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An *in silico* approach to evaluate the polyspecificity of methionyl-tRNA synthetases

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ABSTRACT

Residue-specific incorporation is a technique used to replace natural amino acids with their close structural analogs, unnatural amino acids (UAAs), during protein synthesis. This is achieved by exploiting the substrate promiscuity of the wild type amino acyl tRNA synthetase (AARS) towards the close structural analogs of their cognate amino acids. In the past few decades, seleno-methionine was incorporated into proteins, using the substrate promiscuity of wild type AARSs, to resolve their crystal structures. Later, the incorporation of many UAAs showed that the AARSs are polyspecific to the close structural analogs of their cognate amino acids and that they maintain fidelity for the 19 natural amino acids. This polyspecificity helps to expand the use of this powerful tool to incorporate various UAA residues specifically through *in vivo* and *in vitro* approaches. Incorporation of UAAs is expensive, tedious and time-consuming. For the efficient incorporation of UAAs, it is important to screen substrate selectivity prior to their incorporation. As an initial study, using a docking tool, we analyzed the polyspecificity of the methionyl-tRNA synthetases (MetRSs) towards multiple reported and virtually generated methionine analogs. Based on the interaction result of these docking simulations, we predicted the substrate selectivity of the MetRS and the key residues responsible for the recognition of methionine analogs. Similarly, we compared the active site residues of the MetRSs of different species and identified the conserved amino acids in their active sites. Given the close similarity in the active site residues of these systems, we evaluated the polyspecificity of MetRSs.

1. Introduction

Aminoacyl tRNA synthetase (AARS) is the central component of the translational apparatus of the cell that exerts control over the accuracy of translation by charging the target tRNA molecule with its cognate amino acid [1]. In the past few decades, it has been reported that the endogenous AARS fails to discriminate between natural amino acids (cognate amino acid) and their close structural analogs, unnatural amino acids (UAAs). This substrate promiscuity of AARS towards these close structural analogs favors the residue-specific incorporation of UAAs into recombinant proteins [1,2]. Using this methodology, more than 100 UAAs were globally incorporated into proteins through *in vivo* and *in vitro* approaches [1,3,4]. This technique is referred to as residue-specific incorporation and facilitates the multi-site incorporation of UAAs. In the *in vivo* approach, auxotrophic cells were used to deprive the specific natural amino acid, which helps allowed efficient UAA incorporation in a controlled manner. A number of analogs of tryptophan, phenylalanine, tyrosine, proline and methionine have been incorporated into proteins with the help of their respective auxotrophs. Recently, a polyauxotrophic cell was used for multi-unnatural amino acid (MUAA) incorporation, in which two or more UAAs were simultaneously incorporated into the protein in a residue-specific manner [5]. These incorporated UAAs are used as probes of protein structure and function; they also expand the function and characteristic features of protein. Currently, analyzing its polyspecificity and developing mutant AARSs with broad substrate specificity for the efficient incorporation of UAAs are interesting targets in the protein-engineering field [6].

Methionyl-tRNA synthetase (MetRS) belongs to the family of class I AARSs that acylate methionyl tRNA with methionine [7]. The *in vivo* and *in vitro* incorporation of 25 methionine analogs into recombinant proteins was carried out using methionine auxotrophic cells and an *in vitro* translation system [8]. Among these methionine analogs, many UAAs have not been incorporated into proteins due to a lack of activity by the endogenous tRNA synthetase. On the other hand, testing the translational activity towards UAA is an expensive, tedious and time-consuming process. Therefore, it would be advantageous to develop an easy and efficient screening technique for identifying the best candidates

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for UAA incorporation. Recently, molecular docking was used as a tool for predicting the most suitable UAA substrate for endogenous tRNA synthetase [9–11]. Molecular docking also aids the development of AARSs with relaxed or altered substrate specificities by extending their activity towards non-reactive substrates [12]. Based on the docking score, docking simulation depicts the geometry of the compound and its binding affinity, which in turn provides information about sterically hindering residues.

This rational designing strategy will greatly enhance the growing number of interesting UAAs for incorporation into proteins. In this study, we carried out a bioinformatics analysis to assess the polyspecificity of wild type MetRS of *Escherichia coli* (*E. coli*) and to identify the crucial residues responsible for the polyspecificity of AARS. We also report the sequential and structural comparison of the catalytic cores of MetRSs belonging to different kingdoms of life and their conformational flexibility after substrate binding. Evaluating the polyspecificity of tRNA synthetase and expanding its substrate specificity by developing a mutant might help to improve the efficient incorporation of UAAs. Therefore, we utilized docking as a tool to analyze and sort the substrate specificity of endogenous MetRSs. Structural insight into the substrate recognition of MetRS and the key residues responsible for interaction and hindrance will help in the rational redesign of its substrate specificity. Based on the docking results, we sorted the substrate specificity of the MetRS with respect to its methionine analogs.

2. Materials and methods

2.1. Active site comparison

The available MetRS crystal structures were retrieved from the Protein Data Bank (PDB); their sequence identities and active sites were compared. The key residues interacting with the co-crystallized substrate (methionine) and its analogs were carefully studied and compared with the active site residues of the MetRSs of other kingdoms. Further, the MetRS protein sequence of all species was retrieved from the National Center for Biotechnology Information (NCBI), and multiple sequence alignment was performed using MEGA5. The methionine-binding residues were compared and analyzed from this multiple sequence alignment.

Later, the conformational changes that occurred in the active site after binding of methionine and its analogs were analyzed by comparison of the closed and open conformations of the MetRS.

2.2. Homology modeling

As the *in vivo* incorporation of methionine analogs is well reported in *E. coli*, *Saccharomyces cerevisiae* and *H. sapiens*, we attempted to carry out docking studies in the MetRSs of these systems [13]. To further expand the study, the protein sequence for wild type MetRS of *S. cerevisiae* was retrieved from the NCBI (GenBank accession number: EDN61849), and the BLASTP algorithm against the PDB was carried out to identify the best template [14]. As we planned to model the protein with its substrate methionine, we chose 1F4L (28% identity) as a template instead of 1RQG (32% identity). Using the advanced modeling technique, the substrate methionine was modeled along with the protein sequence. Before constructing the model, a sequence alignment was generated using ClustalX; this was compared with the results obtained from the align2d tool of MODELLER and manually checked [15]. The overall structural and stereochemical qualities of the protein were assessed using the SAVES server [16]. Finally, the 3D structure was docked with the methionine analogs.

2.3. Preparation of ligands

Three-dimensional structures of methionine and the reported methionine analogs were retrieved from the Pubchem compound database (Fig. 1). Hydrogens and Kollman charges were added to the ligands using the Python Molecular Viewer and exported for the docking study as .mol2 files.

2.4. Molecular docking simulation

An initial docking calculation of methionine was performed for the apo- and holo-conformation of wild type MetRS (PDB IDs: 1PG2 and 1QQT) using GOLD [17–19]. Further docking calculations were performed for methionine and its analogs with the holo-form of the *Ec*MetRS and the *Sc*MetRS. Prior to the docking calculations, all hydrogens and Gasteiger charges were assigned to the 3D structures of the MetRS using the Python Molecular Viewer, and the non-polar hydrogens were added and merged. Prior to the protein preparation, co-crystallized methionine and adenylate were removed from the PDB structure file. The grid size was set to 10 Å centered on the *XYZ* coordinate of the methionine. A Lamarckian genetic algorithm was employed as a search parameter, and for each compound, a 50 GA run was carried out. For each GA run, 25,000,000 (long) evaluations were performed for a population size of 150. The operator weights for crossover, mutation and migration were set as the default parameters. The best-docked conformation was selected based on the interaction (hydrogen bond interaction with Leu13 and Asp52), the orientation in the active site and the docking score (GOLD fitness score). The docked complexes of best ranking solutions were exported in PDB format for further analysis.

2.5. Post docking analysis

The binding mode and interactions between the compounds and protein were analyzed using Pymol [20] and compared with the available methionine co-crystallized structures (PDB ID: 1F4L).

2.6. Virtually generated methionine analogs

To further extend our studies, we retrieved some isostructural methionine analogs from the Pubchem compound database using the Pubchem structure search with the help of the 2D smile of methionine. Amino acid-like isostructural analogs were then chosen for analysis, and we carried out the docking analysis with the *Ec*MetRS as described earlier.

3. Results and discussion

The main goal of this work is to study the polyspecificity of the MetRS towards methionine analogs and to compare the variations in the active site residues of the reported polyspecific MetRSs from *S. cerevisiae* and *E. coli*. This might help in the rational designing of new MetRS with broad substrate specificity, which can facilitate the incorporation of methionine analogs into recombinant proteins.

3.1. Active site of EcMetRS

The active site of the *Ec*MetRS catalyzes the charging of the appropriate tRNA with methionine. The methionine-binding site of the *Ec*MetRS is made up of a hydrophobic pocket enfolded by the residues Ala12, Leu13, Tyr15, Trp253, Ala256, Pro257, Tyr260, Ile297 and His301. Among these, His301 has been reported as contributing to the unambiguous recognition of the sulfur atom in methionine. Trp253 and Tyr15 control the conformational flexibility and help the binding of the methionine substrate [21].

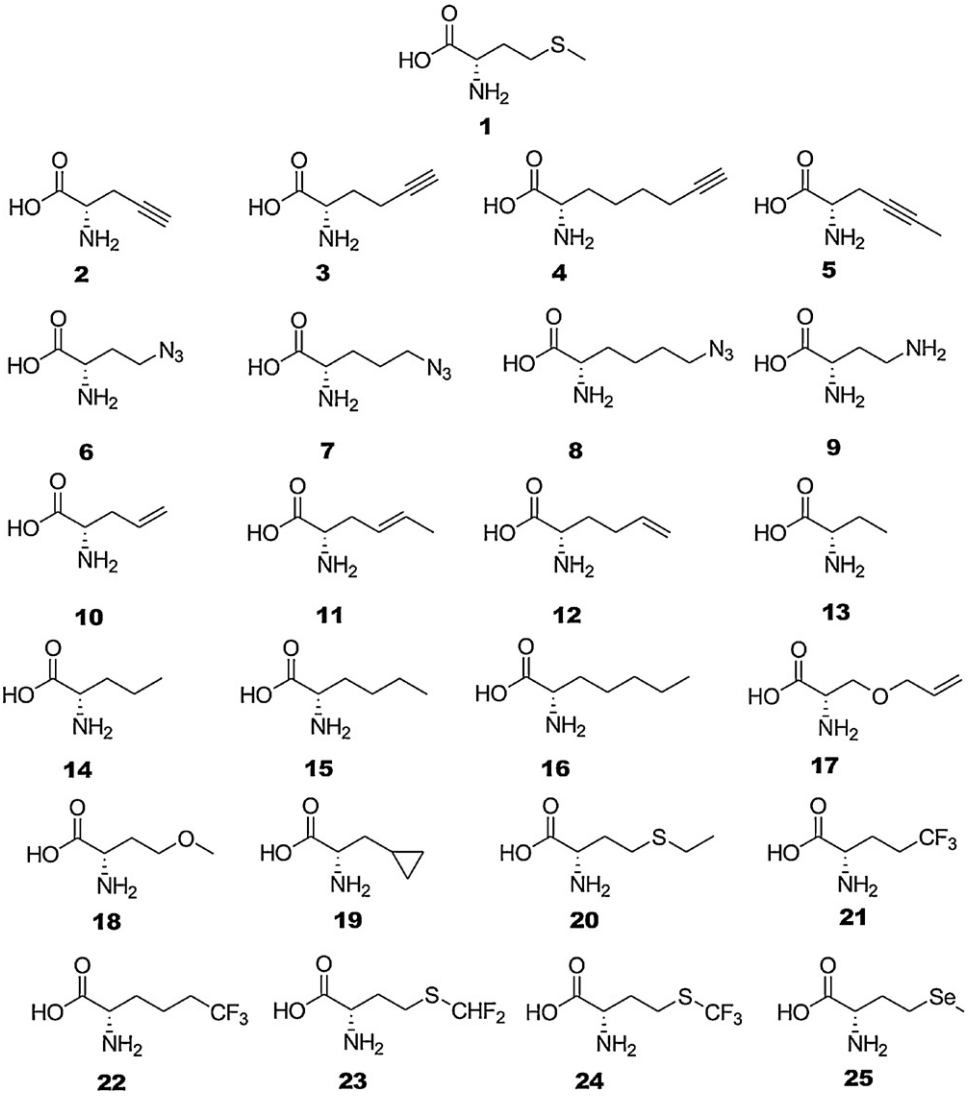

Fig. 1. Chemical structure of methionine and its reported analogs used in the docking study.

3.2. Active site comparison of the different species

We retrieved all available crystal structures of the MetRSs from the PDB, structurally aligned the crystal structures and compared their active site residues (Table 1). Additionally, we also carried out a multiple sequence alignment for the sequences with the *Ec*MetRS and identified the sequence similarities and identities (Supplementary Table 1). To analyze the conserved nature of the active site residues in the MetRSs of different species, we retrieved all of the MetRS sequences from the NCBI database (5686 protein sequences). Due to the variation in their length and amino acid sequences, we were not able to determine multiple sequence alignments for all of the primary structures obtained. Hence, we manually checked the motifs containing methionine-binding residues. As the residues W253 and Y15 control the conformational flexibility of the MetRSs, they are highly conserved in the MetRS sequence of all species. Similarly, Asp52, which is responsible for the recognition of the amino nitrogen of the methionine substrate, is also conserved in the protein sequence of all species. Along with these, Tyr260 is also conserved in the primary structure of all species, and the variations in the active site residues are included in Table 1.

3.3. Conformational changes in the active site

Prior to the docking study, we analyzed the active site of the MetRS that undergoes a conformational change after the binding of methionine. It is reported that the residues Trp253 and Tyr15 control the volume of the methionine-binding pocket and thereby play a part in determining the specificity of the MetRS for methionine. We superimposed and analyzed the open (MetRS) and closed (Met: MetRS) conformation of the *Ec*MetRS. We found that Tyr15 interacts with the amino nitrogen of the methionine substrate by forming a π-cation interaction. On the other hand, Phe300 forms a π–π interaction with Trp253 and in turn helps Trp253 maintain its hydrophobic interaction with methionine, whereas in the case of open (apo) conformation, Tyr15 and Trp253 flip towards the other side of active site (Fig. S1).

The initial study shows that the closed form of MetRS accommodates the methionine substrate well. In contrast, the absence of the docking solution in open form shows that the MetRS was not able to accommodate the substrate methionine. This is due to the conformation versatility of the side chains Tyr15 and Trp253, which maintain hydrophobic interactions with the active site. The change in the orientation of Trp253 and Tyr15 fails to facilitate the

Table 1
Comparison of the active site residues of MetRS of different organisms.

Organism	A12	L13	P14	P257	G259	Y260	I297	H301
E. coli	A	L	P	P	G	Y	I	H
E. coli mutant	A	S	P	P	G	L	I	L
H. sapiens/*S. cerevisiae*	A	L	P	T	G	Y	N	H
Pyrococcus furiosus	A	L	P	P	G	Y	N	H
Methanocaldococcus jannaschii	A	L	A	P	G	Y	I	H
Thermus thermophilus [29]	P	I	Y	L	N	Y	I	H
Mycobacterium smegmatis [30]	P	I	A	L	N	Y	I	H
Variation in the active site	A, P, G	L, I, W, S	P, A, T, S, Y, F	P, L, V, T	G, N, A, E	Y, L	I, N	H, L

active interaction of the methionine in the binding site of the open form of MetRS, whereas the closed form allows the interaction of the methionine by forming hydrogen bond interactions with Asp52 and Leu13. The hydrogen bond interaction of the main chain amino group of substrate methionine with the carbonyl oxygen of Leu13 and side chain oxygen of Asp52 is the key interaction for amino acid recognition.

To analyze the conformation flexibility in the active site for residues other than Trp253 and Tyr15, we compared the crystal structures of MetRS co-crystallized with the substrate methionine and its analogs (difluoromethionine and trifluoromethionine). This analysis showed that the interactions and orientation of residues in the closed form of the active site appear to have no conformation changes when binding methionine analogs.

3.4. Homology modeling of S. cerevisiae

Until now, *E. coli*, *S. cerevisiae* and mammalian systems were reported for the residue-specific incorporation of UAAs. To analyze the polyspecificity of *S. cerevisiae* and mammalian MetRSs, we attempted docking studies for these MetRSs as well. As the docking study with open form showed a different docking solution, we developed a model structure that included the methionine substrate. Based on the active site comparison, it is clear that the amino acids in the active sites of *S. cerevisiae* and *H. sapiens* were identical. Hence, the interaction and geometry of the ligands will be the same for both, that is, the polyspecificity will be same for both. Thus, we modeled only *S. cerevisiae* MetRS and validated this model structure (Figs. S2 and S3).

3.5. Docking methionine analogs with the MetRS

A set of 25 methionine analogs was tested for the translation efficiency of the endogenous MetRS through a docking study. Among them, the bacterial host exhibited translational activity for methionine analogs such as selenomethionine, telluromethionine, azidohomoalanine (AHA), homopropargylglycine (HPG) and norleucine (Nle) [22,23]. To confirm this, docking was carried out with the closed form of MetRS, and the docked complexes were analyzed with the reported crystal structure and compared for both systems. The active site comparison of different species shows that the methionine-binding site is highly conserved in most of the species evaluated (Table 1). Among them, the active site of *S. cerevisiae* varies from *E. coli* by two residues, P257 and I297.

The best docking complexes were screened based on the criteria that the amino nitrogen of the analog formed hydrogen bond interactions with Leu13 and Asp52 and that they had the same geometry of the substrate methionine. From the analysis, the docking result of the *E. coli* MetRS supports the translational activity of the reported analogs; this result was compared with that of the *S. cerevisiae* system. The methionine analogs, selenomethionine and telluormethionine shows better gold fitness score with *Sc*MetRS and *Ec*MetRS. This correlates wells with the experimental data [22,23]. This suggests that these analogs could be substrate for *Sc*MetRS also.

3.5.1. Saturated and unsaturated methionine analogs

The methionine-binding pocket of MetRS is composed of hydrophobic amino acids that closely interact with hydrophobic amino acids. From the docking result, the methionine analogs with shorter side chains (2, 10, 13, and 19) exhibit lower GOLD fitness scores due to fewer energetically favorable interactions with the active site residues (Table 1), suggesting that those candidates as poor substrates for MetRS. Methionine analogs with alkyl chains longer than methionine have a high GOLD fitness score due to the van der Waal's interactions generated through steric hindrance with the active site residues (8, 20, 23 and 24). The presence of an additional methyl group in ethionine (20) compared to methionine provides a good interaction with little steric hindrance in the active site, which helps the efficient recognition of ethionine by MetRS; however, all other analogs (8, 23 and 24) create high steric hindrance in the active site and thereby have a high GOLD fitness score. These compounds are not good substrates for the MetRS. The docked complex of the methionine analogs with the active site of methionyl tRNA synthetase shows that the Tyr260, Leu13 and Ala256 have steric clash with methionine analogs (Fig. S4). By developing point mutants at the residues Tyr260 and Ala256, we can efficiently incorporate these analogs into the recombinant proteins. As the methionine-binding site is located in a hydrophobic pocket, it accommodates the saturated methionine analogs well in the active site. Among them, Nle and HPG show a moderate interaction score for MetRS (Fig. 2).

3.5.2. Fluorinated methionine analogs

The incorporation of fluorinated analogs manipulates the electronic environment of the protein. Its high electronegativity is used for isosteric replacement of hydrogen, which enables it to be used as a biological probe. Fluorinated analogs have been shown to cause a substantial increase in the stability of proteins with respect to thermal and chemical denaturation [24]. It is reported that the analog trifluoronorleucine is not a good substrate for the wild type MetRS because it could not be well accommodated in the active site. However, difluoromethionine and trifluoromethionine are well accommodated in the active site compared to the trifluoro- and difluoro-analogs of norleucine and show the same orientation as that of the reported crystal structure (Fig. 2).

3.5.3. Azido- and amino-containing methionine analogs

Azido-containing analogs are extensively used in protein engineering for developing modified proteins with bioconjugation activity. The azido-containing protein can undergo azido-alkyne cycloaddition and can crosslink with alkyne-containing compounds or other proteins [23]. Earlier, three azido analogs of methionine were analyzed for their incorporation using an *in vitro* approach. Among them, AHA was reactive with the endogenous tRNA synthetase, whereas the azidonorleucine (ANL) and compound 7 were not reactive to the wild type *Ec*MetRS [25]. Tirrell et al.

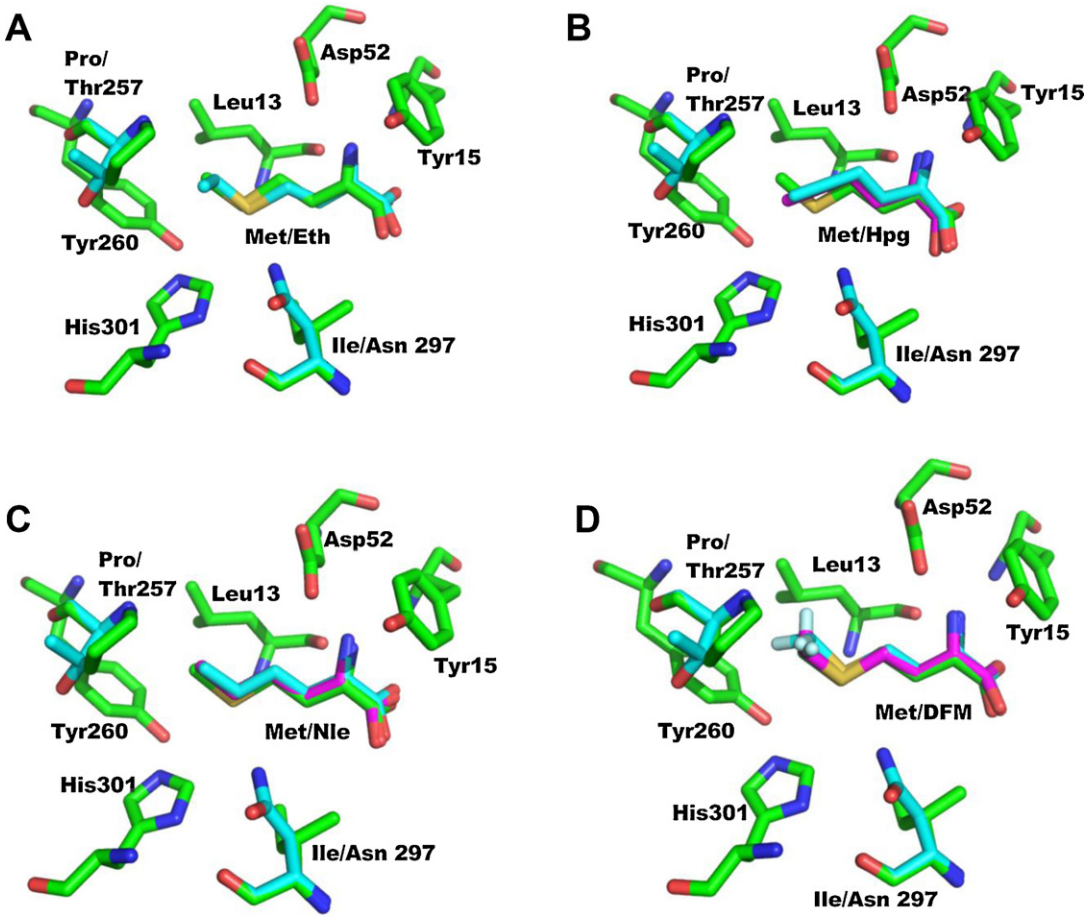

Fig. 2. Top ranked solutions of methionine and their analogs in the active site of the *Ec*MetRS and the *Sc*MetRS. Overlay of docked complexes of methionine analogs of the *Ec*MetRS (magenta) with the *Sc*MetRS (cyan) with the methionine co-crystallized MetRS (PDB ID – 1F4L). Binding modes of (A) ethionine, (B) HPG, (C) Nle and (D) difluoromethionine superimposed with methionine. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and his co-workers modified the MetRS by altering its substrate specificity, thereby enabling the residue-specific incorporation of ANL, which led to the development of a new tool: Bioorthogonal Noncanonical AminoAcid Tagging (BONCAT).

Our docking result supports earlier reports by forming the best GOLD fitness score and docking solution for the best substrates. It also suggests that the azido group of ANL [25] (Fig. S5) creates steric hindrance with His301 and Tyr260, whereas the AHA in the active site results in a good GOLD fitness score (Table 2).

3.5.4. *Virtually generated methionine analogs*

Based on our earlier reports, it is clear that the AARSs are polyspecific for the close structural analogs of their substrates. However, the efficiency of translation depends on the close structural similarity of the analogs with the natural substrates and their accommodation in the active site of AARS. In recent years, some proteins could not accommodate all reported isostructural analogs into their structure, so it is important to develop novel UAAs for different cognate amino acids. Therefore, it is necessary to develop novel methionine analogs with functional groups that are extensively used as biophysical probes in drug delivery systems and bio-orthogonal reactions [26]. Accordingly, we retrieved more methionine analogs from the Pubchem compound database and screened them for *in silico* MetRS-binding activity (Table 3). The goal of this study was to computationally predict good substrates for MetRS. For this purpose, we chose the docking tool as a method for the rapid screening of UAAs for MetRS. If the AARSs are found to be non-reactive to the UAAs, then we can evolve AARS mutants through a rational approach using the available docking information.

The docking study of virtually generated analogs with the *Ec*MetRS suggests the substrate selectivity of the analogs (Table 3). Comparing the GOLD fitness score of the reported analogs with these virtually generated analogs showed that the methionine analogs containing cyanide and free thiol resulted in better GOLD fitness scores than HPG and Nle. From this docking analysis, it is clear that these compounds might also be substrates for the MetRS. On the other hand, boronated amino acids are extensively used in drug delivery systems, and site-specific *in vivo* incorporation of boronated amino acid into proteins has been reported [26,27]. Our docking results show that boronated amino acids have better GOLD fitness scores than HPG, so this analog might also be a better substrate for MetRS and could be incorporated through *in vivo* and *in vitro* approaches. On the other hand, photo-methionine, a diazirine-containing methionine analog, had reduced incorporation into recombinant proteins when evaluated for residue-specific incorporation in mammalian cells; this observation supported our docking studies [28]. Based on the poor gold fitness score, our study suggests that this diazirine-containing analog is a poor substrate for the *Ec*MetRS. The interactions between the novel functional groups containing methionine analogs, including compounds with the MetRS active site, are shown in Fig. 3. Tyrosine 260 and Leu13 interact with the diazirine group of the methionine analog 11855603, and mutating these with Phe and Ala might reduce steric clash and facilitate the incorporation of this analog. The GOLD fitness score directly predicts the binding activity, and an increase in GOLD

Table 2
Docking results of methionine and its isostructural analogs with the *Ec*MetRS and the *Sc*MetRS. The ligands that could not be accommodated in the active site are represented by hyphens. S – substrate; NS – not substrate; PS – poor substrate.

S.No.	Compound name	Pubchem ID	GOLD fitness score			
			*Ec*MetRS		*Sc*MetRS	
1	Methionine	6137	48.04	S	42.87	S
2	Propargylglycine	95575	34.66	NS	–	NS
3	Homopropargylglycine (HPG)	15837543	40.46	S	38.04	S
4	2-Amino-4-azidobutanoic acid	–	40.03	S	39.87	S[a]
5	2-Aminohex-4-ynoic acid	187846	40.90	S	–	NS
6	Azido homoalanine (Aha)	147481	48.02	S	46.00	S[a]
7	2-Amino-5-azidopentanoic acid	11593474	51.03	NS	44.95	S[a]
8	Azidonorleucine (ANL)	16666241	51.00	NS[a]	48.47	NS[a]
9	Diaminobutyric acid	470	–	NS	33.93	NS
10	Allylglycine	14044	35.21	PS/NS	–	NS
11	Cis 2-aminohex-4-enoic acid	168747	39.96	S	–	NS
12	2-Aminohex-5-enoic acid	15664956	40.76	S	–	NS
13	Alpha-aminobutyric acid	6657	31.39	NS	–	NS
14	Norvaline	824	–	NS	–	NS
15	Norleucine(Nle)	9475	41.88	S	37.12	PS
16	2-Azanylheptanoic acid	227939	44.95		36.96	PS
17	2-Amino-3-prop-2-enoxypropanoic acid	22595592	–	NS	–	NS
18	Methoxinine	18713	–	–	–	–
19	Cyclopropylalanine	6951383	37.26	PS	–	NS
20	Ethionine	25674	49.57	S[a]	45.03	S[a]
21	Trifluoronorvaline	253377	–	NS	–	NS
22	Trifluoronorleucine	228893	–	NS	–	NS
23	Difluoromethionine	360816	48.01	S[a]	41.18	S[a]
24	Trifluoromethionine	251951	47.93	S[a]	41.16	S[a]
25	Selenomethionine	–	46.76	S	42.80	S
26	Telluromethionine	–	38.97	S	34.91	S

[a] Represents compounds creating steric hindrance could be accommodated through mutation in the active site.

fitness score suggests the higher substrate selectivity of the compound. From this study, we suggest some substrates for the MetRS, which might help in engineering genetically novel proteins with novel functions.

In summary, we have successfully predicted the polyspecificity of the *Ec*MetRS and the *Sc*MetRS through docking approaches that correlates with the earlier experimental reports. This shows that the wild type tRNA synthetases are polyspecific to the close structural analogs of their cognate amino acids. This helps to encode a broad range of amino acid analogs into recombinant proteins, and this range of analogs can also be expanded through the mutation of active site residues. This polyspecific MetRS might aid in developing a large library of proteins with unusual functional properties. We also carried out a comparative study on the active site residues of the MetRSs in the NCBI database. We identified the conserved nature of the residues and possible amino acid substitutions in the evolution of various species. In this study, we analyzed the substrate specificity of MetRSs towards their methionine analogs. From this study, it is clear that ethionine is the best substrate for both the *Ec*MetRS and the *Sc*MetRS. An initial docking analysis prior to the incorporation of UAAs into nascent proteins might help to provide insight into the incorporation efficiency of UAAs. This study confirms the broad substrate specificity and affinity for 25 methionine analogs. We also showed some novel methionine analogs that can

Table 3
Docking results of methionine and its virtually generated isostructural analogs with the *Ec*MetRS and the *Sc*MetRS. The ligands that could not be accommodated in the active site are represented by hyphens. S – substrate; NS – not substrate; PS – poor substrate.

S. No.	Pubchem ID	GOLD fitness score			
		*Ec*MetRS		*Sc*MetRS	
27	3857	46.59	S	42.63	S
28	11228825	39.33	PS	37.27	S
29	49866509	47.61	S	–	NS
30	53914190	–	NS	–	NS
31	179394	–	NS	–	NS
32	11855603	55.12	NS[a]	48.16	NS[a]
33	1512651	38.83	PS/NS	–	NS
34	10197763	45.15	S	43.14	S[a]
35	440770	39.96	S	–	NS
36	20371845	42.63	S[a]	40.24	S
37	21126383	40.54	S	38.64	S
38	21295102	40.20	S	–	NS
39	55285958	36.96	PS/NS	34.80	PS/NS
40	55301070	41.59	S[a]	–	NS
41	389	–	NS	38.33	S
42	19751625	31.67	NS	–	NS
43	441443	36.42	PS/NS	–	NS
44	91552	40.07	S	37.87	S
45	10419348	45.50	S	–	NS

[a] Represents compounds creating steric hindrance could be accommodated through mutation in the active site.

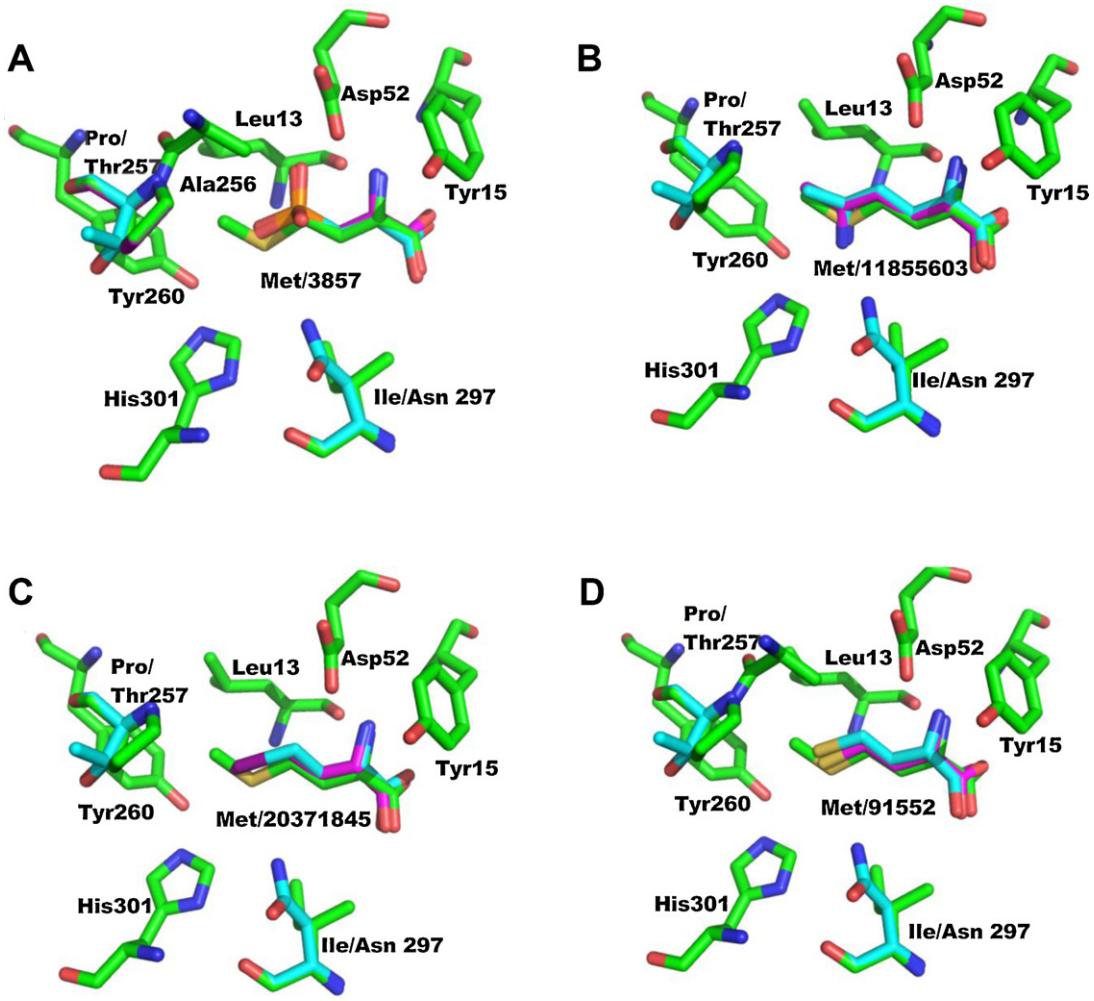

Fig. 3. Top ranked solutions of virtually screened methionine analogs in the active site of the *Ec*MetRS and the *Sc*MetRS. Overlay of docked complexes of methionine analogs of the *Ec*MetRS (magenta) with the *Sc*MetRS (cyan) with the methionine co-crystallized MetRS (PDB ID – 1F4L). Binding modes of (A) 3857, (B) 11855603, (C) 20371845 and (D) 91552 superimposed with methionine.

be used as substrates for endogenous tRNA synthetases. Although the polyspecificity of MetRSs has been reported, mutations in the active site residues will help to extend the broad range of substrates; this can help in the synthesis of large libraries of proteins containing UAAs.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jmgm.2012.11.005.

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