# Structural analyses of the ribosome by hybrid approach

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## Need of hybrid approach

#### X-ray crystallography Cryo-electron microscopy 50S A-site finger EF-Tu · GDP EF-G·GTP deacylated tRNA L1 proteir Ternary complex (iii)L1 protubera Solution: flexibly fit high-resolution structure into low-resolution map F-G · GDP Molecular Dynamics Flexible Fitting High resolution (3-5Å) Medium resolution (typically 5-10Å)

Crystal packing makes it difficult to capture different functional states 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.



#### 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.



## 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

Collaboration with Joachim Frank from Columbia U. Li et al. *Nat. Comm.* (2013) 4:1477.



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



#### MDFF with implicit solvent -Better model and faster equilibration



#### Structure of Ribosome-bound Trigger Factor

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

**Atomic model** 

#### **Cryo-EM density**



Collaboration with Roland Beckmann of U. of Munich

#### Motion-affected densities and dynamics of trigger factor



Goal: Quantitatively correlate MD-generated density to motionwashed-out density





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



## Ionization of RNA systems - *cionize* plugin

- Ions are known to be important for stability of RNA structures during simulation; especially Mg<sup>2+</sup>
- " "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



## Ionization of RNA systems - protocol for ionization



## 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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ile <u>S</u> ettings		Hel			
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Log file:	dowser.log				



#### Structure checking - chirality

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

-		
	C4' C1'	
	C3' C2'	



## 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



	chiral	lity					
					<u>H</u> elp		
Check pro	tein or nucleic acid structur	e for c	hirality	errors			
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PR0 72	HA CA N C CB	P	P1	н	yes		
ILE 188	HA CA N C CB	P	P1	н	yes		
THR 115	HB CB CA OG1 CG2	Р	P1	н	yes		
ILE 188	HB CB CA CG1 CG2	P	P1	H	yes /		
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R	Reset chirality plugin				ucky		

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

<u>http://www.ks.uiuc.edu/Training/Tutorials/science/structurecheck/</u> <u>tutorial\_structurecheck-html/</u>

