Supporting Information

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Functionalized 3D Architected Materials via Thiol-Michael Addition and Two-Photon Lithography

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Supporting Information

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NMR Data:

PETTA/SiOMe:

\[ \begin{align*}
^{1}H \text{ NMR (500 MHz, Chloroform-}d) & \quad \delta 6.37 (d, J = 17.3 \text{ Hz, } 3\text{H, } CH_{2}=CHCO_{2}), 6.07 (dd, J = 17.3, \text{ 10.5 Hz, } 3\text{H, } CH_{2}=CHCO_{2}), 5.84 (d, J = 10.9 \text{ Hz, } 3\text{H, } CH_{2}=CHCO_{2}), 4.25-4.14 (m, 8H, CCH_{2}O), 3.52 (s, 9H, SiOCH_{3}), 2.70 (t, J = 7.3 \text{ Hz, } 2\text{H, } SCH_{2}CH_{2}CO_{2}), 2.57 (t, J = 7.2 \text{ Hz, } 2\text{H, } SCH_{2}CH_{2}Si), 1.64 (m, 2H, SCH_{2}CH_{2}CH_{2}Si), 0.74 – 0.66 (m, 2H, SCH_{2}CH_{2}CH_{2}Si). \\
^{13}C \text{ NMR (125 MHz, Chloroform-}d) & \quad \delta 171.44 (1 \text{ C, } OC=OCH_{2}CH_{2}S), 165.51 (3 \text{ C, } OC=OCH), 131.83 (3 \text{ C, } H_{2}C=CH), 127.63 (3 \text{ C, } H_{2}C=CH), 62.54 (4 \text{ C, } CCH_{2}O), 50.57 (3 \text{ C, } SiOCH_{3}), 42.14 (1 \text{ C, } CCH_{2}O), 34.94 (1 \text{ C, } OC=OCH_{2}CH_{2}S), 34.62 (1 \text{ C, } SCH_{2}CH_{2}CH_{2}Si), 26.63 (1 \text{ C, } OC=OCH_{2}CH_{2}S), 22.88 (1 \text{ C, } SCH_{2}CH_{2}CH_{2}Si), 8.54 (1 \text{ C, } SCH_{2}CH_{2}CH_{2}Si). 
\end{align*} \]

PETTA/OH:
\(^1\)H NMR (500 MHz, Chloroform-\(d\)) \(\delta\) 6.40 (d, \(J = 17.3\) Hz, 3H, \(CH_2=CHCO_2\)), 6.10 (dd, \(J = 17.3, 10.4\) Hz, 3H, \(CH_2=CHCO_2\)), 5.87 (d, \(J = 10.5\) Hz, 3H, \(CH_2=CHCO_2\)), 4.32-4.10 (m, 8H, \(CCH_2O\)), 3.74 (t, \(J = 5.7\) Hz, 2H, \(SCH_2CH_2OH\)), 2.81 (t, \(J = 5.6\) Hz, 2H, \(SCH_2CH_2CO_2\)), 2.73 (t, \(J = 5.9\) Hz, 2H, \(SCH_2CH_2CO_2\)), 2.65 (m, 2H, \(SCH_2CH_2OH\)).

\(^{13}\)C NMR (125 MHz, CDCl\(3\)) \(\delta\) 171.63 (1 C, OC=OCH\(2\)CH\(2\)S), 165.65 (3 C, OC=OCH), 131.94 (3 C, H\(2\)C=CH), 127.68 (3 C, H\(2\)C=CH), 62.73 (4 C, CCH\(2\)O), 60.93 (1 C, SCH\(2\)CH\(2\)OH), 42.25 (1 C, CCH\(2\)O), 35.77 (1 C, OC=OCH\(2\)CH\(2\)S), 34.76 (1 C, SCH\(2\)CH\(2\)OH), 26.95 (1 C, OC=OCH\(2\)CH\(2\)S).

**PETTA/Octane:**

\(^1\)H NMR (500 MHz, Chloroform-\(d\)) \(\delta\) 6.39 (d, \(J = 17.3\) Hz, 3H, \(CH_2=CHCO_2\)), 6.08 (dd, \(J = 17.3, 10.5\) Hz, 3H, \(CH_2=CHCO_2\)), 5.85 (d, \(J = 11.7\) Hz, 3H, \(CH_2=CHCO_2\)), 4.33–4.04 (m, 8H, CCH\(2\)O), 2.72 (t, \(J = 7.2\) Hz, 2H, \(SCH_2CH_2CO_2\)), 2.59 (t, \(J = 7.2\) Hz, 2H, \(SCH_2CH_2CO_2\)), 2.48 (t, \(J = 7.4\) Hz, 2H, \(SCH_2CH_2C_6H_{13}\)), 1.54 (p, \(J = 7.5\) Hz, 2H, \(CH_2\)), 1.43–1.30 (m, 2H, \(CH_2\)), 1.25 (1.30-1.15, 8H, \(CH_2\)), 0.85 (t, \(J = 6.9\) Hz, 3H, \(CH_3\)).
$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 171.52 (1 C, OC=OCH$_2$CH$_2$S), 165.56 (3 C, OC=OCH), 131.86 (3 C, H$_2$C=CH), 127.67 (3 C, H$_2$C=CH), 62.69 (4 C, CCH$_2$O), 42.18 (1 C, CCH$_2$O), 34.69, 32.20, 31.88, 29.59, 29.26, 28.95, 26.89, 22.72, 14.18.

PETTA/CH$_2$CF$_3$:

\[
\begin{align*}
\text{H NMR (500 MHz, Chloroform-}d) &\text{ $\delta$ 6.40 (d, } J = 17.3 \text{ Hz, 3H, CH$_2$=CHCO$_2$), 6.09 (dd, } J = \\
&\text{17.3, 10.5 Hz, 3H, CH$_2$=CHCO$_2$), 5.86 (d, } J = 11.7 \text{ Hz, 3H, CH$_2$=CHCO$_2$), 4.32 - 4.03 \text{ (m,} \\
&\text{8H, CCH$_2$O), 3.22 - 3.02 \text{ (m, 2H, SCH$_2$CF$_3$), 2.95-2.87 \text{ (m, 2H, SCH$_2$CH$_2$CO$_2$), 2.70 - 2.60} \\
&\text{ (m, 2H, SCH$_2$CH$_2$CO$_2$).}
\end{align*}
\]

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 170.94 (1 C, OC=OCH$_2$CH$_2$S), 165.63 (3 C, OC=OCH), 131.92 (3 C, H$_2$C=CH), 127.68 (3 C, H$_2$C=CH), 124.72 (1 C, CF$_3$), 62.72 (4 C, CCH$_2$O), 42.24 (1 C, CCH$_2$O), 34.29 (1 C, OC=OCH$_2$CH$_2$S), 28.07 (1 C, OC=OCH$_2$CH$_2$S).

$^{19}$F NMR (282 MHz, CDCl$_3$) $\delta$ -66.50 (3F, CF$_3$).

PETTA/N-BOC:

\[
\begin{align*}
\text{H NMR (500 MHz, Chloroform-}d) &\text{ $\delta$ 6.36 (d, } J = 17.3 \text{ Hz, 3H, CH$_2$=CHCO$_2$), 6.06 (dd, } J = \\
&\text{17.3, 10.5 Hz, 3H, CH$_2$=CHCO$_2$), 5.83 (d, } J = 11.7 \text{ Hz, CH$_2$=CHCO$_2$), 5.06 (s, 1H, NH),
\end{align*}
\]
4.30 – 4.01 (m, 8H, CCH₂O), 3.30-3.18 (m, 2H, SCH₂CH₂NH), 2.73 (t, J = 6.3 Hz, 2H, SCH₂CH₂CO₂), 2.64-2.54 (m, 4H, CH₂SCH₂CH₂CO₂), 1.38 (s, 9H, CCH₃).

¹³C NMR (125 MHz, CDCl₃) δ 171.22 (1 C, OC=OCH₂CH₂S), 165.50 (3 C, OC=OCH), 155.81 (1 C, NHCO₂), 131.81 (3 C, H₂C=CH), 127.58 (3 C, H₂C=CH), 79.36 (1 C, OC(CH₃)₃), 62.62 (4 C, CCH₂O), 42.15 (1 C, CCH₂O), 39.74 (1 C, CH₂NH), 34.56 (1 C, OC=OCH₂CH₂S), 32.25 (1 C, SCH₂CH₂NH), 28.40 (3 C, CH₃), 26.57 (1 C, OC=OCH₂CH₂S).

PETTA/N-BOC(Cys):

¹³C NMR (125 MHz, CDCl₃) δ 171.47 (2 C, OC=OCH₂CH₂S, CO₂CH₃), 165.57 (3 C, OC=OCH), 155.21 (1 C, NHCO₂), 131.87 (3 C, H₂C=CH), 127.63 (3 C, H₂C=CH), 80.21 (1 C, OC(CH₃)₃), 62.62 (4 C, CCH₂O), 53.31 (1 C, CHNH), 52.67 (1 C, OCH₃), 42.20 (1 C, CCH₂O), 34.44 (1 C, OC=OCH₂CH₂S), 28.34 (3 C, CH₃), 27.48 (1 C, OC=OCH₂CH₂S).

PETTA/PFP:
\[ \text{\textsuperscript{1}H NMR (500 MHz, Chloroform-d) \( \delta \) 6.41 (d, } J = 17.3 \text{ Hz, 3H, } \text{CH}_2=\text{CHCO}_2), 6.10 \text{ (dd, } J = 17.9, 9.9 \text{ Hz, 3H, } \text{CH}_2=\text{CHCO}_2), 5.88 \text{ (d, } J = 10.4 \text{ Hz, 3H, } \text{CH}_2=\text{CHCO}_2), 4.34 - 3.99 \text{ (m, 8H, CCH}_2\text{O), 3.09 (t, } J = 7.0 \text{ Hz, 2H, SCH}_2\text{CH}_2\text{CO}_2).} \]

\[ \text{\textsuperscript{13}C NMR (125 MHz, CDCl}_3 \) \( \delta \) 170.60 (2 C, OC=OCH}_2\text{CH}_2\text{S, CO}_2\text{CH}_3), 165.62 (3 C, OC=OCH), 150-136 (5 C, CF), 132.01 (3 C, H}_2\text{C}=\text{CH), 127.65 (3 C, H}_2\text{C}=\text{CH), 62.53 (4 C, CCH}_2\text{O), 42.24 (1 C, CCH}_2\text{O), 34.74 (1 C, OC=OCH}_2\text{CH}_2\text{S), 29.89 (1 C, OC=OCH}_2\text{CH}_2\text{S).} \]

\[ \text{\textsuperscript{19}F NMR (282 MHz, CDCl}_3 \) \( \delta \) -132.14, -151.58, -160.44.} \]

\[ \text{PETTA/LCF:} \]

\[ \text{\textsuperscript{1}H NMR (500 MHz, Chloroform-d) \( \delta \) 6.40 (d, } J = 17.3 \text{ Hz, 3H, } \text{CH}_2=\text{CHCO}_2), 6.10 \text{ (dd, } J = 17.4, 10.4 \text{ Hz, 3H, } \text{CH}_2=\text{CHCO}_2), 5.86 \text{ (d, } J = 10.4 \text{ Hz, 3H, } \text{CH}_2=\text{CHCO}_2), 4.33 - 4.07 \text{ (m, 8H, CCH}_2\text{O), 2.80 (t, } J = 7.0 \text{ Hz, 2H, SCH}_2\text{CH}_2\text{CO}_2), 2.77 - 2.70 \text{ (m, 2H, SCH}_2\text{CH}_2\text{CO}_2), 2.64 (t, } J = 7.0 \text{ Hz, 2H, SCH}_2\text{CH}_2\text{CF}_2), 2.48-2.30 \text{ (m, 2H, SCH}_2\text{CH}_2\text{CF}_2).} \]
$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 171.19 (1 C, O=OCH$_2$CH$_2$S), 165.65 (3 C, O=OCH), 131.91 (3 C, H$_2$C=CH), 127.70 (3 C, H$_2$C=CH), 117.58 (1 C, CF$_3$), 116.12 (1 C, CF$_2$), 115.54 (1 C, CF$_2$), 115.27 (1 C, CF$_2$), 111.08 (1 C, CF$_2$), 110.30 (1 C, CF$_2$), 62.74 (4 C, CCH$_2$O), 42.27 (1 C, CCH$_2$O), 34.40 (1 C, O=OCH$_2$CH$_2$S), 31.96 (1 C, CH$_2$CF$_2$), 27.09 (1 C, O=OCH$_2$CH$_2$S), 22.91 (1 C, SCH$_2$CH$_2$CF$_2$).

$^{19}$F NMR (282 MHz, CDCl$_3$) $\delta$ -80.83 (3F, CF$_3$), -114.43, -121.94, -122.91, -123.47, -126.18.

PETTA/BM:

$^1$H NMR (500 MHz, Chloroform-d) $\delta$ 7.30 (d, $J = 3.9$ Hz, 2H, CCHCH$_2$), 7.27 – 7.20 (m, 3H, aromatic), 6.41 (d, $J = 17.3$ Hz, 3H, CH$_2$=CHCO$_2$), 6.10 (dd, $J = 17.3$, 10.5 Hz, 3H, CH$_2$=CHCO$_2$), 5.87 (d, $J = 11.9$ Hz, 3H, CH$_2$=CHCO$_2$), 4.31 – 4.09 (m, 8H, CCH$_2$O), 3.71 (s, 2H, SCH$_2$C), 2.63 (d, $J = 7.0$ Hz, 2H, SCH$_2$CH$_2$CO$_2$), 2.53 (m, 2H, SCH$_2$CH$_2$CO$_2$).

$^{13}$C NMR (125 MHz, Chloroform-d) $\delta$ 171.42 (1 C, O=OCH$_2$CH$_2$S), 165.63 (3 C, O=OCH), 138.04 (1C, SCH$_2$C), 131.96 (3 C, H$_2$C=CH), 128.96 (2C, CH), 128.70 (2C, CH), 127.70 (3 C, H$_2$C=CH), 127.29 (1C, CH), 62.62 (4 C, CCH$_2$O), 42.20 (1 C, CCH$_2$O), 36.37 (1C, SCH$_2$C), 34.33 (1 C, O=OCH$_2$CH$_2$S), 26.17 (1 C, O=OCH$_2$CH$_2$S).
Two-photon lithography details:

Table S1. Details of the geometries used for each photoresist used in the two-photon lithography experiments. The volume shrinkage exhibited by each photoresist is also indicated.

<table>
<thead>
<tr>
<th>Photoresist</th>
<th>Geometry</th>
<th>Volume Shrinkage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PETTA/CH$_2$CF$_3$</td>
<td>Octet</td>
<td>8.5</td>
</tr>
<tr>
<td>PETTA/LCF</td>
<td>Tetrakaidecahedron</td>
<td>11.7</td>
</tr>
<tr>
<td>PETTA/PFP</td>
<td>Reinforced Cube</td>
<td>5.3</td>
</tr>
<tr>
<td>PETTA/SiOMe</td>
<td>Octahedron</td>
<td>5.0</td>
</tr>
<tr>
<td>PETTA/OH</td>
<td>Body Centered Cubic</td>
<td>5.6</td>
</tr>
<tr>
<td>PETTA/Octane</td>
<td>Icosahedron</td>
<td>5.6</td>
</tr>
<tr>
<td>PETTA/N-BOC</td>
<td>Shifted Octahedron</td>
<td>13.6</td>
</tr>
<tr>
<td>PETTA/N-BOC(Cys)</td>
<td>Diamond</td>
<td>12.9</td>
</tr>
<tr>
<td>PETTA/BM</td>
<td>Hexagon</td>
<td>5.5</td>
</tr>
</tbody>
</table>

X-Ray Photoelectron Spectroscopy:

Figure S1 shows the XPS spectra of all the polymer plates fabricated via two-photon lithography. Due to the abundance of carbon and oxygen in the polymer, both in the backbone of the structure and from adventitious surface contaminants, the intensity axis was plotted on a log scale to minimize the differences in the peak heights between the carbon/oxygen peaks and that of the other elements present. Plates fabricated using the functional photoresists all exhibited the S 2p peak (~165eV), strong evidence that the functional group is present on the surface of the plate. The F 1s (~ 688eV), N 1s (~400eV) and Si 2p (~100eV) peaks detected in the plates containing fluorine, nitrogen and silicon respectively are also indicative of this.
Figure S1. XPS survey spectra of a) PETTA/LCF, b) PETTA/PFP, c) PETTA/CH$_2$CF$_3$, d) PETTA/N-BOC(Cys), e) PETTA/N-BOC, f) PETTA/SiOMe, g) PETTA/BM, h) PETTA/OH, i) PETTA/Octane and j) PETTA. Intensity scale was plotted on a log scale to clearly show all detected peaks, regardless of the intensity differences between them.
Orange II Amine Test:

In acidic solutions of Orange II, the positively charged protonated amines on the surface of the PETTA/NBOC plates are reversibly bound to the negatively charged sulfonated Orange II dye via electrostatic interactions. On subsequent immersion in basic media, the amines become deprotonated and release the Orange II molecules into solution. The quantity of desorbed dye can then be determined by measuring the absorbance of the solution and comparing it to solutions of known Orange II concentrations.\cite{53} Assuming each Orange II molecule binds to only one amine, the surface density of amines can then be determined. Figure S2 shows both a deprotected PETTA/NBOC plate and a control PETTA plate after immersion in the Orange II acidic solution followed by washing. Visual inspection of both plates indicated that the Orange II dye only bound to the amines on the surface.

![Figure S2. PETTA plate (left) and deprotected PETTA/NBOC plate (right) after immersing in the Orange II acidic solution and washing. The lack of color in the PETTA control plate and the orange PETTA/NBOC plate indicates that the Orange II molecules were successfully bound to the amines on the PETTA/NBOC surface.](image)

By measuring the absorbance of Orange II solutions of various concentrations at 480 nm, a calibration curve can be constructed that would allow us to extrapolate the concentration of an unknown solution from its absorbance. Figure S3 shows the calibration curve constructed using solutions of concentration 0.01 mg/mL to 0.0005 mg/mL.
Figure S3. Calibration curve constructed using the absorbance of Orange II solutions of concentrations 0.01 mg/mL to 0.0005 mg/mL.

Thus, by measuring the absorbance of the desorbed Orange II molecules in the solutions containing the PETTA/NBOC plates, the concentration of Orange II molecules could be determined using the calibration curve. As shown in Table S2, since the volume of solutions and the areas of the PETTA/NBOC plates were known, the surface density of the amine functional groups could be determined.

Table S2. Surface density of amine functional groups on the PETTA/NBOC plates.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Absorbance</th>
<th>Concentration (mg/mL)</th>
<th>Volume of solution (mL)</th>
<th>Area of plate (um²)</th>
<th>Surface density (molecules/um²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0738</td>
<td>0.00114</td>
<td>1.785</td>
<td>9449476</td>
<td>3.9 x 10^8</td>
</tr>
<tr>
<td>2</td>
<td>0.0647</td>
<td>0.00079</td>
<td>1.785</td>
<td>9056216</td>
<td>2.9 x 10^8</td>
</tr>
<tr>
<td>3</td>
<td>0.0919</td>
<td>0.00184</td>
<td>1.730</td>
<td>12516904</td>
<td>4.7 x 10^8</td>
</tr>
<tr>
<td>4</td>
<td>0.0800</td>
<td>0.00138</td>
<td>1.730</td>
<td>9449476</td>
<td>4.6 x 10^8</td>
</tr>
<tr>
<td>5</td>
<td>0.0724</td>
<td>0.00109</td>
<td>1.620</td>
<td>9427004</td>
<td>3.4 x 10^8</td>
</tr>
</tbody>
</table>

The average surface density of amines was determined to be approximately 3.9 ± 0.7 x 10^8 molecules um⁻².
Confocal Fluorescence Microscopy:

From Figure S4a, it can be seen that due to the design of the PETTA/N-BOC structure, a self-shadowing effect was observed where emitted light from the bottom half of the sample was blocked by the top half of the sample. However, where there was no overlap of structure, i.e. the top half of the PETTA/N-BOC structure and the entire PETTA/N-BOC(Cys) structure, a uniform intensity could be seen. This is much clearly emphasized in the supporting videos.