Chem-E8135 Microfluidics and BioMEMS
24.3.2021

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Definition of BioMEMS

BioMEMS:

Biological or Biomedical Micro-Electro-Mechanical Systems
Also devices without electro-mechanical components, (DNA and protein arrays) are often categorized under BioMEMS

Bashir 2004: ”devices or systems that are used for processing, delivery, manipulation, analysis, or construction of biological and chemical entities

Lab-on-a-chip (LOC) and micro-total analysis systems (µTAS): bioanalytical systems scaled down and integrated onto a single chip.

Dimensions

MEMS devices have dimensions ranging from 100 nm to 1 mm

Diameter of DNA alpha helix: 2 nm
Proteins: 1- tens of nanometers scale
Viruses: 30-100 nm scale
Bacteria: 0.2-10 µm
Mammalian cells: 10-30µm

BioMEMS applications

MEMS sensors for the biological systems, no biocomponent included

- Pressure sensors e.g. blood or intraocular pressure sensors
- Inertial sensors e.g. defibrillators, pacemakers
- Hearing-aid transducer

MEMS sensors having a functional biocomponent

- Diagnostic and analytical devices
- Medical applications
- Tools for research

Driving forces towards BioMEMS 1.

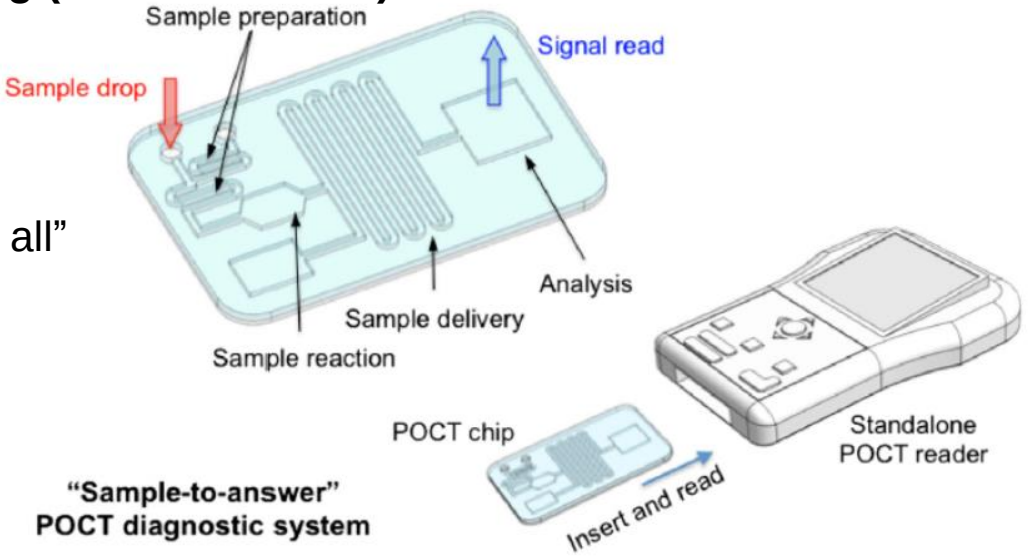
Diagnostics

Demand for higher-quality medical care:

- **Point-of-care testing, home testing (COVID-19 case!)**
- Preventive care
- Personalized medicine
- Continuous monitoring

Solutions needed for ”Medical care to all”

- Simple, user-friendly (easy-to-use)
- Minimally invasive procedures
- Low-cost
- Minimal sample and waste
- No contamination



Jung 2015 **Fig. 1.** Schematic illustration showing a desired POCT diagnostic system having the “sample-to-answer” capability.

Other diagnostic/ analytical application fields: Industry, food safety and quality, environment)

Why microsystems for diagnostics?

A typical microfluidic system moves, mixes and controls small volumes of fluids (nanolitres)

components include: needles, channels, pumps, mixers, valves, sensors, filters, dispensers, reservoirs

A typical point-of-care diagnostic microfluidic system handles biofluids as a sample. Device performs sampling, sample preparation, analyte detection, signal amplification (data analysis and results display)

Microscale diagnostic systems enable:

Miniaturization

Lower reagent, sample and power consumption
Faster analysis
Portable, no cross-contamination with disposable µ-devices

Automation

higher throughput
controlled/closed devices: decreased risk of contamination of the end user
better control of assay parameters
reduces needs for highly skilled personnel as end users

Integration (the main challenge at the moment)

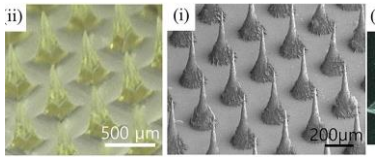
sampling,sample preparation, analysis, detection, data transmission
multiplexed analysis

Driving forces towards BioMEMS 2.

Medical applications

Examples of commercial products (according to the survey of Yole Developpement)

- Kidney dialysis monitoring (pressure sensors)
- Hearing aids (silicon microphones, microelectrodes)
- Smart pills for intestinal imaging
- Microneedles for transdermal drug delivery



Du et al 2019, Yu et al 2015

A major challenge: integration of diagnostic devices with therapeutic devices

implantable devices (passive / **responsive**)

BioMEMS tools for drug development (Tiina Sikanen 10.3.2021):

- Automated, multiplexed, high-throughput screens for drug candidate libraries
- Cells, organs, multiorgans –on-a-chip

Driving forces towards BioMEMS 3.

Research tools

For different omics

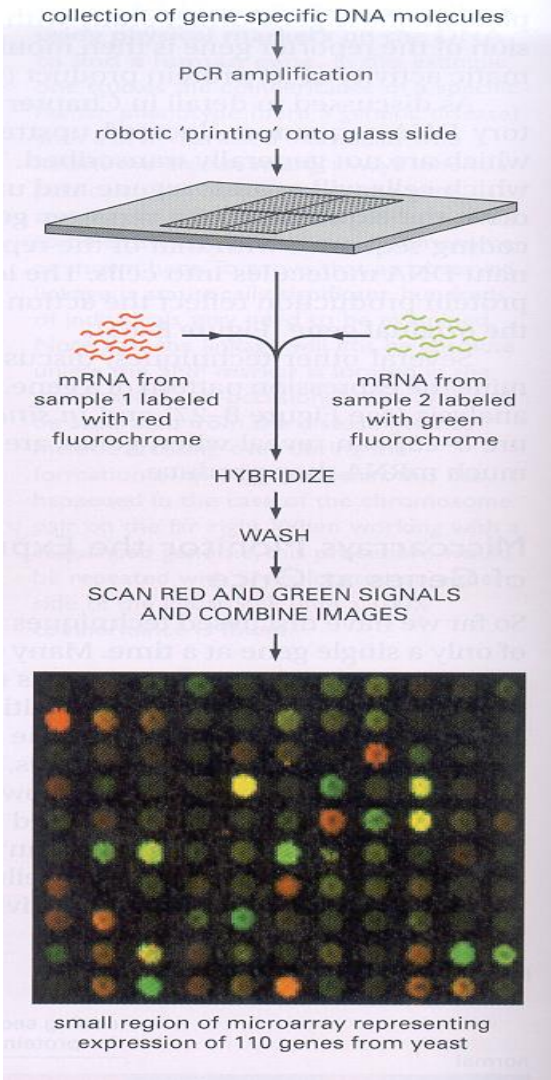
Genomics, peptidomics, transcriptomics, proteomic, glycomics, lipidomics etc.

High-throughput studies possible

e.g normal versus cancer cells

Evolution-on- a-chip

e.g. antibiotic resistance



Alberts et al.

Driving forces towards BioMEMS 4.

Novel / recent possibilities

Single cell analysis / function in a real time

Measurements of mRNA, protein, chemical profiles, oxygen, pH
Whole genome sequencing
Also as a function of controlled stimulus
Understanding of basic cellular functions (e.g. apoptosis, differentiation,) and their implications on disease states (e.g. malignacy)

Novel biological findings: exosomes (extracellular vesicles produced by cells, intensive research ongoing around the functions of exosomes and their potential in e.g. diagnostics)

Driving forces towards BioMEMS 4.

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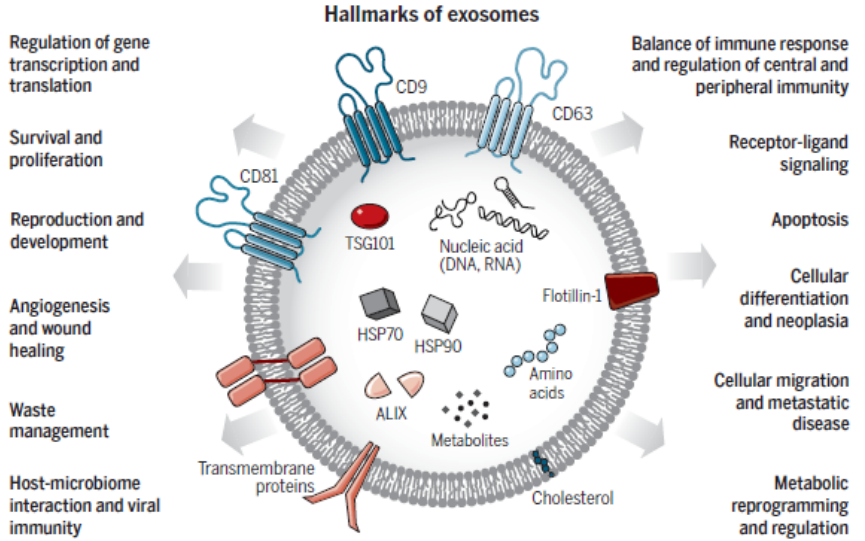


Kalluri and DeBlau, 2020

Specific features introduced to MEMS by biomolecules

BIOMOLECULES AND CELLS ARE SUBJECTED TO A NON-NATURAL ENVIRONMENT!

Bioapplications

Applications are miniaturized systems or they contain novel solutions

Properties of the samples and analytes vary a lot!
Nucleic acids, proteins, small molecules.
Clinical samples, food or environmental samples

Fabrication / construction approaches

Maintain the functionality of the biomolecules (dryness-, temperature-, pH-sensitive)
Preserve stability in biointerface (e.g. immobilization) and in packaging (e.g during lidding)

Biocompatibility (interaction of the material with e.g.human body)

Concern for *in vivo* devices (experience from e.g. pace makers) and to some extend also wearables

Safety issues (toxins, microbes)

prevention of contamination / infection
material choices, costs, life cycle of the device, waste management

Nucleic acids in microsystems as components or target analytes

Nucleic acids

DNA versus RNA

DNA in procaryotes and eucaryotes
RNA in viruses (also DNA)
Genes and non-coding sequences
Notice the differencies in stability between DNA and RNA, single versus double stranded

Location

Eukaryotes (humans, animals, plants): DNA is in nucleus (membrane-bounded intracellular compartment), tightly packed in chromosomes
Prokaryotes (e.g.bacteria): no compartment for DNA, additional small genetic elements: e.g. plasmids
Viruses: DNA/RNA packed inside the virus coat
Notice the location and packing mode of the genetic element when designing BioMEMS applications for nucleic acid detection, pay attention especially to the sample preparation requirements

DNA in BioMEMS

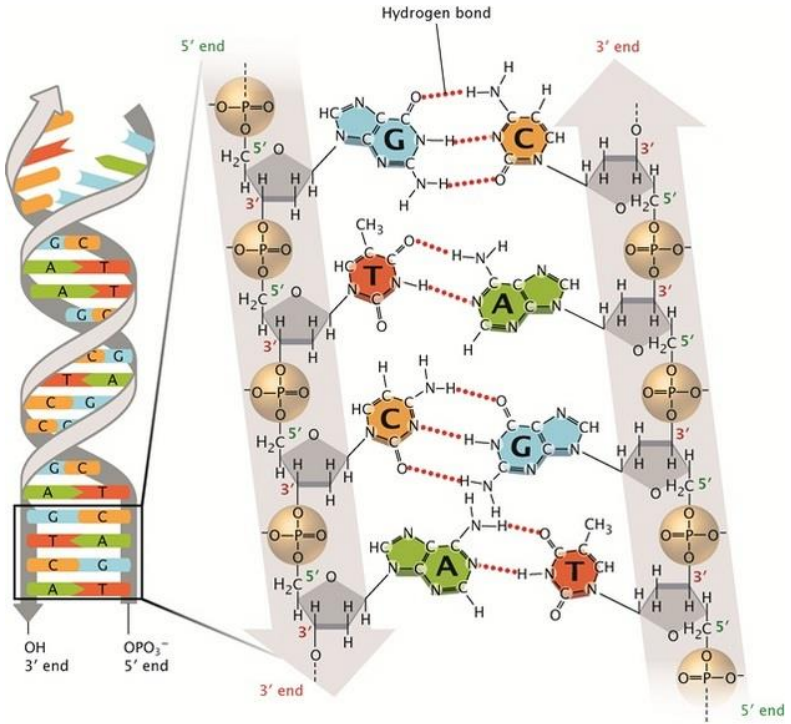
Self-complementarity is a unique feature!
Utilized in identification, immobilisation, construction, isolation, amplification

Convenient to handle (stable)

Many protocols and BioMEMS device constructions
Isolation, digestion, amplification, hybridization, detection

Compatible with many materials
Single stranded DNA/RNA may have unspecific interactions to surfaces/materials

The double-helix structure of DNA



Double strand (ds), single strand (ss)
Long and narrow molecule
Linear or circular by nature
3D molecule by engineering (aptamers)

Single DNA strand recognizes
a complementary sequence
on another single strand of DNA=
Hybridization

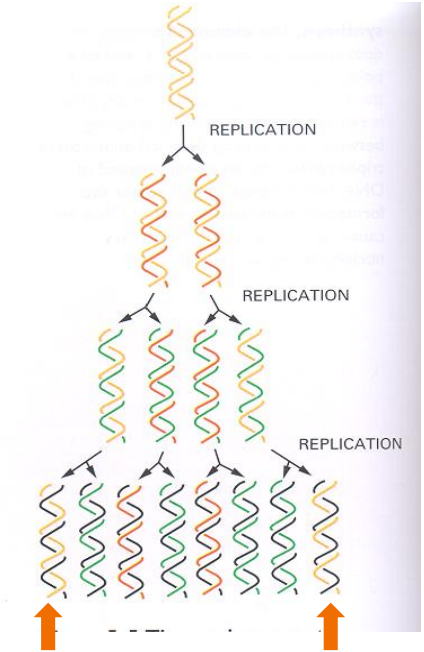
- **Important in amplification (PCR) and constructs (targetting, aptamers, Origamis)**
- **Very specific!**

Pray, 2008

Base pairs: adenosine (A) – thymine (T), cytosine (C) – guanine (G)

Replication (important in PCR applications)

Semiconservative = both DNA strands act as a template for new DNA
Aim: identical copies
Amount of DNA: 2^n , n= cycles of replication



Parental strands

Alberts et al

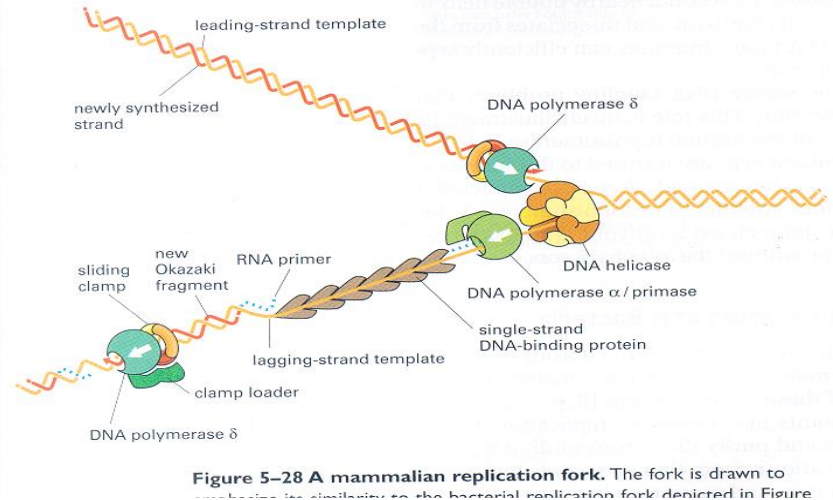


Figure 5–28 A mammalian replication fork. The fork is drawn to

***In vivo*: natural conditions, many assisting proteins are included**

***In vitro* (PCR): non-natural conditions (temperature, buffer, thermostable polymerases primers)**

Primer: a small sequence of a single-stranded DNA. Attaches to the template DNA and initiates replication

BioMEMS example: DNA extraction - from whole blood

Whole blood contains red blood cells, white blood cells (many different types, sizes vary), platelets, plasma (proteins, lipids and small molecules)

DNA is in the nucleus of white blood cells.

One way to extract DNA from whole blood is to **trap the white blood cells by a microfilter** (while everything else flows through), then **lyze** (break down) **the white cells and extract DNA**

Fig. 6. Biological protocol steps for DNA purification silicon chip.

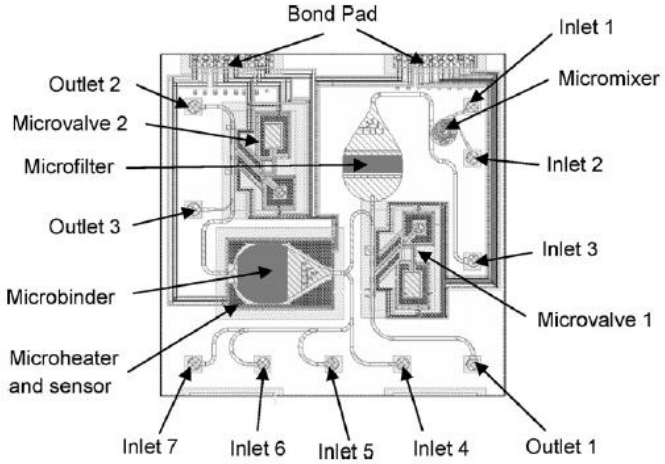
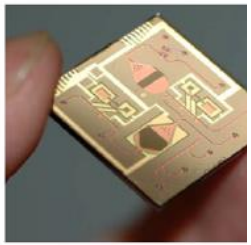


Fig. 4. The integrated DNA extraction chip, the dimension of the chip is 2 cm × 2 cm.



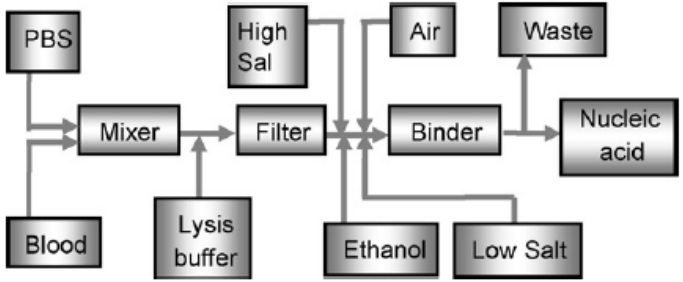


Fig. 7. Flow diagram for DNA extraction.

Ji et al. 2007

BioMEMS example: DNA analysis, sizing, separation

Determination of the size of DNA or DNA fragments cutted by restriction enzymes

Electrophoresis separates DNA fragments based on their size.
The nucleic acids are transported in a capillary or microfluidic channel by an electric field
Calibration ladder as a reference.

Commercially available (e.g. Agilent2100Bioanalyzer, LabChip GX (PerkinElmer))
Benefits: lower consumption (1 µl sample and reagents)
high sensitivity (0.1 ng fragments)
reduced assay time (several samples in 30 min)
high degree of automation and data handling

Agilent DNA chip

Application potential:
-Research tool
search for positive clones, quality check for DNA (purity, uniformity)
-Forensic studies
Genetic fingerprinting: highly variable repeating sequences,
identification of human remains, paternity testing etc

BioMEMS example: Genome analysis of a single cell (Marcy et al 2007)

Amplification of a genome of a single cell

Motivation:

Not all interesting and relevant micro-organisms can be cultivated

Need for analysis of the genome of individual cells isolated from e.g. soil, geysirs

Multiple Displacement Amplification (MDA)

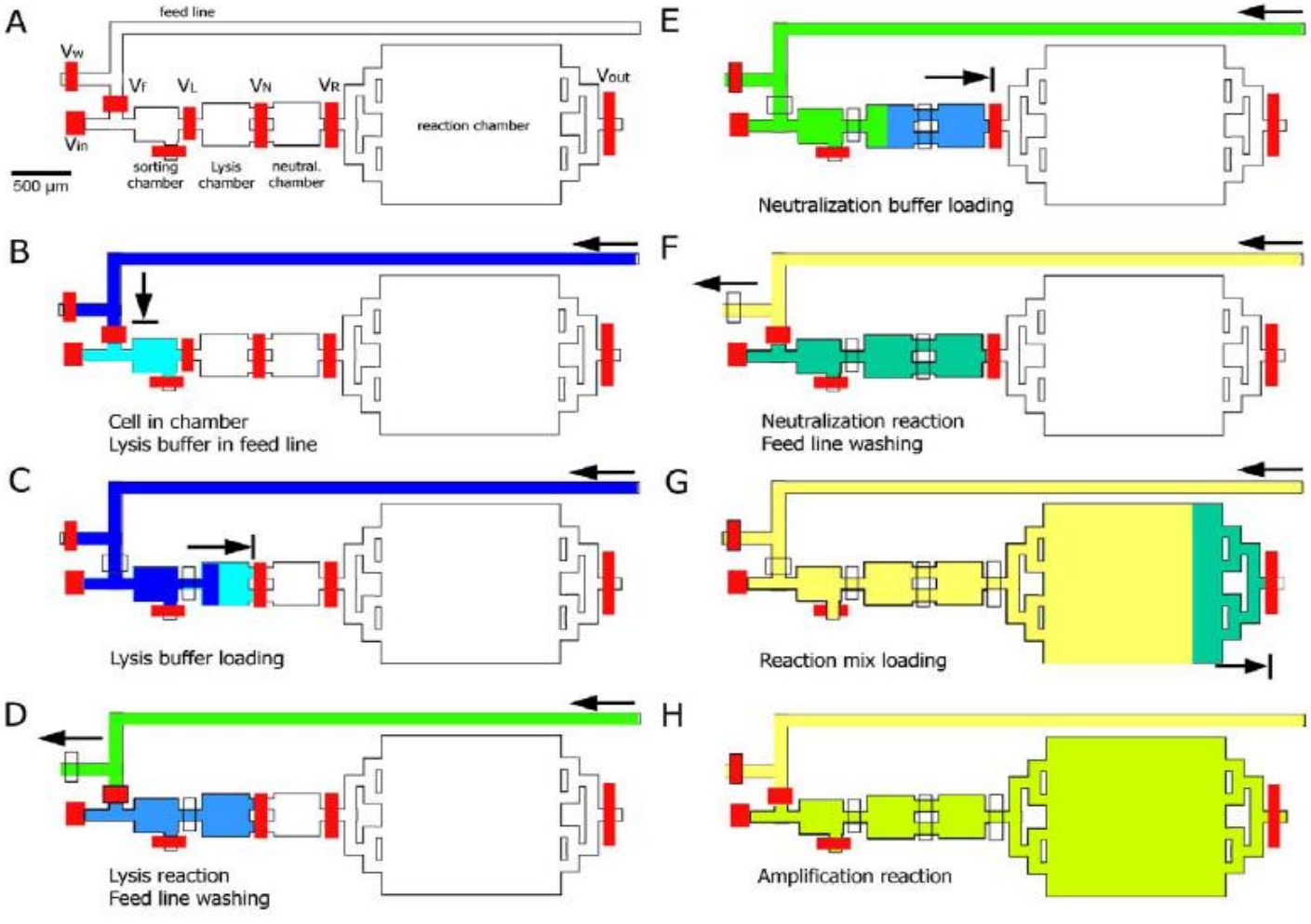
60 nl reactions, 10^7 copies of the genome

Compared to standard 50µl amplification reaction

Benefits:

Less non –specific DNA synthesis and amplification bias (these are drawbacks of MDA in standard scale)

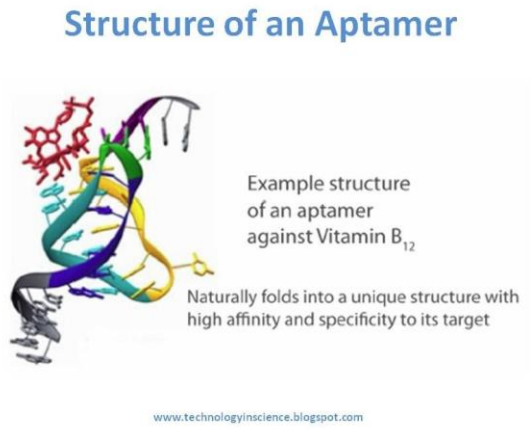
High quality material to the sequencing of the genomic DNA



Marcy et al. 2007

BioMEMS example: DNA as a construction material

- Examples in nanoscale studies/demonstrations
- DNA hybridization makes possible to design 2-3D structures from DNA and also to functionalize the constructs
- **DNA origames**
  - Biosensing, enzyme cascades drug delivery
- **Aptamers**



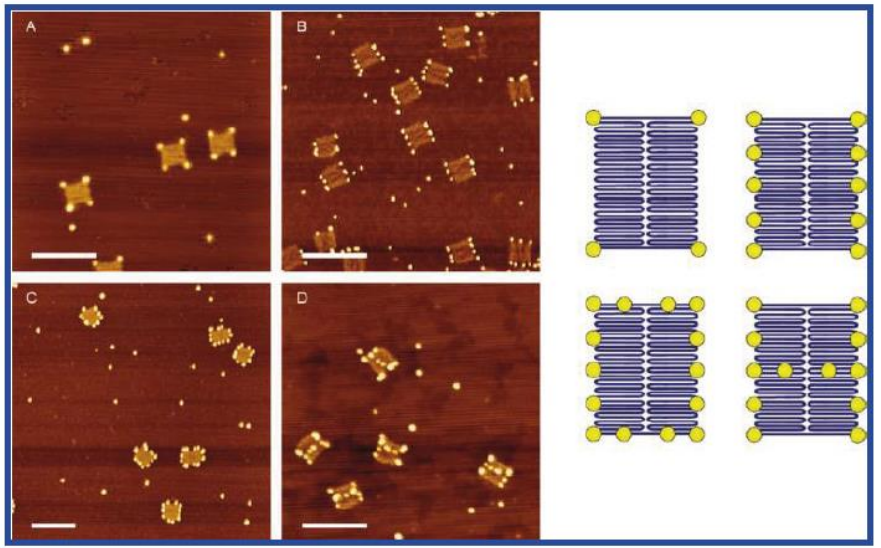
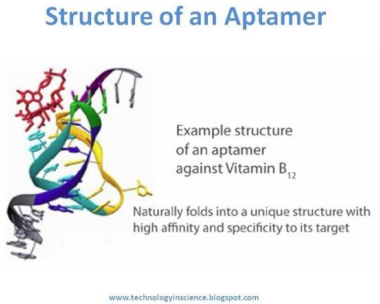


Figure 1. AFM images of different AuNP assemblies on mica: (A) four corners, (B) a pair of parallel bars, (C) ring, and (D) H shape. The patterns are programmed by extending different staples with the same complementary sequence, permitting the use of the same AuNP–DNA conjugates for all assemblies. All scale bars are 250 nm. The size of the particles appears enlarged, due to convolution with the AFM tip. Schematic on the right shows the designed structures.

Pilo-Pais et al. 2011



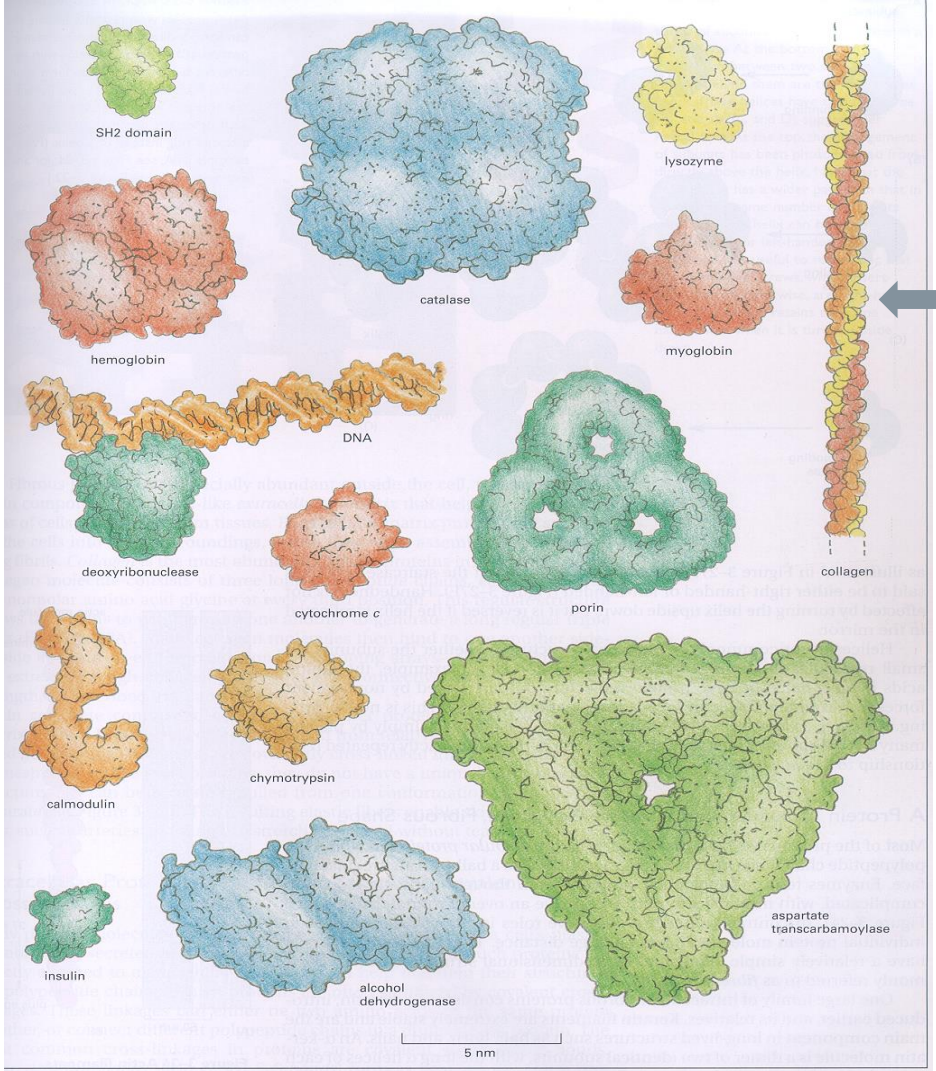
Potential use in diagnostic platforms as a recognition element in sensors

- Single stranded DNA, isolated from a library of synthetic oligonucleotides
  - Affinity (µM to pM) and specificity towards a target molecule

- High stability (DNA), small size (proximity to sensor surface, low unspesific binding), easy and reproducible synthesis *in vitro*, simple to modify, suitable for different detection modes (optical, electrochemical etc)

Recent review Marrazza (2017) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5371778/

Proteins in microsystems as components or target analytes



Proteins have a huge structural variability!

Examples of different sizes and shapes of proteins

Variables: size, shape, surface charge, stability, aggregation tendency

Proteins have different interactions and functionality (binding, catalysis, structural component)

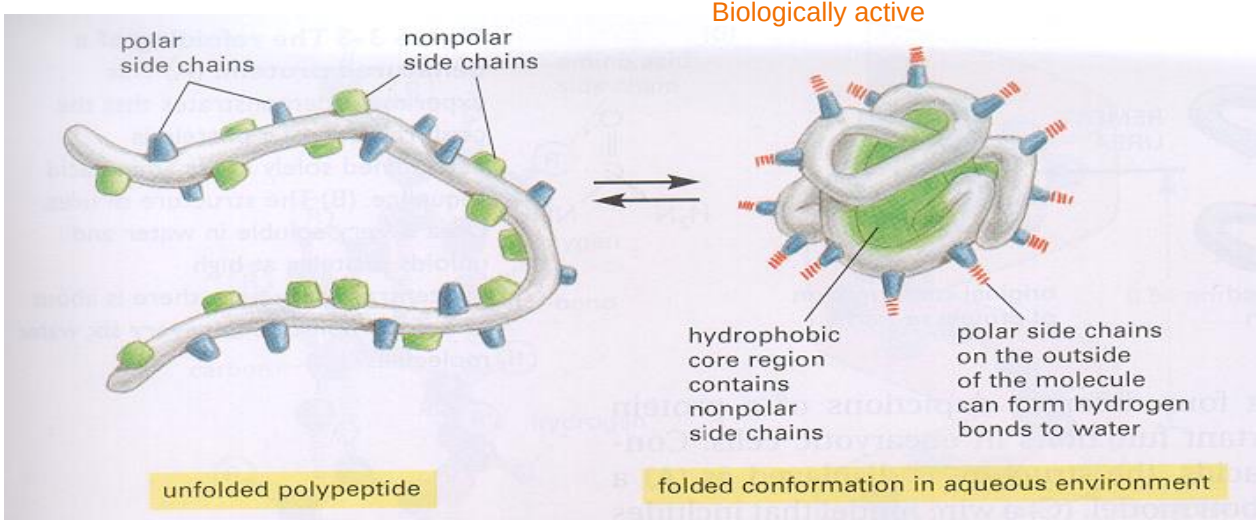
In vivo most of the cellular proteins are in contact with other proteins = protein complexes

Alberts et al.

Stability

IMPORTANT: In BioMEMS systems the proteins are not in their natural environment.
Disturbances have effect on the stability and functionality of the proteins

Biologically active

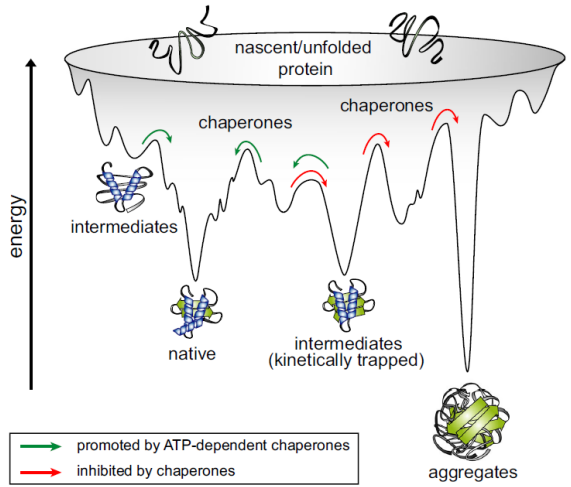


Conformation of lowest energy

Alberts et al.

Folding / refolding of proteins

In vivo: Folding starts during the synthesis
of the polypeptide chain
chaperones = assisting proteins
Uncorrectly folded proteins are degraded or form aggregates



Balchin et al. 2020

Important in BioMEMS when e.g. printed and dried proteins are used: is the protein able to refold by itself and in which conditions?

E.g. for the binding capacity of the sensing surface of the BioMEMS device it is important that all binding proteins are functional. Unfunctional binding proteins cause decrease in sensitivity.
In case of reusable or continuously used BioMEMS devices the functionality of the binding proteins need to be ensured

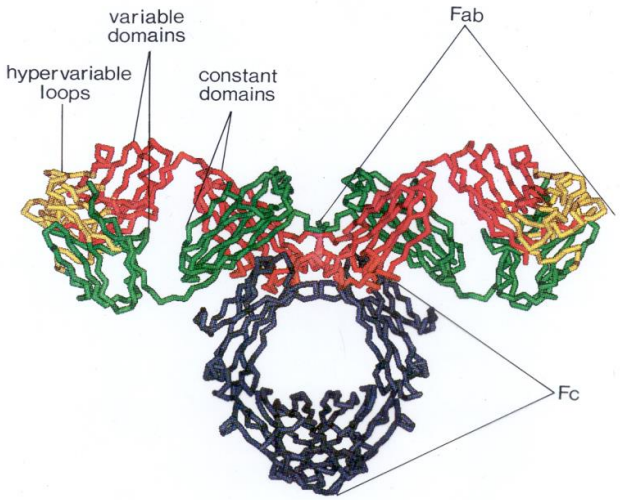
Microfluidics/BioMEMS for proteins

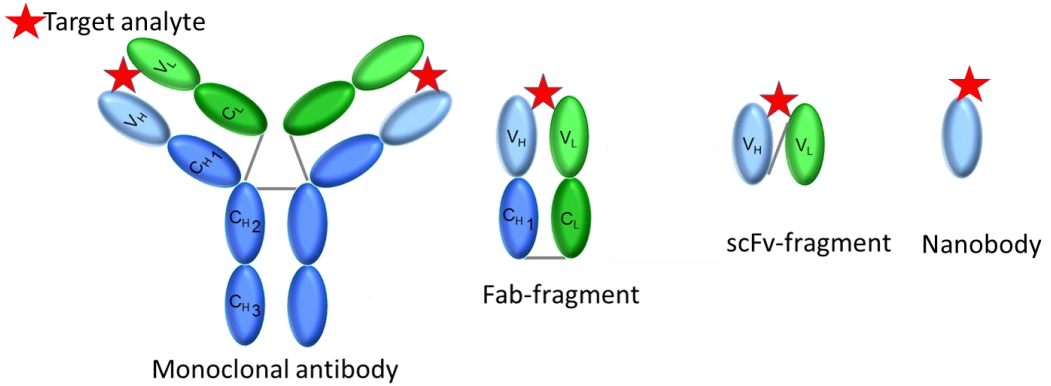
Proteins as targets (single or multiple proteins or proteome)

Proteins as tools (capture or detection molecules)

Antibodies are the most widely used binding molecules for capture and detection

Small antibody fragments (Fab, scFv, nanobody) bring advantages to BioMEMS



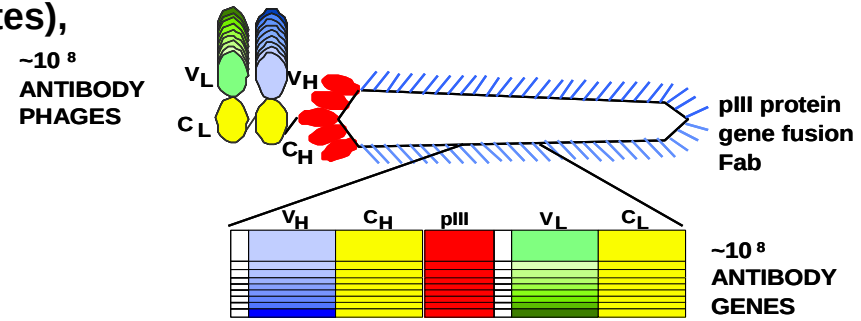


Antibodies as an example of widely used proteins in microsystems

Introduction to antibodies

Produced by mammalian immune system (B-lymphocytes), protection against invaders

e.g. viruses, foreign proteins, bacteria



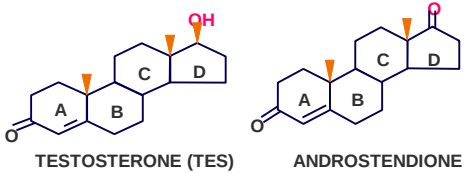
Enormous potential to recognize a wide variety of molecules (diversity)

Antibody gene libraries (size >10^8 clones)

High specificity of the recognition

low molecular weight compounds, peptides, proteins, oligosaccharides, cells, viruses, inorganic materials/surfaces

VTT



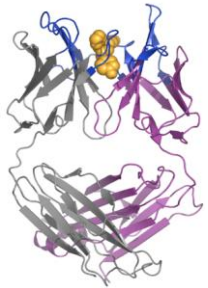
High affinity binding

equilibrium reaction

association – dissociation phases

fM affinities by mutagenesis, <nM from immunizations

VTT



Antibody fragment

Antigen= substance that can induce antibody production *in vivo* (infection/immunisation)

Hapten= small compound which in itself cannot elict the immune response but as a protein conjugate becomes immunogenic.

Recombinant antibodies (only the antigen binding domains, gene available, produced in microbes)

Microsystems introduce a non- natural environment for antibodies! Materials, liquid composition (buffer versus e.g. serum), supporting molecules are missing, temperature

Protein engineering makes possible to improve the properties of the recombinant antibodies for BioMEMS

Conserved structure

lots of structural and sequence data = knowledge-based approach

in vitro evolution techniques

mutant libraries, selection in harsh conditions and screening

Affinity and specificity

association-dissociation behaviour makes the difference

fM affinities by mutagenesis (corresponds to biotin-avidin interaction)

Stability

extended half-life, stability in various conditions, resistance to surface denaturation or dry atmosphere

also non-engineering options (e.g chemical cross- linking)

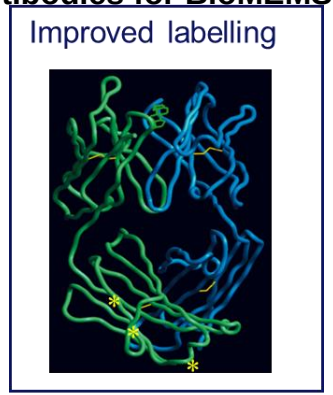
Labeling

additional and designed sites for labels

Immobilisation

oriented, site-directed,

single residues (Cys for gold), aminoacid tags (e.g. histidines), binding domains



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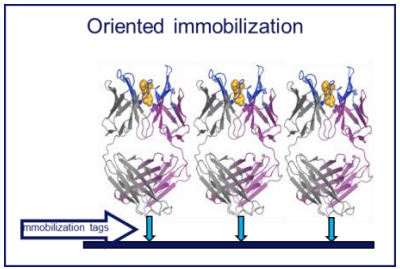


VTT

Examples of antibodies in BioMEMS

Soluble assay format: measuring the concentration of the analyte (here MMP-8, biomarker for inflammation of gums)

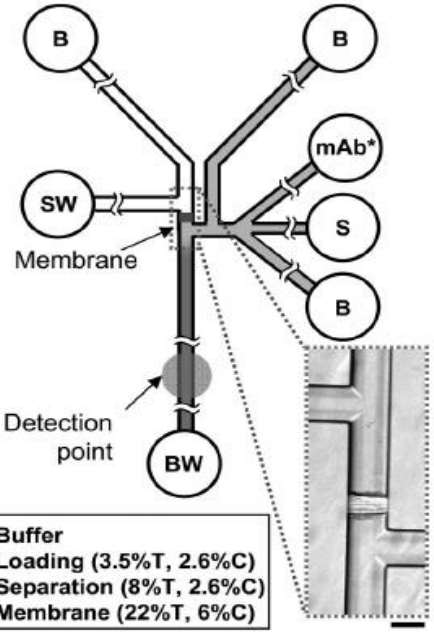
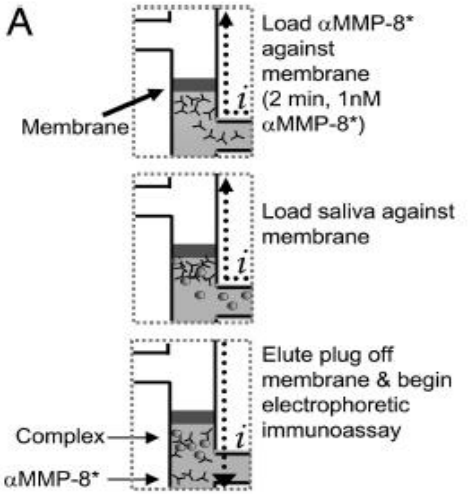
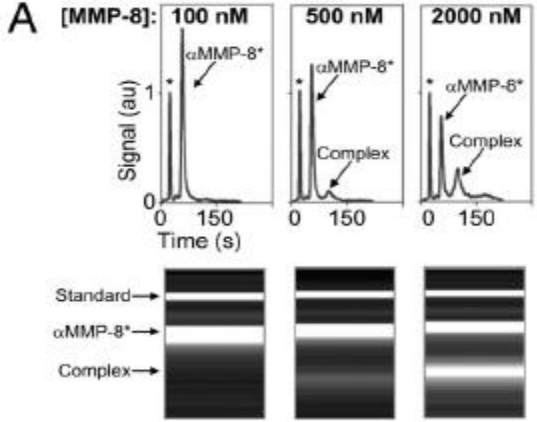


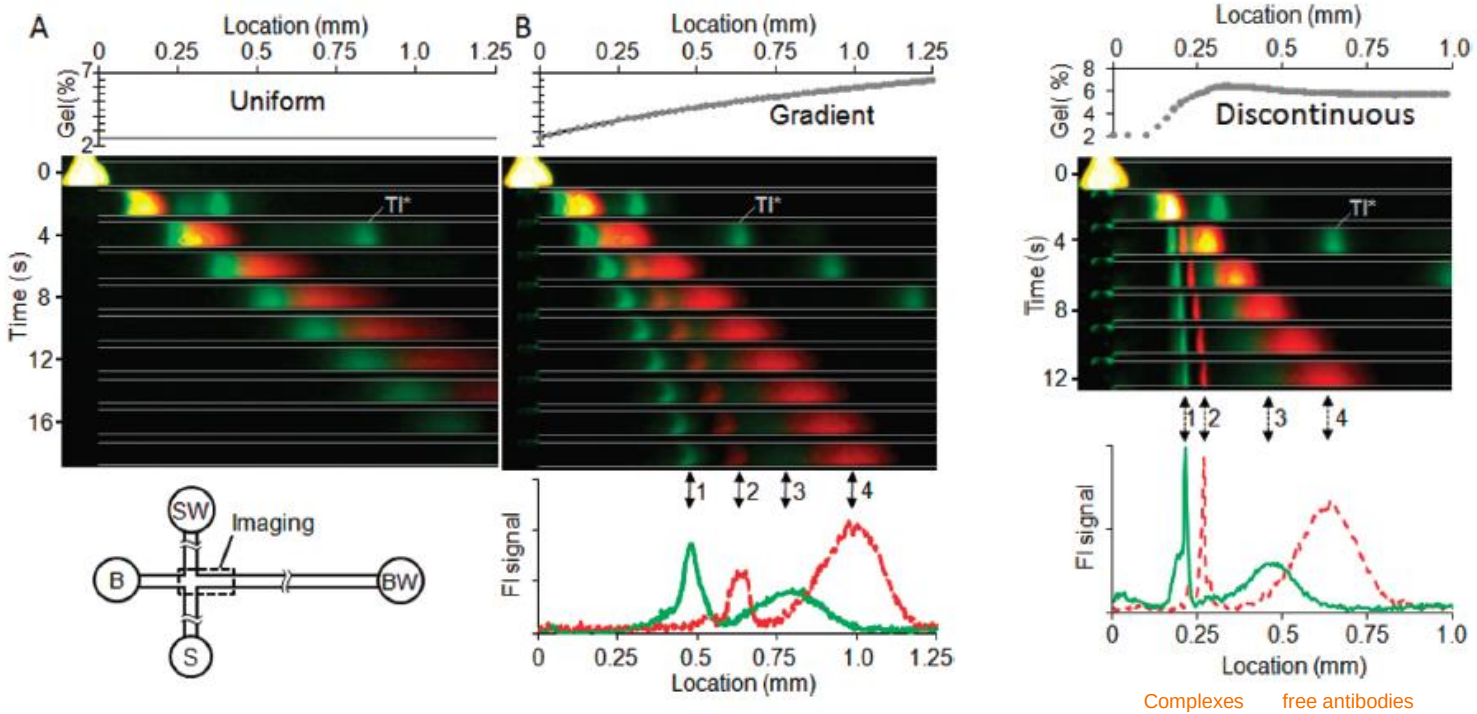
Fig. 1. μCEI device layout. Fluid wells are labeled according to contents as follows: S, sample; B, buffer; SW, sample waste; BW, buffer waste; mAb*, fluorescently labeled monoclonal antibody to MMP-8. Polyacrylamide gel composition is indicated by grayscale shading (%T and %C are percentage of total acrylamide and bis-acrylamide cross-linker, respectively). *Inset* shows a 40× bright-field image of the size-exclusion membrane. (Scale bar, 100 μm.)





Herr 2007

Antibodies in BioMEMS, soluble assay example 2

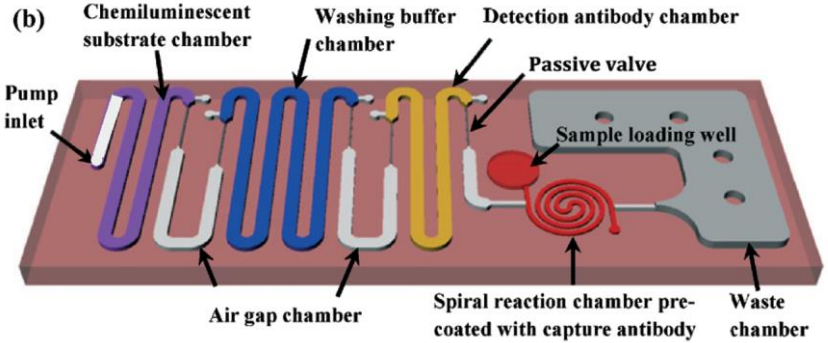


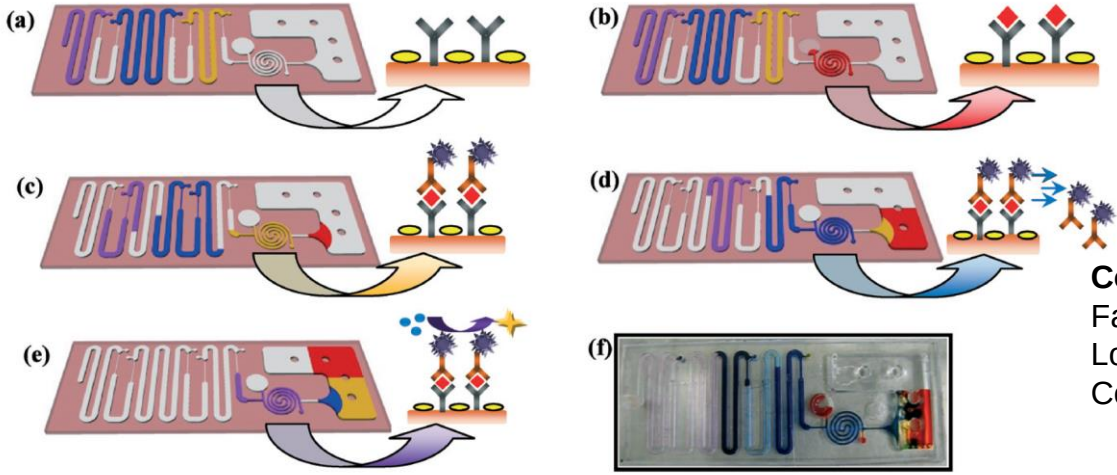
Two analytes detected simultaneously
350µm separation channel needed
Quantitative assay completion in <10 s
LODs: CRP 11ng/ml, TNFα 40ng/ml

Hou, 2010

Antibodies in BioMEMS

Point-of-care test for TSH (thyroid stimulating hormone)



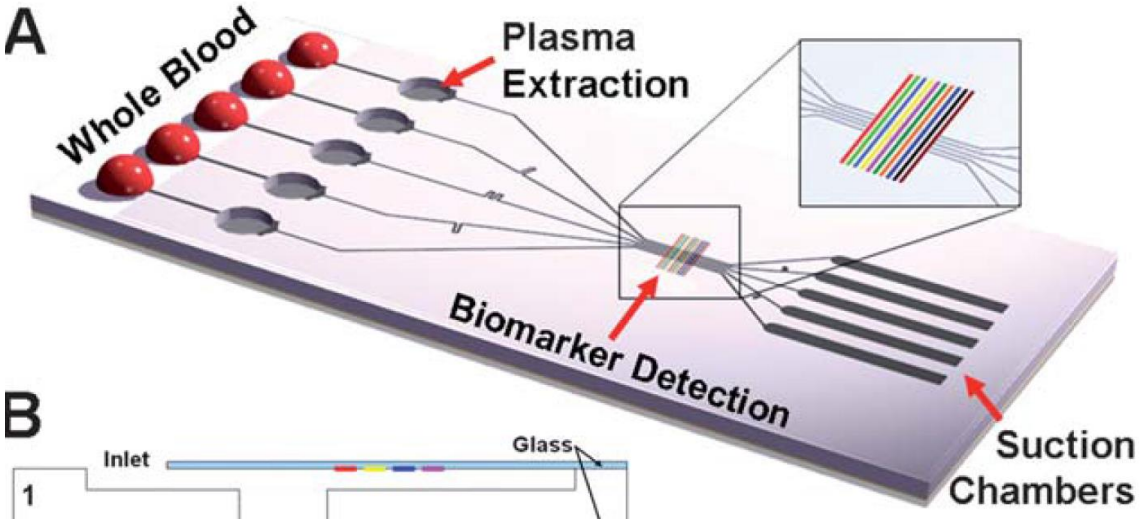


Compared to ELISA:
Faster, less interventions
Lower reagent consumption
Comparable sensitivity

Jung et al. 2013

Antibodies in BioMEMS

Diagnostics: Self-powered integrated microfluidic blood analysis system (SIMBAS)



- Integrated blood plasma separation and multiplexed assays
- 5µl of whole blood

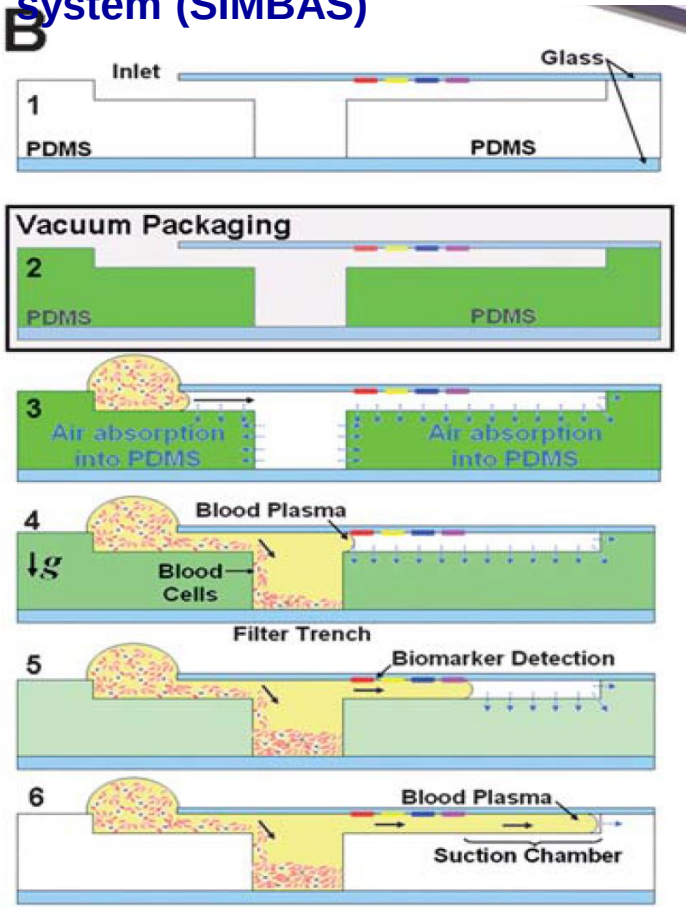
- Self priming degassing-driven flow technique (generated when device is removed from its vacuum packing)

- An example:
  - Pattern of 15µm bars of avidin, spiked blood with fluorescently labelled biotin
  - Picomolar detection concentrations achieved

Details next slide

Dimov et al. 2011

Diagnostics: Self-powered integrated microfluidic blood analysis system (SIMBAS)



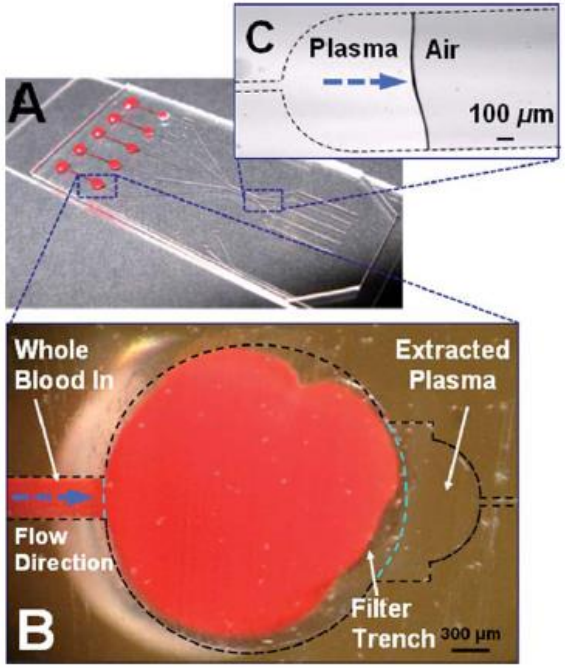


Fig. 2 Degas-driven flow is generated when the SIMBAS device is removed from a low-pressure environment. (A) Fast and effective plasma separation of 5 separate whole-blood samples by (B) filter trench (cylindrical cavity) and gravity-driven blood cell sedimentation generates blood-cell-free plasma in the self-priming untethered SIMBAS, see ESI† Movie SF1. (C) Plasma fills the suction chamber.

The lid can be removed and bound analytes further analysed (PCR/MS)

Dimov et al 2011

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