Structural insight to thymidylate kinase of *Streptococcus mitis* : a potential common drug target of infective endocarditis

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Key points

- The incidence of infective endocarditis (IE) represents the fourth leading cause of life-threatening infectious disease with a yearly incidence of 15,000 to 20,000 new cases.
- *Streptococcus, Enterococcus, Legionella, Staphylococcus, Brucella, tropheryma, Coxiella, Bortonella, Nocardia, Chlamidia, Neisseria, HACEK and Mycoplasma* are the causative organisms of IE.
- *Streptococcus mitis* NCTC 12261, *Enterococcus faecalis* V583 and *Staphylococcus aureus* subsp aur JH9 were identified as three predominant pathogens in IE patients of SVIMS hospital and explored for common novel drug targets from available whole genome sequences through comparative genomic approach, subtractive genomic approach and metabolic pathway analysis.
- Thymidylate kinase plays a vital role in pyrimidine metabolism in deoxyribonucleic acid (DNA) synthesis and was identified as novel common potential drug target against IE.
- In the present study, computational tools were utilized to gain insight on physico-chemical, structural and functional aspects of Thymidylate kinase of *Streptococcus mitis* (the most predominant IE pathogen in SVIMS hospital). Active site of modeled thymidylate kinase was determined for assisting structure based drug discovery.

Material and methods

Sequence retrieval and analysis

JCVI CMR · KEGG · DEG: Database of Essential Genes

Template searching

BLAST Home

RCSB PDB PROTEIN DATA BANK

Target-template alignment

- Comparative genomic approach
- Subtractive genomic approach
- Pathway analysis

Modeller

Model generation incorporating substrate

- PDB ID: 2CCJ
- Identity: 49%
- Similarity: 70%
- Query Coverage:91%

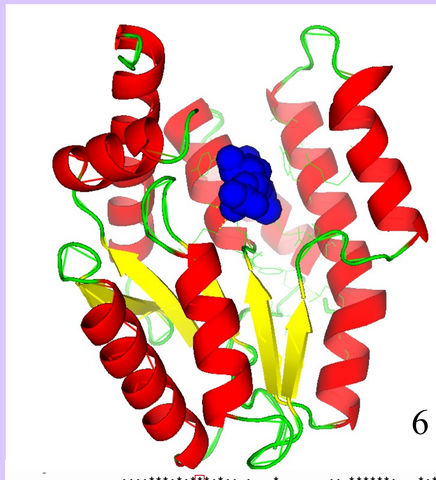
Model validation

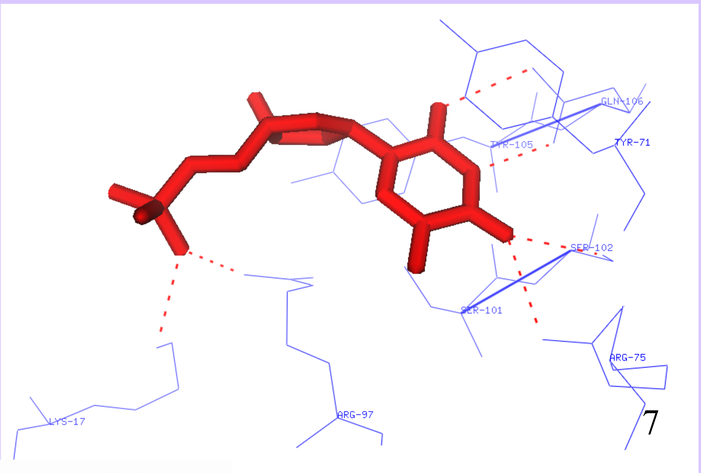
- Procheck
- ProSA
- ProQ
- DOPE analysis
- Superpose

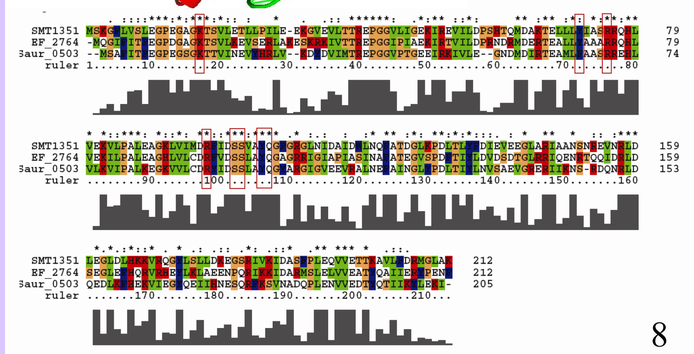
Active site detection

- Substrate incorporated model analysis.
- MSA
- Literature correlation

Active site prediction







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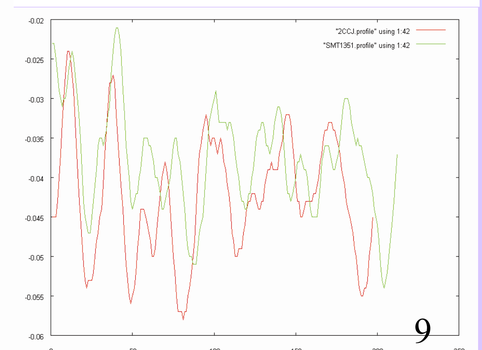
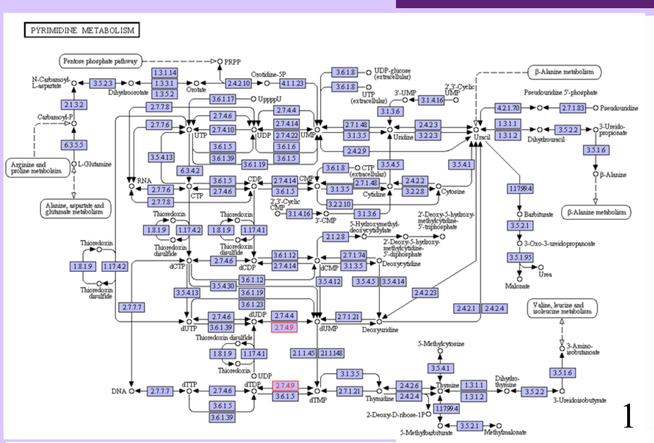
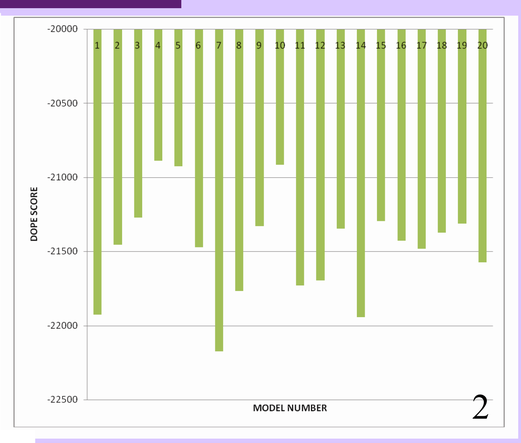
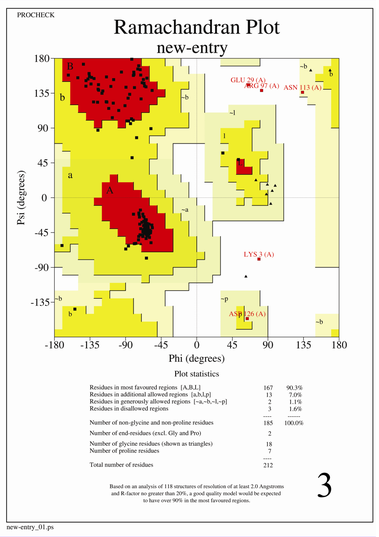


Figure: (6) Model in complex with substrate TMP (7) Interactions of TMP at thymidylate kinase active site (8) MSA showing conserved active site residues(9) DOPE correlation of thymidylate kinase model with template .

Results and Discussion







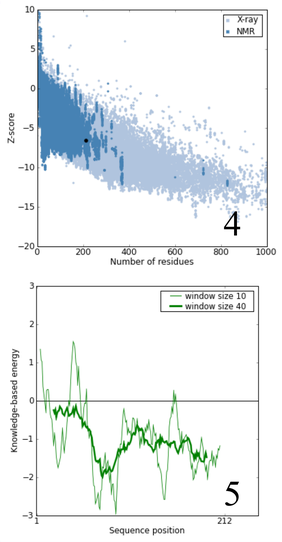


Figure: (1) Pathway analysis of thymidylate kinase (2) Plot with DOPE score for twenty 3D model (3) Procheck report of thymidylate kinase final model (4) ProSA Z score plot (5) ProSA energy plot.

Analysis	Target	Template
Procheck	90.3%	93.0%
ProSA	-6.65	-5.98
ProQ	4.624	4.383
DOPE score	-22175.62	-24144.96
Active site residues	K17, Y71, R75, R97, S101, S102, Y105, Q106	K17, E37, F66, R70, R92, S95, S96, Y100, Q101
C-alpha RMSD	0.25 Å	——
Overall RMSD	0.67 Å	——

Conclusion

- Designing drug molecule against thymidylate kinase would certainly stop DNA synthesis in the pathogen by blocking pyrimidine metabolism pathway. Thus, pathogen would not be able proliferate in the host. Therefore, thymidylate kinase as a drug target would overcome potential concerns of drug resistance against IE causing organism.
- From the twenty models of thymidylate kinase generated by Modeller based on satisfaction of spatial restraints, the final model selected based on DOPE score, was further assessed by various structure validation methods which affirmed the reliable conformation of the model. This model will enlighten us about structure, function relationship and will aid in rational drug design.
- Moreover, the amino acid residues of active site being conserved across the three organisms identified in SVIMS hospital demand special attention during rational drug development exercises.
- Finally, homology model in complex with its natural substrate (TMP) can be explored through ligand based virtual screening to propose potential novel inhibitors against IE.

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