# Mechanistic Insights into Sensitization and Desensitization of IFNα Signaling and Effects on Patient Treatment Strategies

## ROSA Worldwide Webinar Series

**Dr. Marcus Rosenblatt**

*Data Analysis and Modeling of Dynamic Processes in the Life Sciences*
Institute of Physics, University of Freiburg, Germany

July 12, 2023

# Outline

## (I) Sensitization in IFNα signaling

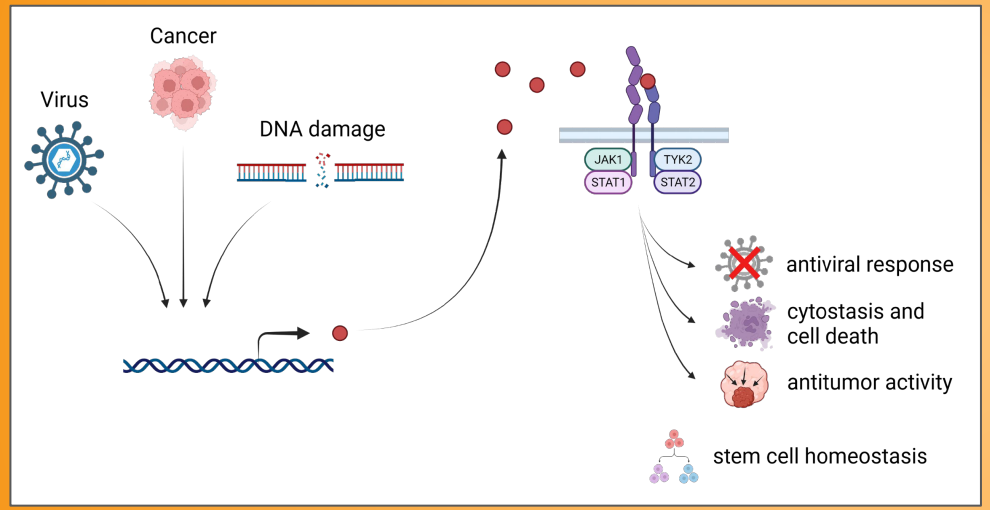
(a) ODE model application ⟷ (b) Methodological development

- Mechanistic insights
- Selected publications from the Timmer group

## (II) Application to Patient-derived Human Hepatocytes (PHH)

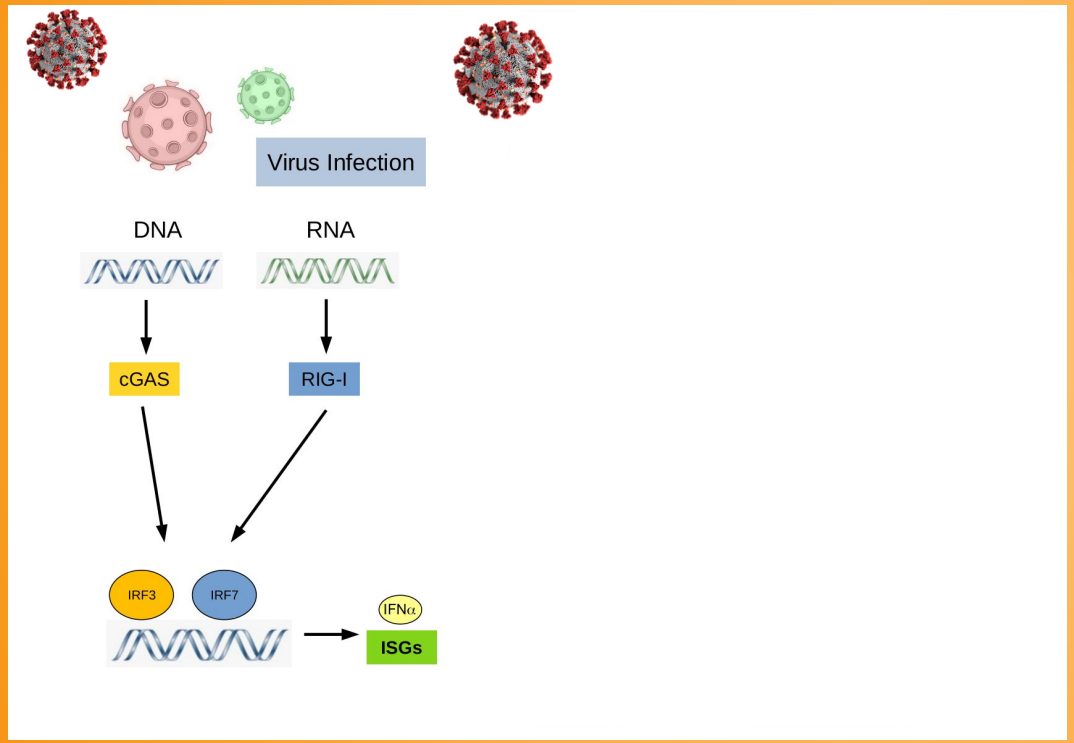
- USP18 as a biomarker for sensitization threshold
- Model adaptations and extensions
- Pharmacokinetics
- Treatment Strategies

# Interferons – why and where they matter?



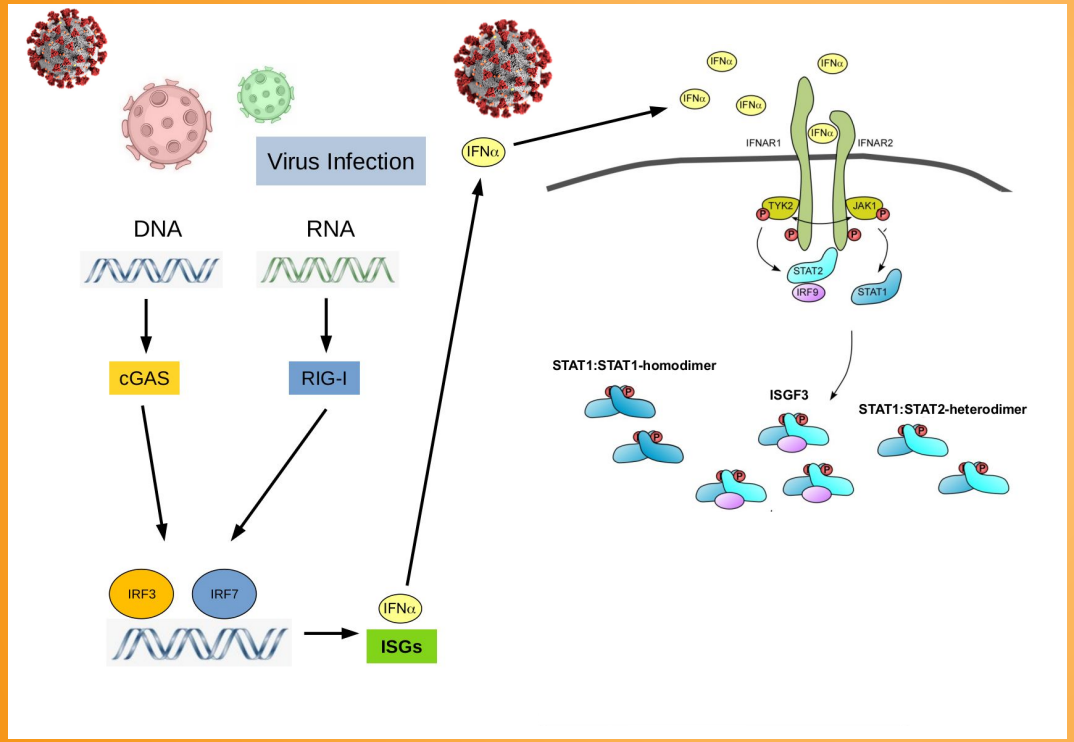

- Therapeutic IFNα used against chronic viral infections (e.g. HBV, HCV), but some patients do not respond
- Signaling pathway is known to desensitize upon activation
- ODE modeling approach to understand the mechanistic origin of desensitization

# IFNα signaling – Part of Innate Immune Response



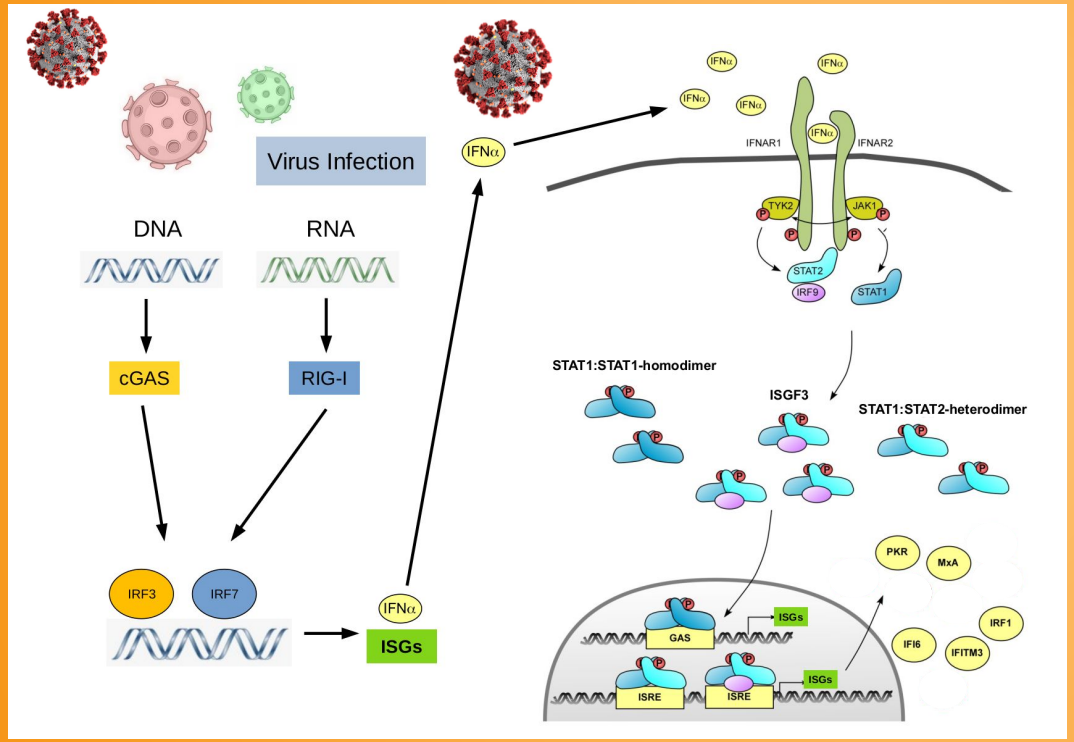

- Pattern recognition receptors sense viral particles
- Interferons (IFNα) and Interferon stimulated genes (ISGs) are activated

# IFNα signaling – Part of Innate Immune Response



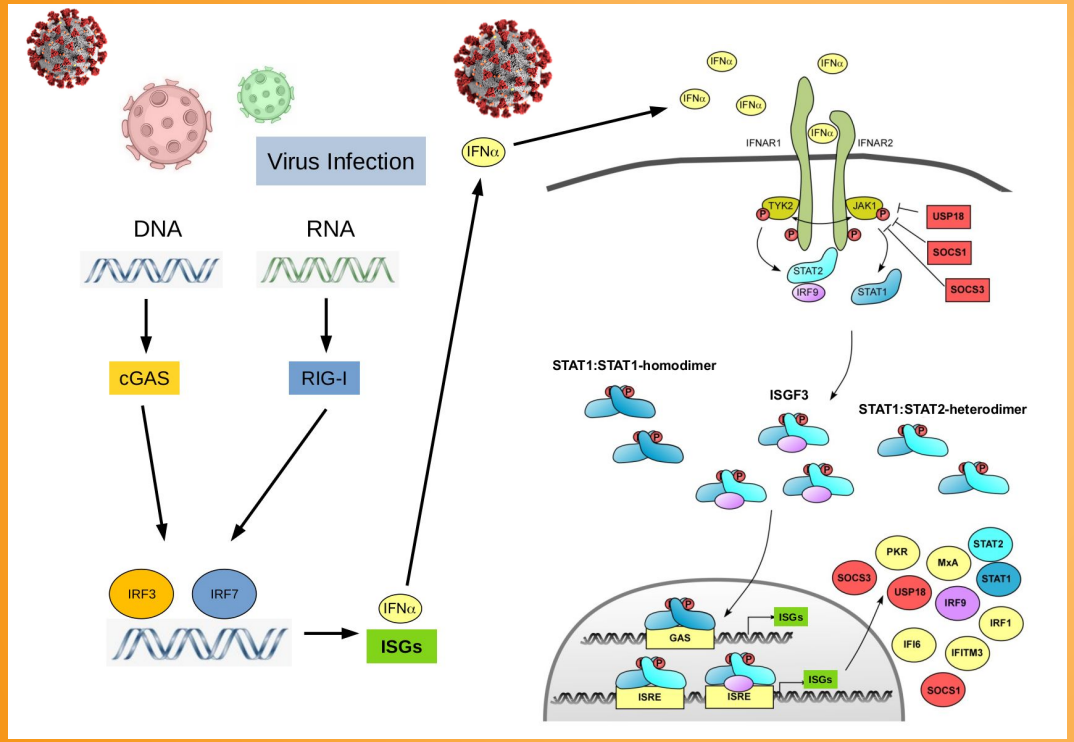

- Pattern recognition receptors sense viral particles
- Interferons (IFNα) and Interferon stimulated genes (ISGs) are activated
- IFNα activates signaling pathway, Formation of STAT complexes

# IFNα signaling – Part of Innate Immune Response



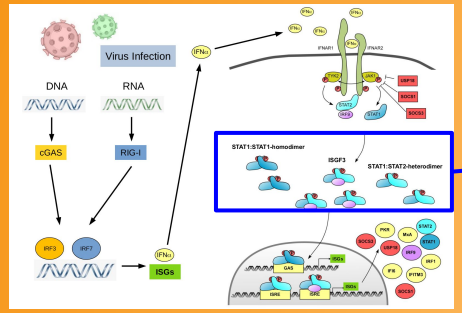

- Pattern recognition receptors sense viral particles
- Interferons (IFNα) and Interferon stimulated genes (ISGs) are activated
- IFNα activates signaling pathway, Formation of STAT complexes
- Binding to **GAS** and **ISRE**, Activation of further ISGs

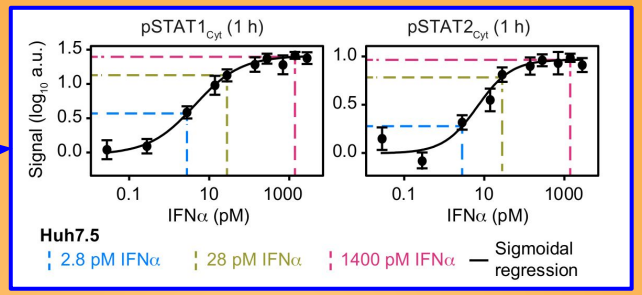
# IFNα signaling – Part of Innate Immune Response




- Pattern recognition receptors sense viral particles
- Interferons (IFNα) and Interferon stimulated genes (ISGs) are activated
- IFNα activates signaling pathway, Formation of STAT complexes
- Binding to **GAS** and **ISRE**, Activation of further ISGs
- Induction of negative feedbacks SOCS1, SOCS3 and USP18
- Induction of positive feedbacks STAT1, STAT2, and IRF9

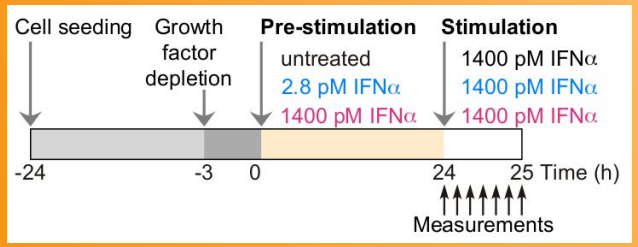
# Pathway sensitization

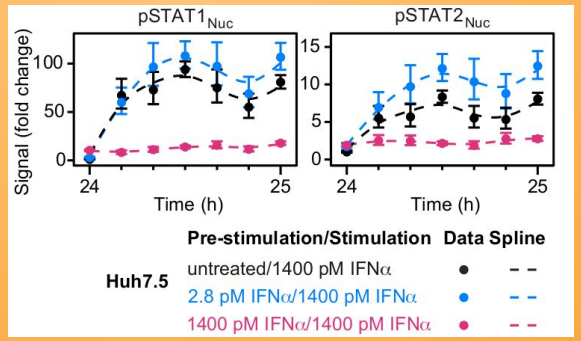



**Definition:** Low dose High dose

## Sensitization Experiments:



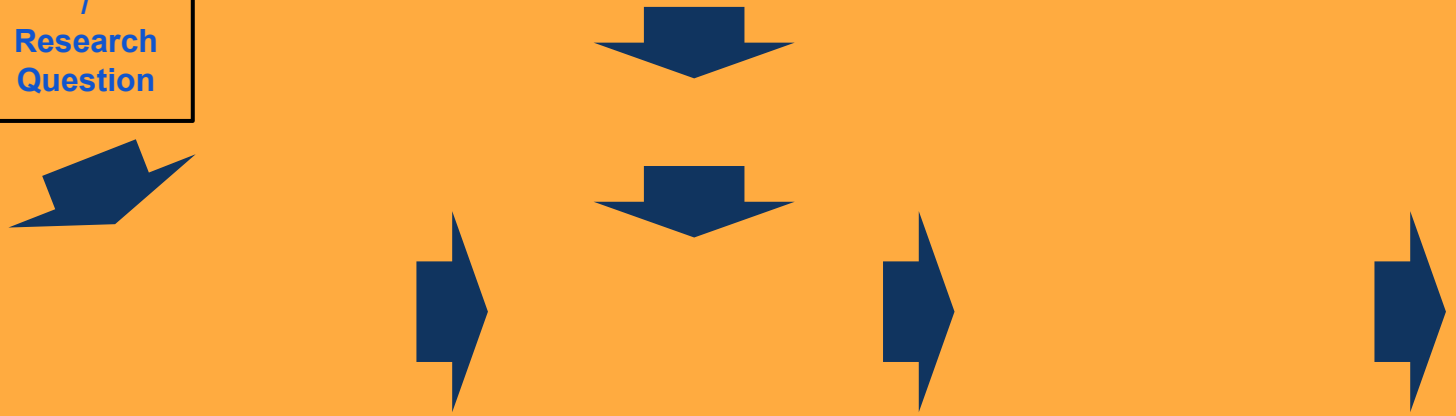




Pre-stimulation with a

- Low dose → Priming / Hypersensitization
- High dose → Desensitization

# A typical modeling project

Biological Hypothesis / Research Question



Biological Validation / Mechanistic and Quantitative Understanding

# A typical modeling project

**Biological Hypothesis / Research Question**

**(Optimal) Experimental Design**

**Experimental Data**

**Preprocessing (e.g. BlotIt )**

**More data / different targets needed**

## I. ODE Model Generation/Building

- Cartoon → Model
- Mechanistic Model
- Michaelis Menten
- Hill-Kinetics
- Linear Chain Trick
- (Structural) Identifiability Analysis
- …

## II. Model Parameter Fitting, (Optimization)

- Observation-Function Definition
- Optimization
- Numerical integration
- LHS-/Waterfall-Plot
- $L_1$ regularization
- …

## III. Uncertainty Analysis / Identifiability Analysis

- Profile Likelihood
- (Practical) Identifiability Analysis
- …

## IV. Model Predictions

- Prediction Profile Likelihood (PPL)
- (Validation)
- …

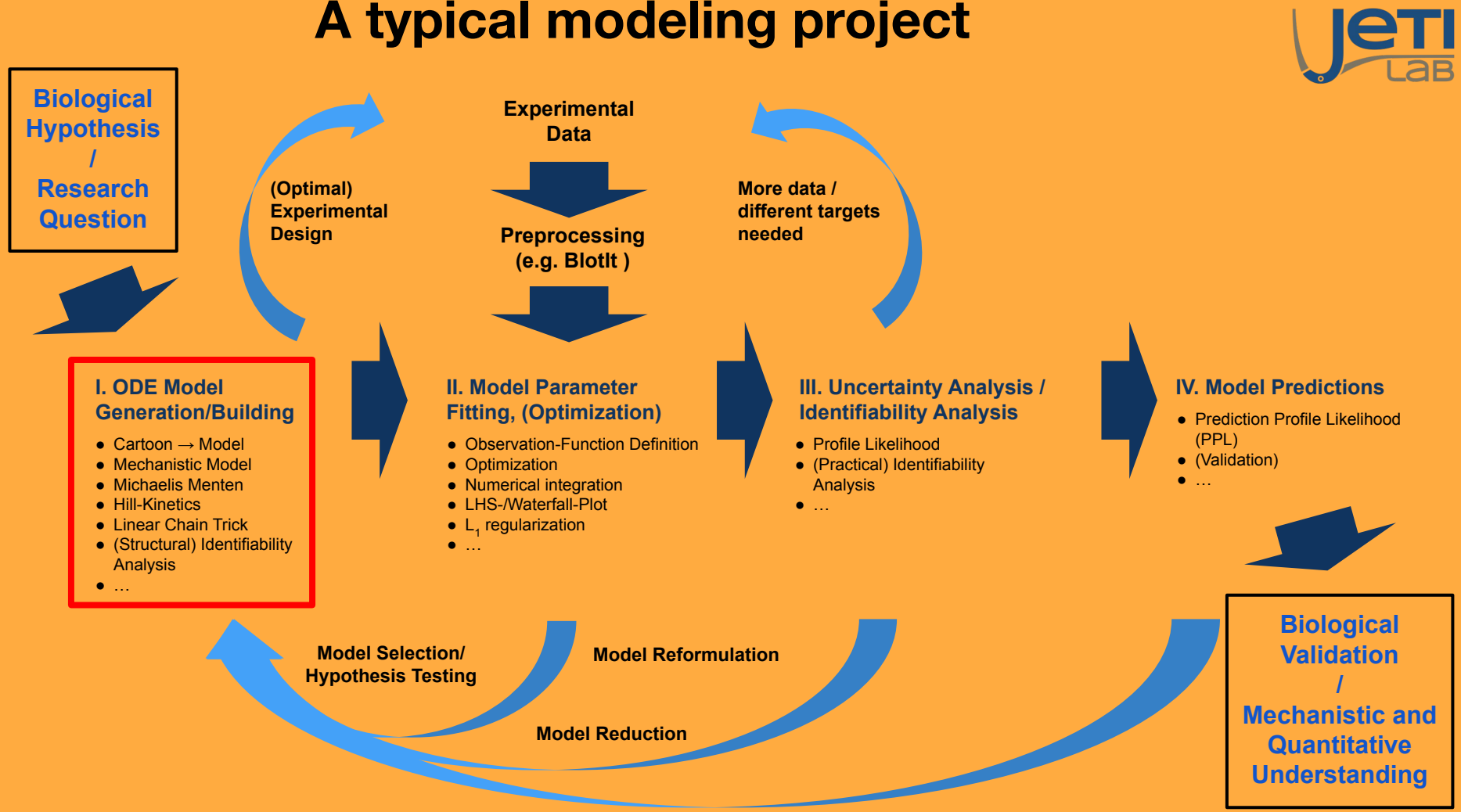
**Model Selection/ Hypothesis Testing**

**Model Reformulation**

**Model Reduction**

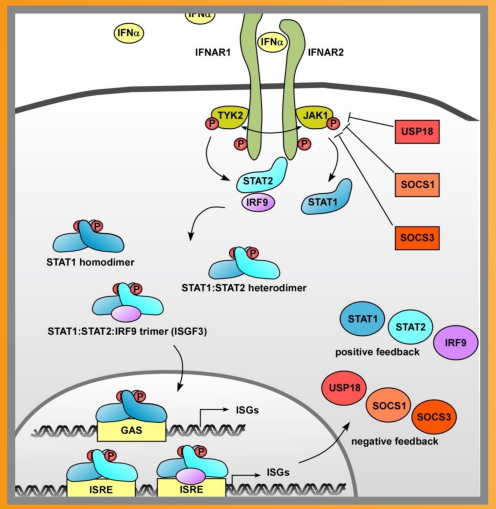
**Biological Validation / Mechanistic and Quantitative Understanding**

# A typical modeling project

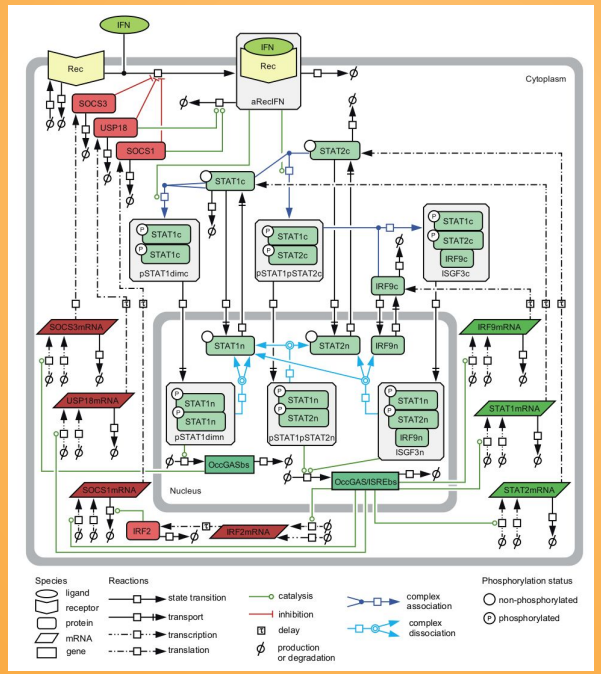


# Formalizing the ODE model

**Biological cartoon**




**Reaction network (SBGN)**




**ODE formulation**

Frequently used rate equations:

- **Mass action:** A + B ⟶ C

  $\dot{A} = \dot{B} = -\dot{C} = -k \cdot A \cdot B$

- **Enzyme kinetics:** E+S ↔ ES ⟶ E+P

  approx. by Michaelis-Menten:

  $\dot{P} = V_{max} \cdot S/(K_M + S)$

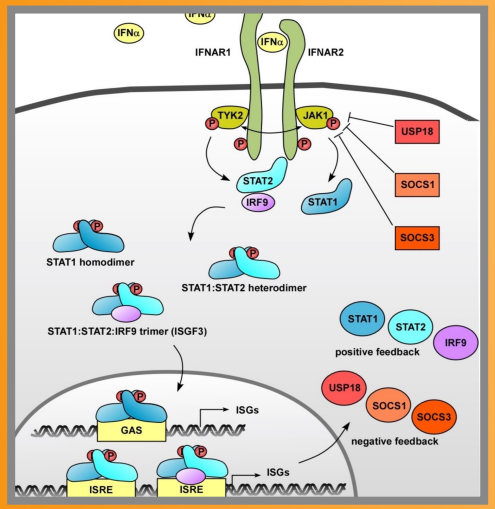
- **Inhibition:** A ⟶ B inhibited by C:

  $\dot{B} = k_1 \cdot A/(1 + k_2 \cdot C)$

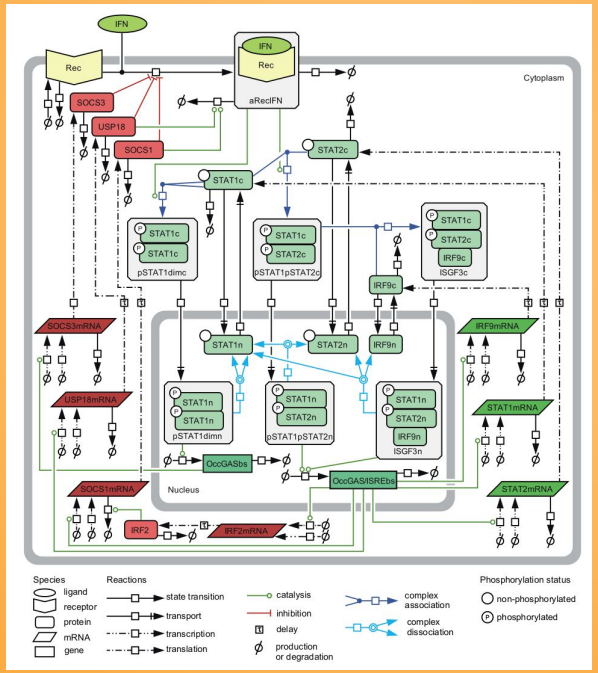
- **Determine unknown parameters by Maximum likelihood estimation**

# Formalizing the ODE model

## Biological cartoon




## Reaction network (SBGN)



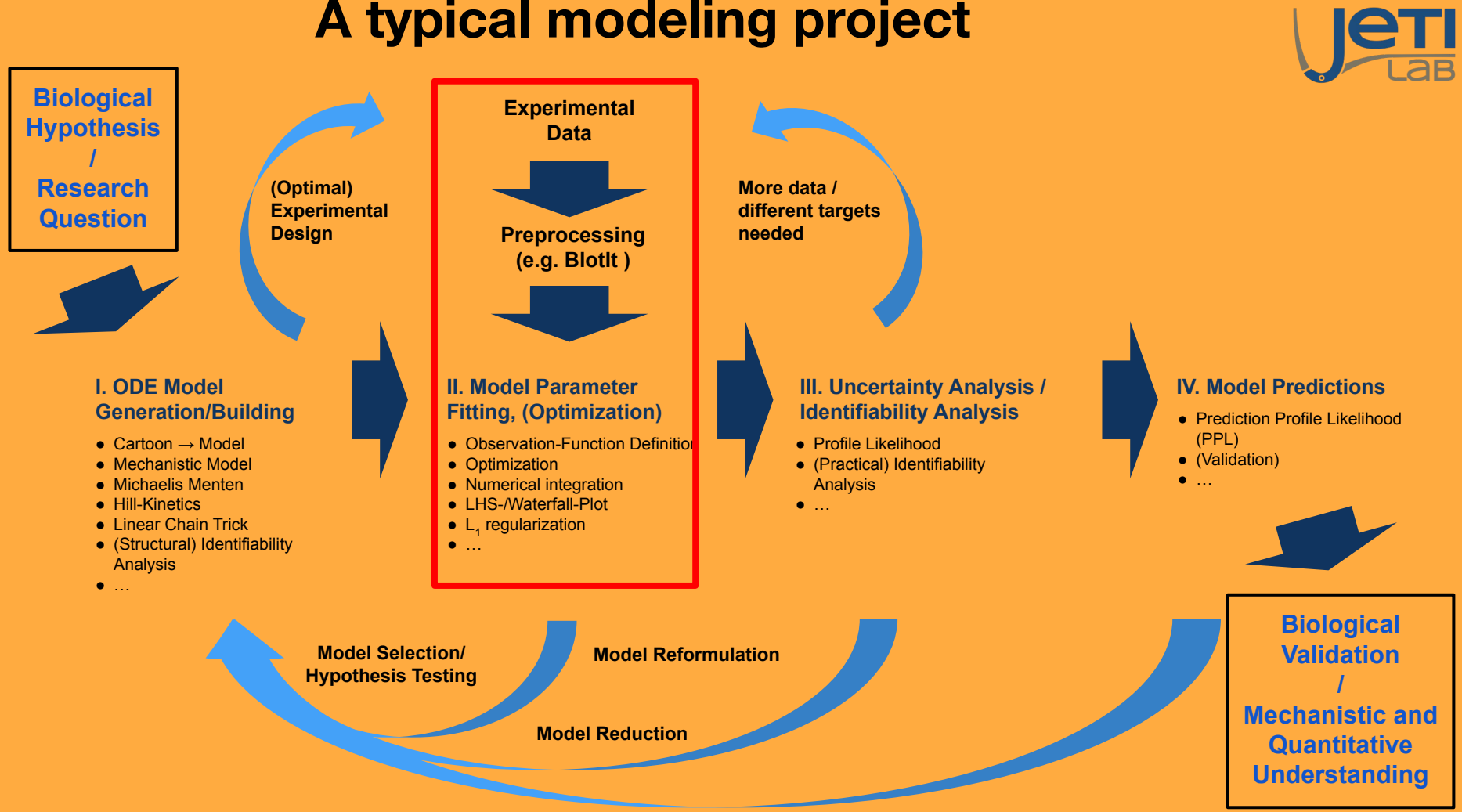

## ODE formulation

| | | | | | |
|---|---|---|---|---|---|
| pSTAT1pSTAT2c | → | pSTAT1pSTAT2n | TrLocSTAT1STAT2c · pSTAT1pSTAT2c | Heterodimer translocation to nucleus | Banninger and Reich, 2004 |
| pSTAT1pSTAT2n | → | STAT1n + STAT2n | decaySTAT1STAT2n · pSTAT1pSTAT2n | Heterodimer decay | Banninger and Reich, 2004 |
| pSTAT1pSTAT2c + IRF9c | → | ISGF3c | BindIRF9c · pSTAT1pSTAT2c · IRF9c | ISGF3 complex formation | Platanias, 2005 |
| ISGF3c | → | ISGF3n | TrLocISGF3c · ISGF3c | ISGF3 translocation to nucleus | Schindler et al., 1992 |

| | | | | | |
|---|---|---|---|---|---|
| ∅ | → | STAT2mRNA | synthSTAT2mRNAbasal | STAT2mRNA basal production | Lehtonen et al., 1997 |
| ∅ | → | STAT2mRNA | synthSTAT2mRNA · OccGAS/ISREbs | STAT2mRNA production by GAS/ISRE | Lehtonen et al., 1997 |
| STAT2mRNA | → | ∅ | hlSTAT2mRNA · STAT2mRNA | STAT2mRNA decay | Lehtonen et al., 1997 |
| ∅ | → | IRF9mRNA | synthIRF9mRNAbasal | IRF9mRNA basal production | Lehtonen et al., 1997 |
| ∅ | → | IRF9mRNA | $\text{synthIRF9mRNA} \cdot \text{OccGAS/ISREbs} \cdot \frac{1}{\text{kmIRF9} + \text{OccGAS/ISREbs}}$ | IRF9mRNA production by GAS/ISRE | Lehtonen et al., 1997 |

- **Determine unknown parameters by Maximum likelihood estimation**

# A typical modeling project



# Calibration of the ODE model

Time derivative of ODE model states x:

$$\dot{x} = f(x, p, u),$$

Observables y:

$$y = g(x, p, t).$$

Minimize weighted residual sum of squares:

$$\chi^2_{\text{res}}(p) = \sum_{k=1}^{m} \sum_{l=1}^{d_k} \left( \frac{y^D_{kl} - g_k(p, t_l)}{\sigma^D_{kl}} \right)^2,$$

$$\hat{p} = \arg\min \left[ \chi^2_{\text{res}}(p) \right].$$

Implementation in modeling toolboxes:

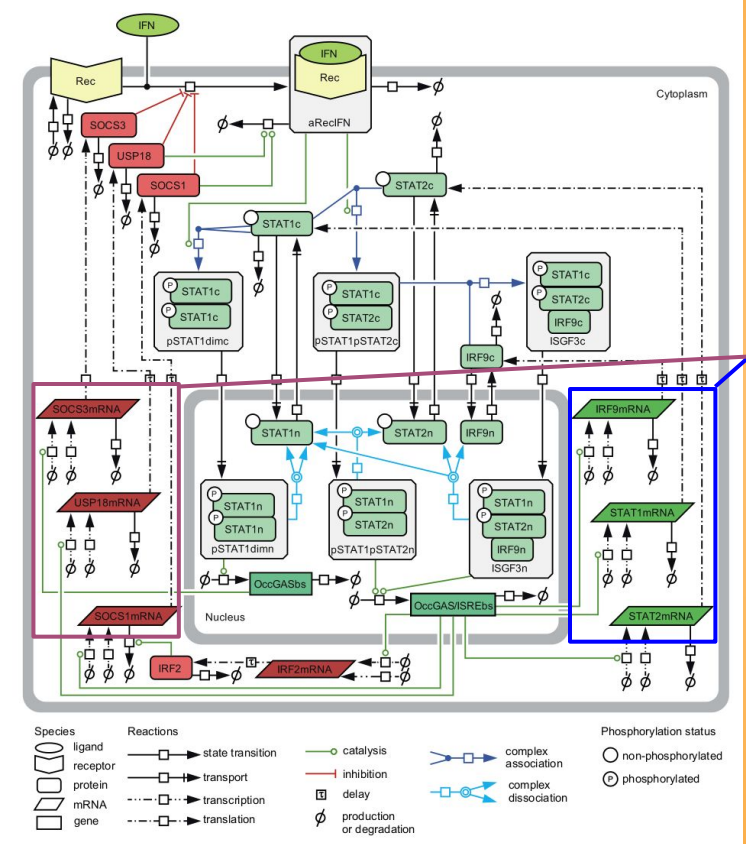
Kaschek et al. “Dynamic modeling, …”
*Journal of Stat. Software* (2019)

**dMod, R**

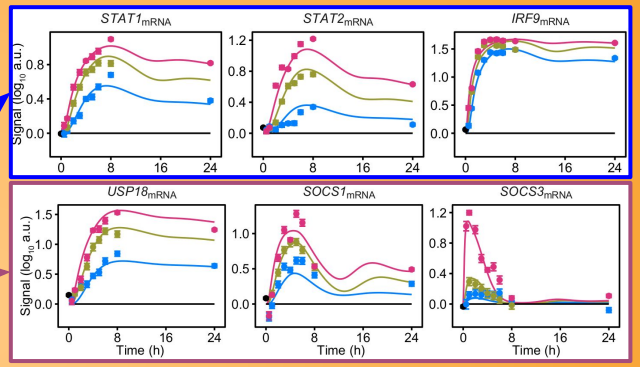
Raue et al. “Data2Dynamics …”
*Bioinformatics* (2015)

**D2D, Matlab**

# Calibration of the ODE model

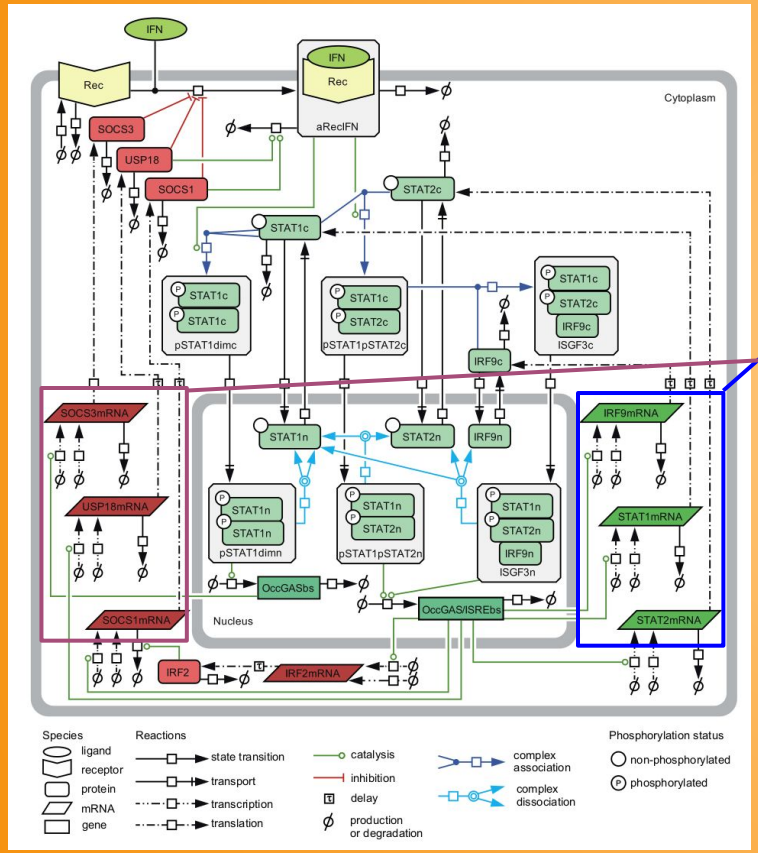



**Time course of mRNA (qPCR):**

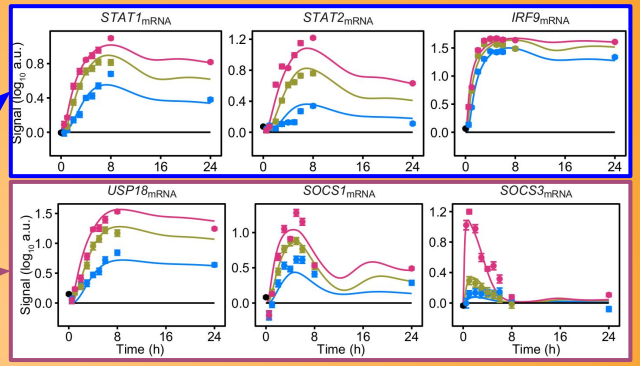



| Huh7.5 Stimulation | Data | Model fit |
|---|---|---|
| untreated | ● | — |
| 2.8 pM IFNα | ● | — |
| 28 pM IFNα | ● | — |
| 1400 pM IFNα | ● | — |

# Calibration of the ODE model



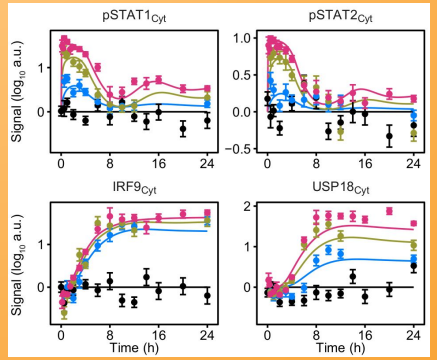

**Time course of mRNA (qPCR):**

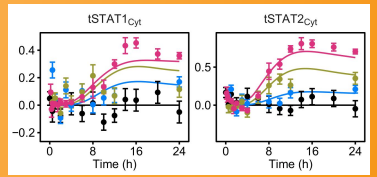



Huh7.5

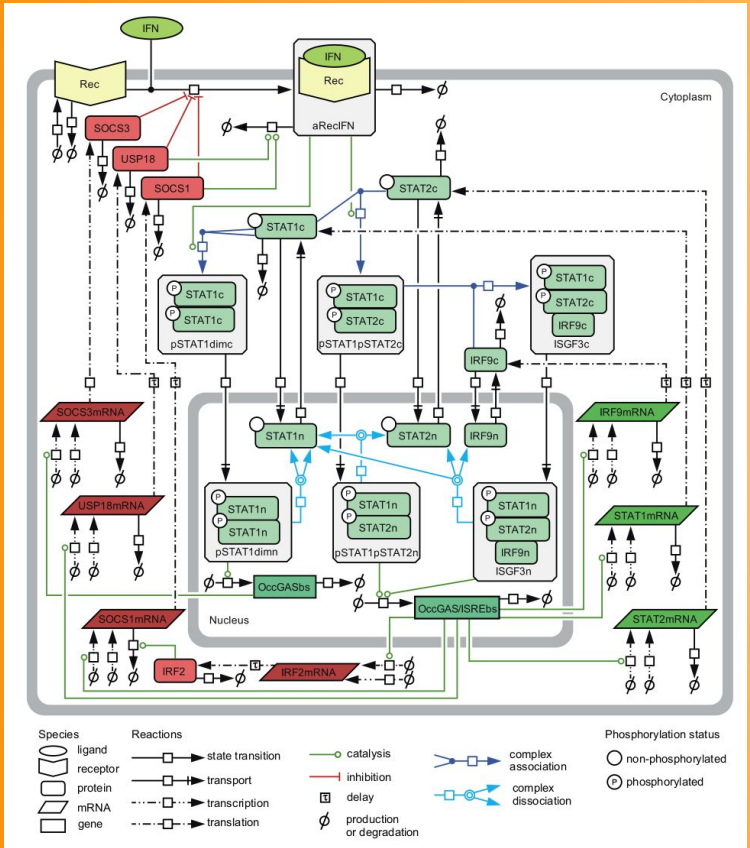
| Stimulation | Data | Model fit |
|---|---|---|
| untreated | ● | — |
| 2.8 pM IFNα | ● | — |
| 28 pM IFNα | ● | — |
| 1400 pM IFNα | ● | — |

**Aligned protein data (Western blot):**



# Calibration of the ODE model




**Model size:**

- 40 dynamic variables
- 85 parameters
- 11700 data points for 20 observables

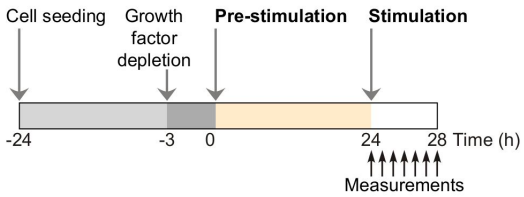
**Measurement techniques:**

- Western blot (WB)
- qPCR
- Mass spec (MS)

**Experimental conditions:**

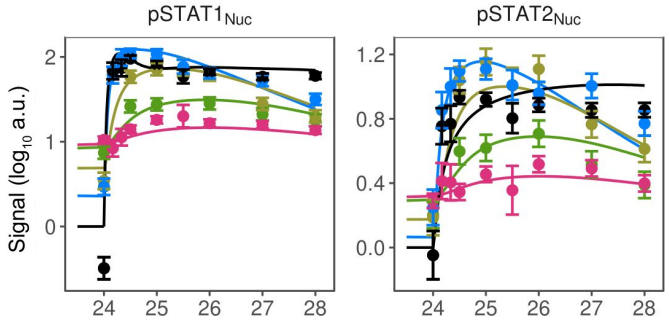
- Time course over 32 hours
- Dose response at 1h, 4h and 24h
- USP18 over-expression and inhibition (siRNA)

# Dose-dependent pathway sensitization

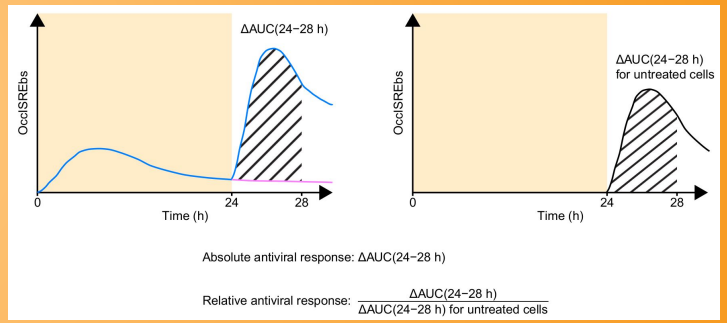



**Huh7.5**

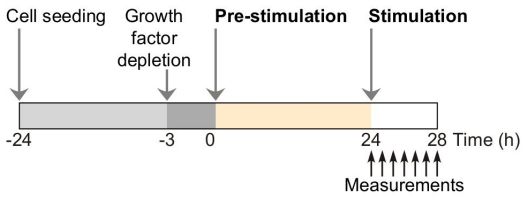
| Pre-stimulation/Stimulation | Data | Model fit |
|---|---|---|
| untreated/1400 pM IFNα | ● | — |
| 2.8 pM IFNα/1400 pM IFNα | ● | — |
| 28 pM IFNα/1400 pM IFNα | ● | — |
| 280 pM IFNα/1400 pM IFNα | ● | — |
| 1400 pM IFNα/1400 pM IFNα | ● | — |

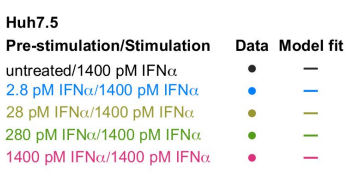


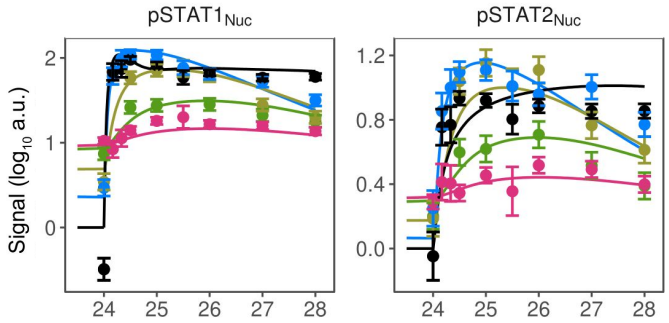

Define a measure for the antiviral response:

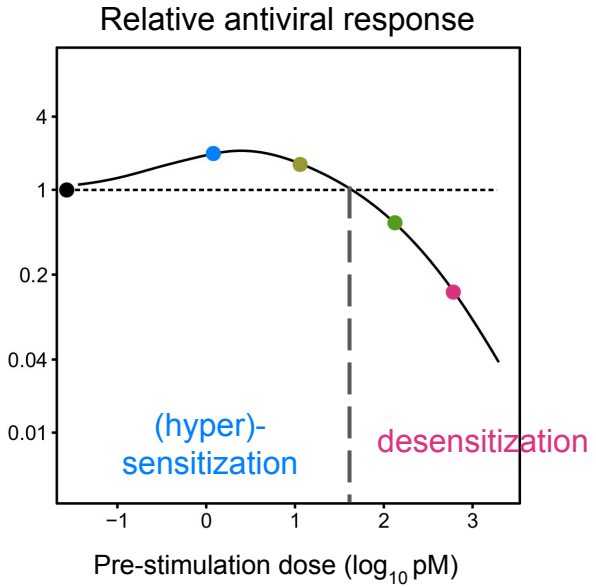


# Dose-dependent pathway sensitization

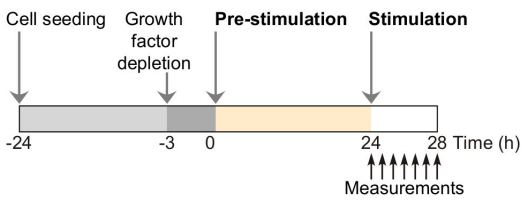


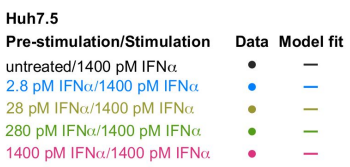



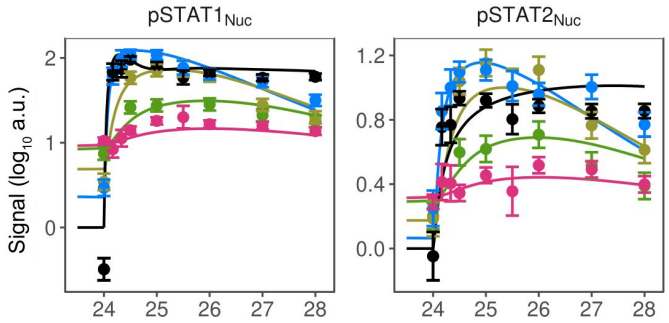



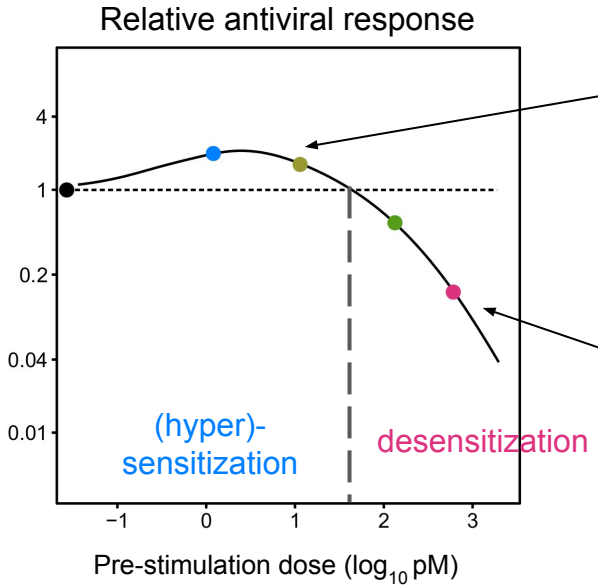



# Dose-dependent pathway sensitization



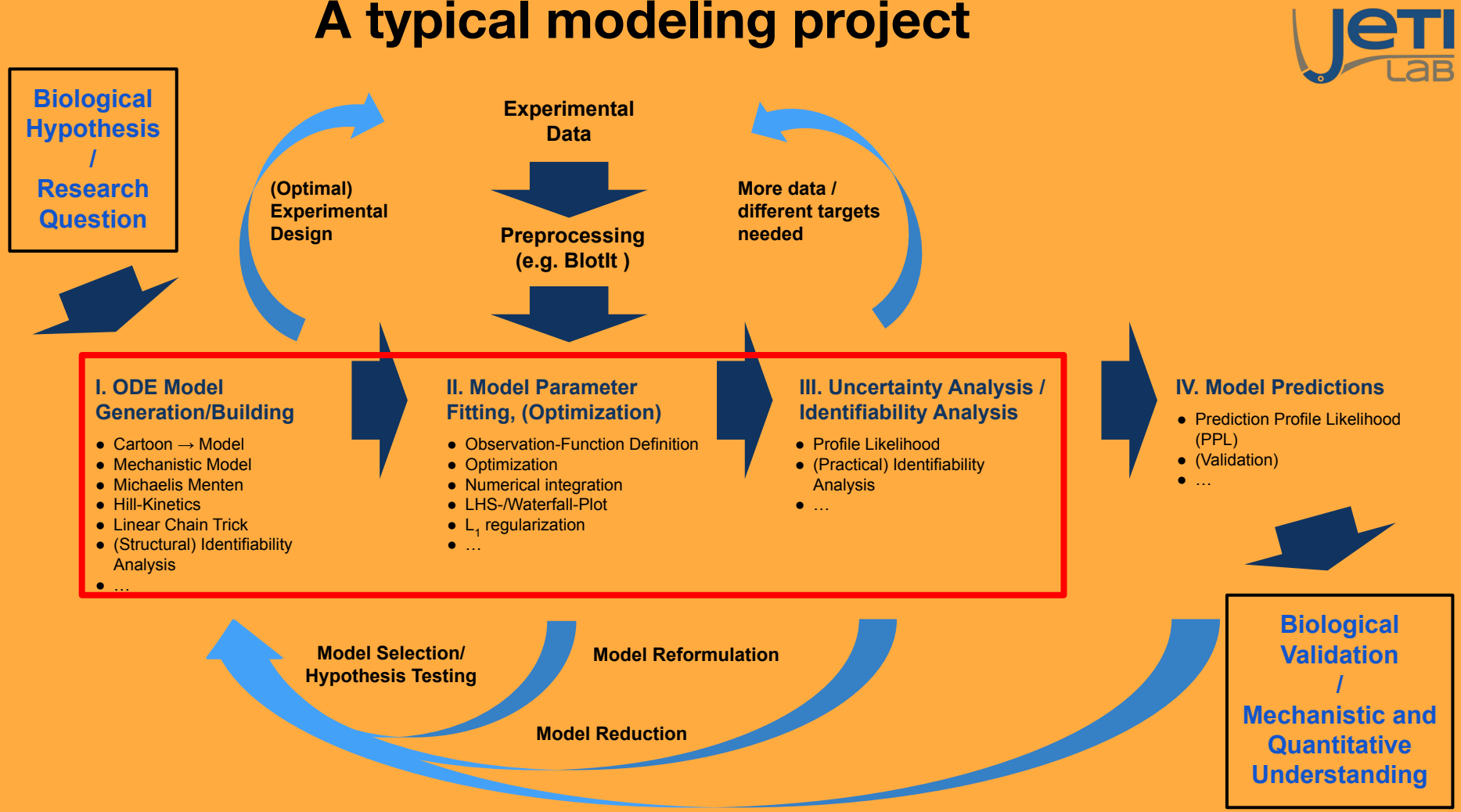




**IRF9, STAT2**

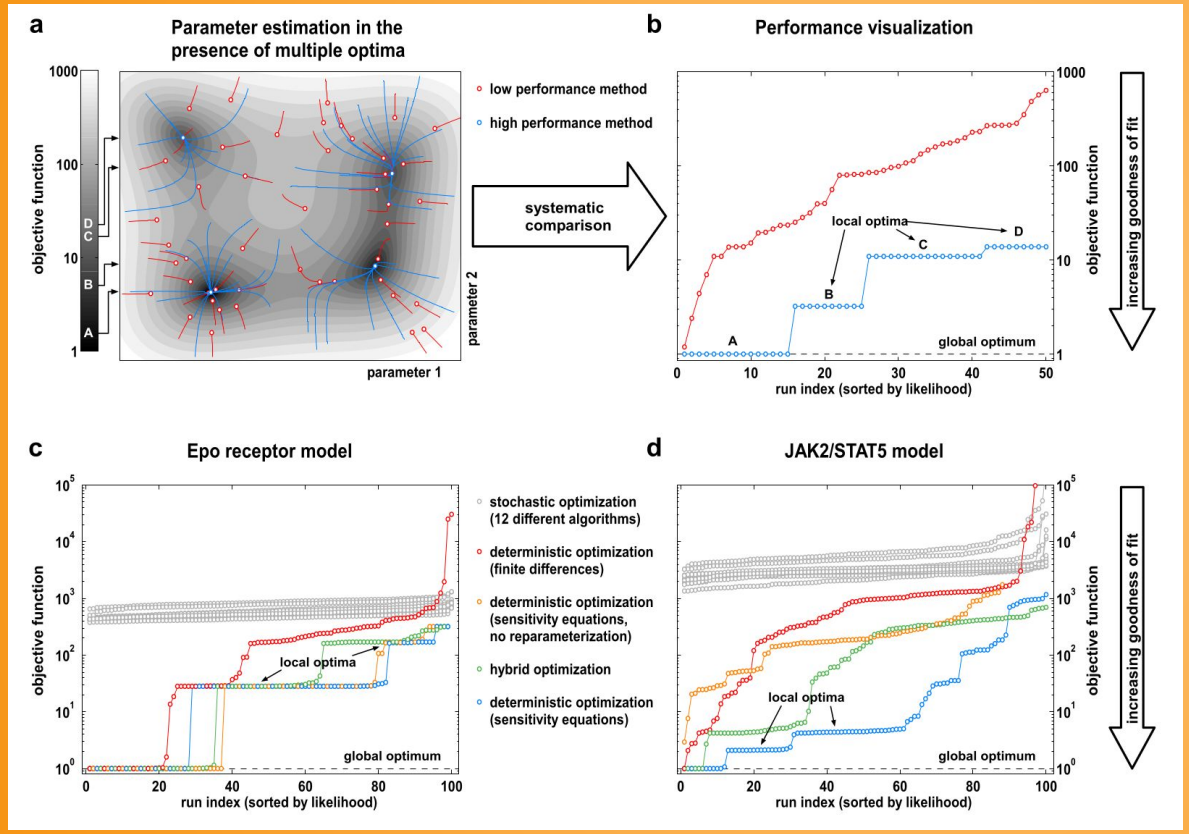
**USP18, SOCS1**

Pathway sensitization is determined by dose-dependent induction of intracellular feedback proteins

# A typical modeling project

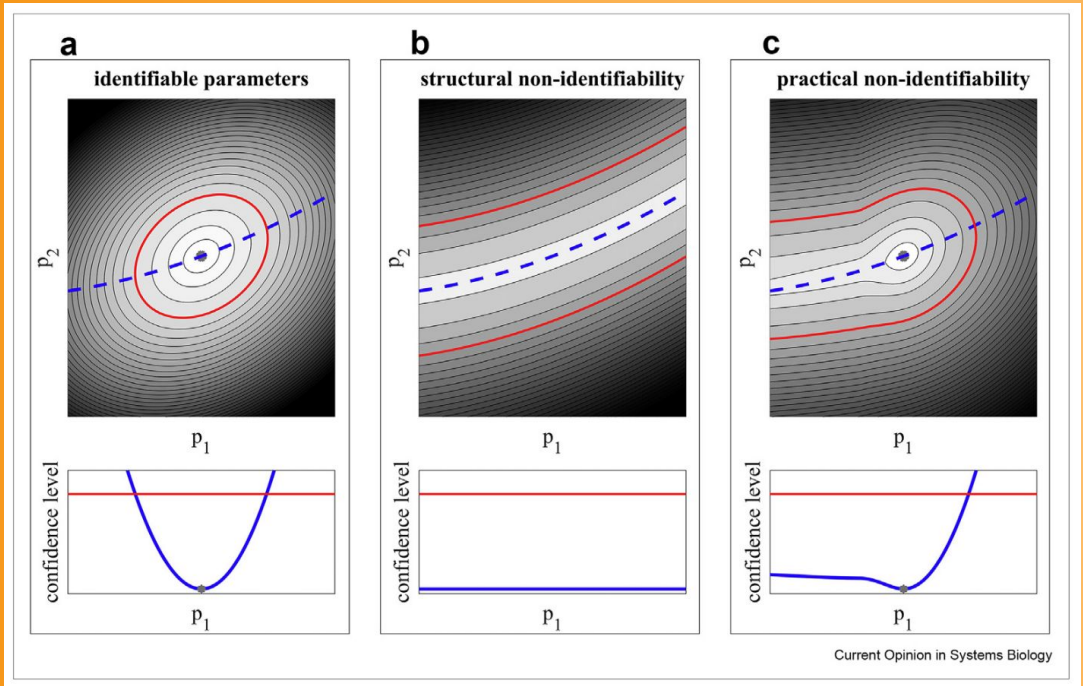


# Numerical optimization



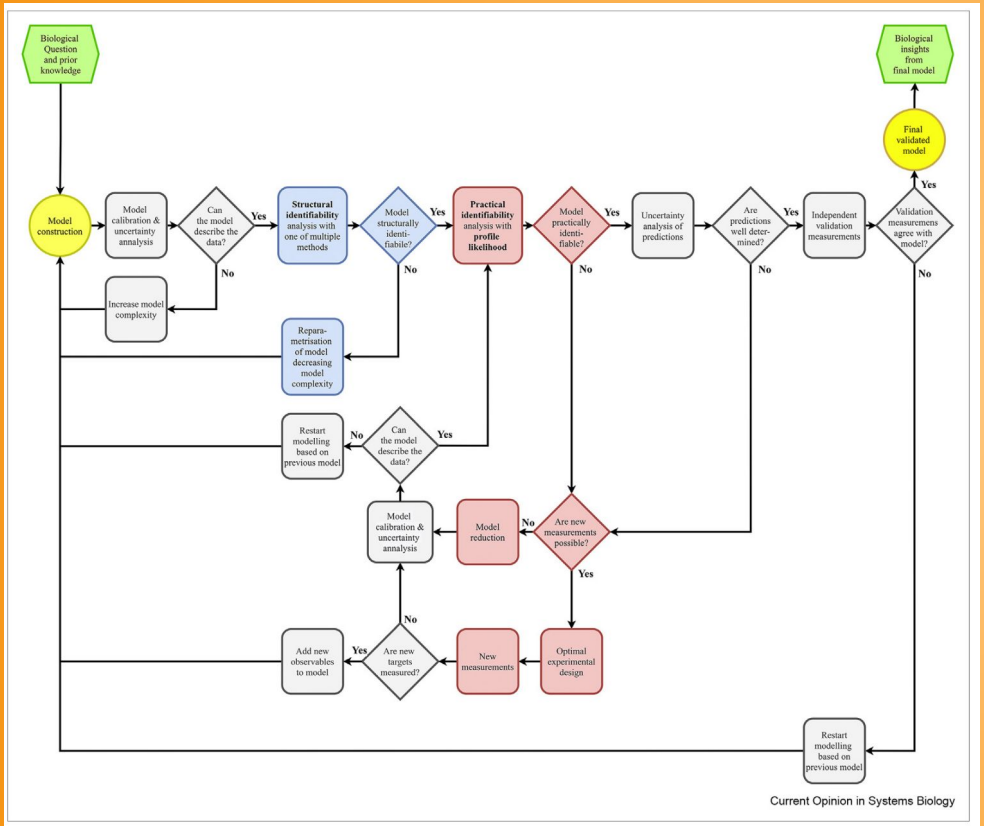

Raue et al. “Lessons Learned …” *PLoS ONE* (2013)

# Identifiability analysis via the Profile likelihood




Raue et al. “Structural and Practical …”
*Bioinformatics* (2009)

# Current Opinion in Systems Biology



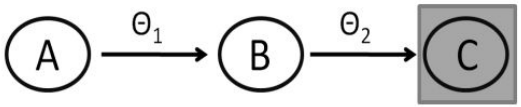

"Tailor the model complexity to the information content of the data"

F.G. Wieland, A.L. Hauber, M. Rosenblatt, C. Tönsing, J.Timmer. (2021)
"On structural and practical identifiability"

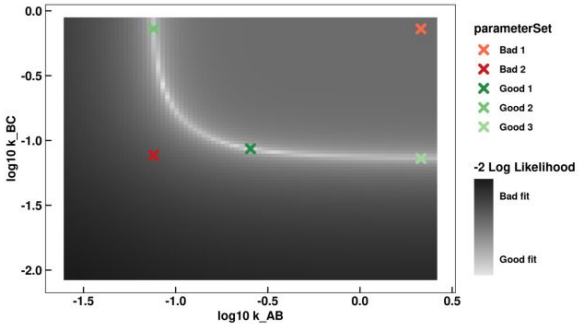
*Current Opinion in Systems Biology*

# The problem of non-identifiability

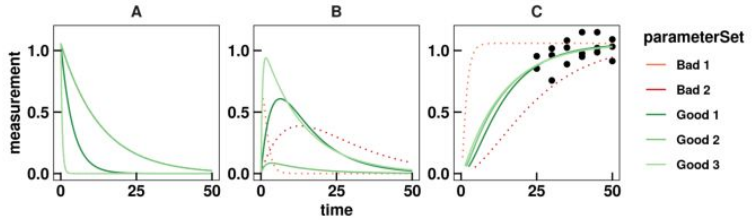
## Toy Model



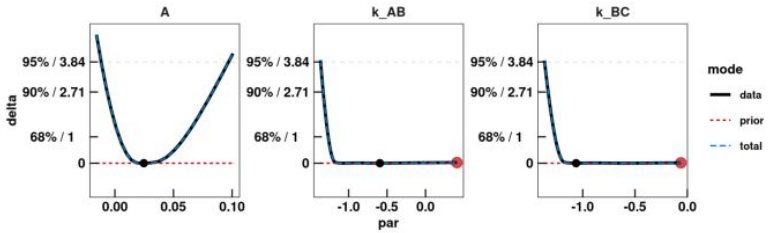

## Likelihood landscape




## Model trajectories



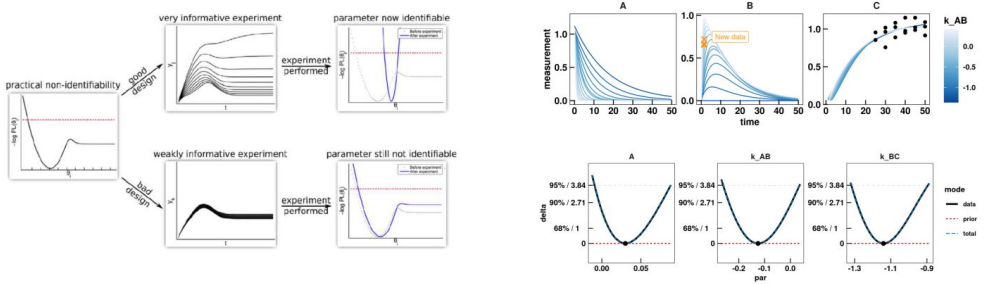

## Profile likelihood




- Values of parameters cannot be determined
- Uncertainty quantification impossible and predictions may become non-meaningful

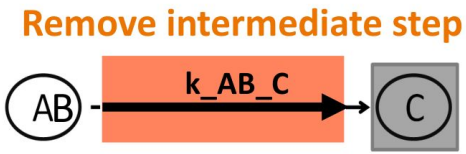
# Curing non-identifiability

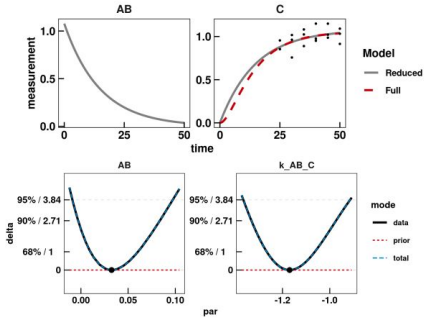
## Strategy 1 More data => Experimental design




Steiert et al. “Experimental Design …”
*PLoS ONE* (2012)

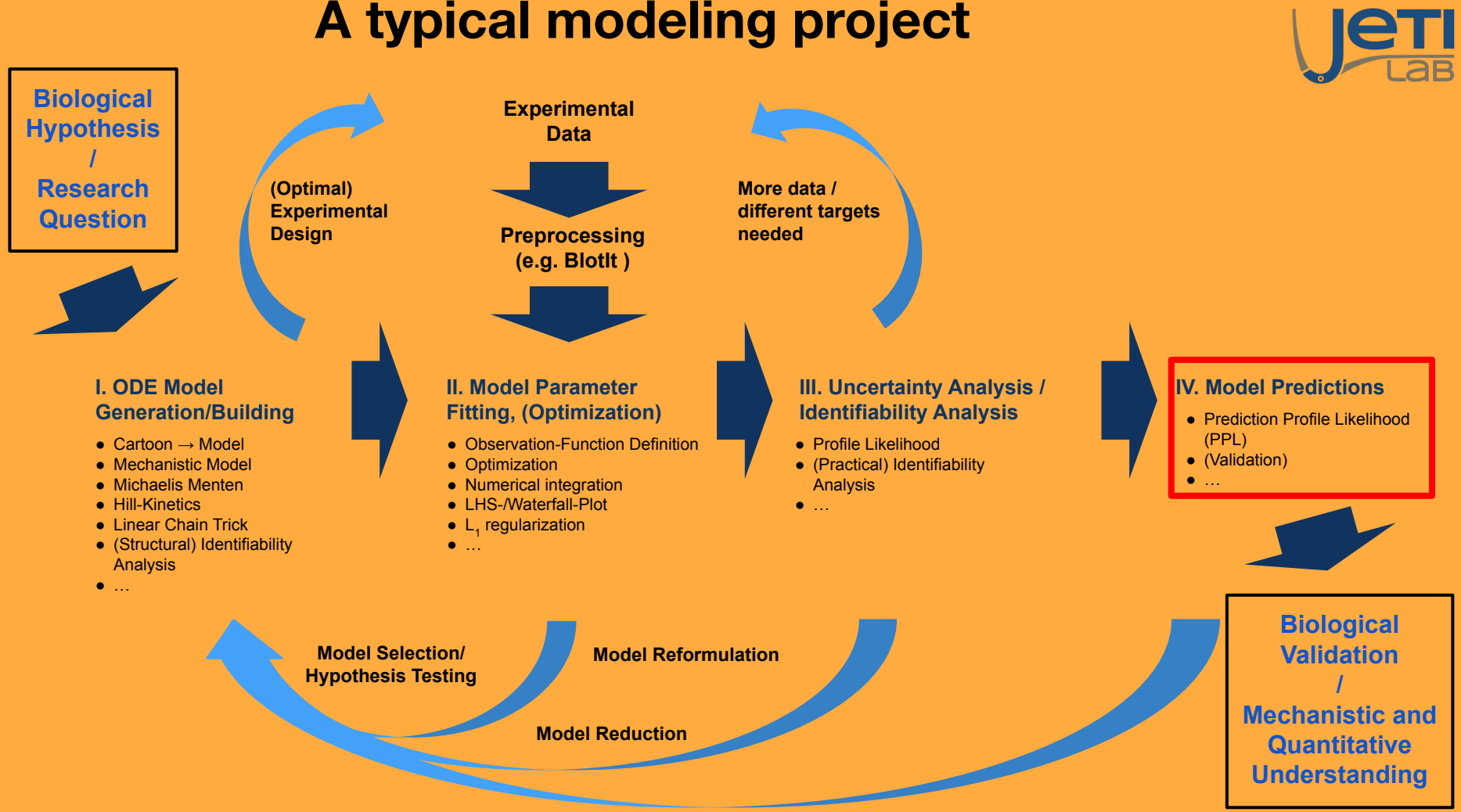
## Strategy 2 Model reduction



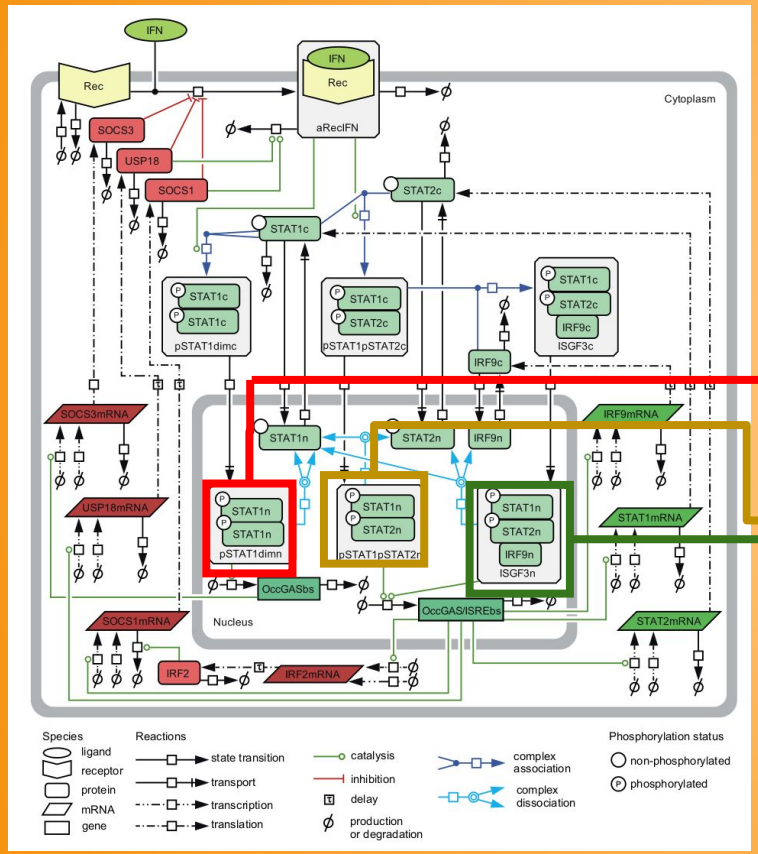



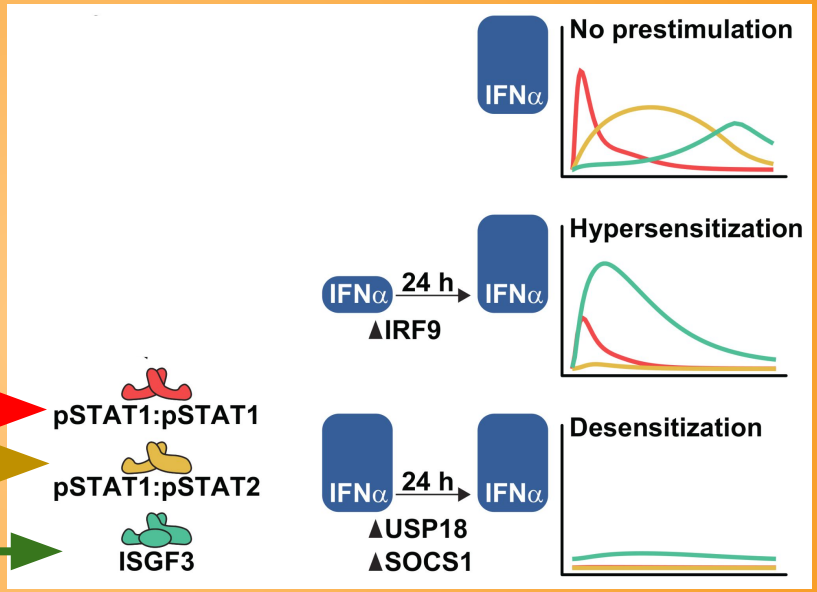

Maiwald et al. “Driving the model …”
*PLoS ONE* (2016)

# A typical modeling project



# Dynamics of Transcription Factors



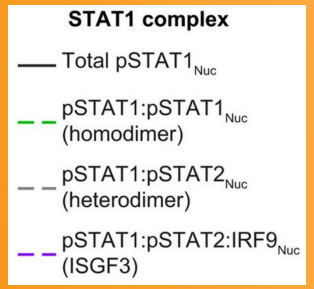



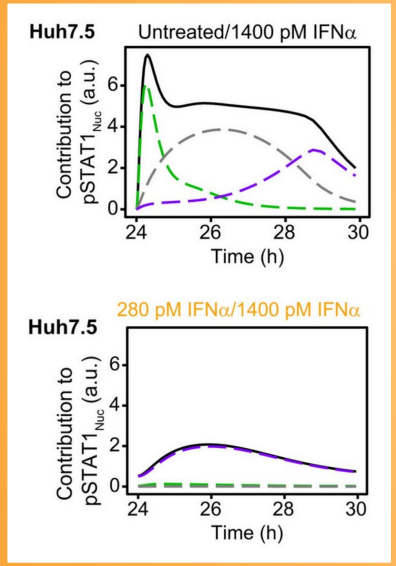

- Calibration by measuring sum of STAT complexes
- Model disentangles dynamics of individual complexes

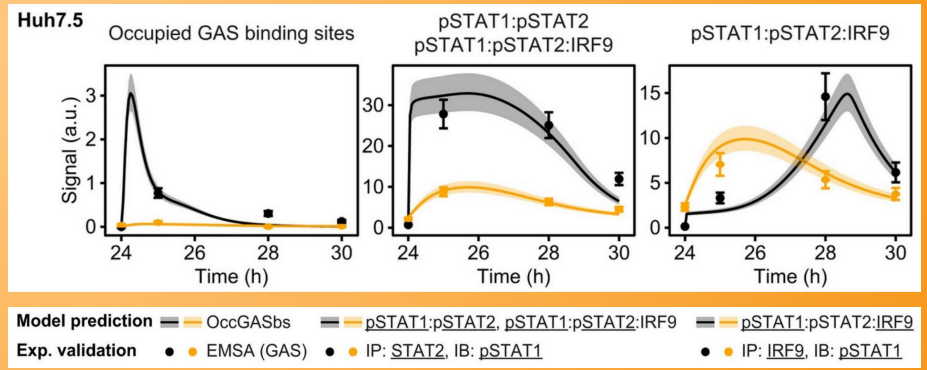
# Dynamics of Transcription Factors

Model prediction uncertainty calculated via prediction profile likelihood
Kreutz et al. “Profile likelihood …”
*FEBS Journal* (2013)

$$\mathrm{PPL}(z) = \min_{p \in \{p \mid g_{\mathrm{pred}}(p) = z\}} \left[\chi^2_{\mathrm{res}}(p)\right],$$



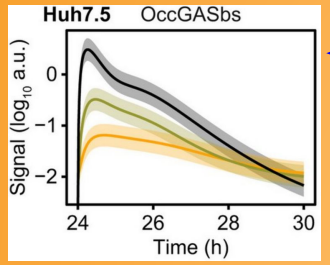



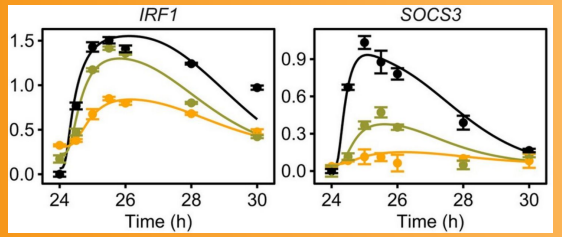



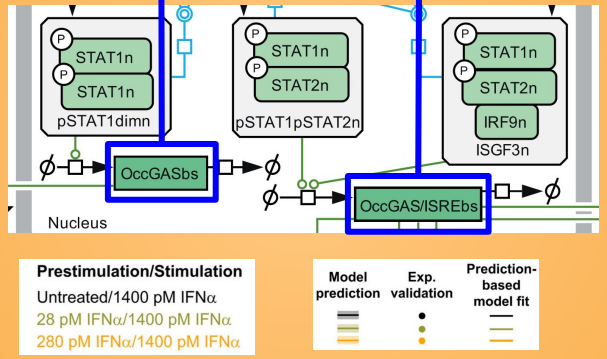

Model correctly predicts dynamics of individual STAT complexes

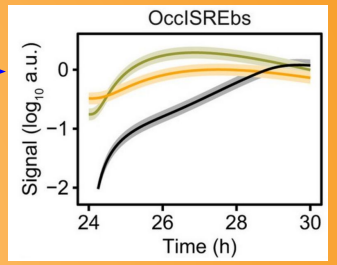
# Dynamics of Gene Expression

**GAS**

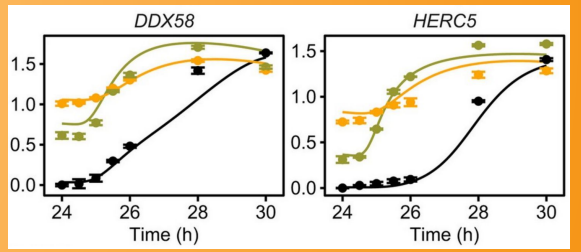


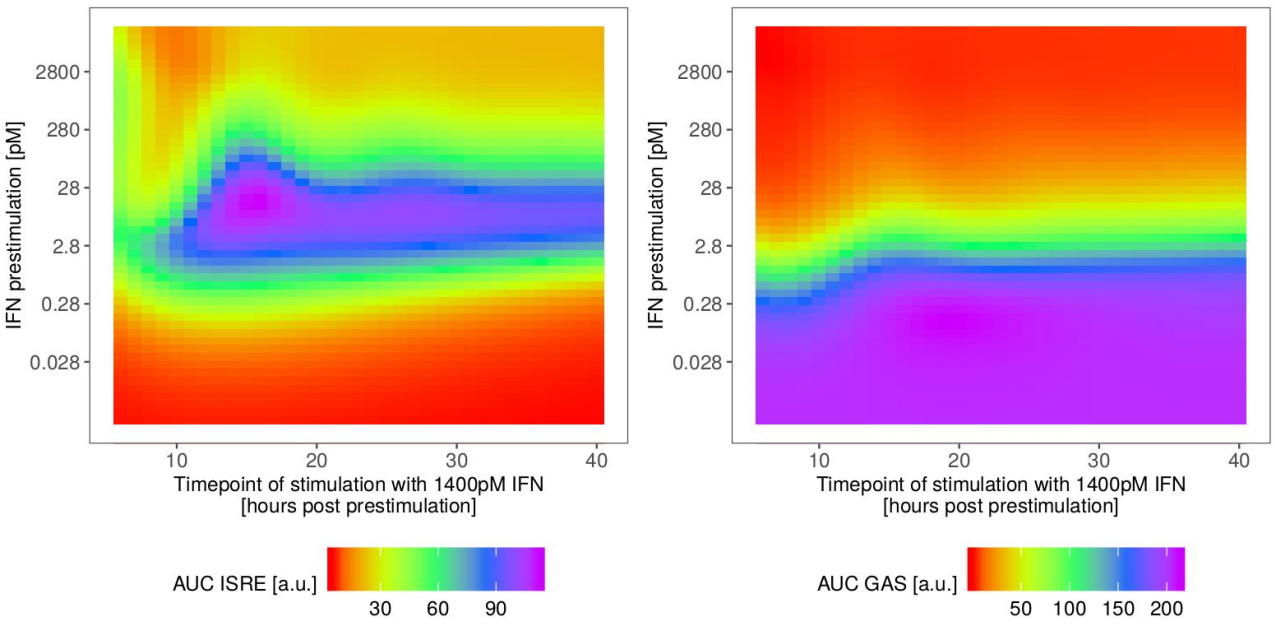




**ISRE**




- Model correctly predicts dose-dependent sensitization at gene level
- **GAS:** no hypersensitization, only desensitization
- **ISRE:** potentially strong hypersensitization, if dose and timing is chosen properly

# Dynamics of Promoter Regions




- Model correctly predicts dose-dependent sensitization at gene level
- **GAS:** no hypersensitization, only desensitization
- **ISRE:** potentially strong hypersensitization, if dose and timing is chosen properly

# Outline

## (I) Sensitization in IFNα signaling

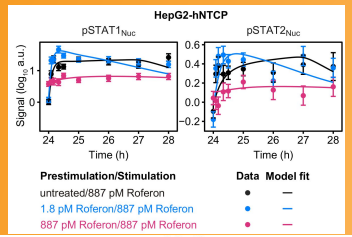
(a) ODE model application ⟷ (b) Methodological development

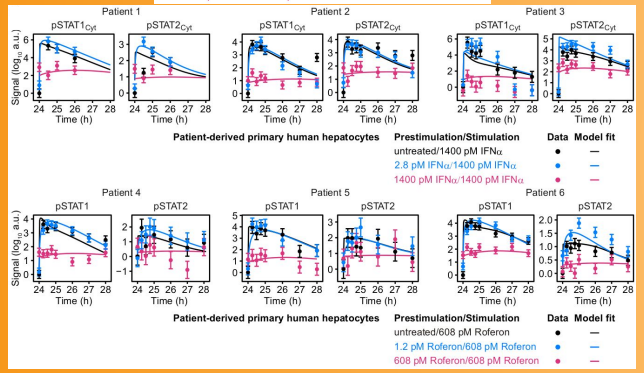
- Mechanistic insights
- Selected publications from the Timmer group

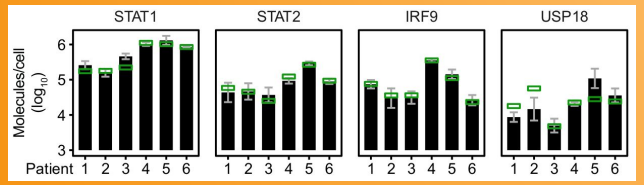
## (II) Application to Patient-derived Human Hepatocytes (PHH)

- USP18 as a biomarker for sensitization threshold
- Model adaptations and extensions
- Pharmacokinetics and treatment strategies

# Model adaptation



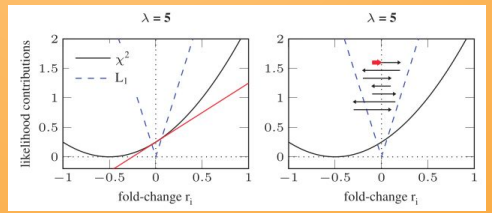



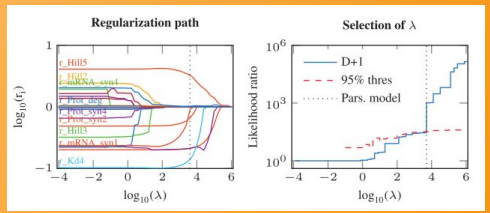





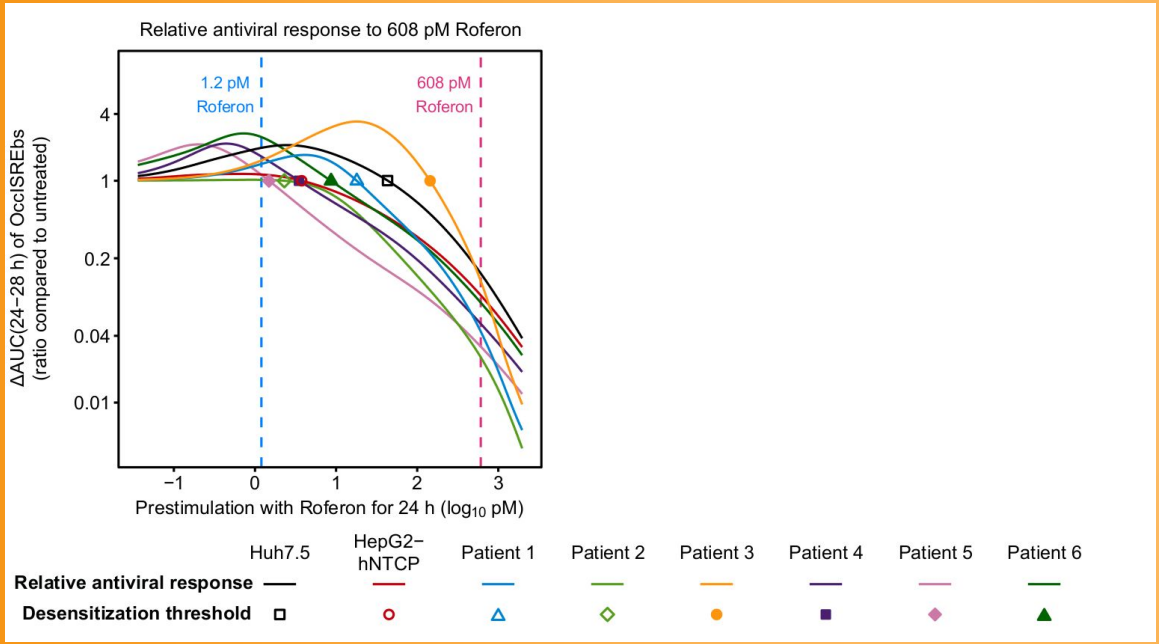
- Sensitization experiments performed for hepatoma cell line HepG2 and primary human hepatocytes (PHH)
- Model structure conserved
- Differences between cell systems are modeled by differences in (a few) system-specific parameters
- Identification by means of $L_1$ regularization

for N=2: Steiert et al. "$L_1$ regularization facilitates …" *Bioinformatics* (2016)
for N>2: Hauber et al. "Uncovering specific mechanisms…" *bioRxiv* (2023)



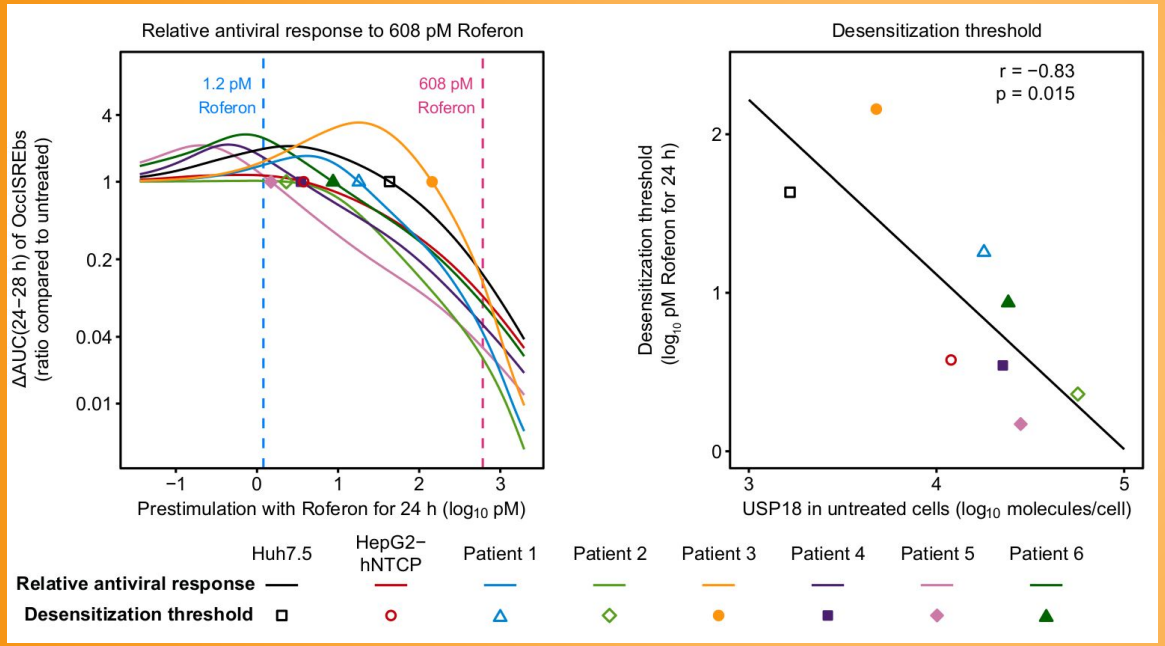



# Pathway sensitization across cell systems




- Characteristic shape of pathway sensitization is conserved across different cell systems
- Exact positioning is determined by abundance of feedback proteins

# Pathway sensitization across cell systems




- Characteristic shape of pathway sensitization is conserved across different cell systems
- Exact positioning is determined by abundance of feedback proteins
- Threshold between sensitization and desensitization determined by the abundance of USP18

# Outline

## (I) Sensitization in IFNα signaling

(a) ODE model application ⟷ (b) Methodological development

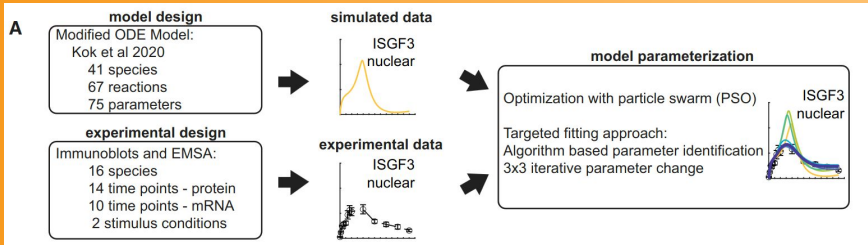
- Mechanistic insights
- Selected publications from the Timmer group

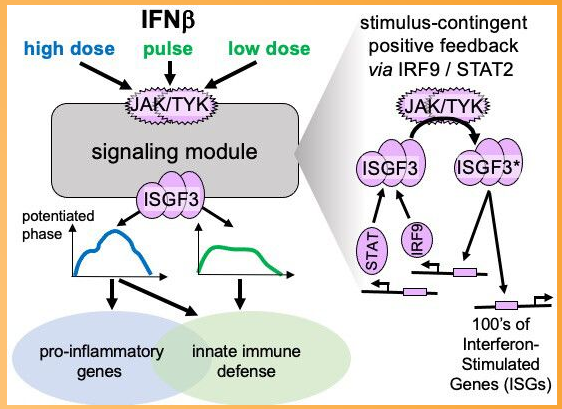
## (II) Application to Patient-derived Human Hepatocytes (PHH)

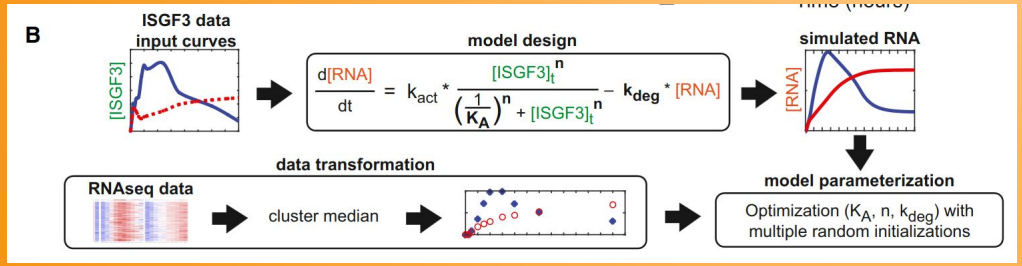
- USP18 as a biomarker for sensitization threshold
- Model adaptations and extensions
- Pharmacokinetics and treatment strategies

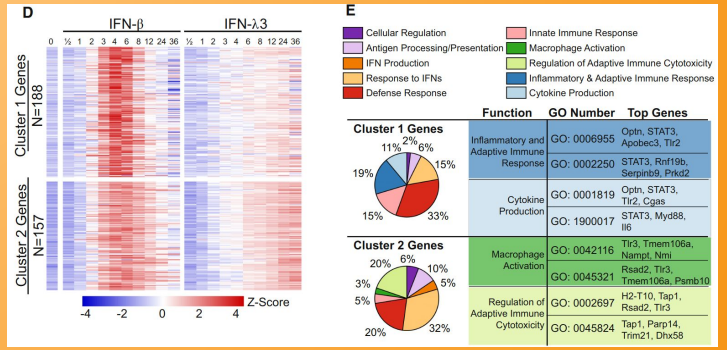
# Link to dynamics of transcriptome

Wilder et al. “A stimulus-contingent feedback …”
*Molecular Systems Biology* (2023)

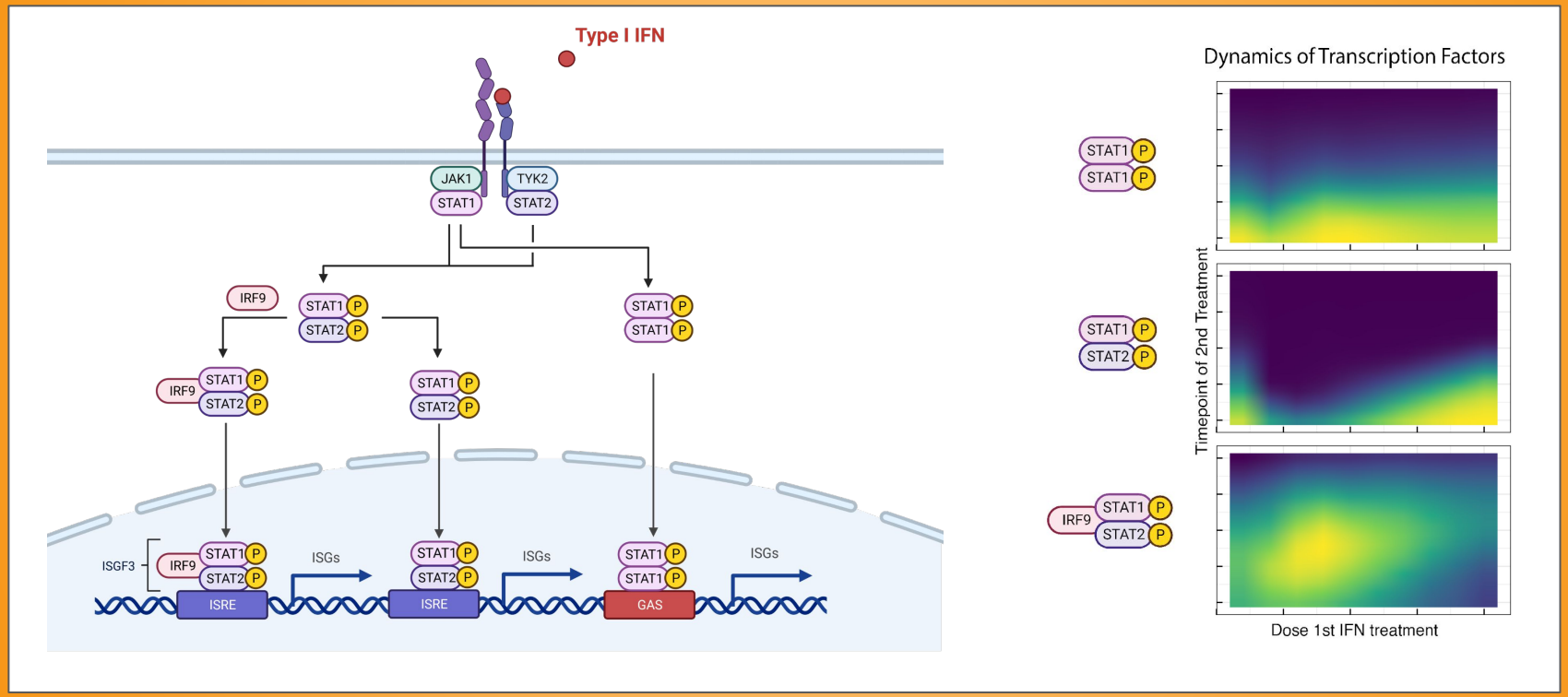


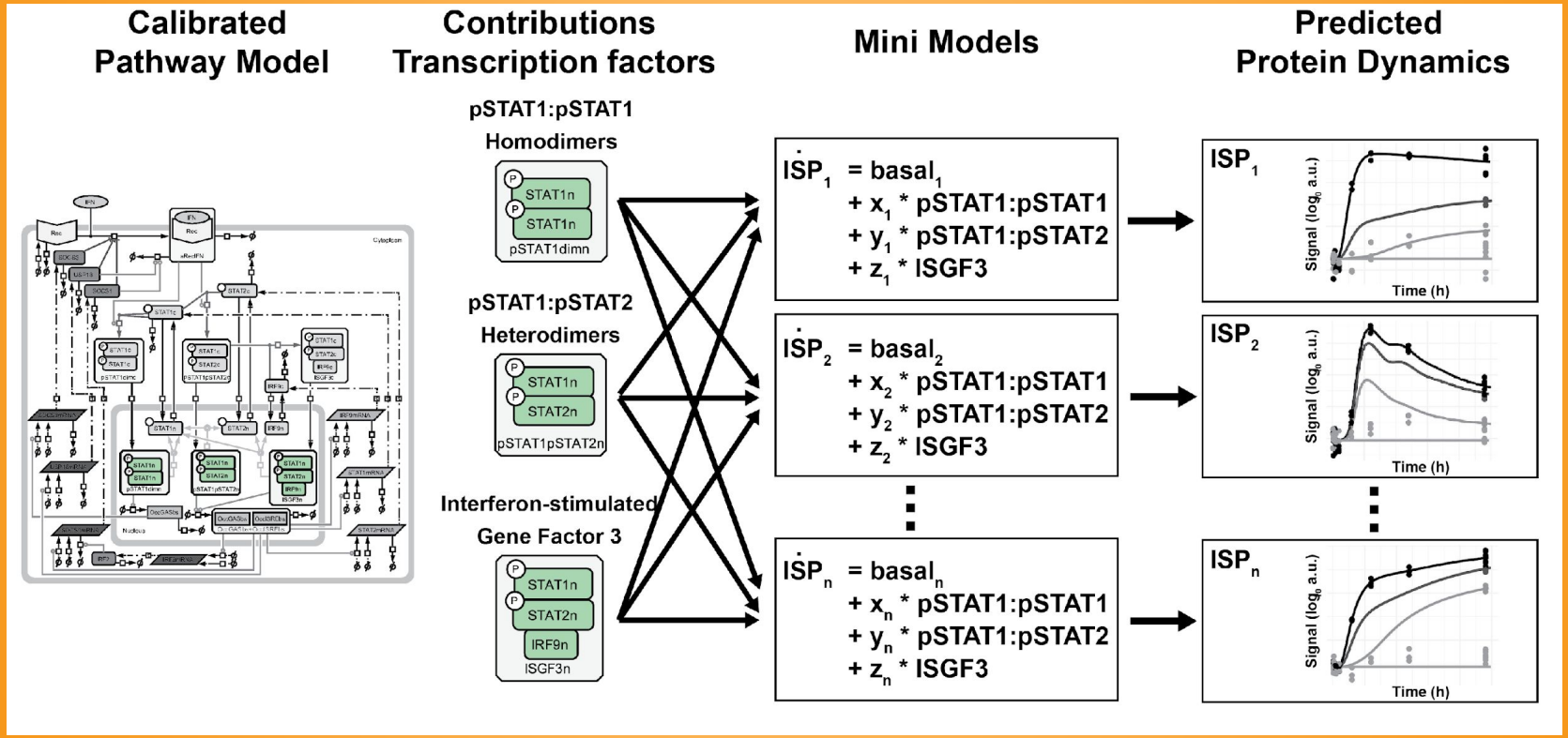



# Sensitization of transcription factor dynamics

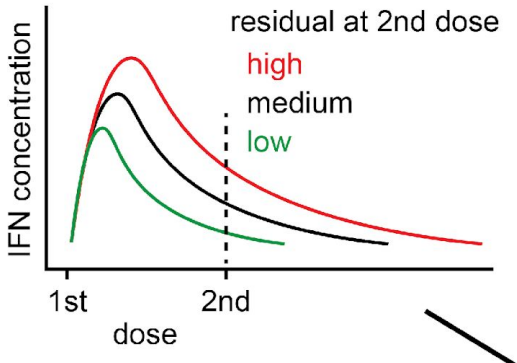


# Dynamics of Gene Response



# Dynamics of Gene Response

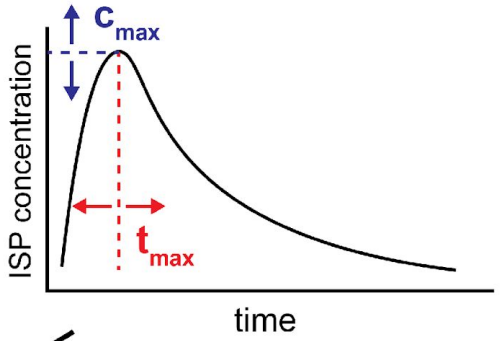
**Pharmacokinetics of Interferon**



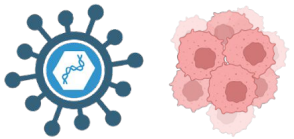

**Mini Models**

$$\dot{ISP}_n = basal_n + x_n * pSTAT1{:}pSTAT1 + y_n * pSTAT1{:}pSTAT2 + z_n * ISGF3$$

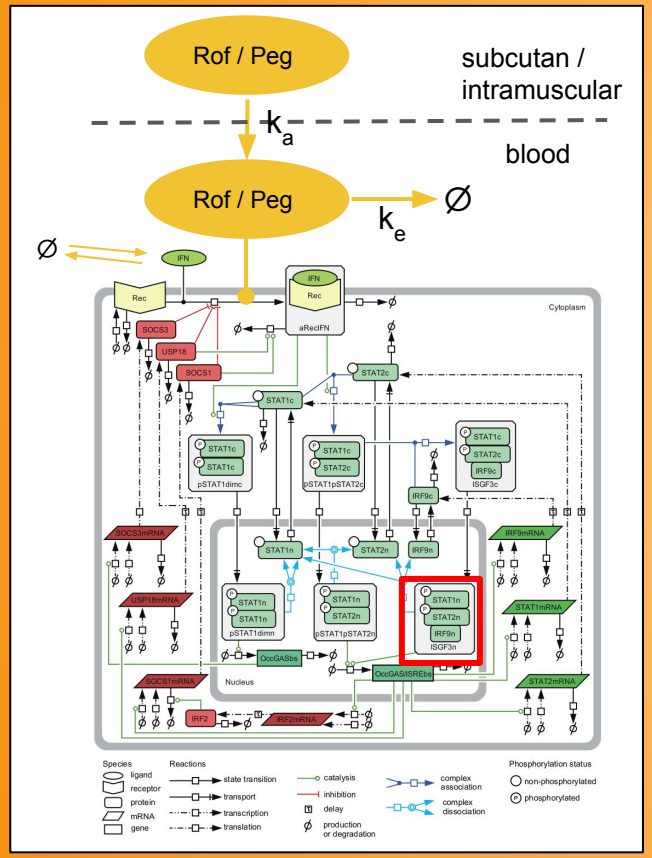
**Fine tuning of Pharmakokinetics of Interferon-stimulated proteins**



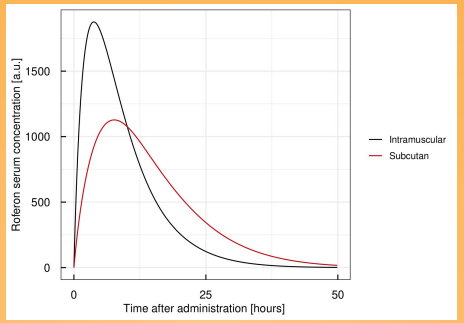

**Patient- & disease-specific dosing regimes**

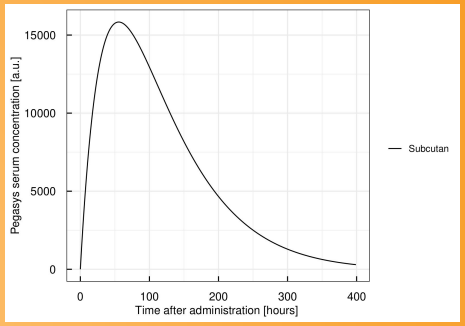


# Link to pharmacokinetic model

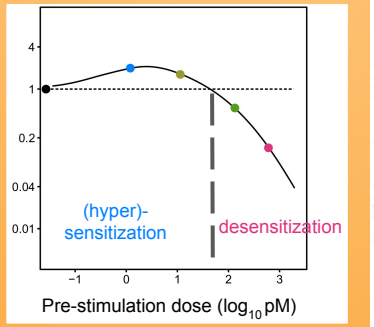


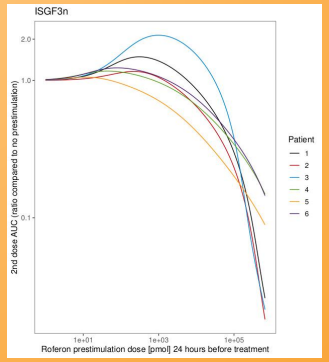

## PK of Roferon and Pegasys:

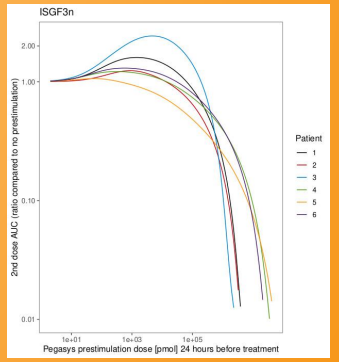


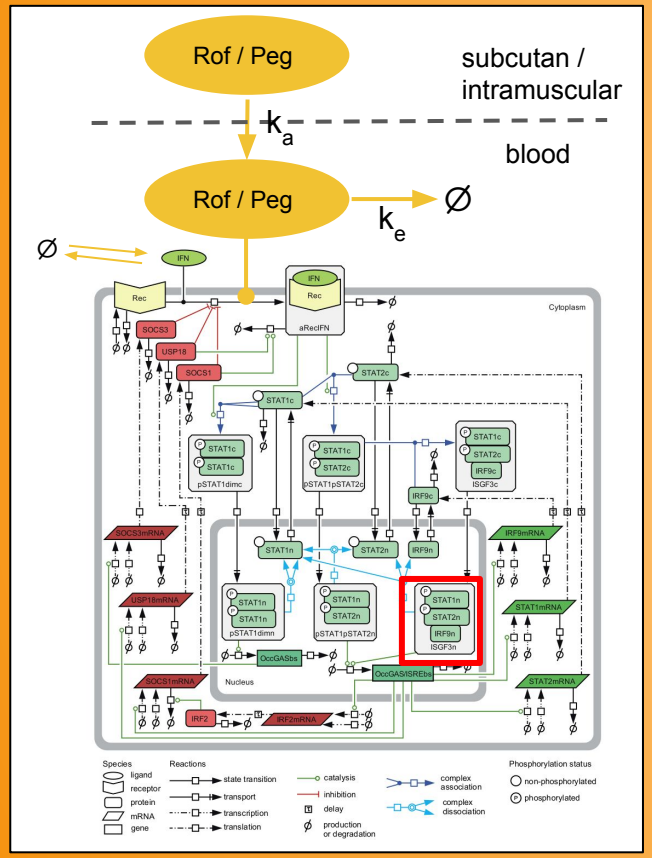

## **Relative antiviral response** (including IFN baseline and PK):

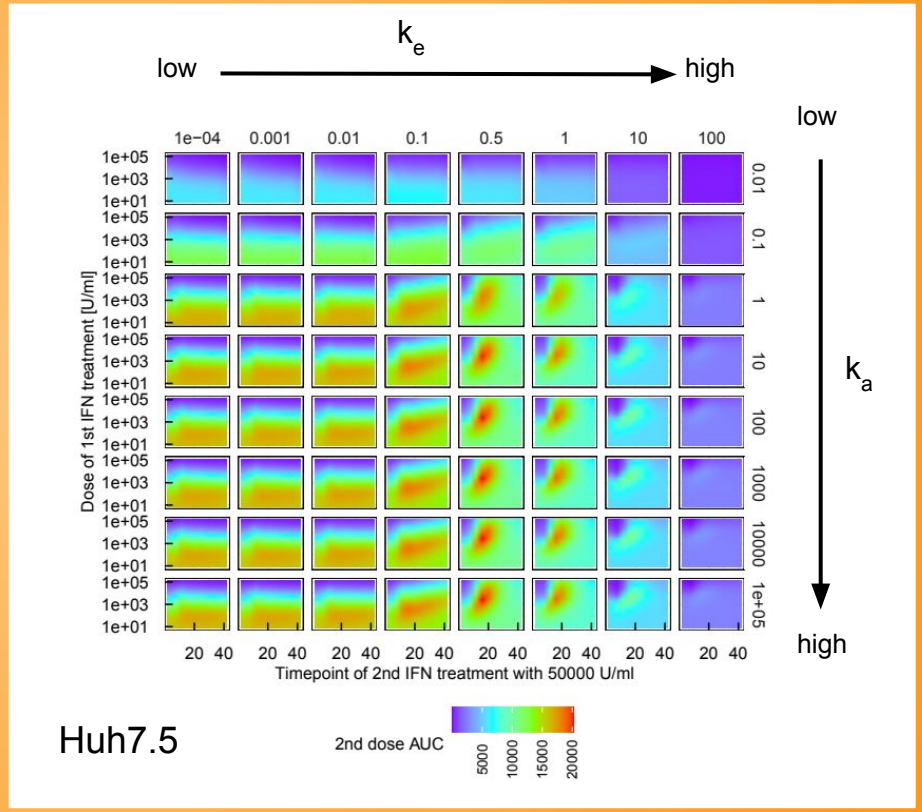


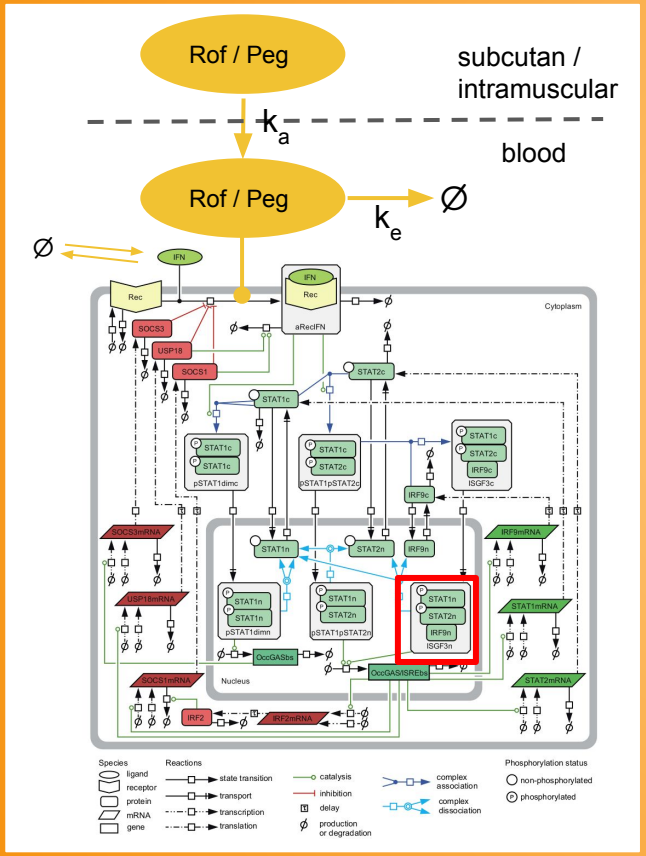



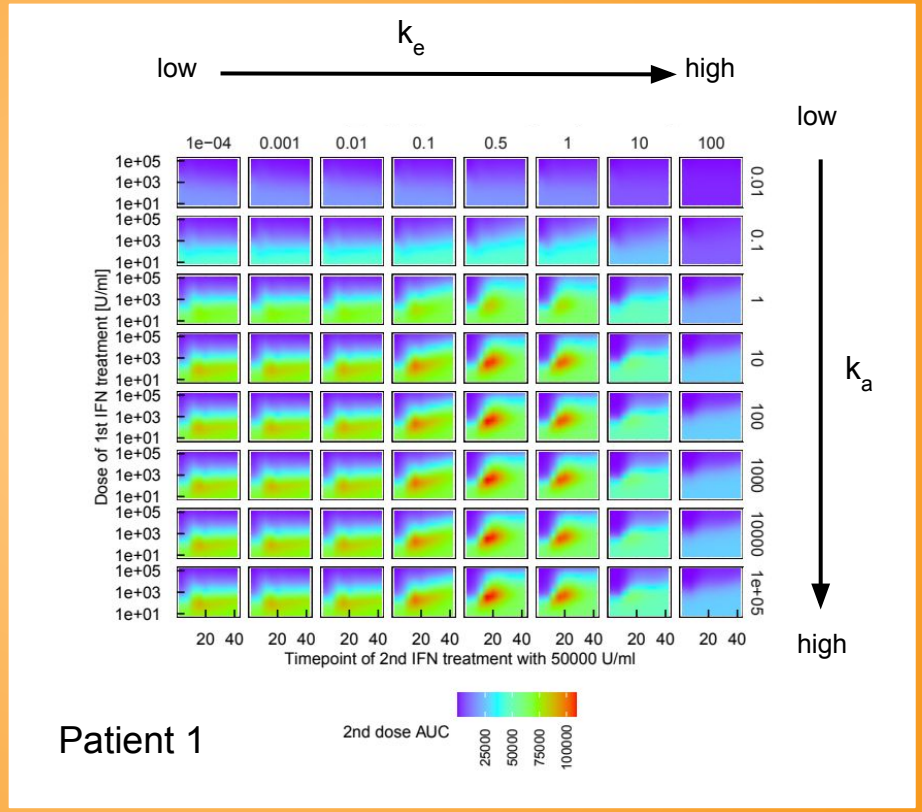
# Link to pharmacokinetic model



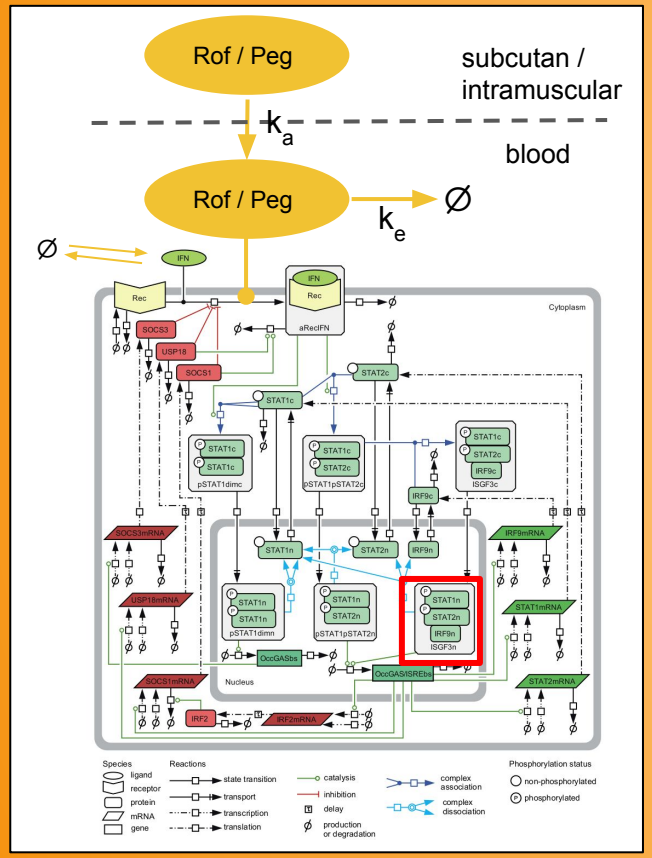



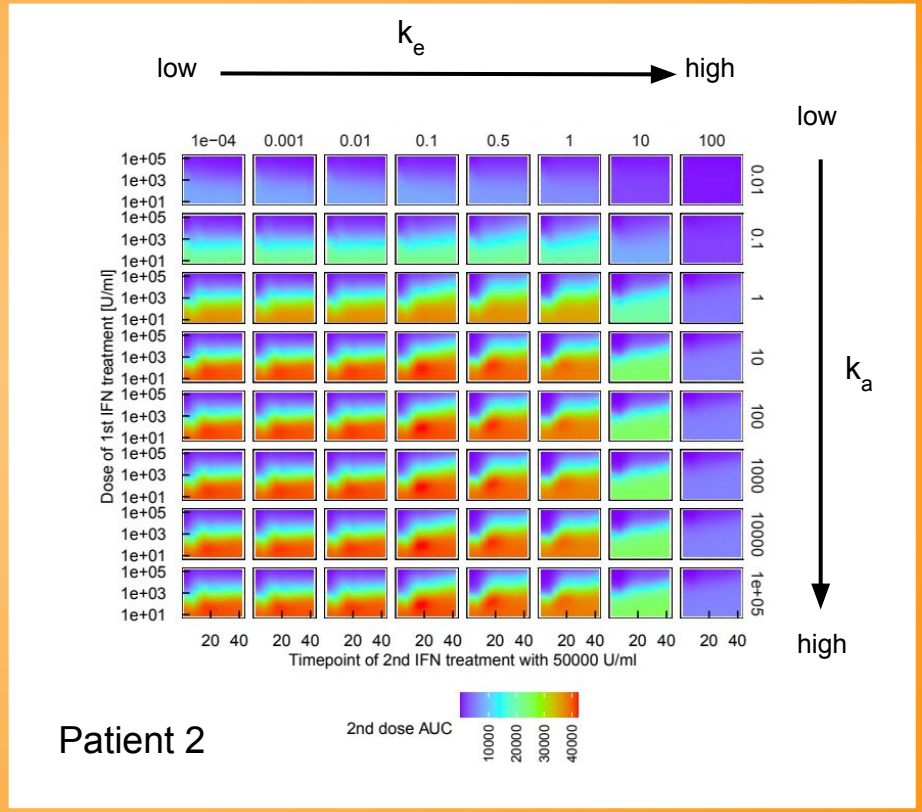
# Link to pharmacokinetic model



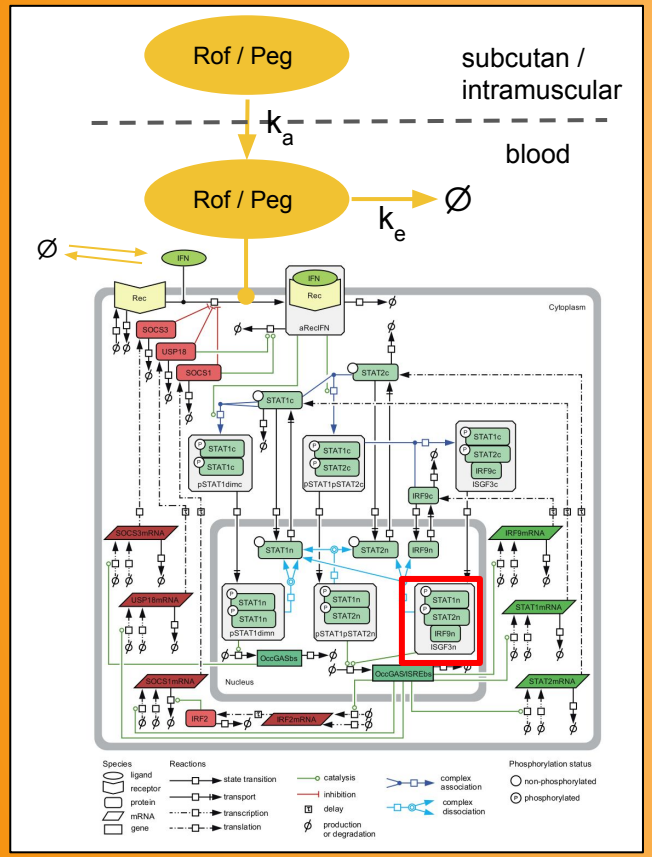



# Link to pharmacokinetic model



# Summary




**Dose-dependent sensitization observable across:**

- Multiple cell systems (including PHH)
- Different stimuli including their PK
- Transcription factors, promoter regions and ISGs
- Dynamic of ISPs (work in progress)

**Patient-individual optimal treatment is influenced by**

- Abundance of feedback proteins
- PK parameters
- Desired readout (ISRE vs. GAS, Target protein)
- But interestingly: Optimal treatment time point is relatively stable

**What next?**

- Optimize response given a multiple treatment scheme
- Compare to clinical application
- More patient data?

# Acknowledgements


Division Systems Biology and Signal Transduction, German Cancer Research Center (DKFZ)

- Till Möcklinghoff
- Frédérique Kok
- Melissa Teusel
- Tamar Nizharadze
- Artyom Vlasov
- Marvin Wäsch
- Barbara Helm
- Marcel Schilling
- Ursula Klingmüller

Department of Hepatobiliary Surgery and Visceral Transplantation, University of Leipzig

- Georg Damm
- Daniel Seehofer

Department of Medicine II, University Hospital Freiburg

- Tobias Böttler

Institute of Physics, University of Freiburg

- Philipp Waibel
- Jacques Hermes
- Christian Tönsing
- Tim Maiwald
- … (Many further group members)
- Jens Timmer

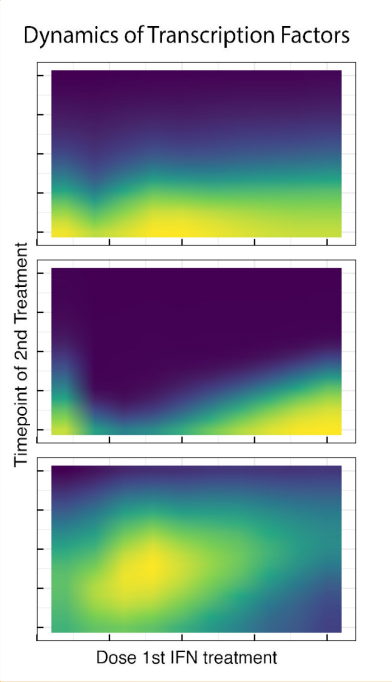
Division Virus-associated Carcinogenesis, German Cancer Research Center (DKFZ)

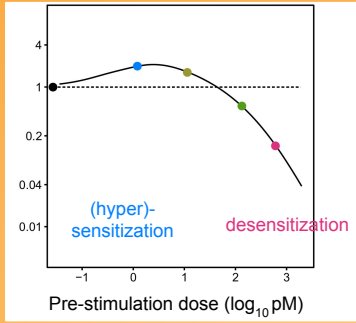
- Christopher Dächert
- Marco Binder
- Ralf Bartenschlager

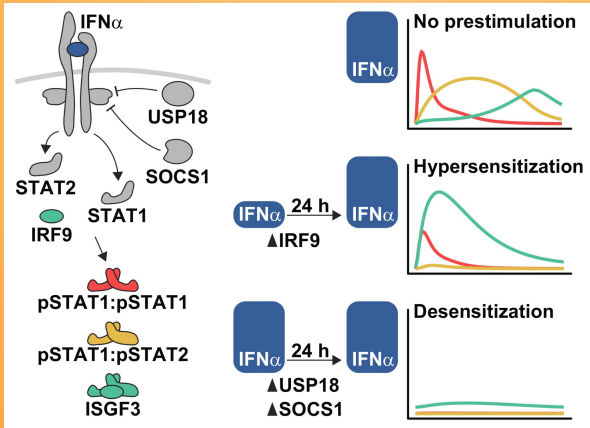
Department of General and Transplantation Surgery, Heidelberg University

- Silvana Tyufekchieva
- Katrin Hoffmann

Funded by DFG Deutsche Forschungsgemeinschaft German Research Foundation




E-Mail: marcus.rosenblatt@gmail.com

Phone: +49 761 203 8530

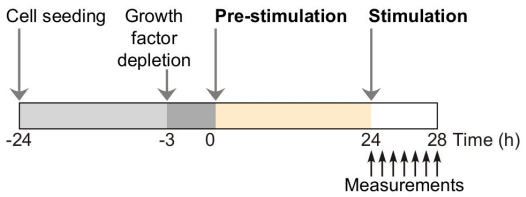
Web: www.sysbio.uni-freiburg.de/mrosen

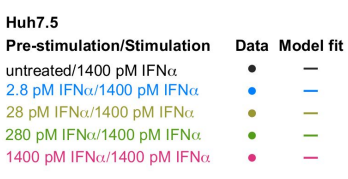
Twitter: @mrf7000,@jetilab

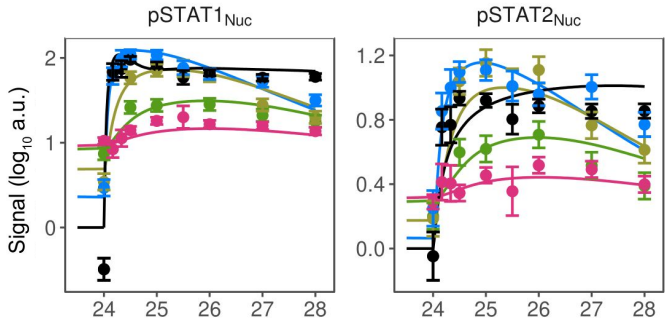
LinkedIn: Marcus Rosenblatt

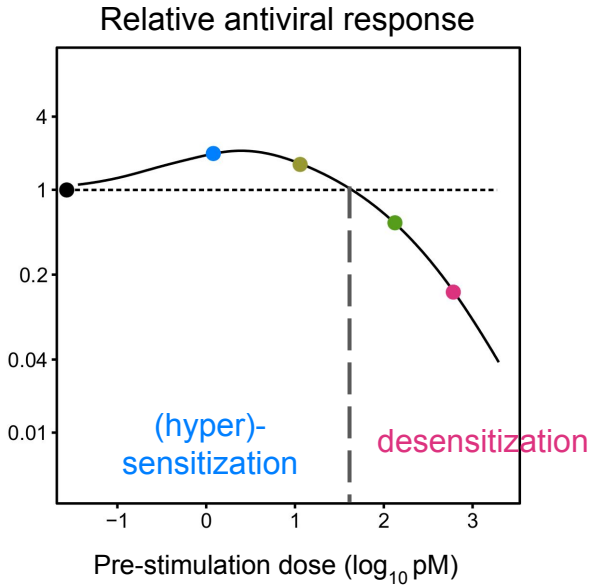
# Back-up slides

# Dose-dependent pathway sensitization

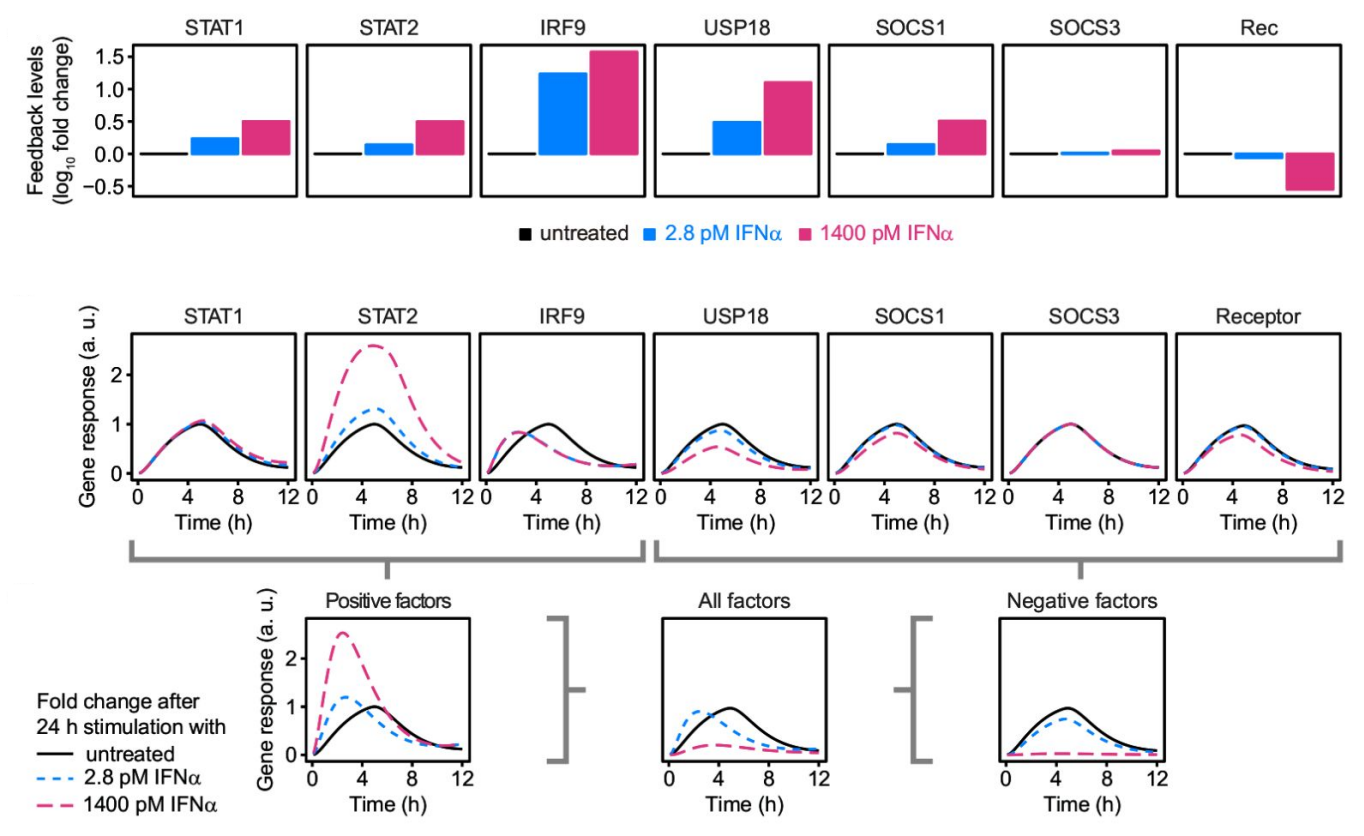


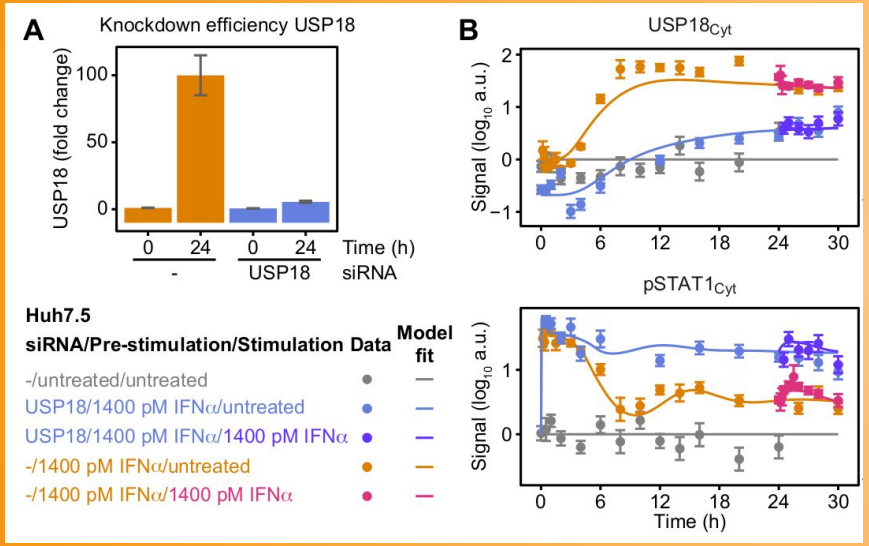



# Disentangle feedback contributions



# The role of USP18

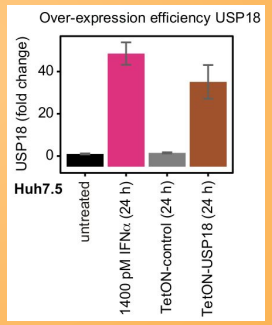
## USP18 knockdown

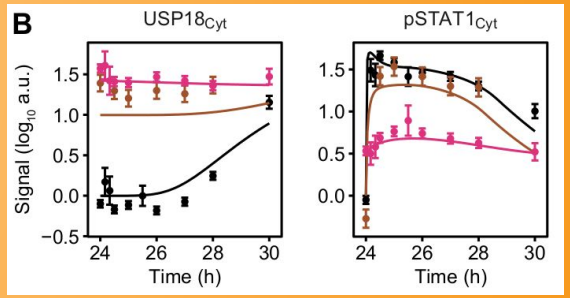



USP18 is a negative regulator

## USP18 over-expression

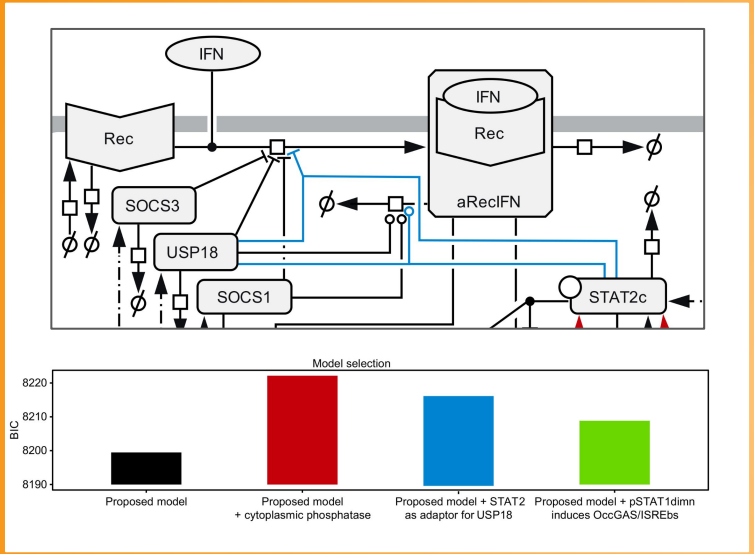
| | Pre-stimulation/Stimulation | Data | Model fit |
|---|---|---|---|
| Huh7.5 | untreated/1400 pM IFNα | ● | — |
| Huh7.5-TetON-USP18 | doxycyclin/1400 pM IFNα | ● | — |
| Huh7.5 | 1400 pM IFNα/1400 pM IFNα | ● | — |



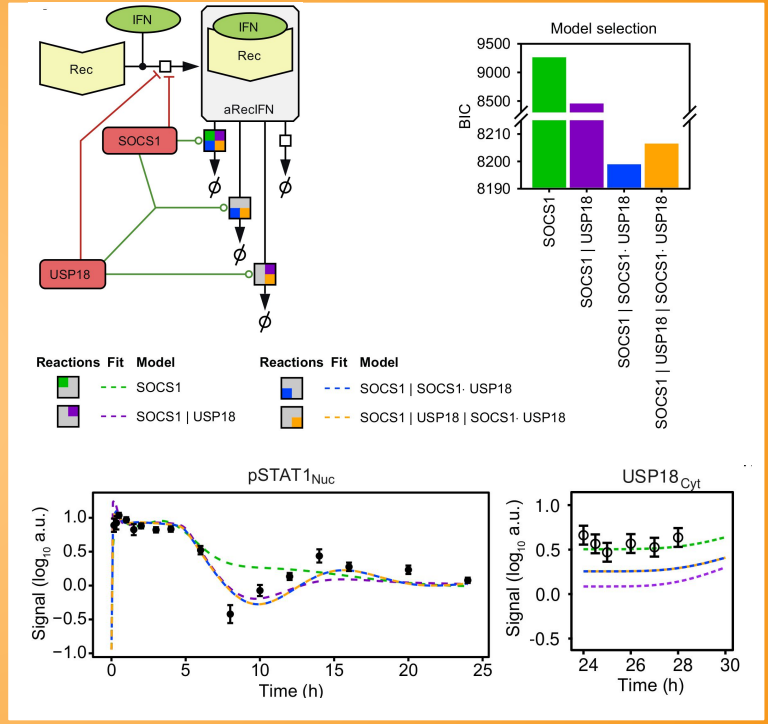




USP18 is not sufficient for desensitization

# Model selection




STAT2 as adaptor for USP18 not necessary




Synergetic effect of SOCS1 and USP18

# Interferons – A very current topic

**Type III interferons disrupt the lung epithelial barrier upon viral recognition**

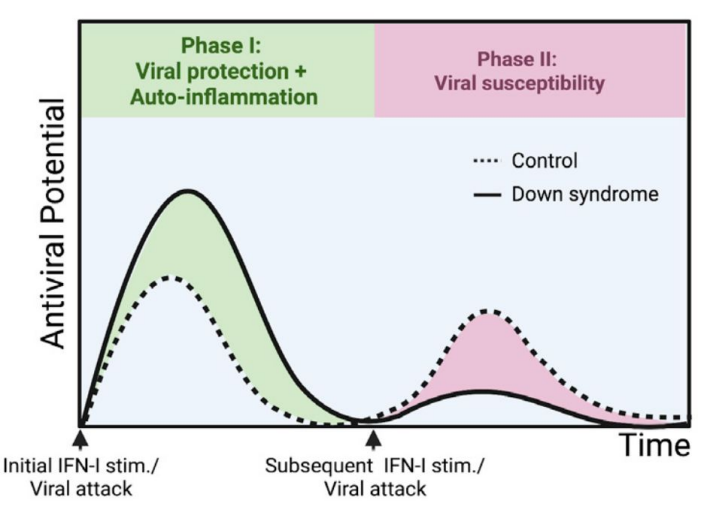
ACHILLE BROGGI, SREYA GHOSH, BENEDETTA SPOSITO, ROBERTO SPREAFICO, FABIO BALZARINI, ANTONINO LO CASCIO, NICOLA CLEMENTI, MARIA DE SANTIS, NICASIO MANCINI, [...], AND IVAN ZANONI +1 authors Authors Info & Affiliations

SCIENCE · 11 Jun 2020 · Vol 369, Issue 6504 · pp. 706-712 · DOI: 10.1126/science.abc3545

"SARS-CoV-2 patients benefit from therapeutic interferons when given early enough"

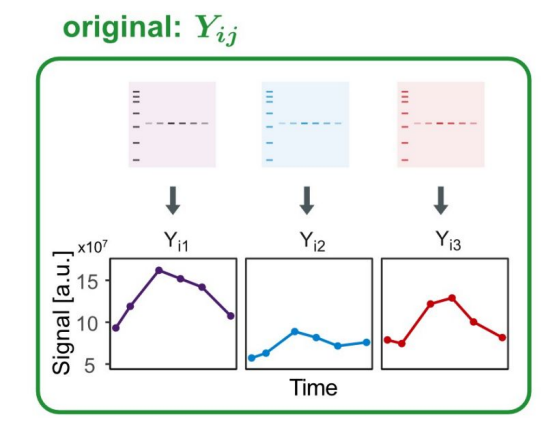
Immunity

**Excessive negative regulation of type I interferon disrupts viral control in individuals with Down syndrome**




Malle et al., 2022, Immunity 55, 2074–2084
November 8, 2022 © 2022 Elsevier Inc.
https://doi.org/10.1016/j.immuni.2022.09.007

# Alignment of biological replicates using BlotIt

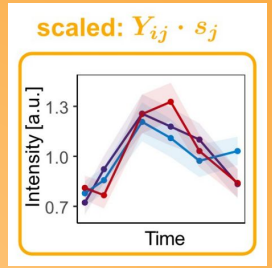



**MODEL**

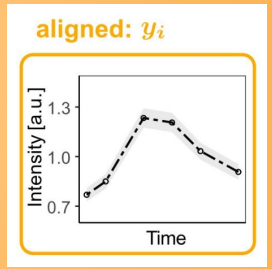
$$Y_{ij} = \frac{y_i}{s_j}$$

**ERROR MODEL**

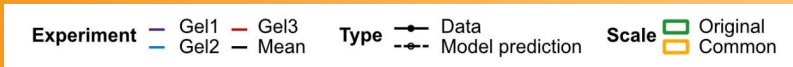
$$\sigma_{ij} = e_{rel} \cdot \frac{y_i}{s_j}$$




- Replicate information
- Use for modeling




- Simple data visualization
- Use for modeling, if low amounts of replicates




Kemmer, Bang et al. “BlotIt – Optimal alignment …”. *PLoS ONE* (2022)

# Analytical steady-state constraints

$$\dot{x} = f(x, p, u),$$

Rosenblatt et al. “Customized steady-state constraints …”
*Frontiers in Cell and Developmental Biology* (2016)