Studies on the emergence of drug resistance in M. Tuberculosis

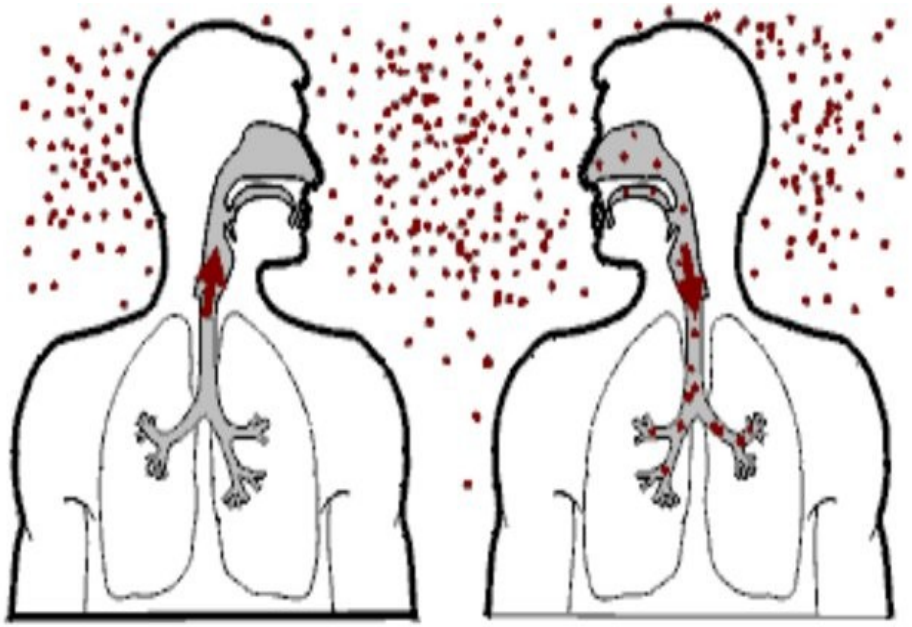
Michał Woźniak
(PhD Student)

Outline

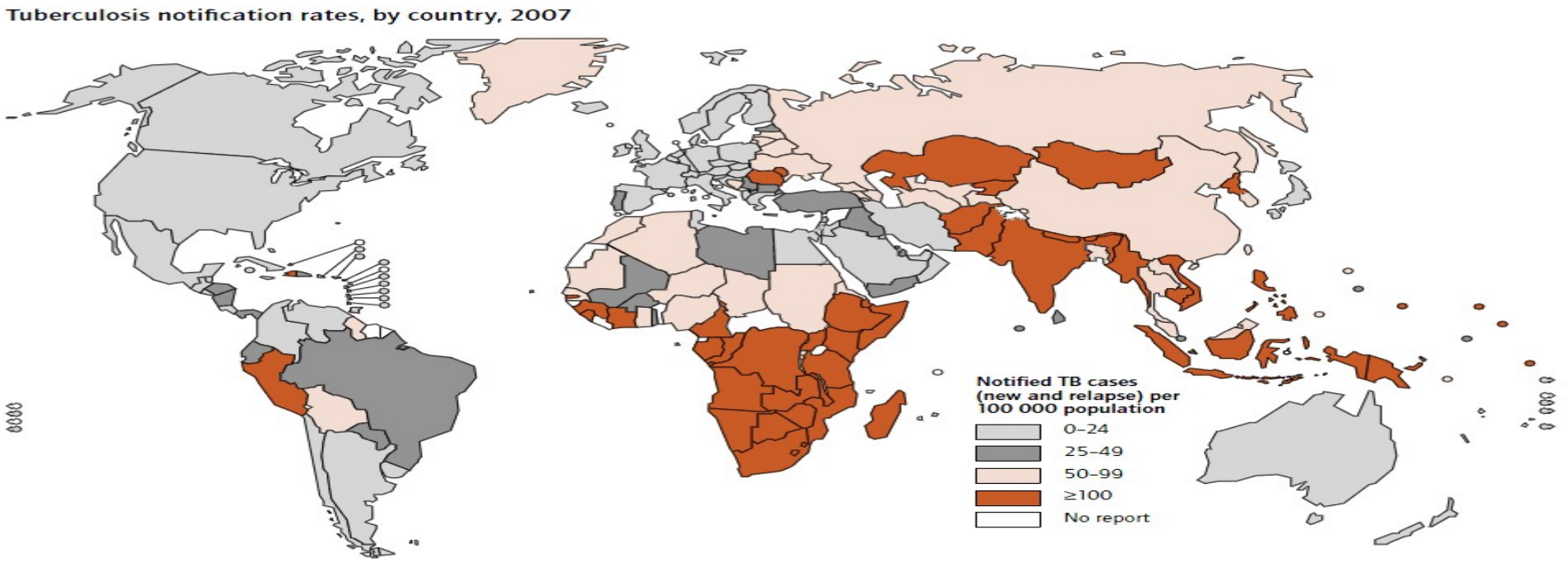
- Introduction about M. Tuberulosis
  - Tuberculosis
  - Drug resistnace
- Raman and Chandra paper
  - Concept of co-targets
  - Schema of experiment
  - Weak points
- My project
  - Formalisation of co-target
  - Results
  - Future works

Tuberculosis – introduction

- Tuberculosis is a common and often deadly infectious disease caused by mycobacteria.
- Tuberculosis usually attacks the lungs.
- Transmission can only occur from people with active (not latent TB).
- In 2006 WHO have launched The Stop TB Strategy and designed some targets to achieve by 2015.

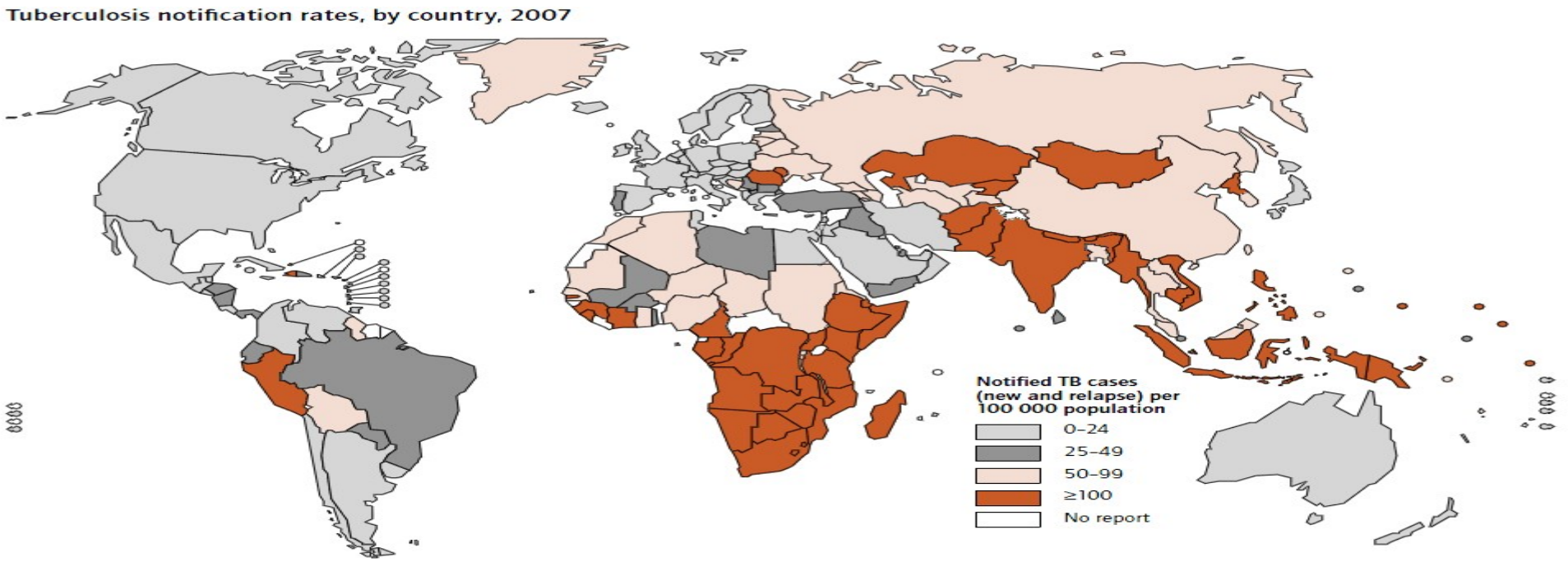


Some WHO statistics and facts



- Tuberculosis (TB) is a major cause of illness and death worldwide, especially in Asia and Africa.
- Globally, 9.2 million new cases and 1.7 million deaths from TB occurred in 2006, of which 0.7 million cases and 0.2 million deaths were in HIV-positive people.
- The most important factor that make people more susceptible to TB infection worldwide is HIV.

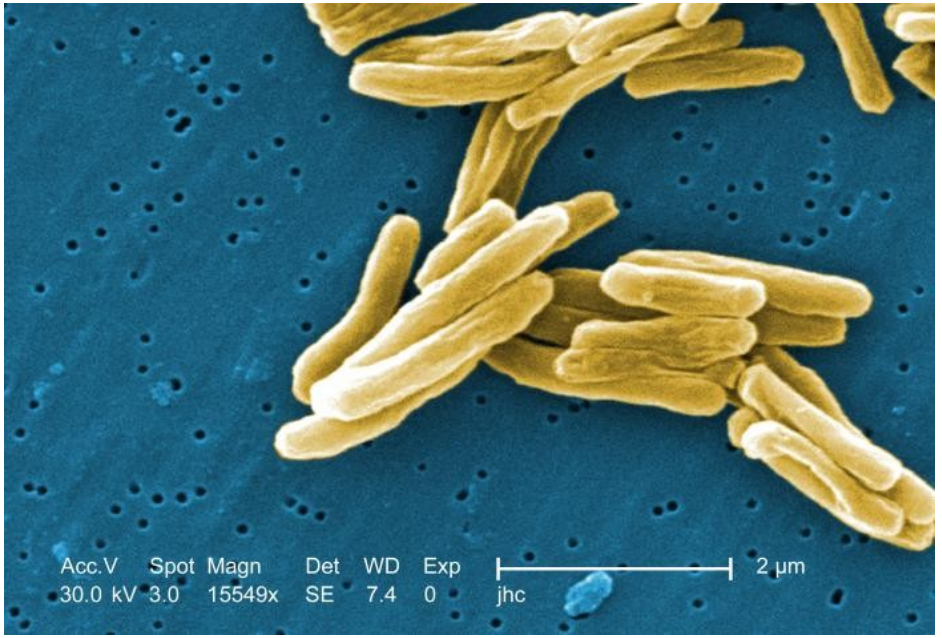
Some WHO statistics and facts



- Tuberculosis (TB) is a major cause of illness and death worldwide, especially in Asia and Africa.
- Globally, 9.2 million new cases and 1.7 million deaths from TB occurred in 2006, of which 0.7 million cases and 0.2 million deaths were in HIV-positive people.
- The most important factor that make people more susceptible to TB infection worldwide is HIV.

M. Tuberculosis

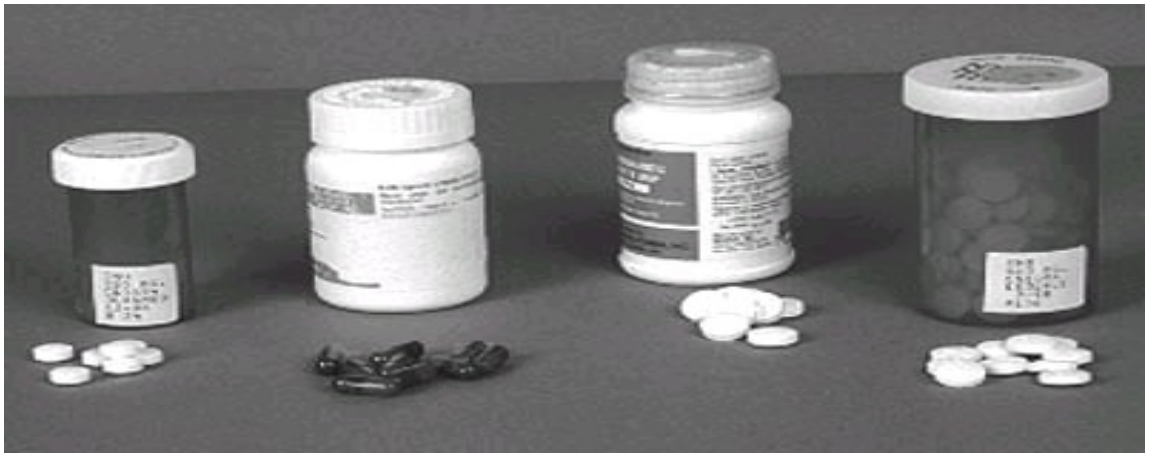
- Mycobacterium tuberculosis (MTB) is a pathogenic bacterial species causative agent of tuberculosis. First discovered in 1882 by Robert Koch.
- M. tuberculosis is a gram positive bacteria, has an unusual, waxy coating on the cell surface (primarily mycolic acid).
- Over 60% of the mycobacterial cell wall is lipid. The lipid fraction of MTB's cell wall consists of three major components, mycolic acids, cord factor, and wax-D.



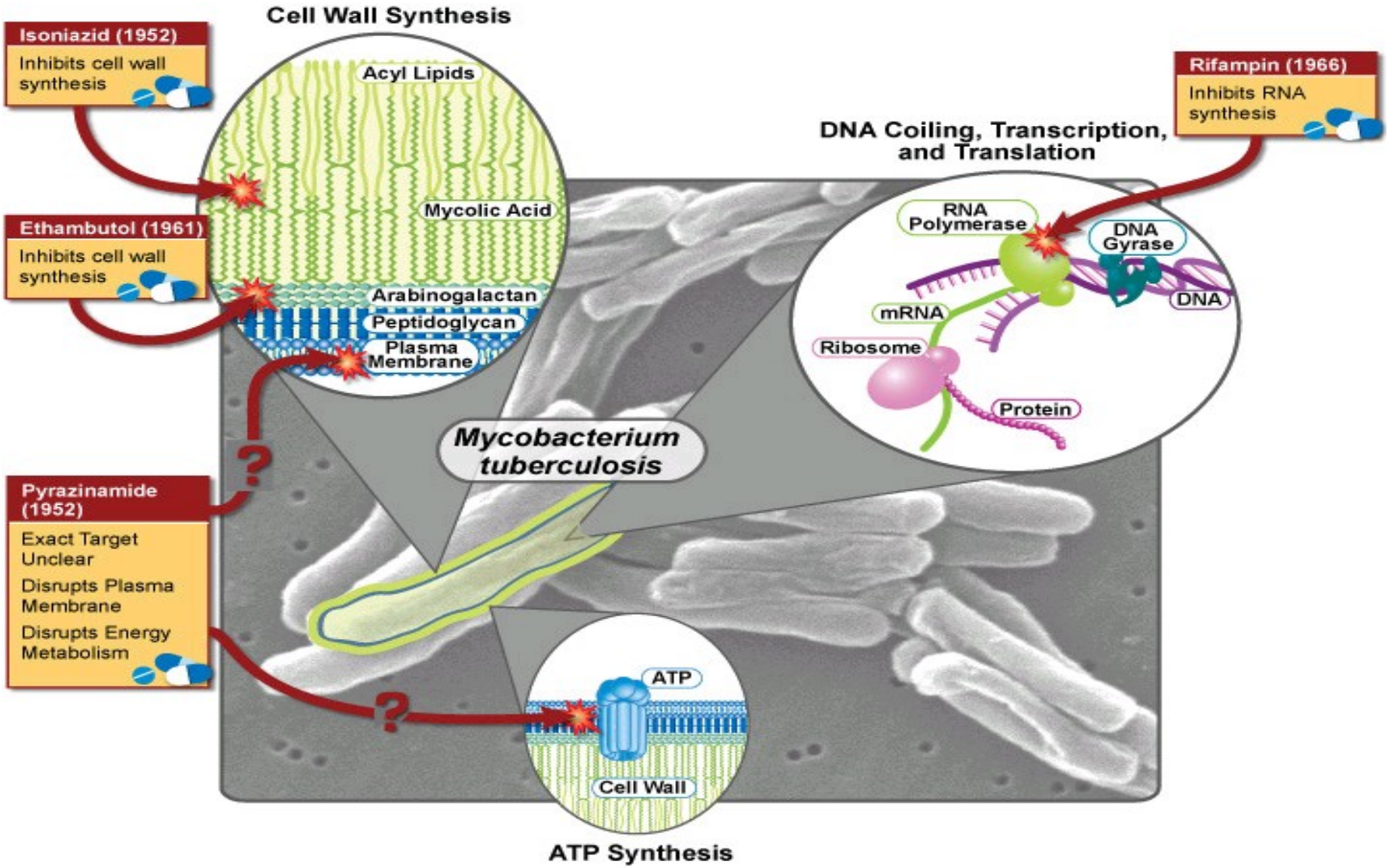
- **Systematic**
  - Kingdom: Bacteria
  - Phylum: Actinobacteria
  - Order: Actinomycetales
  - Suborder: Corynebacterineae
  - Family: Mycobacteriaceae
  - Genus: Mycobacterium
  - Species: M. tuberculosis

Poziomy oporności tuberculosis

- **MDR (**multidrug resistant) -szczep oporny przynajmniej na isoniazid i rifampin. (WHO definition)
- **XDR** (extensivedrug resistant) **-** szczep MDR, dodatkowo oporny na przynajmniej jeden lek z rodziny fluoroquinoloni I przynajmniej jeden lek second-line injectable agent (amikacin, kanamycin or capreomycin). (WHO definition)
- **TDR** (totally drug resistant) – szczep MDR oporny na wszystkie leki z rodziny fluoroquinoloni oraz wszystkie second-line.

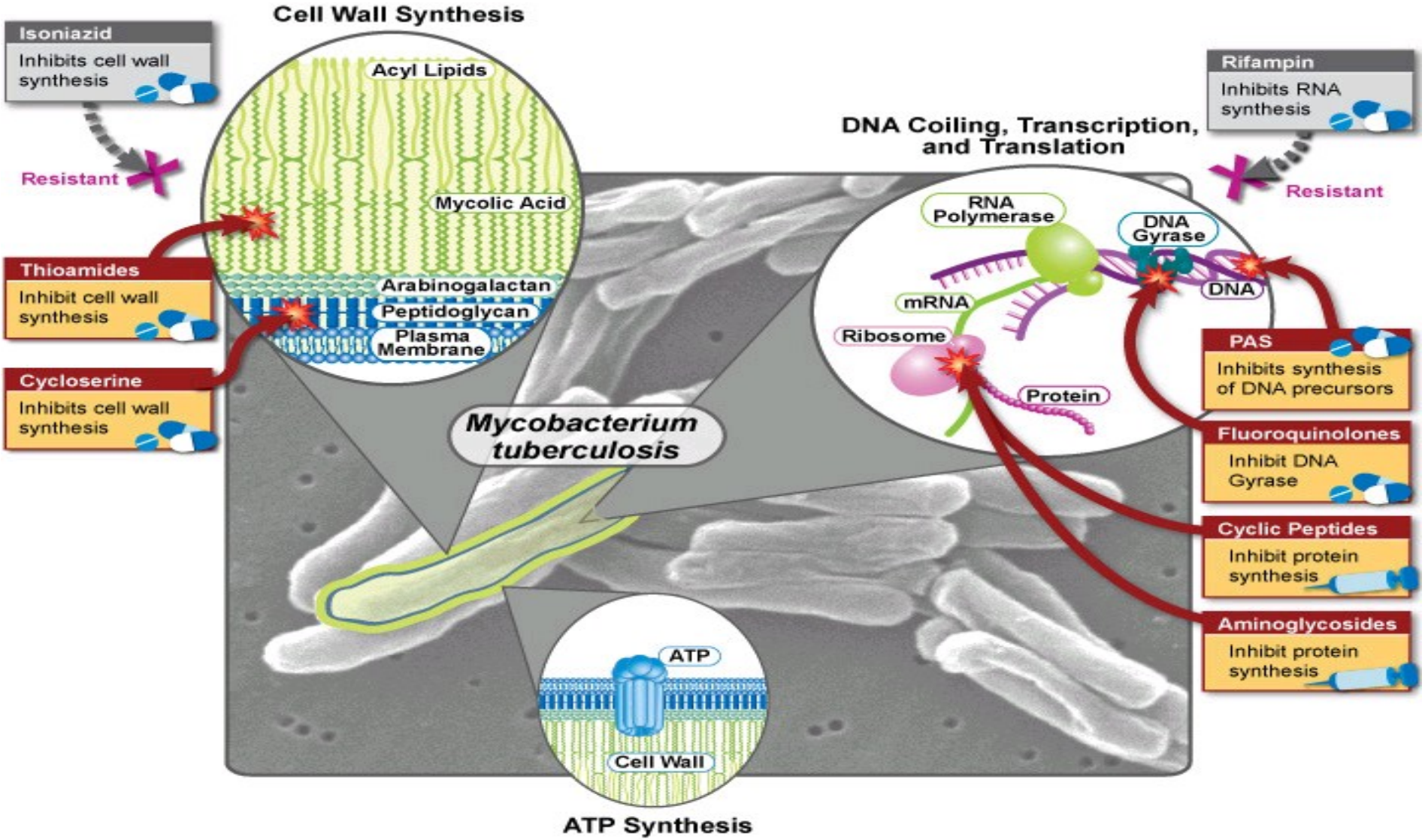


Action of first line drugs



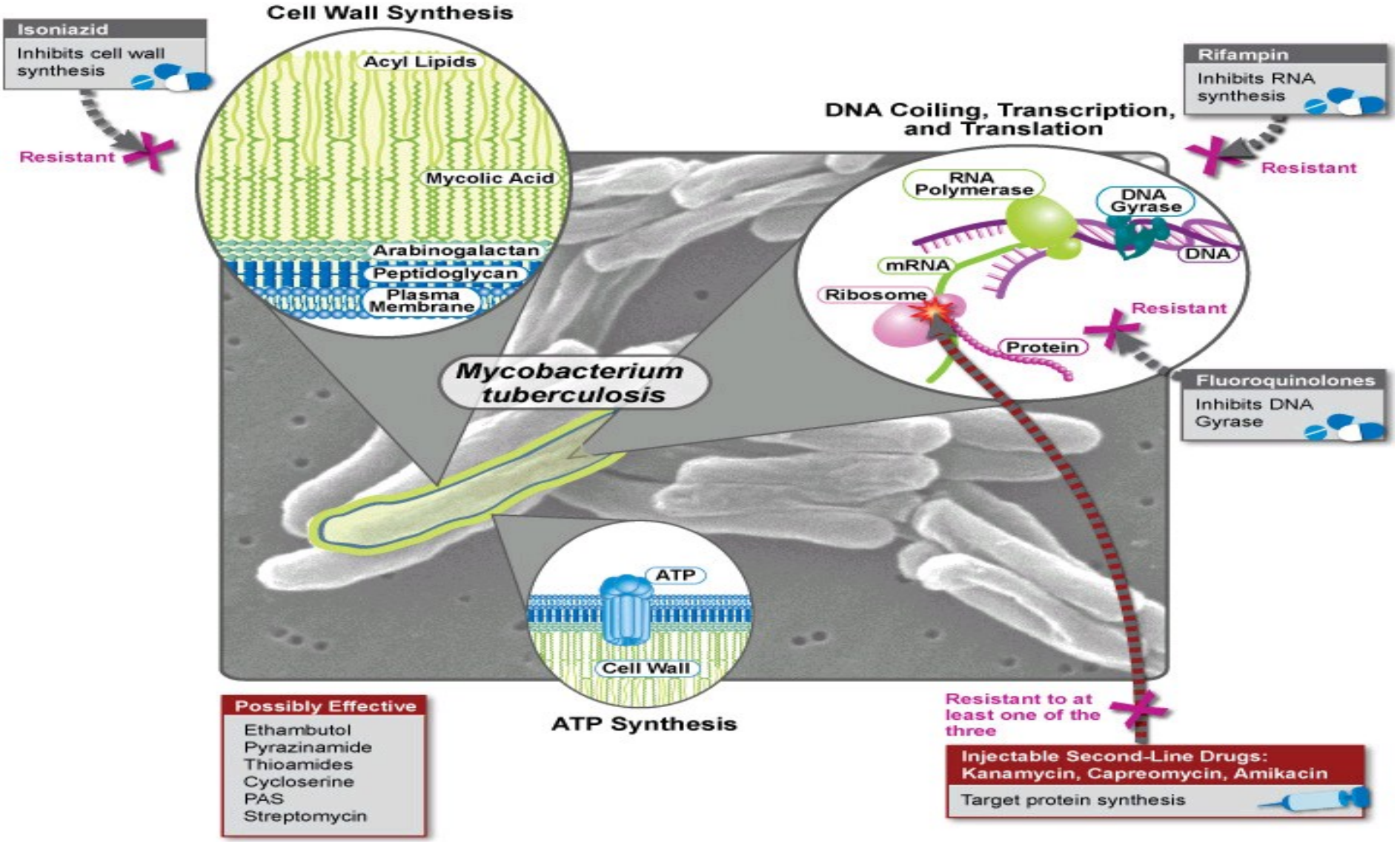
- Source: http://www3.niaid.nih.gov/)

Action of drugs on MDR



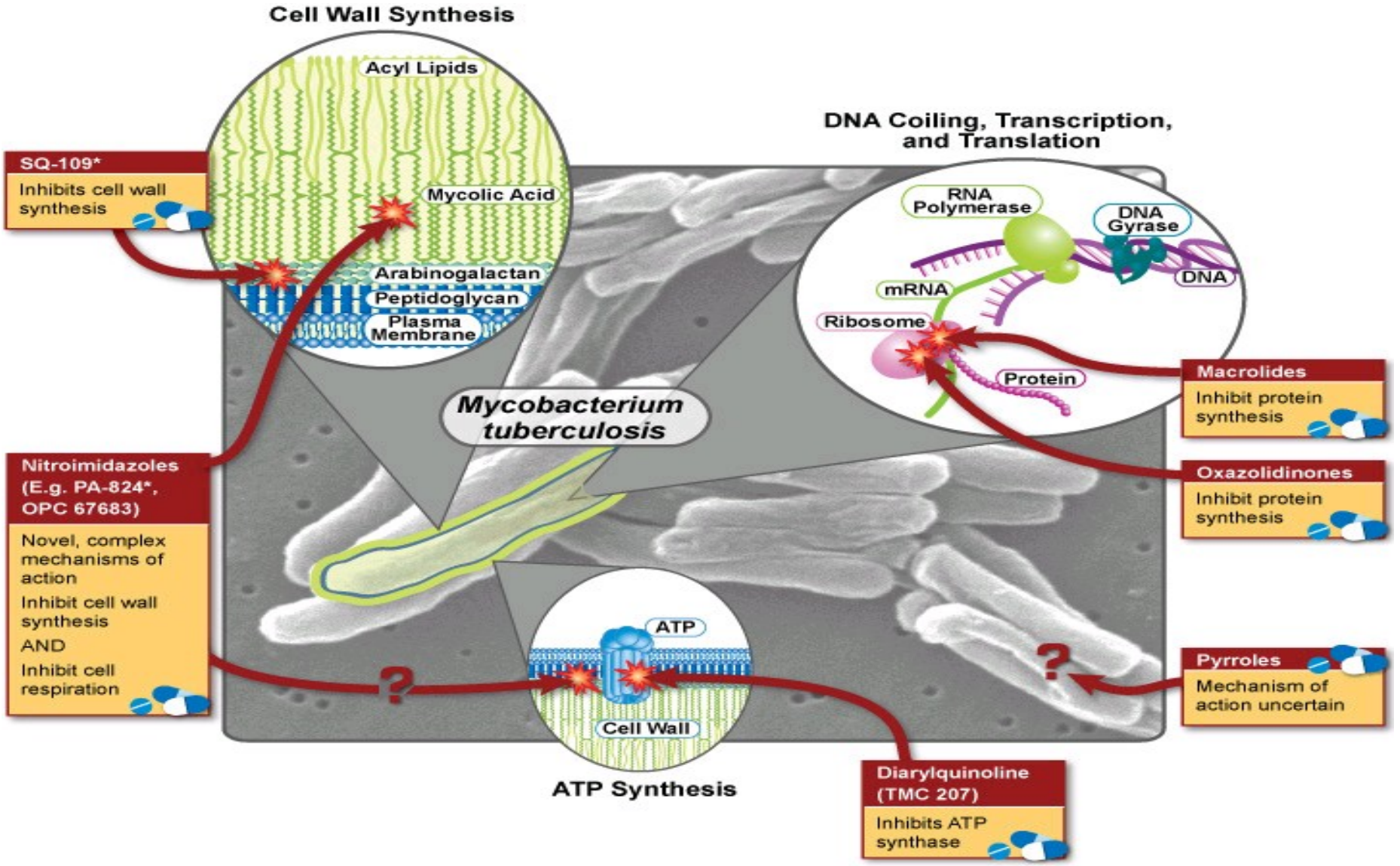
- Source: http://www3.niaid.nih.gov/)

Action of drugs on XDR



- Source: http://www3.niaid.nih.gov/)

Action of TB-drugs under development



- Source: http://www3.niaid.nih.gov/)

Summary points

- The emergence of drug resistance is a common problem for fighting with a variety of pathogens causing infectious diseases.
- Drug resistant tuberculosis has continued to spread internationally and is a serious problem.
- The current approaches to combating resistance resulted in only a limited success due to low level of understanding of how resistance emerges upon treatment.

Mechanisms of the emergence of drug resistance

- Spontaneous mutations
- Mutation induced by some stress and SOS repair mechanism (accelerates the process of the emergence resistance even 200 times)
- Horizontal gene transfers

Common mechanisms of drug resistance

- Drug inactivation or modification of drug molecules
- Changing the destination of attachment of the drug
- Reduced accumulation of drug, by reducing the transmission of cell wall or increased efficiency of the mechanism of efflux pumps,
- The creation of alternative metabolic pathways.

Paper introducing the concept of co-target

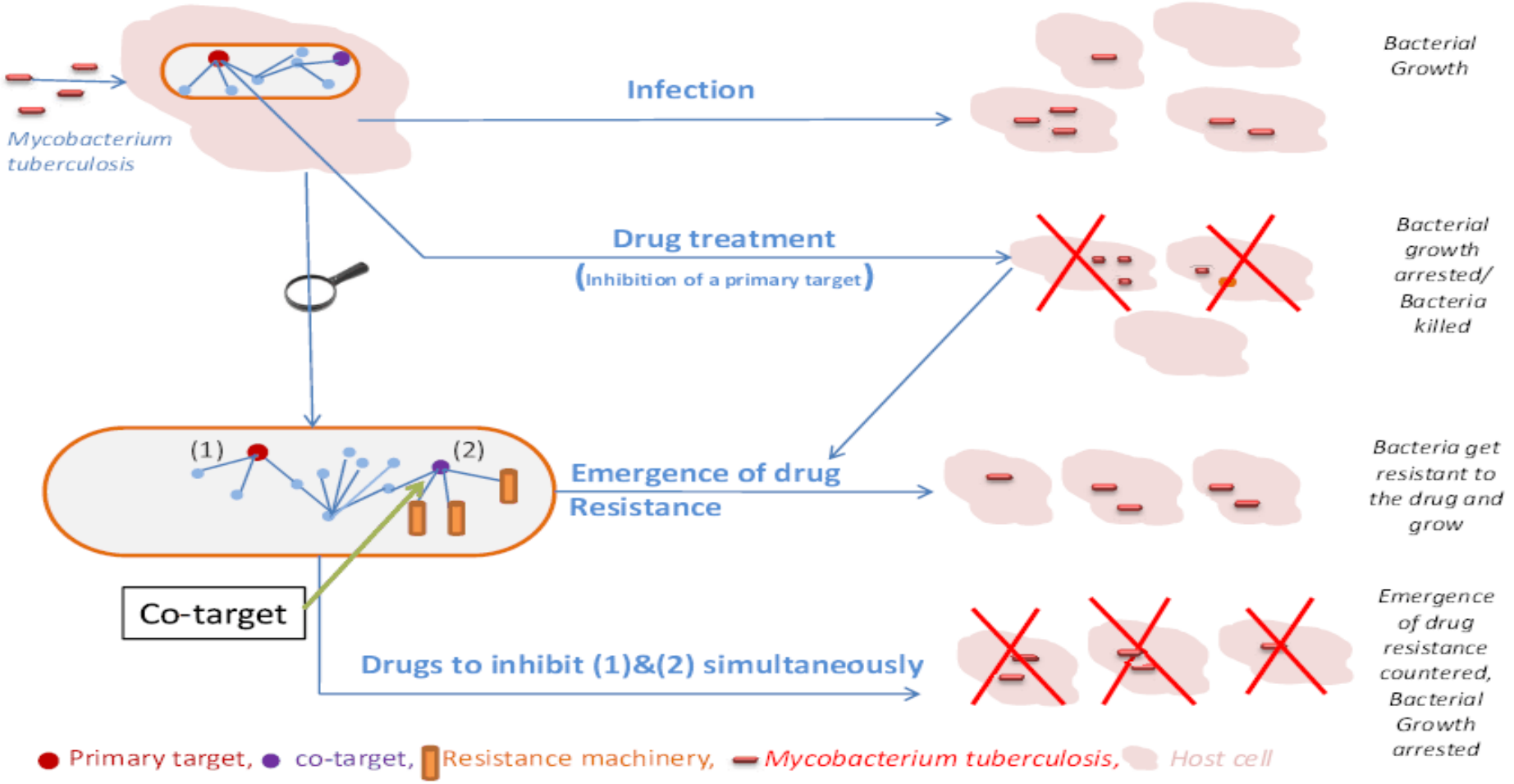
- Mycobacterium tuberculosis interactome analysis unravels potential pathways to drug resistance

  Karthik Raman, Nagasuma Chandra

  2008 BMC Microbiology

The idea of co-target

- co-targets being defined as protein(s) that need to be simultaneously inhibited along with the intended target(s), to check emergence of resistance to a given drug. (Raman and Chandra 2009)



The schema of R&C experiment

STRING protein–protein linkages
gene neighbourhood, fusion, co-occurence, co-expression, experimental evidence, databases, text mining

Construction of *M. tuberculosis* Interactome
3,925 nodes, 29,664 undirected edges

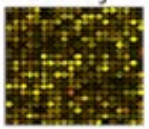
Source: Drug Targets

Identification of 'source' and 'sink' proteins

Sink: Curated Resistance Proteins
74 proteins — SOS:9, Cytochromes:36, Pumps:25, Horizontal Gene Transfer:4

Network of shortest paths: MAP-RES
616 nodes, 1,683 edges

Microarray data



Scoring and ranking of shortest paths

Edge centrality

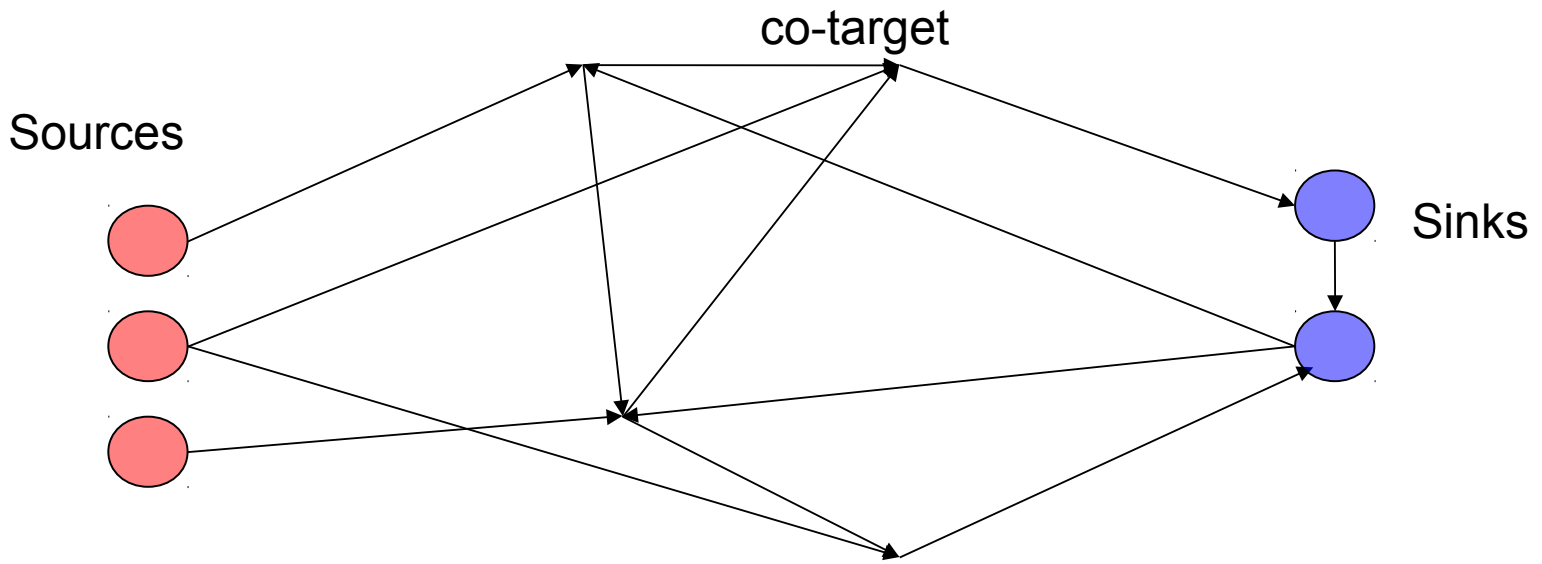
Analysis of shortest paths

Identification of pathways to resistance

'Co-targets' as a new concept to counter resistance

Define of source and sink (R&C)

- **Sources** – a set of proteins that are drug targets (26 proteins on MAP Mycolic Acid Pathway)
- **Sinks** – a set of proteins that are involved in resistance mechanisms (74 proteins divided on 4 subgroups: SOS, HGT, Cytochromes, pumps)
- **Network** – PPI from STRING 7



Key results obtained by Raman and Chandra

- Scored paths indicate most credible pathways for the emergence of drug resistance (often appearing on the shortest paths)
- Several proteins that are potentially good co-targets

Weak points of Raman and Chandra paper

- Has not been presented any method allows to verify the correctness and significance of results (co-targets and pathways)
- The use of approach based on shortest paths poorly corresponds to the parallelism of biological processes
- Has not been proposed any automatic method which allows indicate co-targets
- Selection of sources and sinks – it is not clear what biological process correspond to the movement of proteins that are drug targets to proteins involved in processes of resistance

Our definition of co-targets based on flow networks

- Main idea based on the assumption is that flow networks are better corresponds to the parallelism of biological processes
- In this method we identify co-targets by remove proteins from network and analyse of changes, in flow. This approach is appropriate to process of inhibition of the proteins that are a drug targets.
- As an input we have: proteins graph, sources and sinks.
- We define co-targets as follows: for a fixed natural number k, k-co-target is a set of k proteins whose removal minimizes the maximal flow.
- In particular, for k = 1, co-target is a protein which removal minimizes the maximal flow.

Implemented tools

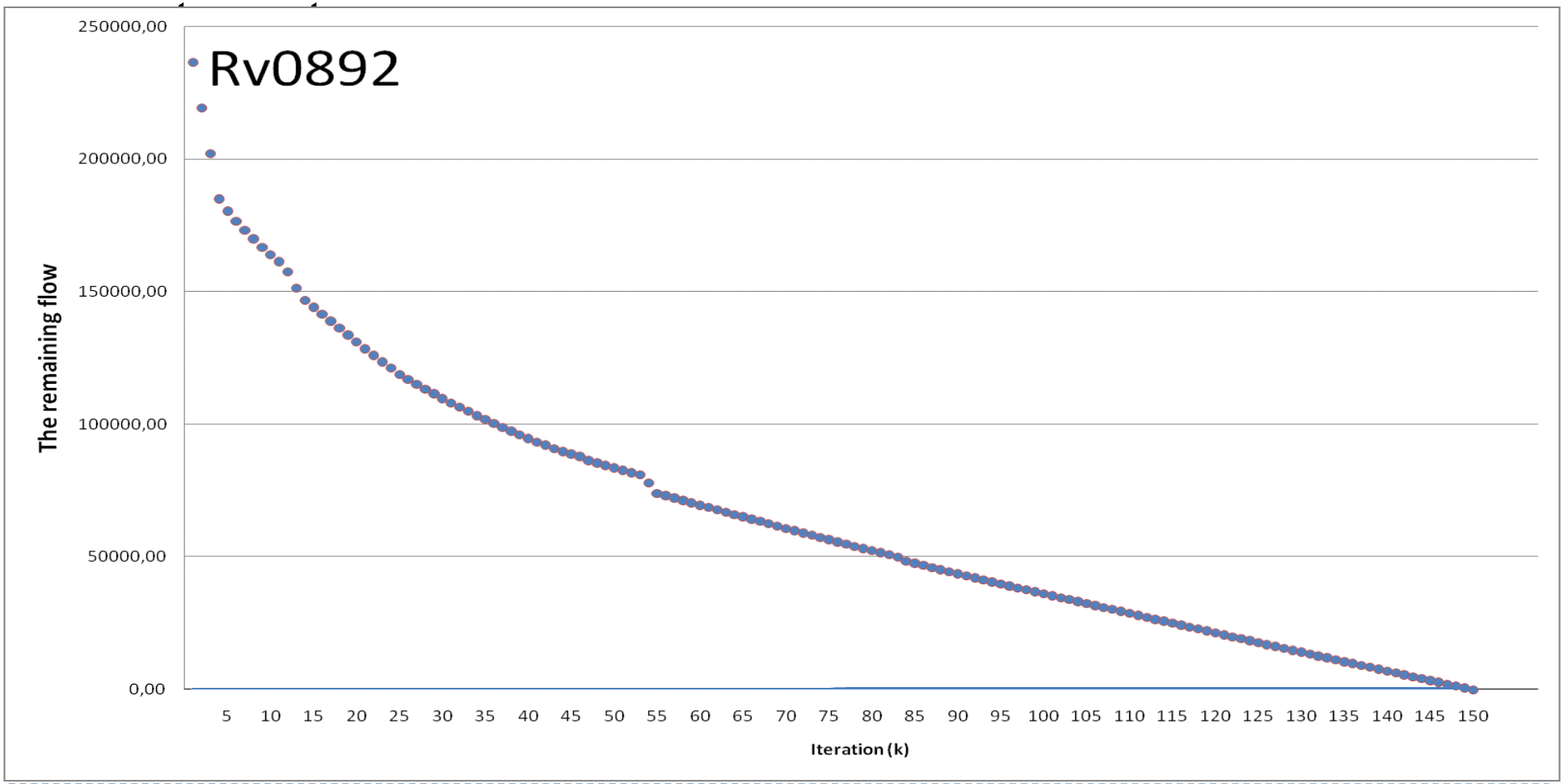
- **Two algorithms:**
  - Algorithm which tests all possible combinations (complexity: {n \choose k} * maxFlow()).
  - Algorithm which calculating the smallest set of proteins whose removal inhibits the flow.
- **Two heuristics** (finding k-co-targets, for each k)**:**
  - Heuristic 1: it starts by finding a node whose removal maximally decreases the flow. Next, iteratively it expands a given set of nodes by adding a new node whose removal maximally decreases the remaining flow.
  - Heuristic 2: it starts by finding a set of proteins whose removal inhibits the flow, and then sorting this set iteratively selects proteins whose removal maximally decreases the remaining flow.

Comparision with R&C - data

- To test my method I prepared few tests to compare with R&C paper
- **Sources** – 26 proteins on MAP (the same to R&C)
- **Sinks** – proteins involved in Cytochromes, efflux pumps, SOS, HGT, and sum all of them.
- **Network** – PPI from STRING 8.1

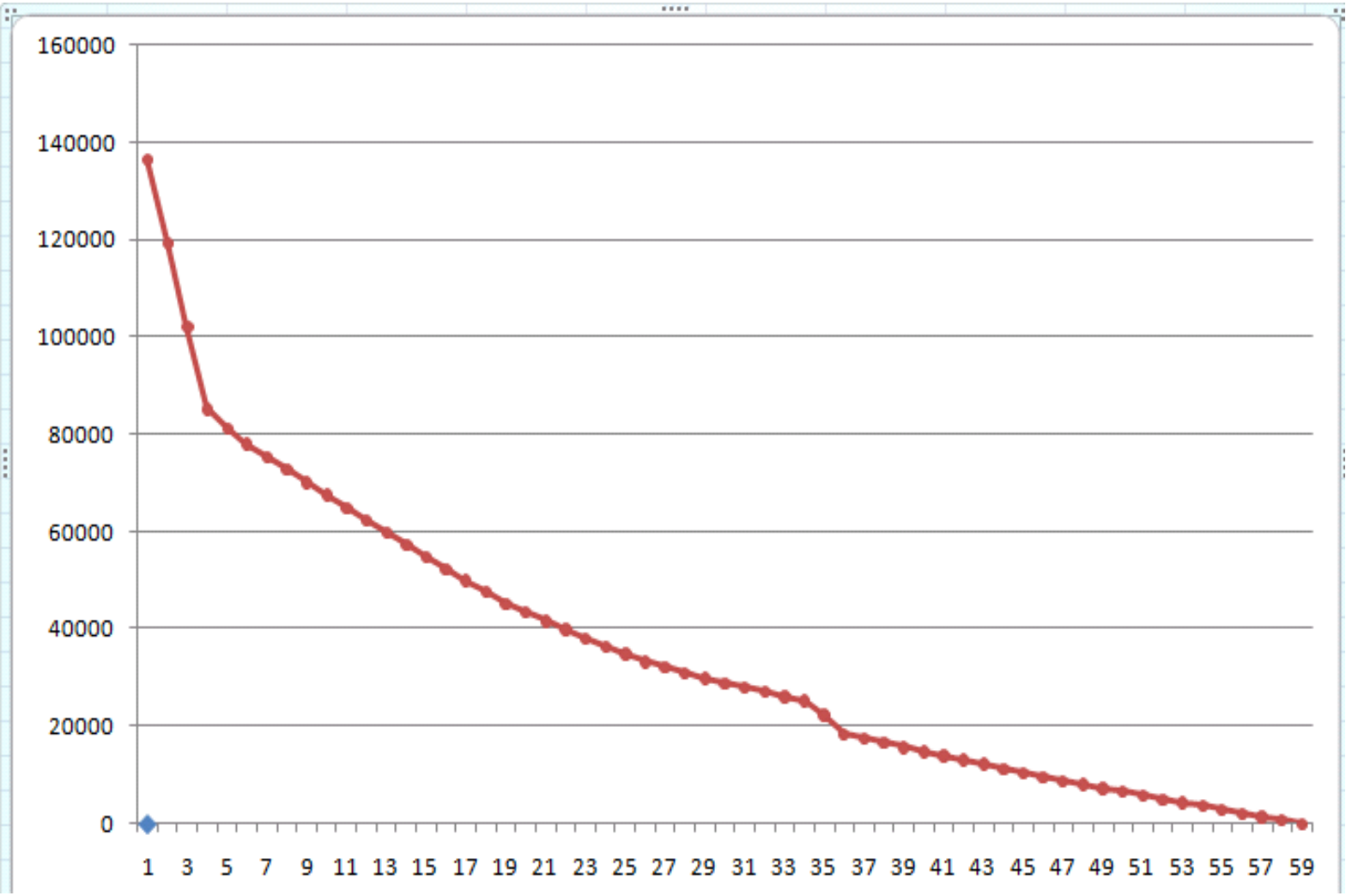
The application of our method, similarly to R&C work.

- The experiment confirmed the protein (Rv0892) identified by Raman and Chandra is a potentially good

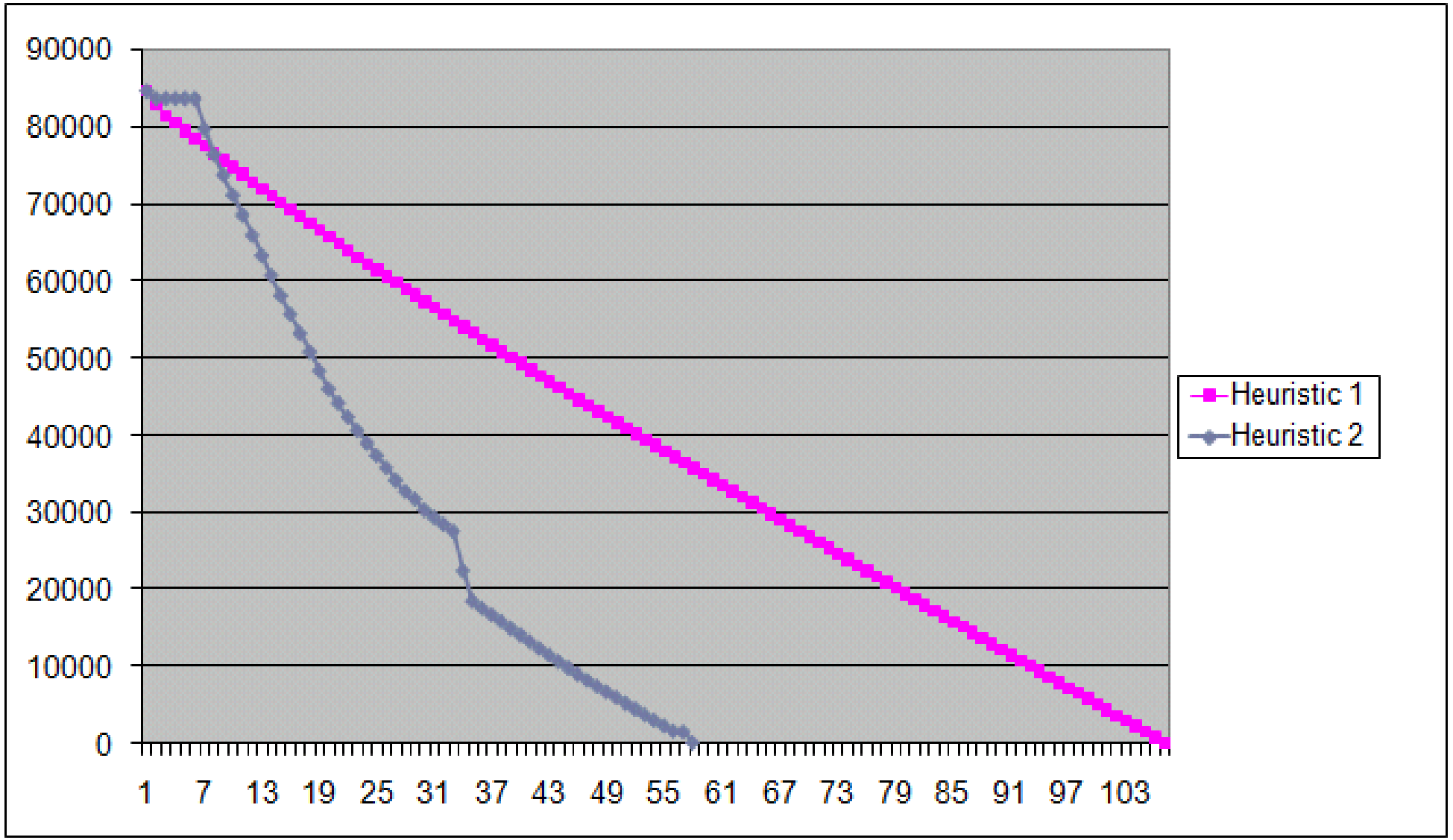


Sinks - Cytochromes

1	Rv1882c	136555
2	Rv3057c	119426
3	Rv0892	102345
4	Rv1895	85264
5	Rv0528	81333
6	Rv0524	78077
7	Rv2195	75381
8	Rv3509c	72778
9	Rv2967c	70178
10	Rv0843	67580
11	Rv2241	64982
12	Rv2496c	62384
13	Rv2497c	59786
14	Rv2199c	57329
15	Rv3003c	54889
16	Rv3002c	52454
17	Rv3470c	50054
18	Rv1820	47654
19	Rv0762c	45311
20	Rv1617	43513
21	Rv1127c	41715
22	Rv0853c	39917
23	Rv2332	38119
24	Rv2987c	36519
25	Rv2988c	34919

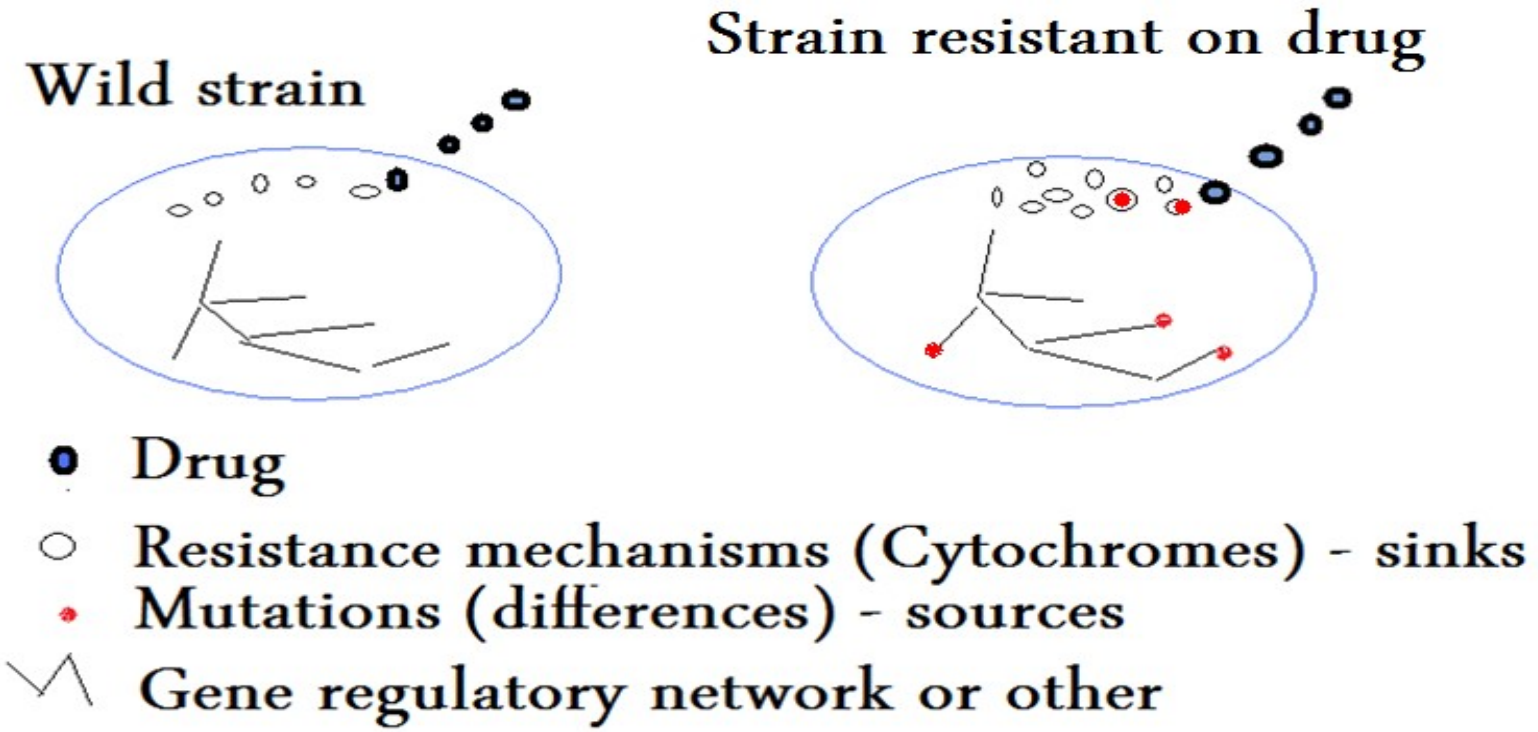


Comparision between heuristics



Selection of sources and sinks

- **sources** are mutated proteins (differences between wild and resistant strains)
- **sinks** are proteins directly involved in drug resistance mechanisms (for example Cytochromes P450 or efflux pumps)



Selection of sources and sinks – new concept

- Sources – mutated drug targets
- Sinks – some important proteins for bacteria, for example: transcript factors or proteins involved in lipid structure formation

Important tasks

- In order to obtain reliable results using the described approach we should solve the problem of choice:
  - Sources
  - Sinks
  - Network
- BigTask: Method which allows to verify the obtained co-targets, possibilities:
  - comparison of results with Raman and Chandra paper
  - clinical experiments examining the dynamics of emerging resistance process in strains with inhibited co-targets (compared to conditions without stress)
  - checking whether the co-targets are related with the emergence of resistance in other species
  - ?? ...

Finding important proteins in bacteria

- Basic idea – the most important proteins are hubs in PPI network
- We can define score for protein based on method of finding co-targets, for $$S(p) = \sum_{\text{source: } s \in V} \sum_{\text{sink: } t \in V} w_{s,t} 1_{\{p \text{ is a co-target}\}}$$
  - This approach taking into account global importance of protein.
  - High correlation between protein degree and this score could be an evidence that my method gives important proteins.
  - Proteins with high score and low degree are potentially interesting for further research.

Identifying of important mutations

- Fully sequenced TB genomes: H37Rv, H37Ra, CDC1551, F11, KZN 1435 (MDR)
- Genome analysis of multi- and extensively-drug-resistant tuberculosis from KwaZulu-Natal, South Africa.

  Thomas R. Ioerger, Sunwoo Koo, Eun-Gyu No, Chen X., Larsen M.H., Jacobs W.R. Jr, Pillay M., Sturm A.W., Sacchettini J.C.

  PLoS, 2009 Nov 5
- *The new completely sequenced and annotated genomes of isolates from patients in Durban (KwaZulu-Natal):*
  - *KZN V4207 (susceptible to drugs)*
  - *KZN V2475 (MDR)*
  - *KZN R506 (XDR).*

Thank you for your attention