

Supplementary Information

Self assembled nanocages from DNA-protoporphyrin hybrid molecules

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Materials:

HPLC purified single strand DNA with 5'-amine modification were purchased from Sigma Aldrich custom oligo synthesis. N,N'-Dicyclohexylcarbodiimide (DCC), N-hydroxysuccinimide (NHS), Protoporphyrin IX (PpIX) were obtained from Sigma Aldrich. All chemicals required for buffer preparation and gel electrophoresis were obtained from either Sigma Aldrich or Alfa aesar and used without further purification. Nanopure water from Millipore was used in all experiments including spectroscopic studies.

Experimental Procedures

DNA sequences used in the study

ODN 1

5'-[C12-NH₂]- TCA GTC AAC AGC-3'

ODN 2

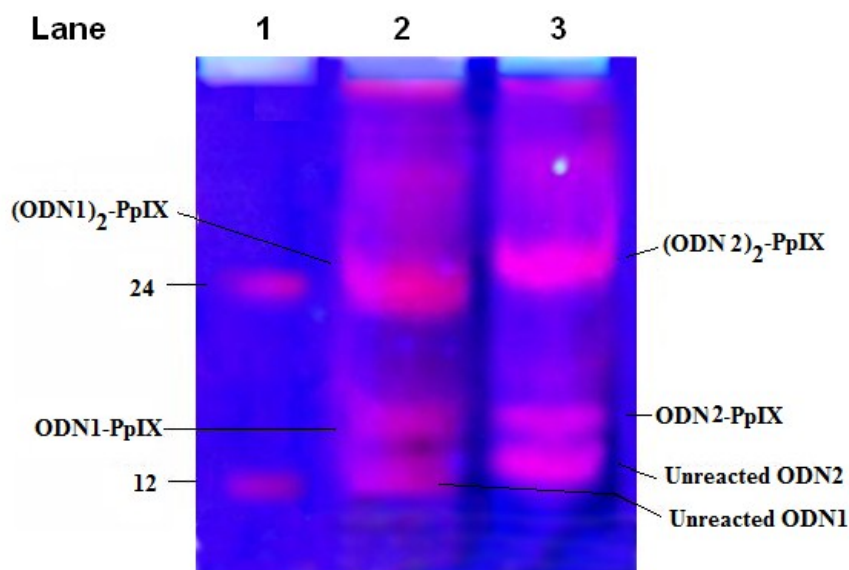
5'-[C12-NH₂]- GCT GTT GAC TGA-3'

Formation of activated PpIX activated ester

A solution of 3,7,12,17-Tetramethyl-8,13-divinyl-2,18-porphinedipropionic acid (PpIX) (20 mg, 35.5 μ mol) in dry DMF (2 ml) was added to a solution of N-hydroxysuccinimide (8.2 mg, 71 μ mol) and DCC (14.6 mg, 71 μ mol) in DMF (1 ml). The mixture was shaken and kept at room temperature for 18 h. The precipitate of dicyclohexylurea was separated by centrifugation and washed with DMF (3 x 2 ml). The DMF solutions were accumulated, to give the disuccinimide ester of 3, 7, 12, 17-Tetramethyl-8, 13-divinyl-2, 18-porphinedipropionic acid.

Conjugation of Protoporphyrin ester to -NH₂ terminated oligonucleotide

5 nmol of oligonucleotide (ODN1/ODN2) was dissolved in the final reaction buffer which comprised of sodium borate buffer at pH 8. The active PpIX ester (2.5 nmol) formed was allowed to react overnight with oligonucleotides at 50 °C. The crude reaction mixture was dialyzed on a 1000 daltons MWCO cellulose acetate membrane and run on 15% PAGE gel containing urea at 200V for 2 h in 1x TTE buffer to visualize the product.

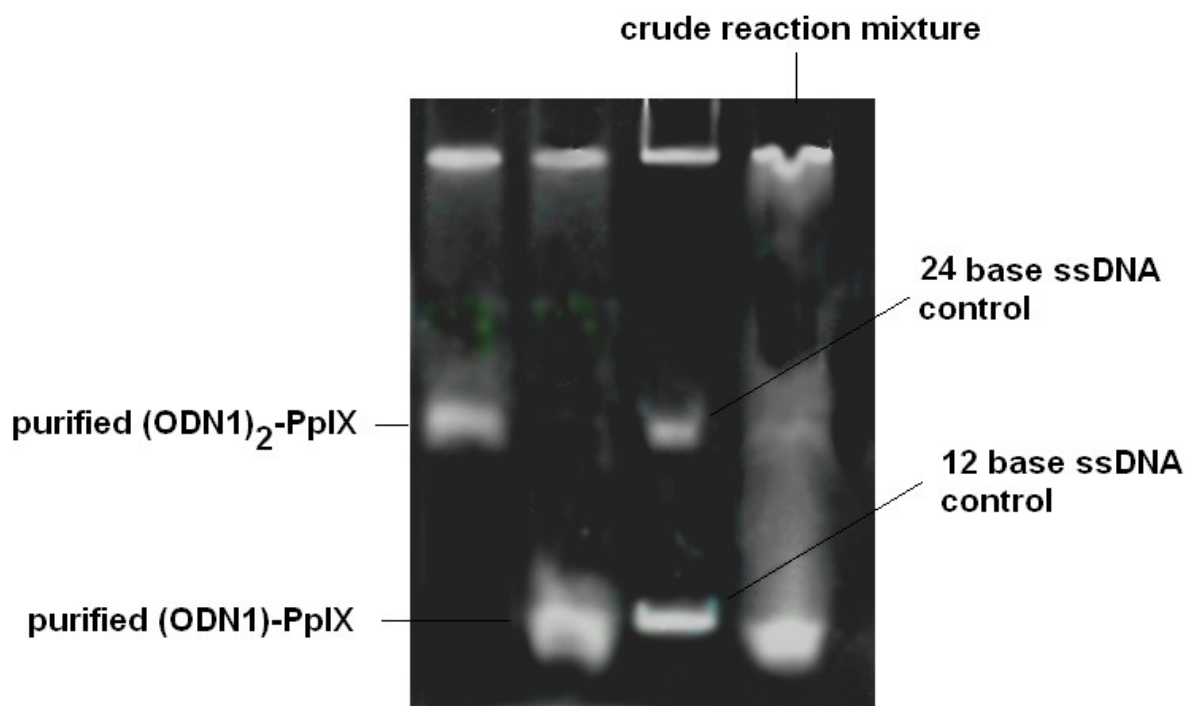


ESI 1: 25% denaturing PAGE showing formation of DNA-PpIX conjugate. Lane 1: 12 and 24 mer control oligomer, Lane 2: Crude reaction mixture (after dialysis) of ODN1 and PpIX, lane 3: Crude reaction mixture (after dialysis) of ODN2 and PpIX

Extraction of Protoporphyrin DNA conjugates from PAGE Gel

After running the gel, the concerned DNA bands were cut into smaller pieces with sharp razor. These gel pieces were soaked in acrylamide gel elution buffer with 10 mM Magnesium Acetate, 0.5 M Ammonium Acetate, 1mM EDTA and 0.1% (w/v) SDS at 37 °C overnight. The elution buffer containing the DNA- PpIX conjugates was transferred to a 1.5 ml microcentrifuge tube leaving the gel debris behind. This was followed by addition of 500 μ l of cold ethanol and 2 μ l of glycogen. This solution was kept at -30 °C overnight, centrifuged at 4 °C for 30 min when a pellet was formed. Then the supernatant is removed carefully leaving the pellet behind. 80% ethanol was added to loosen the pellet followed by

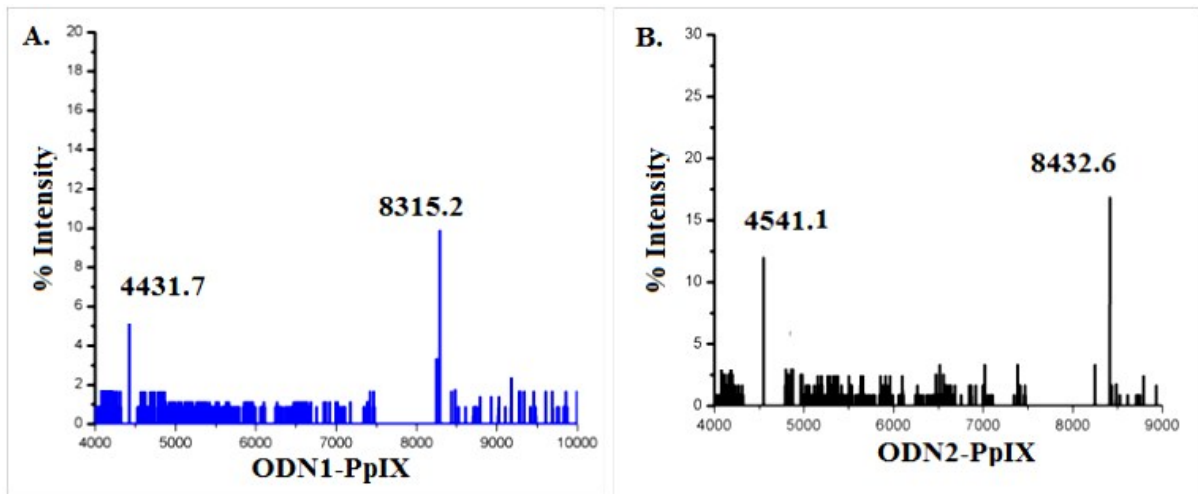
centrifugation. Again the supernatant is discarded the pellet then air-dried and dissolved in sodium phosphate buffer for further experiments.



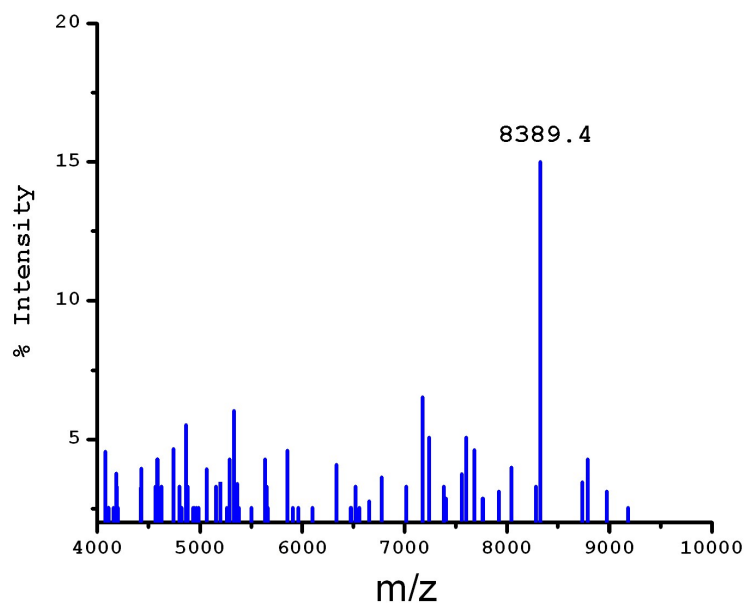
ESI 2: SYBR Gold stained 20% denaturing PAGE showing purified (ODN1)₂-PpIX that are used for MALDI-ToF and self assembly after PAGE extraction and purification.

MALDI-TOF-Mass Analysis of DNA-PpIX conjugates

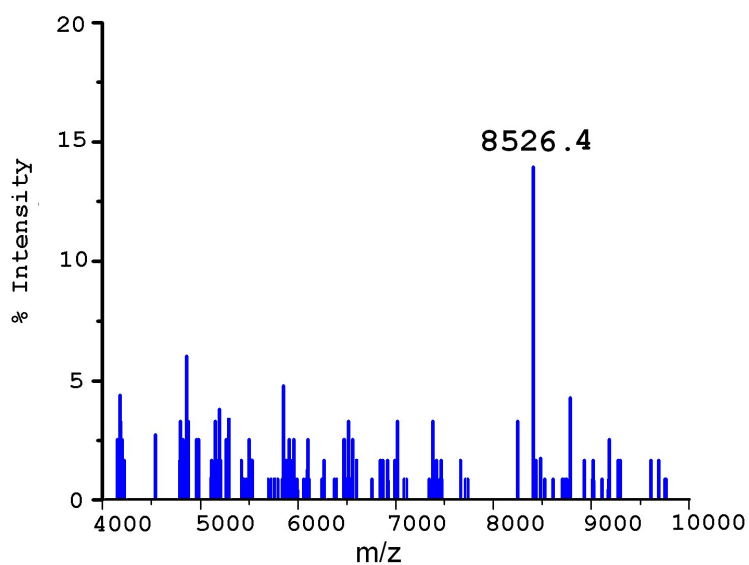
The reaction mixture of DNA and PpIX were purified by repeated ethanol wash and subjected to molecular weight determination by Autoflex II MALDI-TOF-MS (Bruker Daltonics, Billerica, MA) using picolinic acid and dibasic sodium citrate as a matrix. Data processing was performed with Flex Analysis Software.



ESI 3 MALDI-ToF spectra of ssDNA-PpIX reaction mixture after purification. (A) ODN1-PpIX ($m/z=4431.7$, theoretical $m/z=4489.4$) and (ODN1)₂-PpIX ($m/z=8315.2$, theoretical $m/z=8416.1$) B) ODN2-PpIX($m/z=4541.1$, theoretical $m/z=4549.3$) and (ODN2)₂-PpIX($m/z=8432.6$, theoretical $m/z=8536.1$)



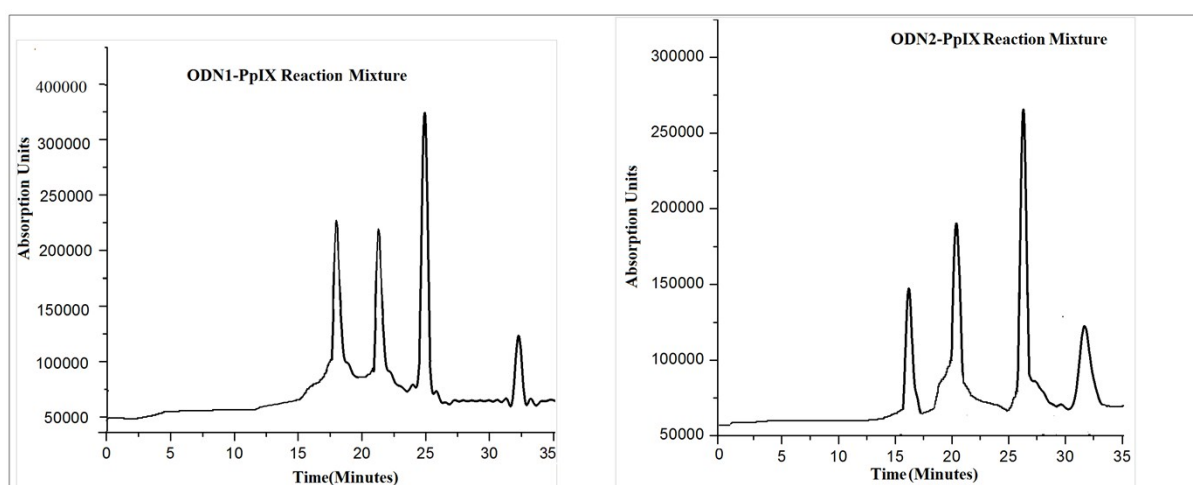
ESI 4 MALDI-ToF spectra of (ODN1)₂-PpIX after PAGE purification that was used to construct nanocages. (m/z = 8389.4 , theoretical m/z= 8416.1)



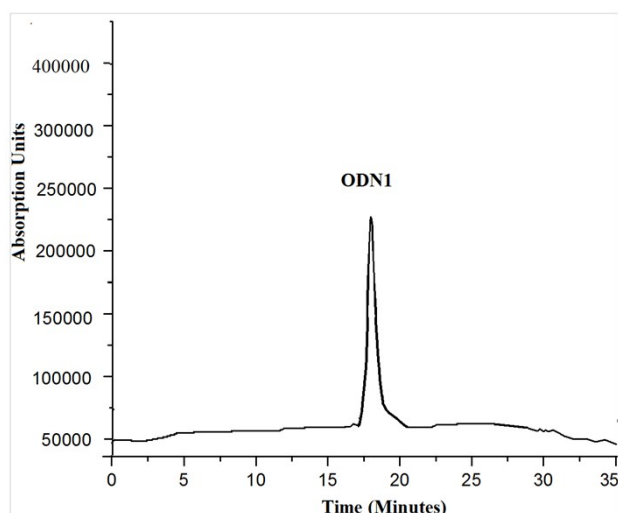
ESI 5 MALDI-ToF spectra of (ODN2)₂-PpIX after PAGE purification that was used to construct nanocages. (m/z = 8526.4 , theoretical m/z= 8536.1)

HPLC analysis of DNA-PpIX conjugates

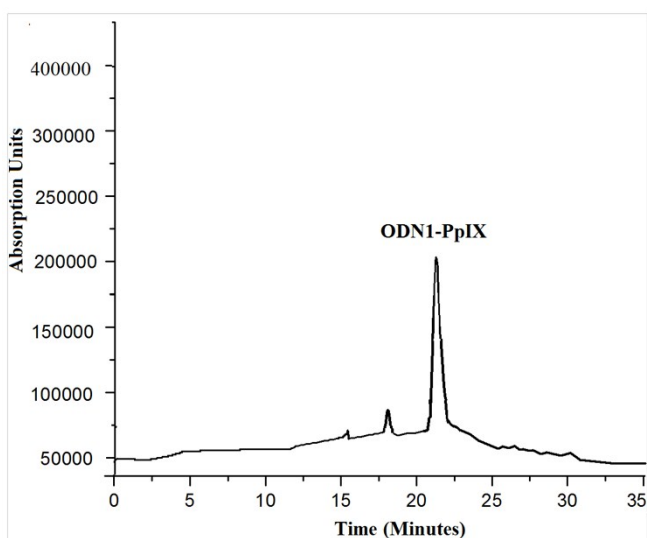
Reverse phase HPLC was done using Enable C18Q 50 x 4.6, 3 μ column. The DNA-Protoporphyrin conjugates were identified by HPLC in solvent A consisting of 0.1 M TEAA buffer and solvent B consisting of acetonitrile /water with linear gradient of 10-100% acetonitrile -water in 50 min. Briefly, reaction mixtures were either gel purified or ethanol washed before introducing them to HPLC. The ODN-PpIX solutions containing 0.1 M triethylamine acetate (TEAA) was heated at 95 °C for 5 min, and then rapidly introduced into the HPLC injector. In ESI2, the first peak from left is the unreacted DNA followed by the single and di-conjugated DNA-PpIX species and the last peak is due to free/unreacted PpIX.



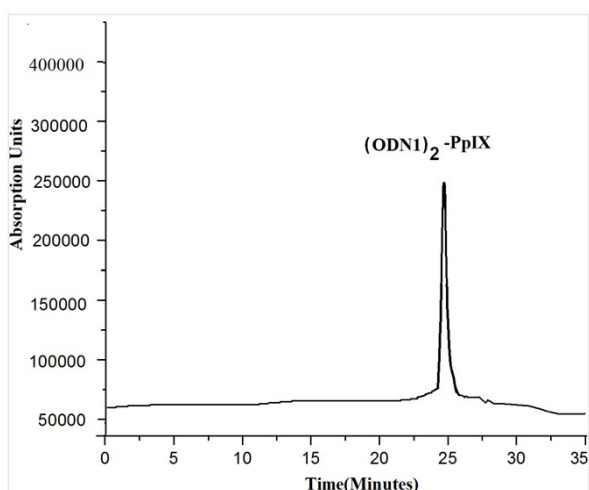
ESI 6 HPLC analysis of reaction products of ODN1-PpIX and ODN2-PpIX



ESI 7 HPLC analysis of reaction products of ODN1



ESI 8 HPLC analysis of PAGE purified ODN1-PpIX



ESI 9 HPLC analysis of reaction products of (ODN1)₂-PpIX

Hybridization of ODN1- PpIX and ODN2- PpIX

(ODN1)₂- PpIX (1 nmol) and (ODN2)₂-PpIX (1 nmol) were taken in equal molar ratio and annealing was performed in presence of 10 mM TE, 10mM magnesium chloride and 250 mM NaCl. The samples were first heated to 90 °C and then slowly cooled to 20 °C with a ramp of 0.1°C/s and then stored at 4 °C.

Thermal Melting Analysis

(ODN1)₂- PpIX were hybridised with (ODN2)₂-PpIX were taken in 1:1 molar ratio in 250 mM NaCl and 10 mM TE buffer and 10 mM MgCl₂ heated to 90 °C to denature the two strands.

Computational Analysis of 2D Nanocages

The carboxy acid derivative of PpIX was sketched using ChemDraw [1], which is a standard software tool for drawing the chemical molecules. The two dimensional structure of PpIX was then converted into mol file which has the information about the atoms, bonds, coordinates of the molecule. In order to generate the three dimensional structure of PpIX molecule, the mol file was then imported to the Schrodinger suite (Schrödinger, LLC, New York, NY, 2014) [2]. The 3D structure of PpIX molecule was then preprocessed, which involves assigning the bond orders and addition of missing hydrogens to the structure. The Maestro's Build panel was employed to create the ssDNA with sequence 5' TCAGTCAACAGC 3' (ODN1). To the 5' end of the ssDNA the amino group was added covalently to form the 5'-aminated ssDNA. This aminated ssDNA was then coupled covalently to the carboxylic acid end group of PpIX molecule through a 12 carbon linker. Correspondingly, the 5'-aminated ssDNA with complementary sequence, 5'GCTGTTGACTGA 3' (ODN2) was also sketched, which was then covalently conjugated to the carboxylic acid ends of another PpIX molecule.

AFM analysis of supramolecular assembly

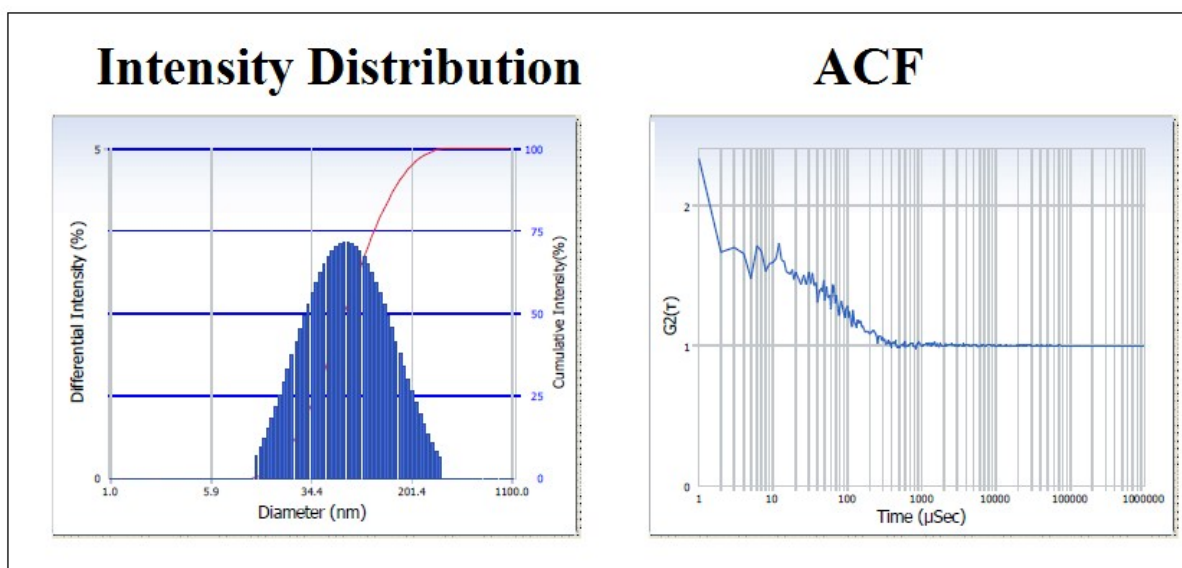
Silicon nitride cantilevers having Force constant 1.2-5.5 N/m were used for the measurements (MiKromash, Bulgaria). The cantilever was oscillating at its resonance frequency ranging from 60-90 kHz. The set point ratio of the cantilever governing the tapping forces varied from 0.2-0.4. The nature of the surface morphology of the nanostructures formed due to the assembly of (ODN1)₂- PpIX and (ODN2)₂-PpIX was observed through Agilent 5500 Atomic Force Microscope (AFM). AFM was operated in semi-contact mode. Samples were prepared by drop casting 20 µL of annealed DNA solution on freshly prepared APS mica of dimensions 1 cm × 1 cm. For functionalization of the mica surface with amino groups (AP-mica), 3 aminopropyltriethoxy silane (APTES) was used which gives mica a positively charged surface. Preparation of APS mica was done by firstly preparing solution of sodium hydroxide in methanol and addition of triethanolamine in a round bottom flask. After this, a precise equivalent of (3-aminopropyl) triethoxysilane is added in a round bottom flask and methanol is evaporated on a rotary evaporator at moderate vacuum for 10 min. The temperature of water bath is raised to 60 °C and the product solidifies to form a crystalline mass following application of vacuum. 1 mM stock is prepared for APS mica preparation.

Circular Dichroism of DNA-Organic Molecule Conjugates and Assembly

Circular Dichroism (CD) was measured at 25 °C in a Jasco J-1500 spectropolarimeter, using a 1-nm bandwidth. CD spectra were averaged over three scans at 100 nm/min to improve the signal-to-noise ratio. A 1 mm path-length quartz cell was used in most cases. The sample compared was hybridized self-complementary oligonucleotides-PpIX and hybridized ODN1-ODN2. (DNA)₂-PpIX conjugates were hybridized in 10 mM TE, 250 mM NaCl and 10 mM MgCl₂ buffer in total volume of 200 μ l and compared with hybridized ODN1-ODN2.

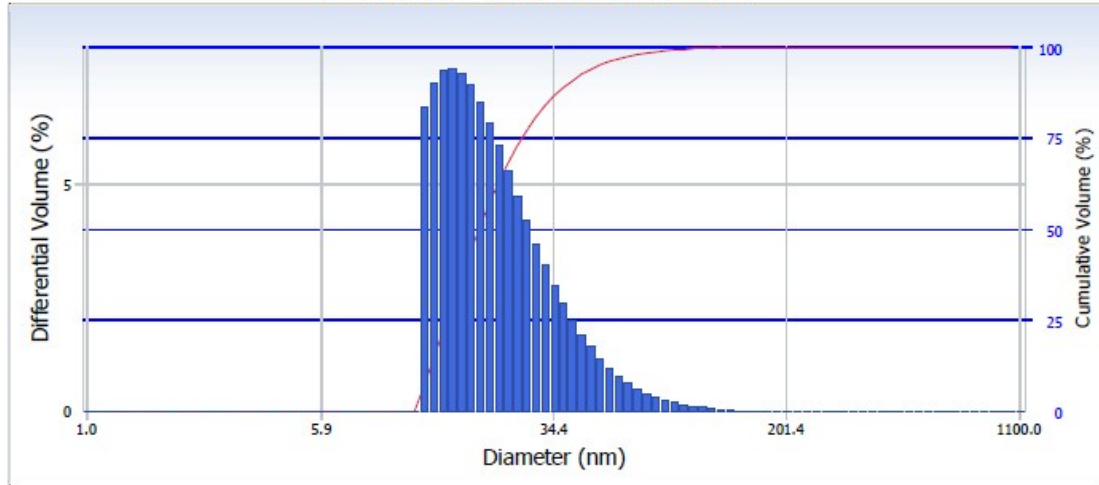
Dynamic light scattering

The solutions were extracted from gel with concentration of 10 μ g/mL and hybridised in 250 mM salt. Control experiments were done with unhybridised DNA. All the solutions were prepared by passing the solutions through centrifugal filters of diameter 0.22 μ m from Millipore. The formation of the DNA nanocages were monitored and analyzed by dynamic light scattering experiments performed on a Particle Size and Zeta Potential Analyzer from Beckman Coulter.



ESI 10 Dynamic light scattering data showing intensity differential intensity vs diameter and G₂(T) vs Time plot of self assembled (ODN1)₂- PpIX and (ODN2)₂- PpIX

Volume Distribution



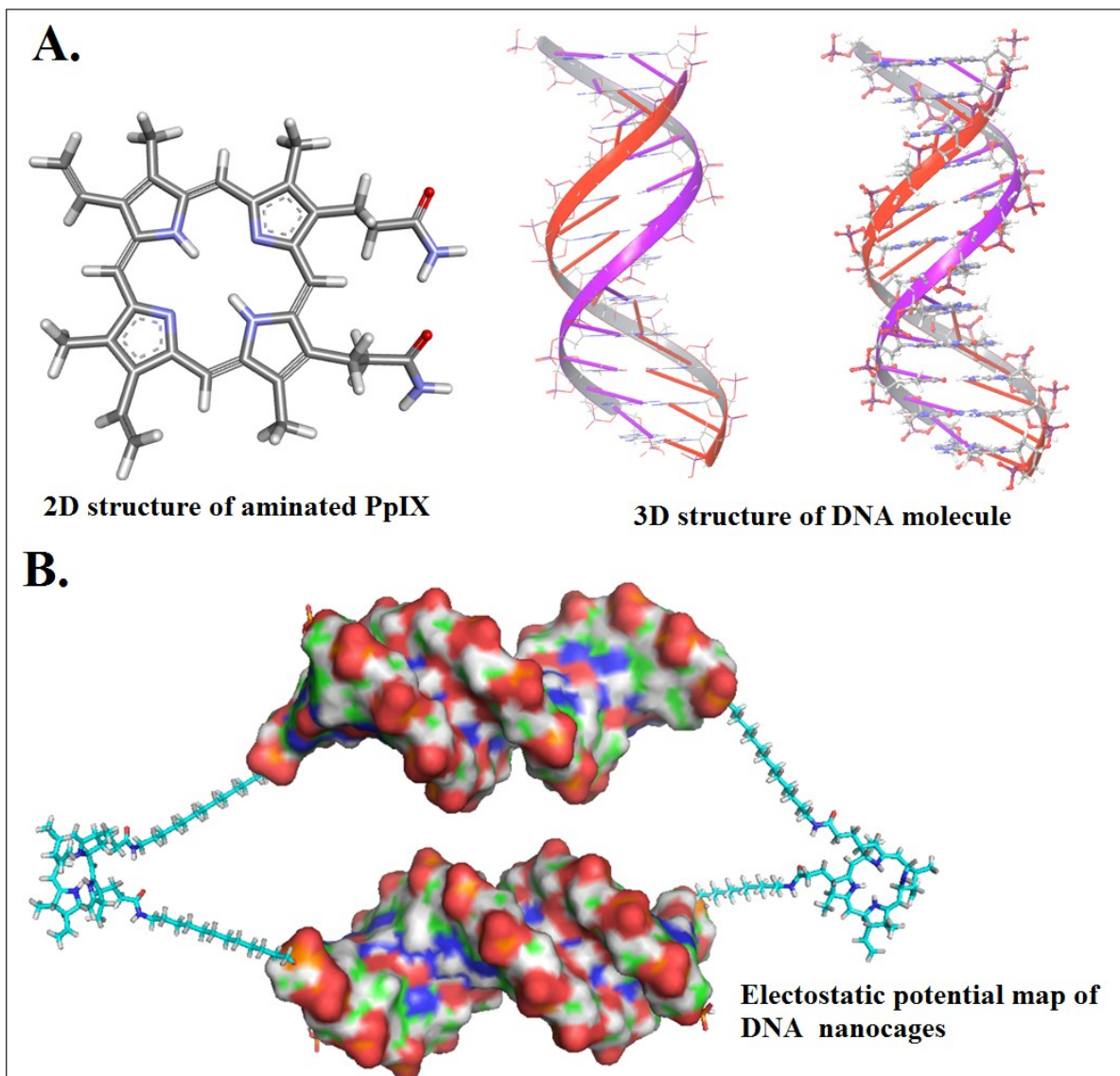
Distribution Results (Contin)

Peak	Diameter (nm)	Std. Dev.
1	81.3	59.8
2	0.0	0.0
3	0.0	0.0
4	0.0	0.0
5	0.0	0.0
Average	81.3	59.8
Residual :	4.525e-002	(N.G)

Cumulants Results

Diameter (d)	: 54.2	(nm)
Polydispersity Index (P.I.)	: 0.308	
Diffusion Const. (D)	: 9.202e-008	(cm ² /sec)
Measurement Condition		
Temperature	: 25.5	(°C)
Diluent Name	: WATER	
Refractive Index	: 1.3327	
Viscosity	: 0.8778	(cP)
Scattering Intensity	: 1587	(cps)

ESI 11 Dynamic light scattering data showing differential volume vs diameter plot of self assembled (ODN1)₂- PpIX and (ODN2)₂- PpIX



ESI 12 Computational studies depicting explicit 2D nanocages formation (A) 2D structure of aminated PpIX and 3D structure of DNA molecule (B) Electrostatic potential map of DNA nanocages

References

- 1 Ultra, ChemDraw. "6.0 and Chem3D Ultra." Cambridge Soft Corporation, Cambridge, USA, 2001.
- 2 Schrödinger Release 2014-3: Maestro, version 9.9, Schrödinger, LLC, New York, NY, 2014.