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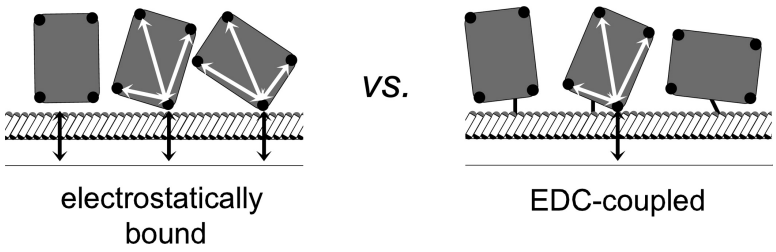

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Electron Conduction across Electrode-Immobilized Neutravidin Bound with Biotin-Labeled Ruthenium Pentaamine

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Since the seminal work of Hill et al.[1] and Kuwana et al.,[2] numerous studies of electrode-immobilized proteins have yielded significant results impacting science and technology.[3−13] Here we report electrochemical properties of neutravidin, a nonelectroactive tetramer containing four opposed biotin binding sites, conjugated with an electroactive biotin derivative (Figure 1). This conjugate, referred to as Neu(Ru)$_4$, was immobilized covalently and noncovalently onto gold electrodes modified by a self-assembled monolayer of 11-mercaptoundecanoic acid (MUA). Cyclic voltammetry indicates noncovalent immobilization favors electrostatic binding of the positively charged Ru(NH$_3$)$_5$ groups to the negatively charged monolayer surface.[15,16] The key result described here is that this electrostatic binding regulates electron-transfer across the immobilized protein in which electrostatically bound Ru(NH$_3$)$_5$ groups mediate heterogeneous electron-transfer to nonelectrostatically bound Ru(NH$_3$)$_5$ groups that are otherwise kinetically inaccessible. Furthermore, negligible intraconjugate electron-transfer occurs (i.e., each conjugate is electrically isolated from its neighbors).

Experimental details are provided in Supporting Information. Briefly, Ru(NH$_3$)$_5$(*N*-[(*N*-[(4-pyridyl)methyl]biotinamide] (Ru(NH$_3$)$_5$biotin) was prepared by reaction of Ru(NH$_3$)$_5$(4 (aminomethyl)pyridine)](PF$_6$)$_2$ with (+)-biotin-NHS ester. Neu(Ru)$_4$ was isolated by size-exclusion chromatography from a solution of neutravidin to which 5:1 mole ratio of Ru(NH$_3$)$_5$biotin was added. For comparison, a second conjugate, referred to here as Neu(Ru)$_1$, was isolated from a solution of neutravidin to which 1:1 mole ratio of Ru(NH$_3$)$_5$biotin was added. Due to the high affinity of neutravidin for biotin derivatives, it is expected that each neutravidin of Neu(Ru)$_4$ is conjugated with four Ru(NH$_3$)$_5$ groups,[17] whereas Neu(Ru)$_1$ represents a mixture in which each neutravidin is conjugated with zero, one, two, three, or four Ru(NH$_3$)$_5$ groups.[17,18] Conjugates were noncovalently immobilized by soaking MUA-modified gold electrodes (7 cm long × 0.05 cm diameter)[15] in conjugate solution. Conjugates were covalently immobilized by EDC-activated amide bond formation (EDC: 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride) between monolayer carboxylic acid groups and neutravidin lysines[18] accomplished by short exposure of MUA-modified gold electrodes in EDC solution prior to soaking in conjugate solution.[19,20]

Figure 2 illustrates typical cyclic voltammetry of the two protein conjugates immobilized on identical MUA-modified gold wire electrodes in low ionic strength electrolyte. In Figure 2, plots a and c correspond to noncovalently and covalently immobilized Neu(Ru)$_4$, respectively. Plot b corresponds to noncovalently immobilized Neu(Ru)$_1$. For each plot, voltammetry was recorded at 5, 10, 20, 50, 100, 200, 500, and 1000 mV/s with 2 mV/s added in plot a. To depict voltammetry over a range of scan rates in a single plot, current is normalized by scan rate yielding capacitance. In each plot, the voltammogram with the smallest peak capacitance corresponds to that recorded at 1000 mV/s as indicated plot a. Ellipsometry using gold-coated silica substrates indicate that each electrode of Figure 2 should have similar protein coverage consistent with a protein monolayer. Adjacent each plot in Figure 2 is an idealized schematic representation of the corresponding conjugate/electrode interface based on interpretation of voltammetry (below). Dark arrows represent relatively fast electron-transfer between electrostatically bound Ru(NH$_3$)$_5$ groups and the electrode, while white arrows represent relatively slow interprotein electron-transfer between electrostatically unbound and bound Ru(NH$_3$)$_5$ groups.[21]

Examination of Figure 2 reveals that the apparent electrode coverage of Neu(Ru)$_4$ is dependent on the immobilization method. Specifically, comparison of cyclic voltammetry recorded at 1000 mV/s of adsorbed Neu(Ru)$_4$ to that of covalently attached Neu-

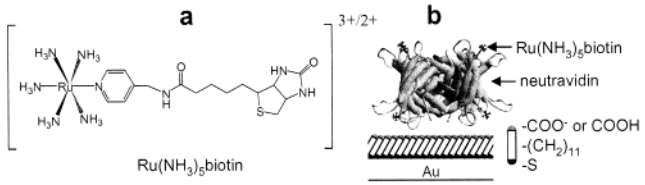

Figure 1. (a) Ru(NH$_3$)$_5$Biotin. (b) Schematic representation drawn to scale comparing the size of a single Neu(Ru)$_4$ (one of its four Ru(NH$_3$)$_5$biotins indicated) to the thickness of a MUA self-assembled monolayer on a gold electrode.[14]

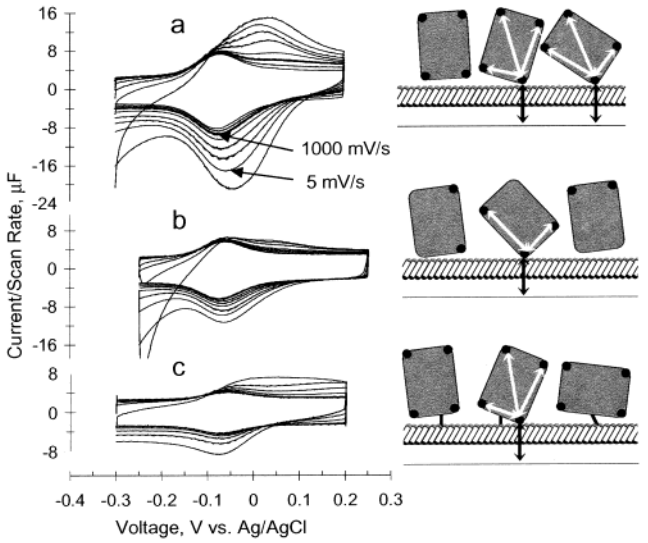

Figure 2. Cyclic voltammetry in 20 mM NaH$_2$PO$_4$ of (a) Neu(Ru)$_4$ noncovalently immobilized on a MUA-modified gold electrode, (b) Neu(Ru)$_1$ noncovalently immobilized on a MUA-modified gold electrode, and (c) Neu(Ru)$_4$ covalently immobilized on a MUA-modified gold electrode. Adjacent each plot is an idealized schematic representation of its corresponding conjugate/electrode interface. Details in text.

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$(Ru)_4$ indicates ~3.1-fold greater integrated anodic peak current (~0.54 vs ~0.17 μC, respectively).[22] Our explanation for this dependency is based on that of Bowden et al.[12,15] describing similar results for cytochrome *c*. Specifically, while both immobilization methods result in a distribution in orientation of $Neu(Ru)_4$, noncovalent immobilization favors, by a factor of ~3.1, orientations in which a $Ru(NH_3)_5$ group is electrostatically bound to the monolayer surface. Electrostatically bound $Ru(NH_3)_5$ groups would have relatively fast rates of interfacial electron-transfer resulting in voltammetric current due to their proximity and/or high frequency of electronic coupling with the underlying electrode.[23] In contrast, nonelectrostatically bound $Ru(NH_3)_5$ groups, although still immobilized on the electrode surface, would be kinetically inaccessible at relatively fast scan rates (i.e., do not contribute to voltammetric current) due to their greater distance and/or lower frequency of electronic coupling with the underlying electrode. In addition, we assert here that covalent immobilization may disfavor the electrostatic binding of $Ru(NH_3)_5$ groups because of positive charge of the EDC-activated intermediate[20] and may reduce frequency of electronic coupling by restricting motion of the immobilized conjugate.[12]

Comparison of cyclic voltammetry recorded at 1000 mV/s of adsorbed $Neu(Ru)_4$ to that of adsorbed $Neu(Ru)_1$ indicates ~2.6-fold greater integrated anodic peak current (~0.54 vs ~0.21 μC, respectively). We interpret this difference to reflect a lower probability for an individual adsorbed $Neu(Ru)_1$ to have an electrostatically bound $Ru(NH_3)_5$ group because it has fewer $Ru(NH_3)_5$ groups on average compared to $Neu(Ru)_4$.

For each electrode with voltammetry depicted in Figure 2, apparent electrode coverage of the conjugate increases with decreasing scan rate. We attribute this phenomenon to the onset of kinetic accessibility of relatively slow interfacial electron-transfer with nonelectrostatically bound $Ru(NH_3)_5$ groups. The ratio of integrated anodic peak current at 5 mV/s to that at 1000 mV/s for each electrode is ~3.1 for adsorbed $Neu(Ru)_4$, ~1.1 for adsorbed $Neu(Ru)_1$, and ~3.6 for covalently attached $Neu(Ru)_4$. Comparison of these ratios is consistent with a smaller ratio of nonelectrostatically bound to electrostatically bound $Ru(NH_3)_5$ groups in the case of adsorbed $Neu(Ru)_1$. Comparison of these ratios also reveals at least two properties of interfacial electron-transfer with the nonelectrostatically bound $Ru(NH_3)_5$ groups of immobilized $Neu(Ru)_4$. Regardless of immobilization method, (1) electrostatically bound $Ru(NH_3)_5$ groups mediate the majority of this electron transfer and (2) negligible electron-transfer among adjacent conjugates occurs over the range of scan rates recorded. We would otherwise expect similar apparent electrode coverage at 5mV/s if nonelectrostatically bound $Ru(NH_3)_5$ groups were exchanging electrons directly with the electrode or with groups on adjacent conjugates until mediated by an electrostatically bound group. Finally, we cannot rigorously rule out large-scale orientational reorganization of immobilized conjugate on the monolayer as causing the increase in apparent electrode coverage with decreased voltammetric scan rate.[13,24] We believe, however, that this is not likely for covalently attached $Neu(Ru)_4$ and that the similarity in the extent of the increase in apparent electrode coverage for adsorbed and covalently attached $Neu(Ru)_4$ (~3.1 and ~3.6, respectively) over the same range of scan rate (1000−5 mV/s) suggests the same underlying mechanism. Furthermore, the concomitant positive shift in potential observed for both immobilization methods is consistent with different environments of electrostatically and nonelectrostatically bound $Ru(NH_3)_5$ groups and environment-sensitivity of formal potentials of ruthenium-amine-based protein labels.[15,25]

Regulated electron conduction within redox proteins and among redox protein assemblies is an important feature of protein function. We believe the ability to engineer electron-conduction across proteins immobilized on electrodes such as that observed here will be an important component toward realization of protein-based approaches to high-density molecular electronic devices and more effective reagentless sensors and photoelectro-/electrocatalysts.

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Supporting Information Available: Experimental details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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