Super-Resolved Optical Mapping of Reactive Sulfur-Vacancies in 2D Transition Metal Dichalcogenides

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Section1. Experimental setup



Figure S1. Schematics of 2D-PAINT setup. Descriptions in Methods and Materials.

Section 2. FIB patterning of MoS₂



Figure S2. AFM height images of two FIB patterned MoS_2 flakes with irradiation dose of 3.4×10^{13} ions/cm². The dashed lines indicate one of the approximate irradiation areas on each flake. The images show that the MoS_2 monolayer in the irradiated areas is not etched away.



Raman shift (cm⁻¹)

Figure S3. Raman scattering spectra of MoS_2 monolayers under different irradiation ion dose with irradiation dose-1 of 3.4×10^{13} ions/cm² and dose-2 of 1.1×10^{14} ions/cm². It shows a downshift of E' peak and upshift of A' peak due to defect-induced phonon confinement as well as the presence of defect-activated peaks at ~ 377 cm⁻¹ at the shoulder of E' peak and LA(M) peak at ~227 cm⁻¹.¹

Section 3. Correlation between localization density and defect density



Figure S4. Correlation between the PL intensity of WS_2 and the cumulative localization density of thiol probes over 5000 frames: (a) PL image of a MOCVD-grown WS_2 monolayer. (b) 2D-PAINT image of FAM-dsDNA70bp-SH binding to WS_2 in the same area in (a). (c) The PL intensity of WS_2 and the cumulative localization density of thiol probes show a strong negative correlation, with the correlation coefficient of -0.59.

Section 4. Non-homogenous FRET rate on large structural defects



Figure S5. Mapping defects with thiol probes directly conjugated to fluorophores (ATTO-SH). (a) PL image of MoS_2 containing large structural defects such as grain boundaries and line defects (appearing as lower PL intensity). (b) 2D-PAINT reconstructed image on the same area shows that the binding events are predominantly detected on the grain boundaries and line defects.

Calculation of distance-dependent relative PL intensity induced by FRET in Fig. 4f.

The calculation of FRET induced distance-dependent PL intensity is performed on a MATLAB GUI of fluorophore lifetime estimation that is published previously.² Since the radiative rate of the fluorophore is independent of the distance, the quantum yield of the fluorophore is proportional to its lifetime. Therefore, the estimated FRET-induced lifetime normalized by the FRET-free lifetime corresponds to the relative intensity of the fluorophore.

The lifetime estimation treats the excited fluorophore as a fast rotating dipole and MoS_2 as a lossy thin film with a complex refractive index. The energy can be transferred by near-field electromagnetic wave coupling. Taken from the literature,³ the complex refractive index of monolayer MOCVD-grown MoS_2 at 520 nm is 4.252+0.865i and the thickness 0.6 nm.

Section 5. Analytical description and simulation of reversible binding process in the presence of fluorescent bleaching

Derivation of equation (1)

The model constructed here describes the reversible binding process of fluorescent thiol labels in solution bound to sulfur-vacancies in a MoS₂ flake. In addition to the reversible binding process, under continuous laser excitation in the total internal reflection mode, the fluorescent thiol labels bound to the surface can get bleached. Intermittent fluorescent signals are observed on the surface, reflecting both binding kinetics and fluorophore bleaching. The following scheme describes this process. The fluorescent thiol probes in solution (B) bind to the unoccupied binding sites on MoS₂ (A) forming fluorescent molecules bound to the defects (AB). The association rate is $k_1 = k_a C [s^{-1}]$, where ka $[M^{-1}s^{-1}]$ is the association rate constant, C [M]the concentration of the thiol probes. The bound molecules get bleached and transform to nonfluorescent form (AB*) with a bleaching rate $k_3 [s^{-1}]$. Assuming the bleaching process does not affect the binding/unbinding process, then both AB and AB* have the same dissociation rate of $k_2 [s^{-1}]$. We are interested in the amount of AB and AB*, denoted as $q_1(t)$ and $q_2(t)$, respectively.

$$A + B \quad \overleftarrow{k_1} \quad AB$$

$$\downarrow k_3$$

$$A + B^* \quad \overleftarrow{k_a C} \quad AB^*$$

The density of unoccupied defect sites on MoS₂ is defined as $Q \ [\mu m^{-2}]$. By TEM imaging, the sulfur-vacancies on MOCVD-grown MoS₂ have a density of 10^4 - $10^5 \ \mu m^{-2}$. The total surface area of the MoS₂ ($S \ [\mu m^2]$) in one measurement is in the order of $10^4 \ \mu m^2$. Hence, we estimate the total number of defect sites $Q \cdot S$ as $10^8 \cdot 10^9$. The amount of fluorescent thiols (B) is denoted as q₃(t). The minimal amount used in experiments is 10^{10} . Therefore, as q₃ >> $Q \cdot S$, we can assume that there are sufficient thiols in the solution and that q₃ is a constant. In addition, since the amount of non-fluorescent molecules (B^{*}) are negligible low in solution, therefore, we ignore A+B*- \rightarrow AB*.

Based on the above assumption, the system of differential equations can be written as

$$\begin{cases} \frac{dq_1}{dt} = k_1 (QS - q_1 - q_2) - k_2 q_1 - k_3 q_1 \\ \frac{dq_2}{dt} = k_3 q_1 - k_2 q_1 \end{cases}$$

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Solving this system of differential equations at steady-state, the detectable binding density on MoS₂ can be then expressed as

$$q = \frac{q_1}{S} = \frac{Qk_1}{k_1 + k_2} \frac{k_2}{k_2 + k_3}.$$

Rearrange, we have

$$q = Q \frac{C}{C + K_{\rm D}} \frac{k_2}{k_2 + k_3}$$
(S1)

where dissociation constant is defined as $K_D = \frac{k_2}{k_a}$ [M], characterizing the affinity between the probe and the defect. Note that equation (S1) is just a classic Langmuir binding equation with an additional factor $\frac{k_2}{k_2 + k_3}$, which describes the fraction of unbleached defect-bound probes.

Simulation of reversible adsorption curve in the presence of fluorophore bleaching

To verify this analytical solution, we set up a simulation in MATLAB based on the random process of binding/unbinding and bleaching with single exponential probability density distributions. The initial state is set as all defect sites are unoccupied. The binding/unbinding process and bleaching process start from t=0. The parameters are set as $k_a = 10^6 \text{ M}^{-1} \text{s}^{-1}$, $k_2 = 10^{-2} \text{ s}^{-1}$, $k_3 = 1 \text{ s}^{-1}$. We extract the number of defect sites of different status at steady state under different probe concentrations. The simulation result is shown in Figure S6, with the BoundON data representing the detectable binding events that resembles the Langmuir adsorption curve.



Figure S6. Simulation results of the number of bindings to surface defect sites at steady state, depicting the number of the fluorescent defect-bound probes (BoundON in blue), the bleached defect-bound probes (BoundBleach in

orange) and the unoccupied defect sites (Unoccupied in yellow). For visibility, BoundON has been multiplied by 100. The parameters are set as $k_a = 10^6 \text{ M}^{-1} \text{s}^{-1}$, $k_2 = 10^{-2} \text{ s}^{-1}$, $k_3 = 1 \text{ s}^{-1}$.

Section 6. Calculation of the standard Gibbs free energy change

The standard Gibbs free energy change can be calculated using classical thermodynamics, *via* $\Delta G^{\circ} = RT \ln(\frac{K_{\rm D}}{C^{\circ}})$, where *R* is the gas constant with the value of 8.314 J·mol⁻¹·K⁻¹ and *T* is the temperature in Kelvin, C° is the molar concentration of the standard reference solution, which is equal to 1 M. For $K_{\rm D} \sim 7 \times 10^{-9}$ M, the corresponding Gibbs free energy change ΔG° is ~ - 47 KJ · mol⁻¹ (19.3 k_BT at room temperature)

Section 7. Photophysics of FAM and ATTO488



Figure S7. The reciprocal of blinking ON time $(1/\tau_{ON} = k_3)$ of fluorophores that are embedded in 1% PVA as a function of excitation power density. The bleaching rate k_b scales with the excitation power density with ATTO488 dyes showing a higher photostability than FAM dyes. Note that the power density is measured at normal incidence.

Although from the single molecule blinking statistics and the photophysics of fluorophore, theoretically, we can obtain the dissociation constant K_D , the accuracy of this approach is questionable due to the fact that the photophysics of the fluorophores is largely affected by the surrounding environment. Even though the 1% PVA has been commonly used as an embedding matrix closely resemble the water environment ($k_3 \sim k_3$ '), in the scenario that $k_2 \ll k_3$, the extraction of $k_2 = 1/\tau_{ON} - k_3' = (k_2+k_3)-k_3'$ would not be accurate enough to give a meaningful estimation of K_D . Instead, from the Langmuir adsorption isotherm, we can extract K_D with high confidence.

Section 8. Extraction of blinking statistics

Blinking statistics were extracted from localization data obtained with the ThunderStorm plugin, which contains super-resolved binding event coordinates, localization uncertainty, frame, intensity, *etc.* The localization data were processed by a home-built MATLAB code. First, we used the algorithm DBSCAN (density-based spatial clustering of applications with noise) to group detected binding events that are spatially within a circle of radius of $3 \times$ localization uncertainty as originated from an identical defect. Then, we count the interevent time (OFF time) and the duration of the event (ON time).

ON time distribution resembles a single-exponential decay as shown in Figure S8a. The average ON time is extracted by fitting the exponential probability density function, $P(t_{ON}) = A \exp(-t_{ON}/\tau_{ON})$, where A_{ON} is the amplitude, τ_{ON} is the average ON time. Using a simplified three-state mode to describe the defect state as discussed in Section 5, $1/\tau_{ON} = k_3 + k_2$.

In the diffusion-limited regime, OFF time distribution resembles a double-exponential decay as shown in Figure S8b. Analytically, the probability density function of OFF time in our system can be expressed as $P(t_{\text{OFF}}) = A \exp(-k_1 t_{\text{OFF}}) - B \exp(-k_2 t_{OFF})$. At the diffusion-limited regime $(k_1 < k_2)$, $P(t_{\text{OFF}}) \sim A \exp(-k_1 t_{\text{OFF}})$. Such diffusion-governed binding process is corresponding to the slow process in the experimentally extracted characteristic OFF time (τ_{OFF}). Therefore, we obtain $1/\tau_{\text{OFF}} = k_1 = k_a$ C. For the fast process, the characteristic time τ_{fast} seems independent of probe concentration. We thus speculate that the fast process reflects the local physics of the fluorophore at the binding site.



Figure S8. Typical ON/OFF time distributions in semi-log scale. (a) ON time distribution shows a single-exponential decay. The average ON time is extracted as 0.31 s by fitting to a single-exponential function. (b) OFF

time distribution follows a double-exponential function. We consider that the slow process reflects the interevent time. The average OFF time is extracted as 43 s. Data were taken with 10 nM ATTO-dsDNA70bp-SH at an excitation power density of \sim 5 W/cm².

Section 9. Extraction of the total density of the chemically active defects with balanced super-resolution optical fluctuation imaging (bSOFI) analysis



Figure S9. bSOFI analysis of the blinking signal from fluorescent thiol probes binding to S-vacancies. (a) SOFI image obtained from 20 000 frames of imaging FAM-dsDNA70bp-SH binding to MoS_2 at 40 nM under excitation power of 19 W/cm². Frame time is 30 ms. (b) Molecular densities are extracted with a different number of frames. The averaged density in the highlighted area in (a) shows saturation at frame numbers above 1 6000. The inset image shows a zoomed-in molecular density map of the selected area (400 by 400 pixels) obtained from 20 000 frames. The pixel refers to the 4th order SOFI pixel, with a size of 105/4 nm. The scale bar in (a) is 5 μ m.

Section 10. Surface characterization of MoS₂



Figure S10. Surface contamination on PMMA-assisted transferred MoS₂. (a) TEM image of MoS₂ monolayer transferred over the opening in SiN_x substrate. The image shows the PMMA residues on MoS₂ (indicated by the arrows). The coverage of PMMA residue on MoS₂ is ~20%. (b and c) AFM height image of MoS₂ transferred on a glass coverslip. The coverage of surface contamination is ~ 19%.

MOCVD grown MoS₂ was transferred onto openings in SiN_x substrates by using poly(methyl methacrylate) (PMMA 450, 8% wt/vol in anisole, spin-coated at 2 500 rpm) and carefully cleaned in hot acetone and isopropanol.⁴ To minimize the interaction of PMMA polymer residues with the electron beam, the samples were additionally annealed in argon at 400°C for 8 h prior to TEM imaging. TEM images of transferred MoS₂ monolayers were acquired using Talos TEM microscope (ThermoFisher Scientific, Hillsboro, Oregon, USA) at an accelerating voltage of 80 kV.

Atomic force microscopy (AFM) measurements were performed using a Dimension FastScan AFM (Bruker) with NanoScope software version 8.15 and a NanoScope V controller (Bruker) operating in Peak Force Quantitative Nano-Mechanics (PF-QNM) mode. The FastScan-B and FastScan-D probes (Bruker, spring constants of 0.8 N/m for FastScan-B and 0.25 N/m for FastScan-D, tip radius of 5 nm) were used. *Ex-situ* measurements were performed in air, while *in-situ* AFM experiments in liquid were performed in hanging meniscus configuration where a capillary bridge is formed upon approach between the hanging droplet (80 μ L) on the AFM head and the droplet (20 μ L) on the sample. To prevent evaporation and corresponding changes in the concentration, two Petri dishes with 2 mL of 400 mM KCl/40 mM Tris buffer were placed in the proximity of the sample. For typical imaging conditions, AFM images were collected at scan frequencies of 1 to 5 Hz, while applying loading up to 100 pN (250 pN) for scan size of 500x500 nm (larger than 1x1µm). All images were flattened and equalized using the WSxM 5.0 software⁵ PMMA coverage was estimated using Digimizer Image Analysis Software.



Figure S11. *In-situ* AFM height image of as-grown MoS₂ surface in the presence of 40 nM FAM-dsDNA70bp-SH in 400 mM KCl/40 mM Tris buffer solution. The gas bubble is pointed with the green arrow, DNA molecules are pointed by red arrows.

Section 11. DFT and *ab-Initio* Molecular Dynamics calculations

1. DFT calculation: solvation effect introduced by COSMO solvation procedure

We first perform the DFT calculation using Material Studio, considering the solvation effect by applying COSMO solvation procedure.

Main Results:

DFT calculation results show that a deprotonated 1-hexanethiol binding to a single sulfur vacancy is thermodynamically preferred with reaction energy of -3.376 eV. The binding is stable as sulfur attached to all three Mo atoms around the monosulfur vacancy with a Mo-S bond distance of 2.403 Å. On the other hand, a protonated 1-hexanethiol tends to adsorb at a monosulfur vacancy with a reaction energy of 9.350 eV. However, it is attached to only one Mo atom at the monosulfur vacancy with a Mo-S bond distance is 2.806 Å, indicating a much weaker binding than the deprotonated thiol.

Method:

DMOL3 module of the Materials Studio software suite was applied for the DFT calculation. The generalized gradient approximation of the Perdew–Burke–Ernzerhof functional type is used for the exchange correlation energy. The optimization was performed using the GGA PBE functional with Grimme's DFT-D dispersion correction. The convergence criteria in total energy and the maximum displacement are 10^{-5} Ha and 0.005 Å with spin unrestricted correction. COSMO solvation procedure was applied to introduce solvation effects. The double numerical plus d-functions (DND)3.5 basis is applied in the basis set.

2. DFT and *ab-Initio* Molecular Dynamics calculation: Solvation effect introduced by adding water molecules in the system

We also performed DFT and AIMD calculations in the presence of water molecules in the system using the VASP package.

Methods:

The barrier calculations are performed by a self-consistent analysis of the Density Functional Theory (DFT)⁶ using the Vienna *ab Initio* simulation (VASP) package.^{7,8} The Perdew-Burke-Ernzerhof (PBE) exchange-correlation functional,⁹ which comes under the Generalized Gradient Approximation (GGA), was used and projected augmented wave (PAW) pseudopotentials with a 500-eV energy cutoff and Gamma-point-centered k-point of $4 \times 4 \times 4$

were used. The barrier for H_3O^+ and deprotonated thiol (C₆H₁₃S) on a sulfur vacancy on MoS₂ is calculated by slowly moving the proton away from the water molecule and towards the sulfur of thiol with the system. Each unit cell consists of 40 molecules of H₂O and 1 molecule of H₃O⁺ placed on a MoS₂ surface with a sulfur vacancy. The simulation box has the dimensions 12.741 × 11.034 × 30 Å³. All the structures are relaxed using an energy convergence criterion of 10⁻⁸ eV.

Ab-Initio molecular dynamics (AIMD)¹⁰ simulations are also performed using VASP on the system described above. All the parameters, including the cut-off energy, k-point grid and the energy convergence criterion are kept unchanged from the DFT simulations. In addition, the AIMD simulations are performed using the canonical ensemble (NVT) at 300 K and 1 atm pressure, with a timestep of 0.5 fs.

Main results

Energies of reaction are calculated for protonated and deprotonated 1-hexanethiol with a sulfur vacancy (V_S) on MoS_2 , in the presence of water in order to check whether the reaction is energetically favourable or not. The system used to calculate these energies is described in Figure S12. The energies of the reaction are calculated to be -3.01 eV and -2.79 eV for protonated and deprotonated thiol, respectively.

AIMD simulations on the V_S -MoS₂ and thiol systems show that, in the case of protonated thiol, the proton is lost immediately after the reaction and it prefers to adsorb on the surface and exists as MoS₂-H_{ads} in the system. The barrier for this proton on the MoS₂ surface to re-attach with the thiol molecule (also the desorption barrier of thiol from V_S -MoS₂) is 2.35 eV.

In acidic conditions, due to the presence of excess H^+ in water, the proton required to desorb thiol might also come from a H_3O^+ molecule. The barrier for an excess proton from H_3O^+ in the solution to attach to thiol is 2.57 eV, which is higher than when the proton comes from the surface.

Additionally, no S-C bond cleavage is observed after thiol reacts with the sulfur vacancy on MoS₂ in AIMD simulations.

Entropy estimation

It has to be noted that the adsorption and the desorption barriers calculated for V_S -MoS₂ do not take entropy into consideration. In order to account for entropic effects, we calculate the entropic barriers using standard statistical mechanics.¹¹ The entropic barriers are calculated for the reaction below, evaluating the change in translational, rotational, and vibrational entropy between the products and the reactants.

$$C_6H_{13}SH + V_S \rightarrow C_6H_{13}SV_S + H_{ads}$$

where V_S is the Sulfur vacancy on the MoS₂ surface and H_{ads} is the hydrogen adsorbed on MoS₂ after the thiol molecule reacts with V_S .

It is observed that the contribution to the free energy due to the entropic effects, $T\Delta S$ is -0.866 eV. This entropic contribution would have to be added to the desorption barrier and subtracted to the adsorption barrier. This effect thus leads to a net decrease of the free energy desorption barrier for thiol to 1.484 eV.



Figure S12. Reaction of deprotonated 1-hexanethiol with V_8 -MoS₂. Sulfur atoms are shown in yellow, carbon in teal, molybdenum in silver, oxygen in red and hydrogen in white.

Section 12. Spatial autocorrelation analysis of Figure 2a

Spatial autocorrelation analysis is performed on the central area (9-by-9 μ m²) of MoS₂ shown in Figure 2a. The Moran's I value was calculated to account for the distribution of the binding events using a MATLAB code,¹² where 0 indicates a completely random distribution and 1 indicates a high level of clustering.¹³ We obtain the global Moran's I value as 0.13, when the size of the weight matrix sets as 29-by-29 pixels (equivalent to 30-by-30 nm², close to the localization uncertainty).

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