Epiphoresis: a nonequilibrium mechanism for fast search on DNA

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Vital biological processes such as genome repair require fast and efficient binding of selected proteins to specific target sites on DNA. Here we propose an active target search mechanism based on “epiphoresis”, the dynamics of DNA-binding proteins up or down gradients in the density of epigenetic marks (biochemical tags on the genome). We focus on a set of proteins that deposit marks from which they are repelled—a case which is only encountered away from thermodynamic equilibrium. For suitable ranges of kinetic parameter values, epiphoretic proteins can perform unidirectional motion and are optimally redistributed along the genome. Importantly, they can also locally open a region of the genome that is collapsed due to self-interactions and “dive” deep into its core, strikingly enhancing the efficiency of target search on such an inaccessible substrate. We discuss the potential relevance of epiphoresis on the repair of DNA damage.

Within the crowded nucleus of a eukaryotic cell or a bacterial nucleoid, it is vital that selected proteins and enzymes can locate their target on the DNA within minutes [1][2], of a specific stimulus. DNA lesions, for instance, occur several thousands of times a day in every cell [3][4]: the requirement for speed of the relevant DNA repair machinery is thus not negotiable.

Passive mechanisms are unlikely to offer the required efficiency: a protein exploring a human chromosome—average size \( \approx 10^8 \) base pairs (bp)—via 1D diffusion along the DNA \( (D_{1D} < 10 \text{ kbp}^2/\text{s}) \) would take over 10 years to find a single target. Purely 3D diffusion within the human nucleus, whose typical size is \( \sim 10 \text{ \mu m} \), is equally impractical. Its limits are apparent by using Smoluchowski’s prediction for diffusion-limited reaction equally impractical. Its limits are apparent by using along the DNA (with high concentration, it cannot provide a viable re-

In this Letter we introduce the concept of epiphoresis—the spontaneous motion of DNA-binding proteins as a result of a self-produced pattern of chemical marks—modifications, such as acetylation or methylation, of histone proteins that, together with DNA, form chromatin in mammalian cells [17]. As these marks provide a layer of inheritable information beyond DNA sequence, they are referred to as epigenetic, hence the name epiphoresis. In the context of epigenetics, biophysical models normally consider a positive feedback loop between the released epigenetic marks and the protein dynamics, leading to accumulation and pattern formation [18][21]. However, the energy input required by active processes allows for negative feedbacks, whereby a protein deposits a mark from which it fleeing. This scenario, which we consider in this work, is reminiscent of chemorepulsion, or negative phoresis in active matter systems, where it leads to co-

The combination of alternate rounds of 3D diffusion in the nucleus and 1D diffusive sliding on the DNA, or fa-

modelled as a truncated Lennard-Jones (LJ)
potential. Provided $\epsilon$ is comparable to $k_B T$, proteins can slide on chromatin, with an effective diffusion coefficient $D_{1D}$ (Fig. 1A, see also Ref. [13]). Larger $\epsilon$ instead leads to cluster formation via the bridging-induced attraction [25].

The proteins we consider here deposit an epigenetic mark on the fibre bead they are bound to at a rate $k_{on}$ (Fig. 1B). This mark, in turn, abrogates the attraction of the protein to the marked beads. Marks are spontaneously lost at rate $k_{off}$, modelling random or active removal. This model harbours a negative feedback, as the mark released by the protein raises, rather than lowers, the potential energy describing fibre-protein interaction. Hence, unlike the case of positive feedback [16, 19], this system cannot be described by an effective equilibrium model. To understand the dynamics that can originate from these microscopic rules, we first consider a simpler 1D model that neglects spatial structure and fluctuations of the chromatin fibre.

1D approximation – As a first approximation, the chromatin fibre can be treated as a straight line. The potential landscape generated by the LJ interaction with the fibre determines the protein dynamics, and is substantially easier to compute for a 1D substrate (see Fig. 2 and SI). In the absence of any mark, a protein sits between two adjacent fibre beads so as to minimise its energy (Fig. 2A). The protein can escape the well by moving in the direction orthogonal to the fibre (vertical axis in Fig. 2). The escape rate $r_{esc}$ can be computed, for $\epsilon > k_B T$, as a Kramers problem [31], and scales as $\sim e^{-2\epsilon/(k_B T)}$. Additionally, a barrier $\epsilon/2$ obstructs the protein motion parallel to the fibre (horizontal axis in Fig. 2), i.e. the thermal diffusion between adjacent potential wells. Kramers’ theory (see SI) yields the effective jump rate for the symmetric random walk the protein performs on the fiber as, $q = \frac{A}{4} \frac{2}{1+e^{\epsilon/k_BT}}$, with $A \simeq 10.6/2\pi$ a numerical factor depending on the potential curvature. Note that, unless otherwise stated, we set $k_B T = \gamma = \sigma = 1$, so that dimensional formulas for rates can be obtained by multiplying those we give here by $k_B T/(\sigma^2 \gamma)$ (see SI).

The diffusion coefficient of the 1D diffusive sliding is then $D_{1D} = q$. In our model, however, a bound protein deposits an epigenetic mark on one of the neighbouring chromatin beads which, by silencing the LJ attraction, reshapes the potential landscape (Fig. 2B) and drives the model away from equilibrium. While the barrier over the marked bead remains unaltered, the one over the unmarked one is tilted, becoming a declivity of size $\epsilon$. From Kramer’s theory, the rate at which the protein slides down the declivity is $q_+ = B \epsilon$, with $B \simeq 22.5/2\pi$. As $q_+/q \simeq 8(1+e^{\epsilon/(k_B T)})$, we expect the protein to move downhill with near-one probability (recall $\epsilon > k_B T$ for Kramers’ theory to hold).

For large enough $k_{on}$, the protein is likely to mark the bead transiently visited thus ending up in the configuration depicted in Figure 2C. As there are two marked beads upstream of the protein, the barrier over the marked bead changes so that the rate of going downstream remains $q_+$ while that of going backward changes to $q_- = C e^{-\epsilon/(k_B T)}$, with $C \simeq 7.5/2\pi$. Therefore, in the typical microscopic sequence of events, an epiphoretic protein binds to the substrate, then it randomly marks one of the two beads on either side and becomes attracted to the other. In doing so, it enters a running state, whereby it makes a step forward towards the unmarked portion of the fibre at rate $q_+ \sim \epsilon$ or jumps backward onto the marked segment at rate $q_- \sim \epsilon e^{-\epsilon/(k_B T)}$. A backward jump would end the running state, forcing the protein off the fibre, hence renormalising the escape rate to $r_{esc} = r_{esc} + q_- \simeq 2q_-$. The relevant lengthscale of this process is the “run length”, i.e. the chromatin segment that the protein explores before detaching.
Figure 3. Epiphoretic collective behaviours. Average number of epiphoretic proteins bound and moving along the substrate for different values of total protein copy number $N$ and mark removal rate $k_{\text{off}}$. The black dashed line marks the limiting protein number $L/l_{\text{trait}}$ discussed in the text: it provides an upper bound for $\langle N_{\text{on}} \rangle$. The inset shows the two-point correlation function in the direction of the protein motion (to the right in the figure).

given by $l_{\text{run}} \sim v/r_{\text{esc}}$, where $v \propto q^+$. More precisely, $l_{\text{run}} = B/2Ce^{\epsilon/k_B T} \sim 3/2e^{\epsilon/k_B T}$.

Mark evaporation does not change this picture, unless it occurs at a rate $k_{\text{off}} \gg q^+$, which we never consider here [32]. It is required, instead, that $k_{\text{on}} \gg q^+$, though 3D simulations show the running state exists even down to $k_{\text{on}} \sim q$. For proteins on chromatin, and reinstating dimensional factors, this translates into $k_{\text{on}} > D_{1D}/\sigma^2$ or $k_{\text{on}} > s^{-1}$ for $D_{1D} \sim 10^{-3} \text{µm}^2/\text{s}$, a bead size $\sigma = 30$ nm and $\epsilon \sim k_B T$. This rate of post-translation modification is compatible, albeit slightly faster, than that of typical proteins that can deposit epigenetic marks, for instance $k_{\text{on}} \approx \min^{-1}\text{s}^{-1}$ for histone acetylation or phosphorylation [33].

The unidirectional motion of a single protein accelerates target search substantially, by enlarging the length-scale covered while bound to the substrate. When multiple epiphoretic proteins are bound on the same fibre, in addition, they interact with each other via the trails of epigenetic marks left on the substrate. This effect emerges from the pair correlation function in Fig. 3 (inset), which is the average density profile seen by a running protein. The downstream peak at short distance is due to collisions with proteins moving in the opposite direction, and the upstream dip to the epigenetic trail and consequent protein depletion. Additionally, due to the forward-backward asymmetry, the resulting effective interaction breaks the action-reaction principle (e.g., a particle in the wake of another is repelled by the latter, without affecting its motion), underscoring the nonequilibrium nature of the model.

The inter-particle interactions are then controlled by the epigenetic marks dynamics: this provides an avenue to set up a cooperative search strategy, which is unavailable to conventional facilitated diffusion. Due to the trail-mediated exclusion between proteins, the average

Figure 4. Kymographs of epiphoretic proteins. Kymographs showing the epigenetic mark dynamics. A 1D model with $M = 1000$, $\epsilon = 2$, $k_{\text{off}} = 0.01$, $k_{\text{on}} = 10$ and $N = 20$ proteins which, when not on the fibre, re-bind to it at rate 0.1. B 3D model with with $M = 1000$, $\epsilon = 4$, $k_{\text{off}} = 0.01$, $k_{\text{on}} = 1$ and $N = 20$ proteins in a $L = 50$ cubic box. C Snapshot from 3D simulations showing epiphoretic proteins (red) and epigenetic marks (cyan) on chromatin (grey).
stranded DNA breaks (DSBs) are often buried within collapsed and inaccessible heterochromatic regions, as \( \sim 70\% \) of our genome is estimated to be in this state [38].

Thus, to explore a more realistic regime for target search during lesion/DSB repair in vivo, we perform simulations where we consider one abundant species of protein bridges that fold the polymer substrate into a collapsed globule [39–41] – modelling, for instance, multivalent HP1 proteins associated with heterochromatin [42]. Once the chromatin fibre is folded by these abundant bridges, we release a trace amount of epiphoretic species. Inspection of the simulations shows that, strikingly, epiphoretic searchers can disrupt bridging-induced collapse and locally open up the chromatin fibre. It is notable that similar phenomenology is observed during DNA repair as large chromatin regions surrounding DSBs swell [43].

In order to quantify epiphoretic search efficiency in this regime we monitor the fraction of beads that are visited each time an epiphoretic protein binds the substrate. Remarkably, we discover that there is a non-monotonic behaviour as a function of \( k_{\text{off}} \) (Fig. 5), which can be explained as follows. For \( k_{\text{off}} \to \infty \) the epigenetic marks evaporate immediately and the searchers only stick to the surface of the polymer globule: this limit is analogous to conventional facilitated diffusion, where a buried target would remain inaccessible to the searchers. In the opposite regime, \( k_{\text{off}} \to 0 \), the fibre swells but the searchers fail to remain attached for long because of the large fraction of non-sticky epigenetic marks. In both these limits, therefore, the fraction of beads visited for each binding event tends to 0 (panel A of Fig. 5).

In the regime of intermediate \( k_{\text{off}} \) we instead observe a qualitatively different phenomenon: searchers can be seen “diving” into the globule during simulations (Fig. 5 and Suppl. Movie 2), locally creating a small opening made of epigenetically marked beads that slowly turn to unmarked. During the turnover time, the searcher is likely to be driven (i.e., to dive) further inside the globule as, on average, the protein sees a gradient of unmarked beads towards the interior. This gradient is actively maintained by the deposition of epigenetic marks, and fuels the descent of the epiphoretic searchers into the core of the globule.

Once a searcher has dived deep enough into a globule it may remain trapped for a long time as there is a large density of unmarked beads which it can stick to. During this time it can explore a large fraction of the polymer contour length by constantly churning the polymer globule from the inside. As a result, the optimum turnover rate \( k_c \) marks a cusp in the fraction of fibre visited per dive as a function of \( k_{\text{off}} \). We further find that for \( k_{\text{off}} > k_c \) searchers spend a very long time attached to the fibre, but cannot make much progress inside the core due to steric effects, whereas for \( k_{\text{off}} < k_c \) the time spent on the fibre after binding is finite (i.e., it tends to zero for sufficiently long simulations, Fig. 5B).

Conclusions – In summary, we have proposed a novel nonequilibrium mechanism for target search within genomes. Inspired by the deposition of epigenetic marks on chromatin and consequent response of certain proteins to the gradient of such marks, we dub this mechanism “epiphoresis”.

In this work we have focussed on a negative and nonequilibrium feedback between the marks and the motion, so that the proteins are repelled from the mark they deposit. We discover that, if mark deposition is sufficiently fast, a single epiphoretic protein can perform unidirectional motion on chromatin, while multiple proteins interact via epigenetically-mediated repulsion, as a result of which they spread out along the fibre with much decreased 1D density fluctuations. Most notably, we found that epiphoresis may provide a generic pathway to accelerate target search, especially in cases where the chromatin fibre is compacted into a globular configuration, as is the case for a large part of the human genome. Under this condition we discover that there exist an optimum evaporation rate of epigenetic marks for which the explo-

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**Figure 5.** A. Average fraction of the fibre visited in a single binding-unbinding, or “diving”, event as a function of \( k_{\text{off}} \) for \( k_{\text{on}} = 0.1 \) and \( \epsilon/k_BT = 5 \). Results are averaged over several diving events and \( 10 – 20 \) independent simulations. B. Fraction of time spent on the fibre over the total simulation time \( \tau/T \) for different observation times \( T \). In A and B the red dot-dashed line highlights a critical \( k_{\text{off}} \) marking the value at which the fraction of covered fibre is maximal and there is a transition in the behaviour of residence time \( \tau/T \). C,D Snapshots of two epiphoretic proteins (red) “diving” into a globule while pushed by their own trail (cyan).
tion of the fibre is fastest. Close to this optimal condition the proteins locally open up the globular chromatin and dive into its core, which is otherwise inaccessible to passive searchers performing facilitated diffusion.

On top of resulting in intriguing nonequilibrium physics, epiphoresis might potentially be biologically relevant in the context of chromatin poly(ADP-ribose) deposition, or PARylation [3]. This is an epigenetic modification deposited by poly(ADP-ribose) polymerase (PARP), a key part of the repair machinery which needs to locate DNA lesions [3]. PARylation has been shown to swell chromatin in vitro [41], and is thought to affect the dynamics of PARP itself, as well as of other proteins, promoting their detachment from the fibre [45]. We therefore conjecture that some parts of PARP function may be related to epiphoresis, and we suggest it would be of interest to test this possibility further. Another experimental pathway that we are keen to explore is the bottom-up design of synthetic epiporetic proteins.

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