

## Mechanical Properties of Self-Assembled Chitin Nanofiber Networks

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### ESI

*Materials:* Chitin squid extract (Industrial Research Ltd-New Zealand) and hexafluoro-2-propanol (HFIP) (Oakwood Products, Inc.) were used as received.

*Chitin Film Fabrication: Cold-Press (CP):* chitin gels were prepared by letting the chitin/HFIP in air for 3-5 days. Then, we put the gels in between the glass slides covered with hardened filter papers (Whatman™) and press firmly with the help of two clips.

*Vacuum Drying (VD):* The solution of chitin/HFIP was drop cast onto a glass petri dish and let dry in a desiccator connected to the house vacuum. The film was ready after 2-3 days.

*Vacuum-assisted Filtration (VF):* In this method, the solution of chitin/HFIP was drop cast into a porcelain funnel covered the PDMS-coated filter paper. PDMS-coated filter paper was prepared by spin coating PDMS on the filter paper on the speed of 500 rpm for 5 seconds and then 5000 rpm for 1 minute. The resulting fresh PDMS-coated filter papers were dried in the oven for 3 hours at 60°C. The porcelain funnel was connected to house vacuum and covered with a glass cover in order to minimize evaporation from the top surface of the chitin film. The drying time for this method was about 1 day.

*Atomic Force Microscopy:* A Veeco Multimode V (Nanoscope IV controller) and Veecoprobes Sb-doped Si cantilevers ( $\rho = 0.01-0.025 \text{ } \Omega \cdot \text{cm}$ ,  $k = 40 \text{ N/m}$ ,  $\nu \sim 300 \text{ kHz}$ ) were used for atomic force microscopy (AFM).

*X-Ray Diffraction:* XRD spectra were obtained at room temperature with a wide-angle X-ray diffractometer ( $2\theta=5-55^\circ$ ) (Bruker D8 Focus) with Cu K  $\alpha$  radiation, operated at 40 KV and 40 MA.

*Fourier Transform Infrared Spectroscopy:* Fourier Transform Infrared (FTIR) spectra were recorded on free-standing substrates with a Bruker vector 33 FTIR spectrophotometer (4000 to 400  $\text{cm}^{-1}$ , 4  $\text{cm}^{-1}$  resolution).

*Mechanical Testing:* Stress vs. strain data of chitin films substrates were recorded with a Shimadzu AGS-X at a rate of 0.1 mm/min. For each fabrication method, 6 chitin strips (3 for each concentration) were cut from the original films for mechanical testing and they were examined carefully by optical microscope for any notches and microscopic cracks. The reported values are the average and the error bars represent the standard deviation for each measurement. Instrumented nanoindentation was performed using the load-controlled Dynamic Contact Module head of an MTS XP Nanoindenter, providing low noise and high resolution sensing (data are recorded for displacement steps of 0.15 nm). Nanoindentation testing was performed in an isolated environment at ambient conditions at a temperature of  $20\pm 1$  °C and relative humidity of  $40\pm 10\%$ . A diamond flat punch of 2050 nm in diameter, fabricated by focused ion beam milling, was brought into conformal contact with a 250 nm thick chitin layer on silicon. Samples were aligned by shallow indentation into an adjacent polymeric layer, scanning of the indent replica by AFM, and tilt correction of the sample from the vector offset of the punch face replica, as described in detail here.<sup>45</sup> Thermal drift was measured for each indentation (generally sub 0.1 nm/s) and linearly corrected for, along with load frame stiffness and sample mounting compliance.

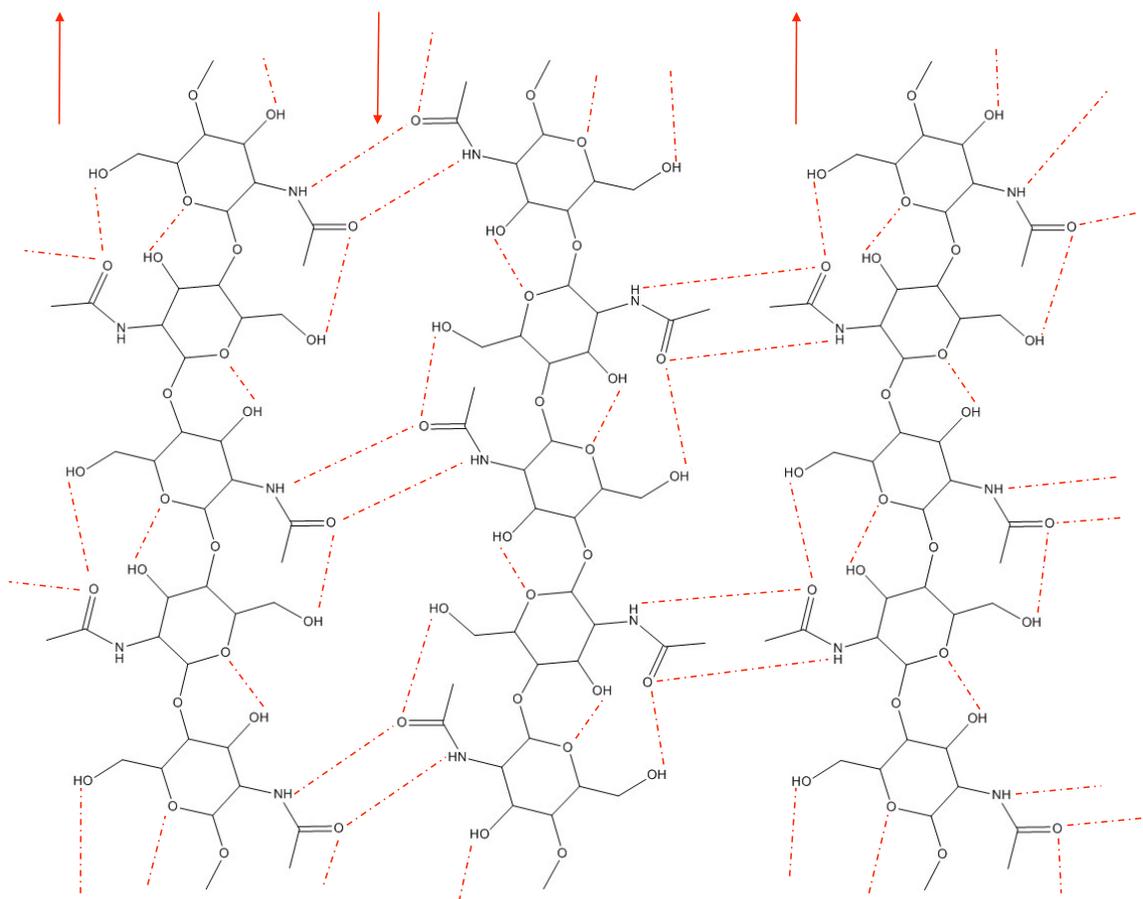


Figure S1. Structure of  $\alpha$ -chitin. The chitin chains are organized antiparallel and they are tightly held by a number of strong intra-sheet and inter-sheet hydrogen bonds.

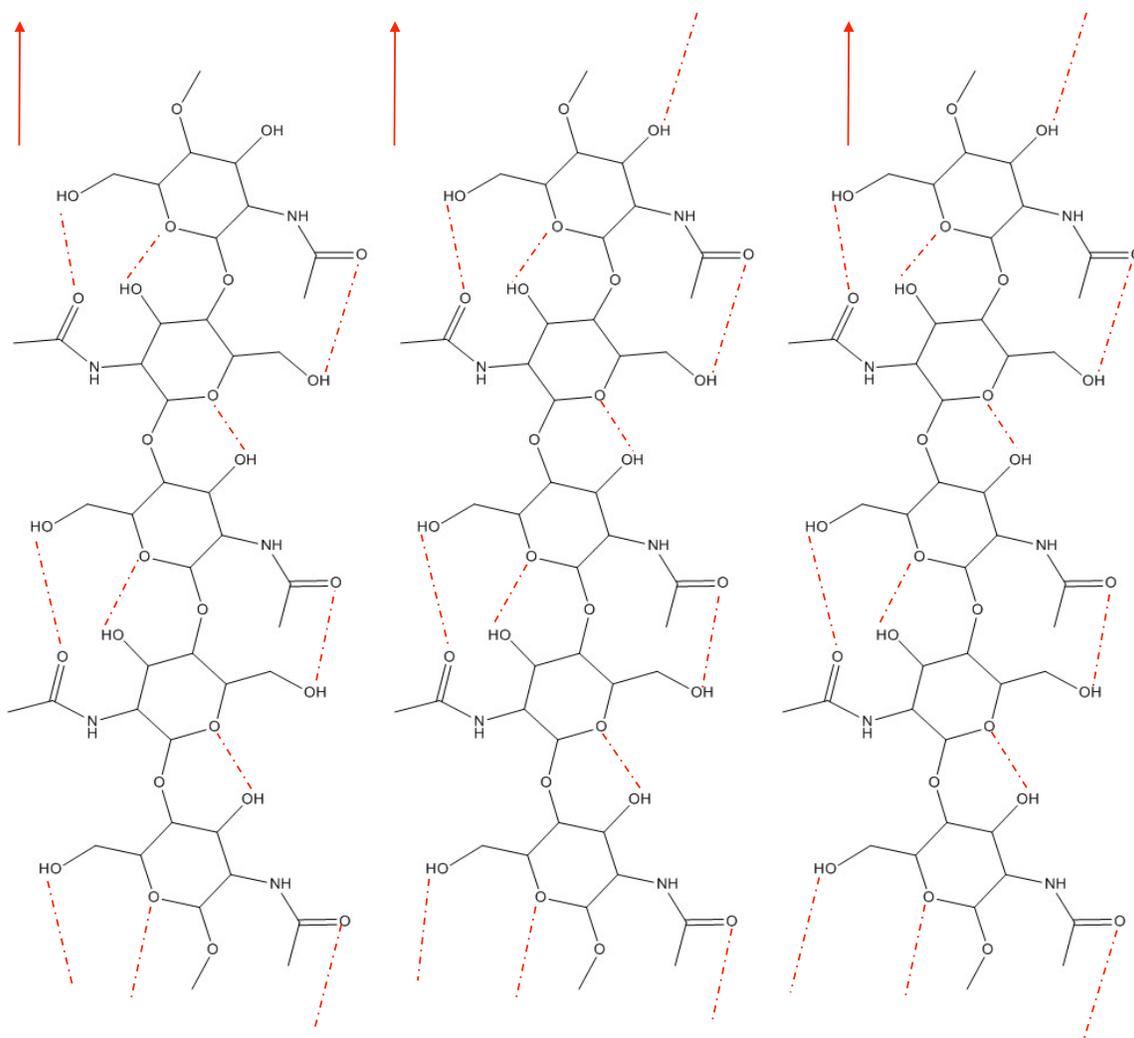


Figure S2. Structure of  $\beta$ -chitin. The chitin chains are organized parallel and they are tightly held by a number of weak intra-sheet hydrogen bonds.