

Strand separation in negatively supercoiled DNA

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Abstract

We consider Benham's model for strand separation in negatively supercoiled circular DNA, and study denaturation as function of the linking difference density $\kappa < 0$. We propose a statistical version of this model, based on bayesian segmentation methods of current use in bioinformatics; this leads to new algorithms with priors adapted to supercoiled DNA, taking into account the random nature of the free energies needed to denature base pairs.

Keywords: Strand separation, statistical mechanics, supercoiled DNA, Bayesian model

Running title: Strand separation

1 Introduction

Initiation of transcription in DNA requires the two strands of the double helix to separate, and strand separation is enhanced in negative supercoiled DNA. Benham(1979,1990,1996) proposes a mathematical model for the process of strand separation, based on statistical mechanics ideas, and develop algorithms to locate interesting sites along the DNA where strand separation or replication is strongly favored (see e.g. Clote and Backhofen(2000)). These computational methods are Metropolis dynamics (Sun *et al*ri(1995)) or exact methods relying on transfer matrices(Fye and Benham(1999)). In a typical state, some base pairs are broken; we are interested in the repartition of the droplets of denatured bonds, and on the nature of the bases situated in these domains. Our aim is to investigate the effect of the number of droplets, of the degree of negative superhelicity of the DNA and of the concentration in A+T bonds on the equilibrium properties of Benham's model. This is the topic of Section 2, where homopolymers are treated according to the number of connected domains of denatured bonds: in Section 2.1.1, we study denaturation when no restriction on the number of domains is imposed, and show that no fully denatured state exists; this is the situation adapted to the algorithms of Fye and Benham(1999). In Section 2.1.2, we study the model when the number of domains r_N is such that $r_N/N \rightarrow 0$, as $N \rightarrow \infty$, where N denotes the number of bases of the DNA. We show the existence of a fully denatured state when the duplex is sufficiently negatively supercoiled. This is the regime where the MCMC of Sun *et al*ri applies. We next turn to copolymers in Section 2.2, and study localized denaturation as function of the proportion of A+T bonds and of the level of negative superhelicity of the DNA. Section 3 focus on the statistical aspects of the model: Section 3.1 translates Benham's model in a Bayesian framework, and Section 3.2 shows how Bayesian segmentation methods of current use in bioinformatics, as presented in Liu and Lawrence(1999), can be of interest in the strand separation problem. This also give new algorithms with priors adapted to supercoiled DNA, which take into account the random fluctuations of the free energies needed to denature A+T and G+C bonds.

In what follows, we consider a spin system based on a circular graph with node set S , $|S| = N$, $N \in \mathbb{N}$, and, for each site $i \in S$, a spin $\sigma_i \in \{-1, +1\}$. Benham's original model deals with a lattice gas, with binary random variables n_i . We use both notations by setting $n_i = (\sigma_i + 1)/2$, and use spins to link this model with known mean field models. The meaning of $n_i = 0$ (resp. $\sigma_i = -1$) is that the bases of the double helix at site $i \in S$ are linked by an hydrogen bond, the link is closed, and $n_i = 1$ (resp. $\sigma_i = +1$) means that this bond is broken, the link is open. Then $n := \sum_{i=1}^N n_i$ denotes the number of open bonds. The partitioning of the DNA in domains allows the linking numbers to be regulated, where the linking number L of a

configuration describes the way the duplex winds about the axis, assuming that the axis of the helix is planar. The twist T is the number of times the duplex revolves about its axis (see e.g. Clote and Backhofen(2000), chap. 6.2). When the DNA is relaxed, the so-called B-DNA state, a segment of N bases produces typically the characteristic linking number $L_0 = N/A$, where the constant A is experimentally situated around 10.4. A negative supercoiled DNA is a configuration obtained from the B-DNA by cutting the strands using topoisomerase of type II; one another piece of the duplex passes through the gap, which is closed afterwards. This process reduces then the linking number of the duplex and forces the circular axis of the helix to wind, producing then a more twisted and compact configuration (see e.g. Lewin(1994)). This supercoiled state permits for example to put the helix in nuclei. Benham's model permits to quantify the way supercoiling enhances strand separation.

Consider a supercoiled DNA with negative linking difference $\alpha = L - L_0 < 0$, imposed during the process. Assume that n base paires are denatured; the helix unwinds locally and thus increases its linking difference to $\alpha + n/A$. Because the strands can rotate around each others, the same process induced also a twist \mathcal{T} , yielding a residual linking difference $\alpha_r = \alpha + n/A - \mathcal{T}$. The total twist \mathcal{T} between separated regions is modeled as

$$\mathcal{T} = \sum_{i=1}^N \frac{n_i \tau_i}{2\pi},$$

where $\tau_i \in \mathbb{R}$ is the local helicity. Benham's idea is to quantify all of these steps with free energy costs. The torsional free energy G_t is given by

$$G_t = \frac{C}{2} \sum_{i=1}^N n_i \tau_i^2,$$

for some stiffness coefficient $C > 0$. Consider a configuration in which n bonds are separated in r runs, that is in r connected components of open bonds. In what follows, $2r$ is the *perimeter of the configuration*.

The free energy cost for separation is modeled as

$$G_s = ar + \sum_{i=1}^N b_i n_i, \quad a > 0, \quad r = \sum_i n_i (1 - n_{i+1}),$$

where the parameters b_i indicate the natures of the bases located at sites $i \in S$: AT links are formed of 2 hydrogen bonds, and GC links consist in 3 hydrogen bonds. If $i \in S$ is associated with an AT link, we set $b_i = b_{AT}$ and $b_i = b_{GC}$ otherwise, with $b_{GC} > b_{AT}$. In the homopolymer case, $b_i \equiv b > 0$. This will act as an exterior magnetic field in the statistical approach; when the bases are chosen at random on the DNA with some law, $\sum_i n_i b_i$ can

be viewed as a random external field. The free energy b needed to break the base pair, and thus to separate a base pair, depends on the inverse temperature β :

$$b = b(\beta) = \Delta H \left(1 - \frac{\beta_m}{\beta}\right), \quad (1)$$

where ΔH is the enthalpy of the reaction and β_m is the inverse temperature associated to the melting temperature T_m . Below T_m , the field b is positive, this is the regime we are interested in. From Benham(1992), the pBR322 DNA is such that $\Delta H_{AT} = 7.25$ kcal/mol, $\Delta H_{GC} = 9.02$ kcal/mol, and the melting temperature T_m follows the law

$$T_m = 354.55 + 16.6 \log(x) + 41F_{GC},$$

where x is some parameter and $F_{GC} = 0$ for AT bonds and $F_{GC} = 1$ for GC bonds. When $x = 0.01$ and $T = \beta^{-1} = 310$ K, the resulting free energies are given by $b_{AT} = 0.255$ kcal/mol and $b_{GC} = 1.301$ kcal/mol.

Long range interactions appear with the fluctuations of the linking difference: it is known experimentally that the energy cost associated with the residual linking difference for supercoiled DNA is given by

$$G_r = \frac{K\alpha_r^2}{2} = \frac{K}{2} \left(\alpha + \frac{n}{A} - T\right)^2.$$

Experimentally, the coefficient K is inverse proportional to the number of bases of the DNA; we thus set

$$K = \frac{K_0}{N}.$$

As N is large, basic statistical reasoning suggests to renormalize the variable n as n/N , to catch the thermodynamical limit. We thus introduce the linking difference density κ by setting

$$\alpha = \kappa N,$$

which is related to the superhelical density s as $s = \kappa A$. The overall free energy takes then the form

$$G = \frac{C}{2} \sum_{i=1}^N n_i \tau_i^2 + N \frac{K_0}{2} \left(\kappa + \frac{n}{2NA} - \frac{1}{N} \sum_{i=1}^N \frac{n_i \tau_i}{2\pi} \right)^2 + \sum_{i=1}^N ((a + 2b_i)n_i - a n_i n_{i+1}).$$

We use the local fields $2b_i$ instead of b_i for notational purpose.

2 Results on denaturation

2.1 The homopolymer approximation

In this paragraph, we suppose that $b_i \equiv b > 0$ and that $\tau_i \equiv \tau \in \mathbb{R}$. Set for convenience

$$M_N = \frac{1}{N} \sum_{i=1}^N n_i, \quad m_N = \frac{1}{N} \sum_{i=1}^N \sigma_i = 2M_N - 1.$$

Then the Hamiltonian of the system becomes

$$G_\tau = N \left(2bM_N + \frac{C\tau^2}{2} M_N + \frac{K_0}{2} \left(\kappa + \left(\frac{1}{2A} - \frac{\tau}{2\pi} \right) M_N \right)^2 \right) + aH_{\text{sing}}/4,$$

where the index τ of G_τ indicates the dependence on the torsion coefficient τ and H_{sing} denotes the Hamiltonian associated with the nearest neighbor ferromagnetic Ising model in dimension 1

$$H_{\text{sing}} = - \sum_{i=1}^{N-1} \sigma_i \sigma_{i+1} = 4r - N.$$

Before introducing the Gibbs measure of the system at inverse temperature $\beta > 0$, let us proceed as in Benham by averaging the system with respect to the torsion coefficient τ . The Boltzman weight should be $\exp(-\beta G_\tau)$. Averaging over $\tau \in \mathbb{R}$ gives the integral $\int_{\mathbb{R}} \exp(-\beta G_\tau) d\tau$, that is the effective Hamiltonian

$$H_1 = N2bM_N + \frac{a}{4}H_{\text{sing}} + N \frac{2\pi^2 CK_0}{4\pi^2 C + K_0 M_N} \left(\kappa + \frac{M_N}{A} \right)^2, \quad (2)$$

(see Fye and Benham(1999)).

2.1.1 Arbitrary large perimeter

In this approximation, we shall consider the behavior of M_N in the thermodynamical limit $N \rightarrow \infty$ under Gibbs measure

$$\pi_{\beta, B}(\sigma) = \exp(-\beta H_1(\sigma)) / Z_N(\beta, B), \quad (3)$$

where $Z_N(\beta, B)$ denotes the related partition function, and where $B = (b_i)_{1 \leq i \leq N}$, $b_i \equiv b$, denotes the exterior field. We use mainly Laplace method bu using the large deviation rate function associated with the ferromagnetic nearest neighbor Ising model in dimension one. Notice the appearance of the magnetization M in the denominator of (2), which is quite unconventional.

Let π_{β_a} be the Gibbs measure at inverse temperature $\beta_a = a\beta/4$ associated with the Hamiltonian H_{sing} . Then, for any function $h : I := [0, 1] \rightarrow \mathbb{R}$,

$$\langle h(M_N) \rangle_{\pi_{\beta,B}} = \frac{\langle h(M_N) \exp(-N\beta F_B(M_N)) \rangle_{\pi_{\beta_a}}}{\langle \exp(-N\beta F_B(M_N)) \rangle_{\pi_{\beta_a}}}, \quad (4)$$

where

$$F_B(y) = b(2y - 1) + G(y), \quad y \in I, \quad (5)$$

and

$$G(y) = \frac{2\pi^2 CK_0}{4\pi^2 C + K_0 y} \left(\kappa + \frac{y}{A} \right)^2.$$

Let μ_N be the law of $m_N(\sigma) = 2M_N(\sigma) - 1$ under Gibbs measure π_{β_a} . Then

$$\langle h(M_N) \rangle_{\pi_{\beta,B}} = \frac{\int_{-1}^{+1} \mu_N(dz) h\left(\frac{1+z}{2}\right) \exp(-\beta N F_B\left(\frac{1+z}{2}\right))}{\int_{-1}^{+1} \mu_N(dz) \exp(-\beta N F_B\left(\frac{1+z}{2}\right))}. \quad (6)$$

Benham(1979) investigates the thermodynamics of supercoiled DNA, by minimizing free energies, and introduces critical thresholds of supercoiling. In this macroscopic approach, it is shown that sufficient negative supercoiling implies local denaturation. In Benham(1996), this work is extended to positively supercoiled DNA ($\kappa > 0$); looking at the various plots contained in this work, we see the appearance of critical linking difference densities above which a positively supercoiled DNA remains intact at temperatures higher than the melting point. Similarly, we introduce the

Definition 1 *The order parameter of the system is*

$$\langle M_N \rangle_{\pi_{\beta,B}}, \quad \text{or} \quad \langle m_N \rangle_{\pi_{\beta,B}},$$

the magnetization of the spin system. We say that the system exhibits phase transitions when there exist critical linking difference densities $\bar{\kappa}_c(B) > \kappa_c(B)$ such that, in the large N limit, $\langle M_N \rangle_{\pi_{\beta,B}} \rightarrow 0$ as $\kappa > \bar{\kappa}_c(B)$, $\langle M_N \rangle_{\pi_{\beta,B}} \rightarrow 1$ as $\kappa < \kappa_c(B)$, and $\lim \langle M_N \rangle_{\pi_{\beta,B}} \in (0, 1)$ otherwise.

Let $\Lambda_N(\lambda)$ be the logarithmic moment generating function

$$\Lambda_N(\lambda) := \ln(\pi_{\beta_a}(\exp(Nm_N\lambda))),$$

with (see e.g. Baxter(1982), p.34)

$$\Lambda_\infty(\lambda) := \lim_{N \rightarrow \infty} \frac{1}{N} \Lambda_N(\lambda) = \ln \left(\frac{e^{\beta_a} \cosh(\lambda) + \sqrt{e^{2\beta_a} \sinh(\lambda)^2 + e^{-2\beta_a}}}{e^{\beta_a} + e^{-\beta_a}} \right).$$

Then, according to Gaertner-Ellis Theorem, (see e.g. Dembo and Zeitouni(1992)) the law of m_N under π_{β_a} satisfies a large deviation principle with good rate function

$$I_{\text{sing}}(z) = \sup_{\lambda \in \mathbb{R}} (\lambda z - \Lambda_\infty(\lambda)),$$

the Legendre transform of Λ_∞ . $\Lambda'_\infty = e^{\beta a} \sinh / \sqrt{e^{2\beta a} \sinh^2 + e^{-2\beta a}}$, and $\Lambda''_\infty = e^{\beta a} \cosh / (e^{2\beta a} \sinh^2 + e^{-2\beta a})$. Λ_∞ is thus strictly convex on \mathbb{R} , and its Legendre transform is essentially smooth (see Theorem 26.3 in Rockafellar(1972)). The derivative $I'_{\text{sing}}(z)$ tends to $+\infty$ when z converges to a boundary point of the domain of I_{sing} . From computation,

$$I_{\text{sing}}(z) = z \ln\left(\frac{ze^{-2\beta} + \sqrt{1+z^2(e^{-4\beta a} - 1)}}{\sqrt{1-z^2}}\right) - \ln\left(\frac{e^{\beta a} \sqrt{1+z^2(e^{-4\beta a} - 1)} + e^{-\beta a}}{\sqrt{1-z^2}(e^{\beta a} + e^{-\beta a})}\right),$$

$|z| \leq 1$, and $I_{\text{sing}}(z) = +\infty$ when $|z| > 1$.

In the special case where $a = 0$, the rate function becomes the entropy

$$I_{\text{sing}}(z) = \frac{1+z}{2} \ln(1+z) + \frac{1-z}{2} \ln(1-z), \quad |z| \leq 1.$$

The integrals appearing in (6) can be estimated through Laplace's method by rewriting the numerator heuristically as

$$\int_{-1}^{+1} dz h\left(\frac{1+z}{2}\right) \exp(-NJ(z)),$$

where we set

$$J(z) := I_{\text{sing}}(z) + \beta F_B\left(\frac{1+z}{2}\right), \quad |z| \leq 1.$$

Then (6) is asymptotically equivalent to

$$\frac{\int_{-1}^{+1} dz h\left(\frac{1+z}{2}\right) \exp(-N(J(z) - \inf_{|z| \leq 1} J(z)))}{\int_{-1}^{+1} dz \exp(-N(J(z) - \inf_{|z| \leq 1} J(z)))}.$$

Theorem 1 \exists a unique $z_* \in (-1, +1)$ minimizing $J(z)$, with

$$\langle M_N \rangle_{\pi_{\beta, B}} \longrightarrow \frac{1+z_*}{2}.$$

The model does not exhibit phase transitions in the sense of Definition 1. This is a consequence of the stiffness of the rate function $I_{\text{sing}}(z)$ as $|z| \rightarrow 1$: $\lim_{|z| \rightarrow 1} (d/dz)I_{\text{sing}}(z) = +\infty$, and the rate function $J(z)$ can not be decreasing in the neighborhood of $z = 1$. The minima of $J(z)$ are thus located in the interior of the unit interval. Suppose that the Ising measure is replaced by some probability measure V_N on Ω_N , such that the law of M_N under V_N satisfies a large deviation principle with strictly convex and smooth free energy function Λ ; then its Legendre transform is essentially smooth, and again, using Varadhan's Theorem, one gets the rate function

$J(z) - \inf_z J(z)$; similarly, the minima of J are located in the interior of the domain of the Legendre transform, and no phase transition occurs.

Proof: Consider the probability measure

$$\nu_N(C) = \frac{\int_C \mu_N(dz) \exp(-N\beta F_B((1+z)/2))}{\int_{-1}^1 \mu_N(dz) \exp(-N\beta F_B((1+z)/2))},$$

for any Borel subset C of $[-1, 1]$. Varadhan's Theorem (see Deuschel and Stroock, Theorem 2.1.10 and exercise 2.1.24) gives that ν_N satisfies a large deviation principle with good rate function $J(z)$. If J attains its infimum at a unique point z_* of the interval, the sequence ν_N converges weakly to the point mass δ_{z_*} . Consider first $F_B(y)$, $y \in [0, 1]$, or equivalently $F_B + 2b\kappa A + b$, which is equal to

$$\frac{2(bA^2 + \pi^2 C)(y - y_0)(y - y_1)}{A^2 (y - y_2)},$$

where

$$y_0 = -\kappa A > 0, \quad y_1 = -\frac{\pi^2 CA}{K_0(bA^2 + \pi^2 C)}(4bA + K_0\kappa) \text{ and } y_2 = -\frac{4\pi^2 C}{K_0}.$$

Notice that $b > 0$ implies that $y_1 > y_2$. The function has a pole at $y = y_2 < 0$, and two roots y_1 and $y_0 > 0$. Then F_B is strictly convex on the half line $(y_2, +\infty)$. Thus J is strictly convex on $[-1, 1]$, with $\lim_{|z| \rightarrow 1} J'(z) = +\infty$. The unique infimum is thus located in the interior of the interval.

2.1.2 Limited perimeter

Computations done in some theoretical studies (see e.g. Benham(1989), p. 268 and Benham(1990), p. 6302) or empirical studies (see e.g. Sun *et alri*(1995, p. 8658)) deal with the behavior of M_N when $2r$ is fixed, or is small. In what follows, we give conditions on the growth of $r = r_N$ ensuring the possibility of phase transitions, that is the possibility for global denaturation when the linking difference density is small enough.

In what follows, we condition on the event $\{\sigma; |\sigma| = 2r_N\}$, where for any configuration σ , $|\sigma|$ denotes the perimeter of σ , with $|\sigma| = |i; \sigma_i = -\sigma_{i+1}| = 2r$, and $H_{\text{sing}}(\sigma) = 2|\sigma| - N$. Classical combinatorics (see e.g. Feller(1971)) shows that the number of configurations of length N with $\sum_{i=1}^N n_i = n$ and perimeter $1 \leq r \leq N/2$ is given by

$$M(n, r) = \frac{N}{r} \binom{n-1}{r-1} \binom{N-n-1}{r-1}.$$

Let $U_{r,N}$ be the uniform probability measure on the subset $\mathcal{C}_{r,N}$ of the cube Ω_N consisting of spins of perimeter $2r$. Let $P_{r,N}$ be the law of M_N under

$U_{r,N}$, with $\text{supp}(P_{r,N}) = \{r/N, \dots, 1 - r/N\}$, given by

$$P_{r,N}(n/N) = \frac{\binom{n-1}{r-1} \binom{N-n-1}{r-1}}{\sum_{r \leq n \leq N-r} \binom{n-1}{r-1} \binom{N-n-1}{r-1}}. \quad (7)$$

Then the average $\langle h(M) \rangle_{\pi_{\beta,B}}$ becomes

$$\frac{\sum_r \exp(-\beta_a r) |\{\sigma\} = 2r\} | \sum_{r \leq n \leq N-r} P_{r,N}(n/N) h(n/N) \exp(-N\beta F_B(n/N))}{\sum_r \exp(-\beta_a r) |\{\sigma\} = 2r\} | \sum_{r \leq n \leq N-r} P_{r,N}(n/N) \exp(-N\beta F_B(n/N))}$$

We will be concerned with integrals of the form

$$\int_0^1 P_{r_N,N}(dy) h(y) \exp(-\beta N F_B(y)),$$

when $r = r_N$ is such that $r_N/N \rightarrow 0$ as $N \rightarrow \infty$.

Lemma 1 *Assume that $2r_N < N$ and that $r_N/N \rightarrow 0$ as $N \rightarrow \infty$. Then*

$$\frac{1}{N} \log \binom{n}{r_N} \rightarrow 0, \quad N \rightarrow \infty, \quad (8)$$

when $n = [\rho N]$, for $0 < \rho \leq 1$. The sequence of probability measures $(P_{r_N,N})_{n \in \mathbb{N}}$ satisfies a large deviation principle with good rate function $I^r : \mathbb{R} \rightarrow [0, +\infty)$ given by $I^r(y) = 0$, $y \in I$, and $I^r(y) = +\infty$, $y \in I^c$.

Proof: The first assertion is a consequence of Stirling's formula. Assume that $A = (a, b) \subset I$. For N large enough, $A \cap \{r_N/N, \dots, 1 - r_N/N\} \neq \emptyset$, and A contains an element ρ_N of the form $\rho_N = [\rho N]/N$ for some $0 < \rho < 1$, with

$$\log P_{r_N,N}(\{\rho_N\}) \leq \log P_{r_N,N}(A) \leq 0.$$

Using (7) and (8), it remains to check that

$$\frac{1}{N} \log \left(\sum_{r \leq n \leq N-r} \binom{n}{r} \binom{N-n}{r} \right) \rightarrow 0.$$

But, as the sum larger than one,

$$\begin{aligned} 0 &\leq \frac{1}{N} \log \left(\sum_{r \leq n \leq N-r} \binom{n}{r} \binom{N-n}{r} \right) \\ &\leq \frac{1}{N} \log \left((N-2r) \sup_{r \leq n \leq N-r} \binom{n}{r} \binom{N-n}{r} \right) \\ &\leq \frac{\log(N)}{N} + \frac{1}{N} \log \left(\sup_{r \leq n \leq N-r} \binom{n}{r} \binom{N-n}{r} \right). \end{aligned}$$

The sequence $\left(\binom{n}{r}\binom{N-n}{r}\right)_n$ attains its supremum when $n = [N/2]$, and the statement is a consequence of (8). A is a I^r -continuity set. When $A \cap I = \emptyset$, $P_{r_N, N}(A) \equiv 0$, which is consistent with $I^r(y) = +\infty$ when $y \notin I$. When A takes the form $A = [-c, \varepsilon]$ with $c > 0$ and $\varepsilon > 0$, $\exists N_0 \in \mathbb{N}$ such that $0 < r_N/N < \varepsilon$, $\forall N \geq N_0$, and the same argument applies. When $A = [-c, 0]$, $P_{r_N, N}(A) \equiv 0$, and we have the inequalities defining the large deviation principle

$$-\inf_{y \in A^0} I^r(y) \leq \liminf \frac{1}{N} \log(P_{r_N, N}(A))$$

and

$$\limsup \frac{1}{N} \log(P_{r_N, N}(A)) \leq -\inf_{y \in \bar{A}} I^r(y). \quad (9)$$

The above arguments show that the upper and lower bounds (9) hold for open and compact sets. The sequence of measures $(P_{r_N, N})$ is supported by the unit interval, and the sequence is exponentially tight. The large deviation principle follows.

When the large deviation principle is satisfied with the flat rate function I^r , Laplace's method gives that the system exhibits a phase transition with respect to the order parameter M_N : the mass of the integral is located near the infimum of the function $F_B(y)$, and therefore, the DNA is completely denatured when the parameters of the problem are such that

$$\inf_{y \in (0,1)} F_B(y) = F_B(1).$$

This occurs for example when the helicity density κ is smaller than a critical density κ_c .

Theorem 2 *The function $F_B : I \rightarrow \mathbb{R}$ attains its infimum at a unique point $y_*(B, \kappa)$. Let*

$$y_0 = -\kappa A > 0, \quad y_1 = -\frac{\pi^2 C A}{K_0(bA^2 + \pi^2 C)}(4bA + K_0\kappa) \quad \text{and} \quad y_2 = -\frac{4\pi^2 C}{K_0}.$$

Let $\bar{\kappa}_c(B)$ and $\kappa_c(B)$ be the smallest roots of the polynomials $P(\kappa) = (y_0 - y_2)(y_1 - y_2) - y_2^2$ and $Q(\kappa) = P(\kappa) - (1 - 2y_2)$, with $\bar{\kappa}_c(B) > \kappa_c(B)$. Set $M_ = y_2 + \sqrt{(y_2 - y_0)(y_2 - y_1)}$. Then i) $\kappa > \bar{\kappa}_c(B)$ implies that F_B is increasing on I with $y_*(B, \kappa) = 0$, ii) $\kappa_c(B) < \kappa < \bar{\kappa}_c(B)$ implies that $y_*(B, \kappa) = M_* \in (0, 1)$, with F_B decreasing below $y_*(B, \kappa)$ and increasing above $y_*(B, \kappa)$, and iii) $\kappa < \kappa_c(B)$ implies that F_B is decreasing on I with $y_*(B, \kappa) = 1$. Let $h : I \rightarrow \mathbb{R}$ be bounded and continuous. Assume that $r_N/N \rightarrow 0$ as $N \rightarrow \infty$. Then*

$$\frac{P_{r_N, N}(h \exp(-N\beta F_B))}{P_{r_N, N}(\exp(-N\beta F_B))} \rightarrow h(y_*(B, \kappa)).$$

Proof: We look for the infimum of F_B on I ; equivalently, we can consider the infimum of $F_B + 2b\kappa A + b$, which is equal to

$$\frac{(2bA^2 + 2\pi^2 C)(y - y_0)(y - y_1)}{A^2 (y - y_2)}.$$

Notice that $b > 0$ implies that $y_1 > y_2$. The function has a pole at $y = y_2 < 0$, and two roots y_1 and $y_0 > 0$. We see that the first part of the theorem (cases i), ii) and iii)) is related to the location of the infimum of the function with respect to $(y_2, 0)$, $(0, 1)$ and $(1, \infty)$. Taking the derivative, we must look for the roots of $y^2 - 2y_2y + y_2(y_0 + y_1) - y_0y_1$, of discriminant $(y_0 - y_2)(y_1 - y_2) \geq 0$, since $y_1 > y_2$, $y_0 > 0$ and $y_2 < 0$, and we get the condition for the largest root M_* . The polynomial $P(\kappa)$ is obtained by imposing $M_* < 0$, and $Q(\kappa)$ by imposing $M_* < 1$. Concerning the last assertion, consider the probability measure

$$\mu_N(A) := \frac{\int_A \exp(-N\beta F_B(y)) P_{r_N, N}(dy)}{\int_I \exp(-N\beta F_B(y)) P_{r_N, N}(dy)},$$

for any Borel subset A . From Varadhan's Theorem (see e.g. Theorem 2.1.10 and exercise 2.1.24 in Deuschel and Stroock(1989) or Theorem II.7.2 in Ellis(1985)) and Lemma 1, the sequence (μ_N) satisfies a large deviation principle with rate function $I_F(y) - \inf_y I_F(y)$, where $I_F(y) = I'(y) + \beta F_B(y)$. $\inf_{y \in \mathbb{R}} I_F(y) = \beta \inf_{y \in I} F_B(y)$, which is realized at a unique element $y_*(B, \kappa)$; μ_N converges then weakly to the point mass $\delta_{y_*(B, \kappa)}$.

2.2 The copolymer case

In the copolymer case, the field $B^N = (b_i)_{1 \leq i \leq N}$ is indexed by a word of length N on the two letters alphabet $\{A + T, G + C\}$. Given B^N , let

$$\rho_N^+(B^N) := \frac{1}{N} \sum_{i=1}^N \mathbb{I}(b_i = b_{AT}),$$

where $\mathbb{I}(\cdot)$ is the indicator function. Given some proportion $\rho^+ \in I$, we shall consider families $(B^N)_{N \in \mathbb{N}}$ of words with $\rho_N^+(B^N) \rightarrow \rho^+$. Let

$$m_N^+(\sigma) = \frac{1}{N} \sum_{i=1}^N \mathbb{I}(b_i = b_{AT}) \sigma_i \text{ and } m_N^-(\sigma) = \frac{1}{N} \sum_{i=1}^N \mathbb{I}(b_i = b_{GC}) \sigma_i,$$

with $m_N = m_N^+ + m_N^-$, and consider the mapping $\Psi_{B^N} : \Omega_N \rightarrow \mathbb{R}^2$ given by $\Psi_{B^N}(\sigma) = (m_N^+(\sigma), m_N^-(\sigma))$, which permits to control the eventual localization of the magnetization. Let V_N be a probability measure on Ω_N , and

let Q_N be the image measure of V_N under Ψ_{B^N} . We consider the behavior of m_N^+ and m_N^- under the Gibbs measure

$$\pi_{\beta, B^N, V_N}(\sigma) = \frac{V_N(\sigma) \exp(-N\beta F_{B^N}(\sigma))}{Z_N(\beta, B^N, V_N)},$$

where we set

$$F_{B^N}(\sigma) = G(M_N(\sigma)) + \frac{1}{N} \sum_{i=1}^N b_i \sigma_i.$$

Let us denote by B_{GC} and B_{AT} the fields associated with the GC and AT homopolymers, with critical linking difference densities $\bar{\kappa}_c(B_{GC})$, $\kappa_c(B_{GC})$, $\kappa_c(B_{AT})$ and limiting proportion of broken bonds $y_*(B_{GC}, \kappa)$ (see Theorem 2).

Theorem 3 *Let $(B^N)_{N \in \mathbb{N}}$ be a sequence of words of $\{b_{AT}, b_{GC}\}^N$ with $\rho_N^+(B^N) \rightarrow \rho^+$, for some $\rho^+ \in I$. Suppose that the family of probability measures Q_N satisfies a large deviation principle with rate function $I_D : \mathbb{R}^2 \rightarrow [0, +\infty)$ given by $I_D(y) = 0$, $y \in D$ and $I_D(y) = +\infty$, $y \in D^c$, where $D = [-\rho^+, \rho^+] \times [-\rho^-, \rho^-]$. Let κ be such that $\kappa_c(B_{GC}) < \kappa < \kappa_c(B_{AT})$. Assume that $\rho^+ > y_*(B_{GC}, \kappa)$: Then denaturation is localized on the AT domain, that is $\langle m_N^+ \rangle_{\pi_{\beta, B^N, V_N}} \rightarrow \rho^+$, and $\langle m_N^- \rangle_{\pi_{\beta, B^N, V_N}} \rightarrow -\rho^-$, where we set $\rho^- = 1 - \rho^+$, the proportion of GC bonds. Conversely, assume that $\rho^+ < y_*(B_{GC}, \kappa)$: Then $\langle m_N^+ \rangle_{\pi_{\beta, B^N, V_N}} \rightarrow \rho^+$, and $\langle m_N^- \rangle_{\pi_{\beta, B^N, V_N}} \rightarrow 2y_*(B_{GC}, \kappa) - 1 - \rho^+ > -\rho^-$.*

Remark 1 *If the parameters of the problem are such that $\bar{\kappa}_c(B_{GC}) < \kappa_c(B_{AT})$ (recall that the fields are temperature dependent, see (1)), and κ is so that $\bar{\kappa}_c(B_{GC}) < \kappa < \kappa_c(B_{AT})$, denaturation is localized on the AT domain, for arbitrary proportion ρ^+ of AT bonds. However, when $\kappa < \bar{\kappa}_c(B_{GC})$, denaturation is localized on the AT domain when $\rho^+ > y_*(B_{GC}, \kappa)$ and denaturation expands beyond the AT domain when $\rho^+ < y_*(B_{GC}, \kappa)$.*

Proof: We must evaluate the asymptotic behavior of

$$\frac{\sum_{\sigma} V_N(\sigma) \exp(-N\beta F_{B^N}(\sigma)) h(m_N^+(\sigma), m_N^-(\sigma))}{\sum_{\sigma} V_N(\sigma) \exp(-N\beta F_{B^N}(\sigma))},$$

for functions h of the two variables (m_N^+, m_N^-) . Notice that

$$\begin{aligned} F_{B^N}(\sigma) &= G(M_N(\sigma)) + b_{AT} m_N^+(\sigma) + b_{GC} m_N^-(\sigma) \\ &= G(M_N(\sigma)) + b_{GC} m_N(\sigma) + (b_{AT} - b_{GC}) m_N^+(\sigma), \end{aligned}$$

Let $F(m^+, m^-) = G(M) + b_{AT} m^+ + b_{GC} m^-$, where $m = m^+ + m^-$ and $M = (m + 1)/2$. We must thus check the behavior of the expectation

$$\mu_N(h) = \frac{\int h(y) \exp(-N\beta F(y)) Q_N(dy)}{\int \exp(-N\beta F(y)) Q_N(dy)},$$

for bounded and continuous functions h . From Varadhan's Theorem, the family of probability measures μ_N satisfies a large deviation principle with rate function $I_F(y) - \inf_y I_F(y)$, where $I_F(y) = I_D(y) + \beta F(y)$. $\inf_y I_F(y) = \beta \inf_{y \in D} F(y)$. If this infimum is realized at a unique point y_* of D , the sequence of measures μ_N converges weakly to the Dirac mass δ_{y_*} . We thus look for the minima of F on D .

$$\begin{aligned} & \inf_{(m^+, m^-) \in D} F(m^+, m^-) \\ &= \inf_{|m| \leq 1} (G(M) + b_{GC}m + (b_{AT} - b_{GC}) \sup_{(m^+, m^-): m^- + m^+ = m} m^+) \end{aligned}$$

Given, ρ^+ , consider m such that $M = (m + 1)/2 \geq \rho^+$, that is $m \geq 2\rho^+ - 1$. Then $\sup_{m^+ + m^- = m} m^+ = \rho^+$ (corresponding to (10) below): when $m^+ = \rho^+$, one obtains $m^- = m - \rho^+$ and the pair (m^+, m^-) is element of D since $m^- = m - \rho^+ \leq \rho^-$ if and only if $m \leq \rho^+ + \rho^- = 1$ and $m^- \geq -\rho^-$ if and only if $m \geq \rho^+ - \rho^- = 2\rho^+ - 1$. Similarly, when m is such that $M \leq \rho^+$, the maximal possible value of m^+ is $m^+ = 2M - \rho^+$ (corresponding to (11) below). In summary the infimum is obtained by taking the minimum between

$$\inf_{M \geq \rho^+} G(M) + b_{GC}m + (b_{AT} - b_{GC})\rho^+, \quad (10)$$

and

$$\inf_{M < \rho^+} G(M) + b_{AT}m + \rho^-(b_{AT} - b_{GC}). \quad (11)$$

We next use the hypotheses. From Theorem 2, $\kappa < \kappa_c(B_{AT})$ implies that $G(M) + b_{AT}m$ attains its infimum when $m_* = 1$, or $M_* = 1$, and is decreasing on the unit interval; (11) becomes

$$G(\rho^+) + b_{AT}(2\rho^+ - 1) + (1 - \rho^+)(b_{AT} - b_{GC}) = G(\rho^+) + \rho^+b_{AT} - \rho^-b_{GC}.$$

Concerning (10), $\kappa > \kappa_c(B_{GC})$ and, from Theorem 2, the function $G(M) + b_{GC}m$ attains its minimum at $y_*(B_{GC}, \kappa)$, and is increasing above this point. Thus

$$\inf_{M \geq \rho^+} G(M) + b_{GC}m = G(\rho^+) + (2\rho^+ - 1)b_{GC},$$

when $\rho^+ \geq y_*(B_{GC}, \kappa)$. Then, both (10) and (11) are minimized for $M = \rho^+$, which is the maximal value of m^+ . Thus $m^+ = \rho^+$ and $m^- = m - m^+ = 2\rho^+ - 1 - \rho^+ = -\rho^-$, as required.

Conversely, assume that $\rho^+ < y_*(B_{GC}, \kappa)$. Then (10) becomes

$$\begin{aligned} & \inf_{M \geq \rho^+} G(M) + b_{GC}m + (b_{AT} - b_{GC})\rho^+ \\ &= G(y_*(B_{GC}, \kappa)) + b_{GC}(2y_*(B_{GC}, \kappa) - 1) + (b_{AT} - b_{GC})\rho^+. \end{aligned}$$

When $M < \rho^+$, the infimum is still given by

$$G(\rho^+) + b_{AT}\rho^+ - \rho^- b_{GC} = G(\rho^+) + b_{GC}(2\rho^+ - 1) + \rho^+ b_{AT} - \rho^+ b_{GC},$$

and one obtains that the minimum is realized when $M = y_*(B_{GC}, \kappa)$, and therefore $m^+ = \rho^+$ and $m^- = m - m^+ = 2M - 1 - m^+ = 2y_*(B_{GC}, \kappa) - 1 - \rho^+$, as required.

In the remaining, we give an example of probability measure V_N on Ω_N such that $Q_N = V_N \circ \Psi_{B^N}^{-1}$ satisfies a large deviation principle with rate function I_D . Given a sequence (B^N) , consider the family of spins σ^N given by $\sigma_i^N = \mathbb{I}(b_i^N = b_{AT}) - \mathbb{I}(b_i^N = b_{GC})$. Given a sequence r_N , consider the restricted Ising measure

$$V_N(\sigma) = \pi_{\beta_a, r_N}(\sigma) = \frac{\mathbb{I}(|\sigma| \leq 2r_N) \pi_{\beta_a}(\sigma)}{\sum_{\sigma \in \Omega_N} \mathbb{I}(|\sigma| \leq 2r_N) \pi_{\beta_a}(\sigma)}.$$

Lemma 2 *Let $(B^N)_{N \in \mathbb{N}}$ be a sequence of words such that $\rho_N^+ \rightarrow \rho^+ \in \mathbb{I}$, and $\sigma^N \in \mathcal{C}_{\bar{r}_N, N}$, for some sequence $(\bar{r}_N)_{N \in \mathbb{N}}$. Assume that $\bar{r}_N + 2 \leq r_N$, and that $r_N/N \rightarrow 0$ as $N \rightarrow \infty$. Then the probability measure $Q_N = \pi_{\beta_a, r_N} \circ \Psi_{B^N}^{-1}$ satisfies a large deviation principle with rate function I_D .*

This is an extension of the homopolymer case: $\bar{r}_N/N \rightarrow 0$ means that the word associated with the DNA is formed of relatively large droplets of A+T bonds alternating with similar droplets of G+C bonds.

Proof: When $r_N/N \rightarrow 0$, (8) implies that $\log(V_N(\tilde{\sigma}^N))/N \rightarrow 0$ for any sequence of spins $(\tilde{\sigma}^N)_N$ with $|\tilde{\sigma}^N| \leq 2r_N$. Now, given an open subset A of D^0 , containing some point $\lambda = (\lambda^+, \lambda^-)$, with $|\lambda^+| < \rho^+$ and $|\lambda^-| < \rho^-$, consider the sequence $\lambda_N = ([N\lambda^+]/N, [N\lambda^-]/N)$, which is in A for $N \geq N_0(A, \lambda)$. Then

$$\exists \tilde{\sigma}^N \in \mathcal{C}_{\bar{r}_N, N} \text{ with } \tilde{r}_N \leq \bar{r}_N + 1 \text{ and } \Psi_{B^N}(\tilde{\sigma}^N) = \lambda_N, \forall N \geq N_0(A, \lambda). \quad (12)$$

Suppose that (12) is true: Then one obtains

$$V_N(\tilde{\sigma}^N) \leq Q_N(\{\lambda_N\}) \leq Q_N(A) \leq 1,$$

and it follows that $\log(Q_N(A))/N \rightarrow 0$ as $N \rightarrow \infty$. In this case, A is a I_D -continuity set. The main property to check is thus (12). Set $\lambda_N^\pm = [\lambda^\pm N]/N$, and define $M_N^\pm = (\lambda_N^\pm + \rho_N^\pm)/2$, where $\rho_N^- = 1 - \rho_N^+$. Choose an origin in the circular DNA at some site i_0 with $\sigma_{i_0-1}^N = -1$ and $\sigma_{i_0}^N = 1$. We can consider the linear string, starting at i_0 , with an A+T droplet, and ending with a G+C droplet. Let $A_1, \dots, A_{\bar{r}_N}$ be the A+T droplets, ordered according to their appearance along the string, and define similarly $G_1, \dots, G_{\bar{r}_N}$. Set $a_j = |A_j|$ and $g_j = |G_j|$, $1 \leq j \leq \bar{r}_N$. The string is

viewed as the juxtaposition of symbols $A_1 G_1 \cdots A_{\bar{r}_N} G_{\bar{r}_N}$. Let T^+ and T^- be defined by

$$T^+ = \min\{1 \leq j \leq \bar{r}_N; \sum_{k=1}^j a_k \geq NM_N^+\},$$

and

$$T^- = \min\{1 \leq j \leq \bar{r}_N; \sum_{k=1}^j g_k \geq NM_N^-\}.$$

Notice that both T^+ and T^- are well defined for N large enough since $\sum_{k=1}^{\bar{r}_N} a_k = N\rho_N^+$, $\sum_{k=1}^{\bar{r}_N} g_k = N\rho_N^-$, $\rho_N^+ \rightarrow \rho^+$, $\rho_N^- \rightarrow \rho^-$, $M_N^+ \rightarrow (\lambda^+ + \rho^+)/2 < \rho^+$ and $M_N^- \rightarrow (\lambda^- + \rho^-)/2 < \rho^-$. Let i_1 be the site situated in A_{T^+} such that

$$|\{i \in A_1 \cup \cdots \cup A_{T^+}; i \leq i_1\}| = NM_N^+,$$

and define similarly i_2 for the G+C domain. Set $\tilde{\sigma}_i^N = +1$ when $i \in A_1 \cup \cdots \cup A_{T^+}$ and $i \leq i_1$, $\tilde{\sigma}_i^N = -1$ when $i \in A_{T^+} \cup \cdots \cup A_{\bar{r}_N}$ and $i > i_1$, $\tilde{\sigma}_i^N = -1$ when $i \in G_1 \cup \cdots \cup G_{T^-}$, $i \leq i_2$, and $\tilde{\sigma}_i^N = +1$ when $i \in G_{T^-} \cup \cdots \cup G_{\bar{r}_N}$, $i > i_2$. Clearly

$$\begin{aligned} \sum_{i \in \cup_{1 \leq k \leq \bar{r}_N} A_k} \tilde{\sigma}_i^N &= NM_N^+ - (N\rho_N^+ - NM_N^+) \\ &= N(2M_N^+ - \rho_N^+) = N\lambda_N^+, \end{aligned}$$

and similarly

$$\sum_{i \in \cup_{1 \leq k \leq \bar{r}_N} G_k} \tilde{\sigma}_i^N = N\lambda_N^-,$$

giving $\Psi_{B^N}(\tilde{\sigma}^N) = \lambda_N$, as required. Next suppose without loss of generality that $T^+ \leq T^-$, then $i_1 < i_2$, and, from construction, $\tilde{\sigma}_i^N = \sigma_i^N$ when $i \leq i_1$, $\tilde{\sigma}_i^N = -\sigma_i^N$ when $i > i_2$ and $\tilde{\sigma}_i^N = -1$ when $i_1 < i \leq i_2$. It follows that $|\tilde{\sigma}^N| \leq 2(\bar{r}_N + 1)$, as required.

Now, consider a Borel subset $A \subset D^c$ in \mathbb{R}^2 . For any sequence of spins $(\tilde{\sigma}^N)$ in Ω_N , $-\rho_N^\pm \leq m_N^\pm(\tilde{\sigma}^N) \leq \rho_N^\pm$, and thus $\Psi_{B^N}(\tilde{\sigma}^N) \in A^c$ for N large enough, that is there exists $N_0(A) \in \mathbb{N}$ such that $Q_N(A) = 0$, $\forall N > N_0(A)$.

3 Statistical approach

3.1 A Bayesian model

In the copolymer case, the field $B^N = (b_i)$ is indexed by a word of length N on the two letters alphabet $\{A+T, G+C\}$. When the perimeter is fixed

by setting $V_N = U_{r,N}$, $\mathcal{C}_{r,N} = \{\sigma \in \Omega_N; |\sigma| = 2r\}$ is a nonlinear code, as a subset of Ω_N , and tools from information theory and bayesian statistics are of great utility for computational issues. We will see that the Hamiltonian of the system models the a posteriori law on the parameter space Ω_N given the observation B^N . First, consider the *a priori* probability measure on the parameter space given by

$$\nu_N(\sigma) = \frac{V_N(\sigma) \exp(-N\beta G(M_N(\sigma))) \prod_{i=1}^N (\exp(-\sigma_i \beta b_{AT}) + \exp(-\sigma_i \beta b_{GC}))}{Z_N(\nu_N)},$$

where $Z_N(\nu_N)$ denotes the related partition function and V_N is an arbitrary probability measure on Ω_N . Notice that ν_N takes into account the level of superhelicity of the DNA through the linking difference density κ , and that ν_N corresponds to the Gibbs measure associated with the homopolymer with field \bar{B} , given by

$$\bar{b} = \frac{b_{AT} + b_{GC}}{2},$$

that is

$$\nu_N(\sigma) = \frac{V_N(\sigma) \exp(-N\beta F_{\bar{B}}(\sigma))}{Z_N(\beta, \bar{B}, V_N)}.$$

The statistical model is as follows: a code word or a parameter σ^0 is chosen at random with law ν_N on $\text{supp}(V_N)$, and is sended through a noisy channel with output alphabet $\{A + T, G + C\}$ and memoryless channel statistics $P(\cdot|\sigma^0)$ given by

$$P(B^N|\sigma^0) = \prod_{i=1}^N p(b_i|\sigma_i^0) = \prod_{i=1}^N \frac{\exp(-\sigma_i^0 \beta b_i)}{\exp(-\sigma_i^0 \beta b_{AT}) + \exp(-\sigma_i^0 \beta b_{GC})}.$$

The output distribution of B^N is

$$q(B^N) = \sum_{\sigma \in \Omega_N} \nu_N(\sigma) P(B^N|\sigma),$$

and the *a posteriori distribution* on the parameter space is the Gibbs distribution

$$\pi_{\beta, B^N, V_N}(\sigma) = \frac{V_N(\sigma) \exp(-N\beta F_{B^N}(\sigma))}{Z_N(\beta, B^N, V_N)}.$$

Notice that

$$E_q(\rho_N^+(B^N)) = 1 - \theta + (2\theta - 1) \langle M_N \rangle_{\nu_N},$$

where $\theta = \exp(-\beta b_{AT}) / (\exp(-\beta b_{AT}) + \exp(-\beta b_{GC})) \geq 1/2$. The law of $M_N(\sigma)$ under ν_N is subject to threshold phenomenon, as shown in Section 2.1. When $V_N = U_{r_N, N}$ with $r_N/N \rightarrow 0$, Theorem 2 gives information on the limiting support of ν_N : $\kappa_c(\bar{B}) < \kappa < \bar{\kappa}_c(\bar{B})$ implies that the law

of M_N under ν_N converges toward the point mass $\delta_{y_*(\bar{B}, \kappa)}$ with $y_*(\bar{B}, \kappa) \in (0, 1)$, and the average proportion of A+T bonds in B^N under q is given by $1 - \theta + (2\theta - 1)y_*(\bar{B}, \kappa) \in [1 - \theta, \theta]$.

Having observed some word B^N , consider the Bayes estimator for σ^0 under quadratic loss $L(\sigma, \sigma') = \|\sigma - \sigma'\|^2$. Bayesian Theory (see e.g. Robert (1992)) gives that the optimal Bayes estimator under quadratic loss is

$$\hat{\sigma}_i = \frac{\sum_{\sigma \in \mathcal{C}_{r,N}} \sigma_i \nu_N(\sigma) P(B^N | \sigma)}{\sum_{\sigma \in \mathcal{C}_{r,N}} \nu_N(\sigma) P(B^N | \sigma)},$$

and the estimated magnetization $\sum_i \hat{\sigma}_i / N$ corresponds to our order parameter $\langle m_N \rangle_{\pi_{\beta, B^N, \nu_N}}$, showing that Benham's model has an interesting statistical content.

In the next Section, we imbed Benham's model in a bayesian segmentation model of current use in bioinformatics, and provide new algorithms with priors adapted to supercoiled DNA.

3.2 Bayesian segmentation for strand separation

3.2.1 A two coins example

Liu and Lawrence(1999) present a Bayesian model for segmentation of biopolymers, and provide algorithms for drawing samples from the various posterior laws of the model, which might be of great interest in the strand separation problem. We start with their *two types of coins* example, to fix ideas. Suppose you know that in a coin tossing game of length N , the first A Bernoulli have a probability of success θ_1 and the next $N - A$ tosses have a probability $\theta_2 \neq \theta_1$ of getting a head. Let y_{obs} be the observed sequence, with h_1 (resp. h_2) heads and t_1 (resp. t_2) tails in the first (resp. second) part of the sequence. The change point A is treated as a missing data, and has some prior law $g(a)$. The likelihood of the observed data is given by

$$L(\theta_1, \theta_2; y_{obs}, A = a) = \theta_1^{h_1} (1 - \theta_1)^{t_1} \theta_2^{h_2} (1 - \theta_2)^{t_2} g(a).$$

In their work, the prior $\pi(\theta)$ for $\theta = (\theta_1, \theta_2)$ is a product measure associated with two independent Beta random variables $B(\theta_1; \alpha_1, \beta_1)$ and $B(\theta_2; \alpha_2, \beta_2)$. Let $p(\theta_1, \theta_2, a, y_{obs})$ be the joint law of all variables, with

$$p(\theta_1, \theta_2, a, y_{obs}) = L(\theta_1, \theta_2; y_{obs}, A = a) \pi(\theta) g(a),$$

and

$$P(A = a, y_{obs}) = \int \int p(\theta_1, \theta_2, a, y_{obs}) d\theta_1 d\theta_2.$$

The following algorithm will converge and give samples from the posterior law of (θ_1, θ_2, A) :

- Fix $A = a$ and θ_2 , and draw θ_1 from its conditional posterior law $P(\theta_1 | \theta_2, A = a, y_{obs})$, to get the new θ_1 ,
- proceed similarly for θ_2 to get the new θ_2 ,
- draw A from its conditional posterior law $P(A = a | \theta, y_{obs})$ proportional to $\prod_{i=1,2} \theta_i^{h_i(a)} (1 - \theta_i)^{t_i(a)} g(a)$, where $h_i(a)$ and $t_i(a)$ are the number of heads and tails contained in the i -th part of the sequence, $i = 1, 2$.

Coming back to the setting of Section 3.1, choose V_N to be $U_{1,N}$, fixing the perimeter to $2r = 2$. Benham's model deals with a circular DNA, and they are N configurations of length a , and two change points. Forgetting for a while this slight difference, we see that the Bayesian model of Liu and Lawrence can be adapted for Benham's model: σ is the missing data or segmentation parameter A , and the prior law $g(a)$ is just the a priori measure ν_N of Section 3.1. Suppose that $\sigma_i = +1$, $1 \leq i \leq A$ and $\sigma_i = -1$, $A < i \leq N$. Set $B^N = y_{obs}$ and fix the parameter θ to

$$\bar{\theta}_1 = \frac{\exp(-\beta b_{AT})}{\exp(-\beta b_{AT}) + \exp(-\beta b_{GC})}, \quad \bar{\theta}_2 = \frac{\exp(\beta b_{AT})}{\exp(\beta b_{AT}) + \exp(\beta b_{GC})} = 1 - \bar{\theta}_1. \quad (13)$$

The model of Section 3.1 can be seen as a particular case of the segmentation model, and the a posteriori law of the change point A is the Gibbs measure π_{β, B^N, V_N} . For the deterministic model with constant $\bar{\theta}$, the last step of the algorithm corresponds to sampling with π_{β, B^N, V_N} . Fye and Benham(1999) give algorithms for strand separation using transfer matrices from statistical mechanics; the method can be applied, thanks to the quadratic form appearing in the prior ν_N (the transfer matrices have complex entries, a consequence of the gaussian transform). The perimeter is not limited, and is penalized by the exponential weight appearing in the one dimensional Ising Boltzman weight $\exp(-4r\beta)$ for a perimeter of $2r$ (notice that this way of penalizing too large perimeters was implemented in a recent work of Ramensky *et al*(2000,2001)). Their method is applicable to situations where the free energies for strand separation b_{AT} and b_{GC} fluctuate: in real situations, chemical reactions can alter these free energies. In the randomized case, the Bayesian segmentation model can also provide an interesting alternative for studying the strand separation problem with random free energies b_{AT} and b_{GC} .

3.2.2 The general case

We adapt the setting of Liu and Lawrence(1999) and Ramensky *et al*(2000,2001), and indicate the main features of the model. The number of

domains or segments of the circular DNA can be limited to r_{\max} . The missing data is the spin σ , with prior law ν_N , given by V_N and the homopolymer of field $\bar{b} = (b_{AT} + b_{GC})/2$, with free energies b_{AT} and b_{GC} given by formula (1). σ can be seen as a juxtaposition of droplets of \pm spins arranged around the discrete circle of length N .

Suppose they are $2r$ domains with r positive droplets, that is containing sites i with $\sigma_i = +1$, and r negative droplets. The parameter θ is here defined given σ . We associate to every positive droplet $\Lambda_{i_0}^+$ with $\sigma_{i_0-1} = -1$, $\sigma_i = +1$, $i_0 \leq i \leq i_k$, and $\sigma_{i_k+1} = -1$ a family of $k+1$ i.i.d. Bernoulli ε_i , with

$$P(\varepsilon_i = A + T) = \theta^{i_0, i_k} \text{ and } P(\varepsilon_i = G + C) = 1 - \theta^{i_0, i_k},$$

of random parameter θ^{i_0, i_k} , which is chosen according to some prior law f^+ . Do the same for negative droplets for a random parameter θ^{j_0, j_l} of prior f^- , with

$$P(\varepsilon_j = A + T) = 1 - \theta^{j_0, j_l} \text{ and } P(\varepsilon_j = G + C) = \theta^{j_0, j_l}.$$

The requirements might be

$$E_{f^+}(\theta^{i_0, i_k}) = E_{f^-}(\theta^{j_0, j_l}) = \bar{\theta}_1,$$

where the constants b_{AT} and b_{GC} appearing in (1) and (13) can be taken as average values. Positive droplets force the sample toward $A + T$ outcomes and conversely for negative droplets. The priors f^\pm can be chosen as in Liu and Lawrence(1999) as Beta laws with the required expectations.

A second way of randomizing the parameters consists in taking, independently for each segment, two positive random variables $b_{AT}(\omega)$ and $b_{GC}(\omega)$, distributed according to some law, which might be motivated from thermodynamics or biochemistry, with average values given by formula (1). Draw $2r$ i.i.d. realizations of these two random variables, and set, for each segment,

$$\theta(\omega) = \frac{\exp(-\beta b_{AT}(\omega))}{\exp(-\beta b_{AT}(\omega)) + \exp(-\beta b_{GC}(\omega))}.$$

The probability to get $A + T$ is $\theta(\omega)$ when the droplet is positive and $1 - \theta(\omega)$ when the droplet is negative.

Let $\pi(\theta|\sigma)$ be the prior law given by the above construction, with joint law $\pi(\theta, \sigma) = \pi(\theta|\sigma)\nu_N(\sigma)$. Then the law of B^N is

$$\begin{aligned} P(B^N) &= \sum_{\sigma \in \Omega_N} \nu_N(\sigma) P(B^N|\sigma) \\ &= \sum_{\sigma \in \Omega_N} \nu_N(\sigma) \int P(B^N|\theta, \sigma) d\pi(\theta|\sigma), \end{aligned}$$

where $P(B^N|\theta, \sigma)$ is the product measure associated with the Bernoulli. The posterior law of interest in the strand separation problem is just $P(\sigma|B^N) =$

$P(B^N|\sigma)/P(B^N)$. This last law is define on the cube Ω_N , of size 2^N . The observables of interest are the number of denatured bonds $M_N(\sigma)$ given by $m_N(\sigma) = 2M_N(\sigma) - 1$, and the restricted magnetization m_N^+ and m_N^- . Information on the localization of denaturation can be obtained by considering the proportion of broken A+T and G+C bonds

$$M_N^\pm = \frac{m_N^\pm + \rho_N^\pm}{2},$$

(see Section 2.2). Bayesian segmentation algorithms like backward sampling, as given in Liu and Lawrence(1999) or Schmidler *et al*(2000), can thus be applied to the strand separation problem to study local denaturation as function of the various parameters, taking into account the random fluctuations of the free energies needed to denature A+T and G+C bonds.

References

- R. Baxter. *Exactly Solved Models in Statistical Mechanics* Academic Press, 1982.
- C. Benham(1979) Torsional stress and local denaturation in supercoiled DNA *Proc. Natl. Acad. Sci. USA*, 76(8):3870-3874
- C. Benham(1989) Mechanics and Equilibria of Superhelical DNA. In *Mathematical Methods for DNA Sequences* Ed. M. Waterman CRC Press.
- C. Benham(1990) Theoretical analysis of heteropolymeric transitions in superhelical DNA molecules of specified sequences. *J. Chem. Phys.*, 92(10):6294-6305
- C. Benham(1992) Energetics of the Strand Separation Transition in Superhelical DNA *J. Mol. Bio.*, 225:835-847
- C. Benham(1996) Theoretical analysis of the helix-coil transition in positively superhelical DNA at high temperature *Phys. Rev. E*,53(3):2984-2987.
- P. Clote and R. Backofen *Computational Molecular Biology: An Introduction* Wiley, 2000.
- A. Dembo and O. Zeitouni *Large Deviations Techniques and Applications* Jones and Bartlett, 1992.
- J. Deuschel and D. Stroock *Large Deviations* Academic Press. 1989.
- R. Ellis *Entropy, Large Deviations and Statistical Mechanics* Springer 1985.
- W. Feller *An Introduction to Probability Theory and Its Applications, Vol. I* Wiley, 1971.
- R. Fye and C. Benham(1999) Exact method for numerically analyzing a model of local denaturation in superhelically stressed DNA *Phys. Rev. E*, 59:3408.
- B. Lewis *Genes V* Oxford University Press. 1994.

- J.S. Liu and C.E. Lawrence(1999) Bayesian inference on biopolymer models *Bioinformatics* 15(1):38-52
- J.S. Liu, A.F. Neuwald and C.E. Lawrence (1999) Markovian structures in biological sequence alignments *JASA* 94:1-15.
- V.E. Ramensky, V.J. Makeev, M.A. Roytberg and V.G. Tumanyan(2000) DNA segmentation through the Bayesian approach *Journal of Computational Biology.* 7(1/2): 215-231
- V.E. Ramensky, V.J. Makeev, M.A. Roytberg and V.G. Tumanyan(2001) Segmentation of long genomic sequences into domains with homogeneous composition with BASIO software *Bioinformatics* 17(11):1065-1066.
- C. Robert *The Bayesian Choice* Springer 1992.
- R.T. Rockafellar *Convex Analysis* Princeton University Press. 1972
- H. Sun, M. Mezei, R. Fye and C. Benham(1995) Monte-Carlo analysis of conformational transitions in superhelical DNA *J. Chem. Phys.* 103(19):8653-8665.
- S.C. Schmidler, J.S. Liu and D.L. Brutlag(2000) Bayesian segmentation of protein secondary structure *Journal of Computational Biology.* 7(1/2): 233-248