

Zn^{2+} , Cd^{2+} and Cu^{2+} Mediated Formation of Amyloid like Fibrils by the Monomers of β -Sheet Rich Peanut Agglutinin

*Khatija Tabbasum and Chebrolu Pulla Rao**

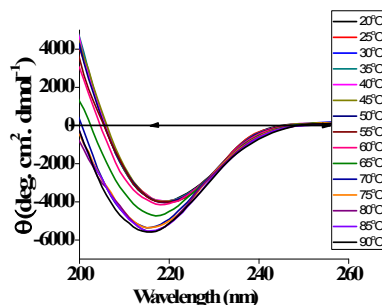
Bioinorganic Laboratory, Department of Chemistry, Indian Institute of Technology Bombay,

Powai, Mumbai 400 076, India. E-mail: cprao@iitb.ac.in

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SI 01 CD of PNA as a function of temperature from 20° to 90° C



SI 02 Absorption and Emission studies in presence of EDTA

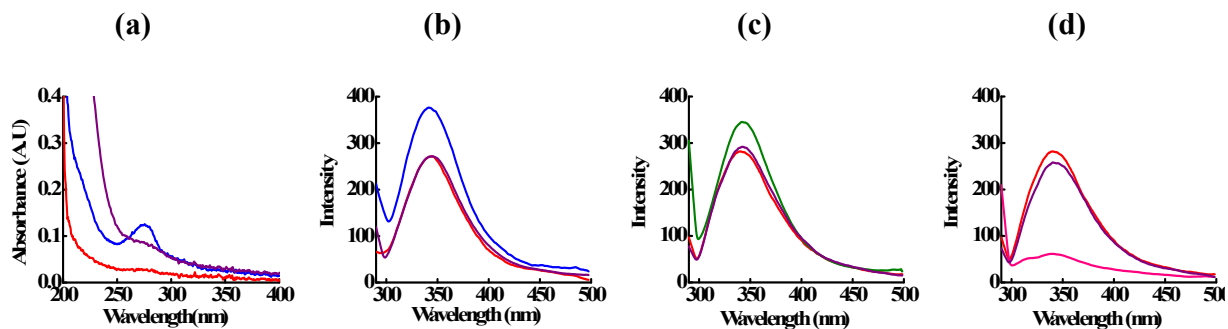


Figure (a) Absorption, and (b, c & d) emission spectra obtained during the titration of {m_PNA + M^{2+} } and this in presence of EDTA. The colour codes are: {m_PNA} (red); {m_PNA+ Zn^{2+} } (blue); {m_PNA+ Cd^{2+} } (green); {m_PNA+ Cu^{2+} } (magenta); {m_PNA+ Zn^{2+} +EDTA} (purple). For all the experiments, protein concentration is 10 μ M and M^{2+} concentration is 0.5 mM in a total volume of 1 ml.

SI 03 CD studies as a function of temperature in presence of EDTA

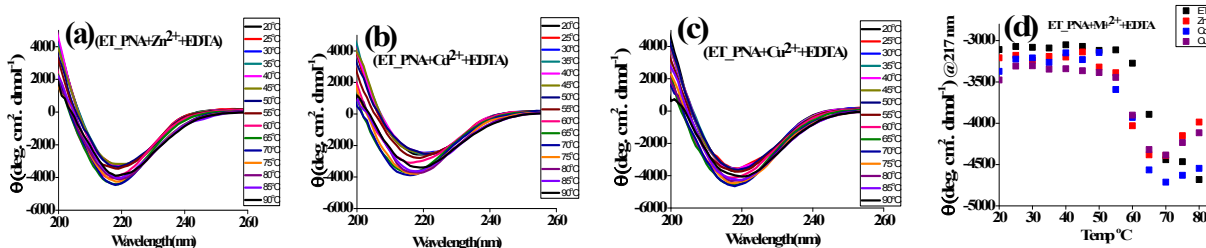


Figure CD spectra observed in the titration of $\{m_PNA + M^{2+} + EDTA\}$ as a function of temperature, where $M^{2+} =$ (a) Zn^{2+} , (b) Cd^{2+} , (c) Cu^{2+} and (d) and elliptic signal of PNA at 217 nm in presence of EDTA as a function of temperature in the temperature range 20 to 90 °C. For all the experiments, protein concentration is 10 μM and M^{2+} concentration is 0.5 mM in a total volume of 1 ml.

SI 04 FTIR studies of ET_PNA in presence of M^{2+} (M=Zn, Cd, Cu):

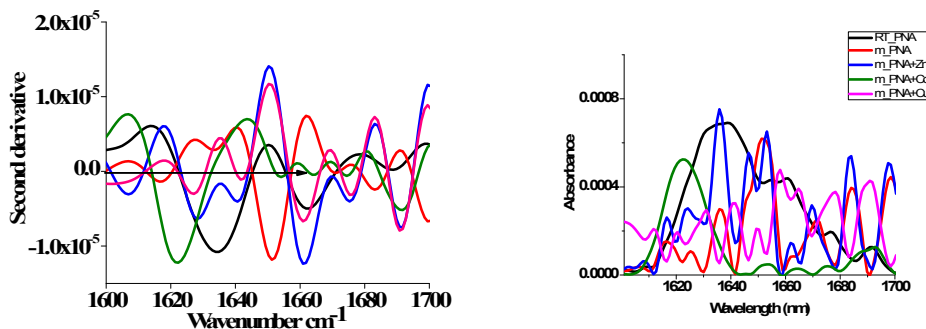
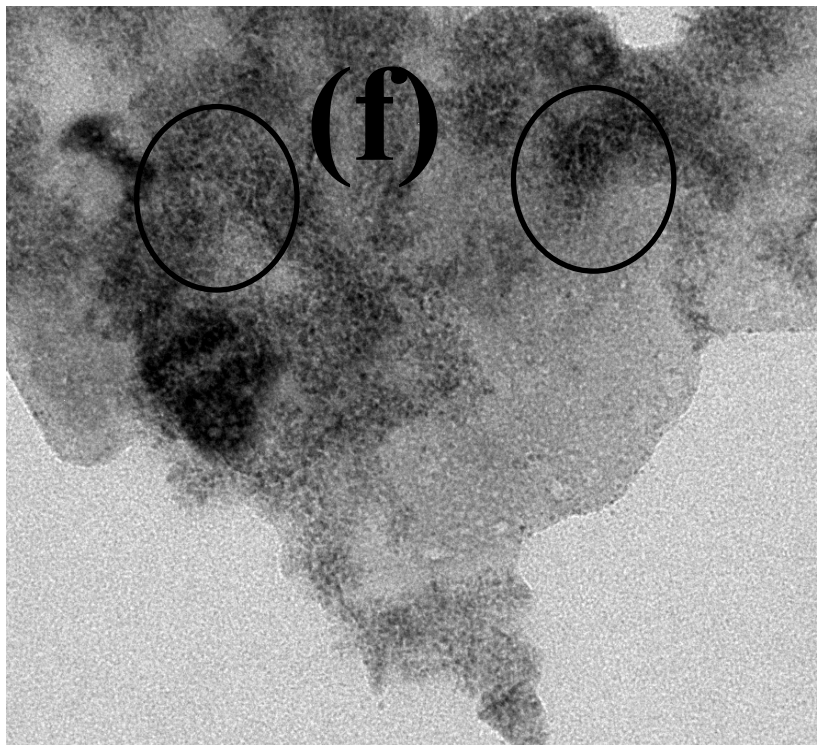
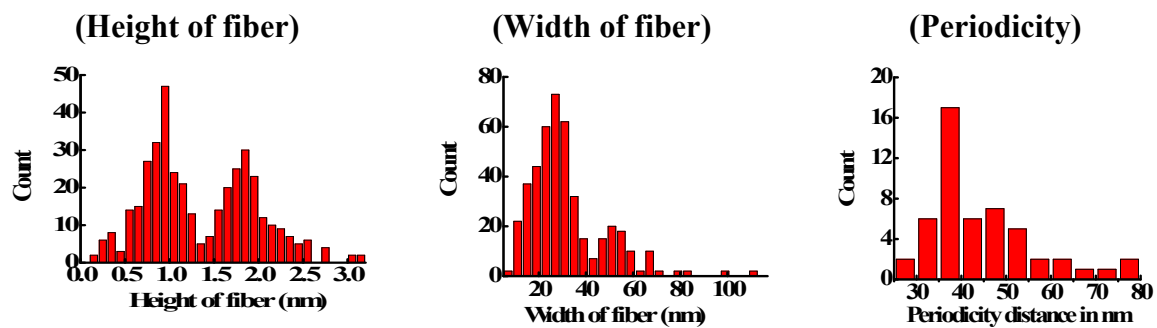


Figure: Second derivative FTIR curves of m_PNA in presence of M^{2+} ($M = Zn, Cd, Cu$). $\{t_PNA\}$ (—); $\{m_PNA\}$ (—); $\{m_PNA + Zn^{2+}\}$ (—); $\{m_PNA + Cd^{2+}\}$ (—); $\{m_PNA + Cu^{2+}\}$ (—). FTIR of the same in Absorbance unit. Protein concentration is 10 μM and M^{2+} concentration is 0.5 mM in a total volume of 1 ml.

SI 05 TEM micrograph showing Protofilaments



SI 06 Statistical Analysis of Fibers



SI 07 Isolation and purification of peanut lectin by Affinity Chromatography

Peanut agglutinin was isolated and purified by affinity chromatography. All the experimental conditions were maintained at 4° C throughout the experiment. 15 gram of defatted powder was extracted overnight in tris-buffer saline (TBS), 25mM, pH=7.4, 0.9% NaCl. The extract was centrifuged at 12000 rpm for 30 min. The supernatant was collected and precipitated with 0-80% ammonium sulfate. Solid ammonium sulfate was added in four parts at regular interval of 15min and whole set up was kept on a magnetic stirrer for 3-4 hours. Pellets were collected by centrifuging at 12000 rpm for 20-30 minutes and dissolved in minimum amount of tris buffer (25ml). These pellets were then dialyzed against the same buffer. The buffer was changed thrice during the dialysis process. The dialyzed protein was loaded on the sepharose column (15cm x 4cm) equilibrated with the same buffer. Reloaded the dialyzed the protein 5-7 times so that protein of interest binds to the affinity column while all non specific and unbound protein get removed during washing with the tris –buffer. The bound protein was eluted with elution buffer and collected in different fraction The fractions whose optical density (OD) was above 0.5 were pooled together and again subjected to 0-80% ammonium sulfate precipitation in order to concentrate the protein. This precipitate was dialyzed against the tris-buffer and stored at 4°C for further use. The protein was run on (15%) SDS PAGE and showed the purified band at 27kDa.

Std. Mwt marker

