Supporting Information

Protein Crystallization in an Actuated Microfluidic Nanowell Device

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This supplementary information contains Figures S-1, S-2, and S-3 which are referred to in the main text in appropriate sections. We show a numerical model illustrating the negative effect omitting the valve has on the concentration gradient over time, as well as the negligible difference in the concentration gradient of lysozyme compared to fluorescein since diffusion coefficients do not significantly impact device filling. Additionally, we show the modeled viscosity profile utilized for the Phycocyanin/PEG crystallization analysis in Figure 5 of the main manuscript and provide specific details of the model.

Figures S-1 shows the concentration changes in the device over a period of 10 days when valves are not included to seal the nanowells. Results were obtained with the *COMSOL* model described in the main manuscript, but allowing diffusion throughout the entire device after filling.

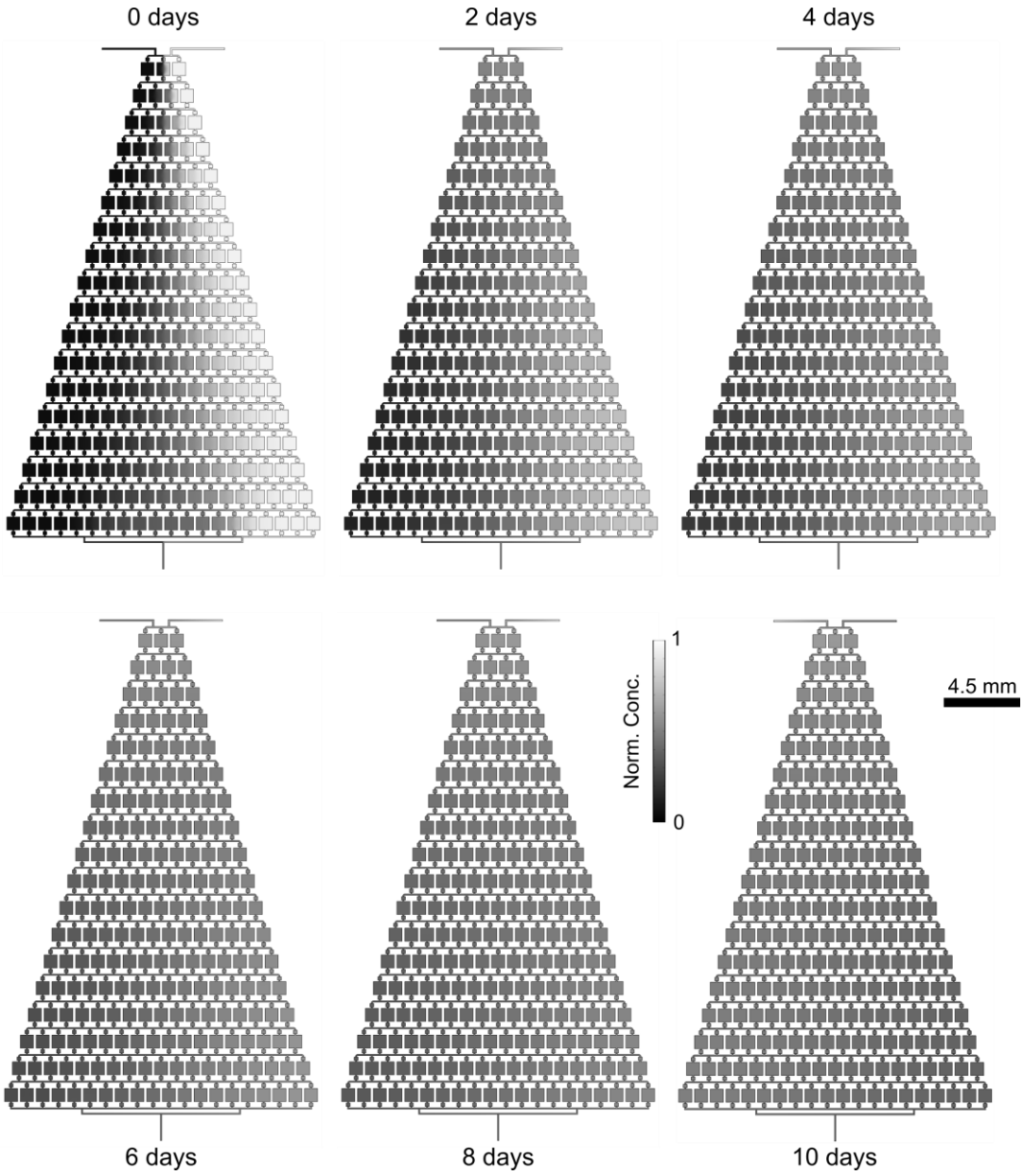

Figure S-1: Modeled concentration profile time series with open valves over 10 days showing a concentration change throughout the device until an equilibrium normalized concentration of 0.5 is eventually reached due to diffusion. This occurs when all channels are open to one another without the valve system. A globally changing concentration gradient would not allow for individual crystallization trials to occur with well-defined conditions.

Figure S-2 shows the normalized concentration distributions of lysozyme (protein) and NaCl (precipitant) as obtained from the *COMSOL* model described in the main manuscript. The concentration gradient created for both the protein and precipitant is clearly visible.

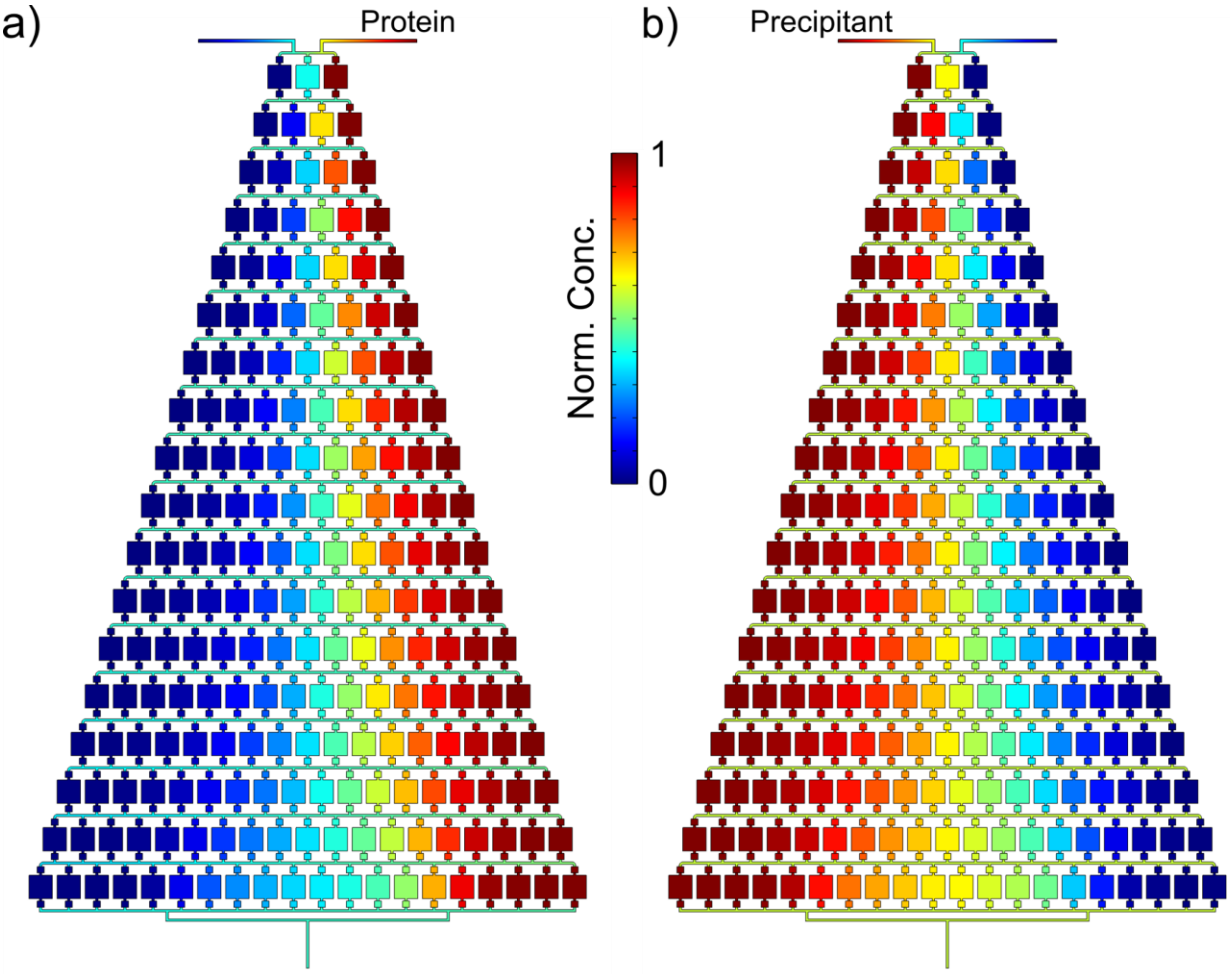

Figure S-2: Modeled concentration profiles of (a) lysozyme and (b) NaCl precipitant with inlets marked accordingly. Models were set up as described in the Experimental section of the main manuscript using a constant value for viscosity (assumed as water).

Figure S-3 shows the viscosity profile obtained with and without the adapted *COMSOL* model taking into account a ~ 6 fold larger viscosity of the PEG-based precipitant compared to the protein solution. Changes to the model defined in the Experimental section of the main manuscript are described below.

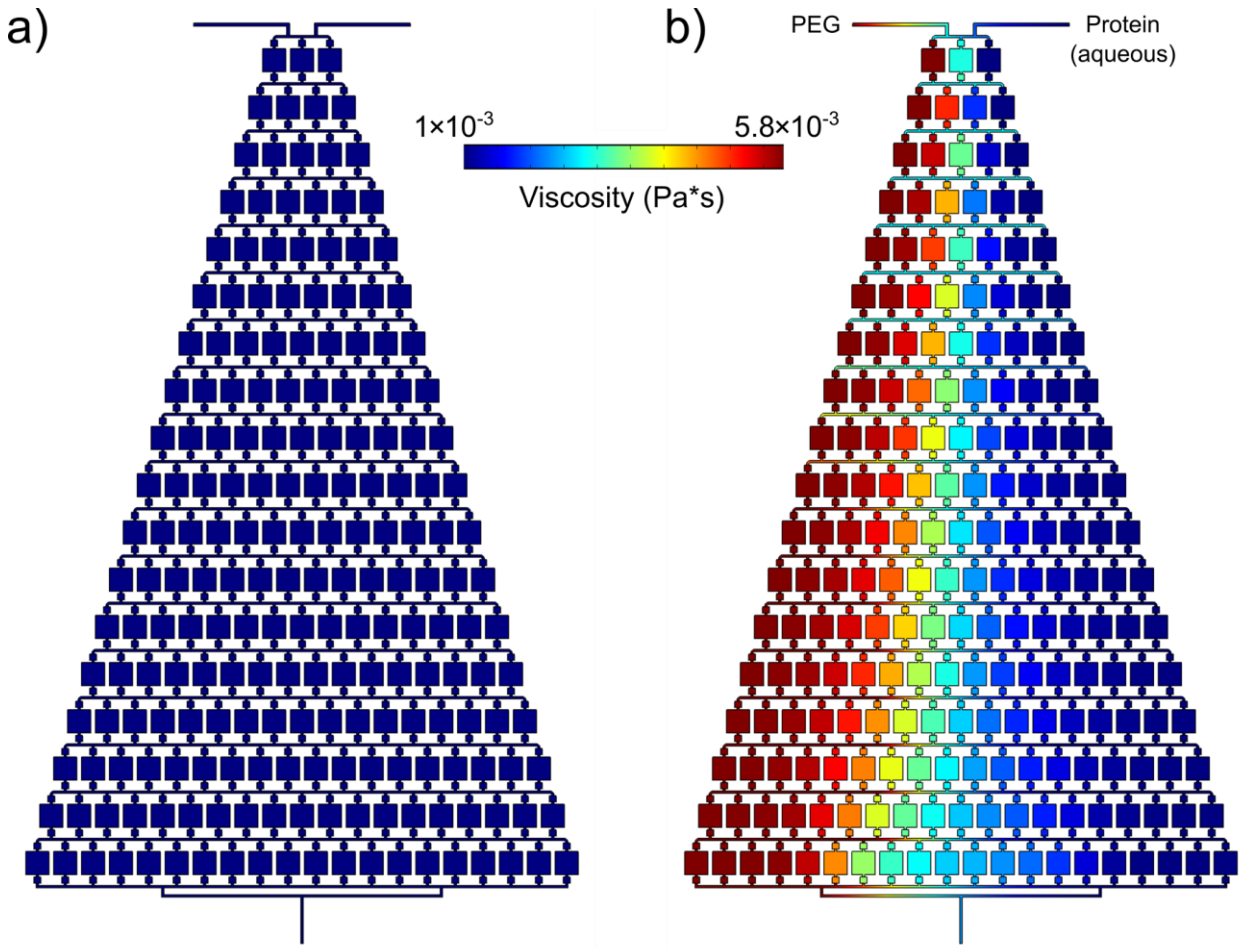

Figure S-3: Modeled viscosity profile (a) without considering any significant viscosity difference between the inlet solutions and (b) considering the significant difference in viscosity between the phycocyanin protein solution and the PEG-based precipitant. Due to viscous resistance, the lower viscosity protein solution has a more significant contribution to the profile.

Modeling was performed as described in the Experimental section of the main manuscript, but the solution viscosity was changed in the *Creeping Flow* module in *COMSOL* from a constant value to the following viscosity blending function:[1]

$$\ln(\eta) = x_1 \ln(\eta_1) + x_2 \ln(\eta_2)$$

where η is viscosity and x is the fraction of the component. The numbers 1 and 2 denote Components 1 and 2, respectively. Component 1 was designated as aqueous phycocyanin protein solution with a viscosity of 0.001 Pa·s and Component 2 was designated as 17.5% w/v PEG3350 with a viscosity of 0.0058 Pa·s as calculated by fitting data from Ninni *et al*.[2]

Appropriate diffusion coefficients were also applied to the *Transport of Diluted Species* module in *COMSOL*. The diffusion coefficient of Phycocyanin[3] was set to 4.73×10^{-11} m^2/s and PEG[4] was set to 1.92×10^{-10} m^2/s.

References

(1) Papaioannou, D.; Evangelou, T.; Panayiotou, C. *J. Chem. Eng. Data* 1991, *36*, 43-46.

(2) Ninni, L.; Burd, H.; Fung, W. H.; Meirelles, A. J. A. *J. Chem. Eng. Data* 2003, *48*, 324-329.

(3) Kato, M.; Lee, W. I.; Eichinger, B. E.; Schurr, J. M. *Biopolymers* 1974, *13*, 2293-2304.

(4) Shimada, K.; Kato, H.; Saito, T.; Matsuyama, S.; Kinugasa, S. *J. Chem. Phys.* 2005, *122*, 244914.