Scaling Laws and Similarity Detection in Sequence Alignment with Gaps

Dirk Drasdo\(^{(1)}\), Terence Hwa\(^{(2)}\), and Michael L"assig\(^{(1)}\)

\(^{(1)}\) Max-Planck Institut f"ur Kolloid- und Grenzfl"achenforschung, Kantstr. 55, 14513 Teltow, Germany

\(^{(2)}\) Department of Physics
University of California at San Diego
La Jolla, CA 92093-0319

Abstract

We study the problem of similarity detection by sequence alignment with gaps, using a recently established theoretical framework based on the morphology of alignment paths. Alignments of sequences without mutual correlations are found to have scale-invariant statistics. This is the basis for a scaling theory of alignments of correlated sequences. Using a simple Markov model of evolution, we generate sequences with well-defined mutual correlations and quantify the fidelity of an alignment in an unambiguous way. The scaling theory predicts the dependence of the fidelity on the alignment parameters and on the statistical evolution parameters characterizing the sequence correlations. Specific criteria for the optimal choice of alignment parameters emerge from this theory. The results are verified by extensive numerical simulations.

Key words: sequence comparison; alignment algorithm; homology; evolution model; optimization

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1 Introduction

Sequence alignment has been one of the most valuable computational tools in molecular biology. It has been used extensively in discovering and understanding functional and evolutionary relationships among genes and proteins. There are two basic types of alignment algorithms: algorithms without gaps such as BLAST and FASTA (Altschul et al., 1990), and algorithms with gaps, for example, the Smith-Waterman local alignment algorithm (Smith and Waterman, 1981). Gapless alignment is widely used in database searches because the algorithms are fast (Altschul et al., 1990) (computational time scales linearly with sequence length), the results depend only weakly on the choice of scoring systems (Altschul, 1993), and the statistical significance of the results is well-characterized (Arratia, et al., 1988; Karlin and Altschul, 1990; Karlin and Altschul, 1993). However, gapless alignment is not sensitive to weak sequence similarities (Pearson, 1991). For a detailed analysis, algorithms with gaps are therefore needed (Waterman, 1989; 1994).

At present, there are two main obstacles to the wider application of these more powerful tools. They require substantially longer computational time than gapless alignments (depending quadratically on the sequence length). As computational power continues its exponential growth at a rate even faster than the growth of genomic information, we expect this constraint to become less stringent in the near future. More importantly, gapped alignments lack a detailed statistical theory assessing the significance of the results. It is this second problem we address in the present paper.

The common algorithms assign a score to each alignment of two or more sequences. The score is based on the number of matches, mismatches, and gaps. Maximization of this score is then used to select the optimal alignment, taken as a measure of the mutual correlations between the sequences. However, it is well known that the optimal alignment of a given pair of sequences strongly depends on the scoring parameters used. The same is true for the fidelity of the optimal alignment, that is, the extent to which mutual correlations are recovered. Hence, the key problem of alignment statistics is to quantify the degree of sequence similarity based on alignment data and to find the scoring parameters producing alignments of the highest fidelity. This problem has been addressed for gapless alignments (Altschul, 1993), based on the knowledge of the exact probability distribution function for the optimal scores in gapless alignments of mutually uncorrelated sequences (Karlin and Altschul, 1990). For algorithms with gaps, however, not even the leading moments of the distribution function have been known so far. Scoring parameters have been chosen mostly by trial and error, although there have been systematic efforts to establish a more solid empirical footing (Benner, 1993; Vingron and Waterman, 1994; Koretke et al., 1996).

To guide the choice of scoring parameters, a quantitative measure of the fidelity of an alignment is necessary. Since the algorithm is designed to detect residual similarities between sequences in a divergent evolution, it is clear that the fidelity measure has to emerge from the underlying evolution process. We use a simple probabilistic evolution model to generate daughter sequences from ancestor sequences by local substitutions, insertions, and deletions. The model is certainly too simple to describe realistic evolution processes, but it allows an
unambiguous identification of inherited mutual similarities between sequences. The fidelity of an alignment is then simply the fraction of the inherited similarities recovered by it. Maximization of the fidelity is used as a criterion to select optimal scoring parameters.

We will not address here algorithmic and computational aspects of alignments. Efficient algorithms are available for parametric and ensemble alignment (Waterman et al., 1992, Gusfield et al., 1992, Waterman, 1994). Our goal is to present a statistical theory of gapped alignments. This theory can then be used to predict optimal scoring parameters appropriate for different classes of inter-sequence correlations.

As is well recognized, the main mathematical difficulty preventing a quantitative statistical characterization of gapped local alignment lies within the global alignment regime. In two recent communications (Hwa and Lässig, 1998; Drasdo et al. 1998), we have shown that the statistical properties in the parameter regime close to the log-linear phase transition line (Waterman et al., 1987; Arratia and Waterman, 1994) of the Smith-Waterman local alignment algorithm are in fact dominated by the statistics of global alignment. This regime is important for biological applications since it has been found empirically to produce “good” alignments (Vingron and Waterman, 1994). It is thus very important to characterize the statistics of global alignment, which is the purpose of this paper. We report a detailed study of the properties of global alignments of mutually uncorrelated as well as correlated sequences by the Needleman-Wunsch (1970) algorithm. The results are used to select optimal scoring parameter to detect sequence correlations generated by the toy evolution process. They can also be incorporated directly into the parameter selection procedure for local alignment (Hwa and Lässig (1998); Drasdo et al., 1998).

The statistical theory of gapped alignments presented here is based on a geometrical approach introduced recently by two of us (Hwa and Lässig, 1996). This approach focuses on the morphology of the optimal alignment paths. The notion of an alignment path (recalled below) provides a very fruitful link to various well-studied problems of statistical mechanics (Kardar, 1987; Fisher and Huse, 1991; Hwa and Fisher, 1994) as has also been noticed by Zhang and Marr (1995). The important statistical properties of alignment paths are described by a number of scaling laws (Hwa and Lässig, 1996; Drasdo et al., 1997) explained in detail below. Their applicability to alignment algorithms is supported by extensive numerical evidence. The resulting scaling theory of alignment has three main virtues:

(i) It distinguishes clearly between universal (parameter-independent) properties of alignments and those depending on the scoring parameters (and hence governing their optimal choice).
(ii) It relates score data of alignments to their fidelity and to the underlying evolutionary parameters characterizing the similarities of the sequences compared.
(iii) Its key statistical averages turn out to be significant for the alignment of single sequence pairs that are sufficiently long.

Statistical scaling theories have also been developed for related optimization problems in structural biology, notably protein folding (Wang et al., 1996; Onuchic et al., 1997).

This paper is organized as follows. In Section 2, we define the evolution process, recall the global alignment algorithm used throughout this paper, and discuss the qualitative aspects
of the geometrical approach. The quantitative theory of alignment starts in Section 3, where we give a detailed description of the alignment statistics for uncorrelated random sequences, and present the power laws governing alignment paths and scores. In Section 4, we turn to sequences with mutual correlations inherited by a realization of our evolution process. We establish a scaling theory that explains the parameter dependence of alignments in a quantitative way. Hence we derive optimal alignment parameters as a function of the evolution parameters, i.e., the frequency of indels and substitutions\footnote{A conceptually similar link between scoring parameters and evolution parameters has been discussed in the context of maximum-likelihood methods (Bishop and Thompson, 1986; Thorne et al., 1991, 1992).}. Furthermore, we show how the evolutionary parameters and the optimal alignment of a given pair of sequences can be deduced from its score data.

2 The geometrical approach to sequence alignment

Evolution model

The evolution process used in this paper evolves from an “ancestor” sequence $Q$ of length $N \gg 1$ whose elements are labeled by the index $i$. The element $Q_i$ is chosen from a set of $c$ different letters. Each letter occurs with equal probability $1/c$, independently of the elements at other positions. Hence, the ancestor sequence is a Markov random sequence. The numerical results presented below are for the case $c = 4$ as appropriate for nucleotide sequences, but for some derivations, it is useful to consider general $c$-letter alphabets.

The evolution process generates a daughter sequence $Q'$ of length $N'$ from the ancestor sequence $Q$. It involves local insertions and deletions of random elements with the same probability $\bar{p}$, and point substitutions by a random element with probability $p$. Insertion, deletion, and substitution events at one point of the sequence are independent of the events at other points. The evolution process can thus be formulated as a Markov process along the sequence (Bishop and Thompson, 1986, Thorne et al., 1991; Hwa and Lässig, 1996). The precise evolution rules used in this paper are given in Appendix A. These rules are such that the average length of the daughter sequence, $\overline{N}$, equals the length $N$ of the ancestor sequence.

A specific realization of this Markov process defines a unique evolution path linking the sequences $Q$ and $Q'$; see Fig. 1(a). However, the same pair of sequences can be linked by different evolution paths. Any evolution path has a number of conserved elements (i.e., elements that are not deleted or substituted at any point of the evolution process). The average fraction of ancestor elements $Q_i$ conserved in the daughter sequence $Q'$ is

$$U(p, q) = (1 - p) (1 - q),$$

where

$$q = \frac{\bar{p}}{1 - \bar{p}}$$

(1)
Fig. 1: (a) A Markov evolution path linking the two sequences \( Q = \{ G, T, A, C, T, G, A, T, G \} \) and \( Q' = \{ G, A, G, T, A, T, C, T, G \} \). Native pairs are marked by bonds with full circles, substitutions by bonds with empty circles. The unpaired letters \( Q_i \) are deleted, the unpaired letters \( Q'_j \) are inserted. (b) A possible alignment between \( Q \) and \( Q' \) with matches \( Q_i = Q'_j \) (full lines), mismatches \( Q_i \neq Q'_j \) (dashed lines) and gaps (unpaired letters). (c) Lattice representation. The evolution path \( R(t) \) corresponding to (a) is marked by circles; there are five native bonds (full circles). The alignment path corresponding to (b) appears as thick line whose solid (dashed) diagonal bonds are matches (mismatches) and whose horizontal and vertical bonds are gaps. It covers three of the five native bonds, producing the fidelity \( F = 3/5 \).

is the effective insertion/deletion rate (see Appendix A). We call these conserved pairs of elements \( Q_i = Q'_j \) native pairs. Their fraction \( U(p,q) \) quantifies the mutual similarity between sequences. In the remainder of this paper, we take \( U \) and \( q \) as the basic parameters characterizing the evolution process. The primary goals of sequence alignment are to identify the native pairs and to estimate the mutual similarity \( U \).

Alignment and Scoring Scheme

We align the sequences \( Q = \{ Q_i \} \) and \( Q' = \{ Q'_j \} \) using the simplest version of the global alignment algorithm by Needleman and Wunsch (1970). A global alignment of two sequences is defined as an ordered set of pairings \( (Q_i, Q'_j) \) and of gaps \( (Q_i, -) \) and \( (-, Q'_j) \), each element \( Q_i \) and \( Q'_j \) belonging to exactly one pairing or gap (see Fig. 1(b)). Any alignment is assigned a score \( S \), maximization of which defines the optimal alignment\(^\text{2}\). We use here the simplest class of linear scoring functions (Smith and Waterman, 1981), with the score given by the total number \( N_+ \) of matches \( Q_i = Q'_j \), the total number \( N_- \) of mismatches \( Q_i \neq Q'_j \), and the total number \( N_g \) of gaps. Hence, the most general such function involves three scoring parameters:

\[
S = \mu_+ N_+ + \mu_- N_- + \mu_g N_g . \tag{3}
\]

However, the optimal alignment configuration of a given sequence pair \( Q \) and \( Q' \) is left invariant if the three scoring parameters are all multiplied by the same factor. Along with

\(^2\) In statistical mechanics, one may think of \(-S\) as an energy that has to be minimized.
the property that $2N_+ + 2N_- + N_g = N$ is conserved in global alignment and the invariance of the alignment configuration to an additive constant to (3), we see that the outcome of global alignment is controlled effectively by a single parameter. Without loss of generality, we may therefore choose to use the scoring function

$$S = \sqrt{c - 1} N_+ - \frac{1}{\sqrt{c - 1}} N_- - \gamma N_g,$$

(4)

which is normalized in such a way that a pairing of two independent random elements has the average score 0 and the score variance 1. The scoring function $S$ depends only on the parameter $\gamma$, which describes the effective cost of a gap over pairing. The optimal alignment depends on $\gamma$ in the regime $\gamma \geq \gamma_0 \equiv 1/(2\sqrt{c - 1})$ (i.e., $2\mu_g > \mu_-$) to which we restrict ourselves in the sequel. For $\gamma < \gamma_0$, it is always favorable to replace a mismatch by two gaps (Waterman et al., 1987), and the alignment is not biological relevant.

The fidelity of an alignment

As discussed above, mutual correlations between the sequences $Q = \{Q_i\}$ and $Q' = \{Q'_j\}$ arise from the set of native pairs $(Q_i = Q'_j)$. The fidelity $\mathcal{F}$ of an alignment can be quantified as the fraction of correctly matched native pairs, see Fig. 1(b). This is an unambiguous measure of the goodness of an alignment, and it will be used below to find optimal alignment parameters. To evaluate $\mathcal{F}$ directly, the native pairs have to be distinguished from random matches $(Q_i = Q'_j)$ involving mutated elements. Hence, the fidelity defined in this way depends not only on the sequences $Q$ and $Q'$ but also on the evolution path linking them. Of course, the evolution path is not known in most applications of sequence alignment. However, the scaling theory discussed below relates statistical properties of $\mathcal{F}$ to alignment data, making it a useful and measurable quantity.

Lattice representation

Any alignment of two sequences $\{Q_i\}$ and $\{Q'_j\}$ is conveniently represented on a two-dimensional $N \times N'$ grid as in Fig. 1(c) (Needleman and Wunsch, 1970). The cells of this grid are labeled by the index pair $(i, j)$. The diagonal bond in cell $(i, j)$ represents the pairing of the elements $(Q_i, Q'_j)$. The horizontal bond between cells $(i, j)$ and $(i, j+1)$ represents a gap $(Q_i, -)$ located on sequence $Q'$ between the elements $Q'_j$ and $Q'_{j+1}$. The vertical bond between cells $(i, j)$ and $(i+1, j)$ represents a gap located on sequence $Q$ between the elements $Q_i$ and $Q_{i+1}$. In this way, any alignment defines a unique directed path on the grid. Using the rotated coordinates $r \equiv j - i$ and $t \equiv i + j$, this path is described by a single-valued function $r(t)$ measuring the displacement of the path from the diagonal of the
alignment grid. A path associated with an optimal alignment is denoted by \( r_0(t) \). For global alignment of typical sequences, the optimal path extends over the entire grid, i.e., it has a length of the order \( N + N' = 2N \). The Needleman-Wunsch dynamic programming algorithm obtains optimal alignment paths by computing the “score landscape” \( S_0(r, t) \) sequentially for all lattice points, where \( S_0(r, t) \) denotes the optimal score for the set of all alignment paths ending at the point \((r, t)\). The version of the algorithm used in this paper is detailed in Appendix B.

In a similar way, any evolution path linking the sequences \( Q \) and \( Q' \) defines a directed path \( R(t) \) on the alignment grid (called evolution path as well) (Hwa and Lässig, 1996). On this path, horizontal and vertical bonds represent deleted and inserted elements, respectively. A fraction \( U \) of the bonds along the evolution path are native bonds representing the native pairs \((Q_i = Q'_j)\). The fidelity of an alignment is then simply the fraction of native bonds that are also part of the corresponding alignment path \( r(t) \), see Fig. 1(c).

**Alignment morphology**

Alignment algorithms are designed to trace the mutual correlations between sequences. As it becomes clear from Figs. 2, the presence of such correlations affects both the morphology of the optimal alignment path \( r_0(t) \) and the associated score statistics. Fig. 2(a) shows the path \( r_0(t) \) for a pair of mutually uncorrelated random sequences. This path is seen to be intrinsically rough; i.e., the displacement has large variations. This “wandering” is caused by random agglomerations of matches in different regions of the alignment grid. Fig. 2(b) shows the corresponding score landscape \( S_0(r, t) \) for a given value of \( t \). The maximum score value occurs at the point \( r_0(t) \) and is seen to be not very pronounced; near-optimal score values occur also at distant points such as \( r_1 \). The statistics of alignment paths and scores for uncorrelated sequences are discussed in detail in Section 3 below.

The optimal alignment path for a pair of mutually correlated sequences (obtained from the evolution process described above) behaves quite differently, as shown in Fig. 2(c). Its wandering is essentially restricted to a “corridor” of finite width centered around around the evolution path \( R(t) \). In this way, the path \( r_0(t) \) covers a finite fraction \( \mathcal{F} \) of the native bonds. The corresponding score landscape is shown in Fig. 2(d). The maximum at \( r_0(t) \) is now very pronounced; all paths ending at points distant from \( r_0(t) \) have a substantially lower score than the optimal path. The alignment statistics of mutually correlated sequence pairs is described in Section 4.

The morphology of the optimal alignment path depends strongly on the choice of the scoring parameter \( \gamma \). As an example, Fig. 3 shows the optimal paths \( r_0(t) \) (dashed lines) for the same pair of correlated sequences with the same underlying evolution path \( R(t) \) (the solid line), and for three different values of \( \gamma \): At small \( \gamma \), the path \( r_0(t) \) follows the evolution path only on large scales. On small scales, variations in the displacement \( r_0(t) \) are seen to be larger than those of \( R(t) \) (Fig. 3(a)). The intrinsic roughness of the optimal alignment path limits its overlap with the evolution path, hence suppressing the fidelity. The fidelity is the highest at some intermediate value \( \gamma^* \), where the alignment path follows the target path
Fig. 2: (a) The optimal alignment path $r_0(t)$ and (b) a slice of the score landscape $S(r, t = 4000)$ for a pair of mutually uncorrelated random sequences. The score maximum is at $r_0$, which defines the endpoint $r_0 \equiv r_0(t = 4000)$ of the optimal path. Similar score values occur also at distant points such as $r_1$. (c) The paths $r_0(t)$ (dashed line), $R(t)$ (solid line) and (d) the score landscape $S_0(r)$ at $t = 4000$ for a pair of sequences with mutual correlations. The score maximum at $r_0$ is now pronounced; all distant points $r$ have a substantially lower score. Hence the fluctuations of the alignment path $r_0(t)$ are confined to a corridor around the evolution path $R(t)$. 
Fig. 3: Optimal alignment paths $r_0(t)$ for the same pair of correlated sequences and three different values of $\gamma$. The evolution path $R(t)$ (solid lines) is the same in all three cases, while the optimal alignment paths $r_0(t)$ (dashed lines) differ. (a) Random fluctuation regime ($\gamma < \gamma^*$). The path $r_0(t)$ has strong fluctuations since the gap cost is low. (b) Optimal alignment parameter $\gamma = \gamma^*$. The fluctuations of the paths $r_0(t)$ and $R(t)$ are of the same order of magnitude. (c) Shortcut regime ($\gamma > \gamma^*$). At high gap cost, the fluctuations of $R(t)$ are dominant, while $r_0(t)$ contains large straight segments.
most closely (Fig. 2(b)). At large $\gamma$, the alignment path contains large straight segments (Fig. 2(c)), which again reduces the fidelity.

A qualitative understanding of this parameter dependence may be gained from an analogy to random walks, regarding $r_0(t)$ as the trajectory of walker following a curvy path $R(t)$. The intrinsic properties of the walker are parametrized by $\gamma$. For small $\gamma$, the the walker is drunk and cannot follow the path $R(t)$ without meandering to its left and right. This is the regime of Fig. 2(a), which we call the random fluctuation regime. For large values of $\gamma$, on the other hand, the walker is lazy and bypasses the larger turns of the path $R(t)$; this is the shortcut regime (Fig. 2(c)). From this analogy, it becomes plausible that a walker who is neither too drunk nor too lazy will follow the path $R(t)$ most closely and thereby achieve the highest fidelity (Fig. 2(b)). Such a criterion for the optimal parameter $\gamma^*$ will indeed emerge from the quantitative theory described in the remainder of this paper.

3 Alignment of Uncorrelated Sequences

A statistical theory of alignment can hardly predict the optimal alignment for a specific pair of sequences. What can be characterized are quantities averaged over realizations of the evolution process for given parameters $U$ and $q$. It will be shown, however, that these ensemble averages are also relevant for the alignment statistics of single pairs of “typical” sequences provided they are sufficiently long. The approach is different from the extremal statistics of the score distribution that has been used to assess the significance of alignment results (Karlin and Altschul, 1990, 1993).

In the absence of mutual correlations (i.e., for $U = 0$), the statistics of alignments is determined by a balance between the loss in score due to gaps and the gain in score due to an excess number of random matches. As discussed by Hwa and Lässig (1996), the corresponding alignment paths belong to a class of systems known in statistical mechanics as directed polymers in a random medium. The statistical properties of directed polymers have been characterized in detail (Kardar, 1987; Huse and Fisher, 1991; Hwa and Fisher, 1994). We now recall the main results and give numerical evidence of their applicability to sequence alignment.

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4 In statistical mechanics, $\gamma$ is the effective line tension of the fluctuating path $r_0(t)$.
5 This is also known as the problem of first passage percolation. A detailed mathematical analysis of the scaling laws presented below can be found in recent works by Licea et al. (1994, 1996). The main difference to alignment statistics is the number of independent random variables on a grid of size $N \times N$. For directed polymers and first passage percolation, this number is of order $N^2$; for alignments of random sequences, it is only of order $N$ (see also Arratia and Waterman, 1994). This difference is, however, irrelevant for the asymptotic scaling behavior (Hwa and Lässig, unpublished). A detailed heuristic discussion of this equivalence in the context of a number of closely related problems is given by Cule and Hwa (1997).
Displacement and score statistics

The displacement $\Delta r_0(t_2 - t_1) \equiv r_0(t_2) - r_0(t_1)$ of the optimal alignment path between two arbitrary points $t_1$ and $t_2$ is found to obey the statistical scaling law

$$ \langle (\Delta r_0(t))^2 \rangle \simeq A^2(\gamma) |t|^{4/3}, $$

the overbar denoting the average over an ensemble of mutually uncorrelated sequence pairs. Eq. (5) is an asymptotic law valid for $(\Delta r_0(t))^2 \gg 1$, i.e., for $t \gg t_0(\gamma) \equiv A^{-3/2}(\gamma)$. It says that the exponent $4/3$ is a very robust feature of the optimal alignment of uncorrelated random sequences, independent of the scoring parameter(s) or even scoring schemes used.

A large gap cost efficiently suppresses the displacement only for the limited range of scales $t < t_0(\gamma)$. On larger scales, the cost of gaps is always outweighed by the gain in score from regions of the alignment grid with an excess number of random matches, leading to the power law (5) with a “universal” exponent. The dependence of the roughness $(\Delta r_0(t))^2$ on the scoring parameters (\gamma in this case) is contained entirely in the amplitude $A(\gamma)$; this dependence is discussed below. The ensemble average (5) also describes the displacement auto-correlation function of the optimal path for a single sequence pair, defined as an average over initial points $t_1$ in an interval $T \gg t$,

$$ \langle (\Delta r_0(t))^2 \rangle = T^{-1} \sum_{t_1=1}^{T} (r_0(t_1 + t) - r_0(t_1))^2. $$

The large displacement fluctuations of the optimal alignment path $r_0(t)$ are accompanied by large variations in its score. For an ensemble of mutually uncorrelated sequences, the score average is asymptotically linear in the length $N$,

$$ \overline{S_0}(N, \gamma) \simeq E_0(\gamma) N $$

for $N \gg 1$, with a monotonically decreasing coefficient function $E_0(\gamma)$. However, the variance of optimal score is described by a nontrivial power law

$$ \langle (\Delta S_0(N, \gamma))^2 \rangle \simeq B^2(\gamma) N^{2/3} $$

which is valid in the asymptotic regime $N \gg t_0(\gamma)$. The dependence on the alignment parameters is again only in the amplitude $B(\gamma)$, while the exponent $2/3$ is universal. The ensemble average can be obtained (up to a $\gamma$-independent proportionality factor) from a single pair of sequences as average in the score landscape $S_0(r, t)$ over a sufficiently long interval $r_1 \leq r \leq r_1 + R$,

$$ \langle (\Delta S_0(t, \gamma))^2 \rangle \sim R^{-1} \sum_{r=r_1}^{r_1+R-1} S_0^2(r, t) - \left( R^{-1} \sum_{r=r_1}^{r_1+R-1} S_0(r, t) \right)^2, $$

see Appendix B. We have verified the scaling laws (5) and (8) numerically for a range of $\gamma$ values; see Figs. 4. The asymptotic behavior is found to set in rather quickly for $t > t_0(\gamma)$. The same scaling has been found for a pair of unrelated cDNA sequences (see also Fig. 4), which justifies our modeling of individual sequences as Markov chains. A more comprehensive study of correlated and uncorrelated cDNA sequences will be presented elsewhere.
Fig. 4: (a) Displacement fluctuations $(\Delta r_0(t))^2$ of the optimal alignment for several values of $\gamma$. Averages over an ensemble of 200 mutually uncorrelated sequence pairs are marked by lines, auto-correlation functions for a single sequence pair of length $N = 10^5$ by squares. (b) Score fluctuations $(\Delta S_0(t))^2$ obtained from the score landscape $\langle S^2 \rangle$ by Eq. (9) for several values of $\gamma$. (c) Displacement auto-correlation function and (d) score fluctuations for a pair of unrelated cDNA sequences (P.lividius cDNA for COLL2alpha gene (Exposito et al., 1995) and Drosophila melanogaster (cDNA1) protein 4.1 homologue (coracle) mRNA, complete cds. (Fehon et al., 1994)). The straight lines indicate the expected power laws given by Eqs. (5) and (8).
Confinement and tilt energies

A related set of power laws govern the change in the average optimal score $S_0$ if the alignment paths are subject to constraints. For example, the constraint $-r_c/2 < r_0(t) < r_c/2$ artificially confines the paths to a strip of width $r_c$ on the alignment grid. This decreases the optimal score $S_0$ since the path $r_0(t)$ is cut off from random agglomerations of matches outside the strip. For long sequences, this confinement cost becomes proportional to $N$, and the average confinement cost per unit of $t$ is

$$\delta E_c(r_c; \gamma) \equiv \frac{\overline{S_0}(r_c; N, \gamma) - \overline{S_0}(N, \gamma)}{N} < 0. \quad (10)$$

It obeys the scaling law

$$\delta E_c(r_c; \gamma) \simeq -C(\gamma) r_c^{-1}, \quad (11)$$

with all the parameter dependence contained in the prefactor $C(\gamma)$. This relation is valid in the asymptotic regime of strong confinement, i.e., for sequences long enough that their unconstrained mean square fluctuations $(\Delta r_0)^2(N)$ exceed the scale $r_c^2$. According to (5), this condition is satisfied for $N \gg r_c^3/2 t_0(\gamma)$.

In a similar way, the alignment may be constrained by restricting both ends of the alignment path to given values of $r$, for example, $r(0) = 0$ and $r(N) = r_0$. This forces an average tilt $\theta = r_0/N$ upon the alignment path, thereby increasing its number of gaps and decreasing its number of matches. The resulting tilt cost is again proportional to $N$ for long sequences, and the average tilt cost per unit of $t$,

$$\delta E_t(\theta; \gamma) \equiv \frac{\overline{S_t}(\theta; N, \gamma) - \overline{S_t}(0; N, \gamma)}{N} < 0 \quad (12)$$

is given by

$$\delta E_t(\theta; \gamma) \simeq -D(\gamma) \theta^2. \quad (13)$$

This power law is valid for long sequences ($N \gg t_0(\gamma)$) and small tilt angles ($\theta^2 < t_0^2(\gamma)$). The scaling form of the confinement and tilt energies has been verified numerically, see Fig. 5.

Parameter dependence

The scaling laws (3), (8), (11) and (13) all have the same structure: they are power laws with universal exponents and parameter-dependent amplitudes. The scaling theory predicts not only the values of the exponents but also universal relations between the amplitudes. We have

$$A^{3/4}(\gamma) \propto B^{-3}(\gamma) \propto C(\gamma) \propto D^{-1/3}(\gamma), \quad (14)$$

the tildes indicating proportionality factors independent of $\gamma$ and of order 1. The amplitudes are monotonic functions of $\gamma$, which become independent of $\gamma$ for $\gamma < \gamma_0$. Their asymptotic behavior for large $\gamma$ can be calculated (Hwa and Lässig, 1996), resulting in $C(\gamma) \sim \gamma^{-1}$. Indeed, we find this amplitude to be well approximated by the form

$$C(\gamma) \sim (\gamma + \text{const.})^{-1} \quad (15)$$
Fig. 5: (a) Confinement cost $\delta E_c(r_c;\gamma)$ for optimal alignment paths in a corridor $-r_c < r_0(t) < r_c$, taken from an ensemble of 200 mutually uncorrelated random sequences. (b) Scaled tilt cost $\delta E_t(\theta;\gamma)/t_0(\gamma)$ as a function of the scaled tilt $\theta t_0(\gamma)$, for the same ensemble of sequences. The curves describe asymptotic power laws with universal exponents and $\gamma$-dependent amplitudes, as given by Eqs. (11) and (13).

Fig. 6: Parameter dependence of the amplitudes $A(\gamma)$, $B(\gamma)$, $C(\gamma)m D(\gamma)$, and $E_0(\gamma)$, together with a fit curve of the form (15).
in the entire interval $\gamma > \gamma_0$. Our numerical data verifying Eqs. (14) and (15) are shown in Fig. 6. We also show numerical results for the average score per unit of $t$, i.e., the function $E_0(\gamma)$ in Eq. (10). We find $E_0(\gamma) \sim C(\gamma)$ approximately.

4 Alignment of Correlated Sequences

Displacement fluctuations of the evolution path

As discussed in Section 2, the mutual correlations between sequences are encoded in their evolution path, which is represented by the evolution path $R(t)$ on the alignment grid. This path has displacement fluctuations due to the random distribution of insertions and deletions, see Figs. 2(c) and 3. However, the statistics of these fluctuations is different from that of the alignment paths discussed in the previous Section. Since the evolution is modeled as a Markov process, the displacement $\Delta R(t_1 - t_2) \equiv R(t_1) - R(t_2)$ has the mean square

$$\overline{\Delta R(t)^2} = q|t|$$

characteristic of a Markov random walk, with $q$ given by Eq. (3). The overbar denotes an ensemble average over realizations of the evolution process with given values of $U$ and $q$. The ensemble average (16) equals the auto-correlation function of a single sufficiently long evolution path $R(t)$ defined in analogy to (3).

We may compare the fluctuations $\overline{\Delta R(t)^2}$ of the evolution path for correlated sequences with the fluctuations $\overline{\Delta r_0(t)^2}$ of the optimal alignment path for uncorrelated sequences (Drasdo et. al., 1997). This defines a scale $\tilde{t}$, where these fluctuations are of the same order of magnitude: $(\overline{\Delta R(t)^2}) = (\overline{\Delta r_0(t)^2}) \equiv \tilde{r}^2$. From Eqs. (3) and (16), we obtain

$$\tilde{t}(\gamma, q) = q^3/A^6(\gamma), \quad \tilde{r}(\gamma, q) = q^2/A^3(\gamma).$$

We call the scales (17) roughness matching scales. For $|t| < \tilde{t}(\gamma, q)$, the displacement of the evolution path exceeds that of the optimal alignment path, while for $|t| > \tilde{t}(\gamma, q)$, the displacement of the alignment path becomes dominant.

Scaling theory for correlated sequences

For sequences with mutual correlations (i.e., $U > 0$), the morphology of the optimal alignment path $r_0(t)$ and the score statistics are more complicated than for uncorrelated sequences since in addition to the random matches, there are now the native matches along the evolution path $R(t)$. Due to these competing score contributions, the problem seems to be beyond the means of any rigorous mathematical approach. However, it turns out that the statistics of weakly correlated sequences is described with remarkable accuracy by the scaling theory developed in the previous Section.

Consider a pair of weakly correlated sequences of length $N \gg 1$ with an optimal alignment of finite fidelity $F > 0$ at a given value of $\gamma$. Since the optimal alignment path $r_0(t)$ and the
evolution path \( R(t) \) have a finite fraction of common bonds, the displacement fluctuations of \( r_0(t) \) remain confined to a "corridor" centered around the path \( R(t) \) (see Fig. 2(c)). The width \( r_c \) of this corridor can be defined by the mean square relative displacement

\[
r_c^2 \equiv \langle (r_0(t) - R(t))^2 \rangle ,
\]

which can again be understood as an ensemble average or equivalently as an average over \( t \) for a single pair of long sequences. To see this equivalence, we note that by Eq. (5), the width \( r_c \) defines a corresponding scale in \( t \) direction, \( t_c = r_c^2 t_0(\gamma) \). One can show that \( t_c \) is a correlation length; i.e., points on the alignment path with \( |t_2 - t_1| > t_c \) are essentially uncorrelated. Averaging over uncorrelated regions of the alignment path generates the ensemble underlying Eq. (18) even for a single pair of sequences if they are sufficiently long, i.e., \( N, N' \gg t_c \).

By confining the alignment path \( r_0(t) \) to a corridor, mutual correlations act as a constraint on its displacement fluctuations. This leads to a score cost as discussed in the Section 3. However, the constraint cost must be outweighed by the gain in score due to the native matches, resulting in a net score gain per unit of \( t \),

\[
\delta E(U, q, \gamma) \equiv \frac{S(N; U, q, \gamma) - S_0(N, \gamma)}{N} > 0 ,
\]

with \( S_0(N, \gamma) \) denoting the average score of uncorrelated sequences.

We now calculate the confinement length \( r_c(U, q, \gamma) \) and the score gain \( \delta E(U, q, \gamma) \) in a variational approach, treating \( r_c \) as an independent variable to be determined a posteriori from an extremal condition. We stress that this approach is not exact; the main approximation consists in treating \( r \) and \( t \) as continuous variables.

The constraint cost per unit of \( t \) imposed by the evolution path \( R(t) \) involves terms of the form discussed in the previous Section: (i) If the path \( r_0(t) \) is confined to a corridor of width \( r_c \) around the fluctuating path \( R(t) \), the tangent to \( r_0(t) \) has a typical tilt \( \theta \sim q/r_c \) with respect to the diagonal of the alignment grid, implying a tilt cost

\[
\delta E_t(r_c; q, \gamma) \sim -D(\gamma) \left( \frac{q}{r_c} \right)^2 .
\]

(ii) The confinement cost to an untilted corridor of width \( r_c \) is \( \delta E_c = C/r_c \). The tilt reduces the effective width of the corridor so that the confinement cost takes the form

\[
\delta E_c(r_c; q, \gamma) \sim -C(\gamma) \frac{1 + q/[C^2(\gamma)r_c]}{r_c} .
\]

On the other hand, the gain in score per unit of \( t \) due to the native matches is simply \( \delta E_n = U \mathcal{F} \), as it is clear from the definition of the fidelity \( \mathcal{F} \). We need to express \( \mathcal{F} \) in terms of \( r_c \). Naively one would expect \( \mathcal{F} \sim 1/r_c \). A detailed analysis shows that this is correct up to a logarithmic correction (Hwa and Nattermann, 1995, Kinzelbach and Lässig, 1995, Hwa and Lässig, 1996) leading to

\[
\delta E_n(r_c; U) \sim U \frac{1 + \log r_c}{r_c} .
\]
The net score gain is the sum of these contributions, $\delta E = \delta E_c + \delta E_t + \delta E_n$. The resulting equation can be simplified by using the scaled variables $x = C/U$, $y = q/U^2$, and $\delta E = \delta E/U$. Absorbing all unknown proportionality factors into their definition, we obtain the scaled energy gain

$$\delta E(r_c; x, y) = -\frac{x}{r_c} - \frac{y}{x} \left( 1 + \frac{y}{x^2} \right) \frac{1}{r_c^2} + \frac{1 + \log r_c}{r_c}.$$ (23)

Maximizing (23) then determines the actual value of $r_c(x, y) = r_c(U, q, \gamma)$:

$$\delta E(x, y) = \max_{r_c} \delta E(r_c; x, y).$$ (24)

Fig. 7 shows numerical data for the fidelity $F(x, y) = F(r_c(x, y); x, y)$ and score gain $\delta E(x, y)$ obtained from single sequence pairs with various values of $U$, $q$ and $\gamma$. As expected from this scaling theory, the data points for different parameter sets $(U, q, \gamma)$ corresponding to the same $(x, y)$ collapse approximately. This data collapse will be useful for similarity detection.

**Alignment parameter optimization**

The numerical fidelity and score patterns of Fig. 7 have clear maxima $F^*(y) \equiv F(x^*(y), y)$ and $\delta E^*(y) \equiv \delta E(x^*(y), y)$, attained at points $x^*(y)$ and $x^*(y)$. Fig. 7 also shows the loci of these maxima, $(x^*(y), F^*(y))$ and $(x^*(y), \delta E^*(y))$, as well as the limit curves $F^*(x, 0)$ and $\delta E^*(x, 0)$ obtained from the scaling theory (i.e., from Eqs. (23) and (24) solved numerically). The theory is seen to predict the functional form of these curves in a reasonable way, except in the region $F \sim 1$ (i.e., $r_c \sim 1$) where the continuum approximation valid in the regime of weak similarity breaks down. (The unknown $\gamma$-independent proportionality factors for the scaling variables $x$, $y$, $\delta E$ and for $F$ have been determined by fits to the data.)

The functions $x^*(y)$, $x^*(y)$, $F^*(y)$, and $\delta E^*(y)$ shown in Figs. 8 (a) and (b) encode in an efficient way the dependence of the fidelity and score maxima on the alignment parameter and on the evolution parameters. Furthermore, it follows from the (numerical) solution of (23) and (24) that the confinement length $r_c^*(y) \equiv r_c(x^*(y), y)$ at the point of maximal fidelity satisfies the approximate relation

$$r_c^*(y) \sim \tilde{r}(x^*(y), y).$$ (25)

in the biologically interesting regime of small $q$ and moderate $U$ ($0 < y < 5$). The optimal confinement length is thus proportional to the roughness matching scale [$17$] at that point. Hence, this scaling theory is in accordance with the qualitative picture of Section 2: At $x^*(y)$, the fluctuations of the optimal alignment path $r_0(t)$ just match those of the evolution path $R(t)$ (see Fig. 2(b)). The shortcut regime (Fig. 2(c)) corresponds to the ascending branch ($x < x^*(y)$) of the fidelity curves in Fig. 7(a), while the random fluctuation regime (Fig. 2(a)) corresponds to the descending branch ($x > x^*(y)$).
Fig. 7: (a) Fidelity $F(x, y)$ and (b) score gain $\delta \varepsilon(x, y)$ obtained from single sequence pairs with various evolution parameters $U, q$ and alignment parameters $\gamma$. The data for different $(U, q, \gamma)$ corresponding to the same $(x, y)$ collapse approximately, as predicted by the scaling theory. The lines are the theoretical loci of the maxima $(x^*(y), F^*(y))$ (short-dashed), $(x^*(y), \delta \varepsilon^*(y))$ (long-dashed) and the theoretical limit curves $F(x, 0)$, $\delta \varepsilon(x, 0)$ (solid).
Fig. 8: Alignments of maximal fidelity and of maximal score gain. Theoretical predictions for the curves (a) $x^*(y)$, $x^s(y)$ and (b) $F^*(y)$, $\delta E^*(y)$, compared to numerical data obtained from fits to the curves of Fig. 7.

**Similarity detection**

The evolution process used in this paper is closely related to a more realistic process for the divergent evolution of two daughter sequences $Q^{(1)}$ and $Q^{(2)}$ from a closest common ancestor sequence $Q$. Modeling the two evolution paths as independent Markov processes with respective parameters $U_1, q_1$ and $U_2, q_2$, one can show that the evolution path linking $Q^{(1)}$ and $Q^{(2)}$ is again a Markov process with parameters $U = U_1 U_2$ and $q = q_1 + q_2 + O(q^2)$.

For practical alignments, however, the evolutionary parameters $U$ and $q$ are unknown. Since they enter the definition of the basic variables $x$ and $y$, knowledge of the optimal parameters $x^*(y)$ and $x^s(y)$ seems to be of little use for applications. However, these parameters can be reconstructed from alignment data, as we will now show for a specific example.

Consider three sequences $Q^{(1)}$, $Q^{(2)}$ and $Q^{(3)}$ related by the evolution tree of Fig. 9(a). The evolutionary distances $\tau_i$ are defined in terms of the mutual similarity coefficients $U_{ij}$ by

$$-\log U_{ij} = \tau_i + \tau_j \quad (i, j = 1, 2, 3). \quad (26)$$

We wish to determine $\tau_1$, $\tau_2$ and $\tau_3$ from pairwise alignments of the sequences. Fig. 9(b) shows the alignment data $\delta E_{ij}$ as defined in Eq. (19) for each of these pairs, plotted as a function of $C(\gamma)$. To fit the data curve $\delta E_{ij}(C)$ to the corresponding scaled score gain curve $\delta E_{ij}(x)$ of Fig. 7(b), we have to divide both axes of the diagram by $U_{ij}$. In this way, we can determine the a priori unknown factors $U_{ij}$, and hence the evolutionary distances $\tau_i$, see Fig. 9(b). For this example, we obtain $U_{12} \approx 0.54$, $U_{13} \approx 0.43$, $U_{23} \approx 0.415$, and $\tau_1 \approx 0.22$, $\tau_2 \approx 0.33$, $\tau_3 \approx 0.55$, which is to be compared with the actual values $\tau_1 = 0.27$, $\tau_2 = 0.38$, and $\tau_3 = 0.61$ used to produce the sequences.

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6 In this example, we use effective indel rates $-\log(1 - q_{ij}) = \Gamma(\tau_i + \tau_j)$ with $\Gamma = 0.2$, but this choice is not crucial.
Fig. 9: (a) Evolution tree linking three sequences $Q^{(1)}$, $Q^{(2)}$, and $Q^{(3)}$. The sequences have evolutionary distances $\tau_1$, $\tau_2$, and $\tau_3$ to the branching point of the tree, as defined by Eq. (26), and have lengths $N_1 \approx N_2 \approx N_3 \approx 5000$. (b) Alignment data $\delta E_{12}$, $\delta E_{13}$ and $\delta E_{23}$ for pairwise alignments of the sequences at different values of $\gamma$, shown as a function of $C(\gamma)$. $\delta E_{12}$, $\delta E_{13}$, and $\delta E_{23}$ obtained by rescaling the raw alignment data by respective factors $U_{12}$, $U_{13}$, and $U_{23}$ such that the maxima of the rescaled curves fall on the theoretical locus $(x^*(y), \delta E^*(y))$ (long-dashed curve, cf. Fig. 7(b)). This determines the a priori unknown similarity coefficients $U_{ij}$, and hence the evolutionary distances $\tau_i$.

Finally, we construct the pairwise alignments of highest fidelity. From Fig. 8(a), they are seen to satisfy the approximate relation $C^*_{ij}/C^*_{ij} \approx x^*_{ij}/x^*_{ij} \approx 1.2$ for $0.1 < y < 4$. With the values $C^*_{12} \approx 0.23$, $C^*_{13} \approx 0.225$, and $C^*_{23} \approx 0.254$ read off from Fig. 9(b) and using Eq. (15) with the constants of Fig. 6, we obtain the optimal alignment parameters $\gamma^*_{12} \approx 1.52$, $\gamma^*_{13} \approx 1.59$, $\gamma^*_{23} \approx 1.25$. The scaled score maxima $\delta E^*_1 \approx 0.26$, $\delta E^*_1 \approx 0.18$, $\delta E^*_2 \approx 0.15$ determine the expected fidelities $F^*_{12} \approx 0.75$, $F^*_{13} \approx 0.58$, $F^*_{23} \approx 0.52$ as seen from Fig. 8(b). They are in good agreement with the actual maxima $F^*_{12} = 0.8$, $F^*_{13} = 0.65$, $F^*_{23} = 0.55$ computed by comparing directly to the evolutionary paths.

5 Discussion

We have presented a statistical scaling theory for global gapped alignments. Alignments of mutually uncorrelated sequences are found to be governed by a number of universal scaling laws: ensemble averages such as the mean square displacement of the alignment path or the variance of the optimal score follow power laws whose exponents do not depend on the scoring parameters. The parameter dependence is contained entirely in the prefactors. This universality is comparable to the diffusion law describing a large variety of random walk processes on large scales, the only parameter dependence being the value of the diffusion constant. In contrast to diffusive random walks, however, we find optimal alignment paths to be strongly non-Markovian on all length scales due to random agglomerations of matches and mismatches. Hence, the exponents take nontrivial values. The scaling laws also govern the displacement statistics of a single alignment path $r(t)$ and the associated statistics of partial scores, which makes these concepts applicable to individual alignment problems.
This scaling theory is also relevant for the statistics of mutually correlated sequence pairs. Two important quantities are the score gain over uncorrelated sequences and the alignment fidelity. Both quantities strongly depend on the evolutionary parameters linking the two sequences and on the alignment parameters. For a simple Markovian evolution model and for linear scoring functions, we have obtained a quantitative description of this parameter dependence. In particular, the alignment parameter of maximal fidelity turns out to be closely related to the parameter of maximal score gain, which makes it possible to construct the alignment of maximal fidelity from a systematic analysis of score data. Moreover, the underlying evolutionary parameters (the mutual similarity $U$ and the effective indel rate $q$) can also be inferred from this analysis.

It is important to understand inhowfar the results of this paper carry over to more refined algorithms for the alignment of realistic sequences. The universal scaling laws for uncorrelated sequences should prove to be very robust under changes of the scoring function (such as scoring matrices distinguishing between transitions and transversions) as well as changes in the sequences (the number of different letters and their frequencies). As corroborated by preliminary numerical results, such changes reduce to a different parameter dependence of the amplitude functions $A, B, C,$ and $D$. In particular, we find the universal scaling laws to be preserved for the alignment of bona fide uncorrelated cDNA sequences, which also validates the Markov model for single sequences. While not affecting the asymptotic universality, some scoring functions (for example, systems with affine gap cost distinguishing between gap initiation and gap extension) may introduce intermediate regimes where the score and fidelity curves are modified. Nevertheless, the fidelity and the score gain remain key quantities of an alignment, and their optimal values are closely related. This makes it possible to construct optimal alignments on the basis of a statistical analysis of score data. This link and the underlying scaling theory are also crucial to the analysis of local alignment algorithms, as we have shown recently (Hwa and Lässig, 1998; Drasdo et al., 1998).

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Appendix A: Evolution model

The Markov process governing the evolution of a daughter sequence $Q'$ from an ancestor sequence $Q$ is specified by the flux diagram of Fig. 10.

The statistical properties of this Markov process are straightforward to compute. Using the notation $t \equiv i + j$ and $R \equiv j - i$, we find $R(t)$ to be a Gaussian random variable with

$$\overline{R(t)} = 0, \quad \overline{R^2(t)} = qt, \quad (27)$$

where $q$ is given by Eq. (2). This implies in particular that the length $N'$ of the daughter sequence is also a Gaussian random variable with

$$\overline{N'} = N, \quad (N' - N)^2 = 2qN. \quad (28)$$

Appendix B: Alignment Algorithm

The dynamic programming algorithm generates the score landscape $S(r, t)$ for all grid points by the recursion relation

$$S(r, t) = \max \left\{ \frac{S(r - 1, t - 1) - \gamma}{S(r + 1, t - 1) - \gamma}, S(r, t - 2) + s(r, t) \right\} \quad (29)$$

with

$$s(r, t) = \begin{cases} \sqrt{c - 1} & \text{if } Q'_{(r+t)/2} = Q_{(r-t)/2} \\ -\frac{1}{\sqrt{c-1}} & \text{if } Q'_{(r+t)/2} \neq Q_{(r-t)/2} \end{cases} \quad (30)$$

This recursion relation is evaluated in a restricted alignment grid shown in Fig. 11, which limits the computing time to a value $\sim T \times L$. The value of $L$ is chosen according to the
Fig. 11: Restricted alignment grid (bounded by thick lines) used for the evaluation of the recursion relation (29). With initial condition (i), the alignment paths are pinned at their initial point (dot) defined to be at $t = 0$. With initial condition (ii), the score is prescribed along the dashed line defined to be at $t = 0$, namely $S(r, t = 0) = 0$.

Two types of initial conditions are used depending on the specific application: (i) $r(t = 0) = 0$ (with $t ≡ i + j$) or (ii) $S(t = 0) = 0$ (with $t ≡ i + j - L/2$). Evaluation of the recursion relation stops at $t = T$. Hence, the optimal alignment path $r_0(t)$ ends at the point $r_0 ≡ r_0(T)$ given by $S(r_0, T) = \min_r S_0(r, T)$. If the score values $S_0(r, T)$ are degenerate for different values of $r$, one of them is chosen at random. The entire path $r_0(t)$ is then found by backtracking it from its endpoint $r_0$. Degeneracies are again resolved by a random choices. This is justified since degenerate optimal paths have a typical distance of order 1 only. For more precise formulations of this “macroscopic uniqueness”, see Fisher and Huse (1991), Hwa and Fisher (1994), Kinzelbach and Lässig (1995).

To compute the unconstrained fluctuations of optimal alignments for uncorrelated sequences, $L$ has to be sufficiently large so that the result becomes independent of it: $L^2 \gg (\Delta r_0(T))^2$. The displacement fluctuations $(\Delta r_0(t))^2$ and the tilt cost $\delta E_t(\theta)$ are evaluated with the pinned initial condition (i); in the latter case, also the endpoint $r_0 = \theta T$ is pinned. The score variance (3) is computed with the initial condition (ii).

On the other hand, the confinement cost $\delta E_c(r_c)$ is determined by choosing $L ≡ r_c$ and $T \gg L^{3/2} t_0(\gamma)$ so that the result becomes independent of $T$ and of the initial condition.

For correlated sequences, we choose $L$ again large enough so that the result becomes independent of it: $L^2 \gg (\Delta R(T))^2 + r_c^2$. For $T \gg t_c$, quantities defined per unit of $t$ such as $F$ and $\delta E$ will also become independent of the initial condition.
References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. 1990. Basic local alignment search tool. *J. Mol. Biol.* **215** (3), 403 – 10.

Altschul, S.F. 1993. A protein alignment scoring system sensitive at all evolutionary distances. *J. Mol. Evol.* **36** (3), 290 - 300.

Arratia, R., Morris, P. and Waterman, M.S. 1988. Stochastic scrabbles: a law of large numbers for sequence matching with scores. *J. Appl. Probab.* **25** 106 – 19.

Arratia, R. and Waterman, M.S., 1994. A phase transition for the score in matching random sequences allowing deletions. *Ann of Appl. Prob. 4*, 200 – 25.

Benner, S.A., Cohen, M.A. and Gonnet, G.H. 1993. Empirical and structural models for insertions and deletions in the divergent evolution of proteins. *J. Mol. Biol. 229* (4), 1065 – 82.

Bishop, M.J. and Thompson, E.A. 1986. Maximum likelihood alignment of DNA sequences. *J. Mol. Biol. 190* (2), 159 - 65.

Cule, D. and Hwa, T. 1998. Static and Dynamic Properties of Inhomogeneous Elastic Media on Disordered Substrate. *Phys. Rev. B.* in press.

Drasdo, D., Hwa, T. and Lässig, M. 1997. DNA sequence alignment and critical phenomena. *Mat. Res. Soc. Symp. Proc.* **263**, 75-80.

Drasdo, D., Hwa, T. and Lässig, M. 1998. A statistical theory of sequence alignment with gaps, submitted to *The Sixth International Conference on Intelligent Systems for Molecular Biology*.

From MEDLINE; 96096722, cf Exposito J.Y., Boute N., Deleage G., Garrone R.. 1995. Characterization of two genes coding for a similar four-cysteine motif of the amino-terminal propeptide of a sea urchin fibrillar collagen. *Eur. J. Biochem.* 234:59-65.

From MEDLINE; 94215495, cf. Fehon R.G., Dawson I.A., Artavanis-Tsakonas S. 1994. A Drosophila homologue of membrane-skeleton protein 4.1 is associated with septate junctions and is encoded by the coracle gene. *Development* 120:545-557.

Fisher, D.S. and Huse, D.A. 1991. Directed paths in a random potential. *Phys. Rev. B 43* (13), 10728 - 10742.

Gusfield, D., Balasubramanian, K., and Naor, D.. 1992. *Proceedings of the Third Annual*
Hwa, T. and Fisher, D.S. 1994. Anomalous fluctuations of directed polymers in random media. Phys. Rev. B 49, 3136 – 54.

Hwa, T. and Lässig, M. 1996. Similarity detection and localization, Phys. Rev. Lett. 76, 2591 - 2595.

Hwa, T. and Nattermann, T. 1995. Disordered induced depinning transition, Phys. Rev. B 51, 455 - 469.

Hwa, T. and Lässig, M. Optimal detection of sequence similarity by local alignment. Proc. of the Second Annual Conference on Computational Molecular Biology (RECOMB98), in press. E-print cond-mat/9712081.

Karlin, S. and Altschul, S.F. 1990. Methods for assessing the statistical significance of molecular sequence features by using general scoring schemes. Proc. Natn. Acad. Scie. U.S.A. 87 (6), 2264 - 8.

Karlin, S. and Altschul, S.F. 1993. Applications and statistics for multiple high-scoreing segments in molecular sequences. Proc. Natn. Acad. Scie. U.S.A. 90 (12), 5873 - 7.

Koretke, K.K., Kutheyschulten, Z., Wolynes, P.G. 1996. Self-consistently optimized statistical mechanical energy functions for sequence structure alignment Prot. Sci. 5, 1043-1059.

Kardar, M. 1987. Replica Bethe ansatz studies of two-dimensional interfaces with quenched random impurities. Nucl. Phys. B 290, 582 - 602.

Kinzelbach, H. and Lässig, M. 1995. Depinning in a random medium. J. Phys. A: Math. Gen. 28, 6535 - 6541.

Licea, C. and Newman, C.M. 1996. Geodesics in two-dimensional first-passage percolation. Ann. Probab. 24, 399 – 410.

Licea, C., Newman, C.M., and Piza, M.S.T. 1996. Superdiffusivity in first-passage percolation. Probab. Theory Relat. Fields 106, 559 – 91.

Onuchic, J.N., LutheySchulten, Z., Wolynes, P.G. 1997. Protein folding funnels: the nature of the transition state ensemble. Ann. Rev. Phys. Chem. 48, 545-600, and references therein.

Needleman, S.B. and Wunsch, C.D. 1970. A general method applicable to the search for
similarities in the amino acid sequence of two proteins. *J. Mol. Biol.* 48 (3), 443 - 53.

Pearson, W.R. 1991. Searching protein sequence libraries: comparison of the sensitivity and selectivity of the Smith-Waterman and FASTA algorithms. *Genomics* 11 (3), 635 - 650.

Smith, T.F. and Waterman, M.S. 1981. Identification of common molecular subsequences. *J. Mol. Biol.* 147, 195 – 7.

Thorne, J.L., Kishino, H. and Felsenstein, J. 1991. An evolutionary model for maximum likelihood alignment of DNA sequence *J. Mol. Evol.* 33 (2), 114 - 24

Thorne, J.L., Kishino, H., and Felsenstein, J. 1992. Inching toward reality: an improved likelihood model of sequence evolution. *J. Mol. Evol.* 34 (1), 3 - 16.

Vingron, M. and Waterman, M.S. 1994. Sequence alignment and penalty choice. Review of concepts, case studies and implications. *J. Mol. Biol* 235 (1), 1 - 12.

Wang, J., Onuchic, J., Wolynes, P.G. 1996. Statistics of kinetic pathways on biased rough energy landscapes with application to protein folding. *Phys. Rev. Lett.* 76, 4861-4864.

Waterman, M.S., Gordon, L. and Arratia, R. 1987. Phase transitions in sequence matches and nucleic acid structure. *Proc. Natl. Acad. Sci. U.S.A.* 84 (5), 1239 - 43.

Waterman, M.S. 1989. In Waterman, M.S, ed., *Mathematical Methods for DNA Sequences*. CRC Press.

Waterman, M.S. Eggert, M. and Lander, E. 1992. Parametric sequence comparisons. *Proc. Natn. Acad. Scie. U.S.A.* 89 (13), 6090 - 3.

Waterman, M.S. 1994. *Introduction to Computational Biology*, Chapman & Hall.

Waterman, M.S. 1994. Parametric and ensemble sequence alignment algorithms. *Bull. Math. Biol.* 56 (4), 743 - 767.

Zhang, M.Q. and Marr, T.G. 1995. Alignment of molecular sequences seen as random path analysis. *J. Theo. Biol.* 174 (2), 119 - 29.