Biological Rule-Based Modeling of Experimental Cell Secretion Data

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***Abstract*—The allergic response in humans results from the crosslinking of IgE-FcϵRI receptor complexes via the binding of IgE antibodies to antigens, which results in cell degranulation. The relationship between cell degranulation and antigen-antibody aggregation was investigated for the shrimp allergen Pen a 1. A biological rule-based model was developed to simulate aggregation of IgE antibodies and the Pen a 1 antigen. The forward rate constant and the crosslinking factor were varied to demonstrate how the model output changes as these model parameters are changed. It was found that the peak of the dose-response curve becomes greater and more defined as the crosslinking factor increases, and that the peak shifts to lower doses as the forward rate constant increases. Parameter scanning was performed to fit the model output to experimental cell secretion data obtained by collaborators, assuming a directly proportional relationship between the two quantities. Four concentrations of allergen-specific IgE were examined: 15 ng/mL, 30 ng/mL, 60 ng/mL, and 120 ng/mL. For each IgE concentration, nine doses of Pen a 1 were examined ranging from 0.0001 ng/mL to 10,000 ng/mL. The average aggregate size was used as the measure of aggregation to compare to the experimental data. It was found that the output of the biological rule-based model fit well to the cell degranulation data.**

***Index Terms*—biological cells, biological processes, biological system modeling, computational biophysics, computational systems biology**

I. Introduction

The allergic response in humans is at least partially initiated by a tyrosine kinase cascade that results from the crosslinking of FcϵRI receptors via allergen-specific IgE antibodies binding to antigens. The IgE antibodies are bound to the receptors. These receptors are located on the surface of human basophils and mast cells. Cell degranulation results in allergy symptoms. Hence, studying the processes of cell degranulation and antigen-antibody aggregation is important for understanding the allergic response.

Computational methods are widely used to study biomolecular interactions due to their complexity. One example of such a method is molecular dynamics, which is useful for simulating atomic motion but is computationally costly for studying longer timescales (on the order of seconds or longer) at which cell signaling events occur [1]. Other methods, including constraint-based modeling and Boolean modeling, tend to only loosely be connected to physicochemical principles (or not at all). Models which are constrained by physicochemical principles are useful due to causality and the ability to measure parameters independently. Models that allow the incorporation of site-specific details and that can overcome the problem of combinatorial complexity are also highly useful for biomolecular simulations.

One technique that meets all of the above requirements is biological rule-based modeling. Biological rule-based modeling is a technique for studying the site dynamics of biomolecular networks [1], which involves representing biomolecular interactions as local rules. With this method, a set of rules, each representing a set of possible reactions and associated with a reaction rate, is specified. For traditional rule-based models, a reaction network is created during simulation from which a set of coupled ordinary differential equations (ODEs) is derived. These equations characterize the rates of change of observables (such as chemical species).

However, the biological rule-based simulator NFsim is stochastic and particle-based [2]. NFsim models individual molecules as particle objects, which are tracked throughout the simulation. NFsim was designed to efficiently model systems with a large number of possible molecular states and interactions. One major advantage of NFsim is its ability to model reaction types, such as aggregation and polymerization, that cannot be modeled using traditional ODE or stochastic simulators. NFsim achieves this because its runtime performance scales with the number of rules rather than the number of reactions or configurations. NFsim models are specified in the BioNetGen Language (BNGL). NFsim was employed to develop the biological rule-based models used in this study.

The researcher used biological rule-based modeling combined with experimental cell degranulation data obtained from collaborators to study the allergic response caused by the shrimp allergen tropomyosin. Shellfish are responsible for many food allergies, and most shellfish species responsible for allergic reactions are crustaceans. Tropomyosin is a protein

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present in all eukaryotic cells; it is an important allergen found in many crustaceans, including crab (Cha f 1), squid (Tod p 1), lobster (Pan s 1, Hom a 1), oyster (Cra g 1), and snail (Tur c 1) [3]. Although there are strong similarities between the various types of tropomyosin, the tropomyosins found in vertebrates are often non-allergenic, and the reason for this difference is not fully understood. There is some evidence that differences between IgE binding regions in vertebrate and invertebrate tropomyosins may play a role [4]. Pen a 1 is a type of tropomyosin molecule found in shrimp. Previous studies have found that tropomyosin elicits an allergic response in at least 80% of shrimp-allergic subjects, and that tropomyosin binds about 75% of shrimp-specific IgE [3].

Currently, experimental data relating to aggregate size and structure, and the effect of the flexibility of the Pen a 1 molecule on aggregation, is not yet readily available. However, a theoretical model can be created that fits a set of cell degranulation data to gain insight into the relationship between the processes of degranulation and antigen-antibody aggregation.

The researcher modeled the experimental dose-response data using a simple rule-based model written in the BNGL language and implemented in the stochastic simulator NFsim in which free allergens are captured by receptors and receptors are cross-linked by Pen a 1 molecules. The "dose" in these experiments refers to the concentration of Pen a 1, and the "response" refers to the percent secretion corresponding to each dose of Pen a 1.

II. Contributions

The contributions of this work are as follows:

1. A biological rule-based model was developed for simulating the process of IgE-FcϵRI receptor cross-linking via the binding of the IgE antibodies to the shrimp allergen Pen a 1.

2. A measure of aggregation was identified for the rule-based model that fits the experimental cell degranulation data well, assuming a directly proportional relationship between degranulation and aggregation.

3. The effect of changing the parameters of the rule-based model on the model output was demonstrated for various doses of Pen a 1.

4. Parameter scanning was used to fit the output of the rule-based model to the experimental cell degranulation data provided by our collaborators.

III. Related Work

A. Rule-Based Modeling

Biological signaling systems are often comprised of macromolecules that can exist in a large number of functionally distinct states. This number scales exponentially with the amount of modification possibilities [5]. One problem that arises when modeling these systems is the specification problem, i.e. how to specify such a large system.

One solution is implicit specification, which involves the coarse-graining of sets of reactions and parameters into rules; the only explicitly specified features in a reaction rule are those which affect the reaction. Rules define the conditions for molecular transformations and interactions, and are associated with rate laws [1]. Some rules define multiple reactions, which means that all of these reactions are associated with the same rate law. The rules can usually be specified independently. Rule-based specification methods include Kappa-calculus [6], BioNetGen [7], ANC [8], and ML-Rules [9]. The Simmune project and the SSC allow the specification of molecules within spatial regions of arbitrary geometries [10].

The rule-based methods can be population-based, particle-based, or hybrid. Population-based methods include ODE/PDE numerical integration as well as the stochastic Gillespie algorithm. In these methods, the application of a rule changes the size of one of the populations, each of which consists of all molecules that share the same state and same species. The system state space can be very large, so methods to reduce it have been introduced [5].

Particle-based rule evaluation involves tracking individual particles (molecules and molecular complexes) through the simulation [1]. This is a network-free method; at any time point, only the existing particles, their states, and the possible reactions for the existing particles are necessary. Spatial particle-based methods include an explicit specification of space, and include SRSim [11] and MCell [12].

Rule-based modeling was used to model cell degranulation in response to Pen a 1 in another work [13]. The differences between these models are described in Section V-B. Also, this study is different from the previous study in its inclusion of an analysis of how the model parameters affect the model output.

B. Experimental Methods

Nanoprobe labeling and transmission electron microscopy (TEM) of cell membranes are used to study aggregation. Methods for the spatial analysis of these nanoprobes, including statistical analysis of clustering, were developed [14]. Quantum dot (QD)-IgE probes that bind FcϵRI have been used to study the mobility of receptors by single-particle tracking [15]. The kinetics of DNP-BSA binding to IgE has been studied by observing fluorescence quenching [16], and the data was analyzed using a mathematical model in which the IgE binding sites are transiently exposed, allowing binding and cross-linking to occur.

IV. Method

A. Cell Degranulation Experiments

Experiments were performed by collaborators [13] to determine the percent secretion of cells in response to the presence of Pen a 1. In these experiments, the shrimp allergen Pen a 1 is used to initiate receptor cross-linking and stimulate degranulation. Receptor cross-linking refers to two or more antibody-receptor complexes binding to a single antigen molecule. Degranulation is the secretion of chemicals such as histamine from the granules (secretory vesicles) of a cell. The "dose" in these experiments refers to the concentration of Pen a 1, and the "response" refers to the percent secretion corresponding to the dose of Pen a 1.

The experiments were conducted in wells each having 0.32 cm^2 of surface area. The cells used in the experiment were human RBL cells created using gene editing. Initially, approximately 34,000 cells were plated the day prior to experiment. The number of cells per well at the time of experiment was measured in two cell count experiments; the average of these two values is 43,063 and is the number used in our calculations of the number of molecules per cell. The volume of media, which contained the allergen-specific IgE, per well was 100 μL.

For each experiment, the cells were primed with allergen-specific IgE for two hours, and Pen a 1 molecules were added at various concentrations. Experiments were performed in which each well contains 15 ng/mL, 30 ng/mL, 60 ng/mL, and 120 ng/mL of tropomyosin specific IgE. For each IgE concentration, the concentration of Pen a 1 molecules was varied from 0.0001 ng/mL to 10,000 ng/mL.

The percent secretion was measured at each order of magnitude of the Pen a 1 concentration. The *percent secretion* is defined as the amount of secretion observed divided by the total secretion possible and multiplied by 100. The total secretion possible is the total amount of secretory granule inside of the cells, which was measured by lysing the cells and measuring the total granule content. Secretion is measured by observing the number of secretory granules released by cells in response to cross-linking of cell receptors.

B. Biological Rule-Based Model

The researcher applied a biological rule-based model written in the BioNetGen Language (BNGL) and implemented in the stochastic simulator NFsim to analyze a set of data obtained from cell degranulation experiments. (The code for this model is presented in the Appendix of this paper.) After performing initial tests of a number of aggregation measures, the researcher determined that one measure, the *average aggregate size*, fits well to the experimental dose-response curve. The researcher defines the *average aggregate size* as the total number of receptors in aggregates divided by the total number of aggregates, where an *aggregate* is defined as any cluster of bound molecules containing two or more receptors.

The researcher used a model in which Pen a 1 has six binding sites that are specific to IgE. Since Pen a 1 has five main binding regions per strand, a six-site model implies that only three of those binding regions are being consistently bound. This may be due to the other two binding regions being less IgE-specific or steric effects causing blockage of other available binding sites. It should be noted that earlier studies of Pen a 1 aggregate size distributions using a 3D rigid-body Monte Carlo method yielded six as the aggregate size with the greatest probability of occurrence at full model resolution for three energy-minimized conformations of Pen a 1 [17], [18], [19].

The researcher used a simple non-geometric method to model the experimental data in order to help develop intuition about antigen-antibody aggregate structure. The biological rule-based model contains a set of rules in which free allergens are captured by receptors and receptors are cross-linked by Pen a 1 molecules. In this model, it is assumed that each Pen a 1 molecule is a dimer with six binding sites, with no steric effects. There are three rules: (1) a rule in which a free (unbound) Pen a 1 molecule binds to a receptor, (2) a rule in which two receptors are cross-linked by a Pen a 1 molecule, and (3) a rule in which an allergen-IgE bond dissociates (see Table I).

TABLE I
TABLE SHOWING THE RULES AND ASSOCIATED RATE CONSTANTS FOR THE RULE-BASED MODEL ASSUMING SIX BINDING SITES PER PEN A 1 MOLECULE. T REPRESENTS A PEN A 1 MOLECULE, E REPRESENTS AN UNBOUND BINDING SITE ON THE PEN A 1 MOLECULE, AND IgE_E REPRESENTS A BINDING SITE BOUND TO AN IGE ANTIBODY.

Rule Number	Reaction Rule	Rate Constant
(1)	T(E,E,E,E,E,E) + IgE $\rightarrow$ T(IgE_E,E,E,E,E,E)	k_{f1}
(2)	T(IgE_E,E) + IgE $\rightarrow$ T(IgE_E,IgE_E)	k_{x1}
(3)	T(IgE_E) $\rightarrow$ T(E) + IgE	k_{r1}

The forward rate constant k_{f1}, which is the rate associated with the first rule, and the cross-linking factor $xfactor$, which is related to the rate associated with the second rule such that $k_{x1} = xfactor \cdot k_{f1}$, are used as free parameters in fitting. It should be noted that the binding sites on a Pen a 1 molecule become more accessible once the molecule binds to a receptor, as the act of binding anchors the molecule close to the cell surface and increases the probability that other binding sites on the Pen a 1 molecule will bind to other receptors. This explains why k_{x1} must be greater than k_{f1}. The dissociation rate k_{r1} is fixed at $KD1 \cdot k_{f1}$ s^{-1} where $KD1 = 10^{-9}M$.

In parallel with the cell degranulation experiments, the researcher modeled cells that were primed with 15 ng/mL, 30 ng/mL, 60 ng/mL, and 120 ng/mL of tropomyosin specific IgE. The number of IgE per cell was obtained from quantifying experimental fluorescence data using flow cytometry [13]. For each IgE concentration, the concentration of Pen a 1 molecules was varied from 0.0001 ng/mL to 10,000 ng/mL. Based on these concentrations and constant parameters derived from the cell degranulation experiments (see Table II for these parameter values), the number of Pen a 1 molecules per cell was calculated for use in the rule-based model (see Table III for the number of IgE used in this model and see Table IV for the number of Pen a 1 molecules used in this model).

TABLE II
TABLE SHOWING CONSTANT PARAMETERS FOR THE RULE-BASED MODEL.

Number of cells per well	43063
Volume of media per well	100 μL

For each Pen a 1 dose, 20 model runs were performed. A single cell or a fraction of a cell was simulated in each model run. Each run took 60 seconds of physical time, as preliminary tests showed that this amount of time is adequate for the system to reach equilibrium. One hundred time steps were used for each run. The computational time of each run

TABLE III
TABLE SHOWING THE CONCENTRATIONS OF IGE AND THE CORRESPONDING NUMBER OF IGE PER CELL FOR THE RULE-BASED MODEL.

IgE concentration (ng/mL)	Number of IgE per cell
15	324
30	716
60	1211
120	2692

TABLE IV
TABLE SHOWING THE CONCENTRATIONS OF PEN A 1 AND THE CORRESPONDING NUMBER OF PEN A 1 PER CELL FOR THE RULE-BASED MODEL.

Pen a 1 concentration (ng/mL)	Number of Pen a 1 per cell
0.0001	2
0.001	19
0.01	194
0.1	1943
1	19426
10	194257
100	1942567
1000	19425673
10,000	194256735

was on the order of seconds to minutes depending on the number of molecules in the model being simulated. The runs were performed on a laptop running the Windows 10 Home operating system with an Intel Core i7-7500U CPU at 2.70 GHz and 16 GB of RAM.

V. EXPERIMENTS, RESULTS, AND DISCUSSION

A. Varying Model Parameters

Firstly, the researcher wants to demonstrate how the curve of the biological rule-based model changes as the model parameters vary. To this end, the researcher performed two experiments: one experiment in which $xfactor$ is varied while k_{f1} is constant, and one experiment in which k_{f1} is varied while $xfactor$ is constant. It should be noted that, in the figures presenting the dose-response curves, continuous lines were added to the data points for the purpose of visualizing the trends in the data.

For the experiment in which $xfactor$ is varied, the researcher tested a model with 120 ng/mL allergen-specific IgE and $k_{f1} = 10^6 M^{-1}s^{-1}$. The researcher ran the model for three different values of $xfactor$: 1000, 10000, and 100000. The resulting dose-response curves are shown in Fig. 1. It can be observed that the peak of the curve grows higher and more defined as $xfactor$ increases. The location of the peak along the x-axis remains the same if only $xfactor$ changes.

For the experiment in which k_{f1} is varied, the researcher tested a model with 120 ng/mL allergen-specific IgE and $xfactor$ = 10000. The researcher ran the model for two different values of k_{f1}: $10^6 M^{-1}s^{-1}$ and $10^9 M^{-1}s^{-1}$. The resulting dose-response curves are shown in Fig. 2. It can be observed that the peak of the curve shifts to lower doses as k_{f1} increases. It can also be observed that the bell-shaped portion of the curve grows wider as k_{f1} increases.

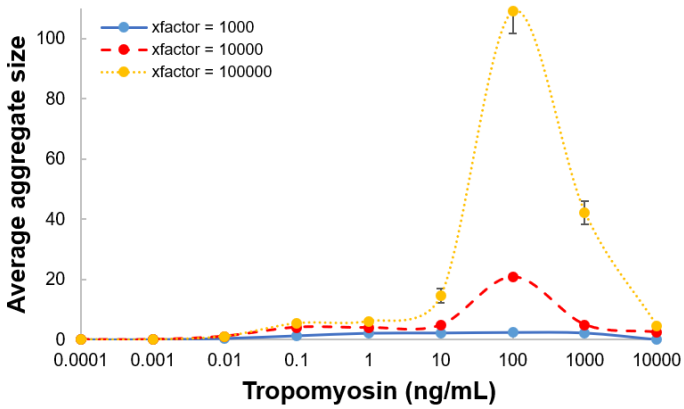

Fig. 1. Average aggregate size generated by the rule-based model for $xfactor = 1000$ (solid blue line), $xfactor = 10000$ (dashed red line), and $xfactor = 100000$ (dotted yellow line) with the tropomyosin concentration on the x-axis, for 120 ng/mL allergen-specific IgE and $k_{f1} = 10^6 M^{-1}s^{-1}$. The error bars represent the standard error for 20 runs of NFsim.

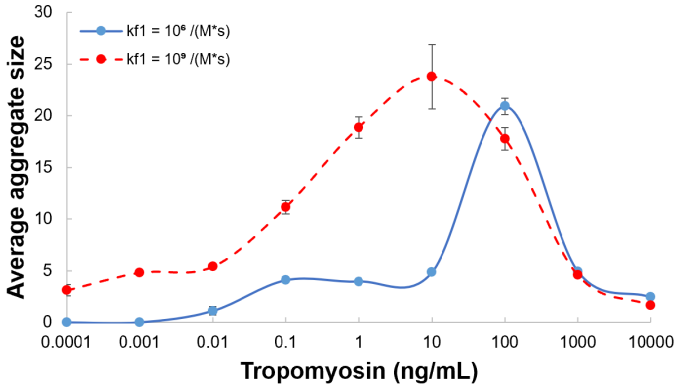

Fig. 2. Average aggregate size generated by the rule-based model for $k_{f1} = 10^6 M^{-1}s^{-1}$ (solid blue line) and $k_{f1} = 10^9 M^{-1}s^{-1}$ (dashed red line) with the tropomyosin concentration on the x-axis, for 120 ng/mL allergen-specific IgE and $xfactor = 10000$. The error bars represent the standard error for 20 runs of NFsim.

B. Fitting Model to Experimental Data

For simplicity, the researcher made the assumption that the measure of aggregation used in this model, namely the average aggregate size, is directly proportional to the experimental cell degranulation data. The researcher sought to find fitting parameters for the model that would result in a reasonable fit of the model output to the experimental cell degranulation data. To this end, the researcher tested $xfactor$ values of 1, 10, 100, 1000, 10000, and 100000, and k_{f1} values of 1, 10, 100, 10^6, and $10^9 M^{-1}s^{-1}$. For the fitting tests, the researcher fixed the allergen-specific IgE concentration at 120 ng/mL. The researcher found that $k_{f1} = 10^9 M^{-1}s^{-1}$ and $xfactor = 10000$ yielded the best fit of the model output to experimental data. The researcher used these model parameters to generate the model output for three other allergen-specific IgE concentrations: 60 ng/mL, 30 ng/mL, and 15 ng/mL.

It should be noted that a fraction of a cell, rather than a whole cell, was modeled for some runs due to computational

TABLE V
TABLE SHOWING THE CELL FRACTION USED IN THE RULE-BASED MODEL FOR EACH ALLERGEN-SPECIFIC IGE AND PEN A 1 CONCENTRATION.

Pen a 1 Concentration (ng/mL)	IgE Concentration (ng/mL)			
	120	60	30	15
0.0001	1.0	1.0	1.0	1.0
0.001	1.0	1.0	1.0	1.0
0.01	0.5	1.0	1.0	1.0
0.1	0.1	1.0	1.0	1.0
1	0.1	0.5	1.0	1.0
10	0.1	0.5	1.0	1.0
100	0.1	0.5	0.5	0.5
1000	0.05	0.05	0.05	0.05
10 000	0.005	0.005	0.005	0.005

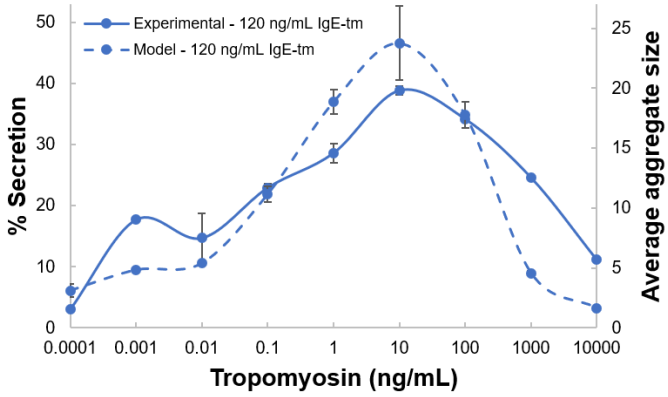

Fig. 3. Average aggregate size generated by the rule-based model (dashed lines) plotted with the experimental percent secretion curves (solid lines) with the tropomyosin concentration on the x-axis, for 120 ng/mL allergen-specific IgE. For this model, $k_{f1} = 10^9 M^{-1}s^{-1}$ and $xfactor = 10000$. The error bars for the model represent the standard error for 20 runs of NFsim. The error bars for the experimental data represent the standard deviation for two experiments.

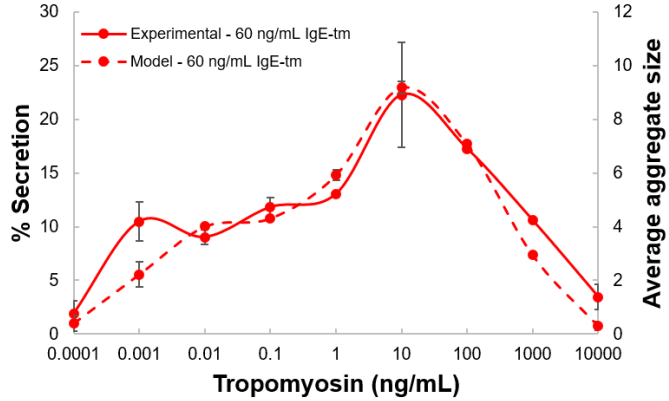

Fig. 4. Average aggregate size generated by the rule-based model (dashed lines) plotted with the experimental percent secretion curves (solid lines) with the tropomyosin concentration on the x-axis, for 60 ng/mL allergen-specific IgE. For this model, $k_{f1} = 10^9 M^{-1}s^{-1}$ and $xfactor = 10000$. The error bars for the model represent the standard error for 20 runs of NFsim. The error bars for the experimental data represent the standard deviation for two experiments.

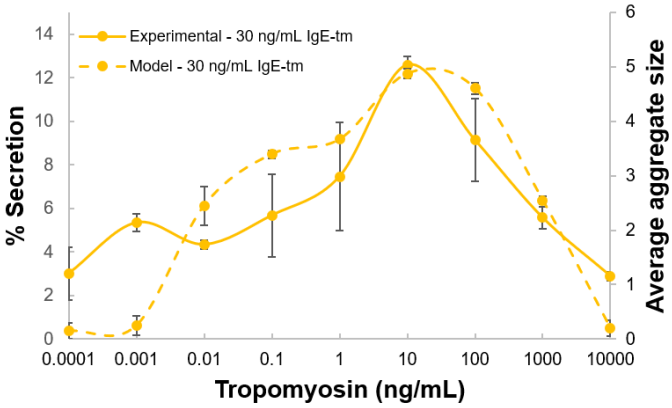

Fig. 5. Average aggregate size generated by the rule-based model (dashed lines) plotted with the experimental percent secretion curves (solid lines) with the tropomyosin concentration on the x-axis, for 30 ng/mL allergen-specific IgE. For this model, $k_{f1} = 10^9 M^{-1}s^{-1}$ and $xfactor = 10000$. The error bars for the model represent the standard error for 20 runs of NFsim. The error bars for the experimental data represent the standard deviation for two experiments.

limitations. For ease of reproducibility, the cell fraction used for each model is displayed in Table V. (A whole cell corresponds to a cell fraction of 1.0.)

The researcher plotted the model output and experimental data on the same plot for comparison. (See Fig. 3 for 120 ng/mL allergen-specific IgE, Fig. 4 for 60 ng/mL allergen-specific IgE, Fig. 5 for 30 ng/mL allergen-specific IgE, and Fig. 6 for 15 ng/mL allergen-specific IgE.)

From these plots, it can be observed that the rule-based model output correlates well with experimental data in regards to the non-zero values of the percent secretion at low doses of tropomyosin, which is an improvement over previous work, which predicted zero secretion at the lowest tropomyosin doses. The model also generally captures the shape of the entire experimental data curve. Specifically, the model correctly predicts that the percent secretion curve gradually rises to its peak from a non-zero value as the tropomyosin dose increases before tapering off. In contrast, the model used in previous work [13] predicts that the curve rises more rapidly to its peak from a value of zero. In particular, the model output for 60 ng/mL allergen-specific IgE corresponds excellently with experimental data (see Fig. 4). It should be noted that the model output for 15 ng/mL allergen-specific IgE does not seem to fit the experimental data as closely as the other models (see Fig. 6). However, the error of the experimental data is particularly large for the 15 ng/mL IgE concentration relative to the model output, meaning there is high variability between the results of the two replicate experiments relative to the model output.

These findings indicate that the measure of aggregation used in this model, namely the average aggregate size measured at equilibrium, may correspond with cell degranulation behavior more closely than the measure used in previous work, namely the time-averaged number of IgE receptors in aggregates. At the very least, analyzing the average aggregate size at equilibrium may provide us with another clue to understanding

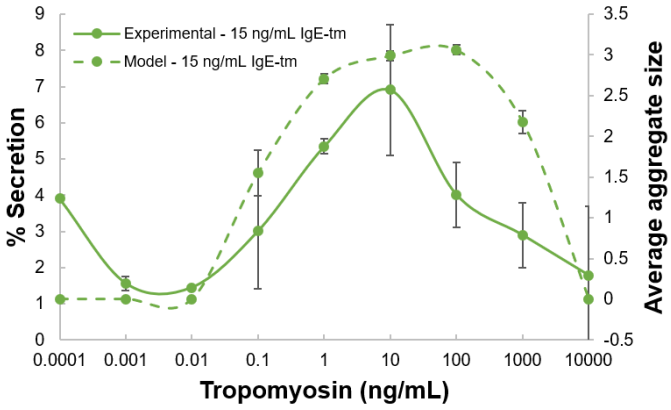

Fig. 6. Average aggregate size generated by the rule-based model (dashed lines) plotted with the experimental percent secretion curves (solid lines) with the tropomyosin concentration on the x-axis, for 15 ng/mL allergen-specific IgE. For this model, $k_{f1} = 10^9 M^{-1} s^{-1}$ and $xfactor = 10000$. The error bars for the model represent the standard error for 20 runs of NFsim. The error bars for the experimental data represent the standard deviation for two experiments.

the relationship between aggregation and cell degranulation.

VI. Conclusion

The researcher found that the biological rule-based model presented in this paper, which simulates the process of antigen-antibody aggregation via the crosslinking of IgE-FcϵRI receptors, generated output that fits reasonably well to the experimental cell degranulation data provided by collaborators. One idea for future work is to continue to improve this model to increase its accuracy for various concentrations of IgE and Pen a 1 molecules. This model assumes a directly proportional relationship between the experimental data and model observable. Another idea for future work is to develop a model without this assumption. This might be accomplished by calculating a different model observable by taking into the account the effect of intracellular events that occur after aggregation but before cell secretion.

Although Pen a 1 has more than six IgE binding sites, the researcher limited the scope of this project to a six-site model based on earlier computational work. Future work could entail evaluating similar models that have more than six binding sites, as the specificity of these sites is unknown.

VII. Appendix

A. BNGL code for biological rule-based model used in the study.

```
# BEFORE YOU BEGIN:
# Make sure that all of the parameter
# values are set correctly for the
# model you want to test.

# These parameters include:
# f - cell fraction
# Pen_a_1_copyNum - number of Pen a 1
#   molecules
# IgE_copyNum - number of IgE
# crosslinking factor - xfactor

begin model

# A model for Pen a 1 interaction with
#   allergen-specific IgE

begin parameters

# fraction of cell to be considered in a
#   (stochastic) simulation
# Obtain from Table V
f 1.0 # [=] dimensionless, 0<f<=1

# Avogadro constant
NA 6.02214e23 # [=] molecules per mol

# volume of extracellular fluid per
#   (RBL-2H3) cell
# multiplied by the cell fraction
# 100 uL of media per well divided by
#   43,063 cells per well
Vecf 2.3e-9*f # [=] L per cell

# copies of Pen a 1 per cell
# Obtain from Table IV
# multiplied by the cell fraction
Pen_a_1_copyNum 19*f

# copies of allergen-specific IgE per cell
# multiplied by the cell fraction
# Obtain from Table III
IgE_copyNum 2692*f

# crosslinking factor
xfactor 10000.0

# rate constants for IgE interaction with
#   an epitope
KD1_nM 10.0 # [=] nM
KD1=KD1_nM*1.0e-9 # [=] M

kf1_Ms 1.0e+9 # [=] /M/s

kf1=kf1_Ms/(NA*Vecf)
#   [=] /(molecule/cell)/s
kr1=KD1*kf1_Ms # [=] /s
kx1=xfactor*kf1_Ms/(NA*Vecf)
#   [=] /(molecule/cell)/s

end parameters

begin molecule types

Pen_a_1(e,e,e,e,e,e)
```

```
IgE(Fab,Fab)

end molecule types

begin seed species

Pen_a_1(e,e,e,e,e,e) Pen_a_1_copyNum
IgE(Fab,Fab) IgE_copyNum

end seed species

begin observables

# total number of receptors
#   (1 Fc receptor per IgE)
Molecules FcRtot IgE()

# number of unclustered receptors
Species FcR1 IgE==1

# number of allergen-induced receptor
#   aggregates
# (i.e., number of clusters containing 2 or
#   more
#   receptors)
Species n_agg_gt1 IgE>1

end observables

begin functions

# average receptor aggregate size = (# of
#   receptors in clusters)/n_agg_gt1
avg_agg_size()=(FcRtot-FcR1)/n_agg_gt1

end functions

begin reaction rules

# capture of free allergen
#   IgE binds an epitope in any region
Pen_a_1(e,e,e,e,e,e)+IgE(Fab)->\
Pen_a_1(e!1,e,e,e,e,e).IgE(Fab!1) kf1

# allergen-mediated receptor crosslinking
Pen_a_1(e!+,e)+IgE(Fab)->\
Pen_a_1(e!+,e!1).IgE(Fab!1) kx1

# dissociation of antigen-antibody bonds
Pen_a_1(e!1).IgE(Fab!1)->\
Pen_a_1(e)+IgE(Fab) kr1

end reaction rules

end model

#ACTIONS

simulate_nf({suffix=>"test",complex=>1,\
gml=>2000000000,print_functions=>1,\
t_start=>0,t_end=>60,n_steps=>100})
```

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