Controlling Surface Wettability for Automated In Situ Array Synthesis and Direct Bioscreening

Weilin Lin, Shanil Gandhi, Alan Rodrigo Oviedo Lara, Alvin K. Thomas, Ralf Helbig, and Yixin Zhang*
Supporting Information

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1. Materials

Acetone, ethanol, Triisopropyl silane (TIPS), Dichloromethane (DCM), 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b] pyridinium 3-oxide hexafluorophosphate (HATU), Dithiothreitol (DTT), DAPI were purchased from VWR International GmbH (Darmstadt, Germany). Polysorbate 20 (Tween 20), N-Methylmorpholine (NMM), N-hydroxysuccinimide (NHS), Chitosan (low molecular weight, 50000 ~ 190000 Da, 75-85% deacetylated, 448869), acetic acid, acetic anhydride, Biotin, Desthiobiotin (D-biotin), 2-Iminobiotin (I-biotin), 4-Dimethylaminopyridine (DMAP), 3,4-Dihydro-2H-pyran-2-methanol, Bovine Serum Albumin (BSA), propionaldehyde, p-anisaldehyde, Benzaldehyde, valeraldehyde, Tert-Butyl Isocyanide, Benzyl Isocyanide, cyclohexyl Isocyanide, 2-Morpholinoethyl Isocyanide were purchased from Sigma Aldrich. N,N’-Diisopropylcarbodiimide (DIC), Fmoc-β-Ala-OH, Fmoc-O2Oc-OH, N,N’-Diisopropylethylamine (DIPEA), Dimethylformamide (DMF), Piperidine, Trifluoroacetic acid (TFA), 2-propanol and all Fmoc protected amino acids were purchased from IRIS Technology GmbH. N-(2-Aminoethyl)-3-aminopropyltrimethoxsilane was purchased from abcr GmbH. Alex Fluor 633 Phalloidin and Dylight550 labeled anti His tag antibody was purchased from life technologies.

2. Equipment

UPLC (Waters, Milford, USA) equipped with the wide pore C8 column (Aeris™ 3.6 μm WIDEPORE XB-C8 200 Å, LC Column 50 × 2.1 mm) and ACQUITY UPLC PDA Detector was used to determine the amino group density on the glass surface. NanoWizard 4 atomic force microscope (JPK, Germany) equipped with BioLever Mini cantilever (Olympus, Japan) was used to measure the thickness of chitosan coating on the glass surface. MultiPep RSI peptide synthesizer (INTAVIS AG, Germany) with a custom-designed flow cell and holder (made by Teflon) and was used to automate synthesis low-density array on the glass surface. Biosynthesizer (S/N: 2377-RPC-BS3.2, GeSiM mbH, Germany) was used to automate the synthesis of the high-density array on the glass surface. ÄKTAprime plus was used to purify the protein.
3. Methods

3.1. Chemical modification of the glass surface (related to figure 1B)

3.1.1. Preparation of amino-functionalized glass

The chemical modification of the glass surface was performed on ordinary microscope glass slides (ROTH Karlsruhe). The slides were cleaned by sonication in MilliQ water (x2) and acetone (x2), alternating for an hour each. This was followed by incubation with 1M KOH in 2-propanol overnight. The cleaned glass slides were rinsed with MilliQ, dried with compressed air, and treated with 100 µL of N-(2-Aminoethyl)-3aminopropyltrimethoxysilane in a silanization/desiccation chamber under vacuum (8 mbar) for 2 hours.

3.1.2. Preparation of chitosan-coated glass surface

(1) The amino-functionalized slides were treated with 0.3 M Succinic anhydride/1 M NMM in DMF for 1 hour, followed by washing in DMF, Milli Q water, and drying with compressed air. This step was repeated one more time.

(2) The carboxyl groups on glass slides were activated with 1 M NHS / 0.3 M DIC in DMF for 1 hour, and this was followed by washing with DMF, ethanol, and dried with compressed air.

(3) The activated glass slides were immersed in 1% (w/v) chitosan/ 1% acetic acid overnight. The slides were incubated in 1% acetic acid (aq.) for 10 minutes, rinsed in 1% acetic acid, and dried with compressed air.
(4) The unreacted amino groups on chitosan were coupled with Fmoc-Gly-OH or Fmoc-βAla-OH by the incubation of glass slides with the mixture of an equal volume of 0.5 M Fmoc-Gly-OH, 0.5 M HATU, and 1.5 M NMM or of an equal volume of 0.5 M Fmoc-βAla-OH, 0.5 M HATU, and 1.5 M NMM. The slides were washed with ethanol, Milli Q water and dried with compressed air. This step was repeated one more time.

(5) The surface density of the amino groups can tune by repeating the steps for chitosan coating (steps 2-4). For application basis, the chitosan coating was done three times to obtain high amino group density.

(6) The free carboxyl groups on the glass surface were converted to hydroxyl groups by repeating the following procedure three times. Activation of the carboxyl groups on glass slides as in step 2, then incubation with ethanolamine for 1 hour, then washing the slides with MilliQ and drying with compressed air.

3.1.3. Linker coupling and introducing amphiphilic nature to the chitosan-coated glass surface

(1) The Fmoc-βAla-OH chitosan-coated glass slide (Fmoc-βAla-chitosan-slide) was incubated 20% piperidine in DMF for 30 mins to remove the Fmoc groups, followed by washing in DMF, Milli Q water and drying with compressed air. Then amino groups on the slide were coupled two times with Fmoc-βAla-OH as described before to obtain Fmoc-βAla-βAla-chitosan-slide.

(2) Repeat step 1 (this section) one time to obtain Fmoc-βAla-βAla-βAla-chitosan-slide.

(3) Repeat step 1 (this section) one more time using Boc-βAla-OH instead of Fmoc-βAla-OH to obtain Boc-βAla-βAla-βAla-chitosan-slide.

(4) The hydroxyl groups on chitosan were coupled to lipid acids (Octanoic acid, Lauric acid, or Palmitic acid) by incubating the slides with a mixture of an equal volume of 0.1 M lipid acid, 0.3 M DIC, and 0.4 M DMAP in DMF overnight.

(5) The Boc protected groups on the slide were removed by incubation with TFA/MilliQ (in a volume ratio of 95:5) for 3 hours, followed by washing in ethanol, Milli Q water, and drying with compressed air.

(6) Then amino groups on the slide (from step 5) were coupled two times with Fmoc-O2Oc-OH as described before to obtain the Fmoc-O2Oc-βAla-βAla-βAla-βAla-chitosan(lipid)-
slide. The Fmoc protection groups on the slide were removed by incubation with 20% piperidine/40% DMF/40% ethanol for 30 mins to remove the fmoc groups, followed by washing in DMF, Milli Q water, and drying with compressed air. Then amino groups on the slide were again coupled two times with Fmoc-O2Oc-OH as previously described to obtain Fmoc-O2Oc-O2Oc-βAla-βAla-βAla-βAla-chitosan(lipid)-slide. These slides after drying were then stored at -20°C for further use in protein-based screening.

(7) The glass slide used for cell-based screening were modified as follows: The amino groups on the slide (from step 5) were incubated with the mixture of an equal volume of 0.05 M Fmoc-PEG2000-OH, 0.05 M HATU, and 0.15 M NMM for 1 hour. The slides were washed with ethanol, Milli Q water and dried with compressed air. This step was repeated one more time. After drying, these slides were stored at -20°C.

(8) **Removal of lipid chain.** The lipid chain on the glass slide surface was removed by hydrolysis of ester bond under 32% ammonia/1M NaCl under constant shaking overnight at 50°C.

3.1.4. Introducing amphiphilic nature to Serine coated glass surface (related to figure S2)

(1) **Synthesis of THP-C16.** The synthesis was performed using DCC and DMAP.\(^1\) Palmitic acid (1 mmol, 256 mg), 3.4-dihydro-2h-pyran-2methanol (1.1 mmol, 114 µL) and DMAP (0.1 mmol, 12.2 mg) was stirred in 10 ml DCM till dissolve. To the mixture, N,N' - Dicyclohexylcarbodiimide (1.5 mmol, 310 mg) in 10 ml DCM was added under stirring drop by drop. The mixture was stirred overnight again. Precipitated urea was filtered off by 0.22 µm PTFE membrane and the filtered solvent was dried by rotary evaporator. The product was loaded in a silica gel column with n-heptane and eluted by n-heptane/ethyl acetate (in a volume ratio of 95:5). The product was confirmed by NMR.

(2) **Fmoc-Ser-OH coupling of amino-functionalized glass (section A).** The coupling was done by the incubation of glass slides with the mixture of an equal volume of 0.5 M Fmoc-Ser-OH, 0.5 M HATU, and 1.5 M NMM overnight. The slides were washed with ethanol, Milli Q water and dried with compressed air. This step was repeated one time. The slides were treated with TFA/MilliQ H₂O (in a volume ratio of 95:5) for 3 hours to remove the tert-butyl ether protection of the serine hydroxyl group.
(3) **THP-C16 coupling of Fmoc-Ser functionalized glass.** The slide was incubated with 10 mM p-Toluenesulfonic acid, 10 mM pyridine, and 150 mM THP-C16 in dichloromethane under shaking overnight.

(4) **Remove of lipid chain.** The lipid chain was removed under TFA cleavage condition

[TFA/DTT/Water/TIPS (in a volume ratio of 88/5/5/2), shaking for 3 hours].

THP-C16 was used in experiments of figure S2 and figure S15.

3.2. **Surface analysis chemical modified glass surface**

3.2.1. **Determination of amino groups density on the glass**

The amino group density on the glass slide was determined from the piperidine-mediated deprotection of the Fmoc group. The concentration of the dibenzofulvene-piperidine (Fmoc deprotection adduct) in the deprotection solution was monitored using UPLC and was calculated by the calibration curves. (Figure S5)

3.2.2. **Dynamic contact angle measurement by mobile phone**

Dynamic contact angle measurement was done on a custom-built stage with a stand to place a smartphone. The droplet aspiration and dispensing on the G1 modified surface with the Lipid groups (C8, C12, and C16), C16 surface after ammonia cleavage, and the unmodified surface was done using an automated syringe dilutor (GeSIM mbH). The dynamic contact angle was measured for three solvents: MilliQ H$_2$O, DMSO, and DMSO/Sulfolane (in a volume ratio of 2:3). The advancing and receding contact angles on the surface were recorded using a smartphone camera. The initial liquid drop volume was set to be 15 μL with the liquid dispensing speed of 2 μLs$^{-1}$. This was followed by aspiration volume of 10μL with the speed of 2 μLs$^{-1}$ and dispensing volume of 10 μL at speed of 2 μLs$^{-1}$. The liquid volume and liquid aspiration/dispensing speed were controlled by the software used for the High-density spot synthesizer. For each surface, three measurements on different locations of a particular surface were performed. The contact angle was analyzed using ImageJ [2].

3.2.3. **Static contact angle measurement by mobile phone**

10 μL of MilliQ H$_2$O or organic solvent (DMSO and DMSO/Sulfolane (in a volume ratio of 2:3)) or glycerol dispensed from the automated syringe dilutor added on the surface of glass slides as above. The photo of the organic droplet was taken by mobile phone with white light on the other side of the slide. The contact angle was analyzed by ImageJ [2]
3.2.4. AFM images of G3 surface (three cycles of chitosan coating)
The glass slide with chitosan coating was scratched with a metal tweezer, then the force mapping imaging (QI mode) with a setpoint of 0.3 nN, z-length of 200 nm was performed to measure the thickness of the coating.

3.3. Ugi reaction (related to figure 2 and S8)
Condition 1 (Figure S8A). Boc-β-Ala, propionaldehyde, and Tert-Butyl isocyanide were prepared in DMF with a final concentration of 0.5 M and were mixed 1:1:1 (volume). 0.3 µL of the mixture was spotted on the G4 surface.

Condition 2 (Figure S8B). Boc-β-Ala, propionaldehyde, Tert-Butyl isocyanide, and piperidine was prepared in DMF with a final concentration of 0.5 M. Propionaldehyde and piperidine were mixed 1:1 (volume), and 0.2 µL of the mixture was spotted at a designed position on the G4 surface. After 10 mins, Boc-β-Ala and Tert-Butyl isocyanide were mixed 1:1 (volume), and 0.2 µL of the mixture was spotted at their respective designed position on the G4 surface.

Condition 3 (Figure S8C). Boc-β-Ala, aldehyde (A1, propionaldehyde; A2, p-anisaldehyde; A3, Benzaldehyde; A4, valeraldehyde), Isocyanide (I1, Tert-Butyl Isocyanide; I2, Benzyl Isocyanide; I3, cyclohexyl Isocyanide; I4, 2-Morpholinoethyl Isocyanide) and piperidine was prepared in DMF to a final concentration of 0.5 M. Aldehyde (A1 or A2 or A3 or A4) and piperidine was mixed 1:1 (volume), and 0.2 µL of the mixture was spotted on their designed position of the G4 surface. After 10 mins, Boc-β-Ala and isocyanide (I1 or I2 or I3 or I4) were mixed 1:1 (volume), and 0.2 µL of the mixture was spotted at their respective designed position on the G4 surface.

Condition 4(Figure 3, S8D and S8E). Boc-β-Ala, aldehyde, Isocyanide, and piperidine was prepared in DMF at a final concentration of 0.5 M, 1 M, 2M, and 1M respectively. Aldehyde (A1 or A2 or A3 or A4) and piperidine were mixed 1:1 (volume), and 0.2 µL of the mixture was spotted on their designed position of the G4 surface. After 10 mins, Boc-β-Ala and isocyanide (I1 or I2 or I3 or I4) were mixed 1:1 (volume), and 0.2 µL of the mixture was spotted at their respective designed position on the G4 surface.

3.4. Automated SPOT synthesis (related to figure S9)
The glass slide was fixed in the flow cell, which is like a swimming pool to wash glass slide by adding organic solvents, and the organic solvents can be drained under negative pressure. The procedures were performed cyclically as follows:
(1) Fmoc deprotection. The Fmoc protective group was eliminated with 20% piperidine in DMF/Ethanol (volume 1/1) for 30 minutes. After this, the glass slide was washed three times with solvent A (50% DMF/50% Ethanol), then three times with Ethanol. The solvents on the slide were dried by vacuum.

(2) Peptide bond formation. A fresh mixture (first cycle 200 nl, following cycle 300 nL) containing an equal volume of 0.5 M Fmoc-amino acid in DMF, 0.5 M HATU in DMF, 1.5 M NMM in DMF is spotted to a predetermined position on the slide. The reaction time is 60 mins. After this, the support is washed three times with solvent B (10% acetic acid/45% DMF/45% Ethanol), then three times with solvent A, then three times with Ethanol. The solvents on the support are dried under a vacuum. The coupling process is repeated one more time.

(3) Capping of unreacted amino groups. The slide was treated with a mixture of an equal volume of Cap A (10% acetic anhydride in DMF) and Cap B (4% DIPEA in Ethanol) for 30 mins. After this, the slide was washed three times with solvent A, followed by three times with Ethanol. The solvents on the slide are dried by vacuum.

At each position, a peptide is synthesized by spotting/coupling amino acids one by one according to the peptide sequence. After all the full-length peptides have been synthesized, the cycles are finished.

For the high-density spot array synthesis, the cycles are the same as low-density spot array but with a lower concentration of fresh coupling reagent (equal volume of 0.15 M Fmoc-amino acid in DMSO, 0.15 M HATU in DMSO, 0.45 M NMM in DMSO) and lower spotting volume (40 pL). The freshly activated amino acids can be used for > 10 mins, while the spotting can be completed in 5 min.

Although EtOH is more polar than DMF, the surface tension of EtOH (22.4 mN m\(^{-1}\)) is lower than that of DMF (37.1 mN m\(^{-1}\)). The low cohesive force of EtOH makes it easier to wet the surface, thus makes the deprotection or capping reagent entering the matrix more efficient.

The glass slide post peptide synthesis is taken out from the flow cell, washed with ethanol, and dried using compressed nitrogen. The glass slide is washed overnight under constant shaking at RT in 2-propanol. On the following day, the cleavage of the side protection groups on amino acids was performed using TFA cocktail: TFA/DTT/Water/TIPS (in a volume ratio of 88:5:5:2) for 3 hours in a sealed bag under constant shaking. The glass slide is washed with ethanol, Milli Q water, and dried using compressed air. The glass
slide is cleaned by washing overnight under constant shaking in 2-propanol. The glass slide was further used for protein incubation or sealed and stored at -20°C for further use.

The peptide sequences that have been synthesized were shown in table S5.

3.5. Small molecular coupling (related to figure 6A)
Biotin, desthiobiotin, and iminobiotin were dissolved in 10 µl of DMSO to a final concentration of 0.2 M. To the biotin, or desthiobiotin or iminobiotin solution, 4 µL of 0.5 M HATU/HOAt and 2 µL of 3 M NMM was added. Then 0.2 µL of the reaction mixture was spotted at designed positions on the glass surface. The iminobiotin solution was cloudy at the beginning and became clear after the addition of HATU/HOAt and NMM.

Fmoc-CsA-COOH was synthesized as described before.[3] Fmoc-CsA-COOH was dissolved in 10 µl of DMSO to a final concentration of 0.1 M. To the solution, 2 µL of 0.5 M HATU/HOAt, and 1 µL of 3 M NMM was added. Then 0.2 µL of the reaction mixture was spotted at designed positions on the glass surface. After capping, the fmoc group was deprotected and the free amino group was coupled to Biotin as described above. The result Biotin-CsA can bind to both NeutrAvidin and Cyclophilin A.

3.6. Protein expression, purification

3.6.1. His-CypA

(1) **His-CypA expression.** His-CypA was expressed and purified as described before.[3]

Single colonies of Escherichia coli (*E. coli*) were picked and inoculated into 20 ml of LB media containing kanamycin (50 µg/ml). The cultures were grown overnight at 37 °C with vigorous shaking. Subsequently, 15 ml of the overnight cultures were inoculated with 1 L of pre-warmed medium (2×YT, 50 µg/ml kanamycin), and incubated at 37 °C with vigorous shaking until OD600 = 0.8 ~ 1 (around 4 hours). The temperature was lower to 25 °C for 30 mins. Expression was induced by adding IPTG to a final concentration of 300 µM and again incubating overnight at and 200 rpm shaking condition. Cells were harvested by centrifuging at 8000 rpm for 20 min and discarding the supernatant.

(2) **Lysis of *E. coli.** To purify the protein, the following method was used. Cells were resuspended in 50 ml buffer A (35 mM Hepes, 500 mM NaCl, 40 mM Imidazole, pH 7.8) containing 1 mM DTT and 1 mM PMSF. The cells were lysis by 3X passing through the French press (EmulsiFlex-C3, AVESTIN) at 4 °C under 1000 psi. The lysate obtained was
centrifuged at 45,000 rpm, 4 °C for 1 h (Beckmann LE-80K ultracentrifuge, Beckmann, Palo Alto, California, USA, rotor SW 45Ti).

(3) **Purification.** The supernatant was collected and loaded on a Histrap HP column at 4 ml min\(^{-1}\), followed by linear gradient change up to 100% Buffer B (35 mM Heps, 500 mM NaCl, 500 mM Imidazole, pH 7.8) in 30 min. The eluted fractions showing peptidylprolyl isomerase (PPIase) activity were concentrated to 500 µL and then were loaded on Superdex 75 at 1.5 ml min\(^{-1}\) in buffer C (35 mM Heps, 200 mM NaCl, 5% glycerol, and 1 mM DTT, pH 7.8). The eluted fractions with enzyme activity were analyzed by LC/MS. High purity fractions were pooled, concentrated, and stored at -80 °C.

### 3.6.2. Recombinant Calcineurin

(1) **Recombinant Calcineurin expression.** Recombinant Calcineurin plasmid was a kind gift from Gunter Fischer. The recombinant CaN was transferred in BL21 (pLysS). Calcineurin was expressed as His-CypA except the used Antibiotics were kanamycin (50 µg ml\(^{-1}\)) and ampicillin (50 µg ml\(^{-1}\)).

(2) **Lysis of *E.coli.*** The lysis of *E.coli* for calcineurin is the same as for His-CypA.

(3) **Purification.** The supernatant was collected and loaded on a Histrap HP column at 4 ml min\(^{-1}\), followed by linear gradient change up to 100% Buffer B in 30 min. The fractions showing activity of RII dephosphorylation were pooled and diluted 10 times in buffer D (35 mM Heps, 3 mM CaCl\(_2\), and 1 mM DTT, pH 7.4) and loaded on Calmodulin Sepharose 4B at 1 ml min\(^{-1}\). The resin was washed sequentially with 50 ml buffer E and 50 ml buffer E with 500 mM NaCl. Protein in the resin was eluted at 1 ml min\(^{-1}\) in buffer F (35 mM Heps, 2 mM EGTA, and 1 mM DTT, pH 7.4). The fractions with enzyme activity were pooled, concentrated, and then were loaded on Superdex 75 at 1.5 ml min\(^{-1}\) in buffer C. The eluted fractions with enzyme activity were analyzed by LC/MS. High purity fractions were pooled, concentrated, and stored at -80 °C.

### 3.7. Protein labeling

The proteins were diluted to around 2 mg ml\(^{-1}\) in PBS, and NaHCO\(_3\) was added to the solution to reach a final concentration of 100 mM. Labeled dyes were dissolved in DMSO with a final concentration of 5 mM. Labeled dyes were added to the proteins and incubated overnight in the cold room with gentle shaking. For
monoclonal ANTI-FLAG® M2 antibody, 5 equivalent of DyLight™ 633 NHS Ester was added. For Neutravidin, 10 equivalent of DyLight™ 633 NHS Ester was added. For His-CypA, 10 equivalent of TAMRA- NHS Ester was added. For calcineurin, 10 equivalent of fluorescein-NHS Ester was added. Tris (1 M, pH 8.0) was added to a final concentration of 100 mM to quench the reaction, and the unreacted dye was removed by the PD10 column.

3.8. Protein binding assay

3.8.1. Antibody binding assay

The cleaved glass slide was pre-blocked in 3% Milk powder in PBST (0.05% Tween 20) overnight at 4°C. On the following day, the stock Monoclonal ANTI-FLAG® M2 antibody labeled with Dylight 633 was diluted to 141 nM using 3% Milk powder in PBST. 100 µL of antibody is incubated by sandwiching it in between a clean glass slide and the peptide synthesized glass slide overnight at 4°C placed in a petri dish. The incubation is done very carefully to avoid the formation of bubbles between the glass slides or moving them. The peptide synthesized glass slide was washed in 100 ml PBST for 1 hour without shaking, then 5 mins Milli Q H2O without shaking, and then dried by lightly dabbing with kimwipes for imaging. The glass slide is then imaged using Bio-Rad ChemiDoc™ XRS+ imaging system using Red Epi illumination (695/55) filter at 20 sec exposure time.

3.8.2. CypA, CaN, and Neutravidin binding assay

The slide was incubated with 100 nM TAMRA-CypA or 500 nM Fluorescein-CaN or 10 µM DyLight633-labelled NeutrAvidin overnight at 4°C as described before. The peptide synthesized glass slide was washed in 100 ml PBST for 1 hour without shaking, 100 ml PBST with 2M NaCl for 1 hour without shaking, then 5 mins Milli Q H2O without shaking, and then dried by lightly dabbing with kimwipes for imaging. The glass slide is then imaged using the Bio-Rad ChemiDoc™ XRS+ imaging system.

3.8.3. The coupling efficiency of synthesized peptides

To test the coupling efficiency of spot synthesis on the glass surface. Long glycine chains from the no glycine (0G) to the 16 glycine chain (16G) (3 repeats each) were synthesized using the Automated SPOT synthesis technique. In the end, each chain was coupled to biotin (0.2M in DMSO), 0.5M HATU (1 eq.), 3 M NMM (3 eq.). After the cleavage procedure, the glass was incubated with 100µL of Dylight633 labeled NeutrAvidin(10 µM) in PBST(0.05% Tween 20) in a petri dish overnight at 4°C as described before. The incubated glass slide was washed in PBST (0.05% Tween 20) in 2M NaCl for 2 hours in 4°C, followed by washing in PBST(0.05% Tween 20) for 2 hours at 4°C. The glass slide was then gently dipped in MilliQ
water for 3 minutes and dried by lightly dabbing with kimwipes for imaging. The glass slide was imaged using the BioRad Imaging system with an exposure time of 1 sec.

3.9. Cell adhesion assay
The L929 cell line (C3H/An connective tissue) was generously provided by the Garbe group (Center for Regenerative Therapies Dresden, ). The cells were cultured in DMEM (Life Technologies GmbH, catalog number 11880028) supplemented with 10% (v/v) heat-inactivated FBS (Biochrom GmbH) and 2mM L-glutamine (Life Technologies GmbH).

For subculturing, the cells were first washed in PBS and then treated with trypsin-EDTA (Life Technologies GmbH). The cells were resuspended in DMEM medium and spun down at 1000 rpm for 3 minutes at room temperature. The pellet was resuspended and transferred into a new T-25 culture flask with a density of 5000 to 20,000 cells/cm².

Primary human umbilical vein endothelial cells (HUVEC) were kindly provided by the Werner group (Max Bergmann Center of Biomaterials, Leibnitz-Institut für Polymerforschung Dresden e.V., werner@ipfdd.de). For cell adhesion, the slide was cut to fit in a 6 well plate. The slide was incubated in 70% ethanol for sterilization and dried inside the cell culture hood. The slide was placed inside the 6 wells plate, then the 2 ml of cells in supplemented DMEM medium with a density of 1 x 10⁵ ml⁻¹ was added. After cell seeding, the 6 wells plate were placed into a cell culture incubator (37 °C and 5% CO₂) and agitated for 10 s every 10 min during the first hour. After the cell was attached (around 2 hours), the supernatant was removed, and the slide was washed with 2 ml supplemented DMEM medium several times till the unattached cells were removed. Then the slide was transferred to a new well with 2 ml supplemented DMEM medium and culture overnight.

The cell on the slide was fixed by adding 1.5 ml of 4% PFA and incubated for 30 mins inside the cell culture incubator. The slide was washed 2 times with PBS. And the cell was stained with Alex Fluor 633 Phalloidin and DAPI as described bellowed. After staining, the cells were imaged using Lionheart LX Automated Microscope (BioTek).

**Alex Fluor 633 Phalloidin staining.** 2 ml of 0.1% Triton X-100 in PBS was added to cover the cells on the slide, then the 6 wells plate was kept in dark for 3–5 minutes, then stain solution was removed and the cells washed 3 times with PBS. 200 μL of 165 nM Alex Fluor 633 Phalloidin in PBS with 1% BSA was added
carefully on the surface of the glass slide and incubated for 60 minutes at room temperature. The cells were washed 3 times with PBS.

**DAPI staining.** 2 ml of 300 nM DAPI stain solution was added to cover the cells on the slide, then the 6 wells plate was kept in dark for 1–5 minutes, then the stain solution was removed and the cells were washed 3 times with PBS.
4. Supporting figures

Figure S1. Serine-based surface coating strategy. PG-linker is coupled to the amino group of serine. Lipid is conjugated to the OH group of serine.
Figure S2. Lipid modification through acid-catalyzed addition reaction to form ether bond using THP-C16. (A) Proton nuclear magnetic resonance of THP-C16. (B) Scheme of the synthesis and hydrolysis of THP-C16 modified surface. (C, D) The resulting ether bond can be cleaved by TFA, causing the switching from a surface with low wettability to DMSO (C) to a surface with high wettability (D).
Figure S3. The stability of the printed droplets on the glass surface. (A) The printed droplets on the glass surface with lower advancing angle (46 ± 1° and 48 ± 2° for DMSO and DMSO/sulfolane, respectively) do not move even when the slide was tilted to 90° and the slide was after 16 cycles of synthesis. This slide was placed vertically in front of a blue background. (B) The printed droplets on the glass surface with a much higher advancing angle (67 ± 2° for DMSO and 66 ± 4° for DMSO/sulfolane) have irregular shifts. Even two spots merged (red arrow).
Figure S4. Ester bond hydrolysis efficiency. (A) Desthiobiotin (Dbt) was coupled to the hydroxyl group. After coupling, the glass slide was treated with ammonia solution for hydrolysis of the ester bond. (B) Desthiobiotin was coupled to the amino group as the positive control. (C) Spot intensities of glass slide stained with Dylight633-labelled NeutrAvidin. (D) Cleavage efficiency calculation.
Figure S5. The concentration of amino group per area was increased by repeating the cycle of chitosan coating. (A) Repeating step i and step ii increased the amount of chitosan on the surface. (B) Calibration curve of piperidine-treated Fmoc-Gly-OH solution in the range of $1.09 \times 10^{-7}$ M to $1 \times 10^{-5}$ M. The adduct absorbance was monitored at 266 nm. The equation ($y = 289.32x - 64.56$) is used to quantify the Fmoc-protected amino group in the coatings. (C) Calculation of the amount of amino group on the glass surface according to the equation from panel B.
Figure S6. AFM images of G3 surface (three cycles of chitosan coating). The thickness is approximately 8.9 nm by the scratch-and-scan method.
**Figure S7. Linker and amino protection group optimization.** (A) To optimize the linker for both solid-phase synthesis and following biochemical assay, the substrate G1 (main figure 1B) was modified with Fmoc protected B4 linker (4 repeats of β-Ala), resulting in substrate G2. After Fmoc deprotection, biotin was coupled to the freed amino group using HATU as activating agent and NMM as the base. The surface was incubated with Dylight633-labelled NeutrAvidin (DL633-NA) and the fluorescent signal was measured (Signal 1). Alternatively, biotin was coupled to G2 after Fmoc deprotection, followed by C16-modification, and the binding of DL633-NA was measured (Signal 2). Alternatively, the substrate G2 with Fmoc-B4 linker was modified with the C16 lipid chain (surface G3). Followed by Fmoc deprotection and biotinylation, the binding of DL633-NA was measured. (Signal 3). Lipid modification causes a moderate decrease of DL633-NA signal, indicating the steric hindrance caused by lipid modification affecting protein–ligand interaction. A remarkable decrease of DL633-NA signal was observed when the lipid-modification was performed before Fmoc deprotection and biotinylation. This indicated that the Fmoc protection group is not stable under the reaction condition of lipid-modification. (B) When the Fmoc protected B4 linker was replaced by Boc-protected B6 linker (6 repeats of β-Ala), the difference among the three methods and resulting signals was diminished. The linker and protection strategy was used in the following preparation of substrates for array synthesis.
Figure S8. Multi-component Ugi reaction on substrate G4 glass surface. (A) Boc-β-Ala/aldoxide/isocyanide (molar ratio 1:1:1) was spotted for Ugi reaction. No fluorescent signal was detected for the spots of Ugi reaction, in contrast to the direct amide bond formation. (B) On the substrate with the amino group, aldehyde/piperidine mixture was first spotted, followed by Boc-β-Ala, or isocyanide, or Boc-β-Ala/isocyanide (molar ratio 1:1). As compared to the positive control, the Ugi reaction showed a reaction yield of 26 %, while no reaction was detected when only carboxylic acid or isocyanide building block was spotted. (C) On the substrate with the amino group, the designed aldehyde/piperidine mixture was first spotted, followed by the designed Boc-β-Ala/isocyanides (molar ratio 1:1) mixture. A1 is propionaldehyde, A2 is p-anisaldehyde, A3 is Benzaldehyde, A4 is valeraldehyde, I1 is Tert-Butyl Isocyanide, I2 is Benzyl Isocyanide, I3 is cyclohexyl Isocyanide, I4 is 2-Morpholinoethyl Isocyanide. The lower coupling yield using I1 as isocyanide may due to the higher volatility of this isocyanide. (D, E) No reaction was detected when missing the isocyanide (D) or the aldehyde (E) building block was spotted.
Figure S9. Scheme of peptide synthesis with full automation. (A) Setup for automated peptide synthesis. The flow cell, O ring, and glass slide are assembled to form a swimming pool to hold the glass slide. (B) Various reaction mixtures can be printed onto the G4 surface using either piezo inkjet printing or contact printing (blue arrow). After each round of coupling reaction, acidic inactivation solvent was added to the pool, to diminish the cross-reaction among spots, followed by washing, capping, and Fmoc deprotection (green arrow). The inactivation, washing, capping, and deprotection solutions can be drained under negative pressure (red arrow). The spotting/coupling can be repeated multiple times to increase the coupling yield after acidic inactivation and washing. (C) In situ peptide array synthesis process. On the G4 surface, activated Fmoc-protected amino acids were spotted at designed positions (I). The unreacted amino acids were washed away and the free amino groups on the surface were capped (II). The protection group (PG, e.g., Fmoc) was removed and the deprotection solution was washed away (III). Activated Fmoc-protected amino acids were spotted at their respective designed positions where the first amino acid was spotted (IV). The unreacted amino acids were washed away and the free amino groups from the first amino acids on the surface were capped (V). The protection group (PG, e.g., Fmoc) of second amino acids was removed and the deprotection solution was washed away (VI). Repeating steps IV, V, and VI until full-length peptides were synthesized. After the side chain deprotection, the final peptide array was presented on the glass surface.
Figure 10. Spot synthesis of biotin (A) and carboxyl fluorescein (B) with various concentrations of the amino group on the spots. Fmoc-Gly-OH and Ac-Gly-OH were mixed with different ratios and spot synthesis. Biotin or fluorescein was coupled to spots. Both signal from DL633-NA and fluorescein decreases with the decrease of amino group on the spot, and the decrease rate is in the same order.
Figure S11. Fmoc deprotection using piperidine in DMF/ethanol. (A) Spreading of piperidine solutions on a G4 surface. With the increasing ratio of ethanol, the solutions (1 µl with 0.1% bromophenol blue) showed increased wettability on the surface. (B) UPLC analysis of in-solution deprotection of Fmoc-Gly-OH. With the addition of ethanol (equal volume mixture of DMF and ethanol), the in-solution Fmoc deprotection was slowed down. Nevertheless, the time of 30 mins is sufficient to remove all the Fmoc protecting groups.
Two dipeptides (top, Boc-βAla-βAla; bottom, Fmoc-βAla-βAla) were synthesized. After the synthesis of the first βAla, the free amino group in image A was capped using DMF/ethanol as solvent, and the free amino group in image B and image C was capped using DMF as solvent. After the synthesis of the second amino acids, Fmoc deprotection was performed. The free amino group in image A was capped using DMF/ethanol as solvent, the free amino group in image B was capped using DMF as the solvent, and the free amino groups in image C were not capped. The Boc protecting group was removed by TFA and the exposed amino group was coupled to dylight 633-NHS. Using DMF/ethanol as solvent showed a slightly better result.
Figure S13. Mass spectroscopy analysis of photo-cleaved PRIETBX (where B is β-Ala and X is a photo-cleavable linker) synthesized on the glass surface. The summary of the theoretical monoisotopic and observed masses is shown in panel F.
Figure S14. Synthesis of peptides with high density. (A) Peptides synthesis with different densities. Red spots, stained with DL633-NA. (B) Spot intensity quantification of image A.
Figure S15. Biotin and iminobiotin coupling on Ser coating slide. (A) The ser coating slide with $(\beta$-Ala)$_4$-(O2Oc)$_2$ as the linker was used for synthesis. Lipid, THP-C16. (B) Biotin and iminobiotin were synthesized by spotting, and the slide was stained with DL633-NA. Due to too low amount of amino group, there is no detectable signal for iminobiotin.
Figure S16. **Cell adhesion on peptide arrays.** Cells can adhere to RGDSP spots (up), but poorly to the peptide-free area and GGDSP spots (bottom). (A, L929; C, Human Dermal Fibroblasts, neonatal; E, Primary human umbilical vein endothelial cells). The panels of B, D, and F are the zoom-in of the panels of A, C, and E, respectively. Blue color, nuclear staining by DAPI; red color, fibronectin staining by Alex Fluor 633 Phalloidin.
**Figure S17. Summary of the properties of different surfaces.** After amphiphilic coating, on-demand array synthesis with full automation and high coupling efficiency has been achieved (on G3 - G6). The amphiphilic surface can be switched to a hydrophilic coating after the synthesis (G7), to make it ideal for protein binding and cell adhesion assays.
5. Supporting tables

**Table S1.** Static contact angles of H$_2$O, DMSO, DMSO/sulfolane (40/60) on 1-round and 3-round chitosan-coated surfaces (three independent experiments).

| Solvent     | Lipid modification | Contact angle 1 | Contact angle 2 | Contact angle 3 |
|-------------|--------------------|----------------|----------------|----------------|
| H$_2$O      | No lipid           | 20             | 19             | 22             |
|             | C8                 | 51             | 55             | 60             |
|             | C12                | 66             | 66             | 67             |
|             | C16                | 70             | 70             | 69             |
|             | C16(amide)         | 22             | 22             | 20             |
|             | C16$^a$            | 73             | 72             | 71             |

| DMSO        | No lipid           | No measurement | No measurement | 12             |
|             | C8                 | 11             | 10             | 12             |
|             | C12                | 21             | 25             | 25             |
|             | C16                | 35             | 33             | 36             |
|             | C16$^a$            | 29             | 31             | 30             |
|             | C16(amide)         | No measurement | No measurement | No measurement |

| Glycerol    | No lipid           | 26             | No measurement | 23             |
|             | C8                 | 25             | No measurement | 23             |
|             | C12                | No measurement | No measurement | No measurement |
|             | C16                | No measurement | No measurement | No measurement |
|             | C16(amide)         | 28             | 28             | 29             |

$^a$ With 3-round chitosan coating

**Table S2.** The surface energy of 1-round chitosan-coated surfaces (calculated from Table S1 based on Owens-Wendt-Rabel & Kaelble Model$^{[4]}$ using OriginPro software).

| Lipid modification | Polar parts of the surface energy (mJm$^{-2}$) | Dispersive parts of the surface energy (mJm$^{-2}$) | Surface energy (mJm$^{-2}$) |
|--------------------|-----------------------------------------------|----------------------------------------------------|------------------------------|
| No lipid           | 56 ± 3                                        | 13 ± 1                                             | 69 ± 3                       |
| C8                 | 23 ± 5                                        | 24 ± 2                                             | 47 ± 5                       |
| C12                | 14 ± 1                                        | 27 ± 1                                             | 41 ± 1                       |
| C16                | 13 ± 1                                        | 24 ± 1                                             | 37 ± 1                       |
| C16$^a$            | 11 ± 2                                        | 28 ± 1                                             | 39 ± 2                       |
| C16(amide)         | 59 ± 2                                        | 11 ± 1                                             | 70 ± 2                       |

$^a$ With 3-round chitosan coating
**Tables S3.** Summary of fluorescence intensity from Ugi reaction.

|                   | β-Ala          | L-Phe          | L-Ile          | L-Met          |
|-------------------|----------------|----------------|----------------|----------------|
| **Propionaldehyde** |                |                |                |                |
| tert-Butyl isocyanide | 4124.10 ± 723.37 | 4742.00 ± 227.04 | 4299.73 ± 499.64 | 3751.93 ± 336.11 |
| Benzyl isocyanide   | 4044.37 ± 1022.23 | 4935.00 ± 318.45 | 4539.07 ± 317.90 | 3660.91 ± 363.46 |
| cyclohexyl isocyanide | 4090.01 ± 366.55 | 4952.17 ± 196.00 | 5018.94 ± 837.10 | 3176.63 ± 81.16  |
| 2-morpholinoethyl isocyanide | 3183.37 ± 164.95 | 4102.20 ± 280.10 | 4416.23 ± 386.71 | 2769.20 ± 253.31 |
| **p-Anisaldehyde**  |                |                |                |                |
| tert-Butyl isocyanide | 3600.48 ± 169.18 | 4262.03 ± 242.36 | 4919.70 ± 308.66 | 3099.87 ± 218.16 |
| Benzyl isocyanide   | 3475.48 ± 306.47 | 4145.46 ± 791.76 | 4469.05 ± 339.54 | 3445.67 ± 484.71 |
| cyclohexyl isocyanide | 4799.16 ± 1166.20 | 4730.88 ± 377.08 | 4565.98 ± 318.02 | 3148.40 ± 372.09 |
| 2-morpholinoethyl isocyanide | 3076.27 ± 126.04 | 3643.75 ± 404.40 | 3812.42 ± 428.25 | 2922.84 ± 526.66 |
| **Benzaldehyde**    |                |                |                |                |
| tert-Butyl isocyanide | 3406.19 ± 799.96 | 4292.94 ± 540.93 | 4163.27 ± 150.14 | 3500.55 ± 710.77 |
| Benzyl isocyanide   | 3547.20 ± 409.37 | 4784.87 ± 340.66 | 4732.80 ± 587.55 | 3527.80 ± 177.93 |
| cyclohexyl isocyanide | 4253.74 ± 755.72 | 4912.87 ± 284.63 | 4307.47 ± 130.82 | 3538.43 ± 409.00 |
| 2-morpholinoethyl isocyanide | 3828.42 ± 656.68 | 4514.98 ± 104.98 | 4180.63 ± 572.87 | 3156.80 ± 288.47 |
| **Valeraldehyde**   |                |                |                |                |
| tert-Butyl isocyanide | 3942.04 ± 164.81 | 5194.23 ± 329.71 | 4425.68 ± 259.14 | 3638.04 ± 298.14 |
| Benzyl isocyanide   | 4078.74 ± 1241.29 | 7797.95 ± 1470.88 | 4492.78 ± 280.34 | 4502.21 ± 815.57 |
| cyclohexyl isocyanide | 3818.55 ± 364.45 | 6066.40 ± 1405.13 | 4219.60 ± 340.33 | 4285.76 ± 225.22 |
| 2-morpholinoethyl isocyanide | 3215.87 ± 145.76 | 4999.81 ± 328.28 | 3881.84 ± 158.25 | 4322.68 ± 218.64 |
| Control, 5454.34 ± 818.32 |            |                |                |                |
**Tables S4.** Structure, the theoretical monoisotopic, and observed masses of Ugi products.

| Structure and formula | Theoretic monoisotopic mass and charge state | Observed mass and charge state |
|-----------------------|--------------------------------------------|-------------------------------|
| C20H38N6O5            | 433.2982 (1+)                               | 433.2988 (1+)                 |
| C34H42N6O6             | 631.3244 (1+)                               | 631.3237 (1+)                 |
| C29H46N6O5             | 559.3608 (1+)                               | 559.3602 (1+)                 |
| C26H49N7O6S            | 588.3543 (1+)                               | 588.3536 (1+)                 |
### Table S5. Summary of peptide sequences.

| Figures | Sequences |
|---------|-----------|
| **Figure 4A** | Biotin-O  
Biotin-GPVIVITO  
Biotin-GPRIEITO |
| **Figure 4B** | Biotin-O  
Biotin-GGGGGGGO  
Biotin-GPVIVITO  
Biotin-GPRIEITO  
Biotin-GGRGDSPO  
Biotin-GGGGIDSPO |
| **Figure 3D** | Biotin-GG  
Biotin-GGGGG  
Biotin-GGGGGGG  
Biotin-GGGGGGGGG  
Biotin-GGGGGGGGGGG |
| **Figure 3E** | Biotin-YL  
Biotin-EELYL  
Biotin-IPEEYL  
Biotin-EEIPEEYL  
Biotin-DFEEIPEEYL  
Biotin-NGDFEEIPEEYL  
Biotin-GGGGNGDFEEIPEEYL  
Biotin-GGGGGGGGNGDFEEIPEEYL |
| **Figure 5** | Biotin-GGGGGGO  
Fluorescein-GGGGGGO |
| **Figure 6A** | Biotin  
Desthiobiotin  
Immobiotin  
Fmoc-CsA-COOH  
AVRHFPRlWLH(CB1)  
HFPRl(CB2)  
Biotin-CsA  
AVRHFPRlWLH(Biotin-CB1)  
HFPRl(Biotin-CB2)  
XYYKDDDDK  
DXYKDDDDK  
DYYKDDDDK  
DYKDDDDK  
DYKDDDXD  
DYKDDDXK  
DYKDDDXX  
HHHHHH |
| **Figure S14** | Biotin-O  
Biotin-GGGGGGO  
Biotin-GPRIEITO  
Biotin-GGGGGGO |

[1] B. Neises, W. Steglich, Angewandte Chemie International Edition in English 1978, 17, 522.
[2] A. F. Stalder, T. Melchior, M. Müller, D. Sage, T. Blu, M. Unser, Colloids and Surfaces A: Physicochemical and Engineering Aspects 2010, 364, 72.
[3] W. Lin, F. V. Reddavide, V. Uzunova, F. N. Gür, Y. Zhang, Anal. Chem. 2015, 87, 864.
[4] D. K. Owens, R. Wendt, J. Appl. Polym. Sci. 1969, 13, 1741.