ICU CRAB Modeling Plan

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1. Modeling Overview and Objectives

1.1 Project Understanding

This project builds an intelligent quorum-quenching system targeting **CRAB** using engineered **EcN**. The system includes **three core layers** (sensing–amplification–execution), a **spatial targeting module** (AHL chemotaxis), a **positive feedback loop**, **multi-scenario visualization modules**, and **multi-level kill switches**, with customized delivery strategies for three infection scenarios: lung, bloodstream, and wound.

1.2 Modeling Objectives

ID	Modeling objective	Corresponding design module
M1	Quantify AHL sensing sensitivity and T7 amplification gain; optimize promoter/RBS strength	Core Pathway: Sensing + Amplification
M2	Analyze the bistable window of the positive feedback loop; determine activation/maintenance thresholds	Positive Feedback Loop Optimization
M3	Predict PvdQ degradation kinetics for 3-OH-C12-HSL	Execution Layer
M4	Simulate chemotaxis of engineered bacteria along AHL gradients	Spatial Enhancement
M5	Build a QS-regulated dynamic growth/dissolution model of CRAB biofilms	Background (biofilm formation mechanism)
M6	Model enzyme–probe competitive kinetics; predict the color-change time point	Visualization (lung/blood/wound)
M7	Predict timed clearance dynamics and safety window of the MazEF kill switch	Kill Switch

ID	Modeling objective	Corresponding design module
M8	Simulate targeted release of pH-sensitive liposomes in the lung	Route of Administration: Pulmonary
M9	Simulate diffusion and mass transfer of AHL/enzymes in core–shell microcarriers	Route of Administration: Wound

2. Model 1: ODE Model for AHL Sensing and T7 Amplification Cascade

2.1 Purpose

Quantify the full signal transduction from extracellular 3-OH-C12-HSL input to high intracellular PvdQ expression. Predict the system's **limit of detection**, **response time**, and **amplification gain**, and provide theoretical guidance for pAbaI* promoter engineering and RBS optimization.

2.2 Biochemical Reaction Network

Sensing layer reactions:

1. Constitutive AbaR expression: $\emptyset \xrightarrow{\alpha_{AbaR}} \mathrm{AbaR}$
2. AHL transmembrane diffusion: $\mathrm{AHL}_{ext} \underset{k_{out}}{\overset{k_{in}}{\rightleftharpoons}} \mathrm{AHL}_{int}$
3. AbaR–AHL complex formation: $\mathrm{AbaR} + \mathrm{AHL}_{int} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \mathrm{AbaR{:}AHL}$
4. pAbaI* activation drives T7 RNAP expression: $\mathrm{AbaR{:}AHL} + \mathrm{pAbaI*} \rightarrow \mathrm{T7\ RNAP}$

Amplification layer reactions:

5. T7 RNAP drives PvdQ expression: $\mathrm{T7\ RNAP} + \mathrm{pT7} \rightarrow \mathrm{PvdQ}$

2.3 ODE System

$$\frac{d[\mathrm{AbaR}]}{dt} = \alpha_{AbaR} - \gamma_{AbaR}[\mathrm{AbaR}] - k_{on}[\mathrm{AbaR}][\mathrm{AHL}_{int}] + k_{off}[\mathrm{AbaR{:}AHL}]$$

$$\frac{d[\mathrm{AHL}_{int}]}{dt} = k_{in}[\mathrm{AHL}_{ext}] - k_{out}[\mathrm{AHL}_{int}] - k_{on}[\mathrm{AbaR}][\mathrm{AHL}_{int}] + k_{off}[\mathrm{AbaR{:}AHL}]$$

$$\frac{d[\mathrm{AbaR{:}AHL}]}{dt} = k_{on}[\mathrm{AbaR}][\mathrm{AHL}_{int}] - k_{off}[\mathrm{AbaR{:}AHL}] - \gamma_c[\mathrm{AbaR{:}AHL}]$$

$$\frac{d[\mathrm{T7}]}{dt} = \alpha_{T7} \cdot \frac{[\mathrm{AbaR{:}AHL}]^n}{K_{d,\mathrm{pAbaI}}^n + [\mathrm{AbaR{:}AHL}]^n} + \beta_{T7} - \gamma_{T7}[\mathrm{T7}]$$

$$\frac{d[\mathrm{PvdQ}]}{dt} = \alpha_{PvdQ} \cdot \frac{[\mathrm{T7}]^m}{K_{d,\mathrm{pT7}}^m + [\mathrm{T7}]^m} + \beta_{PvdQ} - \gamma_{PvdQ}[\mathrm{PvdQ}]$$

Where:

- α_{AbaR}: constitutive AbaR expression rate (set by a weak constitutive promoter)

- k_{on}, k_{off}: binding/unbinding rate constants for AbaR and AHL
- $K_{d,\mathrm{pAbaI}}$: half-saturation constant for pAbaI* activation by AbaR:AHL
- n: Hill coefficient reflecting pAbaI* cooperativity
- $\alpha_{T7}, \alpha_{PvdQ}$: maximal transcription rates
- β_{T7}, β_{PvdQ}: basal leak expression rates
- γ_x: degradation rates (including dilution, $\gamma_x = \delta_x + \mu$, with μ the growth rate)

2.4 Key Parameter Estimates

Parameter	Meaning	Estimate
k_{on}	AbaR–AHL binding rate	$10^6\ \mathrm{M^{-1}s^{-1}}$
k_{off}	AbaR–AHL unbinding rate	$10^{-2}\ \mathrm{s^{-1}}$
$K_{d,\mathrm{pAbaI}}$	pAbaI* activation threshold	10–100 nM
n	Hill coefficient	1.5–2.5
α_{T7}	maximal T7 RNAP expression rate	50 nM/min
γ_{T7}	T7 RNAP degradation rate	0.02 $\mathrm{min^{-1}}$
Amplification gain	T7 cascade	$> 100\times$

These values can be refined from the literature; the above are internet-based estimates.

2.5 Analysis Methods

1. **Steady-state analysis**: set derivatives to zero and solve for the dose–response $[\mathrm{PvdQ}]_{ss}$ vs. $[\mathrm{AHL}_{ext}]$
2. **Dynamic simulation**: under step input (sudden exposure to CRAB environment), compute response time t_{90} (time to reach 90% of steady state)
3. **Parameter scanning**: scan key parameters such as α_{AbaR}, $K_{d,\mathrm{pAbaI}}$, n, etc., and plot heatmaps to find optimal design space

3. Model 2: Bistability Analysis of the Positive Feedback Loop

3.1 Purpose

The positive feedback loop described in the design uses T7 RNAP to drive expression of an additional AbaR copy, forming a "**self-amplifying**" mechanism. This model aims to:

- Determine the **activation threshold** $[\mathrm{AHL}]_{on}$ and **maintenance threshold** $[\mathrm{AHL}]_{off}$
- Quantify the **hysteresis window** $\Delta = [\mathrm{AHL}]_{on} - [\mathrm{AHL}]_{off}$
- Optimize the RBS strength of the extra AbaR copy to maximize Δ

3.2 Extended ODE

On top of Model 1, add a positive feedback term:

$$\frac{d[\mathrm{AbaR}]}{dt} = \underbrace{\alpha_{AbaR}}_{\text{constitutive}} + \underbrace{\alpha_{AbaR,fb} \cdot \frac{[\mathrm{T7}]^m}{K_{d,\mathrm{pT7,AbaR}}^m + [\mathrm{T7}]^m}}_{\text{positive feedback (T7 drives extra AbaR)}} - \gamma_{AbaR}[\mathrm{AbaR}] - k_{on}[\mathrm{AbaR}][\mathrm{AHL}_{int}] + k_{off}[\mathrm{AbaR{:}AHL}]$$

Where $\alpha_{AbaR,fb}$ is the maximal expression rate of the extra AbaR driven by T7 via pT7, determined by the RBS strength of the extra AbaR copy.

3.3 Bistability Analysis Method

Phase plane analysis:

Define two key variables $x_1 = [\mathrm{AbaR{:}AHL}]$, $x_2 = [\mathrm{T7}]$, and plot the nullclines:

$$\text{Nullcline 1:} \quad \frac{d[\mathrm{AbaR{:}AHL}]}{dt} = 0$$
$$\text{Nullcline 2:} \quad \frac{d[\mathrm{T7}]}{dt} = 0$$

When the two nullclines intersect at **three points**, the system exhibits bistability (two stable states + one unstable saddle point).

3.4 RBS Optimization Strategy

Scan $\alpha_{AbaR,fb}$ (corresponding to different RBS strengths) to find the best value that:

$$\max \Delta = [\mathrm{AHL}]_{on} - [\mathrm{AHL}]_{off}$$

While satisfying the constraint:

$$\text{s.t.} \quad \text{Metabolic Burden}([\mathrm{AbaR}]_{ss,\mathrm{ON}}) < \text{Tolerance Threshold}$$

Metabolic burden can be approximated by total protein expression flux:

$$\text{Burden} \propto \alpha_{AbaR} + \alpha_{AbaR,fb} \cdot f([\mathrm{T7}]) + \alpha_{T7} \cdot g([\mathrm{AbaR{:}AHL}]) + \alpha_{PvdQ} \cdot h([\mathrm{T7}])$$

3.5 Expected Outputs

- **S-shaped bifurcation diagram** showing the bistable interval
- **Hysteresis window vs. RBS strength** curve to guide RBS library screening
- **Noise robustness analysis** via Gillespie SSA to validate single-cell bistability stability

4. Model 3: PvdQ Enzyme Kinetics and AHL Degradation Model

4.1 Purpose

Quantify PvdQ degradation efficiency on 3-OH-C12-HSL and predict, at a given engineered bacterial density, the **time required to reduce AHL below the threshold**.

4.2 Enzyme Kinetics

As an acyl transferase, PvdQ follows Michaelis–Menten kinetics:

$$v = \frac{V_{max}[\mathrm{AHL}]}{K_m + [\mathrm{AHL}]}$$

Where:

- $V_{max} = k_{cat} \cdot [\mathrm{PvdQ}]_{total}$
- K_m (PvdQ for 3-OH-C12-HSL): reported ~ **1–10 μM**
- k_{cat}: ~ **5–50 s^{-1}** (to be experimentally validated)

4.3 Environmental AHL Dynamics

Consider competition between continuous AHL production by CRAB and degradation by PvdQ:

$$\frac{d[\mathrm{AHL}_{ext}]}{dt} = \underbrace{r_{AHL} \cdot N_{CRAB} \cdot f(\mathrm{QS\ state})}_{\text{CRAB AHL production}} - \underbrace{\frac{k_{cat}[\mathrm{PvdQ}]_{ext}[\mathrm{AHL}_{ext}]}{K_m + [\mathrm{AHL}_{ext}]}}_{\text{PvdQ degradation}} - \underbrace{\delta_{AHL}[\mathrm{AHL}_{ext}]}_{\text{natural decay/diffusion}}$$

Where:

- r_{AHL}: AHL production rate per CRAB cell
- N_{CRAB}: CRAB cell density
- $f(\mathrm{QS\ state})$: regulatory function for AHL synthesis under QS positive feedback
- $[\mathrm{PvdQ}]_{ext}$: extracellular PvdQ concentration (depends on secretion efficiency)

4.4 Comparison of PvdQ Secretion Strategies

The design evaluates three secretion strategies (OmpA, PelB, HlyA). In the modeling framework, assign each a secretion efficiency η_{sec}:

$$\frac{d[\mathrm{PvdQ}]_{ext}}{dt} = \eta_{sec} \cdot [\mathrm{PvdQ}]_{int} \cdot N_{EcN} - \gamma_{PvdQ,ext} \cdot [\mathrm{PvdQ}]_{ext}$$

Secretion system	Estimated η_{sec} range	Features
OmpA (Sec pathway)	0.1–0.3	Requires signal peptide cleavage; possible folding delay
PelB (Sec pathway)	0.1–0.4	Better suited for periplasmic secretion
HlyA (Type I)	0.3–0.7	Direct transmembrane secretion; no periplasmic transport

Simulate AHL clearance time across different η_{sec} to guide experimental choice.

4.5 Expected Outputs

- **AHL concentration–time decay curves** under different EcN densities and PvdQ levels
- **Critical EcN density** required for effective quorum quenching
- **Secretion strategy comparison plots** of AHL clearance time

5. Model 4: PDE Model for Chemotactic Spatial Targeting

5.1 Purpose

Simulate directional migration of engineered bacteria under AHL gradients, quantify how **chemotaxis efficiency** affects bacterial enrichment near CRAB biofilms, and evaluate the targeting gain of the chimeric Tar–AbaR receptor.

5.2 Keller–Segel Chemotaxis Model

Use the classic Keller–Segel PDE model:

$$\frac{\partial b}{\partial t} = \underbrace{D_b \nabla^2 b}_{\text{random diffusion}} - \underbrace{\nabla \cdot (\chi(c)\, b\, \nabla c)}_{\text{chemotactic migration}} + \underbrace{\mu_b\, b \left(1 - \frac{b}{b_{max}}\right)}_{\text{bacterial growth}}$$

$$\frac{\partial c}{\partial t} = D_c \nabla^2 c + \underbrace{r_{AHL} \cdot \rho_{CRAB}(x)}_{\text{AHL release by CRAB}} - \underbrace{\frac{k_{cat}[\mathrm{PvdQ}(b)] \cdot c}{K_m + c}}_{\text{AHL degradation by PvdQ}} - \delta_c\, c$$

Where:

- $b(x,t)$: engineered EcN density (cells/volume)
- $c(x,t)$: AHL concentration
- D_b: bacterial diffusion coefficient (~ 10^{-6} cm^2/s)
- $\chi(c)$: chemotactic sensitivity function
- D_c: AHL diffusion coefficient (~ 5×10^{-6} cm^2/s)
- $\rho_{CRAB}(x)$: spatial distribution of CRAB (high density in biofilm core)

5.3 Chemotactic Sensitivity Function

Based on receptor occupancy theory:

$$\chi(c) = \chi_0 \cdot \frac{K_d}{(K_d + c)^2}$$

Where:

- χ_0: maximal chemotactic sensitivity (related to chimeric receptor expression level)
- K_d: dissociation constant of the chimeric Tar–AbaR receptor for 3-OH-C12-HSL

This function peaks at $c = K_d$, meaning chemotaxis is most sensitive at intermediate AHL concentrations—consistent with the design goal of enriching near the **biofilm edge** where gradients are steepest.

5.4 Geometry

Infection scenario	Geometry	Boundary conditions
Wound infection	2D plane (wound surface)	No-flux boundary (hydrogel confinement)
Lung infection	quasi-1D (radial from airway lumen to wall)	Airway wall biofilm as AHL source
Bloodstream infection	3D spherical symmetry (OMV delivery)	Spherical biofilm core

5.5 Simulation Comparison: No Chemotaxis vs Chemotaxis

Define the **Targeting Enrichment Factor (TEF)**:

$$\mathrm{TEF} = \frac{\int_{\Omega_{biofilm}} b(x, t_{eq})\, dx}{\int_{\Omega_{total}} b(x, t_{eq})\, dx} \Bigg/ \frac{|\Omega_{biofilm}|}{|\Omega_{total}|}$$

$\mathrm{TEF} = 1$ indicates uniform distribution; $\mathrm{TEF} \gg 1$ indicates effective enrichment near the biofilm.

5.6 Expected Outputs

- **Spatiotemporal heatmaps** of engineered bacterial density and AHL concentration
- **TEF vs time curves** comparing chemotaxis vs no chemotaxis
- **Chemotactic gain factor**: the fold increase of effective PvdQ concentration near biofilms

6. Model 5: Dynamic Model of CRAB Biofilms

6.1 Purpose

Build a QS-regulated biofilm growth–maturation–dissolution model linking QS signal concentration to biofilm state, to evaluate quorum-quenching treatment efficacy.

6.2 Framework

Divide CRAB into three subpopulations:

- P: planktonic cells
- B: biofilm-embedded cells
- E: EPS matrix (extracellular polysaccharides, etc.)

$$\begin{aligned}
\frac{dP}{dt} &= \mu_P P \left(1 - \frac{P}{P_{max}}\right) - \underbrace{k_{attach} \cdot f_{QS}(c) \cdot P}_{\text{QS-regulated attachment}} + \underbrace{k_{detach} \cdot B}_{\text{detachment to planktonic}} - \delta_P P \\
\frac{dB}{dt} &= k_{attach} \cdot f_{QS}(c) \cdot P + \mu_B B \left(1 - \frac{B}{B_{max}}\right) - k_{detach} \cdot B - \delta_B B \\
\frac{dE}{dt} &= \underbrace{r_E \cdot f_{QS}(c) \cdot B}_{\text{QS-regulated EPS synthesis}} - \underbrace{\delta_E \cdot E}_{\text{natural EPS degradation}}
\end{aligned}$$

QS regulation function:

$$f_{QS}(c) = \frac{c^{n_{QS}}}{K_{QS}^{n_{QS}} + c^{n_{QS}}}$$

When PvdQ lowers environmental AHL c, $f_{QS}(c) \to 0$, leading to:

- reduced attachment of planktonic cells into biofilms
- reduced EPS synthesis
- collapse of biofilm steady state

6.3 Relationship Between Resistance and Biofilms

Model the biofilm-enhanced antibiotic resistance factor as:

$$\mathrm{MIC}_{effective} = \mathrm{MIC}_{planktonic} \cdot \left(1 + \phi \cdot \frac{E}{E + K_E}\right)$$

Where ϕ is the maximal enhancement factor (10–1000× per the design), and K_E is the EPS half-saturation constant.

6.4 Expected Outputs

- **Biofilm growth curves**: $B(t)$ with vs without quorum quenching
- **Combination therapy predictions**: quorum quenching + antibiotics killing efficiency
- **Therapeutic window**: required PvdQ level to reduce MIC to clinically achievable range

7. Model 6: Competitive-Substrate Kinetic Model for the Visualization Module

7.1 Purpose

All three visualization strategies rely on **PvdQ's competitive substrate preference** between natural AHL and artificial probes. This model quantifies competition and predicts the **critical AHL concentration** and **time window** for color change.

7.2 Competitive Michaelis–Menten Kinetics

PvdQ faces two substrates simultaneously (natural AHL and artificial Probe):

$$v_{AHL} = \frac{k_{cat,AHL} \cdot [\mathrm{PvdQ}] \cdot [\mathrm{AHL}]}{K_{m,AHL}\left(1 + \frac{[\mathrm{Probe}]}{K_{m,Probe}}\right) + [\mathrm{AHL}]}$$

$$v_{Probe} = \frac{k_{cat,Probe} \cdot [\mathrm{PvdQ}] \cdot [\mathrm{Probe}]}{K_{m,Probe}\left(1 + \frac{[\mathrm{AHL}]}{K_{m,AHL}}\right) + [\mathrm{Probe}]}$$

Key design principle: $K_{m,AHL} \ll K_{m,Probe}$ (PvdQ has much higher affinity for native AHL), therefore:

- At high AHL: $v_{AHL} \gg v_{Probe}$, probe remains intact → colorless
- At low AHL: v_{Probe} increases significantly → dye release

7.3 Scenario-Specific Parameters

Scenario	Probe type	Pigment product	Readout
Wound	BpsA substrate (glutamine) + cI regulatory switch	Indigoidine	Dressing turns blue
Lung	Sulfonated indoxyl probe	Indigo Carmine	Blue sputum
Bloodstream	PEG-shielded prodrug probe	Resorufin	Red urine

Wound specialization: includes cI repressor regulation; couple cI degradation dynamics:

$$\frac{d[\mathrm{cI}]}{dt} = \alpha_{cI} \cdot \frac{[\mathrm{AbaR{:}AHL}]^n}{K_d^n + [\mathrm{AbaR{:}AHL}]^n} - \underbrace{(\gamma_{cI} + \gamma_{ssrA})}_{\text{intrinsic + ssrA-tag accelerated degradation}} \cdot [\mathrm{cI}]$$

BpsA expression repressed by cI:

$$\frac{d[\mathrm{BpsA}]}{dt} = \alpha_{BpsA} \cdot \frac{K_{R,cI}^{n_R}}{K_{R,cI}^{n_R} + [\mathrm{cI}]^{n_R}} - \gamma_{BpsA}[\mathrm{BpsA}]$$

Bloodstream specialization: PEG shielding removal is cleaved by CRAB biofilm-associated enzyme CpaA:

$$\frac{d[\mathrm{Probe}_{active}]}{dt} = k_{CpaA} \cdot [\mathrm{CpaA}] \cdot [\mathrm{Probe}_{PEG}] - v_{Probe}$$

7.4 Expected Outputs

- **AHL vs pigment output dose–response curves** to determine the “color-change threshold”
- **Treatment timeline**: quantify time delay from AHL decrease to color-change trigger
- **Effect of cI ssrA tag on response speed**: parameter scan of ssrA degradation rate in the wound scenario

8. Model 7: Timed Clearance Model for the MazEF Kill Switch

8.1 Purpose

Predict the kill switch **timed clearance window** (time to arabinose depletion) and **emergency clearance response time** (kill rate after doxycycline induction), ensuring engineered bacteria survive during therapy and die completely afterward.

8.2 ODE Model

Arabinose metabolism:

$$\frac{d[\mathrm{Ara}]}{dt} = -\frac{V_{max,Ara} \cdot [\mathrm{Ara}]}{K_{m,Ara} + [\mathrm{Ara}]} \cdot N_{EcN}$$

MazE (antitoxin) expression:

$$\frac{d[\mathrm{MazE}]}{dt} = \alpha_{MazE} \cdot \frac{[\mathrm{Ara}]^{n_A}}{K_{Ara}^{n_A} + [\mathrm{Ara}]^{n_A}} - \gamma_{MazE}[\mathrm{MazE}] - k_{bind}[\mathrm{MazE}][\mathrm{MazF}]$$

MazF (toxin) expression:

$$\frac{d[\mathrm{MazF}]}{dt} = \underbrace{\alpha_{MazF,const}}_{\text{weak constitutive}} + \underbrace{\alpha_{MazF,Dox} \cdot \frac{[\mathrm{Dox}]^{n_D}}{K_{Dox}^{n_D} + [\mathrm{Dox}]^{n_D}}}_{\text{doxycycline induction}} - \gamma_{MazF}[\mathrm{MazF}] - k_{bind}[\mathrm{MazE}][\mathrm{MazF}]$$

MazEF complex (inactive):

$$\frac{d[\mathrm{MazE{:}MazF}]}{dt} = k_{bind}[\mathrm{MazE}][\mathrm{MazF}] - \gamma_{EF}[\mathrm{MazE{:}MazF}]$$

Cell survival:

$$\frac{dS}{dt} = -k_{kill} \cdot \max\left([\mathrm{MazF}]_{free} - [\mathrm{MazF}]_{threshold},\, 0\right) \cdot S$$

Where $[\mathrm{MazF}]_{free} = [\mathrm{MazF}] - [\mathrm{MazE{:}MazF}]$ (killing is triggered when free MazF exceeds the threshold).

8.3 Key Design Parameters

Parameter	Design requirement	Constraint source
Initial $[\mathrm{Ara}]_0$	premixed concentration in hydrogel	ensure sufficient MazE during therapy (e.g., 24 h)
γ_{MazE}	MazE is naturally unstable	MazE half-life ~ 30 min (literature)
γ_{MazF}	MazF is more stable	MazF half-life ~ 2–4 h
$\alpha_{MazF,const}$	weak constitutive “baseline toxin”	cannot be too high to avoid premature killing
RBS strength (MazE)	mentioned as optimization target	ensure MazE > MazF under normal conditions

8.4 Simulation Scenarios

Scenario A: automatic timed clearance

- Initial: sufficient $[\mathrm{Ara}]_0$, $[\mathrm{Dox}] = 0$
- As time progresses, arabinose is metabolized
- Plot: $[\mathrm{Ara}](t)$, $[\mathrm{MazE}](t)$, $[\mathrm{MazF}]_{free}(t)$, $S(t)$
- **Key outputs**: survival window $T_{survival}$ and killing half-life $t_{1/2,kill}$

Scenario B: emergency doxycycline clearance

- Add doxycycline at $t = t_{emergency}$
- MazF rapidly upregulates and overwhelms residual MazE
- **Key output**: time from dosing to 99% killing $T_{99\%}$

Scenario C: parameter robustness

- Scan $[\mathrm{Ara}]_0$ and α_{MazE} (RBS strength)
- Plot “safe operating space” heatmap: x-axis survival window, y-axis killing reliability

8.5 Expected Outputs

- **Arabinose decay curve** and corresponding **cell survival** trajectory
- **Safe operating space plot** to guide hydrogel arabinose premix concentration
- **Emergency clearance time** to validate doxycycline override response speed

9. Model 8: pH-Sensitive Liposome Pulmonary Drug Release Model

9.1 Purpose

The design states that pH-sensitive liposomes (CHEMS/DOPE) remain stable in healthy lung tissue (pH 7.0–7.4) but rapidly destabilize and release drug in the acidic microenvironment of CRAB infection sites (pH 5.5–6.5). This model quantifies the **pH-**

dependent release rate.

9.2 Model Equations

Liposome membrane stability vs pH:

CHEMS protonation determines membrane stability. Let liposome integrity be $I(t) \in [0, 1]$:

$$\frac{dI}{dt} = -k_{release}(\mathrm{pH}) \cdot I$$

Model the pH dependence of $k_{release}$ as a sigmoidal function:

$$k_{release}(\mathrm{pH}) = k_{max} \cdot \frac{1}{1 + 10^{n_{pH}(\mathrm{pH} - \mathrm{pH}_{crit})}}$$

- pH_{crit}: critical pH (~6.0–6.5, near CHEMS pKa)
- n_{pH}: transition steepness
- k_{max}: maximal release rate constant

PvdQ release dynamics:

$$\frac{d[\mathrm{PvdQ}]_{released}}{dt} = k_{release}(\mathrm{pH}) \cdot I(t) \cdot [\mathrm{PvdQ}]_{encap,0}$$

9.3 Spatial Distribution Model

Combine lung anatomy with a 1D reaction–diffusion model (from airway center to wall):

$$\frac{\partial [\mathrm{PvdQ}]}{\partial t} = D_{PvdQ} \frac{\partial^2 [\mathrm{PvdQ}]}{\partial r^2} + \mathrm{Source}(r, \mathrm{pH}(r))$$

Where $\mathrm{pH}(r)$ is acidic near CRAB biofilms (inner layer) and gradually returns to neutral toward the airway lumen.

9.4 Expected Outputs

- **pH–release curves**: PvdQ release time course at different pH values
- **Therapeutic efficiency comparison**: pH-sensitive vs conventional liposomes in healthy vs infected tissue (selective release ratio)
- Recommendations for optimal liposome composition parameters

10. Model 9: Diffusion Model for Core–Shell Microcarriers in Wound Hydrogels

10.1 Purpose

The dual-layer hydrogel system must ensure:

1. External AHL can **diffuse in** to the core–shell microcarrier and trigger the gene circuit
2. Produced PvdQ and pigments can **diffuse out** to function
3. Engineered bacteria (1–2 μm) are **physically retained** and cannot escape

This model quantifies mass transfer in the core–shell structure.

10.2 Modeling Framework

Spherically symmetric diffusion model (three-layer structure):

$$\frac{\partial C_i}{\partial t} = \frac{1}{r^2}\frac{\partial}{\partial r}\left(D_{i,j} \cdot r^2 \frac{\partial C_i}{\partial r}\right) + R_i(r)$$

Where i denotes different species (AHL, PvdQ, pigment), and j denotes different regions:

Region	Radius range	Diffusion coefficient	Reaction term
Core (Ca-alginate + EcN)	$0 < r < R_{core}$	$D_{i,core}$	gene circuit expression
Shell (QCS–alginate PEC)	$R_{core} < r < R_{shell}$	$D_{i,shell}$ (dense membrane; low permeability for macromolecules)	none
External hydrogel matrix	$r > R_{shell}$	$D_{i,gel}$	none

Size selectivity:

Model the effective MWCO of the PEC shell via:

$$D_{i,shell} = D_{i,0} \cdot \exp\left(-\lambda \cdot \frac{R_{H,i}}{R_{pore}}\right)$$

- $R_{H,i}$: hydrodynamic radius of species i
- R_{pore}: pore size of the PEC membrane
- AHL ($M_W \approx 300$ Da, $R_H \sim 0.5$ nm) → passes freely
- PvdQ ($M_W \approx 80$ kDa, $R_H \sim 3$ nm) → passes slowly (needs validation for sufficient diffusion)
- EcN ($\sim 1-2$ μm) → fully retained

10.3 Expected Outputs

- **AHL penetration time** from outside to core to ensure sensing is not overly delayed
- **PvdQ release kinetics** from core to outside to evaluate onset of therapeutic action
- **Bacterial escape risk assessment**: quantitative validation of PEC membrane retention for micron-scale particles

Key Performance Indicators (KPIs)

KPI	Definition	Target
Sensing sensitivity	minimum $[\mathrm{AHL}_{ext}]$ to trigger the system	< 100 nM
Response time t_{90}	time for PvdQ to reach 90% steady state	< 2 h
AHL clearance time t_{clear}	time for AHL to drop below QS threshold	< 6 h
Hysteresis window Δ	difference between maintenance and activation thresholds	> 50 nM

KPI	Definition	Target
Targeting enrichment factor TEF	local concentration enhancement due to chemotaxis	> 5×
Color-change delay	time from treatment completion to color change	< 1 h
Self-destruction window $T_{survival}$	engineered bacteria survival time	24–72 h (tunable)
Emergency clearance time $T_{99\%}$	time to 99% killing after Dox	< 4 h