Ensemble Multiple Sequence Alignment of Proteins (DRAFT)

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Abstract

The multiple sequence alignments computed by an aligner for different settings of its parameters, as well as the alignments computed by different aligners using their default settings, can differ markedly in accuracy. Parameter advising is the task of choosing a parameter setting for an aligner to maximize the accuracy of the resulting alignment. We extend parameter advising to aligner advising, which in contrast chooses among a set of aligners to maximize accuracy. In the context of aligner advising, default advising selects from a set of aligners that are using their default settings, while general advising selects both the aligner and its parameter setting.

In this paper, we apply aligner advising for the first time, to create a true ensemble aligner. Through cross-validation experiments on benchmark protein sequence alignments, we show that parameter advising boosts an aligner’s accuracy beyond its default setting for virtually all of the standard aligners currently used in practice. Furthermore, aligner advising with a collection of aligners further improves upon parameter advising with any single aligner, though surprisingly the performance of default advising on testing data is actually superior to general advising due to less overfitting to training data.

The new ensemble aligner that results from aligner advising is significantly more accurate than the best single default aligner, especially on hard-to-align sequences. This successfully demonstrates how to construct out of a collection of individual aligners, a more accurate ensemble aligner.

keywords: Multiple sequence alignment, parameter advising, aligner advising, accuracy estimation, ensemble methods.

1 Introduction

While it has long been known that the multiple sequence alignment computed by an aligner strongly depends on the settings for its tunable parameters, and that different aligners using their default settings can output markedly different alignments of the same input sequences, there has been relatively little work on how to automatically choose the best parameter settings for an aligner, or the best aligner to invoke, to obtain the most accurate alignment of a given set of input sequences.

Automatically choosing the best parameter setting for an aligner on a given input was termed by Wheeler and Kececioglu [1], parameter advising. In their framework, an
We very briefly summarize prior work on alignment advising. Wheeler and Kececioglu [1] introduced the notion of parameter advisors; Kececioglu and DeBlasio [4] investigated the construction of alignment accuracy estimators, resulting in the Facet estimator [5]; and DeBlasio and Kececioglu [8] investigated how to best form the set of protein sequences for which a correct reference alignment is known, and hence for which the alignment of highest estimated accuracy. Analogously, we call automatically choosing the best aligner for a given input, aligner advising.

To make this concrete, Figure 1 shows an example of advising on a benchmark set of protein sequences for which a correct reference alignment is known, and hence for which the true accuracy of a computed alignment can be determined. In this example, the Facet estimator of DeBlasio and Kececioglu [5] is used to estimate the accuracy of two alignments computed by the Opal [1] and MUMMALS [3] aligners. For these two alignments, the one of higher Facet value also has higher true accuracy as well, so an advisor armed with the Facet estimator would in fact output the more accurate alignment to a user.

For a collection of aligners, this kind of advising is akin to an ensemble approach to alignment, which selects a solution from those output by different methods to obtain an effect a new method that ideally is better than any individual method. Ensemble methods have been studied in machine learning [7], which combine the results of different classifiers to produce a single output classification. Typically such ensemble methods from machine learning select a result by voting. In contrast, an advisor combines the results of aligners by selecting one via an estimator value.

In this paper, we extend the framework of parameter advising to aligner advising, and obtain by this natural approach a true ensemble aligner. Moreover as our experimental results show, the resulting ensemble aligner is significantly more accurate than any individual aligner.
parameter choices for an advisor, called an *advisor set*, developing an efficient approximation algorithm for finding a near-optimal advisor set for a given estimator. All this prior work applied parameter advising to boosting the accuracy of the Opal aligner [6]. In contrast, this paper applies parameter advising to all commonly-used aligners, and aligner advising to combine them into a new, more accurate, ensemble aligner.

To our knowledge, the only prior work on combining aligners is by Wallace, O’Sullivan, Higgins, and Notredame [9] on the M-Coffee aligner, Collingridge and Kelly who developed the MergeAlign [10] tool and by Muller, Creevey, Thompson, Arendt, and Bork [11] on the AQUA tool. AQUA chooses between an alignment computed by MUSCLE [12] or MAFFT [13] based on their NorMD [14] score. Our prior work [4] shows that for choosing the more accurate alignment, the NorMD score used by AQUA is much weaker than the Facet estimator used here for aligner advising. M-Coffee uses a standard progressive alignment heuristic to compute an alignment under position-dependent substitution scores whose values are determined by alignments from different aligners. MergeAlign produces a consensus alignment of several alignments of the same input sequences but constructing a partial order alignment graph that represents all of the alignments, the path with the highest support is then returned. As Section 3.3 later shows, when run on the same set of aligners, M-Coffee and MergeAlign are both strongly dominated by the ensemble approach of this paper.

**Plan of the paper**

In the next section, we review our approach to learning an alignment advisor. An advisor selects aligners and parameter values from a small set of choices that is drawn from a larger universe of all possible choices. Section 2.2 describes how we construct this universe of aligners and their parameter choices for advisor learning. Section 3 then experimentally evaluates our approach to ensemble alignment on real biological benchmarks.

**2 Methods**

**2.1 Learning an alignment advisor**

To make the paper self-contained, we briefly review our prior work on how to learn an alignment advisor. We first review the concept of *parameter advising*, which requires an estimator of alignment accuracy and a set of parameter choices for the advisor, and then summarize our prior techniques for learning both an estimator and an advisor set. In Section 3, we apply these techniques for the first time to *aligner advising* to yield a new ensemble aligner.

**2.1.1 Parameter advising**

The goal of parameter advising is to find the parameter setting for an aligner that yields the most accurate alignment of a given set of input sequences. The accuracy of a computed alignment is measured with respect to the “correct” alignment of the sequences (which often is not known). For special benchmark sets of protein sequences, the gold-standard alignment of the proteins, called their reference alignment, is usually obtained through structural alignment by finding the best superposition of the known three-dimensional structures of the proteins. Columns of the reference alignment that contain a residue from every protein in the set (where a residue is the amino acid at a particular position in a protein), and for which the residues in the column are all mutually close in space in the superposition of the structures, are called core columns.
Runs of consecutive core columns are called core blocks, which represent the regions of the structural alignment with the highest confidence of being correct. Given such a reference alignment with identified core blocks, the accuracy of a different, computed alignment is the fraction of the pairs of residues aligned in the core blocks of the reference alignment that are also aligned in the computed alignment. (So a computed alignment of 100% accuracy completely agrees with the reference on its core blocks, though it may disagree elsewhere.) The best computed alignment is one of highest accuracy, and the task of a parameter advisor is to find a setting of the tunable parameters of an aligner that yields an accurate output alignment. This setting can be highly input dependent, as the best choice of parameter values for an aligner can vary for different sets of input sequences.

When aligning sequences in practice, a reference alignment is almost never known, in which case the true accuracy of a computed alignment cannot be measured. Instead our parameter advisors rely on an accuracy estimator $E$ that for an alignment $A$, gives a value $E(A)$ in the range $[0, 1]$ that estimates the true accuracy of alignment $A$. An estimator should be efficiently computable and positively correlated with true accuracy.

To choose a parameter setting, an advisor takes a set of choices $P$, where each parameter choice $p \in P$ is a vector that assigns values to all the tunable parameters of an aligner, and picks the choice that yields a computed alignment of highest estimated accuracy.

Formally, given an accuracy estimator $E$ and a set $P$ of parameter choices, a parameter advisor tries each parameter choice $p \in P$, invokes an aligner to compute an alignment $A_p$ using choice $p$, and then selects the parameter choice $p^*$ that has maximum estimated accuracy:

$$p^* \in \text{argmax}_{p \in P} \{E(A_p)\}.$$  

Since the advisor runs the aligner $|P|$ times on a given set of input sequences, a crucial aspect of parameter advising is finding a small set $P$ for which the true accuracy of the output alignment $A_{p^*}$ is high.

To construct a good advisor, we need to find a good estimator $E$ and a good set $P$. The estimator and advisor set are learned on training data consisting of benchmark sets of protein sequences for which a reference alignment is known. The learning procedure tries to find an estimator $E$ and set $P$ that maximize the true accuracy of the resulting advisor on this training data, which we subsequently assess on separate testing data.

Note that the process of advising is fast: for a set $P$ of $k$ parameter choices, advising involves computing $k$ alignments under these choices, which can be done in parallel, evaluating the estimator on these $k$ alignments, and taking a max. (Section 3.8 gives actual running times.) The separate process of training an advisor, by learning an estimator and an advisor set as we review next, is done once, off-line, before any advising takes place.

### 2.1.2 Learning an accuracy estimator

Kececioglu and DeBlasio present an efficient approach for learning an accuracy estimator that is a linear combination of real-valued alignment feature functions, based on solving a large-scale linear programming problem. This approach resulted in the Facet estimator, which is currently the most accurate estimator for parameter advising.

This approach assumes we have a collection of $d$ real-valued feature functions $g_1(A), \ldots, g_d(A)$ on alignments $A$, where these functions $g_i$ are positively correlated with true accuracy. The alignment accuracy estimator $E$ is a linear combination of these functions, $E(A) = \sum_{1 \leq i \leq d} c_i g_i(A)$, where the coefficients $c_i$ specify the
Fig 2. Relationship of estimators to true accuracy. Each point in a scatterplot corresponds to an alignment whose true accuracy is on the horizontal axis, and whose value under a given estimator is on the vertical axis. Both scatterplots show the same set of 3,000 alignments (randomly sampled from the more than 209,000 alignments generated by the experiments in Section 3) under the accuracy estimators Facet [4] and TCS [15].

estimator $E$. When the feature functions have range $[0, 1]$ and the coefficients form a convex combination, the resulting estimator $E$ will also have range $[0, 1]$. Facet uses a collection of five feature functions, many of which make use of predicted secondary structure for the protein sequences [4]. Figure 2 shows the relationship to true accuracy of both the Facet and TCS [15] estimators.

A parameter advisor uses the estimator to effectively rank alignments, so an estimator just needs to be monotonic in true accuracy. The difference-fitting approach learns the coefficients of an estimator that is close to monotonic by fitting the estimator to differences in true accuracy for pairs of training alignments.

Let function $F(A)$ give the true accuracy of alignment $A$, and set $\mathcal{P}$ be a collection of ordered pairs of alignments from training data, where every pair $(A, B) \in \mathcal{P}$ satisfies $F(A) < F(B)$. Difference fitting tries to find an estimator $E$ that increases at least as much as accuracy $F$ on the pairs in $\mathcal{P}$, by minimizing the amount that $E$ falls short. Formally, we find the estimator $E^*$ given by the vector of coefficients $c^* \in \mathbb{R}^d$ that minimizes

$$\sum_{(A,B) \in \mathcal{P}} w_{AB} \max \left\{ \left( F(B) - F(A) \right) - \left( E(B) - E(A) \right), 0 \right\},$$

where $w_{AB}$ weights the above error for a pair $(A, B)$. Finding the optimal coefficients $c^*$ can be reduced to solving a linear programming problem as follows.

**** EDIT THIS: All pairs from a particular benchmark such that $F(A) + F(B) + 0.005$ limit of 30,000 per benchmark weight pairs evenly within benchmark, then across benchmark bins.

The linear program has a variable $c_i$ for each estimator coefficient, and an error variable $e_{AB}$ for each pair $(A, B) \in \mathcal{P}$. The constraints are $c_i \geq 0$ and $\sum_i c_i = 1$, which ensure the coefficients form a convex combination, together with $e_{AB} \geq 0$, and

$$e_{AB} \geq \left( F(B) - F(A) \right) - \left( E(B) - E(A) \right).$$

(Note that the expressions $E(A)$ and $E(B)$ are linear in the variables $c_i$, while the quantities $F(A)$ and $F(B)$ are constants.) The linear program then minimizes the objective function $\sum_{(A,B) \in \mathcal{P}} w_{AB} e_{AB}$.

For the linear program to be of manageable size for a large number of training alignments, the set $\mathcal{P}$ of pairs must be quite sparse. Kececioglu and DeBlasio [4]
describe how to find a good sparse set \( P \) together with a good set of weights \( w_{AB} \) by an efficient graph algorithm.

Learning an accuracy estimator with \( d \) feature functions using a set \( P \) of \( p \) pairs, involves solving the above linear program with \( p + d \) variables and \( \Theta(p + d) \) inequalities. Evaluating the Facet estimator on an alignment with \( m \) sequences and \( n \) columns, after secondary structure has been predicted for the protein sequences, takes \( \Theta(m^2 n) \) time.

2.1.3 Learning an advisor set

DeBlasio and Kececioglu [8,17] present an efficient approximation algorithm for learning a near-optimal set of parameter choices for an advisor that uses a given estimator. The approximation algorithm follows a greedy strategy, so we call the sets found by the approximation algorithm greedy sets, in contrast to exact sets that are optimal for the training data, and which can be found by exhaustive search for small instances. These greedy sets tend to generalize better than exact sets, with the remarkable behavior that the greedy sets often outperform exact sets on testing data [8].

The problem of learning an optimal set \( P \) of parameter choices for an advisor is formulated as follows. Let \( U \) be the universe of possible parameter choices that might be included in advisor set \( P \). (Section 2.2 describes how we construct the universe \( U \) for aligner advising.) The training data is a collection of reference alignments \( R_i \), one for each benchmark \( B_i \), and a collection of alternate alignments \( A_{ij} \), where each alignment \( A_{ij} \) is computed by running the aligner on the sequences in benchmark \( B_i \) using parameter choice \( j \in U \). By comparing each alternate alignment to the reference alignment for its benchmark, we can measure the true accuracy \( a_{ij} \) of each alignment \( A_{ij} \).

For a candidate set \( P \subseteq U \) of parameter choices for an advisor that uses estimator \( E \), the set of parameter choices from \( P \) that could potentially be output by the advisor on benchmark \( B_i \) is

\[
\mathcal{O}_i(P) = \arg\max_{j \in P} \{ E(A_{ij}) \},
\]

where the argmax gives the set of parameter choices \( j \in P \) that are tied for maximizing the estimator \( E \) on the benchmark. The advisor could output any parameter choice from \( \mathcal{O}_i(P) \), as all of them appear equally good under the estimator, so we assume the advisor selects a choice uniformly at random from this set. Then the expected accuracy achieved by the advisor on benchmark \( B_i \) using parameter set \( P \) is

\[
A_i(P) = \frac{1}{|\mathcal{O}_i(P)|} \sum_{j \in \mathcal{O}_i(P)} a_{ij},
\]

where again \( a_{ij} \) is the true accuracy of alignment \( A_{ij} \).

In learning an advisor set \( P \), we seek a set \( P \) that maximizes the advisor’s expected accuracy \( A_i(P) \) on the training benchmarks \( B_i \). Formally, we want a set \( P \) that maximizes the objective function

\[
f(P) = \sum_i w_i A_i(P),
\]

where \( i \) indexes the benchmarks, and \( w_i \) is the weight placed on benchmark \( B_i \). (The benchmark weights correct for sampling bias in the training data, as discussed in Section 3.) In words, we want to find an advisor set \( P \subseteq U \) that maximizes the expected accuracy of the parameter choices selected by the advisor, averaged across weighted training benchmarks.

DeBlasio and Kececioglu [8] prove that for a given bound \( k \) on the size of advisor set \( P \), finding an optimal set \( P \subseteq U \) with \(|P| \leq k \) that maximizes objective \( f(P) \) is NP-complete.
**Greedy sets** While this NP-completeness result implies it is unlikely we can efficiently find an optimal advisor set, there is a natural greedy algorithm that is guaranteed to efficiently find a near-optimal set. For any constant ℓ, the optimal advisor set of cardinality at most ℓ can be found in polynomial time by exhaustive search. The following procedure Greedy builds on this idea to find a near-optimal advisor set for cardinality bound k, by starting with an optimal set of size at most ℓ, where ℓ ≤ k, and greedily augmenting it.

```plaintext
procedure Greedy(k, ℓ) begin
    Find an optimal subset P ⊆ U of size |P| ≤ ℓ that maximizes f(P).
    \( \tilde{P} := P \)
    \( \tilde{\ell} := |P| \)
    for cardinalities \( \tilde{\ell} + 1, \ldots, k \) do begin
        Find parameter choice \( j^* \in U - \tilde{P} \) that maximizes \( f(\tilde{P} \cup \{j^*\}) \).
        \( \tilde{P} := \tilde{P} \cup \{j^*\} \)
        if \( f(\tilde{P}) \geq f(P) \) then
            \( P := \tilde{P} \)
    end
    output \( P \)
end
```

DeBlasio and Kececioglu [8] prove that procedure Greedy is an \((\ell/k)\)-approximation algorithm for finding an optimal advisor set, for any constant ℓ with \( \ell \leq k \).

Learning a greedy advisor set for cardinality bound k when \( \ell = 1 \), which is the value we use in practice, for the Facet estimator on a universe of \( u \) parameter choices and a training set of \( t \) benchmarks, takes \( \Theta(k^2ut) \) time.

**Oracle sets** A useful notion in parameter advising introduced by Wheeler and Kececioglu [1] is the concept of an oracle, which is a perfect advisor that has access to the true accuracy of an alignment. For a given advisor set \( P \), an oracle selects parameter choice \( \arg\max_{P \in P} \{F(A_p)\} \), where again function \( F \) gives the true accuracy of an alignment. (Equivalently, an oracle is an advisor that uses the perfect estimator \( F \).) An oracle always picks the parameter choice that yields the highest accuracy alignment.

While an oracle is impossible to construct in practice, it gives a theoretical limit on the accuracy achievable by advising with a given set. Furthermore, if we can find the optimal advisor set for an oracle for a given cardinality bound k, which we call an oracle set, then the performance of an oracle on an oracle set gives a theoretical limit on how well advising can perform for a given bound k on the number of parameter choices.

Kececioglu and DeBlasio [4] show that finding an oracle set is NP-complete, and give the following integer linear program for finding an optimal oracle set. In our prior notation, an optimal oracle set for cardinality bound k is a set \( P \subseteq U \) with \( |P| \leq k \) that maximizes \( \sum_i w_i \max_{P \in P} \{F(A_p)\} \). To formulate this as an integer linear programming problem, let \( y_j \) for all \( j \in U \), and \( x_{ij} \) for all benchmarks \( B_i \) and all \( j \in U \), be integer variables that either have the value 0 or 1, where \( y_j = 1 \) iff parameter choice \( j \) is selected for oracle set \( P \), and \( x_{ij} = 1 \) iff choice \( j \) is used by the oracle on benchmark \( B_i \). The constraints are \( 0 \leq x_{ij} \leq y_j \leq 1 \), \( \sum_j y_j \leq k \), and for each benchmark \( B_i \) the constraint \( \sum_j x_{ij} = 1 \). The objective function is to maximize \( \sum_i w_i \sum_j x_{ij} a_{ij} \). An optimal solution to this integer linear program gives an optimal oracle set \( P^* = \{j \in U : y_j = 1\} \).

Learning an optimal oracle set of cardinality k, for a universe of \( u \) parameter choices and a training set of \( t \) benchmarks, involves solving the above integer linear program.
with $\Theta(n_t)$ variables and $\Theta(n_t)$ inequalities. Kececioglu and DeBlasio show that using the CPLEX integer linear programming solver, this formulation permits finding optimal oracle sets in practice even for cardinalities up to $k = 25$.

2.2 Constructing the universe for aligner advising

We extend parameter advising with a single aligner to aligner advising with a collection of aligners, by having the choices in the advisor set now specify both a particular aligner and a parameter setting for that aligner. To specify the universe that such an advisor set is drawn from during learning, we must determine what aligners to consider, and what parameter settings to consider for those aligners.

2.2.1 Determining the universe of aligners

For default aligner advising, where the advisor set consists of distinct aligners, each using their default parameter setting, we learned advisor sets over a universe containing as many of the commonly-used aligners from the literature as possible. Specifically, the universe for default advising consisted of the following 17 aligners: Clustal[18], Clustal2[19], Clustal Omega[20], DIALIGN[21], FSA[22], Kalign[23], MAFFT[13], MUMMALS[3], MUSCLE[24], MSAProbs[25], Opal[1], POA[26], PRANK[27], Probalign[28], ProbCons[29], SATé[30], and T-Coffee[31].

2.2.2 Determining the universe of parameter settings

For general aligner advising, we created an parameter universe for each of the aligners we used for default aligner advising, the full list of parameters used is found in Table 1. For each aligner, we enumerated parameter settings by forming a cross product of values for each of its tunable parameters. We determined the values for each tunable parameter by one of two ways. For aligners with web-server versions (namely Clustal Omega and ProbCons), we used all values recommended for each parameter. For all other aligners, we chose either one or two values above and below the default value for each parameter, to attain a cross product with less than 200 parameter settings. If a range was specified for a numeric parameter, values were chosen to cover this range as evenly as possible. For non-numeric parameters, we used all available options.
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| Total       | 2338               |                   |         |                             |                 |                 |

*a* Parameter settings retrieved from the Clustal Omega webserver at EBI ([www.ebi.ac.uk/Tools/msa/clustalo](http://www.ebi.ac.uk/Tools/msa/clustalo)).

*b* sp: sum-of-pairs score; spf: dimer approximation of sum-of-pairs score; spm: input dependent (sp if input is less than 100 sequences, spf otherwise); dp: dynamic programming score; ps: average profile sequence score; xp: cross profile score.

*Default values for the gap open penalty are -2.9 when the log-expectation profile is chosen, -1439 for sum-of-pairs using PAM200, and -300 for sum-of-pairs using VTML240. Alternate values are multiples of this default value.*

*e* MULTIALS is distributed with 29 precomputed hidden Markov models, each of which is associated with a setting of three tunable parameters.

*f* The substitution matrices used by Opal are shifted, scaled, and rounded to integer values in the range [0, 100] (BLOSUM62, BLOSUM75, BLOSUM90).

*Parameter settings retrieved from the ProbCons web-server at Stanford ([probcons.stanford.edu](http://probcons.stanford.edu))."
3 Results and Discussion

We evaluate the performance of advising through experiments on a collection of protein multiple sequence alignment benchmarks. A full description of the benchmark collection is given in [4], and is briefly summarized below. The experiments compare the accuracy of parameter and aligner advising to the accuracy of individual aligners using their default parameter settings.

The benchmark suites used in our experiments consist of reference alignments that are largely induced by performing structural alignment of the known three-dimensional structures of the proteins. Specifically, we use the BENCH suite of Edgar [33], supplemented by a selection of benchmarks from the PALI suite [34]. The entire benchmark collection consists of 861 reference alignments.

As is common in benchmark suites, easy-to-align benchmarks are highly over-represented in this collection, compared to hard-to-align benchmarks. To correct for this bias when evaluating average advising accuracy, we binned the 861 benchmarks in our collection by difficulty, where the difficulty of a benchmark is its average accuracy under three commonly-used aligners, namely Clustal Omega, MAFFT, and ProbCons, using their default parameter settings. We then divided the full range [0, 1] of accuracies into 10 bins with difficulties [(j−1)/10, j/10] for j = 1, . . . , 10. The weight $w_i$ of benchmark $B_i$ falling in bin j that we used for training is $w_i = (1/10)(1/n_j)$, where $n_j$ is the number of benchmarks in bin j. These weights $w_i$ are such that each difficulty bin contributes equally to the advising objective function $f(P)$. Note that with this weighting, an aligner that on every benchmark gets an accuracy equal to its difficulty, will achieve an average advising accuracy of roughly 50%.

3.1 Parameter advising

We first examine the results of parameter advising for a single aligner using the Facet estimator. We learned the coefficients for Facet by difference fitting on computed alignments obtained using the oracle set of cardinality $k=17$ found for the parameter universe for each aligner. (We trained the estimator on an oracle set of this cardinality to match the size of the universe for default aligner advising.) Given this estimator, we constructed greedy advisor sets for each aligner.

Figure 3 shows the accuracy of parameter advising using greedy advisor sets of cardinality $k \leq 15$, for each of the 10 aligners in Table 1 under 12-fold cross-validation. The plot shows advising accuracy on the testing data, averaged over all benchmarks and folds.

Almost all aligners benefit from parameter advising, though their advising accuracy eventually reaches a plateau. While our prior work [8] showed that parameter advising boosts the accuracy of the Opal aligner, Figure 3 shows this result is not aligner dependent.

3.2 Aligner advising

To evaluate aligner advising, we followed a similar approach, constructing an oracle set of cardinality $k=17$ over the union of the universe for default advising from Section 2.2.1 and the universe for general advising from Section 2.2.2, learning coefficients for Facet using difference fitting, and constructing greedy sets using Facet for default and general advising.
Fig 3. Accuracy of parameter advising using Facet. The plot shows advising accuracy for each aligner from Table 1 using parameter advising on greedy sets with the Facet estimator learned by difference fitting. The horizontal axis is the cardinality of the advisor set, and the vertical axis is the advising accuracy on testing data averaged over all benchmarks and folds, under 12-fold cross-validation.

Fig 4. Aligner advising and parameter advising using Facet. The plot shows default and general aligner advising accuracy, and parameter advising accuracy for Opal, MUMMALS, and Probalign, using the Facet estimator. The horizontal axis is the cardinality of the advisor set, and the vertical axis is advising accuracy on testing data averaged over all benchmarks and folds under 12-fold cross-validation.
Fig 5. Aligner advising and parameter advising using TCS. The plot shows default and general aligner advising accuracy, and parameter advising accuracy for Opal, MUMMALS, Probalign, and ProbCons, using the TCS estimator. The horizontal axis is the cardinality of the advisor set, and the vertical axis is advising accuracy on testing data averaged over all benchmarks and folds under 12-fold cross-validation.

Figure 4 shows the accuracy of default and general advising using greedy sets of cardinality $k \leq 15$, along with the three best parameter advising curves from Figure 3 for Opal, Probalign, and MUMMALS. The plot shows advising accuracy on testing data, averaged over benchmarks and folds.

The dashed red curve in Figure 4 also shows the accuracy of Opal for parameter advising with greedy sets computed over an alternate universe of much more fine-grained parameter choices. To construct this alternate universe, we first started with a set of over 16,000 parameter settings, and for each training fold chose the 15 parameters with highest average accuracy on each difficulty bin. Unioning these choices across the bins, and removing duplicates, gave a universe of 147 to 150 settings for each fold. Note that the dashed curve for parameter advising with Opal, using greedy sets from these finer universes for each fold, essentially matches the accuracy of general advising at cardinality $k \geq 4$.

3.2.1 Testing the significance of improvement

To test the statistical significance of the improvement in default advising accuracy over using a single default aligner, we used the one-tailed Wilcoxon sign test [35]. Performing this test in each difficulty bin, we found a significant improvement in accuracy ($p < 0.05$) on benchmarks with difficulty $(0.3, 0.4]$ at all cardinalities $2 \leq k \leq 15$, and on benchmarks with difficulty at most 0.4 at cardinality $6 \leq k \leq 9$.

We also tested the significance of the improvement of default advising over the best parameter advisor at each cardinality $k$ (namely MUMMALS for $k \leq 4$ and Opal for $k \geq 5$), and found that at cardinality $k \geq 5$ there is again significant improvement ($p < 0.05$) on benchmarks with difficulty $(0.3, 0.4]$. 
Fig 6. Accuracy of aligner advising compared to M-Coffee. The plot shows average accuracy for aligner advising using Facet, and meta-alignment using M-Coffee, on oracle sets of aligners. Performance on the default M-Coffee set of six aligners is indicated by large circles on the dotted vertical line. The horizontal axis is cardinality of the oracle sets, and the vertical axis is average accuracy on testing data over all benchmarks and folds under 12-fold cross-validation.

3.2.2 Advising with an alternate estimator

We also evaluated in the same way parameter advising and aligner advising on greedy sets using the TCS estimator [15] (the best other estimator for advising from the literature). Figure 5 shows results using TCS for parameter advising (on the four most accurate aligners), and for general and default aligner advising. Note that while TCS is sometimes able to increase accuracy above using a single default parameter, this increase is smaller than for Facet; moreover, TCS often has a decreasing trend in accuracy for increasing cardinality.

3.3 Comparing ensemble alignment to meta-alignment

Another approach to combining aligners is the so-called *meta-alignment* approach of M-Coffee [9] (described in Section 1). M-Coffee computes a multiple alignment using position-dependent substitution scores obtained from alternate alignments generated by a collection of aligners. By default, M-Coffee uses the following six aligners: Clustal2, T-Coffee, POA, MUSCLE, MAFFT, Dialign-T [32], PCMA [36], and ProbCons. The tool also allows use of Clustal, Clustal Omega, Kalign, AMAP [37], and Dialign-TX. Figure 6 shows the average accuracy of both M-Coffee and our ensemble approach with Facet, using the default aligner set of M-Coffee (the dotted vertical line with large circles), as well as oracle sets constructed over this M-Coffee universe of 13 aligners.

3.4 Advising accuracy within difficulty bins

Figure 7 shows advising accuracy within difficulty bins for default aligner advising compared to using the default parameter settings for the three aligners with highest
Fig 7. Accuracy of default aligner advising, and aligners with their default settings, within difficulty bins. In the bar chart on the left, the horizontal axis shows all ten benchmark bins, and the vertical bars show accuracy averaged over just the benchmarks in each bin. The accuracy of default advising using the Facet estimator is shown for the greedy sets of cardinality $k = 5$, along with the accuracy of the default settings for Probalign, Opal, and MUMMALS. The bar chart on the right shows accuracy uniformly averaged over the bins. In parentheses above the bars are the number of benchmarks in each bin.

average accuracy, namely MUMMALS, Opal, and Probalign. The figure displays the default advising result from Section 3.2 at cardinality $k = 5$. The bars in the chart show average accuracy over the benchmarks in each difficulty bin, as well as the average accuracy across all bins. (The number of benchmarks in each bin is in parentheses above the bars.) Note that aligner advising gives the greatest boost for the hardest-to-align benchmarks: for the bottom two bins, advising yields an 8% increase in accuracy over the best aligner using its default parameter setting.

3.5 Generalization of aligner advising

The results thus far have shown advising accuracy averaged over the testing data associated with each fold. We now compare the training and testing advising accuracy to assess how our method might generalize to data not in our benchmark set.

Figure 8 shows the average accuracy of default and general aligner advising on both training and testing data. Note that the drop between training and testing accuracy is much larger for general advising than for default advising, resulting in general advising performing worse than default advising though its training accuracy is much higher. This indicates that general advising is strongly overfitting to the training data, but could potentially achieve much higher testing accuracy. Additionally, there is a drop in training accuracy for default advising with increasing cardinality, though after its peak an advisor using greedy sets should remain flat in training accuracy as cardinality increases when using a strong estimator.
**3.6 Theoretical limit on advising accuracy**

An *oracle* is an advisor that uses a perfect estimator, always choosing the alignment from a set that has highest true accuracy. To examine the theoretical limit on how well aligner advising can perform, we compare the accuracy of aligner advising using Facet with the performance of an oracle. Figure 8 shows the accuracy of both default and general aligner advising using greedy sets, as well as the performance of an oracle using oracle sets computed on the default and general advising universes. (Recall an oracle set is an optimal advisor set for an oracle.) The plot shows advising accuracy on testing data, averaged over all benchmarks and folds. The large gap in performance between the oracle and an advisor using Facet shows the increase in accuracy that could potentially be achieved by developing an improved estimator.

**3.7 Composition of advisor sets**

Table 2 lists the greedy advisor sets for both default and general advising for all cardinalities $k \leq 10$. A consequence of the construction of greedy advisor sets is that the greedy set of cardinality $k$ consists of the entries in a column in the first $k$ rows of the table. The table shows these sets for just one fold from the 12-fold cross-validation. For general advising sets, an entry specifies the aligner that is used, and for aligners from the general advising universe, a tuple of parameter values in the order listed in Table 1. The two exceptions are MUMMALS, whose 6-tuple comes from its predefined settings file, and whose last three elements correspond to the three parameters listed in Table 1 and MSAProbs, whose empty tuple stands for its default setting. It is interesting that other than MSAProbs, the general advising set does not contain any aligner’s default parameter settings, though its values are close to the default setting.
Fig 9. Accuracy of aligner advising using a perfect estimator. The plot shows advising accuracy for default and general aligner advising, both on oracle sets for a perfect estimator, and on greedy sets for the Facet estimator. The horizontal axis is the cardinality of the advisor set, and the vertical axis is advising accuracy on testing data averaged over all benchmarks and folds under 12-fold cross-validation.

### Table 2. Greedy Default and General Advising Sets

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<td>Opal</td>
<td>Opal (VTML200, 45, 2, 45, 45)</td>
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<td>3</td>
<td>Probalign</td>
<td>Opal (BLSM62, 70, 3, 45, 42)</td>
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<td>MUSCLE</td>
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<td>6</td>
<td>T-Coffee</td>
<td>MSAProbs ()</td>
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<td>7</td>
<td>PRANK</td>
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<td>DIALIGN</td>
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<td>10</td>
<td>ProbCons</td>
<td>Opal (BLSM62, 45, 2, 45, 42)</td>
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3.8 Running time for advising

We compared the time to evaluate the Facet estimator on an alignment to the time needed to compute that alignment by the three aligners used for determining alignment difficulty: Clustal Omega, MAFFT, and ProbCons. To compute the average running time for these aligners on a benchmark, we measured the total time for each of these aligners to align all 861 benchmarks on a desktop computer with a 2.4 GHz Intel i7 8-core processor and 8 Gb of RAM. The average running time for Clustal Omega, MAFFT, and ProbCons was less than 1 second per benchmark, as was the average running time for Facet. As stated in Section 2.1.2, the time complexity for Facet is linear in the number of columns in an alignment, and should take relatively less time than computing an alignment for benchmarks with long sequences; the standard benchmark suites tend to include short sequences, however, which are fast to align. This time to evaluate Facet does not include the time to predict protein secondary structure, which is done once for the sequences in a benchmark, and was performed using PSIPRED version 3.2 with its standard settings. Secondary structure prediction with a tool like PSIPRED has a considerably longer running time than alignment, due to an internal PSI-BLAST search during prediction; on average, PSIPRED took just under 6 minutes per benchmark to predict secondary structure.

3.9 Further research

An important question left to explore is how to learn advisor sets that have improved generalization. While greedy advisor sets for general aligner advising achieve very high accuracy on training data, this does not translate to similar accuracy on testing data due to overfitting. Standard techniques from machine learning for improving generalization like regularization do not apply here, as the number of parameters for each aligner and the number of choices in the advisor set are both fixed. Applying this advising framework to DNA and RNA sequence alignment also seems fruitful.

4 Acknowledgements

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References


