Electronic Supplementary Material (ESI) for ChemComm.

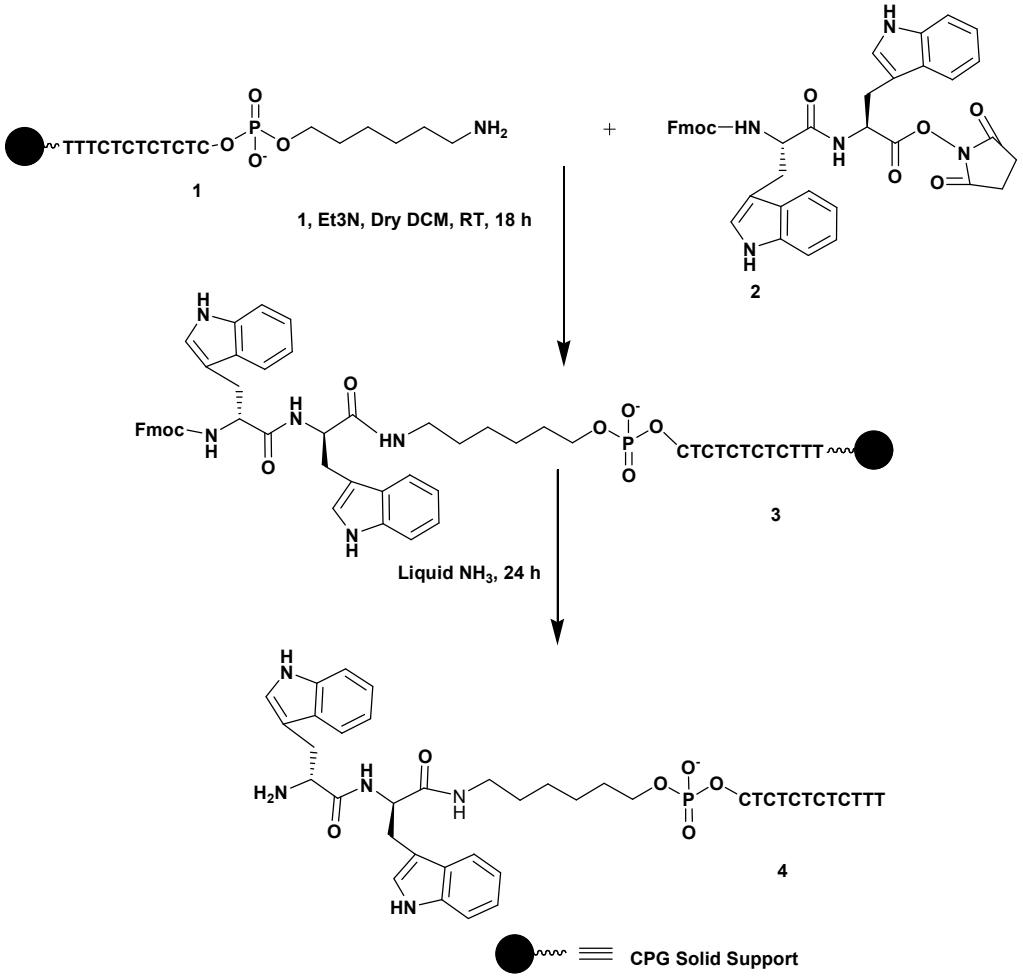
Label-free, Optical Sensing of DNA-Ditryptophan Hybrids Supramolecular Assembly

Nidhi Gour, [†a] Jancy Nixon Abraham[†a], Mohamed Chami[b], Axel Castillo[a], Sandeep Verma[c] and Corinne Vebert-Nardin[a,*]

General: Dichloromethane extra dry, N,N-dimethylformamide, sodium chloride, triethylamine were purchased from Acros Organics (Geel, Belgium). *N*,*N*′-dicyclohexylcarbodiimide, *N*-hydroxysuccinimide, sodium bicarbonate and dialysis tube (cut-off 2000 M_w) were supplied by Sigma Aldrich (Buchs, Swizerland). The ammonia solution 35% was purchased from Fisher Scientific SA (Wohlen, Switzerland). Fmoc-WW-OH was prepared by conventional solution phase synthesis. 5'-CTCTCTCTCTTT-3' modified at the 5' end with an amine group through a hexyl spacer was purchased from Eurogentec (Belgium). WW-PEG was purchased from Genescript (Piscataway, New Jersey, USA). ESI mass spectra were recorded on 11HTS PAL -LC10A-API 150Ex. The analytical HPLC was equipped with a LC-Net II/ADC transmitter, a Jasco UV-2077 detector and a Jasco PU-980 pump.^{1}H NMR spectra were recorded using a 300 –MHz Brucker NMR spectrophotometer in $CDCl_3$ containing small amounts of TMS as internal standard.

Synthesis and Characterization:

In brief, the Fmoc protected acid group of WW-OH was first activated with N-hydroxysuccinimide according to a previously reported methodology.[1] The activated ester thus synthesized was then used for coupling to the free 5'-amine group of a 12-mer nucleotide sequence 5'-CTCTCTCTCTTT-3' modified at the 5'-end through a C_6 hexyl linker whereas the 3'-end was still bound to the controlled pore glass (CpG) solid support. Solid phase synthesis was employed to avoid tedious chemistry or purification steps. Finally the solid support and protecting groups were removed by treatment with liquid ammonia. The resulting compound was characterised by ^{1}H NMR, ESI MS and analytical HPLC as reported in previous studies.[2]



Scheme S1: Synthetic route of the WW-CTCTCTCTCTTT hybrid (WW-DNA) **(4)**

Conjugation of active N-hydroxy-succinimide ester Fmoc WW-OH (2) Activation of Fmoc WW-OH was performed according to a previously reported methodology**.** [1]In a two necked round bottom flask N-hydroxy-succinimide (23 mg, 1.2 eq) and FmocWW-OH (100 mg, 1 eq) were dissolved in dry dichloromethane (20 mL) and stirred on ice under nitrogen atmosphere for 10 minutes. N,N'-dicyclohexylcarbodiimide (46 mg, 1.2 eq) was dissolved in dry dichloromethane (5 mL) and added to the reaction mixture. The stirring was continued for 1 h on ice and subsequently overnight at room temperature. The white precipitate of N,N'-dicyclohexylurea was filtered off. The filtrate was washed with 10% sodium bicarbonate yielding a brine solution further dried over anhydrous sodium sulphate.

The filtration was followed by solvent evaporation yielding 80 mg of product (52% reaction efficiency). Rf=0.5 (10 % methanol/dichloromethane). ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm) : 6.88-7.41 (m, 12H), 7.46-7.54 (m, 2H), 7.75-7.78 (m, 2H), 8.0-8.02 (t, 2H), 6.12-6.14(d, H), 5.3 (m, H) 5.2-5.22 (m, H), 4.32-4.26(m, H), 4.1-4.2 (m, H) 3.67-3.71 (m, H), 3.48-3.57 (m, H), 3.22-3.45 (m, 2H), 305-3.14(m, 2H).

Coupling of Fmoc-WW active ester with a C_6 amino-modified nucleotide sequence bound to the CpG resin (3) Compound **2** (180 mg) was added in a 2 mL reactor. Subsequently, 50 mg of solid CpG-resin bound 5'-CTCTCTCTCTTT-3' nucleotide sequence modified at the 5'-end by an amino group through a hexyl C_6 spacer (**1**) (50 mg, 2 μM, 1 eq) was added to the reactor. To this, 1.5 mL of dry DCM and 45 μL of triethylamine were added. The reactor was subsequently closed and shaked overnight at room temperature. The 2 mL reactor is fixed at the bottom to a filter which prevents filtration of the resin out that enables that of solvents. All residual solution was thus drained from the reactor and the resin washed thoroughly with DCM and DMF and subsequently again with DCM to remove any unreacted peptide and other side products using syringe pressure filtration. Finally the resin was dried in presence of nitrogen and weighed in an eppendorf tube.

Removal of the CpG solid support (4) The nucleotide sequence bound to the solid support obtained in step 3 (40 mg) was transferred to an eppendorf tube and subsequently 1 mL of ammonia solution was added and the resulting mixture kept in a shaker maintained at 40 ^{o}C for 24 h. The solution was then filtered to remove the resin and the supernatant collected. Subsequently this solution was lyophilized in vaccuum. The dried powder was dissolved in nuclease free water and non polar impurities settled by coprecipitation. Subsequently the solution was filtered through 0.45 micron filter membranes and the resulting solution lyophilized again. Yield 5 mg (20%). The resulting compound was characterized by ESI MS and analytical HPLC as reported in previous studies. The lyophilized WW-DNA hybrid was dissoled at a concentration of 1 mg mL^{-1} and passed through 0.45 micron filters and 200 μL of this solution was analyzed by reverse phase HPLC (C-16 analytical column, flow rate of

1 mL min^{-1} in an isochratic gradient of methanol with 10 mM ammonium acetate, detection at 260 and 280 nm). A single sharp peak of **4** evidences the purity of the compound. ESI-MS mass spectra of **4** was taken by dissolving 1 mg of the nucleotide-peptide conjugate in 500 µL water, 490 µL acetonitrile and 10 µL triethylamine. m/z (ESI-MS) M-2H/2= expected: 2031.53. Observed: 2031.1, M-3H/3=expected: 1354.02; observed: 1353.8, M-4H/4= expected: 1015.26, observed: 1015.5; M-5H/5= expected: 812.01, observed: 812.2, M-6H/6=676.5 expected: 676.5.

Methods

Sample preparation

4 mg mL^{-1} of **4** was dissolved in 1 mL water (1 mM). It was subsequently diluted with nuclease free water 2 times (0.5 mM, 2 mg mL^{-1}). A dilution series was prepared from the 1 mM solution by adding nuclease free water to dilution ratio of 2, 4, 8, 16, 32, 64, 128 and 256 respectively. To study the role of hydrogen bonding WW-C_5T_7 (1 mM) sequences were also co-incubated with 10 mM and 100 mM urea respectively and the fluorescence intensity monitored.

Hybridization experiments with WW-$(CT)_5T_2$ conjugates were carried out by incubating the complementary DNA sequence 5'-AAAGAGAGAGAG-3' ($A_2(AG)_5$) in increasing concentration. To 0.5 mM of conjugate **4** in 100 mM NaCl, $A_2(AG)_5$ sequences were added in increasing amount such that the final concentration of $A_2(AG)_5$ was 0.005 mM (1%); 0.05 mM (10%), 0.25 mM (50%), 0.5 mM (100%) and 1 mM (200%). As a control we also studied WW-$(CT)_5T_2$ unspecific hybridization in presence of the non-complementary 5'-CTCTCTCTCTTT-3' ($(CT)_5T_2$) sequence. A similar series than with the complementary sequence was prepared by adding the $(CT)_5T_2$ sequence in increasing amount from 0.005 mM to 0.5 mM..

Ditryptophan self-assembly

1 mg of WW was dissolved in 10µL of HFIP followed by addition of 500 µL of water. This was incubated at 37 ^oC for 24 hours to induce self-assembly.

Scanning Electron Microscopy (SEM)

A 20 µL aliquot of the fresh sample was dried at room temperature on silicon wafers. Dried samples were coated with gold for 20 s in a Jeol JFC-1200 Fine coater. Subsequently, SEM images were acquired on a Jeol 6510LV microscope, equipped with a tungsten filament gun, operating at WD 10.6 mm and 10 kV. The stock solution of WW-$(CT)_5T_2$ was prepared by dissolving 4 mg of compound in 1 mL nuclease free water to achieve a 1 mM WW-$(CT)_5T_2$ solution. The pH 7.5 of the solution was measured.

Transmission Electron Microscope (TEM)

For TEM, 5 µL of fresh sample of a specimen (2 mg mL^{-1}, 0.5 mM) incubated for 1 h in nuclease free water was placed on a carbon coated 400-mesh copper grid. After sample drying, the sample was imaged without staining directly with a Tecnai G2 electron microscope operating at 120 kV. Staining is not required for nucleotide-based self-assemblies since the sequences are composed of electron dense phosphate groups.

For cryo-TEM, 4 µL aliquot of sample was adsorbed onto holey carbon-coated grid (quantifoil, Germany), blotted with Whatman 1 filter paper and vitrified into liquid ethane at -178 °C using a vitrobot (FEI company, USA). Frozen grids were transferred onto a Philips CM200-FEG electron microscope using a Gatan 626 cryo-holder. Electron micrographs were recorded at an accelerating voltage of 200 kV and a nominal magnification of 50000 x, using a low-dose system (10 $e^-/Å^2$) and keeping the sample at -175°C.

Fluorescence spectroscopy

WW-DNA fluorescence intensity measurements were performed by exciting samples at 300 nm and measuring emission intensities between 310 nm and 500 nm using a fluorescence spectrophotometer (Fluoromax – 2, Company: JOBIN YVON-SPEX, New Jersey, USA). About 50 µL of samples were analyzed using a quartz cuvette of 3 mm path length.

Circular Dichroism Studies

Circular dichroism (CD) spectra of the WW-DNA and C_5T_7 sequence (100 μM) were recorded on a JASCO J-815 CD spectrometer (Jasco Corporation, Tokyo, Japan) at 25 °C using a band width of 5.0 nm, a data pitch of 0.2 nm, a scanning speed of 100 nm min^{-1} and a response time of 4 s. A 1 mm quartz cuvette was used for measurements. Three scans of duplicate samples were measured and averaged

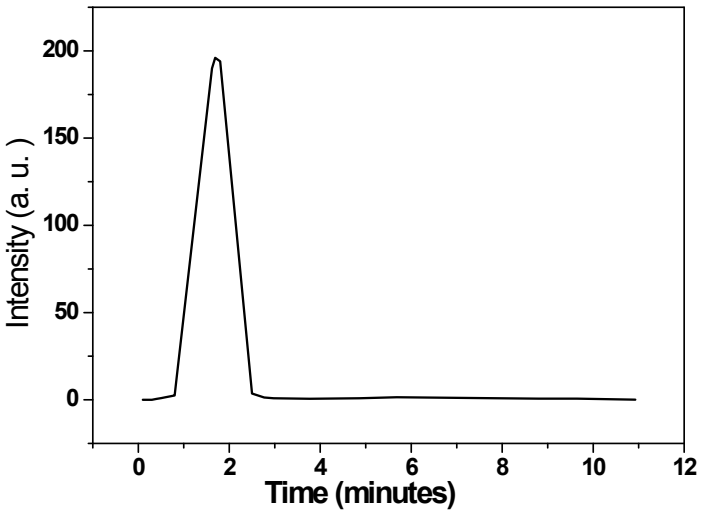


Figure S1: Analytical HPLC of **4** in methanol with 10 mM ammonium acetate at a flow rate of 1mL min^{-1}.

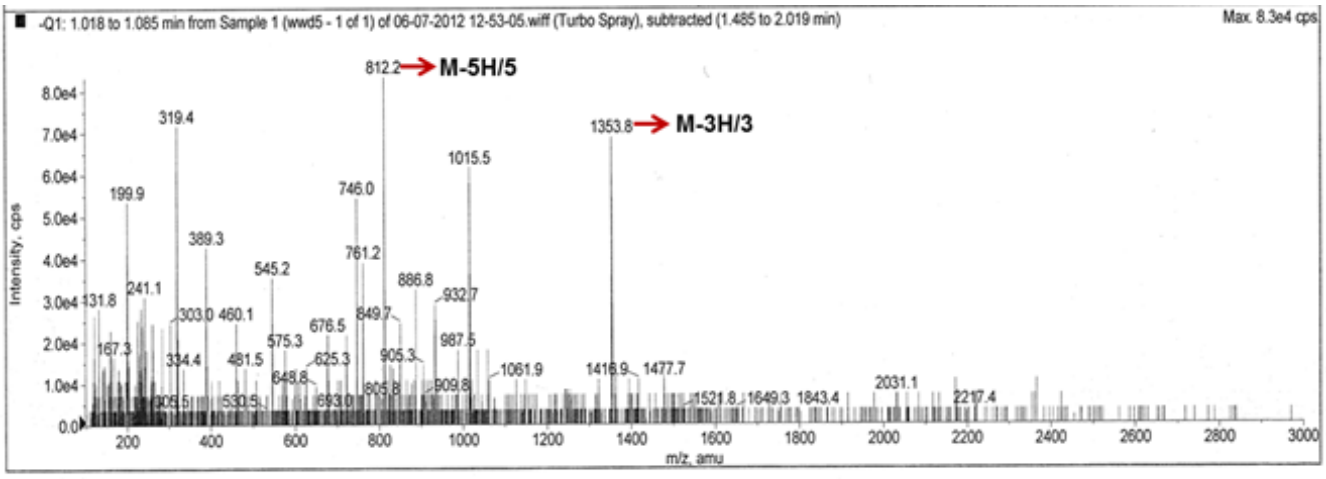


Figure S2: ESI-MS of **4**

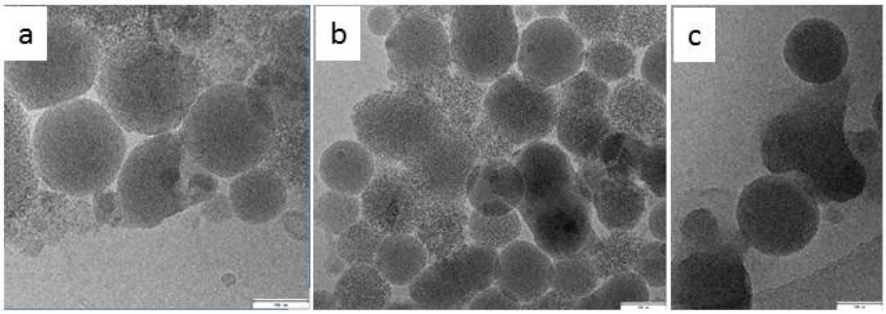


Figure S3: Morphological characterization of the WW-DNA spherical structures by cryo-transmission electron microscopy (scale bar is 100 nm)

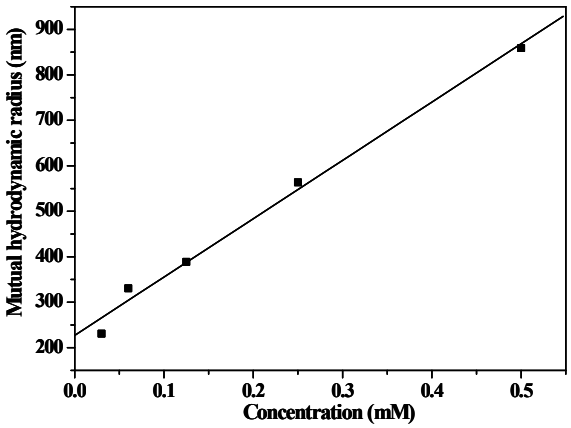


Figure S4 : Concentration dependent DLS monitoring of the size of the self-assembled WW-DNA

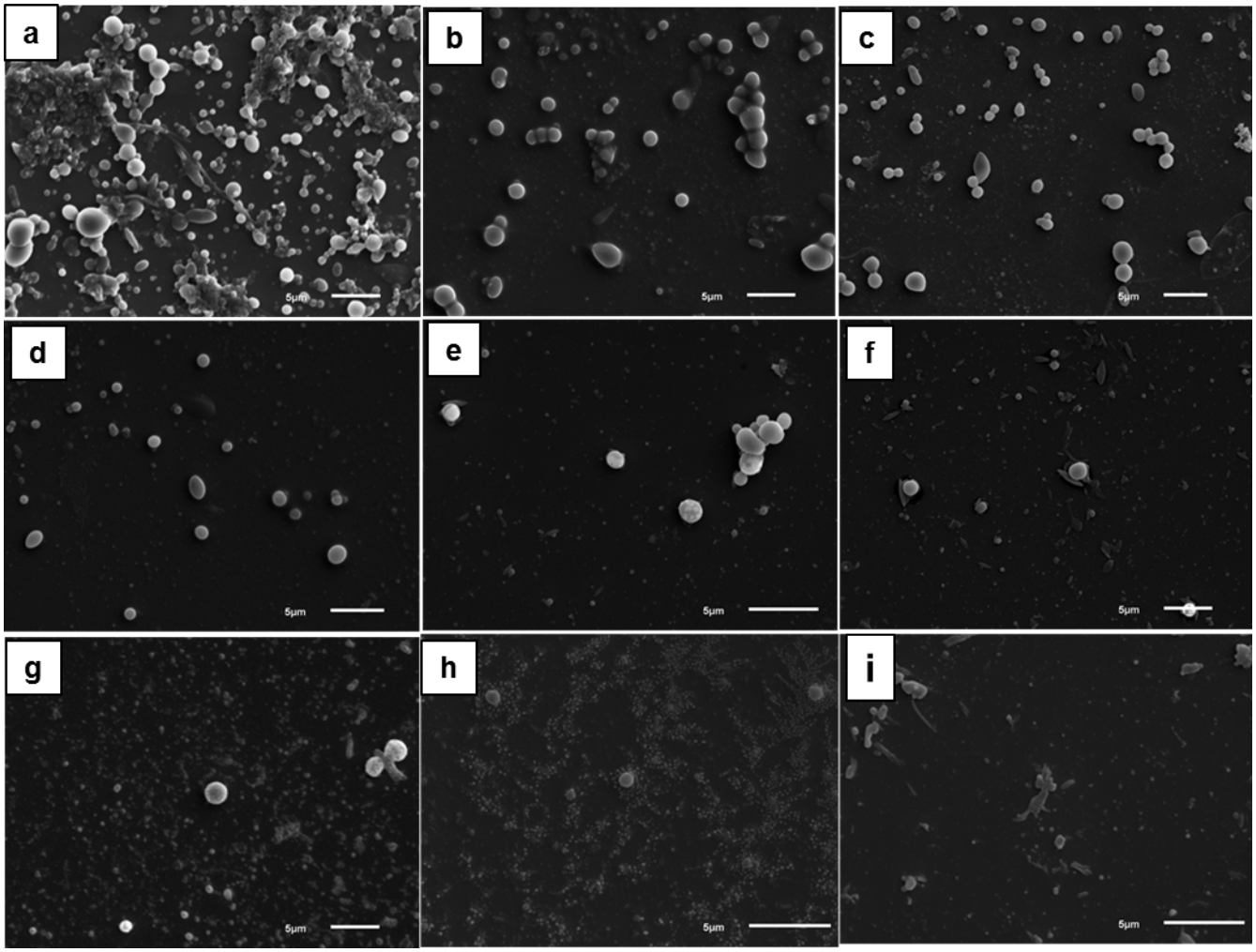


Figure S5: SEM of WW-DNA at different dilutions observed at similar magnification (5µm scale). (a) 1 mM; (b) 0.5 mM; (c) 0.25 mM; (d) .0.13 mM; (e) 0.06 mM; (f) 0.03 mM (g) 0.015 mM (h) 0.008 mM (i) 0.004mM

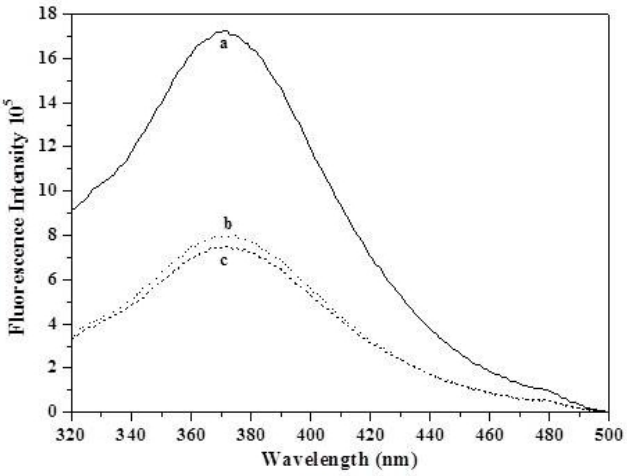


Figure S6: Fluorescence spectroscopy in presence of urea (a) WW- DNA (0.5 mM), incubation with (b) 5 mM urea, (c) 50 mM urea.

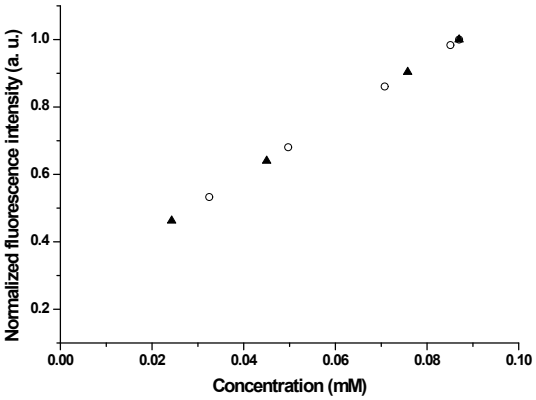


Figure S7: Fluorescence spectroscopy upon hybridization with both the complementary and non-complementary nucleotide sequence. A decrease of the fluorescence intensity is observed upon hybridization in presence of the ▲) complementary and ○) non complementary sequence (as a function of the concentration of unreacted WW-DNA).

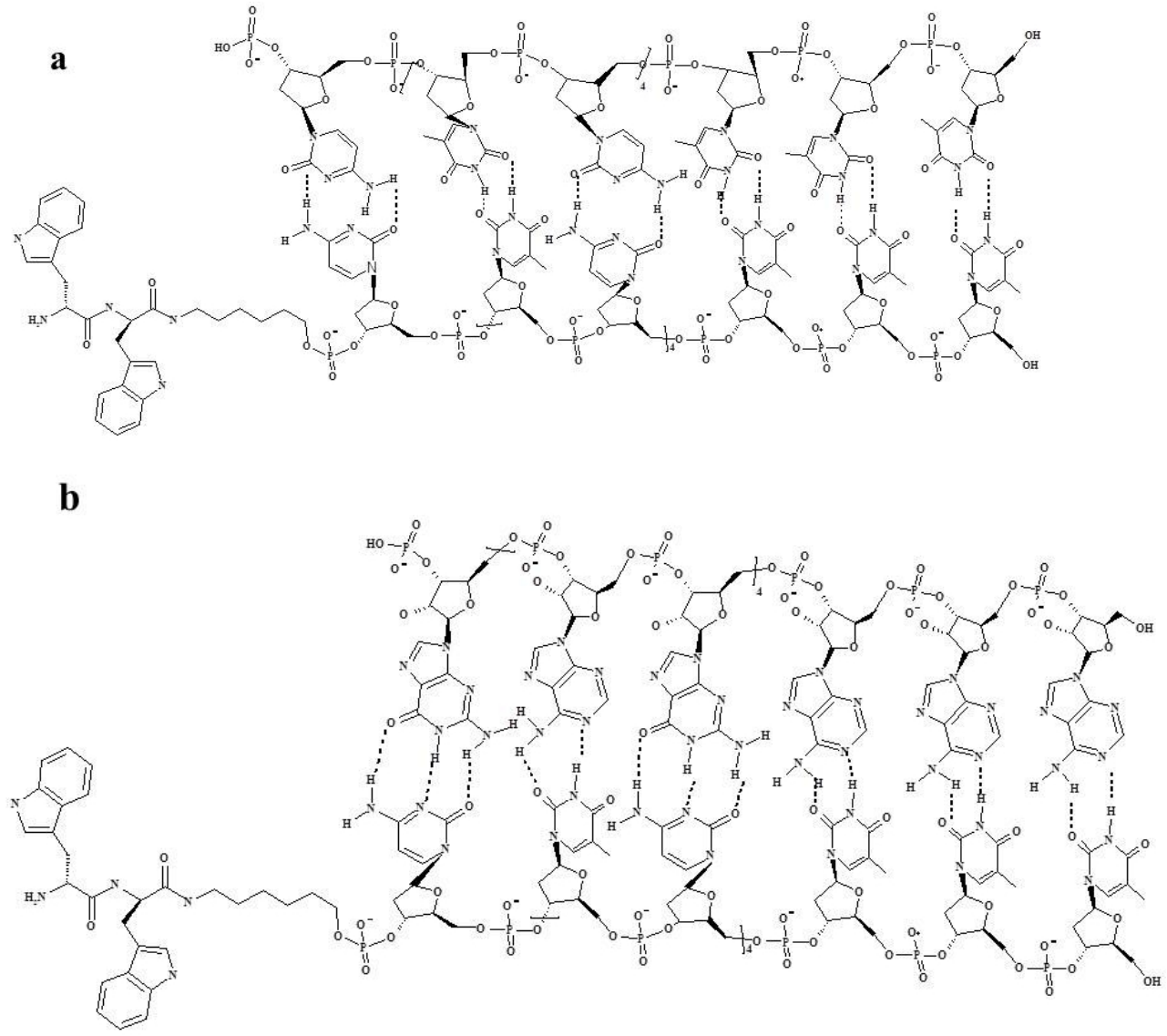


Figure S8: Non- specific and specific hydrogen bonding engaged between either (a) non-complementary and (b) complementary nucleic acid strands

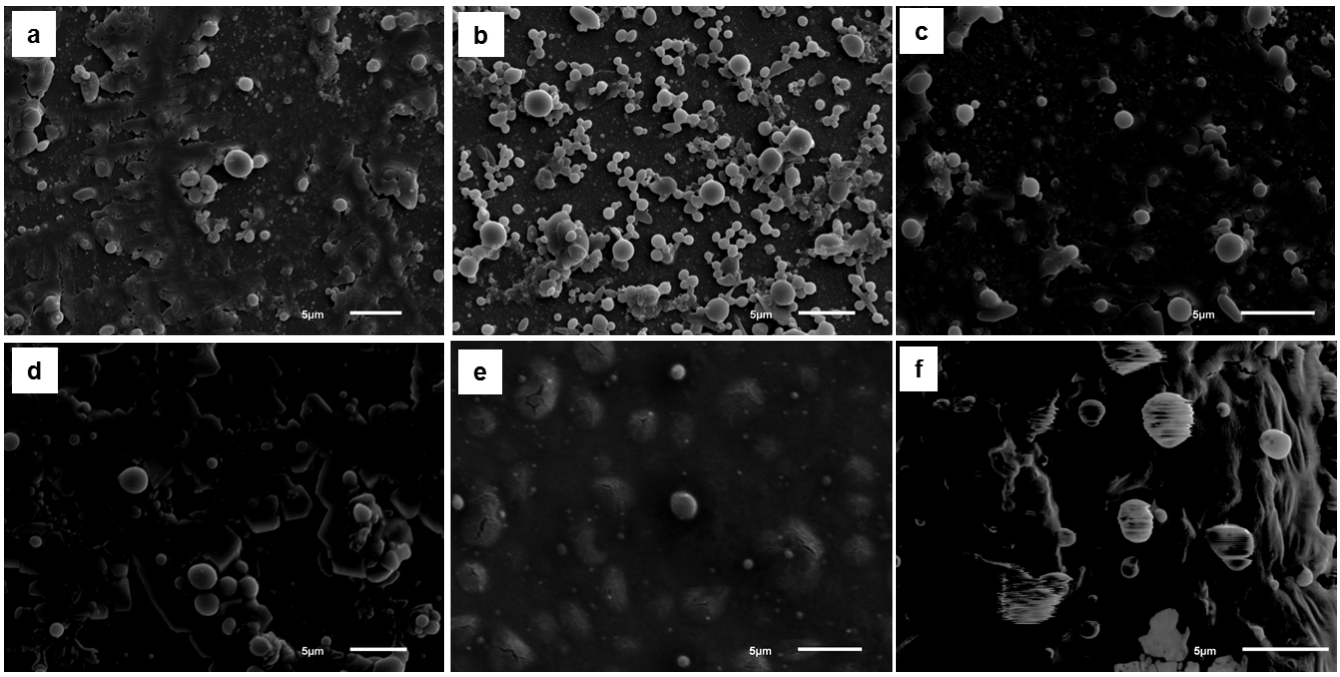


Figure S9. (a) WW-DNA in 100 mM NaCl; (b) WW-DNA in 100 mM NaCl and 1% complementary DNA; (c) WW-DNA in 100 mM NaCl and 10% complementary DNA; (d) WW-DNA in 100 mM NaCl and 50% complementary DNA; (e) WW-DNA in 100 mM NaCl and 100% complementary DNA; (f) WW-DNA in 100 mM NaCl and 200% complementary DNA.

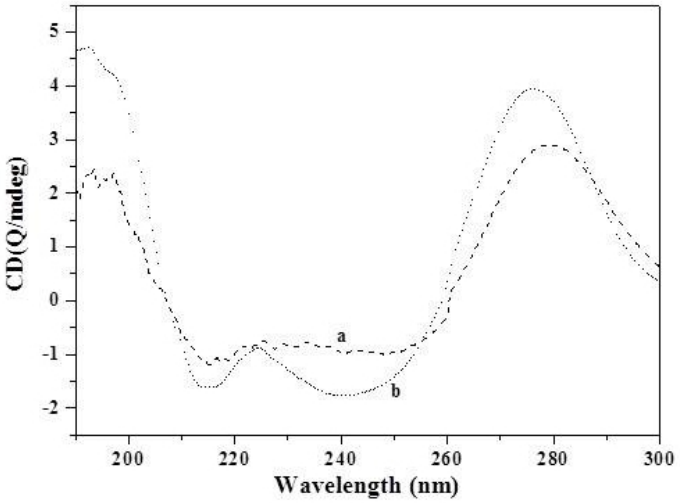


Figure S10: Circular dichroism of a) the single stranded nucleotide sequences and b) assembled WW-DNA hybrid

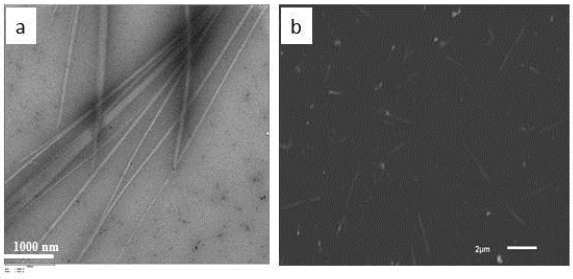


Figure S11: Straight fibrils assembled by WW-PEG

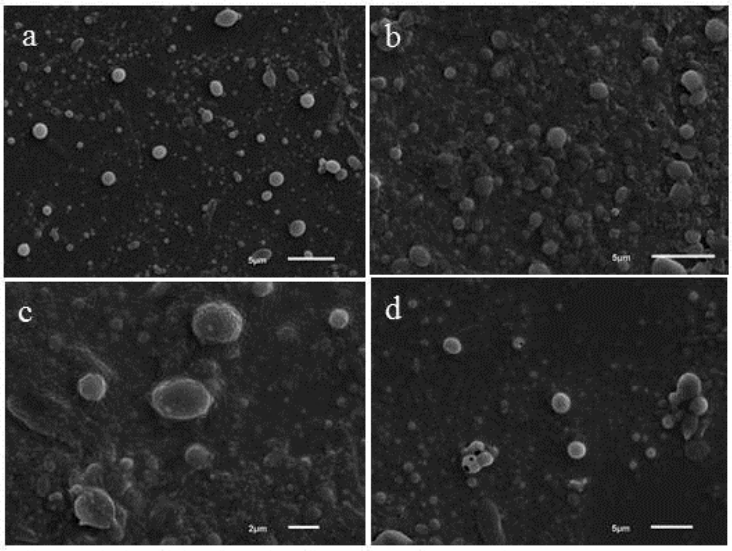


Figure S12: pH effect: SEM imaging of the a) pristine WW-DNA spherical structures (0.5 mM) at pH 7.5. b) the pH is decreased to 5.5 and c) 3.5) and d) 1.5: before rupture, the surface properties are affected[3]

References:

[1] N. Gour, C. S. Purohit, S. Verma, R. Puri, S. Ganesh, *Biochem. Biophys. Res. Commun*. 2009*,* **378***,* 503-506.

[2] N. Gour, D. Kedracki, I. Safir, K. X. Ngo, and C. Vebert-Nardin, *Chem.Commun.* 2012, **48**, 5440–5442.

[3] K. B. Joshi and S. Verma, *Angew. Chem. Int. Ed*. 2008, **47**, 2860-2863