

cAMP - *N*ano-TRF Detection Assay

Properties and Read-Out

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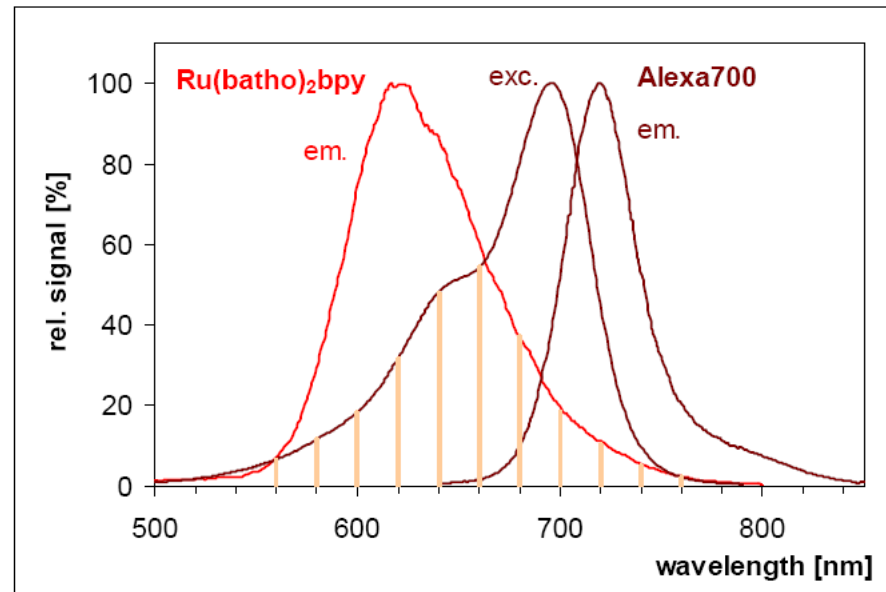
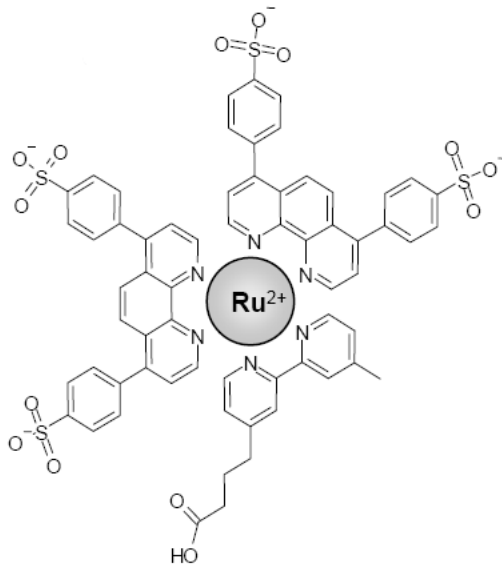
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Outline

1. Ruthenium Complex – basis of Nano-TRF assay technology
2. Instruments
3. cAMP-Nano-TRF product features

Ruthenium-based TR-FRET assay technology

Ruthenium-label with interesting physical properties



lifetime property:

$$\tau = 3.2 \mu\text{s}$$

spectral properties:

$$\lambda_{\text{exc}} = 450 \dots 470 \text{ nm (visible, blue)}$$

$$\lambda_{\text{em}} = 600 \dots 630 \text{ nm (visible, red)}$$

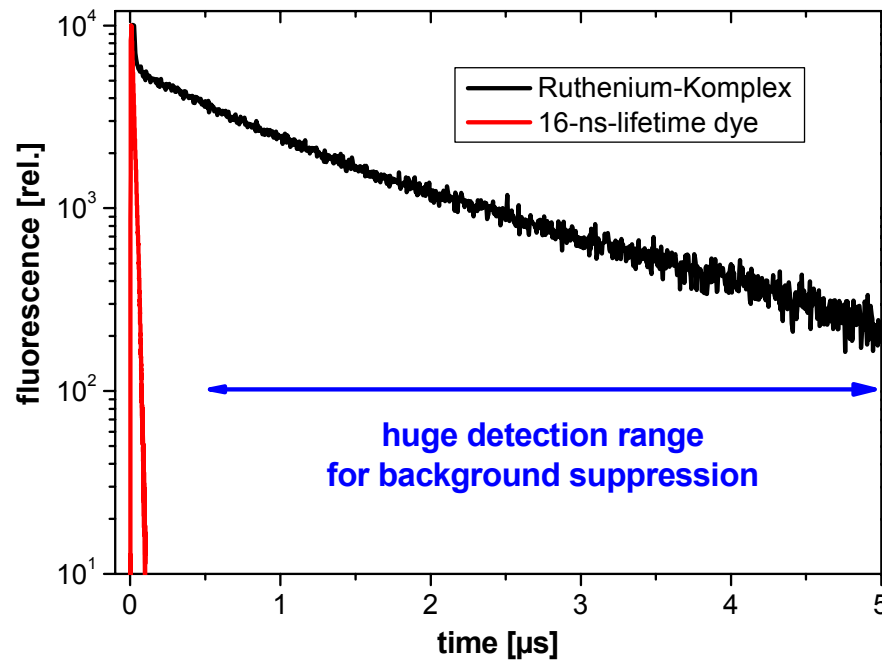
overlap with red-absorbing dyes

Key advantages of Ruthenium label

lifetime: $\tau = 3.2 \mu\text{s}$



- sensitive time-gated detection
- efficient background suppression



excitation with visible (blue) light

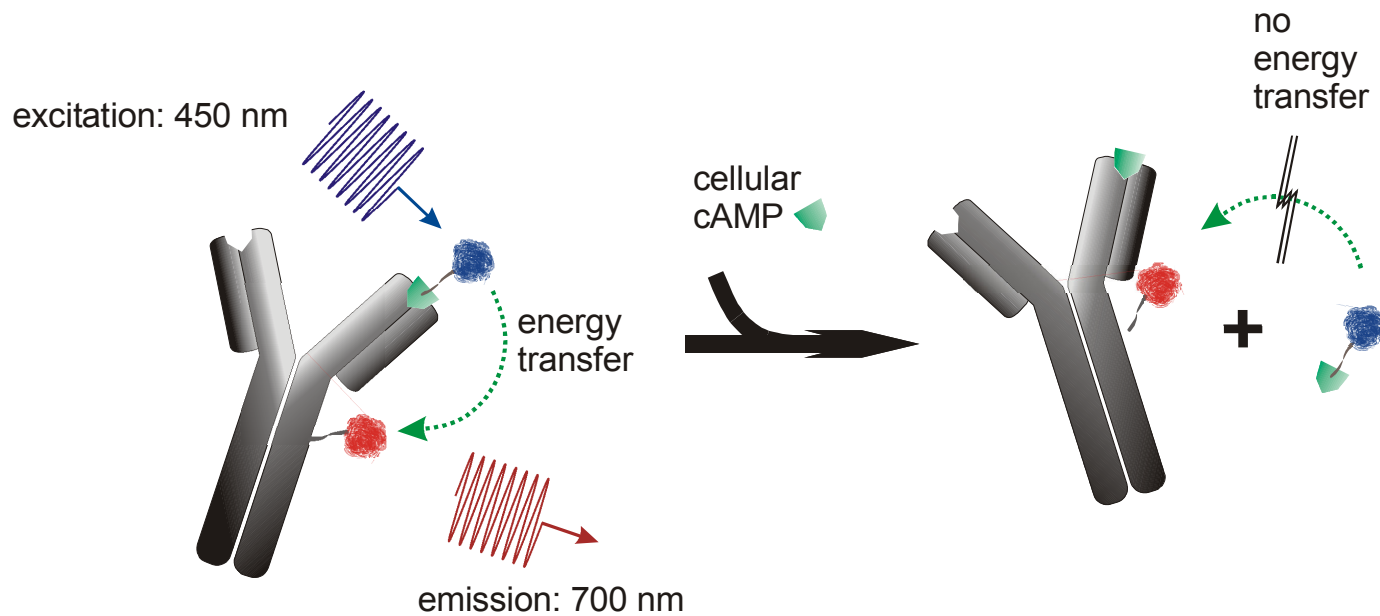




- minimised light absorption of (coloured) compounds
- minimised probability of compound fluorescence
- 4 times reduced interference due to light scattering



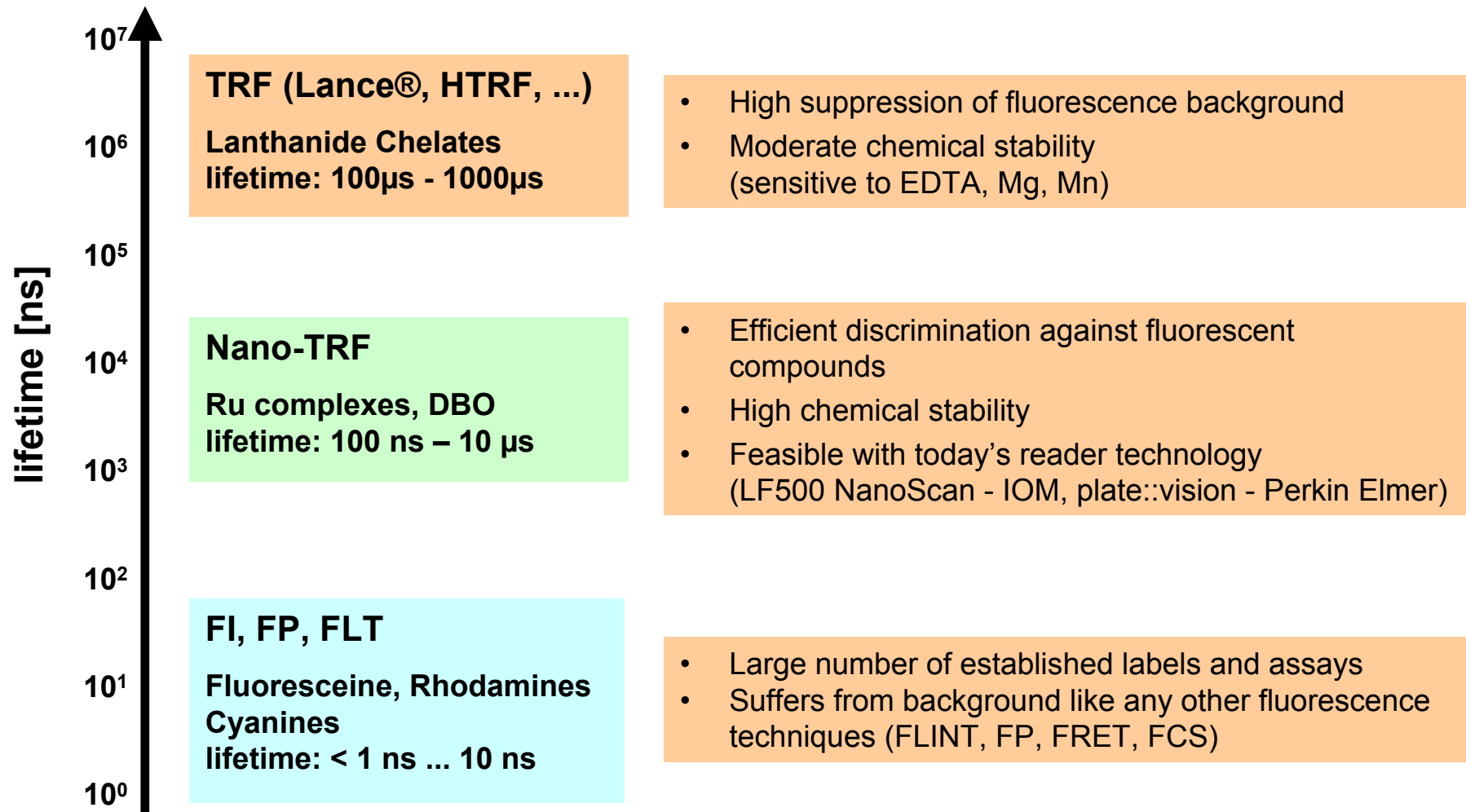
less interference with compounds and assay components

competitive cAMP assay principle



-  = cAMP labeled with Ru²⁺ complex
-  = Alexafluor700® as acceptor

How compares Nano-TRF to other detection modes?



Considerations for Nano-TRF read-out

lifetime of donor: $\tau = 3.2 \mu\text{s}$ and excitation with visible (blue) light



- excitation of samples with nanosecond light pulses
- excitation at 470 nm (355 nm)
- light source may be:
 - Nd:YAG laser (OPO output @ 470 nm or @ 355 nm)
 - Nitrogen/dye laser
 - Nanosecond flash lamp

lifetime of FRET signal: $\tau = 100 \text{ ns}$



- time-gated signal detection in ~ 100-ns-gates
- sensitive signal detection with PMT or Intensified CCD

LF502 NanoScan FLT – Nano-TRF reader with additional value



- Perfectly supports Nano-TRF
- Supports common fluorescence assay methods (FI, TRF, HTRF)
- Opens up two new assay dimensions:
 - FLT - Fluorescence lifetime
 - Nano-FP - Time-resolved Fluorescence Polarisation

it's versatile and flexible !

The NanoScan supplies:

- immediately a second read-out parameter to your existing FI assay.
- additional information to improve existing assay performance.
- the basis for FLT-based assays.

Today's Nano-TRF read-out instruments

	LF502 NanoScan FLT IOM	Plate::Vision Perkin Elmer
field of application	HTS, assay development	HTS, uHTS
sequence of measurement	serial, well by well	parallel, 96 wells simultaneously
plate formats	up to 1536	up to 1536
detector	two PMT's in two channels	Intensified CCD camera
light source	Nitrogen/dye laser (337 ... 785 nm, 2.5 ns)	Nd:YAG laser (355 nm, 6 ns) OPO (470 nm)
detection (filters)	380 ... 850 nm	450 ... 770 nm
temporal resolution	1 ns ... 10 ms lifetimes	ns and μ s lifetimes

cAMP assay kit design



small kit: 5,000x

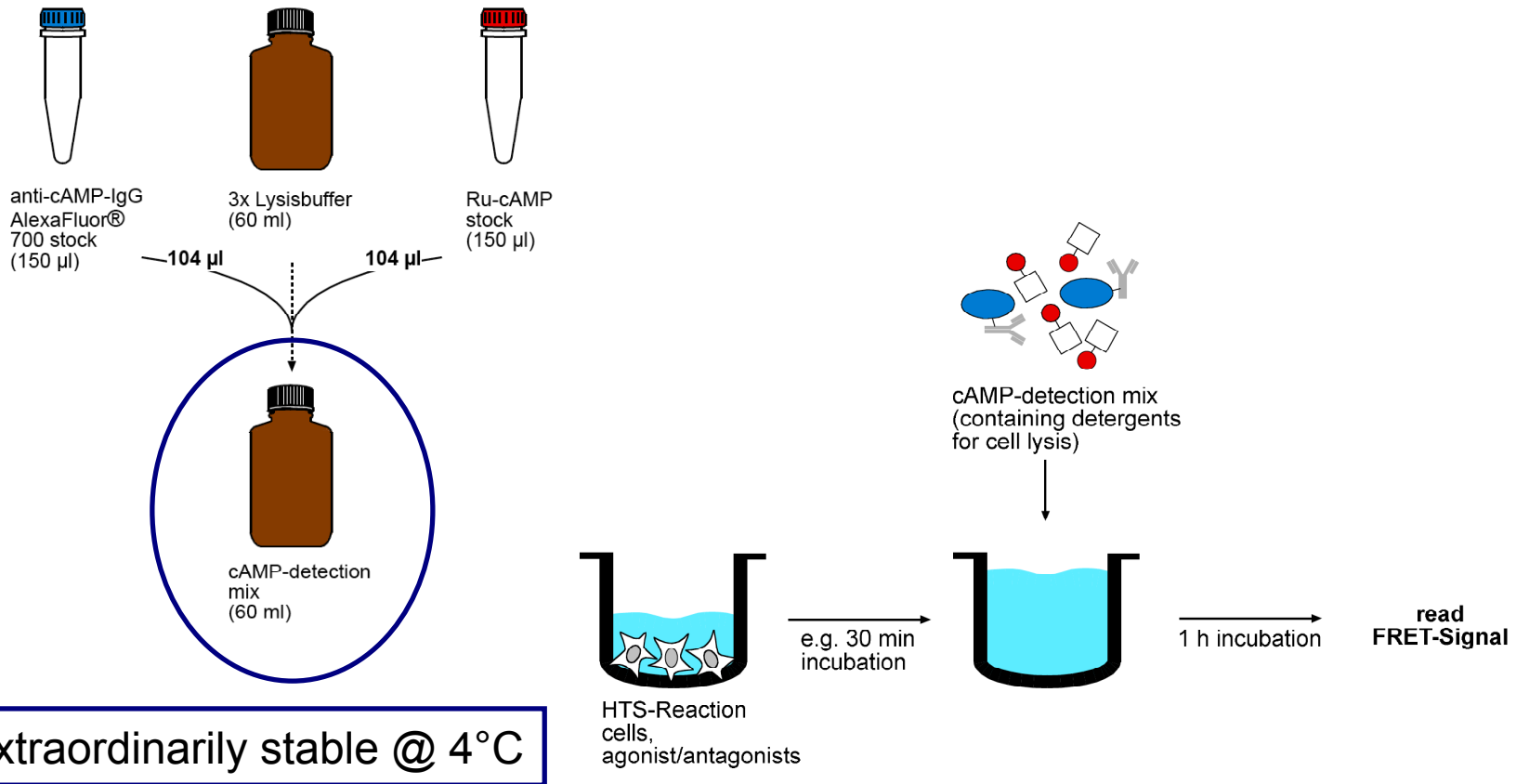


large kit: 44,000x

- produced by Roche Diagnostics, Mannheim, Germany
- launched as commercial product at SBS 2008 (St. Louis)
- worldwide distributed by IOM
our partner in US is Innovative Instruments Inc. (Wake Forest, NC)

cAMP assay kit handling

convenient assay protocol – mix and ready-to-use



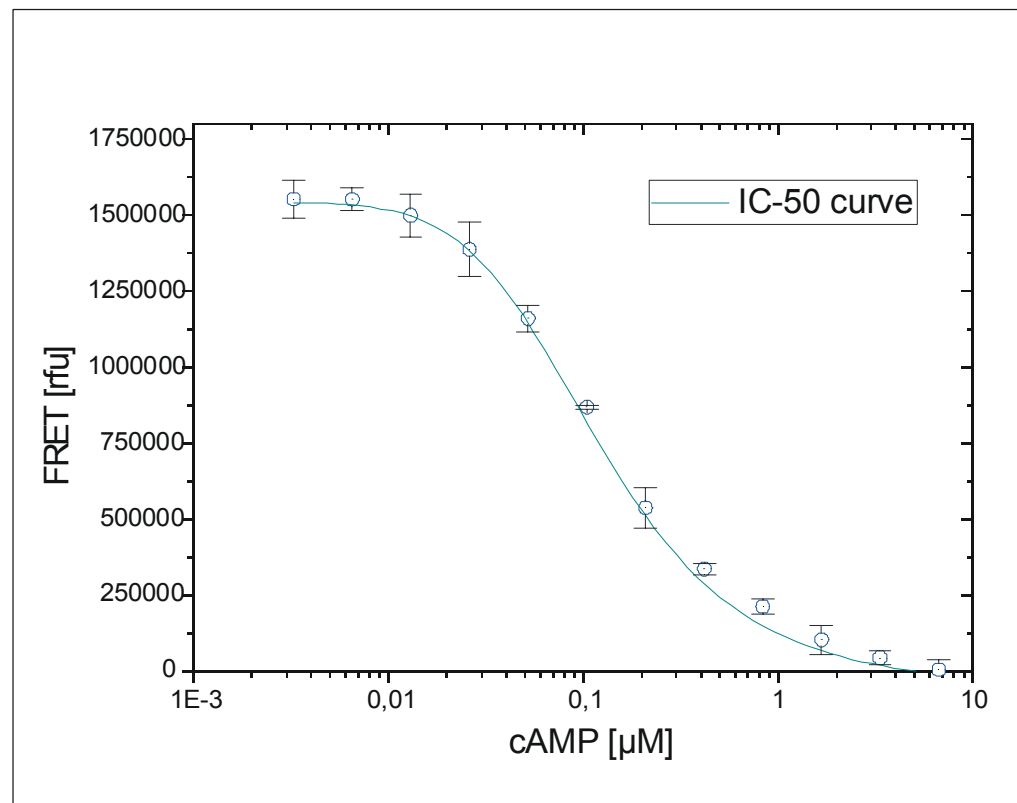
cAMP immunoassay read-out example

Readout, 2 channel end point measurement

Excitation: 470 nm

Emission Ch1: 630 nm, delay 1.5 μ s, gate: 3.5 μ s

Emission Ch2: 700 nm, delay 200 ns, gate: 300 ns



cAMP immunoassay main properties

main properties

- Competition immunoassay for cell-based HTS
- assay works with membrane preparations
- Nano-TRF detection principle suppressing compound/background fluorescence
- EC50 = 80 nM, high dynamic range, good linearity, signal stable up to 24 h
- convenient assay protocol, one step reagent addition
- working solution stable under ambient conditions (4°C) for at least 2 months
- insensitive against EDTA, Mg²⁺, Mn²⁺
- insensitive against high ATP concentrations

**visit us at booth 322
thank you for attention !**

Our offer:

- ***contact us for a free demo kit***
- ***demonstration of assay and LF502 NanoScan in your laboratory***