VIP Nano Stir Bars

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Stirring in Suspension: Nanometer-Sized Magnetic Stir Bars**

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Rapid mixing is essential for achieving effective chemical and biological reactions. Because passive diffusion is a slow process, it is often necessary to agitate or stir a solution. Generating such disturbance must involve an external input of energy, for example, transduction of magnetic field energy through the use of commercial magnetic stir bars. But such use is impractical for the tiny channels and droplets, which are of great importance for lab-on-chip applications[1] and microliter bioassay.[2] The main challenge lies in the fabrication of low-cost stir bars that are sufficiently small but still able to transduce external energy for the mixing. Moreover, the stir bars should be introduced, operated, and extracted with ease.

In microfluidic research, a number of methods have been developed to improve mixing. For a lamellar flow confined in a channel, turbulence can be induced by forcing the solution through a complex winding channel,[1b,3] or by pulsed injection of additional solution/bubbles from the side channels,[4] thermal gradients can be used to induce convection,[2c,5] direct physical agitation can be achieved using ultrasound or piezoelectric transducers,[6] and in-channel stirring was achieved using magnetic turbines that were fabricated by lithographic methods.[7] These mixing schemes were often fabricated together with the channel systems, because it is too difficult to introduce them after the fabrication.

In static microdroplets, however, transducing external energy for mixing remains a challenge, particularly in arrays of microdroplets. Ultrasonication and violent stirring can break up the droplets, while thermal gradients across tiny droplets are impractical. To date, magnetic stirring is still the most convenient option. Micro-sized stir bars have been reported, for example, linear chains of polymer beads embedded with magnetic nanoparticles (NPs),[8] star-shaped micro-stirrers made by soft lithography,[9] and cobalt-based magnetic bars cut by laser micromachining.[10] Because of gravitational and magnetic attraction, micro-sized stir bars tend to stir only at the bottom of the vessel,[10] leaving most part of the solution unstirred. While facile and scalable synthesis remains a challenge, the main problem of micro-sized stir bars is still their size: they are too large to remain suspended, but too small to churn up the whole solution.

Herein, we report a simple and scalable method for fabricating magnetic stir bars, which are tunable from 75 nm–1.4 μm in width and up to around 17 μm in length (Figure 1). They are straight single-line chains assembled from 40 nm magnetic NPs and then preserved in silica shells of variable thickness. These rigid magnetic chains showed immediate response to a common magnetic stir plate and can be easily recovered. Being small and in large number, they can remain suspended and stir independently in all parts of the solution. These nano stir bars can be readily dispensed for parallel stirring of droplets down to picoliter in volume (4 picoliter).

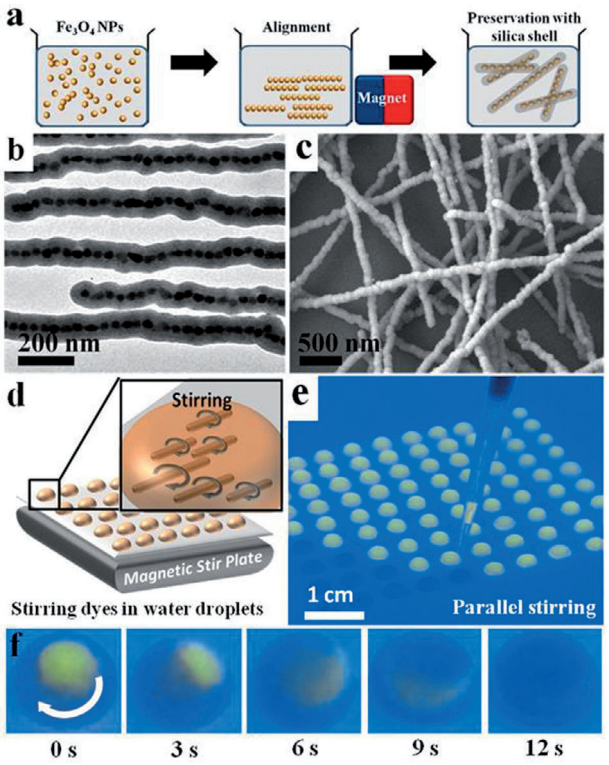

Figure 1. a) The synthesis of nano stir bars. b) TEM and c) SEM images of nano stir bars. d) Schematic representation and e) photograph showing parallel stirring of droplets (5 μL) on a common magnetic stir plate. f) Photographs showing the dispersing of dye solution (rhodamine 6G) in a water droplet (30 μL) with stirring nano stir bars (see also Video S2 in the Supporting Information).

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In a typical synthesis, oleic acid stabilized Fe_3O_4 NPs[11] ($d = 40$ nm) were first modified with citric acid to make them soluble in water.[12] After purification, these NPs were dispersed in water/2-propanol mixture ($v/v = 3{:}5$). To align the NPs and simultaneous encapsulate them in silica,[13,14] tetraethyl orthosilicate (TEOS) and ammonia were added to the solution and the reaction vessel was immediately placed close to a magnet. After incubated overnight, the products were isolated and purified by means of centrifugation.

The product nano stir bars were too small to be observed by the naked eye, but remarkably, droplets containing them "blinked" in sync to the rotating magnetic field (Video S1 in the Supporting Information). The "blinking" appeared more obvious when thicker magnetic chains were used, suggesting that it was caused by light scattering. As the rigid chains spun in unison, their different scattering efficiency in the transverse and longitudinal directions caused the "blinking" of the entire droplet.

As shown in the transmission electron microscopy (TEM) image (Figure 1 b), the dark Fe_3O_4 NPs formed linear single-line chains, which were coated with a grayish silica shell. The gaps between the NPs were barely noticeable, suggesting that the aggregation had probably occurred at the initial stage before the silica encapsulation. In this method, changing both the solvent and the surface ligand of the as-synthesized Fe_3O_4 NPs were important for promoting aggregation and achieving their alignment (Figure S1). In images where the nano stir bars were stacked together (e.g., Figure 1 c), they were straight and rigid. There was no obvious morphological change after stirring, which was remarkable considering the sheer forces involved in the process.[15] It appeared that the silica shells were critical in maintaining their structural integrity. In control experiments without silica encapsulation, the resulting NP chains were much shorter and often in randomly curved conformations (Figure S1a).[16]

To observe the effects of stirring, we added 3 μL of fluorescent dye (rhodamine 6G, 0.25 mM) to static water droplets (30 μL) containing nano stir bars of approximately 200 nm in width (Figure 1 f and Video S2). Under UV irradiation ($\lambda = 254$ nm), the initial droplet was dark, thus the homogenization of the bright dye can be clearly observed. When stirring was off, complete dispersion occurred at about 100 s after the addition of dye, whereas it took only 12 s when stirring was on. From the video, it can be observed that the bright cloud of dye actually swirled around in the droplet along the direction of stirring, almost in the same way swirling is induced by a typical macroscopic stir bar. However, the swirling was much less pronounced in that there was no whirlpool formation.

There are several possible modes of stirring: the nano stir bars may either rotate individually at their local positions, or "swimming" around like a school of fish, or a combination of these two motions.

While the "real" stirring was too fast to be observed, we designed a setup using an optical microscope to directly observe the stirring in slow motion: a droplet containing nano stir bars was positioned on a fixed platform between two opposing magnets, which were placed on a plate beneath the platform (Figure 2 a). As the plate was manually spun, the

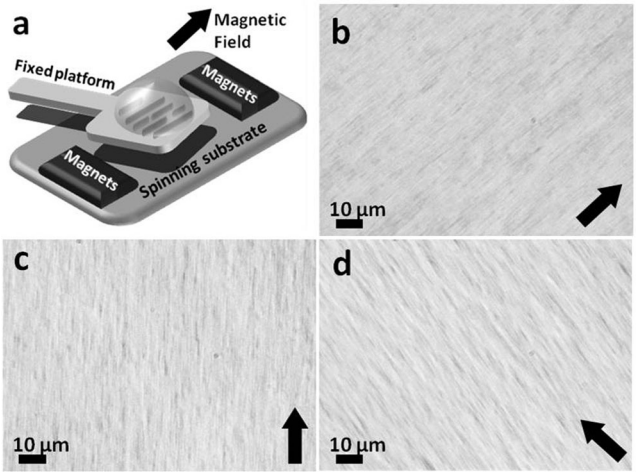

Figure 2. a) Schematic illustration of the setup for observing nano stir bars during their slow stirring. b)–d) Typical optical microscopy images showing the alignment of nano stir bars in accordance to the rotating magnetic field (black arrows). See also Video S3.

movement of the nano stir bars can be directly observed. From the images and video (Figure 2 b–d and Video S3), the nano stir bars were too small to be clearly distinguished. But we can still discern their alignment from the fiber-like appearance, which was always aligned in the direction of the magnetic field (i.e., rotating at the same speed). Hence, it appeared that the nano stir bars were all individually rotating at their local positions. It is logical to expect a similar rotating motion under faster stirring conditions. Indeed, this was supported by the "blinking" phenomenon discussed above.

However, the swirling of dyes in the droplet (Figure 1 f) is indicative of a concerted motion of the entire droplet, as opposed to local agitation of the solution. The rate of swirling appeared much slower than that of the rotating magnetic field. Probably, the rotating nano stir bars can induce their surrounding solution to move along a same direction, causing slow swirling of the entire droplet. When the magnetic field was manually spun at a low speed, the concerted motion of the solution was negligible. Though we cannot directly observe the nano stir bars during fast stirring, there are good reasons to believe that they were being carried along with the slow swirling solution, while they were rotating individually.

To view the magnetic alignment in greater detail, we dried the droplets containing nano stir bars when the stirring was either on or off (Figure 3). The resulting samples were characterized by scanning electron microscopy (SEM). When stirring was off, the nano stir bars in the dried sample were all aligned in the direction of magnetic field. In contrast, when stirring was on, the nano stir bars stacked with each other in a crisscross fashion, as if they were trapped during their rotation at different points in the final stage of drying. Most importantly, the nano stir bars did not arrange head-to-tail like a school of fish, a result consistent with the above analyses.

The smallest nano stir bars in our system (average 75 nm in width and 4 μm in length) were still larger than the typical

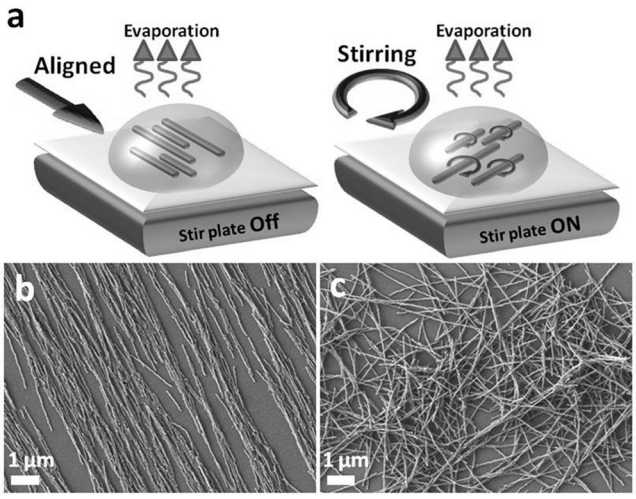

Figure 3. a) Schematics illustrating the drying of water droplets containing nano stir bars. SEM images of the dried samples when the stirring was b) off; or c) on.

colloidal particles, thus, when there was no stirring, they usually sediment out after several hours (5–10 h). But remarkably, they remained almost permanently suspended in solution (for at least 24 h) when the stirring was on (Figure 4 a,b), probably because of their constant agitation. As such, they can stir all parts of the solution, unlike larger stir bars which tend to stir only at the bottom (or on top[9]) of the solution. To make a fair comparison, we synthesized large stir bars (ca. 1.4 μm in width and ca. 10 μm in length) by coating a thick layer of silica, making them close to but still smaller in size than the typical micro-sized magnetic stir bars.[8e,10] While these larger stir bars were slightly more effective in terms of stirring, they were still far too small to create a whirlpool. At this size, most of these stir bars sediment out after about 10 min even when the stirring was on (Figure 4 c,d). Most of

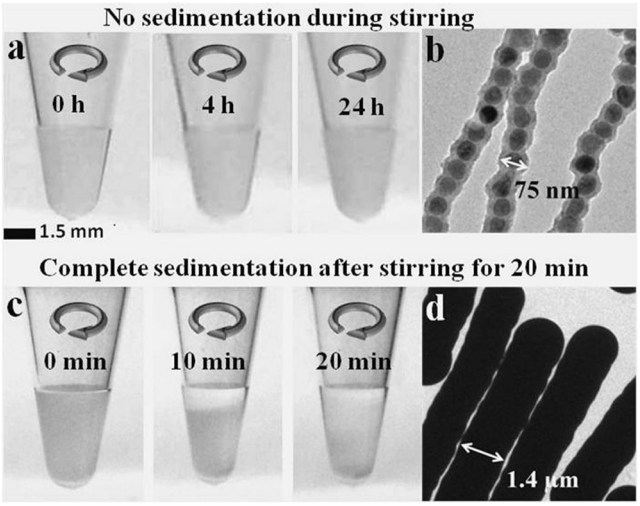

Figure 4. Photographs showing the sedimentation process of the following 30 μL solutions when stirring was on: a) suspension of nano stir bars of approximately 75 nm in width; and c) suspension of "large" nano stir bars of approximately 1.4 μm in width. Typical TEM images of the nano stir bars are shown in (b) and (d), respectively.

the micrometer-sized stir bars reported were in the range of 30–400 μm in width;[7,9,10] they should sediment more quickly than our largest stir bars.

The stir bars in our systems were small and thus, they were made and used in large numbers. On the basis of the average 97 NPs per stir bar, we estimated that there were about 410 million stir bars in the stirring experiments in Figure 1 f (30 μL droplets). Hence, the amount of the dispensed nano stir bars can be easily controlled according to their concentration. This is an advantage over similar systems, where ensuring at least one micro stir bar per droplet is a challenge. On the other hand, extracting the nano stir bars was also easy: As illustrated in Figure 5, a static droplet (8 μL) containing

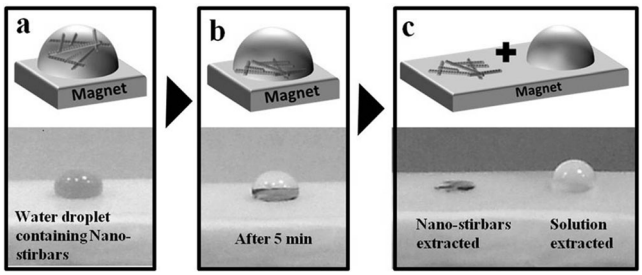

Figure 5. Schematic representations (top) and photographs (bottom) illustrating the extraction of nano stir bars: a) a water droplet (8 μL) containing nano stir bars; b) after incubation for 5 min on a magnet; c) extraction of nano stir bars from the droplet (see also Video S4).

nano stir bars (200 nm in width) was placed on a piece of parafilm on top of a static magnet. After 5 min, the cloudy brownish droplet turned clear and the stir bars were all attracted to the bottom of the droplet. The clear solution on top was moved away using a micropipette (Figure 5 a–c, Video S4), allowing the recovery of both the solution and stir bars.

As a proof of concept, we tried to introduce nano stir bars for stirring inside picoliter droplets, which were generated by channeling water into the flow of olive oil using a simple cross-flow microfluidic chip (Figure 6 a). The resulting water-in-oil emulsion was characterized by optical microscope. By increasing the flow rates, tiny droplets could be generated ($d = 20$ μm, Figure 6 b,c), but their monodispersity was not as good as that obtained at a low flow rate (Figure S10). For easy observation, nano stir bars of approximately 200 nm in width were used in low concentration. They were dispensed into the droplets as part of the initial water phase. As shown in Figure 6 c and Video S5, the nano stir bars in the droplets were initially aligned to the magnetic field but were induced to rotate when the stirring was turned on. Such stirring would not be possible without the extremely small size of the stir bars.

In summary, we have developed a new method for fabricating the world's smallest magnetic stir bars. Their small size turned out to be of great importance: The nano stir bars can be used for stirring inside extremely small droplets; they can remain suspended and stir all parts of the solution; they can be easily confined inside a droplet instead of being pulled out by an external magnetic field, they can stir

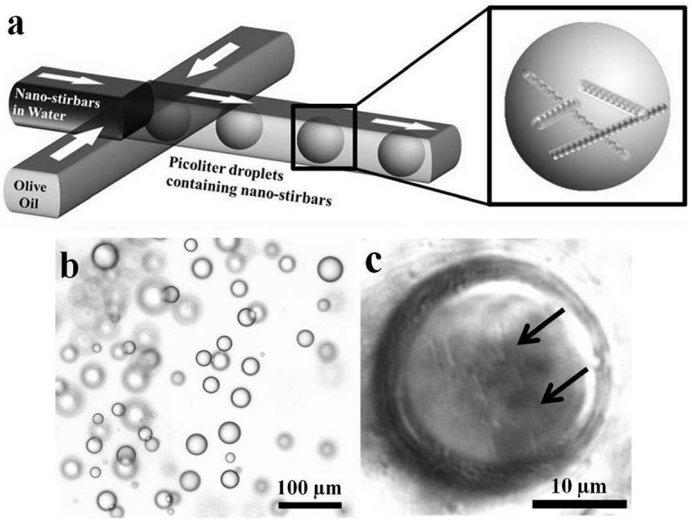

Figure 6. a) Schematic illustrating the formation of picoliter droplets containing nano stir bars by using a cross-flow microfluidic system. b) Optical image of the suspended water droplets in oil. c) High resolution optical images of a picoliter droplet containing nano stir bars (black arrows). See them stirring in Video S5.

effectively without breaking up the droplet, and finally, they can be easily fabricated in great numbers, allowing economic usage, easy dispensing, and effective stirring.

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[1] a) L. D. Mao, H. Koser, *Nanotechnology* **2006**, *17*, S34; b) A. J. deMello, *Nature* **2006**, *442*, 394; c) J. M. Ottino, S. Wiggins, *Science* **2004**, *305*, 485; d) S. Haeberle, R. Zengerle, *Lab Chip* **2007**, *7*, 1094.

[2] a) H. B. Lee, K. Oh, W. H. Yeo, T. R. Lee, Y. S. Chang, J. B. Choi, K. H. Lee, J. Kramlich, J. J. Riley, Y. J. Kim, J. H. Chung, *Microfluid. Nanofluid.* **2012**, *12*, 143; b) B. Hadwen, G. R. Broder, D. Morganti, A. Jacobs, C. Brown, J. R. Hector, Y. Kubota, H. Morgan, *Lab Chip* **2012**, *12*, 3305; c) C. Luni, H. C. Feldman, M. Pozzobon, P. De Coppi, C. D. Meinhart, N. Elvassore, *Biomicrofluidics* **2010**, *4*, 034105.

[3] a) C.-Y. Lee, C.-L. Chang, Y.-N. Wang, L.-M. Fu, *Int. J. Mol. Sci.* **2011**, *12*, 3263; b) N.-T. Nguyen, Z. Wu, *J. Micromech. Microeng.* **2005**, *15*, R1.

[4] a) Y. K. Suh, S. Kang, *Micromachines* **2010**, *1*, 82; b) G. Arnaud, I. Glasgow, N. Aubry, *Mech. Res. Commun.* **2006**, *33*, 739; c) I. Glasgow, N. Aubry, *Lab Chip* **2003**, *3*, 114; d) I. Glasgow, S. Lieber, N. Aubry, *Anal. Chem.* **2004**, *76*, 4825.

[5] B. Xu, T. N. Wong, N. T. Nguyen, Z. Z. Che, J. C. K. Chai, *Biomicrofluidics* **2010**, *4*, 044102.

[6] a) G. G. Yaralioglu, I. O. Wygant, T. C. Marentis, B. T. Khuri-Yakub, *Anal. Chem.* **2004**, *76*, 3694; b) Z. Yang, S. Matsumoto, H. Goto, M. Matsumoto, R. Maeda, *Sens. Actuators A* **2001**, *93*, 266; c) S. S. Guo, S. T. Lau, K. H. Lam, Y. L. Deng, X. Z. Zhao, Y. Chen, H. L. W. Chan, *J. Appl. Phys.* **2008**, *103*, 094701; d) L. Johansson, S. Johansson, F. Nikolajeff, S. Thorslund, *Lab Chip* **2009**, *9*, 297; e) D. Ahmed, X. L. Mao, J. J. Shi, B. K. Juluri, T. J. Huang, *Lab Chip* **2009**, *9*, 2738.

[7] a) L. Liang-Hsuan, R. Kee Suk, L. Chang, *J. Microelectromech. Syst.* **2002**, *11*, 462; b) K. S. Ryu, K. Shaikh, E. Goluch, Z. Fan, C. Liu, *Lab Chip* **2004**, *4*, 608; c) Y. Tian, Y.-L. Zhang, J.-F. Ku, Y. He, B.-B. Xu, Q.-D. Chen, H. Xia, H.-B. Sun, *Lab Chip* **2010**, *10*, 2902.

[8] a) R. Calhoun, A. Yadav, P. Phelan, A. Vuppu, A. Garcia, M. Hayes, *Lab Chip* **2006**, *6*, 247; b) I. Petousis, E. Homburg, R. Derks, A. Dietzel, *Lab Chip* **2007**, *7*, 1746; c) T. Roy, A. Sinha, S. Chakraborty, R. Ganguly, I. K. Puri, *Phys. Fluids* **2009**, *21*, 027101; d) S. H. Lee, D. van Noort, J. Y. Lee, B. T. Zhang, T. H. Park, *Lab Chip* **2009**, *9*, 479; e) S. L. Biswal, A. P. Gast, *Anal. Chem.* **2004**, *76*, 6448; f) H. Singh, P. E. Laibinis, T. A. Hatton, *Langmuir* **2005**, *21*, 11500; g) T. Franke, L. Schmid, D. A. Weitz, A. Wixforth, *Lab Chip* **2009**, *9*, 2831.

[9] Y. Zhang, T. H. Wang, *Microfluid. Nanofluid.* **2012**, *12*, 787.

[10] D. De Bruyker, M. I. Recht, A. A. S. Bhagat, F. E. Torres, A. G. Bell, R. H. Bruce, *Lab Chip* **2011**, *11*, 3313.

[11] J. Park, K. J. An, Y. S. Hwang, J. G. Park, H. J. Noh, J. Y. Kim, J. H. Park, N. M. Hwang, T. Hyeon, *Nat. Mater.* **2004**, *3*, 891.

[12] M. Lattuada, T. A. Hatton, *Langmuir* **2006**, *23*, 2158.

[13] Y. Hu, L. He, Y. Yin, *Angew. Chem.* **2011**, *123*, 3831; *Angew. Chem. Int. Ed.* **2011**, *50*, 3747.

[14] a) Y. J. Wong, L. Zhu, W. S. Teo, Y. W. Tan, Y. Yang, C. Wang, H. Chen, *J. Am. Chem. Soc.* **2011**, *133*, 11422; b) T. Chen, G. Chen, S. Xing, T. Wu, H. Chen, *Chem. Mater.* **2010**, *22*, 3826.

[15] X. Shen, L. Chen, D. Li, L. Zhu, H. Wang, C. Liu, Y. Wang, Q. Xiong, H. Chen, *ACS Nano* **2011**, *5*, 8426.

[16] a) P. Y. Keng, B. Y. Kim, I.-B. Shim, R. Sahoo, P. E. Veneman, N. R. Armstrong, H. Yoo, J. E. Pemberton, M. M. Bull, J. J. Griebel, E. L. Ratcliff, K. G. Nebesny, J. Pyun, *ACS Nano* **2009**, *3*, 3143; b) M.-R. Gao, S.-R. Zhang, J. Jiang, Y.-R. Zheng, D.-Q. Tao, S.-H. Yu, *J. Mater. Chem.* **2011**, *21*, 16888; c) R. R. Qiao, C. H. Yang, M. Y. Gao, *J. Mater. Chem.* **2009**, *19*, 6274; d) Y. Lu, L. Dong, L.-C. Zhang, Y.-D. Su, S.-H. Yu, *Nano Today* **2012**, *7*, 297; e) M.-J. Hu, Y. Lu, S. Zhang, S.-R. Guo, B. Lin, M. Zhang, S.-H. Yu, *J. Am. Chem. Soc.* **2008**, *130*, 11606; f) M. Hu, B. Lin, S. Yu, *Nano Res.* **2008**, *1*, 303; g) Z. He, S. H. Yu, X. Zhou, X. Li, J. Qu, *Adv. Funct. Mater.* **2006**, *16*, 1105.

