Flow Induced Dispersion Analysis for Quantification of Proteins and Protein Ligand Interactions under Native Conditions

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[2]Statens Serum Institut

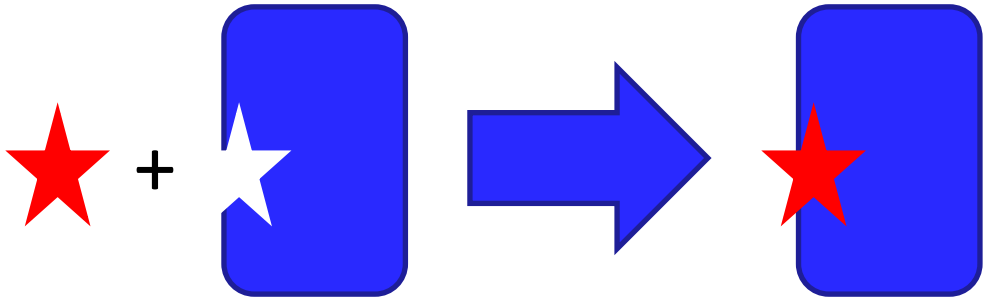
[3]FIDA-Tech Aps

Agenda

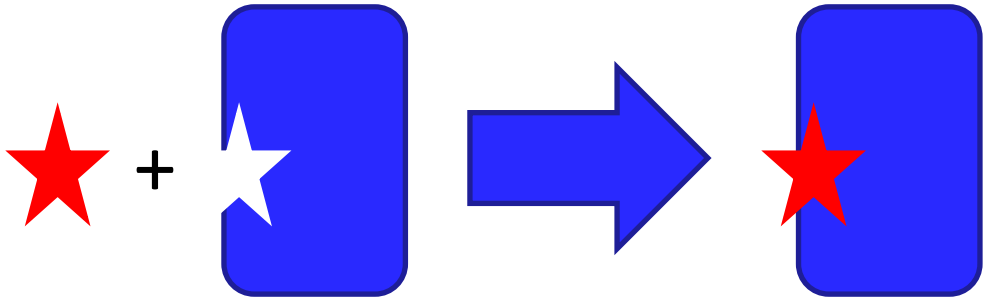
Measuring **hydrodynamic size** and **diffusivity** of proteins using Taylor dispersion analysis (**TDA**) in capillaries

Protein (protein-ligand) quantification by flow induced dispersion analysis (**FIDA**)

Protein (protein-ligand) quantification (ligand binding assays)



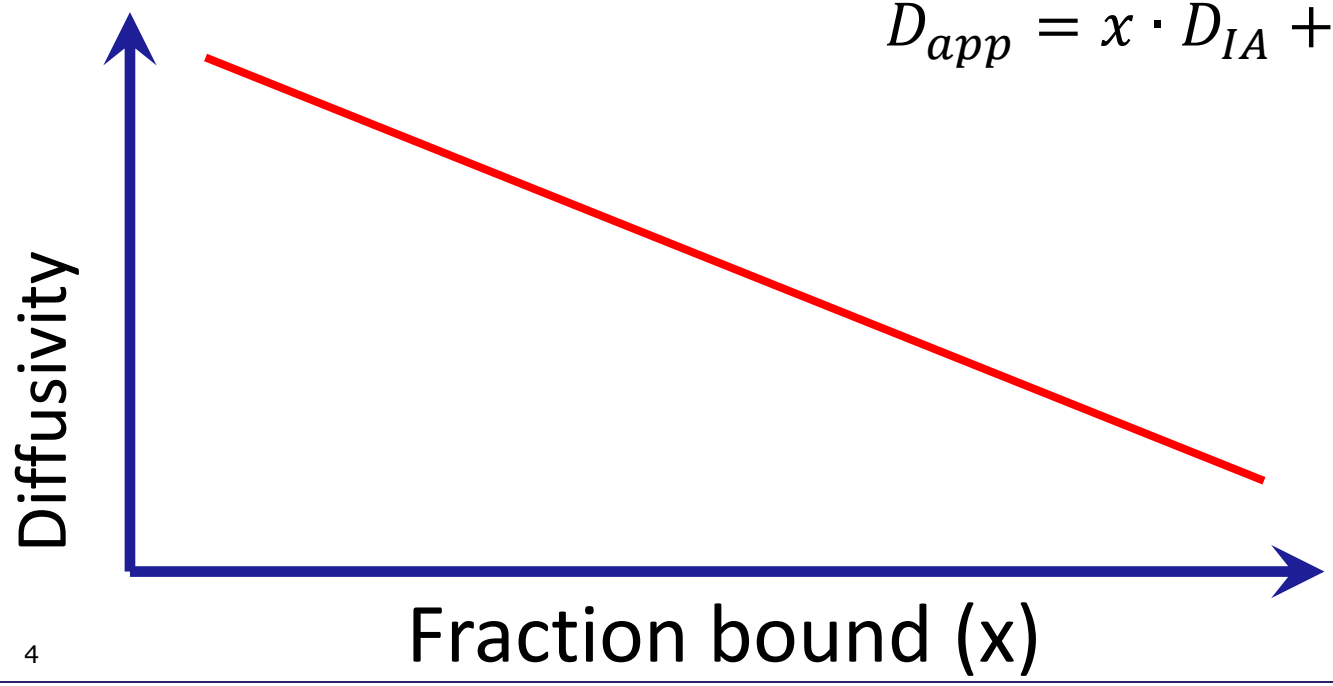
Indicator	+	Analyte	=	Complex
I	+	A		IA

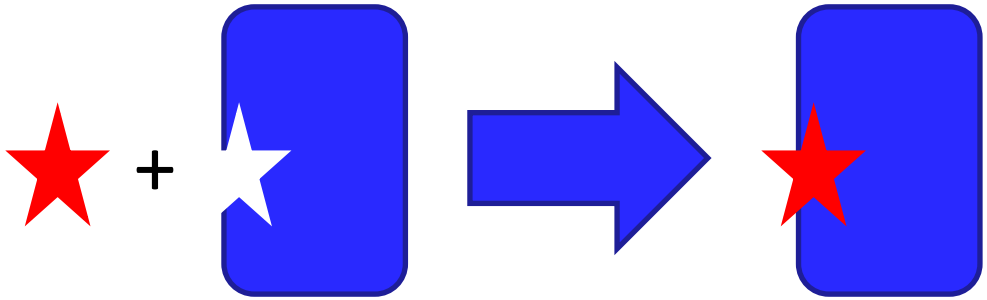


Indicator + Analyte = Complex

I + A IA

$$D_{app} = x \cdot D_{IA} + (1 - x) D_I$$

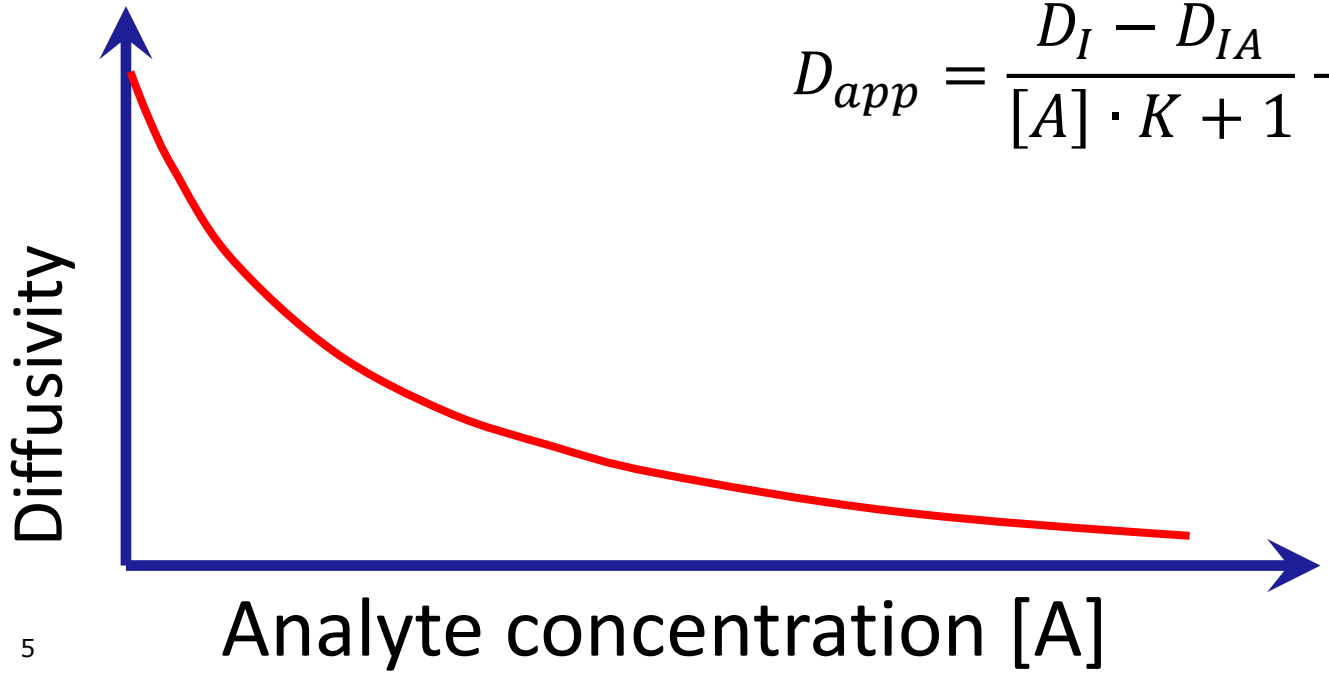


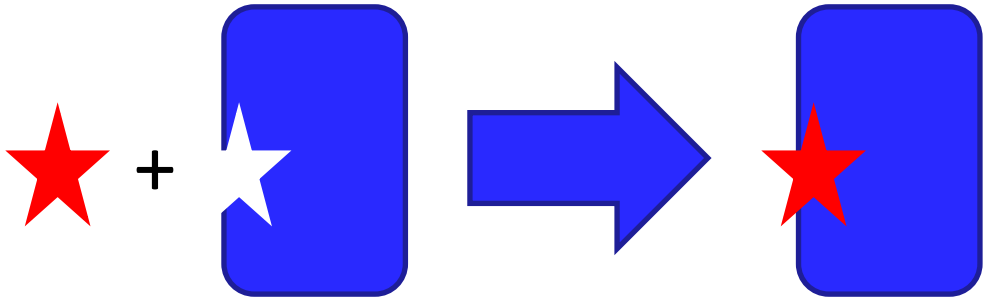


Indicator + Analyte = Complex

I + A IA

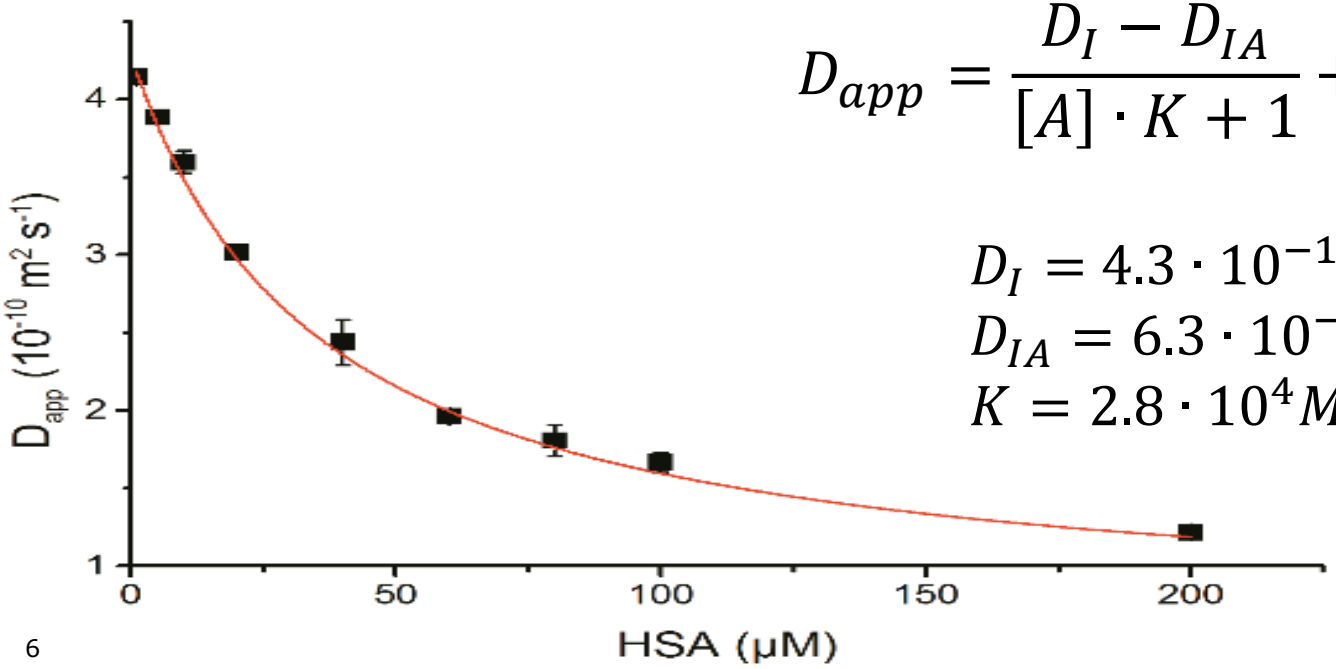
$$D_{app} = \frac{D_I - D_{IA}}{[A] \cdot K + 1} + D_{IA}$$





Fluorescein + HSA = Complex

I + A = IA



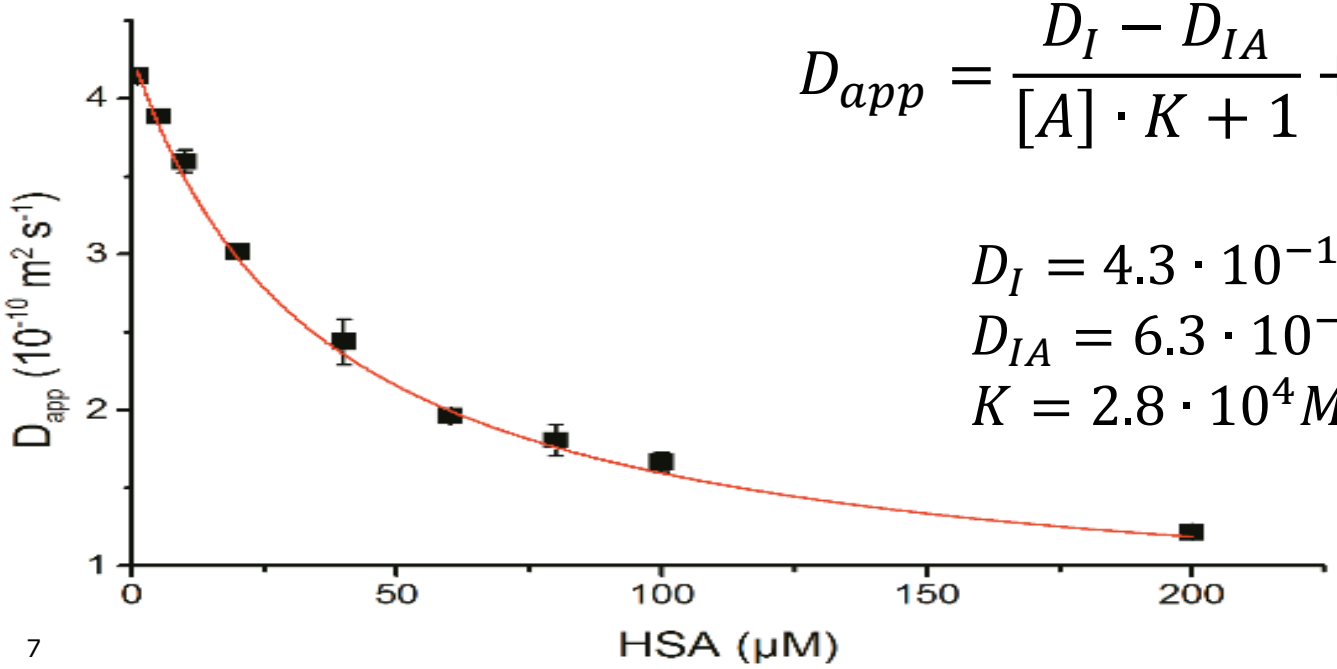
$$D_{app} = \frac{D_I - D_{IA}}{[A] \cdot K + 1} + D_{IA}$$

$D_I = 4.3 \cdot 10^{-10} m^2 s^{-1}$

$D_{IA} = 6.3 \cdot 10^{-11} m^2 s^{-1}$

$K = 2.8 \cdot 10^4 M^{-1}$

Plasma sample	HSA (g/l)	
	FIDA	BCP
1	33	32
2	30	27
3	32	32



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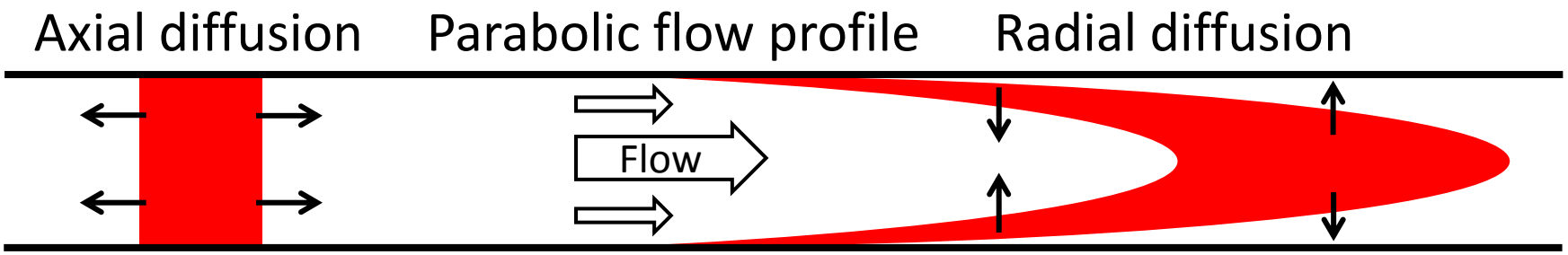
Nicklas N. Poulsen, Nina Z. Andersen, Jesper Østergaard, Guisheng Zhuang, Nickolaj J. Petersen and Henrik Jensen; *Flow Induced Dispersion Analysis Rapidly Quantifies Proteins in Human Plasma Samples* *Analyst*, **2015,** *140*, 4365-4369.

Taylor Dispersion Analysis

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



Theory: Taylor, G. *Proceedings of the Royal Society A: Mathematical, Physical and Engineering Sciences* **1954**, *225*, 473-477.
Taylor, G. *Proceedings of the Royal Society A: Mathematical, Physical and Engineering Sciences* **1953**, *219*, 186-203.

Taylor Dispersion Analysis

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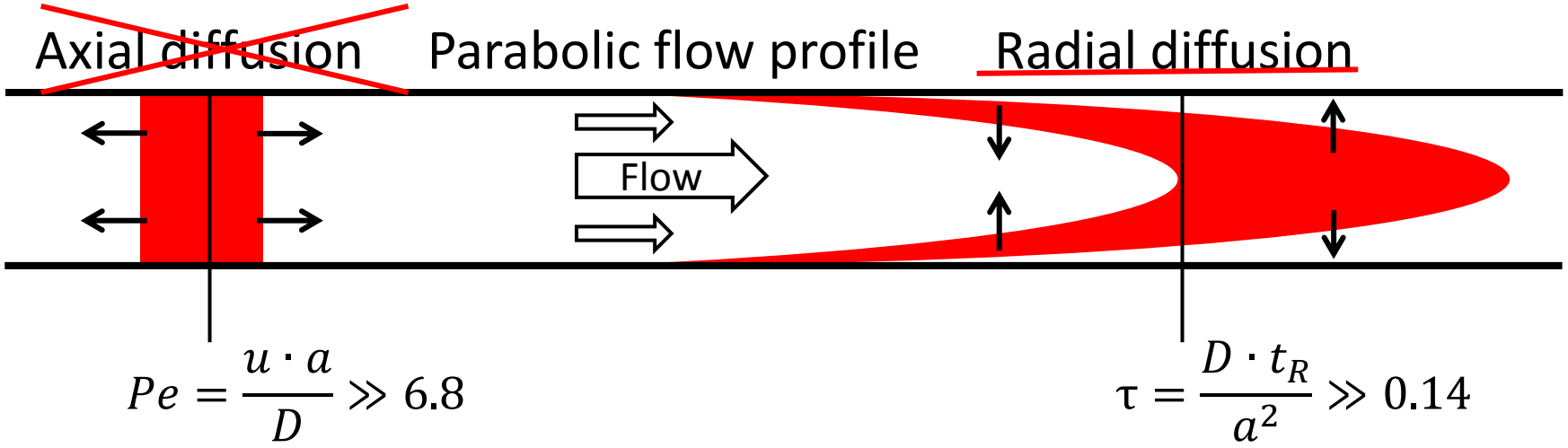
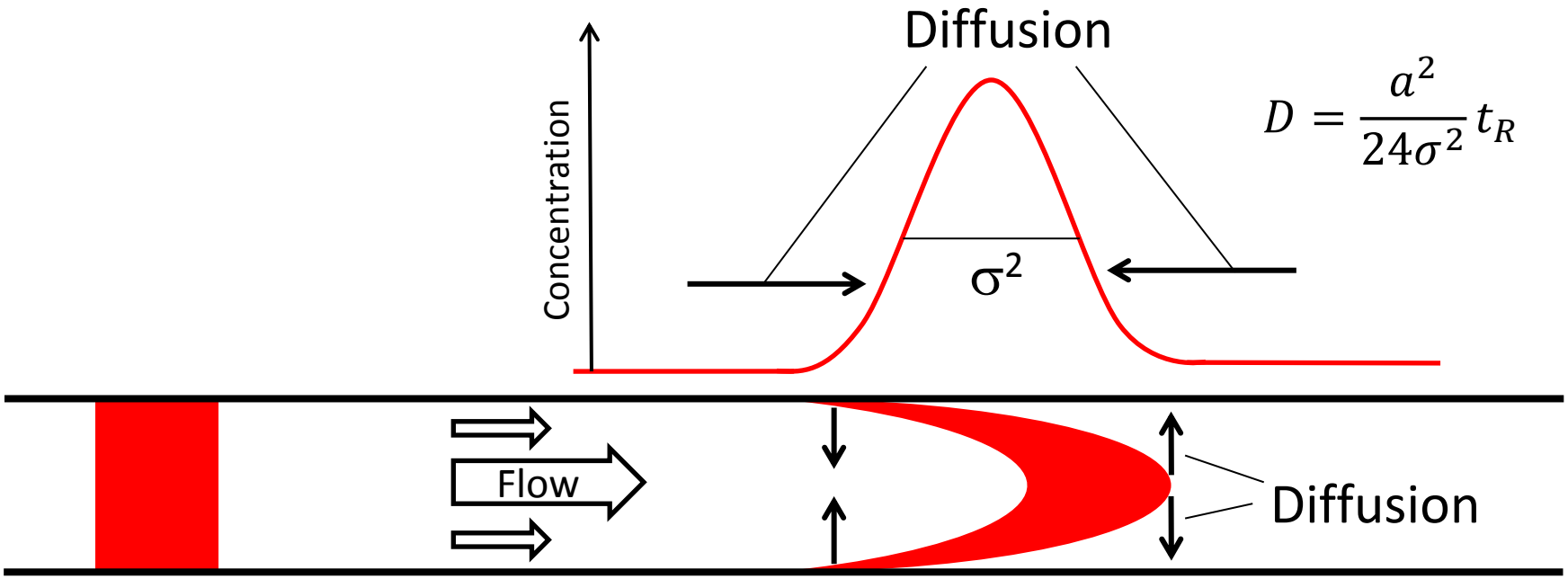


Experimental (capillaries):

Bello et al., Science, **1994**, 266, 773
Cottet, H.; Biron, J.-P.; Martin, M. *Analytical Chemistry* **2007**, *79*, 9066-9073
Sharma et al., Anal. Chem., **2005**, *77*, 806-813.

Taylor Dispersion Analysis



Hydrodynamic radius: $R_H = \frac{k_B \cdot T}{6 \cdot \pi \cdot \eta \cdot D}$

TDA - Finite element based modeling

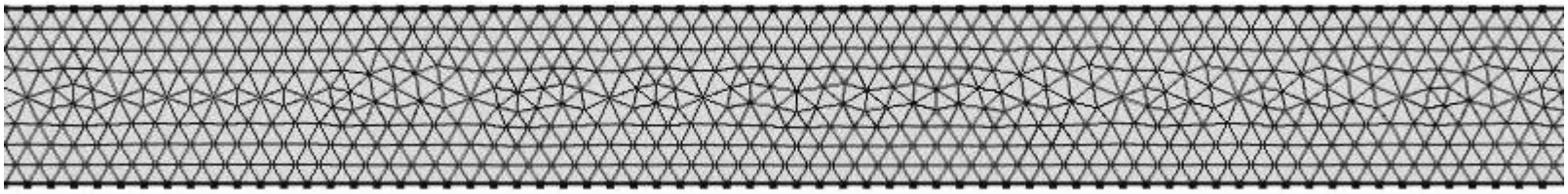
FEM based modeling allows numerical computations of coupled **physics** in complex **geometries**
(**complex formation – including kinetics**)

”**Physics**” could be diffusion (Ficks laws of diffusion), convection (for example a hydrodynamic flow described by the Navier –Stokes equation) and chemical reactions

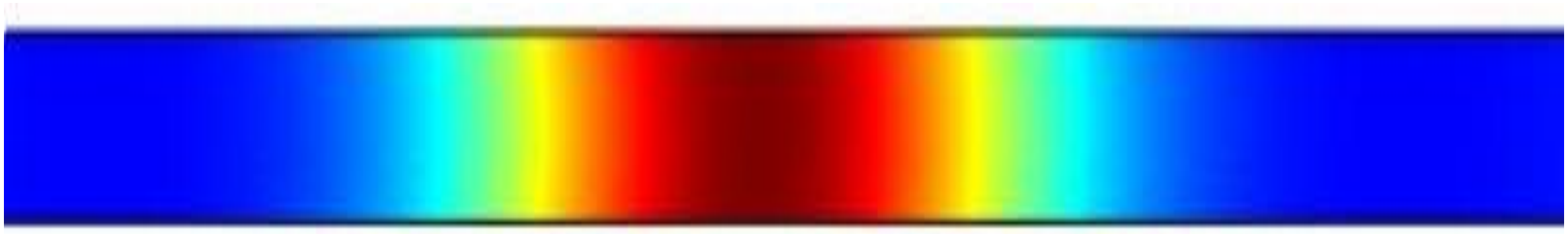
”**Geometry**” defines the border of the solution to the coupled physics.

TDA - Finite element based modeling

Why ***Finite*** element?



Physics are usually based on (coupled) **differential equations** which require **boundary conditions** to be solved (physical boundaries, initial concentrations, etc)



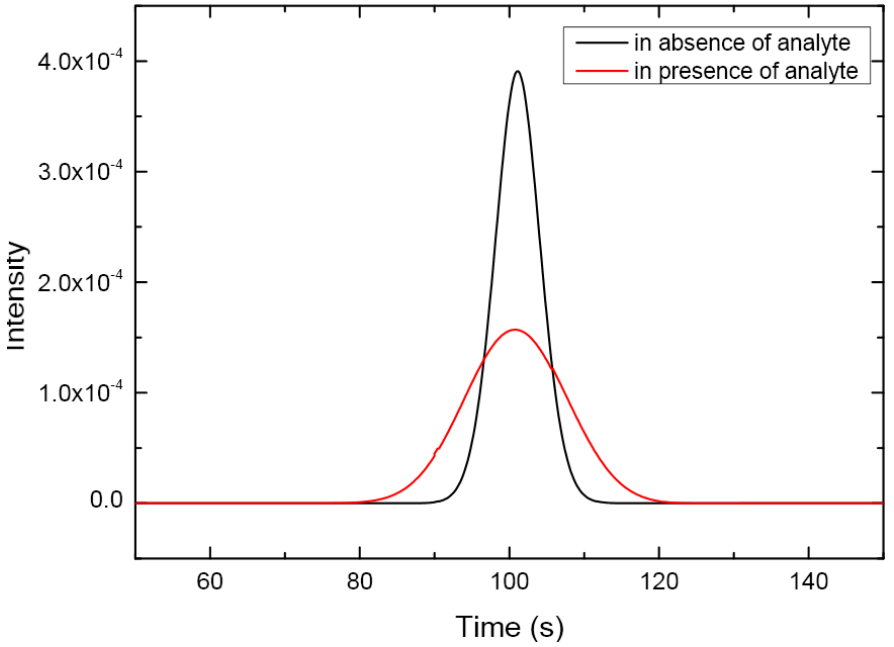
TDA - Finite element based modeling

Visual illustration of Taylor dispersion

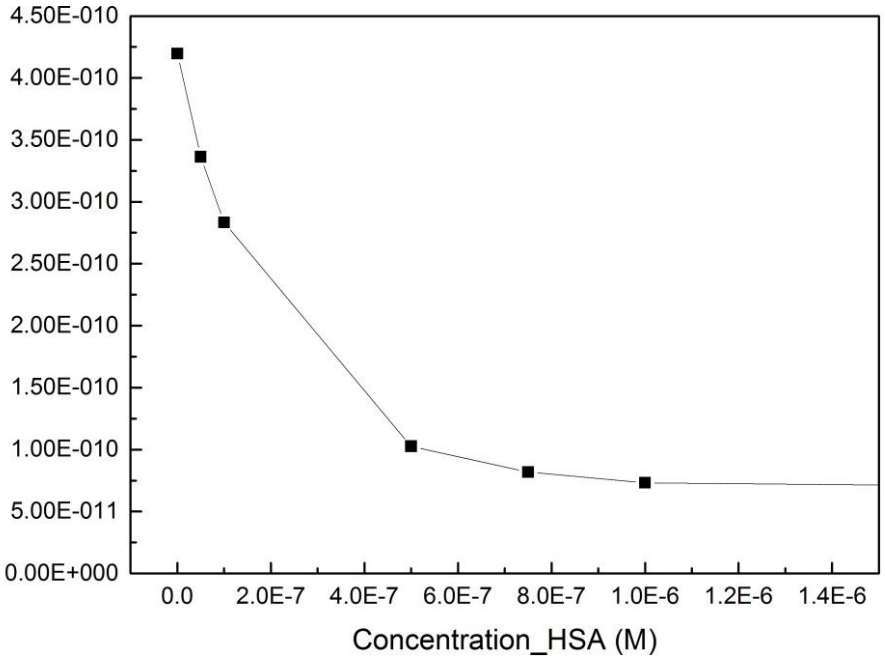
Simulating the effect of an interacting ligand

FEM simulations describes the impact of binding on dispersion

Taylorgrams corresponding to Fluorescein in the presence and absence of Human Serum Albumin (HSA)

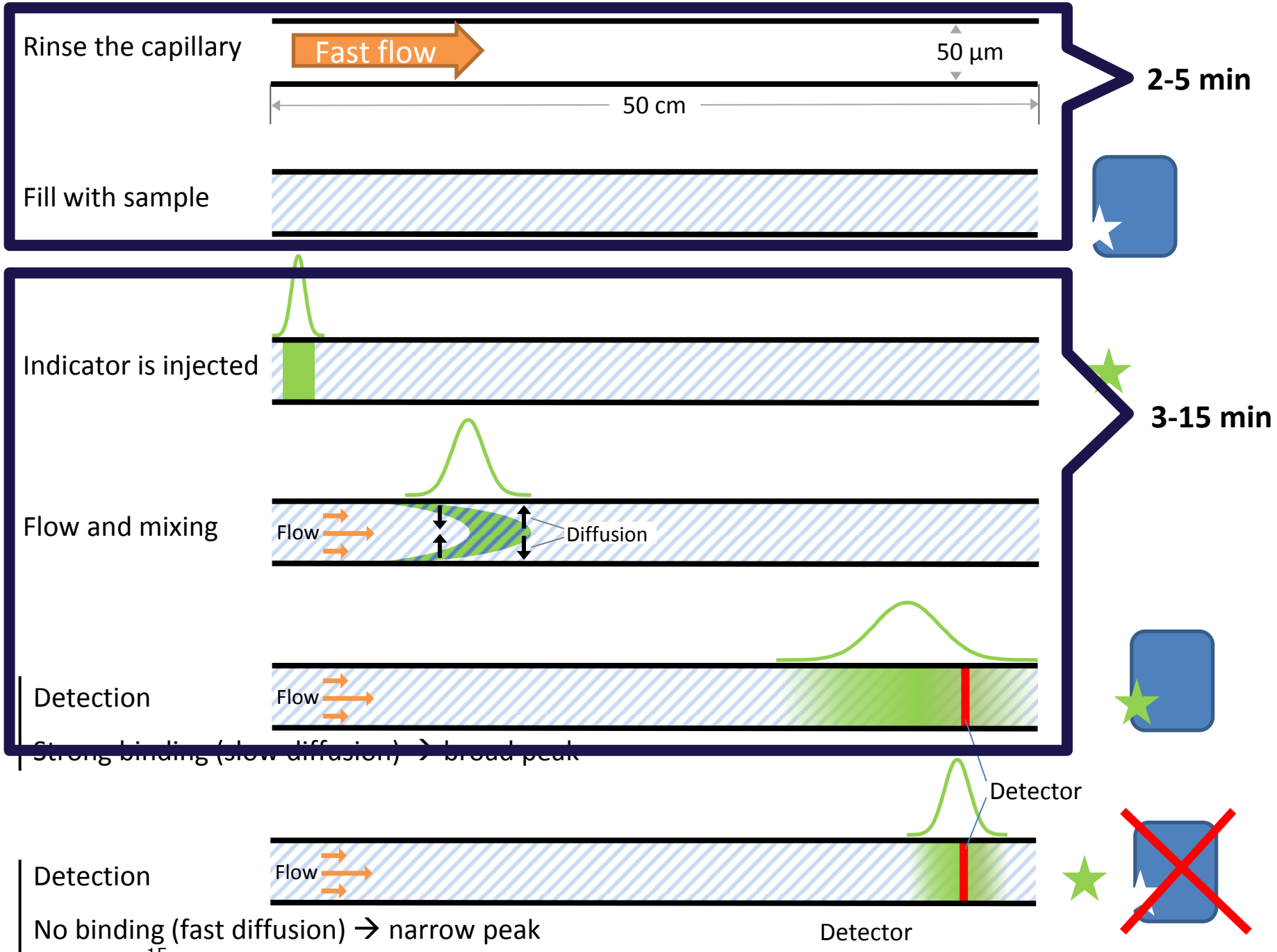


Simulated Standard curve / binding curve

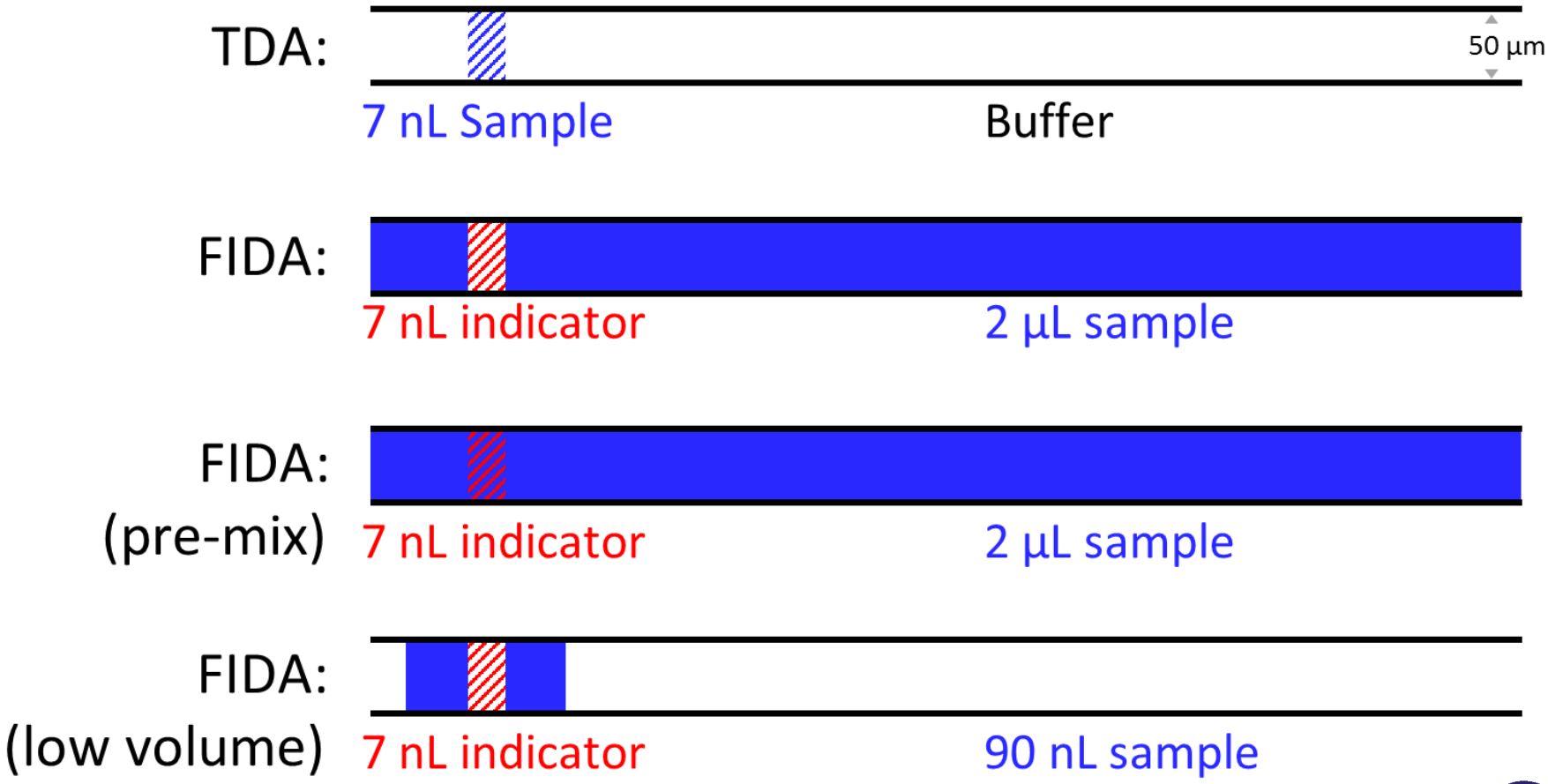


$$K = \frac{k_{on}}{k_{off}} = 6.25 \cdot 10^4 \ (\mathrm{m^3 mol^{-1}})$$

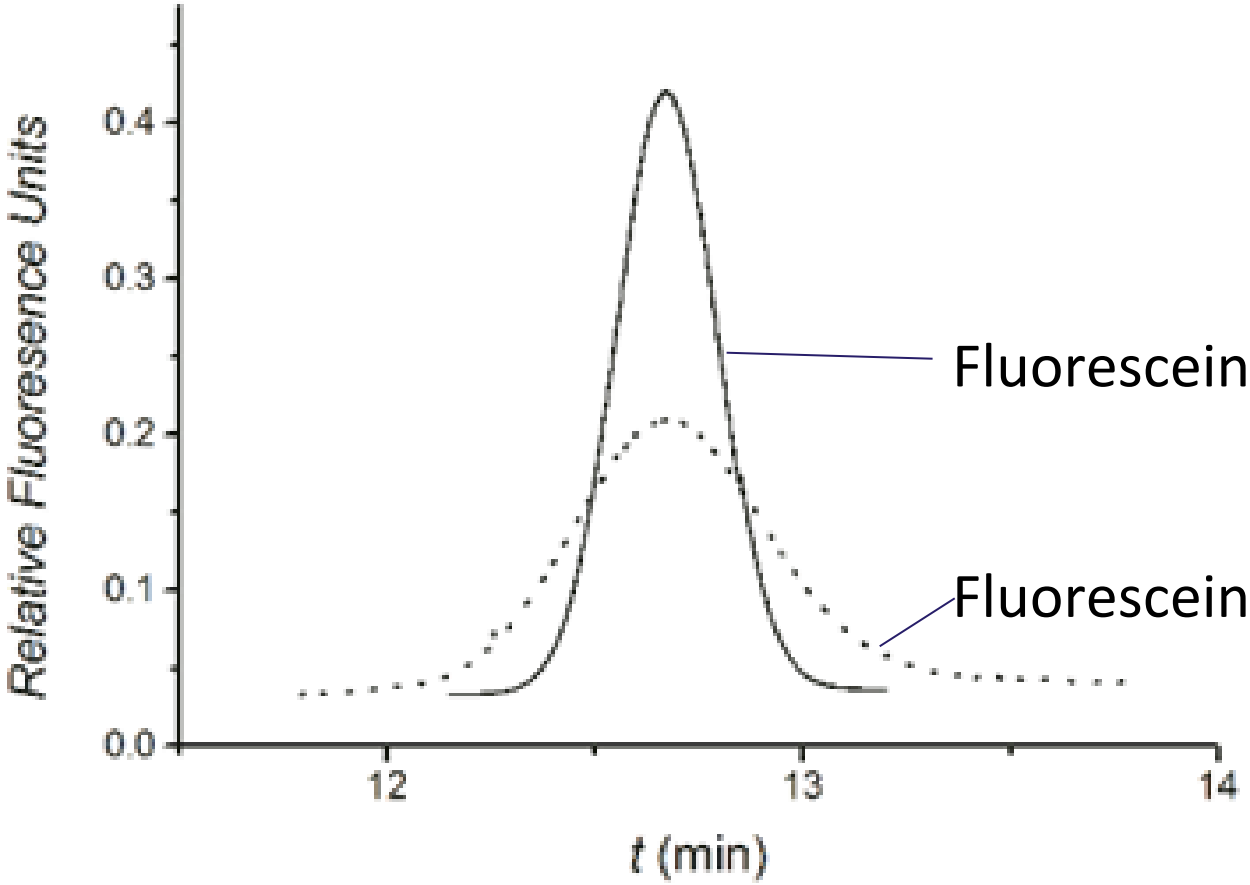
Analyte concentration 10^{-4} M, ligand (indicator) concentration is 50 nM, $D(\text{ligand}) = 4.25 \cdot 10^{-10}$ m^2/s, $D(A) = 6.1 \cdot 10^{-11}$ m^2/s

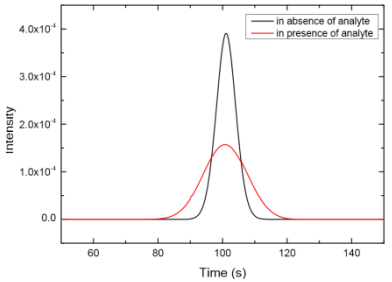

Rinse the capillary
Fast flow
50 µm
50 cm
2-5 min
Fill with sample
Indicator is injected
3-15 min
Flow and mixing
Flow
Diffusion
Detection
Flow
Detector
Detection
Flow
No binding (fast diffusion) → narrow peak
Detector

Injection procedures



Flow Induced Dispersion Analysis





Nicklas N. Poulsen, Nina Z. Andersen, Jesper Østergaard, Guisheng Zhuang, Nickolaj J. Petersen and Henrik Jensen;
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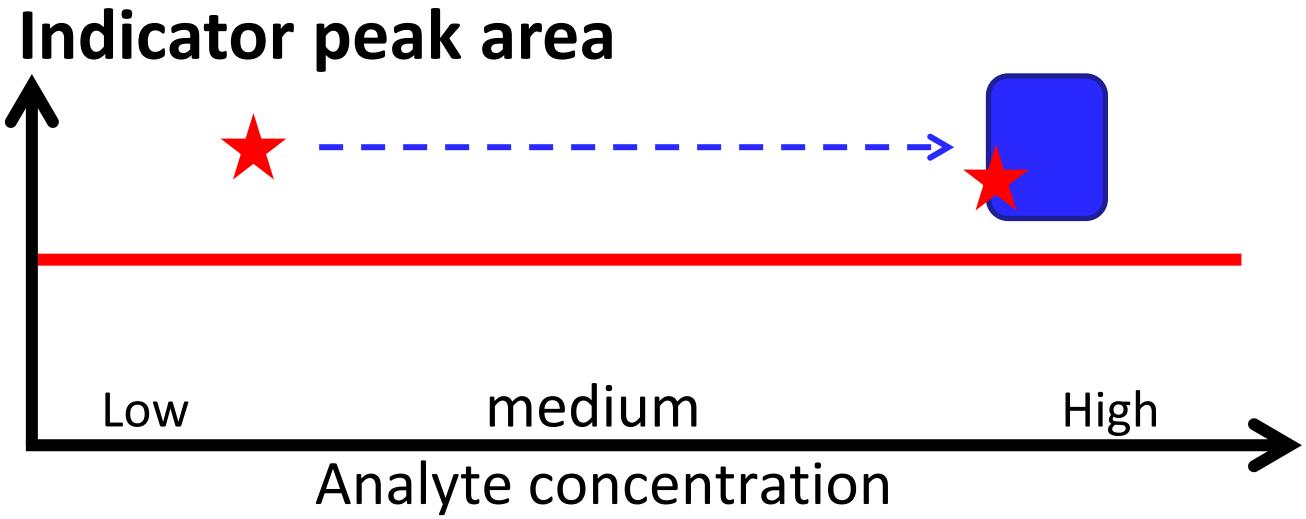
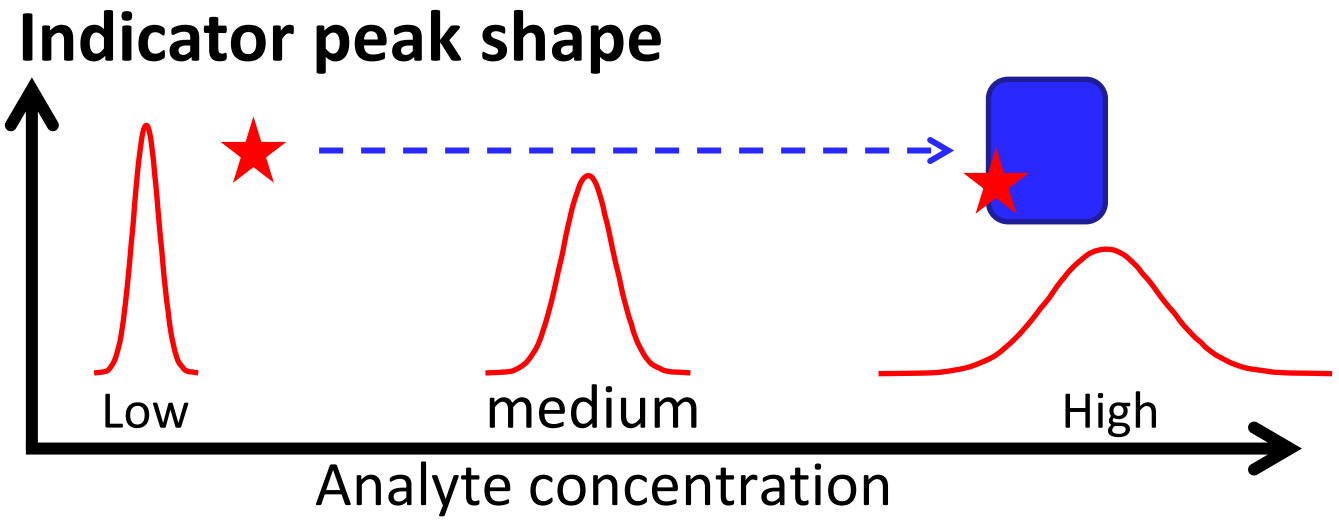
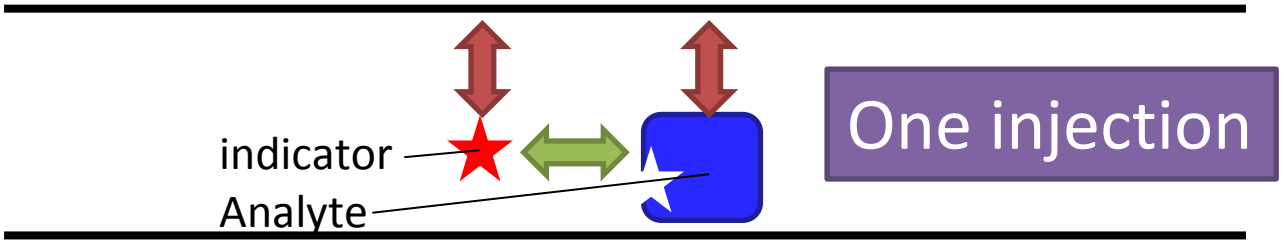
Flow Induced Dispersion Analysis





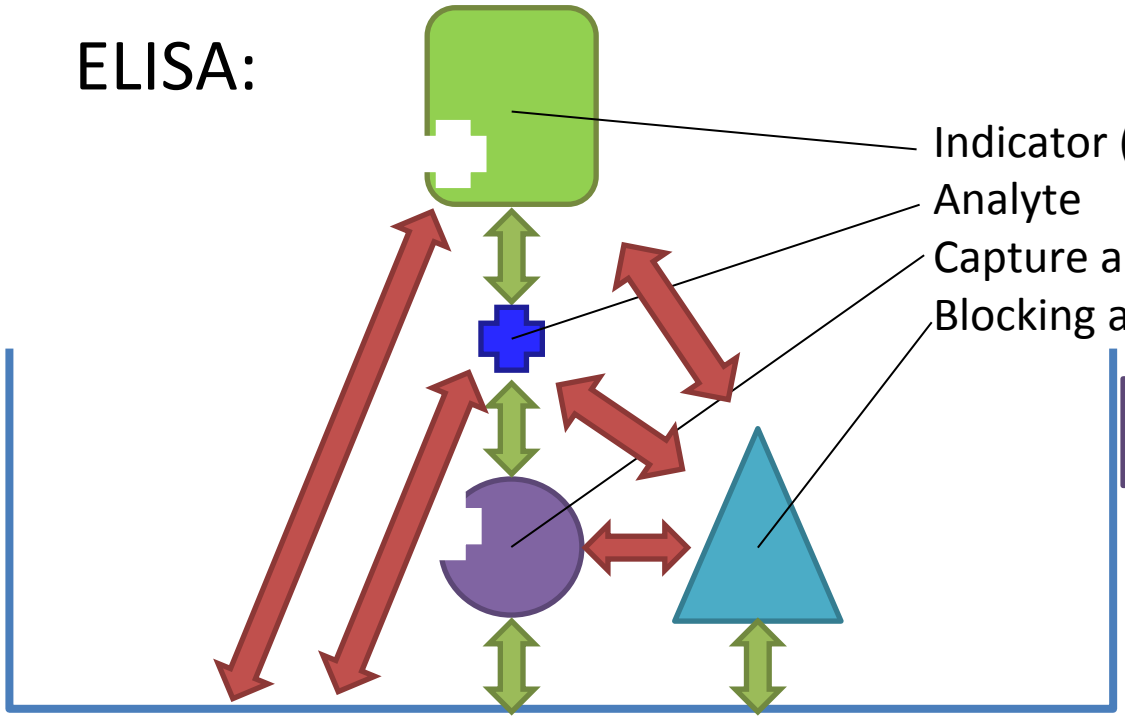
Interactions in FIDA and ELISA

FIDA:



One injection

ELISA:

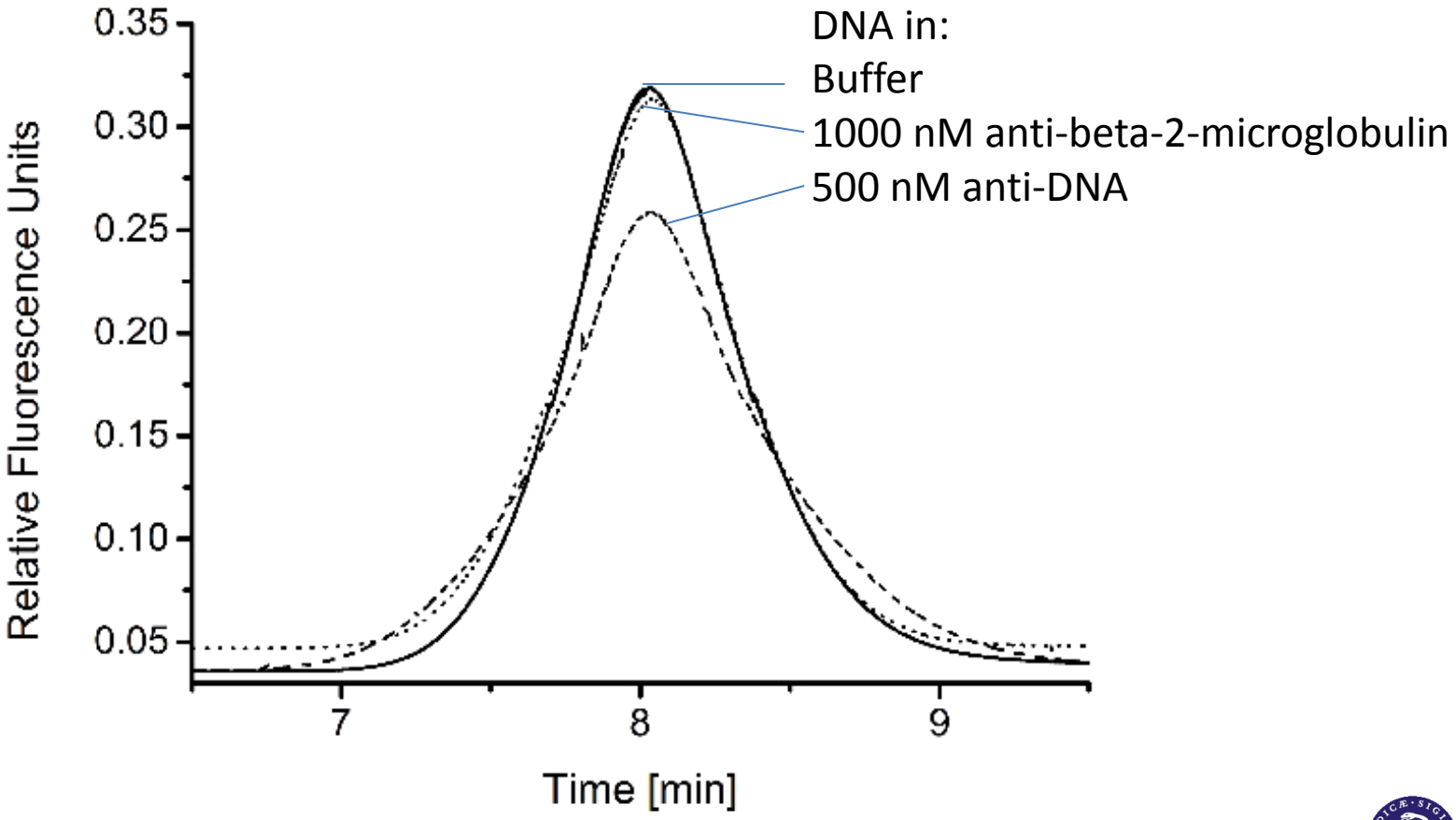


Many handling steps

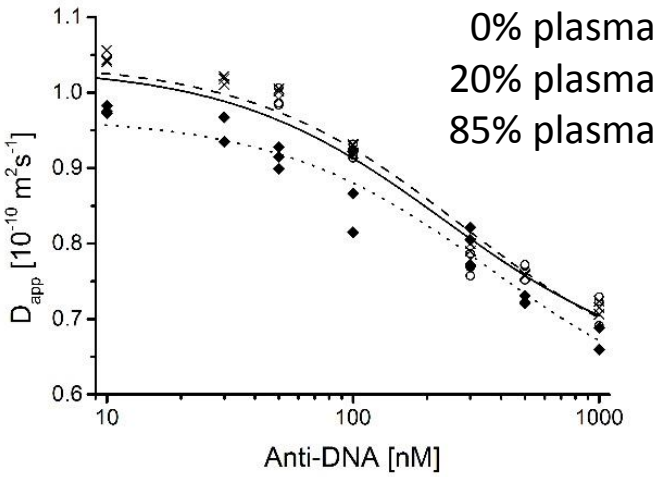
19

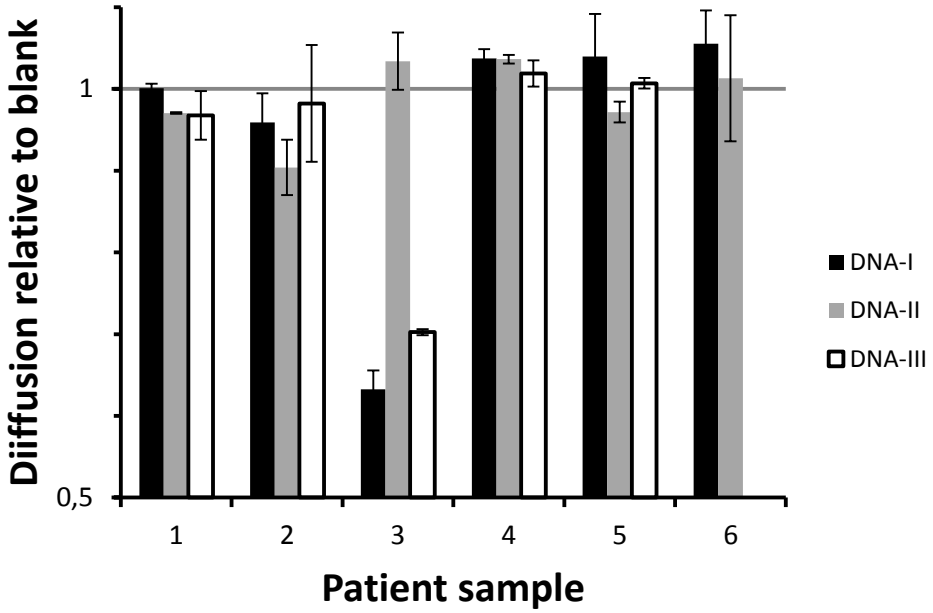
Detection of antibodies against dsDNA

dsDNA + Ab = dsDNA-Ab



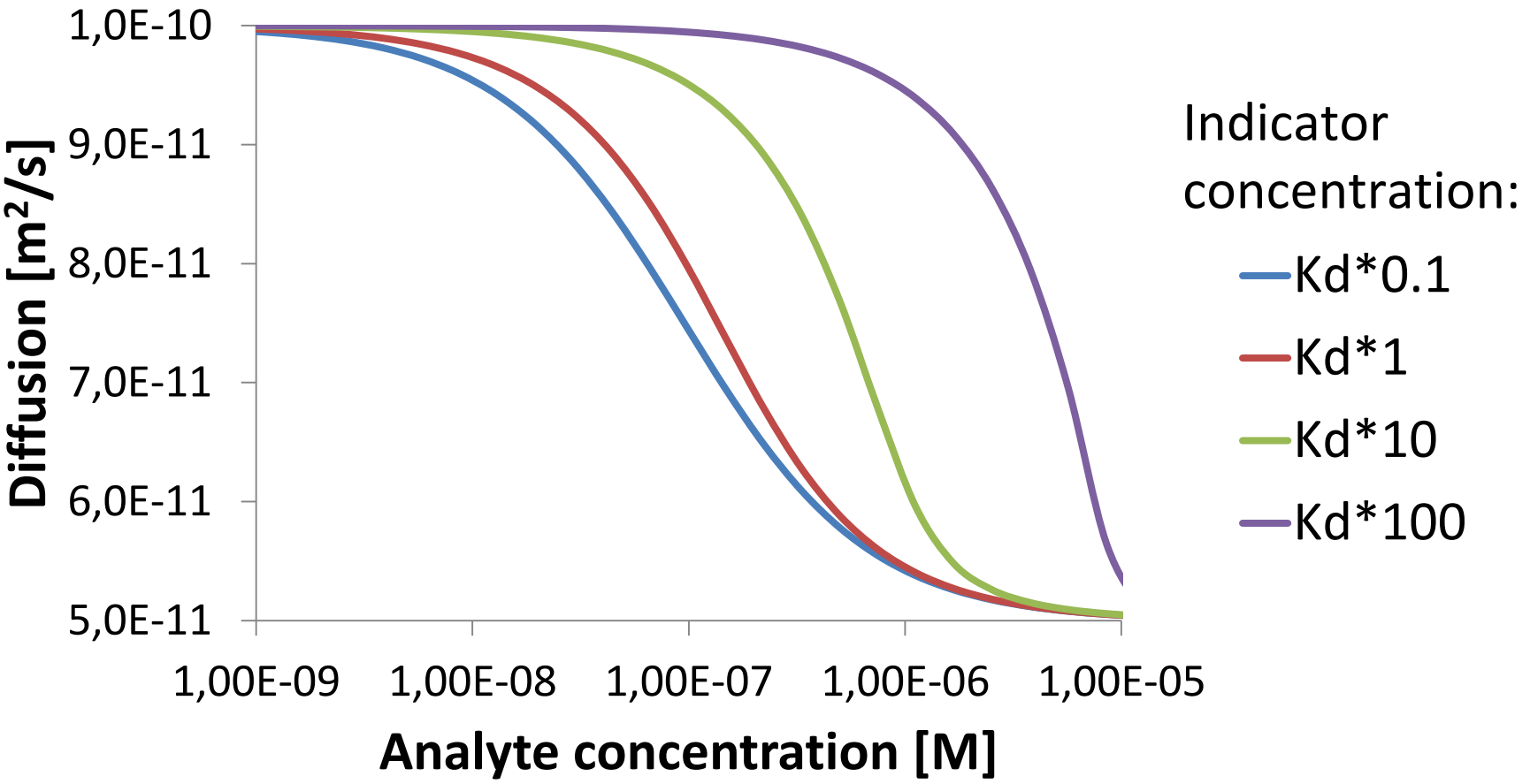
Detection of antibodies against DNA





Nicklas Poulsen, Morten Pedersen, Jesper Ostergaard, Nickolaj Petersen, Christoffer Nielsen, Niels Heegaard, Henrik Jensen;
Flow-Induced Dispersion Analysis for Probing Anti-dsDNA Antibody Binding Heterogeneity in Systemic Lupus Erythematosus Patients: Toward a New Approach for Diagnosis and Patient Stratification
Analytical Chemistry, **2016**, *88*, 9056-9061.

Dynamic range can be adjusted



Assumptions: fast binding kinetics, fast mixing, no dilution

Software based assay development (using dedicated software or excel)

Detection of antibodies against ABeta

Antibody based therapies for Alzheimers

The A-beta peptide is responsible for development of plaques (aggregates) leading to Alzheimers Disease

A-beta 40 is fluorescently tagged and used as ligand (indicator).

Detection of antibodies against ABeta

K_d = 1.2 nM (10% plasma)
R(complex)=5,8 nm
(built-in quality control)

May be used as standard curve

Effect of ”anti-drug antibody” on response - potential for immunogenicity testing

Conditions:

Abeta 5nM

Abeta binding antibody 20nM

Varying concentrations
of anti-IgG (Fc) antibody.

Work in progress – preliminary data

General IgG levels (indicator: Anti-IgG, fluorescently tagged – two interacting antibodies)

Anti-IgG + IgG = Anti-IgG-IgG

Work in progress – preliminary data

Flow Induced Dispersion Analysis is relevant for:

- Protein **binding** (protein – protein interactions) and **quantification**
- Detection in solution under **native** conditions (pM-mM)
- Rapid assays (minutes)
- Swift (*in silico*) assay development (hours – days)
- Low sample volumes (nL – μL)
- Built-in quality control – high level robustness
- Size determination of affinity complexes (1-300 nm)
- Qualitative detection of aggregates

Proof of concept demonstrated for:

Small molecule interactions
(*J. Am. Chem. Soc*., **2010**, *132*, 4070-4071.)

Small molecule – protein interaction
(*Analyst*, **2015,** *140*, 4365-4369.)

Protein – protein interaction
(*Analytical Chemistry*, **2016**, *88*, 9056-9061)

Immune responses (SLE)
(*Analytical Chemistry*, **2016**, *88*, 9056-9061)

Acknowledgements

Danish Research Council – Technology and Production Science

Carlsberg Foundation (equipment)

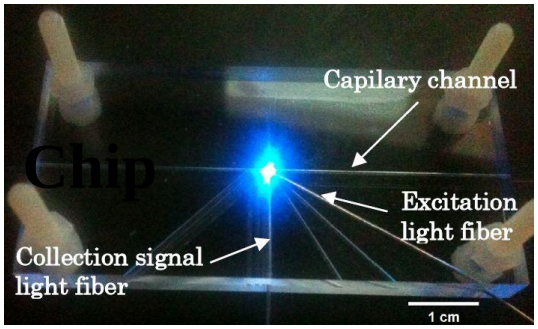
Lundbeck Pharma A/S (Rene Holm, Lars Pedersen, Abeta project)

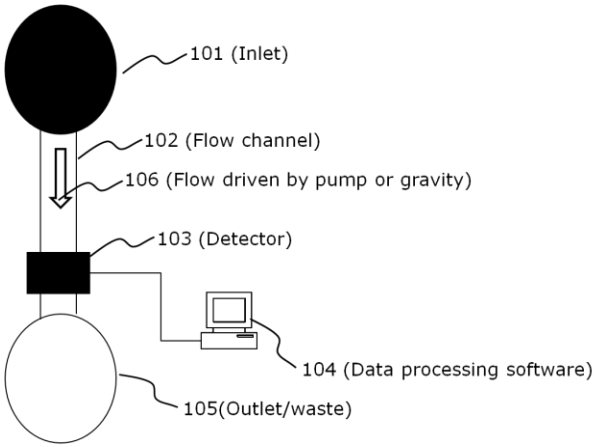
University of Copenhagen

Technical University of Denmark (DTU – Peter Heegaard)

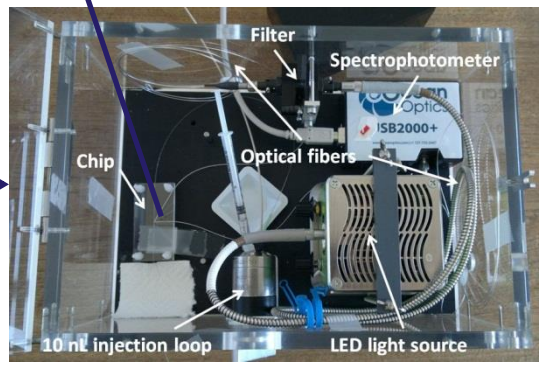
FIDA-Tech Aps

Future direction: Integrated microfluidic system





Lab-based instrumentation



Lab-rack – work in progress

Integrated micro-fluidics, sample pre-treatment, detection and data analysis