Novel anti-Stokes lanthanide-doped nanoparticles and multicolor FRET mechanisms for single-molecule DNA sequencing (LantaSEQ)

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DNA sequencing has become an indispensable tool in clinical diagnosis and life sciences, for example in biotechnology, evolutionary research or in genetic profiling of microorganisms. In the future, a high impact is expected in personalized healthcare because many diseases such as cancer have a genetic component, and DNA sequencing of a personal genome will improve the risk assessment. An early assessment of individual drug responses can help avoid adverse drug reactions and optimize therapies. Knowledge of the DNA sequence will also help to improve the selection of new biochemical targets for drug discovery. Single-molecule DNA sequencing is highly desirable for molecular diagnostics because of (i) simplified DNA template preparation, (ii) higher throughput and speed, (iii) longer read lengths and thus reduction of the costs compared to conventional sequencing techniques.

Current single-molecule DNA sequencing techniques, however, suffer from several disadvantages that can be circumvented by employing the unique photophysical features of novel lanthanide doped luminescent nanomaterials (LnNP) which (1) efficiently emit short-wavelength light under NIR excitation with large anti-Stokes shifts of >300 nm. Under these conditions, no other sample components are excited and the background signal due to autofluorescence and light scattering is eliminated. (2) LnNP are very photostable and – in contrast to quantum dots – do not blink. Consequently, such new nanoparticles provide a constant and permanent donor signal for FRET, which is essential for reading long DNA sequences continuously. (3) LnNP emit multiple narrow emission bands under single wavelength excitation, such that a single luminescent nanoparticle can be used as a donor for FRET to four different acceptor dyes, which are required to identify individual dNTPs.

While these are most promising features, **single-molecule sequencing based on lanthanide doped nanoparticles is not a trivial task and has never been realized before**. Towards this target application, the project will work to

- optimize the **optical readout instruments for single LnNP detection and wide-field microscope imaging**.
- synthesize and evaluate **new LnNP designs** (by optimizing the doping, and core-shell compositional architectures) to make them as bright as possible and as sensitive to acceptor molecules as possible.
  - optimize the **surface conjugation** of the polymerase to minimize the distance between the UCNP and polymerase and prepare or **isolate nanoreaders** consisting of a single nanoparticle and a single polymerase only.
  - employ the nanoreader for **sequencing of DNA test strands.**

The project outcome will be a functional prototype of the new DNA sequencing technology (LantaSEQ).