*applied sciences*

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*Supplementary Materials*

# Polyethylene Terephthalate Micro/Nano-Plastics Induce Structural and Conformational Changes in Cedar Pollen Proteins: Spectroscopic and Molecular Dynamics Evidence

**Tochukwu Oluwatosin Maduka *, Qingyue Wang *, Christian Ebere Enyoh, Miho Suzuki, Weiqian Wang and Md. Sohel Rana**

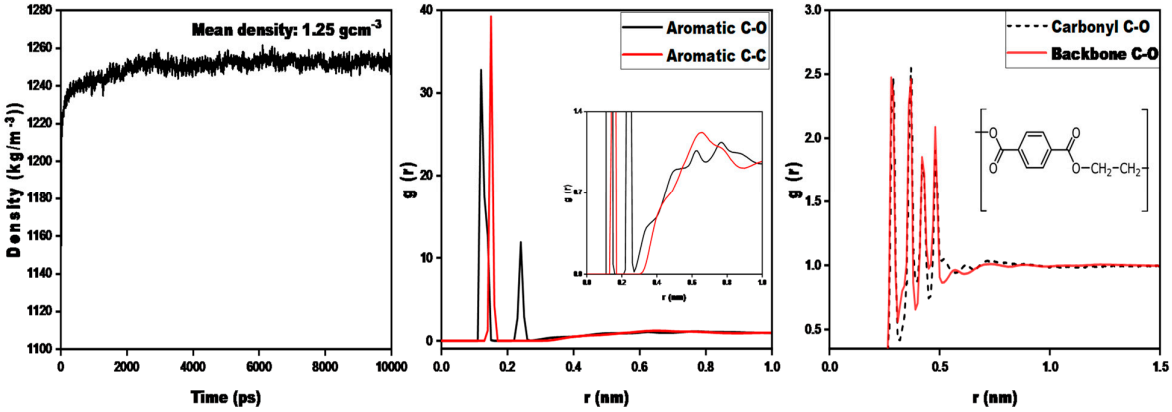



**Figure S1. Radial distribution function (RDF) and density profiles of the amorphous PET system.** **a)** Plot of density vs time and radial distribution functions (RDFs) of PET slab. **b)** Aromatic C-O and C-C (the insert reveals the peaks between 0.5 – 1 nm). **c)** Backbone and carbonyl C-O.

The PET slab density remained stable throughout production (1.2499 ± 0.0067 g·cm$^{-3}$), indicating equilibration in the model. Adsorption analyses therefore reflect interactions with this equilibrated amorphous surface. The radial distribution function (RDF), g(r), describes how atomic density varies as a function of distance from a reference atom. It provides insight into both short-range ordering (local bonding and packing) and long-range structural organization within a material. RDF analysis was performed to verify the structural integrity of the generated PET slab, confirm correct chemical bonding, and evaluate whether the polymer exhibits realistic amorphous packing prior to conducting protein–surface adsorption simulations. RDFs were computed for key PET atom pairs (aromatic C–C, aromatic C–O, backbone C–O, carbonyl C–O) (Table S1, Figure S1). The aromatic C–C RDF showed a distinct peak at 0.150 nm (g(r) = 39.2), and aromatic C–O at 0.120 nm (g(r) = 32.8), reflecting expected intramolecular bond distances. Backbone C–O and carbonyl C–O pairs exhibited broader peaks at 0.28–0.37 nm (g(r) ≈ 2.5), confirming correct ester linkages in the polymer backbone. Secondary peaks observed at 0.6–0.8 nm (g(r) = 1.1–1.2) indicate short-range inter-chain packing consistent with amorphous PET. For all atom pairs, g(r) approached 1.0 beyond ~1.0 nm, confirming the absence of long-range order. Overall, the RDF analysis verifies that the PET model exhibits chemically accurate bonding and realistic amorphous packing, supporting its use for subsequent protein–surface adsorption simulations.

**Table S1.** Summary of the major RDF peaks for each atom pair. These distances confirm that the simulated PET slab maintains realistic intramolecular and intermolecular geometries.

| Atom Pair | Peak (nm) | g(r) | Description |
|---|---|---|---|
| Aromatic C–C | 0.15 | 39.25 | Intramolecular C–C bond |
| Aromatic C–O | 0.12 | 32.78 | Intramolecular C–O bond |
| Backbone C–O | 0.28 | 2.47 | Ester linkage |
| Carbonyl C–O | 0.29 | 2.55 | Ester carbonyl |
| Aromatic C–C | 0.66 | 1.22 | Inter-chain correlation |
| Aromatic C–O | 0.77 | 1.14 | Inter-chain correlation |

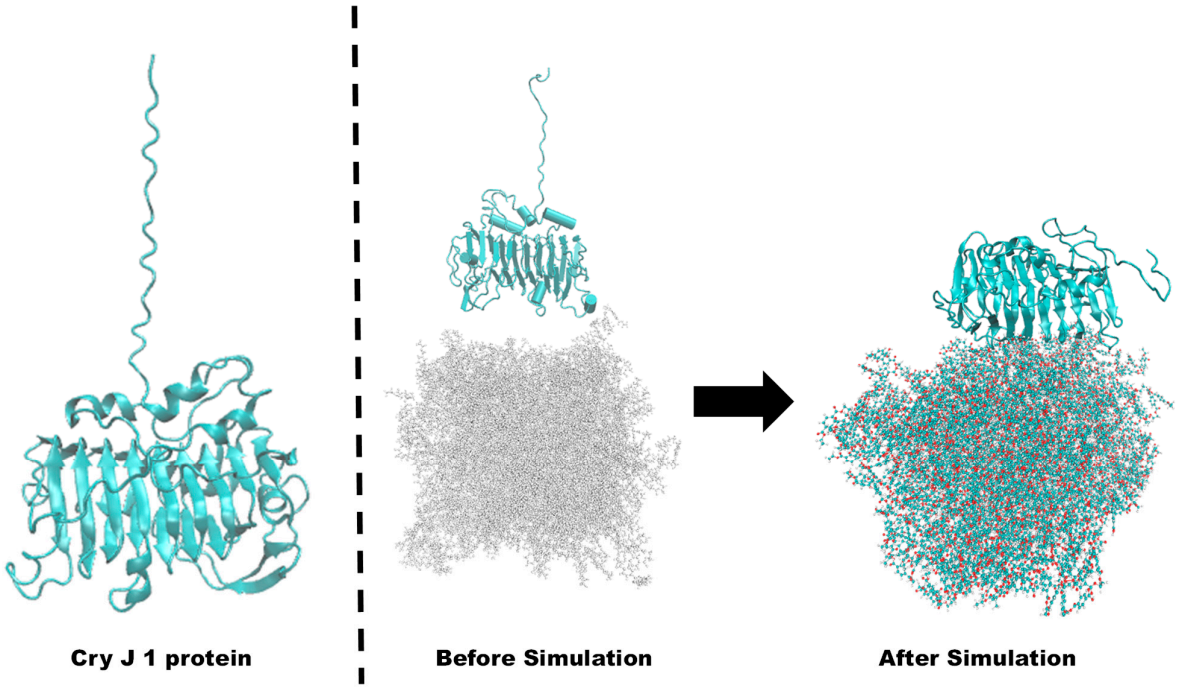



**Figure S2. Three-dimensional structure of the Cry j 1 allergen used for molecular dynamics simulations.**

The Cry j 1 protein structure used in this study is shown as a ribbon representation, highlighting its overall fold and secondary structural elements. The structure is the high-confidence AlphaFold model (pLDDT=94) used for all simulations and processed prior to simulation by standard protonation and energy minimization procedures. Major secondary structure features, including β-sheet-rich regions associated with allergenic stability, are preserved and serve as the basis for subsequent adsorption and conformational analyses.

The Cry J 1 was placed so that at least one of its major T-cell epitopes would be the one touching the PET surface when they make contact. After simulation, we checked when they made their initial contact. The goal of the initial contact was to identify when the protein first interacted with the PET surface. We observed that after 800 ps, the protein and the PET came together to interact, and they remained together till the simulation ended.

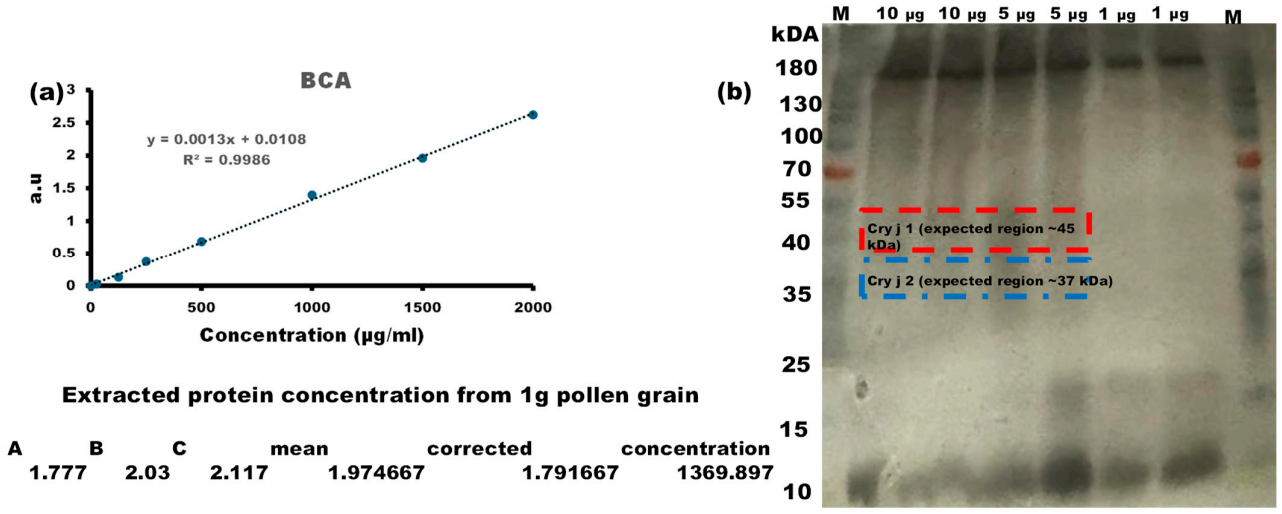



**Figure S3. SDS-PAGE analysis confirming extraction of cedar pollen proteins.** *(a) BCA calibration graph (b) Silver-stained SDS-PAGE of crude cedar pollen protein extracts. Protein samples at increasing mass were separated on a 4–20% Tris–glycine gel and visualized by silver staining. Molecular weight markers are shown on both sides. Shaded regions indicate the expected migration ranges of Cry j 1 (~45 kDa) and Cry j 2 (~37 kDa) based on comparison with the molecular weight marker. From the right, lanes correspond to increasing protein loads of the cedar pollen extract: 1 µg, 5 µg, and 10 µg, respectively. Signals in these regions appear diffused rather than as discrete bands, reflecting the heterogeneous nature of the crude pollen protein extract.*

Crude cedar pollen proteins were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) to obtain a qualitative electrophoretic profile of the extracted protein mixture. Protein samples were prepared under denaturing conditions and separated on a 4–20% Tris–glycine gradient gel, followed by visualization using silver staining. Silver-stained SDS-PAGE revealed a heterogeneous electrophoretic pattern characterized by diffuse protein signals spanning a broad molecular weight range, consistent with the complex and non-purified nature of pollen protein extracts. Comparison with the molecular weight marker indicated the presence of protein signals in the regions corresponding to the reported molecular weights of the major cedar pollen allergens Cry j 1 (~45 kDa) and Cry j 2 (~37 kDa). These signals appeared as broad, unresolved regions rather than sharply defined bands and increased in intensity with increasing protein loading. The diffuse migration behavior observed in these molecular weight regions is consistent with previous reports describing cedar pollen allergens as exhibiting heterogeneity arising from isoform diversity, post-translational modifications (e.g., glycosylation), and non-uniform SDS binding when analyzed in crude extracts (Yasueda et al., 1983). As such, SDS-PAGE in this study was used solely for qualitative assessment of the protein extract, and no definitive allergen identification was inferred from electrophoretic mobility alone. Confirmation of specific allergen identity would require immunochemical or mass spectrometric analysis, which was beyond the scope of this work.

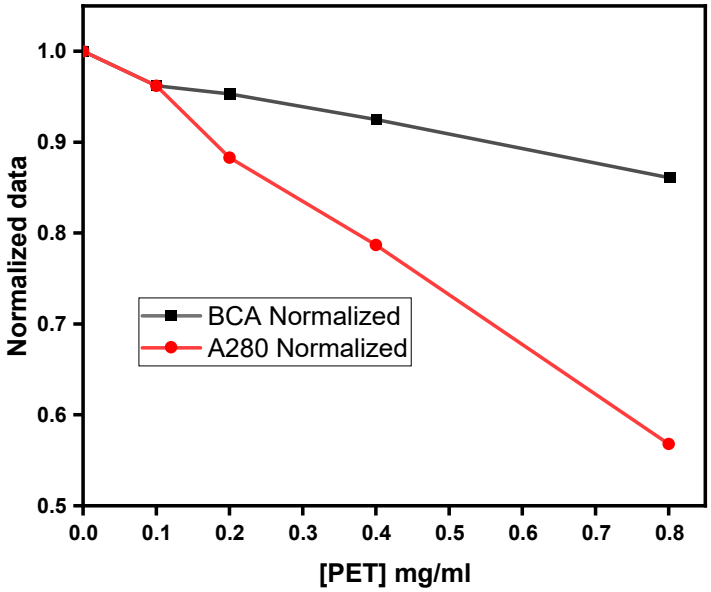



**Figure S4. Normalization of BCA and UV–visible absorbance signals for protein quantification.**

Protein concentrations of cedar pollen extracts were quantified using both the bicinchoninic acid (BCA) assay and UV–visible absorbance at 280 nm. To ensure consistency between colorimetric and spectroscopic measurements, raw BCA and UV absorbance values were normalized to their respective maximum signals. The strong linear correspondence between normalized BCA and UV signals confirms reliable protein quantification and indicates minimal interference from non-protein chromophores. This normalization procedure enabled accurate comparison of protein concentrations across experimental conditions, including adsorption and spectroscopic analyses.

Figure S4. **Normalized plot of BCA (total protein) vs A280 (aromatic proteins)**

Selectivity metric: Ratio = $\frac{A280}{[Protein]} \times 1000$

Normalized Values: Allows direct comparison despite different units/scales

BCA Normalized: $C/C_0$ (where $C_0$ = 275.92 μg/mL)

A280 Normalized: $A/A_0$ (where $A_0$ = 0.5384)

**Table S2. Calculated Binding Parameters.**

| [PET] (mg/mL) | Protein Bound (μg/mL) | % Total Protein Bound | % Aromatic Signal Bound |
|---|---|---|---|
| 0 | 0 | 0 | 0 |
| 0.1 | 10.38 | 3.76 | 3.78 |
| 0.2 | 13.07 | 4.73 | 11.74 |
| 0.4 | 20.77 | 7.52 | 21.29 |
| 0.8 | 38.46 | 13.94 | 43.16 |

Percent binding: % Bound = $\frac{C_0 - C}{C_0} \times 100$

where $C_0$ = 275.92 μg/mL (protein) or 0.5384 (A280)

**Table S3. Adsorbed Amount (Q) calculation.**

| [PET] | C (μg/mL) | $C_0$ - C | Q (μg/mg) |
|---|---|---|---|
| 0.1 | 265.54 | 10.38 | 103.8 |
| 0.2 | 262.85 | 13.07 | 65.35 |
| 0.4 | 255.15 | 20.77 | 51.93 |
| 0.8 | 237.46 | 38.46 | 48.08 |

Adsorbed amount: $Q = \frac{C_0 - C}{[PET]}$

Langmuir isotherm: $Q = \frac{Q_{max} \cdot C}{K_d + C}$

Where $C_0$ = 275.92 μg/mL

Q = amount adsorbed (μg protein/mg PET)

C = equilibrium concentration (μg/mL)

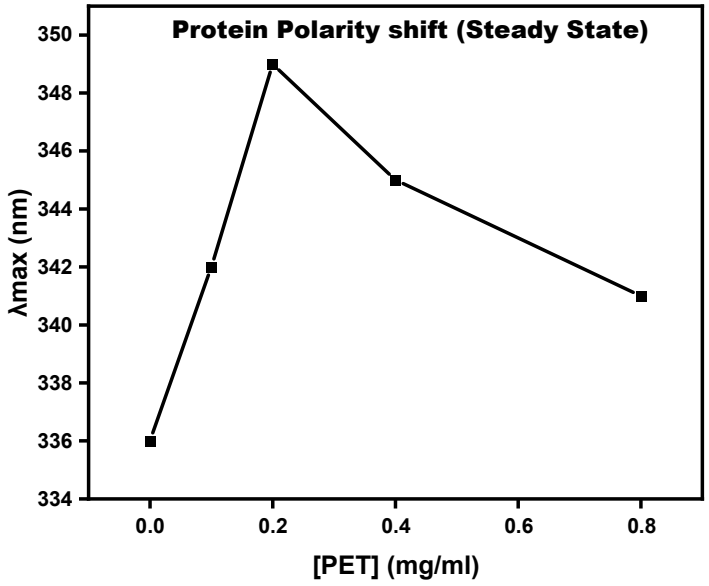



**Figure S5. Fluorescence emission red shift in cedar pollen proteins following interaction with PET microplastics.**

Intrinsic fluorescence emission spectra of cedar pollen proteins were recorded upon excitation at 295 nm. Interaction with PET microplastics induced a reproducible red shift in the emission maximum accompanied by fluorescence quenching. The emission maximum red-shifted to 349 nm at 0.2 mg/mL PET, indicating maximum solvent exposure of aromatic residues at this concentration. The red shift reflects increased solvent exposure and microenvironmental changes in aromatic residues, particularly tryptophan and tyrosine, consistent with adsorption-induced conformational rearrangements.

These spectroscopic changes support partial surface accommodation of pollen proteins on PET without global unfolding.

## References

Yasueda, H., Yui, Y., Shimizu, T., & Shida, T. (1983). Isolation and partial characterization of the major allergen from Japanese cedar (Cryptomeria japonica) pollen. *Journal of Allergy and clinical immunology*, *71*(1), 77-86.