Biomolecular interactions on the cell level

S2004
Methods for characterization of biomolecular interactions – classical versus modern

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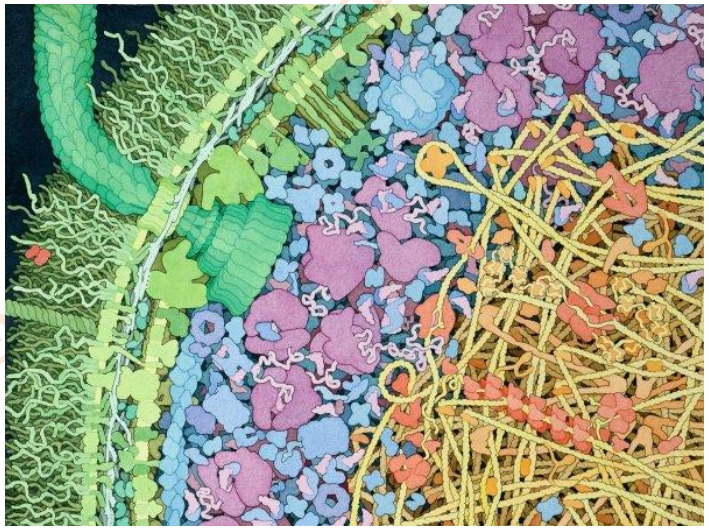
Work with cells

Positives

- Real environment
- All components present – metabolites, protein subunits,...
- (No purification)



Negatives

- Inhomogeneity
- High background/interference
- Cell culture contamination
- Instrument cleaning
- Safety

Calorimetry – whole cells experiments

- Study of energy changes during microbial growth – **isothermal** (not titration!) **calorimetry**.

- Non-destructive method, sample (cells) can be used after experiment.
- Monitoring for hours or days.

Thermochimica Acta 555 (2013) 64–71

Contents lists available at SciVerse ScienceDirect

Thermochimica Acta

journal homepage: www.elsevier.com/locate/tca

Review

Microbial growth and isothermal microcalorimetry: Growth models and their application to microcalorimetric data

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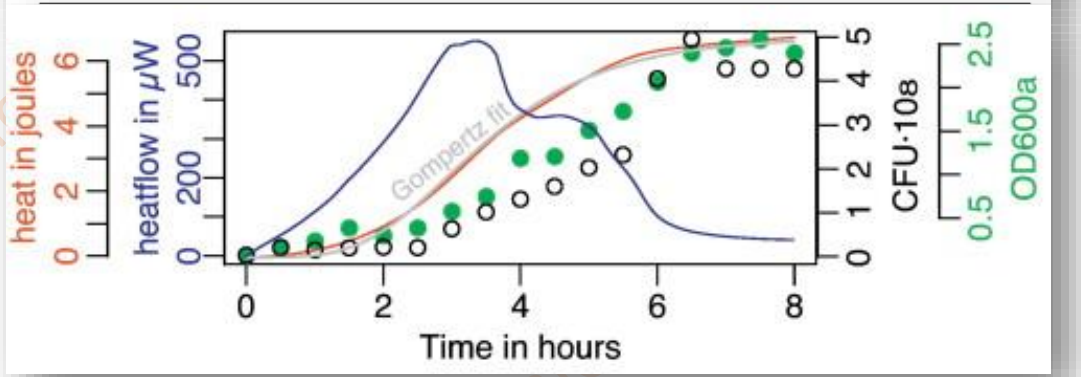
[b] Laboratory for Biomechanics and Biocalorimetry, University of Basel, c/o Biozentrum – Pharmazentrum, Klingelbergstrasse 50-70, CH-4056 Basel, Switzerland



MINIREVIEW

Use of isothermal microcalorimetry to monitor microbial activities

Olivier Braissant, Dieter Wirz, Beat Göpfert & Alma U. Daniels

Laboratory of Biomechanics and Biocalorimetry, Biozentrum/Pharmazentrum, University of Basel, Basel, Switzerland

Calorimetry – whole cells experiments

- Study of the interactions – **isothermal titration calorimetry**.
- Example:

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ScienceDirect

Thermochimica Acta 458 (2007) 34–37

thermochimica acta

www.elsevier.com/locate/tca

Calorimetric investigations of the effect of polymyxin B on different Gram-negative bacteria

Jörg Howe*, Malte U. Hammer, Klaus Brandenburg

Forschungszentrum Borstel, Leibniz-Zentrum für Medizin und Biowissenschaften, Laborgruppe Biophysik, D-23845 Borstel, Germany

Available online 19 January 2007

Calorimetry – whole cells experiments

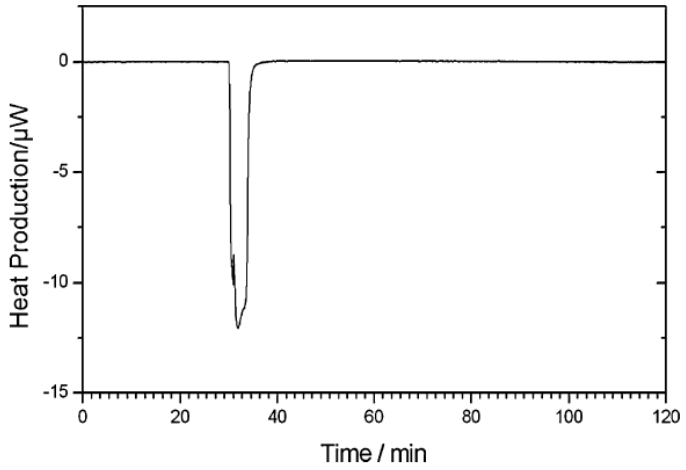


Fig. 2. Isothermal calorimetric titration of 0.8 mmol polymyxin B to a bacterial suspension with an optical density of 0.5, which corresponds to a bacterial concentration of 10^6 bacteria/ml.

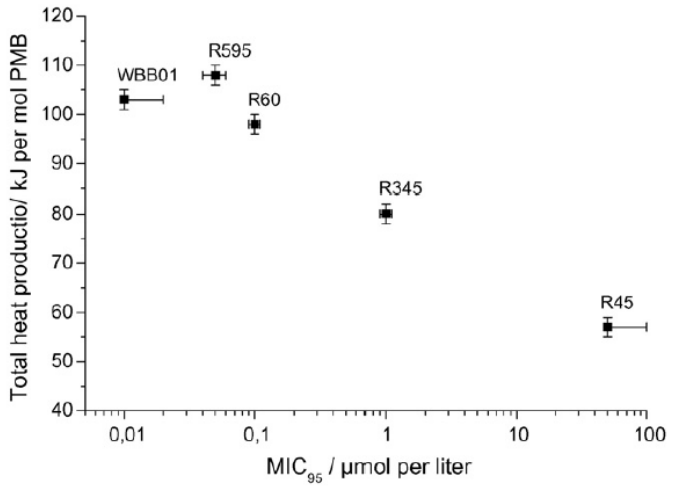


Fig. 3. Heat production of different bacteria. The heat production was plotted versus the minimum inhibitory concentration of the different bacterial strains (WBB01: *E. coli*; R595, R60, R345: *Salmonella minnesota*; R45: *Proteus mirabilis*).

Table 1
Molar enthalpies of different bacterial strains treated with polymyxin B at 37 °C

Bacterial strain	Enthalpy (kJ/mol)
Salmonella minnesota R595	−108 ± 1
Escherichia coli WBB01	−103 ± 1
S. minnesota R345	−80 ± 1
S. minnesota R60	−98 ± 1
Proteus mirabilis R45	−57 ± 1
Buffer (control)	+8

The final PMB concentration was 0.01 mM.

Results – molar enthalpies!

Calorimetry – whole cells experiments

- Study of the interaction – **isothermal titration calorimetry**

- Interaction is temperature-dependent (change in membrane fluidity).
- Metabolic activity of bacteria – blank experiment with **inactive** bacteria necessary.
- Disinfection of the machine necessary.
- Specialized calorimetres preferable.

Titration Ampoule

The removable ampoules offer a level of practicality unmatched in the industry. TAM-ITC ampoules are easily removed and cleaned outside of the instrument. Competitive designs feature fixed cells which require thorough cleaning between experiments, and preclude visual inspection. The open vessels of TAM-ITC ampoules also allows solid suspensions, solid matrixes with attached living cells, macromolecules, etc. to be loaded into the reaction vessel. This allows ligand binding to the solid system to be measured. There is no possibility for this kind of matrix experiment to be run on competitive fixed-cell instruments.

In TAM-ITC different sizes of syringes ranging from 100 µL to 2.5 mL are available. The injections volumes/flow are controlled by a high precision syringe pump. Each pump can support two syringes. In addition, two pumps can be attached to one titration ampoule which is useful for studying enzyme kinetics. This option is not available in competitive designs.

Titration Calorimetry

Isothermal Titration Calorimetry requires the highest level of calorimetric sensitivity and stability, efficient titrant delivery, and a user-friendly platform which facilitates easy cleanup and rapid turnaround. Employing unique and proprietary technology, the TAM III is the ideal system for Isothermal Titration Calorimetry and excels in the most demanding ITC applications such as ligand binding.

The TAM Isothermal Titration Calorimetry (TAM-ITC) system consists of a nanocalorimeter, 1 ml removable titration ampoule with stirring facilities, and a precision syringe pump for efficient titrant delivery. The TA Instruments nanocalorimeter is the most sensitive calorimeter available for the TAM III, and can readily detect microjoule level heat flow. In power compensation mode, the response time of the calorimeter is optimized, and the temperature of the sample is held virtually isothermal. This is a major benefit over competitive designs, in which the sample temperature is allowed to drift during titration.

TA Instruments

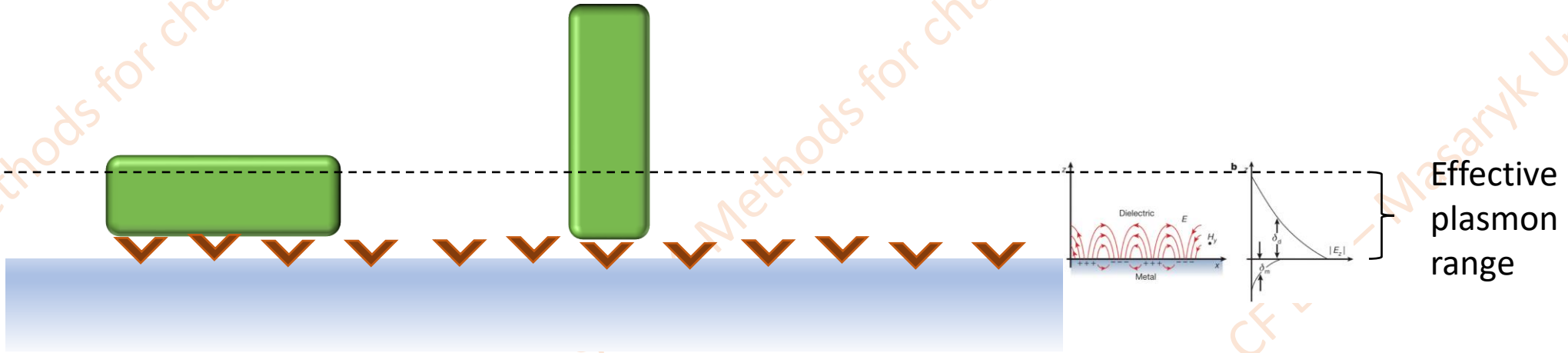
Microcalorimetry

SPR – whole cells experiments

- **Bacteria** and **eukaryotic cells** interactions.
- Immobilization necessary.
- **Low flow rate** needed – cells diffuse much more slowly
- **Problems**:
  - **complexity** of whole cell interaction with their biological ligand
  (simple binding constant equations cannot be applied, arbitrary/apparent K_Ds)
  - **changes** of the **living** cells during time
  - **variability** within population (changes in resonance signals,).

SPR – whole cells experiments

- Cells are large compared to SPR effective range (app 300 nm from the sensorchip surface)
- Different **distances** of captured cells can influence the response
  - The instrument only detects the portion of the cell within the detection range
  - The signal from the binding of whole cells is lower than expected



SPR – whole cells experiments

- Could be used for bacteria and also eukaryotic cells interactions.

- Example:

Analytical Biochemistry 508 (2016) 73–77

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Whole cell-based surface plasmon resonance measurement to assess binding of anti-TNF agents to transmembrane target

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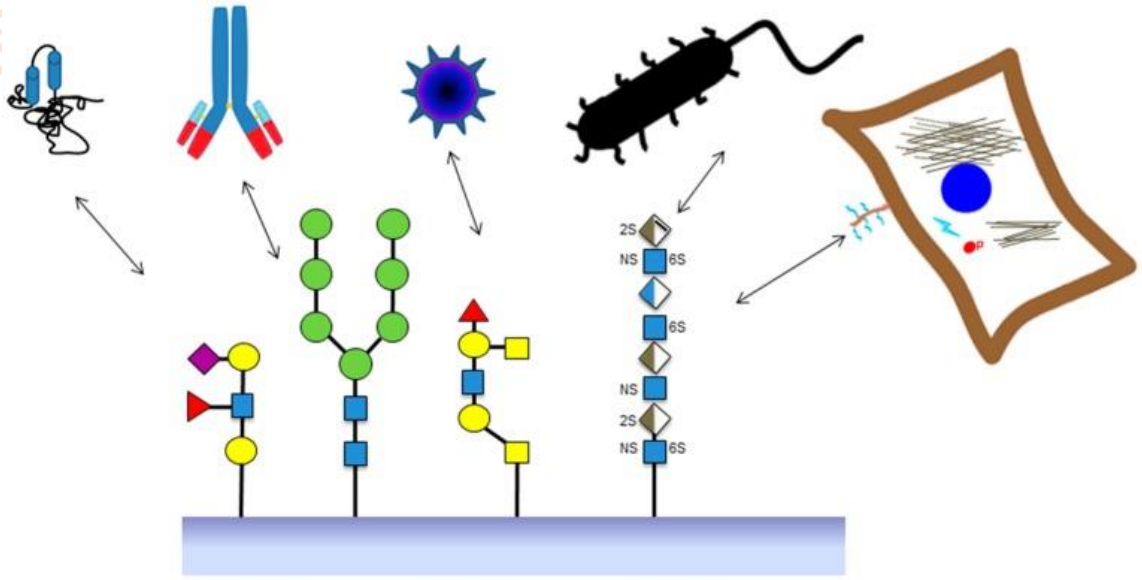
Arrays
Example: Glycoarray Technologies



Microarrays 2016, 5, 3;
doi:10.3390/microarrays5010003

Immobilization of glycans

- Non-covalent non-specific adsorption
- Electrostatic interactions
- Covalent immobilization

- **Detection of bound cells**
  - Cell-permeant fluorescent nuclei staining dyes (SYTO 62)
  - Live/dead assays (Calcein AM and ethidium homodimer)

High throughput!

microarrays

MDPI

Review

Glycoarray Technologies: Deciphering Interactions from Proteins to Live Cell Responses

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Received: 22 October 2015; Accepted: 14 December 2015; Published: 4 January 2016
Academic Editor: Massimo Negrini

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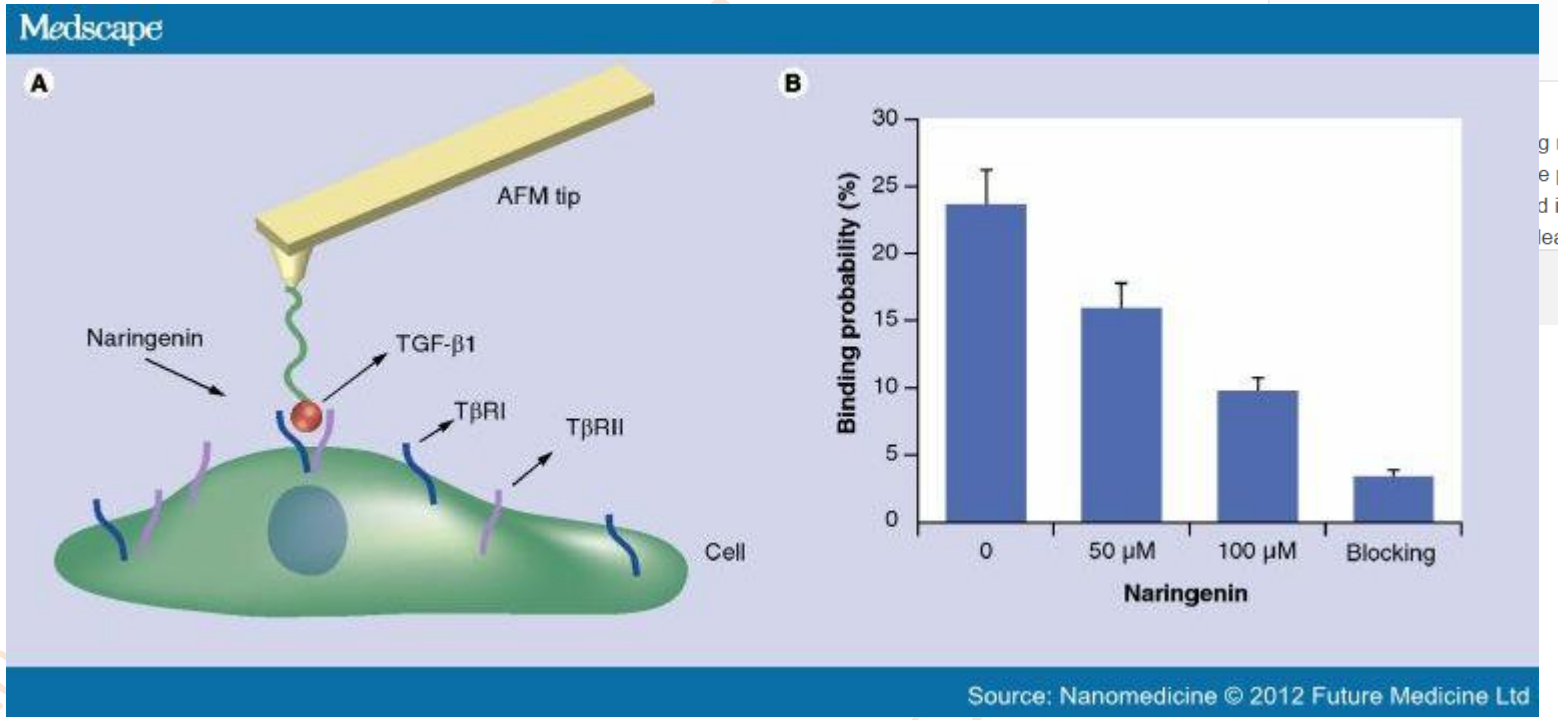
Abstract: Microarray technologies inspired the development of carbohydrate arrays. Initially, carbohydrate array technology was hindered by the complex structures of glycans and their structural variability. The first designs of glycoarrays focused on the HTP (high throughput) study of protein–glycan binding events, and subsequently more in-depth kinetic analysis of carbohydrate–protein interactions. However, the applications have rapidly expanded and now achieve successful discrimination of selective interactions between carbohydrates and, not only proteins, but also viruses, bacteria and eukaryotic cells, and most recently even live cell responses to immobilized glycans. Combining array technology with other HTP technologies such as mass spectrometry is expected to allow even more accurate and sensitive analysis. This review provides a broad overview of established glycoarray technologies (with a special focus on glycosaminoglycan applications) and their emerging applications to the study of complex interactions between glycans and whole living cells.

AFM

- Atomic force microscopy
- Scanning of (cell) **surface** with modified probe



Nanomedicine

Living Cell Study at the Single-molecule and Single-cell Levels by Atomic Force Microscopy

Xiaoli Shi; Xuejie Zhang; Tie Xia; Xiaohong Fang

Disclosures

Nanomedicine. 2012;7(10):1625-1637.

Print

AFM Force Measurement

Owing to the limitation of AFM imaging on living mammalian cells, AFM force mode has become an essential and more popular mode than imaging in living cell studies. With AFM force mode, quantitative information on cellular interactions at the single-molecule level can be obtained.

The schematic illustration of the principle of AFM force mode is shown in Figure 1B. In principle, the tip is held over the substrate and the piezo scanner, which is installed to control the tip or the
g relative to the sample vertically up and down (approaching and retracting) in
e piezo scanner firstly moves to let the tip press onto the sample surface. Then,
d is reached, the direction of motion is reversed and the piezo scanner is
leave the surface of the sample. The tip leaves the surface to complete a

Flow cytometry

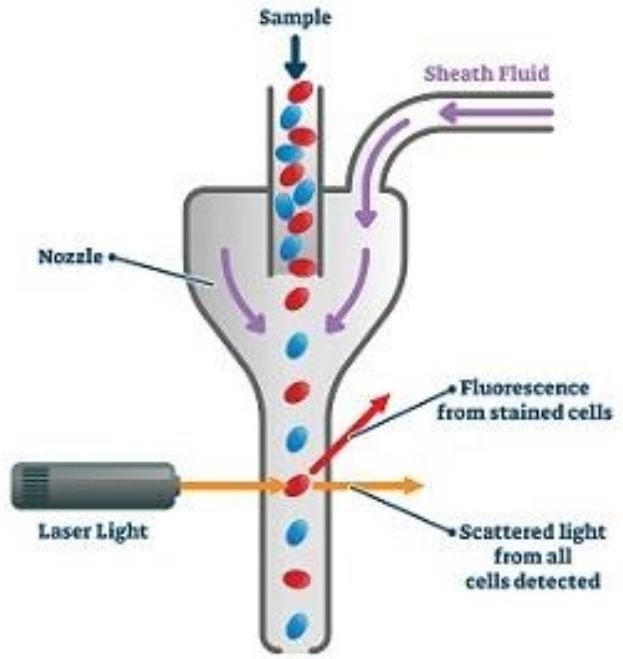
- Detection of individual cells in flow system
- Fluorescence & light scattering
- Individual protein labeling – co-occurence

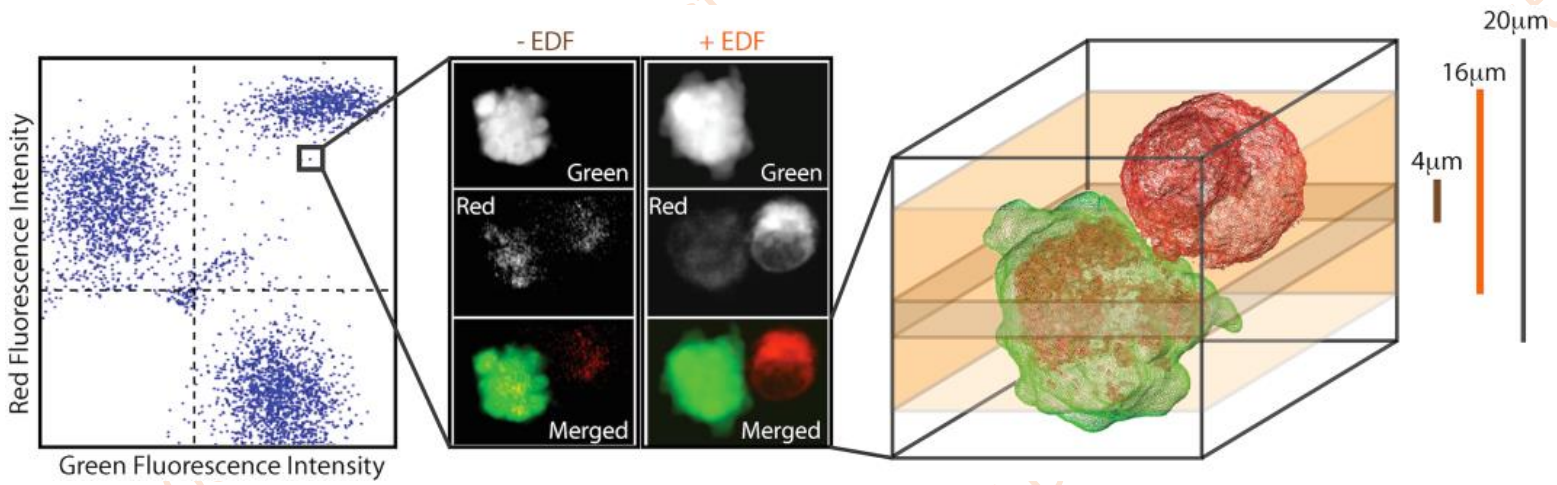
Cell Interaction Analysis by Imaging Flow Cytometry

Cristian Payés, José A. Rodríguez, Sherree Friend and Gustavo Helguera

Additional information is available at the end of the chapter

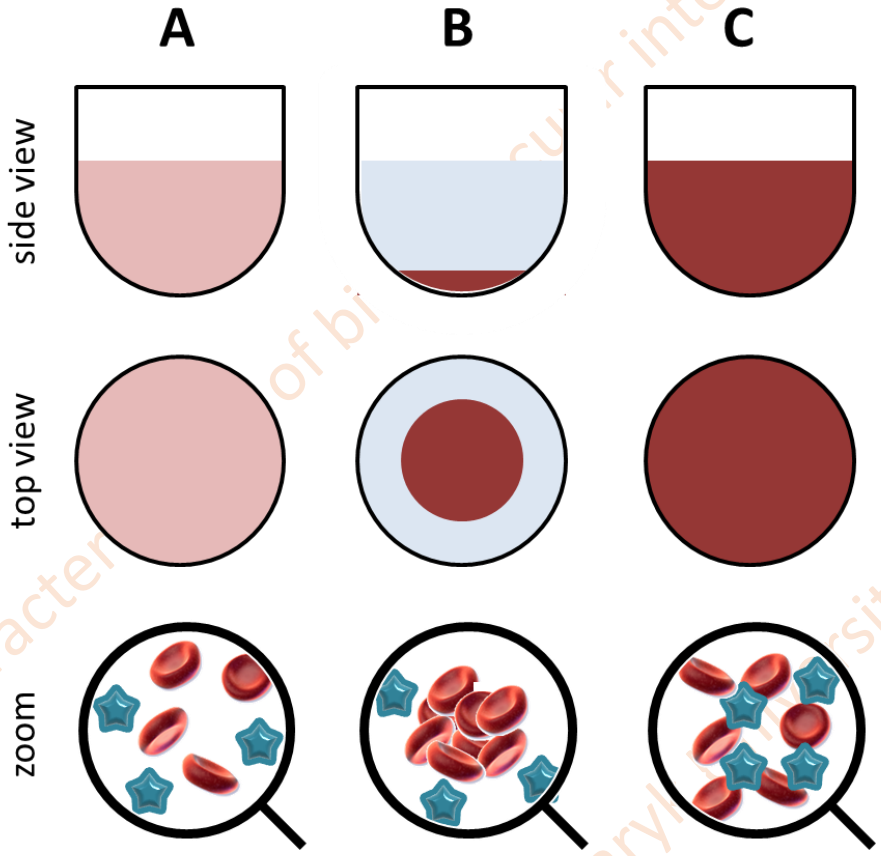
http://dx.doi.org/10.5772/51147

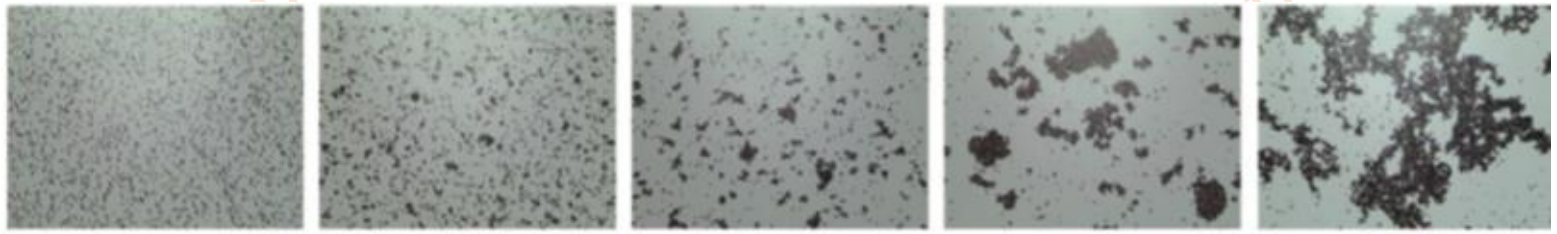




Agglutination/precipitation methods

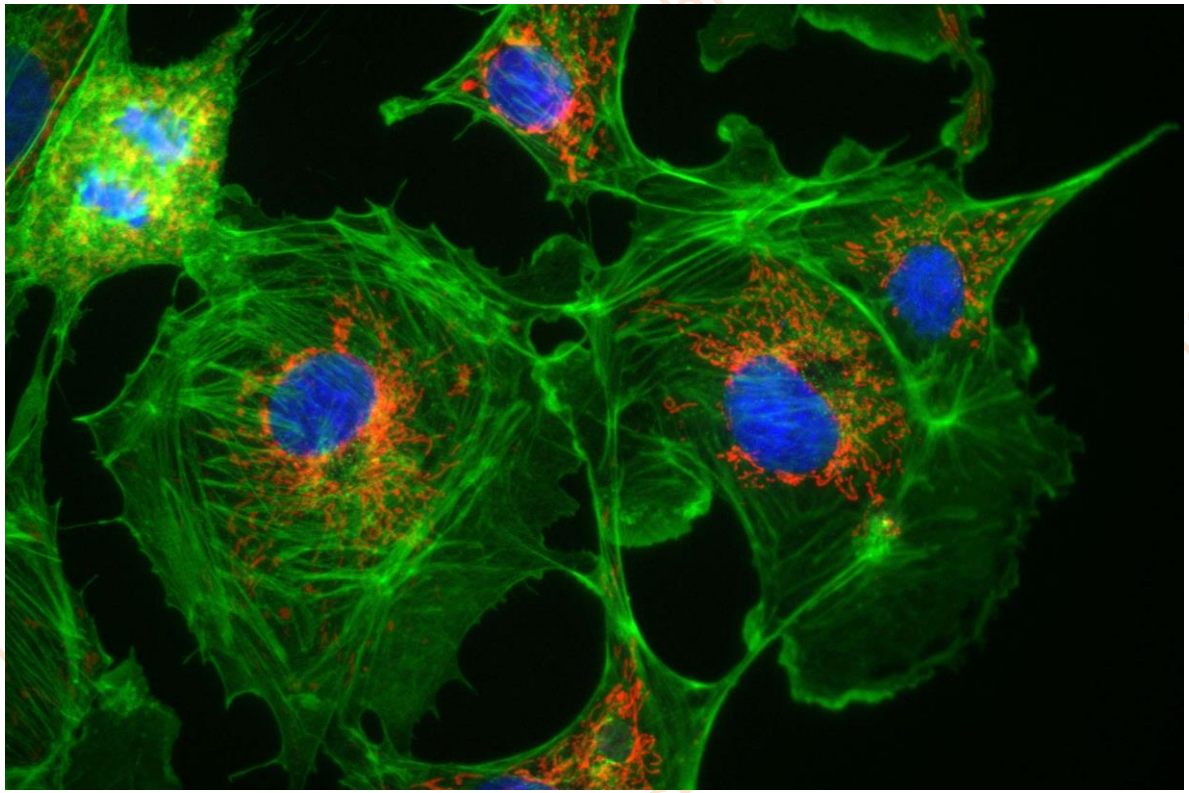
- One binding partner on the cell surface
- Second binding partner multivalent
- Interaction detected as **cell agglutination** (macroscopic in well, microscopic under microscope)
- **Red blood cells** (hemagglutination), yeast, latex beads





Microscopy/fluorescent microscopy

- Labeled probe (protein, antibody,…)
- Interaction and localization



Some not suitable techniques

- **BLI** – confusing interpretation, shaking
- **MST** – cells motility, heterogeneity
- **AUC** – cell size – too heavy = too fast sedimentation
- **DLS** – sedimentation, low resolution
- **TSA, DSF, DSC** – heating necessary

Biomolecular
Interaction and
Crystallization Core Facility

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