Journal Name

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Unidirectional Supramolecular Self-Assembly inside Nanocorrals *via in situ* STM Nanoshaving

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Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Self-assembly of an alkylated diacetylene derivative is spatially confined *via in situ* scanning tunneling microscopy (STM) nanoshaving inside covalently modified highly ordered pyrolytic graphite (CM-HOPG). In contrast to unconstrained self-assembly that occurs randomly along three thermodynamically equivalent surface lattice directions, spatially confined assemblies are shown to form along chosen substrate orientations. Experimental statistics suggest two mechanisms for breaking the rotational degeneracy of the surface. First, the assembly orientation is biased *via* lateral confinement inside nanocorrals that do not match the substrate symmetry. Second, an interaction between the assembling molecules and the STM tip during nanoshaving guides 2D crystal nucleation and growth. The results presented here open new possibilities to regulate and orient self-assembled architectures *via in situ* nanomechanical manipulation techniques and provide mechanistic insights into the process.

Introduction

Spontaneously organized nanostructures occurring on two-dimensional (2D) surfaces are of fundamental interest for the generation of novel catalytic,^{1, 2} separation,³ and charge transport⁴⁻⁷ materials. For many of these surfaces to be functional, in particular in organic electronics, long range and defect free ordering over macroscopic length scales is required. However, due to the stochastic nature of nucleation events, molecular monolayers emerging from self-assembled organic building blocks often suffer from multidomain formation, thus limiting their applicability. Efforts to control molecular orientation at surfaces comprise the use of an electric⁸ or magnetic⁹ field, template assisted assembly,¹⁰⁻¹³ and liquid flow based methods.¹⁴ Nevertheless, further development and implementation of molecular alignment techniques demands for a fundamental understanding of the alignment principles at the molecular level.

The use of spatial confinement is a promising yet less explored approach to control molecular orientation. For instance, the morphology of nanolayered polymer films evolves towards single crystalline lamellae for shrinking layer thicknesses.¹⁵ Similarly, crystallization of organic compounds confined within the cylindrical pores of polymer monoliths,^{16, 17} electrochemically etched silicon,¹⁸ or anodized aluminum oxide¹⁹ templates leads to the preferred formation of nanocrystals aligned with their fast-growth axis parallel to the pore direction. As a means of expanding orientation control, 2D molecular self-assembly methods have followed this confinement trend. Several strategies have been reported to generate lateral

confinement, including the use of monolayer deep etch pits,²⁰ stepped/periodically oxidized surfaces,^{10-13, 21} and narrow terraces.²²

Scanning probe lithography, especially when performed *in situ*, *i.e.* in the presence of molecular building blocks, has emerged as a most powerful tool to create and characterize confined 2D assemblies.²³⁻²⁸ In this approach a sacrificial blocking layer is locally shaved off by the probe, and molecules suspended in solution can adsorb and assemble on the exposed surface area. Previous *in situ* STM nanoshaving studies on graphite, both by our group and other groups, have shown a preferential molecular adsorption orientation inside the exposed surface area.^{29, 30} Given the highly-constrained environment, especially during the initial stages of nanoshaving, it was hypothesized that geometric constraints placed on the assembly process bias its orientation. Recently, AFM imaging of sodium dodecyl sulfate (SDS) at the water/graphene interface revealed a similar molecular alignment of the surface assemblies.³¹ As SDS molecules were free to assemble, confinement effects could be excluded. Instead, the authors hypothesized that the SDS molecules interact with the AFM probe during imaging, which effectively breaks the threefold rotational substrate symmetry and aligns the molecules on the surface. Beebe *et al.* suggested that such an interaction might also influence surface assemblies during STM imaging.³² To date, it remains uncertain if and to what extent a tip-molecule interaction plays a role during *in situ* nanoshaving and how important spatial constraints are for unidirectional self-assembly at surfaces.

Here, we aim to elucidate this enigma by performing a statistical investigation of several key variables associated with *in situ* STM nanoshaving on covalently modified highly ordered pyrolytic graphite (CM-HOPG). Covalent surface functionalization is achieved using aryl diazonium chemistry.^{33, 34} Following a previously reported protocol, 3,5-bis-*tert*-butylbenzenediazonium (3,5-TBD) cations are reduced electrochemically *via* cyclic voltammetry, yielding a dense

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Electronic Supplementary Information (ESI) available: detailed description of the experimental designs and additional data. See DOI: 10.1039/x0xx00000x

monolayer of covalently attached 3,5-TBD molecules to the graphite substrate (Figure 1b).^{29, 35} The CM-HOPG is subsequently topped with a saturated solution of 10,12-pentacosadiynoic acid (PCDA, Figure 1c) in 1-phenyloctane (1-PO). Finally, the STM probe is inserted in the solution and covalently bound molecular units are locally shaved off by operating the tip in close proximity to the surface. In this way, spatially confined areas of pristine graphite, *i.e.* nanocorrals, are exposed that are available for adsorption and self-assembly of PCDA (Figure 1a).

Experimental

Grafting of 3,5-TBD on HOPG

3 mg 3,5-bis-*tert*-butylaniline (TCl, >98%) was dissolved in 5 mL aqueous hydrochloric acid (Honeywell-Fluka, 50 mM), and 100 μ L aqueous NaNO₂ (0.1 M) was added to initiate the diazotization reaction. The solution was shaken for 90 s before injection into a lab-built single-compartment three-electrode cell (HOPG working electrode area: 38.5 mm², Pt wire counter electrode, Ag/AgCl/3 M NaCl reference electrode). Cyclic voltammetry was performed using an Autolab PGSTAT101 potentiostat (Metrohm_Autolab BV, The Netherlands). Each experiment consisted of three voltage cycles (potential limits: 0.6 V to -0.35 V) with a scan rate of 0.1 V/s (Figure S1). Prior to each experiment the HOPG (ZYG grade, Advanced Ceramics Inc., Cleveland, USA) was freshly cleaved using scotch tape. After modification, the CM-HOPG surface was rinsed with Milli-Q water to remove any physisorbed material from the surface. High purity water (Milli-Q, Millipore, 18.2 M Ω cm, TOC < 3 ppb) was used for preparation of the aqueous solutions.

STM experiments

A saturated solution of PCDA (Sigma-Aldrich, $\geq 97\%$) was prepared by adding 10 mg of solid to 1 mL of 1-phenyloctane (Acros Organics, 99%). The solution was repeatedly heated at 70 °C and sonicated before use. The solution was dropcasted on the CM-HOPG surface at room temperature (20 – 22 °C). STM experiments were performed at room temperature using a PicoLE (Agilent) STM system operating in constant current mode. STM tips were cut manually from a Pt/Ir wire (Advent Research Materials, 80%/20%, diameter 0.25 mm) and inserted in the liquid. Imaging parameters are indicated in the figure captions and are denoted by V_{sample} for sample bias, and I_t for tunneling current. Nanoshaving was performed in a line-wise fashion using the PicoLITH v.2.1 software package. In all experiments, the fast shaving axis was oriented parallel to the graphite zigzag direction (high symmetry direction), and the slow shaving direction was orthogonal to this. The nanoshaving parameters are specified for every individual experiment, and are denoted by V_{tip} for tip bias, I_t for tunneling current, v_{tip} for in-line tip speed, and *line spacing* for vertical distance between consecutive shaving lines. All images were processed using the Scanning Probe Imaging Processor (SPIP) software (Image Metrology ApS). The high resolution STM image was corrected for drift using an image of the underlying graphite lattice as a reference (obtained under the same conditions, except for $V_{\text{sample}} = -0.001$ V and $I_t = 200$ pA).

Figure 1. (a) Schematic illustration of *in situ* STM nanoshaving of an equilateral triangular corral inside covalently modified HOPG. The fast shaving axis is oriented along one of the high symmetry axes of graphite (yellow arrows) and is orthogonal to the slow shaving axis. (b) Molecular structure of a 3,5-bis-*tert*-butyl aryl covalently linked to graphite. (c) Molecular structure of 10,12-pentacosadiynoic acid (PCDA). (d) STM image of PCDA self-assembly at the 1-phenyloctane/HOPG interface ($V_{\text{sample}} = -0.8$ V, $I_t = 100$ pA). The high symmetry axes of graphite are represented by black arrows. (e) Tentative molecular model corresponding to the STM image in (d). (f-h) Representative STM images of triangular corrals displaying diagonal left, parallel, and diagonal right assemblies of PCDA, respectively. The parallel bright lines correspond to rows of diacetylene groups (imaging: $V_{\text{sample}} = -0.8$ V, $I_t = 60$ pA; nanoshaving: $I_t = 200$ pA, $V_{\text{tip}} = 0.001$ V, $v_{\text{tip}} = 0.4$ μ m/s, and *line spacing* = 0.6 nm). (i) Statistics on the orientation of PCDA self-assembly as deduced from 187 equilateral triangular corrals. The dashed line represents the theoretically expected outcome based on three thermodynamically equivalent surface directions.

Statistical blocking of experiments

While the PicoLith software allows to accurately control the nanoshaving parameters, there remain a number of variables that we cannot control or monitor. These variables include, but are not limited to, the morphology of the STM probe, the CM-HOPG surface, the amount of liquid deposited on the sample, *etc.* In order to reliably identify effects caused by the parameters of interest it was therefore needed to block the other variables as much as possible. Specifically, a complete data set was acquired for a distinct number of values of the independent variable in one continuous run, *i.e.* on a specific CM-HOPG substrate and without changing the STM tip or adding additional PCDA solution. In order to generate a sufficiently large data set within each blocked experiment, it was necessary to perform nanoshaving in a 4 x 4 matrix format. Furthermore, to account for variations that occur during the course of one blocked experiment, the independent variables under investigation were varied randomly, either within a matrix or from one matrix to another. Random sequences were generated using www.random.org. Every experiment was performed at least twice to check for repeatability.

in the trends. A detailed description of the experimental layout for each of the experiments in this work is provided in the supporting information.

Data analysis.

The area occupied by each of the three possible rotational PCDA structures was measured from the raw images for every corral using the SPIP software. The corrals were subsequently defined by the dominant assembly orientation (rotational structure occupying the major fraction of the corral area) for further analysis. Chiral mirror structures along the same HOPG symmetry axis were treated as having the same orientation due to the small angle separating them ($6.6 \pm 0.6^\circ$). Nanocorrals that still contained one or more grafted molecules after nanoshaving were excluded from analysis, as were corrals in which more than one third of the corral area was occupied by contamination (origin unidentified).

Results and discussion

10,12-pentacosadiynoic acid (PCDA) was selected to evaluate supramolecular self-assembly inside *in situ* fabricated nanocorrals. As shown in Figure 1d, PCDA molecules self-assemble in linear rows (lamellae) at the solution/HOPG interface.^{29, 36} Due to strong, directional hydrogen bonding between the carboxylic acid head groups, PCDA lamellae always appear as dimers of head-to-head oriented rows. The diacetylene unit, which corresponds to the brightest part of the lamellae in Figure 1d, is furthermore crucial for molecular recognition and high-fidelity patterning.^{37, 38} The threefold rotational surface symmetry renders three thermodynamically equivalent lattice orientations for PCDA self-assembly. Surface induced chirality furthermore leads to the formation of two mirror structures along each of these lattice orientations. Unit cell parameters have been reported elsewhere.²⁹

In order to spatially confine PCDA self-assembly, nanocorrals were created inside CM-HOPG by means of STM nanolithography, *i.e.* nanoshaving. Nanoshaving was performed by reducing the tip-sample bias and increasing the tunneling current, which brings the STM probe closer to the surface. Under these conditions, the mechanical force exerted on the grafted molecules is raised sufficiently to detach them from the graphite surface. Once detached from the surface, the aryl species most likely diffuse in solution or adsorb on the STM tip.³⁹ Previous studies have shown that pristine graphite is regenerated after nanoshaving,³⁵ but the details of the detachment mechanism are still under investigation.

Orientationally biased PCDA self-assembly

Nanoshaving was performed *in situ*, *i.e.* in the presence of a saturated solution of PCDA in 1-PO. To demonstrate the concept of orientationally biased self-assembly, we first nanoshaved equilateral triangular corrals. The fast shaving axis was carefully oriented along one of the high symmetry axes of graphite, while the corrals were gradually exposed along the slow shaving axis from the triangle apex towards the base, as is schematically illustrated in Figure 1a. As such, the rotational symmetry of the corral (C_3) matches that of the substrate during and after the shaving process. Immediately after nanoshaving, self-assembly of PCDA lamellae was observed with no

noticeable structural differences compared to self-assembly on bare HOPG. Determined by the relative orientation of the PCDA lamellae with respect to the slow nanoshaving axis, we classify three assemblies: diagonal left (-60° w.r.t. slow shaving axis), parallel (0° w.r.t. slow shaving axis), and diagonal right ($+60^\circ$ w.r.t. slow shaving axis) assemblies, as shown in Figure 1f-h, respectively. An evaluation of 187 equilateral triangular corrals showed that not all three assembly orientations occur with equal probability (Figure 1i). Instead, assemblies in which the lamellae are parallel to the slow shaving axis, *i.e.* parallel assemblies, were preferred. Alternatively, it can be said that PCDA molecules tend to assemble with their long axis parallel to the fast shaving axis. Since equilateral triangles have a threefold rotational symmetry, confinement induced lateral constraints are equal along all three crystallographic surface directions and can thus not be the origin of orientationally biased PCDA self-assembly in this case. In order to elucidate the underlying mechanism(s) for alignment, a statistical investigation of several key variables associated with the *in situ* nanoshaving procedure is presented in the following paragraphs.

Nanocorral apex angle

The influence of lateral constraints on the assembly process was investigated in more detail by changing the triangle apex angle. Acute and obtuse triangles were created to explore their differences compared to the equilateral corrals. In order to exclude confinement effects caused by corral area variations, the height of each triangle was adjusted to keep the total corral area approximately constant (Figure S3). Representative triangular corrals for each investigated apex angle are shown in Figure 2a-d. While parallel assemblies were preferred for all apex angles, statistical analysis of approximately 500 corrals revealed a minimum alignment probability inside corrals having an apex angle close to 60° . To rationalize the occurrence of a minimum alignment probability in equilateral triangles, we must consider how lateral constraints apply to different assembly orientations. When the nanocorral apex angle is 60° a structure is created with threefold rotational symmetry, matching that of the substrate. The assembling PCDA molecules thus experience no orientation dependent constraints. However, the lack of rotational symmetry in acute or obtuse apex angle corrals results in lateral constraints that affect parallel assemblies differently from diagonal assemblies (Figure S6). Lateral constraints thus appear to influence the alignment probability through corral symmetry conditions.

While the alignment reaches a minimum inside equilateral triangles, the assembly orientation remains significantly biased. This bias cannot be explained by lateral constraints which are equal along all three equivalent lattice directions. Our observations thus suggest the existence of an additional alignment mechanism. Following previous work of Hong *et al.*,³¹ we consider the existence of a tip-molecule interaction that helps to align PCDA molecules. Forces exerted on the assembling molecules *via* such an interaction will be spatially anisotropic due to the line-wise movement of the tip, thereby stabilizing parallel assemblies and/or destabilizing diagonal assemblies. Overall, it can thus be concluded that the PCDA assembly orientation is dependent on the triangle apex angle, but that the STM probe likely introduces an additional alignment mechanism.

Figure 2. (a-d) STM images of *in situ* created nanocorrals with four different apex angles. The fast shaving axis was chosen to be parallel to a high symmetry axis of HOPG. The slow shaving axis was oriented orthogonal to the fast shaving axis and ran from the triangle apex towards the base (imaging: $V_{\text{sample}} = -0.8$ V, $I_t = 60$ pA; nanoshaving: $I_t = 200$ pA, $V_{\text{tip}} = 0.001$ V, $v_{\text{tip}} = 0.4$ $\mu\text{m/s}$, and *line spacing* = 0.6 nm). (e) Probability of parallel PCDA self-assembly as a function of corral apex angle. The black, dashed line represents the theoretical, unconstrained self-assembly outcome. Three individual experiments were performed containing 158, 180, and 154 nanocorrals, respectively.

Nanoshaving tip speed and line spacing

In order to better understand how the STM tip affects *in situ* created assemblies, the tip speed during nanoshaving was altered (Figure 3a). Equilateral triangular corrals were created with six different tip speeds and the propensity of parallel assemblies was monitored. Analysis of more than 1200 corrals clearly showed an increased prevalence of parallel assemblies for faster tip speeds as shown in Figure 3b. Previous AFM-based nanografting studies have shown how tip speed determines the stringency of lateral constraints, thereby impacting binary and ternary self-assembled monolayers of thiol derivatives on gold.^{40–42} Nevertheless, in this study we purposefully created equilateral triangular corrals and shaving was started from the apex so that lateral constraints were equal along all graphite high symmetry axes at any moment in time. Lateral constraints can thus hardly explain the observed tip speed trend. Instead, we assume that a faster tip motion intensifies the stabilizing/destabilizing effects exerted on the assembling molecules by the STM probe. Hence, it can be understood that faster shaving speeds give rise to more efficient alignment.

To confirm that the tip motion rather than the shaving time per corral causes the observed alignment behavior, an experiment was designed in which the tip speed was held constant at 1 $\mu\text{m/s}$, but the total shaving time was decreased by lowering the number of executed shaving lines per corral, *i.e.* increasing the vertical spacing (*h*) between two consecutive shaving lines (Figure 3a). A nanoshaving

line spacing ranging from 0.6 nm to 2.4 nm was selected, which corresponds to a shaving time ranging from 7.0 s to 1.8 s. Naturally, upon further increasing the line spacing a point was reached where no longer all TBD molecules were removed, as is illustrated in Figure S10. The exact line spacing at which incomplete nanoshaving was observed depended on the specific STM tip being used. In the line spacing range tested here, complete shaving was achieved in most cases (Figure S11). Throughout the whole study, corrals that still contained one or more grafted molecules were excluded from analysis. As shown in Figure 3c, no clear effect of line spacing on the propensity of parallel assemblies was observed. This result confirms that the shaving time per corral does not necessarily affect the assembly orientation. Rather, the increased prevalence of parallel assemblies with higher tip speed must be caused by the faster motion of the tip itself.

Tunneling parameters during nanoshaving

Thus far, the importance of a tip-molecule interaction during *in situ* nanoshaving was established and its relation to tip speed was discussed. Nevertheless, the nature of this interaction remains largely obscured. Previous studies have demonstrated that the electric field between tip and sample during STM operation can effectively impact surface assemblies^{8, 43}. It should thus be considered whether the electric field during *in situ* nanoshaving contributes to the observed molecular alignment of PCDA

Figure 3. (a) Schematic representation of tip speed (v_{tip}) and line spacing (h) for nanoshaving an equilateral triangle. (b) Probability of observing parallel PCDA self-assembly as a function of the nanoshaving tip speed. The total time for shaving a given corral is shown on top of the graph. Three individual experiments were conducted containing 378, 462, and 367 corrals, respectively. Nanoshaving conditions: $I_t = 200$ pA, $V_{\text{tip}} = 0.001$ V, and line spacing = 0.6 nm. (c) Influence of the line spacing on the probability of parallel PCDA assembly. The total time needed to create a corral is given on top of the graph. 277 and 325 corrals were investigated in two individual experiments, respectively. Nanoshaving conditions: $I_t = 200$ pA, $V_{\text{tip}} = 0.001$ V, and $v_{\text{tip}} = 1$ $\mu\text{m/s}$.

assemblies. To this end, the set point current was varied from 75 pA to 500 pA, while the applied tip bias was held constant at +0.01 V. An equilateral triangular geometry was again chosen to minimize lateral confinement effects. The probability of parallel assemblies as function of set point current was studied for approximately 1000 corrals (Figure 4a). A strikingly high alignment probability was observed for a set point current of 300 pA. However, this behavior seems to be caused by one data set entirely (experiment 1) and was not reproducible. Overall, no clear trend can be observed and we cannot conclude that the set point current affects molecular alignment. Next, a complementary experiment was performed in which the set point current was held constant at 200 pA, but the applied tip bias was varied from +0.001 V to +0.5 V. Nearly 900 equilateral triangles were investigated, but no convincing effects on the assembly orientation were revealed (Figure 4b). Finally, nanoshaving was performed with inversed polarity, *i.e.* negative tip bias and positive sample bias. Under these conditions of inversed polarity, a preferential formation of parallel assemblies was again observed (Figure 4c). Alignment thus occurs irrespective of bias polarity. In conclusion, it appears that neither the applied bias nor the current during nanoshaving have a clear impact on the PCDA assembly process. Consequently, it is unlikely that the tip-molecule interaction is mediated by the electric field.

Within the investigated ranges of applied bias and current during nanoshaving, nanocorral formation was not always successful. Indeed, shaving off the grafted molecules requires that the STM tip is operated sufficiently close to the CM-HOPG substrate. In general, we can distinguish three different cases of incomplete shaving: (i) one or multiple grafted molecules remain, (ii) only a fraction of the intended corral was created, or (iii) no corral was created at all (Figure S15). The relative occurrence of each case of incomplete nanoshaving was monitored as a function of set point current and applied tip bias and is shown in Figures S13 and S15, respectively. It is important to note that no clear threshold demarcating nanoshaving from imaging conditions was observed. Rather, higher applied biases and/or smaller set point currents made nanoshaving less probable. Remarkably, once the STM started shaving it generally continued to do so for all ensuing corrals. Possibly, part of the

detached aryl species adsorb on the tip during the shaving process making it bulkier and therefore more likely to keep shaving.

Origin of tip-induced unidirectional PCDA self-assembly

Since the effect of the STM tip on the assembling PCDA molecules cannot reasonably be attributed to the electric field during shaving, other mechanisms should be considered. Owing to the large polarizability of metal atoms in the STM probe (Pt and Ir), van der Waals forces between PCDA molecules and the tip are expected to be strong. It is thus reasonable to assume that a direct attractive force is exerted on the assembling molecules when the STM tip is moved closely over the surface. Due to the line-wise movement of the tip during nanoshaving this interaction can be understood to align molecules at the surface.

An alternative mechanism for molecular alignment is a tip-induced flow. While the liquid on top of our sample is static, the movement of the STM tip inside the droplet could generate a microscopic shear flow. Frictional dragging forces generated by such a flow are known to induce molecular alignment at surfaces.¹⁴ The strength of this shear flow, and thus the extent of (de)stabilization of PCDA assemblies will depend on the shaving tip speed, which fits our observations well, *i.e.* the assembly orientation is biased more with increasing tip speed, but is independent of the total shaving time.

The importance of tip morphology in aligning molecules at the surface should also be considered. Most experiments display rather different alignment probabilities for individual experiments (*e.g.* apex angle and tip speed data). As each individual experiment is carried out with a different STM tip and CM-HOPG sample, we attribute the observed variability mainly to these two factors. Given the importance of a tip-molecule interaction in the alignment mechanism, the geometric shape of the tip is believed to be an important, but hardly controllable, parameter for alignment. In addition, the presence of a bulky STM tip during nanoshaving places steric constraints on the assembly process. The tip morphology will necessarily also have an impact on the extent of these constraints.

Finally, investigations of *in situ* nanoshaving with long alkanes (not shown) and alkylated isophthalic acid derivatives⁴⁴ indicate that the alignment behavior is more generally applicable to molecules having a long axis.

Figure 4. Effect of nanoshaving tunneling conditions on PCDA self-assembly in equilateral triangular corrals. (a) Probability of parallel assembly as a function of set point current. Two individual experiments were performed containing 446 and 553 corrals, respectively. Nanoshaving conditions: $V_{tip} = 0.01$ V, $v_{tip} = 0.4$ $\mu\text{m/s}$, and $line\ spacing = 0.6$ nm. (b) Probability of parallel PCDA assembly as a function of the magnitude of the applied tip bias. 572 and 326 corrals were investigated in two individual experiments, respectively. Nanoshaving conditions: $I_t = 200$ pA, $v_{tip} = 0.4$ $\mu\text{m/s}$, and $line\ spacing = 0.6$ nm. (c) Relative occurrence of PCDA assembly orientations upon nanoshaving with reversed polarity, i.e. negative tip and positive sample bias. 173 corrals were examined. Nanoshaving conditions: $V_{tip} = -0.001$ V, $I_t = 200$ pA, $v_{tip} = 1$ $\mu\text{m/s}$, and $line\ spacing = 0.6$ nm. The dashed line represents the theoretical distribution for unconstrained self-assembly.

Self-assembly conditions during *in situ* nanoshaving

Besides the observed alignment behavior, a strong interaction with the STM tip potentially has other important consequences for the assembly process. Compared to naturally formed assemblies in which molecules diffuse freely towards and on the surface, the STM tip might actively transfer molecules to the area being nanoshaved. It should thus be considered that the STM tip itself is a possible source of PCDA molecules for self-assembly inside *in situ* created nanocorrals, similar to dip-pen lithography.⁴⁵ To demonstrate this,

Figure 5. Schematic of the dipping process, followed by nanoshaving on a dry CM-HOPG substrate. After nanoshaving, features characteristic of PCDA self-assembly were observed inside the nanocorrals as shown by the STM image. Nanoshaving was performed with the fast shaving axis parallel to a high symmetry axis of HOPG and with the following parameters: $I_t = 200$ pA, $V_{tip} = 0.001$ V, $v_{tip} = 0.4$ $\mu\text{m/s}$, and $line\ spacing = 0.6$ nm. Imaging conditions: $I_t = 60$ pA, $V_{sample} = -0.8$ V.

we performed an experiment in which the STM tip was dipped inside a saturated solution of PCDA in 1-PO for 60 s prior to nanoshaving on a dry CM-HOPG sample. Subsequent STM imaging, shown in Figure 5, displayed the formation of PCDA assemblies inside the nanocorrals. Compared to *in situ* nanoshaving, this approach suffered from instabilities during nanoshaving and/or imaging. Nevertheless, this experiment indicates the efficient adsorption of PCDA molecules on the STM probe, which is believed to actively transfer molecules to the nanoshaved areas.

As a result of lateral confinement inside the corrals and tip effects, the kinetics of nucleation and growth during *in situ* nanoshaving likely differ extensively from the natural self-assembly process. On top of that, corral edges might (dis)favor nucleation events. However, throughout our experiments the kinetics of self-assembly were too fast to track and self-assembly was consistently observed inside the corrals upon the first image taken after the shaving process. In order to slow down the assembly process, experiments at lower concentration are currently being carried out. Further, prolonged scanning did not reveal any changes to the domain structures indicating that they are kinetically stable. Remarkably, given the existence of a tip-molecule interaction, the assemblies did not reorient under both nanoshaving nor imaging conditions (Figure S16 and S17). Overall, it can be concluded that once PCDA assemblies have formed, they are generally stable against interactions with the STM probe. Hence, tip-induced alignment must effectively occur during 2D crystal nucleation and early growth of assemblies that have not yet reached a critical stability on the surface.

Conclusions

In conclusion, we have shed light on the interplay of several effects involved in molecular self-assembly occurring in confined spaces *via in situ* nanoshaving at the liquid-solid interface. Corrals of various rotational symmetries were created and statistical analysis revealed how lateral confinement biases the assembly orientation in those corrals that do not match the substrate symmetry. In addition, a tip-molecule interaction is believed to impact crystal formation during nucleation and early growth, especially at faster shaving tip speed.

Together, these mechanisms represent a powerful method for breaking the rotational degeneracy of symmetry-related lattice orientations enabling anisotropic supramolecular self-assembly. In the future, insights and control over the tip morphology may further push the efficiency of these methods. Coupling this approach with existing technologies such as dip-pen lithography and scanning probe arrays is expected to advance its scalability and applicability. This work highlights the role that compartmentalization and nanomanipulation may play in the creation and orientation of complex molecular surface architectures.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work has received funding from the Fund of Scientific Research–Flanders (FWO), KU Leuven Internal Funds, and the European Research Council under the European Union's Seventh Framework Programme (FP7/2007-2013)/ERC Grant Agreement No. 340324. L.V. thanks the FWO for the fellowship received. B. H. acknowledges the Belgian American Educational Foundation and the FWO.

Notes and references

1. F. Sedona, M. Di Marino, D. Forrer, A. Vittadini, M. Casarin, A. Cossaro, L. Floreano, A. Verdini and M. Sambi, *Nat. Mater.*, 2012, **11**, 970-977.
2. D. Nguyen, G. Kang, N. H. Chiang, X. Chen, T. Seideman, M. C. Hersam, G. C. Schatz and R. P. Van Duyne, *J. Am. Chem. Soc.*, 2018, **140**, 5948-5954.
3. C. Liu, E. Park, Y. Jin, J. Liu, Y. Yu, W. Zhang, S. Lei and W. Hu, *Angew. Chem.*, 2018, **57**, 8984-8988.
4. A. M. S. Esmail, C. R. Staddon and P. H. Beton, *J. Phys. Chem. C*, 2016, **120**, 15338-15341.
5. M. A. Mezour, O. Voznyy, E. H. Sargent, R. B. Lennox and D. F. Perepichka, *Chem. Mater.*, 2016, **28**, 8322-8329.
6. Z. Y. Xia, F. Leonardi, M. Gobbi, Y. Liu, V. Bellani, A. Liscio, A. Kovtun, R. J. Li, X. L. Feng, E. Orgiu, P. Samori, E. Treossi and V. Palermo, *ACS Nano*, 2016, **10**, 7125-7134.
7. R. Phillipson, C. J. L. de la Rosa, J. Teyssandier, P. Walke, D. Waghay, Y. Fujita, J. Adisojoso, K. S. Mali, I. Asselberghs, C. Huyghebaert, H. Uji-i, S. De Gendt and S. Feyter, *Nanoscale*, 2016, **8**, 20017-20026.
8. A. Cristadoro, M. Ai, H. J. Rader, J. P. Rabe and K. Mullen, *J. Phys. Chem. C*, 2008, **112**, 5563-5566.
9. J. D. Mougous, A. J. Brackley, K. Foland, R. T. Baker and D. L. Patrick, *Phys. Rev. Lett.*, 2000, **84**, 2742-2745.
10. J. Wang, I. Kaur, B. Diaconescu, J. M. Tang, G. P. Miller and K. Pohl, *ACS Nano*, 2011, **5**, 1792-1797.
11. A. Saywell, J. Schwarz, S. Hecht and L. Grill, *Angew. Chem.*, 2012, **51**, 5096-5100.
12. B. Cirera, Y. Q. Zhang, J. Bjork, S. Klyatskaya, Z. Chen, M. Ruben, J. V. Barth and F. Klappenberger, *Nano Lett.*, 2014, **14**, 1891-1897.
13. R. Otero, Y. Naitoh, F. Rosei, P. Jiang, P. Thosttrup, A. Gourdon, E. Laegsgaard, I. Stensgaard, C. Joachim and F. Besenbacher, *Angew. Chem.*, 2004, **43**, 2092-2095.
14. S. L. Lee, Z. Y. Yuan, L. Chen, K. S. Mali, K. Mullen and S. De Feyter, *J. Am. Chem. Soc.*, 2014, **136**, 4117-4120.
15. R. M. Michell and A. J. Muller, *Prog. Polym. Sci.*, 2016, **54-55**, 183-213.
16. B. D. Hamilton, I. Weissbuch, M. Lahav, M. A. Hillmyer and M. D. Ward, *J. Am. Chem. Soc.*, 2009, **131**, 2588-2596.
17. J. M. Ha, B. D. Hamilton, M. A. Hillmyer and M. D. Ward, *Cryst. Growth Des.*, 2012, **12**, 4494-4504.
18. A. Henschel, P. Kumar, T. Hofmann, K. Knorr and P. Huber, *Phys. Rev. E*, 2009, **79**, 4.
19. Q. Jiang, C. H. Hu and M. D. Ward, *J. Am. Chem. Soc.*, 2013, **135**, 2144-2147.
20. D. L. Patrick, V. J. Cee and T. P. Beebe, *Science*, 1994, **265**, 231-234.
21. Q. T. Fan, J. Y. Dai, T. Wang, J. Kuttner, G. Hilt, J. M. Gottfried and J. F. Zhu, *ACS Nano*, 2016, **10**, 3747-3754.
22. D. G. de Oteyza, E. Barrena, H. Dosch and Y. Wakayama, *Phys. Chem. Chem. Phys.*, 2009, **11**, 8741-8744.
23. S. Xu and G. Y. Liu, *Langmuir*, 1997, **13**, 127-129.
24. B. Torun, B. Ozkaya and G. Grundmeier, *Langmuir*, 2012, **28**, 6919-6927.
25. M. V. Lee, K. A. Nelson, L. Hutchins, H. A. Becerril, S. T. Cosby, J. C. Blood, D. R. Wheeler, R. C. Davis, A. T. Woolley, J. N. Harb and M. R. Linford, *Chem. Mater.*, 2007, **19**, 5052-5054.
26. D. Takajo, T. Nemoto, O. Franco, H. Kurata and S. Isoda, *Jpn. J. Appl. Phys.*, 2006, **45**, 2091-2094.
27. M. J. J. Coenen, T. Khoury, M. J. Crossley, B. L. M. Hendriksen, J. Elemans and S. Speller, *ChemPhysChem*, 2014, **15**, 3484-3488.
28. T. M. T. Huynh, T. H. Phan, O. Ivasenko, S. F. L. Mertens and S. De Feyter, *Nanoscale*, 2017, **9**, 362-368.
29. L. Verstraete, J. Greenwood, B. E. Hirsch and S. De Feyter, *ACS Nano*, 2016, **10**, 10706-10715.
30. D. Takajo, T. Nemoto, H. Kurata, S. Isoda, H. Ozaki and Y. Mazaki, *Thin Solid Films*, 2003, **438**, 428-432.
31. L. Hong, T. Nishihara, Y. Hijikata, Y. Miyauchi and K. Itami, *Sci. Rep.*, 2018, **8**, 8.
32. S. P. Sullivan, A. Schmeders, S. K. Mbugua and T. P. Beebe, *Langmuir*, 2005, **21**, 1322-1327.
33. M. Delamar, R. Hitmi, J. Pinson and J. M. Saveant, *J. Am. Chem. Soc.*, 1992, **114**, 5883-5884.
34. D. Belanger and J. Pinson, *Chem. Soc. Rev.*, 2011, **40**, 3995-4048.
35. J. Greenwood, T. H. Phan, Y. Fujita, Z. Li, O. Ivasenko, W. Vanderlinden, H. Van Gorp, W. Frederickx, G. Lu, K. Tahara, Y. Tobe, H. Uji-i, S. F. L. Mertens and S. De Feyter, *ACS Nano*, 2015, **9**, 5520-5535.
36. C. Guo, J. D. Xue, L. X. Cheng, R. C. Liu, S. Z. Kang, Q. D. Zeng and M. Li, *Phys. Chem. Chem. Phys.*, 2017, **19**, 16213-16218.
37. Y. Xue and M. B. Zimmt, *J. Am. Chem. Soc.*, 2012, **134**, 4513-4516.
38. Y. Yang and M. B. Zimmt, *Langmuir*, 2015, **31**, 12408-12416.
39. L. Scudiero and K. W. Hipps, *J. Phys. Chem. C*, 2007, **111**, 17516-17520.
40. J. J. Yu, Y. H. Tan, X. Li, P. K. Kuo and G. Y. Liu, *J. Am. Chem. Soc.*, 2006, **128**, 11574-11581.
41. D. L. Bu, T. J. Mullen and G. Y. Liu, *ACS Nano*, 2010, **4**, 6863-6873.

ARTICLE

Journal Name

42. D. L. Bu, S. Riechers, J. Liang and G. Y. Liu, *Nano Res.*, 2015, **8**, 2102-2114.
43. G. Velpula, J. Teyssandier, S. De Feyter and K. S. Mali, *ACS Nano*, 2017, **11**, 10903-10913.
44. J. Seibel, L. Verstraete, B. E. Hirsch, A. M. Bragança and S. De Feyter, *J. Am. Chem. Soc.*, 2018, **140**, 11565-11568.
45. K. Salaita, Y. H. Wang and C. A. Mirkin, *Nat. Nanotechnol.*, 2007, **2**, 145-155.