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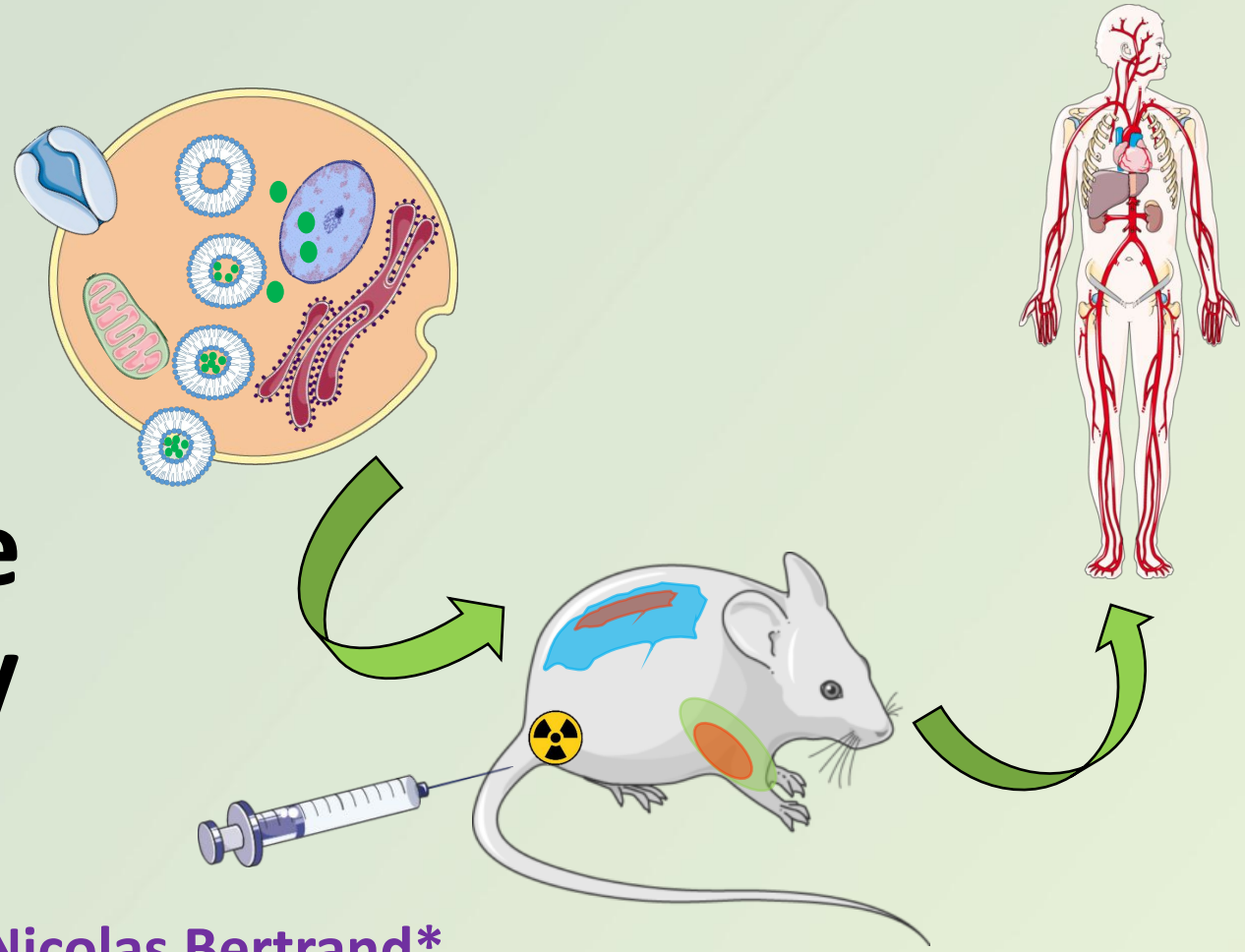

Comparing labelling methods to study the *in vivo* pharmacology of nanomaterials

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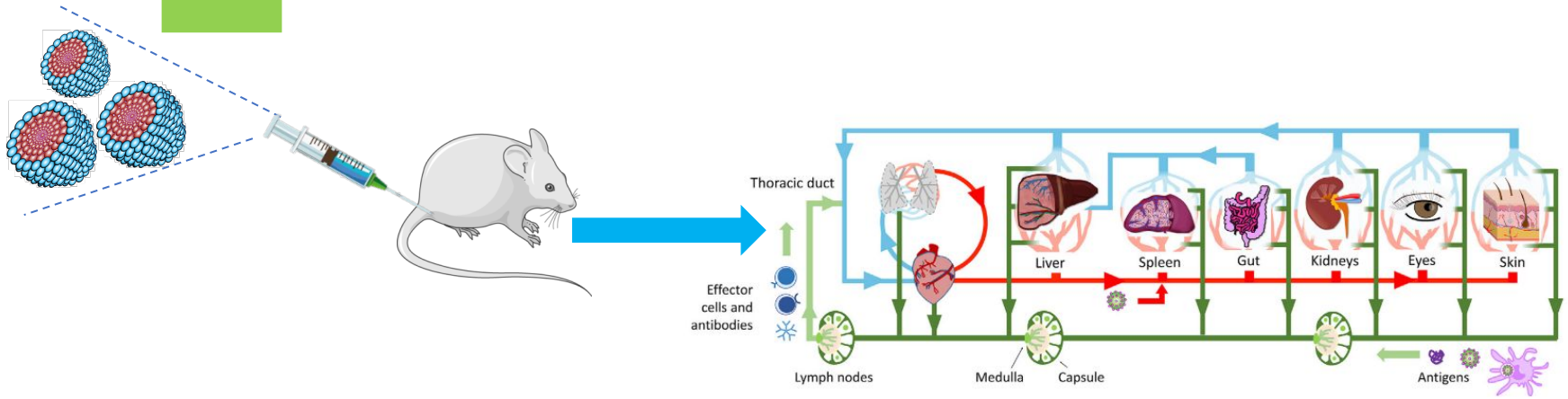
Laval University, Qc, Canada

CRS 2022 Annual Meeting & Expo

July 11 – 15, 2022 | Montreal Congress Center, Montreal Canada

Advanced Delivery Science

Pharmacology and pharmacokinetic of nanomaterials

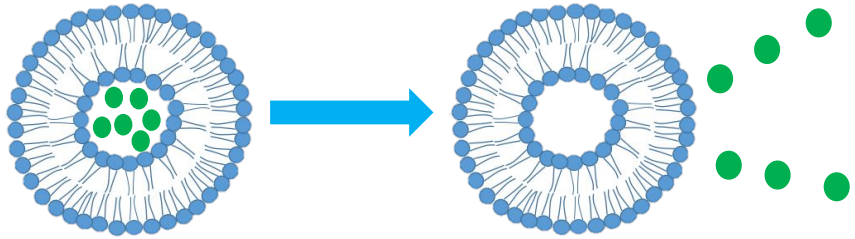


Drug Products, Including Biological Products, that Contain Nanomaterials

Guidance for Industry

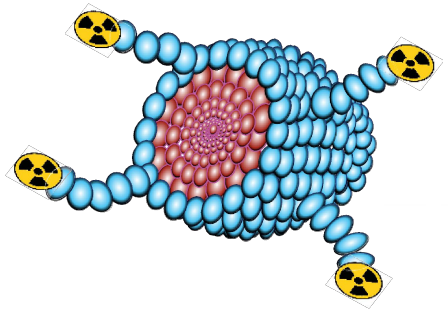
FDA U.S. FOOD & DRUG ADMINISTRATION

Drugs

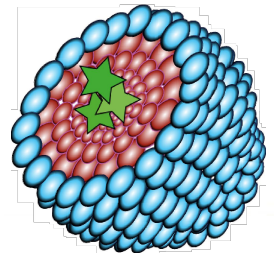


Carrier

Covalent label



Non-covalent label

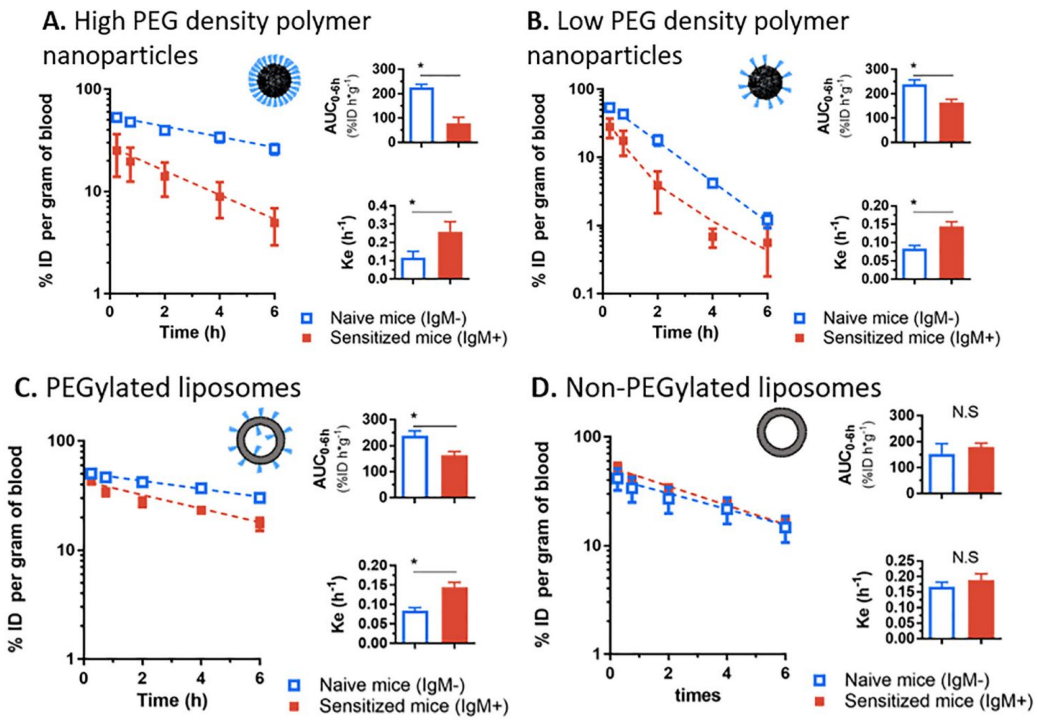


CRS Controlled Release Society

Viana IMO, et al., *Acta Pharmaceutica Sinica B* **2021** , 11 (4) ; 852-870.

Radioactivity and fluorescence can be used to track nanoparticles

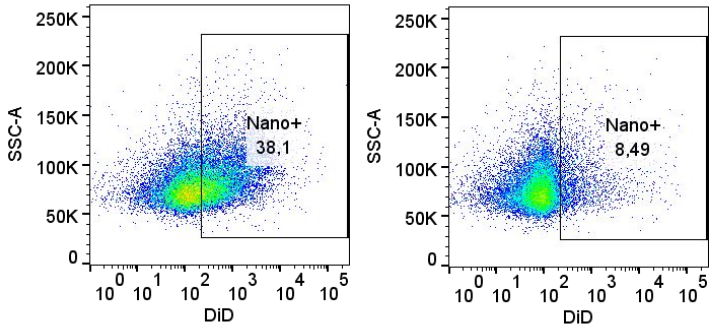
Radioactivity

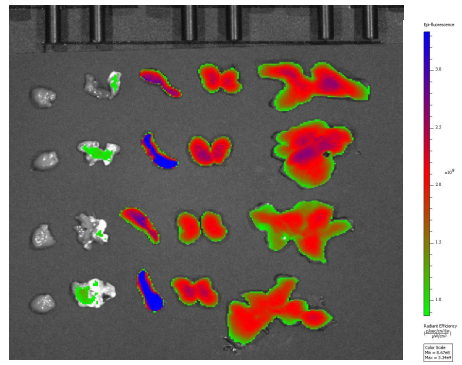


Poster #345

- Quantitative method
- ^{3}H and ^{14}C require scintillation counting
- Expensive and regulated

Fluorescence





- Compatible with various analytical methods (imaging, flow cytometry, microscopy)
- More accessible
- Semi-quantitative

Grenier, P., et al., *Journal of Controlled Release* **2018,** *287*, 121-131.

Viana IMO, et al., *Nanoscale* **2020,** *12* (36), 18875-18884.

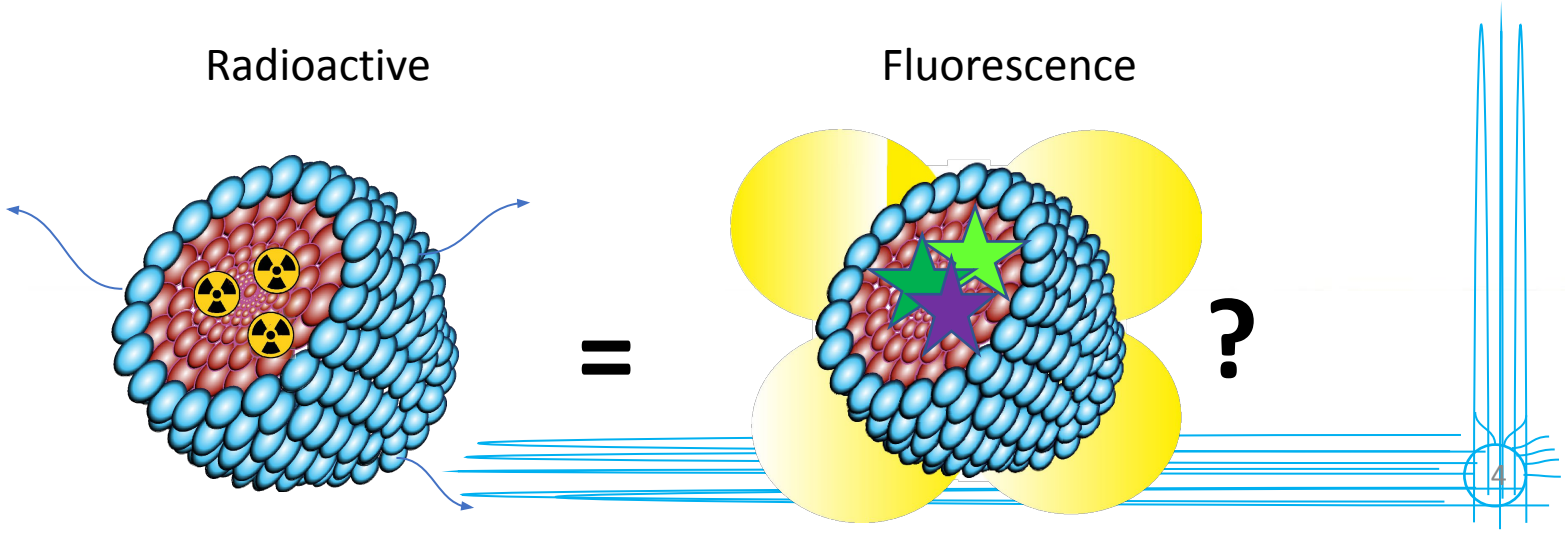
Objectives

Hypothesis

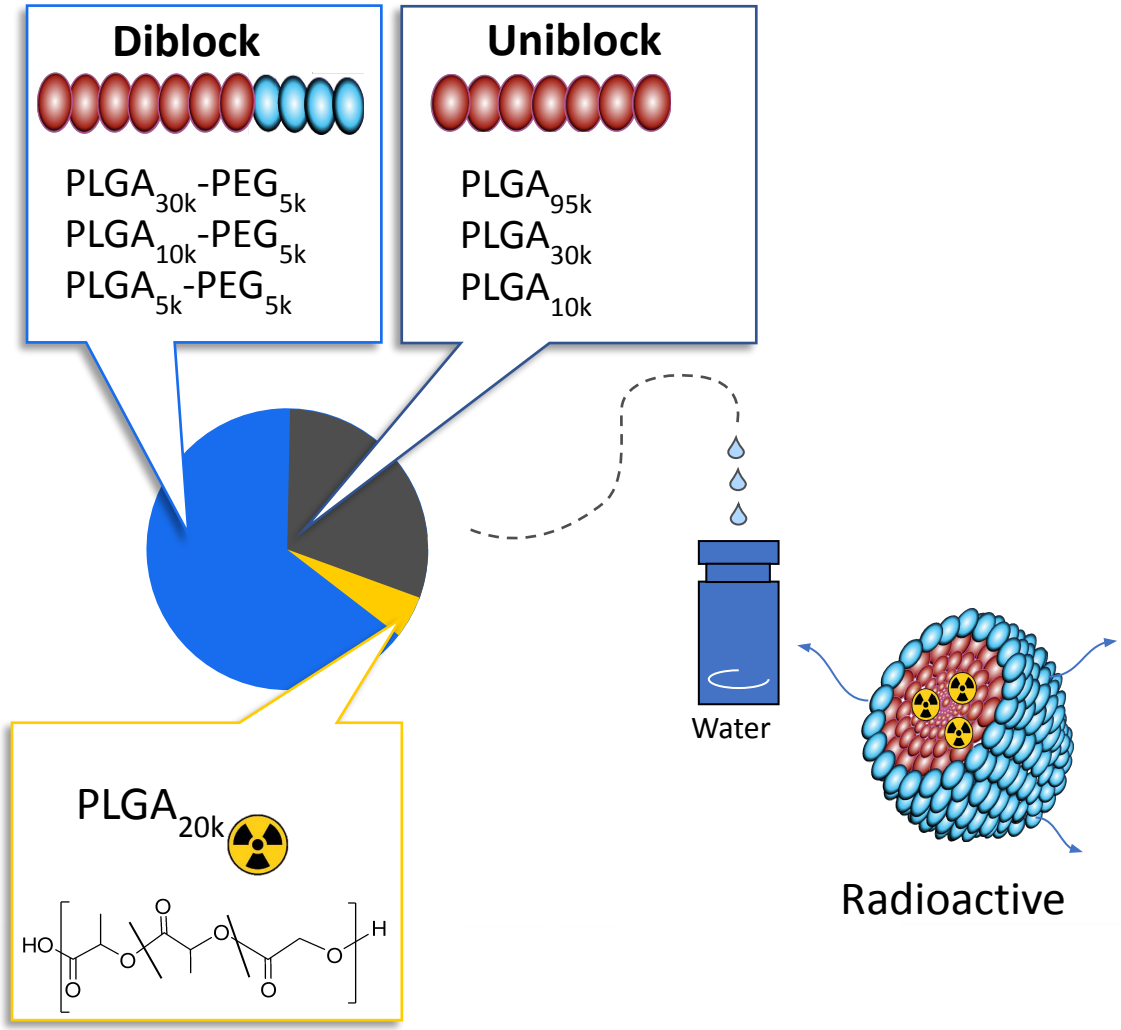
The choice of fluorophores used will impact the result of the pharmacokinetic study

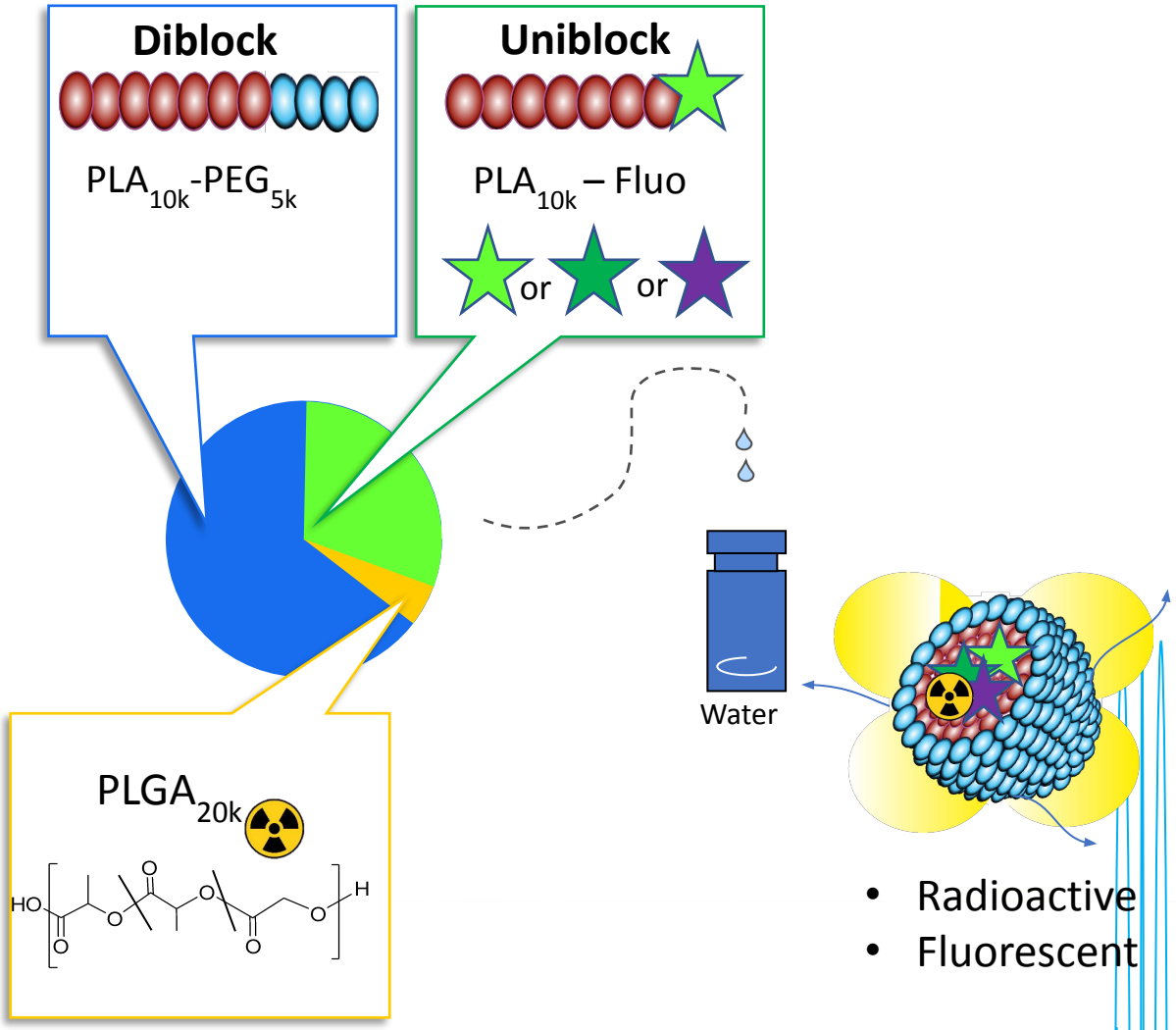
Objectives

- Make a dual-labelled system with radioactive and fluorescent labels
- Evaluate the correlation between radioactive and fluorescent signals *in vivo*
- Determine which fluorophore is more suitable to study the fate of nanoparticles



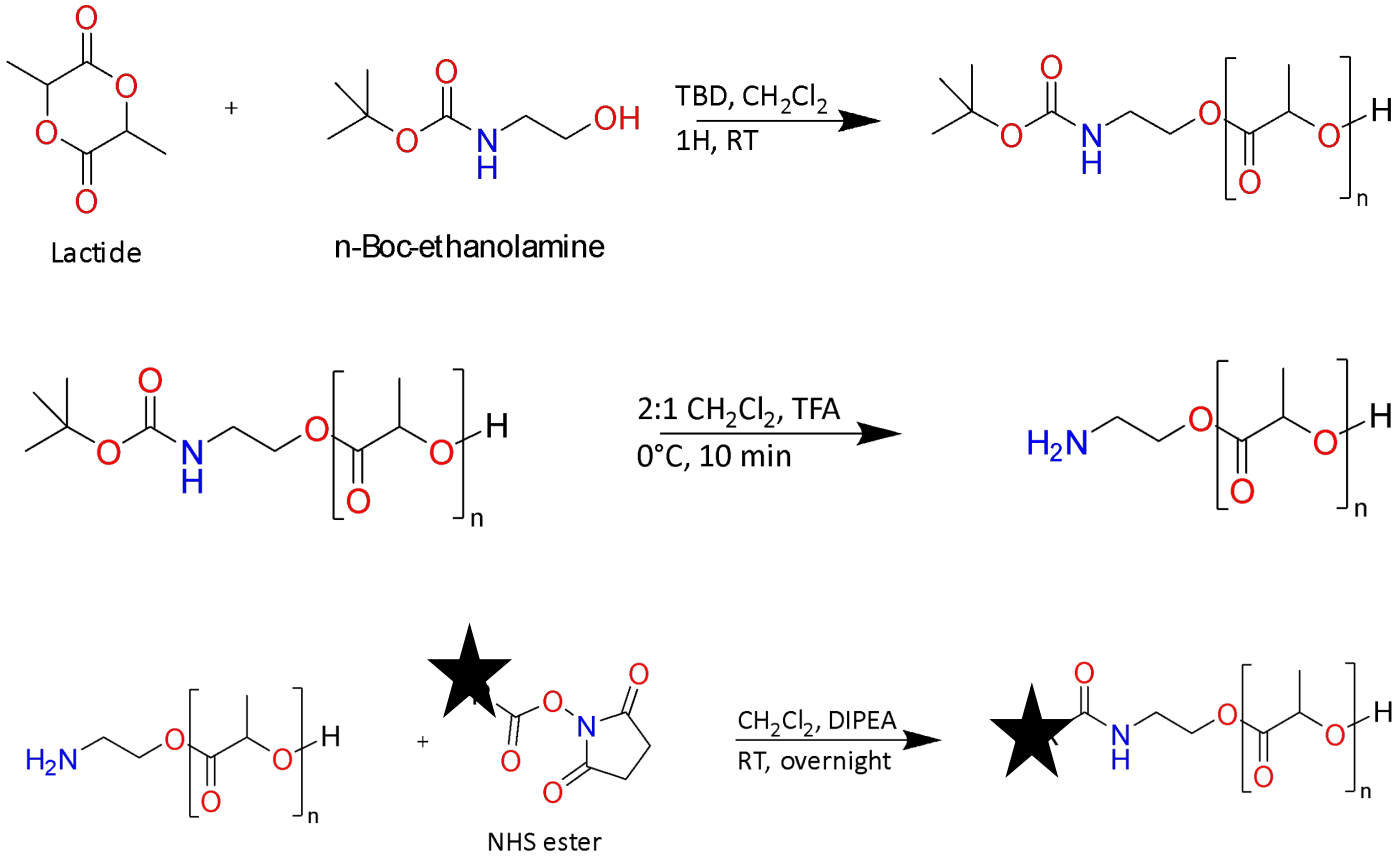
Polymeric nanoparticles are made by nanoprecipitation





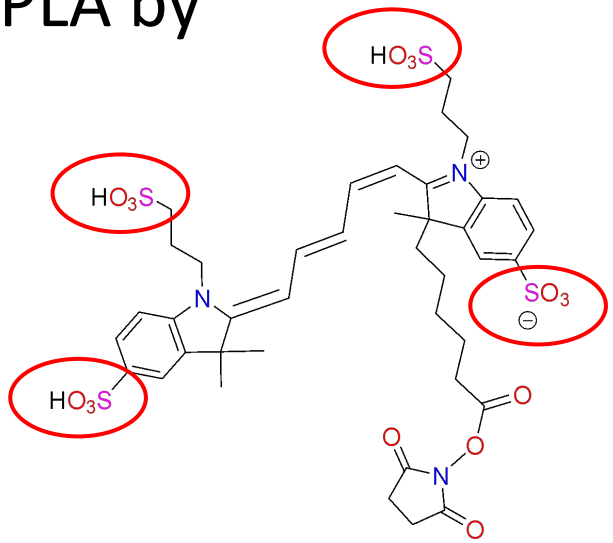
Bertrand, N., et al., *Nature Communications* **2017,** *8* (1), 777.

Fluorophores can be covalently attached to PLA by using an amide bond

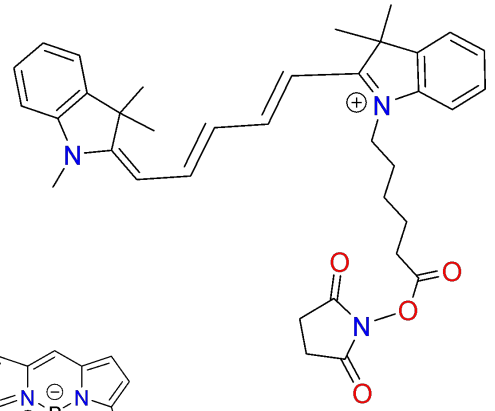


Purification : 4 x precipitations in cold diethyl ether

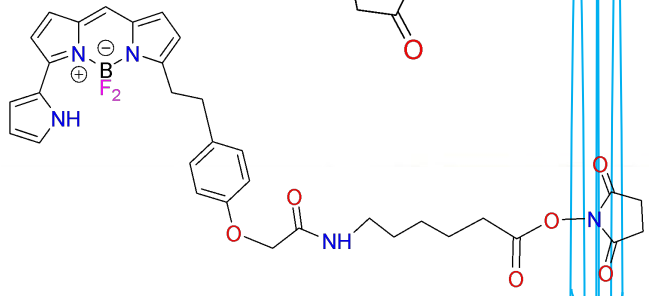
AZ647



Cyanine 5 (Cy5)

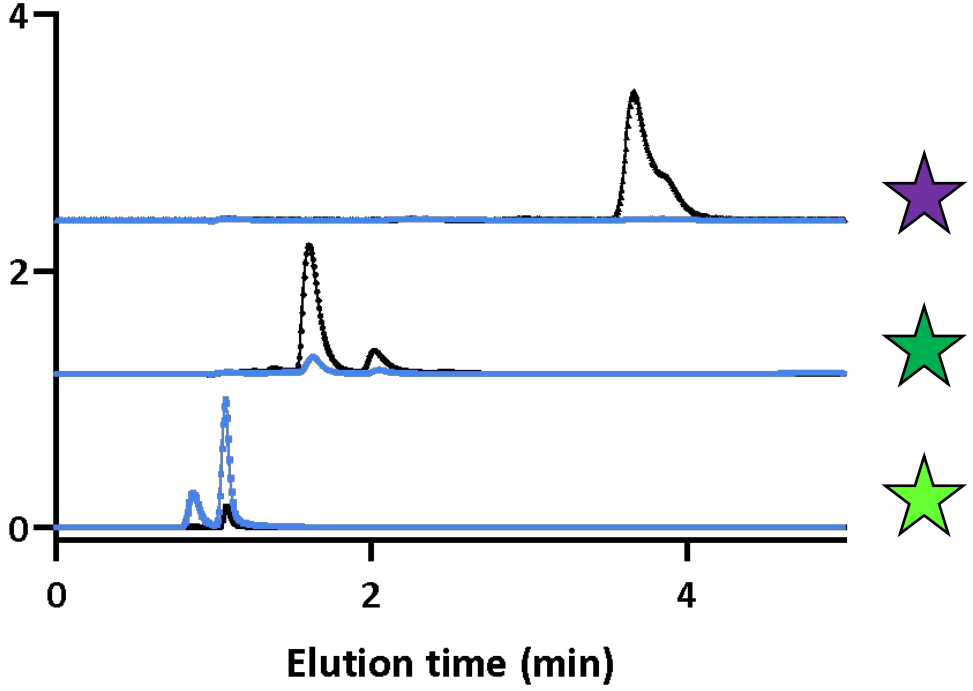


BODIPY650



The partition coefficient of the dyes can be measured by chromatography (HPLC)

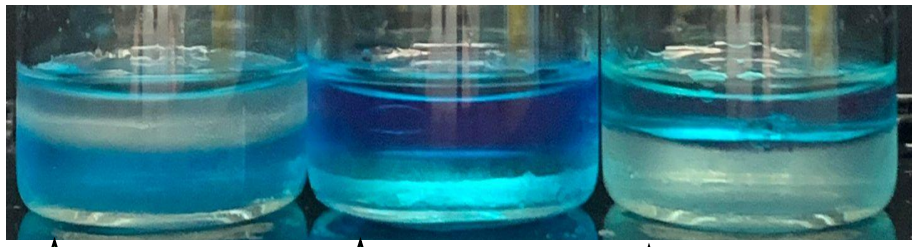
HPLC chromatograms for the three NHS-dyes in octanol and water (C18, MeOH/water 71:29)



Octanol

Water

Hydrophilicity

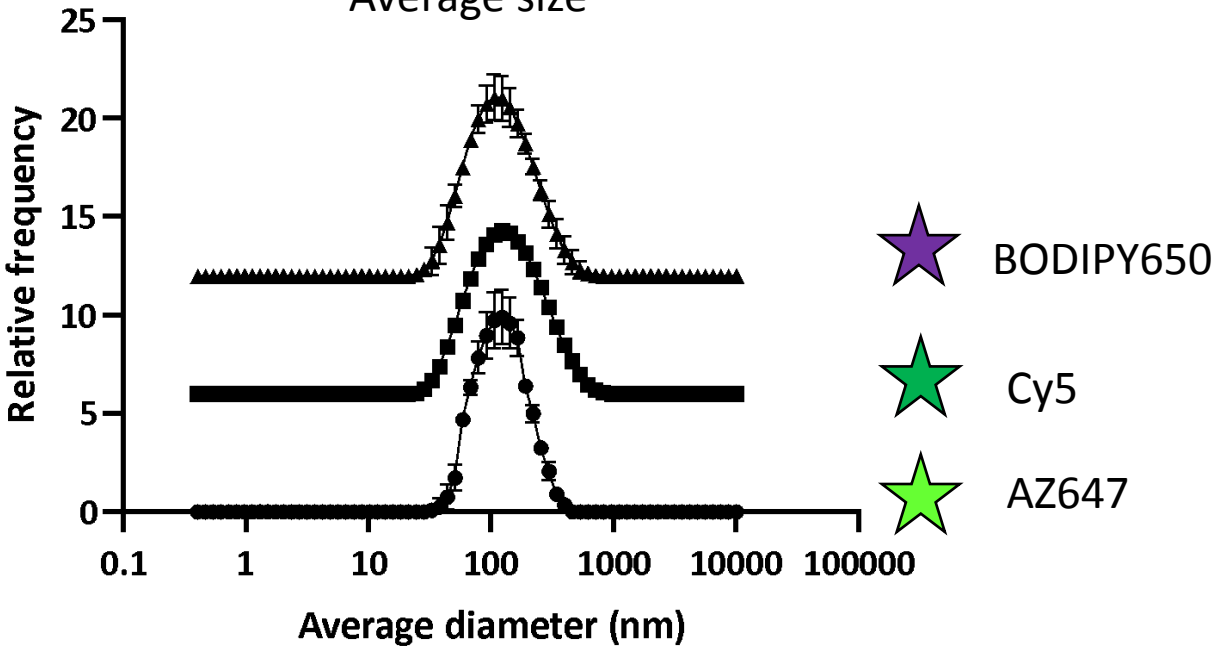


AZ647 Cy5 BODIPY650

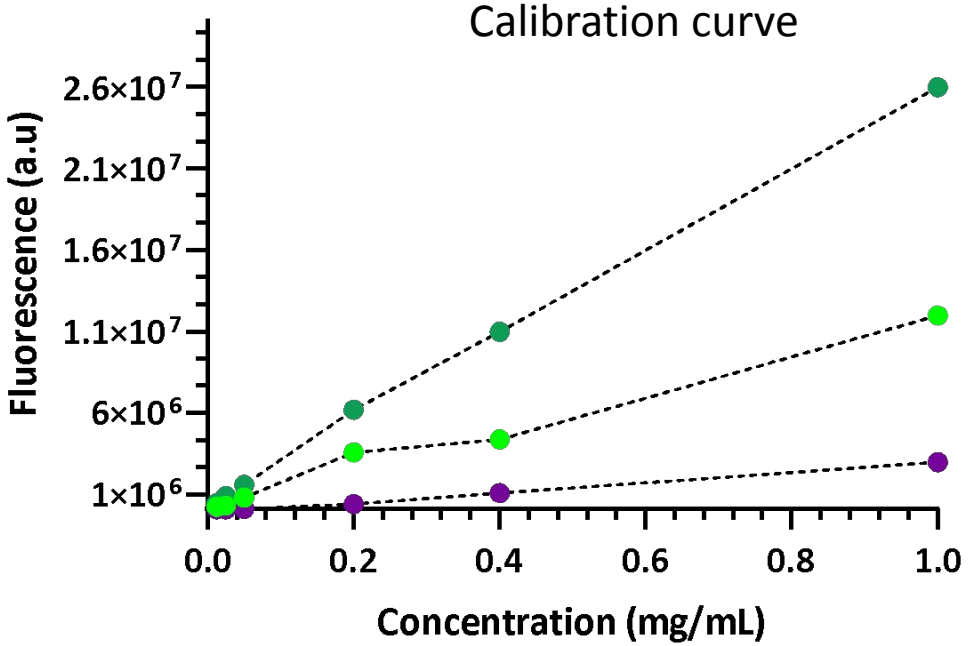
Dye	%octanol	%water
AZ647	2	98
Cy5	93	7
BODIPY650	99	1

All nanoparticles are comparable in terms of size, radioactivity and fluorescence

Average size



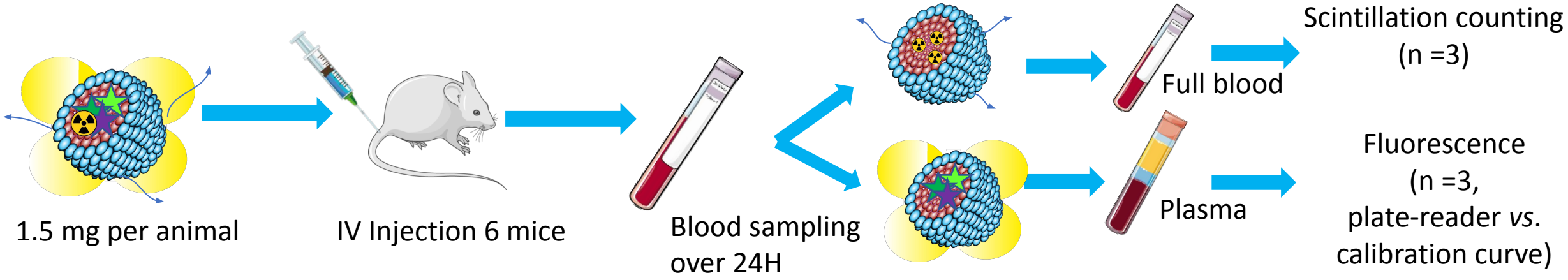
Fluorescence of nanoparticles – Calibration curve



Radioactivity

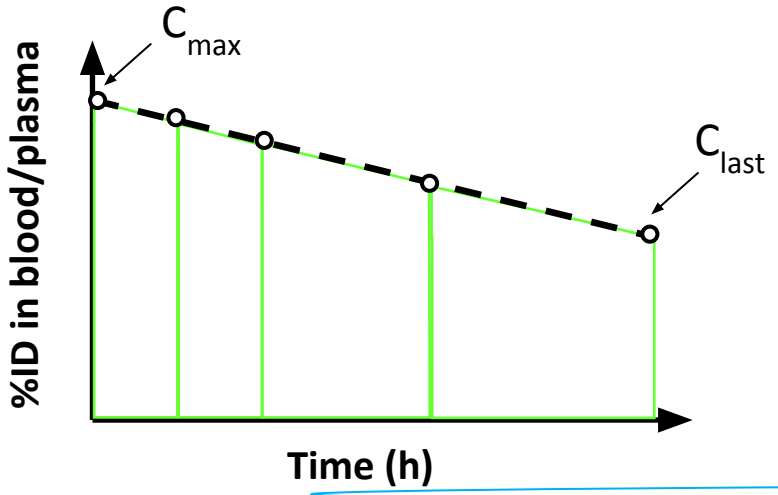
Dye	DPM/100 µL
AZ647	134,930
Cy5	131,045
BODIPY650	131,725

Design of the pharmacokinetic study

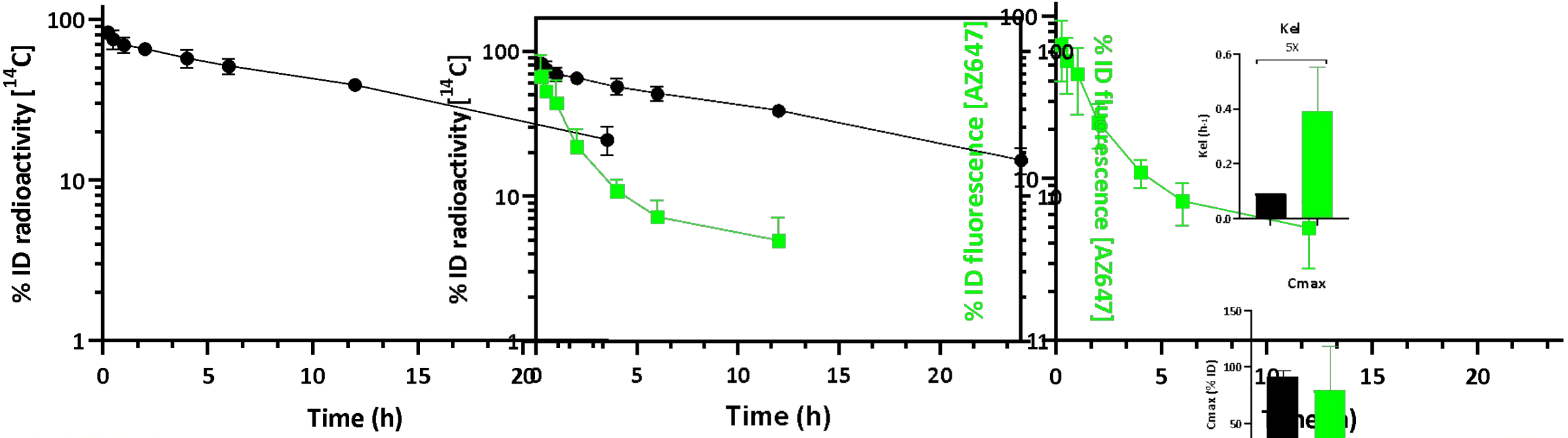


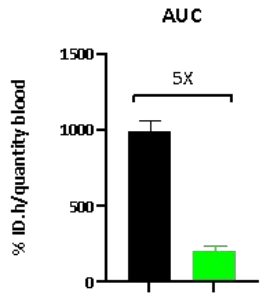
AUC = trapezoidal method

Kel = Cl/Vd = Dose/($AUC_{0\text{-inf}}$ x Vd)



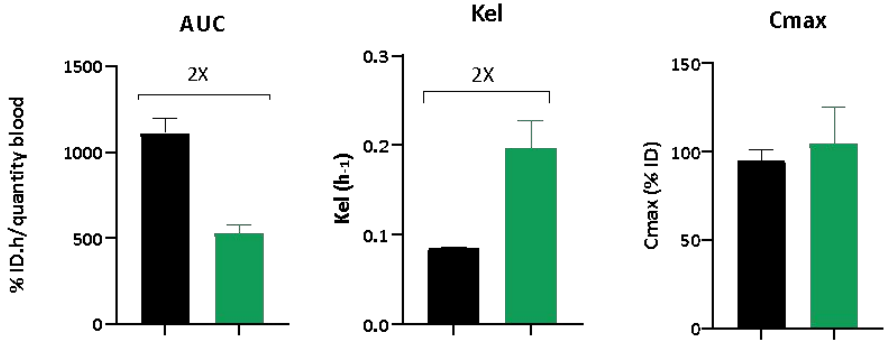
Results – Pharmacokinetic experiment with AZ647

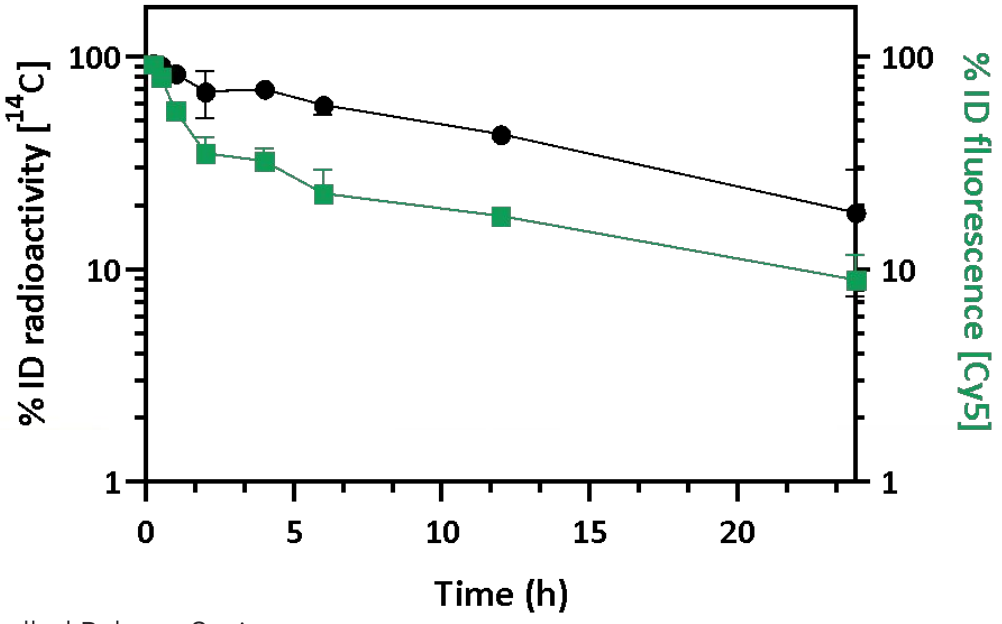




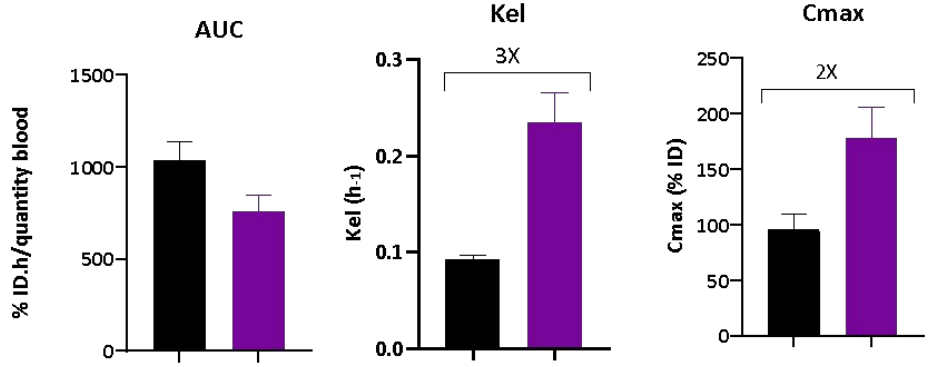
Result- Pharmacokinetic of Cy5 and BODIPY650

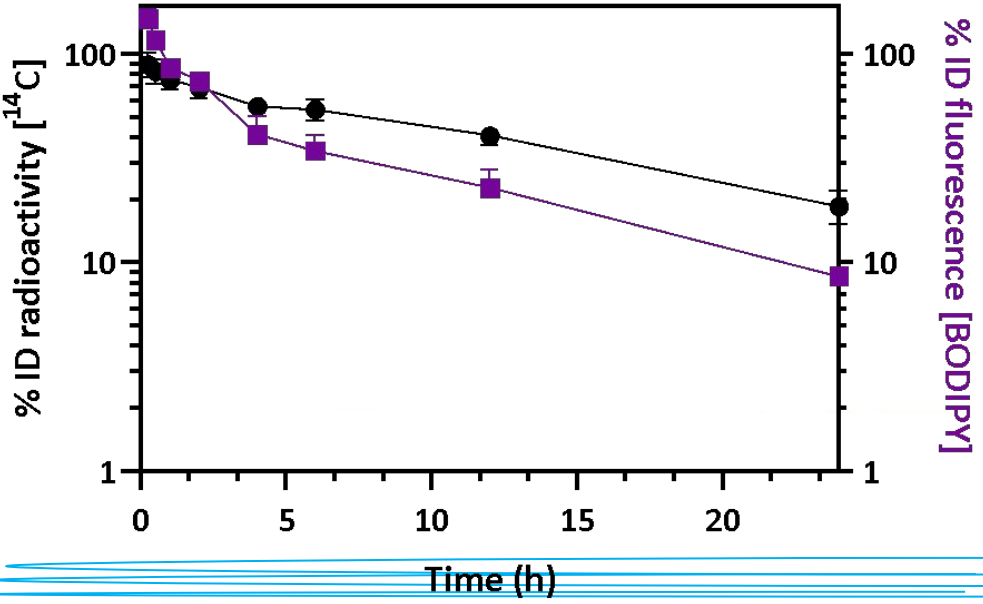
Cy5





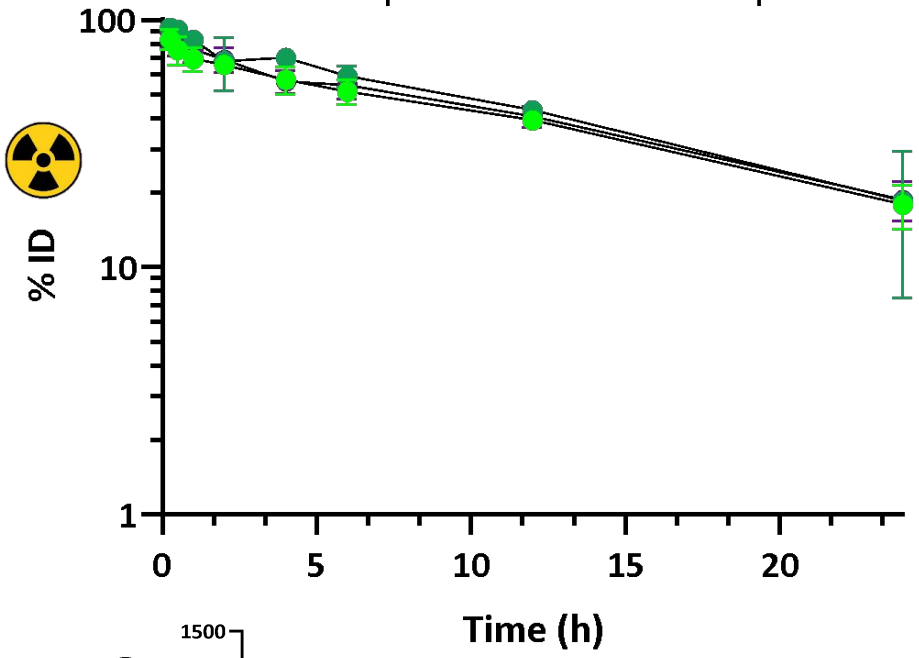
BODIPY650

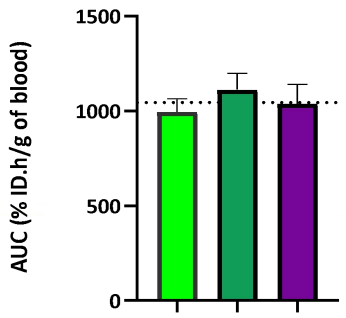




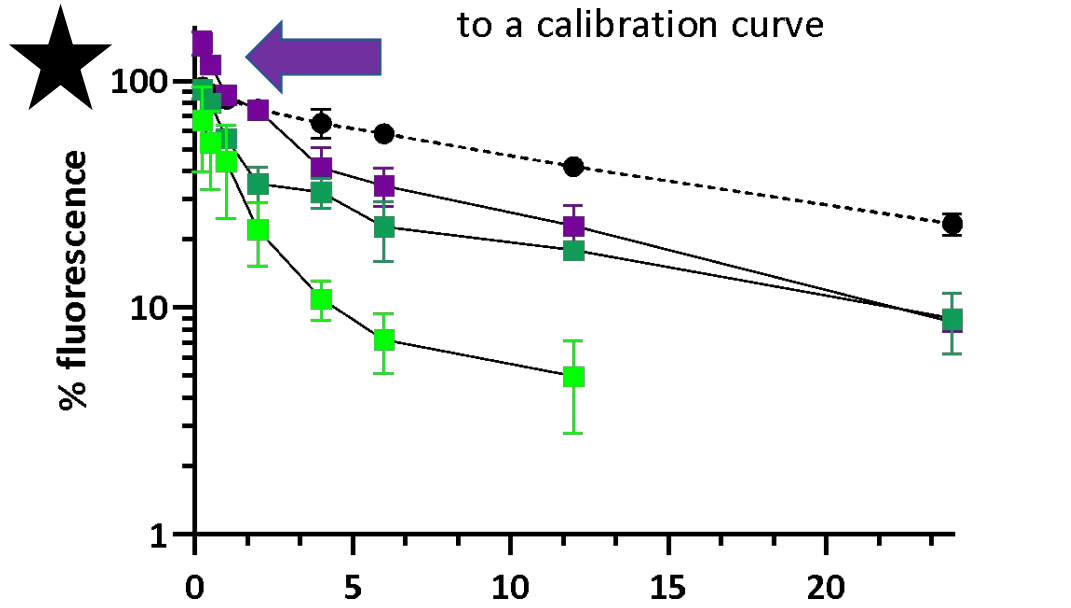
Summary of the PK analysis

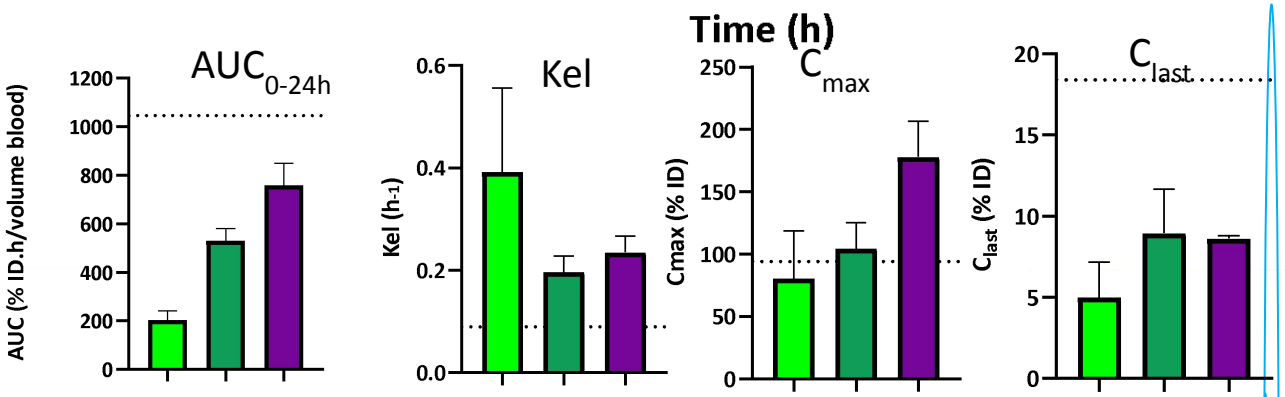
Pharmacokinetic in radioactivity is independent of the fluorophore





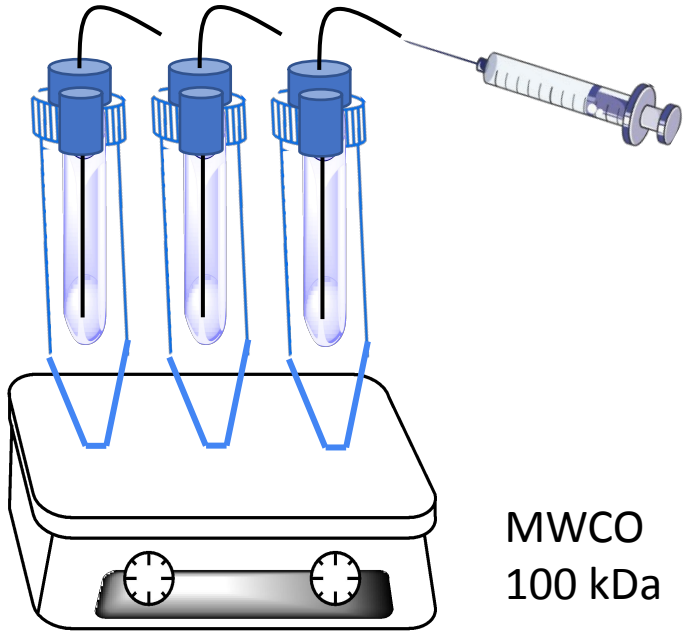
Pharmacokinetic in fluorescence when compared to a calibration curve



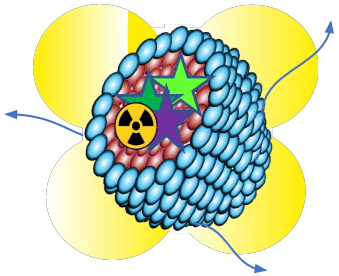


Cy5 is close to radioactive values for 3 out of 4 PK parameters (not AUC)

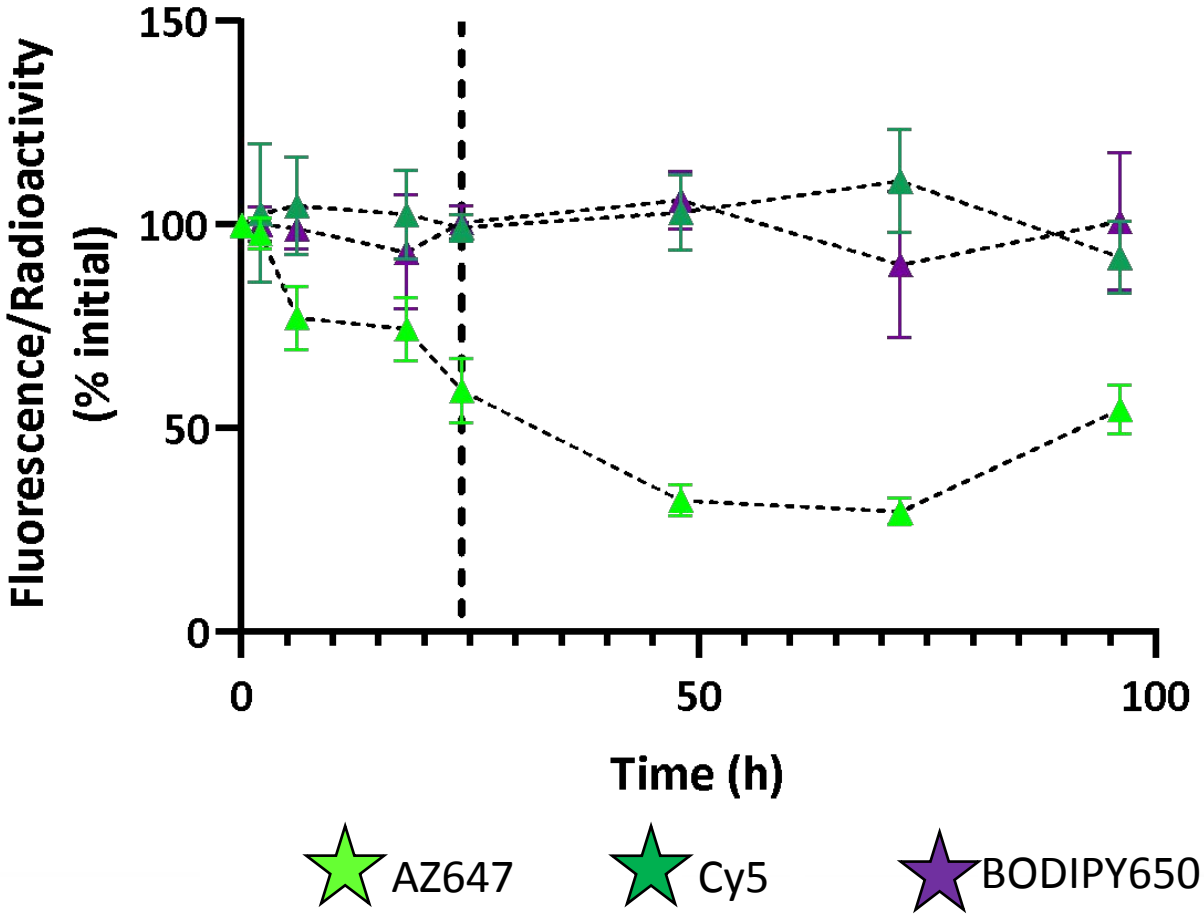
The release of the dye from the nanoparticles can be studied *in vitro*



MWCO
100 kDa



Released fluorescence



AZ647 appears to be released from the nanoparticles faster than the more hydrophobic dyes

Conclusion and perspectives

Conclusions :

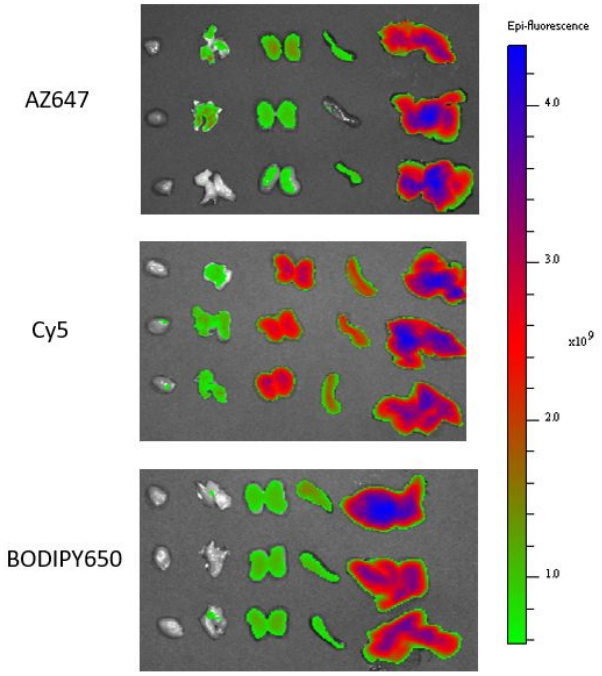
- ❑ The hydrophilicity of the dye impacts the results of the pharmacokinetic;
- ❑ Fluorescence PK analysis appears to underestimate AUC for all fluorophores;
- ❑ The Cyanine 5 dye shows better correlation with the radioactive signal.

Limitations :

- ❑ The purification of fluorescent polymers was standardized, but not optimized;
- ❑ The nanoparticles did not have the same fluorescence intensity;
- ❑ Only one type of nanoparticle was investigated.

Perspectives :

- ❑ Understand how the fluorophore impacts the biodistribution results;
- ❑ Confirm the validity of Cy5 in other conditions.



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