Supporting Information

Agresti et al. 10.1073/pnas.0910781107

SI Text

Optics. A 488 nm solid state laser (Newport) is reflected off of a laser-line dichroic mirror (Semrock) into the back port of a standard inverted fluorescence microscope (Motic) where the arc-focusing optics have been removed. The fluorescence cube in the microscope contains only a 650 nm long-pass dichroic mirror (Semrock) to reflect both the excitation and emission light. The excitation is focused by a microscope objective on to the flow channel of the sorting device at the point adjacent to the gap between the electrodes. Emitted fluorescence is collected by the same objective and reflected out the back of the microscope through the laser-line dichroic and a 590 ± 30 nm filter. The filtered light is focused by a 25 mm focal length planoconvex lens through a ~0.5 mm hole formed by a closed iris onto the detection window of a photomultiplier tube (Hamamatsu). Using this system, we achieve nanomolar sensitivity. Although this is adequate for our current applications, we are aware that the optics could be easily optimized for sensitivity by using a higher N.A. objective, a more sensitive detector, and better stray light shielding.

Kinetics. For the screens between rounds to evaluate enrichment, and the activity screens of single mutants, we diluted induced cells to a final density of 2×10^5 mL^{-1} in a total volume of 200 μL using the same buffer and substrate concentrations as the drop screens: PBS containing 0.1% BSA, 0.5 mM mannose with substrate concentrations of 100 μM Amplex Ultrared (AUR) and 300 μM H_2O_2. We added the substrates to start the reaction and measured the initial rate of absorbance increase at 568 nm.

To determine the kinetic constants, we measured the initial rates of yeast-displayed enzymes as above at different AUR concentrations between 0.015 and 1.5 mM. We used 300 μM H_2O_2, as in the drop screens, which we know to be well above the K_M for the enzyme. In this way, the H_2O_2 is saturating, and we measure the pseudo-first-order rate constants. We determine k_{cat} and K_M by plotting initial rates as a function of AUR concentration and fitting to the Michaelis-Menten equation (Fig. S1): $V = (k_{cat}ES)/(K_M + S)$, where V is the initial reaction rate, E is the enzyme concentration, and S is the substrate concentration. We convert the rates to turnovers per second by using the known extinction coefficient of the AUR oxidation product ($57,000$ M^{-1} cm^{-1}) and an enzyme concentration of 10 pM, which represents $\sim 1.2 \times 10^5$ cells each displaying $\sim 10^4$ copies of the enzyme (Table S1). We measured the display levels using flow cytometry[5], and found differences of less than 10% for all mutants.

Justifications of Costs for Table 1

Robots and Plates. We screened 2×10^7 cells in the first round of each generation followed by three or four further round for generation one or two, respectively, where we screened 2.4×10^6 cells each, for a total of $\sim 6 \times 10^7$ total reactions. We calculate the total number of days to complete this using robotics by considering $1/s \times 20$ h/day $= 72,000$/day: equal to 833 days. We calculate the total number of plates as two wells for each reaction, one for growth, one for assay= 1.2×10^8 wells ~300, 000 384-well plates. We calculate the cost of 384-well plates by considering that VWR.com sells plain polystyrene plates at $262 for 100 plates with academic discount. Assume a discount for buying in large numbers ~$2.00 per plate. The total cost of tips, assuming five 200 μL tips for each sample (pick colony, load growth medium, load cell suspension into reaction tube, load substrate, stop reaction). This is conservative because for this particular study, one would have needed to grow the cells, then change the medium to induce the cells, which would take at least two more tips per sample. The cost of VWR.com, house-brand tips with academic discount is $474 for 9,600 10–250 μL tips = 0.049 each, then assume a further discount for buying in large numbers ~0.04. AUR costs $32/mg from Invitrogen. With a molecular weight of 300 g/mol, this works out to 1×10^{-8} moles per reaction at 9.6×10^6 per mole, so $0.096 per reaction. We estimate that a robotic system capable of this level of throughput would require accessories such as plate stackers, incubators, plate readers, a rail system to connect them, and development software. We conservatively estimate a cost of $750,000 for such a system. Amortized over 5 years, this is $410 per day, or $112,000 for the ~2 years that this study would have taken.

Microfluidics. We fabricate 16 drop makers and 8 sorters on each glass slide. The total cost for one polydimethylsiloxane device is approximately $3 assuming ~10 replicas for each new mold, and we use, at most, one-eighth of a device for each experiment. Adding in the cost of a few feet of tubing and rounding up brings us to $0.50. The optics system consists of a low-cost fluorescence trinocular microscope (Motic); $1,500, some filters, mirrors, and optical mounts; $1,500, a photomultiplier tube; $1,000, a computer and labview system $3000, and a laser. We have used sub-$300 "laser pointer" diode lasers (Wicked Laser) with good success in the system, but we assume $3,000 for a laser to bring the total cost to $10,000. Except for the laser and the single cheapest component, the computer, these are all durable goods that will last far more than 5 years, but to be conservative, we amortize them all over 5 years. We estimate 7 h of instrument time, for a total of $1.70. The cost of a few tips, eppendorf tubes, and 150 μL of AUR is less than $0.50.

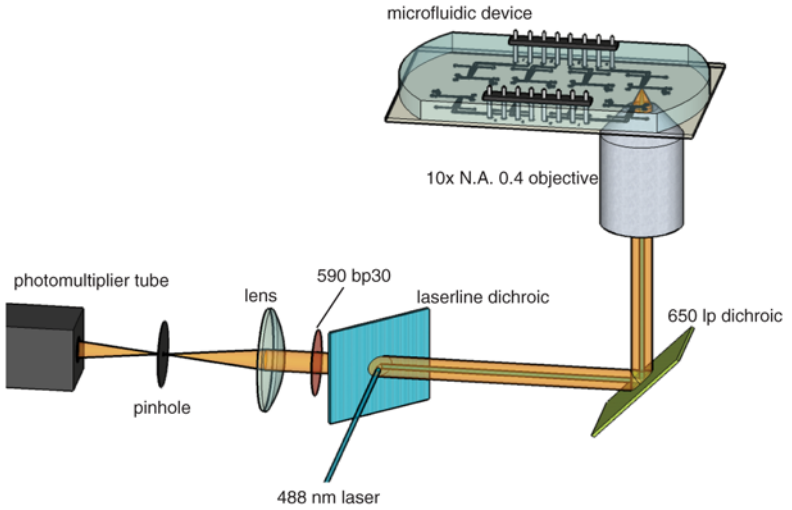


Fig. S1. Schematic of the optical design. A 488 nm laser reflects off of a laser-line dichroic and into the back port of an inverted microscope which contains a 650 long-pass dichroic in a filter cube. The laser is focused on to the device with an objective, and emitted light is collected with the same objective. The emitted light passes through both dichroics and through a 590 bandpass filter before being focused through a pinhole and onto the detection window of a photomultiplier tube. The Kohler illumination light of the microscope is filtered with a 650 nm long-pass filter so that the illumination light passes through the 650 dichroic and the experiment can be seen with the eyepiece and camera port of the microscope without affecting the detection by the photomultiplier tube.

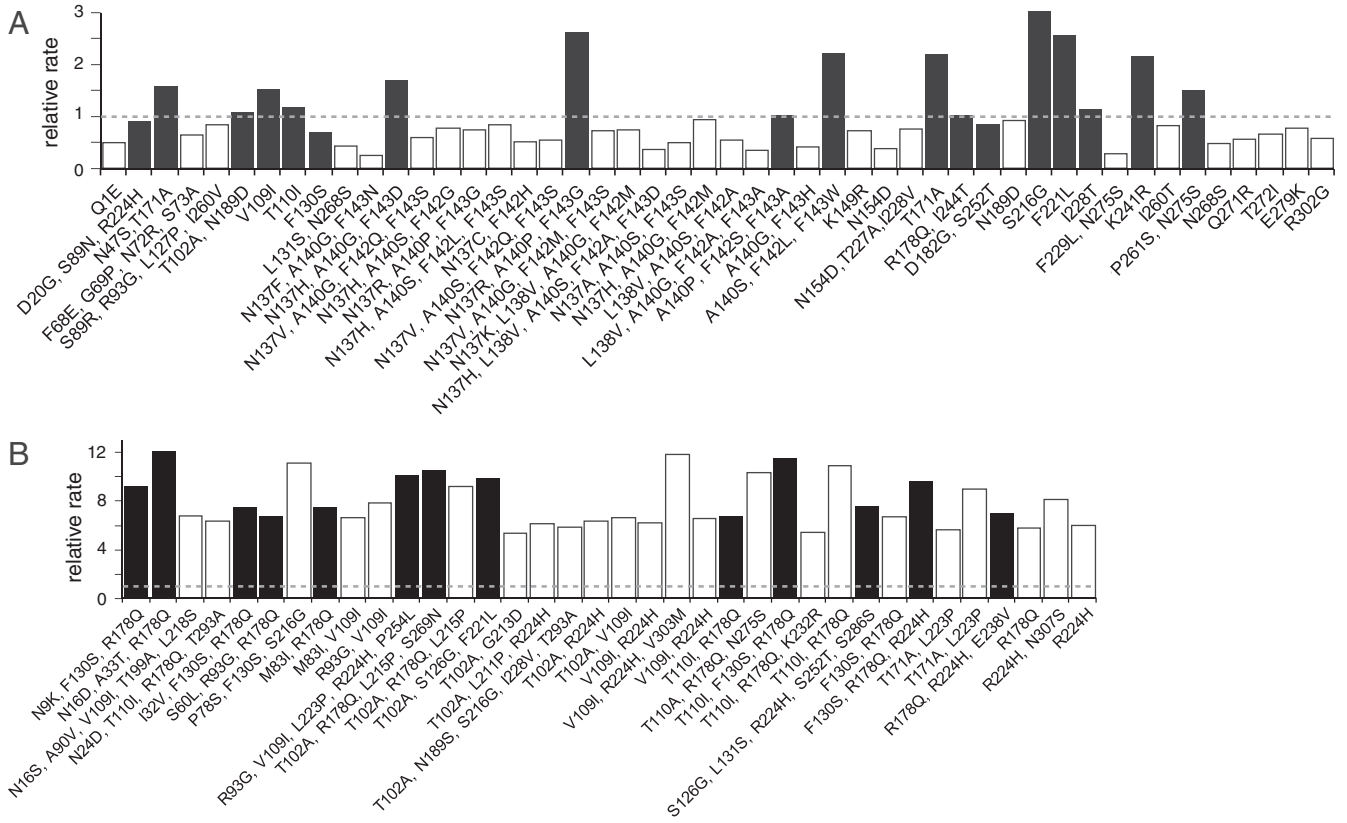


Fig. S2. Unique mutants. (*A*) The 50 unique first-generation mutants (28 from the epPCR library and 32 from the saturation library). We estimate the total number of unique sequences remaining in the populations after enrichment to be 73 [95% confidence interval (CI): 30, 209] and 68 (95% CI: 45, 160) for the epPCR and saturation libraries respectively. The surface-displayed enzyme activities are assayed in multiwell plates using the same substrate conditions as the drop-based screen. The black bars represent clones that were picked as founders of the second-generation. (*B*) The 31 unique mutants from the second-generation libraries. We measured kinetic parameters of the mutants of those with black bars (*SI Text*).

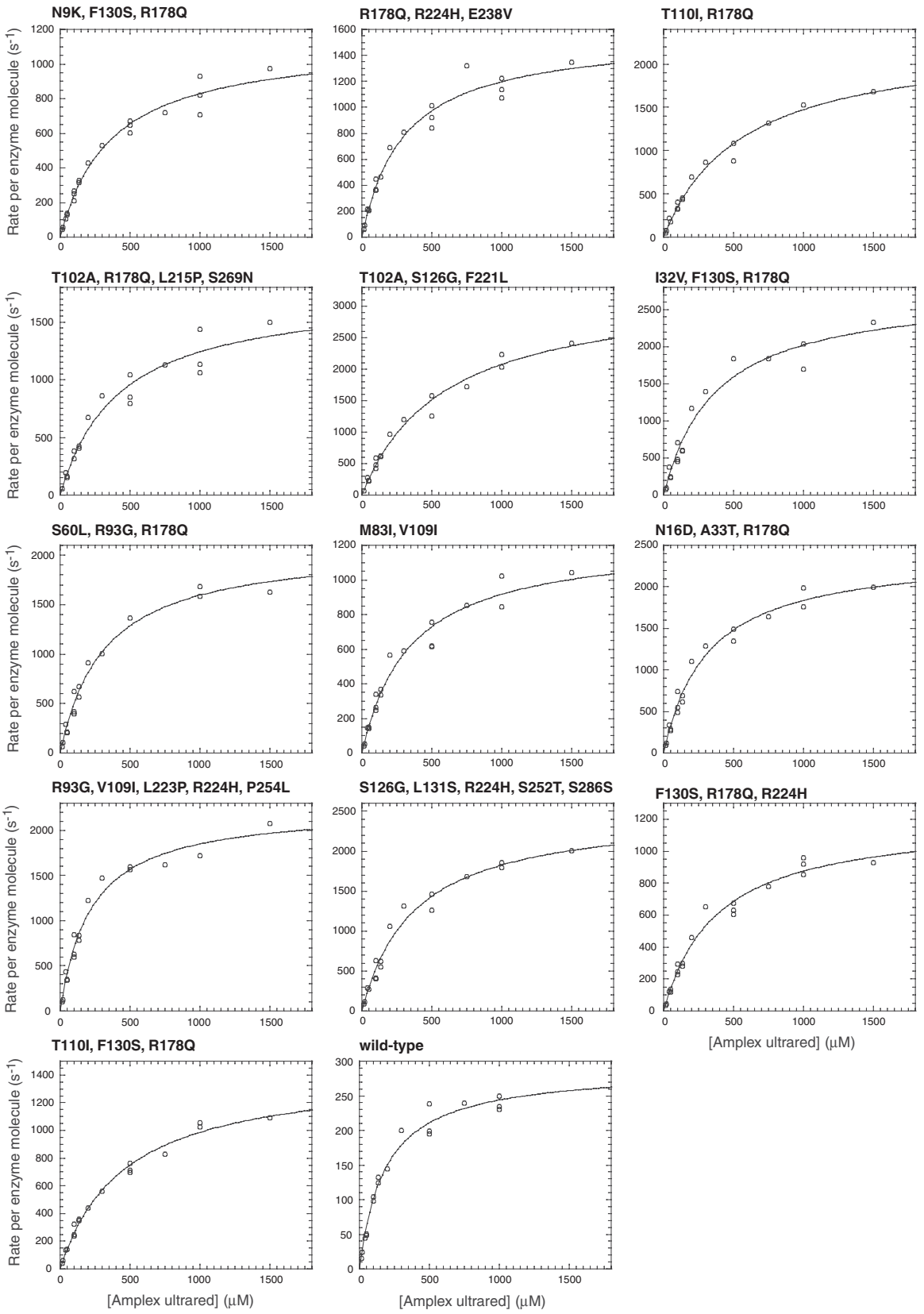


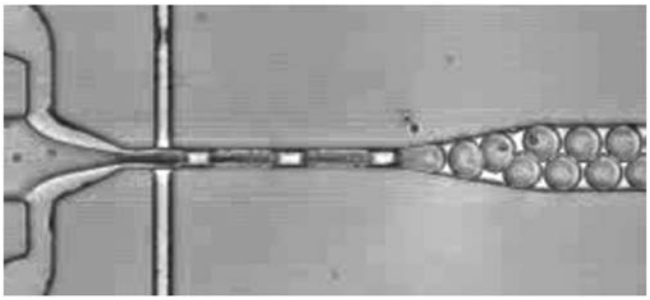
Fig. S3. Michaelis-Menten plots of the top 13 mutants. Extracted values for k_{cat} and K_M are in Table S1.

Table S1. Kinetic constants of selected mutants

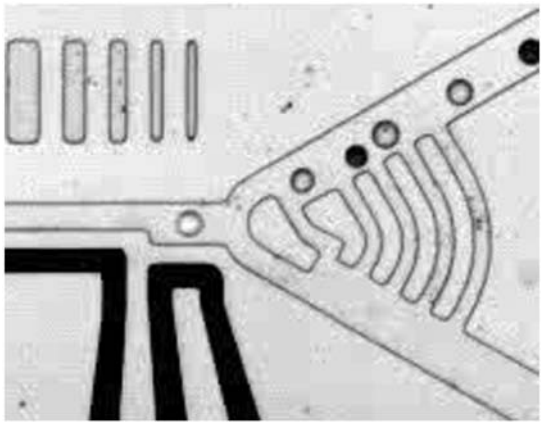
Amino acid substitutions	k_{cat}, s^{-1}		K_M, μM		k_{cat}/K_M, M^{-1} s^{-1}
N9K, F130S, R178Q	2,268	±109	366	±45	6.2×10^6
R178Q, R224H, E238V	3,119	±151	301	±39	1.0×10^7
T110I, R178Q	4,600	±266	561	±67	8.2×10^6
T102A, R178Q, L215P, S269N	3,531	±277	422	±80	8.4×10^6
T102A, S126G, F221L	6,599	±409	588	±77	1.1×10^7
I32V, F130S, R178Q	5,536	±395	369	±65	1.5×10^7
S60L, R93G, R178Q	4,194	±188	314	±35	1.3×10^7
M83I, V109I	2,467	±148	345	±52	7.2×10^6
N16D, A33T, R178Q	4,829	±215	315	±36	1.5×10^7
R93G, V109I, L223P, R224H, P254L	4,509	±202	219	±26	2.1×10^7
S126G, L131S, R224H, S252T, N286S	5,017	±262	373	±48	1.3×10^7
F130S, R178Q, R224H	5,363	±413	381	±71	1.4×10^7
T110I, F130S, R178Q	2,875	±109	457	±40	6.3×10^6
Parent	578	±20	185	±19	3.1×10^6
Literature value[1]	860	±38	108	±13	8.0×10^6

Kinetic constants of 13 mutants from generation two. K_M values are for the substrate AUR. All values are measured under pseudo-first-order conditions with saturating concentrations of the oxidizing substrate H_2O_2, and are therefore considered *apparent*. Numbers after "±" are the standard error.

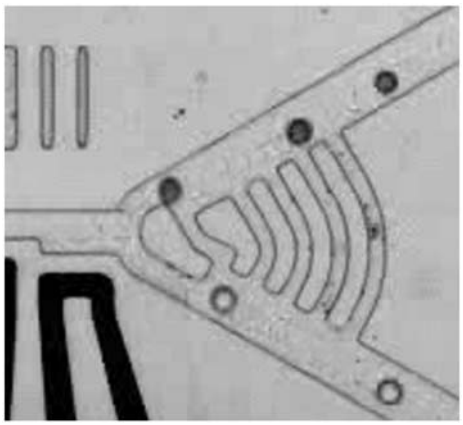
[1]Fruk L, Muller J, Niemeyer CM (2006) Kinetic analysis of semisynthetic peroxidase enzymes containing a covalent DNA-heme adduct as the cofactor. *Chemistry*, 12(28):7448–7457.



Movie S1. Encapsulation of yeast in drops. A suspension of yeast cells displaying the enzyme horseradish peroxidase on their surface is combined with a second aqueous stream containing the fluorogenic substrate AUR to form drops with ~23 μm diameter. Drop rate, ~2, 000/s. Capture rate, 8, 000/s.
Movie S1 (AVI)



Movie S2. An emulsion containing a mixed population of light and dark drops of ~23 μm in diameter is flowed into the drop-sorter device. Light drops contain 1 mM fluorescein and dark drops contain 1% wt/wt bromophenol blue in water. "Sorting off": In the absence of any external force, all drops flow into the waste channel.
Movie S2 (AVI)



Movie S3. An emulsion containing a mixed population of light and dark drops of ~23 μm in diameter is flowed into the drop-sorter device. Light drops contain 1 mM fluorescein and dark drops contain 1% wt/wt bromophenol blue in water. "Sorting on": When a fluorescent drop passes the laser, it triggers a voltage to be applied to the electrodes, and the drop flows into the keep channel. Capture rate: 4, 000 frames/s^{-1}.
Movie S3 (AVI)