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Controlled protein assembly on a switchable surface†

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The strategy presented in this work supplies a general method of controlling protein assembly on a switchable low-density SAM, which may open a new way to design functional biocomposite films for biosensors or protein chips.

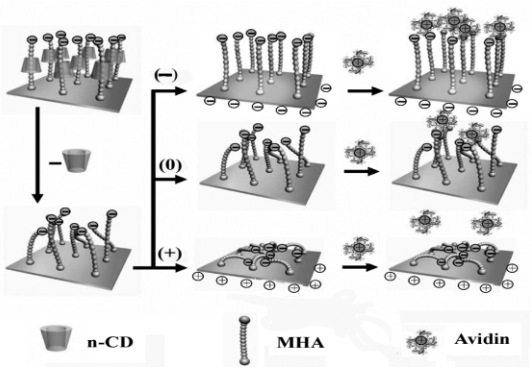
Design of functional surfaces has drawn increasing attention for its diverse applications in controlled assembly,[1] designed wettability alternation,[2] and programmed adsorption of proteins[3] or cells.[4] Various switchable surfaces initiated by external factors, including photon,[5] charge,[6] pH,[7] temperature[8] and chemical or electrochemical energy,[9,10] have been extensively studied. Among this pioneering work, the low density self-assembly monolayer (LD-SAM) generated on Au for reversible switching represents the most attractive discovery.[10,11] The preparation of the LD-SAM is considered to be a key step to ensure the bending of alkanethiol controlled by the applied potential. The solution in the mentioned work involves assembling synthetic *capped* alkanethiolates on Au, followed by dissociating the *cap*, *i.e.* the bulky globular groups, to create the LD-SAM. The thus-prepared functional surface is predicted to have potential applications in dynamic regulation of macroscopic properties, *e.g.* biocompatibility, which triggered our inspiration to expand it to protein assembly on an LD-SAM.

In this work, an alternative method is introduced to generate the LD-SAM by assembling a pre-formed inclusion complex (IC), *i.e.* cyclodextrin (CD)-wrapped alkanethiolate, on Au, followed by unwrapping the CD from the anchored IC. The IC between 16-mercaptohexadecanoic acid (MHA) and α-, β- or γ-cyclodextrin[12] (abbreviated as *n*-CD-MHA, n = 1, 2 or 3 represents α, β or γ in turn) acts as the space-filling group of the SAM. Thus, the LD-SAM is created by removing the CD. Furthermore, controlled protein assembly has been achieved on an LD-SAM by potential-driven adsorption of two fluorescent-labeled avidins.

Firstly, the inclusion complex, *n*-CD-MHA, was synthesized and characterized by ^{1}H-NMR spectra, in which the formation of *n*-CD-MHA was confirmed by significant up-field shift for protons in the apolar alkyl chain.[13] The truncated cone shape for α-, β- and γ-CD offered the corresponding spatial dimensions of *ca.* 1.47 nm^2, 1.84 nm^2 and 2.65 nm^2,[14] somewhat larger than the smallest spatial dimension ($\sim$ 0.65 nm^2) reported by Lahann.[10] Then, the *n*-CD-MHA-SAM was prepared *via* covalent binding following the normal SAM procedure.[15] Thus, the packing density of the resulting SAM was controlled by the spatial dimension of *n*-CD, and hence was expected to provide sufficient conformational freedom for the bending of MHA on Au.[11] The unwrapping procedure, *i.e.* the removal of *n*-CD from the SAM, was accomplished by dissociating the bond *n*-CD–MHA with ethanol through the solvent effect.[16] The removal of *n*-CD was monitored by quartz crystal microbalance (QCM), impedance (IMP) and MALDI-TOF-MS. The LD-SAM, with the desired space for the bending of MHA, was fabricated as illustrated in Scheme 1.

QCM measurement data definitely reflect the quantitative removal of *n*-CD from the surface. Table 1 shows the frequency shift (Δf), the corresponding mass change (ΔM) and the calculated surface coverage (Θ) for α-, β- and γ-CD-derived LD-SAM-*n*. The surface coverage (Θ) for LD-SAM-*n* referring to high-density SAM (HD-SAM) shows an obvious decrease from 100% to 61.2%, 45.3% and 29.2%, respectively. This could be reasonably attributed to the removal of CD from the surface. In addition, MALDI-TOF-MS measurement of the surface remarkably reveals the presence and absence of *n*-CD with the appearance and disappearance of the peaks at *m*/*z* 995, 1158 and 1320, corresponding to the sodium adducts of α-, β- and γ-CD, respectively.[17]

Then, the electric potential triggered reversible switching properties, *e.g.* the wettability, of the resulting MHA-SAM-*n* were investigated by contact angle measurements.[13] A remarkable dissimilarity in contact angles of a polar medium on MHA-SAM-*n* was observed, *e.g.* the contact angle of buffer (pH = 7.4) on MHA-SAM-2 was measured as about 55° at E_{appl} = 0.3 V and as about 22° at E_{appl} = −0.3 V, which strongly implies that the surface transition did occur between the hydrophilic (as MHA in straight) and hydrophobic (as MHA in bent) states. Therefore, the surface charge of the LD-SAM could be controlled in two ways, *i.e.* one was fully negatively charged or hydrophilic, with the carboxyl-terminated thiol facing straight towards the solution at negative applied potential (termed as (−) or *on* state), the other was neutral or hydrophobic as well, with the bent chain being exposed to the



Scheme 1 The idealized illustration for the preparation of LD-MHA-SAM-*n*, the transition for anchored MHA at applied potentials and the subsequent protein assembly at E_{appl} = −0.3 V (−), +0.3 V (+) and the open circuit state (0). The cartoon was inspired by Lahann *et al.* [*Science*, 2003, **299**, 371–374].

Table 1 Characterization of LD-MHA-SAM-*n* with QCM[a,b,c,d]

Type of SAM	Δf_1 (Hz)	ΔM_1 (nmol cm^{-2})	Δf_2 (Hz)	Γ_2 (nmol cm^{-2})	Θ (%)
MHA-SAM-1	94.2	−0.616	−104.4	2.08	61.2
MHA-SAM-2	76.7	−0.430	−55.3	1.54	45.3
MHA-SAM-3	54.1	−0.266	−30.6	0.99	29.2

[a] All data are the average of three measurements with relative standard deviation (RSD) less than 10%. [b] Δf_1 is the frequency shift before and after dissociation of *n*-CD, ΔM_1 represents the mass loss for the corresponding *n*-CD calculated from Δf_1. [c] Δf_2 is the frequency shift for the relevant LD-SAM-*n* referring to bare Au. [d] Γ_2 is the surface concentration for LD-SAM-*n* deduced from Δf_2, Θ represents the surface coverage of LD-SAM-*n* calculated from $\Theta = \Gamma_2/\Gamma_0 \times 100\%$, where Γ_0 ($\sim$ 3.40 nmol cm^{-2}) is the surface concentration for HD-SAM.

† Electronic supplementary information (ESI) available: preparation and characterization of LD-MHA-SAM-*n* and the assembled protein films. See http://www.rsc.org/suppdata/cc/b4/b400776j/

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solution driven by positive potential (termed as (+) or *off* state). The two-way state could be switched reversibly as required by simply changing the applied potential, which could also be confirmed by cyclic scanning in buffer containing a redox probe.

Afterwards, selective protein adsorption was demonstrated on the thus-prepared switchable surface. Two kinds of fluorescent-labeled avidin were employed as the model charged proteins to demonstrate the controlled assembly process. One was avidin (A-821, Sigma), a highly cationic glycoprotein with an isoelectric point (IP) of ~ 10.5, the other was streptavidin (A-11230, Sigma), a nonglycosylated protein with a near-neutral IP (~ 6.3). The assembly of the two proteins on MHA-SAM-*n* was performed in PBS buffer (pH 7.4) at an applied potential of 0.3 V and −0.3 V (*vs.* SCE), respectively.[18] From either QCM or fluorescence spectra (FL) data, distinctly opposite assembly behaviors for the two proteins were observed at two controlled potentials. Figure 1 shows the relative FL emission intensity and mass change for the two assembled proteins at the state of (0), (+) and (−), respectively. For example, the emission intensity for avidin-LD-SAM-1 assembled at (−) for 30 min was about 4.9 times that for assembly at (+). Similarly, FL emission intensities for avidin-LD-MHA-SAM-2 and -3 assembled at (−) were about 5.0 and 4.6 times those for assembly at (+) for 30 min, respectively. These findings were basically consistent with the calculated protein loadings from measured QCM data as shown in the ESI.

Thus, the switchable surface showed a unique feature of controlled assembly for the positively charged avidin. While for streptavidin assembly on LD-MHA-SAM-*n*, smaller emission intensity was observed at (−) compared with those at (0) and (+), corresponding to less protein attachment. In contrast, at an applied potential of (+), about 1.7, 1.5 and 1.4 times the protein loadings were found than for those at (−) for streptavidin-LD-MHA-SAM-1, -2 and -3, respectively. So, the opposite assembly performances for avidin at the two states strongly suggests the occurrence of surface transition upon switching the applied potentials, and also a similar performance for streptavidins was observed. As a comparison, the protein assembly on a bare Au surface which resulted solely from the electrostatic adsorption or repulsion was only about 5–10% of that on LD-MHA-SAM-*n* when the same potential was applied, which suggests that it was the conformation of MHA-SAM-*n*, instead of the static electricity on bare Au,[19] which dominated the protein attachment. Furthermore, Table 2 shows that the applied potential has no effect on protein adsorption for HD-SAM, while for LD-SAM-1, significant mass loading was observed at (+), (0) or (−). So, we can conclude that it is the potential-controlled chain-bending, instead of the protonation for terminal carboxylic acid groups, which contributes to the selective protein adsorption. The dissimilarity of the surface loading for these two proteins is believed to be mainly due to the charge status difference originating from their IPs.

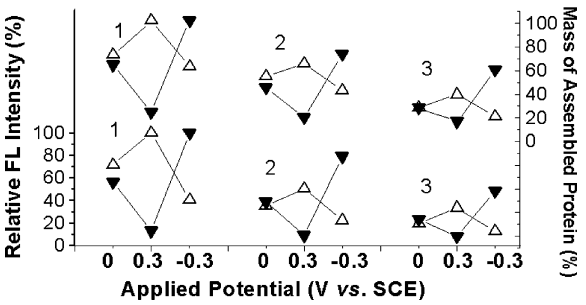

Fig. 1 Avidin (▼) and streptavidin (△)-derived LD-MHA-SAM measured by FL spectrometry and QCM. Inserted 1, 2 and 3 represent LD-MHA-SAM-1, -2 and -3, corresponding to the SAMs originating from α-, β- and γ-CD. All FL intensity and mass data were the mean values from three measurements and are normalized to the maximum values of each. 0 on the *x*-axis represents the surface state at open circuit.

Table 2 Comparison of protein adsorption for LD-SAM and HD-SAM[a,b]

Types and items		Avidin			Streptavidin		
		+	0	−	+	0	−
LD	Δf (Hz)	19.2	63.2	104.3	49.7	34.5	29.3
	Γ_I	122.2	402.2	664.3	316.3	219.5	186.4
HD	Δf_0 (Hz)	135.3	137.1	140.7	75.0	71.7	70.4
	Γ_{II}	861.4	872.7	895.8	477.3	456.2	448.1

[a] All data are the average of three measurements with the RSD less than 10%. Δf and Δf_0 are the frequency shifts after protein adsorption. Γ_I and Γ_{II} (ng cm^{-2}) are the corresponding surface concentrations of protein. [b] LD represents LD-SAM-1and HD represents HD-SAM.

In summary, we have demonstrated controlled protein assembly on a switchable surface. We believe that it might lead to versatile applications, *e.g.* controlling protein adsorption/release in a functionalized capillary or microfluidic channel, or design of intelligent protein chips.

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