Application of droplet microfluidics to high-throughput screenings

Elena De Cecco

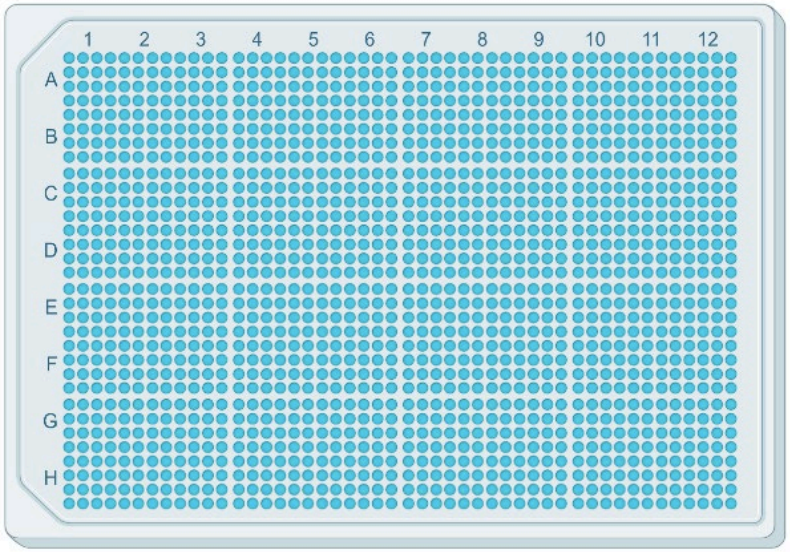
20.04.21

High-throughput screening in multiwell plates

Spatial separation

Sequential addition of reagents

Low volumes
(2 µL)



Additional
manipulations
to the samples

Analysis

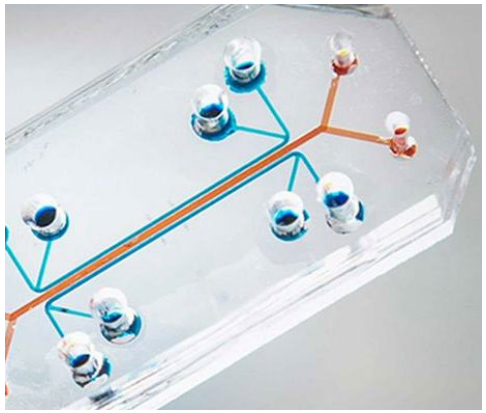
Sample cleanup

Collection of aliquots

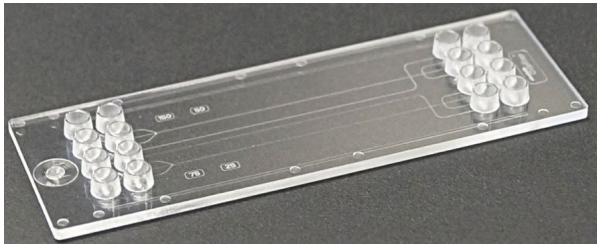
Throughput: >10^5 samples per day

Can we further increase the throughput?

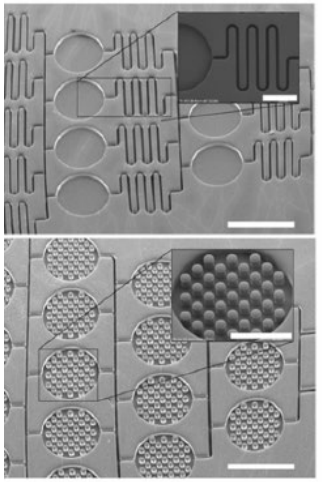
Throughput: >10^3 droplets formed per second (kHz)!



Organ-on-chip

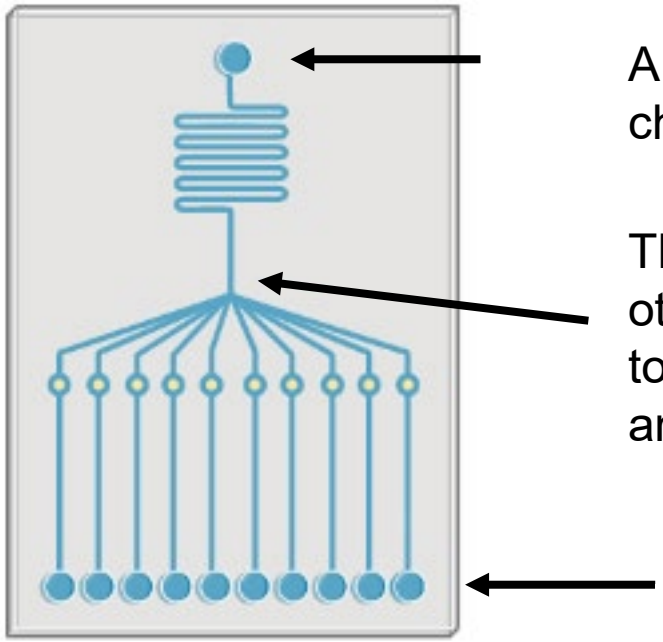


Chip with sorting unit



Chip with cell culture chambers

Microfluidics for HTS

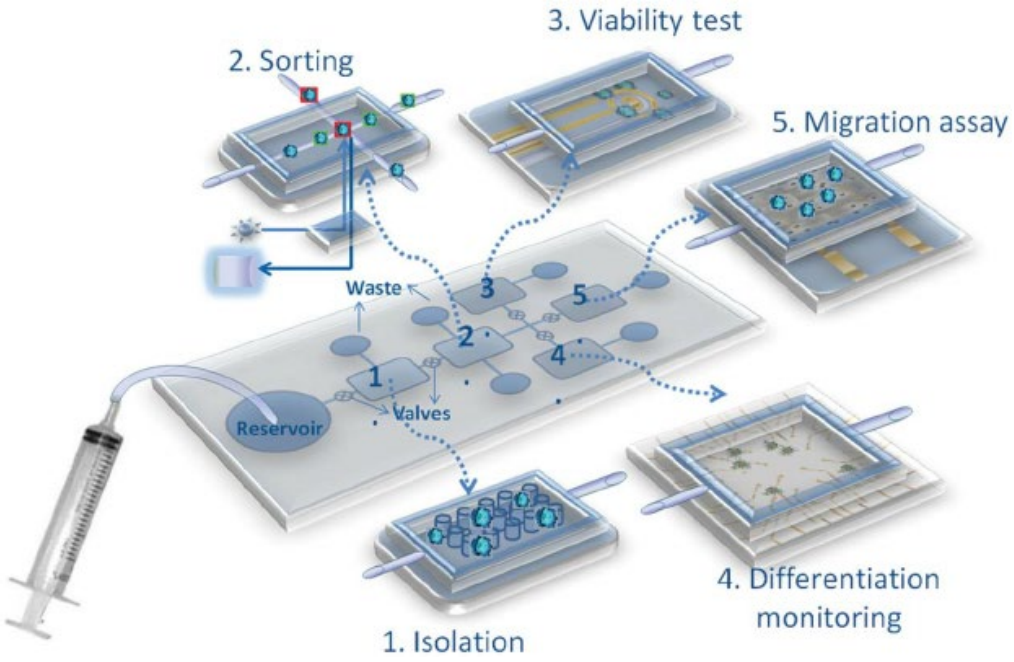


A microfluidic chip is a series of micro-channels molded into silicone or polymers.

The micro-channels are connected to each other to achieve the desired function, and to the external environment through inlet and outlet holes.

Lab-on-chip indicates a miniaturized device that integrate multiple operations in a single chip.

First attempts at realizing lab-on-chip date back to the 80s!

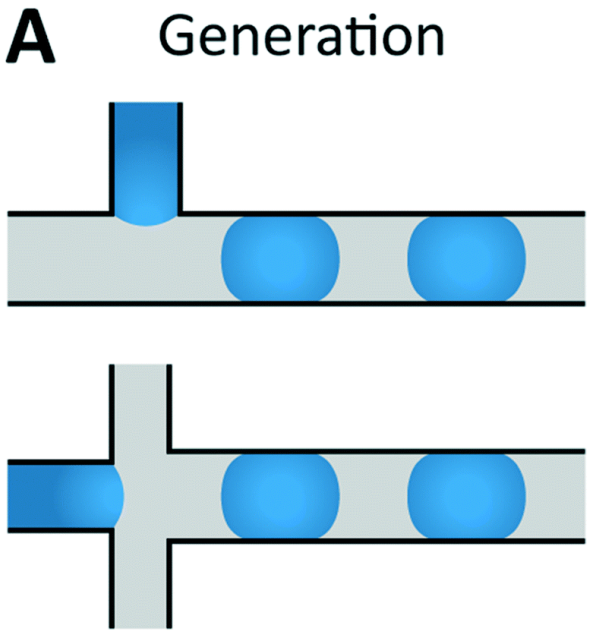


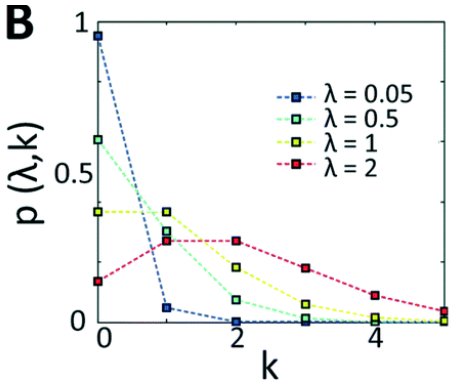
1. Spatial separation

Achieved by breaking fluids into droplets using a T-junction or a flow focusing geometry.

Droplets volumes range from single femtoliters to tens of nanoliters

Multiple substances can be encapsulated in the same droplets



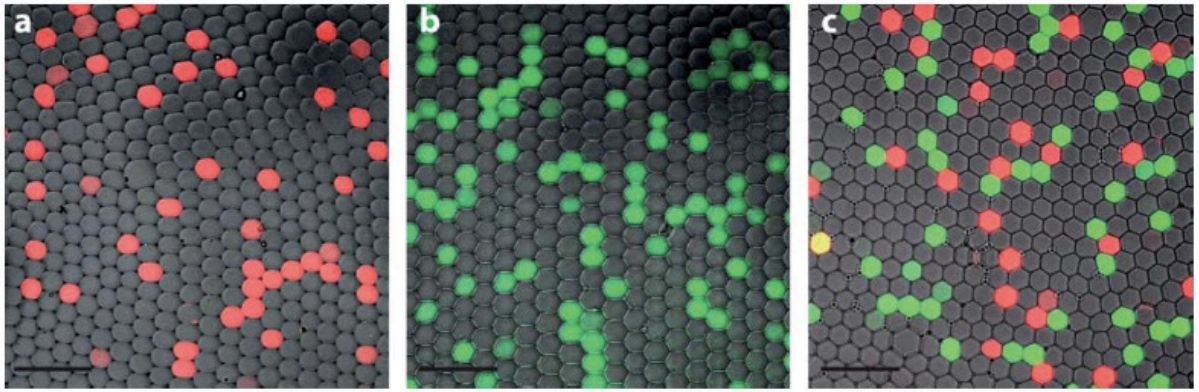


Frequency of analyte encapsulation follows Poisson's distribution → the majority of the generated droplets are empty!

How to maintain spatial separation between droplets? → use of surfactants

1) Limit droplet merging and chemical transfer

2) Ensure stability in a range of temperatures, allowing reactions such as PCR



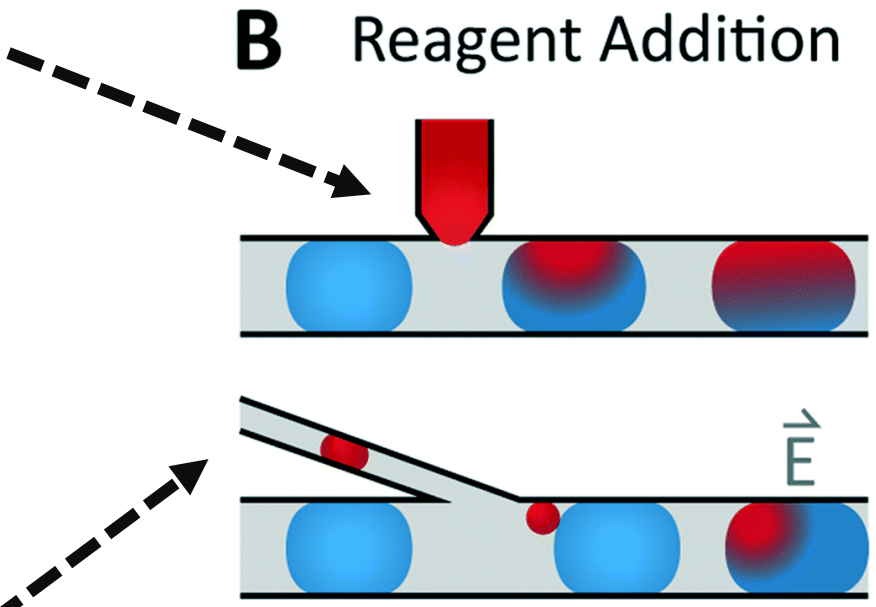
Lab Chip, 2012, 12, 422

PCR amplifying the wt version (red) or the mutated version (green) of a gene.

2. Reagent addition

Pico-injectors: reagent is in brief contact with a continuous flow of droplets, which merge with it and are subsequently resegmentated

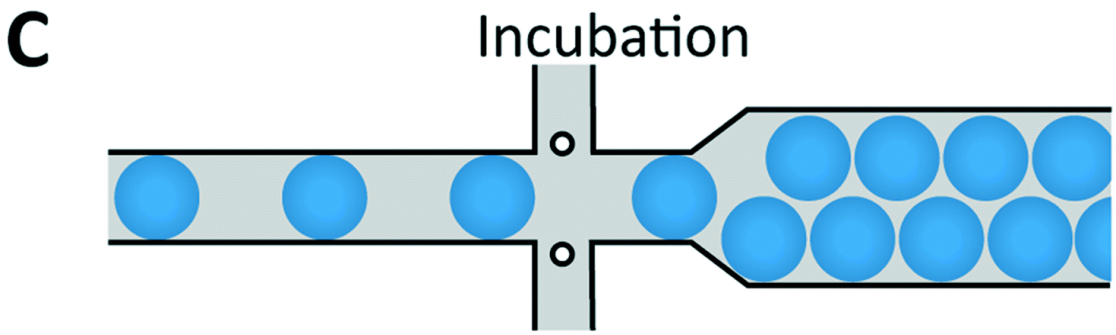
Drawback: all the droplets receive the same reagents.



Droplet pairing and merging: Combine two libraries of droplets to produce a combinatorial set of samples

3. Sample incubation

Sample incubation is crucial to allow the desired reaction to occur



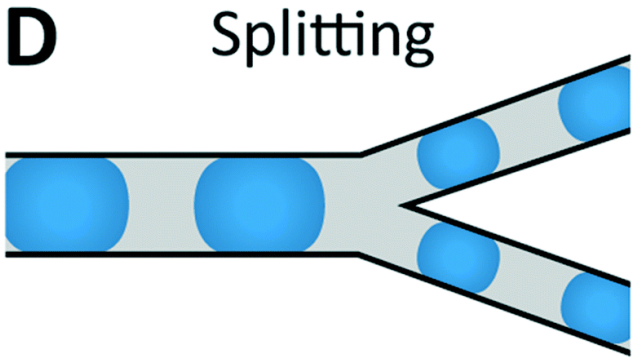
1. Remove droplets from the device and store them in bulk

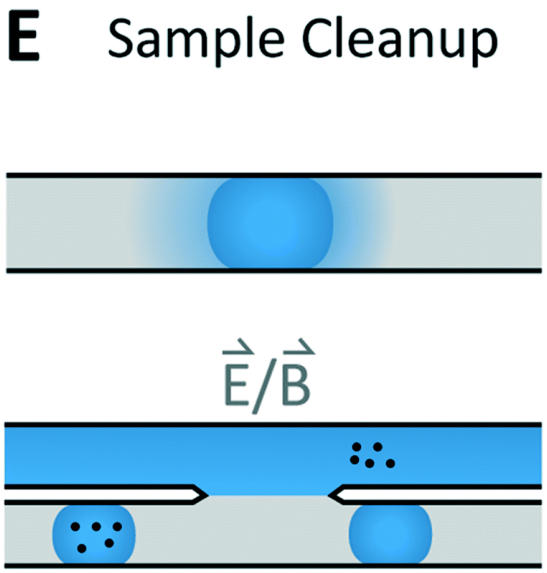
2. Reduce fluidic velocity on chip and increase the on-device time

3. Channel expansion and integration of delay lines

4. Sampling and cleanup

Droplets are splitted into smaller portions by using bifurcated channels, acoustic waves or electrostatic forces

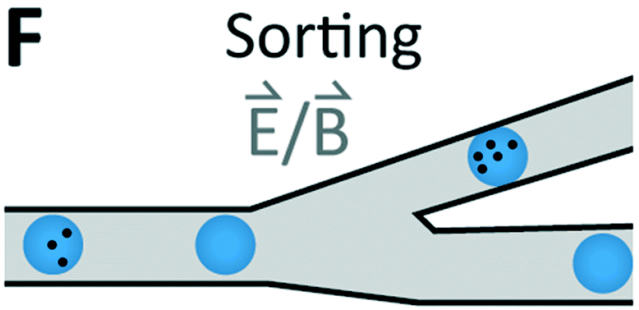




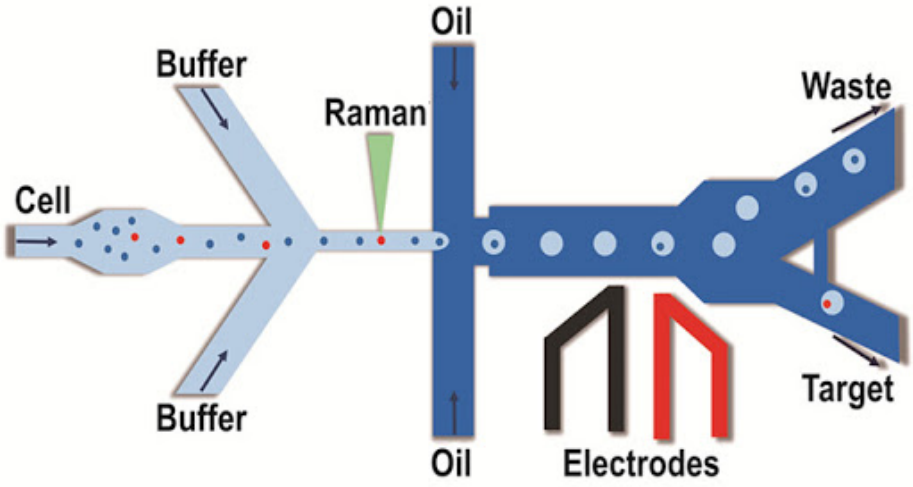
Target analytes are extracted from the droplets by solid or liquid phase extraction

5. Sorting

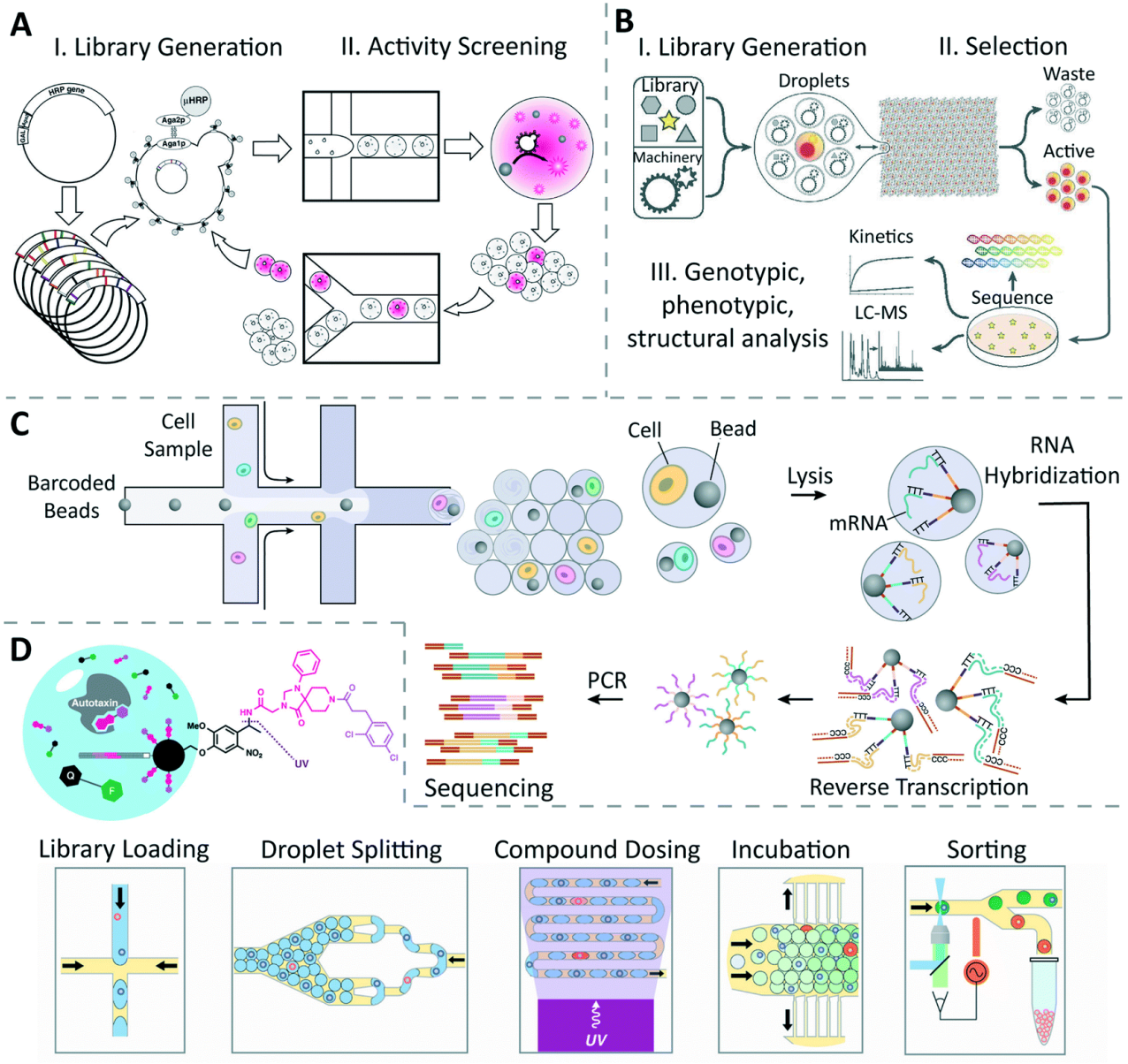
Droplets sorting is required when the samples need to be further manipulated after analysis

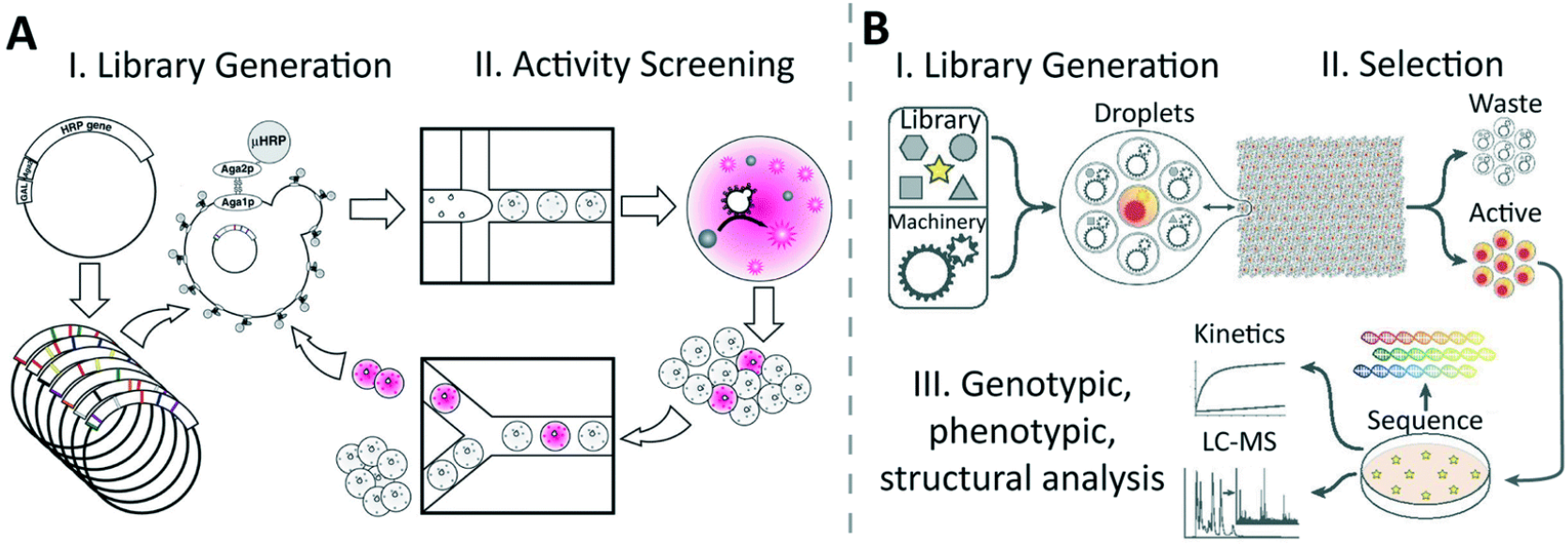


The most common strategy is dielectrophoresis (DEP), where an electric field deflects droplets to exit via a different channel



Applications of microfluidics in HTS

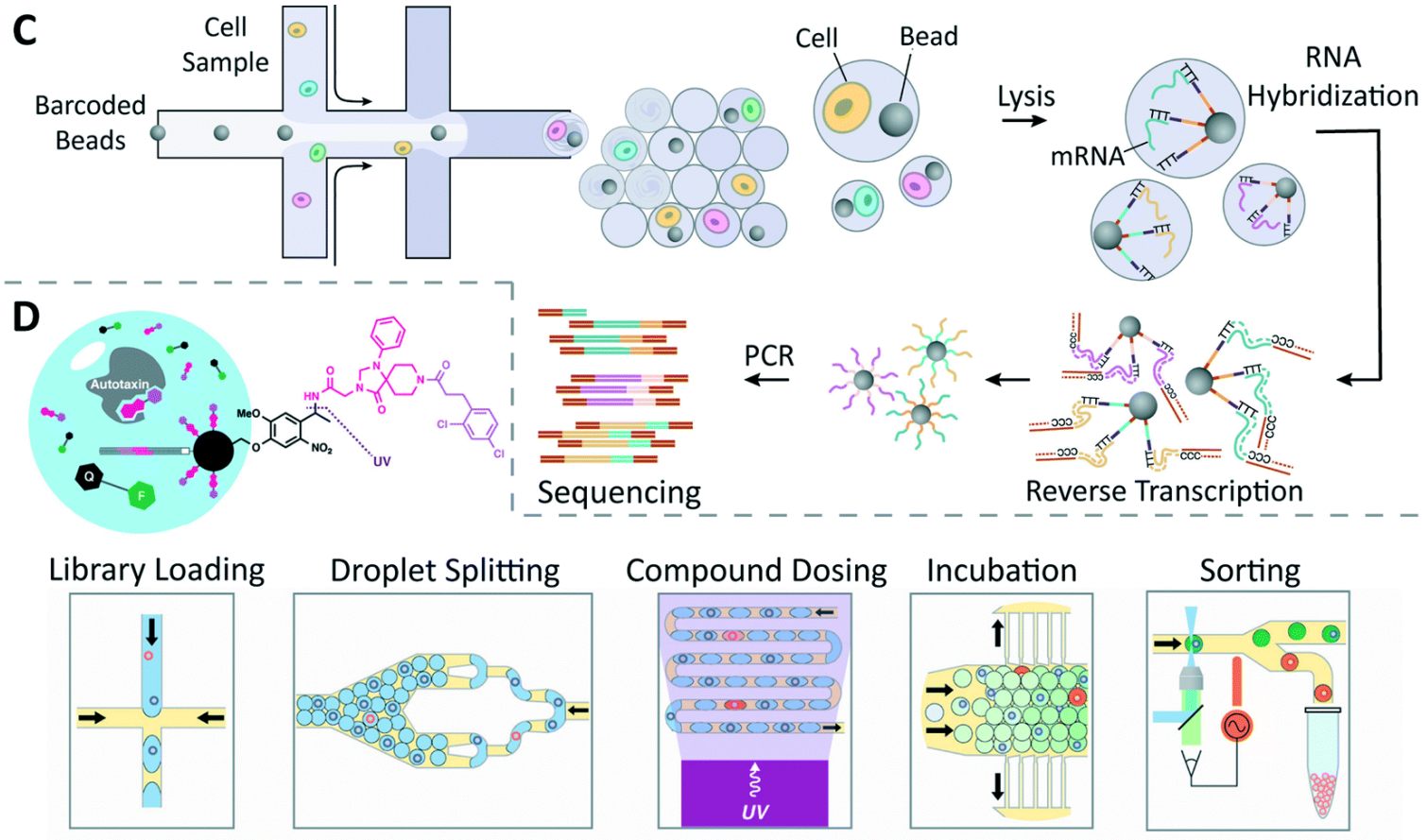




Protein engineering and biocatalysis screens using fluorescence-based assays

Identification of bacteria producing valuable compounds

Single cell isolation and sequencing to identify rare cell populations



HTS of libraries of chemical compounds

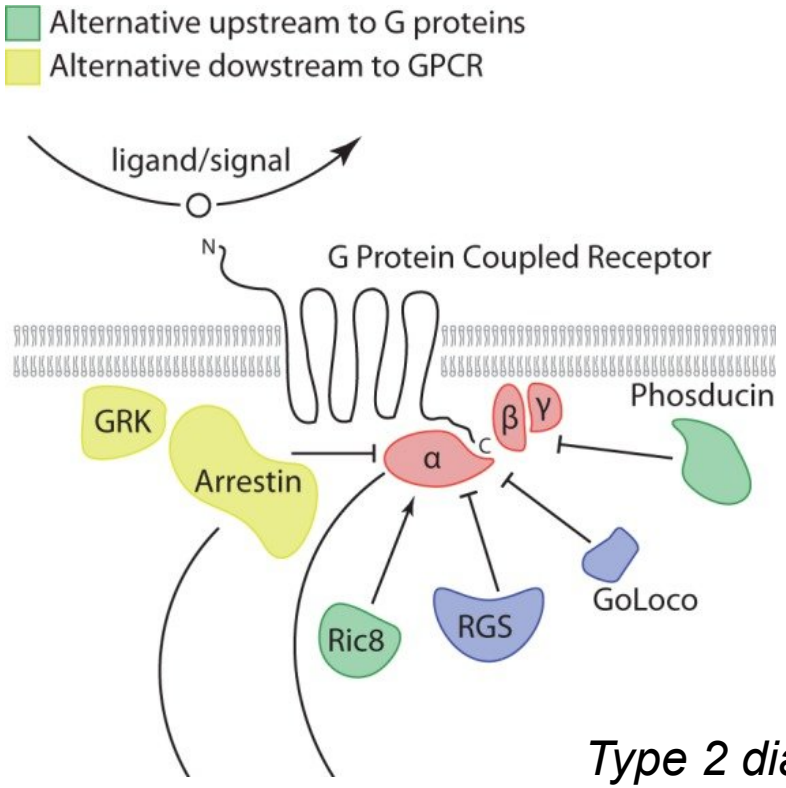
SCIENTIFIC REPORTS

OPEN

High-throughput identification of peptide agonists against GPCRs by co-culture of mammalian reporter cells and peptide-secreting yeast cells using droplet microfluidics

Received: 5 April 2019
Accepted: 15 July 2019
Published online: 29 July 2019

Kenshi Yaginuma[1], Wataru Aoki[1,2,3], Natsuko Miura[4], Yuta Ohtani[1], Shunsuke Aburaya[1,5], Masato Kogawa[6,7], Yohei Nishikawa[6,7], Masahito Hosokawa[3,8], Haruko Takeyama[6,7,8] & Mitsuyoshi Ueda[1,2]



GPCRs mediate many diverse signalling pathways in response to a variety of external stimuli.

Abnormal activity of GPCRs is linked to multiple human diseases, and therefore they represent valuable druggable targets.

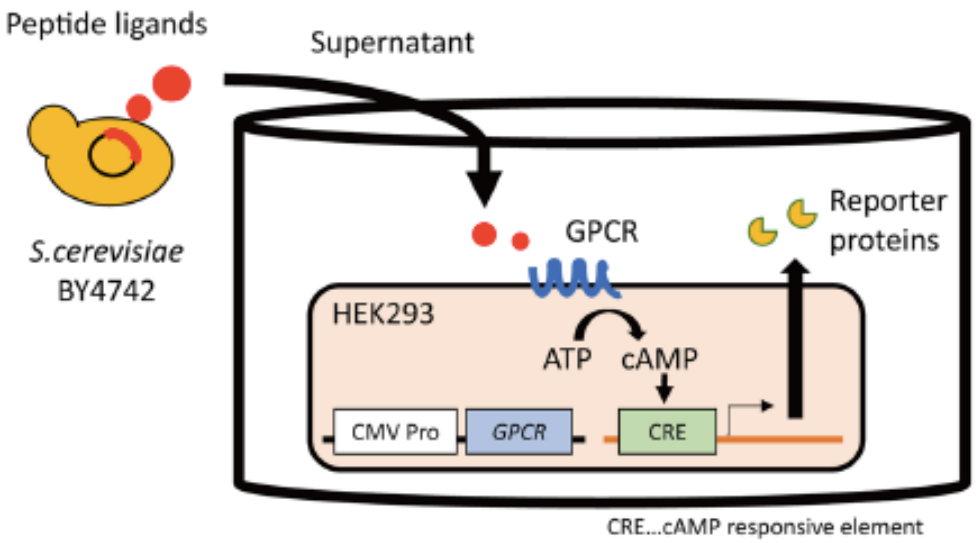
Type 2 diabetes *Asthma* *Epilepsy*

Cancer *Arthritis* *HIV*

→ How can we identify drug targets that act as functional ligands of GPCRs in a high-throughput manner?

Functional cell-based assay

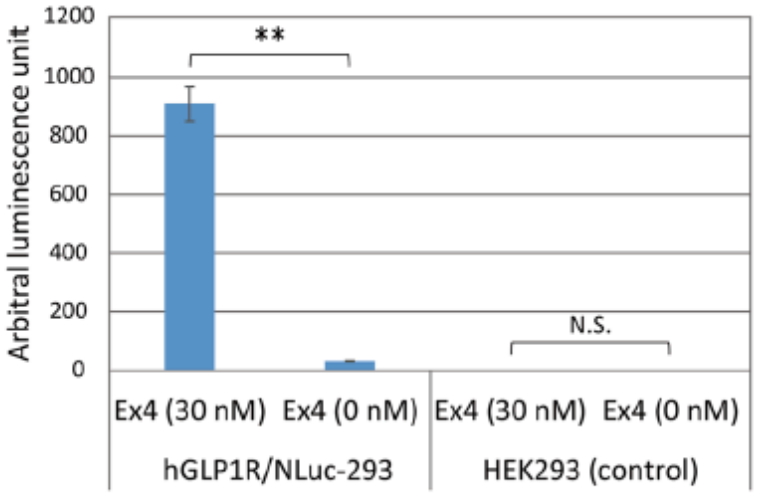
GPR: Human glucagon-like peptide-1-receptor (**hGLP1R**)

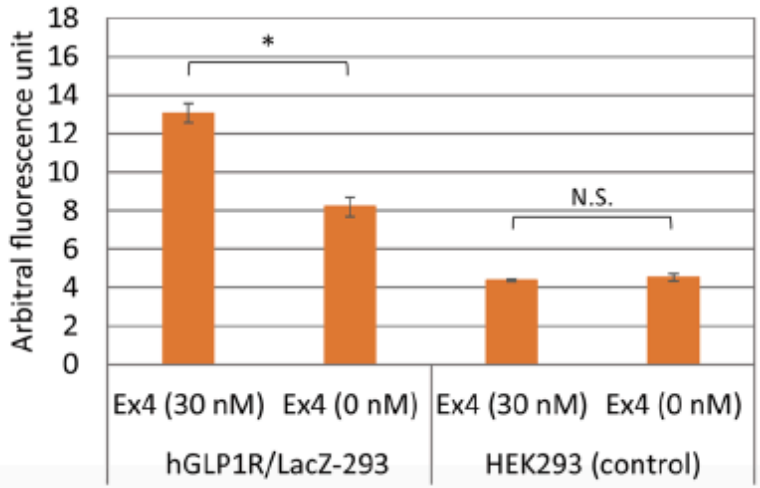


Yeast cells producing and secreting peptides are put in contact with HEK293 cells expressing hGLP1R

When hGLP1R is activated by one of the peptides, it produces cAMP, which in turn activates a reporter protein through a CRE element

Choosing the reporter element



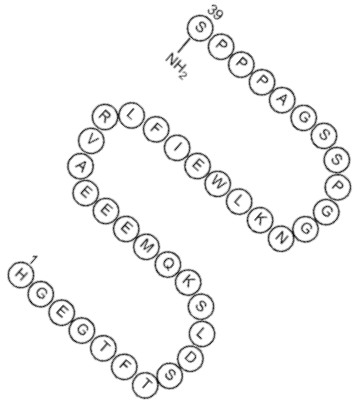


NanoLuc vs. LacZ

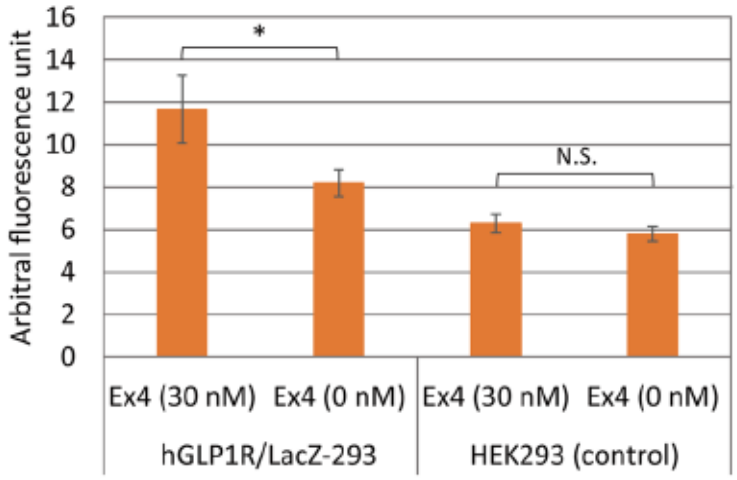
Luciferase has two intrinsic problems:

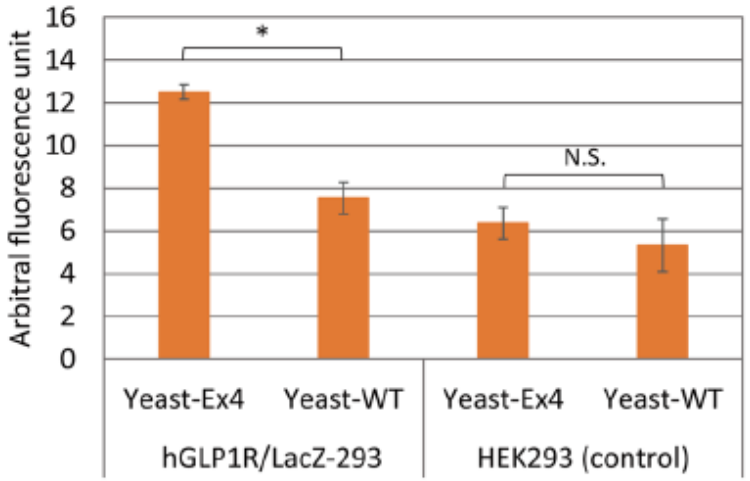
- Limited amount of substrates in droplet impairs detection of luminescence
- Slow rate of photon production

Although the signal:noise ratio is lower, LacZ was chosen as reporter system.



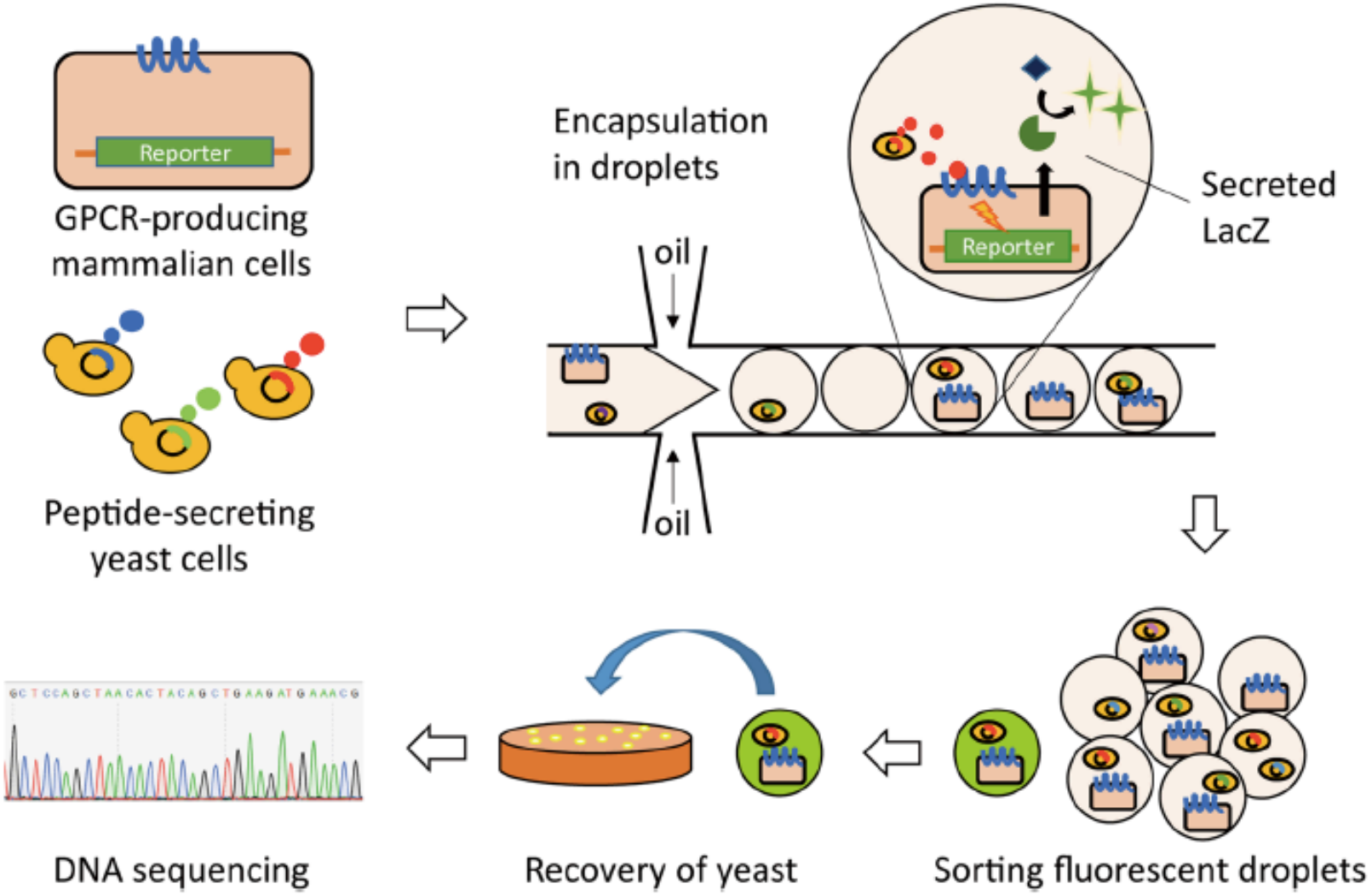
Exendin-4, a 39-aa peptide, is used as positive control to activate hGLP1R



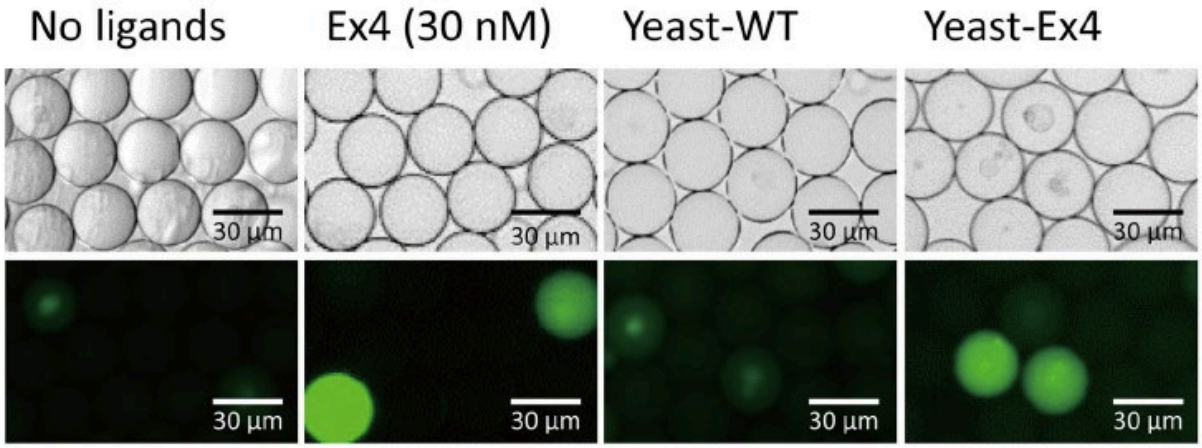


Signal is detectable when the cells are in contact with yeast cells secreting Exendin-4.

Workflow of high-throughput screening



Proof-of-concept using exendin-4



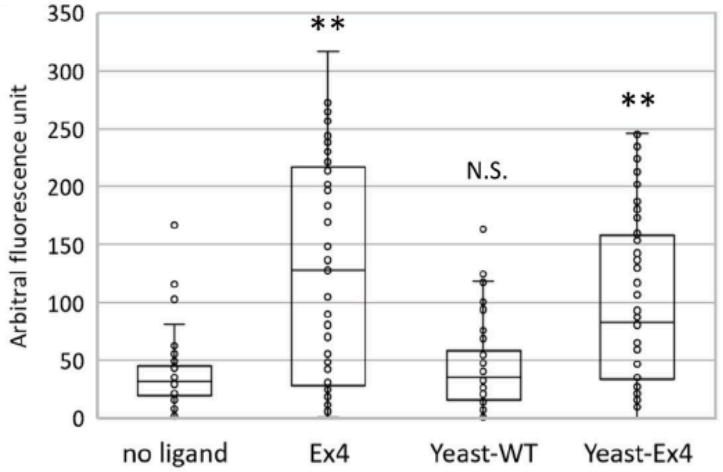
Signal is present only when reporter cells are encapsulated either with free Ex4 or yeast-Ex4

Encapsulation of one reporter cell: 6.55%

Encapsulation of a yeast cell: 11.29%

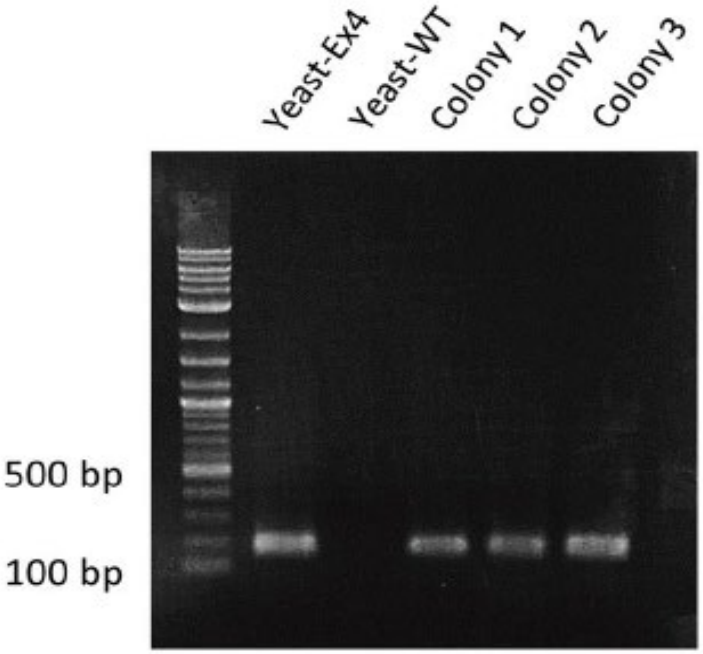
Co-encapsulation: 0.74%

Many droplets where Ex4 was present were not fluorescent



Droplets production rate: 10’000/s

Fluorescent droplets were sorted out of the microfluidic device and the yeast cells were isolated and cultured in agar plates



Colony PCR confirmed that the positive droplets contained indeed yeast cells secreting Ex4

Proof-of-concept → confirmed

High-throughput screening

Library generation

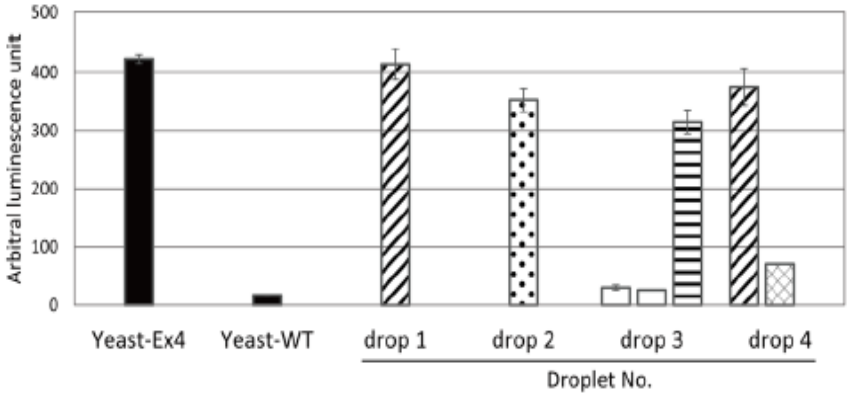
The two N-terminal amino acids of Ex4 are responsible for GLP1R activation

Random mutagenesis (gap repair cloning) to generate a library of mutated Ex4

Original Ex4 appears at probability of 0.2% in the mutated library and is used as positive control

100’000 droplets analysed

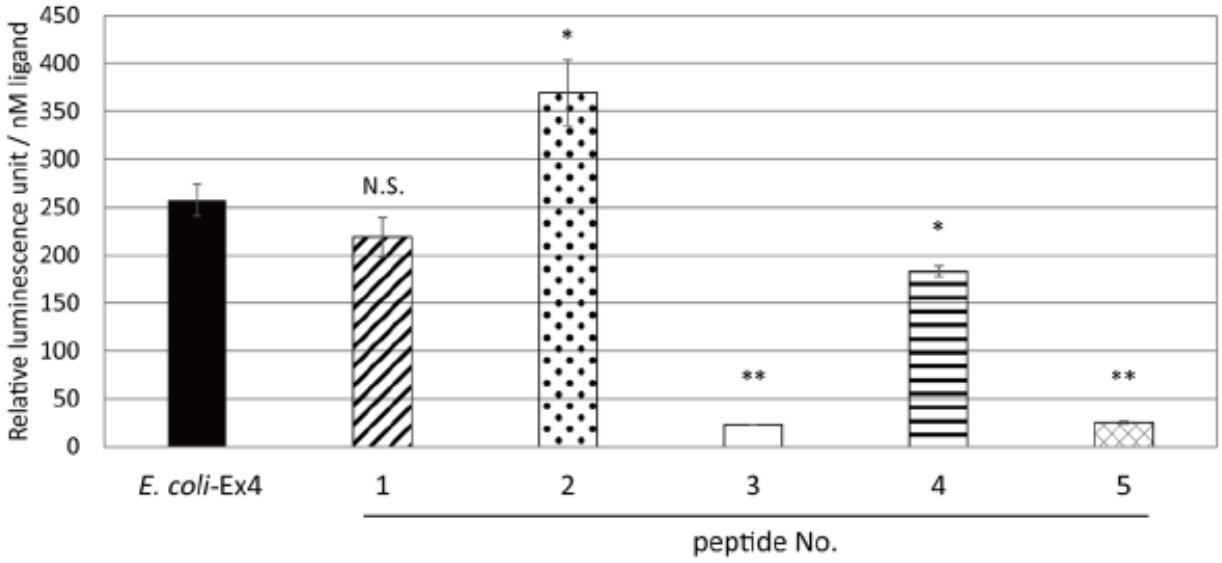
6 positives → 4 yeast clones cultured



4/4 secrete a functional ligand

Sequencing

Influence of post-translational modifications of yeast was excluded by producing the peptides in E.coli and assessing their activity.



Peptide variant 2 showed a slightly higher agonistic activity than Ex4, which is one of the strongest agonists of GLP1R.

Conclusion

The use of a microfluidic device allowed the fast and high-throughput screening of many variants of the agonistic peptide Exendin-4.

Water-in-oil droplets allow the temporary co-incubation of mammalian cells and yeast cells secreting peptides.

Still many optimizations are possible:

- Improve the speed of droplets production
- Improve the efficiency of co-encapsulation
- Couple a fluorescence-activated cell sorting device to improve droplets sorting and further processing.

nature biotechnology

LETTERS

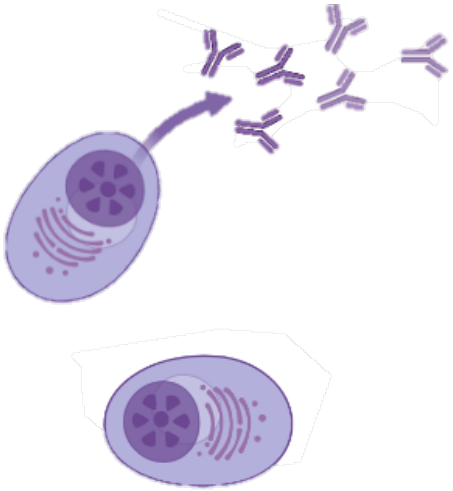
https://doi.org/10.1038/s41587-020-0466-7

Check for updates

High-throughput single-cell activity-based screening and sequencing of antibodies using droplet microfluidics

Annabelle Gérard[1,18], Adam Woolfe[1,17], Guillaume Mottet[2,17], Marcel Reichen[1,17], Carlos Castrillon[2,3,4,17], Vera Menrath[1,17], Sami Ellouze[1,17], Adeline Poitou[1,17], Raphaël Doineau[1,3,4,11,17], Luis Briseno-Roa[1,12], Pablo Canales-Herrerias[2,3,5], Pascaline Mary[6], Gregory Rose[6], Charina Ortega[6], Matthieu Delincé[6], Sosthene Essono[6], Bin Jia[7,13], Bruno Iannascoli[2], Odile Richard-Le Goff[2], Roshan Kumar[6], Samantha N. Stewart[6], Yannick Pousse[1], Bingqing Shen[1], Kevin Grosselin[1,4], Baptiste Saudemont[2,4], Antoine Sautel-Caillé[4], Alexei Godina[4], Scott McNamara[1], Klaus Eyer[5,14], Gaël A. Millot[8], Jean Baudry[5], Patrick England[9,10], Clément Nizak[4], Allan Jensen[1,15,18], Andrew D. Griffiths[4,18] ✉, Pierre Bruhns[2,18] ✉ and Colin Brenan[1,6,16,18] ✉

Target-specific IgG discovery



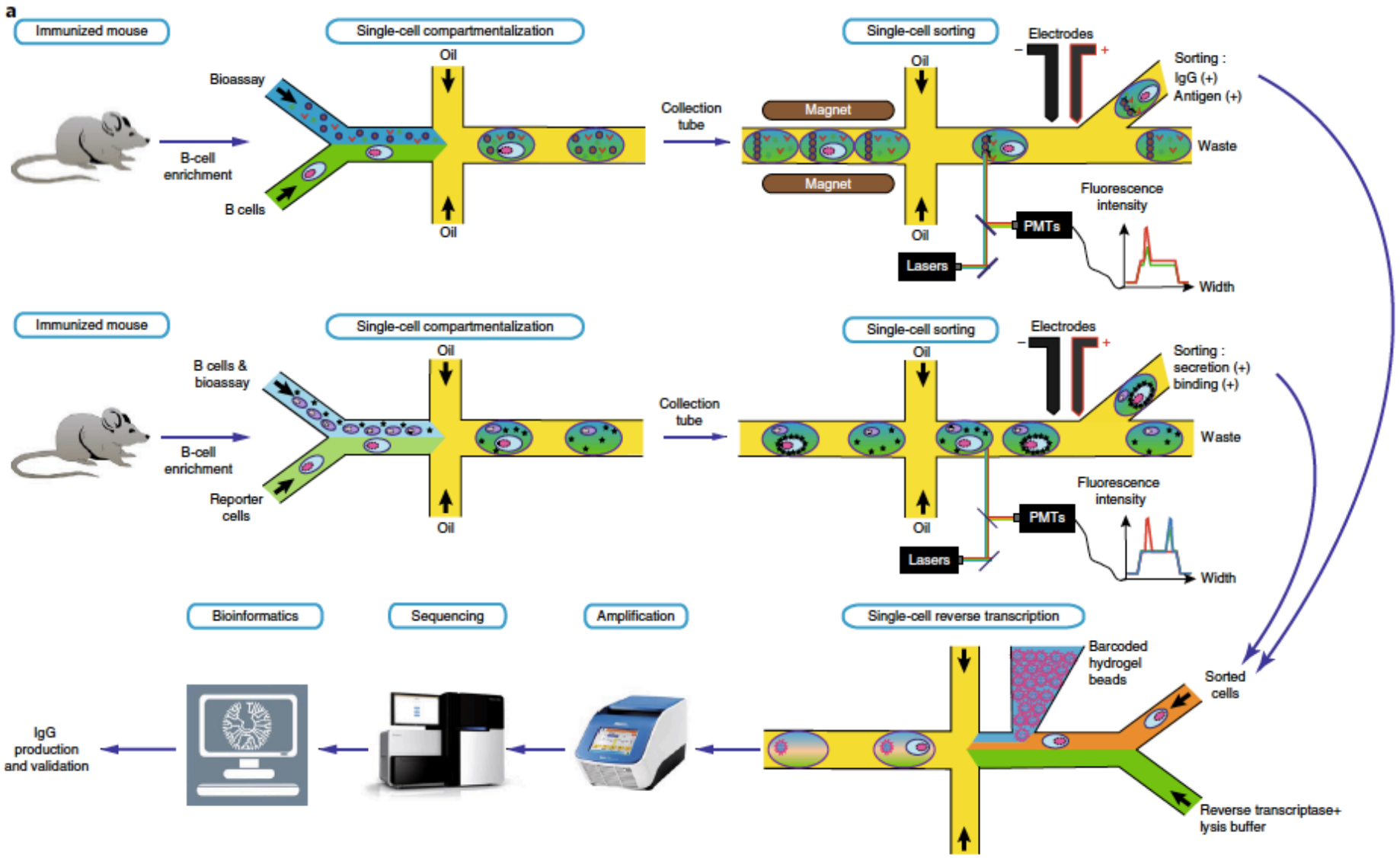
IgGs are secreted by circulating plasmablasts and tissue-resident plasmacells and are present as different variants.

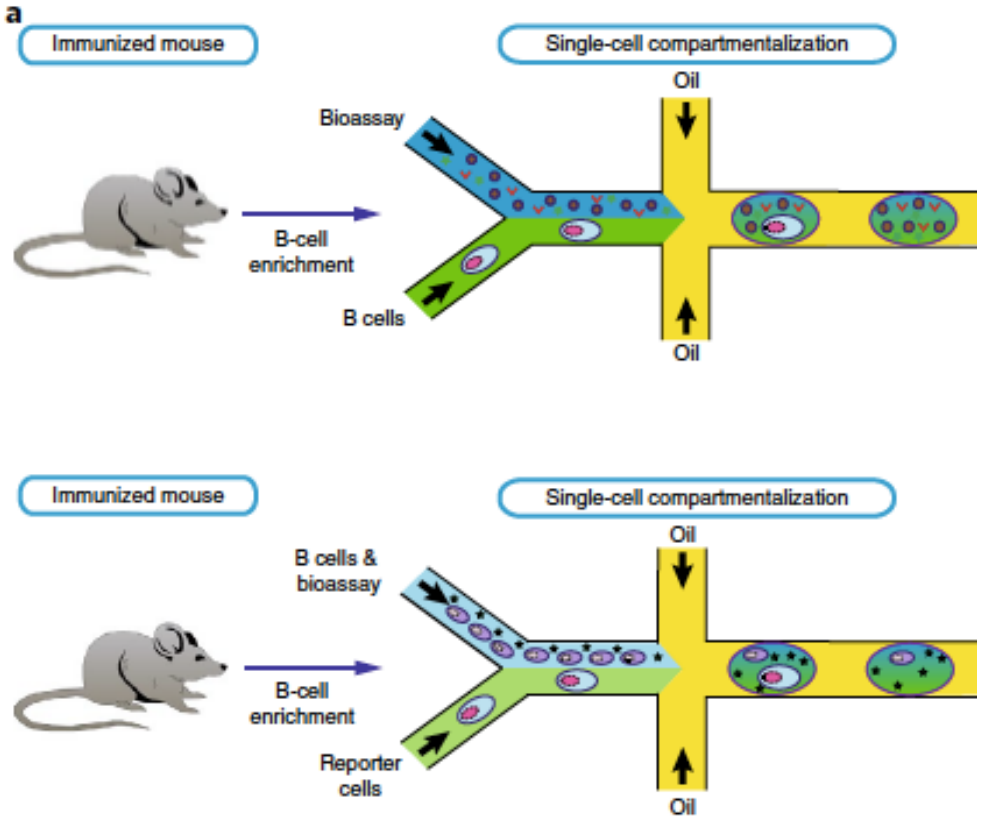
Obtain antibodies for therapeutic use.

Yeast and phage display are still the most useful techniques to identify therapeutic antibodies.

However, V_H-V_L pairing is not always preserved and the technology is low throughput

CelliGO workflow





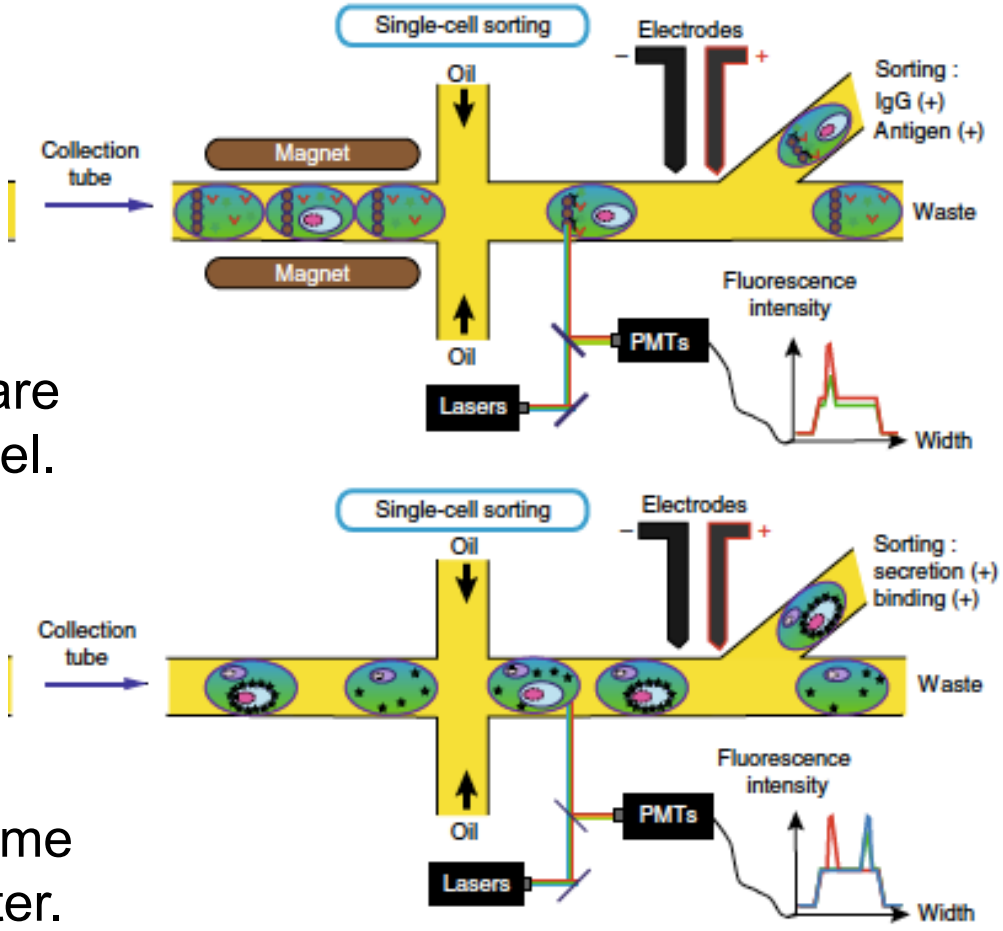
STEP 1

Non-immortalized B cells are streamed into the device together with a target antigen, and then trapped into oil-in-water droplets

STEP 2

Droplets that are considered as positive to the specific bioassay are then sorted into a different channel.

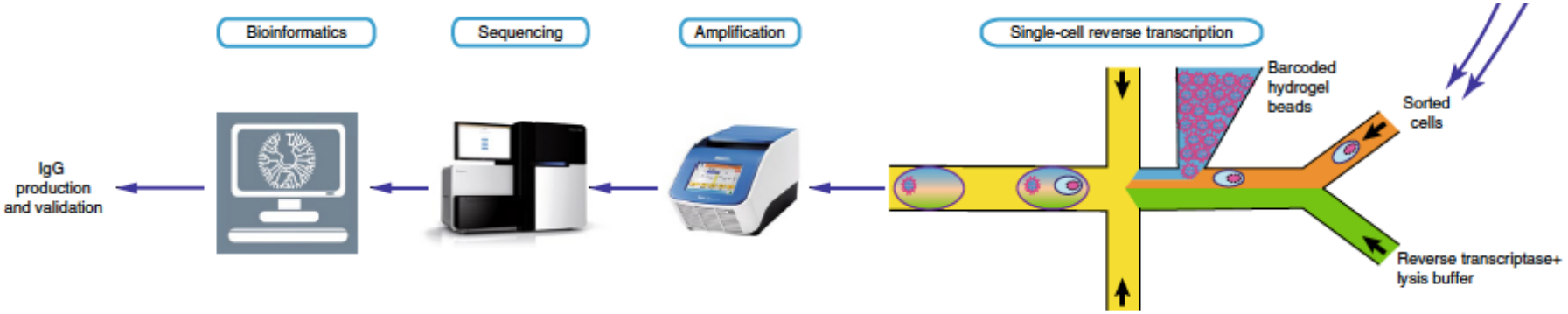
The bioassay usually involves some sort of fluorescence-based reporter.



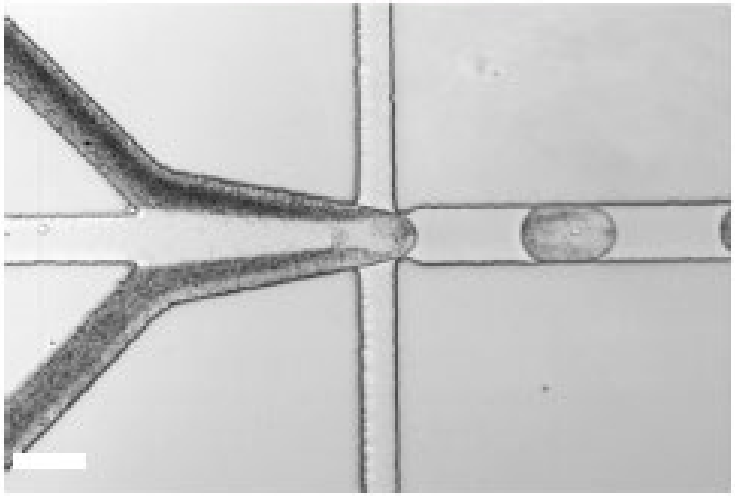
STEP 3

Sorted droplets are then coupled each one with a barcoded bead.

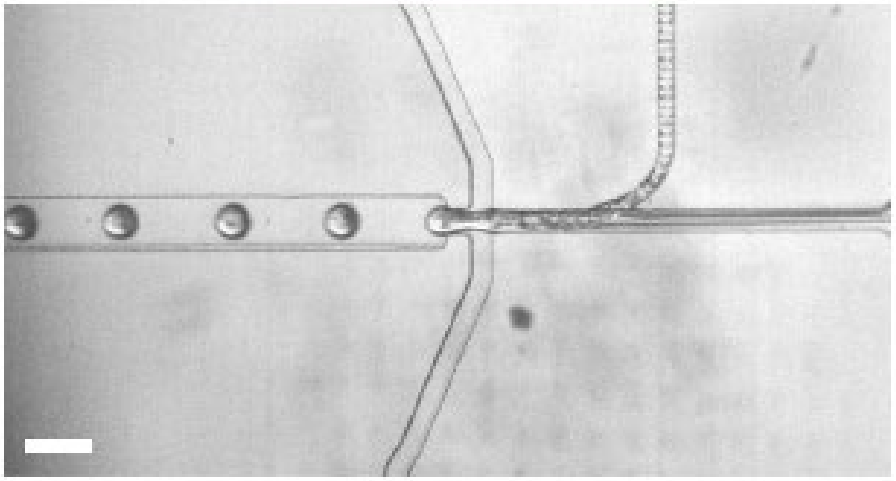
After cell lysis and reverse transcription, V_H - V_L cDNAs coming from the same cell will have a hydentical barcode, allowing for correct V_H-V_L pairing



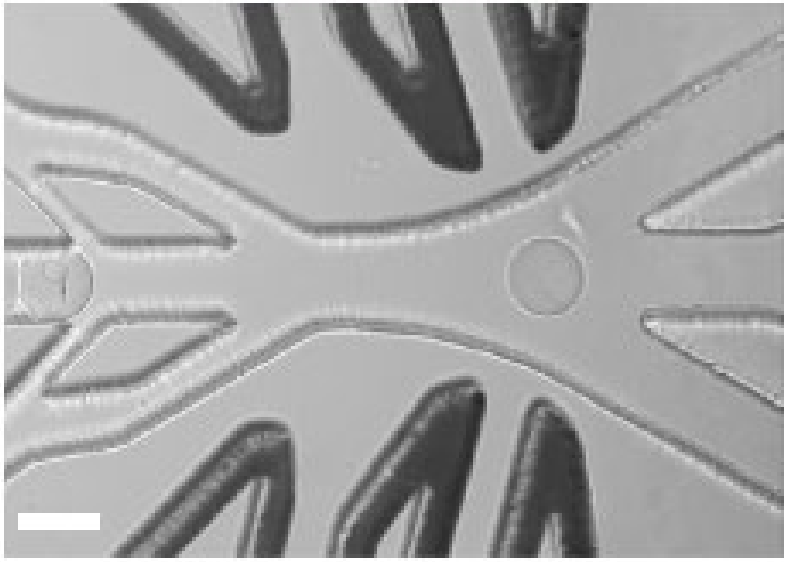
Droplet production



Cell + bead compartmentalization



Droplet sorting

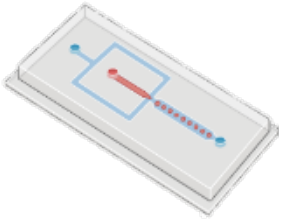


Just some representative pictures…

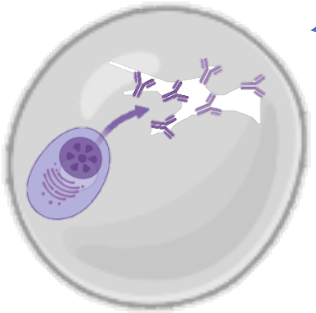
CelliGO system was used to screen B-cells repertoires against **tetanus toxoid (TT)**, **GPI enzyme** involved in cancer progression and metastasis, and the **transmembrane protein TSPAN8**

Immunization

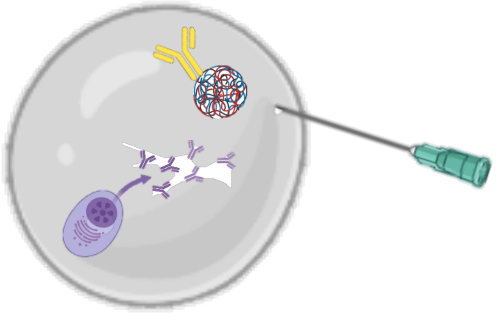
B cells isolation and enrichment



Encapsulation in droplets using flow focusing technology (4-11 million droplets, 40 pL each)

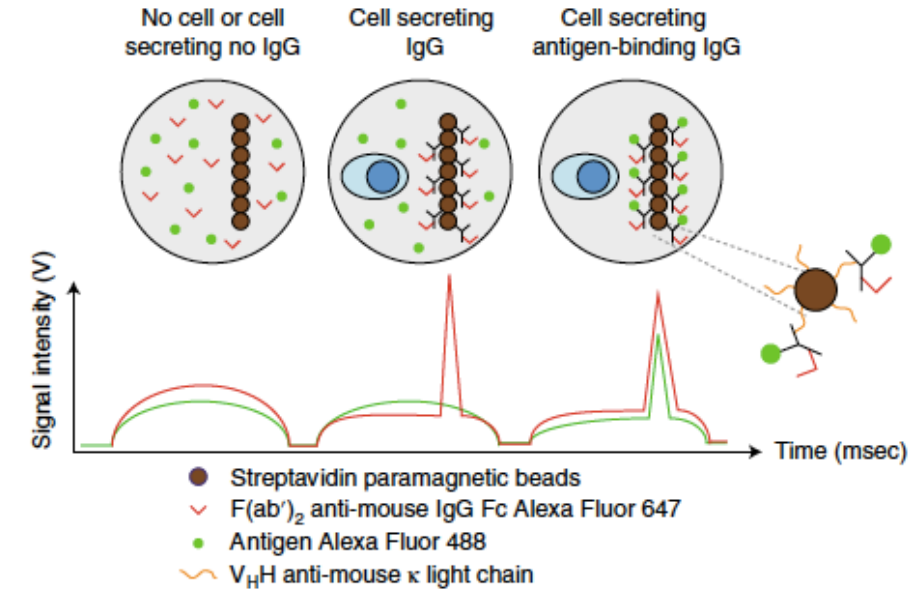


60 minutes incubation to allow antibody secretion



Injection of magnetic nanoparticles coated with anti-mouse antibodies

Magnetic nanoparticles capture all IgGs and are organized in a row by a magnetic field

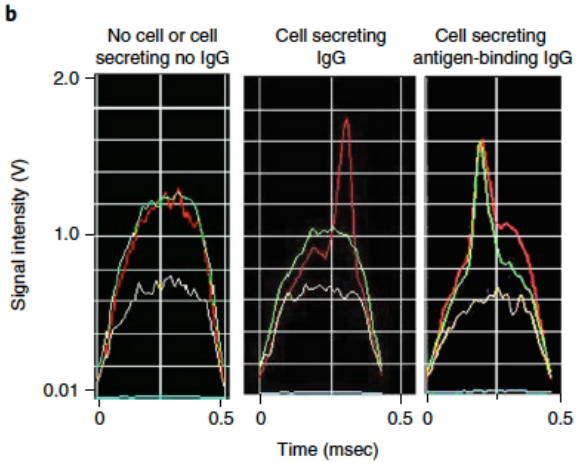


Two fluorophores added:

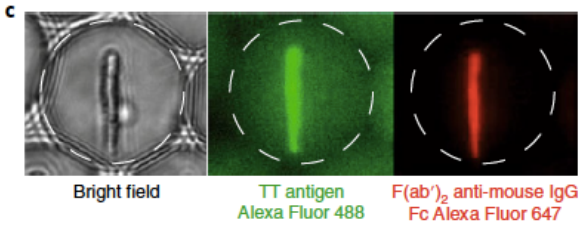
Alexa647: recognizes IgGs

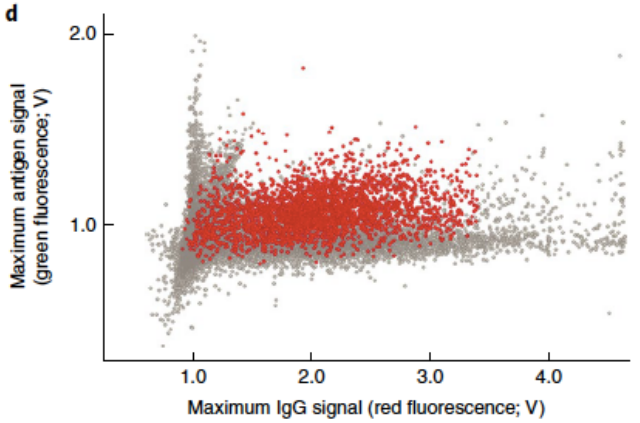
Alexa488: recognizes antigen-specific IgGs

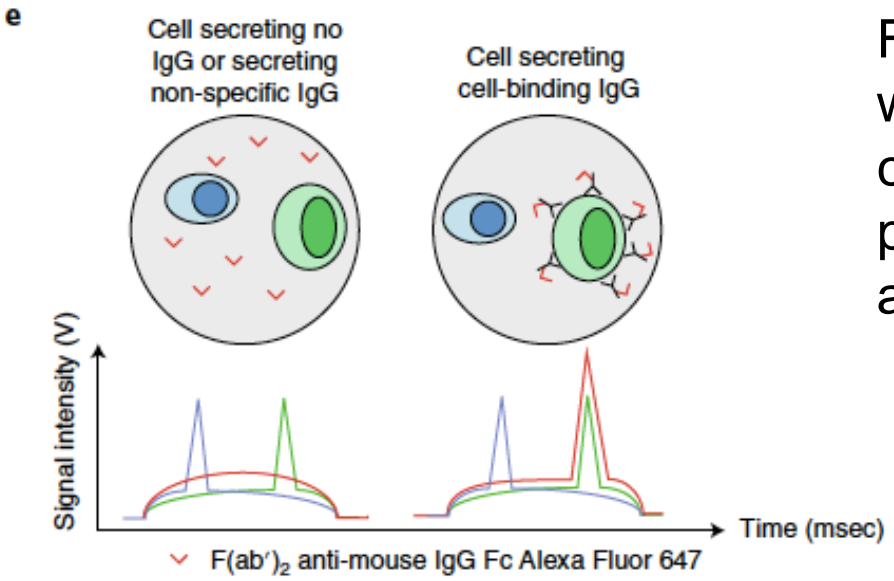
These fluorophore-conjugated antibodies bind IgGs and relocate them onto the beads



Positive droplets are re-scanned in another device and then sorted out

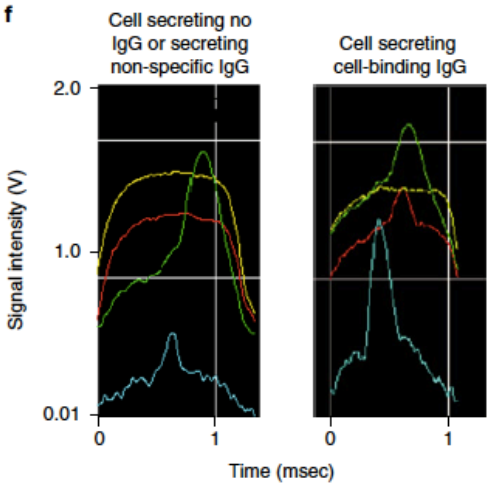


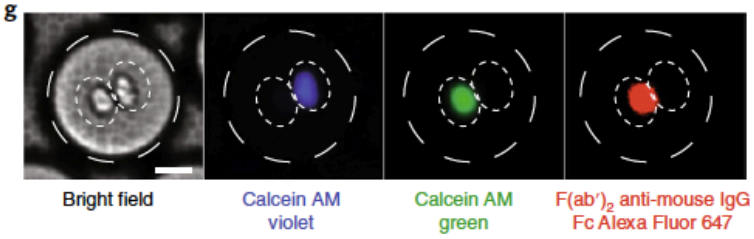




For membrane antigens, splenocytes were co-encapsulated with reporter cells expressing the antigen and then probed with a fluorescent anti-mouse antibody

Droplets positive for the fluorescent secondary antibody were sorted out and analysed.

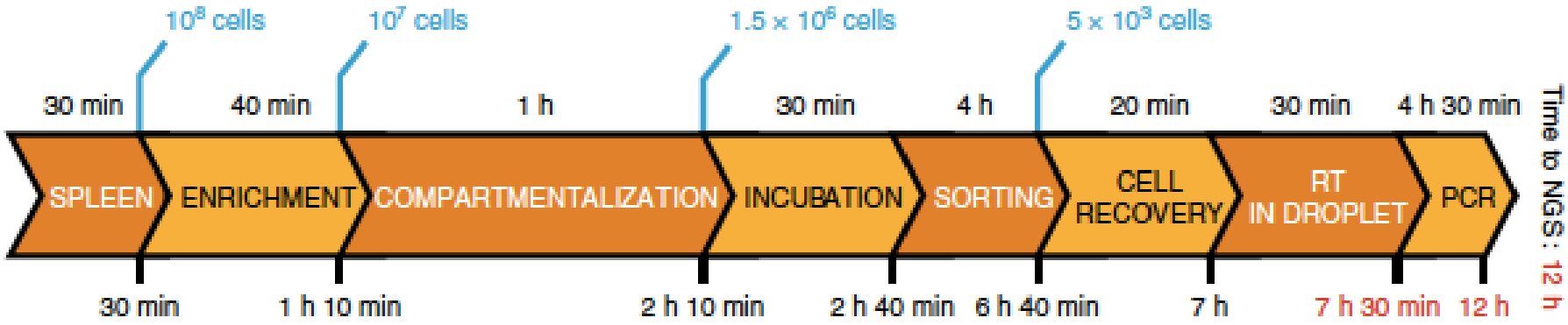




Timeline

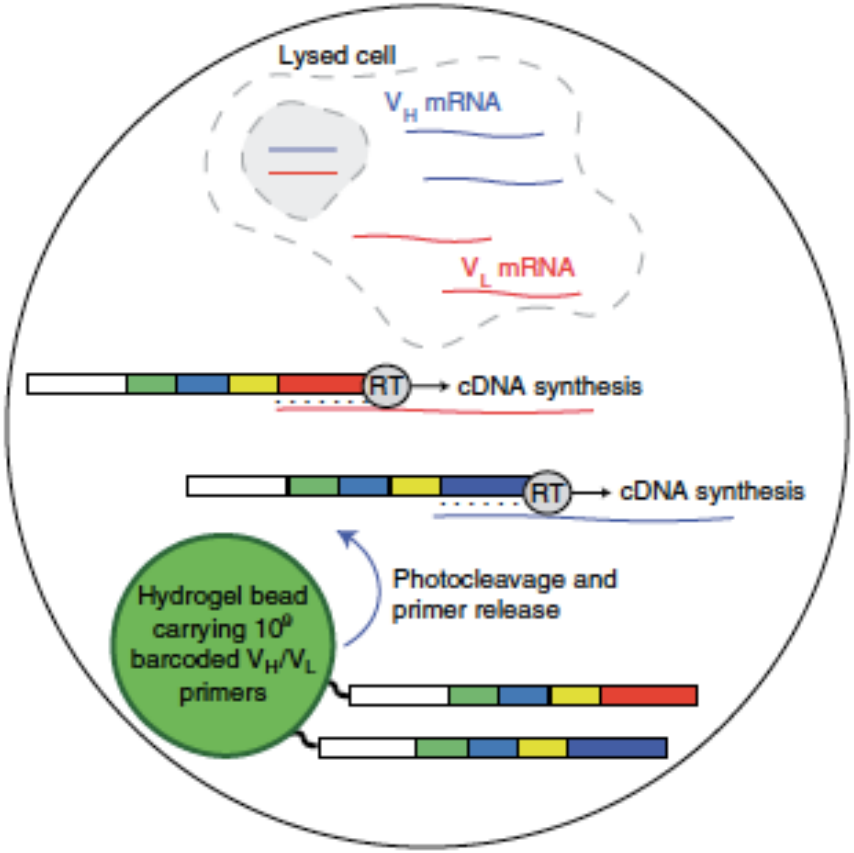
Droplets sorted: from 3500 to 22’000

More than 50% of the recovered cells were still secreting IgGs



The whole sorting process took less than 6 h → 79% viability and very good recovery of alive cells

Barcoded RT-qPCR and bioinformatic analysis

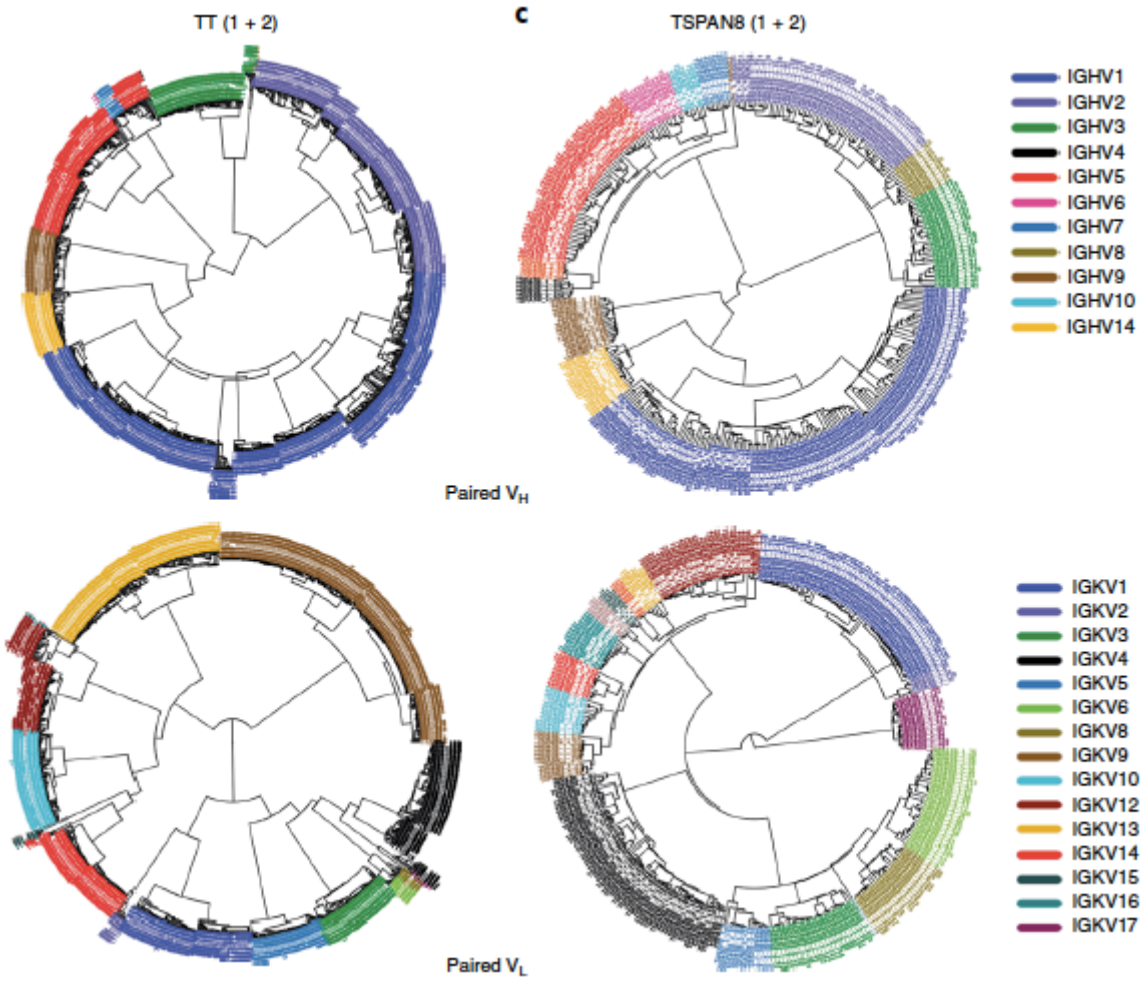


Sorted cells are co-encapsulated with hydrogel beads coupled to 10^9 random primers for V_H and V_L amplification

70% of the droplets contained a single cell and a single pair of primers

Cells are lysed and primers are released from the hydrogel beads by photocleavage

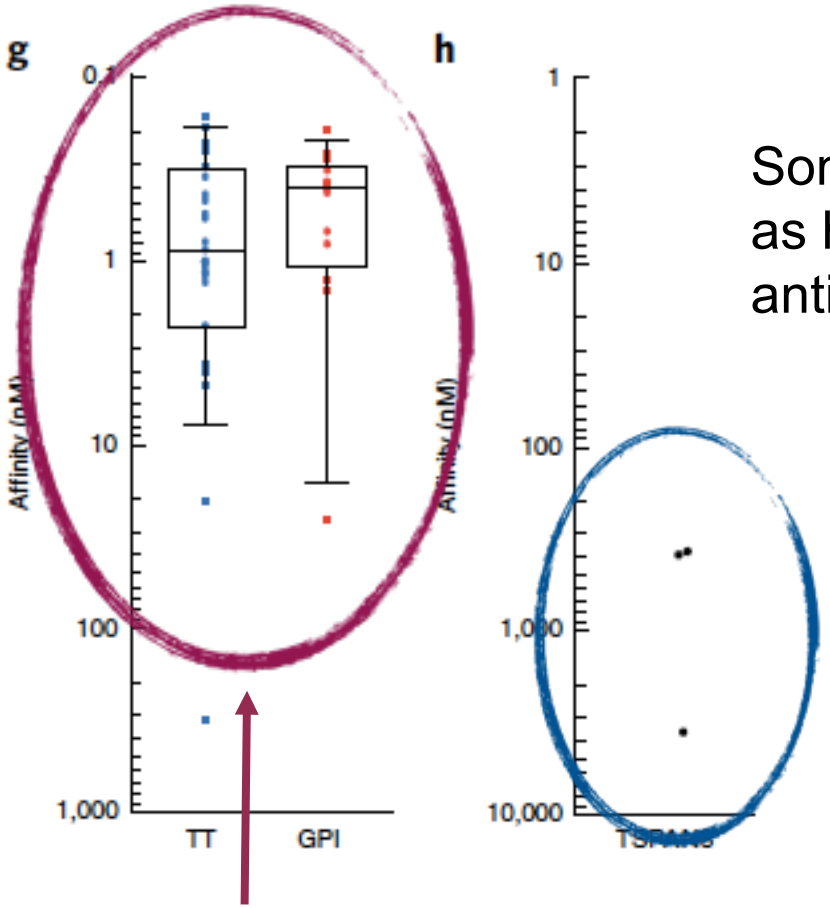
Amplification of cognate V_H and V_L sequences



Non-redundant V_H – V_L pairs were clustered into IgGs clonotypes that derive from a common ancestor and have undergone ricombination and affinity maturation.

IgGs were highly diverse and covered multiple V families, indicating differences in the response against the same antigen

Antibody validation



Almost all tested IgGs reacted against TT or GPI antigens with affinities ranging from 0.1 nM to 300 nM.

Some of the identified IgGs were produced as human IgG1s and tested against their antigens in ELISA assays.

Only 3 IgGs showed a measurable affinity for TSPAN8.

IgGs showed a lower frequency of somatic hypermutation, implying that they did not undergo a full process of affinity maturation.

Conclusions

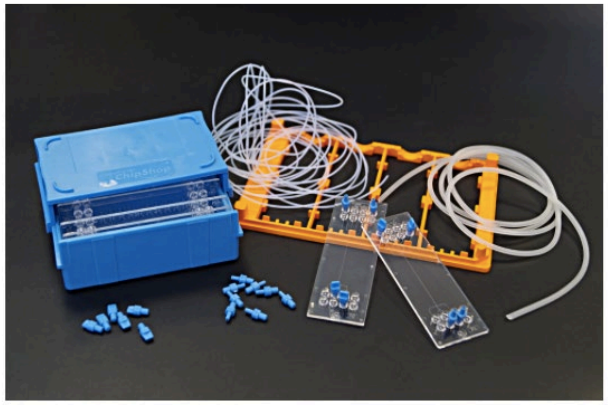
- The CelliGO pipeline allows a deep mining of IgGs repertoire by HTS of millions of IgG-secreting cells using picoliter-sized droplets.
- Screening is based on both the phenotype and the genotype of the secreted IgGs.
- The platform allowed the identification of many more non-redundant IgGs compared to other methods (i.e. yeast display).
- Both soluble and membrane-bound antigens can be used.
- Also low-affinity antibodies can be isolated.
- Thanks to barcoding, V_H-V_L pairing is not lost.

Why are they not so widely used?

Affordable prices

Product Number	Chip Format	Channel Dim [mm] W	H	L	Material		Price [€/chip] 1+	10+	30+
05-0146-0102-01	SBS-titerplate	2	0.15	18	PMMA		89	69	49
05-0147-0102-03	SBS-titerplate	2	0.15	18	PC		89	69	49
05-0148-0102-07	SBS-titerplate	2	0.15	18	PS		89	69	49
05-0149-0102-05	SBS-titerplate	2	0.15	18	Zeonor		89	69	49
05-0160-0102-01	SBS-titerplate	2	0.15	18	PMMA	hydrophilized	108	88	68
05-0161-0102-03	SBS-titerplate	2	0.15	18	PC	hydrophilized	108	88	68
05-0162-0102-07	SBS-titerplate	2	0.15	18	PS	hydrophilized	108	88	68
05-0163-0102-05	SBS-titerplate	2	0.15	18	Zeonor	hydrophilized	108	88	68

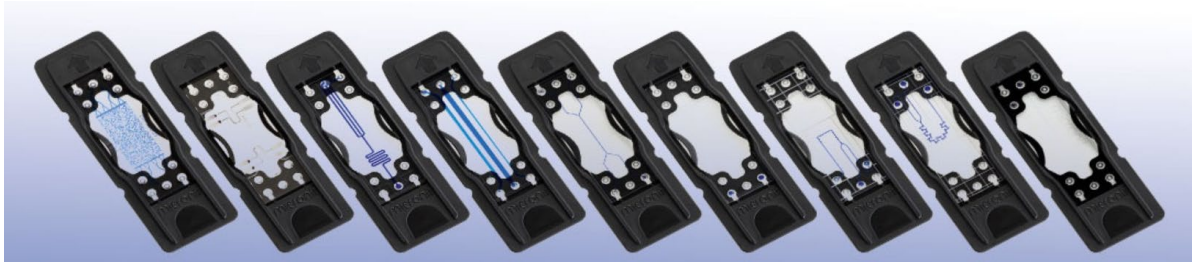
From Fluigent website



Product Code	Price
10001109	277.50€

Microfluidic-chipshop.com

Many different chips available



Cell culture

Organoids

Chip-PCR

Molecular biology

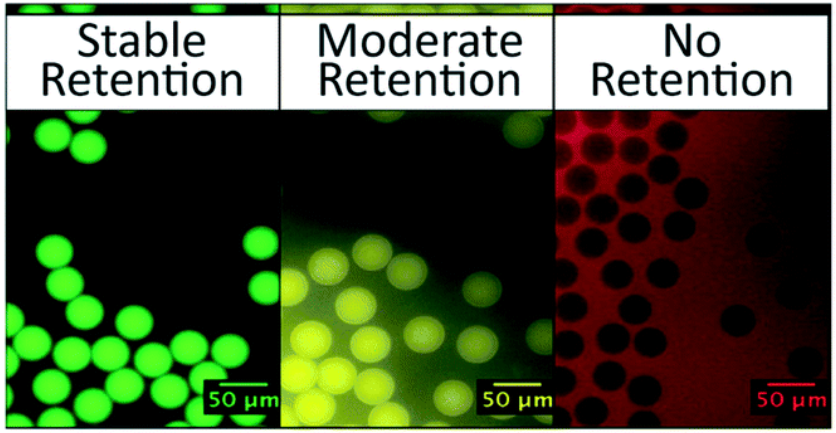
Challenges in droplets HTS

Table 1 Critical challenges to droplet HTS and their common solutions

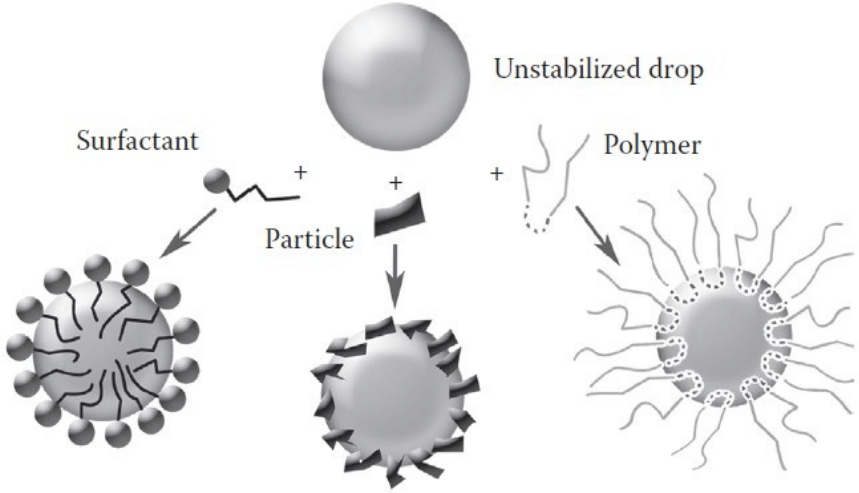
Challenge	Synopsis	Current resolutions
Multi-device integration	Tethering multiple unit operations induces droplet shearing and merging	Gentle droplet reinjection[29,57]
		Single device integration[14,58]
Molecular transport	Small molecules may transport between droplets	Dendritic,[59] nanoparticle[60] surfactar
		Substrate derivatization[61]
Library generation	Compound libraries are difficult to dose into droplet populations	Combinatorial droplet merging[33,34]
		Bead associated libraries[62]
Droplet tracking	Droplet identities are difficult to trace back to original sample information	Fluorescently barcoded beads[63]
		DNA encoded particles[64]
Droplet analysis	Label-free droplet interrogation techniques are lacking	Raman detection[65]
		Mass spectrometry[31,66]

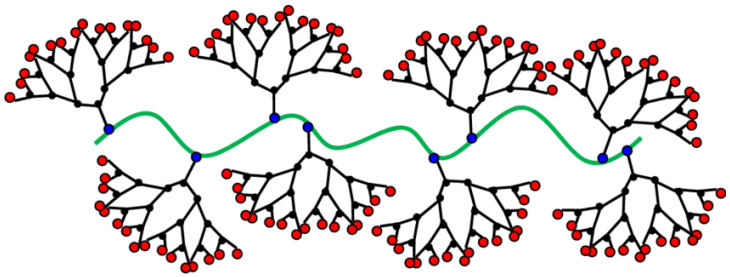
Molecular transport

Cross-talk of analytes might occur between droplets, complicating assays involving small fluorescent molecules

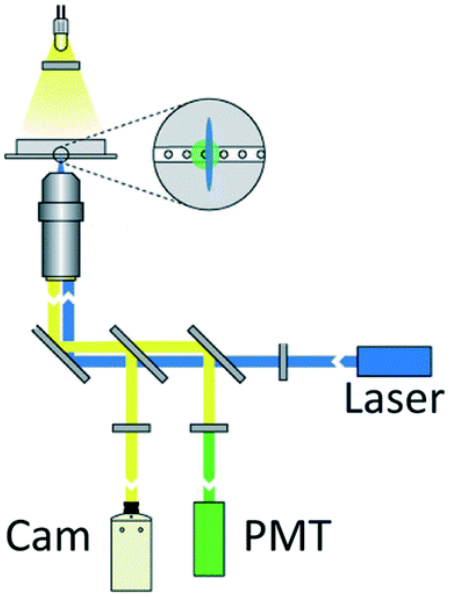


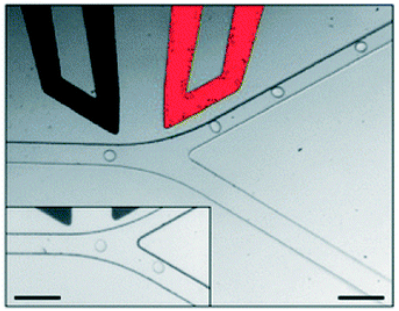
Use of surfactants or dendronized polymers and chemical modification of fluorophores (addition of permanently charged sulfonated groups) facilitate analyte containment within droplets





Droplet analysis





Optical analysis is the most used analysis technique and relies on laser-mediated detection of fluorescent molecules

Fluorescent detection measured by a photomultiplier triggers a pulse that deflects the droplet into a collection outlet channel

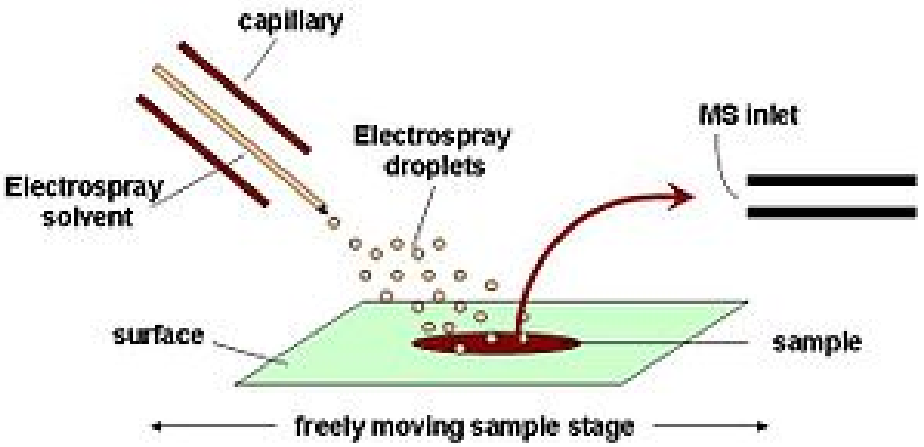
Absorbance measurements: applicable only to strong signals due to high background

Raman spectroscopy and NMR analysis have been tested but not yet applied

What if there is no fluorescent molecule?

Label-free detection with mass spectrometry

Electrospray Ionization (ESI)



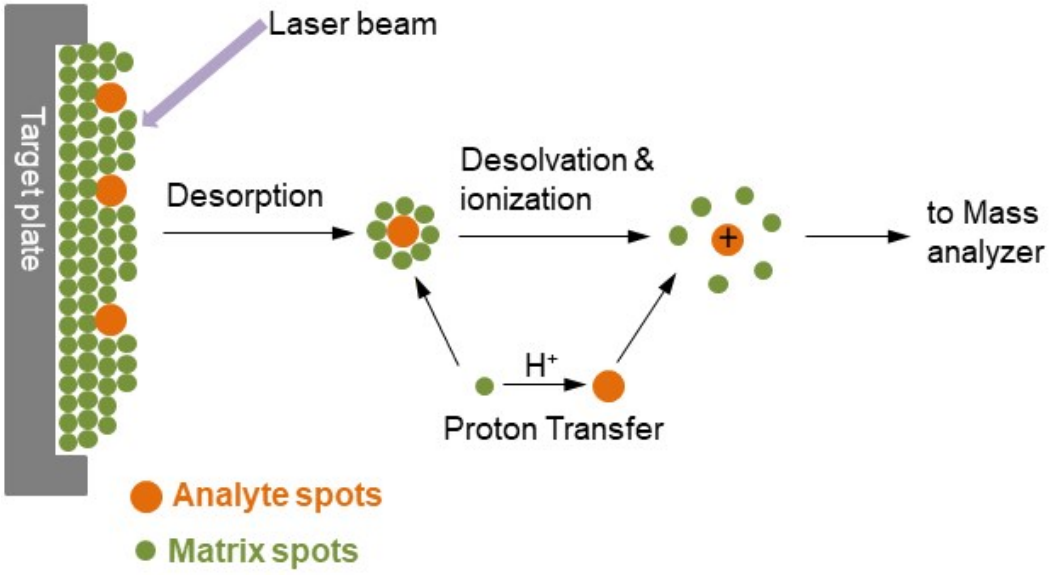
ESI can be easily integrated into microfluidic devices

Drawbacks

Low throughput of analysis (Hz) compared to droplets processing (kHz)

Size limitations: the smallest droplets that can be aalysed are nL-sized

Matrix-assisted laser desorption ionization (MALDI)



Droplets are spotted onto a matrix prior to analysis

Can be adapted to smaller droplets size

Non-destructive → samples can be further processed

Drawbacks

Low throughput

Surfactants that stabilize droplets might impair MALDI analysis


THANK
YOU !