

New Far-Red emitting Lipid-Polymer probes for Lipid Bilayer Imaging

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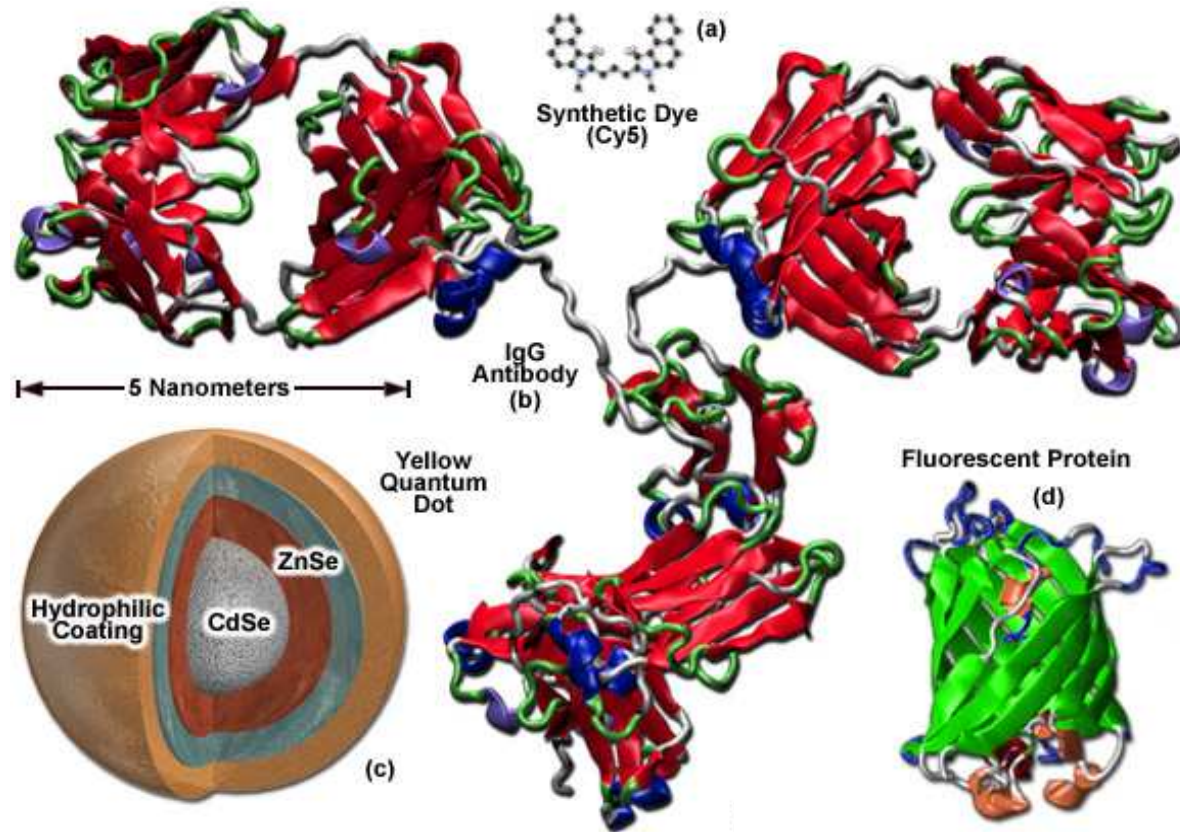
1- Laboratoire Joliot-Curie, ENS de Lyon

& Laboratoire Ingénierie des Matériaux Polymères, INSA-Lyon

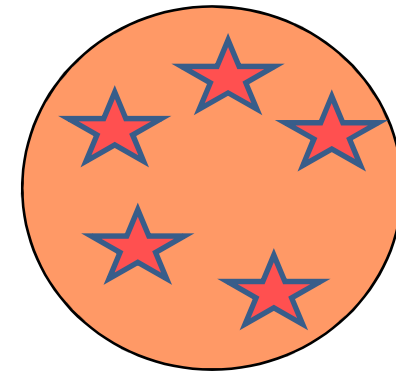
2- Laboratoire de Chimie, ENS de Lyon

3- Laboratoire de Virologie, ENS de Lyon

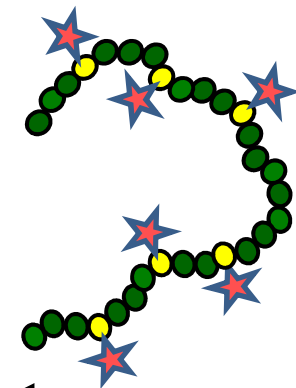
Relative sizes of fluorescent probes



Polymer probes

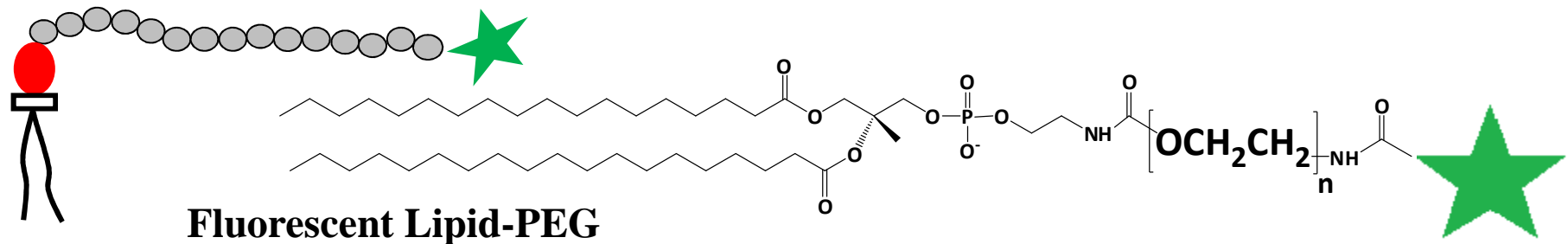
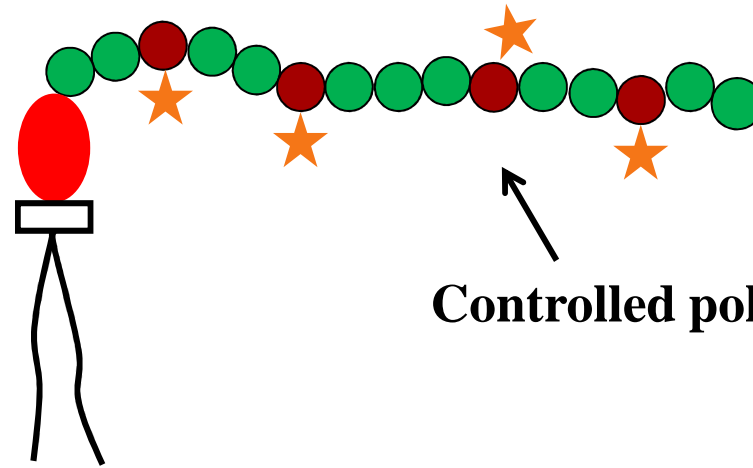
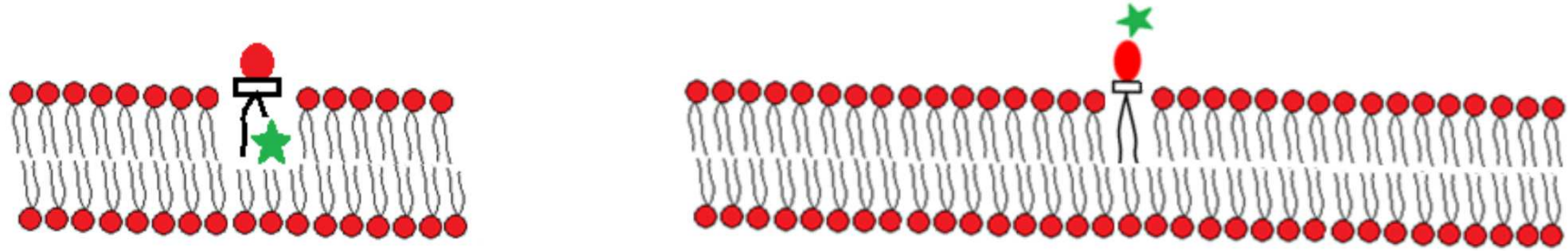


80 - 200 nm

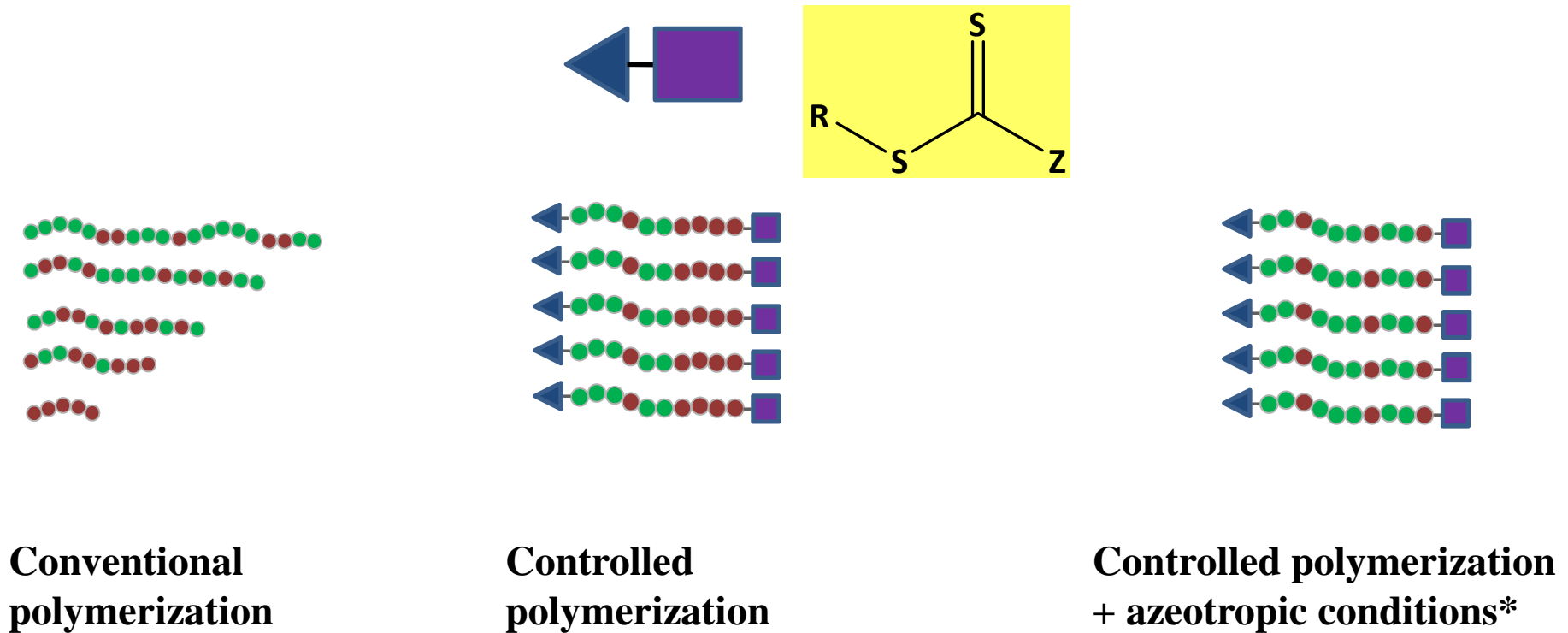


5 - 30 nm

New fluorescent **Lipid-Polymer** probes



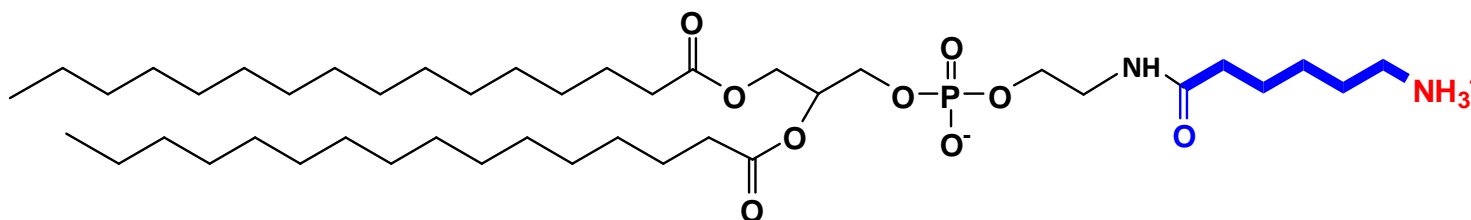
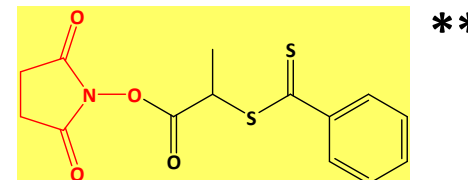
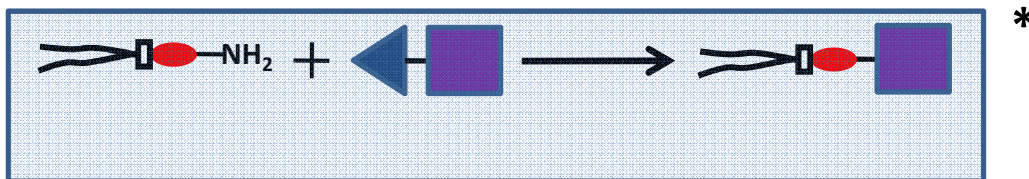
What is a **controlled** polymerization ?



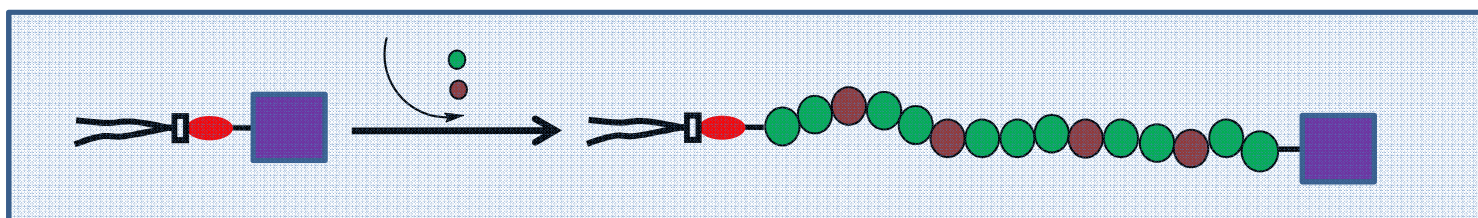
**Full control of size, composition and microstructure
+ 1 (or 2) function(s) precisely localized at each chain-end**

* A. Favier, M-T. Charreyre et al. *Polymer* (2004), 45, 7821-7830

Introduction of a phospholipid end-group



1,2-Dipalmitoyl-*sn*-glycero-3-Phosphoethanolamine-N-(Hexanoylamine)



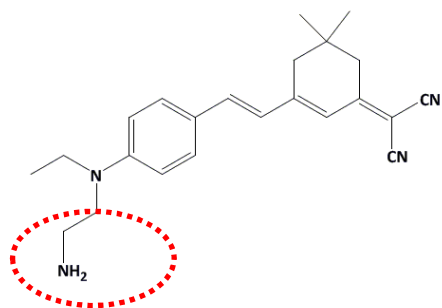
Hydrophilic monomer
Reactive monomer

Lipid-coPolymer
6 000 \leftrightarrow 33 000 g.mol⁻¹

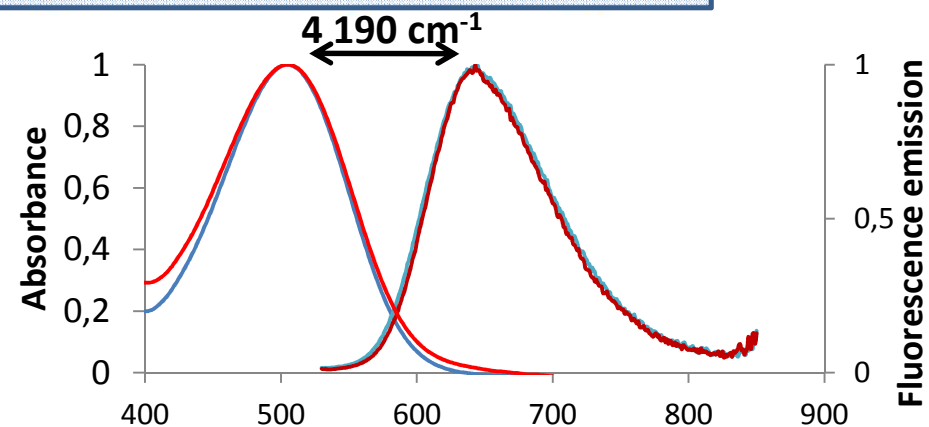
* M. Bathfield, M-T. Charreyre et al. *Macromolecules* (2008) 41, 8346-8353

** M. Bathfield, M-T. Charreyre et al. *JACS* (2006) 128, 2546-2547

Binding of far red chromophores



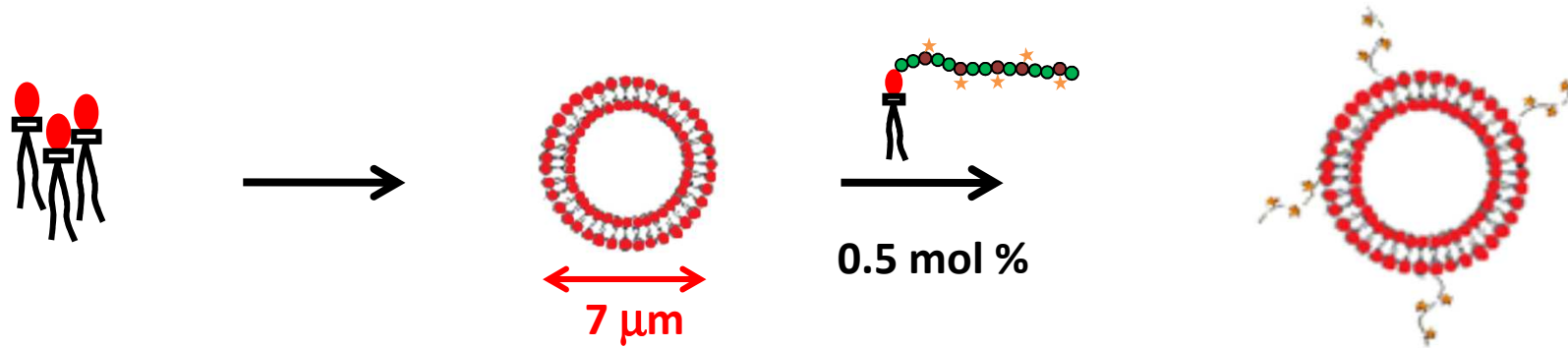
Dicyanoisophorone* family



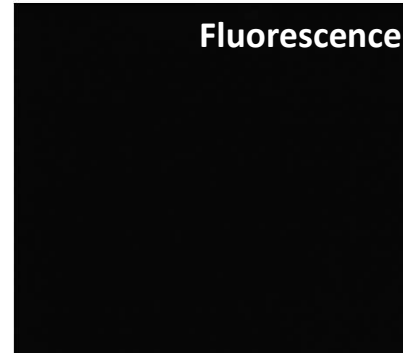
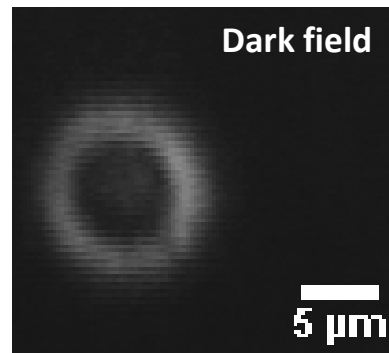
		$\lambda_{\text{Abs max}}/\lambda_{\text{Em max}}$	chromophores per polymer	Brightness
CHCl ₃	Free chromophore	505/644	/	1 400
	33K-9AEM	505/645	9	15 000
	33K-38AEM	502/650	38	50 300
Eau	8K-2H	506/688	2	1 900
	20K-4H	508/688	4	4 400
	33K-9H	501/690	9	13 000

* J. Massin, Y. Bretonnière et al. *Chem. Mater.* (2011), 23, 862-873

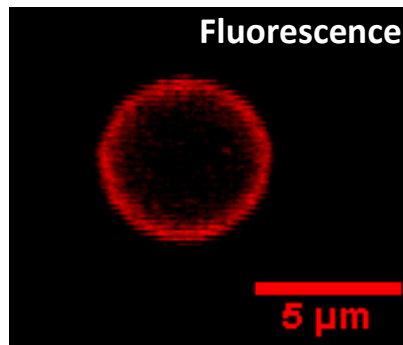
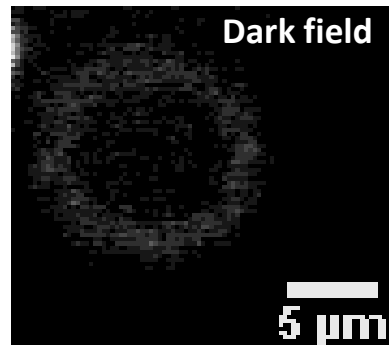
Insertion tests in model lipid bilayers (**GUV**)



1st step

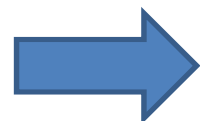
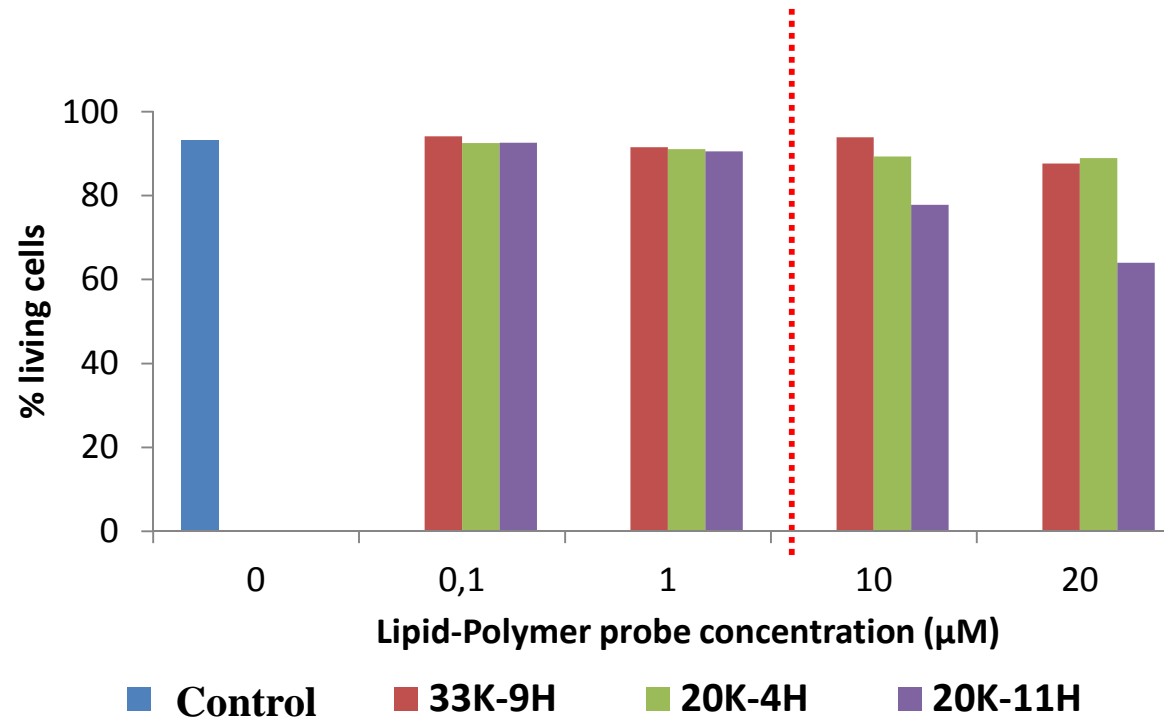
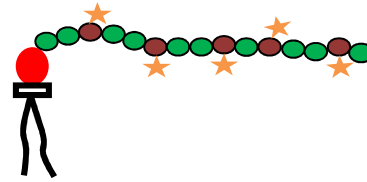


2nd step



Cytotoxicity tests (flow cytometry)

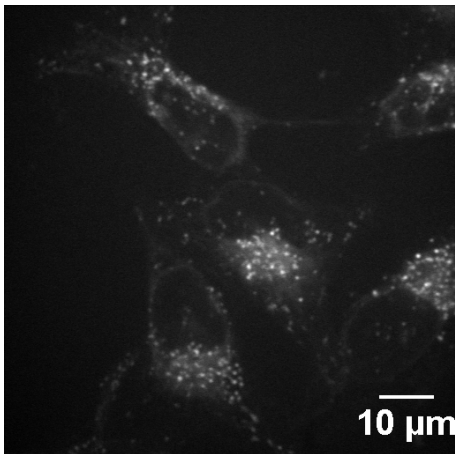
T-Lymphocytes
after 7h of incubation



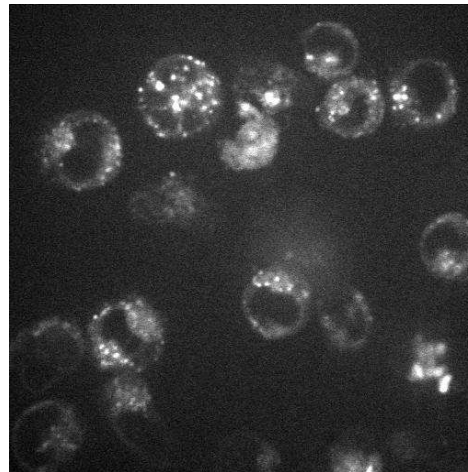
No cytotoxicity at usual concentrations

Internalization tests in **living cells**

HeLa cells
Polymer probe = 1 μ M



T-Lymphocytes
Polymer probe = 1 μ M



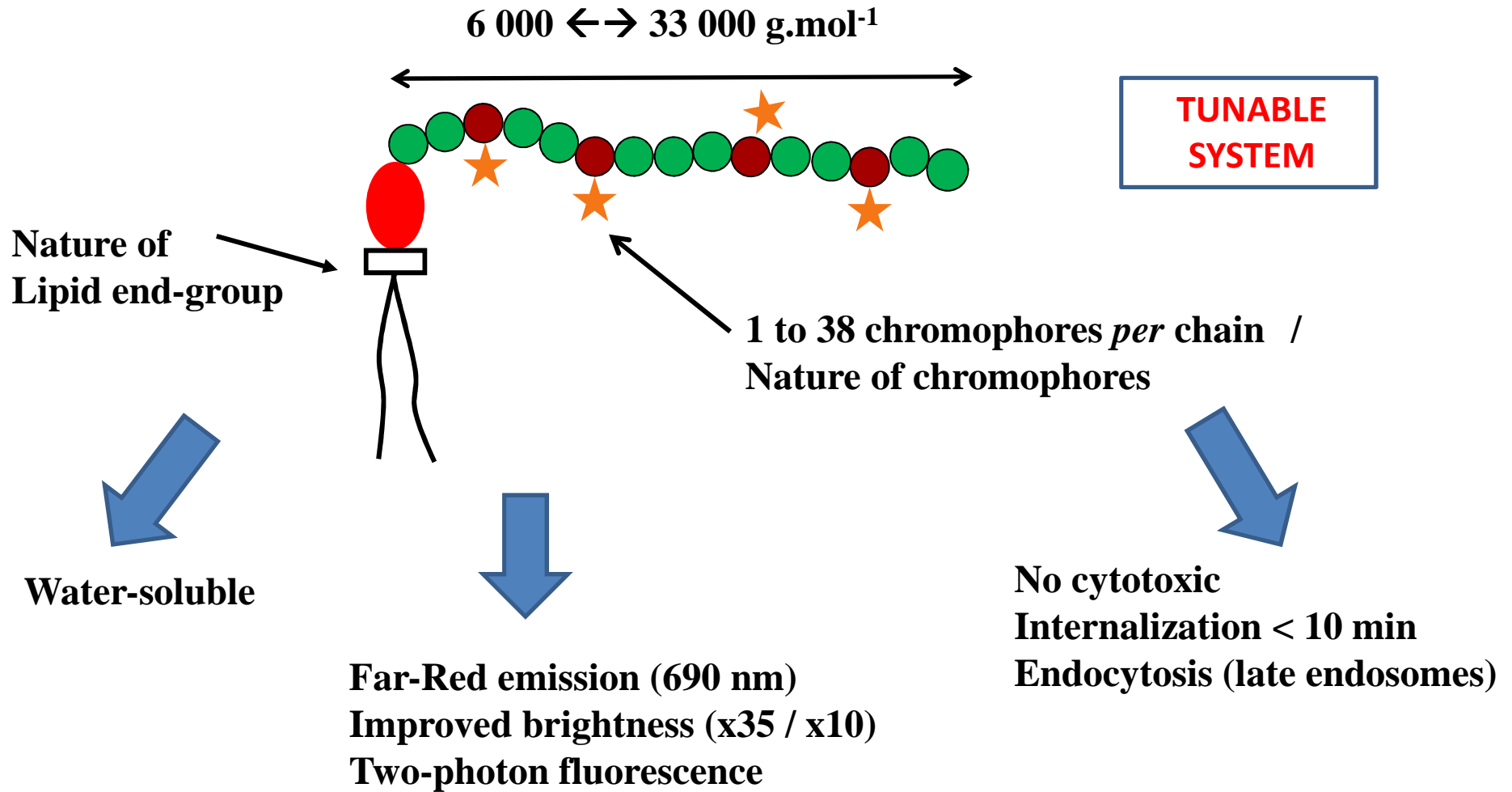
LEICA DMI 4000
“spinning disk”
confocal microscopy

PLATIM – ENS Lyon

Internalization (< 10 min) without using any carrier
Highly fluorescent (> 0.1 μ M)
Resistance to photobleaching
Localized in late endosomes

... on going: labeling of viral particles

Conclusions



Two-photon chromophore-polymer probes for bio-imaging
Photosensitizer-polymer probes for photodynamic therapy (PDT)