Supporting Information for

3D Motion of DNA-Au Nanoconjugates in Graphene Liquid Cell EM

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Text S1-S3, Figure S1, Table S1-S2,Videos S1-S4

Text S1

The linker of a constant length

Below shows how the assumption of two Au nanoparticles bridged by a linker of a constant length leads to the observed same diffusivities for free Au nanoparticles and dimers:

For motion statistics, in general one can regard a dimer as two nanoparticles connected by a spring with a spring constant k. The Langevin equation describing the Brownian motion of particle 1 in the dimer will be:

$$m\dot{v}_1(t) = -\gamma v_1(t) + \eta(t) - k[r_1(t) - r_2(t)]$$

where $v_1(t)$ is the velocity of the particle, and m denotes the particle's mass. $-\gamma v_1(t)$ describes the viscous force acting on the particle proportional to the particle's velocity (Stokes' law). $\eta(t)$ is a noise term showing the effect of fluid molecule collisions. $-k[r_1(t) - r_2(t)]$ describes the force exerted from the spring connecting this particle with the other particle in the dimer. $r_1(t)$ is the position vector of particle 1.

The solution of this Langevin equation is:

$\dot{r}_1(t) = -\nabla U + \sqrt{2D}R(t)$. $-\nabla U$ describes the contribution from the spring. D is the diffusion of free individual particles, $R(t)$ is the displacement of this particle.

Thus, $r_1(t) - r_1(0) = \int_0^t dt'[-\nabla U(t') + \sqrt{2D}R(t')]$

The time-average of this displacement will be:

$$\langle |r_1(t) - r_1(0)|^2 \rangle = \langle \int_0^t dt' \int_0^t dt''[\nabla U(t')\nabla U(t'') + 2DR(t')R(t'')] \rangle$$

$$\langle |r_1(t) - r_1(0)|^2 \rangle = 2Dt + \int_0^t dt' \int_0^t dt'' \langle \nabla U(t') \nabla U(t'') \rangle$$

$$\langle |r_1(t) - r_1(0)|^2 \rangle = 2Dt + k^2 \int_0^t dt' \int_0^t dt'' \langle (r_1(t') - r_2(t'))(r_1(t'') - r_2(t'')) \rangle$$

The inter-particle distance fluctuation at $t = t'$ is decoupled from $t = t''$ since the linker between particles is rigid on this time scale. So no memory effect of the interparticle distance will be expected.

$$\int_0^t dt' \int_0^t dt'' \langle (r_1(t') - r_2(t'))(r_1(t'') - r_2(t'')) \rangle = 0$$

Accordingly: $\langle |r_1(t) - r_1(0)|^2 \rangle = 2Dt$, the same as that for free individual particles.

Text S2

Dosage calculation

1. The suitable imaging magnification: we imaged our samples at a magnification of 29000. At this magnification, the size per pixel is 0.38 nm, which allows sub nanometer resolution in determining the position of Au nanoparticles. The view of the screen is then 380.07×380.07 nm^2, populated with around 20 particles in total. In this case, we can have sufficient sample statistics for each group of nanocrystals in the same liquid pocket.

2. Calculation of dosage at 29000× magnification: A TEM image was taken under the same sample imaging conditions but with both aperture and sample extracted. The electron flux in this TEM image shows the total dosage for the whole graphene liquid cell. We use 10 counts in the CCD camera as 1 electron, and in a typical experiment, dosage is calculated as follows:

$$dosage = \frac{total\ pixel\ intensity}{10 \times area \times exposure\ time} = \frac{8.74 \times 10^9}{10 \times (380.07\ nm)^2 \times 0.5\ s} = 120\ e^-/(\text{Å}^2 \cdot s)$$

This dosage allows the required contrast from Au nanocrystals at this magnification since the liquid background fluctuation smeared out the signal (see **Figure 3**)

Text S3

Experimental Methods and Materials

Gold Nanoparticle Preparation: Citrate-stabilized gold nanoparticles were purchased from Ted-Pella, Inc. (Redding, CA). The listed approximate size and concentration were 5 nm in diameter and 83 nM. We first stirring such nanoparticles solutions with an excess (1 mg per mL) of bis(p-sulfanatophenyl)phenyl-phosphine (BSPP from Strem Chemicals, Inc., Newburyport, MA) overnight. Later, we collected the nanoparticles, now coated with BSPP by centrifuging the solution at 3000 rpm for 15 mins. We then removed the supernatant solution and resuspended the nanoparticles in BSPP solution (1 mg BSPP/1mL deionized water). Based upon the particle size and optical extinction ($\epsilon_{520nm} = 2.3 \times 10^7 M^{-1} cm^{-1}$), we obtained the nanocrystal concentration from optical absorption spectrum measurement. Our stock solution was concentrated to 2 μM, and at ambient temperature when not in use.

Preparation of single-stranded (ss) DNA: We obtained our ssDNA of desired lengths from Integrated DNA Technologies Inc. (Coralville, IA). The 5' ends were modified with hexylthiol disulfide linkers, and then ssDNA were resuspended in 10mM Tris buffer containing 0.5mM EDTA in a ~100 μM concentration.

Preparation of Au- ssDNA conjugates: In one reaction, we mixed 70 μL stock solution of Au nanoparticles, 1.5 μL ssDNA stock solution, 10 μL BSPP (5 mg/mL in deionized water), with 10 μL 500 mM NaCl solution, and had them react overnight without stirring at room temperature. The reaction mixture was then mixed with 10 μL of 20 mM poly(ethylene glycol) methyl ether thiol (MPEG) and reacted for 2 hours to replace the BSPP ligands on the Au nanoparticles. The solution then went through high performance liquid chromatography (HPLC) to collect Au nanoparticles with one and two ssDNA on the surface separately, depending on the desired nanoconjugate structure.

Preparation of dimers and trimers: For dimers, Au-ss DNA monoconjugate with complementary DNA sequence were mixed in equal amount and left reacting at room temperature overnight. Then reacted solution went through an Agarose gel (3 wt% in deionized water) in electrophoresis to obtain Au-ds DNA conjugates, dimers, from unreacted Au-ss DNA monoconjugate. For trimers, the only difference was then the middle Au nanoparticles were conjugated with two ssDNA molecules, and then hybridized with Au-ss DNA monoconjugate twice in amount. The electrophoresis was done in 0.5 x tris-borate-EDTA (TBE) buffer. The gels were run for 1 hour at 125 V, after which several distinct bands were visible. We extracted dimers, or trimers, by cutting the gel at the end of the proper band and immersing the gel piece in 0.5 x TBE buffer and 200 mM NaCl solution. The obtained Au-dsDNA nanoconjugate solution was stocked in fridge when not in use.

Preparation of graphene covered TEM grids: The base TEM grids are Quantifoil R1.2/1.3 300 Mesh Au holey carbon TEM grids purchased from SPI supplies. We followed a modified procedure of the previously published method to transfer graphene onto the base TEM grids [RS1: Regan W et al (2010) A direct transfer of layer-area graphene *Appl. Phys. Lett.* 96:

113102]. In short, TEM grids were pressed against graphene grown on Cu foil via chemical vapor deposition. A drop of isopropanol was cast on the Cu foil to immerse the pressed TEM grids to bind them with graphene as isopropanol evaporates. Then the Cu foil together with attached TEM grids up towards the air were put on the surface of a sodium persulfate (Sigma Aldrich) solution (10 mg/10mL) which etched away the Cu foil, leaving TEM grids coated with graphene. Such TEM grids were subsequently rinsed with deionized water three times to get rid of the etching solution, and were left dry for further use.

Loading liquid sample into graphene liquid cell: We laid one graphene coated TEM grid onto a hard substrate such as a glassslide with the graphene side facing upwards. One tiny droplet (diameter ¼~1/3 of the TEM grid diameter) of the aqueous solution was deposited onto the center of the TEM grid. Then the other graphene coated TEM grid was put from above to cover the bottom TEM grid and the droplet. During the slight evaporation of water after 10 mins in the air, the top and bottom TEM grids were brought into close contact and kept the rest of the solution intact and sealed.

Microscopy

The TEM imaging was performed on LaB6 Tecnai G2 S-TWIN TEM at 200 kV. An accelerating voltage of 200 kV was used to obtain enough contrast and also to balance between avoiding damaging graphene layer and lowering radiation damage. We set 0.1 seconds of exposure time, 0.9 seconds of read out time, and therefore 1 seconds of entire frame time to acquire time-serial images.

Supplementary Figures and Tables

Figure S1: Aqueous solution wrapped by graphene layers. (A) The stability of a liquid pocket determined by the change in the average intensity of captured image over time. From the plot, the intensity stays quite consistent for the first 2 mins and then increases abruptly, indicating that the sample region being observed becomes thinner We interpreted this liquid pocket thinning as a result of liquid escaping from the graphene layer, probably because of the 200 kV imaging voltage higher than knock on voltage of graphene (80 kV). (b) The size distribution of liquid pockets, all in the range of micrometer. The inset is a low magnification TEM of one liquid pocket.

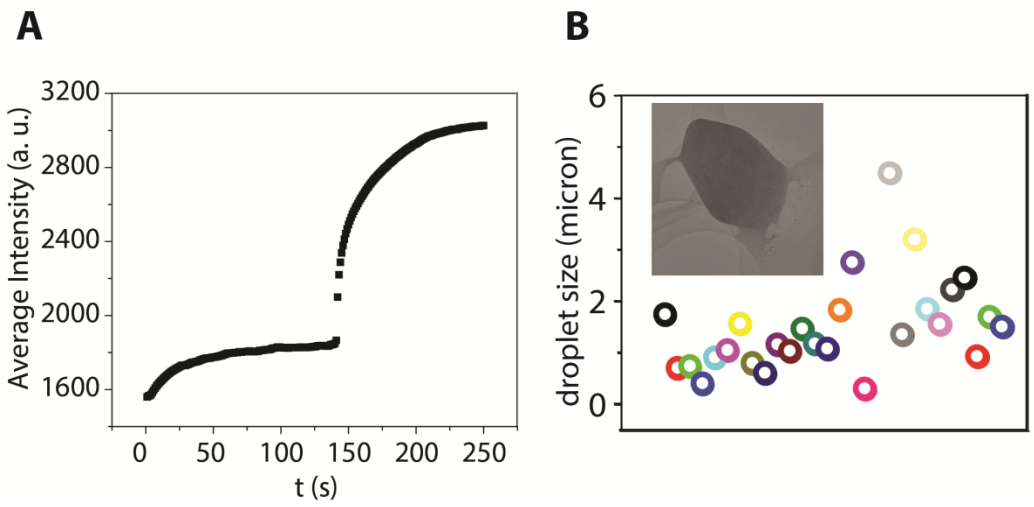

Table S1 The diffusivities of nanoparticles in a liquid cell. D is calculated as a fourth of the slope of $MSD - t$ curve. D_{pre} is calculated from the Stokes-Einstein equation for a spherical particle diffusing in the Brownian fashion $D = \frac{k_B T}{6\pi\eta r}$, where T is 298 K as the heating effect from the beam is negligible, η is the viscosity of the bulk liquid. It is obvious the nanoparticles move six or seven orders of magnitude slower than what is predicted from a bulk liquid environment.

Liquid	Liquid cell type	nanocrystal diameter (nm)	Particle diffusivity D (nm^2/s)	"predicted" diffusivity D_{pre} (nm^2/s)	D_{pre}/D
Aqueous buffer (in this paper)	Graphene liquid cell	5	1.7	$8.7x10^4$	$5.1x10^7$
water glycerol mixture	Si_3N_4 liquid cell	5	0.065	$4x10^5$	$6.4x10^6$
oleyamine	Graphene liquid cell	0.27	1.1	$1.6x10^7$	$1.5x10^7$

Table S2 Runs of iterative reconstruction methods. l_1 and $l_2$are the initial inputs of the code, $Stdl(_3)$, the standard deviation of l_3 from each frame, serves as the evaluation factor of the choice of inputs. For round 3 and 4, $Stdl(_3)$ converges to a minimum value. And then the remodeling of motions shown in Figure 2 is performed using this set of inputs, as well as an average l_3.

rounds	l_1(nm)	l_2(nm)	Std (l_3) (nm)	Std (α) (degree)
1	9.1	9.5	1.19	14.6
2	9.1	9.1	1.07	13.2
3	8.8	9.1	1.01	12.7
4	8.7	9.1	1.01	12.7

Supplementary Videos

Video S1 The movie shows a rotating dimer (see **Scheme 1** for selected images). The frame rate 10/sec and the movie is played at 10× real time. The red traces show the center-of-mass positions of the particle in previous five frames.

Video S2 The TEM movie (left) and 3d dynamics reconstruction (right) of the trimer (see **Figure 3**). The frame rate is 5/sec and the movie is played at 5× real time. In the right 3d plot, the moving trimer composed of yellow spheres shows its motion in 3d, with red arrow highlighting its rotational motion. The dark circular shapes following this trimer are the projections calculated from this trimer, and match with the left TEM movie.

Video S3 The movie shows how a pyramid structure composed of four Au nanoparticles got broken at the end of the imaging time. The frame rate is 5/sec and the movie is played at 5× real time.

Video S4 The movie shows how free Au nanoparticles and dimers behave in a convection flow of the liquid (see **Figure 5**). The frame rate is 5/sec and the movie is played at 5× real time.