Netherlands Organisation for Scientific Research

Proposal

Speeding up biomolecular processes by a molecular sled

Groningen, 2014

1. Title of research proposal:	
Speeding up biomolecular processes by a molecular sled	
2. Summary of research proposal	
The short peptide of adenovirus that is called pVIc has the remarkable ability to slide over DNA. Here, I exploit this property to target the catalytic chemotherapeutic agents to DNA and improve drug action. Secondly, I employ the molecular sled functionalized with fluorescent dyes as novel, non-toxic and non-covalent probe and staining reagent for nucleic acids.	
3. Main applicant * This person will also be the contact person for the project	
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4. Supervisors (group leaders)	
Name (Title(s), first name, surname):	Prof. Dr. Andreas Herrmann
Organisation (institution, department, group):	Polymer Chemistry and Bioengineering group, Zernike Institute for Advanced Materials, University of Groningen

Description of challenge

DNA-protein interactions play a fundamental role in many biological processes, such as transcription, DNA repair, replication and recombination. For decades, it has been known that instead of relying only on three-dimensional diffusion to associate with a target on DNA, many of these proteins reduce the dimensionality of this search process to speed up recognition [1]. Every time the protein associates with the DNA, it transiently diffuses along the DNA in a one-dimensional fashion and thus drastically increases the number of sampled DNA positions per unit time (Figure 1). It then dissociates from the DNA, diffuses through solution to rebind at an entirely different region, and again searches a stretch by one-dimensional diffusion. This combination of three- and one-dimensional diffusion gives rise to a drastic increase in the effective bimolecular association rate constant that describes the association kinetics of the protein with its target.

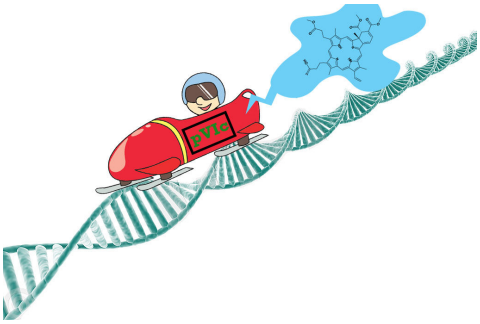

Fig. 1: *pVIc sliding alond DNA chain.*

A notable example of the process described above is the selective interaction of oligopeptide pVIc with DNA and the subsequent transport of the peptide along the DNA chain [2]. Due to its small size (only 11 amino acids), pVIc can be conveniently synthetically modified, making it an ideal candidate to transfer the features of a molecular sled to other biochemical and biomedical problems. In this proposal, I investigate a new method of improving drugs that act on DNA and I develop a non-toxic DNA labelling method using pVIc as fluorescent reporter.

One of the fields that require highly selective delivery of drugs to DNA is anticancer photodynamic therapy (PDT) [3]. The mechanism of action of PDT relies on the production of radicals, which can attack any part of the cell, but DNA cleavage plays the central role in this process. Modification of drugs used for PDT with pVIc should increase the efficiency of DNA cleavage because drugs will be targeted to and move along the DNA chain (Figure 2). The same holds true for anticancer drugs that intercalate. Drugs bound to pVIc should show enhanced catalytic action on DNA, which might lead to an improved therapeutic effect. I present an elegant way to turn a protein function that is threatening human health during viral infection into a therapeutic tool for cancer therapy.

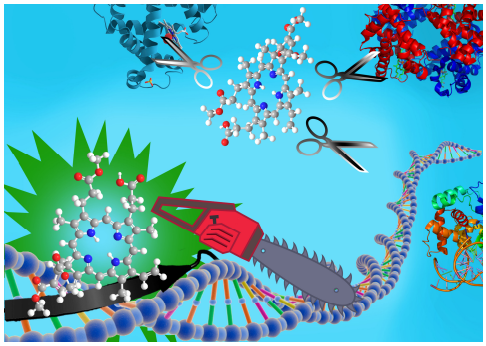

Fig. 2: *Difference in the behaviour of free and pVIc bound drug molecules.*

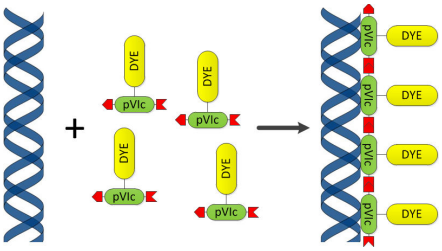

Fig. 3: *Scheme of formation polymer-like chain from dye-modified pVIc molecules.*

Another big challenge is the non-covalent modification of DNA with dyes [5]. Using chromophores modified with pVIc for DNA detection and labelling would have many advantages in comparison with other widely used methods, such as radioactive labelling and labelling by intercalating fluorescent dyes. First of all, non-disruptive labelling should be non-toxic and appears very promising for *in vivo* cellular imaging of DNA and chromatin structures. Secondly, using this method, I could detect double-stranded DNA on DNA chips, which are widely used in diagnostic tests. High-density labelling can be achieved by synthesizing a polymer-like chain from dye-modified pVIc monomers with end groups that react to form a large macromolecule as shown in Figure 3.

[1] A. D. Riggs, S. Bourgeois, M. Cohn, *J. Mol. Biol.* **1970**, 53, 401.
[2] W. F. Mangel, M. L. Baniecki, W. J. McGrath, *Cell. Mol. Life Sci.* **2003**, 60, 2347.
[3] Y. Pommier, E. Leo, H. Zhang, C. Marchand, *Chem. Biol.* **2010**, 17, 421.
[4] P. B. Alper, M. Hendrix, P. Sears, C.-H. Wong, *J. Am. Chem. Soc.* **1998**, 120, 1965.
[5] G. Patonay, J. Salon, J. Sowell, L. Strekowski, *Molecules* **2004**, 9, 40.

Proposed solution for overcoming the described challenge

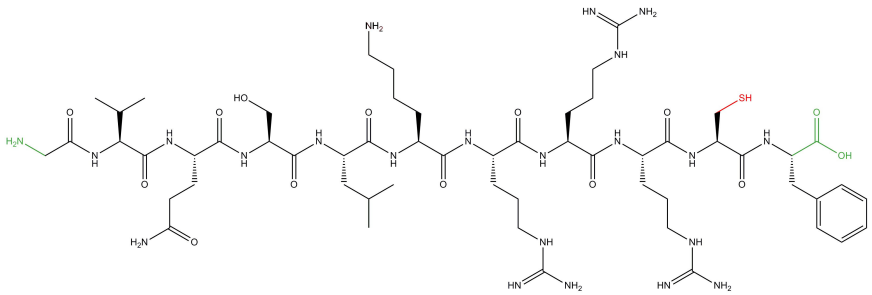

Fig. 4: *Structure of oligopeptide pVIc. Group which can be easily modified are shown in red, end groups - in green.*

Anti-cancer drug-pVIc conjugates

The oligopeptide pVIc can be chemically modified easily without a loss of ability to slide along DNA. In the first part of my project, I plan to modify pVIc with anticancer drugs. I will investigate Porphyrin derivatives (Verteporfin, Photofrin, Temoporfin, Talaporfin, etc.) as a representative of the drugs used for photodynamic therapy (PDT) [1] and Bleomycin as a representative of an intercalating anticancer agent [2] (Figure 5).

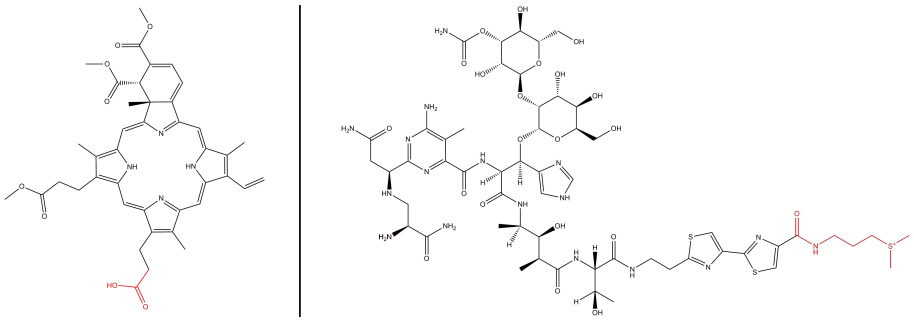

Fig. 5: *Verteporfin (left) and Bleomycin (right) structures. Groups which can be easily modified are shown in red.*

The presence of reactive functional groups in the chemical structure of these drugs gives us the opportunity for regioselective high-yield modification with pVIc (Figure 6). High-performance liquid chromatography (HPLC), nuclear magnetic resonance spectroscopy (NMR), and mass spectrometry will be used for conjugate purification and characterization. To measure the ability of the pIVc-modified drugs to damage DNA more efficiently than the unmodified ones I will employ gel electrophoresis. As DNA substrate for the conjugates I will utilize supercoiled plasmid DNA that shows a different electrophoretic mobility than DNA containing a nick or a double strand break, which are the resulting products of drug action [3]. From these experiments, I can draw conclusions about quantitative and qualitative changes in the behaviour of DNA damage of oligopeptide-modified drugs.

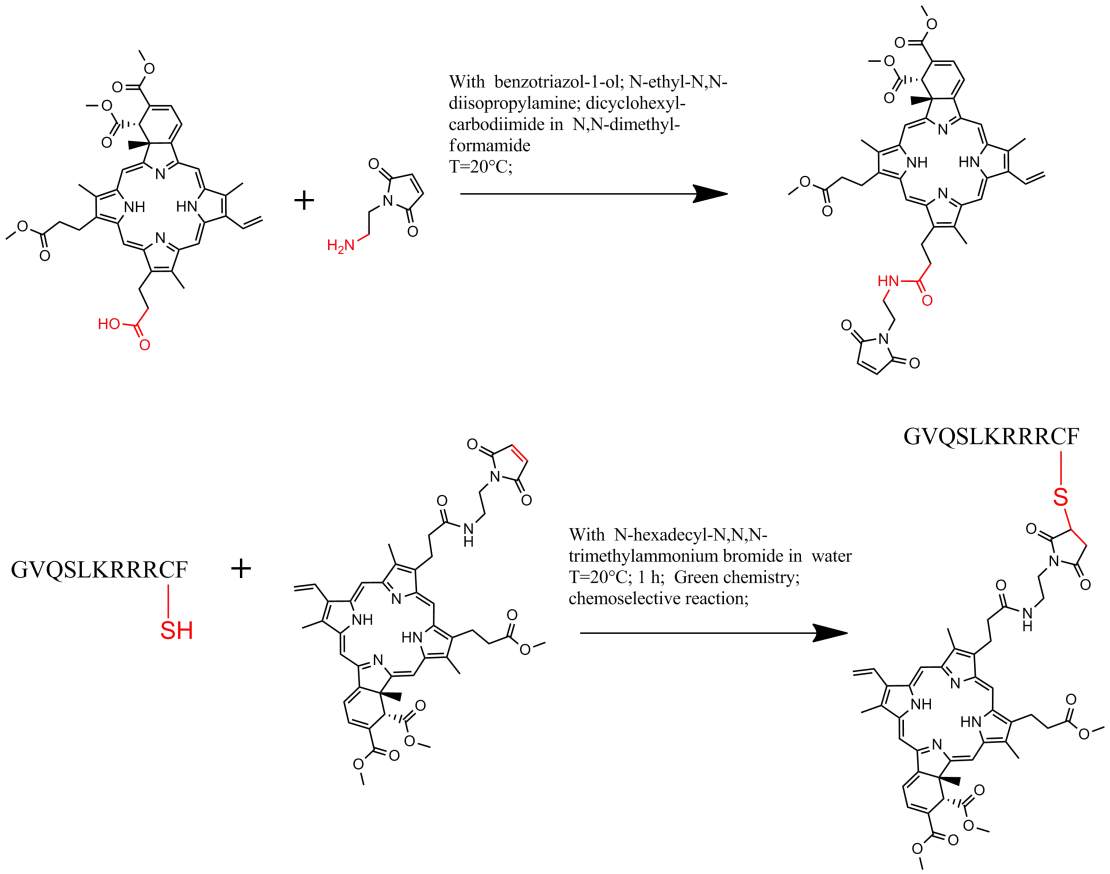

Fig. 6: *Modification of Verteporfin with N-(2-Aminoethyl)maleimide and pVIc.*

Labelling DNA with pVIc

For selective fluorescent labelling and imaging of the DNA chain, I will use pVIc modified with dyes (ATTO and Alexa Fluor dyes, cyanine, rhodamine and fluorescein derivatives) (Figure 7). These dyes exhibit high absorption coefficients, high fluorescent quantum yields, high photochemical stabilities [4] and can be easily conjugated to the oligopeptide. The procedures described above for drug modification will also be used for purification and confirmation of peptide-dye conjugates. For the generation of a highly fluorescent staining reagent, I will create a non-covalently bound polymer-like chain along the DNA double helix. For that purpose the well-known click chemistry will be employed [5]. I will introduce azide group to the N- and triple bound to the C-terminus of pVIc while an internal cysteine residue will be used

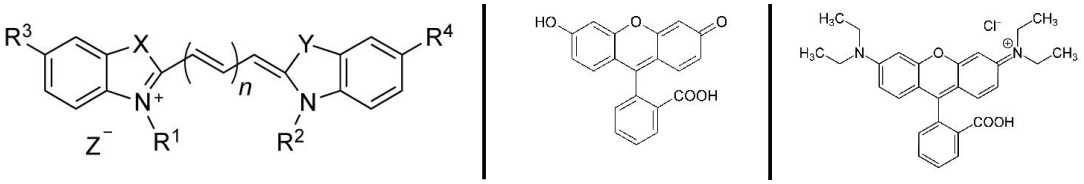

Fig. 7: *Cyanine derivatives (left), Rhodamine (centre) and Fluorescein (right) structures.*

as anchoring point for the fluorescent dyes. Polymerization of this monomer along DNA will be achieved by adding metal copper and copper salt (Figure 8). For investigation of the DNA samples, I will use absorption and emission spectroscopy as well as total-internal-reflection fluorescence microscopy (TIRF) [6] to image the fluorescence signals originating from polymer-like chains of dye-modified oligopeptides arranged along the DNA double helix. Further investigations will be dedicated to measure the detection limit of double-stranded DNA on DNA chips.

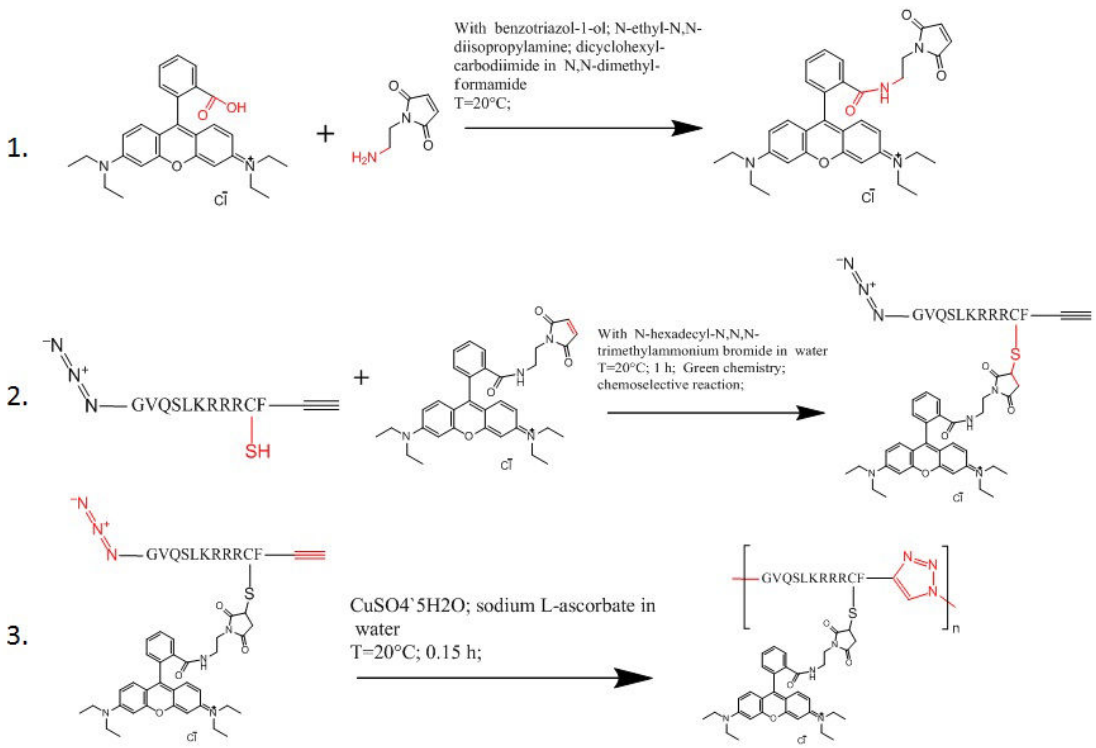

Fig. 8: *Modification of Rhodamine with N-(2-Aminoethyl)maleimide and pVIc. Click chemistry for formation polymer-like chain.*

[1] L. J. Scott, K. L. Goa, *Drugs & Aging* **2000**, 16, 139.
[2] C. A. Claussen, E. C. Long, *Chem. Rev.* **1999**, 99, 2797.
[3] Q. Li, W. R. Browne, G. Roelfes, *Inorg. Chem.* **2011**, 50, 8318.
[4] H. Zheng, X.-Q. Zhan, Q.-N. Bian, X.-J. Zhang, *Chem. Commun.* **2013**, 49, 429.
[5] M. Wilchek, E. A. Bayer, *Anal. Biochem.* **1988**, 171, 1.
[6] D. Axelrod, N. L. Thompson, T. P. Burghardt, *J. Microsc.* **1983**, 129, 19.

Research plan for project

Taking into account that the duration of this project is 4 years, I divided my research plan in four parts. The tasks for each period are indicated in the chart below.

	1 year	2 year	3 year	4 year
Synthesis of pVIc and modified pVIc for click chemistry				
Modification of pVIc with drugs and dyes				
Purification and confirmation of chemical structures of conjugates				
Fabrication of DNA chips				
Measuring of DNA cleavage of drugs modified with pVIc				
Studying the application of dye-modified pVIc for DNA imaging				
Processing and writing down the obtained results				

For this project, I will purchase peptide synthesizer (CS Bio Company Inc.), oligopeptide pVIc (GeneCust Europe), phosphoramidites and modificators for DNA (Glen Research), DNA samples (New England Biolabs), amino acids (Sigma-Aldrich Co., GeneCust Europe), substrates for DNA chips (AMS Biotechnology (Europe) Ltd.), dyes (rhodamine and fluorescein - Sigma-Aldrich Co., ATTO dyes - ATTO-TEC, Alexa Fluor dyes - Life Technologies), drugs (Selleck Chemicals and Medchemexpress). The remaining part of the budget will be used to buy peptide synthesizer, chemical reagents, solvents, and other consumables.
All chemical modifications, purifications and gel electrophoresis will be done in the laboratory of Prof. Dr. Andreas Herrmann (Polymer Chemistry and Bioengineering group, Zernike Institute for Advanced Materials, University of Groningen). Mass spectrometry will be carried out in the Interfaculty Mass Spectrometry Center (University of Groningen, University Medical Center Groningen). NMR, TIRF and absorption and emission spectroscopy will be conducted in the group of Prof. Dr. Antoine M. van Oijen (Single-Molecule Biophysics group, Zernike Institute for Advanced Materials, University of Groningen).

Proposed budget	
Salaries	€ 109200
Peptide synthesizer	€ 50000
Chemical reagents and solvents	€ 60000
Conferences and travels	€ 5000
Other Costs	€ 25800
Total budget	€ 250000
Main hosting university	University of Groningen

Declaration and signature

☒ By submitting this proposal form, I declare that I have completed this form truthfully and completely.

Date: 31.07.2014

Name: Konstantin Balinin

Signature: