University of Groningen

Speeding up biochemistry by molecular sledding along DNA

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2016

Link to publication in University of Groningen/UMCG research database

Chapter 1

Introduction

Most chemical processes that require more than one molecular component rely on three-dimensional diffusion for the reactants to meet each other and undergo chemistry. The idea of reducing the dimensionality of the search space as a means to speed up association has a long and vivid history. Initially, more than half a century ago, this principle was put forth as a mere hypothesis that did not seem to hold any significance in biology or chemistry. The extensive theoretical and experimental treatment it later received, however, suggested that a number of naturally occurring processes might utilise search processes along two- or one-dimensional paths to speed up biomolecular association. The most notable realisation of this principle in nature is one-dimensional sliding along DNA, a process used by various proteins to locate their targets on DNA in a much more rapid manner than by using three-dimensional diffusion. Here, we provide a brief description of the history of the problem, mainly focusing on sliding along DNA. We provide and compare simple theoretical descriptions of association kinetics in three-dimensional *vs.* one-dimensional space. Further, we describe a particular natural system (adenovirus maturation) in which proteins use one-dimensional sliding along DNA. We explain how unique biochemical processes within adenovirus inspired us to develop novel molecular tools to speed up biochemical reactions.

1.1. One-dimensional diffusion along DNA

At a rate of tens of thousands of mutations per day, every individual cell in our body experiences changes in its DNA caused by exposure to ultra-violet (UV) radiation from the sun, spontaneous oxidative processes, mutagenic chemicals, etc.[1] Every single second, genomic integrity – and thus cellular health – relies on the activity of DNA-repair proteins. The challenge faced by these proteins is finding individual, damaged DNA base pairs amidst $6 \cdot 10^9$ undamaged ones (the total amount of genomic DNA inside each cell). If such proteins were too slow in recognising and repairing the damage, the genomically compromised cells would have the chance to rapidly grow into tumours, sealing our fate the very moment DNA damage has occurred. Yet, we know that the continuous generation of DNA damage does not instantly lead to problems. A key molecular reason is that repair proteins make use of specialised search mechanisms by which the damaged DNA can be very rapidly located. Instead of solely relying on three-dimensional (3D) diffusion, DNA repair proteins combine it with one-dimensional (1D) diffusion along DNA, which allows them to scan hundreds or thousands of base pairs on DNA instead of only a few per binding event (Fig. 1.1). Such dynamic confinement of proteins on DNA prevents them from floating around DNA in vain.
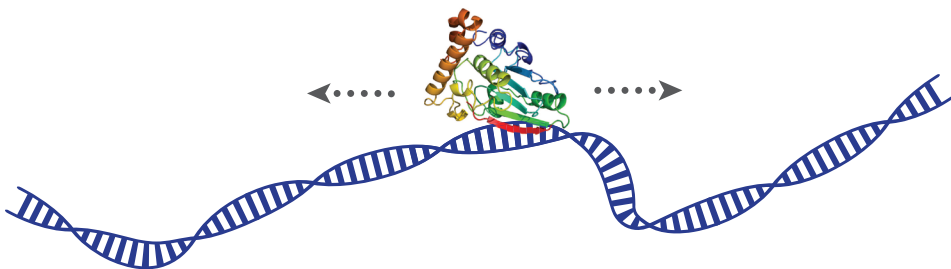

Figure 1.1 | Protein sliding along DNA. Sliding (1D diffusion) of DNA-interacting proteins along DNA allows them to find and process their targets faster than relying only on 3D diffusion.

Repair proteins are not the only class of proteins that exhibit 1D DNA sliding behaviour. The problem of target search on DNA has a rich and vivid history that goes back more than half a century and was initially put forth as a purely theoretical notion. "*A drunk man will find his way home, but a drunk bird may get lost forever*" – this statement is how Shizuo Kakutani (Japanese-born American mathematician, 1911–2004) summarised the theorem of Polya (Hungarian mathematician, 1887–1985), which states that a random walker confined to 1D or 2D is guaranteed to find a stationary target, wherever it is, but in 3D there is some chance he never will.[2,3] This theorem served as a qualitative rationale for the proposal of Trurnit[4] and Bucher[5] who hypothesised that the efficiency of certain membrane-bound enzymes might be enhanced if their aqueous substrates could diffuse in 2D along membrane surfaces.[6] However, the theorem of Polya does not relate to diffusion times or association rates, it deals only with encounter probabilities. Adam and Delbrück commented that the validity of the Trurnit and Bucher hypothesis is dependent on the ratio between 2D and 3D diffusion. They estimated this ratio and further assessed the hypothesis in a quantitative manner in 1968. In particular, they proved that the surface-bound traps will react with bulk-phase reactants with higher reaction rates on the condition that the reactants first get absorbed to the surface and then diffuse in 2D before associating with the trap.[7] The first experimental evidence of this concept was reported in 1970, when Riggs et al. found that the association rate of *lac* repressor to its binding site is two orders of magnitude higher than predicted by association via 3D diffusion in solution.[8] As a result of these two seminal papers, great interest in 1D diffusion in biological processes arose. Since then, search processes with reduced dimensionality have been extensively studied both theoretically and experimentally. However, it was not until the emergence of single-molecule techniques that we could directly observe rapid 1D movement of molecules along DNA. Kabata et al. (1993) were the first to visualise a fluorescently labelled protein, in their case *Escherichia coli* RNA polymerase (RNAP), one-dimensionally moving along λ-DNA 'belts'.[9] Subsequently, in 1999

Harada et al. used fluorescence microscopy to detect RNAP diffusing in a 1D fashion over mechanically stretched DNA.[10]

Text box 1 | Total Internal Reflection Fluorescence (TIRF) microscopy. To visualise the behaviour of single molecules using fluorescence microscopy, one labels them with fluorescent dyes, also known as fluorophores or chromophores, and makes use of their property to emit light upon excitation. Lasers are used to excite the fluorophores and their Stokes-shifted emission in longer wavelengths is detected by a sensitive Electron-multiplying Charged-Coupled Device (EM CCD) camera. The characteristic feature of TIRF microscopy is that the angle of incidence of the laser beam is chosen such that it undergoes total internal reflection at the interface created by a microscope coverslip and an aqueous buffer solution above it. As a result, the laser beam is unable to penetrate the aqueous volume but rather an exponentially decaying wave is created within a ~100 nm layer of buffer solution, which allows one to excite only the molecules within a very small excitation volume of a few femtoliters. This reduction of excitation volume significantly attenuates the background fluorescence due to high concentrations of the molecules at hand or possible contaminants in solution. Using TIRF microscopy, one can achieve signal-to-noise ratios several orders of magnitude higher as compared to conventional wide-field microscopy. In this study, I extensively made use of TIRF microscopy; a detailed description of the experimental set-up is given in Chapter 2.
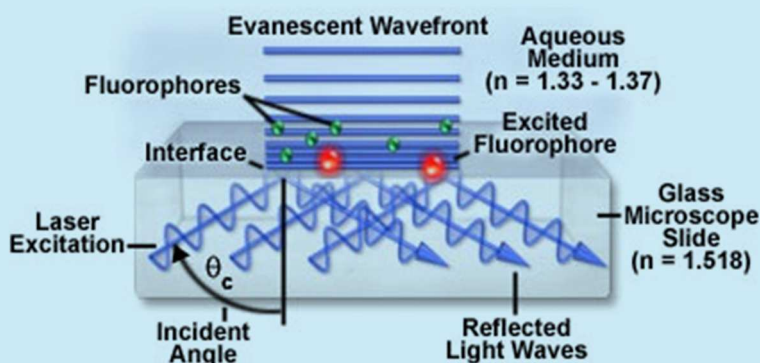
Courtesy of Michael W. Davidson, Florida State University

In the years following, fluorescence microscopy was used extensively to observe fluorescently labelled proteins on stretched DNA molecules. To reduce fluorescence background sufficiently to allow the imaging of the fluorescence of individual fluorophore with high contrast, total internal reflection fluorescence (TIRF) microscopy became widely accepted (Text box 1). The selective observation of fluorescently labelled proteins close to the surface of a microscope coverslip and the mechanical stretching of DNA molecules attached to the same surface became powerful tools for the study of DNA-protein interactions. Using these approaches, or slightly modified versions thereof, more and more proteins from different classes were discovered to diffuse along DNA in a 1D fashion:

- *lac* repressor, a transcription factor whose role is to bind to a specific operator sequence (*lacO*) and prevent the transcription of certain bacterial genes by RNAP. As mentioned above, the experimentally observed rates of target location by *lac* repressor are two orders of magnitude higher than predicted by 3D diffusion. This observation suggested an alternative mechanism of target search that was eventually experimentally confirmed to be 1D sliding in 2006 by Cox and colleagues.[11]

- Rad51, a protein that is involved in homology search during eukaryotic homologous recombination of DNA during double strand break repair. The analysis of single-molecule trajectories of Rad51 proteins moving along DNA confirmed that the movement was an unbiased 1D random walk.[12]

- hOgg1, human oxoguanine DNA glycosylase 1, a base-excision repair protein that locates its targets (8-oxoguanine lesions) by 1D diffusion as confirmed by Blainey et al. Analysing the salt dependence of the diffusion coefficient of hOgg1, the authors concluded that indeed 1D sliding and not hopping was the mechanism of the observed movement.[13]

- T7 RNAP, a highly promoter-specific T7 bacteriophage RNA polymerase whose ability to slide along DNA was detected by Kim et al.[14]

- Msh2-Msh6, a heterodimer protein complex responsible for postreplicative mismatch repair (MMR) in Saccharomyces cerevisiae and humans. Mismatch repair complex Msh2-Msh6 must interrogate thousands of base pairs of undamaged DNA to find the mispaired bases and small insertion/deletion loops and repair them. The MMR complex was shown to 1D slide along DNA.[15]

- p53, a crucial transcription factor that functions as a tumour suppressor in multicellular organisms. p53 is able to detect DNA damage and arrest the cell-cycle to allow DNA repair proteins to attempt to fix the damage. If DNA damage turns out irreparable, p53 induces apoptosis. It had been suggested that p53 uses 1D diffusion to locate the damaged sites on DNA but the first experimental evidence confirming that p53 can indeed slide along DNA was obtained in 2008 by Tafvizi et al.[16]

This list is far from complete; during recent years a wide range of proteins have been found to perform 1D sliding along DNA. These observations have prompted the idea that 1D diffusion of proteins along DNA is an intrinsic property of all proteins that have a DNA-binding interface. To determine the extent to which 1D mechanisms contribute to target search, direct observations of RNAP binding to its promoter were performed *in vitro* in two independent studies.[17,18] Both studies concluded that the dominant association process was direct binding of the RNAP to its promoter by 3D diffusion mechanism. The authors suggested a number of possible reasons as to why 1D sliding is not the main contributor, one of which that 1D sliding could play a role of importance only for those proteins that are present in low copy numbers in the cell. And indeed, subsequent studies of the influence of 1D sliding for various concentrations of RNAP revealed that the acceleration effect corresponding to 1D diffusion of proteins along nonspecific DNA can be obtained by 3D diffusion and simply increasing the protein concentration.[19]

Recently, researchers have begun to visualise 1D diffusion mechanisms inside the cell. The *lac* repressor, a very low-copy number protein that became the canonical example of a 1D DNA slider, was confirmed to undergo 1D diffusion

along DNA *in vivo*.[20,21] The majority of previous studies of protein sliding along DNA were conducted *in vitro* and relied on some sort of DNA stretching to visualise 1D movement of proteins. This situation, however, is not representative of the real conformation of DNA in a cell with its genomic DNA in a compacted state. In particular, it was unclear how 1D sliding could occur in a situation with a high local concentration of DNA. To elucidate the relative contributions of 1D sliding and 3D diffusion of proteins *in vivo*, real-time single-molecule observations of protein diffusion in live cells were conducted. The authors studied *lac* repressor fluorescently labelled with a yellow fluorescent protein (YFP) and concluded that the protein spends ~90% of its time 1D diffusing along nonspecific DNA while covering tens of base pairs in one binding event. The authors theoretically estimated that 1D sliding accelerates promoter search by the lac repressor by a factor of 40 as compared to the situation in which the repressor would non-specifically bind DNA without sliding, and thus confirmed the relevance of sliding to a cellular context.[21]

1.2. Association rate estimations: 3D *vs* 1D

In a fairly straightforward manner, one can estimate the validity of the hypothesis that 1D search can increase association rate constants. In what follows, we will estimate the 3D association rate (Smoluchowski rate) of a protein, exemplified here by the *lac* repressor, with its target and compare it to the experimentally measured values.[22,23] The association of a protein with its target can be represented as $P + S \leftrightarrow PS$. With the assumption that the binding of a protein to its target is irreversible, the rate of complex *PS* formation can be described by the following equation

$$\frac{dC_{PS}}{dt} = k_a C_P C_S,$$

where C_{PS}, C_P and C_S denote the concentrations of *PS*, *P* and *S* respectively, and k_a is the biomolecular association rate constant. The diffusion-limited

biomolecular reaction rate constant for the case of protein-DNA association can be defined as

$$k_a = 4\pi D_{3D} ba \,,$$

where D_{3D} is the 3D diffusion coefficient of the protein, assuming the DNA site to be immobile compared to the protein. The parameter $b = 0.34\,nm$ is the spacing between the base pairs of DNA. Note that displacement by a single base pair leads to a completely different DNA sequence that is unrecognisable by the protein. The parameter a represents the percentage of the site-specific surface of the protein and can be assumed to be relatively large $a \approx 0.2 - 0.5$ because proteins have large reactive interfaces and besides proteins will tend to orient correctly upon approaching the DNA as a result of electrostatic interactions between basic amino acids and negatively charged DNA backbone. Using the equation for k_a mentioned above, we can estimate its value in case of a diffusion limited process taking the 3D diffusion coefficient in the range of $D_{3D} = (1-5) \cdot 10^{-6}\,cm^2 s^{-1}$. The resulting association rate is $k_a \approx 10^8\,M^{-1} s^{-1}$. However, the experimentally measured association rate k_a of the *lac* repressor protein to its binding site yielded $k_a \approx 10^{10}\,M^{-1} s^{-1}$, which is a 100 times higher than the predicted 3D association rate.[8]

   In light of a 100-fold discrepancy between this theoretical limit and the experimentally observed rate constant, Riggs et al. proposed an alternative search mechanism, referred to as facilitated diffusion, which provides the increase in speed.[8] Their idea was that while searching for its target, a protein repeatedly attaches to and detaches from DNA and, once bound non-specifically, slides along the DNA, performing a 1D random walk. Once detached from DNA, the protein diffuses three-dimensionally through solution to randomly bind to another site on DNA and perform another round of sliding (Fig. 1.2). The protein-to-DNA attachment is maintained by the electrostatic interactions between positively charged sites on the protein molecules with negatively charged DNA. The energy

of this binding interaction is typically in the range of 10-15 k_bT (at physiological salt concentration).

If one considers a combination of 3D and 1D diffusion, the association rate k_s of a DNA-binding protein can be related to the Smoluchowski association rate k_{Smol} using the expression $k_s = k_{Smol} \tilde{n} \tau_{3D} / (\tau_{1D} + \tau_{3D})$, where $\tilde{n}$ is the average sliding length $\tilde{n} \approx \sqrt{D_{1D} \tau_{1D}}$ in units of base pairs that a protein covers in one binding event, and τ_{3D} and τ_{1D} are the average times a protein spends during one round diffusing in 3D and 1D respectively.[22,23] From the equation for k_s it is obvious that non-specific binding to DNA results in reaction deceleration by a factor of $\tau_{3D} / (\tau_{1D} + \tau_{3D})$, which is compensated by the increase in the number of visited base pairs per one round of search from 1 to $\tilde{n}$. This latter effect is called the antenna effect by Hu et al.[24]
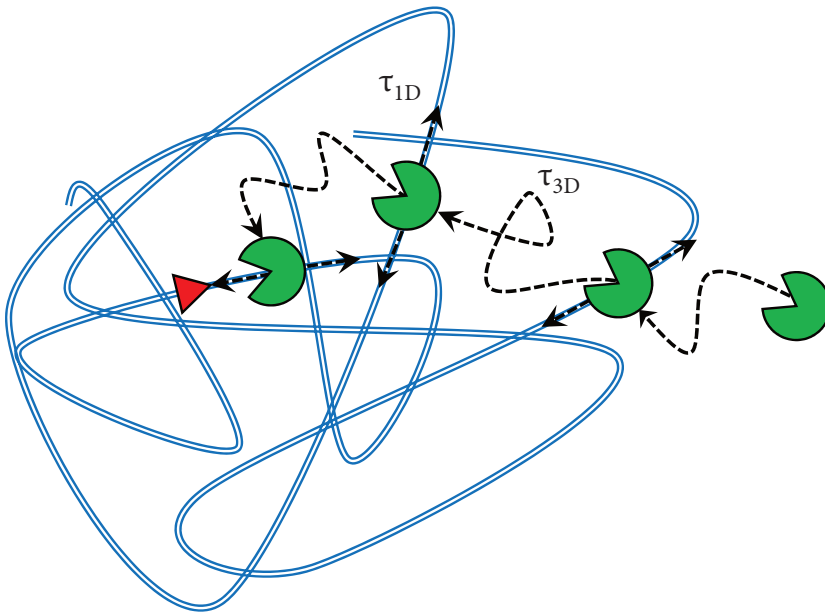

Figure 1.2 | Protein-DNA search problem. A protein locates its targets by alternating rounds of 1D sliding along the DNA and 3D diffusion around the DNA. The duration of each round is τ_{1D} and τ_{3D} correspondingly.

It can be found that the maximum rate constant is achieved when $\tau_{1D} = \tau_{3D}$ by setting $dk_s/d\tau_{1D} = 0$ and assuming that D_{1D} is independent of τ_{1D}. However, theoretical estimates[22] of the factor $\tau_{3D}/(\tau_{1D} + \tau_{3D})$, taking into account the affinity estimates of bacterial DNA-binding proteins to DNA and high DNA concentration inside a bacterial cell, lie in the range of $10^{-4} - 10^{-1}$, corresponding to a situation in which the protein spends most of its time diffusing in 3D. This value is corroborated by measurements *in vivo*[20], but clearly is far from the optimal theoretical one. Moreover, the sliding length is estimated in the *in vitro* experiments and lies in the range of $10^2 - 10^3$ bp but is likely to be less *in vivo*.[21]

Even though the simple theoretical estimations described above provide clues about the role of 1D diffusion in association acceleration, they fail to show that a significant facilitation can be achieved by a combination of 1D and 3D diffusion. Since Riggs et al. proposed a DNA sliding mechanism as a means for proteins to speed up search (in line with the original suggestion by Adam and Delbrück in 1968) various theoretical models have been proposed. Initially, the model of Riggs et al. was studied both experimentally and theoretically by Von Hippel, Berg and colleagues. In their seminal work, the authors proposed several possible mechanisms of reaction speed-up, including one-dimensional (1D) random walk along DNA, 1D hopping on DNA, 3D jumping and intersegmental transfer. Their model was later revisited by Halford and Marko, who significantly simplified it by introducing the mean sliding length as a main parameter.[25] A similar result was obtained by Klenin et al. starting from first principles.[26] Later, several analytical[24,27-29] and simulation[30-34] models were proposed and currently it is generally accepted that a combination of 3D and 1D diffusion contribute to target search. However, a comprehensive theoretical description is still not available. In this thesis, we use a mean-field approach to formulate a general theoretical model to elucidate the role of 1D sliding in reaction acceleration and quantify the contributions of different reaction mechanisms of product formation involving DNA.

1.3. 1D sliding in Adenovirus

Parts of this thesis describe experimental work on proteins involved in the maturation of adenovirus, a naturally occurring system that relies on the diffusion along DNA. A key property of this system that has enabled much of the work described here is the fact that 1D sliding is facilitated by a short peptide of only 11 amino acids long. We will discuss here the basic biological and biochemical properties of this system.

Adenoviruses[35] are nonenveloped (without an outer lipid bilayer) icosahedral viruses with a diameter of 90-100 nm (Fig. 1.3a) that contain a double-stranded DNA genome. Many distinct adenoviral serotypes exist that cause different illnesses, the most common being a common cold (10% of the cases in young children), gastroenteritis, conjunctivitis and cystitis. The vast majority of infections is mild, however, deaths have been reported depending on the virus serotype (the deadliest being serotype 14 with a mortality rate of up to 18%)[36] and the state of the patient's immune system. Adenovirus infection is usually transmitted in droplets of respiratory or ocular secretions, but faecal-oral transmission is also possible.

Late in adenovirus infection, immature viruses are assembled with multiple copies of different precursor proteins. Subsequently, a 23-kDa adenovirus protease (AVP) is activated and cleaves the precursor proteins to produce mature, infectious viruses. The activation of AVP is determined by the presence of two cofactors inside immature viral particles. One of the cofactors is pVIc, an 11-amino acid peptide (GVQSLKRRRCF), which is cleaved by AVP from the C-terminus of virion precursor protein pVI. Cys10' of pVIc subsequently forms a disulphide bond with Cys104 of AVP to yield a covalent AVP-pVIc complex. The second cofactor is the entire viral DNA of ~36-kbp. The two cofactors drastically increase the enzyme efficiency k_{cat}/K_m, where k_{cat} is a catalytic rate constant and K_m is a Michaelis constant.[37-39] The relative k_{cat}/K_m of AVP is increased 110-fold in the presence of adenovirus DNA and 1130-fold in the presence of pVIc.
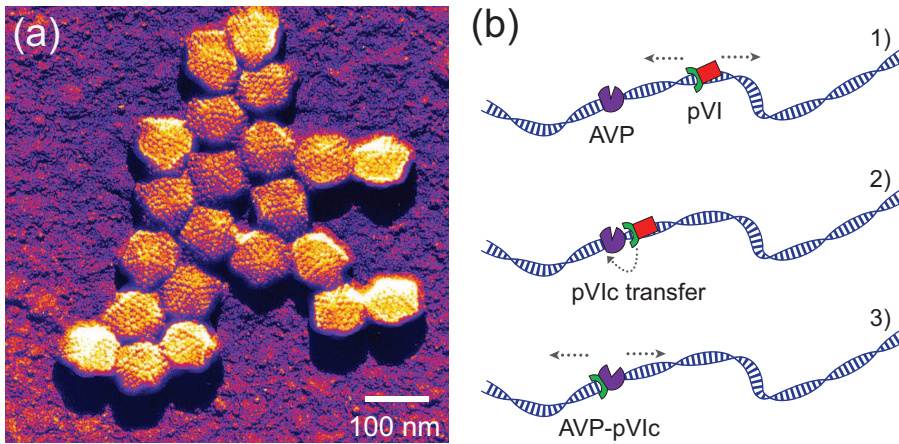
Figure 1.3 | Adenovirus and its activation during maturation. (a) Colorised electron micrograph of adenovirus (Copyright Linda M. Stannard, University of Cape Town). (b) AVP activation as a result of precursor protein pVI cleavage.

AVP together with both cofactors exhibits a k_{cat}/K_m increase of more than 15000-fold. However, a question remains: how can only ~50 fully active AVP's cleave numerous copies of the precursor proteins at ~1900 processing sites in a tightly packed viral capsid? The additional challenge is that the 3D diffusion of AVP and precursor proteins is decreased 6 orders of magnitude due to DNA mass action and tight packing of the virus (DNA concentration > 500 g/L).

The first solution to this problem was proposed in 1993.[40] The authors hypothesised that AVP travels within adenovirus by means of 1D sliding along DNA. While for a long time this idea remained a hypothesis, recent biochemical and single-molecule studies of adenovirus proteins by the group of Walter Mangel support the notion of proteins 1D diffusing within adenovirus.[41-44] These experiments have led to the following picture of the AVP activation cascade. At the start of maturation, AVP is randomly distributed along the viral DNA and unable to move along the DNA (Fig. 1.3b, panel 1). The precursor of protein VI (pVI) is bound to DNA as well but as opposed to the AVP, it is able to one-dimensionally diffuses along DNA. By doing so, pVI eventually encounters AVP, triggering the proteolytic cleavage of pVI at its carboxyl terminus (Fig. 1.3b,

panel 2). This reaction liberates the 11-amino acid pVIc peptide, which binds tightly to the AVP. The resulting AVP-pVIc complex is now able to rapidly diffuse in a 1D fashion along the viral DNA (Fig. 1.3b, panel 3). This 1D movement allows the AVP-pVIc complex to rapidly scan the viral genome and target the large number of protease targets that are distributed along the DNA.

## 1.4. Scope of this thesis

The recent biochemical and single-molecule work by Walter Mangel and colleagues strongly suggests that 1D sliding is responsible for the proteolytic reactions within adenovirus. However, this elegant picture still requires some solid biophysical evidence that adenovirus proteins locate their targets by 1D sliding. Therefore, we aspired to reproduce the processes unravelling within the virus *in vitro*. We anchored linear fragments of DNA to the top surface of a microscope coverslip using a biotin-streptavidin interaction and stretched the DNA molecules by applying a laminar flow over the surface. We studied the behaviour of fluorescently labelled AVP, pVI and pVIc using total-internal-reflection fluorescence (TIRF) microscopy to image the fluorescence signals coming from the individual proteins moving along or binding to the DNA. Building upon our initial data as for the behaviour of the individual components that take part in AVP activation, our goal is to observe the full AVP activation process in real time. Using these experiments, it is our goal to directly test the hypothesis that proteolytic cleavage of pVI by AVP results in a transfer of the pVIc molecular sled to AVP and thus yields AVP in a state in which it can freely diffuse along DNA. These experiments will show how nature utilises the concept of small, transferable modules to allow proteins to rapidly search along DNA (Chapter 2).

Subsequently, inspired by nature's elegant solution of the target search problem we undertook a biomimetic approach, i.e. we attempted to use the principle of 1D sliding along DNA to accelerate the association between an arbitrary pair of binding partners. We chose the canonical association between

biotin and streptavidin as a model system and used FRET as experimental read-out. For this system, we demonstrated a decrease in the reaction time of more than one order of magnitude by having the reactants slide along DNA and reduce the dimensionality of search. In this system, DNA acts as a catalyst and its speed-up effect was characterised for different DNA concentrations and lengths. After establishing the proof-of-principle reaction we extended our approach to the Polymerase Chain Reaction (PCR), a very common reaction and a laboratory technique vital to a large number of biotechnological processes. pVIc was attached to PCR primers and the kinetics of standard PCR reactions accelerated by reducing the reaction time by tens of percents. Significantly fewer PCR cycles were needed to achieve the same amplicon concentration when primers with pVIc modification are employed (Chapter 3).

Text box 2 | Förster (Fluorescence) Resonance Energy Transfer (FRET). This technique is based on nonradiative energy transfer from an excited fluorophore, called donor, to another fluorophore in its vicinity, called acceptor, whereupon the latter emits fluorescence. For FRET to occur the fluorophores need to be in close proximity (< 10 nm); the efficiency E of the process strongly depends on the distance R between them: $E = \left[1 + \left(R/R_0 \right)^6 \right]^{-1}$, where R_0 is a parameter that depends on the chosen pair of fluorophores (usually ~5–7 nm). Labelling interacting molecules or even parts of one molecule with donor and acceptor fluorophores and measuring the FRET efficiency changes allows one to gain access to subnanometer distance changes. In this study, I employed time-course FRET measurements to study the kinetics of the association between biomolecules. A detailed description of the experimental set-up is given in Chapter 3.
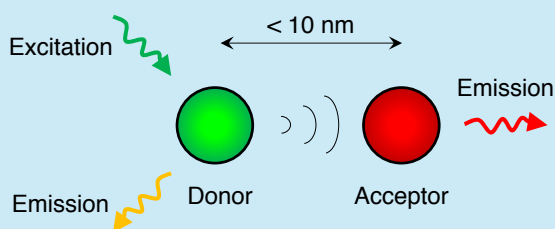
Text box 3 | Polymerase Chain Reaction (PCR). A critical technique in medicine, biotechnology and research, PCR is used to create thousands to millions of identical copies of a particular DNA sequence from several or even a single DNA molecule. PCR consists of a number of cycles, during each of which a piece of DNA is doubled using the DNA copying enzyme – DNA polymerase. During each PCR cycle, the reaction is initiated by small pieces of DNA called primers, which need to find and bind to the region on the DNA that needs to be amplified. Since this process involves searching for a particular sequence on DNA, we attempted to improve PCR by introducing a new reaction pathway in the reaction – 1D sliding along DNA. In this study, we functionalised PCR primers by DNA sliding peptides and demonstrated a PCR speed-up of 15-27%. The experimental details are presented in the PCR part of Chapter 3.
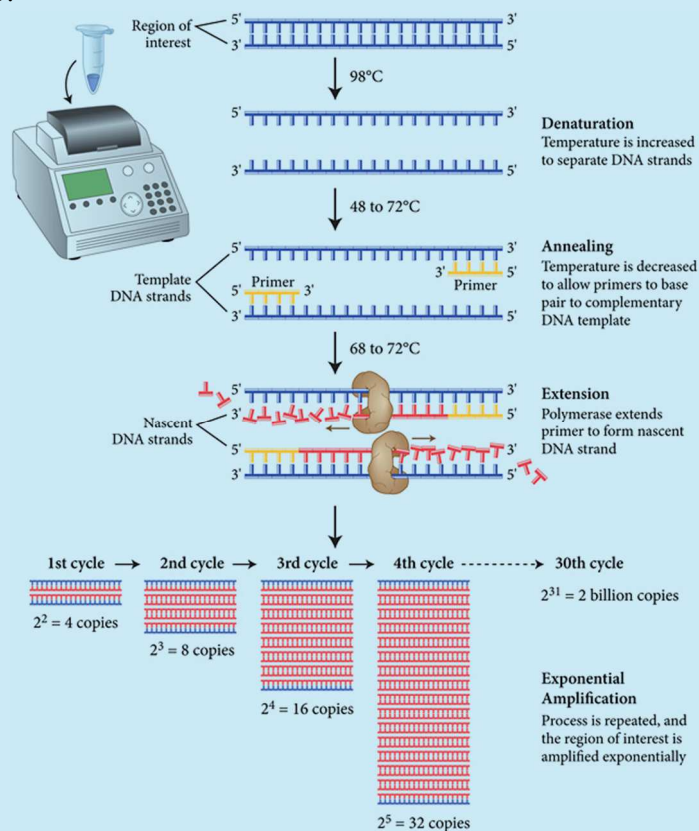
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To better understand the nature of the acceleration effect we observed in our experiments, we developed a kinetic model of a bimolecular reaction in solution with linear extended sinks (i.e. DNA) that can intermittently trap molecules in a solution. We use a mean-field approach to describe the behaviour of pVIc-functionalised reactants in solution with DNA. The model describes the results of our proof-of-principle biotin-streptavidin reaction remarkably well. Moreover, we calculated the relative contributions of different reaction pathways to the total association and conclude, that under optimal search conditions 1D sliding becomes the driving force of reaction product formation (Chapter 4).

To demonstrate the practical applicability of our approach even further, we attempted to increase the efficiency of two biochemical systems with the help of the pVIc molecular sled. The two systems were DNA polymerases and DNA topoisomerase inhibitors (antibiotics and antineoplastics). For this purpose, we expressed a widely used polymerase with a pVIc moiety attached to it. Regarding the antimicrobial agents and anticancer drugs that interfere with the bacterial or cellular DNA machinery, we chemically conjugated them to the molecular sled pVIc. In both cases the addition of pVIc is hypothesised to improve the polymerase or drug efficiency due to the fact that the 3D diffusion is reduced to a 1D search process. Initial compounds and conjugation strategies did not bring about the expected efficiency enhancement. This work will serve as a basis for future efforts in this direction (Chapter 5).

Summarising, the experiments described in this thesis represent a series of projects that not only help us understand the molecular principles underlying the role of the adenovirus pVIc peptide in adenovirus maturation, they also aid the development of novel approaches to put the sliding ability of pVIc to use in a variety of biotechnologically relevant systems.

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