Fluidic Analytics

Monitoring of SMALP-nanodisc formation by microfluidic diffusional sizing

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Application Scientist

SMALP 2020 Web-conference

Outline

Introduction to Microfluidic Diffusional Sizing

SMALP nanodisc formation

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fluidity one-w

- **Changes in protein size**
- **Protein–Protein/Lipid/DNA interactions**
- **Protein sizing in crude backgrounds**

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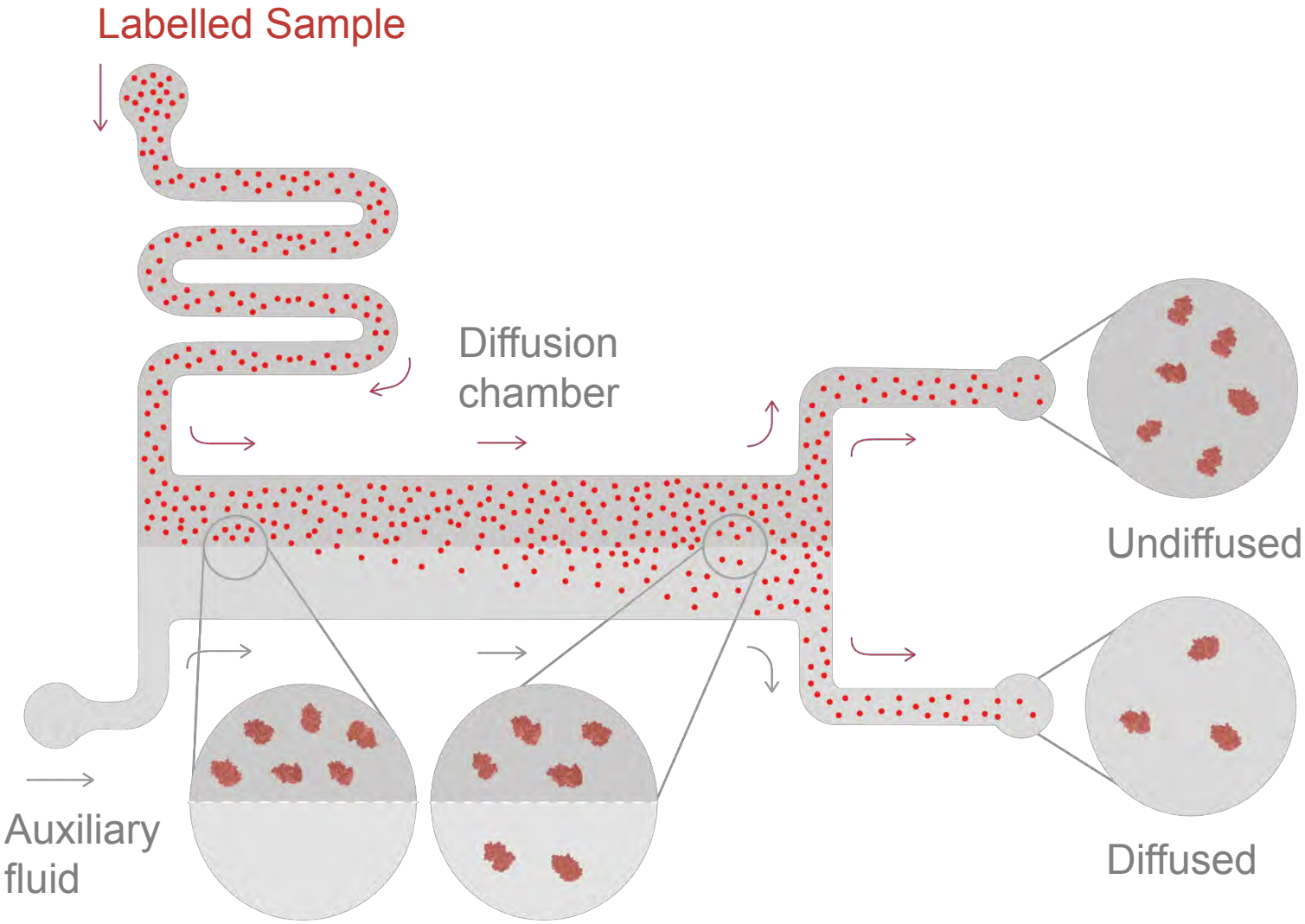
How it Works: Microfluidic Diffusional Sizing (MDS)



fluidity one-w

Ratio diffused/undiffused

↓ "3 / 6"

Average R_h = 1.8 nm

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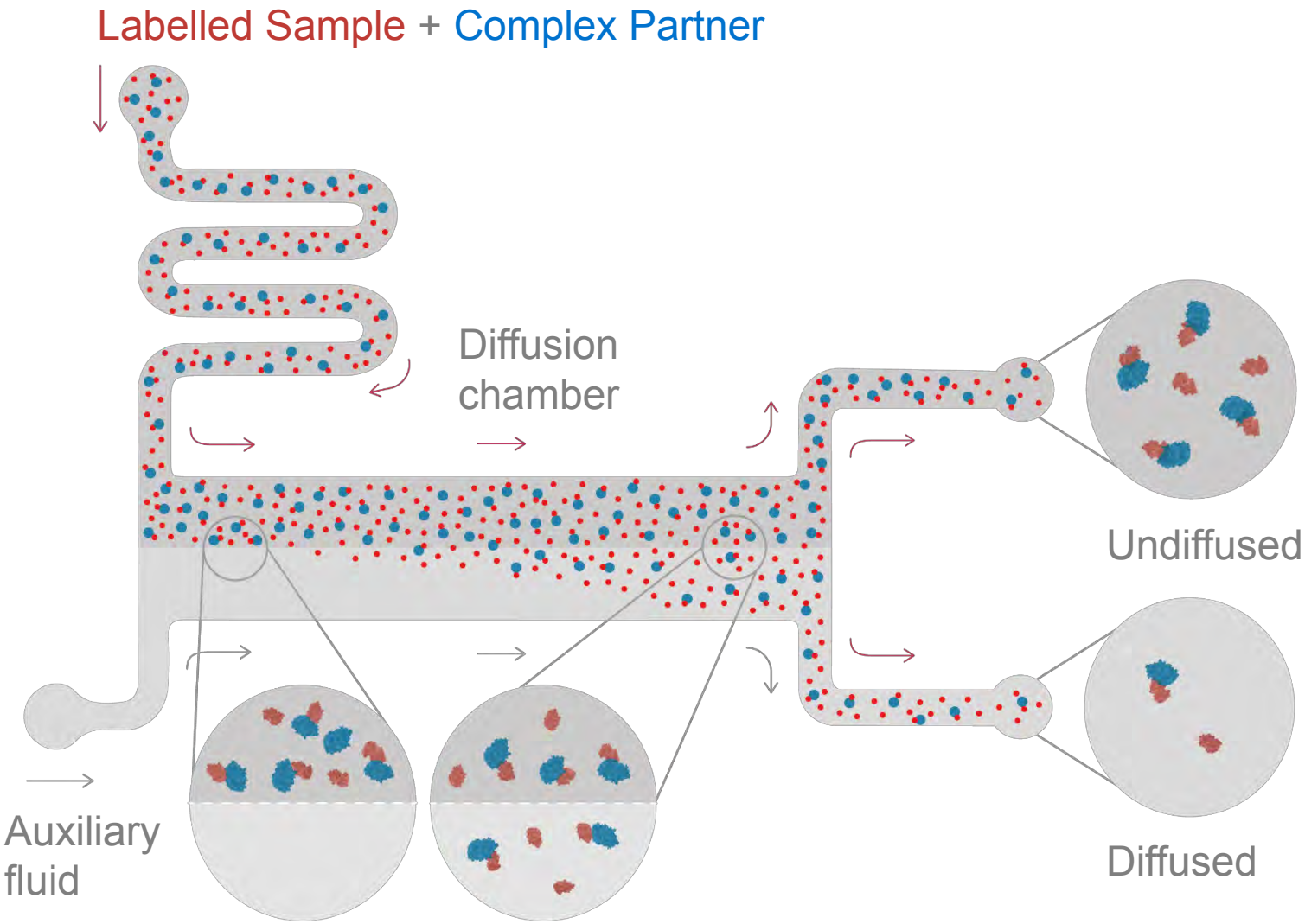
How it Works: Detecting interactions using MDS



fluidity one-w

Ratio diffused/undiffused

↓ "2 / 6"

Average R_h = 13 nm

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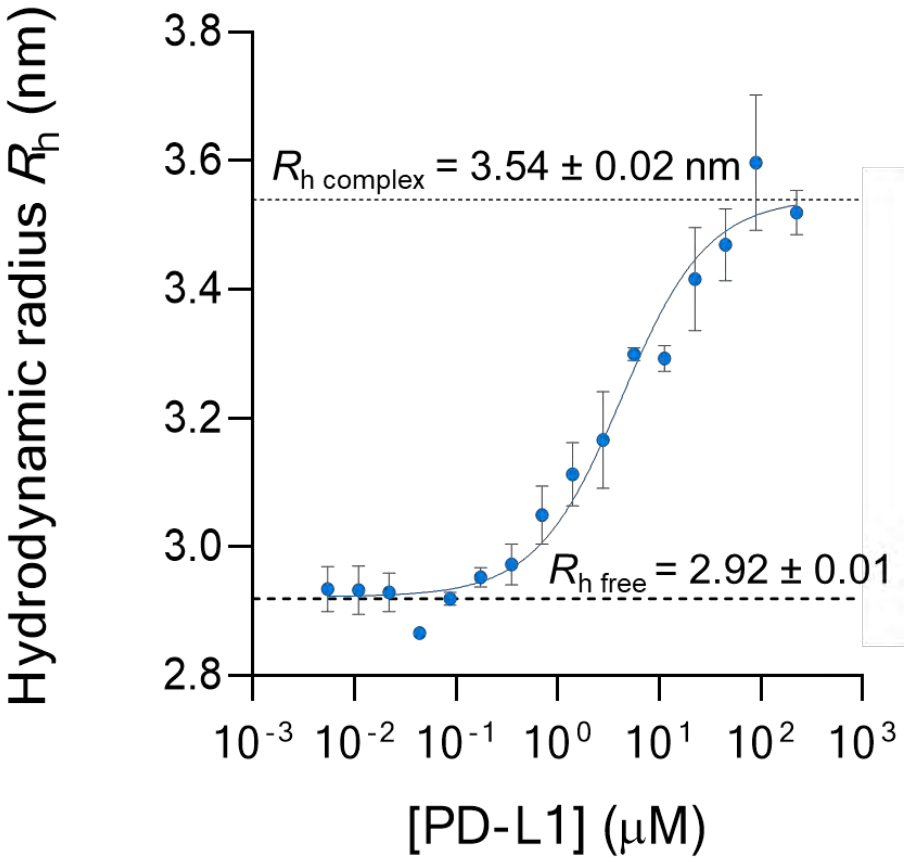
PD-1/PD-L1 interaction: comparison of Fluidity-One W with ITC, SPR and MST



Method	K_D (µM)	Reference
Fluidity One-W	**4.0**	
ITC	2.2	Cheng *et al.*, *JBC*, 11771 (2013)
SPR equilibrium	3.9	Zhang *et al.*, *Immunity*, 337 (2004)
SPR equilibrium	0.7	Latchman *et al.*, *Nat. Imm.* 261 (2001)
MST	7.2	Magnez *et al.*, *Sci. Rep.* 17623 (2017)

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SMALP nanodisc formation

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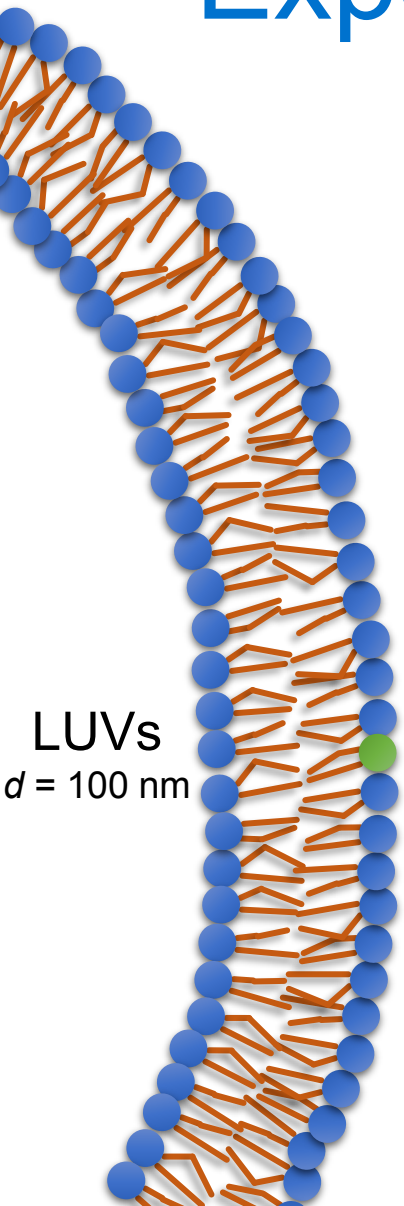
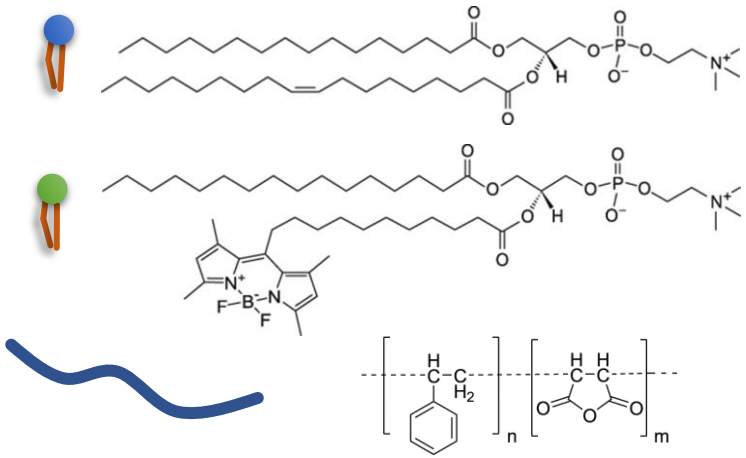
Experimental design



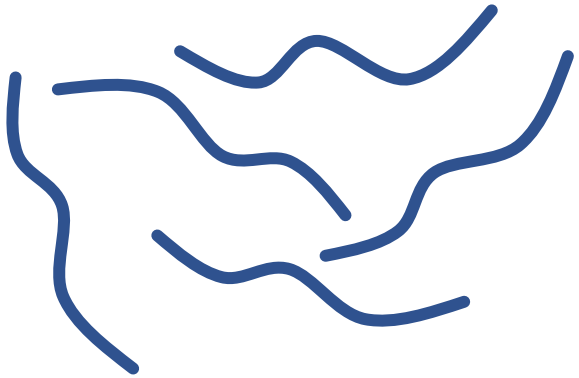
LUVs
d = 100 nm

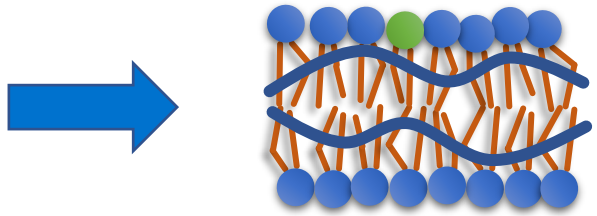


POPC

TopFluorPC®
(1 mol%)

SMA 3:1





Biochimica et Biophysica Acta (BBA) - Biomembranes
Volume 1862, Issue 6, 1 June 2020, 183215

Microfluidic diffusional sizing probes lipid nanodiscs formation

Mehdi Azouz [a], Mathilde Gonin [a], Sebastian Fiedler [b], Jonathan Faherty [b], Marion Decossas [a], Christophe Cullin [a], Sandrine Villette [a], Michel Lafleur [c], Isabel D. Alves [a], Sophie Lecomte [a], Alexandre Ciaccafava [a]

Advantages of MDS

- Other approaches: DLS, ITC, ^{31}P NMR, cryo-EM

MDS:

- Low amounts of sample (nM to µM concentrations)
- Easy to use
- Observe nanodisc formation in crude backgrounds

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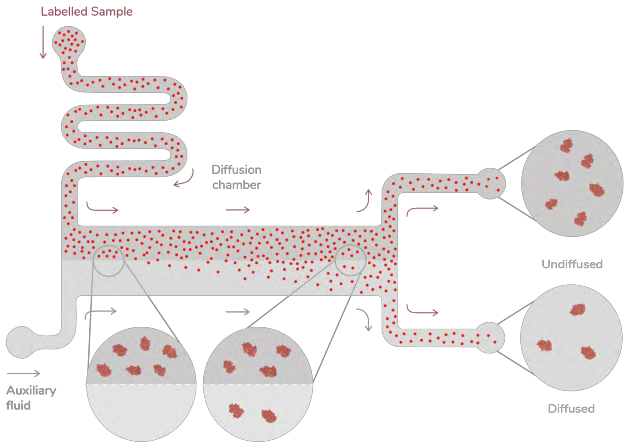
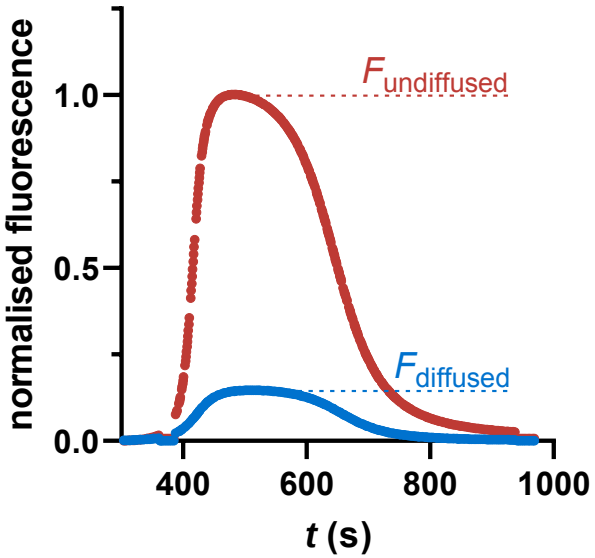
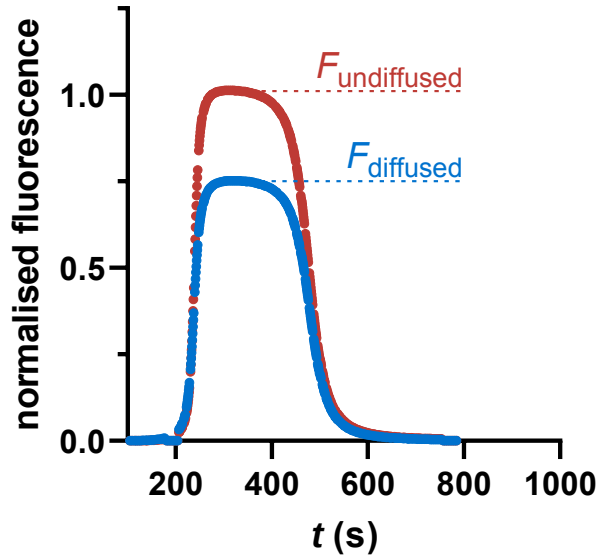
MDS detects nanodisc formation



intact LUVs



SMALP nanodiscs



$$SR = \frac{F_{undiffused}}{F_{diffused}}$$

SR = 0.15

R_h = out of range

SR = 0.75

R_h = 4.3 nm

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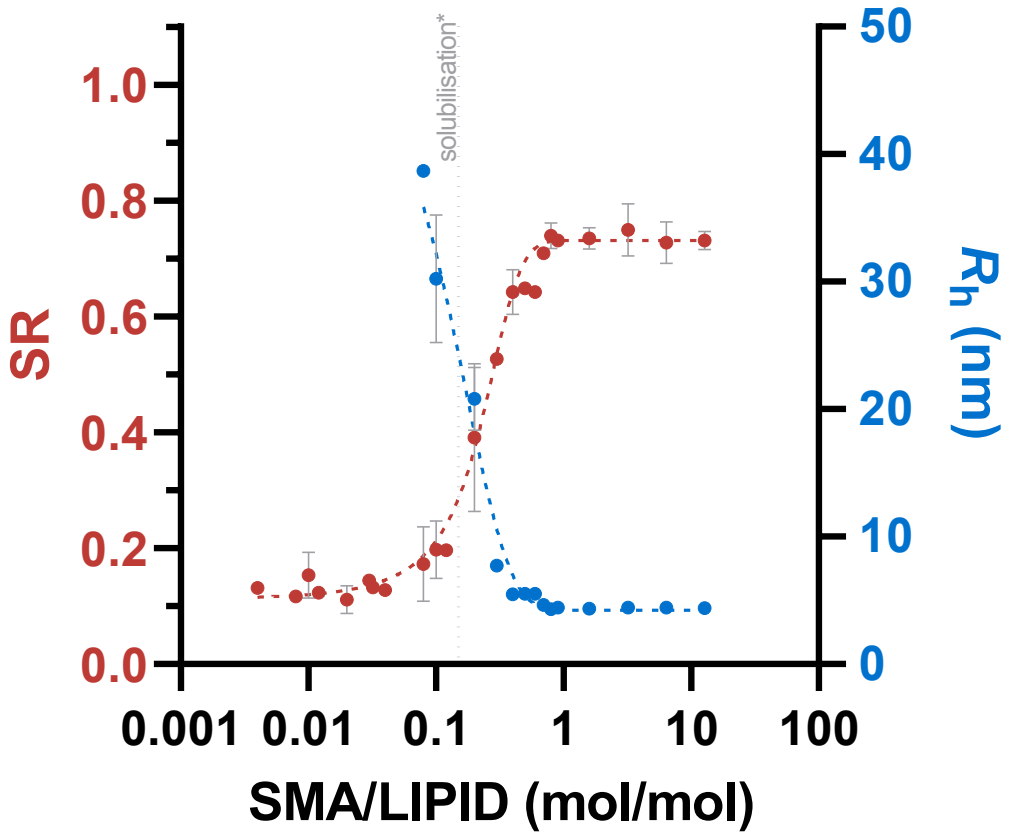
Changes in nanodisc size



- **Monitor changes in nanodisc size**
- **Adjust nanodisc size in line with experimental requirements**
- **With fluorescently labelled membrane proteins:**
  - **Confirm nanodisc size**
  - **Measure biomolecular interactions**

* ^{31}P NMR: Vargas *et al. Nanoscale* (2015) **7**, 20685–20696

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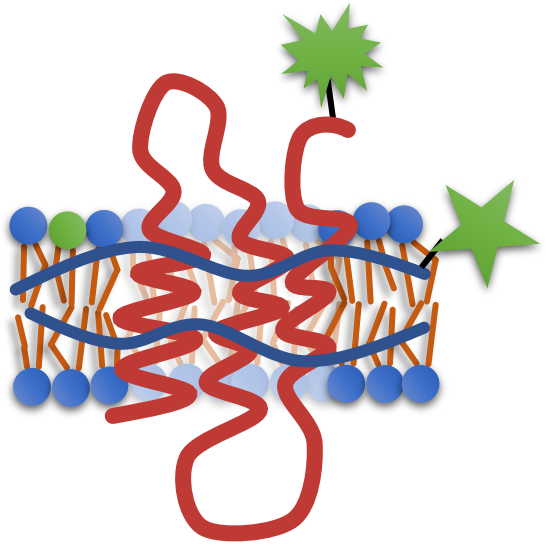
What is next?

- **Membrane proteins extracted from biological membranes**

  → no purification required

- **Measure protein–protein interactions in complex samples**



- **Please get in touch: welcome@fluidic.com**

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