Luminescent probes for sensitive and specific optical microscope imaging

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The detection and localization at microscopic level of analytes present at very low concentration in single cell and tissue samples is one of the most exciting challenges for bioanalytical chemistry. Fluorescence microscopy is commonly used for the sensitive localization of analytes in biological samples through immunohistochemical and in situ hybridization reactions with fluorescent labeled probes. However, this technique suffers from two main drawbacks that reduce the sensitivity, i.e. the autofluorescence of the sample and the light scattering in the apparatus.

We previously demonstrated that chemiluminescent enzyme-labeled probes allow the sensitive localization of DNA sequences and antigens, due the high specificity of the chemiluminescent reactions and low background noise. Chemiluminescence also provides high spatial resolution and the possibility of quantifying the amount of analyte present on a given surface area.

Time-resolved fluorescence (TRF) microscopy is a promising alternative to conventional fluorescence microscopy that allows for vanishing of the sample autofluorescence. Due to their peculiar photophysical properties, luminescent lanthanide chelates are the most suitable labels for this technique. Unfortunately, only a few, expensive, commercially available labels have been developed up to now. We have designed and synthesized new stable Eu$^{3+}$ and Tb$^{3+}$ lanthanide chelate labels suitable for binding to primary amino groups. These Eu$^{3+}$ and Tb$^{3+}$ complexes are characterized by long luminescence lifetimes (0.6 and 1.5 ms) and high emission quantum yields (0.08 and 0.30), thus resulting particularly suitable for TRF applications. Preliminary experiments on model samples proved the suitability of these labels for TRF imaging microscopy. Immunohistochemistry experiments are in progress, using either biospecific probes labeled with the chelate or biotinylated probes revealed by a complex between streptavidin and a labeled biotinylated polymer, in order to assess the potentials of this new family of lanthanide labels for TRF imaging purposes.