Looking for the heartbeat of cellular networks

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Our cells' molecules form an intricate network of interactions. Today's techniques, however, can only be used to measure individual molecular reactions outside the cells. Since molecular concentrations are much higher in cells than in the laboratory, scientists suspect that the kinetics of molecular reactions in living cells differ substantially from external probes.

"We expected the cellular reaction speed to be higher," confirms LMU biophysicist Professor Dieter Braun. "However, our novel optical approach showed that - depending on the length of the strands - the coupling of DNA-strands inside living cells can be both faster and slower than outside." Data yielded from living cells are highly valuable for the development of models to understand the complex interactions as well as pathological processes in biological cells. Braun and his team now plan to probe a variety of molecular reactions in living cells, visualizing the heartbeat of cellular networks. (*PNAS* online, 14 November 2009)

In their work, the scientists investigated the hybridization - the coupling and de-coupling - of two DNA-strands, which they introduced into living cells. To determine the reaction time constant they used an infrared laser to induce temperature oscillations of different frequencies in the cell and measured the concentration of the reaction partners, namely of coupled and de-coupled DNA. At low frequencies, these concentrations followed the temperature oscillations, whereas at higher frequencies they experienced a phase delay and oscillated with diminished amplitude. Both delay time and amplitude decrease, were evaluated to obtain the

reaction time constant.

The team determined the concentrations using the so-called fluorescent energy transfer (FRET), which takes place between two chromophores at a certain spatial distance. They applied a FRET pair to the DNA-strands such that energy transfer occurred only if the strands were coupled. The chromophores were excited with a stroboscopic lamp and a CCD camera registered time and amplitude of the fluorescence, thus visualizing the concentration alterations with a spatial resolution of about 500 nanometres. The experiments revealed that DNA-strands comprising 16 units, the so-called bases, showed a sevenfold higher reaction speed compared to values determined outside living cells.

12-base DNA-strands, on the other hand, reacted times five times slower than outside cells. This is a surprising result, since kinetics of molecular reactions has been assumed to be always faster inside cells, where much higher molecular concentrations prevail. "Apparently cells modulate the reaction speed in a highly selective way," says Braun. "The measurements provide valuable insight into in vivo kinetic data for the systematic analysis of the complexity of biological cells," adds Ingmar Schön, who conducted the demanding experiments. The scientists are now planning to probe a wide variety of molecular reactions in living cells, visualizing the heartbeat of cellular networks.

More information: "Hybridization Kinetics is Different Inside Cells," Ingmar Schoen, Hubert Krammer, Dieter Braun, *PNAS* online, 14 November 2009

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