Fidabio

FIDA Technology

In-solution technology for analysis of complex molecular interactions

No restrictions on sample matrix or buffer.
Absolute measurements.
Nanoliter sample volumes.

One technology to measure:

- Affinity (K_D)
- Kinetics (k_{on} & k_{off})
- Quantity
- Sample Quality Control

FEATURES & BENEFITS 01

Robustness & Versatility

Speed & efficiency

- **Minimal sample consumption**
  - nL – uL range.
- **Walk-away automation**
  - 2x 50 vials or 2x 96 well plates.
- Up to 24 data points in <30 min.
- Up to 8 binding curves with 8 data points in triplicates, each within 4 hours of unattended operations.

Easiness of use & versatility

- Simple software interface.
- Data delivered as tables, plots, and real-time monitoring of the signal.
- Purified or un-purified samples.
- Built-in assay control.
- No limits on buffers, detergents or ionic strength.

Measurement specifications

- **Dissociation constant :**
  - pM to mM
- **Size detection**
  - Hydrodynamic radius between 0.5-500 nm
- Three interchangeable detectors: 480, 640 & UV (280)
- Detection signal-to-noise ratio of >30 (3-fold increase compared to current state-of-art detectors)

Temperature control

- **Measurement chamber:**
  - 15-55°C
  - 59-131°F
- **Autosampler**
  - 5-55°C
  - 41-131°F
- Can be controlled individually in each of the two tray holders and in the capillary compartment.

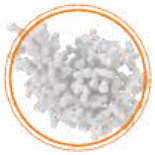
WORK WITH ANY BUFFER COMPOSITION THAT FITS YOUR GOALS

No restrictions related to detergent usage, ionic strength, temperature or sample pH.

This eliminates common research constraints, minimises assay development time and expand the scope of biological systems You can characterise.

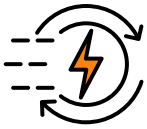
TECHNOLOGY CHARACTERISTICS 02

FIDA Measurements



Size, Hydrodynamic radius (R_h)

- First Principle measurement of hydrodynamic radius in nanometers.
- Range: 0,5 nm – 500 nm Rh.
- Can detect size changes smaller than 5%.



In-solution kinetics

- k_{on} & k_{off}
- No non-specific binding issues
- Seconds to minutes
- No steric hindrance to high density immobilised ligands

Fluorescence detection

- Multiple wavelengths available.
- Labelled or label free assays: 480 nm, 640 nm or UV detector.

Binding Related Intensity Change (BRIC)

- Measure change in fluorescence intensity as a result of binding events - at constant temperature.
- In parallel with size measurement, BRIC signal is obtained for all measurements.

Lambda Dynamics

- Measure the change in emission wavelength that occurs when binding affects the fluorophore's environment.
- Does not require changing the temperature to observe shifts in the emission wavelength.

Sample Quality Control Module & Reporting Tool

- 8 Quality Control Parameters for each sample
- Custom-made reports that fit your workflow
- Export data in multiple formats

NO ENVIRONMENTAL RESTRICTIONS

Seamlessly operate in complex matrices including fermentation media, plasma or serum. With FIDA there is no need for purification, which allows You to save your sample material and time.

03 MULTIPLE SIMULTANEOUS & INSTANT READOUTS

8 quality control parameters

Fida Software automatically includes Quality Control to each measurement taken. This increases the level of transparency and supports troubleshooting and assay optimisation. Our QC Module allows for building and exporting custom-made reports, which facilitates smooth workflows and data storage.

Structural integrity & functionality

- Size measured as hydrodynamic radius.
- Validate your protein stability
- Get insight into folding/unfolding and conformational changes.
- Automated binding curves and equilibrium Kd's are obtained by loading the autosampler with your titrations.

Viscosity

- Every measurement you take provides viscosity data.
- Viscosity compensation

PDB Correlator

- Use the absolute size as a firm reference point.
- Compatible with Protein Data Bank, Pymol or AlphaFold.

Heterogeneity (PDI)

- PDI Index allows for checking the heterogeneity of your sample.

Labelling efficiency

- Option of measuring size of up to 3 species in solution.
- Can e.g. reveal the percentage of free vs. conjugated fluorophore in your sample when you choose to use Fida 1 for labelled assays.

Sample Loss

- Transparently exposed
- Troubleshoot efficiently

Aggregation

- Protein/particle aggregates are clearly detectable and quantifiable whilst still leaving the core signal useful for standard measurement.

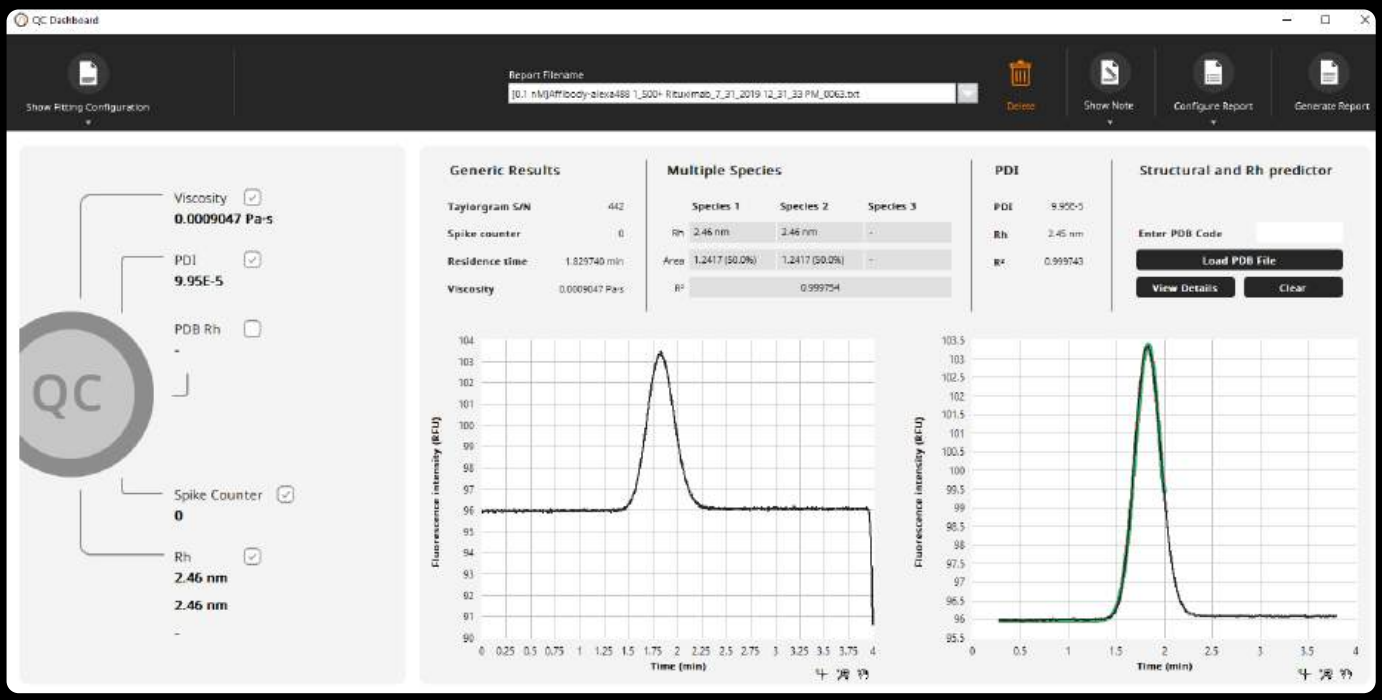
Stickiness

- The shape of the core signal will reveal any stickiness of your binding partners or your binding complexes.
- The core signal is useful for standard measurement despite of the stickiness.

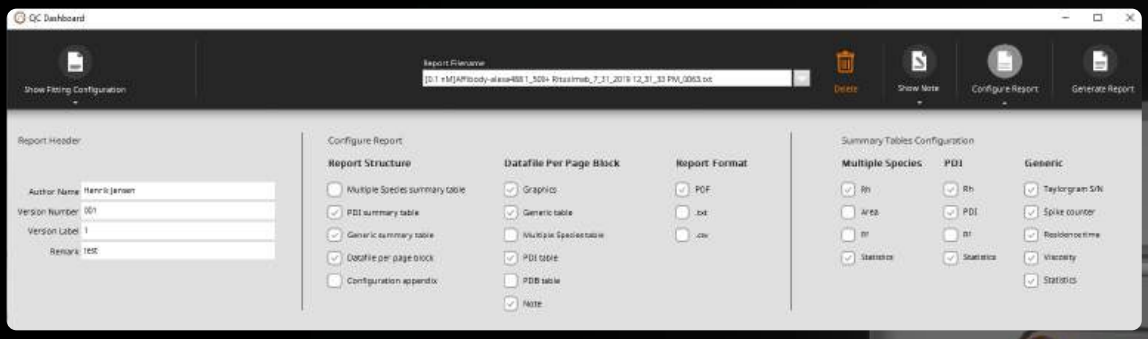
Sample Quality Control Module

CUSTOMISABLE QUALITY CONTROL REPORTS

Reports that meet your requirements



Easy to implement in your workflow.



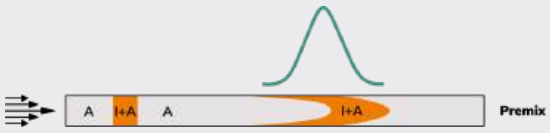
With Fidabio Quality Control Module you can custom make and export Quality Control reports of your samples. The data can be exported as a PDF report file with graphs included, or a .txt file, which is easily processed by any data analysis software.

04 IN-SOLUTION KINETICS

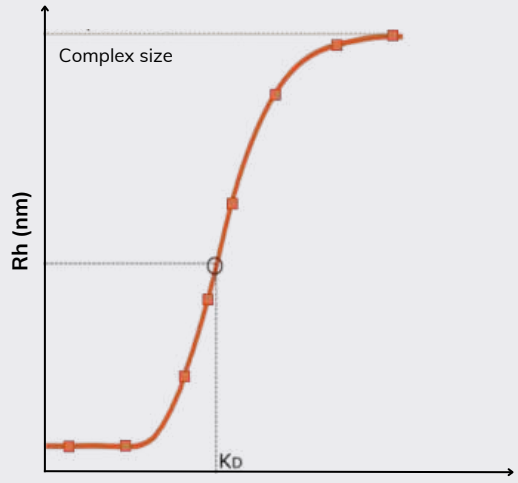
FIDA KINETICS TAKE ADVANTAGE OF THE TIME DEPENDENT ASPECT OF DIFFUSIVITY - THE VARIABILITY OF THE PRESSURE DRIVEN FLOW

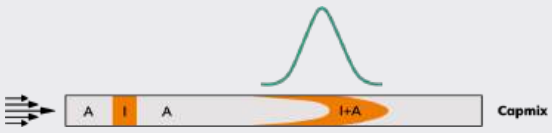
THIS IS HOW IT LOOKS LIKE IN PRINCIPLE:

Mixing principles (inside of the capillary)

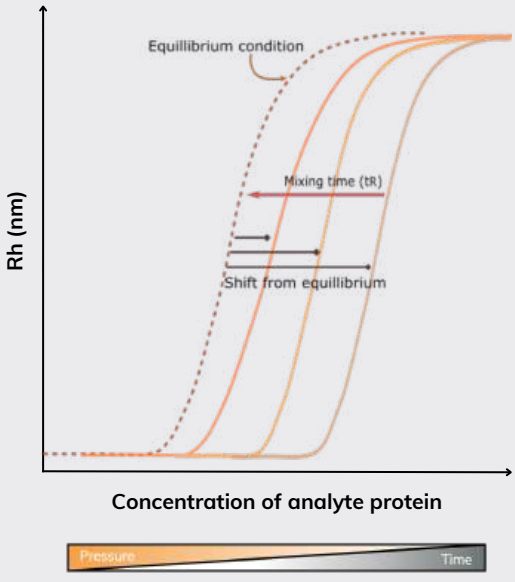


EQUILIBRIUM BINDING CURVE





SHIFTED KINETICS CURVE



Which can be represented as:

$$Dapp(t) = \underbrace{D_I + (D_{AI} - D_I) \cdot \frac{A_T}{AT + K_d}}_{\text{"At equilibrium"}} + \underbrace{(D_I - D_{AI}) \cdot \frac{AT}{A_T + K_d} \cdot e^{-(k_{off} + k_{on} \cdot A_T) \cdot t}}_{\text{"Time-dependent"}}$$

HOW WILL YOU BENEFIT FROM IN-SOLUTION KINETICS?

05

No environmental restrictions

Seamlessly operate in **complex matrices including fermentation media, plasma or serum - no purification required.**

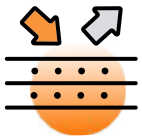
Avoid non-specific binding

No steric hindrance to high density immobilised ligands
No non-specific binding issues
No risk of re-binding

No restrictions on detergents, ionic strengths, temperature, pH etc.

Minimise assay development time
Expand the scope of biological systems you can characterise
Increase environmental relevance

No need for regeneration



With FIDA there is no surface chemistry involved.
Eliminate the risk of denaturing immobilised protein
Rapidly determine slow off rates for high affinity interactions

No surface mass transport limitations

No surface mass transport limitations at high concentrations or slow flow conditions. This allows you to extend your research capacities.

Detect Strong & Weak Binders

FIDA is capable of measuring kinetics of both strong and weak interactions in-solution.

06 APPLICATIONS LANDSCAPE

In-solution validation of structural information.

PDB correlator

The Fida 1 readout (Rh) provides an absolute size measure (in nanometres), which is directly linked to protein structure and function.

Fida 1 software, includes a PDB correlator, which directly compares measurements obtained in solution or under native conditions (Rh) with structures obtained from X-ray, Cryo-EM, NMR, or AlphaFold.

COMPLEX INTERACTIONS

Bi-/Multi-specifics

- Assessment of multiple Kd's in complex binding events.
- Option of **deconvoluting multi-complex** binding events.
- Cooperativity factor.

Stoichiometry/ Oligomeric state

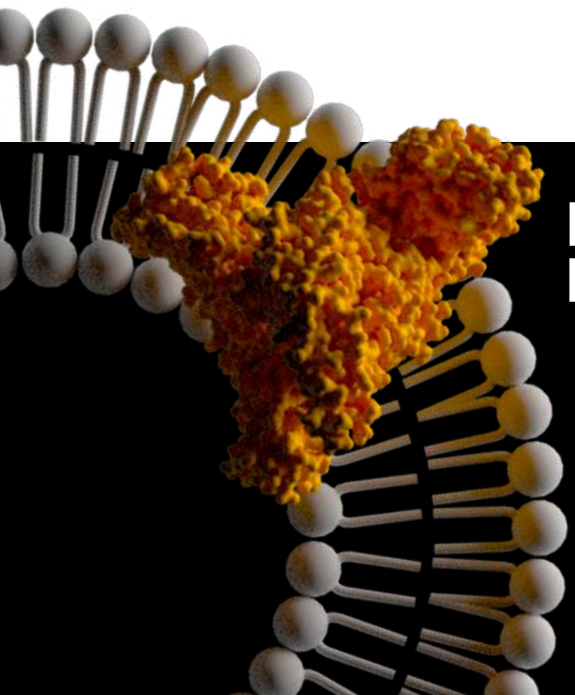
- Absolute read-out in nanometres facilitates reliable insights into stoichiometry of binding events.

Protacs/Molecular glues

- Ternary complex formation **in buffer and in cell lysate.**
- Assessment of **individual Kd's** in the ternary complex.
- Assessment of "**fraction bound**" of your protein
- Cooperativity factor.
- Ubiquitination assay.

STRUCTURAL BIOLOGY

- Structural biology.
- Cryo-EM and x-ray sample QC at high concentrations.
- PDB correlator.
- Nanobody binding.



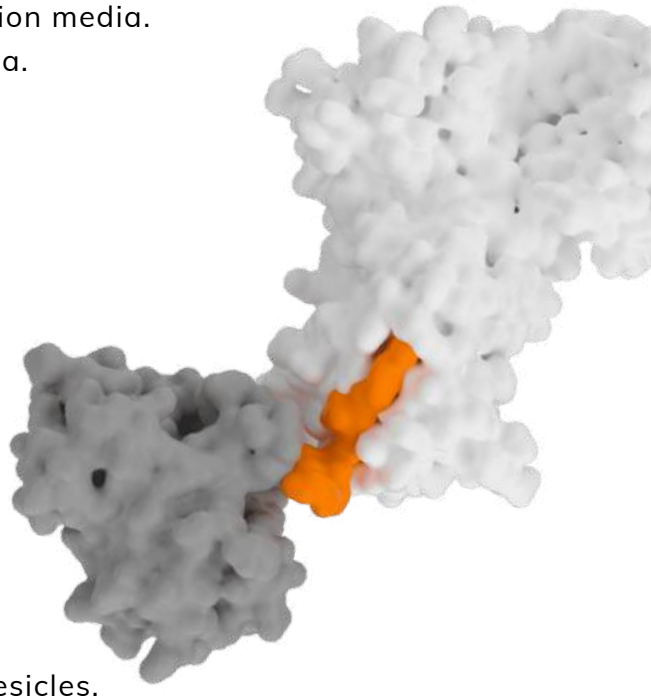
MEMBRANE PROTEINS

Characterization & screening

- Detergent screening uses minimal sample amount in a rapid automated assay.
- Binding assays with unpurified membrane preps.
- Particularly for crude membrane preps, it is significant that Fida 1 enables you to keep your samples at 5°C in the autosampler whilst performing your actual measurement at room-temperature.

QUANTIFICATION

- **Biomarkers** in plasma/serum/cell lysate.
- **Auto-antibodies** in plasma/serum.
- **Bioprocessing** - Therapeutic proteins in fermentation media.
- **Nanoparticles** in plasma/serum/fermentation media.



PARTICLES

- Particle size.
- Polydispersity index.
- Aggregation.
- Binding characterization.

CELL & GENE THERAPY

- gRNA-nuclease complex formation.
- gRNA-nuclease **Kd** determination.
- **Shield protein** characterisation on drug delivery vesicles.

CLONE SELECTION

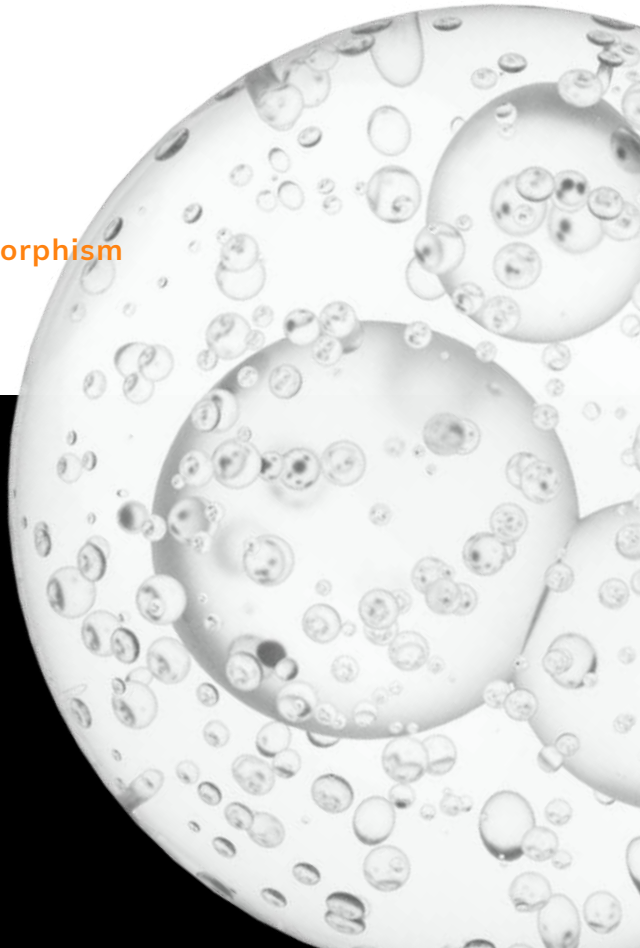
- Clone selection using FIDA is based on **both titre and affinity.**
- Both parameters can be obtained **simultaneously**, without purification of the cell supernatant/cell lysate.

AMYLOID FIBRILS

- In-depth fibril aggregation kinetics
- Thermodynamic characterisation of **amyloid polymorphism**
- Robust sizing and binding

LIQUID-LIQUID PHASE SEPARATION

- Phase diagrams.
- Dilute-phase concentration.
- Kd of LLPS modulating components.
- Relative droplet size distribution.
- Droplet to amyloid transition.



07 FIDA IN A NUTSHELL

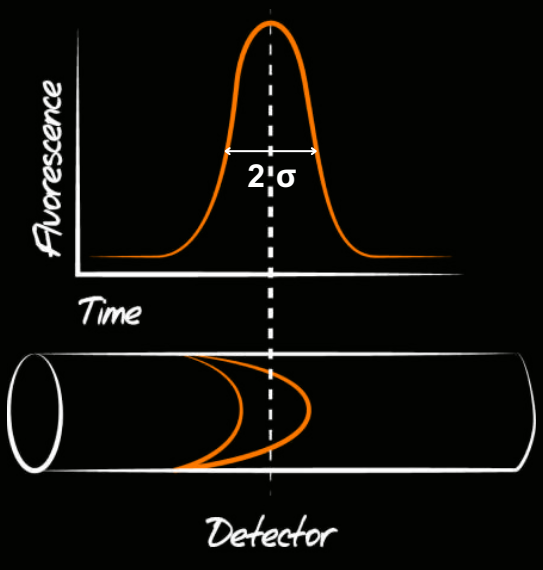
FIRST PRINCIPLE THINKING

FIDA technology is a "1st Principle" technology. It means that FIDA does not dependent on a priori assumption or on empirical calibration. It uses first principles of physics and fluid mechanics to analyse the movement of particles in a fluid. This brings simplicity and robustness straight into the users' lab.

HOW DOES IT WORK?

FIDA measures fluorescence of particles in the laminar flow and analyses their dispersion over time, which allows for calculation of the hydrodynamic radius of a particle of interest. The two basic principles used are Taylor Dispersion and Laminar Flow.

FLOW
INDUCED
DISPERSION
ANALYSIS



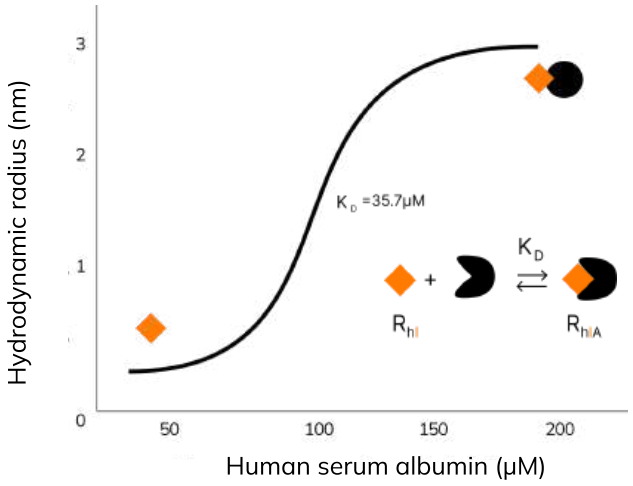
The sample of interest is passed through a thin capillary. Due to the difference in velocity between the walls and centre of the capillary, the sample shapes into a parabolic profile. Molecules diffuse radially, away from the flow axis. The fluorescence emitted by the molecules is acquired as a Gaussian signal by a high sensitivity detection system and is plotted against time. The size of the molecules in the sample determines their radial diffusivity, which in turn defines the extent of sample's dispersion.

FIDA can detect size changes smaller than 5%.

Scan to see how it works!

A DIVE INTO DETAILS

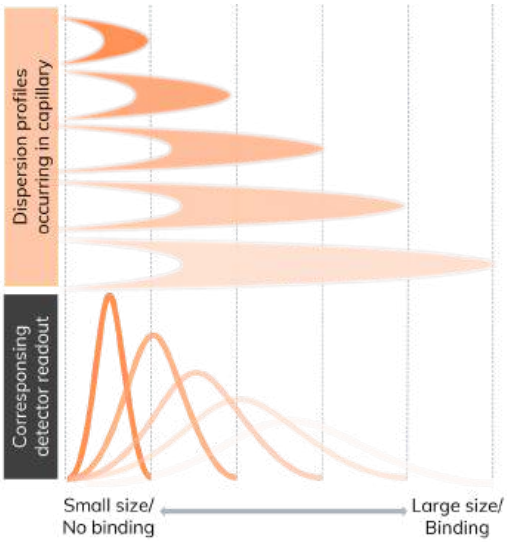
Visit us on fidabio.com to access our literature, knowledge base or simply send us a question.
We are always happy to jump on discovery calls!



As molecules bind the hydrodynamic radius of the complex increases.

With titrating series affinity is easily retrieved.

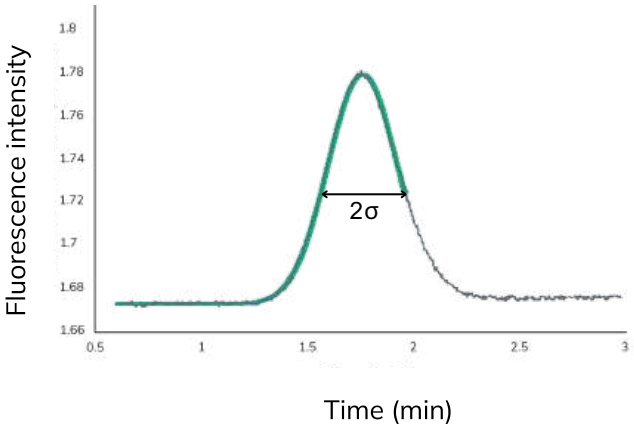
Diffusivity of a molecule or complex is inversely proportional to its hydrodynamic radius.
Thus, for larger complexes the diffusivity is slower and for small complexes faster.



FIDA is based on Taylor's dispersion phenomenon.

$$D = \frac{a^2}{24\sigma^2} t_r$$

$$R_h = \frac{k_b \cdot T}{6 \cdot \pi \cdot \eta \cdot D} t_r$$



09 TECHNICAL SPECIFICATIONS

and instrument characteristics

Detection technology	Fluorescence - multiple wavelengths available: UV (label free), 480, 640
Size accuracy	5%
Kinetics	sec-hrs
Dissociation constant (*K*):	pM - mM
Size detection	Rh of 0.5 - 500nm
Signal-to-noise ratio	> 30
Assay control	Built-in Quality Control parameters
Sample capacity per run	Up to 2 x 96 samples
Pressure range	1 - 3500 mBar
Autosampler temperature control	5°-55°C (41°-131°F)
Capillary chamber temperature control	15°-55°C (59°-131°F)
Capillary types	Fused silica; dynamic coatings or permanently coated
Power	120-240VAC, 50/60Hz
Operating system	Windows

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