Structural Basis for the Enzymatic Incorporation of a Hydrophobic Artificial Base Pair into DNA

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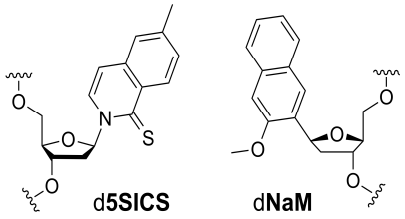
The genetic alphabet is comprised of two base pairs, and the development of a third, unnatural base pair would enhance the potential of numerous chemical and biological applications based on DNA. Therefore, the development of an artificial base pair which is processed by DNA polymerases with efficiencies and selectivities comparable to the natural base pairs is an important task. The Romesberg group at the Scripps Research Institute in California developed the artificial hydrophobic base pair d**NaM**-d**5SICS** which is one of the most efficiently replicated unnatural base pairs identified to date.[1] Its pairing is based only on hydrophobic and packing forces and in free duplex DNA it forms a cross-strand intercalated structure.[2,3] This mode of pairing maximizes packing interactions but the structure is difficult to reconcile with efficient polymerase recognition. In collaboration with the Romesberg group we aim to investigate the structural basis for the incorporation and elongation of d**NaM**-d**5SICS** by crystallographic studies using the well characterized KlenTaq DNA polymerase as a model.



The d**5SICS**-d**NaM** unnatural base pair

[1] D. A. Malyshev, K. Dhami, H. T. Quach, T. Lavergne, P. Ordoukhanian, A. Torkamani, F. E. Romesberg, *Proc. Natl. Acad. Sci. U.S.A.* **2012**, 109, 12005-12010.
[2] K. Betz, D. A. Malyshev, T. Lavergne, W. Welte, K. Diederichs, T. J. Dwyer, P. Ordoukhanian, F. E. Romesberg, A. Marx, *Nature Chem. Biol.* **2012**, 8, 612-614
[3] D. A. Malyshev, D.A. Pfaff, S. I. Ippoliti, G. T. Hwang, T. J. Dwyer, F. E. Romesberg, *Chem. Eur. J.* **2010**, 16, 12650-12659.

Target identification of a small molecule rescuing monastrol-induced spindle defects

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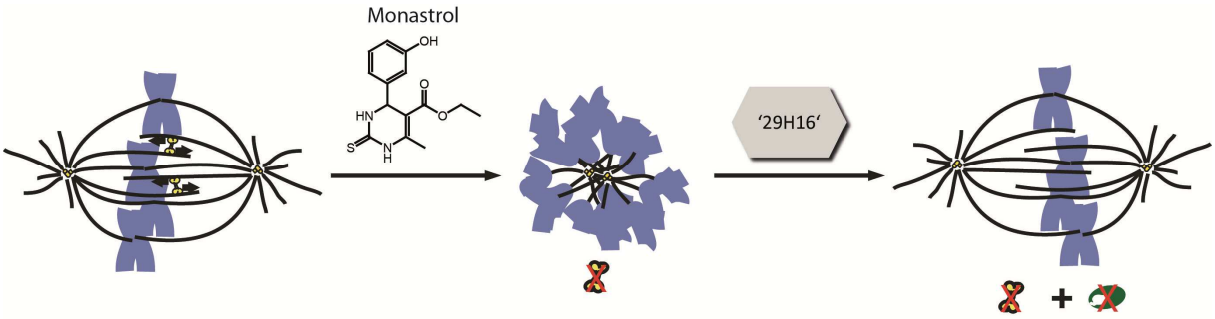

During mitosis, accurate segregation of DNA into two daughter cells is achieved by the dynamic function of a microtubule-based bipolar structure, the mitotic spindle. A major driving force for spindle pole separation is the kinesin Eg5. Small molecule inhibitors of Eg5 such as monastrol induce collapse of the bipolar spindle. There is increasing evidence for further players that contribute to the formation and maintenance of the bipolar spindle whose identity and mode of action, however, remain elusive.

In a phenotype-based screen, we identified a compound, termed 29H16, that rescued spindle bipolarity in Eg5-inhibited cells. Notably, 29H16 also restored the bipolar shape of the spindle in *Xenopus* egg extract supplemented with monastrol indicating that the target of 29H16 is conserved in evolution. To identify the molecular target of 29H16, we first verified the rescue by live-cell imaging showing that a considerable percentage of cells could overcome the monastrol-induced mitotic arrest and divide again upon treatment with 29H16. Next, we optimized an assay which allows the assessment and quantification of the biological activity of 29H16. This assay was used to test commercially available derivatives of 29H16 to gain information on structure-activity-relationship. We further extended the derivative library by synthesizing additional structures and could find both more potent derivatives and a position in the molecule that tolerates modification. Using this knowledge, we immobilized the small molecule and applied affinity chromatography coupled mass spectrometry to identify potential biological targets of 29H16.

We aim to use 29H16 as a means to dissect the process of spindle formation in cells. This goal implies the identification of the molecular target of 29H16. In our work, we characterized the effect of 29H16 on mammalian cells and evaluated the structural requirements for its function. Using the tools we have generated, we aim to identify interaction partners and validate their role in assembly of a bipolar spindle.

Monitoring Kinetics of Enzymatic ATP Consumption by EPR Spectroscopy

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Life uses ATP in cells to supply processes with energy. It also is an important signaling molecule that helps regulating the metabolism of cells and transmits extracellular signals to trigger intracellular processes. Therefore, many enzymes use ATP as an energy or phosphate transferring substrate. Due to this ubiquitous occurrence, the kinetics of enzymatic consumption of ATP is of high interest. Unfortunately, existing methods of investigation are hindered by their need of auxiliary reagents, since these might interfere with the enzymatic process, and thus invalidate the results.

In this work a new tool to monitor the kinetics of enzymatic consumption of ATP by applying EPR spectroscopy is presented. For this purpose doubly spin labeled ATP analogues have been synthesized. Their inter-spin distance ranges up to 3.8 nm as determined by DEER. Due to the high flexibility of the linkers this distribution is very broad and has considerable probability below 1.5 nm. Hence, the corresponding cw EPR spectrum exhibits dipolar broadening as long as the ATP remains intact. After enzymatic cleavage of the ATP, both labels are separated spatially and the dipolar broadening vanishes. The spectrum of a sample with only a fraction of the ATP split is a superposition of the spectra of these two extremes. After proper data analysis, the ratio between cleaved and intact ATP can be extracted from the spectrum. By measuring time resolved cw EPR spectra, the kinetics of ATP consumption are studied.

Thus, one can investigate the inhibition and stimulation of enzymes when consuming ATP without the need for auxiliary reagents. In contrast to fluorescence spectroscopy, EPR can also be applied in opaque media.