

## On the use of modelling coupled antagonistic enzymes to aid in temporal programming of pH and PVA-borate gelation

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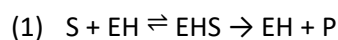
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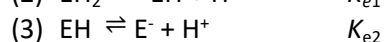
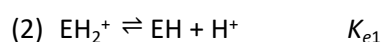
### Supplementary information

#### 1. Dependence of enzyme rate on pH

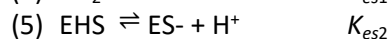
For an enzyme reaction with Michaelis-Menten kinetics:



where S is the substrate, P is product and EH is free enzyme; a typical enzyme bell-shaped rate-pH curve can be explained by the presence of two acid equilibria for the free enzyme:<sup>1</sup>



and for the enzyme-substrate complex:



Taking these equilibria account, the rate expression becomes:

$$(6) \quad v_0 = \frac{V_{max}[S]}{K_M \left( 1 + \frac{K_{e2}}{[H^+]} + \frac{[H^+]}{K_{e1}} \right) + [S] \left( 1 + \frac{K_{es2}}{[H^+]} + \frac{[H^+]}{K_{es1}} \right)}$$

where  $V_{max}$  is the maximum rate and  $K_M$  is the Michaelis constant. It is assumed for urease and esterase that  $K_{e1} = K_{es1}$  and  $K_{e2} = K_{es2}$  (or that  $K_M$  is independent of pH but  $V_{max}$  is not) so that the pH-dependent rate expression is given by  $v_0'$ :<sup>2</sup>

$$(7) \quad rate_{pH} = \frac{v_0'}{v_0} = \frac{1}{\left(1 + \frac{K_{es2}}{[H^+]} + \frac{[H^+]}{K_{es1}}\right)}$$

The bell-shaped pH dependence of the rate used in the simulations is shown in Figure S1 for urease and esterase.

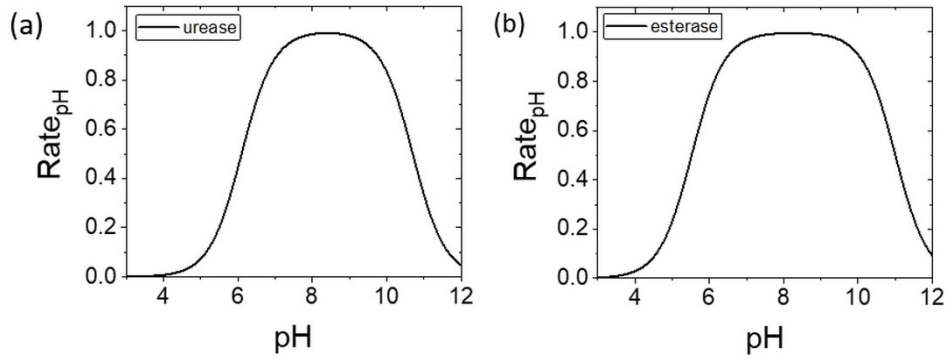


Figure S1. Dependence of rate on pH (equation 7) for (a) urease with  $K_{es1} = 8 \times 10^{-7}$  and  $K_{es2} = 2 \times 10^{-11}$  and (b) esterase with  $K_{es1} = 2 \times 10^{-6}$  and  $K_{es2} = 2 \times 10^{-11}$ .

## 2. Dependence of pH pulse on esterase concentration

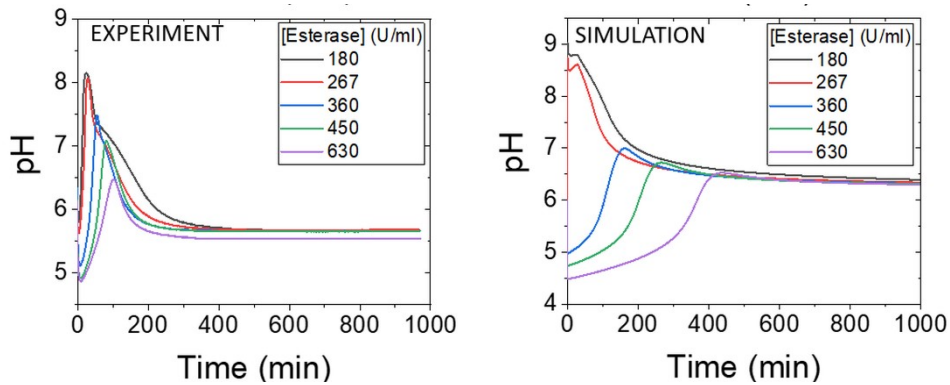


Figure S2. pH-time plots for variations of esterase in experiments with urease in watermelon seed powder (0.045 g/ml), [PVA] = 4 wt%, [boric acid] = 4 mM, [urea] = 0.667 M and simulations with [urease] = 182 U/ml, [ethyl acetate] = 1.08 M, [urea] = 0.667 M, [boric acid] = 4 mM.

## 3. Dependence of pH pulse on boric acid concentration and varying PVA concentration

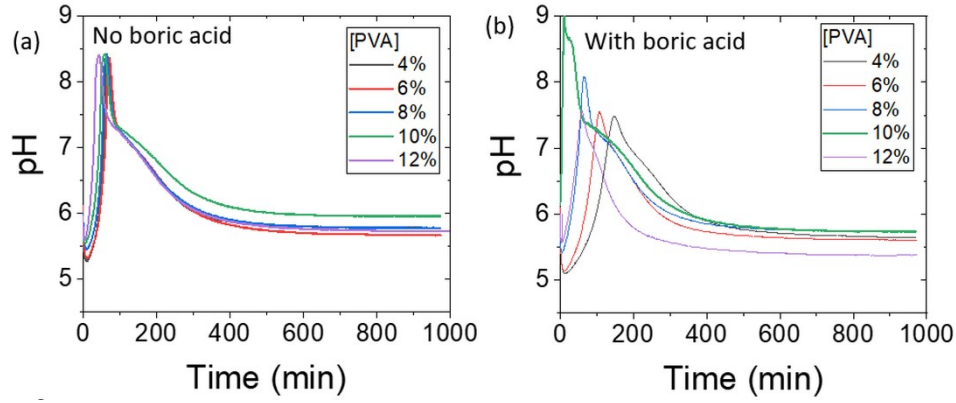


Figure S3. pH-time plots for experiments with urease in watermelon seed powder (0.045 g/ml), esterase (0.015 g/ml), [PVA] = 4 wt%, [urea] = 0.667 M and simulations with [urease] = 182 U/ ml, [esterase] = 270 U/ ml, [ethyl acetate] = 1.08 M, [urea] = 0.667 M, [boric acid] = 4 mM.

#### 4. XPP ode file for simulations

```
#rate constants min-1
#--Esterase Enzyme catalysed reaction with EA = ethyl acetate
#--Urease Enzyme catalysed reaction with U = urea
#--Boric Acid and PVA reaction with P = diol in PVA and PB = monoPVA complex

U'=-k1a*E1*U/((1+Kes2a/H+H/Kes1a)*(KMa*(1+BOH3/KI)+U*(1+(U/Ks)))*(1+(NH4/Kp)))
NH3'=2*k1a*E1*U/((1+Kes2a/H+H/Kes1a)*(KMa*(1+BOH3/KI)+U*(1+(U/Ks)))*(1+(NH4/Kp)))+k5*NH4-
k5r*NH3*H
NH4'=-k5*NH4+k5r*NH3*H
CO2'=k1a*E1*U/((1+Kes2a/H+H/Kes1a)*(KMa*(1+BOH3/KI)+U*(1+(U/Ks)))*(1+(NH4/Kp)))-
k6*CO2+k6r*H*HCO3
HCO3'=k6*CO2-k6r*HCO3*H-k7*HCO3+k7r*CO3*H
CO3'=k7*HCO3-k7r*CO3*H

EA' = -(k1b*E2*EA/(1+Kes2b/H+H/Kes1b)*(KMb+EA))
CH3COOH' = (k1b *E2*EA/(1+Kes2b/H+H/Kes1b)*(KMb+EA))-k2*CH3COOH+k2r*CH3COO*H
CH3COO' = k2*CH3COOH-k2r*CH3COO*H
CH3CH2OH' = (k1b *E2*EA/(1+Kes2b/H+H/Kes1b)*(KMb+EA))-3*CH3CH2OH+k3r*CH3CH2O*H
CH3CH2O' = k3*CH3CH2OH-k3r*CH3CH2O*H

H' = k2*CH3COOH-k2r*CH3COO*H+k3*CH3CH2OH-k3r*CH3CH2O*H+kw-kwr*H*OH+k5*NH4-
k5r*NH3*H+k6*CO2-k6r*HCO3*H+k7*HCO3-k7r*CO3*H+k8*BOH3-k8r*BOH4*H
OH' = kw-kwr*H*OH

BOH3'=-k8*BOH3+k8r*BOH4*H
BOH4'=k8*BOH3-k8r*BOH4*H-k9*P*BOH4+k9r*PB
P'=-2*k9*P*BOH4+2*k9r*PB
PB'=k9*P*BOH4-k9r*PB

aux pH=-log(H)/log(10)

par k1a=1.9e-4,E1=184,KMa=8e-3,Kes1a=8e-7,Kes2a=2e-11,KI=1e-4, Ks=3, Kp=2
par k1b =7e-5, E2=267, KMb=1e-3, Kes1b=2e-6, Kes2b=1e-11
par k2=4.38e7, k2r=2.7e12, k3=7.5e-5, k3r=6e11
par kw=6e-3, kwr=6e11
par k5=1.44e3,k5r=2.58e12,k6=2.22,k6r=4.74e6,k7=1.62e2,k7r=3e12,
```

```
par k8= 3.476e2,k8r=6e11,k9=200, k9r=1
```

```
#initial conditions
```

```
init EA=1.08,CH3COO=0.04291,CH3COOH=0.02639,CH3CH2OH=0,CH3CH2O=0,H=9.976814e-6,OH=1.002324e-9
```

```
init U=0.667,NH3=0,NH4=0,CO2=,HCO3=0,CO3=0,BOH3=4e-3,BOH4=0, P=0.45
```

```
#numerical stuff
```

```
@ total=1000,dt=0.1,tol=1e-12, atol=1e-8, meth=cvode
```

```
@ xplot=t,yplot=pH,xhi=1000,ylo=4.5,yhi=8
```

```
@ maxstor=10000000
```

```
done
```

## References

1. R. Chang, *Physical Chemistry for the Biosciences*, University science books, US, 2005.
2. B. Krajewska and S. Ciurli, *Plant Physiol. Biochem.*, 2005, **43**, 651-658.