Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics

SUPPLEMENTARY INFORMATION

Comparing the Interactions of DNA, Polyamide (PNA) and Polycarbamate Nucleic Acid (PCNA) Oligomers with Graphene Oxide (GO)

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Content

General

PCNA and PNA sequences were synthesized manually using Solid Phase Peptide Synthesis and the DNA sequences were made using a Bioautomation Mermade4 DNA synthesizer. All the oligomers were purified by RP-HPLC on a C18 column and characterized by MALDI-TOF spectrometry. MALDI-TOF spectra were obtained on a Voyager-De-STR (Applied Biosystems). CHCA (α-Cyano-4-hydroxycinnamic acid) matrix was used to analyze PCNA/PNA samples and THAP (2,4,6-trihydroxy acetophenone)/ammonium citrate were used to analyze the DNA samples. UV absorbance and melting studies were performed on a Varian Cary 300 UV-VIS spectrophotometer. Circular Dichroism (CD) analysis was performed on a JASCO J-715 spectrophotometer using a cell of 10mm pathlength. Spectra were recorded as an accumulation of 5 scans using a scan speed of 200nm/min, resolution of 1.0 nm, band-width 1.0 nm and a response of 1 sec. Spectra were smoothened and plotted using OriginPro 6.1. X-ray powder diffraction studies were performed using CuKα (λ = 1.54 Å) radiation on a Philips PW1830 instrument operating at 40 kV and a current of 30 mA at room temperature. Diffraction patterns were collected at a step of 0.020 (2θ) after background subtraction with the help of a linear interpolation method. The samples were prepared as thin films on glass substrates. All Raman spectroscopy measurements were carried out at room temperature on an HR 800 Raman spectrophotometer (Jobin Yvon Horiba, France) using monochromatic radiation emitted by a He–Ne laser (632.8 nm) operating at 20 mW. The experiment was repeated three times to verify the consistency of the spectra. The samples were prepared as thin films on glass substrates. Fluorescence experiments were performed on Varian's Cary Eclipse Fluorescence Spectrophotometer. Fluorescence emission intensity was recorded from 480 to 700nm, with a maxima observed at 520nm, using the excitation wavelength of 470nm. CD and fluorescence experiments were performed in 10mM sodium phosphate buffer containing 10mM NaCl at pH=7.2. The fluorescence of the complexes was recorded at ~6 ^{o}C.

Synthesis and characterization of graphene oxide

Graphene oxide (GO) was prepared from graphite powder according to modified Hummers' methods.[1,2]

Accordingly, graphite powder (1g) was put into sulfuric acid (25mL). $KMnO_4$ (3g) was added at 0°C gingerly under stirring. After addition of $KMnO_4$, the ice-bath was removed and the suspension was stirred at 35°C for 30min. Deionized water (46 mL) was added slowly, and the temperature of the suspension was increased to 98°C and kept for 15min, then the suspension was further diluted to 140mL with warm deionized water. Upon treating with 30% H_2O_2 (1mL), the colour of the suspension changed from black to yellow. The yellow suspension was separated by centrifugation and washed repeatedly with HCl: water (1: 10). The final product was dried over phosphorus pentoxide *in vacuo* for two days.

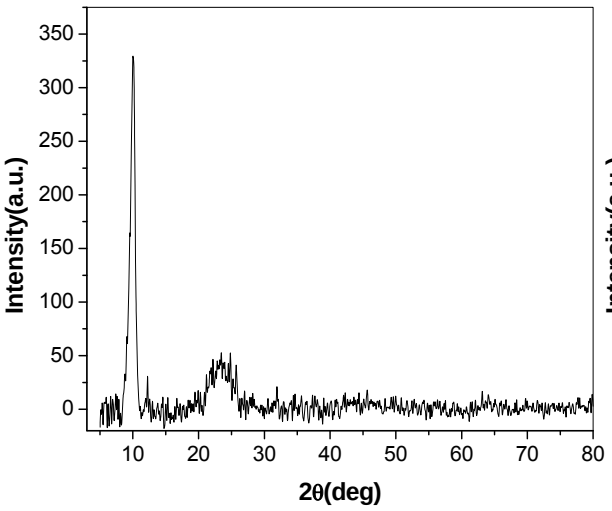


Fig. 1 XRD spectrum of graphene oxide.

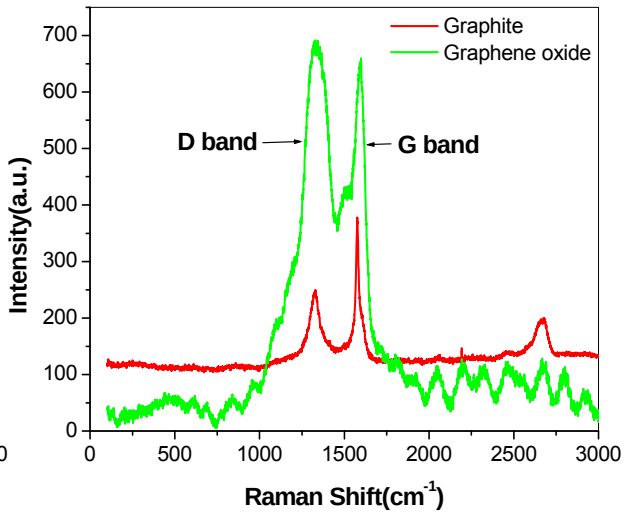


Fig. 2 Superimposed Raman spectra of graphite (red) and graphene oxide(green).

The material was characterized by XRD and Raman spectroscopy. As shown in **Fig.1**, material contains a major peak at 11.3°, characteristic peak of GO along with minor peak at 24°, characteristic peak of partially oxidized/unoxidized graphite.

Raman studies of the material (**Fig. 2**) showed a significant drop in the G-band intensity of oxidized material, ensuing from the breaking of the graphitic structure during oxidation. A good increase in the I_D to I_G ratio from 0.67 to 1.04 was observed.

The UV absorbance scan of the material shows a maxima at 225nm (**Fig.3**), which is a characteristic of GO.

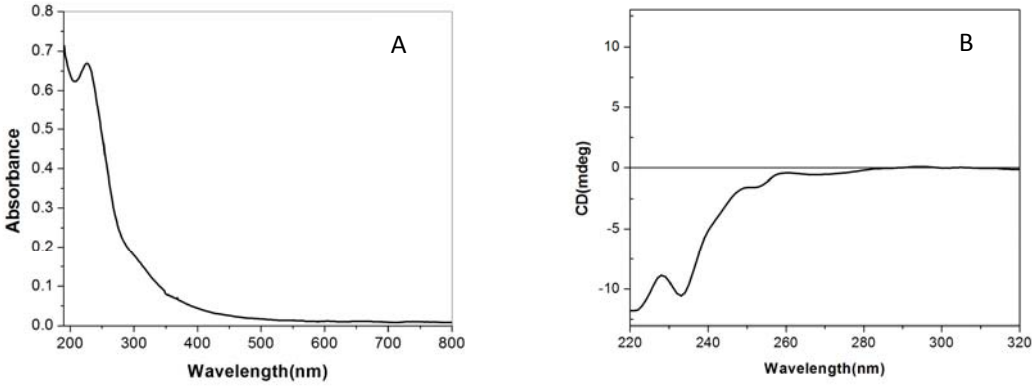
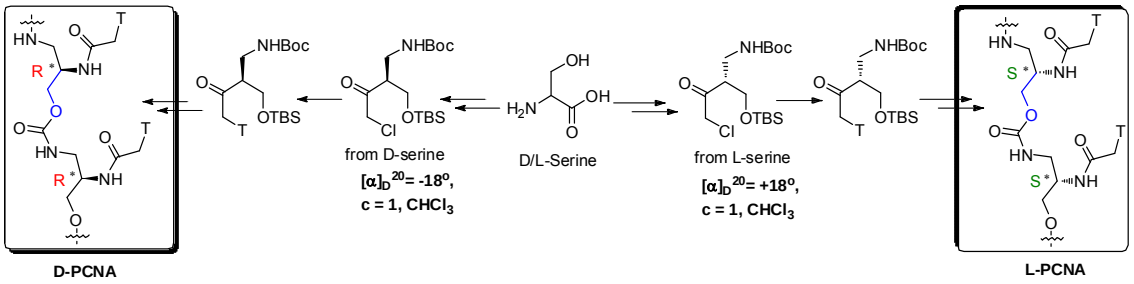


Fig.3 UV absorbance scan of GO (A) and CD spectrum of GO (B)

Synthesis of PCNA, PNA and DNA sequences

The L-PCNA T-monomer was synthesized according to our earlier published procedures,[3] starting from L-serine. For the D-PCNA T-monomer, the same series of reactions was followed, starting from D-serine (**Scheme 1**). These L/D-PCNA T-monomers were used in solid phase synthesis on MBHA resin using L-lysine as a linker, to get PCNA **III/IV** oligomers.



Scheme 1 Synthetic route for L- and D-PCNA monomers and oligomers.

The PNA **II** sequence was synthesized using the Boc-protection strategy on Merrifield resin, using β-alanine as the linker.

All these sequences were labelled with carboxyfluorescein at the N-terminus on the solid support prior to cleavage. They were cleaved from the solid support using TFA-TFMSA, purified by RP-HPLC and characterized by MALDI-TOF spectrometry.

As a control sequence, DNA **I** was synthesized on a Bioautomation Mermade-4 DNA synthesizer. Carboxyfluorescein was introduced as the label at the 5'-end by using the commercially available phosphoramidite (Glen Research). The complementary DNA sequence and the non-complementary sequence (un-labelled) were similarly synthesized. All the sequences were RP-HPLC purified and MALDI-TOF characterized.

UV melting and gel retardation assay of PCNA III& IV with DNA V

The complexation of PCNA **III**& **IV** with complementary DNA **V** was studied by UV melting. The oligomers and DNA **V** (1μM each) were mixed together in 10mM sodium phosphate buffer (pH=7.2) containing 10mM NaCl. The samples were annealed by heating at 90°C for 2min, followed by slow cooling to room temperature and refrigerated at 4°C overnight prior to the experiment. The T_m values obtained are an average of three independent measurements and are accurate to ±1°C. The UV absorption at 260nm was monitored as a function of temperature, when sigmoid transitions were observed. The T_m values were obtained from the peak of the first derivative plots (**Fig. 4**).

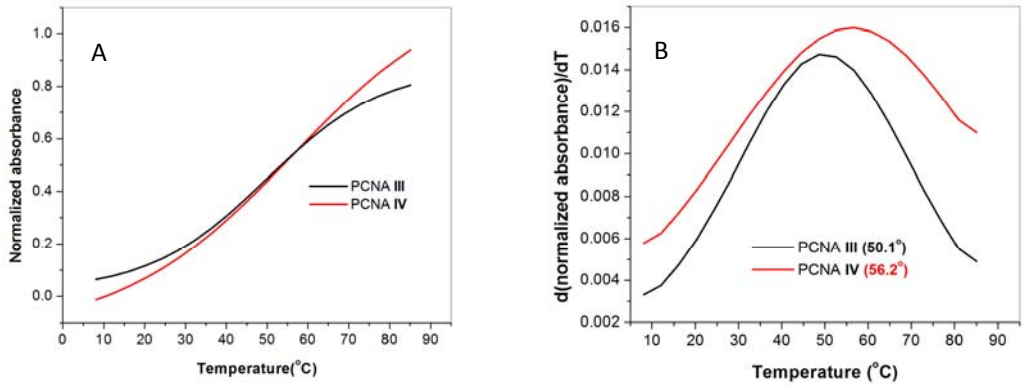


Fig. 4 UV melting profile (A) and First derivative plots (B) of PCNA **III** & **IV** with DNA **V**.

Complexation of PCNA **III** & **IV** with complementary DNA **V** was also studied by gel retardation assay. The labelled oligomers and DNA **V** (0.35mM each) were mixed together in 10mM sodium phosphate buffer (pH=7.2) containing 10mM NaCl. The samples were annealed by heating at 90°C for 2min, followed by slow cooling to room temperature and refrigerated at 4°C overnight. Prior to loading on the gel, the samples were mixed with an equal volume of 40% sucrose in TBE buffer (pH 8.0). Bromophenol blue (BPB) was used as the tracer dye separately in an adjacent well. Gel electrophoresis was performed on a 15% non-denaturing polyacrylamide gel (acrylamide:bis-acrylamide, 29:1) at constant power supply of 200V and 10 mA, until the BPB migrated to three-fourth of the gel length. During electrophoresis the

temperature was maintained at ~7ºC. The complexes were visualized by UV-shadowing. The gel was subsequently stained with ethidium bromide and observed by UV-transillumination. The complexes of PCNA with DNA **V** were found to be retarded to a much greater extent in comparison to the corresponding DNA-**I-CF**:DNA **V** complex (**Fig. 5**).[4]

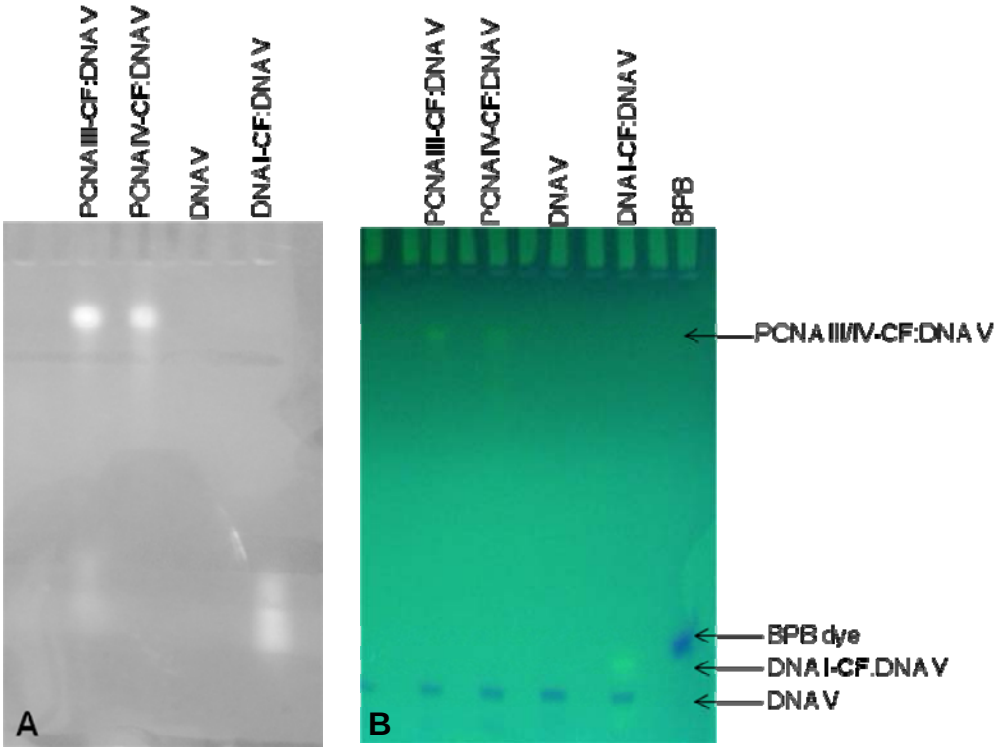


Fig.5 15% non-denaturating PAGE analysis of complexes of PCNA **III-CF** and **IV-CF** and DNA **I-CF** with complementary DNA **V**. A. after staining with ethidium bromide B. Visualization by UV-shadowing

Fluorescence spectra of PCNA IV-CF with GO and the effect of non-complementary DNA VI

The addition of even a large excess (3μM) non-complementary DNA **VI** was found to be unable to restore the fluorescence of **IV-CF** (**Fig. 6**.).

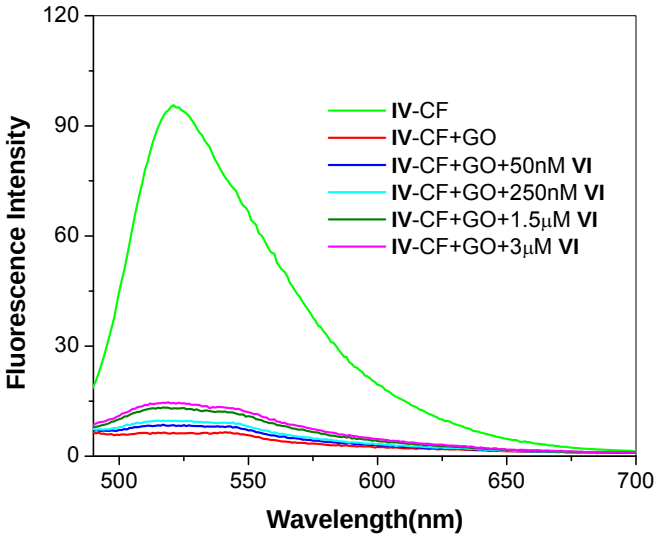


Fig. 6 Fluorescence spectra showing the effect of non-complementary DNA **VI** on the complex of **IV-CF** with GO.

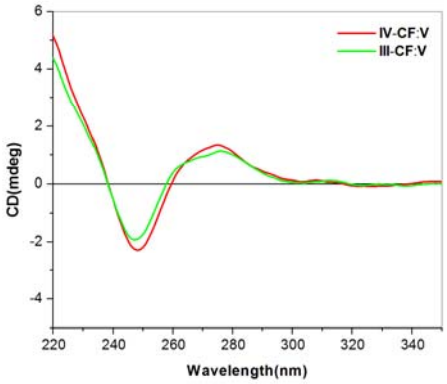
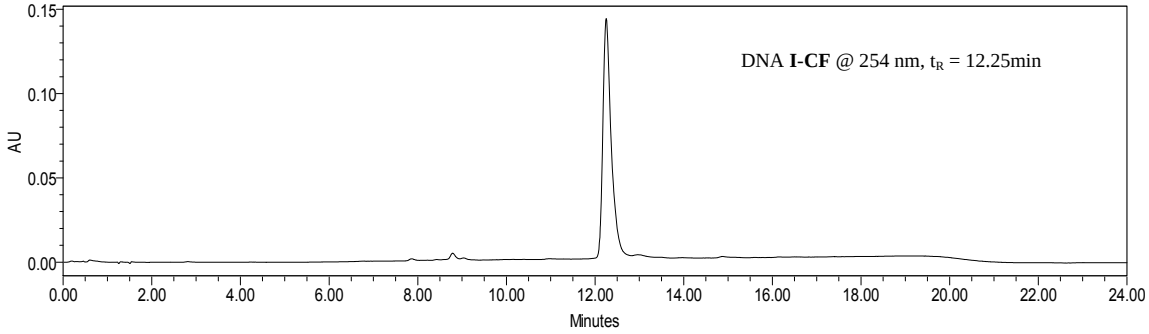
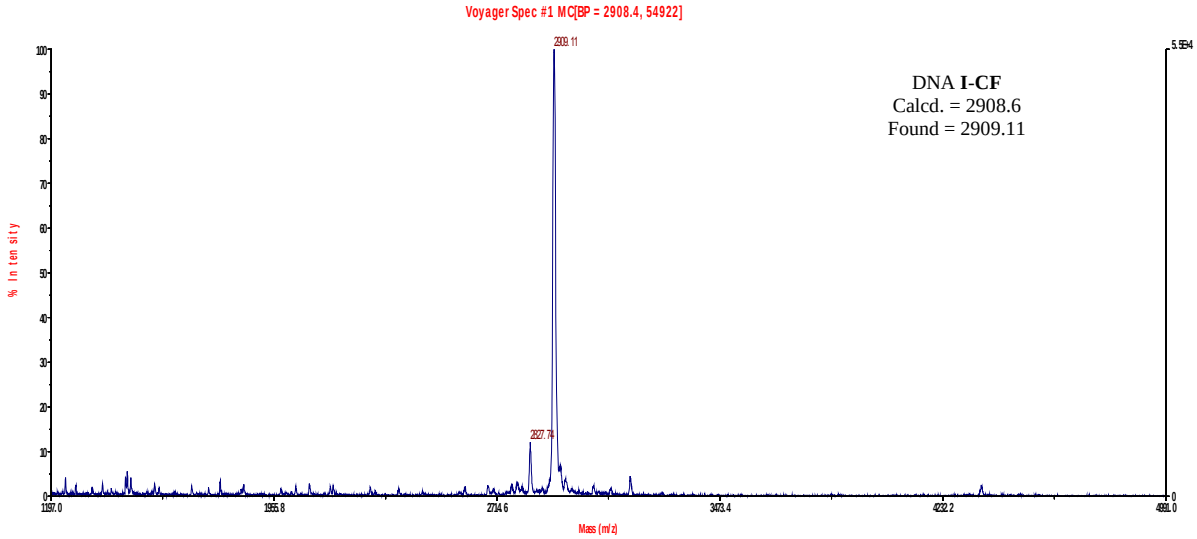


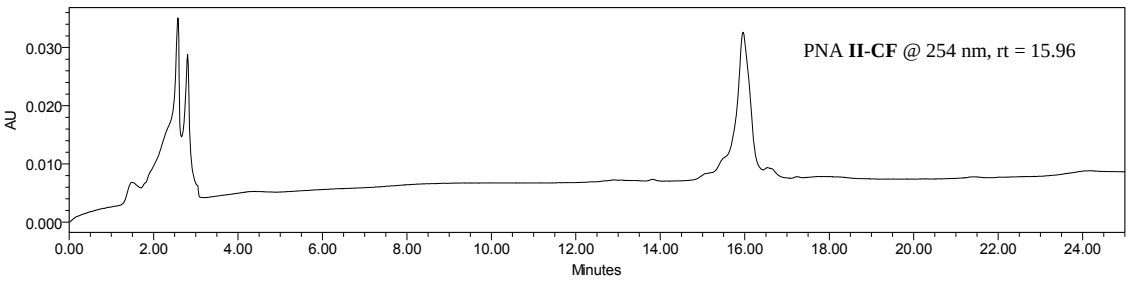
Fig. 7 CD spectra of PCNA-T8 (**III**-**CF** and **IV**-**CF**) complexes with complementary DNA **V**.

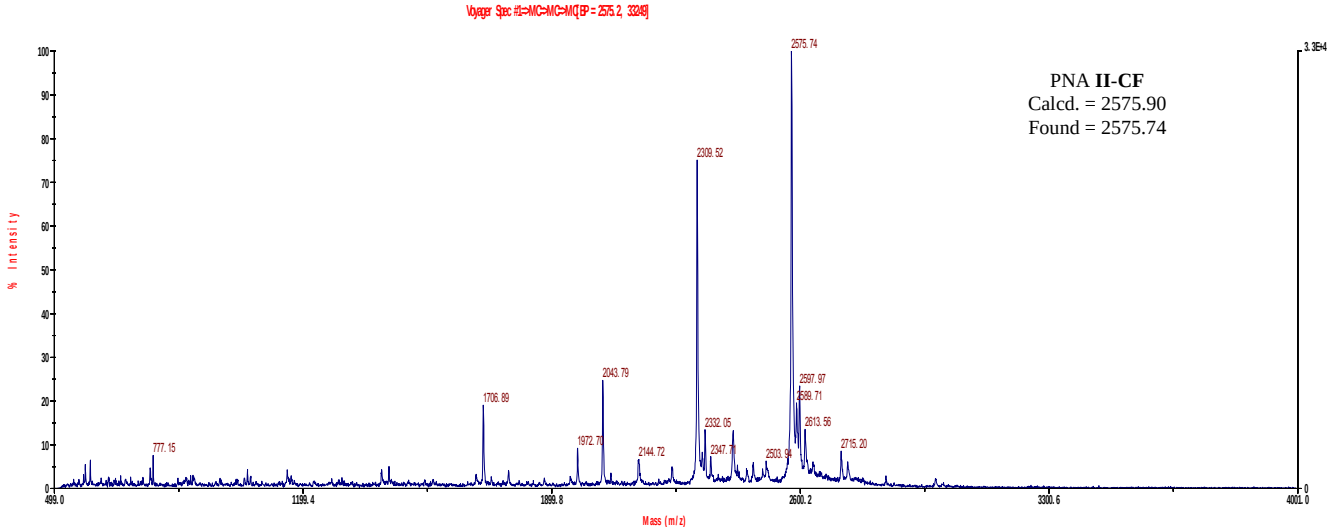
HPLC and MALDI-TOF spectra of DNA I-CF



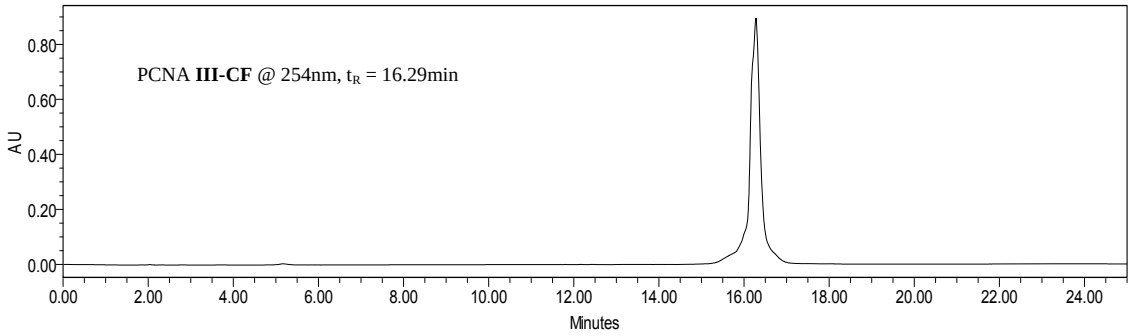


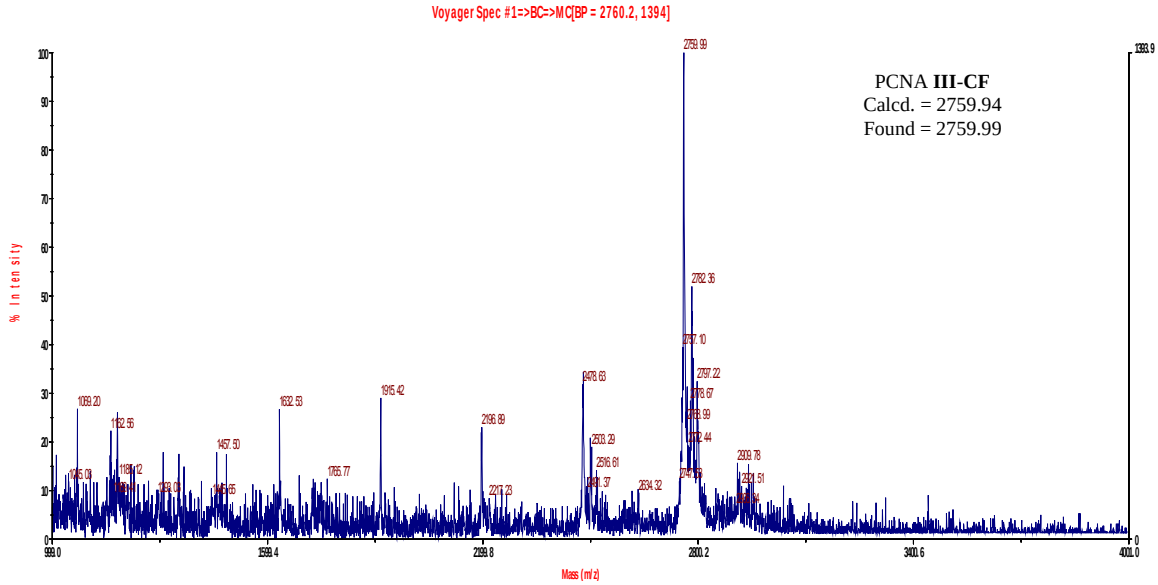
HPLC and MALDI-TOF spectra of PNA II-CF



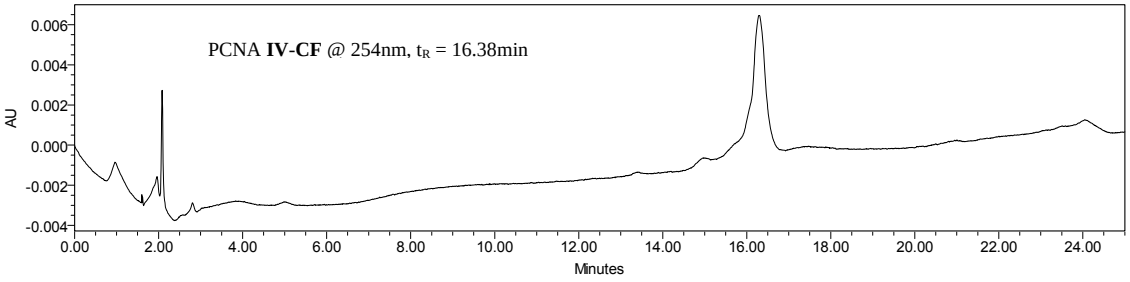


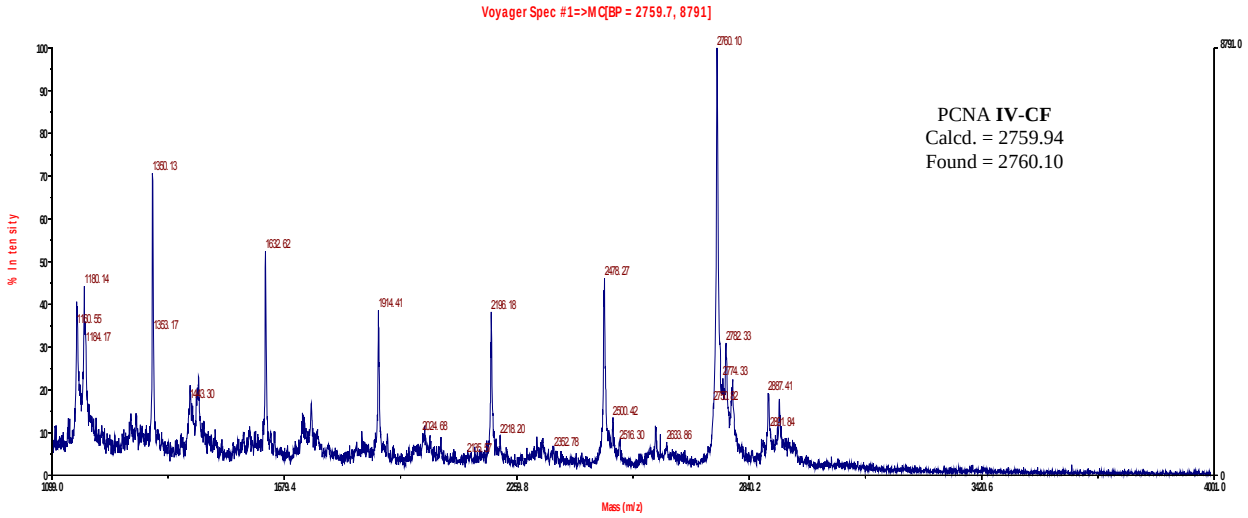
HPLC and MALDI-TOF spectra of PCNA III-CF



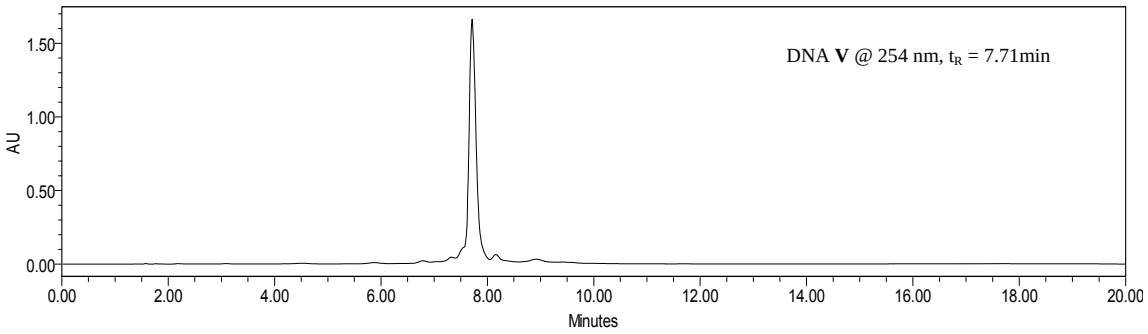


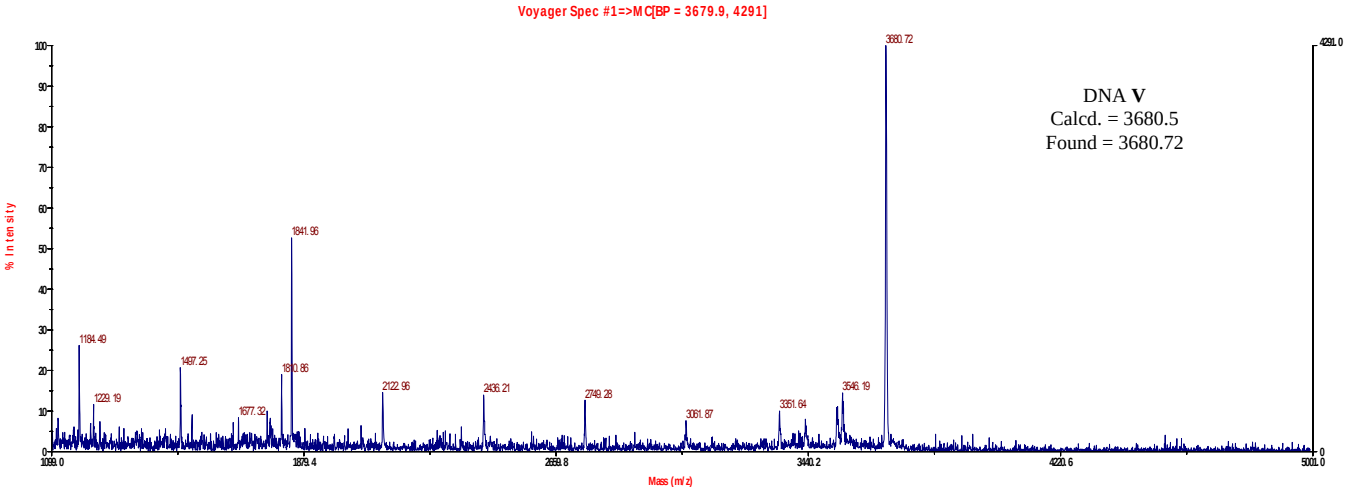
HPLC and MALDI-TOF spectra of PCNA IV-CF



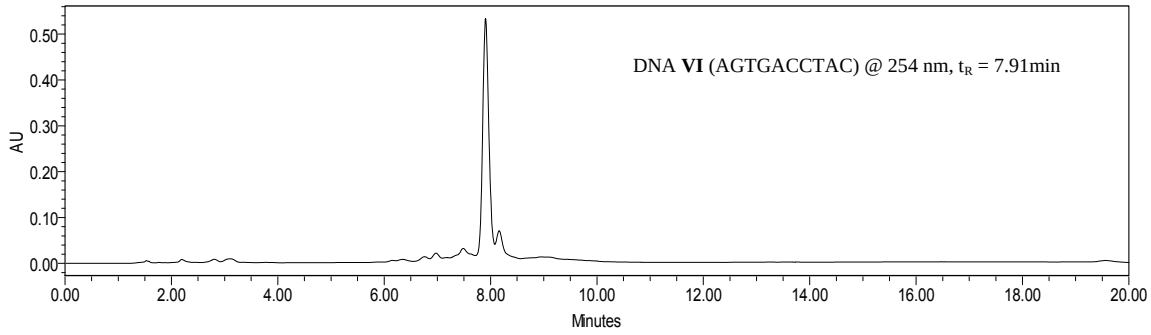


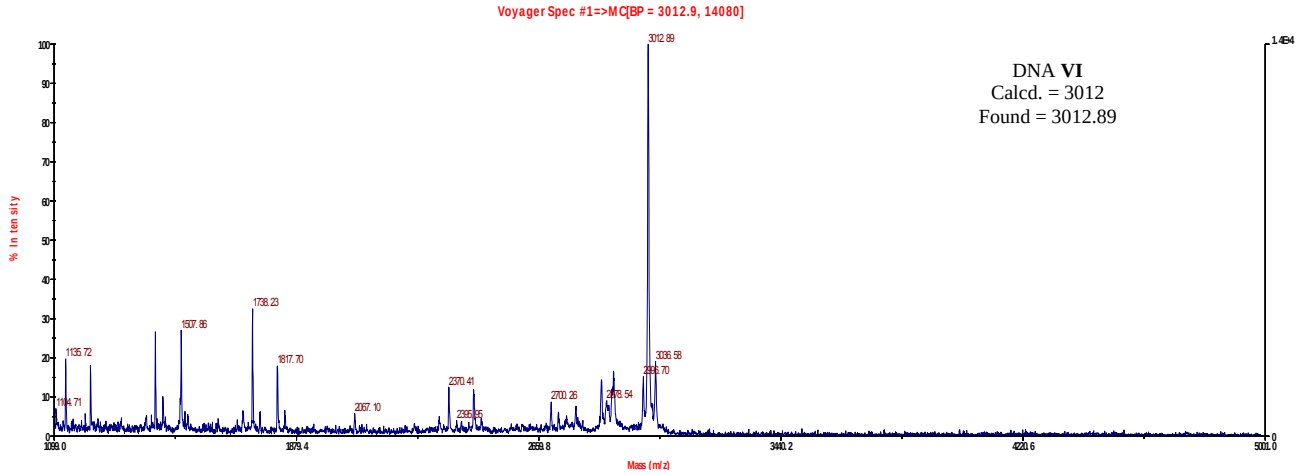
HPLC and MALDI-TOF spectra of DNA V



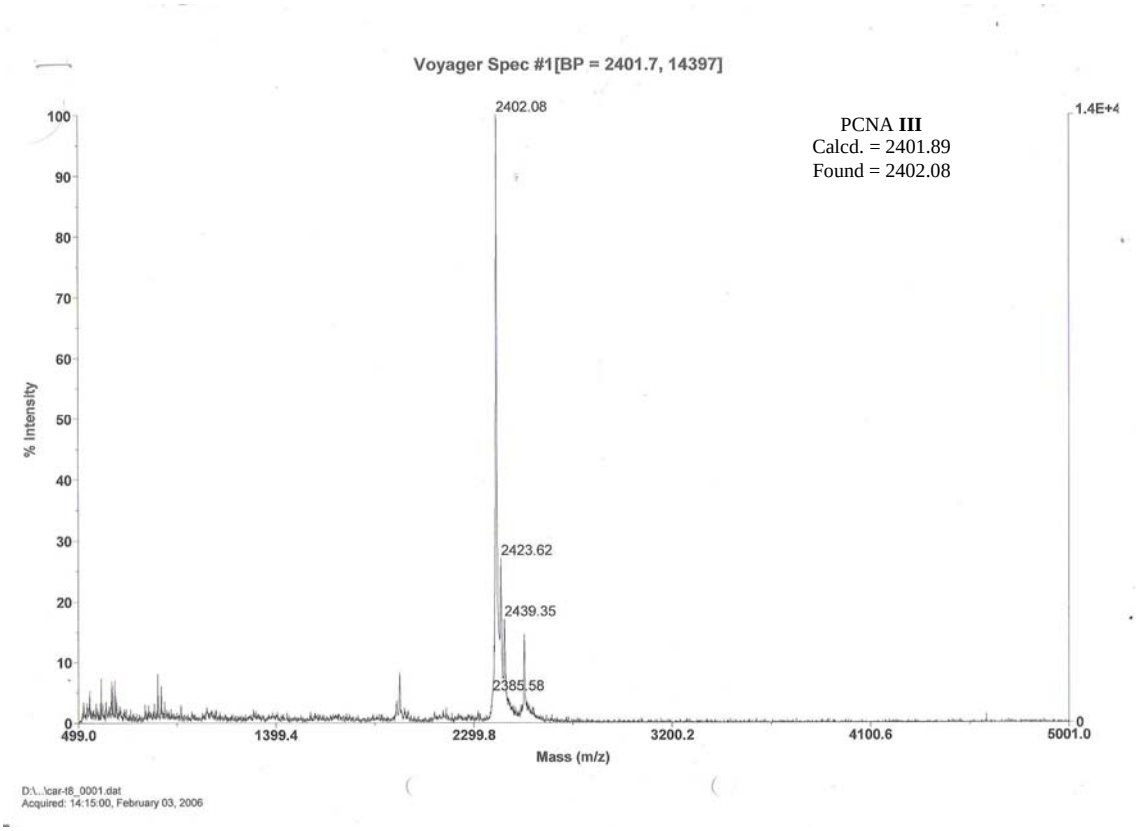


HPLC and MALDI-TOF spectra of DNA VI

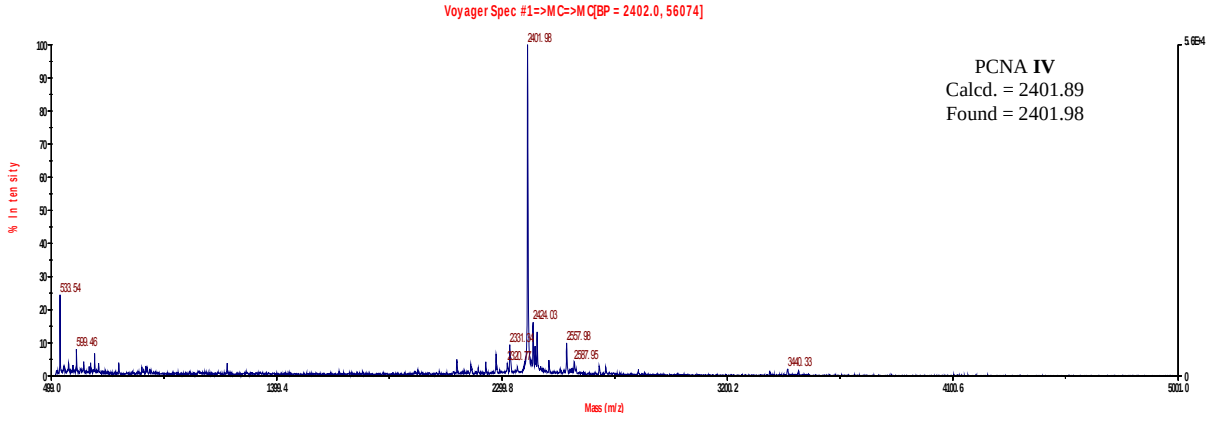




MALDI-TOF spectra of PCNA III & IV



PCNA **III**
Calcd. = 2401.89
Found = 2402.08



PCNA **IV**
Calcd. = 2401.89
Found = 2401.98

References

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