

Stereochemistry induced differential cell behaviours on chiral polymer surfaces

Electronic Supplementary Information

I. Methods.

A. Synthesis and characterization of the chiral polymer brush films.

Materials:

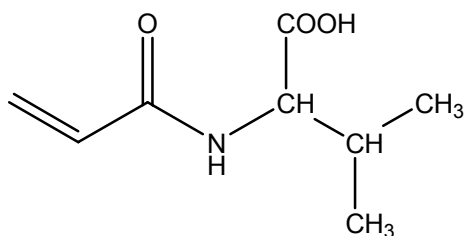
Acryloyl chloride (96%, Aldrich) was stored at 4 °C. L-valine (98%, Aldrich), D-valine (98%, Aldrich), L-alanine (99%, Aldrich), D-alanine (98%, Sigma), L-leucine (98%, Sigma), D-leucine (99%, Aldrich), 3-aminopropyltrimethoxysilane (ATMS, 97%, Aldrich), Cu(I)Br (99.998%, Alfa Aesar), pentamethyl diethylene triamine (PMDETA, 99 %, Aldrich), bromoisobutyryl bromide (97%, Alfa Aesar), α -bromoisobutyric acid (98%, Aldrich) and other general solvents were used as received. Toluene and dichloromethane were dried sufficiently by molecular sieves (Fluka). Silicon wafer was purchased from Silicon Materials Corporation (Germany). De-ionized water (18.2 M Ω cm, MilliQ system) was used.

Instruments:

The AFM investigation was conducted using a Nanoscope IIIa instrument (DI, USA) in the tapping mode. Water contact angles (CAs) were measured on an OCA20 instrument (DataPhysics, Germany) at appointed temperature with saturated humidity. De-ionized water droplets (about 1 μ L) were dropped carefully onto the surface. An average CA value was obtained by measuring the same sample at four different positions. Mass spectrometry (MS) was performed on a matrix-assisted laser/desorption ionization time-of-flight mass spectrometer (MALDI-TOF-MS). UV-Vis absorption spectra were obtained by Cary 50 spectrometer (Varian, Australian) with a 1-cm path-length quartz cuvette. CD spectra were recorded on a Jasco 810 CD spectropolarimeter with a 1-cm path-length quartz cuvette. XPS spectra

were recorded on a ESCALAB 250 from Thermo VG Scientific. The ellipsometer was a VASE Ellipsometer by J.A. Woollam Co., Inc (USA).

Synthesis and Characterization of N-acryloyl-L(D)-valine monomer:



Chemical Formula: $C_8H_{13}NO_3$

Exact Mass: 171.0895

Molecular Weight: 171.1937

L(D)-valine (1.71 g, 10 mmol) were dissolved in 10 mL 1 N NaOH, and with stirring at 0 °C, acryloyl chloride (1.36 g, 15 mmol) were added dropwise within 30 min. The pH of the reaction mixture was held by addition of 2 N NaOH at about 10.0 by use of pH-stat at the beginning of the reaction and was allowed to decrease

to neutrality within 1 hour. Then the reaction was moved to room temperature. After 1 hour the solutions were acidified to pH 2.0 by addition of 2 N HCl, and the monomer were extracted with ethyl acetate (4×30 mL) and the organic phase was dried with anhydrous sodium sulphate. After filtering and evaporate to remove most part of solvent, the concentrated solution was standing overnight for crystallization. Colorless granular crystals were obtained. The yield was above 90%. The crystal monomers were firstly characterized by HRMS (MS-MALDI, m/z): $[M + H]^+$: 172.0976, and $[M + Na]^+$: 194.0800 for *N*-acryloyl-L-valine; $[M + H]^+$: 172.0968, and $[M + Na]^+$: 194.0791 for *N*-acryloyl-D-valine. (MS-MALDI was performed by Department of mass spectrometry at Organic Chemistry Institute of University of Muenster, by Dr. Heinrich Luftmann.) Single crystals with suitable dimension were then selected for single crystal X-ray diffraction analysis. Crystallography data are shown in Table S1 for *N*-acryloyl-L-valine and Table S2 for *N*-acryloyl-D-valine. (The single crystal X-ray structure determination was performed at X-ray crystallography of Institute of Organic Chemistry, University of Muenster, by Dr. Roland Fröhlich.)

Table S1. Crystal Data and Details of Data Collection for *N*-acryloyl-L-valine

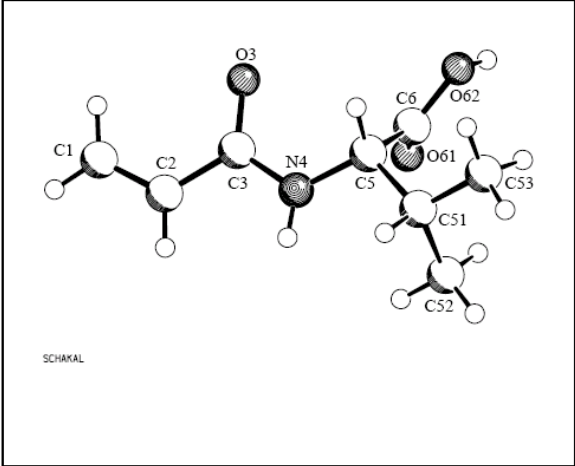
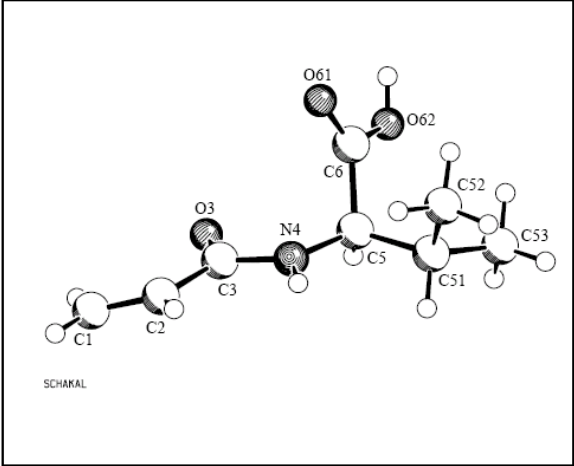
Name of the Compound		Structure solved by
4185.FRO [WX01]		Roland Fröhlich
Structural Formula		Chemical Formula
		C ₈ H ₁₃ NO ₃
		Cell Constants with Standard Deviations
		a [Å] = 8.5362(1) b [Å] = 10.0436(1) c [Å] = 11.4607(1) α [°] = 90.00 β [°] = 90.00 γ [°] = 90.00 V [Å ³] = 982.574(17) T [°C] = -50
Molecular Weight	Crystal Colour	Crystal Size
Mr = 171.19 g mol ⁻¹	colourless	0.40 x 0.25 x 0.25 mm
Molecules per Unit Cell	Calculated Density	
Z = 4	D _{calcd} = 1.157 g cm ⁻³	F(000) = 368 e
Crystal System	Space Group	Reflections Used for Cell Param.
Orthorhombic	P2 ₁ 2 ₁ 2 ₁ (No. 19)	CCD data collection
Wavelength	Monochromator/Filter	
λ = 1.54178 Å	graphite	[(sinΘ)/λ] _{max} = 0.60 Å ⁻¹
Absorption Coefficient	Method of Absorption Correction	Absorption Correction
μ = 7.38 cm ⁻¹	HKL2000	min: 75.7 % max: 83.7 %
Total No. of Reflections Collected	Data Measured	No. of Independent Reflections
13372	± h ± k ± l	1751
Internal Consistency	No. of Observed Reflections	No. of Refined Parameters
R _{av} = 0.041	1717	146
		Error of Fit
R = 0.037	R _w ² = 0.092	0.858
Final Maximum Shift/Error	Final Difference Fourier	Enantiopol Parameter
0.001	ρ = 0.20 (-0.23) e Å ⁻³	0.1(4)
Isotropic Extinction Coefficient	Remarks	
	isopropylgroup splitted over three positions 0.31:0.18:0.51	

Table S2. Crystal Data and Details of Data Collection for *N*-acryloyl-D-valine

Name of the Compound		Structure solved by
4269.FRO [WX 02]		Roland Fröhlich
Structural Formula		Chemical Formula
		C ₈ H ₁₃ NO ₃
		Cell Constants with Standard Deviations
		a [Å] = 8.5527(1) b [Å] = 10.0172(1) c [Å] = 11.4591(1) α [°] = 90.00 β [°] = 90.00 γ [°] = 90.00 V [Å ³] = 981.748(17) T [°C] = -50
Molecular Weight	Crystal Colour	Crystal Size
Mr = 171.19 g mol ⁻¹	colourless	0.35 x 0.15 x 0.10 mm
Molecules per Unit Cell	Calculated Density	
Z = 4	D _{calcd} = 1.158 g cm ⁻³	F(000) = 368 e
Crystal System	Space Group	Reflections Used for Cell Param.
Orthorhombic	P2 ₁ 2 ₁ 2 ₁ (No. 19)	CCD data collection
Wavelength	Monochromator/Filter	
λ = 1.54178 Å	graphite	$[(\sin\Theta)/\lambda]_{\max} = 0.60 \text{ Å}^{-1}$
Absorption Coefficient	Method of Absorption Correction	Absorption Correction
μ = 7.39 cm ⁻¹	HKL2000	min: 78.2 % max: 93.0 %
Total No. of Reflections Collected	Data Measured	No. of Independent Reflections
5718	$\pm h \quad \pm k \quad \pm l$	1726
Internal Consistency	No. of Observed Reflections	No. of Refined Parameters
R _{av} = 0.045	1606	131
		Error of Fit
R = 0.054	R _w ² = 0.140	0.963
Final Maximum Shift/Error	Final Difference Fourier	Enantiopole Parameter
0.000	ρ = 0.41 (-0.26) e Å ⁻³	0.3(5)
Isotropic Extinction Coefficient	Remarks	
0.016(2)	Enantiomer could not be determined!	

Synthesis and Characterization of chiral polymer films on flat silicon substrates:

A clean silicon substrate was firstly treated to generate surface hydroxy groups. After dried under a nitrogen flow, it was immersed and heated to reflux in toluene with 5 wt.-% ATMS for 3 h to obtain surface -NH_2 groups. Then it was rinsed with toluene and dichloromethane, dried, and immersed in dichloromethane with pyridine (2% v/v). Bromoisobutyryl bromide was added dropwise into above solution at 0 °C, and the mixture was left for 1 h at this temperature, and then at room temperature for 12 h. The substrate was cleaned with dichloromethane and toluene. Polymerization was achieved by immersing the dried substrate in a degassed solution of *N*-acryloyl-L(D)-valine monomer (4 mmol) in a 1:1 (v/v) mixture of H_2O and MeOH (8 mL) containing Cu(I)Br (23 mg) and PMDETA (0.10 mL) for 3 h at 60 °C (the obtained polymer brushes were denoted as L-PV and D-PV, respectively). Under these conditions, the film thickness was about 12 ± 2 nm, which was determined by ellipsometry as show in Figure S1. With this method, the chemical compositions of the L-PV films were approximately equal to D-PV films, which were measured by XPS as show in Figure S2 (Ellipsometry and XPS were performed at nanoAnalytics GmbH in Center for Nano Technology, by Dr. Andreas Schäfer).

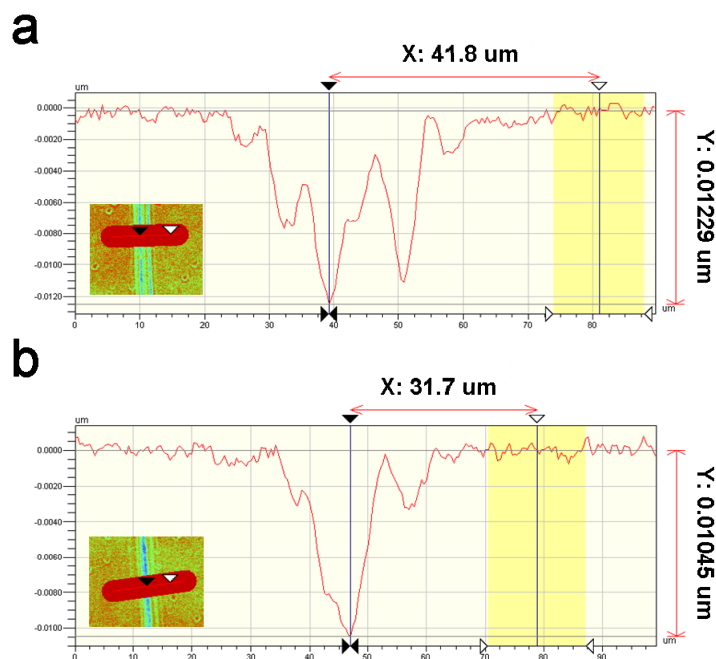


Figure S1. Thickness of polymer films for (a) poly(*N*-acryloyl-L-valine); (b) poly(*N*-acryloyl-D-valine)

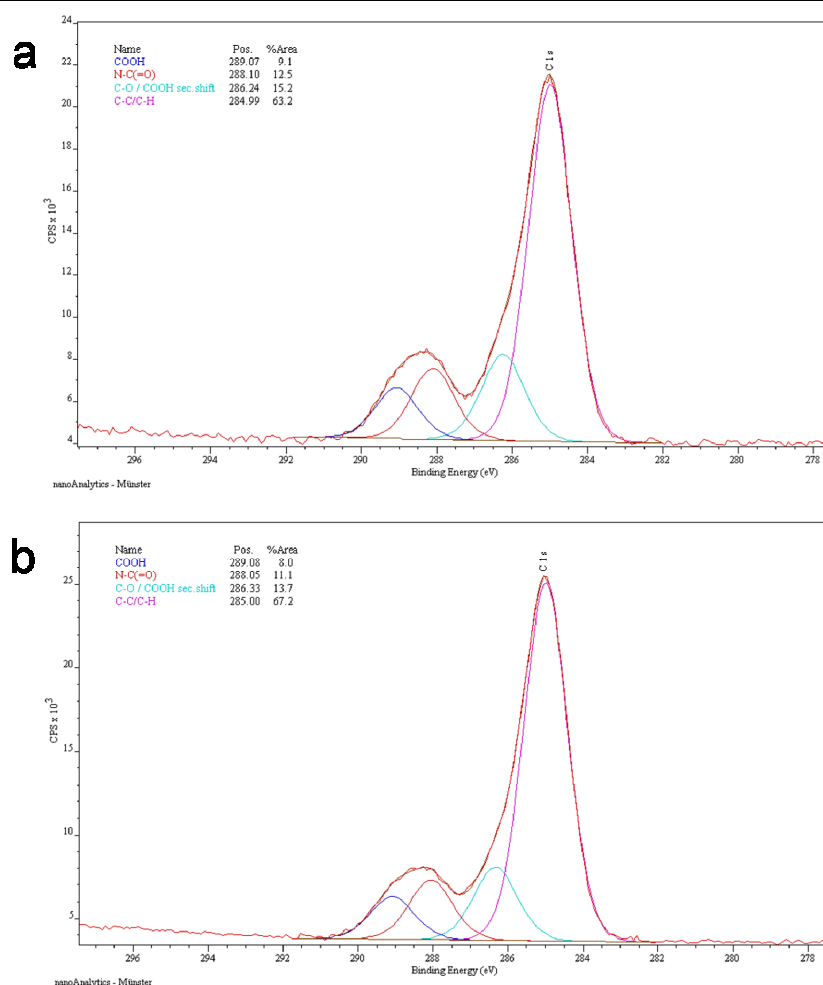


Figure S2. XPS spectra of polymer films for (a) poly(*N*-acryloyl-L-valine); (b) poly(*N*-acryloyl-D-valine)

Synthesis and Characterization of bulk polymer in solution (as reference):

Polymerization was achieved by adding α -bromoisobutyric acid as initiator in a degassed solution of *N*-acryloyl monomer (8 mmol) in a 1:1 (v/v) mixture of H₂O and MeOH (4 mL) containing Cu(I)Br (23 mg) and PMDETA (0.10 mL) for 3 h at 60 °C under N₂ flow. The polymer was purified by alternate treatments between methanol (dissolution) and acidic water (pH 2.0, precipitate). White powders were finally obtained after centrifugation. With this method, the molecular weight of the polymers are about $M_w = 2.0 \times 10^3$ g/mol. These bulk polymers will act as reference for the characterization of chiral properties of the polymers. The solutions of L(D)-Monomer or L(D)-polymers with the same concentration were firstly obtained by using UV-Vis

absorption spectra (Figure 1-b-inserts), then the CD spectra (Figure 1-b) were recorded. Ellipticity (θ , m degrees) was converted to molar ellipticity $\Delta\epsilon = \theta / (32980 \times C \times L)$, where C is molar concentration (mol/liter), and L is path length (cm). (The measurement of CD was performed at Institute of Food Chemistry, University of Muenster, by Ph.D. Tanja Welsch.)

B. Cell culture experiments:

Surface preparation for cell culture:

Surfaces with chiral polymer brush films and silicon (control) were immersed respectively in methanol for 24h, ethanol for 24h, and pure water at 80 °C for 3h twice, then dried under a flow of nitrogen. The samples were immersed in the 75 % medical ethanol solutions for several hours and then washed by phosphate buffered saline (PBS) solution before the experiments.

Cell culture:

African green monkey SV-40 transformed kidney fibroblast cell line COS-7 was cultured in Dulbeccos Modified Eagles Medium (DMEM) (GIBCO, Germany) supplemented with 10% fetal calf serum (FCS) (PAA Laboratories, Germany), 2 mM L-Glutamine (PAA Laboratories, Germany), 100 U/mL penicillin and 100 µg/mL streptomycin (Sigma, USA).

The mouse brain endothelial cell line bEnd.3 was cultured in the same medium as COS-7 cells.

The cells were digested with 0.25% trypsin-EDTA solution (Sigma, USA) in PBS (pH 7.4) and seeded at suitable cell culture plates (Corning, Germany) till they reached appropriate coverage of plates and showed typical cell morphology. Then, the cells were again detached by trypsinization and cultured on the chiral polymer brush films at 5×10^4 COS-7 cells /well and 1×10^5 bEnd.3 cells /well in 24-well plates (Corning, Germany) for cell culture experiments. The cultures were incubated at 37 °C in a humidified air with 5% CO₂ and the observations were made at 10min, 1h, 24h and 48h time points. The medium was changed every three days. All experiments were performed at least 6 times.

Visualization of cells on the chiral polymer brush films:

The CellTracker™ Green CMFDA (5-chloromethylfluorescein diacetate) (C2925, Invitrogen, Germany) was used for long-term tracing of living cells. It was dissolved in high-quality DMSO to a final concentration of 10 mM. Then, the stock solution was diluted to a final working concentration of 20 μ M in serum-free medium (SFM). When the cells have reached the desired confluence, the medium was removed from the dish and the prewarmed CellTracker™ dye working solution was added. After incubation of the cells for 15-45 minutes under growth conditions, the dye working solution was replaced by fresh, prewarmed medium and the cells were incubated for another 30 minutes at 37°C. Afterward, the fluorescently labeled cells were separated, counted and seeded on the sample surfaces at specific cell concentrations. Then after appointed culture time, the samples were washed with warm PBS and fixed on 4% (PFA) paraformaldehyde /PBS solution for 30 minutes. Following another three time's washing, the fluorescently labeled cells on surfaces were immersed with resinous mounting medium and covered by coverslips, which were ready for fluorescence microscopy.

Cells were visualized by fluorescence microscopy using an Axioskop 2 plus (Zeiss, Germany). At least 5 micrographs were taken from random areas of each surface.

Cell attachment was studied at 10 minutes and 1h. The cell numbers of the acquired images were processed using ImageJ (open source image analysis software, downloaded from <http://rsb.info.nih.gov/ij/index.html>). Each cell was fit to the shape of an ellipse using the “analyze particle” command and the number of cells within each image was counted.

For longer cultivation time, the area ratio of the surface (defined as A_r) occupied by cells and the integral fluorescent intensity (defined as I_f) were used to characterize the cellular behaviour. Volocity (Improvision) and Photoshop CS software were used for image processing of the cell surface area and total fluorescent intensity. Data are based on a minimum of six independent experiments and controls for each stage. (Improvision Volocity software was provided and the calculation was helped by Dr.

Rui Benedito and Prof. Ralf H. Adams at the Department of Tissue Morphogenesis, Max-Planck-Institute for Molecular Biomedicine, Muenster.)

Statistical analysis was performed with SigmaPlot 11.0. The student's t-test was used to compare the data for the composite and control samples. Values were considered significant at $P < 0.05$.

As the synthesis of chiral polymer brush films were based on silicon substrates, silicon wafers were used in the cell culture experiments as control. Results showed that the cells were equally spread around on silicon substrates during the whole incubation. At 1h, cells were distributed separately with round morphology, similarly to the cells on the D(L)-PV surfaces. At longer cultivation time (24h, 48h), the cell cultures looked similar to those on normal, but uncoated cell culture plates, but dissimilar to cell cultures on the two chiral polymer brush surfaces.

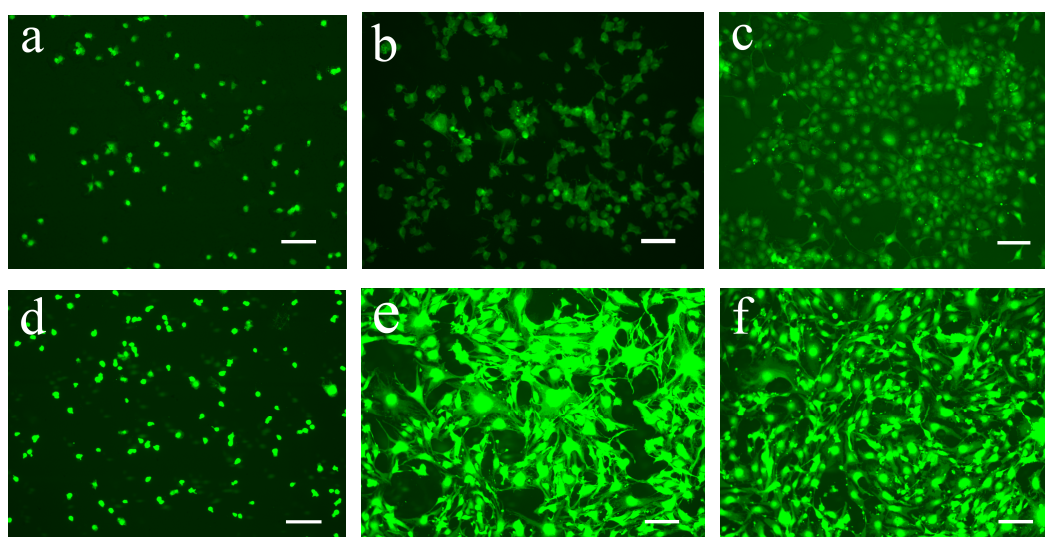


Figure S3. Cell culture experiments on silicon surfaces. (a-f) Typical fluorescent images of COS-7 (a, b, c) and bEnd.3 (d, e, f) on silicon wafers at different incubation stages. (a, d) 1 hour; (b, e) 24 hours; (c, f) 48 hours. Scale bar: 100 μm .

Scanning electron microscopy (*SEM*): After 24 hours of incubation at 37° C in 5% CO₂, the samples were washed twice with warm (37° C) PBS. After that, the cells were pre-fixed with 2% paraformaldehyde (PFA) and 2% glutaraldehyde (GA) in a 1:1 mixture of cell culture medium and cacodylate buffer for 10 minutes. Afterwards, fixation was processed in 2% PFA, 2% GA in 0,1M cacodylate buffer for 2h, which

was followed by 5 washing-steps with A.dest for about 15 minutes. The cells were dehydrated in an ascending ethanol series of 30%, 70%, 96% ethanol, and absolute ethanol, respectively. Then the samples were sputtered with Au for SEM observation.