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Deconvoluting Subensemble Chemical Reaction Kinetics of Platinum–Sulfur Ligand Exchange Detected with Single-Molecule Fluorescence Microscopy

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Supporting Information

ABSTRACT: The subensemble kinetics of a platinum–sulfur covalent chemical reaction at the solution/surface interface of a model industrial catalyst support was examined using single-molecule fluorescence microscopy (SMFM) and was found to exhibit biexponential first-order kinetic behavior. The observed kinetics was a convolution of the observation probability and chemical reaction rate. These results suggest that deconvolution strategies may be broadly important for obtaining accurate chemical reaction kinetics with SMFM.

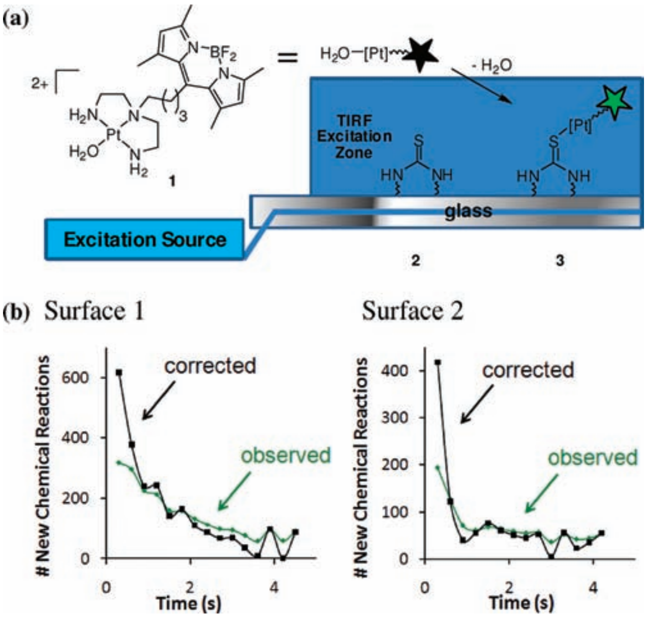

Figure 1. (a) Experimental schematic showing TIRF excitation of molecules on the surface only. (b) Number of new reactions per time: apparent instantaneous rate of the chemical reaction (green) and calculated corrected rate of the chemical reaction (black) for two different samples. Each data point corresponds to the number of new reactions detected in a 300 ms frame.

Single-molecule fluorescence microscopy (SMFM) techniques are a promising way to examine chemical reactivity at the solution–surface interface, such as occurs in heterogeneous catalysts, where the spatial reactivity distribution traditionally complicates analysis. Hofkens[1–7] have probed reactions on chemocatalytic particles, and Chen et al.[8–10] have investigated turnover distributions of individual gold nanoparticles. We recently reported a SMFM technique that correlated the chemical reactivity on silicon oxide surfaces, a common industrial catalyst support, with surface topography characterized by atomic force microscopy.[11,12]

These examples highlight the need for accurate methods to quantitatively determine the chemical reactivity of spatially different multimicrometer regions of surfaces.[13] These multimicrometer regions may contain a small total number of observed chemical reactions; i.e., these regions would be characterized by subensemble reaction kinetics. We herein present an empirical method for deconvolution of subensemble reaction kinetics obtained through SMFM as applied to a ligand-exchange reaction at platinum on an inorganic surface. The observed kinetics reflect the simultaneous occurrence of two processes: the observation probability and the underlying kinetics of the chemical reaction. We consider the method and system described here a useful case study for the wider adoption of SMFM techniques to probe spatial chemical reactivity distributions of inorganic surfaces via subensemble kinetic techniques.

Figure 1 shows a schematic of our experiment. A BODIPY-tagged (dien)platinum complex, **1**, becomes immobilized on a glass surface modified with *N*,*N*′-[3-(triethoxysilyl)propyl]-thiourea through platinum–sulfur covalent bond formation to form **3**.[11,12,14] This functionalized surface was selected to model triethoxysilyl-modified silica, which is a widely used catalyst support.[15] In total internal reflectance fluorescence (TIRF)[16,17] mode, only fluorophores immobilized on the surface are detected, because fluorophores in solution are not excited or are diffusing rapidly.[17] Thus, the appearance of one fluorescence signal at a location on the surface characterizes the individual chemical reaction of one platinum complex with one thiourea ligand on the surface. Images were obtained continuously, with 300 ms exposure per frame. The injection time of complex **1** onto the surface was defined as $t = 0$.

The apparent chemical reaction of (dien)platinum **1** with the thiourea surface was examined for two circular regions with radii of 65 μm. The instantaneous rate of the chemical reaction was defined as the number of new complexes of **3** that were detected in a given 300 ms frame that had not been detected in previous frames. Figure 1b (green data points) shows the number of new complexes of **3** that were detected in each 300 ms frame versus time (i.e., the apparent instantaneous rate of the chemical

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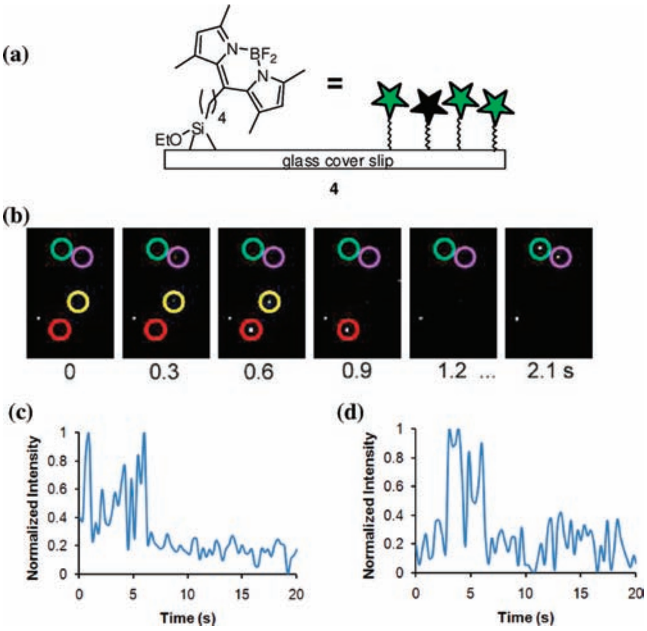

Figure 2. Tethered BODIPY control experiment. (a) Structure of tethered BOPIPY 4. (b) Fluorescence images of surface at different times. Circles mark examples of single molecules that are not fluorescent and therefore not detected in initial frame(s). (c) Time trace of the red-circled single molecule in *b*, 20 s. Data from the first 2.1 s corresponds to the fluorescence image in part *b*. (d) Time trace of a molecule that was in a dark state and detected at $t = 2.7$ s.

reaction vs time). The data in Figure 1b are from two different samples. The small numbers of reactions occurring under these conditions resulted in significant fluctuations in the reaction rate per frame; however, the overall trend of the exponential decay of the reaction rate with time was apparent (Figure 1b).[18,19]

The data presented in Figure 1b (green data points) provided a lower limit for the initial chemical reaction rate on the thiourea surface; more chemical reactions could have occurred that were not detected if the fluorophores were in dark states due to blinking or quenching[20] during the frame in which the reaction occurred. Blinking of organic fluorophores and quenching of these fluorophores through interactions with inorganic surfaces are well-documented processes that depend significantly on the nature of the surface and reaction conditions such as the solvent and temperature.[21−25] These temporary dark states produced the possibility of a delay in observation of the chemical reaction, i.e., that a reaction would not be observed until a later frame than the one in which it occurred (or may escape observation altogether).

In order to deconvolute the rate of the chemical reaction from a possible observation delay in our system, the imaging delay for a population of BODIPY[26,27] fluorophores covalently tethered to the surface was examined (**4**; Figure 2a). The covalent tether meant that a fixed number of BODIPY-tagged molecules was present throughout the experiment because fluorophores could not associate or dissociate. If there was no observation delay in the system, all molecules should be detected in the first frame, a condition that was not observed. This control experiment confirmed that some molecules produced a fluorescent signal only after the first frame(s), and therefore observation of these molecules was delayed (Figure 2b).

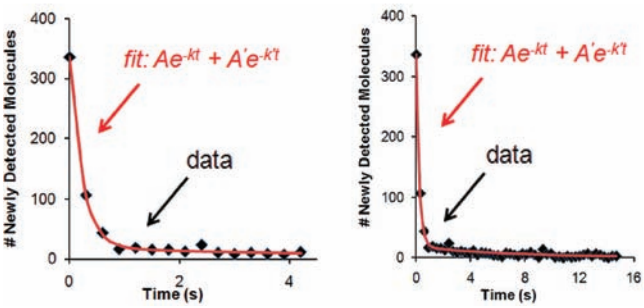

Figure 3. Control: Number of molecules of **4** detected for the first time in that frame per time, showing an empirical two-exponential fit (red line). The first 15 data points (left) and 50 data points (right) were fit to differentiate between monoexponential and biexponential kinetic processes.

Fluorescence time traces for individual molecules showed the characteristic blinking patterns that are well-established fingerprints of single molecules (examples, Figure 2c,d).[28,29] The surface images and time traces in Figure 2 show that the molecules spend significant time in nonfluorescent states and therefore that the observation delay is not simply an artifact of postdata acquisition analysis.[30] Thus, in any given frame, a subset of the molecules were undetectable by SMFM.

An empirical mathematical model for the delay in imaging was developed using the data from surface-tethered BODIPY **4**, with the goal of employing this model to deconvolute the kinetics of the platinum−sulfur ligand-exchange reaction. Figure 3 shows the number of molecules observed for the first time in that frame versus time. The sum of two exponential decays, $Ae^{-kt} + A'e^{-k't}$ ($A = 320$ events·s^{-1}, $k = 4.3$ s^{-1}; $A' = 18$ events·s^{-1}, $k' = 0.15$ s^{-1}), provided a satisfactory fit for the data ($p < 0.0001$ compared to monoexponential decay), representative of at least two processes dictating the probability of detection: a rapid one that dominated at early time and a slower one that dominated at longer time (see the Supporting Information for details and *F* values).

Notably, this result indicates that observation delay *alone* can produce multiexponential kinetics, and therefore care needs to be taken when considering whether apparent multiexponential subensemble kinetics represent underlying heterogeneous chemical processes or the kinetics of the observation process. Multiexponential kinetic behavior of chemical and physical processes has been observed previously on the single-molecule level and has been attributed to surface physical or chemical heterogeneity.[31,32] A probability model where molecules interacted with each other, leading to self-quenching, was considered as a potential physical basis for this two-exponential empirical fit. Less than 1% of the detected molecules, however, were within 1 μm of each other,[11] making significant photophysical interactions between fluorophore-tagged molecules unlikely.

This empirical probability of detection was used to deconvolute the chemical reaction kinetics shown in Figure 1b (see the Supporting Information, section XIV, for details). This empirical model assumed that the deconvolution factors were similar in both the tethered BODIPY and platinum−sulfur systems. The two graphs in Figure 1b show the corrected platinum−sulfur ligand-exchange rates observed on two different samples. Nonspecific physisorption of the BODIPY fluorophore to the surface accounted for less than 2% of surface attachment in the initial period of the reaction,[12] and thus the initial chemical reaction points were examined for the kinetic fit. After deconvolution, the ligand-exchange reaction followed a decay in the reactivity rate consistent with first-order kinetic processes: both biexponential and monoexponential decay with a constant fit the data equally well, indicative of a slower process dominating at longer reaction times (e.g., surface 2 rate = $300e^{-6.3t} + 81e^{-0.26t}$ or rate = $330e^{-4.7t} + 43$).[33] While these multiple processes may represent heterogeneous chemical reaction kinetics,[31] the results described above suggest that it may also represent an observation delay process involving multiexponential kinetics slightly different from those of the tethered BODIPY **4** used in the deconvolution process.

The outcome of this deconvolution was that the detected chemical reaction rate undercounted the actual rate at early reaction times, wherein more reactions occurred than were detected, and overcounted the reaction rate at late reaction times, wherein reactions that occurred in earlier frames but were detected later became a significant contribution.

In summary, a platinum—sulfur ligand-exchange reaction on a model industrial catalyst support exhibited biexponential first-order kinetic trends on the subensemble level. A deconvolution process provided a more accurate comparison of the platinum—sulfur ligand-exchange chemistry on the surfaces of two samples by revealing and correcting for the undercounting of events at early reaction times and overcounting of events at longer reaction times. While the specific equation and mathematical model for deconvolution are likely to be fluorophore- and environment-specific, these results suggest that deconvolution strategies may be broadly useful for accurately determining chemical reaction rates with SMFM.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Experimental details including synthetic procedures, image acquisition procedures, microscopy setup, and method of deconvolution. This material is available free of charge via the Internet at http://pubs.acs.org.

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