Structural analyses of the ribosome by hybrid approach

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Need of hybrid approach

X-ray crystallography

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

Cryo-electron microscopy

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

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

Multistep fitting protocol of RNA-protein complex

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

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.

Structure of Tet(O)-bound ribosome in 9.8 Å cryo-EM map
MDFF with implicit solvent -
Better model and faster equilibration

Purple: implicit solvent
Black: explicit solvent

Structure of Ribosome-bound Trigger Factor

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

Cryo-EM density

Atomic model

Collaboration with Roland Beckmann of U. of Munich

7.7 Å

Motion-affected densities

MDFF
Motion-affected densities and dynamics of trigger factor

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

7.7 Å

16 Å

35 Å
Hybrid simulations of the ribosome-trigger factor-complex

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$^{2+}$

- “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


http://www.ks.uiuc.edu/Research/gpu/#ionplacement
Ionization of RNA systems
- protocol for ionization

crystal ions  cionize  autoionize
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

http://www.ks.uiuc.edu/Research/vmd/plugins/dowser/
RNA systems have many chiral centers

One may introduce chiral errors during modeling
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
