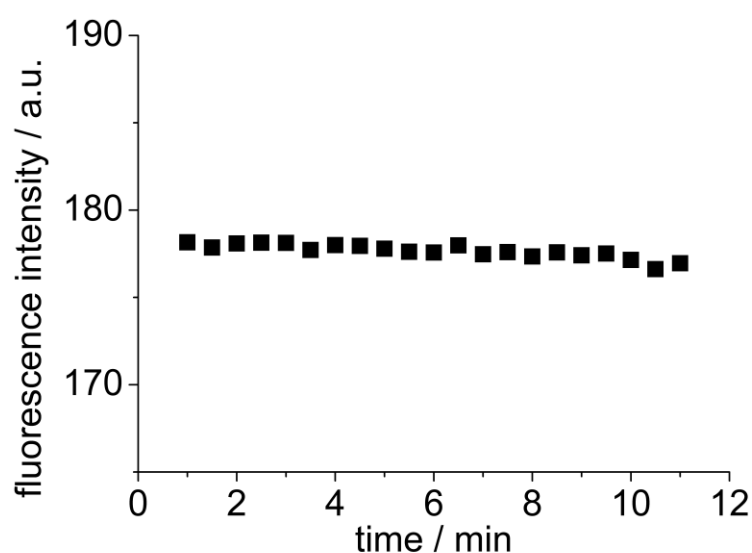


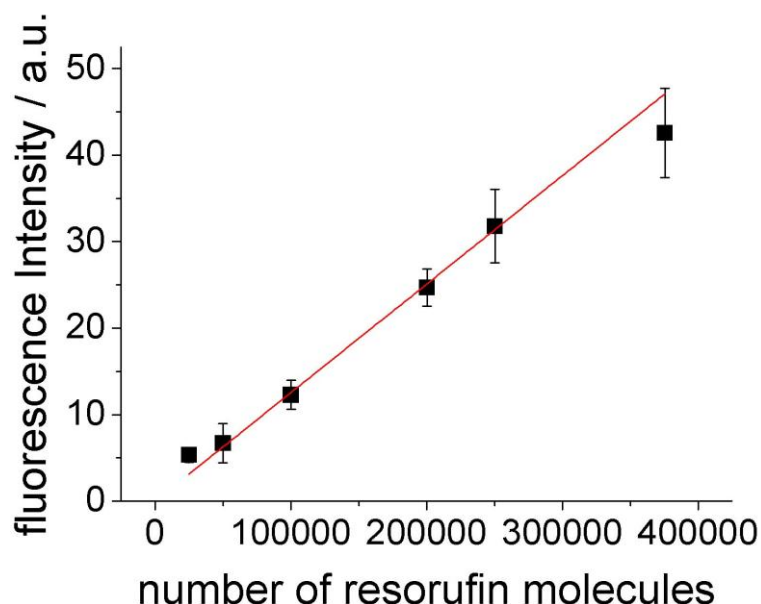
Electronic Supplementary Information

Single molecule kinetics of horseradish peroxidase exposed in large arrays of femtoliter-sized fused silica chambers

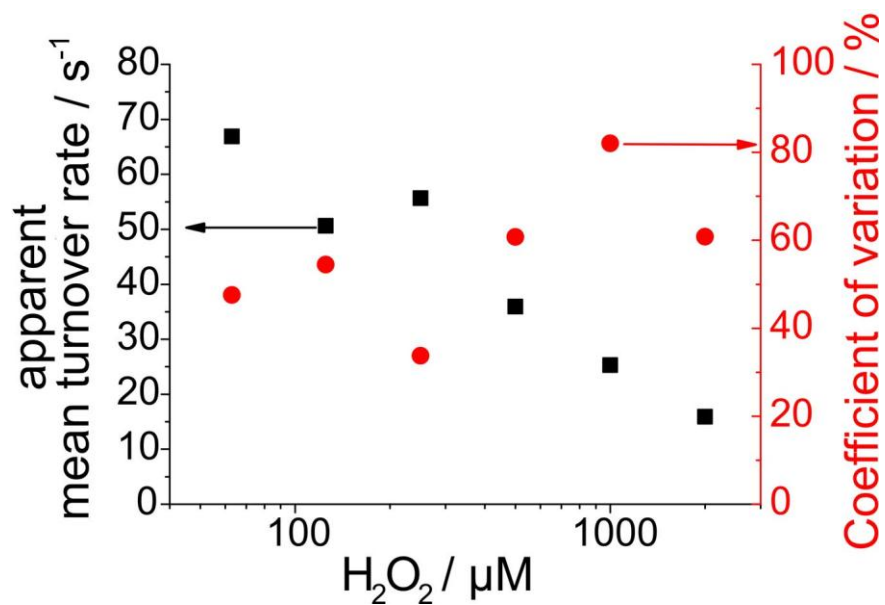
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Supporting Figure 1. Photobleaching of a 1 μM resorufin solution enclosed in the femtoliter array under low power excitation (neutral density filter (ND) = 32, exposure time of 1 s, images taken every 30 s).



Supporting Figure 2. Calibration plot of the fluorescence intensity in femtoliter chambers. Standard solutions (1, 2, 4, 8, 10 and 15 μM) of resorufin were enclosed in the array. The red line is a linear regression of the data points (three measurements / standard deviation). The number of resorufin molecules in a well was calculated by: $\text{FI} = 0.00125 \times (\#\text{molecules})$



Supporting Figure 3 Summary of the mean turnover rates (black) and the respective coefficients of variation (red) of the Gaussian distributions shown in Figure 3.

Supporting Movie 1: Time-lapse of resorufin formation by individual HRP molecules in the femtoliter array. Sequential fluorescence images were taken with a sCMOS camera every 30 s with low excitation light (ND = 32) and an exposure time of 1 s. Chambers that contain a single molecule of HRP can be clearly identified. Three images of this movie are shown in Figure 2a.