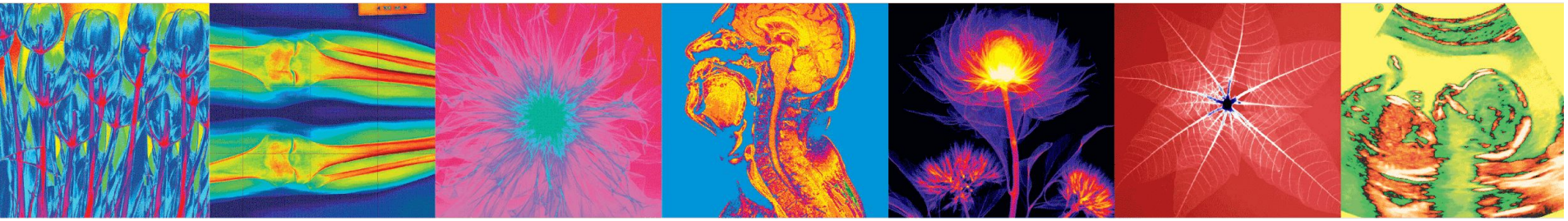
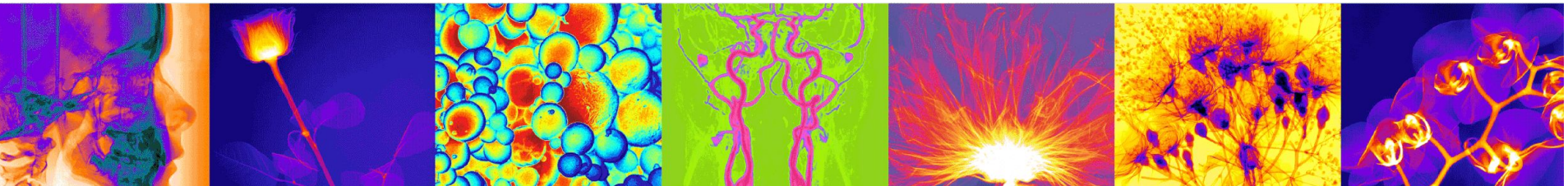


Microfluidic production of monodisperse phase-change nanodroplets for enabling ultrasound imaging and therapy: In vitro and in vivo assessment

Samir Cherkaoui

CRS 2022 Annual Meeting
July 11-15 2022, Montreal, Canada

BRACCO
LIFE FROM INSIDE

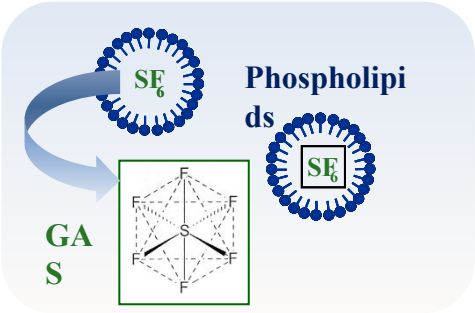


Gas filled microbubbles for CEUS

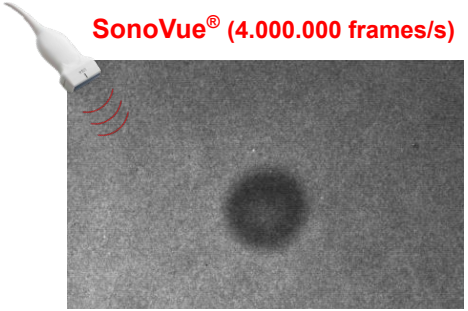
SONOVue®
Sulphur Hexafluoride

BRACCO
LIFE FROM INSIDE

Microbubbles structure



- **Gas stabilized by a lipid shell**
- **Diameter < 8 µm** (smaller than RBC)

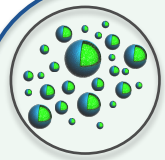


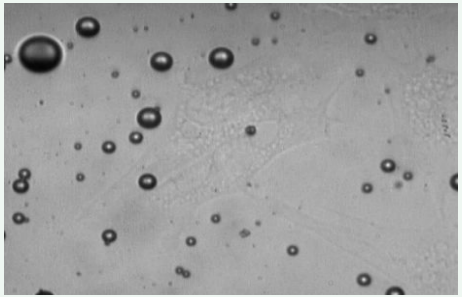
- **Highly compressible**
- **Oscillate in response to the incoming ultrasound wave**

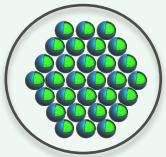
Nico de Jong et al., Thorax Center, Erasmus University Rotterdam

CEUS: Contrast Enhanced UltraSound

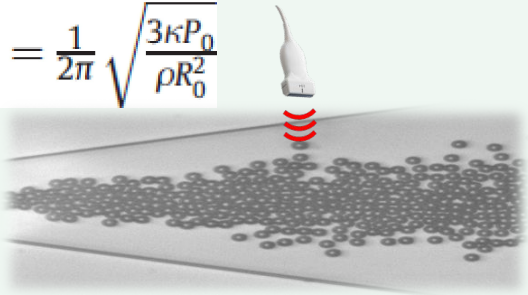
Monodisperse *vs* Polydisperse MBs





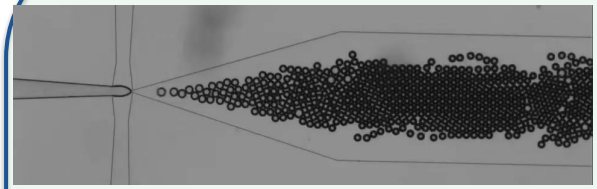


$$f_0 = \frac{1}{2\pi}\sqrt{\frac{3\kappa P_0}{\rho R_0^2}}$$



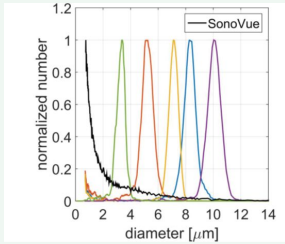
Segers et al. 2017
Helbert et al. 2020

MICROFLUIDIC DEVICE



Shell composition :
DSPC/DPPE-PEG5000 (9/1) @50°C
Production rate :
200-500x10^6 MSB/mL & 9 mL of product/h

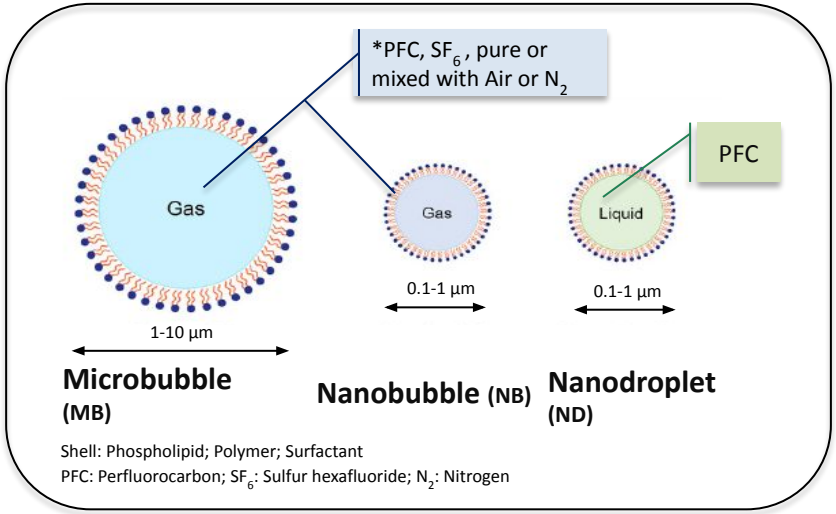
$$d_b \propto D\left(\frac{Q_g}{Q_l}\right)^{0.4}$$



- **Monodisperse microbubbles** as optimal **cavitation nucleus** for US applications
- **15-40 times higher acoustic efficiency** compared to regular polydisperse MB

NDs: New frontiers beyond vessels

Nano/micron-sized US responsive agents

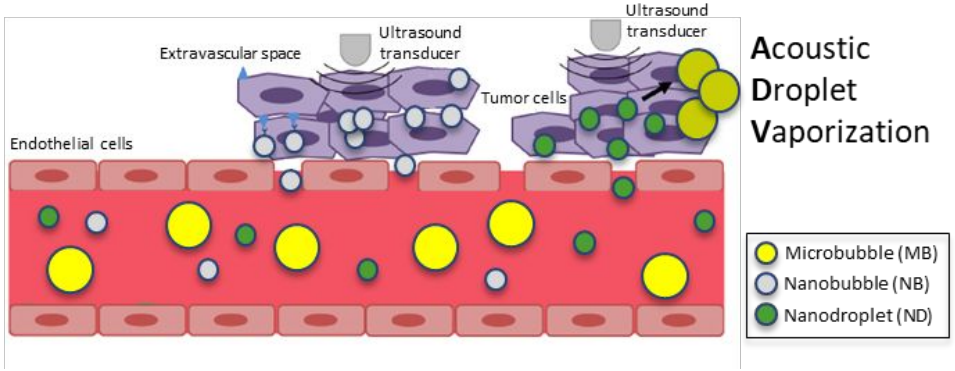


Ultrasound Contrast Agent - MBs

- Short in vivo circulation time due to gas core
- Remain only in the blood circulation due to large size

Main advantages over microbubbles

- **Extravasation** and **tumor accumulation** via either EPR* effect (NB/ND)
- Improved **stability** and **longer residence time** (ND)
- **Selectively activated**, both spatially & temporally (ND)
  –> Acoustic Droplet Vaporization Shift "On/Off"



** EPR Enhanced Permeability Retention*

Top Down

Bottom Up

Nanodroplets production

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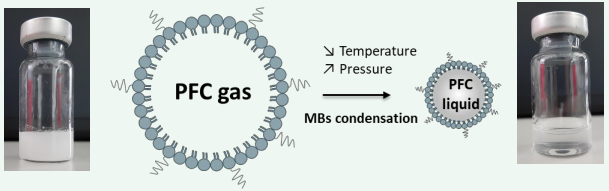
Microbubbles condensation procedure

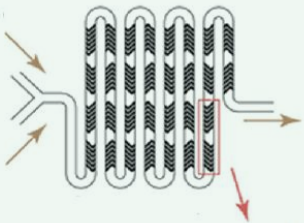
- Use of **highly volatile** compounds : C_4F_{10} (b.p. -2°C) or C_3F_8 (b.p. -36°C)
- Ability to **modify and manipulate** microbubbles prior to condensation
- NDs size/distribution depends on **initial MBs**
- **Low** NDs **concentration**



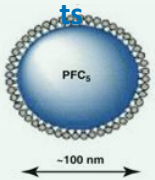
Microfluidic technology (NanoAssemblR benchtop)

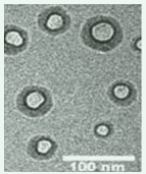
Organic phase :
- Shell molecules
- PFC in EtOH

Aqueous phase



Nanodroplets





- **Process parameters**
  - Total Flow Rate (TFR)
  - Flow Rate Ratio (FRR)
- **Formulation parameters**
  - Encapsulating shell
  - Perfluorocarbon core

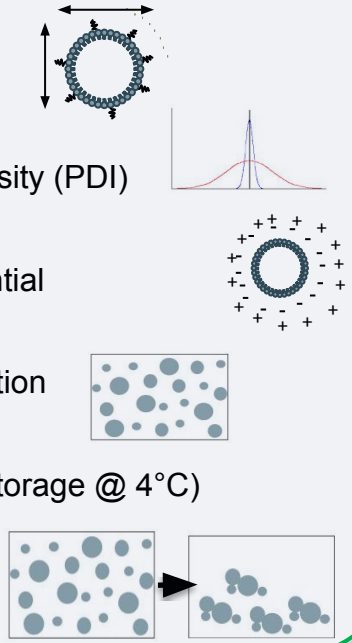
Targeted size
< 400 nm & PDI≤ 0.2

Formulation of various types of PFC nanodroplets in terms of composition and size

PFC-NDs characterization

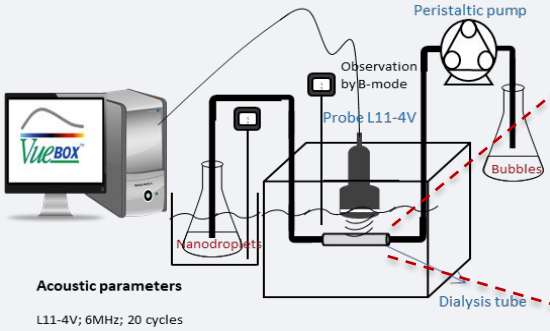
PHYSICOCHEMICAL

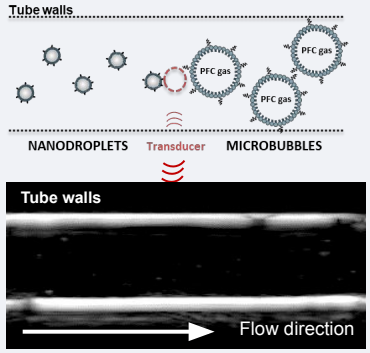
- ✔ Size
- ✔ Polydispersity (PDI)
- ✔ Zeta Potential
- ✔ Concentration
- ✔ Stability (storage @ 4°C)



ACOUSTICAL

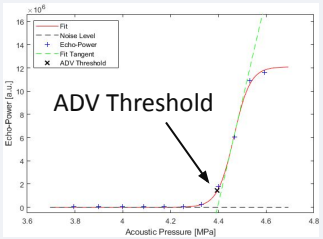
Home-made ADV setup





Increase of the echogenicity with the MBs production

- An ultrasound probe (6 MHz) is used to activate and image the NDs vaporization for a real-time imaging
- The acoustic pressure increase every 10 seconds until the observation of the nanodroplets vaporization into MBs
- The VueBox™ software is used to measure the echopower values from B-mode imaging videos and thus to determine the ADV threshold



VueBox™ - Matlab fit

Melich et al. 2020

Results with Perfluoropentane NDs

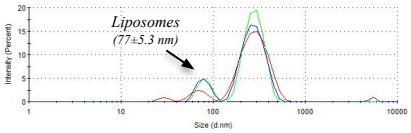
Shell nature effect on NDs characteristics and ADV threshold

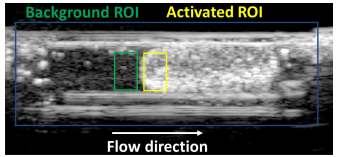
PHYSICOCHEMICAL

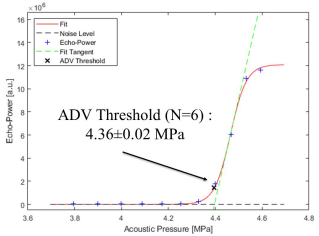
ACOUSTICAL

LIPIDS
DPPC:DSPEG-PEG2000

Size	286 ± 6 nm
Polydispersity index	PDI: 0.294
Zeta potential	-32.2 ± 2.4 mV

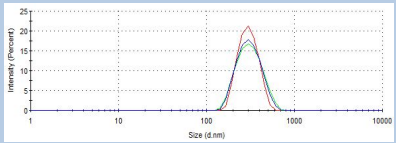


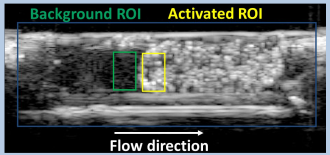


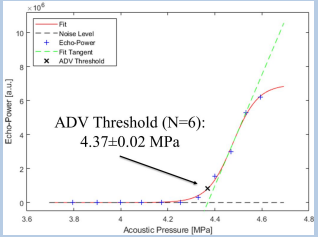


FLUORINATED SURFACTANTS
Zonyl FSO

Size	287 ± 2 nm
Polydispersity index	PDI: 0.078
Zeta potential	-10.0 ± 2.2 mV

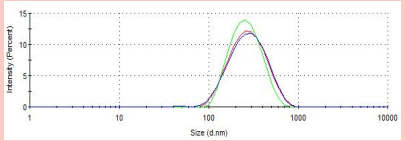


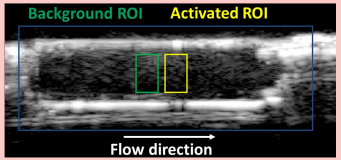


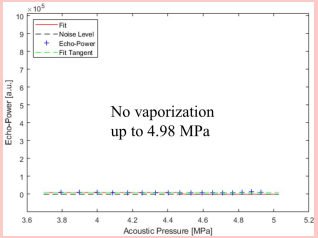


POLYMERS
PLGA-PVA

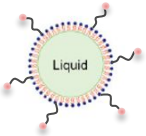
Size	291 ± 14 nm
Polydispersity index	PDI: 0.163
Zeta potential	-13.9 ± 2.6 mV







In vivo behavior of F-NDs



C_4F_{10}
Lipids
Lipids functionalized with Cyanine 5.5

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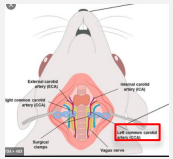
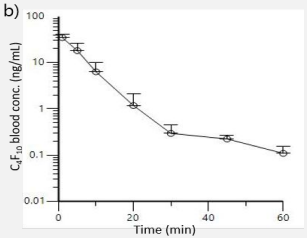
LIFE FROM INSIDE

C_4F_{10} Assay



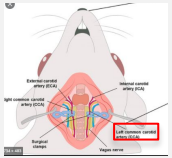
- **IV injection** in the tail vein of rat (n=5) : 8.0×10^9 Particles/kg
- **Blood samples** collected in heparinized **tubes from** a catheter inserted in the left **carotid artery**



✔Distribution half-life (Tα ½) corresponding to **3.1-fold** that of MBs of similar composition[1]

1. Schneider et al., 2011

Fluorescence detection



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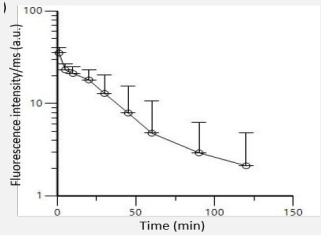
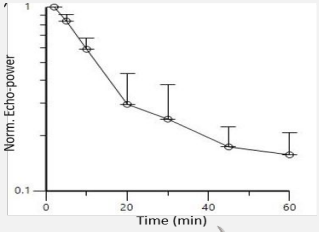


✔Tα ½ has shown a longer time of **24 min**
✔ Due to the **circulating remanent fluorescence** into the blood from F-NDs but also from F-liposomal and other F-lipidic structures

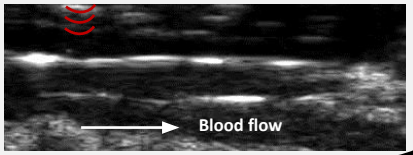
Vaporization detection

- **IV injection** in the tail vein of rat (n=5) : 8.0×10^9 Particles/kg
- **L11-4v probe – Verasonics**
- Positioned **longitudinally** on the **abdominal vein**
- **6 seconds of vaporization** for different time intervals until 60 min



✔ **Bi-phasic decay**
✔ In vivo :
Tα ½ = 9 min

***In vivo* B-mode video**



Take home message

- Microfluidic technology : rapid, robust, tunable and scalable platform for designing and **manufacturing monodisperse PFC-NDs**
- Custom-made **acoustic set-up:** powerful and versatile tool for **ADV threshold determination** and **real-time monitoring of NDs** (Conversion to gaseous microbubbles)
- F-ND **in vivo circulation behavior** was addressed using **3 experimental procedures**
  - ✔ **Longer circulation time** of lipid-coated NDs confirmed
  - ✔ F-NDs **accumulation in organs** (mainly liver and spleen) was observed by the fluorescence evaluation and confirmed by the vaporization
- New opportunities for **US extravascular theranostic** applications

Acknowledgements

Bracco Suisse, Geneva site, Switzerland

Thank you !

Romain Melich
Philippe Bussat
Anne Lassus
David Lazarus
Alexis Vivien Emmanuel Gaud
Patricia Emmel
Isabelle Tardy
Thierry Bettinger

Tim Segers
Guillaume Lajoinie
Michel Versluis

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