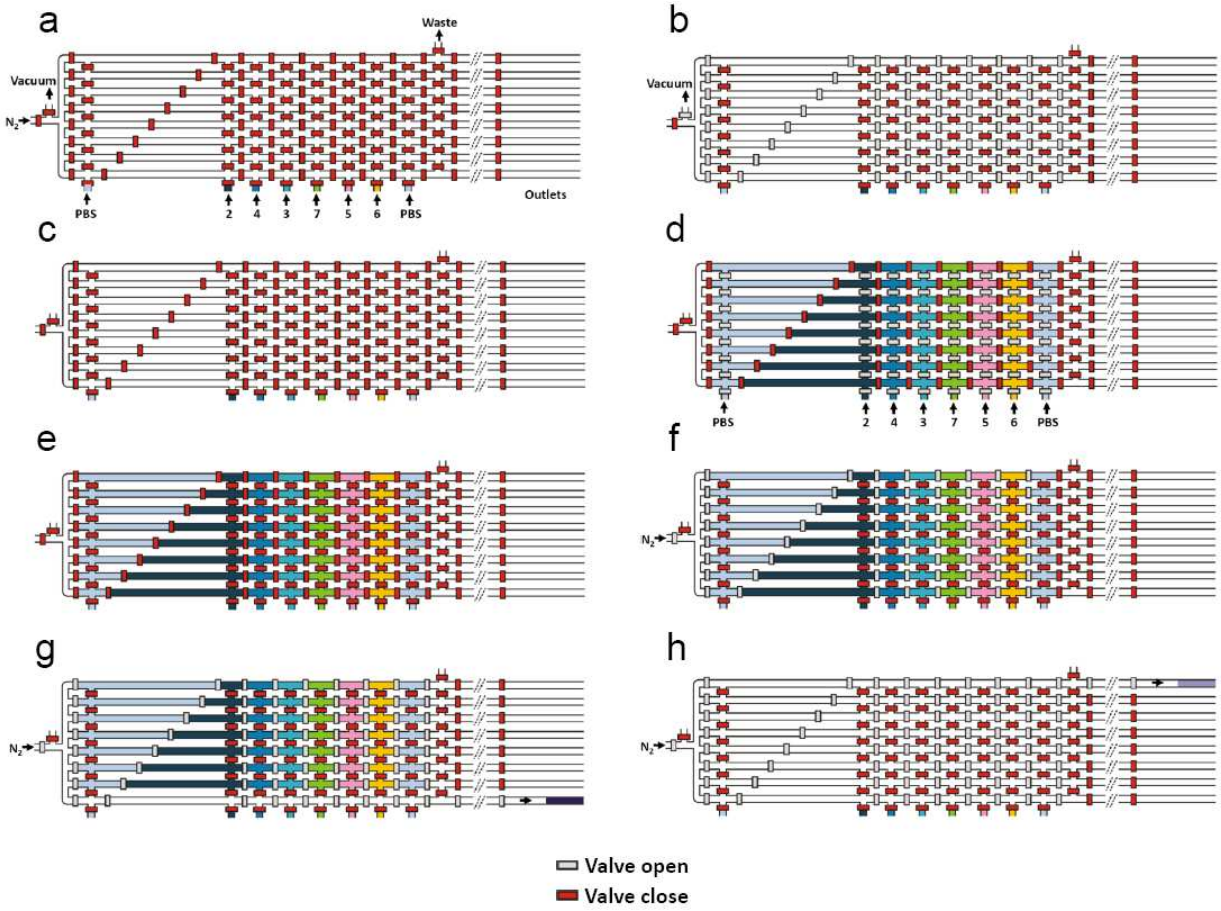


Figure S2. Schematic diagrams of CIM workflow.

2.4. AIM workflow

The reagent preparation process in AIM is illustrated in Fig. S3 , which consisted of ten sequential steps for performing the adjustment of two reagent concentrations by two individual units *via* the cooperation of valves. Similar to CIM, the slugs are digitally controlled and mixed along the tubing.

The reagents with desired concentration could be produced by each cycling through the sequence of valves states in Fig. S3 under the control of a digital program. (a) open vacuum valve and dividing valves: apply vacuum (-22 inHg) to release the back pressure of fluidic channels to ensure that all the reagent filling chambers are on the same vacuum level (it takes *ca.* 150 ms); (b) close dividing valves: forming the desired

volume chambers to be ready for filling the reagents (it takes *ca.* 50 ms) and at the same time the rest of channels are still under vacuum; (c) open reagent inlet valves: the reagent is filled into the chambers by using 30 psi loading pressure (it takes *ca.* 150 ms); (d) close inlet valves: re-close to seal the reagent chambers (it takes *ca.* 50 ms); (e) open dividing valves: form a bigger chamber with vacuum (it takes *ca.* 50 ms); (f) open the valve of PBS: PBS is filled into the bigger chamber to dilute the reagent by using 30 psi positive pressure (it takes *ca.* 150 ms); (g) close the inlet valve of PBS (it takes *ca.* 50 ms); (h) open nitrogen valve to be ready to drive the slug out of chip (it takes *ca.* 50 ms); (i) open the valve at the end of reagent 5 channel: drive diluted reagent 5 to degas module by 8-10 psi nitrogen (it takes *ca.* 150 ms); (j) open the valve at the end of reagent 6 channel: drive diluted reagent 6 to degas module by 8-10 psi nitrogen (it takes *ca.* 150 ms). (k) close both of the microvalves at the end of each microchannel: initialize microfluidic device (it takes *ca.* 50 ms). The total time (from step a to step k) to generate eight slugs is *ca.* 1.1 s.

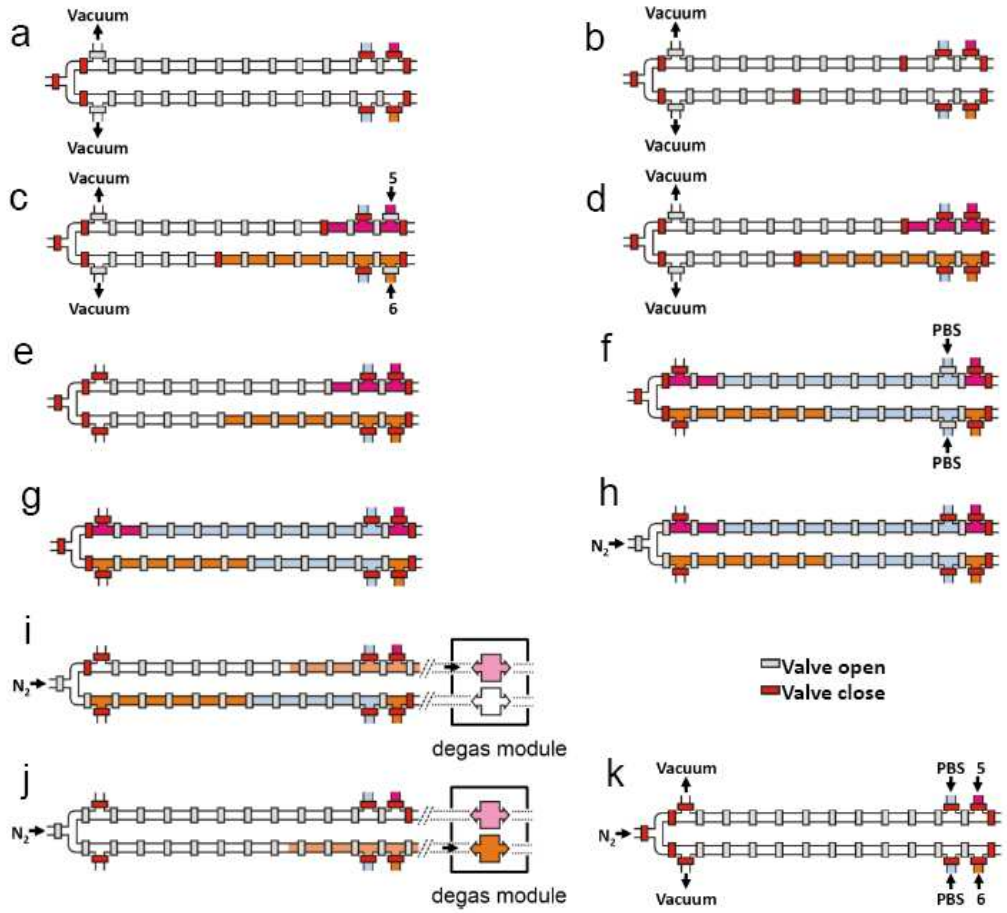


Figure S3. Schematic diagrams of the workflow of AIM.

Cross contaminations between reagent and PBS wasn't observed by food dye. Each cycle of running the

steps is *ca.* 1s and produces *ca*. 240 nL desired concentration reagent for each unit. By controlling to use the different dividing valve at step b-d, the reagent concentration (reagent 5 and 6) could be tuned separately.

2.5. Degas Module

Reagents **5** and **6** with different concentrations were provided by AIM. In order to remove the nitrogen of reagents **5** and **6** which was introduced by AIM, two degas modules for removing the gas of reagents **5** and **6** from AIM and delivering them to CIM were developed (Fig. S4).

Each degas module is composed of two collection vials, two vents, two nitrogen sources, one reagent inlet connected from AIM and one reagent outlet connected to CIM. These two collection vials play two different roles: one collect the diluted reagent from AIM and the other provide desired concentration reagent to CIM synchronously. As shown in Fig. S4, the valves (c2 and c5) are opened and vial A is used to collect the reagent from AIM. The reagent is filled into vial A through c2 and the gas is vented through c5. At the same, the degas module could provide the degassed reagent to CIM from vial B by opening valves (c8 and c3). The 30 psi compressed nitrogen is injected into vial B and the reagent is driven to CIM. Vial A and vial B could be in turn to play the roles of collection and delivery by using this method.

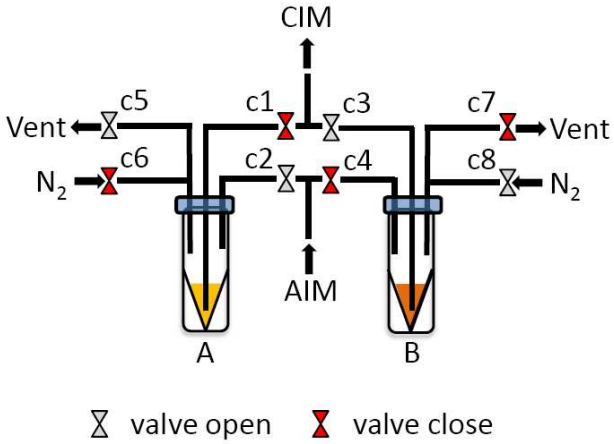


Figure S4. Working mechanism of degas module: degas module A is connected to AIM for collecting the diluted reagent; degas module B is connect to CIM for providing the reagent with the desired concentration.

3. Microscope settings, imaging processing and data analyzing

The 24-well plate was mounted onto a Nikon TE2000S inverted fluorescent microscope with a CCD camera (Photomatrix, Cascade II), X-Cite 120 Mercury lamp, automatic stage, and filters for three fluorescent channels (W1 (DAPI), W2 (EGFP and AO) and W3 (PI)). Following image acquisition, MetaMorph (Molecular Devices, Version 7.5.6.0) was used to quantify EGFP expressed cells. The Multi-Wavelength Cell Scoring module of the

Metamorph software allows image analysis. A nuclei counting application in the module allows us to calculate the total cell number. In order to determine the gene transfection efficiency, the EGFP-expressed cell number was counted by the MetaMorph program that distinguishes the transfected cells from the non-transfected cells. The fluorescence intensity difference between the regular cultured cells and background around 200~300 is measured as a baseline. The cell fluorescence intensity difference between cells and background above 300 are recognized as the transfected cells. The gene transfection efficiency was obtained by the EGFP-expressed cell number divided by the total cell number.

4. Cell viability assay

To determine the number of dead and live cells after transfection, two fluorescent dyes were used. Propidium iodide (PI) is a fluorescent dye that cannot pass through intact cell membranes but readily passes through damaged membranes and binds with DNA. PI fluorescence was bright red when exposed to UV light. The presence of PI in a cell indicates that the cell membrane integrity has been compromised and that the cell is severely damaged. Red fluorescent cells are judged non-viable. Acridine orange (AO) is a fluorescent dye that readily passes through all cell membranes and stains the cytoplasm and nucleus bright green when exposed to UV light. Green fluorescent cells are judged viable. All dyes were used in accordance with the manufacturer's directions. First, we added 1 mL of 10× AO solution and 1 mL of 10 × PI solution into 8 mL of PBS in a 15 mL tube to make the AO/PI working solution. Then, we added approximately 0.5 mL AO/PI working solution into each well. Using the fluorescence microscope, we took images and evaluated the viability of the cells transfected by TAT/RGD-DNA⊂SNPs and the cells cultured in the normal medium after 48 h. There were no significant differences in viability, which suggested that the toxicity of TAT/RGD-DNA⊂SNPs were negligible for *in vitro* transfection studies

References

(1) Wang, H.; Chen, K.-J.; Wang, S.; Ohashi, M.; Kamei, K.-i.; Sun, J.; Ha, J. H.; Liu, K.; Tseng, H.-R. *Chem. Commun.* **2010**, *46*, 1851-53.

(2) Whitesides, G. M. *Nature* **2006**, *442*, 368-73.

(3) Xia, Y. N.; Whitesides, G. M. *Angew. Chem. Int. Ed.* **1998**, *37*, 551-75.

(4) Unger, M. A.; Chou, H. P.; Thorsen, T.; Scherer, A.; Quake, S. R. *Science* **2000**, *288*, 113-16.

(5) Lee, C. C.; Sui, G. D.; Elizarov, A.; Shu, C. Y. J.; Shin, Y. S.; Dooley, A. N.; Huang, J.; Daridon, A.;

Wyatt, P.; Stout, D.; Kolb, H. C.; Witte, O. N.; Satyamurthy, N.; Heath, J. R.; Phelps, M. E.; Quake, S. R.; Tseng, H. R. *Science* **2005**, *310*, 1793-96.

(6) Wang, J. Y.; Sui, G. D.; Mocharla, V. P.; Lin, R. J.; Phelps, M. E.; Kolb, H. C.; Tseng, H. R. *Angew. Chem. Int. Ed.* **2006**, *45*, 5276-81.

(7) Wang, Y.; Lin, W.-Y.; Liu, K.; Lin, R. J.; Selke, M.; Kolb, H. C.; Zhang, N.; Zhao, X.-Z.; Phelps, M. E.; Shen, C. K. F.; Faull, K. F.; Tseng, H.-R. *Lab on a Chip* **2009**, *9*, 2281-85.

(8) Lin, W.-Y.; Wang, Y.; Wang, S.; Tseng, H.-R. *Nanotoday* **2009**, *4*, 470-81.

(9) Song, H.; Chen, D. L.; Ismagilov, R. F. *Angew. Chem. Int. Ed.* **2006**, *45*, 7336-56.

(10) Rhee, M.; Burns, M. A. *Langmuir* **2008**, *24*, 590-601.