Molecular dynamics study of macrolide binding to the *E. coli* ribosome

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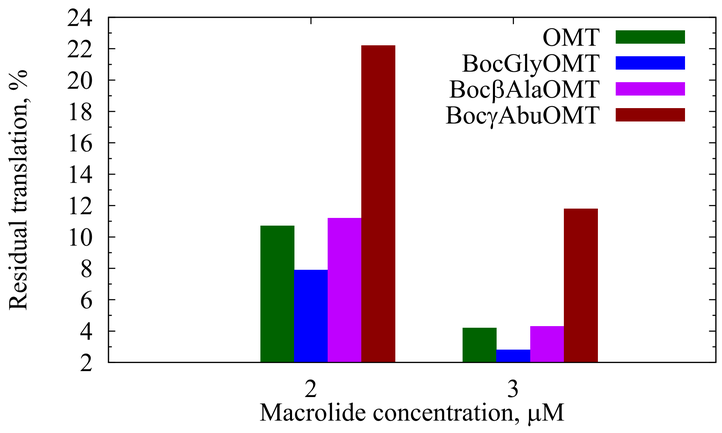
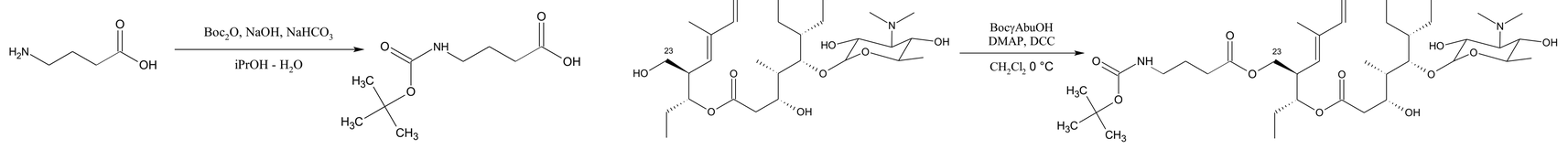
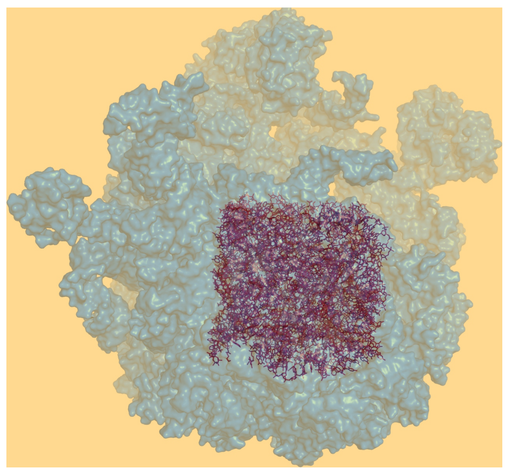
A number of derivatives of the macrolide antibiotic 5-O-mycaminosyltylonolide (OMT) containing N-acylated amino acid or peptide residues were synthesized in order to study potential sites of nascent peptide - ribosomal tunnel interactions. These OMT derivatives showed high although different abilities to inhibit the firefly luciferase synthesis in vitro. Unexpectedly, Boc-Gly-OMT appeared to be the most potent inhibitor of protein synthesis among OMT-derivatives. Molecular dynamics (MD) simulation of complexes of these compounds, as well as the parental antibiotic tylosin with the large ribosomal subunit of *E.coli* allowed us to obtain a plausible explanation of differences in their inhibitory activity. In addition, the MD study resulted in the detection of a new putative site of interaction of the nascent polypeptide chain with the RT walls.

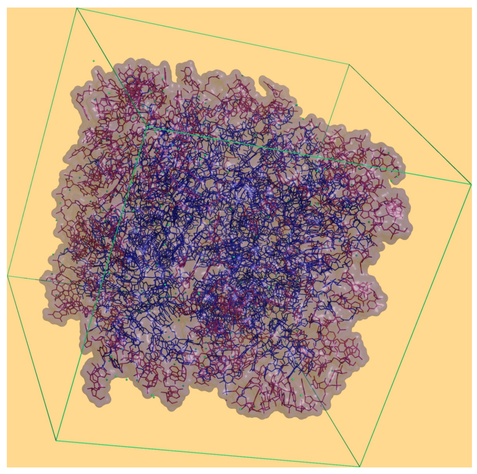




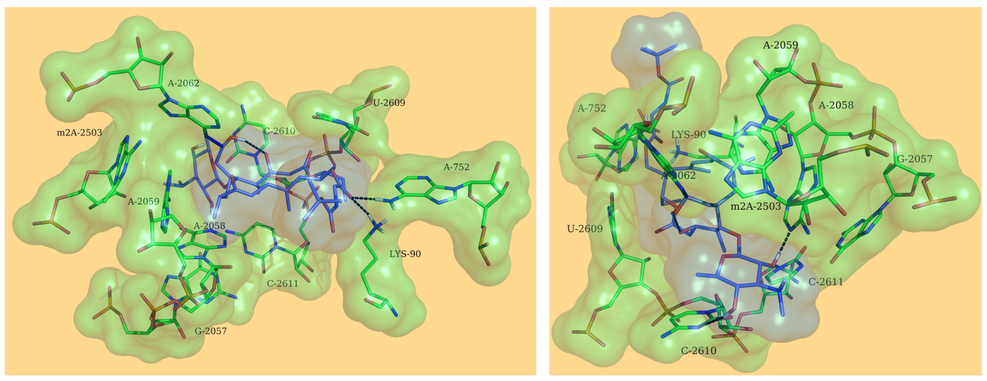
Scheme of synthesis of the amino acid derivative of OMT (BocγAbu-OMT). Figure on the left demonstrates the potency of OMT derivatives to inhibit translation of firefly luciferase mRNA in vitro in *E.coli* S30 extract. Inhibitory activity decreases with lengthening of hydrocarbon chain in an amino acid residue; BocGlyOMT is the most active inhibitor, more potent then OMT.



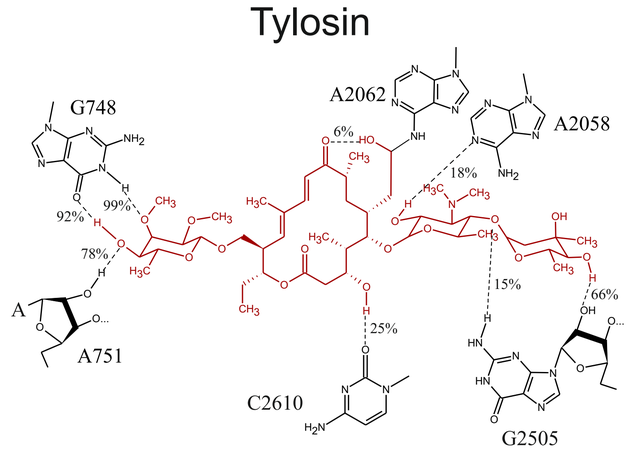
The cubic area with 7.2 nm edge used for molecular dynamics simulation (on the left, red) was selected from *E. coli* large ribosomal subunit (PDB ID 3OFR) containing modifications in 23S rRNA. Residues that had at least one atom within 0.1 nm from face of selected fragment of the ribosome were positionally restrained (on the right, red).*



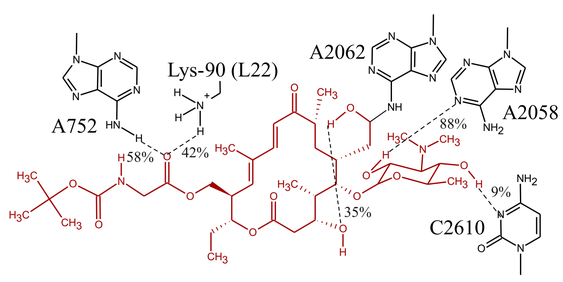
Residues A2058, A2059 and C2611 form “hydrophobic cave”, in which macrolide's lacton ring enters.



Tylosin

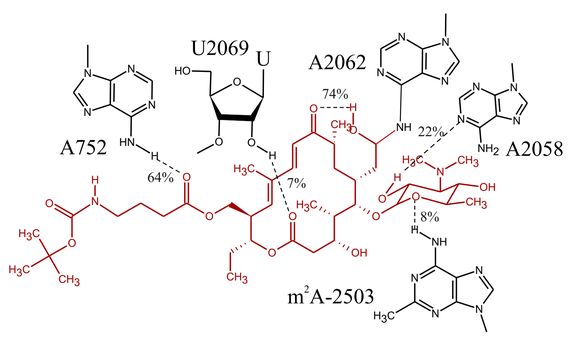


BocGlyOMT

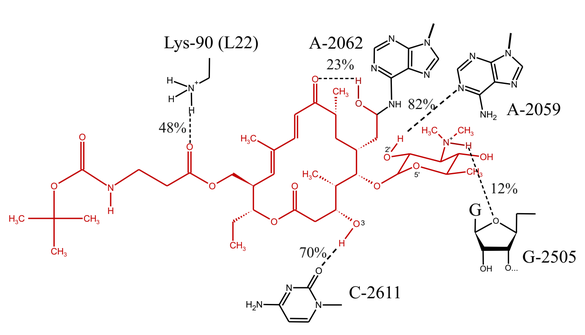


Tylosin derivatives were found to form the hydrogen bond between O2'H of the mycaminose residue and N1 of A2058. Also all derivatives form intramolecular hydrogen bond between OH-group of carbinolamino-bridge connecting a macrolide with A2062 and either O3 or O9 atoms of macrolactone ring. Amino acid residues in BocGlyOMT and BocβAlaOMT form the hydrogen bond with Lys-90 of the ribosomal protein L22. In addition, BocGlyOMT forms the stable H-bond with A752.

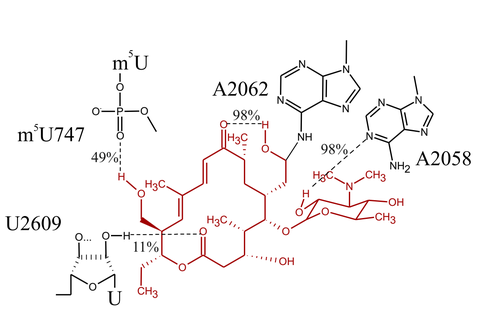
BocγAbuOMT



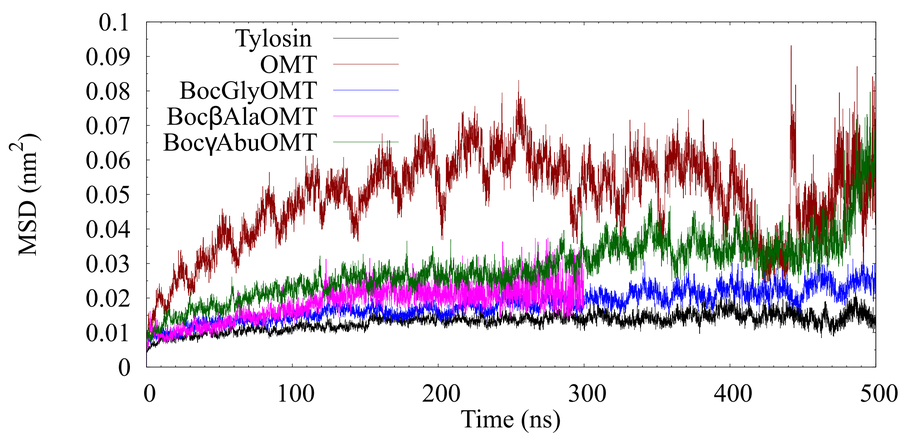
BocβAlaOMT

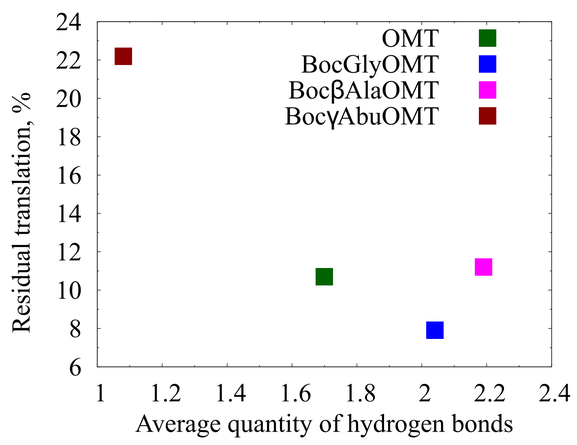


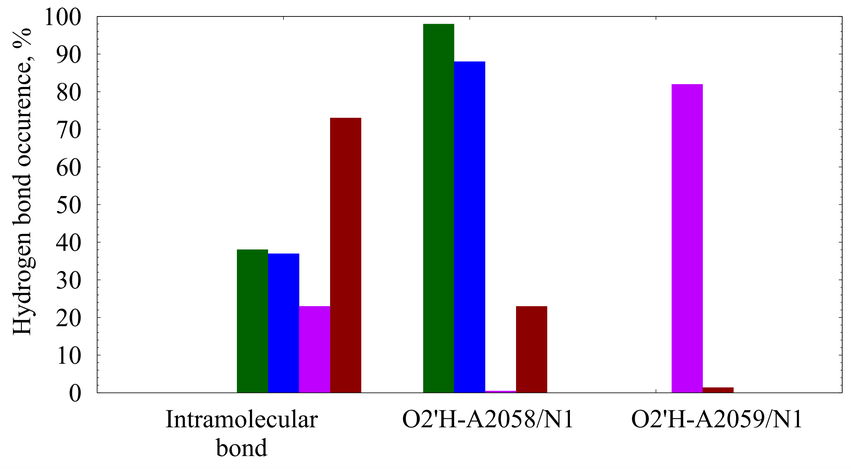
OMT



Inhibitory activity of OMT derivatives positively correlates with average number of hydrogen bonds formed.







Conclusion

Molecular dynamic simulation of complexes of tylosin, OMT and its derivatives with the ribosome allows to explain variations in their ability to inhibit translation and, in particular, high inhibitory activities of N-acylglycyl-contaning compounds. In addition, it allows to evaluate a putative mode of interactions of the backbone of a growing polypeptide chain with the ribosomal exit tunnel.

* All MD simulations and analysis of MD trajectories were carried out using the GROMAC] 4.5.4 software package and parm99sb force field. Calculations were performed using Lomonosov” supercomputer facilities provided by the Moscow State University Computing Center.

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1. M.S. Svetlov, A. Kommer, V.A. Kolb, A.S. Spirin, Protein Sci. 15 (2006) 242–247.
2. Poulsen, S.M., Kofoed, C., Vester, B. (2000) J. Mol. Biol., 304,471–481