Investigating effects of local environment on bottlebrush conformations using super-resolution microscopy

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Supporting Information

Table of Contents

1. Solvent vapor annealing chamber

To perform *in situ* experiments on solvent effects on bottlebrush conformations, a custom solvent vapor annealing chamber was built to fit directly on the microscope setup. Photographs and diagrams detailing this setup are found in Figure S1.

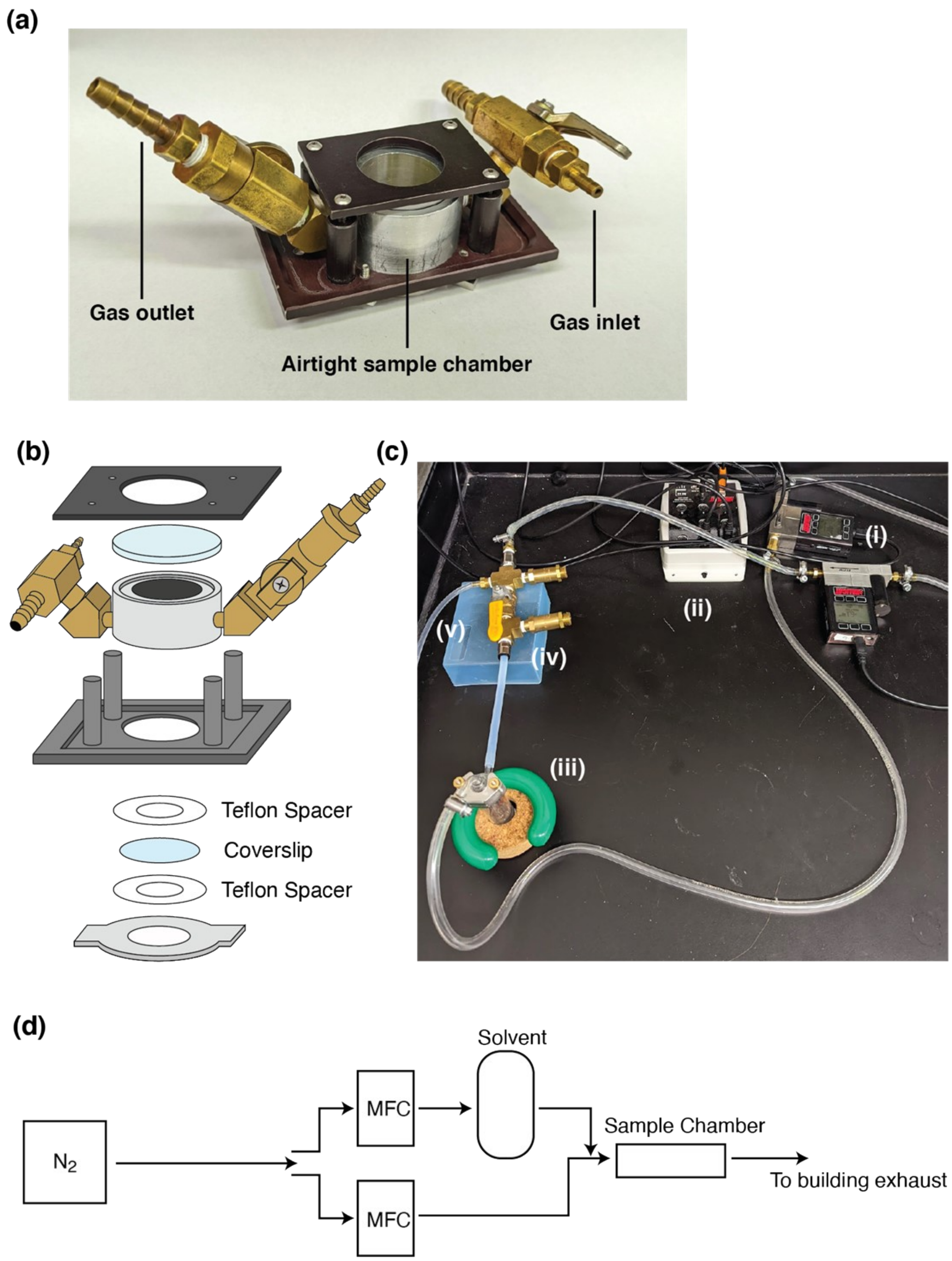

Figure S1: **(a)** Custom coverslip holder for *in situ* imaging on microscope. This chamber is airtight and allows for swelling samples with solvent. **(b)** Deconstructed view of coverslip holder. The coverslip is inserted between two sheets of Teflon for cushion against the hard metal of the chamber. The chamber is hold together tightly by screws. **(c)** Picture of the solvent vapor set up. The setup is comprised of (i) two MFCs, (ii) a connection to a computer, (iii) a gas washing bottle (Wilmad LabGlass) that holds the solvent, (iv) a connecting joint between the dry and solvent vapor lines, and (v) a line to the sample chamber. **(d)** Flow diagram depicting solvent vapor through the microscope sample chamber. In-house nitrogen is flowed through to two mass flow controllers (MFCs). One MFC leads directly to the sample chamber, while the other flows through a solvent reservoir to produce saturated gas within the system. These two lines combine before reaching the chamber. An outlet from the chamber flows directly to the building exhaust.

2. GPC of Cleaved Side Chains

The side chains were cleaved by methanolysis of the ester group along each chain, as previously described.[1,2] Traces for the unlabeled bottlebrushes are shown in Figure S2.

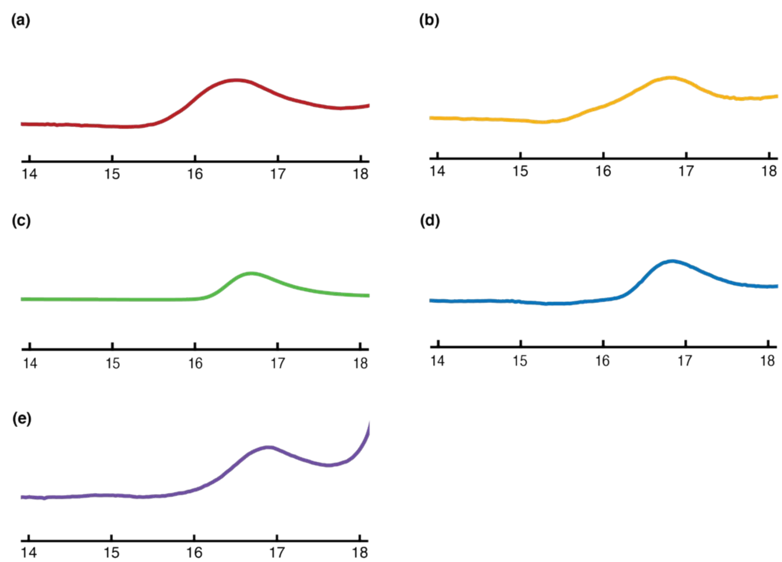

Figure S2: GPC traces of cleaved unlabeled bottlebrush polymers. Polymers were run in chloroform as the mobile phase. The number average molecular weights and dispersities of each are **(a)** M_{sc} = 4,020 g/mol, $Đ_{sc}$ = 1.23, **(b)** M_{sc} = 2,700 g/mol, $Đ_{sc}$ = 1.82, **(c)** M_{sc} = 2,100 g/mol, $Đ_{sc}$ = 1.58, **(d)** M_{sc} = 1,000 g/mol, $Đ_{sc}$ = 1.23, and **(e)** M_{sc} = 750 g/mol, $Đ_{sc}$ = 1.36.

3. Quartz Crystal Microbalance Measurements

Quartz crystal microbalance (QCM) data was used to confirm the swelling ratio at each solvent condition used for *in situ* imaging. QCM measures the change in frequency as the mass of the sample changes. QCM measurements were taken as the piezoelectric quartz crystal was encased within an airtight gas chamber, where the conditions within the chamber could be varied using a similar solvent/MFC described in Figure S1. Figure S3 shows the chamber setup.

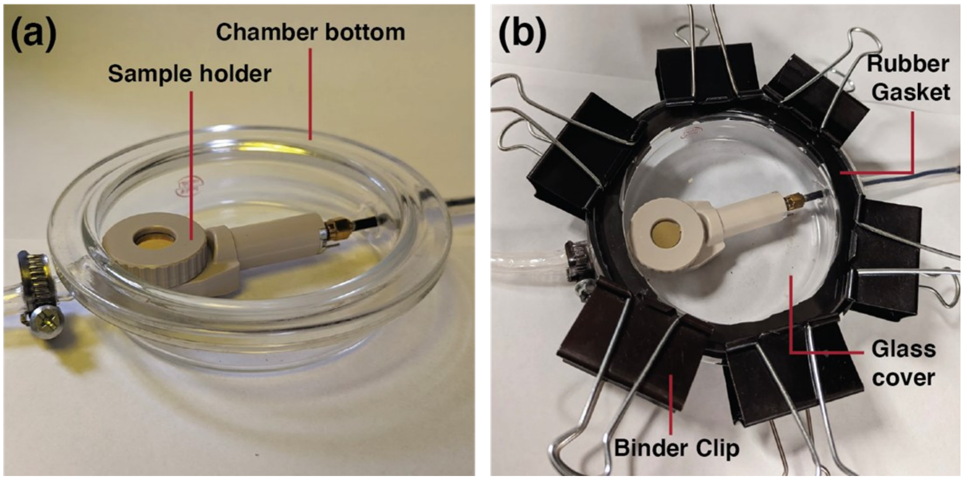

Figure S3: Images of the solvent chamber used for QCM measurements for swelling films. (a) provides an unobstructed view of the chamber. Inside is the holder for the crystal, where it can be directly connected to the instrument. (b) shows the enclosed chamber, which is held tight with binder clips. A gasket is used to ensure an airtight system.

Figure S4 displays an example QCM experiment. Here, a 300 nm thick PMMA film is swollen with toluene at various increments to capture the mass change. The experiment is first equilibrated until it reaches a stable reading. Once equilibrium is reached, the solvent conditions are changed. Here, the percentage values reflect the relative vapor pressure of toluene in the nitrogen flow. After each solvent condition is adjusted, then kept at this value for approximately 20 hours. The mass increase equilibrates at approximately 10 hours. Based on this time scale, we using Fick's laws on diffusion determine that a 40 nm thick film, which reflects imaging conditions, should reach equilibrium under the same concentration conditions within one hour. We have chosen to ensure equilibrium by swelling films for *in situ* imaging experiments for 2 hours.

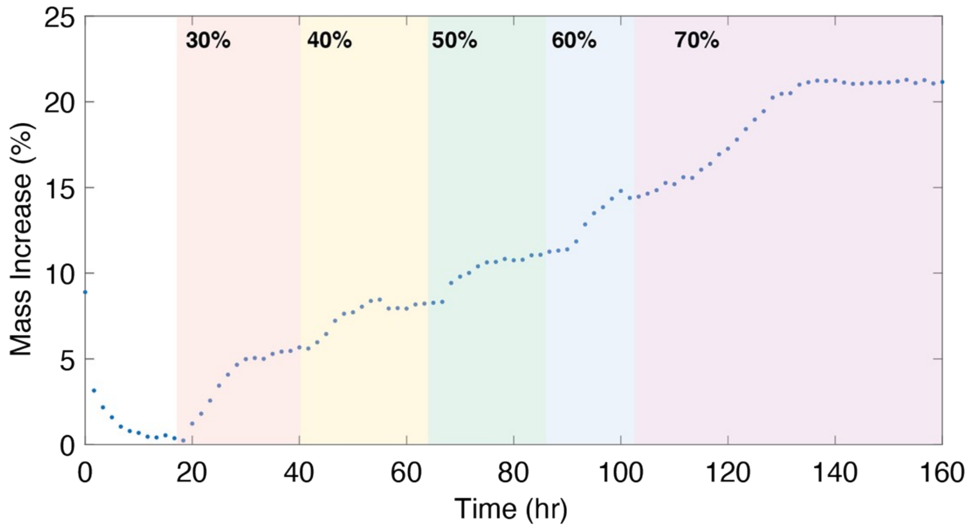

Figure S4: Representative QCM data for a swelling experiment. Here, a film of 300 nm thickness is swelled incrementally by toluene, with relative pressure of toluene ranges from 30%-70%.

4. Super-resolution Images Plotted as Heat Maps

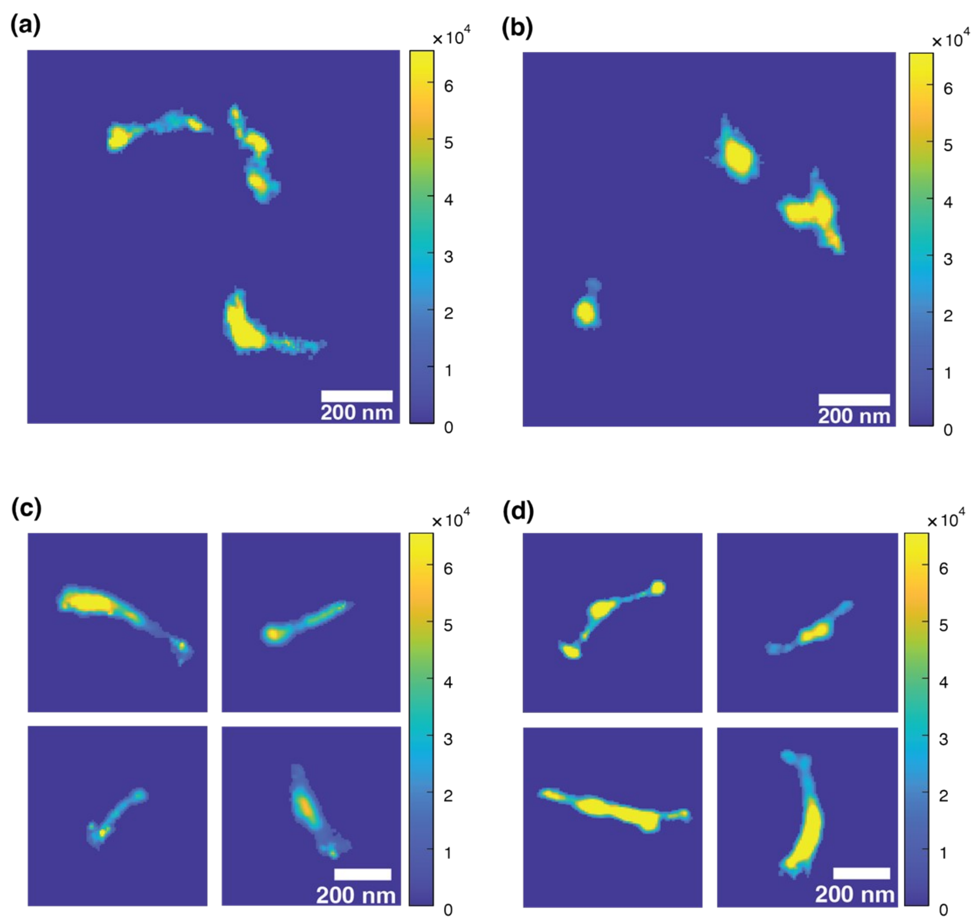

Figure S5: Super-resolution images from Figures 1 and 5 as heat maps. (a) and (b) represent bottlebrush polymers in a linear polymer matrix before and after swelling by toluene, respectively. (c) shows representative images of some bottlebrush features within a linear matrix swollen by toluene. (d) displays representative images of bottlebrushes in a bottlebrush matrix. Color bars are in arbitrary intensity units.

Comment [JC]: Reviewer 2, Comment 2

References

(1) Chan, J. M.; Wang, M. Visualizing the Orientation of Single Polymers Induced by Spin-Coating. *Nano Lett.* **2022**. https://doi.org/10.1021/acs.nanolett.2c01830.

(2) Chan, J. M.; Kordon, A. C.; Zhang, R.; Wang, M. Direct Visualization of Bottlebrush Polymer Conformations in the Solid State. *Proc. Natl. Acad. Sci.* **2021**, *118* (40), e2109534118, 1–9. https://doi.org/10.1073/pnas.2109534118.