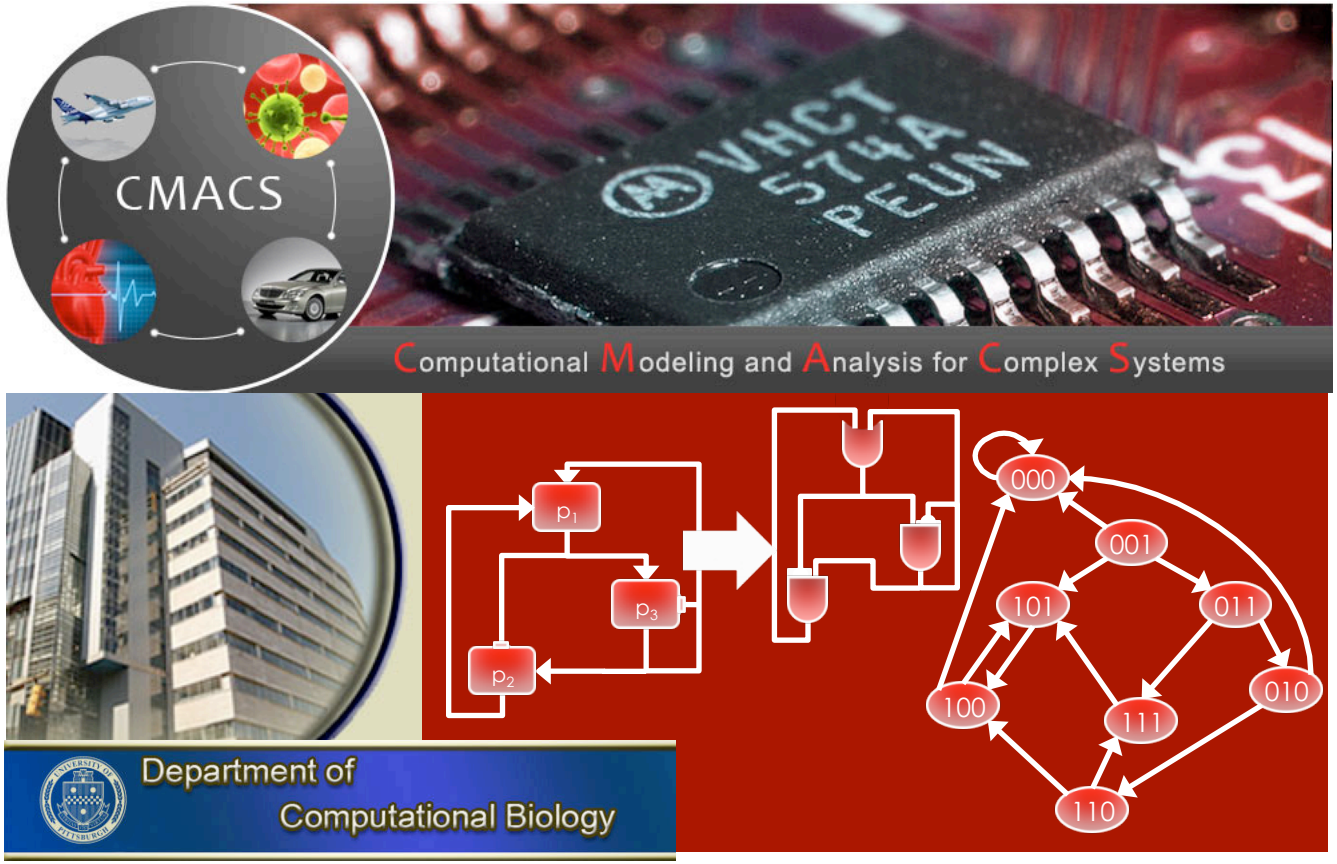
PI Meeting

University of Maryland

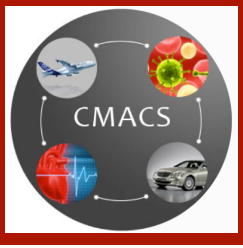
April 2011



A model for T cell differentiation

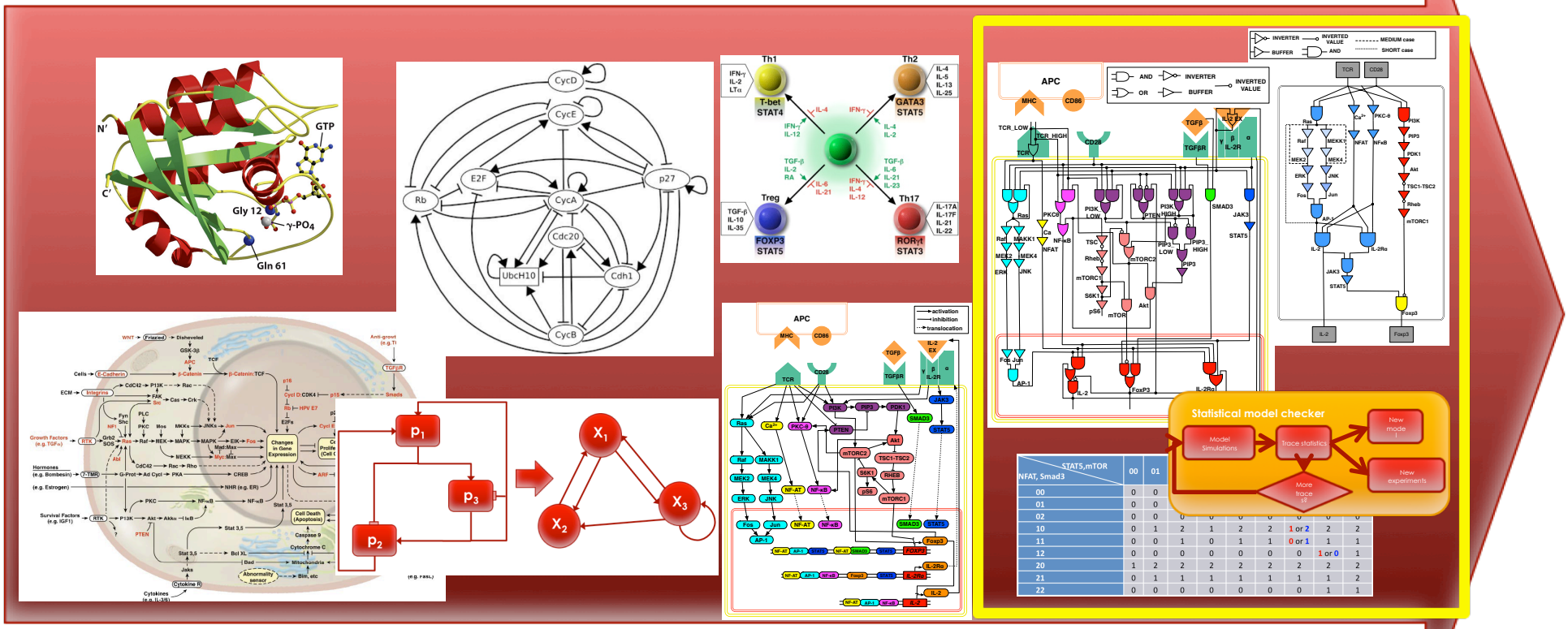
Natasa Miskov-Zivanov
University of Pittsburgh

Acknowledgements



- **Faeder Lab:**
  - Department of Computational and Systems Biology, School of Medicine, University of Pittsburgh
    - John Sekar, James Faeder
- **Morel Lab:**
  - Department of Immunology, School of Medicine, University of Pittsburgh
    - Michael Turner, Penelope Morel
- **Clarke Lab:**
  - Computer Science Department, Carnegie Mellon University
    - Paolo Zuliani, Haijun Gong, Qinsi Wang, Edmund Clarke

Timeline



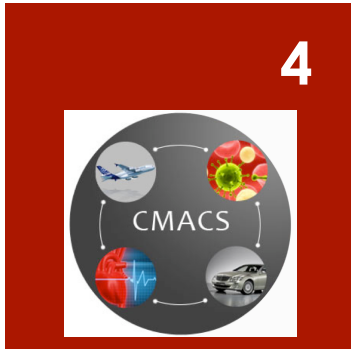
Kickoff
October 2009

NSF Meeting
March 2010

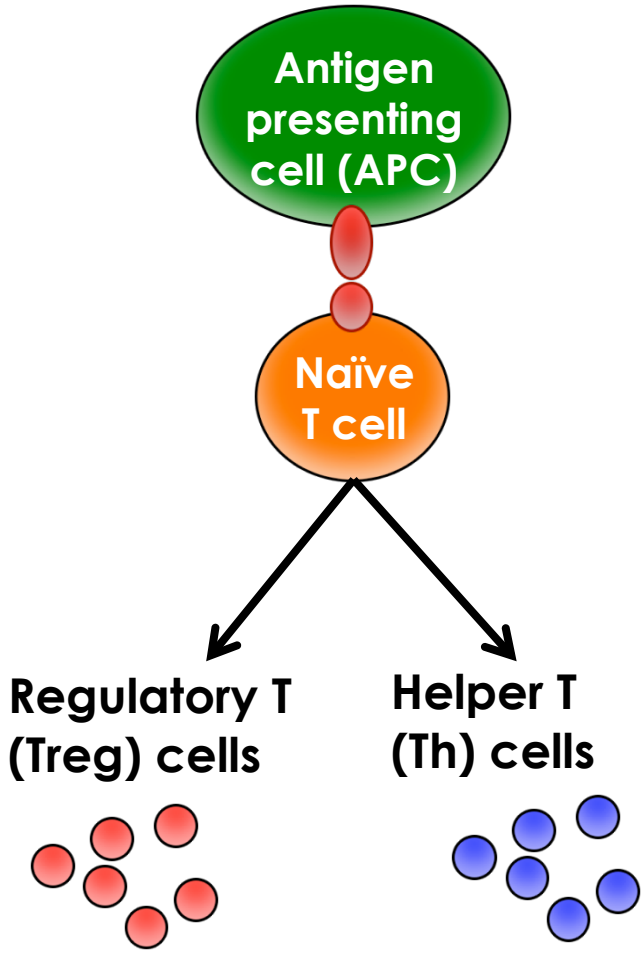
PI Review
October 2010

PI Meeting
April 2011

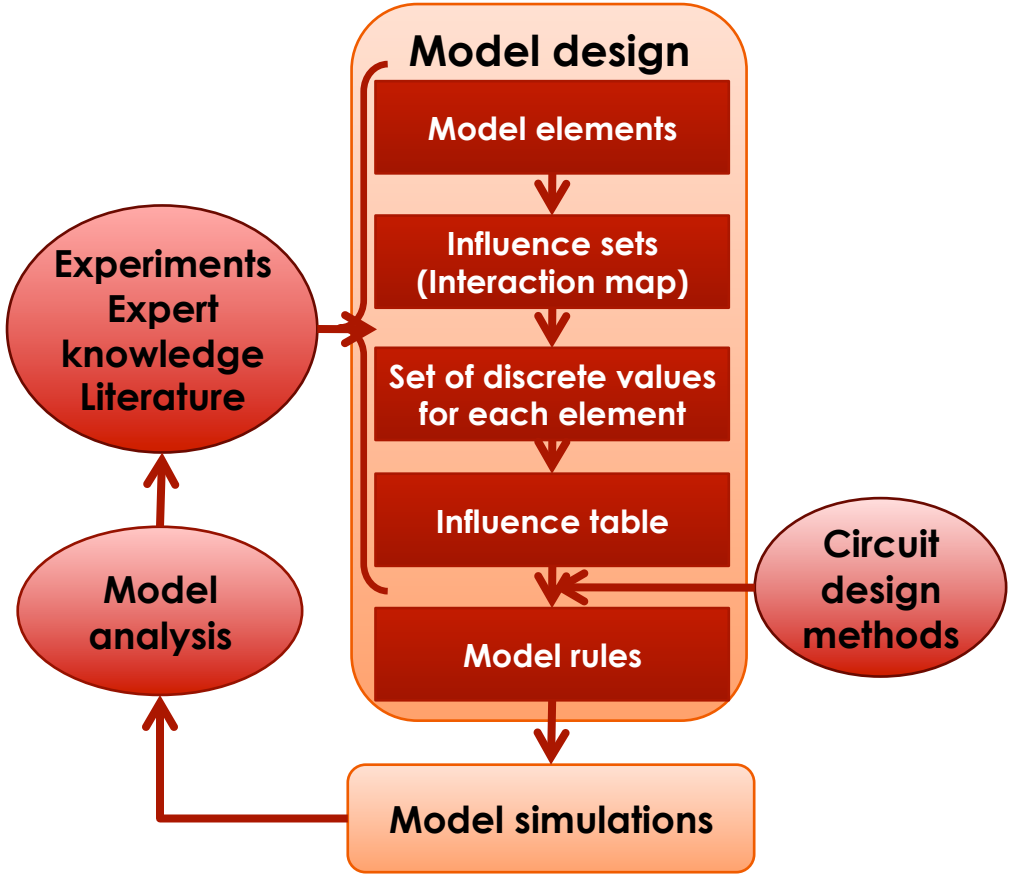
Today's talk



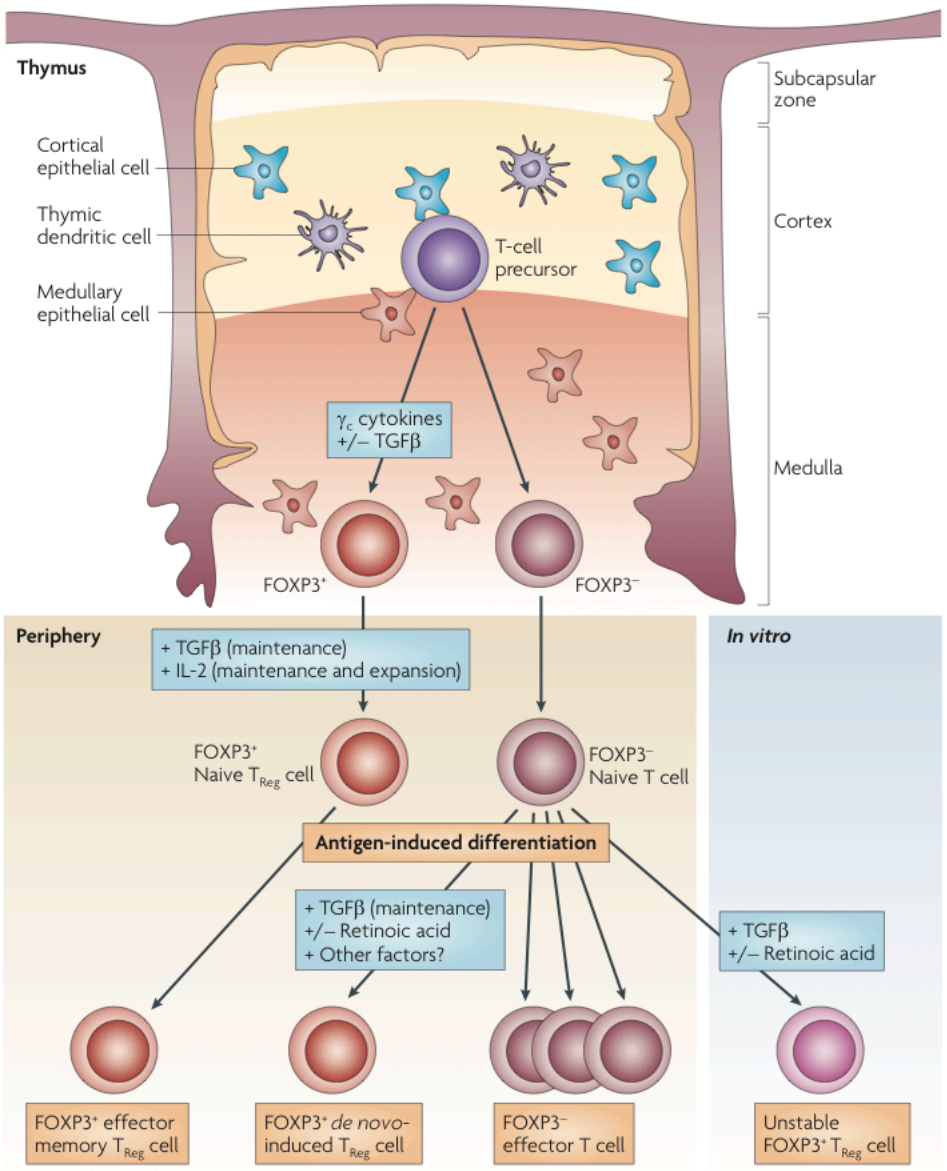
System



Methodology

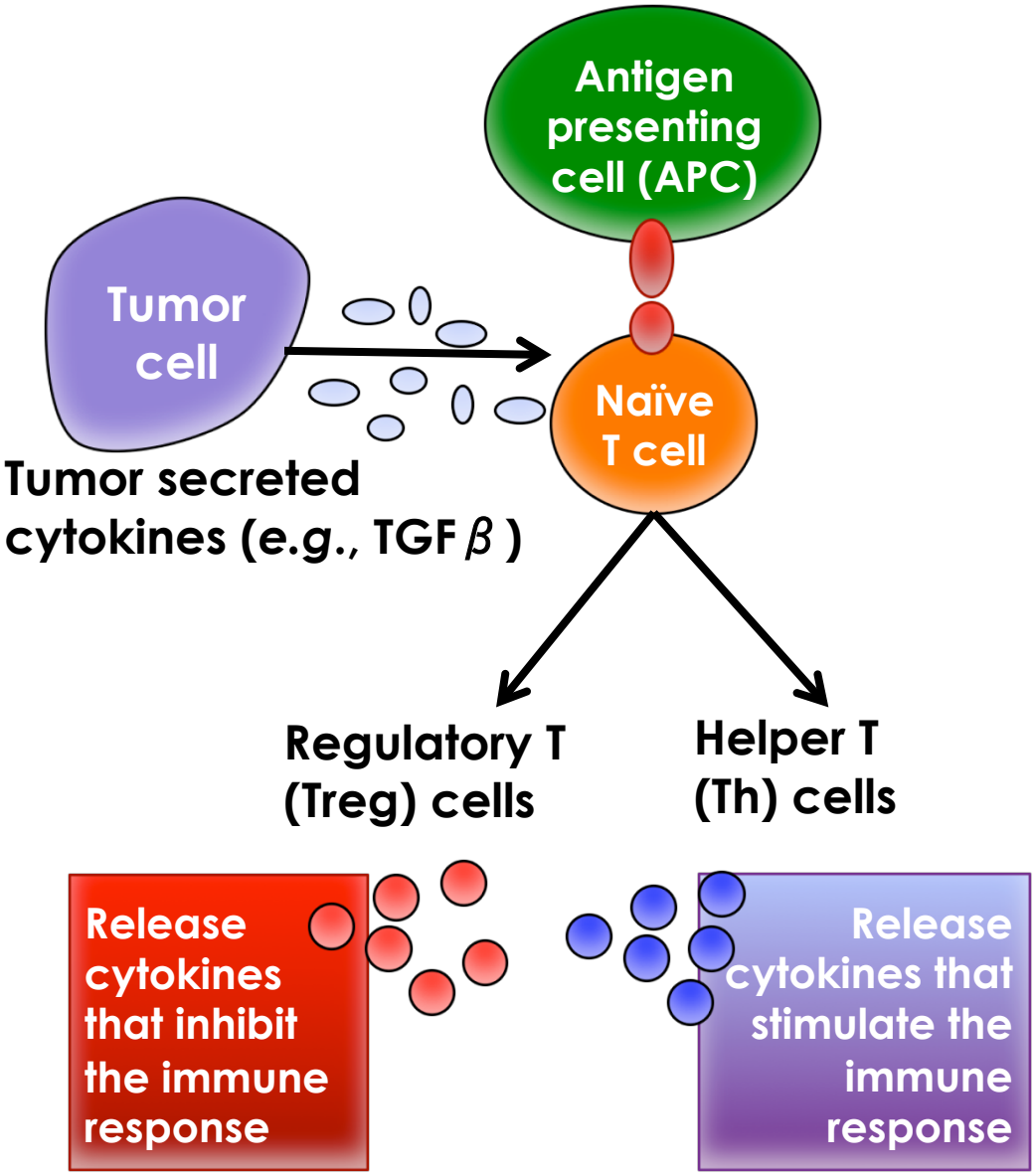


Origins of Regulatory T cells (Treg)

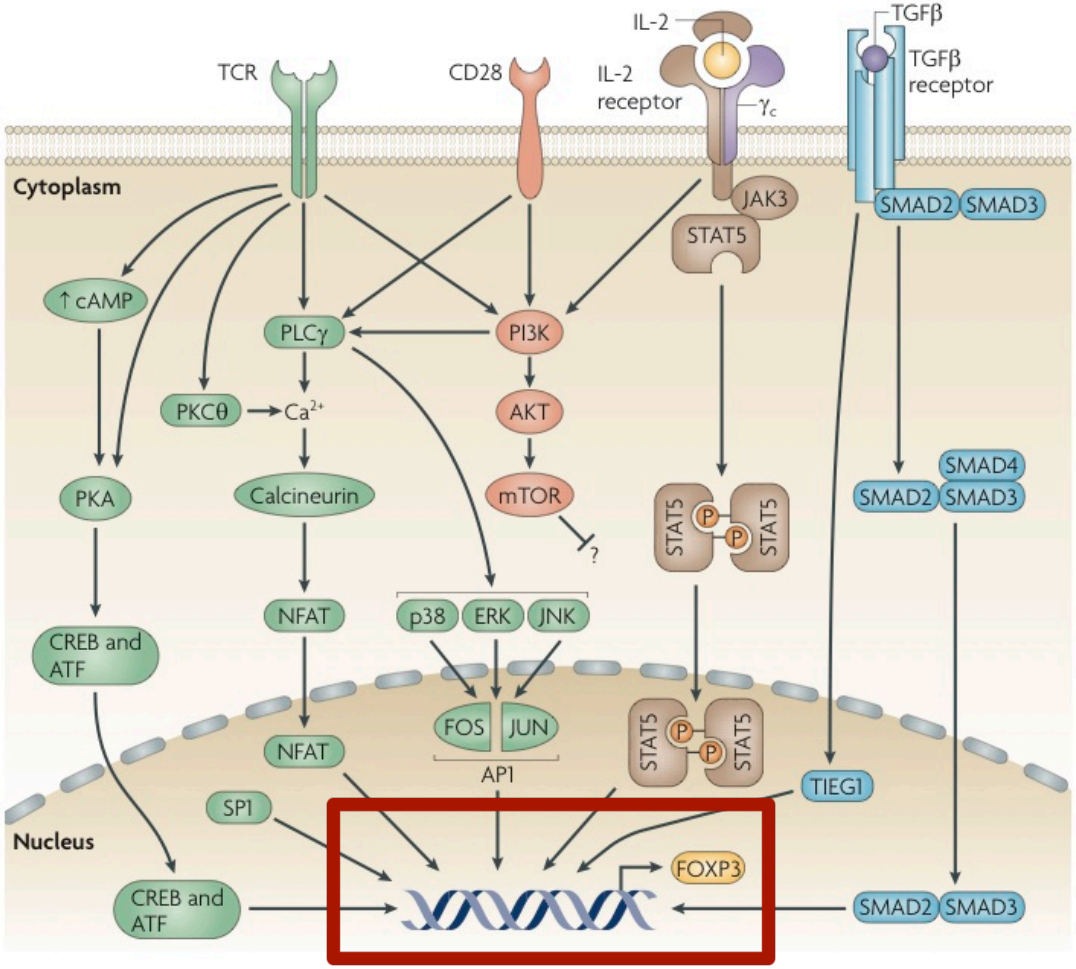


- Treg cells mediate antigen-specific suppression of T cell activation
  - Play a key role in maintaining tolerance
- Naïve T cells can be converted into Treg cells in the periphery
  - High therapeutic potential

Role in cancer



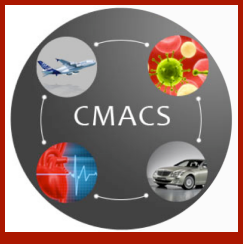
Determinants of differentiation



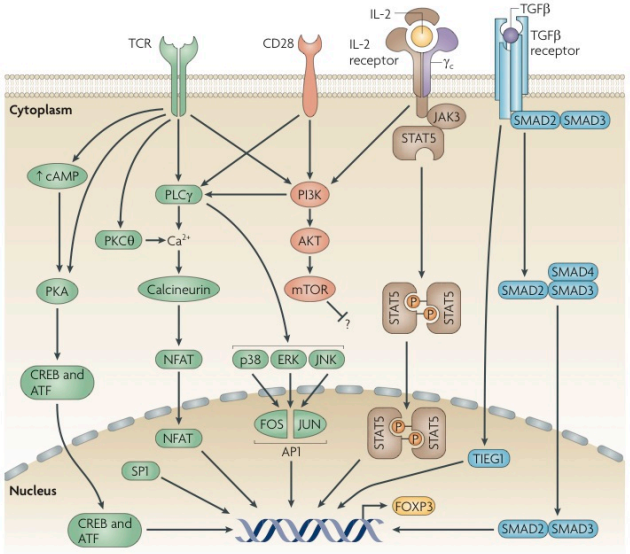
- Determine whether known mechanisms are sufficient to explain experimental observations
- Foxp3 transcription factor is critical for Treg function

Huehn et al. *Nat. Rev. Immunol.* **9**, 83-89 (2009)

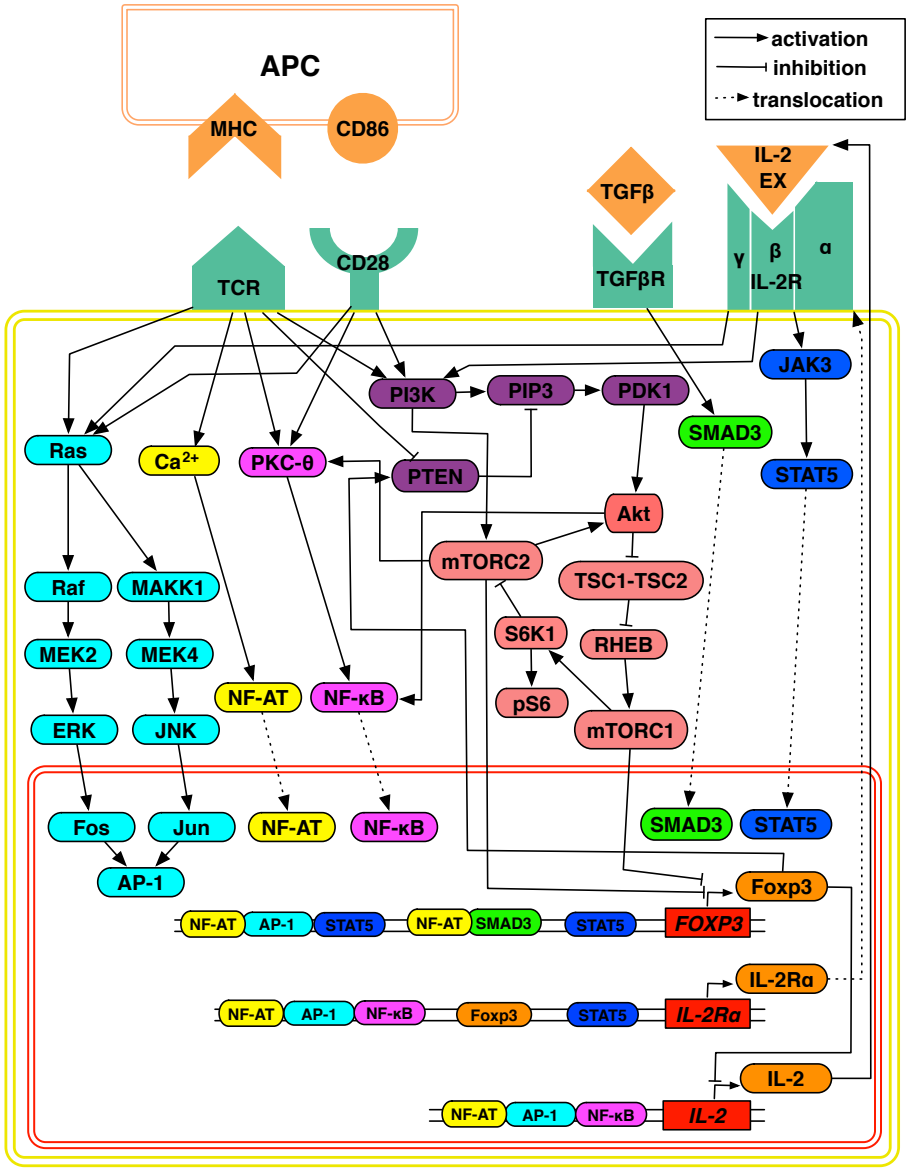
Challenges for Modeling

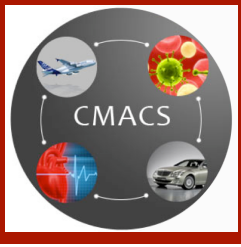


- Large number of components and interactions
- Rapidly evolving list of important components and interactions
  - structural uncertainty in the model
- Involvement of multiple processes
  - signaling
  - gene regulation
  - protein expression
  - (cell division)



Network model



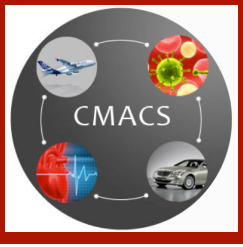


Model elements

Influence sets (Interaction map)

- **Receptors:**
  - T cell receptor (TCR)
  - Co-stimulation through CD28
  - IL-2 receptor (IL-2R)
  - TGF β receptor (TGF β R)
- **Transcription factors:**
  - AP-1, NFAT, NF κ B, SMAD3, STAT5
- **Genes:**
  - IL-2, CD25, Foxp3
- **Other important elements:**
  - PTEN, PI3K, PIP3, PDK1,
  - Akt, mTORC1, mTORC2,
  - TSC1-TSC2, Rheb, S6K1, pS6

Influence sets



Element	Influence set
PI3K	TCR, CD28, IL-2, IL-2R
Akt	PDK1, mTORC2
mTORC1	Rheb, PKC-θ
mTORC2	PI3K, S6K1
Foxp3	NFAT, AP-1, STAT5, Smad3
IL-2	NFAT, AP-1, NFκB, Foxp3
CD25	NFAT, AP-1, NFκB, STAT5, Foxp3
STAT5	IL-2, IL-2R
NFκB	PKC-θ, Akt
Smad3	TGFβ, Akt, mTORC1
PIP3	PI3K, PTEN
Ras	TCR, CD28, IL-2, IL-2R

Element	Influence set
AP-1	Fos, Jun
ERK	Ras
JNK	Ras
Fos	ERK
Jun	JNK
NFAT	Ca
Ca	TCR
PDK1	PIP3
TSC1-TSC2	Akt
Rheb	TSC1-TSC2
S6K1	mTORC1
pS6	S6K1

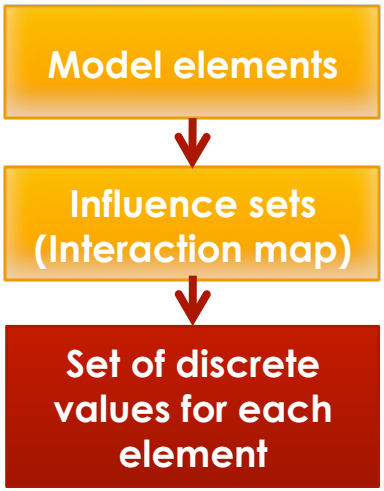
Model elements

↓

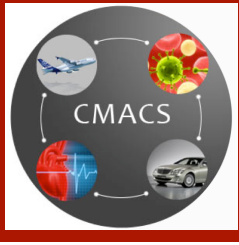
Influence sets (Interaction map)

Circuit design: Variables

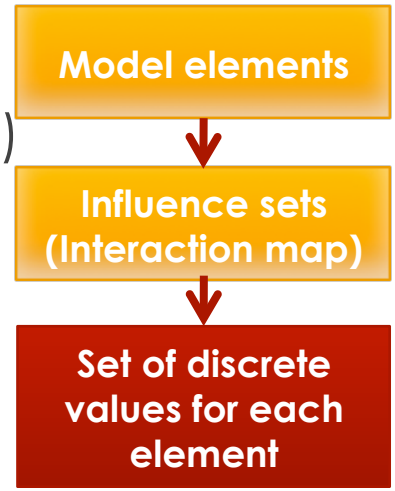
- Number of values for variables
  - Example: three levels for modeling TCR necessary
    - No antigen
    - Low antigen dose
    - High antigen dose



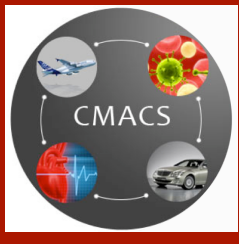
Circuit design: Variables



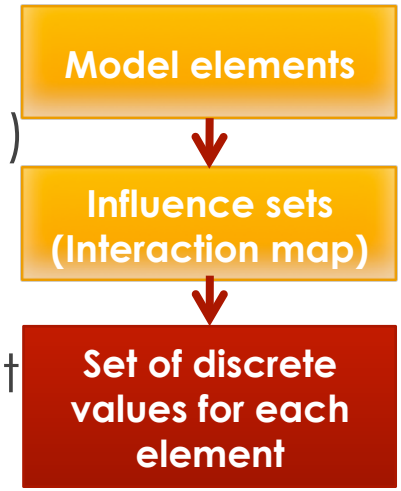
- Number of values for variables
  - Example: three levels for modeling TCR necessary
    - No antigen (TCR_LOW = 0, TCR_HIGH = 0)
    - Low antigen dose (TCR_LOW = 1, TCR_HIGH = 0)
    - High antigen dose (TCR_LOW = 0, TCR_HIGH = 1)
    - encoded with two Boolean variables



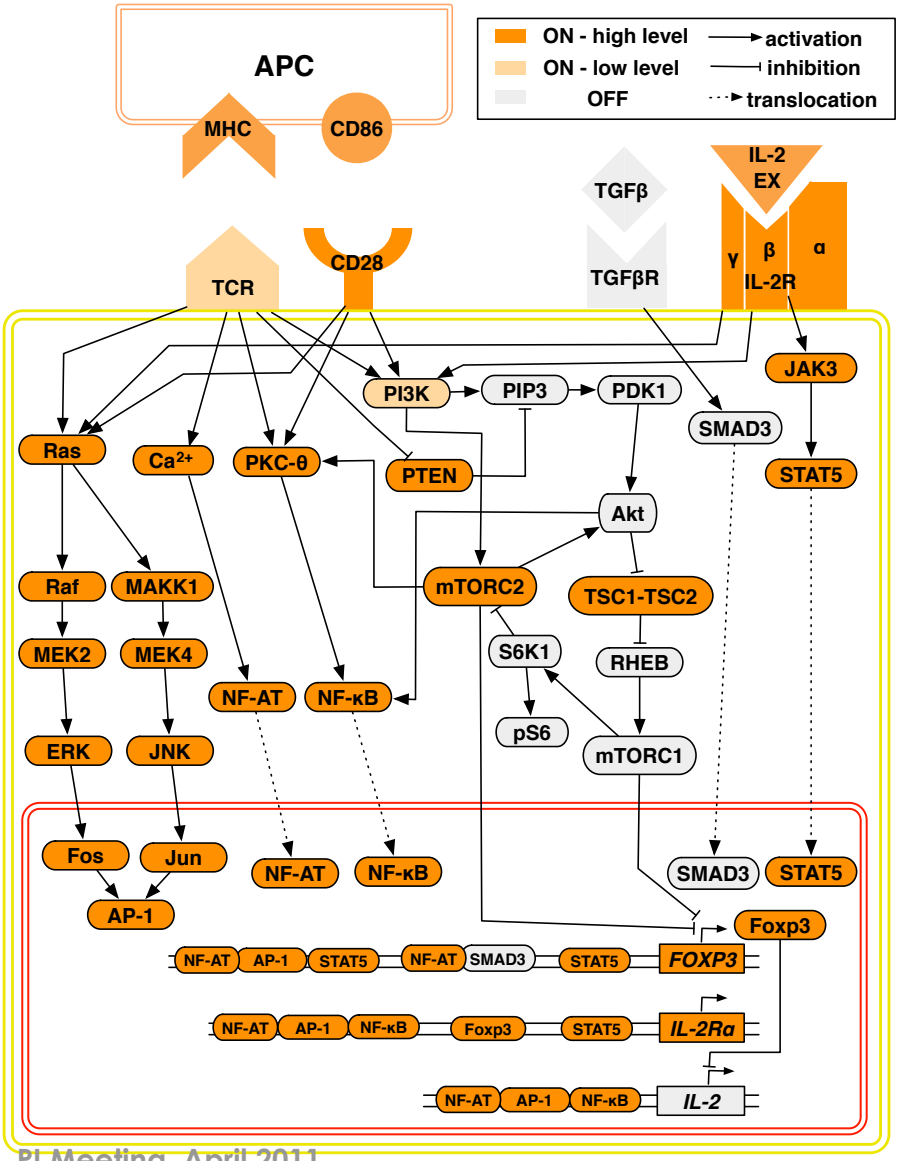
Circuit design: Variables



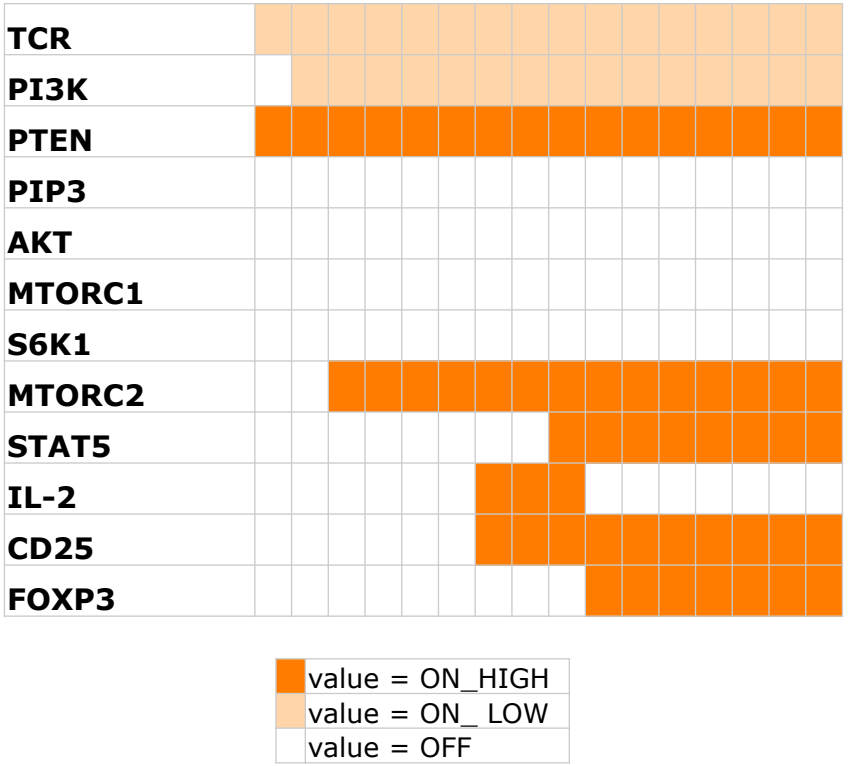
- Number of values for variables
  - Example: three levels for modeling TCR necessary
    - No antigen (TCR_LOW = 0, TCR_HIGH = 0)
    - Low antigen dose (TCR_LOW = 1, TCR_HIGH = 0)
    - High antigen dose (TCR_LOW = 0, TCR_HIGH = 1)
    - encoded with two Boolean variables
  - Example: three levels for modeling PI3K necessary
    - Low and high level of PI3K have different impact on mTORC2



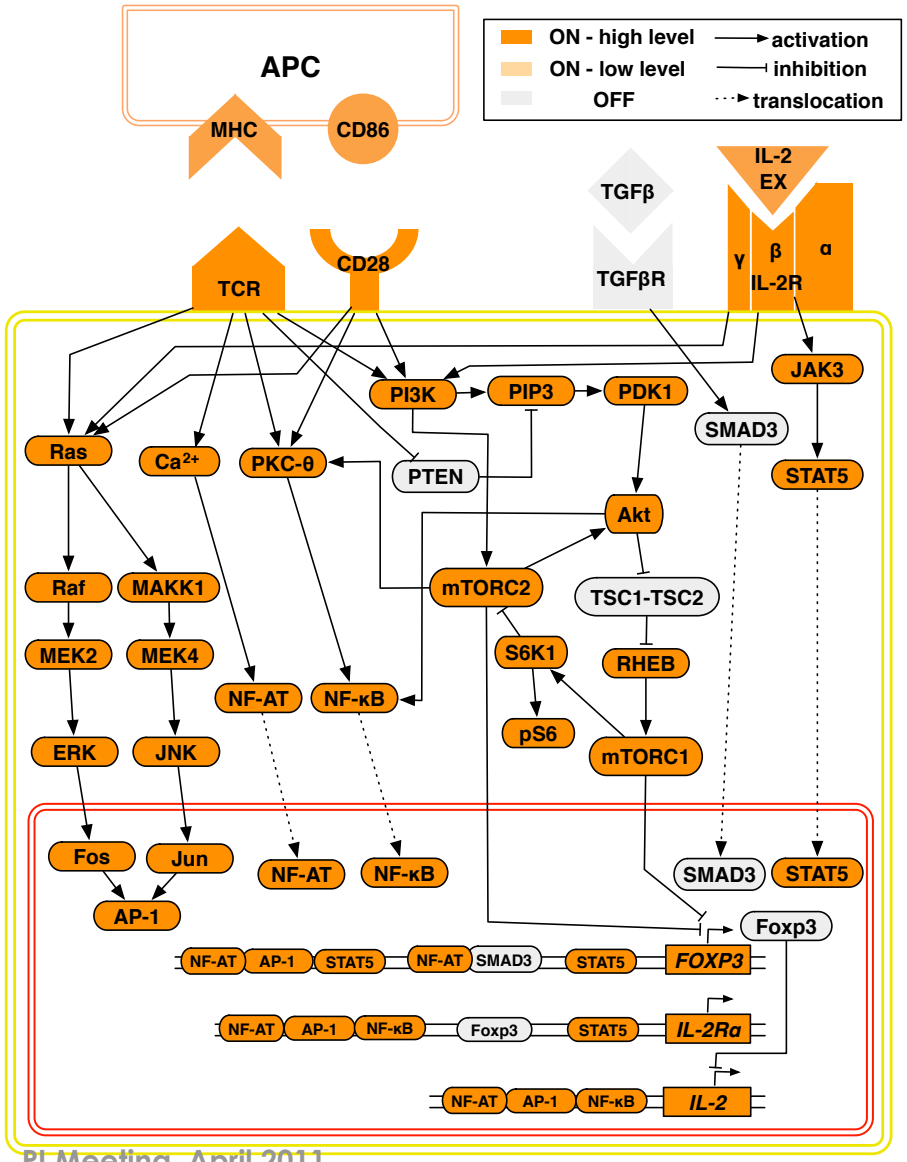
Low Antigen Dose Trajectory



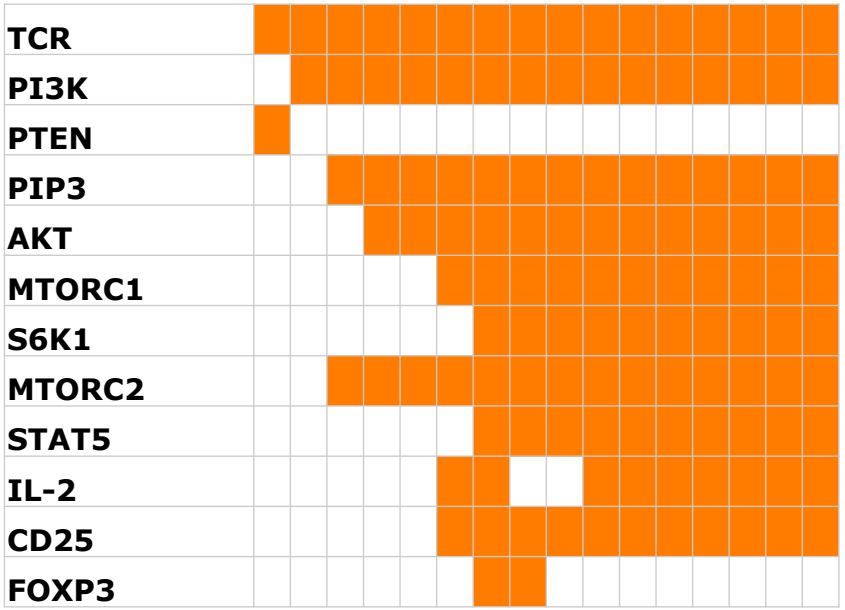
Trajectory Summary

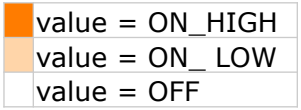


High Antigen Dose Trajectory

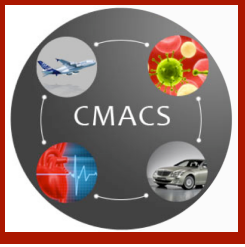


Trajectory Summary





Circuit design: Influence tables



Example 1:
2-level mTORC1

PKC-Θ \ Rheb	0	1
0	0	1
1	0	1

Example 2:
3-level PI3K, 2-level mTORC2

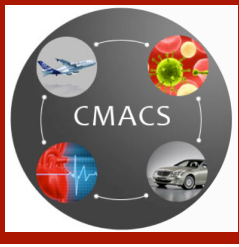
S6K1 \ PI3K	0	1	2
0	0	1	1
1	0	0	1

Example 3: 3-level Foxp3

NFAT, Smad3 \ STAT5,mTOR	00	01	02	10	11	12	20	21	22
00	0	0	0	0	1	2	0	1	2
01	0	0	0	0	0	1	0	1 or 0	1
02	0	0	0	0	0	0	0	0	0
10	0	1	2	1	2	2	1 or 2	2	2
11	0	0	1	0	1	1	0 or 1	1	1
12	0	0	0	0	0	0	0	1 or 0	1
20	1	2	2	2	2	2	2	2	2
21	0	1	1	1	1	1	1	1	2
22	0	0	0	0	0	0	0	1	1

*: Rule 1, *: Rule 2

Example 1: 2-level mTORC1



PKC-Θ \ Rheb	0	1
0	0	0
1	0	1

mTORC1' = Rheb and PKC-θ

'and' rule means both are necessary for activation

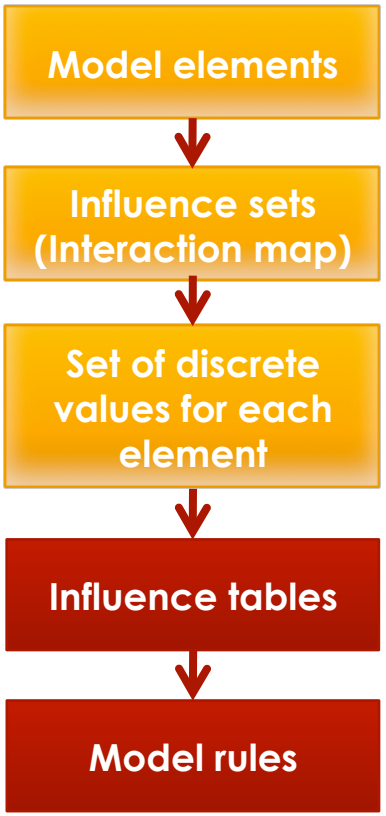
PKC-Θ \ Rheb	0	1
0	0	1
1	0	1

mTORC1' = Rheb

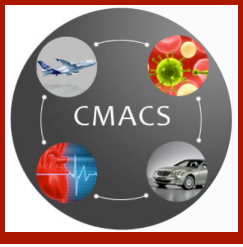
PKC-Θ \ Rheb	0	1
0	0	1
1	1	1

mTORC1' = Rheb or PKC-θ

'or' rule means either one is sufficient for activation



Example 1: 2-level mTORC1



Rheb is the activator, PKC-Θ only strengthens the signal

PKC-Θ \ Rheb	0	1
0	0	0
1	0	1

mTORC1' = Rheb and PKC-θ

'and' rule means both are necessary for activation

PKC-Θ \ Rheb	0	1
0	0	1
1	0	1

mTORC1' = Rheb

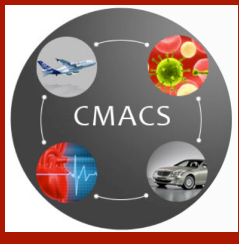
PKC-Θ \ Rheb	0	1
0	0	1
1	1	1

mTORC1' = Rheb or PKC-θ

'or' rule means either one is sufficient for activation

- Model elements
- Influence sets (Interaction map)
- Set of discrete values for each element
- Influence tables
- Model rules

Example 1: 2-level mTORC1



Rheb is the activator, PKC-Θ only strengthens the signal

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0	0	1
1	0	1

mTORC1' = Rheb

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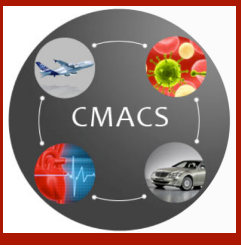

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PKC-Θ \ Rheb	0	1
0	0	0
1	0	1

mTOR… ' = Rheb and PKC-θ

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PKC-Θ \ Rheb	0	1
0	0	1
1	0	1

mTORC1' = Rheb

PKC-Θ \ Rheb	0	1
0	0	1
1	1	1

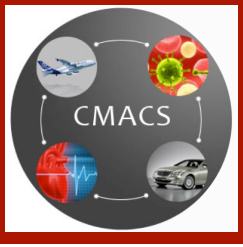
mTORC1' = Rheb or PKC-θ

'or' rule means either one is sufficient for activation

CASE I: include this rule in the model

CASE II: increase the number of values to represent mTORC1

Example 2:
3-level PI3K, 2-level mTORC2



S6K1 \ PI3K	0	1	2
0	0	1	1
1	0	0	1

S6K1 = 0

PI3K_LOW \ PI3K_HIGH	0	1
0	0	1
1	1	X

S6K1 = 1

PI3K_LOW \ PI3K_HIGH	0	1
0	0	1
1	0	X

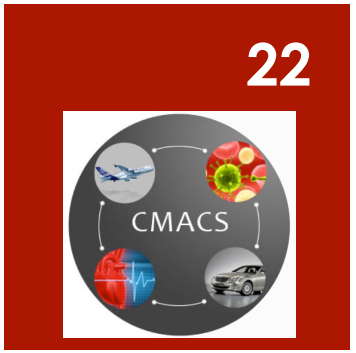
mTORC2' = PI3K_HIGH or (PI3K_LOW and not S6K1)

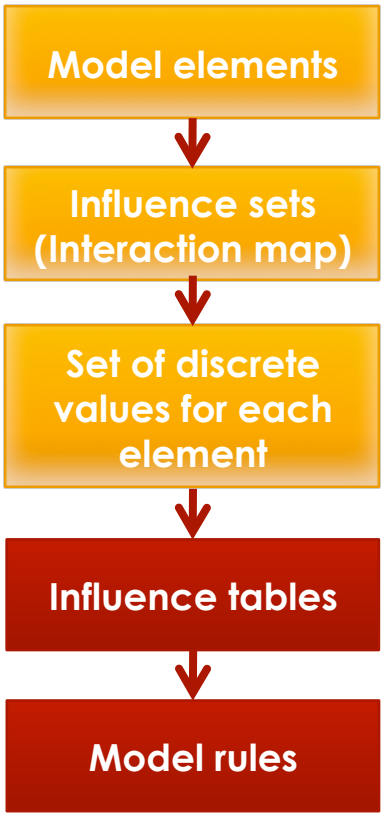
- Model elements
- Influence sets (Interaction map)
- Set of discrete values for each element
- Influence tables
- Model rules

Example 3: 3-level Foxp3

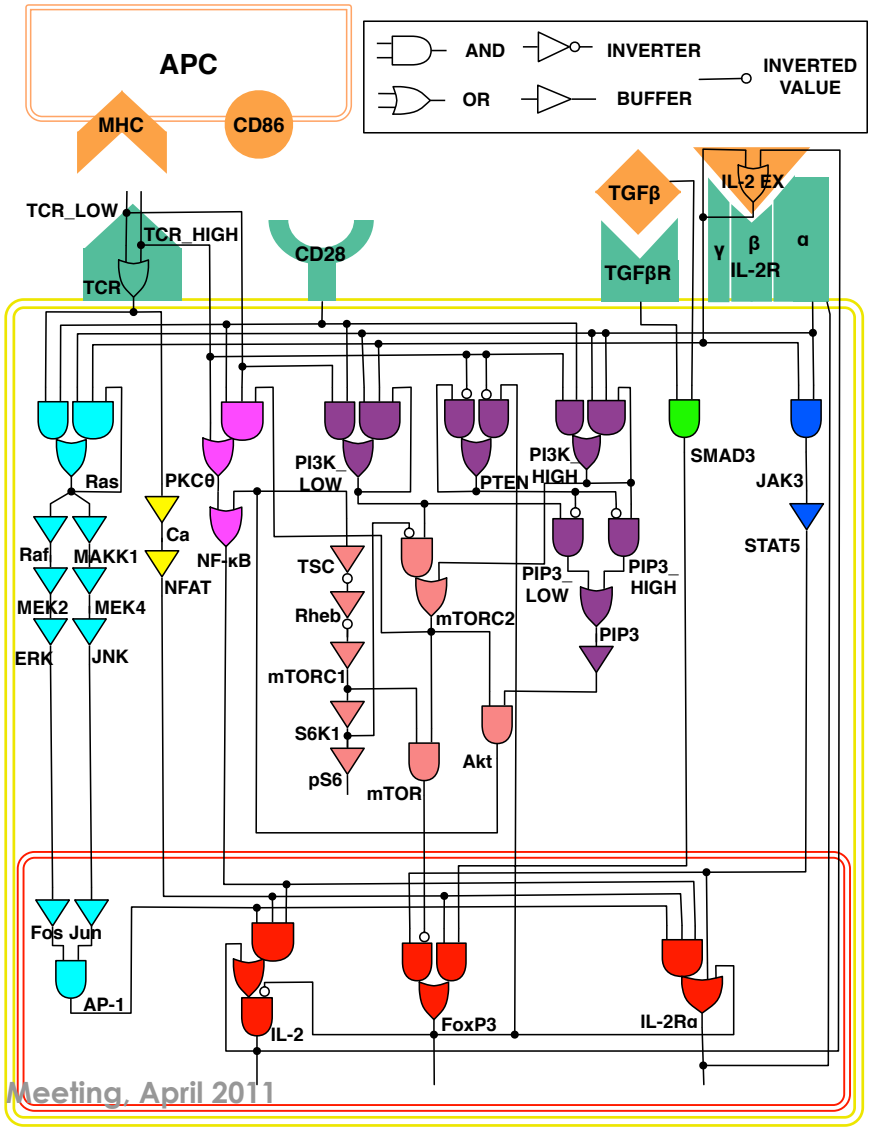
STAT5,mTOR / NFAT, Smad3	00	01	02	10	11	12	20	21	22
00	0	0	0	0	1	2	0	1	2
01	0	0	0	0	0	1	0	1 or 0	1
02	0	0	0	0	0	0	0	0	0
10	0	1	2	1	2	2	1 or 2	2	2
11	0	0	1	0	1	1	0 or 1	1	1
12	0	0	0	0	0	0	0	1 or 0	1
20	1	2	2	2	2	2	2	2	2
21	0	1	1	1	1	1	1	1	2
22	0	0	0	0	0	0	0	1	1

FOXP3_HIGH' = (STAT5_LOW and AP1NFAT_HIGH and not MTORC1_HIGH and not MTORC1_LOW) or (STAT5_HIGH and AP1NFAT_HIGH and not MTORC1_HIGH and not MTORC1_LOW) or (STAT5_LOW and AP1NFAT_LOW and SMAD3_LOW and not MTORC1_HIGH and not MTORC1_LOW) or (AP1NFAT_HIGH and SMAD3_LOW and not MTORC1_HIGH and not MTORC1_LOW) or (AP1NFAT_HIGH and SMAD3_HIGH and not MTORC1_HIGH and not MTORC1_LOW) or (STAT5_LOW and SMAD3_HIGH and not SMAD3_LOW and not MTORC1_HIGH and not MTORC1_LOW) or (STAT5_HIGH and SMAD3_HIGH and not SMAD3_LOW and not MTORC1_HIGH and not MTORC1_LOW) or (AP1NFAT_LOW and SMAD3_HIGH and not MTORC1_HIGH and not MTORC1_LOW) or (STAT5_HIGH and not STAT5_LOW and AP1NFAT_HIGH and not AP1NFAT_LOW and SMAD3_HIGH and not SMAD3_LOW and MTORC1_LOW) or (STAT5_HIGH and AP1NFAT_LOW and SMAD3_LOW and not MTORC1_HIGH and not MTORC1_LOW)

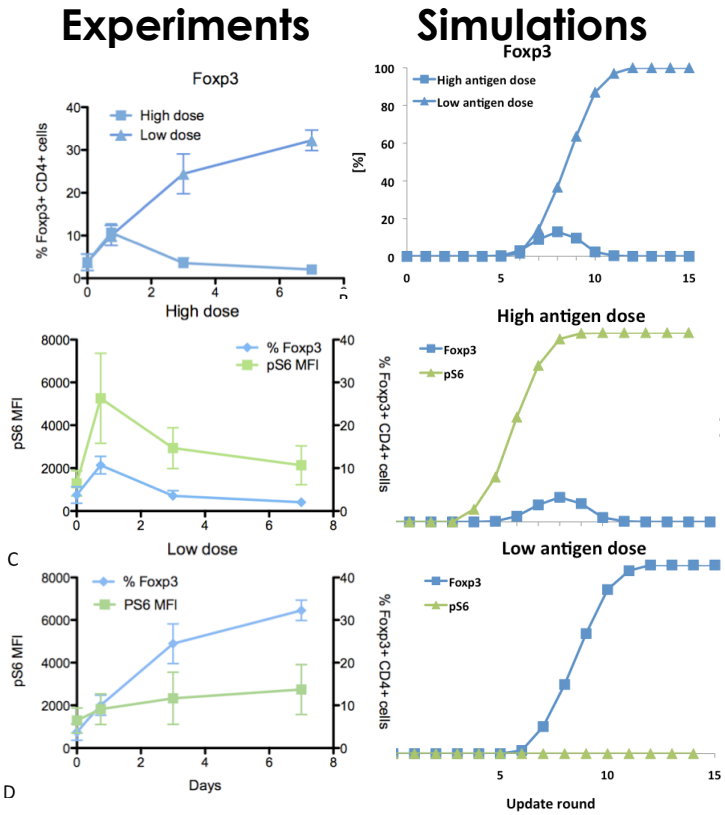




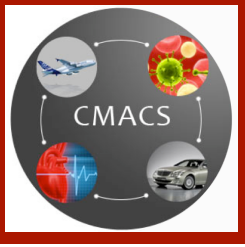
Circuit Model of T Cell Differentiation



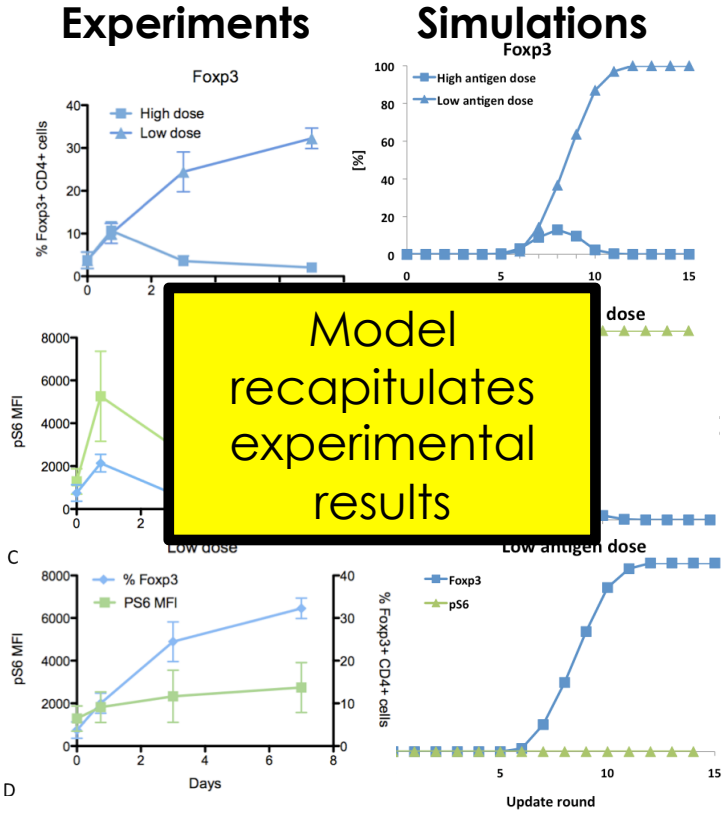
- Computable model of cell dynamics
- Two simulation modes
  - **Synchronous**
    - Variables updated simultaneously
    - *Deterministic*
  - **Asynchronous**
    - Variables updated one at a time in random order
    - *Stochastic*



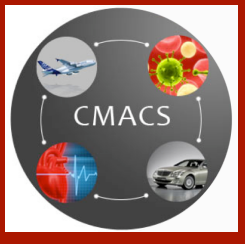
Four scenarios



- High antigen dose
- Low antigen dose

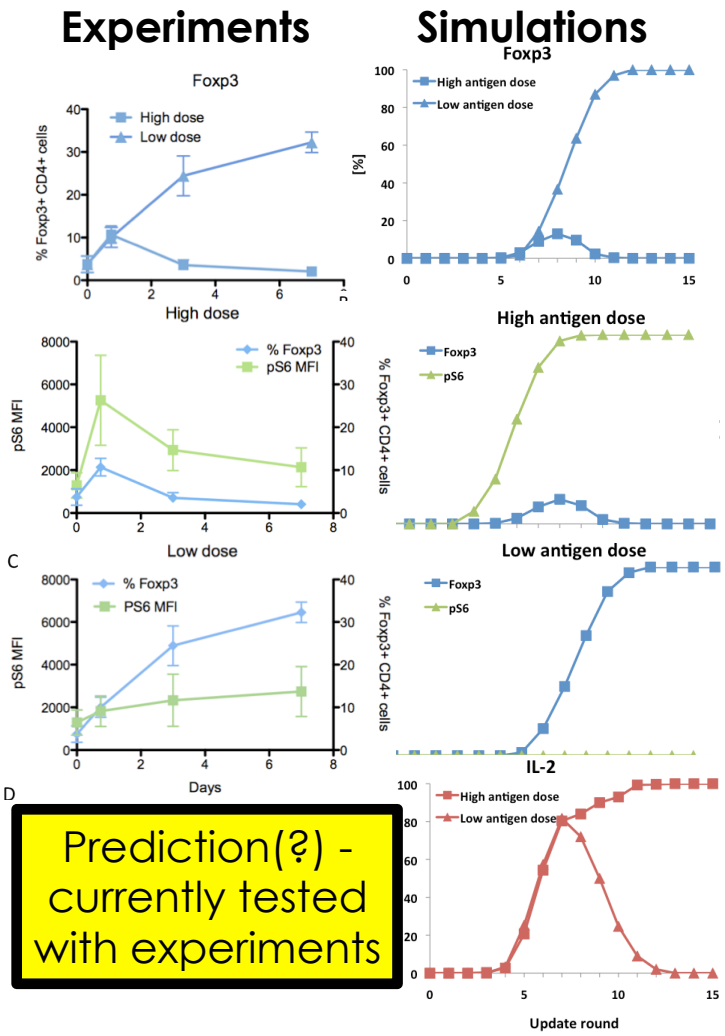


Four scenarios



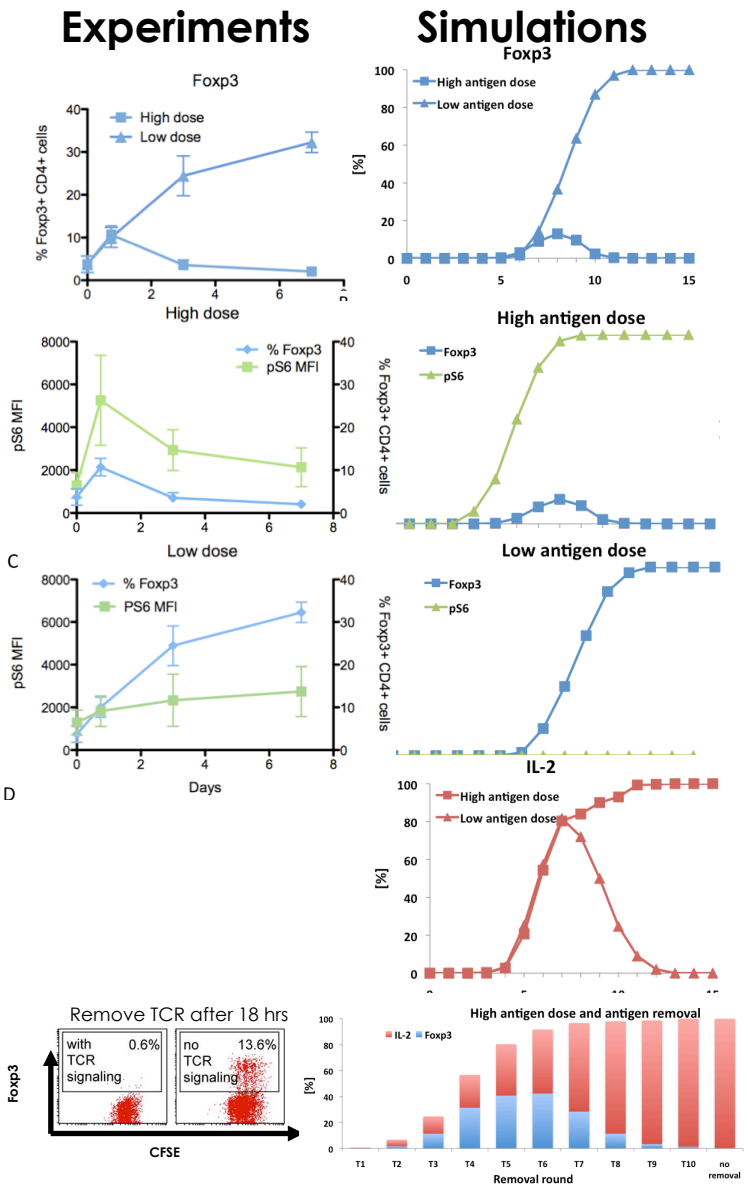
- **High antigen dose**
- **Low antigen dose**

Four scenarios



Prediction(?) - currently tested with experiments

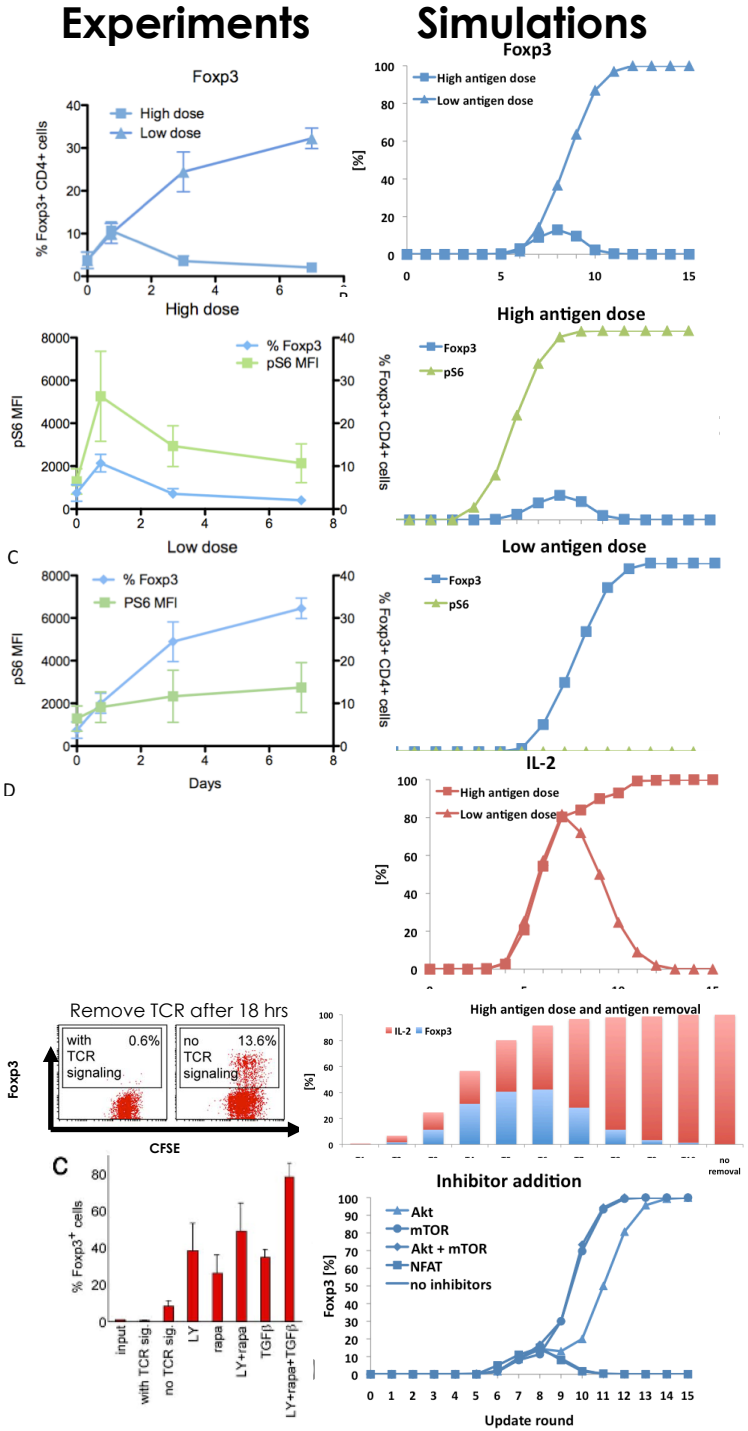
- High antigen dose
- Low antigen dose



Four scenarios

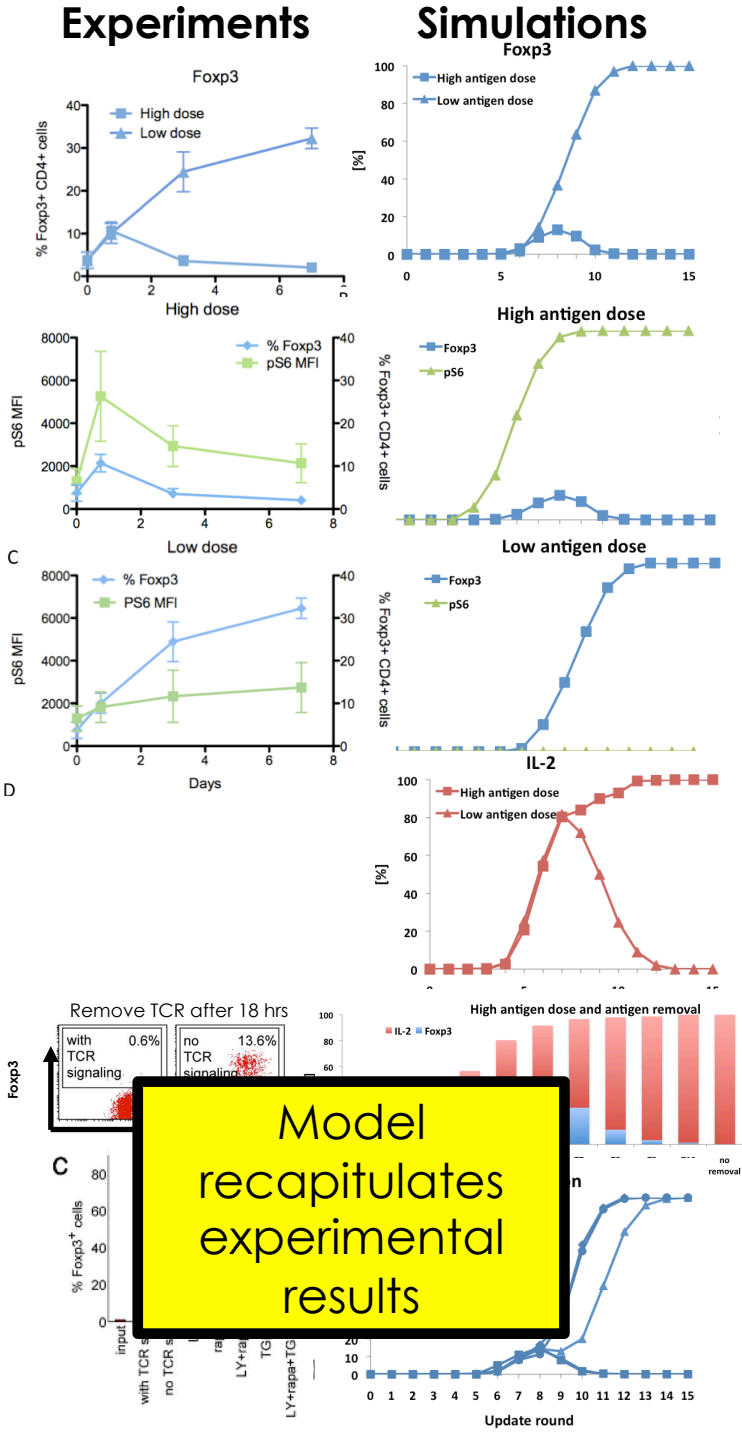
- High antigen dose
- Low antigen dose
- **High antigen dose, then removed**

Four scenarios



- High antigen dose
- Low antigen dose
- High antigen dose, then removed
- **High antigen dose, then inhibitors added**

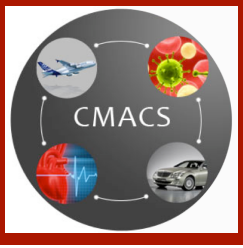
Four scenarios



Model recapitulates experimental results

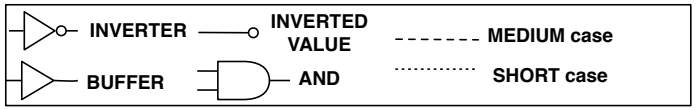
- High antigen dose
- Low antigen dose
- High antigen dose, then removed
- **High antigen dose, then inhibitors added**

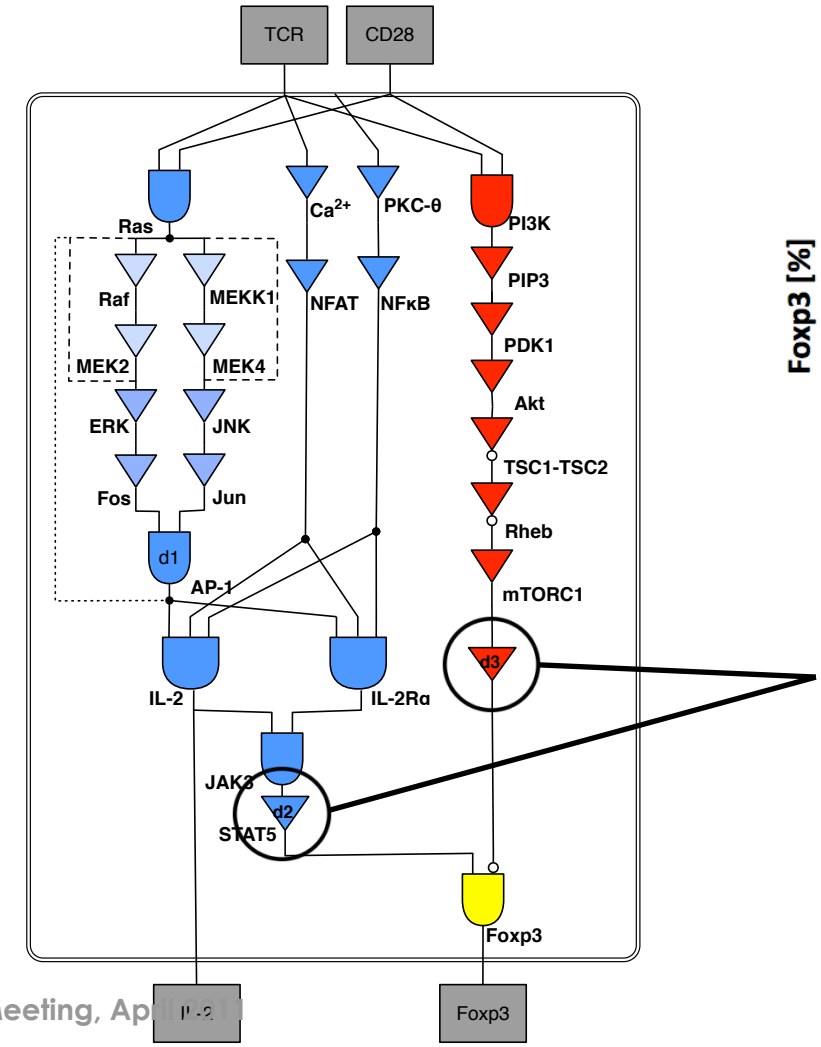
Analysis of Circuit Delays

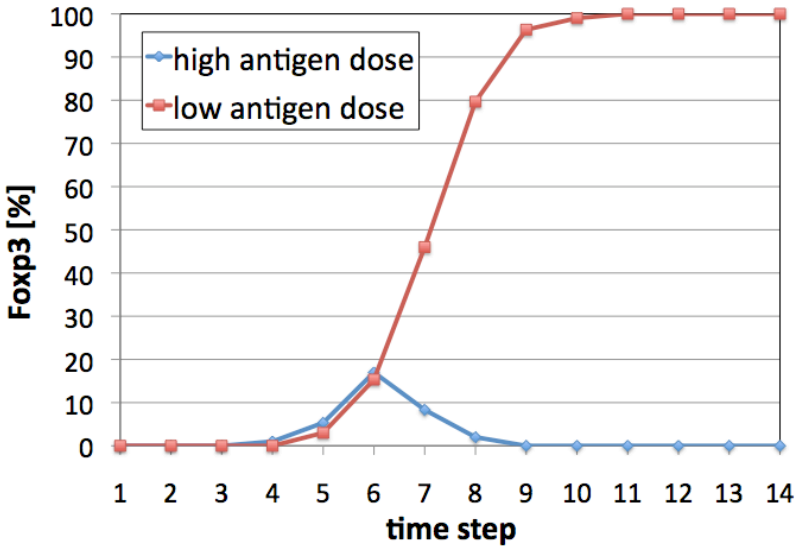


- Model simulations point to the importance of timing in Foxp3 activation and fate selection

Analysis of Circuit Delays

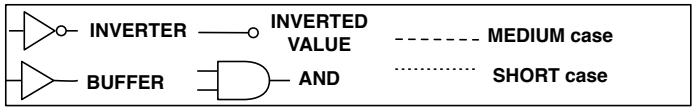


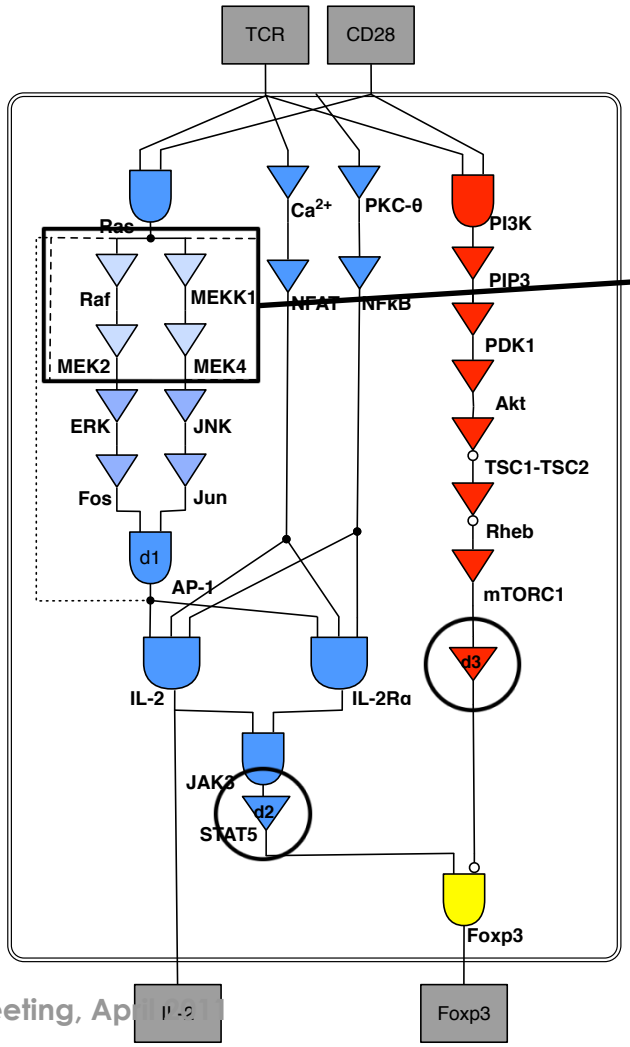


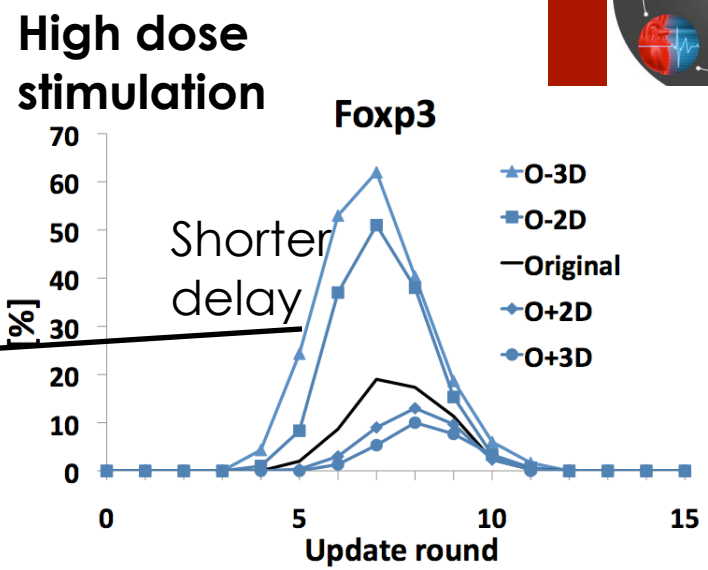


Race determines whether Foxp3 will be expressed with high dose stimulation

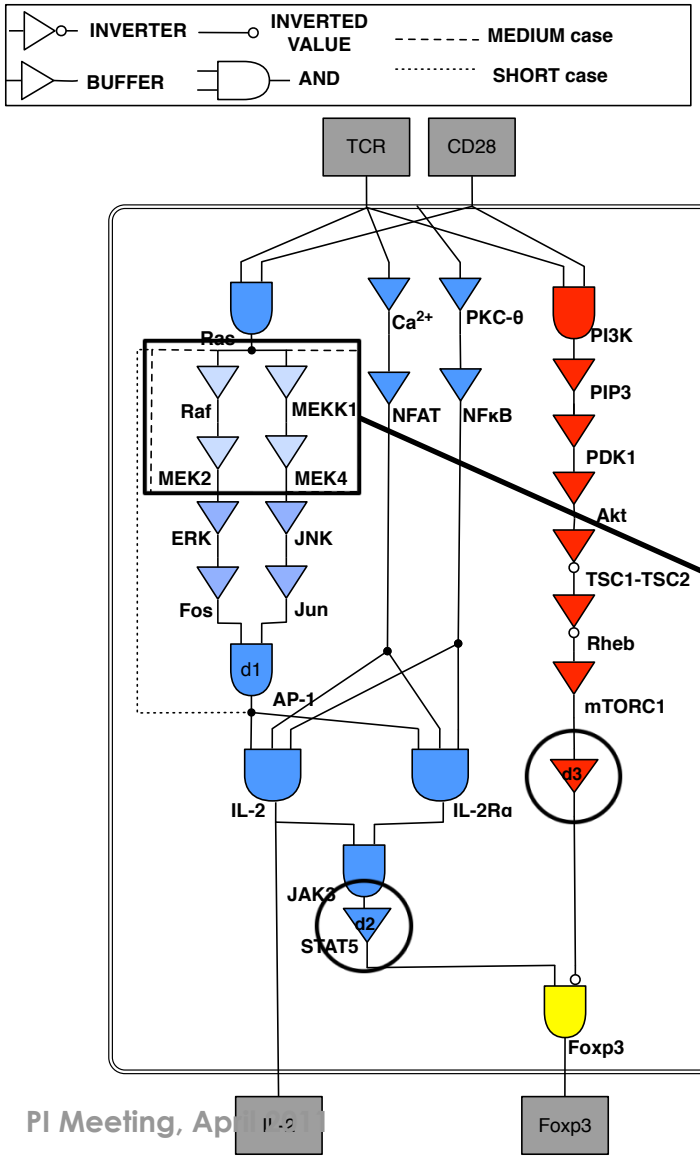
Analysis of Circuit Delays



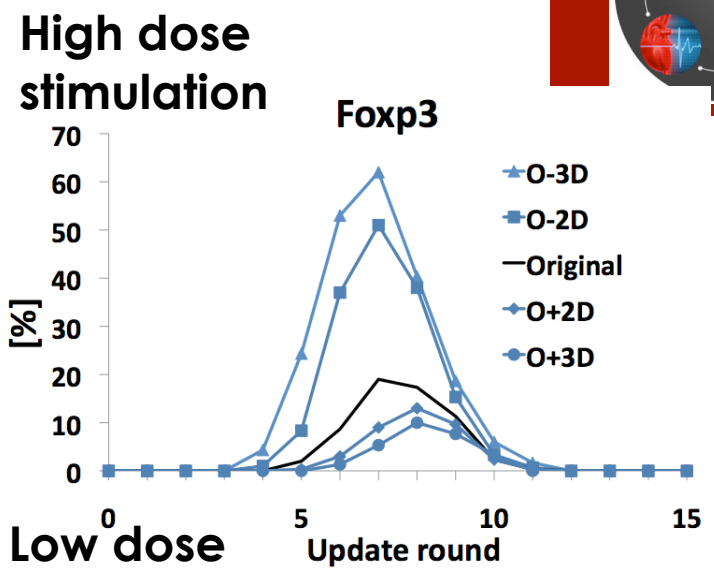


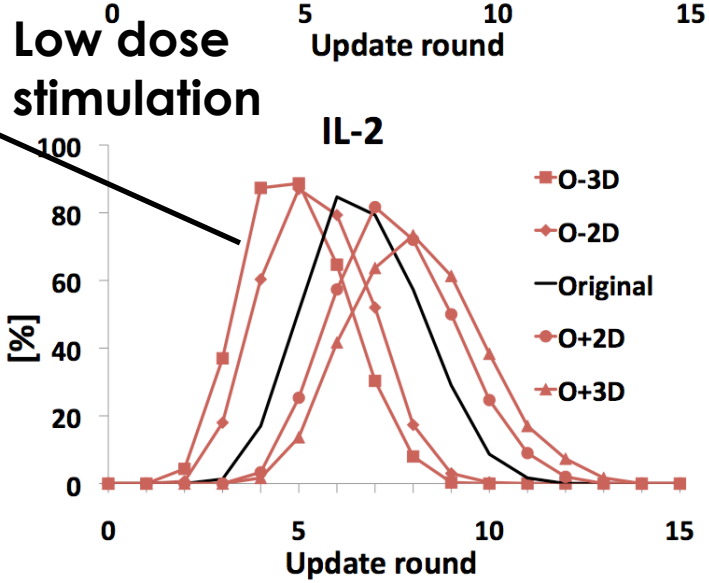


Analysis of Circuit Delays

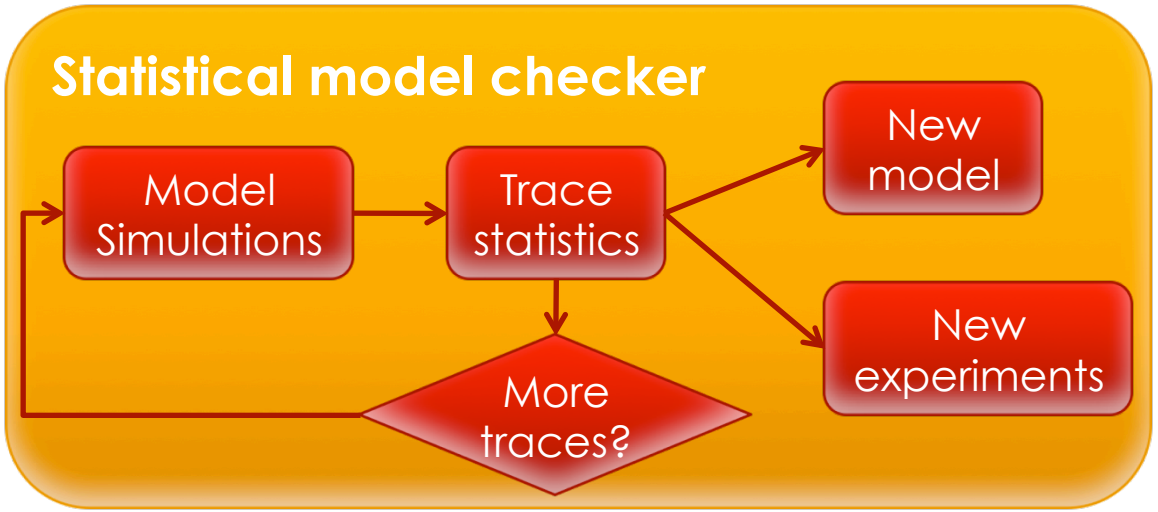


Shorter delay





Further system studies

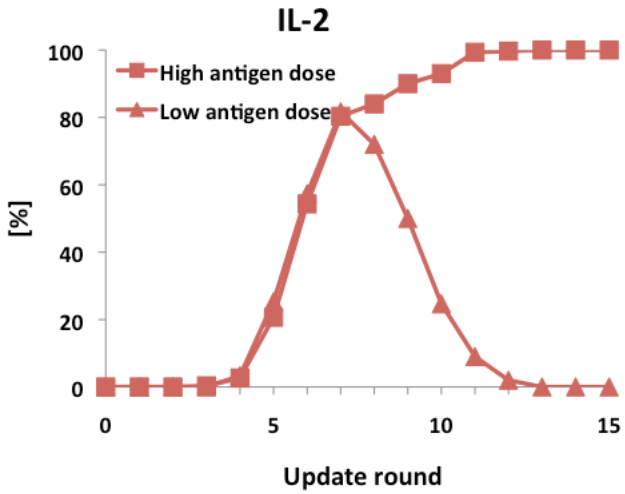


- Low antigen those query:

Does IL-2 always go to 1?

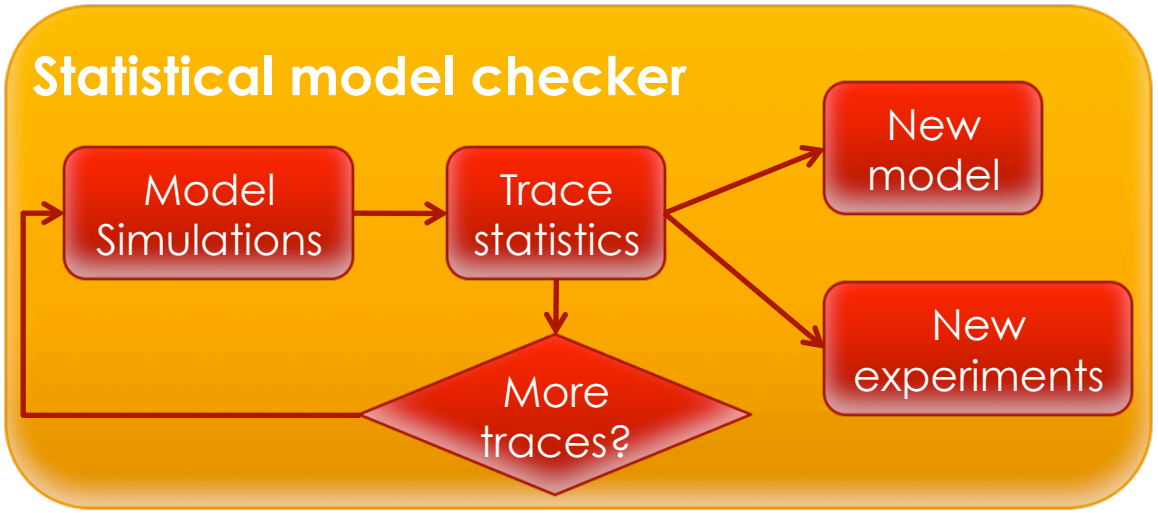
Property: F[20] (IL2 == 1)

Test: BEST 0.001 0.999



Result: estimated probability close to 1

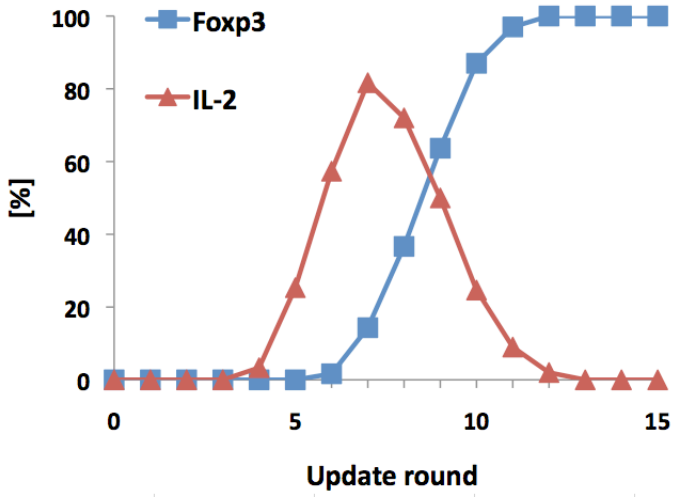
Further system studies



- Low antigen those query:
  - **Probability that IL-2 stays at 0 before Foxp3 becomes 1**?

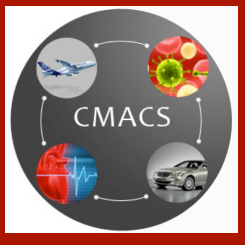
    Property: (IL2 == 0) U[15] (FOXP3 == 1)

    Test: BEST 0.0001 0.999



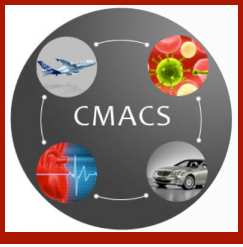
Result: estimated probability = 0.00147– **rare event**

Further system studies



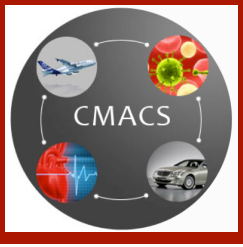
- More queries:
  - High antigen dose:
    Probability of STAT5 being activated before mTORC2
  - Low antigen dose:
    Number of steps IL2 stays active before Foxp3 activation
  - Antigen removal:
    Probability of initial CD25 oscillations
    Probability of PTEN activation
    Probability of initial PTEN and Foxp3 oscillation

Further system studies

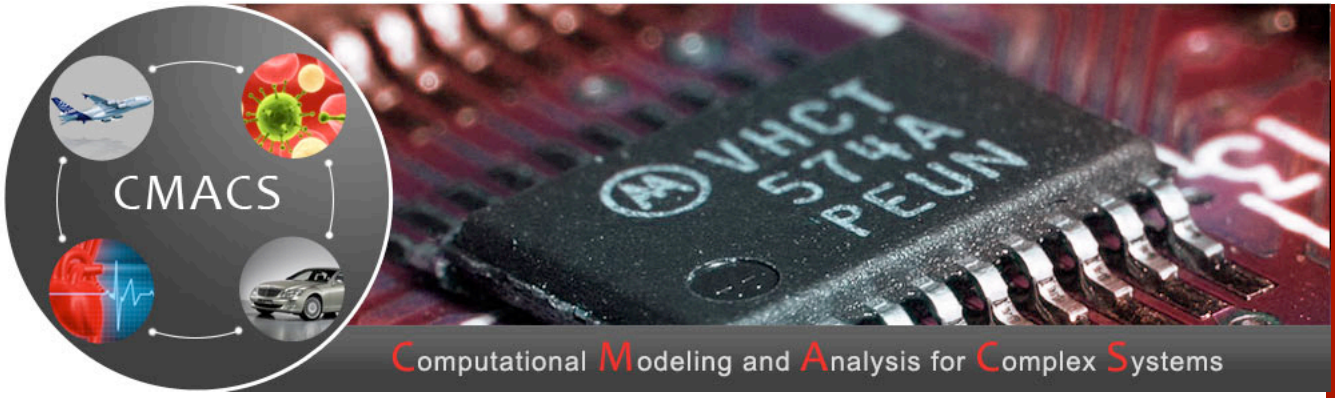


- Next step: **Multi-valued model**
  - Studying simulation results complex and time consuming
  - Many interesting properties to test, for example:
    Effects of different stimulation vs. co-stimulation levels
    Effects of PKC-Θ on mTORC1
    Dumped oscillations in negative mTORC1/mTORC2 loop

Conclusions



- Logical modeling approach allows development of comprehensive models of cell fate
  - Model of peripheral T cell differentiation recapitulates a wide range of experimental observations
  - Circuit analysis reveals key elements of the mechanism for Foxp3 expression
    - Timing of STAT5 vs. mTOR
    - Critical role of PTEN
    - Negative feedback between mTORC1 and mTORC2
- **Logical modeling + Statistical model checking**
  - Gain further insights about the systems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Computational Modeling and Analysis for Complex Systems

Thank you!