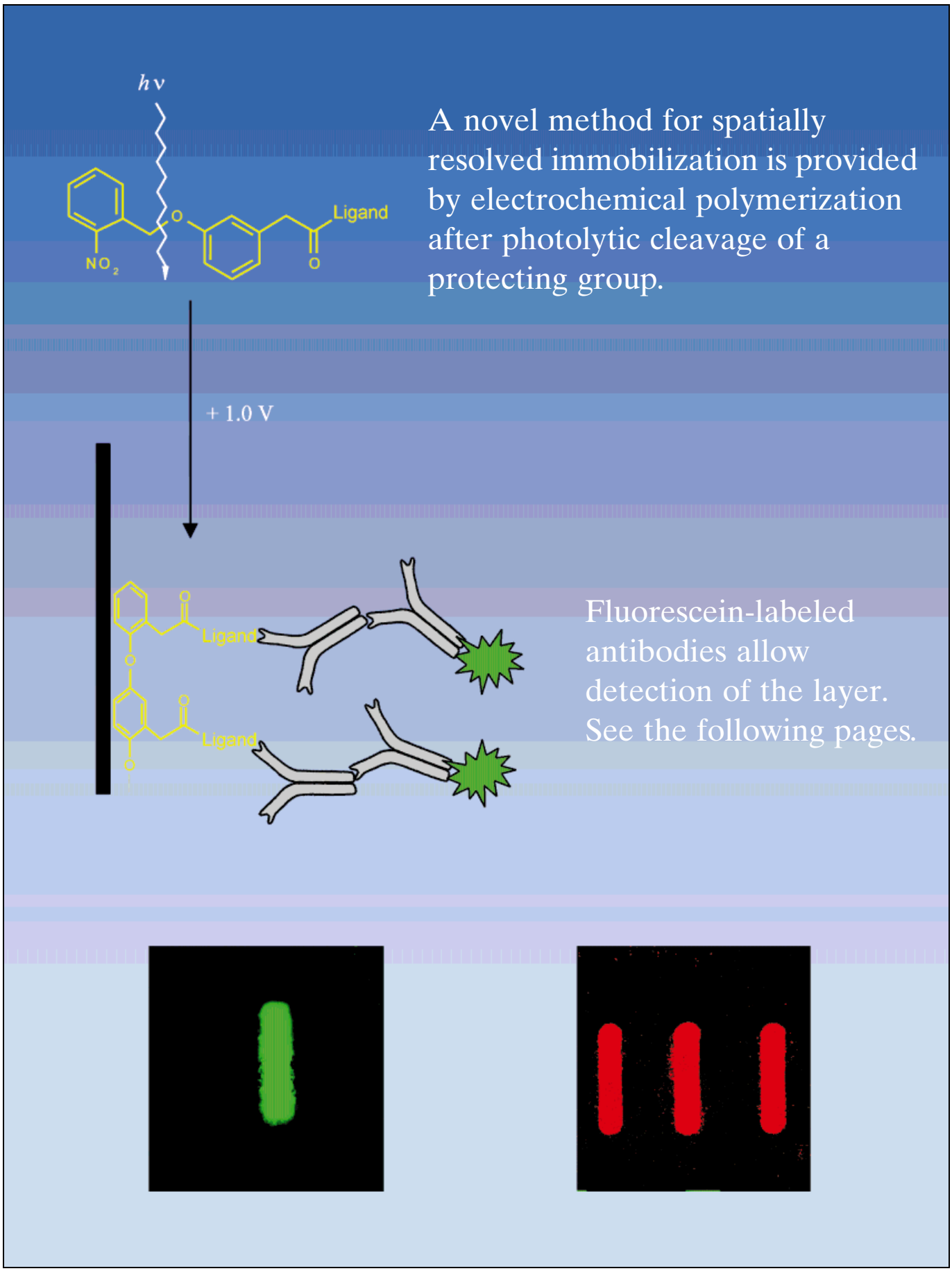

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Spatially Resolved Immobilization of Peptides by Electrochemical Polymerization after Photolytic Cleavage of a Protecting Group**

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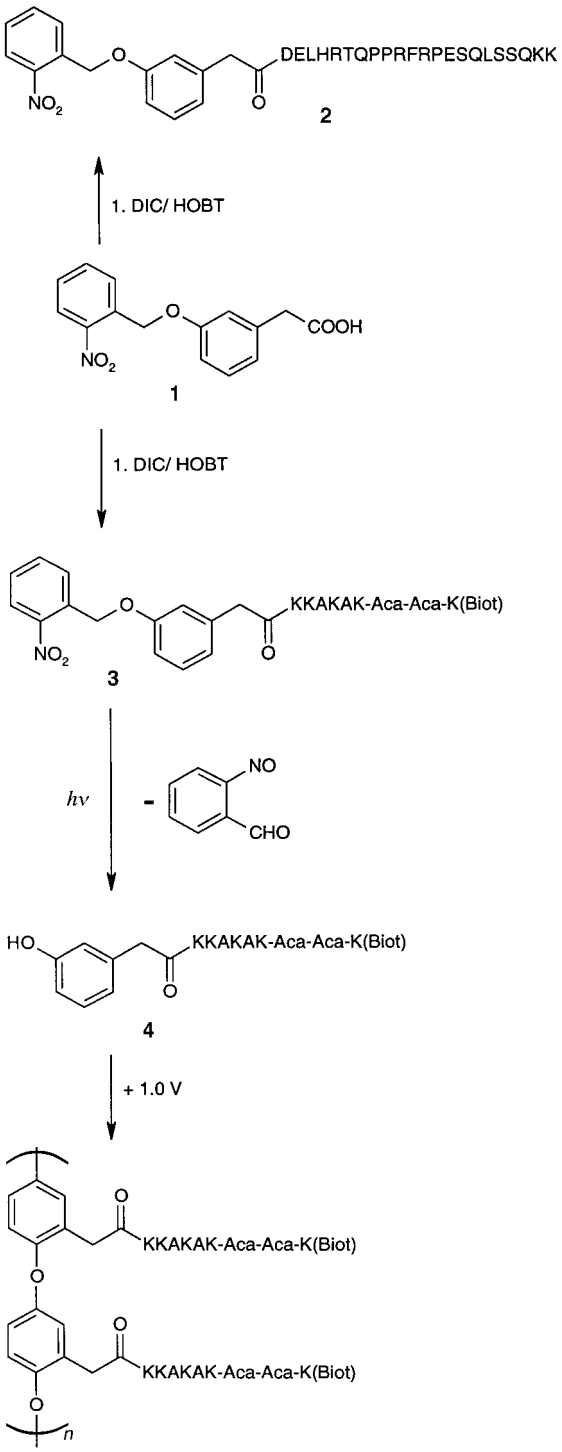
Biopolymer-modified surfaces are used in biosensoring, diagnostics, cell adhesion, and nanotechnology.[1, 2] Microstructured immobilization of macromolecules on solid supports is accomplished by photolithography of photoactive groups. For example, peptides and nucleotides have been synthesized with spatial resolution on a glass surface by the mask technique.[3] Proteins have been bound selectively to surfaces with photoactive benzophenones, diazarines,[4] and phenyl azide derivatives,[5] and photoactive groups have been immobilized on gold with self-assembled monolayers of alkanethiols.[6] Caged biotin, a biotin derivative that does not bind avidin, can be bound covalently to a substrate and induced photochemically to bind to avidin with the mask technique.[7] Morgan et al.[2] immobilized avidin on a gold substrate coated with thiol groups and incubated it with photobiotin (a nitroaryl azide derivative of biotin). The photobiotin was activated to bind covalently to antibodies by photolithography. The same principle underlies the direct binding of photobiotin to a carbon substrate.[1]

We report here a new and generally applicable method for the immobilization of antibodies, receptors, macromolecules, and compounds of low molecular weight on light-addressable sites of a conducting substrate. We have combined photolithographical techniques with the electropolymerization of a biopolymer on a conducting surface. In previous work we demonstrated that peptides with N-terminal 3-hydroxyphenylacetic acid (HPA) can be polymerized anodically through the phenol groups and then bound firmly to the surface.[8] Peptide functionalization of surfaces of glassy carbon electrodes (GCEs) and platinum electrodes was confirmed with fluorescein-labeled antibodies.[8] A particular advantage of this method is that further reagents are unnecessary, and the polymerization is dependent only upon the applied potential and the electrolytes.

The novel feature of the new method is that the phenolic hydroxyl group of HPA is protected from oxidative polymerization by temporary protection with the 2-nitrobenzyl (Nbz) group (Scheme 1), and polymerizable monomer is released only after irradiation. The Nbz group can be cleaved selectively by photolysis, is chemically inert, fulfills the conditions for solid-phase synthesis, and has been used to mask tyrosine.[9, 10] The peptide sequences were prepared by automated multiple synthesis on a solid phase and coupled at the N terminus with the photolabile protected 3-hydroxyphenylacetic acid (Nbz–HPA, **1**)[11] in the final step.



Scheme 1. Synthesis of the Nbz–HPA–peptides **2** and **3** as well as the photolytic deprotection of **3** to **4**, and its electrooxidative polymerization. Aca = 6-aminocaproic acid.

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An electrochemical flow cell was constructed to achieve spatially resolved electrochemical polymerization (Figure 1). Flushing with a solution of the Nbz-protected peptides **2** and **3** fills the chambers of a plastic mask, and thus diffusion of the deprotected monomer into neighboring regions is prevented.

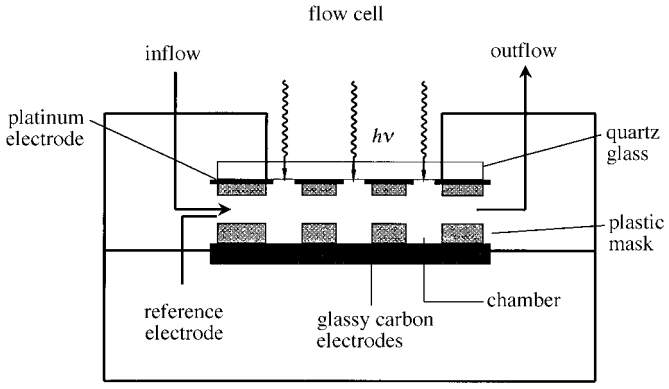

Figure 1. Flow cell for spatially resolved electropolymerization after photolytic cleavage of the Nbz protecting group. The individual chambers have a width of 250 μm.

Gaps in the platinum counterelectrode determine which chambers are irradiated. A potential of 1.0 V is applied to the GCEs. The process is illustrated in Figure 2: The Nbz–HPA–peptide **2** with a photolabile protecting group is situated between the two electrodes. Gaps in the platinum electrode allow the chambers to be irradiated individually to cleave the protecting group. If a constant potential of 1.0 V is applied, the deprotected HPA–peptide can be polymerized electrochemically at the GCE through the HPA groups.

The spatially resolved immobilization of **2** was visualized in the fluorescence microscope by sequential incubation with antipeptide antibodies and fluorescein-labeled secondary antibodies (Figure 3). In the case of biotinylated Nbz–HPA–peptide **3** spatially resolved coating was confirmed with

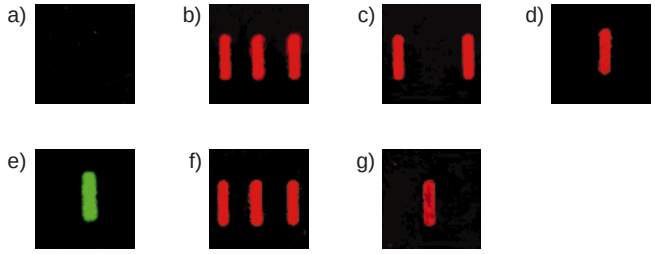

Figure 3. Spatially resolved immobilization of peptides on a GCE by electropolymerization. The chambers of the flow cell were filled with solutions of the Nbz–HPA–peptides **2** and **3**, a potential of 1.0 V was applied for 5 min, and the individual chambers (250 μm × 1200 μm) were irradiated with UV light (280 nm, 13 W cm^{-2}) for 1 min according to the desired pattern. The electropolymerized layer was detected under a fluorescence microscope by fluorescein-labeled antibodies or streptavidin. No immobilization on the electrodes is detected without irradiation (a). Photolytic cleavage of the Nbz protecting group and electropolymerization of peptide **4** with different patterns. Detection with rhodamine-labeled streptavidin (b, c, d) and with fluorescein-labeled streptavidin (e). Solution of **2** with irradiation of all three chambers or the middle chamber, detection with antibodies and rhodamine-labeled secondary antibodies (f, g).

fluorescein-labeled streptavidin. To confirm spatially resolved electropolymerization, the three chambers of the flow cell were filled with solutions of **2** or **3**, a potential of 1.0 V was applied for 5 min, and the respective chambers were irradiated with UV light for 1 min according to the desired pattern. The GCEs were treated with fluorescein-labeled streptavidin or antibodies, rinsed after 90 min, and dried. As a negative control, product **3** in phosphate-buffered saline (PBS) was filled into the flow cell, and a potential was applied (1.0 V, 5 min). To demonstrate that the Nbz-protected HPA–peptide **3** was not polymerized electrochemically, the nonirradiated chambers were incubated directly with fluorescein-labeled streptavidin. No coating was observed by fluorescence microscopy (Figure 3a). In contrast, spatial resolution with the HPA–peptide **4** was clearly visible in the regions where light had initiated the cleavage of the protecting group of the Nbz–HPA–peptide and electropolymerization had been facilitated. Product **2** was similarly bound to the GCE in a spatially resolved manner. In one instance all three chambers were irradiated (Figure 3f), in another the middle chamber only (Figure 3g).

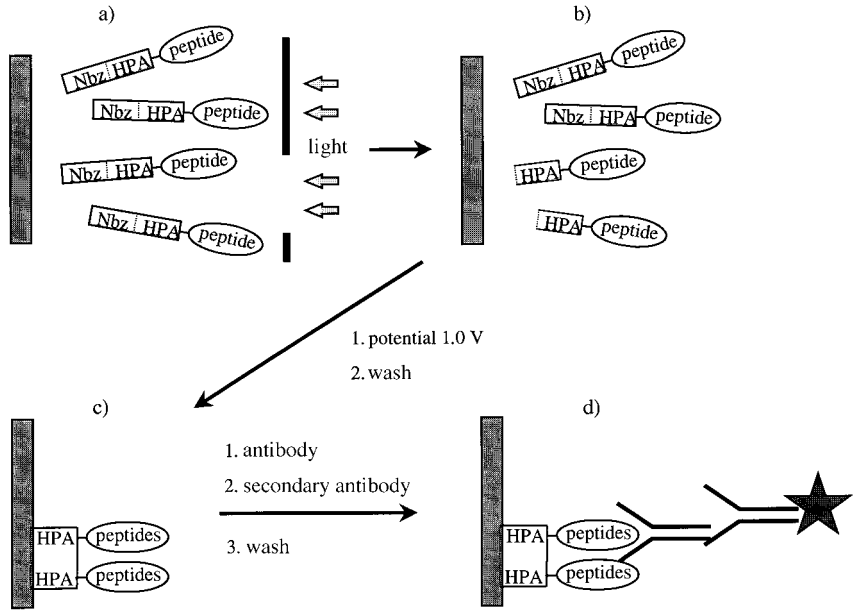

Figure 2. Spatially resolved immobilization of antibodies by electropolymerization. a, b) The photolabile Nbz group is specifically removed; c) application of a potential of 1.0 V leads to electropolymerization of the HPA–peptide; d) recognition of the immobilized peptides by means of antipeptide antibodies and fluorescein-labeled secondary antibodies.

We were thus able to apply locally addressed peptides to conducting substrates by electropolymerization following photolytic cleavage of the Nbz protecting group (Figure 3). The chambers with their current width of 250 μm will be miniaturized in further refinements of the method. Immobilization of macromolecules or

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chemoreceptors can lead to integration in routine analytical procedures. In addition, an application as a multianalytical sensor with a flow injection system is planned. When associated with highly diverse cyclopeptide libraries[12] that may also be immobilized electrochemically, the method offers particular possibilities in chemosensoring for rapid analysis by pattern recognition. The spatially resolved adhesion of nerve cells and the growth of axons are possible with peptides derived from laminin that may also be polymerized electrochemically with retention of their biological function.[13]

Experimental Section

2, 3: Multiple parallel peptide syntheses (each 15 μmol) on Wang resin with 9-fluorenylmethoxycarbonyl (Fmoc) amino acids and activation with *N,N'*-diisopropylcarbodiimide (DIC). Product **1**[11] (8.6 mg, 0,03 mmol) and 1-hydroxybenzotriazole (HOBT; 4.6 mg, 0.03 mmol) in DMF (200 μL) were coupled with 1.5 M DIC in DMF (20 μL, 0.03 mmol) for 4 h.

Cleavage from the resin: trifluoroacetic acid/water/triisopropylsilane (95/2.5/2.5; 500 μL; 3 h), Nbz–HPA–peptide precipitated with diethyl ether, centrifuged, dissolved in *tert*-butyl alcohol/water (4/1), and lyophilized. **2**: ESI-MS: $m/z = 1467.7$ $[M+2H]^{2+}$; **3**: ESI-MS: $m/z = 1523.6$ $[M^{+}+H]$.

The photochemical cleavage of **3** was investigated by UV spectroscopy and HPLC. At the start of the reaction an absorption maximum for the peptide is present at 214 nm, and for the protecting group at 275 nm. During photolysis a further maximum for the cleaved protecting group appears at 315 nm. Within 1 min 70 % of protected peptide **3** is cleaved, and after 5 min complete conversion into the free phenolic end group had occurred. These results indicate a stoichiometric formation of **4** (Scheme 1). A homogeneous product was also obtained with **2**; that is, the amino acids do not participate in detectable side reactions. However, Trp, Tyr, and His residues are partially oxidized during electropolymerization, but this does not have consequences for the practical use of peptide-functionalized surfaces, for example, for ELISA. To ensure that the Nbz–HPA–peptides **2** and **3** and the by-products released during photolysis cannot be oxidized, peptides **2**–**4** were measured by differential pulse voltammetry. This showed that electrochemical oxidation occurred with the photochemically deprotected HPA–peptide **4** at 0.6 V, whereas **2** and **3** were electrochemically inert at this potential.

After electrochemical polymerization by photolytic cleavage of the protecting group of the Nbz–HPA–peptide (1.0 V, 5 min), the GCE is rinsed with water. To block nonspecific binding sites 1 % BSA/PBS (BSA = bovine serum albumine) is applied (500 μL per electrode). After 2 h a solution of streptavidin (rhodamine- or fluorescein-labeled) in 0.1 mg mL^{-1} PBS is applied. After 2 h incubation the electrode is washed. The antibody-coated electrode is treated with a secondary antibody (rhodamine-labeled) in 0.1 mg mL^{-1} PBS and washed after 2 h.

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Solution of the Crystal and Molecular Structure of Complex Low-Symmetry Organic Compounds with Powder Diffraction Techniques: Fluorescein Diacetate**

Kenneth D. Knudsen,* Philip Pattison, Andrew N. Fitch, and Robert J. Cernik

Data from modern X-ray and neutron diffractometers and improvements in computational techniques have allowed increasingly complex crystal structures to be solved from powders. The use of synchrotron radiation has had a marked impact, and provides very narrow and accurately positioned diffraction peaks. The largest structures solved are of inorganic compounds, and sequential radiation with X-rays and neutrons was exploited to locate heavier and then lighter atoms. Example include $Ga_2(HPO_3)_3 \cdot 4H_2O$[1] and $La_3Ti_5Al_{15}O_{37}$[2] with 29 and 60 atoms, respectively, in the asymmetric unit. For organic compounds, the structures solved to date are simpler; for example, the structure of chlorothiazide[3] with 17 atoms was determined by direct methods. Various algorithms have also been developed to locate the positions of known molecular fragments within the unit cell.[4–9] Here we report on the structure solution of fluorescein diacetate ($C_{24}H_{16}O_7$). All 31 carbon and oxygen atoms were accurately located, without employing any prior knowledge of the molecular

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