Original Article

Identification of vaccine candidate proteins in Ureaplasma urealyticum causing infertility

Shilpa Jeevappa Shiragannavar, Shivakumar B. Madagi
Department of Studies and Research in Bioinformatics and Biotechnology, Karnataka State Akkamahadevi Women's University, Vijayapura, Karnataka, India

Address for correspondence:
Shilpa Jeevappa Shiragannavar, Karnataka State Akkamahadevi Women's University, Vijayapura, Karnataka, India.
E-mail: shilpa.shiragannavar@gmail.com

Abstract

Background: *Ureaplasma urealyticum* has gained resistance to number of antibiotics and has been of the greatest concerns nowadays. The treatment options remain extremely low due to the increased levels of intrinsic resistance gained by the pathogen. **Aim:** The present study focuses on designing a peptide-based vaccine as there is no vaccine available for the pathogen. **Materials and Methods:** All the protein sequences of pathogen were collected and examined using various *in silico* methods to identify the most immunogenic proteins. The study identifies the proteins which are antigenic in nature which induce the immune response, which lends to quick response of immune system on reinfection. The study describes peptide-based vaccine against *U. urealyticum* using molecular docking and molecular dynamics simulation approach. **Results:** The study identifies novel putative vaccine candidate proteins that are antigenic, membrane bound and non-allergenic. **Conclusion:** The results of the study imply that the vaccine candidate proteins identified may bring about vigorous enduring defensive immunity against *U. urealyticum*.

Key words: Docking, epitope, *Ureaplasma urealyticum,* vaccine

INTRODUCTION

Ureaplasma urealyticum is a Gram-negative bacterium frequently found in urogenital tracts of humans[1] that is usually associated with mycoplasmas. The unique feature of it is the ability to generate adenosine triphosphate (ATP) by hydrolysis of urea.[2] The bacterium is often a part of normal flora in the reproductive tract of sexually active men and women.[3] Many a times women with this infection experience fertility problems, the untreated infections can render to infertility.[4] Ureaplasma species can cause acute urethritis and have been associated with bacterial vaginosis, preterm birth, and neonatal respiratory disease.[5] A number of reports discuss the association of *U. urealyticum* with prematurity-linked conditions such as preterm labor, premature rupture of the fetal membranes, placental invasion, and intra-amniotic infection as well as chorioamnionitis, postpartum, and postabortal fever.[6] The infected mothers can transmit the pathogen to their babies, and the newborns which get infected are prone to diseases such as meningitis, pneumonia, and respiratory tract disorders. The untreated infections move towards the upper genital tract and cause cervical discharge and may result in secondary infertility.[7] *Ureaplasma* are self-replicating organisms that colonize in human and have the capacity to utilize urea for generation of ATP.[8]

Access this article online

Quick Response Code:

Website: www.ijstd.org

DOI: 10.4103/ijstd.IJSTD_7_19

How to cite this article: Shiragannavar SJ, Madagi SB. Identification of vaccine candidate proteins in *Ureaplasma urealyticum* causing infertility. Indian J Sex Transm Dis 2021;42:95-100.

Submitted: 31-Jan-2019 **Revised:** 07-Feb-2019
Accepted: 18-Dec-2020 **Published**: 27-Jul-2021

However, a significant relationship existed between *U. urealyticum* and *Mycoplasma hominis* and male infertility.[9] There are a number of pathogens known to contribute to male infertility; the two types that most commonly occur are genital *Ureaplasma*[10] and *Mycoplasma*. They are ubiquitous resulting in colonization of the genitalia by sexual contact. Several studies have demonstrated that *U. urealyticum* and *M. hominis* play an etiologic role in male infertility, with these infections changing parameters of semen such as spermatozoa density and motility.[11]

The current treatment options include commonly prescribed antibiotics, and very few are effective against the bacterial infections. Nowadays, bacteria have gained resistance for the antibiotics; therefore, vaccination can be more effective way for treating the bacterial infections.[12] With the data from sequencing projects and advances in proteomics and genomics, the field of vaccine design and development has emerged to be more promising.[13] Hence, in the current work, various *in-silico* tools are used to obtain the immunogenic proteins from the bacteria which significantly reduce the time and cost in developmental process.[14] All the proteins of the bacteria under study were collected and screened for antigenicity, surface accessibility and allergenicity. Further structural and functional analysis of the proteins was performed as these antigenic, outer membrane proteins are important in therapeutic interventions. Finally, an attempt was made to design an effective vaccine, and the results of the study offer novel vaccine candidate proteins for development of vaccine against *U. urealyticum*.

MATERIALS AND METHODS

Protein sequence retrieval, identification of antigenic proteins, evaluation of allergenic, subcellular localization, and functional analysis of proteins

All the 646 protein sequences of *U. urealyticum* were retrieved in FASTA format from UniProt Proteome database *www.uniprot.org*.[15] Antigenic proteins of *U. urealyticum* were identified using VaxiJen v2.0.[16] Four hundred and thirty-five sequences were identified as antigenic proteins.

The overall methodology adopted for the study is shown in the Figure 1. The antigenic proteins were further evaluated for their allergenicity using AlgPred.[17] The subcellular localization predictions of *U. urealyticum* protein sequences were done using SOSUI-GramN tool[18] as the pathogen lacks cell wall. Then, the structural and functional analysis was performed for the sequences by ProtParam and InterPro[1] for obtaining the physical and chemical parameters of the proteins and identification of the conserved domains and important sites in the proteins.

Two-dimensional, three-dimensional protein structure modeling and validation of the models

The two-dimensional structure prediction for the sequences was performed using Self-Optimized Prediction Method with Alignment. Moreover, the three-dimensional (3D) structures were obtained by protein modeling using Swiss-Model. The models were then subjected to energy minimization using Swiss-PDB Viewer. The quality of the homology model was then validated using online tools such as Rampage and Procheck.

Molecular docking and molecular dynamics simulation studies

Protein–drug interaction study was performed using AutoDock, and MetaPocket[19] was used to get the active sites of the protein to obtain site-specific interactions. The binding-free energy of the protein–drug complexes was noted, and the extent of interactions between them was viewed with UCSF Chimera.[20] The entire molecular dynamics simulation study for the complexes was accomplished in CHRAMM. NAMD and VMD are file compatible with CHARMM.[21] Both are popular molecular dynamics programs with high-performance simulation for large biomolecules.

RESULTS

A total of 646 protein sequences of *U. ureaplasma* were collected from the UniProt Proteome database. The sequences were set to series of screening for obtaining efficient vaccine candidates. Out of all proteins of the pathogen, 435 protein sequences in the study were screened out based on the antigenicity score. All the proteins with higher prediction scores were considered as antigenic and were selected for further analysis. AlgPred allows prediction of allergens based on similarity and mapping of IgE epitopes with any region of protein. SOSUI-GramN tool was used for screening the protein sequences; the tool predicted outer and inner membrane proteins and extracellular proteins. Table 1 gives the localization, antigenicity, and allergenicity scores for the proteins.

Due to the lack of crystal structure of the proteins, 3D structures were predicted using Swiss-Model and the

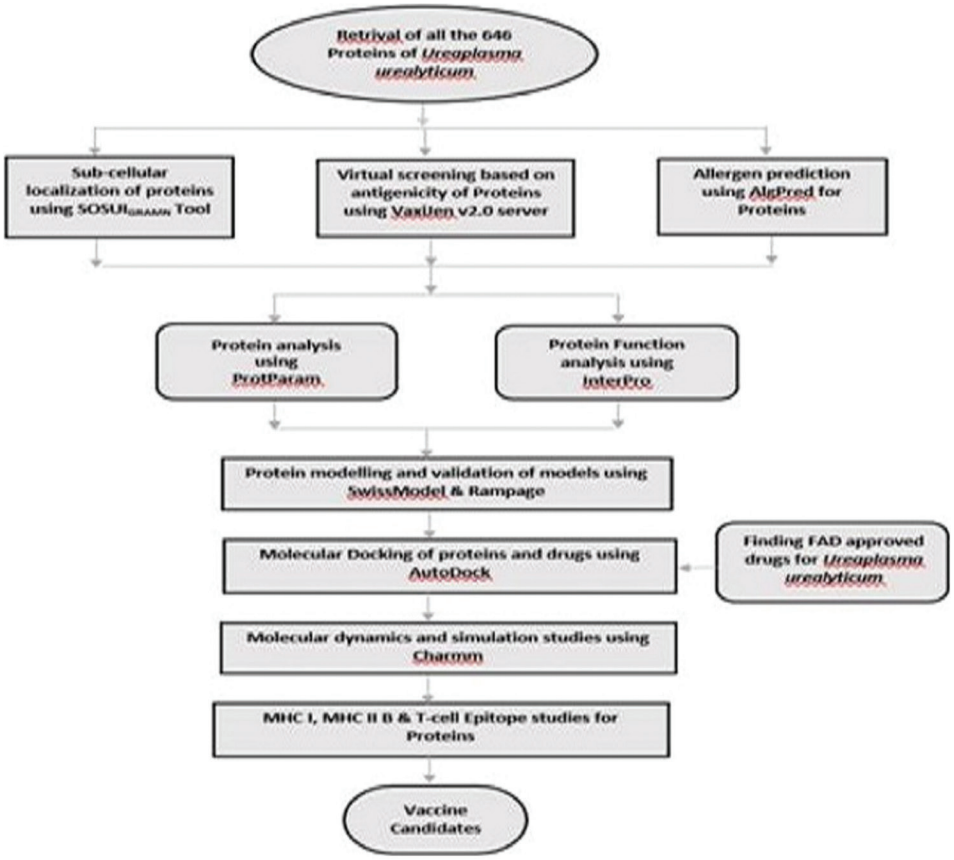

Figure 1: Flowchart summary of protocols used for the process of vaccine design

structures were validated using Rampage and Procheck which produce the PostScript Ramachandran plots given in Figure 2; the results of validation are given in Table 2. Figure 3 shows 3D structure prediction results using Swiss-Model with QMEAN-3.52.

The drug, doxycycline, which is usually given for the urea plasma infection[22] treatment was used for the molecular docking analysis. To obtain the site-specific interactions between protein and doxycycline, the active sites for the proteins were predicted using MetaPocket. Table 2 gives the values of XYZ coordinates, binding energy, and inhibition constant for the vaccine candidate proteins. The AutoDock results were then subjected for molecular dynamic simulations studies using CHARMM. Figure 4 gives the distance and histogram graphs of simulation studies for the vaccine candidate protein molecules. The straight lines in the distance graphs indicate good interactions between proteins and drug molecule. MHC-I and MHC-II was done using the ProPred,[23] as shown in Figure 5.

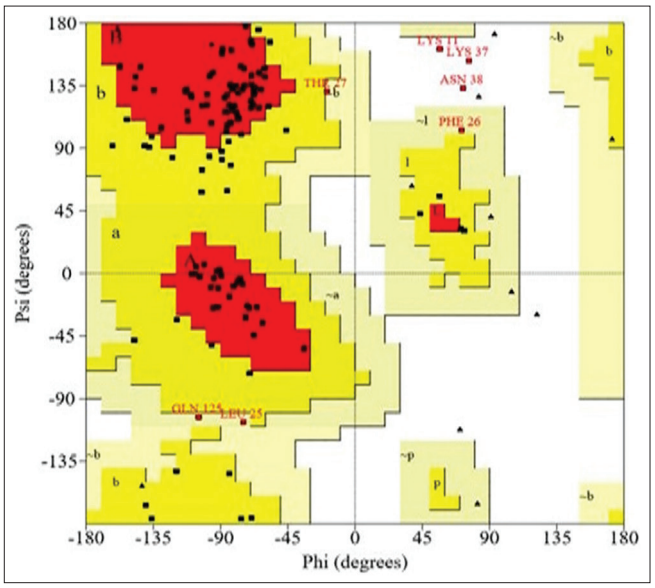

Figure 2: Ramachandran plot analysis of the protein using Procheck

DISCUSSION

50S ribosomal protein L2 is evolutionarily highly conserved protein, which is involved in catalysis of peptide bond formation during transcription. Furthermore, it brings about association between 50S and 30S subunits and helps in binding of tRNA to active and peptide formation sites in the ribosomes.[24] Another ribosomal protein, the 30S ribosomal subunit protein, performs the two basic

functions during protein synthesis, and it ensures the accuracy during translation by avoiding the mismatch codon between aminoacyl-tRNA and mRNA. It also helps in translocation by moving the tRNA to the next codon that is associated with mRNA.[25] Translation initiation factor IF1 is a highly conserved protein and is an important element in prokaryotic translation as it stimulates the initiation phase of protein synthesis. IF1 enhances the rates of 70S ribosome dissociation and subunit association but does not change the equilibrium position. IF1 is also involved in enhancing the association and dissociation of the ribosomal subunits; hence, the protein is essential for keeping the organism viable.[26] The mitochondrial inner membrane harbors three protein translocases. Presequence translocase and carrier translocase are essential for importing nuclear-encoded proteins. Biogenesis of numerous metabolite carriers depends on OXA, although they are not imported by the presequence pathway.[27]

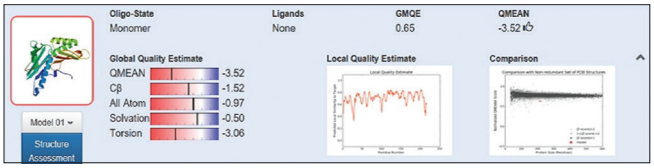

Figure 3: Swiss-Model results for the protein 30S ribosomal protein with QMEAN-3.52

CONCLUSION

Adequate treatment options for the infectious pathogens help in timely cure of the patients. The current study identifies the potential vaccine candidates for the pathogen that brings about effective immune responses against the pathogen. The current treatment options include antibiotics but the pathogen has gained resistance to the available drugs and may reinfect. Thus, vaccination would be an effective way of treating the STIs. In the current study, the effort is made to design the vaccine against the pathogen *U. urealyticum*.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

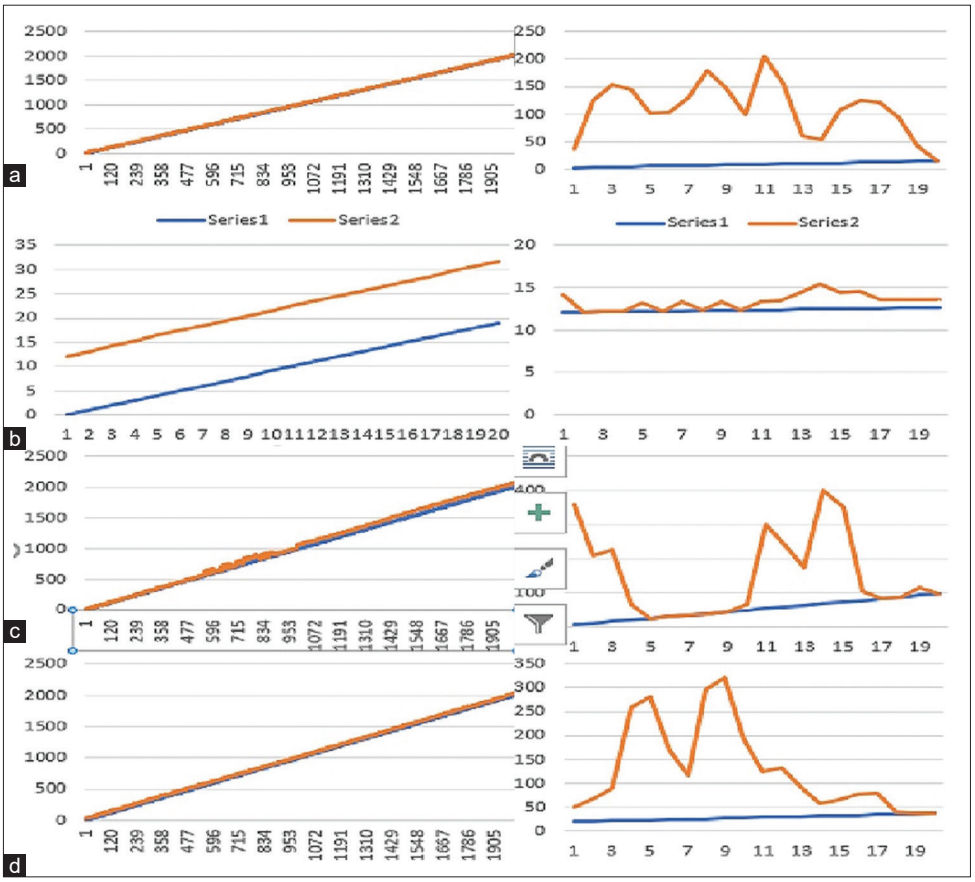

Figure 4: Distance and histogram graphs of simulation studies for the vaccine candidate protein molecules, (a) 50s ribosomal protein, (b) 30s ribosomal subunit S3, (c) Translation initiation factor, (d) Membrane protein OxaA. The straight lines in the distance graphs indicate the good interactions between proteins and the drug molecule

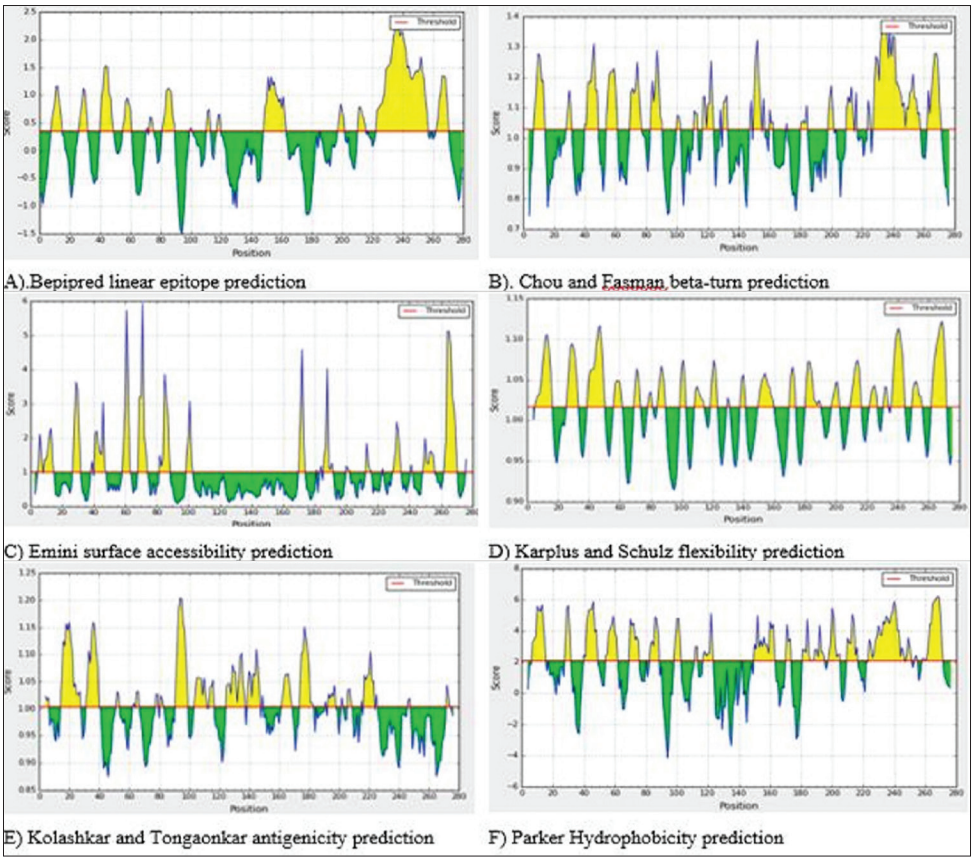

Figure 5: B-cell epitope studies with different methods were performed for all the vaccine candidate proteins, and the results for 50S ribosomal protein L2 are shown above. (A) BepiPred linear epitope prediction. (B) Chou and Fasman beta-turn prediction. (C) Emini surface accessibility prediction. (D) Karplus and Schulz flexibility prediction. (E) Kolaskar and Tongaonkar antigenicity prediction. (F) Parker hydrophobicity prediction

Table 1: Antigenicity, localization and allergenicity scores for the proteins

Protein name	VaxiJen score	Subcellular localization	Allergenic score (threshold 0.4)
50S ribosomal protein	0.5513	Extracellular	−0.777
30S ribosomal subunit S3	0.5172	Cytoplasmic	−0.787
Translation initiation factor	0.5463	Soluble-protein	−1.072
Membrane protein OxaA	0.7323	Inner-membrane	−0.697

Table 2: Three-dimensional model validation, active sites of proteins with their binding energy, and inhibition constants

Protein name	Percentage validation	*X*	*Y*	*Z*	Estimated free energy of binding (kcal/mol)	Estimated inhibition constant (KnM)
50S ribosomal protein L2	90.02	45.46	−8.665	−40.484	−8.98	262.54
30S ribosomal protein S3	93.2	13.62	19.337	−38.135	−7.97	1.45
Translation initiation factor IF-1	91.4	0.321	10.105	−0.543	−9.23	170.82
Membrane protein OxaA	90.6	10.735	47.345	−124.311	−6.81	10.26

REFERENCES

1. Horner P, Donders G, Cusini M, Gomberg M, Jensen JS, Unemo M. Should we be testing for urogenital *Mycoplasma hominis, Ureaplasma parvum* and *Ureaplasma urealyticum* in men and women? a position statement from the European STI Guidelines Editorial Board. Journal of the European Academy of Dermatology and Venereology. 2018 Nov; 32(11):1845-51.
2. Philip F, Ha EE, Seeliger MA, Frohman MA. Measuring phospholipase D enzymatic activity through biochemical and imaging methods. InMethods in enzymology 2017 Jan 1; Vol. 583, 309-325.
3. Liu J, Wang Q, Ji X, Guo S, Dai Y, Zhang Z, Jia L, Shi Y, Tai S, Lee Y. Prevalence of *Ureaplasma urealyticum, Mycoplasma hominis, Chlamydia trachomatis* infections, and semen quality in infertile and fertile men in China. Urology. 2014 Apr 1;83(4):795-9.
4. Larsen B, Hwang J. Mycoplasma, Ureaplasma, and adverse pregnancy outcomes: a fresh look. Infectious diseases in obstetrics and gynecology. 2010; 10-15.
5. Cunningham SA, Mandrekar JN, Rosenblatt JE, Patel R. Rapid PCR detection of *Mycoplasma hominis, Ureaplasma urealyticum* and *Ureaplasma parvum* internat. J. Bact. 2013;2013:1-7.

6. Taylor-Robinson D. The role of mycoplasmas in pregnancy outcome. Best practice and research Clinical obstetrics and gynecology. 2007 Jun 1;21(3):425-38.
7. Dev T, Taneja N, Juyal D, Dhawan B, Gupta S. Upper genital tract infection due to *Ureaplasma urealyticum*: Etiological or syndromic management? Indian Journal of Dermatology, Venereology, and Leprology. 2017 Jul 1;83(4):489-492.
8. Beeton ML, Spiller OB. Antibiotic resistance among Ureaplasma spp. isolates: cause for concern? Journal of Antimicrobial Chemotherapy. 2016 Oct 20: 425-430.
9. Gdoura R, Kchaou W, Ammar-Keskes L, Chakroun N, Sellemi A, Znazen A, Rebai T, Hammami A. Assessment of *Chlamydia trachomatis, Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma hominis*, and *Mycoplasma genitalium* in semen and first void urine specimens of asymptomatic male partners of infertile couples. Journal of andrology. 2008 Mar 4;29(2):198-206.
10. Huang C, Zhu HL, Xu KR, Wang SY, Fan LQ, Zhu WB. Mycoplasma and ureaplasma infection and male infertility: a systematic review and meta-analysis. Andrology. 2015 Sep; 3(5):809-16. doi: 10.1111/andr. 12078. [PMID: 26311339].
11. Arya OP, Tong CY, Hart CA, Pratt BC, Hughes S, Roberts P, Kirby P, Howel J, McCormick A, Goddard AD. Is *Mycoplasma hominis* a vaginal pathogen? Sexually transmitted infections. 2001 Feb 1;77(1):58-62.
12. Kılıç D, Başar MM, Kaygusuz S, Yılmaz E, Başar H, Batislam E. Prevalence and treatment of *Chlamydia trachomatis, Ureaplasma urealyticum,* and *Mycoplasma hominis* in patients with non-gonococcal urethritis. Jpn J Infect Dis. 2004;57(1):17-20.
13. Grandi G. Antibacterial vaccine design using genomics and proteomics. Trends in biotechnology. 2001 May 1;19(5):181-8.
14. Purvis K, Christiansen E. Infection in the male reproductive tract. Impact, diagnosis and treatment in relation to male infertility. International Journal of Andrology. 1993 Feb; 16(1):1-3.
15. Apweiler R, Bairoch A, Wu CH, Barker WC, Boeckmann B, Ferro S, Gasteiger E, Huang H, Lopez R, Magrane M, Martin MJ. UniProt: the universal protein knowledgebase. Nucleic acids research. 2004 Jan 1;32(suppl_1): 115-9.
16. Doytchinova IA, Flower DR. VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC bioinformatics. 2007 Dec 1;8(1):4.
17. Saha S, Raghava GP. AlgPred: prediction of allergenic proteins and mapping of IgE epitopes. Nucleic acids research. 2006 Jul 1;34(suppl_2):202-209.
18. Imai K, Asakawa N, Tsuji T, Akazawa F, Ino A, Sonoyama M, Mitaku S. SOSUI-GramN: high performance prediction for sub-cellular localization of proteins in gram-negative bacteria. Bioinformation. 2008;2(9):417-421.
19. Eckstein J. ISOA/ARF drug development tutorial. InInstitute for the Study of Aging, Alzheimer Research Forum 2005 Feb. 10-15.
20. Goddard TD, Brilliant AA, Skillman TL, Vergenz S, Tyrwhitt-Drake J, Meng EC, Ferrin TE. Molecular visualization on the holodeck. Journal of molecular biology. 2018 Oct 19;430(21):3982-3996.
21. Best RB, Zhu X, Shim J, Lopes PE, Mittal J, Feig M, MacKerell Jr AD. Optimization of the additive CHARMM all-atom protein force field targeting improved sampling of the backbone ϕ, ψ and side-chain $\chi 1$ and $\chi 2$ dihedral angles. Journal of chemical theory and computation. 2012 Sep 11;8(9):3257-3273.
22. Romano N, Tolone G, Ajello F, La Licata R. Adenosine 5'-triphosphate synthesis induced by urea hydrolysis in Ureaplasma urealyticum. Journal of bacteriology. 1980 Nov 1;144(2):830-2.
23. Ba J, Wu S. ProPRED: A probabilistic model for the prediction of residual defects. In Mechatronics and Embedded Systems and Applications (MESA), IEEE/ASME International Conference; 2012. p. 247-51.
24. Diedrich G, Spahn CM, Stelzl U, SchaÈfer MA, Wooten T, Bochkariov DE, Cooperman BS, Traut RR, Nierhaus KH. Ribosomal protein L2 is involved in the association of the ribosomal subunits, tRNA binding to A and P sites and peptidyl transfer. The EMBO Journal. 2000 Oct 2;19(19):5241-50.
25. Carter AP, Clemons WM, Brodersen DE, Morgan-Warren RJ, Wimberly BT, Ramakrishnan V. Functional insights from the structure of the 30S ribosomal subunit and its interactions with antibiotics. Nature. 2000 Sep;407(6802):340-8.
26. Cummings HS, Hershey JW. Translation initiation factor IF1 is essential for cell viability in *Escherichia coli*. Journal of bacteriology. 1994 Jan 1;176(1):198-205.
27. Stiller SB, Höpker J, Oeljeklaus S, Schütze C, Schrempp SG, Vent-Schmidt J, Horvath SE, Frazier AE, Gebert N, van der Laan M, Bohnert M. Mitochondrial OXA translocase plays a major role in biogenesis of inner-membrane proteins. Cell metabolism. 2016 May 10;23(5):901-8.