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Structural aspects of quadruplexes as potential therapeutic targets

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Structural investigations into quadruplexes, formed from guanine rich sequences found in the human chromosome, and development of small molecule ligands that can stabilize these structures have been the main focus of our research. The targeting of the telomere, a G-rich hexanucleotide repeat sequence TAGGGT, found at the end of all mammalian chromosomes has lead to the development of a series of ligands that can effectively inhibit the enzyme telomerase.
Critically a 3' single stranded overhang, located at the end of the chromosome, is the substrate for the ribonucleoprotein telomerase. Folding of these telomeric repeats into higher order DNA structures inhibits access of telomerase and prevents telomere maintenance. Additionally, the binding of other associated proteins that form part of the telosome, are also disrupted leading rapidly to chromosomal damage, such as chromosomal fusions and apoptosis. We will discuss our research into the folding topologies that are available to these telomeric sequences and other target sequence found in the chromosome, and the design of selective ligands that target these novel topologies. This is of particular importance with the identification of quanine rich sequences that have been shown to form stable quadruplex structures within promotor regions of onogenes. The structural diversity of the quadruplex structures formed from these G-rich DNA sequences will also be reviewed.

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A comparison of palindrome and pseudopalindrome cutters

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Type II restriction endonucleases are very diverse in sequence, yet nearly all of them share the fold and the metal-dependent catalytic mechanism. Most are active as dimers that match the two-fold symmetry of their recognition sequences. Although thousands of restrictases have been described, fewer than 20 crystal structures are available. To date, mostly "palindrome-cutters" that recognize *true* palindromes and generate overhangs with an *even* number of bases have been studied in detail. Based on these data, a few general rules have been deduced: (a) "phenotype" predicts "genotype", (b) changes in the recognition sequence, which do not affect the cleavage pattern, require mutations, but no alterations in quaternary structure, (c) changes in the cleavage pattern require radically different dimerization modes. Interestingly, recent data indicate that "pseudopalindrome-cutters" that recognize *pseudo*palindromic sequences and generate overhangs with an *odd* number of bases violate these rules, at least in some cases. Pseudopalindrome cutters are similar to palindrome cutters, especially in their dimerization modes, yet generate different cleavage patterns. We have selected the pseudopalindrome cutter Ecl18kI (/CCNGG) and the related palindrome cutter NgoMIV (G/CCGGC) for a detailed comparison. A high resolution structure of NgoMIV with DNA has been reported previously [1]. We have now crystallized Ecl18kI with DNA and solved the structure at high resolution [2]. The comparison of the cocrystal structures shows that Ecl18kI and NgoMIV use a conserved "recognition module" to interact with their target sequences as predicted. To accommodate the extra nucleotides (N) at the center, Ecl18KI flips them out from the DNA duplex. This flip and the accompanying DNA kink cause a register shift by 1 bp making the distances between scissile phosphates in the NgoMIV and Ecl18kI cocrystal structures nearly identical. Ecl18kI is the first example of a restriction endonuclease that flips nucleotides to achieve specificity for its recognition site.

[1] Deibert, M., Grazulis, S., Sasnausjas, G., Siksnys, V., and Huber, R. (2000) *Nat Struct Biol* **7**, 792-799.
[2] Bochtler, M., Szczepanowski, R.H., Tamalaitis, G. Grazulis, S., Czapinska, H., Manakova, E., and Siksnys, V. (2006) EMBO J. 25, 2219-2229.