Conjugation chemistry on magnetic particles

Aim:

Development and optimization of different protocols for conjugation of oligonucleotides to magnetic particles. Magnetic particles will be combined with rolling circle amplification products (RCP) to be used in a biosensor that uses magnetic read-out. This method will mainly be used for infectious diagnostics.

Background:

A padlock probe is a linear oligonucleotide comprising two target-specific sequences at both ends and a linker segment containing sequences for amplification and identification. The probe is designed to bring the two ends into juxtaposition upon hybridization to the target sequence. This conformation allows the oligonucleotide to be circularized with great target specificity by a DNA ligase. Signal amplification can be achieved by rolling circle amplification (RCA) of the reacted padlock probes. The concatemers of DNA circles produced by RCA can then be digested, ligated into new circles and subjected to another round of RCA, known as circle-to-circle amplification (C2CA).

Binding of RCP to conjugated magnetic particles will have an effect on the Brownian relaxation frequency. This change can be detected using a portable instrument for dynamic magnetic analyses (DynoMag).

Currently used methods in the lab include sulfo-SMCC and SANH coupling chemistry. Sulfo-SMCC chemistry uses a crosslinker containing an NHS-ester and a maleimide group to conjugate to a free amine-group and an activated thiol-group, respectively. SANH contains an NHS-ester and a hydrazine group which can bind to an amine group and an aldehyde group, respectively. If time allows a third conjugation chemistry using EDC can be optimized.

The project will focus mainly on optimizing conjugation chemistries, but it will also be required to make RCP in order to evaluate the performance of magnetic particles in the DynoMag instrument. A fluorometer will be used to evaluate the coverage of oligonucleotides on the magnetic particles. All DynoMag measurements will be performed at Ångström laboratory.

The project will be undertaken in Mats Nilsson's group at the department of immunology, genetics and pathology at the Rudbeck laboratory in Uppsala.

Duration:

20-30 hp. Start: as soon as possible or on agreement

Competence:

Chemical or biotechnical (or equivalent) background is a requirement. Experience in conjugation chemistry is desired, but not necessary. Interest in biosensors is an advantage.

For more information, or to submit an application (letter and CV), please contact:

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