achieved by soaking the beads in concentrated ammonia at room temperature for 24 h. Cyclic compounds were purified by preparative reverse-phase HPLC using a gradient of 0–20 % of acetonitrile over 20 min at a flow rate of 3 mL min$^{-1}$. Analytical data are given in Table 1.

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## Kinetics of a Reversible Covalent-Bond-Forming Reaction Observed at the Single-Molecule Level**

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The examination of single molecules can reveal detail that is obscured in observations of ensembles. A great deal of recent work at the single-molecule level has focussed on biological problems;[1–6] there has been less emphasis on chemistry, especially the making and breaking of covalent bonds. Seminal experiments have resulted in the observation of individual reaction steps catalyzed by enzymes[7] and ribozymes.[8] But, this work has employed optical techniques that are restricted to the use of fluorescent substrates or the investigation of nanoscale movements.[9] We have previously used electrical recording, which does not suffer from these limitations, to visualize individual noncovalent interactions occurring in the lumen of a protein pore.[10] Others have used electrical recording to study individual unidirectional covalent-bond-forming reactions[11] and a reversible thermal isomerization.[12] Herein, we demonstrate that reversible covalent-bond-forming chemistry can be observed by electrical detection. Reversible covalent-bond-forming chemistry at the single-molecule level has not been observed previously by any means.

To demonstrate our approach, we examined the reaction of organoarsenic(III) compounds with thiols, a reaction of considerable importance in the toxicology of environmental contaminants, chemotherapeutic agents, and chemical weapons.[13–21] The reactions of organoarsenic(III) compounds with dithiols to form stable cyclic 1,3-dithia-2-arsolanes have been thoroughly investigated (Figure 1 a, upper).[22–25] Such reactions are essentially irreversible with formation constants of the order of $K_f = 10^7\,\text{M}^{-1}$.[26,27] But, we reasoned that reactions with monothiols would be reversible (Figure 1 a, lower); indeed, unstable adducts of organoarsenic(III) compounds with monothiols are known, although their chemistry has been hardly studied.[28,29]

In our experiments, organoarsenic compounds were present in solution, while thiols were incorporated onto the lumenal face of the α-hemolysin protein pore by site-directed muta-


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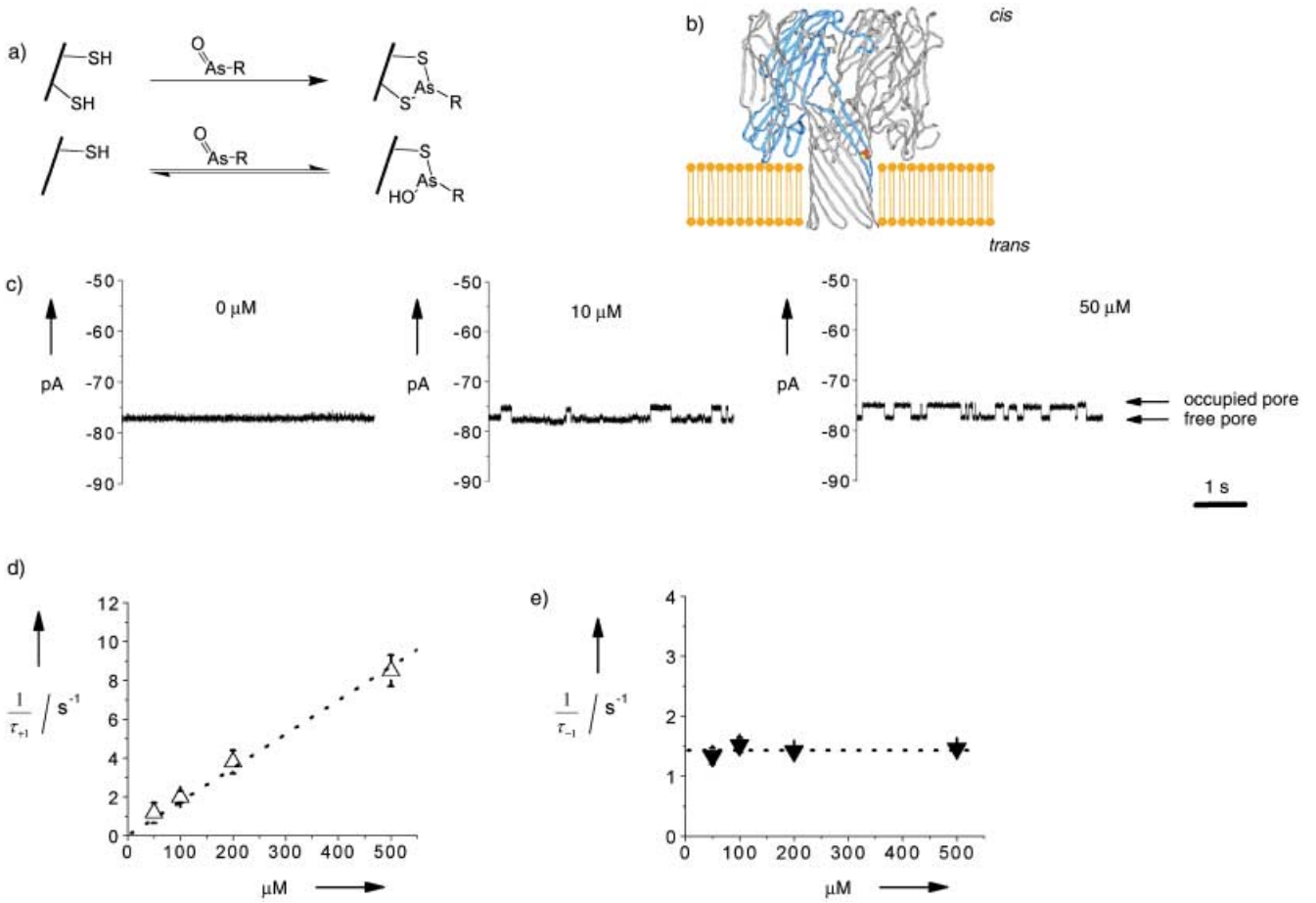


Figure 1. Reversible reactions in which covalent bonds are formed within a single protein pore. a) Upper: organoarsenic(III) compounds react with dithiols to form stable cyclic 1,3-dithia-2-arsolanes. Lower: organoarsenic(III) compounds react with monothiols to form unstable adducts. b) Molecular graphics rendition of the $P_{SH}$ pore. The α-hemolysin pore is a heptamer. In $P_{SH}$, one of the subunits has been replaced with a mutated subunit (blue) containing a cysteine residue (red, with S atom in yellow) in place of the naturally occurring threonine-117. The current flowing through single $P_{SH}$ pores was measured by planar bilayer recording. Arsenic compounds were added to the *trans* side of the bilayer. c) Interaction of 4-sulfophenylarsane oxide with the $P_{SH}$ pore. Single-channel recordings were carried out, samples are shown, at −50 mV with 2 M KCl, 80 mM 3-(4-morpholinyl)-1-propanesulfonic acid (MOPS), 100 μM ethylenediaminetetraacetate (EDTA), pH 8.4, in both chambers. Similar results were obtained with 2 M KCl, 80 mM sodium phosphate, pH 8.4. Current traces in the presence of 0, 10, and 50 μM 4-sulfophenylarsane oxide are shown. Current levels for the free and covalently modified pore are indicated. d) Plot of the reciprocals of the mean inter-event intervals ($\tau_{+1}$, △) versus 4-sulfophenylarsane oxide concentration. e) Plot of the reciprocals of the mean residence time ($\tau_{-1}$, ▼) versus 4-sulfophenylarsane oxide concentration. In d) and e), values of $\tau_{+1}$ and $\tau_{-1}$ were obtained by fitting dwell-time histograms to single exponential functions. For a simple bimolecular interaction: $\tau_{-1} = 1/k_{-1}$; $\tau_{+1} = 1/k_{+1}[R]$, where [R] is the free reagent concentration.

genesis. For example, a version of the α-hemolysin pore ($P_{SH}$) was constructed in which the side-chain of a single cysteine residue at position 117 projects into the lumen of the transmembrane β-barrel (Figure 1b). The chemistry occurring at cysteine was observed by monitoring fluctuations in the ionic current flowing through a single $P_{SH}$ pore in a planar bilayer (Figure 1c). $P_{SH}$ itself had a unitary conductance of 1.54 ± 0.03 nS ($n = 7$, where $n =$ the number of experiments performed) in 2 M KCl). In the presence of 4-sulfophenylarsane oxide,[30] a model reactant, steps were observed during which the current was reduced by 2.5 ± 0.3 pA ($n = 7$). These events were not observed when 4-sulfophenylarsane oxide was added to a bilayer containing the wild-type α-hemolysin pore, which contains no cysteine residues. The events were eliminated either by replacing the arsenic-containing solution with buffer or by the addition of 2,3-disulfanylpropanol (1.5 mM to 10 μM arsenic species).

The approach permits the rapid evaluation of kinetic constants under conditions of dynamic equilibrium.[31] The steps, which represent individual covalent couplings of 4-sulfophenylarsane oxide to form a noncyclic monothiol adduct (Figure 1a, lower), had a mean duration of 702 ± 38 ms ($n = 6$). Plots of the mean lifetime of the adduct ($\tau_{-1}$) and the mean inter-event intervals ($\tau_{+1}$) versus the concentration of 4-sulfophenylarsane oxide are suggestive of a simple bimolecular interaction (Figure 1d,e).[31] Forward and reverse rate constants derived from the $\tau$ values ($k_{+1} = 20 \pm 3 \times 10^3\,\text{M}^{-1}\text{s}^{-1}$; $k_{-1} = 1.4 \pm 0.1\ \text{s}^{-1}$) yielded a formation constant $K_f = 1.4 \pm 0.3 \times 10^4\,\text{M}^{-1}$ at 24 °C ($n = 4$).

Additional arsenic species were tested with the $P_{SH}$ pore and they yielded signals with diverse amplitudes and mean dwell times. These data suggest that a wealth of chemical information should be accessible through the approach disclosed here. Because the observations are made under conditions of dynamic equilibrium, the time resolution is limited only by the sampling time of the system, which can be less than 50 μs. The approach is not limited by a requirement for a particular class of molecules, such as fluorophores.

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Further, the geometry of the lumen of the $\alpha$-hemolysin pore will allow the examination of several effects important in organic chemistry, such as the properties of neighboring groups introduced by mutagenesis or targeted chemical modification. Although the present system is restricted to aqueous chemistry on a protein surface, the general principle might be extended to alternative detection methods that work in different environments.

In addition, the electrical signals from reversible covalent-bond-forming reactions are rich in information and could be employed in stochastic sensing[10] to detect organoarsenic species (such as the vesicant lewisite, 2-chlorovinyldichloroarsane) and other reactive molecules, such as nerve and mustard "gases". Because different patterns of residues can be engineered within the lumen of the α-hemolysin pore, the approach should be adaptable to a wide range of chemical systems. Finally, chemical modification at cysteine has played a major role in determining the structures of ion channels in different conformational states.[32–34] It seems likely that reversible chemical modification at cysteine, during single-channel recording, will be valuable in this area too.

Experimental procedures for the preparation of the $P_{SH}$ pore, the synthesis of 4-sulfophenylarsane oxide, and single channel recording are available in the Supporting Information.

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## Hydroboration of Coordinated Dinitrogen: A New Reaction for the $N_2$ Ligand that Results in Its Functionalization and Cleavage**

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An essential process in nitrogen fixation is cleavage of molecular nitrogen.[1] Several studies using model systems have confirmed that metal nitrides can be formed from coordinated dinitrogen providing that at least six electrons are available to formally reduce the $N_2$ unit to two $N^{3-}$ ligands.[2] To date, all well-characterized molecular examples of metal nitride formation from dinitrogen have involved intermediates containing multiple metal atoms. Therefore, comparisons have been made to the polymetallic FeMoco active site in nitrogenase enzymes, and activated iron surfaces in the Haber process.[3] Herein we report a completely new way to form metal nitrides from coordinated dinitrogen that involves simple organoborane addition to a dinuclear tantalum dinitrogen complex. Because this result is quite unrelated to either biological or industrial nitrogen fixation, we suggest that a new paradigm for both cleaving and functionalizing coordinated dinitrogen is now available.

We have reported the facile preparation of $[\{(NPN)Ta\}_2(\mu\text{-}H)_2(\mu\text{-}\eta^1{:}\eta^2\text{-}N_2)]$ (**1**; where NPN = $(PhNSiMe_2CH_2)_2PPh$),[4] from the spontaneous reaction of $N_2$ gas with the dinuclear tantalum(IV)–hydride precursor $[\{(NPN)Ta\}_2(\mu\text{-}H)_4]$. Equation (1) shows the 1:1 reaction between dark brown **1** and a THF solution of 9-borabicyclononane (9-BBN), which proceeds to completion over a few hours at room temperature to give orange $[\{(NPN)Ta(H)\}(\mu\text{-}H)_2(\mu\text{-}N_2\text{-}BC_8H_{14})\{Ta(NPN)\}]$ (**2**), in almost quantitative yield.

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