Applications of FIDA in Protein Science

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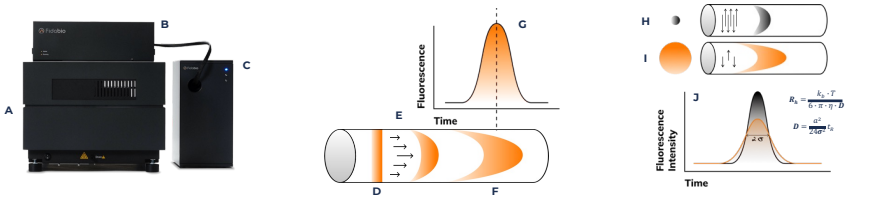
PEAK PROTEINS
A SYGNATURE DISCOVERY BUSINESS

1 Flow-Induced Dispersion Analysis

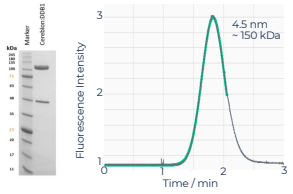
- **FIDA** allows **rapid measurement** of **protein size (R_H)**
- **Proteins** and **complexes** are **analysed in solution** - no need for immobilization
- Samples can be **fluorescently-labelled** or **label-free** using intrinsic fluorescence
- In some applications it can use as little as **40 nL of sample per run**



Meet Fida1. This precision instrument, comprising an autosampler (**A**), capillary chamber (**B**), and detector (**C**), uses carefully controlled pressure to load a 75 micron x 1 meter capillary with assay buffer. Next, it injects a 40 nL sample plug (**D**) that embarks on a journey through the capillary under laminar flow (**E**). As the plug traverses the capillary it's distorted by the different flow rates (**F**), producing a Gaussian peak (**G**) when it passes through the detector window. While the plug moves along, the molecules in it engage in radial diffusion. The smaller ones (**H**) zip around and are barely impacted by laminar flow, while the larger molecules (**I**) take it slow and feel the flow rate variations more intensely. The width of the eluted peak (**J**) is proportional to the diffusion rate, so we can determine the hydrodynamic radius of the sample by measuring the peak width. Figures adapted from fidabio.com

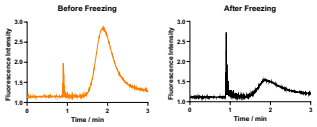
2 Soluble Proteins

FIDA allows rapid protein QC without needing tags or labels



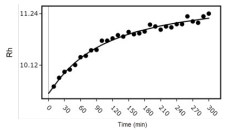
- **Rapid measurement** of protein size
- R_H can **confirm complex formation**
- Sample concentration from 0.25 – 20+ mg/ml

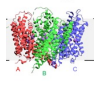
- Proteins can aggregate and complexes fall apart over time - or on freezing
- FIDA can check that samples still look as they should in as little as **6 minutes**
- **40 nL sample consumption** lets you analyse the exact aliquot you're using
- **Aggregates** appear as spikes



3 Membrane Proteins

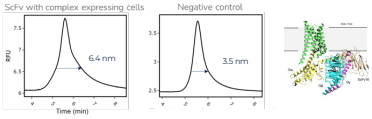
FIDA has no buffer restrictions, enabling work with detergent-solubilised membrane proteins with just a few tweaks to the method





- **Complex disassociation / protein unfolding** could be measured by an **increase in R_H**
- **Temperature-controlled autosampler** gave confidence that this was a real effect
- 15-minute run times allowed **rapid analysis** of samples and **real-time method optimization**

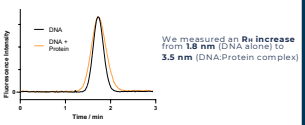
- FIDA can be applied to **complex, challenging systems**
- **Functional** GPCR:G-Protein complexes can be detected using a **fluorescently labelled antibody**
- Despite **sticky proteins, excess antibody and detergent** we can detect these complexes in **crude cell lysates**



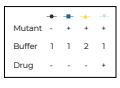
4 Affinity Determination

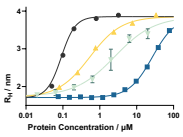
FIDA can measure the affinity Protein:Protein or Protein:Biomolecule interactions

- An existing Fluorescence Anisotropy assay was transferred to FIDA
- Proof-of-concept in **under 1 hour** using FAM-labelled DNA



We measured an R_H **increase** from **1.8 nm** (DNA alone) to **3.5 nm** (DNA:Protein complex)



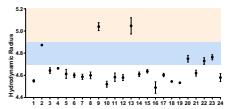


- **Dose-response** effect observed and **K_D measured**
- Buffer modifications, point mutant, and compound binding **effects can be compared**

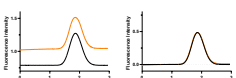
5 Small Structural Changes

FIDA can sometimes measure Protein:Ligand interactions – you just need a change in size

- Compound binding to target induces a **small structural change** increasing R_H by **0.4 nm**
- Batches of 24 compounds were screened at a single concentration
- Automatic **background correction** means fluorescent compounds don't disrupt the analysis
- **Label-free analysis** used **5 µL protein** at 0.5 mg/mL – only **2.5 µg protein per compound**
- Measured size change **was consistent with crystal structures solved at Peak Proteins** in parallel
- Changes in affinity can be distinguished from changes in effect by follow-up titration studies



FIDA measures a **physical property** so we can **eliminate misleading results** (such as compounds that **trigger aggregation**) leaving you with **true hits**



High background signal is automatically corrected without affecting the measurement

A versatile platform for protein characterisation

- FIDA enables rapid, in-solution measurement of protein size, behaviour and stability – often with minimal sample consumption
- Strategic experiment design allows us to study both functional and structural properties using a single instrument
- FIDA complements our robust protein production, structural characterisation and mass spectrometry platforms, offering valuable insights into protein behaviour

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