Single molecule detection with metal nanoparticles : Towards resolving dynamic fluctuations of macromolecular complexes

I- Fibroblast Growth Factor (FGF) ligand-receptor signalling complex

II- Development of a quantitative method using metal nanoparticles to detect biological entities at a single molecule resolution

A) Why NP are good candidates as highly sensitive labels for single molecule biochemistry

B) Requirements to use metal nanoparticles to label proteins

- Soluble and highly stable in physiological condition
- No aspecific interaction, no ligand exchange
- Specific & stoichiometric attachment to the protein of interest (1 NP : 1 protein)

C) Applications

- Stoichiometry of the FGFR1:FGF2:HS complex *in vitro*
- Imaging in living cell

D) Conclusion

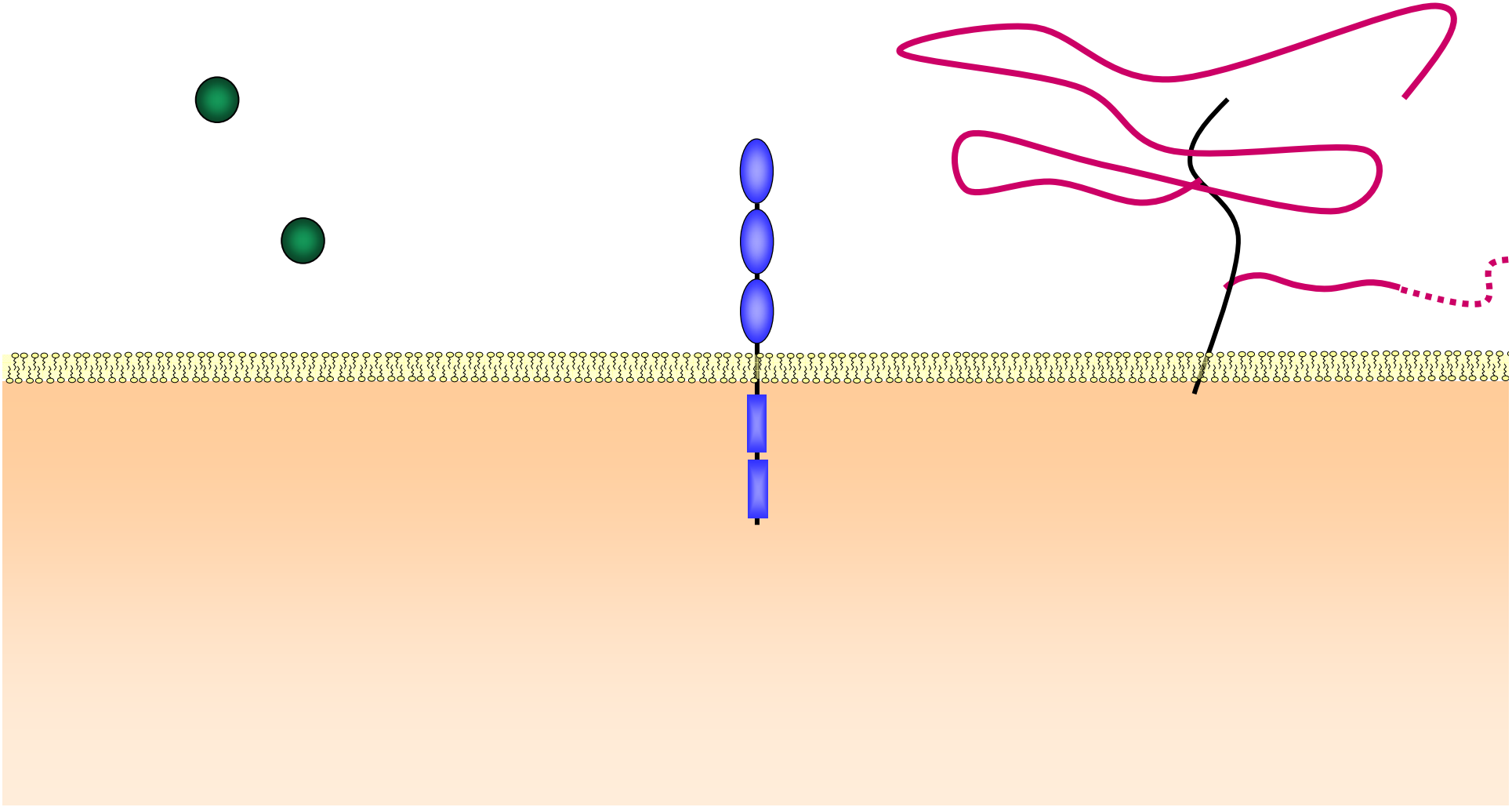
FGF LIGAND-RECEPTOR SIGNALLING COMPLEX

Main actors

FGF
~ 30 FGFs

FGFR
~ 48 isoforms

HSPG
Many sequences



FGF LIGAND-RECEPTOR SIGNALLING COMPLEX

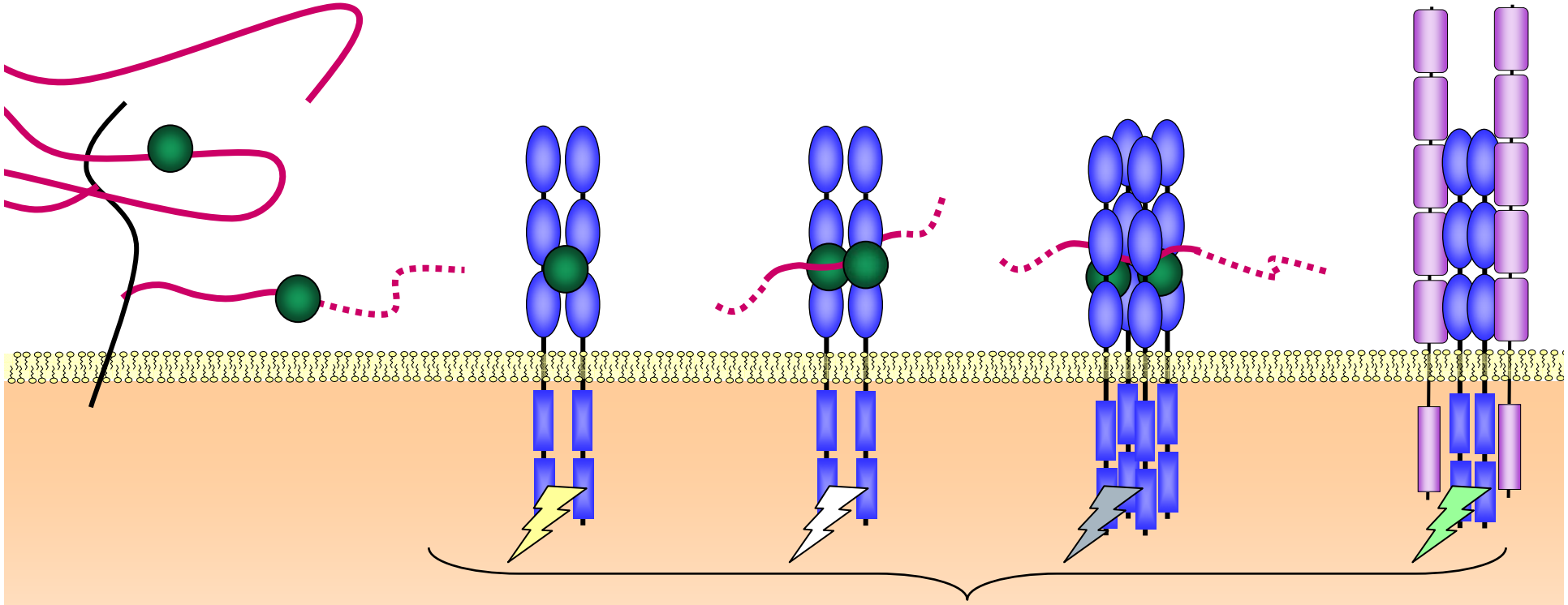
Combinatorial assembly of signalling complexes

FGF
~ 30 FGFs

FGFR
~ 48 isoforms

HSPG
Many sequences

Other molecules



Specific signals integrated by the cell

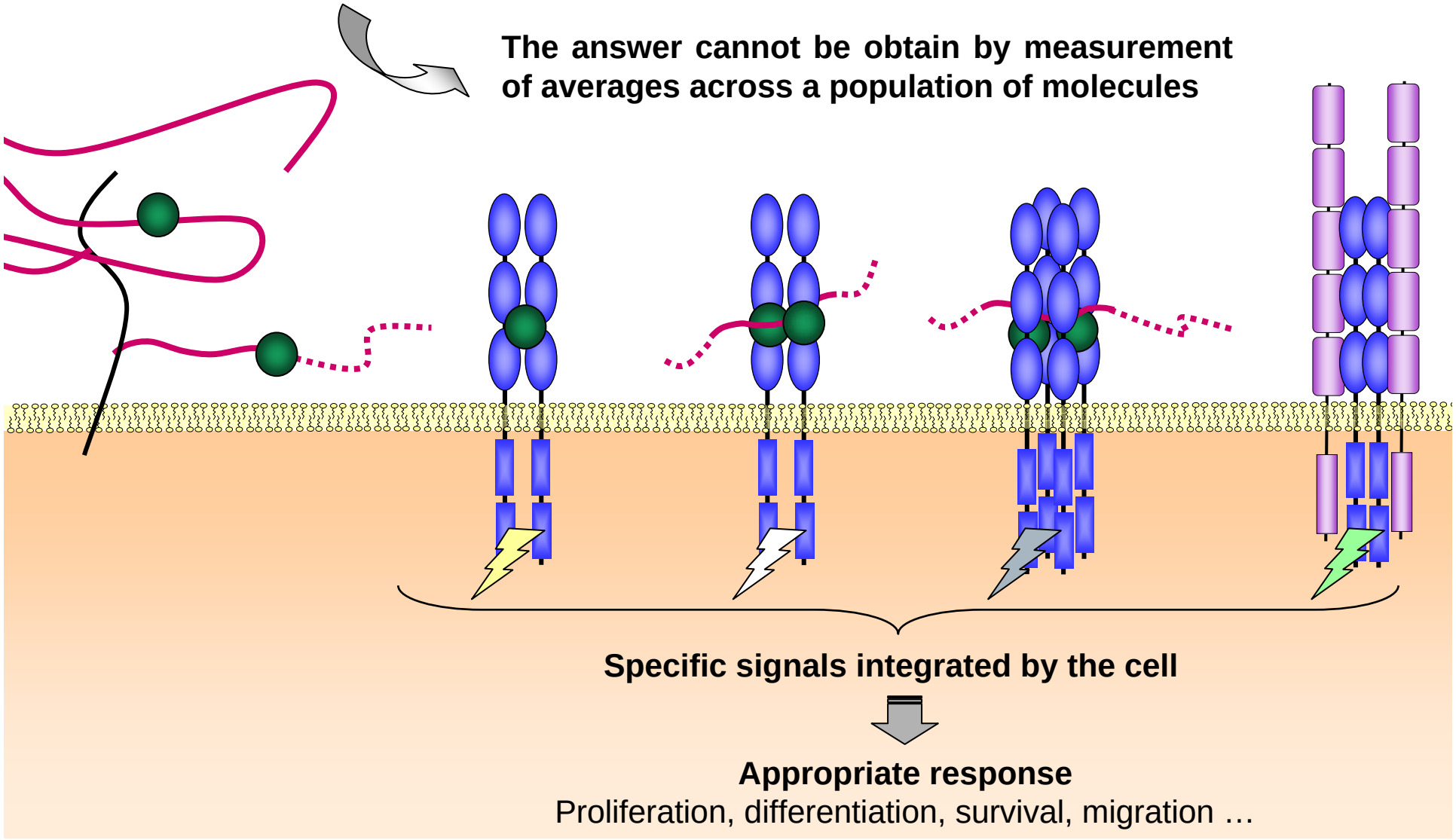
Appropriate response
Proliferation, differentiation, survival, migration …

FGF LIGAND-RECEPTOR SIGNALLING COMPLEX

Combinatorial assembly of signalling complexes

Molecules involved in the complex ? Stoichiometry ? Dynamic ?

The answer cannot be obtain by measurement of averages across a population of molecules



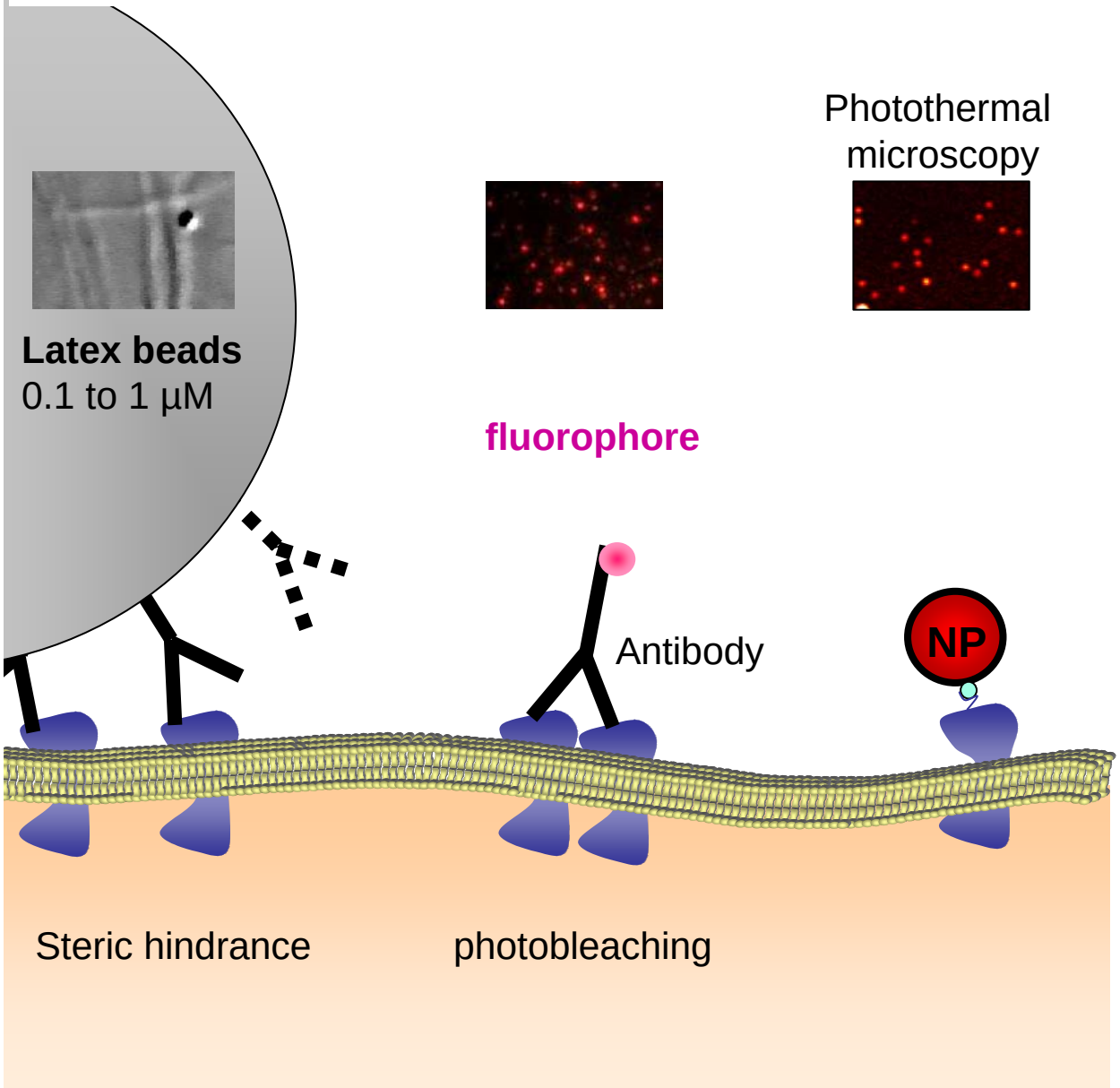
Specific signals integrated by the cell

Appropriate response

Proliferation, differentiation, survival, migration …

A- Single molecule detection with metal nanoparticles : Perfect label ?

Need to be able to detect / follow at a single molecule resolution in physiological conditions



Photothermal microscopy

B. Lounis's group (bordeaux)

Boyer D. *et al*., (2002),
Berciaud S. *et al*., (2004).
Lasne D. *et al*., (2006).

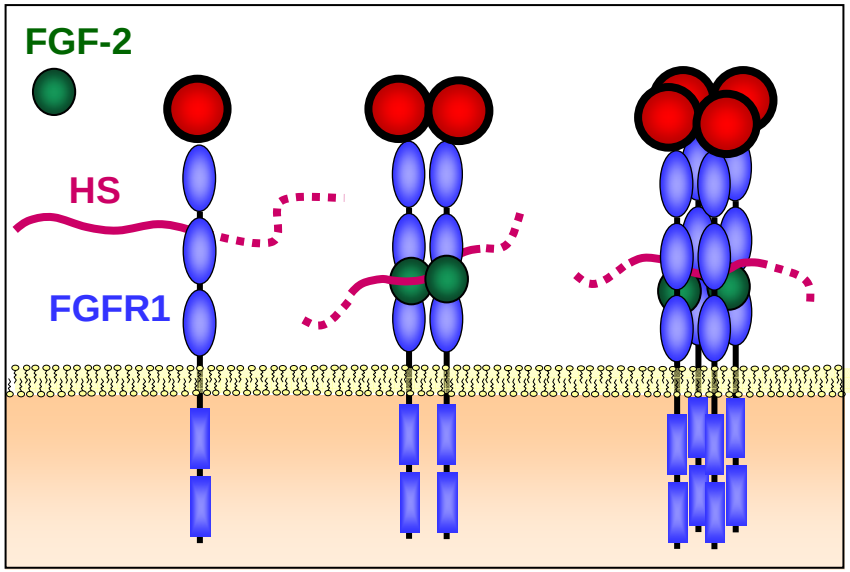
Detection & tracking of single metal NP as small as 2 nm

metal nanoparticles

Perfect label?

No photobleaching
No steric hindrance

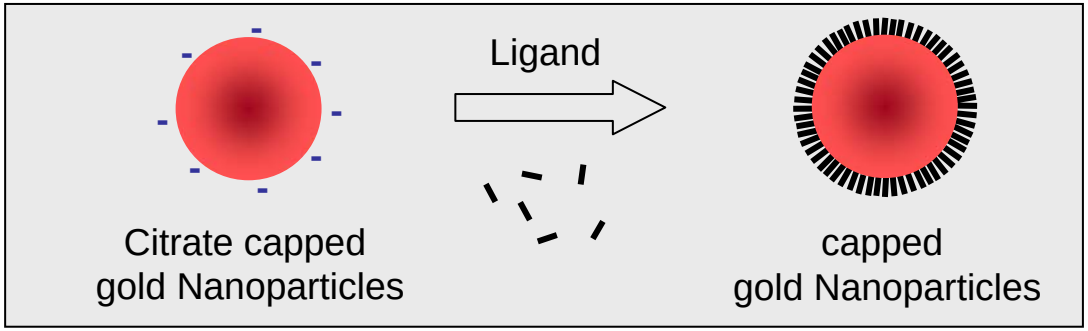
Thus, the possibility of linking such a powerful sensor to biological molecules provides a completely new perspective for the development of quantitative techniques to allow a wide range of applications, such as solving the stoichiometry and the dynamics of interaction of molecular assemblies.



B - Requirements to use metal nanoparticles to label proteins for quantitative analysis :

- **Soluble and highly stable in physiological condition**
- **No aspecific interaction, no ligand exchange**
- **Specific & stoichiometric attachment to the protein of interest (1 NP : 1 protein)**

1) Solubility & stability in physiological condition

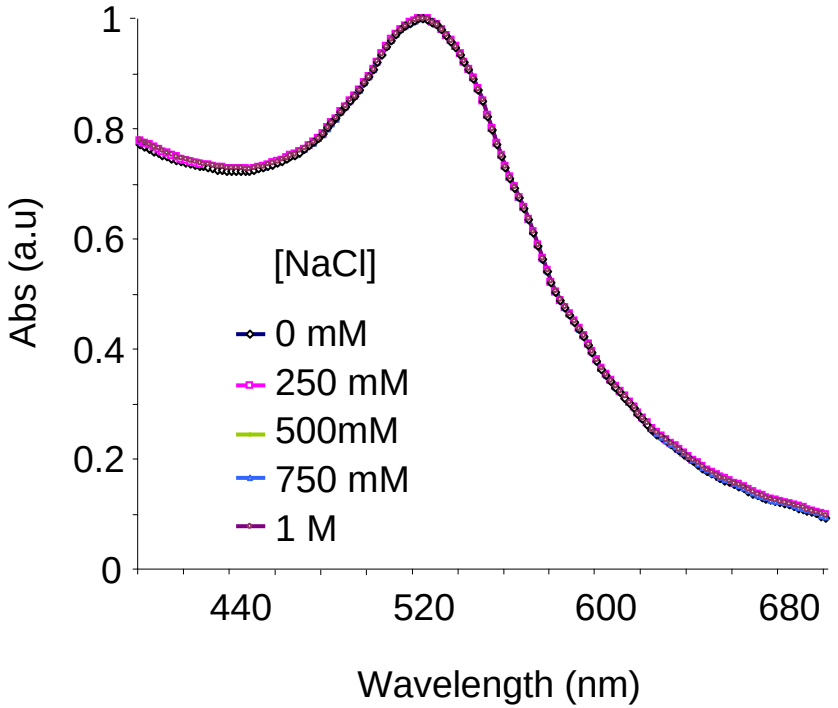


New ligand shell matrices

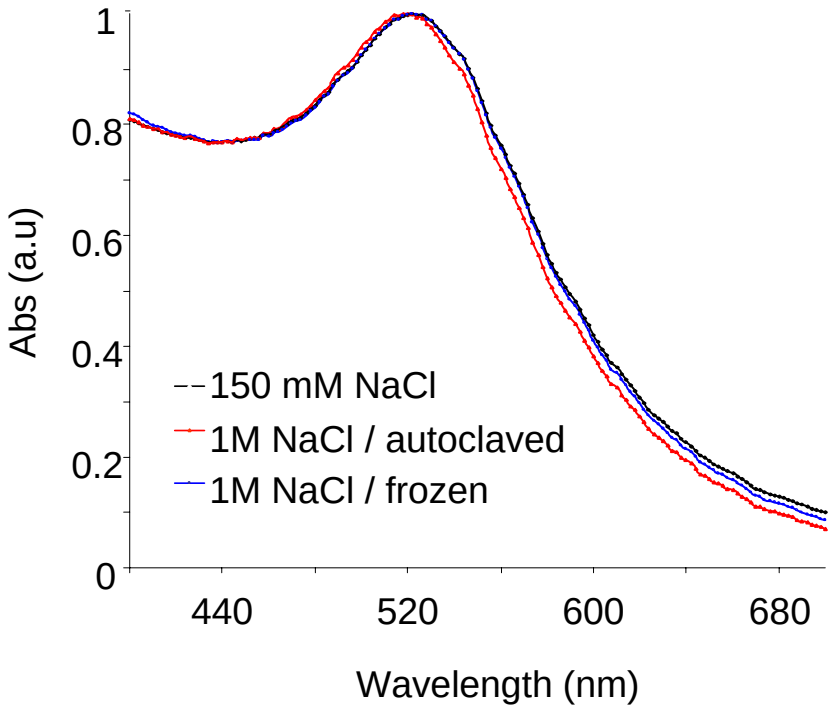
Duchesne et al., 2008 (in preparation), Awaiting patent filing

New ligand shell capped G-NPs

Stability in presence of NaCl

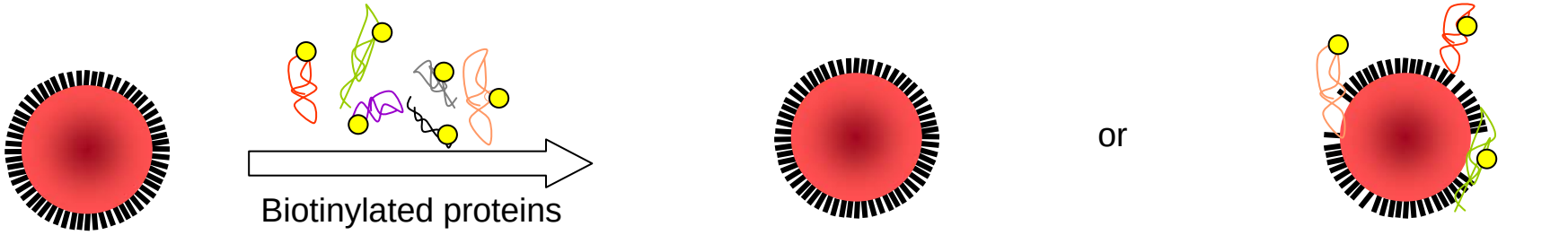


Stability in 1M NaCl following autoclaving and freezing treatment



⇨ New ligand shell capped NPs are soluble in physiological conditions and highly stable

2) No ligand exchange or aspecific binding



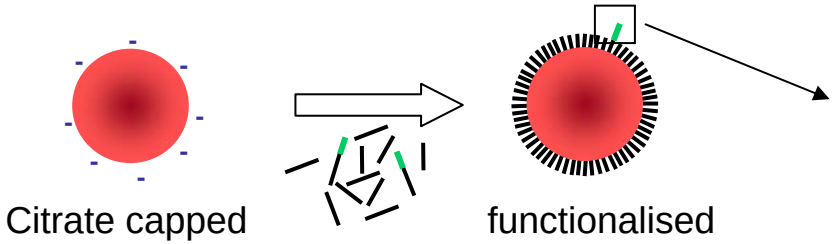
	Highly stable new ligand shell capped nanoparticles			Less stable capped NPs
Capped-NPs with	**CALNNxxxBiotin**	**CVVVTxxxBiotin**	**Biotinylated proteins**	**Biotinylated proteins**
Strepavidin- agarose →				

⇨ No ligand exchange or aspecific binding for new ligand shell capped NPs

3) Specific & stoichiometric attachment to the protein of interest

Easy to introduce a recognition function and to control its number on the surface of the NP

R. Levy *et al.*, 2005

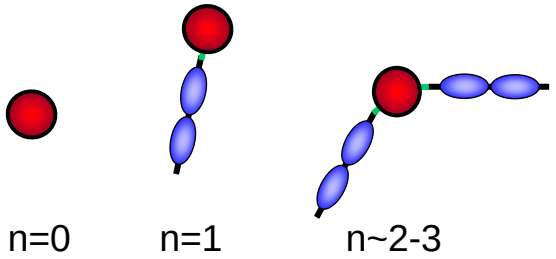


TrisNiNTA function : Affinity for polyhistidine tag

R. Tampé's group, Germany

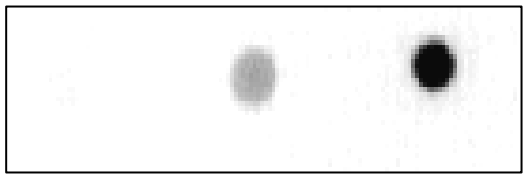
Lata S. *et al*., (2005).
Tinazli A. *et al*., (2005).

polyHis-FGFR1 + **NP-TrisNiNTA**



Same amount of NPs dotted onto the membrane

Detection of the FGFR

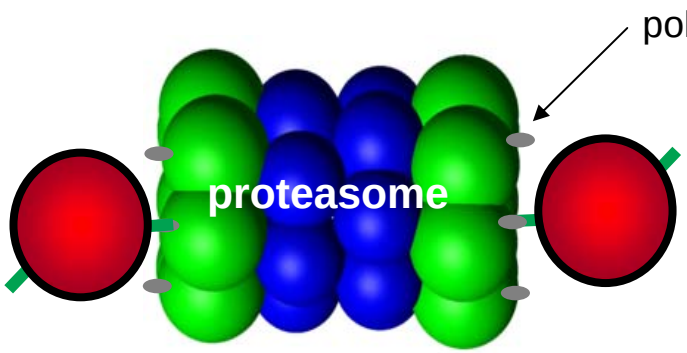


Dot-blot

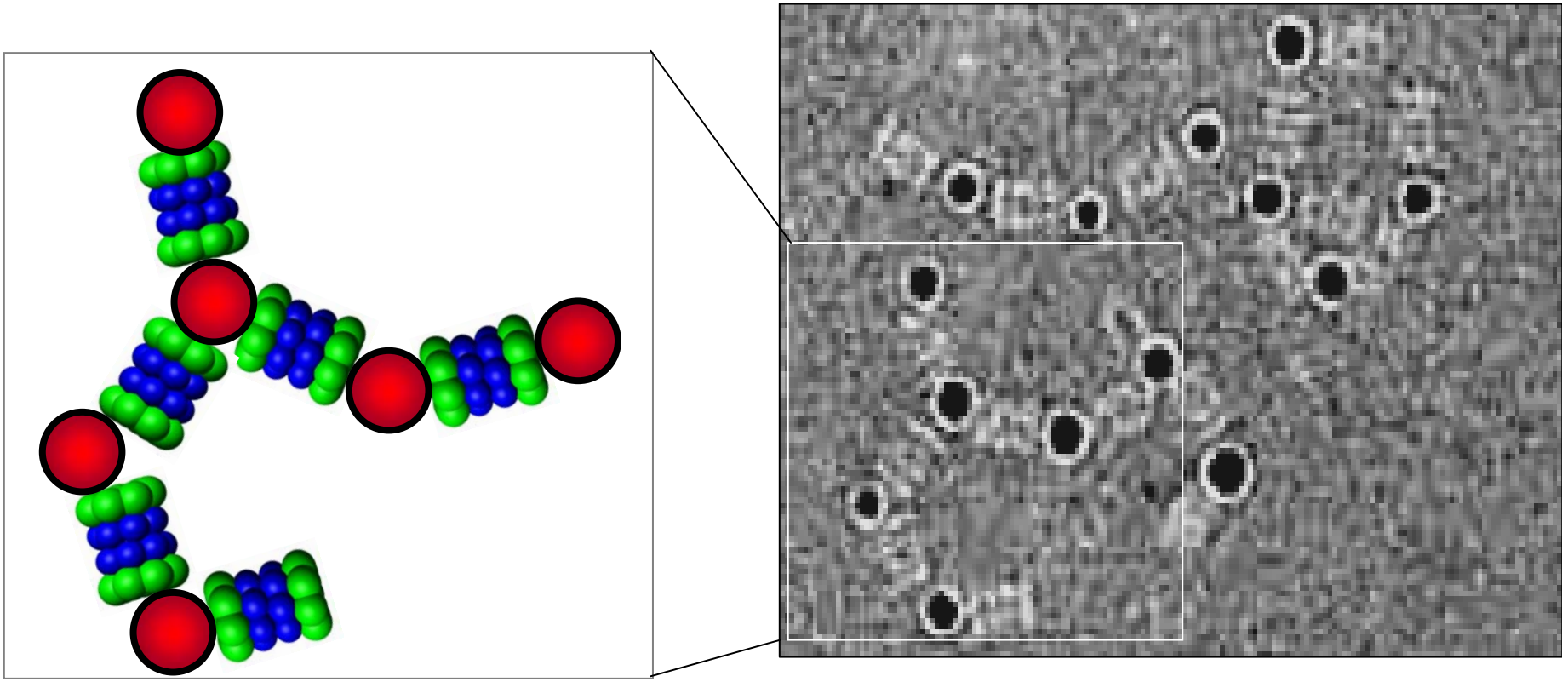
⇨ No aspecific binding of NPs (n=0) with polyHis-FGFR1

⇨ Specific binding of polyHis-FGFR1 to TrisNiNTA-NP n=1 and n~2-3

Specific labelling of polyHis tagged proteasome (collaboration with R. Tampé's group)



New ligand shell capped NP
n~2-3 TrisNiNTA

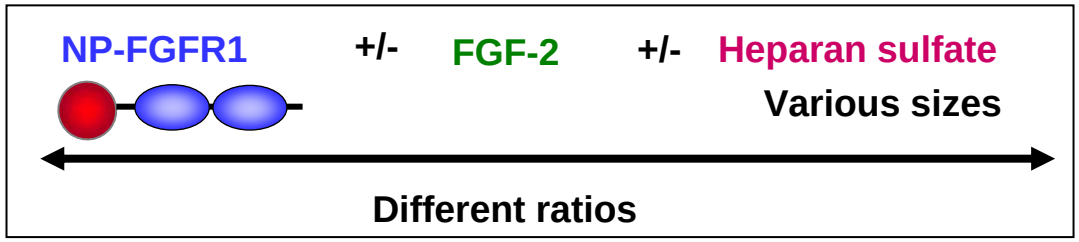


Electron microscopy picture, courtesy K. Schulze

APPLICATION : QUANTITATIVE IMAGING USING THE NEW LIGAND SHELL CAPPED NPs AND PHOTOTHERMAL MICROSCOPY

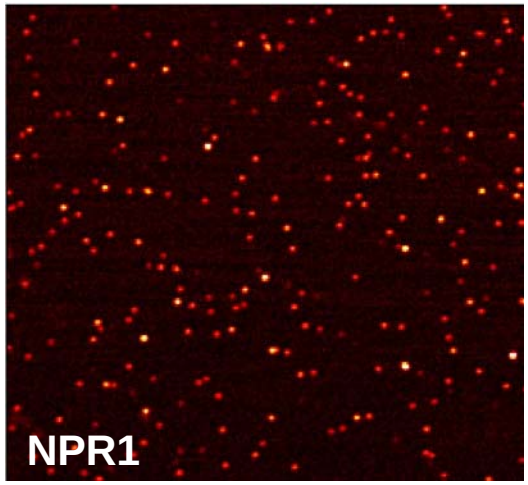
1) Stoichiometry of the FGFR1:FGF2:HS complex

B. Lounis's lab
V. Octeau, D. Lasne



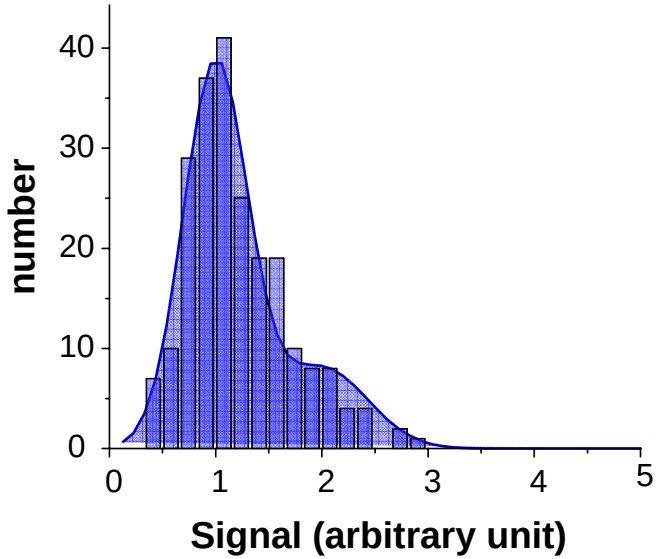
Photothermal imaging (LISNA)

2 nearby particles = 2x intensity + redshift



Signal distribution

NPR1



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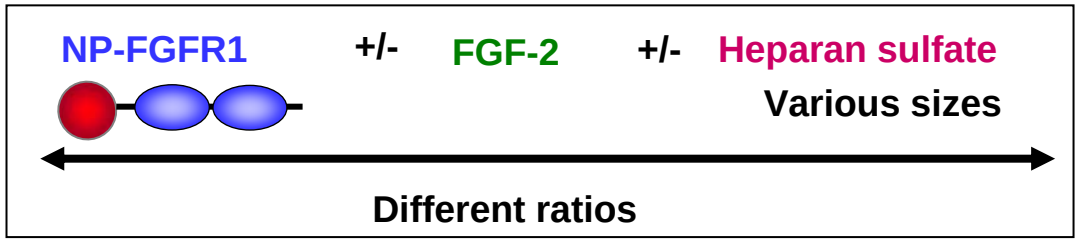

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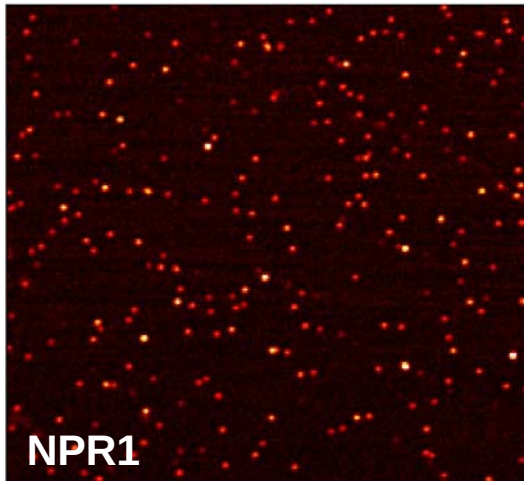
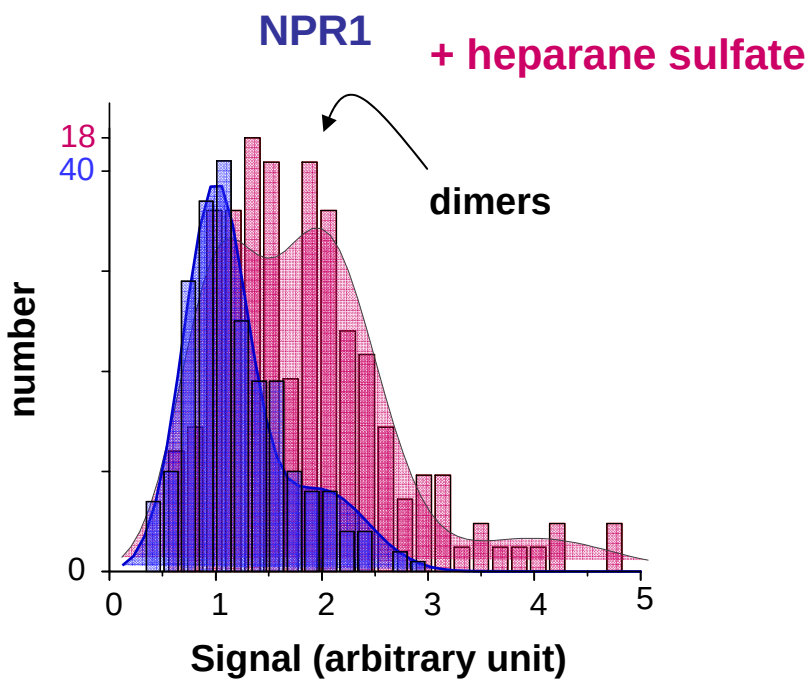
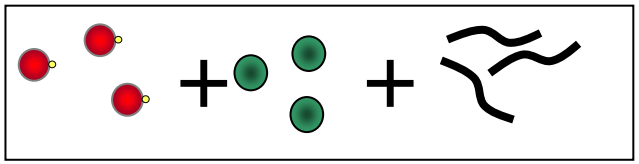


Signal distribution



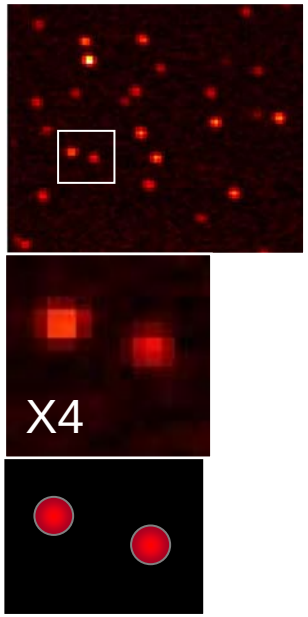
1) Stoichiometry of the FGFR1:FGF2:HS complex

NP n=1 TrisNiNTA + FGF + HS



1:1:1

Photothermal imaging (LISNA)



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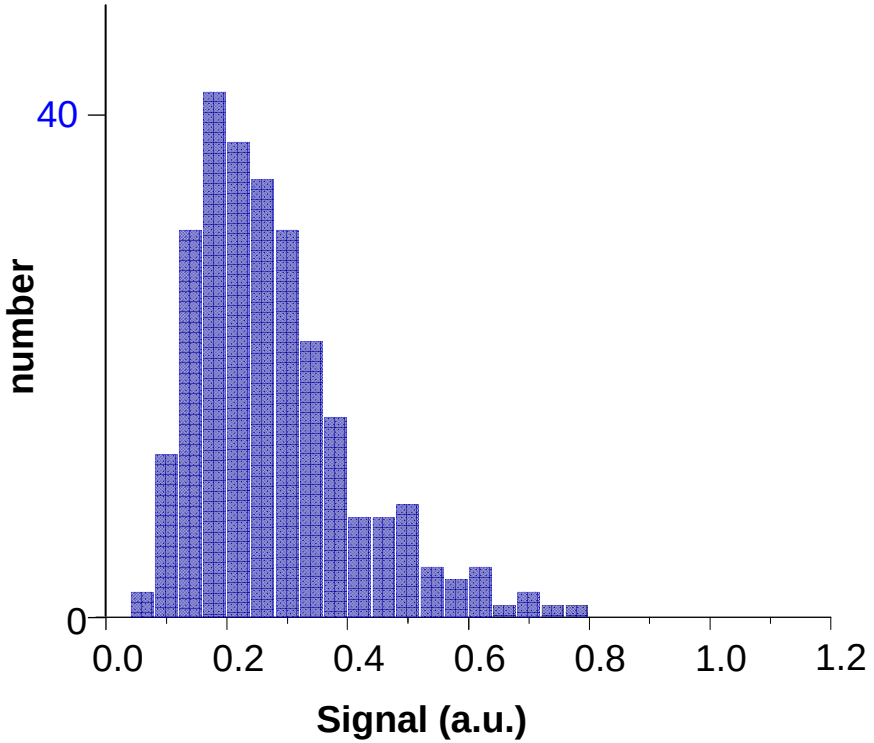

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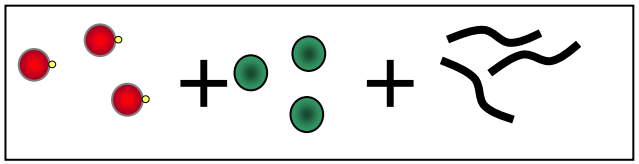

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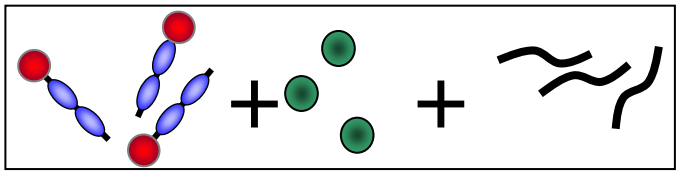
NP-FGFR1 + FGF + HS



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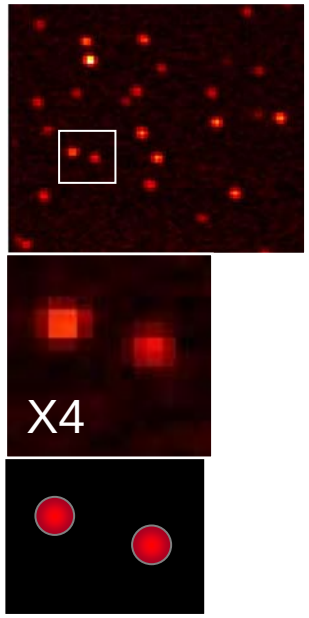
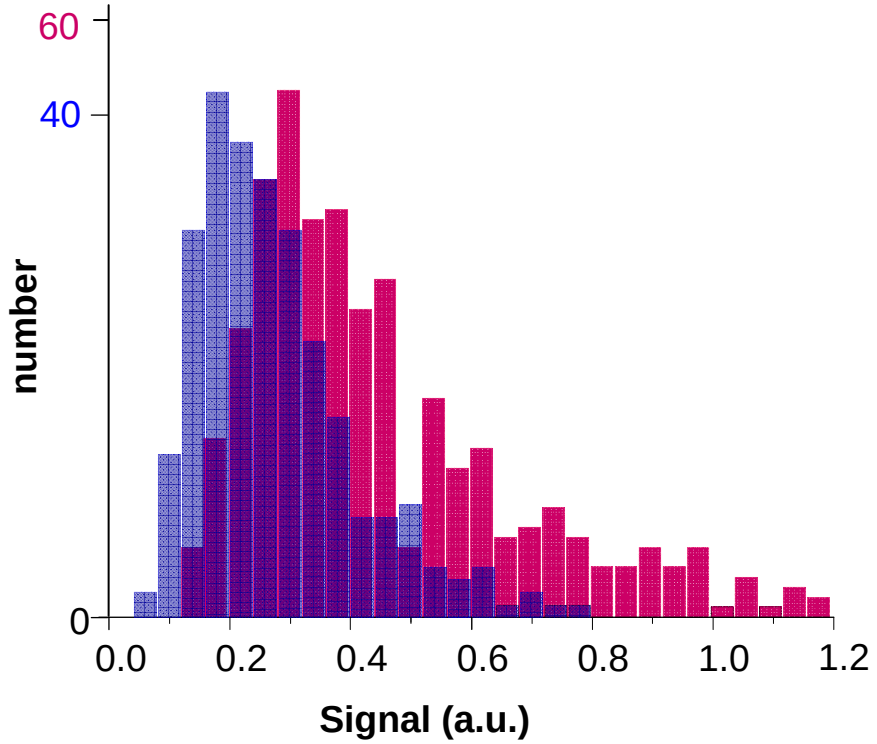


Signal distribution



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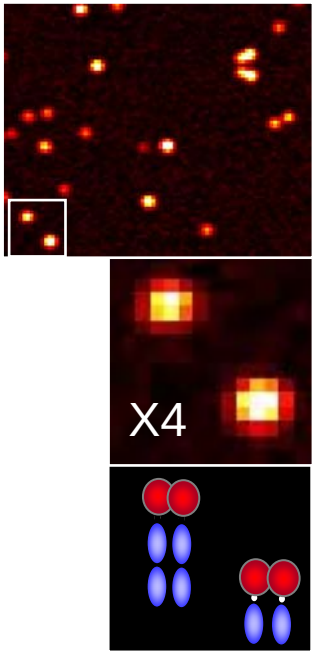
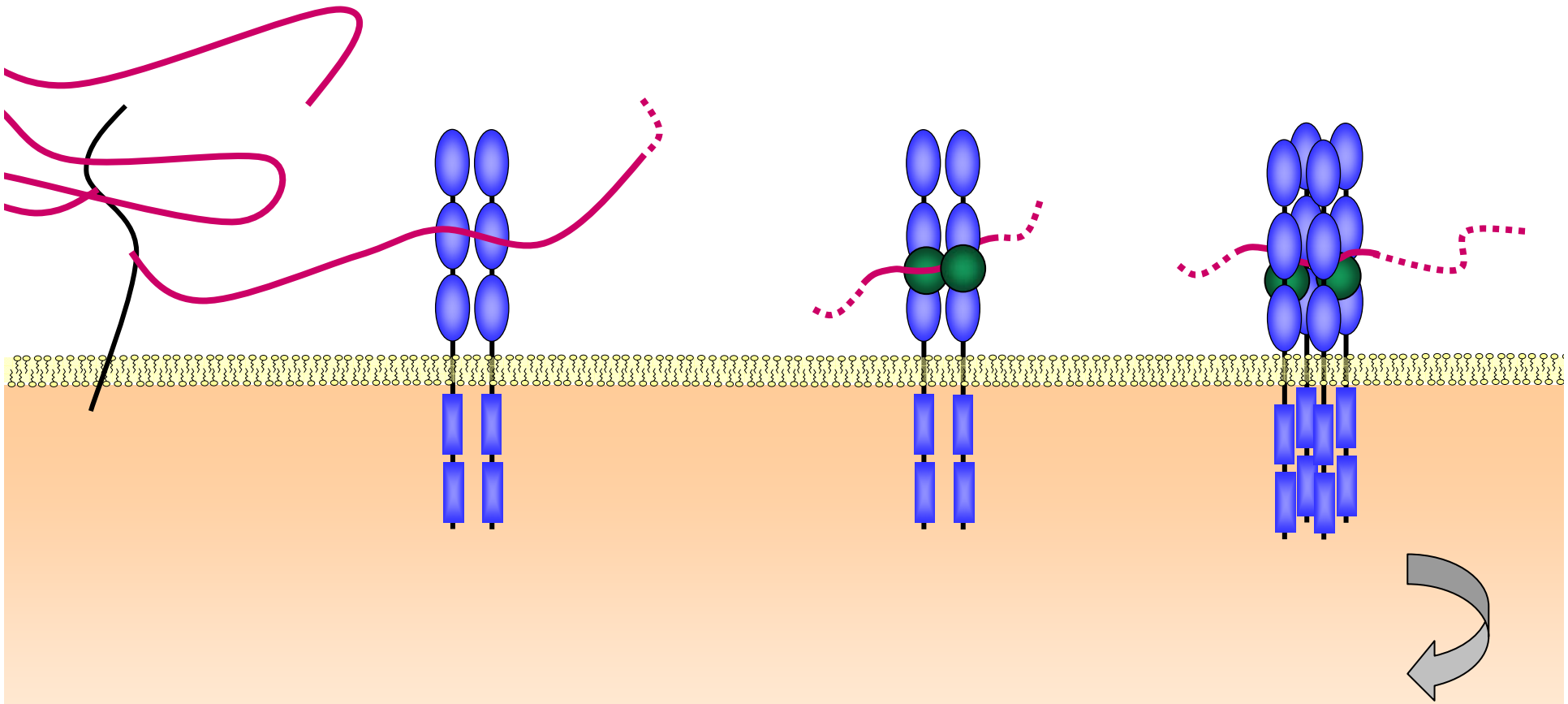


FGF LIGAND-RECEPTOR SIGNALLING COMPLEX

Novel insights into the assembly of the FGF ligand-receptor system will enable the elaboration of the first model of FGFR oligomerisation



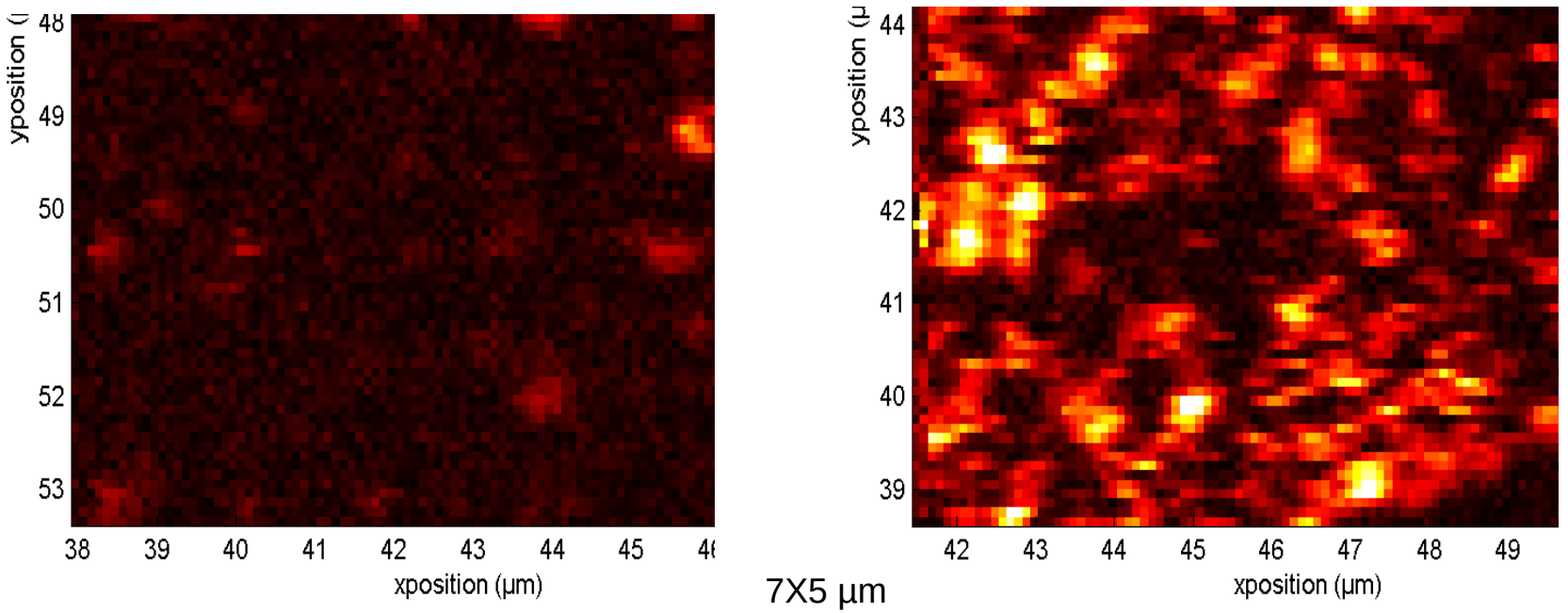
First evidence for higher order complexes (tetramers)

2) Photothermal imaging, in living cells, of membrane receptors

COS cells + NP-TrisNiNTA n=2-3

Non transfected

Transfected with 10xHis membrane protein



7X5 µm

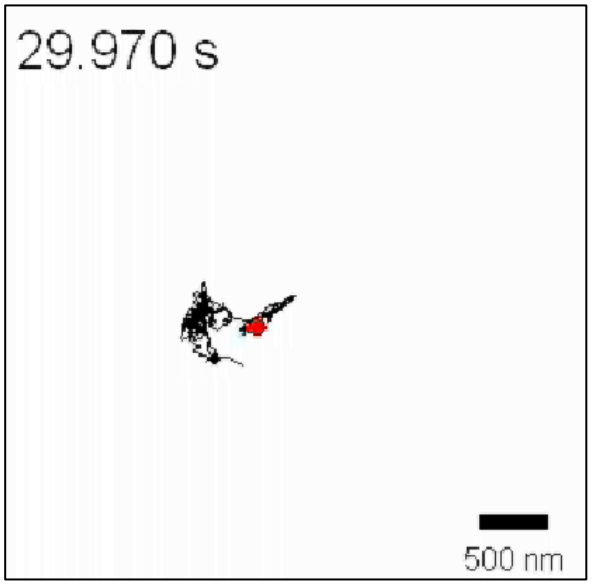
Collaboration with D. Choquet & G. Giannone, University of Bordeaux

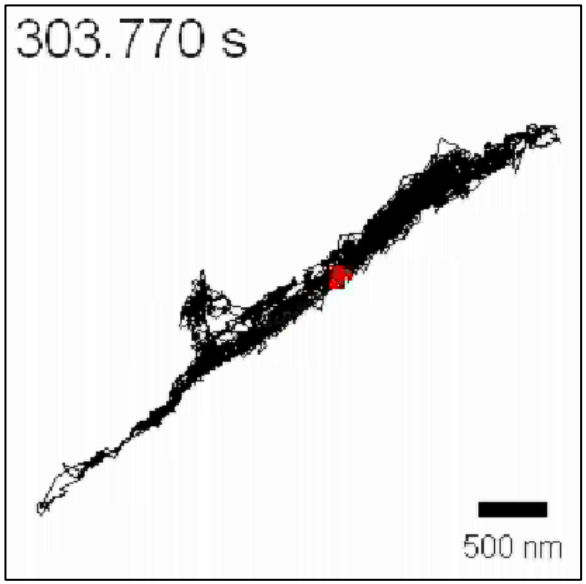
Picture courtesy of D. Choquet & G. Giannone

⇨ Validation of the use of TrisNiNTA new ligand shell capped NPs *in vivo*

Long-term dynamics are accessible

Nano “GPS”: single nanoparticle tracking





(SnapT)

5 nm NPs Coupled to IgG targeted against a membrane receptor (neurone)

Lasne D. *et al*., (2006).

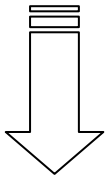
D) Conclusion:

The future is bright

New nanoparticle ligand shell extremely stable in biological environments

+

Harness the optical properties of NPs through photothermal microscopy



Resolution of the dynamics of complex biological molecular systems

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Centre for Nanoscale Science

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Robert Tampé
Katrin Schulze
Helge Groβmann

University of Leeds & Birmingham, UK
Sarah H. Harris
Geoff Wells

University of Hokkaido, Japan
Kazuyuki Sugahara

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