Electronic Supplementary Material (ESI) for Soft Matter.

Solvent Triggered Irreversible Shape Morphism of Biopolymer Films

Amrita Rath[a], *P.M. Geethu*[b], *Santhosh Mathesan*[a], *Dillip K. Satapathy*[b] *and Pijush Ghosh*[a*]

[a] Indian Institute of Technology Madras, Nanomechanics and Nanomaterials Laboratory,
Solid Mechanics Group, Department of Applied Mechanics and Soft Matter Center,
Chennai- 600 036, Tamil Nadu, India

[b]Indian Institute of Technology Madras, Soft Condensed Matter and Biological Physics Laboratory, Department of Physics and Soft Matter Center, Chennai-600 036, Tamil Nadu, India

SUPPLEMENTARY DOCUMENT

S.1. EXPERIMENTAL SECTION

MATERIALS

Chitosan powder (Degree of deacetylation > 90%, viscosity-(100-200) cps, medium molecular weight), were supplied from SRL Pvt. Ltd. (India). Hydroxyapatite nanopowder, < 200 nm size (BET), Glutaraldehyde (25% aqueous solution), and Acetic acid, > 99.7 % were purchased from Sigma Aldrich (India, Bangalore). Resorcinol crystals were purchased from R.K. Chemicals (India, Chennai). Petri dish of size ϕ35, 15 mm thickness was used for pouring chitosan solution to form a film.

CHITOSAN FILM PREPARATION FOR FOLDING EXPERIMENT

Solvent casting method was used to prepare free standing chitosan films. Chitosan solution (1.5% w/v) was prepared by dissolving 150 mg of CS powder in 10 ml of acetic acid (1% v/v). The solution was stirred by keeping over a hot plate magnetic stirrer at ~ 45^{o} C for 2 hours forming a homogenous solution. Cross-linked CS films were prepared by adding glutaraldehyde concentration (300 μL) as a cross-linking agent to the above prepared pure CS solution. The cross-linked CS solution was immediately poured into the petri dish before it forms too viscous. The solution was kept in an oven for 12 hours for the solvent to evaporate, leaving behind a film of ~80 μm thickness.

The obtained films were cut into small rectangular size of 0.15 cm x 0.7 cm dimensions. The entire characterization of the folding behavior mentioned in this work is performed with a rectangular strip of film which undergo a symmetrical folding The prepared film was placed on solvent medium and folding behavior was captured using an optical microscope placed perpendicular to the film. Goniometer setup was used for characterizing the folding behavior. All the experiments were repeated for atleast 3 times. The details of the experimental setup is mentioned in our previous paper [1].

AROMATIC ALCOHOL SOLUTION PREPARATION

Aromatic alcohol solution was prepared by adding resorcinol crystals into water. Molar solution of 2M, 3M, 4M, 6M and 8M were prepared by adding 1.1 g, 1.65 g, 2.2 g, 3.3 g and 4.4 g respectively, to 5 mL of water. The mixture is stirred for about 30 min by placing over a magnetic stirrer plate till a homogenous solution of light reddish colour is formed. This solution is used for all the folding and dielectric spectroscopy experiments.

The rational behind choosing resorcinol as a trigger solvent was to study the competitive interaction with water which has also a similar reactive -OH group that form H-bond with the reactive sites of chitosan, thus affecting the folding behavior. Resorcinol being one of the simplest aromatic alcohol which closely resembles OH group and benzene ring in amino acids was helpful in understanding the folding behavior as well as the underlying mechanism. Later this behavior can be extended to detect the presence of amino acids or any other biomolecules of similar molecular structures to detect abnormality in the living being.

FILM PREPARATION FOR DIELECTRIC SPECTROSCOPY EXPERIMENT

Cross-linked chitosan film of thickness was cut in a circular shape of radius 20 mm for performing DRS experiment. Aromatic alcohol solutions of four different molarity, i.e, 2M, 6M, 8M and 10M were prepared. The sample was soaked in AA solution of varying molarity for about 5 min till it reaches saturation. The surface of the film was gently tapped with a dry tissue paper to remove excess water from it. The film was then placed in between two gold plated electrodes of a parallel plate capacitor for the dielectric tests. The experiments were conducted for at least 3 times to ensure repeatability.

DETAILS OF DIELECTRIC MEASUREMENTS

Dielectric measurements were carried out using Novocontrol Alpha-A High Resolution Dielectric Analyzer with active sample Dielcell ZGS as test interface. Samples were subjected to an oscillating voltage of 1V peak-to-peak in the frequency range from 1 Hz to 40 MHz and all the measurements were recorded at ambient temperature. Solvent casted films of chitosan samples were used for measurements, where samples were placed between two electrodes (diameter 20 mm) of a parallel plate capacitor and the frequency dependent dielectric spectra were recorded. Complex impedance Z* is obtained from the measured voltage and current across the sample. From the complex impedance Z*, the real and imaginary parts of the permittivity were calculated using the relation given by Eq. (1),

$$\varepsilon^*(\omega) = \varepsilon'(\omega) - i\varepsilon''(\omega) = \frac{1}{i\omega C_0 Z^*} \tag{1}$$

where, ω is the angular frequency and C_0 is the empty cell capacitance. The electrical conductivity of the samples were estimated using the relation given by Eq. (2),

$$\sigma^* = i\omega\varepsilon_0\varepsilon^* \tag{2}$$

Dielectric modulus is given by Eq. (3),

$$M^*(\omega) = \frac{1}{\varepsilon^*} \tag{3}$$

SIMULATION SECTION

MODEL BUILDING

In this work, five models were built and analyzed by applying molecular dynamics simulation. These five models consist of i) a single chain chitosan polymer (CS), ii) chitosan chain solvated in water (CS-W), chitosan chain solvated in a mixture of water and AA solution of varying molarity, i.e. iii) 2M (CS-2M), iv) 10M (CS-10M) and v) 20M (CS-20M). All the simulations were performed using LAMMPS [2], a molecular dynamics program. The visual molecular dynamics (VMD) [3] was used for visual aid and to analyze the output trajectory. CHARMM [4] force field parameters for proteins were used for all the simulations, whereas for solvent TIP3P [5] model of water was used.

A single polymer chain of chitosan consisting of 75 repeating units was built using Material Studio 6.0 [6]. The rest of the models were built using packmol [7] to generate an amorphous system in which an equilibrated chitosan chain was packed in a box consisting of 1500 water molecules for CS-W model and 1500 water molecules along with 54, 270 and 540 number of AA molecules for CS-2M, CS-10M and CS-20M models, respectively. The simulation box for all the models was 40 Å × 40 Å × 40 Å. A model for CS-W, CS-2M and CS-20M generated in packmol is shown in **Figure** 1.

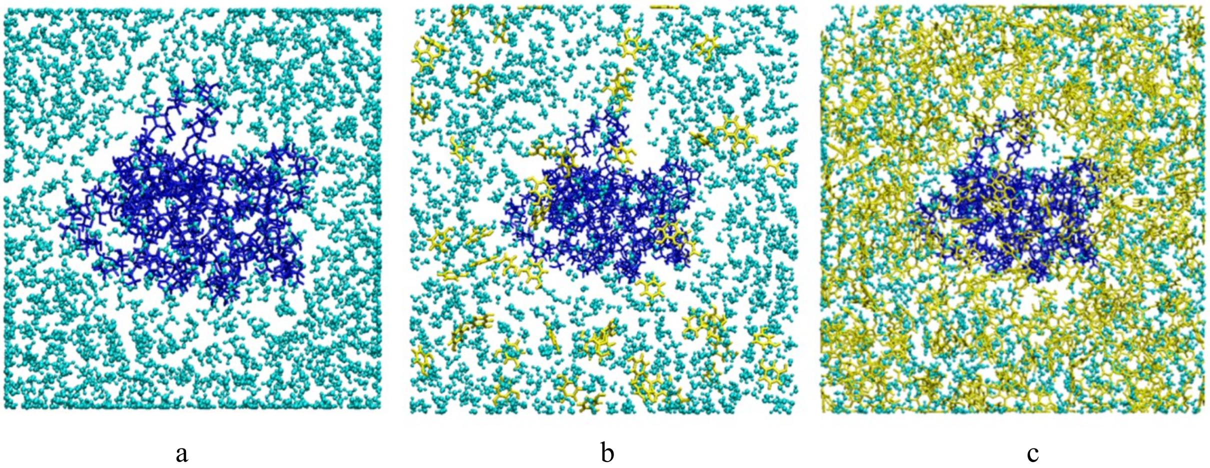

Figure 1. A snapshot of initial structure for (a) CS-W, (b) CS-2M and (c) CS-20M model (Chitosan-Blue; Water-Cyan; Aromatic alcohol-Yellow).

SIMULATION PROCEDURE

The initial structure was optimized using conjugate gradient minimization algorithm. The segmental minimization was performed in which one segment of the structure was fixed while allowing the rest of the structure to minimize. This was followed by a simulation using *NVE* ensemble, with a constant temperature maintained by using Langevin dynamics for 20 ps with a time step of 0.5 fs. The system was equilibrated using NPT ensemble and temperature was increased from 10 K to 300 K in step of 100 K. After each increment the system was relaxed for

50 ps using NPT ensemble. The temperature and pressure was maintained by using Nose/Hoover [8] thermostat and barostat respectively. After reaching 300 K, the simulation was carried out with a time step of 1 fs for 200 ps. The Particle Mesh Ewald (PME) [9] method was used for calculation of long range electrostatic interaction energy. The cut off distance of 10 Å was used for all non-bonded interactions between atoms. The periodic boundary conditions (PBC) was used to eliminate boundary effects from the simulations.

STEERED MOLECULAR DYNAMICS

The SMD simulations were carried out for all the five different models. It provides the dynamics of the unfolding mechanism and its effect on the polymer structure in the presence and absence of AA and water. The center of the mass of the polymer chain was pulled by using harmonic spring in the direction of the applied force vector as shown in **Figure 2**.

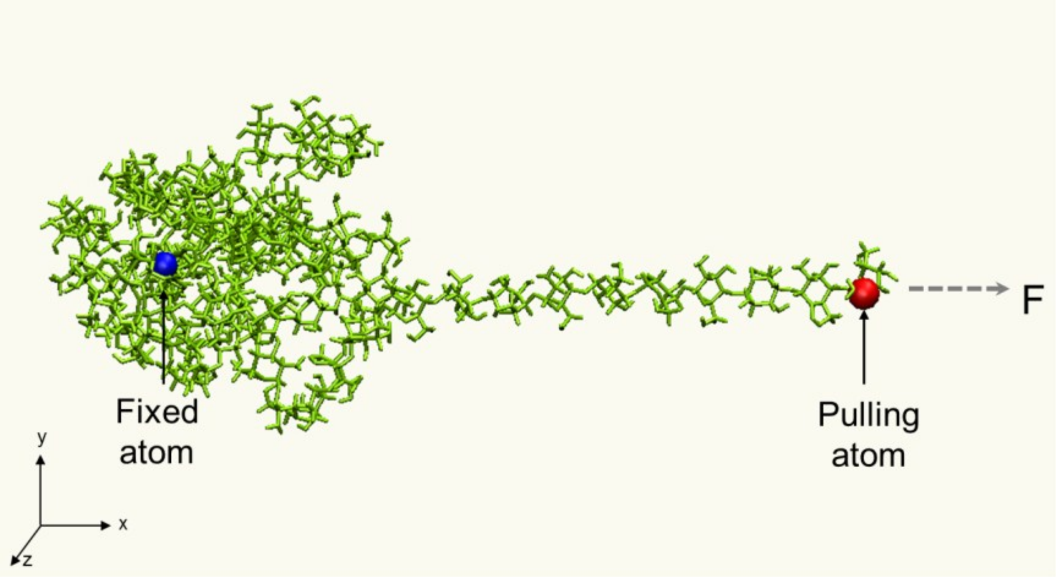

Figure 2. Single chain chitosan polymer (CS) model depicting the pulling of the atom in the direction of the force vector; blue colour atom is constrained at one end and red colour atom getting pulled through a virtual spring attached to it.

The stiffness of the spring need to be carefully chosen for SMD. A very high stiffness result in the absorbance of the energy by the spring rather than the load being taken by the polymer chain. Whereas, very low stiffness cause low mechanical stability of the polymer chain resulting in more noise during stretching. Therefore, simulations were carried out by using spring constant, k = 5, 7 and 10 kcal/mol/Å^2 [10,11] and at velocity, v = 0.1, 0.5, 1 and 5 Å/ps [12,13] to investigate the suitability of these constants. At the end, k= 7 kcal/mol/Å^2 and v = 0.5 Å/ps, the system was found to be suitable, thus used for all the calculations. Prior to running the SMD simulation, the structure was equilibrated at 300 K using NVT ensemble for 50 ps with 0.5 fs time step. The 2D PBC was maintained to ensure non-overlapping of the periodic boundary during pulling of the polymer chain. The simulation was performed for three different initial structures of the single chain chitosan polymer. For the first initial structure, the system was heated stepwise to a temperature of 300 K and the equilibrated structure is used for SMD simulation. Whereas, for the other structures, the system temperature was raised to 500 K and 800 K respectively and then gradually cooled to 300 K. The structure was equilibrated at 300 K for 200 ps with a time step of 1 fs. Further, to ensure repeatability, the simulation is performed at three different initial configurations and the results represent the average of three simulations.

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