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Setting up a Meta-Threading Pipeline for High-Throughput Structural Bioinformatics: *e*Thread Software Distribution, Walkthrough and Resource Profiling

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Abstract

*e*Thread, a meta-threading and machine learning-based approach, is designed to effectively identify structural templates for use in protein structure and function modeling from genomic data. This is an essential methodology for high-throughput structural bioinformatics and critical for systems biology, where extensive knowledge of protein structures and functions at the systems level is prerequisite. *e*Thread integrates a diverse collection of algorithms, therefore its deployment on a large multi-core system necessarily requires comprehensive profiling to ensure the optimal utilization of available resources. Resource profiling of *e*Thread and the single-threading component algorithms indicate as wide range of demands with respect to wall clock time and host memory. Depending on the threading algorithm used, the modeling of a single protein sequence of up to 600 residues in length takes minutes to hours. Full meta-threading of one gene product from *E. coli* proteome requires ~12h on average on a single state-of-the-art computing core. Depending on the target sequence length, the subsequent three-dimensional structure modeling using *e*Thread/Modeller and *e*Thread/TASSER-Lite takes additional 1-3 days of computing time. Using the entire proteome of *E. coli*, we demonstrate that parallel computing on a multi-core system follows Gustafson-Barsis' law and can significantly reduce the production time of *e*Thread. Furthermore, graphics processor units can speedup portions of the calculations; however, to fully utilize this technology in protein threading, a substantial code development is required. *e*Thread is freely available to the academic and non-commercial community as a user-friendly web-service at http://www.brylinski.org/ethread. We also provide source codes and step-by-step instructions for the local software installation as well as a case study demonstrating the complete procedure for protein structure modeling. We hope that genome-wide high-throughput structural bioinformatics using *e*Thread will significantly expand our knowledge of protein structures and their molecular functions and contribute to the thriving area of systems biology.

Keywords: Protein structure prediction; Protein function annotation; Template-based modeling; Protein meta-threading; Structural bioinformatics; Proteome-scale modeling; High-performance computing

Abbreviations: GPU: Graphics Processor Unit; HPC: High-Performance Computing; PBS: Portable Batch System; SVM: Support Vector Machines

Introduction

In modern biological sciences, the focus has shifted from the study of individual molecules to the exhaustive exploration of molecular interactions at the systems level. This new paradigm has given rise to the rapidly developing domain of systems biology [1], which lies at the intersection of life and computer sciences. Systems biology is facilitated by whole genome sequencing [2] that routinely generates large datasets of protein sequences; nevertheless, the molecular structures and functions of many of these sequences often remain unknown. Computational methods for protein structure and function prediction are expected to bridge the gap between the number of known sequences and the number of fully annotated gene products, which are requisite for systems biology applications. Amongst many computational techniques developed over the past years, the most accurate algorithms in this field build on homology, i.e. they use information inferred from related proteins. Sequence-based methods can provide useful structural and functional information for a subset of target proteins; however, these algorithms typically require a high sequence identity to already annotated proteins to maintain a high accuracy [3]. As might be expected, this reduces the coverage of suitable targets, since for many proteins no close homologues are available in the public databases, eg. the Protein Data Bank [4]. It has been demonstrated that relaxing the safe sequence similarity thresholds in sequence-based function annotation may lead to high levels of mis-annotation [5].

To address this issue, a number of techniques have been developed to search for low-sequence identity templates that can be used to construct the structural model of a target protein and to subsequently infer its molecular function. This is a major goal of contemporary structural bioinformatics, which aims at the high-throughput modeling of all gene products across the entire proteomes of various organisms in the so-called "twilight zone" of sequence similarity [6]. Protein threading [7] represents the latest trend in the development of template identification and alignment algorithms. These techniques have the desired capability to effectively deal with the complex and equivocal relations between protein sequence, structure and function, which are the major obstacles for standard bioinformatics approaches. Such

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a structure-oriented approach holds a considerable promise to speed up genome-wide protein annotation [8], which certainly will have impact on many areas of modern molecular, cell and systems biology. It has a great potential to overcome the limitations of more traditional sequence-based approaches; however, at the cost of a significantly increased demand for computational resources.

In particular, meta-threading techniques are widely used in structural bioinformatics. These methods operate by considering outputs from a variety of individual threading algorithms; the combined predictions have a higher chance to be accurate than those produced by a single method. An example of such an approach is recently developed *e*Thread, which integrates ten state-of-the-art single-threading algorithms additionally supported by machine learning to provide a unified resource for protein structure and function modeling [9]. Here, the scientific challenge is to efficiently combine multiple algorithms to significantly increase the overall accuracy over the individual methods and to push the envelope of systems-level template-based protein structure modeling and functional annotation. Meta-threading pipelines also render significant challenges at the level of practical implementation and the optimal utilization of computing resources. These can be considered as heterogeneous collections of algorithms and computational techniques that may significantly differ from each other in terms of the required wall clock time, memory usage, I/O operations and network bandwidth. They also may or may not share common input files or the access to external data libraries (eg. template libraries), and often employ a complicated system of dependencies between individual jobs. To the best of our knowledge, the majority of currently implemented meta-threading pipelines are available as pseudo-gateways, i.e. web interfaces that query several publicly available CGI servers over the Internet using simple web tools such as wget or curl.

A local installation of the meta-threading pipeline on a high-performance computing (HPC) platform provides the most reliable and robust solution for high-throughput structural bioinformatics [10]. However, running a diverse collection of algorithms on a large multi-core system necessarily requires comprehensive resource profiling to ensure the optimal utilization of available resources. In this communication, we describe the stand-alone software distribution of *e*Thread, which can be deployed on any modern Linux-based HPC system. We perform a comprehensive resource profiling, analyze the computational requirements and discuss the achievable models of parallelism. We also touch on the possibility of accelerating the computations by graphics processors (GPUs). Finally, we provide a case study to demonstrate the practical application of this software on production platforms. *e*Thread is freely available for academic and non-commercial users at www.brylinski.org/ethread.

Materials and Methods

Overview of *e*Thread pipeline

*e*Thread is a meta-threading procedure that combines predictions from ten state-of-the-art single-threading algorithms: COMPASS [11], CS/CSI-BLAST [12], HHpred [13], HMMER [14], pfTools [15], pGenThreader [16], SAM-T2K [17], SPARKS [18], SP3 [18] and Threader [7], see table 1. It also uses NCBI BLAST [19] for the construction of sequence profiles and PSIPRED [20] for the prediction of secondary structure. Currently, two structure modeling algorithms can be used in *e*Thread to construct the three-dimensional structures of target proteins: Modeller [21] and TASSER-Lite [22]. *e*Thread/Modeller requires template pre-clustering, which is calculated by MaxCluster. In addition to target-to-template alignments generated by *e*Thread, *e*Thread/TASSER-Lite needs long-range inter-residue contacts, which can be predicted for a given target sequence using *e*Contact (included in the *e*Thread software distribution). Moreover, both modeling protocols employ several popular tools for protein structure modeling, eg. Jackal [23] and Pulchra [24]. Typically, multiple models are generated for a given target sequence. To rank them and assign the prediction confidence, we developed *e*Rank, which uses individual scoring functions provided by Modeller [21] and TASSER-Lite [22], as well as DFIRE [25], fr-TM-align [26], TM-score [27], DSSP [28] and Stride [29]. The complete list of structure-based tools required by *e*Thread is shown in table 2. *e*Thread, *e*Contact and *e*Rank also employ several machine learning models to improve prediction accuracy; we use two software packages that offer various Support Vector Machines (SVM) algorithms: LIBSVM [30] and SVM^{rank}[31], see table 3.

Physical testing systems

The primary testing system is HP Proliant DL 180 G6 server which has 2 Intel Xeon E5645 6-core processors running at 2.4GHz and it is equipped with 48GB of memory. Additionally, the following three systems were used for the benchmarking of GPU-BLAST: 1) dual Intel Xeon E5620 4-core processor running at 2.4GHz, equipped with 24GB RAM and NVIDIA Tesla M2050, 2) dual Intel Xeon E5540 4-core processor running at 2.5GHz, equipped with 24GB RAM and NVIDIA Tesla M2070, and 3) single Intel Xeon E55404-core processor running at 2.5GHz, equipped with 36GB RAM and NVIDIA Tesla C2075.

Simulated multi-core systems

We constructed 18 virtual multi-core systems, equipped with 6, 8, 12, 16, 24 and 32 computing cores, and 1, 2 and 4GB of RAM per core. We also designed a simple job scheduling system that assigns jobs to the computing cores using the following rules: 1) the total number of concurrently running jobs must be less or equal to the number of cores, 2) the total memory for running jobs cannot exceed the host shared

Software	Purpose	Link
COMPASS	Protein threading/fold recognition	http://prodata.swmed.edu/compass/compass.php
CS/CSI-BLAST	Protein threading/fold recognition	http://toolkit.tuebingen.mpg.de/cs_blast
HHpred	Protein threading/fold recognition	http://toolkit.tuebingen.mpg.de/hhpred
HMMER	Protein threading/fold recognition	http://hmmer.janelia.org
NCBI BLAST	Sequence alignment	http://blast.ncbi.nlm.nih.gov/Blast.cgi
pfTools	Protein threading/fold and motif recognition	ftp://lausanne.isb-sib.ch/pub/software/unix/pftools/pft2.3/README
pGenThreader	Protein threading/fold recognition	http://bioinf.cs.ucl.ac.uk/psipred/?program=mgenthreader
PSIPRED	Secondary structure prediction	http://bioinf.cs.ucl.ac.uk/psipred
SAM-T2K	Protein threading/fold recognition	http://compbio.soe.ucsc.edu/papers/sam_doc/sam_doc.html
SPARKS/SP3	Protein threading/fold recognition	http://sparks.informatics.iupui.edu/index.php?pageLoc=Services
Threader	Protein threading/fold recognition	http://bioinf.cs.ucl.ac.uk/software_downloads/threader

Table 1: Sequence-based tools. Components of the *e*Thread pipeline for protein threading and sequence analysis.

Software	Purpose	Link
DFIRE	Protein conformation free energy score	http://sparks.informatics.iupui.edu/hzhou/dfire.html
DSSP	Secondary structure assignment	http://swift.cmbi.ru.nl/gv/dssp
fr-TM-align	Protein structural alignment	http://cssb.biology.gatech.edu/fr-tm-align
Jackal	Protein structure modeling	http://wiki.c2b2.columbia.edu/honiglab_public/index.php/Software:Jackal
MaxCluster	Protein structure comparison and clustering	http://www.sbg.bio.ic.ac.uk/~maxcluster/index.html
Modeller	Protein structure modeling	http://salilab.org/modeller
Pulchra	All-atom reconstruction from the backbone Cα atoms	http://cssb.biology.gatech.edu/PULCHRA
Stride	Secondary structure assignment	http://webclu.bio.wzw.tum.de/stride
TASSER-Lite	Protein structure modeling	http://cssb.biology.gatech.edu/TASSER-Lite
TM-score	Protein structure similarity measure	http://zhanglab.ccmb.med.umich.edu/TM-score

Table 2: Structure-based tools. Components of the *e*Thread pipeline for protein structure modeling and analysis.

Software	Purpose	Link
LIBSVM	Support Vector Machines for classification and regression	http://www.csie.ntu.edu.tw/~cjlin/libsvm
SVM^{rank}	Support Vector Machines for ranking	http://www.cs.cornell.edu/people/tj/svm_light/svm_rank.html

Table 3: Machine learning tools. Machine learning algorithms used by *e*Thread for various classification and ranking tasks.

memory, and 3) jobs are prioritized, where the priority of a job is the product of its memory utilization and computing time. This allocation system can be considered as a simplified version of the widely used Portable Batch System (PBS).

Datasets

The resource profiling is carried out on a dataset of 275 proteins randomly chosen from the original *e*Thread benchmarking dataset [9]. These proteins were selected to uniformly populate 11 bins with 25 structures in each bin. The bins evenly span the range of the target sequence length between 50 and 600 residues.

As a benchmarking dataset for the simulated systems-level modeling, we selected the complete proteome of *Escherichia coli* K-12 [32], which comprises 4,646 gene products 50-600 amino acids in length. *e*Thread meta-threading pipeline employ ten individual threading algorithms, thus the total number of jobs needed to process *E. coli* proteome is 46,460. The expected memory consumption and computing time for each job was calculated based on meta-threading profiling results obtained on the primary testing system.

Results

Profiling of meta-threading components

Individual single-threading components of the *e*Thread pipeline significantly differ from each other with respect to the wall clock time and memory consumption. Both resources are typically limited on many HPC systems; for example, currently the largest HPC cluster in the state of Louisiana, Queen Bee (http://www.loni.org/systems/), allows jobs to run for up to 48 hours and features 8 computing cores and 8GB of RAM per node.

The results of meta-threading resource profiling on the primary testing system are shown in figures 1 and 2. Figure 1A shows the average wall clock time required for each single-threading component method. In all cases, except for HHMER, the simulation time scales well with the target sequence length; however, the algorithms differ with respect to the total CPU time. The least expensive algorithms, CSI-BLAST, pfTools and HMMER, require at most minutes, whereas the most expensive Threader typically needs several hours to complete the calculations. *e*Thread uses two template libraries: full-chain (11,468 structures) and domain-only (10,013 structures). Figure 1B reports the percentage of time spent on threading through a particular library; due to the number and length of template structures, the chain library requires slightly longer simulation times. Furthermore, several algorithms use PSI-BLAST to construct a sequence profile for a given target, which is often the most time consuming step. Blue pie slices in figure 1B show that for HHpred, COMPASS and pGenThreader, 88%, 62% and 51% CPU cycles are used up by the sequence profile construction, respectively.

Individual threading algorithms also differ with respect to the memory utilization, see figure 2A. CSI-BLAST, pfTools, Threader and HMMER require the least amount of memory; whereas, HHpred, COMPASS and pGenThreader, all of which employ PSI-BLAST for the construction of sequence profiles, need significantly more RAM. Figure 2B shows that for HHpred, COMPASS and pGenThreader, 86%, 78% and 93% of the memory was utilized during the sequence profile construction, respectively. SAM-T2K has the highest memory requirement because it launches BLASTP, which loads a large sequence library into memory. The actual threading calculations use only ~2% of the memory; however, throughout 96% of the simulation time (Figure 1B, SAM-T2K). Furthermore, in most cases, the required memory scales well with the target protein length. It is also important to note that all these algorithms do not depend on each other therefore can be efficiently processed in parallel.

Simulated multi-core system running meta-threading

We conduct a simple computer experiment to show that meta-threading pipelines follow Gustafson-Barsis' law, which states that computations involving arbitrarily large data sets can be efficiently parallelized [33]. Figure 3 presents the simulated operation of three virtual systems equipped with 8, 16 and 32 cores, and 1GB of RAM per core, processing 46,460 individual threading jobs for *E. coli* proteome. The CPU utilization is almost constantly 100% throughout the operation time, with the exception for two larger systems, where a few high memory jobs initially saturated the available host memory and blocked the remaining cores for a short period of time, see figures 3B and 3C. Moreover, because of the job prioritization, high memory jobs, e.g. SAM-T2K, pGenThreader and COMPASS, as well as long jobs, e.g. Threader, were selected for execution before other threading algorithms.

The total computer time required for completing meta-threading against *E. coli* proteome is shown in figure 4. For instance, one needs 6 years, 303 days and 17 hours to complete the calculations on a single computing core. Using 100 nodes of the aforementioned Louisiana

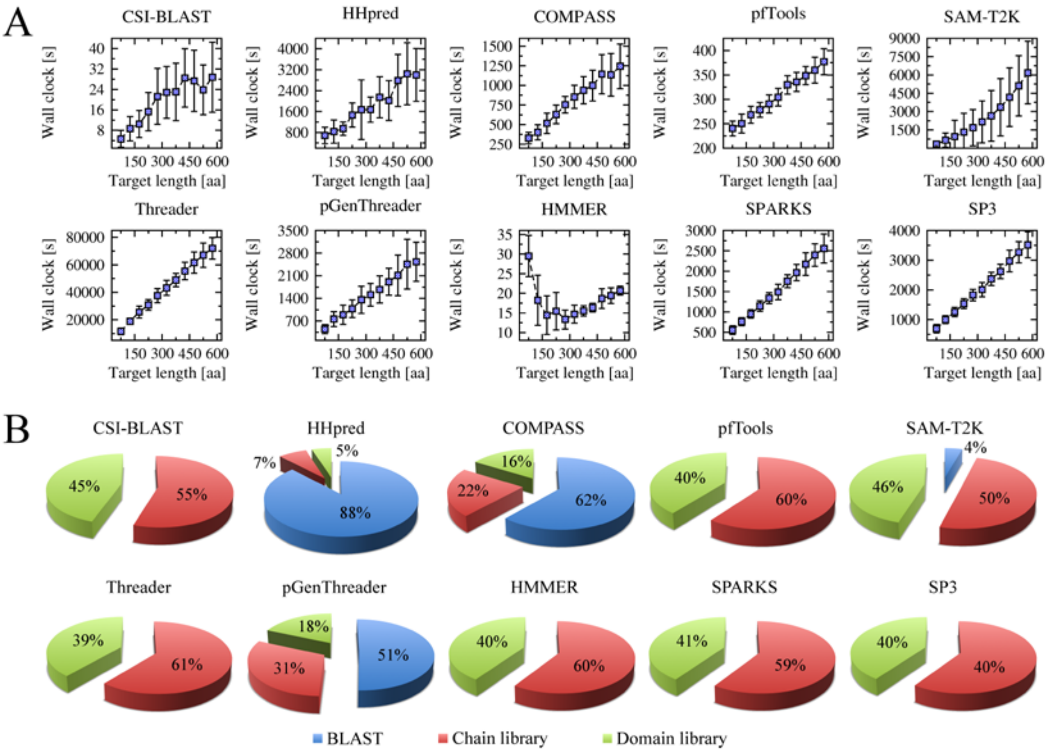


Figure 1: Resource profiling for threading component methods. (**A**) Average ± standard deviation wall time for individual components of *e*Thread plotted as a function of the target protein length; (**B**) percentage of wall time used by individual components of *e*Thread to thread against chain and domain template libraries. Profile construction using NCBI BLAST is benchmarked independently from threading when possible.

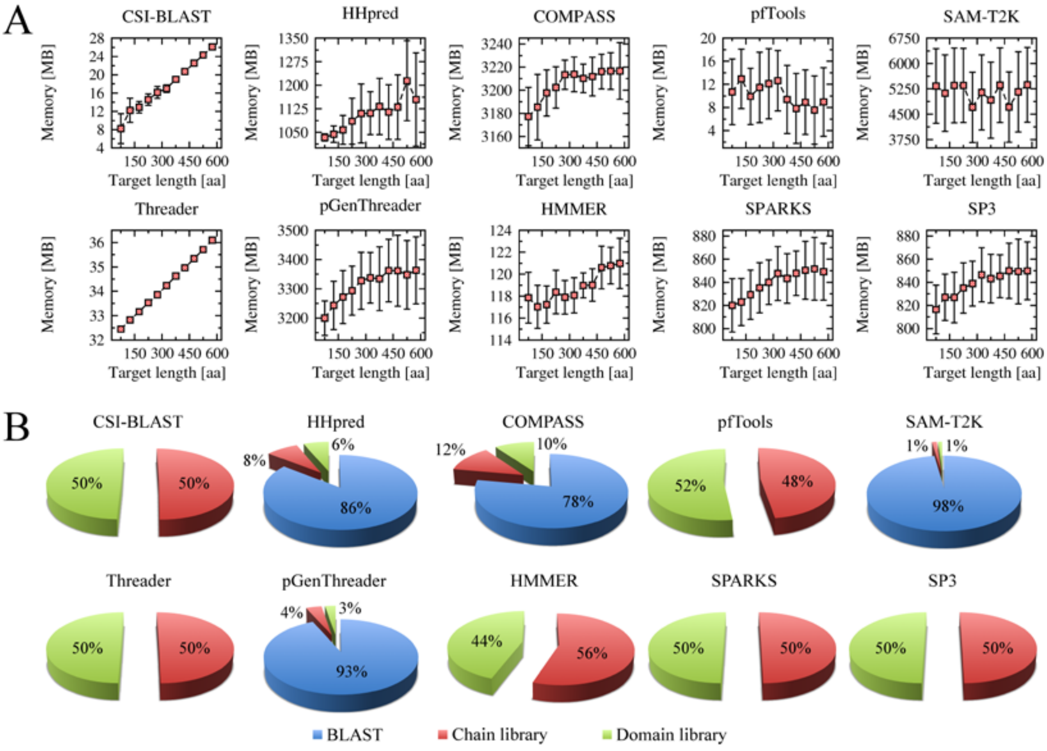


Figure 2: Resource profiling for threading component methods. (**A**) Average ± standard deviation memory consumption for individual components of *e*Thread plotted as a function of the target protein length; (**B**) percentage of memory used by individual components of *e*Thread to thread against chain and domain template libraries. Profile construction using NCBI BLAST is benchmarked independently from threading when possible.

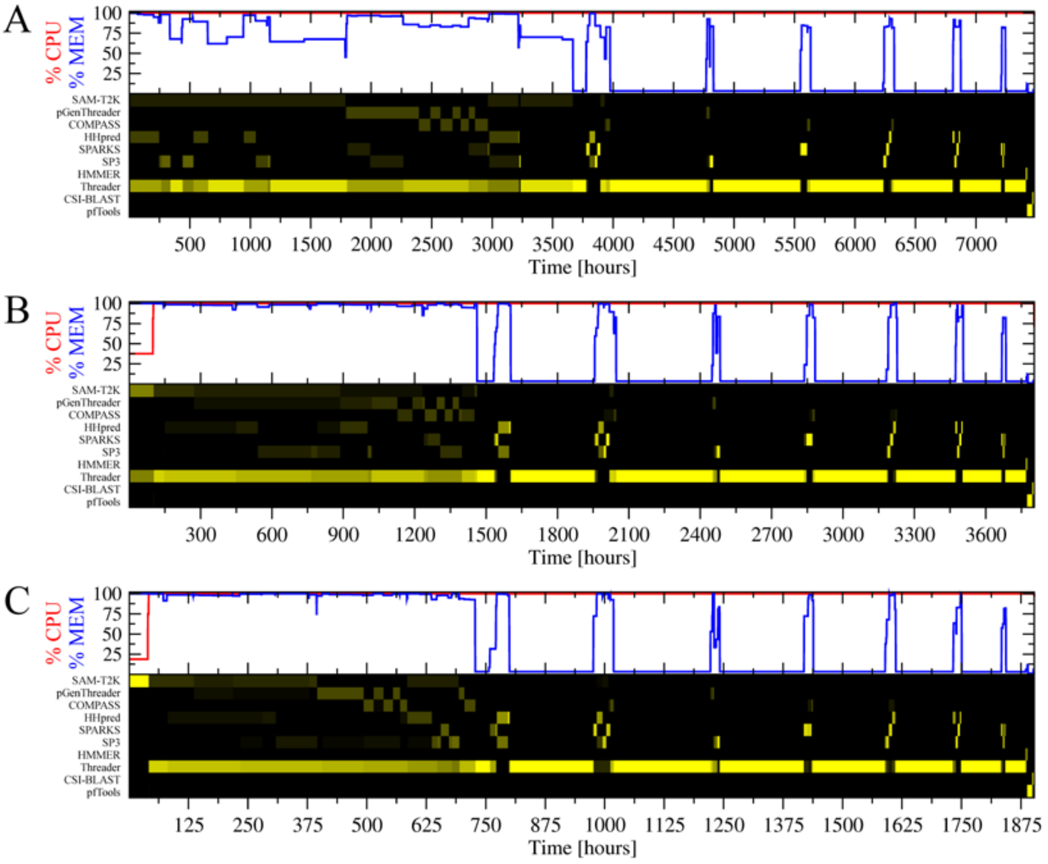


Figure 3: Simulated queuing system dispatching threading jobsfor *E. coli* proteome. Calculations are virtually processed using (**A**) 8 cores/8GB RAM, (**B**) 16 cores/16GB RAM, and (**C**) 32 cores/32GB RAM. For each composite figure, the top pane shows the utilization of computing cores and memory as a function of the machine operation time. The bottom pane shows the fraction of cores assigned to individual threading algorithms at a given time; color intensity matches the fraction: black – 0.0 (no cores), bright yellow – 1.0 (all cores).

HPC cluster Queen Bee, each equipped with 8 cores and 8GB of RAM, the entire *E. coli* proteome could be processed in 3 days and 3 hours. However, increasing the size of the host shared memory does not shorten the simulation time. This is because the computations are dominated by low memory jobs, eg. Threader. Figure 3A demonstrates that the memory was fully utilized only throughout around 50% of the total computing time, which leaves a substantial room to maneuver job allocation. Figure 4 inset shows that a meta-threading pipeline closely follows Gustafson-Barsis' law, i.e. doubling the number of CPU cores shortens the computing time by a factor of 2. We identify three factors responsible for this performance: 1) a large set of diverse protein threading jobs, 2) a considerable margin for the memory utilization on modern HPC systems, and 3) the lack of dependencies between individual jobs. Consequently, proteome-wide meta-threading applications are perfectly parallelizable at the task level.

Profiling of *e*Thread

*e*Thread is a meta-predictor that integrates outputs from individual threading components. It operates in two modes: structural and functional. The former identifies the most confident structural templates and constructs consensus target-to-template alignments for use in protein structure modeling. The latter additionally evaluates selected templates for the utility in function assignment and considers a variety of protein molecular functions: ligand, metal, inorganic cluster, protein and nucleic acid binding. Figure 5 shows that the memory required by *e*Thread is well correlated with the target protein length, whereas the average wall clock time is characterized by larger standard deviations; this is because the simulation time also depends on the number of identified templates. Moreover, including the functional component fourfold increases the wall clock time and the memory

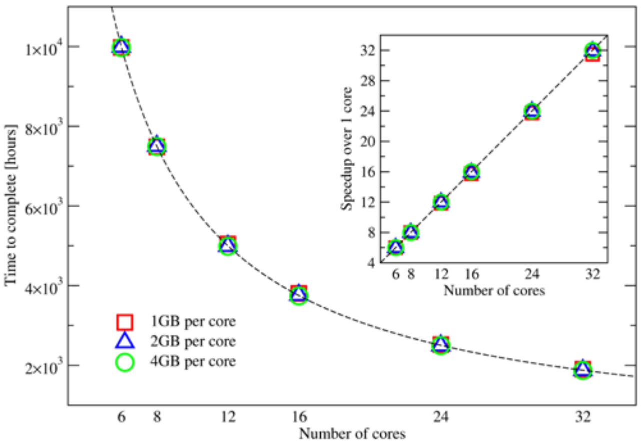


Figure 4: Time required for completing meta-threading against *E. coli* proteome. Completion time is estimated by a simulated job scheduling system for virtual machines equipped with 6, 8, 12, 16, 24 and 32 computing cores, and 1, 2 and 4 GB of RAM per core. Inset: speedup over 1 core for multi-core virtual systems.

consumption. Nevertheless, *e*Thread does not require significant computing resources. Running in a structural (functional) mode, in most of the cases, it completes within 30 (80) minutes and consumes up to 0.6 (2.3) GB of memory.

Profiling of protein structure modeling

In *e*Thread, the three-dimensional structural models of target proteins can be constructed using two modeling protocols: Modeller [21] and TASSER-Lite [22]. Resource profiling for *e*Thread/Modeller are shown in figure 6A. Both simulation time and the memory used are correlated with the target protein length. This protocol has relatively low hardware requirements; even long target sequences typically need less than 0.5GB of RAM and up to 50 hours of CPU time. Compared to *e*Thread/Modeller, *e*Thread/TASSER-Lite requires significantly more resources, see figure 6B. The duration of structure assembly and refinement simulations in TASSER-Lite is limited to 48 hours, so the total wall clock time does not keep growing exponentially for sequences longer than 300 residues. Both modeling protocols profiled here, *e*Thread/Modeller and *e*Thread/TASSER-Lite, typically generate full chain models within 1-3 days on a single computing core.

*e*Thread/Modeller comprises two modeling stages: structure assembly by Modeller followed by model ranking using *e*Rank. There is very little overhead resulting from *e*Rank; it completes within seconds, therefore it is not included in the profiling results. In contrast, structure modeling using *e*Thread/TASSER-Lite consists of four consecutive stages: residue contact prediction using *e*Contact, threading by Prospector [34], structure assembly/refinement by TASSER and model ranking by *e*Rank. As shown in figure 6C, structure assembly and refinement is the most computationally intense and consumes 80% of the total CPU time. TASSER-Lite also includes additional threading using Prospector. This modeling stage is the most memory intense and

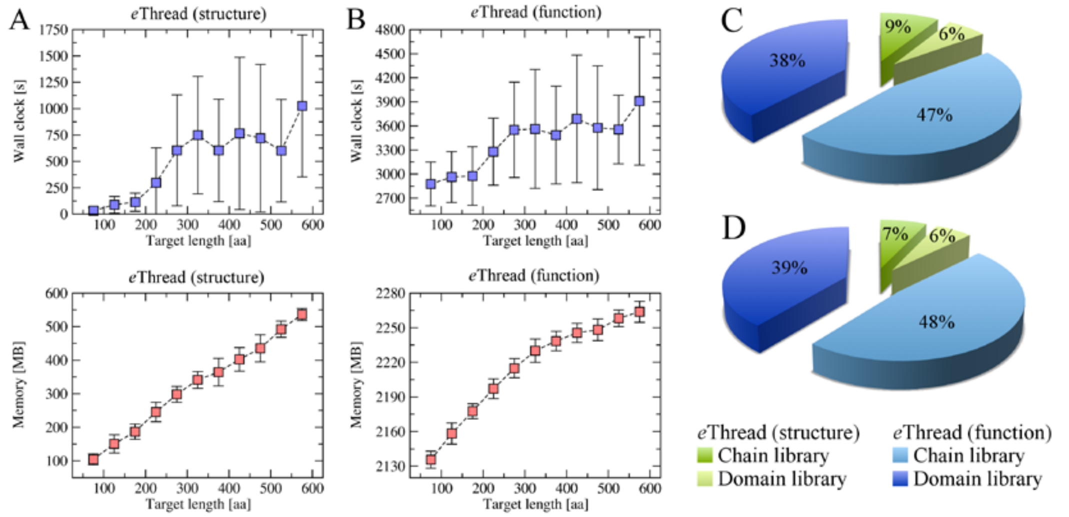


Figure 5: Resource profiling for *e*Thread. Average ± standard deviation wall time and memory consumption for the identification of (**A**) structural and (**B**) functional templates by *e*Thread plotted as a function of the target protein length. Percentage of (**C**) wall time and (**D**) memory used by *e*Thread to identify structural and functional templates in chain and domain libraries.

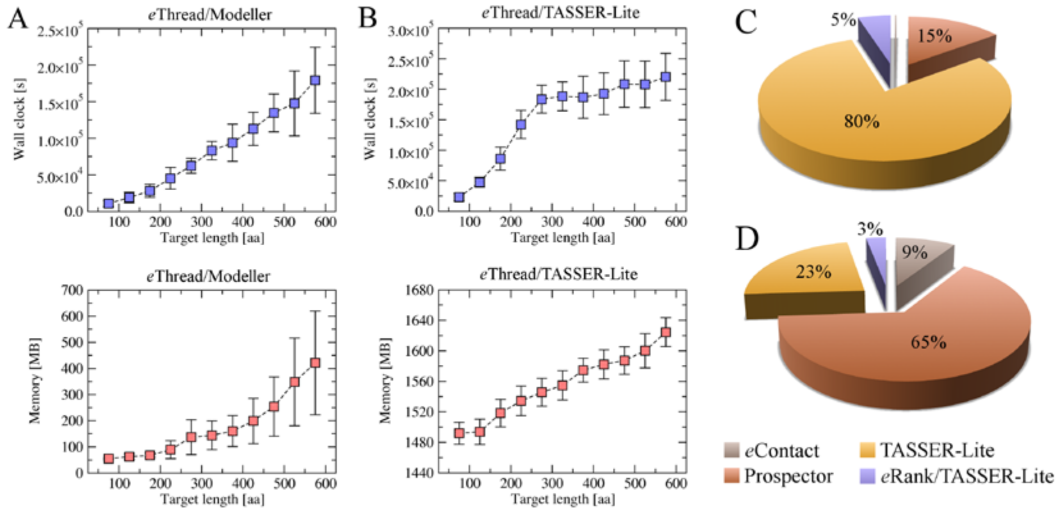


Figure 6: Resource profiling for protein structure modeling. Average ± standard deviation wall time and memory consumption for the model construction using (**A**) *e*Thread/Modeller and (**B**) *e*Thread/TASSER-Lite plotted as a function of the target protein length. Percentage of (**C**) wall time and (**D**) memory used bythe individual components of the *e*Thread/TASSER-Lite modeling protocol: *e*Contact, Prospector, TASSER-Lite and *e*Rank/TASSER-Lite.

accounts for 65% of the total memory utilization of up to 1.65GB, see figure 6D. *e*Contact, which predicts long-range inter-residue contacts before TASSER simulations get started, and *e*Rank, which ranks the constructed models, extend the modeling time by only 5% and have relatively small memory requirements compared to the remaining modeling stages.

Potential for GPU acceleration

Heterogeneous HPC systems that include massively parallel graphics processors (GPUs) are quickly becoming popular, mainly because of their remarkably high performance-to-cost ratio. Consequently, GPU-accelerated supercomputers show an exponential growth in the Top500 ranking, with 52 systems powered by NVIDIA Tesla GPUs currently on the list compared to only 10 systems in 2010. Bioinformatics and systems biologyare examples of many rapidly developing research areas that are moving towards heterogeneous computing architectures [35]; GPU implementations of several popular bioinformatics tools have been reported recently. Bioinformatics software available for GPUs include both sequence, e.g. CUDA-BLASTP [36], GPU-BLAST [37], CUDASW++ [38] and GHOSTM [39], as well as structure alignment algorithms, e.g. ppsAlign [40] and TM-score-GPU [41]. Most of the component methods integrated into the *e*Thread pipeline doesn't have a GPU implementation, with the exception for BLASTP, which is used by SAM-T2K. To the best of our knowledge, GPU-BLASTP has not been benchmarked against its serial CPU version in a meta-threading environment.

Figure 7 shows wall clock times for serial NCBI BLASTP compared to that provided by GPU-BLAST [37] collected on three systems equipped with different GPU cards. Interestingly, the speedup depends on the target sequence length; this is likely due to the overhead caused by transferring the library data to the accelerator. Longer sequences require more calculations, thus the parallel processing by multiple GPU cores results in significantly shorter simulation times compared to the serial version. The speedup starts at 1.4, 1.5 and 1.7 for sequences 50-100aa, and reaches 2.1, 2.2 and 2.5 for sequences 550-600aa on Tesla M2050, M2070 and C2075 card, respectively. Without the laborious porting of the source codes of individual protein threading algorithms to CUDA, the construction of sequence profiles using PSI-BLAST would be the next logical step to speed up the entire pipeline by accelerating HHpred, COMPASS and pGenThreader; however, it is not currently available. Nevertheless, the Authors of GPU-BLAST noted that PSI-BLAST can be implemented on the GPU similarly to BLASTP and similar speedups can be expected [37]. Once available, it could give a boost to meta-threading pipelines by moving the sequence profile construction to GPU accelerators.

Software walkthrough and a case study

*e*Thread is freely available to the academic community. Web-based *e*Thread provides a user-friendly interface for a fast and easy access to the entire software package. Once the target amino acid sequence is submitted with selected structure prediction options, the modeling results can be downloaded to a local machine or displayed directly on the website, see figure 8 for a snapshot of prediction results. In figure 8A, the top-ranked structural model predicted using *e*Thread/Modeller is visualized in the Astex Viewer Java applet [42]. The estimated TM-score of 0.753 for this model suggests a high modeling confidence. The Ramachandran plot in figure 8B shows that 87.8% amino acids in the predicted target backbone reside in the most favorable region (colored in red); here, a threshold of 90% is commonly accepted to define high quality models. The web server also provides other results from the model quality check by PROCHECK [43] with respect to main-chain and side-chain parameters, e.g. peptide bound planarity, bad non-bonded interactions and Cα tetrahedral distortion. These may help users assess how a predicted structure compares with well-refined experimental structures (Figure 8C).

In addition to the web-based service, we also provide the source code of *e*Thread allowing users to install the software package and build protein structures locally. There are three major steps for the local setup including the installation of: 1) required Perl modules, 2) third-party software for single-threading algorithms, and 3) *e*Thread

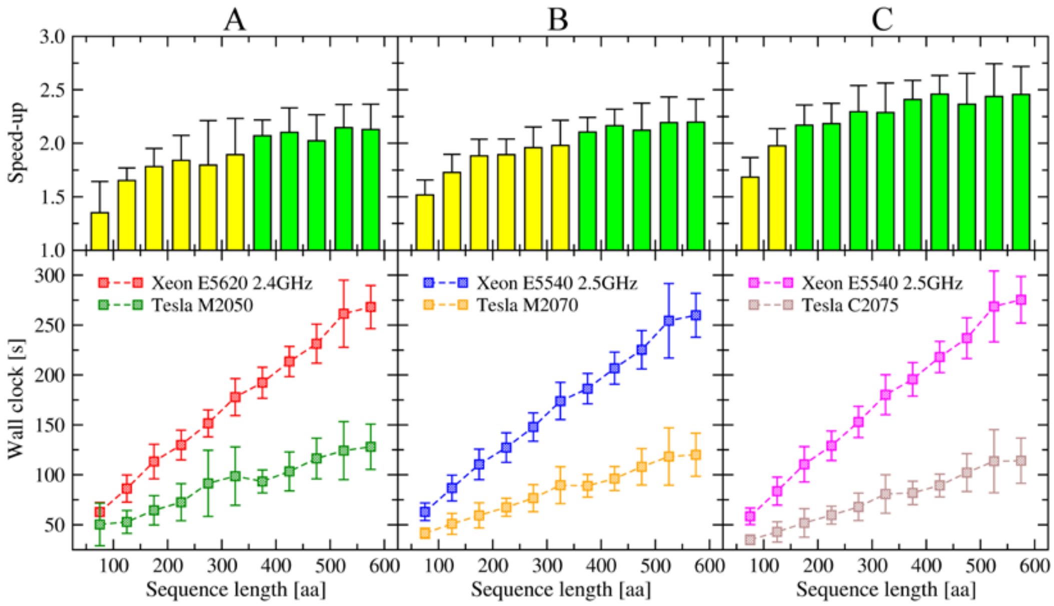


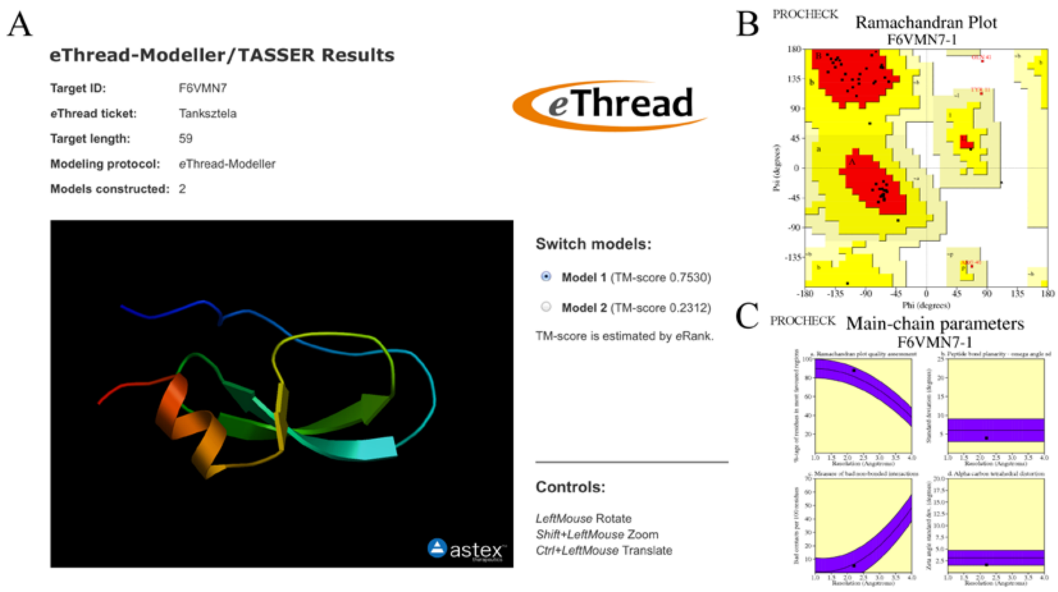
Figure 7: Wall clock time and speedups of parallel GPU-BLASTp relative to serial NCBI BLASTp. Performance is evaluated on three testing systems: (**A**) Xeon 2.4GHz/M2050, (**B**) Xeon 2.5GHz/M2070, and (**C**) Xeon 2.4GHz/C2075. For each sequence length bin, the average value with the corresponding standard deviation is plotted. In the top pane, speedups below and over 2 are colored in yellow and green, respectively.

software and the corresponding threading libraries. We note that all third-party programs are free for academic and non-commercial use; however, users are responsible for obtaining software licenses and complying with legal and other requirements. Upon the completion of the software installation, all single-threading tools as well as *e*Thread will be available locally for the identification of structural templates and the construction of target-to-template alignments. Finally, structural modeling protocols, Modeller [21] and TASSER-Lite [22], can be used to build the three-dimensional model for a given target sequence followed by a simple all-atom refinement using molecular mechanics.

As a proof of concept, we use *e*Thread to build a structural model for the 59-residue fragment of an uncharacterized protein from domestic horse [44] (UniProt ID: F6VMN7), for which the experimental structure is unavailable. The first step is the construction of threading alignments. We start with obtaining the amino acid sequence of F6VMN7in FASTA format from UniProt [45]. Next, we deploy each protein threading/fold recognition algorithm with the sequence of F6VMN7 as an input to identify suitable templates and to generate the corresponding target-to-template alignments. Structural templates are selected from both full-chain and domain-only libraries. Because different threading algorithms generate target-to-template alignments in different formats, the output files are converted to the *e*Thread format using conversion scripts included in the *e*Thread software distribution. Next, the target-to-template alignments constructed by individual threading algorithms are concatenated as one of the input files for *e*Thread. In addition to this input, two other files, the target sequence in FASTA format and the threading libraries, are required to generate the final consensus alignments. As the second step, we use Modeller and TASSER-Lite separately to build structure models from *e*Thread alignments. Typically, more than one model is predicted, therefore model construction is followed by ranking and quality assessment. Specifically, for *e*Rank/Modeller, three input files, including the target F6VMN7 sequence and two files generated by PSIPRED and *e*Thread, are required to rank the constructed models and assign TM-scores as confidence estimates. In figure 9A, the top-ranked *e*Thread/Modeller model is shown in PDB format and its molecular structure is visualized in VMD [46]. For *e*Thread/TASSER-Lite, the inter-residue contacts are first predicted using *e*Contact; subsequently, residue contacts, target sequence and *e*Thread alignments are used as input to construct the three-dimensional model. Similar to *e*Rank/Modeller, *e*Rank/TASSER-Lite is then deployed to rank protein models and assign the modeling confidence. The resulting top-ranked model constructed for F6VMN7 by *e*Thread/TASSER-Lite is shown in figure 9B. Figure 9C shows the global superposition of the top-ranked models generated by *e*Thread/Modeller and *e*Thread/TASSER-Lite. Both models are remarkably similar to each other (RMSD is 0.74Å), which indicates a high confidence for the structure modeling of this target. However, there exist some differences. For example, the model built using *e*Thread/Modeller (yellow) has longer beta sheets compared to that using *e*Thread/TASSER-Lite (green), with approximately 27% and 13% of residues assigned by STRIDE [29] to theβ-sheet conformation, respectively. It suggests that despite the likely correct global topology, the model constructed by *e*Thread/TASSER-Lite may require more rigorous local structure refinement to improve the secondary structure content [9].

Conclusion

Systems biology is emerging as a promising discipline in the field of biology. Powered by modern computer technologies, it aims to help comprehend molecular interactions at the systems level. Towards this goal, acquiring extensive knowledge of protein structures and their functions is essential. Continuing advancements in sequencing technologies spark off the rapid accumulation of gene and gene product sequences; yet, the annotation of these sequences is falling far behind. Therefore, a high-throughput protein annotation is a daunting task in bioinformatics. Up to date, various computational tools have been developed to reach this goal. Different from sequence-based methods that heavily depend on high sequence identity to already annotated protein sequences, structure-based methods are making headway in function inference in the “twilight zone” [6] of sequence identity. Consequently, the genome-wide coverage of annotated proteins can be systematically expanded. Among many template-based approaches,



eThread-Modeller/TASSER Results

Target ID: F6VMN7
eThread ticket: Tanksztela
Target length: 59
Modeling protocol: eThread-Modeller
Models constructed: 2

Switch models:

● Model 1 (TM-score 0.7530)
○ Model 2 (TM-score 0.2312)

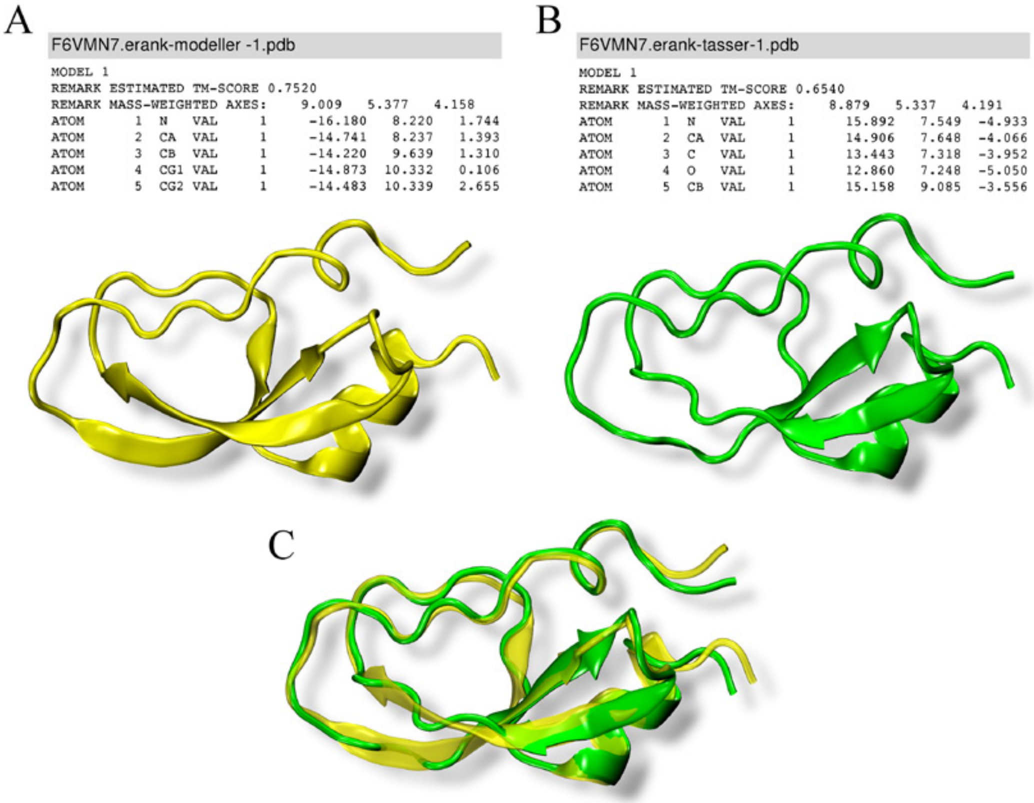
TM-score is estimated by eRank.

Controls:

LeftMouse Rotate
Shift+LeftMouse Zoom
Ctrl+LeftMouse Translate

A
eThread
astex
B
PROCHECK
Ramachandran Plot
F6VMN7-1
Psi (degrees)
Phi (degrees)
180
135
90
45
0
-45
-90
-135
-180
-135
-90
-45
0
45
90
135
180
B
b
A
a
L
l
p
~b
~a
~l
~p
C
PROCHECK
Main-chain parameters
F6VMN7-1
a. Ramachandran plot quality assessment
b. Peptide bond planarity - omega angle sd
c. Measure of bad non-bonded interactions
d. Alpha carbon tetrahedral distortion
%-age of residues in most favoured regions
Standard deviation (degrees)
Bad contacts per 100 residues
Zeta angle standard dev. (degrees)
Resolution (Angstroms)
1.0
1.5
2.0
2.5
3.0
3.5
4.0

Figure 8: Snapshot of a sample result page from the *e*Thread webserver. (**A**) The constructed models are visualized using AstexViewer. The stereochemical quality of the predicted models is assessed by PROCHECK using (**B**) Ramachandran map and (**C**) main-chain parameters graphs.



F6VMN7.erank-modeller -1.pdb

```
MODEL 1
REMARK ESTIMATED TM-SCORE 0.7520
REMARK MASS-WEIGHTED AXES:     9.009   5.377   4.158
ATOM      1  N   VAL     1     -16.180   8.220   1.744
ATOM      2  CA  VAL     1     -14.741   8.237   1.393
ATOM      3  CB  VAL     1     -14.220   9.639   1.310
ATOM      4  CG1 VAL     1     -14.873  10.332   0.106
ATOM      5  CG2 VAL     1     -14.483  10.339   2.655
```

F6VMN7.erank-tasser-1.pdb

```
MODEL 1
REMARK ESTIMATED TM-SCORE 0.6540
REMARK MASS-WEIGHTED AXES:     8.879   5.337   4.191
ATOM      1  N   VAL     1      15.892   7.549  -4.933
ATOM      2  CA  VAL     1      14.906   7.648  -4.066
ATOM      3  C   VAL     1      13.443   7.318  -3.952
ATOM      4  O   VAL     1      12.860   7.248  -5.050
ATOM      5  CB  VAL     1      15.158   9.085  -3.556
```

Figure 9: Sample results of top-ranked models in PDB format visualized in VMD. The models are constructed by (**A**) *e*Thread/Modeller and (**B**) *e*Thread/TASSER-Lite. Structure alignment between the two predicted models is shown in (**C**).

protein meta-threading is of particular interest, primarily because this approach integrates multifaceted factors to enhance the prediction accuracy. Towards this effort, we developed *e*Thread that combines ten single-threading algorithms supported by machine learning to identify suitable templates for the prediction of protein structure and function [9].

The heterogeneous collection of algorithms used in *e*Thread creates a challenge for the optimal utilization of system resources. In this communication, we thoroughly profile *e*Thread and its component methods in terms of the total wall clock time and memory consumption, as well as the resource distribution at major computing stages. The profiling results show that the total CPU time and memory utilization differ dramatically among single-threading methods; yet, the overall resource required typically scales well with the target protein length. Furthermore, in a simple experiment using several simulated multi-core systems, we show that meta-threading pipelines closely follows Gustafson-Barsis' law [33], thus systems-level applications, eg. genome-wide modeling of protein structure and function, are exemplary tasks for large computer clusters.

In addition to parallel computing using multiple CPU cores, we also examine whether using a GPU-accelerated platform would shorten the production time. The benchmarking results are encouraging; however, to significantly speed up protein meta-threading pipelines requires a substantial code development and porting individual algorithms to CUDA. Here, one of the most promising targets for GPU computing is PSI-BLAST, which is used by several component methods. A GPU implementation of this algorithm could significantly accelerate the entire meta-threading pipeline. Similarly to GPU computing, alternative technologies, such as Intel Many Integrated Core architecture, also hold a considerable promise to speed up bioinformatics applications.

We provide a user-friendly web service freely to the academic community and non-commercial users; we also provide source code of *e*Thread, which can be deployed locally on a high-performance computing platform for high-throughput protein structure and function modeling. The web-based gateway, stand-alone software, benchmarking results and datasets, as well as documentation and illustrative tutorials are available at www.brylinski.org/ethread.

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