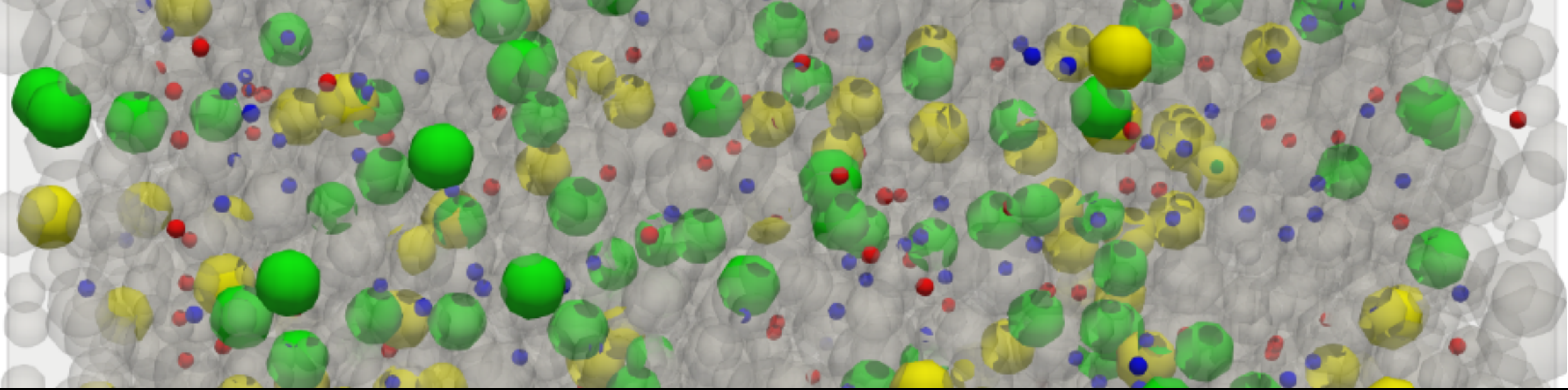
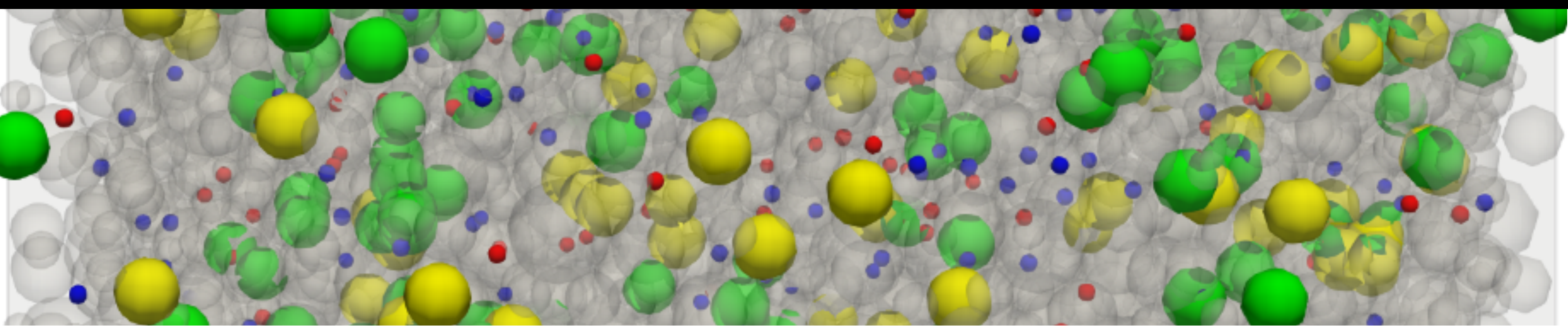


Modeling and efficient algorithms to unravel the effects of crowded metabolism

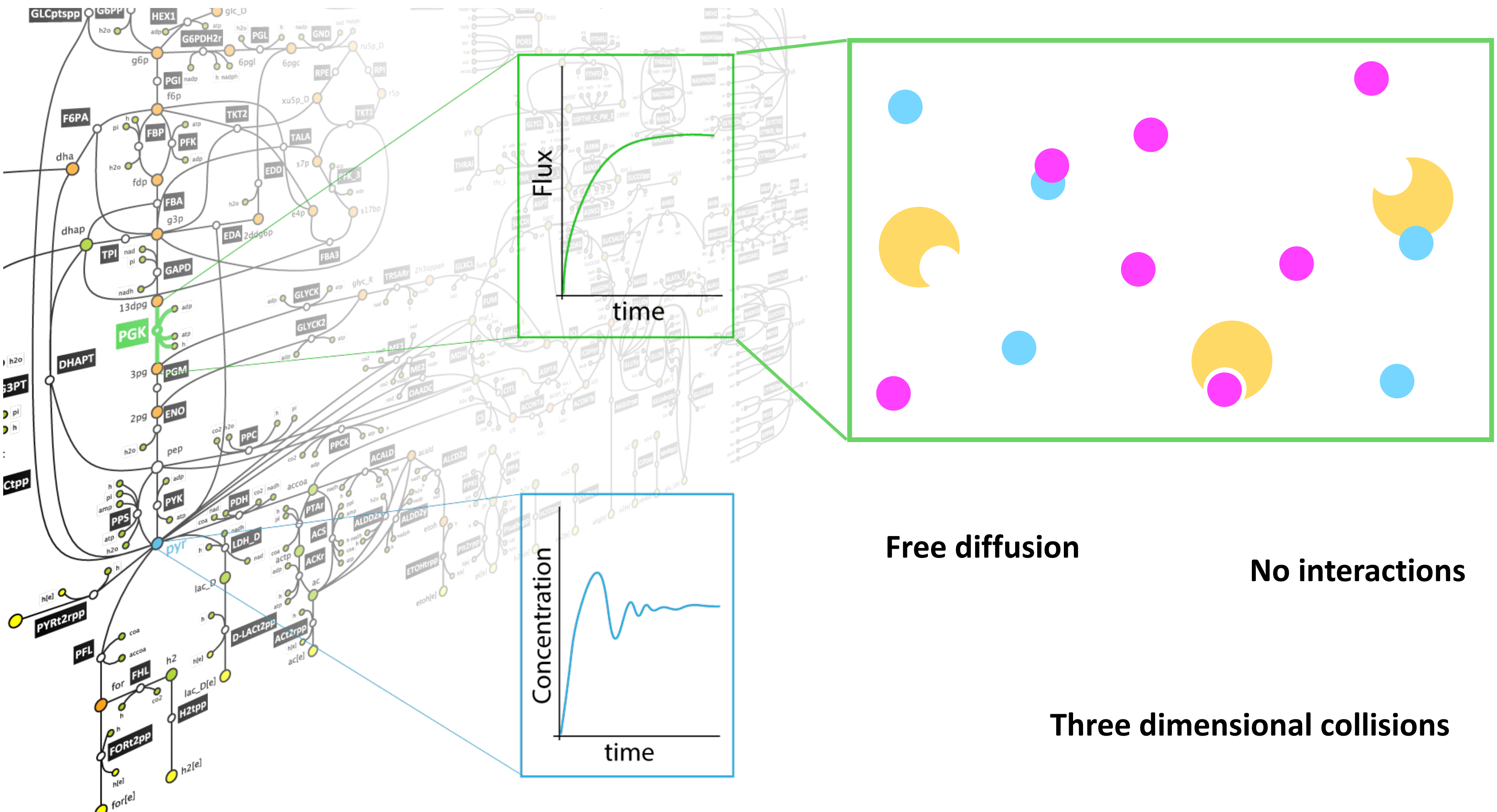


LCSB
LABORATORY OF COMPUTATIONAL SYSTEMS BIOTECHNOLOGY

Daniel Weilandt
Sept. 2018

EPFL
ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

Motivation



Free diffusion

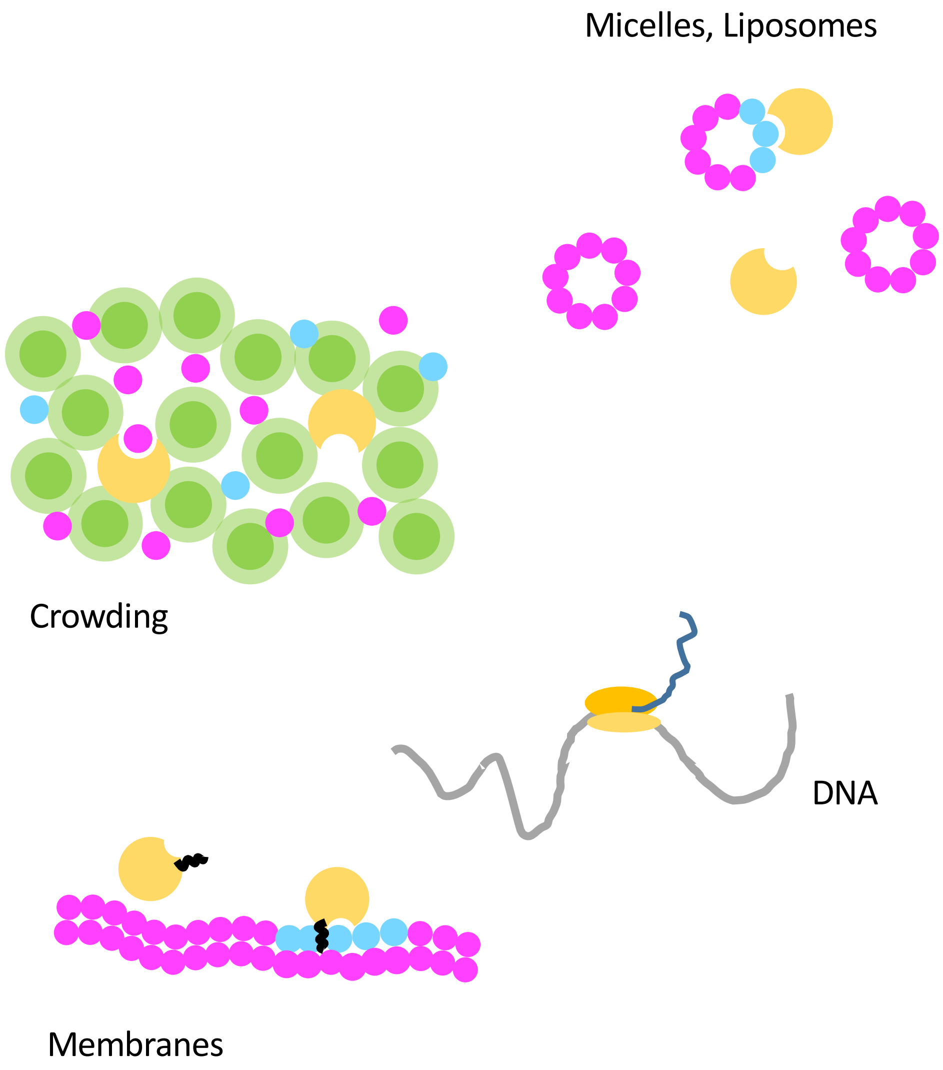
No interactions

Three dimensional collisions

Well mixed

Motivation

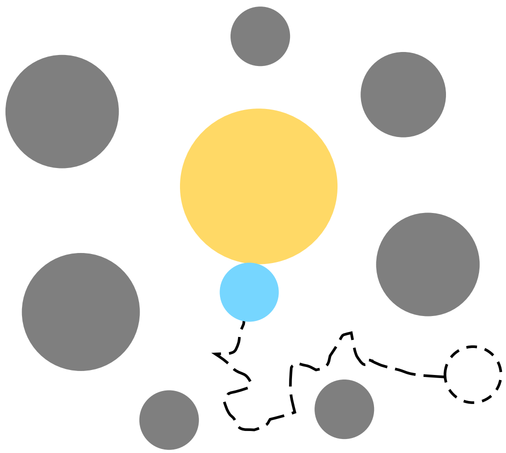
Goodsell, D.S., *Biochemistry and Molecular Biology Education*, **2009**. 37(6)



Generalized Elementary kinetics

$$S + E \underset{k_{1,b}}{\overset{k_{1,f}}{\rightleftharpoons}} ES \underset{k_{2,b}}{\overset{k_{2,f}}{\rightleftharpoons}} E + P$$

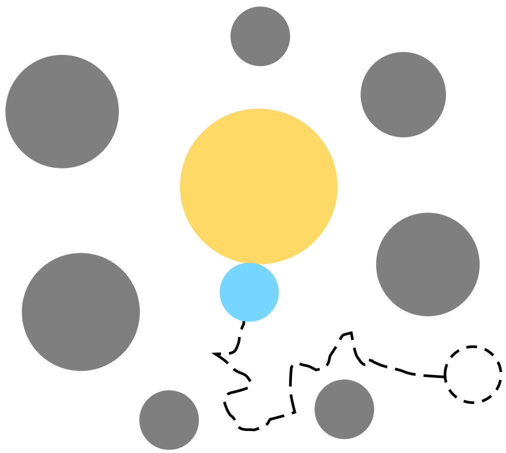
Generalized Elementary kinetics



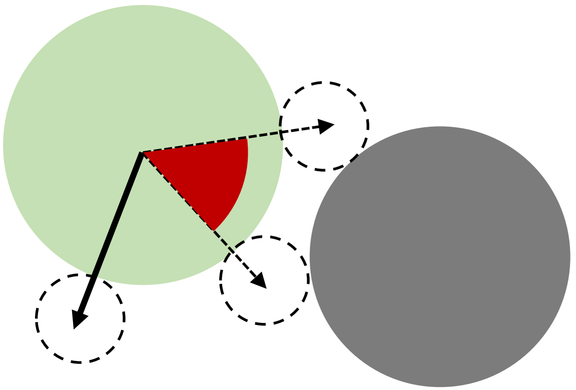
Altered collision dynamics

$$S + E \underset{k_{1,b}}{\overset{k_{1,f}}{\rightleftharpoons}} ES \underset{k_{2,b}}{\overset{k_{2,f}}{\rightleftharpoons}} E + P$$

Generalized Elementary kinetics



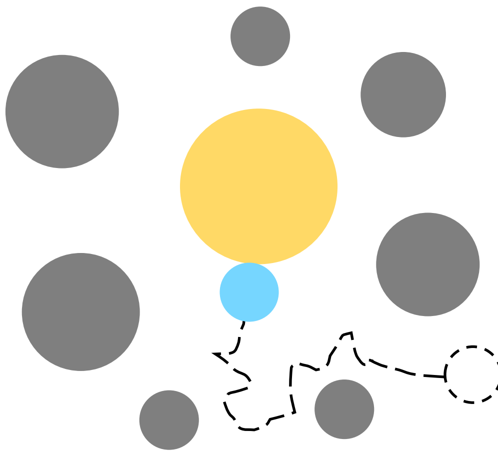
Altered collision dynamics



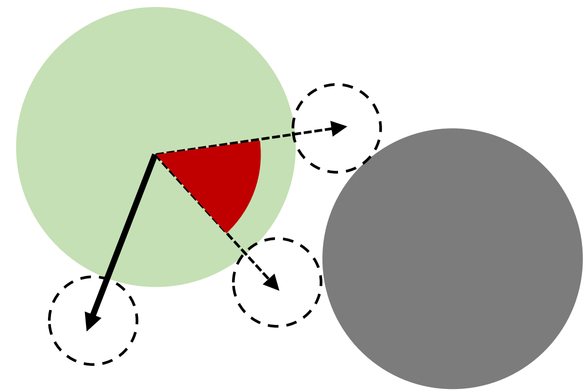
Altered dissociation dynamics

$$S + E \underset{k_{1,b}}{\overset{k_{1,f}}{\rightleftharpoons}} ES \underset{k_{2,b}}{\overset{k_{2,f}}{\rightleftharpoons}} E + P$$

Generalized Elementary kinetics



Altered collision dynamics



Altered dissociation dynamics

$$S + E \underset{k_{1,b}}{\overset{k_{1,f}}{\rightleftharpoons}} ES \underset{k_{2,b}}{\overset{k_{2,f}}{\rightleftharpoons}} E + P$$

Change in effective rate constant

Generalized Elementary rates kinetics

$$S + E \underset{k_{1,b}}{\overset{k_{1,f}}{\rightleftharpoons}} ES \underset{k_{2,b}}{\overset{k_{2,f}}{\rightleftharpoons}} E + P$$

Assume an **average effective rate**

$$\log\left(k_{j,eff}(\phi)\right) = \log\left(k_{j,0}\right) + \zeta_j$$

Dilute rate constant **Correction function**

For a ζ_j **linear** with respect to all **logarithmic concentrations:**

$$\log\left(\frac{k_{j,eff}}{k_{j,0}}\right) = \alpha_{i,j} \sum_{i=1}^{N} \log\left(\frac{[X_i]}{[X_i]_{ref}}\right) + \beta_j$$

Reference concentration state

Generalized Elementary rates kinetics

$$S + E \underset{k_{1,b}}{\overset{k_{1,f}}{\rightleftharpoons}} ES \underset{k_{2,b}}{\overset{k_{2,f}}{\rightleftharpoons}} E + P$$

Assume an **average effective rate**

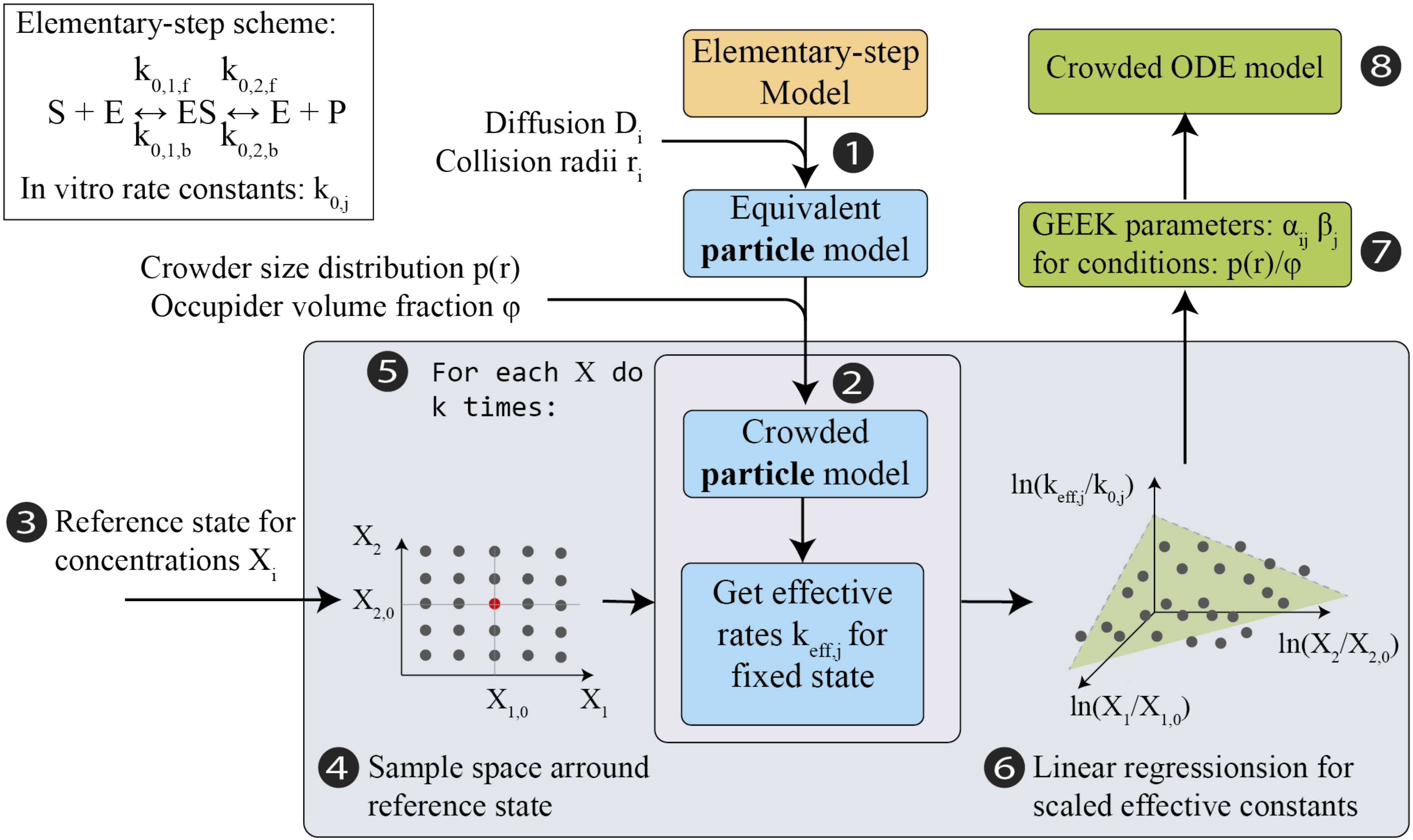
$$\log\left(k_{j,eff}(\phi)\right) = \log\left(k_{j,0}\right) + \zeta_j$$

Dilute rate constant **Correction function**

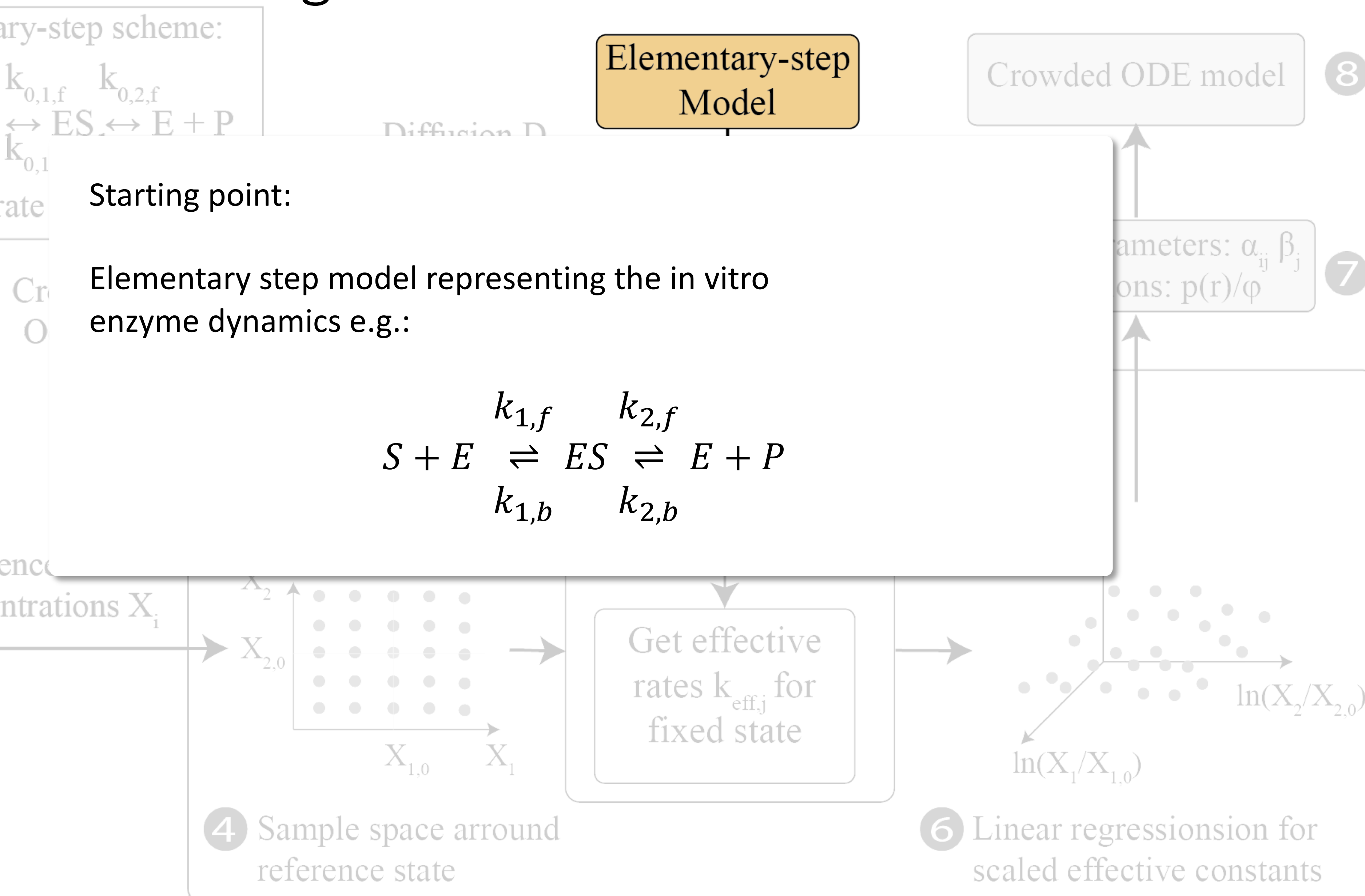
Approximate effective rate constant:

$$k_{j,eff}(\phi) = k_{i,0} e^{\beta_j} \prod_{i=1}^{M} \left(\frac{[X_i]}{[X_i]_{ref}}\right)^{\alpha_{i,j}}$$

Modeling framework



Modeling framework



Starting point:

Elementary step model representing the in vitro enzyme dynamics e.g.:

$$S + E \underset{k_{1,b}}{\overset{k_{1,f}}{\rightleftharpoons}} ES \underset{k_{2,b}}{\overset{k_{2,f}}{\rightleftharpoons}} E + P$$

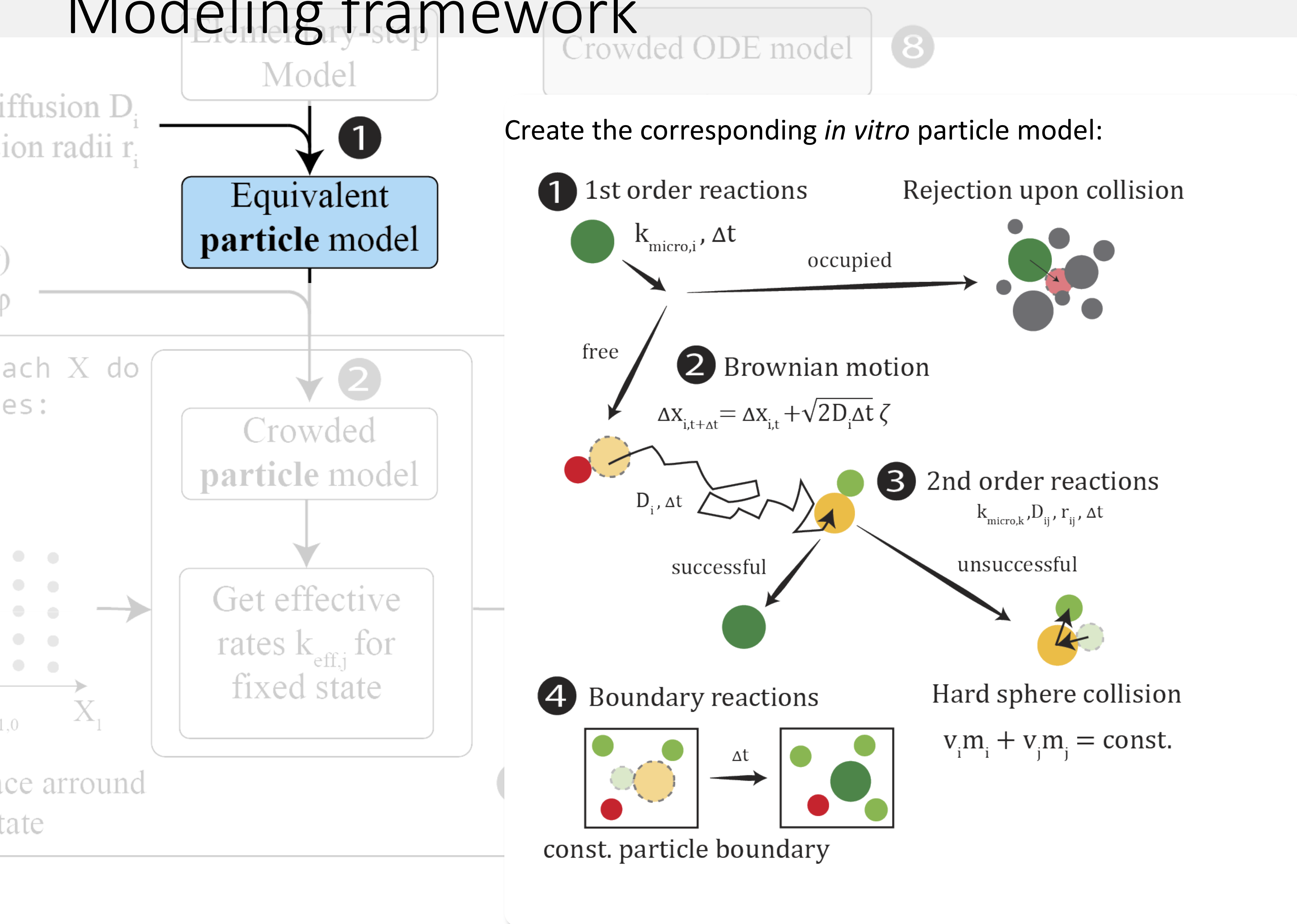
Modeling framework

Reaction mechanism

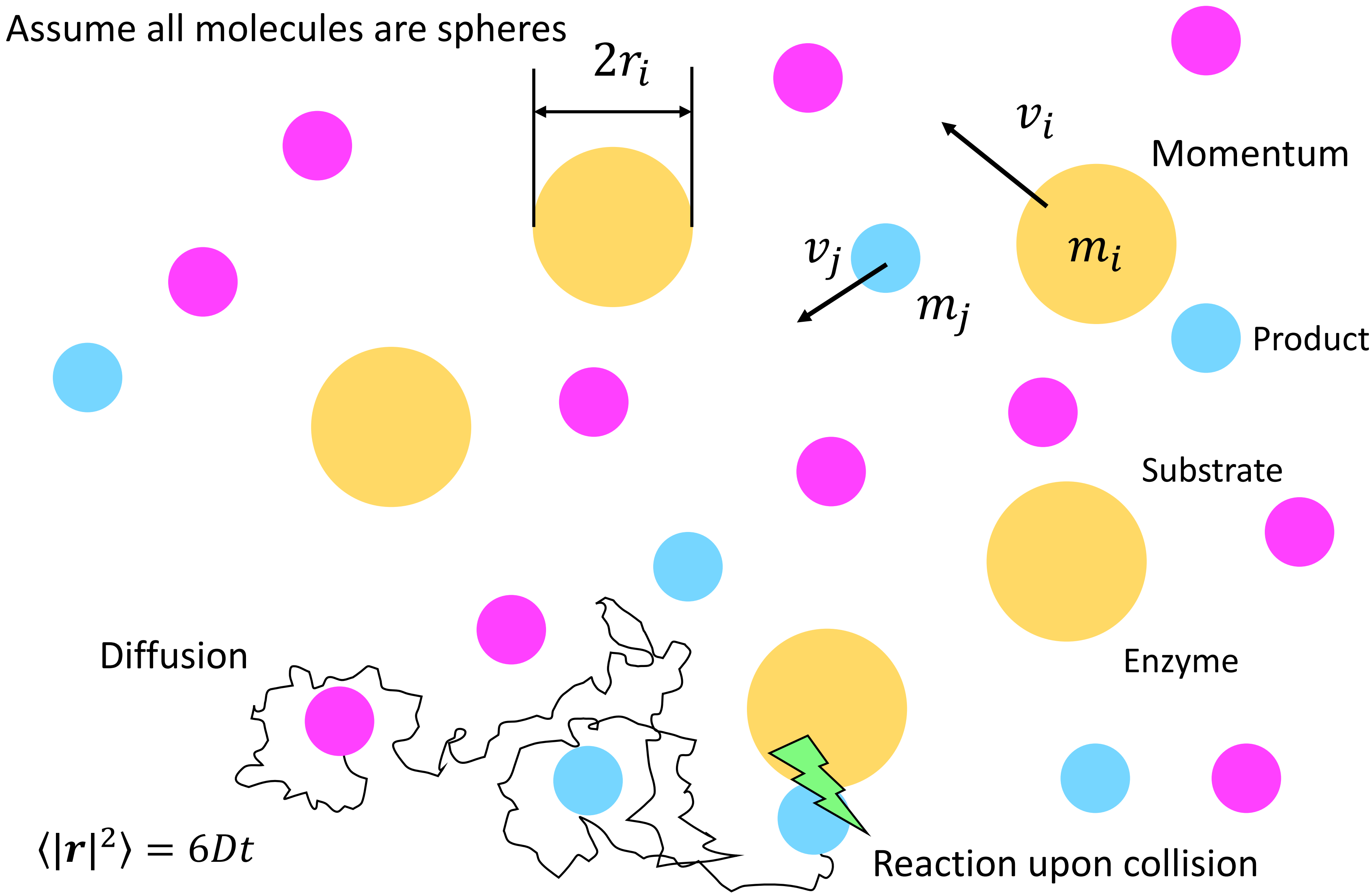
$$\mathrm{g2p{+}pgm} \underset{k_{1,b}}{\overset{k_{1,f}}{\rightleftharpoons}} \mathrm{pgm\!:\!g2p} \underset{k_{2,b}}{\overset{k_{2,f}}{\rightleftharpoons}} \mathrm{pgm + g3p}$$

Michaelis-Menten parameters		Rate constants	
K_m g3p	210 µM	**$k_{1,f}$**	15.2×10^5 $s^{-1}M^{-1}$
K_m g2p	97 µM	**$k_{1,b}$**	10 s^{-1}
k_{cat} g3p to g2p	22 s^{-1}	**$k_{2,f}$**	22 s^{-1}
k_{cat} g2p to g3p	10 s^{-1}	**$k_{2,b}$**	32.9×10^5 $s^{-1}M^{-1}$

Modeling framework



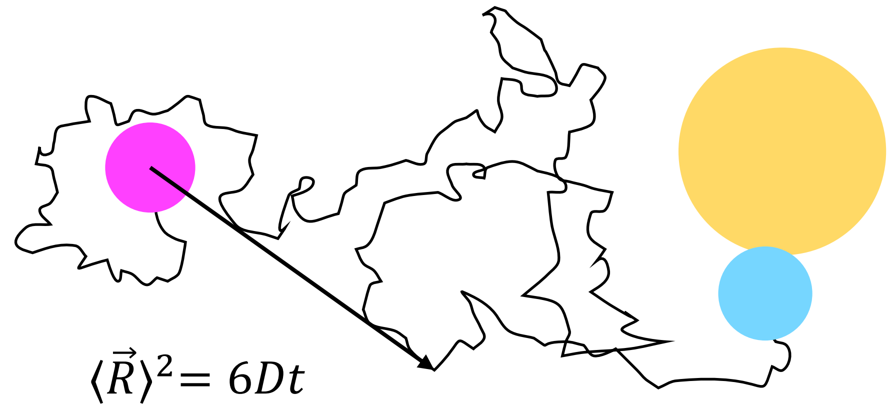
Particle models

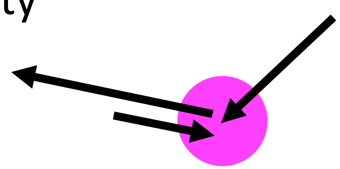


Diffusion

Brownian motion:

The random motion of a particle
in a viscous medium
(microscopic definition of diffusion)





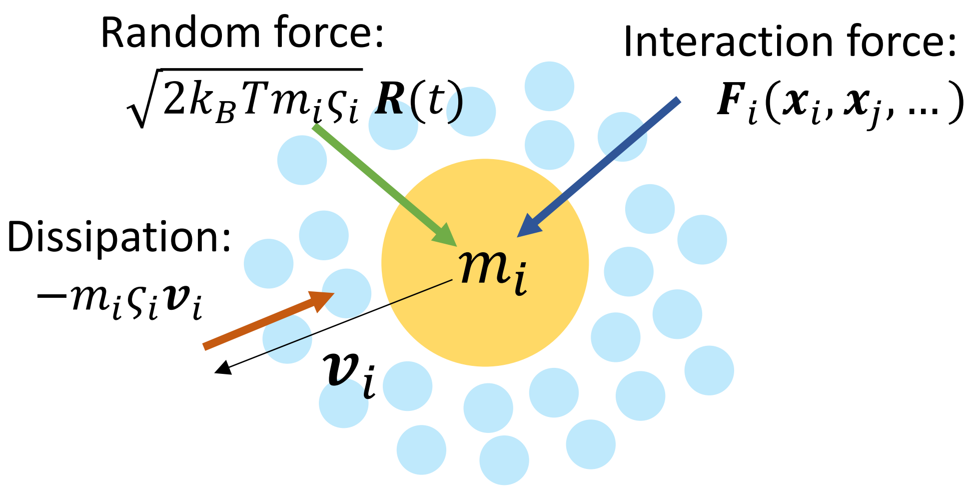
Relation between collision forces
and drag force is the diffusion coefficient

D

Diffusion

Langevin thermostat:

$$m_i \frac{d^2 \boldsymbol{x}_i}{dt^2} = \boldsymbol{F}_i(\boldsymbol{x}_i, \boldsymbol{x}_j, \dots) - m_i \varsigma_i \frac{d\boldsymbol{x}_i}{dt} + \sqrt{2k_B T m_i \varsigma_i}\, \boldsymbol{R}(t)$$



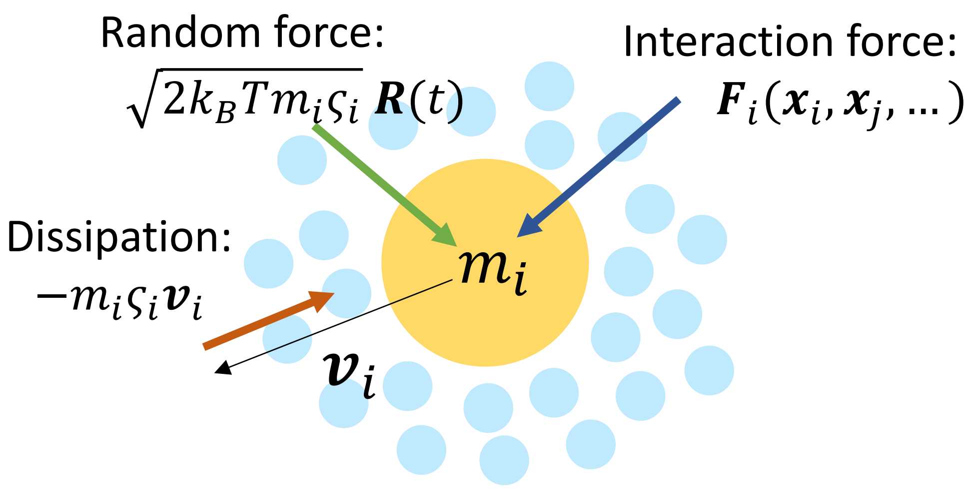
ς_i	Specific drag coefficient
$k_B T$	Thermal energy

[3] A. Brünger et al., 1984, 105 (5) 495-500.

Diffusion

Langevin thermostat:

$$m_i \frac{d^2\boldsymbol{x}_i}{dt^2} = \boldsymbol{F}_i(\boldsymbol{x}_i, \boldsymbol{x}_j, \dots) - m_i\varsigma_i \frac{d\boldsymbol{x}_i}{dt} + \sqrt{2k_B T m_i \varsigma_i}\,\boldsymbol{R}(t) \qquad m_i \gg \quad \rightarrow \quad \frac{d^2\boldsymbol{x}_i}{dt^2} \ll$$



ς_i Specific drag coefficient

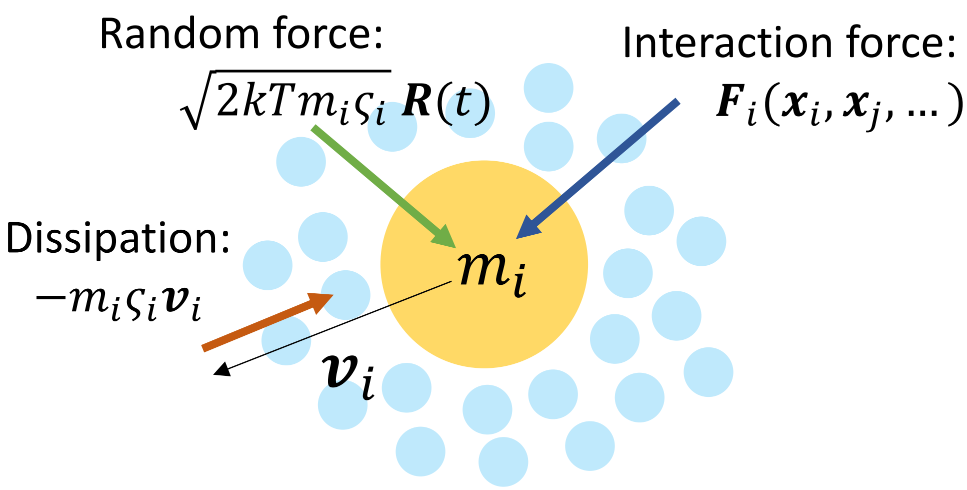
$k_B T$ Thermal energy

[3] A. Brünger et al., 1984, 105 (5) 495-500.

Diffusion

Overdamped langevin thermostat:

$$\frac{d\boldsymbol{x}_i}{dt} = \frac{\boldsymbol{F}_i(\boldsymbol{x}_i, \boldsymbol{x}_j, \ldots)}{m_i \varsigma_i} - \frac{\sqrt{2k_B T m_i \varsigma_i}}{m_i \varsigma_i} \boldsymbol{R}(t) \qquad m_i \gg \quad \rightarrow \quad \frac{d^2\boldsymbol{x}_i}{dt^2} \ll$$



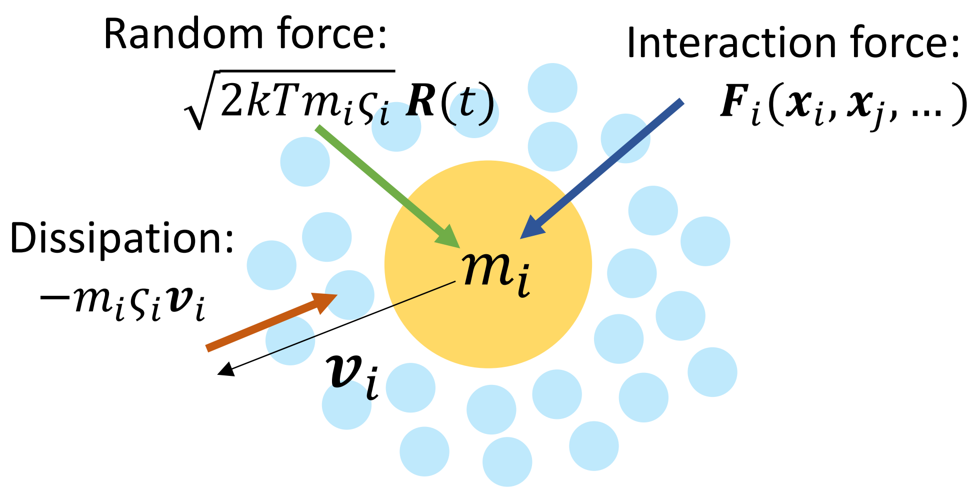
[3] A. Brünger et al., 1984, 105 (5) 495-500.

Diffusion

Overdamped langevin thermostat:

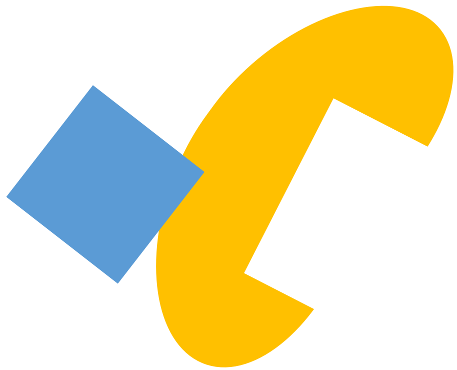
$$\frac{d\boldsymbol{x}_i}{dt} = \frac{\boldsymbol{F}_i(\boldsymbol{x}_i, \boldsymbol{x}_j, \dots)}{m_i\varsigma_i} - \frac{\sqrt{2k_BTm_i\varsigma_i}}{m_i\varsigma_i}\boldsymbol{R}(t) \qquad m_i \gg \quad \rightarrow \quad \frac{d^2\boldsymbol{x}_i}{dt^2} \ll$$

$$\frac{d\boldsymbol{x}_i}{dt} = \boldsymbol{F}_i(\boldsymbol{x}_i, \boldsymbol{x}_j, \dots)\frac{k_BT}{D_i} - \sqrt{2D_i}\boldsymbol{R}(t) \qquad D_i = \frac{k_BT}{m_i\varsigma_i} \quad \frac{\text{Thermal Exitiation}}{\text{Local dissipation}}$$



[3] A. Brünger et al., 1984, 105 (5) 495-500.

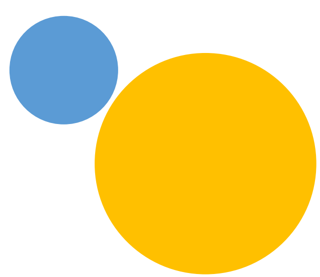
Enzymatic reactions



Unsuccessful collision

Successful collision

Only a part of the collisions is successful

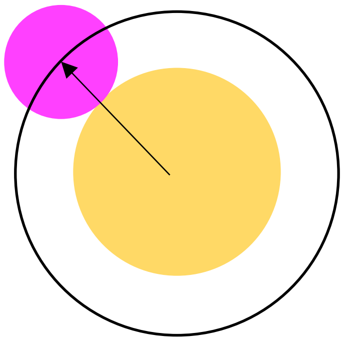


How do can we account for this?

Enzymatic reactions

Collision dynamics:

Reaction volume



a) Reaction determined by the random number p

$$p \leq 1 - e^{\frac{\kappa_{ij}\Delta t}{I_n}}$$

microscopic reaction rate constant

effective reaction volume

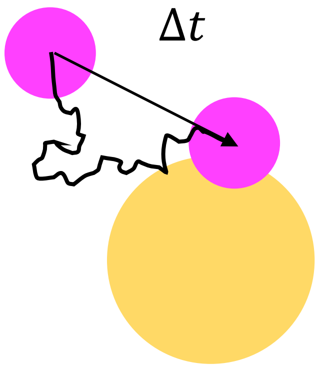
Contact surface

Microscopic reaction rate:

Probability that volume of particle i and j is absorbed at their contact boundary

Reaction volume:

Volume within the contact boundary



Effective reaction volume:

Correction on for all possible path in Δt the particle could have taken.
(Necessary for explicit discrete time integration.)

Morelli, M. J. and P. R. ten Wolde (2008), Journal of Chemical Physics, **129**(5).

Enzymatic reactions

Collision dynamics:

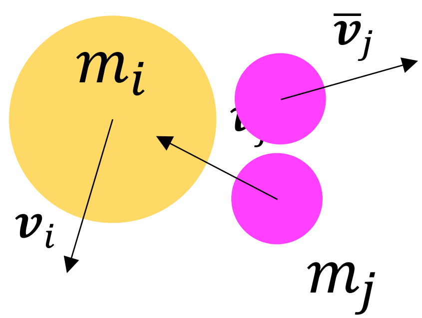
a) Reaction determined by the random number p

$$p \leq 1 - e^{\frac{\kappa_{ij}\Delta t}{I_n}}$$

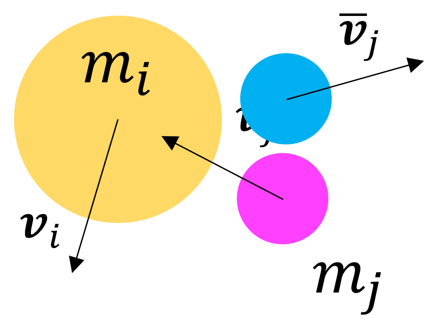
b) Rebound according to momentum conservation:

$$\boldsymbol{v}_i m_i + \boldsymbol{v}_j m_j = \overline{\boldsymbol{v}}_i m_i + \overline{\boldsymbol{v}}_j m_j$$

b)

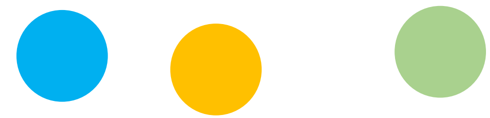


a) + b)



Relation to mass action

$$S + E \xrightarrow{k} ES$$



If every collision is successful:

Diffusion limited reaction rate for bimolecular reactions [1] :

$$\gamma_{ij} = 4\pi(D_E + D_S)(r_E + r_S)$$

$$k = \gamma_{ij}$$

D_S, r_S D_E, r_E

Only a part of the collisions is successful [2] :

$$k \approx \left(\frac{1}{\kappa_{ij}} + \frac{1}{\gamma_{ij}}\right)^{-1}$$

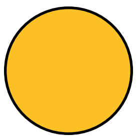
κ_{ij} the **microscopic rate** or probability of a successful collision per time

[1] Smoluchowski, M., 1927, 2 (1) 595-639.
[2] Collins, F.C. & G.E. Kimball, 1949, 4(4) 425-437.

Modeling framework

Reactant data

PGM:

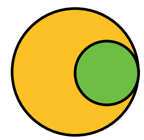


$r_E = 3.87\ \mathrm{nm}$

$D_E = 84.8\,\frac{\mu\mathrm{m}^2}{s}$

$m_E = 61\ \mathrm{kDa}$

PGM:g2p :

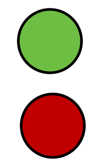


$r_E = 3.87\ \mathrm{nm}$

$D_E = 84.8\,\frac{\mu\mathrm{m}^2}{s}$

$m_E = 61\ \mathrm{kDa}$

g3p/g2p:

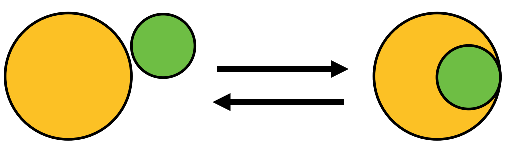


$r_E = 1.11\ \mathrm{nm}$

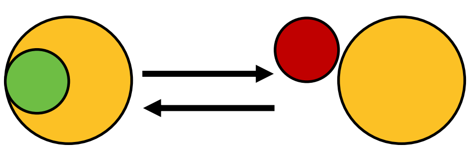
$D_E = 940\,\frac{\mu\mathrm{m}^2}{s}$

$m_E = 0.168\ \mathrm{kDa}$

Microscopic rate constants

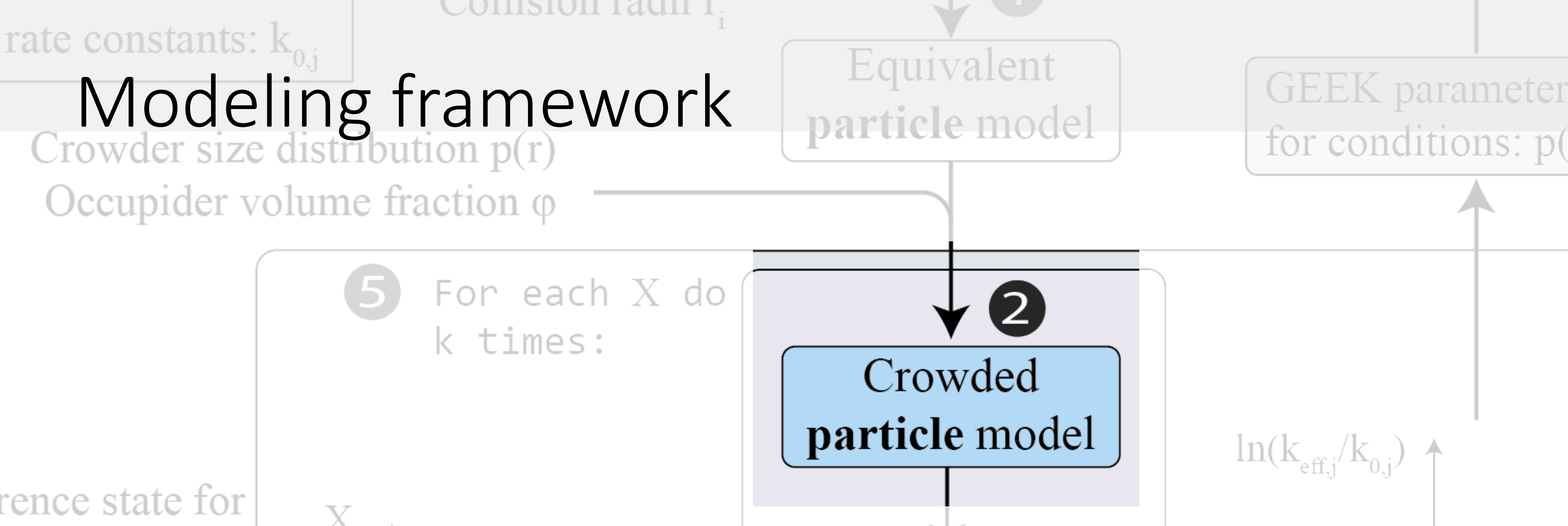


$k_{1,f}$	$15.7 \times 10^5\ s^{-1}M^{-1}$
$k_{1,b}$	$10\ s^{-1}$



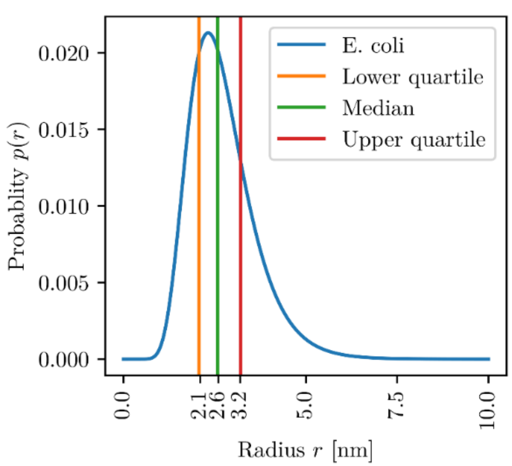
$k_{2,f}$	$22\ s^{-1}$
$k_{2,b}$	$34.0 \times 10^5\ s^{-1}M^{-1}$

Modeling framework



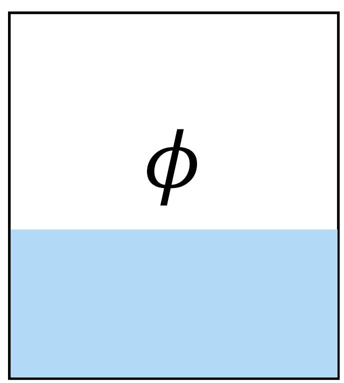
Integrate crowding into the particle model

Size distribution



+

Volume occupancy



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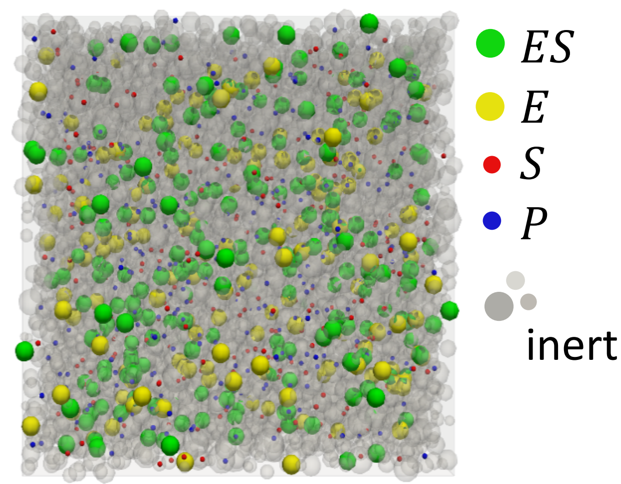
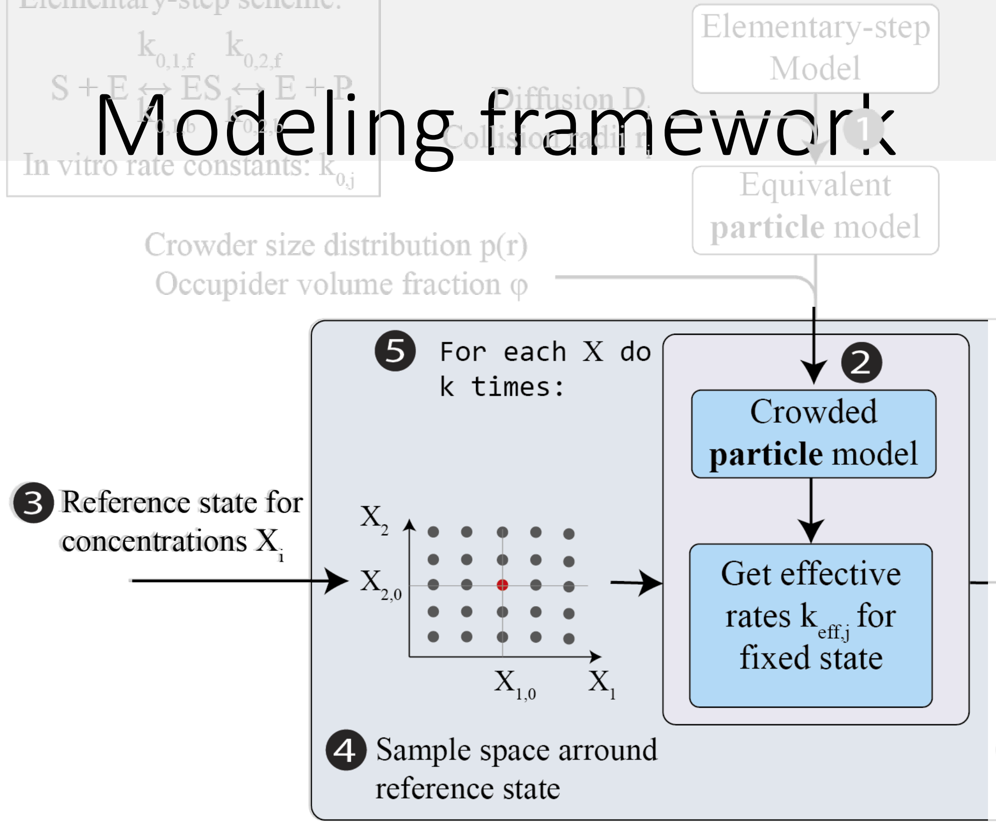
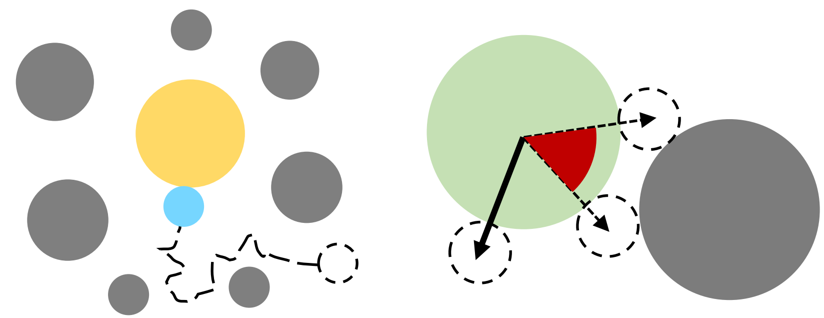
Crowded particle model



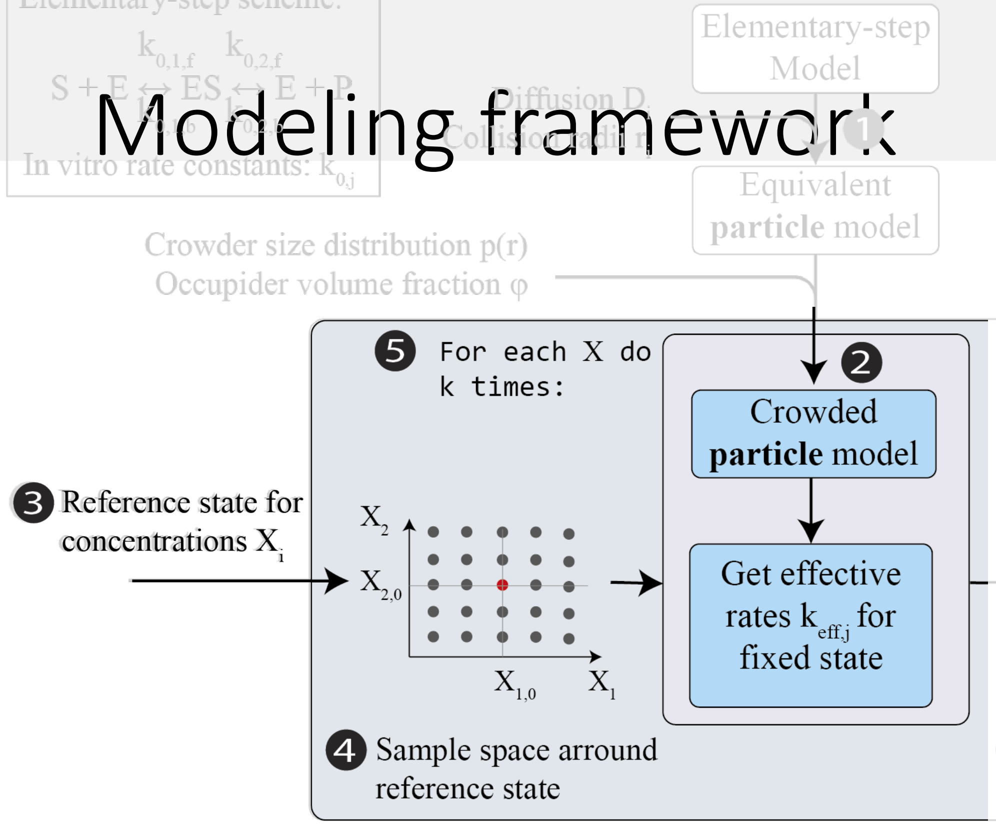
Modeling framework



Measure the effective association and dissociation rates in the crowded particle simulation:



Modeling framework



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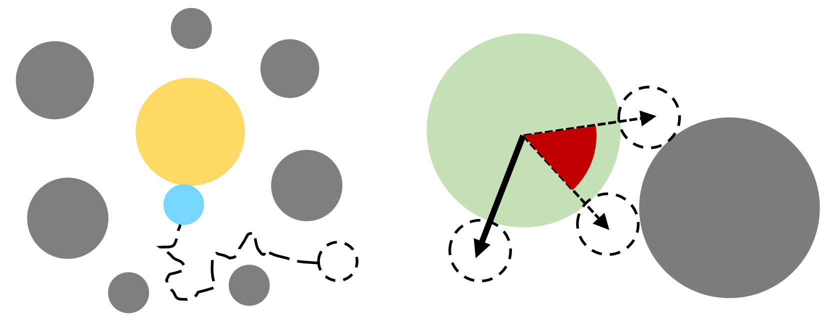
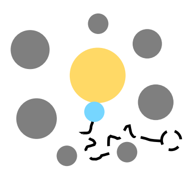


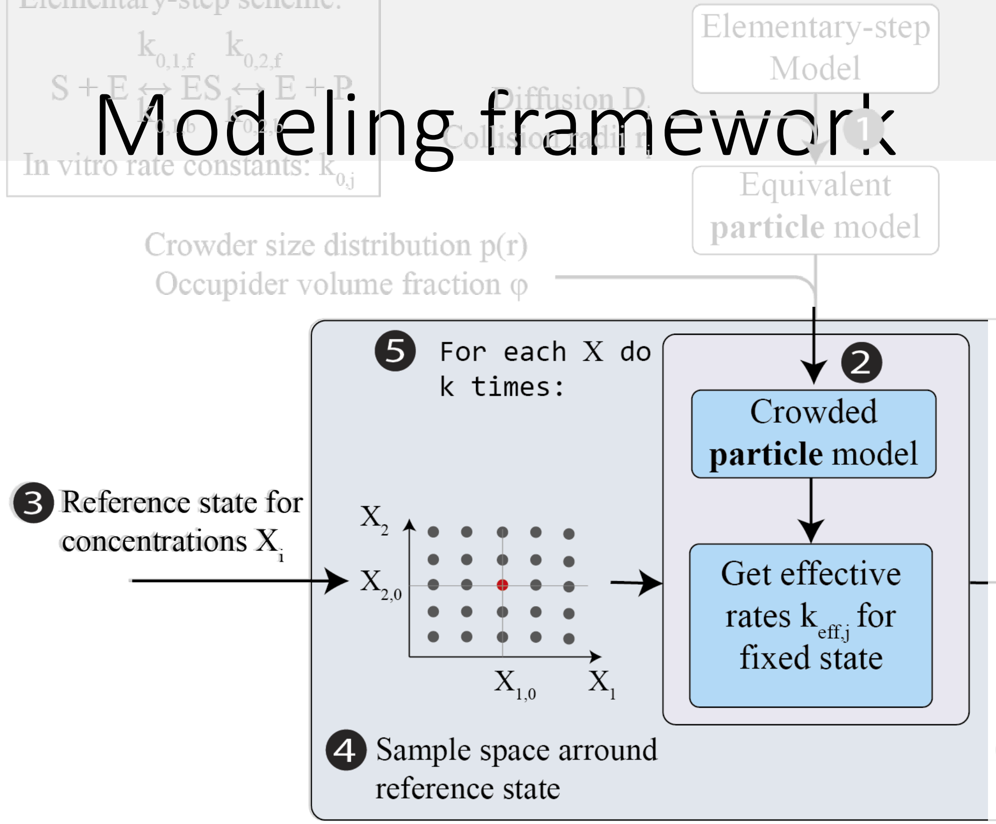
Bimolecular rate constants:



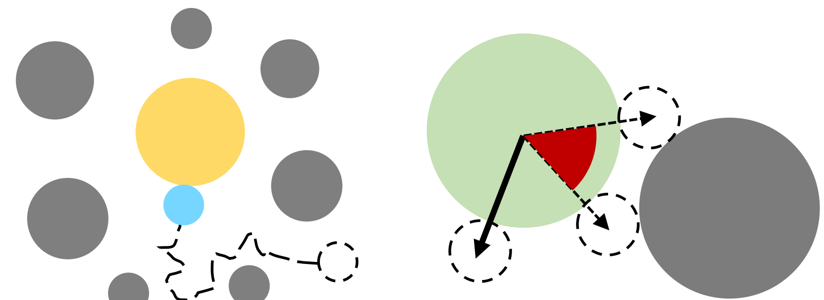
$$\langle z_{A,B} \rangle = \frac{c_N(t, t+\Delta t)}{\Delta t}$$

$$k_{bi,eff} = \frac{\langle z_{A,B} \rangle}{N_A N_B}\left(1 - \exp\left(-\frac{k_{micr}\Delta t}{V_{eff}}\right)\right)$$

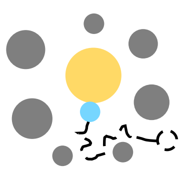
Modeling framework



Measure the effective association and dissociation rates in the crowded particle simulation:



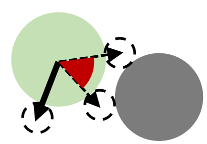
Bimolecular rate constants:



$$\langle z_{A,B} \rangle = \frac{c_N(t, t+\Delta t)}{\Delta t}$$

$$k_{bi,eff} = \frac{\langle z_{A,B} \rangle}{N_A N_B}\left(1 - \exp\left(-\frac{k_{micr}\Delta t}{V_{eff}}\right)\right)$$

Monomolecular rate constants:



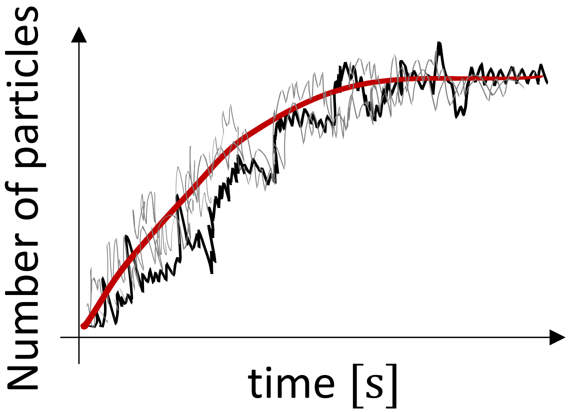
$$\langle \omega \rangle = \frac{1}{NM}\sum_{i,j}^{N,M} \omega_{ij}$$

$$k_{mon,eff} = k_0 \langle \omega \rangle$$

Modeling framework

Classical Hard Sphere Brownian reaction dynamics:

- Computational intensive for reaction rate limited reactions $\frac{t_{computational}}{t_{simulation}} \sim \frac{1\ \mathrm{Month}}{0.1\ s}$
- Collisions in the order of $< \ 1\ \mathrm{ns}$
  Reaction time scale $> 1s$
- Only information about a single realization



Modeling framework

Classical Hard Sphere Brownian reaction dynamics:

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- Collisions in the order of $< \ 1 \text{ ns}$
  Reaction time scale $> 1s$

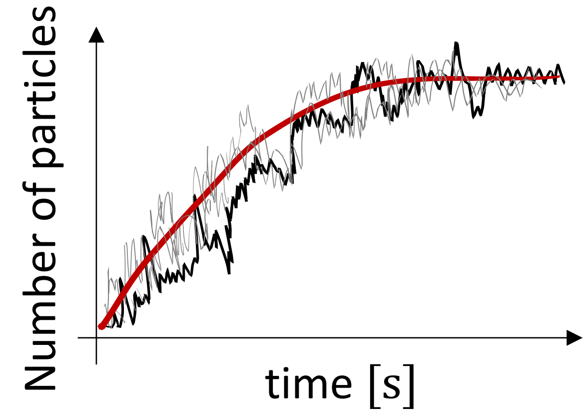
- Only information about a single realization

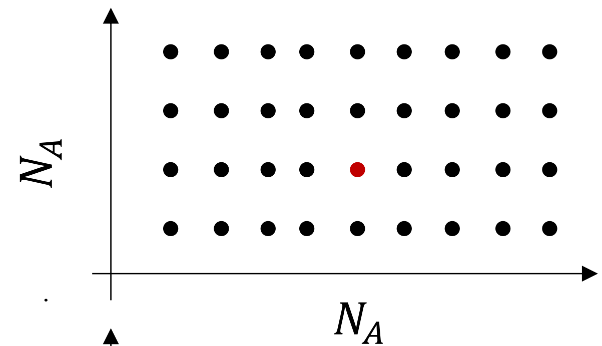
Effective rate assessment:

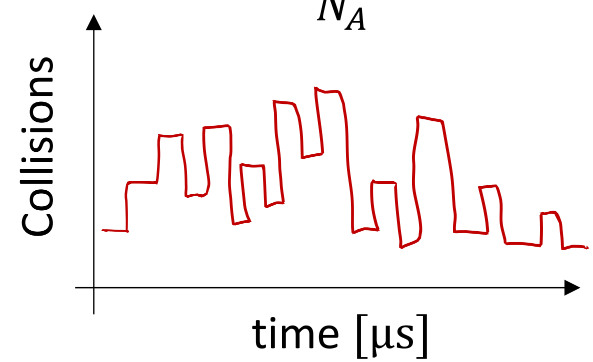
- Asses the effective collision frequency for a given state, i.e. $\{N_A, N_B, N_C, \dots\}$
  ~ a minute / state

- Discretization of the state space

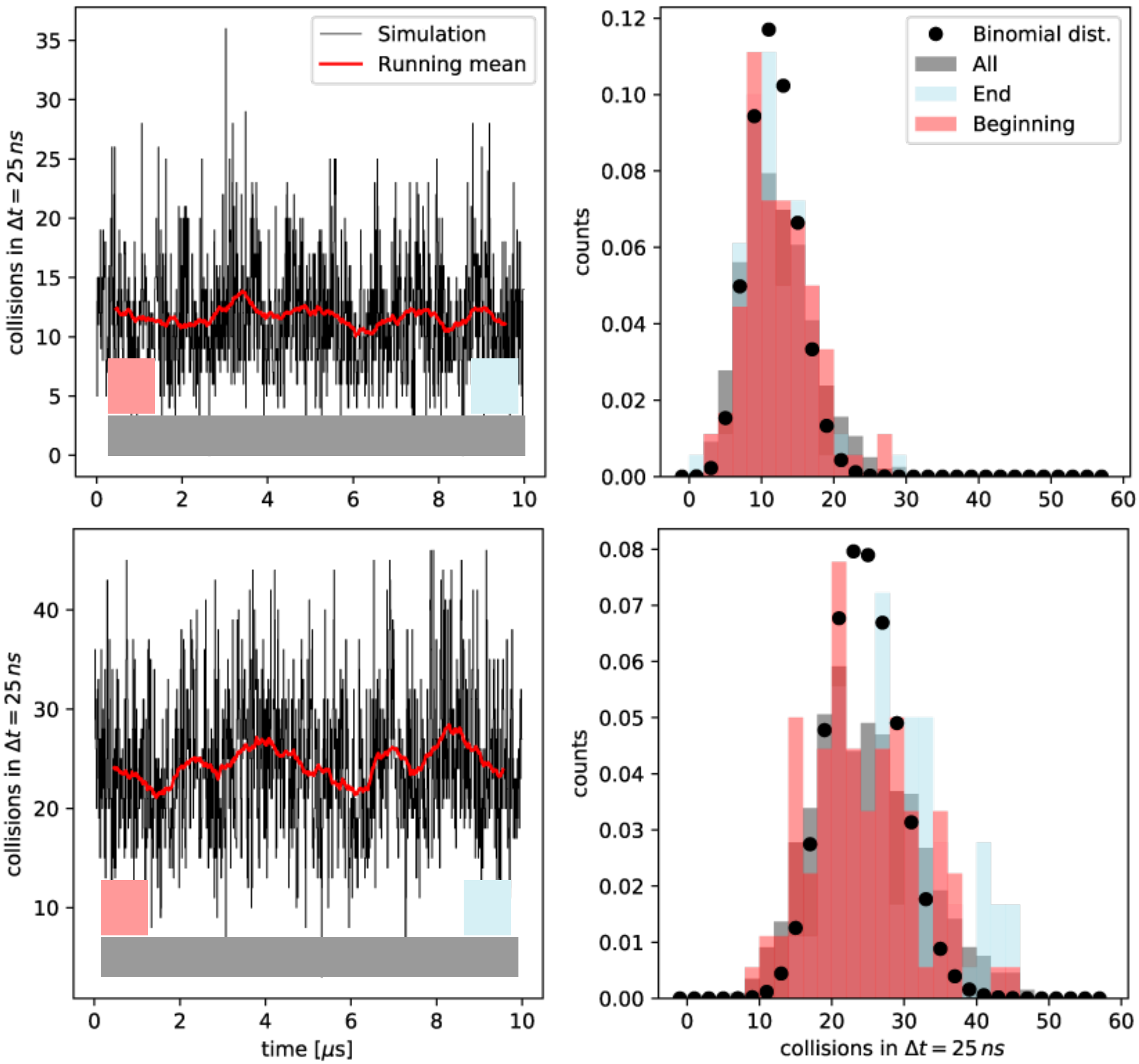
- Information about on the effective reaction rate in every state







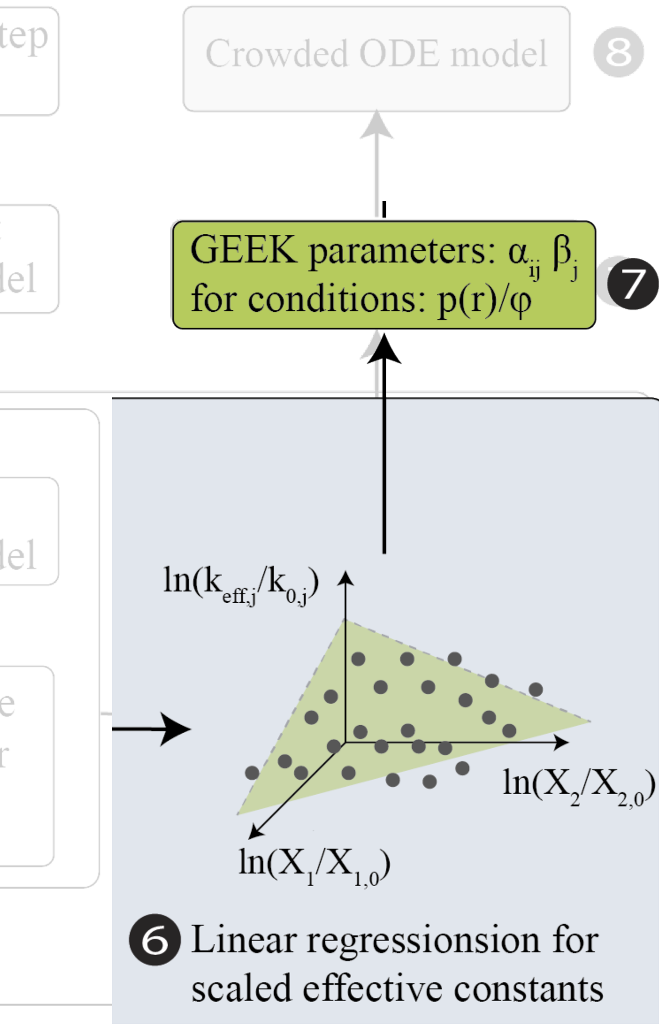
Modeling framework



0 % inert volume fraction

40 % inert volume fraction

Modeling framework



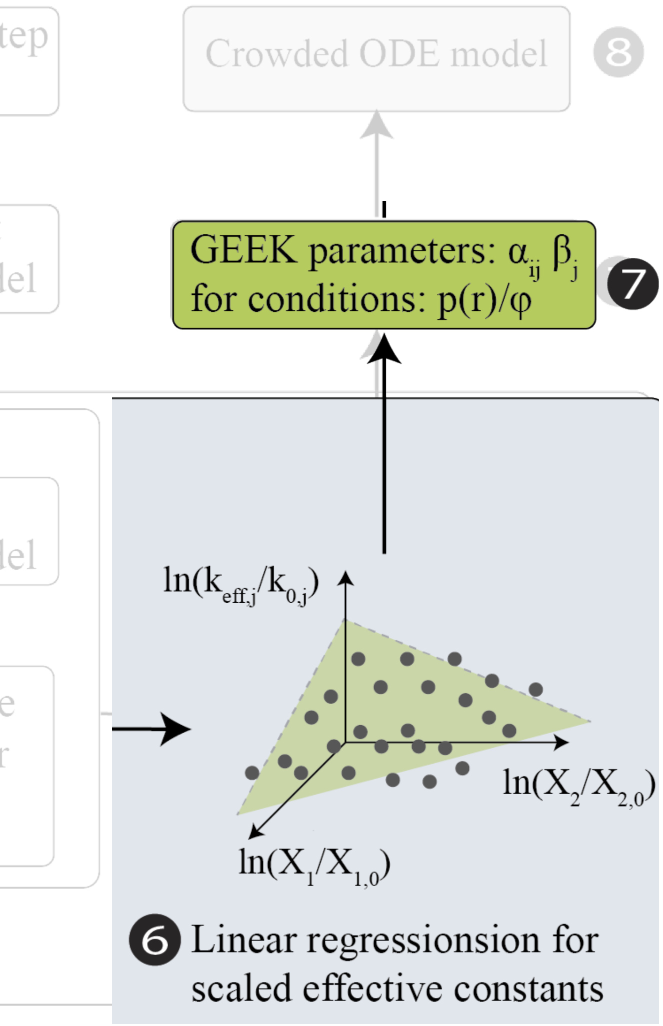
Extract GEEK parameters from logarithmic scaled rate constants:

$$\log\left(\frac{k_{j,eff}}{k_{j,0}}\right) = \sum_{i=1}^{N} \alpha_{i,j} \log\left(\frac{[X_i]}{[X_i]_0}\right) + \beta_j$$

GEEK reaction rates:

$$k_{j,eff}(\phi) = k_{i,0} e^{\beta_j} \prod_{i=1}^{N} \left(\frac{[X_i]}{[X_i]_{ref}}\right)^{\alpha_{i,j}}$$

Modeling framework



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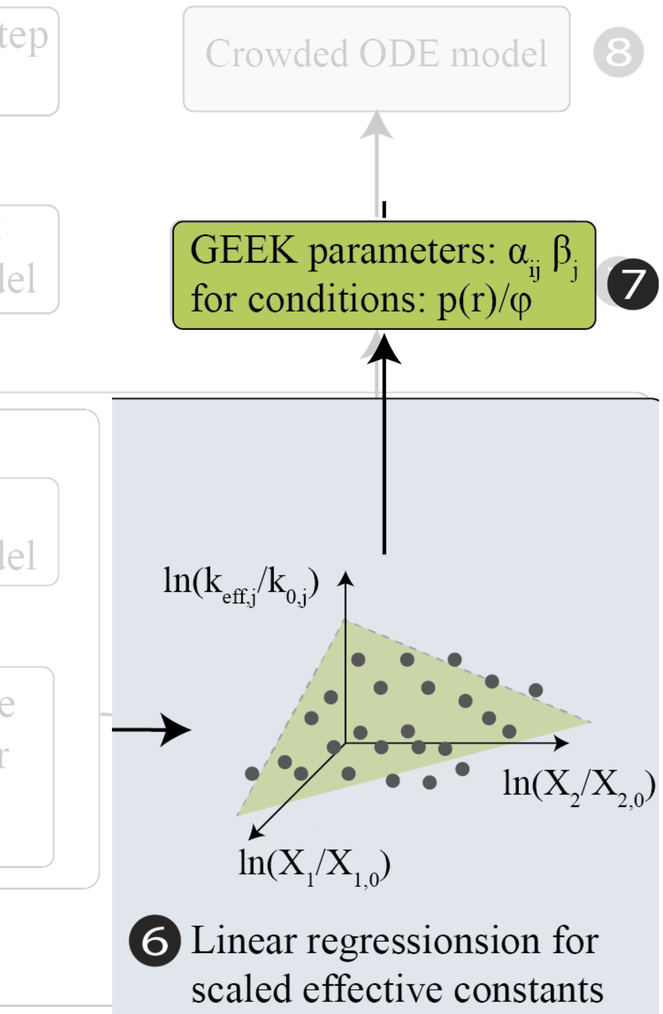
Direct effect of crowding

Modeling framework



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GEEK reaction rates:

$$k_{j,eff}(\phi) = k_{i,0}\, e^{\beta_j} \prod_{i=1}^{N} \left(\frac{[X_i]}{[X_i]_{ref}}\right)^{\alpha_{i,j}}$$

Direct effect of crowding

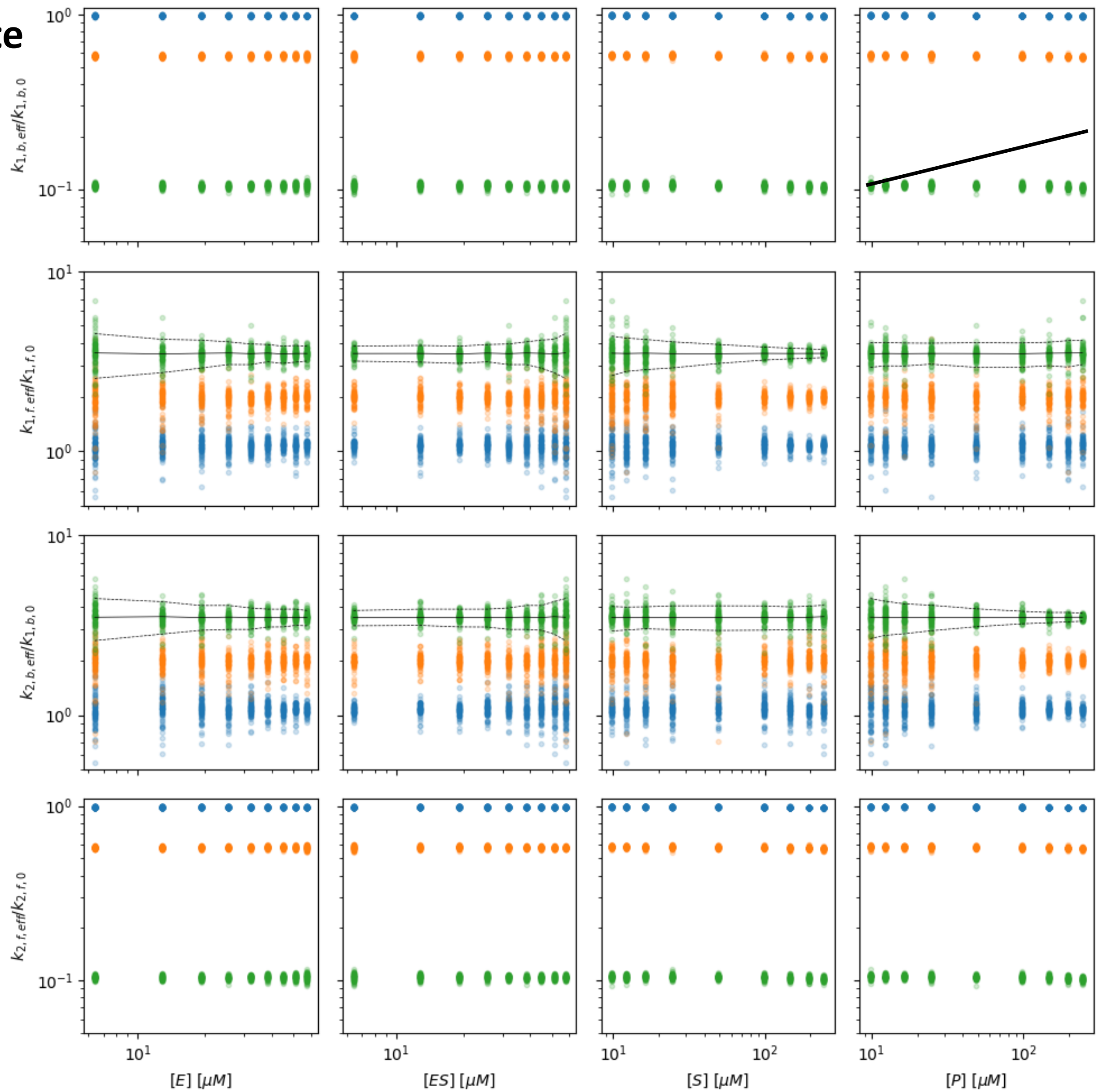
Coupled effect of crowding and concentration

$\alpha_{i,j} > 0$ Synergistic effect

$\alpha_{i,j} < 0$ Inhibiting effect

Modeling framework

Relative rate constant



$$\alpha_{ij}[X_i] + \beta_j$$

Inert volume fraction

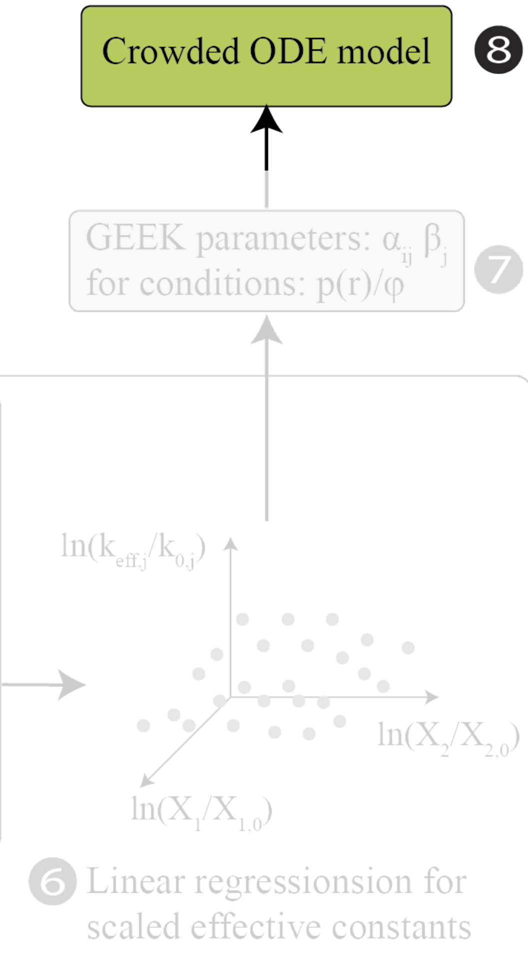
0 %

30 %

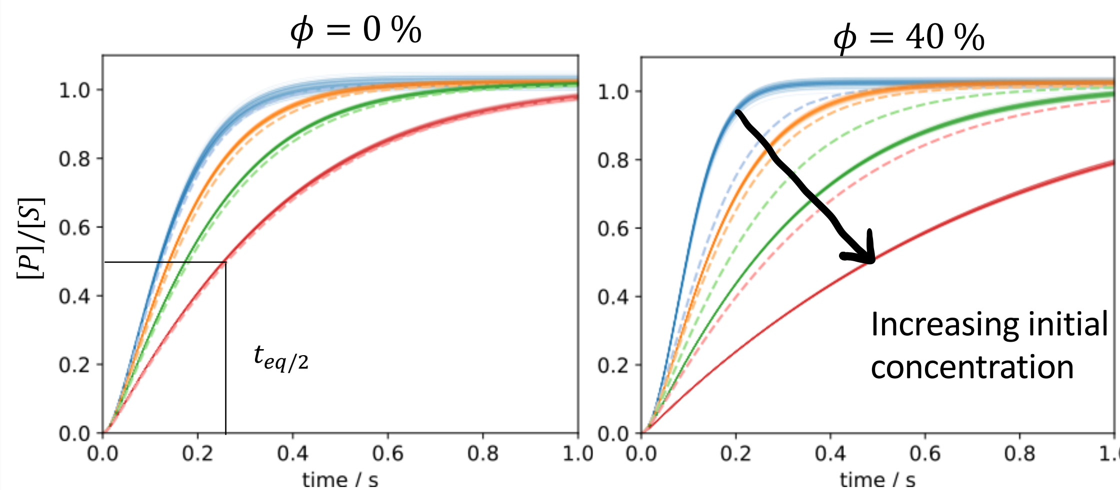
50 %

concentrations

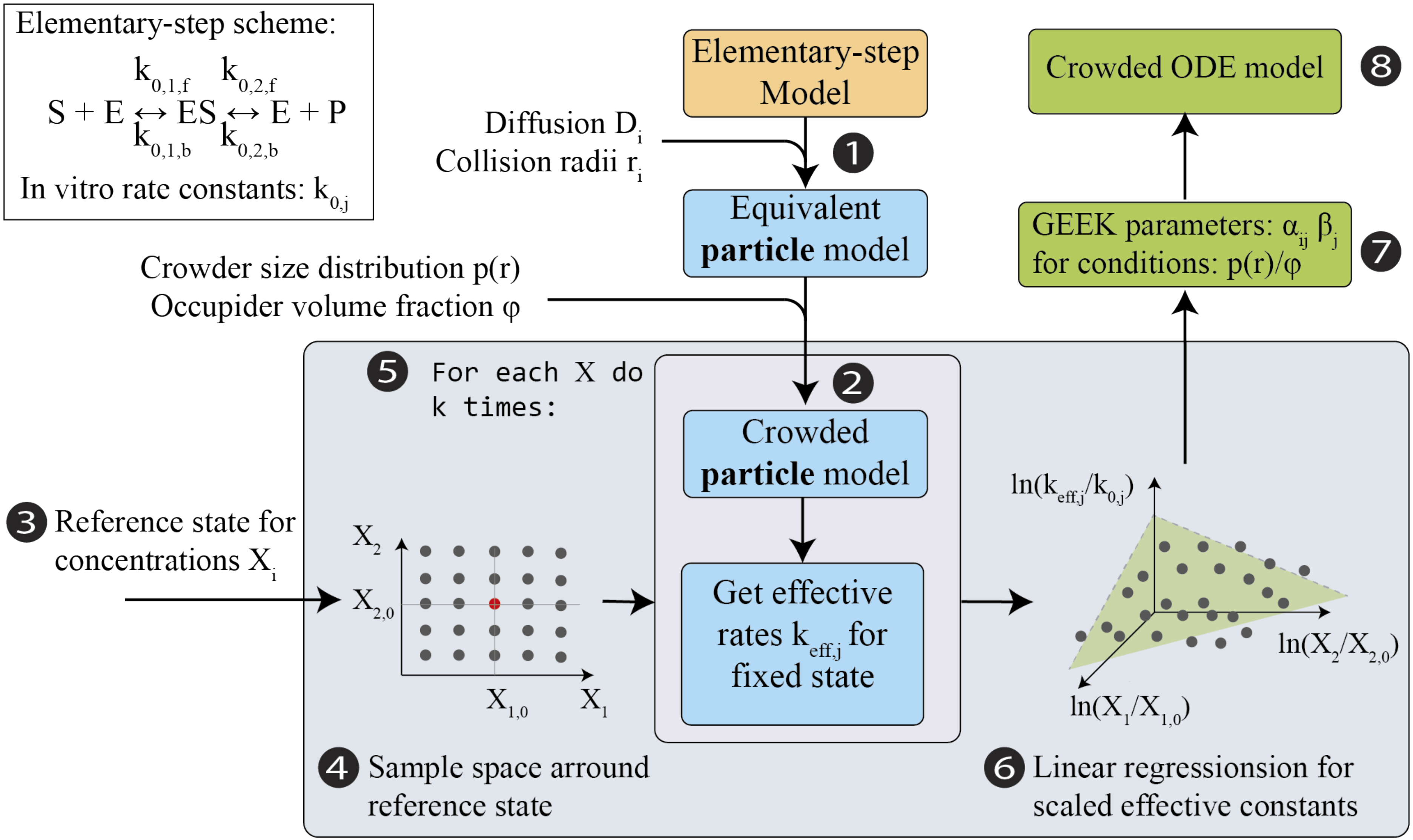
Modeling framework



Crowded ODE models:

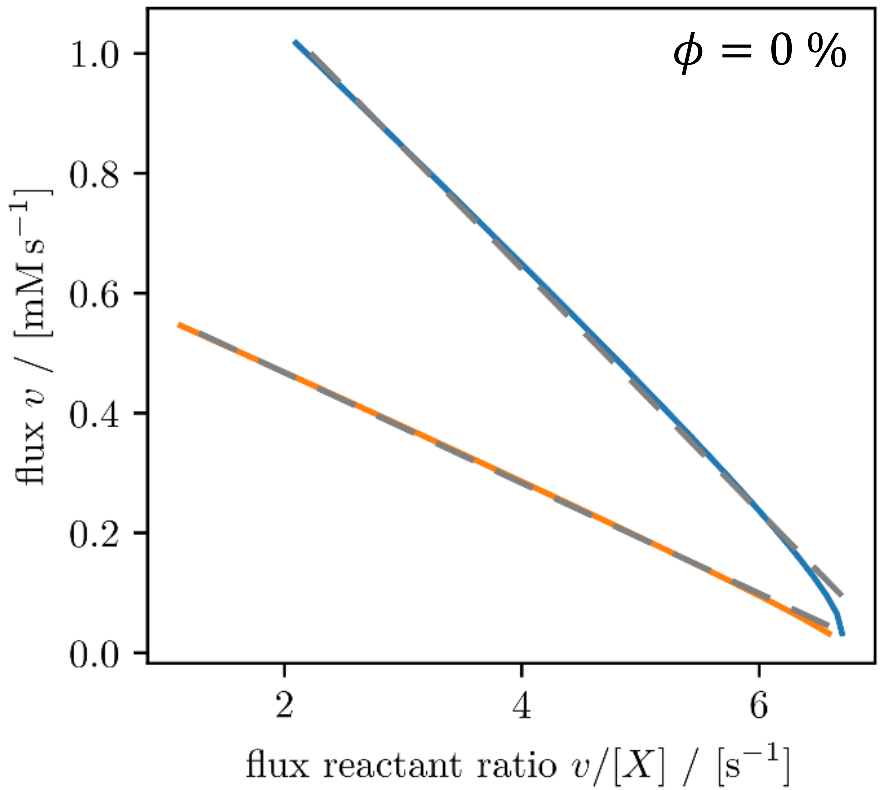


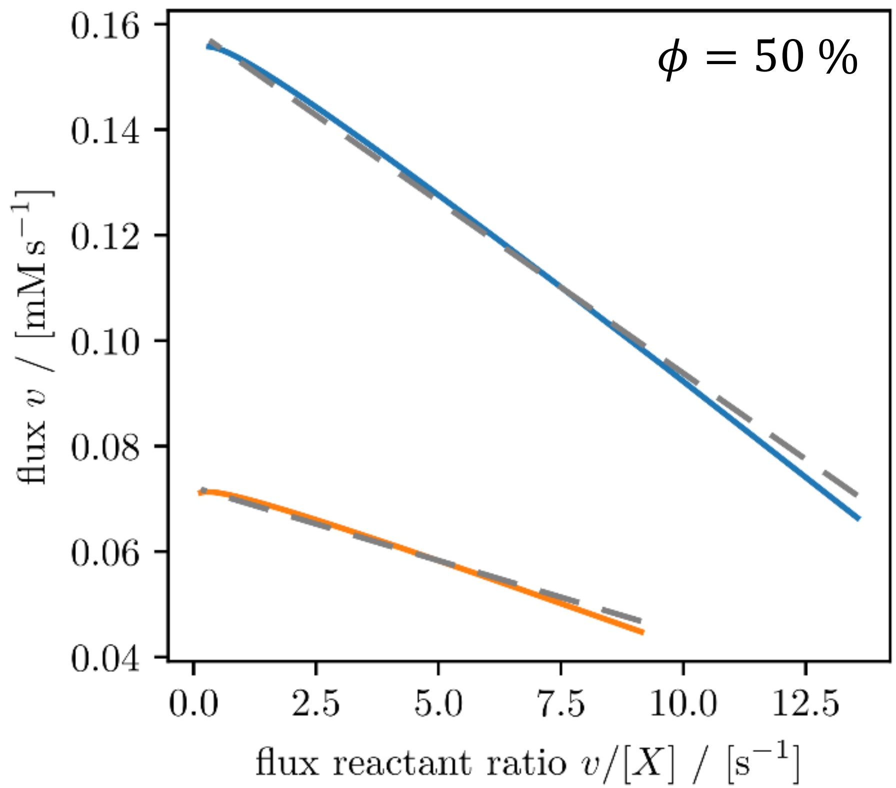
Modeling framework



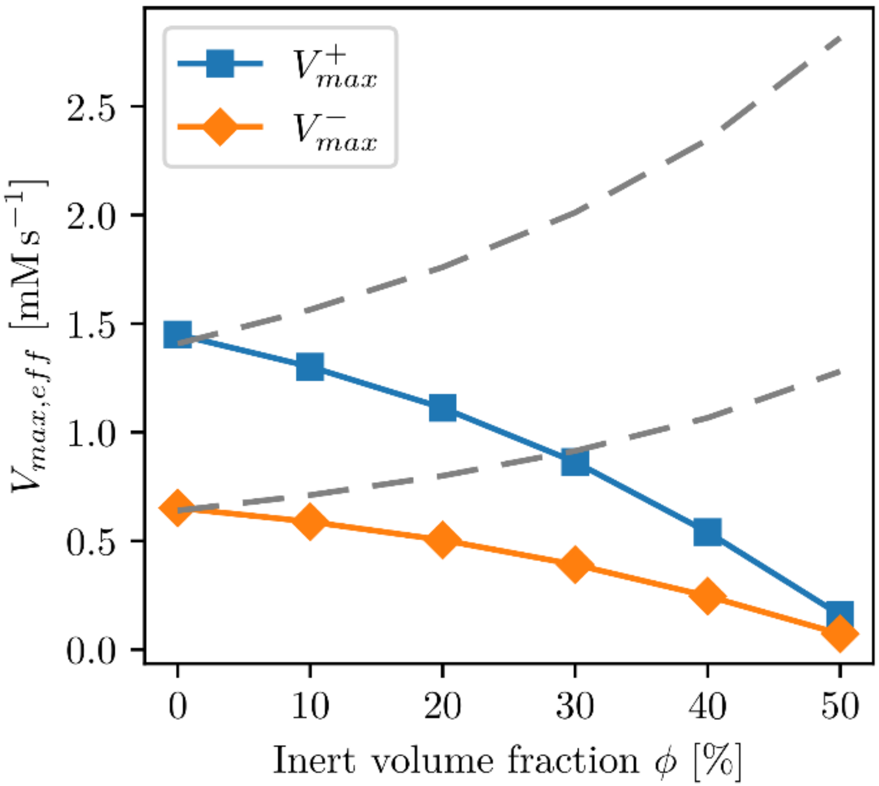
Effects on the MM-Kinetics

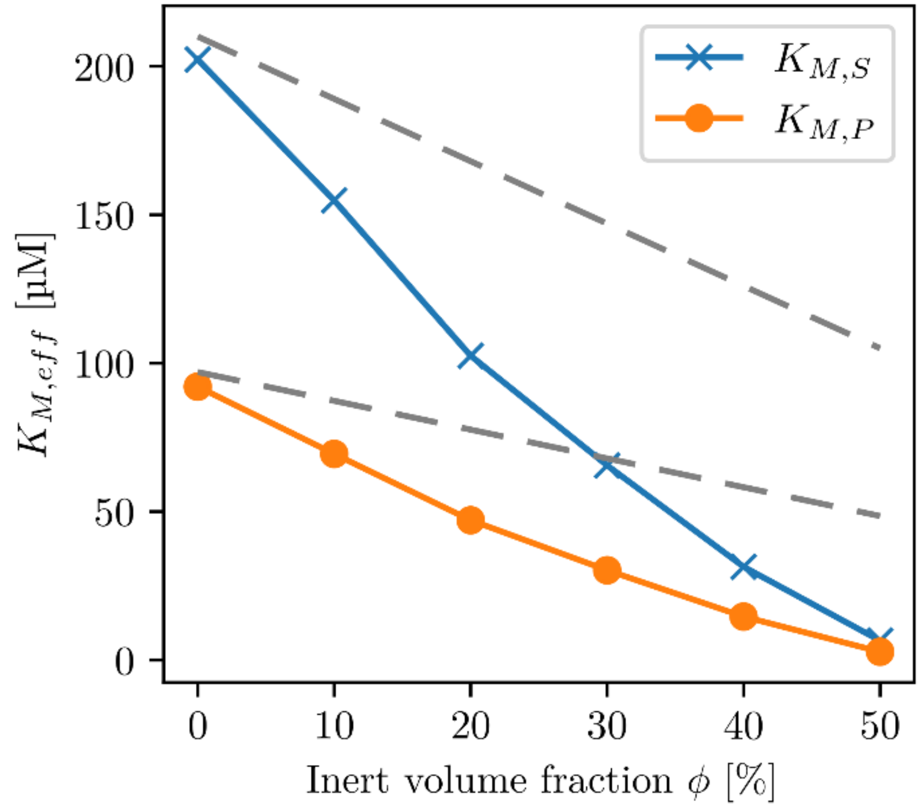
$$v = -K_{m,X}\frac{v}{[X]} + V_{max}$$

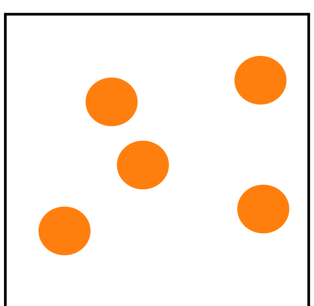




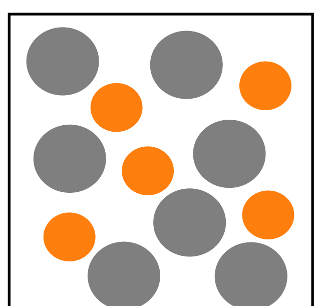
Effects on the MM-Kinetics







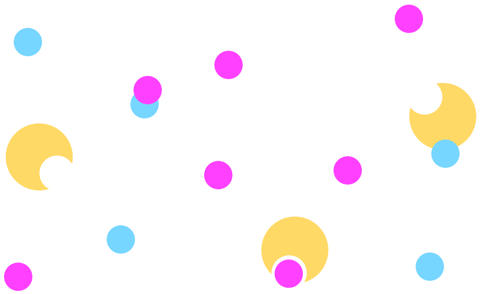
V_0
C_0



$$\left.\begin{aligned} V &= V_0(1-\phi) \\ C &= \frac{C_0}{1-\phi} \end{aligned}\right\} \text{Volume scaling}$$

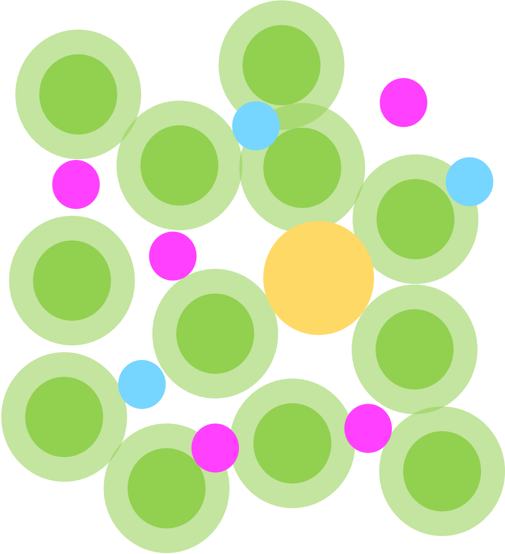
Effects on the MM-Kinetics

$$k_{mic} \ll \gamma_{ij}$$

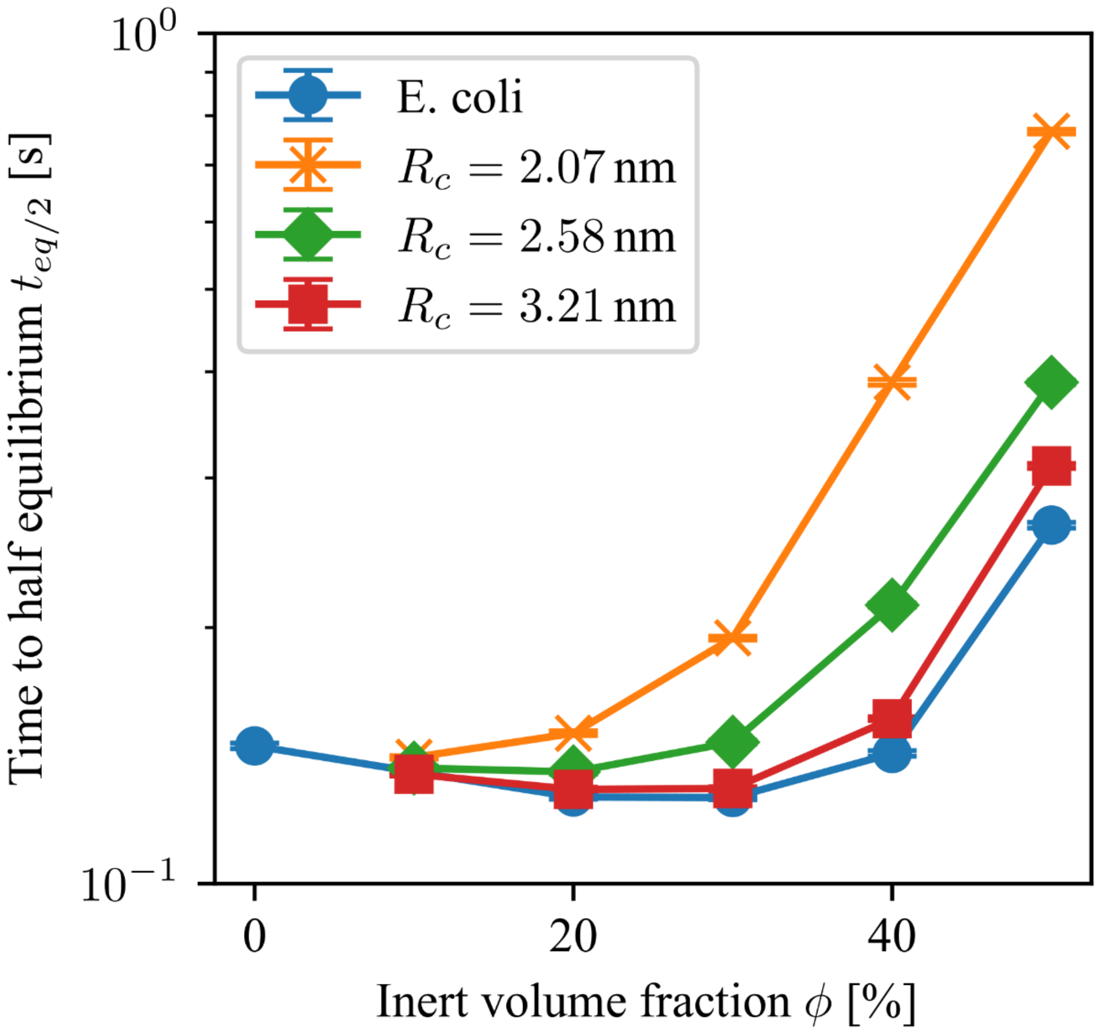


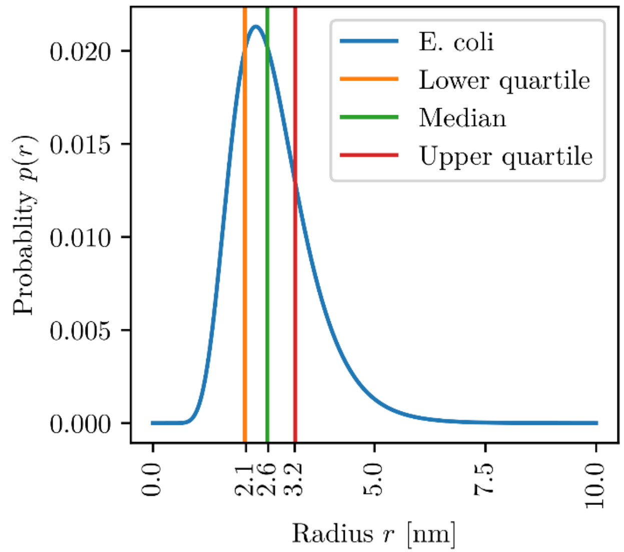
Effects on the MM-Kinetics

$$k_{mic} \ll \gamma_{ij}$$

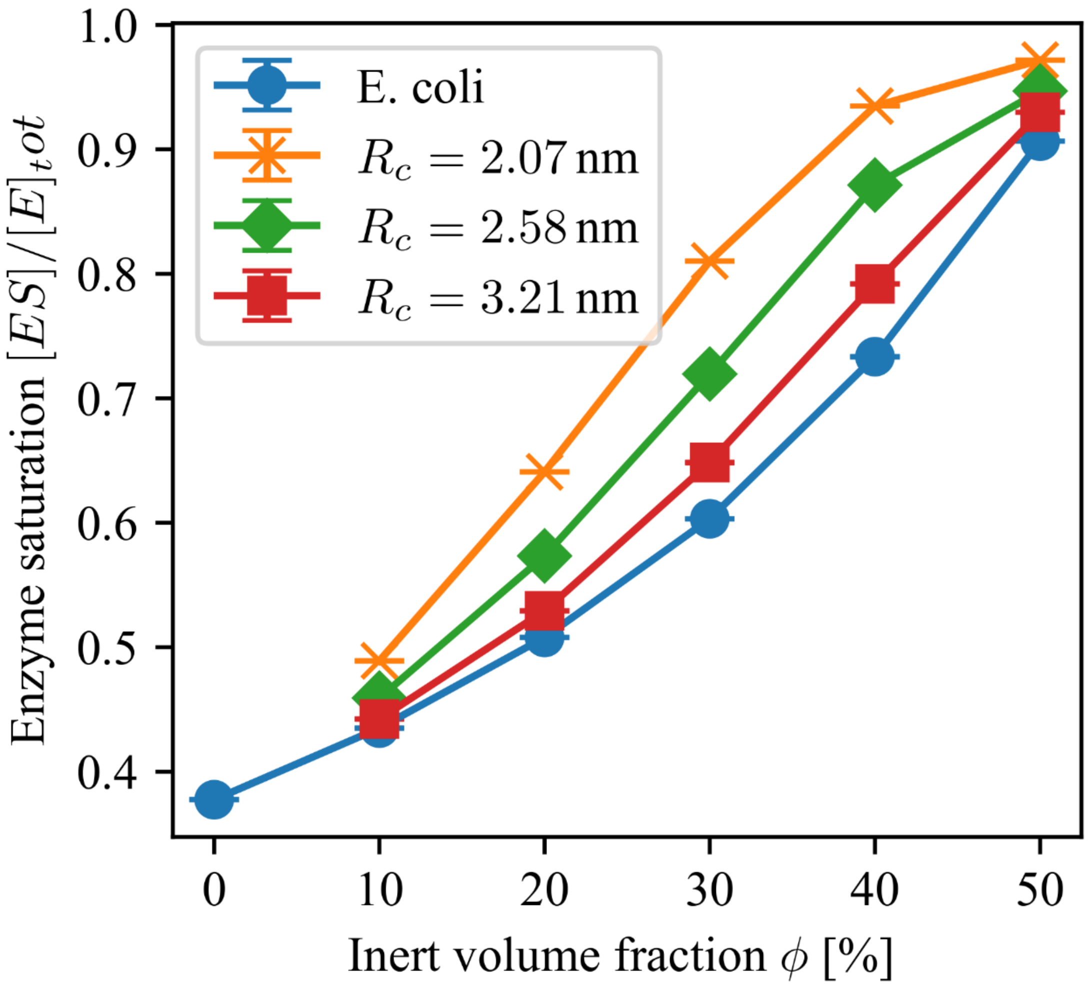


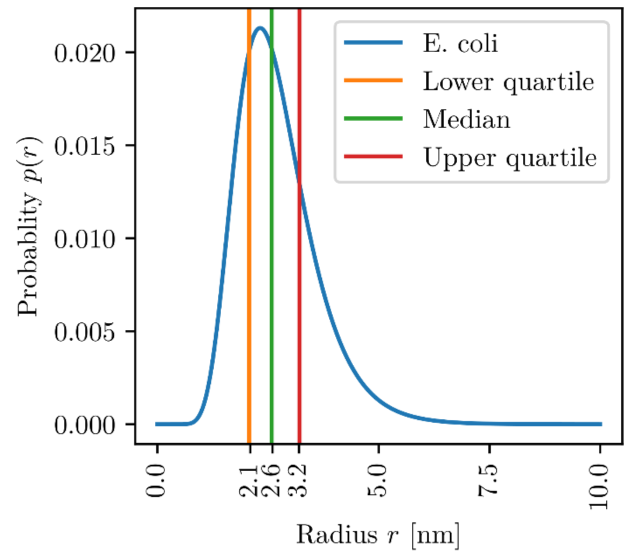
Influence of the crowder-size



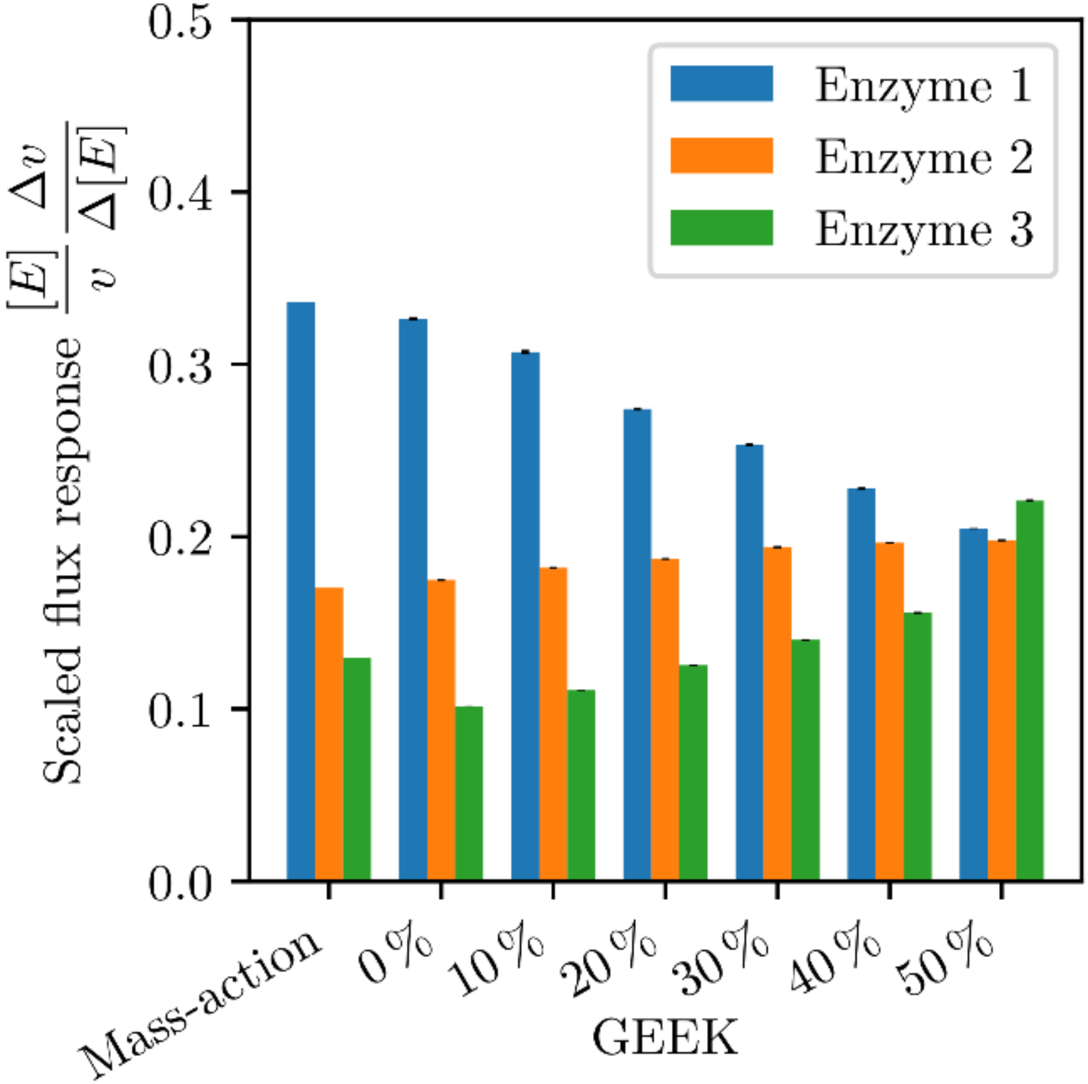


Influence of the crowder-size





Consequences for linear-pathways



Enzyme 1 Enzyme 3

$$X_1 \rightleftharpoons X_2 \rightleftharpoons X_3 \rightleftharpoons X_4$$

Enzyme 2

$$\left.\begin{aligned} X_1 &= 245\ \mu M \\ X_4 &= 49\ \mu M \end{aligned}\right\} \text{const.}$$

Enzyme 1:

$$K_{M,S} = 210\ \mu M$$
$$K_{M,P} = 291\ \mu M$$
$$V_{max}^{+} = 1.4\ mM/s$$
$$V_{max}^{-} = 0.2\ mM/s$$

Enzyme 2:

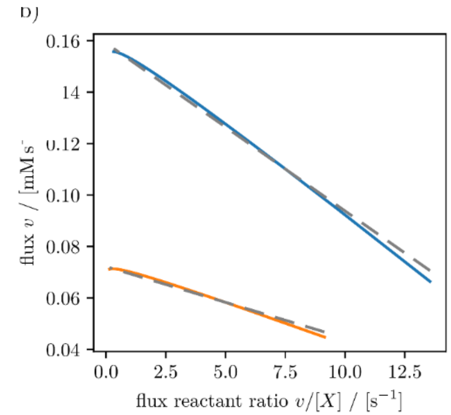
$$K_{M,S} = 210\ \mu M$$
$$K_{M,P} = 194\ \mu M$$
$$V_{max}^{+} = 1.4\ mM/s$$
$$V_{max}^{-} = 0.3\ mM/s$$

Enzyme 3 (PGM):

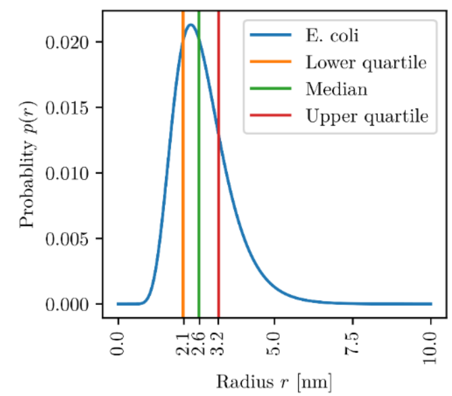
$$K_{M,S} = 210\ \mu M$$
$$K_{M,P} = 97\ \mu M$$
$$V_{max}^{+} = 1.4\ mM/s$$
$$V_{max}^{-} = 0.5\ mM/s$$

Conclusions

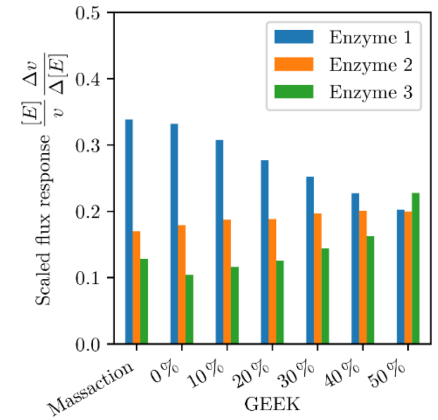
- Concentration dependent Michealis-Menten parameters



- Crowder population depend effects on the MM- parameters



- Control in linear pathways is redistributed

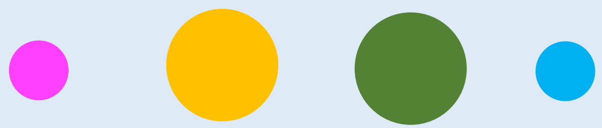


Software architecture

Reaction mechanism

$$S + E \underset{k_{1,b}}{\overset{k_{1,f}}{\rightleftharpoons}} ES \underset{k_{2,b}}{\overset{k_{2,f}}{\rightleftharpoons}} E + P$$

Reactant data D_i, r_i, m_i



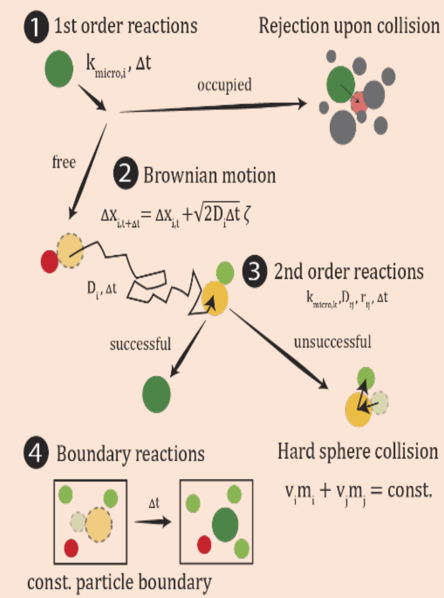
Crowding/Spatial model

OPENBREAD

Python wrapper for HSBRD

HSBRD with OPENFPM

C++ Library
Hardsphere Brownian
Reaction dynamic
Based on the OPENFPM
framework



Effective rate constants

GEEK

Process simulation data to parameterize:

$$k_{j,eff}(\phi) = k_{i,0} e^{\beta_j} \prod_{i=1}^{M} \left(\frac{[X_i]}{[X_i]_{ref}} \right)^{\alpha_{i,j}}$$

Symbolic expression(Sympy)

$$\vec{v}_{eff}(\vec{X}, \vec{p}\,)$$

$$\vec{p} = [\vec{k}_{eff}, \phi, \ldots\,]$$

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Reaction mechanism

$$S + E \underset{k_{1,b}}{\overset{k_{1,f}}{\rightleftharpoons}} ES \underset{k_{2,b}}{\overset{k_{2,f}}{\rightleftharpoons}} E + P$$

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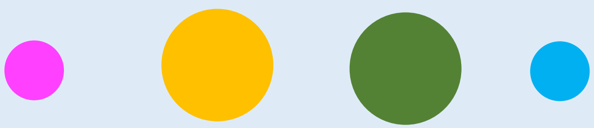

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Available very soon on github

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Effective rate constants

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Process data to parameterize:

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$$k_{j,eff}(\phi \ldots \frac{[X_i]}{[X_i]_{ref}}\Big)^{\alpha_{i,j}}$$

Symbolic expression(Sympy)

$$\vec{v}_{eff}(\vec{X}, \vec{p}\,)$$

$$\vec{p} = [\vec{k}_{eff}, \phi, \ldots]$$

Thank you for your attention