SUPPLEMENTARY INFORMATION

S.1. Control of surface chemistry through the development of the probe/backfiller co-deposition model.

We chose to deposit SH-PEO-PNA (the probe) and SH-PEO 2KDa (the backfiller) using a co-deposition strategy, in which both the probe and the backfiller are deposited simultaneously from a binary solution mixture. In order to finely control the final surface composition at the end of the deposition process in terms of relative probe/backfiller surface coverage, we developed and validated a mathematical model for molecule deposition prediction, based on the Langmuir adsorption equation.

S.1.1 Thiol adsorption onto gold: background

The adsorption kinetics of thiols is described by the Langmuir adsorption equation\(^1,2\):

\[
\frac{d\theta}{dt} = k_a (1 - \theta)C - k_d \theta
\]

where \(\theta\) is the surface coverage expressed as fraction (unitless), \(1 - \theta\) is the surface portion uncovered by the thiol, \(t\) is the molecule grafting time, \(C\) is the thiol bulk concentration [\(\text{mol} \cdot \text{L}^{-1}\)], \(k_a\) and \(k_d\) are the adsorption and desorption rate constants.

Integrating and rearranging Eq. S.1.1, we obtain the following expression, which can be applied to a specie experimental kinetics to obtain its adsorption rate constants\(^1,2\):

\[
\theta(t) = 1 - \exp(-k_{obs} \cdot t)
\]

where \(k_{obs} = k_a C + k_d\) and \(k_d << k_a\)
The above adsorption rate constants can be estimated experimentally from the analysis of their deposition kinetics, as described in other works for thiols or alkanethiols and for PEOs.

**The co-deposition model**

When competitive surface adsorption of two different species (specie 1 and specie 2) from a binary mixture solution is occurring, the uncovered surface fraction seen by one species can be written as:

\[ 1 - \theta_1 - \theta_2 \]  

(S.1.3)

where \( \theta_1 \) and \( \theta_2 \) are the coverage fraction for specie 1 and specie 2, respectively.

Thus, the simultaneous adsorption mechanism of two molecules can be described by the following system.

\[
\begin{align*}
\frac{d\theta_1}{dt} &= k_{a_1} (1 - \theta_1 - \theta_2) C_1 - k_{d_1} \theta_1 \\
\frac{d\theta_2}{dt} &= k_{a_2} (1 - \theta_2 - \theta_1) C_2 - k_{d_2} \theta_2
\end{align*}
\]

(S.1.4, S.1.5)

where \( \theta_1 \) and \( \theta_2 \) are the coverage fraction for specie 1 and specie 2.

Re-arranging the above equations we get:

\[
\begin{align*}
\frac{d\theta_1}{dt} &= -(k_{a_1} C_1 + k_{d_1}) \theta_1 - k_{a_1} C_1 \theta_2 + k_{a_1} C_1 \\
\frac{d\theta_2}{dt} &= -k_{a_2} C_2 \theta_1 - (k_{a_2} C_2 + k_{d_2}) \theta_2 + k_{a_2} C_2
\end{align*}
\]

(S.1.6, S.1.7)

and then:

\[
\begin{pmatrix}
\dot{\theta}_1 \\
\dot{\theta}_2
\end{pmatrix} = \begin{pmatrix}
-k_{a_1} C_1 - k_{d_1} & -k_{a_1} C_1 \\
-k_{a_2} C_2 & -k_{a_2} C_2 - k_{d_2}
\end{pmatrix} \begin{pmatrix}
\theta_1 \\
\theta_2
\end{pmatrix} + \begin{pmatrix}
k_{a_1} C_1 \\
k_{a_2} C_2
\end{pmatrix}
\]

(S.1.8)

\[
\begin{pmatrix}
\dot{\theta}_1 \\
\dot{\theta}_2
\end{pmatrix} = \begin{pmatrix}
\alpha & \beta \\
\gamma & \delta
\end{pmatrix} \begin{pmatrix}
\theta_1 \\
\theta_2
\end{pmatrix} + \begin{pmatrix}
-\beta \\
-\gamma
\end{pmatrix}
\]

(S.1.9)

Solving the calculation, we get the following equations for surface coverage of specie 1 and surface coverage of specie 2:

\[
\theta_1(t) = -c_1 \beta e^{\lambda_1 t} - c_2 \beta e^{\lambda_2 t} + \frac{\beta}{\lambda_+} (\lambda_+ + \lambda_- - \alpha - \gamma)
\]

(S.1.10)
\[ \theta_2(t) = c_1(\alpha - \lambda_+) e^{\lambda_+ t} + c_2(\alpha - \lambda_-) e^{\lambda_- t} + \frac{(\alpha - \lambda_)(\alpha - \lambda_-) + \gamma \alpha}{\lambda_+ \lambda_-} \]  

(S.1.11)

Where:

- \( \lambda_\pm \) are the eigenvalues:
  \[
  \lambda_\pm = \frac{\alpha + \delta \pm \sqrt{(\alpha - \delta)^2 + 4 \gamma \beta}}{2} 
  \]  
  (S.1.12)

- \( \alpha, \beta, \gamma, \delta \) are the components of the following matrix:
  \[
  \begin{pmatrix} \alpha & \beta \\ \gamma & \delta \end{pmatrix} = \begin{pmatrix} -k_{a_{1}}C_{1} - k_{d_{1}}C_{1} \\ -k_{a_{2}}C_{2} - k_{d_{2}}C_{2} - k_{d_{2}} \end{pmatrix} 
  \]  
  (S.1.13)

- Defining the initial conditions (i.e. \( \theta(t = 0) = 0 \)), the coefficients \( c_1, c_2 \) result:
  \[
  c_1 = \frac{\alpha + \gamma - \lambda_-}{\lambda_+ (\lambda_+ - \lambda_-)} 
  \]  
  (S.1.14)

\[
  c_2 = \frac{\alpha + \gamma - \lambda_+}{\lambda_- (\lambda_- - \lambda_+)} 
  \]  
  (S.1.15)

**S.1.2. Experimental**

Measurement of the adsorption constant rates for different thiol compounds.

The \( K_{obs}[s^{-1}] \) and \( K_a \) values of the thiol reagents were obtained by measuring the \( \varphi \neq 0^\circ \) GC-SPR response (resonance angle shifts) as a function of the time of deposition from solutions at known concentrations. We derived the surface coverage (\( \theta(t) \)) at each deposition time by calculating the ratio between the resonance angle shift at the defined deposition time and the maximum resonance angle shift obtained at the end of the process. The plots of \( \theta(t) \) as a function of deposition time (\( t[s] \)) (Figure S.1) were fitted using Eq. (S.1.2) to calculate \( K_{obs} [s^{-1}] \) values and then and \( K_a \) ones (Table S.1). Four thiolated molecules were tested, namely the SH-PNA and SH-PEO\(_{5KDa}\)-PNA probes, the mPEO\(_{2KDa}\) backfiller and its higher MW analogue (5KDa, SH-mPEO\(_{5KDa}\).\(^{16} \)
Figure S.1. Adsorption kinetics of all the molecules tested. The surface coverage fraction $\theta(t)$ is the ratio between the resonance angle shift at a particular deposition time and the maximum resonance angle shift.

Table S.1 The thiol adsorption rate constants calculated

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Concentration [M]</th>
<th>$k_{obs} \times 10^5$ [s$^{-1}$]</th>
<th>$k_a \times 10^2$ [L · mol$^{-1}$ · s$^{-1}$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH-PEO$_{25KDa}$</td>
<td>0.00093</td>
<td>1.86±0.34</td>
<td>1.83±0.02</td>
</tr>
<tr>
<td>SH-PNA-mPEO$_{25KDa}$</td>
<td>0.00082</td>
<td>1.55±0.18</td>
<td>1.88±0.02</td>
</tr>
<tr>
<td>SH-PNA-PEO$_{5KDa}$</td>
<td>0.0005</td>
<td>0.52±0.07</td>
<td>1.05±0.002</td>
</tr>
<tr>
<td>HS-PNA</td>
<td>0.25</td>
<td>1.59±0.33</td>
<td>6.35±0.02</td>
</tr>
</tbody>
</table>

Prediction of binary mixture compositions to control surface composition after co-deposition

The theoretical co-deposition model (§ S.1.2) was implemented in the Mathematica Software. For each binary mixture to be co-deposited, the experimental $K_{obs}$ and $K_a$ values of the thiols were inputted in the formula, and by setting the specie concentration in solution and the functionalization time, we were able to tune and predict the resulting surface coverage for each thiol species.

Validation of the co-deposition model

The validity of the model described in the previous sections was confirmed by a Toluidine Blue O (TBO) test, a method commonly adopted for evaluating the amount of carboxyl groups (-COOH) anchored on a surface$^{17,18,19}$. 
We functionalized nanostructured grated gold surfaces using five mPEO_{2KDa}/SH-PEO_{5KDa-COOH} (Laysan Bio, Arab, AL, USA) mixtures, whose composition was predicted by the mathematical elaboration to generate surface compositions with pre-defined amounts of the COOH-carrying PEO_{5KDa} (Table S.2). We then carried out the TBO test on the final surfaces to measure the number of HS-PEO_{5KDa-COOH} molecules anchored to them. Table S.2 shows the predicted and experimental values.

**Table S.2.** Predicted and experimental number of HS-PEO_{5KDa-COOH} molecules adsorbed onto the grated gold surface.

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Surface coverage fraction predicted(*)</th>
<th>PEO_{5KDa-COOH} Predicted number of molecules [N/nm²]</th>
<th>Solution concentration <a href="*">mM</a></th>
<th>PEO_{5KDa-COOH} Number of molecules from TBO test [N/nm²]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0</td>
<td>1.0</td>
<td>0.00</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>0.25</td>
<td>0.75</td>
<td>0.20</td>
<td>0.18</td>
</tr>
<tr>
<td>3</td>
<td>0.50</td>
<td>0.50</td>
<td>0.41</td>
<td>0.43</td>
</tr>
<tr>
<td>4</td>
<td>0.75</td>
<td>0.25</td>
<td>0.62</td>
<td>0.85</td>
</tr>
<tr>
<td>5</td>
<td>1.0</td>
<td>0.0</td>
<td>0.82</td>
<td>1.0</td>
</tr>
</tbody>
</table>

(*) Parameters derived from the co-deposition theoretical model. For the SH-PEO_{5KDa-COOH} adsorption constant the same value of SH-mPEO_{5KDa} one was adopted.

The results confirmed the validity of the theoretical model. The same approach was then used to control the surface composition of all the sensing surfaces adopted for this work, selecting according to the algorithm the relative solution concentration of the PNA-SH/SH-PEO_{2KDa}, or SH-PEO_{5KDa-PNA}/SH-PEO_{2KDa} binary mixtures to achieve the desired surface compositions.

**S.2. Comparison between glass substrate-based and gold substrate-based fluorescence DNA hybridization experiments**

*Figure S.2.* Fluorescence image obtained upon hybridization of Cy3-DNA-c with the glass surface functionalized with the DNA-probe or the flat gold surface functionalized with the HS-PEO5KDa-PNA/HS-mPEO2KDa mixture at final 0.1 surface ratio.
S.3. Preparation of MT PCR amplicons

AmpliTaq Gold 360 DNA Polymerase kit and dNTPs were purchased from Applied Biosystem (Life Technologies, Milan, Italy). Cy3 dCTP was purchased from GE Healthcare (Little Chalfont, UK).

To generate the MT 224 bp PCR amplicon, 200 ng of DNA extract were PCR amplified using the following reagent final concentrations: 1X PCR buffer, 1.5 mM MgCl$_2$, 200 µM dNTPs, 500 µM of both mFw (5’-CGCAGACGTTGATCAACATCCGGC-3’) and mRev (5’-GGTTTCGATCGGGCACATCCGGC-3’) primers, 1 unit of Taq polymerase. The following thermal cycle was performed: 5’ at 95 °C; 40 cycles of 30’’ at 95 °C, 30’’ at 58 °C and 1’ at 70 °C; 7’ at 70°C and hold at 4°C. The PCR product was purified with silica spin column (PureLink PCR Purification Kit, Life Technologies) and run on Agilent Bioanalyzer with DNA chip (Agilent Technologies, Santa Clara, CA) to check fragment integrity, dimensions and amount. Cy3 labelled wild type MT PCR amplicon (Cy3-PCR amplicon) was obtained by amplifying genomic DNA in the same conditions, but using a mix of dNTPs including Cy3 dCTP. Fluorophore incorporation was verified spectrophotometrically (NanoPhotometer, Implen, München, Germany).

References


16 A. Sonato, D. Silvestri, G. Ruffato, G. Zacco, F. Romanto, M. Morpurgo, Quantitative control of poly(ethylene oxide) surface antifouling and biodetection through azimuthally enhanced grating coupled-surfaceplasmon resonance sensing, 286 (2013) 22-30

