

## **Will Genetic Material Soon be Decoded more quickly?**

### **Single-molecule sequencing: The foundation has been laid**

The sequence of the human genome has been extensively determined. In order to understand the relationships between individual genome segments and specific diseases, for example, the appropriate sequences have to be identified, characterized, and examined for mutations. "This is all too tedious with conventional sequencing methods," explains Susanne Brakmann of the University of Leipzig. "However, if we were successful in sequencing single DNA molecules, we could "read" substantially longer fragments, which would allow us to compile the sequence data orders of magnitude more quickly." Together with Sylvia Löbermann of the Max Planck Institute for Biophysical Chemistry in Göttingen, Brakmann has just gained another milestone toward this goal.

The principle is this: DNA is a double-helix consisting of two complementary strands, which are held together by the pairing of their components, the nucleotide bases A and T, as well as G and C. The two strands can be separated, and one of the single strands acts as a stencil for the generation of a copy -- incorporating nucleic bases that have been marked with different fluorescent dyes. Just recently, the researchers also discovered an enzyme that could synthesize correct copies from bulky, labeled nucleic bases (Angew.Chem. 2001, 113, 1473 - 1476). When coupled to tiny polymer spheres, the labeled DNA molecules can be isolated. In the next step, one nucleotide after the other has to be sliced, salami fashion, from the end of the DNA molecule, and identified. Spectrometric methods for the identification of individual fluorescent molecules have existed for the last ten years. One barrier to their use has, until recently, been the lack of a "salami knife", an enzyme that can rerelease the fluorescent nucleotides, because the labeled DNA is very unwieldy and also winds differently than the unlabeled original.

Initially, all of the "salami knives", or exonucleases, failed. Instead of testing further "knives", the researchers varied the conditions of the separation; by means of the addition of the solvent dioxane, the solubility of the DNA can be raised, improving the separation mechanism of

the selected enzyme, *E. coli* exonuclease III. Also, if only two of the four types of nucleotides are labeled, the DNA is less unwieldy. If the experiment is repeated with all possible permutations, a complete sequence analysis should also be obtained. "The foundations for single-molecule sequencing have now been laid," Brakmann states optimistically. "Fully automated instruments could discover individual variations in gene segments, and could possibly decipher up to one million nucleotides a day."