Fluorescence intensity measurements are among the most often applied methods for detection of biomolecules, due to their versatility and high detection sensitivity. A more advanced method is the detection of the luminescence decay time (lifetime) of fluorescent labels. This technology enables the interaction of biomolecules to be determined quantitatively by making use of only one label. This advantage can be exploited to simplify many assays by reducing the number of incubation and washing steps, making immobilization or toxic substrates redundant. Thus, it is much simpler than FRET (fluorescence resonance energy transfer), which requires two fluorescent conjugates with specific spectral specifications to detect their interaction. Moreover, the determination of lifetime is independent of fluorescence intensity, concentration (in a first approximation), and light source intensity.

We present direct homogenous assays. Three exemplary systems are shown: biotin - streptavidin (small molecule - protein), streptavidin - biotinylated bovine serum albumin (BSA) (protein - protein), and oligonucleotide - complementary oligonucleotide (hybridization assay). Whenever an affinity binding takes place, e.g. between streptavidin and biotin, or between complementary oligonucleotides, this is indicated by a significant change in the lifetime. The change in lifetime is directly dependent on the relative concentrations of the binding partners. All binding curves show a concentration dependent range.

Lifetime is directly dependent on the relative concentrations of the binding partners. All measurements were performed in the 96 well microplate under standard conditions on a LF 401 NanoScan microplate reader from IOM. Excitation/emission occurred at 505/630 nm. The signal for one datapoint was averaged over 64 laser pulses. Moreover, the determination of lifetime is independent of fluorescence intensity, concentration (in a first approximation), and light source intensity. The fluorescence lifetime increases with increasing amounts of streptavidin (LOD in the shown experiment: 1.7 \times 10^{-8} \text{ mol/L}).

Fluorescence intensity measurements are not affected by the fluctuation of the excitation source, stray light, or the concentration of the fluorophore. Fluorescence lifetime measurements are not affected by the fluctuation of the excitation source, stray light, or the concentration of the fluorophore.

### Covalent Labels

<table>
<thead>
<tr>
<th>Label name</th>
<th>(\lambda_{	ext{abs}}) [nm]</th>
<th>(\lambda_{	ext{em}}) [nm]</th>
<th>Lifetime of the free label in buffered system</th>
<th>Lifetime of the conjugate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromone</td>
<td>546</td>
<td>545</td>
<td>&lt; 0.1 ns</td>
<td>0.4 ns**</td>
</tr>
<tr>
<td>P503</td>
<td>506</td>
<td>600</td>
<td>&lt; 0.1 ns</td>
<td>2.8 ns**</td>
</tr>
<tr>
<td>P537</td>
<td>537</td>
<td>580</td>
<td>&lt; 0.1 ns</td>
<td>2.3 ns**</td>
</tr>
</tbody>
</table>

*conjugated to biotin
**conjugated to human serum albumin

**conjugated to human serum albumin

### Summary

We show three exemplary fluorescence decay time assay systems using three different covalent labels. The luminescence decay time of the label is dependent on its micro environment. Therefore it is possible to detect binding events via the change in lifetime. The experiments were chosen to show the suitability of lifetime both in hybridization and protein assays. The great advantage of using fluorescence lifetime in comparison to absorbance or emission intensity measurements in binding assays is that only one labeling procedure is necessary. This means a reduction of both assay time and costs for labels and analytes. Fluorescence lifetime measurements are not affected by the fluctuation of the excitation source, stray light, or the concentration of the fluorophore.

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**IOM Innovative Optische Messtechnik GmbH, Rudower Chaussee 29, D-12489 Berlin, Germany**

**Complementary Oligonucleotides**

An amino-linked 15-mer oligonucleotide is labeled with Chromeon P503. The graph shows the binding curve of a hybridization assay, used for the determination of the 15-mer counter strand. (LOD: \(1.0 \times 10^{-7} \text{ mol/L}\)).

**Streptavidin - Biotin (protein – small molecule)**

The graph shows the binding curves of an assay for the determination of streptavidin. Constant amounts of Chromeon 546-biotin were mixed with increasing quantities of streptavidin. Whenever an affinity binding takes place, e.g. between streptavidin and biotin, or between complementary oligonucleotides, this is indicated by a significant change in the lifetime. The change in lifetime is directly dependent on the relative concentrations of the binding partners.

**Streptavidin - biotinylated BSA (protein – protein)**

The graph shows the binding curve of an assay for the determination of biotinylated BSA using streptavidin labeled with the fluorophore Chromeon P537.

**Fluorescence intensity measurements**

Fluorescence intensity measurements are among the most often applied methods for detection of biomolecules, due to their versatility and high detection sensitivity. A more advanced method is the detection of the luminescence decay time (lifetime) of fluorescent labels. This technology enables the interaction of biomolecules to be determined quantitatively by making use of only one label. This advantage can be exploited to simplify many assays by reducing the number of incubation and washing steps, making immobilization or toxic substrates redundant. Thus, it is much simpler than FRET (fluorescence resonance energy transfer), which requires two fluorescent conjugates with specific spectral specifications to detect their interaction. Moreover, the determination of lifetime is independent of fluorescence intensity, concentration (in a first approximation), and light source intensity.