Mechanistic Model Demonstrates Importance of Autocrine IL-8 Secretion by Neutrophils

Wangui Mbuguiro[1,2] & **Feilim Mac Gabhann**[1,2]

[1] Department of Biomedical Engineering, Johns Hopkins University & School of Medicine, Baltimore, MD, USA;
[2] Institute for Computational Medicine, Johns Hopkins University, Baltimore, MD, USA

Summary

IL-8 (CXCL8) is a chemoattractant and pro-angiogenic factor that promotes both disease & homeostasis throughout the body, largely through neutrophil activation.

Endometriosis – Neutrophil recruitment, macrophage activation, angiogenesis, proliferation [4]
Ovulation – neutrophil recruitment (folliculogenesis) [5]

Irritable Bowel Disease – Neutrophil recruitment (cytokine release)
Protection – Neutrophil migration (antimicrobials), angiogenesis

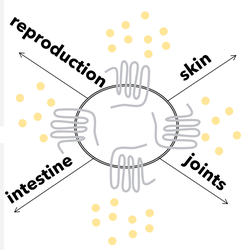


Figure adapted from [3]

Psoriasis – Keratinocyte activation, neutrophil recruitment
Wound Healing – Skin fibrosis & scar formation

Arthritis – Neutrophil recruitment, angiogenesis, pain
Pain Reduction – Neutrophil recruitment (endogenous opioids)

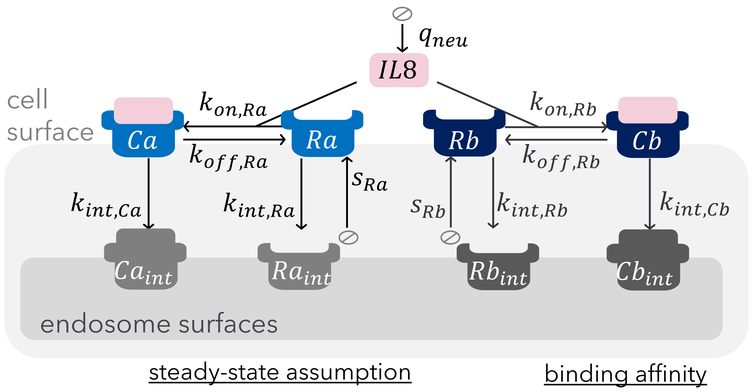
Model Construction: We create a computational model to characterize the regulation of receptors on neutrophils by IL-8. This model predicts changes to proteins based on **mechanisms** (i.e. the combined effects of processes on receptors and ligands on neutrophils).

Results: Through modeling and simulating experiments from a previous study, we identify: neutrophil IL-8 secretion rate (11 molecules/cell/s), receptor internalization rates (0.7–5*10^{-4} s^{-1}), and unbinding rates (10^{-4} s^{-1}). This secretion rate is consistent with published estimates [1,2], and the latter rates have not been previously reported. Our modeling predicts that this rate of neutrophil secretion can decrease the level of IL-8RB available on neutrophils by up to 10% in pM-range environments.

Future Work: This model can be expanded to include additional cell types and processes in order to explore IL-8 signaling in blood and tissues.

Model Construction

Neutrophil IL-8 signaling occurs through two G-protein coupled receptors: IL-8RA (CXCR1) and IL-8RB (CXCR2). We model IL-8 secretion, binding to and unbinding from receptors, and receptor trafficking to and from the cell surface using 9 coupled ordinary differential equations with 11 parameters (rate constants). These equations predict how many receptors are available (Ra and Rb) and bound to IL-8 (Ca and Cb) on neutrophils, and how many are internalized ($Ra_{int}, Rb_{int}, Ca_{int}, Cb_{int}$) over time.



steady-state assumption

$s_{Ra} = k_{int,Ra}[Ra]_{t=0}$
$s_{Rb} = k_{int,Rb}[Rb]_{t=0}$

binding affinity

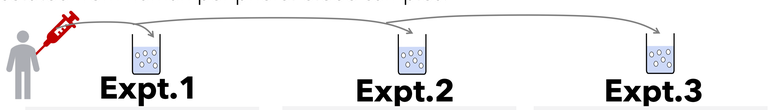
$K_D = \frac{k_{off,R}}{k_{on,R}}$ $K_{D,Ra} = 168\ pM$, $K_{D,Rb} = 31\ pM$ ($K_D's$ from [2])

Example Equation:

$$\frac{d}{dt}[Ra(t)] = +s_{Ra} - k_{on,Ra}\,[IL8(t)][Ra(t)] + k_{off,Ra}\,[Ca(t)] - k_{int,Ra}[Ra(t)]$$

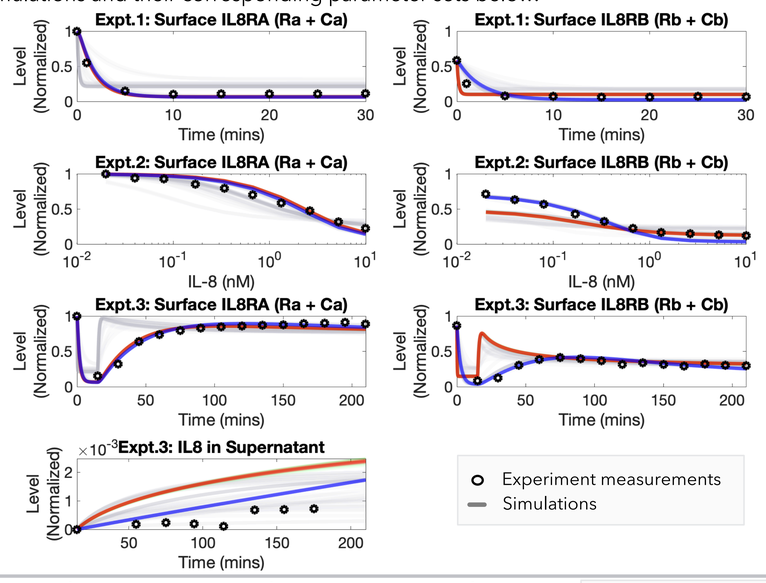
Overview of Experimental Data

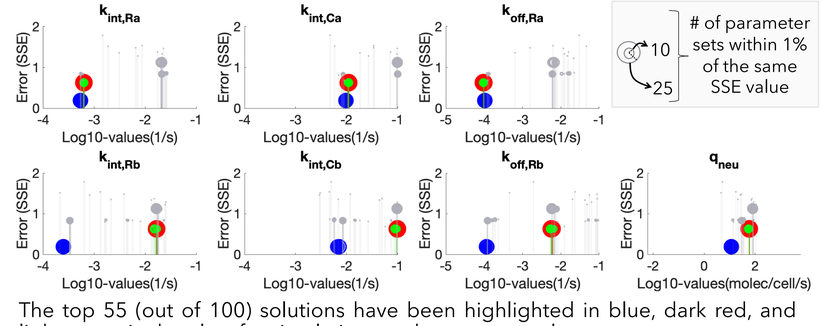
We constructed and parametrized our model using data from experiments done by Chuntharapai & Kim [2]. These experiments were conducted using neutrophils isolated from human peripheral blood samples.



Parameter Optimization

After constructing the model equations, we performed optimization to identify the unknown parameters. We used an optimization solver in MATLAB to find parameter sets that minimize the sum of squared error **(SSE)** between measurements from the published experiments and model simulations. We performed optimization using 100 different initial parameter guesses. We then plotted the resulting 100 optimal simulations and their corresponding parameter sets below.

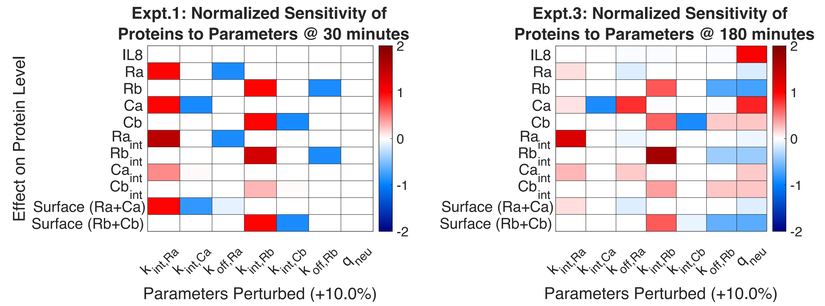




The top 55 (out of 100) solutions have been highlighted in blue, dark red, and light green in the plots for simulations and parameter values.

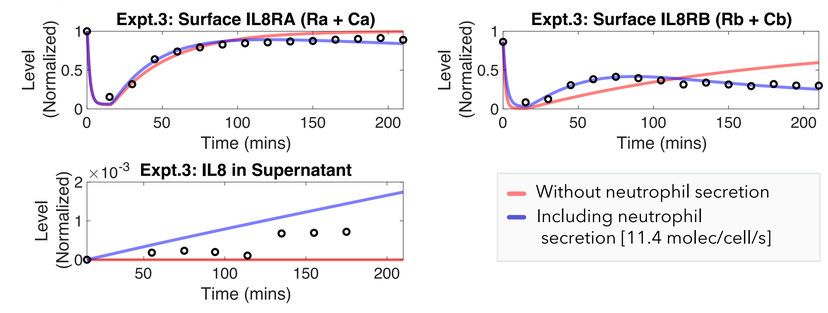
Sensitivity of Proteins to Processes

To assess how responsive each protein is to individual process rates (parameters), we did univariate sensitivity analysis. This showed that autocrine IL-8 secretion can affect the levels of all proteins in the system. Sensitivity was calculated and normalized, such that a value of "+1" means a 10% increase to a parameter caused a 10% increase in protein level (relative to the level for the original parameter value).

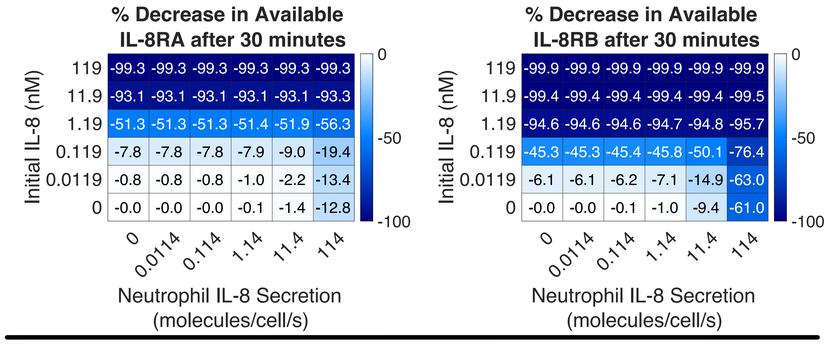


Role of Secretion

To explore the significance of IL-8 secretion, we constructed an alternative model where no IL-8 secretion can occur ($q_{neu} = 0$), but all other processes are present. We then performed 100 optimizations as described. The best optimization for this model fit experiments 1 & 2 well (not shown here) but not experiment 3.



We simulated experiment 1, varying two conditions: (1) the initial level of IL-8 in culture, and (2) the ability of neutrophils to secrete IL-8. These simulations predict that in environments with 0~1 nM IL-8, IL-8 secretion by neutrophils will affect the level of unbound IL-8RB on the surface of neutrophils, with little effect on IL-8RA.



References:
[1] Fujishima S, Hoffman AR, Vu T, Kim KJ, Zheng H, Daniel D, et al. *J Cell Physiol*. 1993;154(3).
[2] Chuntharapai A, Kim KJ. *J Immunol*. 1995;155(5):2587–94.
[3] Russo RC, Garcia CC, Teixeira MM, Amaral FA. *Expert Rev Clin Immunol*. 2014;10(5):593–619.
[4] Ahn SH, Monsanto SP, Miller C, Singh SS, Thomas R, Tayade C. *Biomed Res Int*. 2015;2015(May):1–12.
[5] Garcia-Velasco JA, Arici A. *Fertil Steril*. 2002;22(3):567–83.

Acknowledgments: This work is supported by the National Science Foundation Graduate Research Fellowship Program. Grant No. DGE1746891 (to WM).