Algorithms for Normalized Multiple Sequence Alignments

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Abstract
Sequence alignment supports numerous tasks in bioinformatics, natural language processing, pattern recognition, social sciences, and other fields. While the alignment of two sequences may be performed swiftly in many applications, the simultaneous alignment of multiple sequences proved to be naturally more intricate. Although most multiple sequence alignment (MSA) formulations are NP-hard, several approaches have been developed, as they can outperform pairwise alignment methods or are necessary for some applications. Taking into account not only similarities but also the lengths of the compared sequences (i.e. normalization) can provide better alignment results than both unnormalized or post-normalized approaches. While some normalized methods have been developed for pairwise sequence alignment, none have been proposed for MSA. This work is a first effort towards the development of normalized methods for MSA. We discuss multiple aspects of normalized multiple sequence alignment (NMSA). We define three new criteria for computing normalized scores when aligning multiple sequences, showing the NP-hardness and exact algorithms for solving the NMSA using those criteria. In addition, we provide approximation algorithms for MSA and NMSA for some classes of scoring matrices.

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1 Introduction

Sequence alignment lies at the foundation of bioinformatics. Several procedures rely on alignment methods for a range of distinct purposes, such as detection of sequence homology, secondary structure prediction, phylogenetic analysis, identification of conserved motifs or genome assembly. On the other hand, alignment techniques have also been reshaped and found applications in other fields, such as natural language processing, pattern recognition, or social sciences [1, 3, 8, 18].
Given its range of applications in bioinformatics, extensive efforts have been made to improve existing or developing novel methods for sequence alignment. The simpler ones compare a pair of sequences in polynomial time on their lengths, usually trying to find editing operations (insertions, deletions, and substitutions of symbols) that transform one sequence into another while maximizing or minimizing some objective function called edit distance [16]. This concept can naturally be generalized to align multiple sequences [28], adding another new layer of algorithmic complexity, though. In this case, most multiple sequence alignment (MSA) formulations lead to NP-hard problems [13]. Nevertheless, a variety of methods suitable for aligning multiple sequences have been developed, as they can outperform pairwise alignment methods on tasks such as phylogenetic inference [21], secondary structure prediction [12] or identification of conserved regions [24].

In order to overcome the cost of exact solutions, a number of MSA heuristics have been developed in recent years, most of them using the so-called progressive or iterative methods [17, 23, 25, 27]. Experimental data suggest that the robustness and accuracy of heuristics can still be improved, however [28].

Most approaches for pairwise sequence alignment define edit distances as absolute values, lacking some normalization that would result in edit distances relative to the lengths of the sequences. However, some applications may require sequence lengths to be taken into account. For instance, a difference of one symbol between sequences of length 5 is more significant than between sequences of length 1000. In addition, experiments suggest that normalized edit distances can provide better results than both unnormalized or post-normalized edit distances [18]. While normalized edit distances have been developed for pairwise sequence alignment [6, 18], none have been proposed for MSA to the best of our knowledge.

In this work, we propose exact and approximation algorithms for normalized MSA (NMSA). This is a first step towards the development of methods that take into account the lengths of sequences for computing edit distances when multiple sequences are compared.

The remainder of this paper is organized as follows. Section 2 introduces concepts related to sequence alignment and presents normalized scores for NMSA, followed by the complexity analysis of NMSA using those scores in Section 3. Next, Sections 4 and 5 describe exact and approximation algorithms, respectively. Section 6 closes the paper with the conclusion and prospects for future work.

2 Preliminaries

An alphabet $\Sigma$ is a finite non-empty set of symbols. A finite sequence $s$ with $n$ symbols in $\Sigma$ is seen as $s(1) \ldots s(n)$. We say that the length of $s$, denoted by $|s|$, is $n$. The (sub)sequence $s(p) \ldots s(q)$ of $s$, with $1 \leq p \leq q \leq n$, is denoted by $s(p:q)$. If $p > q$, $s(p:q)$ is the empty sequence, whose length is zero, and it is denoted by $\varepsilon$. We denote the sequence resulting from the concatenation of sequences $s$ and $t$ by $st$. A sequence of $n$ symbols $a$ is denoted by $a^n$. A $k$-tuple $S$ over $\Sigma^*$ is called a $k$-sequence and we write $s_1, \ldots, s_k$ to refer to $S$, where $s_i$ is the $i$-th sequence in $S$. Let $\Sigma := \Sigma \cup \{-\}$, where $\not\in \Sigma$ and the symbol $-$ is called a space. Let $S = s_1, \ldots, s_k$ be a $k$-sequence. An alignment of $S$ is a $k$-tuple $A = [s'_1, \ldots, s'_k]$ over $\Sigma^*$, where (a) each sequence $s'_h$ is obtained by inserting spaces in $s_h$; (b) $|s'_h| = |s_h|$ for each pair $h, i$, with $1 \leq h, i \leq k$; and (c) there is no $j$ in $\{1, \ldots, k\}$ such that $s'_1(j) = \ldots = s'_k(j) = \varepsilon$. Notice that $k$-tuples over $\Sigma^*$ are written enclosed by square brackets “[ ]”. The sequence $[s'_1(j), \ldots, s'_k(j)]$ is the column $j$ of the alignment $[s'_1, \ldots, s'_k]$. We denote the column $j$ of the alignment $A$ by $A(j)$ and by $A[j_1:j_2]$ the columns $j_1, j_1 + 1, \ldots, j_2$ of $A$. We say that the pair $[s'_1(j), s'_1(j)]$ aligns in $A$ or, simply, that $s'_h(j)$ and $s'_i(j)$ are aligned in $A$, and $|A| = |s'_i|$ is the length of the alignment $A$. It is easy to check that $\max_i\{|s_i|\} \leq |A| \leq \sum_i |s_i|$.
An alignment can be used to represent editing operations of insertions, deletions and substitutions of symbols in sequences, where the symbol - represents insertions or deletions. An alignment can also be represented in the matrix format. Thus, alignments $[aaa-, ab-, -cac]$ and $[-aaa-, ab-, -ca-c]$ of $aaa, ab, cac$ can be represented respectively as

$$
\begin{bmatrix}
  a & a & a & - \\
  a & b & - & - \\
  - & c & a & c \\
\end{bmatrix}
\quad \text{and} \quad
\begin{bmatrix}
  - & a & a & - \\
  a & b & - & - \\
  - & c & a & - \\
\end{bmatrix}.
$$

Let $I = \{i_1, \ldots, i_m\} \subseteq \{1, \ldots, k\}$ be a set of indices such that $i_1 < \cdots < i_m$ and let

$$A = [s_1 ^{i_1}, \ldots, s_k ^{i_m}]$$

be an alignment of $S = s_1, \ldots, s_k$. We write $S_I$ to denote the $m$-tuple $s_{i_1}, \ldots, s_{i_m}$. The alignment of $S_I$ induced by $A$ is the alignment $A_I$ obtained from the alignment $A$, considering only the corresponding sequences in $S_I$ and, from the resulting structure, removing columns where all symbols are -. In the following example, $A = [aaa-, ab-, -cac]$ is an alignment of $aaa, ab, cac$ and

$$
\begin{bmatrix}
  a & a & a \\
  a & b & - \\
\end{bmatrix}
$$

is an alignment of $aaa, ab$ induced by $A$. We denote by $A_S$ the set of all alignments of $S$.

For a problem $P$, we call $I_P$ the set of instances of $P$. If $P$ is a decision problem, then $P(I) \in \{\text{Yes, No}\}$ is the image of an instance $I$. If $P$ is an optimization (minimization) problem, there is a set $\text{Sol}(I)$ for each instance $I$, a function $v$ defining a non-negative rational number for each $X \in \text{Sol}(I)$, and a function $\text{opt}_v(I) = \min_{X \in \text{Sol}(I)} \{v(X)\}$. We use $\text{opt}$ instead of $\text{opt}_v$ if $v$ is obvious. Let $A(I)$ be a solution computed by an algorithm $A$ with input $I$, and $A(I) \geq \text{opt}(I)$. We say that $A$ is an $\alpha$-approximation for $P$ if $A(I) \leq \alpha \text{opt}(I)$, for each $I \in I_P$, with $\alpha \geq 1$. We say that $\alpha$ is an approximation factor for $P$.

The alignment problem is a collection of decision and optimization problems whose instances are finite subsets of $\Sigma^*$ and $\text{Sol}(S) = A_S$, for each instance $S$. For scoring alignments, is called criterion for $P$ and we call $v[A]$ the cost of the alignment $A$. The $v$-optimal alignment $A$ of $S$ is such that $v[A] = \text{opt}(S)$. Thus, we state the following general optimization problems using the criterion $v$:

**Problem 1 (Alignment with criterion $v$).** Given a $k$-sequence $S$, with $k \in \mathbb{N}$, find a $v$-optimal alignment of $S$.

We also need the decision version of the alignment problem with criterion $v$, where we are given a $k$-sequence $S$ and a number $d \in \mathbb{Q}_\geq$, and we want to decide whether there exists an alignment $A$ of $S$ such that $v[A] \leq d$.

Usually an alignment $v$ is defined from a scoring matrix $\gamma$ is a rational matrix such that the elements in $\Sigma$. are indices of its rows and columns. For $a, b \in \Sigma$, and a scoring matrix $\gamma$, we denote by $\gamma_{a \rightarrow b}$ the entry of $\gamma$ in line $a$ and column $b$. The value $\gamma_{a \rightarrow b}$ defines the score for a substitution if $a, b \in \Sigma$, for an insertion if $a = -$, and for a deletion if $b = -$. The entry $\gamma_{- \rightarrow -}$ is not defined.

Given a scoring function $v_\gamma$ for alignments that depends on a scoring matrix $\gamma$, we say that two scoring matrices $\gamma$ and $\rho$ are equivalent considering $v$ when $v_\gamma[A] \leq v_\gamma[B]$ if and only if $v_\rho[A] \leq v_\rho[B]$ for any pair of alignments $A, B$ of sequences $s, t$. If $\rho$ is a matrix obtained from $\gamma$ by multiplying each entry of $\gamma$ by a constant $c > 0$, then $v_\rho[A] = c v_\gamma[A]$ and $v_\rho[B] = c v_\gamma[B]$, which implies that $\gamma$ and $\rho$ are equivalent. As a consequence, when the scoring function is $v_\lambda$, or $v_\gamma$, and it is convenient, we can suppose that all entries of $\gamma$ are integers instead of rationals, according to the definition.

A $k$-vector $\vec{j} = [j_1, \ldots, j_k]$ is a $k$-tuple, where $j_i \in \mathbb{N} = \{0, 1, 2, \ldots\}$. We say that $j_i$ is the $i$-th element of $\vec{j}$. The $k$-vector $\vec{0}$ is such that all its elements are zero. If $\vec{j}$ and $\vec{h}$ are $k$-vectors, we write $\vec{j} \leq \vec{h}$ if $j_i \leq h_i$ for each $i$; and $\vec{j} < \vec{h}$ if $\vec{j} \leq \vec{h}$ and $\vec{j} \neq \vec{h}$. A sequence of $k$-vectors $\vec{j}_1, \vec{j}_2, \ldots$ is in lexicographical order if $\vec{j}_i \leq \vec{j}_{i+1}$ for each $i$. 

ISAAC 2021
Consider $S = s_1, \ldots, s_k$ a $k$-sequence with $n_i = |s_i|$ for each $i$ and $\tilde{n} = [n_1, \ldots, n_k]$. Let $V_{\tilde{n}} = \{\tilde{j} : \tilde{j} \leq \tilde{n}\}$ be the set of all $k$-vectors $\tilde{j}$ such that $\tilde{j} \leq \tilde{n}$. For example, if $k = 3$ and $\tilde{n} = [1, 2, 1]$, then $V_{\tilde{n}} = \{(x, y, z) : x, y, z \in \mathbb{N}, x \leq 1, y \leq 2, z \leq 1\}$. Notice that if $n_i = n$ for all $i$, then $|V_{\tilde{n}}| = (n+1)^k$.

Define $S(\tilde{j}) = s_1(j_1), \ldots, s_k(j_k)$ a column $\tilde{j}$ in $S$ and we say that $S(1 : \tilde{j}) = s_1(1 : j_1), \ldots, s_k(1 : j_k)$ is the prefix of $S$ ending in $\tilde{j}$. Thus, $S = S(1 : \tilde{n})$. Besides that, if $A$ is an alignment and $\tilde{v} = [j_1, \ldots, j_n]$, then $A[\tilde{v}] = A(j)$.

Denote by $B^k$ the set of $k$-bit vectors $[b_1, \ldots, b_k]$, where $b_i \in \{0, 1\}$ for each $i$. Now, for $\tilde{b} \leq \tilde{j}$, define $\tilde{b} \cdot S(\tilde{j}) = [x_1, \ldots, x_k] \in \Sigma^k_\pm$ such that $x_i = s_i(j_i)$ if $b_i = 1$ and $x_i = -1$ otherwise. Therefore, given an alignment $A$ of $S(1 : \tilde{j})$, there exists $\tilde{b} \in B^k$, with $\tilde{b} \leq \tilde{j}$, such that $A[|A|] = \tilde{b} \cdot S(|\tilde{i}|)$. In other words, if $\tilde{n} = [n_1, \ldots, n_k]$ and $\tilde{b} = [b_1, \ldots, b_k]$, we have $b_i = 1$ if and only if $s_i(n_i)$ is in the $i$-th row of the last column of $A$. We also define the operation $\tilde{j} - \tilde{b} = [j_1 - b_1, \ldots, j_k - b_k]$. Notice that $|B_k| = 2^k$.

Consider a scoring matrix $\gamma$. Let $s, t \in \Sigma^*$, with $|n| = |s|, m = |t|$. A simple criterion for scoring alignments using the function $v_{A_{\gamma}}$ follows. For an alignment $[s', t']$ of $s, t$ we define

$$v_{A_{\gamma}}[s', t'] = \sum_{j=1}^{\gamma_{s'j} - \gamma_{t'j}}.$$ 

We say that $v_{A_{\gamma}}[s', t']$ is a $v_{A_{\gamma}}$-score of $s, t$. The optimal function for this criterion is denoted by $optA_{\gamma}$ and an alignment $A$ of $s, t$ is called an $A$-optimal alignment of $s, t$ if $v_{A_{\gamma}}[A] = optA_{\gamma}(s, t)$.

Now, suppose that $n \geq m$. Needleman and Wunch [20] proposed an $O(n^2)$-time algorithm for computing $optA_{\gamma}(s, t)$. If $optA_{\gamma}$ is a Levenstein distance, Masek and Paterson [19] presented an $O(n^2/\log n)$-time algorithm using the “Four Russian’s Method”. Crochemore, Landau and Ziv-Ukelson [11] extended this result for real arrays, describing an $O(n^2/\log n)$-time algorithm. Indeed, there is no algorithm to determine $optA_{\gamma}(s, t)$ in $O(n^{2-\delta})$-time for any $\delta > 0$, unless SETH is false [7]. Andoni, Krauthgamer and Onak [2] described a nearly linear time algorithm approximating the edit distance within an approximation factor $\text{poly}(\log n)$. Later, Chakraborty et al. [10] presented an $O(n^{2-2/7})$-time $\alpha$-approximation for this problem, where $\alpha$ is constant.

Marzal and Vidal [18] defined another criterion for scoring alignments of two sequences called $v_{N_{\gamma}}$-score, which is a normalization of $v_{A_{\gamma}}$-score, as follows:

$$v_{N_{\gamma}}[A] = \begin{cases} 
0, & \text{if } |A| = 0, \\
|v_{A_{\gamma}}[A]|/|A|, & \text{otherwise}.
\end{cases}$$

The optimal function for this criterion is $\text{opt}N_{\gamma}(s, t) = \min_{A \in A_{\gamma}} \{v_{N_{\gamma}}[A]\}$, and an $N$-optimal alignment $A$ of $s, t$ is such that $v_{N_{\gamma}}[A] = \text{opt}N_{\gamma}(s, t)$.

A naive dynamic programming algorithm was proposed by Marzal and Vidal [18] to obtain an $N$-optimal alignment of two sequences in $O(n^3)$-time. Using fractional programming, Vidal, Marzal and Aibar [26] presented an algorithm with running time $O(n^3)$, requiring $O(n^2)$-time in practice, similarly to the classical (unnormalized) edit distance algorithm. Further, Arslan and Egecioglu [6] described an $O(n^2 \log n)$-time algorithm to solve this problem.

Let $L$ be an $A$-optimal alignment of maximum length of 2-sequence $S = s, t$, with $|s| = n$ and $|t| = m$. Considering $\tilde{n} = [n, m]$ and $\tilde{b}$ a bit vector such that $A[|A|] = \tilde{b} \cdot S(\tilde{n})$, the length of a maximum length $A$-optimal alignment of $S(1 : \tilde{n} - \tilde{b})$ must be $|A| - 1$. Thus, the maximum length $L(n, m)$ can be found by a dynamic programming formula as following:

$$L(0, 0) = 0, \quad L(0, j) = j, \quad L(i, 0) = i,$$
\[
L(i, j) = \max \begin{cases} 
L(i - 1, j), & \text{if } d(i, j) = d(i - 1, j) + \gamma_{s(i)\rightarrow} \\
L(i, j - 1), & \text{if } d(i, j) = d(i, j - 1) + \gamma_{t(j)\rightarrow} \\
L(i - 1, j - 1), & \text{if } d(i, j) = d(i - 1, j - 1) + \gamma_{s(i)\rightarrow t(j)}
\end{cases} + 1, i, j > 0,
\]

where \(d(i, j) = \text{opt} A_\gamma(s(1:i), t(1:j))\). Therefore, the maximum length A-optimal alignment of \(s, t\) can be obtained in \(O(nm)\)-time. The following theorem shows that we can propose a simple approximation algorithm to find an A-optimal alignment of maximum length.

\textbf{Theorem 2.1.} Let \(s, t\) be sequences of lengths \(n, m\), respectively, and let \(L(n, m)\) be the maximum length of an A-optimal alignment of \(s, t\). Then,

\[
\text{opt} A_\gamma(s, t)/L(n, m) \leq 2 \text{opt} N_\gamma(s, t),
\]

and it can be computed in \(O(n^2)\)-time if \(n = m\). Moreover, this ratio is tight, i.e., for any positive rational \(\varepsilon\), there exists a scoring matrix \(\gamma\), sequences \(s, t\) and an A-optimal alignment of \(s, t\) with maximum length \(A\) such that \(\text{opt} A_\gamma(s, t)/|A| = \text{vA}_\gamma[A]/|A| = (2 - \varepsilon) \text{opt} N_\gamma(s, t)\).

\textbf{Proof.} Let \(A\) be an A-optimal alignment with maximum length computed by the heuristic above in \(O(nm)\)-time and space. Let \(B\) be an N-optimal alignment. Thus, \(\text{vA}_\gamma[A] \leq \text{vA}_\gamma[B]\).

Moreover, \(|B| \leq n + m \leq 2 \max(n, m) \leq 2|A|\), that is, \(|A| \geq |B|/2\). Therefore, \(\text{vN}_\gamma[A] = \frac{\text{vA}_\gamma[A]}{|A|} \leq \frac{\text{vA}_\gamma[B]}{|B|/2} = 2 \text{opt} N_\gamma(s, t)\).

We present now two sequences and a scoring matrix \(\gamma\) such that the solution given by the heuristic is at least \(2 - \varepsilon\) times the vN-\(\gamma\)-score of an N-optimal alignment, for any \(\varepsilon\) in \(Q_\gamma\).

Let \(\Sigma = \{a, b\}\), \(\gamma\) be a scoring matrix such that \(\gamma_{a\rightarrow} = \gamma_{b\rightarrow} = 1/\varepsilon\) and \(\gamma_{a\leftarrow} = \gamma_{b\leftarrow} = 2/\varepsilon - 1\) and \(a^\alpha, b^\beta \in \Sigma^*, n \in \mathbb{N}^*.\) Observe that the \(vN_\gamma\)-score of any alignment of \((a^\alpha, b^\beta)\), where \([a, b]\) is aligned in \(k\) columns, is \(2n/\varepsilon - k\). Thus, \(\text{opt} A_\gamma(a^\alpha, b^\beta) = \min_{1 \leq k \leq n} \{2n/\varepsilon - k\} = 2n/\varepsilon - n = (2/\varepsilon - 1) n\) which implies \([a^\alpha, b^\beta]\) is the A-optimal alignment with maximum length. Since \(\text{opt} N_\gamma(a^\alpha, b^\beta) \leq \text{vN}_\gamma([a^\alpha-n, -b^\beta]) = 1/\varepsilon\), it follows

\[
\text{opt} A_\gamma(s, t)/\text{vA}_\gamma(s, t) = (2/\varepsilon - 1) n/n = (2 - \varepsilon)/\varepsilon \geq (2 - \varepsilon) \text{opt} N_\gamma(a^\alpha, b^\beta).
\]

We define now classes of scoring matrices. The usual class of scoring matrices \(M^C\) has the following properties: for all symbols \(a, b, c \in \Sigma\), we have (a) \(\gamma_{a\rightarrow b} > 0\) if \(a \neq \emptyset\), and \(\gamma_{a\rightarrow b} = 0\) if \(\emptyset = b\); (b) \(\gamma_{a\rightarrow b} = \gamma_{b\rightarrow a}\) and (c) \(\gamma_{a\rightarrow c} \leq \gamma_{a\rightarrow b} + \gamma_{b\rightarrow c}\). The class \(M^\gamma\) of scoring matrices is such that for all symbols \(a, b, c \in \Sigma\), we have (a) \(\gamma_{a\rightarrow a} = \gamma_{a\rightarrow a} > 0\); (b) \(\gamma_{a\rightarrow b} > 0\) if \(a \neq \emptyset\), and \(\gamma_{a\rightarrow b} = 0\) if \(a = \emptyset\); (c) if \(b \leq a \rightarrow c\rightarrow a\), then \(\gamma_{a\rightarrow b} = \gamma_{a\rightarrow c}\); (d) \(\gamma_{a\rightarrow b} \leq \gamma_{b\rightarrow a} + \gamma_{b\rightarrow c}\); and (e) \(\text{min}\{\gamma_{a\rightarrow c}, \gamma_{a\rightarrow b} + \gamma_{b\rightarrow c}\} \leq \gamma_{a\rightarrow b} + \gamma_{b\rightarrow c}\). Moreover, the class \(M^\gamma\) is such that (a) \(M^\gamma \subseteq M^C\) and (b) \(\gamma_{a\rightarrow a} \leq 2\gamma_{b\rightarrow a}\) for each \(a, b \in \Sigma\).

For a set \(S\), we say that the \(\gamma\)-function \(f : S \times S \rightarrow \mathbb{R}\) is a metric on \(S\) if, for all \(s, t, u \in S\), the distance \(f\) satisfies: (1) \(f(s, s) = 0\) (reflexive); (2) \(f(s, t) > 0\) if \(s \neq t\) (positive); (3) \(f(s, t) = f(t, s)\) (symmetry); and (4) \(f(s, u) \leq f(s, t) + f(t, u)\) (triangle inequality).

If a given criterion \(\nu\) depends on a scoring matrix \(\gamma\) and it is a metric on \(\Sigma^*\), we say that the scoring matrix \(\gamma\) induces a \(\nu\)-distance on \(\Sigma^*\). Sellers [22] showed that matrices in \(M^C\) induce an \(\text{opt} A_\gamma\)-distance on \(\Sigma^*\) and Araujo and Soares [5] showed that \(\gamma \in M^\gamma\) if and only if \(\gamma\) induces an \(\text{opt} A_\gamma\)-distance on \(\Sigma^*\). Furthermore, \(\gamma \in M^\gamma\) if and only if \(\gamma\) induces an \(\text{opt} N_\gamma\)-distance on \(\Sigma^*\). Figure 1 shows the relationship between these classes.

\textbf{2.1 \(vSP_\gamma\)-score for \(k\) sequences}

Consider a scoring matrix \(\gamma\). Let \(S = s_1, \ldots, s_k\) be a \(k\)-sequence and \(A = [s'_1, \ldots, s'_k]\) be an alignment of \(S\). The criterion \(vSP_\gamma\), also called \(SP\)-score, for scoring the alignment \(A\) is

\[
vSP_\gamma[A] = \sum_{h=1}^{k} \sum_{i=h+1}^{k} vA_\gamma[A_{(h,i)}].
\]
We define $\text{optSP}_\gamma$ as the optimal function for the criterion $vSP_\gamma$. An alignment $A$ of $S$ such that $vSP_\gamma[A] = \text{optSP}_\gamma(S)$ is called $vSP_\gamma$-optimal alignment. Regardless its decision or optimization version, we call this the multiple sequence alignment problem (MSA). Formally,

**Problem 2 (Multiple sequence alignment).** Let $\gamma$ be a fixed scoring matrix. Given a $k$-sequence $S$, find a $vSP_\gamma$-optimal alignment of $S$.

In order to compute $\text{optSP}_\gamma$, we extend the definition of $vSP_\gamma$ considering a column of an alignment $A = [s'_1, \ldots, s'_k]$ as its parameter. Thus, $vSP_\gamma(A(j)) = \sum_{i < h} \gamma_{s'_i(j) \rightarrow s'_h(j)}$ assuming that $\gamma_{\rightarrow \rightarrow} = 0$ and

$$\text{optSP}_\gamma(S) = \text{optSP}_\gamma(S(1:\bar{n})) = \min_{\bar{b} \in B^k, \bar{\gamma} \leq \bar{j}} \{ \text{optSP}_\gamma(S(1:\bar{n} - \bar{b})) + vSP_\gamma[\bar{b} \cdot S(\bar{n})] \}.$$  \hspace{1cm} (2)

Recurrence (2) can be computed using a dynamic programming algorithm, obtaining $D(j) = \text{optSP}_\gamma(S(1:j))$ for all $j \leq \bar{n}$. This task can be performed by generating all indexes of $D$ in lexicographical order, starting with $D(\bar{0}) = 0$, as presented in Algorithm 1.

**Algorithm 1** $vSP_\gamma$-optimal alignment of $S$.

- **Input:** $S = s_1, \ldots, s_k \in (\Sigma^*)^k$
- **Output:** $\text{optSP}_\gamma(S)$

1. $D(\bar{0}) \leftarrow 0$
2. for each $j \leq \bar{n}$ in lexicographical order do
3. $D(j) \leftarrow \min_{\bar{b} \in B^k, \bar{\gamma} \leq \bar{j}} \{ D(j - \bar{b}) + vSP_\gamma[\bar{b} \cdot S(\bar{j})] \}$
4. return $D(\bar{n})$

Suppose that $|s_i| = n$ for each $i$. Notice that the space to store the matrix $D$ is $\Theta((n+1)^k)$ and thus Algorithm 1 uses $\Theta((n+1)^k)$-space. Besides that, Algorithm 1 checks, in the worst case, $\Theta(2^k)$ entries for computing all entries in the matrix $D$ and each computation spends $\Theta(k^3)$-time. Therefore, its running time is $O(2^k k^3(n+1)^k)$. Observe that when the distances between sequences are small, not all entries in $D$ need to be computed, such as in the Carrillo and Lipman’s algorithm [9].

### 2.2 $V^\gamma$-score for $k$ sequences

In this section we define a new criteria to normalize the $vSP_\gamma$-score of a multiple alignment. The symbol aligned to the same symbol does not contribute to the definition of scoring, and thus this entry is not defined. However, as all the criteria are additive, it is convenient to consider $\gamma_{\rightarrow \rightarrow} = 0$. The new criteria for aligning sequences takes into account the length of the alignments according to the following:

$$V^\gamma[A] = \begin{cases} 0, & \text{if } |A| = 0, \\
\frac{vSP_\gamma[A]}{|A|}, & \text{otherwise}, \end{cases}$$  \hspace{1cm} (3)
We show a polynomial time reduction from MSA that NMSA check in polynomial time on the length of Lemma 3.1.

We define \( \text{optNSP}_z \) as the optimal function for the criterion \( V_3^z \). An alignment \( A \) of \( S \) such that \( V_3^z[A] = \text{optNSP}_z(S) \) is called \( V_3^z \)-optimal alignment. Moreover, regardless its decision or optimization version, we establish the criterion \( V_3^z \) for the normalized multiple sequence alignment problem (NMSA-z), for \( z = 1, 2, 3 \). Formally,

\[ \text{Problem 3 (Normalized multiple sequence alignment with score } V_3^z \text{). Let } \gamma \text{ be a fixed scoring matrix and } z \in \{1, 2, 3\}. \text{Given a } k\text{-sequence } S, \text{ find a } V_3^z\text{-optimal alignment of } S. \]

3 Complexity

We study now the complexity of the multiple sequence alignment problem for each new criterion defined in Section 2. We consider the decision version of the computational problems and we prove NMSA-z is NP-complete for each \( z \) when the following additional restrictions for the scoring matrix \( \gamma \) hold: \( \gamma_{a \rightarrow b} = \gamma_{b \rightarrow a} \) and \( \gamma_{a \rightarrow b} = 0 \) if and only if \( a = b \) for each pair \( a, b \in \Sigma \). Elias [13] shows that, even considering such restrictions, MSA is NP-complete.

We show a polynomial time reduction from MSA to NMSA-z.

Consider a fixed alphabet \( \Sigma \) and a scoring matrix \( \gamma \) with the restrictions above. Let \( \sigma \notin \Sigma \) be a new symbol and \( \Sigma^\sigma = \Sigma \cup \{\sigma\} \). Let \( G \) be a fixed (constant) positive integer such that each entry in \( \gamma \) is at most \( G \). We define a scoring matrix \( \gamma^\sigma \) such that \( \gamma^\sigma_{a \rightarrow b} = \gamma_{a \rightarrow b}, \gamma^\sigma_{a \rightarrow \sigma} = \gamma_{\sigma \rightarrow a} = G \) and \( \gamma^\sigma_{\sigma \rightarrow \sigma} = 0 \), for each pair \( a, b \in \Sigma \).

For an instance \((S = s_1, \ldots, s_k, C)\) of MSA, let \( S^L = s_1 \sigma \sigma^L, \ldots, s_k \sigma^L \), where \( L = \max_j \{|s_i|\} \) and \( N = \binom{k}{2} M \). Define \( l \) as the tail length of an alignment \( A \) if \( A[i,j] = \sigma \) for each \( i = 1, \ldots, k \) and \( j = l + 1, l + 2, \ldots, |A| \), i.e., every symbol in the last \( l \) columns of \( A \) is \( \sigma \). We say that an alignment of \( S^L \) is canonical if its tail length is \( L \). If \( A = [s_1^\sigma \sigma^L, \ldots, s_k^\sigma \sigma^L] \) of \( S^L \). The two following results are useful to prove Theorem 3.3, which is the main result of this section. The proofs of these two lemmas can be found in [4].

\[ \text{Lemma 3.1. There exists a canonical alignment of } S^L \text{ which is } V_3^z\sigma \text{-optimal for each } z. \]

\[ \text{Lemma 3.2. If } C \geq k^2 MG, \text{ then MSA}(S, C) = \text{NMSA-z}(S^L, C^z) = \text{Yes, for each } z. \]

\[ \text{Theorem 3.3. NMSA-z is NP-complete for each } z. \]

**Proof.** Given a \( k\)-sequence \( S \) over \( \Sigma^\sigma \), an alignment \( A \) of \( S \) and a integer \( C \), it is easy to check in polynomial time on the length of \( A \) that \( V_3^z[A] \leq C \), for \( z \in \{1, 2, 3\} \), and then NMSA-z is in NP.

Consider now \( C^1 := C^2 := C/L, C^3 := C/(\binom{k}{2}) L \) and \( L := Nk^2 MG \) and then we prove that MSA(S, C) = Yes if and only if NMSA-z(S^L, C^z) = Yes for each \( z \in \{1, 2, 3\} \). If \( C \geq k^2 MG \), the Lemma 3.2 holds trivially. Thus, we assume \( C < k^2 MG \). Suppose that MSA(S, C) = Yes and, hence, there exists an alignment \( A \) such that \( v\text{SP}_z[A] \leq C \):

\[ \begin{align*}
V_3^1[A^L] &= \frac{\text{vSP}_z[A^L]}{|A^L|} \leq \frac{\text{vSP}_z[A^L]}{L} = \frac{\text{vSP}_z[A]}{L} \leq \frac{C}{L} = C^1, \\
V_3^2[A^L] &= \frac{\sum_{h=1}^{k} \text{vA}_z[A^L_{[h,i]}]}{|A^L_{[h,i]}|} \leq \frac{\sum_{h=1}^{k} \text{vA}_z[A^L_{[h,i]}]}{L} \leq \frac{\text{vSP}_z[A^L]}{L} \leq \frac{C}{L} = C^2,
\end{align*} \]
\[
V^3_\gamma[A^L] = \frac{vSP_{\gamma^*}[A^L]}{N + L} \leq \sum_{h=1}^{k-1} \sum_{i=h+1}^k |A^L_{[h,i]}| \leq \frac{vSP_{\gamma^*}[A^L]}{N + L} = \frac{vSP_{\gamma^*}[A^L]}{L} \leq \frac{C}{L} = C^3,
\]

where the first inequality in each equation follows since either \(A^L\) or each alignment induced by \(A^L\) has length at least \(L\) and the second inequality follows since \(vSP_{\gamma^*}[A] \leq C\). Thus, if \(\text{MSA}(S, C) = \text{Yes}\) then \(\text{MSA}^{-\gamma}(S^L, C^2) = \text{Yes}\).

Suppose that \(\text{MSA}^{-\gamma}(S^L, C^2) = \text{Yes}\). It follows from Lemma 3.1 that, for each \(z\), there exists a canonical alignment \(A^L\) such that \(V^2_{\gamma^*}[A^L] \leq C^2\). Thus, considering \(V^3_{\gamma^*}[A^L] \leq C^3\), we have

\[
vSP_{\gamma}[A] = vSP_{\gamma^*}[A^L] = (N + L) \frac{vSP_{\gamma^*}[A^L]}{N + L} \leq (N + L) \frac{vSP_{\gamma^*}[A^L]}{|A^L|} = (N + L) V^3_{\gamma^*}[A^L]
\]

which the first equality holds since \(A^L\) is canonical, the first inequality holds since \(|A^L| \leq N + L\) and the second and the third inequalities hold by hypothesis. Considering \(V^3_{\gamma^*}[A^L] \leq C^2\), we have

\[
vSP_{\gamma}[A] = vSP_{\gamma^*}[A^L] = (N + L) \frac{vSP_{\gamma^*}[A^L]}{N + L} = (N + L) \frac{vSP_{\gamma^*}[A^L]}{N + L} \leq (N + L) \frac{vSP_{\gamma^*}[A^L]}{|A^L_{[h,i]}|} = (N + L) \frac{vA_{\gamma^*}[A^L_{[h,i]}]}{N + L}
\]

where the first equality holds since \(A^L\) is canonical, the first inequality holds since, for each \(h, i, |A^L_{[h,i]}| \leq N + L\), and the second and the third inequalities hold by hypothesis. And finally, considering \(V^3_{\gamma^*}[A^L] \leq C^3\), we have

\[
vSP_{\gamma}[A] = vSP_{\gamma^*}[A^L] = (N + \binom{k}{2} L) \frac{vSP_{\gamma^*}[A^L]}{N + \binom{k}{2} L} \leq (N + \binom{k}{2} L) \frac{vSP_{\gamma^*}[A^L]}{|A^L_{[h,i]}|} = (N + \binom{k}{2} L) \frac{vSP_{\gamma^*}[A^L]}{|A^L_{[h,i]}|} \leq (N + \binom{k}{2} L) C^3
\]

where the first equality holds since \(A^L\) is canonical, the first inequality holds since the sum of lengths of two sequences induced by a canonical alignment is at most \(N + \binom{k}{2} L\) and the second and the third inequalities hold by hypothesis.

Therefore, if \(\text{MSA}^{-\gamma}(S^L, C^3) = \text{Yes}\) then \(vSP_{\gamma}[A] < 1 + C\), for any \(z \in \{1, 2, 3\}\). Since the entries in the scoring matrix are integers, we have that \(vSP_{\gamma}[A]\) is an integer. And since \(C\) is an integer, it follows that \(vSP_{\gamma}[A] \leq C\).
4 Exact algorithms

4.1 NMSA-1

Let $S = s_1, \ldots, s_k$ be a $k$-sequence and $A = [s'_1, \ldots, s'_k]$ be an alignment of $S$. As defined in Equation (3), $V^1_n[A]$ takes into account the length of $A$, and the optimal function is given by $\text{opt}V^1_n(S) = \min_{A \in \mathcal{A}_n} \{V^1_n[A]\}$. The $V^1_n$-optimal alignment of $S$ is an alignment $A$ such that $V^1_n[A] = \text{opt}V^1_n(S)$. Thus, in NMSA-1 we are given a $k$-sequence $S$ and we want to compute $\text{opt}V^1_n(S)$ for a fixed matrix $\gamma$. We can solve NMSA-1 by calculating the minimum SP-score considering every possible length of an alignment. That is, we compute the entries of a table $D$ indexed by $V_2 \times \{0, 1, \ldots, N\}$, where $N = \sum_{i=1}^k |s_i|$. The entry $D(\vec{v}, L)$ stores the score of an alignment of $S(\vec{v})$ of length $L$ with lowest SP-score. Notice that $D(\vec{0}, 0) = 0$. The running time of Algorithm 2 is $O(D_0 \times \gamma)$, plus the running time of line 7. Each entry of $D$ indexed by $\vec{v}, L \in \mathbb{R}$ is computed in $\Theta(2^k \times N)$. Therefore, the table entries can be calculated as:

$$D(\vec{v}, L) = \begin{cases} 0, & \text{if } \vec{v} = \vec{0}, L = 0, \\ \infty, & \text{if } \vec{v} = \vec{0}, L \neq 0 \text{ or } \vec{v} \neq \vec{0}, L = 0, \\ \min_{\vec{b} \in \mathcal{B}_k} \{D(\vec{v} - \vec{b}, L - 1) + \gamma \cdot \vec{v} \cdot \vec{b} \cdot S(\vec{v})\}, & \text{otherwise}. \end{cases}$$

Table $D$ is computed for all possible values of $L = 0, \ldots, N$. Consequently, $\text{opt}V^1_n(S) = \min_L \{D(\vec{0}, L) / L\}$ is returned. Algorithm 2 describes this procedure more precisely.

Algorithm 2 $V^1_n$-optimal alignment of $S$.

**Input**: $k$-sequence $S = s_1, \ldots, s_k$ such that $n_i = |s_i|

**Output**: $\text{opt}V^1_n(S)$

1. $D(\vec{0}, 0) \leftarrow 0$
2. for each $L \neq 0$ do $D(\vec{0}, L) \leftarrow \infty$
3. for each $\vec{v} \neq \vec{0}$ do $D(\vec{v}, 0) \leftarrow \infty$
4. for each $0 < \vec{v} \leq \vec{n}$ in lexicographical order do
5. for each $L \leftarrow 1, 2, \ldots, N$ do
6. $D(\vec{v}, L) \leftarrow \min_{\vec{b} \in \mathcal{B}_k} \{D(\vec{v} - \vec{b}, L - 1) + \gamma \cdot \vec{v} \cdot \vec{b} \cdot S(\vec{v})\}$
7. return $\min_L \{D(\vec{0}, L) / L\}$

Suppose that $n_i = |s_i| = n$ for each $i$. Notice that the space to store the matrix $D$ is $\Theta(N(n+1)^k)$. The time consumption of Algorithm 2 corresponds to the time needed to fill the table $D$ up, plus the running time of line 7. Each entry of $D$ can be computed in $O(2^k \times n)$-time. Therefore, the algorithm spends $O(2^k \times n \cdot (n+1)^k)$-time to compute the entire table $D$, since $D$ has $\Theta(N(n+1)^k)$ entries. Line 7 is computed in $\Theta(2^k)$-time. Therefore, the running time of Algorithm 2 is $O(2^k \times n \cdot (n+1)^k + \Theta(N) = O(2^k \times n \cdot (n+1)^k)$. If $N = kn$, it follows that the total running time is $O(2^k \times n \cdot (n+1)^{k+1})$.

4.2 NMSA-2

Let $S = s_1, \ldots, s_k$ be a $k$-sequence and $A = [s'_1, \ldots, s'_k]$ be an alignment of $S$. As defined in Equation (4), $V^2_n[A]$ takes into account the length of the induced alignment $A$, and the optimal function is given by $\text{opt}V^2_n(S) = \min_{A \in \mathcal{A}_n} \{V^2_n[A]\}$. The $V^2_n$-optimal alignment of $S$ is an alignment $A$ such that $V^2_n[A] = \text{opt}V^2_n(S)$. Then, in NMSA-2 we are given a $k$-sequence $S$ and we want to compute $\text{opt}V^2_n(S)$ for a fixed matrix $\gamma$.

Let $\vec{L} = [L_1, L_2, \ldots, L_k]$ be a $k$-sequence indexed by sets of two integers $\{h, i\}$ such that $1 \leq h < i \leq k$ and $L_{hi}$ denotes the element of $\vec{L}$ of index $\{h, i\}$. The lengths of the induced alignments by an alignment can be represented by a vector...
Let $\vec{L}$. Thus, if $A$ is an alignment and $|A_{[h,i]}| = L_{hi}$ for each pair $h, i$, we say that $\vec{L}$ is the induced length of $A$. For a $k$-sequence $S = s_1, \ldots, s_k$, where $n_i = |s_i|$ for each $i$, we define $L = \{|L| = L_{12}, L_{13}, L_{23}, \ldots, L_{(k-1)k} : 0 \leq L_{hi} \leq n_h + n_i\}$. Note that if $n$ is the length of each sequence in $S$, then $|L| = (2n + 1)^{\binom{k}{2}}$. Let $\vec{b} = [b_1, \ldots, b_k]$ be a $k$-bit vector. Overloading the minus operator “$-$”, we define $\vec{L} - \vec{b}$ to be a $\binom{k}{2}$-vector $\vec{L}'$ which is obtained from $\vec{L}$ and from $\vec{b}$ such that, for each pair $h, i$, we have $L'_{hi} = L_{hi}$ if $b_h = b_i = 0$, and $L'_{hi} = L_{hi} - 1$, otherwise. Observe that if $\vec{L}$ is the induced length of an alignment $A$ of $S(\vec{v})$ and $\vec{b}$ is a $k$-bit vector such that $\vec{b} \cdot S(\vec{v})$ is the last column of $A$, then $\vec{L}' = \vec{L} - \vec{b}$ is the induced length of the alignment $A(1 : |A| - 1)$.

Let $\vec{\gamma}$ be a vector of $\binom{k}{2}$ scoring matrices indexed by two integers $\{h, i\}$, with $1 \leq h < i \leq k$. We denote by $\gamma^{(hi)}$ the element of $\vec{\gamma}$ with index $\{h, i\}$. Then, we have $\vec{\gamma} = [\gamma^{(12)}, \gamma^{(13)}, \gamma^{(1k)}, \gamma^{(23)}, \gamma^{(2k)}, \ldots, \gamma^{((k-1)k)}]$, and define the $\vec{\gamma}$-SP-score of $A$ as $vSP_\vec{\gamma}[A] = \sum_{h=1}^{k-1} \sum_{i=h+1}^{k} vA_{\gamma^{(hi)}}[A_{[h,i]}]$. If we define the $\vec{\gamma}$-SP-score of a vector $\vec{\sigma}$ as $\sum_{i=1}^{s} \sum_{h=1}^{k} \gamma^{(hi)}_{\sigma}$, then we can alternatively calculate the $\vec{\gamma}$-SP-score of the alignment $A$ as $vSP_\vec{\gamma}[A] = \sum_{j} vSP_\vec{\gamma}[A(j)]$.

### 4.2.1 Computing $optV^2_\vec{\gamma}$

In this section we describe an algorithm in two steps for computing $optV^2_\vec{\gamma}$ for a given $k$-sequence $S$: in Step 1 we consider the particular case where we have three sequences, and in Step 2 we treat the general case.

**Step 1: $k = 3$**

Let $S = s_1, s_2, s_3$ be a 3-sequence. Suppose that we have induced lengths $\vec{L} = [L_{12}, L_{13}, L_{23}]$ of a $V^2_\vec{\gamma}$-optimal alignment $A$ of $S$. In consequence, we have that $L_{hi} = |A_{[h,i]}|$ for each pair $h, i$. Notice that knowing the lengths $L_{12}, L_{13}$ and $L_{23}$ does not imply knowing the $V^2_\vec{\gamma}$-optimal alignment $A$. In general, we cannot even infer what $|A|$ is. For example, the alignments

$$
\begin{bmatrix}
s_1(1) & s_1(2) & - \\
s_2(1) & - & s_2(2) \\
- & s_3(1) & s_3(2)
\end{bmatrix}
$$

have different lengths but same induced lengths for $s_1, s_2, s_3$, where $|s_1| = |s_2| = |s_3| = 2$ and $L_{12} = L_{13} = L_{23} = 3$. However, if we know $\vec{L} = [L_{12}, L_{13}, L_{23}]$, we have

$$
optV^2_{\vec{\gamma}}(S) = \min_{A \in AS_\vec{\gamma} : L_{hi} = |A_{[h,i]}|} \{ \sum_{h=1}^{k-1} \sum_{i=h+1}^{k} vA_{\gamma^{(hi)}}[A_{[h,i]}] / L_{hi} \}
$$

$$
= \min_{A \in AS_\vec{\gamma}} \{ \sum_{h=1}^{k-1} \sum_{i=h+1}^{k} vA_{\gamma^{(hi)}}[A_{[h,i]}] \},
$$

where $\gamma^{(hi)}$ is a scoring matrix obtained by multiplying the elements of $\gamma$ by $1 / L_{hi}$. Since we guarantee it is the induced length of an alignment $V^2_\vec{\gamma}$-optimal, we fix $\vec{L}$ and compute $\vec{\gamma}$ in order to calculate the entries of a table $D_{\vec{\gamma}}$, such that

$$
D_{\vec{\gamma}}([v_1, v_2, v_3] \vec{L} = [L_{12}, L_{13}, L_{23}]) = \min_{A \in AS_\vec{\gamma} : L_{hi} = |A_{[h,i]}|} \{ \sum_{h|s<h} vA_{\gamma^{(hi)}}[A_{[h,i]}] \}
$$

corresponds to the score of an alignment with the lowest $\vec{\gamma}$-SP-score when the induced length is $\vec{L}$. The table $D_{\vec{\gamma}}$ can then be computed using the following recurrence

$$
D_{\vec{\gamma}}(\vec{v}, \vec{L}) =
\begin{cases}
0, & \text{if } \vec{v} = \vec{0}, \vec{L} = \vec{0}, \\
\infty, & \text{if } \vec{v} = \vec{0}, \vec{L} \neq \vec{0} \text{ or } \vec{v} \neq \vec{0}, \vec{L} = \vec{0}, \\
\min_{\vec{b} \in B_k, 0 \leq \vec{b} \leq \vec{L}} \{ D_{\vec{\gamma}}(\vec{v} - \vec{b}, \vec{L} - \vec{b}) + vSP_{\vec{\gamma}}[\vec{b} \cdot S(\vec{v})] \}, & \text{otherwise},
\end{cases}
$$

where $\vec{b} \leq \vec{L}$ is also an overloading, meaning that $\vec{L} - \vec{b} \geq \vec{0}$. 


In this case, if \( \vec{L} \) is the induced length of a \( V^2_3 \)-optimal alignment of \( S \), then \( \text{opt}V^2_3(S) = D_{\vec{L}}(\vec{u}, \vec{L}) \). If each sequence has length \( n \), then the total space to store the table \( D_{\vec{L}} \) is \((2n + 1)^2 \cdot (n + 1)^3 = \Theta(n^6)\). When \( \vec{L} \) is unknown, the computation must be repeated for each \( \vec{L} \in \mathbb{L} \), but the space can be reused and no additional space required. If \( \vec{L} \) is known, the algorithm runs to compute all entries of \( D_{\vec{L}} \). As \( D_{\vec{L}} \) has \( \Theta(n^6) \) entries and each entry takes \( O(1) \)-time to be computed, the total time spent is \( O(n^6) \). If \( \vec{L} \) is unknown, the time needed to compute \( \vec{L} \) must be multiplied by the total of elements in \( \mathbb{L} \) which is \((2n + 1)^3\). Therefore, in the latter case, the total time is \( O(n^6 \cdot (2n + 1)^3) = O(n^9) \).

**Step 2: \( k > 3 \)**

Algorithm 3 is a natural extension of the algorithm described in Step 1. Given a scoring matrix \( \gamma \) and an induced length \( \vec{L} \), let \( \gamma \times \vec{L} = [\gamma^{(1)} \ldots, \gamma^{(k(k-1))}] \) be the vector of \( \binom{k}{2} \) scoring matrices, where \( \gamma^{(h,i)} \) is obtained dividing each entry of \( \gamma \) by \( L_h \) for each \( h < i \).

**Algorithm 3 \( V^2_3 \)-optimal alignment of \( S \).**

**Input:** A \( k \)-sequence \( S = s_1, \ldots, s_k \) such that \( n_i = |s_i| \)

**Output:** \( \text{opt}V^2_3(S) \)

1. for each \( \vec{L} \in \mathbb{L} \) do
2. \( D_{\vec{L}}(\vec{0}, \vec{0}) \leftarrow 0 \)
3. for each \( \vec{L} \neq \vec{0} \) do \( D_{\vec{L}}(\vec{0}, \vec{L}) \leftarrow \infty \)
4. for each \( \vec{v} \neq \vec{0} \) do \( D_{\vec{L}}(\vec{v}, \vec{0}) \leftarrow \infty \)
5. \( \vec{\gamma} \leftarrow \gamma \times \vec{L} \)
6. for each \( 0 \leq \vec{v} \leq \vec{\bar{v}} \) in lexicographical order do
7. \( \vec{L} \leftarrow \vec{L} \) in lexicographical order do
8. \( D_{\vec{L}}(\vec{v}, \vec{L}) = \min_{\vec{L} \in S} \{ D_{\vec{L}}(\vec{v}, \vec{L}) \} + v \cdot SP(\vec{v}, \vec{S}(\vec{L})) \}
9. return \( \min_{\vec{L} \in S} \{ D_{\vec{L}}(\vec{0}, \vec{L}) \} \)

For \( k \) sequences of length \( n \), Algorithm 3 needs \((2n + 1)^2 \cdot (n + 1)^k \) space to store the table \( D_{\vec{L}} \). For each of the \((2n + 1)^2 \) values \( \vec{L} \in \mathbb{L} \), table \( D_{\vec{L}} \) is recalculated. Since the computation of each entry takes \( O(2^k k^2) \)-time, the total time is

\[
O(2^k k^2 \cdot (2n + 1)^2 \cdot (n + 1)^k) = O((1 + 1/(2n + 1))^k (2n + 1)^k k^2) .
\]

If \( k \leq 2n + 1 \), the total time can be written as \( O((2n + 1)^{k^2} k^2) \), since \((1 + 1/k)^k \leq e = 2.718281828 \ldots \) Note that \((1 + 1/(2n + 1))^k \leq (1 + 1/k)^k \leq e \) is also constant.

### 4.3 NMSA-3

Let \( S = s_1, \ldots, s_k \) be a \( k \)-sequence and \( A = [s'_1, \ldots, s'_k] \) be an alignment of \( S \). As defined in Equation (5), \( V^3_3[A] \) takes into account the length of \( A \), and the optimal function is given by \( \text{opt}V^3_3(S) = \min_{A \in A_S} \{ V^3_3[A] \} \). The \( V^3_3 \)-optimal alignment of \( S \) is an alignment \( A \) such that \( V^3_3[A] = \text{opt}V^3_3(S) \). Then **NMSA-3** is defined as follows: for a fixed matrix \( \gamma \), given a \( k \)-tuple \( S \), determine \( \text{opt}V^3_3(S) \).

#### 4.3.1 Computing \( \text{opt}V^3_3 \)

Here, each entry \( D(\vec{v}, L) \) of \( D \) stores the SP-score of an alignment \( A \) of the prefix \( S(\vec{v}) \) with the lowest SP-score, such that \( \sum_{i \leq h} |A_{c(i,h)}| = L \). If \( \vec{b} \) is a \( k \)-vector, define \( ||\vec{b}|| = \sum_{i \leq h} b_i \). Notice that if \( \vec{v} - \vec{b} \) is the last column of an alignment \( A \) and \( L = \sum_{h=1}^{k} \sum_{i=h+1}^{k} |A_{i,h}| \)
is the sum of lengths of the alignments induced by $A$. Thus, the sum of lengths of the alignments induced by $A(1:|A| - 1)$ is $L - \|\vec{b}\|$. Therefore,

$$D(\vec{v}, L) = \begin{cases} 
0, & \text{if } \vec{v} = \vec{0}, L = 0, \\
\infty, & \text{if } \vec{v} = \vec{0}, L \neq 0 \text{ or } \vec{v} \neq \vec{0}, L = 0, \\
\{D(\vec{v} - \vec{b}, L - \|\vec{b}\|) + vSP_{\gamma}(\vec{b}, S(\vec{v}))\}, & \text{otherwise}.
\end{cases}$$

Algorithm 4 provides more details about the procedure for computing $optV^3$.

**Algorithm 4** $V^3$-optimal alignment of $S$.

| Input: | $k$-sequence $S = s_1, \ldots, s_k$ such that $n_i = |s_i|$ |
| Output: | $optV^3(S)$ |
| 1: | $D(\vec{0}, 0) \leftarrow 0$ |
| 2: | for each $L \neq 0$ do $D(\vec{0}, L) \leftarrow \infty$ |
| 3: | for each $\vec{v} \neq \vec{0}$ do $D(\vec{v}, 0) \leftarrow \infty$ |
| 4: | for each $0 < \vec{v} \leq \vec{n}$ in lexicographical order do |
| 5: | for $L \leftarrow 1, 2, \ldots, N(k - 1)$ do |
| 6: | $D(\vec{v}, L) \leftarrow \min_{\vec{b} \in \mathcal{B}^k} \min_{0 \leq \vec{b} \leq \vec{v}, \|\vec{b}\| \leq L} \{D(\vec{v} - \vec{b}, L - \|\vec{b}\|) + vSP_{\gamma}(\vec{b}, S(\vec{v}))\}$ |
| 7: | return $\min_L \{D(\vec{n}, L)/L\}$ |

Assume that all sequences in $S$ have length $n$. The table $D$ is computed for all possible values of $L = 1, \ldots, (k^2)/2)$ of $n$ and, $nk^2 - nk$ and, after this, we determine $optSP(S) = \min_L \{D(\vec{n}, L)/L\}$. Thus, Table $D$ needs space equivalent to $(nk^2 - nk + 1) \cdot (n + 1)^k = \Theta(k^2(n + 1)^{k+1})$. Since the time required to determine each entry of $D$ is $O(2^k k^3)$, the running time of Algorithm 4 is $O(2^k k^4(n + 1)^{k+1})$.

## 5 Approximation algorithms for MSA and NMSA-2

Gusfield [15] described a 2-approximation algorithm for MSA. It assumes that $\gamma \in M^C$. In this section, we adapt Gusfield’s algorithm, proposing a 6-approximation algorithm for MSA when $\gamma \in M^A$ and a 12-approximation algorithm for NMSA-2 problem when $\gamma \in M^N$.

We consider here a generic function $v$ to score an alignment of a $2$-sequence such that $opt(s, s) = 0$ (identity) and $opt(s, t) = opt(t, s)$ (symmetry), where $opt(s, t)$ is the score of a $v$-optimal alignment of a $2$-sequence $s, t$. Notice that $vA_s$ and $vN_s$ have these properties when $\gamma \in M^A$ and $\gamma \in M^N$, respectively. Let $S$ be a $k$-sequence and $A$ in $\mathcal{A}_S$ be an alignment. We define $V$ and $OPT$ as functions such that $V[A] = \sum_{i=1}^{k-1} \sum_{i=h+1}^{k} v[A_{[i,j]}]$ and $OPT(S) = \min_{A \in \mathcal{A}_S} V(A)$. Thus, a $V$-optimal alignment is an alignment $A$ such that $V[A] = OPT(S)$.

Let $c$ be an integer with $1 \leq c \leq k$. A star $X$ with center $c$ of $S = s_1, \ldots, s_k$, also called a $c$-star, is a collection of $k - 1$ alignments: alignment $X_h = [s^h_c, s_k^h]$ of $s_h, s_c$, for each $h < c$, where $v[s^h_c, s^h_k] = v[s^h_c, s^h_k]$; and alignment $X_h = [s^h_c, s^h_k]$ of $s_c, s_h$, for each $h > c$, where $v[s^h_c, s^h_k] = v[s^h_c, s^h_k]$. The set of all $c$-stars is denoted by $\mathcal{X}_c$. The score of the $c$-star $X$ is $cStar(X) = \sum_{h \neq c} v[X_h]$ and a $v$-optimal star is one whose score is $optStar(S) = \min_{X \in \mathcal{X}_c \in \mathcal{N}\{cStar(X)\}}$. Notice that $optStar(S) = \min_{cStar(S) = \min_{X \in \mathcal{X}_c \in \mathcal{N}\{cStar(X)\}}} \{ \sum_{h \neq c} vStar(s_h, s_c) \}$, and if $v = vA_S$, and $\gamma \in M^A$, $optStar(S)$ can be computed in $O(k^2 n^2)$-time, and if $v = vN_S$, and $\gamma \in M^N$, $optStar(S)$ can be computed in $O(k^2 n^3)$-time when $|s_i| \leq n$, for each $s_i$ in $S$.

We say that an alignment $A$ of a $k$-sequence $S$ and a $c$-star $X$ of $S$ are compatible ($A$ is compatible with $X$ or $X$ is compatible with $A$) in $S$ when either $A_{(h,c)}$ or $A_{(c,h)}$ is equal to $X_h$, for each $h$. It is easy to obtain, from an alignment $A$ and $c$ in $\mathcal{N}$, the unique $c$-star $X$
that is compatible with \( A \). On the other hand, it is known from Feng and Doolittle [14] that one can find an alignment \( A \) compatible with a given c-star \( X \) in \( O(kn) \)-time, where \( |s_i| \leq n \) for each sequence \( s_i \) in the \( k \)-sequence \( S \). In this case, there exists one or more compatible alignments with \( X \).

The next result is a straightforward consequence of a result from Gusfield [15], where \( v \) is the scoring function. See the proof in [4].

**Lemma 5.1.** Given a \( k \)-sequence \( S \), \( \text{optStar}(S) \leq (2/k) \text{OPT}(S) \).

From now on, we consider \( v = v_A \), or \( v = v_N \), and \( \gamma = M^A \) or \( \gamma = M^N \), respectively. Let \( s, t \in \Sigma^* \) be sequences. Suppose that \( A = [s', t'] \) is an alignment of \( s, t \). We say that a column \( j \) is *splittable in \( A \) if \( s'(j) \neq \gamma \) and \( t'(j) \neq \gamma \) and \( \min \{ \gamma, \gamma' \} \leq \gamma \). Let \( J := \{ j_i \in \mathbb{N} : 1 \leq j_i < \cdots < j_m \leq |A| \text{ and } j_i \text{ is splittable in } A \} \). An \( A \)-splitting is the alignment
\[
\begin{bmatrix}
  s'(1:j_1-1) & s'(j_1) & s'(j_1 + 1:j_2 - 1) & s'(j_2) & \cdots & s'(j_m + 1:|A|) \\
  t'(1:j_1-1) & t'(j_1) & t'(j_1 + 1:j_2 - 1) & t'(j_2) & \cdots & t'(j_m + 1:|A|)
\end{bmatrix}.
\]

We say that \( J \) is required to split \( A \). The following proposition is used to check properties of an \( A \)-splitting. See its proof in [4].

**Proposition 5.2.** Consider \( \gamma \in M^A \) and \( a, b \in \Sigma \). If \( \gamma_a \rightarrow \gamma \neq \gamma_b \rightarrow \gamma \), or \( \gamma_a \rightarrow \gamma \neq \gamma_b \rightarrow \gamma \), then \( \gamma_a \rightarrow \gamma = \gamma_b \rightarrow \gamma \).

Let \( X = \{ X_1, \ldots, X_c-1, X_c+1, X_k \} \) be a c-star. The \( X \)-star splitting is the c-star \( Y = \{ Y_1, \ldots, Y_{c-1}, Y_{c+1}, Y_k \} \), where \( Y_j \) is the \( X_j \)-splitting for each \( j \). The next result shows that the \( v \)-score of the star \( Y \) is bounded by the \( v \)-score of the star \( X \) when \( \gamma \in M^A \) and \( v = v_A \), or \( \gamma \in M^N \) and \( v = v_N \). Thus, as a consequence of Preposition 5.2, we have the following lemma. The formal proof can be seen in [4].

**Lemma 5.3.** Let \( S = s_1, \ldots, s_k \) be a \( k \)-sequence, \( X \) be a c-star of \( S \), \( Y \) be the \( X \)-star splitting and \( v \) be a function to score alignments. Consider \( \gamma \in M^A \) and \( v = v_A \), or \( \gamma \in M^N \) and \( v = v_N \). Then, \( Y \) is also a c-star and \( c \text{Star}(Y) \leq 3 c \text{Star}(X) \).

**Proof sketch.** As a consequence of \( \gamma \in M^A \) and Proposition 5.2, we have that \( Y \) is also a c-star. Let \( h \in \{1, \ldots, k\} \) and \( J \) be a set required to split \( X \). We prove that \( v_A[A_h[Y_h]] \leq 3 v_A[A_h[Y_h]] \) and \( v_N[A_h[Y_h]] \leq 3 v_N[A_h[Y_h]] \) hold since \( \gamma \in M^A \) when \( v = v_A \) and \( \gamma \in M^N \) when \( v = v_N \), and \( J \subseteq \{1, 2, \ldots, |A|\} \). Therefore, in these cases, we have
\[
c \text{Star}(Y) = \sum_{h \in J} v[Y_h] = \sum_{h \in J} v[Y_h] + \sum_{h \in J} v[Y_h] \leq 3 \sum_{h \in J} v[X_h] = 3 c \text{Star}(X).
\]

Notice that the time consumption for computing an \( X \)-splitting from \( X \) is \( O(kn) \) when \( |s_i| \leq n \), for each \( s_i \) in \( S \). Considering a star  \( X = s_1, \ldots, s_k \), there can exist many compatible alignments with a \( v \)-star \( Y \) which is a \( X \)-splitting. Let \text{COMPATIBLEALIGN} be a subroutine that receives the c-star \( Y \) and returns an alignment \( A \) compatible with \( Y \). It is quite simple: if symbols \( s_h(j_1) \) and \( s_c(j_2) \) are aligned in \( X_h \), they are also aligned in \( A \); otherwise, \( s_h(j) \) aligns only with \( - \) in \( A \). This property is enough to guarantee the approximation factor of \( \text{MSA} \) and \( \text{NMSA-2} \).

Let \( Q_{\text{max}} := \max_{a \in \Sigma} \{ \gamma_a \rightarrow \gamma \rightarrow \rightarrow \gamma \} \) and consider the following result, whose proof can be found in [4].
Proposition 5.4. Let \( S \) be a \( k \)-sequence, \( X \) be a \( c \)-star of \( S \) and \( Y \) be a \( X \)-starsplitting. Assume that \( \gamma \in \mathbb{M}^A \) and that \( \text{COMPATIBLEALIGN}(Y) \) returns \( A = [s'_1, \ldots, s'_k] \). If \( h \neq c \) and \( i \neq c \), we have that

(i) \( \gamma_{s'_h(j) \rightarrow s'_i(j)} \leq \gamma_{s'_h(j) \rightarrow s'_i(j)} + \gamma_{s'_h(j) \rightarrow s'_i(j)} \) for each \( j = 1, \ldots, |A| \), and

(ii) \( v_{N,\gamma}[A_{h,i}] \leq 2Q_{\max} \) when \( \gamma \in \mathbb{M}^N \).

Proposition 5.4 is an auxiliary result to show the following lemma.

Lemma 5.5. Let \( S \) be a \( k \)-sequence, \( X \) be a \( c \)-star of \( S \), \( Y \) be a \( X \)-starsplitting and \( \text{COMPATIBLEALIGN}(Y) = A \). Then, for each \( h < i, h \neq c \) and \( i \neq c \),

(i) \( v_{A,\gamma}[A_{h,i}] \equiv v_{A,\gamma}[A_{h,c}] + v_{A,\gamma}[A_{c,i}] \) when \( \gamma \in \mathbb{M}^A \), and

(ii) \( v_{N,\gamma}[A_{h,i}] \leq 2(v_{N,\gamma}[A_{h,c}] + v_{N,\gamma}[A_{c,i}]) \) when \( \gamma \in \mathbb{M}^N \).

Proof. Let \( A = [s'_1, \ldots, s'_k] \) and \( Z = \{ j : s'_h(j) \neq - \) and \( s'_h(j) = s'_i(j) = - \} \). We have \( v_{A,\gamma}[A_{h,i}] \equiv v_{A,\gamma}[A_{h,c}] + v_{A,\gamma}[A_{c,i}] - \sum_{j \in Z} (\gamma_{s'_h(j) \rightarrow s'_i(j)} + \gamma_{s'_h(j) \rightarrow -}) \) from a consequence of Proposition 5.4. Besides, since \( \gamma \in \mathbb{M}^A \), we have that \( \gamma_{s'_h(j) \rightarrow s'_i(j)} + \gamma_{s'_h(j) \rightarrow -} > 0 \). It implies that (ii) is proven.

For proving (ii), observe first that, by definition of \( \mathbb{M}^N \), we have that \( Q_{\max} \leq \gamma_{s'_h(j) \rightarrow s'_i(j)} + \gamma_{s'_h(j) \rightarrow -} \) for every \( j \). Furthermore, following these statements, we have that

\[
v_{N,\gamma}[A_{h,i}] = \frac{v_{A,\gamma}[A_{h,i}]}{|A_{h,i}|} \leq \frac{v_{A,\gamma}[A_{h,i}] + v_{A,\gamma}[A_{c,i}]}{|A_{h,i}| + |Z|} \leq \frac{2(v_{A,\gamma}[A_{h,c}] + v_{A,\gamma}[A_{c,i}])}{|A_{h,i}| + |Z|}.
\]

where the first inequality of (6) is a consequence of Proposition 5.4 and the second inequality follows since every entry of \( \gamma \) is nonnegative, (7) follows from the result in the first paragraph and \( |A_{h,i}| = |A_{h,i,c}| - |Z| \), (8–9) follow as a consequence of the definition of \( Q_{\max} \), and as a consequence of \( |A_{h,i}| \leq |A_{h,i,c}| \) and \( |A_{c,i}| \leq |A_{h,i,c}| \).

We can now describe the approximation algorithm (Algorithm 5).

Algorithm 5: Approximation algorithm for MSA and NMSA-2.

Input: \( k \)-sequence \( S = s_1, \ldots, s_k \)
Output: \( v[A] \), where \( A \) is an alignment of \( S \), and \( v_{SP,\gamma}[A] \leq 6\text{optSP}(S) \) if \( v = v_{A,\gamma} \) and \( \gamma \in \mathbb{M}^A \), and \( v_{SP}^2[A] \leq 12\text{optNSP}(S) \) if \( v = v_{N,\gamma} \) and \( \gamma \in \mathbb{M}^N \).

1. Let \( X \) be a \( v \)-optimal star of \( S \) with center \( c \)
2. Compute the \( X \)-splitting \( Y \)
3. \( A \leftarrow \text{COMPATIBLEALIGN}(Y) \)
4. \( \text{return } v[A] \)
Clearly, Algorithm 5 is correct. Furthermore, Lemmas 5.1, 5.3 and 5.5 are auxiliary to prove its approximation factor. Since the running time of COMPATIBLEALIGN is $O(k^2n)$, a straightforward running time analysis allows us to state the following theorem. The detailed proof is presented in [4].

**Theorem 5.6.** Let $S = s_1, \ldots, s_k$ be a $k$-sequence and $\gamma$ be a scoring matrix. Then, Algorithm 5 computes $v[A]$ correctly:

1. in $O(k^2n^2)$-time such that $vA, \gamma \leq 6 \text{opt}_\gamma(S)$, if $v = vA, \gamma$ and $\gamma = M^A$, or
2. in $O(k^2n^3)$-time such that $v2, \gamma \leq 12 \text{opt}_\gamma(S)$, if $v = vN, \gamma$ and $\gamma \in M^N$, where $A$ is the alignment of $S$ computed by the algorithm.

### 6 Conclusion and future work

We presented and discussed several aspects of normalized multiple sequence alignment (NMSA). We defined three new criteria for computing normalized scores when aligning multiple sequences, showing the NP-hardness and exact algorithms for solving the NMSA-z given criterion $V^z_\gamma$ for each $z$. In addition, we adapted an existing 2-approximation algorithm for MSA when the scoring matrix $\gamma$ is in the common class $M^C$, leading to a 6-approximation algorithm for MSA when $\gamma$ is in the broader class $M^A \supseteq M^C$, and to a 12-approximation for NMSA-2 when $\gamma$ is in $M^N \subseteq M^A$, a slightly more restricted class compared to $M^A$ such that the cost of a deletion for any symbol is at most twice the cost for any other.

This work is an effort to expand the boundaries of multiple sequence alignment algorithms towards normalization, an unexplored domain that can produce results with higher accuracy in some applications. In future work, we will implement our algorithms in order to verify how large are the sequences that our algorithms are able to handle. Also, we plan to perform practical experiments, measuring how well alignments provided by our algorithms and other MSA algorithms agree with multiple alignment benchmarks. In addition, we intend to measure the accuracy of phylogenetic tree reconstruction based on our alignments for simulated and real genomes. Finally, we will work on heuristics and parallel versions of our algorithms in order to faster process large datasets.

### References


