Controlled Molecular Assembly of Tetrazine Derivatives on Surfaces

Shuo Wang¹, Shuang Liu², Audrey Sulkanen¹, Joseph M. Fox³, Xinqiao Jia^{2*} & Gang-yu Liu^{1*}

¹Department of Chemistry, University of California, Davis, CA 95616, ²Department of Material Science and Engineering, University of Delaware, Newark, DE 19716, ³Department of Chemistry and Biochemistry, University of Delaware, Newark, DE 19716

*Corresponding authors: gyliu@ucdavis.edu; xjia@udel.edu Introduction

limitation makes it difficult to achieve molecular assembly by design

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While self-assembly is relatively well-known and widely used to form hierarchical structures and thin film coatings, controlled assembly is less known and utilized. Our prior work has demonstrated the concept of controlled assembly of macromolecules such as star polymers [molecular weight M_w) ~383 kDa, hydrodynamic radius $R \sim 13.8$ nm] in droplets. This work extends this concept to smaller molecules, in this case, poly(ethylene glycol) bis-tetrazine (PEGbisTz, M_w 8.1 kDa, R ~1.5 nm). The key to controlled molecular assembly is to first deliver ultrasmall volumes (sub-fL) of solution containing PEG^{bis}Tz to a substrate. The solvent evaporates rapidly due to the minute volume, thus forcing the assembly of solute, whose overall size and dimension are dictated by the initial liquid geometry and size. Using prepatterned surfaces, this work revealed that the initial liquid shape can be further tuned, and we could control the final assembly of solute such as PEGbisTz molecules. The degree of control was demonstrated by varying the micropatterns and delivery conditions. This work demonstrated the validity of controlled assembly for PEG-bisTz and enables

three-dimensional (3D) nanoprinting of functional materials. The technology has promising applications in nanophotonics, nanoelectronics, nanocomposite materials, and tissue engineering.



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using SA alone. Thus, new approaches need to be developed to reach "molecular assembly by design," thereby enabling production of a wider range of structures and materials with designed properties and functions.

Controlled assembly to produce zero-dimensional (0D) assemblies has been demonstrated by our team using macromolecules, including star polymers [molecular weight (M_w) of 383 kDa,

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Assembly of molecules into micro- and nanoscale structures by design still poses a great challenge.¹ Selfassembly (SA) of molecules provides one good means to assemble molecules into mesoscale structures, such as self-assembled monolayers (SAMs),²⁻⁴ phase-separated polymers,^{5,6} molecular brushes,⁷ block copolymers,⁸ and lipid bilayers.^{9,10} While powerful, SA is limited to structures or molecular assemblies that are favored thermodynamically.^{11,12} This

hydrodynamic radius of 13.8 nm].¹³ The study indicated that factors governing the solute assembly in ultrasmall droplets significantly differs from that of larger droplets because ultrasmall droplets exhibit

ultrafastevaporation; and therefore, the initial droplet geometry and concentration of the solute play dominant roles in dictating the final assembly of solute molecules. The applicability of this methodology to the assembly of low M_w molecules, such as small linear polymers, has become our new task. Extending the concept of controlled assembly to smaller molecules is significant for several reasons: they are generally easier to synthesize, less expensive, more stable, and more readily available than macromolecules. Thiswork applies the concept of controlled assembly to poly(ethyleneglycol)bistetrazine(PEG-bisTz), with Mwof8.1kDaand dynamic radius near 1.5 nm.14,15 Functionalized PEGs have been widely used for surface modification and for the synthesis of complex molecules and networks.^{16,17} Moreover. the tetrazine functionality within the molecule is known to participate in tetrazine-ligation with trans-cyclooctene (TCO) derivatives.¹⁵ These bio-orthogonal inverse electron demand Diels-Alder reactions occur fast and require no additional catalysts.^{18–22} These reactions are also clean, producingnitrogengasastheonlyby-product,^{18–22} andthusare utilized as an important tool for important tool for producing extracellular matrix mimetic hydrogels and fibers. 15,23,24 TofurtheradvancetheapplicationsofPEG-bisTzandthreedimensional (3D) nanoprinting technology, this study demonstrates the concept of controlled molecular assembly of PEG-bisTz from 0D to 3D, combining our methodology of using ultrasmall droplets (sub-aL) and surface patterning. The level of control reported in this work paves the way for future applications including 3D nanoprinting, aswellasprogrammableandengineeredmeanstoproduce complex and hierarchical structures of materials.25-27

Experimental Methods Materials

Glass slides (22 mm × 22 mm × 0.25 mm) were purchased from Thermo Fisher Scientific (Pittsburgh, PA). Sulfuric acid (H₂SO₄, 95.0%-98.0%), hydrogen peroxide (H₂O₂, 30% aqueous solution), ammonium hydroxide (NH₄OH, 30% aqueous solution). tetrahydrofuran (THF), and toluene (99.8%) were purchased from Sigma-Aldrich (St. Louis, MO). N-(6-Aminohexyl)aminopropyltrimethoxysilane (AAPTMS) was purchased from Gelest Inc., (Morrisville, PA). Ethanol (99.5%) was purchased from Koptec (King of Prussia, PA). Milli-Q water (MQ water, 18.2 M Ω ·cm at 25 °C) was produced by a Milli-Q water purification system (EMD Millipore, Billerica, MA). Nitrogen gas (99.999%) was purchased from Praxair, Inc. (Danbury, CT, King of Prussia, PA). Deuterated chloroform (CDCl₃) was purchased from Cambridge Isotopes (Tewksbury, MA).

Synthesis of PEG-bisTz and C12-bisTCO

PEG-bisTz and dodecane bis-trans-cyclooctene (C12bisTCO) used in this study were synthesized following our previously reported methods using PEG-diamine with a $M_{\rm w}$ of 7.5 kDa and 1,12-diaminododecane,

respectively.15,28,29

Preparation of surfaces as support for material delivery

Glass slide substrates were first cleaned following established protocols.³⁰ Briefly, slides were soaked in piranha solution for 1 h, and then rinsed with a copious amount of MQ water. The slides were then treated with a basic bath [mixture of NH₄OH, H₂O₂, and H₂O at a ratio of 5:1:1 (v/v/v) for 1 h at 70 °C and then rinsed with a copious amount of MQ water followed by drying under nitrogen gas. Cleaned slides were modified using silane chemistry according to an established protocol.³¹ SAMmodified glass slides were prepared as follows: slides were heated at 70-80 °C in a sealed Teflon container (100 mL) containing 200 μL of AAPTMS for 2 h, then rinsed with ethanol and MQ water, and then dried again under nitrogen gas.13 PEG-bisTz coated glass slides were prepared as follows: 20 μ L of 2.5 \times 10^{-4} M PEG-bisTz aqueous solution was deposited by 100 μL Eppendorf pipette (SigmaAldrich, St. Louis, MO) on AAPTMSmodified glass surfaces and dried under ambient conditions for 30 min.

Contact angle measurement

Contact angle data of water and PEG-bisTz solution were both collected for the modified substrates with a VCA Optima Contact Angle Measurement system (AST Products, Billerica, MA) per standard protocols. For water contact angle measurement, typically, a 2 μ L drop of MQ water was placed on the substrate using a highperformance liquid chromatography (HPLC) needle. At least three different positions per sample were studied to confirm the surface wettability. For PEG-bisTz solution contact angle measurement, an identical procedure was applied except that MQ water was replaced with

 2.5×10^{-4} M PEG-bisTz aqueous solution.

Integrated atomic force microscopy and microfluidic device

A microfluidic system (FluidFM Bot; Cytosurge AG, Glattbrugg, Switzerland) was integrated with an atomic force microscopy (AFM) assembly, allowing precise position and movement control, and an inverted optical microscope (IX73; Olympus America, Center Valley, PA).^{32,33} Solution delivery was carried out using a FluidFM Nanopipette (CYPR/001511; Cytosurge AG) with a 300 nm opening. The cantilever resembled typical AFM silicon probes: 200 µm long, 36 µm wide, and 1.5 µm thick. The spring constant was 2 N/m. The nanopipette was made with a microchannel connected to a reservoir and controlled by a precise pressure controller. The pressure control system enabled a pressure application range from –800 to +1000

mbar at a 1 mbar precision. Initially, 1 μ L of PEG-bisTz solution was filled into the probe's 2 μ L reservoir using a Hamilton 7000 series syringe (Hamilton, Reno, NV).

AFM imaging

PEG-bisTz structures deposited on the surface were dried at room temperature for 30 min before being characterized by AFM (MFP-3D; Oxford Instrument, Santa Barbara, CA). Silicon probes (AC 240-TS; Olympus America, Central Valley, PA) with a force constant of 1.7 N/m and resonant frequency of 70 kHz were used. Topography images were acquired using tapping mode. The driving frequency and damping were set at 70 kHz and 40%, respectively. Images were analyzed using Asylum MFP-3D software on an Igor Pro 6.12 platform. Particularly, the initial volume of droplets deposited on the AAPTMS surface was calculated based on the total number of PEG-bisTz molecules in the spherical cap (total solid volume calculated based on the AFM measured height and diameter per individual PEG-bisTz volume) and the initial concentration in the solution.

Results and Discussion

Controlled assembly of PEG-bisTz in individual ultrasmall droplets

The surfaces used were clean glass modified by SAMs of AAPTMS. The water contact angles on this SAM measured 55.0° ± 1.2°, which is consistent with known values for high-quality SAMs with amine termini.³⁴ The contact angle of 2.5 \times 10⁻⁴ M PEG-bisTz aqueous solution measured 47.0° \pm 1.3°. Thus, the SAM surface is both hydrophilic and "solvophilic." The structure of PEG-bisTz (M_w = 8.1 kDa) is depicted in Figure 1. The reduced contact angle of PEG-bisTz solution compared with water is likely due to the stronger interaction of PEG-bisTz solution with the AAPTMS SAMs than pure water. This strong attachment of PEG-bisTz to the AAPTMS surface was also verified independently by subjecting a dried droplet of PEGbisTz on AAPTMS to a thorough rinsing with water. Despite the hydrophilic nature of PEG, an approximately 1 nm thick disk of PEGbisTz remained after the rinse, demonstrating the stability of the PEGbisTz assembly on the AAPTMS surface. These thin layers of PEGbisTz exhibited strong adhesion to the AAPTMS surface, as also revealed by consistent and stable contact angle measurements.

Delivery of an ultrasmall droplet was achieved with the microfluidic–AFM delivery system, as illustrated schematicallyinFigure1.Thelateralpositionofdeliverywasprecisely controlledwithamovementrangeof240mm×74mmand precision of 100 nm. An inverted optical microscope with a 10X objective was utilized to monitor the position of the probe and solution delivery. The vertical or z movement moved with 4 nm precision and was independent from lateral movement. The glass slides were placed on the sample stage, above which the probe was mounted to the vertical assembly controlling z movement. Typical probesurface contact during delivery is also shown in Figure 1, where the contact

DOI: 10.31635/ccschem.021.202101088 CCS Chem. 2021, 3, 1789–1799 force was measured and controlled via similar means as conventional AFM with a deflection configuration.^{13,32,35} The square pyramidal tip tilted 11° from the surface normal. The nanopipette probe had a 300 nm diameter pore located at the probe apex connected to a microchannelwithintheprobebody.Themicrochannelwas connected to a small reservoir where the PEG-bisTz aqueous solution was stored. This setup enabled delivery of aqueous droplets as small as 0.4 attoliter (aL).¹³

First, we demonstrated that the geometry and size of the individual and concrete assemblies can be controlled with PEG-bisTz molecules tightly packed within. Under a contact force of 40 nN, delivery pressure of 200 mbar, and contact time of 0.2 s, 250 aL droplets of PEG-bisTz solution were delivered on the AAPTMS SAM surfaces, as shown in Figure 2a. An expanded view of one feature is displayed in Figure 2b, where PEG-bisTz molecules assembled into a tiny spherical cap with height and base diameter of 73 and 860 nm, respectively. Further expansion of a 500 nm × 500 nm area atop the cap (Figure 2c) shows a relatively smooth morphology decorated by 16 bumps approximately 1.8 nm in height with width ranging from 15 to 35 nm. These bumps are likely due to clustering of PEG-bisTz molecules. This geometry and the packing of the PEG-Tz solid can be rationalized by the initial liquid droplet geometry and the evaporation mechanism: (a) the contact angle of the droplet, 47°, dictated the overall geometry of the droplet and contact "footprint," that is, a short spherical cap and circle, respectively, and (b) the solution-surface interaction also determined the evaporation profile, in this case, fast and constant-area evaporation. Since the quantity of tetrazine in the droplet exceeded a monolayer within the footprint, the final feature was a short spherical dome, with PEG-bisTz molecules closely packed, and interdigitated within, as shown in Figure 2. The 860 nm diameter base of the cap was dictated by the initial spreading of the droplet, and the number of PEG-bisTz molecules within the mound was determined by the concentration and volume. The intrafeature morphology is also consistent with clustering of PEG-bisTz because of constant-area evaporation, 36,37 that is, freezing of PEG-bisTz in the dynamically confined space. These observations are highly reproducible, as we made 11 × 11 arrays of PEG-bisTz assemblies (Figure 2a),



Figure 1 | Schematic diagram of an integrated atomic force microscope (AFM) with microfluidic delivery probe. An enlarged view illustrates the probe–surface contact. The chemical structure of solute, PEG-bisTz, is also shown. The inset at the lower left is a scanning electron microscopy (SEM) image of the nanopipette apex with a 300 nm aperture.

Scale bar is 500 nm.

where feature dimensions are within 2%, all have spherical cap geometry, and their morphologies resemble that shown in Figure 2c. These observations indicate that upon using ultrasmall droplet and rapid evaporation, spherical cap-shaped assemblies were produced with tightly packed, internal PEG-bisTz molecules. In other words, the initial droplet shape dictates the outcome of the individual and concrete assemblies of molecules due to fast evaporation. The concept of controlled assembly using small liquid droplets was first reported by our team using macromolecules (e.g., star polymers).¹³ This result extends the concept to a much smaller molecule, PEG-bisTz, which has a hydrodynamic radius of 1.5 nm.

The robustness of our approach was demonstrated by forming PEG-bisTz spherical caps with various sizes by varying delivery parameters such as contact time (t) and pressure (p). Results shown in Figure 3 correlate the increase of base and height with the increasing contact time. At contact force of 40 nN and delivery

pressure of 200 mbar, a 5 × 6 array of PEG-bisTz droplets with periodicity of 7 μ m was delivered on an AAPTMS glass surface. From the bottom to the top row in Figure 3a, the contact time increased during initial delivery, resulting in larger droplets, and therefore larger spherical caps, as summarized in Table 1. The quantitative plot is shown in Figure 3b, where both the base and height increased with increasing contact time. These observations indicate that the size of these individual and concrete 0D structures of PEG-bisTz can be controlled by varying the amount of material delivered in our approach.

Controlled assembly of PEG-bisTz during continuous delivery of solution along lines

With knowledge of the 0D delivery conditions and the corresponding geometry of the resulting PEG-bisTz features, we moved forward to delivering solutions while moving the surface along a line, thereby creating a one-



Figure 2 | (a) An atomic force microscopy (AFM) topographic image of an array of PEG-bisTz spherical caps. (b) An expanded view of a single feature as indicated in (a). The top inset is the corresponding cursor profile. (c) A further expanded view from the region as indicated in (b).

Figure 3 | (a) Atomic force microscopy (AFM) topographic image of an array of PEG-bisTz spherical caps created with various dwell times from 0.1 to 5 s under pressure of 200 mbar. (b) Plot showing the variation of the dimensions of PEG-bisTz spherical caps with dwell time.

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(µm)



Table 1 The PEG-bisTz Spherical Caps Formed via Controlled Assembly in Correlation With Contact Time							
Dwell Time (s)	Pressure (mbar)	Force (nN)	Volume (aL)	Height (nm)	Base Diameter (µn		
0.10	+200	40	82 ± 5	51 ± 2	0.59 ± 0.06		
1.33			474 ± 23	94 ± 2	1.04 ± 0.05		
2.56			1046 ± 45	131 ± 4	1.32 ± 0.07		
3.78			1241 ± 58	139 ± 3	1.41 ± 0.09		
5.00			1633 ± 69	157 ± 5	1.49 ± 0.07		

dimensional (1D) trajectory of solute upon drying. One could conceptually understand the 1D delivery as a delivery of droplets continuously, in contrast to delivery of discrete droplets in OD. Therefore, the OD delivery conditions were used as a guide for the desired cross-section and height of lines in the 1D delivery. As material was delivered along the 1D trajectory, the printing speed, pressure, and contact force were kept constant. Figure 4a shows an array of four lines formed at 5 $\mu\text{m}/\,\text{s}$ under a contact force of 40 nN and delivery pressure of 200 mbar using a 2.5 × 10⁻⁴ M PEG-bisTz aqueous solution. These lines were 1.13 \pm 0.02 μm wide and 45 \pm 3 nm tall, with a fan-shaped cross-section. In Figure 4b, the line arrays were produced under identical condition as that in Figure 4a, except we changed the delivery direction by 90°. Comparison of the two arrays is further displayed in Figure 4c, where the geometry and intra-line morphology are nearly identical. The base width and height of the lines printed at 0° and 90° were 1.13 \pm 0.02 μ m and 45 \pm 3 nm, 1.15 \pm 0.03 μ m and 46 \pm 3 nm, respectively. Therefore, the delivery of PEG-bisTz solution showed little dependence on the printing direction, in contrast to the printing of highly viscous materials such as photopolymers.³² These results were reproduced 40 times along various printing directions. We attribute the lack of dependence on printing direction to the low viscosity of PEG-bisTz solution, which is close to 1.0016 mPa·s, similar to that of water.38,39 The liquid geometry, upon delivery, is dictated primarily by the volume of liquid and surface tensions at the printing sites, in contrast to photopolymers, whose viscosity measure 6000 -13,500 mPa·s and that depend on delivery geometry such as directions.³² In this low

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viscosity situation, the resulting molecular assembly could be understood as equivalent to a two-step process: production of a liquid line with arched cross section, followed by rapid drying of solvent leading to the final assembly. Therefore, the initial liquid line shape and geometry dictate the overall size and geometry of the final assembly of solid.

The control over 1D delivery of liquid was also demonstrated by producing lines with various widths by tuning the delivery conditions. When movement speed was decreased while keeping load and pressure constant, lines with increasing width and height were produced, as shown in Figure 4d and quantified in Table 2. At contact force of 40 nN and delivery pressure of 200 mbar, nine PEG-bisTz lines with spacing of 10 μ m were delivered on an AAPTMS glass surface. From left to right in Figure 4d, the printing speed decreases, resulting in wider and taller lines, from 1.58 μ m and 55.8 nm to 2.84 μ m and 100.8 nm for base width and height, respectively. This dependence of line height and width on printing speed provides a 200 mbar.



Figure 4 | (a) An atomic force microscopy (AFM) topographic image of four PEG-bisTz lines from an array of 20 lines with periodicity of 5 μ m. (b) An AFM topographic image of four PEG-bisTz lines from an array of 20 lines with periodicity of 5 μ m. The stage movement in (b) is perpendicular to that in (a). (c) Combined cursor profiles from the lines indicated in (a) (red) and (b) (blue), and the schematic diagram indicates the movement directions. (d) AFM topographic image of an array of nine PEG-bisTz lines formed with increasing speed, under the delivery pressure of

means to control the size of the 1D assemblies and demonstrates the robustness of this technique. As printing speed decreases, the contact time at each delivery point increases, which is equivalent to a larger droplet being delivered at each point and results in a larger crosssection collectively along the line. With rapid, constantarea evaporation at each point, a line of solute with uniform cross-section is produced. In other words, controlled assembly was successfully demonstrated for PEG-bisTz systems to form 1D features: lines and line arrays along designed trajectories.

Table2 The PEG-bisTz Nanolines Formed via Controlled Assembly,
in Correlation with the Moving Speed of the Probe along the Line

Printing		Contact		Base
Speed	Pressure	Force	Height	Diameter
(µm/s)	(mbar)	(nN)	(nm)	(µm)

2	200	40	100.8 ± 0.6 2	.84 ± 0.06	
3	200	40	98.5 ± 0.6 2.25 ± 0.03		
4	200	40	93.6 ± 0.5 2.05 ± 0.02		
5	200	40	89.4 ± 0.5	1.95 ± 0.03	
6	200	40	81.7 ± 0.4	1.76 ± 0.03	
7	200	40	76.6 ± 0.5	1.76 ± 0.02	
8	200	40	71.1 ± 0.6	1.76 ± 0.03	
9	200	40	65.6 ± 0.6	1.65 ± 0.04	
10	200	40	55.8 ± 0.6	1.58 ± 0.04	

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The impact of surface patterning on the assembly of PEG-bisTz

Given the significance of the initial liquid shape and geometry, we have attempted various means to control the initial delivery, one of which is to use prepatterned surfaces. To investigate the effect of surface patterning on the controlled assembly of PEG-bisTz, two sets of perpendicular line arrays were printed within the same area, resulting in a grid of intersecting lines. The first set of lines was an array of 10 PEG-bisTz lines printed on an AAPTMS-glass surface and then dried under ambient conditions for 30 min. The delivery conditions were 200 mbar, 5 µm/s, and 40 nN contact force, which produced solid and homogeneous lines. Each linewas 100 µm long, 45 ± 2 nm tall, 1.12 ± 0.04 µm wide, with interline periodicity of 5 µm. Under the same conditions and design, another array of PEG-bisTz lines was printed perpendicular to the first set of lines. Figure 5a reveals a 25 \times 25 μ m area of the final structure of intersecting line arrays. The first set of lines contained continuous and homogeneous PEG-bisTz lines on an AAPTMS SAM, which was expected. The second array, however, contained broken lines, with a bulge at each

intersection, despite that both arrays were produced under identical conditions. Cursor plots along the two primary directions are displayed in Figure 5b, where the differences are clearly revealed quantitatively. The first set of lines were homogenous continuous lines with height and width of 45 ± 2 nm and $1.12 \pm 0.04 \mu$ m. The second set of lines were broken lines with bulges, with a bulge-to-bulge distance of $1.72 \pm 0.06 \mu$ m, where there was no material deposition. Figure 5c shows an angled 3D AFM image of a representative bulge at a cross section. This bulge feature measured 117 nm high, with perpendicular short axis and long axis widths of 1.82 and 3.71 μ m, respectively.



Figure 5 | (a) Atomic force microscopy (AFM) topographic image of a representative area of PEG-bisTz mesh. (b) Cursor profile of two cursors in (a). (c) 3D display of the expanded green square in (a). (d) Schematic diagram of droplet delivery on self-assembled monolayer (SAM). (e) Schematic diagram of continuous line delivery. (f) Schematic diagram illustrating the difference between hypothetical solution distribution and actual distribution, with the darker color line representing the dried line from the first printed set.

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Since PEG-bisTz solutions were delivered under identical conditions in both line arrays, the differences in the resulting structures were attributed to the prepatterning of the surface. In the case of the first array, the surface region had relatively uniform AAPTMS-glass SAM. In the case of the second array, the surface contained solid line arrays of PEG-bisTz surrounded by the AAPTMS SAM. Figures 5d–5f rationalize the impact of patterned surfaces on the resulting line geometry of PEG-bisTz. In Figure 5d, the geometry of a droplet of PEG-bisTz solution on an AAPTMS SAM is shown: a spherical cap with the contact angle of 47°. Rapid and constant-area evaporation led to assembly of PEGbisTz molecules into a short spherical cap, as discussed in Figure 2. Figure 5e illustrates the geometry of PEG-bisTz solution upon continuous delivery along a straight line on the AAPTMS SAM surface, assuming no evaporation. This cylindrical cap can be conceptually considered as the merging of discrete droplets into a line. In reality, constant-area evaporation at each point during delivery is equivalent to constant-area evaporation from the liquid line shown in Figure 5e, leading to a solid line with homogeneous height and width. Assuming no interference from the PEG-bisTz lines underneath, delivery linearly along the perpendicular direction should result in a liquid with the shape shown in Figure 5f (left). The assumption is incorrect in practice, due to stronger affinity between PEG-bisTz solution and PEG-bisTz lines than that with the AAPTMS surfaces. In fact, the PEG-bisTz solution exhibits a contact angle of 38.0 ± 1.2° atop dried PEG-bisTz, smaller than that on AAPTMS SAM (47.0 ± 1.3°). Such interactions led to accumulation of the solution at the cross-section region, as illustrated in Figure 5f (right), again assuming no evaporation. Finally, constant-area

The robustness of the aforementioned mechanism was also demonstrated experimentally, as shown in Figure 6. By changing the surface patterns, for example, decreasing the interline spacing or periodicity of the PEG-bisTz lines on AAPTMS surfaces, the geometry of the PEGbisTz solution delivered during printing of the second set of line arrays could change accordingly. With the identical printing condition as that in Figure 5a, three sets of PEG-bisTz line arrays were first produced with the interline spacing of 15, 10, and 5 $\mu\text{m},$ respectively. Each line measured ${\sim}100~\mu m$ long, 45 \pm 2 nm tall, and 1.12 \pm 0.04 μ m wide. The delivery of the second set of line arrays above the three prepatterned surfaces also followed the same conditions as those in Figure 5a. The periodicity of the second set of line arrays matched that of the lines on surfaces, that is, with the interline spacing of 15, 10, and 5 $\mu\text{m},$ respectively. As shown in Figure 6a-c, the resulting three grids reveal clear differences in material redistribution, that is, the heterogeneity of the second set of line arrays decreased. The cursor profiles in Figure 6d compare the heights of the peaks along the lines, 278 ± 10 , 166 ± 6 , and 114 ± 3 nm, with the decrease of the periodicity of the line patterns underneath. The short axis base widths also decreased, from 4.91 ± 0.16, 3.01 \pm 0.07, to 1.77 \pm 0.04 μ m, while the long axis base widths also decreased from 4.61 ± 0.03 , 4.35 ± 0.11 , to $3.64 \pm 0.08 \mu$ m. These trends are consistent with the concept of solution redistribution due to the surface patterns underneath, that is, less liquid accumulation at the cross-sectional region with decreasing interline spacing underneath.

Furthermore, we have demonstrated that our controlled delivery approach could be combined with surface chemical reactions to



Figure6 | Atomic force microscopy (AFM) topographic images of a representative region of the three PEG-bisTz grids with periodicity of (a) 15, (b) 10, and (c) 5 μ m, respectively. Each line in the first array was printed diagonally from topleft to bottom-right in each frame. Each line in the second array was produced from lower left to upper right in each frame. (d) Cursor profiles as indicated in images (a), (b), and (c), respectively.

evaporation led to the broken line geometry of the second arrays of PEG-bisTz lines. Redistribution of liquid droplets due to micropatterns on surfaces have been reported previously.⁴⁰ Our results indicate that the impact of surface patterns goes beyond 0D, for example, surface patterns underneath directly impact the geometry of lines.

produce designed functionalities. Following the same protocols as that in Figure 6b, an array of 10 PEG-bisTz lines were produced on AAPTMS glass. Each line was ~100 μ m long, 45 nm tall, and 1.2 μ m wide, with interline spacing of 10 μ m. Upon drying for 30 min under ambient conditions, the surfacewas immersed into a toluene solution containing 2.5 × 10⁻⁴ M C12-bisTCO for



Figure 7 | (a) A 20 \times 20 μ m atomic force microscopy (AFM) topographic image from the printed area. (b) Ligation between surface bound PEG-bisTz and C12-bisTCO in toluene solution form multiblock copolymers.¹⁵

30 min, followed by toluene rinsing. The lines measured 1.3 nm tall and 1.2 μm wide, as shown in Figure 7a (line features inside the red oval enclosure). These lines were shorter than the initial PEG-bisTz lines deposited. The reduced height from 45 to 1.3 nm was due to the Tz– TCO ligation shown in Figure 7b that occurred at the interface of PEG-bisTz solid and C12-bisTCO solution. Because of the reaction, the products located at the outermost solid–solution interaction dissolved into the toluene, exposing fresh PEG-bisTz solid to the C12bisTCO solution above. The cycle of reaction-dissolution perpetuated until reaching the PEG-bisTz attached to the glass surface, where the product remained immobilized on the surface due to the strong attachment of PEG-bisTz to the surface. The surrounding AAPTMS SAM region is known to exhibit little reactivity toward TCO, thus we anticipated only physisorbed C12-bisTCO molecules atop or interdigitated with the AAPTMS molecules.²²

Next, we created a second array of PEG-bisTz lines, perpendicular to the first, following identical printing conditions as that in the first line array. The new lines, as indicated by the blue arrows in Figure 7a, were continuous and relatively homogeneous crossing the unpatterned surface region, with height and base width of 62 nm and 1.35 μ m, respectively. Compared with the first array of lines before the C12-bisTCO treatment (45 ± 2 nm height, 1.12 ± 0.04 μ m width), the second array of lines are taller and wider by 17 nm and 0.15 μ m, respectively. This increase in material deposition is mainly attributed to the changes in molecule–surface interactions: from PEGbisTz with AAPTMS to PEG-bisTz with mixed AAPTMS and C12-bisTCO. During the deposition of the second line

array, PEG-bisTz molecules reacted with the C12-bisTCO molecules immobilized on the surface, thus remaining on site, and leading to structures shown in Figure 7a upon drying. At the regions crossing the first set of line bulges were seen, with a height of 98 nm and a base width of 2.14 μ m. In comparison to the lines on the unpatterned region, the bulges at the cross-sections are 0.79 μ m wider. This widening is significantly less than in the experiments shown in Figure 6b (1.89 μ m), where all delivery conditions remained the same. This observation

is again attributed to the changes in molecule–surface interactions. In the delivery of PEG-bisTz in the experiments shown in Figure 6b, the PEG-bisTz solution interact with AAPTMS surface at the unpatterned area, and with the PEG-bisTz solid lines at the cross section. As such, the liquid "redistributed," leading to thin lines and wider bulges at the cross sections. In the delivery of PEGbisTz in the experiments shown in Figure 7a, the PEGbisTz solution interacted mainly with the C12-bisTCO functionalities due to our treatments, though the density or coverage of C12-bisTCO functionality is higher at the cross sections. As such, the widening effect is less than that in Figure 6b.

Conclusion

Assembly of molecules into micro- and nanoscale structures by design has largely relied on SA of molecules. This work introduces

controlled assembly of molecules as a new means to address this challenge. The principle of this approach is based on delivery of ultrasmall volumes of solution on surfaces using combined AFM and microfluidic delivery. The solvent evaporates rapidly due to the minute volume, thus forcing the assembly of solute, whose overall size and dimension are dictated by the initial liquid geometry and size. Using prepatterned surfaces, this work revealed that the initial liquid shape can be further tuned, and as such, we can control the final assembly of the solute, such as PEG-bisTz molecules. Varieties of structures of PEG-bisTz were produced from 0D to 3D, whose geometry and size could be controlled. Patterns can also be transferred through chemical reaction with C12-bisTCO. This work introduces the concept of controlled assembly of molecules and paves the way to achieving 3D nanoprinting using materials in general. Work is in progress to produce functional 3D nanostructures by design. Beyond the new scientific concept of controlled assembly, the results shall impact technology development, including nanophotonics, nanoelectronics, nanocomposite materials, and tissue engineering.

Conflict of Interest

There is no conflict of interest to report.

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