Structural analyses of the ribosome by hybrid approach

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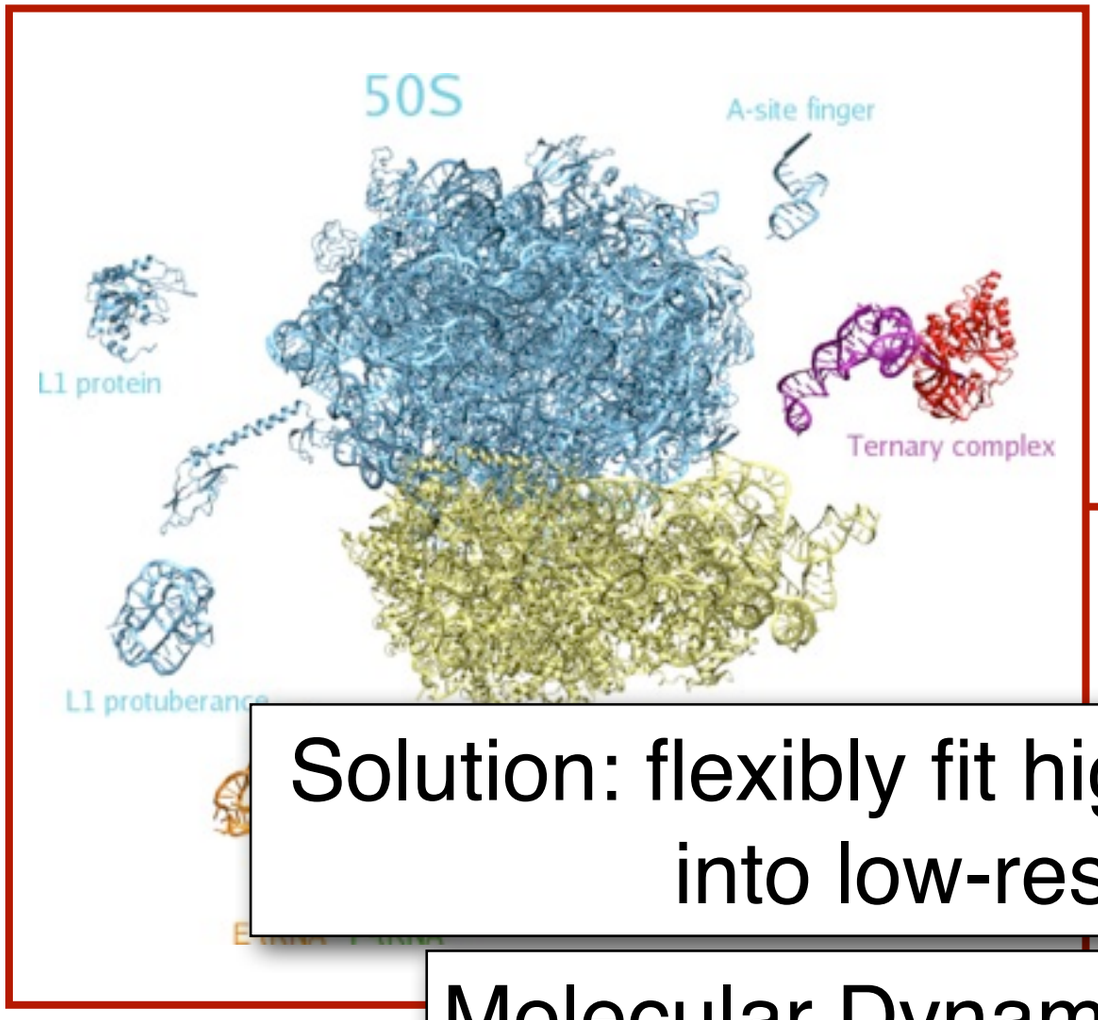
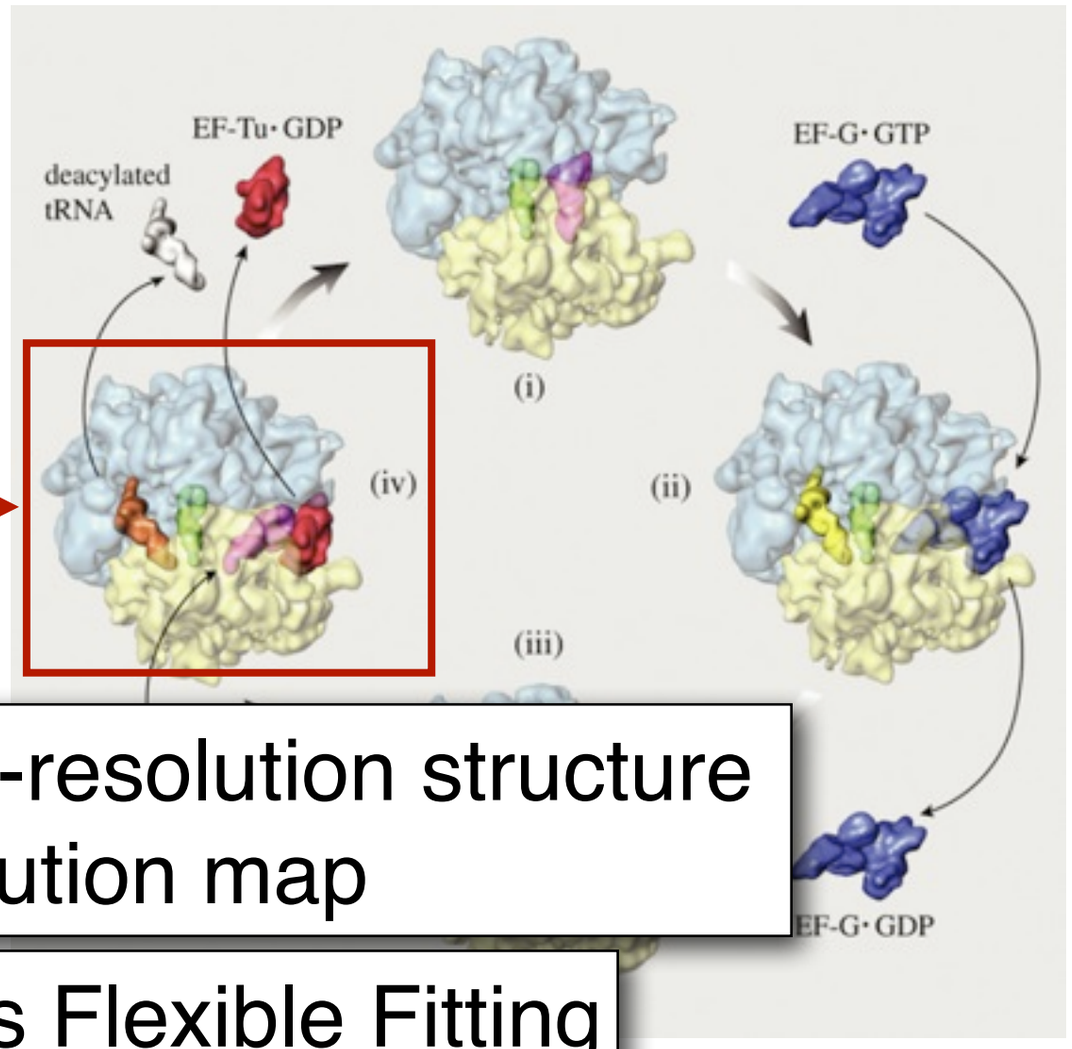
Need of hybrid approach

X-ray crystallography



Cryo-electron microscopy



Solution: flexibly fit high-resolution structure into low-resolution map

Molecular Dynamics Flexible Fitting

High resolution (3-5Å)
Crystal packing makes it difficult
to capture different functional states

Medium resolution (typically 5-10Å)
Many functional states can be obtained

Right figure reproduced from: Frank (2004) The dynamics of the ribosome inferred from cryo-EM, in Conformational Proteomics of Macromolecular Architectures.

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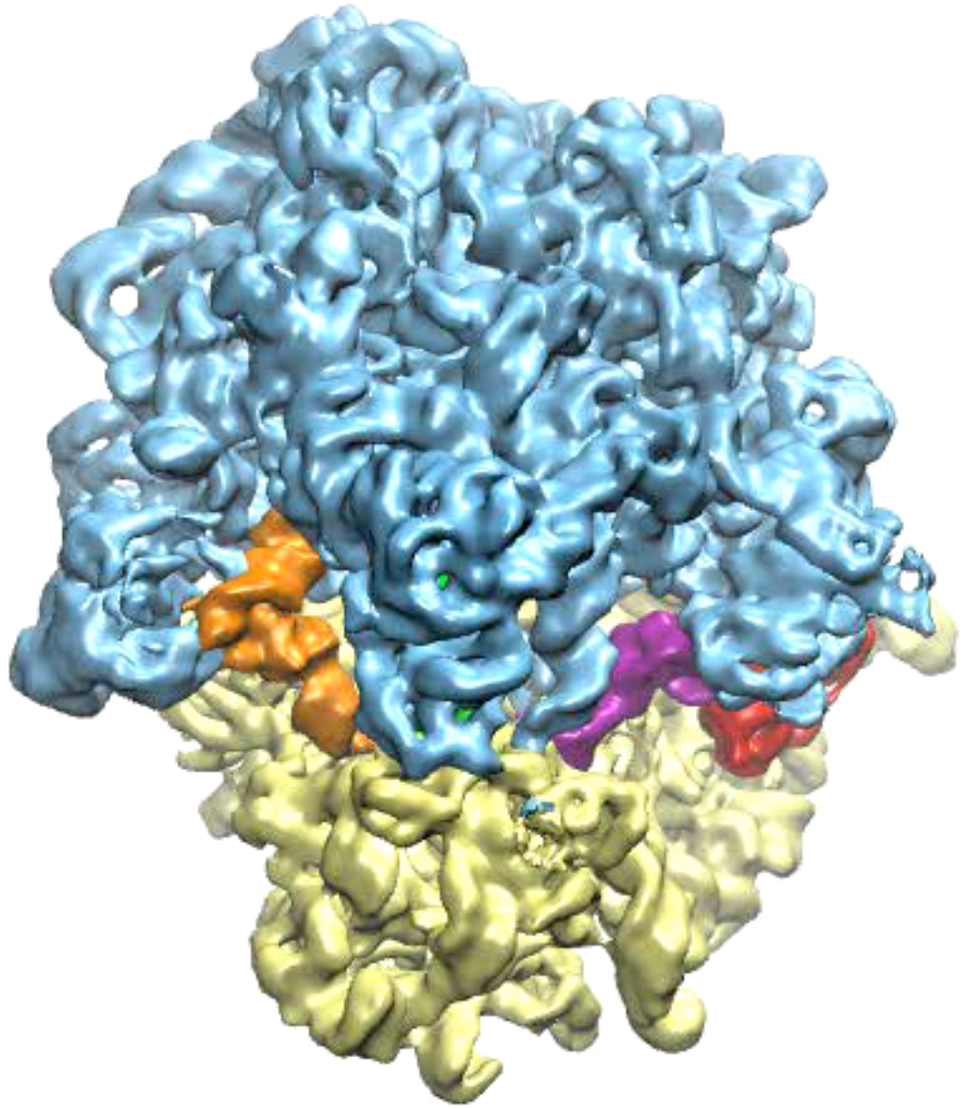
Multistep fitting protocol of RNA-protein complex

Cryo-EM map of the *E. coli* ribosome at 6.7-Å resolution



[1] Trabuco et al. *Structure* (2008) 16:673-683.
[2] Villa et al. *PNAS* (2009) 106:1063-1068.

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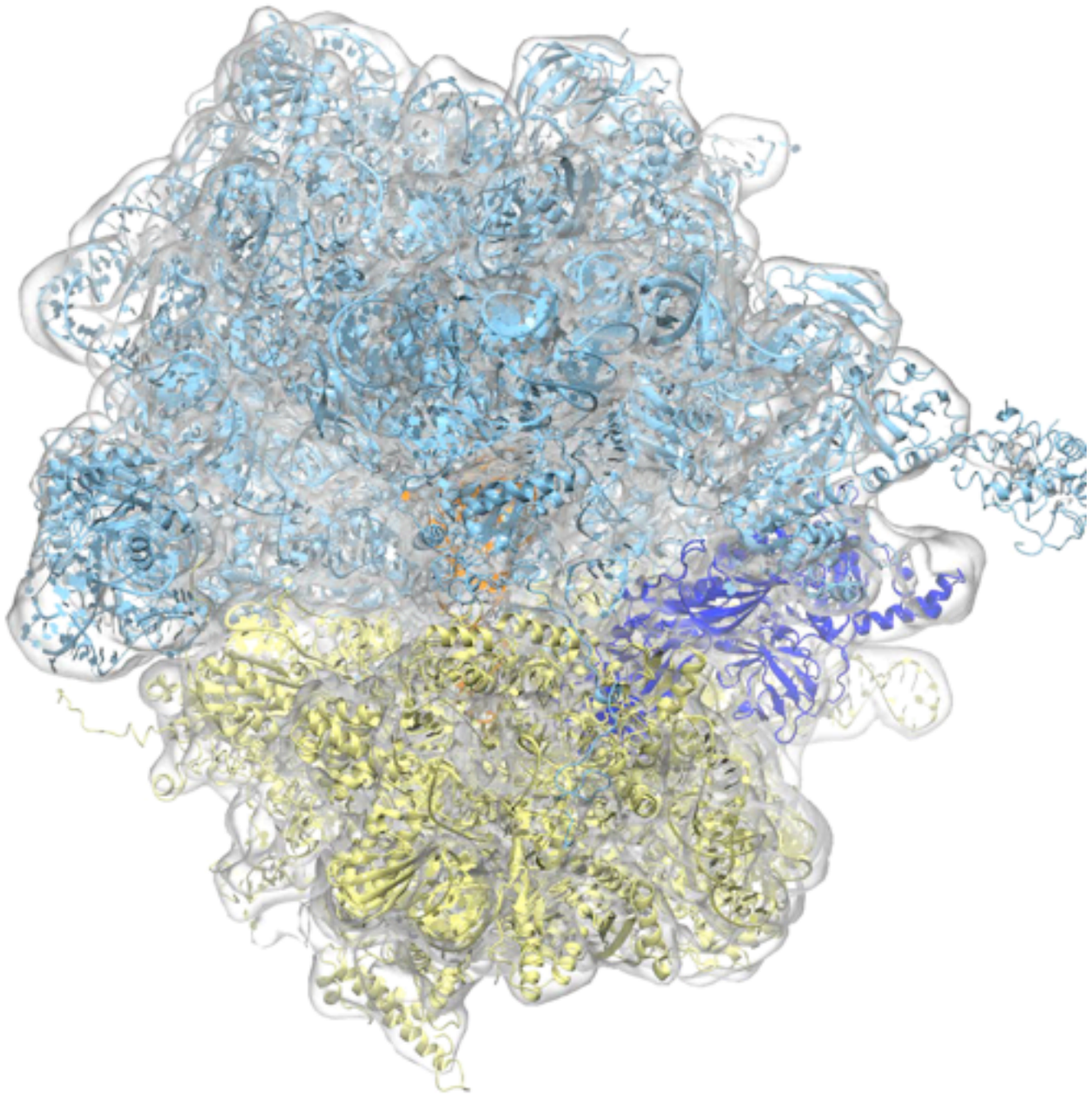
Mechanism of bacterial resistance to antibiotics

- Many antibiotics target bacterial ribosome, e.g. Tetracycline (Tc)
- Tet(O) binds to ribosome to remove Tc, promote resistance
- Applied MDFF to obtain ***first*** atomic model of Tet(O)-bound ribosome
- Used **homology model** of Tet(O)
- MDFF with implicit solvent



Structure of Tet(O)-bound ribosome in 9.8 Å cryo-EM map

Collaboration with Joachim Frank from Columbia U.

Li et al. ***Nat. Comm.*** (2013) 4:1477.

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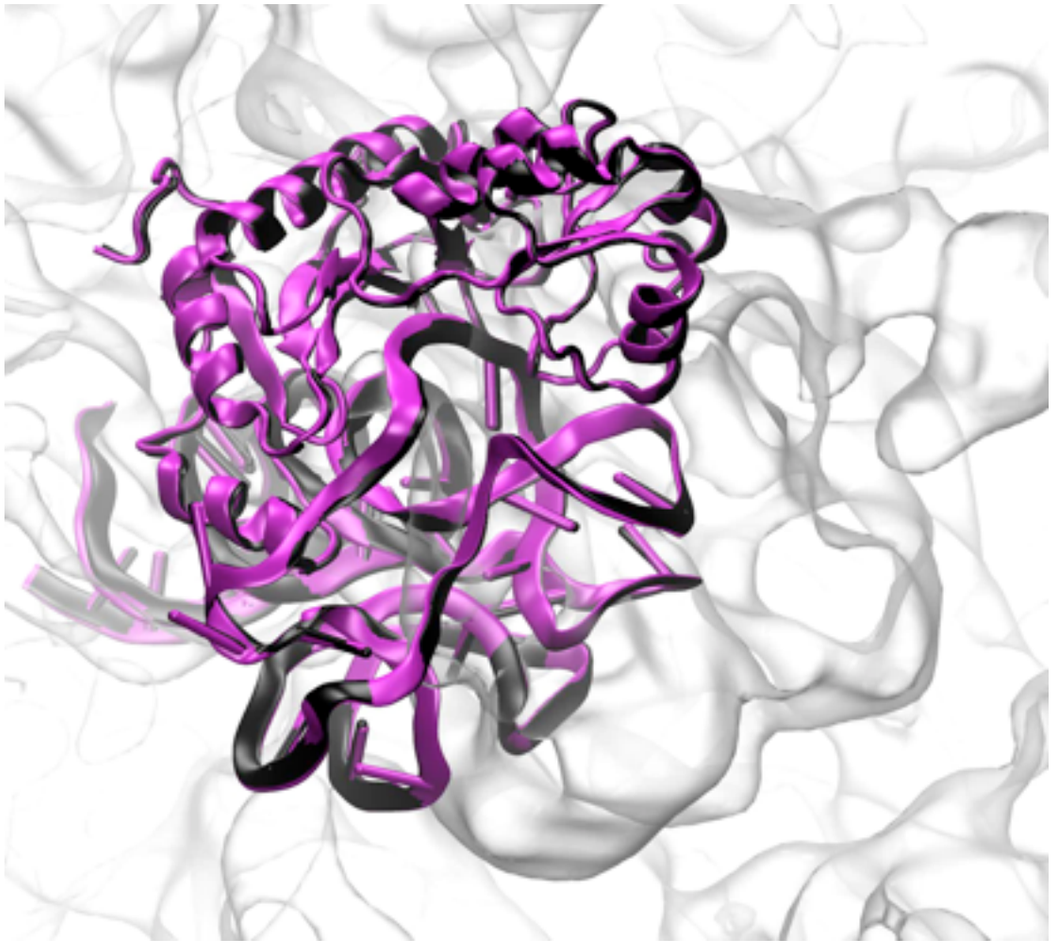
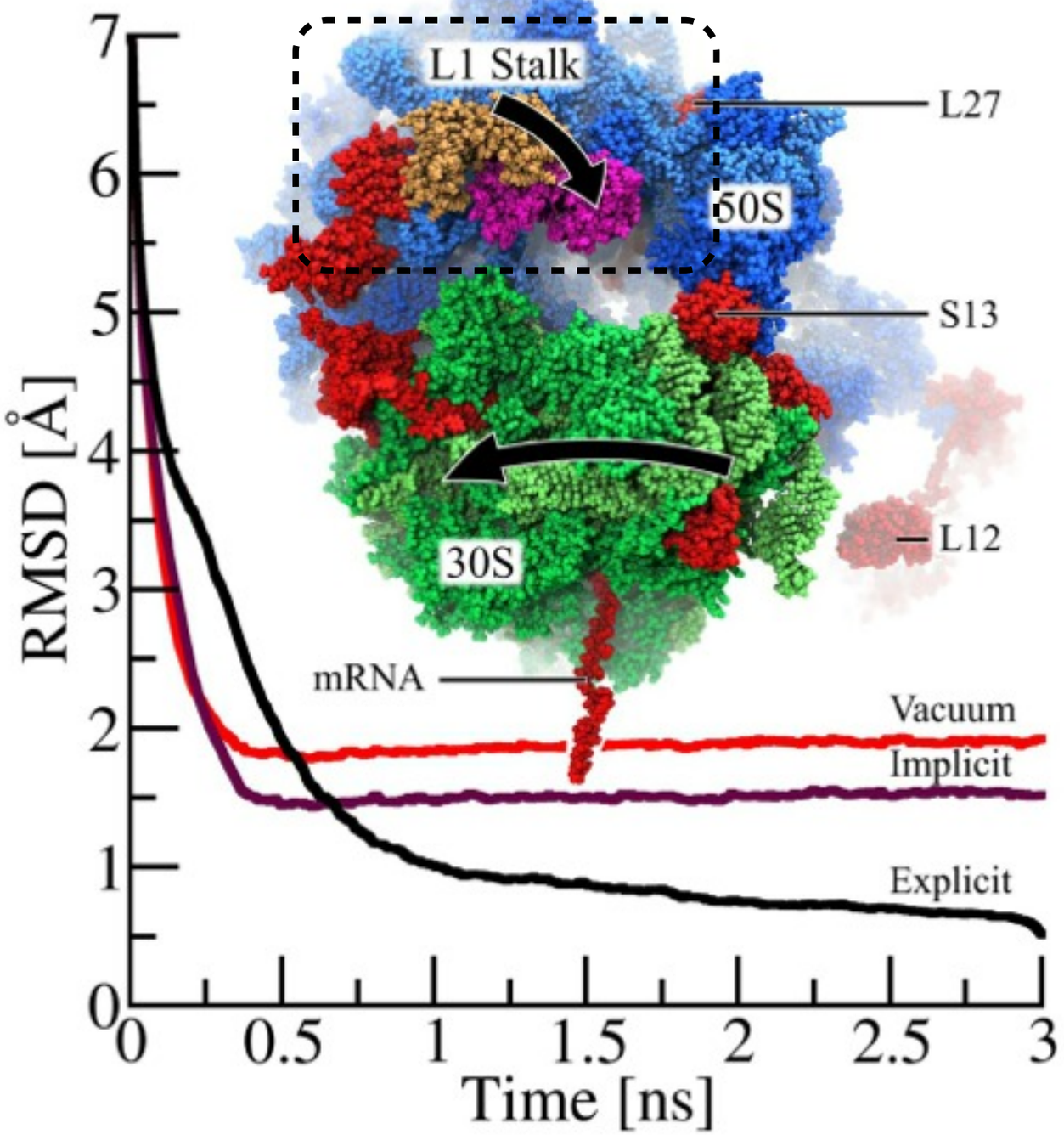
MDFF with implicit solvent - Better model and faster equilibration



Purple: implicit solvent

Black: explicit solvent



Tanner et al. ***J. Chem. Theo. Comp.*** (2011) 7:3635-3642.

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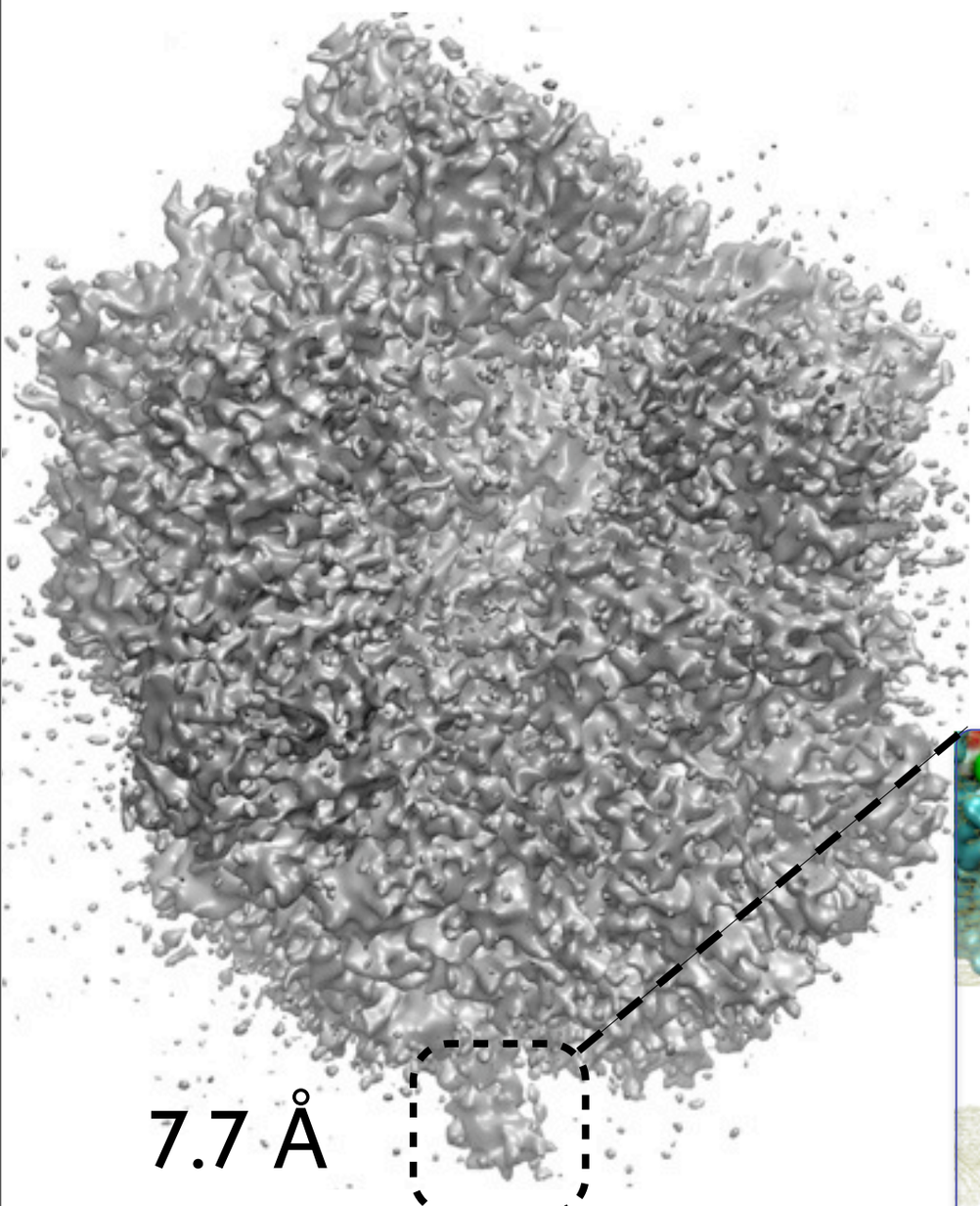
Structure of Ribosome-bound Trigger Factor

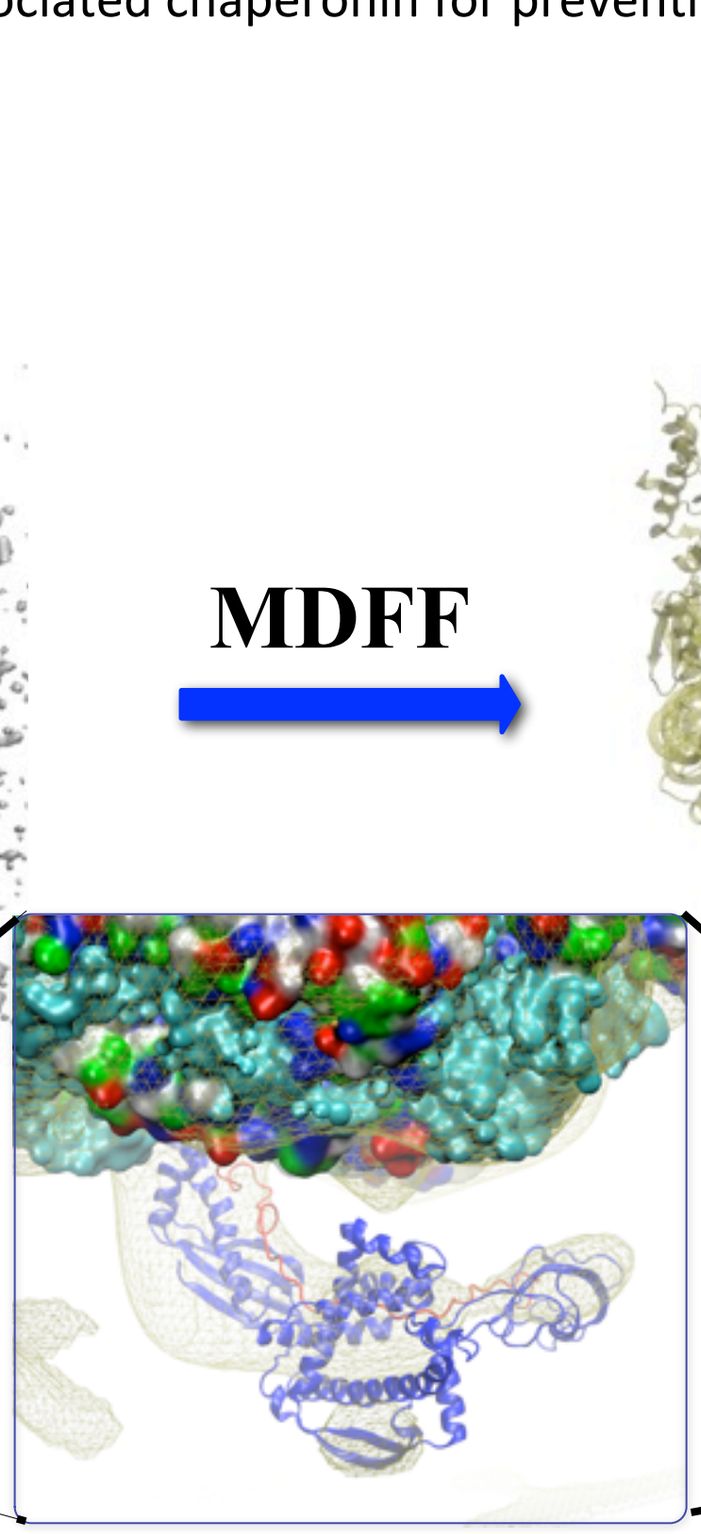
Trigger factor is a ribosome-associated chaperonin for preventing misfolding of nascent chain

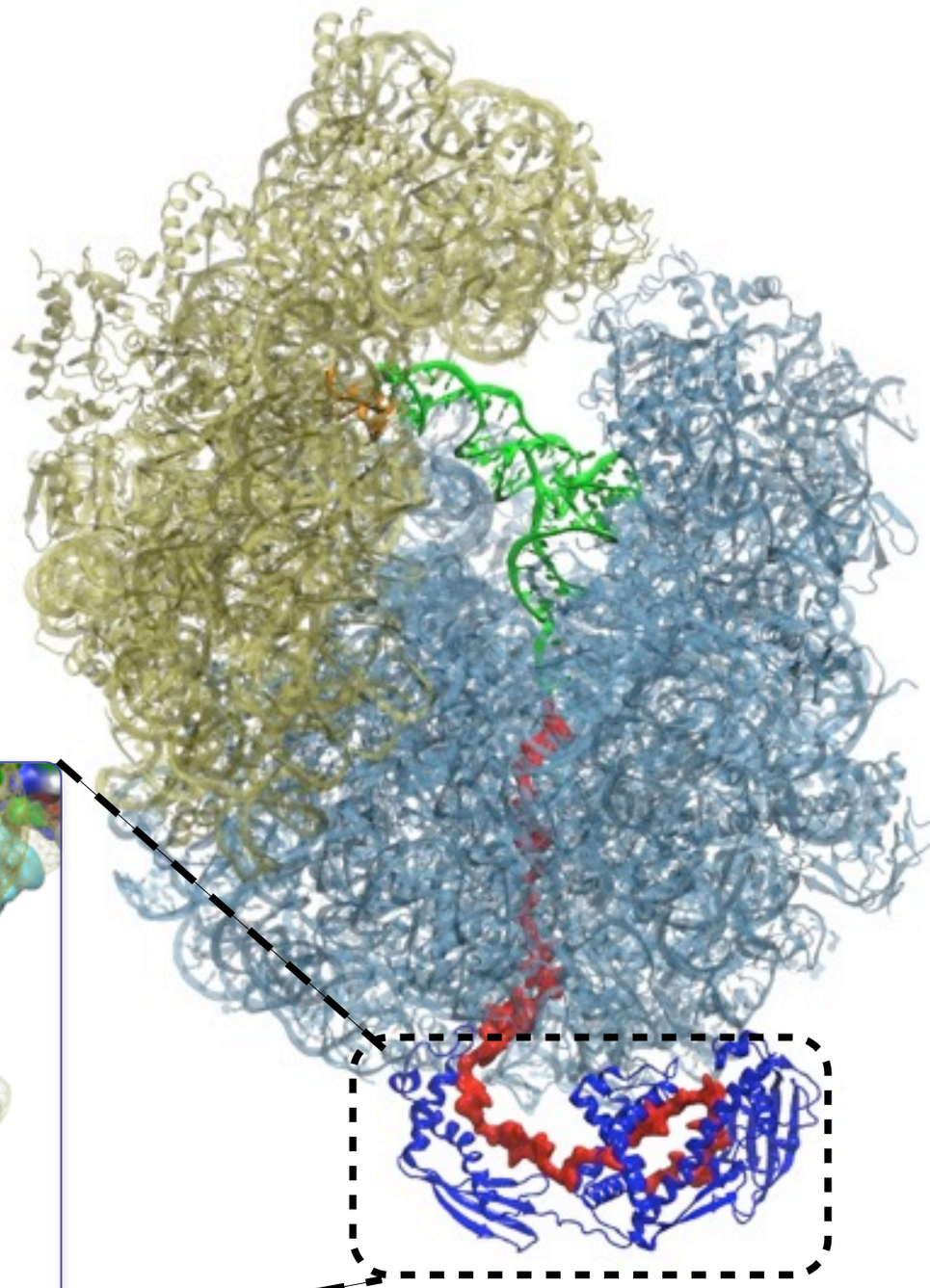
Cryo-EM density

Atomic model

MDFF



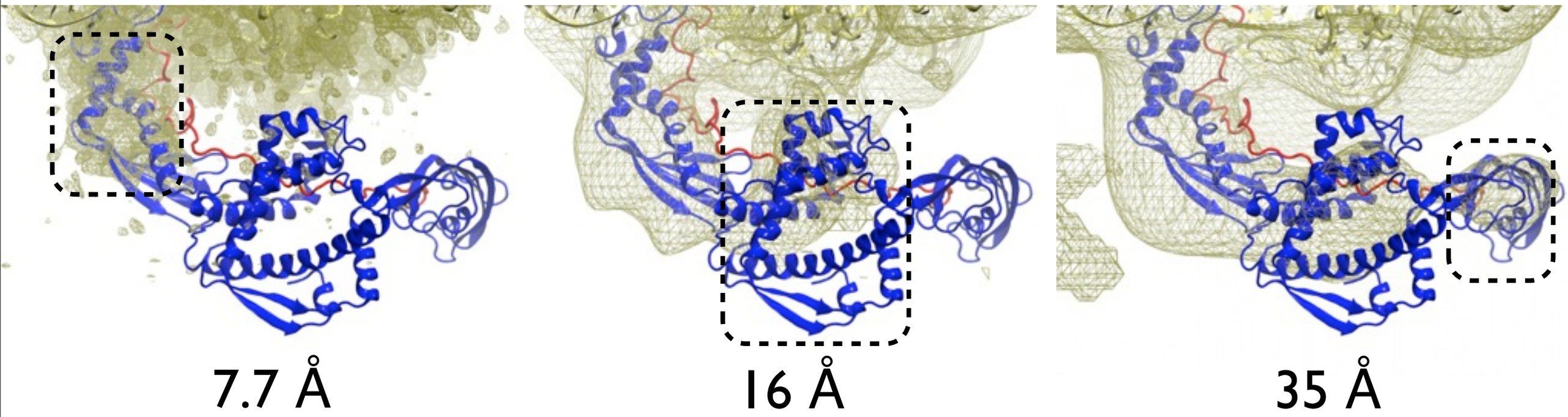




Motion-affected densities

Collaboration with Roland Beckmann of U. of Munich

Motion-affected densities and dynamics of trigger factor



7.7 Å 16 Å 35 Å

Goal: Quantitatively correlate MD-generated density to motion-washed-out density

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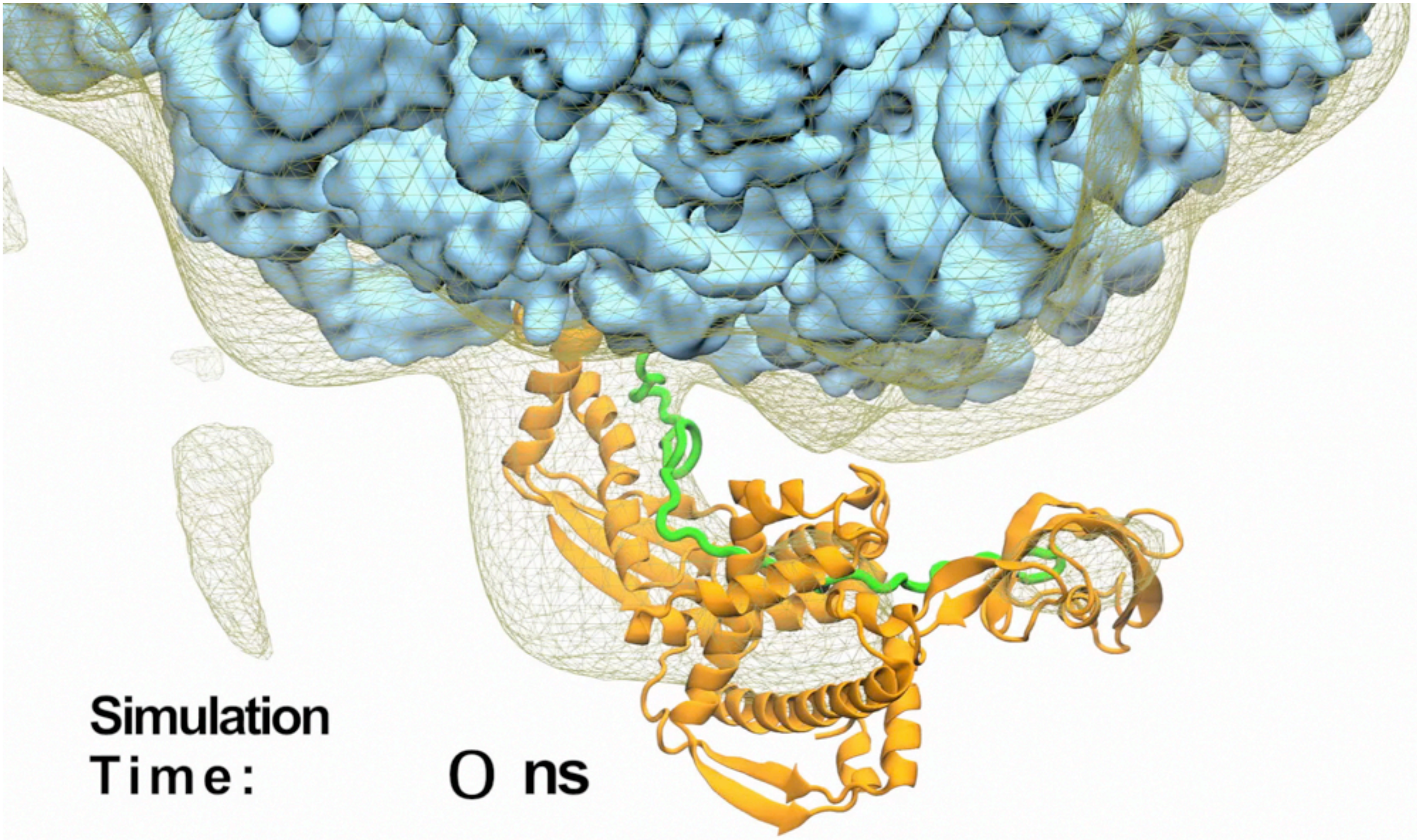

Hybrid simulations of the ribosome-trigger factor-complex

PACE hybrid forcefield: Han et al. ***J. Chem. Theo. Comp.*** (2012) 8:4413-4424.

Useful VMD plugins for RNA simulations

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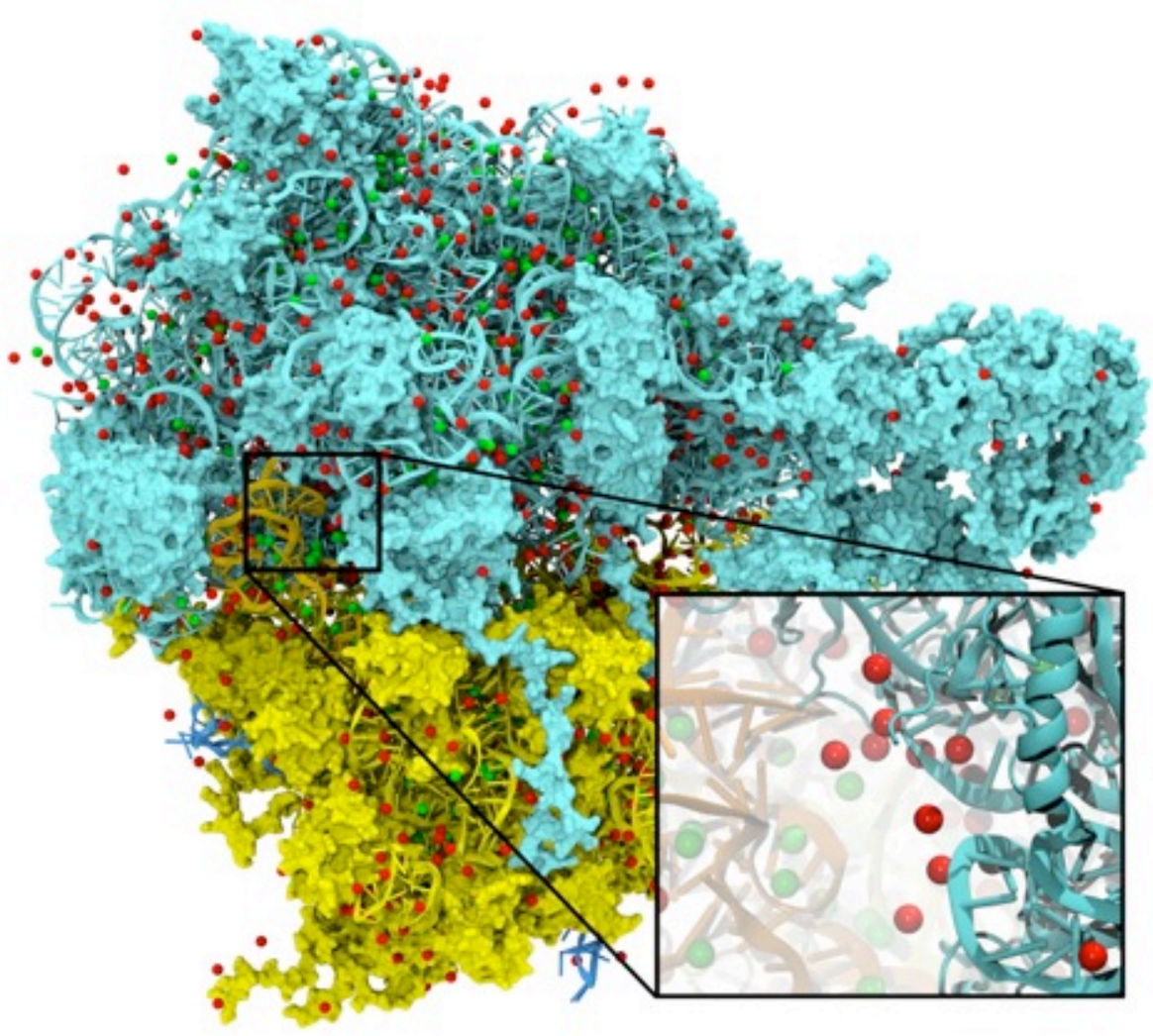
Ionization of RNA systems - *cionize* plugin

- Ions are known to be important for stability of RNA structures during simulation; especially Mg^{2+}
- "*cionize*" plugin in VMD
- Calculate Coulomb potential and place ions at global minima *iteratively*
- GPU accelerated, 26-44 times faster; scale linearly with size of systems



Stone et al. *J Comp Chem* (2007) 28:2618-2640.

http://www.ks.uiuc.edu/Research/gpu/#ionplacement

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Ionization of RNA systems
- protocol for ionization

crystal ions

cionize

autoionize

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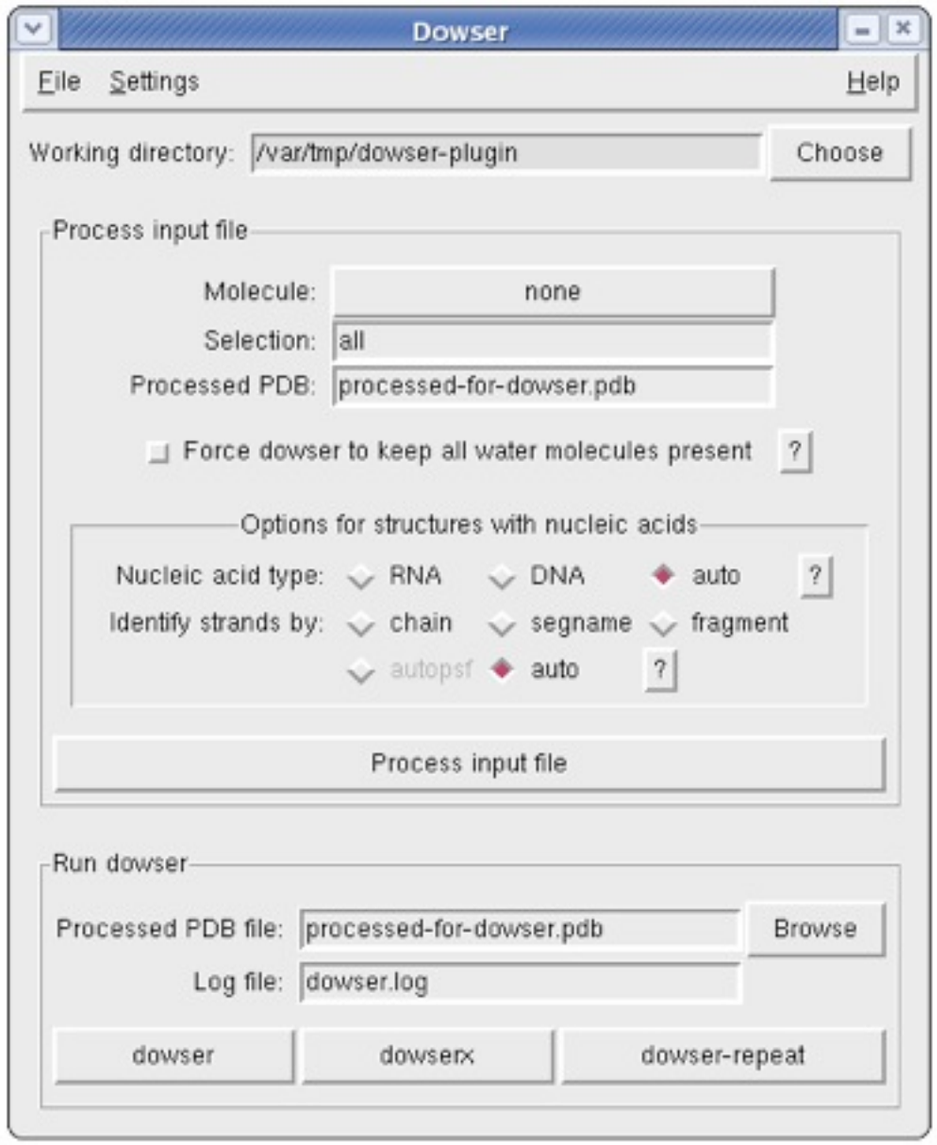
Solvation of RNA systems - Extension of Dowser

- Dowser - a software to find cavities and crevices and place water
- VMD graphical interface
- Extended to RNA systems

Dowser

VMD solvate



Gumbart et al. *Structure* (2009) 17:1453-1464.

http://www.ks.uiuc.edu/Research/vmd/plugins/dowser/

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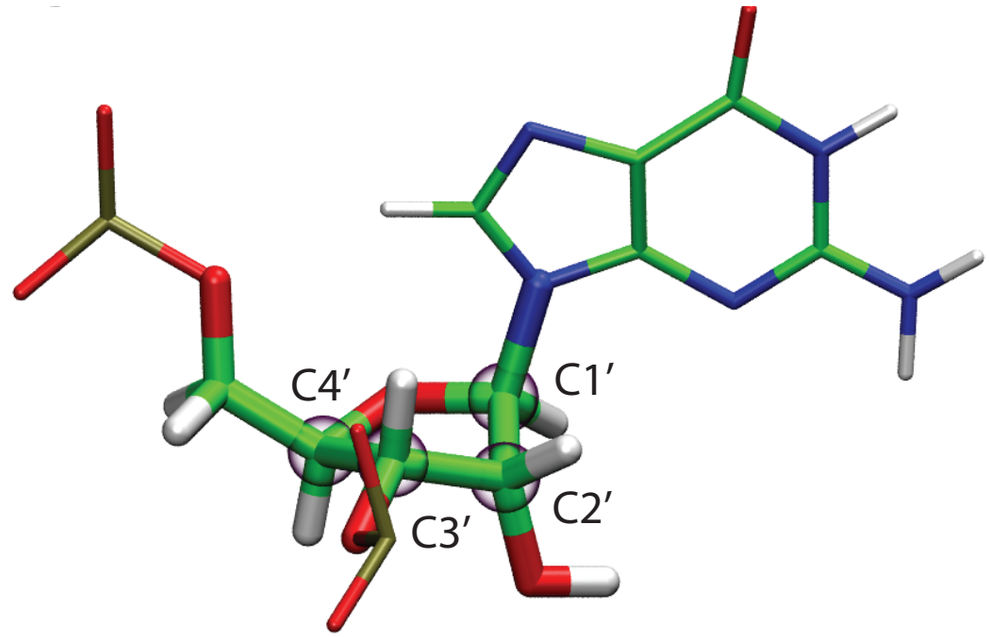
Structure checking
- *chirality*

- RNA systems have many chiral centers
- One may introduce chiral errors during modeling



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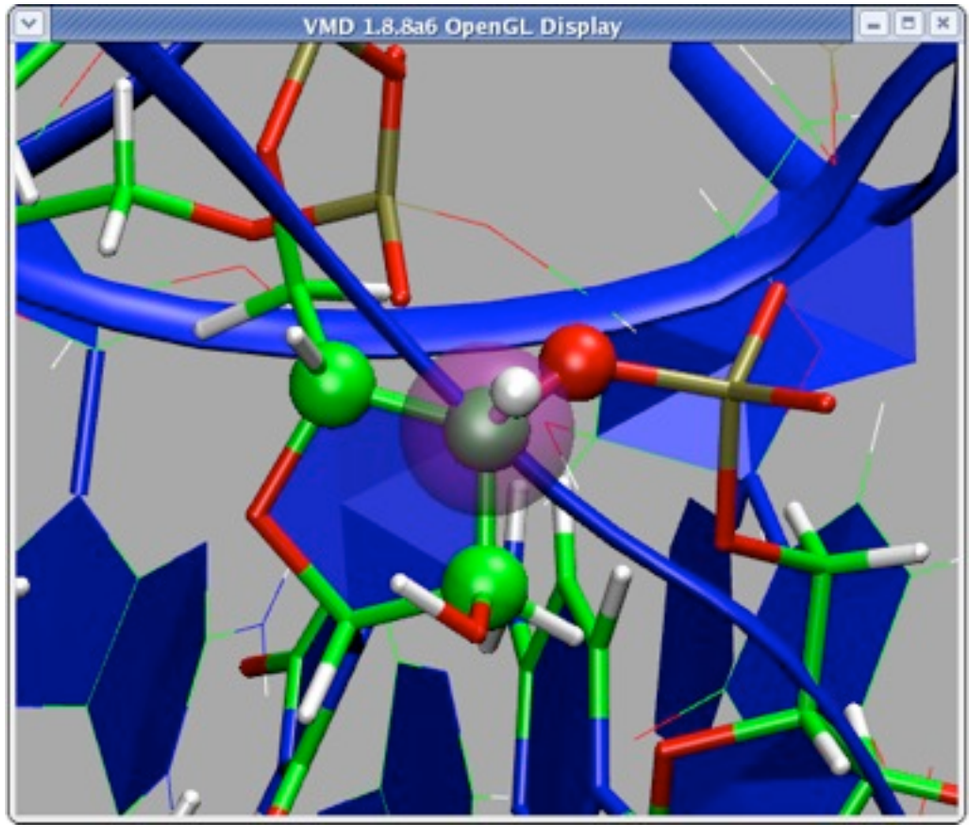
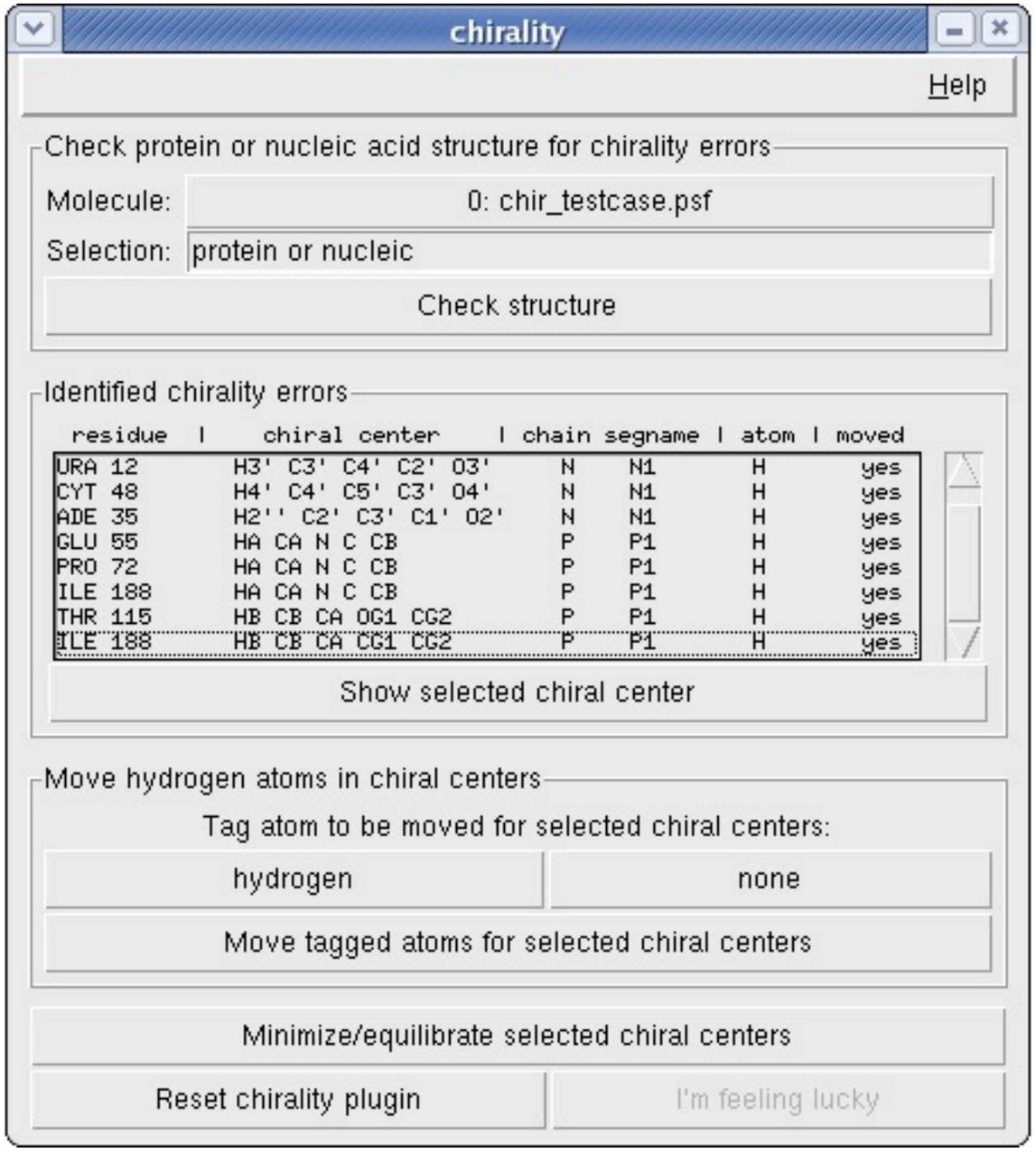
Structure checking - *chirality*

- New VMD plugin - *chirality* to check for chiral errors
- Using VMD to visualize them and NAMD to fix them
- Generate restraint files to preserve chirality during simulation
- Demo & tutorial





Schreiner et al. *BMC Bioinformatics* (2011) 12:190.

http://www.ks.uiuc.edu/Training/Tutorials/science/structurecheck/tutorial_structurecheck-html/

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