Electronic Supporting Information (ESI) for:

Hierarchically Patterned Striped Phases of Polymerized Lipids: Toward Controlled Carbohydrate Presentation at Interfaces

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Experimental Details

Materials. Chloroform (≥99.5 % purity), undec-10-ynoic acid (95 %), dec-1-yne (98 %), iodine (99.8 %), copper iodide (99.5 %), morpholine (99 %), potassium hydroxide, hydroxylamine hydrochloride (98 %), ethylamine (70 % (*v*/*v*) solution in water), sulfuric acid (95.0–98.0 %), sodium thiosulfate, and sodium sulfate, were all purchased from Sigma Aldrich (St. Louis, MO) and used as received. Absolute ethanol (100 % purity) was purchased from Decon Laboratories, Inc. (King of Prussia, PA) and used as received. Methanol, diethyl ether (anhydrous), hexanes, THF, and toluene were purchased from Fisher Scientific (Hampton, NH) and used as received. Silica gel was purchased from Macherey-Nagel (Bethlehem, PA) and used as received. 1,2-Bis(10,12-tricosadiynoyl)-*sn*-glycero-3-phosphoethanolamine (diyne PE, >99.0 % purity), 1,2-bis(10,12-tricosadiynoyl)-*sn*-glycero-3-phosphocholine (diyne PC, >99.0 % purity), and 1,2-distearoyl-*sn*-glycero-3-phosphoinositol (ammonium salt) (PI, >99.0 % purity) were purchased from Avanti Polar Lipids (Alabaster, AL) and used as received. Commercially available fatty acids 10,12-tricosadiynoic acid (TCDA, ≥ 98.0 % purity) and 10,12-pentacosadiynoic acid (PCDA, ≥ 97.0% purity) were purchased from Sigma-Aldrich (St. Louis, MO), and 10,12-nonacosadiynoic (NCDA, >97.0% purity) was purchased from Tokyo Chemical Industry Co., Ltd. (Montgomeryville, PA). All fatty acids were dissolved in chloroform and filtered through 0.2-μm syringe filters to eliminate oligomers prior to use. For preparation of the poly(dimethylsiloxane) (PDMS) elastomer stamps, SYLGARD 184 silicone elastomer kits containing base and curing (crosslinking) agent were purchased from Dow Chemical Company (Midland, MI). When water was experimentally required, Milli-Q water (≥18.2 MΩ·cm resistivity) was used. Ultrahigh purity nitrogen (UHP N_2, 99.999 % purity) was purchased from Indiana Oxygen Company (Indianapolis, IN). Lipids were deposited on 1 cm × 1 cm highly oriented pyrolytic graphite (HOPG) substrates (MicroMasch, Watsonville, CA), which were freshly cleaved immediately prior to transfer. All initial steps in the transfer process were carried out under UV-filtered light to prevent polymerization in solution. PELCO conductive liquid silver paint, standard SEM pin stub mounts, and double-coated carbon conductive tape were purchased from Ted Pella, Inc. (Redding, CA). Silicon wafers photolithographically patterned with arrays of 5 μm × 5 μm × 5 μm recessed cubes with a 10 μm pitch were provided by Prof. Wei-Ssu Liao (National Taiwan University).

General procedure for synthesis of 1-iododec-1-yne. Synthesis was carried out using a modification of previously published procedures,[1] described briefly here. A solution of morpholine (44 mmol) in toluene (34.8 mL) was treated with iodine (6.16 mmol), shielded from light and stirred for 1 h at 45 °C. A solution of dec-1-yne (4.4 mmol) in toluene (3.48 mL) was then added and the reaction mixture stirred continuously at 45 °C for 1 h. The reaction mixture was cooled to room temperature and filtered to remove the iodomorpholine salt. The filtrate was poured over a mixture of diethyl ether

(50 mL) and a saturated aqueous solution of $Na_2S_2O_3$ (50 mL) and shaken until the organic layer was colorless. The organic layer was separated, washed again with a saturated aqueous solution of $Na_2S_2O_3$ (50 mL), dried over anhydrous Na_2SO_4, filtered, concentrated, and purified via column chromatography, with hexane as an eluent, to afford a 1-iododec-1-yne as a colorless oil (typical yield ~70 %).

Synthesis of 10,12-henicosadiynoic acid (HCDA). Synthesis was carried out using a modification of published procedure,[1-2] described briefly here. Undec-10-ynoic acid (1.9 mmol) was dissolved in THF (14 mL) and CuI (0.43 mmol) was dissolved in 70% (*v*/*v*) ethylamine in water (14 mL). The undec-10-ynoic acid solution and the CuI solution were combined with ethanol (14 mL). Subsequently, 1 M KOH in water (6 mL) was added to the reaction mixture along with hydroxylamine hydrochloride (0.33 mmol). The reaction was cooled to 0 °C. A solution of 1-iododec-1-yne (5.1 mmol) dissolved in THF (10 mL) was then added dropwise, causing a precipitate to form. The reaction was allowed to warm to room temperature and proceed for a further 24 h. If the solution turned blue, additional aliquots of hydroxylamine hydrochloride were added. The reaction was quenched by the addition of a 10% aqueous solution of sulfuric acid to achieve neutral pH (typical required volume ~4 mL). Crude product was extracted with diethyl ether (3 × 50 mL) and then washed with water (3 × 50 mL) and brine (3 × 50 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and the ether was removed by rotary evaporator. The crude product was purified by recrystallization from hexanes to yield a fatty acid with an internal diyne, as a white solid (typical yield ~30 %).

Preparation of PDMS Stamps. Stamps were prepared by mixing SYLGARD 184 silicone elastomer base and curing (crosslinking) agent at the desired ratio (*e.g.* 10:1 *m*/*m*, or as described in the manuscript text). After the components were thoroughly mixed (approximately 5 min), the mixture was poured onto a photolithographically etched silicon wafer resting in a petri dish. The mixture was then deaerated in a vacuum desiccator until no bubbles remained. Subsequently, the petri dish was placed in an oven to allow the PDMS to cure for 24 h at 60 °C. After curing, crosslinked PDMS was peeled from the silicon wafer and cut to the desired size using a razor blade. PDMS stamps were cleaned by soaking them in Milli-Q water for 1 h, followed by sonication in a 1:1:1 (*v*/*v*/*v*) mixture of ethanol, methanol, and Milli-Q water for 30 min. The sonication step is crucial. Following sonication, stamps were placed in an oven for 1 h at 60 °C to allow residual polar solvent mixture to evaporate. Stamps were then soaked in hexanes for 6 h, replacing the hexanes every 2 h. Finally, the stamps were dried for 24 h at 60 °C and placed, pattern side up, in a covered petri dish prior to use. The cleaning procedure was repeated in preparation for each use of the stamp.

Ultraviolet ozone (UVO) plasma processing to increase PDMS stamp hydrophilicity. PDMS stamps hydrophilicity was increased using a Herrick PDC-3XG Plasma Cleaner with an oxygen flow rate of 150 cc/min and the RF level set to high for 60 min, unless otherwise stated in the manuscript.

Inking of PDMS Stamp. For inking, a cleaned PDMS stamp was first rinsed briefly with ethanol and blown dry with UHP N_2. The patterned surface of the stamp was immersed in a solution of the chosen amphiphile. Solutions of amphiphiles were prepared first at 2.5 mg/mL in either $CHCl_3$ (for phospholipids) or 3:2 (*v:v*) hexane:IPA (for fatty acids). The solution was then dissolved to the desired concentration (stated in the manuscript) with ethanol. This procedure was followed in order to maintain amphiphile solubility, while achieving a relatively low concentration of hexanes and $CHCl_3$ in the inking solution, since these solvents are known to swell PDMS and distort features. After 30 s of immersion in the dilute lipid solution in the carrier solvent mixture, the stamp was removed, blown dry with UHP N_2 and placed pattern side up for 1 h at room temperature to allow additional carrier solvent to evaporate from the stamp.

Transfer of amphiphiles from PDMS to HOPG. After inking and subsequent drying for 1 h, the patterned side of the PDMS stamp was brought into contact with a freshly cleaved HOPG substrate, using one of the methods described below. In the 'flat contact' method, the stamp was lowered gently onto the HOPG surface. The PDMS stamp typically wet the HOPG surface; light tapping pressure with tweezers was applied to restore contact if needed. PDMS–HOPG contact was maintained for 30 s (unless otherwise specified) before the stamp was carefully lifted from the surface. In the 'rolled contact' method, the stamp was mounted on a copper cylinder 2.54 cm in diameter, 6.8 cm in length, and 300 g in mass. Double-sided tape was placed around the diameter of the copper cylinder, and the back side of the stamp was affixed to the tape. In one fluid motion (typically lasting approximately 3 s), the stamp was rolled across the surface of a freshly cleaved HOPG substrate. After both 'flat contact' and 'rolled contact' transfers, the functionalized HOPG was placed under a hand-held UV lamp (254 nm, 8 W) for 1 h with ~2 cm between the lamp and the substrate, to induce diyne photopolymerization, stabilizing the transferred molecular layer.

SEM imaging. Molecular layers on HOPG were imaged using a Teneo VS SEM (FEI Company, Hillsboro, OR). Images were acquired at a working distance of ~5 mm using the segmented in-lens T3 detector. A beam current of 3.2 nA was selected for optimal image resolution, utilizing a 32-μm diameter aperture with an accelerating voltage of 5 kV. All substrates were affixed to standard SEM pin stub specimen mounts with double-sided conductive carbon tape. To further enhance substrate–mount conductivity, a small amount of colloidal silver paint (PELCO, Ted Pella, Inc.) was applied along the perimeter of the substrate, providing electrical contact with the underlying pin stub.

AFM imaging. All AFM measurements were performed under ambient conditions using a Bruker MultiMode AFM (Bruker Instruments, Billerica, MA) equipped with an E scanner. The cantilever oscillation phase shift was carefully monitored to ensure the tip was engaged in the attractive mode to improve imaging of lamellar domains. The setpoint ratio was typically maintained between 0.4 and 0.7 and was rarely decreased below 0.4 to avoid tip sweeping effects.

Image analysis. Images were processed using Gwyddion scanning probe microscopy data visualization and analysis software[3] and ImageJ analysis software[4] to perform median line corrections, plane flattening, scar artifact removal, and contrast adjustment. Transfer fidelity and domain area measurements were performed using Adobe Photoshop to identify domain boundaries and calculate transfer coverage.

Energy minimization. Software packages Maestro and Macromodel (Schrödinger, Cambridge MA) were used, respectively, to visualize molecular structures and to perform force field minimizations. Models were minimized using the OPLS_2005 force field, with extended cutoffs for Van der Waals, electrostatic, and hydrogen bonding interactions. The dielectric constant of the simulation was set to 80.1. Minimizations were performed using the Polak-Ribiere conjugate gradient (PRCG) algorithm and gradient method with 50000 runs and a convergence threshold of 0.05.

Comparison of PCDA transfer from PDMS stamps with base:crosslinker ratios of 10:5 to 10:1

Because the local surface roughness of HOPG is lower than that of Au substrates commonly used in microcontact printing of alkanethiols, we examined whether this led to differences in PDMS rigidity required for optimal molecular transfer to HOPG. The elastomer base and curing (crosslinking) agent are typically mixed in a 10:1 (*m*/*m*) ratio for transfer of alkanethiols to Au; here, we prepared PDMS stamps with ratios from 10:5 to 10:1. High crosslinker ratios (*e.g.* 10:5) produce more rigid stamps with high elastic moduli, possibly useful for improving stamping fidelity of small features, given the limited need of the stamp to deform on the fairly flat HOPG substrate. Simultaneously, high curing agent ratios have been observed in other systems to limit the ability of the stamp to absorb molecular ink. Stamps were cleaned as described in the Experimental Methods, and an ink solution of 1.1 mM PCDA in the carrier solvent mixture was applied. Fig. S1 shows SEM images of PCDA transferred to HOPG from the three stamps. Stamps prepared with a base:crosslinker ratio of 10:5 (Fig. S1a,b) produce a high degree of molecular deposition both inside and outside the contact area. The intermediate 10:2 ratio (Fig. S1c,d) produces desirable transfer characteristics: a high degree of striped phase coverage in the contact area, with limited transfer outside the contact area. In general, stamps prepared at a ratio of 10:1 (Fig. S1e,f) resulted in a somewhat increased range of transfer outside the stamp contact area, and in some cases, increased PDMS deposition (black spots) within the contact area. However, overall both 10:2 and 10:1 base:crosslinker ratios produce

reasonable transfer, and in many cases we tested stamps prepared in both ratios, for comparison with common stamp preparation conditions used in the assembly of standing phases on gold.

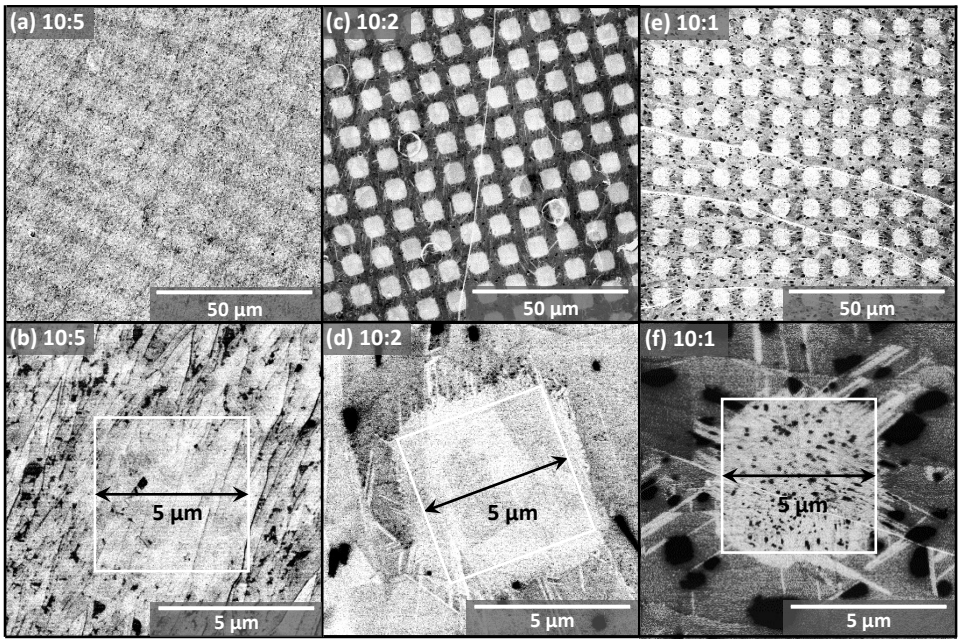


Fig. S1. SEM images of PCDA transferred to HOPG from PDMS stamps prepared at elastomer base:crosslinker ratios of (a,b) 10:5, (c,d) 10:2, and (e,f) 10:1.

Representative SEM images for microcontact transfer of lipids to HOPG at concentrations from 2.1 – 0.045 mM

Previously, ink concentration has been found to be an important factor in producing high density molecular coverage in the stamp contact area, while limiting transfer outside the contact area, with concentrations in the range of 1–10 mM producing optimal transfer for standing phases, depending on the structure of the molecular ink. In lying-down striped phases, molecular footprints are much larger than for similar molecules assembled in a standing orientation (*e.g.*, 154 Å^2 for PCDA in a lying-down phase *vs.* ~25 Å^2 when assembled as a standing phase), requiring, in the case of PCDA, ~1/6 as many molecules to transfer per unit surface area. Fig. S2 illustrates SEM images of HOPG substrates that have been exposed to PDMS stamps carrying PCDA in carrier solvent at concentrations ranging from 2.1 to 0.045 mM, using the conditions described above. Patterns of squares representing deposited PCDA appear in higher contrast due to enhanced electron scattering relative to the conductive HOPG substrate, in agreement with previous SEM images acquired from PCDA monolayers assembled through LS transfer.[5-6] Higher-resolution SEM imaging of a single contact area at each concentration (Fig. 3b,d,f,h) illustrates that at 1.1 mM, the entire contact area is functionalized with PCDA, with a narrow

band of continuously functionalized surface up to 600 nm outside the contact area, and a low fractional coverage of long narrow molecular domains between contact areas. At 0.045 mM PCDA in the ink solution (Fig. S2g,h), the contact area is only partially functionalized, although individual domains are larger (typical length 1–2 μm) than those observed for transfer at 1.1 mM PCDA, which is reasonable given that lower monomer concentrations result in fewer but larger molecular islands in the submonolayer island nucleation and growth model. For this transfer condition, substantial areas of PDMS deposition (black spots) are also observed in the contact area. Even at 2.1 mM PCDA in the transfer solution, some PDMS deposition can be observed; the amount of PDMS impurity deposited can vary from transfer to transfer. Overall, 2.1 mM PCDA produced the greatest extent of PCDA transfer outside the contact area. Based on these findings, we utilized amphiphile ink solutions prepared with 1.1 mM alkyl chain concentrations (*i.e.,* 1.1 mM PCDA; 0.5 mM diyne PC), unless otherwise described in the manuscript.

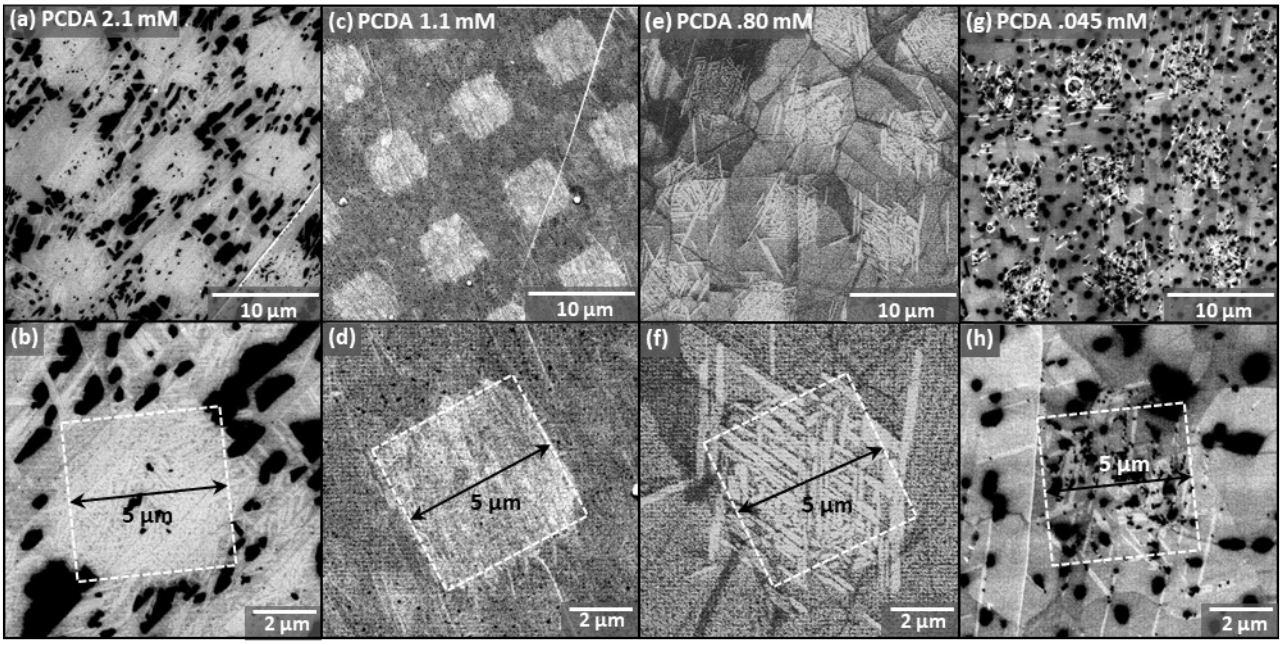


Fig. S2. SEM images of PCDA transferred to HOPG from solutions containing (a,b) 2.1 mM PCDA, (c,d) 1.1 mM PCDA, (e,f) 0.80 mM and (g,h) 0.045 mM PCDA in ethanol.

Comparisons of AFM and SEM images to examine orientation of transferred molecules

Because molecular domains produced by microcontact printing are relatively small (edge lengths *ca.* 100 nm), we utilized AFM imaging in addition to SEM imaging to characterize transfer, in order to examine the density of molecular domains produced under different transfer conditions. Fig. S3 compares SEM and AFM micrographs of microcontact printed

squares of PCDA produced using 1.1 mM PCDA in ethanol. We have previously observed that striped monolayers of diacetylene amphiphiles can exhibit cracking defects following polymerization, which are emphasized in SEM images (presumably due to further polymerization and restructuring under the electron beam).[5] The presence of these defects makes it possible to infer the directionality of molecular rows within ordered domains. Cracking defects of this type were observed in SEM images of diynoic acids deposited by microcontact printing, pointing to the assembly of ordered lamellar phases; AFM imaging was additionally utilized to quantify the presence of any areas of standing phase molecules based on topographic height (up to 3 nm for standing phases; 0.5–1.0 nm for typical lying down phases, dependent upon molecular orientation).

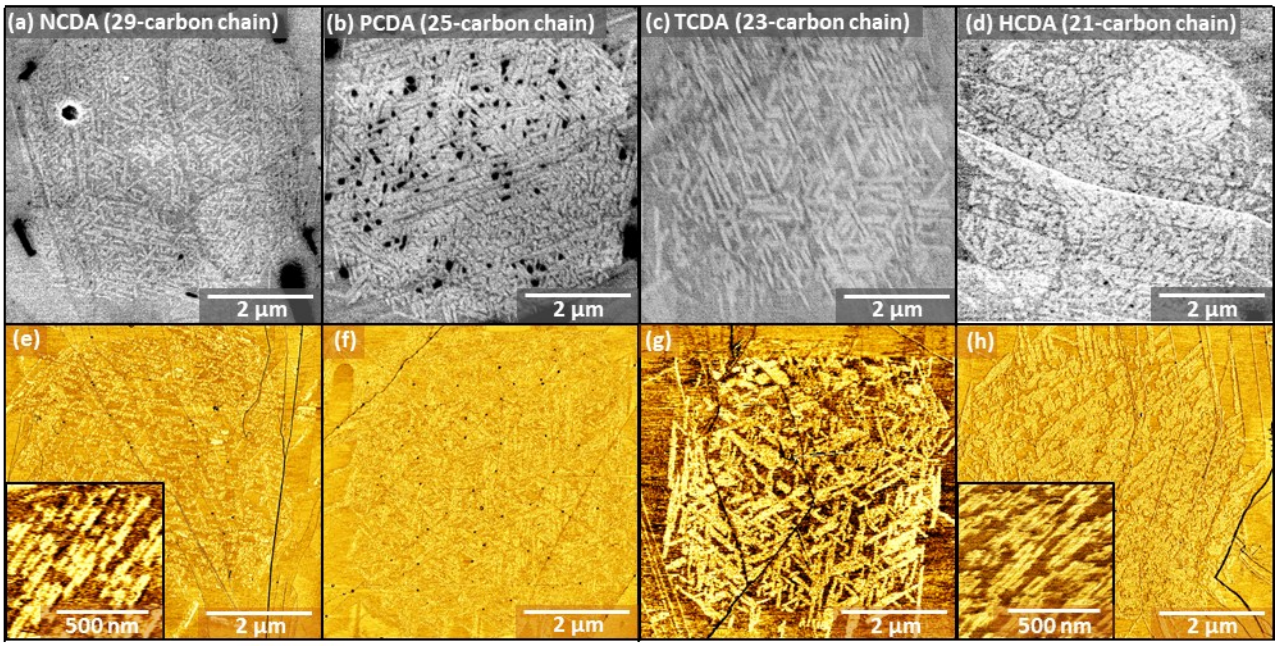


Fig. S3. SEM and AFM images of microcontact printed diynoic acids with chains 29 to 21 carbons in length: (a,e) 10,12-nonacosadiynoic acid (NCDA, 29-carbon chain); (b,f) 10,12-pentacosadiynoic acid (PCDA, 25-carbon chain); (c,g) 10,12-tricosadiynoic acid (TCDA, 23-carbon chain); (d,h) 10,12-henicosadiynoic acid (HCDA, 21-carbon chain).

Figure S4 shows example SEM and AFM images of phospholipids deposited on HOPG by microcontact printing, to illustrate the distinction between standing and lying-down phases. Diyne PE was deposited using a flat contact geometry, with a contact time of 30 s; diyne PC was deposited utilizing rolled contact. Both phospholipids were deposited from a 0.55 mM transfer solution. The bright contrast in the SEM images of diyne PE (Fig. S4a,b) is characteristic of amphiphiles assembled in a standing phase, and is consistent with height profiles observed in AFM topography images. Diyne PC,

deposited utilizing the rolled contact geometry, exhibits primarily ordered striped phases in the stamp contact areas (see manuscript for diyne PC images).

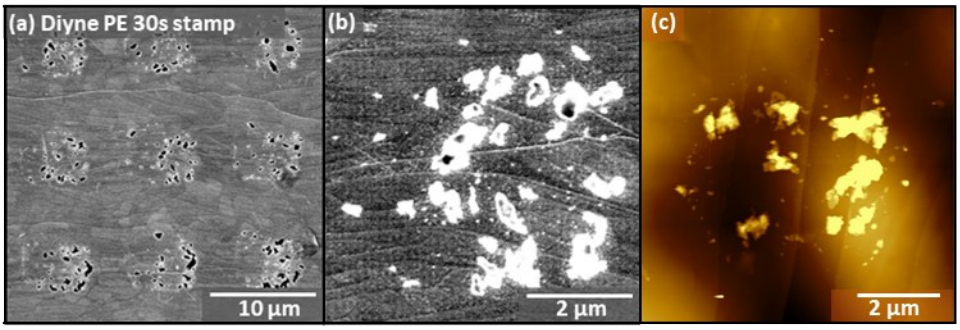


Fig. S4. (a,b) SEM and (c) AFM images of diyne PE transferred to HOPG using a conventional flat stamping geometry, illustrating transfer of standing phase.

Comparison of HOPG surfaces brought into contact with un-inked PDMS stamps prepared at elastomer base:crosslinker ratios of 10:5 to 10:1

For optimizing the delivery of amphiphiles to the substrate, stamps with a range of base:crosslinker ratios (10:5 to 10:1) (*m*/*m*) were examined. Fig. S5 compares the transfer of PDMS impurities. Stamps were exposed to just the solvent components of the ink solution and allowed to dry as described in the Experimental Methods. Areas of dark contrast in SEM images of substrates prepared in this way (such as those in Fig. S5) were consistent with those observed following transfer of single-chain and dual-chain amphiphiles. The extent of impurity transfer varied; there was no observed correlation with base:crosslinker ratio. Fig. S6 shows AFM phase micrographs of substrates exposed to 1.1 mM PCDA during transfer and illustrates the deposition of PDMS on the HOPG surface.

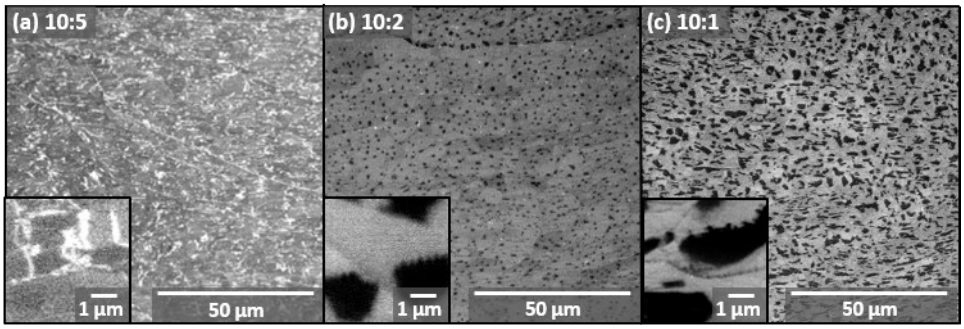


Fig. S5. SEM images of HOPG placed in contact with PDMS stamps prepared at (a) 10:5, (b) 10:2, and (c) 10:1 ratios of elastomer base to curing agent and exposed to solvent only.

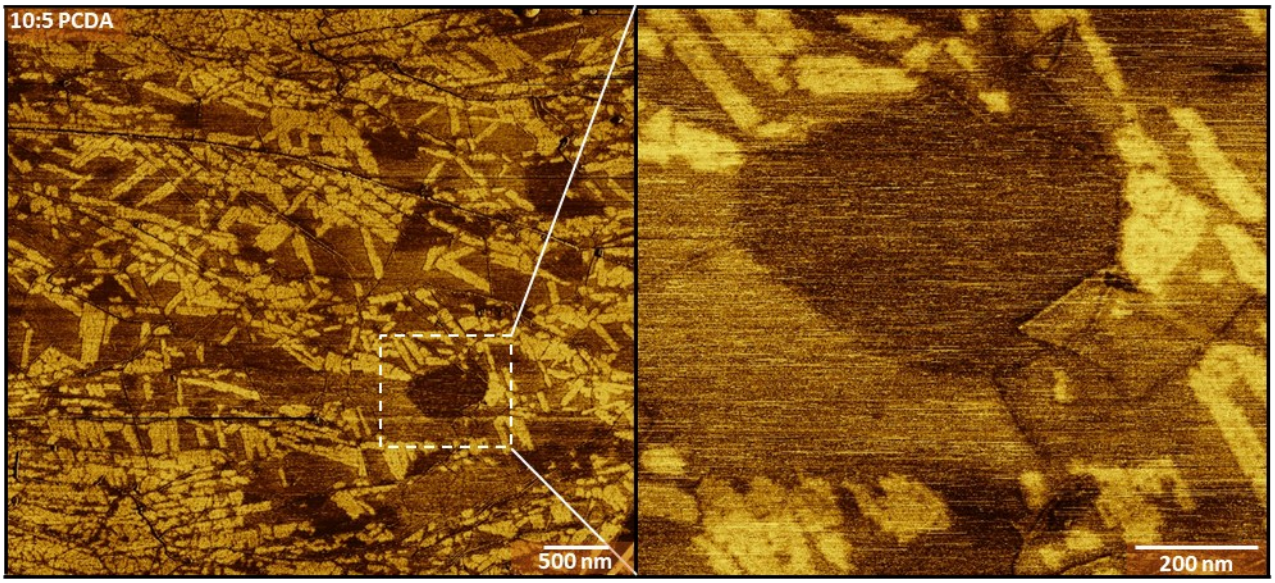


Fig. S6. AFM images of PCDA transferred at 1.1 mM concentration in the ink solution, with a stamp prepared at 10:5 base:crosslinker ratio, resulting in transfer of both striped phase PCDA domains (light regions), and PDMS impurities (dark regions).

Representative images of 10:1 base:crosslinker ratio of 0.5 mM diyne PC

The manuscript shows representative images of diyne PC transferred from PDMS stamps prepared at a 10:2 base:crosslinker ratio. Fig. S7 shows representative images of diyne PC transferred from stamps prepared at a 10:1 base:crosslinker ratio; similar images were used to calculate domain number densities and diffusion distances.

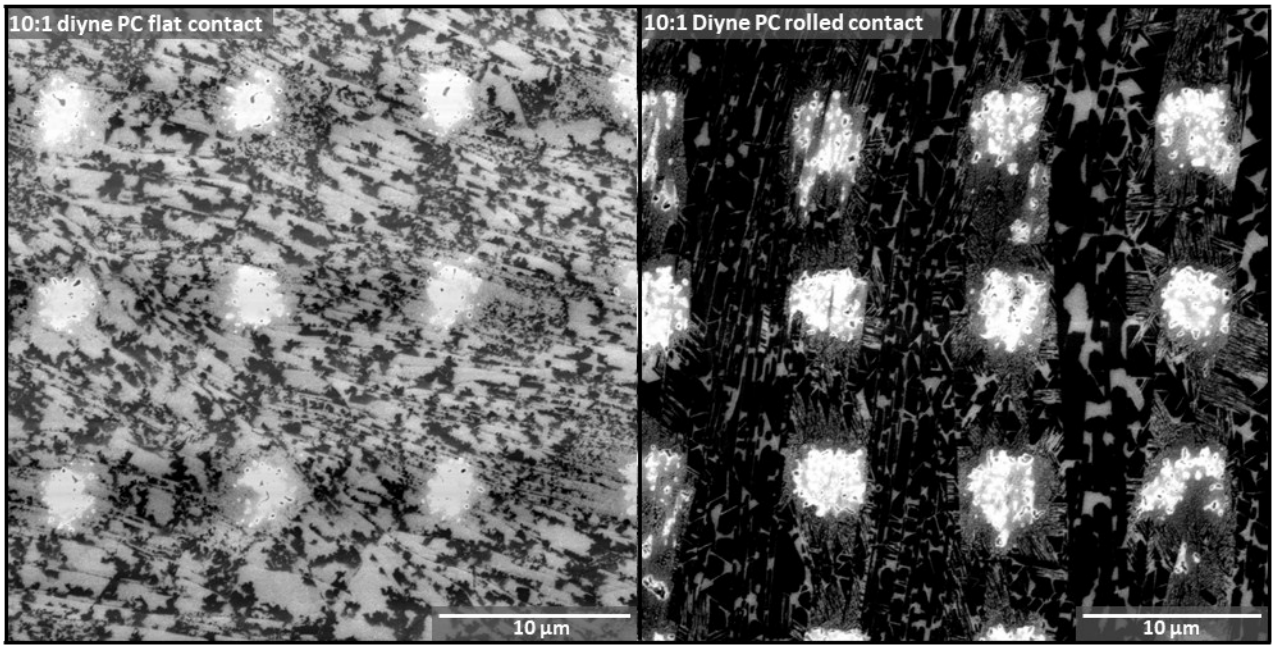


Fig. S7. SEM images of HOPG after rolled and flat contact transfer of diyne PC.

Image segmentation and analysis examples

Fig. S8 illustrates how manual image segmentation was performed to compare the assembly of striped phases and standing phases for the tested molecules (*e.g.* PCDA, diyne PE) using transfer conditions described in the manuscript and Experimental Methods. The figure illustrates an SEM image of diyne PC transferred to HOPG using a PDMS stamp prepared at a 10:1 base:crosslinker ratio, using rolled transfer. Striped phases can typically be identified based on rectangular domain geometries with linear edges, and/or the presence of long linear defects within the domain that appear during SEM imaging. The percent of the transfer resulting in striped phase domains is calculated by taking the difference of the area of the standing phase (Fig. S8c) from the total area occupied by the lipids (Fig. S8b). This gives the area of striped phase, which can then be divided by the total lipid-functionalized area to give the percentage of striped phase coverage. The diffusion distance was calculated by taking the total area occupied by the lipid (Fig. S8b) and subtracting the theoretical contact area (25 μm^2). This difference is the overfill (or underfill), which can then be used to calculate the diffusion distance.

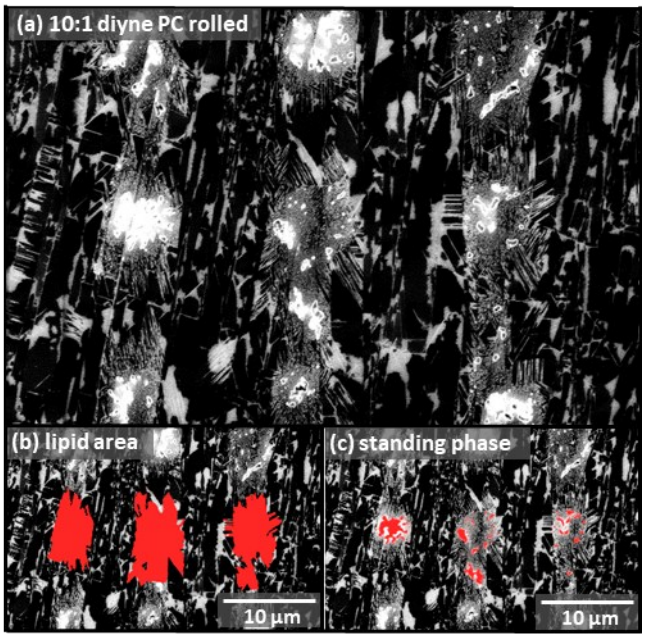


Fig. S8. Sample SEM images showing segmentation (red highlighted areas) utilized for calculations of % striped phase (*vs.* standing phase) molecular transfer.

Molecular domains created through microcontact printing are frequently small relative to size scales that are straightforward to identify utilizing SEM images. Thus, for some image analyses, we utilized AFM images, which typically provide higher resolution at smaller scales. Fig. S9 shows example AFM images of NCDA and HCDA transferred to HOPG using PDMS stamps prepared at 10:2 base:crosslinker ratios. Red lines indicate domains tabulated for number density measurements.

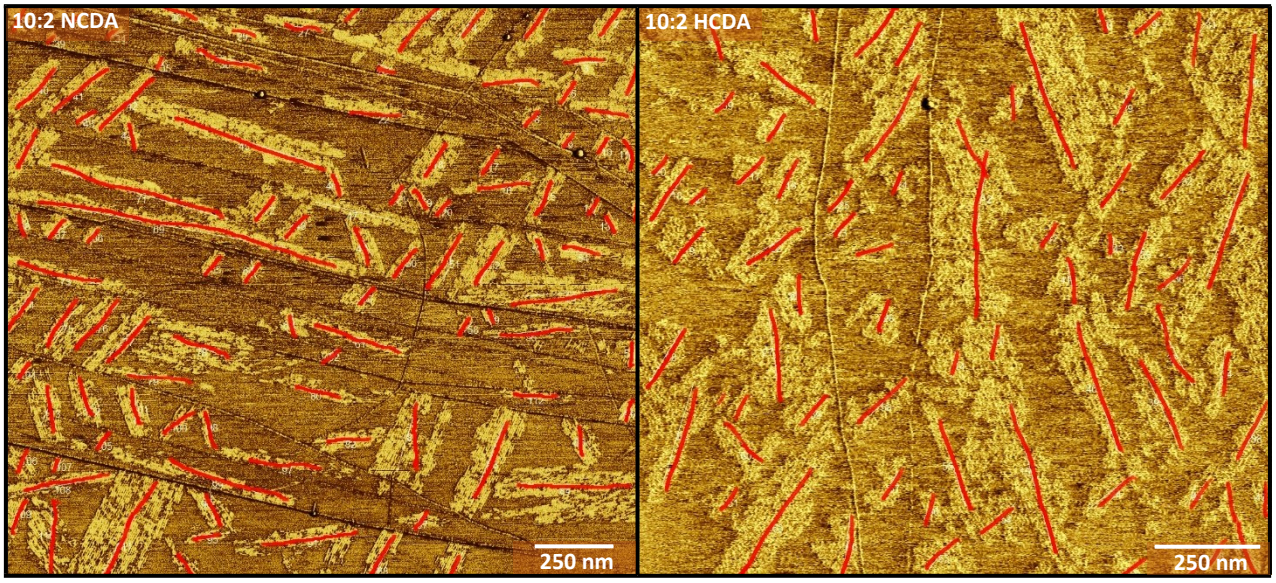
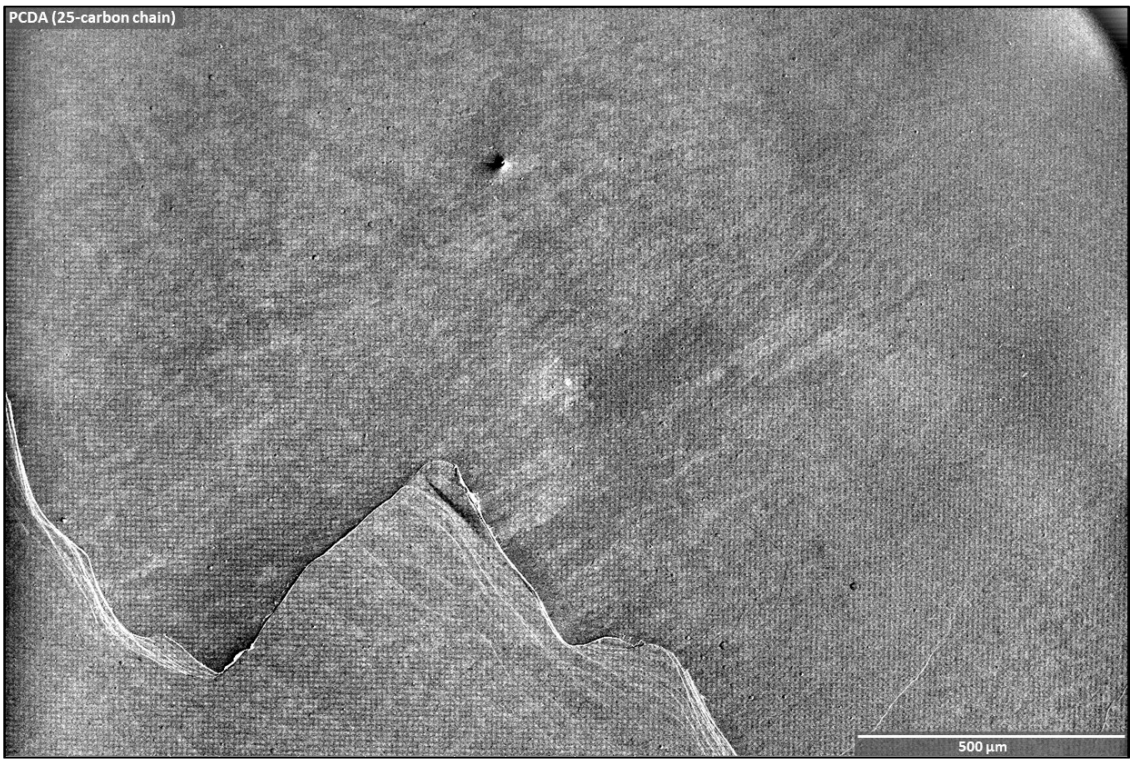


Fig. S9. AFM images showing example domain number density measurements (red lines highlighting each domain) utilized for average domain number density per μm².

Large-scale versions of SEM images shown in main manuscript

In the main manuscript, several SEM images are shown at small scale to facilitate comparison between molecular transfer conditions. Here, we show SEM images of larger areas of the surface and/or larger image sizes, to increase visibility of features within individual images. Fig. S10 shows a mm-scale area of the HOPG surface with areas of transferred PCDA striped phase. The square pattern is faintly visible at this scale, in addition to a large set of HOPG terraces in the lower left quadrant of the image; such features are common on cleaved HOPG. Fig. S11 shows an image of a section of the surface from Fig. S10, illustrating the degree of fidelity of pattern transfer, and the presence of narrow linear molecular domains



(brighter) and amorphous impurities (darker) in the regions between stamp contact areas.

Fig. S10. SEM images of HOPG after stamping with PDMS, illustrating long-range patterning and surface defects common on cleaved HOPG substrates.

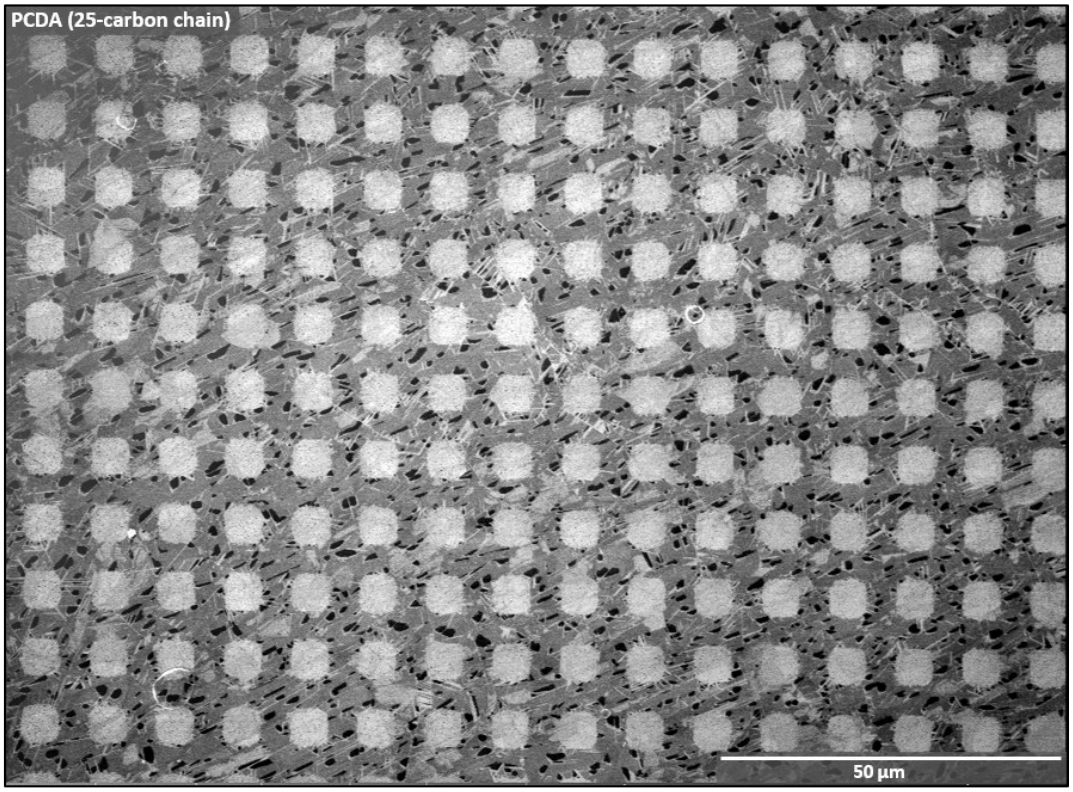


Fig. S11. SEM image of HOPG after stamping with PDMS, illustrating rounded edges of square features in stamp following transfer, and narrow linear molecular domains extending between stamped areas.

Larger versions of images in main manuscript illustrating fidelity and quality of transfer.

In the main manuscript, individual square areas of deposited NCDA, PCDA, and HCDA are shown in Fig. 4. Here, Fig. S12–15 show larger areas around the selected squares for visual comparison. Larger scale images of Diyne PE, Diyne PC, and PI from Fig. 5-7 are shown here in Fig. S16-21.

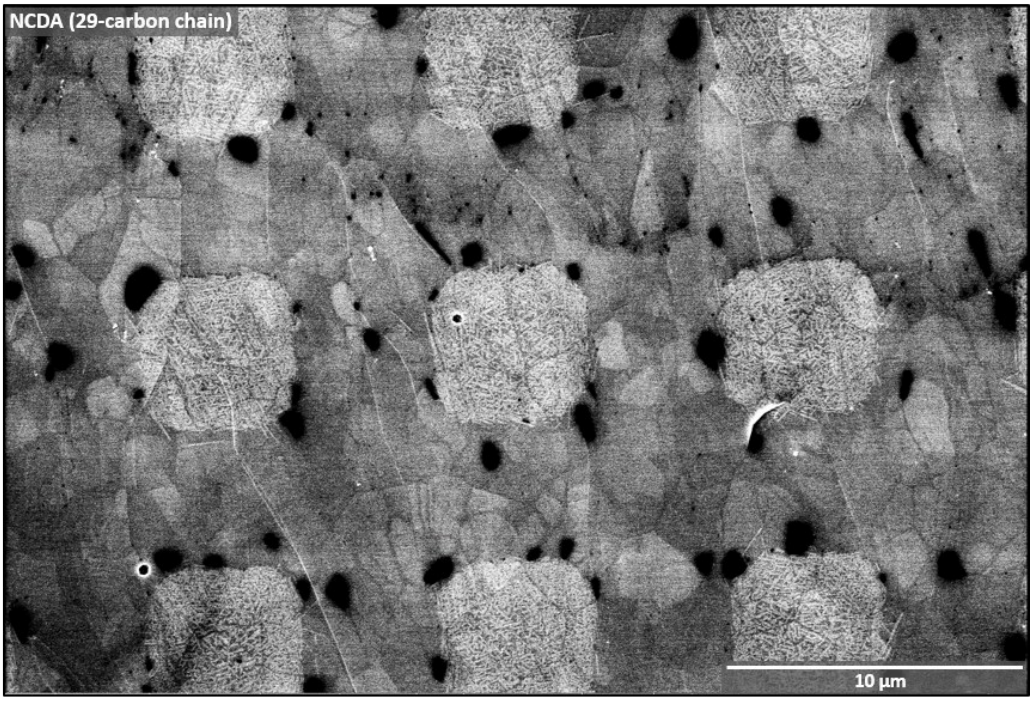


Fig. S12. SEM image of HOPG after stamping with NCDA.

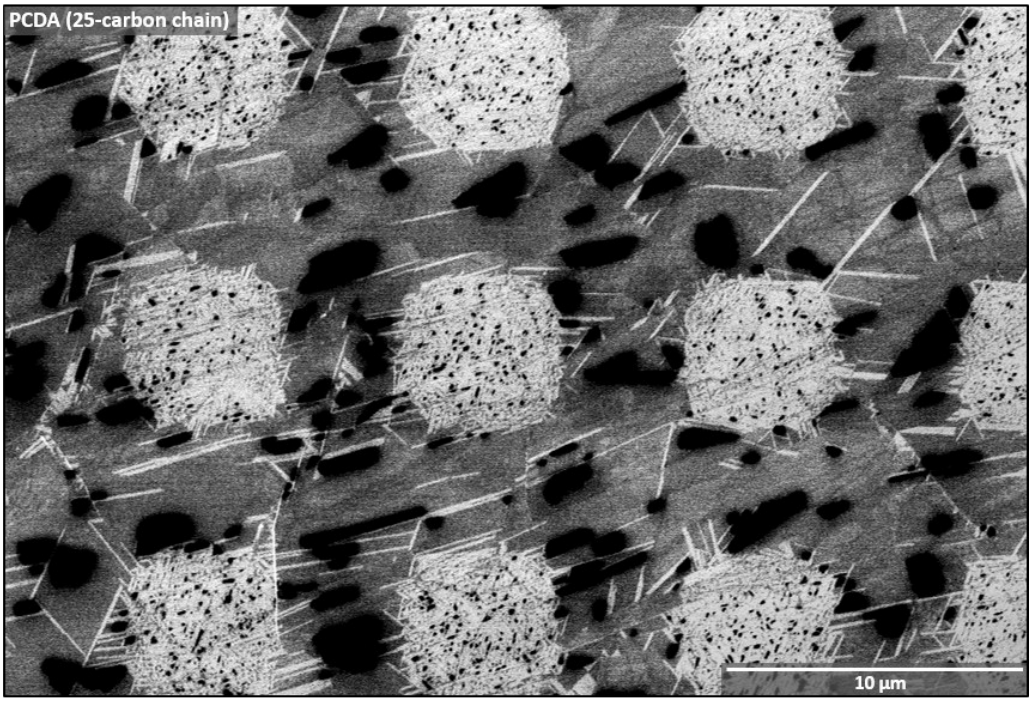


Fig. S13. SEM image of HOPG after stamping with PCDA.

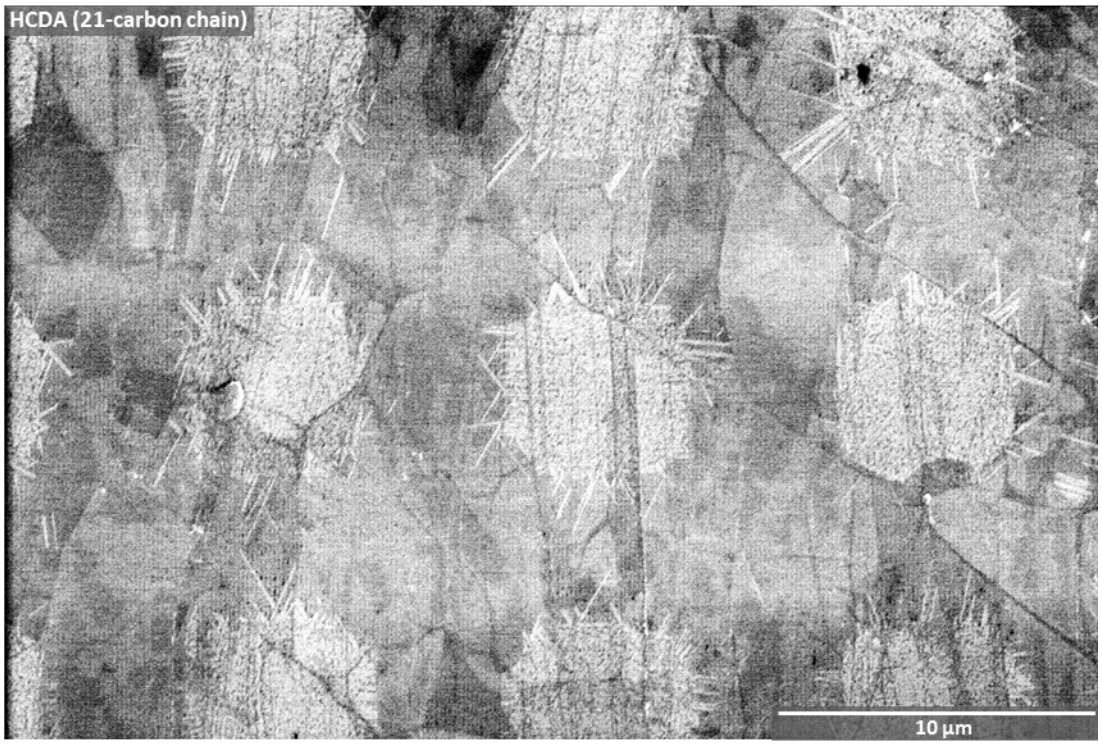


Fig. S14. SEM image of HOPG after flat contact stamping with HCDA.

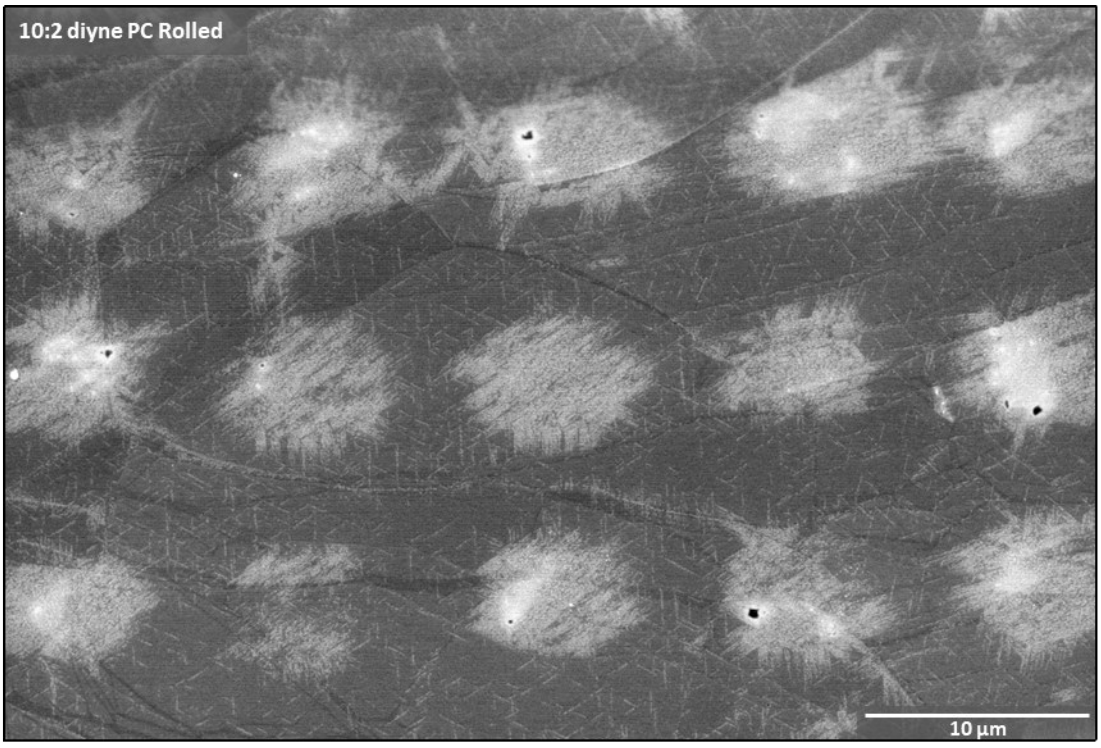


Fig. S15. SEM image of HOPG after rolled contact stamping with Diyne PC.

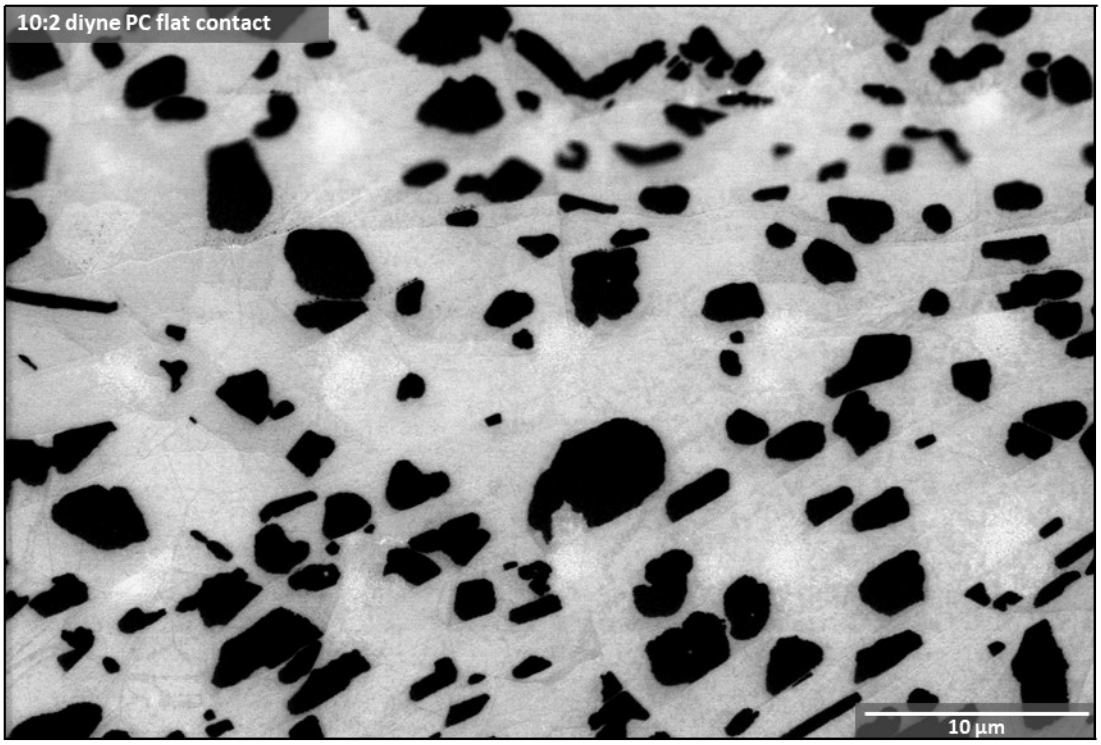


Fig. S16. SEM image of HOPG after flat stamping with Diyne PC.

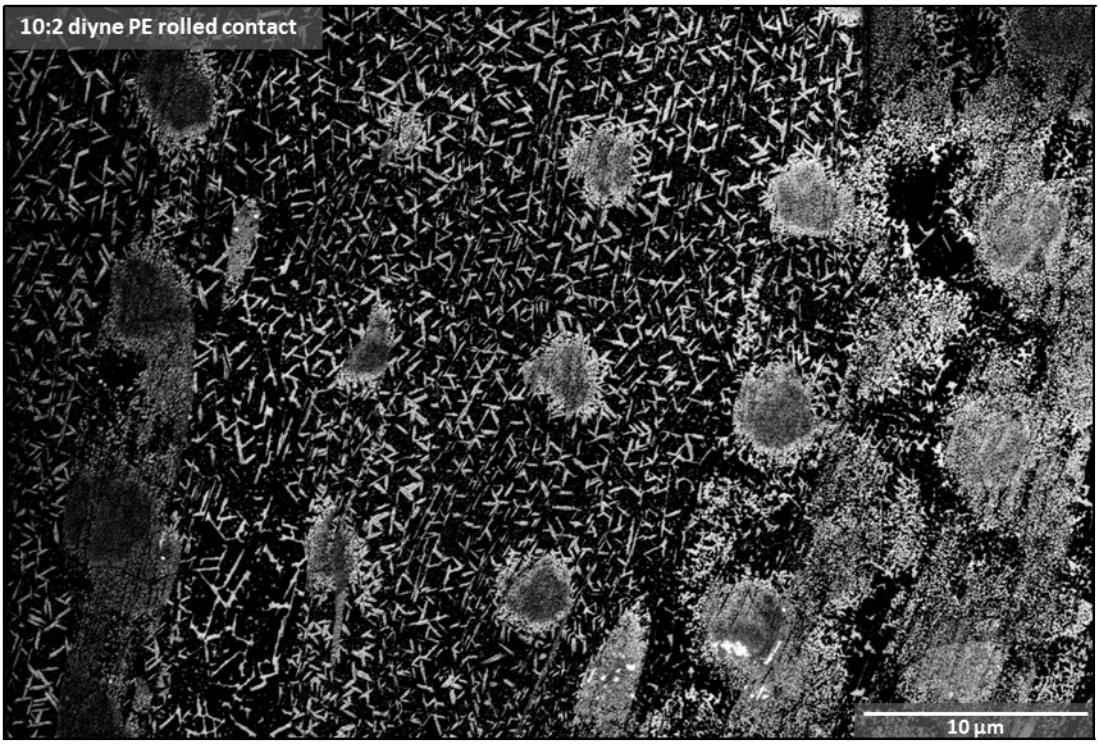


Fig. S17. SEM image of HOPG after rolled contact stamping with Diyne PE.

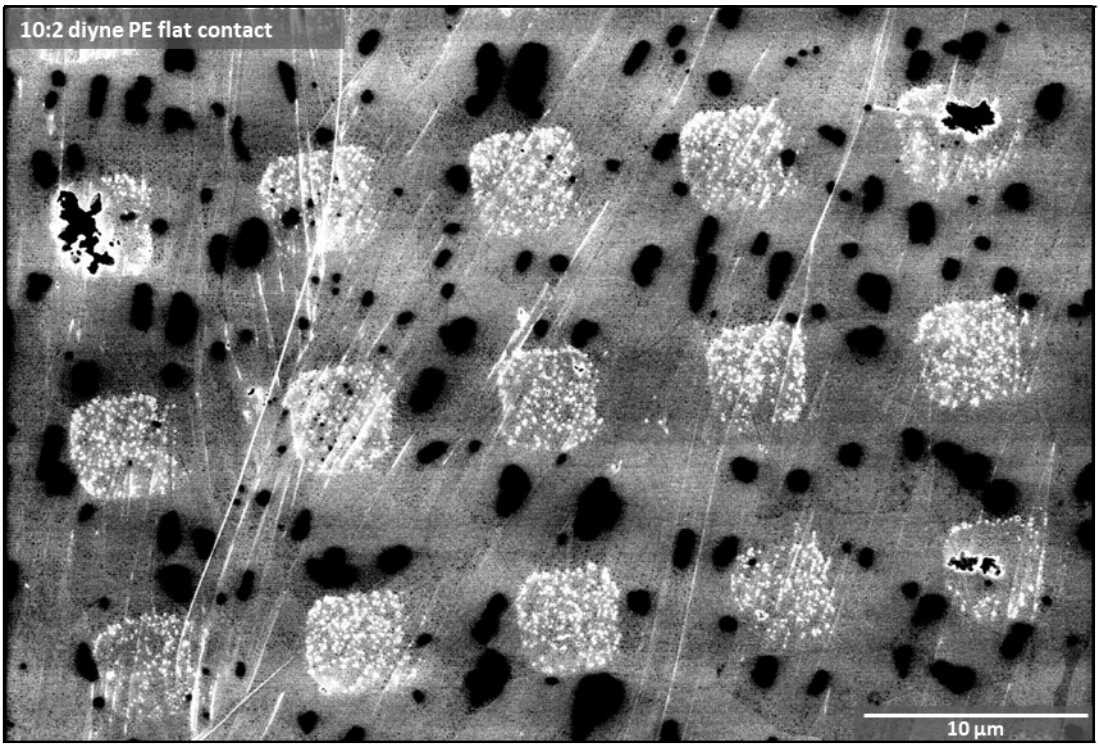


Fig. S18. SEM image of HOPG after flat stamping with Diyne PE.

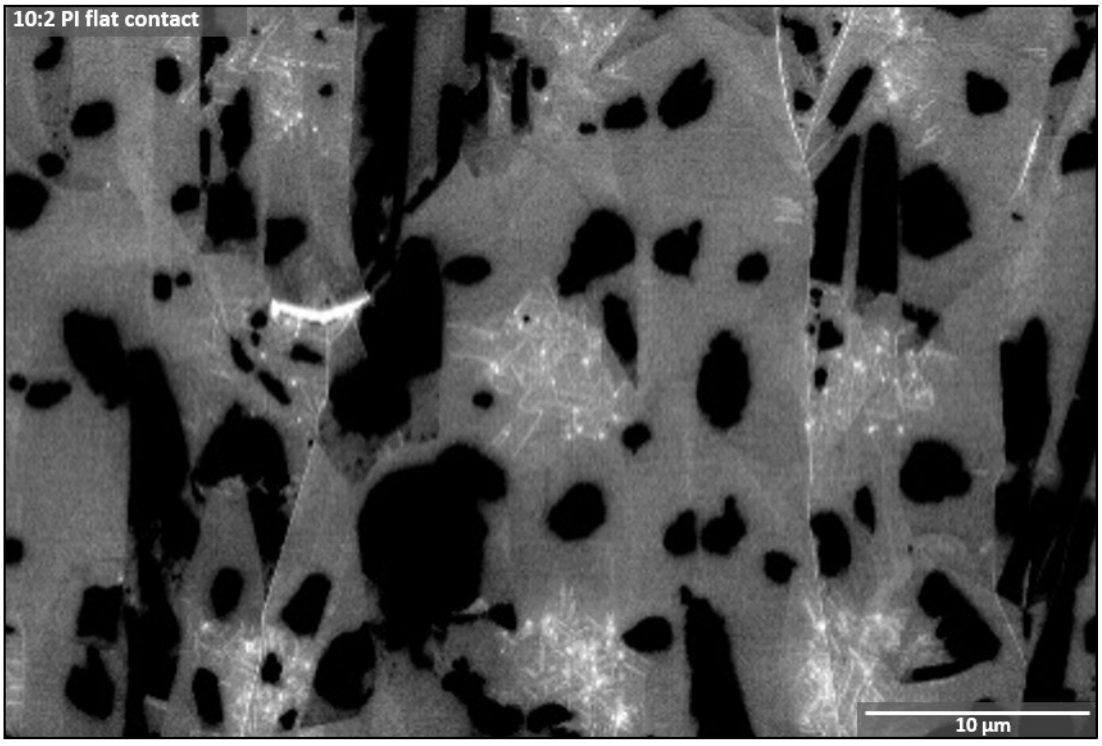


Fig. S19. SEM image of HOPG after flat stamping with PI.

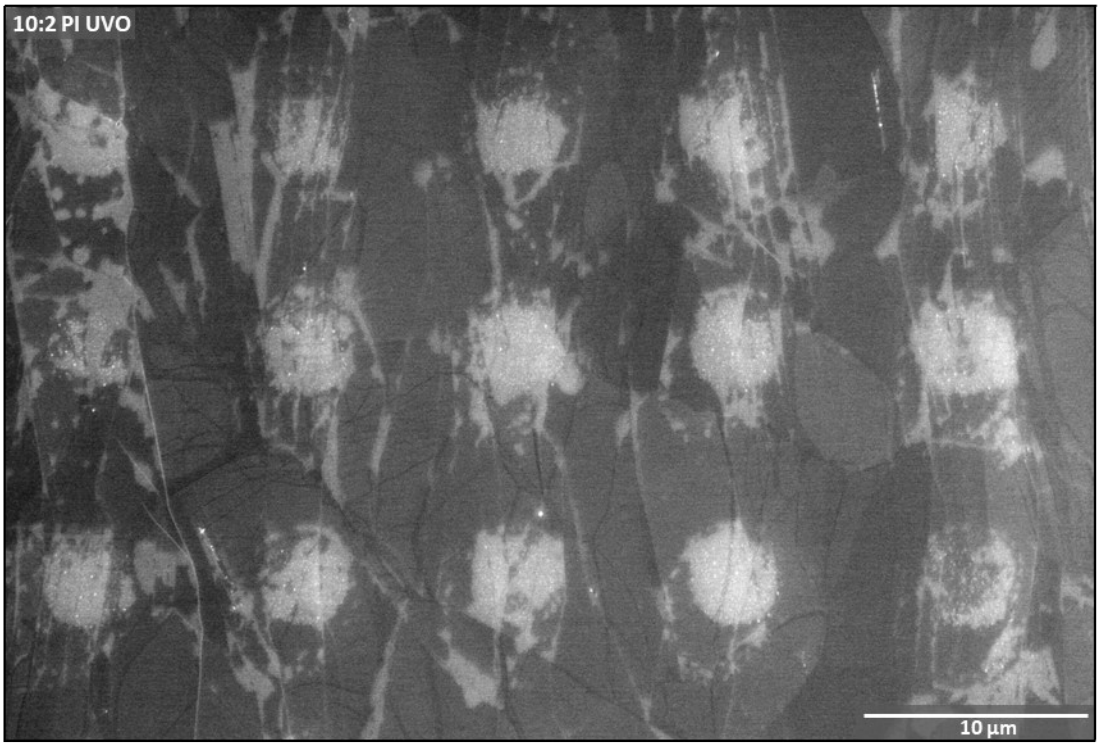


Fig. S20. SEM image of HOPG after flat + UVO contact stamping with PI.

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