Published in final edited form as:

ACS Appl Mater Interfaces. 2019 April 17; 11(15): 13874–13880. doi:10.1021/acsami.8b07101.

Redox Engineering of Cytochrome c using DNA Nanostructure-Based Charged Encapsulation and Spatial Control

Zhilei Ge^{†,‡}, Zhaoming Su[§], Chad R. Simmons[†], Jiang Li[‡], Shuoxing Jiang[†], Wei Li[†], Yang Yang[†], Yan Liu^{*,†}, Wah Chiu[§], Chunhai Fan^{*,†,‡}, and Hao Yan^{*,†}

[†]Center for Molecular Design and Biomimetics, The Biodesign Institute, Department of Chemistry and Biochemistry, Arizona State University, Tempe, Arizona 85287, United States

[‡]Division of Physical Biology & Bioimaging Center, Shanghai Synchrotron Radiation Facility, Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800, China

[§]National Center of Macromolecular Imaging, Department of Bioengineering and Department of Microbiology and Immunology, James H. Clark Center, Stanford University, Stanford, California 94305, United States

Abstract

Three-dimensional (3D) DNA nanostructures facilitate the directed self-assembly of various objects with designed patterns with nanometer scale addressability. Here, we report the enhancement of cytochrome c (cyt c) redox activity by using a designed 3D DNA nanostructure attached to a gold electrode to spatially control the position of cyt c within the tetrahedral framework. Charged encapsulation and spatial control result in the significantly increased redox potential and enhanced electron transfer of this redox protein when compared to cyt c directly adsorbed on the gold surface. Two different protein attachment sites on one double stranded edge of a DNA tetrahedron were used to position cyt c inside and outside of the cage. Cyt c at both binding sites show similar redox potential shift and only slight difference in the electron transfer rate, both orders of magnitude faster than the cases when the protein was directly deposited on the gold electrode, likely due to an effective electron transfer pathway provided by the stabilization effect of the protein created by the DNA framework. This study shows great potential of using structural DNA nanotechnology for spatial control of protein positioning on electrode, which opens new routes to engineer redox proteins and interface microelectronic devices with biological function.

Graphical Abstract

*Corresponding Authors: hao.yan@asu.edu., fchh@sinap.ac.cn., yan_liu@asu.edu.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acsami.8b07101](https://doi.org/10.1021/acsami.8b07101).

Experimental and methodologic information on oligonucleotide sequences, conjugation of protein to oligonucleotides, DNA tetrahedron preparation, cryo-EM/ET specimen preparation and data analysis, polyacrylamide gel electrophoresis, UV-vis and circular dichroism spectroscopy, and electrochemical measurements (PDF)

Notes

The authors declare no competing financial interest.

Keywords

DNA tetrahedron; framework nucleic acids; cytochrome c; protein engineering

INTRODUCTION

DNA has emerged as an exceptional molecular building block for nanoconstruction due to its predictable conformation and programmable intra- and intermolecular Watson–Crick base-pairing interactions.^{1,2} Unlike protein engineering and polypeptide construction that require sophisticated computer algorithms for the prediction of folding patterns,^{3–5} DNA directed assembly could facilitate the design and organization of proteins with specific functions and high structural accuracy^{6–11} and organization of other heteroelements such as virus capsids,¹² nanoparticles,^{13–15} and carbon nanotubes,¹⁶ leading to unique and improved functional properties such as increased enzyme-cascade activities or shifts of surface plasmon resonance controlled by specific arrangement of nano-particles.^{17–22} Structural DNA nanotechnology has opened up tremendous opportunities in diagnosis, therapeutics, catalysis, and synthetic biology.

Assembly of redox active enzymes onto solid electrodes has been extensively explored to achieve efficient redox activity, both kinetics and thermodynamics, on the electrode for detection, energy, and environmental applications.^{23–29} One commonly used method is binding thiolated derivative of proteins onto gold electrodes to form self-assembled monolayers based on specific thiol–gold interaction. However, suboptimal electron transfer efficiency was observed due to lack of precise spatial control of the protein conformation on the electrode and inconsistent specimen preparation. Recent advances in DNA nanotechnology^{30,31} make it possible to assemble them into well-defined 2D/3D architecture with precise modulation of interprotein distances and to better control the relative protein position to the electrode, hence improving the kinetics (electron transfer rate) and the thermodynamics (redox potential) of surface-bound proteins.

Among all the DNA nanostructures, the DNA tetrahedron is an ideal molecular scaffold which has been developed as a sensitive detection platform in bioanalysis or a drug carrier in cancer therapy^{32–36} due to its high mechanical rigidity and a well-defined, stable 3D structure. This 3D DNA tetrahedron nanostructure can be assembled rapidly and reliably with high yields, readily immobilized onto surfaces with high stability, ordered orientation, and well-controlled lateral spacing, which offer a readily available system for surface-confined functional nanostructures.^{37–42} When deposited on a gold electrode, the distance of the encapsulated proteins with the electrode and the electric fields for protein could be

controlled, spatial positioning of protein relative to the tetrahedron could be realized, and the spurious lateral interactions between the proteins on the electrode can be effectively prevented, which makes the electron transfer from the redox proteins to the electrode more efficient. These structural features are particularly important for hosting redox active molecules for electrochemical applications. Cytochrome c (cyt c), a fully electrochemically studied heme protein, was chosen to report the electrochemical behavior of engineering of redox protein, including redox potential and electron transfer rate.

Herein, we report a framework nucleic acid (FNA) caged cyt c following a previous design⁴³ that, when attached to a gold electrode, displayed an engineering of redox potential with a 300 mV positive shift and a greatly enhanced electron transfer efficiency with more than 100-fold higher ET rate compared to cyt c adsorbed directly on the electrode.⁴⁴ We used cryo-EM single particle analysis (SPA) and cryo-electron tomography (cryo-ET) subtomogram averaging along with atomic force microscopy (AFM), dynamic light scattering (DLS), and gel electrophoresis to comprehensively validate the successful attachment of the cyt c with the DNA nanocage with a control of position.

RESULTS AND DISCUSSION

This DNA tetrahedron (Figure 1) was hierarchically assembled from 3 thiolated DNA fragments of 63 nucleotides (63-nt) and one amino-modified DNA fragment of 63-nt, which were mixed in stoichiometric equivalents in buffer, heated, and then rapidly cooled to 4 °C. The thiol of cysteine in cyt c was used for site-specific conjugation to the 5' end of an amino-modified oligonucleotide, which was attached via the bispecific linker sulfo-SMCC (Figure 1b). This protein–DNA conjugate was combined with the other three thiolated-oligonucleotides to form the tetrahedral cage with the protein attached to one edge of the cage and an opposite surface of the cage modified with three thiol groups at each of the vertices position (Figure 1a). Because the edges of the cage are not free to rotate around the DNA helical axis due to the double connections to their neighboring edges (Figure 1c), we could precisely design the position of the nick point on the DNA helix where an amino-modified group was attached. By shifting the position by 5 nucleotides, or one-half of a helical turn, the protein could be attached either inside or outside the tetrahedron with different orientation relative to the electrode.^{43,45,46} Once the DNA cage was deposited on the gold electrode, it was expected to assume a well-defined orientation with the three thiol-groups at each of the vertices facing down on to the gold electrode,^{32,33} providing orientation and distance control between cyt c and the electrode (Figure 1a).

The formation of DNA tetrahedron was confirmed using cryo-EM SPA⁴⁷ at 17 Å resolution (Figure 1d and S2). The vacant space inside the DNA tetrahedron (T_0) is sufficient for encapsulation of a cyt c.

DNA tetrahedron with cyt c attached at two different positions T_1 (inside) and T_2 (outside) were assembled separately. Their UV absorbance spectra (Figure S8) suggested that each DNA nanocage contained one cyt c molecule. In the native polyacrylamide gel electrophoresis (Figure 1e), the purified T_1 or T_2 had slightly slower mobility compared with the purified DNA tetrahedron T_0 due to a higher molecular mass. T_1 migrates slightly faster

than T₂ due to the more compact shape. This confirmed the formations of two different configurations of the DNA–cyt c tetrahedron as designed. DLS statistical analysis of the diameter of T₀, T₁, and T₂ (Figure S3) showed that mean diameters of T₀, T₁, and T₂ were 10.6 ± 0.7 , 12.2 ± 1.2 , 14.5 ± 5.0 nm, respectively. The statistical analysis of the AFM measurements showed that mean diameters of T₀, T₁, and T₂ were 16.0 ± 1.0 nm (24 particles), 16.2 ± 1.4 nm (22 particles), and 15.5 ± 1.9 nm (51 particles), respectively, which were bigger than the DLS analysis mainly effected by the AFM tip size.

We collected cryo-EM images of both T₁ and T₂, and found that while T₁ was well-monodispersed, T₂ particles were more crowded under cryo-EM condition and not suitable for further cryo-EM data processing (no additional density that could be attributed to cyt c was observed in T₂ raw images as shown in Figure S4). Thus, we used Cryo-ET subtomogram averaging to validate the encapsulation of cyt c inside the DNA cage of T₁, which resulted a density map of occupied tetrahedron at ~ 38 Å resolution (Figure 1f, right, and Figure S5). The cutaway view showed disappearance of the cavity inside the tetrahedron, indicating successful encapsulation of cyt c inside the DNA cage.

It is well-known that thiolated ssDNA spontaneously forms self-assembled monolayers on gold electrodes.^{48–51} Previous studies have demonstrated that these surface-confined DNA strands are predominantly well-aligned nearly vertical to the electrode surface,^{52,53} although strand entanglement is hard to avoid. The DNA tetrahedron used here each has three thiolmodified vertices to anchor to the gold electrode surface. With the protein covalently linked to one upright arm and the C-terminus of the protein pointing toward the attachment site, the DNA caged proteins are expected to have a unique orientation with respect to the normal of the gold electrode. The center of the heme group embedded in the protein of both T₁ and T₂ are estimated to have comparable distance to the electrode surface, ~ 3 – 4 nm. A submonolayer of T₁ or T₂ on gold electrode was achieved, and the rest of the surface was covered by binding of short thiolated molecules.

Cyclic voltammetry (CV) analysis of the submonolayer T₁ and T₂ on the gold electrode revealed a pair of clearly visible redox peaks, which represented an exchange of electrons between cyt c and the gold electrode (Figure 2). The position of the oxidation peak was at ~ 0.36 V (vs Ag/AgCl (3 M)), which is more positive than the previously observed in other relevant cyt c studies (0.26–0.29 V vs SHE), depending on the solution conditions.^{54,55} We also compared the CV scans with those of three other control samples: bare gold electrode, submonolayer of T₀ on electrode, and SMCC conjugated T₀ on the same electrode (Figure S12). All of the control samples did not contain cyt c; their CV scans showed no significant peaks that corresponded to those observed for T₁ and T₂ (Figure 2). These experiments confirmed that the peaks observed in the CV curves of T₁ and T₂ could only be assigned to the redox of cyt c. We also conducted several control experiments with different arrangements of cyt c on gold electrode to explain the redox potential shift (Figure S13). In the case that cyt c was directly attached to the electrode surface, the redox peak position was observed at approximately 0.027 V (vs Ag/AgCl (3 M)), which was consistent with the previously observed values.^{54,55} The difference peak positions between T₁ and control groups (Figure S13) once again certified the integrity of DNA tetrahedron–cyt c complex on electrodes. When we attach the cyt c protein to the end of a DNA helix extended away from

the top vertex of the DNA tetrahedron, no redox peak was observed (Figure S13b). This was due to the much longer distance between the protein and the electrode of >10 nm.

Comparing the direct attachment of cyt c to the electrode with cyt c encapsulated in the DNA tetrahedron, the positive redox peak shift was attributed to the negatively charged environment provided by the DNA nanostructure surrounding the cyt c. Similar redox potential shift had been previously observed on DNA-bound proteins, which was ascribed to the charged environment of proteins.⁵⁶

To confirm that introducing DNA nanostructures to the heme group will cause a decrease of the electron density of the heme macrocycle and change the polarity of the heme environment, thus shifting the redox potential, we conducted UV-vis and circular dichroism spectra analysis to probe the microenvironment of the heme group. Based upon the UV-vis spectroscopy analysis, the DNA-cyt c conjugate was typically in the oxidized form with nearly the same spectral characteristics of the wild-type cyt c without TCEP treatment (Figure 3a, left panel), with the Soret peak at ~410 nm and a broad heme absorbance in the range of 510–560 nm. In the fully reduced state of cyt c (black trace in Figure 3a, right panel), the Soret peak shifts to 416 nm. The Soret band shifts are primarily a response to changes in the electrostatic field surrounding the heme.^{57,58} This result indicated that although during the DNA-cyt c conjugation procedure the protein had been exposed to overnight TCEP treatment, unlike the reduced cyt c (due to treatment), the cyt c in the conjugates could be reoxidized after the reaction with DNA. Moreover, the absorbance peak intensities at 520 and 550 nm (correspond to the Fe²⁺ state of the heme group) in the reduced DNA-cyt c conjugate decreased, compared with that of the wild-type cyt c, and the ratio of absorbance values at 550 and 520 nm was lowered to 1.00 from 1.61 (Figure 3a, right panel). These observations were similar to the spectral features of the mutant cyt c that had the redox potential shifted up to ~0.41 mV (vs SHE).⁵⁹ All these spectral changes implied that the DNA modified cyt c had similar structural perturbation in the microenvironment of the heme group that made the redox potential more positive.

CD spectroscopy of both the wild-type cyt c and DNA-cyt c conjugate was then performed in both the Soret and far-UV regions (Figure 3b). The Soret region (450–350 nm) of the wild-type protein exhibited the characteristic negative Cotton effect previously reported in cyt c.^{60,61} However, the DNA-cyt c conjugate spectrum revealed an increased negative Cotton effect, with a Soret CD spectrum similar to that of a partially unfolded cytochrome. Far-UV (250–180 nm) CD spectrum of the wild-type protein showed characteristic α -helical content with troughs at 222 and 208 nm, while the DNA-cyt c conjugate showed a decreased trough at 222 nm and an increased trough at 208 nm, indicative of a perturbed α -helical structure. These results indicated that the introduction of negatively charged oligos (63 nt) at residue Cys108 destabilized the dipole moment of the C-terminal α -helix and could have possibly caused partial unfolding in this region of the protein. This destabilization effect is consistent with our electrochemical measurements.

It was previously reported that decreasing the electron density of the heme macrocycle and changing the polarity of the heme environment will cause significant increase in redox potential.⁵⁹

In the study reported here, the highly negatively charged DNA oligos were placed close the C-terminus of the cyt c. UV-vis absorbance and circular dichroism spectra analysis (Figure 3) confirmed that introducing DNA nanostructures to the cyt c cause similar structural perturbation to the microenvironment of the heme group as that in the protein mutant, leading to a change of the redox potential.

Next, alternating current voltammetry (ACV) was used to analyze the electron transfer of T₁ and T₂ with the electrode. ACV is more sensitive than CV for probing electro-active species at an electrode surface. Similar ACV curves were obtained for both samples, with the peaks centered at ~0.36 V vs Ag/AgCl reference electrode (Figure 4), consistent with the oxidation peak positions obtained in the CV measurements.

The rate constants of electron transfer (ET), k , were obtained by plotting the ratio of the ACV peak current to the background current (I_p/I_b) vs the ACV frequency,⁶² resulting in $k_{T1} = 32.4 \pm 1.9 \text{ s}^{-1}$ and $k_{T2} = 22.5 \pm 2.4 \text{ s}^{-1}$ (Figure 4). Compared with that of yeast cyt c adsorbed directly on the gold electrode,⁴⁴ these ET rates were about 100–200-fold higher. This may be due to a control of the specific protein orientation and distance on the electrode by hosting the protein on a DNA nanostructure, which prevents random binding of the protein to the electrode.

Because the peak positions in cyclic voltammetry measurements are very similar for both T₁ and T₂ (0.38 V for T₁ and 0.36 V for T₂), the electric fields inside and outside of the tetrahedron (T₁ and T₂) should be similar to each other. Because when cytochrome c was placed outside the tetrahedron, it was still surrounded by the edges of neighboring tetrahedrons due to the high surface coverage on electrode, which is just the same as T₁ when cytochrome c was placed inside the tetrahedron. The electric fields did not play an important role in differentiating the electron transfer rate in the two cases, although the peak potential of T₁ (0.38 V) has a slightly more positive shift than that of T₂ (0.36 V).

The two configurations of cyt c-DNA tetrahedron structures had a measurable difference in their ET rates, with T₁ faster than T₂. The average distances between the center of the heme group in cyt c to the electrode are expected to be comparable in these two cases, ~3–4 nm; however, T₂ has a flexibility much higher than that of T₁ due to the external attachment position on the DNA nanocage, which gives the protein in T₂ more translational and rotational freedom, and thus it has a much wider distance distribution. The difference in the ET rates might result from an effective electron transfer pathway provided by the stabilization effect of the protein created by the DNA frame the different heme orientations relative to the electrode controlled by the DNA tetrahedron host and/or the different distances distribution of the proteins to the electrode.

CONCLUSION

In conclusion, we studied not only the kinetics (electron transfer rate) but also the thermodynamics (redox potential) of surface-bound DNA tetrahedron-cyt c complex and found that DNA tetrahedron-cyt c complex had a 100–200-fold higher ET rate, and the redox potential of cyt c was positively shifted as much as ~300 mV when encapsulated in the

DNA tetrahedron. Such a huge shift of redox potential would not be possible without the highly charged environment provided by the tetrahedral framework, which again provides unambiguous evidence for the integrity of the complex on the surface.

By modulating the relative orientation and proximal distance of the protein with respect to the electrode surface in 3D framework, we not only combined several parameters (electrostatic surrounding, distances between the heme and electrode, DNA-mediated charge transfer) to regulate electron transfer to cytochrome c with an effective electron transfer pathway between electro-active proteins and electrode in a non-native environment but also provided a new method to engineer the redox potential via DNA encapsulation with as much as ~300 mV positive shift. We believe that nanofabrication of functional proteins scaffolded by DNA nanostructures^{63,64} could serve as valuable model systems which lead to stabilization/protection of the proteins in a non-native environment and realize better manipulations of their activities for new technological development. This DNA-directed protein-assembly technology will represent a unique direction for the applications of structural DNA nanotechnology, and it will also create new opportunities for a diverse range of technological development, ranging from biofuel cell production to the fabrication of bioelectronic devices.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

H.Y. and Y.L. acknowledge the funding support from DOD-Navy-ONR MURI award (W911NF-12-1-0420), Army Research Office grant (W911NF-11-1-0137), and the National Science Foundation (Grant 14030615). C.F. gratefully acknowledges the National Natural Science Foundation of China (Grants 21329501 and U1532119), National Key R&D Program of China (Grants 2016YFA0400900 and 2016YFA0201200), and the Key Research Program of Frontier Sciences (Grant QYZDJ-SSW-SLH031), the Open Large Infrastructure Research of CAS, Chinese Academy of Sciences. W.C. and Z.S. gratefully acknowledge funding support from National Institutes of Health (Grants NIH P41GM103832 and P50GM103297).

REFERENCES

- (1). Seeman NC Nucleic acid junctions and lattices. *J. Theor. Biol* 1982, 99 (2), 237–247. [PubMed: 6188926]
- (2). Seeman NC Nanomaterials Based on DNA. *Annu. Rev. Biochem* 2010, 79, 65–87. [PubMed: 20222824]
- (3). Plaxco KW; Simons KT; Baker D Contact order, transition state placement and the refolding rates of single domain proteins. *J. Mol. Biol* 1998, 277 (4), 985–994. [PubMed: 9545386]
- (4). Kuhlman B; Dantas G; Ireton GC; Varani G; Stoddard BL; Baker D Design of a novel globular protein fold with atomic-level accuracy. *Science* 2003, 302 (5649), 1364–1368. [PubMed: 14631033]
- (5). Razeghifard R; Wallace BB; Pace RJ; Wydrzynski T Creating functional artificial proteins. *Curr. Protein Pept. Sci* 2007, 8(1), 3–18. [PubMed: 17305556]
- (6). Wilner OI; Weizmann Y; Gill R; Lioubashevski O; Freeman R; Willner I Enzyme cascades activated on topologically programmed DNA scaffolds. *Nat. Nanotechnol* 2009, 4 (4), 249–254. [PubMed: 19350036]

- (7). Fu JL; Liu MH; Liu Y; Woodbury NW; Yan H Interenzyme Substrate Diffusion for an Enzyme Cascade Organized on Spatially Addressable DNA Nanostructures. *J. Am. Chem. Soc* 2012, 134 (12), 5516–5519. [PubMed: 22414276]
- (8). Niemeyer CM; Koehler J; Wuerdemann C DNA-directed assembly of bienzymic complexes from in vivo biotinylated NAD(P)H: FMN oxidoreductase and luciferase. *ChemBioChem* 2002, 3 (2–3), 242–245. [PubMed: 11921405]
- (9). Delebecque CJ; Lindner AB; Silver PA; Aldaye FA Organization of Intracellular Reactions with Rationally Designed RNA Assemblies. *Science* 2011, 333 (6041), 470–474. [PubMed: 21700839]
- (10). Flory JD; Simmons CR; Lin S; Johnson T; Andreoni A; Zook J; Ghirlanda G; Liu Y; Yan H; Fromme P Low Temperature Assembly of Functional 3D DNA-PNA-Protein Complexes. *J. Am. Chem. Soc* 2014, 136 (23), 8283–8295. [PubMed: 24871902]
- (11). Zhang F; Jiang SX; Wu SY; Li YL; Mao CD; Liu Y; Yan H Complex wireframe DNA origami nanostructures with multiarm junction vertices. *Nat. Nanotechnol* 2015, 10 (9), 779–784. [PubMed: 26192207]
- (12). Stephanopoulos N; Liu MH; Tong GJ; Li Z; Liu Y; Yan H; Francis MB Immobilization and One-Dimensional Arrangement of Virus Capsids with Nanoscale Precision Using DNA Origami. *Nano Lett.* 2010, 10 (7), 2714–2720. [PubMed: 20575574]
- (13). Mirkin CA; Letsinger RL; Mucic RC; Storhoff JJ A DNA-based method for rationally assembling nanoparticles into macroscopic materials. *Nature* 1996, 382 (6592), 607–609. [PubMed: 8757129]
- (14). Alivisatos AP; Johnsson KP; Peng XG; Wilson TE; Loweth CJ; Bruchez MP; Schultz PG Organization of ‘nanocrystal molecules’ using DNA. *Nature* 1996, 382 (6592), 609–611. [PubMed: 8757130]
- (15). Zhang JP; Liu Y; Ke YG; Yan H Periodic square-like gold nanoparticle arrays templated by self-assembled 2D DNA nanogrids on a surface. *Nano Lett.* 2006, 6 (2), 248–251. [PubMed: 16464044]
- (16). Maune HT; Han SP; Barish RD; Bockrath M; Goddard WA; Rothmund PWK; Winfree E Self-assembly of carbon nanotubes into two-dimensional geometries using DNA origami templates. *Nat. Nanotechnol* 2010, 5 (1), 61–66. [PubMed: 19898497]
- (17). Sonnichsen C; Reinhard BM; Liphardt J; Alivisatos AP A molecular ruler based on plasmon coupling of single gold and silver nanoparticles. *Nat. Biotechnol* 2005, 23 (6), 741–745. [PubMed: 15908940]
- (18). Maye MM; Nykypanchuk D; Cuisinier M; van der Lelie D; Gang O Stepwise surface encoding for high-throughput assembly of nanoclusters. *Nat. Mater* 2009, 8 (5), 388–391. [PubMed: 19329992]
- (19). Cheng WL; Campolongo MJ; Cha JJ; Tan SJ; Umbach CC; Muller DA; Luo D Free-standing nanoparticle superlattice sheets controlled by DNA. *Nat. Mater* 2009, 8 (6), 519–525. [PubMed: 19404241]
- (20). Wang J; Chao J; Liu H; Su S; Wang L; Huang W; Willner I; Fan C Clamped Hybridization Chain Reactions for the Self-Assembly of Patterned DNA Hydrogels. *Angew. Chem., Int. Ed* 2017, 56 (8), 2171–2175.
- (21). Kahn JS; Trifonov A; Cecconello A; Guo W; Fan C; Willner I Integration of Switchable DNA-Based Hydrogels with Surfaces by the Hybridization Chain Reaction. *Nano Lett.* 2015, 15(11), 7773–7778. [PubMed: 26488684]
- (22). Golub E; Albada HB; Liao W-C; Biniuri Y; Willner I Nucleoapzymes: Hemin/G-Quadruplex DNAzyme–Aptamer Binding Site Conjugates with Superior Enzyme-like Catalytic Functions. *J. Am. Chem. Soc* 2016, 138 (1), 164–172. [PubMed: 26652164]
- (23). Lvov Y; Ariga K; Ichinose I; Kunitake T Assembly of Multicomponent Protein Films by Means of Electrostatic Layer-by-Layer Adsorption. *J. Am. Chem. Soc* 1995, 117 (22), 6117–6123.
- (24). Minteer SD; Liaw BY; Cooney MJ Enzyme-based biofuel cells. *Curr. Opin. Biotechnol* 2007, 18 (3), 228–234. [PubMed: 17399977]
- (25). Cooney M; Svoboda V; Lau C; Martin G; Minteer SD Enzyme catalysed biofuel cells. *Energy Environ. Sci* 2008, 1 (3), 320–337.

- (26). Fruk L; Niemeyer CM Covalent Hemin–DNA Adducts for Generating a Novel Class of Artificial Heme Enzymes. *Angew. Chem., Int. Ed* 2005, 44 (17), 2603–2606.
- (27). Fruk L; Müller J; Weber G; Narváez A; Domínguez E; Niemeyer CM DNA-Directed Immobilization of Horseradish Peroxidase–DNA Conjugates on Microelectrode Arrays: Towards Electrochemical Screening of Enzyme Libraries. *Chem. - Eur. J* 2007, 13 (18), 5223–5231. [PubMed: 17393544]
- (28). Li L; Wang L; Xu Q; Xu L; Liang W; Li Y; Ding M; Aldalbahi A; Ge Z; Wang L; Yan J; Lu N; Li J; Wen Y; Liu G Bacterial Analysis Using an Electrochemical DNA Biosensor with Poly-Adenine-Mediated DNA Self-Assembly. *ACS Appl. Mater. Interfaces* 2018, 10 (8), 6895–6903. [PubMed: 29383931]
- (29). Li M; Guo X; Li H; Zuo X; Hao R; Song H; Aldalbahi A; Ge Z; Li J; Li Q; Song S; Li S; Shao N; Fan C; Wang L Epitope Binning Assay Using an Electron Transfer-Modulated Aptamer Sensor. *ACS Appl. Mater. Interfaces* 2018, 10 (1), 341–349. [PubMed: 29241329]
- (30). Zhang Y; Tsitkov S; Hess H Proximity does not contribute to activity enhancement in the glucose oxidase–horseradish peroxidase cascade. *Nat. Commun* 2016, 7, 13982. [PubMed: 28004753]
- (31). Zhao Z; Fu J; Dhakal S; Johnson-Buck A; Liu M; Zhang T; Woodbury NW; Liu Y; Walter NG; Yan H Nanocaged enzymes with enhanced catalytic activity and increased stability against protease digestion. *Nat. Commun* 2016, 7, 10619. [PubMed: 26861509]
- (32). Pei H; Lu N; Wen Y; Song S; Liu Y; Yan H; Fan C A DNA Nanostructure-based Biomolecular Probe Carrier Platform for Electrochemical Biosensing. *Adv. Mater* 2010, 22 (42), 4754–4758. [PubMed: 20839255]
- (33). Ge Z; Lin M; Wang P; Pei H; Yan J; Shi J; Huang Q; He D; Fan C; Zuo X Hybridization Chain Reaction Amplification of MicroRNA Detection with a Tetrahedral DNA Nanostructure-Based Electrochemical Biosensor. *Anal. Chem* 2014, 86 (4), 2124–2130. [PubMed: 24495151]
- (34). Li J; Pei H; Zhu B; Liang L; Wei M; He Y; Chen N; Li D; Huang Q; Fan C Self-Assembled Multivalent DNA Nanostructures for Noninvasive Intracellular Delivery of Immunostimulatory CpG Oligonucleotides. *ACS Nano* 2011, 5 (11), 8783–8789. [PubMed: 21988181]
- (35). Walsh AS; Yin H; Erben CM; Wood MJA; Turberfield AJ DNA Cage Delivery to Mammalian Cells. *ACS Nano* 2011, 5 (7), 5427–5432. [PubMed: 21696187]
- (36). Ge Z; Pei H; Wang L; Song S; Fan C Electrochemical single nucleotide polymorphisms genotyping on surface immobilized three-dimensional branched DNA nanostructure. *Sci. China: Chem* 2011, 54 (8), 1273–1276.
- (37). Lin M; Wang J; Zhou G; Wang J; Wu N; Lu J; Gao J; Chen X; Shi J; Zuo X; Fan C Programmable Engineering of a Biosensing Interface with Tetrahedral DNA Nanostructures for Ultrasensitive DNA Detection. *Angew. Chem., Int. Ed* 2015, 54 (7), 2151–2155.
- (38). Lu N; Pei H; Ge ZL; Simmons CR; Yan H; Fan CH Charge Transport within a Three-Dimensional DNA Nanostructure Framework. *J. Am. Chem. Soc* 2012, 134 (32), 13148–13151. [PubMed: 22809010]
- (39). Mitchell N; Schlapak R; Kastner M; Armitage D; Chrzanowski W; Riener J; Hinterdorfer P; Ebner A; Howorka S A DNA Nanostructure for the Functional Assembly of Chemical Groups with Tunable Stoichiometry and Defined Nanoscale Geometry. *Angew. Chem., Int. Ed* 2009, 48 (3), 525–527.
- (40). Zhu D; Pei H; Yao G; Wang L; Su S; Chao J; Wang L; Aldalbahi A; Song S; Shi J; Hu J; Fan C; Zuo X A Surface-Confined Proton-Driven DNA Pump Using a Dynamic 3D DNA Scaffold. *Adv. Mater* 2016, 28 (32), 6860–6865. [PubMed: 27218679]
- (41). Yang F; Zuo XL; Li ZH; Deng WP; Shi JY; Zhang GJ; Huang Q; Song SP; Fan CH A Bubble-Mediated Intelligent Microscale Electrochemical Device for Single-Step Quantitative Bioassays. *Adv. Mater* 2014, 26, 4671–4676. [PubMed: 24729272]
- (42). Pei H; Liang L; Yao G; Li J; Huang Q; Fan C Reconfigurable Three-Dimensional DNA Nanostructures for the Construction of Intracellular Logic Sensors. *Angew. Chem., Int. Ed* 2012, 51, 9020–9024.
- (43). Erben CM; Goodman RP; Turberfield AJ Single-molecule protein encapsulation in a rigid DNA cage. *Angew. Chem., Int. Ed* 2006, 45 (44), 7414–7417.

- (44). Tarlov MJ; Bowden EF Electron-Transfer Reaction of Cytochrome c Adsorbed on Carboxylic-Acid Terminated Alkanethiol Monolayer Electrodes. *J. Am. Chem. Soc* 1991, 113 (5), 1847–1849.
- (45). Goodman RP; Schaap IAT; Tardin CF; Erben CM; Berry RM; Schmidt CF; Turberfield AJ Rapid chiral assembly of rigid DNA building blocks for molecular nanofabrication. *Science* 2005, 310 (5754), 1661–1665. [PubMed: 16339440]
- (46). Kato T; Goodman RP; Erben CM; Turberfield AJ; Namba K High-Resolution Structural Analysis of a DNA Nanostructure by cryoEM. *Nano Lett.* 2009, 9 (7), 2747–2750. [PubMed: 19492821]
- (47). Tang G; Peng L; Baldwin PR; Mann DS; Jiang W; Rees I; Ludtke SJ EMAN2: an extensible image processing suite for electron microscopy. *J. Struct. Biol* 2007, 157 (1), 38–46. [PubMed: 16859925]
- (48). Herne TM; Tarlov MJ Characterization of DNA probes immobilized on gold surfaces. *J. Am. Chem. Soc* 1997, 119 (38), 8916–8920.
- (49). Levicky R; Herne TM; Tarlov MJ; Satija SK Using self-assembly to control the structure of DNA monolayers on gold: A neutron reflectivity study. *J. Am. Chem. Soc* 1998, 120 (38), 9787–9792.
- (50). Okahata Y; Matsunobu Y; Ijiri K; Mukae M; Murakami A; Makino K Hybridization of Nucleic-Acids Immobilized on a Quartz Crystal Microbalance. *J. Am. Chem. Soc* 1992, 114 (21), 8299–8300.
- (51). Lao RJ; Song SP; Wu HP; Wang LH; Zhang ZZ; He L; Fan CH Electrochemical interrogation of DNA monolayers on gold surfaces. *Anal. Chem* 2005, 77 (19), 6475–6480. [PubMed: 16194115]
- (52). Steel AB; Herne TM; Tarlov MJ Electrochemical quantitation of DNA immobilized on gold. *Anal. Chem* 1998, 70(22), 4670–4677. [PubMed: 9844566]
- (53). Hirst J; Armstrong FA Fast-scan cyclic voltammetry of protein films on pyrolytic graphite edge electrodes: Characteristics of electron exchange. *Anal. Chem* 1998, 70 (23), 5062–5071. [PubMed: 9852788]
- (54). Rodkey FL; Ball EG Oxidation-Reduction Potentials of the Cytochrome c System. *J. Biol. Chem* 1950, 182 (1), 17–28.
- (55). Lisdat F; Ge B; Scheller FW Oligonucleotide-modified electrodes for fast electron transfer to cytochrome c. *Electrochem. Commun* 1999, 1 (2), 65–68.
- (56). Bartels PL; Zhou A; Arnold AR; Nunez NN; Crespilho FN; David SS; Barton JK Electrochemistry of the [4Fe4S] Cluster in Base Excision Repair Proteins: Tuning the Redox Potential with DNA. *Langmuir* 2017, 33 (10), 2523–2530. [PubMed: 28219007]
- (57). Franzen S; Boxer SG On the origin of heme absorption band shifts and associated protein structural relaxation in myoglobin following flash photolysis. *J. Biol. Chem* 1997, 272 (15), 9655–9660. [PubMed: 9092494]
- (58). O'Brien E; Holt ME; Thompson MK; Salay LE; Ehlinger AC; Chazin WJ; Barton JK The [4Fe4S] cluster of human DNA primase functions as a redox switch using DNA charge transport. *Science* 2017, 355(6327), eaag1789. [PubMed: 28232525]
- (59). Lett CM; Guillemette JG Increasing the redox potential of isoform 1 of yeast cytochrome c through the modification of select haem interactions. *Biochem. J* 2002, 362, 281–287. [PubMed: 11853535]
- (60). Rafferty SP; Pearce LL; Barker PD; Guillemette JG; Kay CM; Smith M; Mauk AG Electrochemical, kinetic, and circular dichroic consequences of mutations at position 82 of yeast iso-1-cytochrome c. *Biochemistry* 1990, 29 (40), 9365–9369. [PubMed: 2174257]
- (61). Myer YP; Saturno AF; Verma BC; Pande A Horse heart cytochrome c: The oxidation-reduction potential and protein structures. *J. Biol. Chem* 1979, 254 (22), 11202–11207. [PubMed: 227862]
- (62). Creager SE; Wooster TT A new way of using ac voltammetry to study redox kinetics in electroactive monolayers. *Anal. Chem* 1998, 70 (20), 4257–4263.
- (63). Fu YM; Zeng DD; Chao J; Jin YQ; Zhang Z; Liu HJ; Li D; Ma HW; Huang Q; Gothelf KV; Fan CH Single-Step Rapid Assembly of DNA Origami Nanostructures for Addressable Nanoscale Bioreactors. *J. Am. Chem. Soc* 2013, 135(2), 696–702. [PubMed: 23237536]
- (64). Fu J; Yang YR; Johnson-Buck A; Liu M; Liu Y; Walter NG; Woodbury NW; Yan H Multi-enzyme complexes on DNA scaffolds capable of substrate channelling with an artificial swinging arm. *Nat. Nanotechnol* 2014, 9 (7), 531–536. [PubMed: 24859813]

Figure 1.

DNA tetrahedron is formed by self-assembly of four oligonucleotides. (a) Three strands are thiol-modified to anchor the DNA tetrahedron onto the gold electrode, and the fourth strand is site specific, covalently linked to cyt c, to form the DNA tetrahedrons named T₁ and T₂. (b) SMCC was used as the bispecific linker between the DNA and the protein, with the distances marked between the terminal end of the DNA and the heme group of cyt c. (c) A simple scheme illustrating the positioning of the protein molecule inside or outside the edge of the DNA tetrahedron. The arrows point to the protein attachment sites at the 5' end of one of the nick points on one of the duplex edge. (d) Cryo-EM 3D reconstruction and comparison between representative class averages (*C*) and corresponding 3D map projections (*P*) of DNA tetrahedron T₀. Twenty angstrom scale bar is shown in panel d. (e)

Left: Native PAGE (6%) characterization of the assembled DNA tetrahedron with cyt c protein inside and outside. Lane 1: T₀; Lane 2: T₁; Lane 3: T₂. Right: Fluorescence gel image of an Alexa 555-labeled cyt c-DNA tetrahedron structure. Lane 2: Alexa 555-labeled T₁; Lane 3: Alexa 555-labeled T₂. Lane M: DNA ladder. (f) Left: Cryo-EM SPA reconstruction of T₀ and the corresponding cutaway views, applied with a Gaussian filter low passed to 38 Å. Right: Cryo-EM subtomogram averaging of cyt c occupied T₁ and the corresponding cutaway views at spatial resolution of 38 Å. Twenty angstrom scale bar is shown in panel f.

Figure 2. Cyclic voltammetry scans of cyt c accommodated by the DNA tetrahedron structure on the gold electrode. (a) T₁. (b) T₂. All CV scans were taken at a scan rate of 50 mV/s using reference electrode Ag/AgCl (3 M).

Figure 3.

(a) Comparison of the UV-vis spectra of the wild-type cyt c (black) and DNA-cyt c conjugate (red) of same concentration in HEPES buffer (2 mM; pH 7.4; 25 °C). Left panel: Both samples were dissolved in HEPES buffer without any treatment. Right panel: Reduced cyt c was prepared from the wild-type cyt c, treated with TCEP overnight with the TCEP subsequently removed the following day. The same DNA-cyt c conjugate spectrum in the left panel is shown for comparison. (b) Comparison of circular dichroism spectra of the wild-type cyt c (black) and DNA-cyt c conjugate (red) in HEPES buffer. Left panel: Far-UV (250–180 nm) region. Right panel: Soret (450–350 nm) region. The CD spectra were scanned at 30 nm/min and averaged from 4 scans.

Figure 4.

Alternating current voltammetry scans and plot of I_p/I_b vs log(frequency). (a) T₁. (b) T₂. The ACV data were collected at 5 Hz and baseline-subtracted. In the I_p/I_b plot, the dots are the experimental data; the solid curves are theoretical fitting following the method reported previously.⁶²