

Extracellular pH imaging of a plant leaf with a polyelectrolyte multilayered nanosheet

Daichi Someya^a, Satoshi Arai^{b, c}, Toshinori Fujie^{d, e*}, and Shinji Takeoka^{a*}

^aDepartment of Life Science and Medical Bioscience, Waseda University, TWIns, 2-2 Wakamatsu-cho, Shinjuku-ku, Tokyo 162-8480 Japan. Email: takeoka@waseda.jp

^bPRIME, Japan Agency for Medical Research and Developing (AMED), Tokyo, 100-0004, Japan.

^cResearch Institute for Science and Engineering, Waseda University.

^dWaseda Institute for Advanced Study, Waseda University, TWIns, 2-2 Wakamatsu-cho, Shinjuku-ku, Tokyo 162-8480 Japan. Email: t.fujie@aoni.waseda.jp

^eJapan Science and Technology Agency, PRESTO, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012 Japan.

Supporting Figures

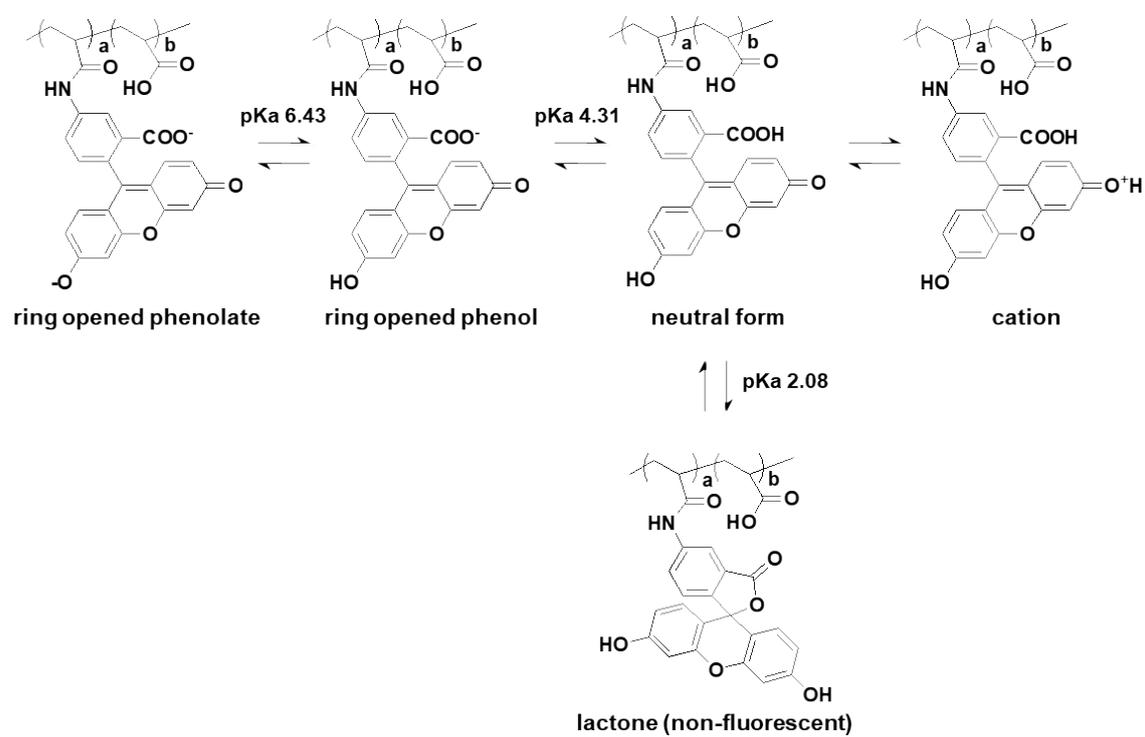


Figure S1 Ionization equilibria of fluorescein conjugated poly(acrylic acid).

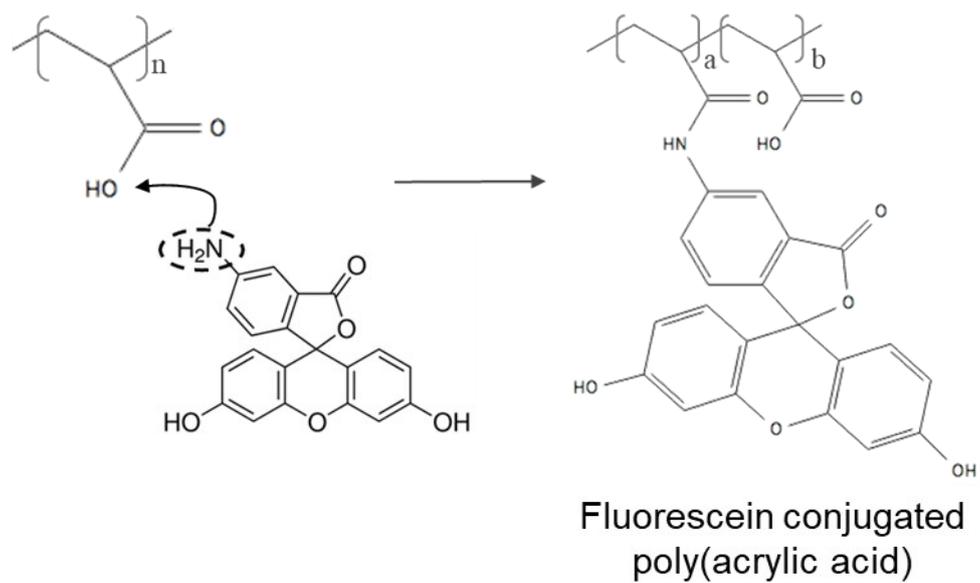


Figure S2 Synthetic route to fluorescein conjugated poly(acrylic acid).

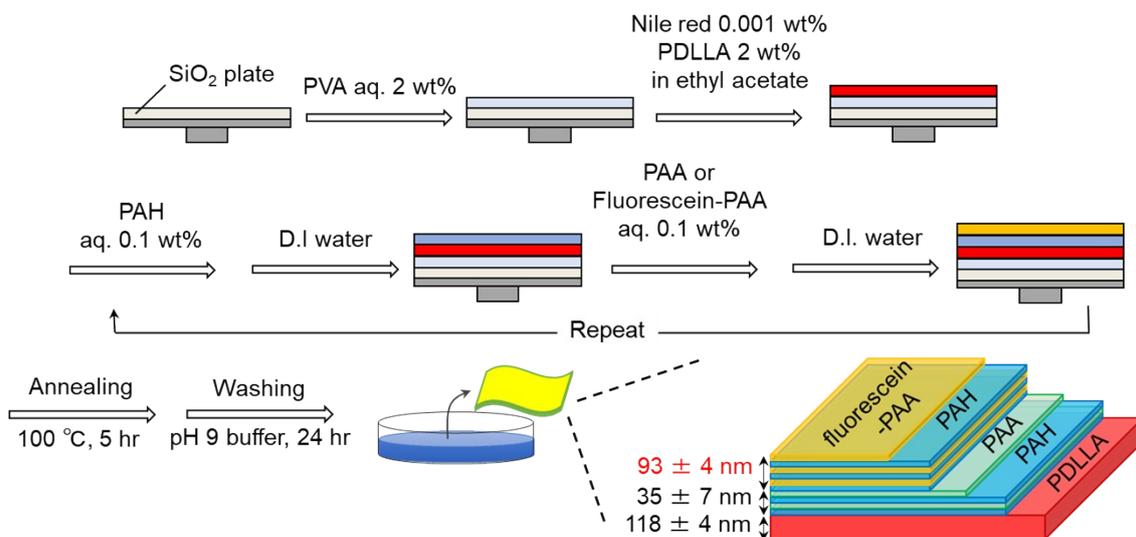


Figure S3 Preparation method of pH sensor nanosheet composed of Nile red embedded PDLLA layer and fluorescein conjugated LbL layers.

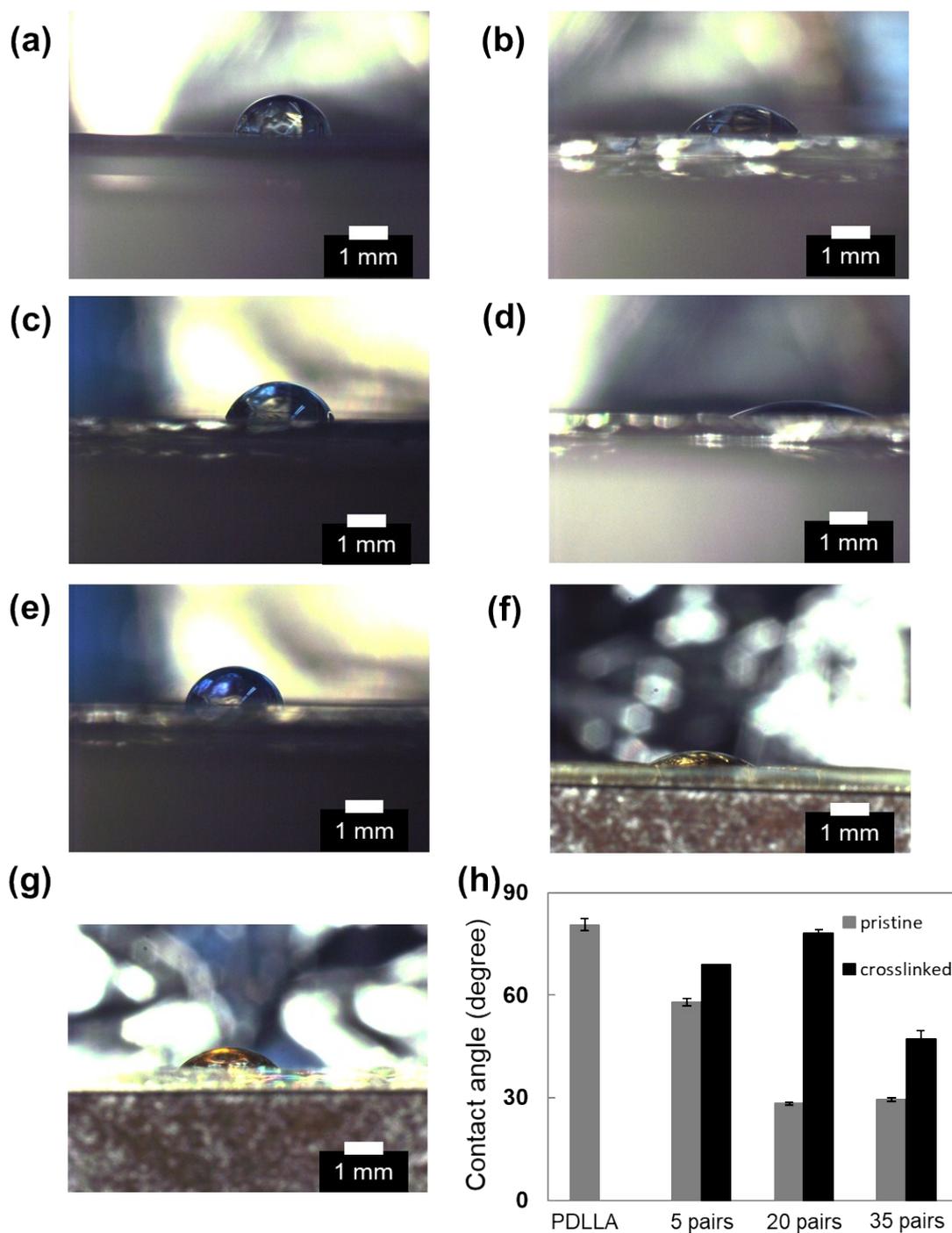


Figure S4 Microscopic images of a water droplet on **(a)** a PDLLA film, **(b)** 5 pairs and **(d)** 20 pairs and **(f)** 35 pairs of pristine LbL films, and **(c)** 5 pairs and **(e)** 20 pairs and **(g)** 35 pairs of LbL films after thermal crosslinking. **(h)** Contact angles of the PDLLA film and different layers of the LbL films analyzed from microscopic images shown in **(a-g)**.

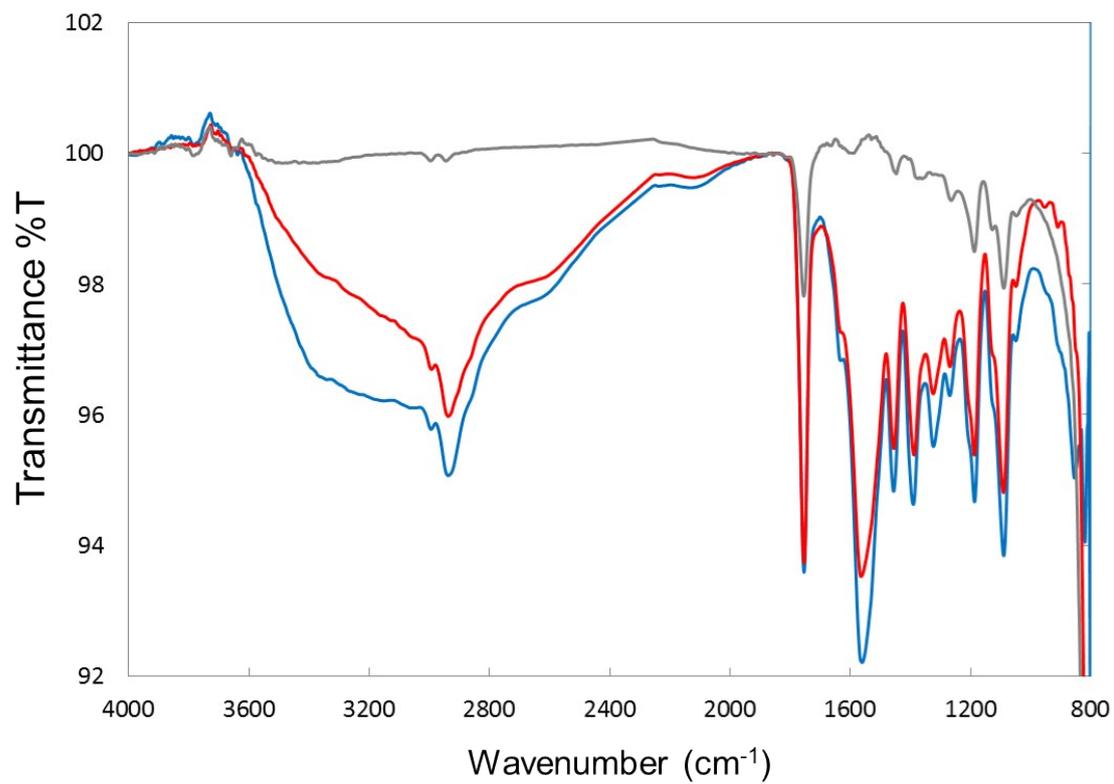


Figure S5 IR spectra of PDLLA nanosheets (grey), fluorescein-conjugated LbL film (blue: before, red: after thermal crosslinking).

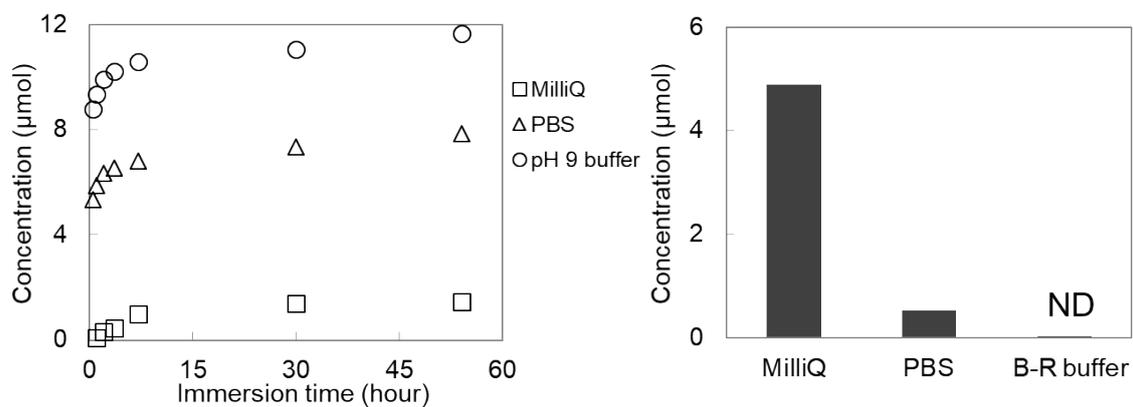


Figure S6 (a) Time-course study of the dye leaching in MilliQ(squares), PBS (triangles), pH9 of B-R buffer (circles) before washing procedures. **(b)** Concentration of Leakage in PBS at 37°C, 50 rpm after washed by MilliQ, PBS and pH9 of B-R buffer.

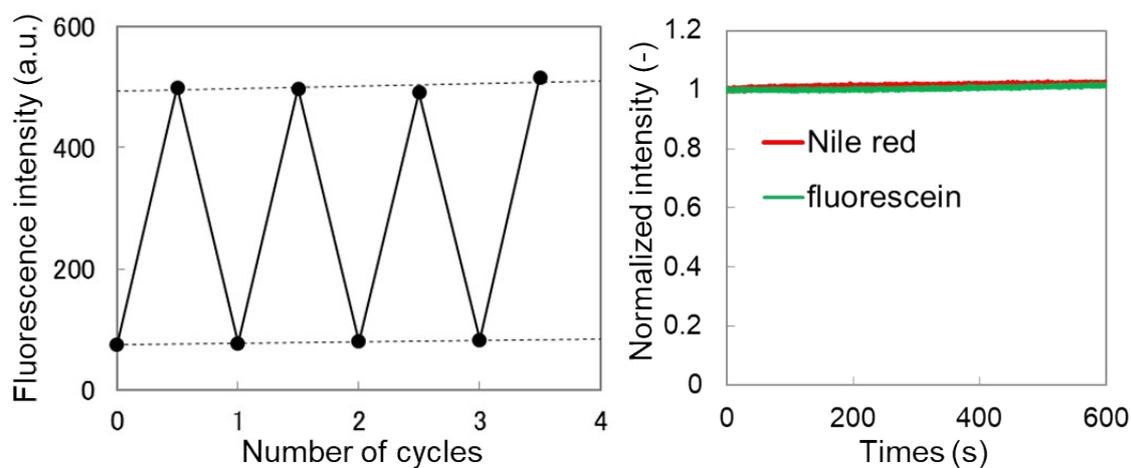


Figure S7 (a) Reversible fluorescent intensity of fluorescein conjugated LbL nanosheets between pH 5 and 9. **(b)** Normalized peak intensity of Nile red embedded PDLLA nanosheets (red) and fluorescein conjugated LbL nanosheets (green) plotted against time.

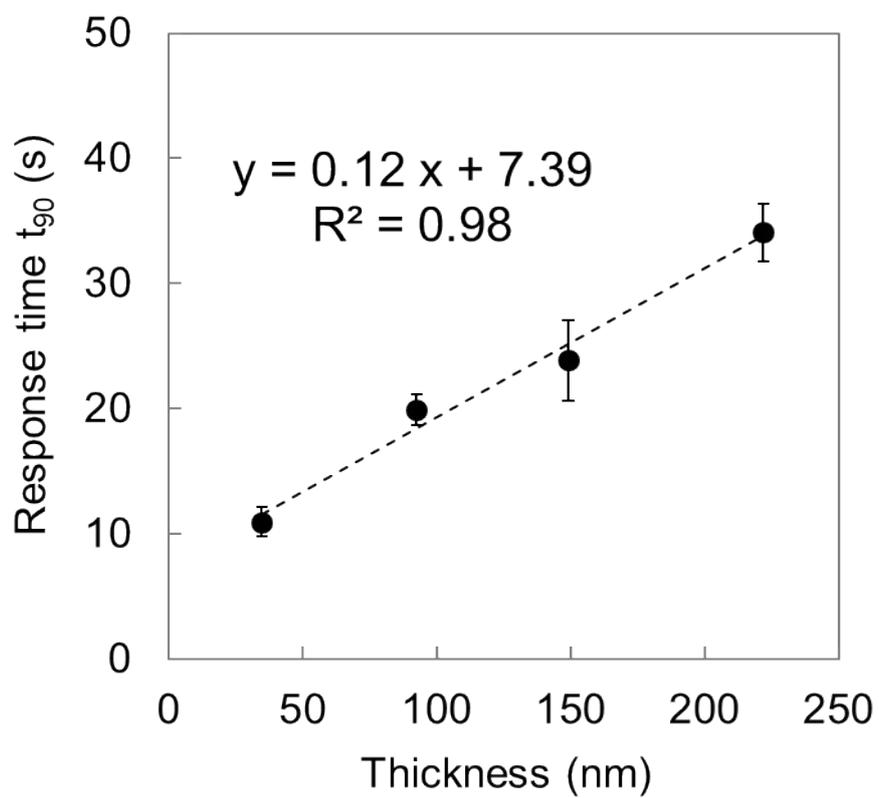


Figure S8 Thickness-dependent response time of the LbL nanosheets (mean \pm SE.).

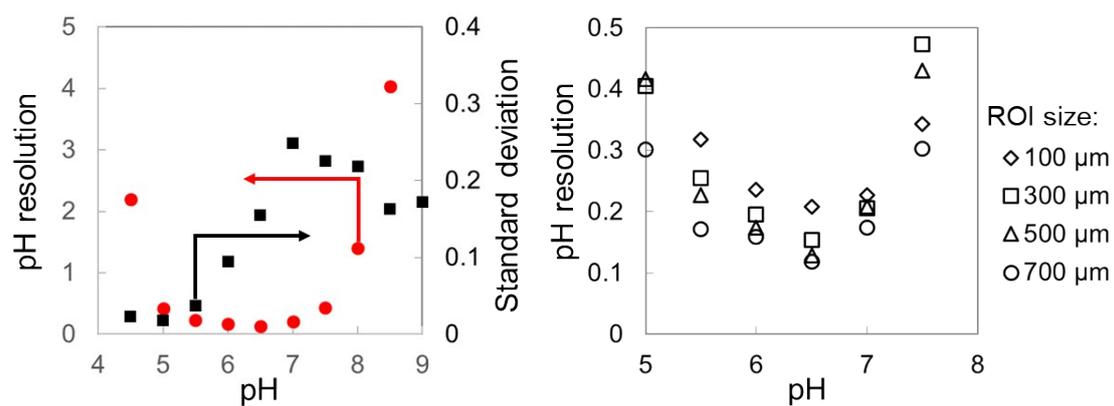


Figure S9 (a) pH resolution and normalized standard deviation plotted against pH 4.5-9 (every 0.5) were calculated by calibration data (ROI size: 500 × 500 μm²). **(b)** pH resolution was plotted against pH of each ROI sizes.

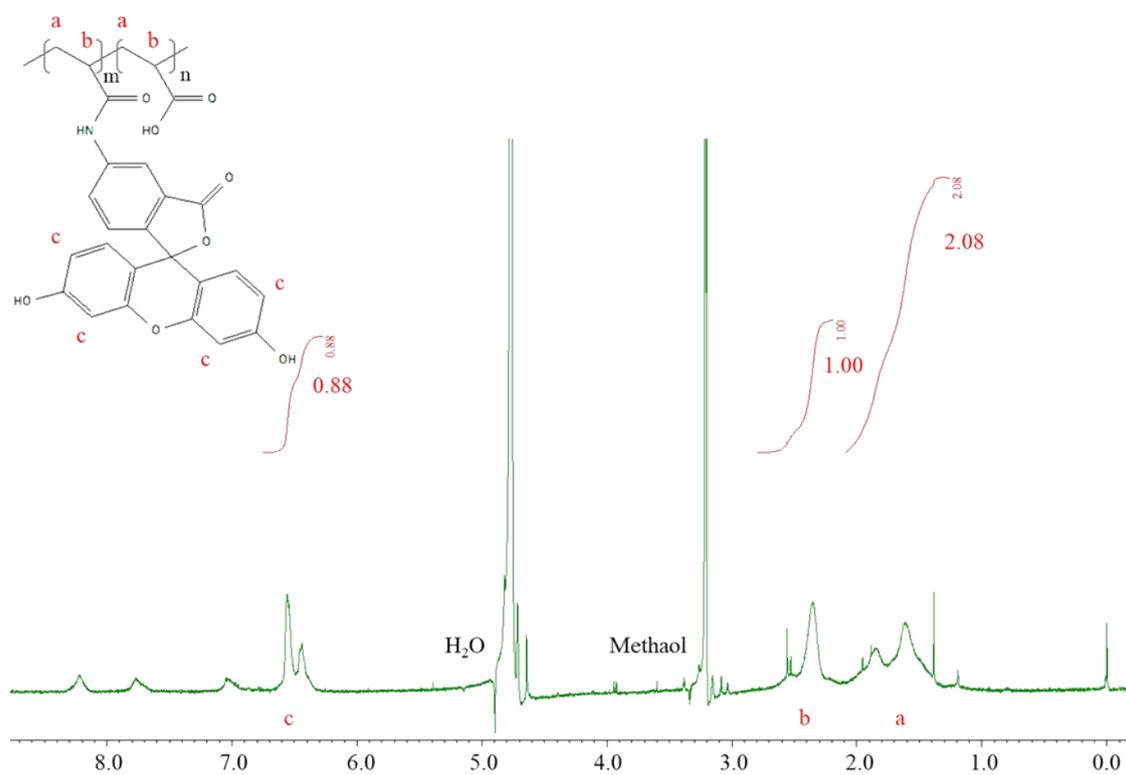


Figure S10 ¹H-NMR spectra of fluorescein conjugated poly(acrylic acid).