Modeling and Experimental Characterization of IgE Receptor Signaling to Develop New Drugs for Allergies

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**Executive Summary**

Allergies are an important health issue in our community. We want to develop a drug to lower the effects of allergies by characterizing the IgE receptor in a mast cell. The mast cell is an immune cell with many receptors on its membrane. Our project focuses on an IgE receptor called FcεR1, which plays a critical role in the allergy signal cascade within the mast cell. The FcεR1 has three protein subunits: the alpha, beta, and gamma chain. In the gamma chain, there are two tyrosines (amino acid) that are important for signaling. The phosphorylation (transfer of a phosphate) of these tyrosines by a kinase called Lyn is known to be important for the initiation of signaling. Then, another kinase called Syk binds to the phosphorylated tyrosines. This action is the very first step to the mast cell signal cascade. However, the timeline and pattern of phosphorylation of each tyrosine is unknown. In our project, we will investigate these unidentified parameters using two independent methods: modeling and experimental biology. These two approaches allow us to gain independent results that will complement each other.

The purpose of the experimentation is to validate and verify the model. The way we do this is by finding an antibody that can detect specific phosphorylation. P0 is no phosphorylation, P1 is the first tyrosine phosphorylated, P2 the second, and P12 both. Using methods of phage display, yeast display, monoclonal analysis, drop assay, immunoprecipitation, and western blot analysis, a P1 antibody was found. A P2 antibody is currently being developed. However, initial experiments did not yield P0 or P12 antibodies, so those experiments will be done again. The P1 antibody was developed and then tested against the gamma subunit. The mast cell signal cascade was initiated with different concentrations of the allergen, then stopped at different times. Next, the gamma subunit was picked out for experimentation. When tested against the antibody, phosphorylation at two minutes was detected, but the antibody did not detect phosphorylation at five minutes. This suggests that P1 is phosphorylated by two minutes and dephosphorylated by five minutes. However, the gamma subunit may or may not have been picked out from the mast cell successfully. These experiments will be repeated to confirm results. All of this data was compared with the simulations of the model.

The purpose of the modeling is to find new drug targets in order to speed up the experimental methodology. This is done using a programming language called BioNetGen which specializes in simulating cellular signaling. BioNetGen is a rule-based programming language which means the programmer writes very simple rules that dictate the interactions of molecules and add rates or parameters which define the scope of the rule. Using this approach, we have made a simulation in which, the allergen or DNP (dinitrophenyl) binds to the IgE receptor or more specifically the fragment antigen-binding (Fab). Next, a receptor localizes the Lyn Kinase which phosphorylates the opposite receptor’s gamma chain. Then, the Syk Kinase binds to the phosphorylated tyrosine of the gamma chain and the cell signaling cascade begins. We input the molecules of interest, rules for interactions, and parameters governing the rules in order to receive specified outputs, such as P1 phosphorylation. In agreement with preliminary experimental data, the model predicts an increase in P1 phosphorylation.
Once the model has been completely validated, it can predict phosphorylation patterns of P2 and P12. Experiments can be done to verify those results as well. Then, the model can design a small molecule that will bind to the phosphorylated gamma. This molecule will prevent the binding of Syk to the phosphorylated tyrosine, and therefore inhibit the signal cascade. This also means that the rest of the cell’s functions would go on normally because the only thing that would be affected is a transient change of a single protein. After the molecule is modeled, experiments will be done to make, develop, and test it against the gamma subunit. If successful, this drug molecule can be tested in animals, and then go through clinical trials. Eventually, it would become a new drug for allergies. This molecule will inhibit the very first steps of the signal cascade, which is before any current medications stop signaling. Therefore, our drug would be more effective and beneficial than any current day medications. In conclusion, by using experimentation as well as modeling, we would develop a new drug for allergies that stops the initial steps of mast cell signaling.
Introduction

The overall purpose of this project is to develop a new drug for allergies. Allergies are an abnormal response by the immune system. Seasonal allergies are usually caused by pollens from grasses, trees, weeds, etc. Other allergies can be caused by foods or animal fur. The symptoms are sneezing, itchy throat, nose, or eyes, nasal congestion, runny nose, coughing, and watery or red eyes. 20% of Americans are affected by allergies whose development is triggered by genetics as well as the environment.

Human body’s reaction to an allergen

During an allergic reaction, an allergen binds to a mast cell in an organism, as shown in figure 1(1). The mast cell’s proteins begin signaling other parts of the cell using phosphorylation. This results in the degranulation of the cell (shown in electron micrograph(2)). Degranulation is the disruption of the mast cell membrane to release histamines, cytokines, chemokines, etc. This is where current medications prevent allergies with the use of antihistamines or steroids.

Current Medications

<table>
<thead>
<tr>
<th>Treatment option</th>
<th>Mechanism of action</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>antihistamines</td>
<td>• blocks action of histamine at receptor</td>
<td>• many different forms of medicines available</td>
<td>• causes drowsiness due to blocking of histamine receptors on nerve and brain cells no side effects</td>
</tr>
<tr>
<td></td>
<td>• competes with histamine for binding</td>
<td>• effective short-term relief</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• displaces histamine from receptor</td>
<td>• not all medicines effective on all people</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• prevents histamine from eliciting a response</td>
<td></td>
<td></td>
</tr>
<tr>
<td>allergy shots/immunotherapy</td>
<td>• desensitization of mediator release from mast cells and basophils to allergen exposure</td>
<td>• one of the best long-term solutions</td>
<td>• have to get shots for 5 years or more not all allergies are cured</td>
</tr>
<tr>
<td>mast cell stabilizers</td>
<td>• blocks a calcium channel essential for mast cell degranulation, so no histamines are released</td>
<td>• many different forms of medicines available</td>
<td>• could have side effects takes several weeks before treatment is felt</td>
</tr>
<tr>
<td>steroids</td>
<td>• blocks cytokines and chemokines at receptor</td>
<td>• could have side effects</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• requires use during asymptomatic seasons</td>
<td></td>
</tr>
<tr>
<td>neti pot/shower</td>
<td>• removes allergens from the body</td>
<td>• does not have long term effects</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• no side effects</td>
<td></td>
</tr>
</tbody>
</table>

Most current medications treat allergies after mast cell degranulation. The drug we would like to develop would stop the cascade during the initial steps of signaling, making it a more effective and beneficial treatment.
**Inside the mast cell**

Figure 2 depicts the signal cascade in the mast cell leading to allergy. The mast cells have many different receptors. Our project focuses on the high affinity receptor for IgE, also known as FcεRI. This specific receptor is important because the IgE molecule was originally evolved to fight parasites. However, in a developed country, humans usually live in a very clean community. This means the IgEs do not have any parasites to fight, so it binds to random substances such as pollens, food, etc. The FcεRI receptor binds to the IgE molecules, which binds to an allergen. When multiple IgE-receptor complexes are co-localized by an allergen, it causes the initiation of the signal cascade in a mast cell.

**Subunits of FcεRI receptor**

The FcεRI receptor has three protein subunits: alpha, beta, and gamma as shown in figure 3. The gamma is considered the key part of the signal cascade. When an allergen is received by the mast cell, two FcεRI IgE receptors are brought together. Phosphorylation, (i.e., the transfer of a phosphate group) of the beta protein’s amino acid tyrosine (yellow circles) localizes the Lyn kinase. This kinase then phosphorylates the gamma of the other receptor. Thus, it is necessary to have at least two receptors bind to the allergen, otherwise the gamma is not phosphorylated. The phosphorylated gamma subunit is then recognized and bound by the Syk kinase, which initiates the signal cascade as shown in figure 2.
Summary of Previous Research

• Gamma subunit phosphorylation is needed for initiation of the signal cascade in a mast cell(5).
• The amount of phosphorylation is dependent upon the concentration of allergen and duration of exposure(5).
• It is not known whether the N-terminal tyrosine (P1) or the C-terminal tyrosine (P2) is phosphorylated during this process.
• If both tyrosines are phosphorylated, the time and duration of phosphorylation for each of the tyrosines are also not known.
• Computational models have been made but confirmation of results has been limited due to lack of reagents for detection of specific phosphorylation sites.

Hypothesis
If we develop a drug that will stop the signal cascade in a mast cell, it would be more effective than current medications.

Challenges: A molecule that can detect and inhibit the signal cascade starting from initial phosphorylation has never been found. This is because detecting a single phosphate within the sea of molecules in the signal cascade is extremely difficult. The only thing differentiating dephosphorylated gamma from phosphorylated gamma is five atoms (PO$_4$). This is also a transient change, not a change in the subunit itself. Finding a molecule that can detect phosphorylation, and therefore inhibit the signal cascade, will be challenging. This is the innovation of our project.

Experimental Design
1) Understand timeline for activation and pattern of gamma phosphorylation
   - We do this using two different methods: modeling and experimentation. The purpose of the modeling is to develop drug targets. The purpose of the experimentation is to validate and verify the model. Combining our efforts will help us reach our overall goal of developing a drug faster than if it was done with only one side of this project. The modeling and experimental biology will also complement and verify each other’s results.

2) Find small molecules that enhance/inhibit the signal cascade
   - Modeling can be used to design small molecules that will inhibit the signal cascade by binding to the phosphorylated gamma subunit. This will prevent the binding of Syk. However, the rest of the cell’s functions will go on normally. Experimental data will provide guidance for the small molecule design. If successful, this molecule will be developed further to become a new drug for allergies. If unsuccessful, a library of small molecules can be scanned and tested against the gamma subunit. The model can narrow down the amount of testing that would have to be done. Once an inhibiting molecule is found, it will be developed to become a new drug for allergies.

3) Develop new drugs for allergies
   - Once the drug has been tested against the gamma subunit, it can been tested in animals. If this is successful, the drug can go into various clinical trials to be tested against humans.
Developing Antibodies

The overall purpose of the experimental side of the project is to validate and verify the model. To do this, experiments will be done to attain antibodies that can detect specific phosphorylation.

Selection of Antibodies using Phage Display

The selection of antibodies requires a target, so gamma subunit peptides were used. A peptide is a part of the gamma subunit (amino acid sequence in table). Synthetic peptides allow labelling, uniform phosphorylation, and availability in higher quantities. Biotin and non biotin labeled and peptides were used for selections and sorting. A library containing $10^{13}$ different antibody genes were cloned into M13 phage virus (7). This library was used for selection of antibodies against each of the peptides. Three rounds of selection were carried out for each peptide. This yielded a mini library of $10^4$ antibodies for each peptide that was more specific. The antibody genes were then amplified using polymerase chain reaction (PCR) and cloned into yeast display vector (8).

Sorting for Antibodies using Yeast Display

In yeast display, antibodies are displayed on baker’s yeast (8). This allows visualization of antibody display and peptide binding using a technology called flow cytometry (9). Flow cytometry is a laser-based technology that records when an antibody is displayed on yeast (x-axis) and recognizing the peptide (y-axis) as shown in Figure 5. This allows sorting of yeast that contain specific antibodies. After three rounds of phage selection, P0, P2, and P12 specific binders had been successfully selected (shown in Figure 6). P2 yeast sorting is ongoing.

<table>
<thead>
<tr>
<th>Fcy-2 phosphorylation sites</th>
<th>ASREKSDAVYTGLNTRNQETYETLKHE</th>
</tr>
</thead>
<tbody>
<tr>
<td>γ-P0</td>
<td>ASREKSDAVYTGLNTRNQETYETLKHE</td>
</tr>
<tr>
<td>γ-P1</td>
<td>ASREKSDAVYTGLNTRNQETYETLKHE</td>
</tr>
<tr>
<td>γ-P2</td>
<td>ASREKSDAVYTGLNTRNQETYETLKHE</td>
</tr>
<tr>
<td>γ-P12</td>
<td>ASREKSDAVYTGLNTRNQETYETLKHE</td>
</tr>
</tbody>
</table>

Phospho-tyrosines in red

Figure 4

Figure 5

Figure 6
The P0 yeast library also had very good P1 binders, so P1 specific antibodies were selected out of this library. Subtractive sorting was used to remove P0 binders out of the P1 library. These antibodies ended up being the best binders, so P1 experimental results are shown in the following figures. After three rounds of yeast sorting, about a hundred P1 and P12 specific yeast colonies were chosen for further analysis out of the $10^4$ libraries for P0 and P12. The P0 antibody library did not yield P0 specific binders.

**Specificity Assay for P1 Antibody after Three Rounds of Yeast Sorting**

![Figure 7](image)

**Monoclonal Analysis and Drop Assay**

Out of the hundred colonies of yeast for P1 and P12, twenty four of each were chosen to be studied individually in a monoclonal analysis. The results showed that each of the P1 clones was specific to the P1 and P12 peptides. The P12 clones were specific to the P12 peptide. Then a drop assay was done. The drop assay is used to find out if the antibodies are binding to the peptide itself or to the biotin it is labelled with for selection and sorting purposes. The drop assay for P1 was successful, the P1 antibodies were binding to the peptide and not the biotin. However, the P12 antibodies were only binding to the biotinylated peptide, so selection or sorting for P12 will need to be done again. PCR fingerprinting and DNA sequencing (shown in Figure 8) was done for the best eight P1 antibodies. This showed that the DNA sequences of the DNA making the proteins were exactly the same. One antibody was sent for DNA sequencing. This was translated into an amino acid sequence of the antibody.

![Figure 8](image)

**Immuno Precipitation and Western Blot Analysis**

The P1 antibody that was selected recognized the peptide successfully, but the peptide is only a segment of the gamma subunit. Therefore, the next experiment focused on testing the ability of the antibody to recognize the phosphorylated gamma subunit. Mast cells were stimulated with different concentrations of DNP-BSA (2nM, 20nM, and 200nM), then the signal cascade was stopped at different times (two minutes and five minutes). This was done by lysing, or exploding, the cell. Then, using an immuno precipitation procedure, the gamma subunit was picked out of the cell lysate to be tested against the antibody. To check that the gamma had successfully been selected out of the lysate, a western blot analysis was done (Figure 9). The gamma had not been selected for the first three samples, and it is uncertain whether it has been picked for samples five
and seven. The next western blot analysis shows whether the antibody is binding to the gamma subunit, and therefore whether the first tyrosine is phosphorylated (Figure 10). In samples four and six, there is definitely a band. Both of these samples were lysed at two minutes, which suggests that there is phosphorylation of P1 at two minutes. There is no band for samples five and seven, indicating that P1 dephosphorylates by five minutes. However, since the results of the immuno precipitation for those samples are unsure, P1 may or may not be phosphorylated by five minutes. These experiments will have to be done again to check the phosphorylation for the first three samples and verify whether or not P1 is phosphorylated at five minutes.

1) basal
2) 0.1 ug/mL DNP at 2 minutes
3) 0.1 ug/mL DNP at 5 minutes
4) 1 ug/mL DNP at 2 minutes
5) 1 ug/mL DNP at 5 minutes
6) 10 ug/mL DNP at 2 minutes
7) 10 ug/mL DNP at 5 minutes
Modeling

Introduction
During an allergic reaction, an allergen binds to a mast cell in an organism. The mast cell’s proteins begin signaling other parts of the cell using phosphorylation. The FceRI receptor binds to IgE molecules, which binds to an allergen. When two IgE molecules are coupled by an allergen, it causes the initiation of the signal cascade in a mast cell. The FceRI receptor has three protein subunits: alpha, beta, and gamma. Phosphorylation of the amino acid tyrosine (Y in figure to the far right) by Lyn kinase is the first step of the signaling. Phosphorylated gamma subunit is then recognized by Syk kinase and the signal cascade continued.

Mathematical models are powerful tools that can be used to understand complex biological systems. Pathway models can be used to narrow down which experimental conditions would be most useful to consider, and can provide insights into the effect of variables on biological systems. Computational models of FceRI have been developed but confirmation of model predictions has been limited due to lack of reagents for detection of specific phosphorylation sites (15). These phosphorylation sites are difficult to detect because the difference between a phosphorylated site and a non-phosphorylated one is only five atoms. In this project, modeling can be used to make experimental design more efficient. This can be done by using the model to predict phosphorylation dynamics and the impact of small molecule inhibitors. Such inhibitors can sometimes produce unexpected repercussions in cell signaling networks, and thus it is desirable to have a model that could predict such effects (16). These capabilities would reduce the cost and time needed to run experiments.

Method
To model the signaling of the IgE receptor, a programming language called BioNetGen Language (BNGL) was used. BNGL is specifically designed for development of chemical kinetic models of cell signaling systems. BioNetGen is a rule-based programming language which means you specify the necessary and sufficient conditions for a reaction to occur, as well as parameters governing the rates of the reactions (10). Traditional approaches for modeling cell signaling systems have many chemical species interacting and require numerous specific statements of the network specification (14). This problem is avoided using rules in the place of interactions which makes the network specification brief and to the point (14). Pictured in Figure 11 are a few of the rules for ligand interactions graphically represented. Below each diagram is the rule as it is written in BNGL. Figure 12 is a full rule map of the rules in our program (13).
The graphical representations of the rules and the actual syntax used in the program below it.

The graphical representation of all the rules in the program.
In our simulation, the allergen or DNP (dinitrophenyl) binds to the IgE receptor or more specifically the fragment antigen-binding (Fab) as shown by the Figure 13. Next, a receptor localizes the Lyn Kinase which phosphorylates the opposite receptor’s gamma chain. Syk then binds to the phosphorylated tyrosine of the gamma chain and the cell signaling cascade begins. We input the molecules of interest, rules for interactions, and parameters governing the rules in order to receive specified outputs, such as P1 phosphorylation.

Figure 13:

**Results**

We have used a rule-based approach to create a program that simulates the phosphorylation dynamics of all of the sites that are detectable experimentally using the antibodies described in the experimental part of this project. A limitation of this simulation is the number of species potentially generated by the model is large enough to require a network-free simulation. This means our results are stochastic and in order to have reliable results we have to run the simulation multiple times. We have run the simulation at concentrations: 2nM, 20nM, and 200nM, from 2 - 5 minutes each 1000 times to eliminate the stochastic nature of the results. In agreement with preliminary experimental data, the model predicts an increase in P1 phosphorylation. In the future, we can use the model to more accurately and conveniently predict phosphorylation pattern at many different concentrations and times to be verified by experimental results and model the effects of inhibiting molecules on signaling cascade to find new drugs for allergy. The model can also be used to predict and to test various molecules to use as inhibitors. Such inhibitors can sometimes produce unexpected repercussions in cell signaling.
networks, and thus it is desirable to have a model that could predict such effects (16). This would narrow down antibody libraries and reduce the cost and time needed to run experiments. Thus, the experimental methodology would be yield results much faster than without the modeling.

![Concentrations of P1 for 5 minutes](image)

**Conclusion**

An antibody that can detect P1 phosphorylation was found. It detected phosphorylation at two minutes and dephosphorylation by five minutes. However, the immuno precipitation results of the five minutes samples are uncertain so those experiments will have to be done again. P2 yeast sorting is ongoing, and alternate selection and sorting procedures will be done to attain P0 and P12 antibodies.

We have run the simulation at concentrations: 2nM, 20nM, and 200nM, from 2 - 5 minutes each. In agreement with preliminary experimental data, the model predicts an increase in P1 phosphorylation. In the future, we can use the model to more accurately and conveniently predict phosphorylation pattern at many different concentrations and times to be verified by experimental results.

Once the model has been completely validated, it can predict the phosphorylation and dephosphorylation of P0, P1, P2, and P12. All of these results can be verified by the experimentation. Once all of that has been finished, the modeling can be used to study the effects of inhibitory molecules to find new drugs for allergies. This will narrow down the number of molecules that will need to be tested by experimentation, leading to faster drug development.
Acknowledgements

We would like to thank Dr. Lily Chylek, Dr. Bill Hlavacek, for modeling project Dr. Andrew Bradbury, Mrs. Leslie Naranjo, Mrs. Nileena Velappan, Dr. Avanika Mahajan, Dr. Bridget Wilson for experimental biology, NMC Biolabs, LANL, UNM-Albuquerque for lab space, equipment, and reagents.

References

13. (Suppl. 8), S3 (16 pages).
Source Code

begin model

#---
# Header:
# Title:
# Author: Alex Ionkov
# Date: 12-November-2015 #...

#---
# References:
# 189(2), 646-658.
#...
#...

begin parameters

NA 6.022e23 celldensity 2e9
Fx 1e-4
Vo Fx/(celldensity)

# Avogadro's number; molecules/mole.
# Cells/L 1e9
# Fraction of cell volume to simulate; unitless.
# Extracellular volume; L/cell.
# Simulated fraction of extracellular volume; L.
# Cytoplasmic volume; L. Value from Faeder et al. # Simulated fraction of cell volume; L.

# concentration

simECFvol Cellvol simCellvol concen

LigTot
Lyn_total 4e5*Fx Syk_total 4e5*Fx IgE_Rec_total 4e5*Fx

Vo*Fx 1.4e-12
Cellvol*Fx 2e-7
NA*Vo*concen

# Ligand binding
kp1 9.2e6/(NA*Vo) #moles per liter and per second kxlink 3.6e-12/(NA*Vo)
km1 0.012

#phosphorylation constants kp2 1.53e-8
km2 0.012

#Syk binding kpS 6e-5 kpSf 6e-3 kmS 0.13

#Lyn phosphorylation of gamma pLb 30
pLg 1

#Lyn (Sh2) binds Lyn(Y508~P) and reverse kf3 10.0
kr3 3.0e-4

#Ligand o/c rates pLambda 9.1e-5 mLambda 7.5e-4

#Lyn phosphorylation of PAG kp8a 1.0e3
kp8b 1.0e3
kp8c 1.0e3

#Lyn binding to PAG(PRS2), reverse kf19a 1.0e5 #1.0e-5
kr19a 30.0
kf19b 1.0e3

#Lyn binding to PAG(Y387_Y417~P), reverse kf20a 3.0e-5
kf20b 1.0e3
kr20b 0.1

#Csk(SH2) binds PAG(Y317~P) kf23 3.0e-7
kr23 3.0e-3

#Csk phosphorylation of Lyn(Y508~0) kp24 1.0e3

#dephosphorylation of Lyn(Y397~P) and (Y508~P) kdp28a 1.0
kdp28b 1.0

#dephosphorylation of PAG kdp30a 1.0
kdp30b 1.0
kdp30c 1.0
end parameters begin molecule types
Lig(DNP-c,o,DPN-o) #ligand, (Y16) antigen for immune sys, molecule
Lyn(SH2,unique,SH3,Y397-0,P,Y508-0-P)
Syk(nSH2,cSH2,kinase)
IgE_Rec(Y1-0-P,Y2-0-P,Fab,Fab,B-0-P)
Lat(Y1-0-P)
Csk(SH2) PAG(PRS1,PRS2,Y317-0-P,Y163_Y181-0-P,Y387_Y417-0-P)

end molecule types

begin seed species
Lyn(SH2,unique,SH3,Y397-0,Y508-0) Lyn_total
Csk(SH2) 3.0e5*Fx PAG(PRS1,PRS2,Y317-0,Y163_Y181-0,Y387_Y417-0) 3.0e5*Fx
Syk(nSH2,cSH2,kinase) Syk_total
Lig(DNP-c,DNP-c) 0.79529919098*LigTot
Lig(DNP-o,DNP-o) 0.01170821657*LigTot
Lig(DNP-o,DNP-o) 0.19299259244*LigTot IgE_Rec(Y1-0,P,Y2-0,Fab,Fab,B-0) IgE_Rec_total

end seed species begin observables
#Molecules LigRecBond Lig(DNP!1).IgE_Rec(Fab!1)
#Molecules SykBondN Syk(nSH2!1,cSH2).IgE_Rec(Y2~P!1)
#Molecules SykBondC Syk(cSH2!1,nSH2).IgE_Rec(Y1~P!1)
#Molecules SykBondTotal Syk(nSH2!1,cSH2!2).IgE_Rec(Y2~P!1,Y1~P!2) Molecules P0Count
IgE_Rec(Y1-0,P,Y2-0)
Molecules P1Count IgE_Rec(Y1-~P!,Y2-0)
Molecules P2Count IgE_Rec(Y2-~P!,Y1-0)
Molecules P12Count IgE_Rec(Y1-~P!,Y2-~P!)
#Molecules RecTot IgE_Rec
Molecules Lyn508 Lyn(Y508-~P!)
Molecules Lyn397 Lyn(Y397-~P!)
Molecules Csk Csk(SH2!+)
#Molecules Pag PAG(Y163_Y181-~P,Y387_Y417-~P)
Molecules Pag PAG(PRS2!+)

end observables

begin reaction rules

# Interactions of Lig #
Lig(DNP-c) <-> Lig(DNP-o) pLambda, mLambda
Lig(DNP-c,DNP-o)+IgE_Rec(Fab)->Lig(DNP-c,DNP-o!1).IgE_Rec(Fab!1) kp1
Lig(DNP-o,DNP-o)+IgE_Rec(Fab)->Lig(DNP-o,DNP-o!1).IgE_Rec(Fab!1) kp1
Lig(DNP-o!+,DNP-o)+IgE_Rec(Fab)->Lig(DNP-o!+,DNP-o!1).IgE_Rec(Fab!1) km1
Lig(DNP-o!1).IgE_Rec(Fab!1)->Lig(DNP-o)+IgE_Rec(Fab) km1

# Lyn Phosphorylation
of both tyrosines, dephosphorylation #

#binding and phosphorylation of Lyn
IgE_Rec(B~0) + Lyn(unique) -> IgE_Rec(B~0!1).Lyn(unique!1) pLb

#binding of phosphorylated beta to SH2 domain of Lyn
IgE_Rec(B~0) + Lyn(unique) -> IgE_Rec(B~0!1).Lyn(unique!1) pLb

# phosphorylation of beta
Lig(DNP!1,DNP!2).IgE_Rec(Fab!1,B~0!3).IgE_Rec(Fab!2,B~0!3).Lyn(unique!3) -> Lig(DNP!1,DNP!2).IgE_Rec(Fab!1,B~0!3).IgE_Rec(Fab!2,B~0!3).Lyn(unique!3) kp2

# phosphorylation of Y1 and Y2
Lig(DNP!1,DNP!2).IgE_Rec(Fab!1,B~0!3).IgE_Rec(Y1~0).Lyn(unique!3) -> Lig(DNP!1,DNP!2).IgE_Rec(Fab!1,B~0!3).IgE_Rec(Y1~0).Lyn(unique!3) kp2

# autoinhibition of Lyn, SH2 domain of Lyn binds C-terminal pY
Lyn(unique,SH3,SH2,Y508~P) <-> Lyn(unique,SH3,SH2,Y508~P) kf3, kr3

# Lyn phosphorylates PAG
Lyn(Y397~P,Y508).PAG(Y387_Y417~0) -> Lyn(Y397~P,Y508).PAG(Y387_Y417~P) kp8a
Lyn(SH2!1,Y397~P,Y508).PAG(Y163_Y181~0,Y387_Y417~P!1) -> Lyn(SH2!1,Y397~P,Y508).PAG(Y163_Y181~0,Y387_Y417~P!1) kp8b
Lyn(SH2!1,Y397~P,Y508).PAG(Y317~0,Y387_Y417~P!1) -> Lyn(SH2!1,Y397~P,Y508).PAG(Y317~0,Y387_Y417~P!1) kp8c

# SH3 domain of Lyn binds PRS2 in PAG
# association, Lyn is free
Lyn(unique,SH3,SH2) + PAG(PRS2,Y387_Y417) -> Lyn(unique,SH3!1,SH2).PAG(PRS2!1,Y387_Y417) kf19a # dissociation
Lyn(unique,SH3!1,SH2).PAG(PRS2!1,Y387_Y417) -> Lyn(unique,SH3,SH2) + PAG(PRS2,Y387_Y417) kr19a # association, Lyn is tethered to PAG (via SH2 domain-pY interaction) # Lyn, already tethered in PAG by SH2, binds PAG via SH3 domain Lyn(unique,SH3,SH2!2).PAG(PRS2,Y387_Y417~P!2) -> Lyn(unique,SH3!1,SH2!2).PAG(PRS2!1,Y387_Y417~P!2) kf19b # SH2 domain of Lyn binds a pY docking site in PAG # association, Lyn is free Lyn(unique,SH3,SH2) + PAG(PRS2,Y387_Y417~P) -> Lyn(unique,SH3,SH2!2).PAG(PRS2!1,Y387_Y417~P!2) kf20a # association, Lyn is tethered to PAG (via SH3 domain-PRS interaction) Lyn(unique,SH3!1,SH2!2).PAG(PRS2!1,Y387_Y417~P) -> Lyn(unique,SH3!1,SH2!2).PAG(PRS2!1,Y387_Y417~P!2) kf20b # release, breaking two-point attachment Lyn(unique,SH3!1,SH2!2).PAG(PRS2!1,Y387_Y417~P!2) -> Lyn(unique,SH3,SH2) + PAG(PRS2,Y387_Y417~P) kr20b # SH2 domain of Csk binds pY317 docking site in PAG Csk(SH2) + PAG(Y317~P) <-> Csk(SH2!3).PAG(Y317~P!3) kf23, kr23 # Csk cis phosphorylates C-terminal Y in Lyn Lyn(Y508~0).PAG().Csk() -> Lyn(Y508~P).PAG().Csk() kp24 # Lyn dephosphorylation Lyn(Y397~P) -> Lyn(Y397~0) kdp28a # A-loop tyrosine Lyn(Y508~P) -> Lyn(Y508~0) kdp28b # C-terminal regulatory tyrosine # Dephosphorylation of PAG PAG(Y317~P) -> PAG(Y317~0) kdp30a # tyrosine in Csk docking site PAG(Y387_Y417~P) -> PAG(Y387_Y417~0) kdp30b # tyrosines in Lyn docking sites PAG(Y163_Y181~P) -> PAG(Y163_Y181~0) kdp30c

# binding of IgE Receptor by Syk#
Syk(nSH2,cSH2)+IgE_Rec(Y2~0)<->Syk(nSH2!1,cSH2).IgE_Rec(Y2~P!1) kpS, kmS
Syk(nSH2!1,cSH2).IgE_Rec(Y2~P!1,Y1~P)<->Syk(nSH2!1,cSH2!2).IgE_Rec(Y2~P!1,Y1~P!2) kpSF, kmS
Syk(nSH2,cSH2)+IgE_Rec(Y1~0)<->Syk(nSH2,cSH2!1).IgE_Rec(Y1~P!1) kpS, kmS
Syk(nSH2,cSH2!1).IgE_Rec(Y2~P,Y1~P!1)<->Syk(nSH2!2,cSH2!1).IgE_Rec(Y2~P!2,Y1~P!1) kpSf, kmS

end reaction rules end model

#ACTIONS
simulate_nf({t_end=>300, n_steps=>10})