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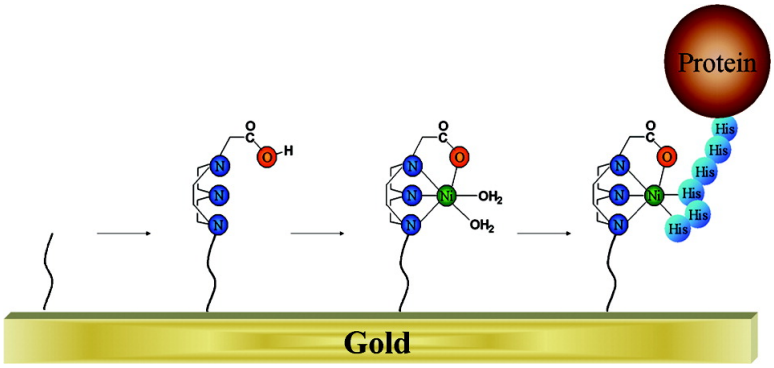

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Controlling Protein Orientation at Interfaces Using Histidine Tags: An Alternative to Ni/NTA

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Since it was first reported almost 30 years ago,[1] nitrilotriacetic acid (NTA)/histidine-tag (HT) technology has become a powerful tool in the biosciences for the single-step isolation and purification of proteins and enzymes modified at the N- or C terminus with a series of histidine residues.[2] Although originally designed for chromatography, NTA/HT technology has also been adapted to a plethora of other applications including surface plasmon resonance (SPR)[3] and electrochemistry,[4] in both academic and commercial domains. These second-generation applications use the perceived specificity of NTA/HT technology to predict and control protein orientation at interfaces.[4]

While the NTA/metal/HT interaction is suitable for protein purification and other transient applications, applications requiring longer-term stability such as biosensors, surface coatings, and binding studies are often compromised by problems related to metal leaching and protein dissociation.[5−7] To overcome these inherent problems, an alternative chelator complex based on the macrocycle triazacyclononane (tacn) was synthesized and evaluated for use as a more generally applicable support for capture of HT biomolecules.[8] Furthermore, since our laboratory is interested in electron transfer between redox-active proteins and electrodes, we have evaluated the influence of orientation on the electrochemical behavior of three redox proteins with diverse physiology, structure, and function: thioredoxin,[9] plastocyanin,[10] and cytochrome P450c17.[11]

Figure 1 illustrates the difference between the chelator used in this study (1-acetato-4-benzyl-triazacyclononane, (Acbztacn[12])) and NTA. The basic scaffold for metal chelation is the same for both compounds, where the immobilized metal is facially ligated by four atoms from the chelating support molecule, leaving two adjacent, unligated metal sites for interaction with histidine residues. (This coordination environment has been previously identified as the most successful template for interaction with histidine residues).[13] However, the Acbztacn ligand offers an additional feature in the tacn ring, which stabilizes the ligand/metal interaction through the macrocyclic effect,[14] reducing complexities associated with metal leaching.

Acbztacn was covalently bound to gold surfaces previously modified with an activated self-assembled monolayer (SAM) (Figure 2). Diffuse reflectance infrared Fourier transform spectroscopy and X-ray photoelectron spectroscopy experiments were conducted at each step during the surface modification protocol to confirm successful binding and to assess the geometric dispersion and orientation of Acbztacn at the modified surface.

SPR experiments (Biacore X) were conducted to analyze metal binding to the immobilized chelator and also to evaluate binding of HT proteins to the metal-treated surface. Identical experiments were also conducted on a commercial Ni/NTA surface (Biacore) for comparison. Several important features were noted. First, once metal ions (Ni(II)) had been added to the Acbztacn-modified surface, standard regeneration conditions (EDTA and/or low pH)[3,6] could not remove the immobilized Ni(II) ions or generate the

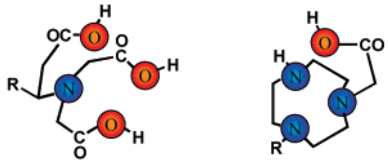

Figure 1. Molecular structures of (a) the NTA ligand and (b) the Acbztacn ligand. Metal coordinating functional groups are enlarged.

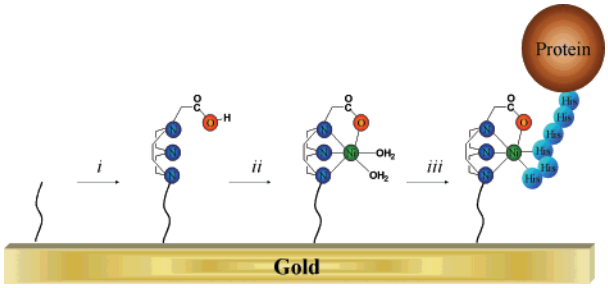

Figure 2. Modification of a gold surface for specific capture of histidine-tagged proteins. (i) Acbztacn ligand is coupled to a SAM; (ii) modified surface is treated with Ni(II). (iii) HT protein is added to the surface, binding specifically to the immobilized metal.

original surface. Second, the sensorgram for the NTA-modified surface treated with Ni(II) ions lost ∼20% of the original binding magnitude over a 24 h period in the presence of standard running buffer (10 mM HEPES, 150 mM NaCl, flow rate 20 μL min^{-1}), confirming earlier reports of metal leaching or bleeding from NTA-modified surfaces.[5−7] Over the same period and under the same experimental conditions, the sensorgram for the Acbztacn-modified surface treated with Ni(II) revealed no change, indicating that the Acbztacn/Ni complex is very stable and may provide an alternative to studies or devices requiring long-term stability. Third, the Acbztacn/Ni-modified surface afforded a much lower component of nonspecific protein binding than the NTA/Ni-modified surface (Table 1). The binding characteristics of three HT proteins were considered at each surface in the presence or absence of Ni(II). Nonspecific binding accounted for 20−50% of the total binding magnitude for the NTA-modified surface. In contrast, nonspecific binding accounted for <5% of the total binding magnitude for the Acbztacn-modified surface. Fourth, while K_D values could be obtained for each protein at an NTA/Ni-modified surface, binding to an Acbztacn/Ni-modified surface revelealed virtually no dissociation, preventing an estimate of K_D at this surface. These results collectively indicate that the Acbztacn surface offers superior selectivity and specificity and a stronger chelator/metal/HT interaction. In addition, it can be inferred that a protein layer bound to an Acbztacn/Ni-modified surface has a >95% homogeneous orientation.

Many modern voltammetric studies attempt to characterize redox active proteins and enzymes while immobilized at surfaces. In some

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Table 1. Comparison of the Nonspecific Binding Magnitude for Histidine-Tagged Thioredoxin (trx), Plastocyanin (pc), and Green Fluorescent Protein (GFP) at NTA- and Acbztacn-Modified Gold Surfaces, Assessed Using SPR[a]

protein	nonspecific binding to NTA/Ni (%)	nonspecific binding to Acbztacn/Ni (%)
HT-trx	47	5
HT-pc	28	4
HT-GFP	21	5

[a] Nonspecific binding, a qualitative parameter, was defined as any interaction other than that between immobilized Ni(II) and the protein−histidine tag. Total binding, comprising both specific and nonspecific binding, was determined by adding protein to the modified gold surface treated with Ni(II). Nonspecific binding was determined by adding protein to the modified surface in the absence of Ni(II). The values above represent the nonspecific binding magnitude as a percentage of the total binding magnitude.

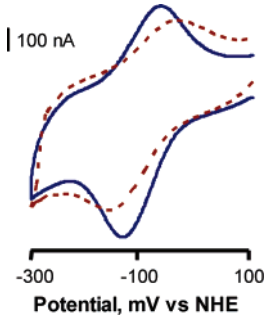

Figure 3. Cyclic voltammograms of trx specifically immobilized at a Acbztacn/Ni-modified gold electrode (bold line) and randomly immobilized at a SAM-modified gold electrode (dotted line). Scan rate = 1 mV s^{-1}; electrode area = 0.04 cm^2; solution conditions = 0.1 M phosphate buffer.

cases, these studies feature very wide peak widths (E_{fwhm}) due to heterogeneous orientation and kinetic dispersion of individual redox sites.[15,16] Since the strategy outlined in this report affords a virtually uniform surface monolayer coverage of protein, it was expected that an Acbztacn/Ni-modified surface would facilitate electron transfer between the electrode and an immobilized redox site with narrow E_{fwhm} values. Three proteins were studied by direct electrochemistry using an Acbztacn-modified surface treated sequentially with Zn(II) ions[17] and protein: thioredoxin (trx), a disulfide/dithiol redox protein that participates in sequential two-electron redox reactions;[9] plastocyanin (pc) a simple electron-transfer protein involved in photosynthesis;[10] and cytochrome P450c17, a complex enzyme involved in a number of important metabolic processes.[11] Rapid electronic communication between the protein redox site and the underlying electrode surface was observed for each protein, not an insignificant achievement since the midpoint potentials range from −300 mV (P450c17) to −100 mV (trx) and +600 mV (pc). Figure 3 (bold line) illustrates a representative cyclic voltammogram obtained for the single-electron reduction of trx.[9] The electron-transfer properties of trx were also considered by an alternate method, where trx was covalently bound to a SAM-modified gold electrode,[18] a procedure that results in an inherently random monolayer of immobilized protein (Figure 3, dotted line). The electron-transfer rates obtained under both conditions indicate the efficacy of electron transfer; for Acbztacn/Ni/trx, $k_s = 8\ s^{-1}$; for SAM/trx, $k_s \approx 0.1\ s^{-1}$ (calculated using the method of Laviron).[19] Furthermore, the E_{fwhm} values obtained for each immobilization constraint emphasize the homogeneity of the immobilized film. At a scan rate of 1 mV s^{-1}, E_{fwhm} for Acbztacn/Ni/trx was 98 mV, marginally higher than the theoretical value of 90.6 mV predicted for a totally homogeneous protein film.[20] In contrast, E_{fwhm} for covalently immobilized trx at the same scan rate was 153 mV, reflecting the composite electrochemical response observed for randomly dispersed protein films. It was also noted that the Acbztacn/Zn/trx protein film was considerably more robust, being electrochemically addressable over a wider range of scan rates for a longer period.

These initial results illustrate the potential for this technology to act as a generic template for fabrication of oriented protein films at surfaces. The template can, in principle, be applied to any surface and/or adapted for any application currently using NTA/Ni technology, in particular those applications currently dealing with problems inherent to NTA/Ni technology. Finally, since the Acbztacn-modified surface appears to offer a wide potential "window" for electrochemical experiments, we are continuing to explore this technology for use in biological sensing, diagnostics, and other applications.

Supporting Information Available: Cyclic voltammograms for plastocyanin and cytochrome P450c17, synthetic and protein purification procedures, and surface modification protocol. This material is available free of charge via the Internet at http://pub.acs.org.

References

(1) Porath, J.; Carlsson, I.; Olsson, I.; Belfrage, G. *Nature* **1975**, *258*, 598−599.
(2) Terpe, K. *Appl. Microbiol. Biotechnol.* **2003**, *60*, 523−533.
(3) Nieba, L.; Nieba-Axmann, S. E.; Personn, A.; Hamalainen, M.; Edebratt, F.; Hansson, A.; Lidholm, J.; Magnusson, K.; Karlsson, A. F.; Pluckthun, A. *Anal. Biochem.* **1997**, *252*, 217−228.
(4) Madoz-Gurpide, J.; Abad, J. M.; Fernandez-Recio, J.; Velez, M.; Vazquez, L.; Gomez-Moreno, C.; Fernandez, V. M. *J. Am. Chem. Soc.* **2000**, *122*, 9808−9817.
(5) Mateo, C.; Fernandez-Lorente, G.; Cortes, E.; Garcia, J. L.; Fernandez-Lafuente, R.; Guisan, J. M. *Biotechnol. Bioeng.* **2001**, *76*, 269−276.
(6) Chaga, G. S. *J. Biochem. Biophys. Methods* **2001**, *49*, 313−334.
(7) Jiang, W.; Graham, B.; Spiccia, L.; Hearn, M. T. W. *Anal. Biochem.* **1998**, *255*, 47−58.
(8) The chelating ligand 1-acetato-4-benzyl-triazacyclonone was synthesised according to the method of Warden et al. Synthetic procedures are available in the Supporting Information.
(9) Johnson, D. L.; Polyak, S. W.; Wallace, J. C.; Martin, L. L. *Lett. Peptide Sci.* **2003**, *10*, 495−500.
(10) Johnson, D. L.; Maxwell, C. J.; Losic, D.; Shapter, J. G.; Martin, L. L. *Bioelectrochemistry* **2002**, *58*, 137−147.
(11) Johnson, D. L.; Conley, A. J.; Martin, L. L. *J.Mol.Endo.* **2004**. Manuscript submitted.
(12) Warden, A.; Graham, B.; Hearn, M. T. W.; Spiccia, L. *Org. Lett.* **2001**, *3*, 2855−2858.
(13) Hainfeld, J. F.; Liu, W.; Halsey, C. M. R.; Freimuth, P.; Powell, R. D. *J. Struct. Biol.* **1999**, *127*, 185−198.
(14) Cabbiness, D. K.; Margerum, D. W. *J. Am. Chem. Soc.* **1969**, *91*, 1.
(15) Clark, R. A.; Bowden, E. F. *Langmuir* **1997**, *13*, 559−565.
(16) Rowe, G. K.; Carter, M. T.; Richardson, J. N.; Murray, R. W. *Langmuir* **1995**, *11*, 1797−1806.
(17) Zn(II) was used in preference to Ni(II) for electrochemical studies because Zn(II) is not electroactive in the potential window utilised by biological redox reactions.
(18) Johnson, D. L.; Thompson, J. L.; Brinkmann, S.; Schuller, K. A.; Martin, L. L. *Biochemistry* **2003**, *10*, 10229−10237.
(19) Laviron, E. *J. Electroanal. Chem.* **1979**, *101*, 19−28.
(20) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods, Fundamentals and Applications*; John Wiley & Sons: New York, 1980.

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