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Research Article

HOMOLOGY MODELING FOR HUMAN ADAM12 USING PRIME, I-TASSER AND EASYMODELLER

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ABSTRACT

Objective: ADAM12 has been implicated in the pathogenesis of various cancers, liver fibrogenesis, hypertension, and asthma, and its elevation or decrease in human serum has been linked to these and other physiological or pathological conditions. Therefore ADAM12 is considered as a significant drug target for various diseases. The experimental 3D structure of ADAM12 is not available. Therefore, present study aims in developing homology model using 3 different softwares and evaluating the best model.

Methods: Homology based 3D model of ADAM12 is constructed using three different softwares namely PRIME, I-Tasser and Easy Modeller. All the predicted models were analyzed and validated by PROCHECK, PROSA, Errat, Verify 3D and Prove.

Results: Homology model predicted from prime showed top results with 84.2% of the residues in the most favorable region, 14.7% in the allowed region, 0.8% in the generously allowed region and 0.3% in the disallowed region. The RMSD between the modeled and the template structure was found to be 0.18 Å. Model developed by prime had the best LGscore of 3.79 and MaxSub of 0.09 indicated that the model is very good. ERRAT, Verify_3D, Prove, ProSA and dDFIRE also confirmed the same.

Conclusion: In this study, homology model was developed for ADAM12 using PRIME, I-Tasser and EasyModeller. The models developed were validated using ERRAT, Verify_3D, Prove, ProSA and dDFIRE. These analyses validated the homology model produced by PRIME is best, robust as well as reliable enough to be used for future study.

Keywords: *ADAM12,* Homology modeling, PRIME, I-Tasser, Easy Modeller

INTRODUCTION

ADAM12 is a member of the greater ADAM family of enzymes: these are multifunctional, generally membrane-bound, zinc proteases. They are about 800 amino acids long and have a unique domain organization, containing pro-metalloprotease, disintegrin, cysteine-rich, transmembrane, and cytoplasmic domains. The ADAMs has structural similarity and 30% sequence identity to snake venom metalloproteases (SVMPs), which cause hemorrhage in snake bite victims. *Yagami-Hiromasa et al* searched for homologs of ADAMs 1 and 2 in a mouse myogenic cell line and identified ADAM 12 (meltrin α)[1]. ADAM 12 showed strong expression in neonatal skeletal muscle and bone. In mouse C2 myoblast cultures, the expression of ADAM 12 became apparent upon muscle cell differentiation [2]. This protein is known also as Meltrin-alpha (approved gene symbol: MLTNA) [3]. ADAM12S and ADAM12L are short and long form, respectively, of the protein and arise by alternative splicing. ADAM12S is a secreted form of the protein consisting of pro, catalytic, disintegrin, cysteine-rich, and EGF domains, whereas the other variant is a membrane form. ADAM12 has been implicated in the pathogenesis of various cancers, liver fibrogenesis, hypertension, and asthma, and its elevation or decrease in human serum has been linked to these and other physiological/pathological conditions [4].

The ADAM12 protein is synthesized as a zymogen in which the prodomain maintains the metalloprotease domain in a latent form. After activation the metalloprotease domain of ADAM12 is catalytically active. ADAM12-S degrades gelatin, collagen type 4, and fibronectin but not collagen type 1 or casein [5]. ADAM12 appears to be involved in myogenesis and to be required for myotube. The protein is expressed largely in mesenchymal cells that give rise to skeletal muscle, bones and visceral organs and may play a role also in osteoblast differentiation and/or function. Significant levels of ADAM12 are expressed in a variety of haematological malignancies, including leukemia, erythroleukemia, lymphoma and myeloma[6]. Roy et al (2004) have purified ADAM12 from the urine of breast cancer patients and reported that increased urinary levels of this protein correlate with breast cancer progression [7]. Hepatocellular carcinomas and liver metastases display higher ADAM-12 than normal liver and benign tumors in liver cancers. ADAM12 expression is associated with tumor aggressiveness and progression [8].

ADAM12 overexpression results in increased tumor take, tumor size, and metastasis in vivo. These findings suggest that ADAM12 may represent a potential therapeutic target in breast cancer [9]. In bladder cancer ADAM-12 levels are unregulated in urine of patients with bladder cancer compared with urine from healthy individuals. After removal of the tumor by surgery, levels of ADAM-12 in urine decrease. ADAM-12 is therefore an interesting biomarker of bladder cancer and hepatic cancer. [10]. ADAM-12 expression is also correlated with tumor aggressiveness and progression, Glioblastoma. Membrane-anchored ADAM-12 is overexpressed in glioblastomas compared to non-neoplastic brain tissues [11].

While a full three-dimensional structure of ADAM12 is not yet available, Wewer et al. have unraveled the gross structural features of the full length ADAM12-S molecule via electron microscopy. The electron microscopic data suggest that the overall protein molecule has a

"Compact clover shaped architecture composed of four globular domains, one of which is the prodomain" [12].

The experimental 3D structure of ADAM12 is not available therefore there is need for the creation of the homology model. Computational approaches can provide homology modeling, which can be further used in molecular dynamic simulations, and automatic docking in order to demonstrate the function of proteins and to illustrate the mode of substrate binding. These types of methods can be used successfully in enzyme–substrate systems and can provide useful information for future studies. The main objective of the present work is to introduce a three-dimensional (3D) model of ADAM12 using 3 different software's namely PRIME (Schrodinger Inc) [13,14], EasyModeller [15,16] and I-Tasser [17,18,19], comparing the results and using the best model developed for future study.

MATERIALS AND METHODS

Homology modeling

Homology modeling refers to constructing an atomic-resolution model of the query (Target) protein from its amino acid

sequence and an experimental three-dimensional structure of a related homologous protein called template protein. The query protein is aligned with the template and the secondary structure is predicted between the two and the model is developed. The primary sequence of the target ADAM12 was obtained from UniProtKB database with a sequence id: O43184, entry name ADA12_HUMAN, sequence length 909aa [20]. Extracellular Topological domain of ADAM12 consists of Disintegrin, EGF like, cysteine switch and cystine rich domain, which is 501 amino acid length, was used for modeling.

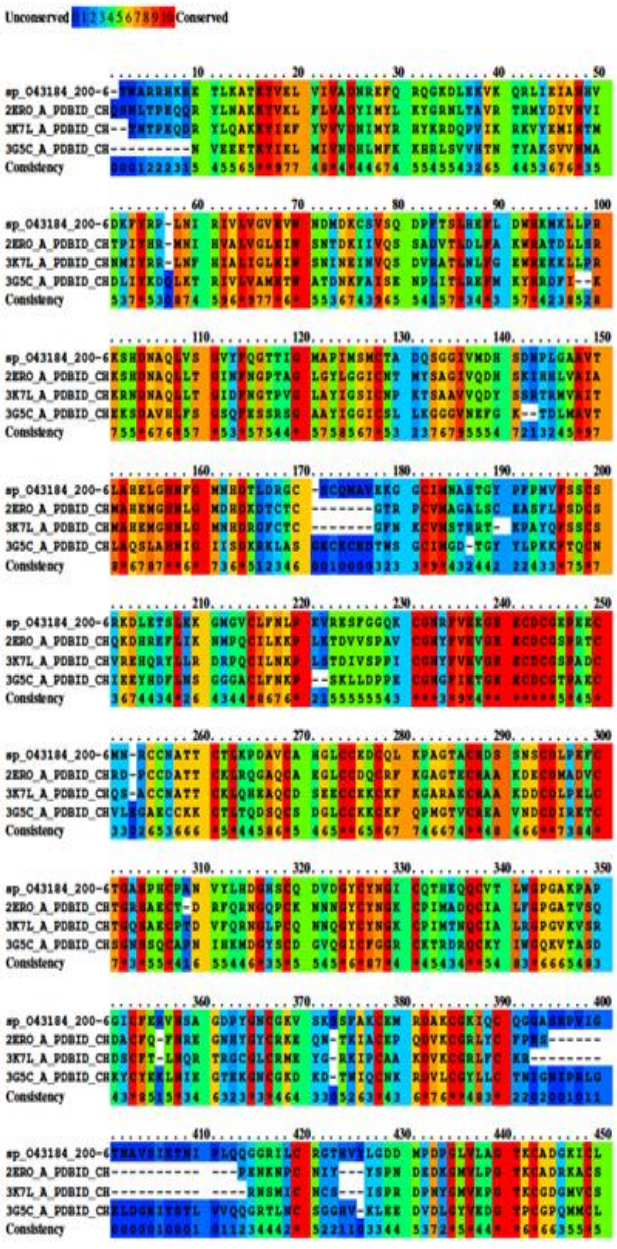

Fig.1: Multiple sequence alignments of the target sequence O43184 with the template sequence (2ERO, 3K7L and 3G5C).

The accuracy of the homology model is related to the degree of sequence identity and similarity between template and target. The selection of a suitable template and an optimal sequence alignment is essential to the success of homology modeling. BLASTp [21] was performed to find a template structure of a known protein from Protein Data Bank (PDB). Template identification was performed using PSI-BLAST [22] to search the non-redundant PDB database. [23] (http://www.rcsb.org/pdb/). The top 6 hits retrieved by the BLASTp program are shown in (Table 1). Multiple sequence alignment of the query and templates were shown in (Fig. 1**)**. The crystal structures of vascular apoptosis-inducing protein-1 (PDB ID:2ERO) and D-domain of snake venom metalloprotease (PDB ID: 3K7L) identified as best with 39% and 38 % sequence identity, therefore both the structures were used as templates to generate the model in Prime. The model was generated using Prime and then the energy was minimized using the OPLS (optimized potentials for liquid simulations) 2005 force-field. The other two softwares used to generate the homology model are I-TASSER and EasyModeller. I-TASSER implements multiple threading algorithms and iterative structure assembly simulations to find optimal sub-fragments within a database structures or within a user-specified structure. EasyModeller is new GUI for Homology modeling using modeller with Tab based logical modeling and extensive error handling steps. EasyModeller allows loading unlimited number of templates with an inbuilt alignment editor. Inbuilt DOPE profile Viewer, Ramachandran Plot viewer, Loop modeling, and basic model optimization makes it user friendly software.

Table 1: Best Hit obtained by PSI-BLAST with the ADAM12 Sequence ID: O43184

Accession	Max score	Total score	Query cover	E value	Identity
2ERO_A	325	325	88%	5.00E-90	39%
3K7L_A	314	314	88%	2.00E-86	38%
3DSL_B	305	305	89%	6.00E-84	39%
2DW0_A	303	303	89%	2.00E-83	38%
3HDB_A	303	303	88%	4.00E-83	39%
3G5C_A	295	295	95%	2.00E-80	36%

Assessment of homology model

The validation of structure model obtained from Prime, I- Tasser and EasyModeller was performed by inspecting the backbone conformation of the modeled structure was calculated by analyzing the phi (φ) and psi (ψ) torsion angles using PROCHECK, as determined by Ramachandran plot. The results were also confirmed using Structural Analysis and Verification Server (SAVES). The ProQ web server [28] (available at Stockholm Bioinformatics Center website: http://www.sbc.su.se/~bjornw/ProQ/ProQ.html) was also used. With ProQ different ranges are given for a model as LGscore>1.5 fairly good model, >2.5 very good model, >4 extremly good model, MaxSub>0.1 fairly good model, >0.5 very good model, >0.8 extremly good model. ERRAT is a protein structure verification algorithm that is especially well-suited for evaluating the progress of crystallographic model building and refinement. The program works by analyzing the statistics of non-bonded interactions between different atom types. This is extremely useful in making decisions about reliability. Verify 3D will provide you with a visual analysis of the quality of a putative crystal structure for a protein and analyzes the compatibility of an atomic model of the protein with its amino acid sequence. Prove Calculates the volumes of atoms in macromolecules. The PROSA test was applied to the final model to check energy criteria against the potential of mean force derived from a large set of known protein structures.

DFIRE is a statistical, potential based program that uses a distance-scaled finite ideal-gas reference state. DFIRE is used to assess non-bonding interactions in the protein model. A lower energy indicates that a model is closer to the native conformation. The root mean square deviation (RMSD) between the main chain atom of the model and the template was calculated by superimposing the structure of the template on the predicted structure of ADAM12 in order to assess the reliability of the model using PyMol.

RESULTS AND DISCUSSION

Homology modeling using PRIME

The model was generated based on the template 2ERO (vascular apoptosis-inducing protein-1) and 3K7L (two elapid snake venom metalloproteases), which has similar structural features with the query protein (Multiple sequence alignment Fig.1.). One advantage of prime over other software's is modeling with the heteroatom. Therefore Metal ions like Zinc and calcium where also used in developing the model. The side chain coordinates

for all non-identical residues were predicted using PRIME. Loop refinement of ADAM12 (6 loops) was carried out and multiple loop conformations were constructed using Prime functionality. Scoring of these conformations was done by side-chain predictions and all-atom minimizations. After completion of model building calculations, the model was further optimized and minimized. The non-template regions were minimized [24]. The final model was energy minimized with a truncated-Newton energy minimization using OPLS_2000 all-atom force field [25] (Fig. 2A.). Every step was checked for improvement in SAVES server and the final model after refinement had the best scores which were used for further validation (Table 2).

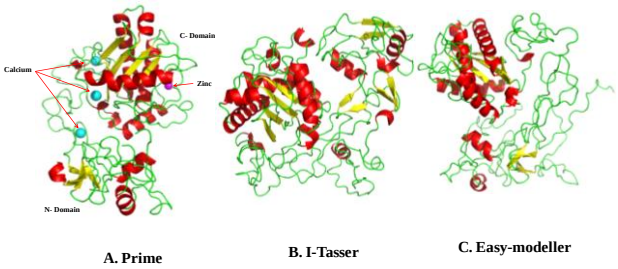

Fig. 2: Ribbon diagram of the modeled ADAM12 with Prime, I-Tasser and EasyModeller, α-Helices, β-strands and loops are colored red, yellow and green, respectively.

Table 2: Comparative values of Procheck, Errat, Verify_3D, Prove in different stages of refinement used in Prime software

Validation		After Modeling	Refine Loop	Minimize	Predict Side Chain
Ramachandran Plot	Allowed	84.5	85.3	84.2	84.2
	Disallowed	0.8	0.3	0.3	0.3
Errat		75.77	77.54	81.65	86.21
Verify_3D		78.26	85.75	85.27	89.61
Prove_z-score		0.834	0.79	0.71	0.71

Table 3: Top Identified structural analogs in PDB Used by I-Tasser to model protein

Rank	PDB Hit	TM-score	RMSD	Identity	Coverage
1	3g5cA	0.949	0.6	0.354	0.954
2	3k7lA	0.616	4.36	0.24	0.758
3	2e3xA	0.603	5.04	0.311	0.79
4	3hdbA	0.595	5.01	0.311	0.776
5	2dw0B	0.589	4.89	0.293	0.762
6	3dslA	0.584	5.05	0.3	0.766
7	2erpB	0.558	4.49	0.276	0.677
8	3k7nA	0.462	2.98	0.255	0.513
9	2eroB1	0.402	2.02	0.368	0.423

Homology modeling using I-TASSER

In this method the target sequences are first threaded using a representative PDB structure library to search for the possible folds by Profile- Profile Alignment (PPA), Hidden Markov Model, PSI-BLAST profiles, Needleman-Wunch and Smith-Waterman alignment algorithms. The top 10 alignments are from the following threading programs MUSTER, dPPAS, Neff-PPAS, PPAS, wdPPAS, SPARKS-X, SP3, HHSEARCH2, PROSPECT2, FFAS03. The PDB ID: 3G5CA had the best Z-score using all the ten algorithms and was used for modeling ADAM12 structure **(Table 3)**. I-TASSER server predicted 5 models from which the model with best C-Score of 1.42 was selected with estimated accuracy of 0.91(TM-Score) and 4.3Å (RMSD) **(Figure. 2B)**. C-score is a confidence score for estimating the quality of predicted models by I-TASSER. It is calculated based on the

significance of threading template alignments and the convergence parameters of the structure assembly simulations.

Homology modeling using EasyModeller

The sequences of target and template (3K7L) were aligned using the align module of EasyModeller. EasyModeller uses model building module of Modeller to build the 3D model. The best model of target was selected on the basis of the internal scoring functions, dope score of Modeller, and Procheck procedure (Laskowski et al., 1993). Then, the chosen model was subjected to energy minimization. The quality of the final model was validated using different validation software's. Figure 2C shows the modeled protein using EasyModeller.

Model validation

Validation of the model including the geometric properties of the backbone conformations, were analyzed using various structure evaluation programs. Ramachandran plot calculations were calculated with PROCHECK program. Ramachandran plot of the three models was shown in Figure. 3. Model from prime indicated that 84.2% of the residues in the most favorable region, 14.7% in the allowed region, 0.8% in the generously allowed region and 0.3% in the disallowed region (Fig. 3A).

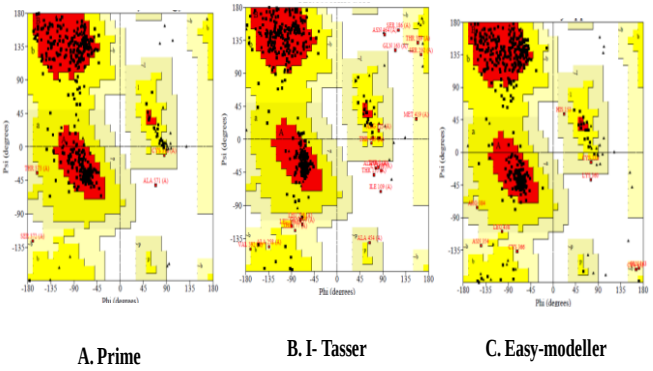

Fig. 3: Ramachandran Plot for the modeled ADAM12 after refinement. The red, yellow and white regions represent the favoured, allowed and the disallowed regions respectively.

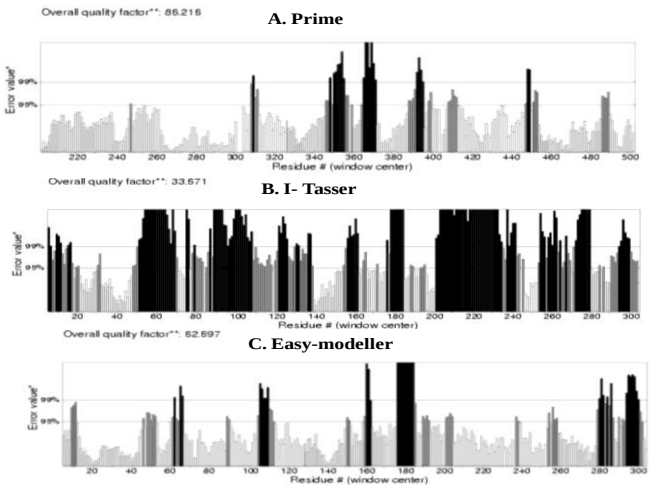

Fig. 4: ERRAT plot of ADAM12 modelled by (a) Prime, (b) I-Tasser, (C)EasyModeller and Overall quality factor or ERRAT score

These results revealed that the majority of the amino acids are in a phi-psi distribution that is consistent with a right-handed α-helix,

and the model is reliable and of good quality. Whereas the other two models did not have such best scores compared with prime (Fig. 3B & 3C). Model developed by prime had ProQ LGscore of 3.79 and MaxSub of 0.09 indicated that the model developed by prime is was very good whereas other two model come in the criteria of fairly

good model with LGscore of 2. ERRAT (Figure. 4), Verify_3D, Prove, ProSA (Fig. 5 and 6), and dDFIRE showed that model developed by Prime was best compared to I-Tasser and Easy model. RMSD between the template and model developed by prime was 0.18 Å (Fig. 7.A) whereas with I-Tasser and Easy model it was 0.46 Å and 1.18 Å (Fig. 7B and 7C). All these results suggest that the model developed by prime is comparatively robust and can be used in subsequent stages of analysis (Table 4). Therefore, the PROCHECK, ERRAT Verify_3D, Prove, ProSA results confirm the quality of predicted 3D structure as more reliable and within an acceptable range.

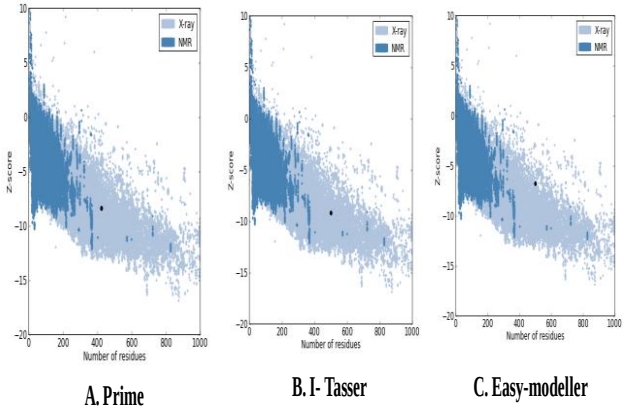

Fig. 5: ProSA-web Z-scores of ADAM12 model (black Spot) in relation to all protein chains in PDB determined by X-ray crystallography (light blue) or NMR spectroscopy (dark blue) with respect to their length

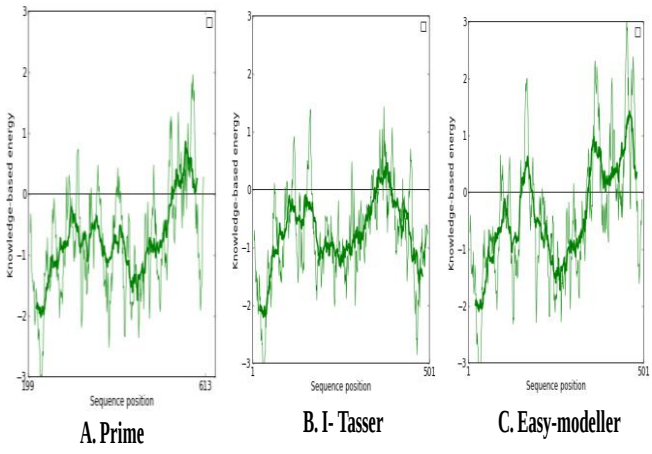

Fig. 6: Residue energy plots of ADAM12 from Prime, I-Tasser and EasyModeller

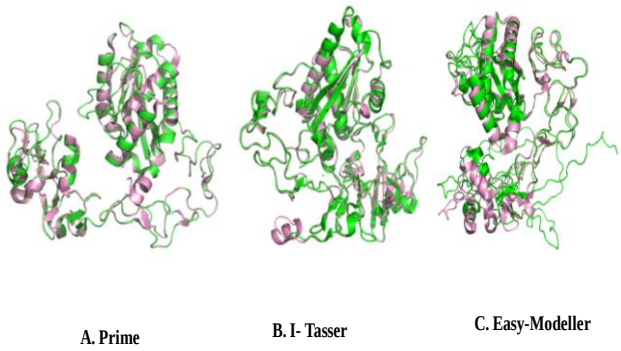

Fig. 7: Superimposition of template (pink) and model protein (green) from with Prime, I-Tasser and EasyModeller.

Table 4: Comparative values of Procheck, ProQ, Errat, Verify_3D, Prove, ProSA Z-scores and dDFIRE, with the RMSD between the Template and Modelled protein of all the Three models.

Validation		Prime	I- Tasser	EasyModeller
Procheck Ramachandran Plot	Allowed	84.2	83.2	84.2
	Additionally allowed	14.7	12.1	13.7
	Generously Allowed	0.8	2.8	1.9
	Disallowed region	0.3	1.9	0.2
ProQ	Predicted LGscore	3.79	2.11	2.07
	Predicted MaxSub	0.09	0.24	0.01
Errat		86.21	33.67	62.69
Verify_3D		89.61	77.69	71.5
Prove_z-score		0.71	0.46	1.03
ProSA Z-score		-8.4	-9.19	-6.83
dDFIRE		-805.53	-845.48	-852.87
RMSD		0.18	0.46	1.18

CONCLUSION

ADAM12 has been implicated in the pathogenesis of various cancers, liver fibrogenesis, hypertension, and asthma, and its elevation or decrease in human serum has been linked to these and other physiological/pathological conditions. Therefore ADAM12 is considered as a significant drug target for various diseases. In the present work, a homology based 3D model of ADAM12 is constructed using three different softwares namely PRIME, I-Tasser and EasyModeller software.

The best models produced by all software's were further assessed by Procheck, ProQ, Errat, Verify_3D, Prove and dDFIRE. Based on the results it can be suggested that, PRIME software produced satisfactory Ramachandran plot statistics, Errat plot quality factor. Moreover, the online validation server (ProSA web) showed that the Z-score and energy of protein folding of the models was in good agreement with the available protein structures in PDB, which favored the overall quality of the structures. These analyses validated the homology model produced by PRIME is robust as well as reliable enough to be used for Drug Discovery.

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