Supporting Information

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SI Methods

On the Number of Enzyme Molecules within Vesicles. We used a low concentration of enzyme molecules (0.5 μM) during the preparation of vesicles to ensure that most vesicles contained only a single molecule. To find out what the actual distribution of enzyme molecule numbers within vesicles was, we conducted the following experiment. We prepared vesicles in the presence of the usual concentration of HRP that contained also fluorescent resorufin molecules, which allowed us to observe the vesicles before adding the substrate (Amplex Red) and to count them. We then performed the standard experiment, as described in the main text, preceded by a photobleaching step that ensured that all resorufin molecules included in the vesicles in advance were destroyed. Counting the number of vesicles in which the signal increased due to enzymatic reaction, we found that they amounted to as many as 40% of the total vesicles. Using Poisson statistics, we calculated, based on this number, that 25–30% of the occupied vesicles might contain more than one enzyme molecule (mostly two). This number is slightly more than we would have expected based on the enzyme concentration used for vesicle preparation. However, it might only affect our conclusions favorably: it can partially explain why the correlation we see between initial velocity and plateau level is not higher than approximately 0.6.

Kinetic Model

Given that the substrate concentration is in excess relative to the total enzyme concentration, $[E]_T$, one can make the following two approximations for the reaction shown in Scheme 1 of the main text:

$$[E]_T = [E] + [ES] + \{ESP] \approx [ES] + [ESP] \quad \textbf{[S1]}$$

and

$$k_1[E][S] - (k_{-1} + k_c)[ES] = 0. \quad \textbf{[S2]}$$

It follows that product formation is determined by the following two coupled differential equations:

$$d[P]/dt = k_c[ES] \quad \textbf{[S3]}$$

$$d[ES]/dt = -k_2[ES][P] + k_{-2}[ESP]. \quad \textbf{[S4]}$$

By assuming that the term in Eq. **S4** that corresponds to dissociation of ESP can be neglected, which will prove to be appropriate for the experimental results, and by then taking the derivative with respect to time of this equation, one obtains:

$$d^2 \ln[ES]/dt^2 = -k_2 d[P]/dt. \quad \textbf{[S5]}$$

Combining Eqs. **S2** and **S5** yields:

$$d^2 \ln[ES]/dt^2 = -k_2 k_c [ES]. \quad \textbf{[S6]}$$

Eq. **S6** can be solved yielding:

$$[ES] = (C_1/2k_c k_2)(1 - \tanh^2(0.5(C_1(C_2 + t)^2)^{1/2})), \quad \textbf{[S7]}$$

where C_1 and C_2 are integration constants that are equal to $2[E]_T k_c k_2$ and 0, respectively, as determined from the initial conditions that at time zero $[ES] = [E]_T$ and $d[ES]/dt = 0$. Upon combining Eqs. **S3** and **S7** one, therefore, obtains:

$$d[P]/dt = k_c[E]_T(1 - \tanh^2(0.5(2[E]_T k_c k_2)^{1/2}t)). \quad \textbf{[S8]}$$

Integration of Eq. **S8** yields:

$$[P] = (2k_c[E]_T/k_2)^{1/2} \tanh(0.5(2[E]_T k_c k_2)^{1/2}t)). \quad \textbf{[S9]}$$

The experimental data can be fitted well to Eq. **S9**. A simulation of the full solution to Eq. **S4** shows, however, that when k_{-2} is finite, but small compared to k_2, a continuous slow increase in P is expected at long times. The trace of Fig. 3*A* and similar traces show that such a slow increase barely exists, implying that $k_2 >> k_{-2}$, as assumed above in relation to Eq. **S4**. According to Eq. **S9**, the concentration of P increases from 0 to a plateau value given by $(2k_c[E]_T/k_2)^{1/2}$. Given that the typical experimental plateau value is approximately 25 molecules, it follows that the value of k_c/k_2 is approximately 300, which shows that also $k_c >> k_{-2}$.

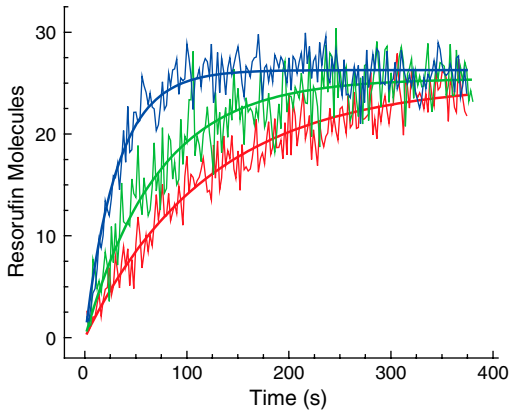

Fig. S1. Sample single-molecule traces at three concentrations of H_2O_2. Red, 50 μM; green, 120 μM; and blue, 1 mM. The traces show a clear dependence of the rate of product formation on this substrate.

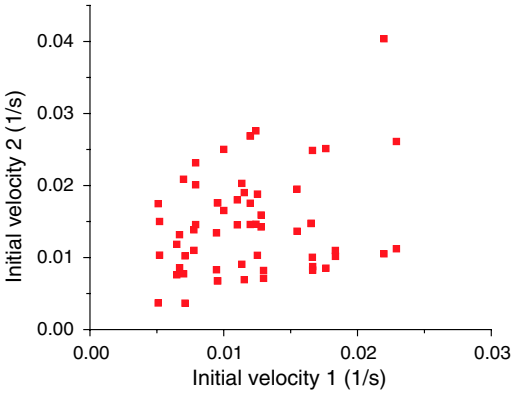

Fig. S2. Correlation plot of consecutive initial velocity values obtained from traces with repeated photobleaching. Traces from approximately 30 vesicles, in which photobleaching was used to repetitively release the enzyme from inhibition (as in Fig. 4 of the main paper), were analyzed, and pairs of initial velocity values were extracted and plotted. Each trace provided three pairs of values. A very weak and statistically insignificant correlation between consecutive initial velocity values was found.

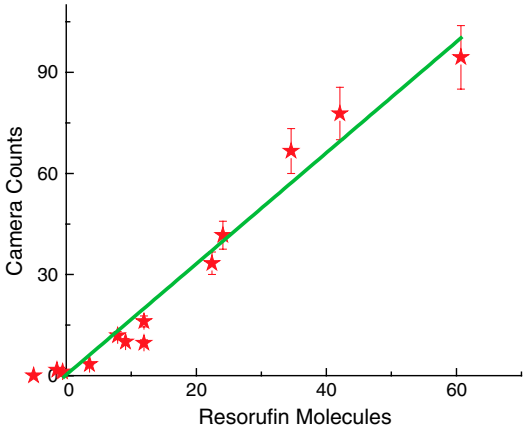

Fig. S3. Calibration curve for resorufin fluorescence within individual vesicles. Vesicles were loaded with varying concentrations of resorufin (1 resorufin molecule ≅ 2 μM), and the fluorescence of surface-tethered vesicles was measured and averaged over multiple vesicles to obtain the numbers in the plot after background subtraction.

Movie S1. The movie shows the gradual increase of the fluorescence of surface-tethered vesicles as individual enzyme molecules encapsulated within them generate more product molecules.

Movie S1(AVI)