Title

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Investigation of the Three-Dimensional Structural Dynamics and Fluctuations of DNA-Nanogold Conjugates by Individual-Particle Electron Tomography

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Abbreviations:

3D, three-dimensional; dsDNA, double-stranded deoxyribonucleic acid; EM, electron microscopy; ET, electron tomography; IPET, individual-particle electron tomography; NG, nanogold; NS, negative-staining; OpNS, optimized negative-staining.

Keywords: 3D structure, DNA-nanogold conjugates, individual-particle electron tomography, IPET

Abstract

DNA base-pairing has been used for many years to direct the arrangement of inorganic nanocrystals into small groupings and arrays with tailored optical and electrical properties. The control of DNA-mediated assembly depends crucially on a better understanding of the three-dimensional (3D) structure of the DNA-nanocrystal hybridized building blocks. Existing techniques do not allow for the structural determination of these flexible and heterogeneous samples. Here, we employed cryo-electron microscopy (cryo-EM) and negative-staining (NS) techniques to investigate the morphology of DNA-nanogold conjugates that were self-assembled from a mixture of an 84-base-pair double-stranded DNA (dsDNA) conjugated with two 5-nm nanogold particles for potential substrates in plasmon coupling experiments. Using NS electron tomography and the individual-particle electron tomography (IPET) reconstruction method, we obtained 3D reconstructed 14 electron density maps at a resolution of ~2 nm from each individual dsDNA-nanogold particle. Using these 3D density maps as constraints, we derived 14 conformations of dsDNA by projected a standard flexible dsDNA model onto the observed EM density maps using Molecular Dynamics (MD) simulations. The variation of the conformations was largely consistent with the variation from liquid solution. Moreover, the IPET approach provides the most complete experimental determination of the flexibility and fluctuation range of these directed nanocrystal assemblies to date. The general features revealed by these experiments can be expected to occur in a broad range of DNA-assembled nanostructures.

Introduction

Organic–inorganic hybridized nanocrystals are a valuable class of new materials that are suitable for addressing many emerging challenges in biological and material sciences [1,2]. Nanogold and quantum dot conjugates have been used extensively as biomolecular markers [3,4], whereas DNA base-pairing has directed the self-assembly of discrete groupings and arrays of organic and inorganic nanocrystals in the formation of a network solid for electronic devices and memory components [5]. Discretely hybridized gold nanoparticles conjugated to DNA were developed as a molecular ruler to detect subnanometer distance

changes via plasmon coupling-mediated variations in dark field light scattering [3,6]. For many of these applications, it is desirable to obtain nanocrystals functionalized with discrete numbers of DNA strands [7,8]. In all of these circumstances, the soft components can fluctuate, and the range of these structural deviations have not previously been determined with a degree of rigor that could help influence the future design and use of these assemblies.

Conformational flexibility and dynamics of the DNA-nanogold conjugates limit the structural determination by X-ray crystallography, nuclear magnetic resonance (NMR) spectroscopy and single-particle electron microscopic (EM) reconstruction, because they do not crystallize, are not sufficiently small for NMR studies and cannot be classified into a limited number of classes for single-particle EM reconstruction. Additionally, the three-dimensional (3D) structure averaged from tens of thousands of different macromolecular particles obtained without knowledge of the macromolecular structural flexibility could result in an absence of flexible domains upon employing the single-particle reconstruction method; for example, two ankyrin repeated regions of TRPV1 were absent in its atomic resolution 3D density map [9].

A fundamental experimental solution to reveal the structure of flexible macromolecules is to determine the structure based on each individual macromolecule itself [10]. Electron tomography (ET) provides high-resolution images of a single object from a series of tilted viewing angles [11]. ET has been applied to reveal the 3D structure of a single section of a cell and an entire bacterium at nanometer-scale resolutions [12]; however, obtaining a 3D reconstruction of an individual macromolecule remains challenging. The first 3D reconstruction of an individual macromolecule, a fatty acid synthetase molecule, was reported by the Walter Hoppe group in 1974 [13]; however, serious doubts have been raised regarding the validity of this structure [14], as this molecule received a radiation dose hundreds of times greater than the reported damage threshold [15]. Whether a structure at an intermediate resolution (1-3 nm) can be achieved from only approximately one hundred low-contrast ET images of an individual macromolecule particle under a low-dose illumination condition remains unclear. Recently, we re-investigated this possibility on simulated ET data, real experimental negative-staining (NS) and electron cryo-tomography (cryo-ET) images [10]. We demonstrated that a 3D structure at an intermediate resolution (1-3 nm) could potentially be achieved from an individual protein particle using our reported approach, individual-particle electron tomography (IPET) [10,16-18]. IPET is an iterative refinement process that contains a series of automatically generated dynamic filters and soft-masks. It does not require a pre-given initial model, class averaging of multiple macromolecules or an extended ordered lattice. However, it can tolerate certain levels of measurement tilt-errors and image distortion to precisely determine the translational parameters by decreasing the reconstruction image size to reduce the effects of measuring tilt-errors and large-scale distortions of ET micrographs on the 3D reconstruction. It can be used to obtain a “snapshot” single-molecule 3D structure at an intermediate resolution and even to reveal the macromolecular dynamics and fluctuation [17].

Here, we employed IPET, cryo-electron microscopy (cryo-EM) and our previously reported optimized negative-staining (OpNS) [19,20] techniques to investigate the morphology and 3D structure of hybridized DNA-nanogold conjugates. These conjugates were self-assembled from a mixture of two mono-conjugates, each consisting of 84-base single-stranded DNA and a 5 nm nanogold particle. The dimers were separated by anion-exchange high-performance liquid chromatography (HPLC) and agarose gel electrophoresis as potential substrates in plasmon coupling experiments.

Results

The TEM grids of the HPLC-purified 84-base-pair double-stranded DNA (dsDNA, the molecular mass was ~52 kDa) and two 5 nm nanogold conjugates (**Extended Data Fig. 1**) were prepared by two methods, *i.e.* electron cryo-microscopy (cryo-EM, native buffer, vitreous ice, no staining) and optimized negative staining (OpNS) [19,20]. Cryo-EM is an often used method to study protein structures under near-native conditions, as it can prevent artifacts induced by fixatives and stains. However, imaging a small

protein (< 100 kDa) has generally much lower contrast, making it challenging to be visualized and even difficult to be 3D reconstructed. In comparison, NS is a historical method for high contrast imaging of small proteins through heavy metal salts which coat the surface of proteins. Induced by heavy metal reaction, conventional NS sample have a potential for artifacts in structure, such as the rouleaux formation of lipoprotein particles [19,21]. We previously investigated this artifact and reported an OpNS refined from the conventional NS protocols [22] via reducing the rouleaux artifact of lipoprotein particles [19,21] using cryo-EM as a control [19]. This OpNS method has also been tested by proteins with known structure, such as 53 kDa cholesteryl ester transfer protein [23], GroEL and proteasomes [20]; flexible proteins with partially known structure have also been tested, such as the IgG1 antibody and its peptide conjugates [16,17]. The heavy metal atoms surrounding the proteins provide greatly increased electron scattering and more radiation damage resistance than only the relatively light atoms of proteins. We used both cryo-EM and OpNS methods to examine the sample under -178°C and room temperature respectively (**Fig. 1**).

Cryo-EM and NS images of DNA-nanogold conjugates

Survey cryo-EM micrographs and OpNS EM micrographs at low magnification (**Fig. 1a and e**) showed that each pair of nanogold particles was near one another. A statistical analysis of 1,032 nanogold particles from cryo-EM micrographs showed that the particles had a diameter of ~63.5 ± 6.7 Å (mean ± standard derivation, std) and a peak population (~25.1%) diameter of 63.6 ± 1.0 Å (black solid line in **Fig. 1c**). This measurement is consistent with those from OpNS, *i.e.*, 606 nanogold particles from NS micrographs showed that the particles had a diameter of ~63.0 ± 6.4 Å (mean ± std) and a peak population (~25.5%) diameter of 62.8 ± 1.0 Å (blue dashed line in **Fig. 1c**).

To quantitatively identify whether the pairs of nanogold particles are strongly linked together by a statistical method, Pearson's correlation coefficients [24] were used and calculated from the cryo-EM and OpNS micrographs. The Pearson's correlation coefficients, $r_{x,x}$ (defined as $r_{x,x} = \sum_{i=1}^{n}(x_i^a - \bar{x}^a)(x_i^b - \bar{x}^b) / \sqrt{\sum_{i=1}^{n}(x_i^a - \bar{x}^a)^2 \sum_{i=1}^{n}(x_i^b - \bar{x}^b)^2}$ for the *x*-axis coordinates of two objects, a and b) and $r_{y,y}$ for the *y*-axis coordinates, were 0.9996 and 0.9976 for cryo-EM, and 0.9984 and 0.9983 for NS-EM results, respectively. The coefficients corresponded well with previous TEM observations of the same sample in liquid solution (i.e., $r_{x,x}$ = 0.934 and $r_{y,y}$ = 0.943) [24]. The high Pearson's correlation coefficients suggests that the pair of nanogold particles are strongly linked together [24].

Higher-magnification cryo-EM images of 24 representative particle pairs (**Fig. 1b**) and higher-magnification NS images of 36 representative particle pairs (**Fig. 1f**) revealed that the polygonal-shaped nanogold particles were bridged by a ~2 nm-width fiber-shaped density. A statistical analysis of the distances among the 516 pairs of cryo-EM nanogold particles yielded a length of 255.3 ± 48.7 Å (mean ± std, measured from the center to center of the nanogold particles) with a peak population (~19.7%) distance of 286.4 ± 10 Å (black solid line in **Fig. 1d**). In comparison, a statistical analysis of the distances between two nanogolds of 303 pairs yielded a length of 245.5 ± 62.6 Å (mean ± std, measured from the center to center of the nanogold particles) with a peak population (~15.6%) distance of 287.0 ± 10 Å (blue dashed line in **Fig. 1d**).

The width and length of the fiber-shaped densities measured from both cryo-EM and NS images were consistent to each other and similar to those measured from liquid solution by SAXS, *i.e.*, 28-30 nm [25]. This dimension is also similar to those of a standard model of 84-base-pair dsDNA (~2 nm wide and ~30 nm long).

Additionally, several pairs of nanogold particles presented abnormally closer to one another in both cryo-EM and NS. The higher contrast NS images showed their fiber-shaped bridging densities appeared thicker, but with lengths ranging from ~20 – 30 nm seemed similar to those of the full length of the dsDNA (**Fig. 1g**). We suspected these particles may be formed by two conjugates, in which each

conjugate lost one of their two containing nanogolds, but met each other and formed a supercoil via their two dsDNA portions. The mass of those complexes is only 53kDa above that of the regular conjugates, which is too small to be identified or isolated by our filtration.

3D reconstruction of an individual DNA-nanogold conjugate

To obtain a 3D structure of the DNA-nanogold conjugates, we employed the IPET technique [10] rather than the conventional single-particle reconstruction method or sub-volume averaging ET method because these conjugates were not guaranteed to share the same structure (DNA is naturally flexible and dynamic in structure). IPET is used to obtain the *ab initio* 3D structure of an individual macromolecular particle from the targeted particle's images themselves acquired from a series of tilt angles using ET (**Fig. 2a**). Unlike conventional single-particle reconstructions, IPET does not require a homogenous population, the averaging of different particles, or a pre-determined initial model [10].

Although the DNA portion in the cryo-EM images could be barely visible under a total maximal dose of ~20$e^-/Å^2$ (beyond this dose limitation, the contrasts rapidly disappeared), this dose limitation prevents us from further tomography data collection on a whole tilt series (~80 micrographs), which limited three-dimensional (3D) reconstruction under this current condition. Thus, we used NS-EM for the IPET reconstructions.

The signal-to-noise ratios (SNRs) of the nanogold portion in the tilt series of images (from -60° to +60° at 1.5° increments) were only ~0.19 to ~0.41 with an average of ~0.31, the overall shape of the DNA was still visible in the tilt series **(Extended Data Video**) and the representative tilt images (**Fig. 2b, left column)**. After contrast transfer function (CTF) correction, the tilt images were iteratively aligned to their global center to achieve a final *ab initio* 3D reconstruction (**Fig. 2b, right panel**). During the iterations, the SNR of the DNA portion gradually increased up to ~2.44 in the final 3D reconstruction. The final 3D showed an overall handcuff-shape (**Fig. 2c**) at a resolution of ~14.7 Å, which was measured based on a Fourier shell correlation (FSC) analysis, i.e., the spatial frequencies at which the curve falls to 0.5 (details given in the method section) (black line in **Fig. 2e**).

To avoid the potential over-estimation of the FSC defined by the resolution of the nanogolds instead of correctly reflecting the resolution of the DNA, we masked out the DNA portion only to repeat the above FSC analyses. The analyses showed, without the nanogolds, the FSC curve is nearly identical to those which contain nanogolds (red vs. black lines in **Fig. 2e)**, suggesting that the nanogold component did not lead to an over-estimation of the resolution of the 3D reconstruction.

The surface of the nanogold particles appeared to be coated with a layer of densities, which may be the polyethylene glycol (PEG) surface protection layer on the particle surfaces (**Fig. 2c**).

Considering that the nanogold particles were in opposite image-contrast to the DNA, we reversed the image-contrast of the final 3D (colored in gold) and overlaid this 3D with its original 3D to display both the DNA and nanogold particles in a same 3D map (**Fig. 2d**). This overlaid map showed the nanogold particles with diameters of ~73.0 and ~72.0 Å that were bridged by a high-density fabric with dimensions of ~242.0 Å × ~18.0 Å × ~18.0 Å (**Fig. 2d**). The surfaces of each nanogold particle were surrounded by irregularly shaped densities, which is possibly thiolated short chain PEG molecules used to stabilize the particles against aggregation at high ionic strength.

Although the resolution of the fabric density was insufficient to determine the orientation and structure of the DNA at the atomic level, its overall shape could be used as a constraint to flexibly dock the standard structure of an 84-base-pair dsDNA into it to achieve a dsDNA conformation. By satisfying both the best fit of the density map and the chemical minimal energy requirements, a dsDNA conformation was

obtained by gently bending the straight dsDNA structure into the density map using molecular dynamics (MD) simulations (**Fig. 3a, and Extended Data Video**).

By repeating the above IPET process, we reconstructed a second 3D density map from another individual DNA-nanogold conjugate (**Fig. 2f-i**). The representative tilt images showed that the DNA was still visible (**Fig. 2f left panel**), and the SNR of DNA portions were ~0.41 to ~ 0.62, with an average of 0.56. Through IPET reconstruction processing, the tilt images were gradually and iteratively aligned to the global center (**Fig. 2f**), and the SNR was gradually increased up to ~3.26 in the final 3D reconstruction (**Fig. 2g**). The overall handcuff-shaped 3D reconstruction had a measured resolution of ~17.1 Å based on the FSC analyses under with and without the nanogold portion conditions (**Fig. 2i**). The overlaid density map from the final 3D density map and its reversed density map (colored in gold) showed that the nanogold particles had diameters of ~71.0 Å and ~65.0 Å that were bridged by a fabric-like DNA density with dimensions of ~191.0 Å × ~20.0 Å × ~20.0 Å (**Fig. 2h**). Again, the nanogold particles were surrounded by irregularly shaped densities of a PEG surface protection layer against aggregation at high ionic strength. We obtained another conformation of the dsDNA by flexibly docking the standard structure of an 84-base-pair dsDNA model into the bridging portion density and following MD simulation for energy minimization **(Fig. 3b)**.

3D reconstructions of a total of 14 individual DNA-nanogold conjugates

Through particle-by-particle 3D reconstructions, a total of 14 conjugates were reconstructed using IPET (**Fig. 3, Extended Data Figs. 2-13 and Extended Data Table 1**). Other conjugates were excluded from the 3D reconstruction because they were overlapping to each other at certain tilt angles, missing tilt images, contained unevenly stained background, or exhibited a poor SNR of the dsDNA portion. The steps leading to the structures of an additional 12 DNA-nanogold conjugates are shown in **Extended Data Figs. 2-13.** The selected 2D projections of their final 3D reconstructions showed a fabric DNA density between two nanogold particles (**Fig. 3c**). The overlaid density maps (reversed maps are colored in gold) (**Fig. 3d and e**) also confirmed that the nanogold particles were connected by densities attributable to dsDNA. The resolutions of their final 3D reconstructions were ~20 Å, which allowed us to flexibly dock the DNA model into them to obtain 12 additional DNA conformations via MD simulations using the CHARMM force field for all MD simulations [26,27] (**Fig. 3e** and **Extended Data Figs. 2c-13c)**.

Although up to ~30% of the fabric-like densities in those maps (**Fig. 3e**) could not be fully observed under the selected contour levels due to various factors, such as uneven staining, image noise and reconstruction errors, these defects had a limited effect on the spacing distribution and the overall shape determination of the dsDNA due to its connectivity. The dsDNA conformations could still be determined from these maps.

Statistical analyses of the structures of the DNA-nanogold conjugates

Aligning the 14 conformations of dsDNA along their first 14 base-pairs yielded a distribution in a shape of a bundle of flowers (**Fig. 3f**). Considering the 14 conformations is insufficient to reveal the full 3D distribution of DNA conformations, only one dimensional distribution analysis was conducted, i.e., the nanoparticle size and DNA length. The histogram of the nanogold particle sizes measured from the 3D reconstructions (the measurement method described in **Extended Data Method section**) showed that the geometric mean of the nanoparticle diameters was 65.7 ± 5.0 Å, which is similar to the diameter measured from the 2D images of the 606 nanogold particles (62.8 ± 1.0 Å at peak population, ~25.5%) **(Fig. 1d and Extended Data Table 1).** The average distance measured from the 3D reconstructions (the measurement method described in **Extended Data Method**) was 291.1 ± 31.9 Å (mean ± std), which was longer than the mean distance measured from the 2D images (245.5 ± 62.6 Å) but similar to the distance of 287.0 ± 10.0 Å at the peak population (~15.6%) **(Fig. 1d)**. Approximately 69% of the distances measured from 2D images were shorter than the peak distance, whereas only ~31% were longer (**Extended Data Table 1**). This uneven distribution of the distance around the distance of the peak

population is likely due to the portion of dsDNA that formed a supercoiled structure that was not reconstructed or measured in the 3D reconstructions. These variations in conformations reflected the conformational flexibility and dynamics of the dsDNA between the two conjugated nanogold particles.

Although the particles flash-fixed by heavy ions and attached to a substrate (carbon film) may cause certain artifacts, such as a preferred orientation, flatness and an uneven staining distribution, the statistical analyses showed that the measured lengths from 303 dimers were highly similar to the same sample measured in solution (**Extended Data Table 2**) [24,25]. In detail, the distances measured using SAXS from the same sample in solution were ~280 Å on average and ~320 Å at the peak population [25], which were ~10-15% longer than those measured from our 2D images, i.e., 245.5 ± 62.6 Å and 287.0 ± 10.0 Å at the peak population (~15.6%) (**Fig. 1d**), as measured from center to center of the nanogold particles. Considering the length measured from the 2D EM micrographs corresponded to the projection distance of the 3D length in solution, the 2D projection is naturally shorter than the 3D object by a factor of π/4 under an isotropic distribution assumption condition. Based on the solution measured distances, their corresponding 2D projection distances should be ~220 Å (π/4 × 280 Å) on average and ~250 Å (π/4 × 320 Å) at the peak population, which were ~10-12% shorter than our measurement from the 2D EM images. Our measured lengths (~246 Å and ~287 Å) were between the 3D lengths (~280 Å and ~320 Å) and the 2D projection lengths (~220 Å and ~250 Å), suggesting our particles have a certain preferred orientation to the carbon film. The mean distance measured in solution via in situ TEM was ~180 Å [24], which was ~26% shorter than the mean measured from our 2D images. Although the short-distance views of the targeted conjugate was specifically chosen for easy tracking and imaging in the in situ experiment, the mean value was still close to the error bar range in our measurement.

Statistical analyses of the bending energy of DNA-nanogold conjugates

The conformations of dsDNA provide an opportunity to study the DNA bending energy. The bending energy can be calculated based on a simple worm-like chain (WLC) model [25,28,29], in which the energy of the dsDNA is simplified to the bending potential while ignoring the distortion potential at the single-base-pair level. The calculation of the bending energy depends on the local bending angles of each base-pair, which is governed by a single parameter for the mechanical response, *i.e.*, the persistence length. Although the persistence length could be extracted by measuring the tangent correlation function for a worm-like chain, the length of our 84 base pair DNA here is too short to derive a persistence length. Thus, the widely used persistence length of 50 nm was used to compute the bending energy as this length is a favorable parameter to describe the dsDNA conformational statistics for constructs composed of tens of base-pairs [25].

To measure the local bending angle (**Fig. 4a**), each of the two nearby base-pairs were first fitted with a small standard cylinder with a fixed length and width (**Fig. 4b and 4c**). Two types of angles can be measured to represent the bending angle of the base-pair, i.e., the angle θ_i, formed between center-to-center directions of three nearby cylinders (**Fig. 4d**) and the angle φ_i, formed between the two axes of nearby cylinders (**Fig. 4e**). The energies of each base-pair in the DNA conformation were calculated and summed to represent the total energy of this DNA conformation. The energy distribution of the 14 conformations showed that the averaged DNA bending energies were ~116 and ~169 kcal/mol based on two types of measured angles (**Fig. 4f and Extended Data Table 2**). The average bending energies were ~2-3 times higher than the bending energy calculated based on a theoretical WLC model prediction on 84-base-pair DNA (~50 kcal/mol at room temperature) [25], suggesting the 14 DNA conformations were more flexible than the prediction.

The parameters may influence the length and bending energy of DNA

Above analyses showed two definition types of bending angles, *i.e.* θ_i and φ_i could cause a ~50% difference on the computed bending energy from the experimental DNA conformations. Considering the EM observed DNA is more flexible than predicted, it is worthy to evaluate whether other factors may also

influence the measurement of the DNA length and bending energy, such as the fluctuation of distal ends, DNA modeling methods, noise bias in the EM density map, initial model bias and temperature. Additional analyses were performed as the following: i) the bending energy based on the central 42 base pair was recalculated. This was because 84 base pair DNA is relatively short, and the ends of the chain exhibit greater fluctuations than the middle portion. The calculation results showed the bending energy is ~15% less than when using all 84 base pairs (**Extended Data Table 2**), suggesting DNA is still more flexible than the WLC prediction. (ii) The initial model of DNA was obtained by manually fitting a standard DNA model to each EM configuration. Considering the manual operation may lead to kinks, which are difficult to be repaired by MD simulation, we used another method to generate a smooth curved model of DNA whose structure was as close as possible to the canonical B-form DNA double helix (**details in Extended Data Method section**). Before conducted any simulation, we calculated its bending energy, which is only about ~10% of the above bending energy, and only about 20% of the WLC prediction, suggesting the smooth DNA is more stiff than the WLC prediction and EM configurations. iii) After submitting this smooth model for energy minimization using the Nanoscale Molecular Dynamics version 2 (NAMD2) software package [30], we found the bending energy was increased by ~4-5 times becoming close to the WLC prediction. iv) However, after submitting the model to further MD simulations for only 0.1 ns, the calculated energy jumped up to ~80% of the energy from the EM configuration, confirming the DNA is more flexible than the WLC prediction. This result suggests the MD simulations may play the key role to increase the bending energy and result in a more flexible DNA model. v) To evaluate how the noise in the density map may influence the bending energy, we conducted MDFF based on the smooth model to constrain its structure with the EM density map. The calculated bending energy immediately jumped up to an even higher level, *i.e.*, ~10% more than that from the EM configurations. This test suggests the MDFF and noise in the EM density could be critical in causing the DNA model to be more flexible than it should be. vi) To avoid a potential influence to the energy from the given initial models, a standard and straight model of DNA was used for MD simulations under the same condition, *i.e.* under 0.15 M physiological salt solution, temperature of 298 K and a pressure of 1 atm. Using NAMD2 for 20 ns of simulation without any constraint for DNA conformational changes, the equilibration of DNA in a box of ~347.3 Å × ~93.4 Å × ~50.8 Å was monitored using the root mean-square deviation (RMSD) by visual molecular dynamics (VMD) [31] (**Extended Data Fig. 14a**). The bending energies (**Extended Data Fig. 14b**) and the distances between the two distal ends (**Extended Data Fig. 14c**) revealed that the system became nearly balanced after 8 ns. The statistical analyses of the bending energies of the DNA in its last 10 ns simulations showed that the averaged energy was 99.1 ± 10.9 kcal/mol with a peak population (~7.1%) energy of 97.4 ± 1.0 kcal/mol based on the bending angles of the φ_i calculation, whereas the average was 152.1 ± 16.1 kcal/mol with a peak population (~4.6%) energy of 151.3 ± 1.0 kcal/mol based on the bending angles of the θ_i calculation (**Extended Data Fig. 14b and d**). This energy is surprisingly similarly to that from the EM configuration (about 10-15% lower) (**Extended Data Table 2**). The statistical analyses of the length between the two distal ends of equilibrated DNA in the last 10 ns of the simulations showed that the average length was 268.5 ± 2.1 Å with a peak population (~18.5%) length of 267.9 ± 0.5 Å (**Extended Data Fig. 14c and e**). The distance is similar to the length at the peak population measured from TEM images. vii) To evaluate how temperature influences the bending energy measurement using MD simulations, the above processes were repeated under a higher temperature, *i.e.*, 310 K instead of 298 K (**Extended Data Fig. 15**). The bending energies calculated under the higher temperature in the last 10 ns showed that the averaged energy was 120.8 ± 14.7 kcal/mol with a peak population (~6.1%) energy of 115.1 ± 1.0 kcal/mol based on the bending angles of the φ_i calculation, whereas the average was 177.9 ± 15.6 kcal/mol with a peak population (~5.1%) energy of 177.5 ± 1.0 kcal/mol based on the bending angles of the θ_i calculation (**Extended Data Fig. 15b and d**). The energy is increased by ~20% from those under 298 K and becomes more similar to that from the EM configuration, suggesting the temperature is related, but not critical. The statistical analyses on the length showed that the averaged length was 269.5 ± 2.9Å with a peak population (~13.3%) length of 271.1 ± 0.5 Å (**Extended Data Fig. 15c and e**), which is similar to those measured from the EM configurations and 298 K MD simulation, suggesting the length is insensitive to

the temperature. viii) To further confirm the length is insensitive to bending energy, one EM configuration with the DNA length of ~241.0 Å was performed by MD simulations under a length constrain (**Extended Data Fig. 16**). This length is close to the mean length of the DNA portion estimated from solution using SAXS [25]. The bending energy in the last 10 ns was 105.8 ± 10.7 kcal/mol with a peak population (~7.3%) energy of 102.7 ± 1.0 kcal/mol based on the bending angles of the φ_i calculation, whereas the average was 163.6 ± 17.4 kcal/mol with a peak population (~5.0%) energy of 158.4 ± 1.0 kcal/mol based on the bending angles of the θ_i calculation (**Extended Data Fig. 16b and c**). This energy is similar to that from the other EM configurations and simulations, suggesting the length can't reflect the bending energy and flexibility of DNA.

Above tests showed, although EM density maps contain noise, the bending energy can be influenced limitedly by the initial models and the EM map configuration. The similar bending energies calculated from different MD simulations and initial models suggested the DNA is more flexible than WLC prediction. However, we cannot exclude those MD simulations may result in DNA which presents more flexibility than WLC prediction.

Discussion

Although the direct imaging of dsDNA has been previously reported using heavy metal shadowing [32,33] and NS methods [34-36], to the best of our knowledge, the 3D structure of an individual dsDNA strand has not previously been achieved. It has been thought that individual dsDNA would be destroyed under the high energy of the electron beam before a 3D reconstruction, or even a 2D image, is able to be achieved. Our NS tilt images showing fiber-shaped dsDNA bridging two conjugated nanogold particles demonstrated that the dsDNA can in fact be directly visualized using EM, which is consistent with the recently reported single-molecule DNA sequencing technique via TEM [36]. The resolutions of our density maps ranged from ~14 Å to ~23 Å, demonstrating that an intermediate-resolution 3D structure can be obtained for each individual macromolecule. This capability is consistent with our earlier report of a ~20 Å-resolution 3D reconstruction of an individual IgG1 antibody using the same approach [16,17].

Notably, a total dose of ~2,000 $e^-/Å^2$ used in our ET data acquisition is significantly above the limitation conventionally used in cryo-EM (~80~100 $e^-/Å^2$), which can be suspected to have certain artifact from radiation damage. In cryo-EM, the radiation damage could cause sample bubbling, deformation and knock-out effects; in NS, only the knock-out phenomena is often observed, in which the protein is surrounded by heavy atoms which were kicked out by electron beam. Since the sample was coated with heavy metal atoms and dried in air, the bubbling and deformation phenomena were not usually observed. The heavy metal atoms which coat the surface of the biomolecule can provide a much higher electron scattering than from a biomolecule only inside lighter atoms. The scattering is sufficiently high to provide enough image contrast at our 120 kV high tension, thus, a further increase to the scattering ability by reducing the high tension to 80 kV may not be necessary for this NS sample. Additionally, the heavy atoms can provide more radiation resistance and allow the sample to be imaged under a higher dose condition. The exact dose limitation for NS is still unknown. The radiation damage related artifact in NS samples is knock-out, which could reduce the image contrast and lower the tilt image alignment accuracy and 3D reconstruction resolution. In our study, a total dose of 2,000 $e^-/Å^2$ did not cause any obvious knock-out phenomena, but provides a sufficiently high contrast for the otherwise barely visible DNA conformations in each tilt series. The direct confirmation of visible DNA in each tilt image is essentially important to us to validate each 3D reconstruction, especially considering this relatively new approach.

Our 3D reconstruction algorithm used an *ab-initio* real-space reference-projection match iterative algorithm to correct the centers of each tilt images, in which the equal tilt angle step for 3D reconstruction of a low contrast and asymmetric macromolecule was used. This method is different from recently reported Fourier-based iterative algorithm, termed equally sloped tomography (EST), in which the

pseudo-polar fast Fourier transform, the oversampling method and internal lattice of a targeted nanoparticle are used to achieve 3D reconstruction at atomic resolution [37].

It is generally challenging to achieve visualization and 3D reconstruction on an individual, small and asymmetric macromolecule by other conventional methods, our method demonstrated its capability for 3D reconstruction of 54 kDa 84-base-pair dsDNA through these studies, IgG1 antibody 3D structural fluctuation [17], peptide induced conformational changes on flexible IgG1 antibody[5], floppy liposome surface binding with 53kDa proteins [38], all suggesting this method could be used to serve the community as a novel tool for studying flexible macromolecular structures, dynamics and fluctuations of proteins, and for catching the intermediate 3D structure of protein assembling.

DNA-based self-assembling materials have been developed for use in materials science and biomedical research, such as DNA origami designed for targeted drug delivery. The structure, design and control require feedback from the 3D structure, which could validate the design hypothesis, optimize the synthesis protocol and improve the reproducible capability, while even providing insight into the mechanism of DNA-mediated assembly.

Contributions: This project was initiated and designed by JS and PA and refined by LZ and GR. JS prepared the conjugates. LZ, HT, ZL and GR prepared the TEM samples and/or acquired the data. LZ and GR processed the data, and LZ solved the IPET 3D structures. XZ, MZ and LZ docked the model, and DL measured the angles, computed/analyzed the energies of dsDNA in solution by the MD simulations. LZ, JS, DL, JS, PA and GR interpreted and manipulated the structures. GR drafted the initial manuscript, which was revised by LZ, JS, XZ, DL, ZL, HT, MZ and PA.

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Competing financial interests

The authors declare no competing financial interests.

Data deposition

The TEM 3D density maps of 14 DNA-nanogold conjugates are available from the EM data bank as EMDB IDs 2948 -2961.

Additional Information

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Figure Legends

Figure 1 | Electron cryo-microscopy (cryo-EM) and negative-staining (NS) electron microscopy images of DNA-nanogold conjugates. (a) Cryo-EM images (vitreous buffer, no staining) of 5-nm

nanogold particles conjugated to 84-base-pair double-stranded DNA (dsDNA) via a 5′ thiol linker. Pairs of nanogold were marked by yellow dashed ovals. (**b**) 24 representative cryo-EM images of the particles of DNA-nanogold conjugates. The polygonal-shaped areas are the nanogold particles, which were bridged by a fiber-shaped density, ~20-30 nm in length and ~2 nm in width. The surfaces of the nanogold particles were coated with a layer of extraneous polyethylene glycol (PEG) for surface protection. **(c)** Histogram of the geometric diameters of 1032 nanogold particles from cryo-EM images and 606 nanogold particles from NS images. **(d)** Histogram of the DNA lengths measured from 516 conjugates from cryo-EM and 303 conjugates from NS. The center-to-center length was measured between the centers of each nanogold particle pair. (**e**) NS images and (**f**) 36 representative NS images of the particles. The polygonal-shaped nanogold particles were bridged by a fiber-shaped density, and their surfaces were coated with a layer of extraneous polyethylene glycol (PEG) for surface protection. (**g**) A few pairs of nanogold particles were significantly closer in distance to each other, whereas their bridging fabric-like densities were thick, likely due to the supercoiling of the dsDNA. Scale bars, 30 nm.

Figure 2 | 3D reconstruction of two representative DNA-nanogold conjugates by individual-particle electron tomography (IPET). (**a**) The OpNS samples of DNA-nanogold conjugates were imaged using electron tomography (ET) from a series of tilt angles (from -60° to +60° at 1.5° intervals). Three targeted particles (yellow circled) with their orthogonal views are indicated by the linked dashed arrows in the three selected ET tilt micrographs. The relative tilt angles are indicated in each image, and the axis of the tilt is vertical to the images. (**b**) Nine representative tilt images of the first targeted individual particle are displayed in the first column from the left (SNR of DNA portion: ~0.31). Using IPET, the tilt images (after contrast transfer function correction, CTF correction) were gradually aligned to a common center for 3D reconstruction via an iterative refinement process. The projections of the intermediate and final 3D reconstructions at the corresponding tilt angles are displayed in the next 4 columns according to their corresponding tilt angles. (**c**) Final IPET 3D density map of the targeted individual particle (SNR of DNA portion: ~2.44). (**d**) The final 3D density map and its overlaid 3D density maps (final map in gray and its reversed map in gold) indicated the overall conformation of the DNA-nanogold conjugates. (**e**) The Fourier shell correlation (FSC) analyses under including (black line) and excluding (red line) nanogold portions (two density maps reconstructed from odd and even numbers of tilt images) revealed that the resolutions of the IPET 3D density map were both ~14.7 Å. (**f-i**) The 3D density map of a second individual DNA-nanogold conjugate was reconstructed from the tilt images (SNR of DNA portion: ~0.56) using IPET. The FSC analysis showed that the 3D reconstruction resolution (SNR of DNA portion: ~3.26) was ~17.1 Å. Scale bars, 20 nm in **a**; 10 nm in **d** and **h**.

Figure 3 | 3D conformations of 14 dsDNA structures obtained by flexibly fitting the double-stranded DNA model onto the EM density maps using targeted molecular dynamics (TMD) simulations. (a) The final density map provided a constraint for the TMD simulations to achieve a new DNA conformation. Four snapshot images during the TMD simulation illustrate the process of flexibly docking the DNA model into the IPET density map to achieve a new conformation of DNA. During this process, the DNA conformation was allowed to change its structure while maintaining its chemical geometry and bonds with local energy minimization. **(b)** The final conformation of the second dsDNA structure was obtained from the second density map by following the same processes. **(c-e)** Gallery of 12 additional conformations from the 3D density maps of an additional 12 DNA-nanogold conjugates reconstructed using IPET. (**c**) Selected projections of the 3D density map of each individual DNA-nanogold conjugate. (**d**) Final 3D density maps of the individual DNA-nanogold conjugates. **(e)** The overlaid density maps of the final 3D reconstruction (gray) and its reversed contrast map (gold) revealed the overall conformation of the DNA-nanogold conjugates. A standard 84-base-pair dsDNA structure was flexibly docked into each density map to achieve the new conformations via TMD simulations. **(f)** Conformational flexibility and dynamics of the DNA-nanogold conjugate. Fourteen conformations of the DNA-nanogold conjugates were aligned together based on their first 14 base-pairs. The distribution of dsDNA is shown from three orthogonal views. Scale bars, 5 nm in **a**; 10 nm in **c**, **d**, and **e**.

Figure 4 | Bending energy distribution of dsDNA. **(a)** dsDNA conformation was obtained by fitting the standard dsDNA model into the IPET 3D density map. **(b)** Schematic model illustrating that the nanogold interacts with the dsDNA and that the dsDNA contains kink regions that carry bending elasticity. **(c)** Cylinder model illustrating the bending angles between two connected base-pairs. The cylinder is defined by the two consecutive dsDNA base-pairs. **(d)** The bending angle can be presented by the angle θ_i, formed by the centers of three consecutive cylinders, or **(e)** by the angle φ_i, formed by the center axes of two consecutive cylinders. **(f)** Based on the two types of measured angles, θ_i and φ_i, the bending energies for each DNA conformation were calculated and plotted based on a simple worm-like chain (WLC) model. The averaged bending energies from the two types of angles are indicated by the dashed lines. The bending energy of the standard DNA model was also calculated and indicated as structure No. 0 as a control.

References

1 Alivisatos, A. P. *et al.* Organization of 'nanocrystal molecules' using DNA. *Nature* **382**, 609-611, doi:10.1038/382609a0 (1996).

2 Mirkin, C. A., Letsinger, R. L., Mucic, R. C. & Storhoff, J. J. A DNA-based method for rationally assembling nanoparticles into macroscopic materials. *Nature* **382**, 607-609, doi:10.1038/382607a0 (1996).

3 Elghanian, R., Storhoff, J. J., Mucic, R. C., Letsinger, R. L. & Mirkin, C. A. Selective colorimetric detection of polynucleotides based on the distance-dependent optical properties of gold nanoparticles. *Science* **277**, 1078-1081 (1997).

4 Wu, X. *et al.* Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots. *Nature biotechnology* **21**, 41-46, doi:10.1038/nbt764 (2003).

5 Zheng, J. *et al.* Two-dimensional nanoparticle arrays show the organizational power of robust DNA motifs. *Nano letters* **6**, 1502-1504, doi:10.1021/nl060994c (2006).

6 Barrow, S. J., Wei, X., Baldauf, J. S., Funston, A. M. & Mulvaney, P. The surface plasmon modes of self-assembled gold nanocrystals. *Nature communications* **3**, 1275, doi:10.1038/ncomms2289 (2012).

7 Claridge, S. A., Liang, H. W., Basu, S. R., Frechet, J. M. & Alivisatos, A. P. Isolation of discrete nanoparticle-DNA conjugates for plasmonic applications. *Nano letters* **8**, 1202-1206, doi:10.1021/nl0802032 (2008).

8 Jones, M. R., Seeman, N. C. & Mirkin, C. A. Nanomaterials. Programmable materials and the nature of the DNA bond. *Science* **347**, 1260901, doi:10.1126/science.1260901 (2015).

9 Liao, M., Cao, E., Julius, D. & Cheng, Y. Structure of the TRPV1 ion channel determined by electron cryo-microscopy. *Nature* **504**, 107-112 (2013).

10 Zhang, L. & Ren, G. IPET and FETR: experimental approach for studying molecular structure dynamics by cryo-electron tomography of a single-molecule structure. *PloS one* **7**, e30249, doi:10.1371/journal.pone.0030249 (2012).

11 Milne, J. L. & Subramaniam, S. Cryo-electron tomography of bacteria: progress, challenges and future prospects. *Nature reviews* **7**, 666-675 (2009).

12 Komeili, A., Li, Z., Newman, D. K. & Jensen, G. J. Magnetosomes are cell membrane invaginations organized by the actin-like protein MamK. *Science* **311**, 242-245, doi:10.1126/science.1123231 (2006).

55313 Hoppe, W., Gassmann, J., Hunsmann, N., Schramm, H. J. & Sturm, M. Three-
554 dimensional reconstruction of individual negatively stained yeast fatty-acid synthetase
555 molecules from tilt series in the electron microscope. *Hoppe-Seyler's Zeitschrift fur*
556 *physiologische Chemie* **355**, 1483-1487 (1974).
55714 Frank, J. *Electron Tomography, Methods for Three-Dimensional Visualization of*
558 *Structures in the Cell*. (Springer, 2006).
55915 Unwin, P. N. & Henderson, R. Molecular structure determination by electron microscopy
560 of unstained crystalline specimens. *Journal of molecular biology* **94**, 425-440 (1975).
56116 Tong, H. *et al.* Peptide-conjugation induced conformational changes in human IgG1
562 observed by optimized negative-staining and individual-particle electron tomography.
563 *Scientific reports* **3**, 1089, doi:10.1038/srep01089 (2013).
56417 Zhang, X. *et al.* 3D Structural Fluctuation of IgG1 Antibody Revealed by Individual
565 Particle Electron Tomography. *Scientific reports* **5**, 9803, doi:10.1038/srep09803 (2015).
56618 Lu, Z. *et al.* Calsyntenin-3 molecular architecture and interaction with neurexin 1alpha.
567 *The Journal of biological chemistry* **289**, 34530-34542, doi:10.1074/jbc.M114.606806
568 (2014).
56919 Zhang, L. *et al.* An optimized negative-staining protocol of electron microscopy for
570 apoE4 POPC lipoprotein. *Journal of lipid research* **51**, 1228-1236,
571 doi:10.1194/jlr.D002493 (2010).
57220 Rames, M., Yu, Y. & Ren, G. Optimized negative staining: a high-throughput protocol for
573 examining small and asymmetric protein structure by electron microscopy. *Journal of*
574 *visualized experiments : JoVE*, e51087, doi:10.3791/51087 (2014).
57521 Zhang, L. *et al.* Morphology and structure of lipoproteins revealed by an optimized
576 negative-staining protocol of electron microscopy. *Journal of lipid research* **52**, 175-184,
577 doi:10.1194/jlr.D010959 (2011).
57822 Ohi, M., Li, Y., Cheng, Y. & Walz, T. Negative Staining and Image Classification -
579 Powerful Tools in Modern Electron Microscopy. *Biological procedures online* **6**, 23-34,
580 doi:10.1251/bpo70 (2004).
58123 Zhang, L. *et al.* Structural basis of transfer between lipoproteins by cholesteryl ester
582 transfer protein. *Nature chemical biology* **8**, 342-349, doi:10.1038/nchembio.796 (2012).
58324 Chen, Q. *et al.* 3D motion of DNA-Au nanoconjugates in graphene liquid cell electron
584 microscopy. *Nano letters* **13**, 4556-4561, doi:10.1021/nl402694n (2013).
58525 Mastroianni, A. J., Sivak, D. A., Geissler, P. L. & Alivisatos, A. P. Probing the
586 conformational distributions of subpersistence length DNA. *Biophysical journal* **97**,
587 1408-1417, doi:10.1016/j.bpj.2009.06.031 (2009).
58826 Foloppe, N. & MacKerell, A. D. All-atom empirical force field for nucleic acids: I.
589 Parameter optimization based on small molecule and condensed phase macromolecular
590 target data. *Journal of computational chemistry* **21**, 86-104, doi:10.1002/(Sici)1096-
591 987x(20000130)21:2<86::Aid-Jcc2>3.0.Co;2-G (2000).
59227 MacKerell, A. D. & Banavali, N. K. All-atom empirical force field for nucleic acids: II.
593 Application to molecular dynamics simulations of DNA and RNA in solution. *Journal of*
594 *computational chemistry* **21**, 105-120, doi:Doi 10.1002/(Sici)1096-
595 987x(20000130)21:2<105::Aid-Jcc3>3.0.Co;2-P (2000).
59628 Bustamante, C., Smith, S. B., Liphardt, J. & Smith, D. Single-molecule studies of DNA
597 mechanics. *Current opinion in structural biology* **10**, 279-285 (2000).

59829 Bustamante, C., Bryant, Z. & Smith, S. B. Ten years of tension: single-molecule DNA
599 mechanics. *Nature* **421**, 423-427, doi:10.1038/nature01405 (2003).
60030 Kale, L. *et al.* NAMD2: Greater scalability for parallel molecular dynamics. *Journal of*
601 *Computational Physics* **151**, 283-312, doi:DOI 10.1006/jcph.1999.6201 (1999).
60231 Humphrey, W., Dalke, A. & Schulten, K. VMD: visual molecular dynamics. *Journal of*
603 *molecular graphics* **14**, 33-38, 27-38 (1996).
60432 Hall, C. E. Method for the observation of macromolecules with the electron microscope
605 illustrated with micrographs of DNA. *The Journal of biophysical and biochemical*
606 *cytology* **2**, 625-628 (1956).
60733 Beer, M. Electron microscopy of unbroken DNA molecules. *Journal of molecular*
608 *biology* **3**, 263-266 (1961).
60934 Zobel, C. R. & Beer, M. Electron stains. I. Chemical studies on the interaction of DNA
610 with uranyl salts. *The Journal of biophysical and biochemical cytology* **10**, 335-346
611 (1961).
61235 Beer, M. & Zobel, C. R. Electron stains. II: Electron microscopic studies on the visibility
613 of stained DNA molecules. *Journal of molecular biology* **3**, 717-726 (1961).
61436 Bell, D. C. *et al.* DNA base identification by electron microscopy. *Microscopy and*
615 *microanalysis : the official journal of Microscopy Society of America, Microbeam*
616 *Analysis Society, Microscopical Society of Canada* **18**, 1049-1053,
617 doi:10.1017/S1431927612012615 (2012).
61837 Scott, M. C. *et al.* Electron tomography at 2.4-angstrom resolution. *Nature* **483**, 444-447,
619 doi:10.1038/nature10934 (2012).
62038 Zhang, M. *et al.* HDL surface lipids mediate CETP binding as revealed by electron
621 microscopy and molecular dynamics simulation. *Scientific reports* **5**, 8741,
622 doi:10.1038/srep08741 (2015).
623