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A facile approach to prepare dual functionalized DNA based material in a bio- deep eutectic solvent

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Experimental Section

Materials

Deoxyribonucleic Acid extracted from Salmon testes in the sodium salt form (CAS No. 9007-49-2, ca. 20 kbp) was purchased from TCI Chemicals, Tokyo, Japan. The DNA was used as received, since the purity of DNA was sufficiently high as determined from optical measurements. The ratio of the absorbance of the DNA stock solution at 260 nm to that at 280 nm was found to be 1.92, which suggested the absence of proteins. [1] Choline chloride, Ethylene Glycol and Isopropyl alcohol was purchased from S D Fine chemicals, Mumbai, India. $FeCl_3.6H_2O$ and $FeSO_4.7H_2O$ was purchased from SISCO Research laboratories Pvt. Ltd, Mumbai, India. Urea was procured from RFCL Ltd., New Delhi, India. NaOH was purchased from Central Drug House (P) Ltd, New Delhi, India. H_2O_2 was purchased from Qualikem Fine Chem Pvt Ltd., Vadodara, India. All chemicals were of analytical grade (AR) and used as received.

Preparation of Fe_3O_4

$FeCl_3.6H_2O$ (5.4 gm) and Urea (3.6 gm) were dissolved in water (200 ml) at 85 ^{o}-90 ^{o}C for 2 hours. The resulted mixture was kept at room temperature to cool it down. After that, $FeSO_4.7H_2O$ (2.8 gm) was added into it. The pH of this mixture was adjusted to 10 by the drop wise addition of 0.1M NaOH solution. The black precipitates of hydroxide were appeared which were placed in an ultrasound at 30 ^{o}-35 ^{o}C for 30 minutes. After that the solution was kept at room temperature with undisturbed condition for 5 hours, the resulting black precipitates was washed and vacuum dried.

Preparation of $H_2.Ti_2O_5.H_2O$

$H_2.Ti_2O_5.H_2O$ was prepared using literature procedure reported by Sutradhar *et al.*(2011). [2] Briefly, 0.01M Titanium isopropoxide (2.84 gm) was dissolved in 10 ml of ethanol followed by drop wise addition of 40 ml of ammonical water with constant stirring at 500 rpm. White precipitates were formed and stirring was continued for another 15 minutes. These precipitates were collected by the centrifugation and washed 4 times with milli Q water. After complete washing, 25 ml of 1.6 molar of aqueous ammonium carbonate solution was added into it with constant stirring. A milky white solution appeared, in which 3 ml of 30% H_2O_2

was added drop wise and the color of the solution became light yellow. This solution was allowed to stir overnight at ambient temperature. The precipitates obtained was collected by the centrifuge and washed with water (3 times) and dried at hot oven for overnight.

Preparation of Bio-based Deep Eutectic Solvent (choCl-EG 1:2)

The Bio-based Deep-eutectic solvent (DESs) were prepared following the method described by Abbott *et al.* (2004). [3] In a typical procedure, both the hydrogen bond donor (HBD) i.e., choline chloride and acceptor (HBA) i.e., ethylene glycol was heated at 80ºC with constant stirring at optimized mole ratio (1:2) under argon atmosphere until homogenous and colourless liquids were formed (2 h).

Preparation of dual functionalized DNA hybrid material.

Various parameters were optimized to prepare DNA hybrid material. In a typical reaction, 75 mg of Salmon testes DNA was added in 3 g of choCl-EG 1:2. This mixture was allowed for simple stirring at room-temperature nearly for 3 h resulting formation of 2.5% *w/w* DNA solution. [4] **A**

Dispersion of 5 mg/ mL of Fe_3O_4 in choCl-EG 1:2 was prepared by ultrasonication for 5 min. **B**

Dispersion of 10mg/mL of $H_2.Ti_2O_5.H_2O$ in choCl-EG 1:2 was prepared by ultrasonication for 5 min. **C**

B was added into **A** followed by ultra sonication for 1/2 h**. D**

C was added into D followed by ultra sonication for 1/2 h. **E**

Into **E**, isopropyl alcohol was added to precipitate out the dual functionalized DNA hybrid material. The hybrid material was isolated by centrifugation followed by washing with IPA for several times. The supernatant was stored and DES was recycled after removing the IPA by evaporation in a rotary evaporator.

Characterizations

Ultrasonication was done on Elma, Ultasonication bath LC 60H (Germany) at 50 Hz. As prepared Fe_3O_4 and $H_2.Ti_2O_5.H_2O$ and DNA-composite was characterized by powder XRD, SEM, TEM and FT-IR spectroscopy. Powder XRD was measured at 298 K on a Phillips X`pert MPD system using CuK_α radiation (λ = 0.15405 nm) with 2θ range from 5 º to 80 º at a scan speed of 0.1 º sec^{-1}. The samples were dispersed in isopropyl alcohol (IPA) and loaded on an aluminum stubs and evaporated to dryness followed by recording their SEM images on a LEO 1430 VP instrument employing accelerating voltage of 18 kV. Samples were dispersed in IPA and loaded on carbon coated copper grids (300 mesh sizes) and the loaded samples were used for the recording their TEM images on a JEOL TEM (Model-JEM 2100, Japan) instrument operated at accelerating voltage of 120 kV. FT-IR spectra were recorded on a Perkin Elmer Spectrum GX, FT-IR system, USA by taking 2.0 mg of sample in 600 mg of KBr. AFM measurements were performed in the semi contact mode using an Ntegra Aura (Nt-Mdt, Russia) instrument at room temperature in air. The height of the images was calculated from different area of each sample using image analysis software 'Nova'. Phae contrast microscopic image was recorded using ordinary light microscope with 100 X magnification (Fine Vision Microscope, India). Circular dichroism (CD) spectra were recorded on a Jasco model J-815 CD Spectrometer, using measurement range at 200–500 nm in tris-HCl

Buffer (pH = 7.2) at a scanning speed of 10 nm/min and band width of 1 nm. The spectra were acquired in a 1.0 cm path-length quartz cuvette at 25 °C. The UV–Vis absorption spectra of DNA at concentration of 0.02 mg/ml in tris-HCl buffer (pH = 7.2) were recorded on a Varian CARY 500 UV-vis-NIR spectrophotometer. The ^{31}P NMR spectra of standard DNA and recovered DNA solution in tris-HCl buffer were recorded at 25 °C on a Bruker Avance 200 MHz spectrometer. The measurements were carried out with a DDO probe at a resonance frequency of 80.96 MHz. The phosphorous chemical shifts of DNA were externally referenced to 85% o-phosphoric acid.

The ratio of peak height to trough depth was calculated using Eq. (1), described by Morris, Rees, and Thom (1980). [5]

$$\text{Peak / trough ratio} = (\theta_{trough} - \theta_{peak} / \theta_{trough}) \quad [1]$$

Antimicrobial study

Bacterial growth inhibition activity was checked using gram negative (*Escherichia coli, Shigella flexineri, Pseudomonas fluorescens*) and gram positive (*Bacillus subtilis*) bacteria in the presence and absence of DNA hybrid material. All bacterial strains were grown in 3 mL of Luria-Bertani broth Miller (Himedia, India) at 37 °C and 120 rpm using single colony from LB agar plate. After 12 hrs growth, OD600nm was adjusted to 1.0. Subsequently, cultures were 200-fold diluted with LB broth and incubated with and without 5 mg/mL of nanocomposite compound at 37°C and 120 rpm for 4 hrs. Each culture was serially diluted with sterile 0.9% physiological saline and 100 uL of solution was spread on LB agar plate. The number of colonies was counted after an overnight incubation at 37°C. Microscopic image of the gram stained bacterial culture before and after treatment with the hybrid material was recorded on an Olympus BX60 microscope fitted with a camera DP72 with 100 X magnification.

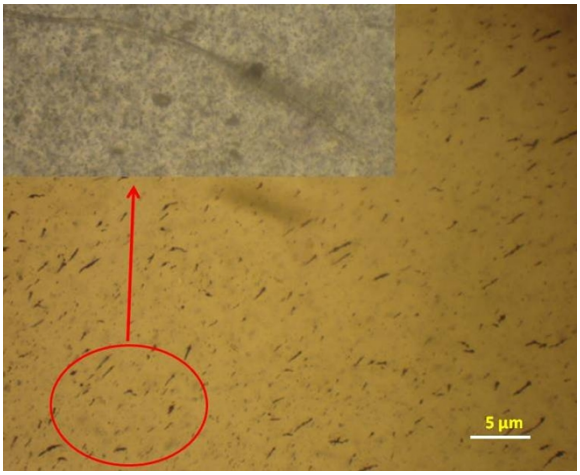

Fig. S1 : Phase contrast optical image (100 X) of Fe_3O_4 particles in DNA solution prepared in choCl-EG 1:2

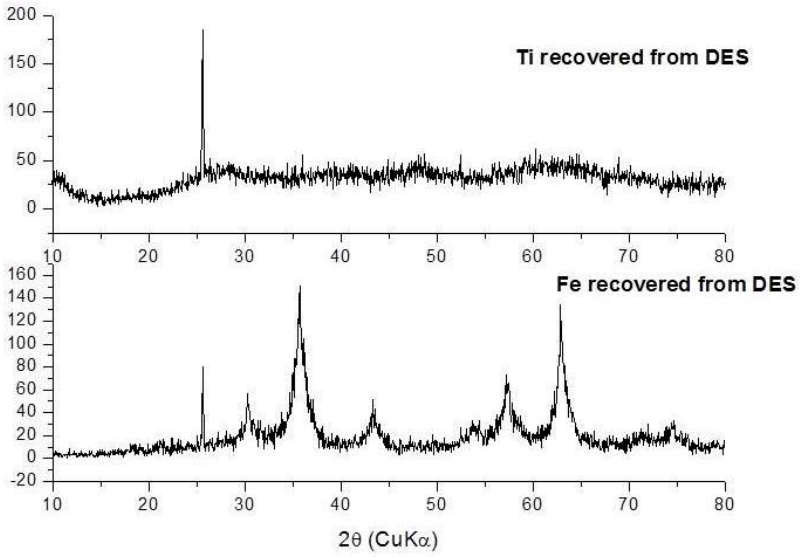

Fig. S2: Powder XRD profile of Fe_3O_4 and $H_2.Ti_2O_5.H_2O$ recovered from choCl-EG 1:2

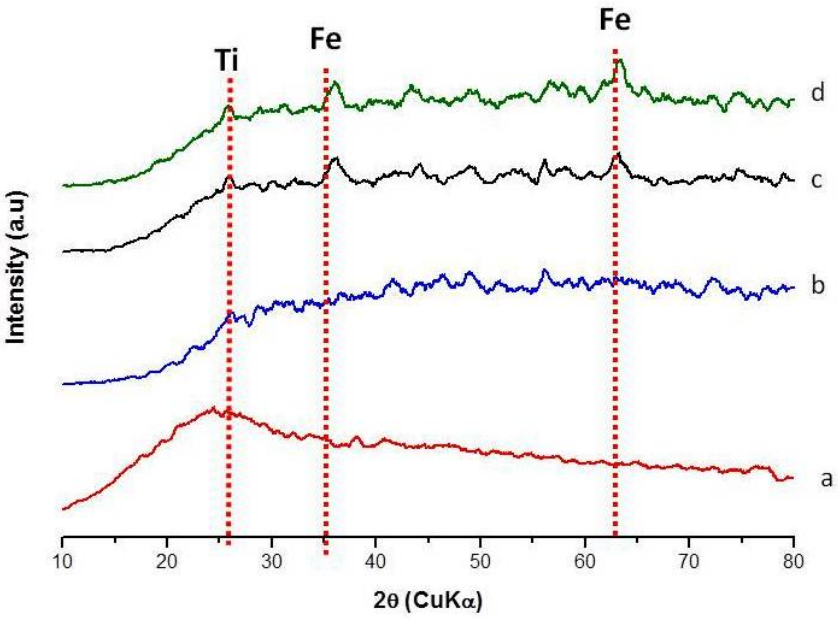

Fig. S3: Powder XRD profile of (a) standard DNA (b) DNA and Fe_3O_4 mixture recovered from DES (c) DNA and $H_2.Ti_2O_5.H_2O$ mixture recovered from DES (d) dual functionalized DNA hybrid material

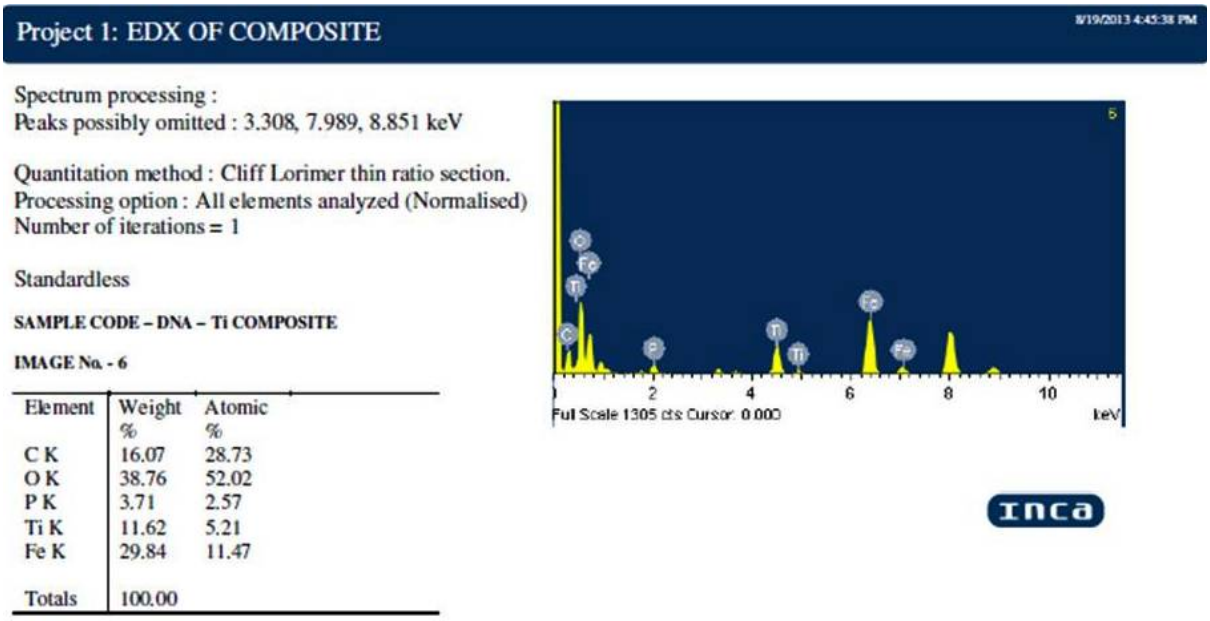

Fig. S4 : TEM-EDX profile of dual functionalized DNA hybrid material.

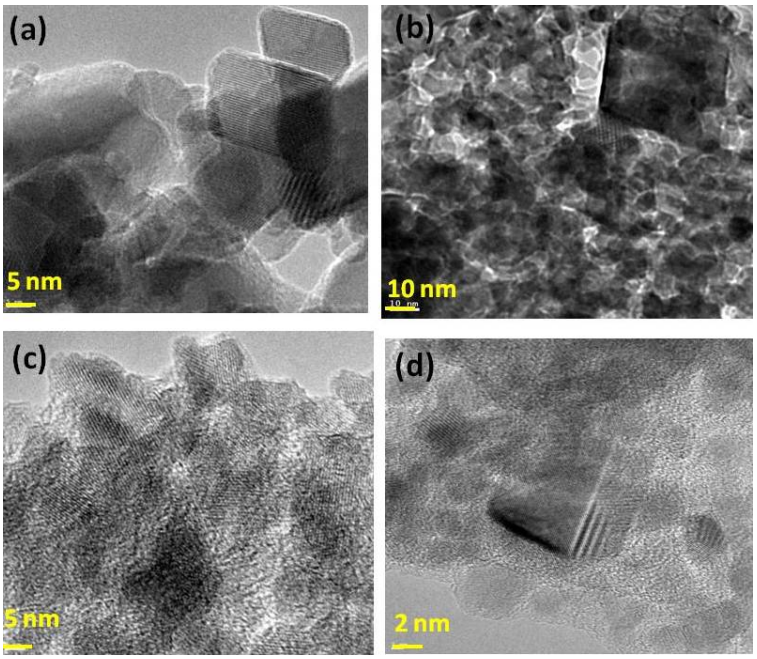

Fig. S5 : TEM image of (a) Fe_3O_4 (b) Ti-Complex (c) DNA and Ti- complex mixture recovered from DES and (d) DNA and Fe_3O_4 mixture recovered from DES

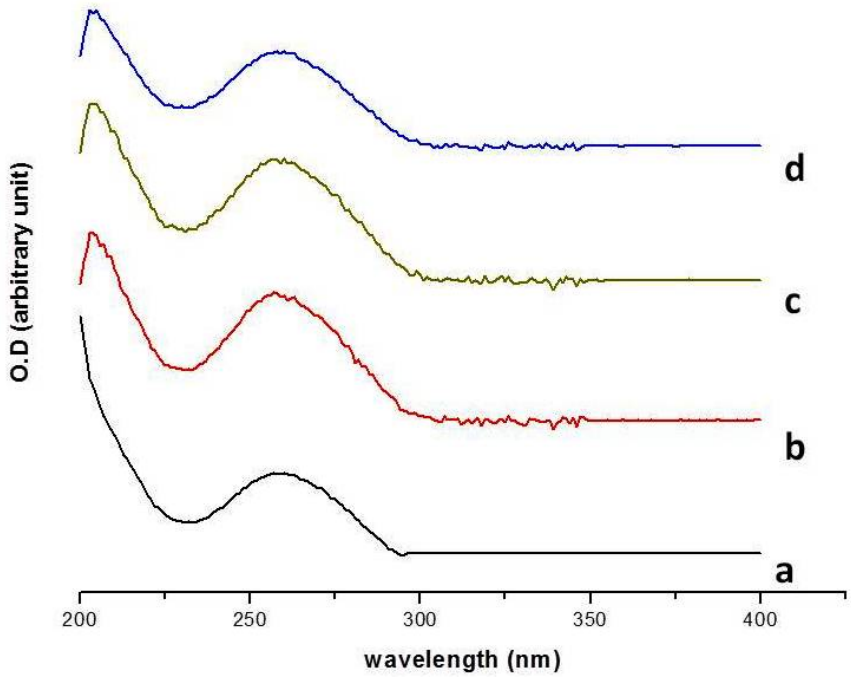

Fig. S6: UV-Vis spectra of (a) standard DNA (b) DNA and Fe_3O_4 mixture recovered from DES (c) DNA and $H_2.Ti_2O_5.H_2O$ mixture recovered from DES (d) dual functionalized DNA hybrid material in Tris.HCl buffer (pH 7.2)

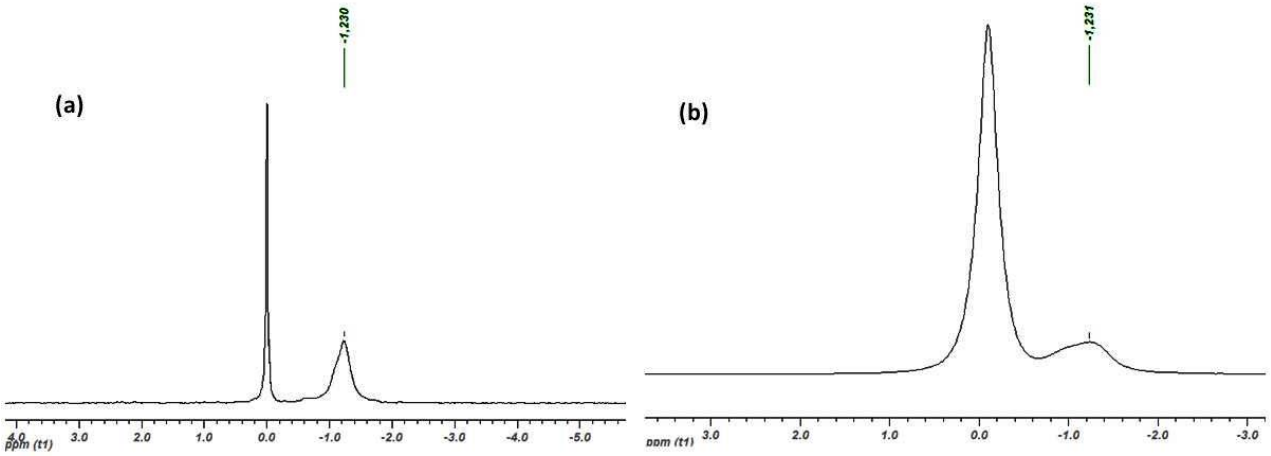

Fig. S7 : ^{31}P NMR spectra of (a) standard DNA and (b) recovered mixture of DNA and Fe_3O_4

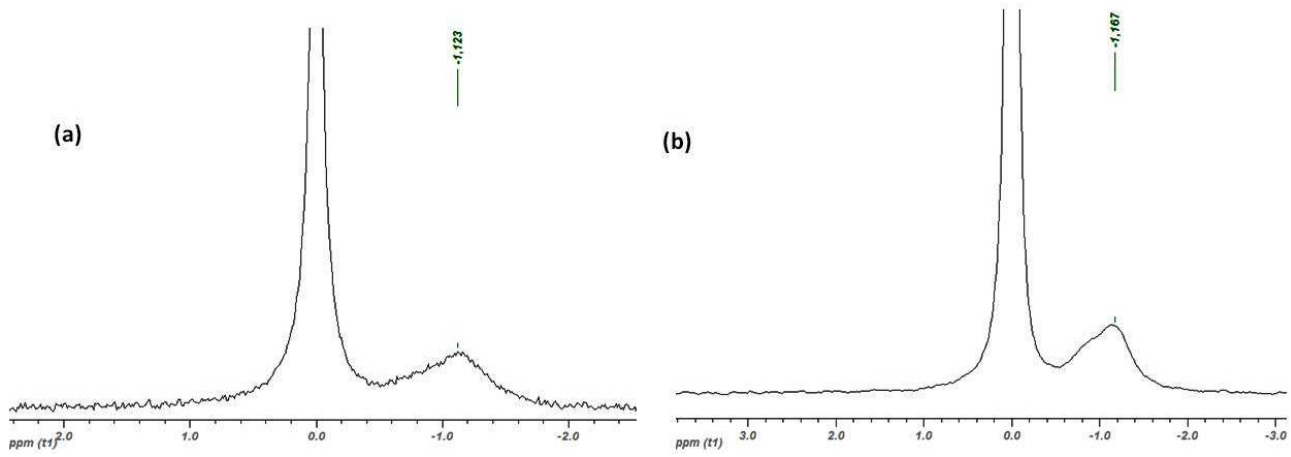

Fig. S8 : ^{31}P NMR spectra of (a) recovered mixture of DNA and $H_2.Ti_2O_5.H_2O$ and (b) DNA based hybrid material.

Table S1- Comparison of colony formation unit (CFU) of *Escherichia coli, Pseudomonas fluorescens, Shigella flexineri* and *Bacillus subtilis* culture with and without (W/O) 5 mg/mL of hybrid material.

Bacterial strains	**W/O**	**5 mg/mL**
E.coli	2.83E+10	0
Ps. Fluorescens	3.1E+11	3.45E+10
S. flexineri	1.52E+11	8.45E+10
B.subtilis	3.3E+10	4.5E+09

W/O = Without hybrid material

Mean of CFU/mL is expressed in logarithmic scale.

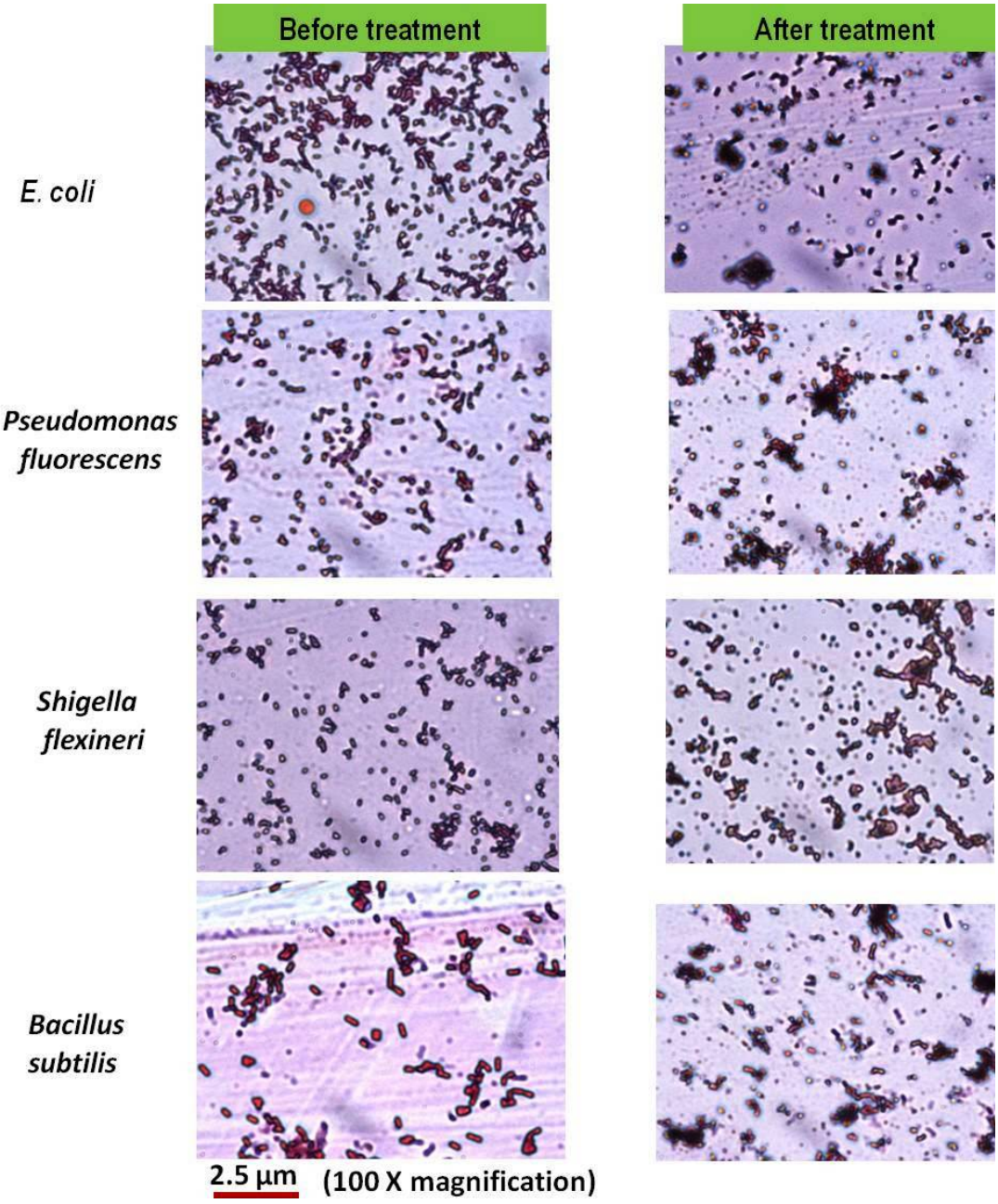

Fig. S9: Optical microscopic images of bacteria colony before and after treatment with the DNA based hybrid material.

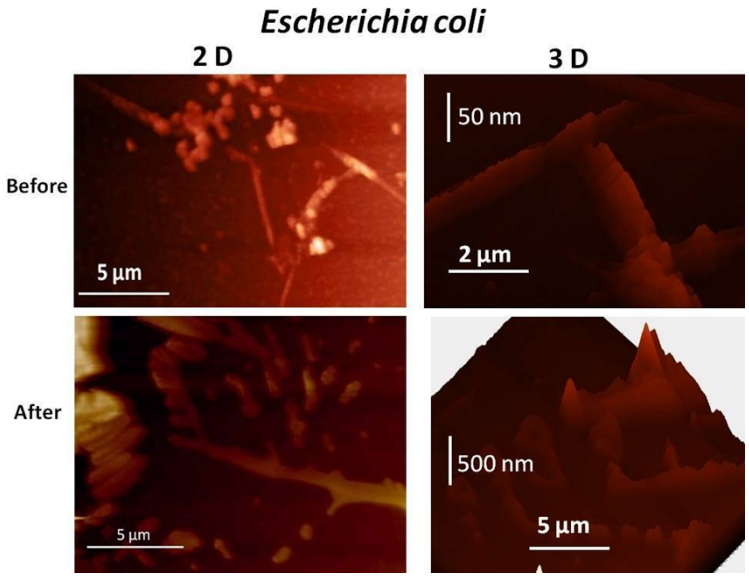

Fig S10 : AFM image of *E. coli* bacteria culture before and after treatment with the dual functionalized DNA nanocomposite.

References:

[1] W. Saenger, Principles of Nuclei Structure; *Springer-Verlag : New York*, 1984.

[2] Narottam Sutradhar, Apurba Sinhamahapatra, Sandip Kumar Pahari, Hari C. Bajaj and Asit Baran Panda, *Chem.Commun.* 2011, 47, 7731-7733.

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[5] E. R. Morris, D. A. Rees and D. Thom, *Carbohydr Res*, **1980**, *81*, 305–314.