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Speeding Up Standard PCR Reactions with a Molecular Peptide Sled

Alexander Turkin[1], Lei Zhang[2], Alessio Marcozzi[2], Walter F. Mangel[3], Andreas Herrmann[2]*, Antoine M. van Oijen[1]*

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Speeding up standard PCR reactions with a molecular peptide sled

Alexander Turkin[1]†, Lei Zhang[2]†, Alessio Marcozzi[2], Walter F. Mangel[3], Andreas Herrmann[2]*, Antoine M. van Oijen[1]*

[1]Single-Molecule Biophysics, Zernike Institute for Advanced Materials, University of Groningen, Groningen, the Netherlands.

[2]Department of Polymer Chemistry, Zernike Institute for Advanced Materials, University of Groningen, Groningen, the Netherlands.

[3] Brookhaven National Laboratory, Upton, NY, U.S.A.

†These authors contributed equally to this work.

*Correspondence to: a.m.van.oijen@rug.nl; a.herrmann@rug.nl.

We demonstrate the use of a DNA-interacting peptide to reduce the dimensionality of diffusional search processes and speed up associations between biological macromolecules by orders of magnitude in the presence of DNA. We show that modifying PCR primers with the peptide sled enables significant acceleration of standard PCR reactions. We further extend our approach to a model system for bimolecular association in which we functionalise binding partners of interest with the sliding peptide and demonstrate a drastic reduction in reaction time.

The crowded intracellular environment presents many challenges for basic molecular processes to occur. Non-specific interactions between proteins hinder diffusional mobility and increase the time needed for binding partners to find each other and associate.[1] Nature displays several examples in which the dimensionality of search processes is reduced to speed up association times[2] **(Fig. 1**). For example, binding partners of certain classes of cell-surface receptors associate with lipid membranes and utilize two-dimensional diffusion to promote association.[3] Many DNA-interacting proteins find specific sequences or lesions in large amounts of nonspecific DNA by performing one-dimensional random walks along the DNA.[4] Every time such a protein associates

with DNA, it transiently diffuses along the duplex and thus drastically increases the number of sampled DNA positions per unit of time. It then dissociates from the DNA, undergoes three-dimensional (3D) diffusion through solution to rebind at an entirely different region and again searches a stretch by one-dimensional (1D) diffusion. The combination of 3D and 1D searches gives rise to a drastic increase in the effective bimolecular association rate constant of the protein with its target.[5,6]

An example of a naturally occurring system in which one-dimensional diffusion along DNA is used to speed up association between two proteins is found in adenovirus.[7,8] During viral maturation, a large number of proteins within a single viral particle need to be proteolytically processed by the adenovirus protease (AVP) before infection of a cell.[9] Tight packing of protein and DNA within the viral particle makes regular 3D diffusion as a mechanism for the protease to travel from one target to the other impossible. Recent work has shown that the AVP protein[10] recruits the short 11-a.a. pVIc peptide (GVQSLKRRRCF)[11], itself a proteolytic product in early maturation, and uses it to slide along the DNA inside the viral particle and thus effectively reduces the search space for the protease from three dimensions to one.[8]

In this work, we demonstrate that the ability of the pVIc peptide to slide along DNA can be used to speed up a much broader class of biomolecular processes than just those occurring *in vivo* and that it can even be used to dramatically improve the speed of common laboratory reactions. In particular, we show its applicability to speed up a standard polymerase chain reaction (PCR) by establishing a significant decrease in the time needed for pVIc-coupled primers to anneal to the template DNA. The exponential amplification of DNA during PCR can be divided into three distinct steps.[12] The first step is the melting of the double-stranded DNA template (**Fig. 2a**), followed by primer annealing and elongation with the polymerase. During the annealing step, primers need to find and hybridise to their complementary target sequence on a template. During this annealing step, the DNA will consist of a mixture of denatured and double-stranded regions, providing a large

variety of structures for the pVIc-primers to interact with and potentially move along, resulting in a reduction of the time needed for the primer to locate and bind to its target sequence.

We covalently coupled (Online Methods) the pVIc peptide to the 5' ends of a pair of PCR primers (primer set I, **Supplementary Table 1**, **Supplementary Fig. 1 and 2**) designed to amplify a 807-bp stretch from a linear double-stranded 1970-bp-long template and used real-time PCR (qPCR) experiments with SYBR Green I fluorescence to report on the kinetics of amplicon formation[13] (**Fig. 2b** and **Supplementary Fig. 3**). The correct length of the PCR product was confirmed by agarose gel electrophoresis (**Supplementary Fig. 4**). Similar results were obtained for PCR experiments employing a different pair of primers (**Supplementary Table 1** and **Supplementary Fig. 5**) and a longer 8669-bp-long circular template M13KO7 (**Supplementary Fig. 6**). The kinetics of amplicon formation were quantified in an unbiased manner by employing a PCR threshold cycle analysis (Online Methods and **Supplementary Fig. 7**). Remarkably, the PCR reaction containing the pVIc-conjugated primers displayed a significant reduction in the number of cycles needed, suggesting the use of a molecular sled as a viable approach to speed up the overall reaction time of PCR. In our experiments we were able to shorten the PCR reaction time by 15% ~ 27%.

To ensure that the increase in speed is not caused by a nonspecific electrostatic association between the four positively charged amino acids in the sliding peptide and the negatively charged DNA backbone, we repeated the same PCR experiments using primers conjugated to a scrambled peptide (S-peptide, SFRRCGLRQVK) containing the same residues in a random order, which presumably affects the sliding behaviour of the peptide yet preserving the net charge. Our qPCR data reveals that use of primers conjugated with this scrambled peptide does not result in a decrease of the number of PCR cycles required for amplification (**Fig. 2b**, S-peptide). The performance of the S-peptide-modified primers is very similar to the unmodified primers. Furthermore, we observed a significant reduction in the number of PCR cycles when using a truncated pVIc variant containing only the last six amino acids of pVIc, four of which are positively charged and are sufficient to

support sliding along DNA (**Fig. 2b**, K-peptide, KRRRCF, **Supplementary Fig. 8**). Finally, we studied the behaviour of the primer modifications under different conditions by varying the annealing time t_A and primer concentration C_{primer} (**Supplementary Fig. 6**). In case of the most stringent conditions (short annealing time, low primer concentration) the effect of the sliding peptides was the most pronounced, confirming the hypothesis that the molecular sled acts in improving the kinetic properties of the annealing process, not the thermodynamic ones. Further work is needed to more precisely define the optimal conditions for sled-induced speed up of PCR. Considering the prevalence of PCR in biological research and diagnostics, we strongly believe that our approach will have large impact in these areas.

To further extend our approach and demonstrate that the ability of the pVIc peptide to perform 1D searches along DNA can be utilised to speed up any biochemical reaction we used the canonical biotin-streptavidin interaction as a model reaction. We couple each of the two reactants to the pVIc molecular sled (**Fig. 3a**) and show that association proceeds more than an order of magnitude faster in the presence of DNA. Fluorescence Resonance Energy Transfer (FRET)[14] was used to monitor the time dependence of the bimolecular association reaction. For simplicity, we refer to the functionalised biotin and streptavidin as reactants *B* and *S*, respectively. Reactant *B* is formed by reacting a maleimide-functionalized biotin with the cysteine Cys10 of Cy3-labelled pVIc in a Michael-addition reaction (Online Methods and **Supplementary Fig. 9** and **10b**). The maleimide and biotin units are connected via a high-molecular weight polyethylene glycol (PEG) linker resulting in a total molecular weight for reactant *B* of 6.7 kDa. This high molecular weight reduces its diffusional mobility and allows us to more easily gain access to the timescale of association. Reactant *S* is prepared by forming a complex between a Cy5-labelled tetrameric streptavidin and an unlabelled biotin-pVIc conjugate (Online Methods and **Supplementary Fig. 10a**). Reactants *B* and *S* were combined in aqueous solution at final concentrations of 150 nM and 37.5 nM, respectively, and FRET between the Cy3 donor and Cy5 acceptor fluorophores was measured (**Supplementary Fig.**

11). **Figure 3b** shows the time dependencies of bimolecular association in the presence of 2686-bp long double-stranded DNA (dsDNA) at different concentrations. Addition of the DNA up to 1 pM did not have a significant effect on the reaction rate, whereas DNA concentrations of higher than 10 pM resulted in a clearly discernable reduction of the reaction time. For a DNA concentration of 300 pM, already after 15 s 99% of the maximum FRET efficiency was achieved.

Figure 3c shows the reaction times as derived from the FRET traces for different DNA concentrations and lengths. For each length, varying from 2,686 to 15 basepairs (**Supplementary Table 2**), the association times decrease by up to 20-fold at higher concentrations of added DNA. Interestingly, the critical concentration for reaction speed up differs for the different DNA lengths: longer DNA fragments are required at lower concentrations than short DNA molecules to achieve the same catalytic effect. This behaviour can be explained by the fact that the critical number of reaction partners associated with DNA is reached at higher DNA concentrations for short fragments and at low DNA concentrations for longer pieces of DNA. Thus, the main parameter that governs the kinetics of reaction is the total base-pair concentration, a unit that describes the total length of DNA per unit volume. This notion is validated by plotting the reaction time against DNA base-pair concentration (**Fig. 3d**), showing the curves cluster together in three distinct regions. These regimes can be understood in terms of the density of reactants on the catalytic DNA molecules. At low base-pair concentrations, the amount of DNA available per reactant is too low to allow for efficient 1D diffusion speeding up their association. In the optimal regime, around 0.1 to 10 ∝M of base pairs, the reactant molecules have sufficient space to slide along DNA to find each other by 1D diffusion. At base-pair concentrations higher than 100 ∝M, the probability for reactants B and S to bind to the same DNA molecule diminishes, resulting in a deceleration of the association reaction.

In an alternative mechanism to explain the increased association rates, DNA-bound reactants bound to the same DNA molecule could be brought into proximity of each other by bending and looping of the DNA duplex. In such a mechanism, the reactants would rely on the conformational

flexibility of the DNA and use the duplex as a scaffold to bring binding partners together. In order to exclude this pathway, we conducted a series of experiments with DNA of four different lengths (50, 100, 150 and 300 bp), which were chosen such that the corresponding DNA looping probability differed significantly from one another.[15,16] Under the low-salt buffer conditions used in this study, DNA molecules of 50 and 100 bp can be regarded as stiff rods whose folding onto itself is excluded (DNA persistence length is estimated to be 250 bp at 2 mM NaCl).[17] In case looping is the main mechanism for reaction speed-up one would expect a considerably lower reaction acceleration in case of 50 and 100 bp long DNA as compared to 300 bp, which is long enough to form loops. However, in all cases we observed the same 20-fold reaction speed-up (**Fig. 3c** and **3d**), confirming that association is not mediated by DNA bending onto itself.

Using a similar reasoning, one could argue that the conformational flexibility of the long PEG linkers attached to both reactants allows reactants that are statically but distally bound to the same DNA to associate without the need for sliding. **Figure 3d** shows, however, a 10-fold increase in reaction time at a reaction stoichiometry as low as 1 reactant per 1,000 base pairs, clearly an average molecular separation too high to be bridged by reactants statically bound to the same DNA. From the considerations above, one can conclude that sliding along DNA, and not just static binding, is responsible for the increase in association rate.

Summarizing, the 11-a.a. DNA-interacting pVIc peptide acts as a molecular sled in speeding up biochemical reactions by introducing a 1D reaction pathway in addition to bimolecular association via 3D diffusion. Our demonstration of the speed up of both a highly generalized reaction and a commonly used laboratory process suggests a wide variety of other potential uses.

Methods

Methods and any associated references are available in the online version of the paper.

Acknowledgements

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Author contributions

A.T. and A.M.v.O. designed the proof-of-concept FRET assay and A.T. performed the experiments. L.Z., A.M. and A.H. designed the PCR speed-up assay, A.M. set up the qPCR procedure and designed the primers and templates for it and L.Z. performed the experiments. A.T., A.M.v.O. and A.H. wrote the paper with input from all other co-authors.

Competing financial interests

The authors declare no competing financial interests.

References

1. Dlugosz, M. & Trylska, J. *BMC Biophys.* **4:3**, 1-9 (2011).

2. Riggs, A.D., Bourgeois, S. & Cohn, M. *J. Mol. Biol.* **53**, 401-417 (1970).

3. Chatterjee, S. & Rothenberg, E. *Viruses*. **4**, 3162-3178 (2012).

4. Redding, S. & Greene, E.C. *Chem. Phys. Lett.* **570**, 1-11 (2013).

5. Berg, O.G., Winter, R.B. & von Hippel, P.H. *Biochem.* **20**, 6929-6948 (1981).

6. Tafvizi, A., Mirny, L.A. & van Oijen, A.M. *ChemPhysChem* **12**, 1481-1489 (2011).

7. Graziano, V. *et al*. *The J. of Biol. Chem.* **288**, 2068-2080 (2013).

8. Blainey, P.C. *et al*. *The J. of Biol. Chem.* **288**, 2092-2102 (2013).

9. Pérez-Berná, A.J. *et al*. *J. Mol. Biol.* **392**, 547-557 (2009).

10. Mangel, W.F., Baniecki, M.L. & McGrath, W.J. *Cell. Mol. Life Sci.* **60**, 2347-2355 (2003).

11. Mangel, W.F., McGrath, W.J., Toledo, D.L. & Anderson, C.W. *Nature* **361**, 274-275 (1993).

12. Kennedy, S. & Oswald, N. *PCR Troubleshooting and Optimization: The Essential Guide.* (Caister Academic Press, Norfolk, UK, 2011).

13. Wilhelm, J. & Pingoud, A. *ChemBioChem* **4**, 1120-1128 (2003).

14. Gadella, T.W.J. *FRET and FLIM Techniques*. (Elsevier Science, Oxford, UK, 2009).

15. Peters, J.P. *et al.*, *Methods Enzymol.* **488**, 287-335 (2011).

16. Du, Q., Smith, C., Shiffeldrim, N., Vologodskaia, M. & Vologodskii, A. *Proc. Nat. Acad. Sci. U.S.A.* **102**, 5397-5402 (2005).

17. Baumann, C.G., Smith, S.B., Bloomfield, V.A. & Bustamante, C. *Proc. Nat. Acad. Sci. U.S.A.* **94**, 6185-6190 (1997).

FIGURE LEGENDS

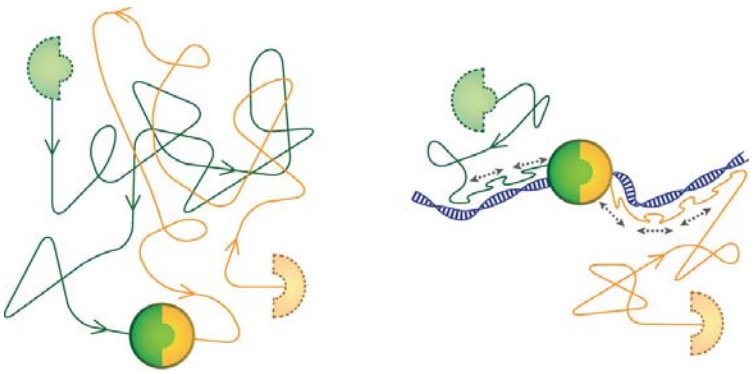


Figure 1 | Speeding up association reactions between biomolecules using the ability of a 'sled' peptide to one-dimensionally (1D) diffuse along DNA. A cartoon depiction of 3D and 1D bimolecular association pathways. Usually, association between molecules occurs as a result of reactants finding each other by diffusion in a 3D fashion through solution (left panel). Addition of a 1D reaction pathway can drastically speed up the reaction by reducing the dimensionality of search (right panel).

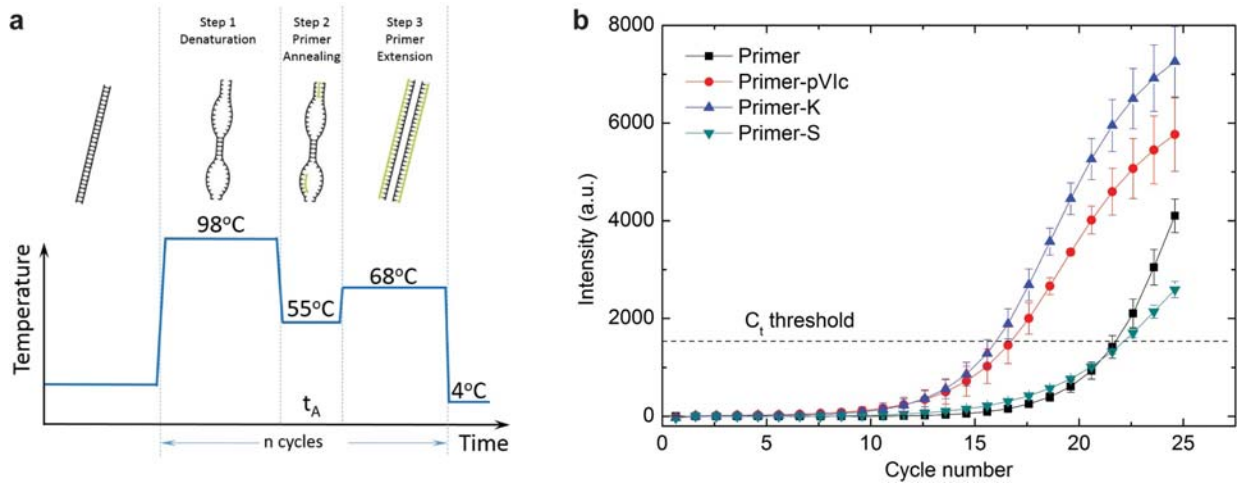


Figure 2 | Speed-up of PCR by sled-modified primers. (**a**) Thermal cycling protocol of the PCR reaction. A PCR cycle that is repeated multiple times consists of three distinct steps: template denaturation, primer annealing and elongation by a DNA polymerase. During the annealing step, primers need to find and hybridise to their complement on a template. Attaching the pVIc sled peptide to PCR primers enhances their ability to more rapidly reach their target sequence, thus speeding up PCR product formation. (**b**) qPCR plot comparing different primer-peptide conjugates. Amplicon formation is reported as SYBR Green I fluorescence measured as a function of PCR cycle. Amplicon formation for different primer-peptide conjugates is shown for annealing time t_A = 1s and primer concentration C_{primer} = 0.125 μM. The C_t threshold values for PCR reactions using unmodified primers, primer-S (negative control), primer-pVIc and primer-K were measured to be 21.7 ± 0.4, 22.1 ± 0.2, 16.7 ± 0.7, and 16 ± 0.5, respectively. The two primers that were modified with sled peptides reduce the number of PCR cycles by 15-27% compared to unmodified primers. Error bars indicate ±SD; n = 3.

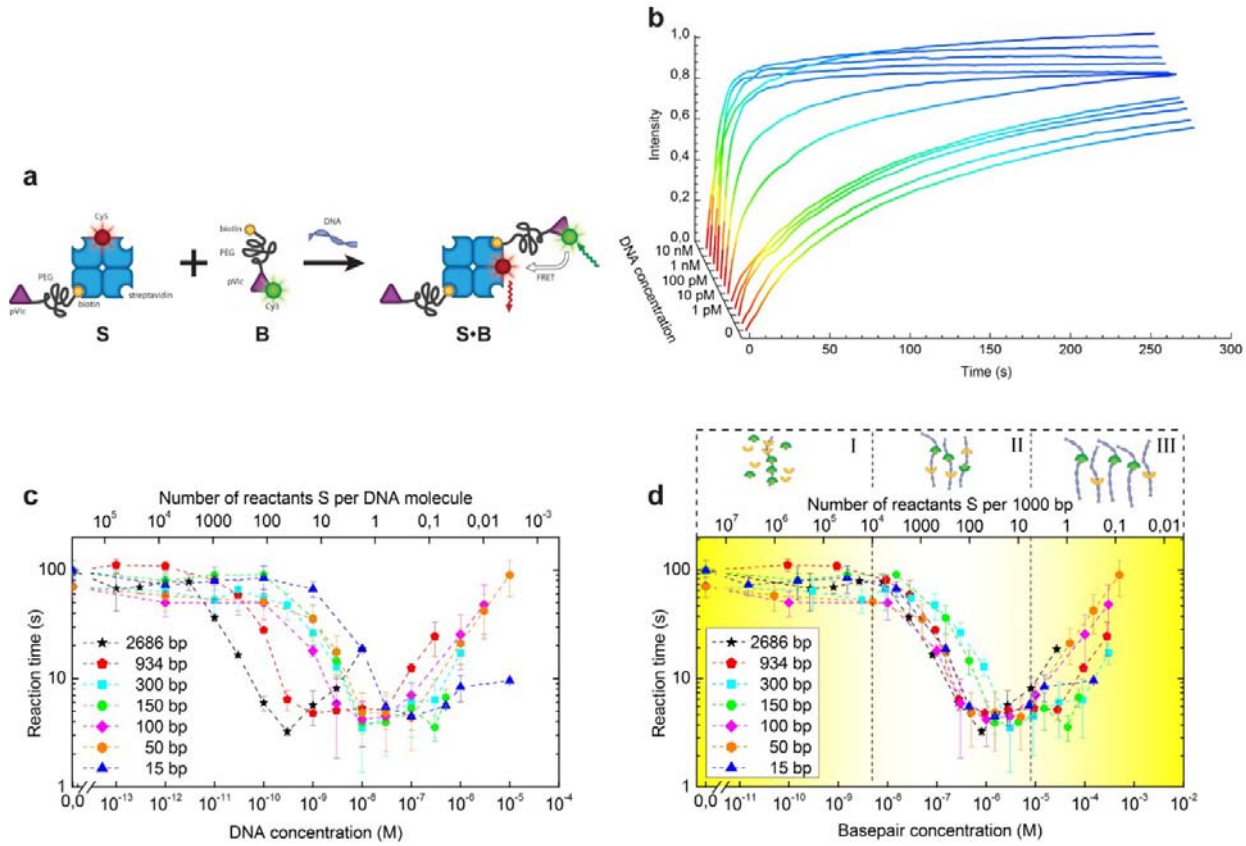


Figure 3 | Speeding up bimolecular association between *S* (sled-conjugated streptavidin) and *B* (sled-conjugated biotin) by DNA of different lengths and concentrations. (**a**) A schematic of the proof-of-concept biotin-streptavidin association reaction. The formation of a complex between Cy5-labeled streptavidin (*S*) and Cy3-labeled biotin (*B*), both modified by the peptide sled pVIc, is studied in the absence and presence of DNA of different lengths and concentrations. Fluorescence Resonance Energy Transfer (FRET) between *S* and *B* is used as experimental readout for complex formation. (**b**) The bimolecular association process is initiated by rapidly mixing *S* and *B* to final concentrations of 37.5 nM and 150 nM, respectively. The formation of complex *S•B* is monitored as a time-resolved detection of FRET. The results are shown for the bimolecular association in the presence of various concentrations of 2686-bp long DNA. The measured rise of the concentration of *S•B* complex can be approximated by an exponential growth $C(t) = C_{max}\left(1 - e^{-t/\tau}\right)$, with τ is the observed characteristic reaction time. (**c**) Reaction times τ as a function of DNA concentration for different DNA lengths. The curves follow the same trend differing only by the range of DNA concentrations at which the reaction speed-up occurs. (**d**) Reaction time τ *vs.* basepair concentration. The dependencies of the reaction time on DNA basepair concentration follow the same trend and the curves cluster together in three regions: I) no speed-up due to an insufficient number of DNA molecules per reactant; II) maximum speed-up with the optimal amount of DNA per reactant; III) reduced speed-up caused by the amount of DNA being so high that the probability of reactants to meet on the same DNA molecule diminishes. Error bars indicate ±SD; n ≥ 3.

ONLINE METHODS

Primer synthesis

Amino modified primers (provided by biomers.net GmbH, **Supplementary Table 1**) were functionalised by the peptides in a two-step reaction.[18,19] First, lyophilised amino modified primers (1.5 mM) were reacted with a 20-fold excess of 4-(N-maleimidomethyl)cyclohexane-1-carboxylic acid 3-sulfo-N-hydroxysuccinimide ester (sulfo-SMCC) linker (Sigma Aldrich) in 0.1 M sodium phosphate buffer (pH 7.6). A stock solution of sulfo-SMCC in DMF was used. The reaction was allowed to proceed in a shaker at room temperature overnight. After incubation, the reaction mixtures were centrifuged three times using 3000 Da cut-off spin-columns (Sartorius stedim biotech) to wash out excess Sulfo-SMCC and other small molecules by Milli-Q water. The resulting solution was lyophilised and the conjugate was used for the next coupling step without further treatment. The coupling efficiency was ~50%. Lyophilized primer-SMCC conjugate was dissolved in 0.1 M sodium phosphate buffer (pH 7.6) to obtain a 200 µM solution. Subsequently, dry peptide of choice (pVIc, K or S, provided by CASLO) were added in a 5-fold molar excess. If the peptide did not dissolve completely DMF was added until a clear solution was visible. The reaction mixture was vortexed at room temperature overnight and the product was purified by reversed-phase chromatography (buffer A (pH 7.5): 0.1 M triethylammonium acetate (TEAT) containing 5% Acetonitrile; buffer B (pH 7.5): 0.1 M TEAT containing 65% Acetonitrile; column: RESOURCE RPC 1 mL; gradient: 0-60%, 50CV; wavelength: 260 nm). Finally, the buffer was exchanged to Milli-Q water by centrifugation in a 3000 Da cut-off spin-column. The products were analysed using polyacrylamide gel electrophoresis (PAGE) (**Supplementary Fig. 1**) and MALDI-TOF mass spectrometry (**Supplementary Fig. 2**). For further use the conjugates were lyophilised. In this reaction step, a coupling efficiency of 30 – 40% was achieved.

PCR reactions

Real time PCR (qPCR) experiment was performed using Bio-Rad iQ5 Real-Time PCR System (Bio-Rad Laboratories, Richmond, USA). 20 µL reaction mixtures contained forward and reverse primers (modified or unmodified) at 0.125 µM, DNA template (10 ng for circular 8669-bp M13KO7 DNA template, 5 ng for short linear 1970-bp DNA template), SYBR Green I (1×), Qiagen fast cycling PCR kit (1×) and Q-solution (1×). All reactions (unless indicated otherwise) were performed in triplicate using the following cycling protocol: initial template melting 98°C (5min); 30 cycles of [98°C (1min), 55.6^{o}C (1s), 68°C (30s)]; 68°C (4min), 4°C (hold). The SYBR Green I fluorescence was monitored at the end of each cycle. It should be noted that the primer annealing temperature used was the one calculated for the original unmodified primers. The modified primers may have different optimal annealing temperature but, nevertheless, for all primers the same annealing temperature was used not to introduce a variable in the experiments. To compare the kinetics of amplicon formation for PCR reactions with different primers we employed the threshold cycle (C_t) analysis. The C_t was set in the exponential phase of amplification using the built-in function of the software for Bio-Rad iQ5 Real-Time PCR System. To confirm that no unspecific amplification occurs and that primer annealing step is essential in the PCR protocol we performed several control experiments (**Supplementary Fig. 3**).

Preparation of reactants

Stock solution of Reactant *B* was prepared as follows. N-terminus Cy3 labelled pVIc (custom peptide synthesis by Bio-Synthesis, Inc), 50 μM, was derivatised at Cys10' in 25 mM PBS buffer (pH 7.3) by adding biotin-PEG-maleimide (5 kDa, Nanocs) to 50 μM. The reaction vial was wrapped in aluminium foil to protect from light and the reaction was allowed to proceed for 5 hours at room temperature on a nutator. The reagent was subsequently stored at $+4^0$C and used as is without further purification over the course of one month. Successful conjugation was confirmed by MALDI/TOF mass spectrometry (**Supplementary Fig. 9**).

Spectrofluorometer assay

The experiments were performed in the sliding buffer, containing 60% wt. glycerol, 10 mM HEPES (pH 7.0), 2 mM NaCl, 20 mM Ethanol, 50 μM EDTA. The reactions were run in a 3mL quartz cuvette (FP-1004, Jasco) on a Jasco FP-8300 spectrofluorometer at 20^oC, continuously stirred at 800 rpm. The excitation and emission wavelengths were 520 nm and 666 nm correspondingly. The reaction read-out was FRET signal detected as a function of time. The solutions of reactants *S* and *B* were prepared 30 minutes before the experiment in the following way. Solution 1 (reactant *S*), final volume 2 mL, was obtained by incubating Cy5-streptavidin (43-4316, Life Technologies) with a two-fold excess of biotin-PEG23-pVIc (custom peptide synthesis by Bio-Synthesis, Inc) in the sliding buffer to render Cy5-streptavidin molecules functionalised with pVIc. Solution 2 (reactant *B*), final volume 1 mL, was prepared from the stock solution and contained biotin-PEG-pVIc-Cy3 in the sliding buffer. Prior to the experiments, DNA of the required length was added at a necessary concentration to both solutions. Solution 1 was transferred into the cuvette and FRET *vs.* time signal acquisition was started. Subsequently, solution 2 was rapidly injected into the cuvette with a syringe. The final reaction mix of a final volume 3 mL contained 37.5 nM Cy5-streptavidin (functionalised by 75 nM biotin-PEG23-pVIc), DNA at a chosen concentration and 150 nM reactant *B*.

FRET traces analysis

The obtained FRET vs. time traces were initially processed using Spectra Analysis subprogram of the Spectra Manager V.2 software package by JASCO Inc. The data were subject to baseline correction, noise elimination and smoothing (binomial interpolation, 6 iterations). Subsequently, the traces were fit with a single exponential function $C(t) = C_{!"\#}\left(1 - e^{!\ /!}\right)$ and reaction times τ were extracted from the fit.

18. Tung, C.H., Rudolph, M. J. & Stein S. *Bioconj. Chem.* **2**, 464-465 (1991).

19. Fraley, A.W. et al. *J. Am. Chem. Soc.* **128**, 10763-10771 (2006).

SUPPLEMENTARY INFORMATION

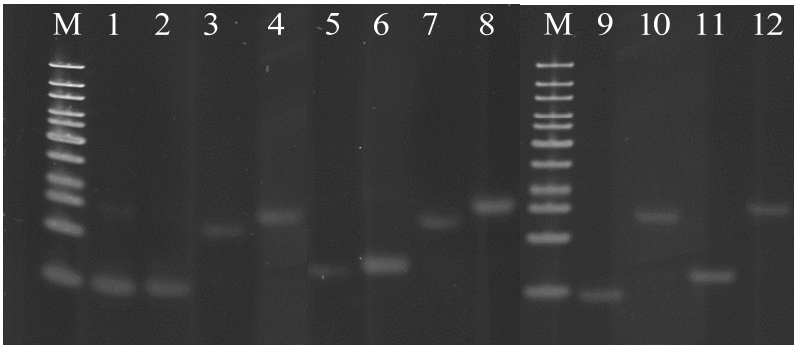
Speeding up standard PCR reactions with a molecular peptide sled

Alexander Turkin[1]†, Lei Zhang[2]†, Alessio Marcozzi[2], Walter F. Mangel[3], Andreas Herrmann[2]*, Antoine M. van Oijen[1]*

[1]Single-Molecule Biophysics, Zernike Institute for Advanced Materials, University of Groningen, Groningen, the Netherlands.
[2]Department of Polymer Chemistry, Zernike Institute for Advanced Materials, University of Groningen, Groningen, the Netherlands.
[3] Brookhaven National Laboratory, Upton, NY, U.S.A.

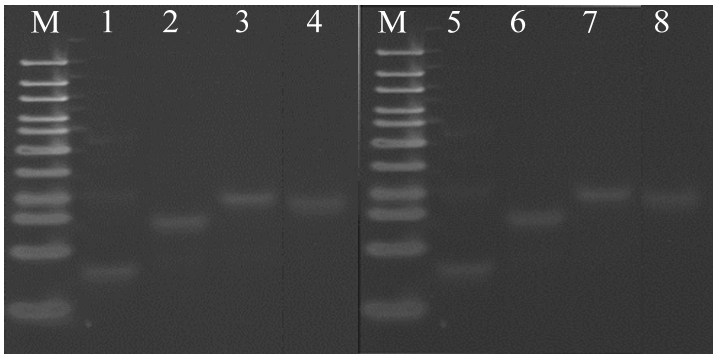
†These authors contributed equally to this work.
*Correspondence to: a.m.van.oijen@rug.nl; a.herrmann@rug.nl

Primer set I



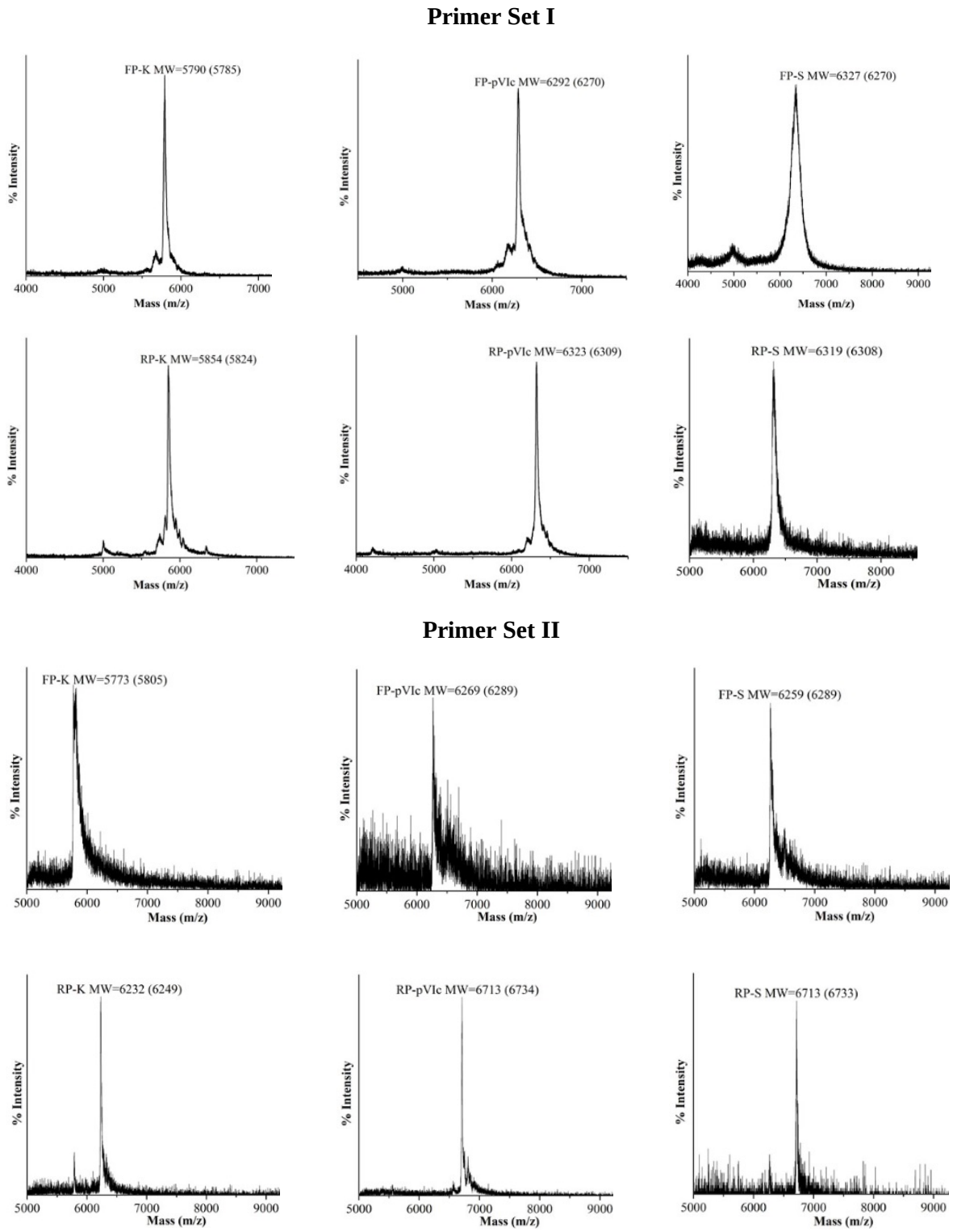
M is DNA ladder for reference (10-300 bp); Lane 1: FP; Lane 2: FP-SMCC linker; Lane 3: FP-K; Lane 4: FP-pVIc; Lane 5: RP; Lane 6: RP-SMCC linker; Lane 7: RP-K; Lane 8: RP-pVIc; Lane 9: FP; Lane 10: FP-S; Lane 11: RP; Lane12: RP-S.

Primer set II

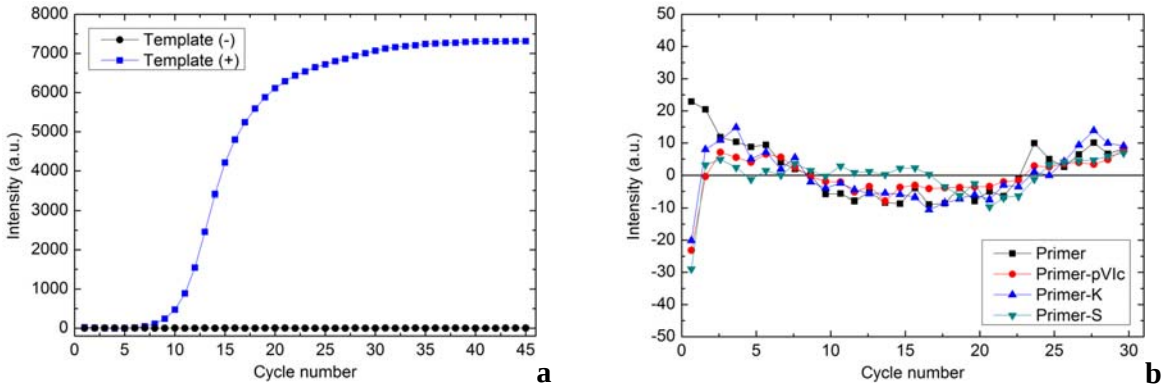


Lane 1: FP; Lane 2: FP-K; Lane 3: FP-pVIc; Lane 4: FP-S; Lane 5: RP; Lane 6: RP-K; Lane 7: RP-pVIc; Lane 8: RP-S.

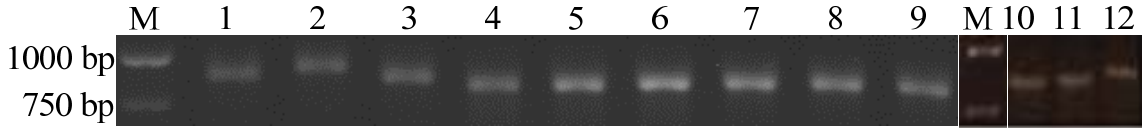
Supplementary Figure 1. Analysis of primer-peptide conjugates by polyacrylamide gel electrophoresis (PAGE) after chromatographic purification. The purity of forward (FP) and reverse (RP) primer-peptide conjugates was analysed using a 20% PAGE gel with subsequent SYBR Gold staining.



Supplementary Figure 2. MALDI-TOF mass spectra of primer-peptide conjugates. The molecular weights (MW) obtained from the measurements are shown above the peaks. The predicted MW are given in brackets.

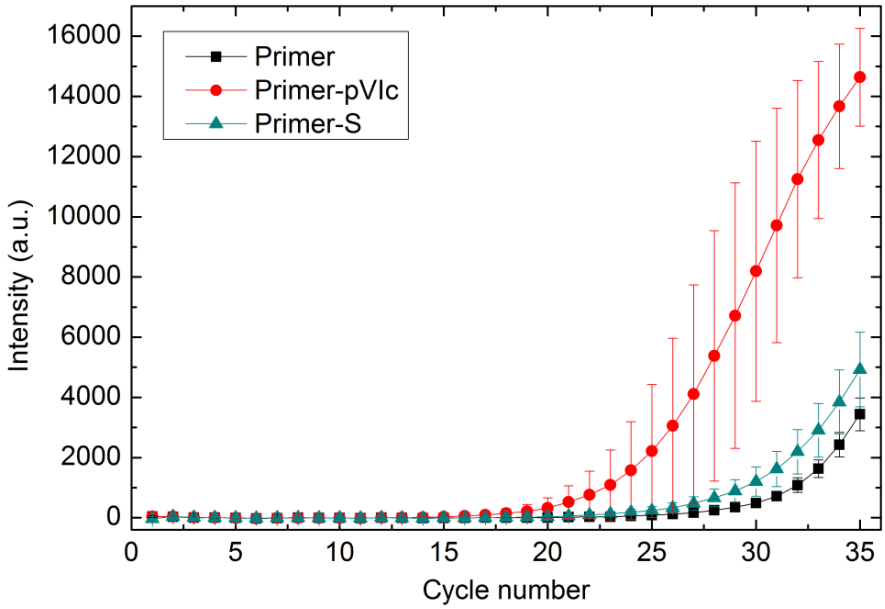


Supplementary Figure 3. qPCR control experiments. (a) Unmodified primers were used in qPCR with and without DNA template to detect if any unspecific DNA amplification occurred. A 20 µL reaction mixture contained pristine forward and backward primer (primer set I) at a concentration of 0.5 µM, 10 ng DNA template (circular dsDNA of 8669 bp length that originated from bacteriophage M13KO7), Sybr Green I (1×), Qiagen fast cycling PCR kit (1×) and Q-solution (1×). The following qPCR protocol was used: 98°C (5min); 45 cycles of [98°C (1min), 55.6^{o}C (15s), 68°C (30s)]; 68°C (4min), 4°C (hold). When the template was omitted, no amplicon generation was detected indicating that unspecific DNA amplification does not occur. **(b)** To confirm that PCR product formation depends on primer hybridisation on the template and that the annealing of modified and unmodified primers does not occur during the elongation step we performed the qPCR experiment without the annealing step during thermal cycling. The PCR conditions were selected as described above for panel **(a)** except for an altered thermal cycling protocol: 98°C (5min); 30 cycles of [98°C (1min), 68°C (30s)]; 68°C (4min), 4°C (hold). No amplicon formation is detected for any of the primers indicating that the annealing step within the thermal cycling protocol is critical for product formation.

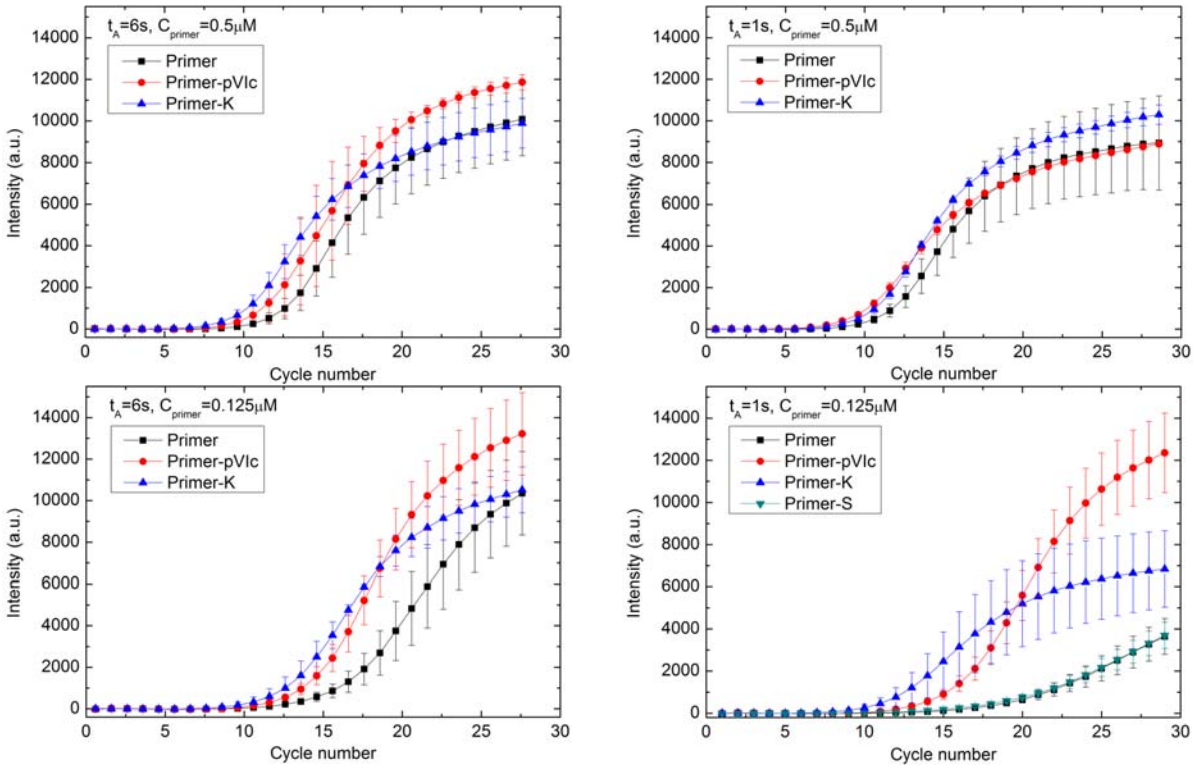


Lane M: DNA ladder; Lane 1-3: amplicon from unmodified primers; Lane 4-6: amplicon from primer-K; Lane 7-9: amplicon from primer-pVIc; Lane 10-12: amplicon from primer-S.

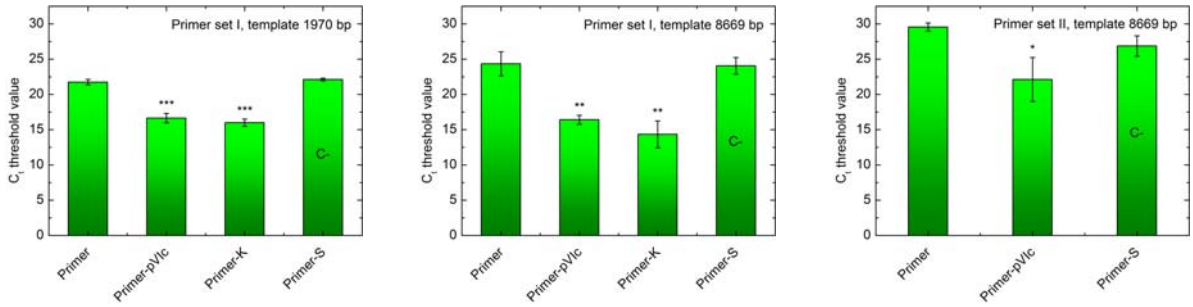
Supplementary Figure 4. Amplicon formation. The formation of the amplicon of the correct length was confirmed using a 2% agarose gel.



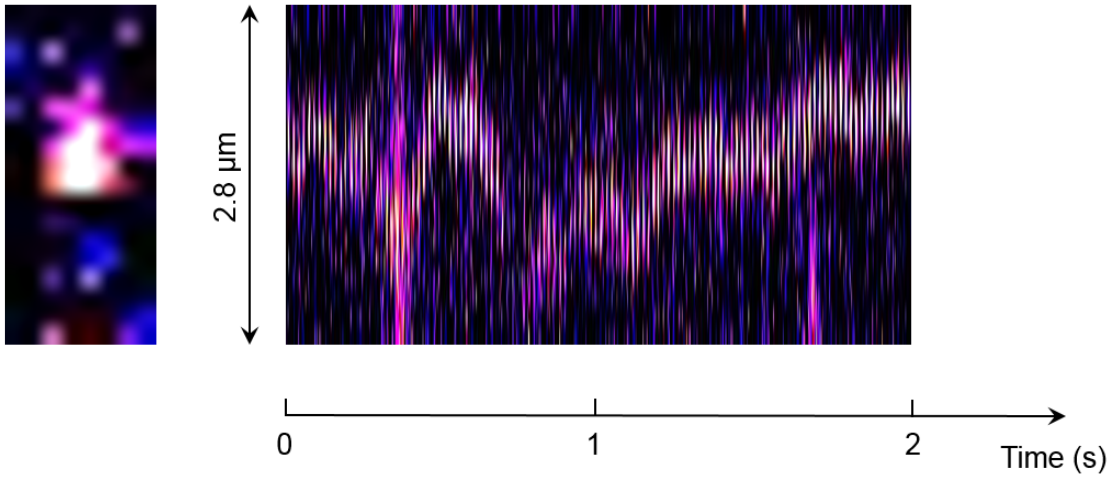
Supplementary Figure 5. qPCR using primer set II. To prove the general applicability of the sliding peptides to speed up PCR we conducted the qPCR experiments with a different set of primers (Primer set II, **Supplementary Table 1**). For qPCR conditions and the template see legend to **Supplementary Fig. 3**. Error bars represent standard deviation based on experiments in triplicate.



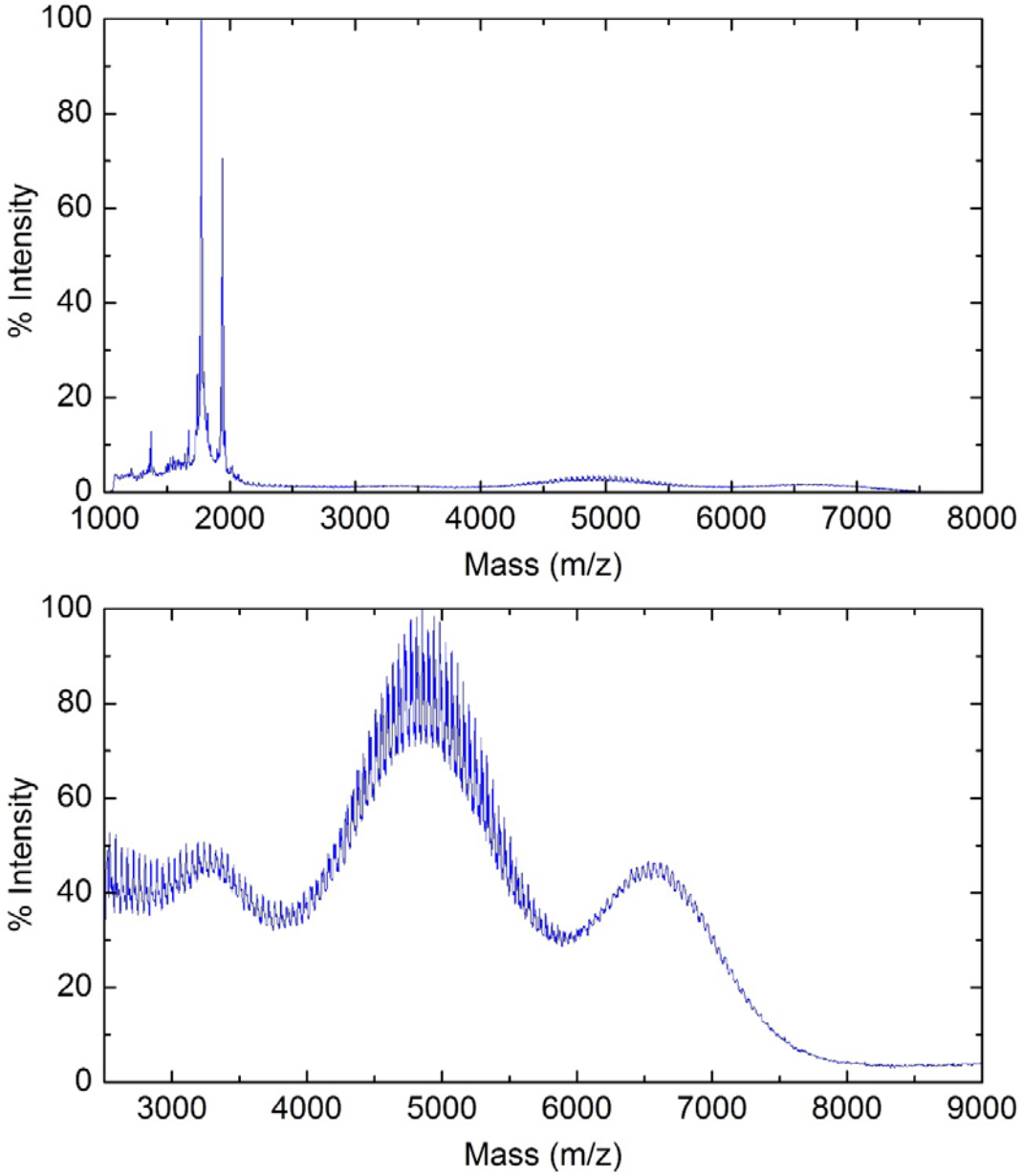
Supplementary Figure 6. Performance comparison of different primer-peptide modifications under different PCR conditions. The qPCR reactions for primer set I, template 8669 bp dsDNA M13KO7 were performed using different annealing times and primer concentrations. The annealing times in qPCR protocol (for protocol see legend to **Supplementary Fig. 3**) were 1 s and 6 s and two primer concentrations were 0.5 μM and 0.125 μM. Low primer concentrations and low annealing times (the most stringent conditions) gave the highest speed-up of PCR amplification compared to higher annealing times and primer concentration. Error bars represent standard deviation based on experiments in triplicate.



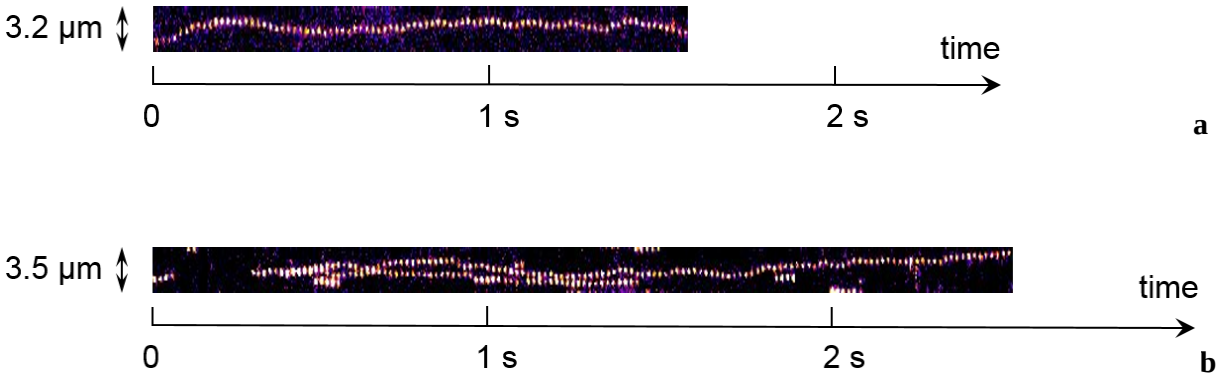
Supplementary Figure 7. Performance comparison of different primer-peptide conjugates in their ability to speed up the PCR. PCR accelerating effect of the sliding peptides was studied for two different pairs of primers and two different templates. Cycle threshold values Ct were extracted from qPCR traces. Statistical test for significance was unpaired t-test with unequal variance. ***$p<0.001$, **$p<0.01$, *$p<0.05$. Error bars represent standard deviation based on experiments in triplicate.



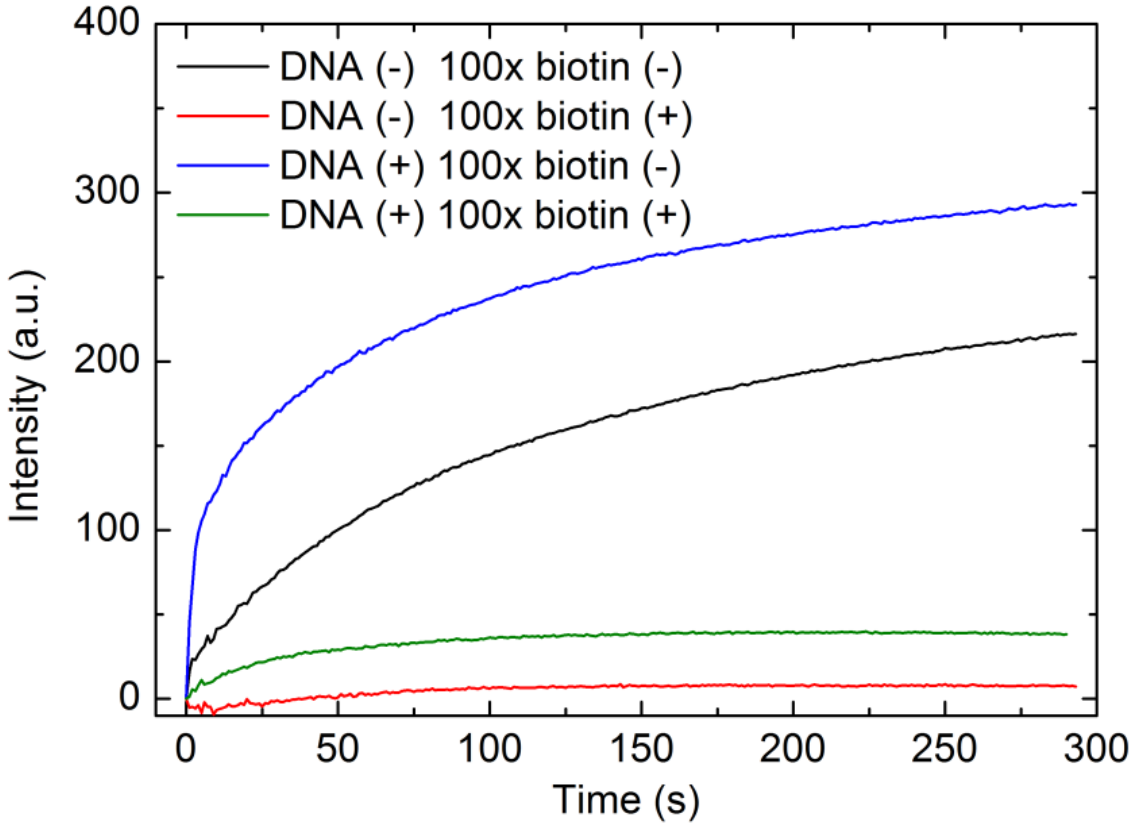
Supplementary Figure 8. Single-molecule experiments on the sliding of K-peptide. To confirm that peptide K (truncated pVIc) retained the ability to slide, single-molecule experiments were performed on this peptide carrying a fluorophore at the cysteine. The average diffusion coefficient $D_{K-peptide} = 22.12 \cdot 10^6 \, {bp^2}/{s}$, which corresponds to the values measured for pVIc (see main text for references).



Supplementary Figure 9. MALDI-TOF mass spectra of reactant *B*. Reactant *B* is a conjugate of biotin and Cy3 labelled pVIc. Both units are connected by a poly(ethylene glycol) (PEG) linker, which exhibits a degree of polymerization $D_P = 100$. Due to the polydisperse nature of the PEG linker a broad mass peak corresponding to reactant *B* is obtained. The calculated molecular weight (MW) of reactant *B* is 6700 g/mol, while the the MW determined by MALDI/TOF is 6650 g/mol. After conjugation reactant *B* was used without further purification because unconjugated Cy3-pVIc would not bind to streptavidin and the unconjugated biotin-PEG while still possessing the ability to bind to streptavidin, would not contribute to the FRET signal.



Supplementary Figure 10. Single-molecule experiments on reactant *S* and *B*. To confirm that pVIc renders reactants *S* and *B* able to slide along dsDNA we performed single-molecule experiments on flow-stretched λ-DNA using a Total Internal Reflection Fluorescence (TIRF) microscope set-up. **(a)** single-molecule trace showing rapid movement of reactant *S* on 1D dsDNA. **(b)** Prior to single-molecule experiments on reactant *B* we first pre-incubated it with Cy5 labelled streptavidin to be able to use a 641 nm (Cy5 excitation) laser instead of a 532 nm (Cy3 excitation) one, which drastically improved the signal-to-noise ratio and allowed us to detect clearly discernable sliding along DNA.



Supplementary Figure 11. Negative controls for the biotin-streptavidin experiment. To confirm that the rising FRET signal originates from the association between reactant *S* and *B* we performed the control experiments for which we pre-incubated reactant *S* with a 100x molar excess of biotin to occupy all binding pockets of streptavidin and prevent reactant *B* from binding to them. The results for two cases are given: without DNA (black and red curves) and with 10 pM dsDNA of 2686 bp long (blue and green curves). A non-zero FRET signal in case of a 100x biotin excess might be explained by non-specific interactions between reactant *S* and *B*.

Supplementary Table 1. Primer sets used in PCR experiments.

Primer set I	Forward:	5'-(NH_2)-CTC ATC GAG CAT CAA-3'	807 bp amplicon
	Reverse:	5'-(NH_2)-ATG AGC CAT ATT CAA-3'	
Primer set II	Forward:	5'-(NH_2)-GCG TTT CCT CGG TTT-3'	831 bp amplicon
	Reverse:	5'-(NH_2)-GTA ATT TAG GCA GAG G-3'	

Supplementary Table 2. Double stranded DNA sequences used in the proof-of-principle reaction studies.

DNA length	DNA sequence
15 bp	5'-GCC TCG CCG CGC CCC-3' (Custom DNA synthesis by Integrated DNA technologies)
50 bp	5'-AGA CAG CAT CGG AAC GAG GGT AGC AAC GGC AAC AGA GGC TTT GAG GAC TA-3' (Custom DNA synthesis by Integrated DNA technologies)
100 bp	5'-ATG CGC CTG GTC TGT ACA CCG TTC ATC TGT CCT CTT TCA AAG TTG GTC AGT TCG GTT CCC TTA TGA TTG ACC GTC TGC GCC TCG TTC CGG CTA AGT AAC A-3' (Custom DNA synthesis by Integrated DNA technologies)
150 bp	5'-TTG AAA AAG CCG TTT CTG TAA TGA AGG AGA AAA CTC ACC GAG GCA GTT CCA TAG GAT GGC AAG ATC CTG GTA TCG GTC TGC GAT TCC GAC TCG TCC AAC ATC AAT ACA ACC TAT TAA TTT CCC CTC GTC AAA AAT AAG GTT ATC AAG TGA-3' (Initially amplified from a kanamycin resistance gene (F primer: 5'-TTG AAA AAG CCG TTT CTG TAA TGA AGG AGA AAA CTC-3', R-primer: 5'-TCA CTT GAT AAC CTT ATT TTT GAC GAG GGG AAA TTA-3') Subsequently amplified using 150mer itself as a template. Purified by ethanol precipitation)
300 bp	5'-AGT GCG ATT AAG CCA GAC ATG AAG ATC AAA CTC CGT ATG GAA GGC AAC GTA AAC GGG CAC CAC TTT GTG ATC GAC GGA GAT GGT ACA GGC AAG CCT TTT GAG GGA AAA CAG AGT ATG GAT CTT GAA GTC AAA GAG GGC GGA CCT CTG CCT TTT GCC TTT GAT ATC CTG ACC ACT GCA TTC CAT TAC GGC AAC AGG GTA TTC GCC AAA TAT CCA GAC AAC ATA CAA GAC TAT TTT AAG CAG TCG TTT CCT AAG GGG TAT TCG TGG GAA CGA AGC TTG ACT TTC GAA GAC GGG GGC ATT TGC-3' (Initially amplified from pNZ-mEos3.2 plasmid (F primer: 5'- AGT GCG ATT AAG CCA GAC ATG AAG ATC AAA CTC CGT-3', R primer: 5'- GCA AAT GCC CCC GTC TTC GAA AGT CAA GCT TCG T-3') Subsequently amplified using 300mer itself as a template. Purified by ethanol precipitation)

934 bp	5’-CGC GAG CAT AAT AAA CGG CTC TGA TTA AAT TCT GAA GTT TGT TAG ATA CAA TGA TTT CGT TCG AAG GAA CTA CAA AAT AAA TTA TAA GGA GGC ACT CAC CAT GGG AAG TGC GAT TAA GCC AGA CAT GAA GAT CAA ACT CCG TAT GGA AGG CAA CGT AAA CGG GCA CCA CTT TGT GAT CGA CGG AGA TGG TAC AGG CAA GCC TTT GAG GGA AAA CAG AGT ATG GAT CTT GAA GTC AAA GAG GGC GGA CCT CTG CCT TTT GCC TTT GAT ATC CTG ACC ACT GCA TTC CAT TAC GGC AAC AGG GTA TTC GCC AAA TAT CCA GAC AAC ATA CAA GAC TAT TTT AAG CAG TCG TTT CCT AAG GGG TAT TCG TGG GAA CGA AGC TTG ACT TTC GAA GAC GGG GGC ATT TGC AAC GCC AGA AAC GAC ATA ACA ATG GAA GGG GAC ACT TTC TAT AAT AAA GTT CGA TTT TAT GGT ACC AAC TTT CCC GCC AAT GGT CCA GTT ATG CAG AAG AAG ACG CTG AAA TGG GAG CCC TCC ACT GAG AAA ATG TAT GTG CGT GAT GGA GTG CTG ACG GGT GAT ATT GAG ATG GCT TTG TTG CTT GAA GGA AAT GCC CAT TAC CGA TGT GAC TTC AGA ACT ACT TAC AAA GCT AAG GAG AAG GGT GTC AAG TTA CCA GGC GCC CAC TTT GTG GAC CAC TGC ATT GAG ATT TTA AGC CAT GAC AAA GAT TAC AAC AAG GTT AAG CTG TAT GAG CAT GCT GTT GCT CAT TCT GGA TTG CCT GAC AAT GCC AGA CGA TAA CTG CAG GCA TGC GGT ACC ACT AGT TCT AGA GAG CTC AAG CTT TCT TTG AAC CAA AAT TAG AAA ACC AAG GCT TGA AAC GTT CAA TTG AAA TGG CAA TTA AAC AAA TTA CAG CAC GTG TTG CTT TGA TTG ATA GCC AAA AAG CAG CAG TTG-3’ (Initially amplified from pNZ-mEos3.2 plasmid (F primer: 5’-CGC GAG CAT AAT AAA CGG CTC TG-3’, R primer: 5’-CAA CTG CTG CTT TTT GGC TAT C-3’) Subsequently amplified using 934mer itself as a template. Purified by ethanol precipitation)
2686 bp	pUC19 vector (N3041L, New England Biolabs)