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In Silico Studies of M. Tuberculosis L-Lysine 6-Monooxygenase (Mbtg) as Potential Target and an Approach for Repurposing First Line Antitubercular Oral Drugs

Swapnil Mishra

Center for Bioinformatics
University of Allahabad, Allahabad, India
Email: swapniliaps@gmail.com

Krishna Kumar Ojha

Department of Biological Sciences
Central University of South Bihar, Patna, India
Email: krisiids @gmail.com

P. N. Pandey

Center for Bioinformatics
University of Allahabad, Allahabad, India
Email: pnpiaps@gmail.com

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Abstract: Tuberculosis deadly bacterial disease caused by Mycobacterium tuberculosis which has affected the almost all part of the world population. A exhaustive work has been done for defending this bacteria but due to emergence of resistant strain of Mycobacterium tuberculosis a search for the target and drugs to counter the bacteria is still on. In this study, we had proposed the repurposing of well known Antitubercular basic drugs against proposed target L-lysine 6-monooxygenase (mbtG) of Mycobacterium tuberculosis. L-lysine 6-monooxygenase is an essential enzyme for mycobactins formation and iron sequestration which in turn is important for the survival of the Mycobacterium. Since, the structure of L-lysine 6-monooxygenase is not solved yet, so a comparative model of the same was predicted through Modeller 9v18 using the template of Lysine monooxygenase (NbtG)from Nocardia farcicinia (4D7E_A). The physico-chemical parameters of the predicted structure were validated using various programmes such as RAMPAGE, Procheck, Verify_3d and ProSA. We have found that Mycobacterium tuberculosis L-lysine 6-

monooxygenase had similar conserved domains as present in the template (4D7E_A). Computational study was done for having an idea about the interactions between basic drugs and proposed target protein. For this we had done molecular docking using AUTODOCK4 software. For comparative analysis of result and hypothesis Flavin Adenine Dinucleotide (FAD) a natural ligand of L-lysine 6-monooxygenase was also docked with the same. Among all Antitubercular oral drugs Streptomycin showed the strongest affinity with fair molecular interactions with proposed target even stronger and better than its own natural ligand. These results depicts that streptomycin might also share the targeting to Mycobacterium tuberculosis L-lysine 6-monooxygenase (mbtG).

Keywords: Mycobacterium tuberculosis, L-lysine 6-monooxygenase(mbtG), Homology Modelling, Molecular Docking, Molecular Dynamics.

1. Introduction

Tuberculosis is a deadly disease caused by M. tuberculosis. Its presence in almost all part of the world is making people sick and dead. According to report of World Health Organisation (WHO), in developing countries 95% reported cases are registered and 25% infected people meet death[1] .For the cure of this disease remarkable work has been done. But due to emergence of the MDR and XDR strain of the bacteria the medication and work against this bacteria is lagging somewhere. L-lysine 6-monooxygenase (mbtG), EC1.14.13.59, to be involved in lipid metabolism and Mycobactins formation. MbtG is Flavoprotein monooxygenase and is required for N-hydroxylation of the acetylated lysines assembly of mycobactins which is important for the iron sequestration[2]. Earlier it was predicted as a potential target for antitubercular leads by many groups[3]. Isonazid ,rifampicin, ethambutol, pirazinamide and streptomycin are the first line and basic oral drugs which are medicated to the people who suffer from tuberculosis. Although a number of new leads are found to be antitubercular and are set for the clinical trial. Here we had proposed an idea to study whether any of these basic drugs interact potentially to our proposed target. So that we can think about repurposing that established drug and also could elaborate the interactions between that particular drug and proposed target. This was done with the help of in silico docking studies between the drugs and proposed target mbtG. So keeping an eye on its importance it was taken for the in silico analysis and studies.

2. Material and Methods

Sequence Analysis

The protein sequence of the mbtG (Accession number AOZ43633) was downloaded from the NCBI database (https://www.ncbi.nlm.nih.gov)[4]. Blastp was done to in order to obtain the most suitable template from PDB (http://www .rcsb.org/pdb)[5] for the comparative prediction of the target protein sequence.A search in the Pfam[6] and Cog database[7] was done to know if conservative domain present in the given sequence of the target.A search in Pfam and COG database showed that proposed target mbtG have K_oxygenase conserved Domain having a Rossanmann fold(pfam13434) and IucD Domain (COG3486) respectively. For determining the essentiality of the given target a search was done against DEG database[8].

3D Structure Prediction and Validation

3D structure of the mbtG was predicted by using comparative modelling with software Modeller[9]. Modeller predicts the structure based on the homology present between the given structures. Validation of the predicted protein was done with the help of Rampage (http://mordred.bioc. cam.ac.uk/ ~rapper /rampage.php)[10], ProsA(Protein Structure Analysis) (https://prosa. services. came.sbg.ac.at/prosa.php)[11], Veify3D (http://services. mbi.ucla.edu/ Verify _3D/)[12]. RAMPAGE generates the ramachandran plot which gives information about the dihedral angles of the residues the protein structure,one can determine the energetically favoured position of each amino acid of the protein backbone. ProSA programme was used for checking the quality of the predicted 3-D model and asses the stability and accuracy of the predicted structure on the basis of Z score and interaction energies between amino acid residues. Verify _3D program checks the suitability of a 3D structure with its own sequence in its location and environment. The ligand binding pocket was determined by Metapocket (http://projects.biotech.tu-dresden.de/metapocket) it compiles the result of many servers which are used for pocket detection in protein and gives the best three top ranked pocket[13]. The pocket was then compared with the pocket of protein structure that was taken as template for model building for the refinement of result through comparison.

Molecular Dynamics Simulation

To access the quality of the modelled protein molecular dynamics simulation was performed on Gromacs package 5.1[14] running on Intel(R) Xeon(R) CPU E31245 @ 3.30GHz machine with 6 GB RAM and running CentOS 7. Structure of modelled protein was used as starting point for MD simulations.

In Silico Approach to Compare the Binding of Basic Antitubercular Drugs against MbtG Predicted Model

A comparative study was done to have an idea about the mode of binding and binding affinity of the tuberculosis basic drugs to the predicted mode of mbtG. This idea can repurpose the drug or can also show if one drug may have action on the proposed target too. The 3D structure file of the taken drugs are downloaded from the Zinc Dtabase(http://zinc.docking.org/).These files were converted into PDB file with the help of Open Babel software[15], it is used for the interconversion of most of the chemical file formats. For the comparison and interaction studies of most potent leads, protein ligand docking was performed between modelled mbtG with AUTODOCK suite[16]. AUTODOCK was used to study the molecular interactions between the ligand and protein. Docking was performed using AUTODOCK with default parameters by taking the mbtG as rigid body using Lamarckian Genetic algorithm. AUTODOCK create 10 poses by default of ligand-protein complex and the result is in the terms of free energy of binding of ligand to the protein while assigning a particular energy for each pose**.** A number of information can be revealed by analysis of the Autodock result such as number of H-bonds formed between ligand and target, interacting residues between ligand and target and inhibitory constant of the ligand etc.

3. Results and discussion

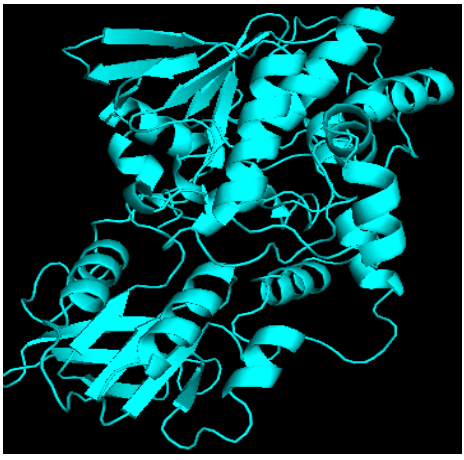
Sequence Analysis

The protein sequence of mbtG (Accession number AOZ43633) was downloaded from the NCBI database. With Blastp there was not any sequence similarity found between the M. tuberculosis mbtG and Humen proteins.Thus mbtG was proposed as a antitubercular drug target. For search of the best template for predictive modelling of mbtG PDB blast was done. Three pdbs 4D7E_A, 3S5w_A,4TLX_A were taken as templates among which 4d7E is chosen as the best template for homology modelling of mbtG. Domain search was done using Pfam and COG database showing K_oxygenase conserved Domain having a Rossanmann fold(pfam13434) and IucD

Domain(Lysine/ornithine N-monooxygenase) (COG3486) respectively.. The essentiality of the taken target was cross checked by DEG database, by doing blastp between mbtG protein sequence and DEG essential protein sequences of M. Tuberculosis which shows a significant match with DEG10100376 essential protein having identity of 93%.

3D Structure Prediction and its Validation

3 D Structure prediction of mbtG was done by Homology Modelling with Modeller9.18 ,choosing 4D7E_A as template. The template was chosen on the basis of Resolution (R=2.4 Å), R free (0.281), Max Score (456), Query Cover (94%) and identity (56%). Total 5 models were generated by Modeller9.18 and the best model was selected on the basis of the minimum dope score. The Model corresponding to the lowest dope energy -46698 was selected among all 5 models for the model validation (Figure1a). Ramachandran plot generated by Rampage server of the 3D structure of the modelled mbtG showed approx. 95% residues in favoured region,3.6% in allowed region and 1.4% in outlier region, depicting that the predicted mbtG structure was reliable(Figure1b). Verify-3D verified that 93.27% of the all residues had an averaged 3D-1D score >= 0.2 in the predicted mbtG structure, indicating that the modelled protein was stable (Figure1c). A comparison of the template protein(4D7E_A) and the modelled protein mbtG was done with the help of ProsA server, which showed that a very little difference in Z score having



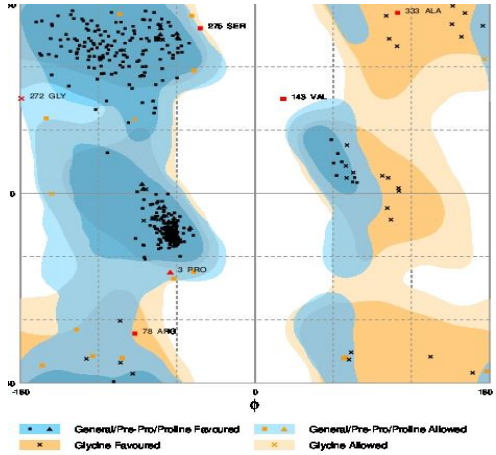


Figure 1(a) **Figure 1(b)**

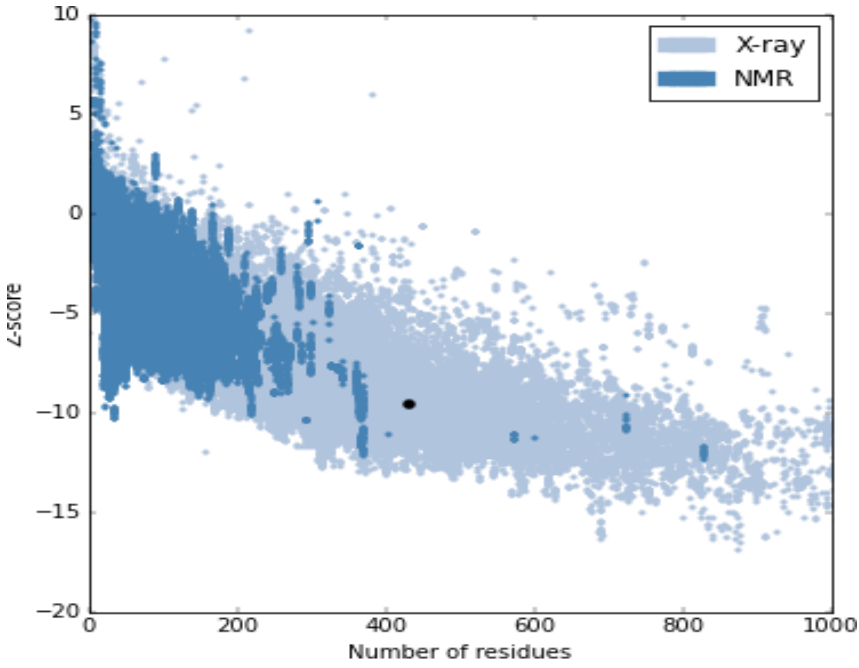


Figure 1(c)

Figure 1(a) The3D Model of the mbtG generated by Modeller viewed by Pymol. Figure 1(b)The Ramachandran Plot of the modelled 3D structure of mbtG generated by Rampage, showing most of the residues in favourable region. Figure 1(c)The plot of modelled target protein, mbtG, generated by ProsA having a reliable Z score of -9.52, -9.32 and -9.52 respectively and it also depicted that the most of the amino acid residues of the predicted model of mbtG had negative interaction energy which signified that the predicted model was stable.

The pocket detection for the binding of ligand into proposed target was done by using MetaPocket which shows almost same residues THR60, Leu401, LYS12, Asp44, Val137 present in the catalytic pocket of the template 4d7E structure.

Molecular Dynamics Studies

Systems were solvated in a cubic box with simple point charge (SPC) water molecules at 10 Å marginal radius at normal PH and neutral medium. Initially the solvent molecules were relaxed while all the solute atoms were

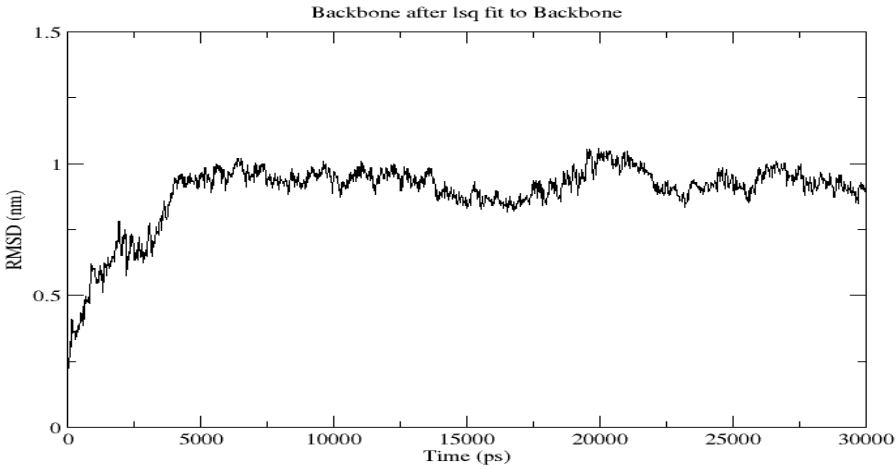


Figure 2: RMSD Plot of predicted model mbtG over the initial structure

harmonically restrained to their original positions with a force constant of 10 kcal/moL for 5000 steps. After this, whole molecular system was subjected to energy minimization for 50000 iterations by steepest descent algorithm. Berendsen temperature coupling method[17] was used to regulate the temperature inside the box. Electrostatic interactions were computed using the Particle Mesh Ewald method[18]. Two small equilibration phases of *NVT* followed by NPT was performed for 100 ps each to properly relax the complex. The temperature was maintained at 300 K and pressure was maintained at 1 atm with the allowed compressibility range of atom. SHAKE algorithm was used to constrain bond lengths involving hydrogen, permitting a time step of 2 fs. Van der Waals and coulomb interactions were truncated at 1.0 nm. The nonbonded pair list was updated every 10 steps and conformations were stored every 0.5 ps. Position restraint simulation for 500 ps was implemented to allow solvent molecules to enter the cavity region of structure[19]. Finally, systems were subjected to MD simulation for 30 ns.

We studied RMSD for all Cα atoms from the initial structure was examined to study the convergence of the protein system. The RMSD is commonly used as an indicator of convergence of the structure towards an equilibrium state. RMSD is merely a distance measure and is most meaningful for low values. In Figure.2, complex structures showed deviation till 5000 ps from their starting structure, resulting in a backbone RMSD of ~0.25 to 1.2 nm during the simulations. After this, native structure retained the maximum deviation till the end of the simulation resulting in the backbone RMSD of ~0.85 to ~1.12 nm, respectively. The RMSD increases to a plateau value initially, this means that the structure of the protein reaches a certain

distance from the reference structure and then keeps that distance more or less same with the reference structure.

In Silico Approach to Compare the Binding of Basic Antitubercular Drugs to Mbtg Model

In this study we had used an approach to propose a comparison of binding and molecular interaction of 5 basic oral antitubercular drugs namely Isonazid ,rifampicin, ethambutol, pirazinamide and streptomycin to proposed target mbtG. MbtG is protein of Flavoprotein family, so Flavin Dinucleotide(FAD) is its natural ligand. Here we tried to compare all ligand binding and interaction with FAD. All these six compounds were downloaded from Zinc Database and were docked against modelled mbtG with Autodock4. The binding energy, H bonds and participating residues of all six compounds were shown (Table1). The results showed that streptomycin is only the drug which had shown a better binding in terms of energy(-6.46) with formation of five H bonds in the docked complex with modelled mbtG than FAD(-6.15). In this way we proposed that streptomycin might also interact strongly to proposed target mbtG. In this way we can propose drug repurposing of streptomycin. The amino acids residues involved in formation of docked complex with FAD are also seen taking part in forming the docked complexes of the compounds and the target mbtG.

Compounds(ZINC Id)	**Binding Energy**	**Number of H Bonds**	**Residues Involved in formation of H Bonds**
Flavin Dinucleotide (ZINC08215434)	-6.15	3 H Bonds	Lys13,Thr210
Isoniazid (ZINC00001590)	-5.09	1 H Bonds	Gly166
Rifampicin (ZINC94313219)	-1.61	1 H Bonds	Gly166
Ethambutol (ZINC19364219)	-5.10	1 H Bonds	Ala12
Pyrazinamide (ZINC00002005)	-3.65	2 H Bonds	Ala12,Lys13
Streptomycin (ZINC08143632	-6.46	5 H Bonds	Lys13,Trp46,Gly166, Thr210,Gly332

Table 1: Comparison of the compounds that were listed for the docking studies against proposed target mbtG. According to the resultant minimum binding energy from Autodock, Streptomycin (in bold) might been seen as repurposing agent.

Residues involved in formation of H bonds in the complex shared fair similarity in each case. Residues with a good frequency of taking part in H bond formation were THR60,Leu401, LYS13,Asp44,Trp46, Val137,Thr210 and Gly166 of mbtG(Figure 3).

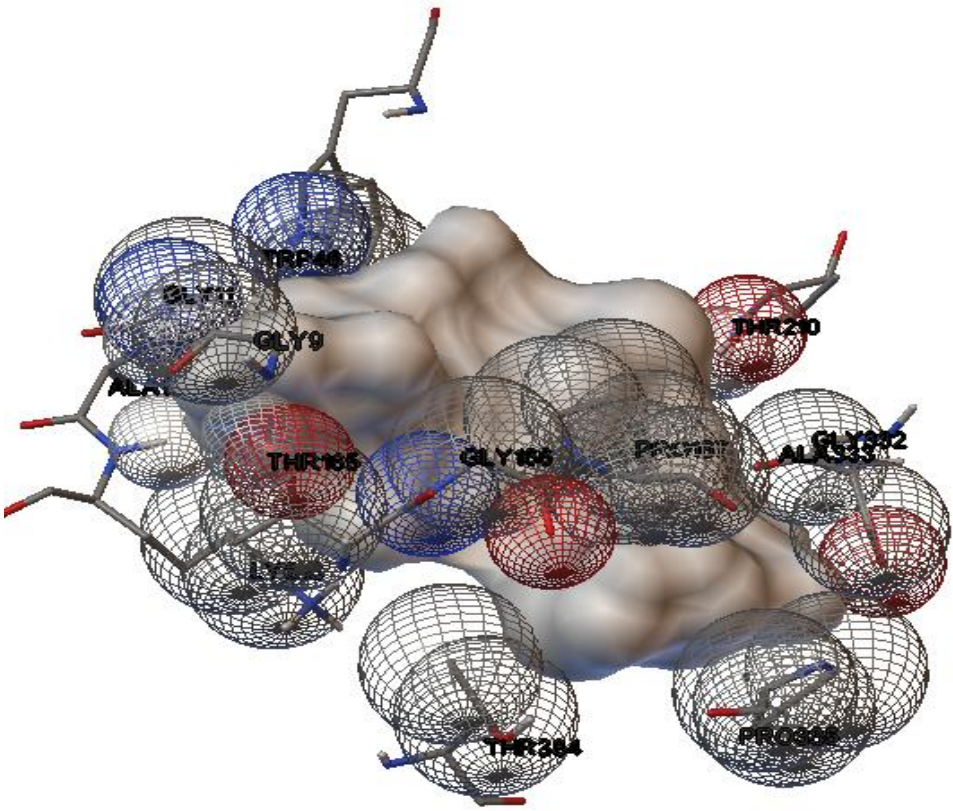


Figure 3: The Figure generated by Autodock showing the interactions between the grey coloured represented in connolly form and nearby amino acid residues of proposed target mbtG are represented by meshed spheres . The H bonds are represented by green colored cylinders.

4. Conclusion

In this study we had taken a target mbtG , which is essential for the survival of M. tuberculosis while persisting in the host. We had predicted the 3D structure and validated it using many servers and programmes. Molecular Dynamics Simulation of 30ns also validates the stability and fair molecular interaction within predicted model. For having an idea for repurposing of the

well known basic oral antitubercular 5 drugs against target mbtG, were taken for docking studies. Streptomycin showed the strongest affinity by attaining minimum binding energy given by Autodock. For comparative analysis of result, the natural ligand FAD was docked with same proposed target mbtG .In this way we can predict that streptomycin along with its established target might also interact strongly to proposed target mbtG.

References

1. Zumla, A., et al., WHO's 2013 global report on tuberculosis: successes, threats, and opportunities, *The lancet*, **382** (2013) 1765-1767.
2. L. E. Quadri, et al., Identification of a Mycobacterium tuberculosis gene cluster encoding the biosynthetic enzymes for assembly of the virulence-conferring siderophore mycobactin, *Chemistry & biology*, **5** (1998) 631-645.
3. S. Anishetty, M. Pulimi, and G. Pennathur, Potential drug targets in Mycobacterium tuberculosis through metabolic pathway analysis, *Computational biology and chemistry*, **29** (2005) 368-378.
4. N. Mulder, G. Mazandu, and H. Rapano, Using host-pathogen functional interactions for filtering potential drug targets in Mycobacterium tuberculosis, *Mycobacterial Diseases*, **3**(2013) 2161-1068.
5. A. D. Baxevanis and B. F. Ouellette, *Bioinformatics: a Practical Guide to the Analysis of Genes and Proteins*, Willey, **43**, 2004.
6. H. M. Berman, et al., The Protein Data Bank, 1999, *in International Tables for Crystallography Volume F: Crystallography of Biological Macromolecules, Springer. p.* (2006) 675-684.
7. E. L. Sonnhammer, S. R. Eddy and R. Durbin, Pfam: a comprehensive database of protein domain families based on seed alignments, *Proteins-Structure Function and Genetics*, **28** (1997) 405-420.
8. R. L. Tatusov, et al., The COG database: an updated version includes eukaryotes, *BMC Bioinformatics*, **4** (2003) 41.
9. R. Zhang, H. Y. Ou and C. T. Zhang, DEG: a database of essential genes, *Nucleic acids research*, **32** (suppl_1) (2004) D271-D272.
10. A. Fiser and A. Šali, Modeller: generation and refinement of homology-based protein structure models, *Methods in enzymology*, **374** (2003) 461-491.
11. S. C. Lovell, et al., Structure validation by Cα geometry: ϕ, ψ and Cβ deviation, *Proteins: Structure, Function, and Bioinformatics*, **50** (2003) 437-450.
12. M. Wiederstein and M. J. Sippl, ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins, *Nucleic acids research*, **35** (2007) 407-W410.
13. D. Eisenberg, R. Lüthy and J. U. Bowie, VERIFY3D: Assessment of protein models with three-dimensional profiles, *Methods in enzymology*, **277** (1997) 396-404.

14. B. Huang, Meta Pocket: a meta approach to improve protein ligand binding site prediction, *OMICS A Journal of Integrative Biology*, **13** (2009) 325-330.

15. J. J. Irwin and B. K. Shoichet, ZINC− a free database of commercially available compounds for virtual screening, *Journal of Chemical Information and Modeling*, **45**(2005) 177-182.

16. N. M. O'Boyle, et al., Open Babel: An open chemical toolbox, *Journal of Cheminformatics*, **3** (2011) 3-33.

17. O. Trott and A. J. Olson, Auto Dock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading, *Journal of Computational Chemistry*, **31** (2010) 455-461.

18. H. J. Berendsen, et al., Molecular dynamics with coupling to an external bath, *The Journal of Chemical Physics*, **81** (1984) 3684-3690.

19. U. Essmann, et al., A smooth particle mesh Ewald method, *The Journal of Chemical Physics*, **103** (1995) 8577-8593.