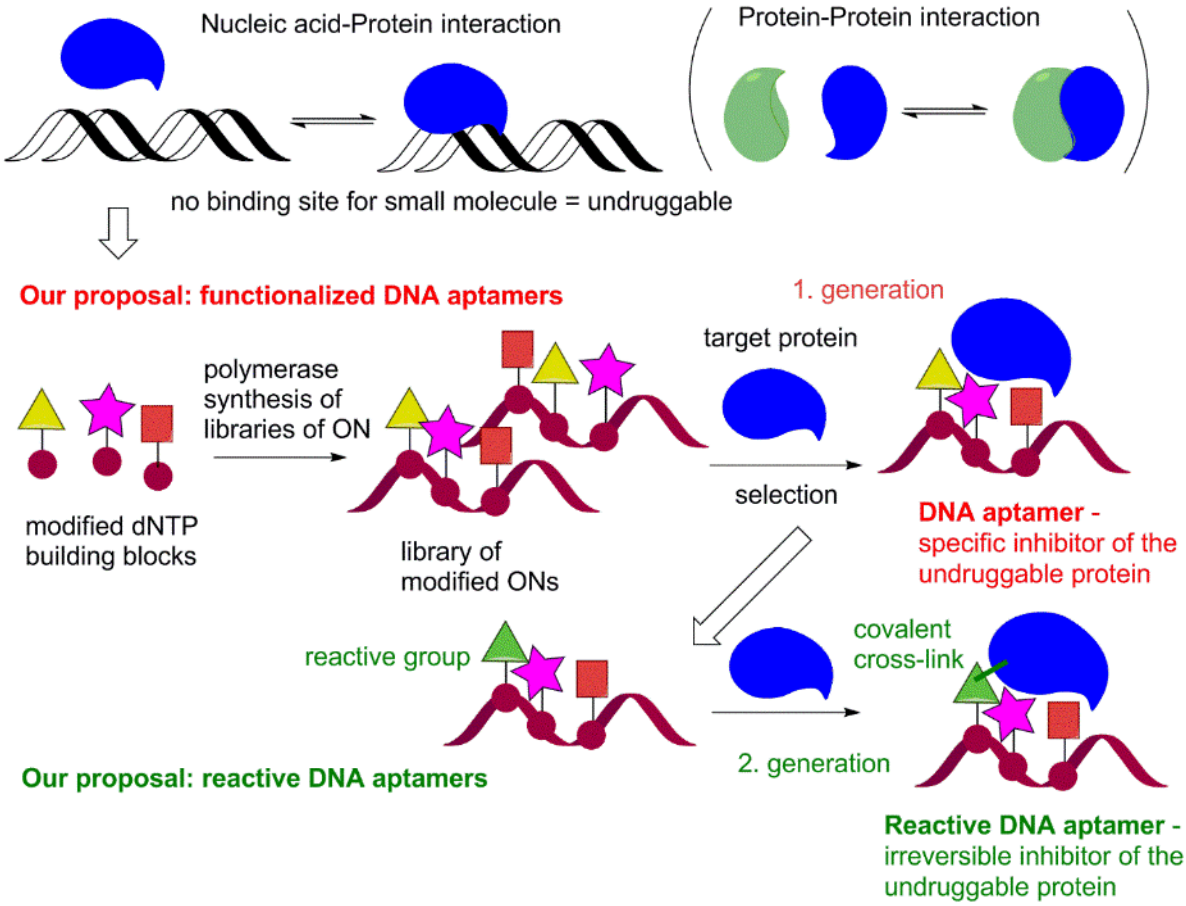
WP3.1. Development of aptamers against oncogenic factors (Hocek)

Research aims

- Design and synthesis of novel modified (d)NTPs bearing diverse functional groups
- Systematic study of polymerase incorporation of the modified nucleotides to DNA or RNA
- Systematic study of incorporation amino-acid specific as well as non-specific reactive groups to DNA or RNA and study of covalent cross-linking with DNA- or RNA-binding proteins
- SELEX-based selection of modified aptamers against target proteins
- Design and synthesis of inherently reactive aptamers against selected proteins (oncogenic factors or viral proteins)
- Testing of cytostatic or antiviral activity of selected aptamers

Research plan and methodology

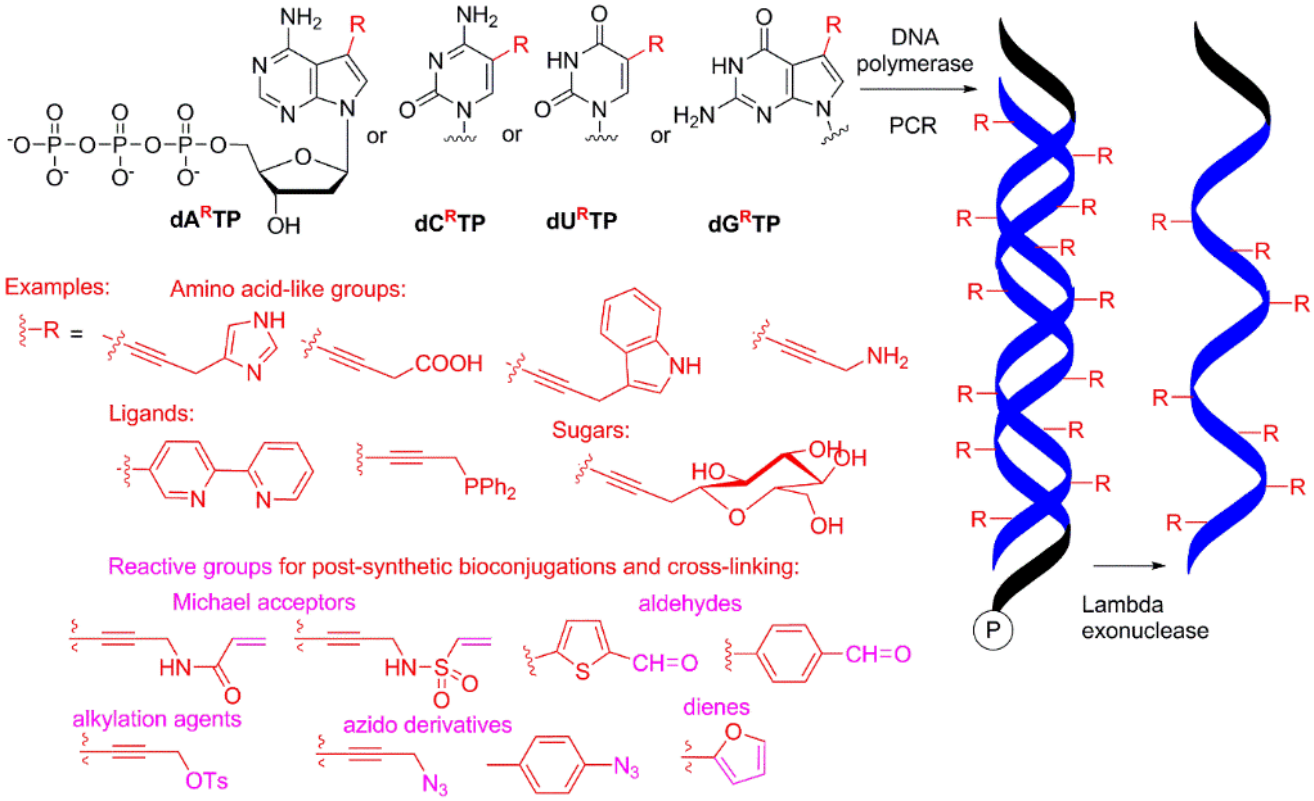
We propose to build on our unique expertise in the design and synthesis of modified dNTP building blocks and in their polymerase incorporation to DNA, in the selection of modified DNA aptamers and, later on, reactive covalent aptamers for targeting of undruggable proteins, in particular proteins involved in protein-DNA or protein-RNA interactions relevant to cancer or viral diseases. However, the aptamer selection and modification approach should be general enough to enable possible applications in selection of aptamers disrupting protein-protein interactions and thus many cross-interactions and collaborations with RP1 can be envisaged.

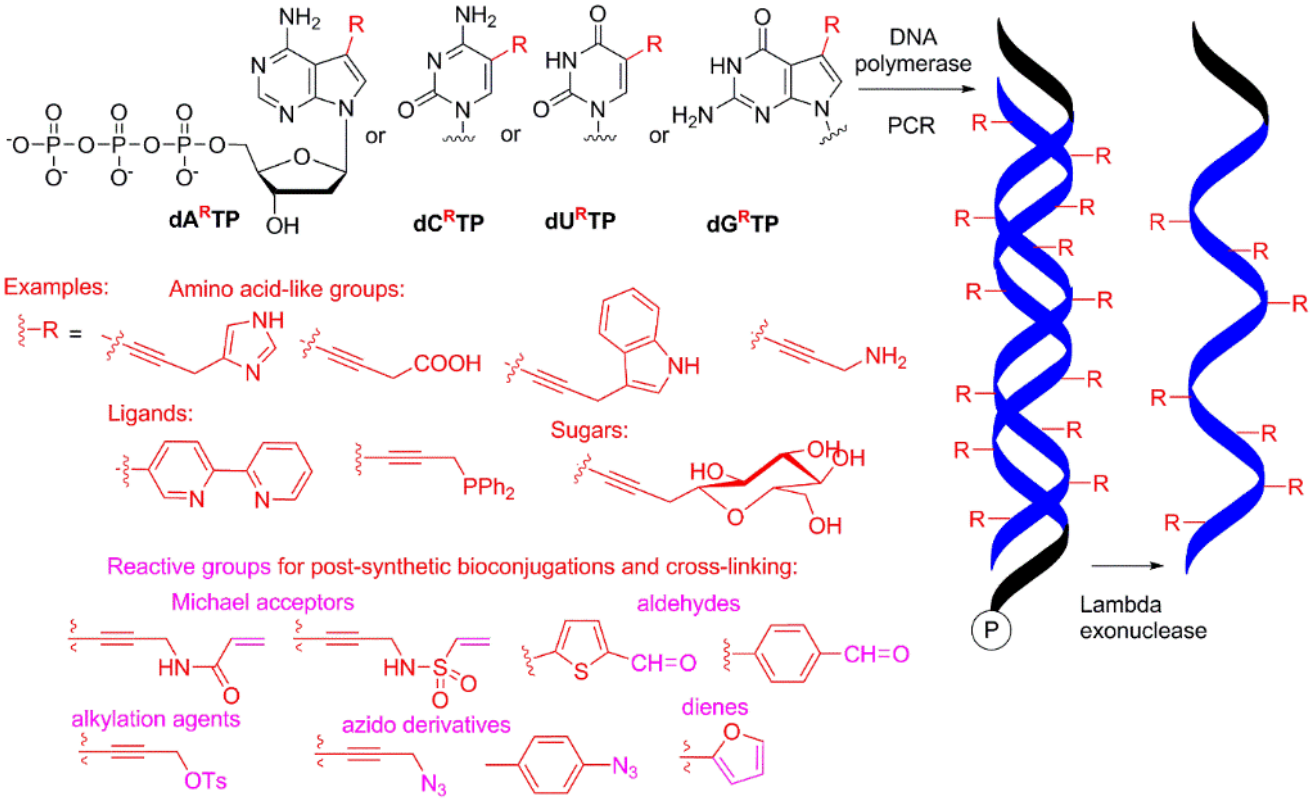


We plan to select several biologically and clinically relevant undruggable proteins (see below) and use them in the selection of modified aptamers. We will heavily collaborate with the group of Dr. Ed Curtis, who has extensive experience in SELEX from his stay at Harvard in the D. R. Liu lab. We also started a collaboration with Prof. Claudia Höbartner from Göttingen, who is an expert in selections and

aptamers who agreed to help us with the methodology of generation of DNA libraries and selections. We plan several research stays of our PhD students in her lab.

We will start with the design of novel modified (d)NTPs bearing diverse functional groups for possible interactions with proteins. We already have a library of >100 different modified dNTPs bearing diverse substituents of different sizes, functional groups (CH=O, NH_2, COOH, N_3, NO_2 etc.) and we will design and synthetize many others bearing other functional groups (including e.g. side chains of amino acids, P- or N-ligands for metal cations, peptides, sacharides etc.). With the modified (d)NTPs in hands, we will systematically test their substrate activity with DNA or RNA polymerases and optimize their polymerase incorporations (by PEX, NEAR, PCR etc.) in order to generate libraries of modified DNA or RNA sequences for the selection experiments.

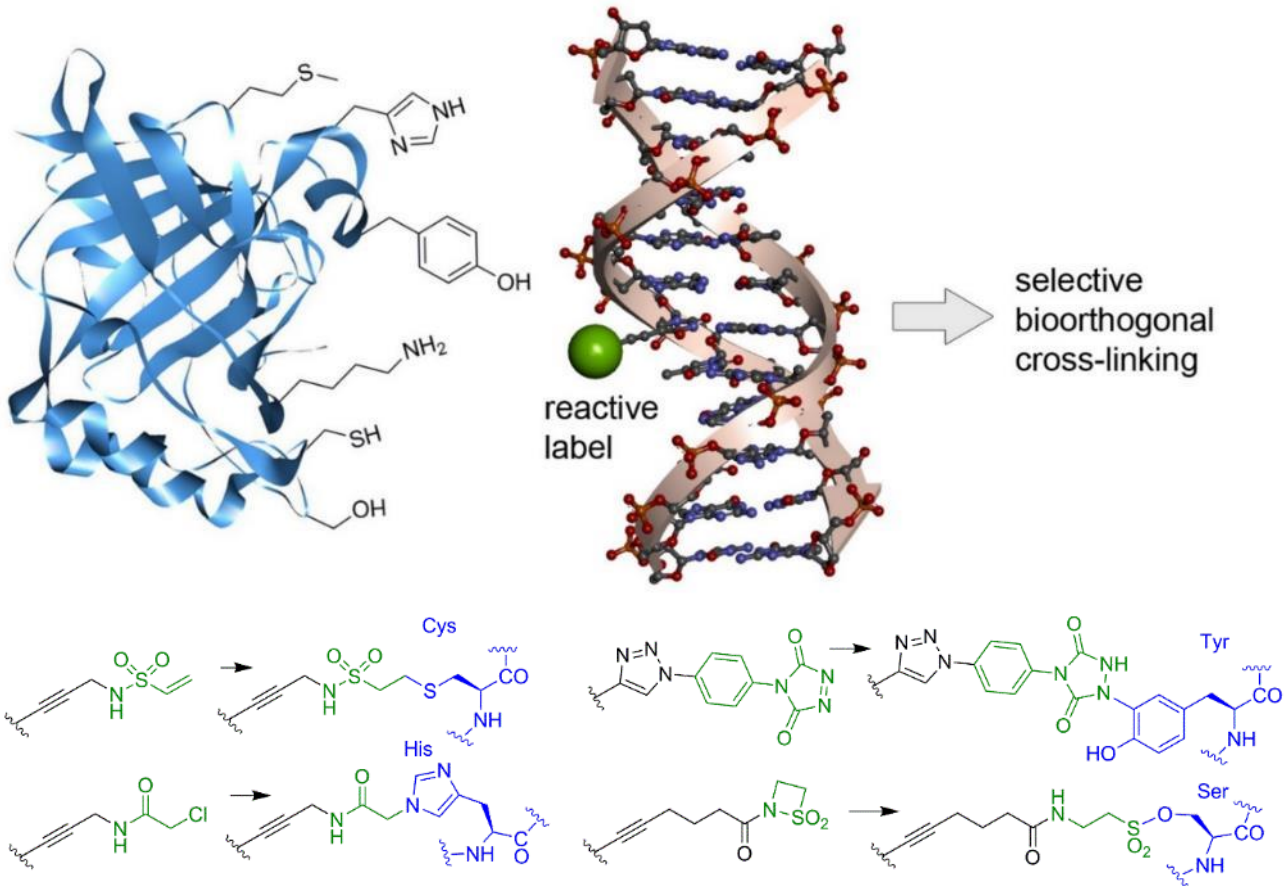




In particular, we will design and prepare (d)NTPs containing chemically reactive groups capable of specific or non-specific cross-linking with amino acid side-chains of proteins. Recently, we published vinylsulfonamide-linked dNTPs and DNA and their cross-linking with Cys-containing peptides and proteins. Within this project, we plan to explore alkylation agents (for cross-linking with His or Cys), diazodicarboxamides or masked diazonium groups (for cross-linking with Tyr), sultams (for cross-linking with Ser or Cys), as well as general reactive groups, i.e. benzophenone or diazirine (for photo-cross-linking).

We will utilize in vitro SELEX approach using proteins containing suitable tags (e.g. His-tag or biotin). Starting from commercial DNA libraries, we will perform PCR using one or several base-modified dNTPs and one 5'-phosphorylated primer. The resulting library of dsDNA PCR products will be treated by Lambda Exonuclease (which specifically digests 5'-phosphorylated DNA strand) to generate a library of base-modified single-stranded ONs. This library will undergo the selection with the target tagged-protein which will be captured after incubation using the affinity chromatography specific to the tag. After washing out the non-bounds DNA, the bound oligonucleotides will be released by denaturation and will undergo sequencing [a new Next Generation Sequencing instrument MinoSeq will be purchased for this purpose] and bioinformatic analysis. The pool of protein-binding ONs will undergoes another round of PCR and selection to further optimize and focus the library of active mutants.

Once we identify specific and efficient aptamers which would bind the target protein, we will re-synthetize the selected aptamers in larger quantities and their binding afinities to the target proteins will be confirmed and quantified (including K_d determination by isotermal titration calorimetry or other relevant method). The selected aptamers will be also tested for their *in vitro* cytostatic and antiviral activity.



Moreover, in collaboration with the Structural Biology Group (Veverka, Řezáčová), we will try to solve the NMR and/or X-ray structure to gain more information for further design. An important part of the characterization of aptamers and their covalent conjugates with proteins will be mass spectrometry analysis in collaboration with J. Cvačka. We will use the newly purchased Orbitrap instrument and try to develop MS methods for analysis of the whole DNA-protein conjugates, as well as the products of the digestion of DNA (nucleotide-linked proteins) and products of digestion of protein (peptide-linked DNA).

Then we will select the most potent and specific aptamers and will further modify them by the incorporation of one or more reactive groups (either specific or non-specific). They will be then used for the study of covalent cross-linking to the target proteins and/or their irreversible inhibition. The successful covalent aptamers will also be tested for in vitro cytostatic or antiviral activity.

Selection of targeted oncogenic factors

The selection of proteins will be based on the possible therapeutic potential and needs unmet by classical approaches. An obvious choice will be oncogenic transcription factors – in collaboration with Dr. Vojtěšek (MOU Brno), we will try to develop inhibitors of oncogenic factors including Myc, FOXM1, NFκB, Jun, delta Np73, HSF1, IRF1 or Fos proteins, which are all involved in oncogenesis or in the resistance of cancer cells. Inhibitors of these factors may clearly have potential in cancer therapy. We will express the factors containing His-tag, use them for selection of aptamers and the best candidates will be then re-synthetized and tested for antitumor activity (newly purchased semipreparative HPLC and flash chormatography systemas will be indispensable for isolation and purification of these modified oligonucleotides).

We aim at identification and synthesis of at least several antitumor aptamers.

In the newly identified biologically active modified oligonucleotide aptamers we will study their hydrolytical stability in serum and cell extracts. Since they will contain chemical modifications, it is possible that they will be sufficiently stable toward hydrolysis. However, in cases when the hydrolytical stability will be limited, we will design and synthesize stable ONs with modified backbone

(phosphorothioates, boranophosphates) or with modified sugar (LNA), which are known to be more stable toward hydrolysis. Also, we will study their biodictribution and transport through the cell membranes and we will explore the established approaches for their transport using liposomes, cell-penetrating peptides or lipophilic conjugates.

This approach is in principle very general and versatile and can be adopted for any other protein of choice in collaboration with other biological groups participating in the project. It will be also used in the development of anti-HBV aptamers - see WP3.2. below.

Research schedule

2018-2022

- Design and synthesis of novel modified (d)NTPs bearing diverse functional groups
- Systematic study of polymerase incorporation of the modified nucleotides to DNA or RNA
- SELEX-based selection of modified aptamers against target proteins

2019-2022

- Systematic study of incorporation amino-acid specific as well as non-specific reactive groups to DNA or RNA and study of covalent cross-linking with DNA- or RNA-binding proteins
- Design and synthesis of inherently reactive aptamers against selected proteins
- Testing of cytostatic or antiviral activity of selected aptamers

Publications and patents

Publications (Jimp)

	Jimp
2017	1
2018	7
2019	7
2020	7
2021	7
2022	6
Total	35

Angewandte Chemie International Edition
Nucleic Acids Research
Chemistry a European Journal
Journal of Medicinal Chemistry
ACS Chemical Biology
Bioconjugate Chemistry
Bioorganic and Medicinal Chemistry
Organic and Biomolecular Chemistry
Journal of Organic Chemistry
ChemBioChem

Patents and patent applications

	Patents (granted)	International patent applications (filed)
2017	0	0
2018	0	0
2019	0	1
2020	0	1
2021	0	1
2022	1	0
Total	1	3

We expect IP protection in the following areas:
New modified aptamers against TFs.
Irreversible covalent antitumor aptamers.

Cooperation with foreign institutions

- University of Göttingen, selection and development of aptamers
- Gilead Sciences, development of antitumor aptamers