A Whole Cell Biofilm Model

A simulation for antibacterial evaluation

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ACS Spring National Meeting

University of Victoria

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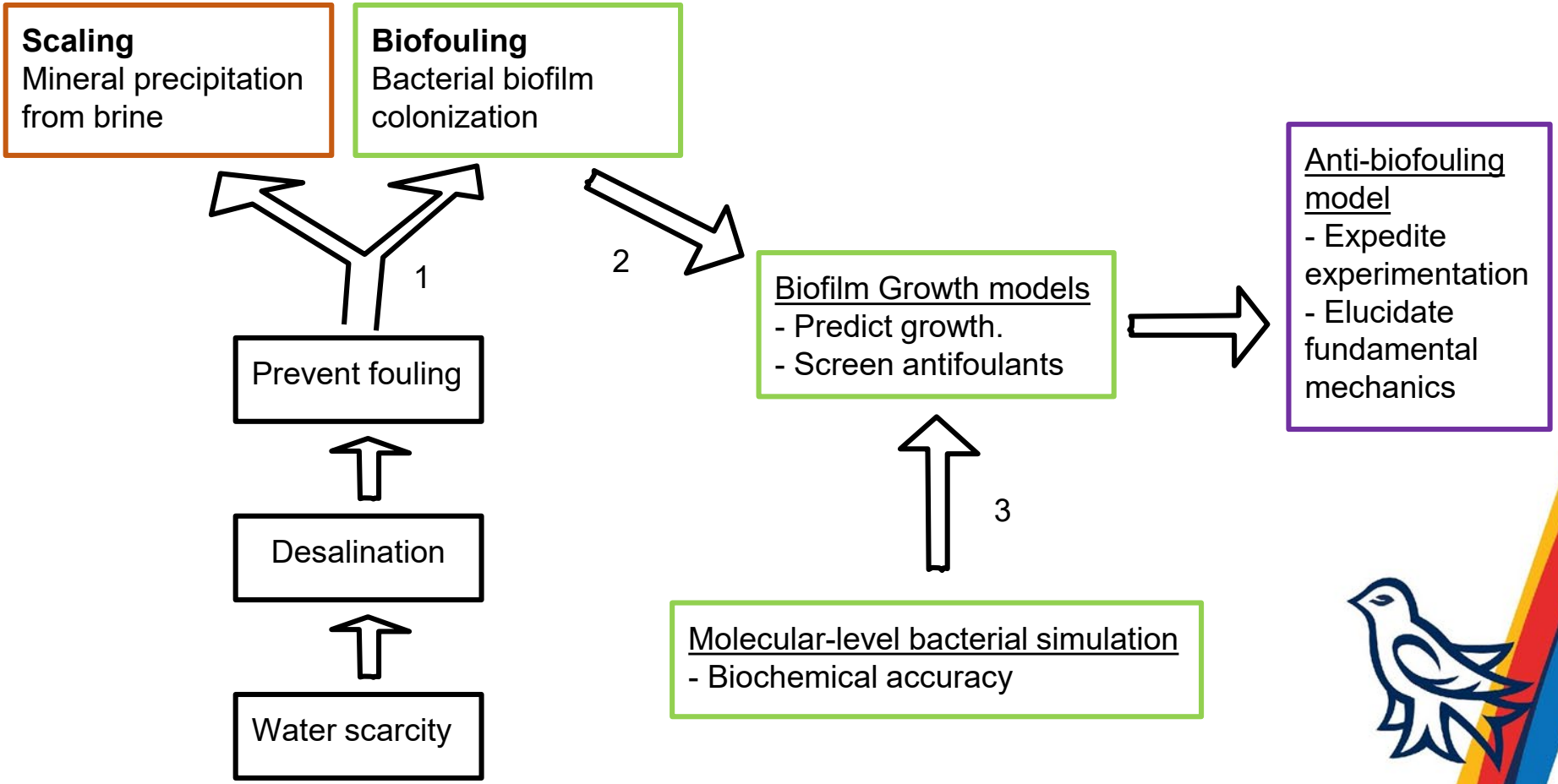
Desalination is hindered by biofouling



Experimental system

1) Can $^1O_2$ prevent biofilm formation?

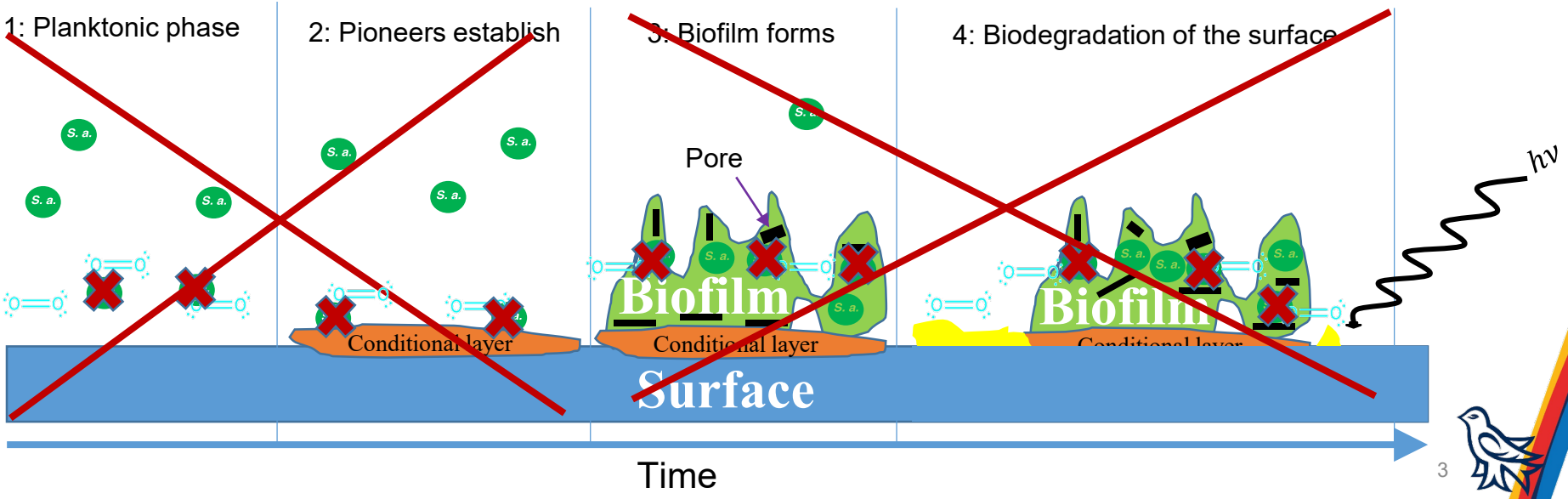
2) Can $^1O_2$ inactivate existing biofilms?

3) What are the requisite $^1O_2$ concentrations?

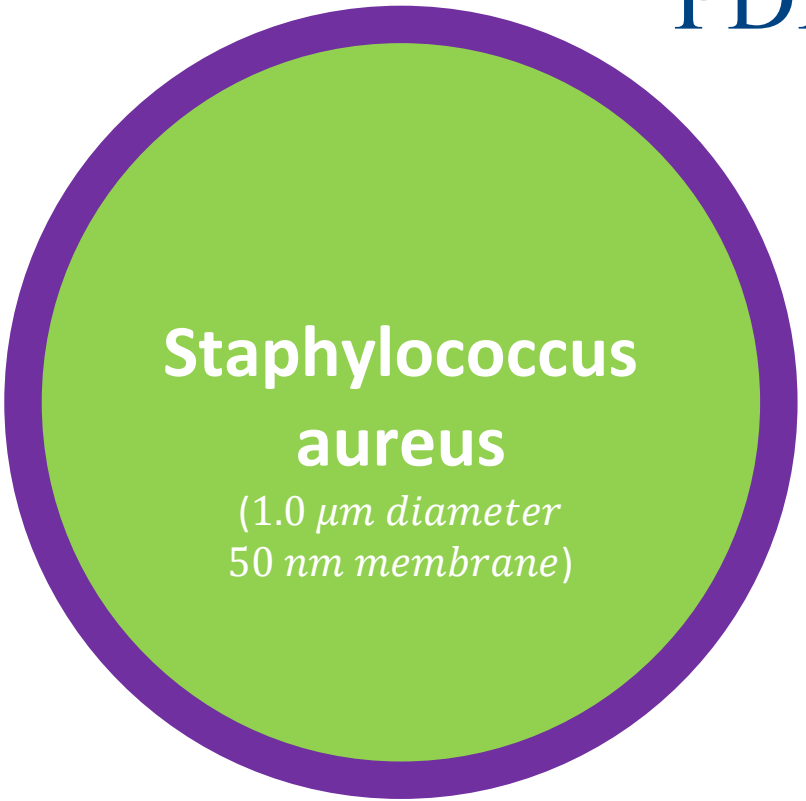
4) What mechanisms cause $^1O_2$ inactivation?

5) Is periodic dosing of $^1O_2$ sufficient for inactivation?

6) Does $^1O_2$ differentially affect (Gram +\-) species?



PDI oxidation – to scale

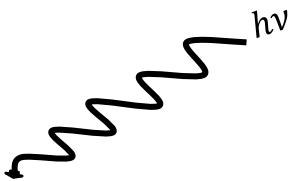


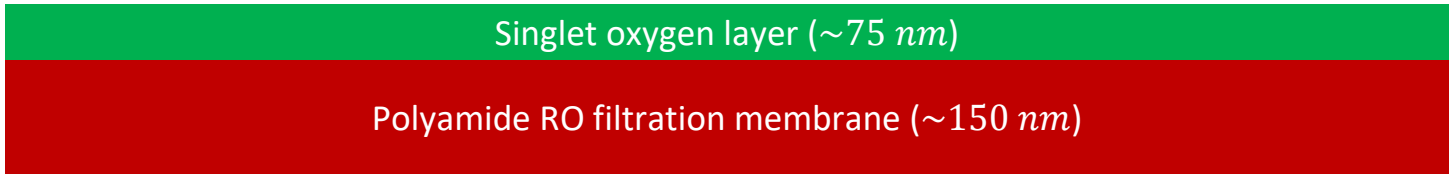
Bacterial dimensions:

S. aureus = *sphere* $[0.5, 1.5]\mu m$
membrane $[20, 80]nm$

P. aeruginosa = *rod* $[0.5, 1]\mu m \; x \; [1, 5]\mu m$
E. coli = *rod* $1\mu m \; x \; 2\mu m$

Human skin thickness $\approx 1.8 \; mm$





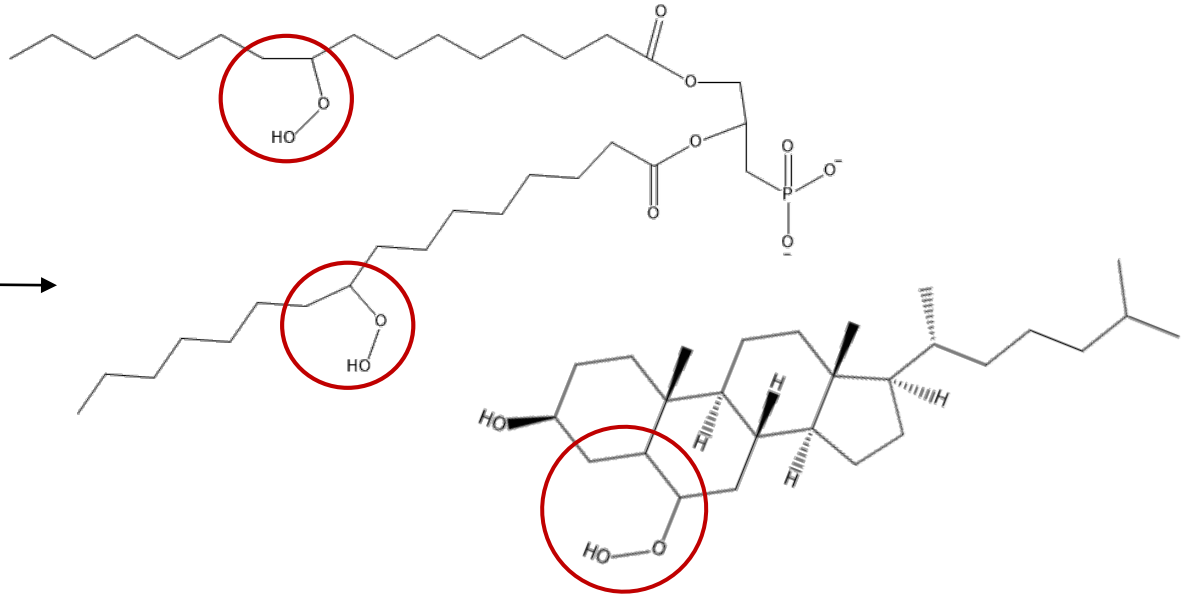
Membrane inactivation

$\sim 15 - 80\ nm$

Bulk solution

Membrane

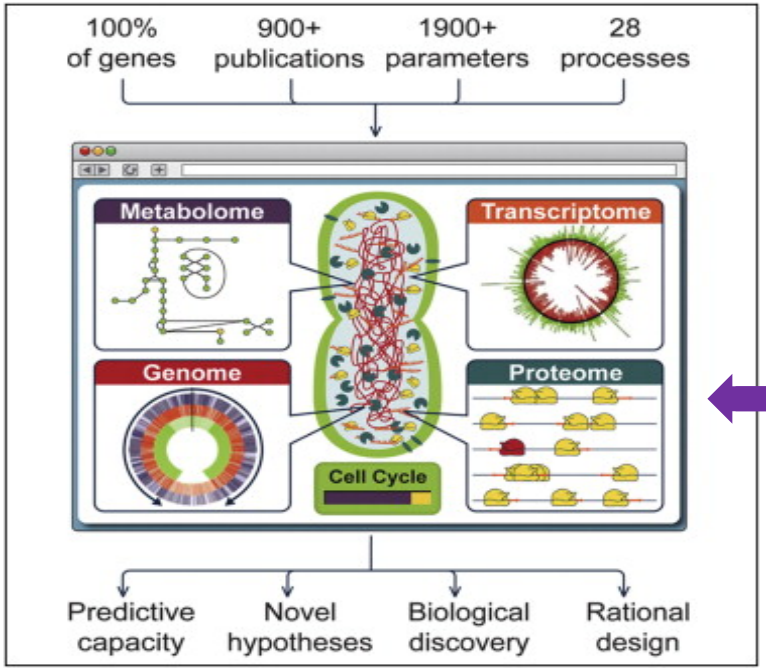
$\sim 70\ nm\ diffusion$

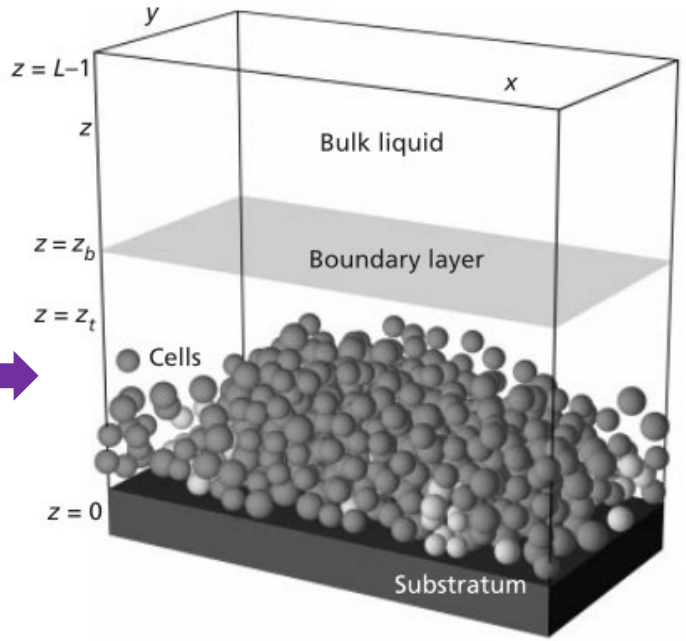


Lysis

Experimental definition of Microbial Death

Computational inspiration





The Whole Cell Model (*Cell*, **2012**)

- Bottom-up biochemical accuracy

Biofilm Models

- Top-down deterministic ODEs

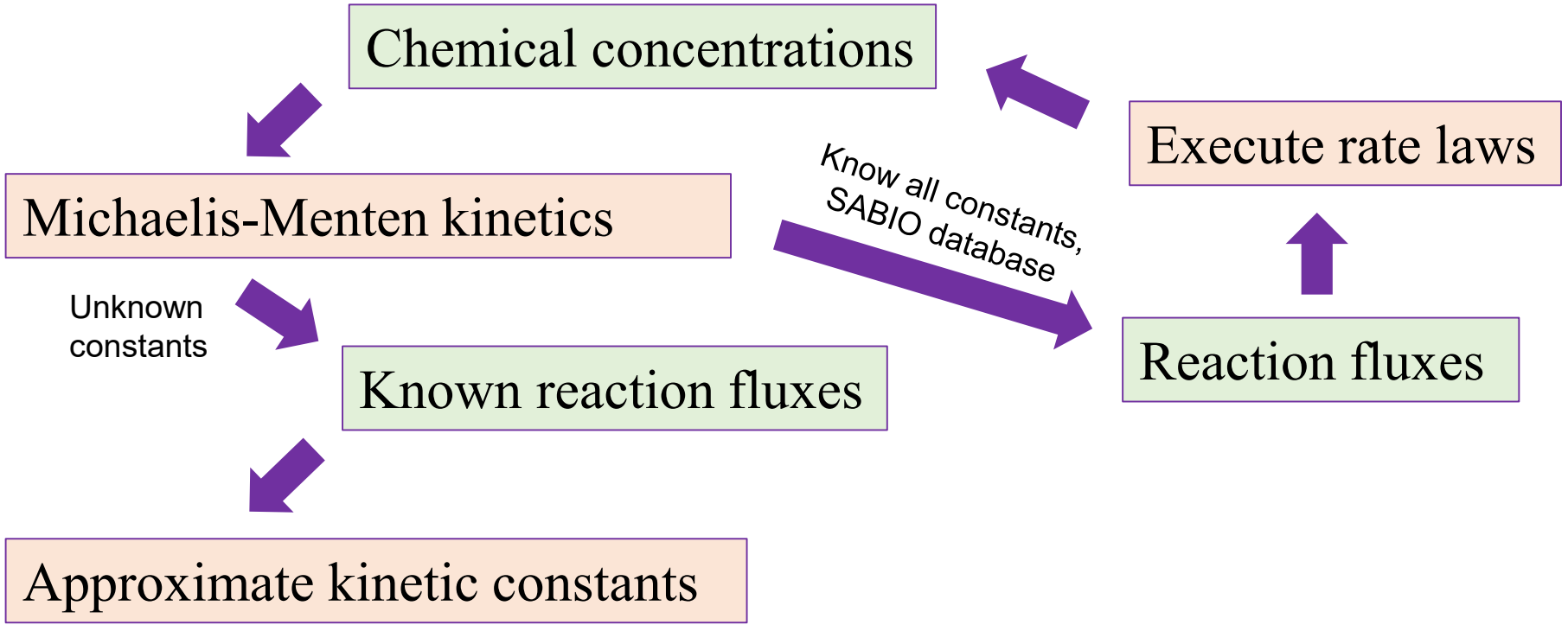
Model intentions

1) Predict results

2) Educate mechanisms

Green: quantity ; Orange: calculation

Kinetic workflow

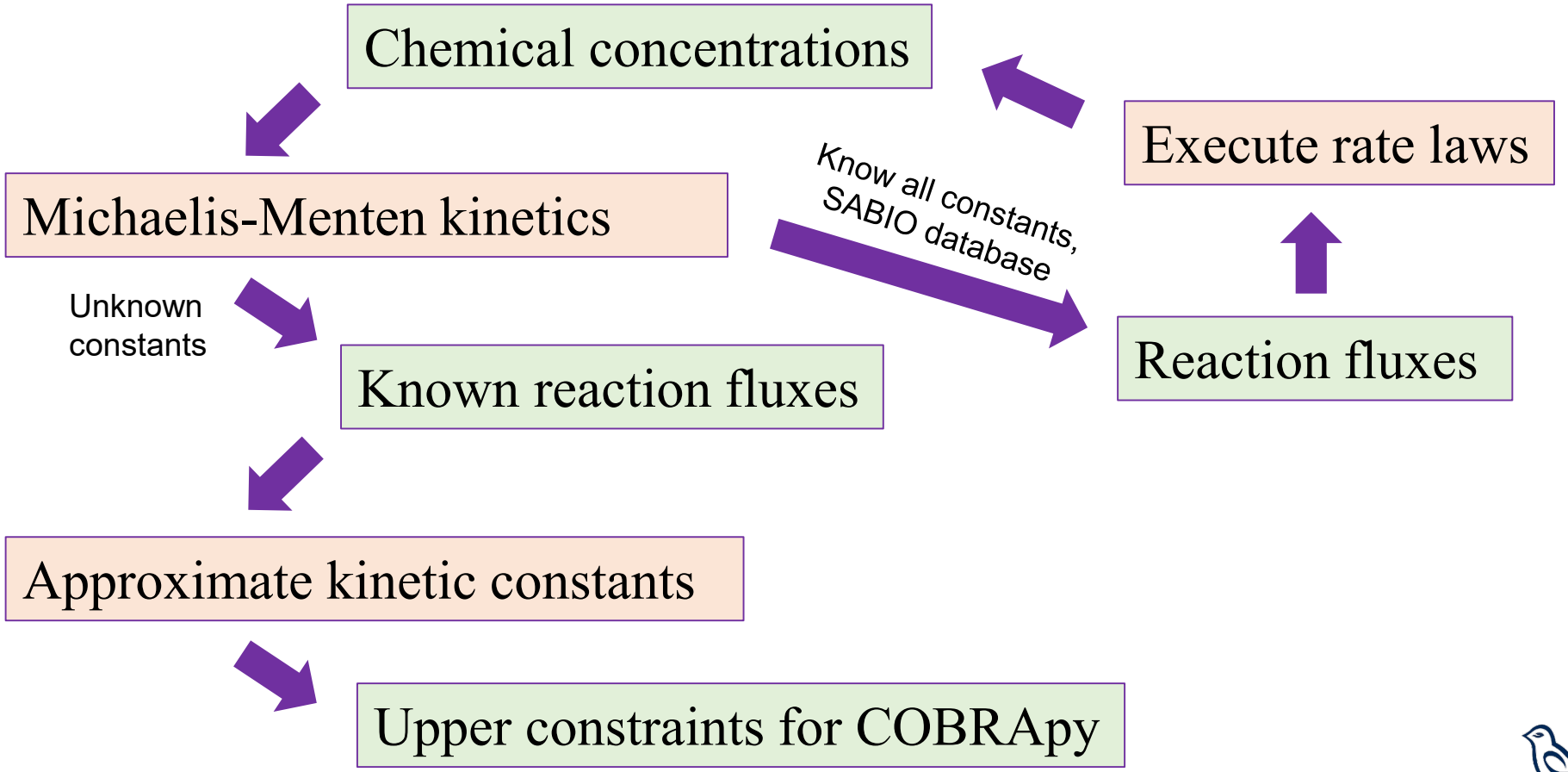


Kinetic approximations

- Quantitative structure-property relationships (QSPRs)
- Thermodynamic proxy
- Potentially negligible missing reactions

Green: quantity ; Orange: calculation

Kinetic workflow



Chemical reaction dynamics “flux”

3) Flux balance analysis (Cobrapy) – linear programming toward a directive

$$\begin{bmatrix} -a & -a & 0 \\ -b & 0 & 0 \\ c & 0 & -c \\ d & -d & 0 \\ 0 & y & 0 \\ 0 & z & -z \\ 0 & 0 & growth \end{bmatrix} \cdot \begin{bmatrix} v_1 \\ v_2 \\ v_3 \end{bmatrix} = 0 \quad [steady\ state]$$

$$maximize\ growth = maximize\ v_3$$

$$aA + bB \rightarrow cC + dD$$
$$dD + aA \rightarrow yY + zZ$$
$$cC + zZ \rightarrow growth$$

Chemical reaction dynamics “flux”

3) Flux balance analysis (Cobrapy) – linear programming toward a directive

- Thermodynamic reaction limits are approximated

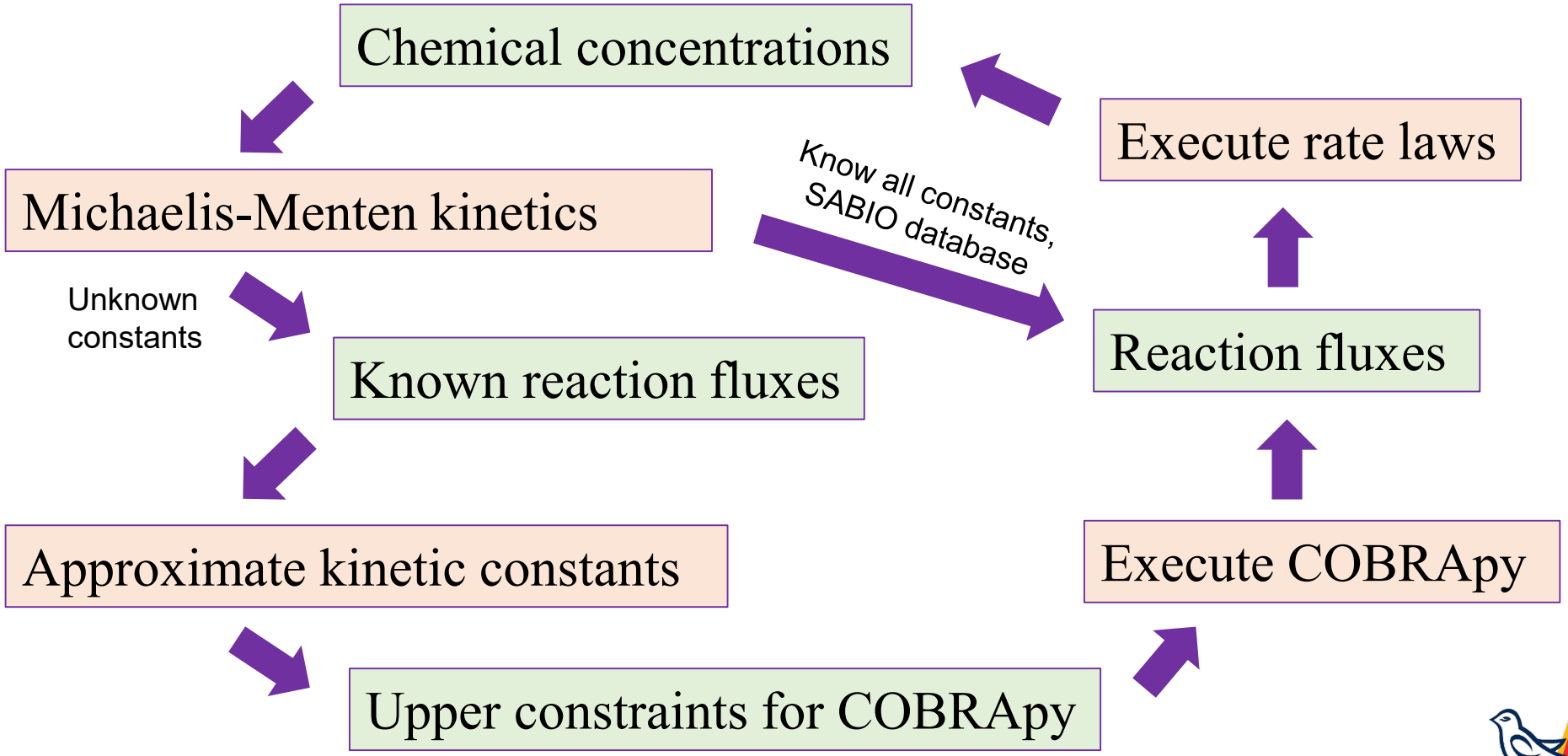
  - $v\ of\ forward\ reactions \subseteq [0,1000]$
  - $v\ of\ reversible\ reactions \subseteq [-1000,1000]$

$$bound_{lower} \leq v_a \ldots \leq bound_{upper}$$

$$aA + bB \rightarrow cC + dD$$
$$dD + aA \rightarrow yY + zZ$$
$$cC + zZ \rightarrow growth$$

Green: quantity ; Orange: calculation

Kinetic workflow



Web scraping and database curation

1) Standardize biochemical databases

- *WholeCellKB.org*
- NIST *Thermodynamics of Enzyme-Catalyzed Reactions*
- *SABIO kinetic database*

2) Partnership with the ModelSEED database.

New directions

1) Approximate the missing kinetic values

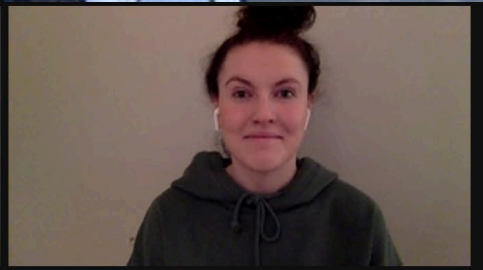
2) Execute a preliminary cellular model

3) Incorporate singlet oxygen and biofilm reactions

4) Visualize through results plots and a GUI

University of Victoria

Green Safe Water Lab



MITACS ACCELERATE


¿Questions?
¿Suggestions?

Kinetic calculations

$$v_1 = \frac{v_{max_1} * [A] * [B]}{(K_{M_a} + 1) * [A] + (K_{M_b} + 1) * [B]} \; ; \; v_{max_1} = k_{cat_1} * [Enzyme_1]_0$$

$$\vdots$$

$$\frac{d[A]}{dt} = v_1 * -a + v_2 * -a_2$$

$$\frac{d[D]}{dt} = v_1 * d + v_2 * -d_2$$

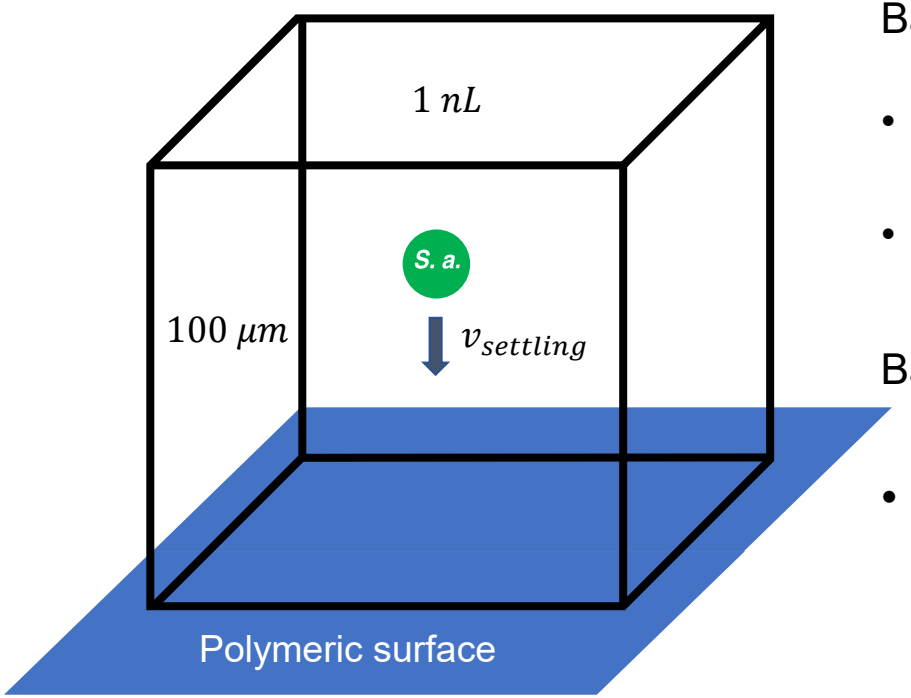
$$\vdots$$

$$aA + bB \xrightarrow{Enzyme_1} cC + dD$$

$$d_2D + a_2A \xrightarrow{Enzyme_2} yY + zZ$$

$$cC + zZ \xrightarrow{Enzyme_3} growth$$

Simulation space with settling



Bacterium $\approx 1\ fL$

- 1 million grid volume cells
- $\frac{bacterium}{simulation\ volume} = \frac{1\ fL}{1\ nL} = \frac{1E-15\ L}{1E-9\ L} = (1E-6) = ppm$

Bacterium = particle

- Stoke's law of terminal velocity

- $v_{settling} = \frac{g*(\rho_{bacterium}-\rho_{water})*d^2_{bacterium}}{18*\mu} \approx 0.25\frac{\mu m}{s}$
- $Re = \frac{\rho_{water}*d_{bacterium}*v_{settling}}{\mu} \approx 4E-9 \ll 2$

Chemical reaction dynamics “flux”

3a) Dynamic FBA (dfba) – Time variability and dependence

- $[A], [B], [C], [D], [Y], [Z]$ are variable over simulation time

```
Set the initial biomass concentration
Set the initial conditions of the environment
From the starting time to the final time
    Based on the current biomass concentration and environmental conditions,
        set the upper and lower bounds of the exchange reactions
    Solve for the maximum growth rate and optimal fluxes
    Update the biomass concentration based on the predicted growth rate
    Update the environmental conditions based on the predicted exchange fluxes
```

$$aA + bB \rightarrow cC + dD$$
$$dD + aA \rightarrow yY + zZ$$
$$cC + zZ \rightarrow growth$$

User-defined parameters

1) Extracellular conditions
- LB broth, as casein and yeast extract ; temperature ; NTUs, et cetera

2) Bacterium species
- *Mycoplasma genitalium* => cell cycle, cell mass\volume, metabolic proportions

3) Inactivation method
- Photodynamic inactivation (PDI) with singlet oxygen
- Organic antimicrobial agents

Approximated parameters for *M. genitalium*

*Quite uncertain

1) $starvation\ proportion = \left(\frac{mass_t}{mass_{t=0}}\right) = \frac{1}{3}$

- The fraction of initial mass below which the bacterium dies

2) ** $translation\ rate = 4\ \left(\frac{codons}{second}\right)$

- The rate at which codons are transcribed into amino acids for protein synthesis

3) ** $enzyme\ halflife = 5000\ (seconds)$

- The rate of enzyme degradation into amino acids for every enzyme

4) $bacterial, electrical\ cell\ potential = 0.2\ (volts)$ *

- Electric cell potential of the bacterium, which is the aggregation of all biochemical reactions

5) $T_{opt} = 310\ °K$

- The optimum incubation temperature, which is the aggregate thermodynamics of the bacterium

6) ** $reaction\ completeness = \frac{reactions\ executed}{possible\ reactions} = 0.9$

- The proportion of necessary reactions to achieve $\left(\frac{Q}{K_{eq}}\right)_{opt}$ that are executed in a timestep

Approximated parameters for *M. genitalium*

7) $Planktonic = 1\,;\ Detached = 0.8\,;\ Biofilm = 0.5\,;\ Persister = 0.00001$

- The relative metabolic reaction rate, which is informed through an interview of a Eukaryotic biologist

8) $** \ vital\ energetic\ proportion = \frac{(energetic\ proportion)_t}{(energetic\ proportion)_{t=0}} = \frac{1}{2}$

- The proportion of energetic chemicals relative to below which the bacterium dies

Assumptions and limitations

1) Homogeneous bulk and cytoplasm

2) Mass balance applies everywhere

3) ** Need-based absorption

- $need = \left(\frac{Q}{K}\right)_{optimum} - \left(\frac{Q}{K}\right)_{current}$
- $\left(\frac{Q}{K}\right)_{optimum}$ is estimated from the incubation $°K$

4) ** Boltzmann distribution to substrates

5) ** Transcription and\or enzymes are negligible

6) Singlet oxygen oxidizes only unsaturated lipids

7) Constant cell cycle times

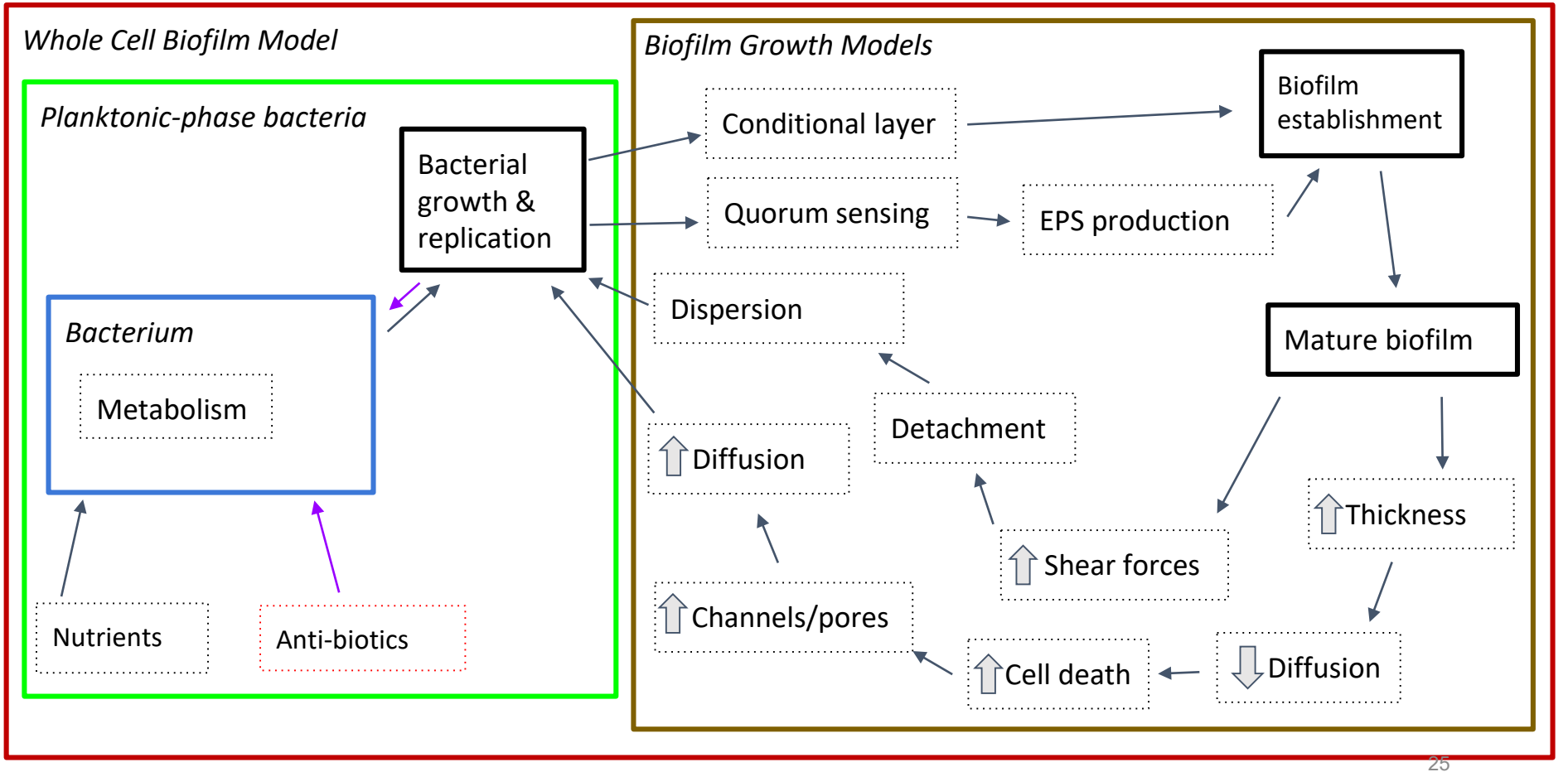
- Only three phases: Interphase, S, and mitosis

8) Replication resets the bacterium

9) $\varrho_{bacterium} = constant$

10) The acquired datasets are accurate

System dynamics



** Membrane flux = mass balance

$$mass_{bacterium,t} = mass_{t=t-1} + mass_{net,t}$$

$$mass_{net,t} = mass_{absorbed,t} - mass_{ejected,t}$$

$$mass_{absorbed,t} = \sum_{i=1}^{a} n_{i,absorbed} * MW_i$$

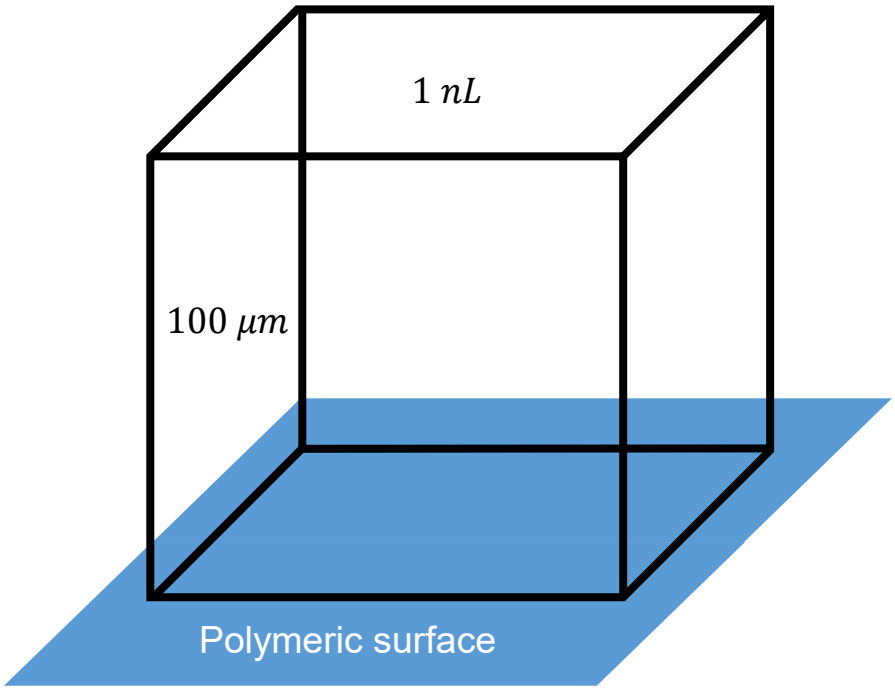
$$a = \#\ absorbed\ molecules$$

$$mass_{ejected,t} = \sum_{i=1}^{z} n_{i,ejected} * MW_i$$

$$z = \#\ ejected\ molecules$$

$$V_{\text{bacterium,t}} = m_{bacterium,t} * \left(\frac{V_{\text{bacterium,0}} \approx 1\ fL}{m_{bacterium,0} \approx 1\ pg} = constant \right)$$

System boundaries



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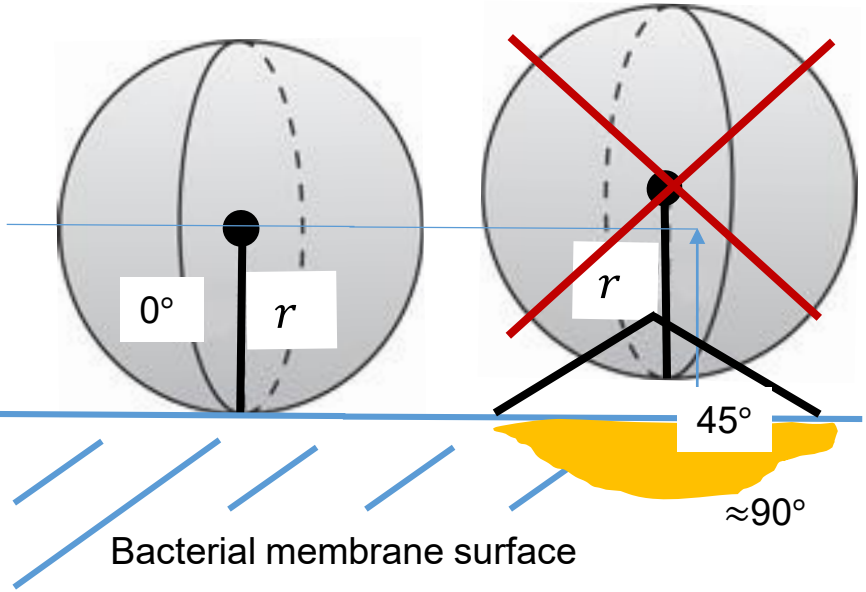
- $\frac{bacterium}{simulation\ volume} = \frac{1\ fL}{1\ nL} = \frac{1E{-}15\ L}{1E{-}9\ L} = (1E-6) = ppm$

- $(\#viable\ bacterium)_{t=0} \approx 2E6\ \left(\frac{CFU}{mL}\right) = 2E9\ \left(\frac{CFU}{L}\right) = 2\ \frac{CFU}{simulation} = 2\ CFU\ ppm$
- $(\#viable\ bacterium)_{t=final} \approx 2E9\left(\frac{CFU}{mL}\right) = 2E3\frac{CFU}{simulation}$

** Proportion of interactions from bulk



$\overrightarrow{V}_{rms} = rms\ velocity\ of\ substrates$

$r = \vec{V}_{rms} * \Delta t$

$Surface\ area\ =\ probability$

$r = maximal\ distance\ for\ a\ substrate\ interaction\ in\ \Delta t$

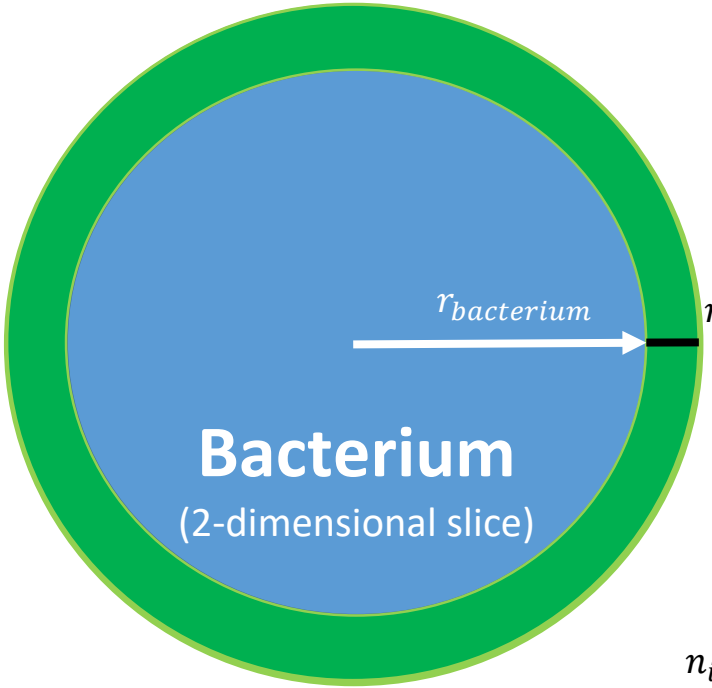
≈ 0 probability of membrane interaction

$\approx \frac{1}{2}$ probability of membrane interaction

$\approx 45°$ average angle between $0°$ and $\approx 90°$

$$average\ probability\ of\ interaction = \bar{P} = \frac{surface\ area\ (intercepted\ spherical\ cap)}{surface\ area\ (sphere)} = 14.6\%$$

** Chemical adsorption



$$V_{shell} = \left(\frac{4\pi}{3}\right) * \left(r^3_{outer} - r^3_{bacterium}\right),$$
$$\text{with } r_{outer} = r_{bacterium} + r_{interaction}$$

$$r_{interaction} = \overrightarrow{\frac{V}{rms}} * \Delta t$$

$$n_{i,\text{interaction}} = [C]_i * V_{shell} * P$$

$$n_{i,\text{absorbed}} = \begin{cases} n_{i,interaction} * \dfrac{\left(\left(\dfrac{Q}{K_{eq}}\right)_{optimum} - \Pi_{i=1}^{x}\left(\dfrac{(Q_i)_{t,C_i}}{(K_i)_{eq,C_i}}\right)\right)}{\left(\dfrac{Q}{K_{eq}}\right)_{optimum}} * \beta_i \\ 0, \quad \Pi_{i=1}^{x}\left(\dfrac{(Q_i)_{t,C_i}}{(K_i)_{eq,C_i}}\right) > \left(\dfrac{Q}{K_{eq}}\right)_{optimum} \end{cases}$$

$$m_{\text{absorbed}} = \sum_{i=1}^{y} n_i * MW_i$$

$C_i = concentration\ of\ bulk\ substrate\ i$
$x = \#\ reactions\ with\ i$
$\beta_i = barrier\ inhibition$
$y = \#\ substrates\ i$

** Optimal thermodynamics

$$\left(\frac{Q}{K_{eq}}\right)_{optimal} = e^{\frac{-n*F*E}{R*T_{opt}}}$$

$\Delta G = Gibbs\ free\ energy$

$\Delta G^0 = Gibbs\ @\ standard\ conditions$

$T_{opt} = optimal\ bacterial\ incubation\ temperature$

$R = gas\ constant$

$$Q = reaction\ quotient = \frac{[product]^n}{[reactant]^m}$$

1) $\Delta G = \Delta G^0 + R * T_{opt} * ln(Q)$
2) $\Delta G = -n * F * E$
3) $\Delta G^0 = -R * T_{opt} * ln(K_{eq})$

$$n = < \frac{e^-}{mol} >$$

$F = Faraday's\ constant$

$E = electrical\ potential\ of\ the\ bacterium$

$$-n * F * E = R * T_{opt} * (\ln Q - \ln K_{eq})$$

$$\left(\frac{-n * F * E}{R * T_{opt}}\right) = \ln\left(\frac{Q}{K_{eq}}\right)$$

$$K_{eq} = equilibrium\ constant = \frac{[product]^n}{[reactant]^m}$$

New directions

1) Thoroughly organize the reaction database
- Categorizing reactions as inter-\intra-compartmental

2) Expand biochemical accuracies
- Introduce quorum sensing reactions

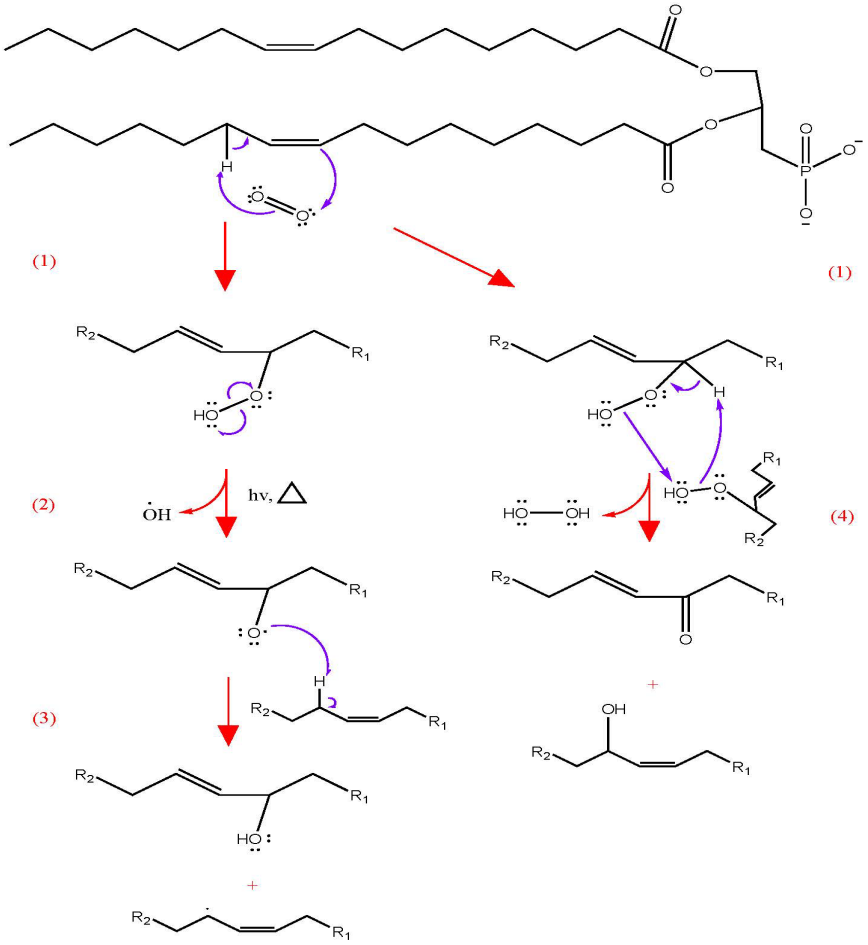
3) Expand the bacterium model into a biofilm model
- Incorporate new functionalities like metabolic states

4) Compare with conventional methods
- Flux balance analysis via cobrapy module

5) Introduce a visual depiction
- Matplotlib plots and tkinter GUI

6) Implement antibiotic reactions
- Parameterize $^1O_2$ reactions

Membrane oxidation mechanisms

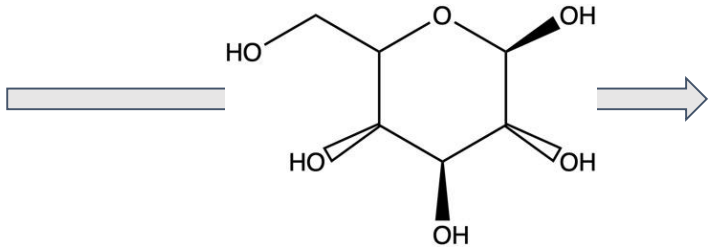


Suspension phase model

Solution elements

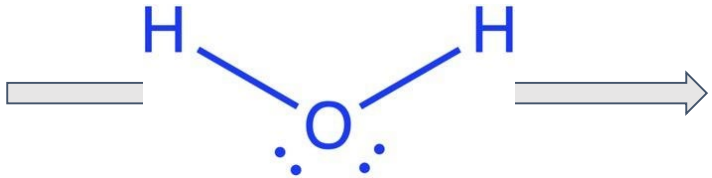
Parameters/equations

Substrate



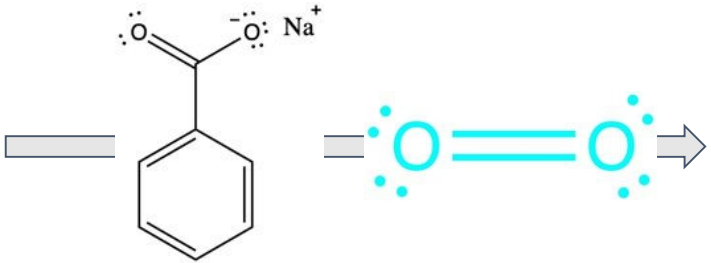
Diffusion - Reaction-diffusion Equations
Fick's law or Cahn-Hilliard equations

Water



Diffusion - Reaction-diffusion Equations
Fick's law or Cahn-Hilliard equations

Anti-foulants



Disrupt Biochemistry -
Quorum sensing
Bacteriostatic
Bactericidal

Diffusion - Reaction-diffusion Equations
Fick's law
Cahn-Hilliard equations

Modeled biofilm and membrane

Biofilm qualities	Parameters/equations
Shape	Shear forces - Digital Biofilm model (2016) Thickness - Biofilm Growth Model (2000 MSU)
Channeled	Porous - Cellular automata algorithm
Membrane	Porous - Biofilm Growth Model (2000 MSU)

Modeled bacteria

S. aureus

Bacterial elements	Parameters/equations
Motility	*Staphylococcus aureus* is not motile
Density limit	Quorum sensing - Frederick et al. 2016 Daughter cell dispersion - Individual-based algorithm
Bacterial growth	Substrate - Monod kinetics - Michaelis-Menten kinetics Anti-foulant - ***Novel***

Biofouling

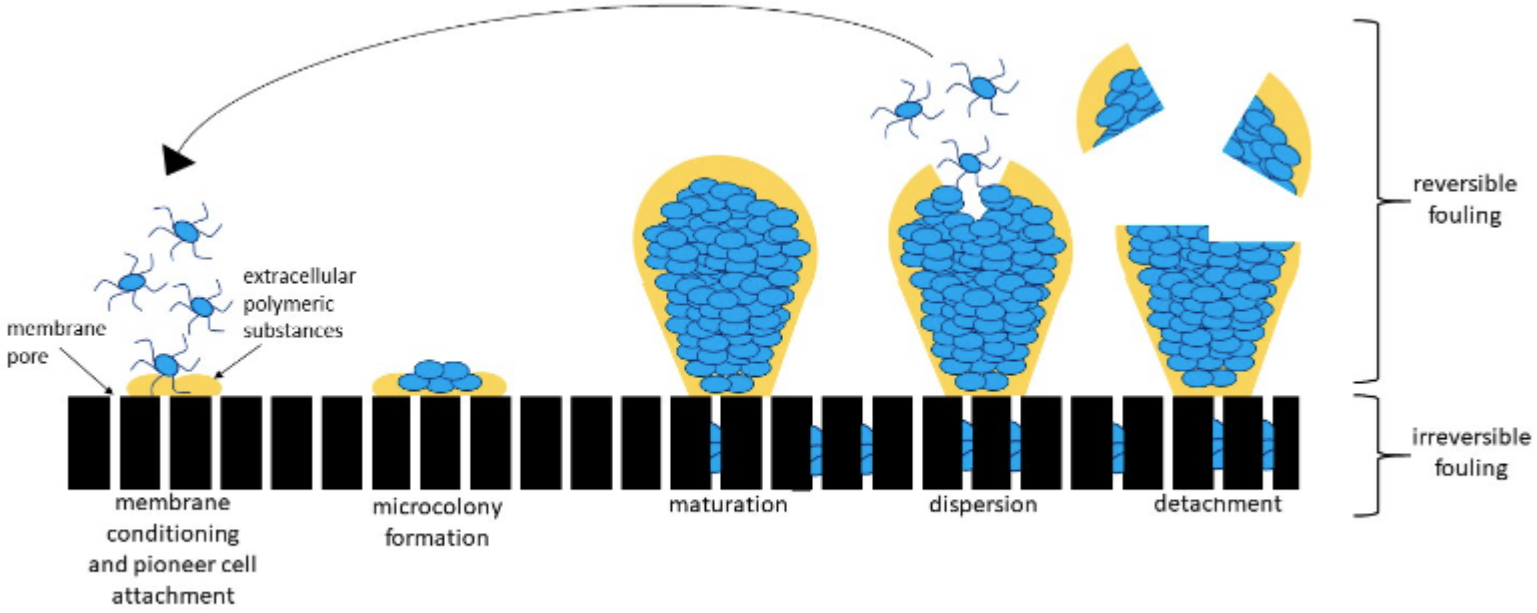
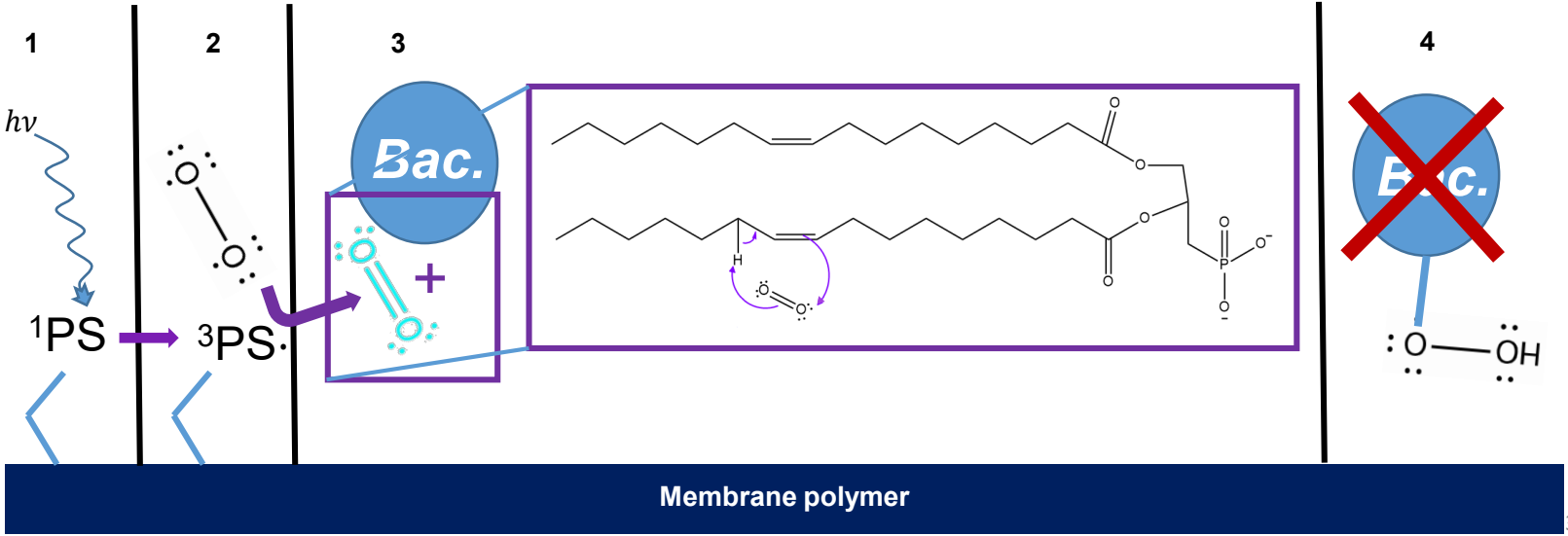


Figure 1. Biofilm growth on a semipermeable membrane (Image Credit: Anna Curtin)

Fundamental questions



Algorithm/Model	Assumptions/limitations	Model contribution
Rittmann model & Biofilm Accumulation model (BAM)	1) The biofilm is composed of dead+active bacteria and water 2) Constant Biofilm growth, [Substrate], and bulk volume	Foundation
Biofilm Growth model (BGM)	1) The biofilm is composed of dead+active bacteria and water 2) Constant Biofilm growth and [Substrate]	Dynamic bulk volume
Digital Biofilm Model (DBM)	1) Biofilms are two-phases: rigid bacteria and malleable EPS 2) Proteins were only modeled in the EPS	Accurate Biofilm composition
Individual-based algorithm	1) Computational demands 2) Bacteria are inelastic spheres 3) Porosity is predestined by net vector daughter cell dispersal	Natural evolution of population growth
Cellular automaton algorithm	1) Heterogeneous bacteria and biofilm 2) Unrealistic quantization of parameters 3) Parameters values can be subjective	Mature biofilm channelling

Whole Cell Biofilm Model

Algorithm/Model	Assumptions/limitations	Model contribution

Whole Cell Biofilm Model

Cellular Automaton algorithm

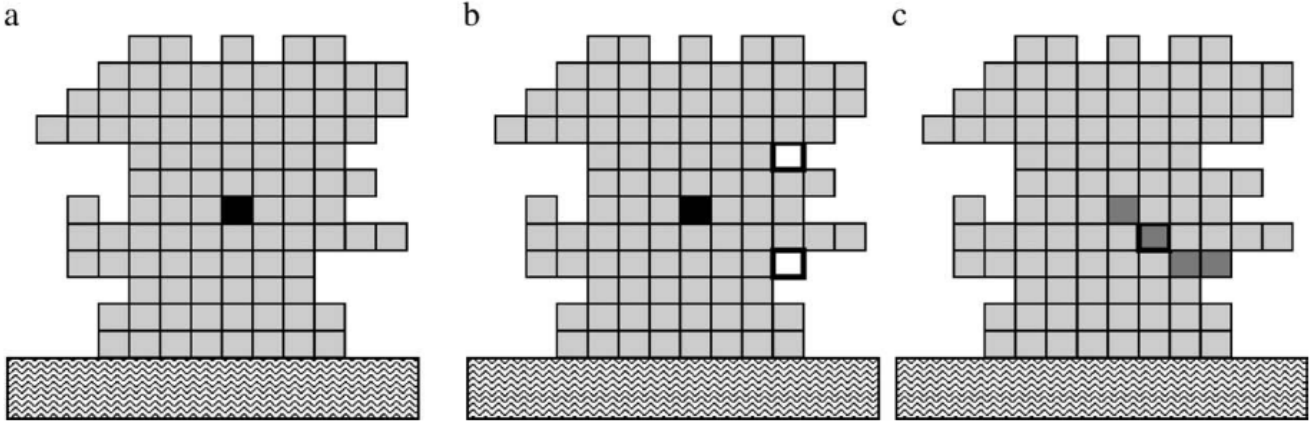
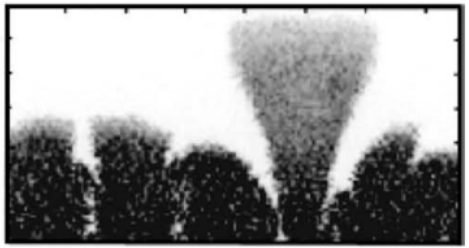


Fig. 2. The CA algorithm used in the UMCCA model. (a) Overflowing compartment, (b) The nearest two equidistant compartments to the overflowing compartment, (c) The algorithm randomly picks one of the two and places the new biomass in a neighboring compartment (bold compartment), while it shoves existing biomass along the path of least resistance.

Cellular Automaton algorithm

- Lattice Cartesian grid
- Stochastic selection of the closest unoccupied cells

c



Individual-based algorithm

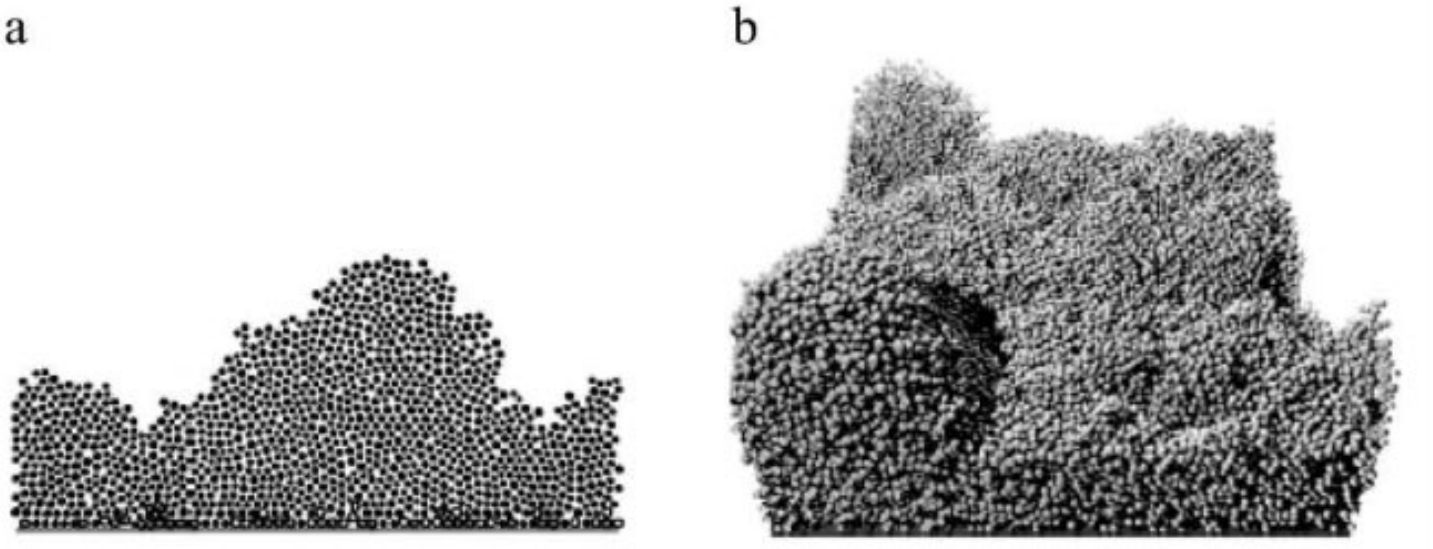


Fig. 3. Sample output of the IbM by Picioreanu et al. [29] in (a) 2-D and (b) 3-D. (c) sample 2-D outpu

Individual-based algorithm

- Dispersal according to the net vector from cellular overlap

Bioassays