Metabolic basis of complex diseases as new targets for novel designed therapies

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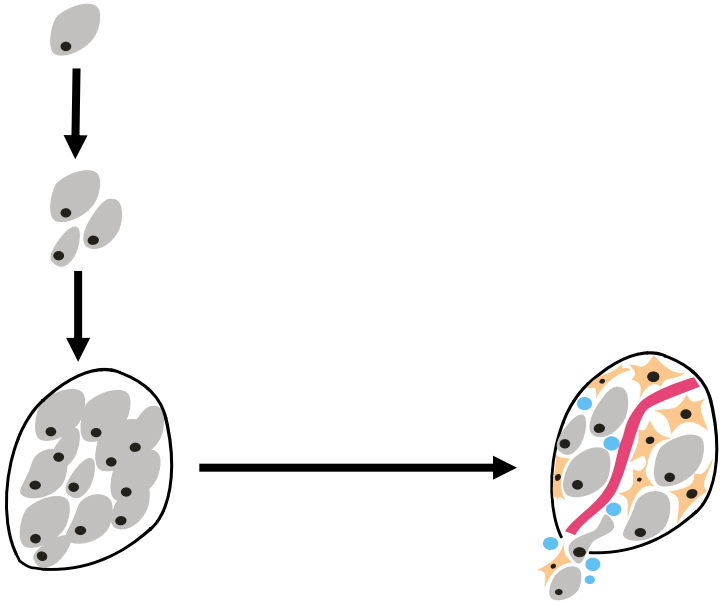
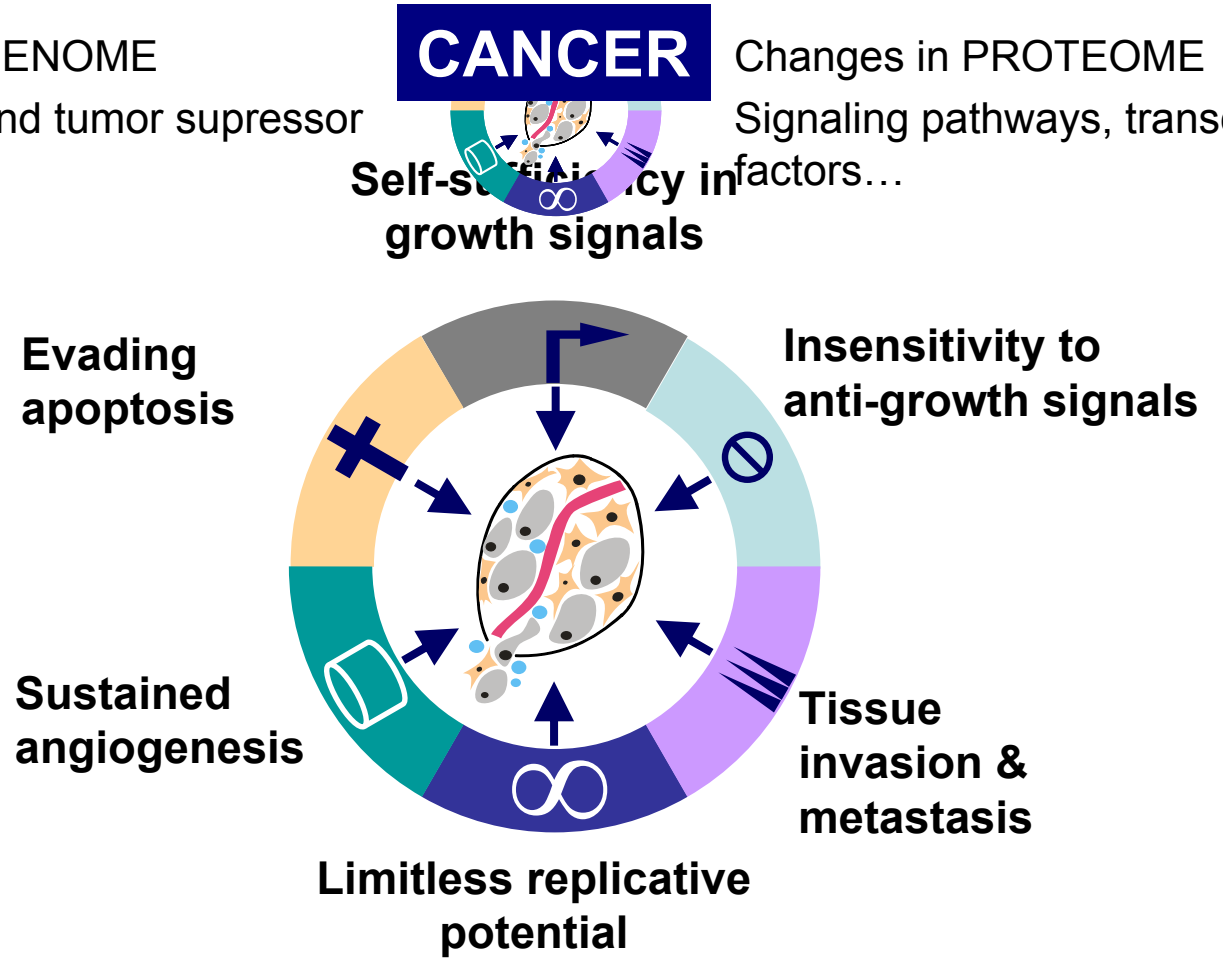
CANCER



Accelerated, disordered and decontrolled proliferation of tissue cells that invades, moves and destroys as well as in a local level as in distance, other health tissues of the organism.

Changes in GENOME

Oncogenes and tumor supressor genes…



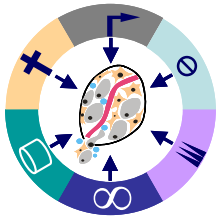
Changes in PROTEOME

Signaling pathways, transcription factors…

Modified of Hanahan and Weinberg, *Cell* 2000

Changes in GENOME

Oncogenes and tumor supressor genes…



Changes in PROTEOME

Signaling pathways, transcription factors…

Alterations in METABOLISM

TUMOR METABOLOME

Are there common alterations in different processes associated to malignant tumor progression?

Is metabolic network reorganization a consequence or a cause of tumor progression?

Could metabolism be used as therapeutic target against tumor progression?

TUMOR METABOLOME

High glucose consumption and lactate production. Warburg effect

Activation of biosynthetic pathways

Expression of isoforms, changes in enzymatic activities and affinities

Vizan et al. Metabolomics 4, 1-12 2008 (Review)

CANCER AS A METABOLIC ALTERATION

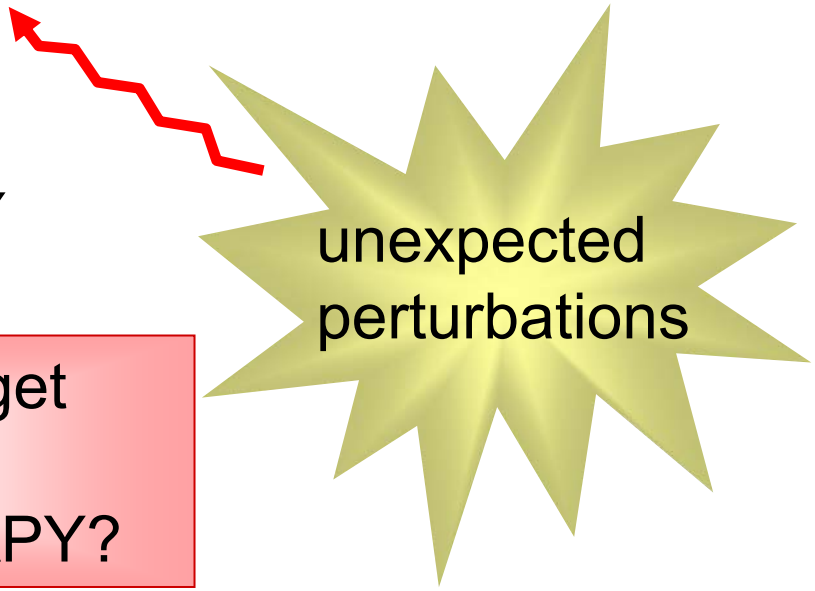
Cancer cells are perfect systems
to invade and parasite other tissues

Robust metabolic profile

⇓

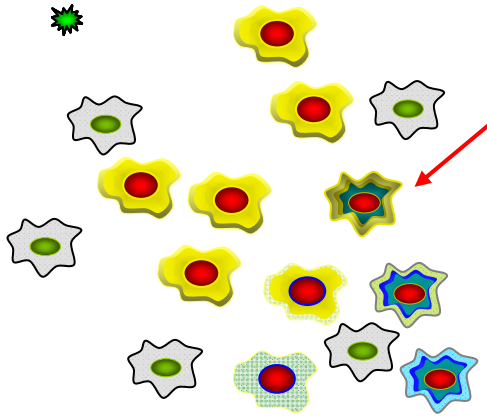
FRAGILITY

Exploitable Target
for
CANCER THERAPY?



MULTIPLE HIT CANCER THERAPY AT METABOLIC LEVEL

Specific molecular target

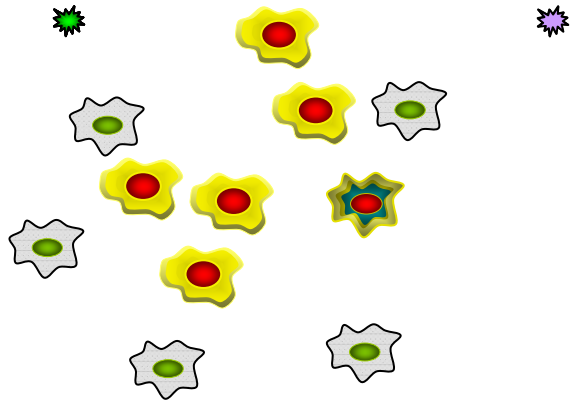


- Heterogeneous populations

↓

Low effective strategy

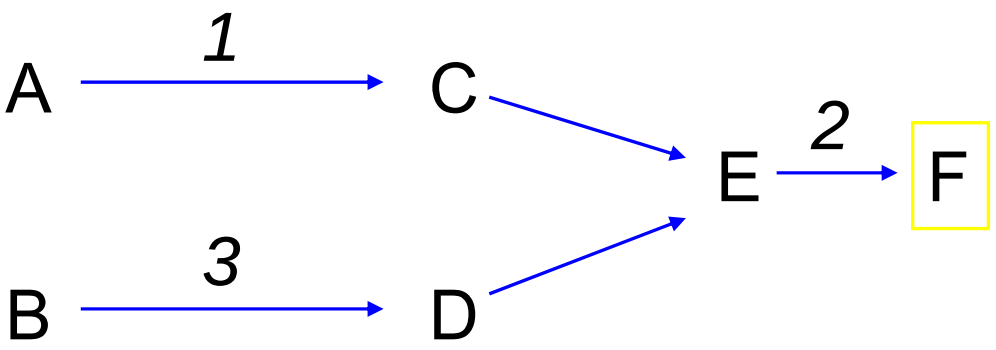
MULTIPLE HIT CANCER THERAPY AT METABOLIC LEVEL



Drug combination

↓

High effective strategy



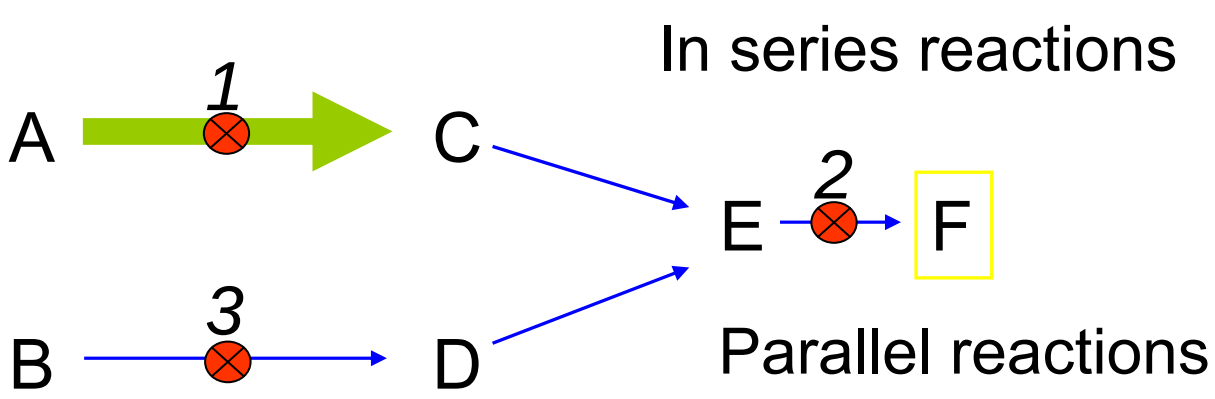
In series reactions

Parallel reactions

MULTIPLE HIT CANCER THERAPY AT METABOLIC LEVEL

- Tumor metabolism robustness counteracts single hits
- Multiple hit strategies can avoid bypass of single inhibitions
- Tumor metabolism response to multiple inhibition is unpredictable

Rational design of new therapeutical combinations is necessary



- Synergy
- Addition
- Antagonism

MULTIPLE HIT CANCER THERAPY AT METABOLIC LEVEL

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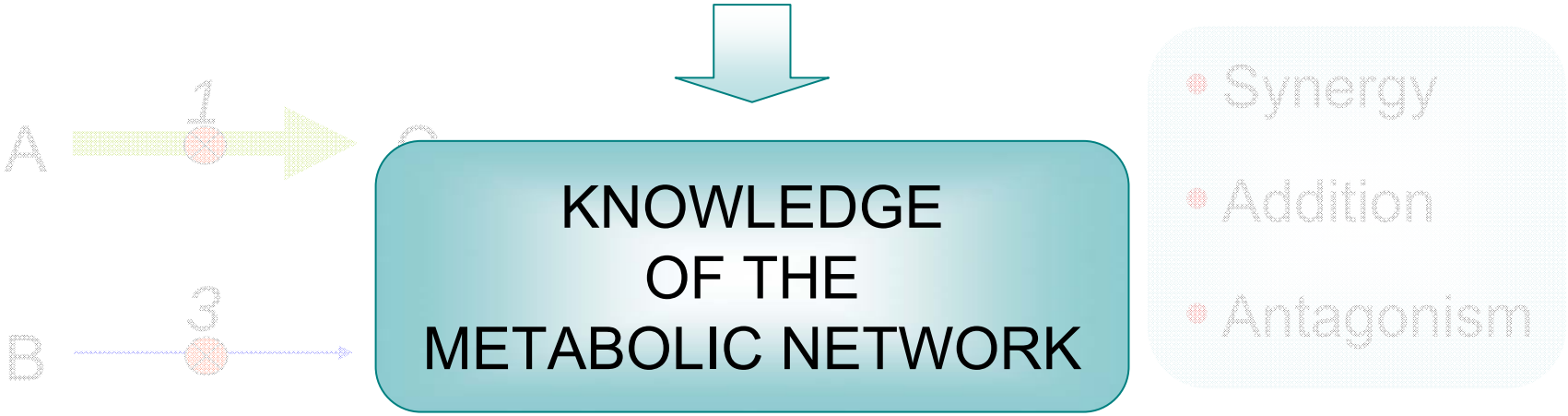
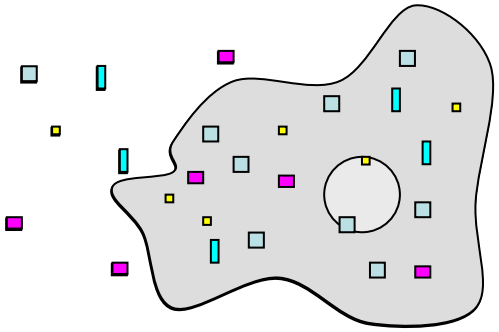
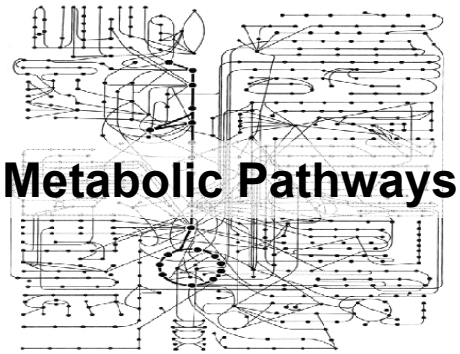


FLUXOMICS FOR THE ANALYSIS OF TUMOR METABOLOME

Metabolomics and Fluxomics are necessary for rational design of new therapeutical combinations



Metabolome



FLUXOME

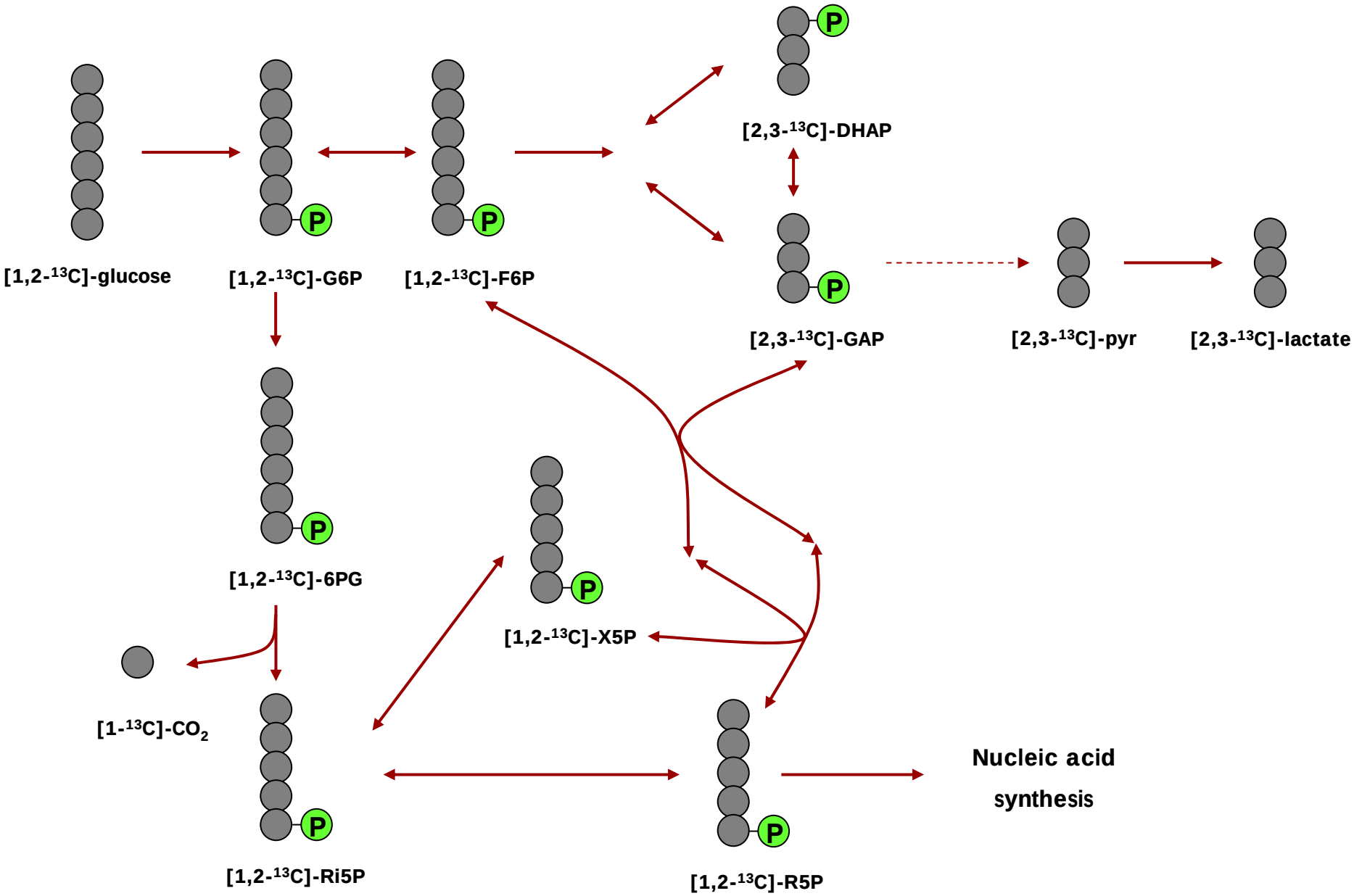
TRACER-BASED METABOLOMICS

- Incubation with stable labeled substrates
- Analysis of isotopomeric distribution
- Analytical platforms: GC/MS, LC/MS, NMR

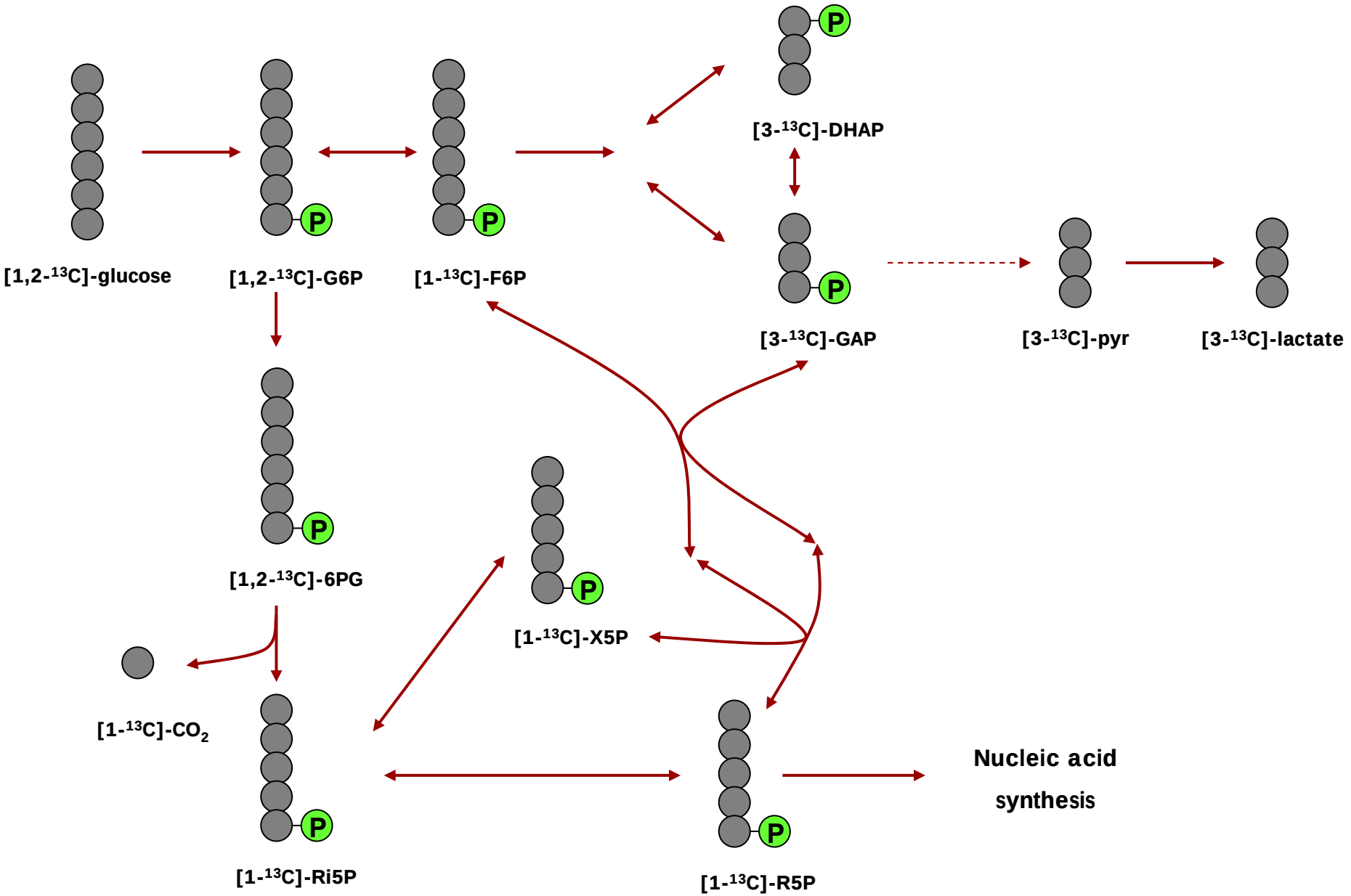
KINETIC MODEL OF DYNAMIC METABOLIC NETWORK

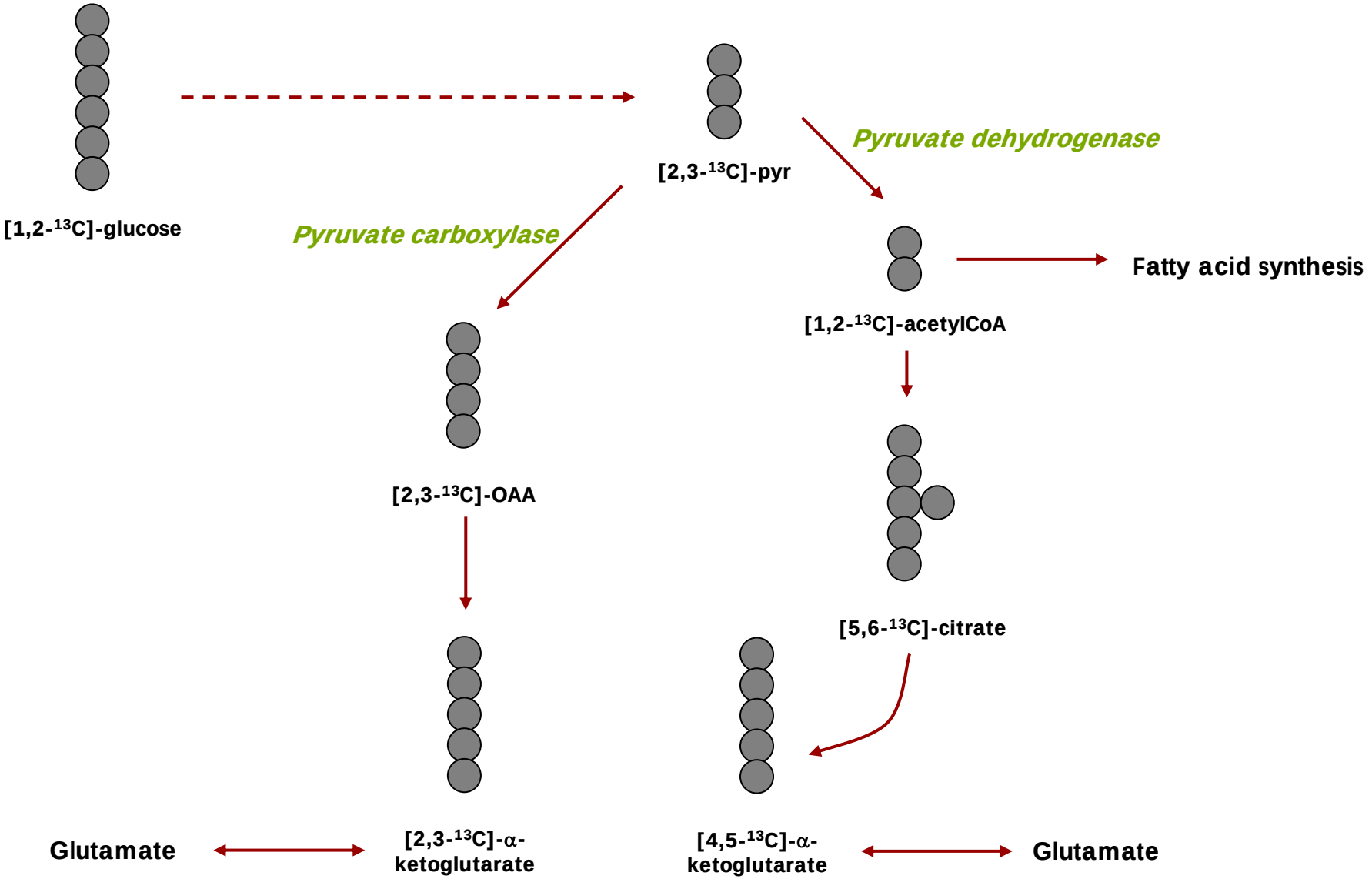
- Analyze and understand the metabolic adaptations supporting cell functions
- Design metabolic interventions in drug development

TRACER-BASED METABOLOMICS AND FLUXOMICS

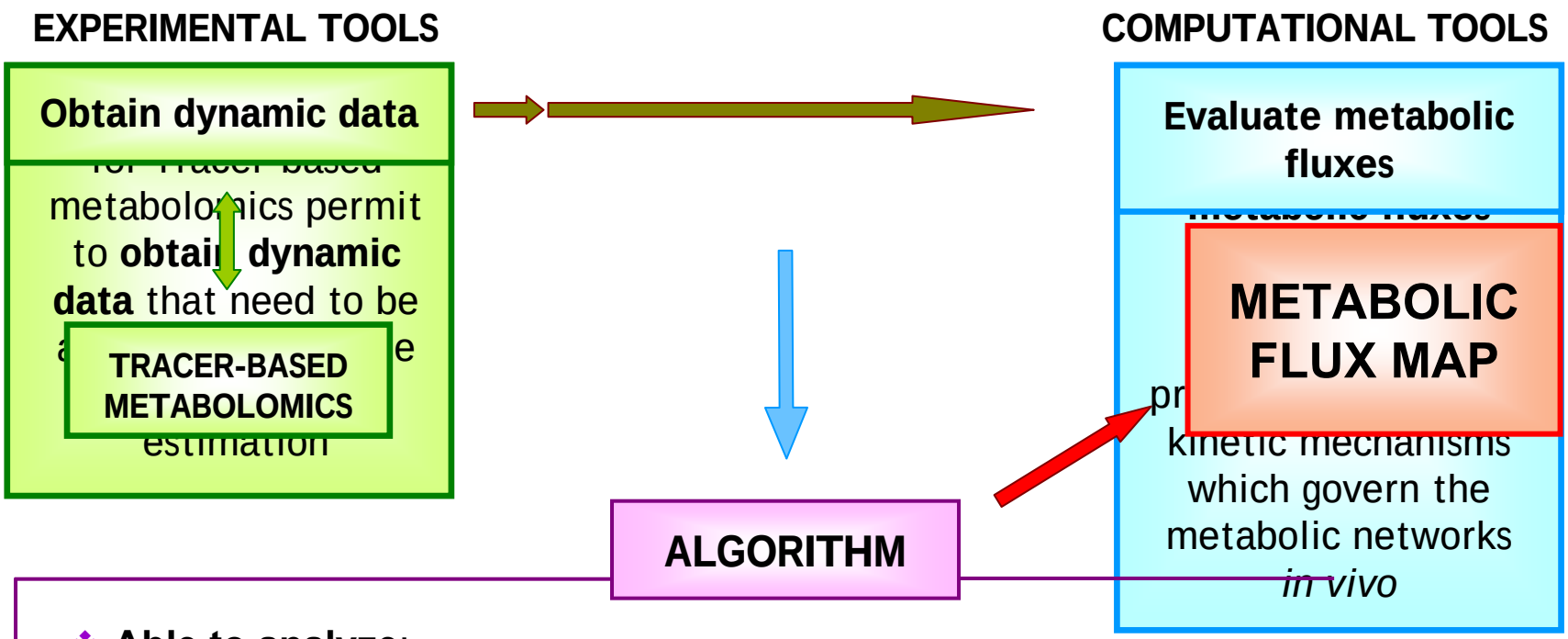


Quantification of flux distribution through metabolic pathways from the analysis using GC/MS of the metabolite isotopomers formed after incubation of cells with [1,2-^{13}C]glucose


[1,2-13C]-glucose
[1,2-13C]-G6P
[1-13C]-F6P
[3-13C]-DHAP
[3-13C]-GAP
[3-13C]-pyr
[3-13C]-lactate
[1,2-13C]-6PG
[1-13C]-X5P
[1-13C]-CO2
[1-13C]-Ri5P
[1-13C]-R5P
Nucleic acid synthesis

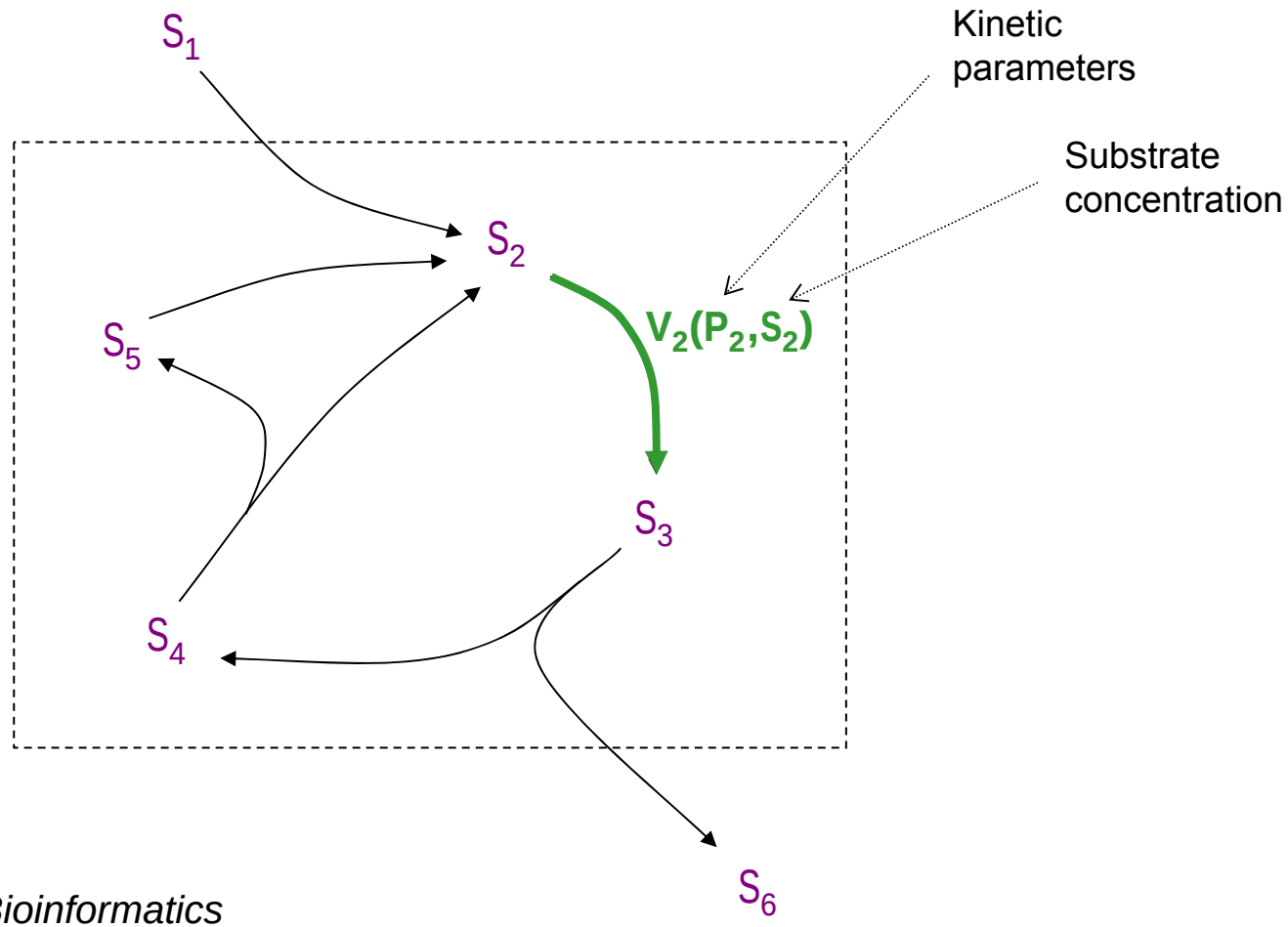

[1,2-13C]-glucose
[2,3-13C]-pyr
Pyruvate dehydrogenase
Pyruvate carboxylase
Fatty acid synthesis
[1,2-13C]-acetylCoA
[2,3-13C]-OAA
[5,6-13C]-citrate
Glutamate
[2,3-13C]-α-ketoglutarate
[4,5-13C]-α-ketoglutarate
Glutamate

AN ALGORITH FOR DYNAMICS ANALYSIS OF THE ISOTOPE TRACER DISTRIBUTION IN METABOLITES



- **Able to analyze**:
  - the isotopic isomer distributions obtained for isotopic non-steady state
  - the non-steady state of metabolism
- **By using** the enzyme kinetic information and the *in vivo* metabolomic data
- **Useful for** applications in Pharmacology, various areas of Biomedicine, and Biotechnology

AN ALGORITH FOR DYNAMICS ANALYSIS OF THE ISOTOPE TRACER DISTRIBUTION IN METABOLITES

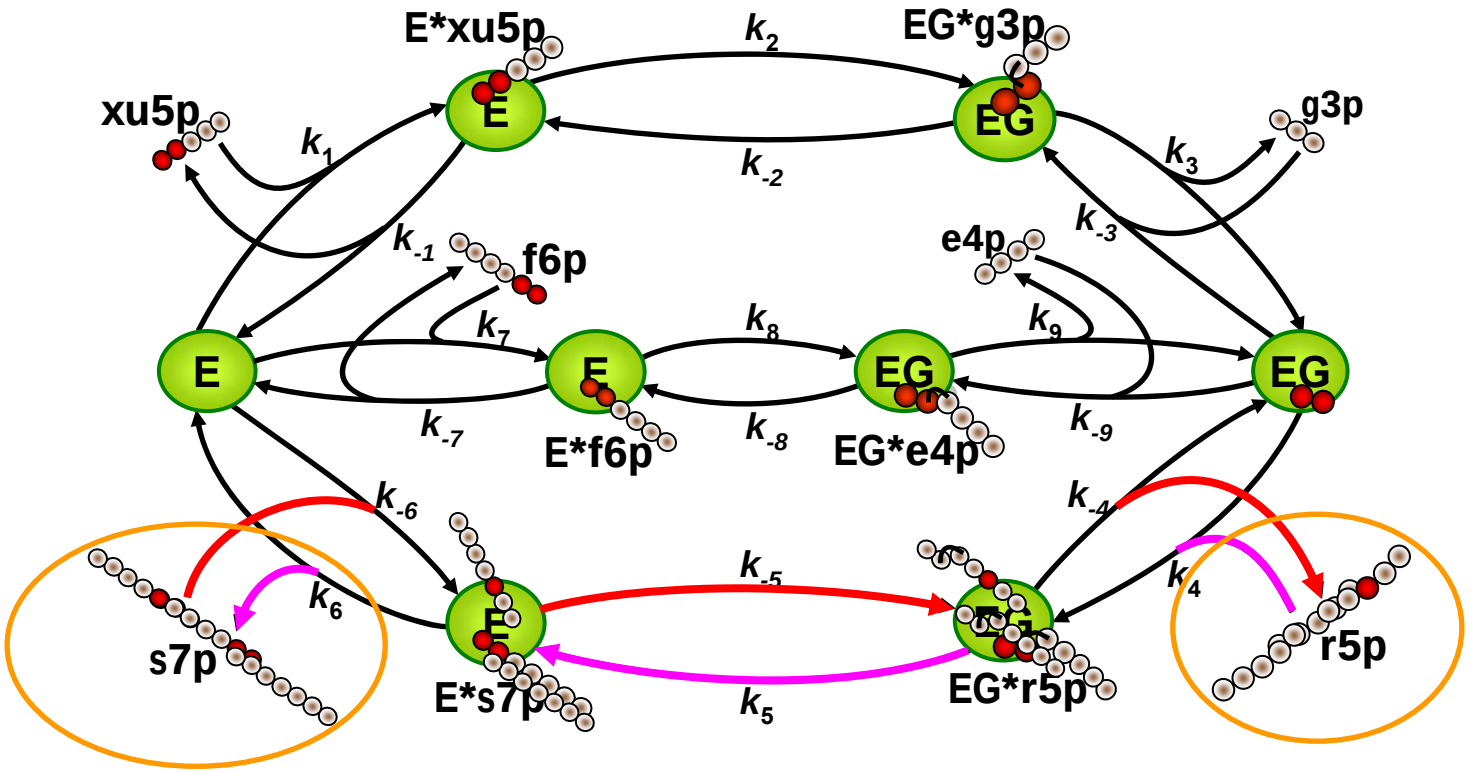


Selivanov *et al*, 2004 *Bioinformatics*
Selivanov *et al*, 2005 *Bioinformatics*
Selivanov *et al*, 2006 *Bioinformatics*

ISOTOPOMER ANALYSIS AND KINETIC MODELS

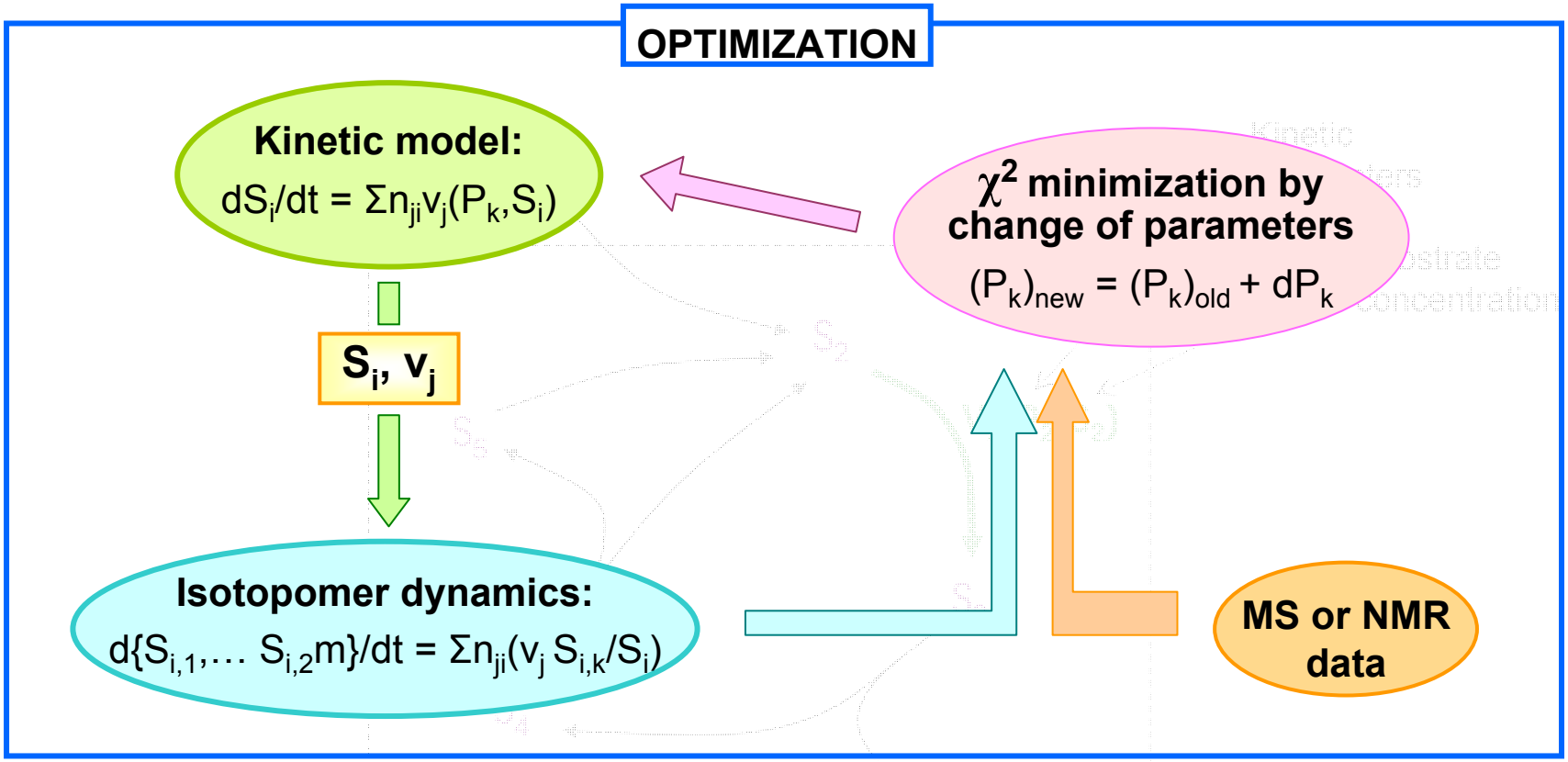
Kinetic models need to take into account the “invisible reactions” and enzymes catalyzing multiple reactions.

TRANSKETOLASE needs elemental step kinetic description in multiple chemical reactions (steps in the network not independent)



Invisible reactions: they change isotopomer distribution but not chemical balance

AN ALGORITH FOR DYNAMICS ANALYSIS OF THE ISOTOPE TRACER DISTRIBUTION IN METABOLITES



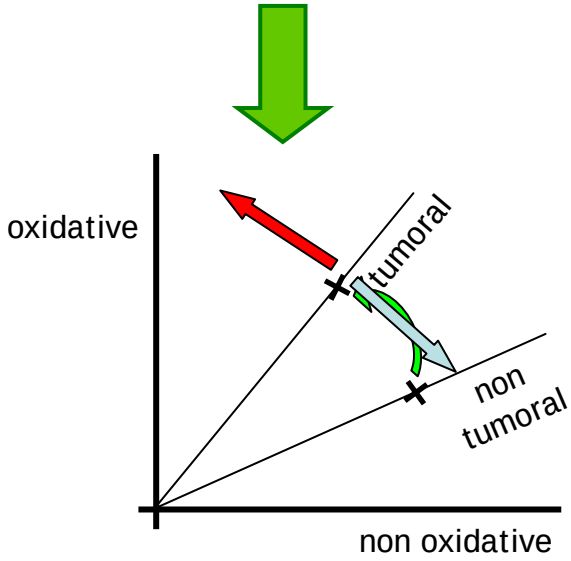
STATISTICAL ANALYSIS $V_j \pm sd,\ P_k \pm sd,\ S_i \pm sd$ → **METABOLIC FLUX MAP**

Selivanov *et al*, 2006 *Bioinformatics*

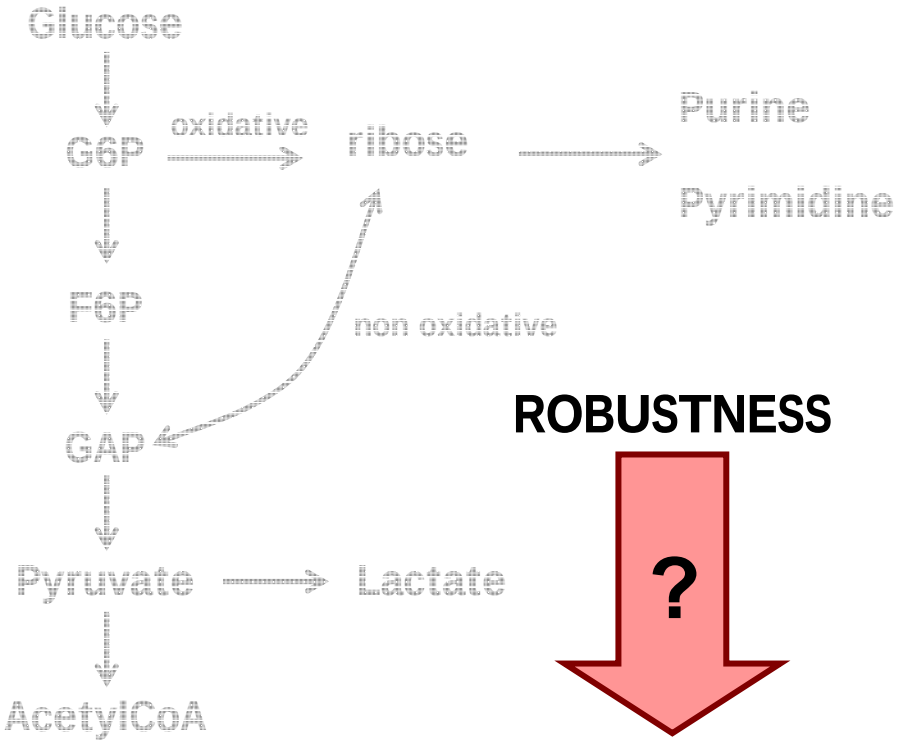
DEVELOPING DRUGS FOR NEW THERAPEUTICAL STRATEGIES AIMING TO DISRUPT METABOLIC ROBUSTNESS OF CANCER CELLS

Exploiting tumoral metabolic adaptation of adenocarcinoma cancer cells for new antitumoral therapies

Pentose-phosphate pathways enhanced

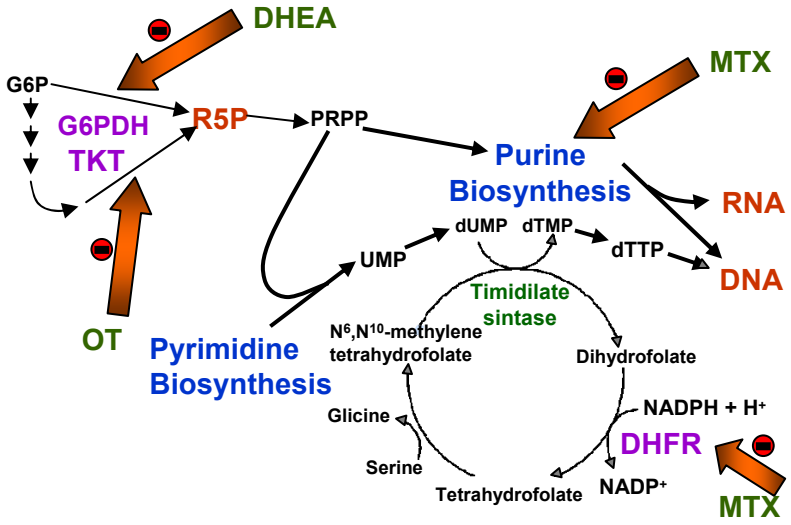


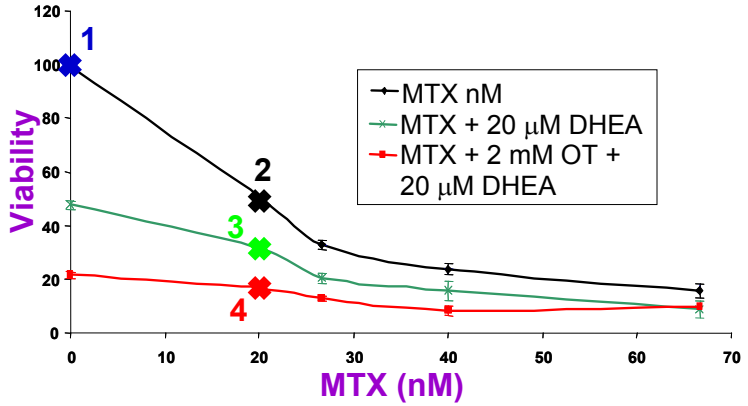
Phase Plane Analysis

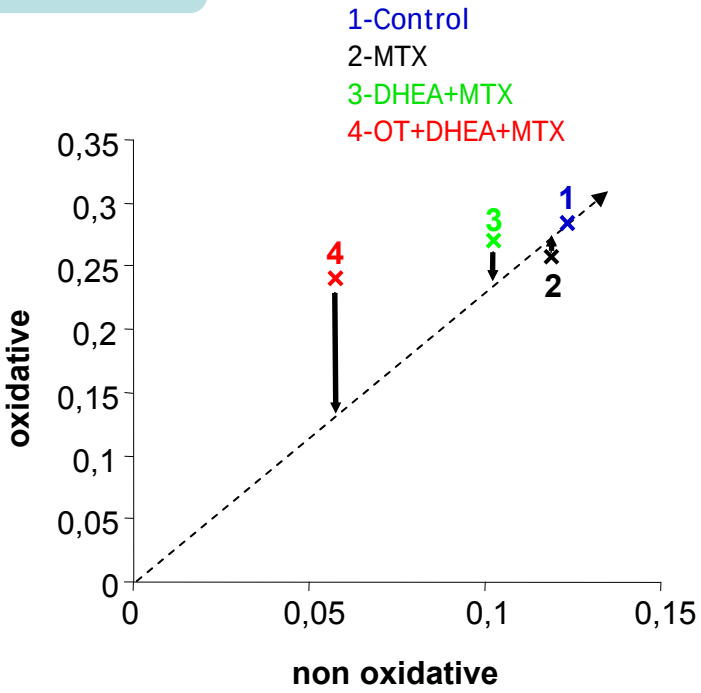


DEVELOPING DRUGS FOR NEW THERAPEUTICAL STRATEGIES AIMING TO DISRUPT METABOLIC ROBUSTNESS OF CANCER CELLS

Multiple hit target strategy to disrupt this balance







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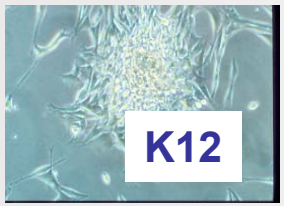
Oxidative/non-oxidative balance is essential to cancer cells and is a possible new target within the cancer metabolic network for novel therapies. Ramos-Montoya *et al,* 2006 *Int J Cancer*

TRANSFORMATION INDUCED BY ONCOGENES: Different single Point Mutations at genetic level can induce different metabolic adaptations

Transfection of NIH-3T3 cells with different single-point mutations in K-*ras* oncogene induces cell transformation



Transfected line	In vitro and in vivo characteristics	Molecular profile
K12 (Codon 12 point mutation)	•**Low apoptotic rate** (resistance to apoptosis and spontaneously formation of spheroids *in vitro*) •**Low mitotic rate** •**High aggressiveness in vivo induced sarcoma**	•**Upregulation of AKT pathway** •**Cyclin B1 downregulation** •**Upregulation of markers for sarcoma aggressiveness** (p53 and *c-myc*)
K13 (Codon 13 point mutation)	•**High apoptotic rate** (apoptotic death when reaching confluence in vitro) •**High mitotic rate** •**Less aggressiveness in vivo induced sarcoma**	•**Downregulation of AKT pathway** •**Cyclin B1 upregulation** •**Downregulation of markers for sarcoma aggressiveness** (p53 and *c-myc*)

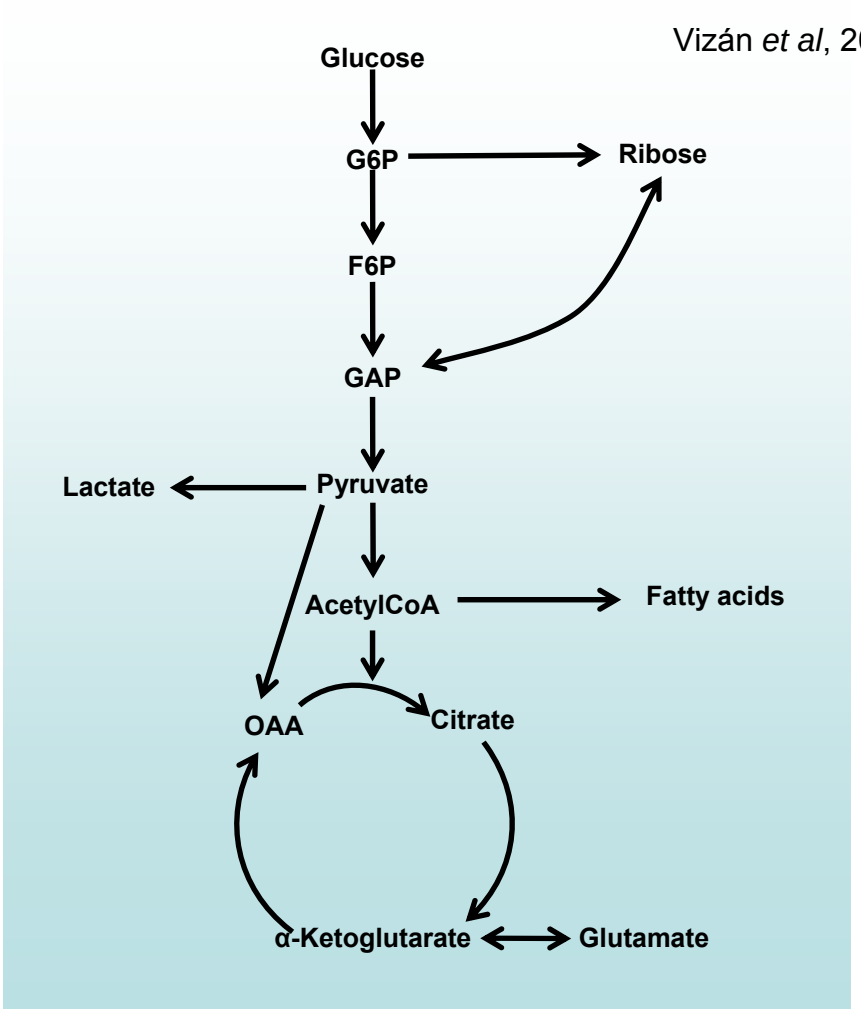
Guerrero *et al*, 2000 *Cancer Res;* Guerrero *et al*, 2002 *FASEB J*

This model is a suitable and elegant way of correlating resistance to apoptosis and tumor aggressiveness with a metabolic pattern, using [1,2-$^{13}C_2$]-glucose tracing

TRANSFORMATION INDUCED BY ONCOGENES: Different single Point Mutations at genetic level can induce different metabolic adaptations

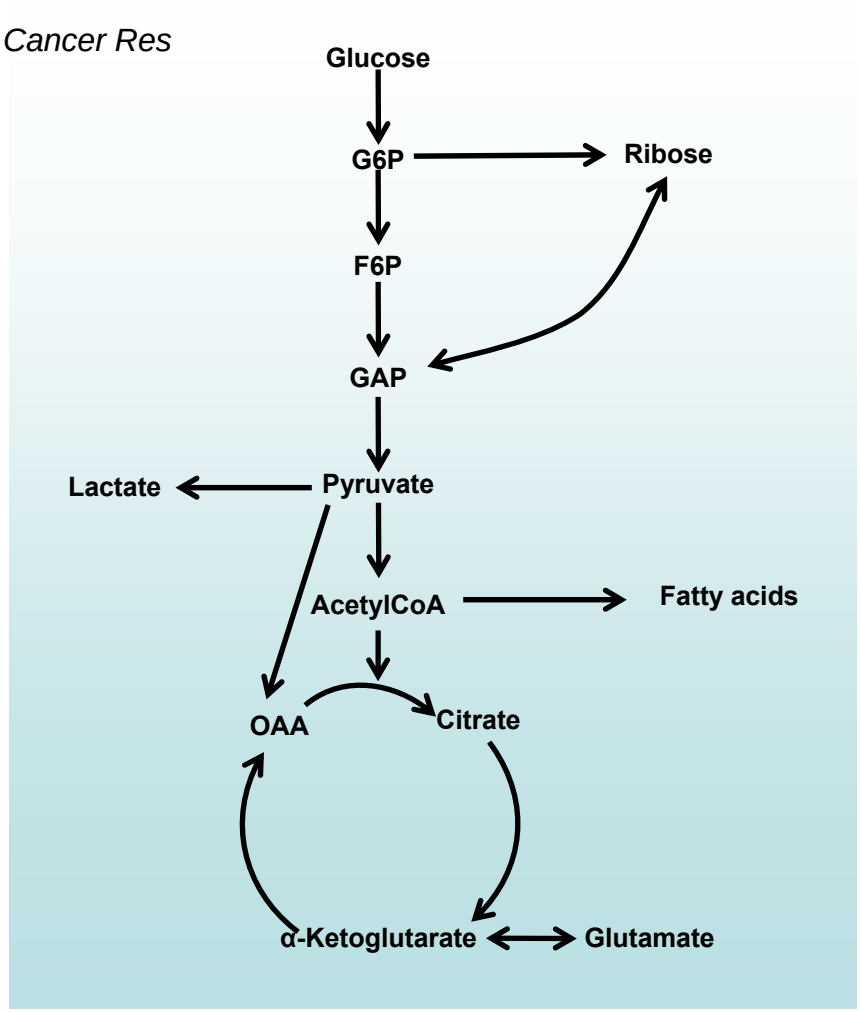
Vizán *et al*, 2005 *Cancer Res*

Metabolic profile of K12 cells



Aggressiveness and apoptotic resistance correlates with high glycolytic rate and decreased mitochondrial metabolism

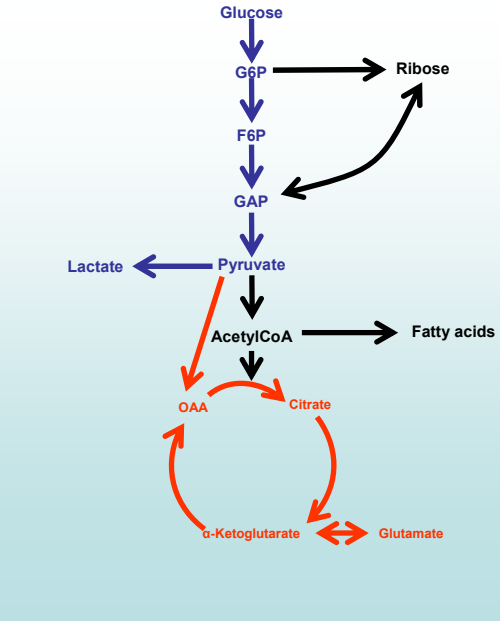
Metabolic profile of K13 cells

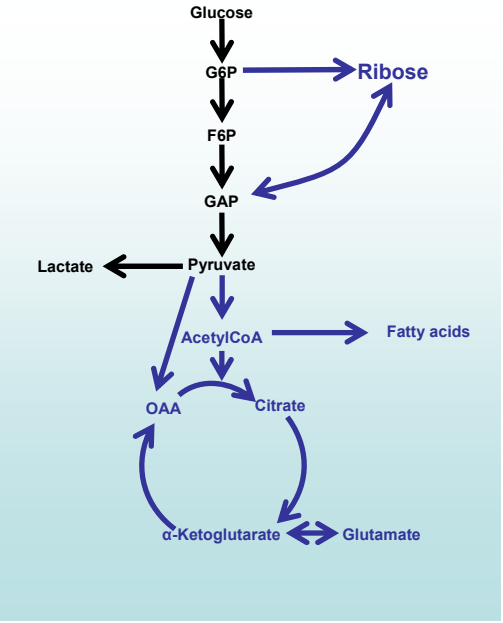


Biosynthetic pathways are activated because of high mitotic rate, but it implies more apoptotic sensitivity

RATIONAL METABOLIC THERAPY: Different metabolic adaptations in cancer cells require specific metabolic intervention

Metabolic profile of K12 cells





Specificity of phenotypic transformations in K12 and K13 mutants are related not only to altered signalling pathways but also to distinct alterations in metabolic profiles.

These metabolic fingerprints should be exploited for a rational drug therapy depending on K-*ras* point mutation.

EXTENDING RATIONAL METABOLIC THERAPY STRATEGIES TO OTHER MULTIFACTORIAL DISEASES SUCH AS COPD

BIOBRIDGE

Integrative Genomics and Chronic Disease Phenotypes: modelling and simulation tools for clinicians

Objective: To develop a modelling environment, which links clinical characteristics with the redox status of muscle cell

COPD patients have an alteration in muscle metabolism:

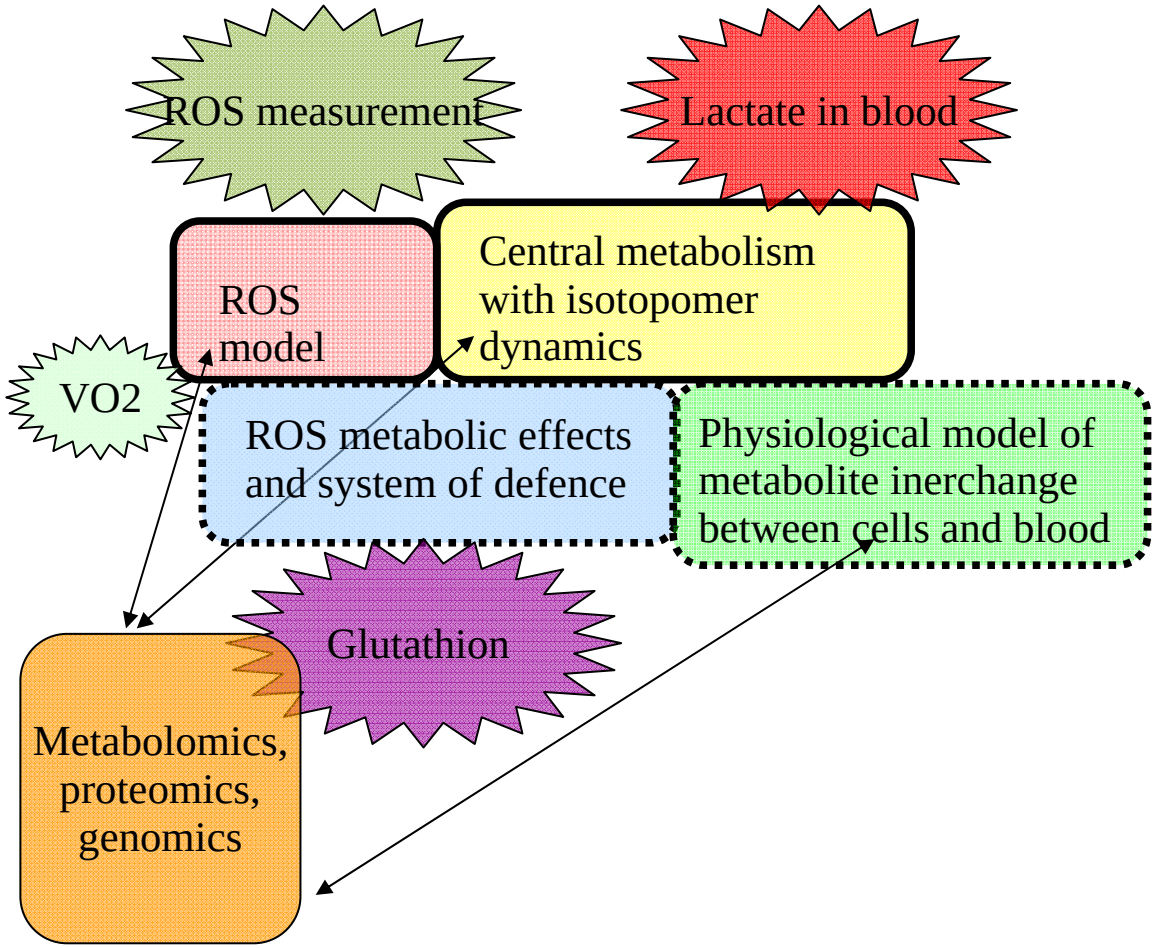
- Mitochondrial dysfunction
- Reduced muscle redox capacity and oxidative stress

Hypothesis:

- Systemic diseases, as COPD, is a consequence of nitroso-redox imbalance
- Important component of redox status is the level of reactive oxygen species (ROS) is produced in mitochondria

The integrated model will give a possibility to analyze simultaneously the data of different levels: from microarrays to lactate content in blood and oxygen consumption for each individual patient.

- Modelling will permit to design specific training programs for COPD patients specially for those with low body mass index.
- Interventions treatments with drugs or nutritional supplements.
- Identification of biomarkers for disease prognosis.



ACKNOWLEDGEMENTS

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Dr. Vitaly Selivanov

Dr. Silvia Marín

Dr. Pedro de Atauri

Dr.Shirley Guzmán

Gema Alcarraz-Vizán

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Former members

Dr. Antonio Ramos-Montoya

Dr. Pedro Vizán

Collaborators

Pneumology service, Institut del Torax, Hospital Clínic-IDIBAPS, University of Barcelona, directed by Dr. Josep Roca

Financial support:

SAF2005-01627 from the Ministerio de Ciencia y Tecnologia of the Spanish Government

BioBridge LSHG-CT-2006-037939 from the European Union (FP6)