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CHARACTERIZING MULTI-DRUG TREATMENTS ON METABOLIC NETWORKS

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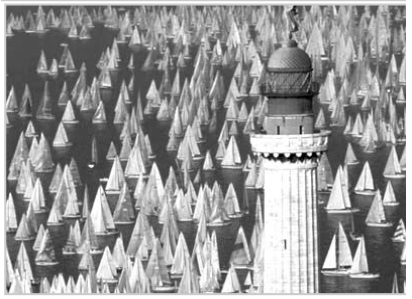

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First and foremost

The real authors of this work:

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SISSA, Trieste
now: ICTP, Trieste

Mattia Zampieri
SISSA, Trieste
now: ETH, Zurich

Outline

- Problem formulation: using drugs to block a desired reaction
- When a drug directly targeting is missing:
  multiple drugs + synergisms
- Methodology behind: bilevel optimization
- Uses of drug synergisms:
  - drug repurposing
  - reduce side effect
- Applications
  - systematic screening for synergisms in metabolic networks
  - human metabolic diseases
  - contrasting two networks: human + cancer cells
- Drug effect on enzyme: from ON/OFF to partial action

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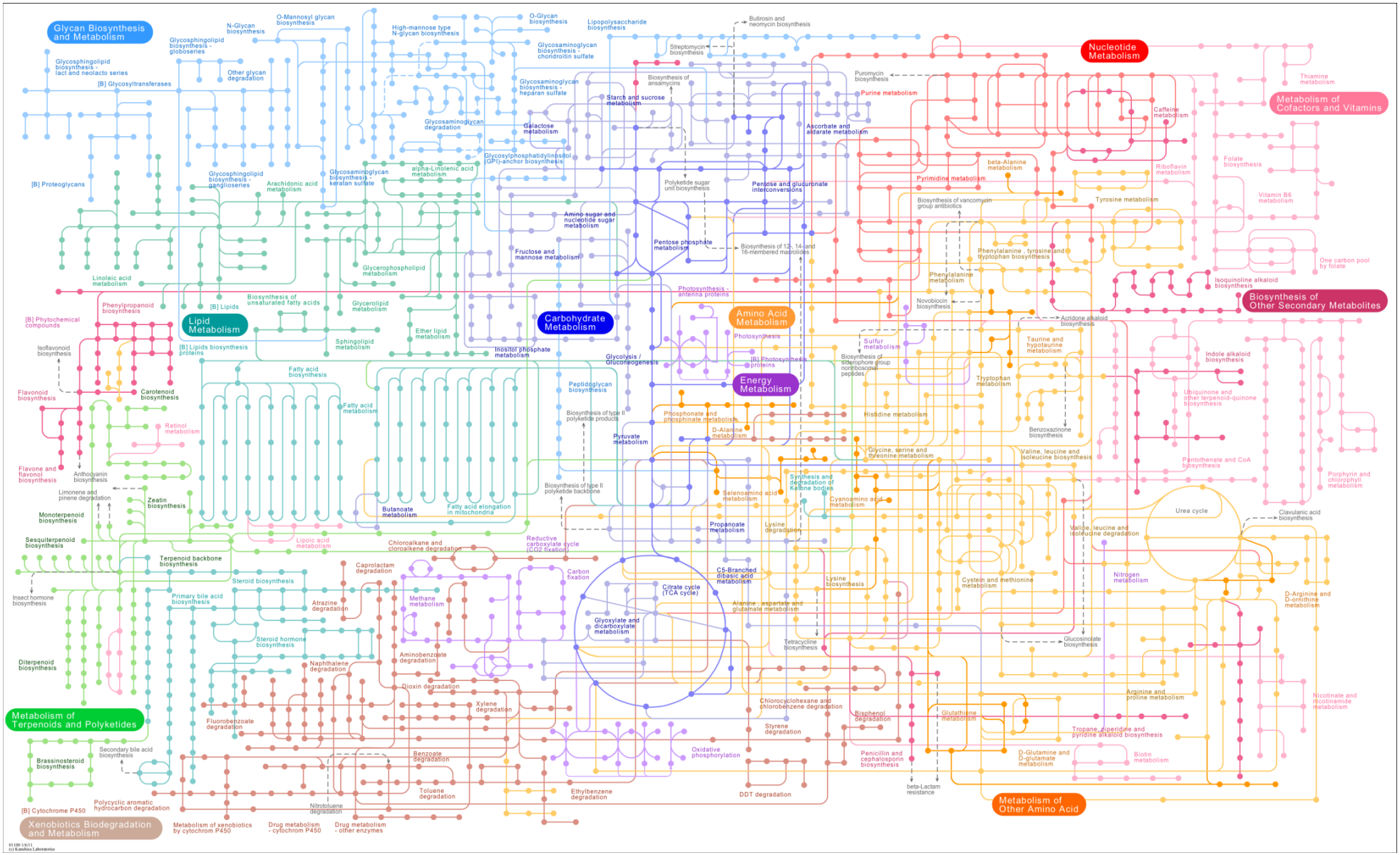
Metabolic Networks

Escherichia Coli: 2383 reactions and 1668 metabolites



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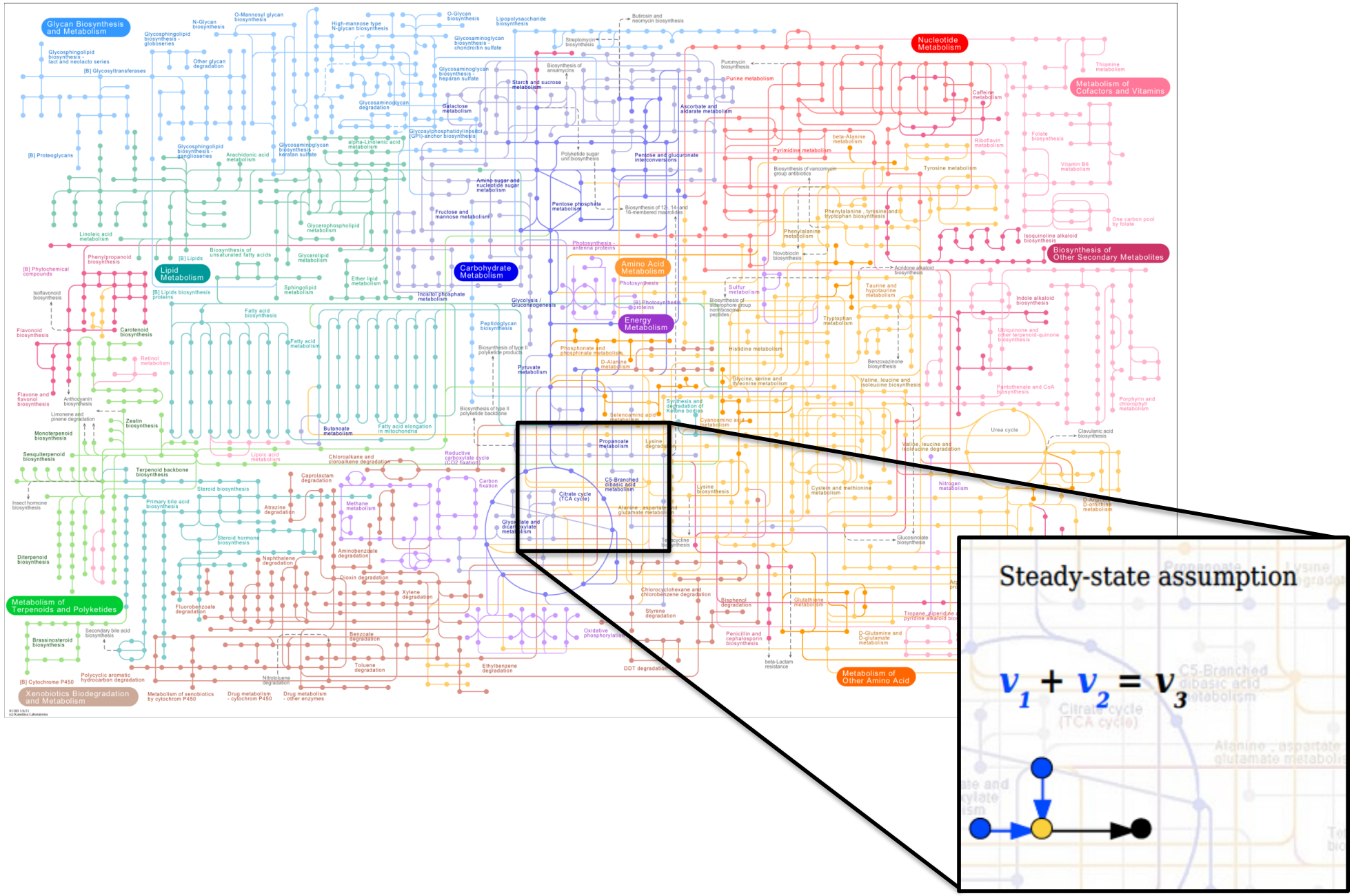
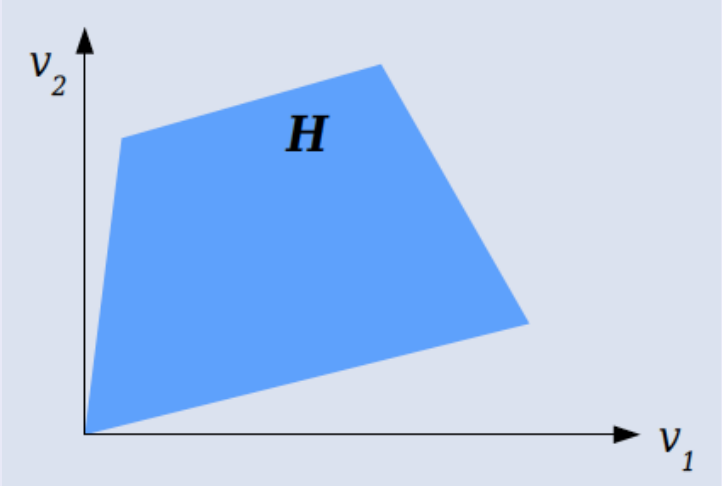
Flux Balance Analysis



Flux Balance Analysis

linear constraints
H = convex polytope of admissible fluxes

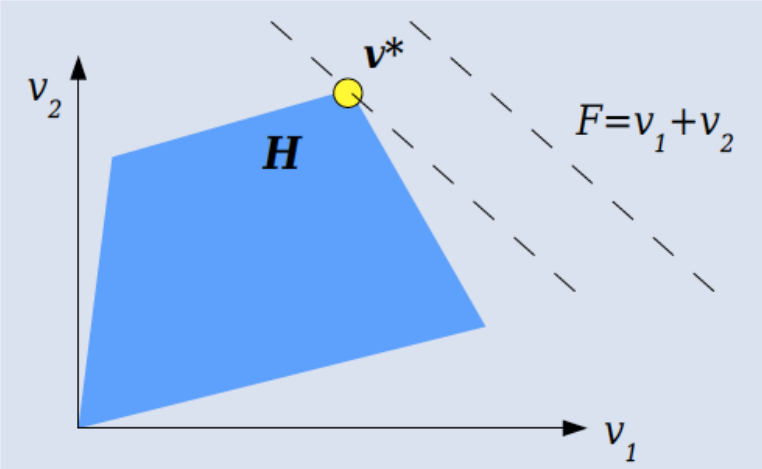
$$S\,v = 0$$
$$0 \le v_i \le U_i$$



optimal flux: LP maximization

$$\max_v F(v) = b^T v$$

$$S\,v = 0$$
$$0 \le v_i \le U_i$$



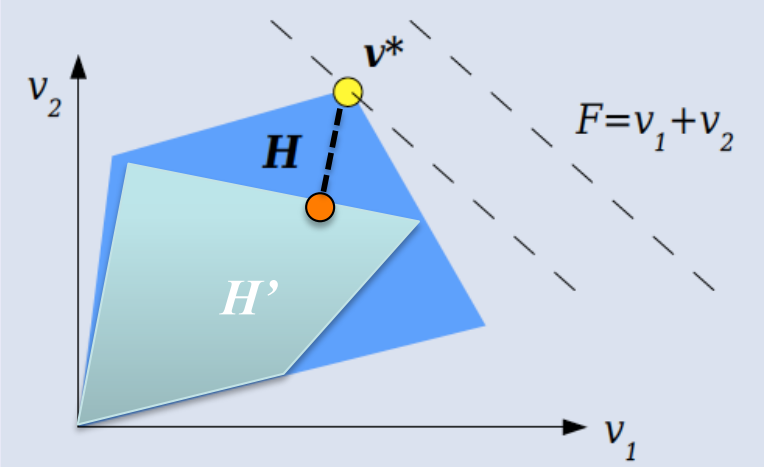
MOMA

perturbed networks: MOMA = Minimization of Metabolic Adjustement

$$\max_v F(v) = b^T v$$

$$S\, v = 0$$

$$0 \leq v_i \leq U_i$$



D. Segre` et. al.
PNAS 2002

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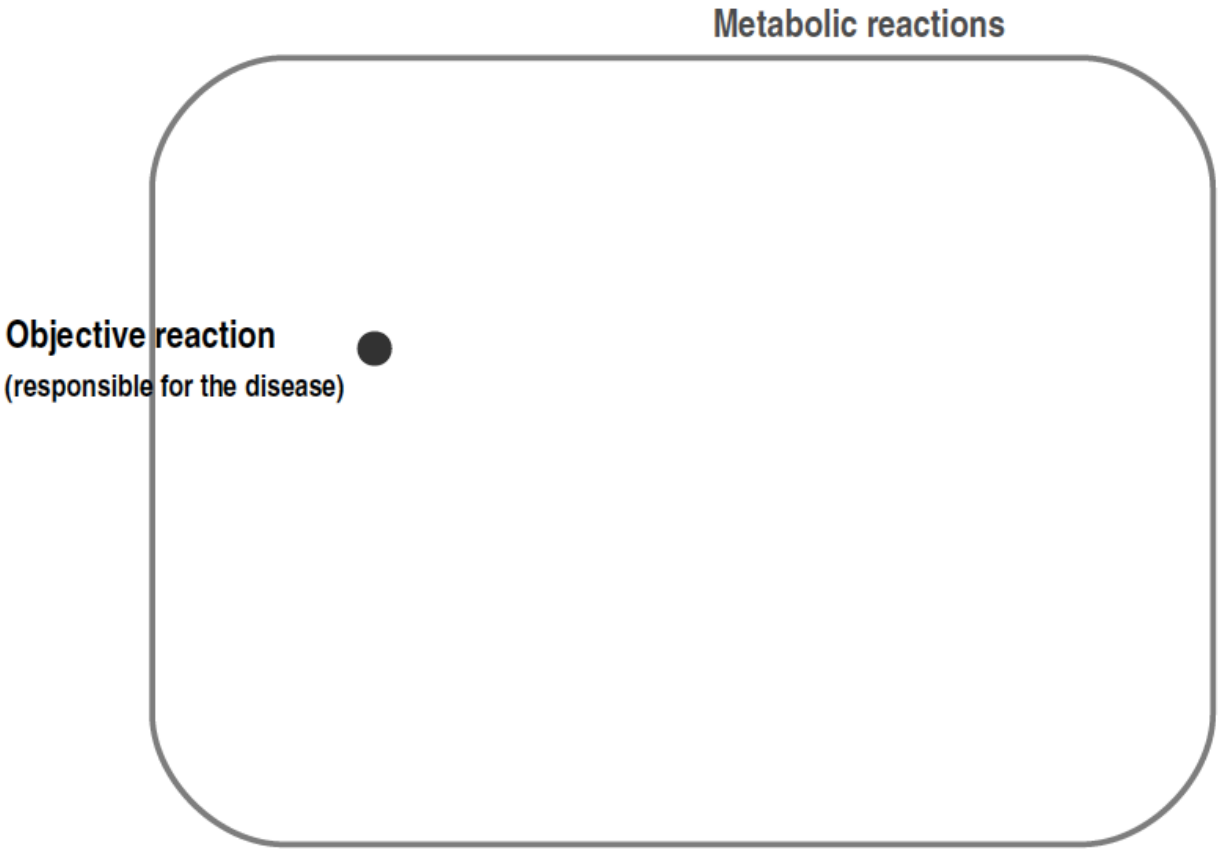
Our Problem

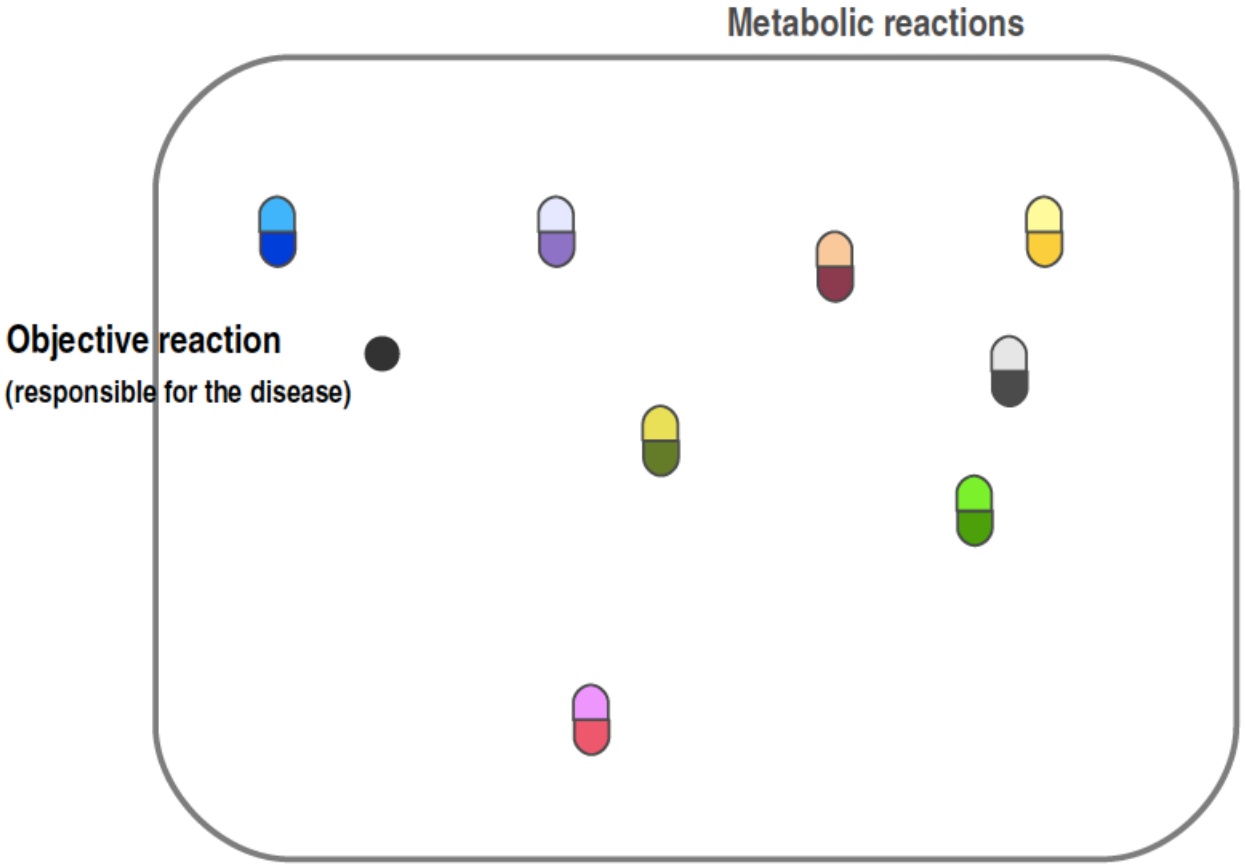
TASK: inhibiting an "objective" reaction, while maintaining viability



Our Problem

TASK: inhibiting an "objective" reaction, while maintaining viability

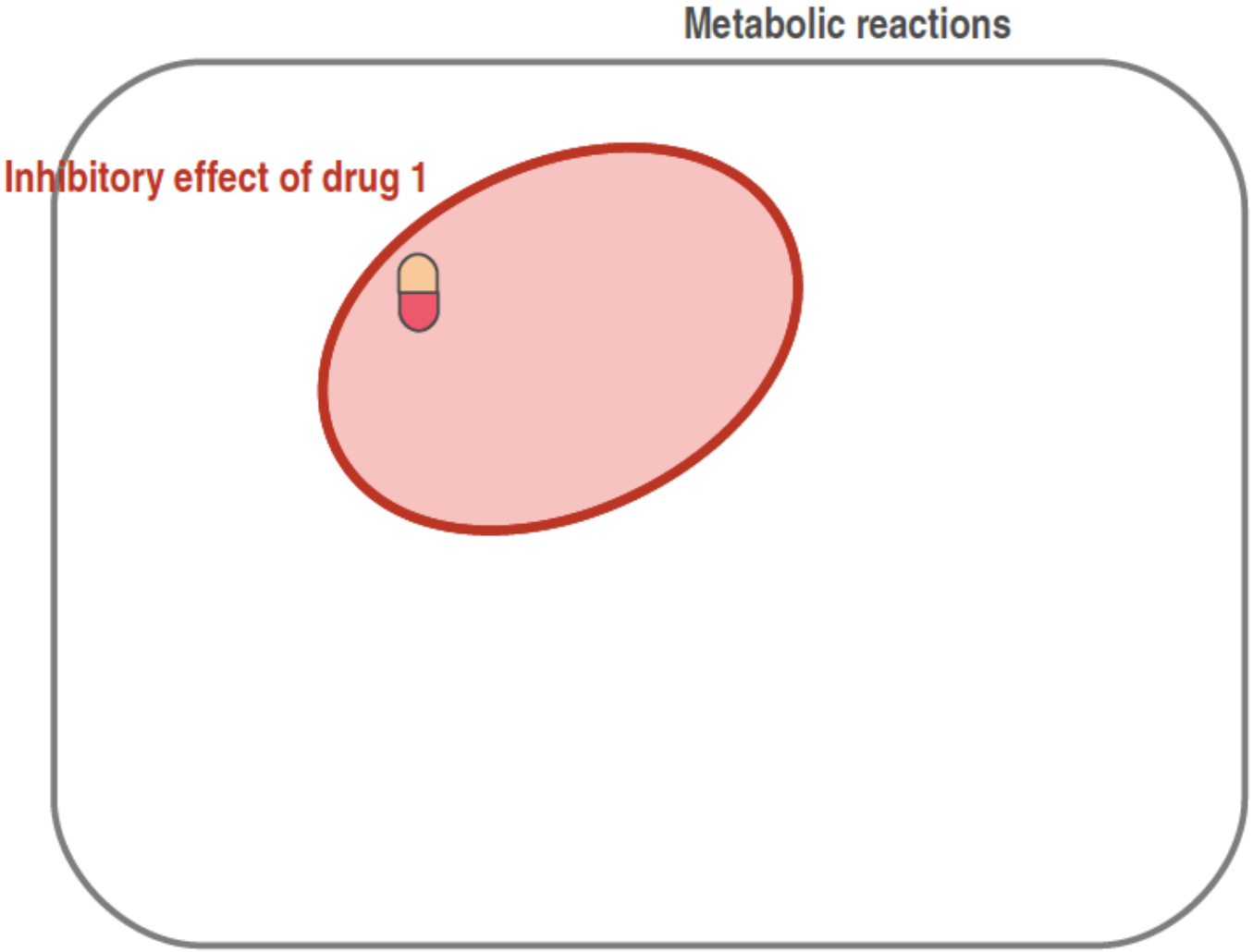
AVAILABLE RESOURCES: drugs targeting enzymes



ASSUMPTIONS:

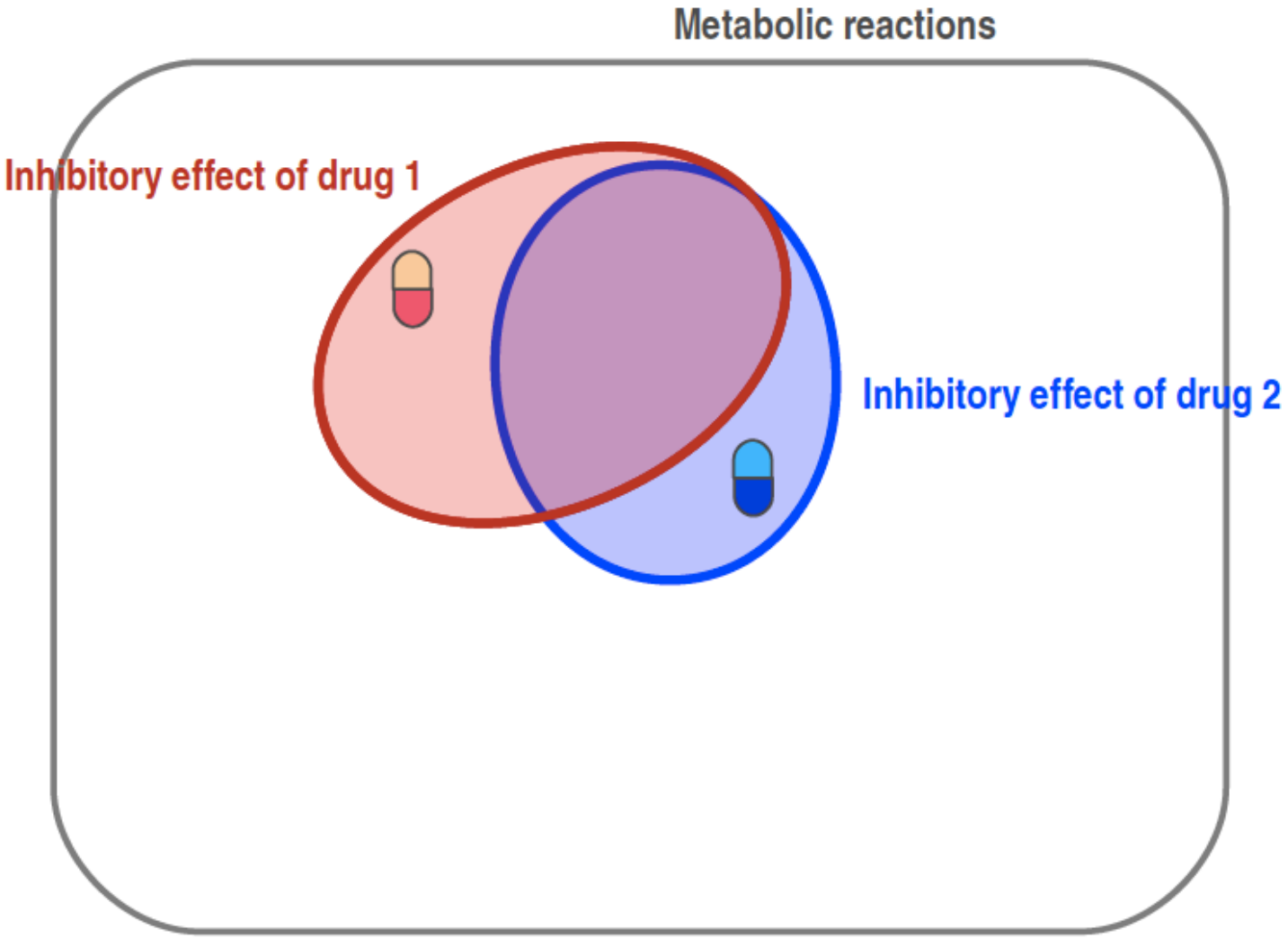
- drug targeting v_{obj} is missing
- drug action is ON/OFF on its targets

Looking for drug synergisms



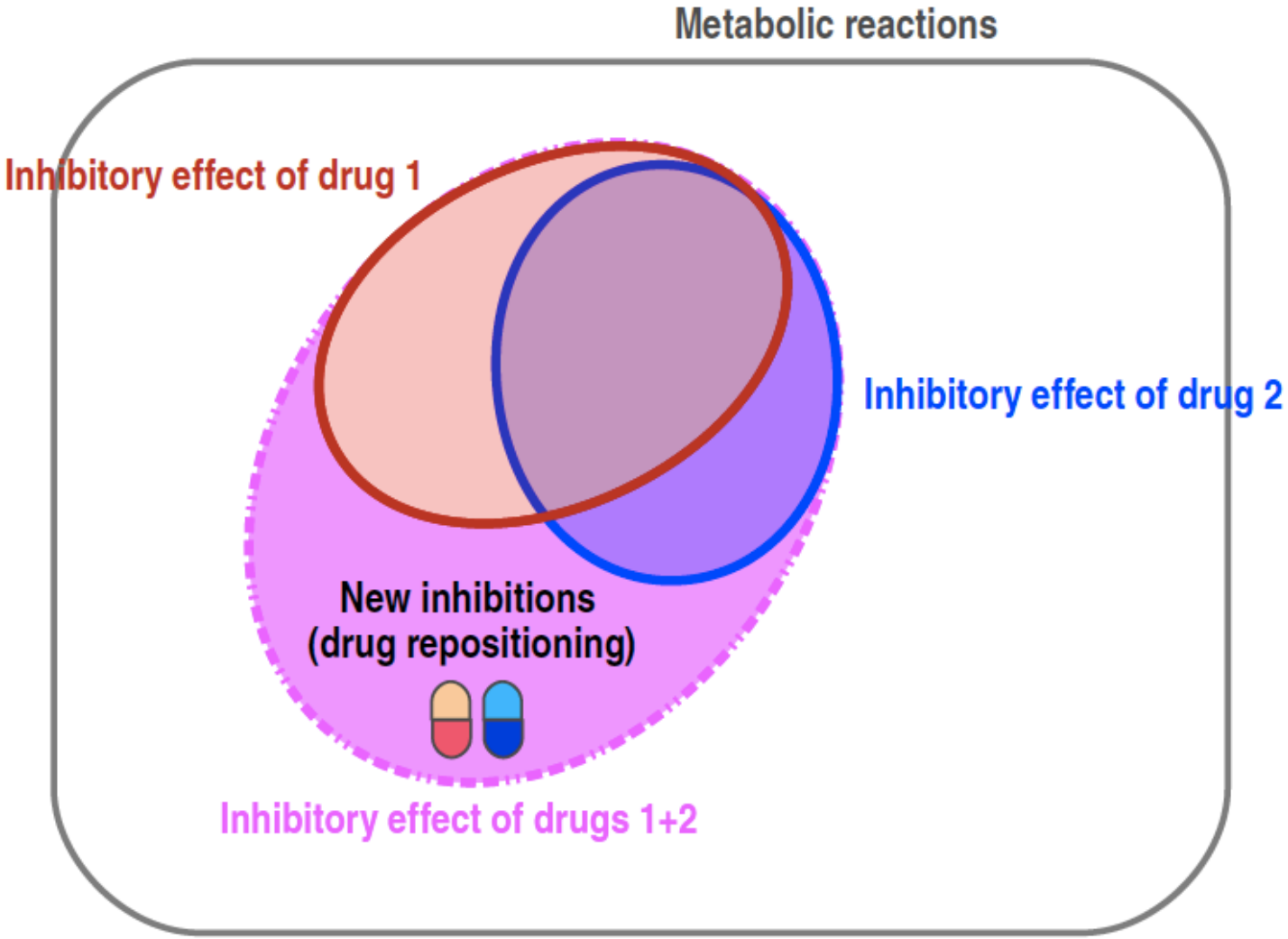
Inhibition = set of reactions affected by the drug

Looking for drug synergisms



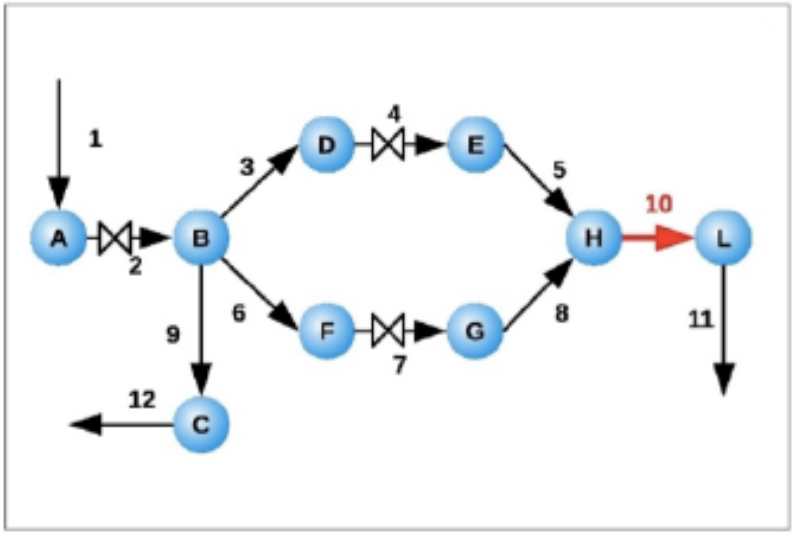
Inhibition = set of reactions affected by the drug

Looking for drug synergisms

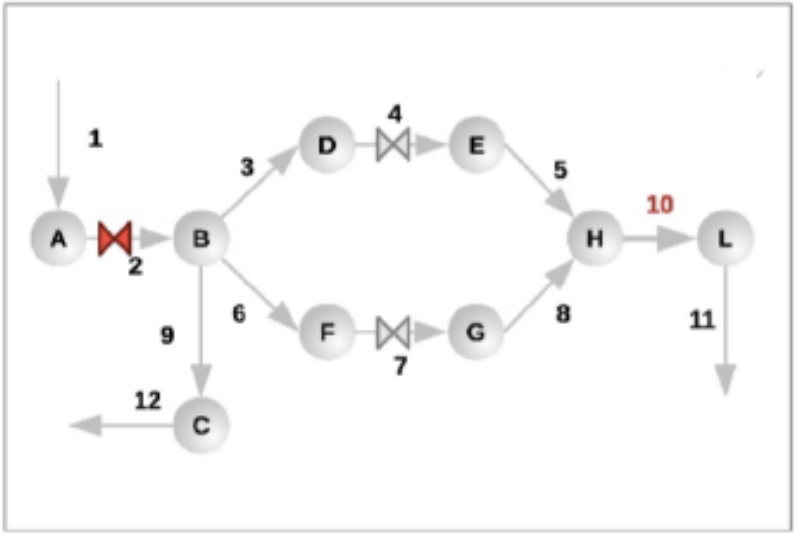


Synergism = set of affected reactions which are "more" than the linear superposition of the effects

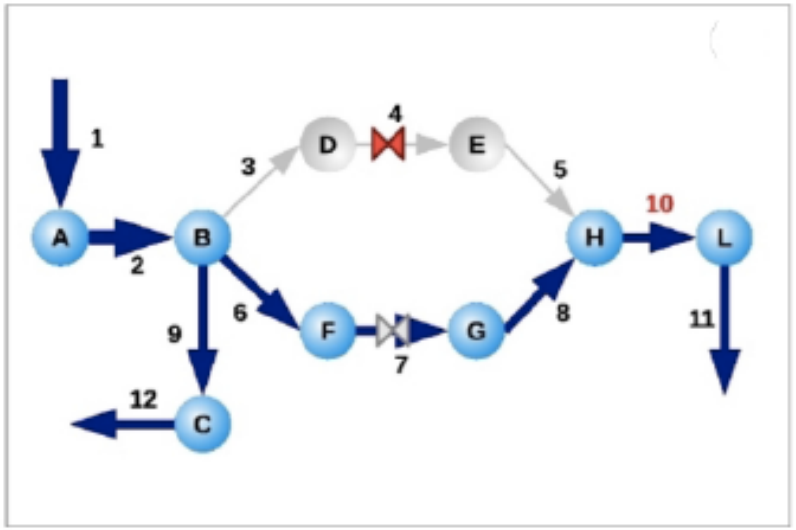
Drug synergisms in FBA



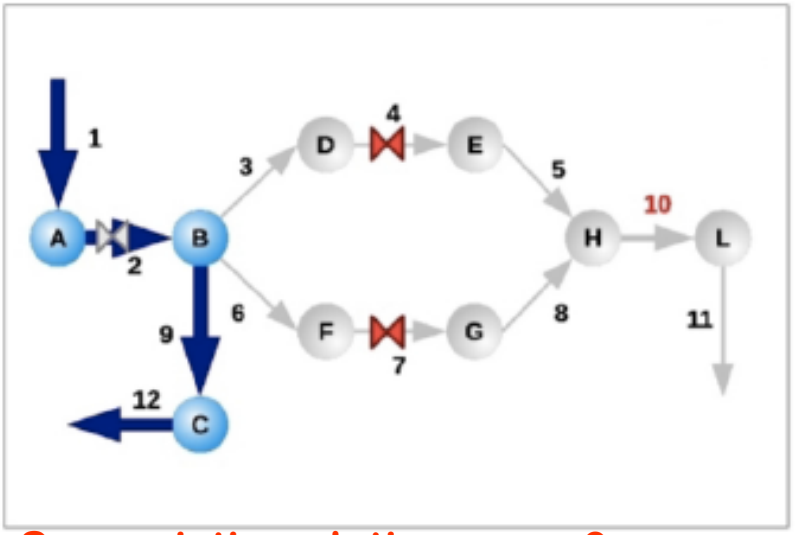
None of the 3 drugs (⋈) inhibits the objective reaction (v_{10})



First solution: $v_{10} = 0$, big side effect



Not a solution: $v_{10} \neq 0$

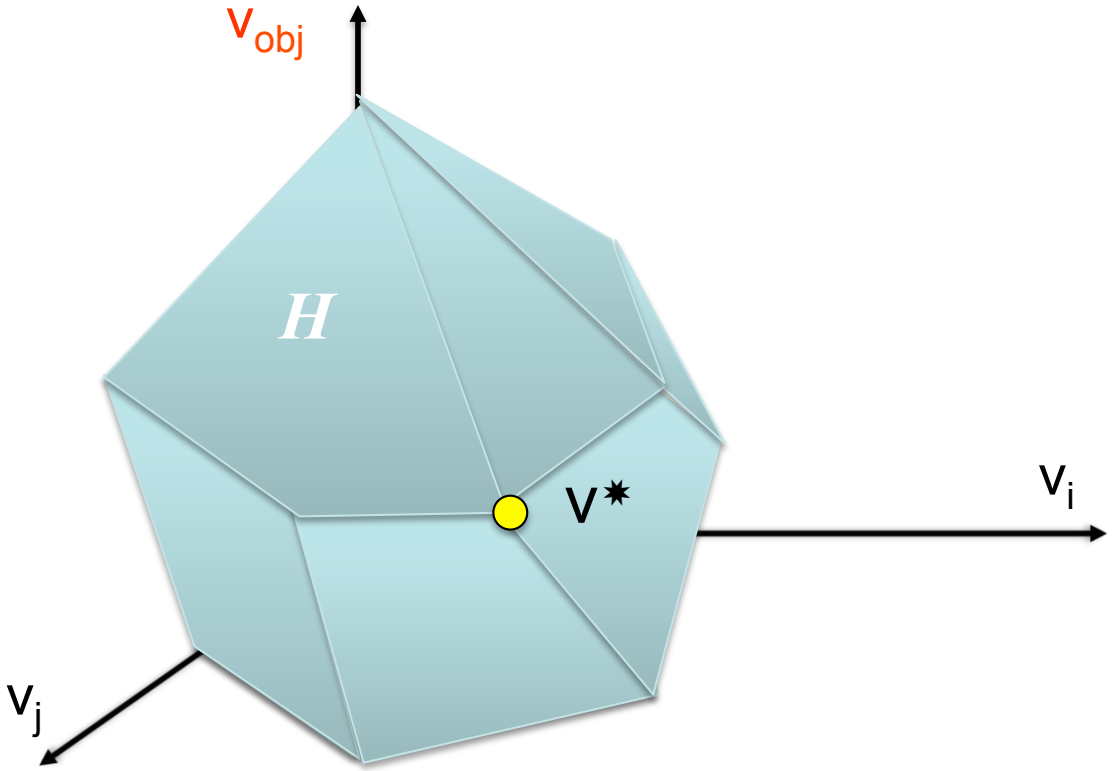


Synergistic solution: $v_{10} = 0$, smaller side effect

Drug action and FBA

starting from “wild type”

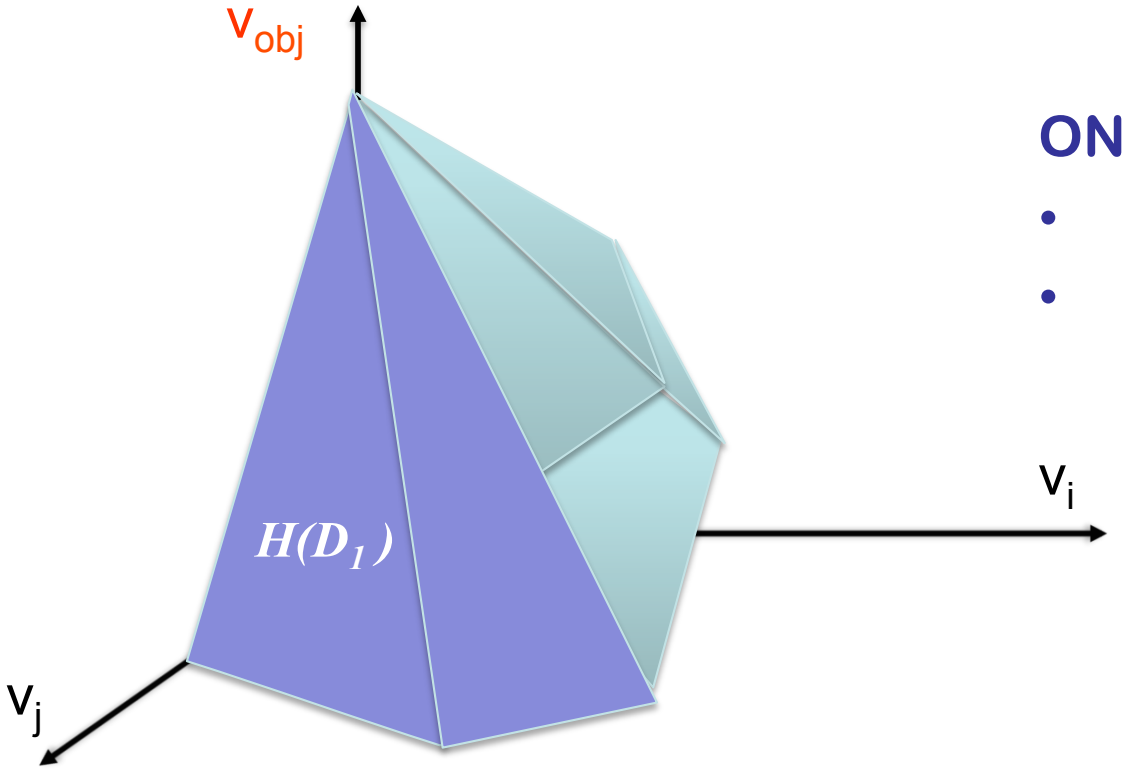
$$\max_v F(v) = b^T v$$
$$S\, v = 0$$



Drug action and FBA

drugs: additional constraints ➔ H reduces to H(D)

$$S\,v = 0$$
$$0 \leq v_i \leq U_i(1 - d_j) \quad d_j \in D$$



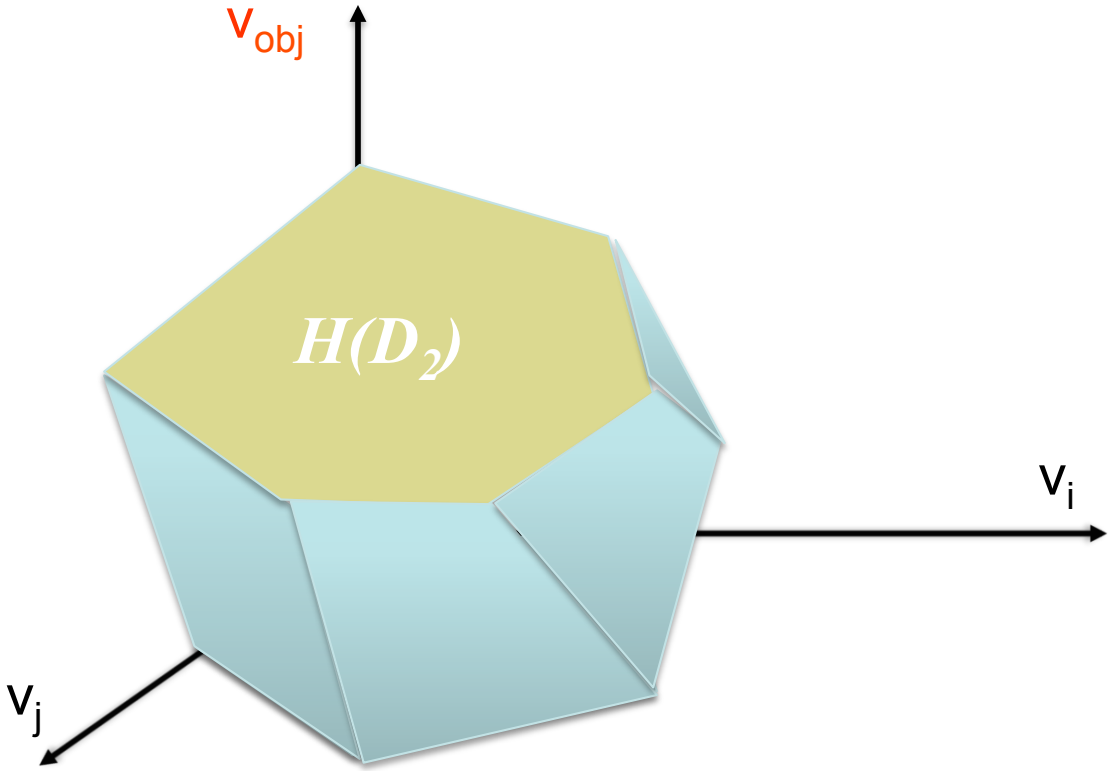
ON/OFF drugs:

- d_j = 0 drug not used
- d_j = 1 drug is used

Drug action and FBA

drugs: additional constraints ➔ H reduces to H(D)

$$S\ v = 0$$
$$0 \leq v_i \leq U_i(1 - d_j) \quad d_j \in D$$



Drug action and FBA

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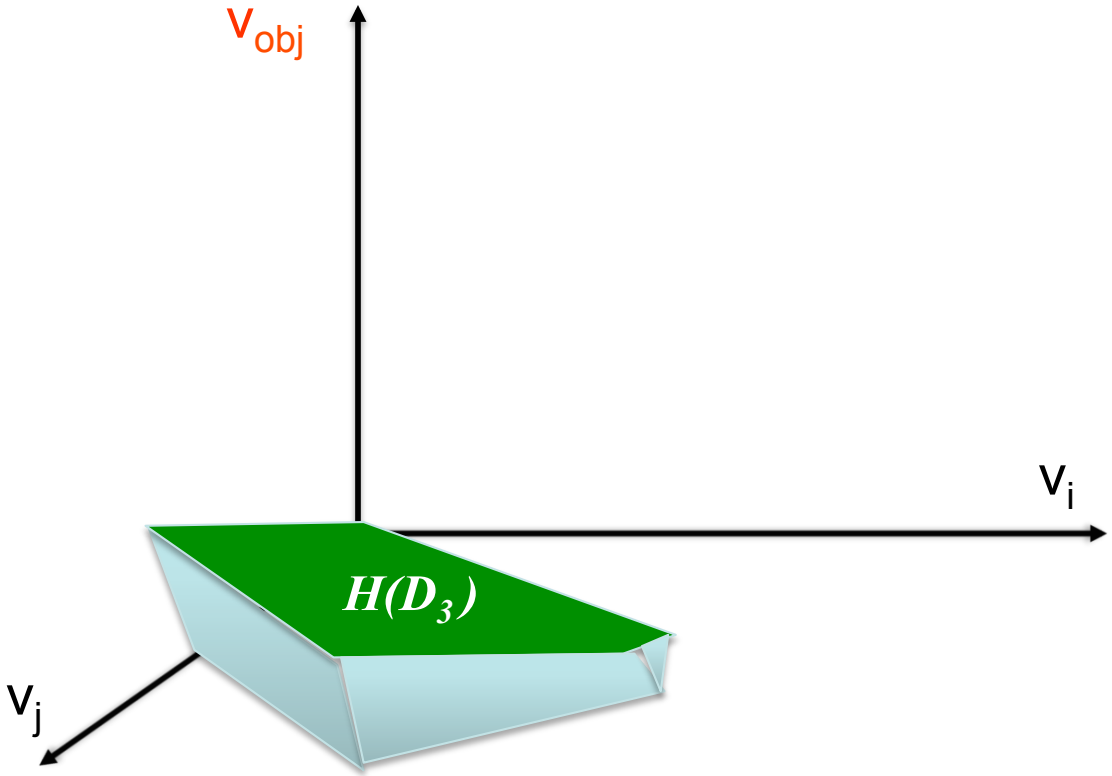


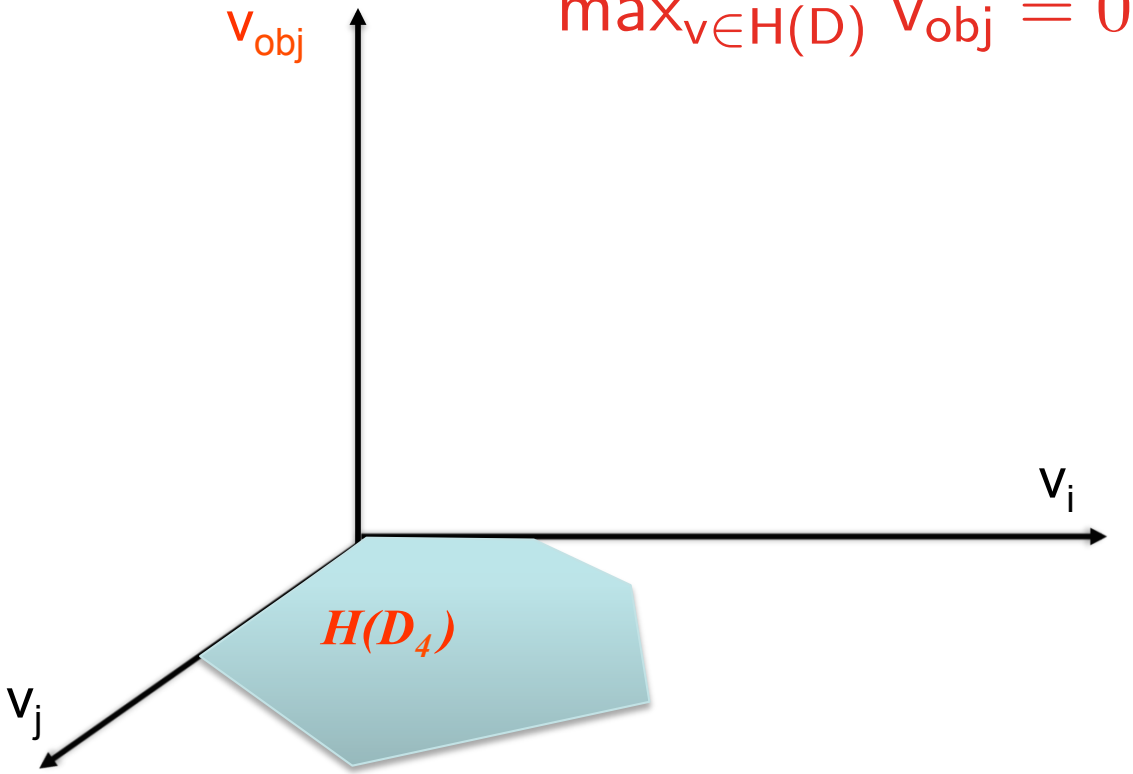
Drug action and FBA

problem is feasible when $v_{obj} = 0$

$$S\ v = 0$$

$$0 \leq v_i \leq U_i(1 - d_j) \quad d_j \in D$$

$$\max_{v \in H(D)} v_{obj} = 0$$

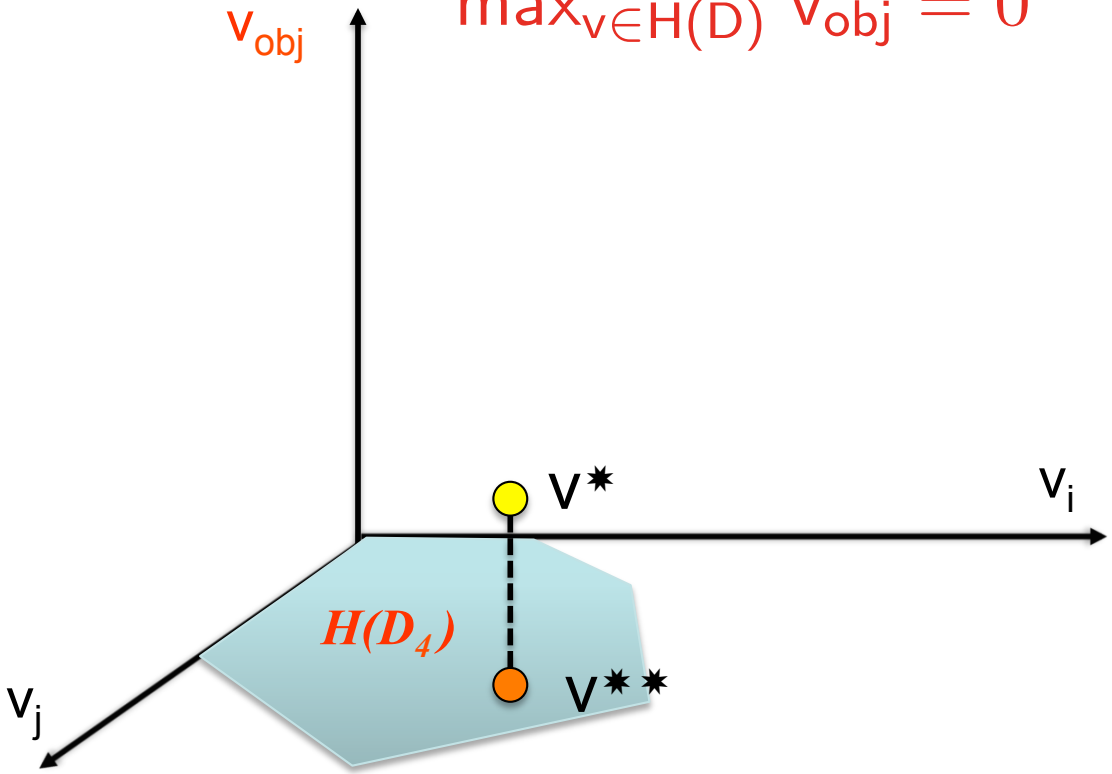


Drug action and FBA

when problem is feasible, optimum is in MOMA sense w.r.t. v✷

$$\min_{d \in D} \|v(d) - v^*\|$$
$$S\,v = 0$$
$$0 \le v_i \le U_i(1 - d_j) \quad d_j \in D$$
$$\max_{v \in H(D)} v_{obj} = 0$$

bilevel optimization problem



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Bilevel optimization

$$\min_{d \in D} \|v(d) - v^*\|$$
$$S\,v = 0$$
$$0 \le v_i \le U_i(1 - d_j)$$
$$\max_{v \in H(D)} v_{obj} = 0$$

OUTER PROBLEM:

Search for the combination of drugs which minimizes the side effect given that max (v_{obj}) = 0 of the inner problem

INNER PROBLEM:

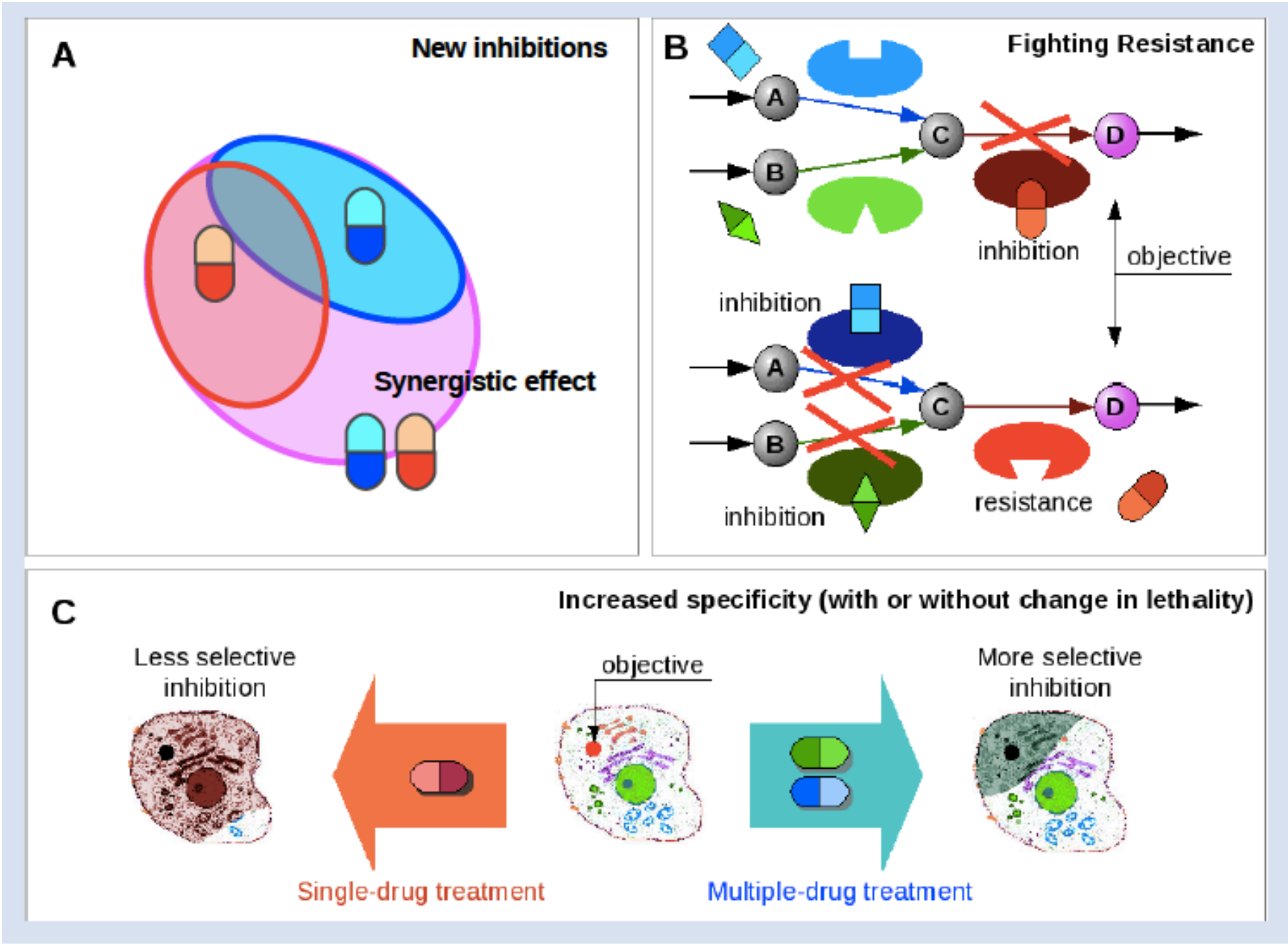
For a fixed set of drugs (provided by the outer problem) it maximizes the v_{obj} flux

Key point: strong duality theory is applicable
➔ inner problem can be reformulated exactly by appending extra constraints to the outer problem

Avoid exhausitve solution
(unfeasible: 40 drugs ➔ a trillion combinations)

Bilevel optimization for metabolic networks: OptKnock (Burgard et. al. 2003), OptORF (Kim et. al., 2010)

Multi-drug problems



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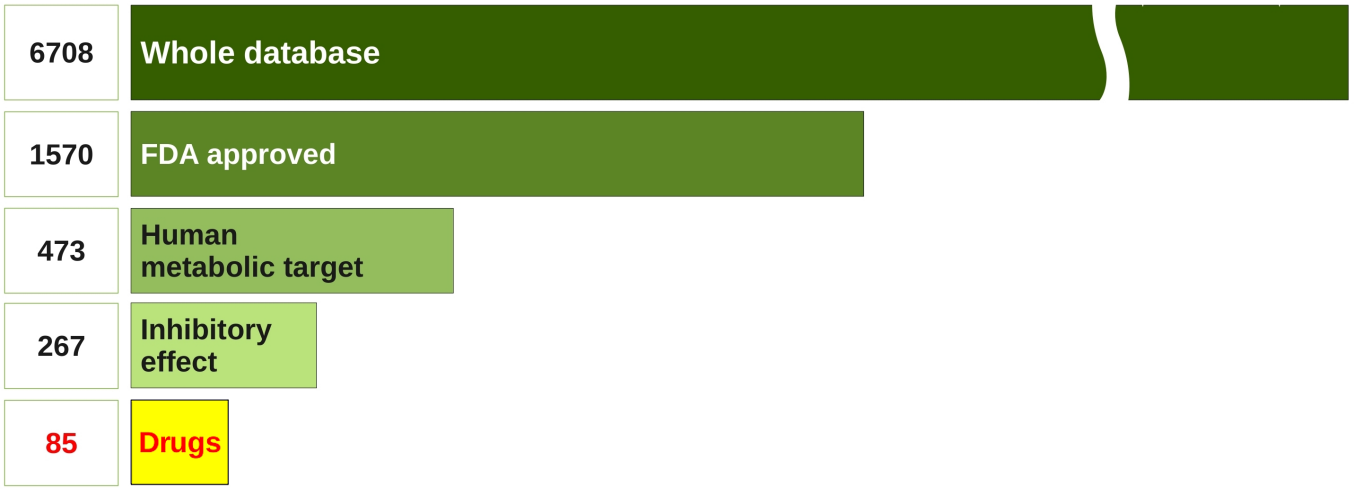
DrugBank / Metabolic networks

Metabolic networks and drugs for various organisms

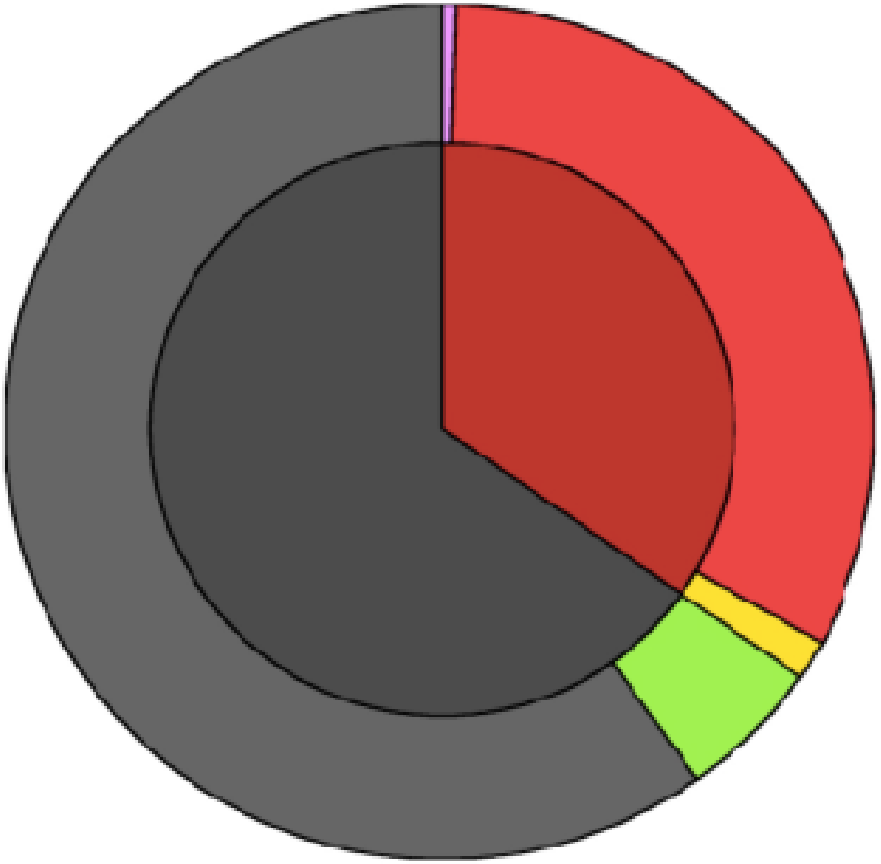
Organism	metabolites	reactions	compart.	drugs
Helicobacter pylori	422	600	2	76
Staphylococcus aureus	455	665	2	76
Methanosarcina barkeri	454	619	2	96
Shewanella oneidensis	528	799	2	124
Mycobacterium tuberculosis	614	964	2	109
Saccaromyces cerevisiae	547	931	3	124
Escherichia coli	1337	2221	3	129
Salmonella typhimurium	1497	2564	3	123

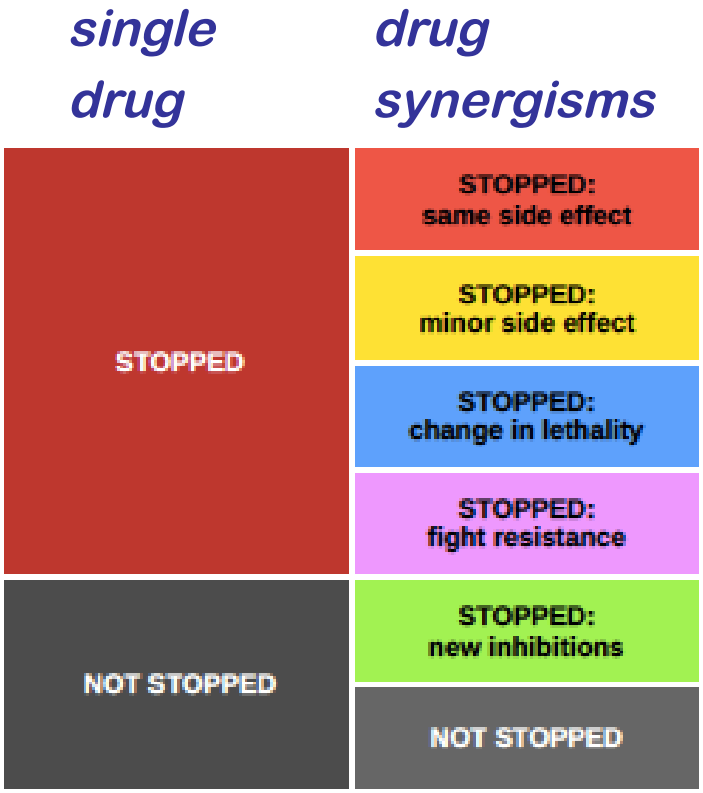
DrugBank for human metabolism



Results for *E.coli*

screening over all metabolic reactions





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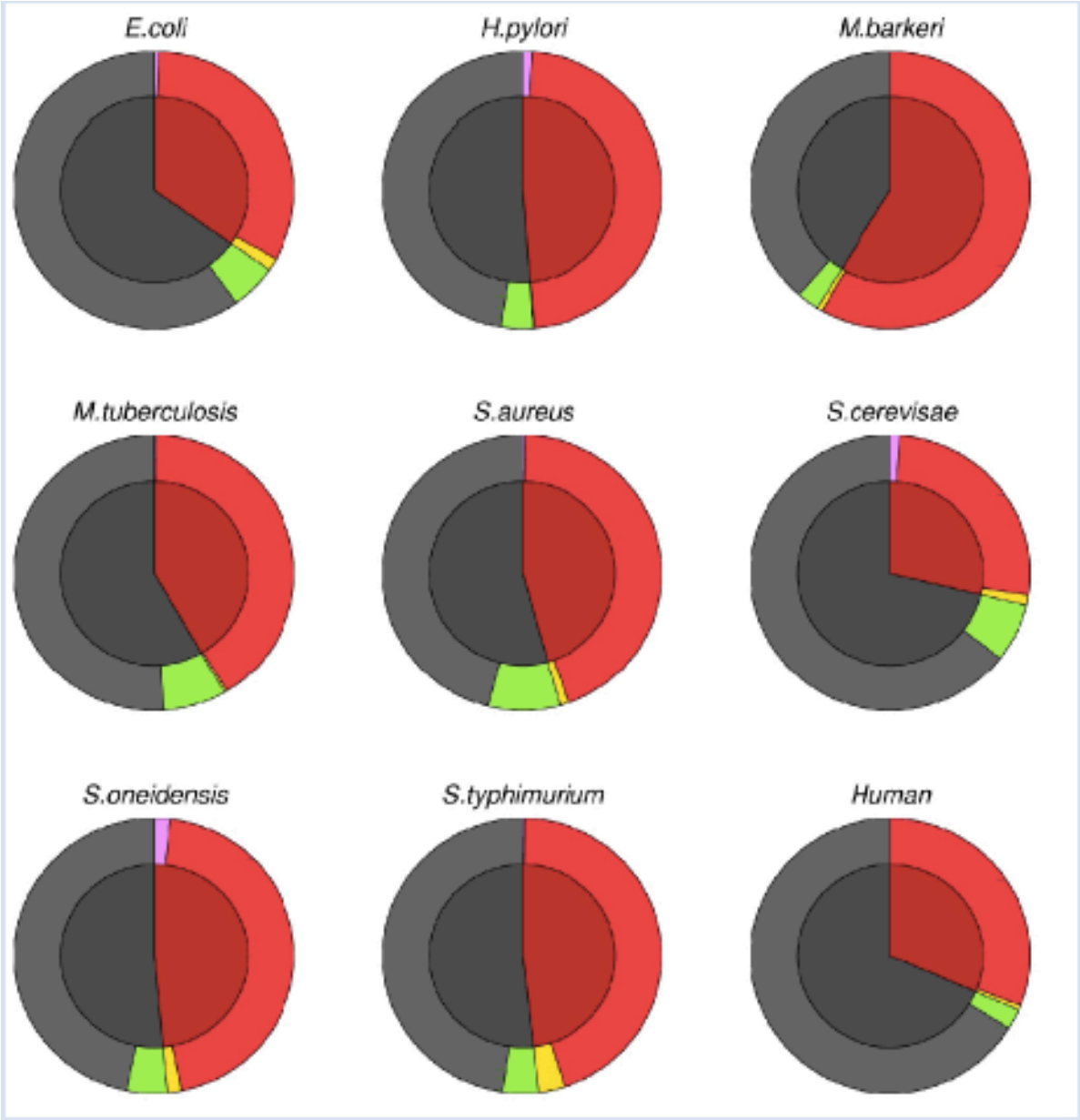
Results for 9 organisms

approx. 10% of new drug repurposing through synergistic actions



Human metabolic network

Human metabolic network (Duarte et al. PNAS 2007)

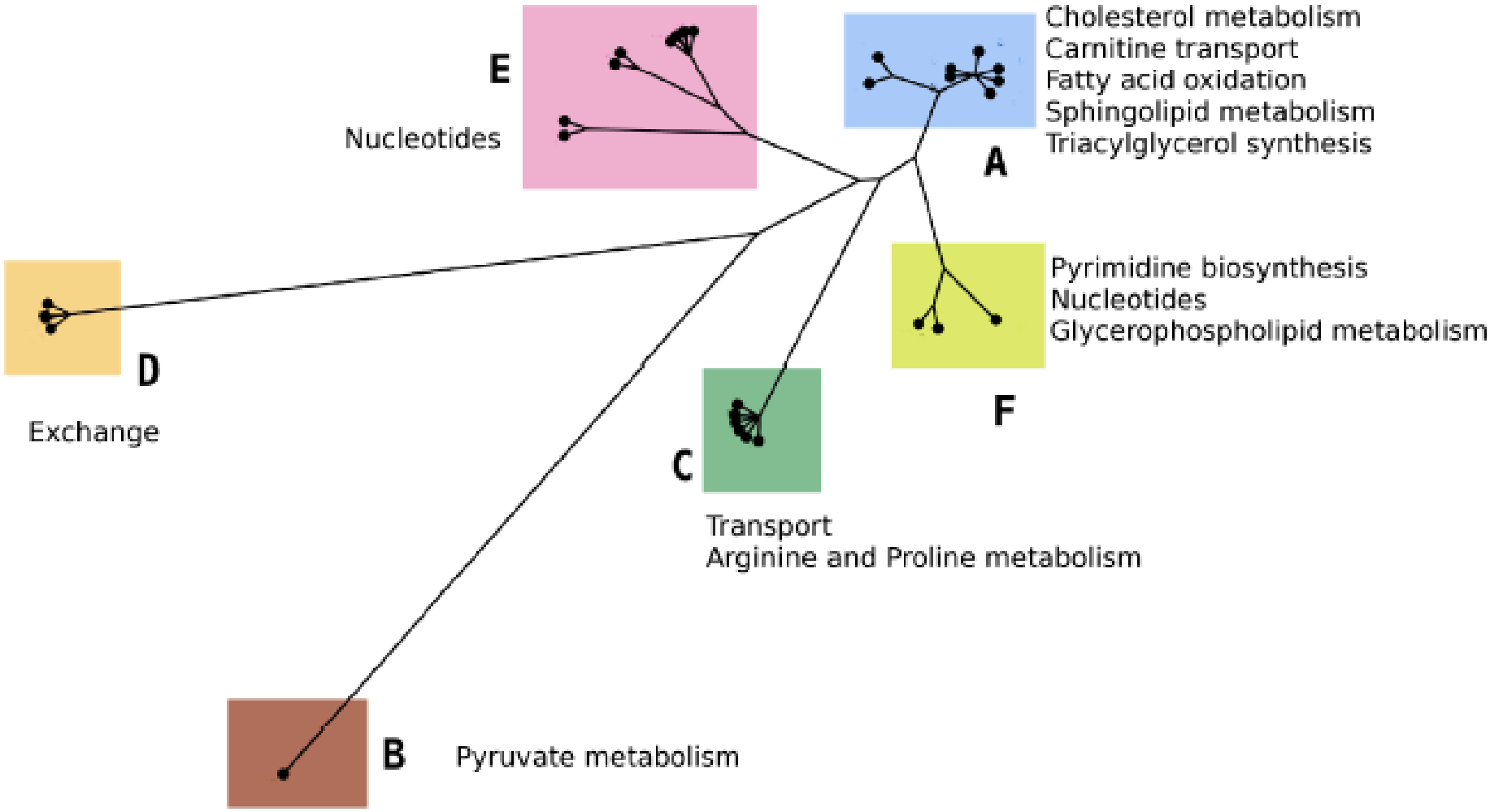
- 2469 reactions
- 1579 metabolites
- 83 pathways
- 85 drugs (or groups of drugs with same targets)

Side effect

- no FBA or similar, hence v^* is not available
- side effect = # of reactions that cannot take place because of the drugs

Screening all v_{obj}: only 32 synergisms exist

Clustering of synergisms



clustering is based on Hamming distance of side effects

Clustering of synergisms

d_1 = Rosiglitazone

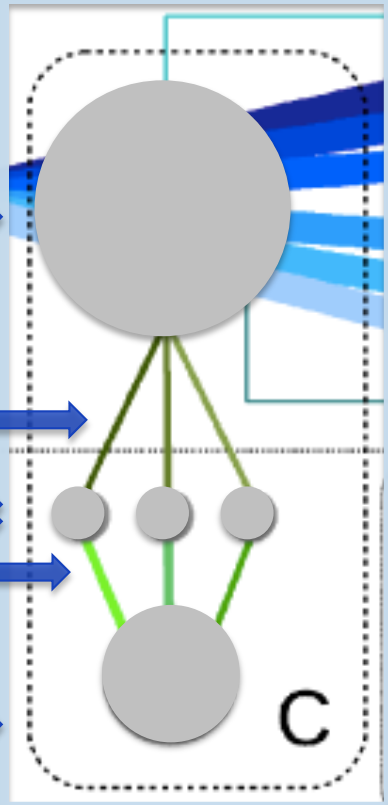
v_{obj} = dihydroceramide desaturase
(metabolic obesity syndrome)

d_2 = Simvastatin

d_2 = Simvastatin

v_{obj} = dihydroceramide desaturase
(metabolic obesity syndrome)

d_1 = Acetylsalicyclic acid



clinical experiments:
significant reduction (30%)
of the intracellular
accumulation of lipids

also undergoing
clinical trials

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All drug synergisms (human)

In red: experimental validations. (combinations of up to 4 drugs: 50 milions)

Drugs	Side eff.	Syn.	ratio	Class
Rosiglitazone (#7) - Cerulenin (#62)	298.9	52	17.3%	A
Rosiglitazone (#7) - Orlistat (#65) -	312.8	52	16.6%	A
Rosiglitazone (#7) - Quinacrine (#36) - Cerulenin (#62) - Tyloxapol (#85)	363.8	91	25.0%	A
Rosiglitazone (#7) - Quinacrine (#36) - Orlistat (#65) - Tyloxapol (#85)	377.7	91	24.0%	A
Rosiglitazone (#7) - Indomethacin (#22) - Cerulenin (#62) - Tyloxapol (#85)	390.6	91	23.2%	A
Rosiglitazone (#7) - Diclofenac (#35) - Cerulenin (#62) - Tyloxapol (#85)	397.5	91	22.8%	A
Rosiglitazone (#7) - Indomethacin (#22) - Orlistat (#65) - Tyloxapol (#85)	404.5	91	22.4%	A
Rosiglitazone (#7) - Diclofenac (#35) - Orlistat (#65) - Tyloxapol (#85)	411.4	91	22.1%	A
Indomethacin (#22) - Fomepizole (#75)	84.7	1	1.1%	B
Naftifine (#43) - Acetylsalicylic acid (#55)	116.0	6	5.1%	C
Acetylsalicylic acid (#55) - Tioconazole (#60)	116.0	6	5.1%	C
Simvastatin/Pravastatin (#4) - Acetylsalicylic acid (#55)	123.9	6	4.8%	C
Rosiglitazone (#7) - Tioconazole (#60)	280.9	6	2.1%	C
Rosiglitazone (#7) - Naftifine (#43)	280.9	6	2.1%	C
Simvastatin/Pravastatin (#4) - Rosiglitazone (#7)	288.8	6	2.0%	C
Carbidopa (#6) - Droxidopa (#24)	93.1	1	1.0%	D
Droxidopa (#24) - Selegiline (#45)	96.1	1	1.0%	D
Droxidopa (#24) - Minaprine (#49)	152.4	1	0.6%	D
Droxidopa (#24) - Zonisamide (#54)	289.7	1	0.3%	D
Mycophenolic acid (#42) - Mercaptopurine (#58)	11.0	5	45.4%	E
Ribavirin (#51) - Mercaptopurine (#58)	23.9	5	20.9%	E
Udenafil (#10) - Mycophenolic acid (#42) - Mercaptopurine (#58) -	18.0	7	38.8%	E
Mycophenolic acid (#42) - Dipyridamole (#57) - Mercaptopurine (#58)	22.0	7	31.8%	E
Udenafil (#10) - Ribavirin (#51) - Mercaptopurine (#58)	30.9	7	22.6%	E
Ribavirin (#51) - Dipyridamole (#57) - Mercaptopurine (#58)	34.9	7	20,0%	E
Theophylline (#18) - Mycophenolic acid (#42) - Mercaptopurine (#58)	41.7	7	16.7%	E
Mycophenolic acid (#42) - Pentoxifylline (#50) - Mercaptopurine (#58)	53.8	7	13.0%	E
Theophylline (#18) - Ribavirin (#51) - Mercaptopurine (#58)	54.6	6	10.9%	E
Pentoxifylline (#50) - Ribavirin (#51) - Mercaptopurine (#58)	66.7	6	8.9%	E
Pentoxifylline (#50) - Arsenic trioxide (#72)	118.2	17	14.3%	F
Cladribirne (#16) - Pentoxifylline (#50)	118.2	17	14.3%	F
Gemcitabine (#30) - Pentoxifylline (#50)	157.8	15	9.5%	F

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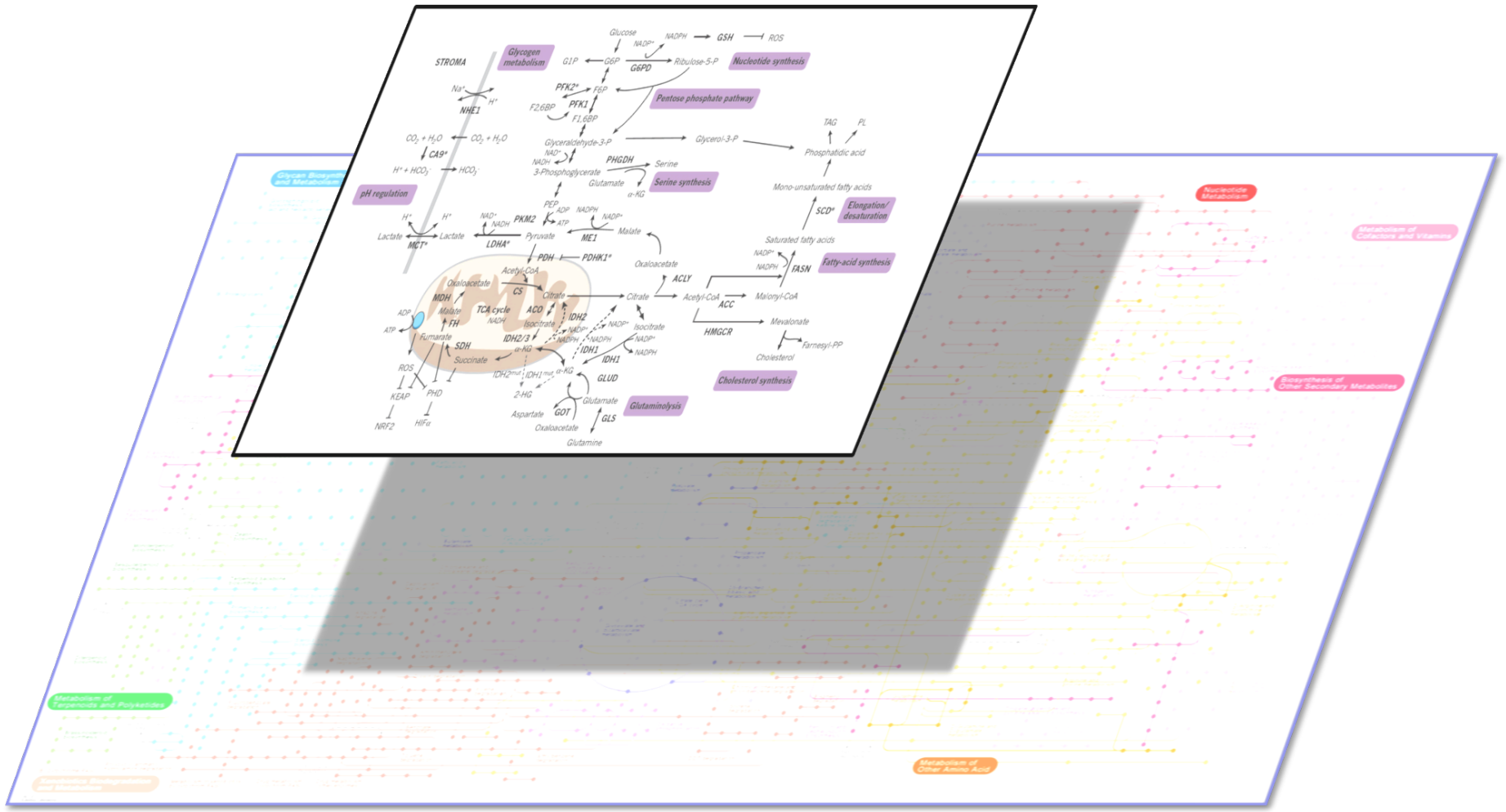
Human vs cancer metabolism

Cancer metabolic network (Folger et al. Mol. Sys. Bio. 2011)



Human metabolic network (generic cell)

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Human vs cancer metabolism

	Human	Cancer
reactions	2469	940
metabolites	1587	654
drugs	85	55

Human vs. cancer metabolic network: a two-network problem

- same enzymes
- different topology
- objective function: tumoral cell biomass
- side effect: # of inhibited reactions on healthy human cells

TASK: use drugs to suppress tumoral growth while having the least side effect on the healthy cells

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Human vs cancer metabolism

drugs (mostly single) suppressing tumoral growth

Solution nr.	Drugs	Side effect $\sigma(h)$
1	Floxuridine (#20)	1
2	Mycophenolic acid (#42)	4
3	Trimethoprim (#29)	5
4	Methotrexate (#11)	5
5	Atovaquone (#69)	6
6	Tyloxapol (#85)	6
7	Ezetimibe (#56)	12
8	Pemetrexed (#41)	15
9	Ribavirin (#51)	17
10	Quinacrine (#36)	22
11	Myo-Inositol (#82)	29
12	Tioconazole (#60)	34
13	Naftifine (#43)	34
14	Simvastatin (#4)	42
15	Leflunomide (#66)	42
16	Auranofin (#59) - Fomepizole (#75)	47
17	Indomethacin (#22)	48
18	Diclofenac (#35)	55
19	Hydroxyurea (#16)	56
20	Arsenic trioxide (#72)	56
21	Gemcitabine (#30)	99

in red: experimentally validated antitumoral drugs

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Human vs cancer metabolism

screening over all reaction as (hypotetical) additional target: 31 new solutions

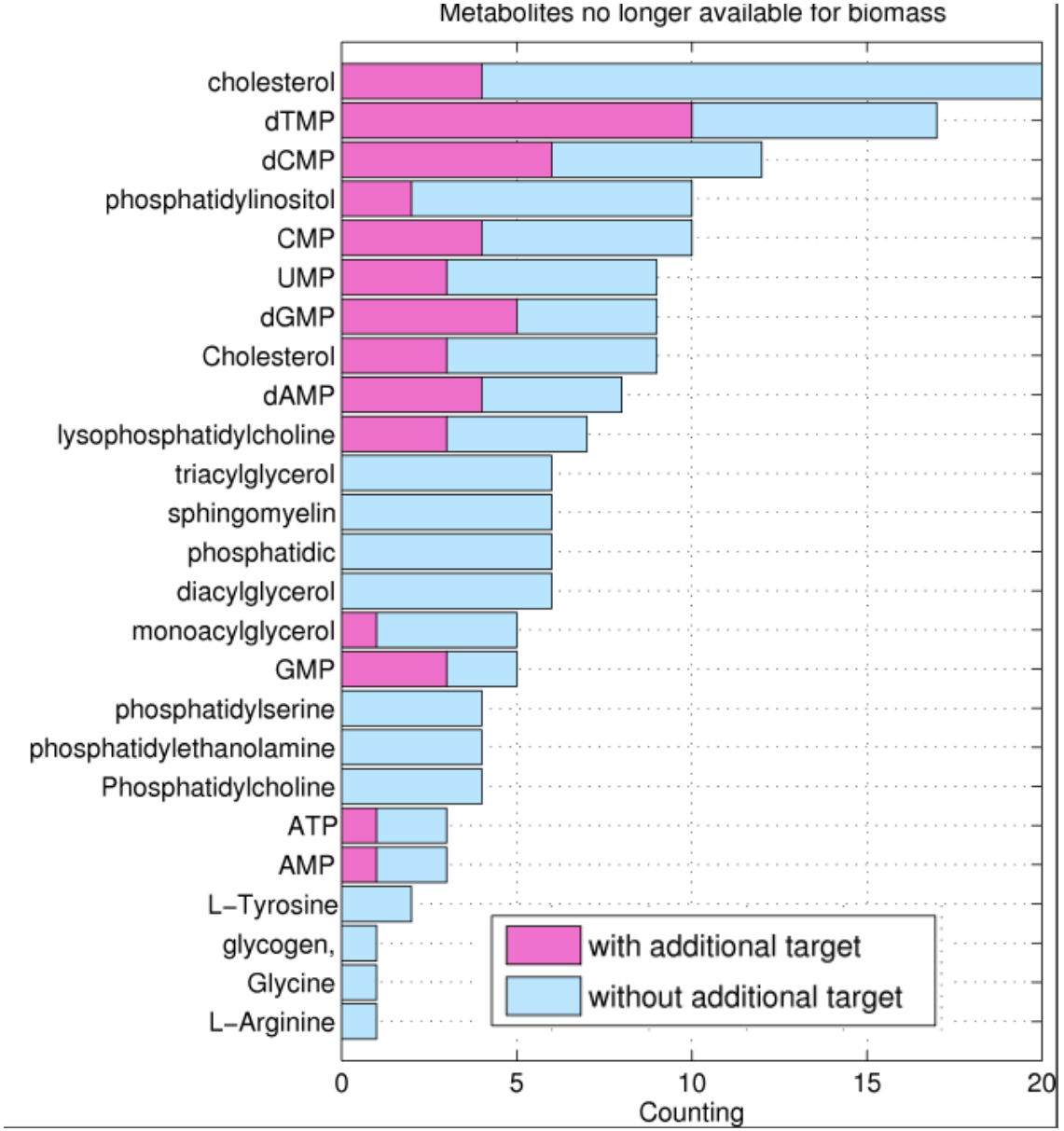
Assuming we can have an hypothetical new drug. What should it be its target in order to have an antitumoral multiple-drug treatement?

Examples of new combinations:

Mimosine + cis-octadecenoic acids [Mahfouz, 1981]

Cerulenine (or Orlistat) + 5,6,7,8-tetrahydro-N^5,N^{10}-carbonylfolic acid [Temple, 1982]

Sulfasalazine + perfluorodecanoic acid (or spiropentaneacetic acid) [Borger 1993, Tserng 1991].



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From ON/OFF to partial inhibition

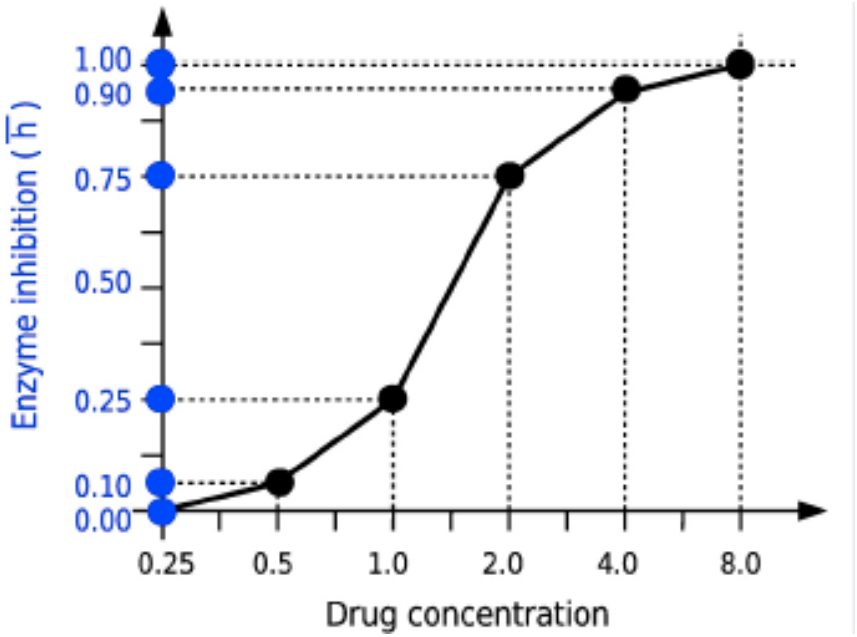
Pros:

- prediction of synergisms
- possibility of including experimental drugs
- contrasting two or more networks
- computational efficiency
- applications
  - drug repurposing
  - strategy against drug resistance
  - antifungal/antibacterial effect
  - non-lethal therapies for commensal bacteria

Cons:

- predicted drug synergisms have restricted variability
- human metabolic network is not tissue-specific
- ON/OFF modeling of drug inhibition

From ON/OFF to partial inhibition



1. Inhibition of an enzyme induced by a drug can be partial
2. Desired inhibition on v_{obj} can be partial
3. Partial activation of v_{obj} can be sought

Partial inhibition

$$V_{obj} < \tau\, V^*_{obj}$$
$$\tau < 1$$

Partial activation

$$V_{obj} > \tau\, V^*_{obj}$$
$$\tau > 1$$

Partial inhibition

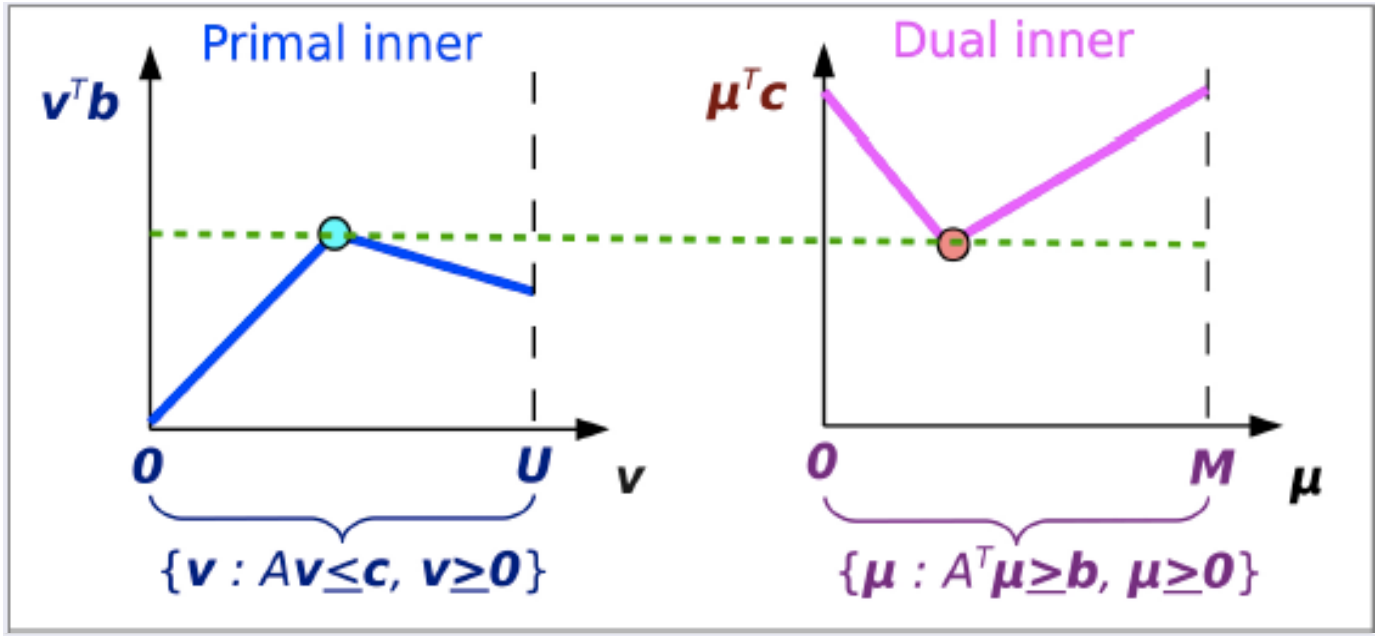
min

max

min

dual

constraints



Strong duality theorem of LP

$$\max\left\{b^T v \ \text{ s.t. } A\,v \leq c,\ v \geq 0\right\} = \min\left\{c^T \mu \ \text{ s.t. } A^T \mu \geq b,\ \mu \geq 0\right\}$$

When this holds: the set defined by

$$\begin{cases} Av \leq c,\ v \geq 0 \\ A^T \mu \geq b,\ \mu \geq 0 \\ b^T v = c^T \mu \end{cases}$$

contains only the optimal solution of the inner problem

Partial inhibition

For our inner problem:

$$\mathsf{A}\ \mathsf{v} \leq \mathsf{c} \quad \Longleftrightarrow \quad \begin{cases} \mathsf{S}\ \mathsf{v} = 0 \\ 0 \leq \mathsf{v}_i \leq \mathsf{U}_i(1 - \mathsf{d}_j) \quad \mathsf{d}_j \in \mathsf{D} \\ \dots \end{cases}$$

where $\mathsf{d}_j \in [0,\ 1]$ is a variable of the outer problem which describe the inhibition by the drug *j*

➔ d is part of vector c i.e., (for some Q and p)

$$\mathsf{c} = \mathsf{Q}\ \mathsf{d}\ + \mathsf{p}$$

➔ from the strong duality theorem

$$\mathsf{b}^T\mathsf{v} = \mu^T\mathsf{Q}\ \mathsf{d}\ + \mu^T\mathsf{p}$$

➔ no longer linear in the variables!

Partial inhibition

ON/OFF inhibition:

When d is a binary variable: $d_j \in \{0,\ 1\}$

➔ exact linearization is possible $z_{ij} = \mu_i d_j$

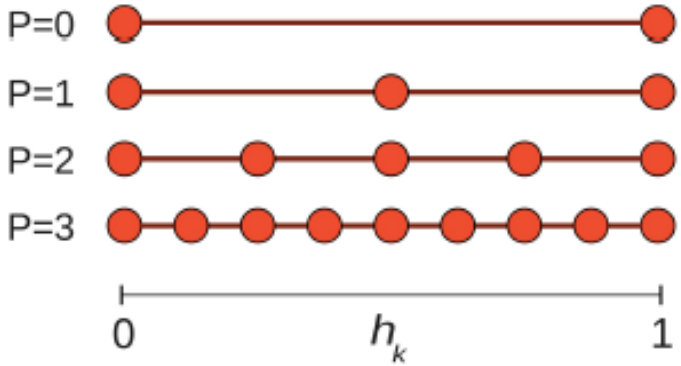
$$\begin{aligned} 0 &\leq z_{ij} \leq M_i d_j \\ \mu_i - M_i(1 - d_j) &\leq z_{ij} \leq \mu_i \end{aligned}$$

(M_i is the upper bound on the dual variable μ_i)

More fine-graded inhibition:

Performing an equipartition of [0, 1] with P+1 binary variables

$$d_j = \frac{x_{j,0}}{2^P} + \sum_{n=1}^{P} \frac{x_{j,n}}{2^n}$$



➔ exact linearization is still possible

Partial inhibition

Effect of partial inhibition on microorganism: MOMA

- Inner problem: duality requires a linear cost function:
  MOMA with L^1 norm

$$\min_{v \in H(D)} \sum_i |v_i - v_i^*|$$

- Outer problem: side effect (still L^1 MOMA)

$$\min_{d \in D} \sum_i |v_i(d) - v_i^*|$$

- Therapeutic requirement: (partial inibition/activation of v_{obj})

$$v_{obj} < \tau\, v_{obj}^* \qquad \tau < 1$$

$$v_{obj} > \tau\, v_{obj}^* \qquad \tau > 1$$

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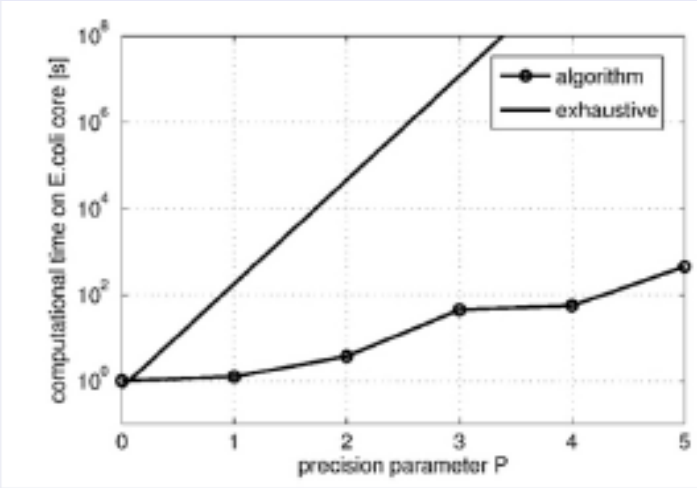
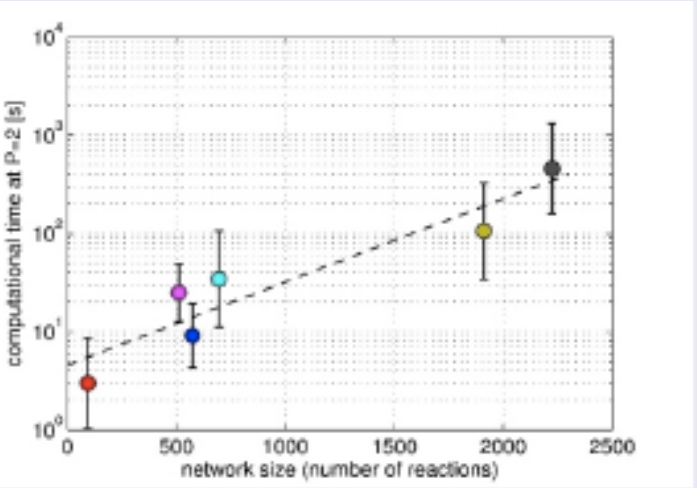
Partial inhibition: evaluation

Comparison of performances

E.coli core

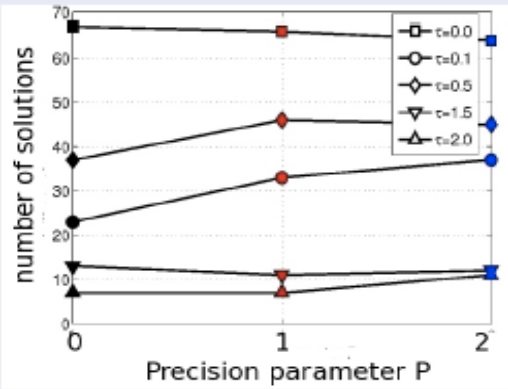


different microorganisms, P=2

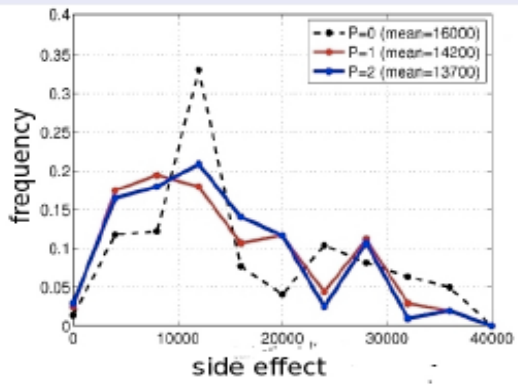


Screening over all *E.coli* reactions

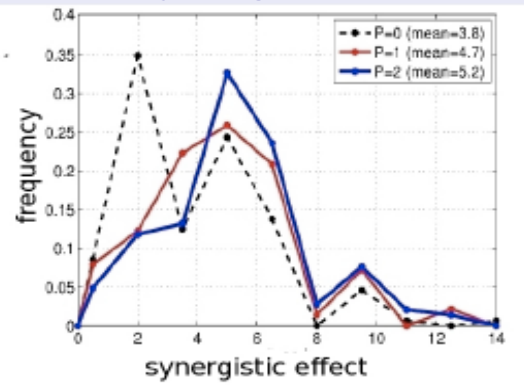
n. solutions



side effect



synergisms



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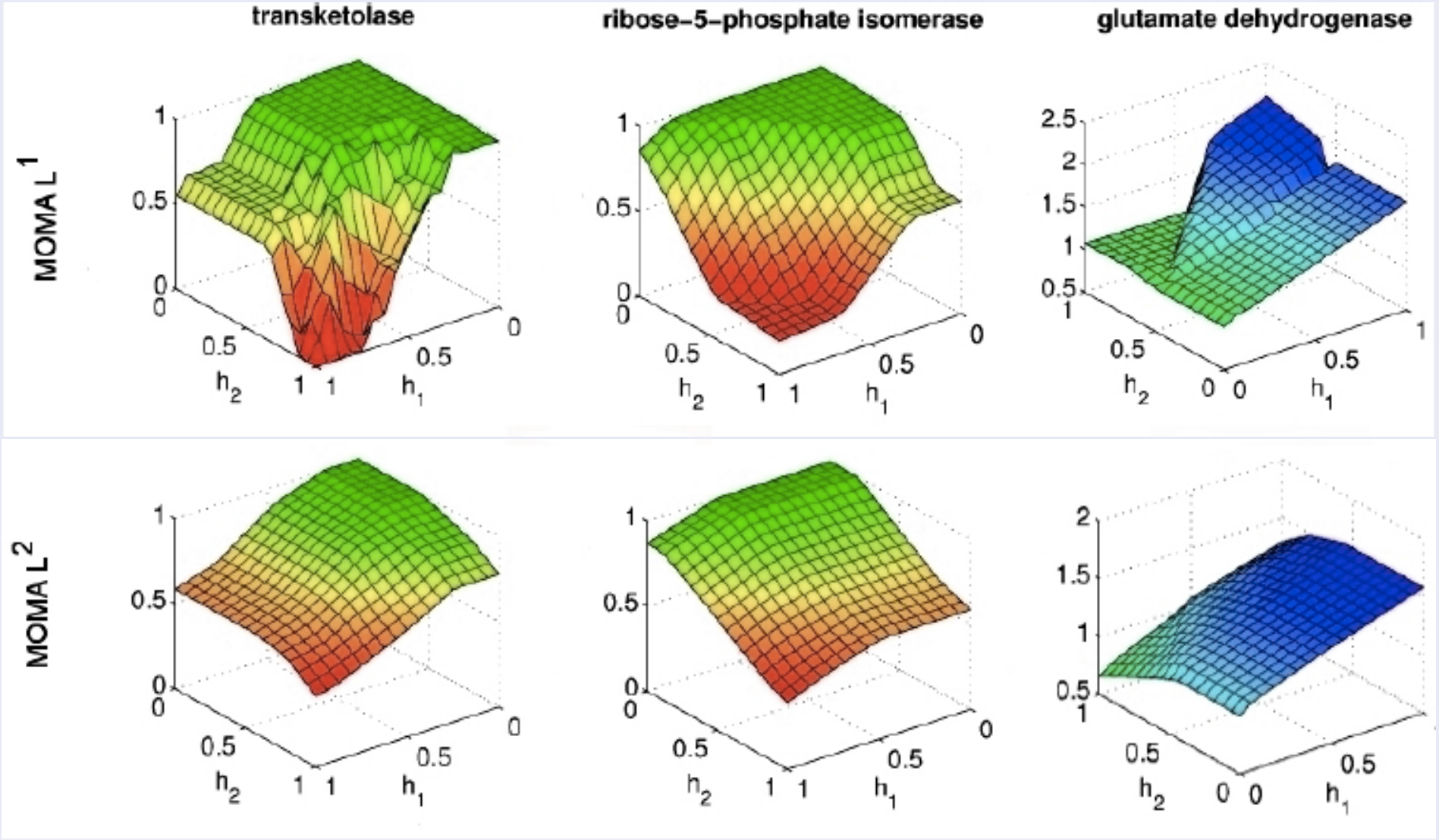
Partial inhibition: evaluation

surfaces of drug interactions: three examples



grid constructed with P = 4

Conclusions

- Drug synergisms:
  - can be determined systematically
  - no need of exhaustive search

- Bilevel optimization:
  - usually NP-hard MILP problem
  - properly addressing the bilinear terms it can still be used
  - also realistic representations of drug action are feasible

- Applications:
  - repurposing of approved drugs (“cheap”)
  - clues on multi-drug effects / potential solutions
  - partial action: all epistatic effects can be sought

THE END

THANK YOU FOR YOUR ATTENTION

Human metabolic drugs

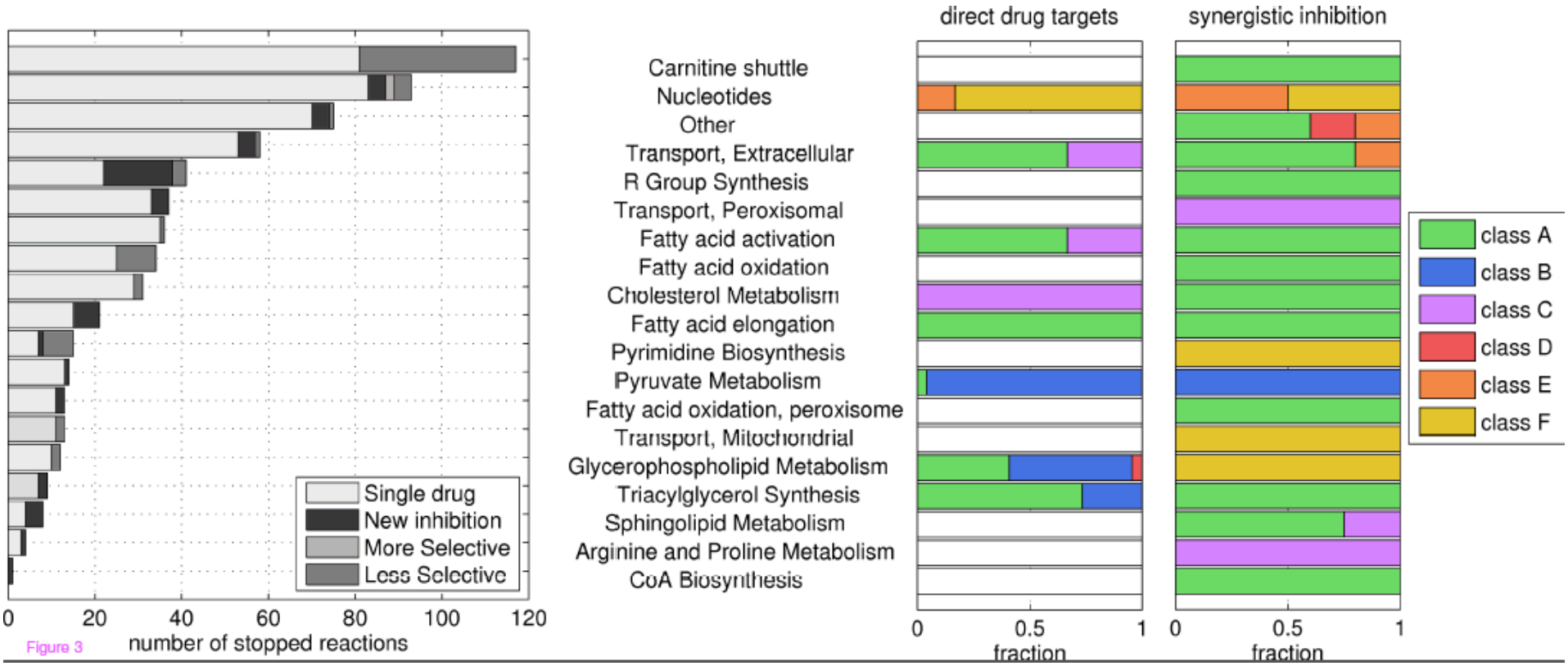


Figure 3