ADVANCED MATERIALS

Supporting Information

for *Adv. Mater.*, DOI: 10.1002/adma.201505197

Ultrasensitive In Situ Fluorescence Analysis using Modulated Fluorescence Interference Contrast at Nanostructured Polymer Surfaces

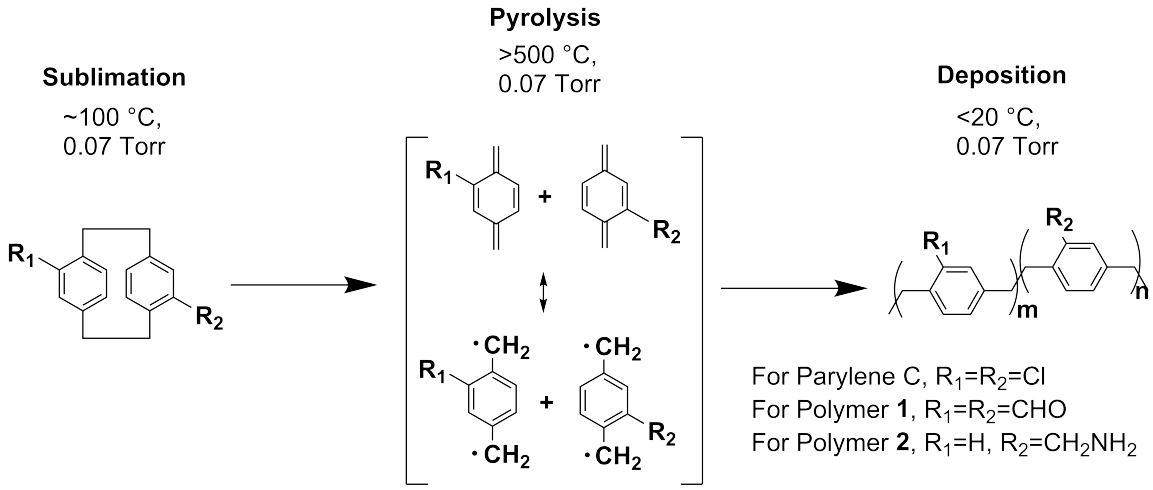
Xiaopei Deng, Siheng He, Fan Xie, Christian Friedmann, Henry Hess, and Joerg Lahann**

Supporting Information

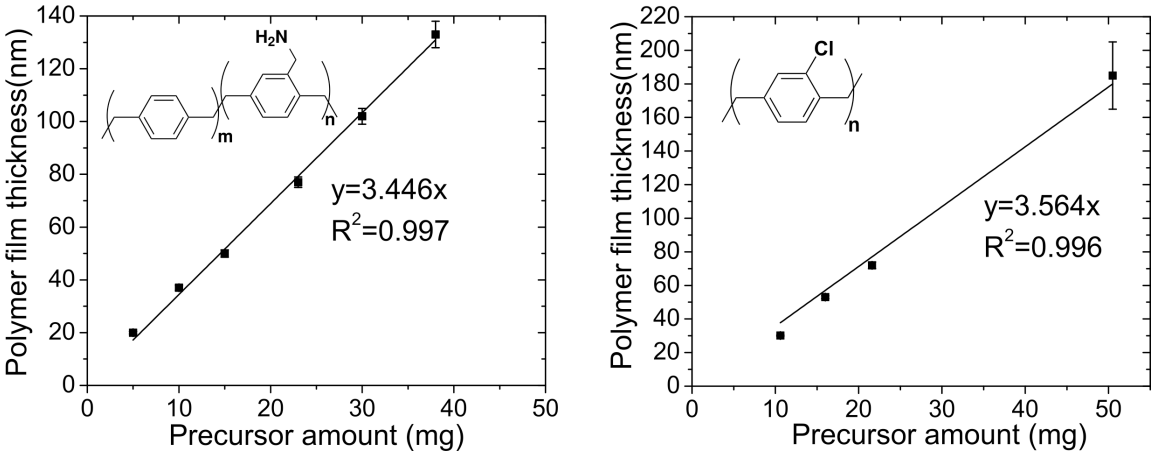
Ultra-sensitive *in situ* fluorescence analysis using modulated fluorescence interference contrast at nanostructured surfaces

Xiaopei Deng, Siheng He, Fan Xie, Christian Friedmann, Henry Hess*, Joerg Lahann*

Supplementary Note 1
CVD polymerization scheme and accurate control of the deposition thickness by precursor feed amount

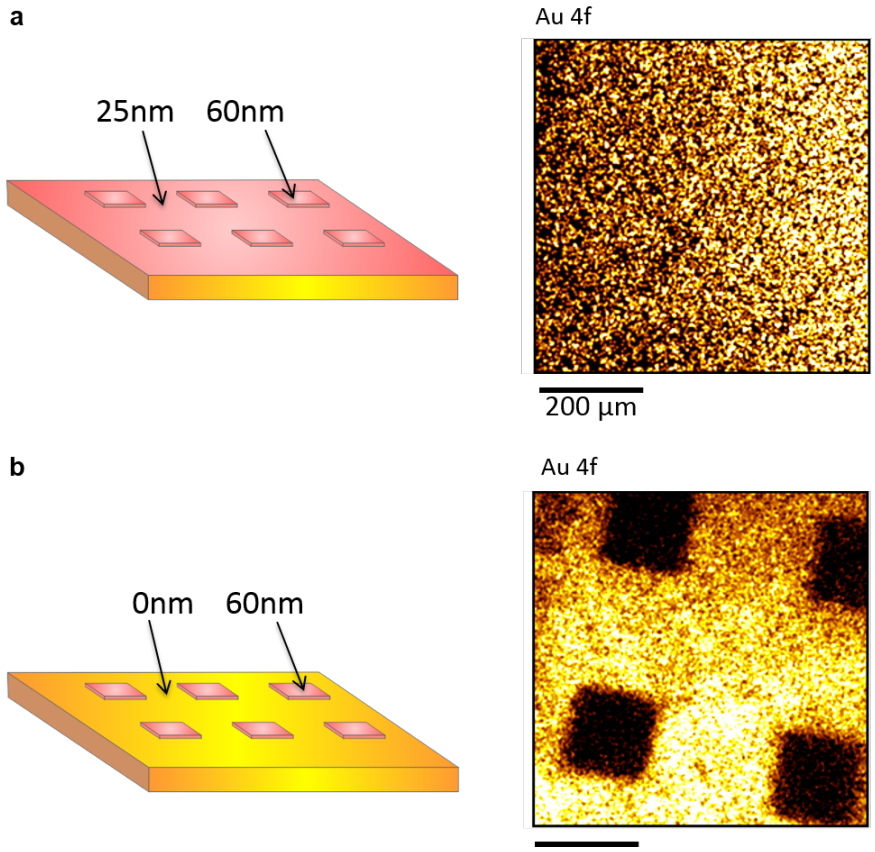


Supplementary Figure 1.1. Chemical vapor deposition polymerization scheme for the three types of polymers with different functional groups used in this study. Poly(4-chloro-p-xylylene) (Parylene C), Poly(formyl-*p*-xylylene) (polymer 1) and poly[(4-aminomethyl-*p*-xylylene)-*co*-(*p*-xylylene)] (polymer 2) were synthesized via CVD polymerization as shown in the scheme. The CVD process was carried out at 0.07 torr, with 20 sccm argon as carrier gas. The precursor was sublimed at 90-110˚C in vacuum and converted into the corresponding diradical by thermal pyrolysis (670˚C) under vacuum. The diradicals then spontaneously adsorbed and polymerized on the cooled (15˚C) substrate placed on top of a rotating stage. The deposition rate was controlled at 0.5 Å/s and the resulted film thickness was controlled by different precursor feed amount (Supplementary Figure 1.2). To generate surfaces with polymer micro-patterns by multiple-step CVD, the same polymer was coated on the surface multiple times with different monomer feeding amount and with the help of PDMS masks.



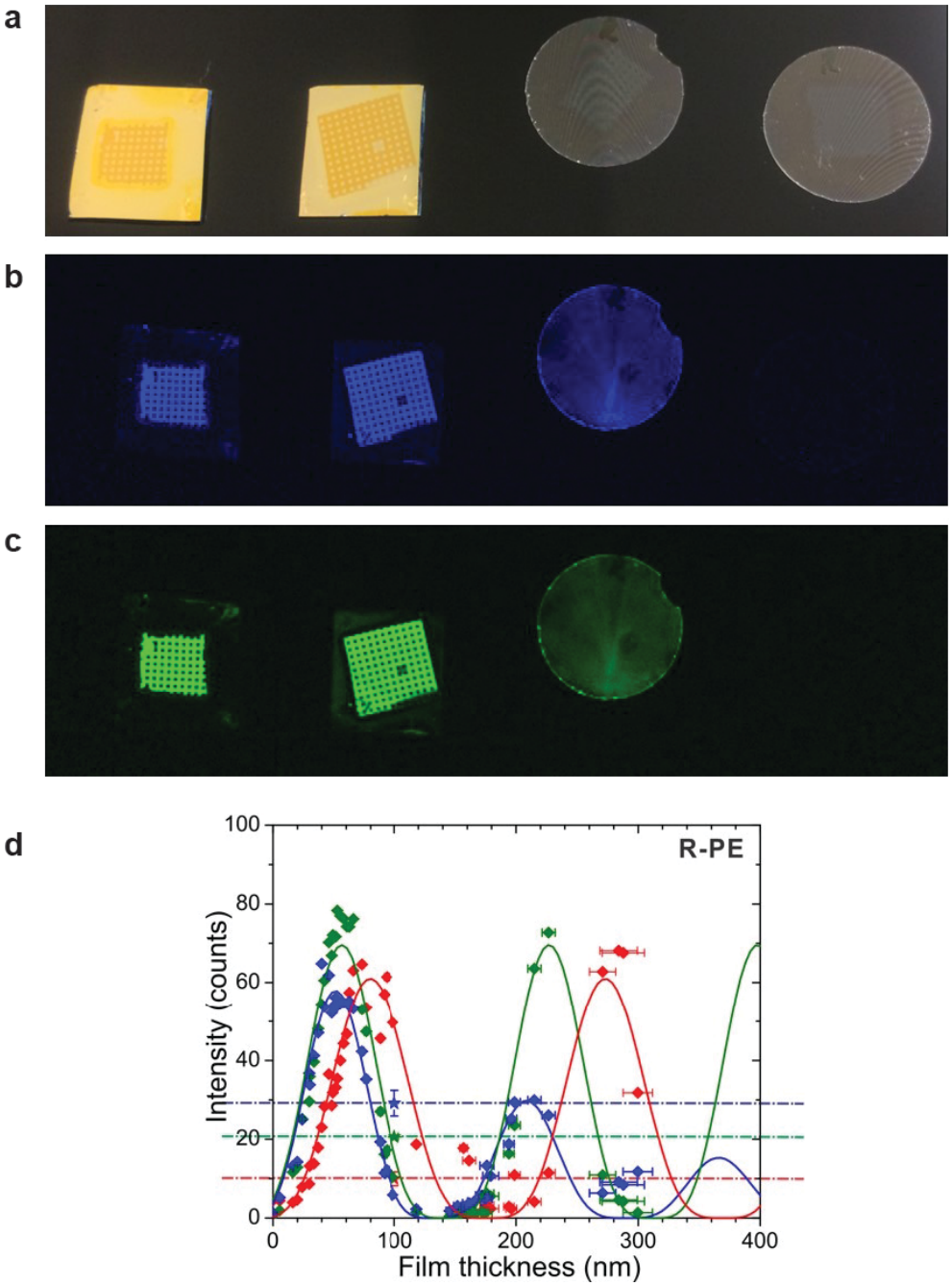
Supplementary Figure 1.2. Relationship of precursor feeding amount and the resulted CVD polymer film thickness for different types of polymers used in this study. (a) Polymer 2; (b) Parylene C. The CVD polymer thickness has a linear relationship with the precursor feed amount when the deposition rate and other parameters are fixed. The relationship of precursor feeding amount and the resulted film thickness for the Polymer 1 is presented in Figure 2c in the main manuscript. The slopes are different as the radicals with different functional groups have different sticking coefficient.

Supplementary Note 2
XPS imaging of the polymer patterned surfaces without or with chemical differences in different pattern regions



Supplementary Figure 2.1. XPS imaging of Au 4f on the CVD polymer patterned on gold surfaces. (a) For the patterned surface with 25nm Parylene C outside the squares and 60 nm inside the squares, no chemical contrast is observed. (b) For the patterned surface with no Parylene C outside the squares and 60 nm inside the squares, clear chemical contrast is observed. The XPS images are presented without processing. Despite the existence of photoelectron background noise and artifacts from the detector (the intensity variation from one side to the other side of the images), the difference is clear between a chemically identical surface with two layers of polymer and a surface with chemical difference in different pattern regions.

Supplementary Note 3
Comparing the intensity change of a fluorescent protein R-Phycoerythrin (R-PE) adsorbed on the CVD polymer coated gold or glass substrates



Supplementary Figure 3.1. Fluorescence intensity comparison of R-phycoerythrin (R-PE) adsorbed on the Parylene C coatings deposited on gold or glass with pattern areas. (a) Photograph of the samples on the stage of the FluorChem M imaging dark room. The polymer thicknesses inside and outside the pattern squares are marked as thickness inside/thickness outside. For the samples from left to right: 126nm/56nm, 167nm/53nm,

150 nm/55nm, 150 nm/55nm. The CVD polymer thicknesses on glass coverslips were estimated by measuring the polymer thicknesses on silicon pieces right next to them. The left three samples were incubated in 8 μg/ml R-PE (a fluorescent protein) for 2 hours, rinsed three times with PBS and three times with water before drying and imaging. The rightmost sample went through no treatment after being coated and patterned by Parylene C. All samples have the same pattern feature size. (b) Fluorescence images under blue excitation light; (c) Fluorescence images under green excitation light. The exposure time for both (b) and (c) is 1 s. Note that the FluorChem M imaging dark room shows the color of the excitation light, which is different from the fluorescence microscope. (d) A series of Parylene C coated gold pieces with different polymer layer thicknesses were incubated in R-PE for 90 min, thoroughly rinsed, air dried. The samples were imaged in the FluorChem M digital dark room with blue, green and red excitation light respectively. The fluorescence intensity from the center areas of the pieces are measured by ImageJ and plotted against the film thickness from the center areas of the samples. Both the experimental data (the diamond dots) and the fitting curve (the solid line) are plotted. The dash-dot lines present the fluorescence intensity of the R-PE adsorbed on Parylene C coated glass pieces.

Supplementary Note 4

Data fitting with the fluorescence interference contrast (FLIC) equation

Following the method of Kerssemakers et al. (PNAS 103(43), 15812-15817 (2006)), and assuming that the reflectivity of the gold surface is nearly perfect for the wavelengths considered here (parameter R=0), the fluorescence intensity *I* on the spacer layer with thickness *h* is fitted with:

$$I(h) = I_0 \sin^4(2\pi n(h + h_0)/\lambda)\exp(-h/\gamma)$$

where I_0 is the maximal fluorescence intensity, n is the refractive index of the spacer layer (here n=1.64 for parylene), h_0 is a parameter which introduces an offset (discussed below) in the intensity modulation, λ is the effective average wavelength of excitation and emission light and γ is the decay parameter which approximates the loss in modulation depth due to the high numerical aperture objective and the finite coherence length of excitation and emission light. The fitting results are shown in Supplementary Table 1.

Supplementary Table 4.1. Fitting parameters of the FLIC equation

	I_0	h_0 (nm)	λ (nm)	γ (nm)
FITC-BSA (Figure 1c)	103.9 ± 3.1	23.4 ± 2.2	475.6 ± 6.7	-
Alexa Fluor 555 Hydrazide, Au (Figure 3h)*	72.8 ± 5.1	19.3 ± 1.7	560 (fixed)	700 (fixed)
Alexa Fluor 555 Hydrazide, Au (Figure 3h)	23.5 ± 1.9	-2.8 ± 3.1	560 (fixed)	700 (fixed)
Alexa Fluor 488 Fibrinogen, Si (Figure 3i)*	110.2 ± 8.3	22.4 ± 1.7	488 (fixed)	700 (fixed)
Alexa Fluor 488 Fibrinogen, Si (Figure 3i)	44.7 ± 5.5	-3.2 ± 3.5	488 (fixed)	700 (fixed)
R-PE with blue excitation light (Supplementary Figure 3.1d)	72.3 ± 3.2	24.8 ± 2.0	516.3 ± 9.1	236.7 ± 32.4
R-PE with green excitation light (Supplementary Figure 3.1d)	69.6 ± 1.7	29.0 ± 2.0	559.9 ± 7.0	-
R-PE with red excitation light (Supplementary Figure 3.1d)	61.0 ± 2.0	16.8 ± 2.5	634.0 ± 11.2	-

* Fit with modified model $I(h) = I_0 \sin^{10}(2\pi n(h + h_0)/\lambda)\exp(-h/\gamma)$

The presence of an offset h_0 of between 15 nm and 30 nm indicates that the light penetrates partially into the surface of the metallic mirror, an effect which is not observed for reflection at a dielectric surface such as silicon. These intriguing differences in the FLIC curves between metallic and dielectric reflectors will be studied in detail in a future publication.

Supplementary Note 5

Experimental details of the specific antibody binding experiment shown in Fig. 5

The patterned surface was incubated in 0.5 mg/ml biotinylated polyethylene glycol (PEG) hydrazide (Thermo Scientific) solution (pH=5) for 4 h. After thorough rinsing with water and PBS solution, 20 μg/ml streptavidin in phosphate buffer (PBS) containing 0.1% bovine serum albumin (BSA) was added and washed away with PBS after 90 min. Subsequently, 10 μg/ml biotinylated mouse epidermal growth factor (EGF) (Life Technologies) in PBS containing 0.1% BSA was added and washed away after 90 minutes. Then the surface was incubated in 10 μg/ml anti-mouse EGF antibody (a rabbit IgG protein) (Abcam) and thoroughly rinsed after 90 minutes. Finally the surface was incubated with 20 μg/ml Alexa 647 conjugated secondary antibody (goat anti-rabbit IgG) (Life Technologies) in goat serum and the specific antibody binding process was monitored in real time under fluorescence microscope.

Supplementary Note 6
Synthesis and characterization of the novel polymer 1

CVD Polymer 1 Precursor Synthesis. All chemicals were purchased from Aldrich and VWR and used as received. Routine monitoring of reactions was performed using silica gel coated aluminium plates (silica gel 60), which were analyzed under UV-light at 254 nm. All NMR spectra were recorded on a *Bruker* Avance III spectrometer as solutions. ^{1}H-NMR: Chemical shifts are given in parts per million (ppm, δ) and are referenced to $CHCl_3$ (7.26 ppm) as internal standards. All coupling constants are absolute values and *J* values expressed in Hertz (Hz). The description of signals include: s = singlet, d = doublet, q = quartet, m = multiplet, dd = doublet of doublets, ddd = doublet of dd, etc. The spectra were analyzed according to first order. ^{13}C-NMR: Chemical shifts are expressed in parts per million (ppm, δ) referenced to $CDCl_3$ (77.0 ppm) as internal standards. ^{19}F-NMR: Chemical shifts were calculated from the spectrometer without internal standard. Mass spectra were recorded on a *Finnigan* MAT95. IR spectra were recorded on a *Bruker* IFS 88. Samples were measured on KBR or directly (ATR).

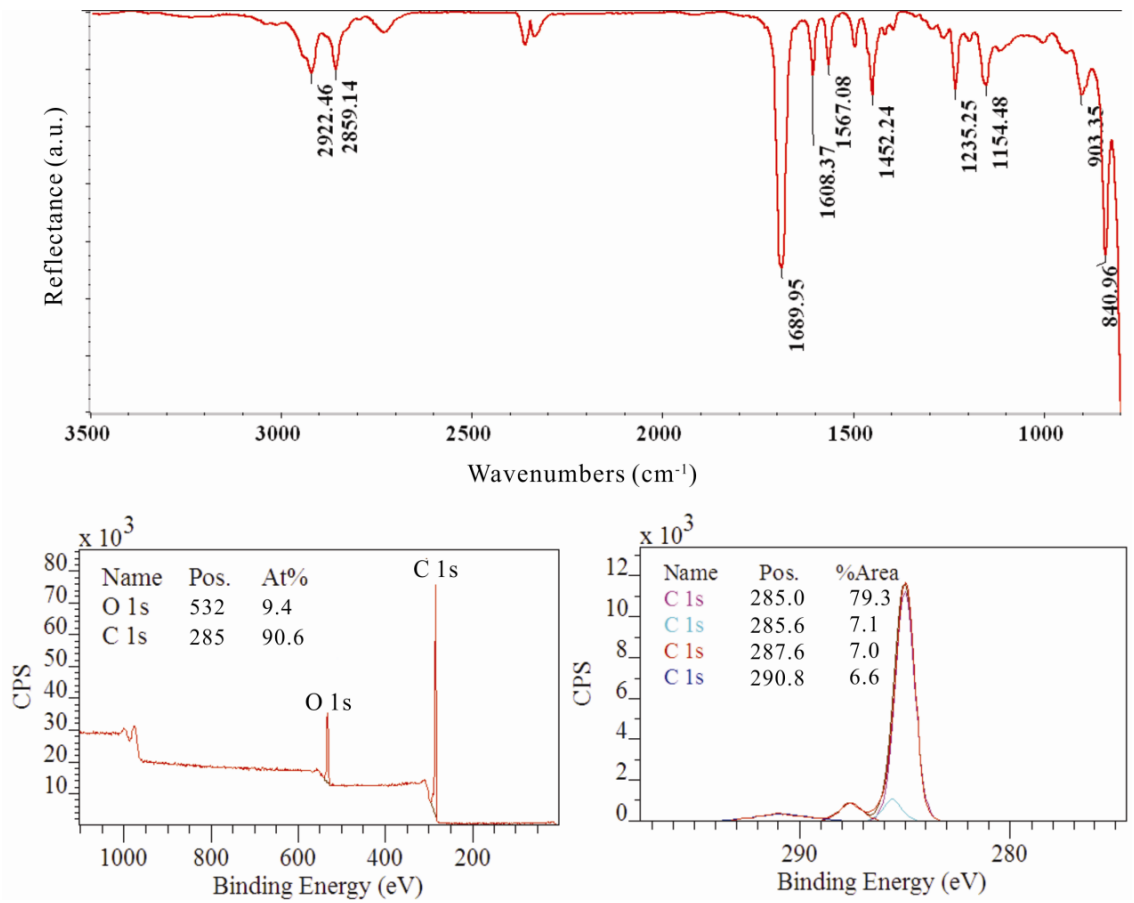
The polymer **1** precursor 4,12-diformyl[2.2]paracyclophane started with the synthesis of the compound 4,12-dibromo[2.2]paracyclophane, which was synthesized by a slightly modified literature procedure[1]. First, bromine (12.4 ml, 38.8 g, 243 mmol) was dissolved in 100 ml tetrachloromethane and 15 ml of this solution was added dropwise to iron powder (250 mg, 4.48 mmol) in a 1 liter round bottom flask. After stirring for 1 h the mixture was diluted with 350 ml tetrachloromethane and [2.2]paracyclophane (25.0 g, 120 mmol) was added. The remaining bromine solution was added dropwise over a period of 3h and stirring remained for 16 h. A saturated aqueous thiosulfate solution (5 ml) was carefully added and the solid was filtered off. The residue was washed with water (100 ml), ethanol (100 ml) and pentane (100 ml), then refluxed in dichloromethane (1200 ml) and filtered again. The solution was now concentrated (to a total volume of 700 ml) and cooled to –30 °C. After filtration the 4,12-dibromo[2.2]paracyclophane compound (12.0 g, 27%) was obtained as a colorless solid. **^{1}H NMR** (500 MHz, $CDCl_3$): 7.15 (dd, *J* = 7.8 Hz, 1.7 Hz, 2H, aromatic), 6.52 (d, *J* = 1.7 Hz, 2H, aromatic), 6.44 (d, *J* = 7.8 Hz, 2H, aromatic), 3.45 (ddd, *J* = 13.1 Hz, 10.4 Hz, 2.4 Hz, 2H, CH_2), 3.16 (ddd, *J* = 12.9 Hz, 10.4 Hz, 5.0 Hz, 2H, CH_2), 2.95 (ddd, *J* = 12.9 Hz, 10.8 Hz, 2.4 Hz, 2H, CH_2), 2.85 (ddd, *J* = 13.1 Hz, 10.8 Hz, 5.0 Hz, 2H, CH_2) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$): 141.2, 138.5, 137.3, 134.1, 128.3, 126.7, 35.4, 32.8 ppm. **FT-IR** (ATR): 2933, 1583, 1535, 1473, 1390, 1186, 1030, 899, 855, 830, 706, 669, 648, 523, 464 cm^{-1}. **m.p.**: 225 °C (lit[1]: 235 °C). **HR-MS (EI)**: 363.9462 (calculated for [M^+], $C_{16}H_{14}Br_2$), 363.9640 (observed). Analytical data agree with literature[1].

Under an argon atmosphere 4,12-dibromo[2.2]paracyclophane (3.51 g, 9.59 mmol) was dissolved in 175 ml THF and cooled to –78 °C. Then *n*-BuLi (21.4 ml, 34.2 mmol, 1.6 M in hexane) was slowly added and the solution was stirred at –78 °C for 9 h. DMF (5.88 ml, 5.53 g, 75.6 mmol) was added dropwise and the solution was slowly warmed to room temperature (over 4 h) and stirred for further 16 h. After addition of sat. NH_4Cl solution (100 ml) the mixture was acidified by 1 m HCl solution. Ethyl acetate (150 ml) was added and the phases were separated. The aqueous phase was extracted with EtOAc (150 ml) and the combined organic phases were dried over $MgSO_4$. After filtration and evaporation of the solvent, the crude product was purified by column chromatography (eluent: CH_2Cl_2) yielding the product (1.13 g, 45%) 4,12-diformyl[2.2]paracyclophane as a colorless solid. **^{1}H NMR** (500 MHz, $CDCl_3$): 9.94 (s, 2H, CHO), 7.05 (d, *J* = 1.9 Hz, 2H, aromatic), 6.64 (dd, *J* = 7.8 Hz, 1.9 Hz, 2H, aromatic), 6.52 (d, *J* = 7.8 Hz, 2H, aromatic), 4.13 (ddd, *J* = 13.3 Hz, 10.5 Hz, 2.4 Hz, 2H, CH_2), 3.29 (ddd, *J* = 13.4 Hz, 10.7 Hz, 2.4 Hz, 2H, CH_2), 3.16 (ddd, *J* = 13.4 Hz, 10.5 Hz, 5.8 Hz, 2H, CH_2), 3.02 (ddd, *J* = 13.3 Hz, 10.7 Hz, 5.8 Hz, 2H, CH_2) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$): 191.9, 142.9, 140.5,

137.0, 136.8, 136.5, 135.2, 34.3, 32.8 ppm. **FT-IR** (ATR): 1670, 1587, 1223, 1137, 862, 721, 649 cm^{-1}. **m.p.**: 233 °C. **EI-MS** [70 eV, m/z (%)]: 264 (84) [M^+], 209 (39) [$C_{15}H_{13}O$], 132 (91) [$C_9H_8O]^+$, 104 (100) [$C_8H_8]^+$. **HR-MS (EI)**: 264.1150 (calculated for [M^+], $C_{18}H_{16}O_2$), 264.1152 (observed). Analytical data agree with literature[2].

CVD Polymerization. Poly(formyl-*p*-xylylene) (polymer **1**) was synthesized *via* CVD polymerization as shown in Supplementary Figure 1.1. The CVD process was carried out at 0.07 Torr, with 20 sccm argon as carrier gas. The precursor was sublimed at 90-110˚C in vacuum and converted into corresponding diradical by thermal pyrolysis (670˚C) in vacuum. The diradicals then spontaneously adsorbed and polymerized on the cooled (15˚C) substrate placed on top of a rotating stage. The deposition rate was controlled at 0.5 Å/s and the resulted film thickness was controlled by different precursor feed amount.

Polymer Coating Chemical Characterization. FTIR spectra of the CVD polymer films were recorded on a Nicolet 6700 spectrometer with the grazing angle accessory (Smart SAGA) with a 80° fixed angle of incidence. XPS spectra were acquired on an Axis Ultra X-ray photoelectron spectrometer (Kratos Analyticals, UK) equipped with a monochromatized AlKα X-ray source. All spectra were calibrated with respect to the non-functionalized aliphatic carbon with a binding energy of 285.0 eV. The FTIR and XPS results are shown in the figure below. The strong IR peak at 1689 cm^{-1} is characteristic of the aldehyde functional group. The XPS data present the surface composition of polymer 1 within the outermost 10 nm. Values generated from the experimental spectra by CasaXPS software are listed in Supplementary Figure 6.1. The survey results indicate that the atom ratios of C and O are 90.6% and 9.4%, respectively. These experimental values are in good accordance with the theoretical calculated values from the chemical structure of polymer 1, which show C 90.0% and O 10.0%. The high resolution C1s region presents 3 carbon atoms with different chemical states: C-C/H at 285.0 eV, C-C=O at 285.6 eV, C=O at 287.6 eV, and the $\pi \rightarrow \pi^*$ signal at 290.8 eV. The atom ratios of the three different carbon species care 79.3%, 7.1%, and 7.0%, respectively. The polymer 1 is stable in common organic solvents, such as acetone and chloroform. It remained intact after assessment of the adhesion by the scotch tape test method[3], confirming excellent adhesion of polymer 1.



Supplementary Figure 6.1. FTIR and XPS spectra of polymer 1.

References

[1] L. Bondarenko, I. Dix, H. Hinrichs, H. Hopf, *Synthesis* **2004,** *16*, 2751.

[2] I. Dix, H. Hopf, T. B. N. Satyanarayana, L. Ernst, *Beilstein J. Org. Chem.* **2010,** *6,* 932.

[3] J. Lahann, *Polym. Int.* **2006,** *55*, 1361.