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Additional discussion

Control reactions with alkaline phosphatase (MW 46 kDa, E.C 3.1.3.1) confirmed that enzymatic acceleration in the VFD arises from the unique attributes of the device. First, identical enzyme-substrate solutions were centrifuged at 14 krpm (17530 *g*) to mimic the introduction of centrifugal forces exceeding levels expected inside the VFD sample tube; no increase in enzymatic activity resulted from centrifugation (Figure S2). Second , as a control for simple vortexing without a thin-film, the enzyme-substrate solution (1.3 mL in a 15.0 mL Eppendorf tube) was vigorously mixed by a laboratory vortexer; again, no rate enhancement resulted from this control (Figure S3). Third, as a control for the VFD transforming the substrate without the enzyme present, the four enzymes were omitted during identical VFD processing conditions that generated a VFD-mediated response; no evidence of substrate conversion to product was observed at the substrate concentrations reported here. The possibility of in situ protein folding and generation of higher surface area particles[1] during processing were also eliminated as follows. The enzyme has the same degree of catalytic competence before and after VFD treatment, eliminating the possibility that the VFD restores correct structure (Figure S4). Furthermore, DLS analysis shows no decrease in particle size following VFD processing (Table S1).

Additionally, the reported acceleration in enzymatic catalysis likely does not result from a temperature increase during VFD processing. We apply short processing times with a dual bearing device to limit temperature variation due to friction. Monitoring by thermal imaging IR camera has shown that the temperature increase for such conditions is <2 °C.[2] In the thermal image below (Figure S1A), we demonstrate that after 1 h of processing the sample tube remains at slightly above ambient temperature with any heat generation being localized to the upper bearing (Figure S1A). Furthermore, the device has been fitted with two O-rings, both on the lower bearing that limits heat transfer from the bearing to the sample tube (Figure S1B). Additionally, experiments were conducted on a 1.30 mL scale to prevent fluid in the sample tube from reaching same height as the the upper bearing, where heat transfer into the fluid could take place (Figure S1C). To further demonstrate the lack of significant heating by the VFD, the solution temperature was measured directly by a thermometer in 1.30 mL of H_2O processed by the VFD at 8 krpm for 1 h. The temperature of the solution rose slightly from 23.0 to 25.0 °C. As an additional consideration, the enzyme DERA can be accelerated on average ≈15-fold compared to the non-VFD-processed control; a temperature driven acceleration of 15-fold would require a dramatic change in temperature (tens of degrees). Such a temperature increase has not been observed. Thus, the reported enzyme rate acceleration likely results from the Faraday waves produced through sample tube vibration, not increased temperature.

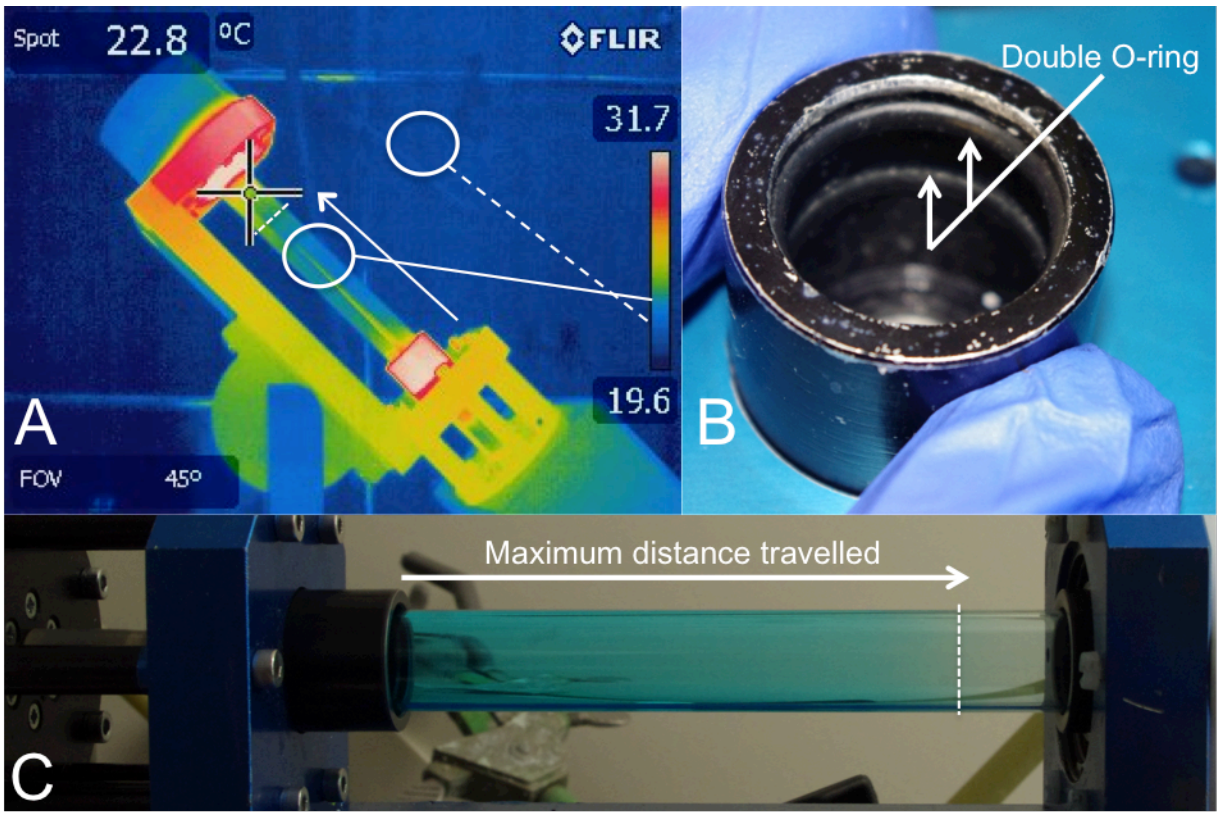


Figure S1. Control experiments and VFD features preventing friction-based temperature increases from impacting enzyme assays. A) A thermal IR (FLIR) image of the device operating at a 8 krpm rotational speed after 1 h of processing. The heat generated is localized to the upper and lower bearing, and the sample tube remains at roughly ambient temperature (23 °C). **B)** The lower bearing contains two O-rings that stop direct contact of the bearing to the sample tube. This design modification limits heat transfer to the sample tube and allows the sample tube to remain at ambient temperature for sustained periods of time. **C)** In this experiment, 1.30 mL of blue food coloring, an identical volume to reactions reported here, was rotated at 8 krpm and the distance the fluid travelled was monitored. This chosen volume ensures processed solutions do not enter into the upper bearing region and become exposed to slightly elevated temperatures.

Centrifuge control

The enzyme-substrate solution (alkaline phosphatase and *p*-nitrophenol phosphate) was subjected to centrifugation in a bench top centrifuge at the indicated rotational speeds for 10 min. A direct comparison was then made between a VFD sample and a centrifuged sample (Figure S2).

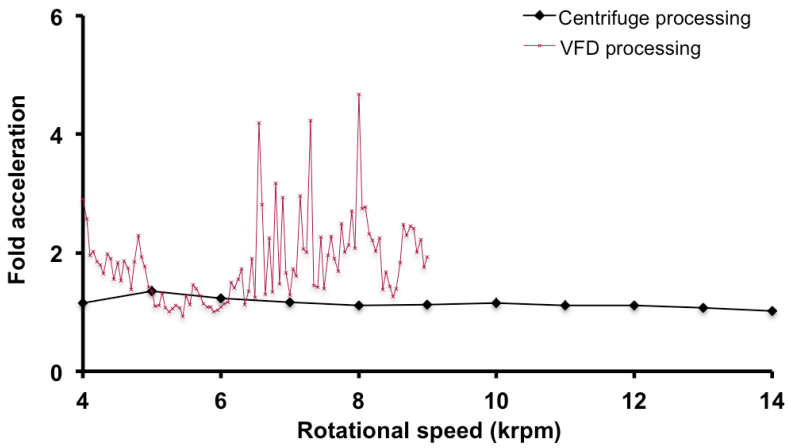


Figure S2 Centrifugation compared to VFD-mediated processing. The centrifuged sample has no observed rate acceleration at any rotational speed.

Control reaction in a conventional vortexer

The enzyme-substrate solution (1.3 mL) (β-glucosidase and 4-nitrophenyl β-D-glucopyranoside) was added to an Eppendorf tube, and vortexed for 10 min at 25 °C before quenching as described below. No increase in enzyme rates comparable to the VFD-processed solution was observed (Figure S3).

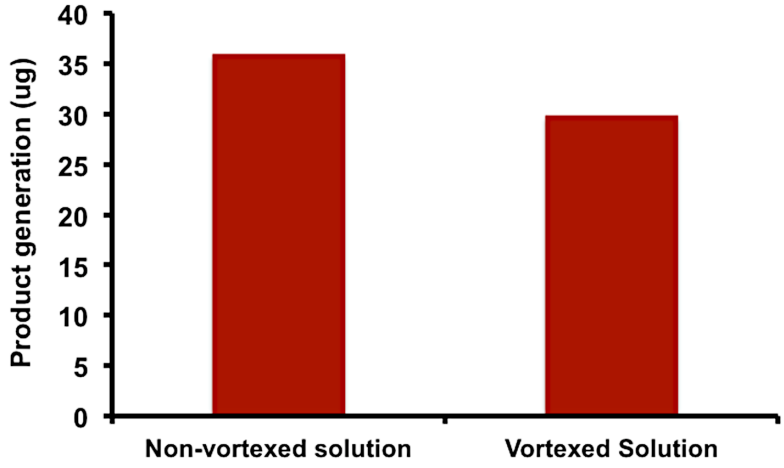


Figure S3 Vortexed enzyme-substrate solution in a conventional bench top vortex. In this experiment, β-glucosidase (77 nM solution, 325 µL) and 4-nitrophenyl β-D-glucopyranoside (0.01 M solution, 975 µL) were used to examine the effects of vortexing on enzyme activity. In this example, the fold acceleration was not calculated, as the conventional vortexer decreases enzyme activity.

VFD-processed *vs*. non-VFD-processed enzyme solutions for analysis in a kinetic assay

To ensure that protein folding was not driving enzymatic acceleration, a comparative kinetic study was performed with VFD-processed and non-VFD-processed β-glucosidase and 4-nitrophenyl β-D-glucopyranoside. In this experiment, β-glucosidase (77 nM solution, 2.00 mL) was

added to a sample tube and rotated at 7850 rpm for 10 min. The enzyme was collected and 0.500 mL of treated enzyme added to 0.500 mL of 0.01 M 4-nitrophenyl β-D-glucopyranoside. The solution was immediately mixed and 100 µL was added to a 96-well micro plate for kinetic analysis. As a control, non-VFD-treated enzyme was also tested under identical conditions. There was no significant difference in activities between VFD-processed and non-VFD-processed enzymes.

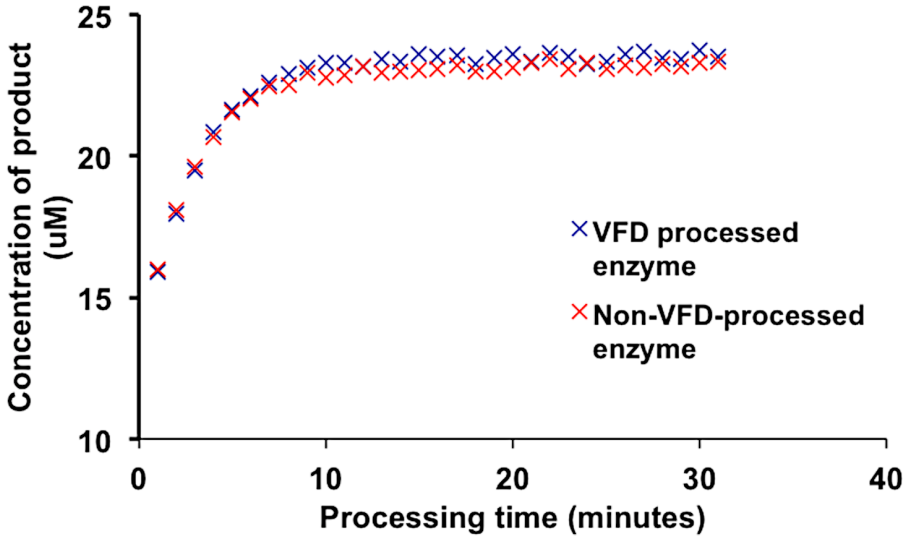


Figure S4 The effect of VFD-processing on the enzyme before the enzymatic catalysis. After 10 min of VFD processing, the conversion of 4-nitrophenyl β-D-glucopyranoside to 4-nitrophenol catalyzed by β-glucosidase was monitored at 351 nm. At this wavelength, the unquenched reaction can be monitored. The concentration of β-glucosidase was 19.3 nM and its substrate 4-nitrophenyl β-D-glucopyranoside was 7.5 mM.

VFD-processed enzyme *vs*. non-VFD-treated enzyme Dynamic Light Scattering (DLS) measurements

To examine particle size after VFD-processing, DLS measurements were performed on VFD- and non-VFD-processed enzyme solutions. β-glucosidase (77 nM solution, 2.00 mL) was added to a sample tube and rotated at 7850 rpm for 10 min. The enzyme was collected and then centrifuged (24154 *g*) for 20 min. The solution was then passed through a syringe filter (0.22 µm, 30 mm diameter) into a DLS cuvette and the measurements performed. The results indicated that there are imperceptible differences between the samples (Table S1).

Table S1 DLS data. The values displayed in the table below compare the VFD-processed β-glucosidase solution to the non-VFD-processed enzyme solution. *PDI* – Polydispersity index, *d.nm* – mean diameter of particles (nm).

Sample	PDI	Peak 1 (d.nm)	Peak 2 (d.nm)
Non-VFD processed	0.491	177.2	5271
VFD-processed	0.431	176.8	5295

Supplemental Materials and Methods

Unless otherwise indicated, all commercially available reagents and solvents were used directly from the supplier without further purification. Technical grade solvents and silica gel (60-120 mesh) were used for column chromatography with visualization accomplished with UV light (254 nm) and/or a potassium permanganate solution (40 g K_2CO_3, 600 mL of water, 6.0 g $KMnO_4$ and 5.0 mL 2.0 M NaOH). ^1H NMR and ^{13}C NMR spectra were recorded at ambient temperature using $CDCl_3$ (7.27 ppm) or D_6-DMSO (2.50 ppm), unless otherwise indicated on a Brüker 400 MHz spectrometer. Chemical shift values are expressed as parts per million (ppm) and *J*-values are in Hertz (Hz). Splitting patterns are indicated as s:singlet, d:doublet, t:triplet, q:quartet, hex:hextet or combination, br.s:broad singlet or m:multiplet. The vortex fluid device (VFD) sample tubes were commercial quality borosilicate glass, with an internal diameter of 17.7 mm, and were cleaned with piranha solution (4:1, sulfuric acid: H_2O_2), rinsed with diH_2O, dried using acetone, and stored in an oven at 160 °C prior to usage. FTIR spectra were collected using Perkin Elmer at 25 °C. Optical rotation was measured using a Perkin Elmer device at RT using a 1.0 dm^3 glass cell. All buffered solutions were prepared with double-deionized water (diH_2O, >18 MV) from a Milli-Q water system (Millipore, Bedford, MA).

Composition of non-VFD solutions

In a 2.0 mL Eppendorf tube, enzyme and substrate in the appropriate buffer (below) were mixed (final volume of 1.3 mL). The reaction proceeded for the indicated length of time and then quenched as described below. A 100 µL aliquot of the reaction mixture was transferred to a 96-well plate (Corning, UV transparent, pathlength of 0.375 cm) and the absorption was measured at the required wavelength (MicroQuant; Biotek Instruments, Winooski, VT).

General VFD-mediate enzyme acceleration

The outside length of the sample tube was lubricated with Dow Corning high vacuum grease before insertion into the VFD. A solution of enzyme and substrate in buffer (1.30 mL) identical to the comparison described above was added to this tube. The sample tube was then stoppered with a B19 Suba Seal™ cap, and the tube rotated about its axis at the specified tilt angle for the indicated length of time. Immediately after rotation, the solution was added to the quenching reagent to terminate the reaction. The tube was then rinsed with the buffer and re-used in subsequent experiments. To measure enzyme activity in this VFD-processed sample, 100 µL of the solution was added to a 96-well plate, and the absorption measured at the specified wavelength (below). A ratio of this activity to activity of the solution from the non-VFD treated sample determined the level of VFD-mediated enzyme enhancement. All enzyme measurements were performed in triplicate unless otherwise stated.

Enzymes, Buffers and Assays

Alkaline phosphatase

Buffer. The enzyme buffer, 1.0 M diethanolamine, was prepared as follows: 140 g of diethanolamine was added to 1.0 L of H_2O, then the pH of the solution was adjusted to pH 9.8 at 25 °C with 5 M HCl. This buffer was further diluted to 1 M diethanolamine, and 500 µL of 1 M $MgCl_2$ was added. The resulting buffer was filtered-sterilized through a 0.22 µm filter (Corning), and stored wrapped in aluminum foil at 4 °C.

Assay. Fast thermosensitive alkaline phosphatase (1.0 µL, 0.11 mM) was added to 10 mL of the diethanolamine buffer to generate an enzyme stock solution (11.1 nM). This solution was made fresh every two hours and stored on ice. Each sample was prepared by combining 0.800 mL of the enzyme stock solution and 0.500 mL of alkaline phosphatase liquid substrate system (pNPP, Sigma-Aldrich, 0.435 mM); this solution (1.3 mL) was added to either an Eppendorf or VFD sample tube. The reaction was incubated at 25 °C for 10 min unless otherwise indicated. After this time period, 4.0 M NaOH (150 µL) was added to quench the reaction. The sample (100 µL) was then transferred to a 96-well micro plate reader, and the absorbance measured at 402 nm (λ_{max}). The molar absorption coefficient of *p*-nitrophenol after the quench described above was 15644 M^{-1} cm^{-1}.

β–Glucosidase.

Buffer. 50 mM sodium acetate, pH 5.0 buffer was prepared as follows: 4.37 g sodium acetate (anhydrous) was dissolved in 1.0 L diH_2O and ≈1.1 mL of glacial acetic acid to generate a buffer of pH 5.0 at 25 °C. The buffer was then filtered-sterilized through a 0.22 µm filter and stored at 25 °C.

Assay. In a 15 mL falcon tube, 5.0 mg of lyophilized β-glucosidase enzyme (Sigma) was re-suspended in 10 mL of 50 mM sodium acetate, pH 5.0 buffer. From this solution, a 100 µL aliquot was transferred to 10 mL of 50 mM sodium acetate, pH 5.0 buffer to create a 77 nM solution. The substrate solution consisted of 0.01 M 4-nitrophenyl β-D-glucopyranoside (31.25 mg) in 10 mL of 50 mM sodium acetate, pH 5.0 buffer. Each sample was prepared by combining 0.325 mL of the enzyme stock solution and 0.975 mL of the substrate stock solution; this solution (1.30 mL total volume) was added to either an Eppendorf or VFD sample tube. The reaction was performed for 10 min unless otherwise indicated. Then, a solution of 0.70 M glycine, NaOH, pH 10.8 buffer (200 µL) was added to quench the reaction. The sample was then transferred to a 96-well micro plate reader, and the absorbance was measured at 405 nm. The molar absorption coefficient of *p*-nitrophenol after the quench described above was 9413 M^{-1} cm^{-1}.

Esterase.

Buffer. 50 mM phosphate, pH 7.0 buffer was prepared as follows: 1.459 g of monosodium phosphate and 3.867 g of dibasic phosphate were mixed in 500 mL of diH_2O. The pH of the

resulting solution was adjusted to pH 7.0 at 25 °C with 5.0 M HCl. The buffer was then filtered-sterilized through a 0.22 μm filter and stored at 10 °C.

Assay. In a 15 mL falcon tube, 64 mg of *p*-nitrophenylacetate was re-suspended in 10 mL of ACS Reagent Select™ grade methanol (Sigma) to generate a stable solution that was stored at 4 °C. Then, 3.0 mL of this solution was added to 100 mL of H_2O with rapid mixing before further dilution with 100 mL phosphate buffer (50 nM, pH 7.0) in generating a 0.052 mM stock solution.

The enzyme solution for esterase was prepared as follows. To a 15 mL falcon tube, 5.0 mg of esterase was added, and dissolved in 10 mL phosphate buffer to create a working solution of 0.806 mM. This stock solution was further diluted to produce a 0.806 nM working solution. Each reaction used 1.10 mL of substrate solution and 0.20 mL of enzyme solution (1.3 mL total volume). Unless otherwise indicated, reactions were incubated for 20 min. To quench the reaction, 1.0 mL of propan-1-ol was added. 100 μL of the sample was transferred to a 96-well reader microtiter plate, and the absorbance measured at 405 nM. The molar absorption coefficient of *p*-nitrophenol after the quench described above was 6423 M^{-1} cm^{-1}.

DERA.

Enzyme assays and also protein production of py-DERA, referred to as DERA, were adapted from previous publications.[3]

Enzyme Production.

Enzyme Assay Buffer. 100 mM bis-tris propane, pH 8.5 buffer was prepared as follows. 28.23 g bis-tris propane was dissolved into 900 mL nanopure water. The solution was adjusted to pH 8.5 at 25 °C with ≈5 mL of conc. HCl and a final volume of 1 L. The solution was filtered-sterilized through a 0.22 μm filter and stored at 25 °C.

Assay. The substrate solution was generated by re-suspending 30 mg of 7-deoxyribosyl-4-methylumbelliferone in 3.0 mL of DMSO. The volume was adjusted to 15 mL with 100 mM bis-tris propane, pH 8.5 to generate a final 6.80 mM solution in 20% DMSO. The substrate solution was wrapped in aluminum foil and stored at 25 °C.

Enzyme Solution. After dialyzing the recombinant py-DERA into 100 mM bis-tris propane, pH 8.5, the protein concentration was determined by A_{280} using (ε = 16305 $M^{-1}cm^{-1}$) and a MW of 30906.5 g/mol. A working stock of 8.33 μM enzyme solution was prepared by either diluting with 100 mM bis-tris propane, pH 8.5 or through microconcentration with a 10 kDa cutoff Amicon Ultra-15 Centrifugal Filter (EMD Millipore, Billerica, MA). The purity of the protein was confirmed by 12% SDS-PAGE (Coomassie brilliant blue stain, Figure S5 below), and the enzyme were assayed with ≥95% homogeneity. For each reaction, 100 μL of the fluorogenic substrate was mixed with 1.2 mL of enzyme solution (1.30 mL total volume). The enzyme reaction was incubated in the VFD or on the bench top for 2 h. To quench the reaction, 30 μL chloroacetaldehyde (≈50% w/v in H_2O) was added. Each sample was measured in triplicate with a total volume of 200 μL in 96-well micro titer plates (96w Costar black/white bottom), and covered with an optically transparent foil (MicroAmp, Applied Biosystems, USA). A fluorescence spectrometer SpectraMax M2 (Molecular

Devices, USA) quantified the release of the 4-methylumbelliferone product; the samples were measured with fluorescence excitation at 360 nm and emission at 470 nm at a constant temperature of 28 °C. The concentration of 4-methylumbelliferone was determined from a calibration curve given below (Figure S15).

Enzymes

Alkaline phosphatase was purchased from Life Technologies (Fast thermosensitive alkaline phosphatase, 1 U/μL, 0.11 mM). β–glucosidase from almonds was purchased from Sigma and Aldrich (Lyophilized powder, 2 U/mg). Esterase from porcine liver was purchased from Sigma and Aldrich (lyophilized powder, ≥15 U/mg protein). These enzymes were used without further purification.

Production of py-DERA

Since py-DERA is not commercially available, the enzyme was prepared using bacterial overexpression as follows.

The gene 2-deoxyribose-5-phosphate aldolase (DERA) from *Plasmodium yoelli* was purchased from Addgene in DH5 alpha cells (Plasmid #25577). The QIAprep Spin Miniprep Kit (Qiagen) was used as directed by the manufacturer to isolate plasmid DNA from an overnight culture of *E. coli* cells. The following PCR parameters and oligonucleotides (Eurofins MWG Operon) were used to amplify the py-DERA gene. iproof DNA Polymerase (BioRad) was used for all PCR amplification steps as directed in the manufacturer's instructions. Ten ng of plasmid #25577 was used as the template for 1 cycle at 95 °C for 1 min, 30 cycles at 95 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min, and 1 cycle at 72 °C for 5 min.

Lig_DERA_Fwd: 5'-GAC GAC GAC AAG ATG GCT AAT TAT ACA GAA AAA TTC GCA GCG TGG TCA G -3'

Lig_DERA_Rev: 5'- GAG GAG AAG CCC GGT TCA TCA CAA TGG ACA TTG AGA AAT AAC TTT TCT CAA TTT TAT CAC TAA TGA TGA TGA -3'.

DNA excised from agarose gels was purified using the QIAquick Gel Extraction Kit (QIAGEN). The purified PCR product was then used with the pET46 Ek/LIC Vector Kit (EMD Millipore Novagen, Billerica, MA, USA) to generate the py-DERA recombinant protein expression vector. *E. coli* TOP10 cells (Invitrogen) were used to isolate the plasmid prior to transformation into other heterologous hosts. Genewiz performed the DNA sequencing.

Expression and purification of py-DERA

The pET46-pyDERA construct was transformed into *E. coli* Rosetta (DE3) cells (Novagen). The transformed cells were transferred to an LB agar plate supplemented with 50 μg/mL kanamycin antibiotic, and incubated at 28 °C for 14-16 h. A seed culture was prepared by inoculating a single colony from the transformation plate in 50 mL of 2YT medium with 50 μg/mL kanamycin antibiotic and shaking the culture at 225 rpm for 14-16 h at 28 °C in a 250 mL baffled

flask. The expression culture was then prepared by inoculating 10 mL of the seed culture in 1.0 L of TB media (12 g Tryptone, 24.0 g yeast extract, 4.0 mL glycerol, 0.17 M KH_2PO_4, 0.72 M K_2HPO_4) with 50 µg/mL kanamycin and shaking the culture at 225 rpm in 28 °C (2.0 L baffled flask). When the optical density of the culture reached A_{600} 0.6, overexpression of py-DERA protein with a C-terminal 6x His-tag was induced through addition of 0.50 mM isopropyl β-D thiogalactopyranoside (IPTG), and the mixture was incubated further for 36 h at 15 °C with shaking at 225 rpm. The cells were harvested and re-suspended in buffer A (50 mM Hepes pH 7.5, 200 mM NaCl, 10 mM imidazole, 10 mM BME, 5% glycerol, 0.5% CHAPS, 250 units benzonase (Sigma), 1.0 mM PMSF, and 1.0 mM benzamidine). The cell lysate was prepared by sonication (Digital Sonifier 450, Branson, USA; std. horn, T <8 °C, 5×10 s pulses, 70% amplitude), followed by centrifugation at 33,*634* g for 60 min to remove cell debris. The supernatant was applied on to Ni^{2+} NTA column pre-equilibrated with equilibration buffer (50 mM Hepes pH 7.5, 200 mM NaCl, 10 mM imidazole, 10 mM BME, 5% glycerol). The column was washed with buffer B (50 mM Hepes pH 7.5, 200 mM NaCl, 30 mM imidazole, 10 mM BME, 5% glycerol), and purified pyDERA protein was eluted using elution buffer (50 mM Hepes pH 7.5, 200 mM NaCl, 100 mM imidazole, 10mM BME, 5% glycerol). Fractions containing purified protein identified by SDS PAGE were pooled and concentrated using microconcentration with a 10 kDa cutoff Amicon Ultra-15 Centrifugal Filter (EMD Millipore, Billerica, MA). The purity of the protein was confirmed using 12% SDS-PAGE (coomassie brilliant blue stain); purification to ≥95% homogeneity was required before subsequent assays. For py-DERA assays the purified recombinant protein was dialyzed in 100 mM Bis-tris propane, pH 8.5. The protein concentrations were determined by A_{280} using 16305 $M^{-1}cm^{-1}$ as the estimated molar extinction co-efficient and 30906.5 g/mol as the MW of the protein (http://www.expasy.org).

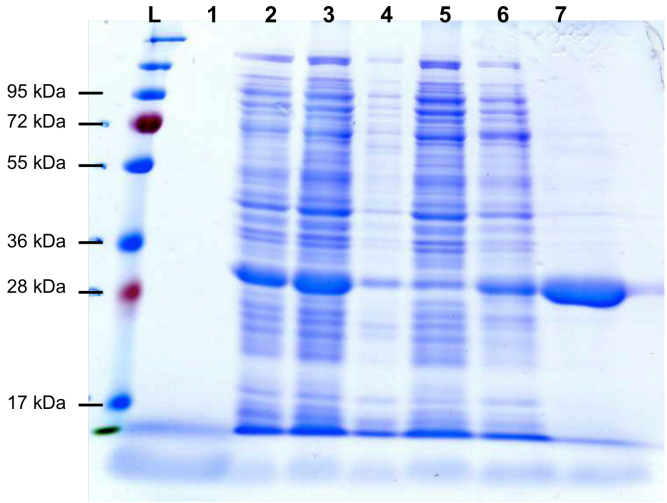


Figure S5 Step-wise purification of DERA. In this 12% Tris-glycine SDS-PAGE, each lane was loaded with 15 µL of sample. **Lane L**. PageRuler Plus pre-stained protein ladder (ThermoFisher Scientific, Waltham, MA). **Lane 1**. 6X SDS loading dye used in all lanes. **Lane 2**. Cell lysate after centrifugation at 15 krpm for 1 h. **Lane 3**. Flow-through after weak anion exchange. **Lane 4.**

Wash following anion exchange chromatography. **Lane 5**. Flow-through from the Ni^{2+} IMAC chromatography purification of the solution visualized in lane 3. **Lane 6**. Wash of the Ni^{2+} IMAC column with buffer B. **Lane 7.** Eluted py-DERA from Ni^{2+} IMAC column with elution buffer (50 mM Hepes pH 7.5, 200 mM NaCl, 100 mM imidazole, 10 mM BME, 5% glycerol). The protein fraction visualized in this lane was dialyzed into the assay buffer before further experiments.

Assessment of protein purity *via* SDS-page

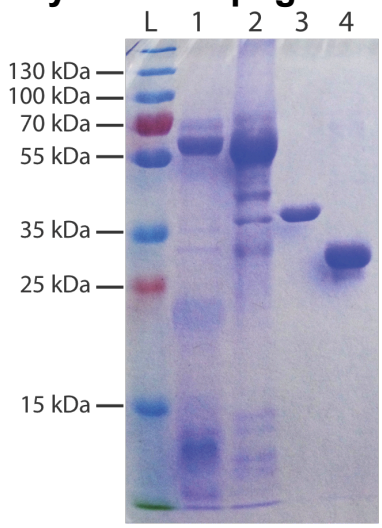


Figure S6 An SDS-PAGE of the enzymes used in this study. In this 12% Tris-glycine SDS-PAGE, each lane was loaded with 5 µL of the indicated enzymes in 6X SDS loading dye. **Lane L**. PageRuler Plus pre-stained protein ladder. **Lane 1**. Lyophilized β−glucosidase (65 KDa) was re-suspended in PBS to generate a 0.154 mM solution. **Lane 2.** Lyophilized esterase (62 KDa) was re-suspended in PBS to generate a 0.161 mM solution (w/v). **Lane 3.** Alkaline phosphatase (36 KDa) was used as purchased from the supplier. **Lane 4**. DERA (30 KDa) was re-suspended in its buffer (as described above) to a final concentration 0.194 mM.

Calculation of alkaline phosphatase concentration and MW

A proprietary enzyme solution, Fast alkaline phosphatase required estimation of its MW by SDS-Page chromatography (Figure S7A) and its concentration by BCA assay (Figure S7B).

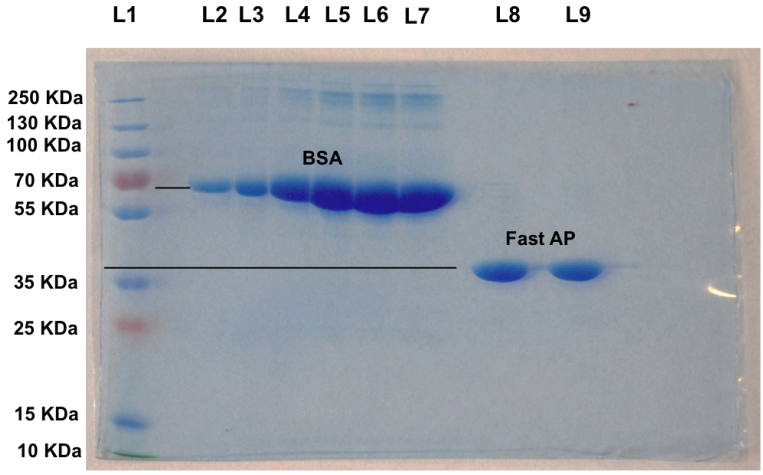


Figure S7A Estimating the MW of commercial Fast alkaline phosphatase. In this 12% Tris-glycine SDS-PAGE, each lane was loaded with 12 µL of protein solution as follows. **L1** PageRuler Plus pre-stained protein ladder. **L2**. 0.45 µM BSA (99% purity), **L3.** 0.75 µM BSA, **L4**. 1.51 µM BSA, **L5.** 3.01 µM BSA, **L6.** 4.52 µM BSA, L7**.** 6.02 µM BSA, and **L8** and **L9.** Identical samples of Fast Alkaline Phosphatase solution. The BSA standards provided an estimation of the enzyme concentration as further confirmed by BCA assay (below). From this experiment, Fast alkaline phosphatase has an estimated MW of 36 KDa.

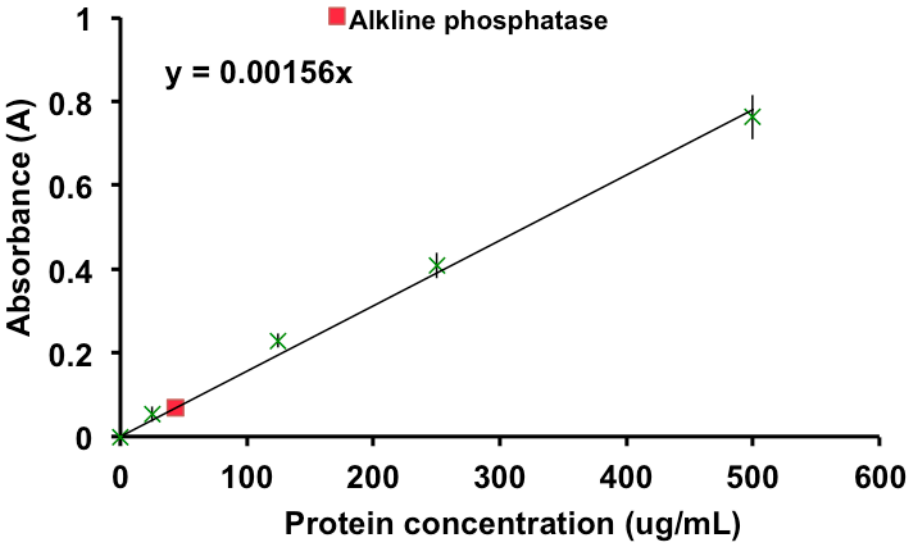
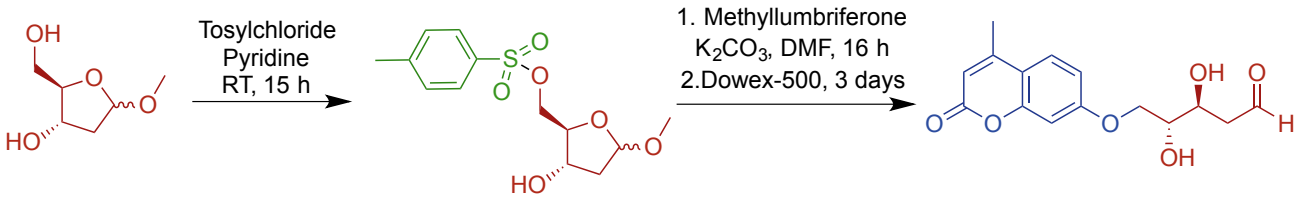


Figure S7B Determining the concentration of Fast alkaline phosphatase as provided by the supplier. For this experiment a BCA assay kit was used (Thermo Scientific). The Fast alkaline phosphatase stock solution was diluted 1:100 solution into PBS to dilute glycerol. The concentration of the stock solution is 0.11 mM.

Synthesis of the fluorophorogenic substrate for the DERA assay



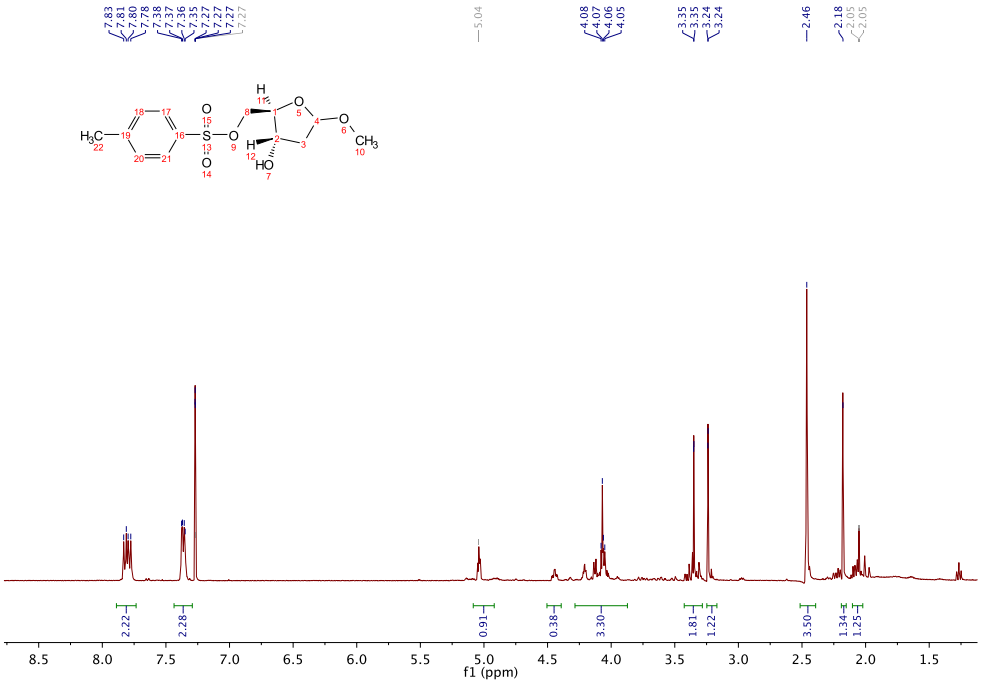
(1) **(2)** **(3)**

The synthesis of the DERA assay substrate (compound **3**) was adapted from the literature[4] as follows.

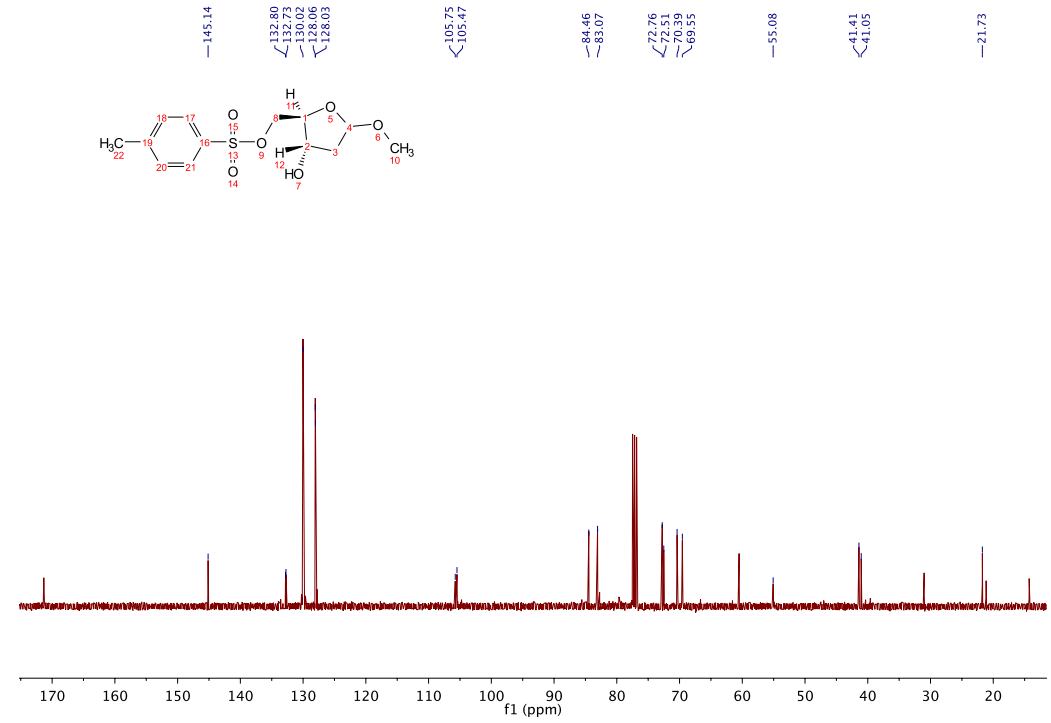
Part 1: Toluenesulfonyl chloride (6.44 g, 33.77 mmol) was added in small portions over the course of one hour to a stirring solution of 1-O-methyl-2-deoxy-D-ribose (5.00 g, 33.77 mmol) in anhydrous pyridine (100 mL). This mixture was stirred vigorously for 15 h. Then, the solvent was removed under reduced pressure to afford a dark gum. The residue was redissolved in EtOAc (150 mL), washed with brine (1.0 M, 50 mL), saturated $NaHCO_3$ (1.0 M, 50 mL), water (50 mL) and then brine again (50 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent removed under reduced pressure to yield a yellow gum. The intermediate product was confirmed by TLC (pure EtOAc, *Rf* – 0.75), and purified by column chromatography (neat EtOAc) to yield crude methyl 5-toluenesulfonyl-2-deoxyribose as an off-white gum, 3.32 g, 33% yield. Compound **2** had ^{1}H and ^{13}C NMR spectra identical to a previous report.[5]

Part 2: Crude methyl 5-toluenesulfonyl-2-deoxyriboside (**2**) (2.00 g, 6.62 mmol) was dissolved in anhydrous DMF (20 mL). K_2CO_3 (1.87 g, 13.24 mmol) and methylumbelliferone (1.47 g, 8.27 mmol) were added to this solution, which was then stirred at 75 °C for 15 h. Next, water (75 mL) was added, and the product extracted with EtOAc (2 x 50 mL). The organic layer was washed with NaOH (0.1 M, 50 mL), water (20 mL) and then dried using anhydrous $MgSO_4$. The product solution was concentrated *in vacuo*, and suspended in acetonitrile (5 mL) and water (15 mL). Dowex-WXD-100 (500 mg) ion exchange resin was added, and the solution stirred for 2 h. Next, the solution was briefly exposed to reduced pressure to remove any generated methanol. The remaining solution was the stirred for two days, filtered and then concentrated under vacuum. The product was purified via column chromatography with an EtOAc:acetone gradient running from 100:0 to 80:20. TLC confirmed the product purity (EtOAc, *Rf - 0.46*). The product **(3)** was isolated as a thick colorless oil, which formed a white foam under vacuum, 440 mg, 24% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.70 – 7.67 (m, 1 H, C<u>H</u>-1,4-enone), 7.01 – 6.95 (m, 2 H, C<u>H</u>-Aryl), 6.29 (d, *J* = 4.00 Hz, 0.30 H, C<u>H</u>$_\alpha$), 6.21 (s, 0.82 H, C<u>H</u>), 6.17 (d, *J* = 4.00 Hz, 0.51 H, C<u>H</u>$_\beta$), 5.43 – 5.39 (0.42 H, C<u>H</u>$_\alpha$), 5.35 – 5.31 (0.46 H, C<u>H</u>$_\beta$), 5.16 (d, *J* = 4.00 Hz, 0.69 H) 4.27 – 3.92 (m, 4 H), 2.89 (s, 0.20 H_α), 2.73 (s, 0.21 H_β), 2.40 (s, 3 H), 2.37 – 2.33 (m, 1 H), 2.00 – 1.61 (br.m, 2 H); ^{13}C NMR; (100.0 MHz, DMSO-d_6) δ_c 161.6, 160.2, 154.7, 153.4 (<u>C</u>H-1,4-enone), 126.5 113.2, 112.4, 111.2, 101.3, 98.0, 97.0 (<u>C</u>H aryl), 82.9, 80.9, 70.9, 70.7, 70.5, 69.0, 42.1, 39.7, 38.9, 30.7 and 18.1; FTIR

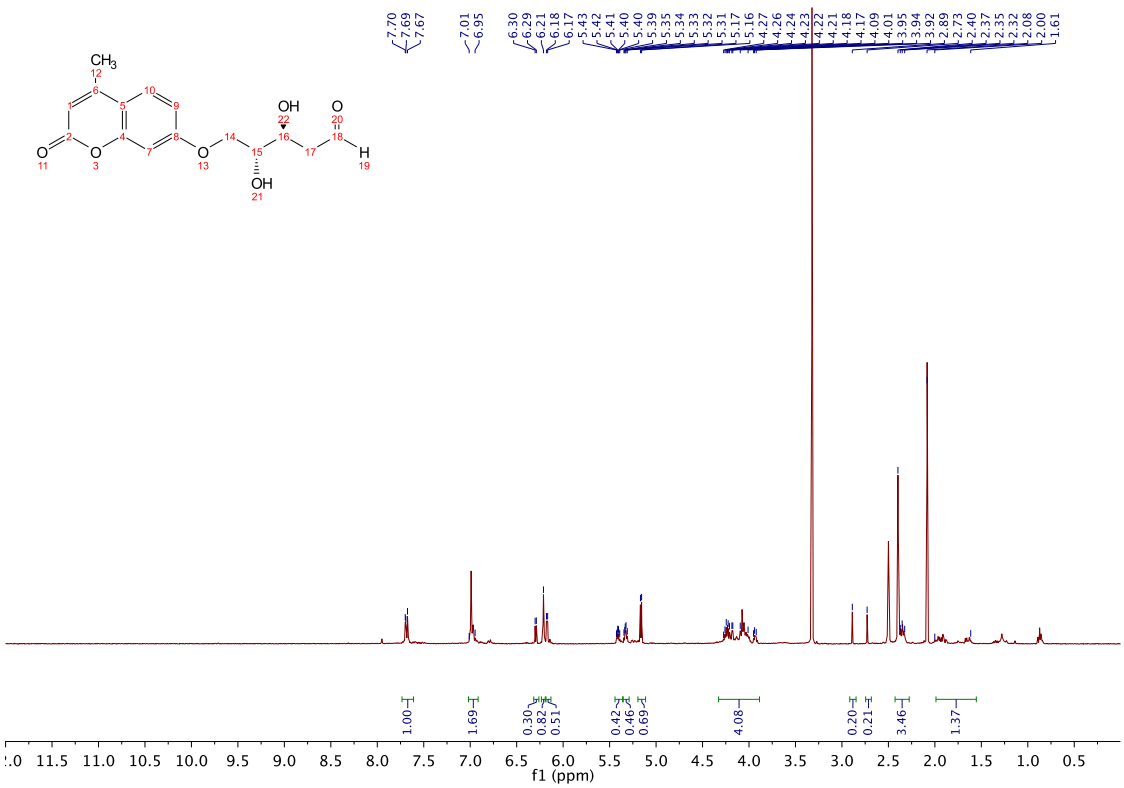
(cm^{-1}) 3400, 2928, 1699, 1608, 1557, 1511, 1427, 1389, 1368, 1281, 1266, 1204, 1149, 1069, 1016, 956, 845, 748, 706 and 636; $[\alpha]^{24}_{D}$ = +29 (c = 0.0016 g/mL, CH_3OH). EIMS calc; 294.1 $[M+Na]^+$, found; 317.1. This compound (**3**) had ^1H and ^{13}C NMR spectra identical to the previous report.[3]



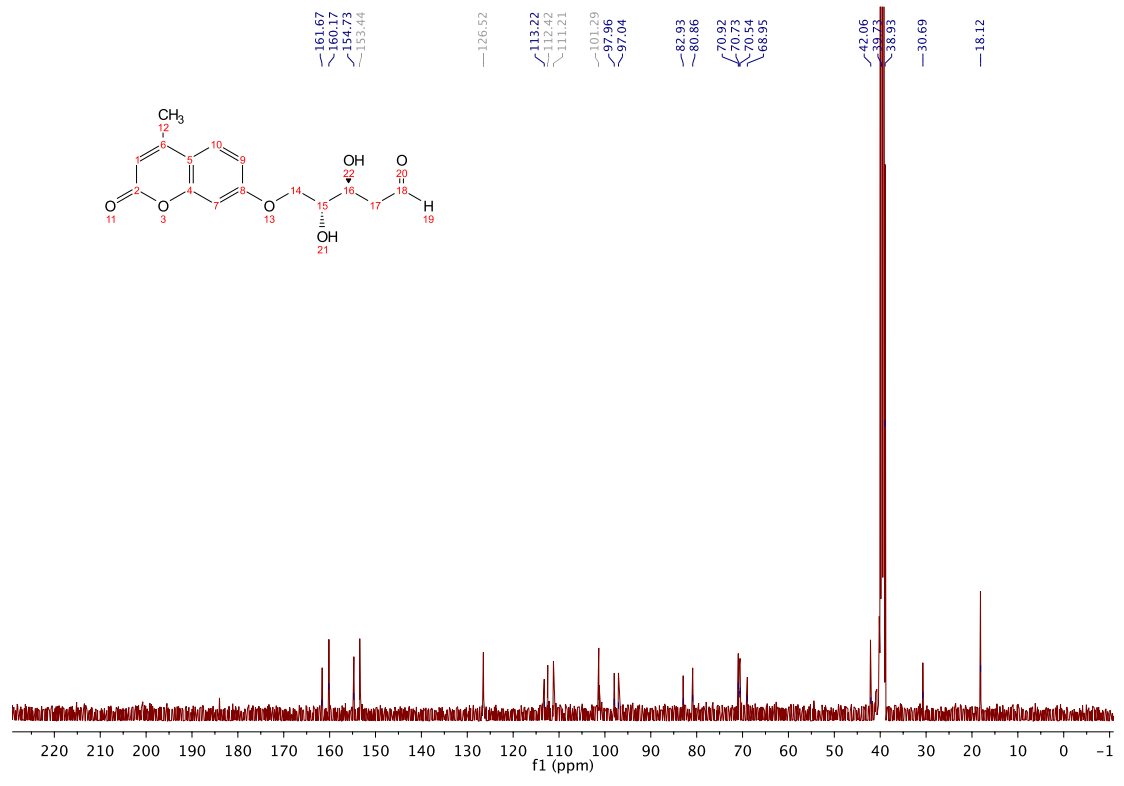
Spectra A ^1H NMR spectra of methyl 5-toluenesulfonyl-2-deoxyriboside (**2**) in $CDCl_3$.



Spectra B ^{13}C NMR spectra of methyl 5-toluenesulfonyl-2-deoxyriboside (**2**) in $CDCl_3$.



Spectra C ^{1}H NMR spectra of the compound **3** in D_6-DMSO.



Spectra D ^{13}C NMR spectra of compound **3** in D_6-DMSO.

Reaction times for VFD-mediated acceleration

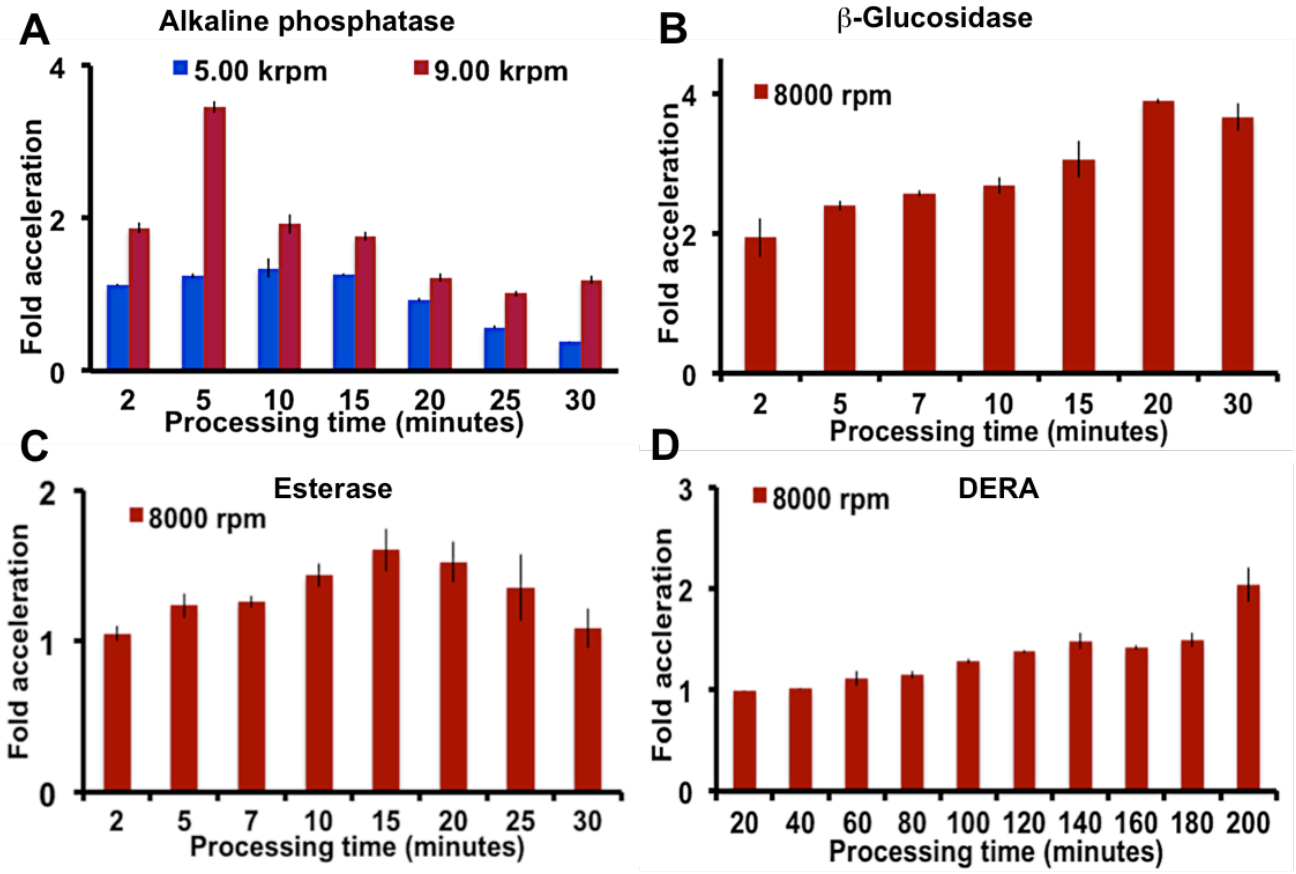


Figure S8 Variation in VFD-mediated reaction times at various rotational speeds for the four enzymes. **(A)** Alkaline phosphatase **(B)** β-glucosidase **(C)** esterase and **(D)** DERA were processed as previously described, and the fold acceleration calculated through comparison to identical enzyme-substrate solutions that were not VFD-processed. Error is indicated as standard deviation around the mean (n=3). The concentration of enzyme and substrate used in the above experiment are as follows (1.30 mL total volume): Alkaline phosphatase (6.77 nM) and its substrate *p*-nitrophenol phosphate (0.167 mM), β-glucosidase (19.3 nM) and its substrate 4-nitrophenyl β-D-glucopyranoside (7.5 mM), esterase (0.12 nM) and its substrate *p*-nitrophenol acetate (44 μM) and DERA (7.69 μM) and its fluorogenic substrate (0.52 mM)

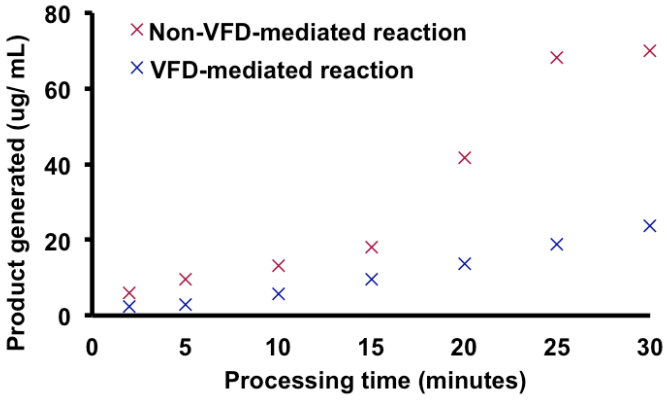


Figure S9 Production of *p*-nitrophenol catalyzed by alkaline phosphatase monitored at 405 nm. Error is indicated as standard deviation around the mean (n=3). The concentrations of alkaline

phosphatase was 6.77 nM) and its substrate *p*-nitrophenol phosphate 0.167 mM. A total volume of 3.0 mL was used in this experiment.

Simultaneously varying enzyme and substrate concentration

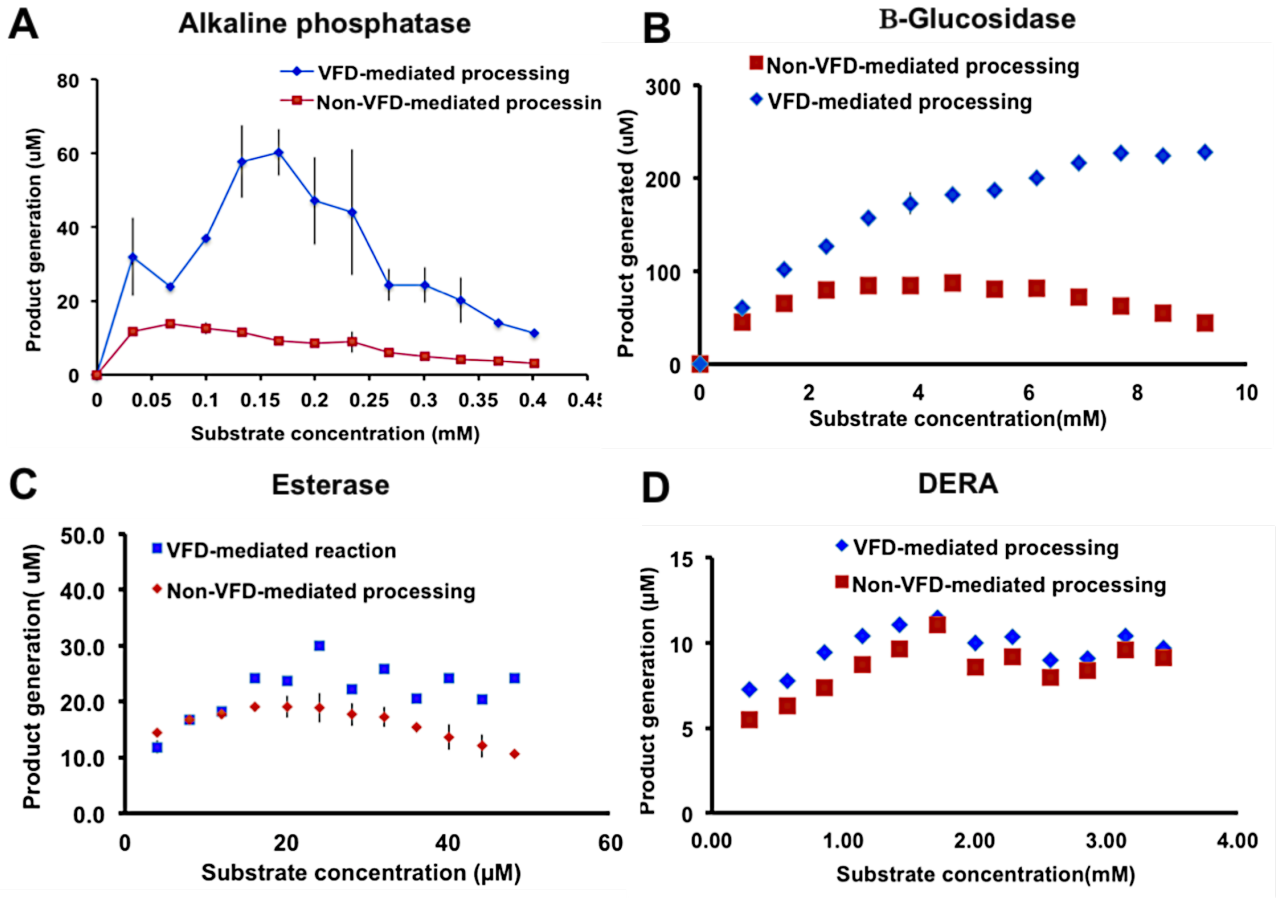


Figure S10 Rapid scanning of reaction space by simultaneously altering the concentrations of substrates and their respective enzymes. In this representation, the amount of product generated is plotted *vs*. the concentration of substrate in the solution. (**A**) alkaline phosphatase, (**B**) β-glucosidase, (**C**) esterase and (**D**) DERA. Error is indicated as standard deviation around the mean (n=3). The rotational speed used for analyzing the effect of substrate concentration on DERA was non-optimized here, hence why little difference is observed. However, variation of the rotational speed (Figure S10C) gives high levels of enhancement.

β-Glucosidase.

This 65 kDa, dimeric β-glucosidase was dissolved in the appropriate buffer (above) to 77 nM, and the substrate stock solution had a concentration of 0.010 M. For the data in Figure 2B and S10, the following concentrations were used with a final volume of 1.30 mL.

Table S2A Enzyme and substrate concentrations for β-glucosidase and 4-nitrophenyl β-D-glucopyranoside.

Enzyme volume (mL)	Enzyme concentration (nM)	Substrate volume (mL)	Substrate concentration (mM)
1.20	71	0.10	0.8
1.10	65	0.20	1.5
1.00	59	0.30	2.3
0.90	53	0.40	3.1
0.80	47	0.50	3.8
0.70	41	0.60	4.6
0.60	35	0.70	5.4
0.50	29	0.80	6.2
0.40	23	0.90	6.9
0.30	17	1.00	7.7
0.20	11	1.10	8.5
0.10	0.5	1.20	9.2

Esterase.

This 62 kDa, trimeric esterase was dissolved in the appropriate buffer (above) to 0.806 nM, and the substrate stock solution had a concentration of 0.052 mM. For the data in Figure 2B and S10, the following concentrations were used with a final volume of 1.30 mL.

Table S2B Enzyme and substrate concentrations for esterase and *p*-nitrophenylacetate.

Enzyme volume (mL)	Enzyme concentration (nM)	Substrate volume (mL)	Substrate concentration (μM)
1.20	0.74	0.10	4
1.10	0.68	0.20	8
1.00	0.62	0.30	12
0.90	0.56	0.40	16
0.80	0.50	0.50	20
0.70	0.43	0.60	24
0.60	0.37	0.70	28
0.50	0.31	0.80	32
0.40	0.25	0.90	36
0.30	0.19	1.00	40
0.20	0.12	1.10	44
0.10	0.062	1.20	48

DERA.

The 30 kDa, dimeric DERA was dissolved in the appropriate buffer (above) to 8.33 μM and the substrate stock solution had a concentration of 6.80 mM. For the data in Figure 2B and S10, the following concentrations were used with a final volume of 1.30 mL.

Table S2C Enzyme and substrate concentrations for DERA and Substrate **3/4**.

Enzyme volume (mL)	Enzyme concentration (μM)	Substrate volume (mL)	Substrate concentration (mM)
1.20	7.69	0.10	0.52
1.10	7.05	0.20	1.05
1.00	6.41	0.30	1.57
0.90	5.77	0.40	2.09
0.80	5.13	0.50	2.62
0.70	4.49	0.60	3.14
0.60	3.85	0.70	3.66
0.50	3.21	0.80	4.18
0.40	2.56	0.90	4.71
0.30	1.92	1.00	5.23
0.20	1.28	1.10	5.78
0.10	0.64	1.20	6.28

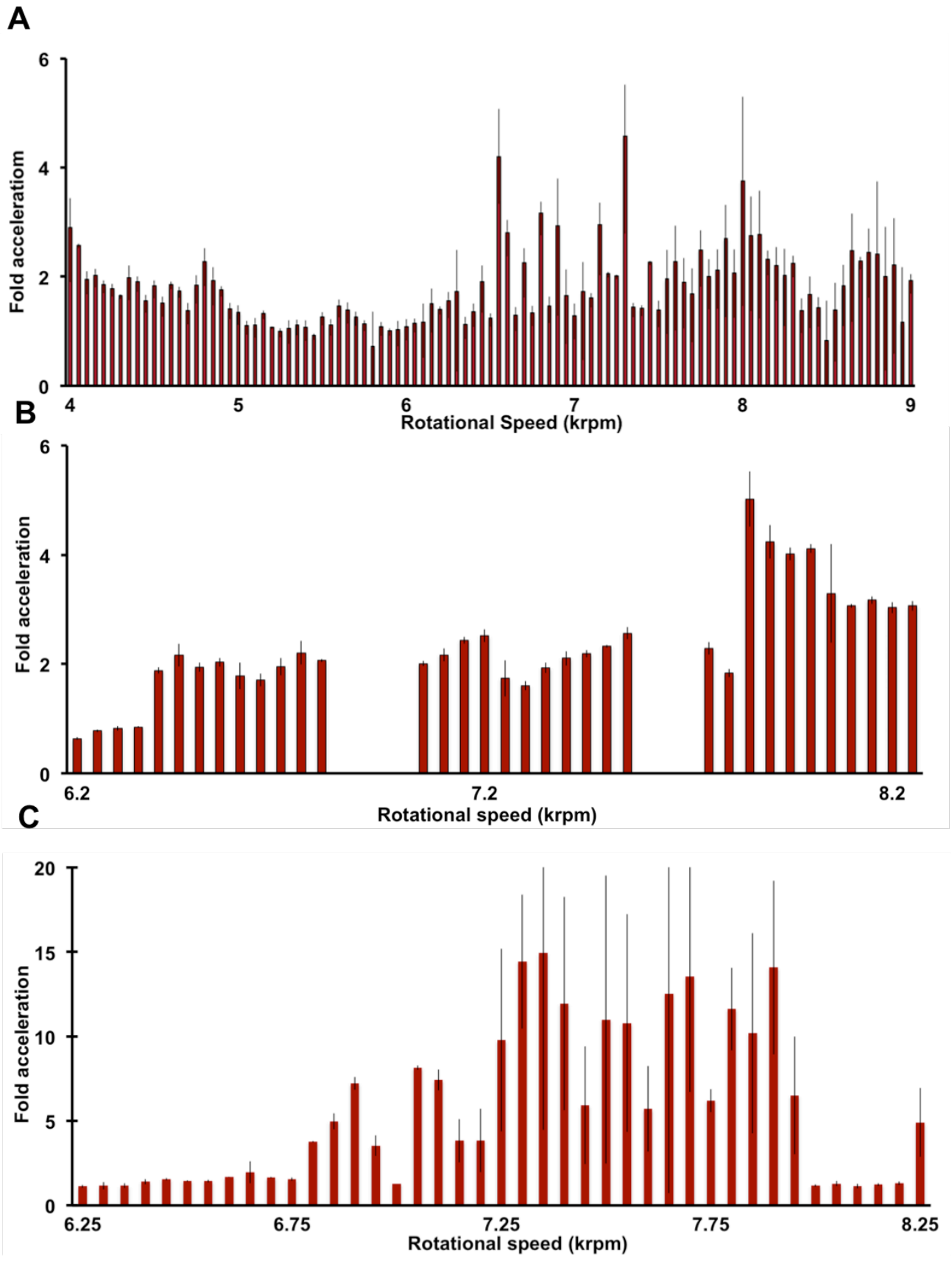
Alkaline phosphatase.

The 36 kDa, dimeric alkaline phosphatase was dissolved in the appropriate buffer (above) to 11 nM, and the substrate stock solution had a concentration of 0.435 mM. For the data in Figure 2B and S10, the following concentrations were used with a final volume of 1.30 mL.

Table S2D.Enzyme and substrate concentrations for alkaline phosphatase and the *p*-nitrophenyl phosphate liquid substrate system.

Enzyme volume (mL)	Enzyme concentration (nM)	Substrate volume (mL)	Substrate concentration (mM)
1.20	10.15	0.10	0.033
1.10	9.31	0.20	0.067
1.00	8.46	0.30	0.10
0.90	7.61	0.40	0.13
0.80	6.77	0.50	0.17
0.70	5.92	0.60	0.20
0.60	5.08	0.70	0.23
0.50	4.23	0.80	0.27
0.40	3.38	0.90	0.30
0.30	2.53	1.00	0.33
0.20	1.69	1.10	0.37
0.10	0.84	1.20	0.40

The effect of rotational speed on enhancement



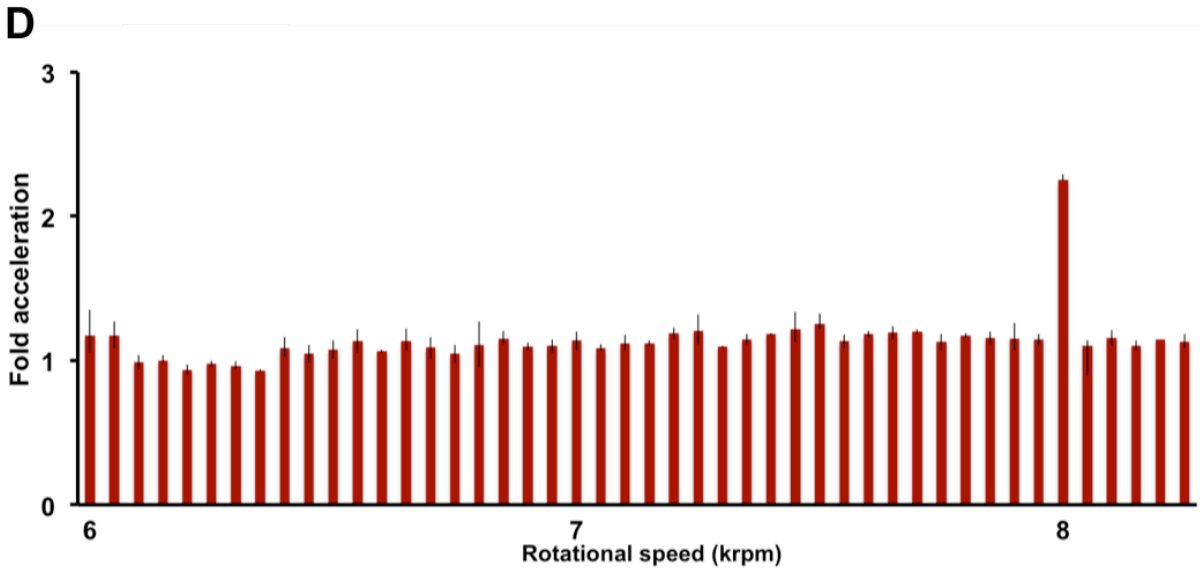


Figure S11 Variation in the rotational speed of the sample tube for the enzymes **(A)** alkaline phosphatase, **(B)** β-glucosidase, **(C)** DERA, **(D)** esterase. β-glucosidase rotational speed scan was conducted at ±250 rpm around the optimal rotational speeds found for alkaline phosphatase in order to rapidly find rotational speeds that mediated VFD-based enzyme acceleration. The rotational speed dependency for VFD-mediated enzyme acceleration is fine and intricate. Though the above rotational speed landscapes are done in 50-rpm increments, the rotational speed dependency of an enzyme is sensitive to ±5 rpm, as detailed in Figure 3. These broad rotational speed scans are performed to allow a rotational speed to be elucidated that can then be further enhanced. Error is indicated as standard deviation around the mean (n=3). The concentration of enzyme and substrate used in the above experiment are as follows: Alkaline phosphatase (6.77 nM) and its substrate *p*-nitrophenol phosphate (0.167 mM), β-glucosidase (19.3 nM) and its substrate 4-nitrophenyl β-D-glucopyranoside (7.5 mM), esterase (0.12 nM) and its substrate *p*-nitrophenol acetate (44 μM) and DERA (7.69 μM) and its fluorogenic substrate (0.52 mM). A total volume of 1.30 mL was used in these experiments

Product inhibition

Product inhibition during VFD processing was examined by the following reaction conditions for alkaline phosphatase.

Phosphate inhibition. The amount of sodium phosphate, dibasic required for the indicated final concentrations was added to the enzyme buffer, and the pH adjusted to 9.8 with 5.0 M HCl. Then, alkaline phosposphatase was added to 0.80 mL of this solution before transfer to the VFD sample tube. *p*-Nitrophenyl phosphate liquid substrate solution (0.50 mL) was next added. The solution was rotated for 10 min at the indicated rotational speeds, and then immediately quenched with 4.0 M NaOH (0.150 mL). Analysis proceeded as previously described.

p-Nitrophenyl inhibition. The amount of *p*-nitrophenol required for the indicated final concentrations was added to the enzyme buffer, and the pH adjusted to 9.8 with 4.0 M NaOH. The experiment proceeded as described above. The absorbance values are higher due to the absorbance of 4-nitrophenoxide in basic solution (Figure S12).

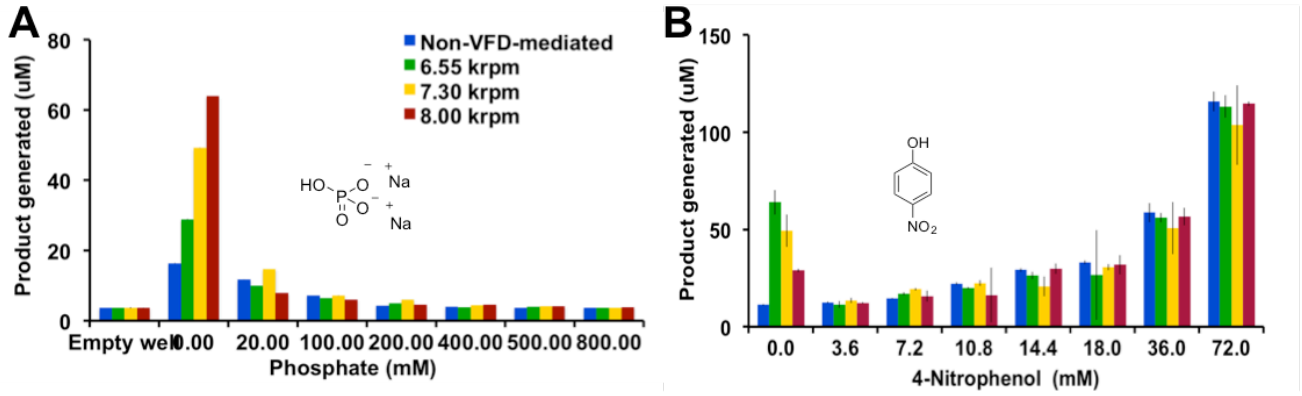


Figure S12 Product inhibition effects on VFD-mediated acceleration of alkaline phosphatase. **(A)** The addition of dibasic phosphate decreases catalytic activity for both VFD-processed and non-VFD-processed enzyme-substrate solutions. **(B)** Similarly, the addition of 4-nitrophenol results in decreased substrate conversion for both VFD and non-VFD conditions. Error is indicated as standard deviation around the mean (n=3). The concentrations of alkaline phosphatase used was 6.77 nM) and its substrate *p*-nitrophenol phosphate 0.167 mM. The total volume used in this experiment was 1.30 mL.

Crowding Experiments

To explore the ability of VFD-mediated processing to overcome steric crowding reagents, two PEG polymers with average MW of 3350 and 8000 g/mol were added to the alkaline phosphatase-substrate solutions as follows. The appropriate quantities of PEG reagent for the indicated final concentrations were dissolved in diethanolamine buffer (total volume of 10.0 mL). Alkaline phosphatase (1.0 µL) was added, and this enzyme solution (800 µL, 11.1 nM) was added to the *p*-nitrophenyl phosphate liquid substrate solution (500 µL, 0.435 mM). After 10 min, the reaction was quenched through addition of 4.0 M NaOH (150 µL). The absorbance of the solution was then measured at 402 nm (Figure S13).

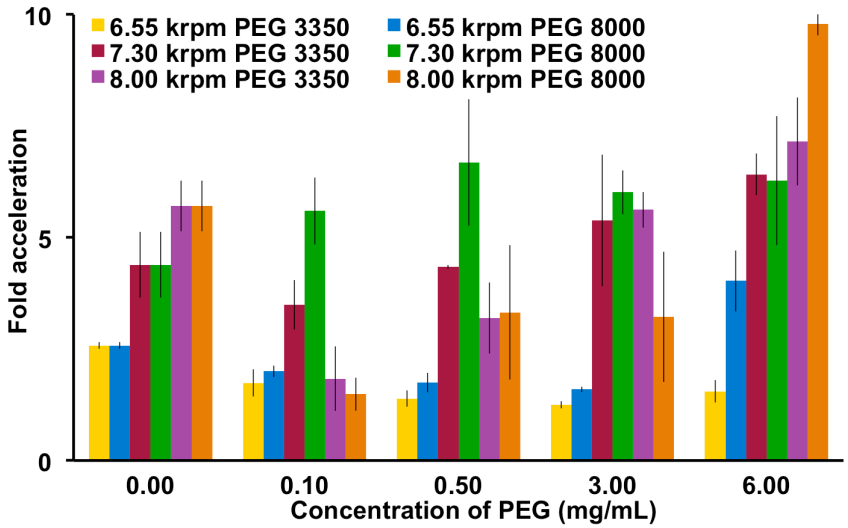


Figure S13 Steric crowding reagents decrease activity of alkaline phosphatase in the non-VFD-processed enzyme-substrate solution. The VFD-mediated reaction, however, demonstrates effective catalysis and a high degree of enzymatic rate acceleration. Error is indicated as standard deviation around the mean (n=3). The concentration of alkaline phosphatase used was 6.77 nM, and the concentration of its substrate, *p*-nitrophenol phosphate, was 0.167 mM. The total volume used in this experiment was 1.30 mL.

Generation and photography of Faraday waves

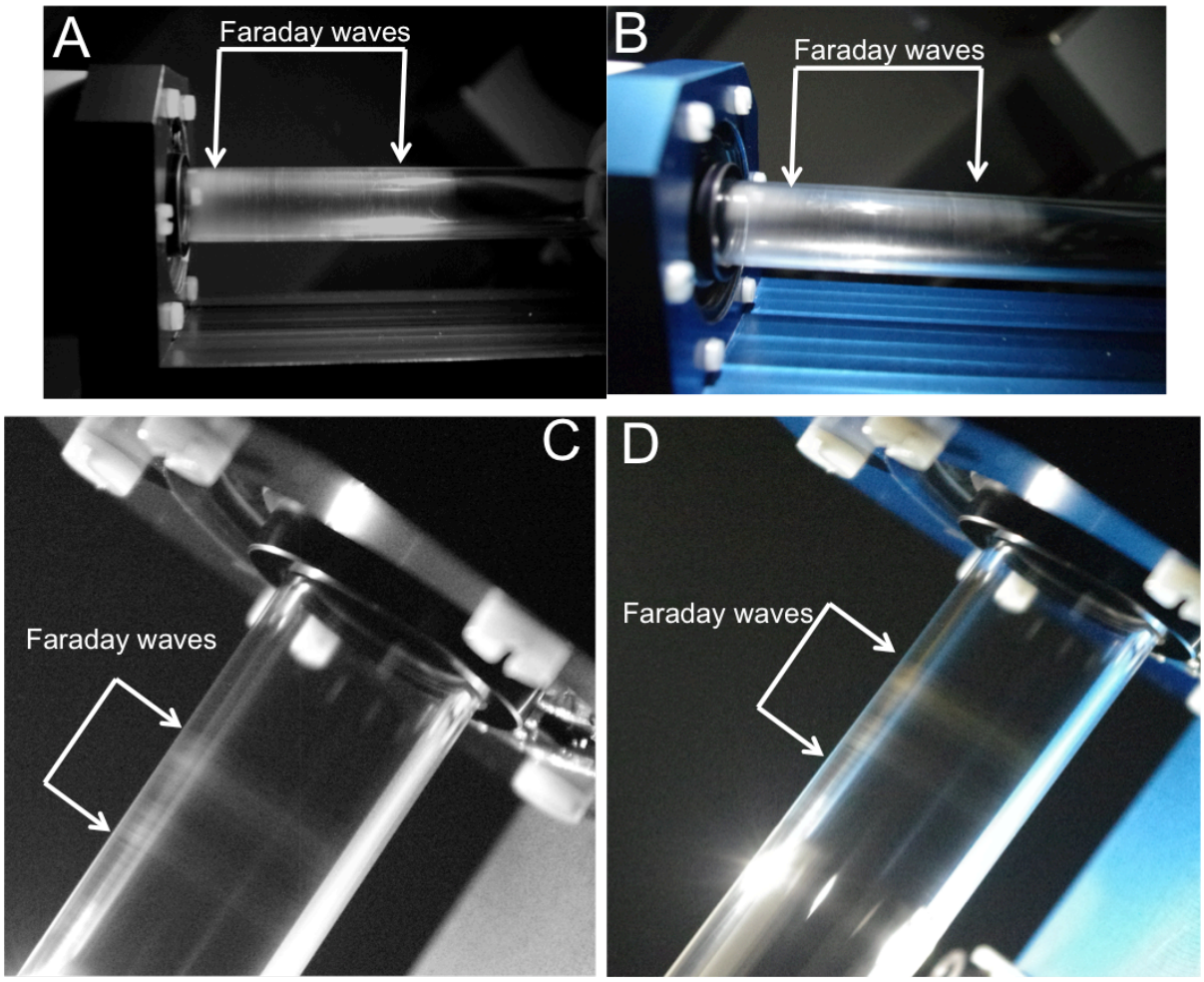


Figure S14 Photographs of the Faraday waves generated in the VFD at different rotational speeds. **(A)** and **(B)** This larger version of the photograph in Figure 1 depicts a Faraday wave generated in the VFD at 3.50 krpm rotational speed with 5.0 mL of water. Shown in black and white for higher levels of contrast. **(C)** and **(D)** Photographs of the Faraday waves generated in the VFD at a 8.00 krpm rotational speed with 3.00 mL of water. Shown in both black and white for high levels of contrast as well as original photograph. Photography conditions taken using a Pentax-*Kr* camera with a 18-55 mm lens, shutter speed; 1/60, exposure; 0.7 EV, focal length; 28.13 mm, with LED lighting. At higher rotational speeds the Faraday waves have much shorter wavelengths.

Measuring the rate of product generation with DERA under VFD-mediated conditions

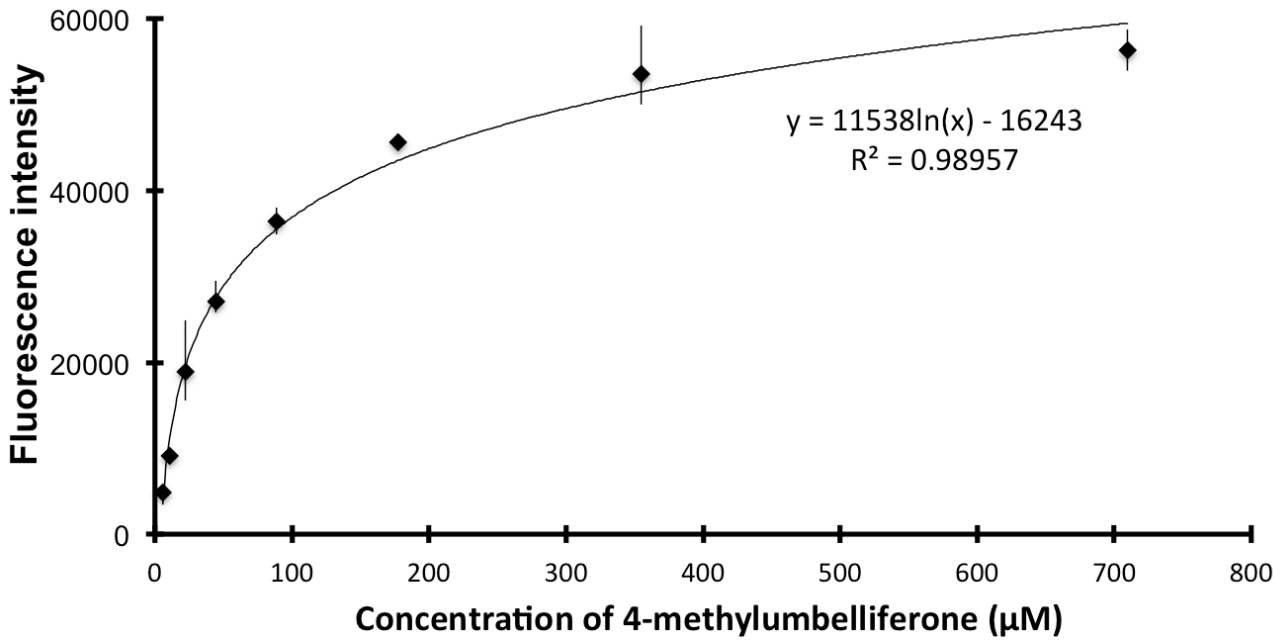


Figure S15 A calibration curve for calculating the quantity of 4-methylumbelliferone, monitored by its fluorescence. This plot was required to determine the rate of product formation from the DERA-catalyzed decomposition pathway, detailed below, Figure S16.

First a standard curve for quantifying concentrations of 4-methylumbelliferone was produced (Figure S15). The indicated concentrations of 4-methylumbelliferone were dissolved in DMSO and then diluted to 100 mL of the bis-tris propane buffer described above (for a final concentration of 1.5% DMSO) in volumetric flasks. To quantify the fluorescence at each concentration, 200 µL solution was transferred to a 96-well microtiter plate, covered with an optically transparent foil, and measured as described above.

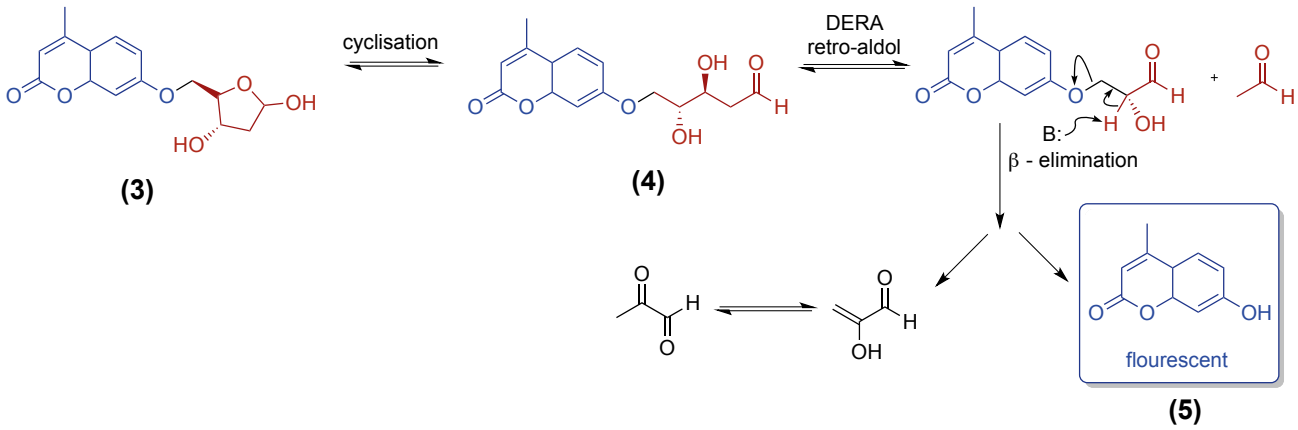


Figure S16 The DERA-catalyzed formation of fluorophore **(5)** by a retro-aldol and subsequent β-elimination reaction.

Michaelis-Menten Kinetics

Non-VFD-mediated kinetics

Conventional Michaelis-Menten measurements were performed as follows. The enzyme solution (β-glucosidase, 9.62 nM) and the substrate at the indicated concentration were mixed (1.30 mL total volume), and incubated for 1, 2, 3 and 4 min. Then, 200 µL of the quench solution (as described above) were added, and the absorbance data collected as previously described. The initial rates of the reaction where calculated and fitted to the Michaelis-Menten equation. The global minimum was found for both V_{max} and K_m to demonstrate that these values have the lowest *sum of squared fitting* (SSR), whilst still maintain a good calculated fit to the data.

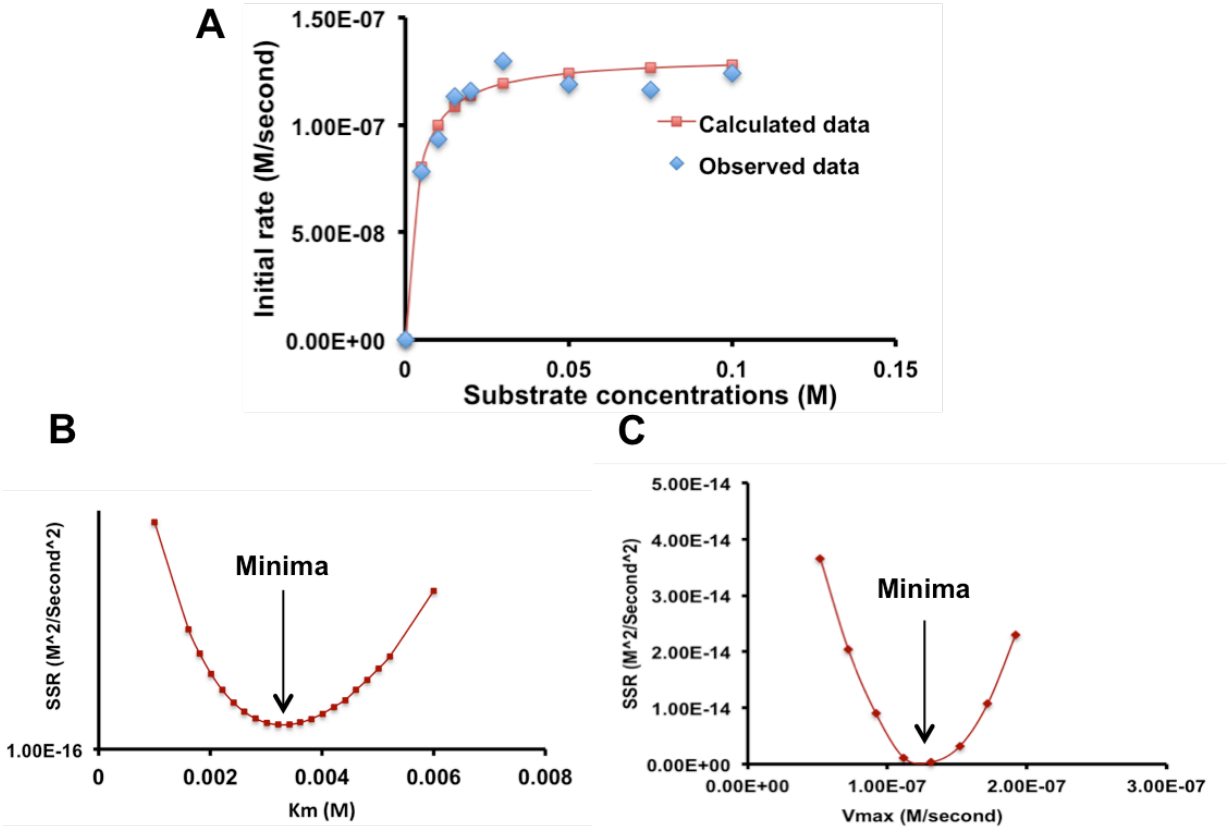


Figure S17 Results from a non-linear regression analysis for non-VFD-mediated Michaelis-Menten kinetics. A non-linear regression analysis was performed using an LSF approach to determine the local minima for both K_m and V_{max}. Results are in good agreement with the Lineweaver-Burk analysis. **(A)** The graph compares the observed data to calculated data using the LSF approach. The model was then fitted to provide the lowest value for the *sum of squared residuals* (SSR) whilst maintaining a good visual fit. Both K_m and V_{max} (**(B)** and **(C)** respectively) were varied independently of each other in finding global minima SSR.

VFD-mediated kinetics

This comparable experiment applied the conditions above with the following modifications necessary for measuring enzyme kinetics in a rapidly rotating tube. The enzyme (β-glucosidase, 9.62 nM) was added to the bottom of VFD sample tube, which had been inserted into the VFD. The substrate solution (775 µL) was then flowed down the side of the sample tube (1.30 mL total

volume); this tube was immediately capped, and rotation began. Thus, the enzyme and substrate solutions remained apart until a fraction of a second before the VFD reached the indicated rotation speed. In order to achieve the indicated time points, the VFD motor was stopped 16 s prior to the end of the required time point; thus, each time point indicates the rotational time within 1-2 s. The quenching and measurement steps then followed the protocol described above.

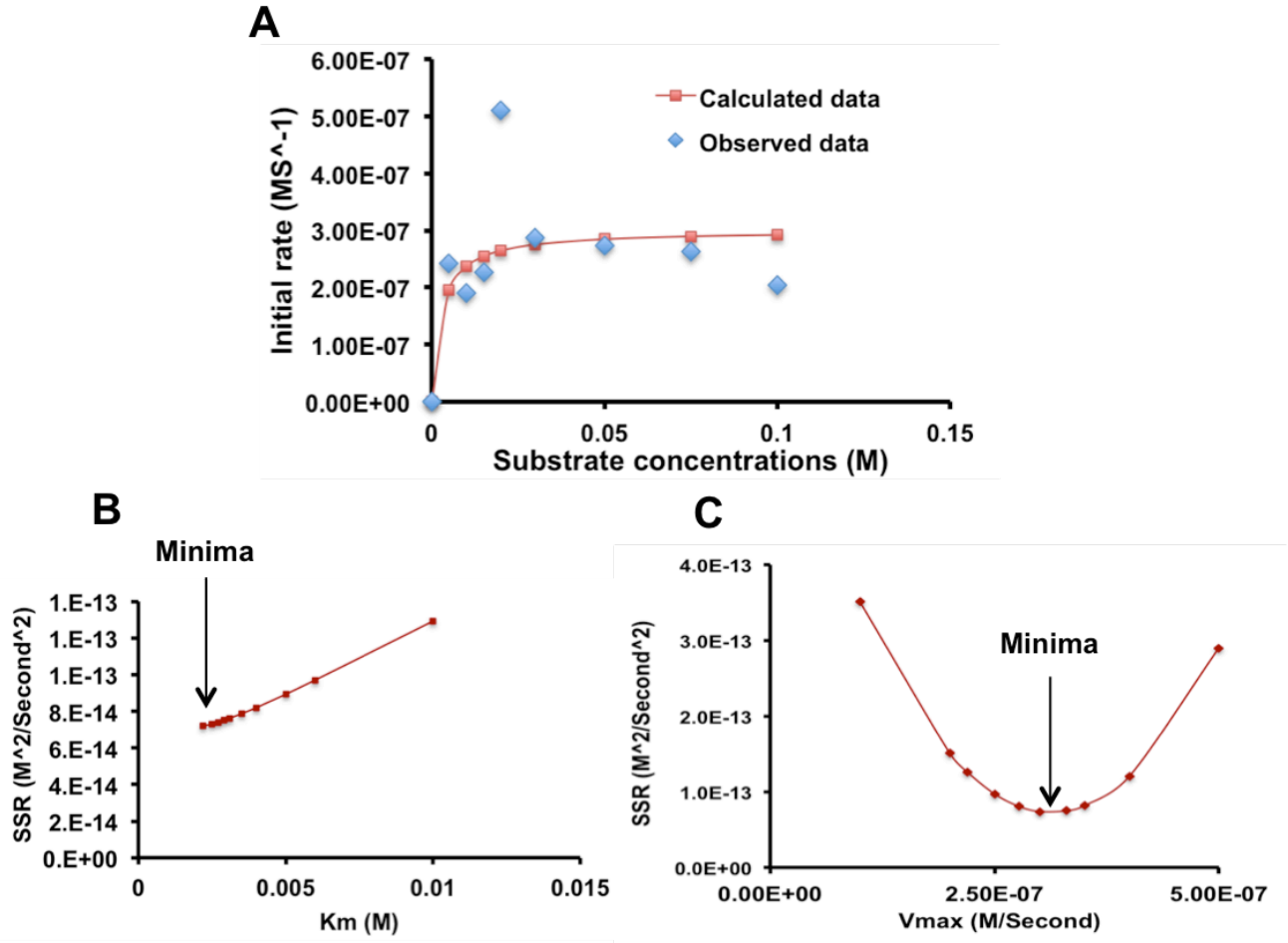


Figure S18 Results from a non-linear regression analysis for VFD-mediated Michaelis-Menten kinetics. A non-linear regression analysis was performed using an LSF approach to determine the local minima for both K_m and V_{max}. Results are in good agreement with the Lineweaver-Burk analysis. **(A)** The graph compares the observed data to calculated data using the LSF approach. The model was then fitted to provide the lowest value for the sum of squared residuals (SSR) whilst maintaining a good visual fit. Both K_m and V_{max} (**(B)** and **(C)** respectively) were varied independently of each other in finding global minima SSR.

Table S3 Michaelis-Menten parameters for the VFD *vs.* non-VFD mediated processing of β-glucosidase and its susbstrate.

Parameter	*Non VFD-mediated reaction*	*VFD-mediated rate reaction*
V_{max} (nM s^{-1})	128 ± 5.71	309 ± 52.4
K_m (mM)	3.76 ± 0.15	2.50 ± 0.44
k_{cat} (s^{-1})	13.4 ± 0.59	32.1± 5.45
k_{cat}/K_m ($mM^{-1}s^{-1}$)	3.55 ± 0.19	13.32 ± 4.03

3D-printed interchangeable plastic collar and sleeve

To maintain the vibrations generating VFD-mediated enzyme acceleration, a 3D printed plastic collar and interchangeable sleeve were created. Thus, a relatively inexpensive consumable part can be worn and replaced to maintain enhancement. Without a collar on the upper part of the VFD, no enhancement is observed, directly linking the vibration of the sample tube within the collar to enzyme acceleration. In addressing this concern, we created a plastic insert that can be replaced after four hours of processing. In this experiment, an 'Airwolf 3D printer' for ABS was used. The plastic density was set to 100% for the interchangeable sleeve and 50% plastic density for the plastic collar. The 3D printing files are available online at www.grabcad.com under 'VFD collar.

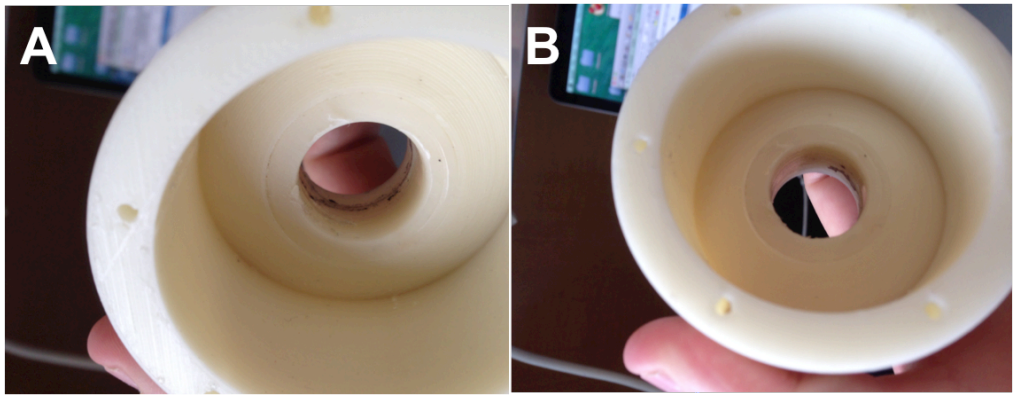


Figure S19 The 3D printed VFD collar with the interchangeable plastic sleeve (center). This approach ensured that the vibrations were maintained. After about 4 h, a new sleeve can be inserted, and the enzyme enhancement maintained.

Kinetic constants for the enzyme-substrate systems in this study

Table S4 Kinetic constants for the enzyme-substrate systems used in this study.

Enzyme and substrate	Kinetic constants	Kinetic measurement conditions
Fast alkaline phosphatase and *p*-nitrophenylphosphate	Unknown	Unknown
β-glucosidase and 4-nitrophenyl β-D-glucopyranoside	K_m - *Literature* - 2.50 mM *This work* - 3.76 mM k_{cat} - *This work* - 804 min^{-1} k_{cat}/K_m - *Literature* - 3.2x10^6 $M^{-1}min^{-1}$ *This work* - 2.1x10^6 $M^{-1}min^{-1}$	*Literature* - 27 °C, pH 5.6, 10 mM piperazine, 20 mM sodium acetate, 0.1 mM EDTA.[6] *This work* - 25 °C, pH 5.0, 50 mM sodium acetate
DERA and substrate **3**	Unknown	Unknown
Esterase and *p*-nitrophenylacetate	K_m - *Literature* - 0.52 mM k_{cat} - *Literature* - 1209 min^{-1} k_{cat}/K_m - *Literature* - 2320 $mM^{-1}min^{-1}$	pH 7.4, 37 °C, 10 mM KH_2PO_4.[7]

Supplemental References

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