PDIpy: Antimicrobial studies with membrane biology

December 16, 2021 – **PacifiChem**

Crossing the Biological Membrane: Frontiers in the Computational Study of Membrane Transport

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Antimicrobial resistance (AMR)

AMR development

Projected 2050

in economic losses

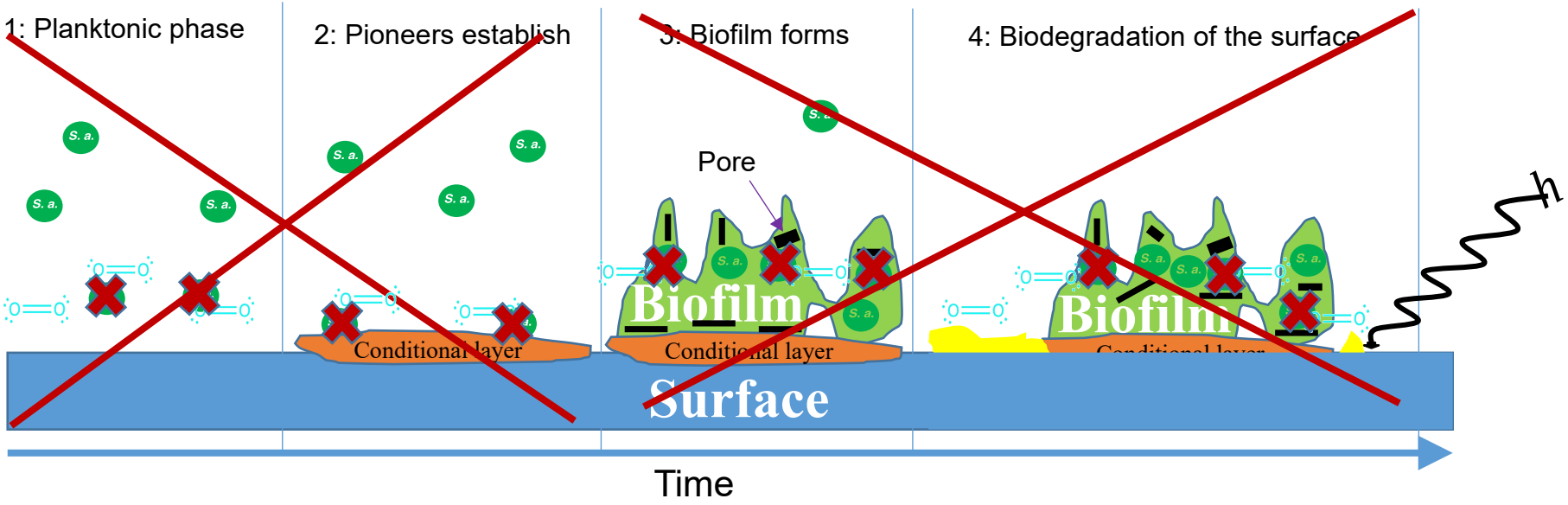
O'Neill, "Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations". **2014**.

Van Boeckel et al., "Reducing antimicrobial use in food animals". *Science*. **2017**. DOI: 10.1126/science.aao1495

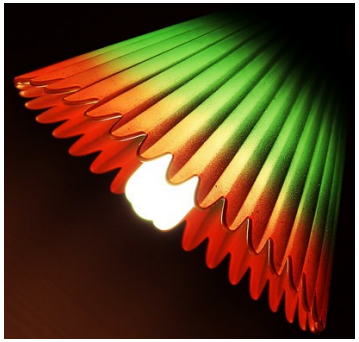
Eggleton, Paul. "The State of the World's Insects". *Annual Review of Environment and Resources.* **2020**. https://doi.org/10.1146/annurev-environ-012420-050035

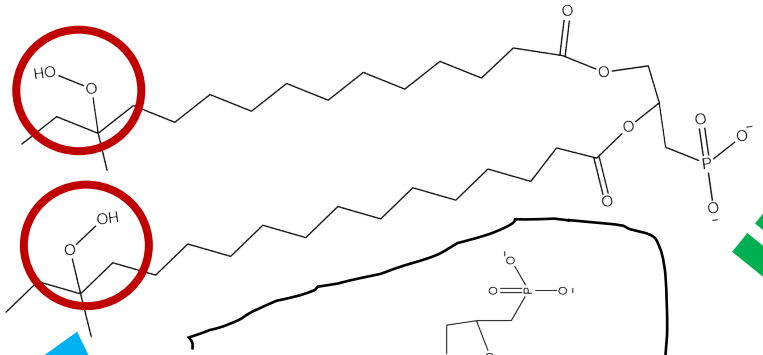
Photodynamic Inactivation/Therapy (PDI/PDT)
- Rapidly achieves >5-log reductions
- Avoids resistance evolution after 1000 generations

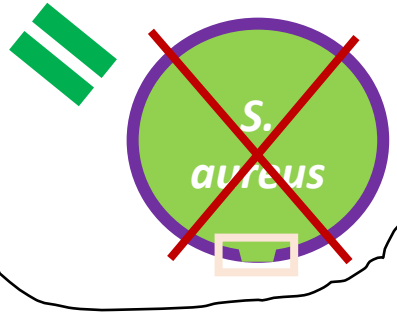
Experimental goal

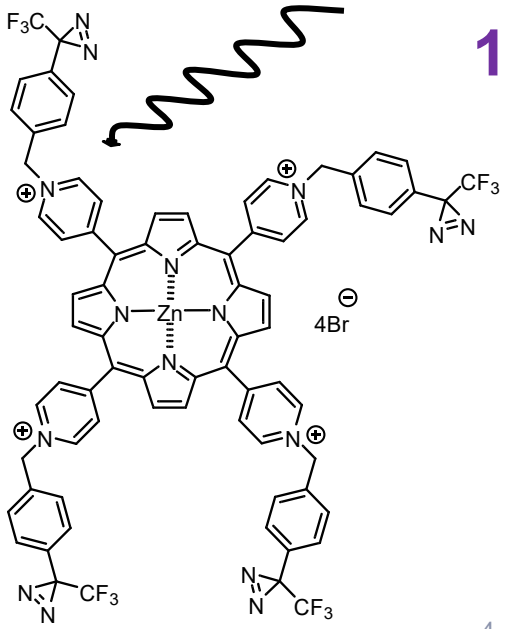


System schema

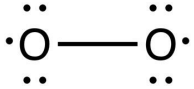








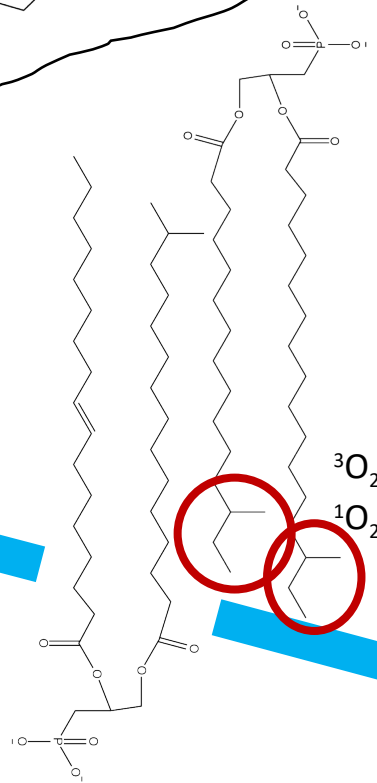
1



$^3O_2$: = -1,0,1; Stable; unreactive

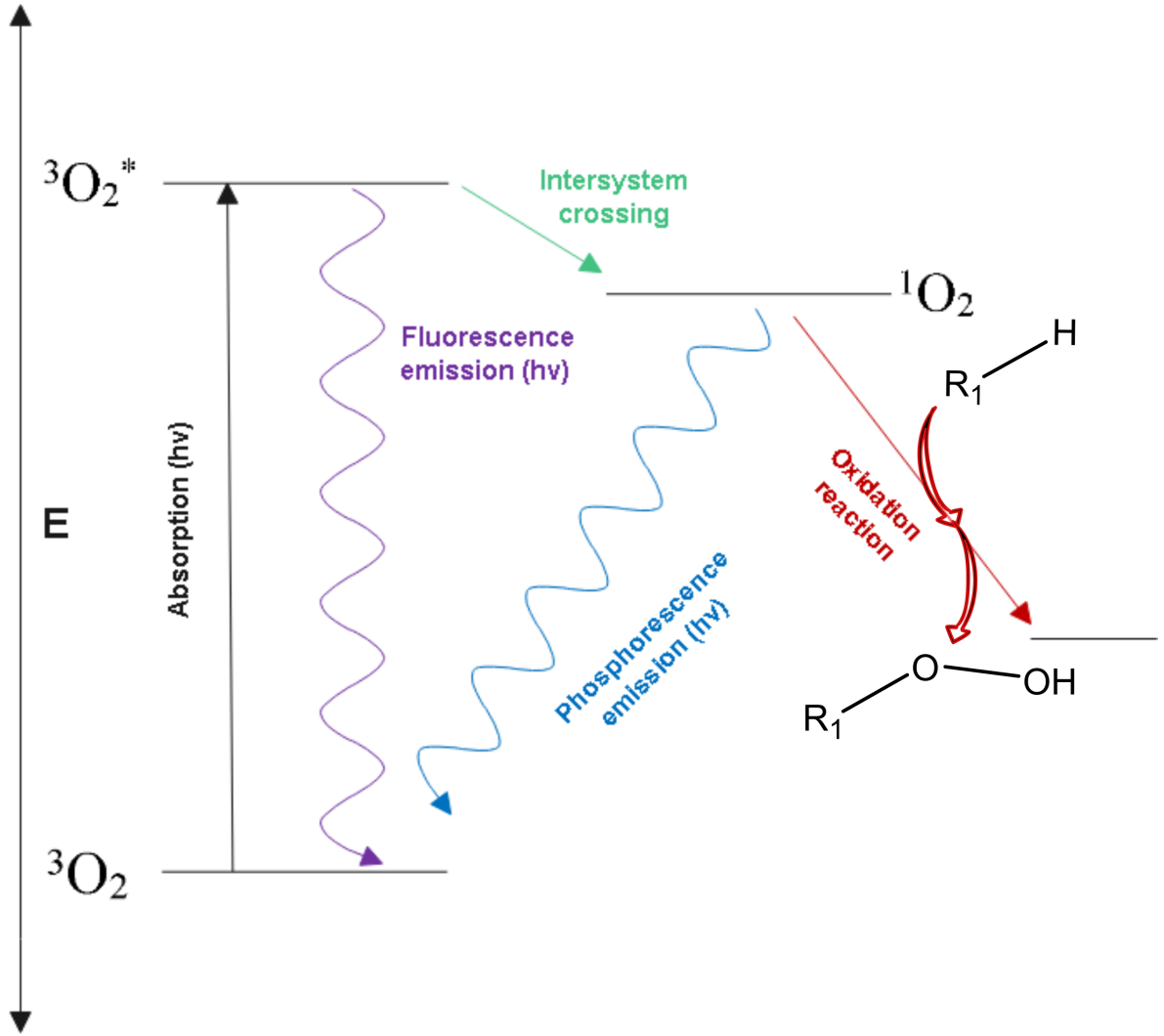
$^1O_2$: = 0; Unstable; reactive

2

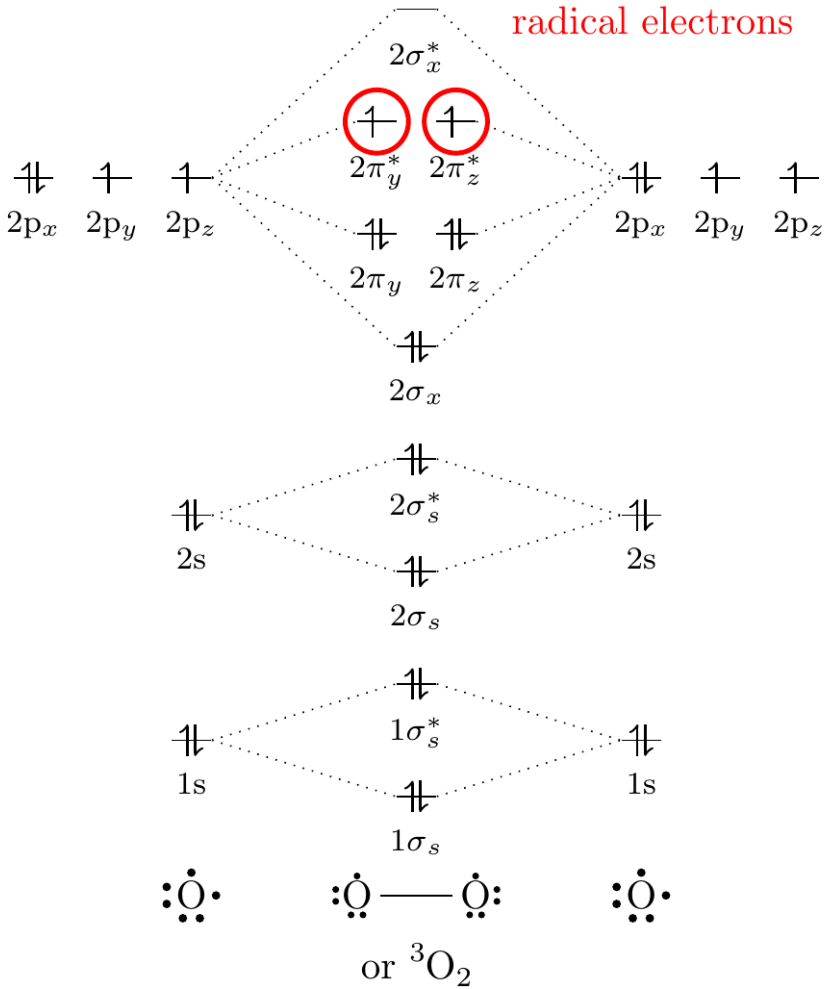


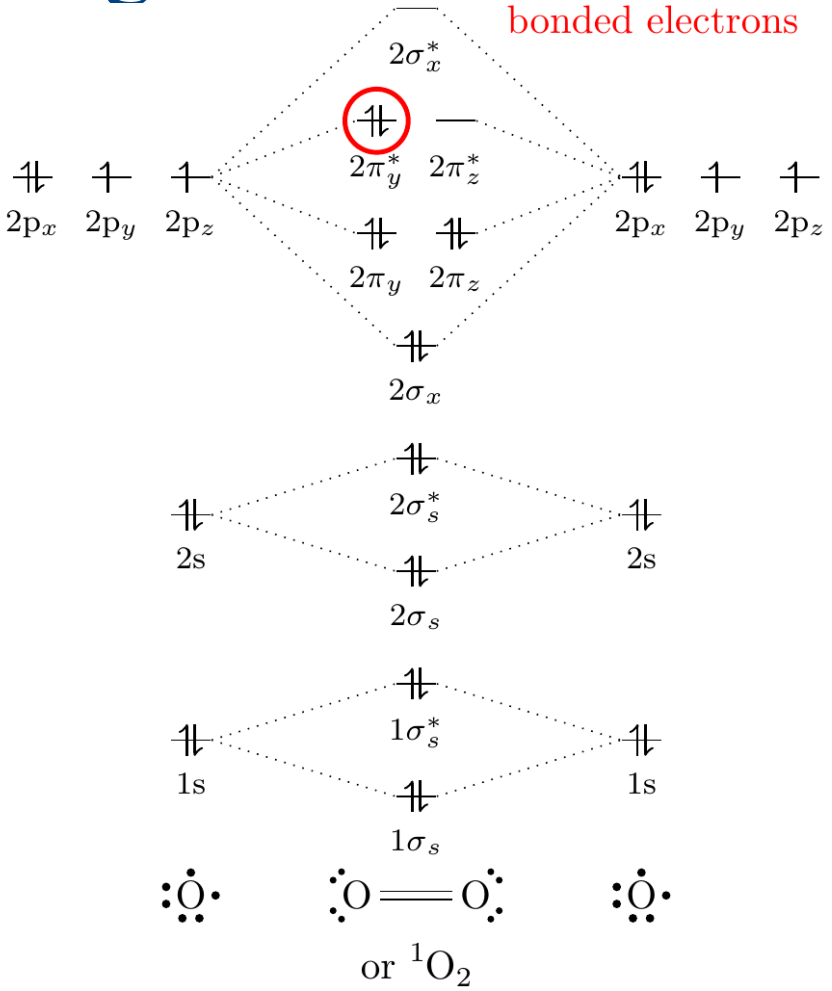
3

Jablonski plot

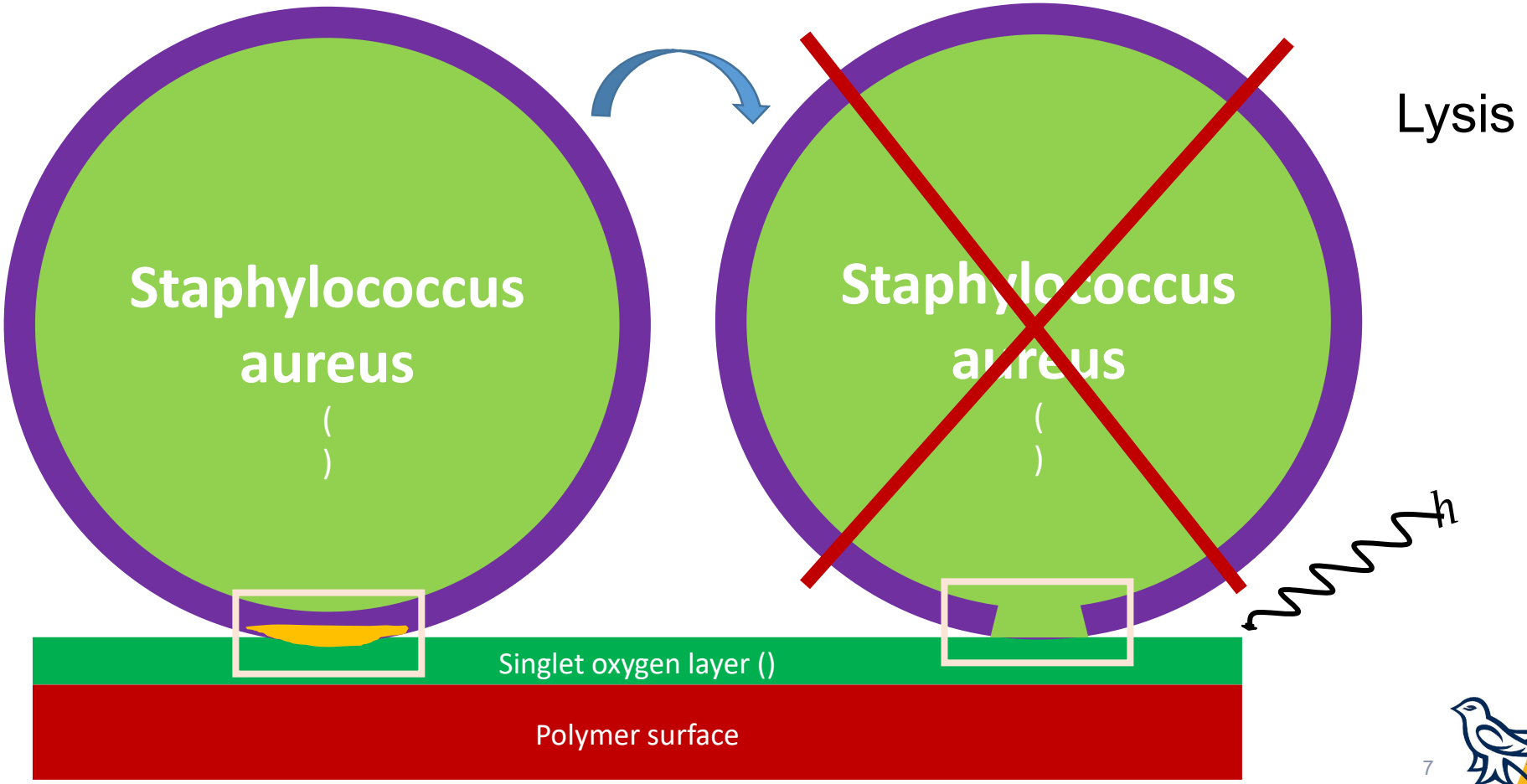


MO diagrams

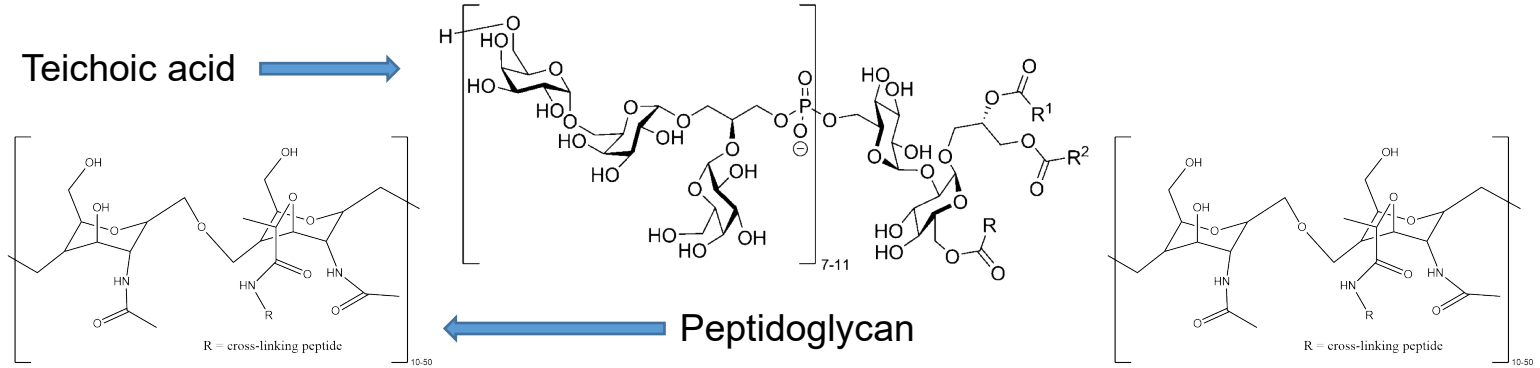

radical electrons
$2\sigma_x^*$
$2\pi_y^*$ $2\pi_z^*$
$2\pi_y$ $2\pi_z$
$2\sigma_x$
$2p_x$ $2p_y$ $2p_z$ $2p_x$ $2p_y$ $2p_z$
$2\sigma_s^*$
$2\sigma_s$
2s 2s
$1\sigma_s^*$
$1\sigma_s$
1s 1s
or $^3O_2$


bonded electrons
$2\sigma_x^*$
$2\pi_y^*$ $2\pi_z^*$
$2\pi_y$ $2\pi_z$
$2\sigma_x$
$2p_x$ $2p_y$ $2p_z$ $2p_x$ $2p_y$ $2p_z$
$2\sigma_s^*$
$2\sigma_s$
2s 2s
$1\sigma_s^*$
$1\sigma_s$
1s 1s
or $^1O_2$

Our approach

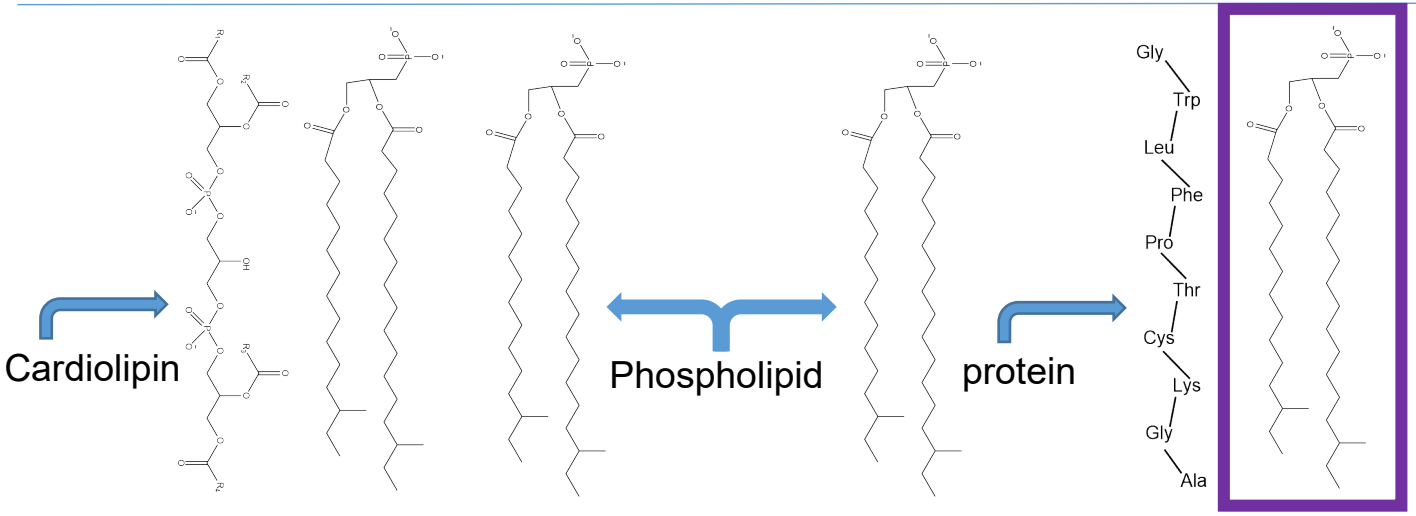


Gram (+) bacterial membrane



Peptidoglycan

20-80 nm

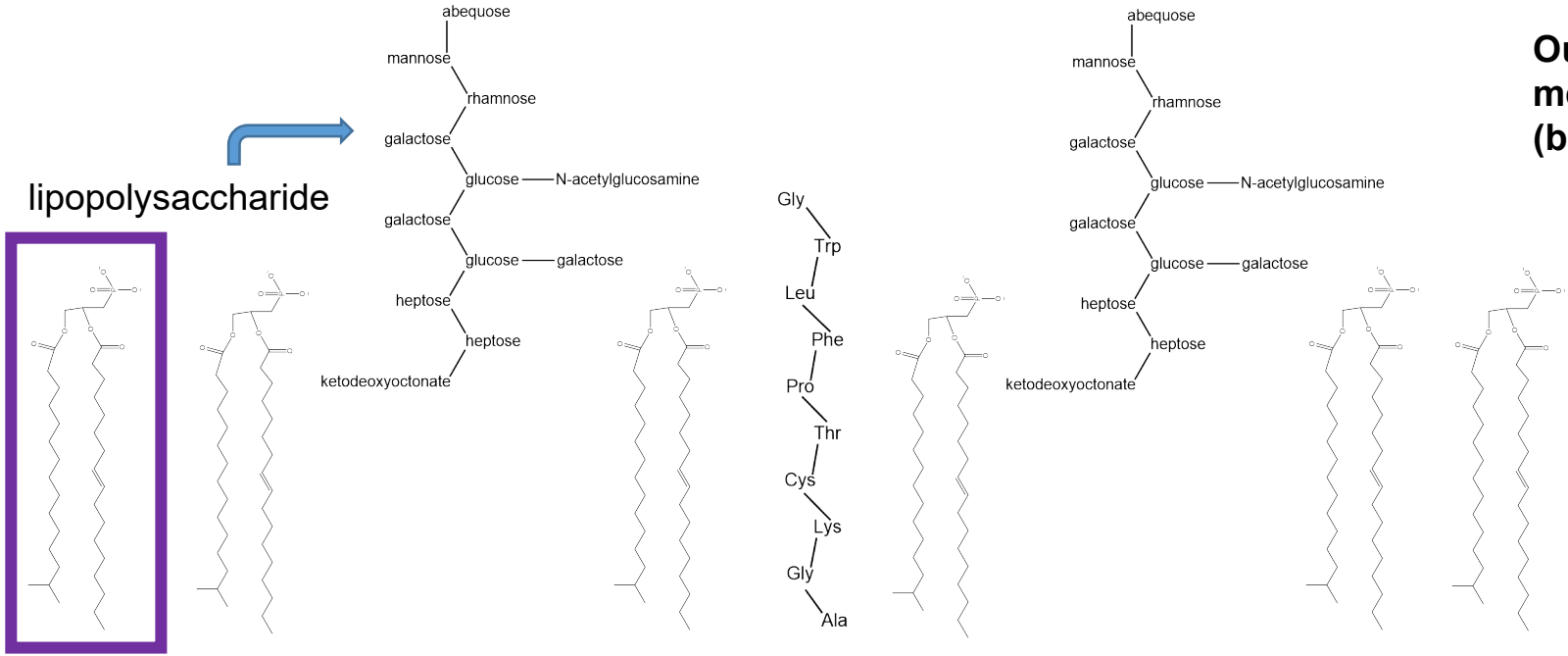


Cytoplasmic membrane (bilayer)

~4 nm

Cytoplasm

Gram (-) bacterial membrane

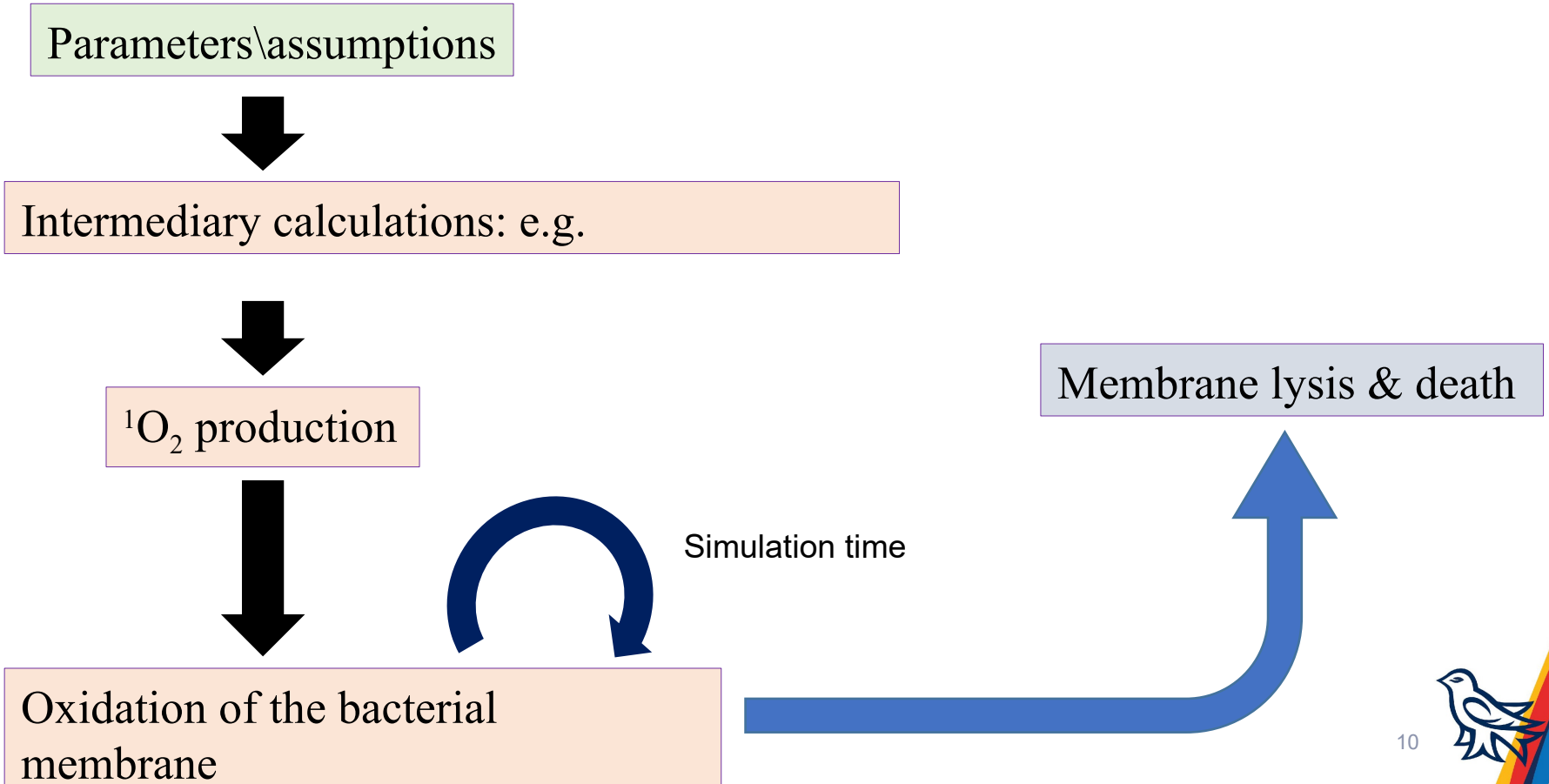


10 nm **Peptidoglycan**

~4 nm **Cytoplasmic membrane**

Cytoplasm

PDIpy workflow



Kinetic system – in Tellurium

1 [ ]h [ ]

2 [ ]h [ ]

3 [ ]+[¿3 2]→[¿1 2]+[ ]¿¿

4 [¿1 2]→[¿3 2]¿¿

5 [¿1 2]+[ ]→[ ]+[¿3 2]¿¿

6 [ ]h [ ]

7 [¿1 2]+[ ]→[ ]+[¿3 2]¿¿

Dissolution equilibrium with the atmosphere

Applicable systems

Photosensitizer

- Surface-bound (moles/area) or dissolved (moles/volume)
- Parameter files Excitation wavelengths, quantum yields, et cetera
- Molecular dimensions volume proportion & concentration near the surface

Bacterial colonization

- Planktonic (CFUs/mL) or biofilm
- Specie fatty acid profile

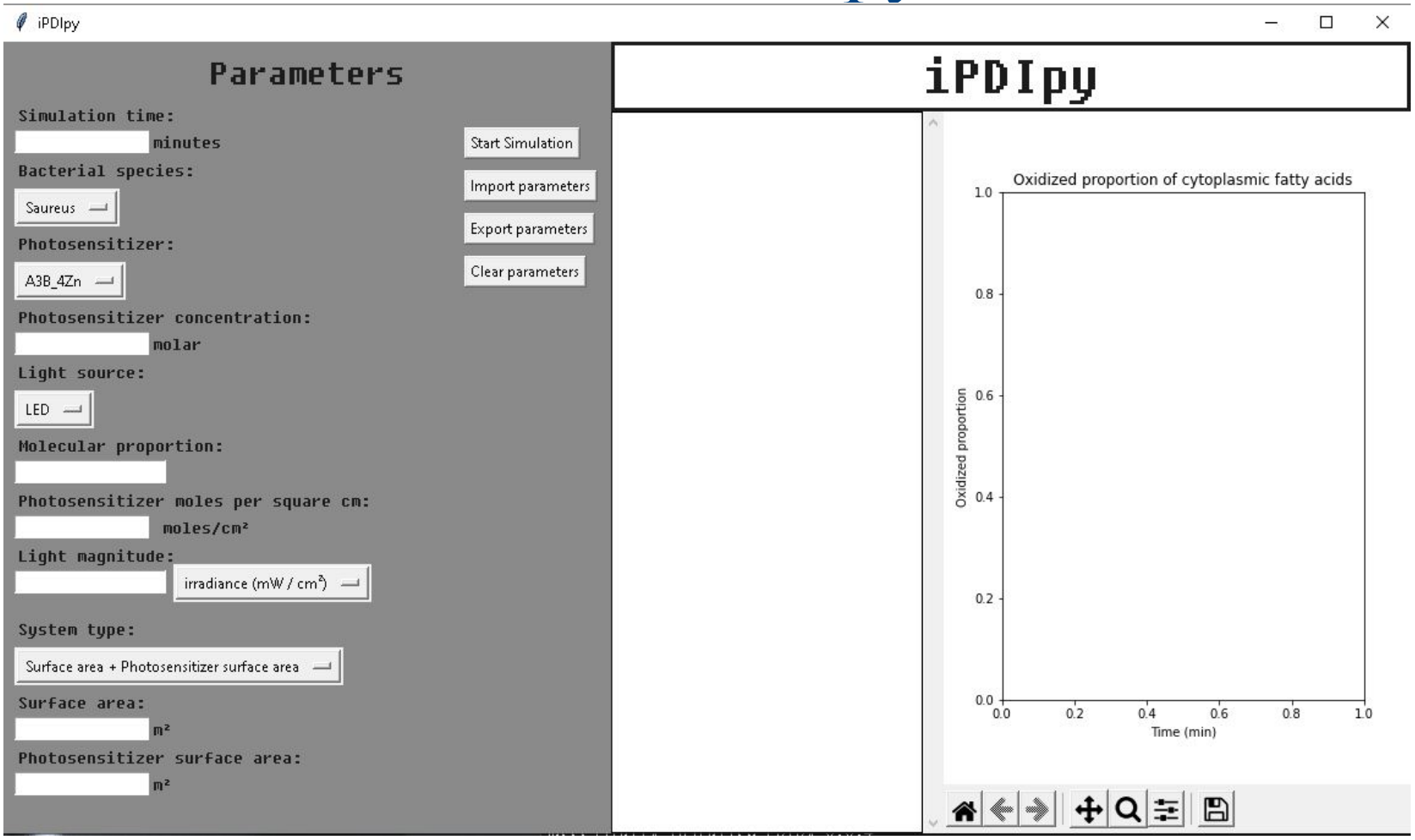
Simulation outputs

2021-12-09-PDIpy-A3B_4Zn-Saureus-8

Name	Date modified	Type	Size
input.omex	09-Dec-21 00:32	OMEX File	3 KB
output.svg	09-Dec-21 00:32	Scalable Vector Gr...	34 KB
parameters.csv	09-Dec-21 00:32	Microsoft Excel C...	2 KB
processed_data.csv	09-Dec-21 00:32	Microsoft Excel C...	2 KB
raw_data.csv	09-Dec-21 00:32	Microsoft Excel C...	7 KB
variables.csv	09-Dec-21 00:32	Microsoft Excel C...	2 KB

Name	Date modified	Type	Size
main.xml	09-Dec-21 05:32	XML Document	2 KB
manifest.xml	09-Dec-21 05:32	XML Document	1 KB
metadata.rdf	09-Dec-21 05:32	RDF File	1 KB
pdipy_oxidation.xml	09-Dec-21 05:32	XML Document	6 KB

iPDIpy


iPDIpy
Parameters
Simulation time:
minutes
Start Simulation
Bacterial species:
Saureus
Import parameters
Photosensitizer:
A3B_4Zn
Export parameters
Clear parameters
Photosensitizer concentration:
molar
Light source:
LED
Molecular proportion:
Photosensitizer moles per square cm:
moles/cm²
Light magnitude:
irradiance (mW / cm²)
System type:
Surface area + Photosensitizer surface area
Surface area:
m²
Photosensitizer surface area:
m²
iPDIpy
Oxidized proportion of cytoplasmic fatty acids
Oxidized proportion
Time (min)
0.0
0.2
0.4
0.6
0.8
1.0

Cross-linked experiment calibration

Parameters (unpublished)

Photosensitizer:

Surface area:

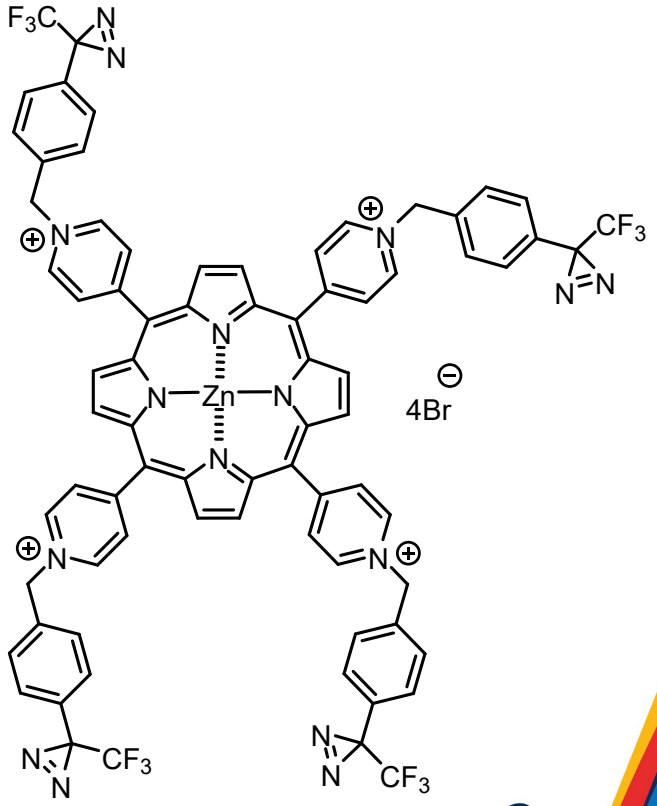
Specie: *S. aureus*

Light: White LED (75 W, 1800 Lumens)

Intensity:

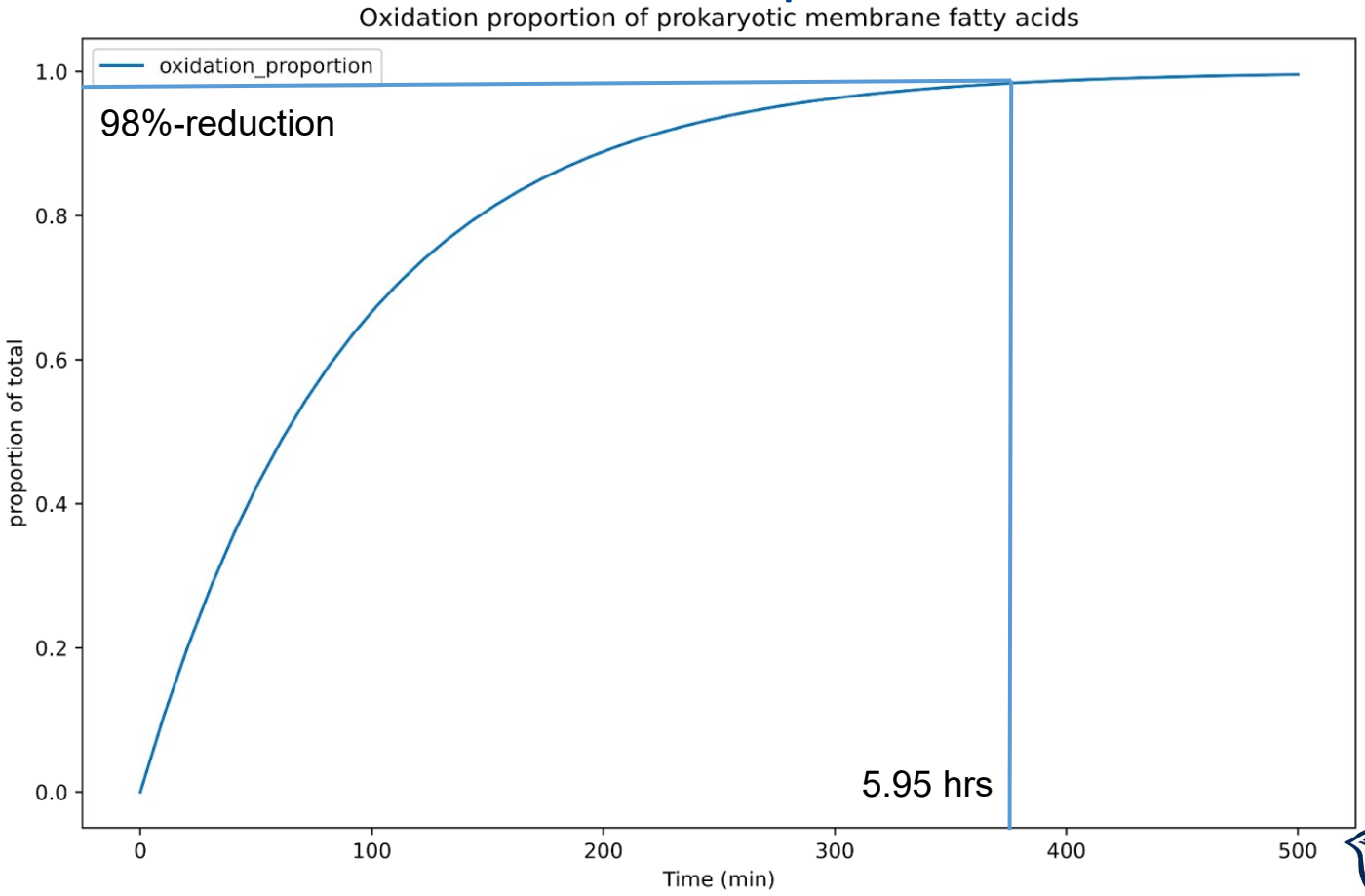
Reduction: reduction

Irradiation time: 6 hours



Calibrated output

time (hr)	oxidation_proportion
0	0
0.34188	0.192462
0.683761	0.347883
1.025641	0.473392
1.367521	0.574746
1.709402	0.656593
2.051282	0.722686
2.393162	0.776058
2.735043	0.819159
3.076923	0.853963
3.418803	0.882069
3.760684	0.904767
4.102564	0.923095
4.444444	0.937896
4.786325	0.949849
5.128205	0.959501
5.470085	0.967295
5.811966	0.97359
6.153846	0.978673
6.495726	0.982777
6.837607	0.986092
7.179487	0.988769
7.521368	0.99093
7.863248	0.992676
8.205128	0.994085
8.547009	0.995224
8.888889	0.996143
9.230769	0.996885
9.57265	0.997485
9.91453	0.997969
10.25641	0.99836
10.59829	0.998675



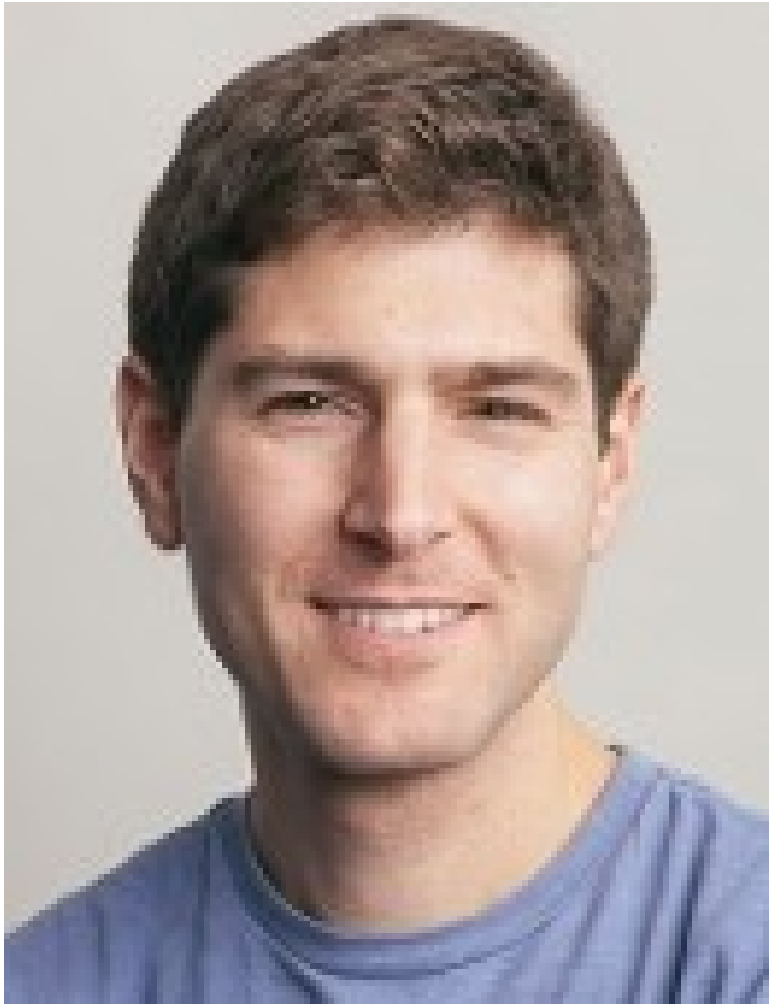
Corresponding content

1) GitHub
https://github.com/freiburgermsu/PDIpy

2) PyPI
https://test.pypi.org/project/PDIpy/

Remaining tasks

1) Validate the software with case studies from literature
2) Compose unit-tests and API documentation
3) Distill iPDIpy into an executable file



Thank you!

Icahn School of Medicine at Mount Sinai

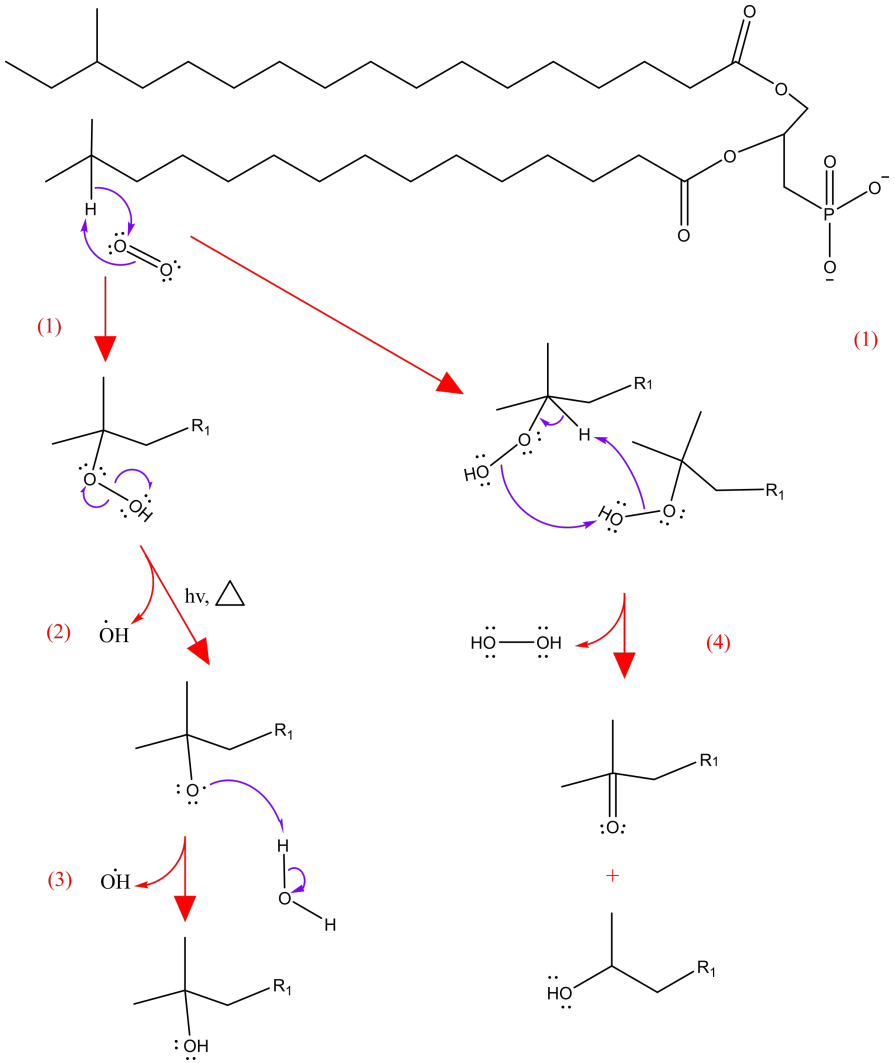
Thank you!

Green Safe Water Lab

Mitacs ACCELERATE

¿Questions?

Chemical mechanism: BCFA oxidation



Photonic calculations

Engineering membrane model

Assumptions

1) Ambient conditions [DO]
2) $^1O_2$ only oxidizes membrane cholesterol and phospholipid fatty acids
3) Membrane lipids and the bacterium are stationary
4) and , average excitation is 513 nm
5) Constant broth and environmental conditions
6) Visible proportion is 10% in incandescent and 50% in LED
7) Prokaryotic phospholipids is 1/3 saturated BCFAs and 2/3 saturated SCFAs
8) First order collision between PS and $^3O_2$ is assumed

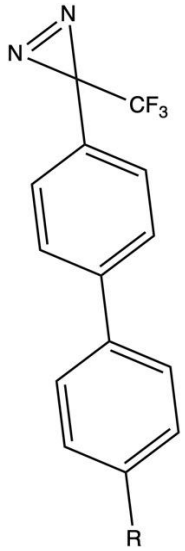
Engineering membrane model

Membrane oxidation proportion calculations

Engineering membrane model

Membrane oxidation proportion calculations continued

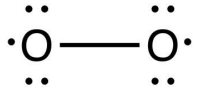
Photochemical process



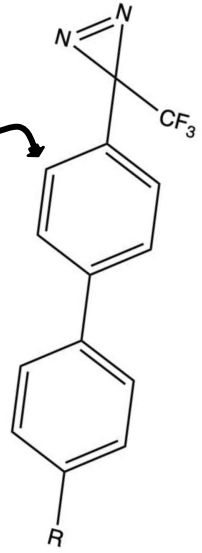
+

Potent antibiotic

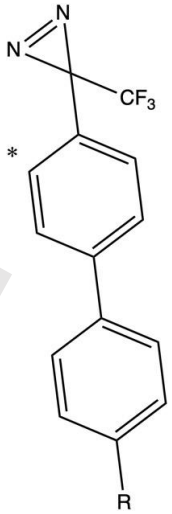
Ambient



UV



Photosensitizer



Type II
Photosensitization

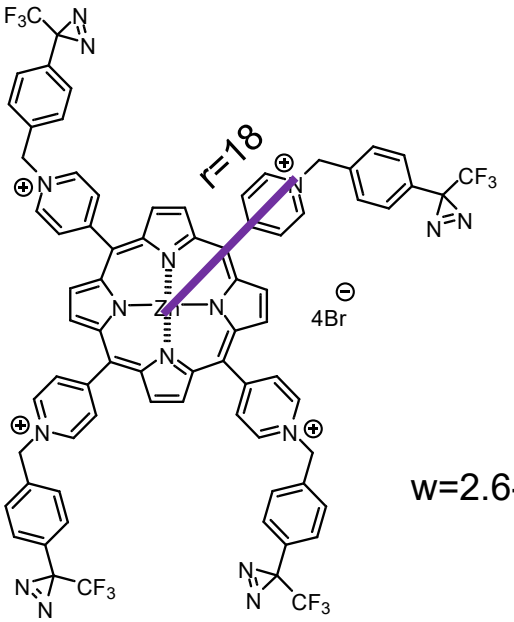
Singlet state $^1O_2$:

- All electrons are paired
- Angular momentum = 0
- Unstable\ ⇧ reactivity

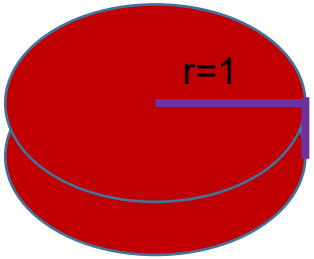
Triplet state $^3O_2$:

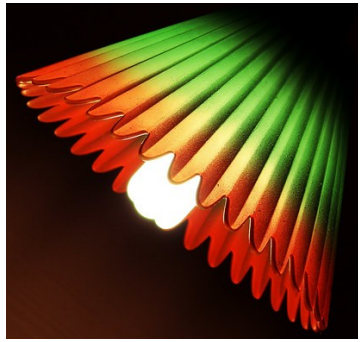
- Two electrons are unpaired
- Angular momentum = -1,0,1
- Stable \ moderate reactivity

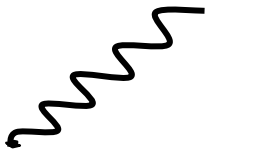
Incident solution volume

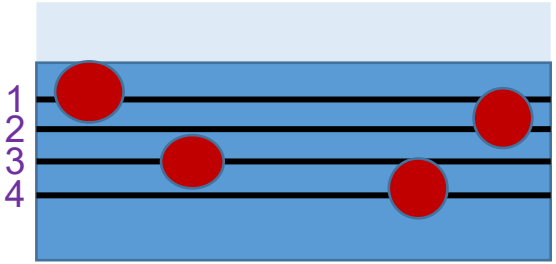


w=2.6-

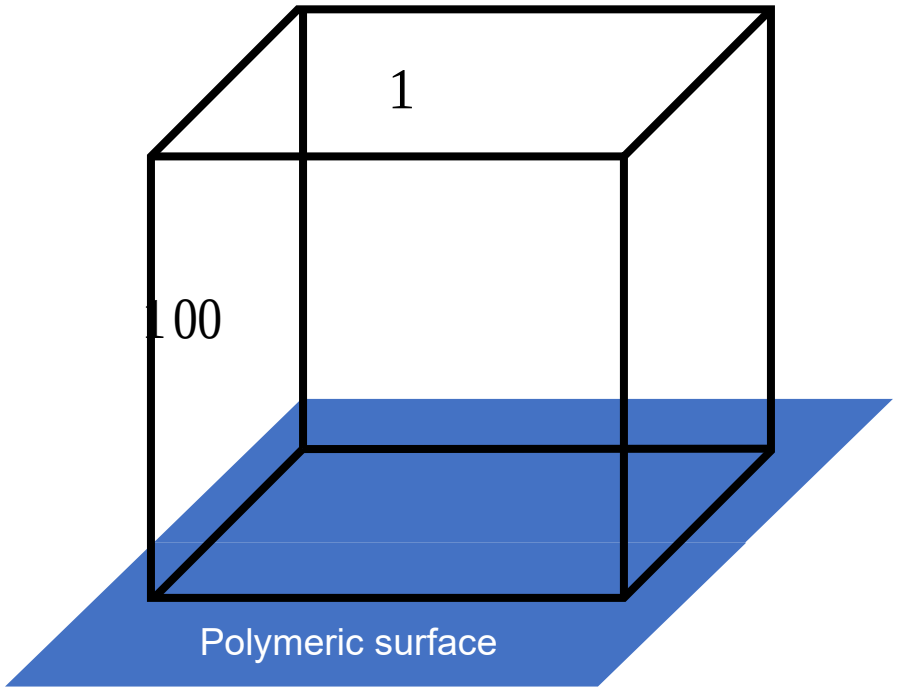








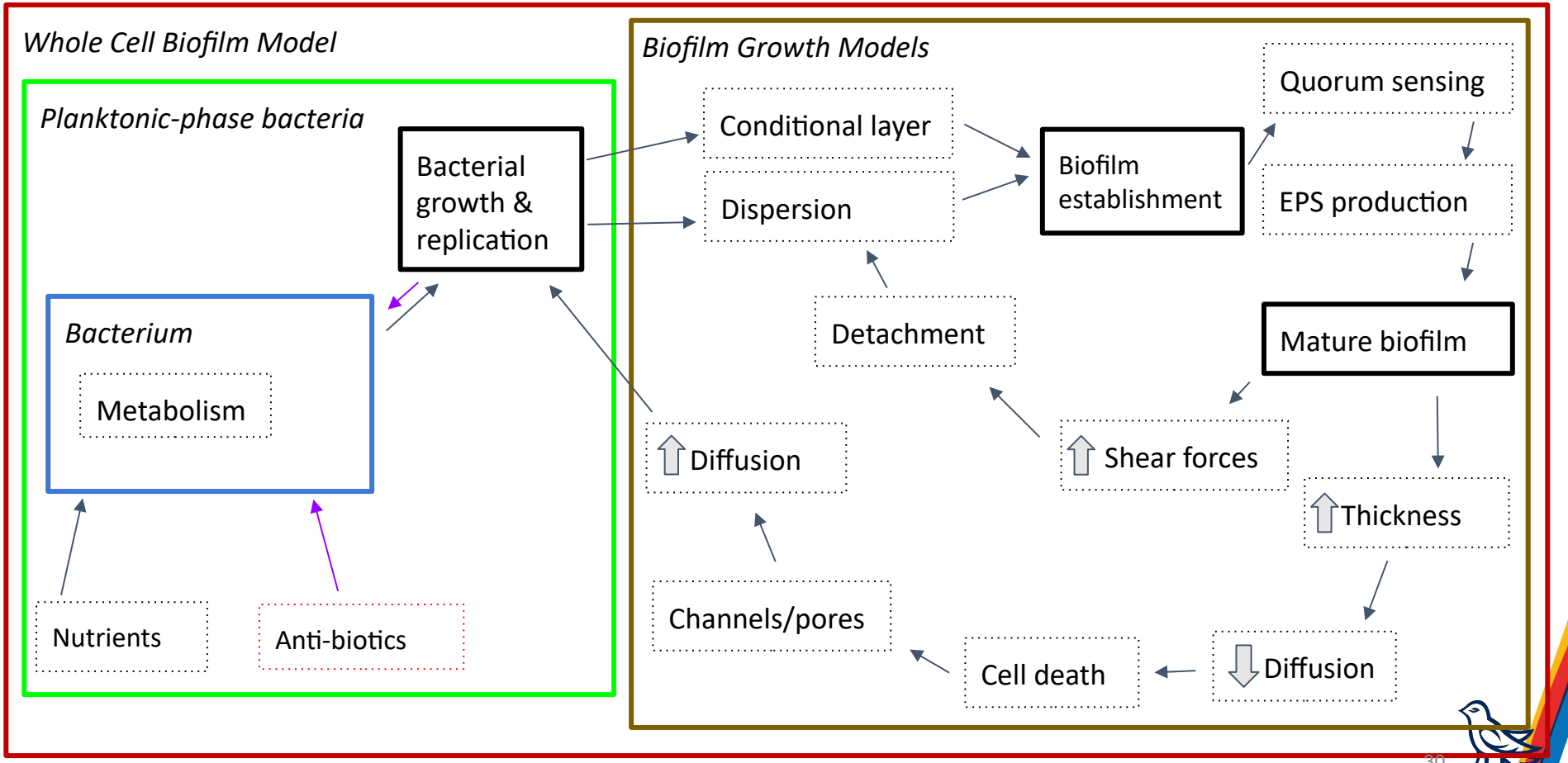
Biofilm software



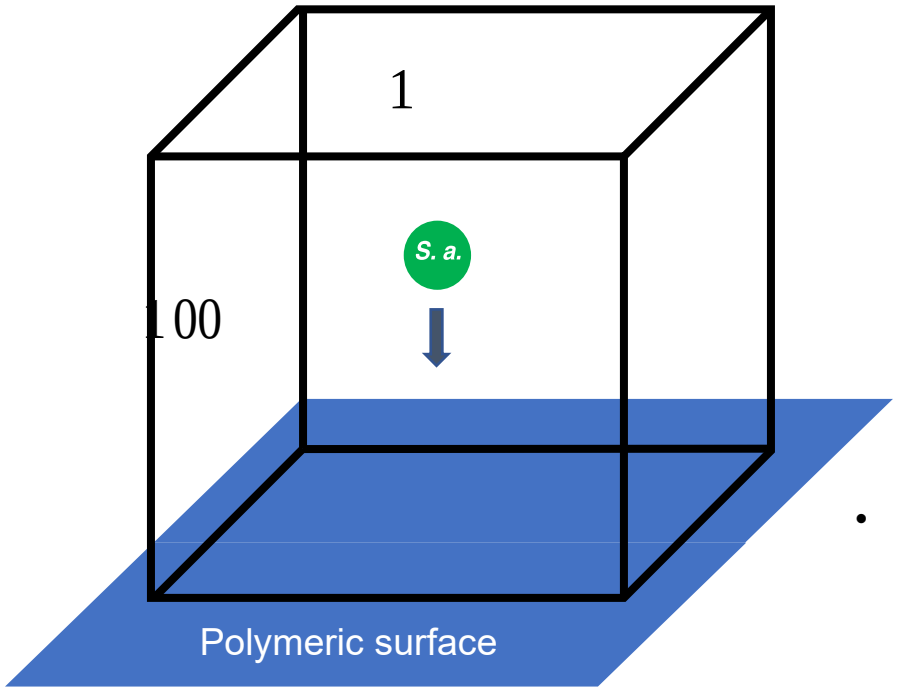
Bacterium

- 1 million grid volume cells

Biofilm conceptual scheme

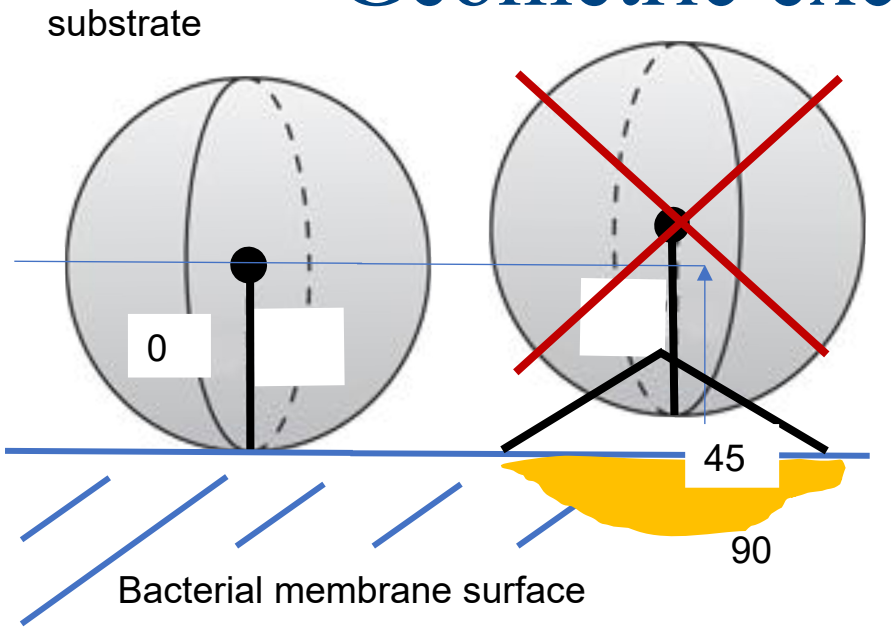


Biofilm software



- Bacterium = particle
  - Stoke's law of terminal velocity for a settling particle

Geometric exchange constraints



probability of membrane interaction

probability of membrane interaction
≈45° average angle between and

$$= \_ = \frac{\left(\quad h \quad \right)}{\left(\quad h \quad \right)} = 14.6\%$$

Qualities to consider

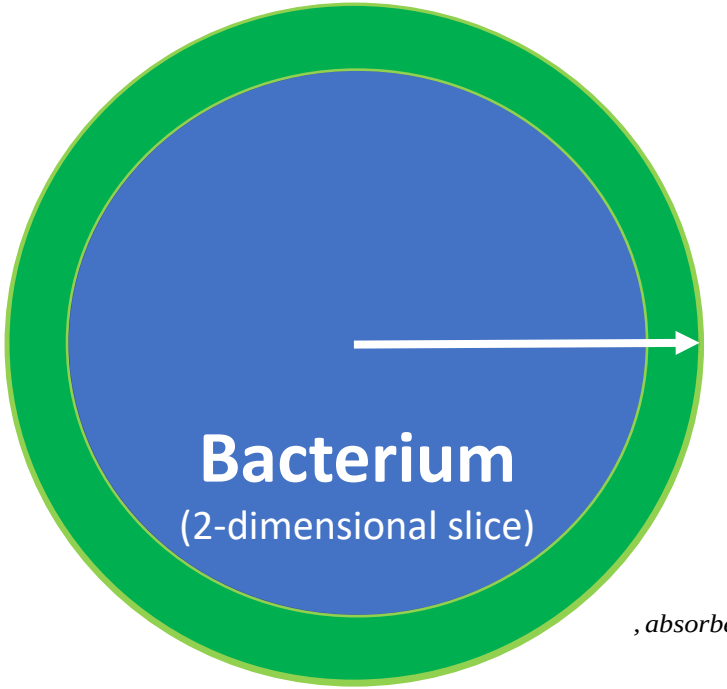
1) Barrier properties
- Rejection
- Absorption delay

1) Bacterium metabolic states
- “Persister”
- Planktonic
- Biofilm
- Detached

2) Behavioral phenomena
- Growth
- Replication
- Wasting

Fundamental bacterial model

Chemical adsorption →



$$= \vec{} * \qquad ,interaction = [\quad] * V_{shell} *$$

$$,absorbed = \begin{cases} , & * \left(\left(\text{——} \right) \right. \qquad - \Pi \; _{=1} \left(\frac{(\quad)\;,}{(\quad)\;,} \right) \\ ¿0, \Pi \; _{=1} \left(\frac{(\quad)\;,}{(\quad)\;,} \right) > \left(\text{——} \right) \end{cases}$$

$$absorbed = \sum_{=1} *$$

Assumptions and limitations

1) Homogeneous bulk and cytoplasm
- Boltzmann distribution of velocities
- Average velocity applies to all substrates

2) Need-based absorption

- is estimated from the incubation

3) Transcription is redundant and negligible

4) Mass balance applies across the membrane

5)

6) Singlet oxygen oxidizes only unsaturation

1) The WCM data describes the one of the smallest known bacterium: *Mycoplasma*

2) Internal limitation to personal computers

Membrane flux

$$\Delta = \Delta_{t=0} + \Delta - \Delta$$

$$\Delta = \sum_{=1} * = ¿$$

$$_{new,t} = , * \left(\frac{V_{bacterium,0} \approx 1}{_{,0} \approx 1} = \right) +$$

New directions

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

2) Expand biochemical accuracies
- Introduce quorum sensing reactions

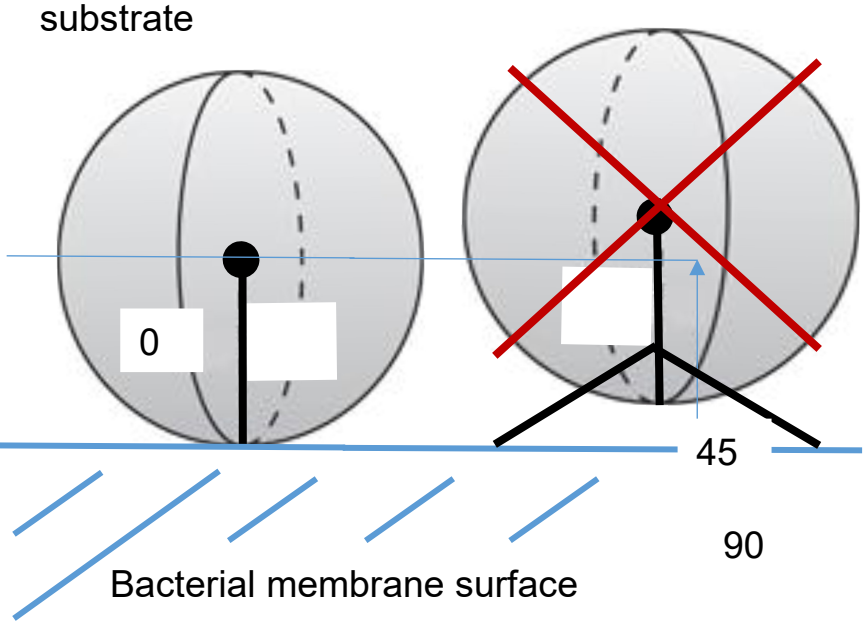
3) Expand the bacterium model into a biofilm model
- Incorporate new functionalities like metabolic states

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

Proportion of absorbed substrates



=

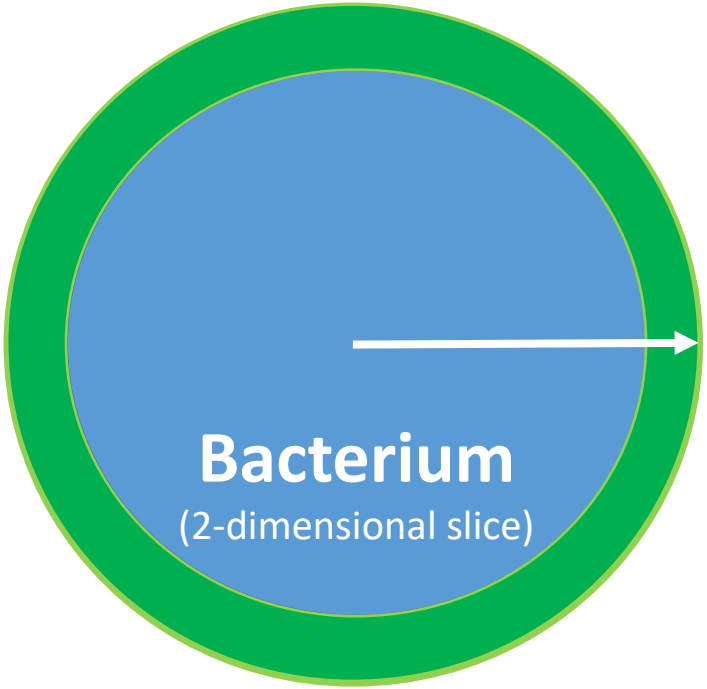
probability of membrane interaction

The angle of interaction ranges between and .

- The average angle is from vertical

$$= = \frac{\left(\quad h \quad \right)}{\left(\quad h \quad \right)} = 14.6\%$$

Chemical adsorption → →



$$,\ available = [C] * V_{shell} *$$

$$,\ absorbed = \begin{cases} , & * \left(1 - \Pi\ _{=1}\left(\frac{(\quad)\ ,}{(\quad)\ ,}\right)\right), (\quad)\ , \leq (\quad) \\ ¿0, \Pi\ _{=1}\left(\frac{(\quad)\ ,}{(\quad)\ ,}\right) > 1 \end{cases}$$

$$absorbed = \sum_{i=1}^{F} *$$

C = extracellular substrate
F = # substrates C
x = # reactions with C

$$= \vec{V} * \Delta$$

Membrane mass balance

$$\Delta = \Delta_{t=0} + \Delta - \Delta$$

$$\Delta = \sum_{=1} *$$

$$_{new,t} = {}_{,} * \left(\frac{V_{bacterium,0} \approx 1}{{}_{,0} \approx 1} \right) +$$

Allometry [eukaryotes?]

E = # ejected metabolites

Octyl gallate – Anti-biofoulant interactions

Table S1. Antifungal activity of compounds tested in *Aspergillus brasiliensis* ATCC16404. MIC (mM), minimum inhibitory concentration, where no fungal growth was visible in RPMI liquid culture; MFC (mM), minimum fungicidal concentration achieving ≥ 99.9% fungal death, determined on recovery agar plate. All compounds that showed some activity at the concentrations tested are highlighted.

	Benzoyl	MIC	MFC	Salicyl	MIC	MFC	Gallyl	MIC	MFC	Root alkyl	MIC	MFC
C0	Benzoic acid	>6.4	>6.4	Salicylic acid	>6.4	>6.4	Gallic acid	>6.4	>6.4	-	-	-
C1	Methyl benzoate	>6.4	>6.4	Methyl salicylate	>6.4	>6.4	Methyl gallate	>6.4	>6.4	Methanol	>6.4	>6.4
C2	Ethyl benzoate	>6.4	>6.4	Ethyl salicylate	>6.4	>6.4	Ethyl gallate	>6.4	>6.4	Ethanol	>6.4	>6.4
C3	Propyl benzoate	>6.4	>6.4	Propyl salicylate	>6.4	>6.4	Propyl gallate	>6.4	>6.4	*n*-Propanol	>6.4	>6.4
C4	Butyl benzoate	>6.4	>6.4	Butyl salicylate	>6.4	>6.4	Butyl gallate	6.4	>6.4	*n*-Butanol	>6.4	>6.4
C5	Pentyl benzoate	>6.4	>6.4	-	-	-	Pentyl gallate	1.6	6.4	*n*-Pentanol	>6.4	>6.4
C6	Hexyl benzoate	>6.4	>6.4	Hexyl salicylate	>6.4	>6.4	-	-	-	*n*-Hexanol	>6.4	>6.4
C8	Octyl benzoate	>6.4	>6.4	Octyl salicylate	>6.4	>6.4	Octyl gallate	0.1	0.4[3]	*n*-Octanol	>6.4	>6.4
C9	-	-	-	-	-	-	Nonyl gallate	1.6	>6.4	-	-	-
C10	-	-	-	-	-	-	Decyl gallate	ND[4]	ND[4]	-	-	-
C12	Dodecyl benzoate	>6.4	>6.4	Dodecyl salicylate	ND[1]	ND[1]	Lauryl gallate	ND[5]	ND[5]	*n*-Dodecanol	>6.4	>6.4
C16	-	-	-	Stearyl salicylate	ND[2]	ND[2]	-	-	-	*n*-Stearol	>6.4	>6.4
C0	Benzamide	>6.4	>6.4	Salicylamide	>6.4	>6.4	Gallamide	>6.4	>6.4	-	-	-
C3	*N*-Propyl benzamide	>6.4	>6.4	*N*-Propyl salicylamide	1.6	>6.4	*N*-Propyl gallamide	1.6	>6.4	*N*-Propyl amine	>6.4	>6.4
C8	*N*-Octyl benzamide	>6.4	>6.4	-	-	-	*N*-Octyl gallamide	0.8	>6.4	*N*-Octyl amine	1.6	>6.4

Incumbent	MIC	MFC
Methyl 4-hydroxybenzoate	3.2	>6.4
Propyl 4-hydroxybenzoate	0.8	>6.4
Octyl 4-hydroxybenzoate	>6.4	>6.4
2-Methoxy-4-hydroxybenzoate	6.4	>6.4
Butylated hydroxyanisole	0.8	>6.4
4-Chloro-3,5-xylenol	0.4	1.6
2-Phenoxyethanol	>6.4	>6.4
Sorbic acid	>6.4	>6.4

[1]Not determined. Precipitated in RPMI liquid medium at 3.2 mM (and fungus grew up to 1.6 mM).
[2]Not determined. Precipitated in RPMI liquid medium at 1.6 mM (and fungus grew up to 0.8 mM).
[3]99.8% fungal death (also 99.8% fungal death at 0.8 mM, while precipitation occurred at 1.6 - 6.4 mM).
[4]Not determined. Precipitated in RPMI liquid medium at 0.4 mM (and fungus grew up to 0.2 mM).
[5]Not determined. Precipitated in RPMI liquid medium at 0.1 mM (and fungus grew up to 0.05 mM).

Predictive simulations will expedite biofilm anti-biofoulant discovery

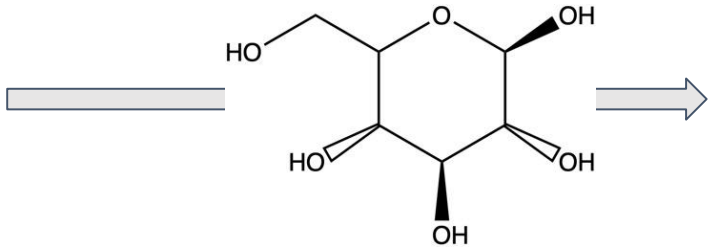
Buckley et al., 2017

Suspension phase model

Solution elements

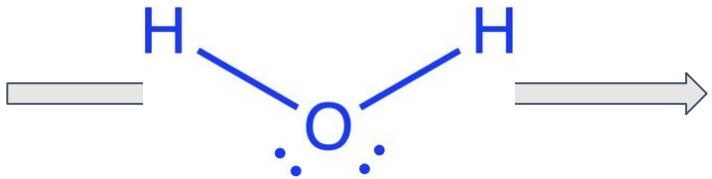
Parameters/equations

Substrate



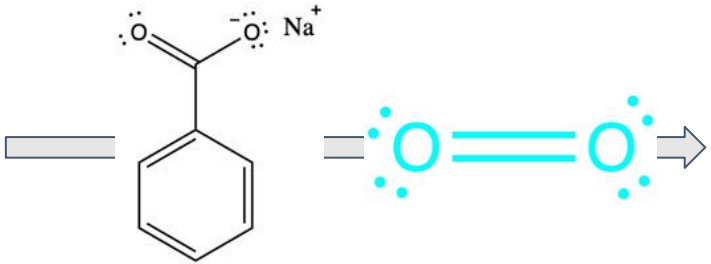
Diffusion - Reaction-diffusion Equations
Fick's law or Cahn-Hilliard equations

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 or Cahn-Hilliard

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***

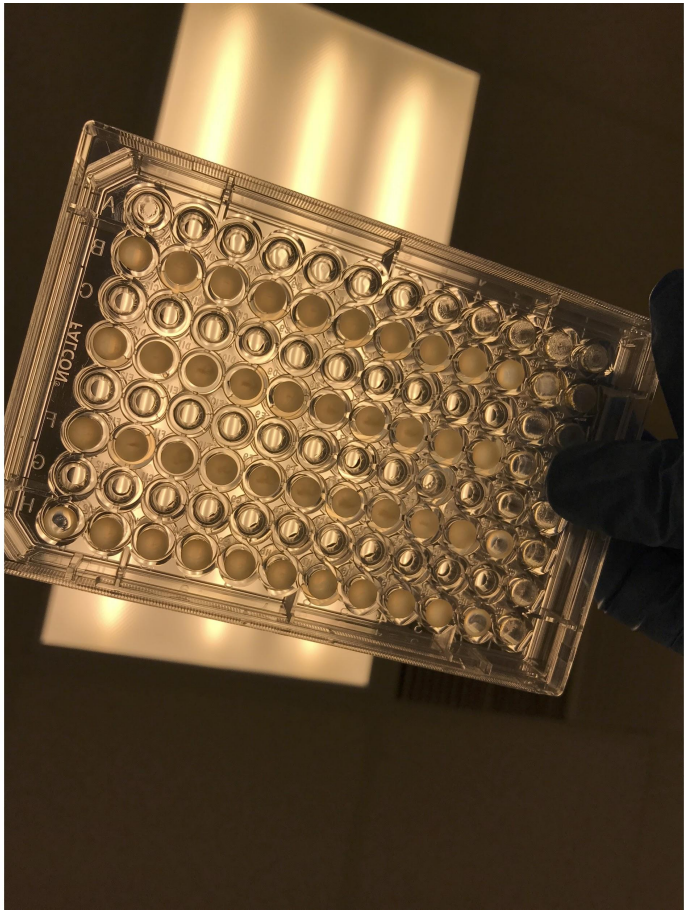
Algorithm/Model	Assumptions/limitations	Model contribution
Rittmann model & Biofilm Accumulation model (BAM)	1) The biofilm is composed of dead+active bacteria and water 2) Constant Biofilm growth, [Substrate], and bulk volume	Foundation
Biofilm Growth model (BGM)	1) The biofilm is composed of dead+active 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

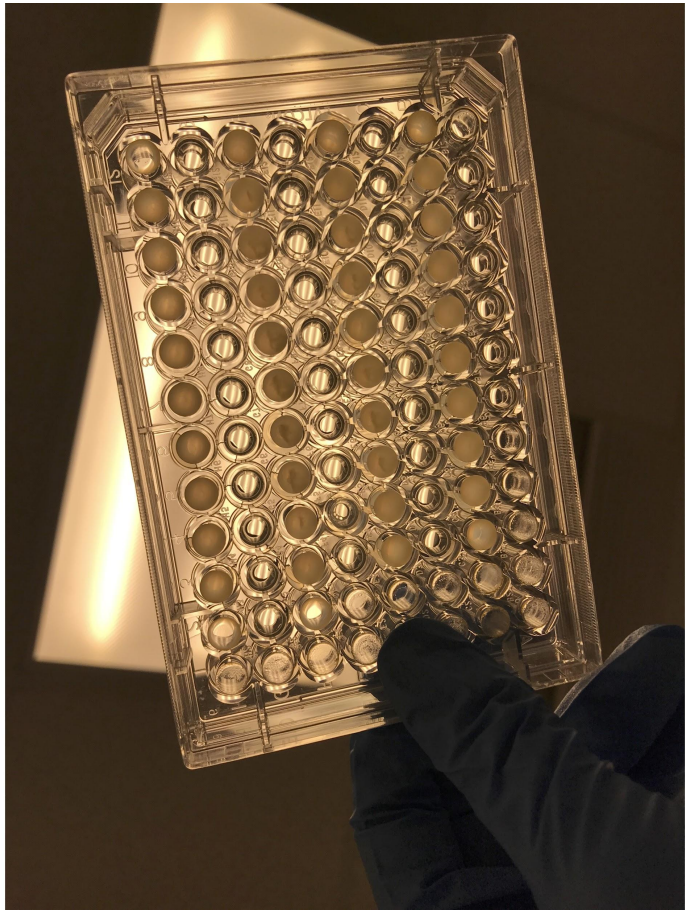
Buckley Biofilm Growth Model

Algorithm/Model	Assumptions/limitations	Model contribution

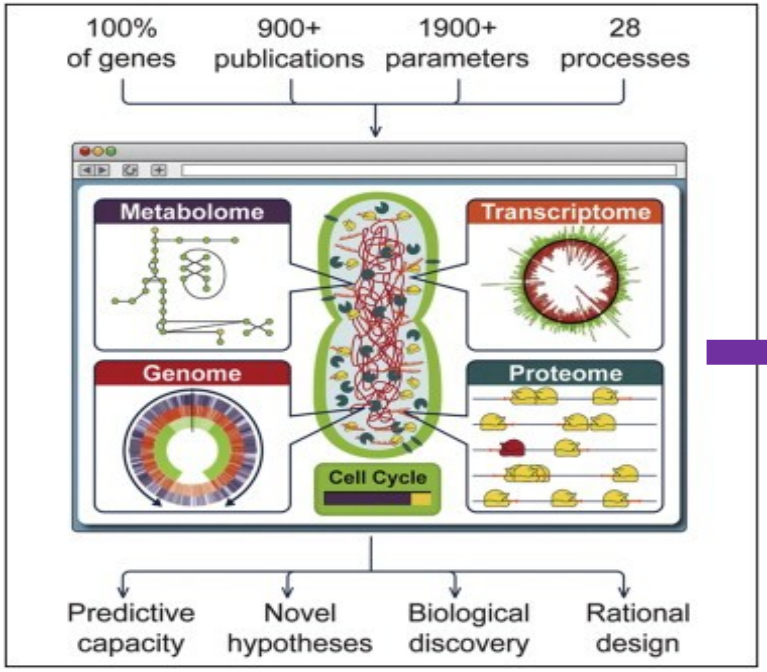
Buckley Biofilm Growth Model

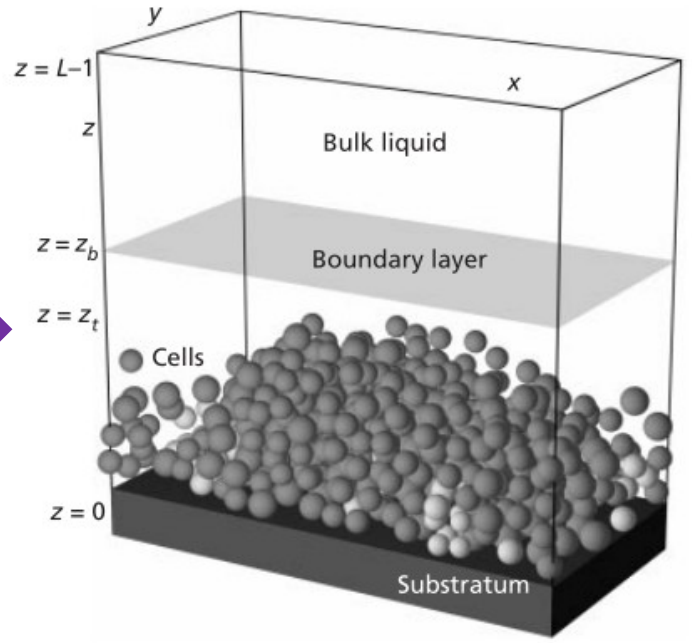
Bioassays





Whole Cell biofilm model inspiration





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

- Dissertation by Jonathan Karr, Stanford
- Citations
- SimTK download: 58,044 / 100,484
- *Nature* (2013, 2020)

Biofilm Models

- Top-down differential equations
- The literature solicits biochemical accuracy