

1 **Impact of polystyrene microplastics on human alternative DNA structures**

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9

10 **Abstract**

11 The widespread contamination of the environment by microplastics (MPs), particularly
12 polystyrene (PS) and polyethylene (PE), poses significant ecological and health concerns.
13 While much attention has been given to the physical impact of MPs, their molecular
14 interactions, particularly with genetic material, remain largely unexplored. This study
15 examines how PS and PE MPs influence the structure and stability of human alternative i-
16 motif (iM) DNA, which plays a key role in regulating gene expression and maintaining
17 genomic integrity. Using biophysical techniques such as UV-Vis spectroscopy, circular
18 dichroism (CD), FT-IR spectroscopy, and thermal melting analysis, our results have
19 demonstrated the effects of PS-MPs and PE-MPs of different sizes on HRAS2 iM DNA. Our
20 results reveal that PS-MPs, particularly those with a 100 nm diameter, strongly interact with
21 HRAS2 iM DNA, causing hypochromism and shifts in the UV-Vis spectra, along with
22 changes in secondary structure observed by CD. FT-IR spectroscopy confirmed alterations in
23 DNA conformation, and thermal melting data showed increased stability of the HRAS2 iM

DNA in the presence of PS-MPs. In contrast, PE-MPs exhibited weaker interactions, likely due to differences in size and surface properties. These findings suggest that PS-MPs, due to their small size, may disrupt the structural integrity of iM DNA, potentially affecting gene regulation. This work highlights the need to consider both the physical and biochemical properties of MPs in assessing their environmental and health risks.

Keywords Microplastic pollutants . alternative DNA structures . biophysical approach . risk assessment . environmental protection

Introduction

Plastic pollution has become a critical global issue of the 21st century, with microplastics (MPs) becoming a ubiquitous contaminant in ecosystems worldwide, from oceans to remote environments. These small particles pose significant threats to both wildlife and human health, as they are increasingly detected in human tissues such as the liver, lungs, and kidneys. Recent studies have revealed the presence of MPs in the human body, acquired through ingestion, inhalation, or skin exposure, raises concerns about their potential toxicological effects [1]. MPs, particularly Polystyrene (PS) and Polyethylene (PE), are widely distributed pollutants in both terrestrial and aquatic environments. PS, commonly found in packaging, disposable products, and insulation materials, accumulates in ecosystems due to its resistance to degradation, with significant concentrations in marine waters and sediments. Similarly, PE, the most produced plastic globally, contributes heavily to MP pollution, especially in urban and coastal regions. Both materials are highly persistent, often detected in remote areas like the Arctic and deep oceans, where they pose risks to organisms through bioaccumulation. These pervasive pollutants, resistant to environmental breakdown, raise concerns over their long-term ecological impact and potential toxicological effects on wildlife and humans [2]. Approximately 3% of the genome is composed of simple DNA

repetitions that are capable of generating alternative (non-B) DNA structures. I-motif (iM) DNA structures are the alternative, dynamic, non-canonical cytosine-rich tetraplex structures, where the base pairing of cytosines occurs through Hoogsteen hydrogen bonding at acidic pH [3]. When found in promoter regions of genes, iMs can function as molecular switches that regulate gene expression [4]. These structures have significant biological roles in cellular processes, including genomic stability, gene transcription, translation, replication, as well as centromere and telomere functions [5].

Recent studies have employed a range of multispectroscopic techniques to examine the interactions between non-canonical DNA structures like iMs and various molecules [6-9]. We have previously published research detailing how microplastics (MPs) influence the structural configurations of human G-quadruplex DNA, another alternative DNA structure [10]. However, to date, there has been no exploration into the interactions between iM DNA structures and MPs, which is critical for understanding the potential negative effects of MPs on human health. Any DNA double strand capable of forming a G-quadruplex in one strand, has the potential to generate iMs on its complementary strand. Therefore, in this study, we aim to investigate the interactions between PS-MPs and PE-MPs with different iM DNAs. Due to their smaller size (100 nm), PS-MPs have the ability to cross the blood-brain barrier. Environmental research regarding the effects of MPs on iM DNAs remains limited, as the molecular mechanisms behind the dynamic interactions between PE/PS-MPs and iM DNAs are still poorly understood. If MPs cause structural changes in human iM DNA, these alterations could affect the functionality of iM DNA. Understanding the molecular processes behind the interactions between PE/PS-MPs and human genetic material is essential for evaluating potential health risks.

This research aims to explore the growing concern of MPs in human health, focusing on their interactions with iM DNAs and the potential biological consequences. By

understanding these interactions, we hope to shed light on the mechanisms through which MPs may contribute to genetic and cellular damage in human tissues and also causes environmental damage. By examining these interactions, this work will provide novel insights into the potential long-term health risks posed by MP exposure. The findings of this study are significant, as this could lead to the development of better risk assessment models for MP exposure and inform strategies to mitigate its potential impact on human health. By employing various biophysical methods such as UV-Vis, CD, FT-IR, and thermal melting, the study can examine alterations at several stages—from molecular binding to changes in DNA structure and stability. This diverse approach ensures an in-depth understanding of the interactions between microplastics and iM DNAs at molecular level, which is essential for assessing the potential health risks posed by MP exposure.

Experimental

Materials, oligonucleotides and stock solution preparation

Sigma Aldrich supplied various iM-forming DNA sequences (HRAS1, HRAS2, VEGF, CMYC, H-Telo), duplex DNA, and polystyrene (PS-MPs) and polyethylene (PE-MPs) microplastics, with sizes ranging from 34–50 μm and 100 nm, respectively. The HRAS1, HRAS2, VEGF, CMYC, and H-Telo sequences are present in the promoter regions of their respective genes, playing key roles in gene regulation and expression. PS-MPs and PE-MPs were thoroughly mixed with Milli-Q water using ultrasonic waves for thirty minutes to achieve final concentrations of 1 mg/ml for both PS-MPs and PE-MPs. Prior to use, the stock solution was sonicated for an additional thirty minutes in a bath with ultrasonic waves. Desalted DNA sequences were dissolved in double-distilled water and stored at 4°C. To form iM DNA and duplex structures, a specific amount of DNA from the main stock was added to the buffer solution, and the mixtures were heated at 95°C for 5 minutes. The solutions were

then allowed to cool gradually to room temperature (25°C) before being kept overnight at 4°C. All experiments were conducted using a buffer solution composed of 1 mM K₂EDTA, 10 mM KH₂PO₄, and 50 mM KCl at pH 5.4 and 7.4, maintained at a temperature of 25°C.

UV-Vis absorption measurements

UV-Visible absorption spectra of iM DNA and duplex DNA, with and without the presence of PS-MPs and PE-MPs, were measured using a Hitachi UH5300 spectrophotometer. The samples were placed in a quartz cuvette with a 1 cm path length. The concentration of PS-MPs and PE-MPs varied from 0 µg/ml to 100 µg/ml, while the DNA concentration remained constant at 2 µM.

Circular Dichroism (CD) spectral measurements

The circular dichroism (CD) spectra of iM and duplex DNA were recorded using a Jasco J-1500 CD Spectrometer, set at a scan speed of 100 nm/min, with and without PS-MPs. The buffer solution was used as a reference for each scan. A 1 mm path-length cuvette was employed, with the HRAS2 iM and duplex DNA concentrations maintained at 20 µM. The concentration of PS-MPs varied from 20 to 100 µg/ml. Each sample was scanned three times to obtain an average CD spectrum. The CD curves were analysed using Origin-2019b software.

Thermal (UV) melting assay

Thermal denaturation and UV-melting studies were conducted using a Hitachi UH5300 spectrophotometer, a Julabo F12 water stirrer, and a 3J1-0104 water pump cell holder with a 1 cm path-length quartz cuvette. Samples were heated at a rate of 1°C/min between 30°C and 98°C. Absorbance values of 10 µM HRAS2 iM and duplex DNA, both

with and without 50 µg/ml PS-MPs, were continuously monitored at 295 nm and 260 nm. The thermal melting curves were normalized and analysed using the curve fitting tool in Origin-2019b software.

Fourier Transform Infrared spectroscopy (FT-IR)

The structural characteristics of the samples were examined using Fourier Transform Infrared (FT-IR) spectroscopy. Specific vibrational peaks in the IR spectra provided distinct identifiers for various iM and duplex DNA conformations. FT-IR spectra of HRAS2 iM and duplex DNA in the presence and absence of PS-MPs were recorded with a Spotlight 400 FT-IR spectrometer (Perkin-Elmer, UK) at room temperature (25°C). The samples were quickly dried on a zinc-selenium plate with an infrared light lamp, and spectra were obtained from over 120 scans at a resolution of 2 cm⁻¹. The spectrum of a blank ATR plate was used as a reference. Spectral profiles in the 800-2500 cm⁻¹ frequency range were analysed using the Baseline Correction method in the Opus 7.5 Bruker Optics application. Measurements were performed on liquid samples.

Statistical analysis

All experiments were conducted in triplicate, and the results were analysed for statistical significance at a confidence level of p<0.05. Graphs and curves were generated and analysed using the OriginPro tool in Origin-2019b software.

Results and discussion

UV-Vis absorption measurements

One powerful approach to exploring the molecular interactions of various iM DNAs is through UV-Vis absorption studies, which provide valuable insights into the binding process [6,11]. In our study, we analyzed the UV-Vis absorption spectra of different iM DNA types (HRAS1, HRAS2, VEGF, CMYC, H-Telo iM DNA, and duplex DNA) both in the presence and absence of PS-MPs and PE-MPs to understand the preferential binding patterns. Both PS-MPs and PE-MPs showed no absorption or emission in the DNA's characteristic wavelength range (220-320 nm), meaning no spectral changes could be attributed to binding interactions between PS/PE-MPs and iM/duplex DNA (**Fig. S1**).

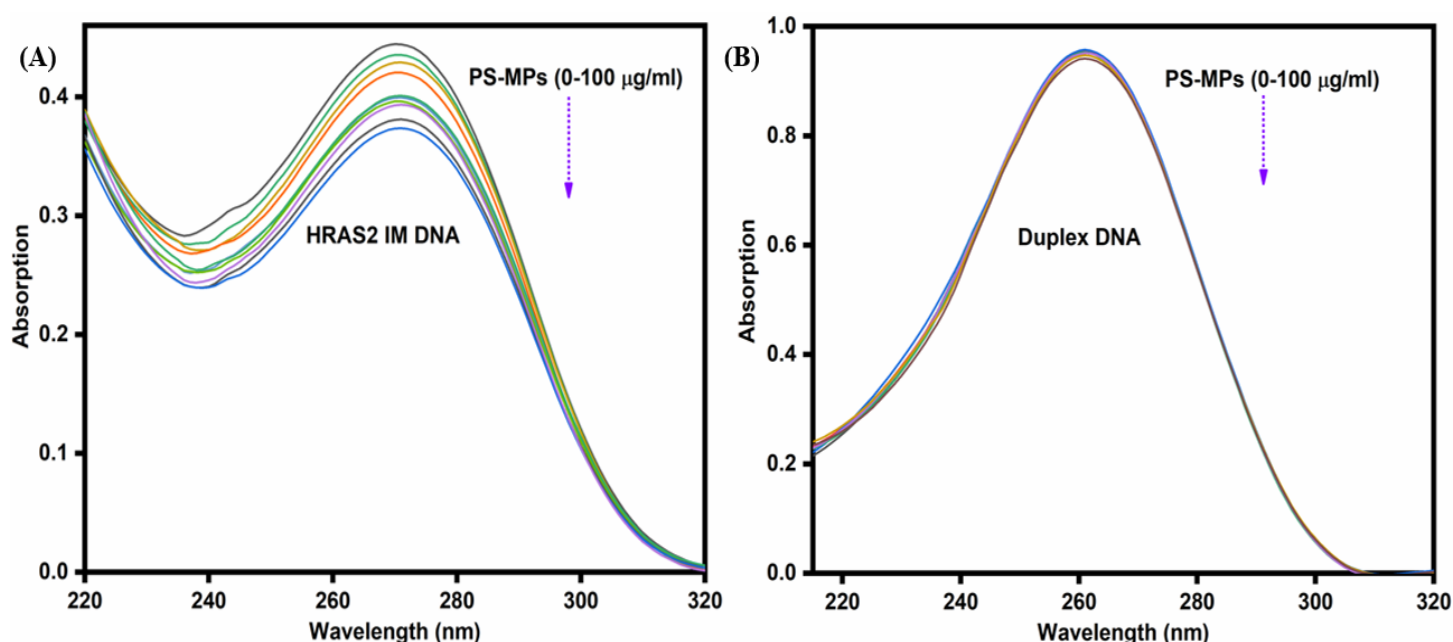


Fig. 1 UV-Vis absorption spectra of [A] HRAS2 iM, [B] Duplex (2 µM) in the absence and presence of successive additions of PS-MPs (0-100 µg/ml) (iM buffer, pH-5.4; Duplex buffer, pH-7.4; temp. 25⁰ C).

When increasing amounts of PS-MPs (ranging from 0 to 100 µg/ml) were added, HRAS2 iM DNA exhibited a notable reduction in absorbance (15.90% hypochromism) along with a significant 5 nm red shift in the absorbance maximum (**Fig. 1A**). This drop in absorption, accompanied by the red shift, suggests a polarity transfer within the PS-MPs, likely due to their selective interaction with HRAS2 iM DNA structures. This degree of hypochromism and red shifting was much more pronounced compared to other PS-MPs and

153 PE-MPs-iM DNA complexes (**Fig. 1B, Fig. S2, Fig. S3, and Table 1**). Further analysis of
 154 UV absorption changes, both increases and decreases, revealed that PS-MPs exhibit strong
 155 binding to HRAS2 iM DNA. Based on these findings, subsequent investigations were
 156 designed to explore this interaction in more detail.

Microplastics (MPs)		Molecular formula and Nature	
Polystyrene Microplastics (PS-MPs)		Chemical formula for polystyrene (PS) is $(C_8H_8)_n$, obtained from styrene. n represents the degree of polymerization.	
Polyethylene Microplastics (PE-MPs)		Chemical formula for polyethylene is $(C_2H_4)_n$, obtained from ethylene (C_2H_4). n represents the degree of polymerization.	
Oligonucleotides (i-Motifs)		Length (bases)	Sequence (5'-3')
1	HRAS2 iM	23	GCCCCCGCCCCCGCCCCGCCCCG
2	HRAS1 iM	23	CGTGCCCTGCGCCCGCAACCCGA
3	VEGF iM	24	GACCCCGCCCCCGGCCCGCCCCGG
4	CMYC iM	28	TCCCCACCTTCCCCACCCTCCCCACCCT
5	H-Telo iM	22	CCCTAACCCCTAACCCCTAACCCCT
6	Duplex	26	CAATCGGATCGAATTCGATCCGATTG
		UV absorption studies	
(MPs-iM DNA interaction)		Hypochromicity (%)	Red shift (nm)
1	PS-MPs+HRAS2	15.90	5
2	PS-MPs+HRAS1	No change	No shifting
3	PS-MPs+VEGF	2.07	1
4	PS-MPs+CMYC	4.93	2

5	PS-MPs+H-Telo	1.79	No shifting
6	PS-MPs+Duplex	No change	No shifting
7	PE-MPs+HRAS2	3.02	No shifting
8	PE-MPs+HRAS1	No change	No shifting
9	PE-MPs+VEGF	2.3	1
10	PE-MPs+CMYC	2.1	No shifting
11	PE-MPs+H-Telo	1.3	No shifting
12	PE-MPs+Duplex	1.2	No shifting

Table 1. Preliminary screening data between polystyrene & polyethylene microplastics and iM forming (HRAS1, HRAS2, VEGF, CMYC & H-Telo iM) and duplex DNA sequences used in this investigation. Spectral parameters (hypochromicity and red shifting) obtained from UV-Vis absorption studies.

160 Circular Dichroism (CD) Spectral Measurements

161 Circular dichroism (CD) spectroscopy is a powerful method for detecting changes in
162 the conformation of iM DNA and identifying its essential characteristics, particularly those
163 influenced by interactions with various substances [12]. In our study, CD spectroscopy was
164 used to investigate dynamic changes and secondary structures of HRAS2 iM and duplex
165 DNA, along with any structural modifications induced by the binding of PS-MPs. It was
166 observed that PS-MPs did not produce any spectral changes in the CD profile within the iM
167 DNA's characteristic wavelength range (240-320 nm), indicating that PS-MPs did not bind to
168 HRAS2 iM DNA (**Fig. S4**).

Without PS-MPs, HRAS2 iM and duplex DNA displayed a positive band at 290 nm and 276 nm, as well as negative bands at 264 nm and 246 nm—these bands are markers for the iM and duplex DNA structures (**Fig. 2**). However, significant changes in the CD profile were observed after adding PS-MPs to HRAS2 iM DNA, particularly in the band intensities (**Fig. 2A**). For duplex DNA, no major changes were detected upon the addition of PS-MPs (**Fig. 2B**). Notably, our analysis revealed substantial alterations in the band intensities of HRAS2 iM DNA as the concentration of PS-MPs increased. The CD analysis suggests that these changes were primarily due to alterations in the secondary structure of HRAS2 iM, without noticeable shifts in the wavelength. Additionally, as the concentration of PS-MPs increased, the band intensity of HRAS2 iM also increased, indicating that higher concentrations of PS-MPs lead to more significant disruptions in its secondary structure. The changes in band intensities primarily reflect structural modifications of HRAS2 iM DNA in the presence of PS-MPs.

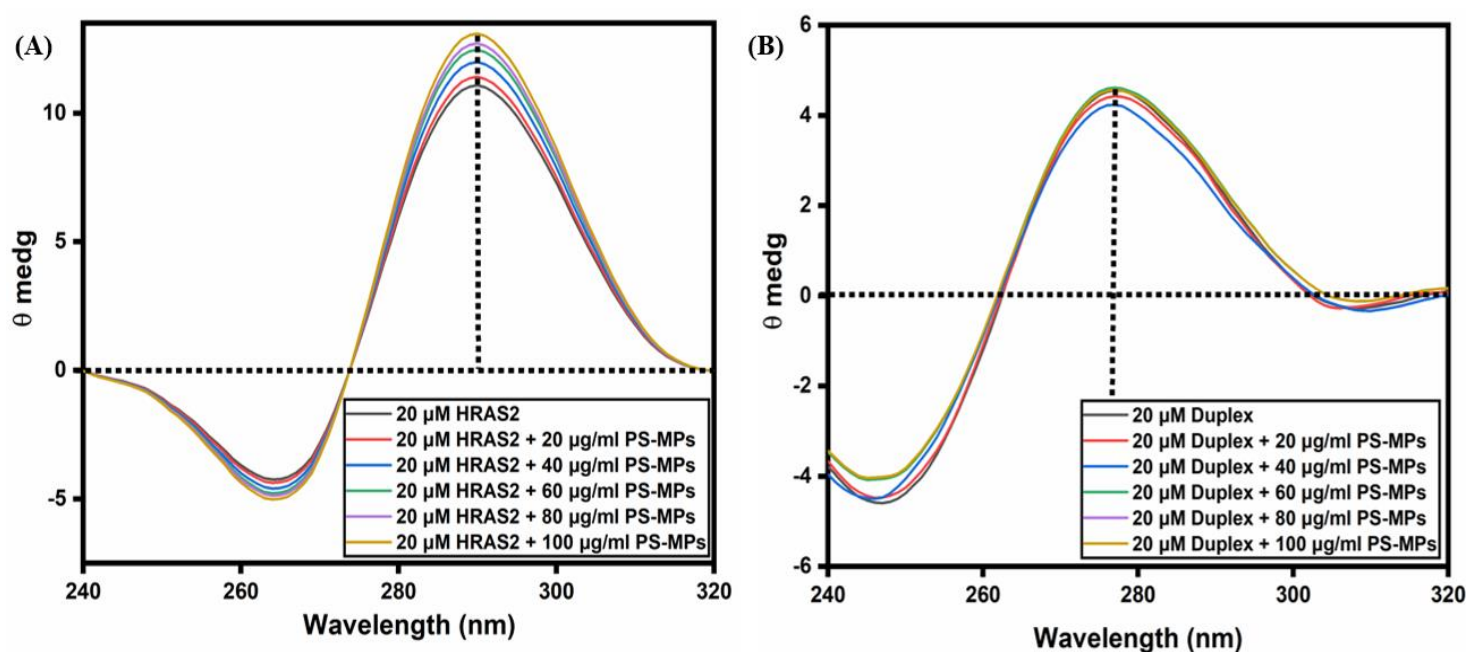


Fig. 2 Circular dichroic spectral profile of [A] HRAS2 iM, [B] Duplex (20 μ M) in the absence and presence of successive additions of PS-MPs (20, 40, 60, 80 & 100 μ g/ml) (iM buffer, pH-5.4; Duplex buffer, pH-7.4; temp. 25⁰ C).

182 Thermal (UV) melting analysis

183 Thermal melting/UV melting experiments were performed to examine how PS-MPs
 184 affect the structural stability of HRAS2 iM and duplex DNA. The melting temperature (T_m)
 185 for each detectable transition was determined by identifying the midpoint of the melting
 186 curve [13]. T_m was estimated using the absorption values of iM DNA bases at 295 nm and
 187 duplex DNA at 260 nm. No melting profile was observed for PS-MPs at 295 nm, indicating
 188 that no spectral changes could be attributed to PS-MPs binding with HRAS2 iM DNA (**Fig.**
 189 **S5**). The melting profiles and temperature variations, with and without PS-MPs, are shown in
 190 **Fig. 3A and 3B**. The addition of 50 $\mu\text{g/ml}$ PS-MPs increased the thermal stability of HRAS2
 191 iM DNA by approximately 3°C (**Fig. 3A**). In contrast, PS-MPs were found to destabilize
 192 duplex DNA, lowering the T_m by 1°C at a concentration of 50 $\mu\text{g/ml}$ (**Fig. 3B**). Thermal
 193 melting analysis further suggests that PS-MPs enhance the stability of HRAS2 iM DNA.

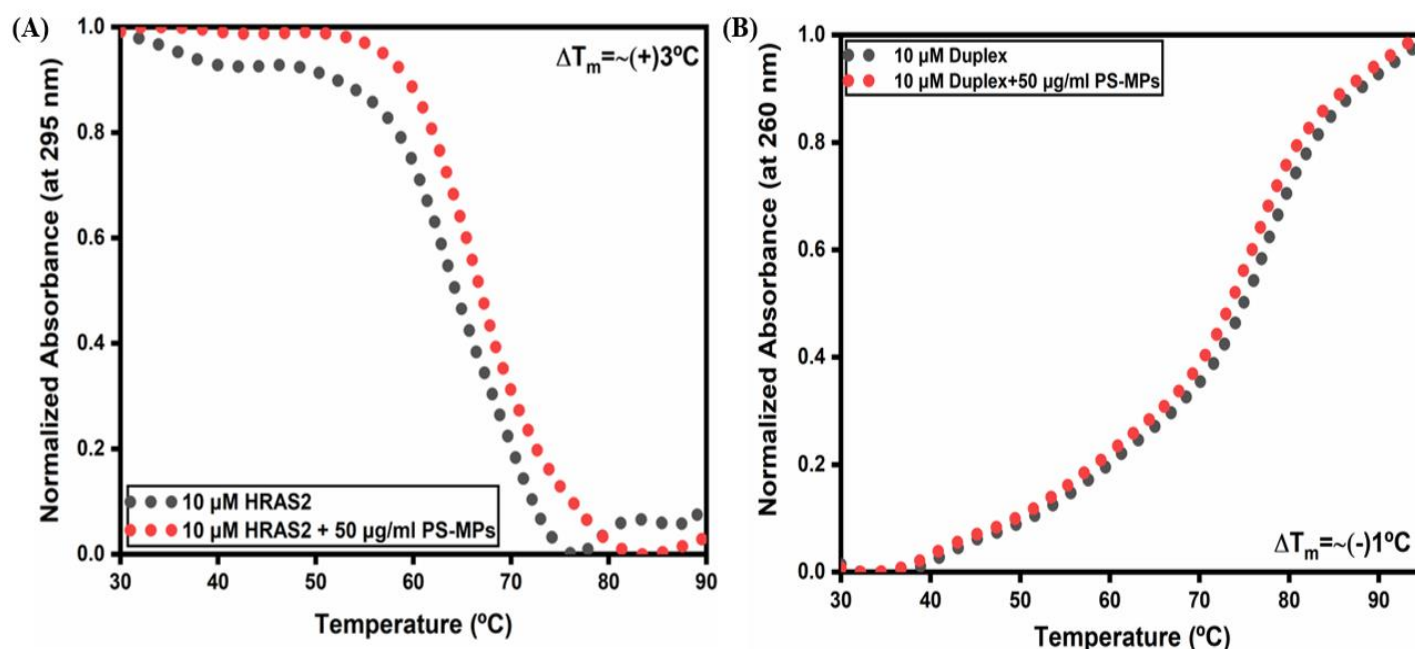


Fig. 3 Thermal melting profiles of [A] HRAS2 iM, [B] Duplex (10 μM) in the absence and presence of PS-MPs (50 $\mu\text{g/ml}$) (iM buffer, pH-5.4; Duplex buffer, pH-7.4; temp. 25°C).

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Fourier Transform Infrared spectroscopic (FT-IR) measurements

To gain a deeper understanding of the rapid structural changes and interpretations that occur upon direct exposure of cytosine-rich iM DNA sequences to illumination, the spectrum variations linked to the dynamics and mechanisms of iM structure formation were monitored using FT-IR spectroscopy [14]. A considerable fluctuation in the spectrum within the 800-2500 cm^{-1} range can be attributed to the vibrational stretches of the in-plane C=C double bonds in the ring structure and the carbonyl groups of the bases. As depicted in Figure 4, FT-IR spectra show peaks between 800 and 2500 cm^{-1} , indicating that the iM DNA structures remain stable even under the hydration conditions tested. It was observed that in the FT-IR analysis, PS-MPs did not exhibit any spectra in the iM DNA's relevant wavenumber range (800-2500 cm^{-1}), suggesting that no spectral changes could be attributed to the binding between PS-MPs and HRAS2 iM DNA. (**Fig. S6**). However, when PS-MPs were added to HRAS2 iM DNA, noticeable conformational alterations took place. The results reveal that the binding of PS-MPs leads to significant shifts in the peak regions of HRAS2 iM DNA (**Fig. 4A**) at the selected hydration level, whereas no such shifts were observed in the peak positions of duplex DNA (**Fig. 4B**). Generally, the infrared spectrum of nucleic acids falls between 2500 and 800 cm^{-1} . This range includes spectral bands linked to various structural elements, such as bases, sugar groups, and phosphate stretches (**Fig. 4**). Due to the exchange of labile protons, water exhibits a strong absorbance around 1,635 cm^{-1} , which necessitates the use of D_2O to minimize spectral interference, causing only slight changes in the nucleic acid base modes. However, H_2O is used in the 1,300–1,575 cm^{-1} range, where IR frequencies associated with base-sugar bending modes, sensitive to glycoside torsion angles, are present. In D_2O , the 1,800–1,500 cm^{-1} region predominantly shows base vibrations, including C=C, C=N, and C=O stretching, as well as in-plane vibrations. The IR signals are highly responsive to changes in base stacking, base pairing, solvent interactions, and metal ion binding due to

the electron-rich nature of the p-double bonds. Structural and conformational changes can be monitored by observing simultaneous shifts in the spectrum and variations in band intensity [15,16]. The pure PS-MPs samples display prominent characteristic peaks at wavenumbers 3026, 2920, 2850 cm^{-1} (Fig. S6). The peaks at 3026 cm^{-1} correspond to the aromatic C–H stretching, while the C=C vinyl group is indicated by these peaks. The characteristic peaks at 2920 and 2850 cm^{-1} are attributed to the asymmetric and symmetric stretching of CH₂, respectively [17].

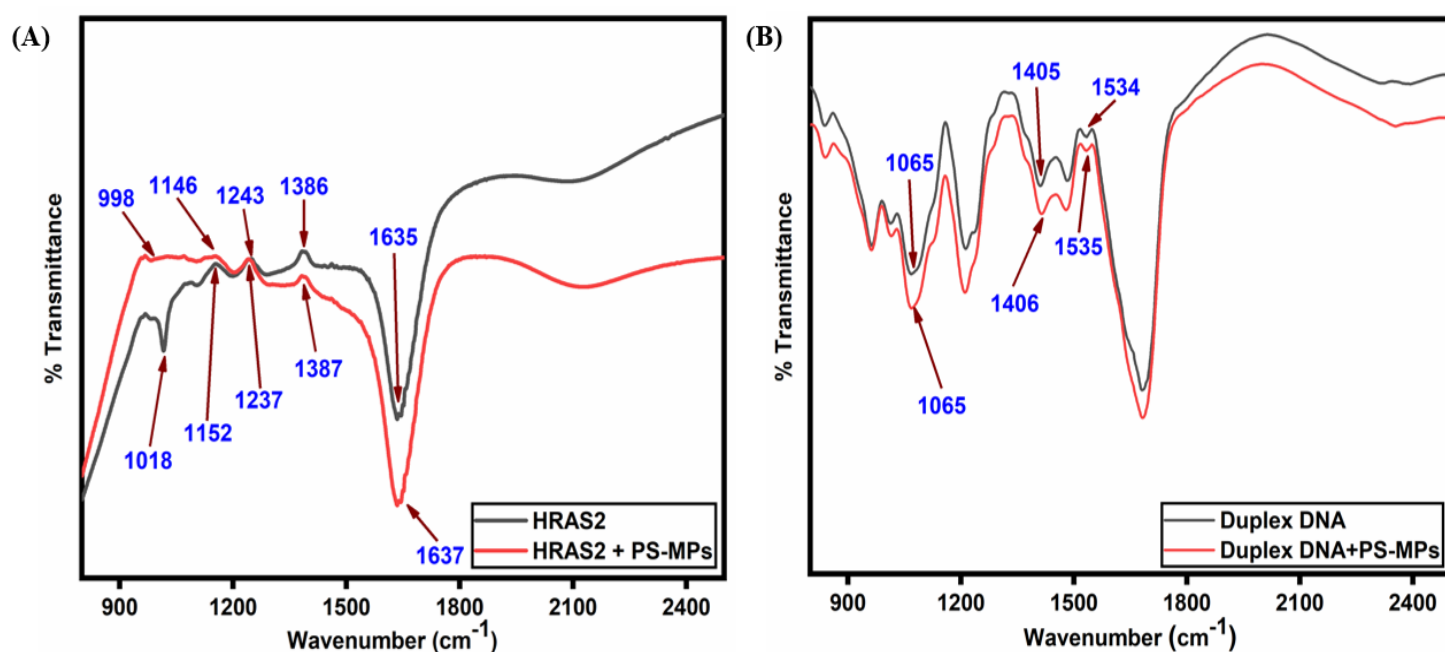


Fig. 4 Fourier Transform Infrared (FT-IR) spectra in the [800–2500] cm^{-1} spectral region of [A] HRAS2 iM, [B] Duplex alone (black line) and [A] HRAS2 iM + PS-MPs, [B] Duplex + PS-MPs (red line). The peak centre of each spectrum is indicated by the arrows (iM buffer, pH-5.4; Duplex buffer, pH-7.4; temp. 25⁰ C).

The interaction between HRAS2 iM DNA and PS-MPs is evident through several changes in the infrared spectrum of the HRAS2/PS-MPs complex, compared to the individual spectra of HRAS2 and PS-MPs. By analysing the spectral shifts in the HRAS2/PS-MPs complex, we were able to assess the effects of this interaction. These results align with our observations of the binding between HRAS2 iM DNA and PS-MPs, where a downshift in the peak occurs and

the phosphate group in the HRAS2 iM DNA backbone is disrupted during the binding. In contrast, fewer changes were observed in the infrared spectrum of the duplex DNA/PS-MPs complex. This suggests that duplex DNA experiences minimal structural alterations when interacting with PS-MPs. These findings are consistent with our circular dichroism (CD) results, which also indicate changes in secondary conformation of HRAS2 iM in the presence of PS-MPs.

Conclusion

Pollutants like microplastics (MPs) have the potential to disrupt cellular function and genomic integrity by infiltrating DNA, leading to strand breaks and genetic mutations. Despite ongoing research, identifying the precise binding locations and interaction mechanisms between the functional groups of MPs and DNA remains a significant challenge. Gaining a comprehensive understanding of these intricate interactions is essential for uncovering the molecular basis of how environmental pollutants like MPs influence DNA. To date, no studies have conducted to explore the interactions between human alternative non-canonical iM DNA and various MPs. In summary, based on our experimental results, PS-MPs demonstrated a stronger affinity and specificity for HRAS2 iM DNA, leading to noticeable alterations in the spectral profile, including hypochromism and a red shift in the UV-Vis absorption spectrum. CD measurements revealed modifications in the secondary structure intensity of HRAS2 iM DNA, while FT-IR spectroscopy highlighted changes in the conformation of the iM DNA upon binding. Furthermore, thermal melting analysis indicated that the presence of PS-MPs enhanced the thermal stability of HRAS2 iM DNA by approximately 3°C. This provides important insights into the potential toxicity of PS-MPs on HRAS2 iM DNA at an early stage. Given their small size, PS-MPs are capable of penetrating cellular membranes and potentially reaching the nucleus, where they could bind to specific iM DNA structures, posing significant risks to vital biological processes. We believe our

257 research offers valuable insights into the potential health risks posed by PS-MPs
258 contamination, thereby advancing our understanding of their impact on human health. These
259 results reveal a new aspect of microplastic pollution: their ability to interact with and
260 potentially modify regulatory DNA structures in organisms. By connecting environmental
261 contaminants to molecular-level effects on genetic material, this study enhances our
262 understanding of the chemical interactions and biological consequences of microplastics in
263 ecosystems. It emphasizes the importance of developing comprehensive environmental
264 chemistry models that consider both the physical presence and the chemical reactivity of
265 emerging pollutants.

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270 **Author Contributions**

271 Sagar Bag: conceptualization; experiment; methodology; formal analysis; software and
272 validation; writing original draft. Souvik Ghosal: formal analysis; validation. Sudipta
273 Bhowmik: conceptualization; formal analysis; funding acquisition; project administration;
274 review and editing.

275 **Data Availability** Data will be made available on request.

276 **Code availability** Code will be made available on request.

277 **Declarations**

278 **Conflicts of interest** The authors declare no conflict of interests.

279 **Consent to participate** Not applicable.

280 **Consent for publication** Not applicable.

281 **Ethics approval** Not applicable.

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Graphical Abstract

