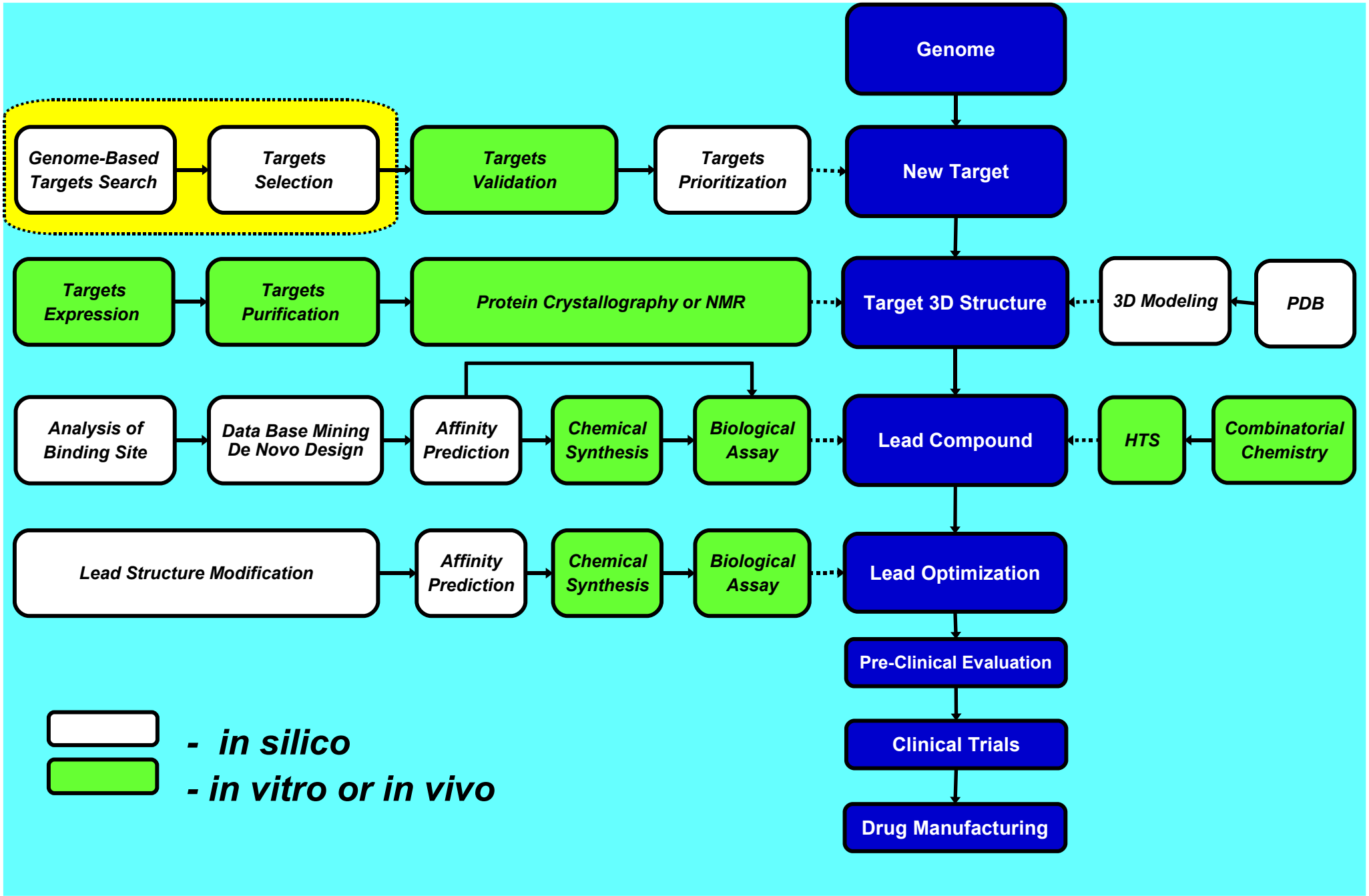
V.N.Orekhovich Institute of Biomedical Chemistry RAMS, Moscow, Russia

PLATFORM «FROM GENE TO LEAD COMPOUND»: INTEGRATION IN SILICO AND IN VITRO TECHNOLOGIES

Prof. A. S. Ivanov

Novosibirsk - 2008

Integral Pipeline “From Gene to Drug”



Comparative genomes analysis

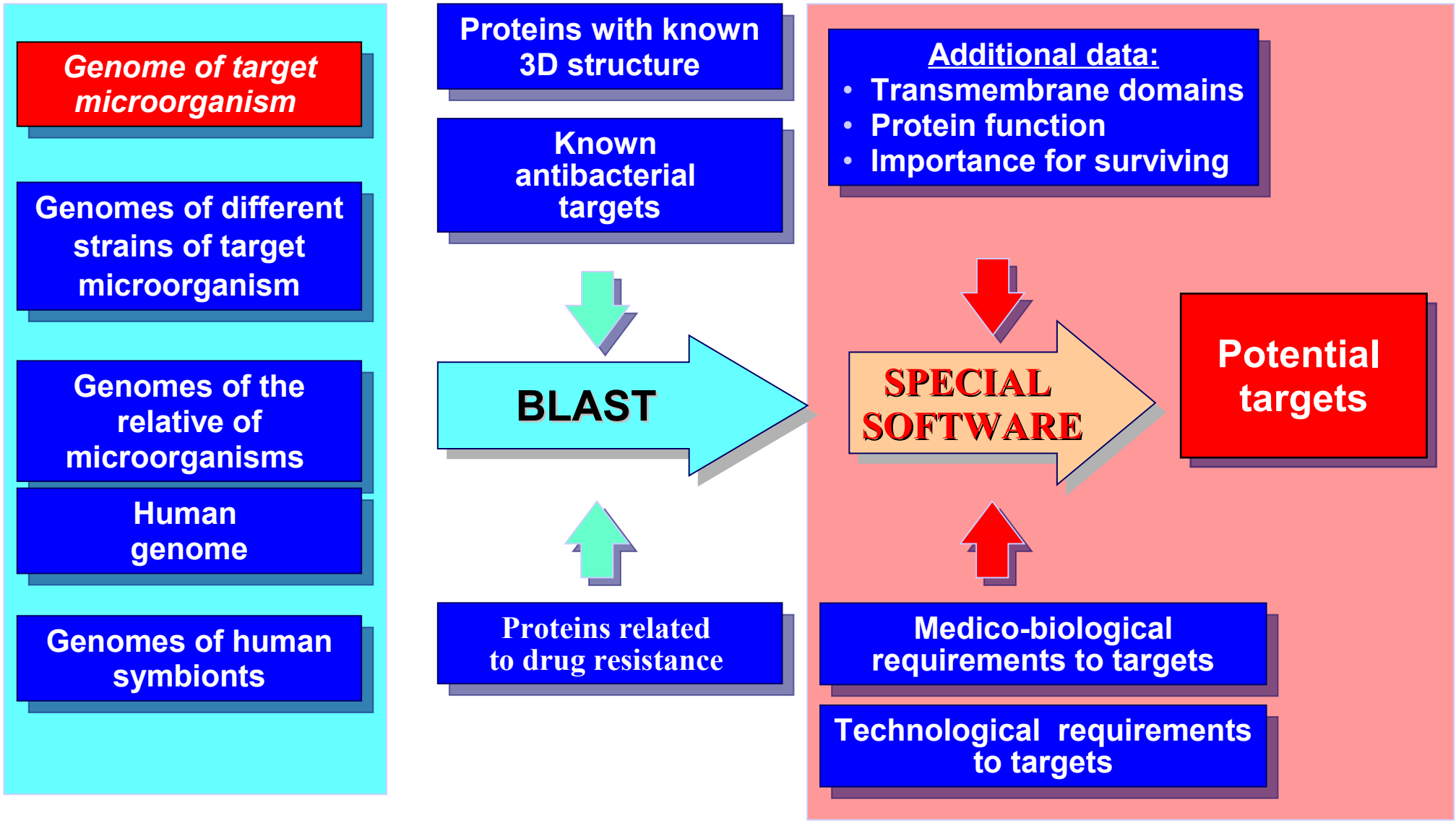
Let's consider the example of molecular targets search for new antibacterial drugs

Requirements of “IDEAL” antimicrobial agent and to its target

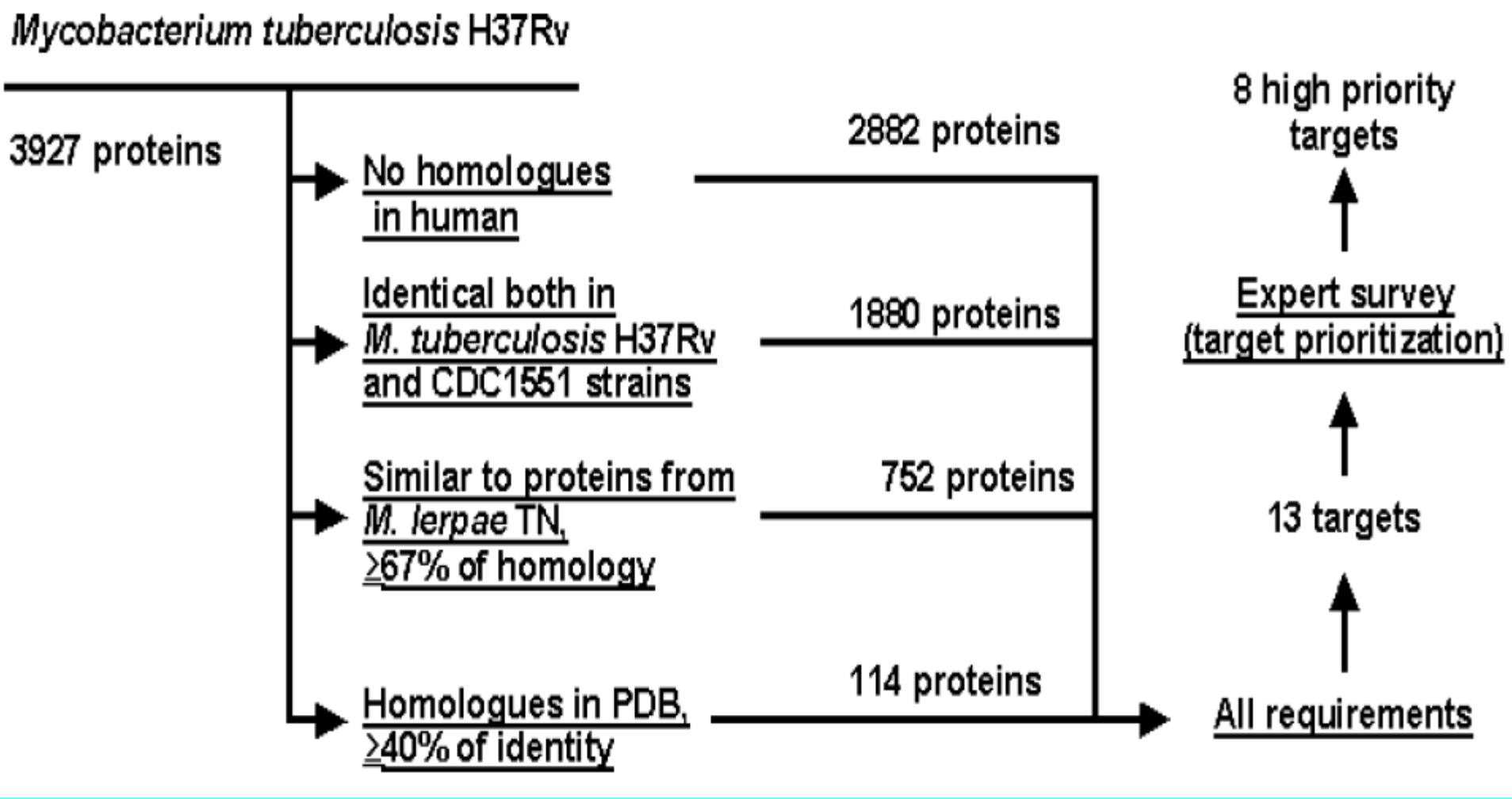
Drug	Target
Biomedical requirements	
Effective suppression of growth and reproduction of micro-organism	Important for growth and reproduction
Lethality to pathogen	Essential for survival
Definite antimicrobial spectrum	Occurs in all target microbial species and strains
Selectivity: minimal host toxicity	Absent in host (human)
Selectivity: minimal alteration of normal microflora	Absent in host's (human) symbiont bacteria
Low risk of resistance	Conserved in all target strains
Technological requirements	
Target-based CADD	Available 3D structure
Definite mechanism of action	Known function

CADD, computer-aided drug discovery.

Comparative genomes analysis



Target selection in genome of Mycobacterium tuberculosis



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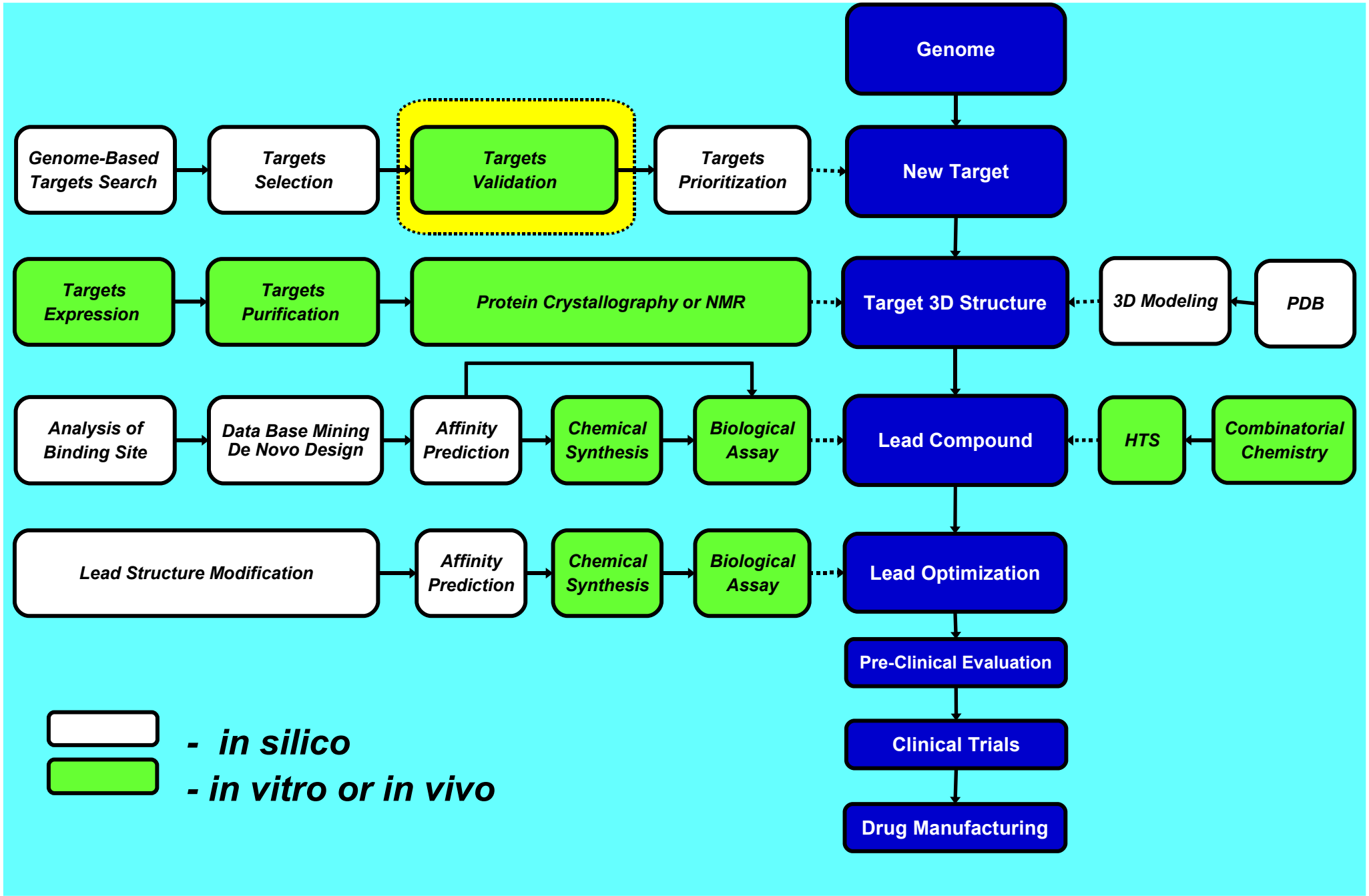
Bioinformatics Platform Development

From Gene to Lead Compound

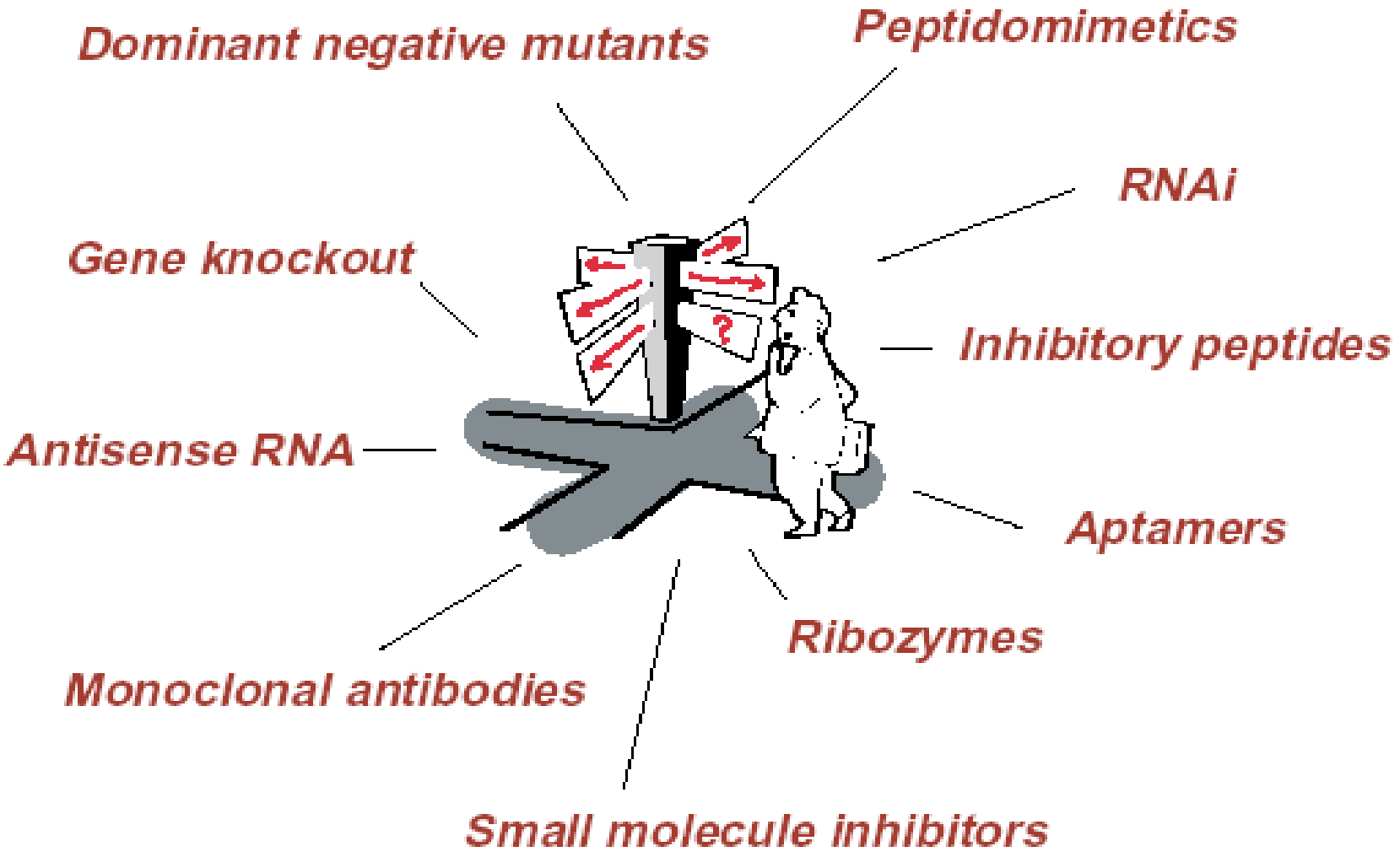
Alexis S. Ivanov, Alexander V. Veselovsky, Alexander V. Dubanov, Vladlen S. Skvortsov

Methods Mol. Biol. 2006, 316, 389-431.

Integral Pipeline “From Gene to Drug”



What method of target validation to choose?



Loss-of-function strategies: Which way to go?

Targets validation approaches

The main tasks of targets validation:

- maximal reduction in the number of potential targets

- obtainment of additional information for target prioritization

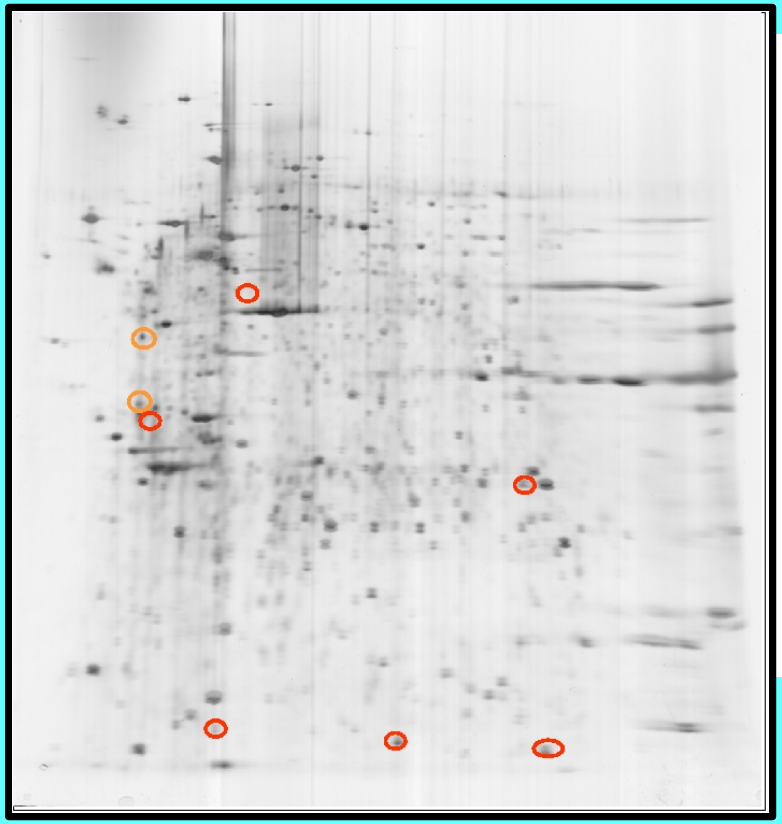
1. Proteomic methods

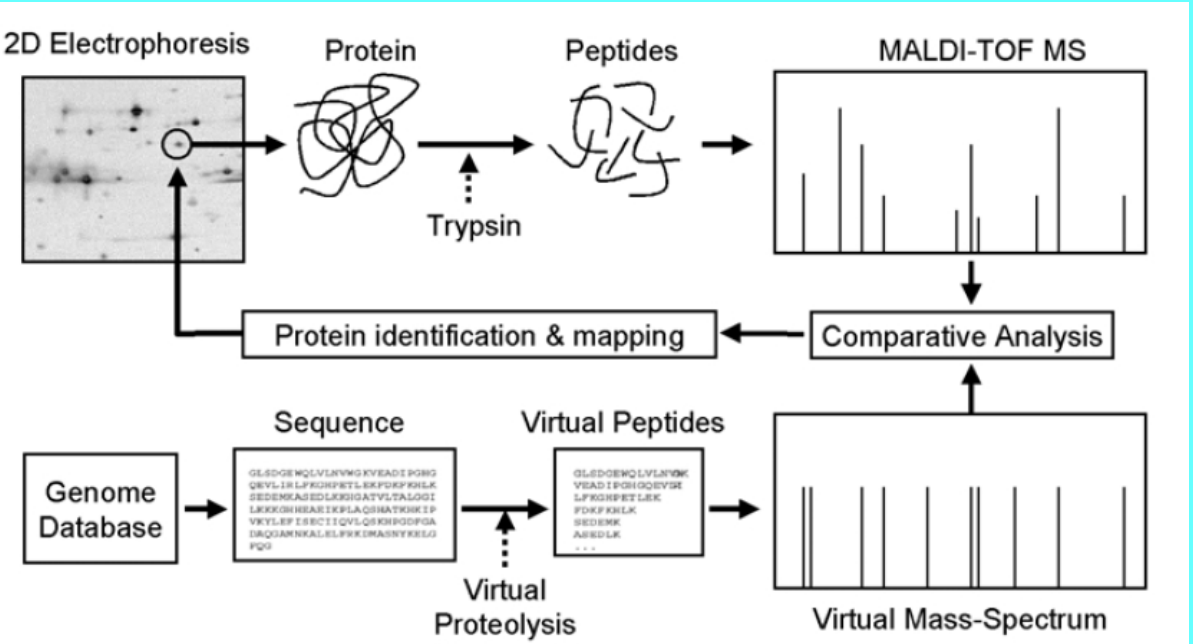
2. Genomic methods

3. Target inactivation

1. Proteomic methods of target validation

- *Examination of target proteins expression*
- *Examination of Target Expression In Different Strains*
- *Analysis of protein-protein interactions*





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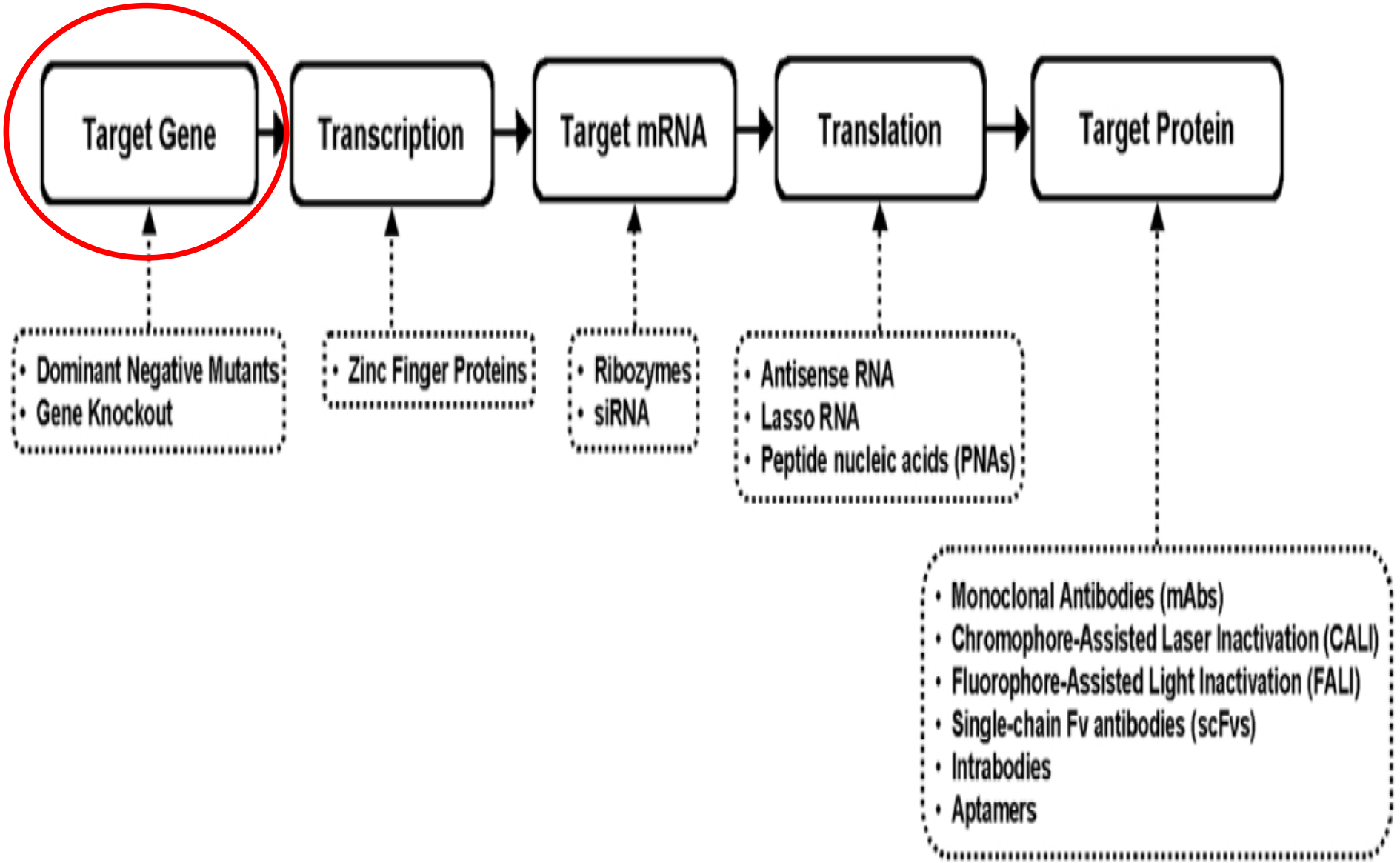

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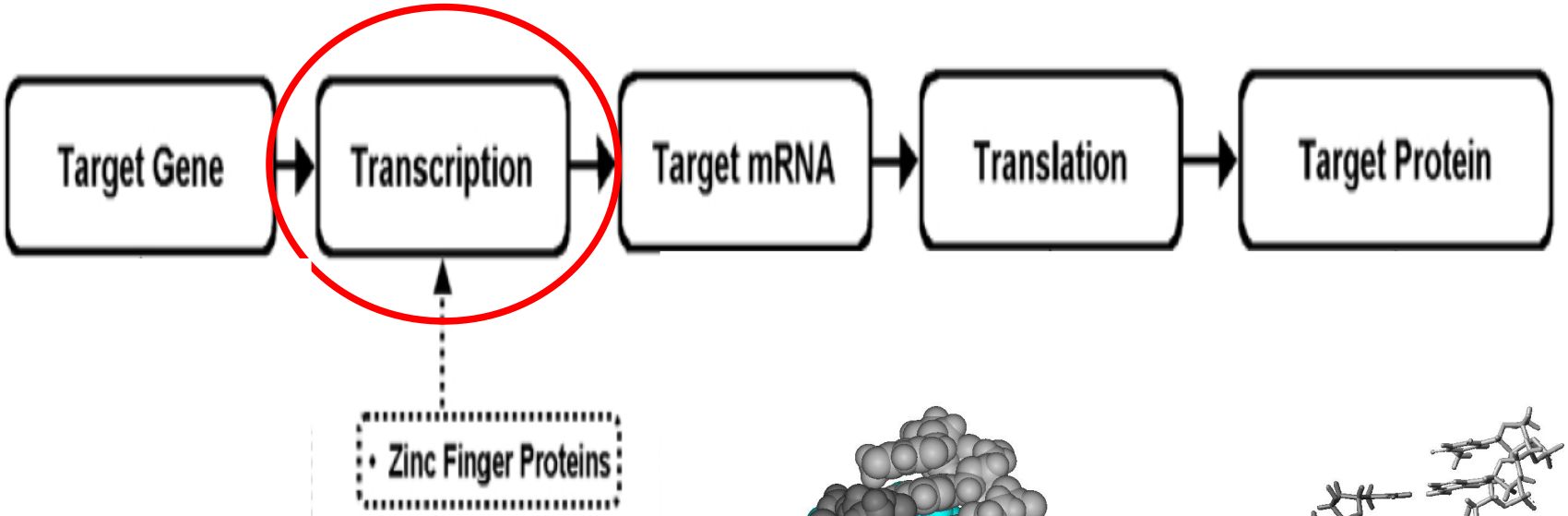

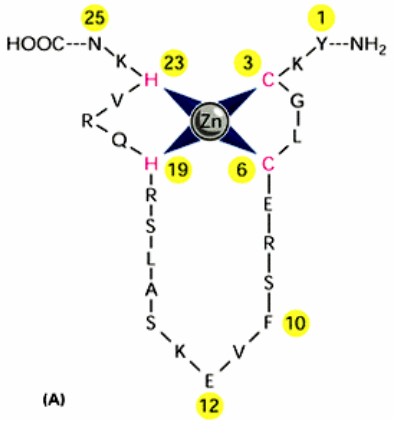

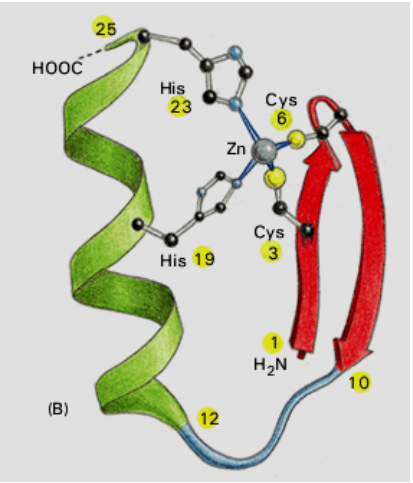

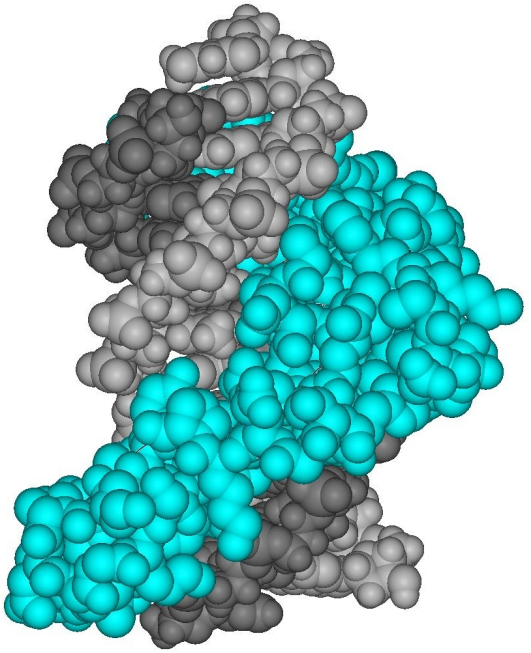

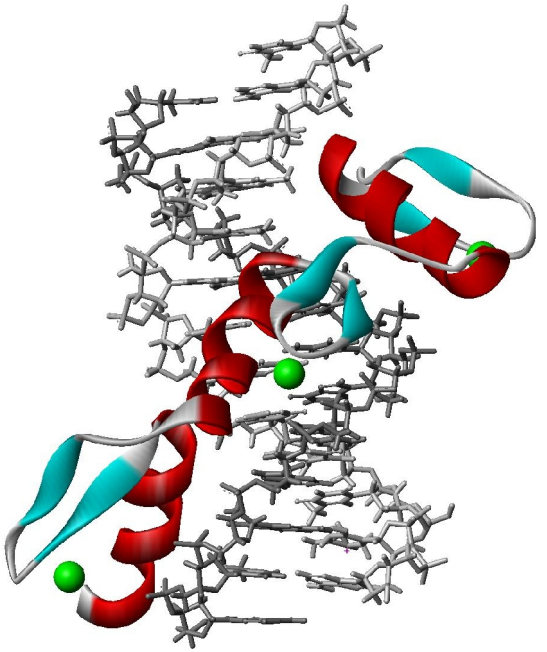

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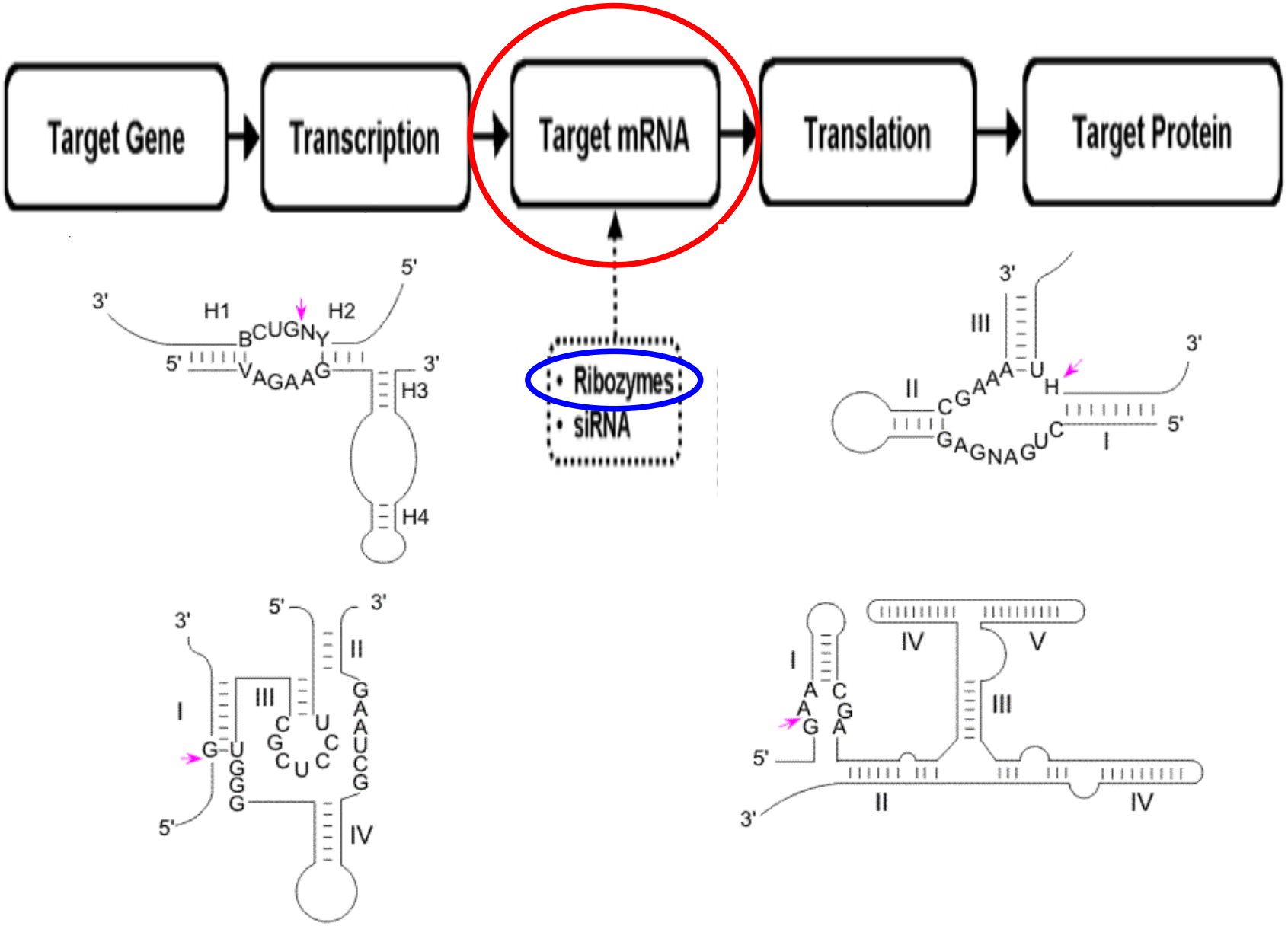

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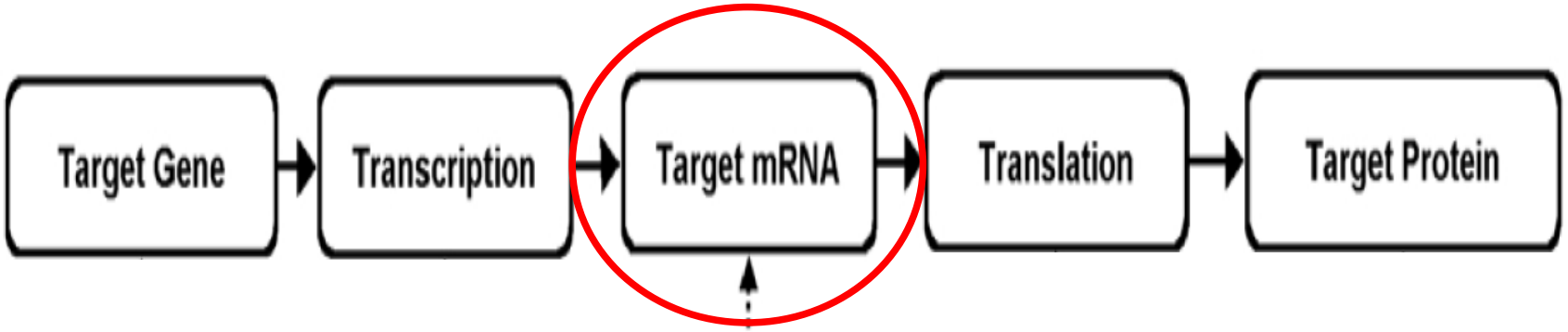
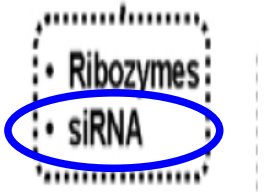
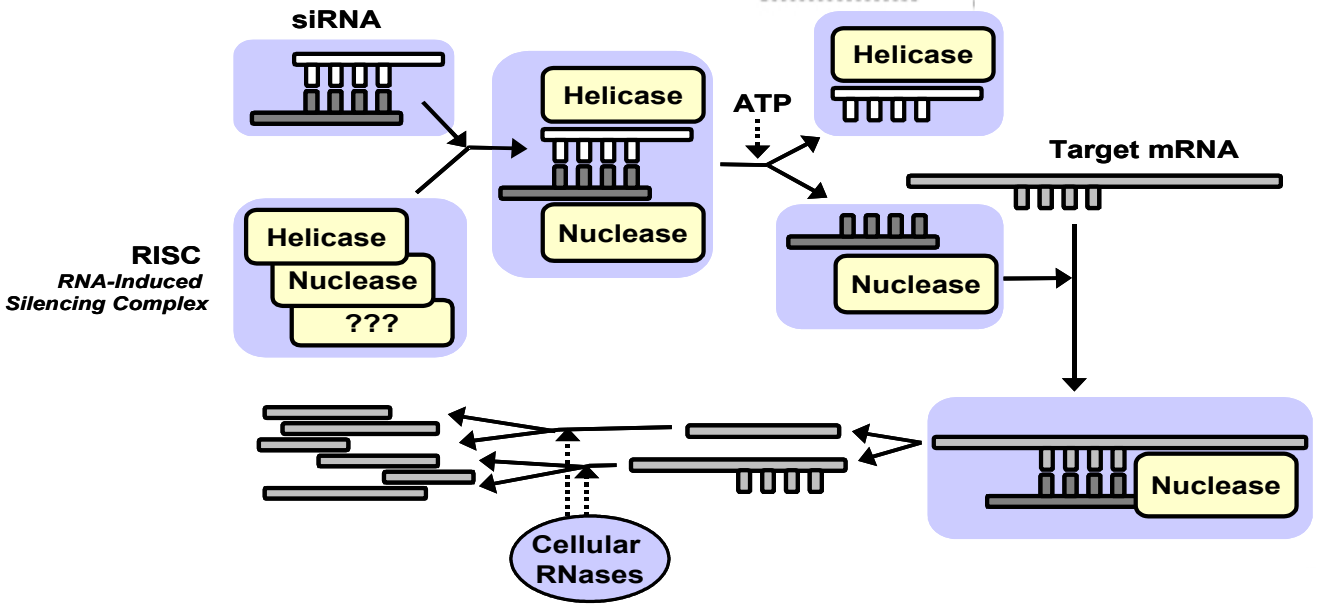


RNA interference



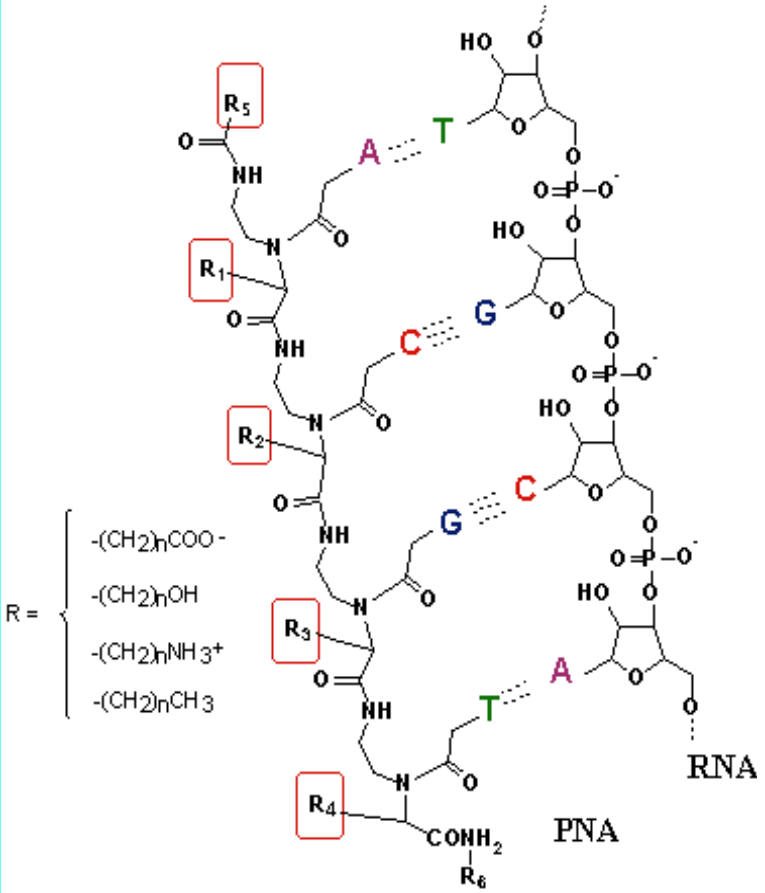


Andrew Fire

Craig C Mello

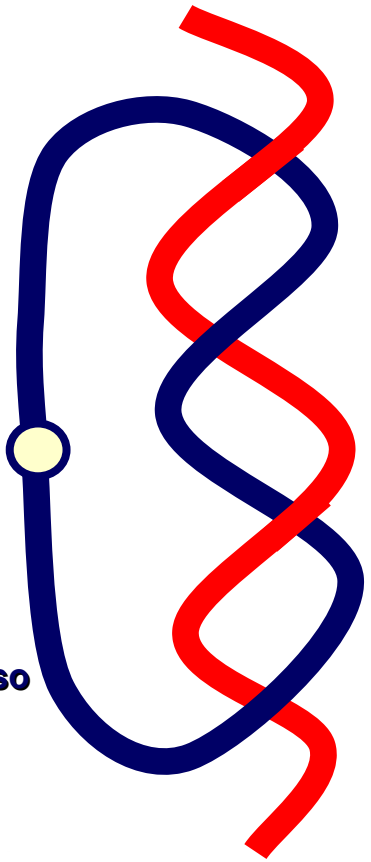
Nobel Prize 2006 in physiology and medicine

2. Genomic methods of target validation



- Antisense RNA
- Lasso RNA
- Peptide nucleic acids (PNAs)

Antisense RNA



RNA Lasso

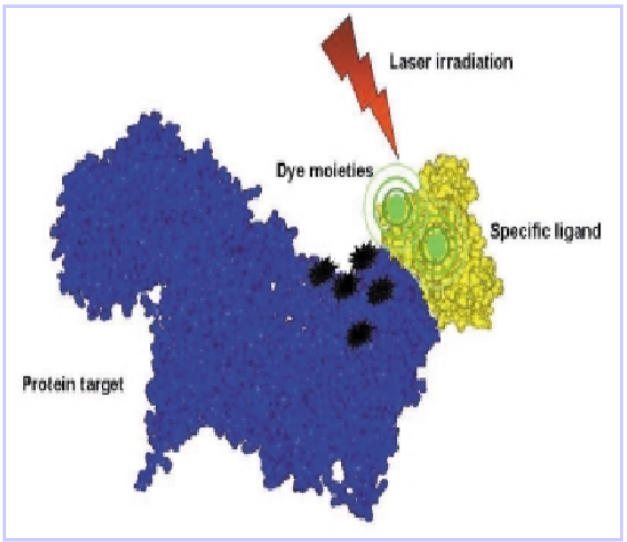
3. Target inactivation

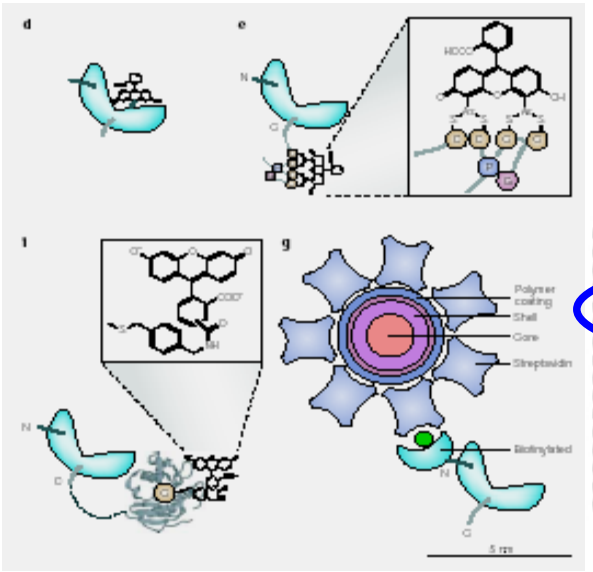
CALI

Chromophore-Assisted Laser Inactivation

FALI

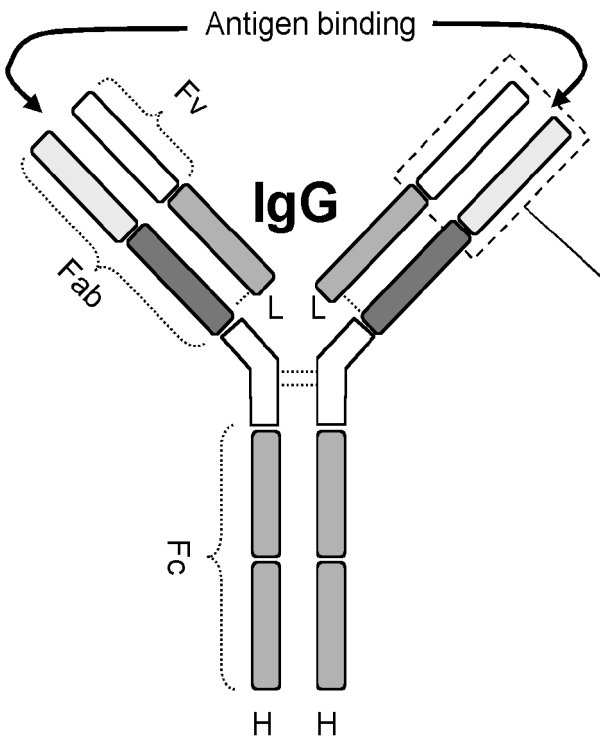
Fluorophore-Assisted Light Inactivation



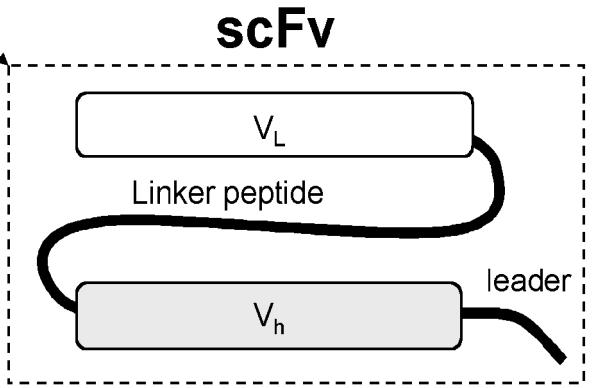


- Monoclonal Antibodies (mAbs)
- Chromophore-Assisted Laser Inactivation (CALI)
- Fluorophore-Assisted Light Inactivation (FALI)
- Single-chain Fv antibodies (scFvs)
- Intrabodies
- Aptamers

3. Target inactivation



Single-chain Fv antibodies



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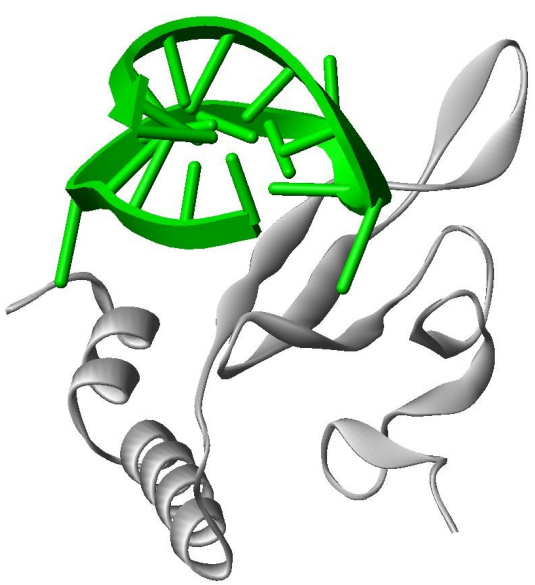

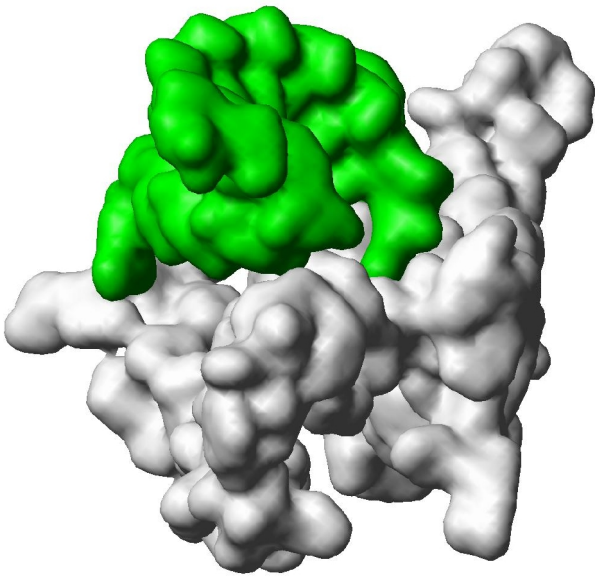

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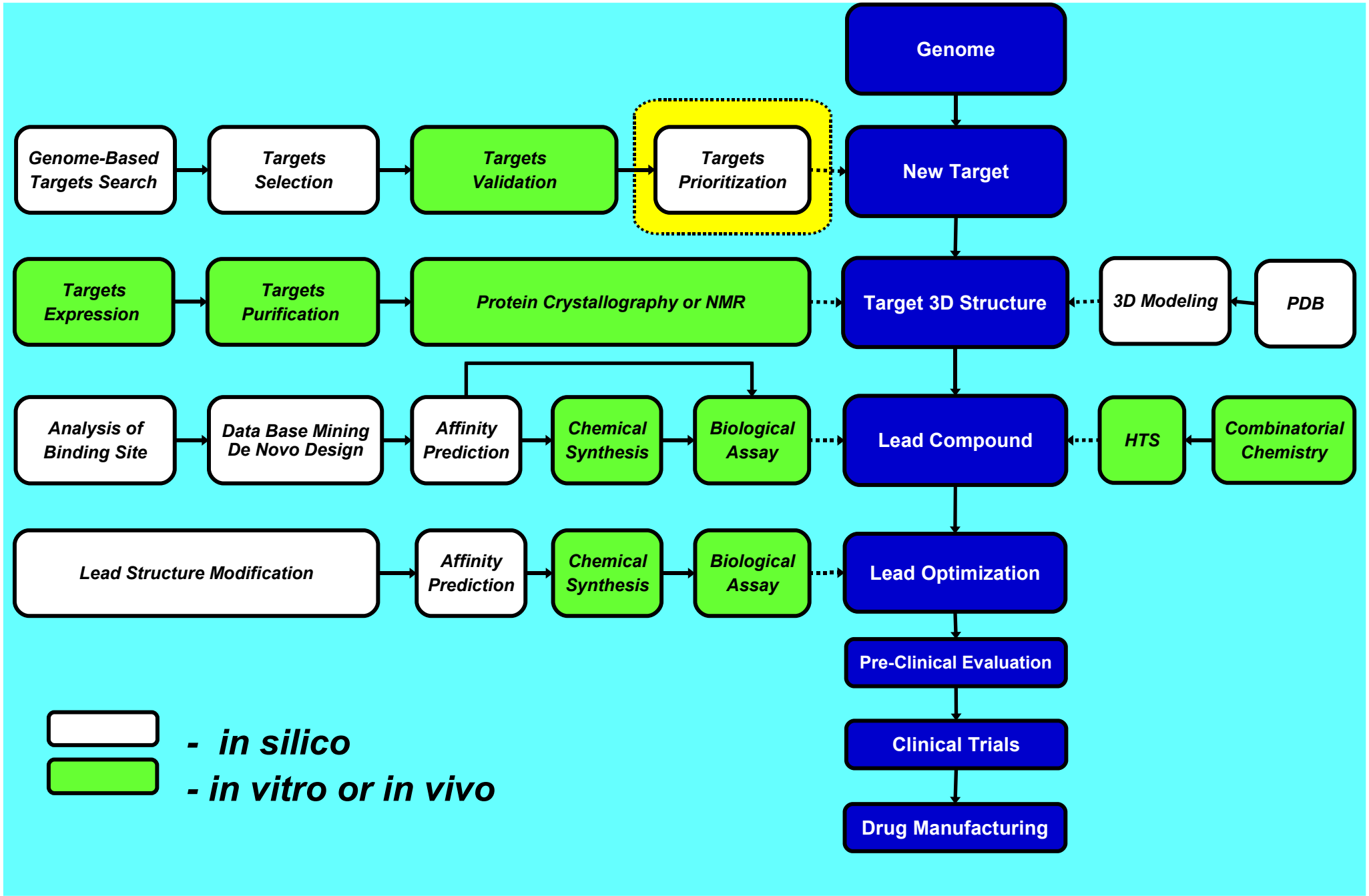

Targets Prioritization

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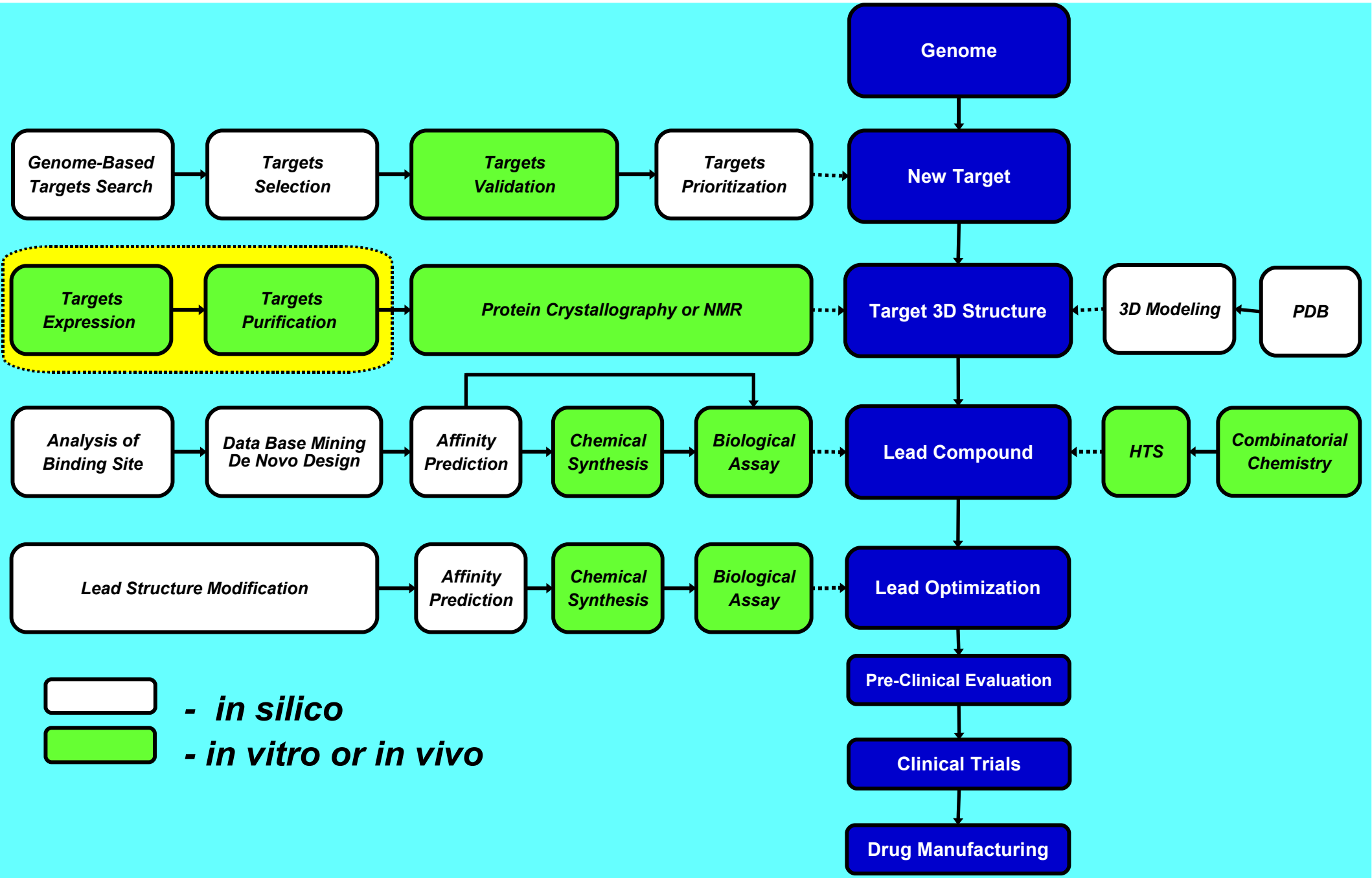


Sample of pure and native protein

Before

Biological material

Homogenization

Separation

Solubilization

Separation

Purification

Now

Gene

Vector

Microorganism

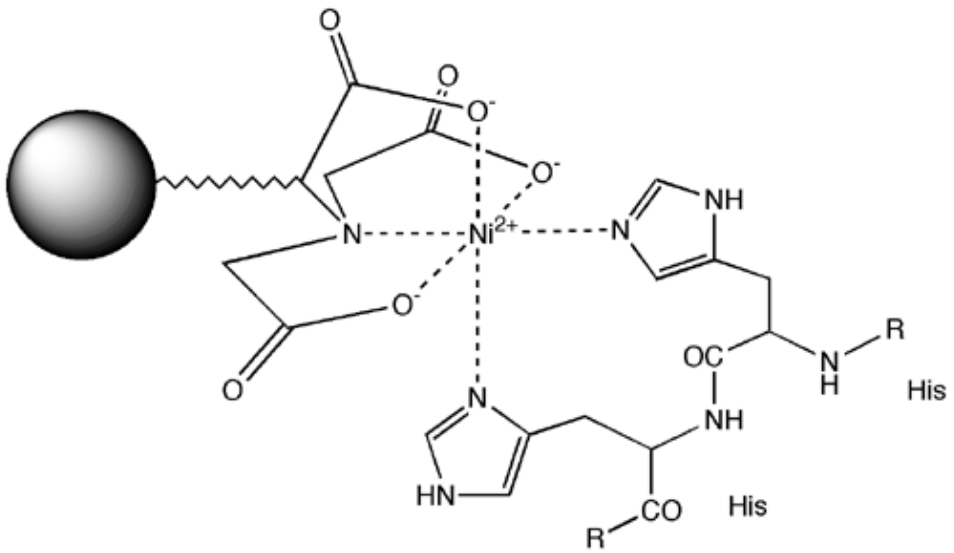
Expression

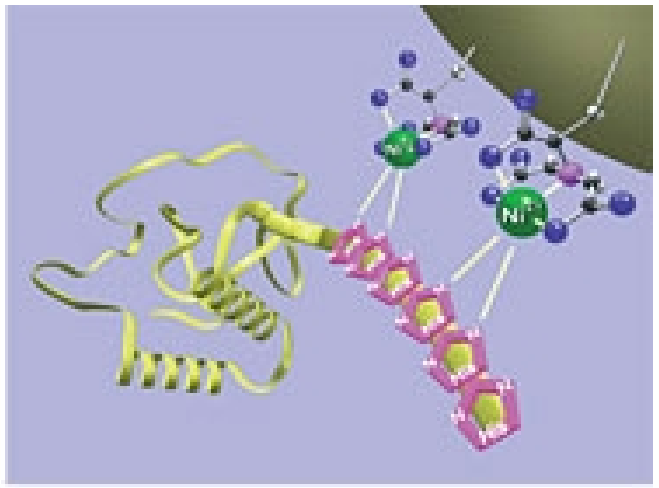
Fust protein purification using special “lable”

Popular «lables»

HisTag (6 x His)

+ Ni $^{2+}$ + NTA



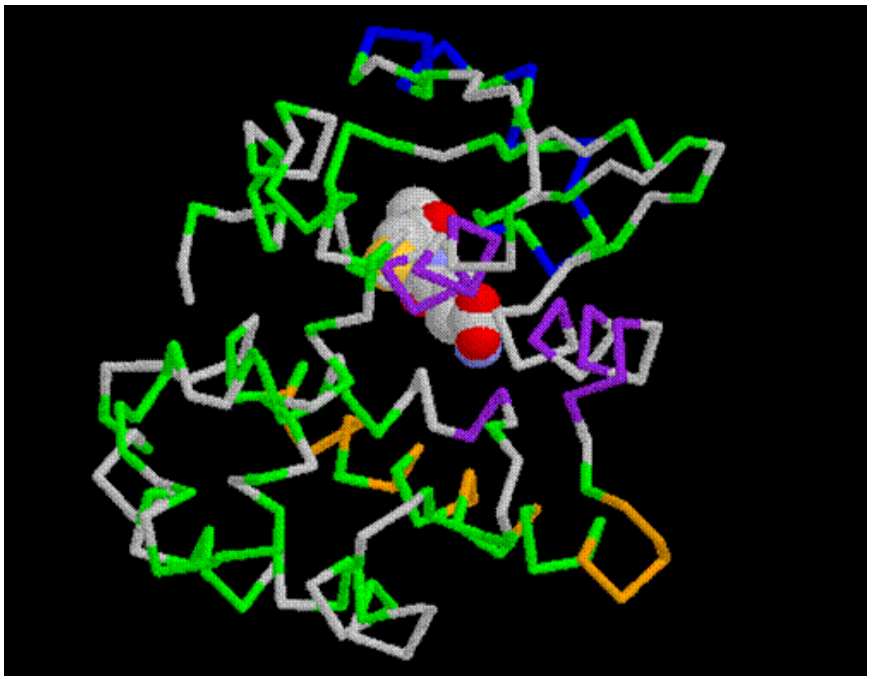


6xHistidine-tag protein binding to Ni-NTA resin

Glutathione S-Transferase (GST)

+

Glutathione (tripeptide = Glu-Cys-Gly)



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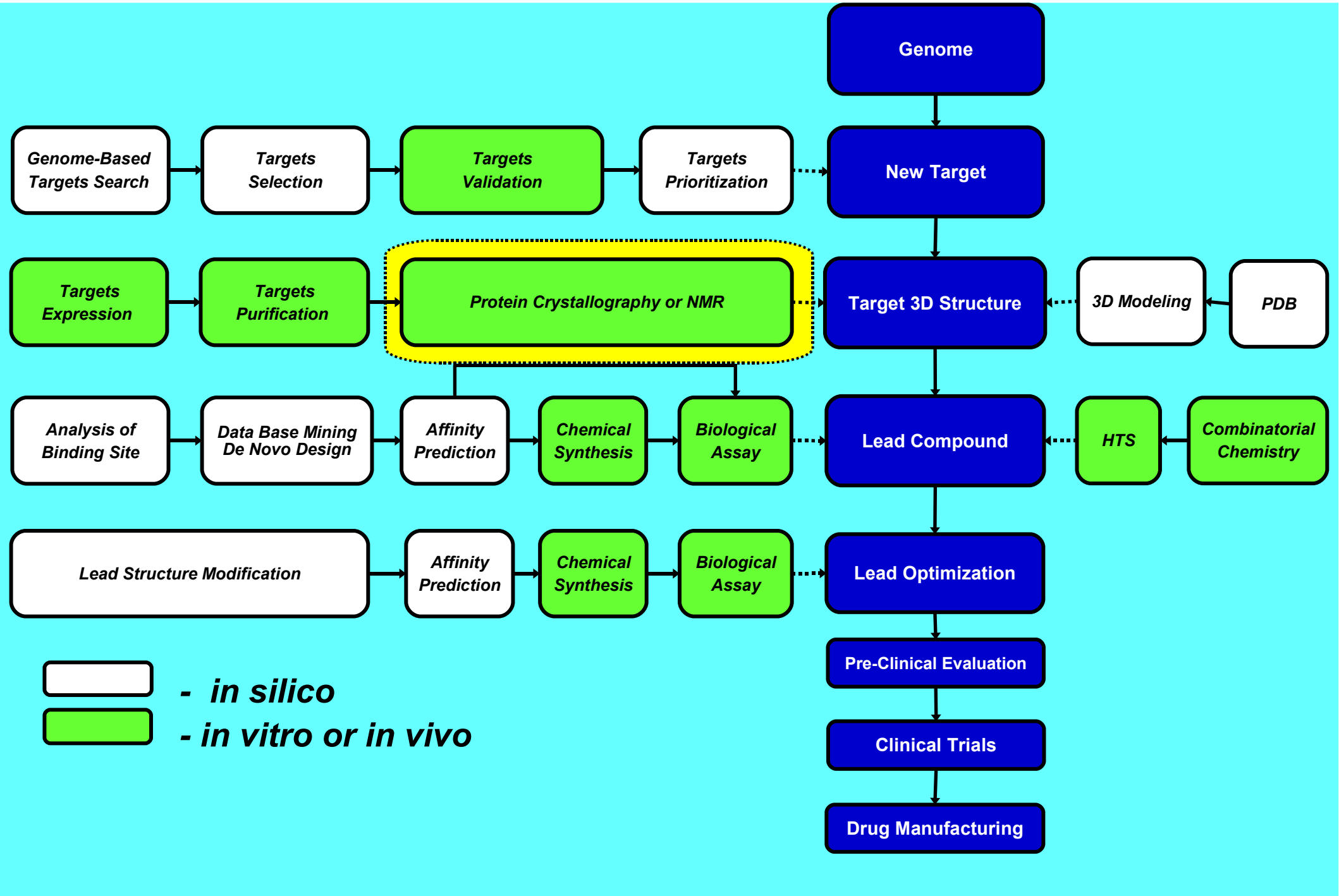
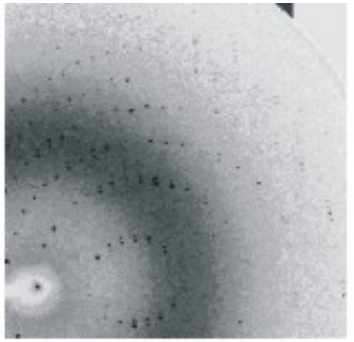
PPAT

Pure and active PPAT from M. tuberculosis

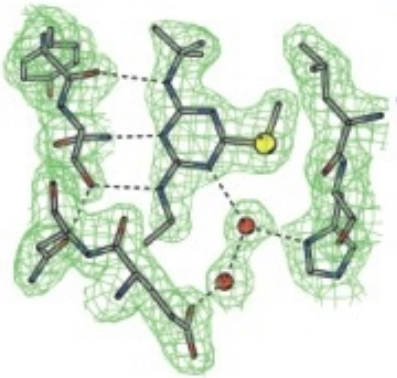
Institute of Bioorganic Chemistry (Moscow)

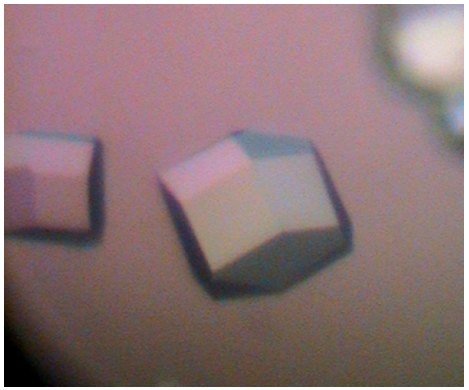
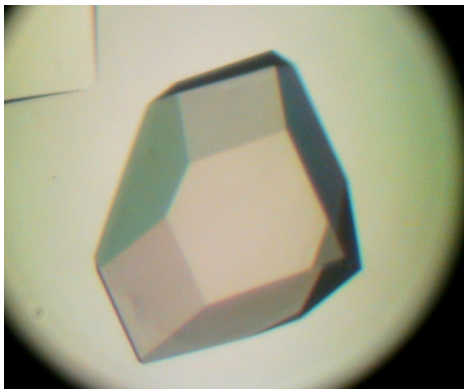
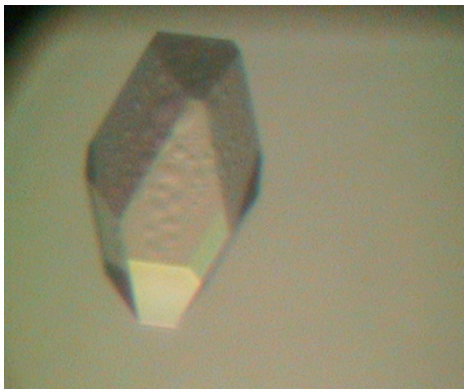
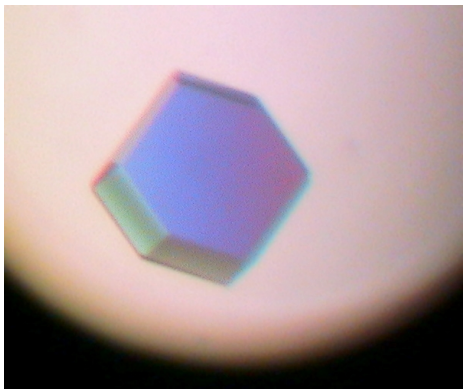
Integral Pipeline “From Gene to Drug”



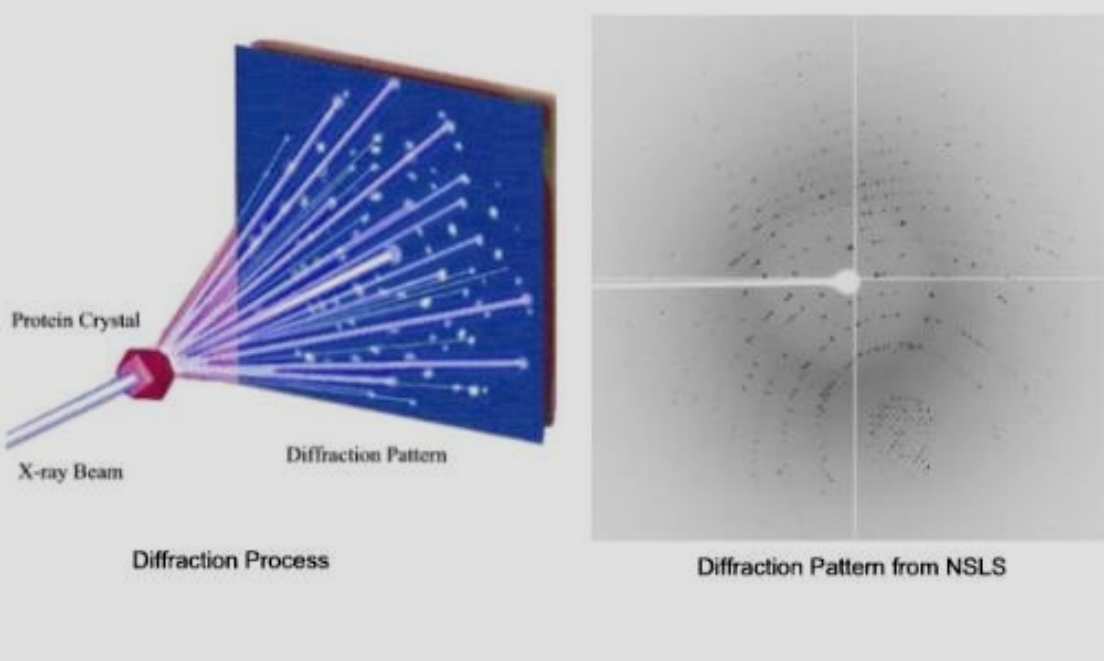


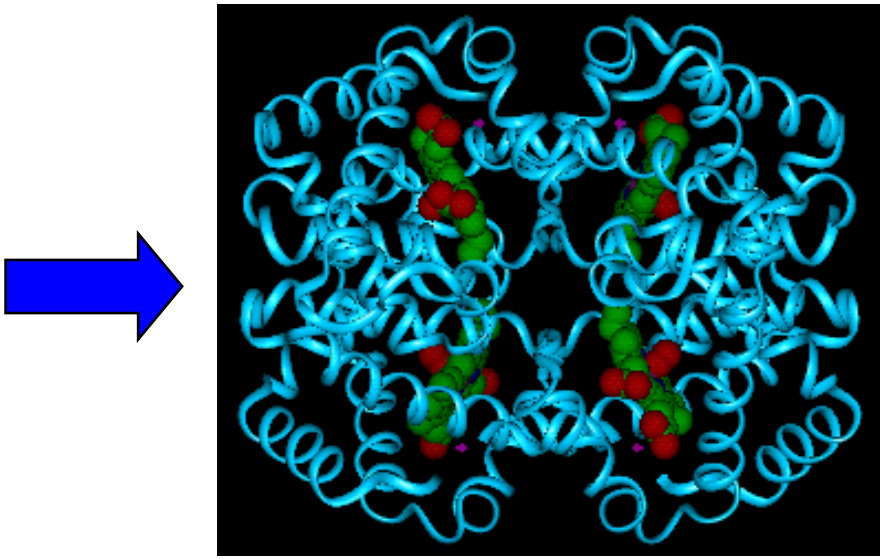
Protein Crystallography



Protein crystallography





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Pure and active PPAT from M. tuberculosis

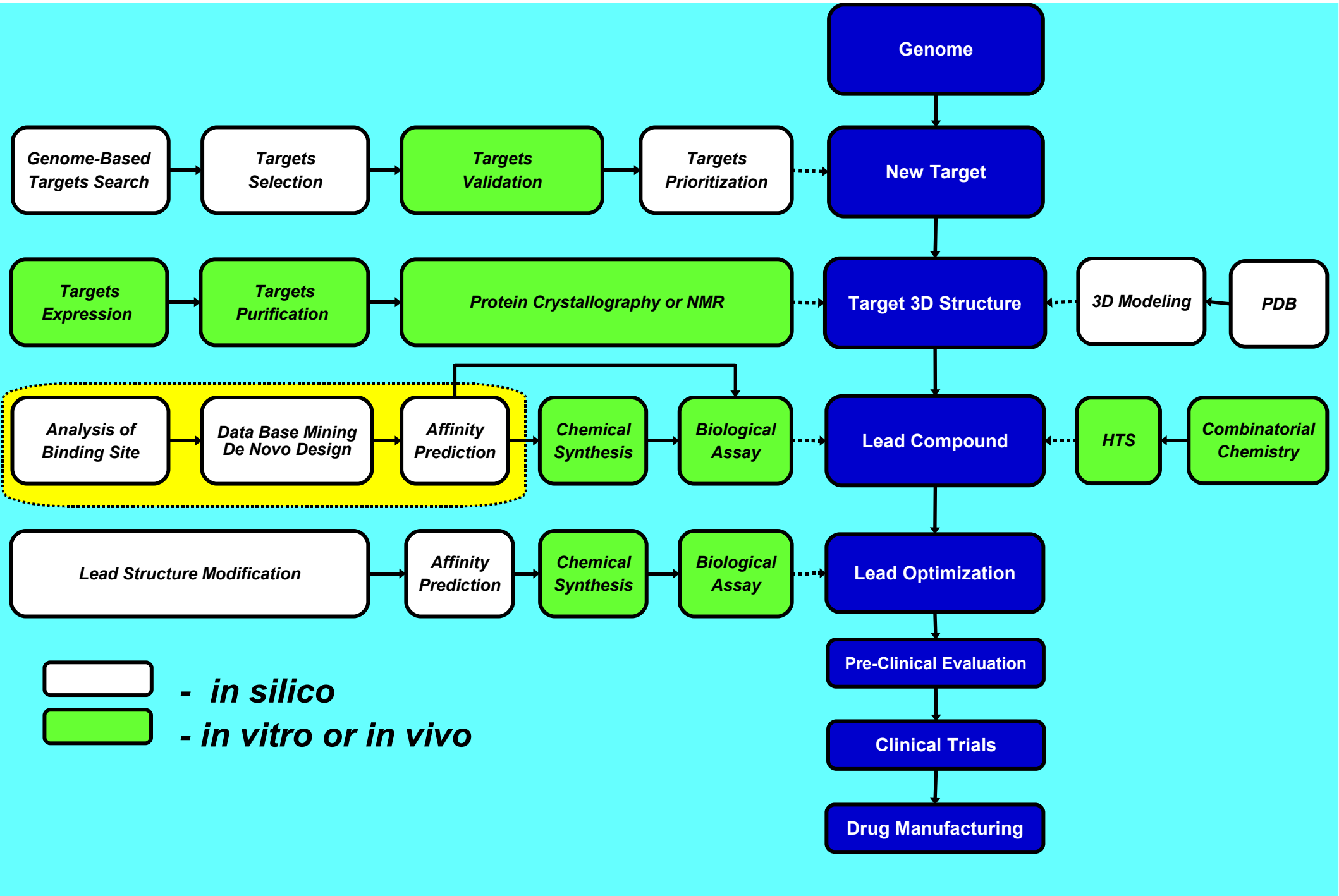
→

Institute of Crystallography (Moscow)

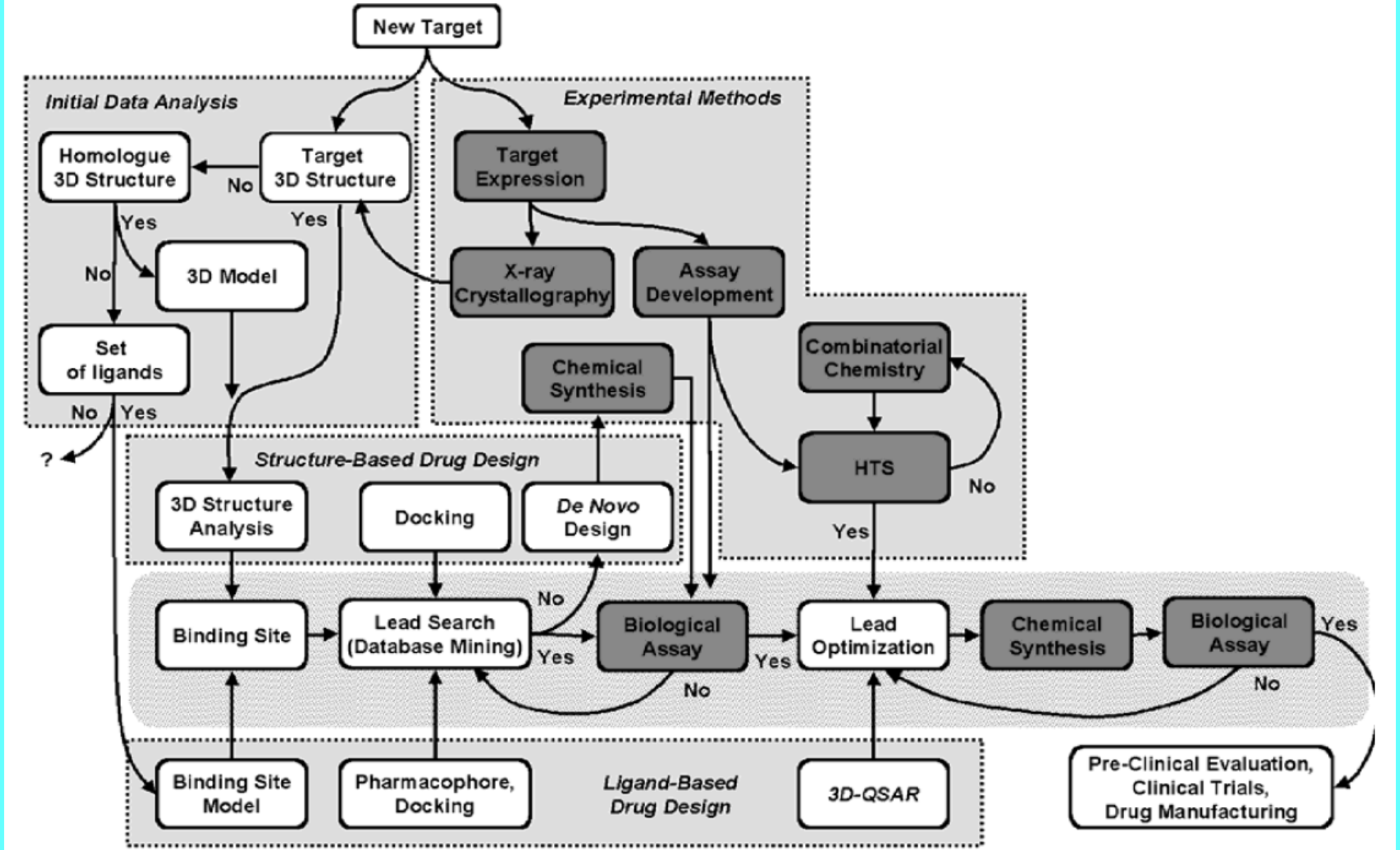
PPAT from *M. tuberculosis*

Institute of Crystallography (Moscow) → ***Crystal Structure of PPAT from M. tuberculosis***

Integral Pipeline “From Gene to Drug”

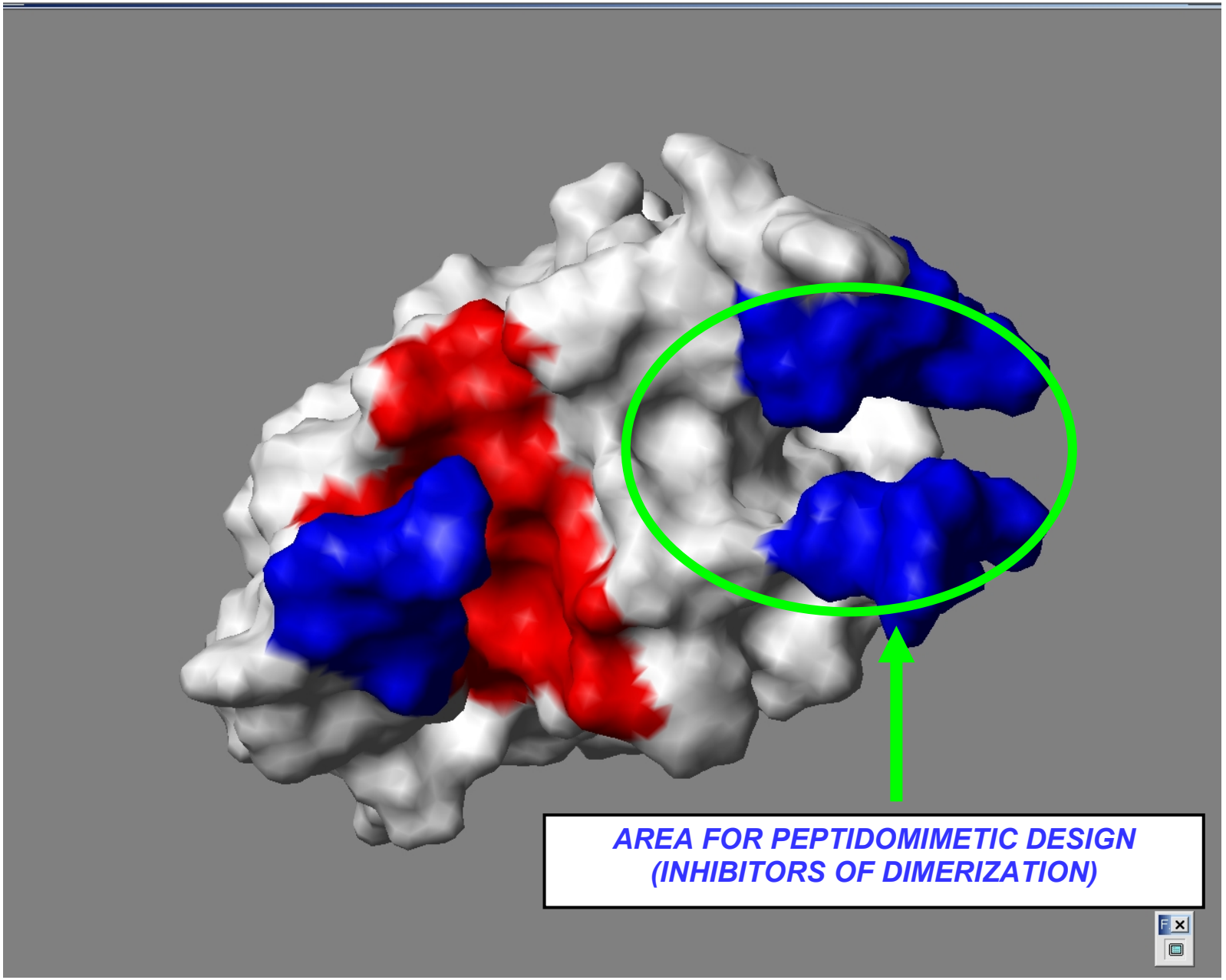


Strategy of Computer-Aided Drug Design

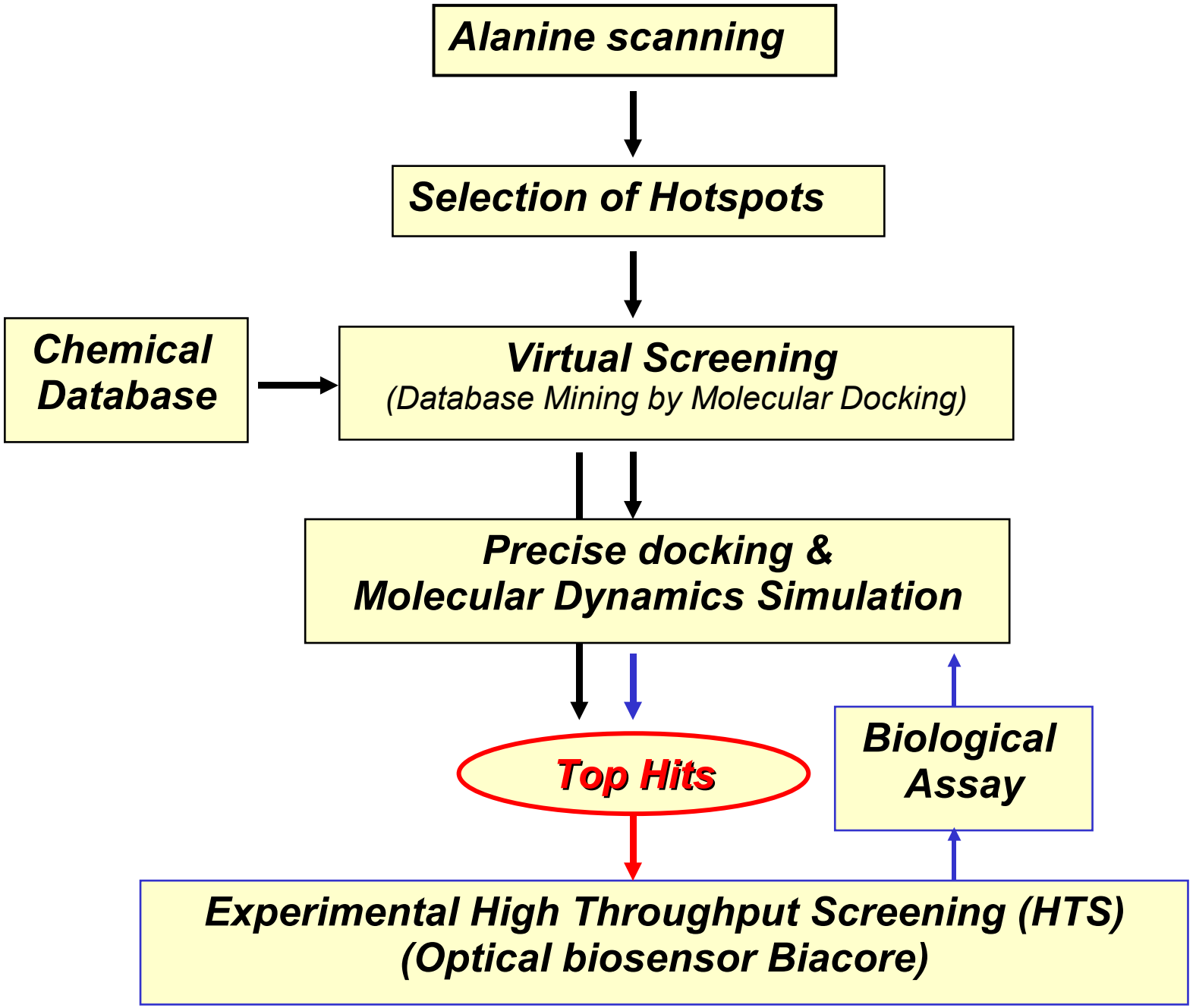


HIV Protease

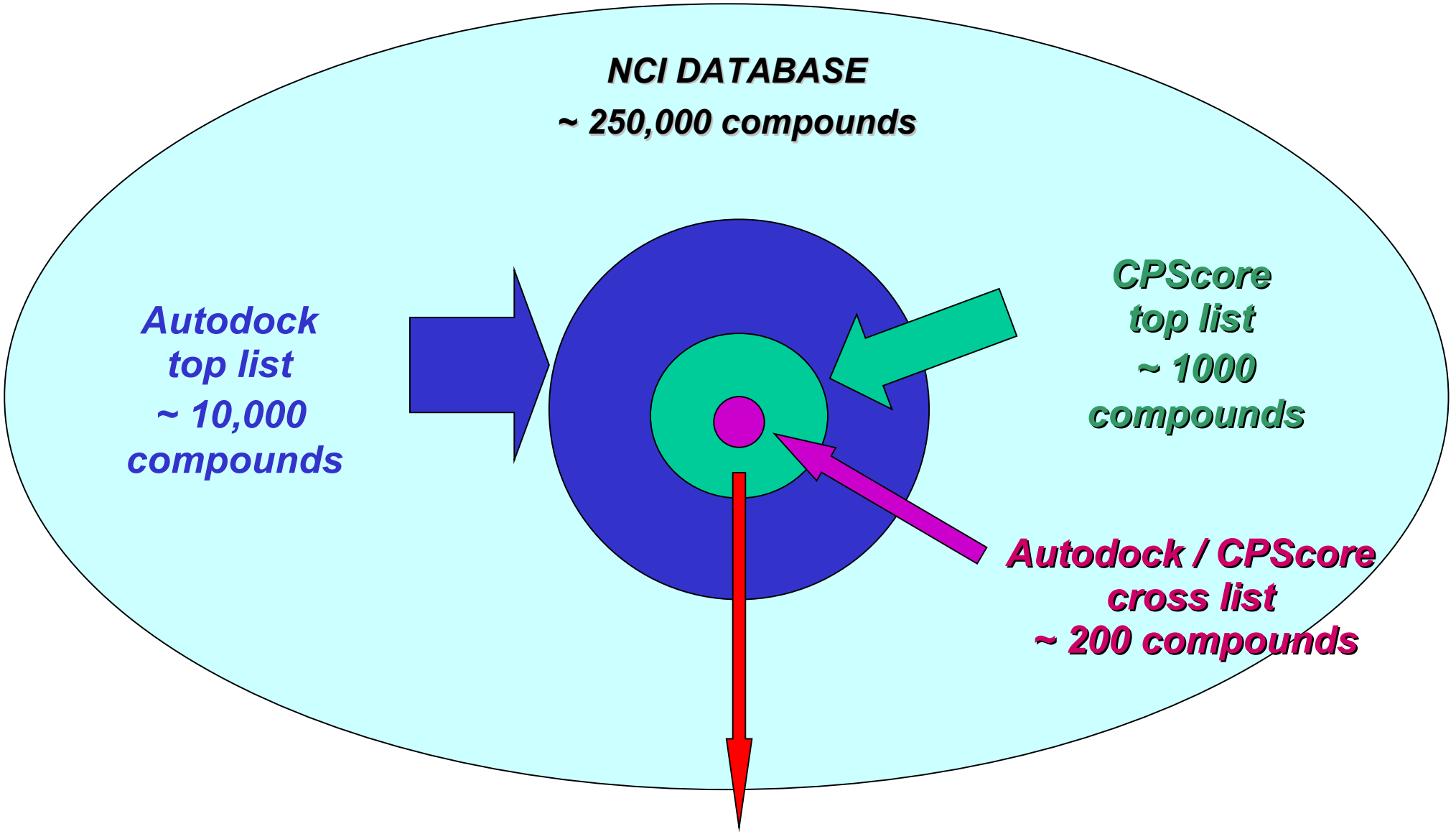
INACTIVE FORM - MONOMER



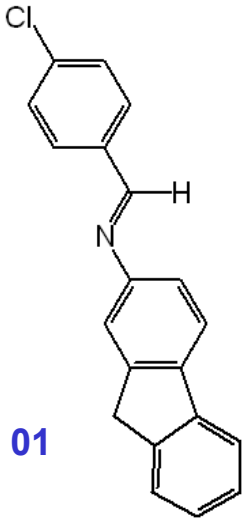
Integral strategy of PPI inhibitors screening



Virtual screening of HIVp inhibitors of dimerization



Top hits from virtual screening of HIVp inhibitors of dimerization



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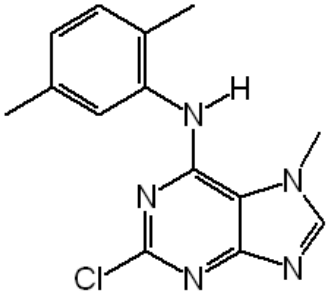

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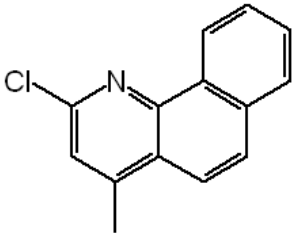

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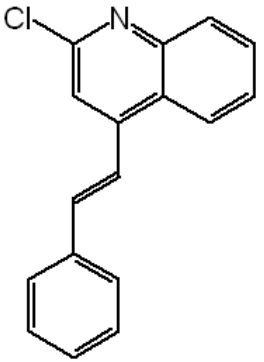

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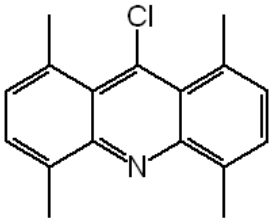

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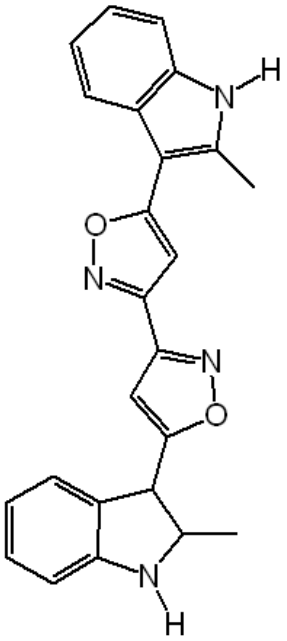

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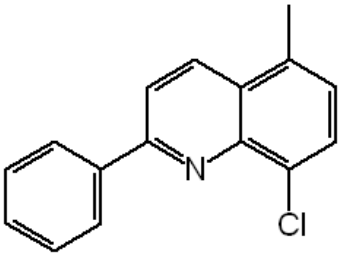

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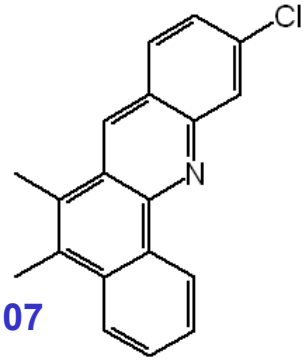

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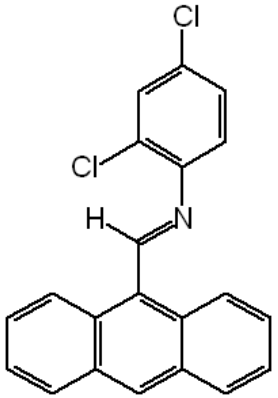

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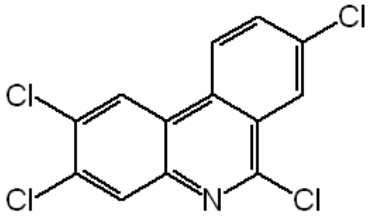

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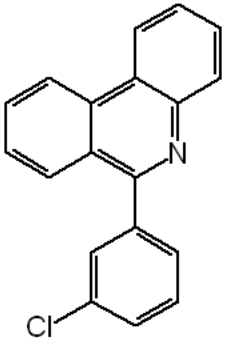

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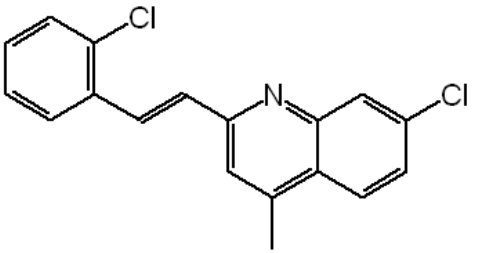
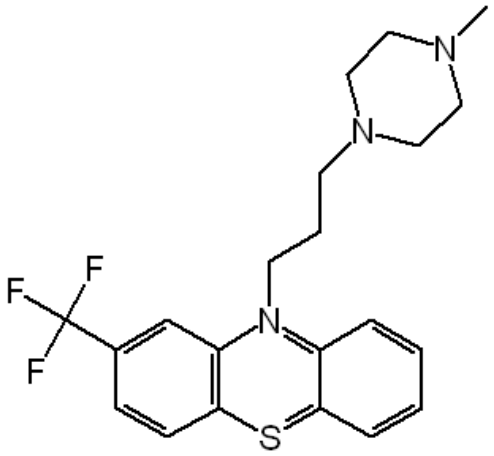


12

Manual selection from lab collection some compounds looked like Top hits



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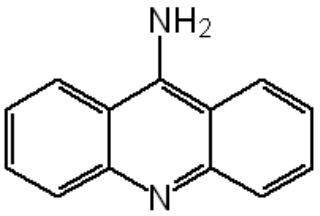

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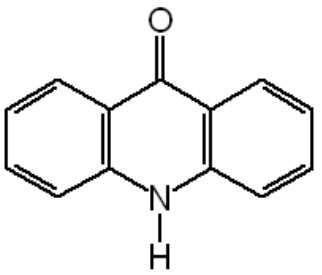

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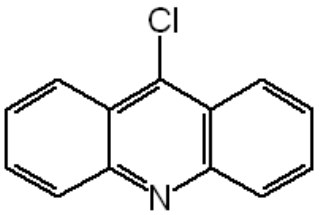

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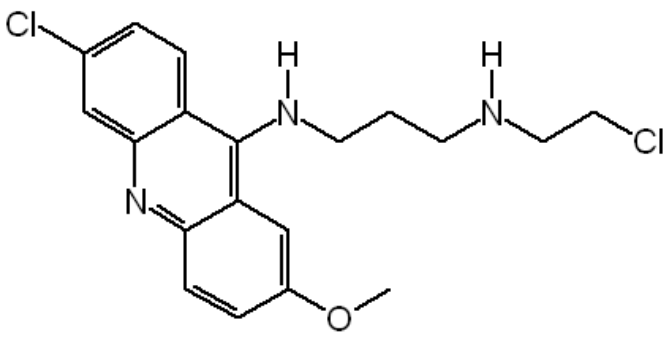

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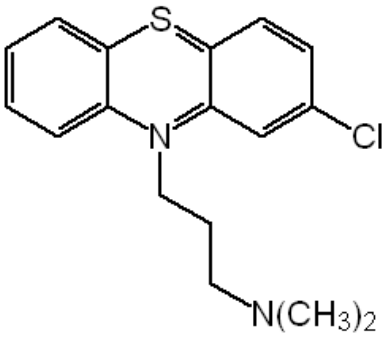
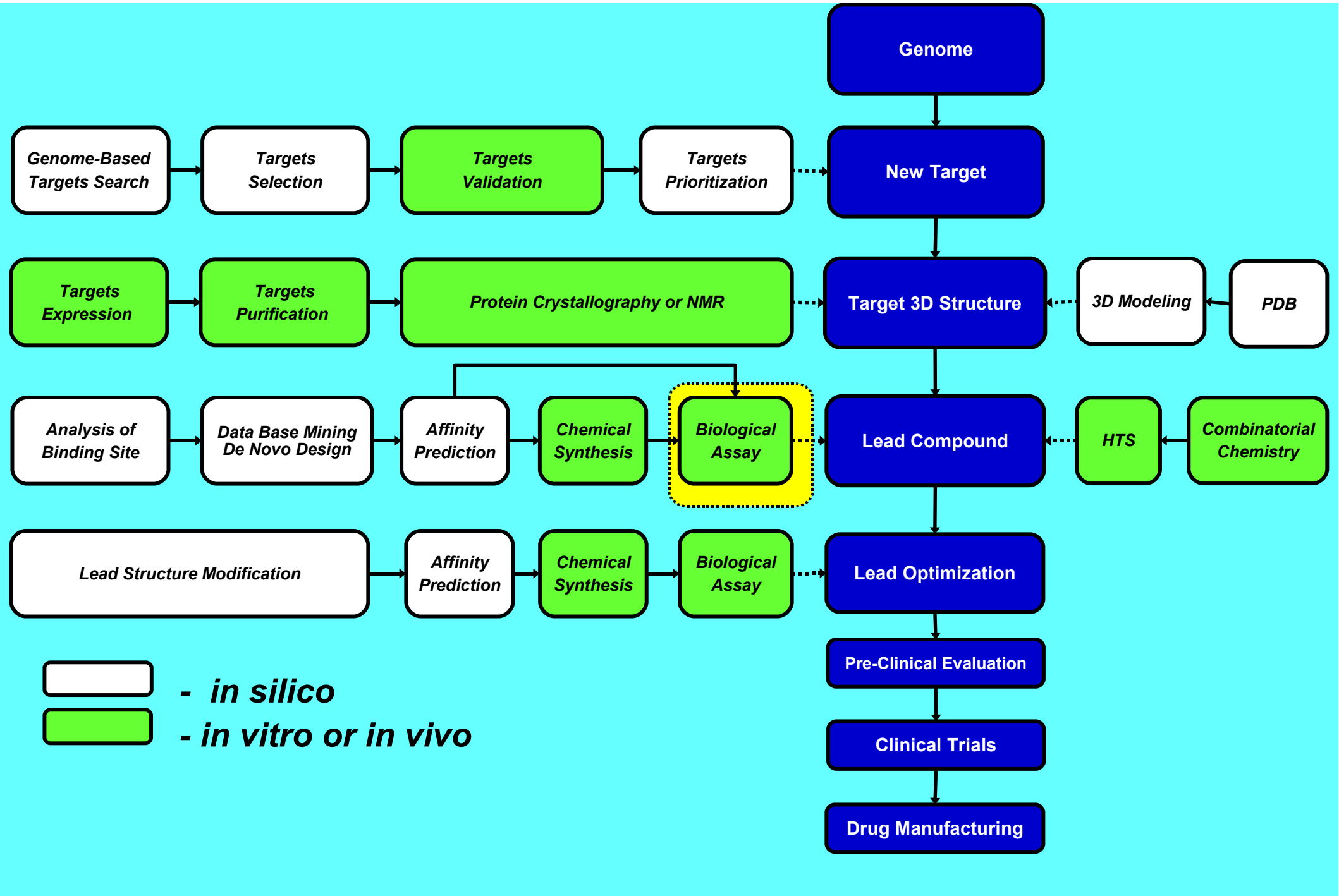


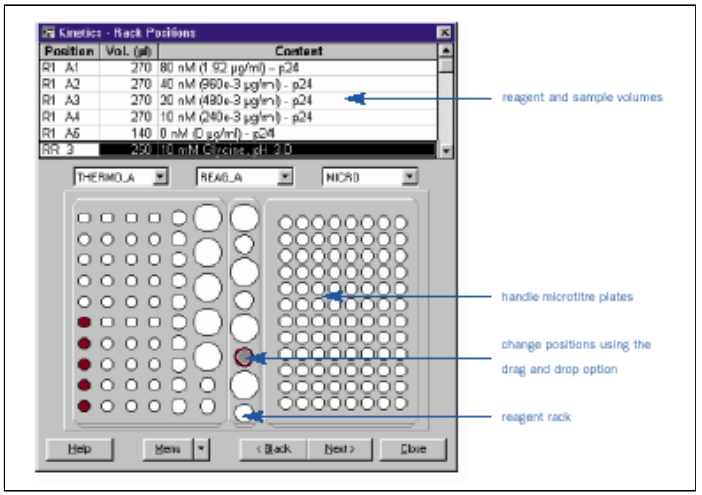
18

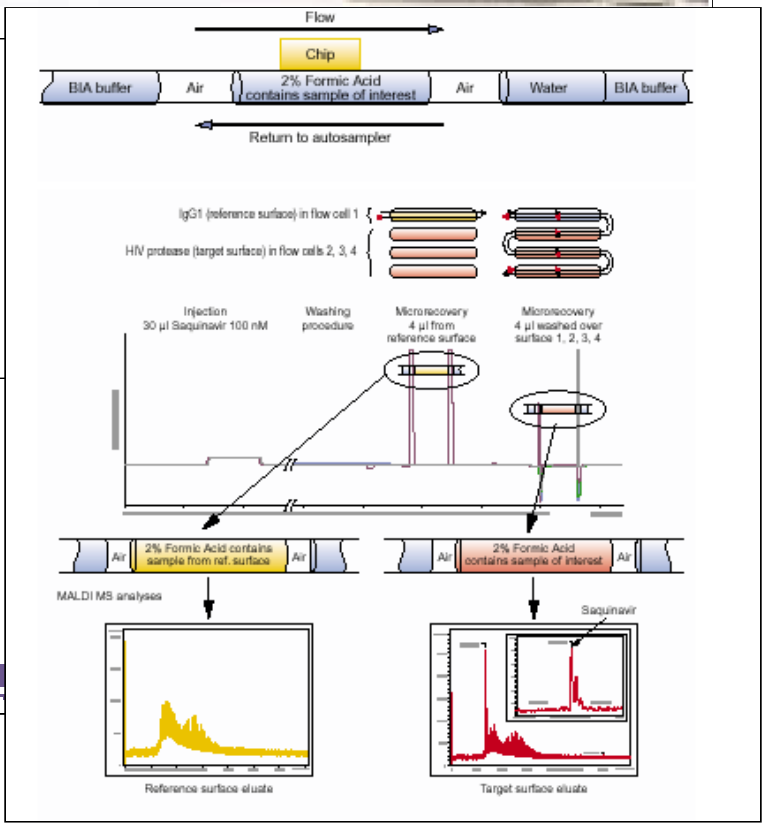
Integral Pipeline “From Gene to Drug”

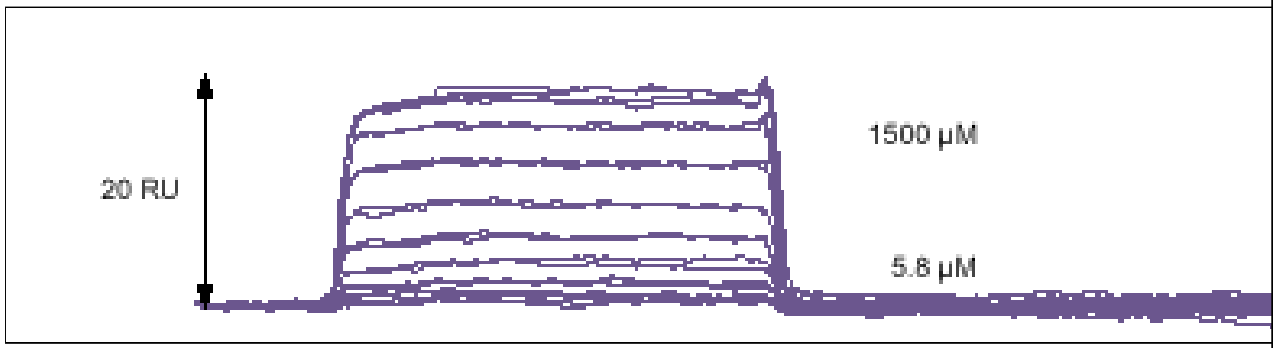


Biological testing in vitro

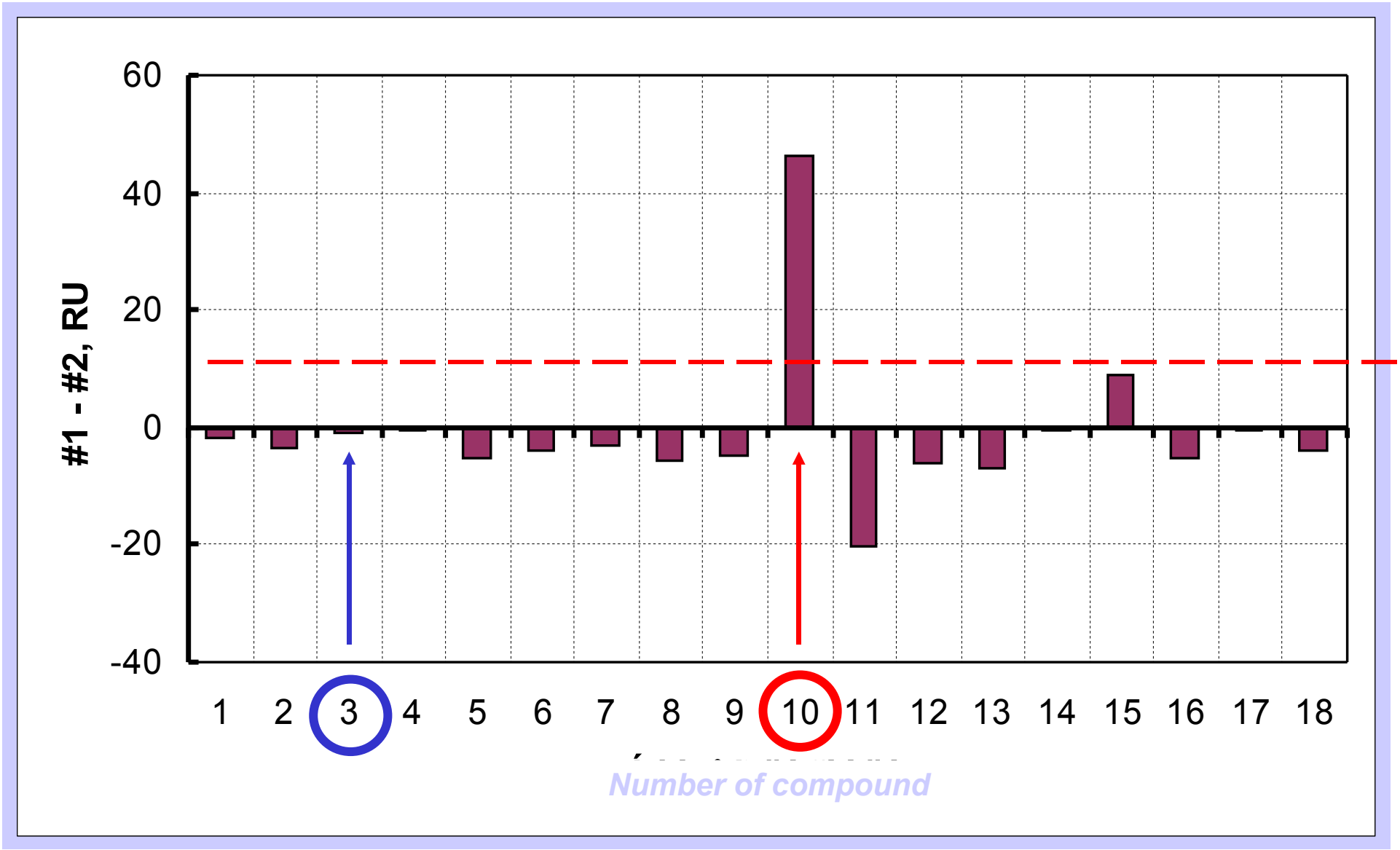
- High sensitivity
- Very small samples
- Short time of analysis
- Different optical chips







In vitro assay for inhibitors of HIVp dimerization

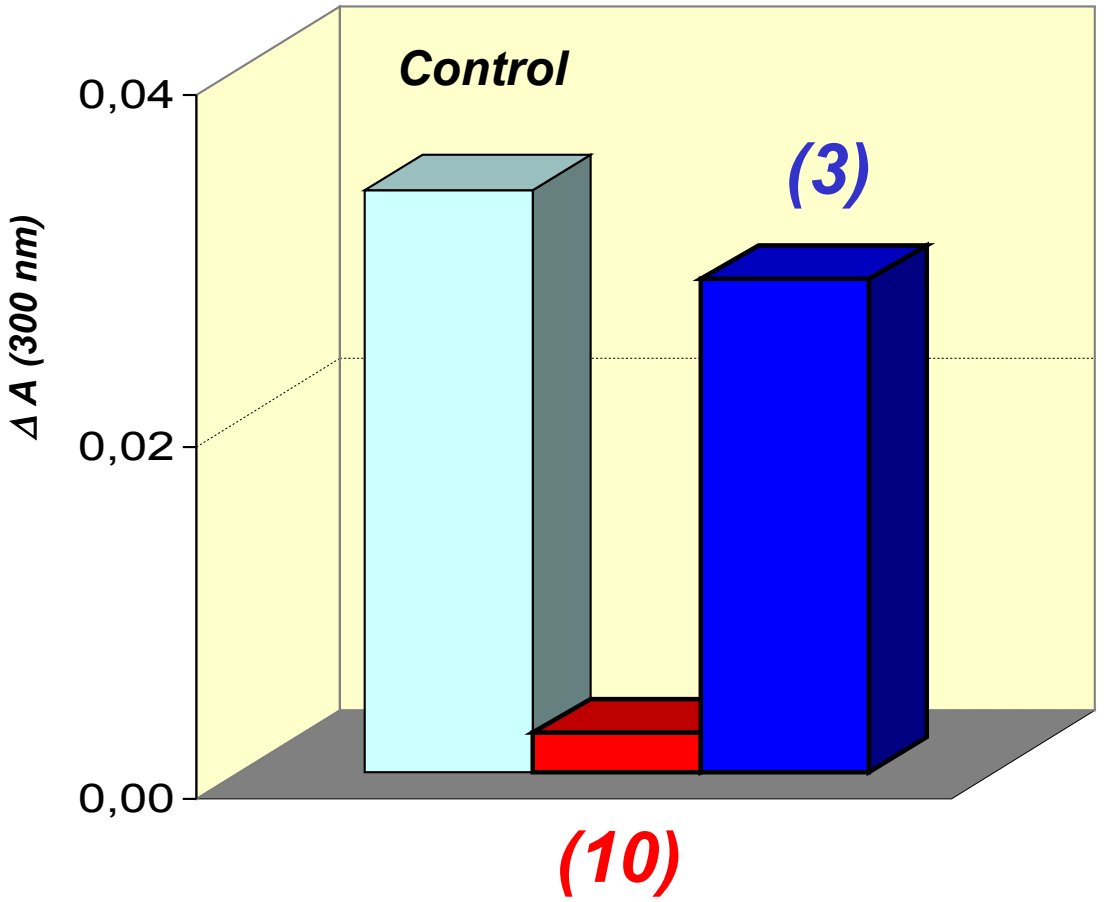


In vitro assay for HIVp inhibition

- ✓ *The ability of selected compounds to inhibit HIVp was assessed with chromogenic peptide substrate:*

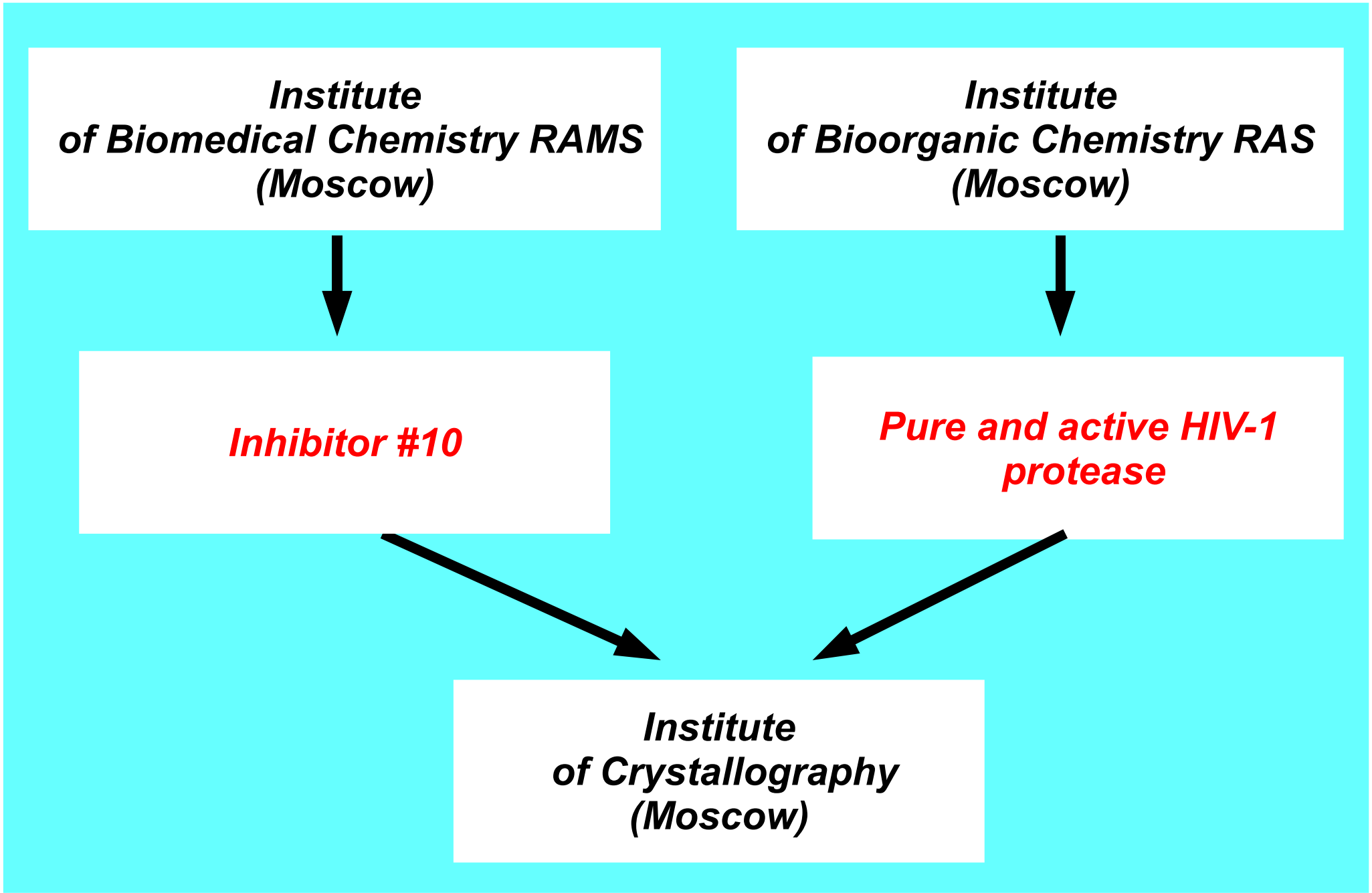
  H-Lys-Ala-Arg-Val-Tyr-p-nitro-Phe-Glu-Ala-Nle-NH_2

- ✓ *<u>Spectrophotometric assay</u> - ΔA (300 nm) is proportional to HIVp activity*



Ligands concentration is 10^{-6} M

Inhibitor of HIV protease dimerization



Thank you for attention!