Supporting Information for:

Agarose Hydrogels Embedded with pH-Responsive Diblock Copolymer Micelles for Triggered Release of Substances

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Synthesis of Homopolymer Poly(2-(*N*,*N*-diisopropylamino)ethyl methacrylate) (PDPAEMA). Ethyl 2-bromoisobutyrate (EBiB, 12.4 mg, 0.0636 mmol), 2-(*N*,*N*-diisopropylamino)ethyl methacrylate (DPAEMA, 2.000 g, 9.390 mmol), CuBr (12.7 mg, 0.0885 mmol), 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA, 21.9 mg, 0.0951 mmol), and acetone (7.014 g) were added into a 25 mL 2-necked round bottom flask with a stir bar. The mixture was degassed by three freeze-pump-thaw cycles and placed in a 35 °C oil bath. The polymerization was monitored by ^{1}H NMR spectroscopy. After the reaction proceeded for 305 min, the reaction mixture was opened to air, diluted with THF, and passed through a basic

alumina column with THF as eluent. The solution was concentrated using a rotary evaporator and analyzed by SEC. The $M_{n,\ SEC}$ and PDI were 5.2 kD and 1.21, respectively, based on the calibration curve of polystyrene standards. The polymer was then precipitated in methanol four times, dried under high vacuum at 55 °C overnight, and analyzed by ^{1}H NMR spectroscopy. The DP of the obtained PDPAEMA is 38, calculated from the monomer conversion determined by ^{1}H NMR spectroscopy analysis on the assumption that the initiator initiation efficiency is 100 %. The polymer is denoted as $PDPAEMA_{38}$. The DP of this homopolymer is very close to that of PDPAEMA in the diblock copolymer PEO_{113}-b-$PDPAEMA_{31}$ used in the fabrication of agarose hydrogels.

Determination of pK_a of $PDPAEMA_{38}$ in Water by Titration. $PDPAEMA_{38}$ (15.8 mg) was dissolved in a 0.1 M HCl solution (volumetric standard solution, 1.761 g). A 0.1 M KOH solution (volumetric standard solution) was injected into the $PDPAEMA_{38}$ solution in 25 μL increments by a microsyringe under the stirring condition. After each injection, the pH of the solution was recorded by a pH meter (Accumet AB15 pH meter from Fisher Scientific, calibrated with pH = 4.01, 7.00, and 10.01 standard buffer solutions) at room temperature. The pK_a of $PDPAEMA_{38}$ was found to be 6.3, determined by taking the pH value at the half-neutralization point on the pH titration curve (Figure S1), which is the same as the pK_a value reported in the literature.[1]

Thermal Stability of PEO-*b*-PDPAEMA Micelles. To study the micelle's thermal stability, a diblock copolymer PEO-*b*-PDPAEMA with a PDPAEMA DP of 36, denoted as PEO_{113}-*b*-$PDPAEMA_{36}$, was made using a similar procedure for PEO_{113}-*b*-$PDPAEMA_{31}$, the block copolymer used in the fabrication of hybrid agarose hydrogels. The $M_{n,SEC}$ and PDI of PEO_{113}-b-$PDPAEMA_{36}$ were 13.6 kDa and 1.07, respectively, determined by using a calibration curve

from polystyrene standards. The same procedure as described in the experimental section was employed to prepare micelles of PEO_{113}-*b*-$PDPAEMA_{36}$ in a 1× pH 7.4 buffer with a concentration of 1.0 mg/g. The micelle solution was then passed through a Millipore hydrophilic PTFE filter. The thermal stability of PEO_{113}-*b*-$PDPAEMA_{36}$ micelles was investigated by dynamic light scattering (DLS). The DLS measurements were performed on a Malvern Zetasizer Nano ZS equipped with a He-Ne 633 nm laser and a temperature controller at a scattering angle of 173°. The temperature was gradually increased from 25 to 65 °C. At each temperature, the sample was equilibrated for 10 min prior to data collection.

At temperatures below 45 °C, two size distributions were observed for the micelle solution of PEO_{113}-*b*-$PDPAEMA_{36}$ with the majority of micelles having an apparent hydrodynamic size (D_h) of 39 nm and constituting > 80 % of the total population by scattered light intensity. The larger-sized distribution at ~ 500 nm is likely due to a small amount of micelle aggregates. Figure S2 shows a representative plot of micelle size distributions at 25 °C. Note that the apparent D_h of the smaller-sized micelles was similar to that of PEO_{113}-*b*-$PDPAEMA_{31}$ (31 nm at room temperature). As the temperature was gradually increased from 25 to 65 °C, the dominant micelle size distribution underwent little change with the average apparent size remaining between 35 and 40 nm (see Figure S3). At 45 °C, a small amount of aggregation occurred, evidenced by the appearance of a third distribution at ~ 5000 nm which persisted at all higher temperatures studied and coincided with a decrease in the intensity of the distribution at ~ 500 nm. The distribution at ~ 5000 nm constituted < 5 % of the total population by scattered light intensity. Figure S4 shows a representative plot of the size distribution of the sample at 65 °C. The 35-40 nm micelle population persisted to be the dominant distribution throughout the studied temperature range, remaining at least of 80 % of the total population by scattered light

intensity. This variable temperature DLS study indicated that the micelles of PEO-b-PDPAEMA are stable in the temperature range from 25 to 65 °C.

Reference

1. Zhou, K.; Wang, Y.; Huang, X.; Luby-Phelps, K.; Sumer, B. D.; Gao, J. *Angew. Chem., Int. Ed.* **2011**, *50*, 6109 –6114.

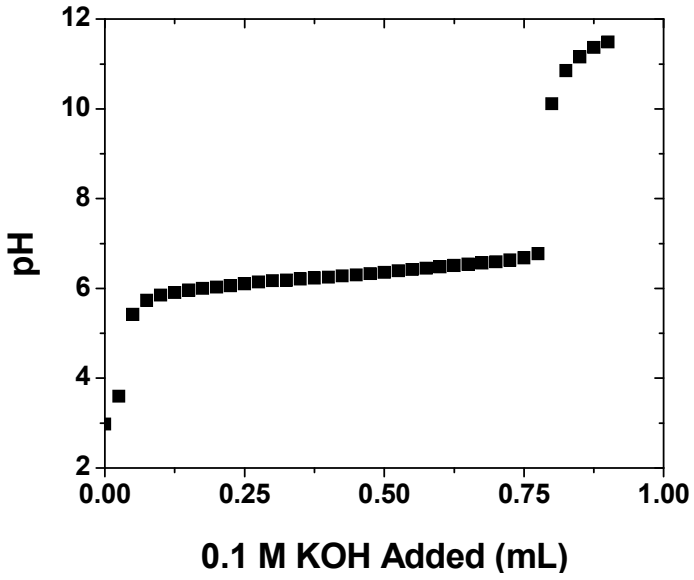

Figure S1. Titration of a solution of homopolymer $PDPAEMA_{38}$ in 0.1 M HCl with a 0.1 M KOH solution.

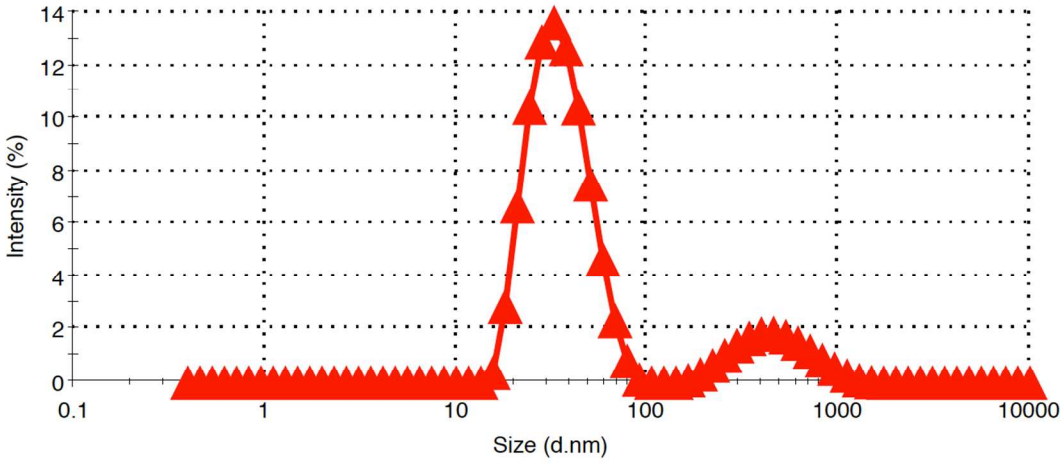

Figure S2. Plot of the size distribution by scattered light intensity from dynamic light scattering analysis at 25 °C of a 1.0 mg/g solution of PEO_{113}-*b*-$PDPAEMA_{36}$ micelles in a 1× pH = 7.4 PBS aqueous buffer.

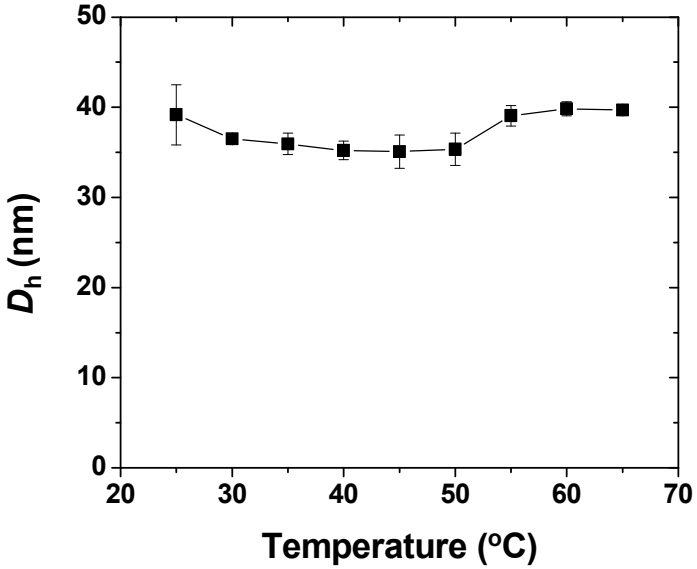

Figure S3. Apparent hydrodynamic diameter (D_h) of the dominant size distribution (> 80% by scattered light intensity) of PEO_{113}-*b*-$PDPAEMA_{36}$ micelles as a function of temperature. Error bars represent the standard deviation.

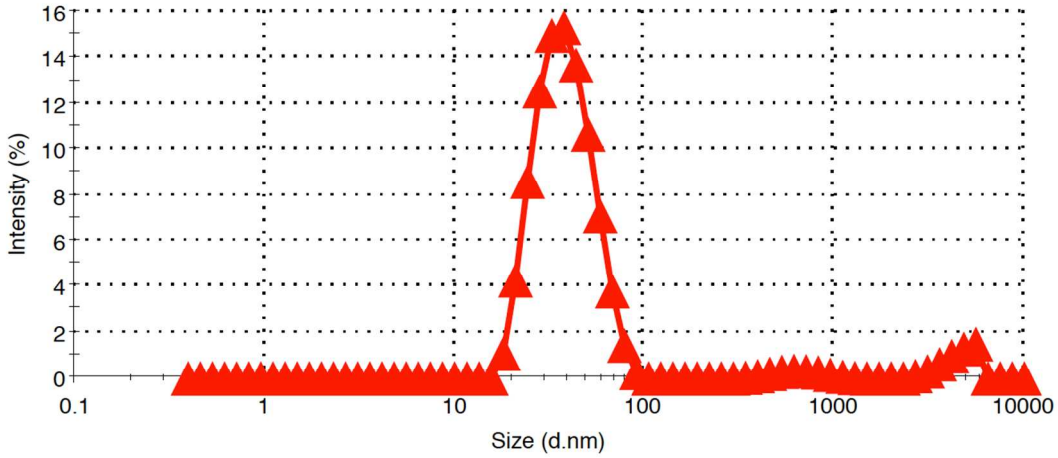

Figure S4. Plot of the size distribution by scattered light intensity from dynamic light scattering analysis at 65 °C of a 1.0 mg/g solution of PEO_{113}-*b*-$PDPAEMA_{36}$ micelles in a 1× pH = 7.4 PBS buffer.

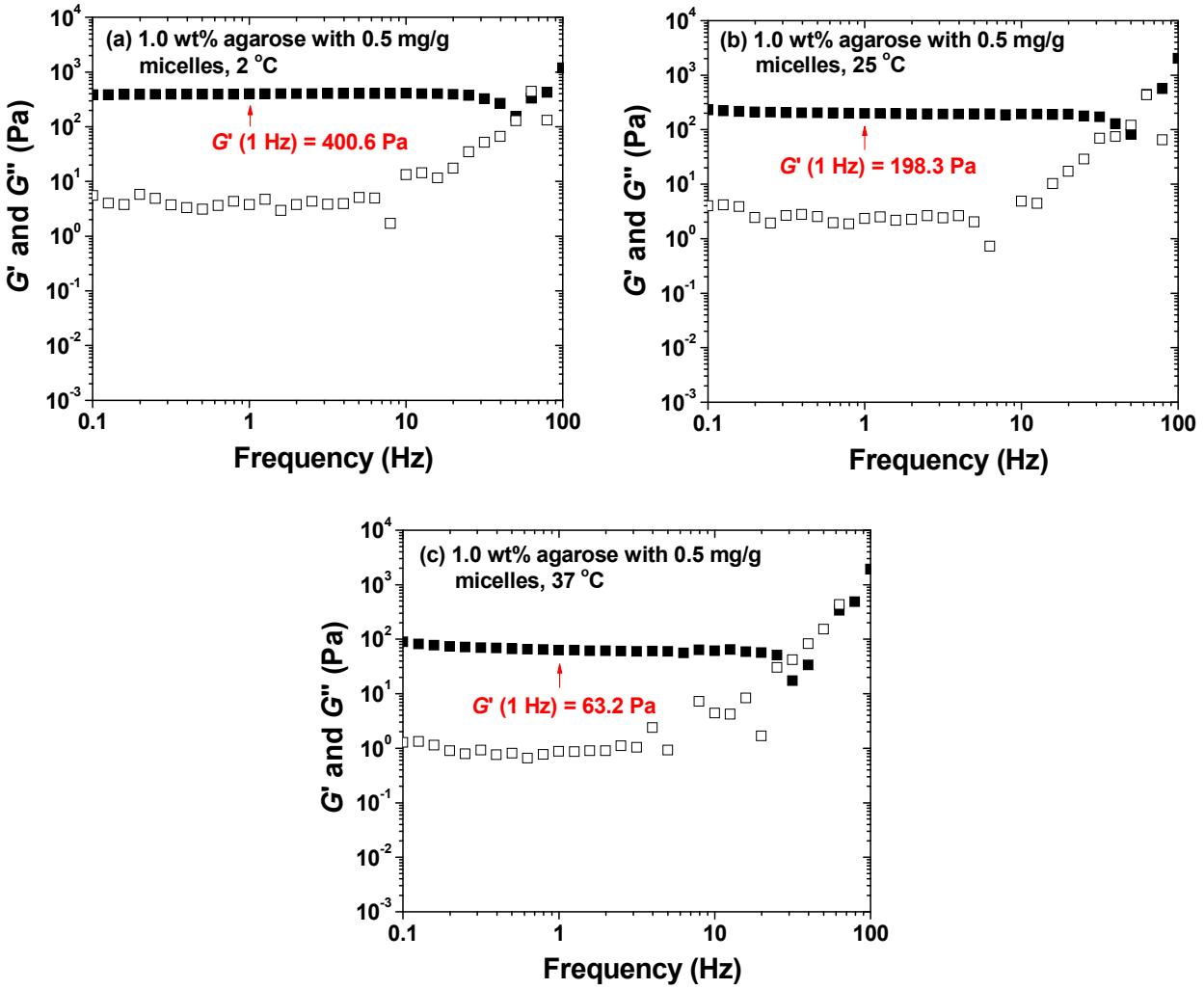

Figure S5. Frequency dependences of dynamic storage modulus G' and loss modulus G'' experiments of a 1.0 wt% agarose hydrogel embedded with 0.5 mg/g PEO_{113}-*b*-$PDPAEMA_{31}$ micelles at (a) 2, (b) 25, and (c) 37 °C. A strain amplitude of 1 % was used.

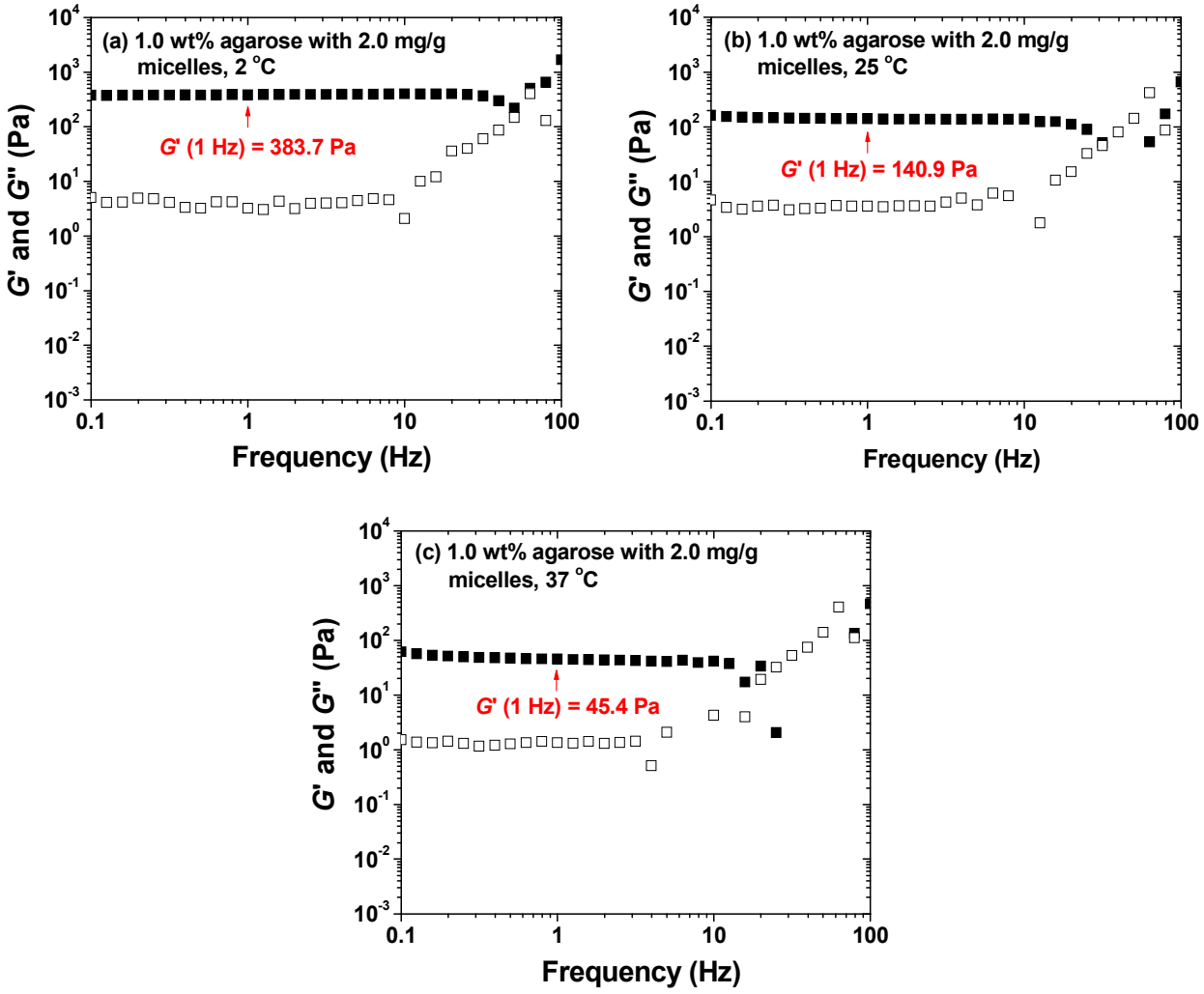

Figure S6. Frequency dependences of dynamic storage modulus G' and loss modulus G'' experiments of a 1.0 wt% agarose hydrogel embedded with 2.0 mg/g PEO_{113}-*b*-$PDPAEMA_{31}$ micelles at (a) 2, (b) 25, and (c) 37 °C. A strain amplitude of 1 % was used.

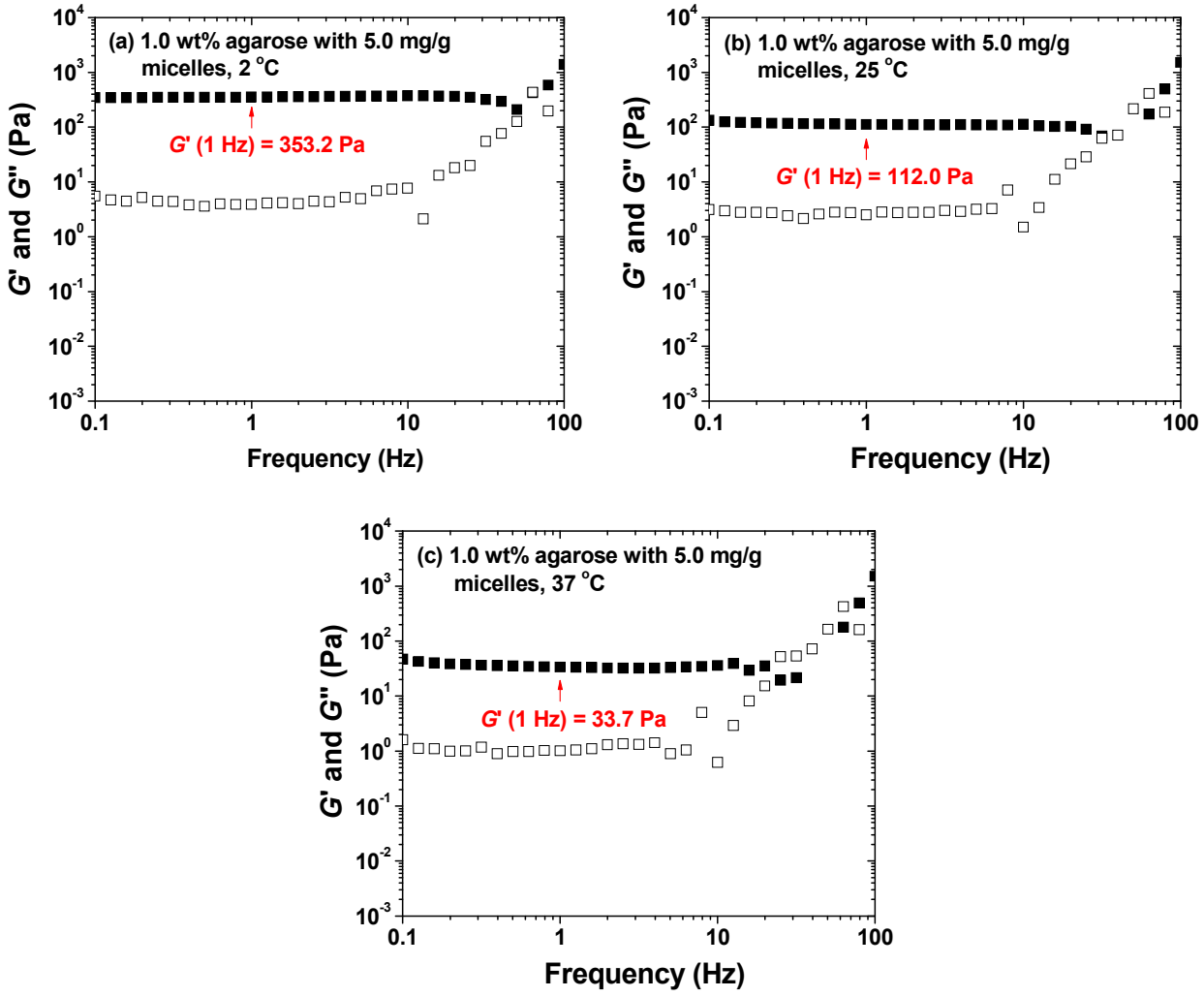

Figure S7. Frequency dependences of dynamic storage modulus G' and loss modulus G'' experiments of a 1.0 wt% agarose hydrogel embedded with 5.0 mg/g PEO_{113}-*b*-$PDPAEMA_{31}$ micelles at (a) 2, (b) 25, and (c) 37 °C. A strain amplitude of 1 % was used.