RUN
Regensburg Center
for Ultrafast Nanoscopy

physikalisches Kolloquium

Prof. Dr. Ralf Seidel
Peter Debye Institute for Soft Matter Physics
Universität Leipzig

Dissecting the target recognition by CRISPR-Cas complexes using single-molecule nanomechanical measurements

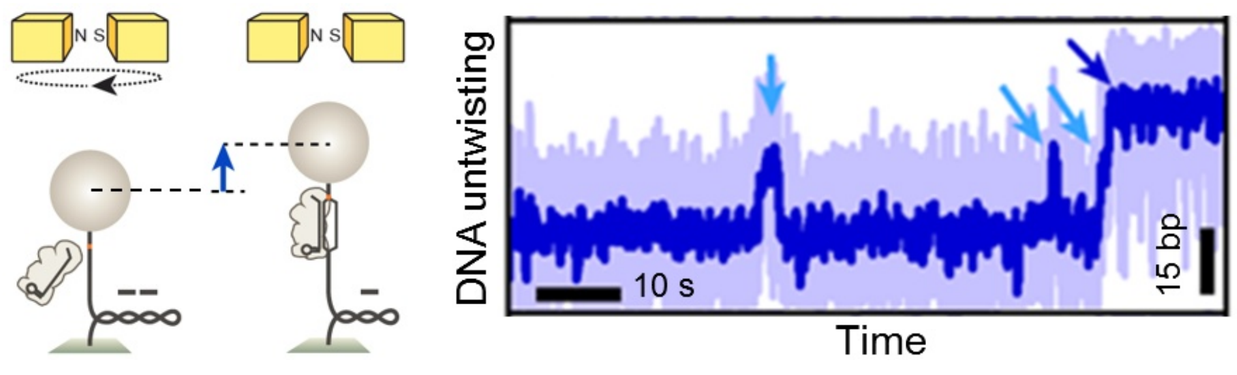
Mo. 18.11.19
16:00 Uhr
Ort: H34

The recently discovered CRISPR-Cas enzymes are promising tools in biotechnology and medicine. These enzymes can be guided to any genetic locus by a short RNA that recognizes desired DNA targets by base pairing. The target recognition is, however, highly promiscuous, such that technologically undesired mispairs between RNA and target DNA occur.

We employ single-molecule nanomechanical measurements [1] and single-molecule imaging [2] to resolve the dynamics of the target recognition process with few base pair resolution [3,4]. We find that this process occurs in sequential base pair steps. Mispairs act as barriers that stall the recognition but that can be overcome by thermal fluctuations.

The target recognition process can be quantitatively described as a random walk in a rugged energy landscape. By establishing fast measurements of DNA untwisting using gold nanoparticle rotors, we can directly infer the energy landscape as well as the time scale of single base pair steps. We think that our thermodynamic model can help to avoid the recognition of wrong targets by CRISPR-Cas complexes in emerging applications.

[1] Huhle et al. Camera-based three-dimensional real-time particle tracking at kHz rates and Ångström accuracy. Nat. Commun. 6, 5885 (2015)
[2] Kemmerich et al. Simultaneous single-molecule force and fluorescence sampling of DNA nanostructure conformations using magnetic tweezers. Nano Lett. 16, 381-386 (2016)
[3] Rutkauskas et al. Directional R-Loop Formation by the CRISPR-Cas surveillance complex Cascade provides efficient off-target site rejection. Cell Rep. 10, 1534-1543 (2015)
[4] Songaliene et al. Decision-Making in Cascade Complexes Harboring crRNAs of Altered Length. Cell Rep. 28, 3157-3166 (2019)



(Left) Target recognition by a CRISPR-Cas complex is probed with negatively twisted DNA in a magnetic tweezers setup. DNA unwinding during target binding absorbs part of the twist resulting in a DNA length increase.
(Right) Time trajectory of the DNA unwinding including unsuccessful (light blue arrows) and successful (dark blue arrow) recognition.