Supplemental Data:

Synthesis of DHAP: Briefly, anhydrous ethanol (500 ml), concentrated sulfuric acid (3 ml, 56 mmol), and triethyl orthoformate (105 ml, 631 mmol) were combined in an oven dried round bottom flask and the solution was refluxed for 30 minutes. The solution was then cooled to 4 ºC and under nitrogen, 4.23 g of dihydroxyacetone dimmer was added every twelve hours over three days. The solution was allowed to stir for an additional full day and anhydrous sodium bicarbonate (19.0 g, 226 mmol) was added. After stirring for 30 minutes, the resulting solution was warmed to room temperature and filtered. The filtrate was concentrated and mixed with ethyl acetate (100 ml) and hexane (400 ml). The resulting white solid was filtered out and washed with hexane before being dried in a desiccator. From this white solid, 10 g were dissolved in anhydrous pyridine (45 ml) and cooled in an ice bath before adding diphenyl phosphorochloridate (22.8 g, 84.8 mmol) dropwise over the course of an hour. After stirring for an hour, the reaction mixture was combined with ethyl ether (250 ml) and washed successively water, cold 1 N hydrochloric acid, water, a saturated sodium bicarbonate solution, and water. After drying with anhydrous sodium sulfate, the ether layer was evaporated. The resulting solid was dissolved in 200 ml of ethanol to which 1.0 g of platinum oxide was added. The solution was hydrogenated under 50 psi of H_2 for approximately one day. The mixture was filtered and the catalyst rinsed with 200 ml of ethanol. The ethanol solution was concentrated under reduced pressure to yield a syrup to which 300 ml of water was added and the pH adjusted to 7.2 with 5 N sodium hydroxide. The solution was lyophilized to a white powder (the DHAP dimer) and stored at room temperature in the dark under vacuum for future use.

Two grams of Dowex 50H resin was washed three times with water, 1 N NaOH, 1 N HCl to remove impurities. The cleaned resin was combined with 0.25 g of DHAP dimer and 10.0 ml of

water and incubated at 65 ºC for four hours with occasional mixing. The resin was filtered away with a glass wool filter and the resulting DHAP solution was stored in 0.5 ml aliquots at -20 ºC. Before using, the pH was raised to ~ pH 6.0 using 2 N NaOH. Typically, the solution was found to be between 60 and 110 mM DHAP.

Quantitation of DHAP: Briefly, 40 mM Tris (pH 8.0), 0.15 mM NADH, and one unit of glycerophosphate dehydrogenase was mixed with 10 µl of a one to ten dilution of the DHAP solution and the reaction was allowed to go to completion while monitoring the absorbance at 340 nm. The total decrease in A_{340} was measured to determine the amount of NADH consumed using an extinction coefficient of 6.22 cm^{-1} mM^{-1}. The amount of NADH consumed during the reaction is equal to the amount of DHAP added to the assay mixture.

H98Q data fit to a substrate inhibition model for a variety of PGA concentrations.

[PGA] (µM)	V_{max}(app) ($mM \cdot s^{-1}$)	K_m(app) (mM)	K_I (mM)
0	0.035	3.8	NA
10	0.075	2.0	3.8
20	0.12	0.40	1.9
50	0.34	0.78	1.9
100	0.91	2.4	2.3
200	0.66	3.6	62
400	0.92	7.0	284

The value of k_{cat} for the "R-state" H98Q enzyme was calculated at 400 µM PGA to be 37 s^{-1}.