Fluidic Analytics

The one-w ith history in the making.

www.fluidic.com

fluidity one-w

WHAT DOES FLUIDITY ONE-W DO?

The Fluidity One-W's unique capabilities allow you to study protein complexes and their formation in crude biological backgrounds such as cell lysates or blood plasma.

Absolute size measurement of protein complexes by the Fluidity One-W helps to confirm the identity of your complex and means you can control for off-target binding and false positive measurements.

Combining minimal sample preparation and automatic K_D calculation, you can confidently analyse your protein at the earliest possible stage, reducing the time for important go/no-go decisions.

- ✓ Fluidity One-W early stage analysis means you can choose the correct experiment sooner, helping you save time and money.
- ✓ Monitor protein binding events entirely in solution, with confidence that you're on target, eliminating any artefacts and allowing you to study protein binding in near native state.

EARLIER STAGE UNDERSTANDING IN A VARIETY OF APPLICATIONS

Analyse protein interactions in complex backgrounds

Study protein–protein, protein–DNA and protein–lipid interactions in biological mixtures such as crude lysates or blood plasma. No surface binding means no artefacts so you can study proteins in near native state with no risk of non-specific binding.

Work with challenging proteins

Because there is no surface attachment, it is possible to study challenging proteins including membrane proteins, multi-protein complexes, and intrinsically disordered proteins.

Determine stoichiometry and conformational arrangement

Absolute size measurements allows you insight into the conformation and stoichiometry of proteins and their complexes.

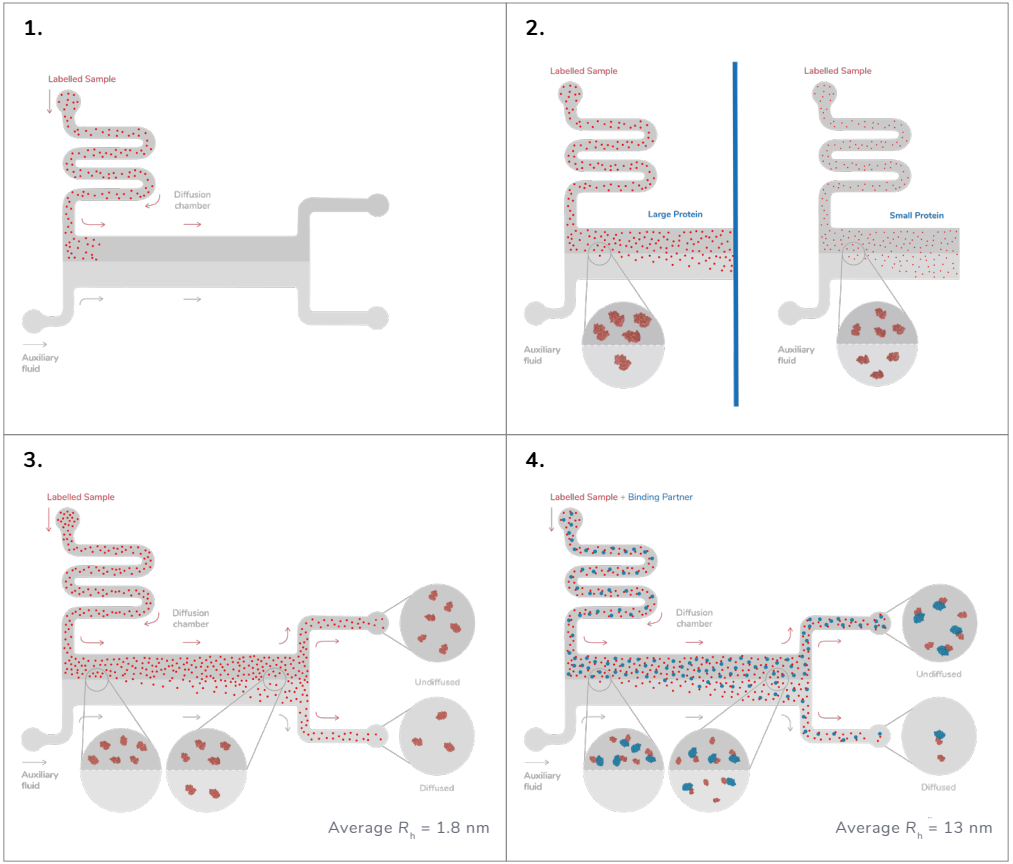
HOW DOES IT WORK?

The Fluidity One-W measures the rate of diffusion of proteins under steady state laminar flow in a microfluidic chip — a technique known as microfluidic diffusional sizing (MDS).

1. To do this, a stream of fluorescently labelled protein is introduced alongside an auxiliary stream.
2. These streams flow in parallel and because there is no convective mixing the only way protein can migrate into the auxiliary stream is by diffusion, the rate of which depends on the size of the protein. Small proteins will diffuse rapidly, and large proteins and aggregates more slowly.
3. At the end, the streams are re-split, and at this point the degree of diffusion is fixed. The quantity of protein in each stream is then determined by the fluorescence from the label. The ratio of the fluorescence between the two streams gives the protein's hydrodynamic radius (R_h).

   Fluidity One-W can measure proteins in buffer and in crude solutions like cell lysates or biological fluids, because only the labelled species is detected.
4. If the test is repeated using a mixture of labelled protein and unlabelled binding partner the degree of binding can be observed, due to the change in average size. Only species including the labelled protein are detected and measured.

   Titrating the binding partner against the labelled protein gives a binding curve and automatically generates a K_D value. The hydrodynamic radius for the unbound protein and protein complex are also calculated.


1.
Labelled Sample
Diffusion chamber
Auxiliary fluid
2.
Labelled Sample
Large Protein
Auxiliary fluid
Labelled Sample
Small Protein
Auxiliary fluid
3.
Labelled Sample
Diffusion chamber
Undiffused
Auxiliary fluid
Diffused
Average R_h = 1.8 nm
4.
Labelled Sample + Binding Partner
Diffusion chamber
Undiffused
Auxiliary fluid
Diffused
Average R_h = 13 nm

UNDERSTAND YOUR PROTEIN EARLIER

Assess binding in native conditions

By measuring your proteins and their interacting partners in a close-to-native, biological background, you can expect more transferable results that accurately represent natural biological processes.

Look at the hard stuff

Solution state measurement coupled with high sensitivity means you can study proteins that are problematic for other systems including membrane proteins, multi-protein complexes, and intrinsically disordered proteins.

STUDY PROTEINS WITHOUT ANY HASSLE

Study proteins in any solution

Work in crude biological mixtures with minimal preparation, while controlling for off-target binding. Gain valuable insights at the earliest possible stage.

Avoid wasted downstream processing

Assess the quality of your protein complex even in early stage fractions, allowing you to optimize protocols early. Use insights on protein size, state and yield to rationalize how you move forward before you begin time consuming purification.

MORE INSIGHTS WITH LESS EFFORT

More for less

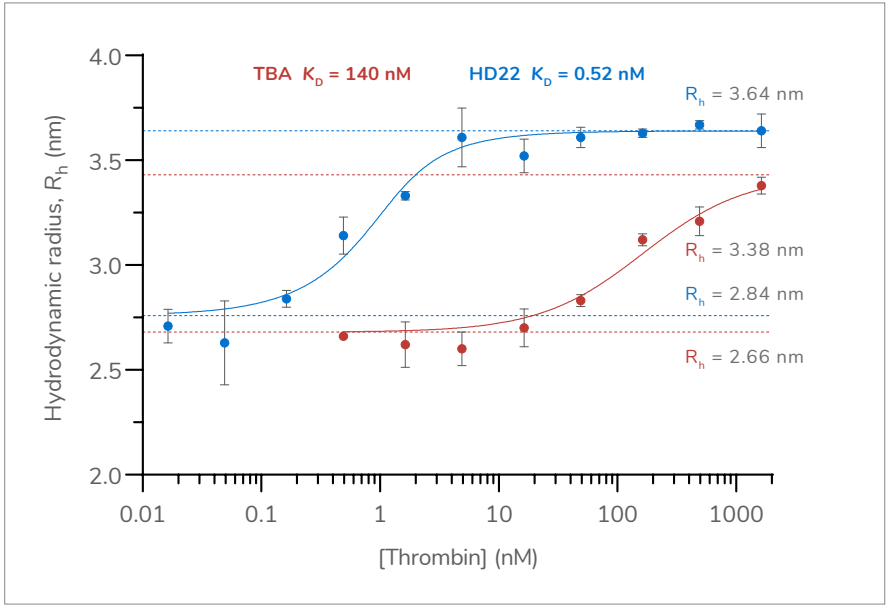
Gain meaningful insights into protein complexes and their formation with less sample and in less time.

Pipette, plug, play

The minimal sample prep, easy-to-use design and wide dynamic range means you can spend less time setting up, and more time generating the data you need.

FLUIDITY ONE-W FEATURES

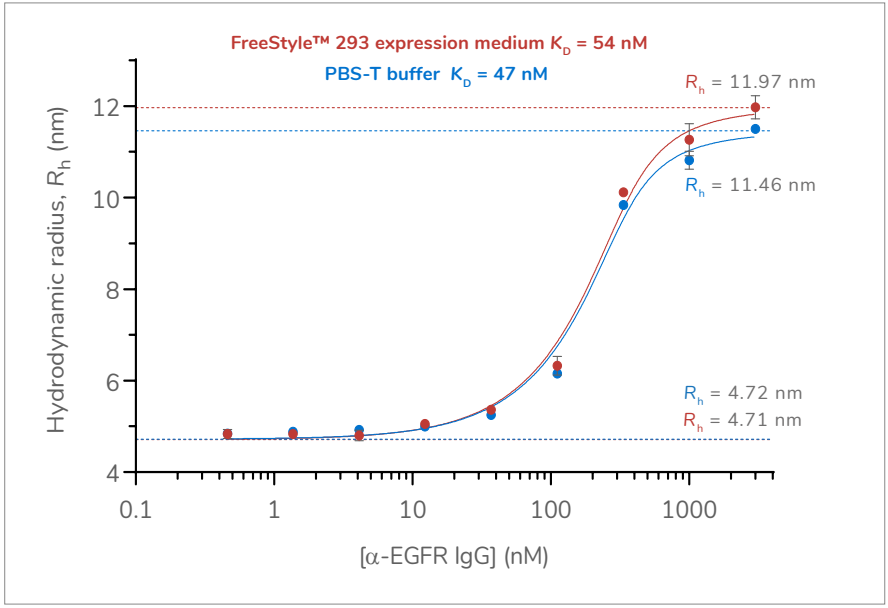
- **In-solution, on target determination of complex formation** in a single measurement using microfluidic diffusional sizing (MDS)
- **Low sample requirement** of just 5 µL per sample
- **High reproducibility and accuracy** even at low concentrations
- **Easy-to-use** interface and consumable management
- **Wide dynamic range** allows you to determine nM to mM K_Ds
- **Obtain an automatically calculated** K_D after a simple titration
- **Disposable single use chips and contained waste** minimize risk of cross contamination between measurements and reduce cleaning and setup times
- **Sizing of any fluorescently labelled molecule** — proteins, lipids, carbohydrates, oligonucleotides, polymers or nanoparticles
- **Broad buffer compatibility** suitable for use with any biologically compatible buffer, even those containing detergents
- **Over the air software updates** deliver ongoing system improvements
- Manufactured under **ISO 9001** quality management system



INTERACTIONS OF PROTEINS WITH SECONDARY MOLECULES

Using microfluidic diffusional sizing (MDS) the Fluidity One-W can calculate the binding affinity (K_D) for protein interactions as well as the hydrodynamic radius (R_h).

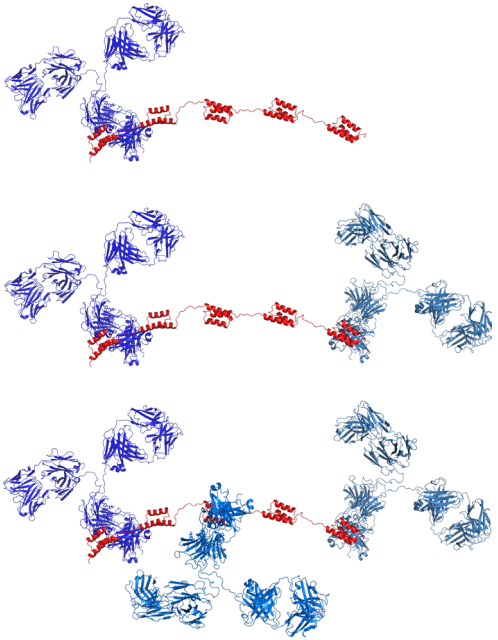
In this graph the serine protease thrombin was titrated against two pre-labelled anti-thrombin aptamers — HD22 and TBA. Each aptamer binds to a different epitope of thrombin; these interactions have previously been well characterised. The binding affinity of each aptamer-protein complex and hydrodynamic radii of each complex and aptamer were identified and are in good agreement with previously reported values.

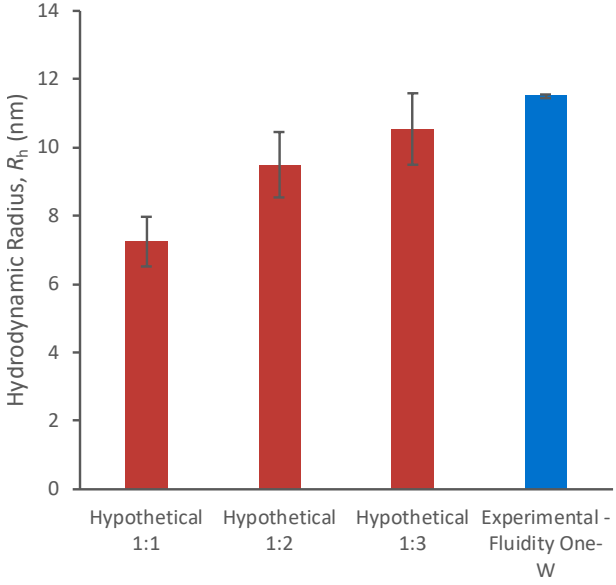


STUDY PROTEIN INTERACTIONS IN COMPLEX BACKGROUNDS

The Fluidity One-W can study protein interactions in crude biological backgrounds, saving time in purification as well as allowing the study of more difficult interactions in their native conditions.

The graph compares the interaction of Protein A (SpA) with immunoglobulin G (IgG) antibody in Phosphate buffer solution with 0.05% Tween 20 (PBS-T) and in FreeStyle™ 293 expression media. It shows there is no significant difference in binding affinity or hydrodynamic radius between each solution.




Hydrodynamic Radius, R_h (nm)
14
12
10
8
6
4
2
0
Hypothetical 1:1
Hypothetical 1:2
Hypothetical 1:3
Experimental - Fluidity One-W

CHARACTERIZING THE STOICHIOMETRY OF PROTEIN COMPLEXES

Characterizing the stoichiometry of protein complexes is essential to fully understand cellular pathways; from fundamental biological processes, to disease pathology and the action of drugs.

In the process of calculating the binding affinity (K_D) of an interaction the Fluidity One-W provides the hydrodynamic radius (R_h) of both the unbound labelled species and the complex. The hydrodynamic radius can be used to infer the stoichiometry of the protein complex and the overall conformational arrangement in solution.

In the models to the left, PyMOL was used to render SpA-IgG complexes at binding ratios of 1:1, 1:2 and 1:3. The red ribbon represents SpA and the blue ribbons represent IgG. Using these models three hypothetical R_h values (red bars) were calculated. These values were compared to the experimentally measured R_h on the Fluidity One-W, in triplicate at 100 nM (blue bar). Error bars of 10% of the predicted R_h values have been added to indicate the minimal uncertainty in the models. The experimental R_h obtained by MDS analysis on the Fluidity One-W agrees with the hypothetical R_h of a 1:3 SpA-IgG complex.

JÖNS JACOB BERZELIUS: THE ONE-WHO HISTORY THANKS

Jöns Jacob Berzelius (20 August 1779 – 7 August 1848) was a Swedish chemist who led a remarkable life that laid the very foundations of how we study science today. Along with Robert Boyle, John Dalton and Antoine Lavoisier, Berzelius is considered to be a founder of modern chemistry. He was a strict empiricist and insisted that any new theory be consistent with the sum of scientific knowledge, similar to the scientific method we use today.

Born in Östergötland Sweden, Berzelius enrolled at Uppsala University where he became a doctor at the age of 23. In 1807 he was appointed professor of medicine and pharmacy at the medical college in Stockholm, which later became the world renowned Karolinska Institute. This is where he spent his career devoted to scientific research. He was also elected as a member of the Royal Swedish Academy of Sciences and was credited with revitalising the Academy and ushering in a second golden era.

Not content with investigating solely his own theories, Berzelius also scrutinized theories put forward by others which were lacking in empirical evidence. To test these theories he developed novel techniques and instruments. One famous example of this was his validation of Joseph-Louis Proust's theory of stoichiometry.

Additionally, the word “protein” was first used by Berzelius, coining the term in his correspondences with the Dutch chemist, Gerdays Johannes Mulder. Mulder went on to be the first scientist to publish a paper with the term "protein" included.

Jöns Jacob Berzelius was a scientist in the truest sense, a man who asked questions about the world around him and worked hard to answer them. We've got a lot in common with Berzelius. The Fluidity One-W was created to allow researchers to break new ground in protein science, and solve the tough biological challenges we face today.

So be more Berzelius. Be another One-Who history thanks.

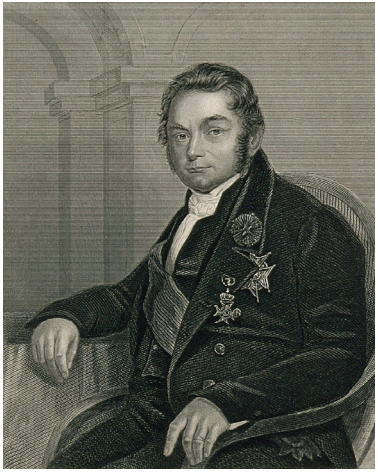


Image: Jöns Jacob Berzelius. Line engraving by C. W. Sharpe after O. J. Soedermark. Credit: Wellcome Collection. CC BY

OUR PEER REVIEWED PAPERS

Yates et al. **Nature Chemistry** 2015;7;802–809 **|** Arosio et al. **ACS Nano** 2016;10;333–341 **|** Arosio et al. **Anal. Chem** 2016;88;3488–3493 Herling et al. **Biophysical Journal** 2016;110;1957–1966 **|** Zhang et al. **ChemBioChem** 2016;17;1920–1924 **|** Lapinska et al. **Phys Chem Chem Phys** 2017;19;23060–23067 **|** Saar et al. **Lab Chip**, 2018;18;162–170 **|** Wright et al. **Biochemistry** 2018;57;3641–3649 **|** Falke et al. **Chemistry and Physics of Lipids** 2019;220;57–65 **|** Scheidt et al. **Science Advances** 2019;5;eaau3112 **|** Gang et al. **Anal. Chem** 2018;90;3284–3290 **|** Macikova et al. **The FEBS Journal** 2019;286;3664-3683 **|** Wright et al. **Analyst** 2019;144;4413-4424

SPECIFICATIONS

Sizing	Range (hydrodynamic radius)	0.7 – 20 nm
	Range (molecular weight)	0.5 kDa – 14 MDa
	Accuracy	± 10%
	Precision	CV < 10%
	Sensitivity	1 nM Alexa Fluor™ 488
Operational Specifications	Sample volume required	5 µL
	Run time	Small proteins and peptides – 8 minutes Large protein – 14 minutes
	Buffer compatibility	Compatible with pure buffer and crude lysates
	Runs per reagent cartridge	96
	Dimensions	40 x 40 x 43 cm
	Detection	Fluorescence
	Label compatibility	GFP, FITC, Alexa Fluor™ 488 and equivalents

SEE THE FLUIDITY ONE-W FOR YOURSELF

Go online to find videos, application notes and more.

Request a demo to try the Fluidity One-W in your own lab today at www.fluidic.com/demo

Fluidic Analytics

+44 (0) 1223 560 432 www.fluidic.com welcome@fluidic.com