

**Supplementary information    Figure S1-4**

**Nanogel containing electrospun nanofibers as a platform for stable loading of proteins**

Asako Shimoda<sup>1, 2</sup>, Yong Chen<sup>3, 4</sup> and Kazunari Akiyoshi<sup>1, 2\*</sup>

<sup>1</sup>Department of Polymer Chemistry, Graduate School of Engineering,

<sup>2</sup> Japan Science and Technology Agency (JST), The Exploratory Research for Advanced Technology (ERATO), Bio-nanotransporter Project, Katsura Int'tech Center, Katsura, Nishikyo-ku, Kyoto 615-8530, Japan.

<sup>3</sup>Ecole Normale Supérieure, CNRS-ENS-UPMC UMR 8640, 24 rue Lhomond, 75005 Paris, France.

<sup>4</sup>Institute for Integrated Cell-Material Sciences, Kyoto University, Kyoto, 606-8501, Japan

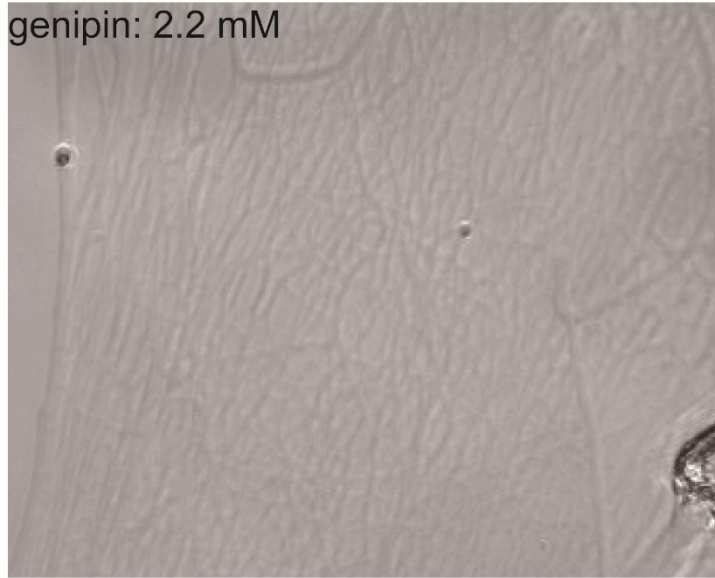
\*Corresponding author: Kazunari Akiyoshi

Department of Polymer Chemistry, Graduate School of Engineering, Kyoto University, Katsura, Nishikyo-ku, Kyoto, 615-8510, Japan

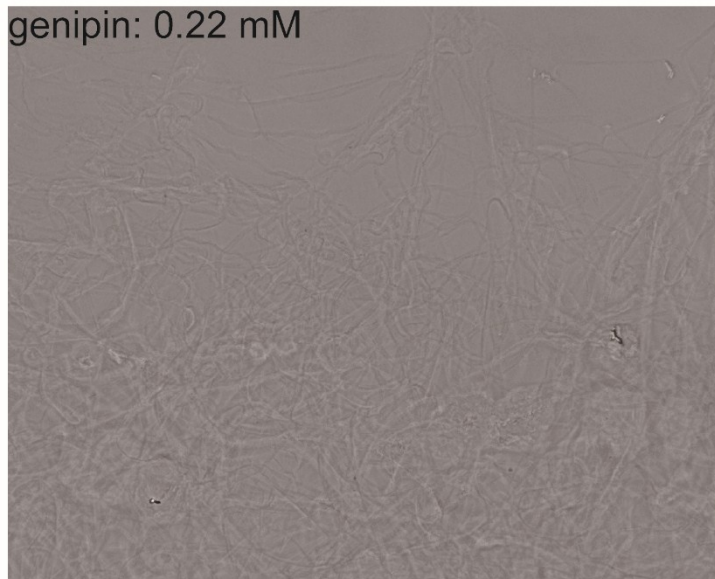
Tel.: +81-75-383-2589; Fax: +81-75-383-2590.

E-mail: akiyoshi@bio.polym.kyoto-u.ac.jp

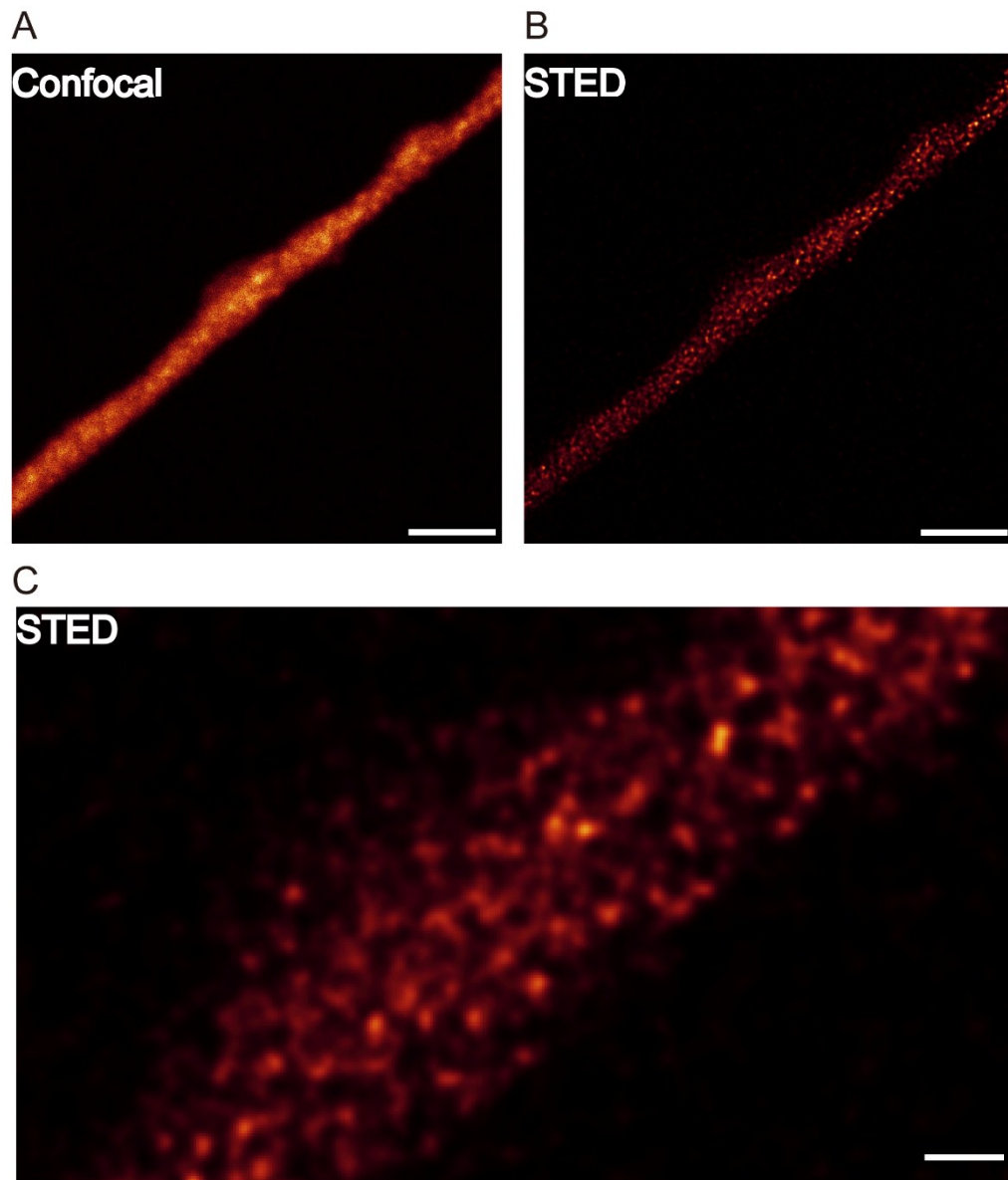
A genipin: 2.2 mM



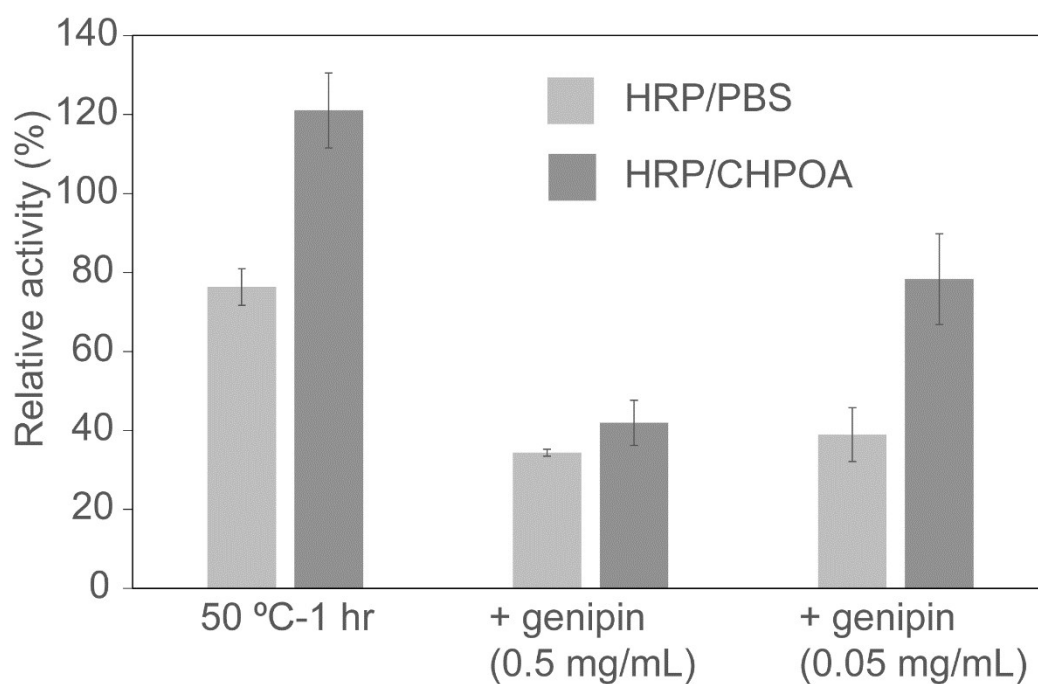
B genipin: 0.22 mM



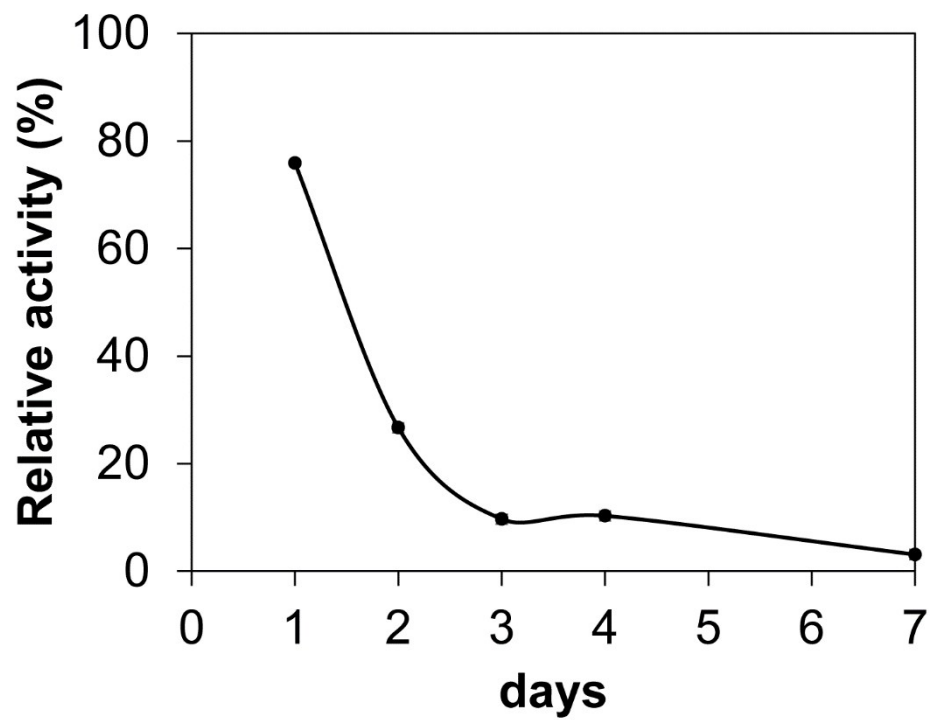
**Supplementary Figure S1.** Genipin-cross-linked CHPOA / gelatin fibers after soaking in distilled water for one day. Genipin concentrations were 2.2 mM (A) and 0.22 mM (B), respectively. Scale bar = 50  $\mu\text{m}$ .



**Supplementary Figure S2.** Confocal (A) and STED (B) images of cross-linked POARh/gelatin fibers. Scale bar = 2.5  $\mu\text{m}$ . STED image was deconvolved by Huygens. (C) High magnification image of Supplementary Figure S2B. Scale bar = 500 nm.



**Supplementary Figure S3.** The effect of genipin concentration on HRP solution with or without CHPOA nanogels. HRP (60  $\mu\text{g/mL}$ ) solution was diluted with or without CHPOA nanogel solution (22.2 mg/mL in 10 $\times$  D-PBS) and was incubated at 37  $^{\circ}\text{C}$  for 24 hr. The samples were heated at 50  $^{\circ}\text{C}$  for 1 hr, and then, genipin in ethanol solution (0.5 or 0.05 mg/mL) was added and incubated for 5 min. The relative HRP activity was measured as described in Experimental Section. The data represents the mean  $\pm$  the standard deviation,  $n = 3$ .



**Supplementary Figure S4.** Thermal stability of free HRP in PBS at 37 °C. The data represents the mean  $\pm$  the standard deviation,  $n = 3$ .