

Fluorescent Hybridization Probes for Sensitive and Selective DNA and RNA Detection

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Fluorescence hybridization sensors, such as molecular beacons and binary probes, containing two or three dyes were designed for selective detection of specific oligonucleotide sequences. These sensors switch in emission wavelength and intensity upon binding to target DNA or RNA. This change in the fluorescence signature allows for ratiometric fluorescence analysis. Molecular beacons consist of two (or three) fluorescence dyes on each end of the oligonucleotide. In the absence of target DNA or RNA the molecular beacon is in its stem-closed form and shows efficient energy transfer from the donor to the acceptor. In the presence of the complementary target DNA the beacon opens, hybridizes to the target, and energy transfer is blocked. Binary probes consist of a donor and acceptor fluorophores that are attached to two different oligonucleotides and serve as resonance energy transfer pair when hybridized to adjacent sites of a target sequence. In the absence of target, both, donor and acceptor, are separated and no energy transfer occurs.

A major problem in sensitive target DNA and RNA detection is luminescence and scattering from the cellular background (autofluorescence). Because autofluorescence usually has decayed within 10 ns, luminescence probes were designed which show delayed fluorescence upon target detection. For example, a Ru complex was used as donor and an organic dye as acceptor in a binary probe. Because energy transfer from Ru triplets to generate singlet excited states of the organic fluorophore is spin-forbidden, a delayed fluorescence (65 ns) of the acceptor was generated. This delayed fluorescence allowed target detection in the presence of strong cellular background by using a time gated fluorescence detection technique.

The photophysical processes in the hybridization probes were thoroughly studied by steady-state and time-resolved luminescence techniques. In addition, two-photon excitation was explored in combination with fluorescence lifetime imaging microscopy.

