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IMPROVING PROTEIN STRUCTURE PREDICTION, REFINEMENT AND QUALITY ASSESSMENT TECHNIQUES

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Abstract: Several novel techniques have been combined to improve protein structure prediction, structural refinement and quality assessment of protein models. We discuss in brief the development of four-body potentials that take into account dense packing and cooperativity of interactions of proteins, and its success. We have developed a method that uses whole protein information filtered through machine learning to score protein models based on their likeness to native structure. Here we consider electrostatic interactions and residue depth, and use these for structure prediction. These potentials were tested to be successful in CASP9 and CASP10. We have also developed a Quality Assessment technique, MQAPsingle, which is a quasi-single-model MQAP, by combining advantages of both "pure" single-model MQAPs and clustering MQAPs. This technique can be used in ranking and assessing the absolute global quality of single protein models. This model (Pawlowski-Kloczkowski) was ranked 3rd in Model Quality Assessment in CASP10. Consideration of protein flexibility and its fluctuation dynamics improves protein structure prediction and leads to better refinement of computational models of proteins. Here we also discuss how Anisotropic Network Model (ANM) of protein fluctuation dynamics and Go-like model of energy score can be used for novel protein structure refinement.

Keywords: protein structure prediction, model quality assessment, structure refinement

1. Introduction

Recent progress in the mass-scale sequencing projects has produced enormous numbers of protein sequences, for which crystallographic structures have not

yet been determined. Additionally, despite the huge investments in high throughput protein crystallography and the important efforts of Protein Structure Initiative (PSI) Centers, the gap between the number of experimentally solved protein structures, and the number of known sequences continues to widen. The knowledge of protein structure is critical to comprehend their function, for understanding of molecular mechanisms of disease, and for development of new generations of medicines based on the computer-aided drug design. Therefore, there is an urgent need to improve the existing computational methods of structure prediction to reach ultimately the accuracy comparable to crystallographic or NMR structure determination resolution. Another extremely important aspect of the improved structure prediction is computational design of completely new proteins with desired properties that haven't been yet created naturally by evolution. In the last decade we have witnessed the rise of synthetic biology, including *de novo* design of proteins that were first theoretically conceived, and then synthesized. Possible improvement of this groundbreaking methodology might have a transformative effect on protein science, biomedicine and engineering in the 21st century. Computational protein structure prediction and design usually lead not only to a single model, but to many alternative models corresponding to local, nonnative energy minima. Thus it becomes critical to develop potentials, scoring functions, model quality assessment and refinement programs that may identify the structural model that is the closest to the native state, and successfully refine it.

Statistical potentials or knowledge based potentials are getting more popular compared to physics based methods. Statistical potentials relay on structural, biological and evolutionary information from structures of experimentally solved proteins deposited in Protein Data Bank (PDB). The "physical" energy functions expresses our current (limited) understanding of the forces guiding the protein folding process. However it has been shown that "statistical" potentials are more successful and useful in CASP [1–6]. The physical energy functions, both atomic-detailed and coarse-grained remain impractical for template-free modeling of proteins, or for models with remote homology because of the inability to reproduce the funnel-like shape of the energy function. On the other hand, statistical potentials have been shown to model the funnel-shape energy landscape for a much larger (and practically useful) range of models. Most of potentials currently used in protein modeling are pair-wise. The one most widely used in the assessment of protein models are the Miyazawa-Jernigan potentials [7–16]. It has been demonstrated that pair-wise potentials are insufficient as a tool for accurate modeling [17]. As an alternative, multi-body potentials are able to take into account more complex three dimensional interactions that have significant contribution to energy in the densely packed protein core. Importantly they can capture the strong cooperativity operative within protein structures [18, 19], and were demonstrated to perform better than two-body potentials. The four-body contact potentials developed by us [20] incorporated sequence information with levels of solvent accessibility of the residues and details of the interactions between

backbones and side chains through a simple geometric construction. We have improved these potentials by combining the four-body sequential [20] with the four-body non-sequential potentials [21] and with short range potentials [22]. We used this optimized potential [23] in the identification of protein native structure, and tested them with a great success in 2010 in CASP9.

An extremely important problem for protein structure prediction is the development of better potentials. For models that are several Angstroms away from the native state, atomic potentials (force-fields) are completely useless and we must rely on coarse-grained potentials. There is a need to develop potentials that could scale continuously from the coarse-grained to the atomic as we approach the native state during the process of refinement of structural models. Additionally almost all potentials used in protein structure prediction are for pairs of interacting points. In our opinion, because of the high cooperativity of interactions of densely packed residues in protein structures, a much better approach is the use of multibody potentials. In 2007 we proposed four body potentials [20] that improve the discrimination of the native structure among decoys. Since four points give the simplest representation of packing in 3D space, our four body potentials reflect the nature of dense packing of residues in protein cores. Recently we have significantly improved these potentials (4B OPT POT) [23] by combining two types of four body potentials (sequential and non-sequential ones) with pair-wise interactions and by optimizing weights of each term using Particle Swarm Optimization [24] (see Table 1).

Table 1. Threading results for optimized 4-body potentials vs. other potentials for modeled targets see [23]; Pearson Correlation Coefficient, Z-score and RMSD of the top ranked models are shown. Both RMSD for template-based and template-fee is lowest for the optimized four-body potentials (4B OPT POT)

Potential	Template-based			Template-free		
	Pearson ρ	Z-score	RMSD	Pearson ρ	Z-score	RMSD
4B OPT POT	0.4	1.33	3.7	0.17	1.3	7.5
BT	0.49	1.5	4.1	0.16	2.14	7.7
4B POT	0.38	1.29	4.6	0.12	2.02	8.4
SKJG	0.43	1.41	4.6	0.14	1.2	9.1
MJ3	0.4	1.29	4.6	0.13	1.98	9.2
VD	0.43	1.4	4.6	0.14	1.7	9.3

We had the opportunity in 2010 to test our approach, and prove their improvements by comparing them with other modeling approaches at CASP9. We have participated in CASP9 as the prediction group 4_BODY_POTENTIALS. According to Nick Grishin, who was the assessor of free modeling techniques at CASP9, 4_BODY_POTENTIALS was one of few most successful groups in free modeling (see Figure 1) despite the fact that we submitted only predictions for 20 FM targets (out of 26 total). Free modeling is the most difficult and most

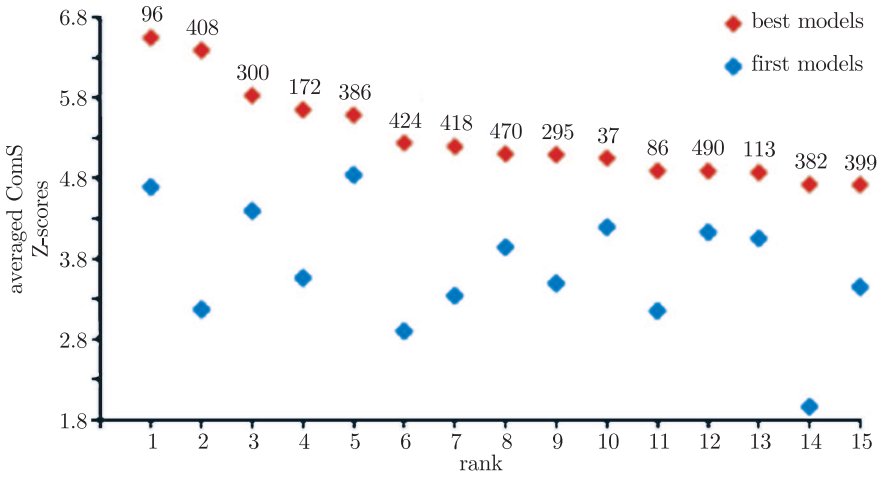

Figure 1. Ranking group performance in free modeling at CASP9. Our 4_BODY_POTENTIALS (no 300) was ranked 3rd both for best models (red) after Zhang (96) and Keasar (408), and for first models (blue) after Dong Xu (MUFOLD, no 386) and Zhang (96) [Courtesy of Nick Grishin]

challenging problem in protein structure prediction, when the sequence of the protein has only a low sequence identity. We obtained predictions at CASP9 from several servers that performed well in CASP8. These servers include Zhang, Baker, Raptor, HHPred, Tasser, Pcons and SAM servers. All the predictions from each of these servers were taken, which amounts to 30 structure predictions in total. Optimized four body potentials were applied to each of these structures and the minimum energy structure was identified as the best fit to the native. However, because of highly coarse-grained nature of these potentials they performed very well for template-free modeling, but their performance in template-based modeling in CASP9 was less satisfactory. Since electromagnetic (EM) interactions are the most important physical forces for biological processes (gravitational, strong and weak nuclear interactions are irrelevant) the detailed consideration of these interactions is of critical importance for successful protein structure prediction. Here we propose a completely new approach to deal with these interactions. All "physical" force-fields contain Coulombic terms (that describe very well also hydrogen bonding) by using predetermined partial atomic charges. The partial atomic charges are either estimated from various experiments (including dipole moment measurements) or from quantum mechanics computations of the electron density for individual amino acids or small peptides. Here we propose a completely different approach with partial atomic charges being parameters that are optimized for the recognition of the native structure among decoys. The hydrophobic interactions that also have EM nature are included in this scheme (24). The combination of the multibody coarse-grained packing potentials with detailed electrostatic interaction has been tested in 2012 in CASP10 and led to excellent results both for template-based modeling and template-free modeling.

Table 2. Ranking of performance of participating groups in prediction of top model in CASP10, for all targets (upper Table), and for template-free modeled targets (lower Table); only top 10 groups (out of 150 total) are shown; from http://predictioncenter.org/casp10/groups_analysis.cgi

#	GR #	GR name	Domains count	SUM Z-score (GDT_TS)	AVG Z-score (GDT_TS)	AVG GDT_TS	No. models ranked 1	No. models in Top 3	No. models in Top10
1.	237	zhang	71	71.293	1.004	49.046	3	8	31
2.	035	s Zhang-Server	71	63.544	0.895	47.825	2	5	21
3.	350	Kloczkowski_Lab	71	61.772	0.870	46.157	5	12	25
4.	489	MULTICOM	71	59.969	0.845	45.966	4	6	15
5.	130	Pcomb	71	59.638	0.840	46.665	3	3	17
6.	267	Pcons	70	58.432	0.835	46.336	2	3	19
7.	114	s QUARK	71	58.076	0.818	47.101	1	6	16
8.	388	ProQ2	71	57.813	0.814	43.943	3	4	18
9.	079	TASSER	71	57.283	0.807	47.248	2	6	21
10.	475	CNIO	71	56.550	0.796	47.049	3	4	16

#	GR #	GR name	Domains count	SUM Z-score (GDT_TS)	AVG Z-score (GDT_TS)	AVG GDT_TS	No. models ranked 1	No. models in Top 3	No. models in Top10
1.	388	ProQ2	14	20.200	1.443	24.421	1	2	9
2.	350	Kloczkowski_Lab	14	16.890	1.206	23.091	1	5	7
3.	315	keasar	13	15.848	1.219	24.366	3	4	5
4.	237	zhang	14	15.411	1.101	23.536	0	1	5
5.	035	s Zhang-Server	14	14.655	1.047	23.100	1	2	5
6.	130	Pcomb	14	13.169	0.941	22.199	1	1	5
7.	294	chuo-repack	14	12.538	0.896	22.402	1	2	4
8.	172	Zhang-IRU	10	12.372	1.237	23.622	1	1	4
9.	114	s QUARK	14	12.279	0.877	22.544	0	2	4
10.	267	Pcons	14	10.582	0.756	21.272	1	1	6

Our prediction group was officially ranked as 3rd (for the top model prediction) for all targets, and 2nd for hard (freely-modeled) targets. See Table 2.

Hence, the proposed combination of coarse-grained potentials that include multi-body packing with detailed electrostatic interactions is the most promising approach to support protein structure prediction methods of the future.

Going further we then combined these multibody potentials with entropies from elastic network models in order to obtain free energies of structures. We have shown that these free energy changes based models improve coarse-grained modeling of protein structure and dynamics [25]. Using these free energy based models we were able to show enhanced selection of native like structures for CASP9 decoys. We were also able to pick the best docking poses from high quality docking poses [26]. The major problem in high-accuracy protein structure modeling based on templates with remote homology is the discrimination of model accuracy. The most successful Model Quality Assessment Programs (MQAPs) according to CASP are those that compare different models (constructed with >100 different programs) with each other and identify the consensus. However, consensus-based prediction is possible only within CASP, since running >100 different methods to collect alternative models is impractical for many reasons, including the unavailability of most CASP methods outside the CASP experiment. Nonetheless, there exist methods that do not use a consensus or utilize only a handful of models, and they have been demonstrated to perform reasonably well in CASP. It has been clearly shown in CASP9 that protein model refinement is an extremely difficult problem in protein structure prediction, and among all participants in this prediction category only two groups were able to improve (and only slightly) the initial starting models provided by protein structure prediction servers, while all other participants only degraded these predictions, mostly because of the inability to discriminate between parts of the model that are "essentially right" and those that need further refinement, and the inability to predict the appropriate direction of modification that would bring the model toward the native structure. Recently a significant progress in structure refinement was achieved by Michael Feig who developed physics-based protein structure refinement through multiple molecular dynamics trajectories and structure averaging using structural constraints [27]. In this paper we present some newly developed Model Quality Assessment techniques as well as structure refinement techniques.

2. Methods

2.1. Protein structure refinement

Getting biologically relevant structural models of proteins is a problem involving several resolution scales. Usually we start the modeling from a coarse-grained model and try to refine it by using more atomic details. For some biological interactions a detailed picture of both the static and dynamic nature of the proteins may be necessary. For this case one would need to refine a model

structure, and select proper candidates from the ensemble of structures generated. For these purposes specifically designed scoring function could be advantageous for a general use. Our goal is to modify our Seder methodology to apply it for the structural refinement.

By construction Seder is a modular algorithm and hence can be modified with relative ease. Simple ways of improving Seder would be to increase the size of the training database to include recently resolved structures and unique decoy models. Since Seder learns from both native and non-native structures, it is uniquely positioned to perform such a task. Another possible improvement for Seder can come by increasing the types of inputs. This can be done by partitioning descriptive features based on the distance involved in their interaction and other unique descriptors. Additionally, higher order expansion of the underlying electrostatic sums that make up Seder can be employed. At its current form, Seder sums information of the first and the third inverse power of distance. These account for interactions involving bare charges and dipole moments. One can expand this description to include higher order moments, capturing the finer scale of interactions.

Thermal motions of atoms in the protein native state, *i.e.* the fluctuations about the minimum of the global free energy, are well reproduced by the simple elastic network models (ENMs) such as the anisotropic network model (ANM). Elastic network models represent protein dynamics as vibrations of a network of nodes in which the spatially close nodes are connected by harmonic springs. These models provide a reliable representation of the fluctuational dynamics of proteins and RNA, and explain various conformational changes in protein structures including those important for ligand binding. A study done by our group presented results where we discuss how elastic network models provides the basis for protein structure refinement [28].

We have developed a novel protein structure refinement procedure based on Anisotropic Network Model (ANM) of protein fluctuational dynamics and Go-like model of energy score. The starting structures were models from past CASP experiments. We changed positions of C-alpha atoms using ANM, creating a new set of 250 structures from the initial model and computed energies of these structures using Go-like energy score. The top 6 coarse-grained structures were fully rebuilt with BBQ and Scrwl4. To remove bond stretches and the excluded volume clashes, short molecular dynamics simulations (up to 10000 steps) were performed with OPLS-AA force field and implicit solvent GBSA-OBC. There was an improvement of RMSD of each of the 6 structures after 50 iterations. For each step, the structures from the last iteration (5 decoys) were taken (except for the first step where only 1 structure is available). Based on these, NM (normal modes) are calculated for every decoy, and 250 new decoys are generated (with the average deviation from the original decoy 1 Å). Then 5 of the best structures are chosen for

further processing. The top 5 decoys are chosen based on Go-like energy functions which are described in the following relationship.

$$E = \sum_{\langle i,j \rangle} E(d^*_{ij})$$
$$E(d^*_{ij}) = \frac{10}{1+\exp\big(5(d^*+1)\big)} + \frac{1}{1+\exp\big(3(-d^*+1)\big)} - 1.0 \tag{1}$$

where $d^* = r_{ij} - r^0_{ij} + 1$.
r_{ij} is an instant distance between i^{th} and j^{th} particles and r^0_{ij} is a distance between i^{th} and j^{th} particles in the native structure.

2.2. Quality assessment techniques

Recently we have developed a method, called MQAPsingle, for ranking and assessing the absolute global quality of single protein models. The current version of MQAPsingle submits the target sequence to the GeneSilico Fold prediction metaserver [29] to collect approximately one hundred of 3D models for the target protein, These models are later used as reference models.

In parallel, the MQAPsingle executes the following three modules. First module predicts secondary sequence, solvent accessibility and contact maps for the target sequence using third-party methods. These predictions are compared with values of the corresponding features calculated directly from the 3D structural models by the DSSP program [30]. These (dis)agreement terms, together with in-house implementation of the DFIRE [31] statistical potential and the number of unsatisfied hydrogen bond donors/acceptors, are used to estimate GDT_TS score [32] of each of the input and reference models. The second module calculates the all possible pairwise comparisons between the input models and the reference models *only*. Two measures of similarity between a pair of models are applied: GDT_TS and Q-score [33, 34], the latter measures the structural similarity between two models by comparing their internal residue distances.

Then, 3D-Jury algorithm [35] is applied to calculate the consensus scores of the input model(s). The third module is based on an assumption that values of "pure" single-model scoring function, on average, decrease as models become more similar to the native structure. Thus, the model that is the closest to the native structure should provide the highest correlation coefficient of a score (provided by such a single-model MQAP) versus distance, when used as the reference in pairwise comparisons with the remaining models [36]. MQAPsingle applies this idea by comparing the input model(s) to the reference models, and then calculating such a correlation coefficient for the input model(s), and then MQAPsingle uses the correlation coefficient as an additional score of the model(s) correctness.Our method uses two sets of such Pearson's correlation coefficients between the single-model based scores provided by the first module and either GDT_TS or Q-score. Finally, to predict the GDT_TS score of the input model(s), the primary scores provided by the above-mentioned three modules and a linear regression algorithm are used.

3. Results and discssion

3.1. Protein structure refinement

We have tested Seder modular algorithm and modifications on the CASP10 hard target T0624. We began by unfolding the protein from its native structure generating 10,000 structures this way. These structures were then used to train a neural network to predict the TM-score to native of a given model. We call this version Custom Seder. In Figure 2 we give the results. DFire 2.0 is a popular knowledge based energy function DFIRE2 [31, 37]. One should note that DFire2 ranks lower energy as more fitting to native and hence the significant positive correlation is misleading, as actually a negative correlation should be observed if DFire2 was useful for this target. Seder [38] is a knowledge based scoring function. It shows some overall correct correlation albeit small. Custom Seder on the other hand shows a significant positive correlation.

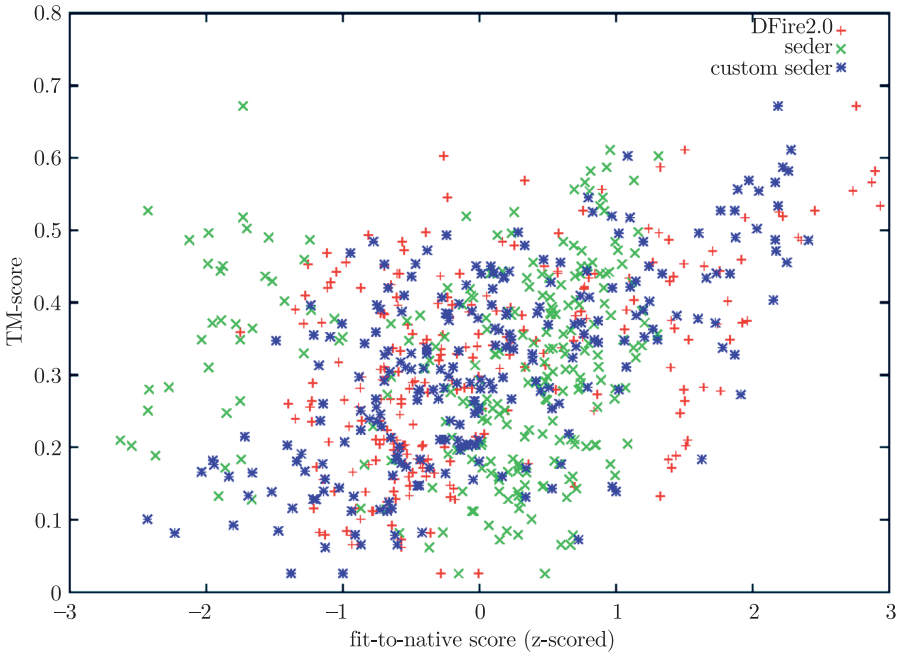

Figure 2. TM-Score as a function of Fit-to-native score; red, green, and blue points correspond to correlations with DFire 2.0 (corr = 0.41), seder (0.10) and custom seder (corr = 0.60) developed for our refinement purposes, respectively

We tried to apply elastic network models to refine structural models using methodology described in [28]. Table 3 shows, for each structure the length, starting RMSD and the RMSD after 50 iterations. dRMSD is also shown. The whole structural refinement process was performed iteratively leading to the improvement of average RMSD from 3.8 Å to 2.6 Å in 50 iterations. Figure 3 shows the RMSD of the models (TR464, TR469, TR517, TR530, TR594, and TR624) with the iteration number. The whole structural refinement process was performed

Table 3. For the 6 structures used, RMSD of the starting structure and the RMSD of the structure after 50 iterations are shown; length and dRMSD values are also shown; it can be seen that for all of the 6 structures RMSD has improved after 50 iterations

Name	length	RMSD(0)	RMSD(50)	dRMSD
TR464	69	3.032	2.06	−0.972
TR469	63	2.181	1.321	−0.86
TR517	159	4.645	4.016	−0.629
TR530	80	1.987	1.332	−0.655
TR594	140	1.817	1.726	−0.091
TR624	69	5.202	2.664	−2.538

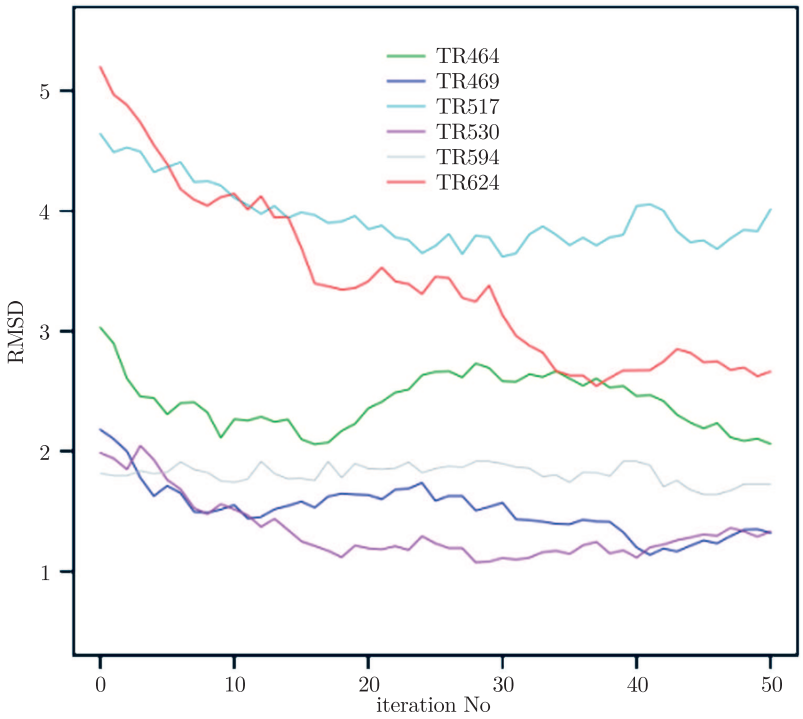

Figure 3. Figure shows the RMSD of the models (TR464, TR469, TR517, TR530, TR594 and TR624) with the iteration number up to 50 iterations; Tte whole structural refinement process was performed iteratively leading to the improvement of average RMSD from 3.8 Å to 2.6 Å in 50 iterations

iteratively leading to the improvement of average RMSD from 3.8 Å to 2.6 Å in 50 iterations. It is clear from this figure the decrease of RMSD with each iteration performed.

3.2. Quality assessment techniques

The current version of MQAPsingle was tested in the latest CASP10 experiment. According to the benchmark published by CASP10 assessors [39],

MQAPsingle was among the best performing MQAPs for ranking models for a given target according to their similarity to the native structure (Figure 4). This approach results in higher accuracy compared to the state-of-the-art single-model MQAPs. Notably, the prediction for a given model is the same regardless if this model is submitted to our server alone or together with other models.

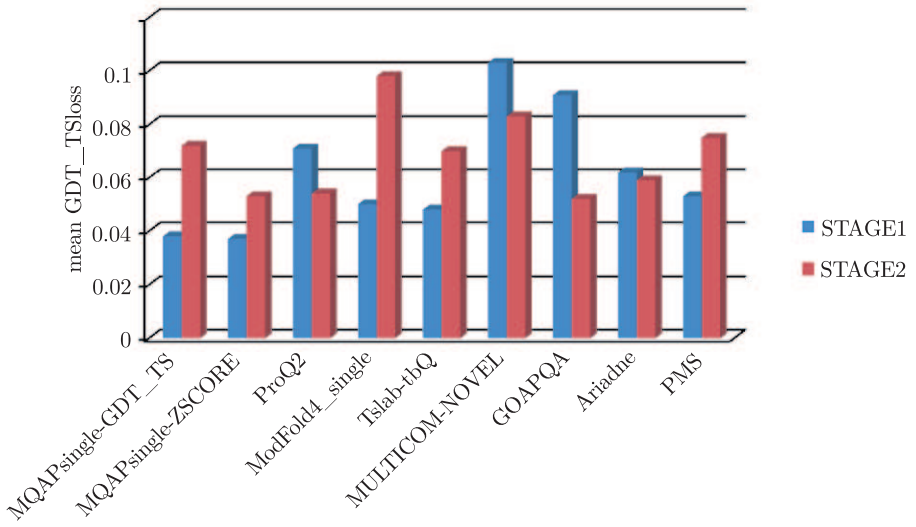

Figure 4. The "best selector" performance of MQAPsingle and the best performing non-clustering QA predictors in CASP10; the average difference in qualities of the top scored model by a QA predictor and the model with the highest GDT_TS score per target, which implies that the better QA selectors of the most accurate models are expected to have lower values of the GDT_TS-loss parameter; the data are presented both STAGE1 and STAGE2 of the CASP10 experiment for single-domain targets

There is still more space for improvements to obtain more accurate MQAP, compared to MQAPsingle in the future, for assessing the quality of a single model It is important to analyze the performance of the all afore-mentioned QA metrics in the context of modelling difficulty. This approach will be a key component in creating specialized MQAPs to use them for free modeling, template-based modeling, or protein refinement. The results of this analysis will be valuable in terms of helping us understand why and how some model features make it very difficult/easy to accurately predict the model quality. We believe that in contrast to clustering MQAPs, MQAPsingle reflects the real life needs of those who want to predict protein structure by using a few protein structure predicting servers, and then choose the model ranked the highest according to a model quality assessment program. We would strongly recommend MQAPsingle (GDT_TS Z-score) for those who would like to select the best models from ensemble of models, regardless their quality. Our main motivation to develop MQAPsingle was the need of a clear distinction between the quality assessment of internal domain structures and the mutual orientation of domains in multi-domain proteins. Nevertheless, we believe

that our benchmark is complementary to that of CASP10 and that the quality assessment of individual domains could be used as a separate step in the structure prediction of multi-domain proteins.

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