Electrochemical switching of the flavoprotein dodecin at gold surfaces modified by flavin-DNA hybrid linkers

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Dodecin from *Halobacterium salinarum* is a dodecameric, hollow-spherical protein, which unspecifically adopts flavin molecules. Reduction of flavin dodecin holocomplexes induces dissociation into apododecin and free flavin. Unspecific binding and dissociation upon reduction were used as key properties to construct an electrochemically switchable surface, which was able to bind and release dodecin apoprotein depending on the applied potential. A flavin modified electrode surface (electrode-DNA-flavin) was generated by direct adsorption of double stranded DNA (ds-DNA) equipped with flavin and disulfide modifications at opposite ends. While the disulfide functionality enabled anchoring the ds-DNA at the gold surface, the flavin exposed at the surface served as the redox-active dodecin docking site. The structures of protein and flavin-DNA hybrid ligands were optimized and characterized by x-ray structural analysis of the holocomplexes. By surface plasmon resonance (SPR) spectroscopy, the adsorption of flavin modified DNA as well as the binding and the electrochemically induced release of dodecin apoprotein could be shown. When the surface immobilization protocol was changed from direct immobilization of the modified ds-DNA to a protocol, which included the hybridization of flavin and thiol modified DNA at the surface, the resulting monolayer was electrochemically inactive. A possible explanation for the strong influence of the surface immobilization protocol on addressing dodecin by the applied potential is that electron transfer is rather mediated by defects in the monolayer than modified ds-DNA. © *2008 American Vacuum Society.* [DOI: 10.1116/1.2965134]

I. INTRODUCTION

Dodecin from *Halobacterium salinarum* is a dodecameric, hollow-spherical flavoprotein, which contains six identical flavin binding pockets.[1,2] In each binding pocket, two flavins are bound as antiparallelly arranged dimers in an aromatic tetrade between two tryptophan residues.[3] The incorporation of isoalloxazine rings between the indol rings of tryptophans leads to efficient quenching of flavin fluorescence.[4,5] Besides native flavins and the flavinlike ligand lumichrome, dodecin binds also artificial flavins such as N(10)-hexylaminoflavin[6] (CofC6) with high affinities. This is possible due to a binding strategy inverse to conventional ligand binding of flavoproteins. As illustrated in Fig. 1, dodecin loads binding energy predominantly on the isoalloxazine substructure, keeping the aliphatic chain rather unrestricted.[7,8] The dodecin binding mode is reflected by dissociation constants (K_d's) for lumiflavin (17.6 nM), riboflavin (35.8 nM), and CofC6 (46.9 nM), which are almost independent of the N(10) substituent at the isoalloxazine moiety.

While dodecin binds ligands with high affinities in the oxidized state, reduction of the flavin ligand induces the dissociation of the holocomplex into apododecin and free flavin. This could be shown by size exclusion chromatography and spectroelectrochemical investigations. The redox cycle is chemically reversible without any aggregation or degradation of the (apo)protein.

These unusual properties were considered as an appropriate functional background for the development of an electrochemically active flavin modified electrode surface with dodecin bound or released depending on the applied redox potential. Starting with electrode surfaces of some mm^2, a further interest will be the miniaturization of this setup down to the single molecule level, as shown in Scheme 1.

Using the dodecin apoprotein as the mobile unit for the realization of this molecular switch, flavin modified molecular wires remained to be synthesized and linked to an electrode. Several materials such as carbon nanotubes,[9,10] organic π-electron systems,[11–13] or DNA (Refs. 14–22) could be suitable for an application as molecular wire. Equipped with a thiol or disulfide tether at the one and a flavin anchor group at the other end, these molecules bind to gold surfaces, exposing the flavin to the solution. In a first approach, DNA was chosen as potential molecular wire because it is cheap

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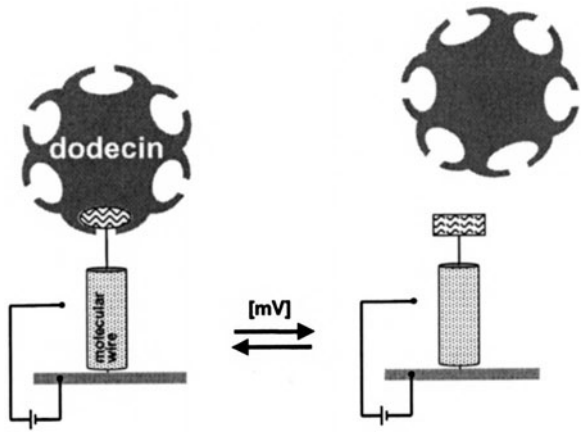


SCHEME 1. Concept of the electrochemical switching of protein states. Flavin-molecular wire hybrids are linked to electrode surfaces. Upon reduction, the steric and electronic constitution of the isoalloxazine submoiety is changed. This dramatically influences the stability of the protein ligand complex and allows one to electrochemically trigger a loaded and an unloaded state.

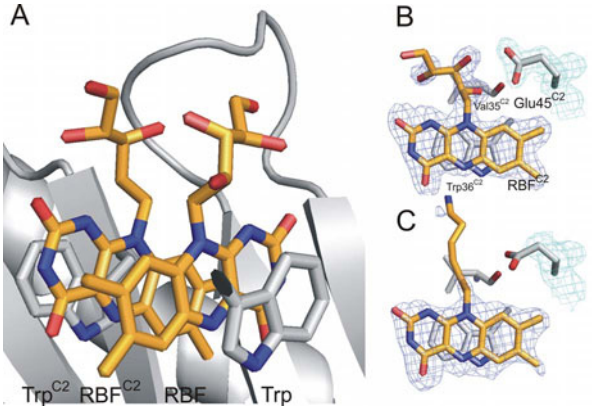


FIG. 1. Dodecin ligand binding. (A) Aromatic tetrade arrangement of an antiparallelly stacked riboflavin dimer (gold) between tryptophans of adjacent monomers. (B, C) Omitted-electron density is shown in blue for ligands (the non-mutated dodecin binding pocket is shown) and Glu45 with the aromatic tetrade reduced to the twofold-related (C2-related) part. Binding of riboflavin (B) in the dodecin binding pocket is mediated by stacking interactions to Trp36 as well as hydrogen bonding of the uracil substructure of isoalloxazine to Gln55 (not shown), and hydrogen bonding of the ribityl side chain to Val35 and Glu45. The synthetic ligand CofC6 (C) is solely bound via the isoalloxazine substructure (Trp36 and Gln55).

and easy to synthesize, and surface modification procedures are well established.[23,24] The intrinsic conductivity of long bare DNA is too low to allow its utilization as molecular wire.[25–28] On the other hand, there are impressive reports about photoinduced electron transfer (ET) through DNA over short distances.[29–31] Furthermore, different groups have independently shown that ET through monolayers of short double stranded DNA (ds-DNA) adsorbed on gold is possible.[14–22] Here, a first approach using ds-DNA of 20 base pairs as potential molecular wire system is presented.

II. RESULTS

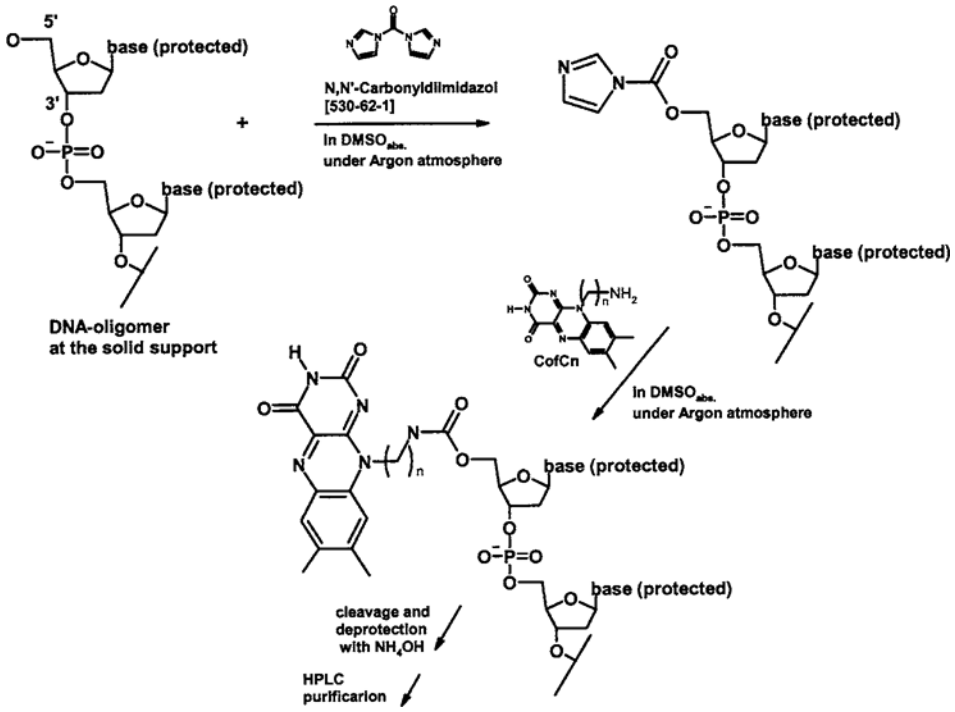
A. Preparation of flavin-DNA-disulfide ligands and protein engineering

N(10)-alkylamino-substituted flavins, synthesized as described previously,[6] were attached to the 5′ end of single stranded DNA (ss-DNA) by a coupling reaction with carbonyl diimidazole.[32] The individual reaction steps of the coupling of the flavins to DNA are depicted in Scheme 2, and further experimental details are presented in the supporting information. Two series of flavin modified ss-DNA oligomers were synthesized. A series of 20-mer flavin-ssDNA oligomers termed CofCn-O20, with a base sequence allowing strong and definite hybridization at room temperature,[33] was prepared for electrode modification, whereas a shorter series of 5-mer flavin-ssDNA oligomers termed CofCn-O5 was required for x-ray structural analysis. The length n of the alkyl chain between DNA and the redox-active isoalloxazine subunit was varied. The complementary ss-DNA strand modified at the 5′ end by a $HO(CH_2)_6S-S(CH_2)_6$ tether for adsorption on Au termed RS-O20 was purchased from *metabion* (Martinsried, Germany).[34–38] The structures of CofCn-O5, CofCn-O20, and RS-O20 are depicted in Fig. 2.

Assuming that a DNA double strand of 20 base pairs exhibits sufficient conductivity, two bottlenecks for ET between gold electrode and flavin remained: (1) the saturated bonds between electrode and DNA and (2) the alkyl chain linking the redox-active isoalloxazine to DNA. A mercaptohexyl tether was employed since tethers of similar length[16,17,19,39] (or even much longer)[17] had been used by other groups when efficient ET through DNA was reported. For a flexible mercaptohexyl tether, the maximum direct distance between the first DNA base pair and the electrode surface is slightly below 13 Å.

In order to improve the ET probability at the second bottleneck, the linker between DNA and isoalloxazine was shortened to a minimum length, which still allowed efficient binding. For this purpose, also the structure of the protein binding site was optimized by two mutations, while the dodecin binding functionality was conserved. First, Glu45 was mutated to alanine to sterically release the isoalloxazine submoiety for more stable aromatic tetrade arrangements. Second, Glu50 and Glu51 were deleted to increase accessibility of the binding pocket and decrease the depth of the entrance channel. Figure 3 depicts a view at one flavin binding site of wild-type dodecin and the new dodecin variant (tE). Protein engineering was guided by x-ray crystallography using the less space consuming CofCn-O5 model compounds. Figure 4 depicts the dodecin variant tE with CofC4-O5 and CofC2-O5 ligands. Within the series of flavin modified oligomers, the ethyl chain of CofC2 was regarded as the shortest linker for high affinity binding to the tE variant.

In x-ray structural analysis, additional binding interactions between CofC2-O5 and tE-mutated dodecin not involving the isoalloxazine moiety were found. As these interactions were expected to stabilize the protein/ligand complex under reductive conditions, CofC4-O20 was used for surface coatings.



SCHEME 2. Covalent linkage of artificial flavins to DNA using carbonyl di-imidazole. The two-step coupling procedure was carried out when the DNA was still at the solid support. After cleavage and deprotection the flavin modified oligomers were purified by HPLC and analyzed by mass spectroscopy.

III. COMBINED ELECTROCHEMICAL AND SURFACE PLASMON RESONANCE STUDIES

A. Direct adsorption procedure

After optimization of the protein structure and the flavin modified oligomers, dodecin was reconstituted at gold surfaces, and electrochemical investigations were performed. Each experimental step was monitored by surface plasmon resonance (SPR) spectroscopy. The gold coated slide (serving as working electrode) was mounted in a flow-through SPR cell equipped with a gold coated counter electrode and a Pt pseudoreference electrode. The $HO(CH_2)_6S-S(CH_2)_6$ tether was applied in its disulfide form without previous conversion into the free thiol.[14,18,34] Following the work of Barton and co-workers,[16,17] CofC4-O20 and RS-O20 were first hybridized in solution and thereafter adsorbed on gold. This straightforward approach, which will be termed *direct adsorption* procedure, resulted in the additional adsorption of the protecting group *R*. The SPR kinetics, i.e., the changes in reflectivity at a fixed angle (within the linear scan range) during the individual steps, is depicted in Fig. 5. After each experimental step the reflectivity was measured at the complete angular range and adjusted to an angle of 30°. In the first step, the flavin and disulfide modified ds-DNA hybrid, termed CofC4-ds20-SR, was allowed to bind at the gold surface during an incubation period of 2 h [Fig. 5(a)]. After rinsing with buffer solution, a nonbinding dodecin variant (termed dA), generated by mutation of Trp36 and Gln55 to

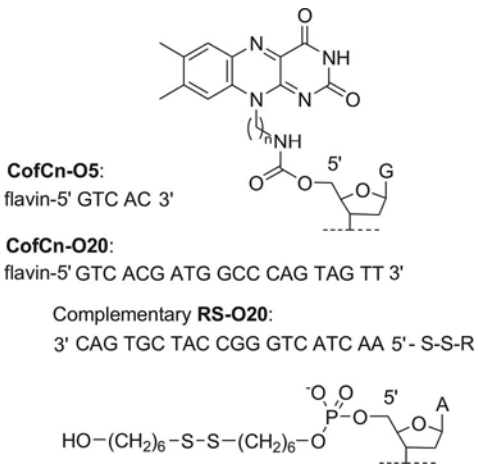


FIG. 2. Structure of the flavin and disulfide modified oligomers CofCn-O5, CofCn-O20, and RS-O20. The structures of the 5′ flavin and the 5′ disulfide modification are shown in detail.

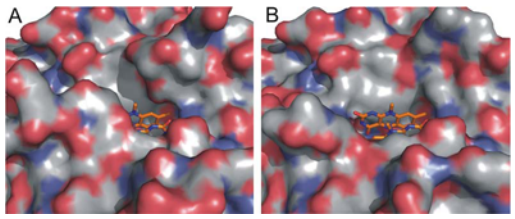


FIG. 3. Access to the dodecin binding pocket. Dodecin apoprotein is shown in surface presentation with the surface colored according to atom type (oxygen red and nitrogen blue). Coordinated waters were omitted for surface calculations. For clarity, just the isoalloxazine anchors (lumiflavin moiety) of ligands are shown. When comparing binding pockets of (a) wild-type and (b) tE dodecin, the increased accessibility of the binding site in (b) is clearly visible.

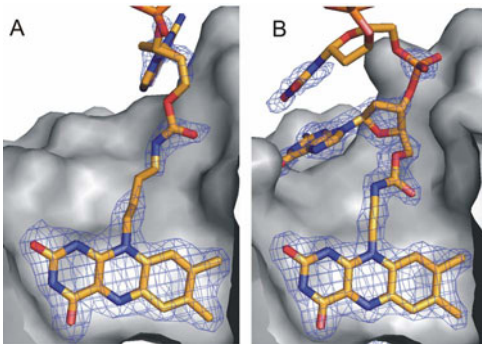


FIG. 4. Complex of apododecin with (a) CofC4-O5 and (b) CofC2-O5 ligands. CofCn-O5 ligands were used as model compounds and soaked into apododecin crystals. Omitted density of ligands is shown in blue. Dodecin is depicted in surface presentation with the binding pocket reduced to the C2-related part. For data collection and refinement statistics of dodecin holocomplexes with CofC4-O5 and CofC2-O5 ligands, see supporting information.

alanine, was added as negative control [Fig. 5(b)]. Subsequently, after rinsing again with buffer, a solution of the binding dodecin variant tE was added [Fig. 5(c)]. While the surface layer thickness did not change during incubation with the dA variant, incubation with the tE-mutated dodecin led to an increase of thickness, implying specific binding of tE dodecin to the CofC4-ds20-S-Au layer. During incubation of the dodecin variants tE and dA, lumichrome was added as a binding mediator (saturating the second position in the binding pocket) in a protein/lumichrome molar ratio of 2:1. Kinetics of protein immobilization was fitted to a Langmuir isotherm. The good agreement of experimental data and fit suggests specific binding and formation of monolayers. At extended incubation times, the kinetics of tE dodecin binding shows some deviation from the ideal behavior, which might be explained by beginning multilayer formation (see supplementary information, Fig. S3). After changing to oxygen-free conditions, a negative potential was applied and subsequently decreased down to a value of −600 mV [versus the Pt pseudoreference electrode, see Fig. 5(d)]. This resulted in the reduction of the flavin moieties followed by the release of the apoprotein.

Between the individual steps of the experiment, the angular scan curves shown in Fig. 6 were collected. According to the Fresnel algorithm, the increase in optical thickness could be translated into a physical thickness. The average thickness of the CofC4-ds20-S-Au layer at the surface was about 3.3 nm. The absorption of the dodecin variant tE resulted in an increase of the layer thickness by 4.3 nm. This value implies that the surface was not covered completely, as a densely packed protein monolayer should result in an average thickness of almost 7 nm, which is the diameter of dodecin.

The CofC4-ds20-S-Au monolayer was analyzed electrochemically on ordinary gold electrodes under oxygen-free conditions. For surface modification, these electrodes were exposed to a solution of CofC4-ds20-SR for a longer period (>24 h). The corresponding flavin reduction wave collected by cyclic voltammetry and the flavin reduction peak detected by square wave voltammetry are depicted in Fig. 7.

The experimental results presented so far show the development of a CofC4-ds20-S-Au modified electrode surface,

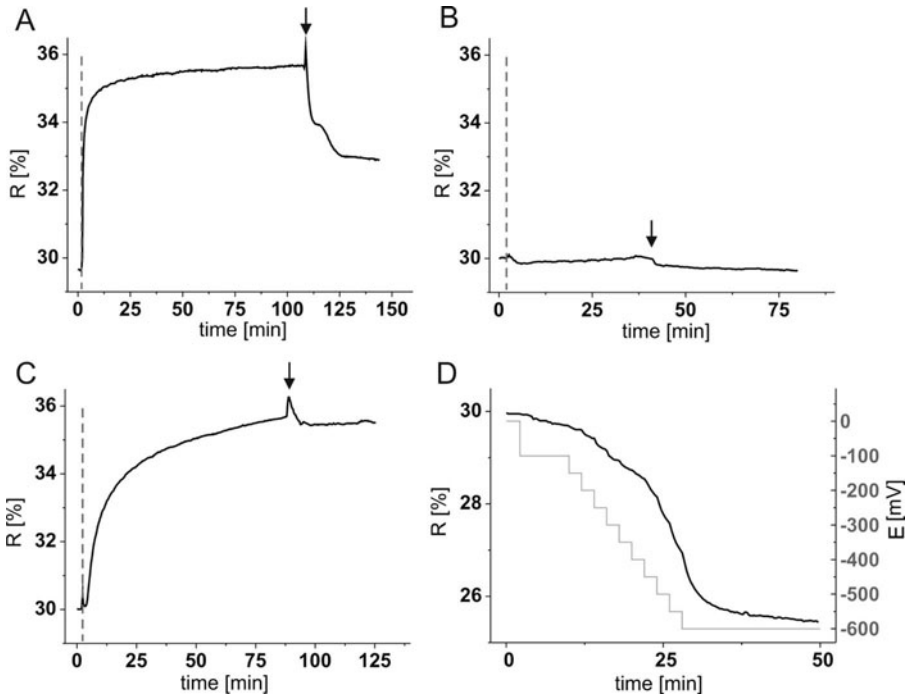


FIG. 5. SPR Kinetics of/after *direct adsorption* of ds-DNA. Change in reflectivity is shown at a fixed angle during (a) adsorption of CofC4-ds20-SR (100 μM), (b) addition of the nonbinding variant dA as negative control (20 μM), (c) addition of the high affinity binding variant tE (20 μM), and (d) electrochemical reduction followed by the release of tE. Dashed lines highlight the start of incubations with CofC4-ds20-SR, dA, and tE. Arrows denote rinsing with protein-free buffers.

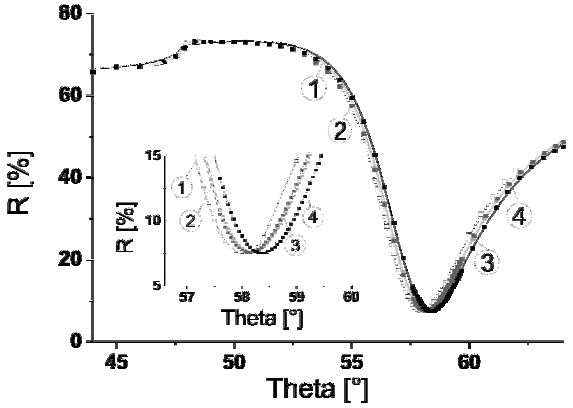


FIG. 6. Angular scan curves of/after *direct adsorption* of ds-DNA. Angular scan curves of the reflectivity are shown, detected between steps of the experiment presented in Fig. 5. The different angular scan curves are labeled as follows: (1) bare gold surface, (2) CofC4-ds20-S-Au monolayer, (3) CofC4-ds20-S-Au monolayer after rinsing with the dA variant, and (4) CofC4-ds20-S-Au monolayer after adsorption of the tE variant. All steps were performed in PBS buffer adjusted to 1*M* NaCl. The inset shows a zoom-in to the angles between 56.5° and 60°.

which was able to bind and release dodecin apoprotein triggered by the redox potential. During further experiments, it turned out that the stability of the CofC4-ds20-S-Au layer was limited. It was possible to bind and release dodecin apoprotein up to three times, i.e., the surface showed symptoms of fatigue (see supporting information, Fig. S1). One reason for this observation could be that protein and/or DNA was of limited electrochemical stability. However, when chemically reducing the tE/CofC4_O5/lumichrome complex in solution by sodium dithionite and monitoring the reductive disassembly into ligands and protein by size exclusion chromatography, no instability or degradation of the components could be recorded.

B. Tarlov procedure

Another reason for the limited lifetime of the electrochemically switchable CofC4-ds20-S-Au monolayer might be the presence of defects. Defects in the monolayer might be formed already during the adsorption of CofC4-ds20-SR. This conclusion is supported by the observation that the CofC4-ds20-SR modified surface was not entirely covered by the apoprotein. It has been pointed out in literature that, besides ss-DNA, also ds-DNA shows a tendency to adsorb nonspecifically on gold.[40] Furthermore, CofC4-ds20-SR may be adsorbed via the isoalloxazine subunit at the gold surface as it was observed for other flavin derivatives.[6,41] Therefore, an alternative surface modification protocol termed *Tarlov* procedure[23,24] was performed, which should reduce defects in the CofC4-ds20-S-Au layer. Accordingly, in the first step, the thiol modified ss-DNA was adsorbed on gold. This was done with RS-O20 and alternatively with the free thiol derivative HS-O20, which was obtained after deprotection with tris(2-carboxyethyl) phosphine according to Ref. 42. Besides the observation that HS-O20 showed faster adsorption kinetics, both layers behaved similar in the following steps. In the second step, a solution of mercaptohexanol was added to release nonspecifically adsorbed ss-DNA and to orient the specifically adsorbed DNA toward the solution in order to enhance the hybridization efficiency. The CofC4-ds20-S-Au monolayer was finally assembled by adding CofC4-O20 in the third step. A consequence of the Tarlov procedure is that the isoalloxazine subunit was never in direct contact with the bare gold surface. The hybridization of DNA at the solid phase is a more complex process than in solution.[36,43] However, the base sequence was chosen to allow hybridization at room temperature only when complete overlap of the complementary ss-DNA strands is guaranteed. Efficient hybridization could be recorded after addition of CofC4-O20 to the complementary Au-S-O20 monolayer. The SPR kinetics during this step and the subsequent reconstitution of dodecin are depicted in Fig. 8 (for the angular scan curves see supporting information, Fig. S2). While no binding could be observed after addition of the dodecin variant dA (negative control), the tE-mutated dodecin bound to the CofC4-ds20-S-Au monolayer. In a characteristic experiment, immobilization of HS-O20 and subsequent treatment with mercaptohexanol increased the surface layer thickness by 3.0 nm. The CofC4-ds20-S-Au monolayer could be fitted to 3.9 nm in thickness after addition of CofC4-O20. Specific binding of

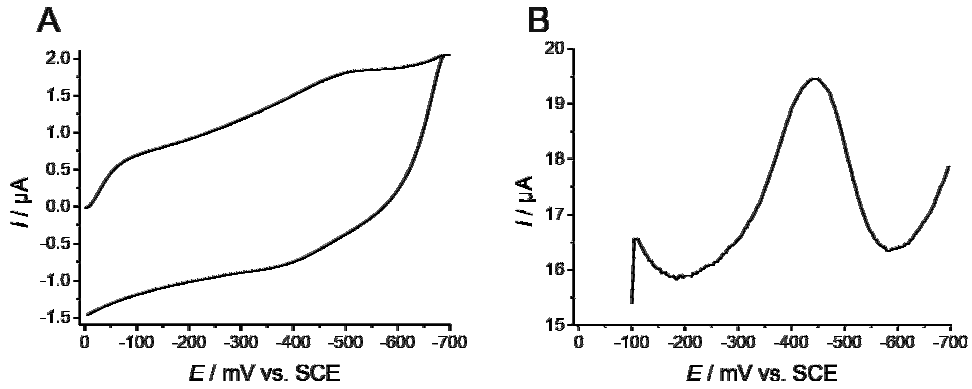


FIG. 7. Flavin reduction at ordinary gold electrodes. The flavin reduction could be detected at ordinary gold electrodes after adsorption of CofC4-ds20-SR (experimental conditions: phosphate buffer 0.1*M* at *p*H 7; NaCl: 0.1*M*). (a) Cyclic voltammogram, scan rate: 500 mV s^{-1} and (b) square wave voltammogram: frequency, 500 Hz; pulse height, 30 mV; step, 4 mV.

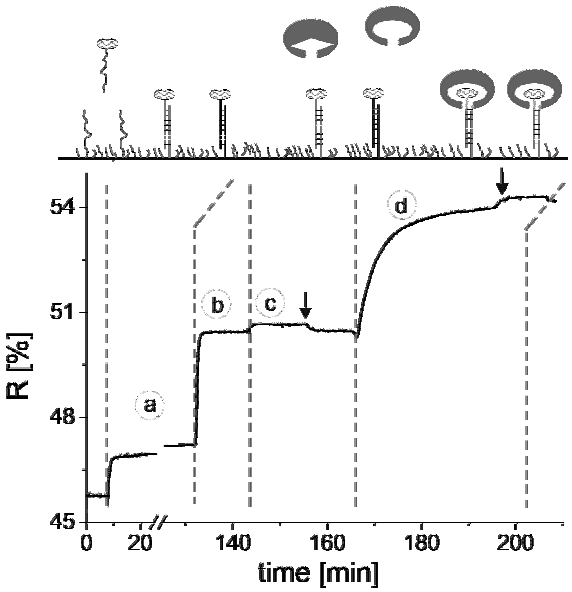


FIG. 8. SPR kinetics of/after surface immobilization according to Tarlov. The characteristic kinetic SPR curve was collected in the linear range of the corresponding surface plasmon scan. HS-O20 at 8 μM [100 mM tris-HCl (*p*H 7.5)] was adsorbed, and a solution of mercaptohexanol (1 mM) was added (data not shown). (a) CofC4-O20 (5 μM) was hybridized at the surface [1*M* NaCl, 10 mM Tris-HCl (*p*H 7.5), 1 mM EDTA]. (b) The CofC4-ds20-S-Au layer was equilibrated to PBS at 1*M* NaCl. (c) Injection of the nonbinding variant dA (20 μM). (d) Injection of the binding variant tE (20 μM). In the protein immobilization steps [(c) and (d)], lumichrome was added in a 0.5 molar ratio to the proteins to ensure the saturation of the second position in the dodecin binding pocket. Dashed lines highlight change of conditions according to the scheme attached; arrows denote rinsing with protein-free buffers. In the next steps negative potentials have been applied (not shown).

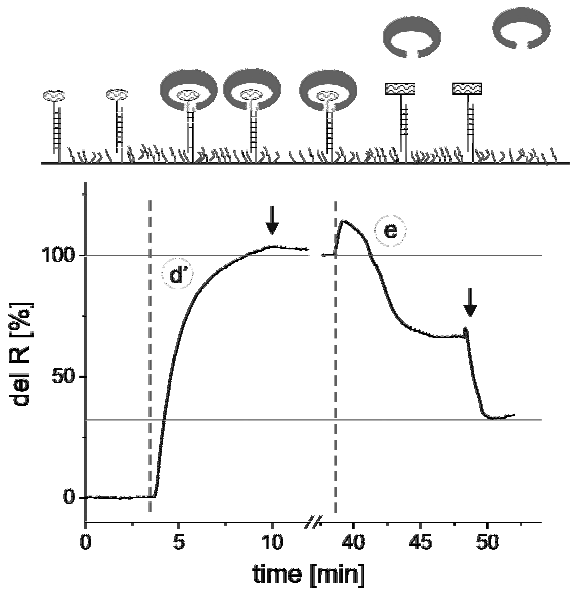


FIG. 9. Change in refractive index during binding of dodecin variant tE and subsequent release by chemical reduction. (*d'*) Injection of dodecin analogously to (d) in Fig. 8. (e) At concentrations of 10 mM sodium dithionite (at *p*H 8), apododecin was released from the gold surface, as illustrated by the decrease in reflectivity. Dashed lines highlight change of conditions according to the scheme attached. First arrow: rinsing with protein-free buffer and equilibration to oxygen-free conditions. Second arrow: re-equilibration to nonreductive conditions.

the tE-mutated dodecin finally added 6.3 nm to the surface, which suggests the formation of a tightly packed monolayer of dodecin particles on the CofC4-ds20-S-Au layer. Kinetics of protein immobilization was fitted by a Langmuir isotherm. Binding of the tE-mutated dodecin, shown in Fig. 8, yielded a k_{on} value of 2.48×10^3 M^{-1} s^{-1} (see also supporting information, Fig. S3). As a decrease in reflectivity upon rinsing the system with tE-free buffer could not be observed, k_{off} was assumed to be very low (details of the SPR analysis are presented in the supporting information).

The dodecin binding surface was equilibrated to oxygen-free conditions, and a negative potential was applied in series of 5 min: a potential of −550 mV was applied in the first 5 min. After going back to zero potential, no release of the apoprotein could be detected. In the next step, the negative potential was decreased to −600 mV. This procedure was continued down to very negative potentials resulting in destruction of the thiolate layer (data not shown). When the Tarlov procedure was performed, the release of the apoprotein by electrochemical reduction was not possible. In order to exclude any protein denaturation which could be induced by tight packing of the tE layer, in an alternative experiment, after dodecin reconstitution, the surface was equilibrated to reductive conditions by addition of sodium dithionite (10 mM). As illustrated in Fig. 9, this resulted in the release of about 70% of the tE apoprotein previously adsorbed, indicating that the release of the immobilized apododecin by reduction was still possible.

Similarly as it was done after direct adsorption of CofC4-ds20-SR, cyclic voltammetry and square wave voltammetry measurements were carried out on ordinary gold electrodes, modified by the Tarlov procedure. In contrast to the direct adsorption procedure, this time the flavin reduction could not be detected. In the absence of the apoprotein, the flavin reduction should have been facilitated (during both procedures) since the isoalloxazine moiety was able to approach the DNA bases, i.e., for ET only the bottleneck between electrode and DNA remained. According to the presented experimental results, the direct adsorption procedure led to an electrochemically switchable CofC4-ds20-S-Au monolayer, which was able to bind and release dodecin apoprotein, whereas the *Tarlov* procedure, which was expected to result in a more ordered monolayer, led to an electrochemically inactive surface.

IV. DISCUSSION

An electrochemically addressable flavin modified electrode surface was developed, which binds and releases dodecin apoprotein depending on the applied redox potential. This was only possible when the CofC4-ds20-S-Au monolayer was formed by direct adsorption, whereas the Tarlov procedure resulted in an electrochemically inactive surface. In both procedures a mercaptohexyl tether was used for adsorption on gold. The length of this tether was within the length scale of tethers, which have been successfully applied

when ET through DNA monolayers was observed.[17] A sequence of 20 bases, which allowed strong and definite hybridization at room temperature, was chosen. A flavin redox center was covalently linked to the ss-DNA when it was still at the solid support, and the amino groups of the bases, which are sensitive against many coupling reagents, were protected. This synthetic procedure was expected to be more controlled than, e.g., the attachment of redox centers after hybridization or adsorption (the hybridized DNA is in equilibrium with its single stranded form, in which additional reactive groups are accessible). It is difficult to compare the results of this work directly with those of other groups who have reported about ET through ds-DNA monolayers on gold[14–22] because important parameters can be varied. (i) The employed thiol tether can vary in length and composition and can be attached to the 3′ or 5′ end of DNA. (ii) The DNA sequence can differ in length and composition, which may also affect the ET and adsorption properties.[31,38,44–47] (iii) The nature of the redox center, its redox potential, and its mode of attachment vary. For instance, one or two electrons can be transferred, and additional protonation steps can be involved in ET. Covalent attachment of redox centers is possible at the end of one of the DNA strands (either at the same strand bearing the thiol modification or the opposite)[18] or as intercalator within the ds-DNA when daunomycin is used.[16,48,49] In contrast to covalently attached redox centers, intercalators have been used quite frequently.[19,20] However, it has been shown that often the majority of the intercalator molecules are adsorbed nonspecifically.[19] Even though the electrochemical response of the specifically adsorbed intercalator molecules may depend on their environment, it must be questioned whether the ET proceeds through DNA or ET is mediated, e.g., by nonspecifically adsorbed intercalators.[40] (iv) ET through DNA monolayers, labeled with covalently attached redox centers, was often studied with the ds-DNA adsorbed directly after hybridization.[15–18] In some cases the adsorption was carried out in the presence of mercaptoalcohol (or disulfide) or a separate mercaptoalcohol incubation step was added.[14,39] The more complicated Tarlov procedure was not applied when ET through DNA monolayers of DNA equipped with covalently linked redox centers has been investigated.[14–18,39]

Assuming that ET proceeds through the base pair stack of DNA[39] and the Tarlov procedure results in a more ordered CofC4-ds20-S-Au layer (as indicated by the strong hybridization event and the more densely packed protein monolayer), ET should be more efficient when the Tarlov procedure is applied. This is, however, in contrast to the experimental results and implies alternative ET pathways. As a result of electrochemical studies on a ds-DNA monolayer of 20 base pairs equipped with a ferrocenyl head group, it has been suggested that ET occurs directly between the ferrocenyl head and the electrode without any contribution of the DNA base stack.[44,50] ET is only possible due to the elastic bending motion of DNA, and an elastic bending diffusion model has been developed.[50] This model is not in contradiction to the observation that single mismatches in the ds-DNA may affect the ET characteristics since the bending elasticity of ds-DNA should be highly sensitive to the base sequence, mismatch, or improper pairing.[50] The elastic bending diffusion model could help to understand why in this work ET was not observed when the Tarlov procedure was applied. In line with this model, the following explanation is suggested: after the formation of a dense and highly ordered monolayer, it should not be possible for the flavin redox centers to approach the electrode surface close enough for ET. This effect is even stronger when the flavin redox centers are captured inside the protein. The flavin redox centers are also captured inside the protein when direct adsorption of CofC4-ds20-SR was performed, but in this case the protein layer is less dense, i.e., the proteins are more flexible and able to approach the electrode surface, at least to some extent. At the same time, a significant number of nonspecifically adsorbed CofC4-ds20-SR molecules are present. The isoalloxazine subunits are adsorbed either directly at the surface or located somewhere between the electrode surface and the reconstituted proteins in a flexible manner. This assembly allows the ET to proceed by hopping between the electrode surface, flexible isoalloxazine redox centers, and the flavins inside the dodecin binding pocket.

V. CONCLUSION

An electrochemically switchable flavin modified electrode surface was developed, which binds and releases dodecin apoprotein depending on the applied redox potential. DNA was evaluated as a possible molecular wirelike linker between the electrode and flavin. The electrochemical addressability of the flavins was only obtained by direct adsorption of CofC4-ds20-SR, whereas the Tarlov procedure resulted in an electrochemically inactive surface. Nevertheless, the experimental results, especially the observation of a strong hybridization event and the selective binding of the dodecin variant tE, indicate that the Tarlov procedure was very efficient. The increase in protein reconstitution by a factor of almost 1.5 by applying the Tarlov procedure implies that this procedure resulted in a more ordered monolayer with a lower amount of defects. A possible conclusion is that ET through the DNA monolayer is catalyzed by defects in the layer, and the DNA based molecular junction between flavin and the electrode is not conductive. The insufficient ET characteristic does not necessarily have to be caused by DNA since a serious drop off in conductivity is expected at the saturated linkers between electrode-DNA and DNA-isoalloxazine. In future experiments it might be worth decreasing the length of the tether between thiol and DNA or attaching the thiol group directly at the DNA base.[51] For the development of a molecular switch, which allows the binding and release of single dodecin apoprotein molecules, other systems than DNA will be examined as molecular wires.[9,13,52] The results obtained in this work indicate that different surface modification procedures should be compared when ET through DNA monolayers is investigated.

Supporting information available.[53] The experimental part (organic synthesis, further experimental details, and a de-

scription of the experimental setup) and additional SPR data are presented.

Accession numbers. The atomic coordinates and structure factors have been deposited in the Protein Data Base (PDB) with ID codes 2vkf (holocomplex with ligand CofC2-O5) and 2vkg (holocomplex with ligand CofC4-O5).

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[53]See EPAPS Document No. E-BJIOBN-3-003803 for further experimental details and a description of the experimental setup. This document can be reached through a direct link in the online article's HTML reference section or via the EPAPS homepage (http://www.aip.org/pubservs/epaps.html).