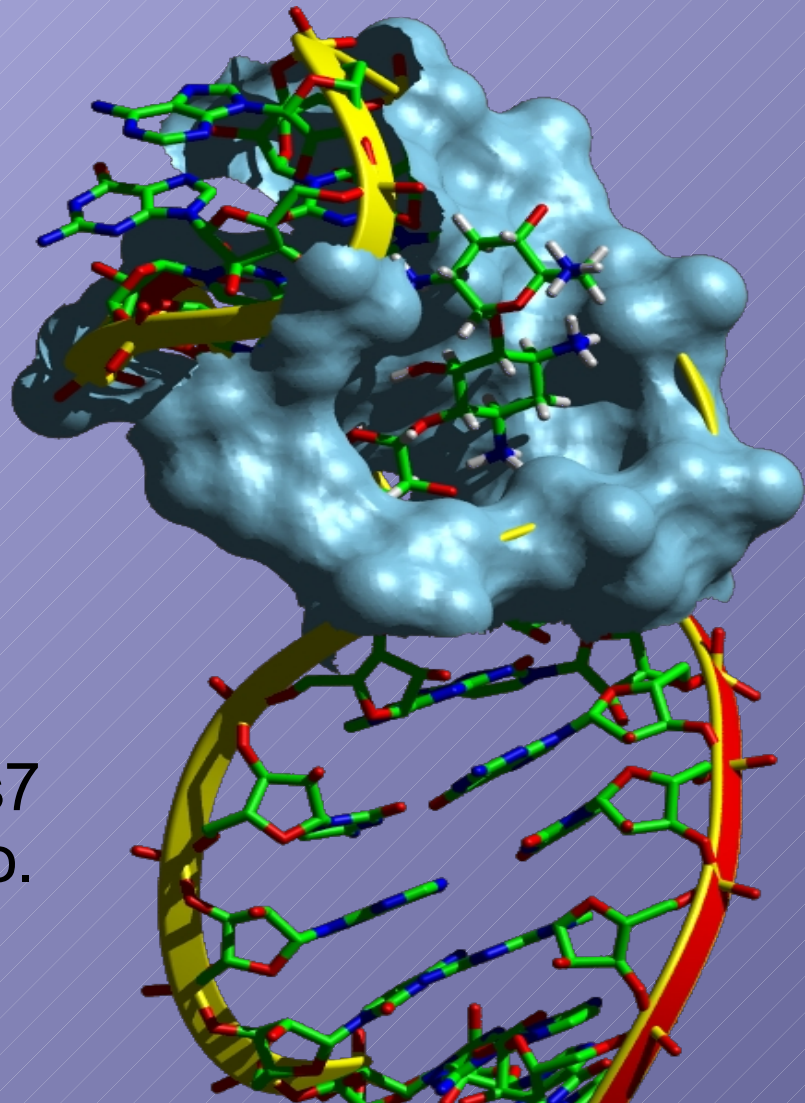
RNA as drug target: docking studies



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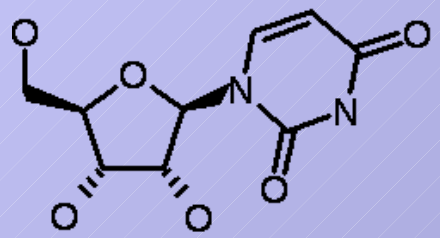
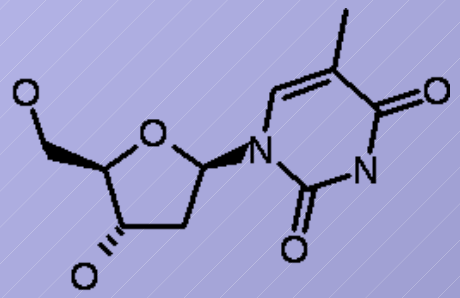
Overview

- Why targeting RNA
- Drug design strategy
- Parametrisation of the scoring function
- First Virtual screening
- Flexibility of target
- Conclusions & Prospects

Why targeting RNA?



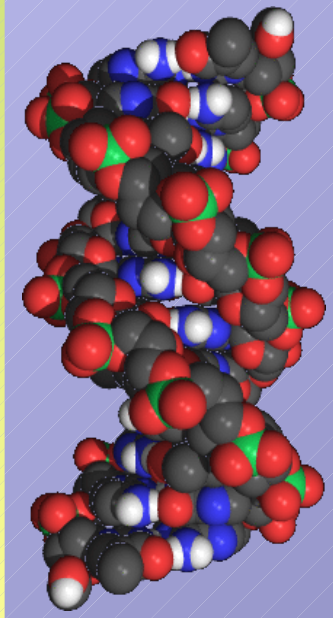
RNA



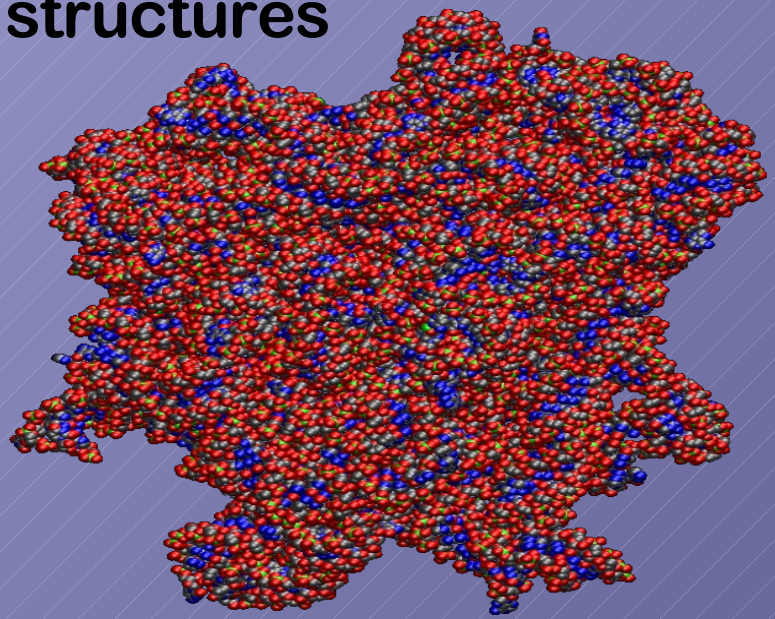
DNA

RNA and DNA are negatively charged molecules

RNA structures
single strand
=> complex 3D structures



DNA structures
double strand
=> mainly Helix



Why targeting RNA?

Biological role of RNA:

- Protein synthesis (mRNA, Ribosome, tRNA)
- Enzyme (Ribozyme)
- RNA is the genome of all retrovirus (HIV, HCV,…)
- RNA can control gene regulation (siRNA)

We need new and original targets

RNA can be one of them

RNA is involved in a lot of biological functions

Different opportunities and effects

RNA have structural domains that are more highly conserved

Slower developpment of drug resistance

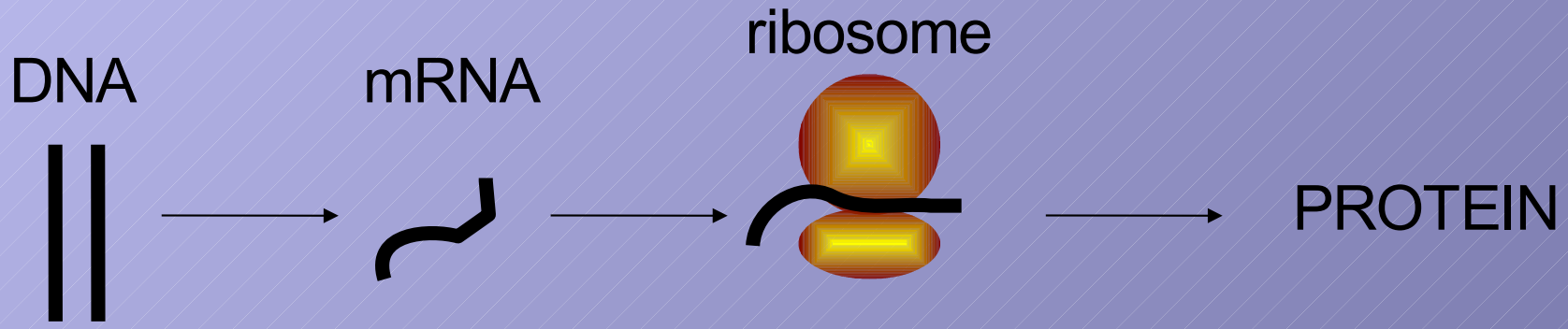
RNA is upstream in translation pathway (protein synthesis)

Inhibiting 1 RNA (ribosome) could prevent ~1000 proteins

Drug-design strategy

Purpose: design antimicrobial compounds

Protein synthesis scheme



Same global scheme for procaryote & eucaryote but 2 different RNA ribosomal fragments:

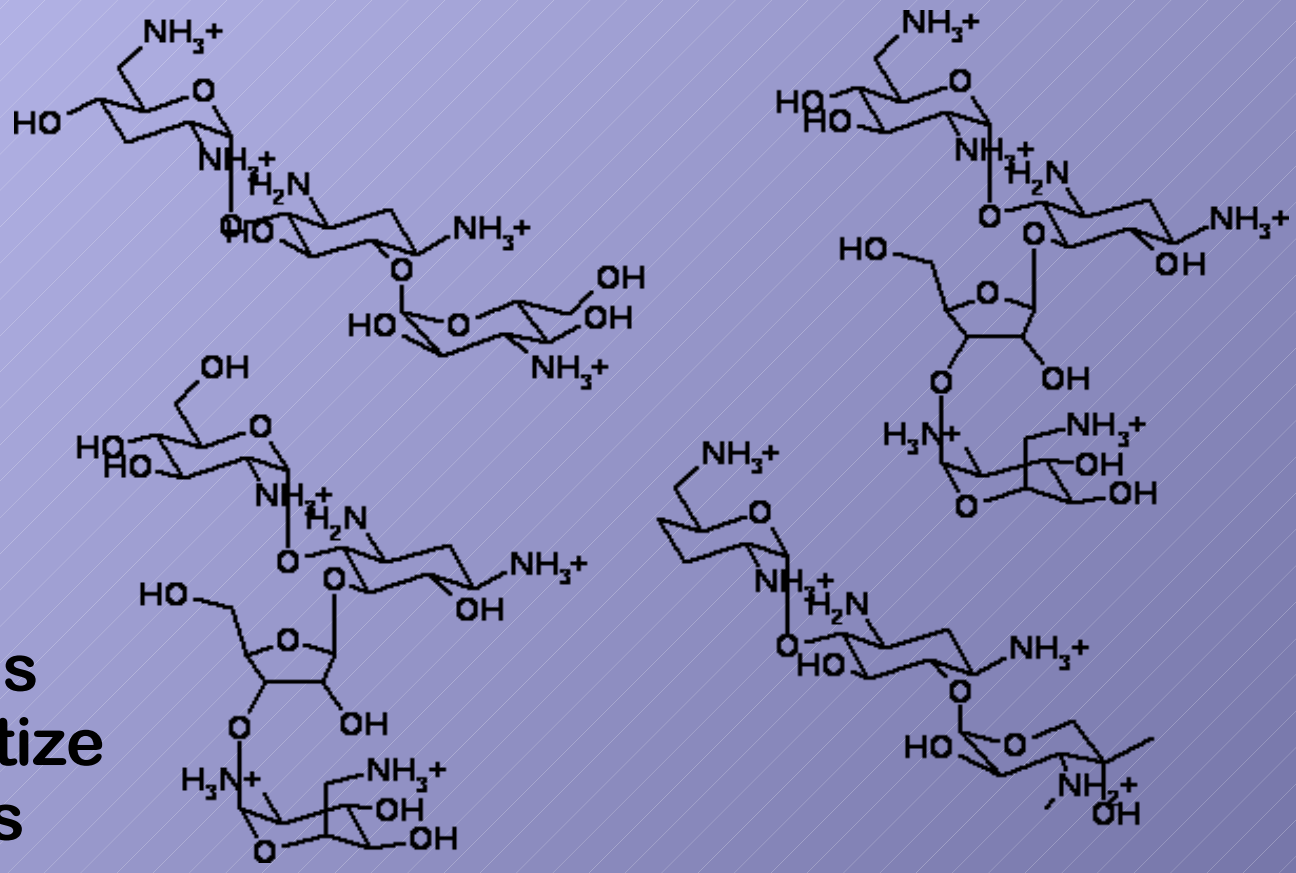
- **RNA 16S (bacteria)**
- **RNA 18S (human)**

We want to design selective ligands against 16S and not 18S

Drug-design strategy

RNA ligands?

Aminoglycosides!



- Natural products
- Good affinity
- Low selectivity
- Ammonium groups
- Difficult to synthetize
- Flexible molecules

Goal:

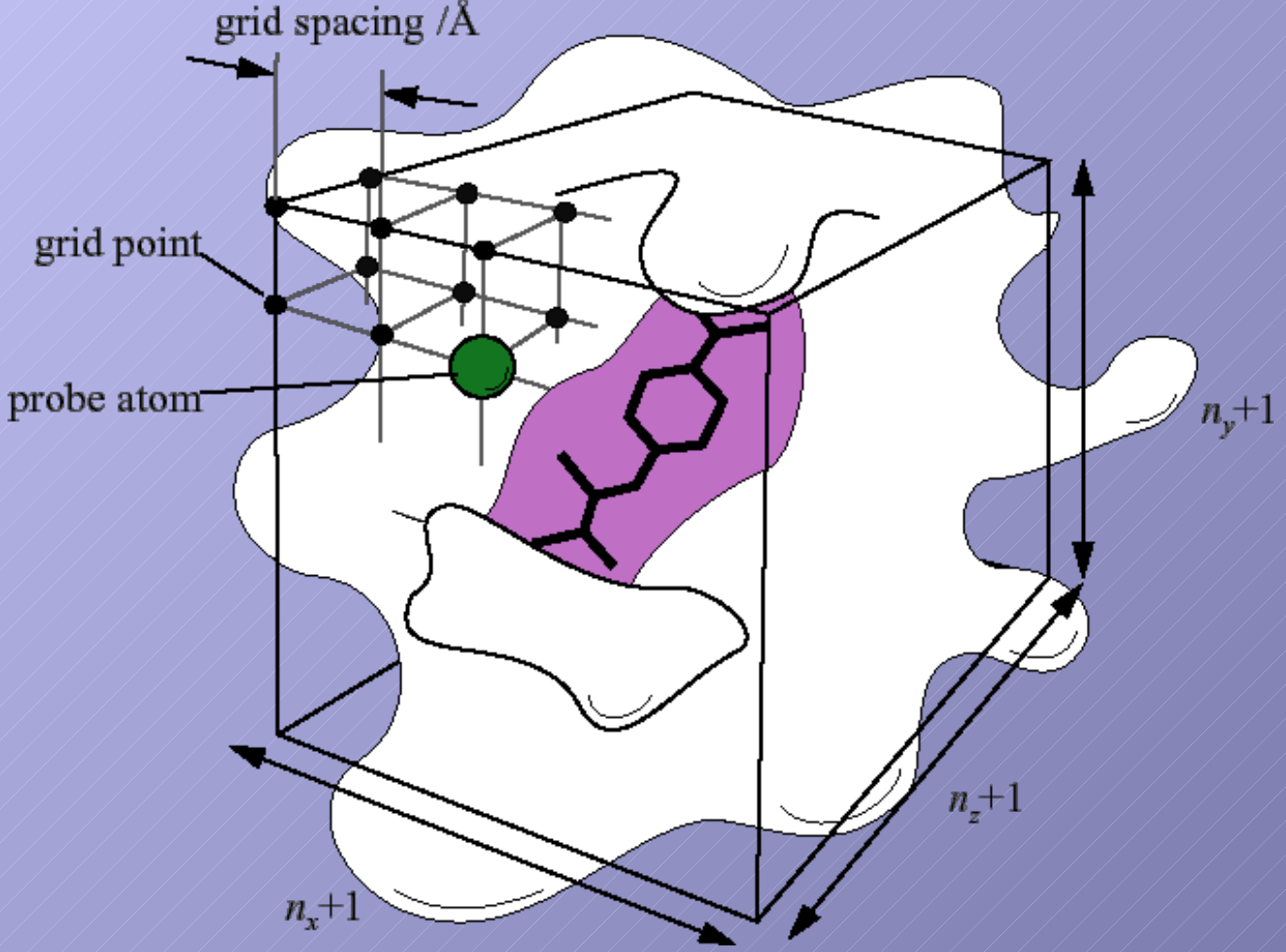
- Take these molecules as scaffold for chemical modifications
- Add a nucleoside to make a triple-base-pair or intercalation

Drug-design strategy

Two questions:

		List of compounds with bioactivity?	
		YES	NO
3D structure of the target?	YES	All	Docking
	NO	QSAR-2D QSAR-3D Pharmacophoric screening	NOTHING

Parametrisation of the scoring function



Autodock (3.0) pre-calculate energy grid maps:
1 for each atoms + electrostatic
Ligands are flexible whereas target is rigid
Genetic Algorithm + Local Search to find ligand position

Parametrisation of the scoring function

$$\Delta G = f_{vdw} \sum \left(\frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{6}} \right) + f_{hbond} \left[\sum E(t) \left(\frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{10}} \right) + E_{Hbond} \right] + f_{elec} \sum \left(\frac{q_i q_j}{\varepsilon(r_{ij}) r_{ij}} \right) + \Delta G_{tors} N_{tors} + f_{solv} \sum \left(S_i V_j + S_j V_i \right) e^{-r_{ij}^2}$$

5 empirical parameters derived from protein/ligand complexes

NOT SUITABLE TO RNA! We need these 5 parameters for RNA

Parametrisation of the scoring function

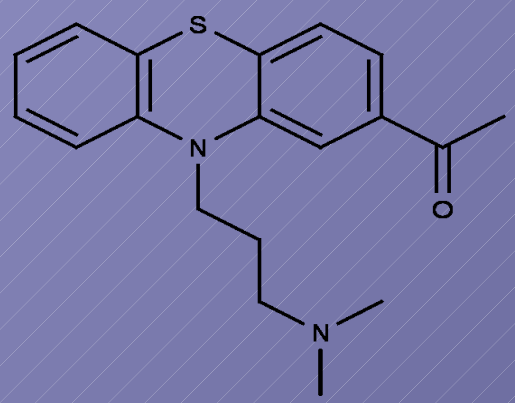
We need RNA/Ligands structures with experimental ΔG

ONLY 8 !

Tobramycin with RNA aptamer I
Tobramycin with RNA aptamer II
Neomycin-B with RNA Tau exon
Neomycin-B with RNA aptamer
Neomycin-B with RNA HIV-1 Tar
Gentamicin C1A with A-site rRNA
Paramomycin with RNA-16S

} aminoglycosides

Acetylpromazine with RNA HIV-1 Tar



Parametrisation of the scoring function

For each structures:

- 31 docking calculations with differents parameters.
- Values are ranged from 0 to 2x of their default value.
- At the end: 248 results

Correct docking:

- Free energy of binding similar to the experimental
- RMSD between experimental and calculated is low

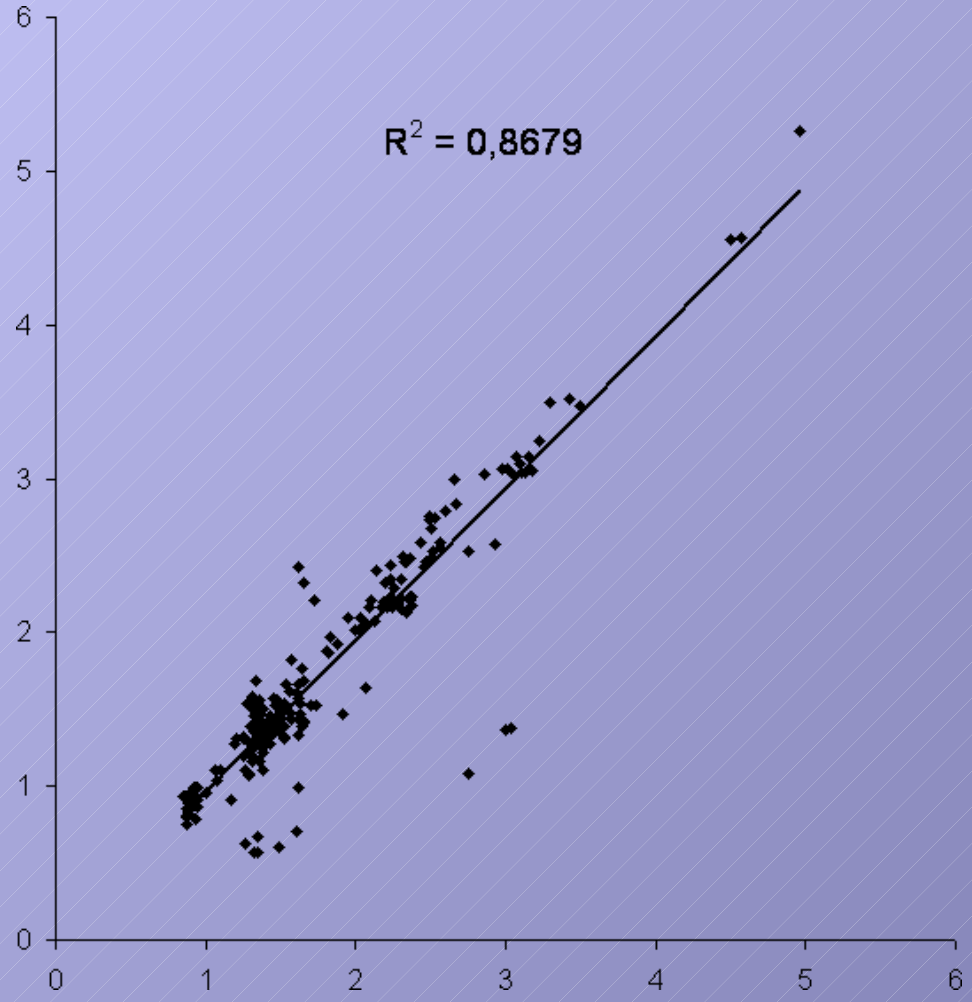
ANN is used to correlate ΔG and RMSD with parameters.

- 1 hidden layers
- Back propagation algorithm
- Leave-One-Out cross-validation

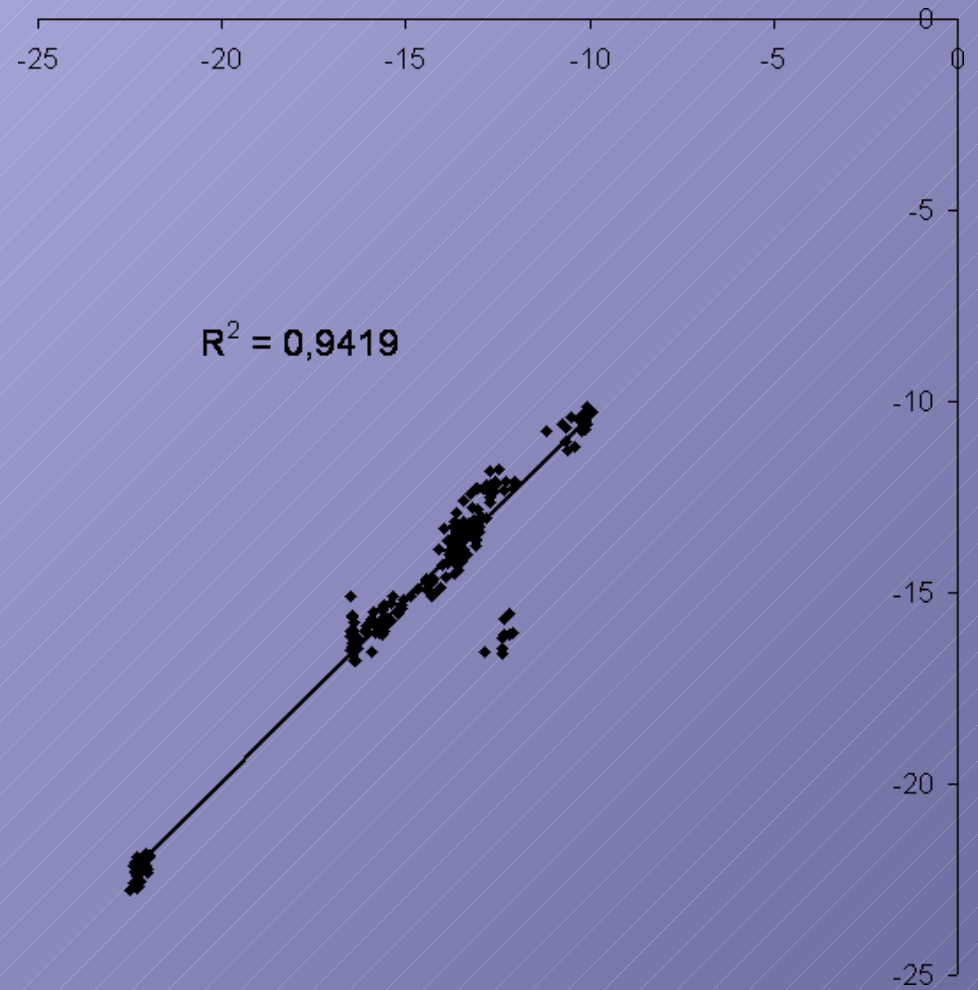
Final RMS error: 0.269 and Final max error: 0.883

Parametrisation of the scoring function

RMSD



ΔG



Parametrisation of the scoring function

With the Neural Network Model

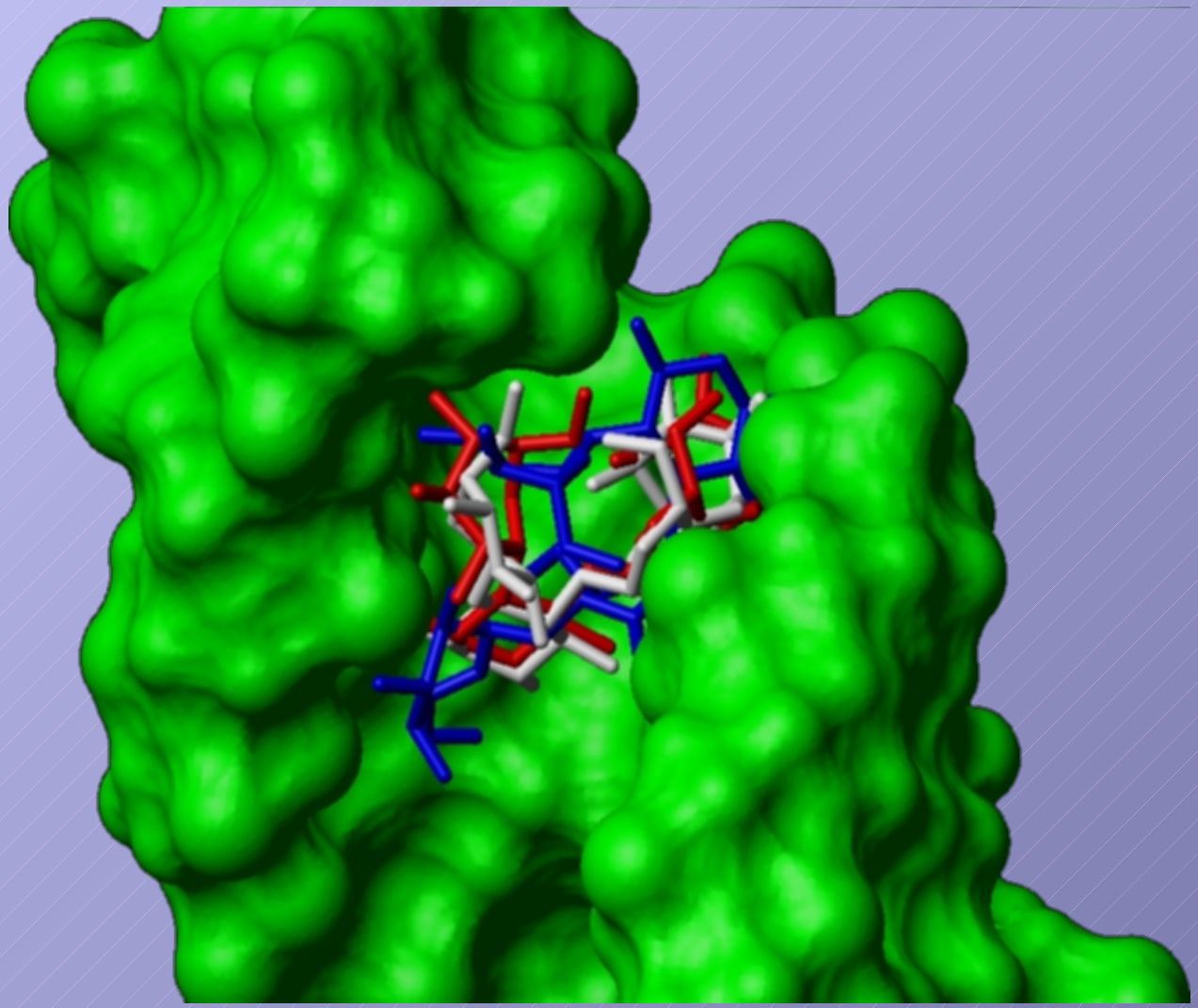
- 80,000 parameters were randomly generated and tested

- Best values were selected
  - as good as possible ΔG agreement
  - as weak as possible RMSD value

	Vdw	Elec	Hbond	Tors	Sol
Autodock	0.1485	0.1146	0.0656	0.3113	0.1711
RNA-Autodock	0.155	0.101	0.056	0.361	0.153

Parametrisation of the scoring function

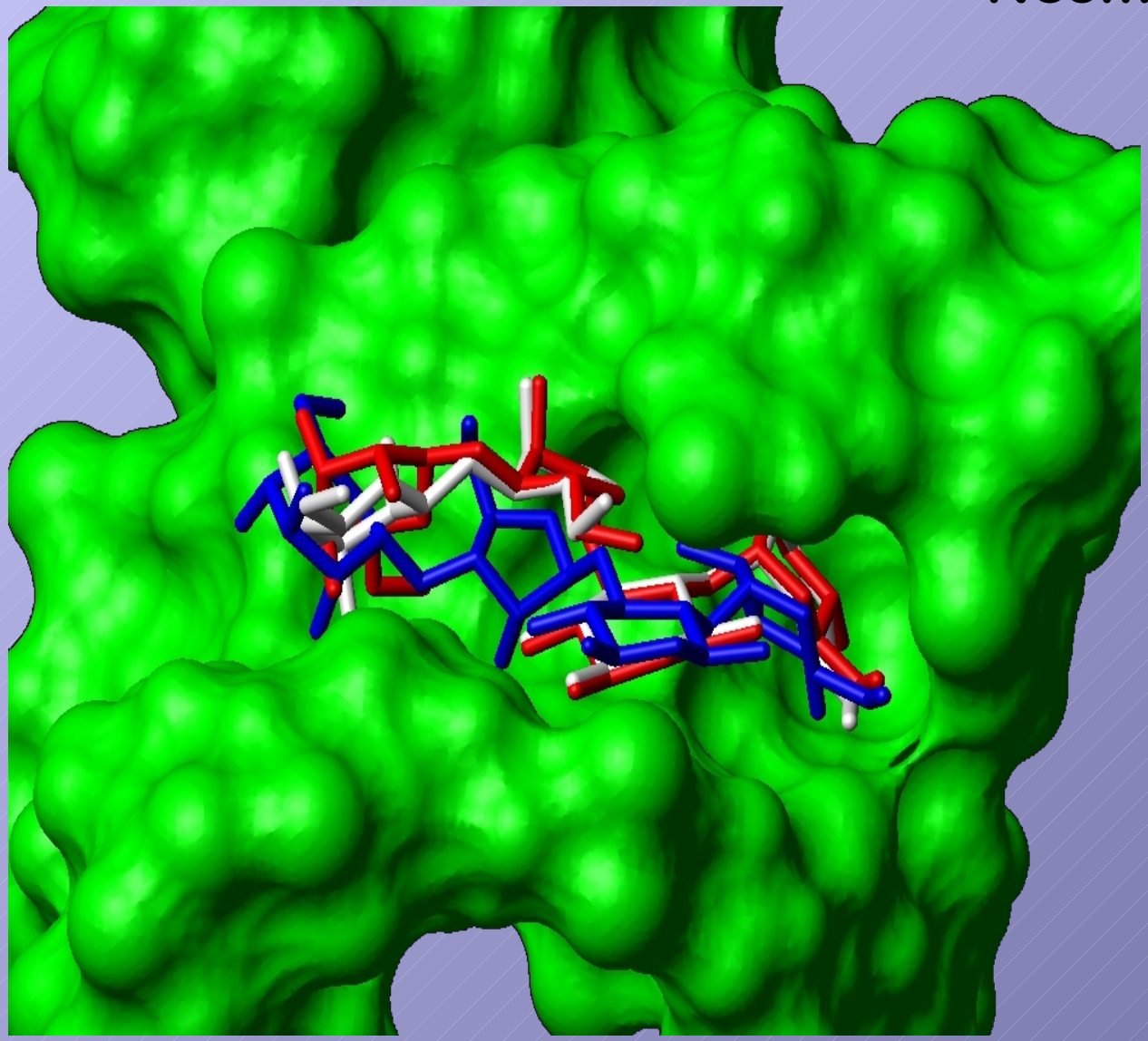
NeomycinB with RNA Tau 10



White: experimental
Blue: autodock
Red: RNA-autodock

Parametrisation of the scoring function

Neomycin with RNA Tar



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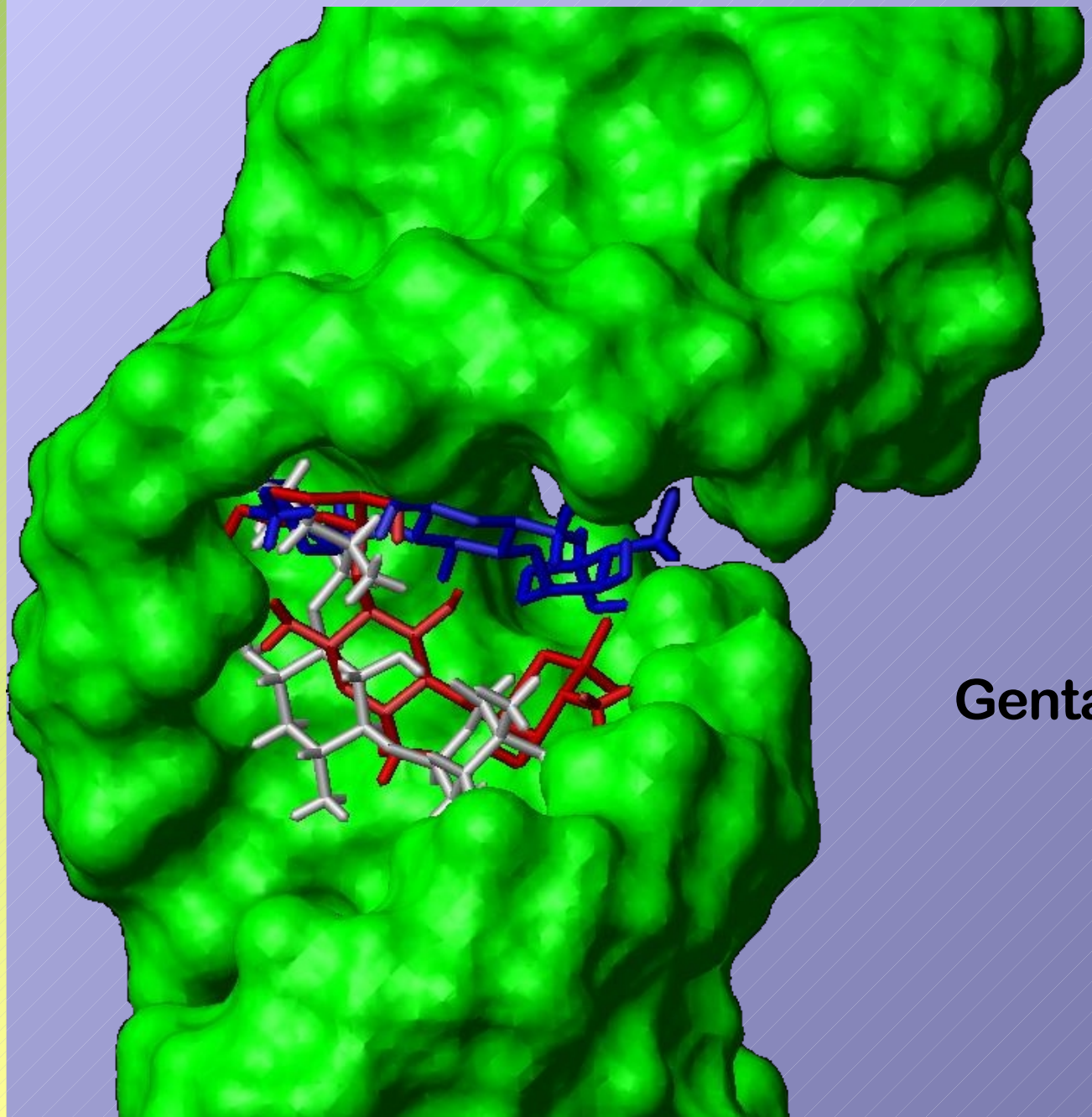
Parametrisation of the scoring function



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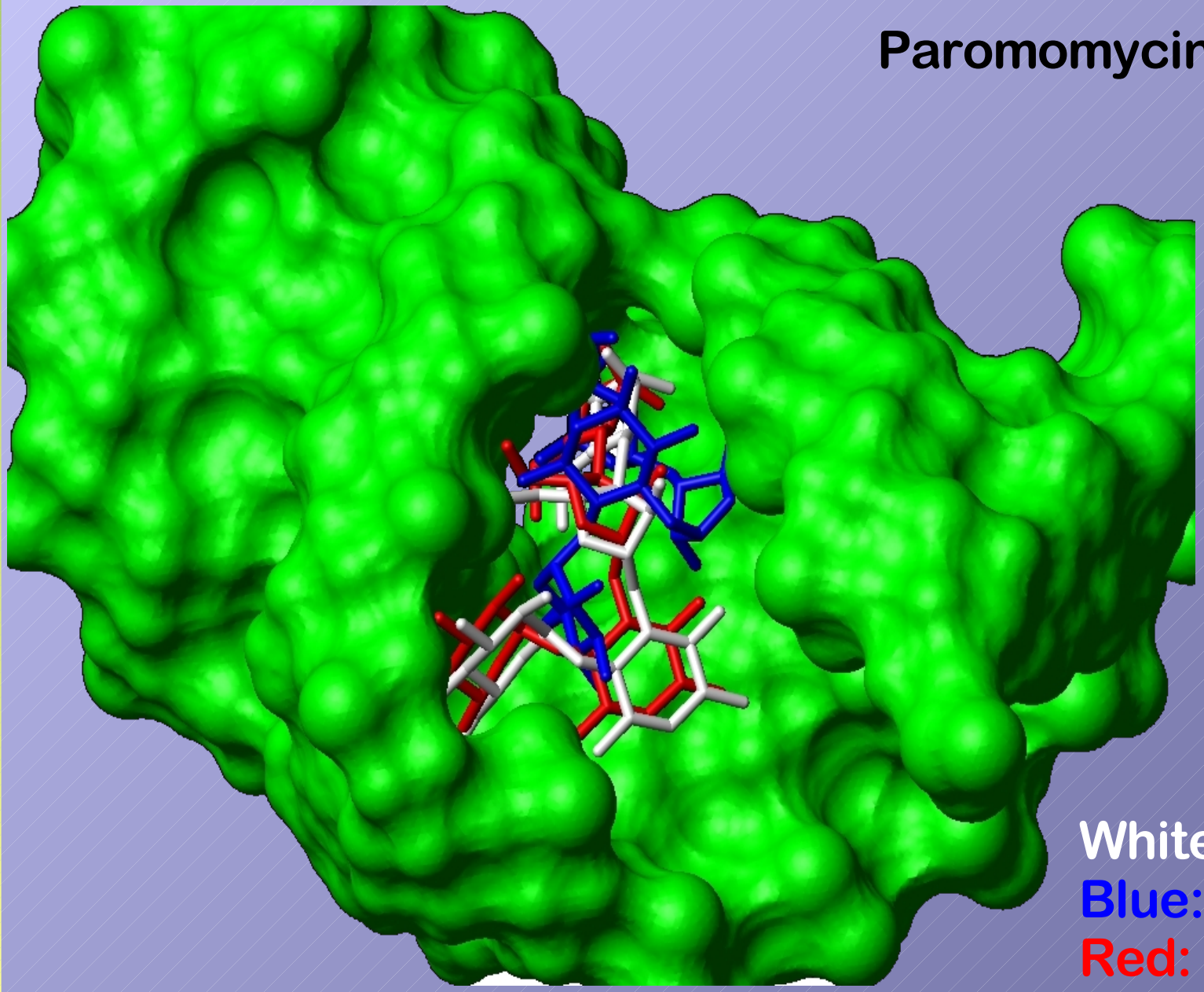
Blue: autodock
Red: RNA-autodock

Gentamycin with RNA-16S

Parametrisation of the scoring function

Paromomycin with RNA-30S

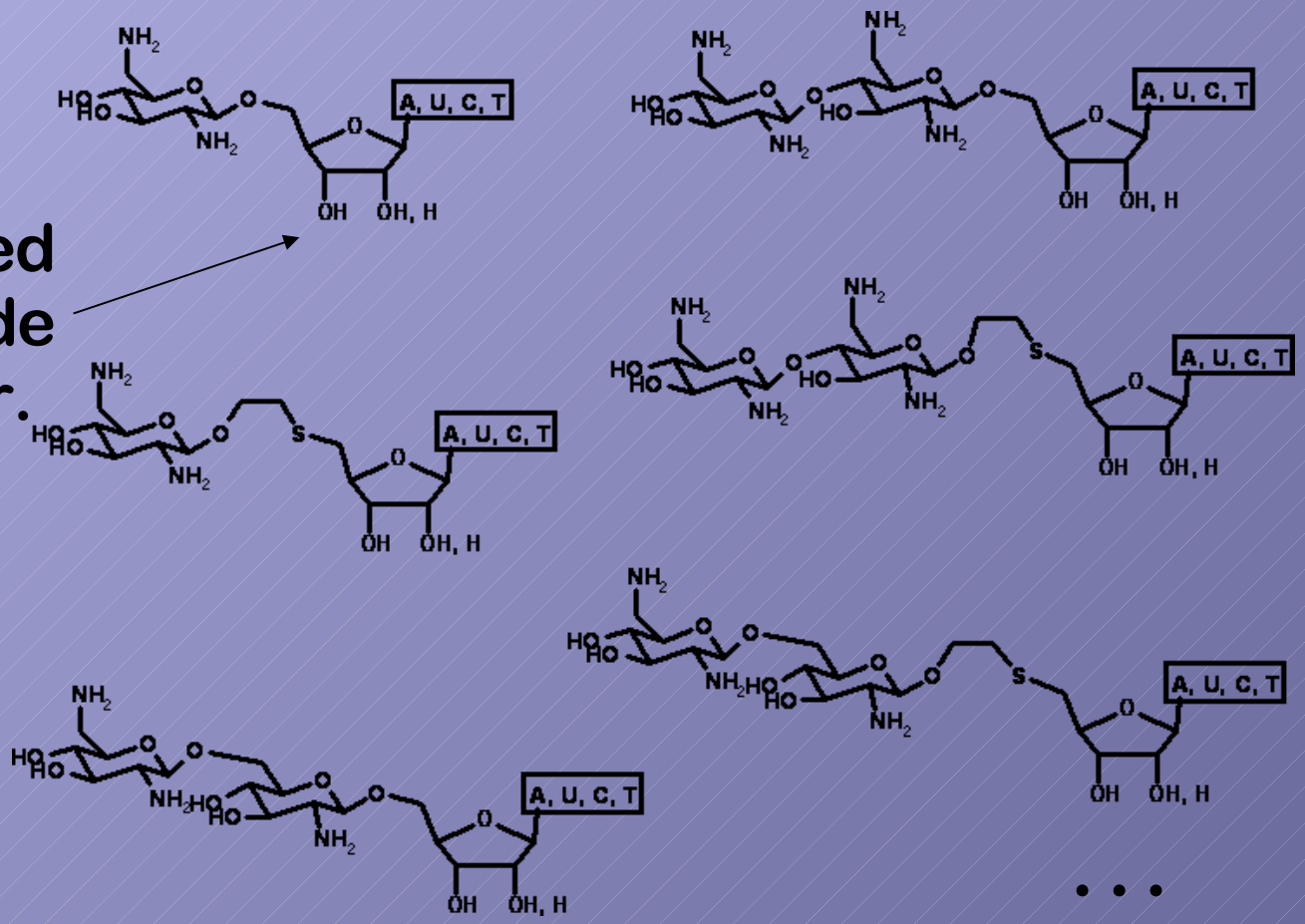


White: experimental
Blue: autodock
Red: RNA-autodock

First Virtual Screening

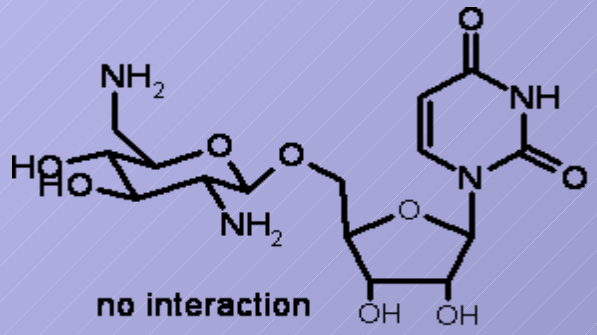
With the new scoring function:
First Virtual Screening with ~300 compounds against 16S
Library created in agreement with the organic chemist group

A nucleoside is linked to the aminoglycoside to form a 3rd base pair.

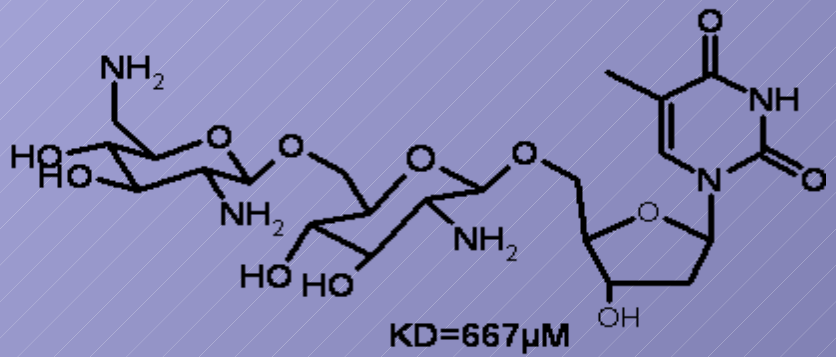


First Virtual Screening

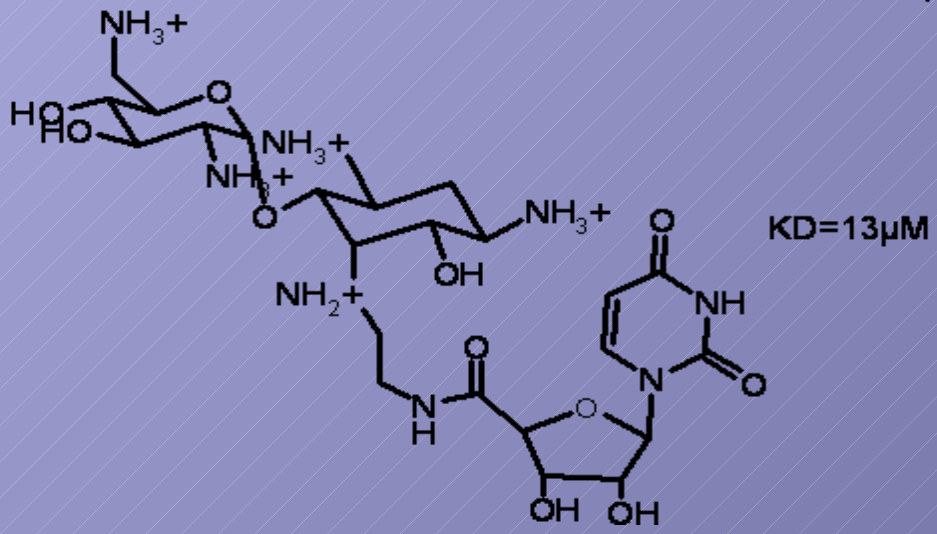
40 compounds were proposed for the synthesis
25 were synthesize (not only belonging to the 40)
17 present micromolar affinity (16&18S) & 8 no interaction.



no interaction



KD=667µM



KD=13µM

First Virtual Screening

Back docking calculation with Autodock for 16S & 18S:

- Understand selectivity
- Find Structure/Activity Relationships

Results:

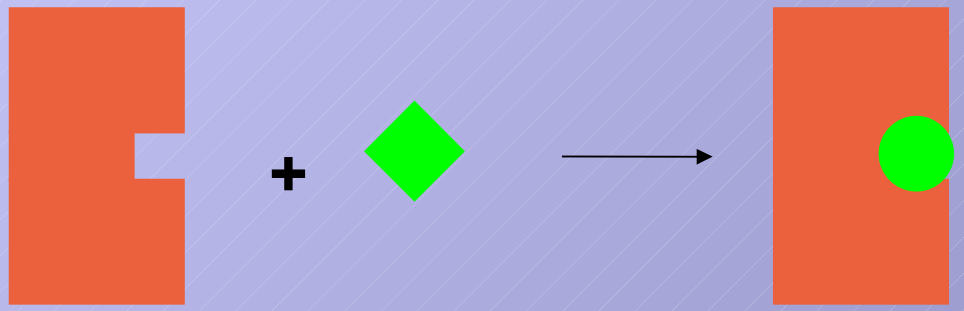
- All ligands without interaction are placed at the end
- Except Neamine (bad pose?)
- Correlation between ΔG calculated and experimental
- R^2= 0.72 for RNA-16S and R^2=0.7 for RNA-18S

But:

- Unable to explain selectivity of 16S *vs* 18S
- Unable to find a 3rd base-pair or intercalation

TARGET FLEXIBILITY

First Virtual Screening



FLEXIBILITY of the target

After a first docking with RNA as rigid target:

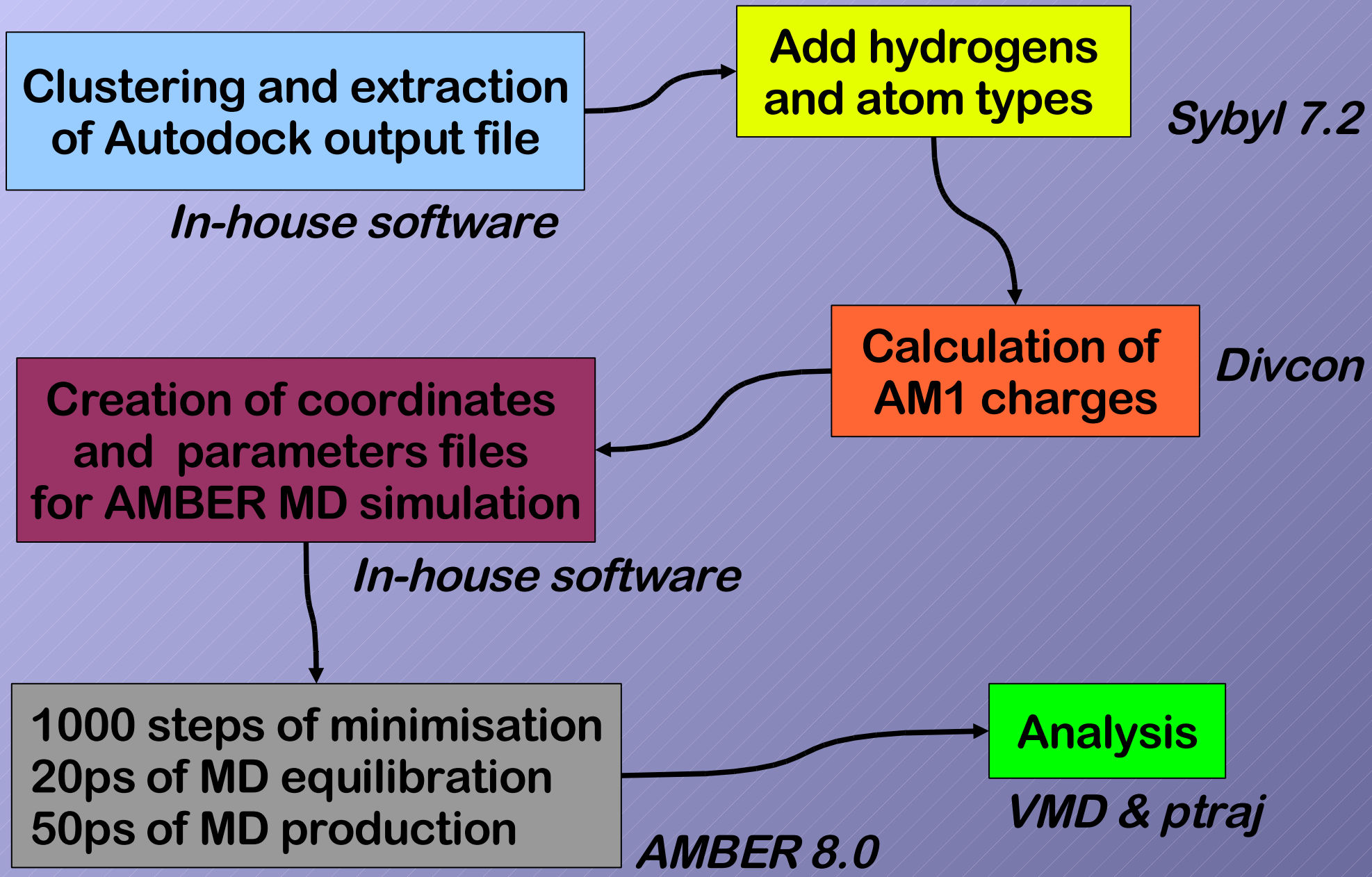
Small Molecular Dynamic simulations with GB implicit solvent (AMBER 8.0)

MD of complexes (~2 hours for each complexes)
MD of target alone (~2 hours)
MD of Ligands alone (~5 minutes for each ligands)

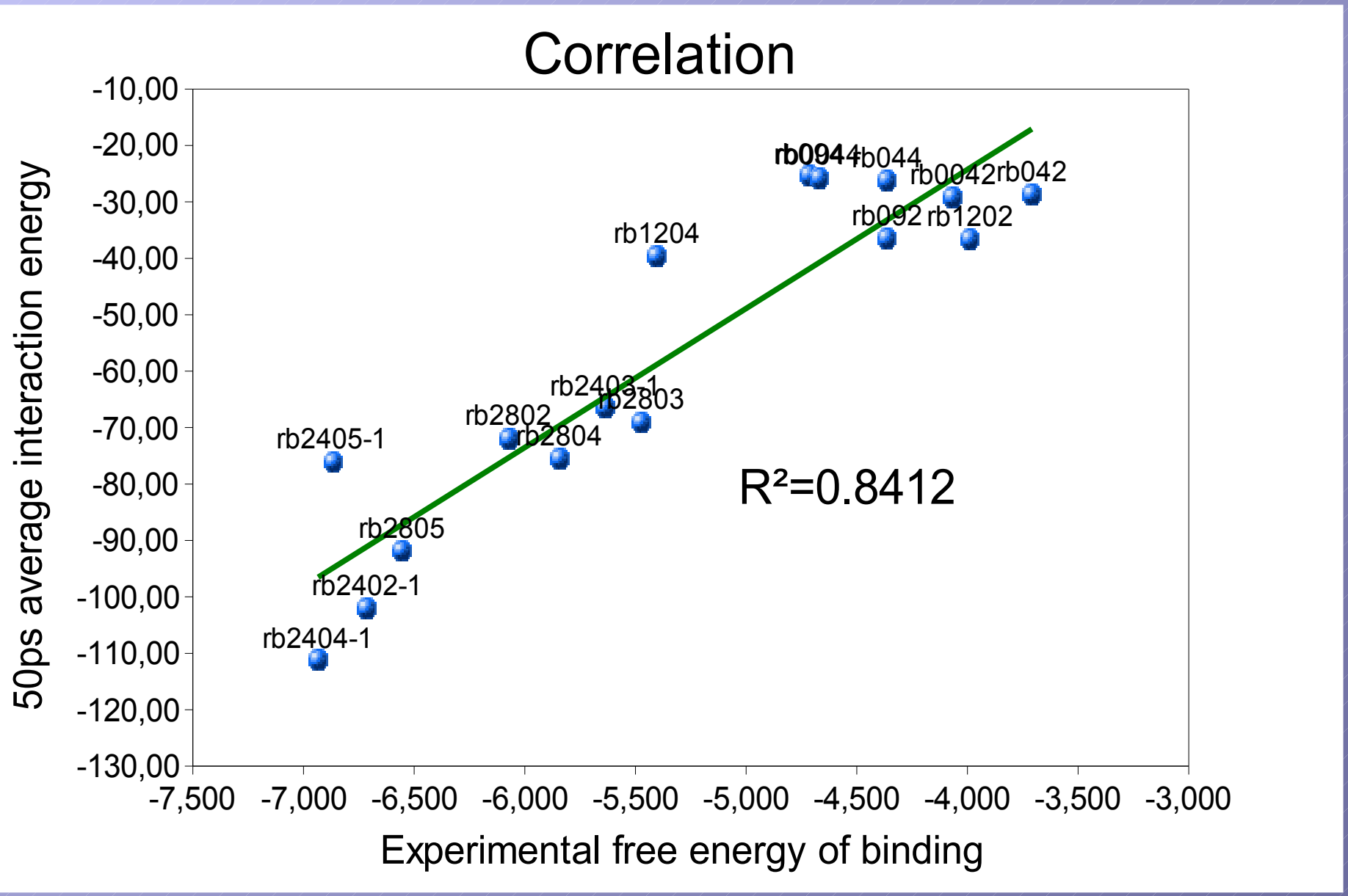
Einteraction = Ecomplex – Etarget – Eligand

Force-fields: parm03 for RNA and gaff for Ligands

Flexibility of the target

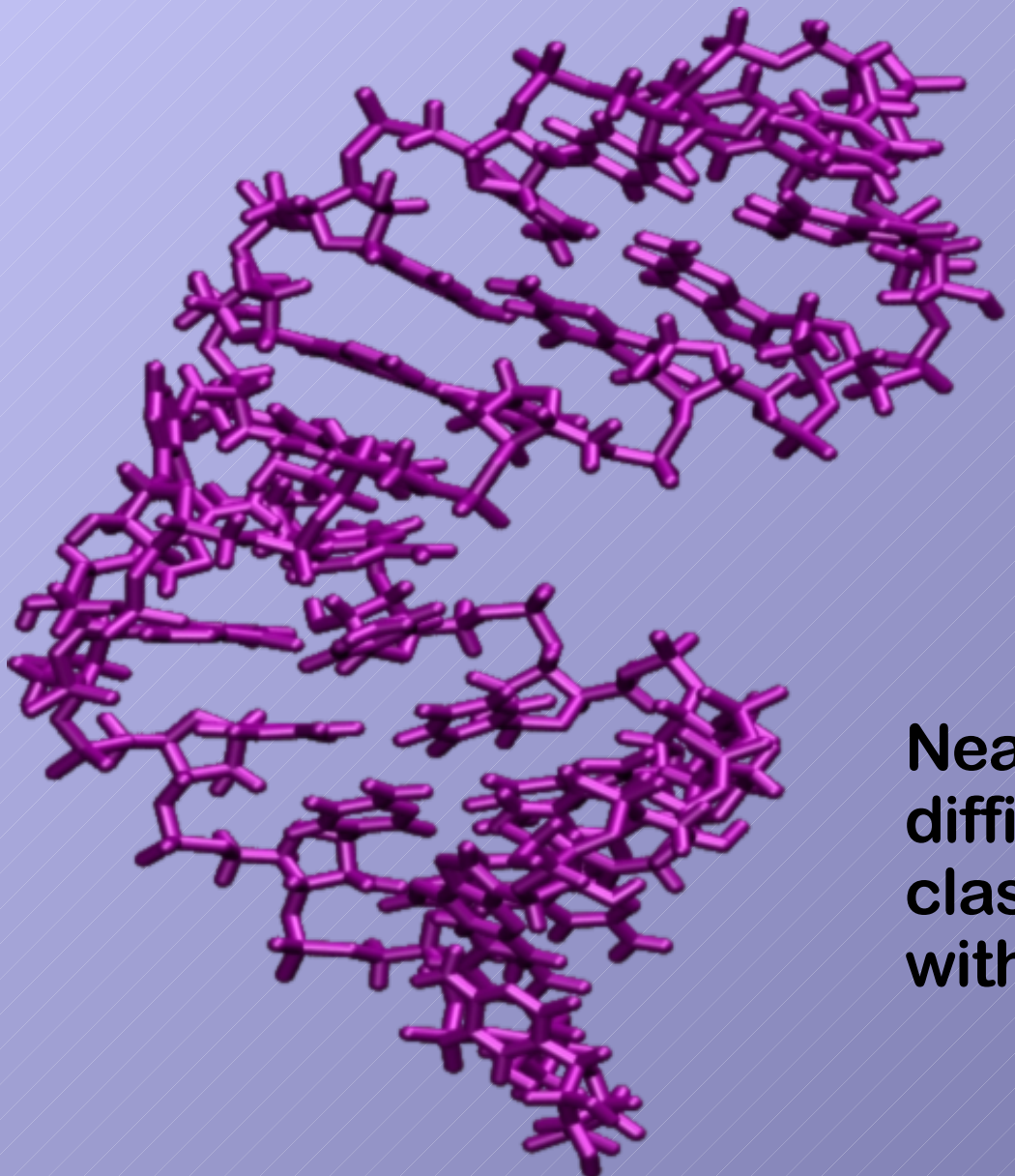


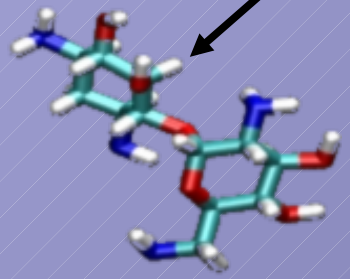
Flexibility of the target



Flexibility of the target

Graphical analysis

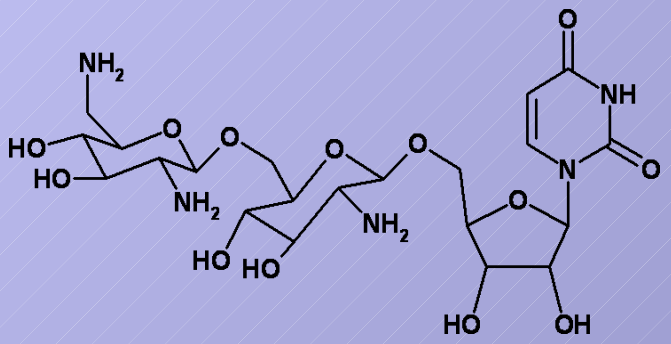




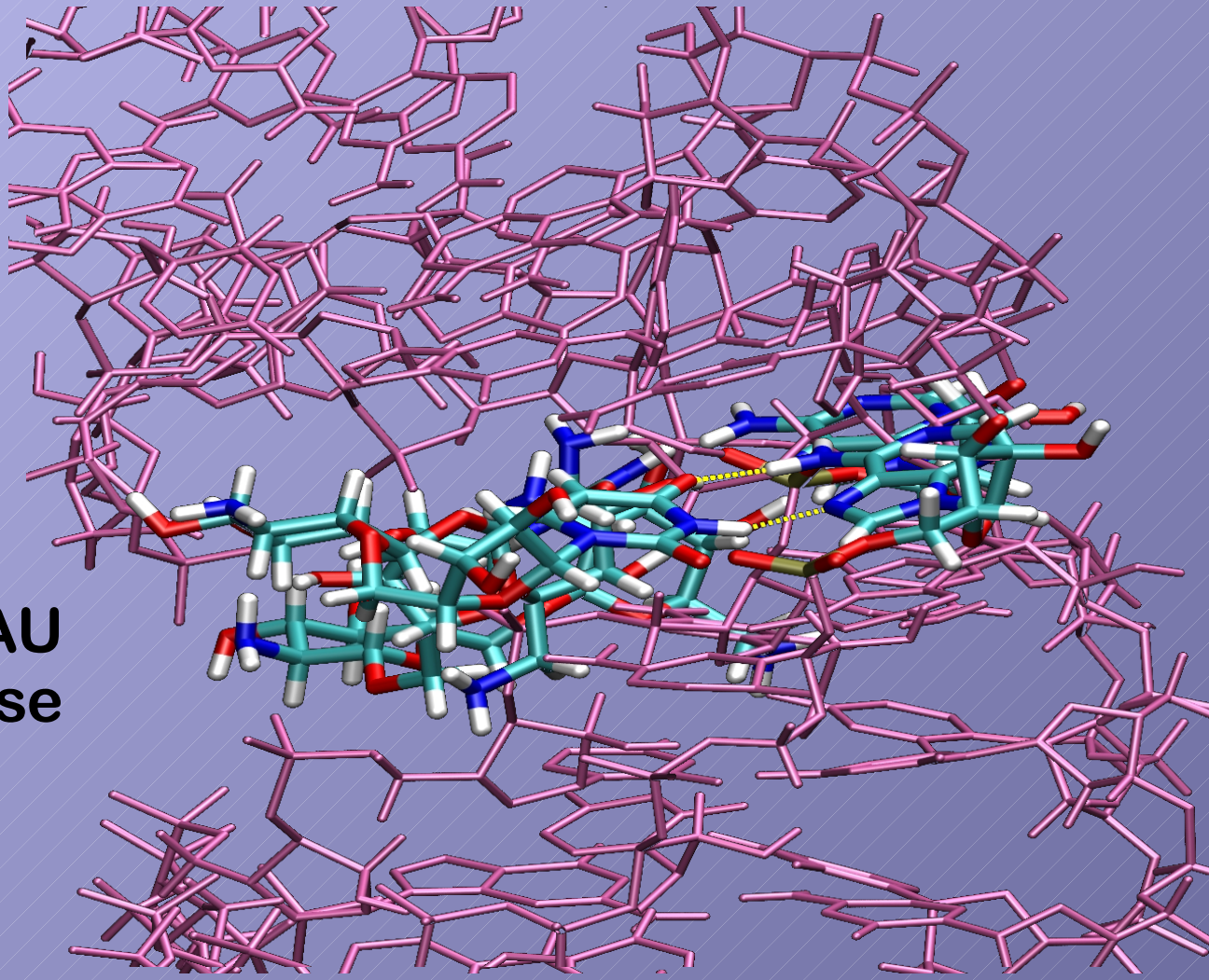
Neamine compound who was difficult to correlate with classical docking is expulsed with MD.

Flexibility of the target

Graphical analysis



Formation of a AU reverse hoogsten base pair



MD permit the adaptation of the target to form a triple base-pair

Conclusions & prospects

Conclusions:

- Design of a specific set of parameters for the scoring function
- Docking is able to discriminate "good" or "bad" ligands
- Docking is limited for RNA because of induced fit
- MD can predict more efficiently free energy of binding
- MD is able to remove "false" docking position
- MD need big computation times not yet applicable for VS

Prospects:

- Finish calculation for RNA 18S
- Extract structure activity relationships
- Limiting MD computation times with SA with restraint?

Aknowledgements

Peking University CHINA, Health science center
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Group of Molecular Modeling & Chemical Information

Thank you for your attention