Systems Biology of the Epo-Receptor

Jens Timmer

Center for Systems Biology
Center for Data Analysis and Modeling
Freiburg Institute for Advanced Studies
Department of Mathematics and Physics
University of Freiburg

Department of Clinical and Experimental Medicine
Linköping University, Sweden

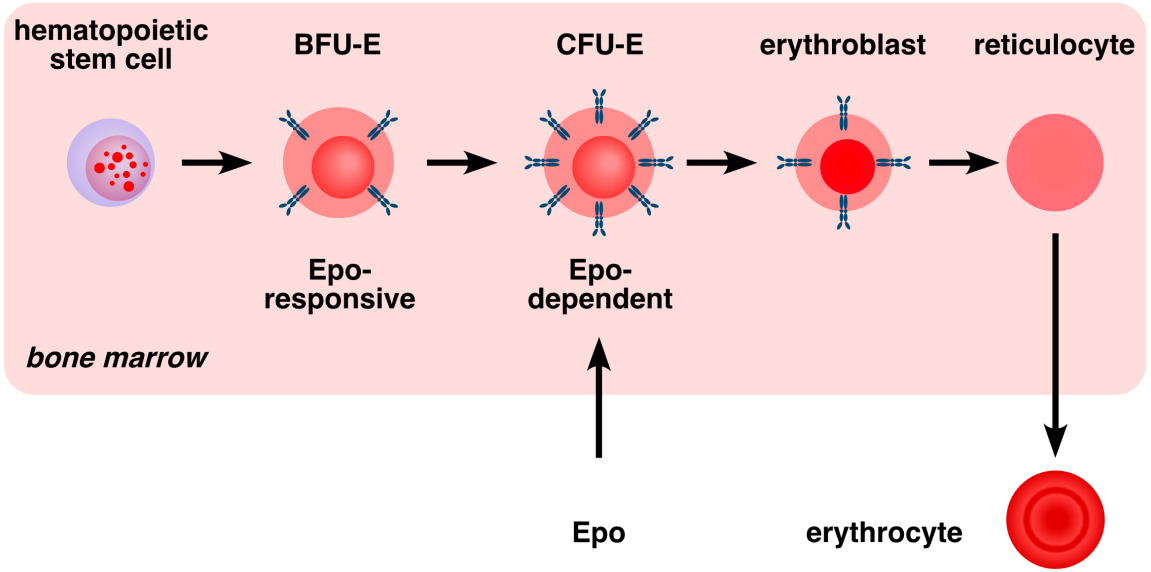
Outline

- Systems Biology
- A dynamical model for the Epo receptor
- Validating the model
- Infering systems' properties
- Understand what is known

Outline

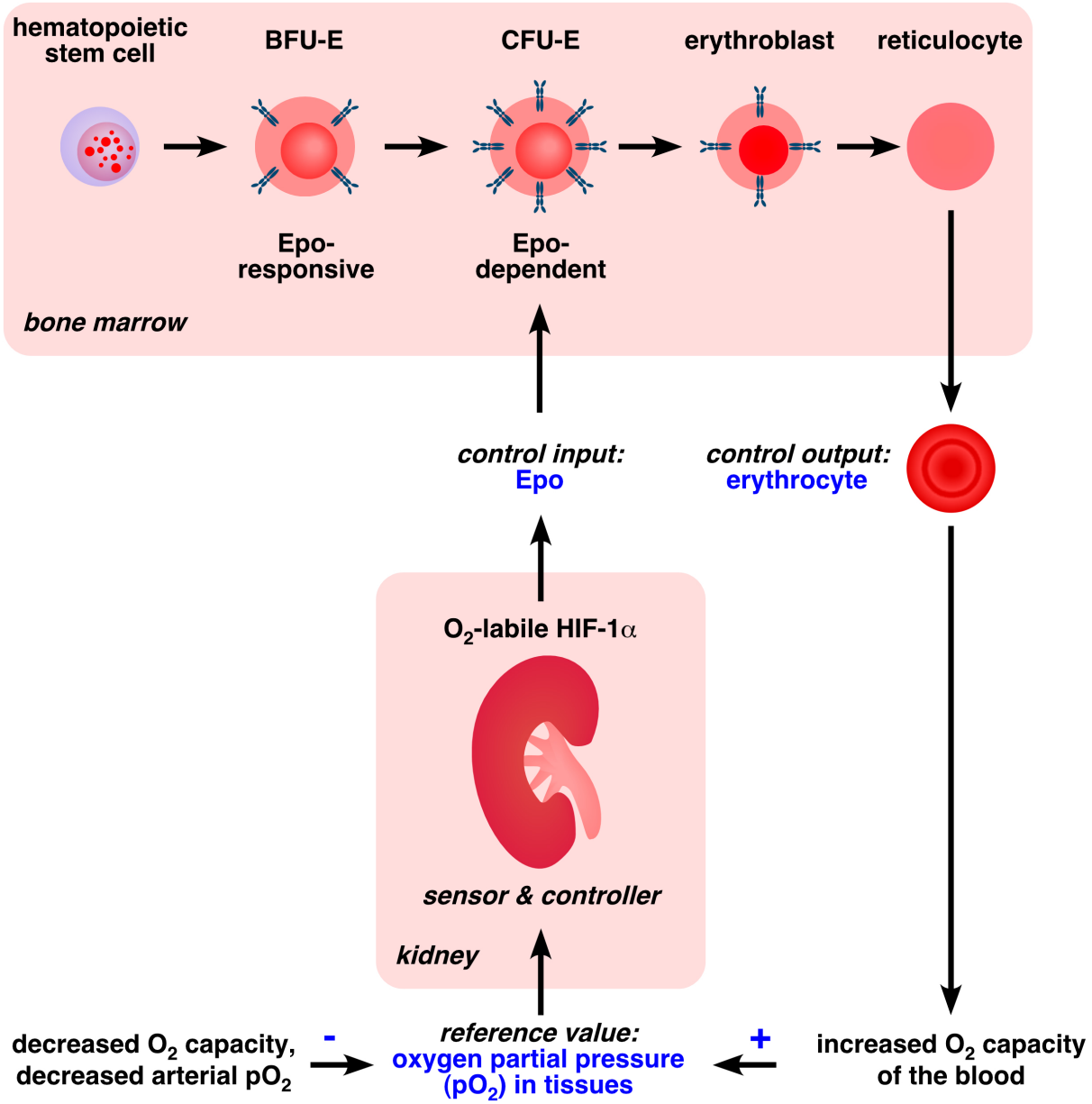
- ~~Systems Biology~~
- A dynamical model for the Epo receptor
- Validating the model
- Infering systems' properties
- Understand what is known
- Latest results

Erythropoiesis - A Closed-Loop Control System



- **Epo:** key regulator of erythropoiesis

Erythropoiesis - A Closed-Loop Control System

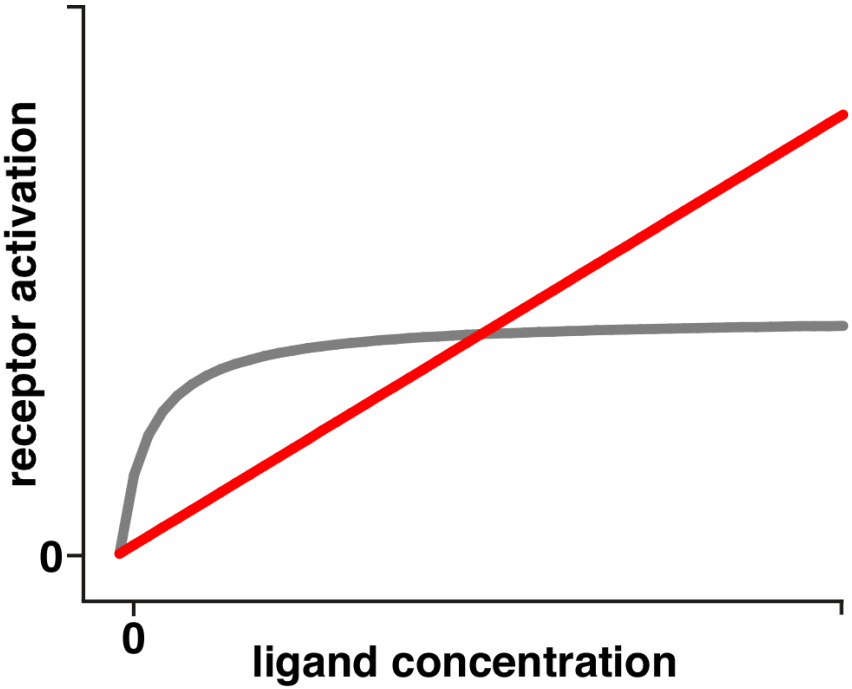


- **Epo:** key regulator of erythropoiesis
- **feedback via red blood cell mass:** establishing a closed-loop control circuit
- **normal conditions:** low levels of plasma Epo

  15 mU/ml
- **hypoxic conditions:** increased Epo levels

  up to 10000 mU/ml

Erythropoiesis - Coping with Different Ligand Concentrations



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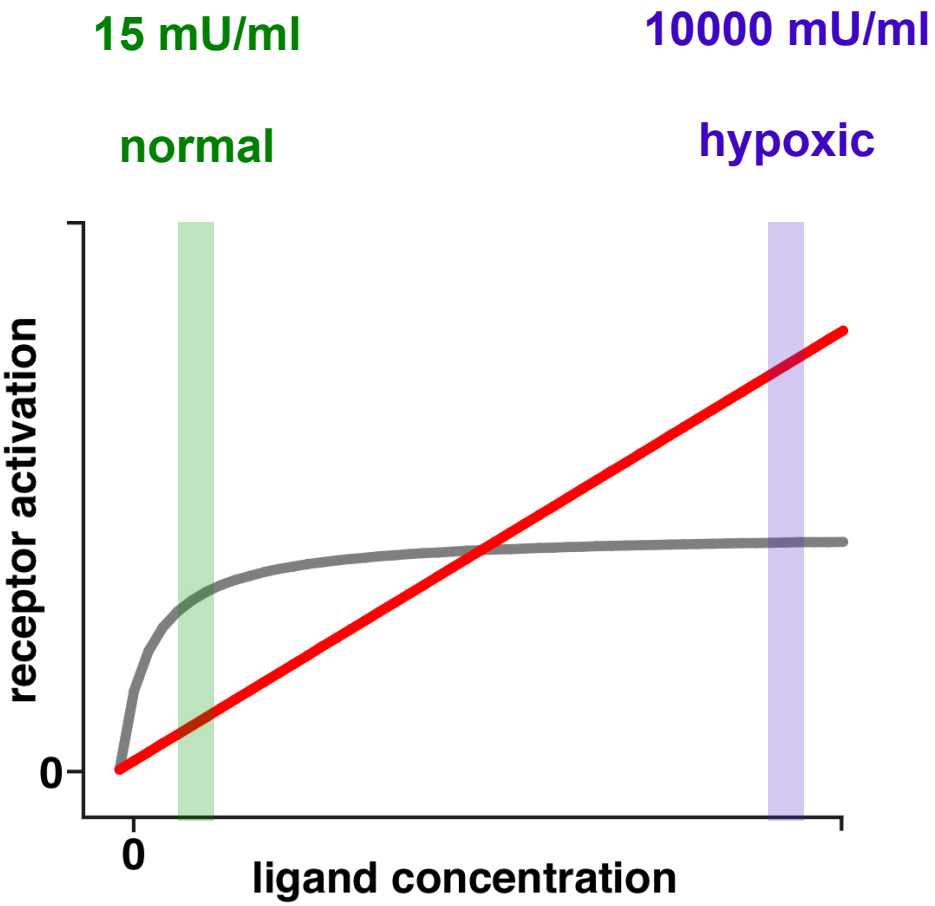
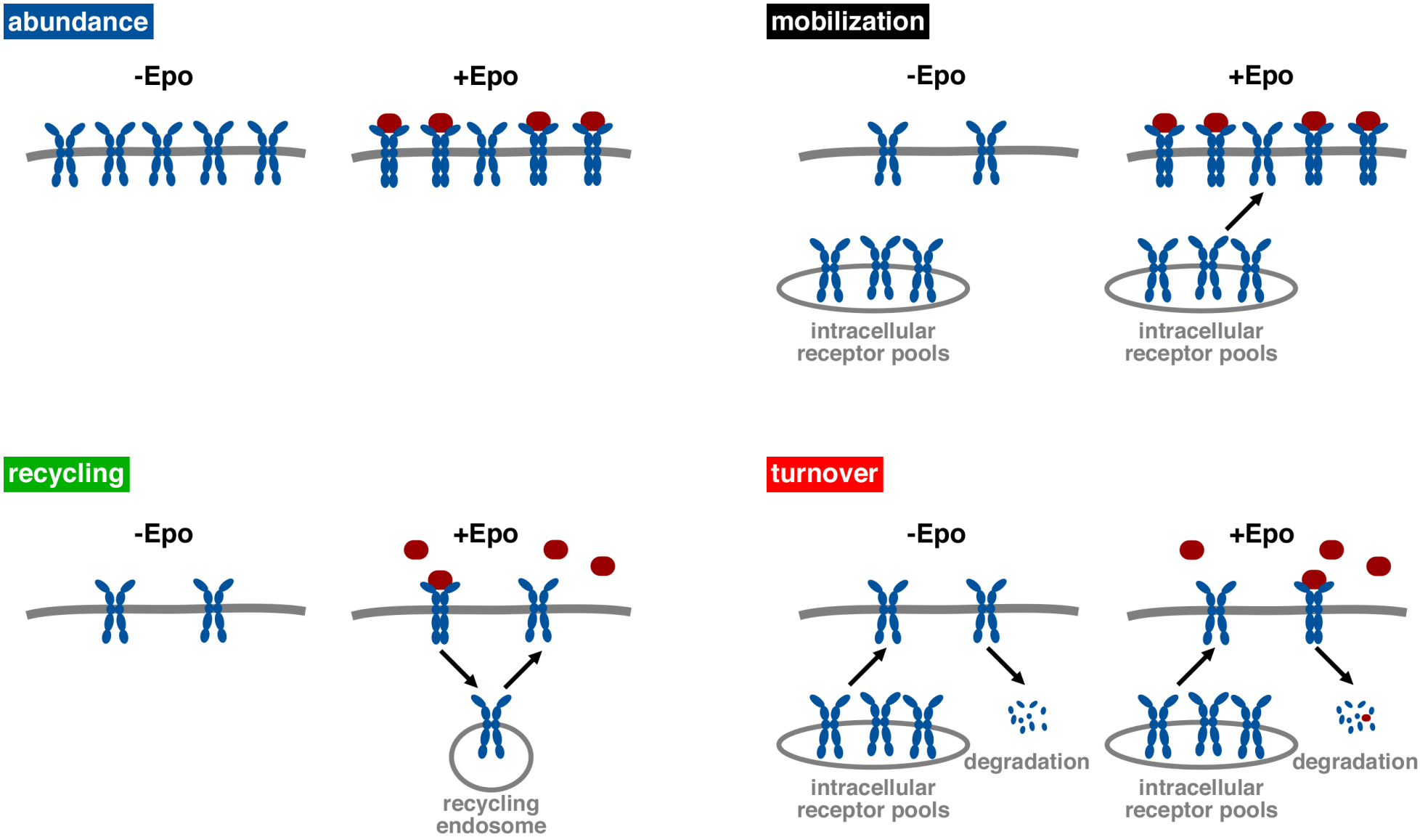


➜ How is ligand-encoded information processed by the EpoR?

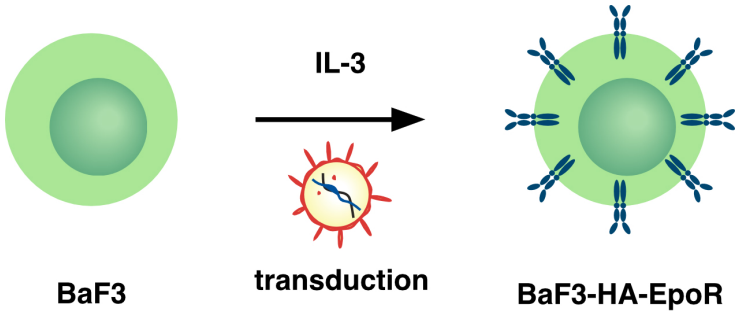
➜ Which dynamic properties of the EpoR facilitate information processing over a broad ligand range?

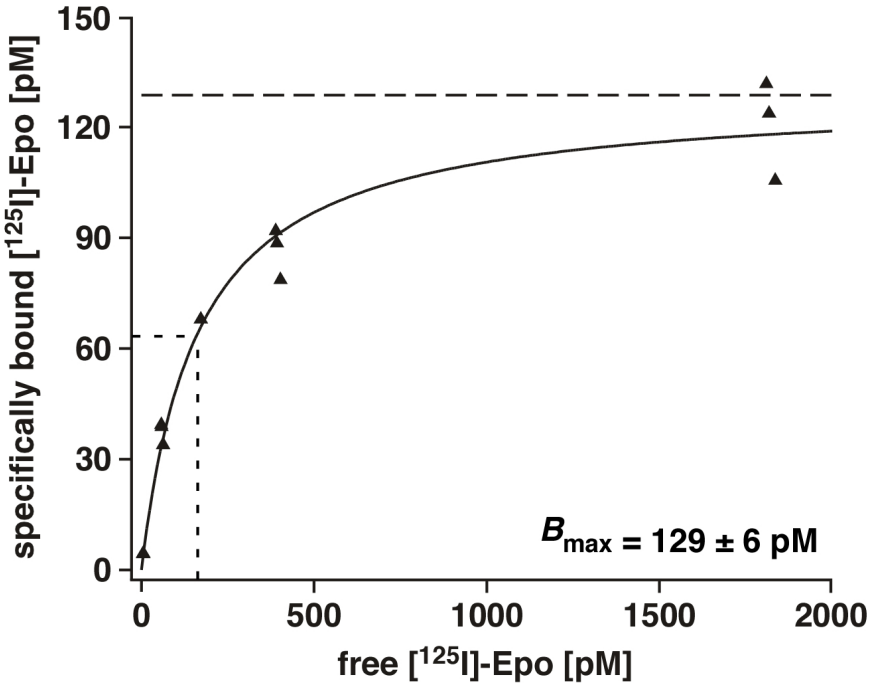
Strategies for Processing Ligand-Encoded Information



Low EpoR Abundance on the Plasma Membrane

lymphoid murine
BaF3-EpoR cell line





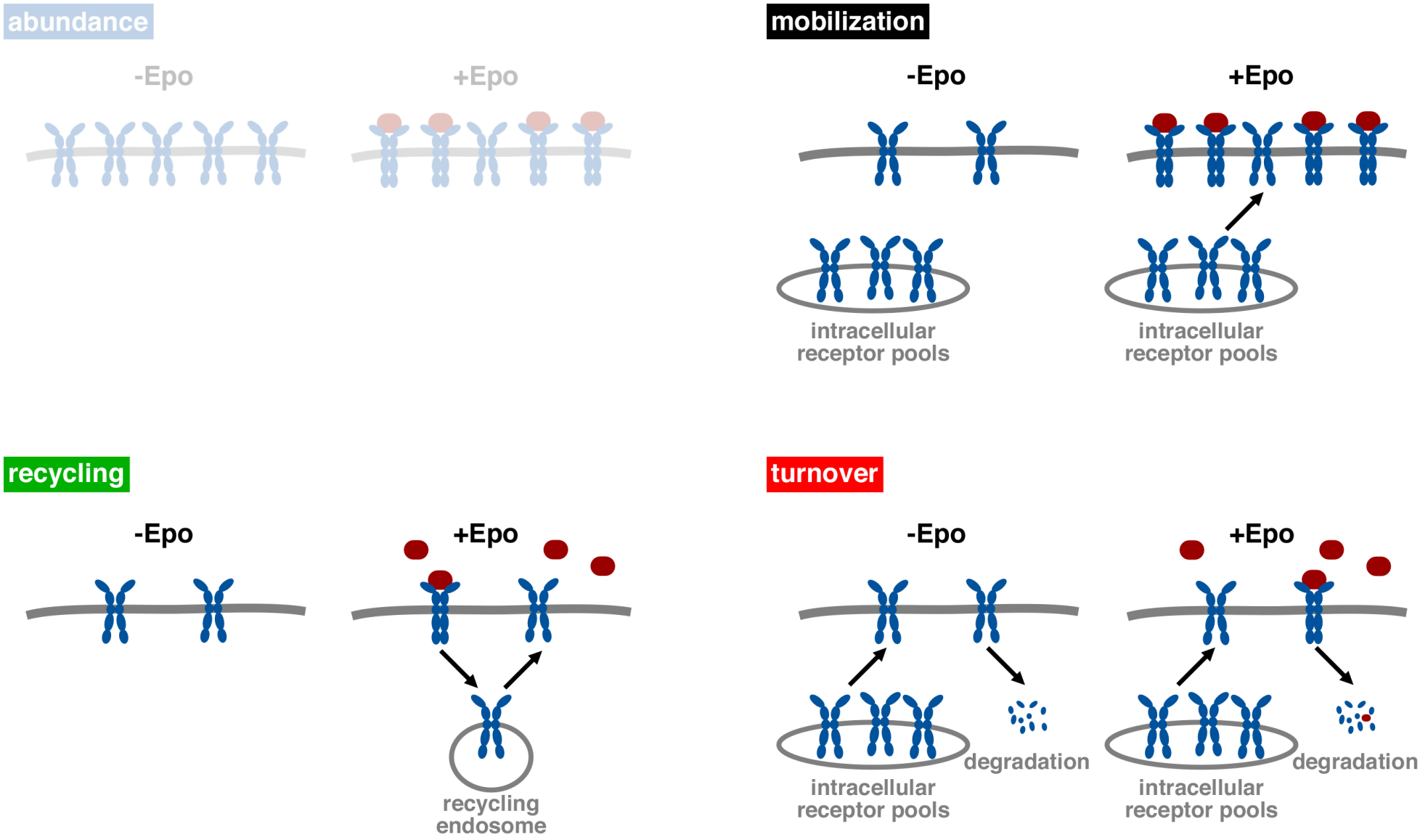
➜ **Epo binding sites:**

- **BaF3-EpoR:**
  appr. 7800
- **primary erythroid progenitor cells:**
  up to 1000

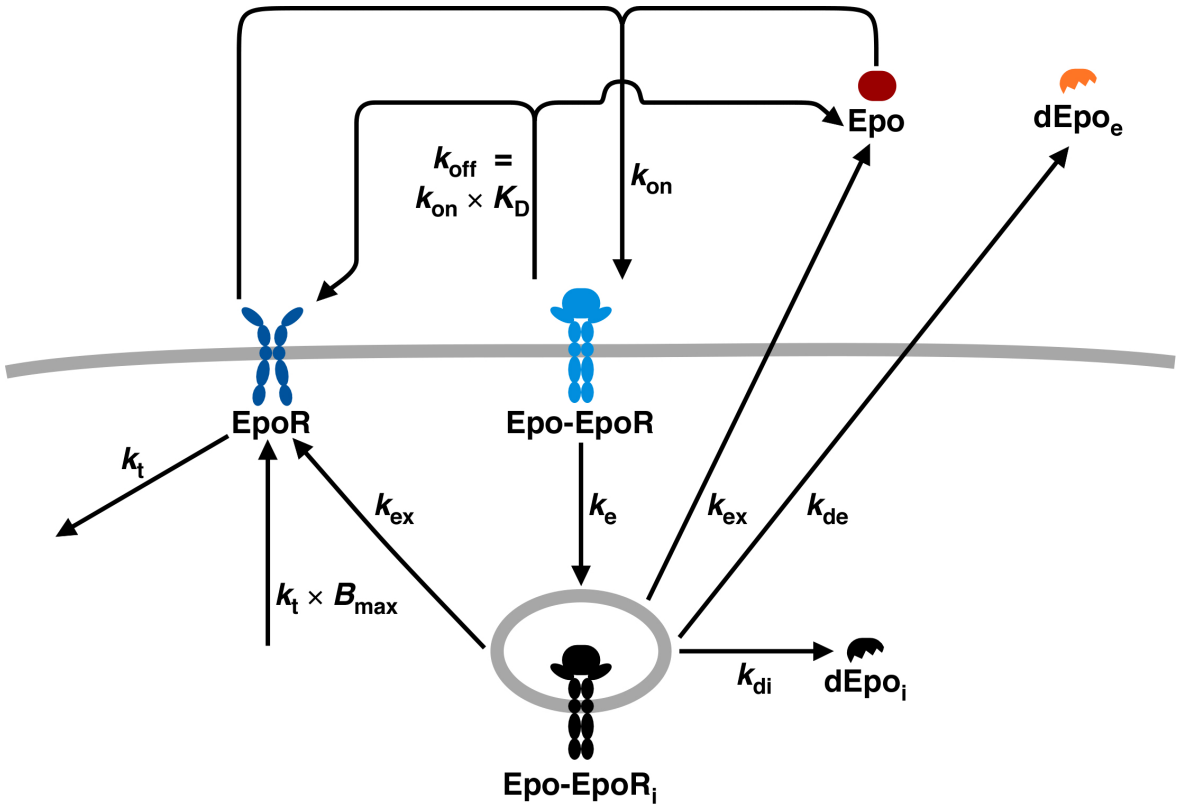
- **EGFR: up to 100000 receptors**

➜ **EpoR abundance excluded as a strategy to cope with large ligand concentrations**

Strategies for Processing Ligand-Encoded Information



Mathematical Model for Epo-EpoR Interaction and Trafficking Kinetics



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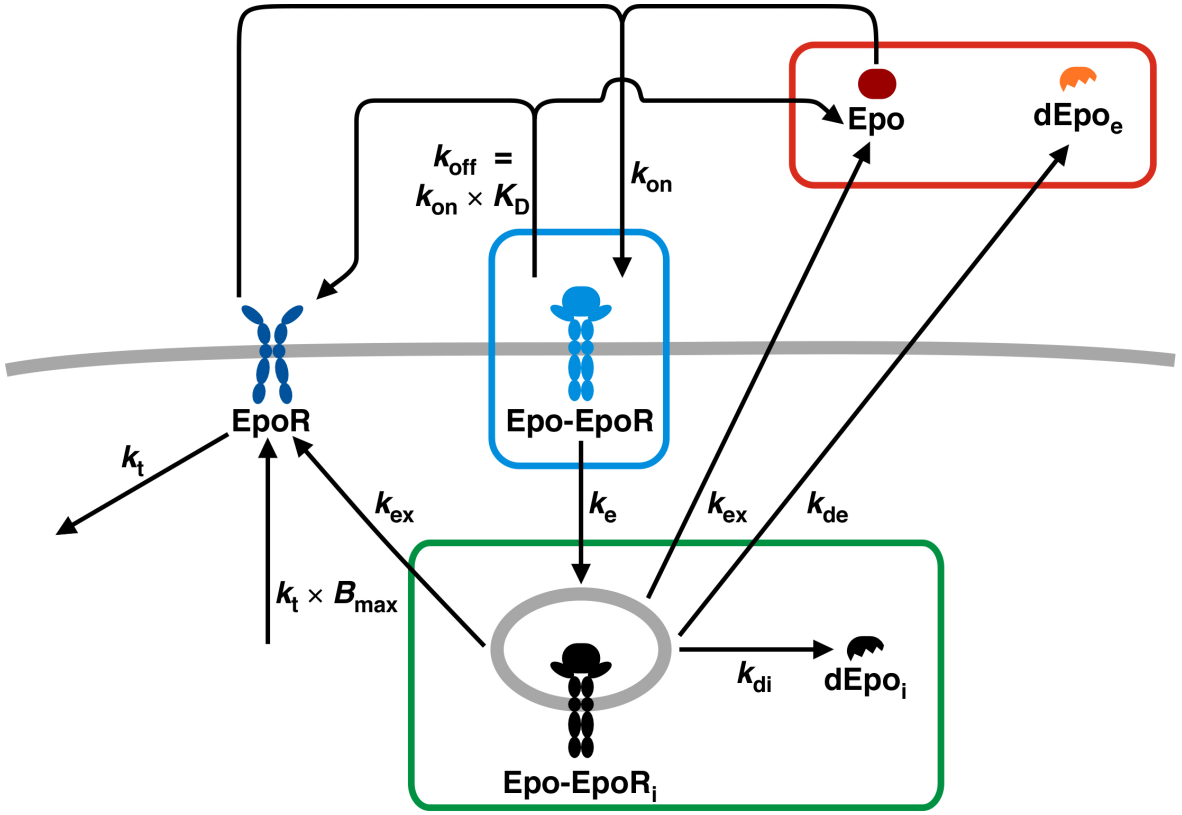
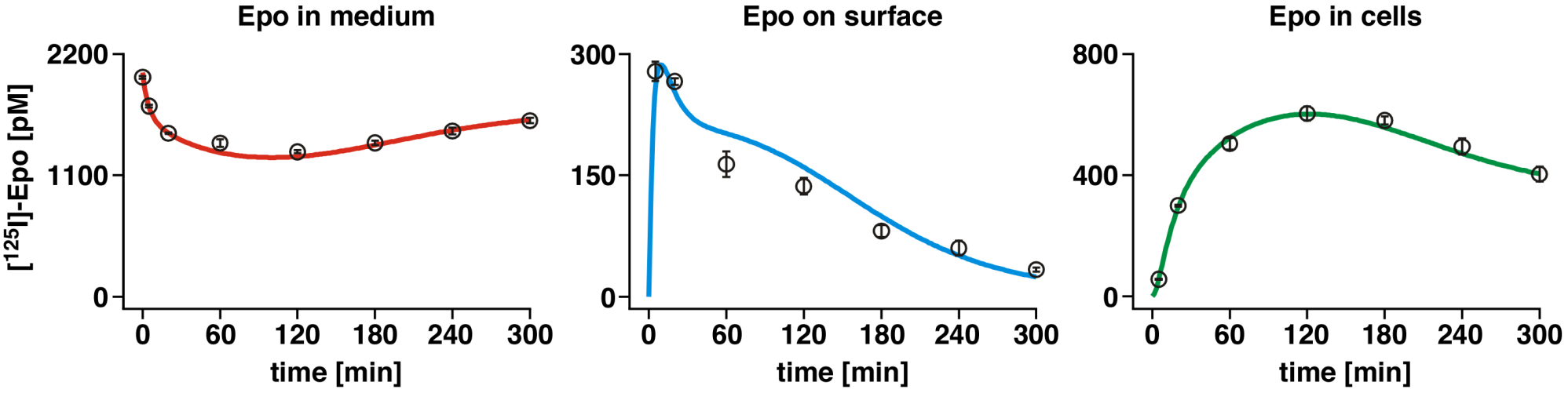


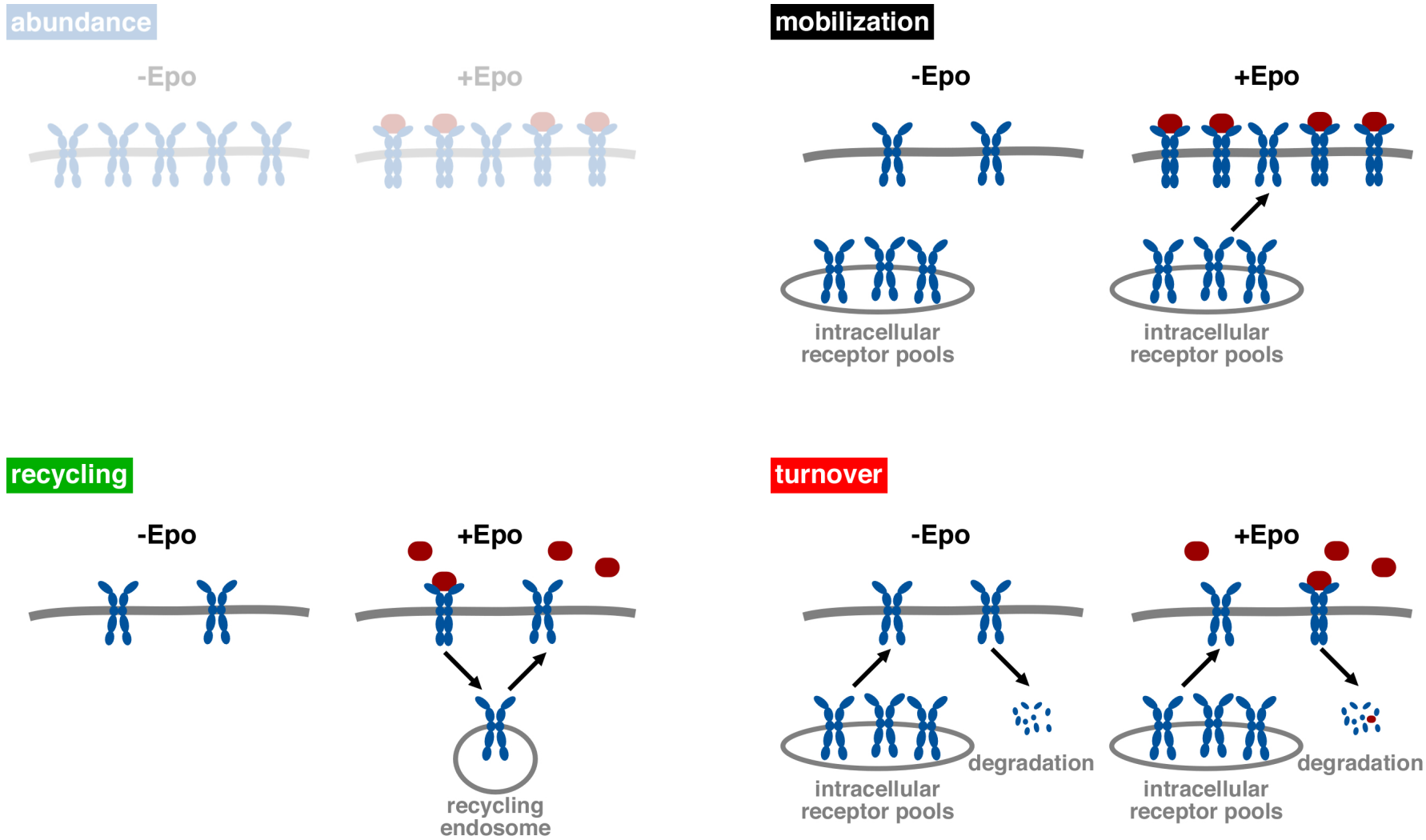
➜ all parameters identifiable with small confidence intervals

➜ allowing for accurate predictions

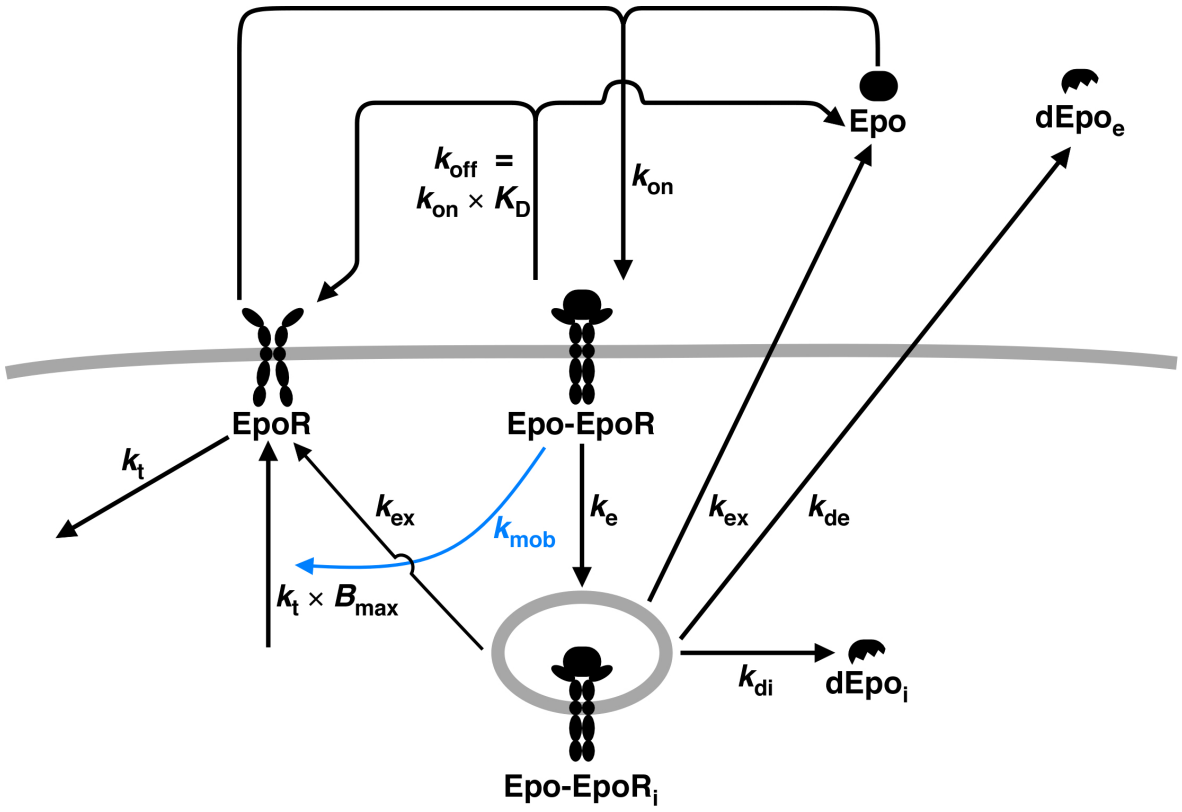
➜ extended model: EpoR mobilization excluded as a major strategy



Strategies for Processing Ligand-Encoded Information



Analysis of Model Including EpoR Mobilization



Model Topology – Core Model / Core Model + k_{mob}

'Core model' / 'Core model + k_{mob}'

Ordinary differential equations ('core model')

EpoR	$\dot{x}_1 = k_t \cdot B_{max} - k_t \cdot x_1 - k_{on} \cdot x_1 \cdot x_2 + k_{off} \cdot x_3 + k_{ex} \cdot x_4$
Epo	$\dot{x}_2 = - k_{on} \cdot x_1 \cdot x_2 + k_{off} \cdot x_3 + k_{ex} \cdot x_4$
Epo-EpoR	$\dot{x}_3 = k_{on} \cdot x_1 \cdot x_2 - k_{off} \cdot x_3 - k_e \cdot x_3$
Epo-EpoR$_i$	$\dot{x}_4 = k_e \cdot x_3 - k_{ex} \cdot x_4 - k_{di} \cdot x_4 - k_{de} \cdot x_4$
dEpo$_i$	$\dot{x}_5 = k_{di} \cdot x_4$
dEpo$_e$	$\dot{x}_6 = k_{de} \cdot x_4$

Ordinary differential equations ('core model + k_{mob}')

EpoR	$\dot{x}_1 = k_t \cdot B_{max} + k_{mob} \cdot x_3 - k_t \cdot x_1 - k_{on} \cdot x_1 \cdot x_2 + k_{off} \cdot x_3 + k_{ex} \cdot x_4$
Epo	$\dot{x}_2 = - k_{on} \cdot x_1 \cdot x_2 + k_{off} \cdot x_3 + k_{ex} \cdot x_4$
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dEpo$_i$	$\dot{x}_5 = k_{di} \cdot x_4$
dEpo$_e$	$\dot{x}_6 = k_{de} \cdot x_4$

Assignment rules

k_{off}	$k_{off} = k_{on} \cdot K_D$

Nonzero initial values

EpoR	$x_1 = B_{max}$ (t=0)
Epo	x_2 (t=0)

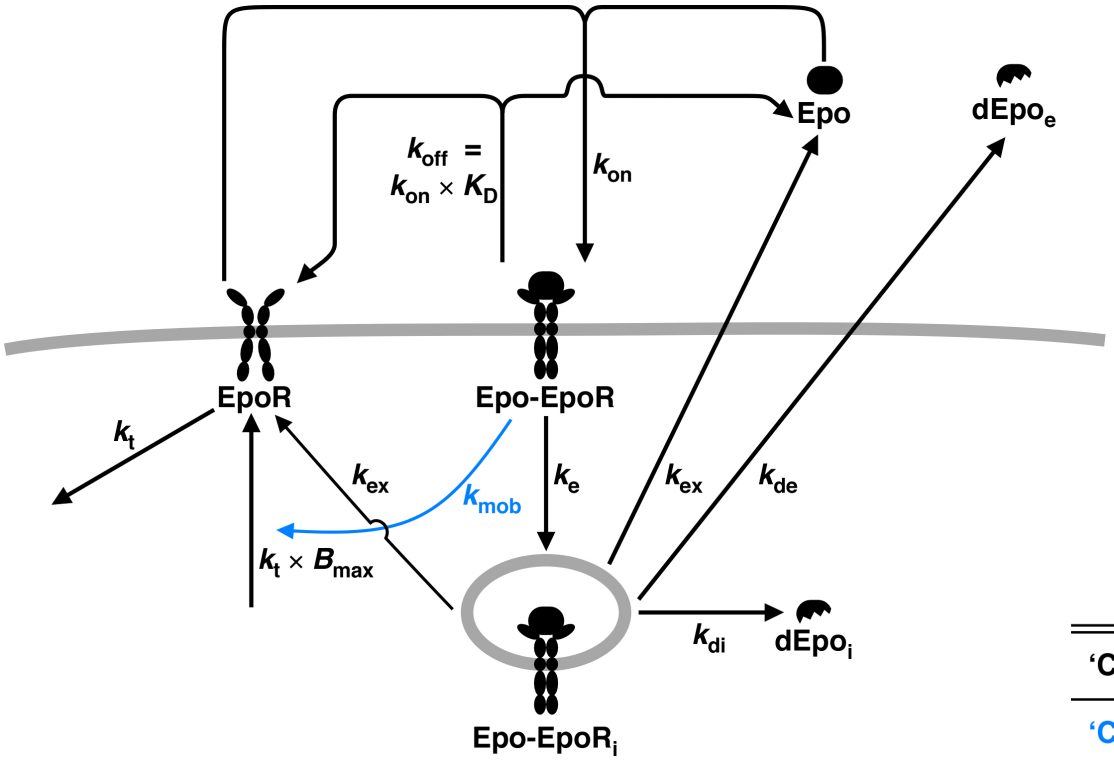
Observables

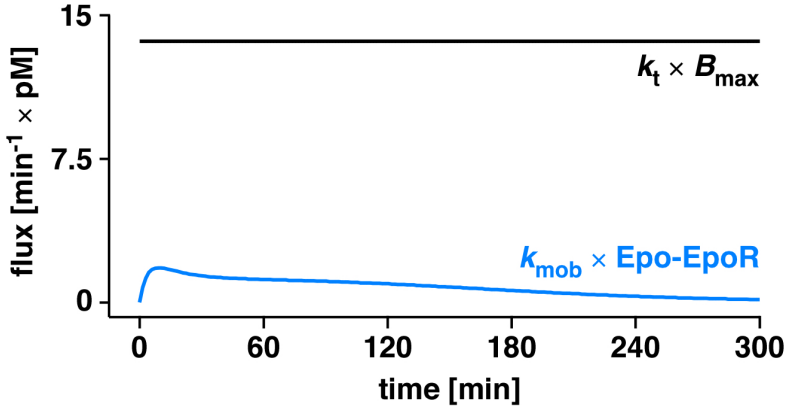
Epo in medium	$y_1 = x_2 + x_6$ (Epo + dEpo$_e$)
Epo on surface	$y_2 = x_3$ (Epo-EpoR)
Epo in cells	$y_3 = x_4 + x_5$ (Epo-EpoR$_i$ + dEpo$_i$)

Parameters

k_t	ligand-independent EpoR endocytosis
k_{mob}	ligand-induced EpoR mobilization
k_{on}	association of Epo and EpoR
k_{off}	dissociation of Epo and EpoR
K_D	dissociation constant for Epo-EpoR
k_e	ligand-induced EpoR endocytosis
k_{ex}	recycling of Epo and EpoR
k_{di}	degradation of ligand-EpoR complexes, remaining intracellular
k_{de}	degradation of ligand-EpoR complexes, secreted extracellular

Analysis of Model Including EpoR Mobilization

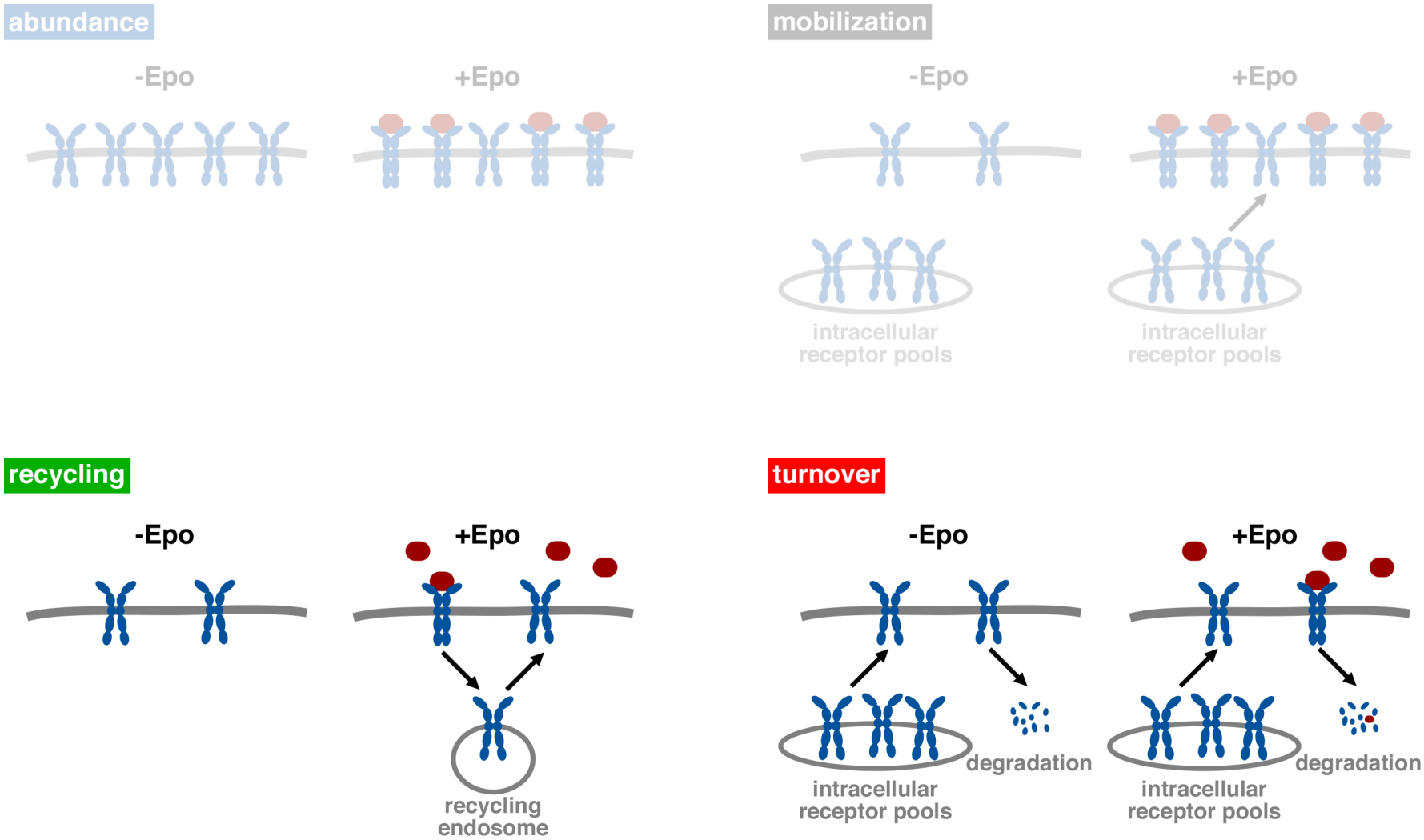




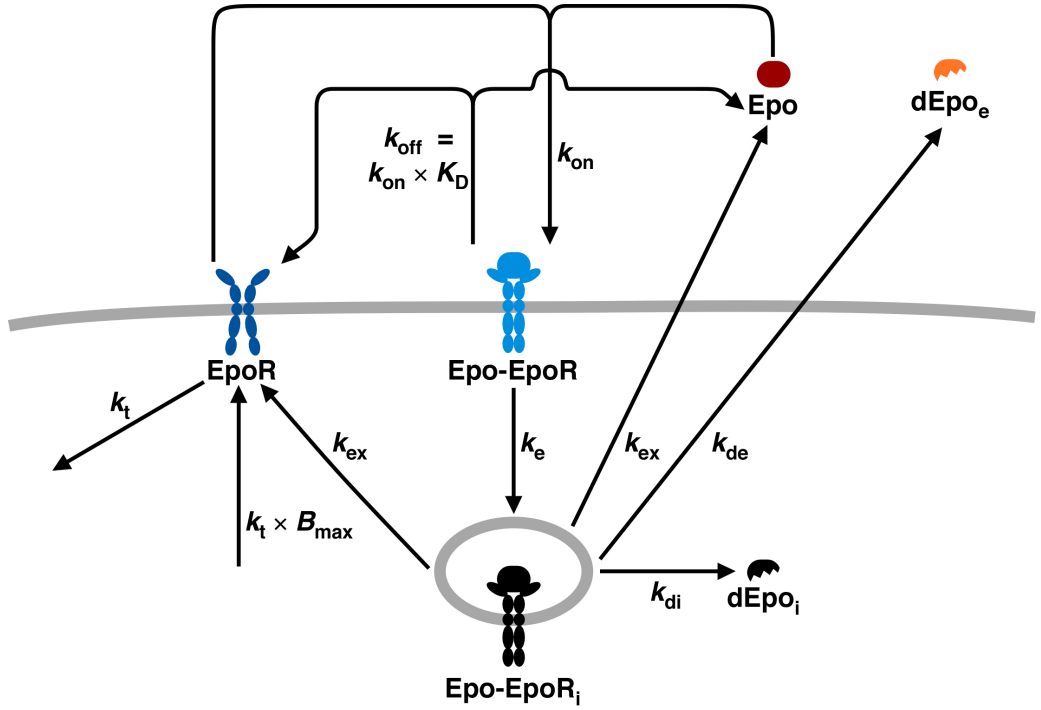
	# of parameters	*p*(LRT)	AIC	BIC
'Core model'	10		172.2	186.9
'Core model + k_{mob}'	11	0.4178	173.6	189.7

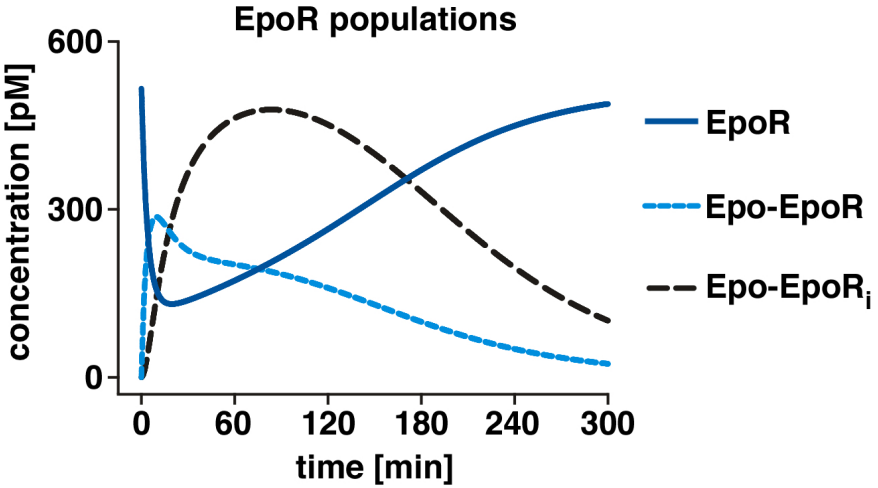
➜ EpoR mobilization excluded as a major strategy to cope with large ligand concentrations

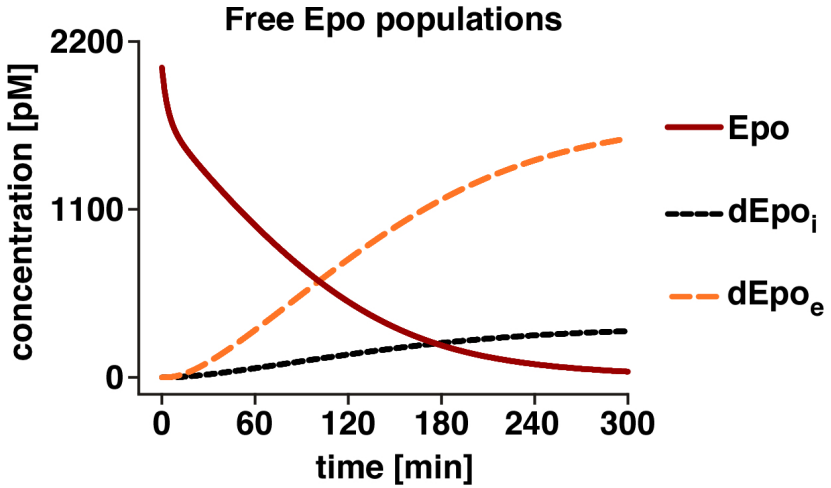
Strategies for Processing Ligand-Encoded Information



Key Properties of the EpoR System



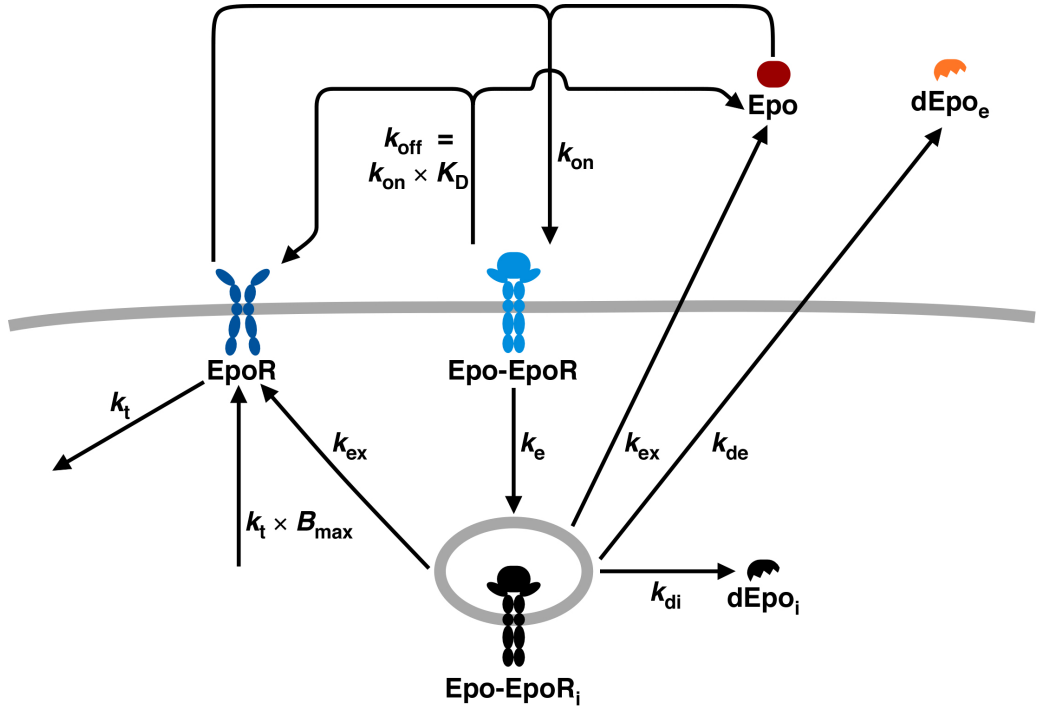


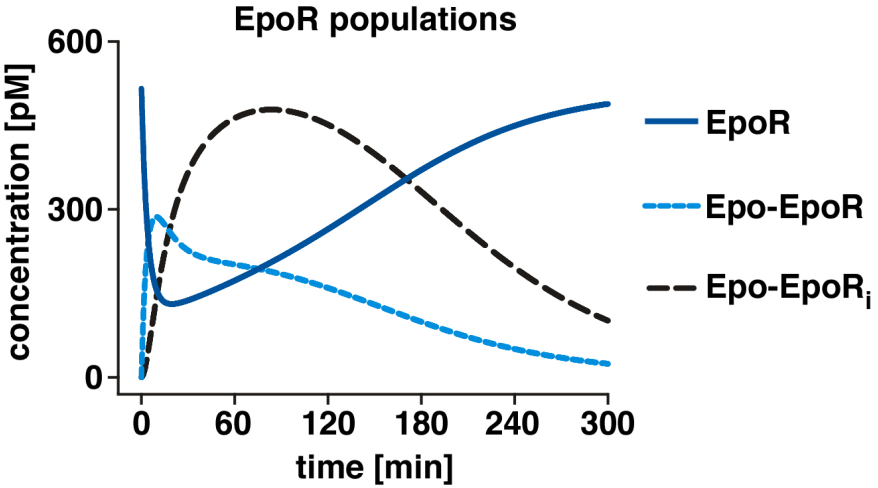


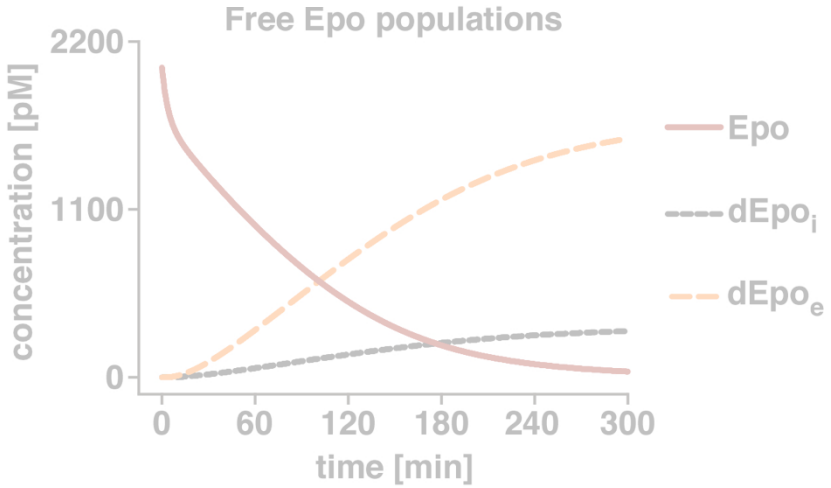
➜ fast recovery of cell surface EpoR

➜ rapid depletion of intact Epo

EpoR Recovery at the Cell Surface - Model Validation



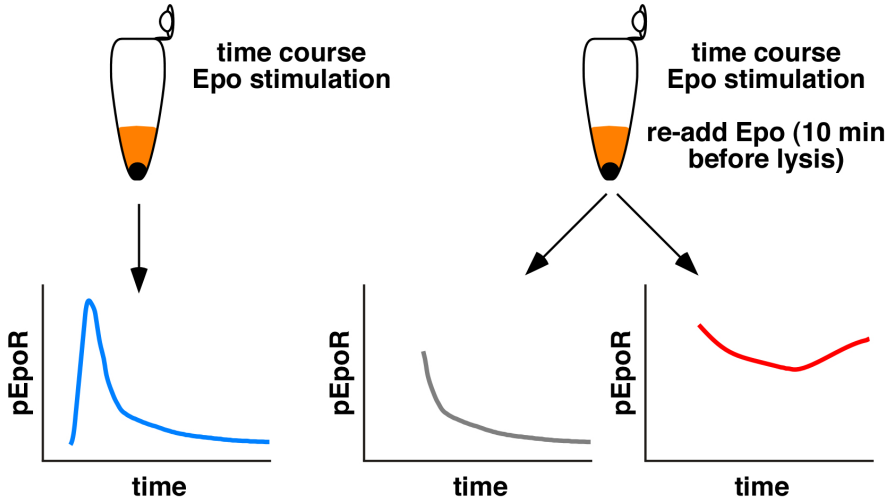


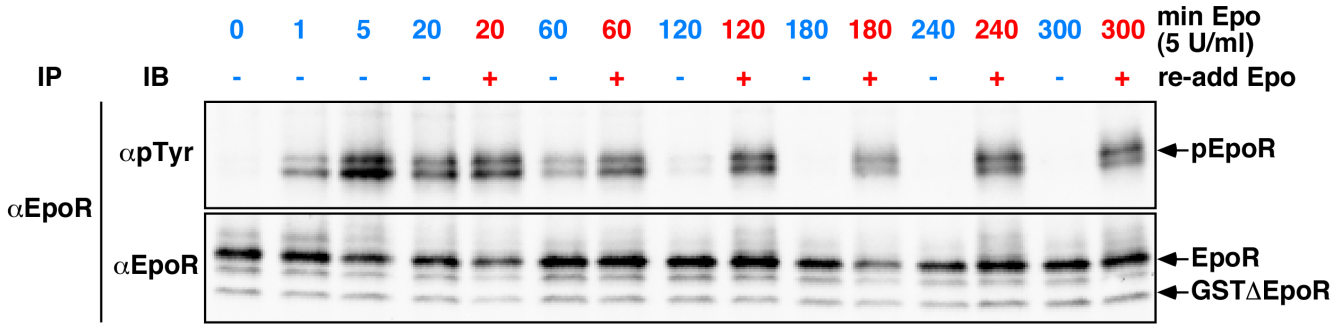


➜ **fast recovery of cell surface EpoR**

➜ **rapid depletion of intact Epo**

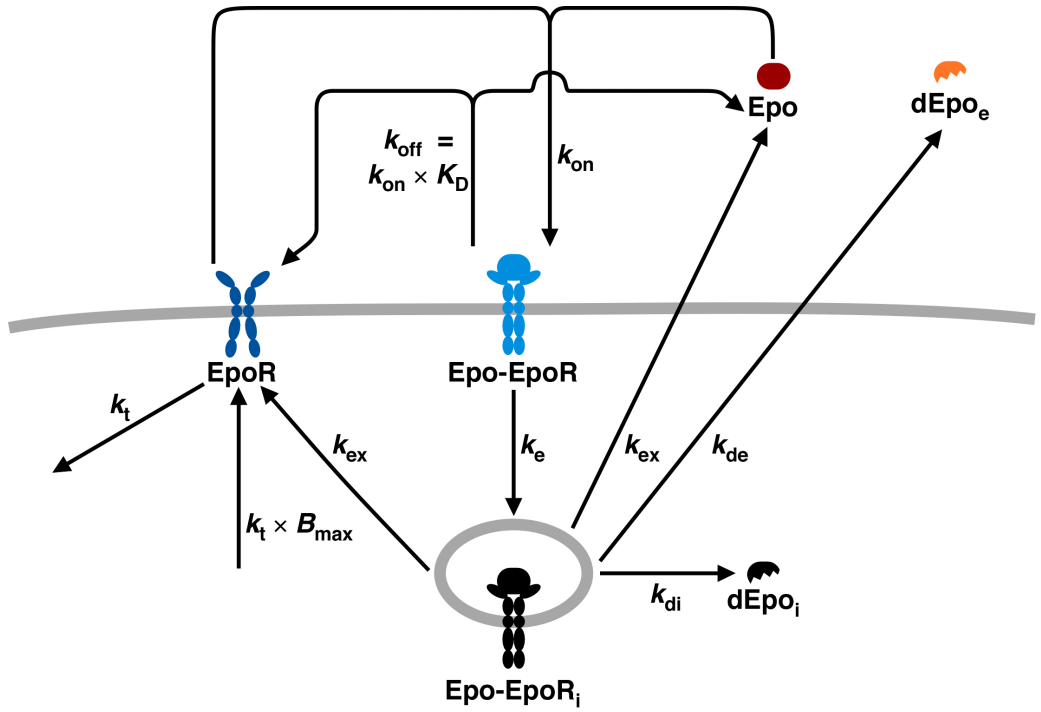
EpoR Recovery at the Cell Surface - Model Validation

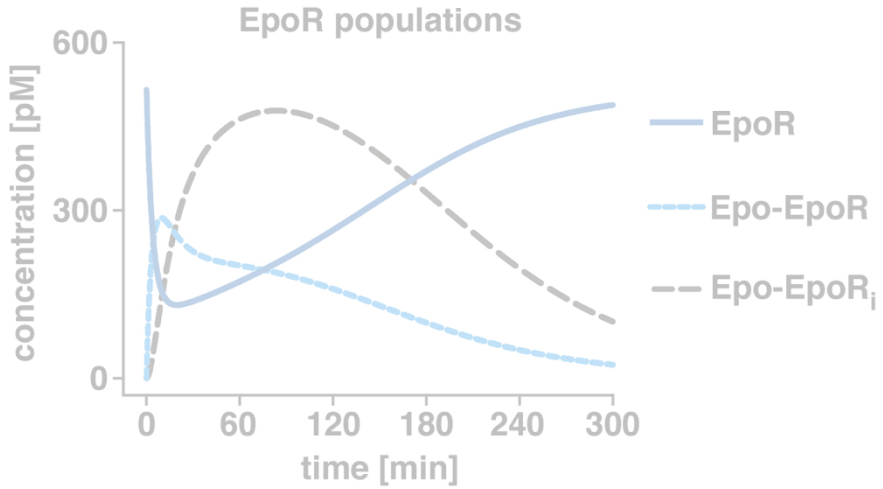




➜ recovery of EpoR, cells remain ligand-responsive

Epo Depletion - Model Validation





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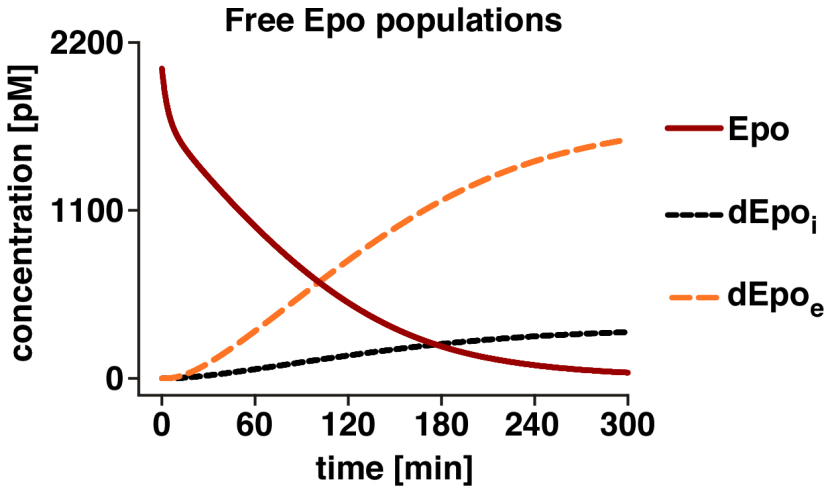
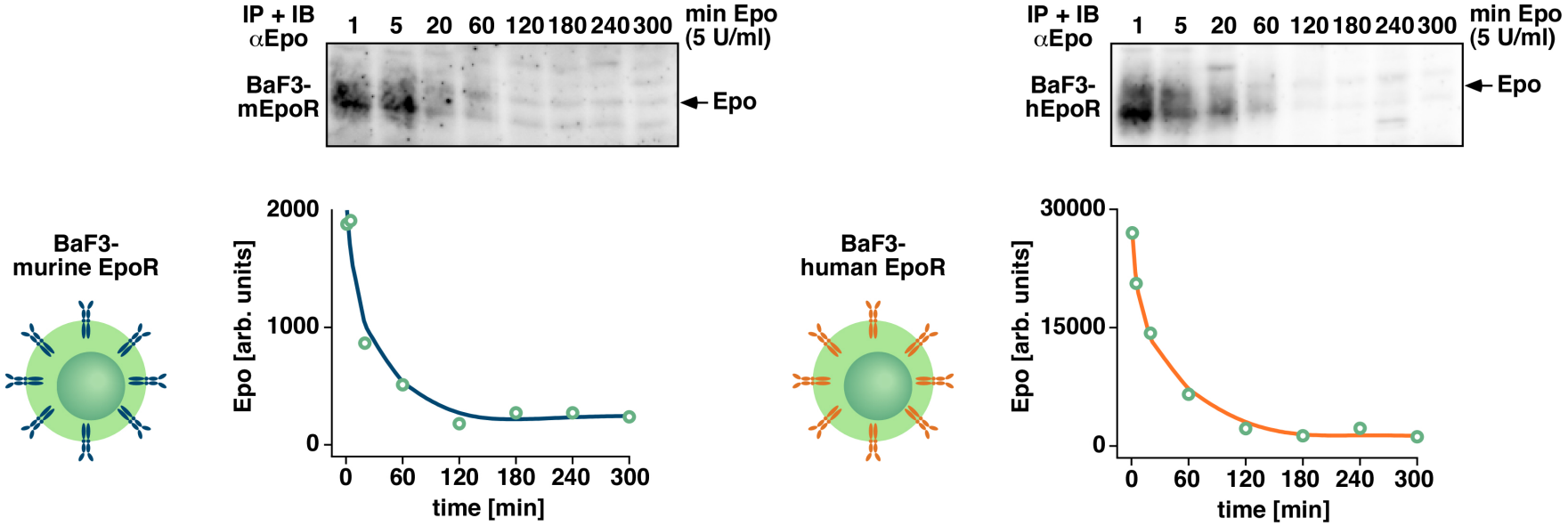


➜ rapid depletion of intact Epo

Epo Depletion - Model Validation by Direct Measurements



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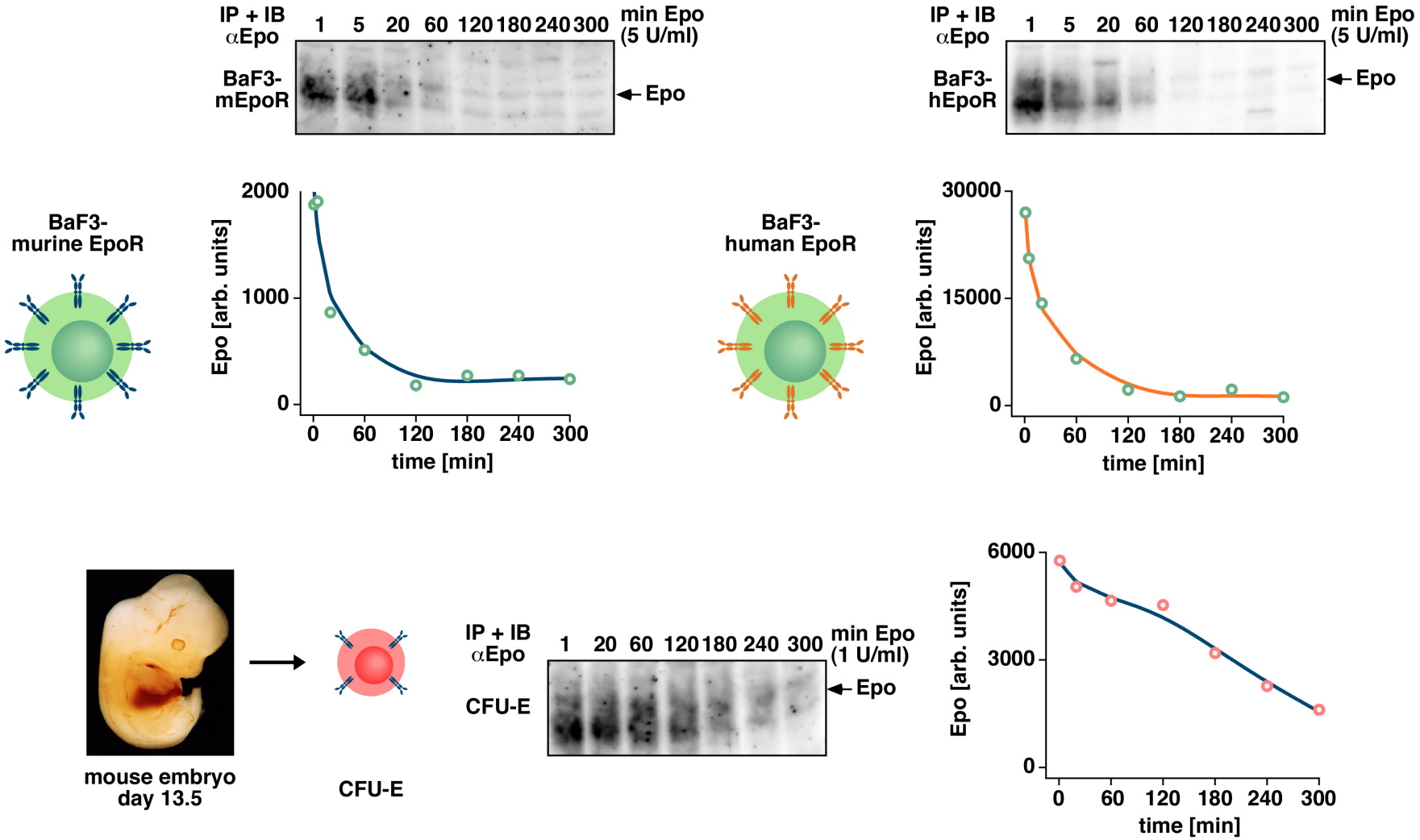
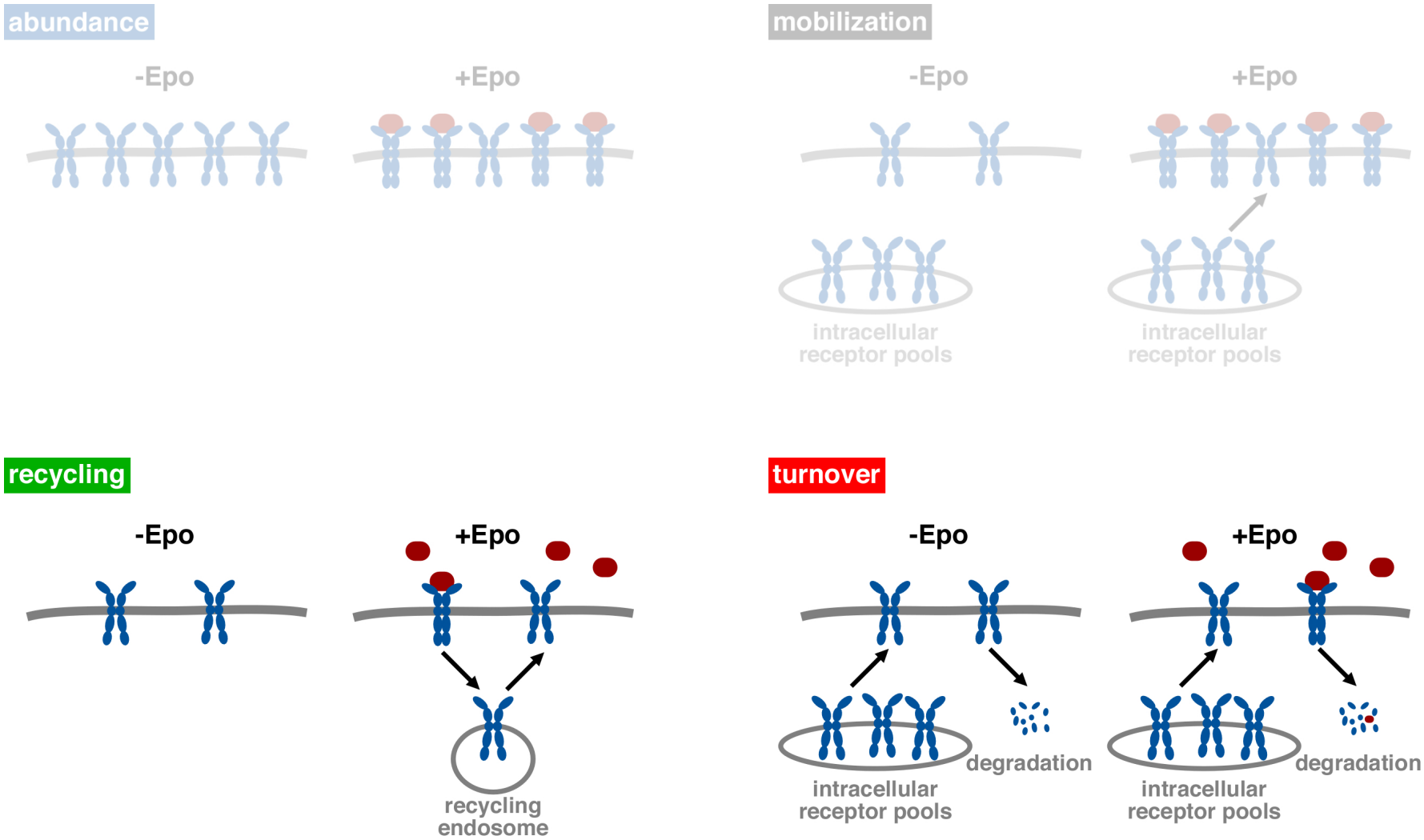


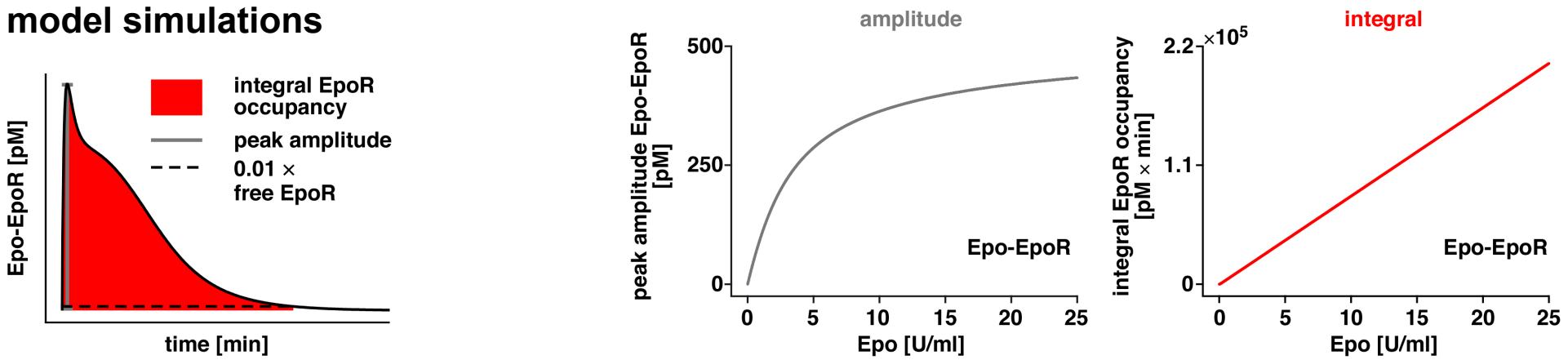
➜ ligand depletion in both murine and human system

➜ regulation of signal initiation by EpoR endocytosis through ligand depletion

Strategies for Processing Ligand-Encoded Information

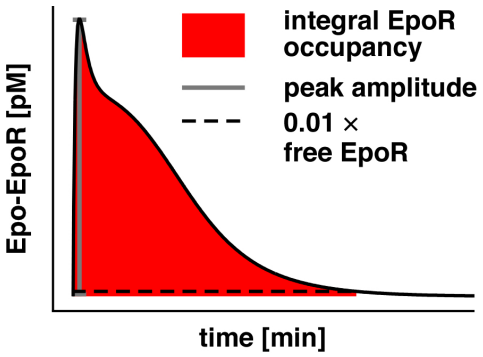


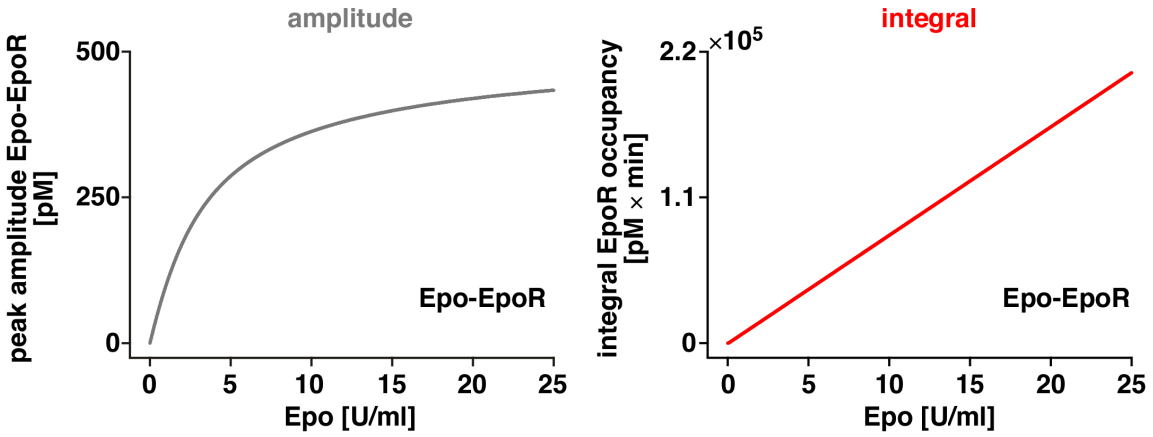
Linear EpoR Signaling for a Broad Range of Epo Levels



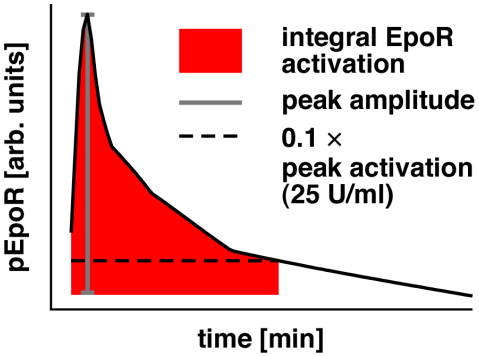
Linear EpoR Signaling for a Broad Range of Epo Levels

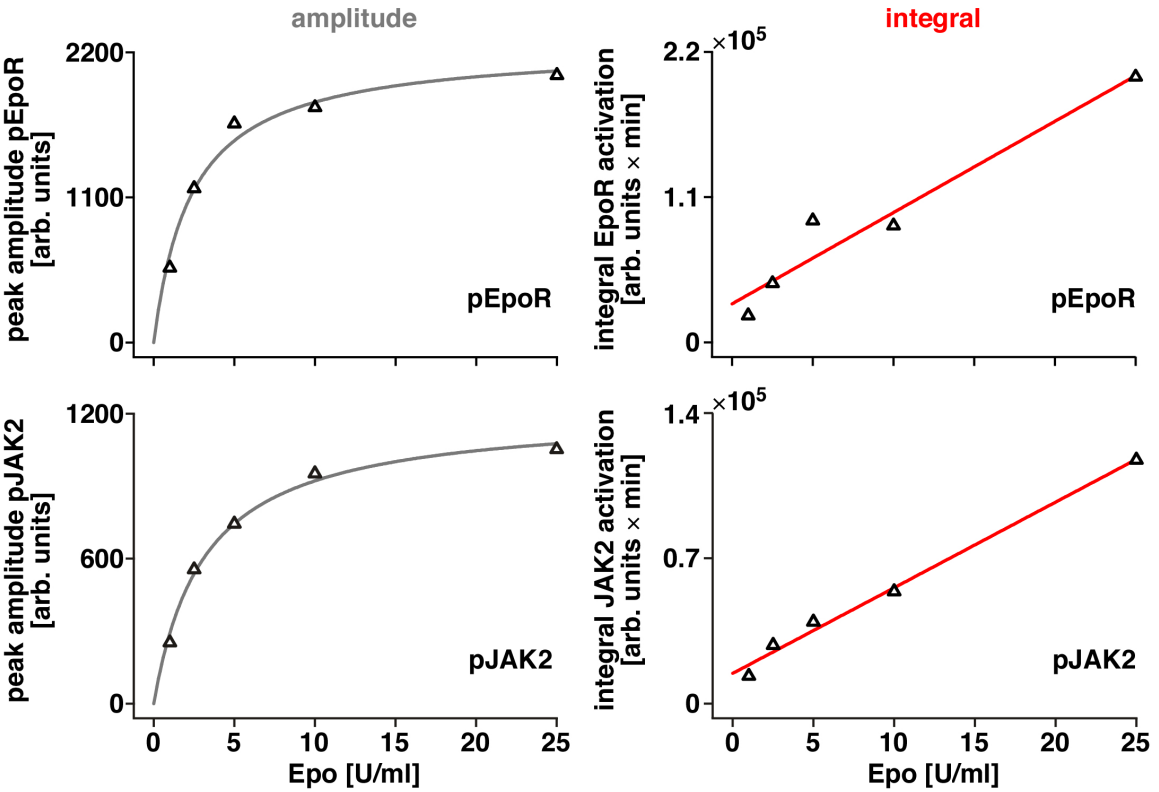
model simulations





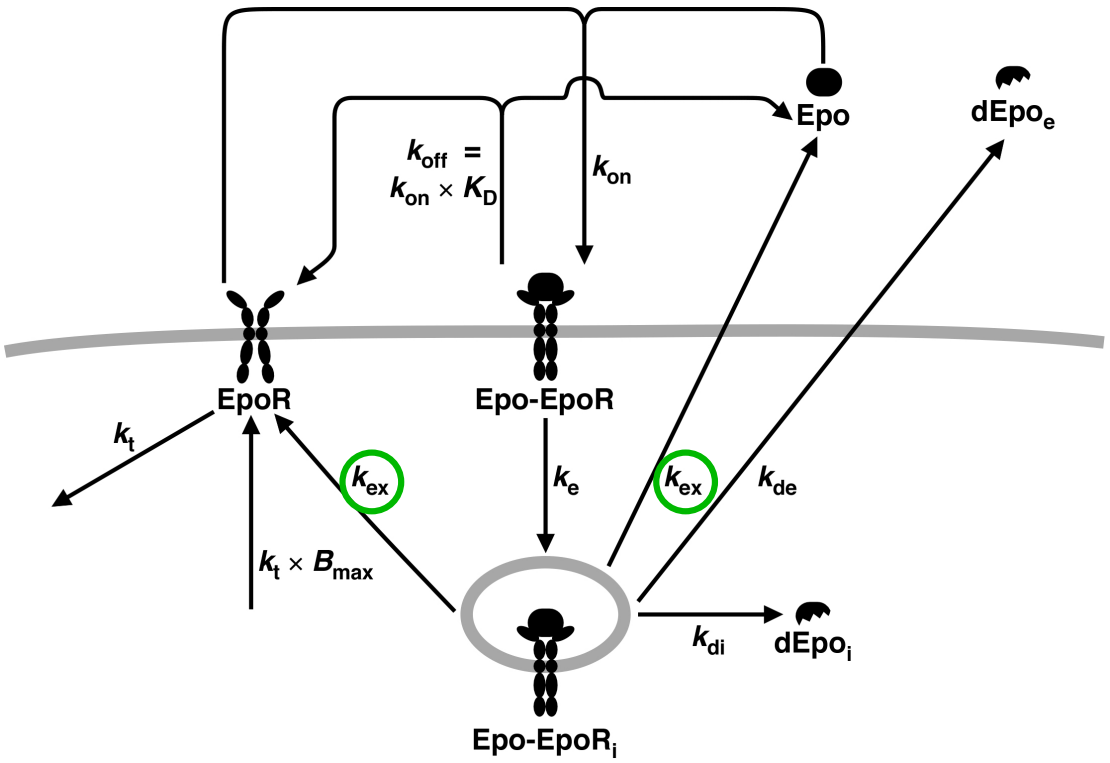
experiments

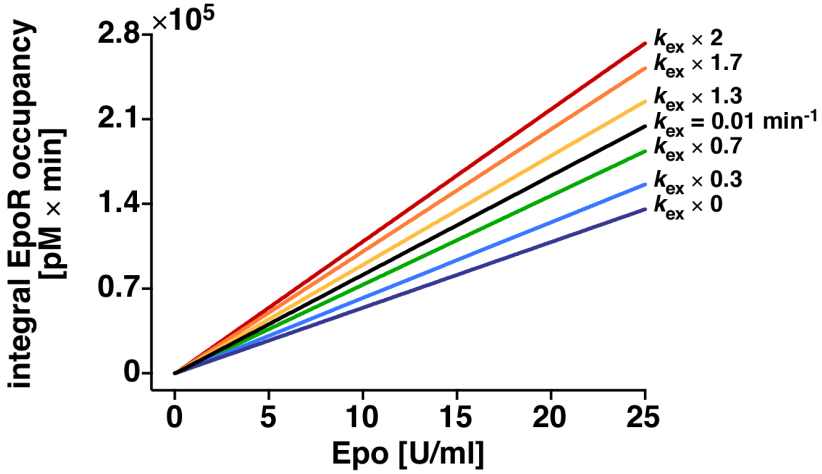




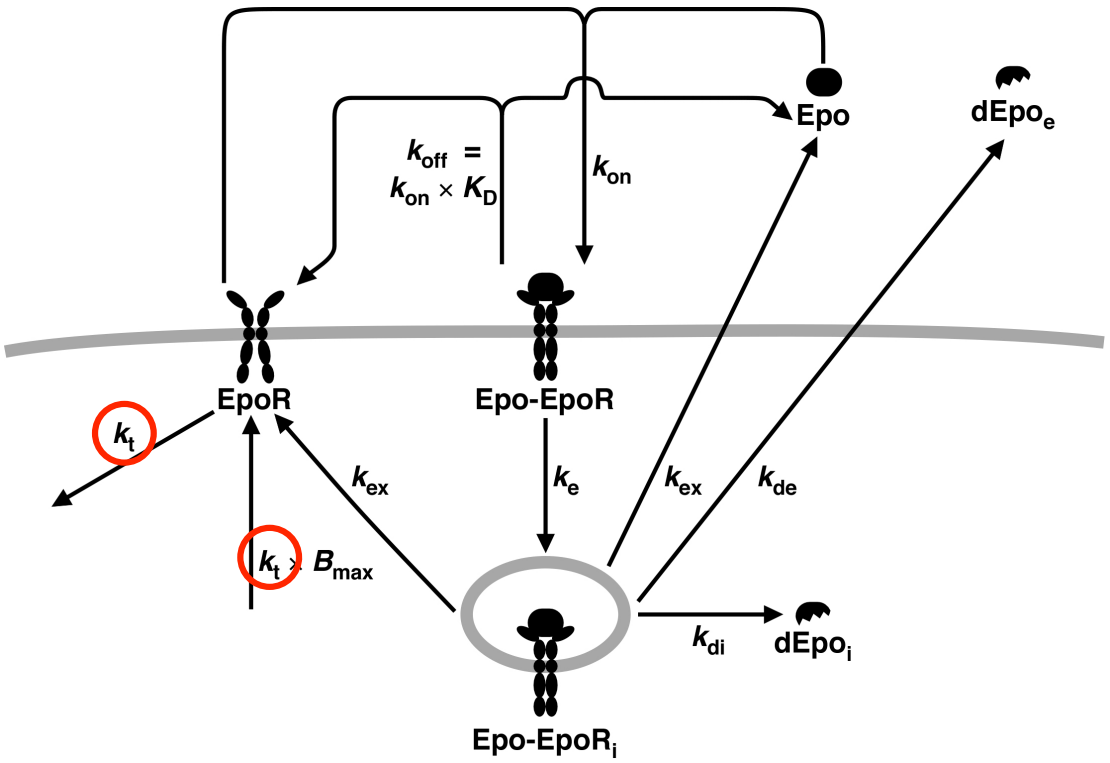
➜ **linear relation of Epo input and integral EpoR activation**

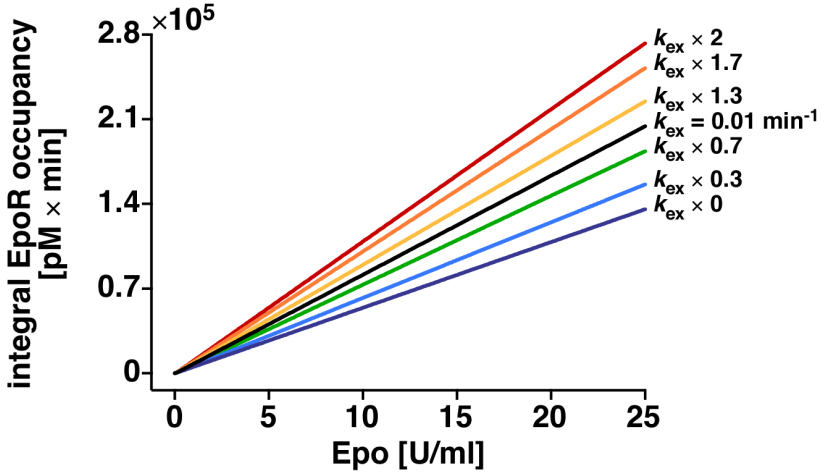
Dependency of Linear Relation

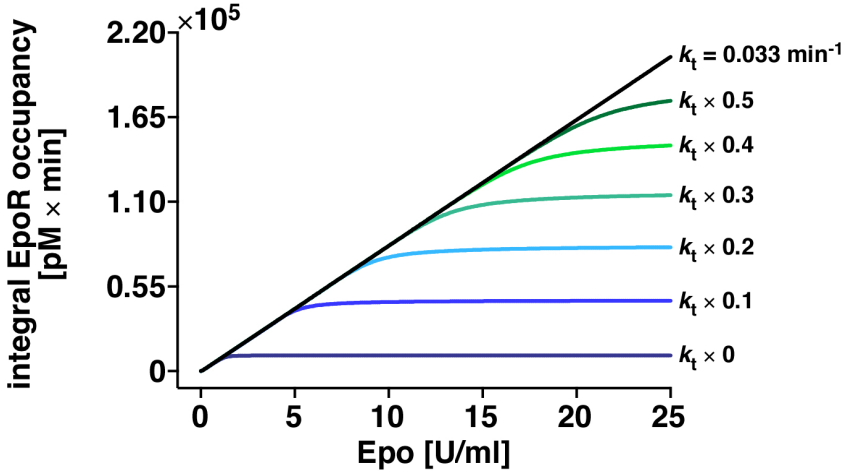




Dependency of Linear Relation on EpoR Turnover

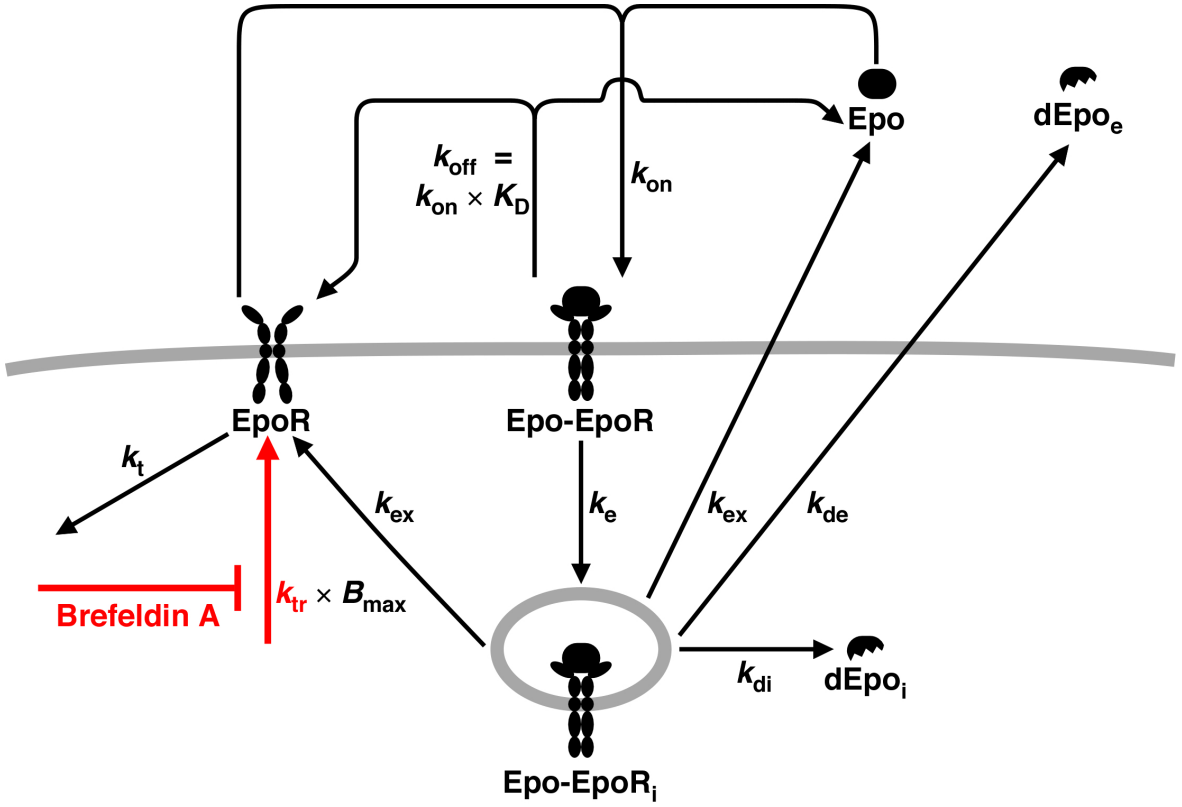


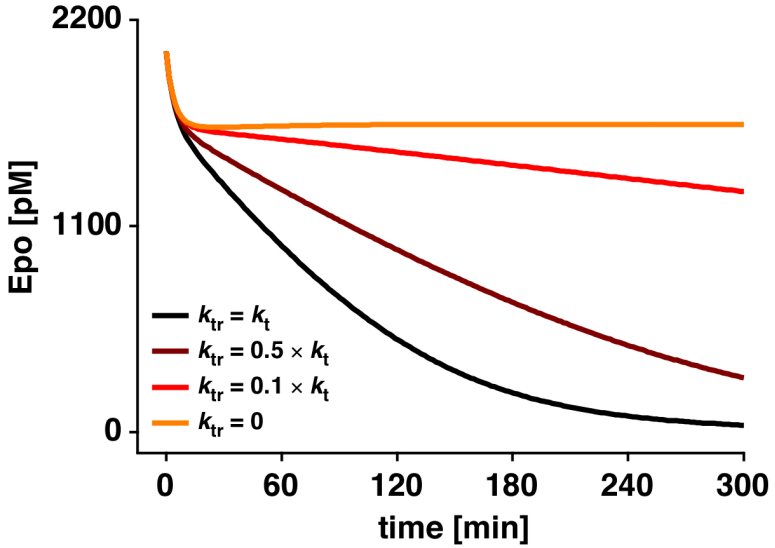




➜ **constitutive EpoR turnover: linear signal integrator**

Contribution of Intracellular EpoR Pools





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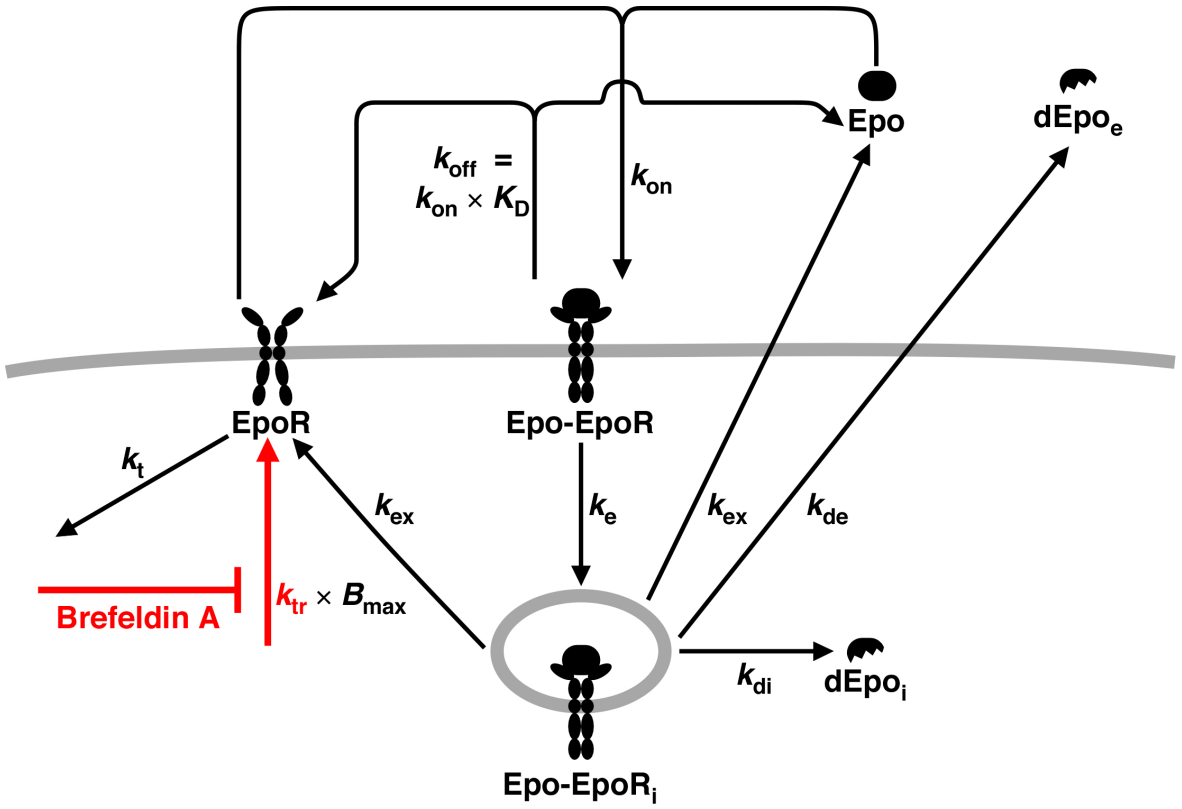
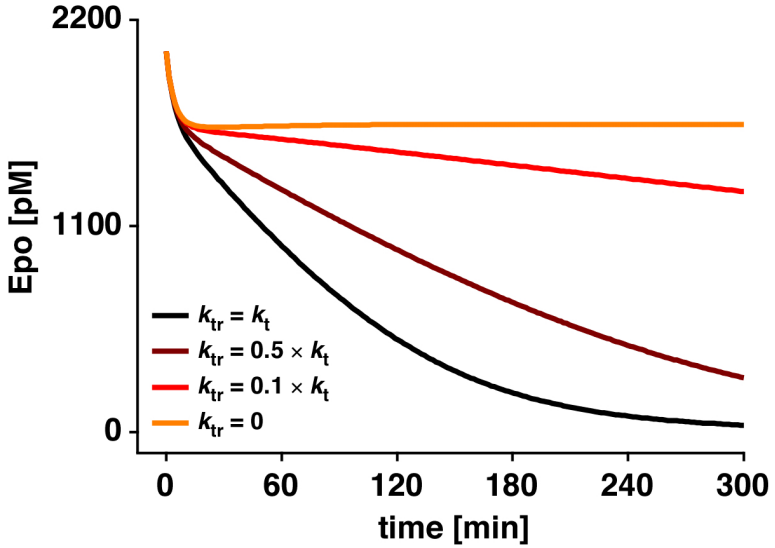
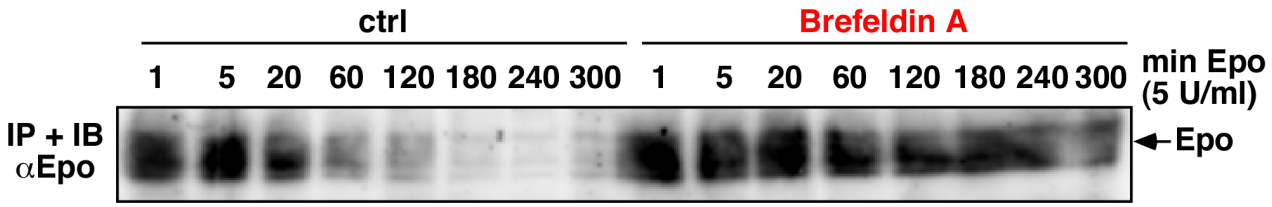
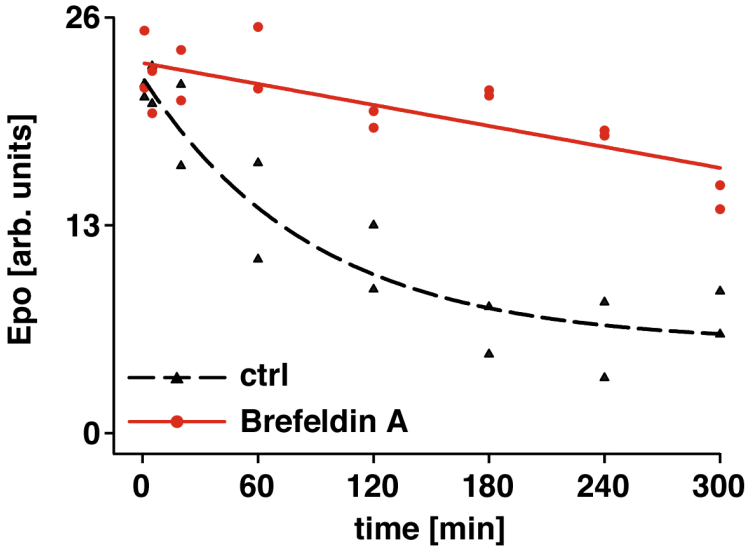






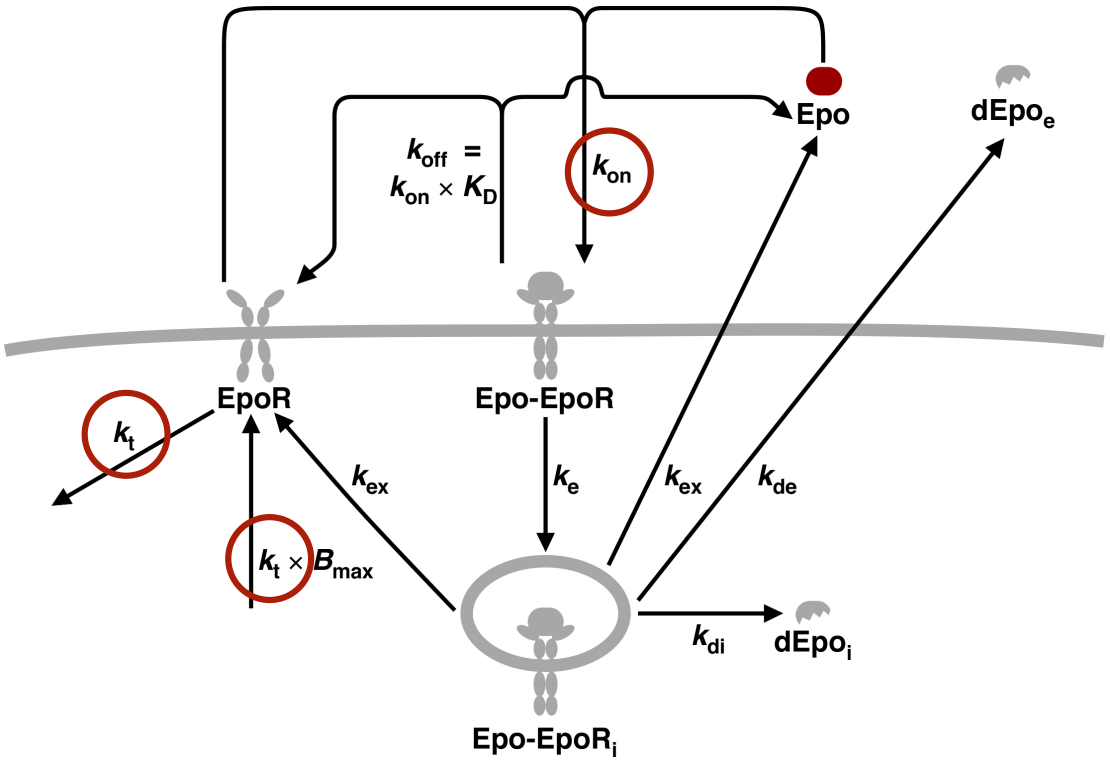


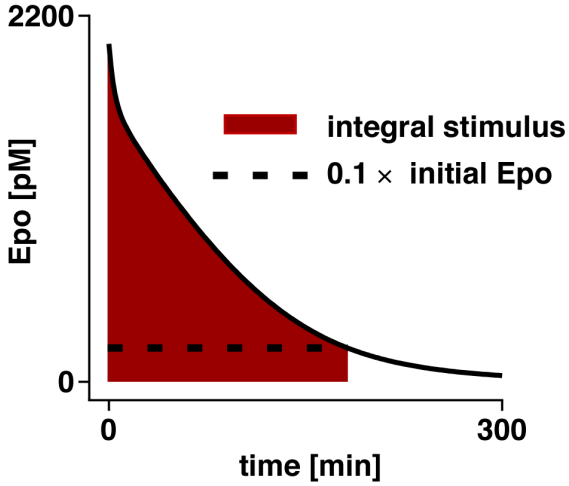
➜ EpoR transport as a prerequisite for sampling and integrating ligand

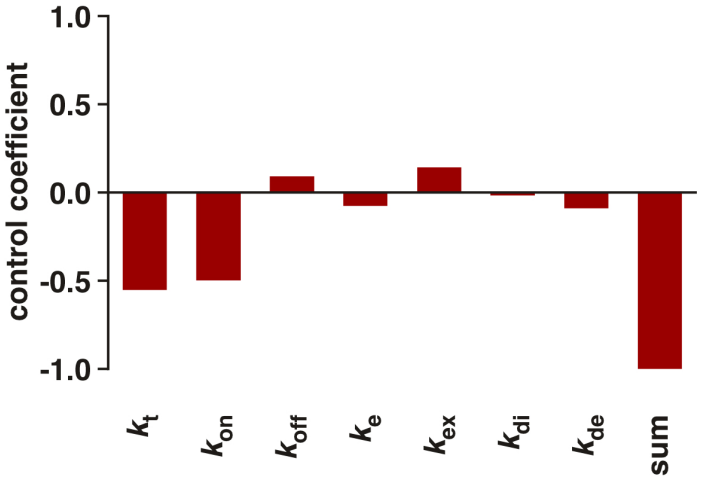
➜ critical role of large pools of newly synthesized EpoR in ER and Golgi

Differential Ligand Binding Properties of Epo Derivatives

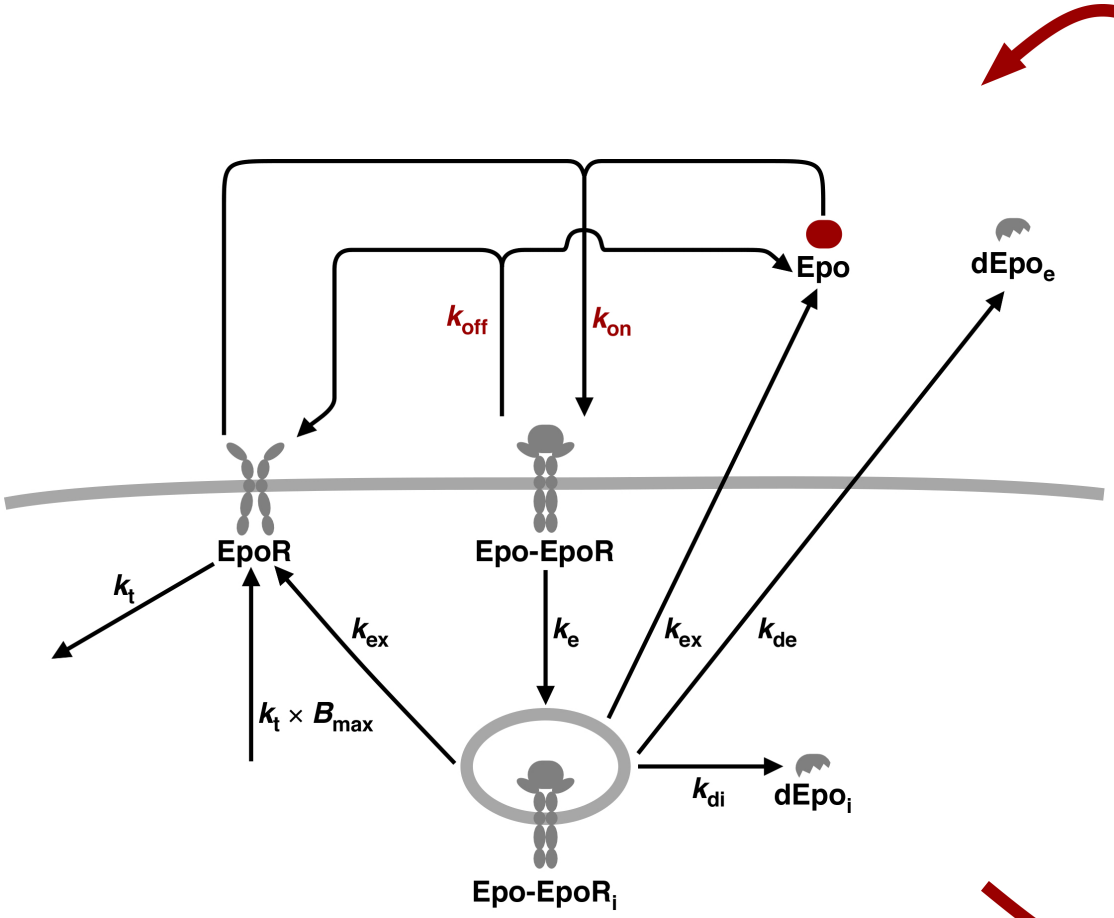
➜ **sensitivity analysis: k_{on} essential ligand binding property for Epo depletion**

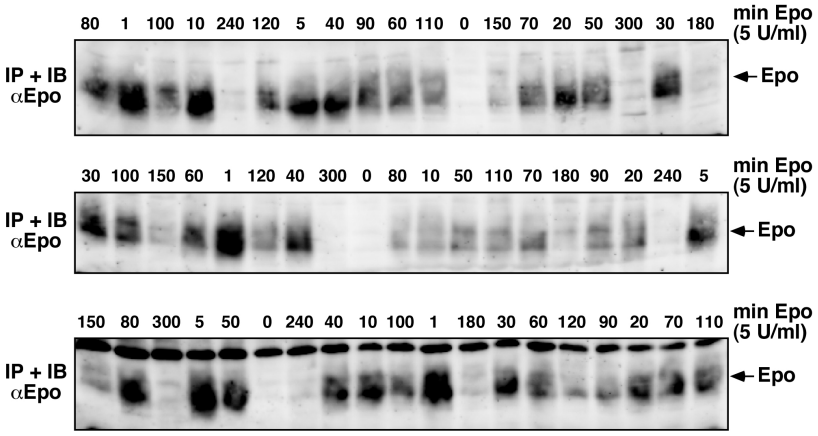


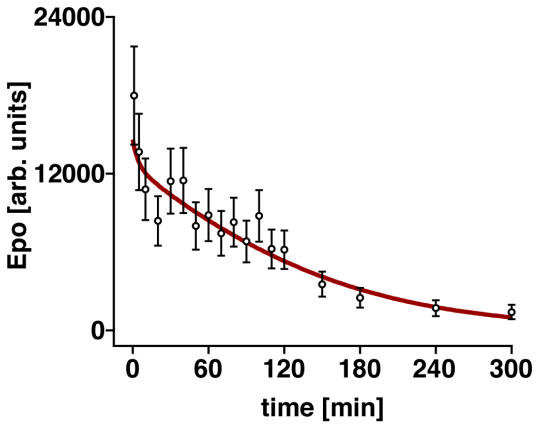




Simulation of Bioactivity and Bioavailability of Epo Derivatives







➜ simulate system dynamics for different k_{on}/k_{off} rate couples

➜ calibrated model employed to estimate k_{on} and k_{off} parameter values by using immunoblot data for Epo and NESP

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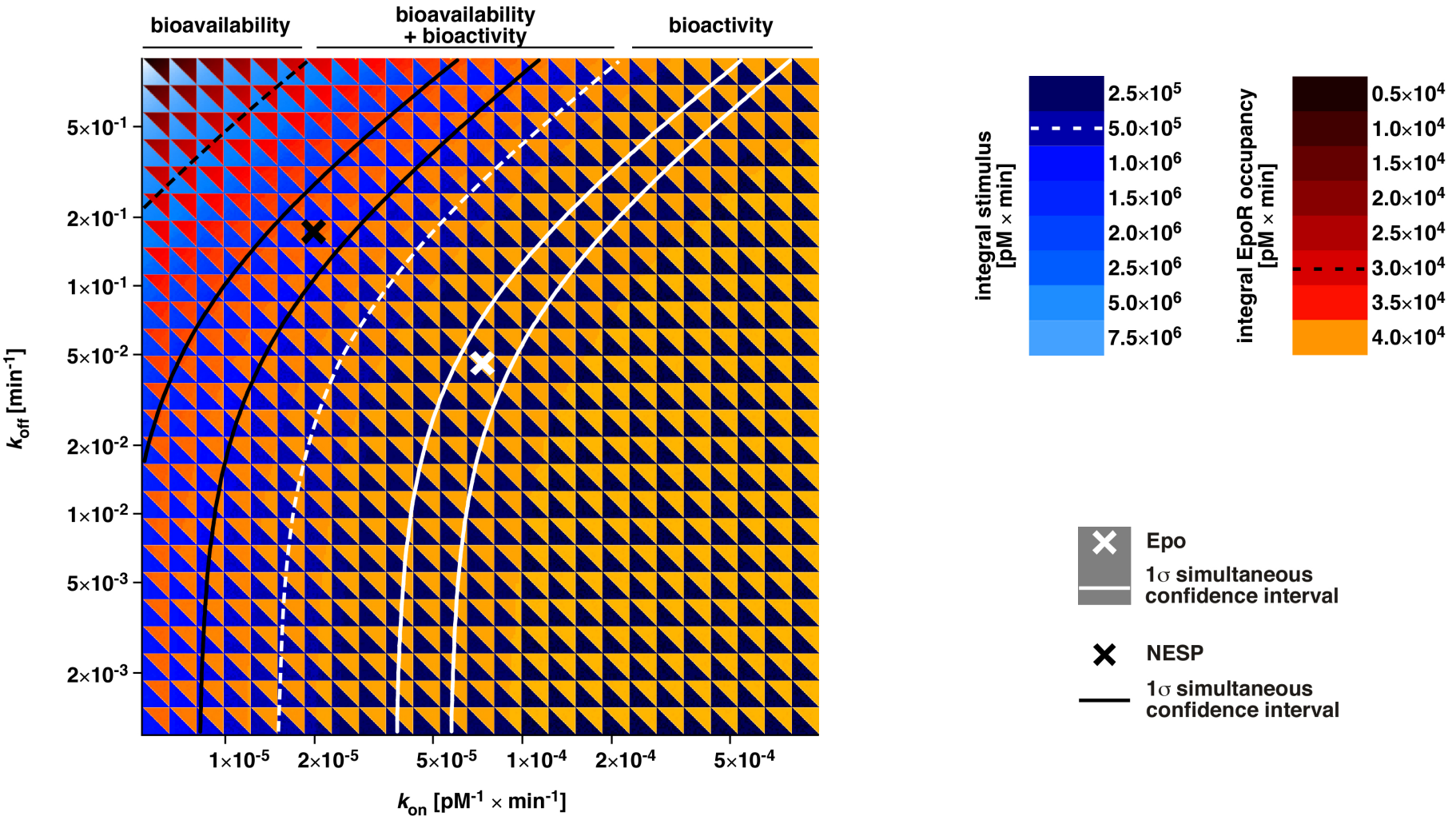


➜ estimation of bioactivity and bioavailability of Epo derivatives via ligand binding kinetics

➜ rapid application, circumvents radioactivity or animal experiments

Generalisation of the Model

- **Different cell types: CFU-E, m/hBaF3, H838**
- **Different ligands: Epoα, Epoβ, NESP, CERA**

$$\dot{x} = f(x, p), \quad x(0) = x_o$$

Different cell types, three possibilities:

- **different x_o: different expression levels**
- **different p: different reaction rates**
- **different $f(.)$: different topology**

Generalisation of the Model

Ansatz: Fit all data by one model, individual parameters for

- **number of receptors**
- **ligand-receptor affinities**

Amount of data: 600 from 22 experimental conditions

Result: It works !

Generalisation of the Model

Number of receptors

CFU-E: 1463 ± 156

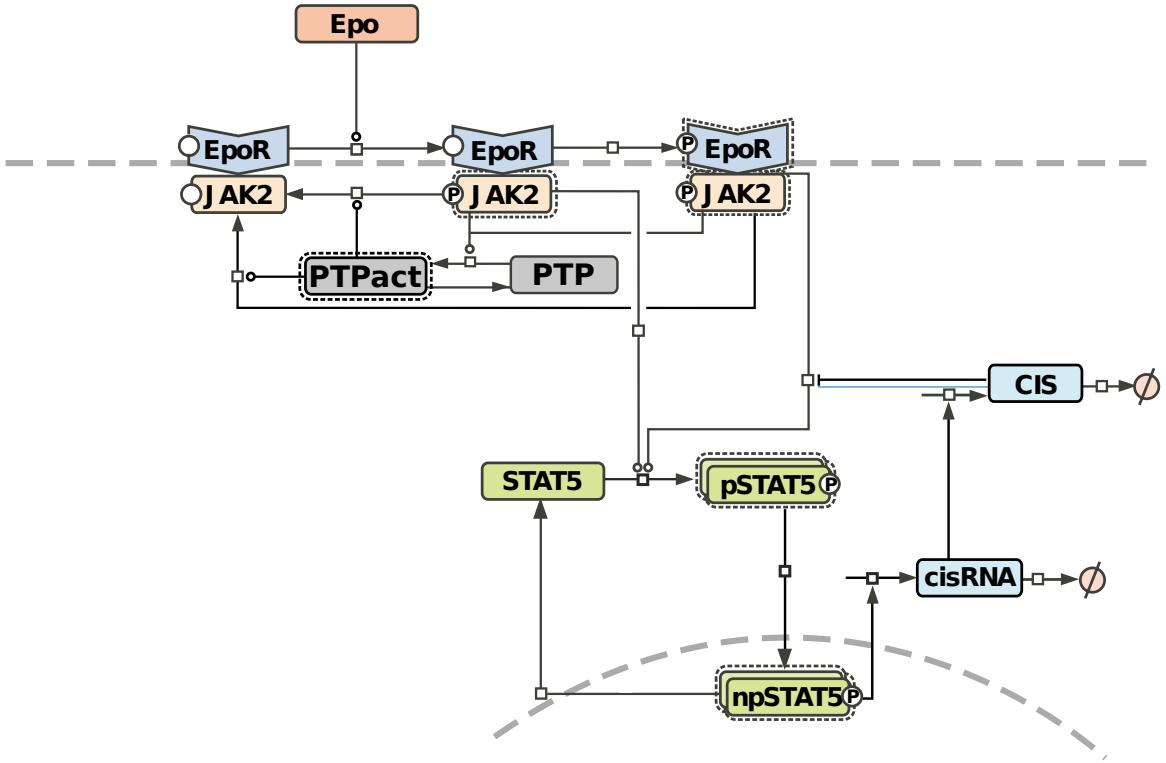
BaF3: 10293 ± 485

H838: 458 ± 46

- **# receptors CFU-E & BaF3 agree with experiments**
- **# receptors for H838 not determinable by experiments**

Looking Downstream

Combine receptor model with STAT5 signaling model

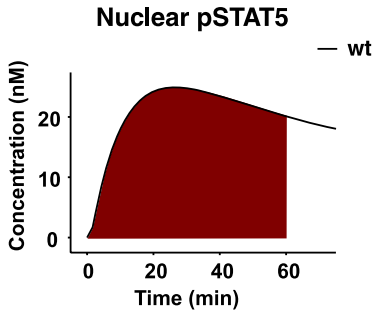


Epo and Cancer

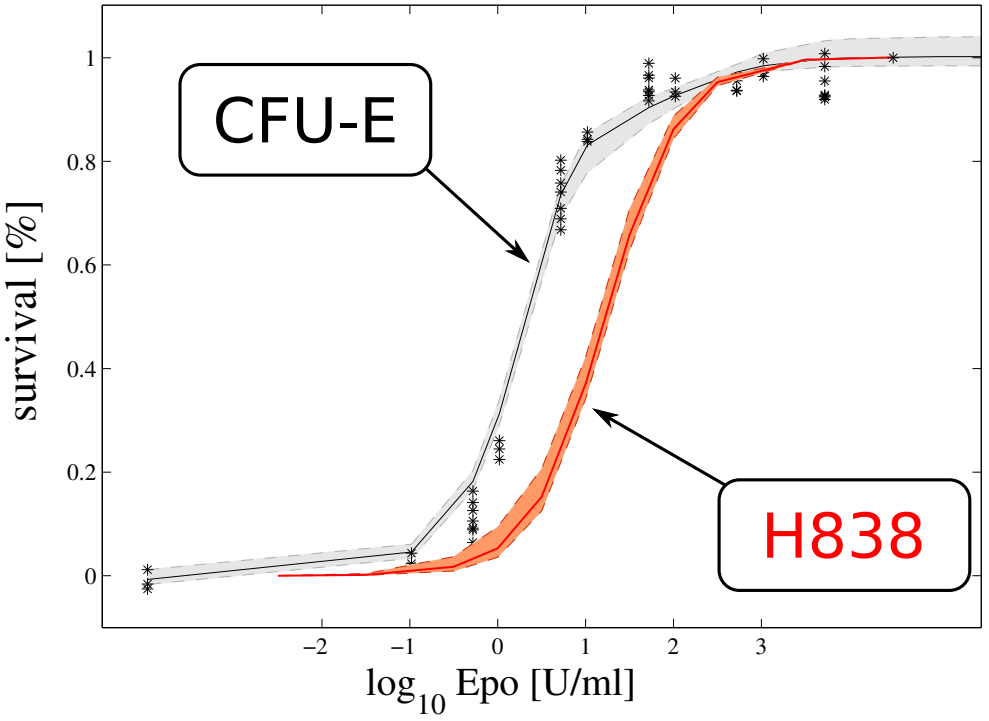
- Epo often applied during chemotherapy to fight anemia
- But, Epo-receptors also expressed on tumor cells

Question: Is there a difference in dosing effects ?

Integral nuclear pSTAT5 determines cell survival



Dosing Effects



Suggests: There is a range of differential effects

Summary

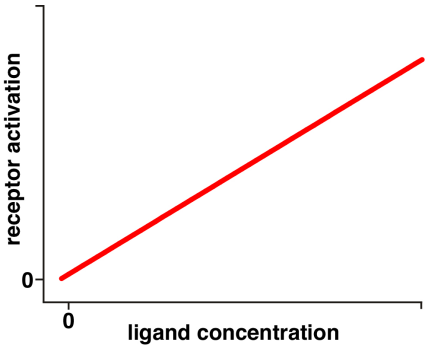
Information processing through EpoR:

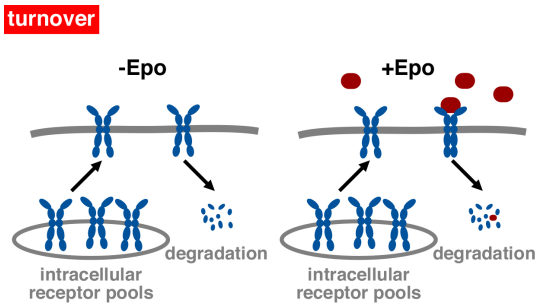
➜ rapid Epo depletion

➜ fast recovery of cell surface EpoR

➜ linear relation of Epo levels
and integral EpoR activation over a
broad range of ligand concentrations

➜ accurate translation of ligand input
into erythrocyte production





V. Becker, M. Schilling, J. Bachmann, U. Baumann, A. Raue, T. Maiwald, J. Timmer, and U. Klingmüller (2010). *Science* 328(5984):1404-1408.

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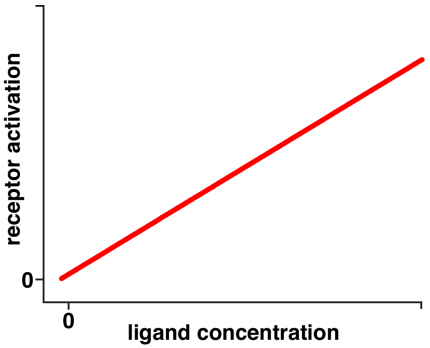

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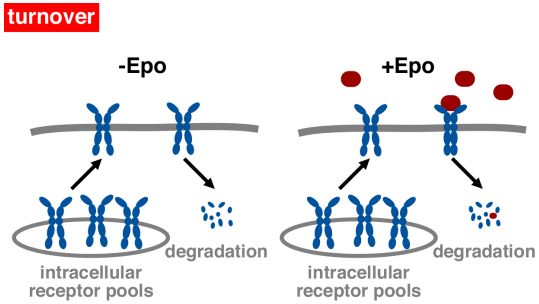
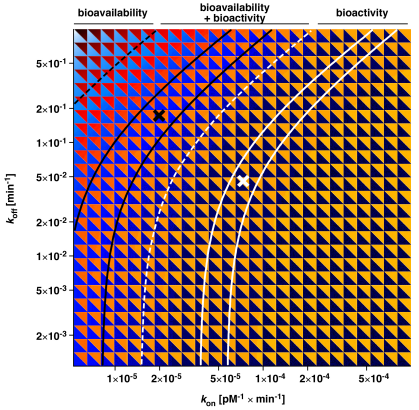


Rational design of therapeuticals and cancer treatment strategies:

➜ estimation of k_{on} and k_{off} rates

➜ identification of risks in Epo treatment of lung cancer patients



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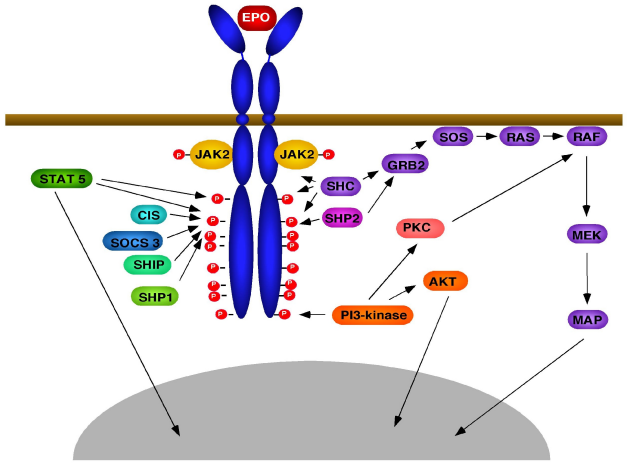
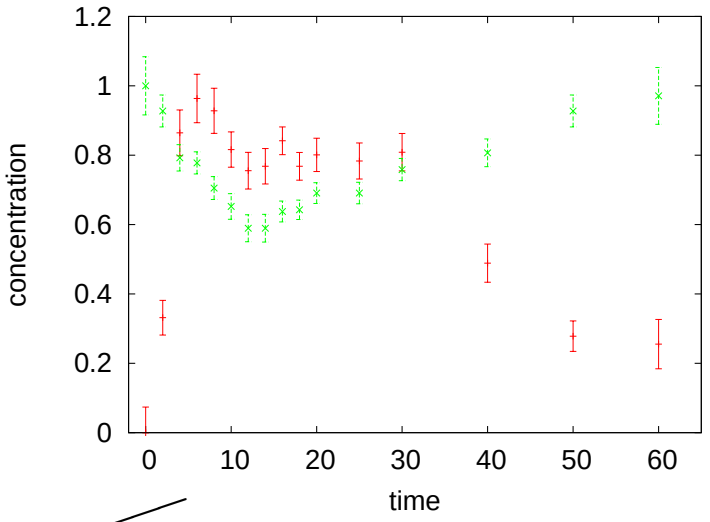
Signal transduction through the Erythropoietin receptor (EpoR)





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

In silico biology

Test the prior knowledge

Understanding systems' properties

Identification of potential drug targets

Acknowledgements

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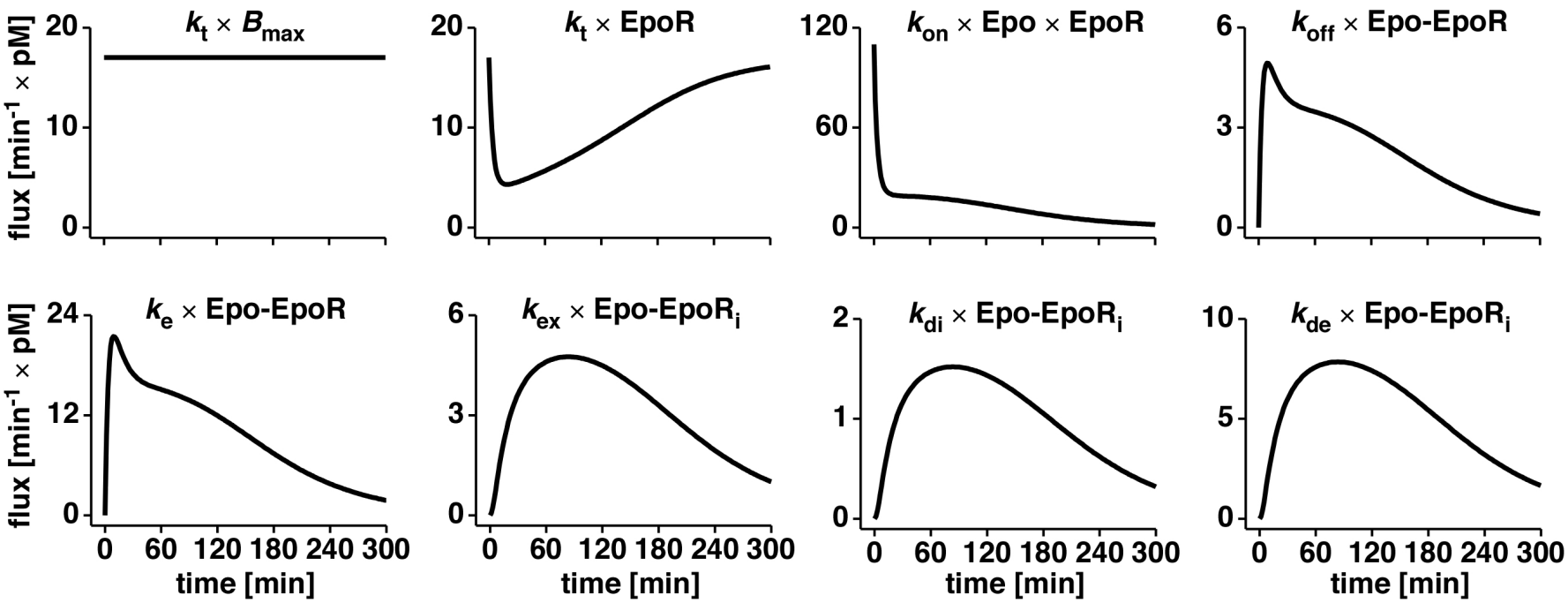
Ute Baumann
Ursula Klingmüller

Flux Analysis Core Model

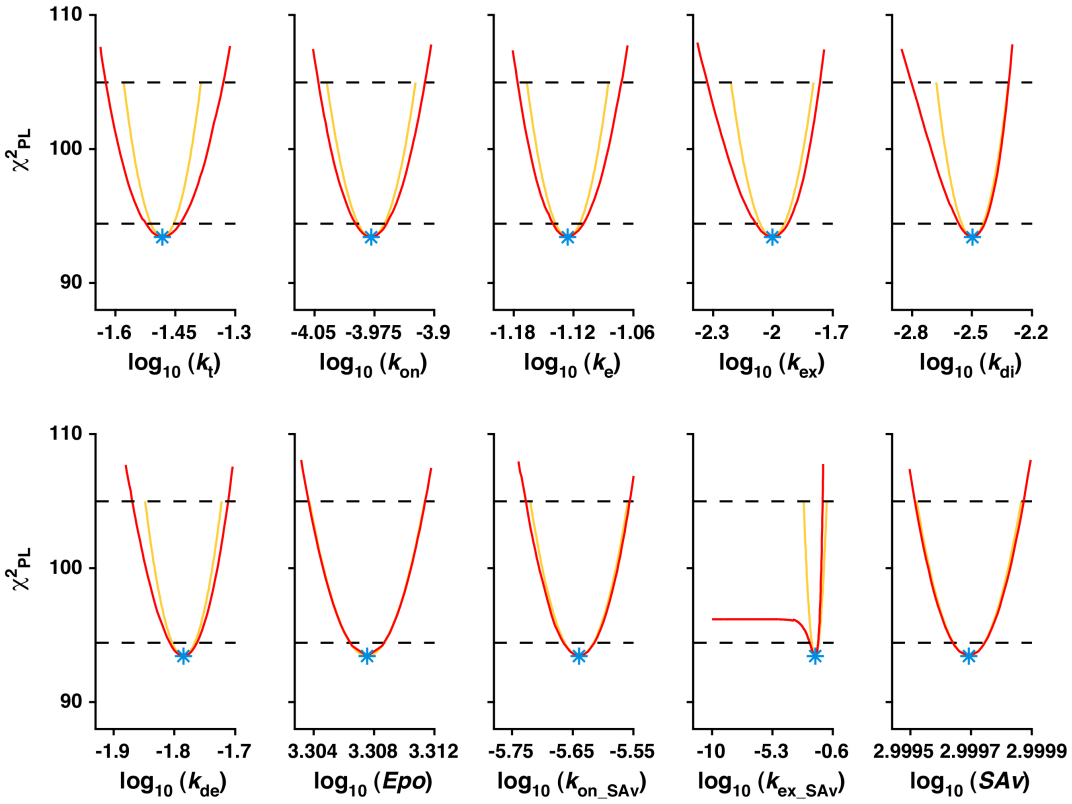


Identifiability Analysis by Profile Likelihood Exploit

$$\chi^2_{PL}(\theta_i) = \min_{\theta_{j\neq i}}\left[\chi^2(\theta)\right]$$

$$\left\{\theta \middle| \chi^2(\theta) - \chi^2\left(\hat{\theta}\right) < \Delta_\alpha\right\} \quad with \quad \Delta_\alpha = \chi^2(\alpha, df)$$

Raue et al. (2009), Bioinformatics



parameter	best fit
k_t [min^{-1}]	0.03294 (+ 0.00356 / - 0.00293) (+ 10.81% / - 8.89%)
k_{on} [pM^{-1} × min^{-1}]	0.10496×10^{-3} ($+ 4.72\times10^{-6}$ / $- 4.68\times10^{-6}$) (+ 4.50% / - 4.46%)
k_{off} [min^{-1}]	0.01721 (+ 0.00077 / - 0.00077) (+ 4.47% / - 4.47%)
k_e [min^{-1}]	0.07483 (+ 0.00277 / - 0.00248) (+ 3.70% / - 3.31%)
k_{ex} [min^{-1}]	0.00994 (+ 0.00195 / - 0.00169) (+ 19.62% / - 17.00%)
k_{di} [min^{-1}]	0.003179 (+ 0.000475 / - 0.000461) (+ 14.94% / - 14.50%)
k_{de} [min^{-1}]	0.01640 (+ 0.00086 / - 0.00083) (+ 5.24% / - 5.06%)
Epo [pM]	2030.19 (+ 5.22 / - 5.21) (+ 0.26% / - 0.26%)
k_{on_SAv} [pM^{-1} × min^{-1}]	2.294×10^{-6} ($+ 1.36\times10^{-7}$ / $- 1.32\times10^{-7}$) (+ 5.93% / - 5.75%)
k_{off_SAv} [min^{-1}]	0.006799 (+ 0.000403 / - 0.000391) (+ 5.93% / - 5.75%)
k_{ex_SAv} [min^{-1}]	0.0110 (+ 0.0076 / - 0.0069) (+ 69.09% / - 62.73%)
SAv [pM]	999.293 (+ 0.120 / - 0.120) (+ 0.01% / - 0.01%)

➜ **good model accuracy:**

- **all parameters identifiable with small confidence intervals**

➜ **allowing for accurate predictions**