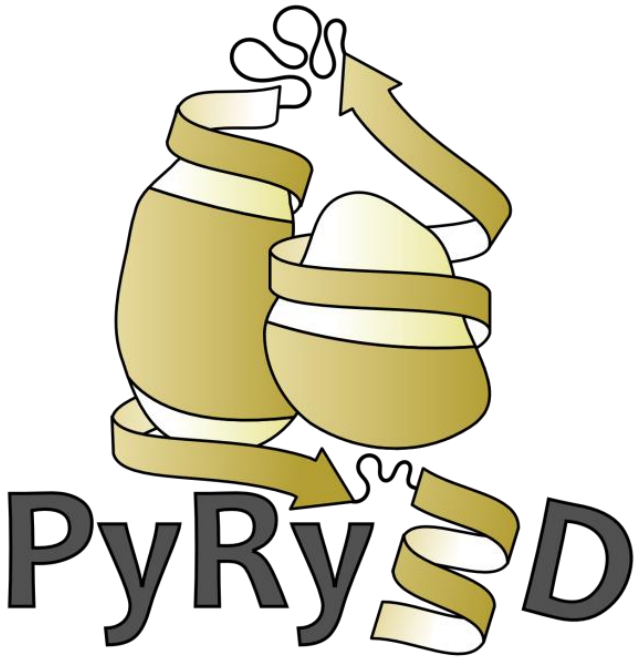
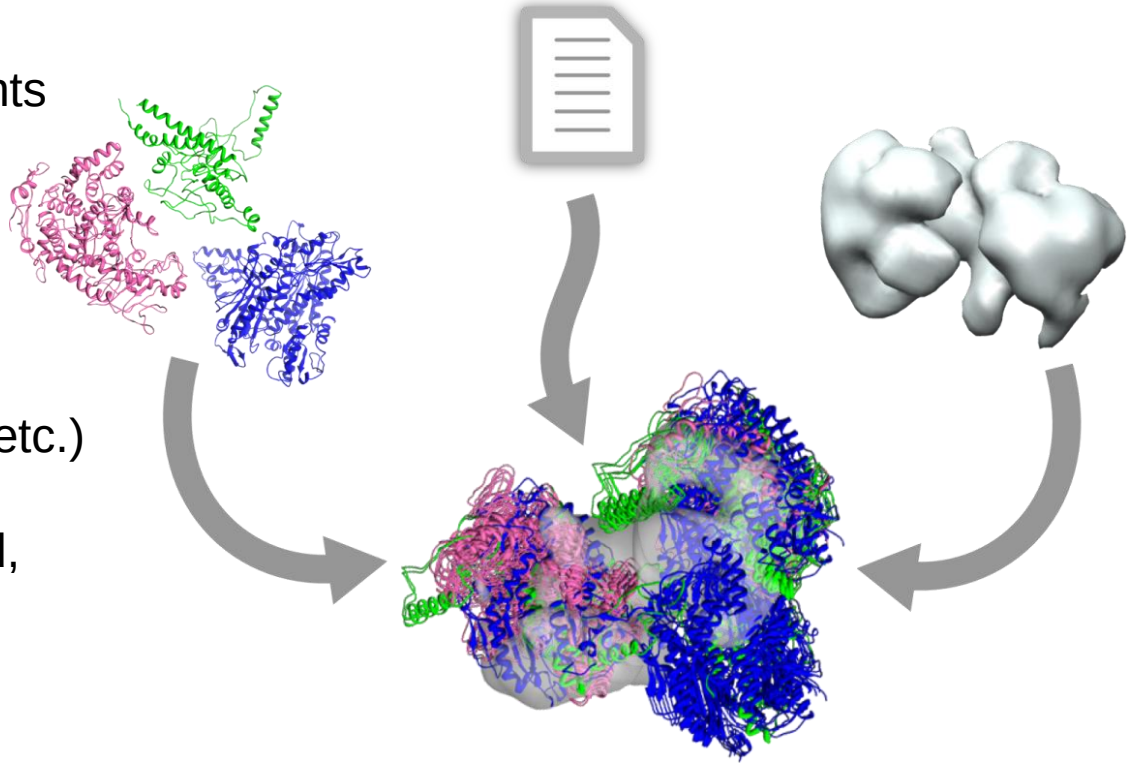


a software tool for low-resolution modeling of large macromolecular complexes

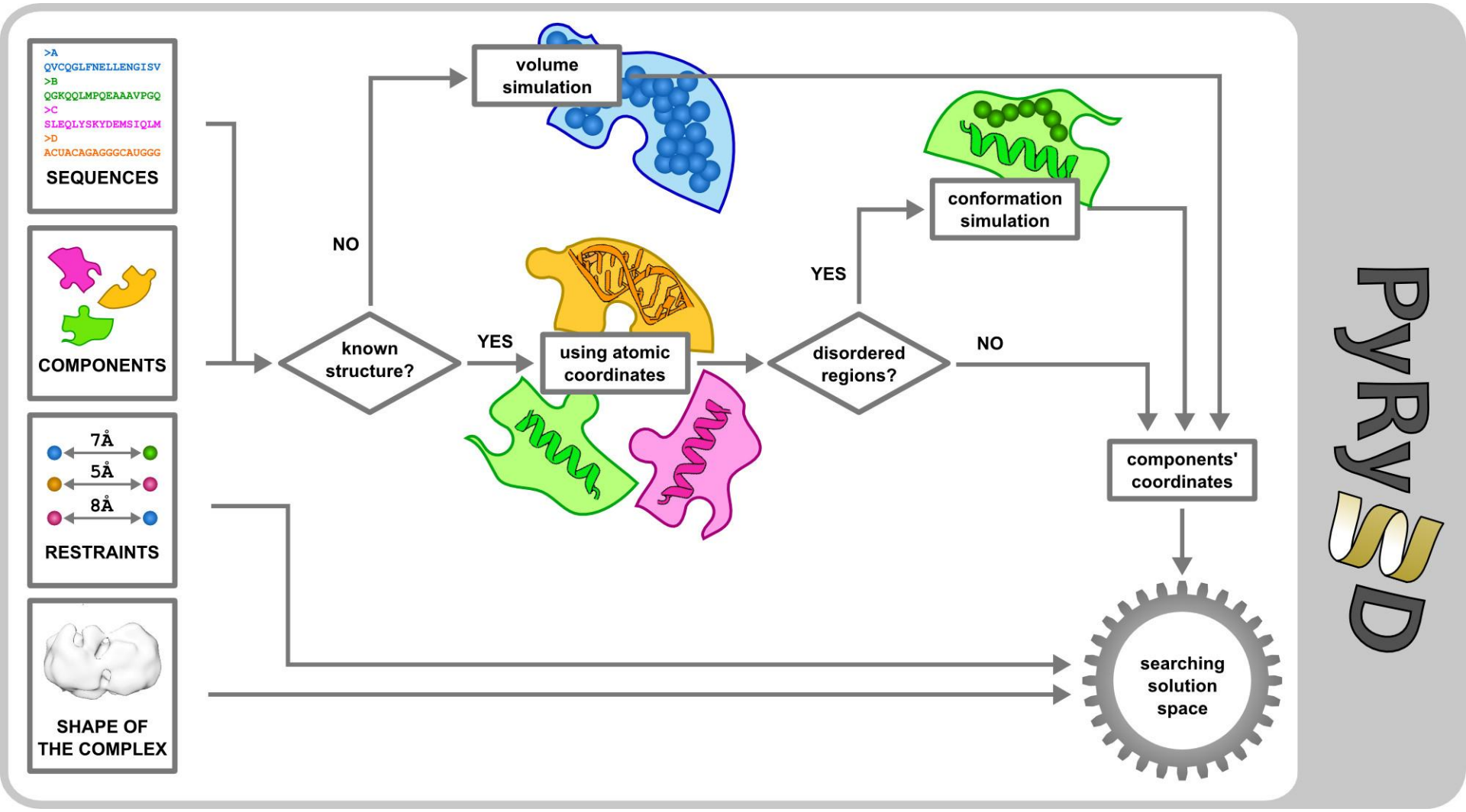
Making sense out of the available data

- sequences of all components
- structures of some components
- models of some components
- disorder / flexibility
- molecule shapes
  (**cryoEM, SAXS, SANS**)
- distance restraints
  (**FRET, EPR, cross-linking**, etc.)
- accessibility
  (enzyme active sites exposed,
  footprinting, etc.)

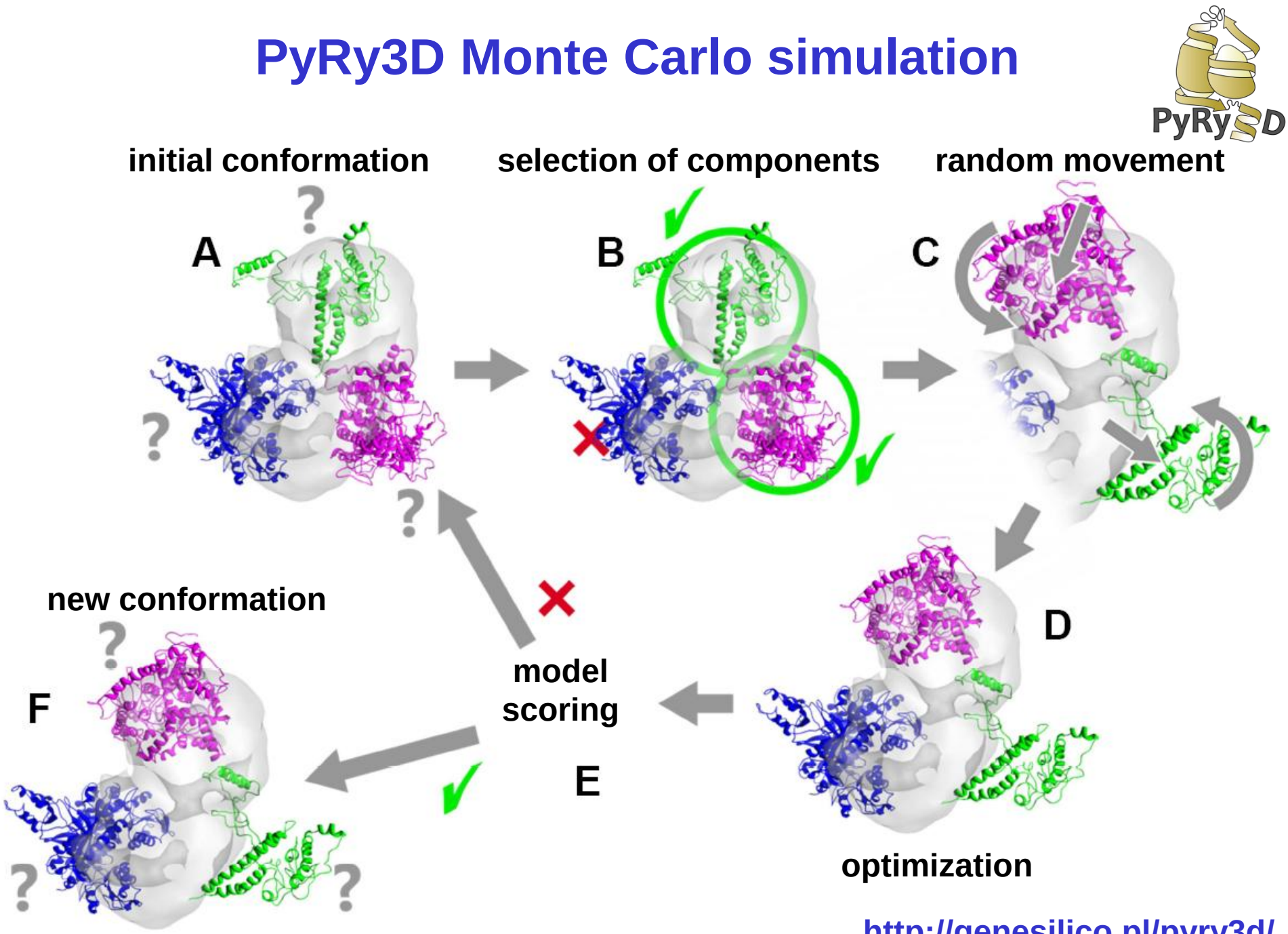


Is it possible to build a 3D model consistent with these data?

Workflow

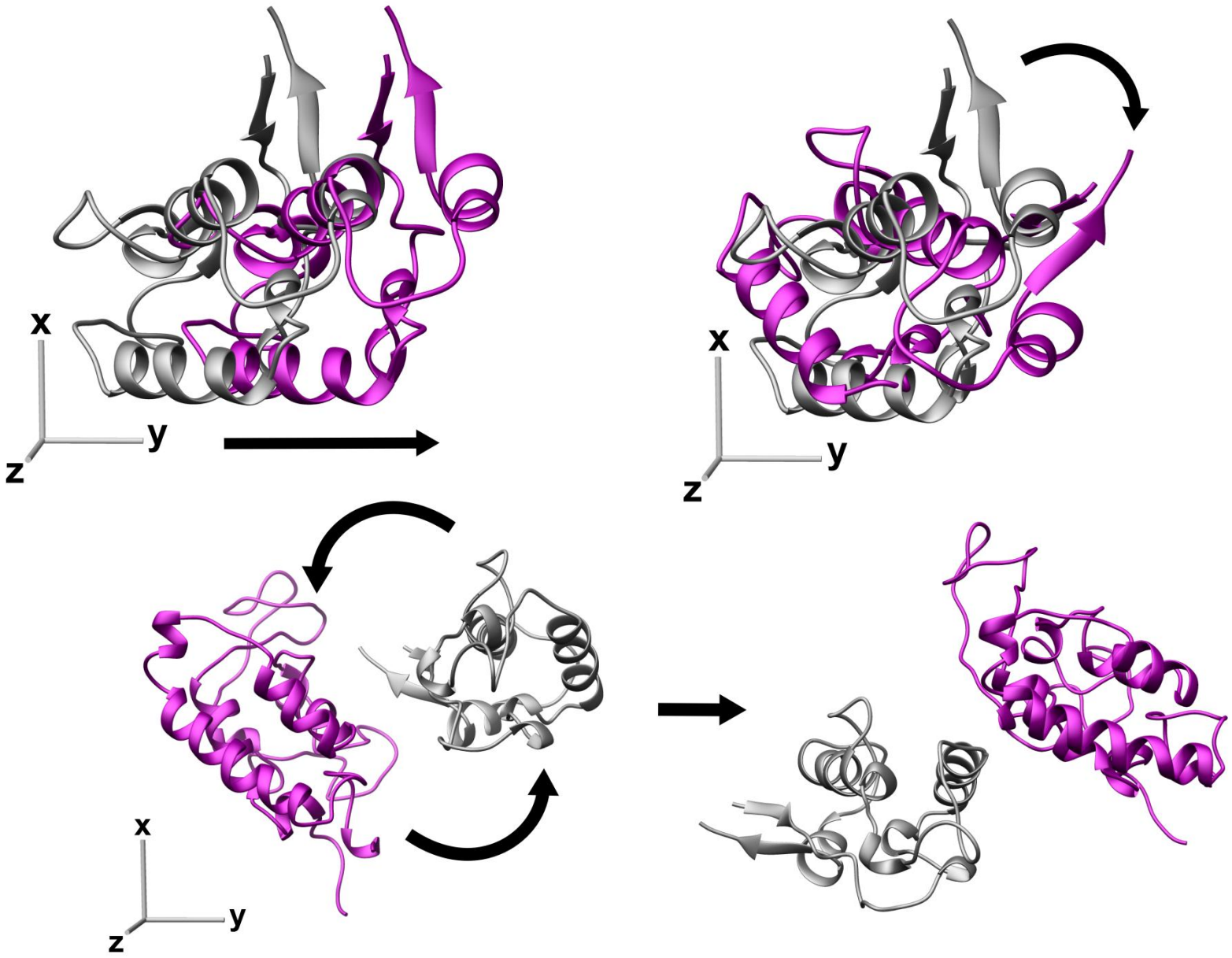

>A
QVCQGLFNELLENGISV
>B
QGKQQLMPQEAAAVPGQ
>C
SLEQLYSKYDEMSIQLM
>D
ACUACAGAGGGCAUGGG
SEQUENCES
COMPONENTS
7Å
5Å
8Å
RESTRAINTS
SHAPE OF THE COMPLEX
known structure?
NO
YES
volume simulation
using atomic coordinates
disordered regions?
YES
NO
conformation simulation
components' coordinates
searching solution space
PyRySD

PyRy3D Monte Carlo simulation

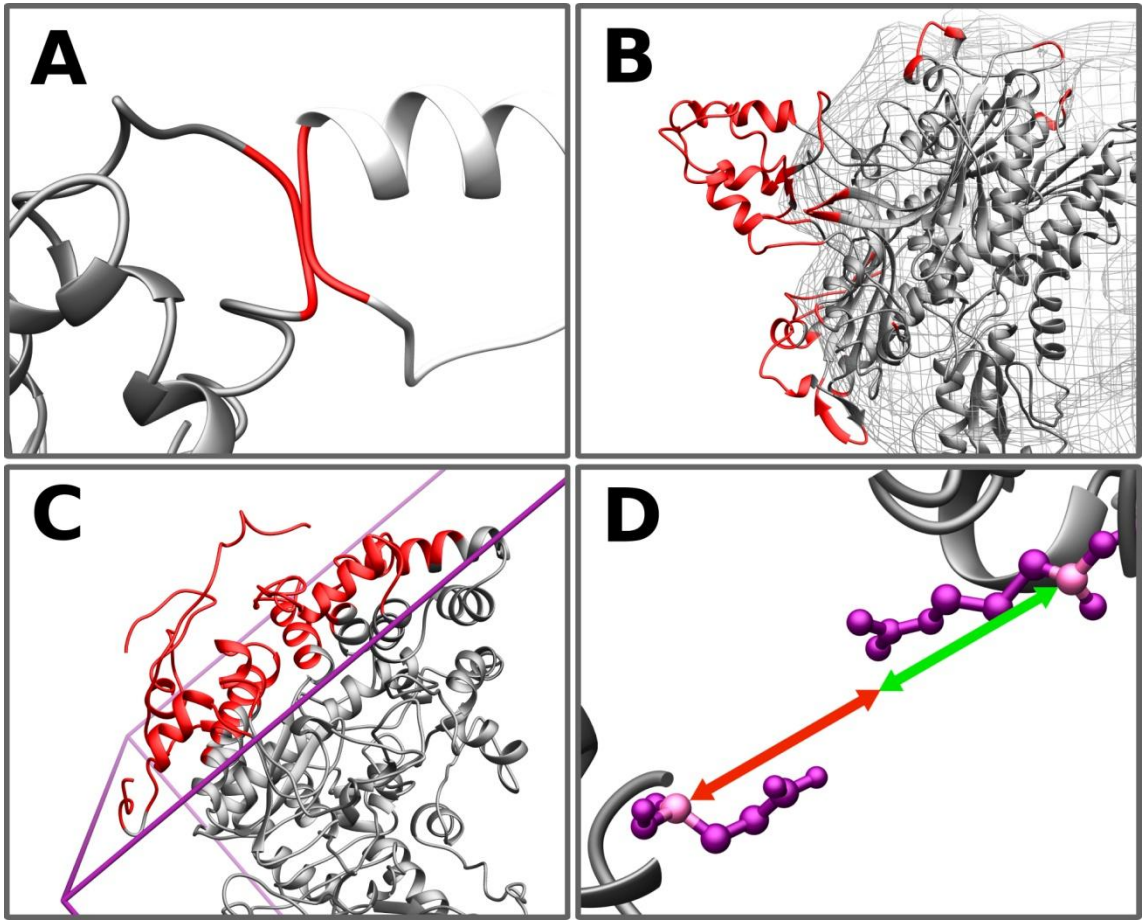


http://genesilico.pl/pyry3d/

Set of movements



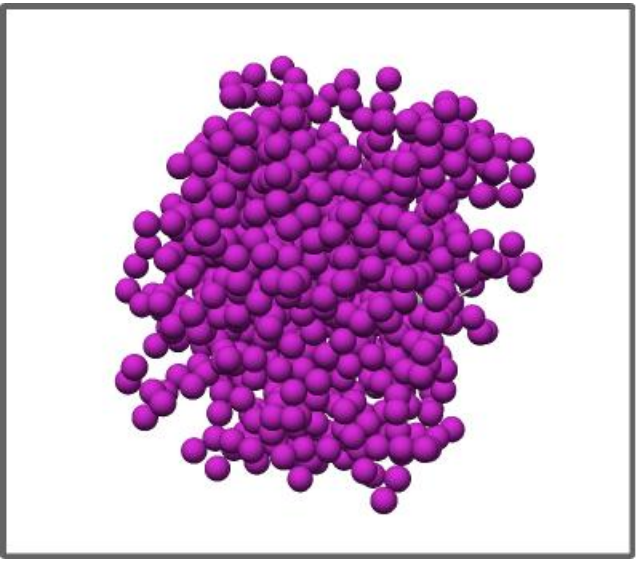
Scoring function

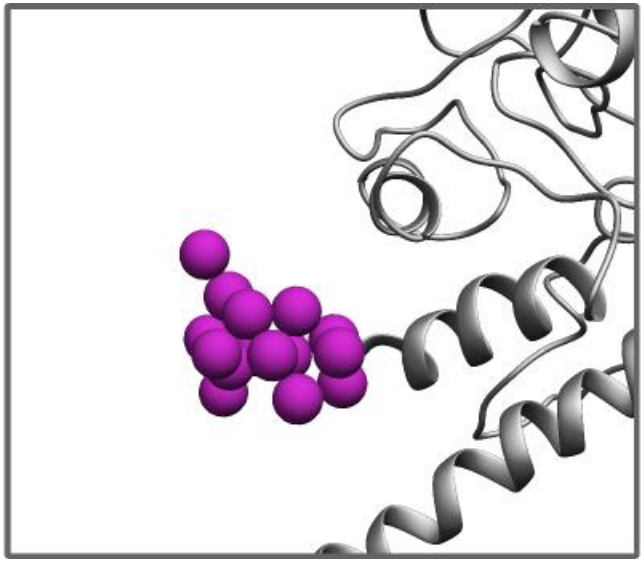


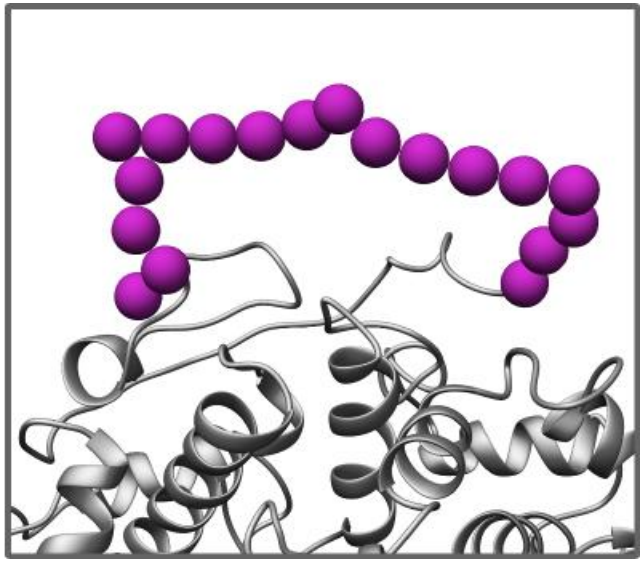
Statistical potentials to evaluate interactions (to be added soon):

- **protein-protein**
- **protein-RNA/DNA**

Including information about flexibility



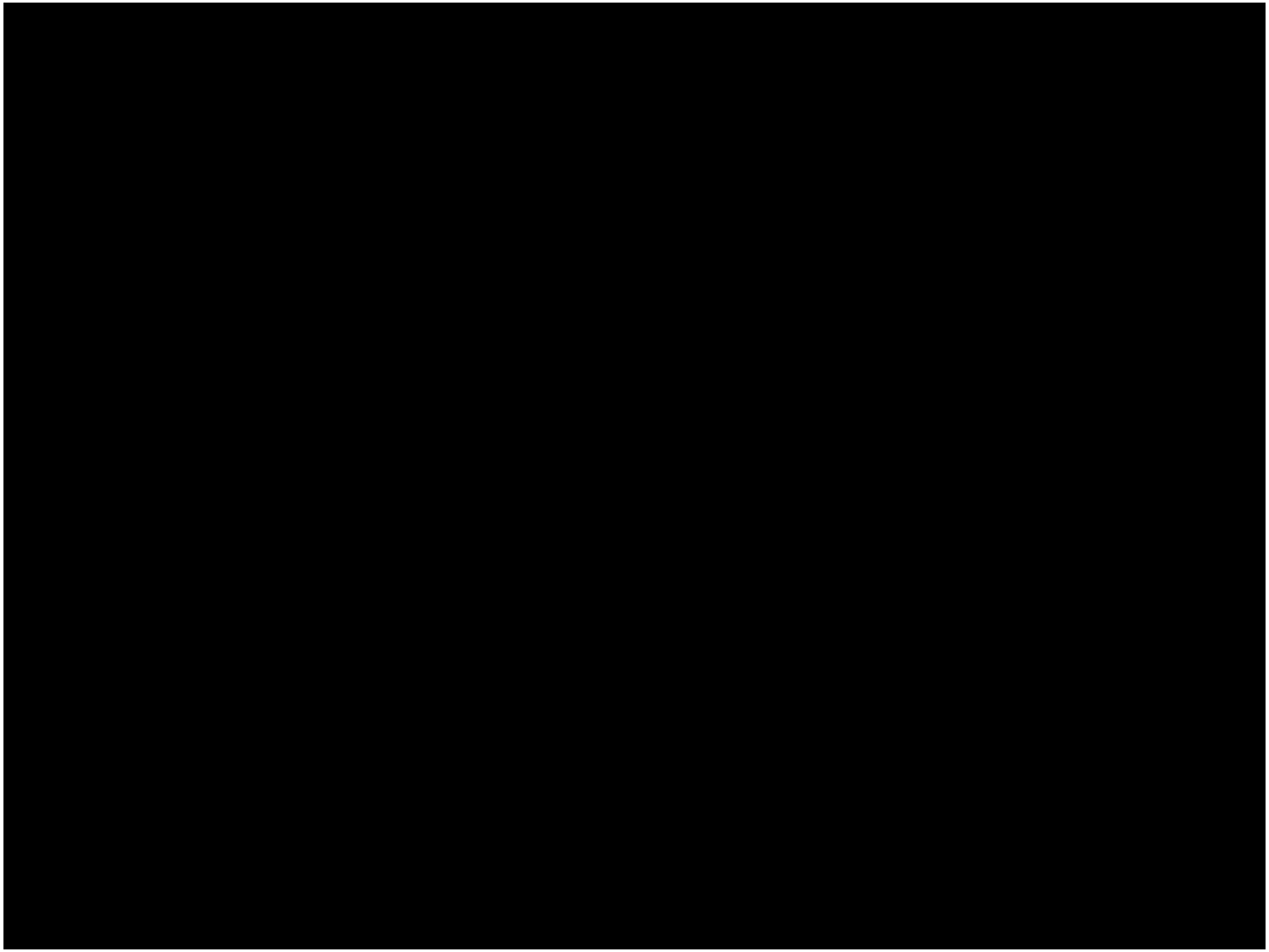




Pseudoatoms are used to build components with:

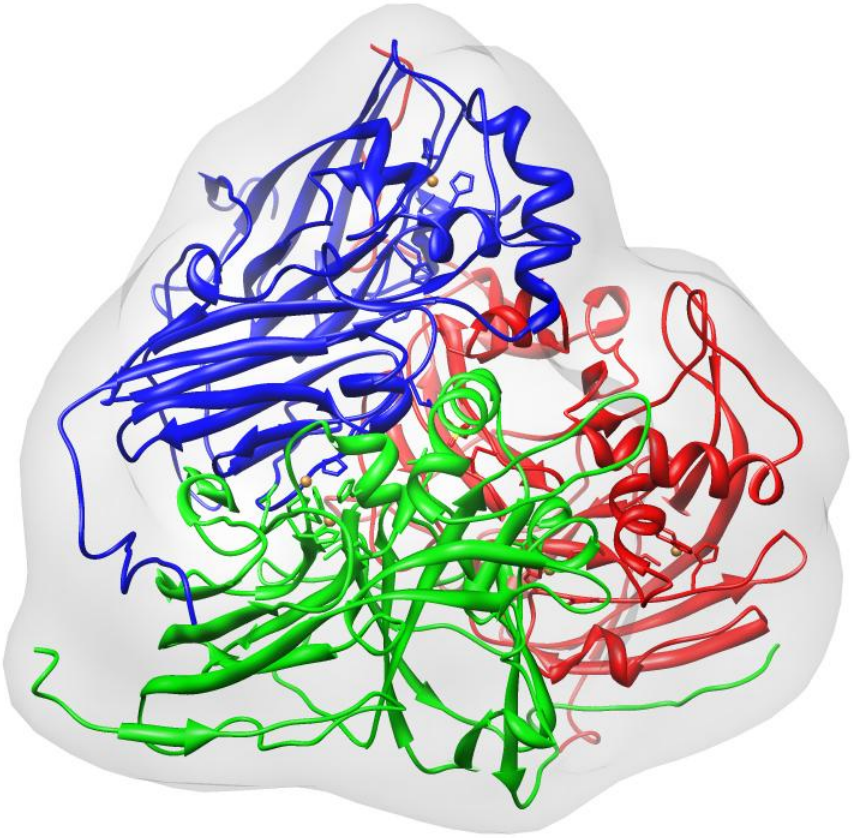
- **no structural data**
- **missing terminal fragments**
- **missing internal fragments**

PyRy3D Monte Carlo simulation
copper-nitrite reductase (1NIC)

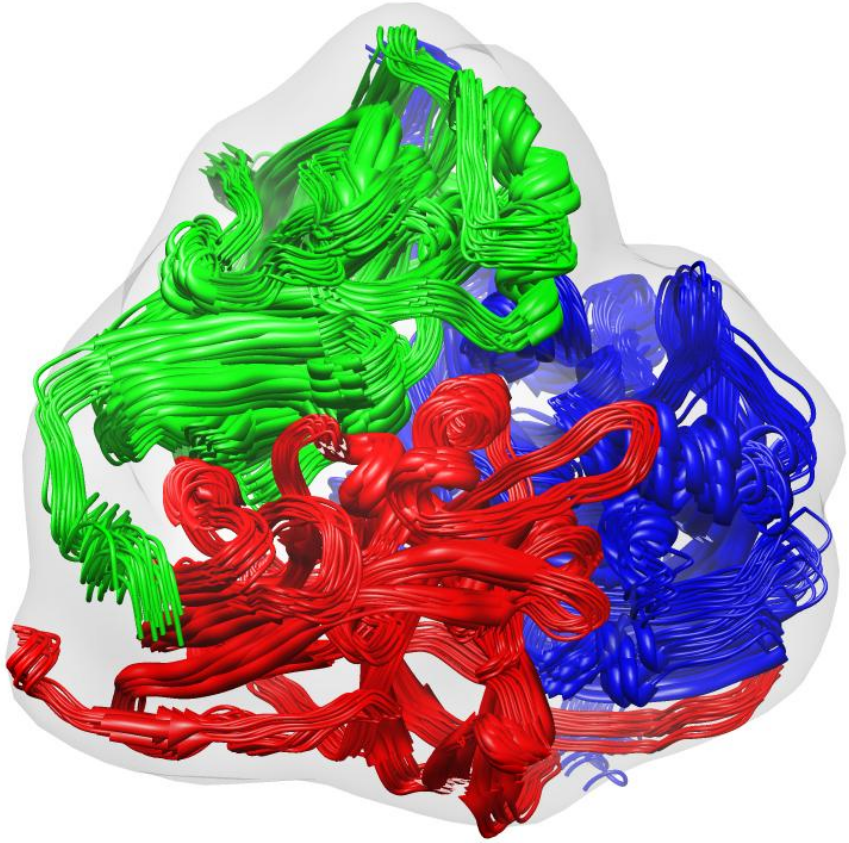


http://genesilico.pl/pyry3d/

Example results:
copper-nitrite reductase (1NIC)



X-ray structure



models consitent
with cryoEM map

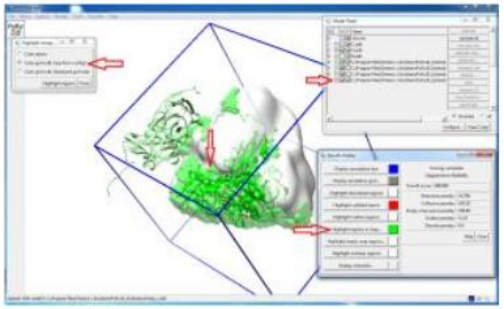
http://genesilico.pl/pyry3d/

PyRy3D usage

PyRy3D command-line (engine)

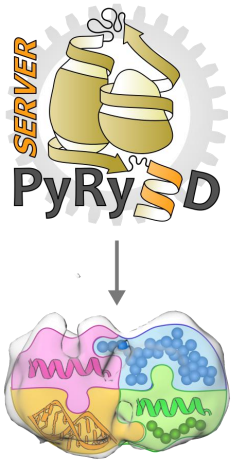
```
E:\pyrky\PyRy3D_v4.2>python pyry3d.py --help

   PyRy3D
   program for macromolecular docking into cryoEM maps

   (c) 2010 by Joanna M. Kasprzak

   usage: python pyry3d.py --help

Usage: pyry3d.py [options]

Options:
  -h, --help            show this help message and exit
  -s FILE, --seq_file=FILE
                        input file with sequences in PASTA format
  -d FILE, --dirfile=FILE
                        input file with structure
  -r FILE, --restr_file=FILE
                        input file with restraints in Filtrest3D format
  -m FILE, --map_file=FILE
                        input file with complex map
  -x FILE, --saxs_file=FILE
                        input file with complex map shape
  -c CONFIG, --config=CONFIG
                        config file with simulation parameters
  -o OUTPUT, --output=OUTPUT
                        write output to file
  -t TRAFLFILE, --traflfile=TRAFLFILE
                        write output to trajectory file
  -f FULLATOM, --to full atom model=FULLATOM
                        write best model in fullaton representation
  -v MOVEHISTORY, --save history of moves=MOVEHISTORY
                        save all moves into a file
  -s SCORE_PLOT, --save plot with complexes scores=SCORE_PLOT
                        save plot with complexes scores
```

PyRy3D Extension (GUI)



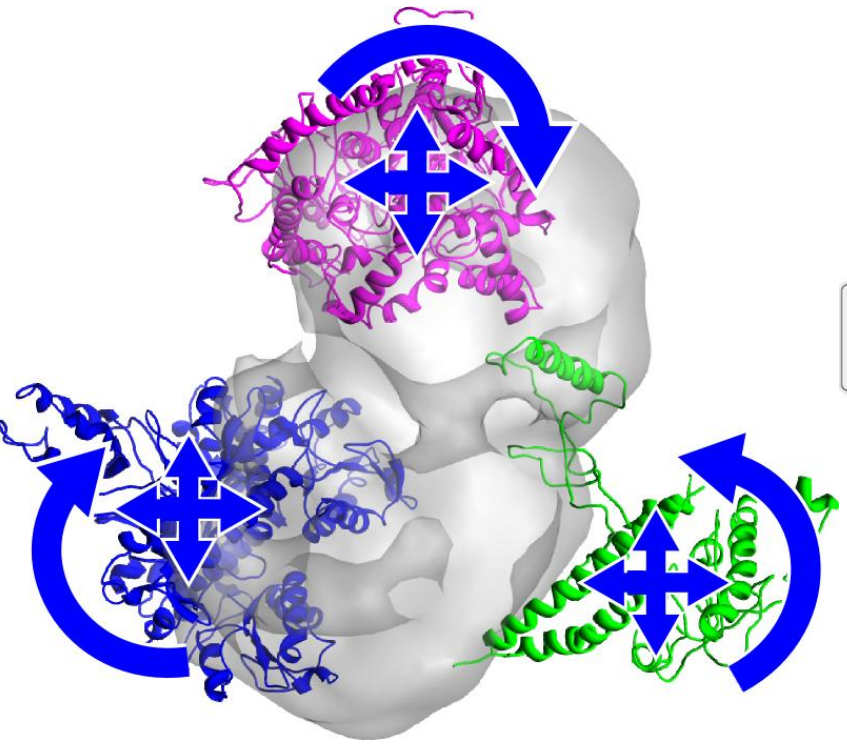
web server

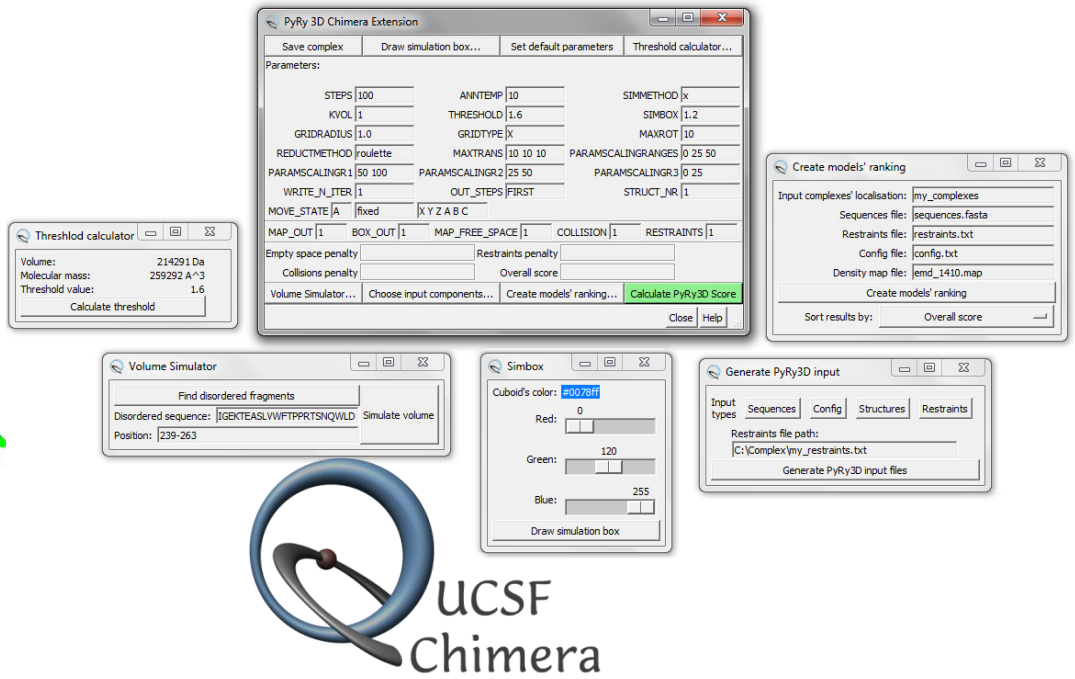
http://pyry3d.icm.edu.pl



http://genesilico.pl/pyry3d/

PyRy3D GUI – UCSF Chimera extension

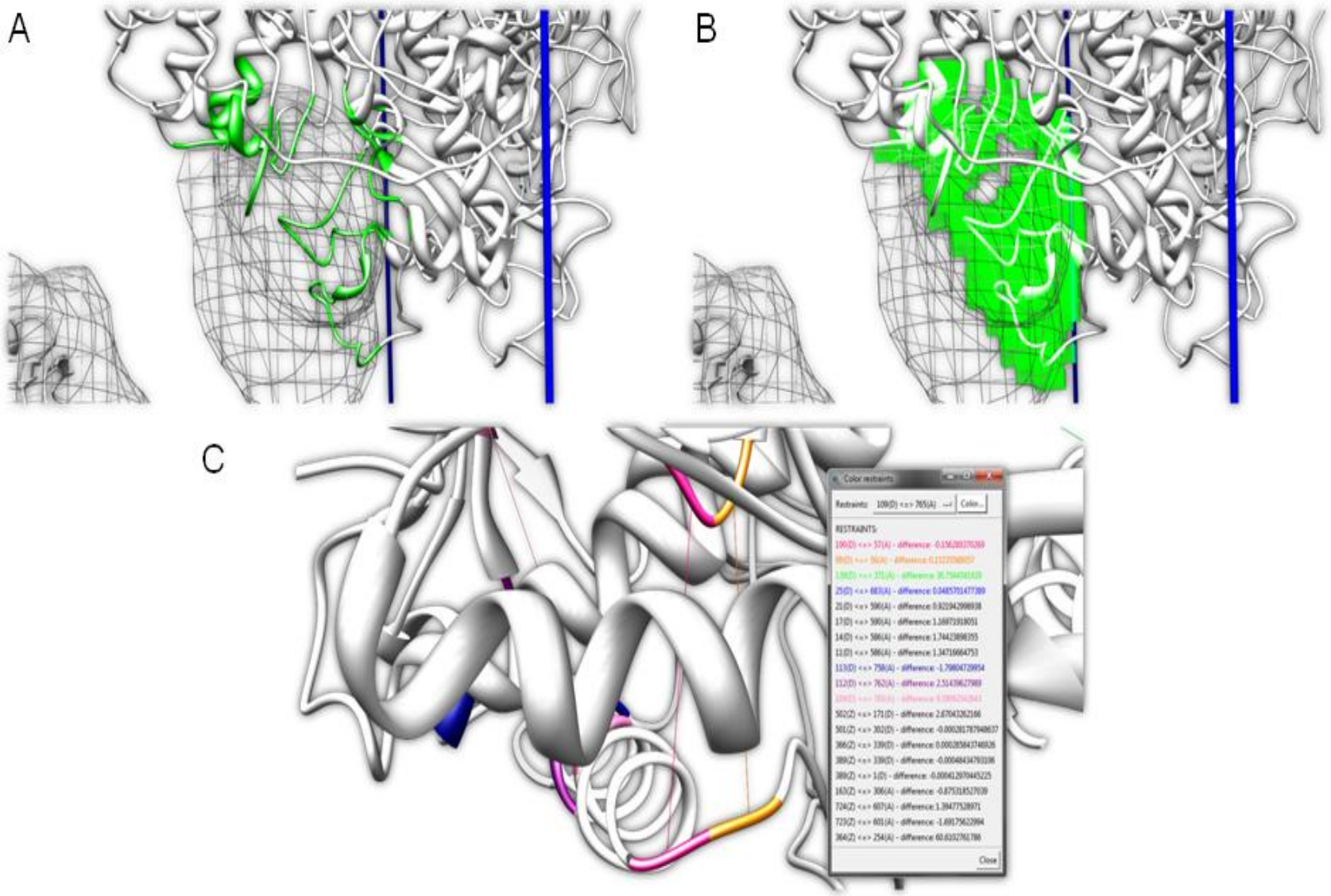




- generate input files for PyRy3D
- run simulations
- rank models
- analyze the scores (e.g. visualize, cluster, rank)
- generate animations
- **and many many more features!!**

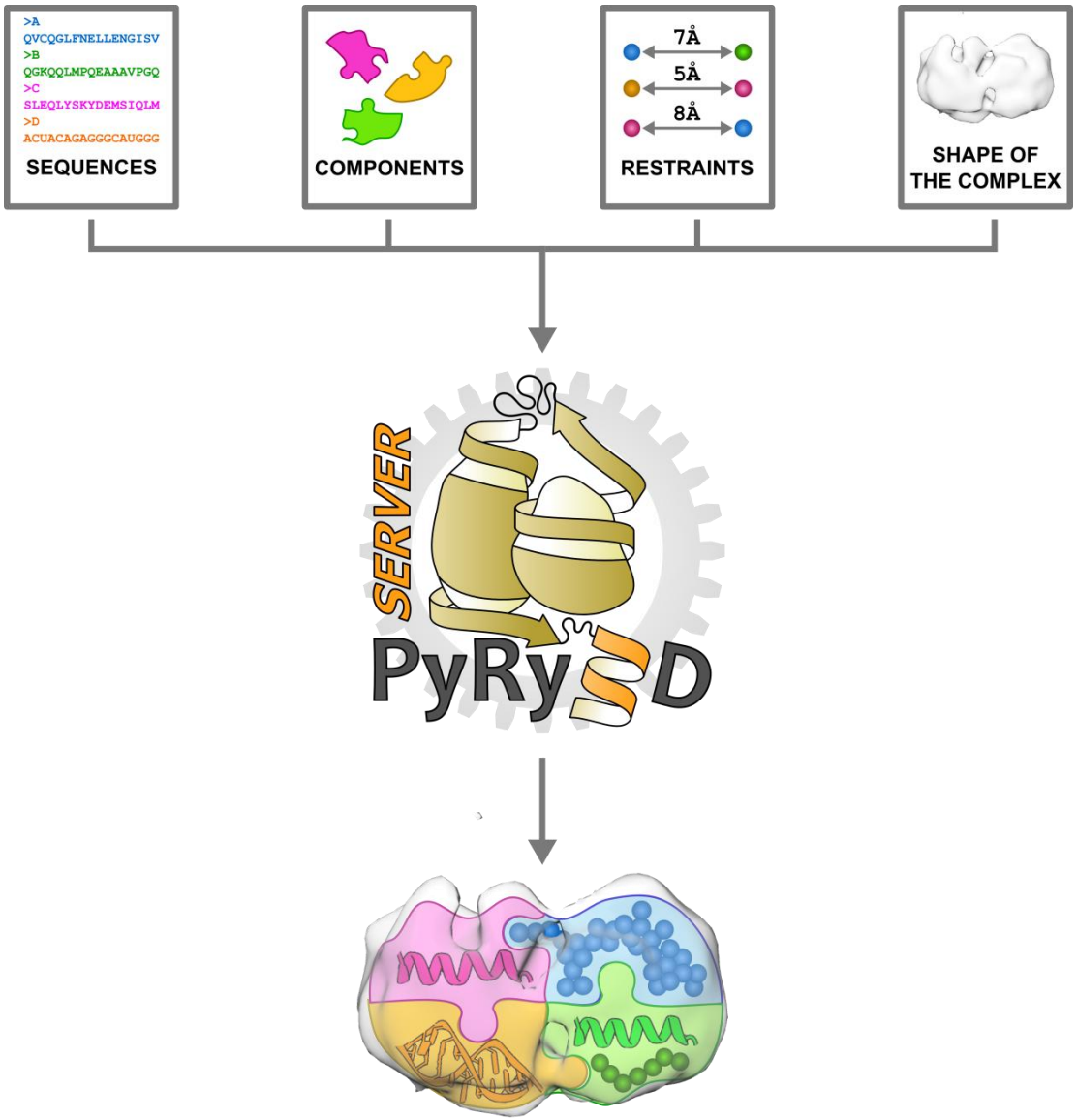
http://genesilico.pl/pyry3d/

PyRy3D GUI – UCSF Chimera extension



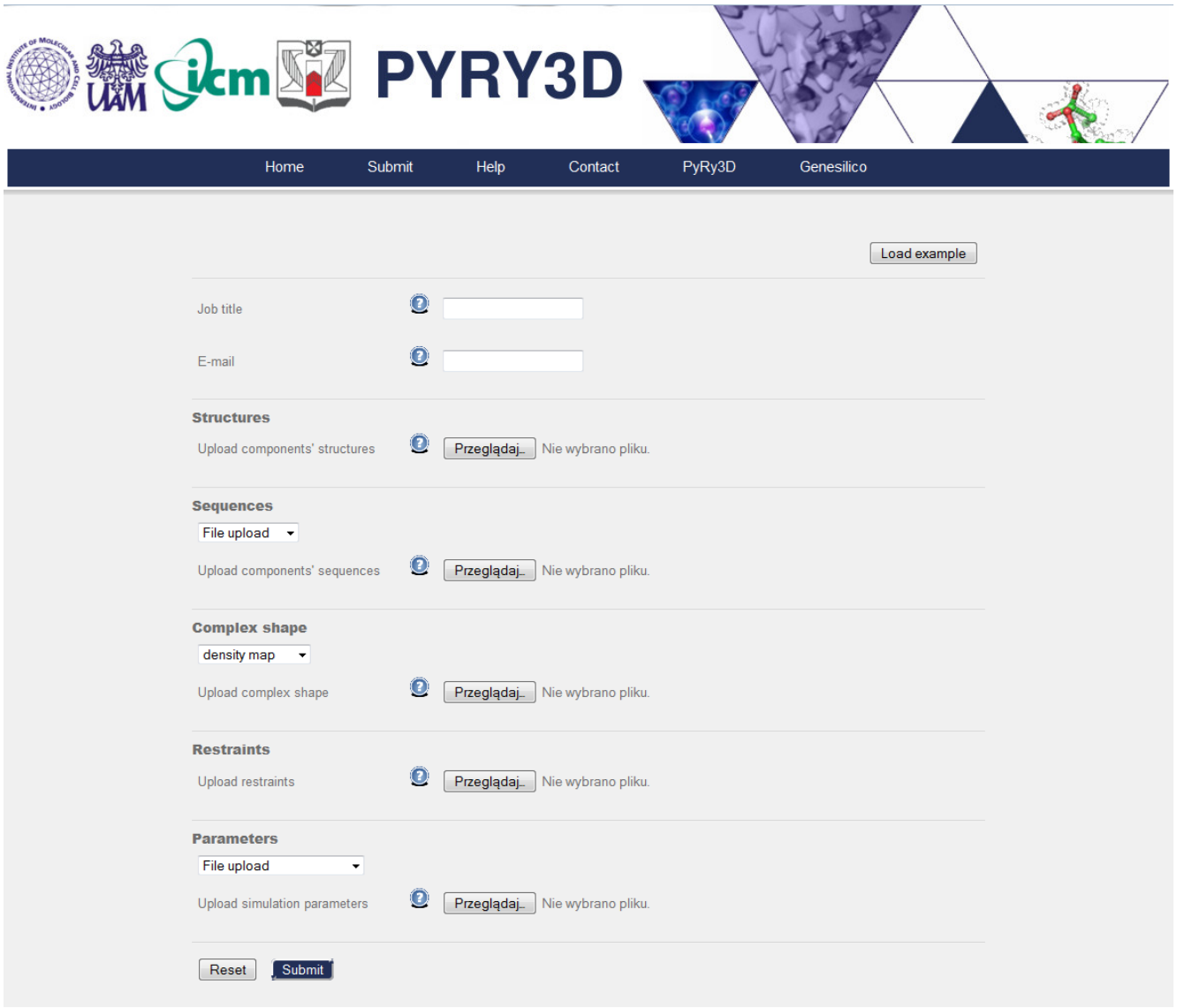
http://genesilico.pl/pyry3d/

PyRy3D server



http://pyry3d.icm.edu.pl

PyRy3D server



http://pyry3d.icm.edu.pl

Highlights of PyRy3D

Easy to use for non-experts, has a graphical user interface to generate input files, set up simulation parameters, visualize the simulation in real time, change parameters during the simulation etc.

Fully scriptable, expert users can write Python scripts for complicated operations

Enables the use of electron density maps and SAXS/SANS data to describe shapes

Enables the use of many different restraints from experiments and predictions

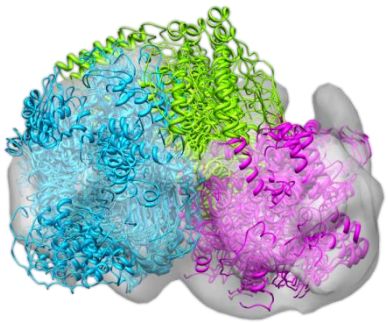
Can use crystal structures, NMR ensembles, theoretical models, arbitrary shapes

Can model disordered segments and sequences without known structure

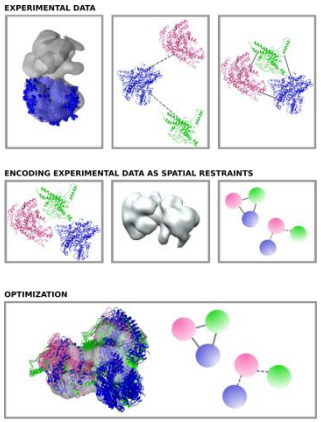
http://genesilico.pl/pyry3d/

Summary

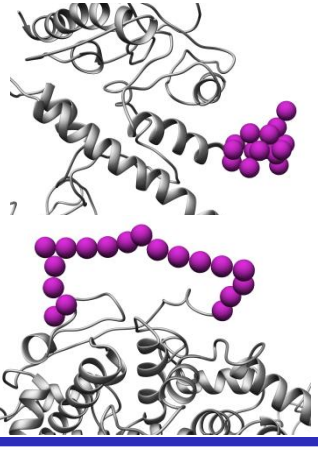
BUILDS LOW-RESOLUTION MODELS



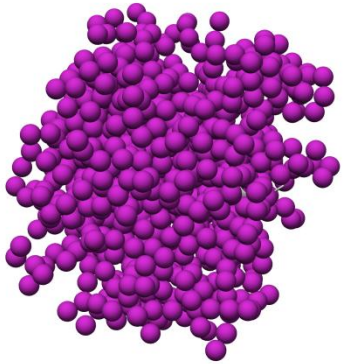
WORKS WITH RESTRAINTS



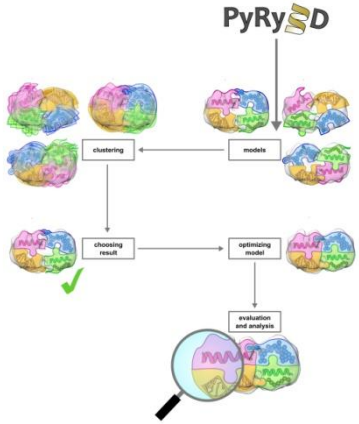
ADDS MISSING FRAGMENTS



SIMULATES VOLUMES



PROVIDES ENTIRE MODELING PIPELINE

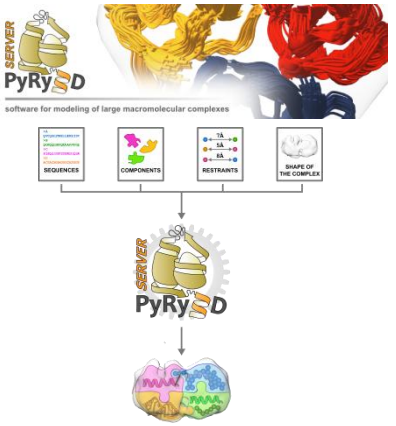


FULLY SCRIPTABLE

http://genesilico.pl/pyry3d

GRAPHICAL INTERFACE

ONLINE SERVER



http://pyry3d.icm.edu.pl

3,2,1 …. START!!!

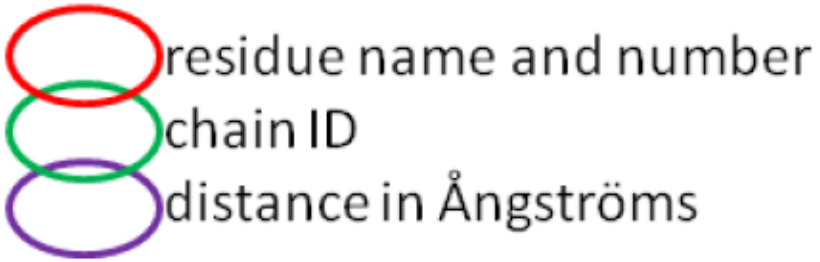
How to prepare input files?

DATA TYPE	FORMAT	COMMENTS
***STRUCTURES**	PDB	• EACH COMPONENT AS ONE CHAIN; • IF DISORDERED REGIONS OCCUR – NUMBERING OF RESIDUES MATTERS
SEQUENCES	MULTI FASTA	• REQUIRED ONLY WHEN DISORDERED REGIONS OCCUR
DENSITY MAP AB INITIO MODEL SAXS CURVE	MRC PDB – DAMMIN/IF .DAT	
RESTRAINTS	FILTREST3D	• POSSIBLE TO ASSIGN WEIGHTS TO RESTRAINTS AND TO COMBINE THEM BY BOOLEAN OPERATORS
CONFIGURATION FILE	TEXT FILE	• ALL PARAMETERS CAN BE MODIFIED HERE

*** required**

Restraints file – extented Filtrest3D format

```
dist (
    (G8) "C" - (U9) "D" (<=1.50)
    (U11) "D" - (C12) "A" (<=1.50)
    (G59) "A" - (A60) "D" (<=1.50)
    (C62) "D" - (C63) "B" (<=1.50)
    (G83) "B" - (U84) "D" (<=1.50)
    (G88) "D" - (C89) "C" (<=1.50)
    )
```



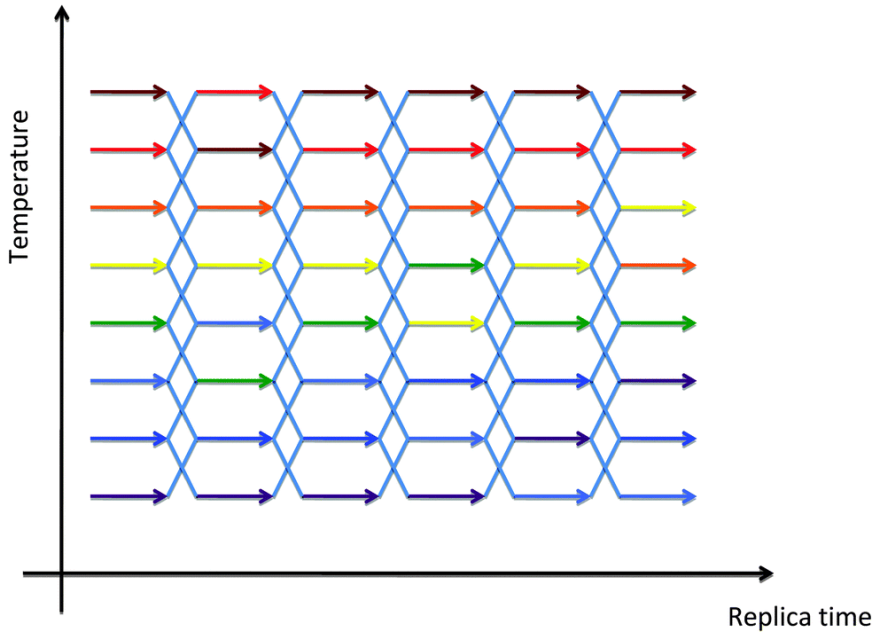
- Boolean operators: AND, OR
- Other types of restraints: PointDistance, SurfaceAccess, Symmetry, Relation

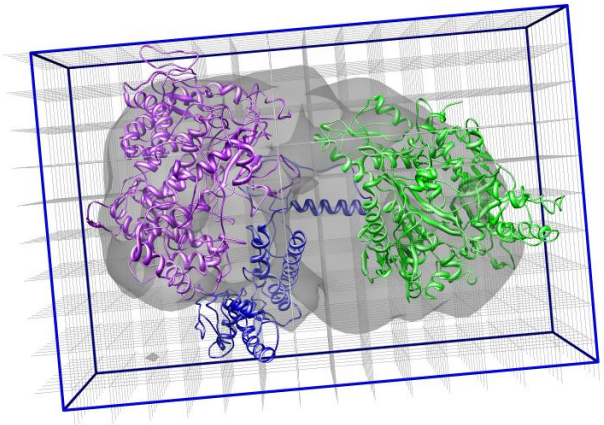
How to set first modeling?

SIMMETHOD SimulatedAnnealing
ANNTEMP 10

STEPS 100
WRITE_N_ITER 10

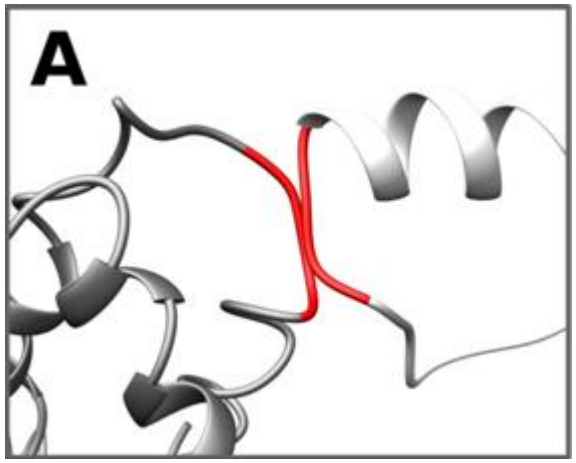
SIMBOX 1.2
GRIDRADIUS 1.0

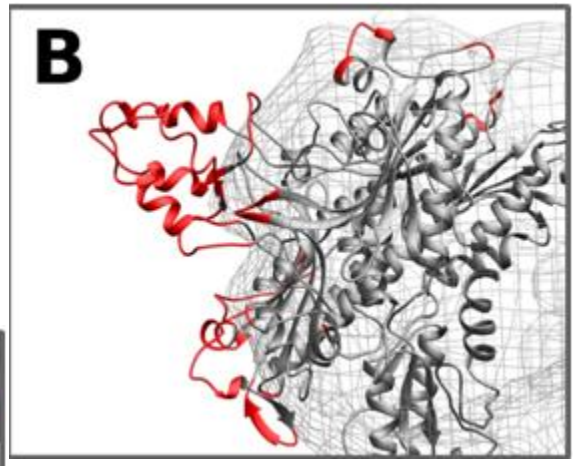




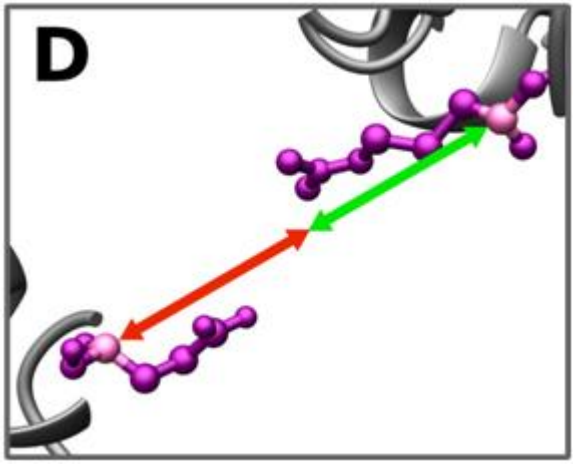
How to set scoring function weights

CLASHES 10 10
CLASHES_ALLATOMS 1 1





OUTBOX 10 10
MAP_FREESPACE 5 1
DENSITY 0 3



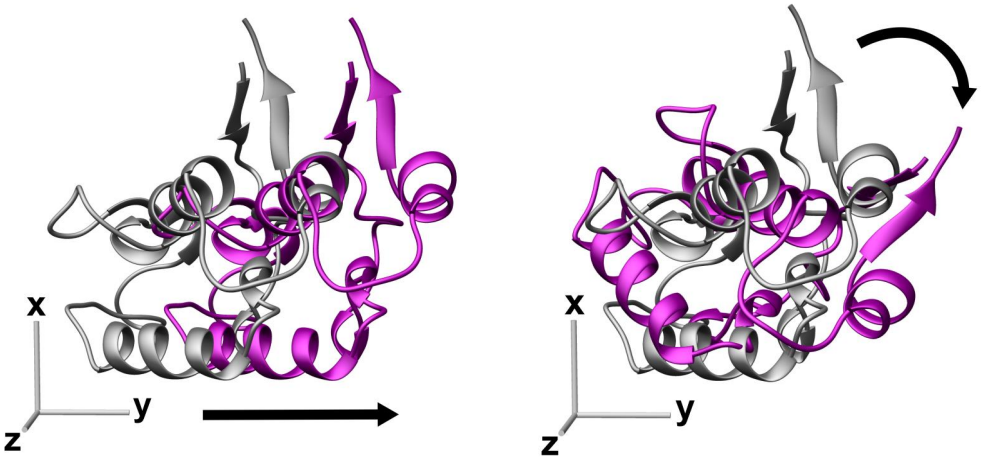
RESTRAINTS 1 1
SYMMETRY 0 0

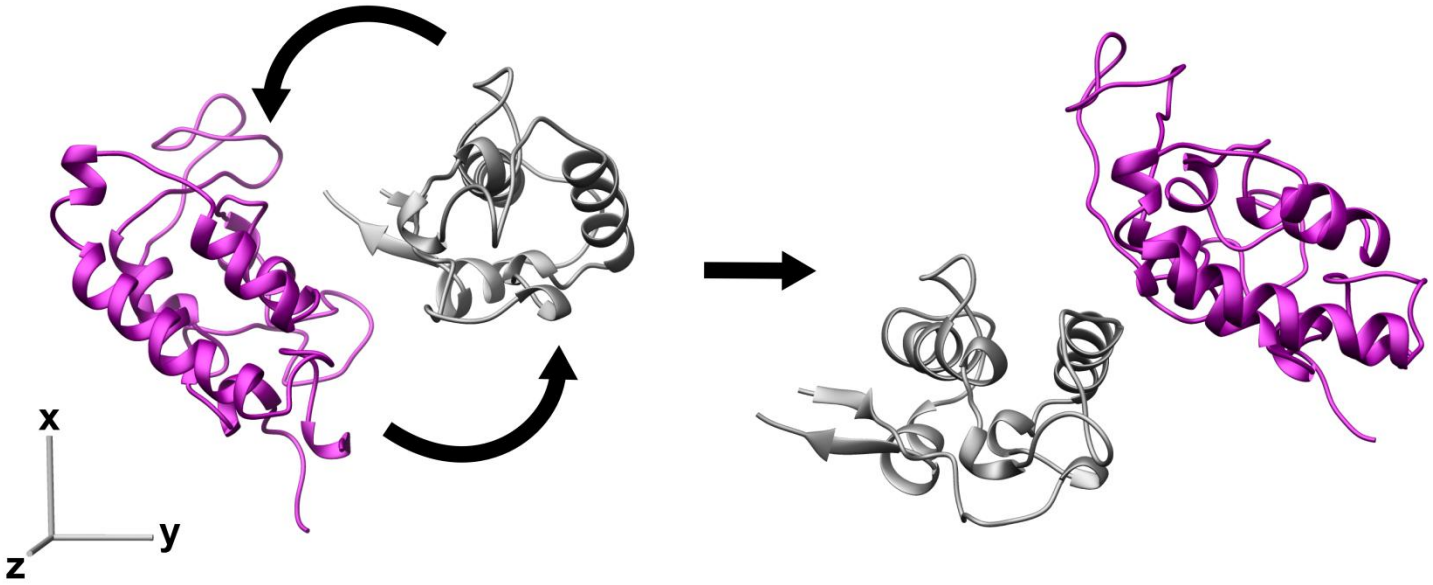
CHI2 1 1
RG 1 1

SCALE_PARAMS ON

How to choose allowed moves

ROTATION_FREQ	0.3
ROTATION_COV_FREQ	0.0
TRANSLATION_FREQ	0.3
EXCHANGE_FREQ	0.4
EXCHANGESAMPLE_FREQ	0.0
SIMUL_DD_FREQ	0.0
TRANSLATION_ALL_FREQ	0.0
ROTATION_WHOLE_FREQ	0.0





PyRy3D team

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Mateusz Dobrychłop
Wojciech Potrzebowski

Witold Rudnicki
Mateusz Susik
Laura Pogorzelska
Rafał Niemiec

Thank you for your attention!