Supporting Information:

Coupled Enzyme Reactions Performed in Heterogeneous Reaction Media: Experiments and Modeling for Glucose Oxidase and Horseradish Peroxidase in a PEG/citrate Aqueous Two-Phase System

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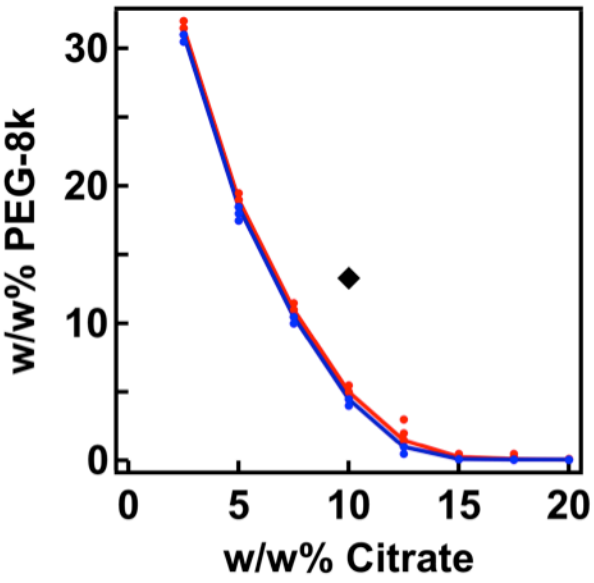
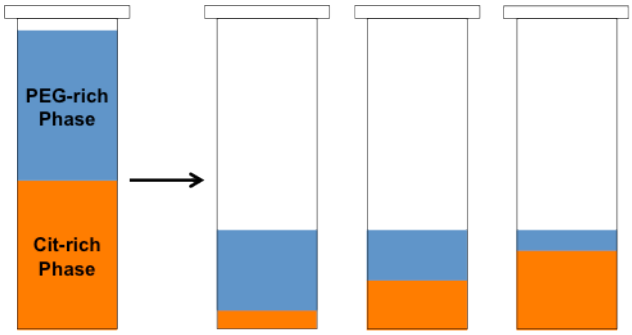
Supporting Table 1. Physical Properties of a 13.3% PEG 8000 and 10% citrate ATPS

Phase	wt% PEG	*Molal PEG	wt% citrate	*Molal citrate	Viscosity (cP)
PEG-rich	23.79 ± 0.09	0.04	3.53 ± 0.09	0.1	38.8 ± 0.3
Citrate-rich	0.6 ± 0.1	0.0008	14.9 ± 0.3	0.6	2.25 ± 0.01

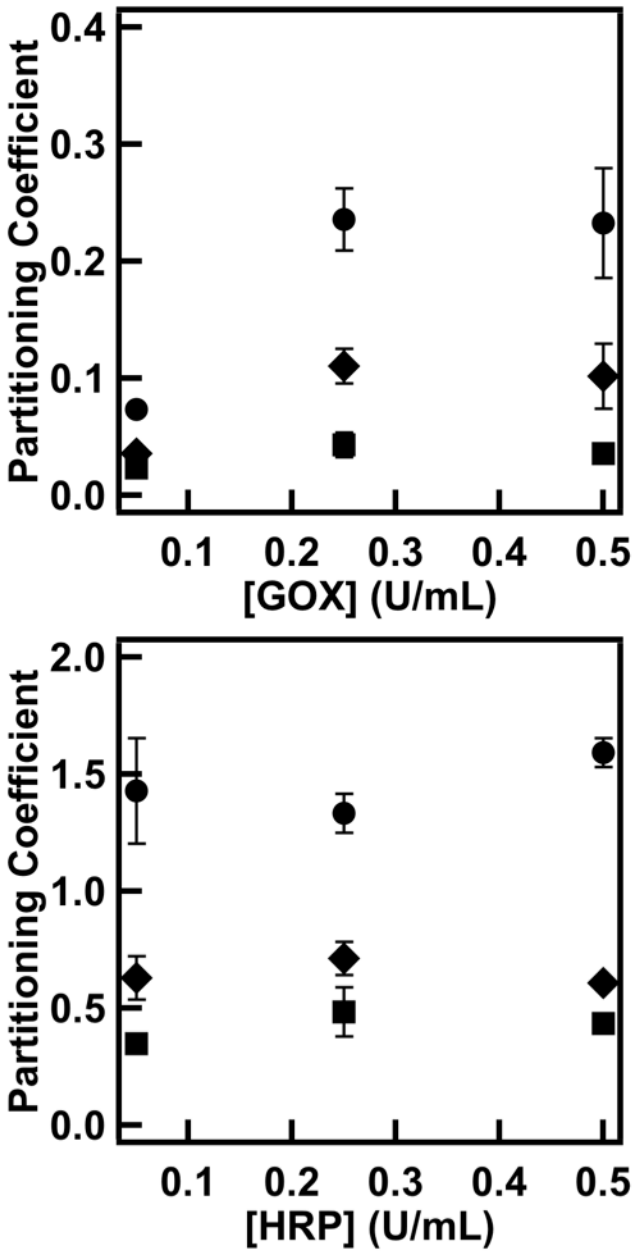
*Molality approximated based on experimentally determined component weight percents



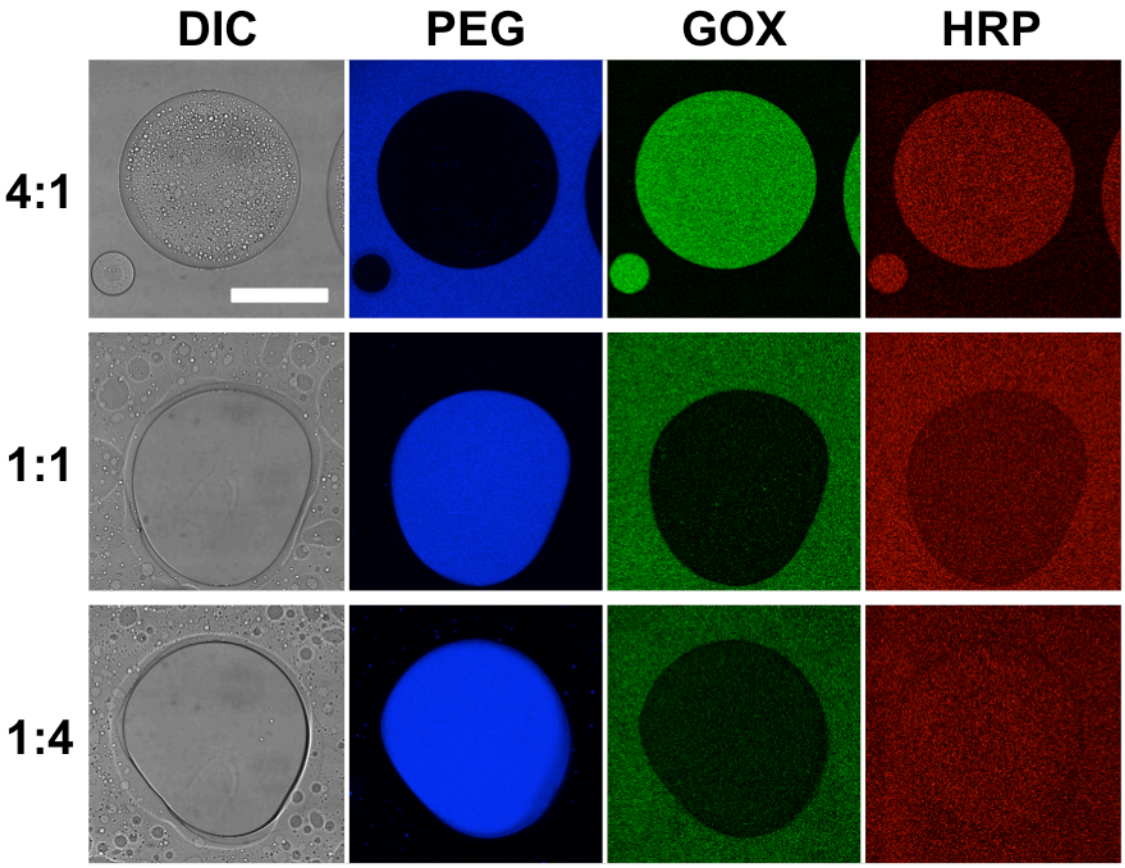
Supporting Figure 1. Phase diagram of PEG-8k and citrate. Samples prepared above the coexistence curve (red) phase separate and were visually turbid whereas those below exist as a single phase (blue). The 13.3% PEG-8k and 10% citrate composition used in this study is marked with a black diamond.



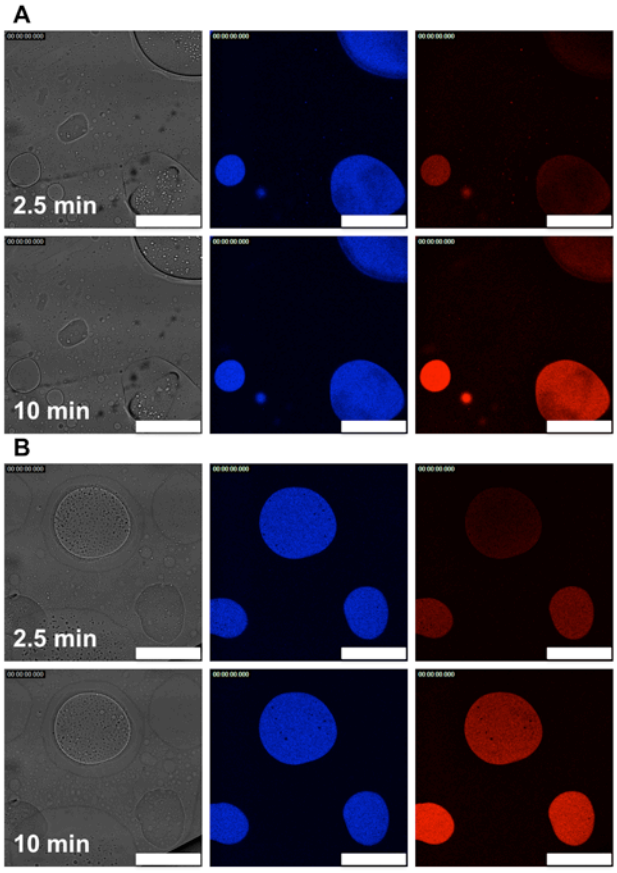
Supporting Scheme 1. After an ATPS is prepared, the phases can be separated and later reconstituted to form PEG:citrate volume ratios. From left to right: 4:1, 1:1, and 1:4.



Supporting Figure 2. The partitioning coefficient of (**A**) GOX and (**B**) HRP was measured while varying enzyme concentration in the experimental volume ratios: 4:1 (squares), 1:1 (diamonds), and 1:4 (circles). GOX partitioning is volume ratio and enzyme concentration dependent, whereas HRP partitioning is volume ratio dependent.



Supporting Figure 3. GOX and HRP partitioned in 4:1, 1:1, and 1:4 PEG:citrate volume ratios. Transmitted light (DIC) images are on the left. Confocal fluorescence images have been false-colored: blue is PEG 5000-Alexa 647, to indicate the location of the PEG-rich phase while green and red channels indicate fluorescence from Alexa 488-labeled GOX and Alexa 546-labeled HRP, respectively. Labeled GOX and HRP were included at 0.5 U/mL. The drop sizes in the figure are not meant to be representative of the entire sample, as the coalescence rate of this ATPS very high and the true droplet diameter while mixing certainly resulted in a smaller drop size distribution; rather it is meant to show the enzyme localization. Notably, the HRP appears to be partitioned relatively equally in the 1:4 system, partitioned slightly in the 1:1, and partitioned the strongest in the 4:1. The GOX is partitioned to the citrate-rich phase for each ratio. Scale bar = 250 μm.

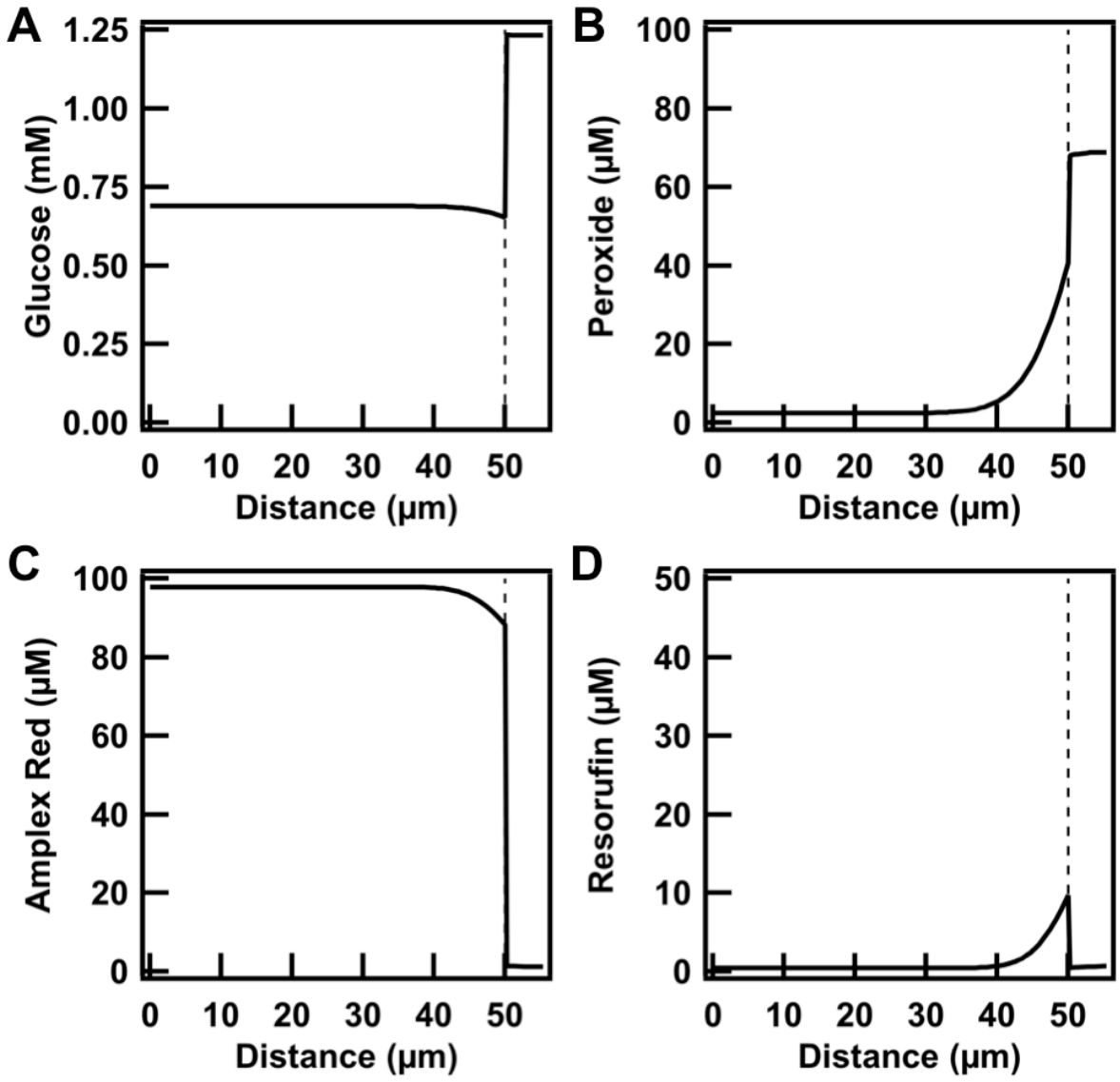


Supporting Figure 4. Assays could not be quantified in real time by confocal microscopy due to background resorufin production. (**A**) Assay conducted in a 1:4 PEG:citrate volume ratio. (**B**) Control without glucose. In both (A) and (B), transmitted light (DIC) images are on the left while blue and red channels correspond to fluorescently-labeled PEG to indicate the PEG-rich phase droplets, and produced resorufin, respectively. It is evident that resorufin is being formed through photooxidation as described previously.[1]

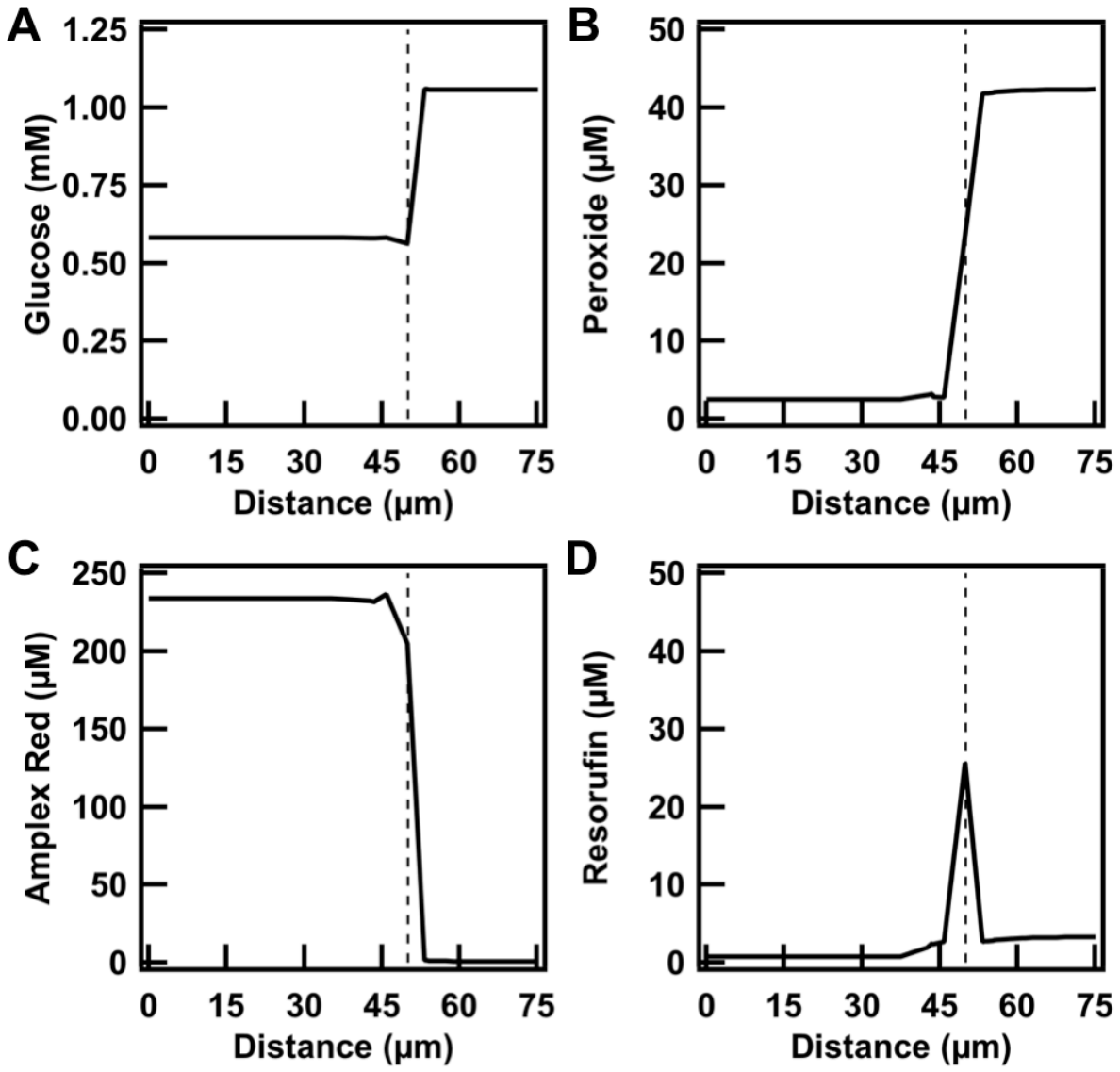
Supporting Table. Definitions of Parameters			
Parameter	Definition	Parameter	Definition
$c_{GOX,j}$	GOX concentration in phase *j*	$D_{g,j}$	glucose diffusion factor in phase *j*
$c_{HRP,j}$	HRP concentration in phase *j*	$D_{p,j}$	peroxide diffusion factor in phase *j*
$c_{g,j}$	glucose concentration in phase *j*	$D_{a,j}$	Amplex Red diffusion factor in phase *j*
$c_{p,j}$	peroxide concentration in phase *j*	$D_{r,j}$	resorufin diffusion factor in phase *j*
$c_{a,j}$	Amplex Red concentration in phase *j*	$C_{g,j}$	non-dimensionalized glucose concentration in phase *j*
$c_{r,j}$	resorufin concentration in phase *j*	$C_{p,j}$	non-dimensionalized peroxide concentration in phase *j*
K_g	glucose partitioning factor	$C_{a,j}$	non-dimensionalized Amplex Red concentration in phase *j*
K_p	peroxide partitioning factor	$C_{r,j}$	non-dimensionalized resorufin concentration in phase *j*
K_a	Amplex Red partitioning factor	ζ	ratio of PEG phase volume to citrate phase volume
K_r	resorufin partitioning factor		

Supporting Table 2. Optimized Parameters from Prepartitioned and Phase Controls

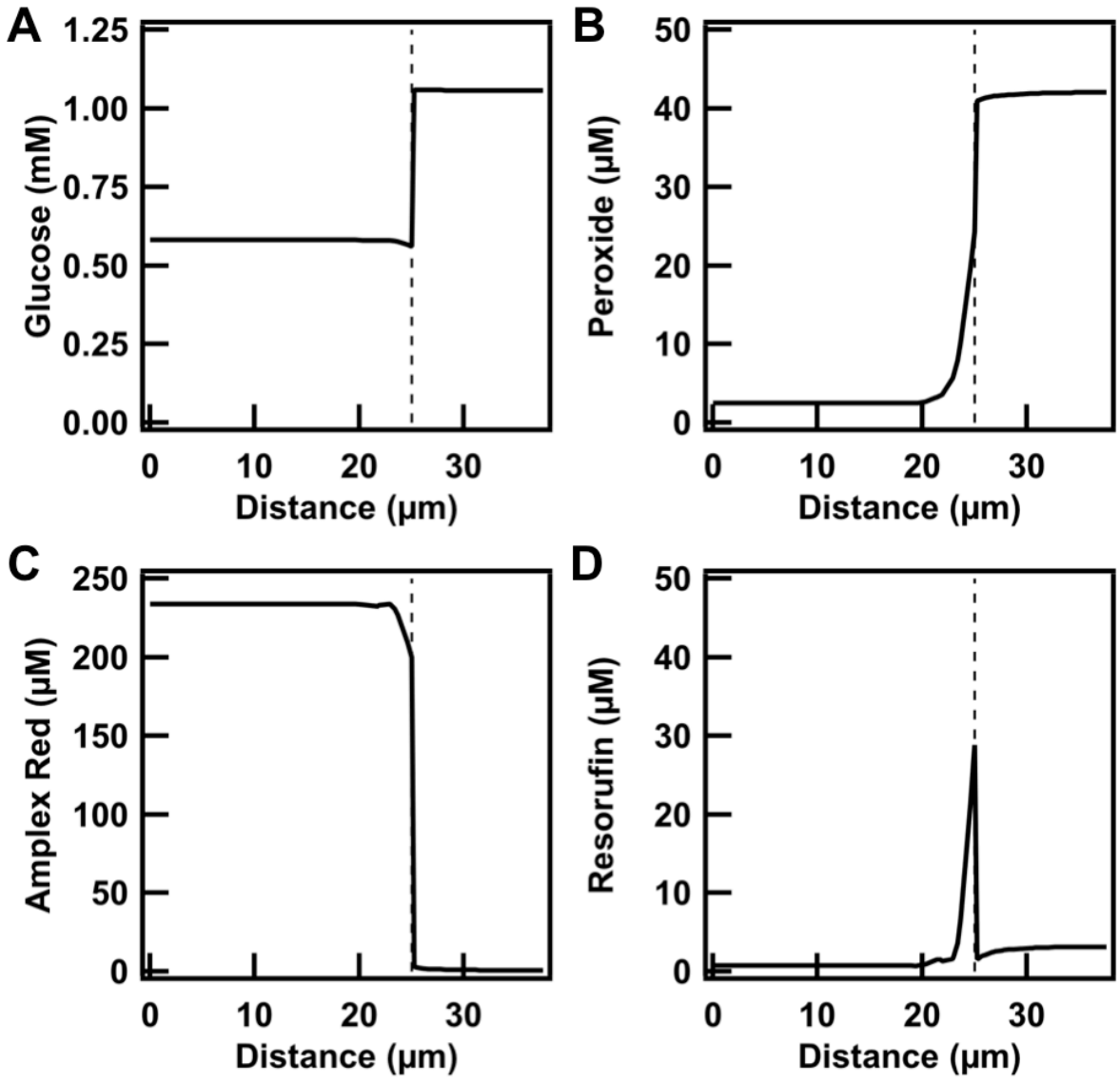
Parameter	Units	PEG-rich phase	Citrate-rich phase
$\frac{K_{cat,2}}{k_0}$	$[\mu M.s]^{-1}$	20.41	150.03
$\frac{k_{12}}{k_0}$	$[\mu M]^2$	5.69	10.21
$\frac{k_1}{k_0}$	$[\mu M]$	0.03	7.86
$\frac{k_2}{k_0}$	$[\mu M]$	0.02	2.16



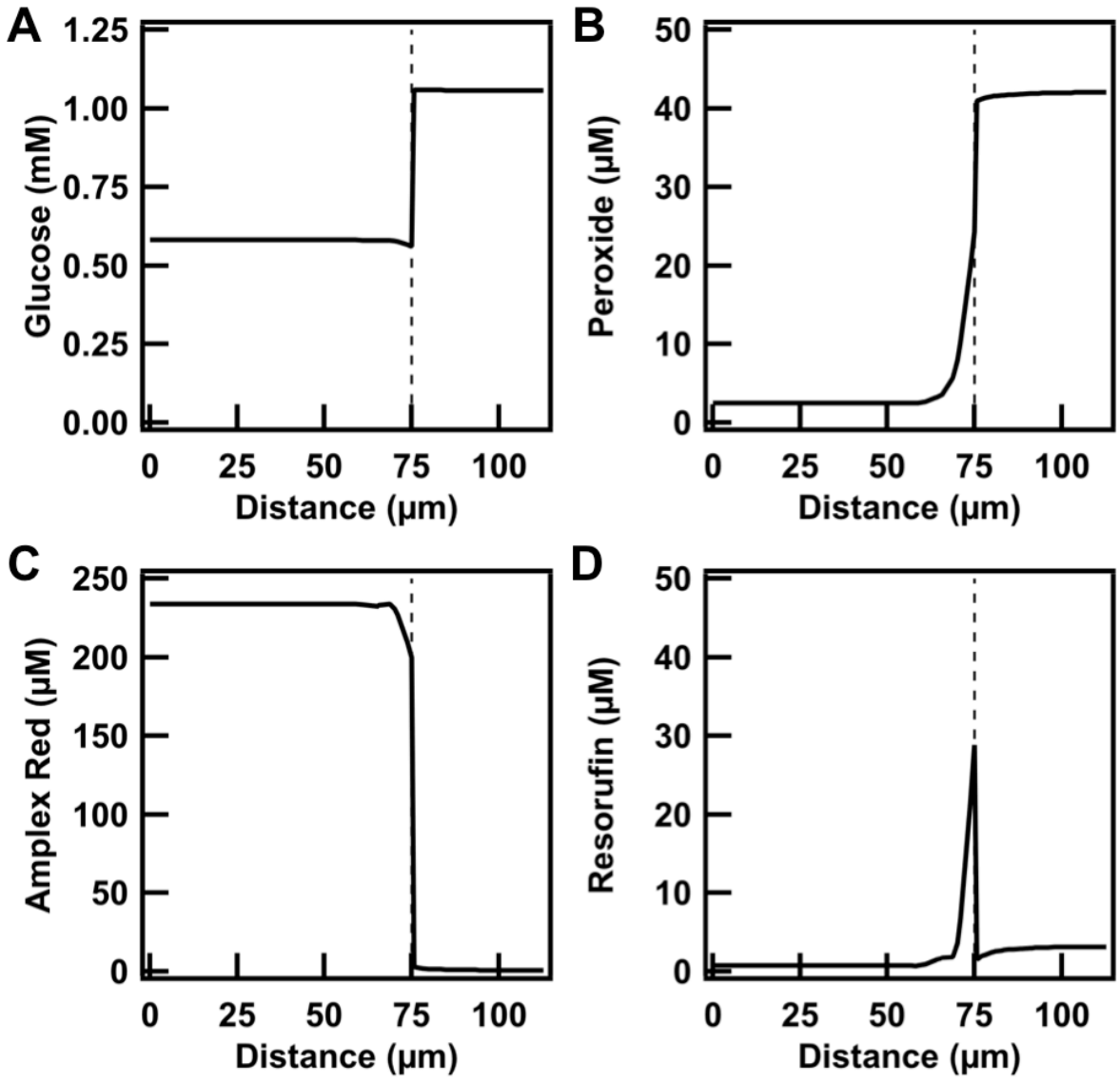
Supporting Figure 5. Concentration profiles at the end of the assay (t = 10 min) within a 1:1 PEG:citrate volume ratio, depicted from the center of a phase droplet outwards. Panels show the various substrates and products: (**A**) glucose, (**B**) peroxide, (**C**) Amplex Red, and (**D**) resorufin profiles display that the concentration throughout the droplet is uniform, except in proximity of the interface. The dotted line represents the 50 μm radius of the phase droplet.



Supporting Figure 6. Concentration profiles within a 1:4 PEG:citrate volume ratio with 100× less diffusivity, depicted from the center of a phase droplet outwards at the end of the assay (t = 10 min). (A) Glucose, (B) peroxide, (C) Amplex Red, and (D) resorufin profiles illustrate that the concentration remains uniform with decreased diffusion. The dotted line represents the 50 μm radius of the phase droplet.



Supporting Figure 7. Concentration profiles of a 25 μm radius phase droplet, within a 1:4 PEG:citrate volume ratio, depicted from the center of a phase droplet outwards at the end of the assay (t = 10 min). (A) Glucose, (B) peroxide, (C) Amplex Red, and (D) resorufin profiles illustrate that the size of the droplet does not significantly alter the uniform distributions. The dotted line represents the 25 μm radius of the phase droplet.

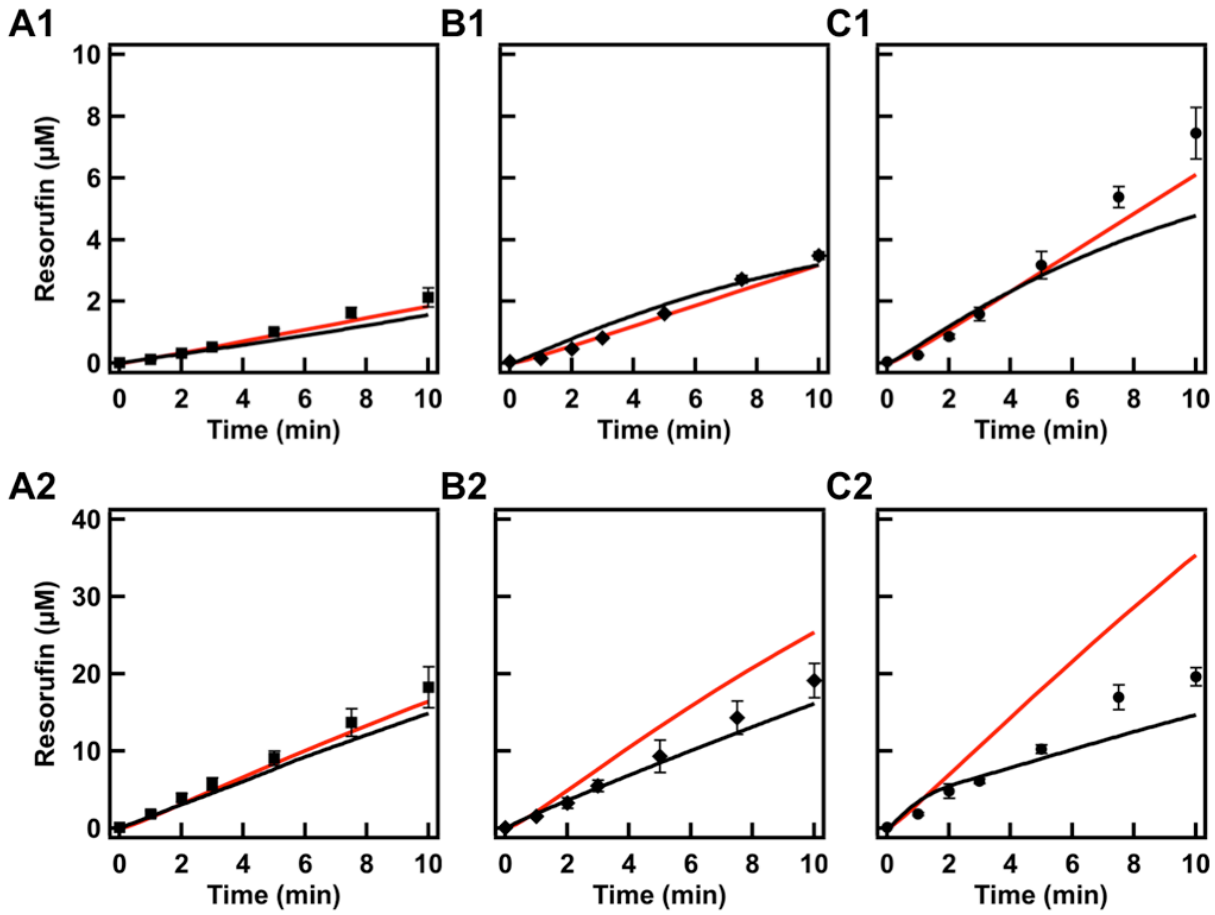


Supporting Figure 8. Concentration profiles of a 75 μm radius phase droplet, within a 1:4 PEG:citrate volume ratio, depicted from the center of a phase droplet outwards at the end of the assay (t = 10 min). (A) Glucose, (B) peroxide, (C) Amplex Red, and (D) resorufin profiles suggest that the assumed 50 μm radius for our experimental data did not affect our predictions. The dotted line represents the 75 μm radius of the phase droplet.

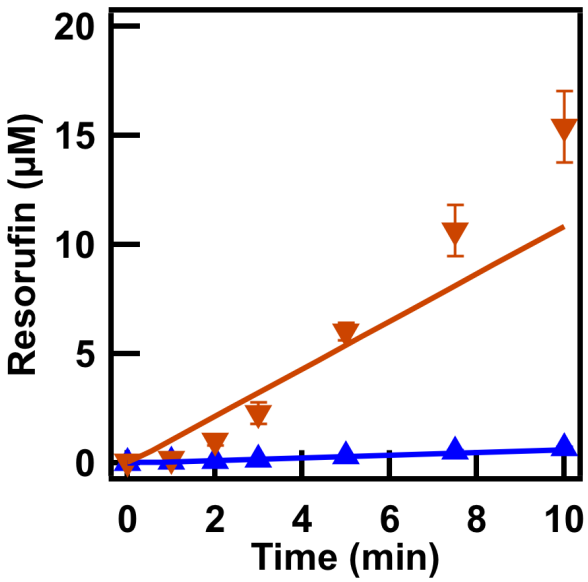
Supporting Discussion 1: To simplify the problem at hand, based on the observation that diffusion is faster than the net reaction rate in the two phases resulting to relatively "flat" concentration profiles (shown in Supporting Figures 5-8), one may be tempted to assume the concentration of all species in each phase is uniform (well mixed system assumption) and that the transfer through the interface is instantaneous. Therefore, the original governing partial differential equations of the system of (9)-(13) may be simplified to an ordinary differential equation set for two compartments.

$$\frac{\partial C_{i,C}}{\partial T}(1+\zeta K_i) = R_{i,C}\left(C_{n,C}\right) + R_{i,P}\left(K_i C_{n,C}\right)\zeta \tag{S.1}$$

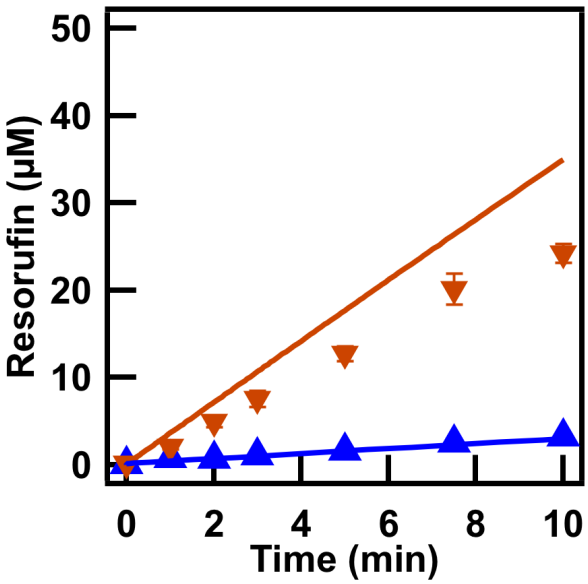
where ζ is the ratio of PEG phase volume to citrate phase volume. Also $R_{i,C}$ and $R_{i,P}$ are the net reaction rate functions as defined in (9)-(12) and $C_{n,C}$ and $C_{n,P} = K_i C_{n,C}$ is a vector of all the species concentrations at the citrate and PEG phases, respectively. The ordinary differential equation system can be employed to obtain species concentrations and draw conclusions. However, one can logically expect the appearance of prediction errors as the underlying assumption is put to the test. Such errors can be observed in Supporting Figure 9.



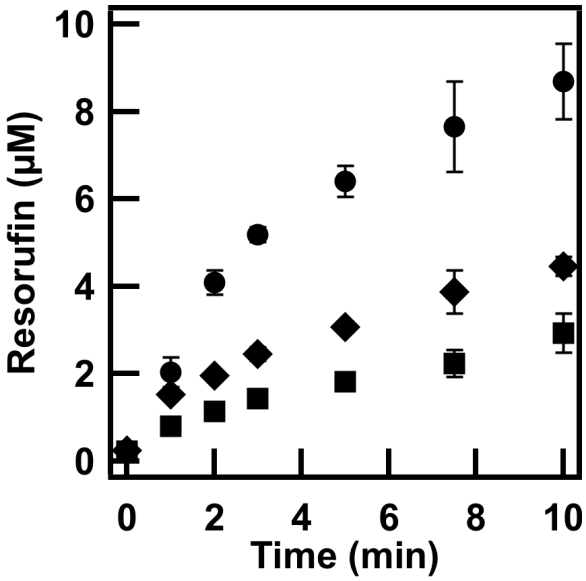
Supporting Figure 9. PEG:citrate volume ratios (**A-**1,2) 4:1, (**B-**1,2) 1:1, (**C-**1,2) 1:4. The assay conditions were 2.1 nM GOX, 1 mM glucose, 50 μM Amplex Red, 0.45 nM or 4.5 nM HRP in (A1, B1, C1) and (A2, B2, C2) correspondingly. The black markers represent the experimental data. Model predictions to experimental ATPS volume ratios with (PDE model of (9)) and without considering diffusion (ODE model of (S.1)) are presented with black and red traces respectively.



Supporting Figure 10. Controls in PEG-rich phase (blue triangles) and citrate-rich phase (orange triangles) are displayed with predictions from the ODE mathematical model (solid lines).



Supporting Figure 11. Sequential assays were performed with a higher HRP concentration of 0.05 U/mL. Controls in PEG-rich phase (blue triangles) and citrate-rich phase (orange triangles) are displayed with predictions from the ODE mathematical model (solid lines).



Supporting Figure 12. Product formation within various samples that after initial vortexing to provide a homogenous mixture were centrifuged so the reaction would have to proceed in the two separated phases, thus providing one interface with substantially less surface area. Several unmixed PEG:citrate volume ratios were used: 4:1 (squares), 1:1 (diamonds), and 1:4 (circles).

REFERENCE

(1) Zhao, B.; Summers, F. A.; Mason, R. P. *Free Radical Biol. Med.* **2012**, *53*, 1080.