SUPPORTING INFORMATION

A Universal Protocol for Photochemical Covalent Immobilization of Intact Carbohydrates for the Preparation of Carbohydrate Microarrays

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1. Limit of detection (LOD) determination.

The LOD concentration is estimated based on the line profiles shown in Figure 4 using a simple equation:

$$c_1/c_2 = h_1/h_2$$

$$LOD = c_2 \cdot h_1/h_2$$

Where c_1 is the LOD concentration, c_2 is the actual detection concentration, h_1 is the peak position at signal-to-noise ratio of 3 and h_2 is the peak height.

2. Determination of surface coverage of carbohydrate on PFPA surfaces.

A measured volume of carbohydrate solution was applied evenly onto PFPA-functioned surface. After drying and immobilizing by UV illumination, the unreacted carbohydrates were removed by soaking the samples in water. By determining the concentration difference using anthrone-sulfuric acid method *(1)* before and after the immobilization, the absolute surface coverage of immobilized carbohydrates can be calculated. Experimental details are described as follows.

A PFPA-modified chip (2.4×2.4 cm^2) was first coated with 100 μL carbohydrate solution (for example, 5 mg/mL mannose in water), dried in vacuum at 0.1 MPa for 5 min and exposed to UV light for 5 min. The chip was then immersed in 1.9 mL water and sonicated for about 5 min at room temperature to remove the un-reacted carbohydrate off the chip. The aqueous solution (1 mL) was transferred to a 25-mL borosilicate glass vial in an ice bath and the anthrone reagent (4 mL) was added dropwise. The solution was then heated in boiling water for 10 min and cooled in tap water. The solution was finally subjected to spectrometric measurement on a TU 1900 UV-Vis spectrophotometer (Beijing Purkinje General Instrument Co., Beijing, China). Depending on the type of carbohydrates, maximal absorbance was measured and was compared to the corresponding calibration curve, which was prepared by plotting the maximal absorbance against the carbohydrate concentration in the range of 31.5-521.9 μM (Figure 1S).

The control experiment without irradiation was done and no difference was observed.

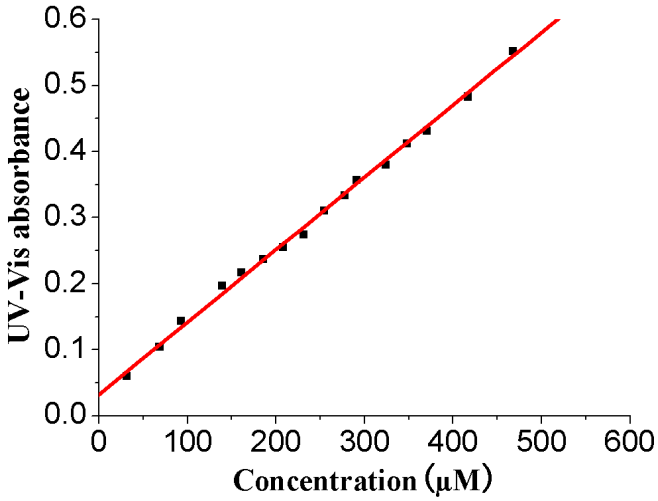

Figure 1S. Standard calibration curve of maximal UV-Vis absorbance (at 624 nm) of mannose against its concentration after anthrone-sulfuric acid reaction.

Table 1S. Surface concentration of mannose immobilized on (a) EDA- and (b) PLL-based PFPA surfaces (2.4×2.4 cm^2).

Chip	Mannose concentration (μM)			Surface concentration (molecules/nm^2)[a]
	before reaction	after reaction	difference	
a	277.78	276.53	1.25	6.5±2.2
b	277.78	272.57	5.21	27.2±5.3

[a] The results were averages of three measurements.

3. XPS characterization of PFPA on EDA- and PLL-based surfaces

X-ray photoelectron spectra were obtained by AlKα radiation at 300 W and base pressure of about 3×10^{-9} mbar with an ESCALab220i-XL electron spectrometer from VG Scientific (VG Scientific Ltd., East Grinstead, Sussex, U.K.). The binding energies were calculated with the C1s line at 284.8 eV from adventitious carbon as a reference. XPS scans exhibited intense peaks corresponding to C 1s (285 eV) and F 1s (688 eV).

The concentration data shown in Table 1S and the F1s spetra can prove the assembling of PFPA on the surfaces. N, O, F atoms on the contribution of surface concentration are expected. As PLLs assemble on the surface in a random conformation, part of amine groups may not protrude outside, the decrease of F1s concentration on surface of PFPA/PLL/MUA/Au showed not all of amine groups were converted into the amide linkage with PFPA. The C 1s spectra were curve-fitted with three peaks as C-C, C-H at 285 eV, C-N at 286 eV and C=O, C-F at 288 eV, which showed the existence of abundant amines and PFPA.

Table 2S. XPS Results of two PFPA-Functionalized Surfaces

element	PFPA/EDA/MUA/Au		PFPA/PLL/MUA/Au		sensitivity factor
	area (P) CPS.eV	concentration (%)	area (P) CPS.eV	concentration (%)	
C_{1s}, 284.8eV	16826.5	47.91	17245.2	49.24	1
Au_{4f}, 83.7eV	190491.6	30.34	178454.1	28.04	17.1
N_{1s}, 400.2eV	10790.6	12.65	12450.6	14.4	2.93
O_{1s}, 532.1eV	3779.1	6.67	3825.7	6.66	1.8
F_{1s}, 687.4eV	1416.4	2.43	2208.1	1.66	4.43

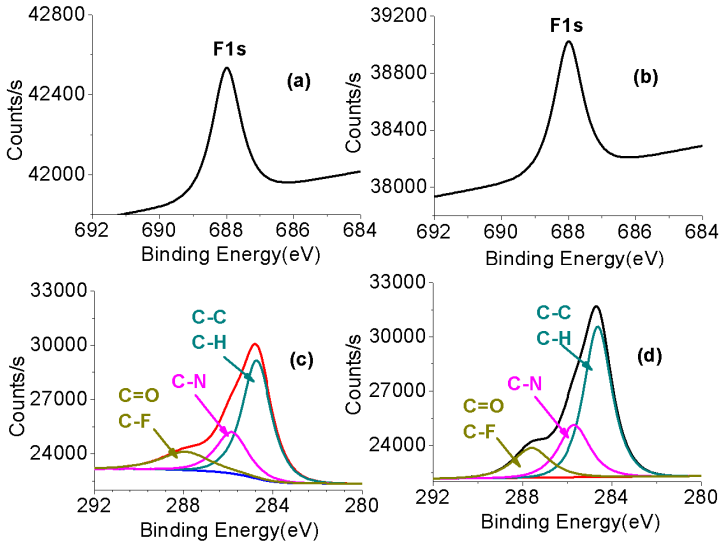

Figure 2S. The XPS F 1s and C 1s analysis of PFPA surfaces using EDA- (a, c), and PLL-based (b, d) chips.

4. Amine density determination of the EDA and PLL surfaces

Each amine chip was allowed to react with 4-nitrobenzaldehyde to form an imine linkage *(2)*. The imine linkage could be hydrolyzed by acid and then released 4-nitrobenzaldehyde, whose aqueous solution has a maximum absorbance at 267nm measured by UV-Vis spectroscopy (Figure 3S). Thus the aldehyde molecules are equivalent to the number of active amine groups on the surface. Then the absolute amine coverage of each surface could be calculated (Table 2S).

Two control experiments were also conducted to prove the reliability of the experiment. Firstly, MUA surface (before amine modification) was subjected to measurement of amines and no UV absorbance was measured at 267nm. Secondly, after the chips were modified with EDA or PLL, they were first incubated with glutaraldehyde instead of 4-nitrobenzaldehyde to block the activity of amine groups, then the amine were measured and no UV absorbance was observed at 267nm. Thus only the aminized surfaces have response to the incubation of 4-nitrobenzaldehyde. The quantity of 4-nitrobenzaldehyde determined from the UV absorbance experiment should be equal to that of active amine groups.

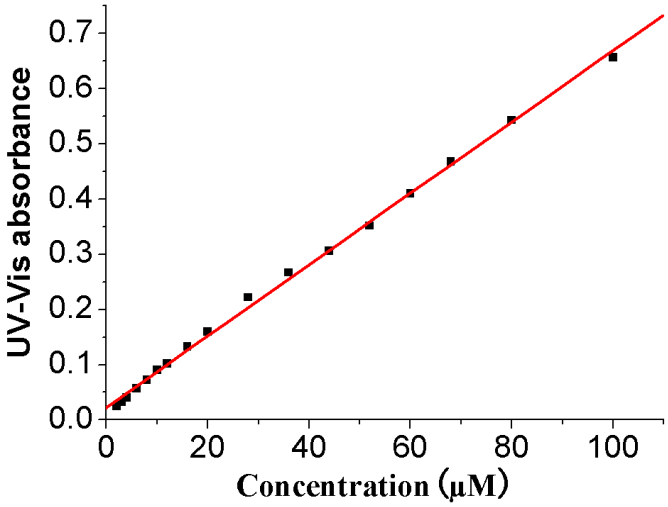

Figure 3S. Standard calibration curve of maximal UV-Vis absorbance (at 267 nm) of 4-nitrobenzaldehyde against its concentration

Table 3S. Surface concentration of amino groups on (a) EDA- and (b) PLL-based surfaces (2.4×2.4 cm^2) measured by UV-Vis spectroscopy at 267 nm.

Sample	concentration (μM)	Surface concentration (molecules/nm^2)[a]
a	15.5	8.1±1.7
b	71.53	37.3±5.3

[a] The results were averages of three measurements.

References

(1) Leyva, A., Quintana, A., Sánchez, M., Rodríguez, E.N., Cremata, J., and Sánchez, J.C. (2008) Rapid and sensitive anthrone–sulfuric acid assay in microplate format to quantify carbohydrate in biopharmaceutical products: method development and validation. *Biologicals. 36,* 134−141.

(2) Moon, J. H., Kim, J. H., Kim, K.-J., Kang, T.-H., Kim, B., Kim, C.-H., Hahn, J. H., Park, J. W. (1997) Absolute Surface Density of the Amine Group of the Aminosilylated Thin Layers: Ultraviolet-Visible Spectroscopy, Second Harmonic Generation, and Synchrotron-Radiation Photoelectron Spectroscopy Study. *Langmuir. 13,* 4305–4310.