frequency change. Monolayers saturated with a biotin–alkaline phosphatase conjugate gave much larger frequency responses, clearly indicating that during the HSV-1 assay the streptavidin monolayer was not saturated with the HSV-1 target strand hybrid.

We have demonstrated that robust, irreversibly bound, functionally active monolayer films of avidin and streptavidin form spontaneously and rapidly on gold and silver surfaces and are useful in a broadly applicable format.

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Supplementary Material Available: Complete experimental details, including procedures for the monolayer formation, biotin binding, enzymatic reactions, and HSV-1 assay (1 page). Ordering information is given on any current masthead page.

Direct Measurement of Vibrational Temperatures in Photoexcited Deoxyhemoglobin on Picosecond Time Scales†

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Recent advances in laser technology allow optical measurements, such as Raman scattering and absorption, to be performed in the picosecond and femtosecond time regimes. Commensurate with these shorter temporal regimes, the molecular dynamics of the system become increasingly complex. In addition to electronic transitions of the chromophore, the decay dynamics of photoexcited molecules are also influenced on subnanosecond time scales by the laser field impinging on the system, dielectric changes of the solvent, and conformation transitions, as well as other short-time phenomena. Power dependent broadening has been observed for the strongest Raman mode, ν_4, of deoxyhemoglobin (deoxy-Hb) using 30-ps, 436-nm excitation pulses.[1] Martin and co-workers[2,3] have recently suggested that, upon absorption of a 355-nm or 580-nm photon, a vibrationally "hot" heme at 500 K results subsequent to photodissociation of CO, which cools substantially in 10 ps. Anfinrud et al.[4] have presented indirect evidence that the heme macrocycles of both deoxy-Hb and HbCO are thermally excited for 1–2 ps after photoexcitation based on base-line shifts in the free CO IR absorption region.

Classical molecular dynamics simulations have been conducted by Henry et al.,[5] to predict the rate at which excess vibrational energy in the heme is dissipated into the protein matrix for myoglobin and cytochrome *c*. In the absence of electronic radiative or nonradiative processes, the energy supplied by a 530-nm photon would result in an excess of vibrational energy and an instantaneous rise in the temperature of the porphyrin. In these heme protein systems at room temperature, complete conversion of the 530-nm photon energy equipartitioned into the vibrational modes of the ground state is estimated to raise the temperature of the

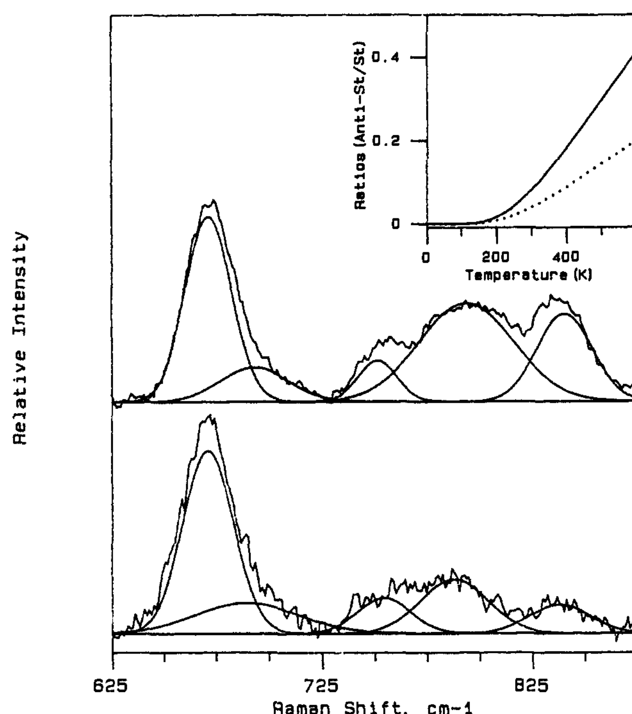

Figure 1. Representative 30-ps resonance Raman spectra of deoxy-Hb (pH ~7.0 in 0.1 M phosphate buffer) obtained by using 436-nm laser pulses. The 436-nm excitation wavelength was generated by anti-Stokes stimulated Raman scattering of the laser second harmonic (532 nm) of an active–passive mode-locked 30-ps Nd:YAG laser operating at 10 Hz. A spinning cell was used in all experiments to minimize the effects of photodegradation. The spectra were generated at a laser fluence of ~4 × 10^9 W/cm^2. The Stokes region of the spectrum (upper spectrum) is shown from 625 to 875 cm^{-1}, with the fits used to extract the intensities. The bands at 671 cm^{-1}, 756 cm^{-1}, and 790 cm^{-1} are ν_7, ν_{16}, and ν_{32}, respectively, of the heme. The shoulder at ~690 cm^{-1} arises from nonresonant scattering from the globin.[13] A propylene glycol mode appears at 841 cm^{-1}. Corresponding fits in the anti-Stokes region yield the analogous band positions and are shown in the lower spectrum. The figure shows the average fit of the 625–875-cm^{-1} regions of the Stokes and anti-Stokes spectra. This yielded a ratio of 8.6% for the areas of the anti-Stokes and Stokes ν_7 bands. The ratios obtained for several fits ranged from 6.7% to 10.2%. This yields temperatures of 300 ± 30 K. The variance in the ratios resulted from changes in the base-line fits and small changes in line widths of the components. The curve fits, however, are nonpathological in that only the number of lines is fixed in the calculation. The inset is a plot of the expected Boltzmann distribution for ν_7 (···) in the absence of resonance and readsorption effects, while the second curve (—) displays the expected behavior of ν_7 in the presence of reabsorption effects and resonance conditions at 436 nm, using the Kramers–Kronig transform technique.

heme by 500–700 K. The dissipation of the vibrational energy into the thermal bath (the protein matrix in this case) is nonexponential in time, with approximately 50% of the loss occurring in 4 ps and the remainder in 20–40 ps. Decay to the ground electronic state in deoxy-Hb occurs in <3 ps.[6] Therefore, investigation of ground-state dynamics is best addressed by utilizing 30-ps excitation pulses, which might generate steady-state populations of the putative vibrationally "hot" species.

Deoxyhemoglobin was chosen for this study to accentuate the effects of the vibrational population changes subsequent to absorption of a 436-nm photon. HbCO has previously been demonstrated to exhibit broadening and shifting of ν_4 subsequent to CO photolysis. The phenomenon was attributed to the anharmonic coupling of ν_4 to thermally populated low-frequency modes of the heme due to absorption of a 580-nm photon.[6] The resulting spectra were deconvoluted from the residual six-coordinate species and subtracted from the equilibrium deoxy-Hb spectrum to obtain the shifted and broadened ν_4 spectrum. The thermal effects observed in deoxy-Hb should be larger than those in HbCO due to the

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absence of a photodissociative pathway for energy dissipation in the molecular system.[7] In addition, the interpretation of the spectra would be greatly simplified by the presence of a single species in solution. This study addresses the vibrational population changes of deoxy-Hb as a result of absorption of a 436-nm and 532-nm photon by examining the anti-Stokes/Stokes intensity ratio of ν_7, the strongest mode in the low-frequency region of the spectrum, as a function of laser fluence using 30-ps time resolution.

Representative transient resonance Raman spectra of both Stokes and anti-Stokes scattering in the 625–875-cm^{-1} region of deoxy-Hb are shown in Figure 1. The picosecond Raman spectrometer used in this study has been described in detail elsewhere.[8] Three Raman-active heme modes, ν_7, ν_{16}, and ν_{32}, at 671, 756, and 790 cm^{-1}, respectively, are observed in this spectral region. The anti-Stokes/Stokes intensity ratio of the totally symmetric heme mode, ν_7, at 671 cm^{-1} is found experimentally to be 8.6%. The spectra were fit with five Gaussian lines. The 841-cm^{-1} propylene glycol line was used to correct for reabsorption effects of the respective scattering frequencies of the Stokes and anti-Stokes lines of ν_7, following the formalism used by Schomaker and Champion.[9]

Three qualitative observations suggest that little or no heating occurs at the heme on the time scale of our experiments. First, the anti-Stokes/Stokes intensity ratios for all modes in the 625–875-cm^{-1} region and the relative intensities of the anti-Stokes heme and solvent bands are independent of laser fluence. If the macrocycle were locally heated by absorption of an incident photon, the anti-Stokes/Stokes ratios of the heme modes would presumably display a power-dependent behavior distinctive from that of the solvent band. Second, spectra obtained with 10-ns pulses at an excitation wavelength identical with that of the 30-ps experiments yield an anti-Stokes/Stokes intensity ratio of 8.6% for the 674-cm^{-1} heme mode. The heme is undoubtedly in thermal equilibrium with the bath on this time scale. Third, a two-pulse protocol using strong 532-nm pump pulses ($\sim 10^{10}$ W/cm^2) temporally and spatially overlapped with the 436-nm probe pulse also resulted in no change in the relative intensities of the anti-Stokes and Stokes heme modes. Collectively, these observations indicate that the macrocycle of deoxy-Hb is not "heated" within the 30-ps pulses.

In order to quantitatively extract vibrational populations (hence Boltzmann temperatures) from resonance scattering intensities, it is necessary to account for differences in resonance cross sections for Stokes and anti-Stokes processes. In the absence of B-term scattering,[10-12] the Kramers–Kronig transform technique provides a useful method of monitoring the population in the presence of complicating resonance effects. The inset in Figure 1 displays the relationship between the Boltzmann temperature and the observed anti-Stokes/Stokes intensity ratio of ν_7 using a Kramers–Kronig transform technique and a first-order resonance Raman cross-section expression, which separates the thermal properties of the absorption line shape from the Bose–Einstein factor of the Raman mode. (For an excellent discussion of the applicability and limitations of the Kramers–Kronig transform technique on heme proteins, see ref 9, 12, and 13.) In the absence of resonance effects, the expected Boltzmann anti-Stokes/Stokes intensity ratio of ν_7 at 300 K is 3.95%. After correction for reabsorption of the scattered light,[9] the transform technique predicts an anti-Stokes/Stokes intensity ratio of 8.2% for ν_7 (see figure caption for details of analysis). On the basis of these studies, it is estimated that the average temperature during 30-ps pulses of the heme active site is 300 ± 30 K.

This study demonstrates that the excess energy resulting from the absorption of a visible photon does not produce Boltzmann heating at the active site of deoxy-Hb. This finding is totally consistent with the qualitative observations of the invariance of the intensity ratios of the anti-Stokes and Stokes lines to laser fluence. Finally, the observed ratios are quantitatively consistent with the ratios expected from application of the Kramers–Kronig transform technique. The simplistic concept of an equipartition of excess energy into vibrational modes of the heme appears to be invalid on this time scale. Further studies will probe the mode-specific relaxation processes (T_1 and T_2) of the heme using femtosecond and picosecond coherent and incoherent spectroscopies to elucidate the role vibrational dynamics plays in the short-time behavior of heme proteins.

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Metal-Assisted CO Insertion Reaction on a New Surface Rhodium Dimer Catalyst Observed by an in Situ Extended X-ray Absorption Fine Structure Technique

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The direct observation of structural changes of active sites at catalyst surfaces in a working state is a fascinating challenge.[1-4] Kitamura-Bando et al. prepared a new SiO_2-attached Rh-dimer catalyst active for C_2H_4 hydroformylation by the reaction between *trans*-$[Rh(C_5Me_5)(CH_3)]_2(\mu\text{-}CH_2)_2$ and surface OH groups of SiO_2.[5] They found that two CO per Rh dimer absorb to form twin-type carbonyls exhibiting IR bands at 2032–1969 cm^{-1} and that the CO insertion into an alkyl group to form acyl proceeds by heating to 423–473 K under vacuum, while the reverse decarbonylation of the acyl group to form twin CO and alkyl occurs under ambient CO at room temperature. This behavior is opposite that observed with Rh-monomer catalysts in homogeneous systems. In the present article, we report the structure change of the SiO_2-attached Rh dimers during reversible CO insertion, which is a key step for hydroformylation, observed by an in situ EXAFS (extended X-ray absorption fine structure) technique.

SiO_2 (Aerosil 300, pretreated at 673 K for 1 h) was impregnated with a pentane solution of *trans*-$[Rh(C_5Me_5)(CH_3)]_2(\mu\text{-}CH_2)_2$[6] for 1 h at room temperature under high-purity Ar, followed by treatment at 313 K for 30 min and at 373 K for 1 h under vacuum to complete the surface reaction of the Rh complex with OH groups of SiO_2. The obtained SiO_2-attached Rh dimers were transferred to an in situ EXAFS cell without exposure to air.

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