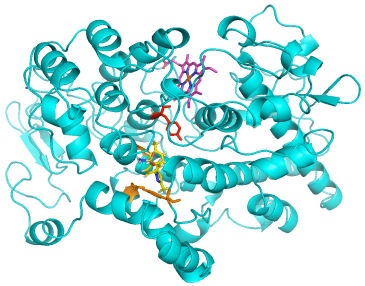
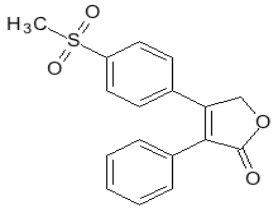
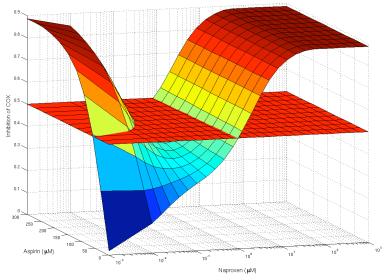




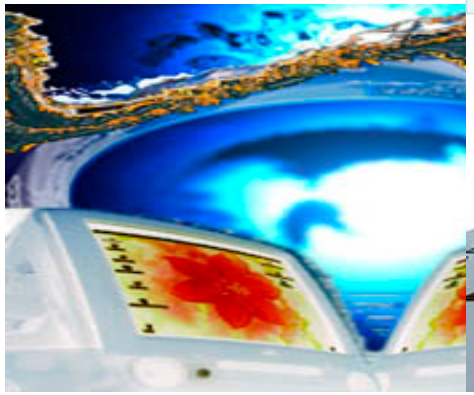


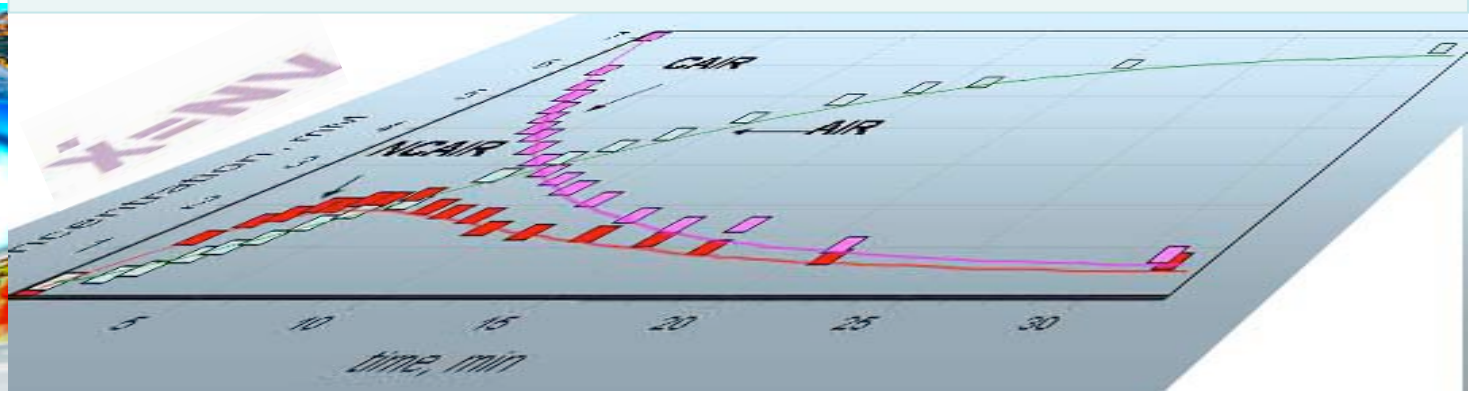
Systems Biology for Drug Discovery

as applied to NSAID safety problem

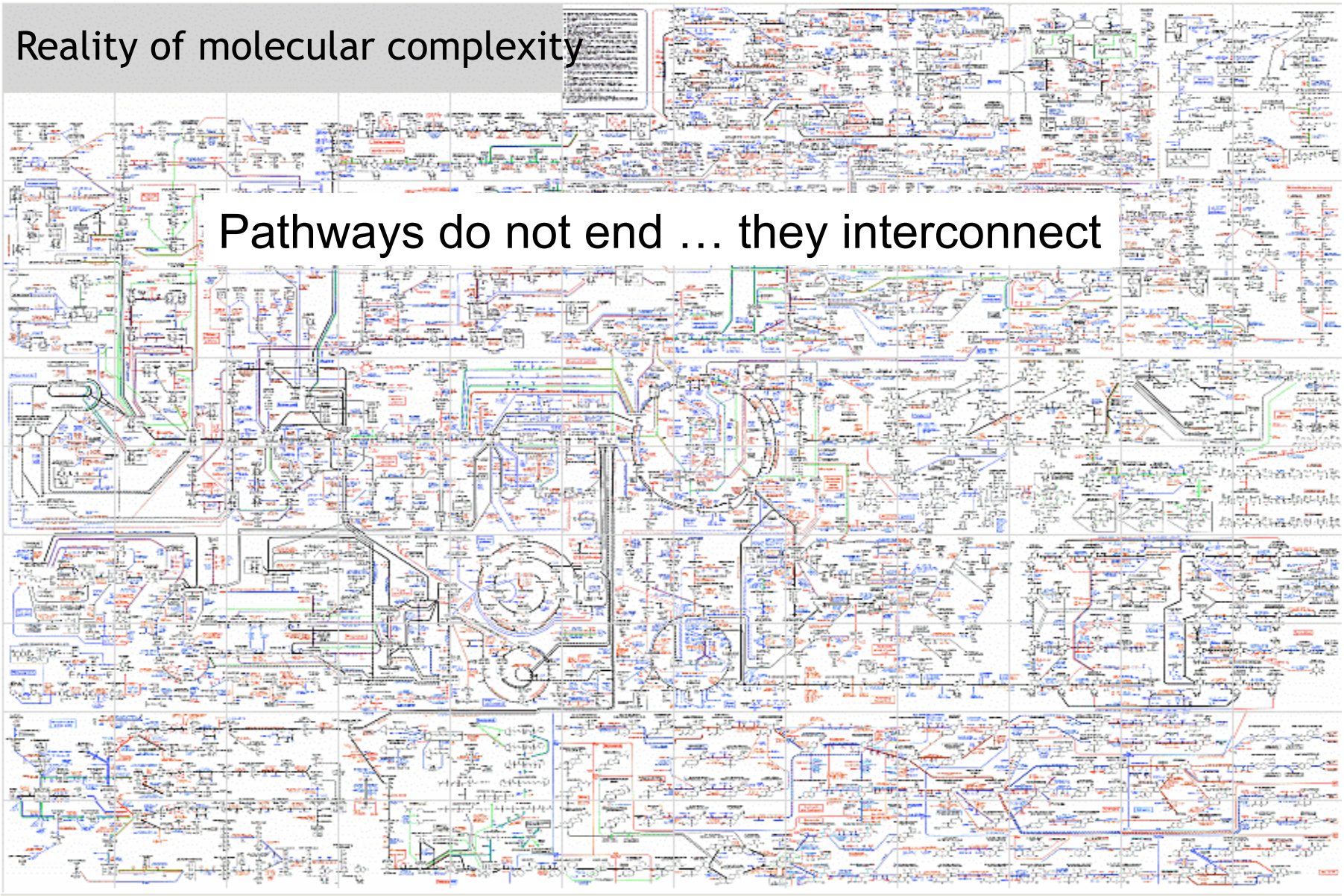
Dr. Galina Lebedeva

12th March 2010





Reality of molecular complexity



Health, disease and therapeutic intervention in terms of network modelling

- Input – output behaviour of biological networks
- Health: balance of input and output, adequate response to perturbation
- Disease: balance is disturbed, due to suppression or activation of certain stages, resulting in inadequate output
- Goal of therapy: to restore the normal balance by targeting key components/checkpoints of networks by drugs
- Therapeutic resistance – loss of the sensitivity of the output to the drug

Key To Future Medical Breakthroughs Is Systems Biology,

Say Leading European Scientists

- ***High Complexity of biochemical networks underlying cell functioning***
- ***If disturbed can result in a disease (diabetes, cancer..- network diseases)***
- ***Conventional approaches of biology are not suitable for the analysis of these elaborate webs of interactions, which is why drug design often fails***
- ***Knocking out one target in a pathway is not productive – disease will by-pass the drug (network robustness)***
- ***Systems Biology approaches (computational modelling and analysis) should be engaged to develop smarter therapeutic strategies and predict drug safety and efficacy.***

ScienceDaily (Jan. 13, 2009)

Systems medicine

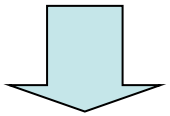
- aimed at exploitation of systems biology approaches to develop prognostic and predictive models for diagnostics and therapeutic applications
- there is not yet an established arsenal of computational approaches, methods, and techniques applicable for model-based diagnostics and design of therapeutic strategies
- specific techniques of model analysis need to be developed to allow for application of general models in the context of therapeutic research.

Kinetic Modeling (KM) Approach

•Takes into account all properties of the biosystem (protein structure, mechanism, stoichiometry, dynamics and regulation)

•Clearly describes the key properties of the biosystem in terms of easily understandable and measurable parameters (Vmax, Km, Kd, Ki, IC50..)

•Reproduces all known responses of the biosystem to external and internal perturbations and therapeutic interventions



Kinetic Modeling in Systems Biology, Demin, Goryanin
Chapman & Hall/CRC, 2008

Kinetic modelling approach

1. Pathway reconstruction and static model development: elucidation of stoichiometry of the network, identifying key cross-talks and regulations
2. Generation of the system of ODEs describing dynamics of the metabolic/signalling network:

   $$dx/dt=N\cdot v(x;e,K)$$

   Here, $x=[x_1,\ldots x_m]$ is vector of compound concentrations and $v=[v_1,\ldots v_n]$ is vector of rate laws
3. Modelling metabolic, signalling and transport processes:

   Detailed description of the catalytic cycles of key proteins, derivation of rate equations
4. Prameterisation of the model (literature, experiments, fitting)
5. Validation of submodels on the base of *in vitro* and cell extract data
6. Validation of the whole model using *in vivo* dynamic data (genomics, proteomics, metabolomics)

Application of the validated model to practical problems:
e.g. in pharmaceutical industry - drug resistance, safety

Practical applications

- Various analyses of the validated model, e.g.:
  - In silico experiments to test various hypothesis on the mechanisms of disease and drug action
    - E.g. test how modifications within the network can affect its input-output behaviour
  - Local and global sensitivity analysis to generate ideas on
    - Drug targets
    - Biomarkers
    - Combination therapies

Challenges in translating theory into clinical practice:

- Extrapolation to a multi-layered context, from molecular to cellular, tissue,… and organism level
- High level of individual variability of the networks, e.g. in cancer – to be addressed by personalised medicine
- Complex dynamical aspects
  - pharmaco-kinetics and pharmaco-dynamics effects,
  - drug scheduling,
  - circadian rhythms…
- Incomplete knowledge on the biological networks underlying disease onset and progression
- limitations imposed by the number of elements which a tractable model can include

Context layers to consider…

- Intracellular microenvironment
  - regulation of protein activity by local concentration of substrates/ products and co-factors…
- Larger network context
  - Subsystem embedded in a larger network is subject to higher level regulation
- Cell-specific protein expression and gene regulation
  - Pathway structure and dynamics vary in different cell types…
- Organ/ organism level effects –
  - Spatial aspects
- ….

Example: Application of Systems Biology Approach to

Drug Safety Problem

"Too many pharmacological agents
have entered into clinical practice
for which considerable and
potential life-threatening outcomes
were recognized only AFTER
a large number of patients had been treated"

Wall Street Journal – Thursday, January 26, 2006 quoting an editorial from same week's New England Journal of Medicine

Non-Steroidal Anti-Inflammatory Drugs

(NSAIDs) safety problem

- **Aspirin**
- **Ibuprofen**
- **Naproxen**
- **Indomethacin**
- **Celebrex …**

- NSAIDs – popular drugs for pain relief and antipyretic, more recently started to be used in cancer and neurophysiology (depression).
- Main target – COX1,2
- COX1 – constitutive, COX2 – induced at inflammation
- Aspirin (targeting both COX1 and COX2)– risk of gastro-intestinal bleeding at medium/high dose
- Selective COX-2 inhibitors (Coxibs) – developed to overcome GI side effects:
  - efficient in pain relief but with new dangerous side effects (heart attacks)
  - Vioxx withdrawal from the market – cost Merck $billions, with ongoing legal costs
  - FDA suggests Vioxx has contributed to >20 000 heart attacks & sudden cardiac deaths during its stay on market
- The exact mechanism of NSAID action, and the origin of many undesirable adverse effects still remain poorly understood.

Non-Steroidal Anti-Inflammatory Drugs

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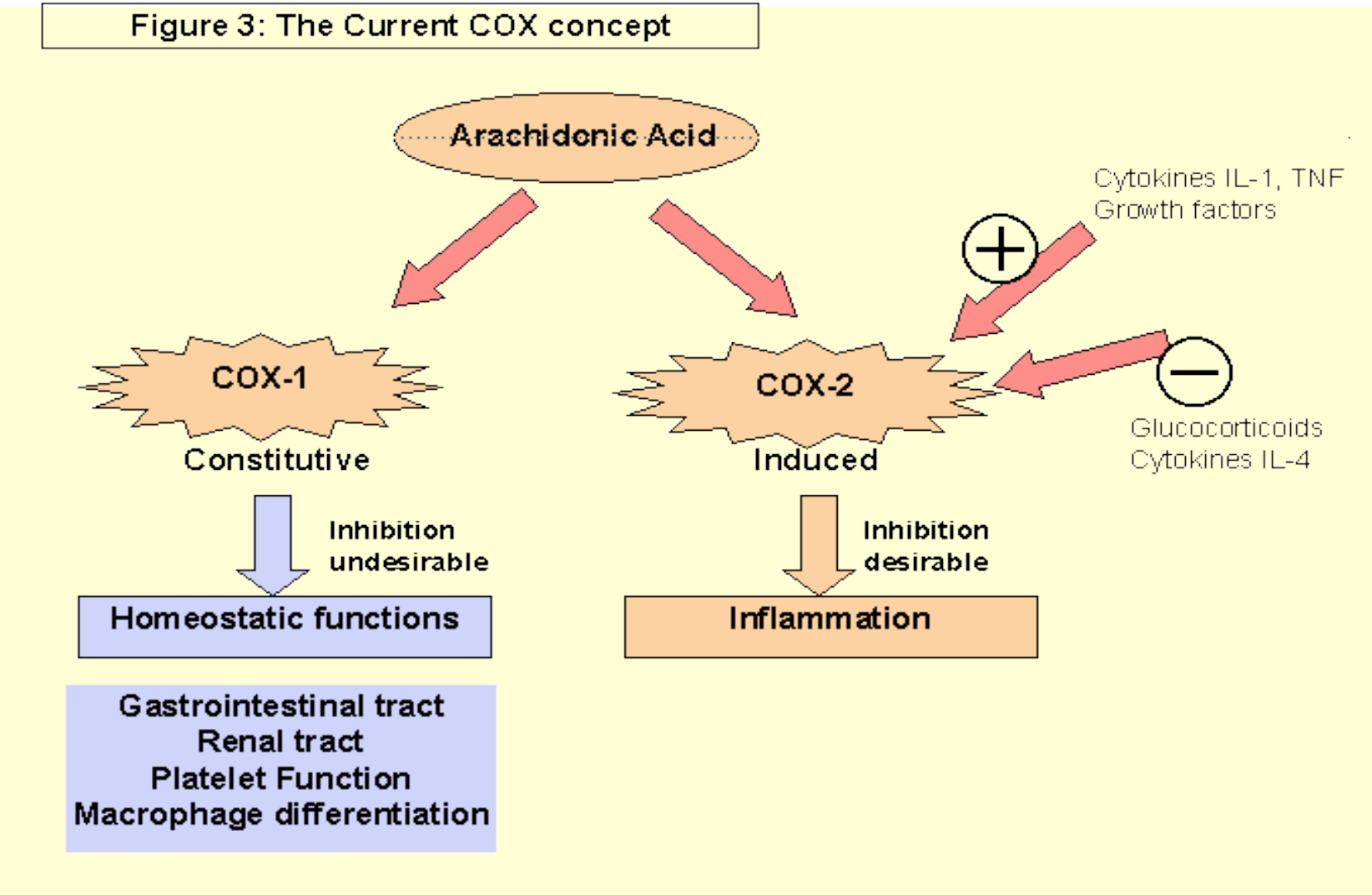
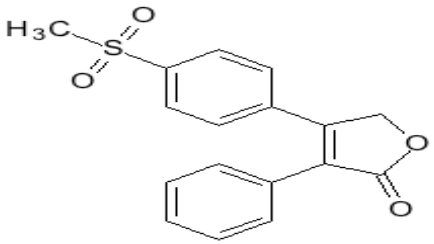




"Vioxx" project

- **Static pathway reconstruction**
- Intracellular level models:
  - Catalytic cycle of Drug Target (COX)
  - NSAID action on COX
  - Upstream and downstream metabolic and signalling pathways involved in response to NSAIDs
- Cell level models
  - NSAID action on platelets
  - NSAID action on endothelium cells
  - Combined platelet-endothelium-plasma
- Model of Blood circulation system
- Coupling with pharmacokinetic profiles

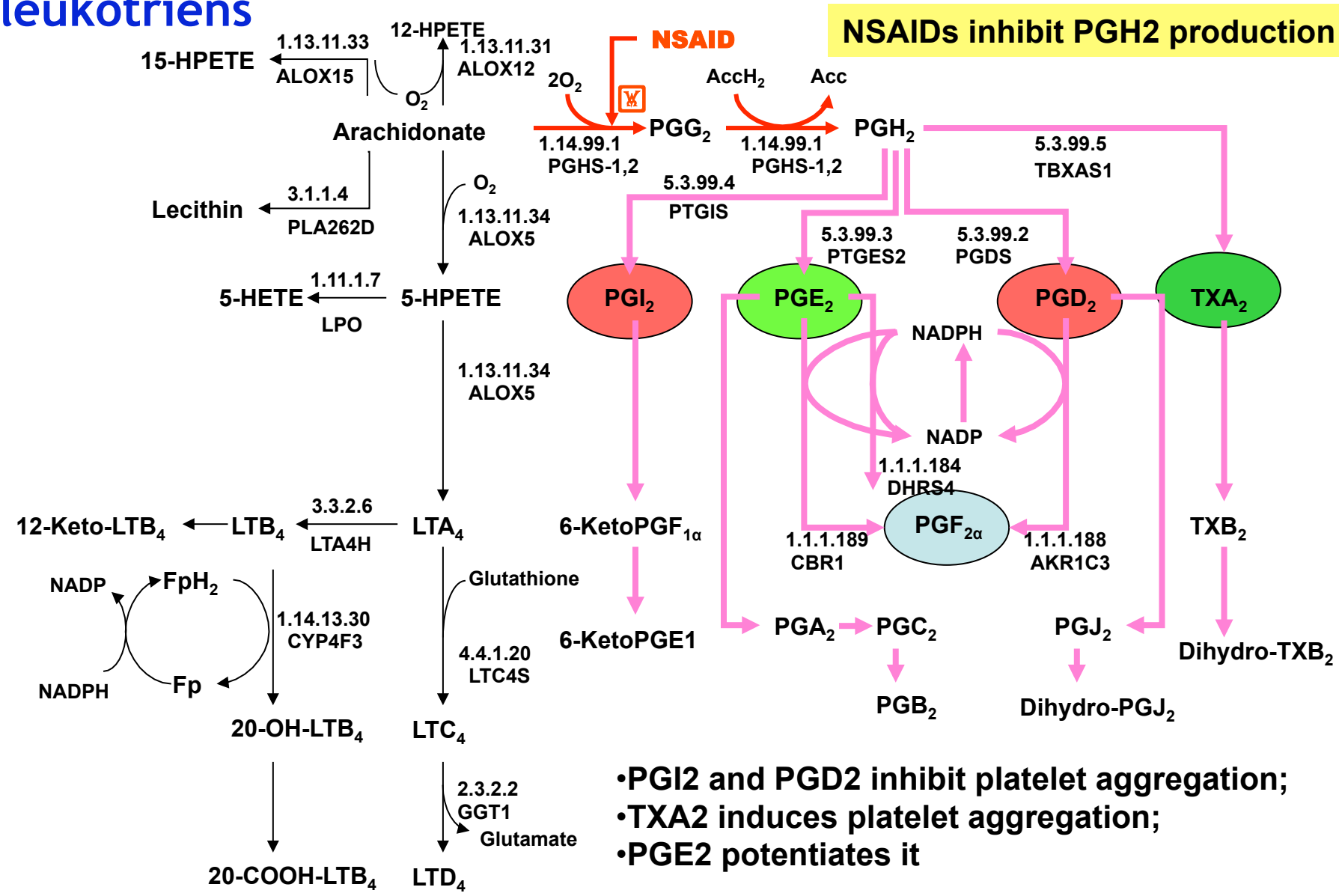
Intracellular

Cell-type – specific

Organ level

Biosystems Informatics Institute, Newcastle
Moscow States University, ISB SPb

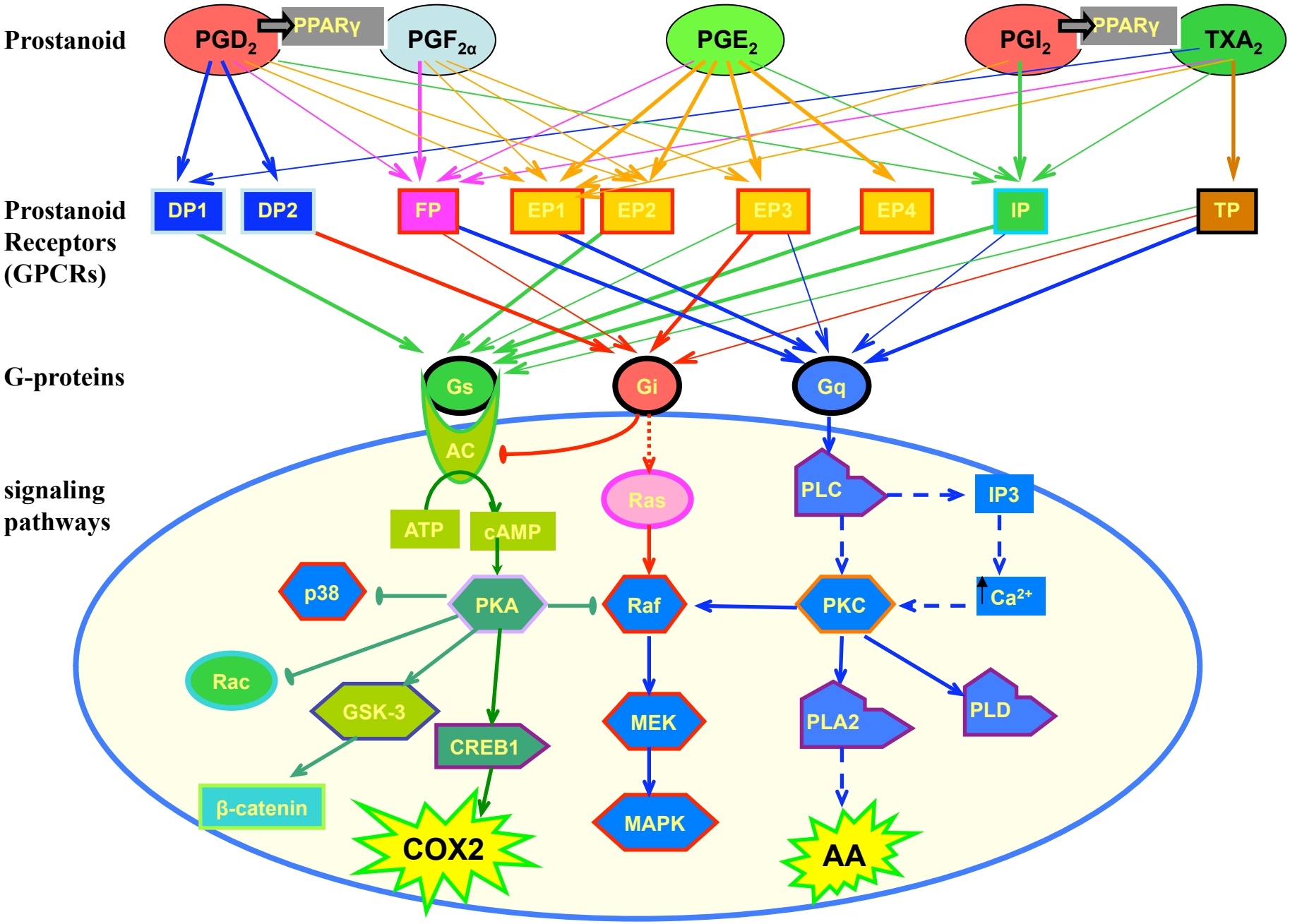
Pathway reconstruction for biosynthesis of prostanoids and leukotriens

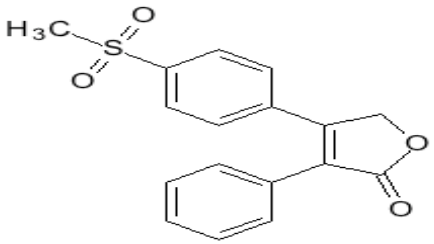


- PGI2 and PGD2 inhibit platelet aggregation;
- TXA2 induces platelet aggregation;
- PGE2 potentiates it

The overall response to NSAIDs results from complex interplay of inductions/inhibitions in different branches of prostaglandin synthesis and further signalling

Pathway reconstruction for prostanoid signalling network





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- Static pathway reconstruction
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  - NSAID action on platelets
  - NSAID action on endothelium cells
  - Combined platelet-endothelium-plasma

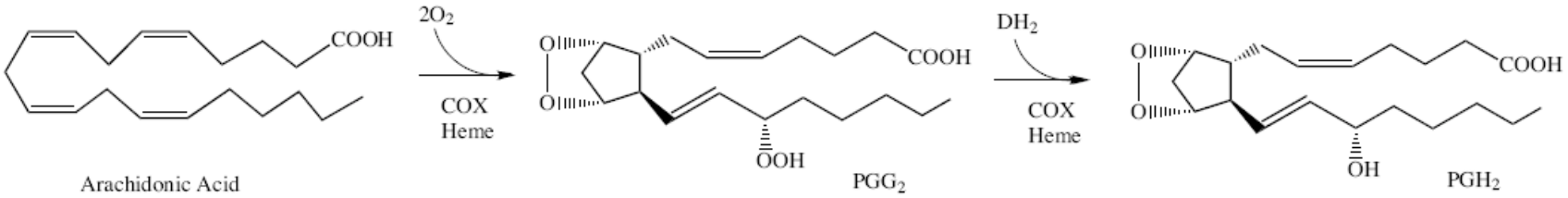
  } **Cell-type – specific**
- Model of Blood circulation system
- Coupling with pharmacokinetic profiles

} **Organ level**

Biosystems Informatics Institute, Newcastle
Moscow States University, ISB SPb

The Cyclooxygenase Reaction

Cyclooxygenase (COX) is a membrane bound enzyme responsible for the oxidation of arachidonic acid to Prostaglandin G2 (PGG2) and the subsequent reduction of PGG2 to prostaglandin H2 (PGH2).



Arachidonic Acid PGG_2 PGH_2

The enzyme has two activities:
Cyclooxygenase and Peroxidase

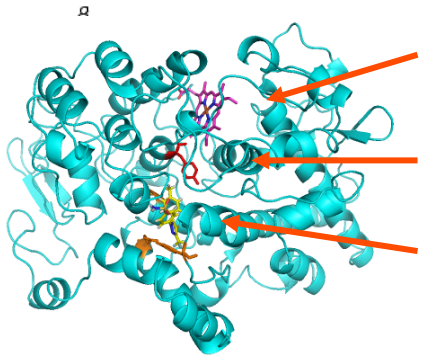
Arachidonic acid + $2O_2$ ⟶ PGG2 + H2O

PGG2 ⟶ PGH2

Kinetic model of COX-1/2 catalytic cycle (as for purified enzyme)

Catalytic mechanism of drug target elucidated and understood

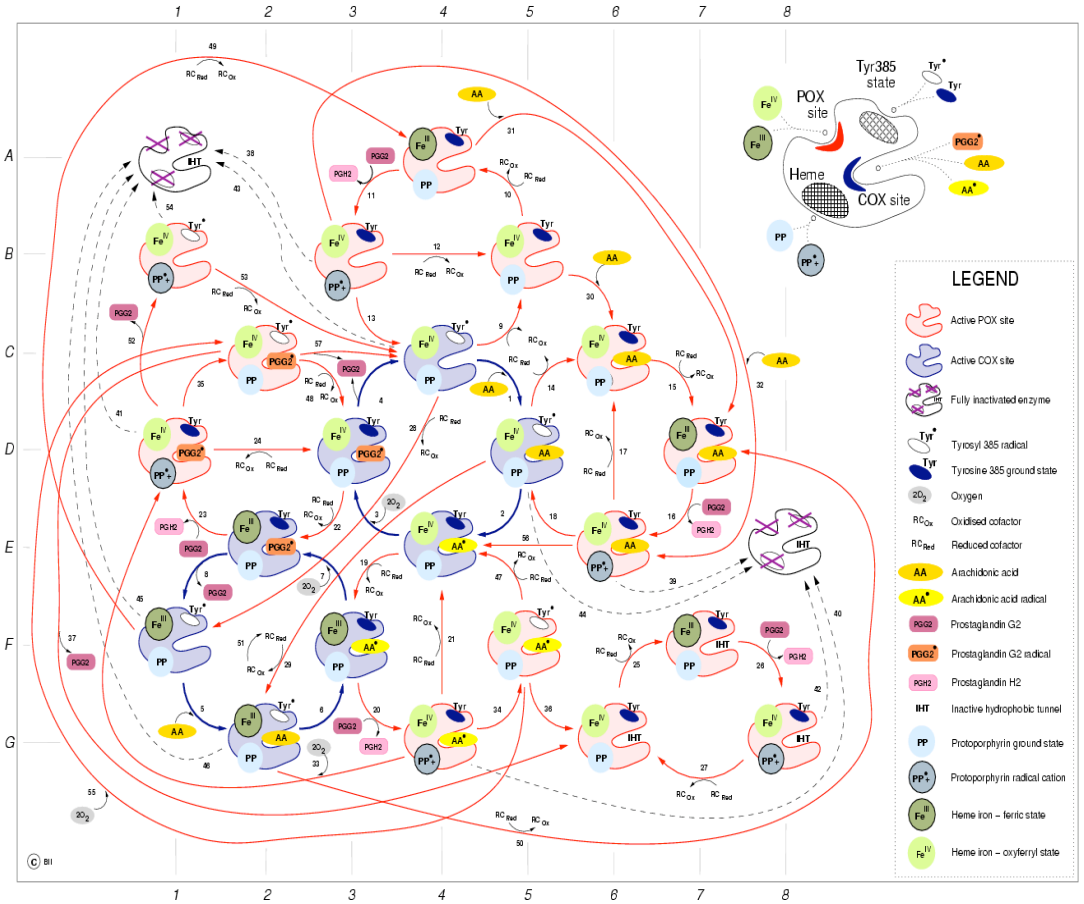
Structural knowledge as a base for mechanistic description of COX catalysis



- **Detailed ODE-based model for COX-1,2 catalysis, with all kinetic parameters accurately cross-validated. (>30 ODE, >60 rate equations, >25 parameters)**

From Model to Simulation

step 1



Transform the scheme into a set of reactions and reaction rates, e.g.:

R_1: C4 → D5

$$v_1 = k1 \cdot C4 \cdot AA$$

R_2: D5 → E4

$$v_2 = k2 \cdot D5$$

. . . . . .

step 2

Write this as set of ordinary differential equations (ODEs), e.g.:

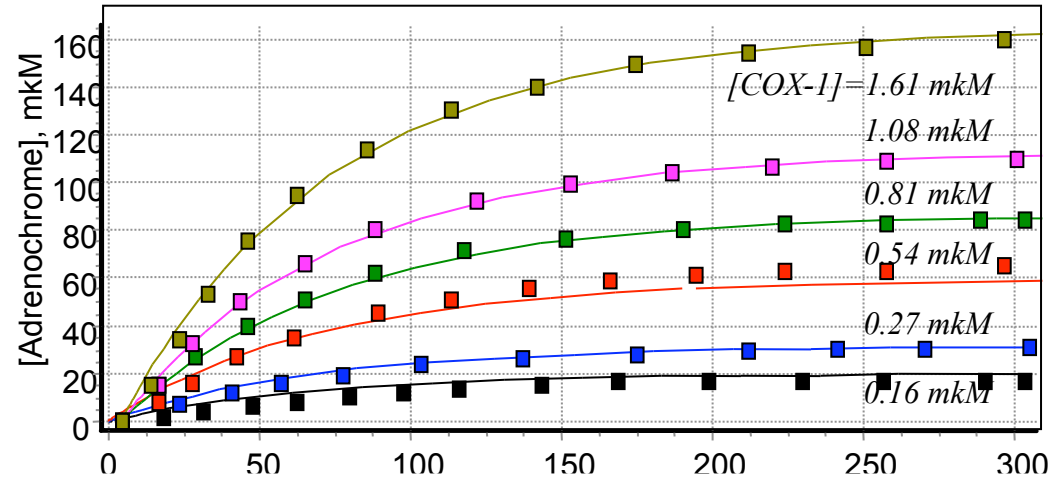
$$\frac{d[C4]}{dt} = v_4 + v_{57} + v_{53} + v_{13} - v_1 - v_9$$

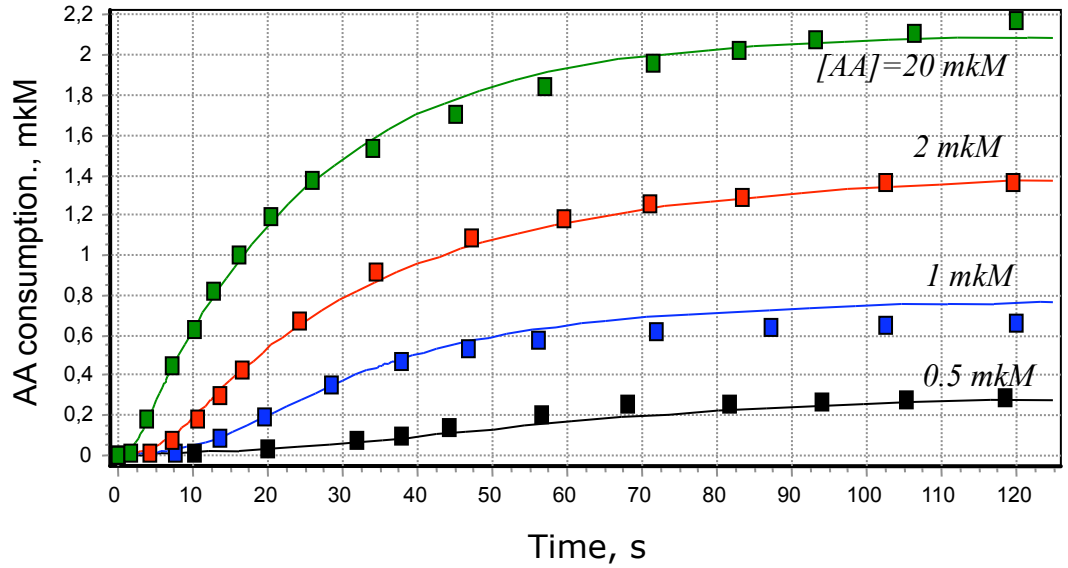
$$\frac{d[D5]}{dt} = v_1 - v_2 - v_{14} - v_{29} + v_{18}$$

step 3

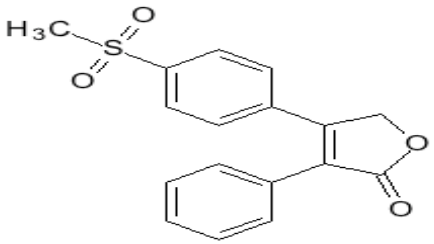
Implement the ODE set in DBSlove or/and MATLAB (via SBML conversion)

Model Validation. Identification of kinetic parameters.





Parameters of the COX catalytic cycle identified on the base of experimental data available from literature



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  Cell-type – specific
- Model of Blood circulation system
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  Organ level

Biosystems Informatics Institute, Newcastle
Moscow States University, ISB SPb

Extended Cox-1/2 Model for Drug Action Modeling

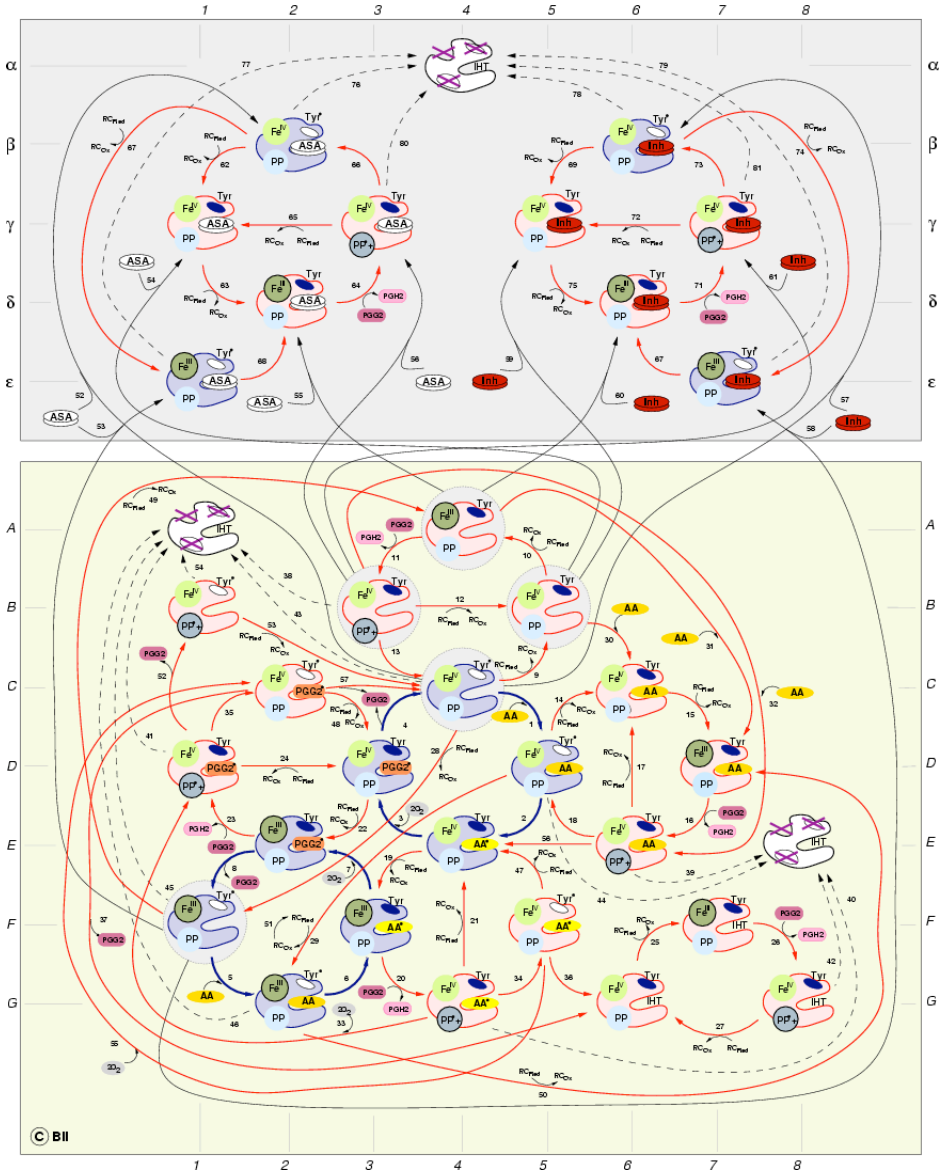


Figure A: Extended Cox-1/2 model. Bottom panel – default Cox-1/2 model; Top panel – inhibition of Cox-1/2 by Aspirin and/or second Inhibitor.

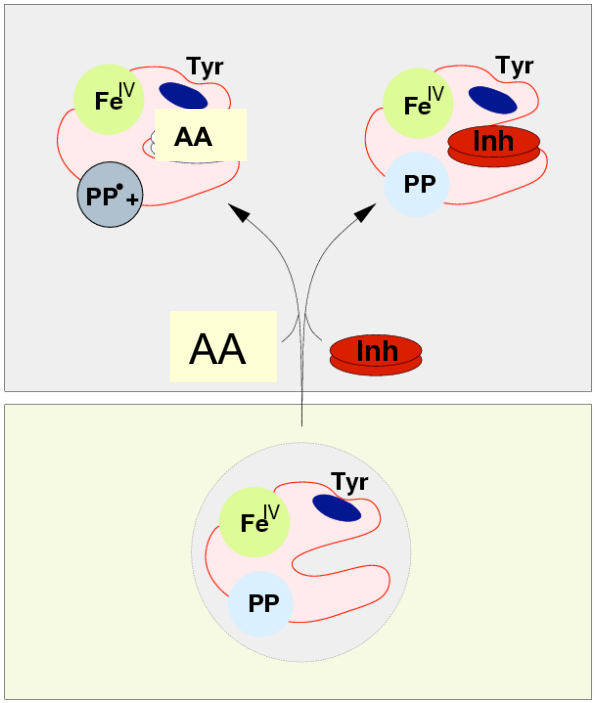


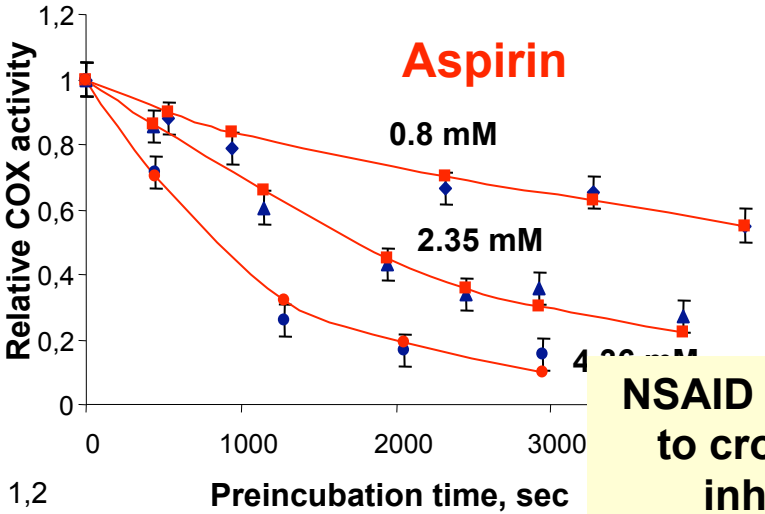
Figure B: Cox cartoon of the simplified Cox1/2 inhibition mechanism.

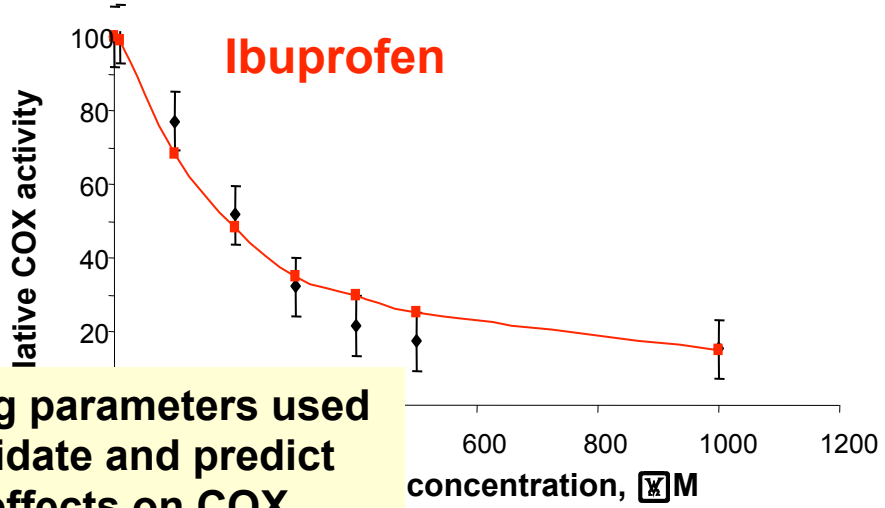
Effects of inhibitors (NSAIDs) introduced to the COX model:

	Time dependence	Reversibility of binding	Selectivity to COX1,2
•Aspirin	+	-	1,2
•Indomethacin	+	+	1,2
•Naproxen	1-,2+	+	1,2
•Diclofenac	+	+	1
•Ibuprofen	-	+	1,2
•Celecoxib	1-,2+	+	2
•Vioxx	1-,2+	+	2

1- COX1; 2 - COX2

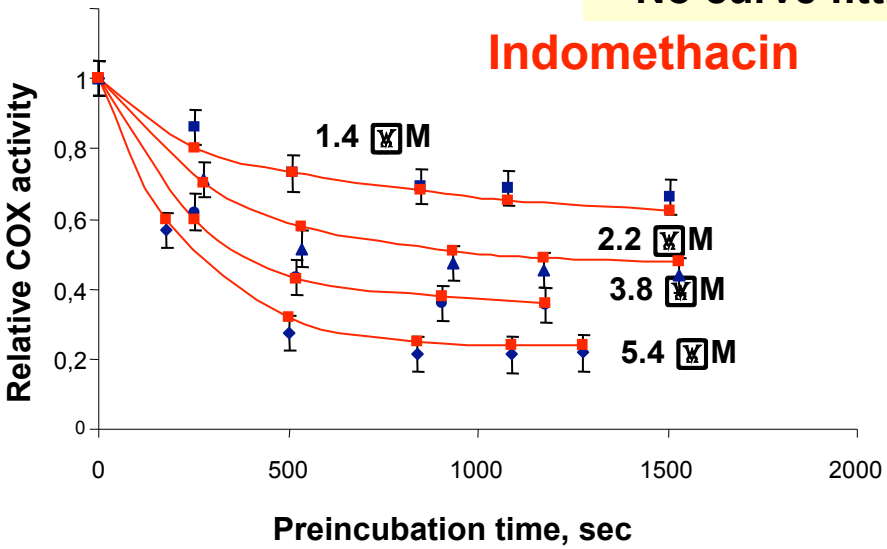
Consistent description of experimental data on inhibitory effects of different NSAIDs

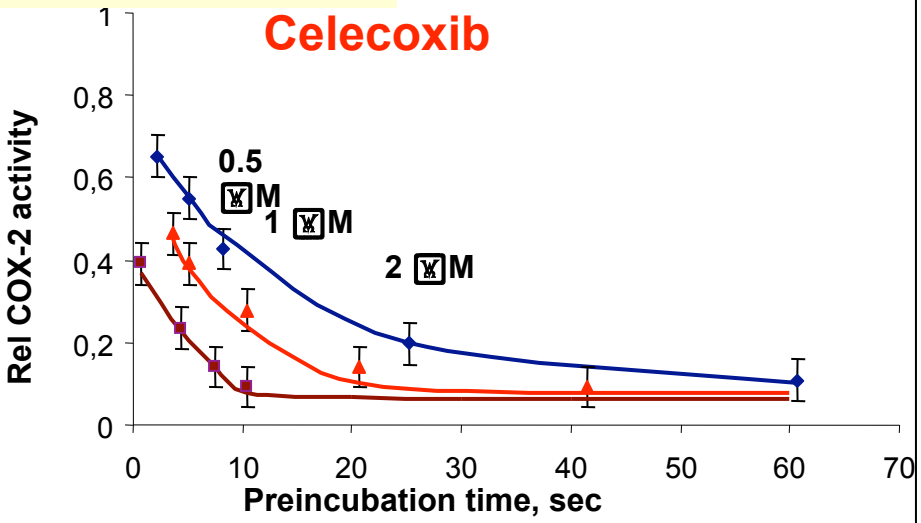




NSAID binding parameters used to cross-validate and predict inhibitor effects on COX

- No curve fitting was employed -





Prediction of NSAID action on target

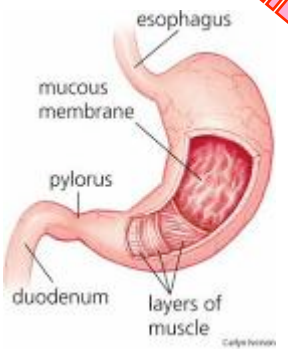
-For both single drug and drug combinations:

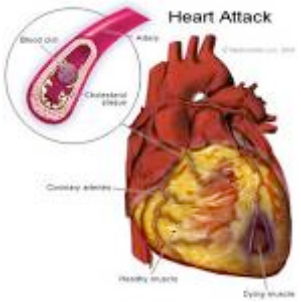
-Aspirin, Ibuprofen, Naproxen, Celecoxib, Indomethacin, Diclofenac,…

High Dose Aspirin
GI side effects

Safer ASA/Coxib Combinations?

High Dose Coxib
CV side effects



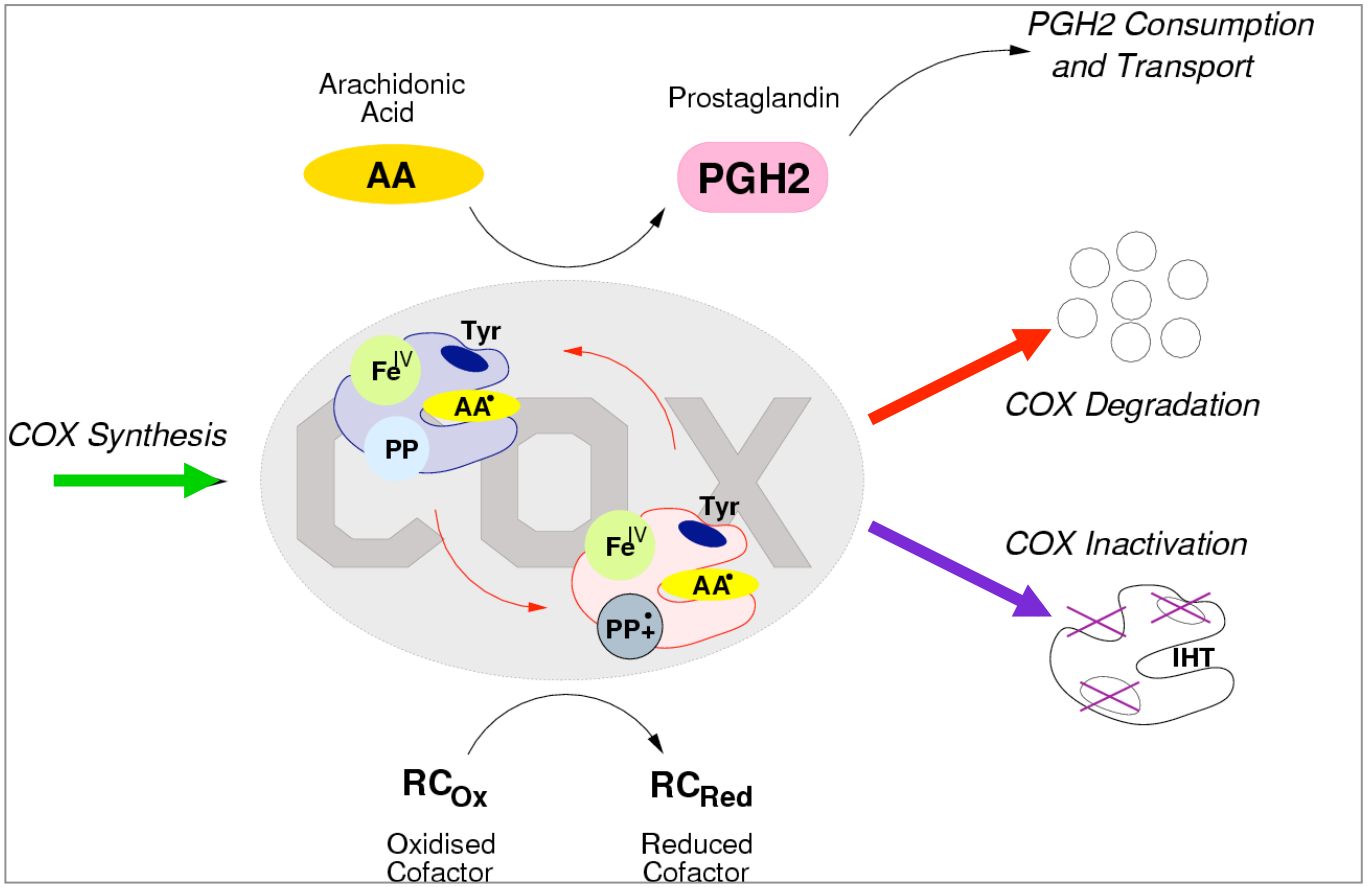


50% inhibition plane

- **Selective COX2 inhibitor can block aspirin effect – experimental phenomena observed, not previously explained**
- **Model based analysis allows for prediction of safer drug combinations**

COX in the context of intracellular micro- conditions

COX catalysis and NSAID effects in real time and within real physiological substrate / product concentration range



Description includes:

- **Detailed catalytic cycle of COX**
- **COX self-inactivation**
- **COX synthesis and degradation**
- **In-fluxes and out-fluxes of substrates and products**

Allows for properties of COX and NSAID inhibition to be translated into in vivo conditions

Intracellular microenvironment controls COX activity and dictates sensitivity to NSAIDs

model allowed to explain/predict many experimental phenomena:

- Discrepancies between *in vitro / in vivo* estimates of IC50 for Aspirin
- Origin of variability of *in vivo* experimental values of Aspirin IC50 – intracellular micro- environmental concentrations of substrates
- Variability in COX-1/COX-2 selectivity – results from intracellular conditions
- Attenuation of ASA effect by Celecoxib

EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES 36 (2009) 122–136

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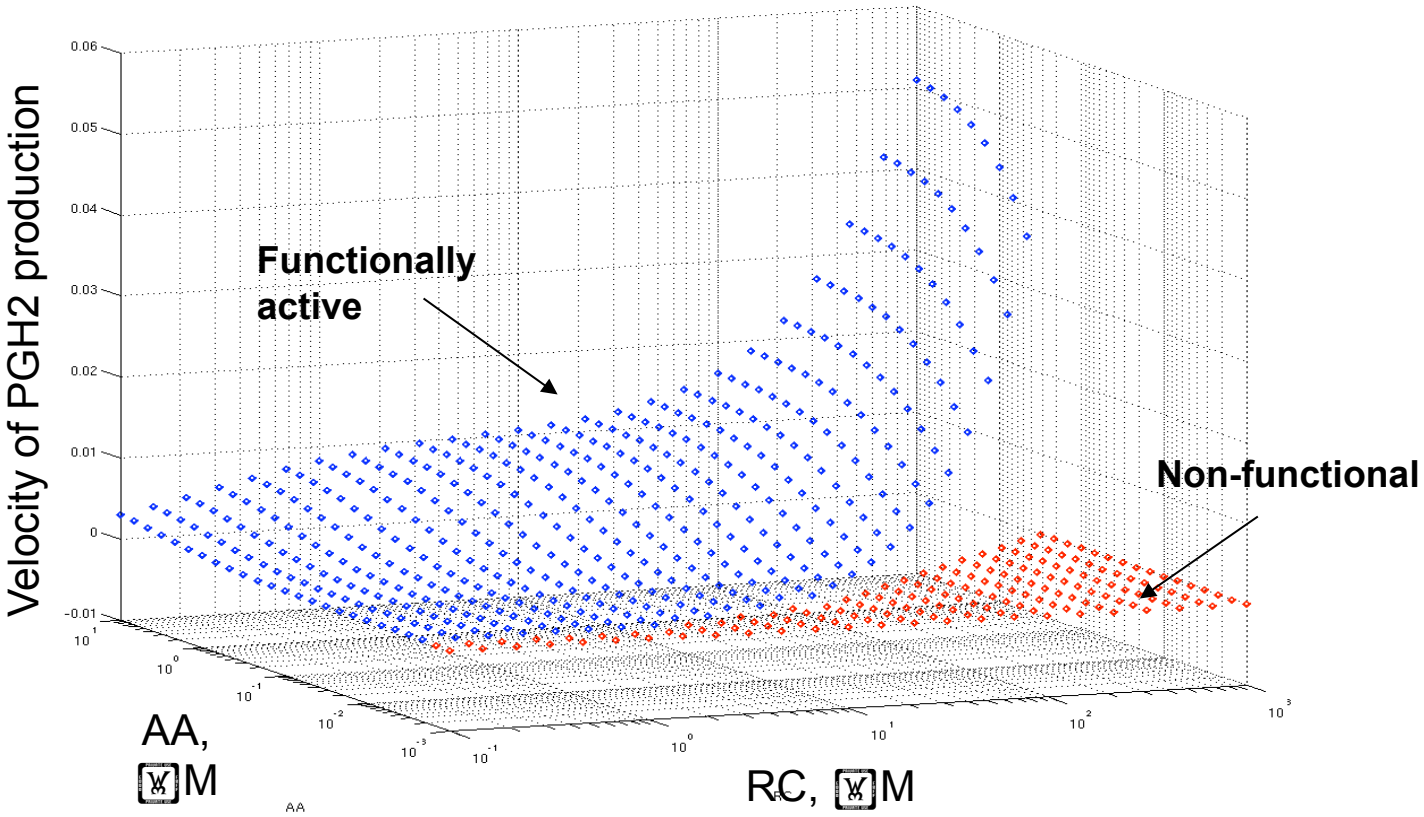
Kinetic modelling of NSAID action on COX-1: Focus on *in vitro/in vivo* aspects and drug combinations

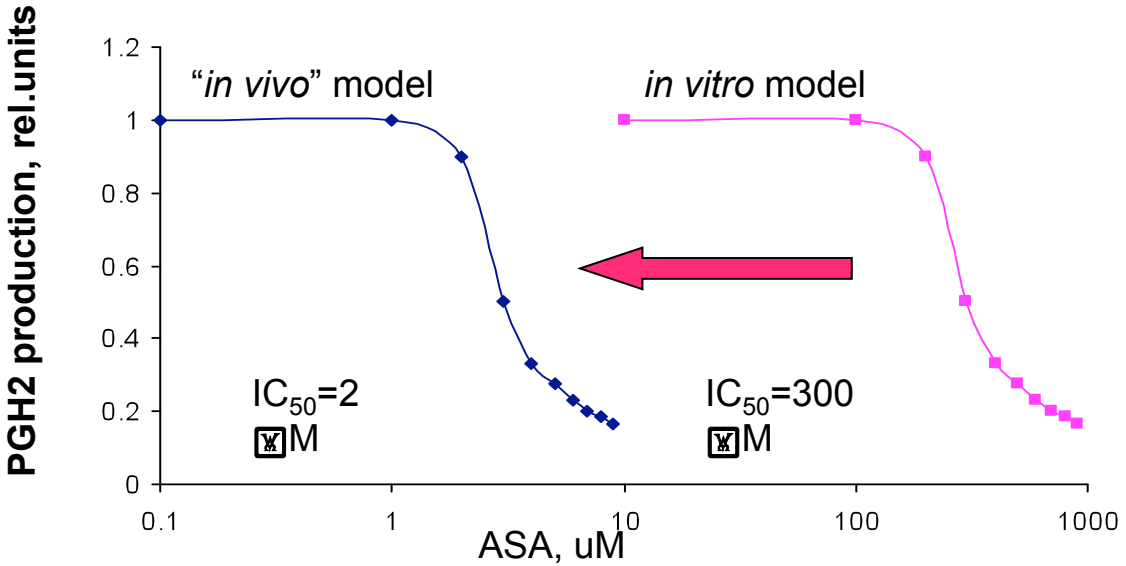
Alexey Goltsov[a,*], *Anton Maryashkin*[b], *Maciej Swat*[c], *Yuri Kosinsky*[b,d], *Ian Humphery-Smith*[e], *Oleg Demin*[c,d], *Igor Goryanin*[a], *Galina Lebedeva*[a,**]

COX activity is controlled by intracellular micro-environmental conditions:

Arachidonic Acid and Reducing Co-substrate concentration



First valid explanation of discrepancies between *in vitro / in vivo* estimates of IC_{50} for Aspirin



Experimental estimates of IC_{50} (M) for Aspirin:

In vivo COX-1

Whole blood assay	1.3 [1],
Platelet	1.3 [2]
Endothelial cells	1.5 [3]
Fibroblasts	2.6 [4]

In vitro COX-1

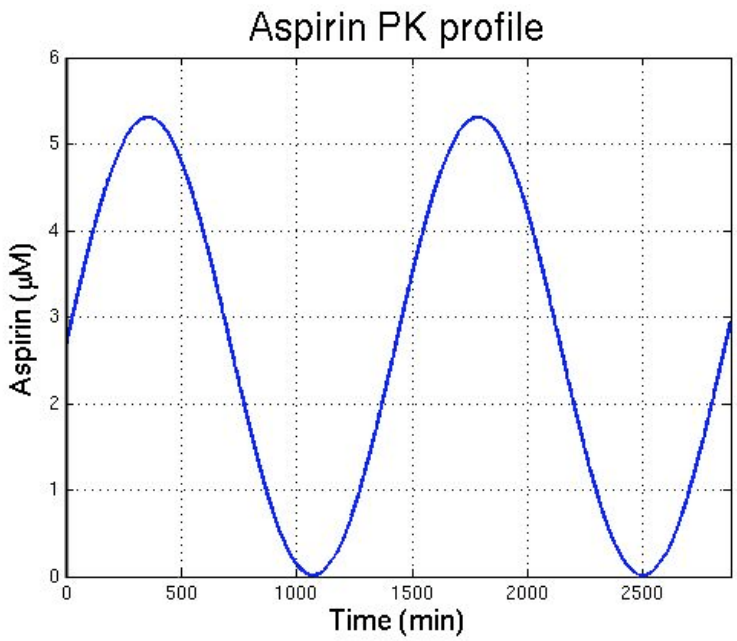
Purified enzyme: 30-200 [5]

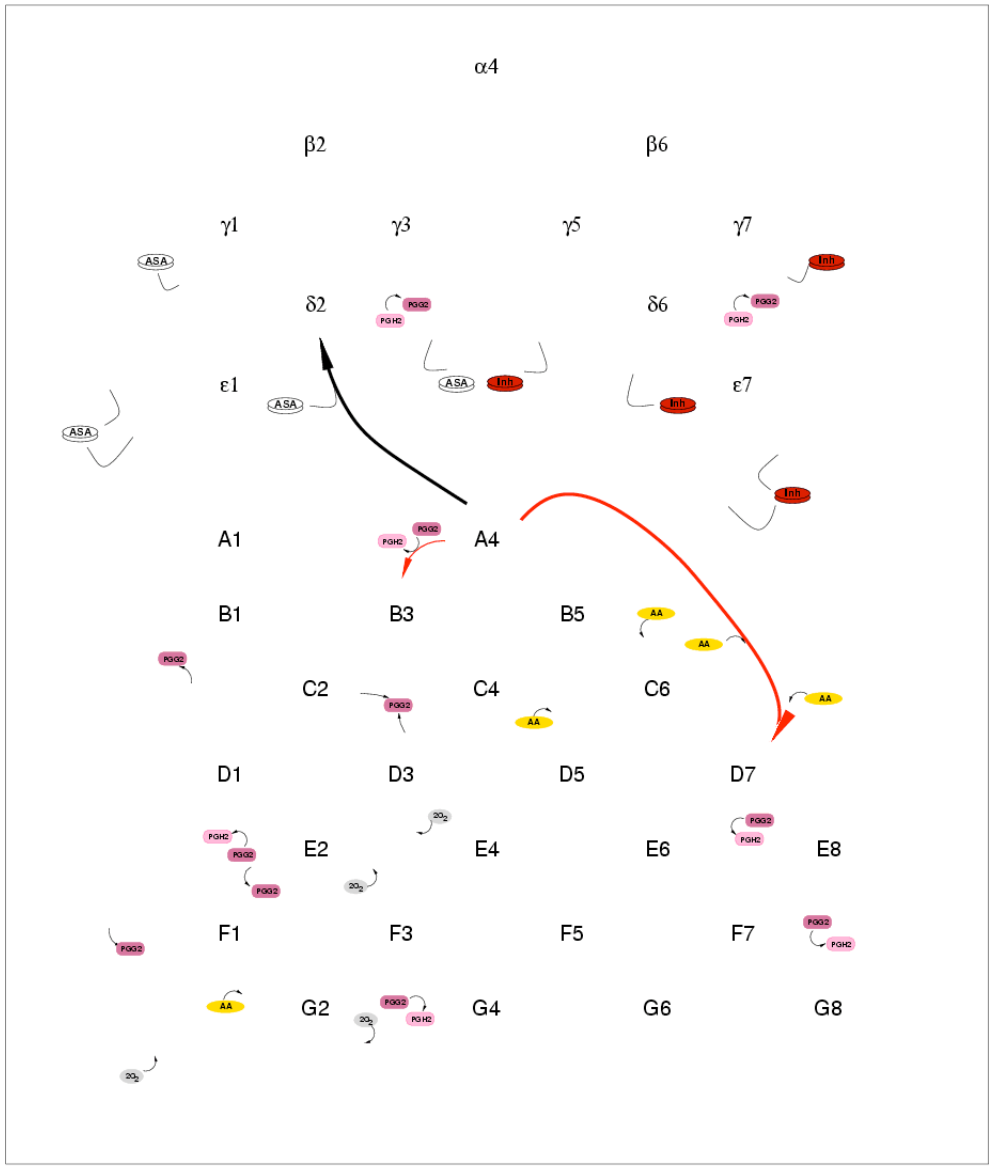
1. Warner T., Giuliano F., et al PNAS 96, 1999, 7563
2. Quellet M., Riendeau D., Percival M. PNAS 98, 2001, 14583
3. Mitchell J. , Akarasereenont P., et al PNAS 90, 1994, 11693
4. Chulada P., Langenbach R. J. Pharm. Exp. Ther. 280, 1997,606
5. Kargman S., Wong E. et al Bioch. Pharm. 52, 1996, 1113

Accumulation of acetylated COX gives rise to additional COX inhibition due to retained peroxidase activity

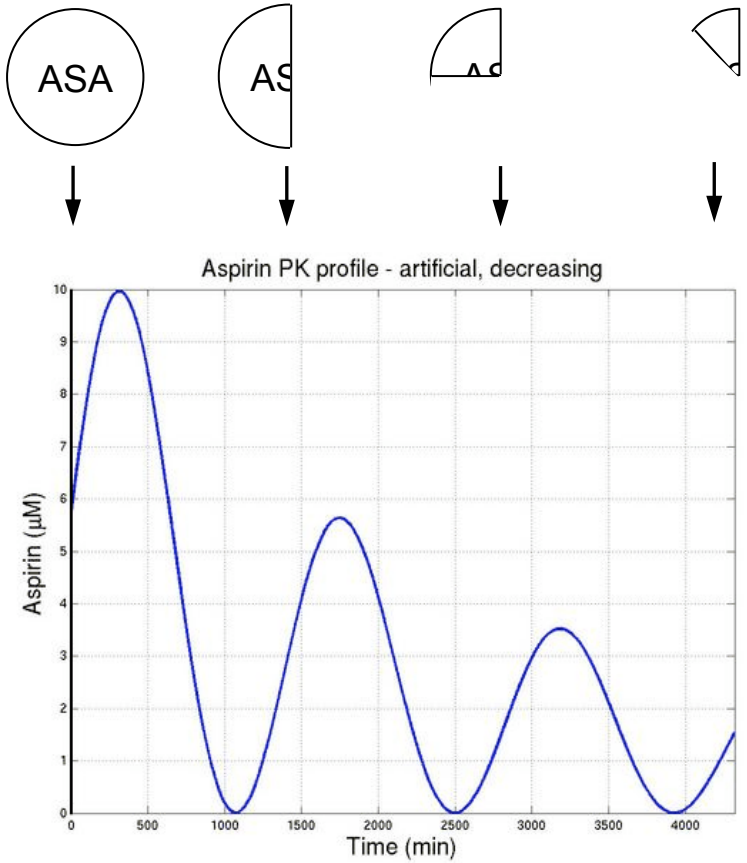
Modelling drug action on target under various regimes of drug administration

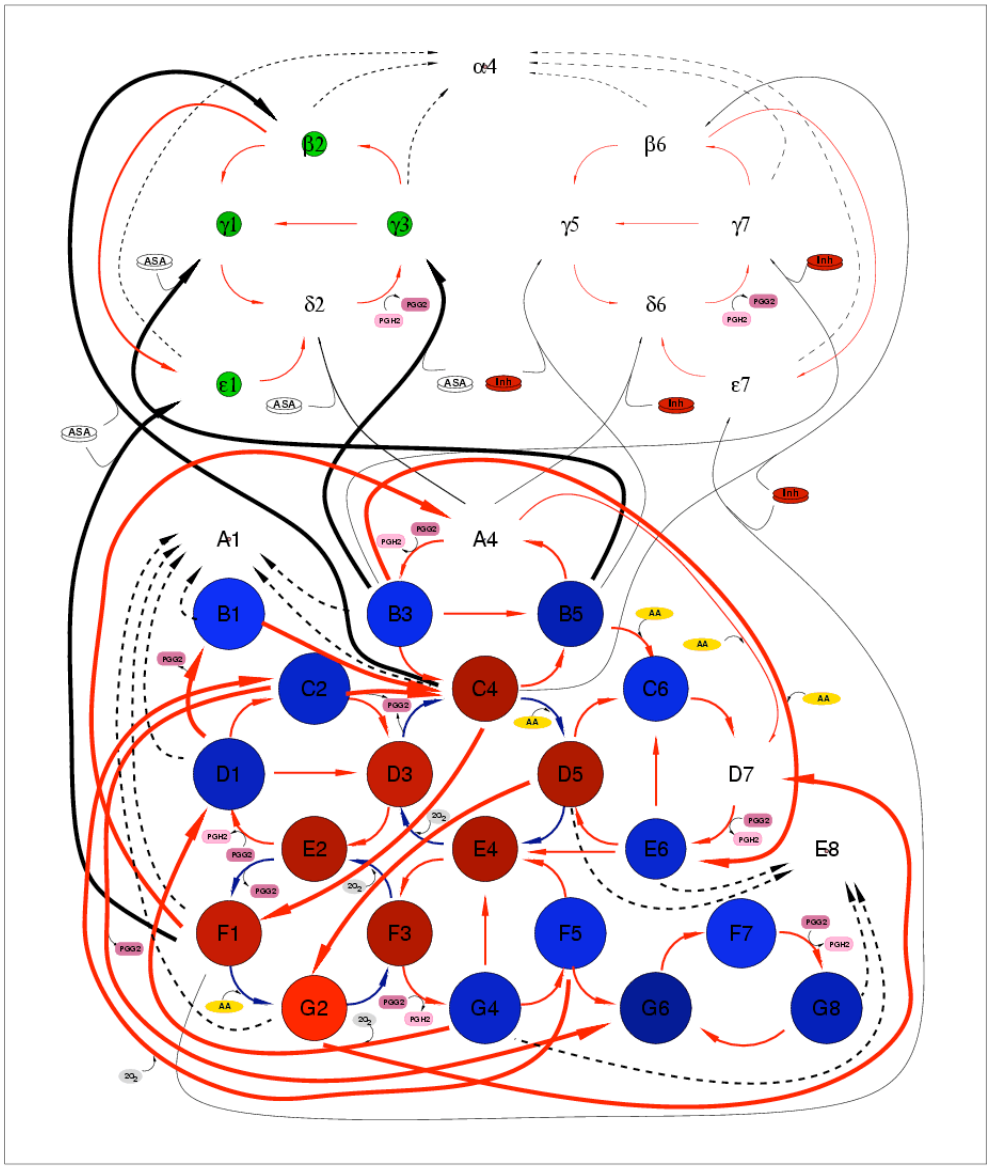
Low dose Aspirin, once daily





A **higher** dose Aspirin reduced with time





Start

1 hours

End

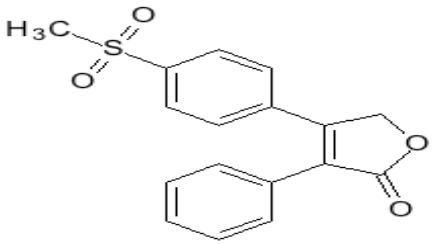
max PGH2
4.8395

max PGG2
0.01041

PGG2

PGH2

Asp = 7.08 uM Inh = 0 uM



"Vioxx" project

- Static pathway reconstruction
- Intracellular level models:
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Intracellular

Cell-type – specific

Organ level

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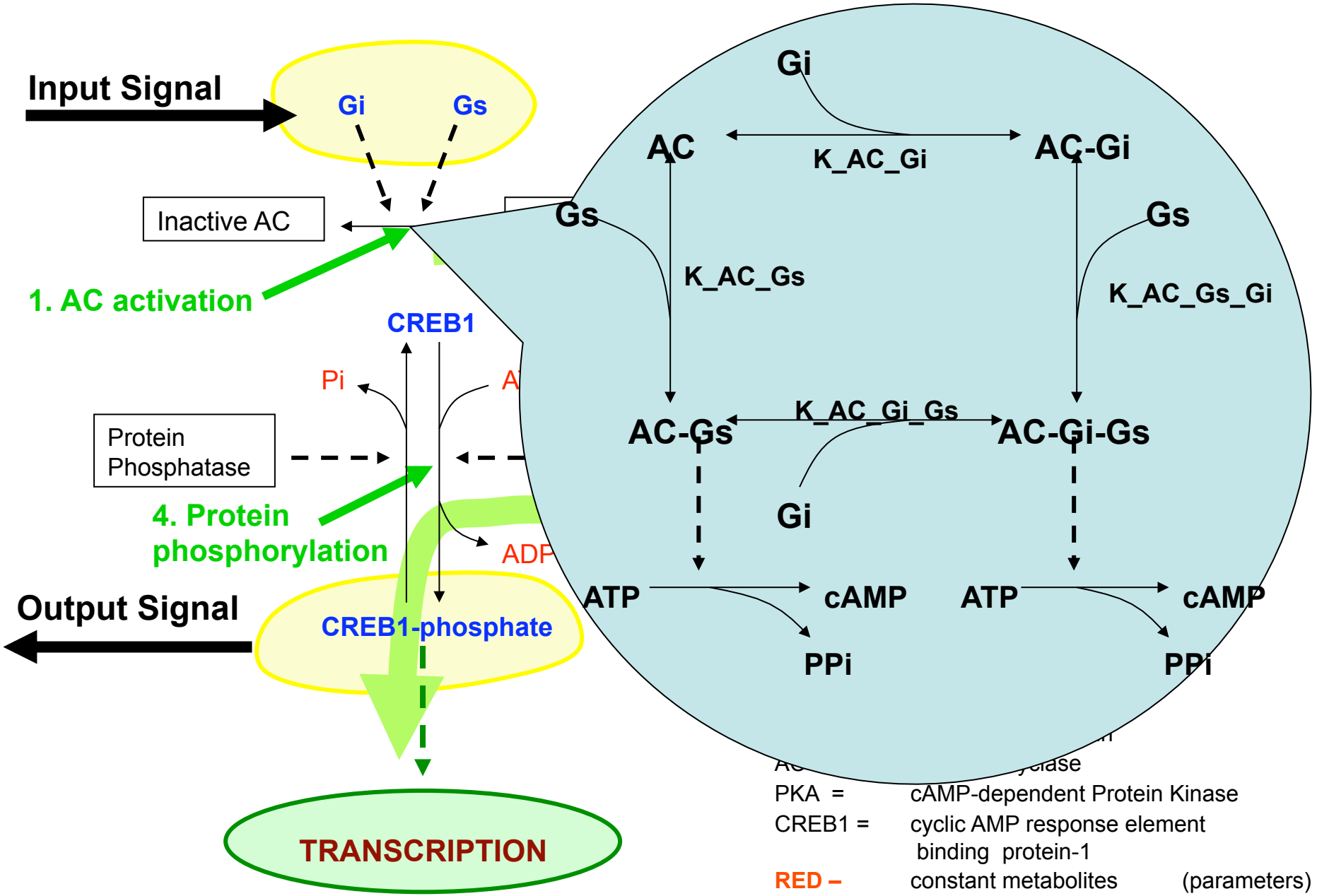
Moscow States University, ISB SPb

Example: Adenylate Cyclase and Protein Kinase A Signalling



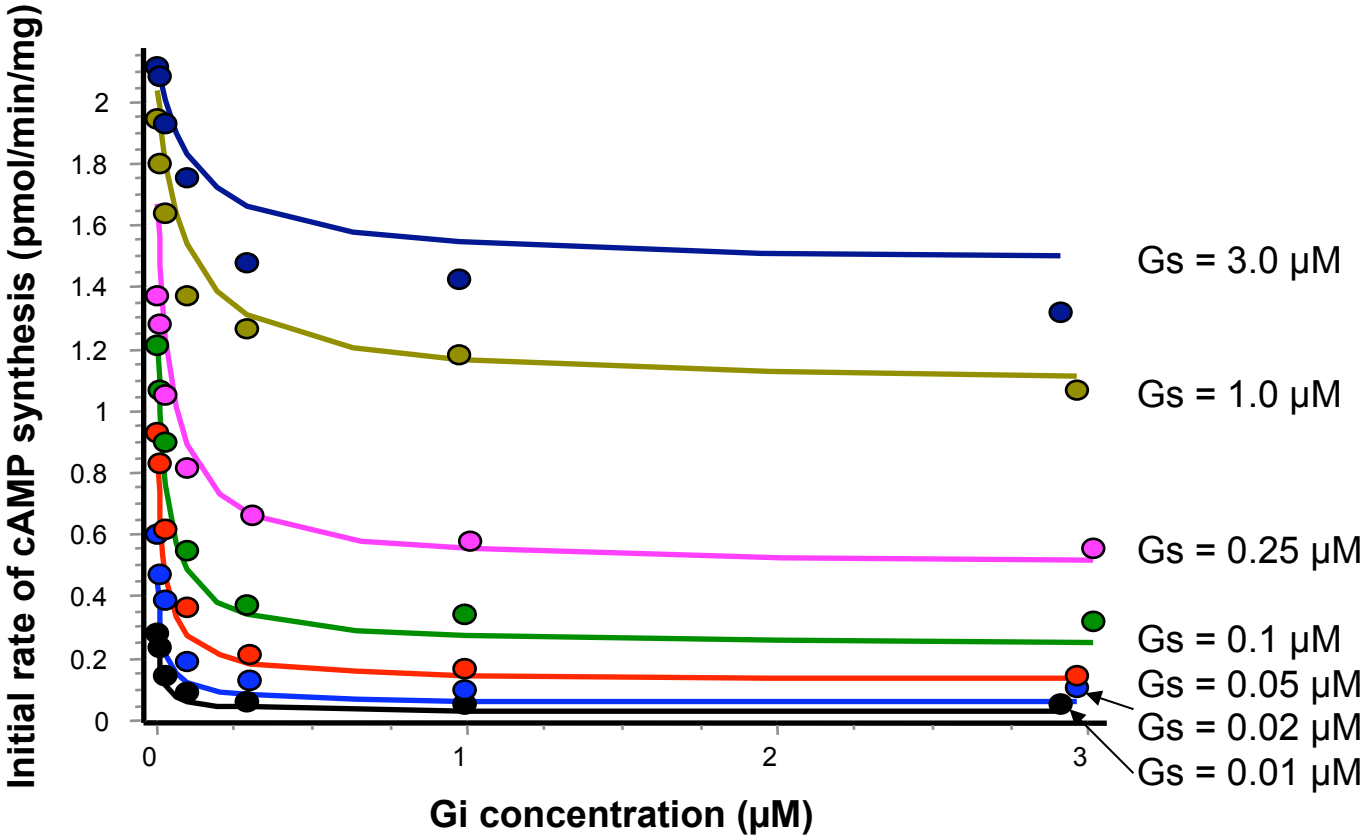
PKA = cAMP-dependent Protein Kinase

CREB1 = cyclic AMP response element binding protein-1

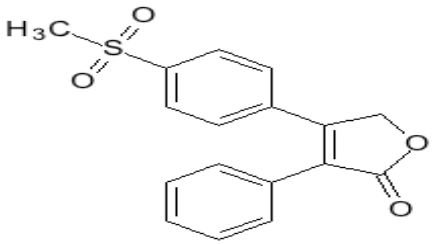
RED – constant metabolites (parameters)

BLUE – variable metabolites

Identification of constants for AC activation model



***** Chen-Goodspeed M, Lukan AN, Dessauer CW; Modeling of Galpha(s) and Galpha(i) regulation of human type V and VI adenylyl cyclase; J Biol Chem. 2005; 280(3):1808-16



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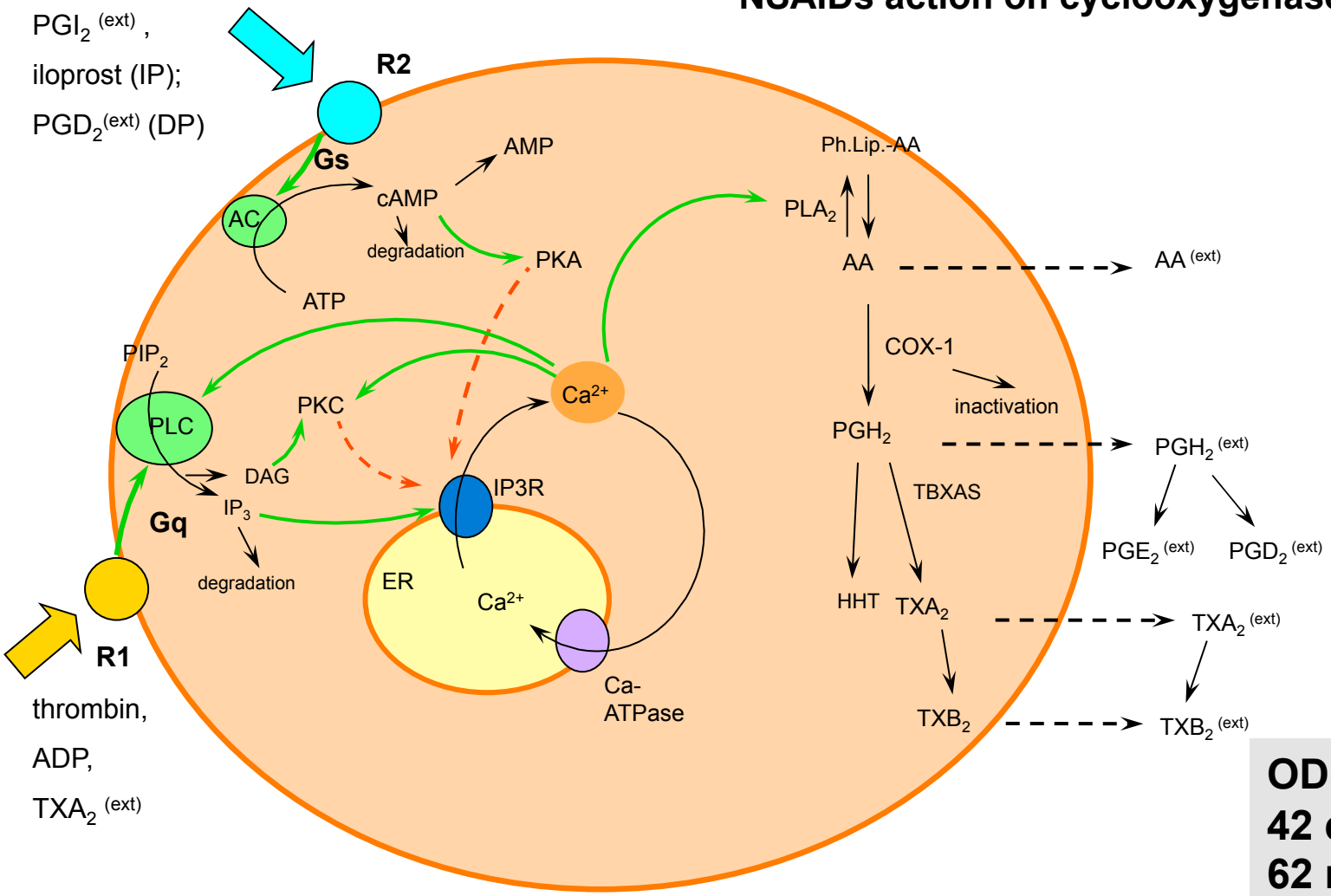
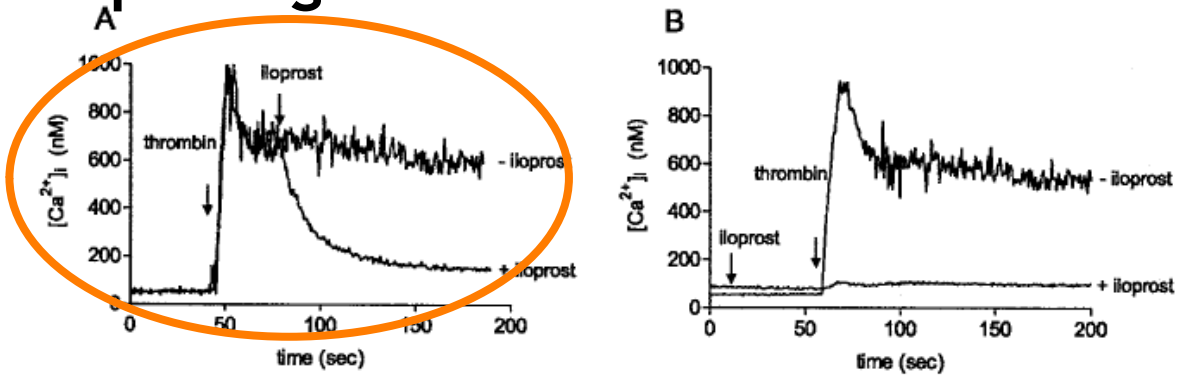
Platelet model

- TXA_2 biosynthesis
- transmembrane transport
- signalling pathways activated by prostanoids
- Ca^{2+} fluxes involved in platelet activation
- NSAIDs action on cyclooxygenase



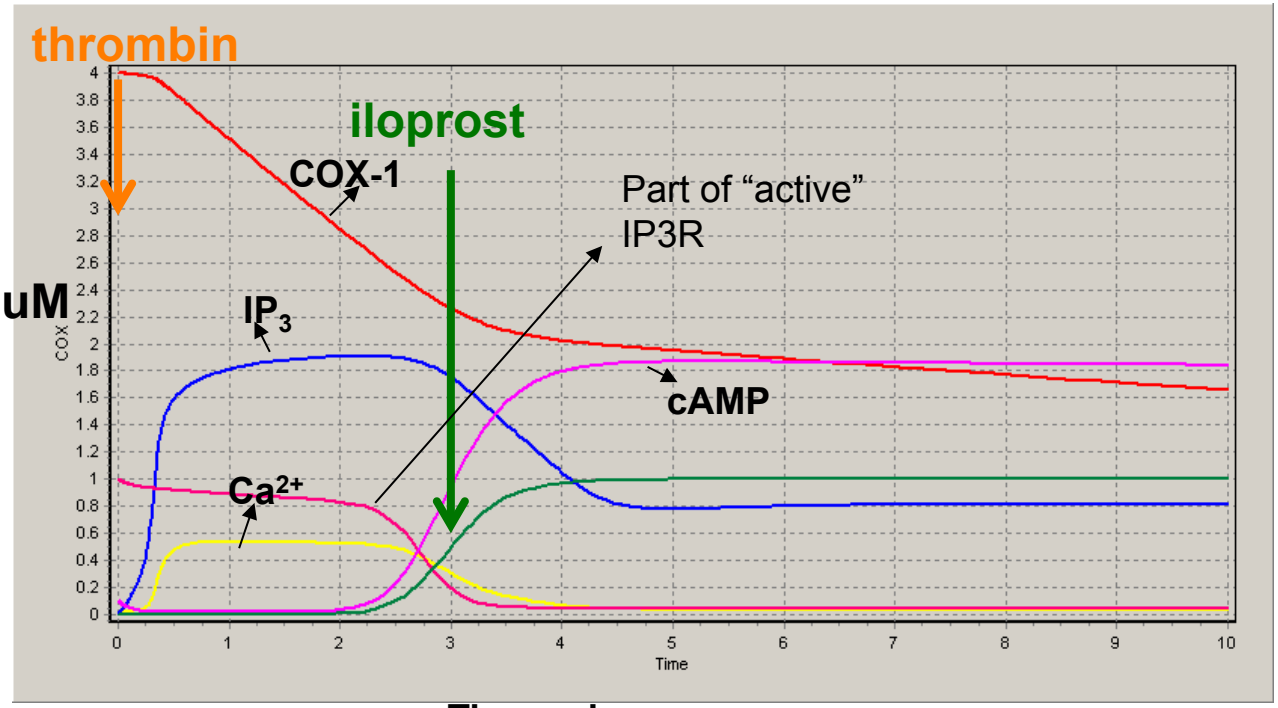
ODE system:
42 equations,
62 rate laws,
152 parameters

Platelet model validated on perturbation experimental data with receptor agonists



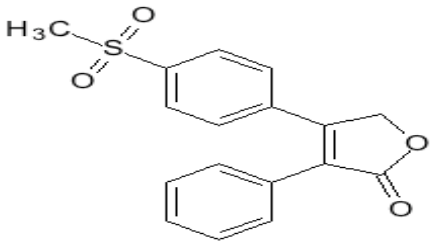
Data from
JBC(2002)277:29321

FIG. 1. **Inhibition of thrombin-induced Ca^{2+} increases by iloprost in platelets.** *A*, Fura-2-loaded platelets were stimulated with thrombin (1 unit/ml) and 30 s later with iloprost (1 μM). *B*, conversely, platelets were first incubated with iloprost (1 μM) and 1 min later stimulated with thrombin (1 unit/ml). Tracings show the increase in $[Ca^{2+}]_i$ measured in the presence of 1 mM extracellular Ca^{2+} and are representative of three observations with similar results.



High dose of iloprost effectively inhibits platelets activation by thrombin

Stimulation of platelets by iloprost leads to cAMP-dependent PKA activation that phosphorilates IP3-dependent calcium channels (IP3R) on endoplasmic reticulum (ER). This results in in the inhibition of IP3R sensitivity to IP3, and a decrease in Ca2+ outflux from ER



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Organ level

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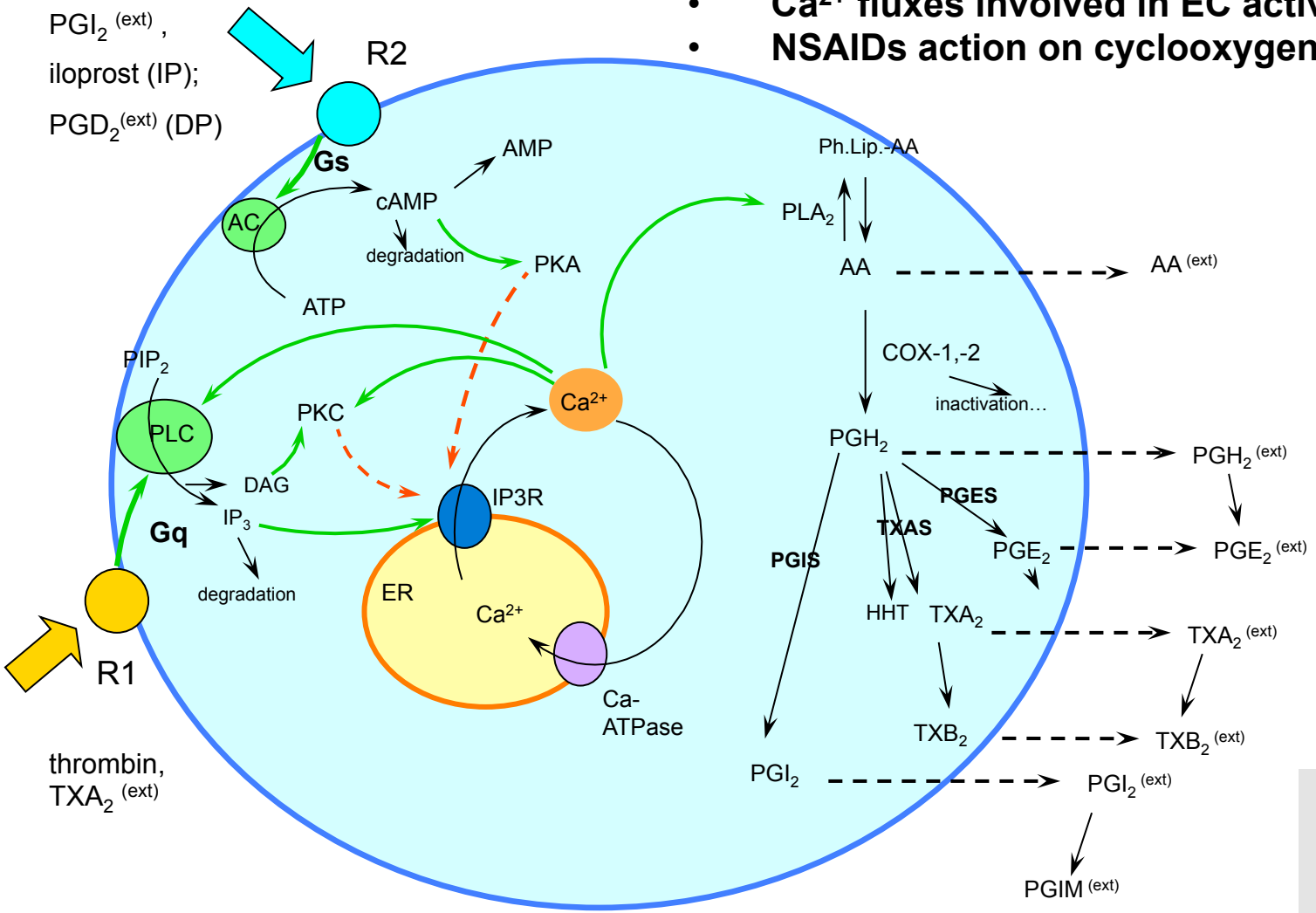
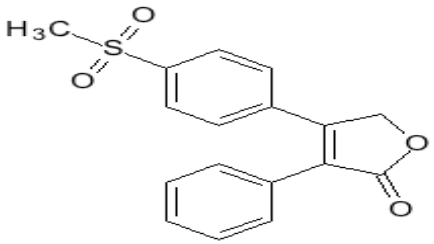
Endothelium cell model

- **prostanoid biosynthesis (PGI_2, PGE_2, TxA_2)**
- **transmembrane transport**
- **signalling pathways activated by prostanoids**
- **Ca^{2+} fluxes involved in EC activation**
- **NSAIDs action on cyclooxygenase**



ODE system:
47 equations,
69 rate laws,
184 parameters



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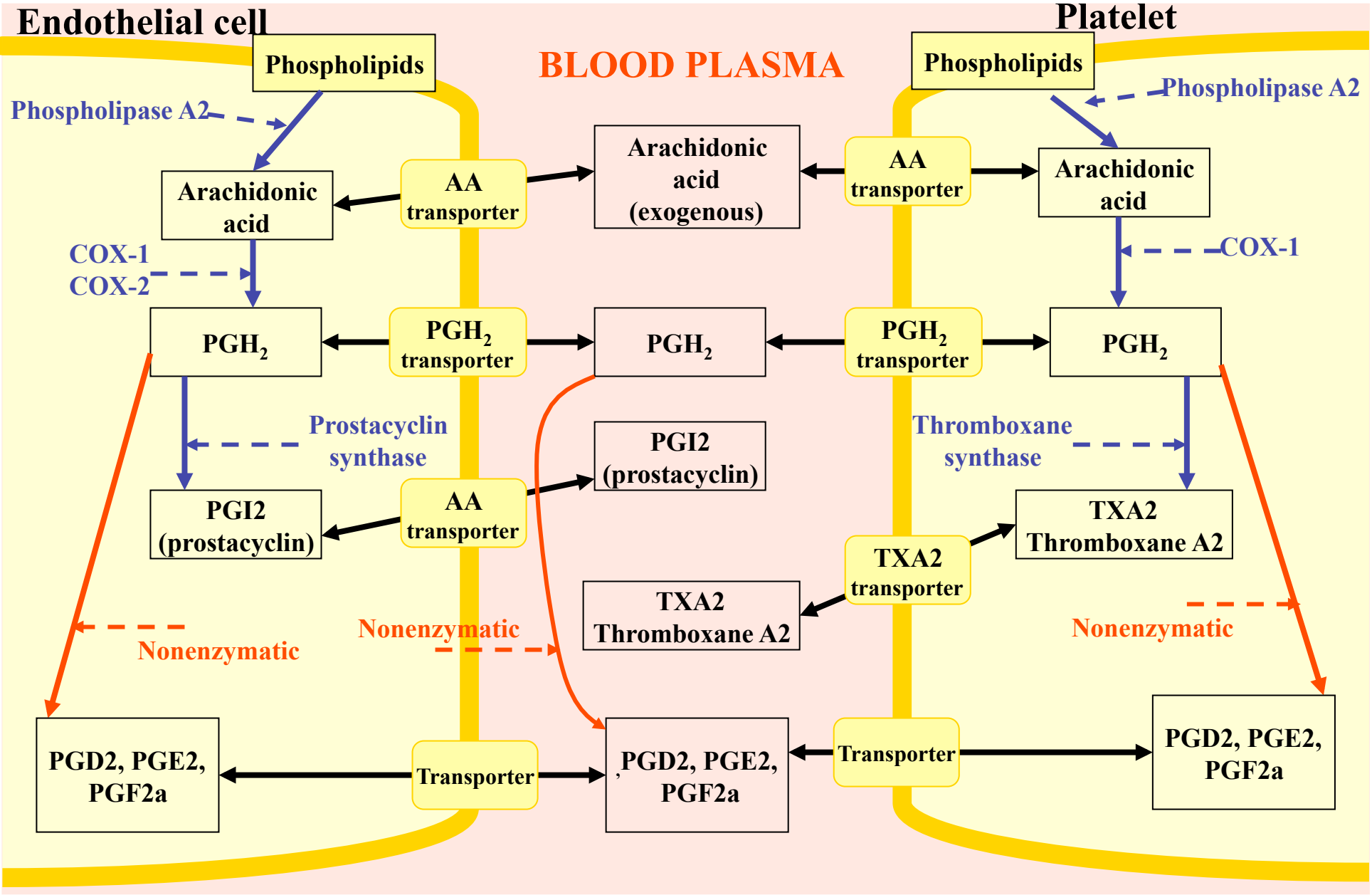
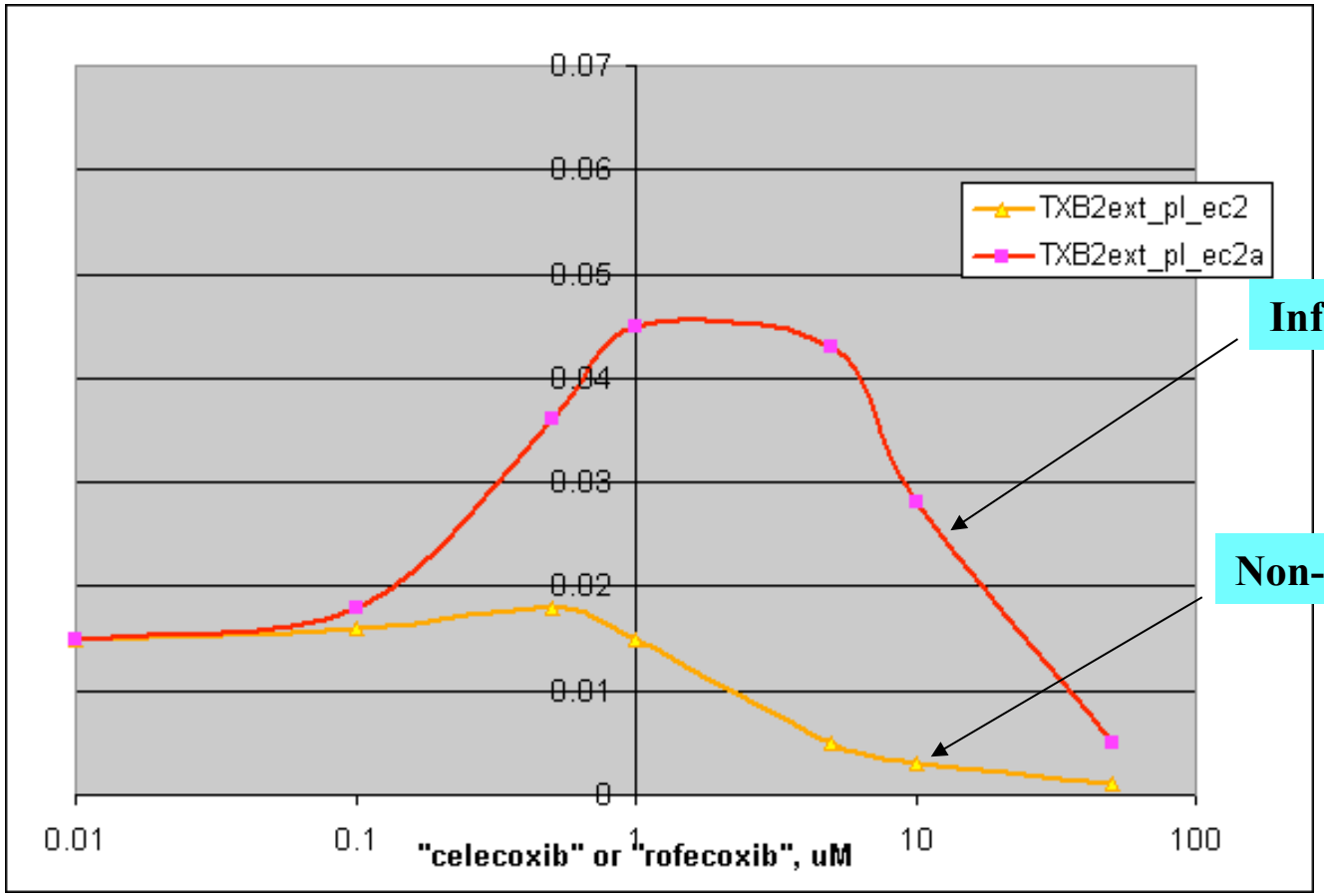
Platelet-Endothelium-Plasma model allows to assess potential cardiovascular risk after NSAID administration



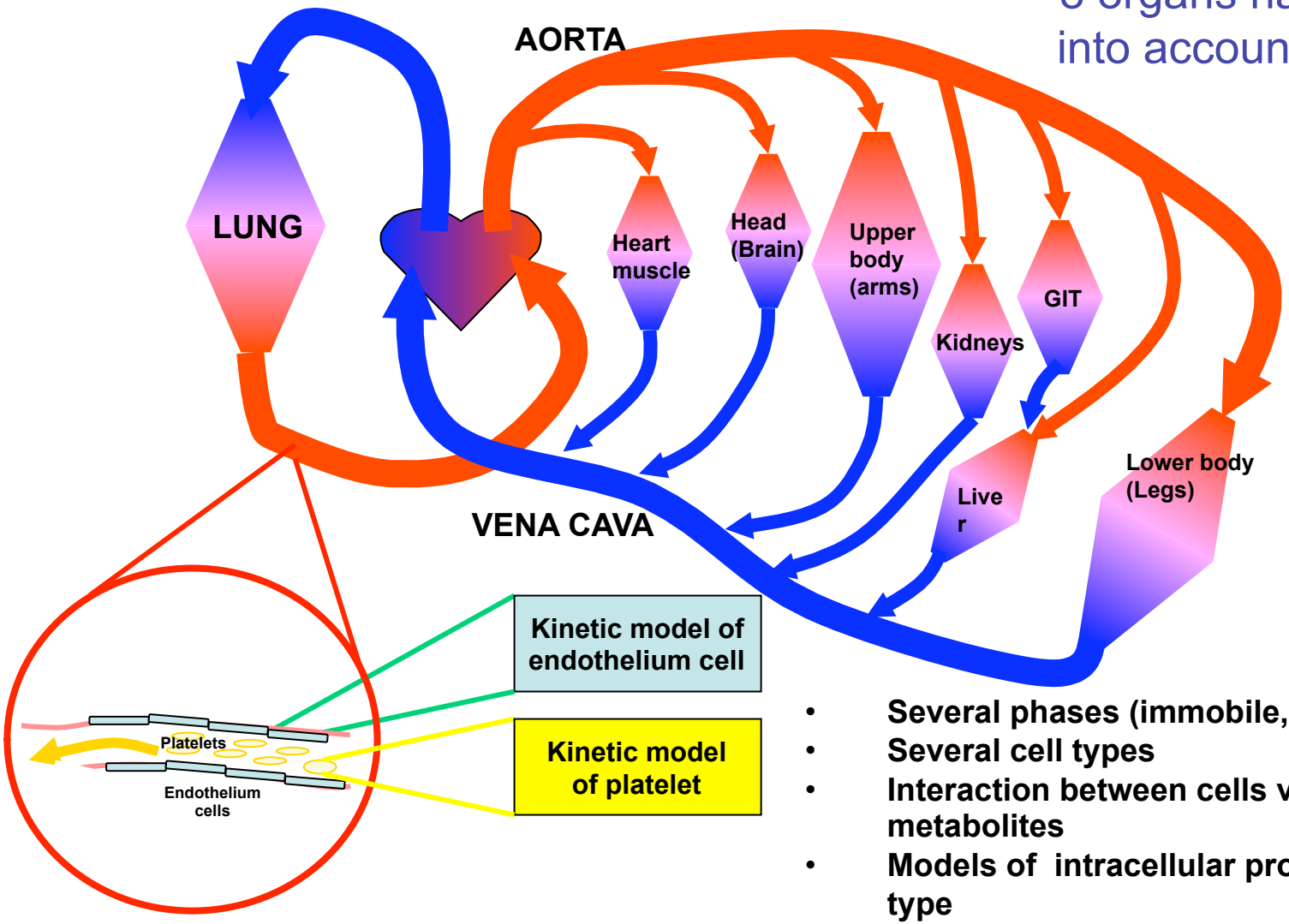
Dose-response of the platelet-endotelium plasma system. Prediction of clotting risks for NSAIDS and combinations



Risk of clot formation increases with increase in COX2 selective inhibitor concentration under inflammatory conditions

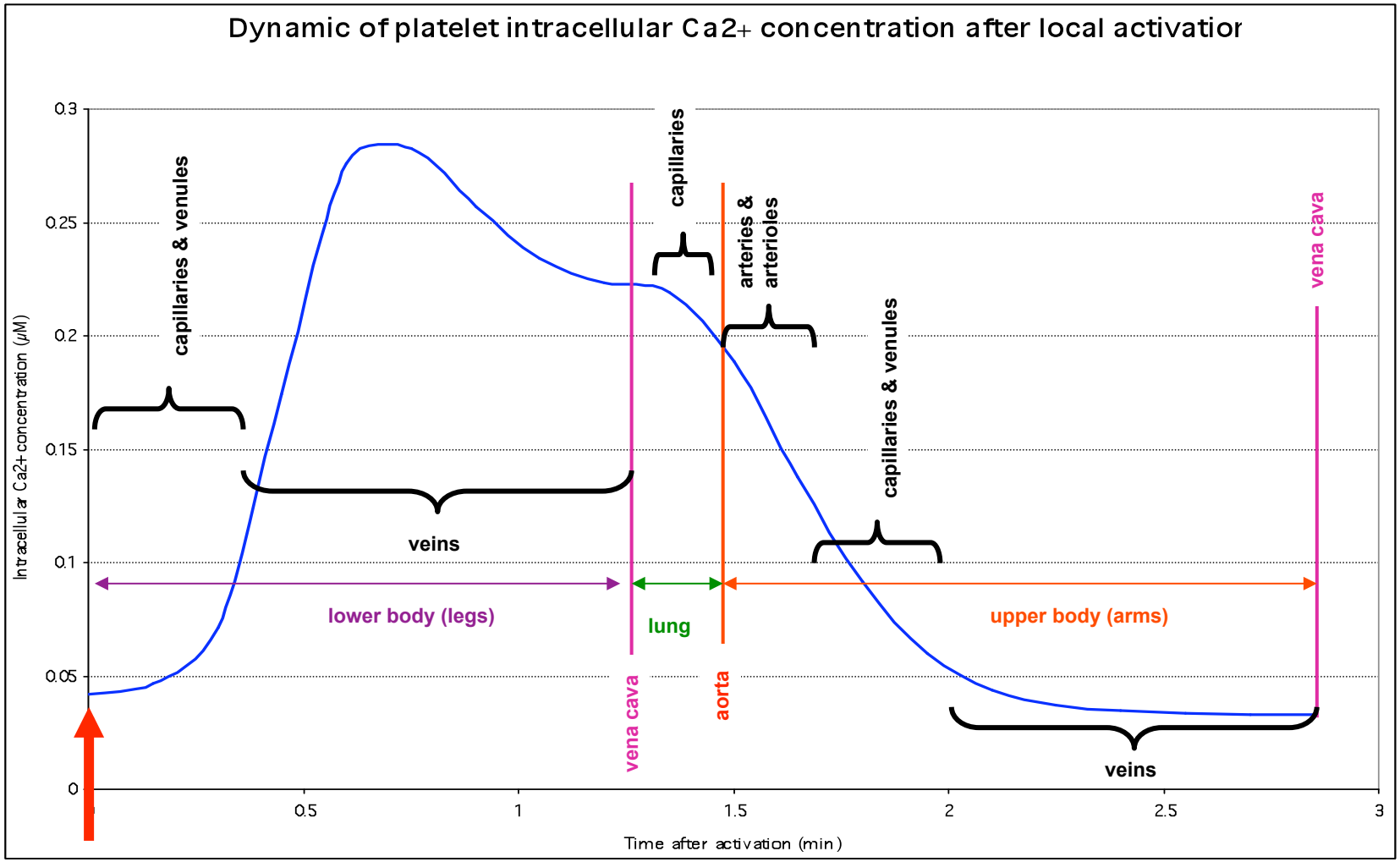
Scaling up to organism level: Blood circulation

8 organs have been taken into account in our models



- Several phases (immobile, mobile,…)
- Several cell types
- Interaction between cells via secreted metabolites
- Models of intracellular processes in each cell type
- Anatomical/geometrical features of the system
- Changes in properties of the cells located at different parts of the system

Monitoring state of platelets in different parts of blood circulation



1) Clotting risks monitored (proportional to intracellular Ca2+ concentration in platelets)
2) Maximal risk of clot formation is observed in leg veins
3) Capillaries of lungs and arms decrease risk of clot formation substantially

Systems Approach applied to drug safety

- *In silico* methods help to understand the efficacy, mode of action and potential dangerous side effects of drugs
- Model-based analysis and prediction of drug safety and efficacy
- Informed advice on drug dosing, scheduling and combinations

Acknowledgement

Dr. Alexey Goltsov

Dr. Maciej Swat

Dr. Oleg Demin

Dr. Yury Kosinsky and team

Dr. Jörg Lippert

Prof. Tim Warner

William Harvey Research Institute

Prof. Igor Goryanin

University of Edinburgh