

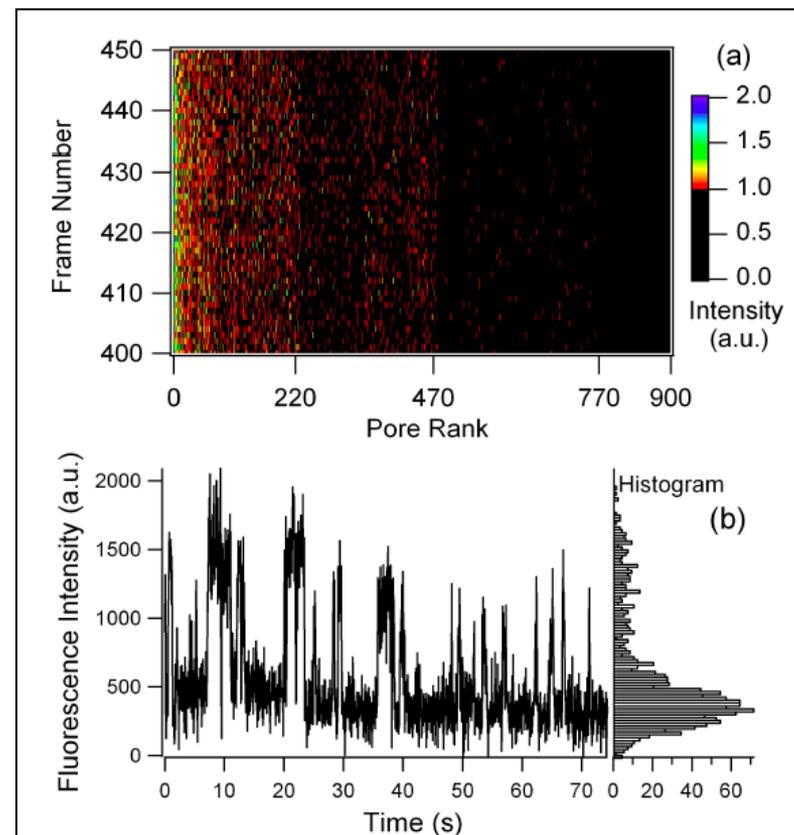


# Zero Mode Waveguide Studies of Single Enzyme Kinetics

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A good deal of effort has gone into defining the conditions under which single molecule spectroscopy can be used to study complex biochemical phenomena, such as enzyme dynamics. Furthermore, there is significant interest in understanding how biomolecules behave under conditions of crowding and confinement in the ultrasmall volumes relevant to nanomanufacturing. We have developed a new modality for zero-mode waveguide (ZMW) studies employing over-etched Au-based ZMWs, which have significant advantages relative to the more commonly encountered Al ZMWs. Furthermore, these platforms are applied to an intrinsically interesting system—the single enzyme dynamics of sarcosine oxidase—an enzyme important for bacterial energy management. Because we identify just those ZMWs that contain exactly one enzyme and because this enzyme is covalently attached to the  $\text{SiO}_2$  floor of the ZMW, one can readily imagine carrying out experiments where the interior structure of the pore can be modified to change the immediate environment of the enzyme molecule in a well-controlled manner. The spectroscopic results themselves speak to enzyme turnover rate distributions, static and dynamic heterogeneity and even allow measurement of off-time distributions for mismatched substrates, *i.e.* L-proline. Taken together these results point the way to realize a powerful new platform for spectroscopic studies of single enzyme dynamics.



**Figure 7.4:** Single molecule intensity trajectories. (a) Intensities binned by color as a function of time (frame number) and pore number with pores ranked by their total integrated intensity. Approximately 2.5 s of a 45 s movie is displayed. (b) (Left) Typical temporal trajectory of a single MSOX molecule catalyzing oxidation of L-proline. (Right) Intensity histogram of the trajectory shown on the left.