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Computational Studies on Physicochemical Properties, Substrate Specificity Prediction and Binding Mode Analysis of Aliphatic Nitrilase

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Abstract

Nitrilase enzyme catalyze conversion of nitrile compounds to its corresponding carboxylic acid and ammonia. With the rise of digital era, the use of computational analysis to revel hidden characteristics of enzymes is continuously growing because of its cost effectiveness and faster output. Highly developed sequencing technology provides an opportunity to conduct virtual studies and explore unique properties and applications of various enzymes. Physico-chemical properties of aliphatic nitrilase evaluated results show quite similar properties of all 13 studied aliphatic nitrilase. The 3D structures of aliphatic nitrilase were generated by using Swiss-Model server and validated using different tools describes that quality of 3D structure of all 13 protein is good. Docking of 13 nitriles with all 13 aliphatic nitrilase was done by using Autodock v4.2.6 software, reveals that cis-cis Muconitrile has highest binding affinity with most of studied protein, while Glycolonitrile and Propionitrile has less binding affinity toward major studied nitrilase.

Keywords

In-silico approach, Nitrilase, cis-cis muconitrile, Molecular docking, Substrate specificity, Binding mode

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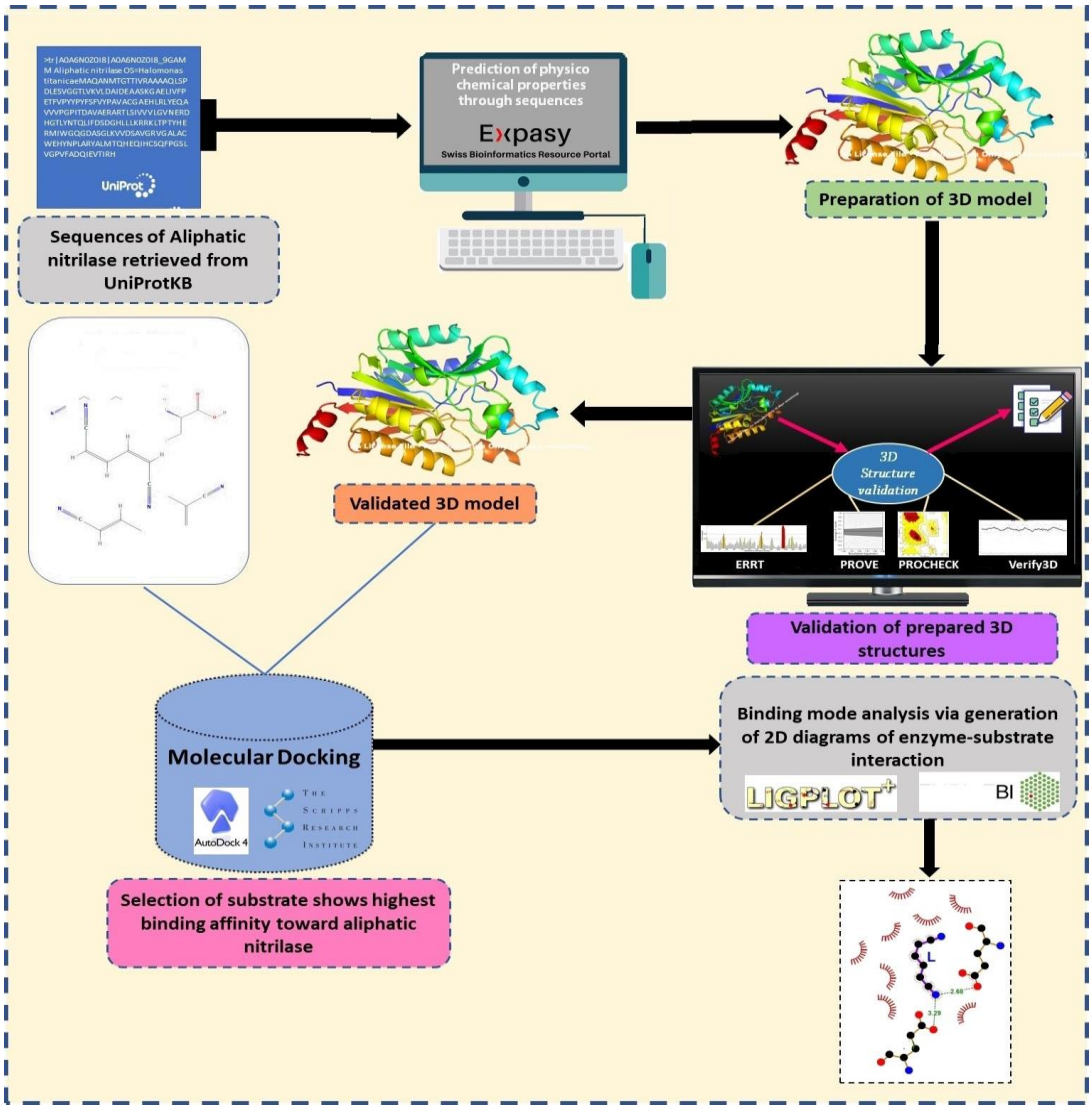


Highlights

- Physico-chemical properties of aliphatic nitrilase was investigated.
- Binding affinity of aliphatic nitriles towards aliphatic nitrilase was studied
- Cis-cis muconontrile shows higher binding affinity with most of studied nitrilase
- Glycolonitrile and Propionitrile has less binding affinity with all nitrilase
- Non-covalent interactions also play an important role in substrate-enzyme binding

1. INTRODUCTION

The organic compound which contains –C≡N functional group are officially called as nitrile compounds, apart from it inorganic compounds which contains –C≡N functional group are called as cyanide compounds [1,2,3]. It is widely present in nature from microorganisms to plant and animals and also synthesized chemically. Currently nitriles are widely used by various industries such as pharmaceuticals, pesticides, herbicides, polymer industries, etc. [4-8]. Various nitrile compounds used for production of herbicides such as bromoxynil, dichlorobenil, ioxynil-ocanoate, etc. [3,6,7,9-14]. In pharmaceutical industries nitrile compounds used for production of drugs such as vildagliptin, letrozole, anastrozole, gallopamils, cilomilast, entacapone, trilostane, etc. [8,15-23]. Many nitrile compounds also used to synthesize important polymers such as polyacrylonitrile, nylon 66, acrylamide, nicotinamide, cyanoverlamide, cyanide butadiene polymers. [3,24-28] On the other side these compounds also used to synthesize carboxylic acid, amines, amides, ketones, esters, etc. [3]

The disposal of nitrile containing compounds into water and soil raises environmental concerns. Need efficient and ecofriendly detoxification and degradation of nitrile compound into less toxic and beneficial products. [29-34] The best way for

degradation of nitrile compounds is biological (enzymatic) degradation, which is economical and ecofriendly, it also has many advantages than chemical degradation. Enzymatic degradation involves nitrile degrading enzymes such as nitrilase, nitrile hydratase and amidase. Nitrilase enzyme catalyze direct conversion of nitrile compounds to its corresponding carboxylic acid and ammonia. Apart from its nitrile compounds can be hydrolyzed by nitrile hydratase enzyme which converts these compounds into its corresponding amides followed by to carboxylic acid and ammonia by using amidase enzyme. Based on substrate specificity nitrilase enzyme classified into three categories: (i) Aliphatic nitrilase, (ii) Aromatic nitrilase and (iii) Arylacetonitriles. Aliphatic nitrilase specifically act on aliphatic nitriles such as acrylonitrile, mucononitrile, malononitrile, valeronitrile, etc. while aromatic nitrilase act on aromatic nitriles such as benzonitrile, cyanopurines, cyanoindole, etc. Apart from it arylacetonitrile preferentially catalyze hydrolysis of arylacetonitriles such as cinnaminonitriles, phenylpropioitrile, etc. [7,35-39] Because of widespread occurrence of nitrile compounds many microorganisms are reported for production of nitrilase enzyme such as *Acinetobacter* sp., *Alcaligenes* sp., *Rhodococcus* sp., Fusarium sp., *Aspergillus* sp., *Burkholderia* sp., etc. [38-45]

With the rise of digital era the use of computational analysis to revel hidden characteristics of enzymes is continuously growing because these methods are cost effective and also gives faster output. Highly developed sequencing technology and its ease of access through various online available databases provides an opportunity to bio-scientists to conduct virtual studies and explore unique properties and characteristics of various enzymes. There are various serves like ExPASy and its Prot Param tool [46], through which we can explore several properties of proteins just by introducing aminoacid sequence such as; aminoacid composition, molecular weight, theoretical pI, negative and positively charged residues, aliphatic index, instability index, grand average of hydropathicity (GRAVY), Extinction coefficient with and without Cys residue, etc. [47-49].

For unknown protein, 3D structure of these proteins can be constructed by using Homology modelling and model construction of Swiss-Model server. Also, there are software (SAVES 6.0 structure validation server and ProSA-web protein structure analysis) [50-57] through which quality of constructed structures can be estimated. We can also predict substrate specificity, binding affinity and binding modes of Enzyme with Ligand (substrate) using in-silico approach [58-61]. So, by combination of all these virtually available servers and software we can conduct very nice study which can explore some hidden characteristics of particular enzyme. Objective of this study is to analyze physico-chemical properties, predict substrate specificity and analyze binding pocket and binding mode of aliphatic nitrilase which obtained from various microbial-resources.

2. MATERIALS AND METHODS

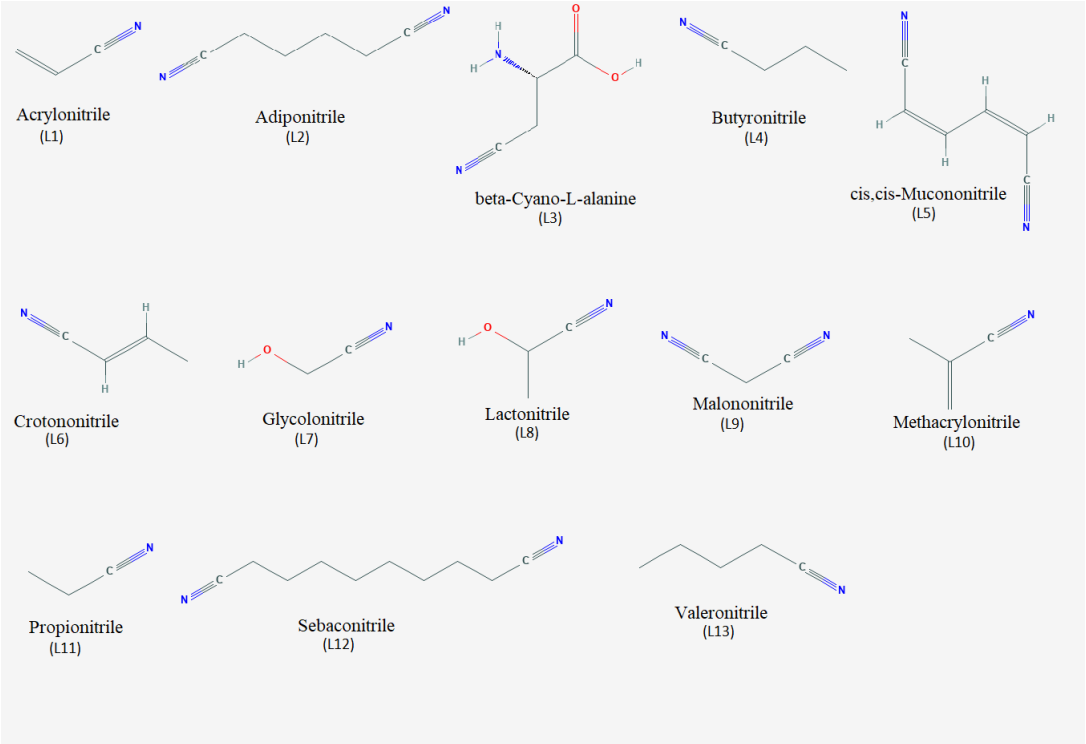
2.1 Collection of datasets

To conduct this study 13 aliphatic nitrilase (enzyme) was selected and it's aminoacid sequence collected from UniProtKB database. List of selected microbial resources with its UniProtKB accession number for aliphatic nitrilase describes in Table 1. These sequences further used for sequence-based analysis, physicochemical properties and to build its 3D models. Apart from it 13 nitrile group containing ligand (substrate) selected from PubChem databases and further converted into 3D format, its 2D representation described in Figure 1.

Table 1 Selected microbial enzyme resource with its UniProtKB accession number for aliphatic nitrilase

No.	Accession no of protein from UniProtKB	Microbial enzyme resource
P1	A0A1Q3QRA0	*Acidobacteriales bacterium*
P2	A0A1M5QJF3	*Candidimonas bauzanensis*
P3	A0A250B6G0	*Gibbsiella quercinecans*
P4	A0A6N0Z0I8	*Halomonas titanicae*
P5	A0A4R1GPC2	*Marinobacterium mangrovicola*
P6	A0A509EBB4	*Methylobacterium symbioticum*
P7	A0A4R3Y4K4	*Methylomonas methanica*
P8	A0A248VI41	*Paraburkholderia aromaticivorans*
P9	A0A0P0RE58	*Paraburkholderia caribensis MBA4*
P10	B1G3B9	*Paraburkholderia graminis ATCC 700544*
P11	U7R0S6	*Photorhabdus temperata J3*
P12	A0A5E7NH36	*Pseudomonas fluorescens*
P13	Q9UYV8	*Pyrococcus abyssi GE5*

Figure 1 Shows 2D representation selected ligands (substrates)



2.2 Analysis of physicochemical properties

Physico-chemical characteristics such as Molecular Weight (MW), theoretical pI, Instability index, aliphatic index, Extinction coefficient with and without Cys, grand average of hydropathicity (GRAVY) of aliphatic nitrilase was evaluated by using Expert Protein Analysis System's (EXPASY's) Prot Param tool, which is proteomic server of swiss institute of bioinformatics [47-49,62]. Prot Param explore these properties by using aminoacid sequence of protein without any additional information. Molecular weight of protein was estimated by sum of molecular weight of all aminoacid present in the sequence and by removing molecular weight of single water molecule from it. Extinction coefficient of aliphatic nitrilase was calculated by using equation 1 [46,47,60]. This study is very important for purification of protein. The stability of protein on the basis of its aminoacid composition, was calculate by using equation 2 [46,60]. Aliphatic index of protein calculated using equation 3 which is based on mole percent and relative volume of side chains [46,63]. GRAVY value of protein calculated by equation 4 [46,61]. Aminoacid composition of all sequences also evaluated by using same tool.

Equation 1: Extinction coefficient = Num (Tyr) x Ext (Tyr) + Numb (Trp) x Ext(Trp) + Numb(Cystine) x Ext(Cystine). Where, Numb = Number of amino acid residue, Ext = Molar extinction coefficient. The absorbance can be calculated using the following formula: Absorbance = Extinction coefficient/Molecular weight. Equation 2: i = L-1, Instability index = (10/L) x Sum DIWV(x[i]x[i+1]). i=1 Where, L is the length of sequence DIWV(x[i]x[i+1]) is the instability weight value for the dipeptide. Starting in position i. Equation 3: Aliphatic index = X(Ala) + a xX(Val) + bx [X(ile) + X(Leu)]. Where, X(Ala), X(Val), Xille), and X(Leu) are mole percent of alanine, valine, isoleucine, and leucine. The a and b are the relative volume of valine side chain (a = 2.9) and of Leu/lle side chains (b = 3.9) to the side chain of alanine. Equation 4: GRAVY = Sum of hydropathy value of al amino acid/No. of amino acid residue in sequence.

2.3 Preparation of Ligand

The ligand 3D structures retrieved from PubChem was converted to .pdb format. To prepare ligand structure for docking, Hydrogen and Gasteiger charges applied to the ligand structures followed by energy minimization in Amber 03 force field using UCSF chimera 1.15 software [47,64,65].

2.4 Homology modelling

The FASTA sequence of protein retrieved from UniProtKB database was then applied to SWISS-Model to predict 3D structure of given protein. The best predicted structure was selected on the basis of appropriate score and ranking of model [47,66,67].

2.5 Preparation of Protein structure

The 3D model structure of all 13 aliphatic nitrilase then subjected to removal of water, unwanted cofactor, ions and ligand followed by addition of hydrogen and kollman charges. Then all structures energy minimized under Amber 03 forcefield using UCSF chimera 1.15 software [68,69].

2.6 Validation of 3D protein structure

The quality of prepared 3D structure of protein was estimated by using various scores and rankings such as ERRAT, Verify3D, Z score. Apart from it Ramachandran plots of all structures was prepared to validate 3D structure. It was done by using SAVES 6.0 structure validation server (ERRAT, Verify3D, Procheck) and ProSA-web protein structure analysis [50-57].

2.7 Molecular Docking

Dock prepared structures of 13 aliphatic nitrilase and 13 ligands (substrates) was then subjected to molecular docking by Autodock 4.2.6 - a molecular docking software of The Scripps research institute using one ligand at a time to protein approach, to evaluate best dockpose, binding affinity and binding energy of protein-ligand interaction. For this all .pdb structure files are converted into. pdbqt format (suitable format for docking by Autodock 4.2.6 software) and then proceed for docking by setting grid box and finally select best dockpose which have lowest binding energy [65,70-73]. By evaluating results of docking, we can predict the substrate specificity and binding affinity of aliphatic nitrilase with particular ligand. Results of Molecular docking was visualized by using Biovia Discovery Studio Visualizer 2021.

2.8 Binding pocket and Binding mode analysis

For deep understanding of protein-ligand interaction and active binding site (binding pocket), best dockpose was imported into LigPlot +v2.2 software EMBL-EBI ligand protein interaction diagrams. It will generate 2D diagrams of protein ligand interactions and gives insight into binding mode and non-covalent interactions that occurs during binding [47,58-61].

3. RESULT AND DISCUSSION

3.1 Analysis of Physico-chemical properties

Physico-chemical properties of aliphatic nitrilase was studied virtually and shows that its molecular weight and no. of aminoacid residues varied from 35 to 36kDa and 262 to 358 residues respectively (Table 2). Another important property of protein is theoretical pI. pI of most of protein is between 5-6. The highest pI 6.27 is of *Methylobacterium symbioticum* and the lowest pI 5.35 is of *Pseudomonas fluorescens*. Instability index of protein sequence varied greatly. Instability index of *Gibbsiella quercinecans*, *Halomonas titanicae*, *Paraburkholderia graminis ATCC 700544, Pseudomonas fluorescens, Pyrococcus abyssi GE5* is below 40 and interpret as highly stable, while of remaining protein is above 40 and become unstable. Lowest Instability index 28.16 is of *Pyrococcus abyssi GE5* while highest 49.49 is of *Paraburkholderia caribensis MBA4*. High aliphatic index shows that this protein can stable in wide temperature range. Aliphatic index of most of protein is between 85-90 indicates that it is highly stable for wide range of temperature. Aminoacid composition of aliphatic nitrilase is very interesting. In all sequences Alanine present in highest amount while Tryptophan present in lowest amount (Table 3). The percentage of Alanine and Tryptophan varied between 8.4-12.5% and 0.4-1.2% respectively.

Table 2 Shows Physico-chemical properties of selected aliphatic nitrilases of different microbial origins

Properties of Protein	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13
No. aminoacid	324	330	333	330	336	358	331	348	328	336	335	324	262
MW (Da.)	35600.46	36012.81	36541.53	35869.85	36789.83	38972.24	36289.51	38007.50	36178.16	36264.26	36863.35	35236.15	29798.34
Total residue (Asp+Glu)	40	37	42	39	42	44	37	41	41	39	34	37	40
Total residue (Arg+Lys)	32	28	31	26	30	39	32	35	33	31	27	25	35
Theoretical pI	5.54	5.49	5.47	5.41	5.46	6.27	6.13	6.01	5.47	5.70	6.00	5.35	5.37
Extinction co-eff. Cys	33140	31650	34630	31775	34630	30160	33265	34295	40005	23045	30410	34630	23380

Extinction co-eff. Cys reduced	32890	31400	34380	31400	34380	29910	32890	33920	39880	22920	29910	34380	23380
Instability Index	46.30	46.72	39.50	37.62	41.60	45.47	43.46	44.70	49.49	33.77	40.10	39.45	28.16
Aliphatic Index	87.31	86.94	90.54	94.33	89.20	86.98	88.43	88.30	85.95	86.87	93.46	88.55	88.17
GRAVY	-0.146	-0.132	-0.162	-0.020	-0.144	-0.233	-0.129	-0.117	-0.195	-0.084	-0.020	-0.054	-0.161

Table 3 Shows aminoacid percent (%) composition of selected aliphatic nitrilases from P1 to P13.

		Aliphatic nitrilases of different microbial origins												
		P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13
AMINO ACIDS	Ala (A)	9.6	10.6	11.1	11.5	12.5	13.1	10.6	11.8	9.8	12.5	8.4	10.8	8.4
	Arg (R)	6.8	5.8	6.9	5.2	5.4	8.7	6.0	6.9	6.7	6.2	3.9	5.2	6.1
	Asn (N)	1.5	1.8	2.1	2.4	3.3	2.0	2.4	2.3	1.8	2.1	3.0	1.2	3.4
	Asp (D)	5.9	5.5	6.0	5.8	5.7	6.4	4.8	4.9	5.5	6.0	4.2	4.9	5.7
	Cys (C)	1.5	1.5	1.2	2.1	1.5	1.4	1.8	1.7	0.6	0.9	2.4	1.2	0.4
	Gln (Q)	4.9	7.0	4.2	4.2	3.6	4.2	4.2	4.3	4.9	5.1	6.0	5.6	1.9
	Glu (E)	6.5	5.8	6.6	6.1	6.8	5.9	6.3	6.9	7.0	5.7	6.0	6.5	9.5
	Gly (G)	8.6	8.5	7.8	7.6	6.5	8.4	7.6	7.5	8.8	9.2	7.5	9.3	6.9
	His (H)	2.5	2.7	3.3	3.9	3.6	3.9	3.3	3.2	2.1	3.0	3.9	3.4	0.8
	Ile (I)	6.5	6.1	5.4	5.5	4.8	3.6	6.6	5.5	5.8	5.7	7.8	5.6	5.7
	Leu (L)	7.7	7.9	8.7	9.7	8.9	8.7	7.3	6.9	7.6	7.4	9.0	8.6	7.6
	Lys (K)	3.1	2.7	2.4	2.7	3.6	2.2	3.6	3.2	3.4	3.0	4.2	2.5	7.3
	Met (M)	2.8	2.4	2.4	2.1	2.4	2.0	2.4	2.9	3.0	3.6	3.0	4.0	3.1
	Phe (F)	3.4	3.6	2.4	2.7	3.0	2.8	3.0	2.9	3.4	3.6	3.9	2.8	6.9
	Pro (P)	4.3	4.5	6.0	5.2	6.2	6.7	6.6	6.6	5.2	4.5	5.1	4.9	4.6
	Ser (S)	6.8	6.7	3.9	4.8	4.8	4.2	4.8	4.6	5.2	5.4	7.8	7.1	3.1
	Thr (T)	5.6	5.5	6.6	6.4	5.1	3.6	6.0	4.9	6.1	5.4	3.9	4.0	4.2
	Trp (W)	0.9	0.9	0.9	0.9	0.9	0.8	0.9	1.1	1.2	0.6	0.9	0.9	0.4
	Tyr (Y)	3.4	3.0	3.6	3.0	3.6	2.5	3.3	2.3	3.7	2.4	2.7	3.7	4.6

	Val (V)	7.7	7.6	8.4	8.2	8.0	8.9	8.2	9.8	8.2	8.0	6.9	7.7	9.5

3.2 Homology modelling

Homology modelling to prepare 3D structures of given aliphatic nitrilase sequences was done by using SWISS-MODEL server. The selection of single best model among proposed is based in its Global Model Quality Estimation (GMQE), identity and Quality Model Energy Analysis (QMEAN) scores. For each protein a structure with high GMQE and identity value is most preferred structure (Figure 2).

3.3 Validation of 3D protein structures

Structure of aliphatic nitrilase retrieved from SWISS-MODEL by homology modelling followed by dock preparation was then subjected to validation through SAVES 6.0 and ProSA, Procheck servers. Various properties and scores such as, ERRAT, Z score, Verify3D score, Ramachandran plot study of prepared 3D structure was analyzed, and quality of structures concluded on the basis of results (Table 4). Procheck results suggest that more than 98% restudies present in allowed and favored regions. G factor of 3D model structures varied between -0.10 to -0.20. Z score of models was checked by using ProSA-web protein structure analysis, it is between -7.5 to -8.5. ERRAT score is also very high, between 85-90 (Table 4). Results indicates that overall quality of 3D model structure of aliphatic nitrilase is good.

Figure 2 Shows 3D structure of selected 13 aliphatic nitrilase generated by homology modelling and numbered as P1 to P13.

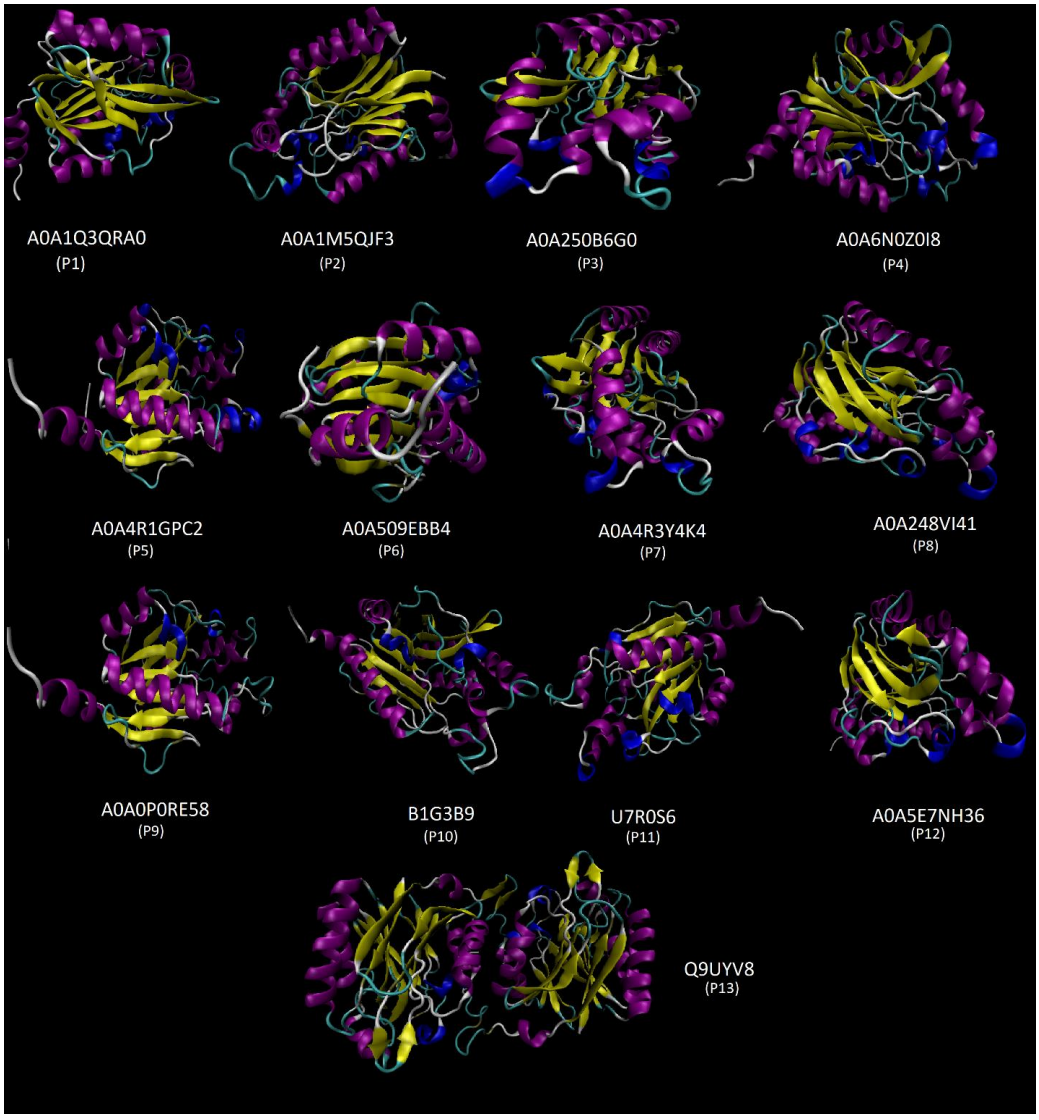


Table 4 Shows results of 3D protein structure validation

	Aliphatic nitrilases of different microbial origins												
Properties	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**	**P10**	**P11**	**P12**	**P13**
ERRAT	89.39	88.90	91.91	91.11	89.41	87.54	85.07	90.26	86.13	91.57	91.82	85.39	91.96
Verify3D	90.39 %	90.53	96.84%	96.81%	95.44%	65.09 %	94.98 %	93.19 %	86.67 %	83.80%	92.14 %	87.46 %	85.82 %
Most favored regions	90.0%	89.3%	90.5%	91.0%	91.2%	92.1%	91.2%	92.1%	91.8%	91.3 %	90.4 %	90.3 %	88.4%
Additional allowed regions	7.1%	7.4%	7.4%	6.9%	6.8%	6.2%	7.5%	5.8%	6.1%	5.8%	7.1%	7.2%	10.7%
Generously allowed Regions	2.1%	2.5%	0.8%	0.8%	0.8%	0.4%	0.4%	0.4%	1.2%	1.7%	1.2%	1.3%	0.2%
Disallowed regions	0.8%	0.8%	1.2%	1.2%	1.2%	1.2%	0.8%	1.7%	0.8%	1.2%	1.2%	1.3%	0.7%
G-factor	-0.13	-0.16	-0.13	-0.13	-0.13	-0.14	-0.09	-0.11	-0.15	-0.14	-0.17	-0.14	-0.20
Z-score	-8.79	-7.6	-8.14	-7.41	-7.63	-7.61	-7.84	-7.84	-8.05	-7.22	-7.75	-7.25	-7.87

3.4 Molecular docking

To evaluate best binding pose and binding affinity of 13 ligand with 13 aliphatic nitrilase molecular docking was done by using Autodock v4.2.6 software of The Scripps research institute. All 13 ligands are docked with each 13 aliphatic nitrilase and from predicted binding pose, a pose with lowest binding energy (docking energy) was selected as a best binding pose for particular pair (Figure 3). Results of all docking listed in Table 5, results suggest that cis-cis Muconontrile (CCM) have highest binding affinity toward most of aliphatic nitrilase while Glycolonitrile and Propionitrile has less binding affinity toward aliphatic nitrilase. Binding energy and contacting aminoacid residues listed in Table 6. CCM docked with *Methylomonas methanica* at -5.44 kcal.mol^{-1} binding energy is the best among all studied proteins. The Pro 71, Thr 72, Val 73, Pro 74, Gly 75, Ile 77, Thr 78, Ile 96 and Leu 109 are present as contacting aminoacid residue.

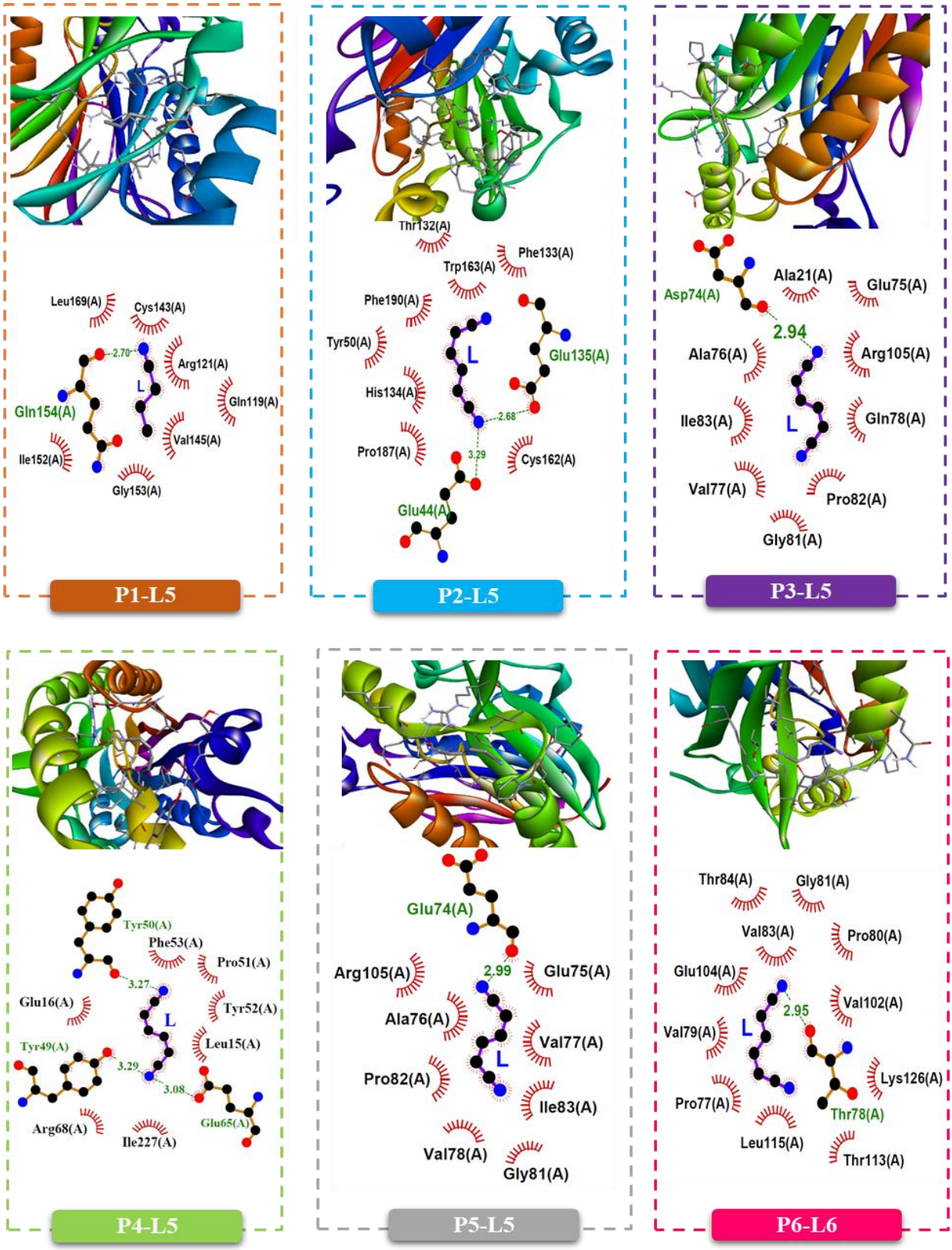


Figure 3 Shows docked pose and binding mode (2D diagram of substrate-enzyme interaction) of cis-cis muconitrile with aliphatic nitrilase (P1 to P6)

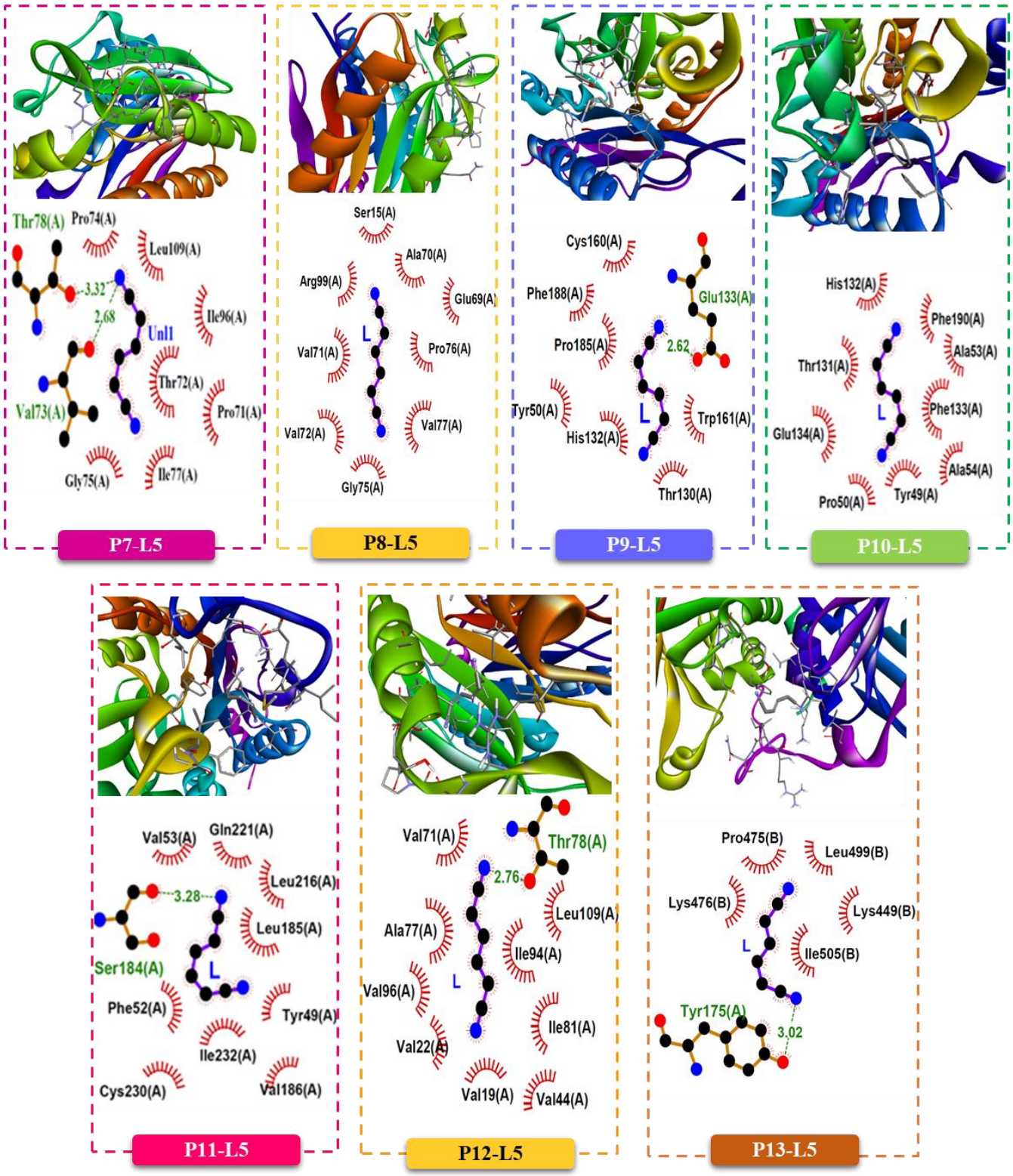


Figure 4 Shows docked pose and binding mode (2D diagram of substrate-enzyme interaction) of cis-cis mucononitrile with aliphatic nitrilase (P7 to P13)

Table 5 Shows efficiency (higher to lower from top to bottom) of ligand to bind with different aliphatic nitrilases, predicted using molecular docking

	Aliphatic nitrilases of different microbial origins												
	P1	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**	**P10**	**P11**	**P12**	**P13**
LIGANDS	3	5	5	5	5	5	5	5	5	12	12	5	5
	5	12	3	3	10	3	2	12	12	5	5	2	3
	12	2	10	12	12	12	13	3	3	3	3	12	2
	6	3	12	2	4	10	10	10	2	2	2	3	10
	2	8	4	10	2	2	12	2	6	13	10	6	6
	10	13	2	4	9	4	4	4	8	6	9	10	9
	4	6	6	6	3	13	6	6	10	10	8	4	8
	13	10	9	9	13	9	3	9	13	1	13	9	11
	9	4	1	13	6	6	9	1	9	8	6	1	13
	8	9	8	1	1	1	1	11	1	11	4	13	1
	1	1	11	8	8	8	8	8	11	4	1	8	4
	11	11	13	11	11	11	11	13	4	9	11	11	7
	7	7	7	7	7	7	7	7	7	7	7	7	12

Table 6 Shows binding energy and contacting receptors residue of cis-cis Mucononitrile with all selected aliphatic nitrilases

Aliphatic Nitrilase	Binding Energy [kcal.mol^{-1}]	Contacting receptors residues
P1	-4.29	Val45, Glu119, Arg 121, Cys 143, Ile 152, Gly 153, Gln 154, Leu 169
P2	-4.66	Glu 44, Tyr 50, Thr 132, Phe 133, His 134, Glu 135, Cys 162, Trp 163, Pro 187, Phe 190
P3	-4.55	Ala 21, Asp 74, Glu 75, Ala 76, Val 77, Glu 78, Gly 81, Pro 82, Ile 83, Arg 105
P4	-5.06	Leu 15, Glu 16, Tyr 49, Tyr 50, Pro 51, Tyr 52, Phe 53, Glu 65, Arg 68, Ile 227
P5	-4.80	Glu 74, Glu 75, Ala 76, Val 77, Val 78, Gly 81, Pro 82, Ile 83, Arg 105
P6	-5.38	Pro 77, Thr 78, Val 79, Pro 80, Val 83, Thr 84, Val 102, Glu 104, Thr 113, Leu 115, Lys 126

3.5 Binding pocket and Binding mode analysis

To fully understand protein ligand interaction and non-covalent (Hydrogen bond and hydrophobic interaction) interaction which contributes to stable binding of protein with ligand, 2D ligand-protein interaction maps was generated by using LigPlot +v2.2 software. It gives information regarding which atom of ligand interacts with which aminoacid residue by which interactions. Ligand atom, receptor atom and distance listed in Table 7. Figure 3 also shows 2D Ligand-protein interaction plots which are generated by LigPlot +v2.2. Binding pocket residues are listed in Table 6 as contacting receptor residues. Total 3 Hydrogen bond (H bond) observed in interaction between CCM and *Halomonas titanicae*, involving Tyr 49, Tyr 50, Glu 65 residues, which is

highest number of H bond observed among all interactions. Binding pocket of respective pair involves mainly Leu 15, Glu 16, Tyr 49, Tyr 50, Pro 51, Tyr 52, Phe 53, Glu 65, Arg 68 and Ile 227 aminoacid residues (Table 6). Interaction of CCM and *Methylomonas methanica* in which lowest binding energy was reported during docking generates two H bond, involves Thr 78 and Val 73 aminoacid residues, its binding pocket formed by Pro 71, Thr 72, Val 73, Pro 74, Gly 75, Ile 77, Thr 78, Ile 96 and Leu 109 residues (Table 6,7).

The main disadvantage of chemical synthesis is generation of substances which are hazardous for human as well as environmental health. So, the main objective of green chemistry is to develop processes which minimize or eliminate generation of hazardous products and satisfy needs of green and clean production and degradation [74,75]. Enzymes (biocatalysts) has very phenomenon characteristics such as catalytic efficiency, low energy consumption, lesser generation of hazardous products, substrate specificity, optical purity, etc. Because of all these properties enzymes emerged as cost effective, environment friendly and sustainable tool which promotes development of sustainability [74,76]. Enzymes are currently used on various industries not only for sustainable and efficient production but also for treatment of waste materials, water and soil. The insight into physico-chemical properties provides benefits for optimal and efficient use of particular enzyme. As discussed above enzymes performed predicts mode of binding of enzyme with its substrate though in silico approach, like for small molecule modulators [90], ligase [91], oxidosqualene cyclase inhibitors [92], cellulase [89], etc. All these studies proved that this is also a best approach to explore hidden characteristics of enzyme, predict suitable substrates and to find newer applications of enzymes.

very specific biochemical tasks, so studies on its substrate specificities and binding mode helps to explore new substrates and application of a particular enzyme.

The first nitrilase was described from barley leaves by Thimann et al. [77] shows conversion of Indole acetonitrile to Indoleacetic acid. Later studies suggest that this enzyme has wide range of substrates [78]. There are very few reports which uses in silico approaches for analysis of physico-chemical properties, substrate specificity and binding mode of nitrilase enzyme. There are lots of studies in which reports substrate specificity of nitrilase (without using is silico approaches) obtained from various microbial resources such as *Acinebacter* sp. AK226 [79], *Alcaligenes faecalis* ATCC 8750 [80], *Bacillus pallidus* Dac521 [81], *Rhodococcus* ATCC39484 [82], etc. Duca et al. [83] performed in vivo analysis of substrate specificity of nitrilase from *Pseudomonas* sp. strain UW4 and found that it is specific for aromatic nitrile compounds. While Mulelu et al. [84] revel substrate specificity, binding pocket and binding mode of Arabidopsis nitrilase.

There are many studies which reports computational analysis of substrate specificity of enzymes such as acyl adenylate [85], methyl transferase [86], enzymes of glycolysis pathway [87], protease [88], amidase [1], cellulase [89], etc. They use in silico approach to predict substrate specificity of enzyme. While there are also some studies which

Table 7 Shows ligand atom and receptor atom which participates in H bond formation and describes distance of this bond

Aliphatic Nitrilase	Ligand atom	Receptor atom	Distance (Å)
P1	N	O Gln154	2.70
P2	N2	OE2 Glu135	2.68
	N2	OE2 Glu44	3.29
P3	N1	O Asp74	2.94
P4	N1	O Tyr50	3.27
	N2	OH Tyr49	3.29
	N2	OE1 Glu65	3.08
P5	N1	O Glu74	2.99
P6	N1	O Thr78	2.95
P7	N1	OG1 Thr78	3.32
	N1	O Val73	2.68
P9	N1	OE2 Glu133	2.62
P11	N1	O Ser184	3.28
P12	N1	OG1 Thr78	2.76
P13	N2	OH Tyr175	3.02

4. CONCLUSION

Knowledge regarding physico-chemical properties of any enzyme is very important for its optimal application and efficient use. Besides experimental methods in this digital era there is opportunity to revel these properties using online available servers and software by in silico approach. Apart from these properties we can also predict substrate specificity, binding affinity and binding mode using in silico approach. Physico-chemical properties of aliphatic nitrilase evaluated by using EXPASY's Prot Param tool, results show quite similar properties of all 13 studied aliphatic nitrilase with only slight variations. A high aliphatic index shows that this protein remains stable at wide range of temperature. The 3D structures of aliphatic nitrilase was predicted by using Swiss-Model server and validated using 4 different tools (ERRAT, Verify3D, Procheck, ProSA) describes that quality of 3D structure of all 13 protein is good. Docking of 13 ligands (substrate) with all 13 aliphatic nitrilase was done by using Autodock v4.2.6 software, reveals that cis-cis Mucononitrile has highest binding affinity with most of studied protein, while Glycolonitrile and Propionitrile has less binding affinity toward major studied protein. Binding between cis-cis Mucononitrile and *Methylomonas methanica* has lowest binding energy among all 13 proteins. The 2D maps of Ligand-protein interaction was generated using LigPlot +v2.2 software and it reveals that non-covalent interactions plays and important role in stable binding of ligand with protein.

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6. CONFLICT OF INTEREST

Authors do not have any kind of conflict of interest

7. AUTHORS APPROVAL

All authors have seen and approved the manuscript

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