Model development for enzymatic reactive crystallization of β-lactam antibiotics: A reaction-diffusion-crystallization approach

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Enzymatic reaction kinetic parameters

Table S1. Reaction kinetic parameters for reaction network of Figure 1 for ampicillin and cephalexin synthesis systems. Adopted from Ref. [11]. K_N is assumed to be a function of pH as $K_N = 0.0011e^{pH \times 0.525}$. The preexponential term for CEX is 0.0005.

API	K_S (M)	K_P (M)	K_N (M)	k_2 (s^{-1})	k_3 (s^{-1})	k_4 (s^{-1})	k_{-4} (s^{-1})	k_5 (s^{-1})	K_{A1}	K_{A2}
Ampicillin	0.38	0.095	0.043	162	44	235	217	9.0	$10^{-7.52}$	$10^{-8.19}$
Cephalexin	0.38	0.057	0.019	162	44	316	217	6.3	$10^{-7.52}$	$10^{-8.19}$

Crystallization kinetic parameters

Table S2. Crystallization kinetics parameters for ampicillin trihydrate and cephalexin monohydrate crystals from Ref. [14,15].

Substrate	k_{B1} ($min^{-1}L^{-1}$)	B_0	k_{B2} ($min^{-1}L^{-1}$)	b	m	s	k_G (μm min^{-1})	g
Ampicillin	5.00×10^{10}	1.27	2.20×10^{9}	0.6	-	1.37	8.95	1.87
Cephalexin	2.54	1.79	2.98×10^{5}	1.0	0.46	-	6.52	2.00

Ampicillin trihydrate crystallization kinetic equations [15]

$$B_1 = k_{B1} \exp\left(\frac{-B_0}{\ln^2 S}\right) \qquad B_2 = k_{B2} M^b (S-1)^s \qquad G = k_G (S-1)^g$$

Cephalexin monohydrate crystallization kinetic equations [14]

$$B_1 = k_{B1} S \exp\left(\frac{-B_0}{\ln^2 S}\right) \qquad B_2 = k_{B2} G^b M^m \qquad G = k_G (S-1)^g$$

b = secondary nucleation mass of crystals exponent
B_0 = primary nucleation constant
g = growth rate exponent
G = crystal growth rate (μm / min)
k_{Bi} = primary/secondary nucleation rate constant (# / min.L)

Acid dissociation equilibrium constants for different species (Ref. [11])

API	PGME	7ADCA	6APA	CEX	AMP	PG
pKa	7.24	6.26	4.83	7.12	7.31	9.14

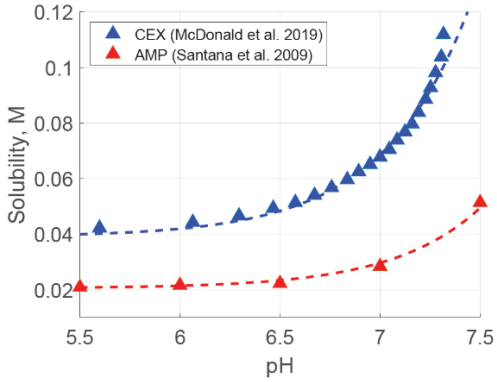
Calculation of cephalexin effective diffusion coefficient

The effective diffusion coefficient of cephalexin was determined using the Grunwald method Ref. [13]. Briefly, a known mass of ChiralVision Immobeads (COV2) were equilibrated with a known concentration of cephalexin in a known volume of solution. After filtration, the Immobeads were placed into a known volume of well-mixed DI water and samples were withdrawn periodically for HPLC analysis. Experimental concentrations as a function of time were used to fit the effective diffusion coefficient according to the equation below, where c_t is the cephalexin concentration at time, t, $c_\infty$is the concentration at infinite time, and R is the average radius of the Immobeads.

$$c_t = c_\infty \left(1 - e^{-\left(\frac{\pi^2 D_{eff}}{R^2}\right)t} \right)$$

Reported thermodynamic solubility of cephalexin and ampicillin

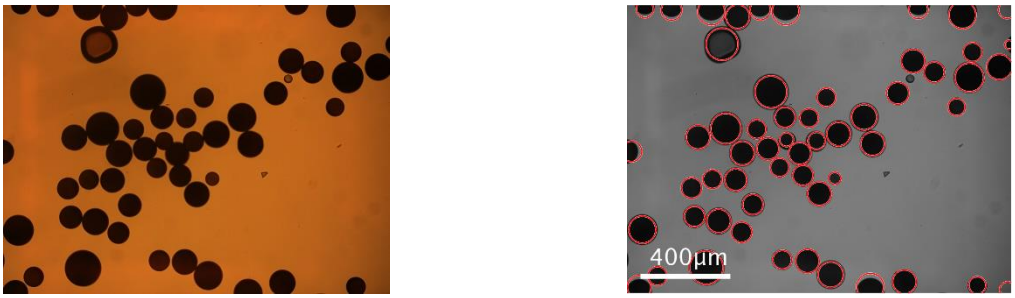
Figure S1. Cephalexin and ampicillin thermodynamic solubility in water at 25 °C as a function of pH [30,14]. Corresponding ionic strengths are not explicitly reported in these studies, however, considering the pK_a values it can be estimated by the API concentration.



Estimation of bead size distribution for Immobead (COV2) and ReliZyme (S) carriers

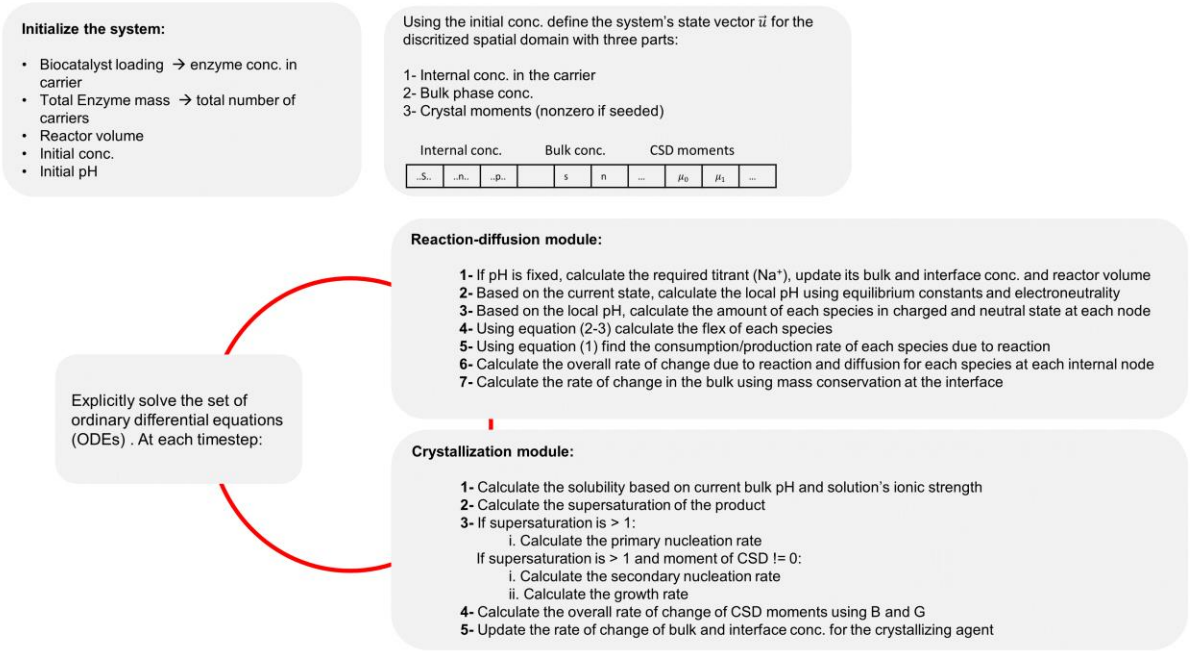
Size distribution of carrier particles was estimated by optical microscopy followed by image analysis in MATLAB. A fixed mass of each bead sample was mixed with water and then sampled with a pipet with enlarged tip for acquiring images under optical microscope. Image analysis was performed with MATLAB and consisted of : 1- loading the image, 2- convert to grayscale, 3- removing the background, 4- convert to binary image, 5- use *strel* and *regionprops* functions to detect disks with the largest size, save their size, and remove them from the image, 6- continue until all objects are removed. For both samples, the obtained distribution closely matched information provided by the vender.

Figure S2. Example of the optical image and processed image for ReliZyme beads. Objects detected on edges were excluded in estimating size distribution.



Numerical simulation framework

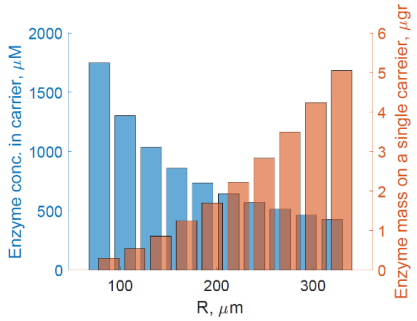
(Code available at: *github.com/GroverGroup/Reactive-Crystallization*)



Calculation of enzyme loading on beads with different size in a carrier sample

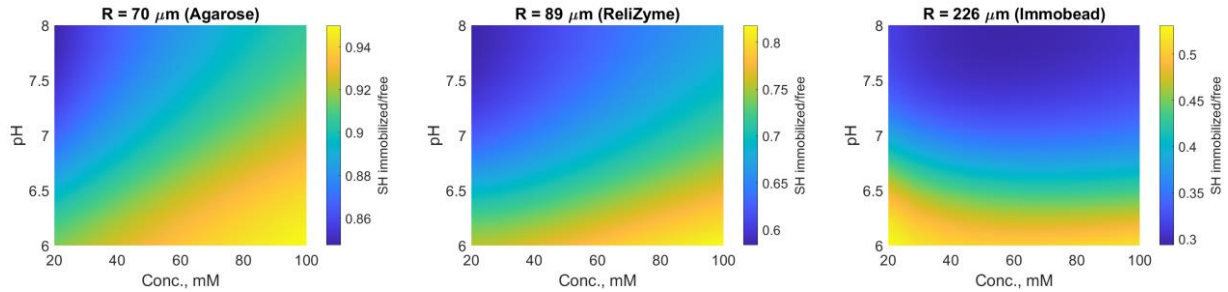
If multiple representative carrier radii are to be considered in the model, a new challenge arises, that is how should enzyme loading be calculated for individual beads. In a sample with a single radius, all carriers would have a similar loading = total mass of enzyme used/total mass of carriers. When multiple bead sizes are to be considered in the model, using above equation for calculating the enzyme loading into each carrier assumes that enzyme immobilization is a size-independent but more thermodynamically driven phenomenon. In other words, all carrier particles have similar enzyme concentration loaded into them driven by the protein concentration in the immobilization solution. Another alternative method for calculating enzyme loading is to assume that to be dependent on the outer surface area of the bead. Smaller carriers in a sample make a larger portion of the total available surface area, but they have a smaller mass compared to larger carriers. This can be the basis for calculating enzyme loading on each carrier particle, i.e., g enzyme / g carrier $= \frac{(A_1 \,.\, R^2)}{(A_2 \,.\, R^3)} = A \,.\, R^{-1}$, where A_1 and A_2 are proportionality constants. The value for constant A and so enzyme loading for each carrier size can be calculated using enzyme mass conservation, total enzyme mass = $\frac{4.\pi.\rho.A}{3} \sum n_j R_j^2$, where n_j is the number of beads for each size bin in a specific bead sample.

Figure S3. Enzyme mass and enzyme concentration in individual beads in a sample of commercial Immobead (COV2) porous carriers, calculated based on assumption of surface-dependent loading.



Initial rate experiments sensitivity to immobilization for ampicillin synthesis

Figure S4. Simulations of SH ratio measurements in initial rate experiments for ampicillin synthesis using immobilized PGA. Simulation conditions are similar to those of Figure 10 in the main text.



Comparison of batch crystal productivity at different pH values for ampicillin and cephalexin crystals.

Figure S5. Comparison of batch crystal productivity at different pH values for ampicillin and cephalexin crystals. Simulations were performed for s0 = 0.3 and n0 = 0.2 M, with total enzyme concentration of 5 μM immobilized on ReliZyme carriers with "high" loading. Dynamic simulation was halted at byproduct concentration of 0.05 M.