Metal oxide semiconductors are promising photocatalysts for chemical transformations relevant to the petroleum industry, such as the partial methane oxidation to produce methanol.[1-2] However, the photon-to-chemical conversion efficiencies of these materials are constrained by low absorption of visible light and low electron and hole mobilities. These limitations can be mitigated if the surface of the metal oxide catalyst possesses sites that are highly active for the chemical transformation of interest. For example, the presence of oxygen vacancies has been shown to enhance catalytic activity in metal oxide semiconductors, including tungsten, molybdenum, and indium oxide.[3-6] Different mechanisms have been proposed to explain this activity enhancement,[3-6] indicating further fundamental research is needed. A major obstacle in understanding the role oxygen vacancies play in catalytic activity is that different metal oxide particles within a single batch exhibit variations in the concentration and distribution of oxygen vacancies. This heterogeneity makes it difficult to correlate specific morphological and structural features with catalytic activity when measurements are made on large groups of particles. In this project, we have used chemically-triggered fluorogenic probes to study the spatial distribution of active regions in individual tungsten oxide ($W_{18}O_{49}$) nanowires using super-resolution fluorescence microscopy. Activation of 3'-(p-aminophenyl) fluorescein (APF) requires photoexcitation above the bandgap of the semiconductor to generate hydroxyl radicals and produce fluorescein, which can be detected in situ at the single-molecule level. The acid-catalyzed condensation of furfuryl alcohol (FA) does not require photoexcitation but instead relies on the presence of either oxygen vacancies or hydroxyl groups at the surface of the nanowires to generate fluorescent oligomers. Through quantitative, coordinate-based colocalization of these two probe molecules activated by the same nanowires, we demonstrate that the nanoscale regions most active for the photocatalytic generation of hydroxyl radicals also possess a greater concentration of oxygen vacancies.

Single-molecule super-resolution microscopy was conducted using total internal reflection fluorescence (TIRF) excitation so that only fluorophores adsorbed near the surface of the coverslip would be detected. $W_{18}O_{49}$ nanowires dispersed on a glass coverslip and immersed in a solution containing APF were illuminated simultaneously with a 405-nm laser to excite the nanowires and a 488-nm laser to excite the fluorescein produced after reaction with photogenerated •OH radicals. By localizing the positions of individual fluorescence bursts captured during the image acquisition, we acquired activity maps for single $W_{18}O_{49}$ nanowires with a localization precision of 22 nm. A representative nanowire is shown in **Figure 1a** in which significant variations in activity are seen along the $W_{18}O_{49}$ nanowire. We next selected furfuryl alcohol (FA) as a probe molecule that can undergo acid-catalyzed condensation to generate fluorescent oligomers (**Figure 1b**). While both oxygen vacancies (i.e., Lewis acid sites) and surface hydroxyl groups (i.e., Bronsted acid sites) can catalyze the condensation of FA, only Lewis acid sites are expected to be active because surface hydroxyl groups are deprotonated at neutral pH. As this reaction does not require photoexcitation of the nanowires, TIRF excitation with a 561-nm laser (i.e., below the bandgap
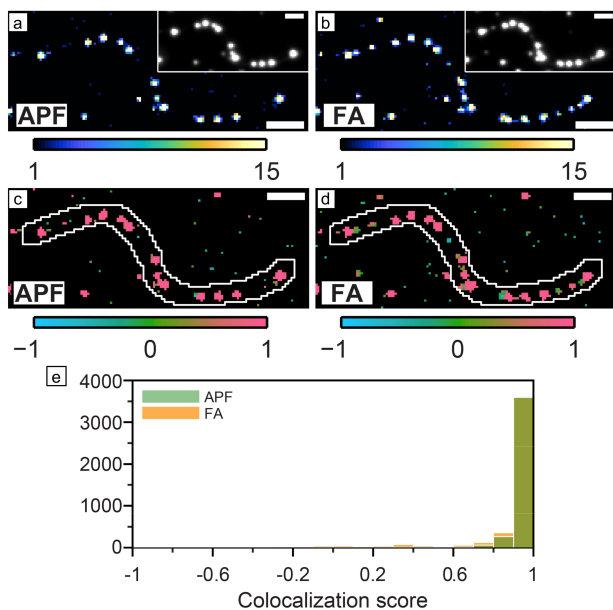
Figure 1. Super-resolution images of the same $W_{18}O_{49}$ nanowire using **(a)** APF as a probe to detect •OH radicals and **(b)** furfuryl alcohol (FA) as a probe to identify Lewis acid sites. Color scale: number of fluorescence bursts per bin. Inset: diffraction-limited images of each imaging condition. Coordinate-based colocalization (CBC) of **(c)** APF and **(d)** FA intensity bursts. Color scale: median colocalization score in each bin ranging from –1 for anti-correlated +1 for perfectly correlated burst distributions. White lines depict boundaries of the nanowire regions. All scale bars are 2 μm. **(e)** CBC scores for APF (green) and FA (orange) bursts within the nanowire regions.

energy of $W_{18}O_{49}$) was used to detect the oligomeric products. We imaged significant variations in activity along the lengths of 33 different $W_{18}O_{49}$ nanowires using both fluorogenic probes. To directly correlate the active regions for photocatalytic •OH radical generation with the distribution of oxygen vacancies (i.e., Lewis acid sites), we sequentially performed single-molecule imaging with both APF and FA as probe molecules on the same $W_{18}O_{49}$ nanowires. A coordinate-based colocalization (CBC) algorithm developed by our collaborator, Prof. Matthew Lew in Electrical and Systems Engineering at Washington University was then used to quantify the spatial correlation of each fluorescent burst from the two probes, yielding a value ranging from –1 for anti-correlated, through zero for random, to +1 for perfectly-correlated spatial distributions. The colocalization maps and the distribution of the CBC scores across a representative $W_{18}O_{49}$ nanowire (**Figure 1c-e**) show that the regions of the nanowire that are more active for generating •OH radicals are also more active for the condensation of furfuryl alcohol. CBC analysis of 33 nanowires shows a high degree of colocalization between the two fluorogenic probes (**Figure 2**). Thus, not only is the catalytic activity heterogeneous along the lengths of individual nanowires, but to a large extent the same segments within each nanowire are active for both reactions.
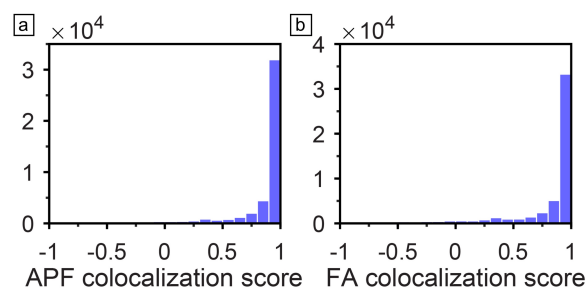
Different mechanisms have been proposed to explain the increased photocatalytic activity for metal oxide particles containing oxygen vacancies. These mechanisms include changes in the electronic structure induced by oxygen vacancies that lead to a narrowing of the band gap,[3, 5] absorption enhancement through the sub-band gap, free carrier absorption,[4] and the activation of molecules adsorbed onto metal ions exposed by oxygen vacancies.[5-6] The high degree of colocalization between •OH radical generation and the presence of oxygen vacancies imaged by single-molecule fluorescence reveals that regions

Figure 2. Accumulated (**a**) APF and **b** FA colocalization scores for 33 initial $W_{18}O_{49}$ nanowires.

containing oxygen vacancies are active sites for water oxidation. Previous density functional theory calculations show that H_2O molecules preferentially bind to tungsten ions exposed by oxygen vacancies at the surface of tungsten oxide, which activates H_2O towards dissociation to H^+ and OH^-.[7] An adsorbed OH^- ion can then be oxidized to an •OH radical using photoexcited holes in the metal oxide. By spatially correlating the nanoscale regions that trigger two fluorogenic probe molecules, we demonstrate that oxygen vacancies are the active site responsible for generating hydroxyl radicals. A manuscript detailing these results is under review. We are currently applying this method to correlate the active regions for both photocatalytic reduction and oxidation reactions in bismuth oxybromide nanoplatelets.

As PI for this project, I received partial support for one summer month. Over the last year, I have presented results from this project during invited talks at the University of Missouri-St. Louis, University of Michigan, Purdue University, Indiana State University, and the ACS National Meeting in San Diego. Travel funds from the project were used to support my conference trip to the ACS meeting. Results from this project will form a major component of my tenure package, which I plan to submit in the summer of 2020. Funds from this grant were used to support the research of two graduate students. One graduate student, currently in his 5th year of graduate school, was supported for the entire year of the project period. He conducted the single-molecule, super-resolution studies described above. He plans to present these results at an ACS meeting in Spring 2020 and will seek a post-doctoral research position following graduation. A second graduate student, currently in his 3rd year, was supported for 3 months during the summer of the project period. During this time, the student learned to conduct single-nanocrystal fluorescence studies and will continue the work of his senior lab-mate.

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