Table ronde MIFOBIO

Agents de contraste, sondes, biosenseurs, nanovecteurs, physique et chimie des fluorophores

Introduction:
Aucun objectif n'avait été fixé par MIFOBIO ...
La table ronde a tourné autour des différentes stratégies de marquage pour différents types de cibles, en essayant de bien mettre en relief les difficultés et avantages associées à chacune d'entre elles.

Programme détaillé des présentations (si des présentations étaient prévues)
Pas de présentation formelle.

Liste des participants (si très différents des inscrits du site ateliers Mifobio)
Une vingtaine de participants. Je ne peux pas dire si les personnes présentes étaient celles inscrites au départ.

Par thèmes abordés : principales conclusions
Voir ci-dessous les notes de la tables rondes

Conclusions générales et perspectives
Les problèmes de marquage sont importants pour un grand nombre d'utilisateurs. Certains peuvent avoir de fausses idées / connaissances incomplètes, ce qui peut mener à des échecs dans les manips qu'on aurait pu éviter. Conclusion : poursuivre ou intensifier cet aspect de la microscopie dans les futures éditions MIFOBIO !

Discussion Notes de la table ronde
Uses :
Protéines fluorescentes (60%)
Molécules chimiques (20%)
NP & QDots (10%)

La somme est > 100

Halo-tags & tandems halo-tags : pb marquages incomplets
Marquages de Protéines : c'est surtout les PFs ?
How about lipids, sugars, nucleic acids ? We still need organic dyes

FAST similar to DNA Paint

Peptidoglycan of bacteria (Streptoccocus) tagged by click chemistry with a modified amino-acid ?

Color multiplexing
Getting more information

FPs not compatible with primary cells and tissues
Organoids are very difficult to perfuse with probes
We need to work with living systems, go to *in vivo*.

Weak interactions and functionality, transient dynamics is important

Super-resolution has also tracking possibilities
Dynamic of the structure?

If any biological material is used, it is a biosensor?
If it senses a biological analyte also?

Biomarker vs biosensor: difference is when we quantify?
SR still mostly restricted to fixed samples
Antibodies and nanobodies, aptamers can be used in live cells but?
Nanobodies can be expressed but you still have to label them
Use toxins to internalize? Tricky...

Unnatural aa, appeared in 2000, and now it’s becoming more easy
Artificial systems for protein synthesis do exist. We should invite someone.

Halo tags? Halo-tag is bigger than FPs
SNAP tag OK should be more specific than Halo tags de
CLIP-tag forget.

The solution to avoid background is fluorogenic agent (needs more than x100 contrast)
FAST: protonation changes in addition to fluorescence enhancement. Be careful about the excitation wavelength. S/N is very good. 2 labelings are possible

Pb: brightness of FAST is average, and lower than good FPs and organic dyes.

Ulrike made a review about comparing probes

FIAsh and ReAsh react non-specifically on thiols

Going red will require bigger probes AND bigger proteins?

Carsten Schulz, Kai Johnsson use unnatural aa

Good protocols to handle chemical probes are critical (solubility protocols)
Biologist users need kits

What about detecting nucleic acids? MS2 is a protein to detect DNA loops: 30 copies are needed
CPPs to deliver probes in cells?

Molecular evolution or directed evolution to find peptides that carried Malachite green inside the cell.

Why not use combinatorial chemistry to find probes that target a single protein directly in the cell?

Spinach: similar to FAST but for nucleic acids.

Labeling lipids is much easier. There are molecules for rafts and change color in ordered vs disordered phase. NR12S. Not good photostability for super-resolution, and not bright enough.

We need to label many different proteins of different kinds. FP requires checking good localization of the FP constructs, absence of impairment of function, interactions...

How can it remain functional with such a big stuff on it?

There are fluorescent molecules to detect NO

FAST would be of interest if the probe brings a new function eg fluorogenicity, ON/OFF switching, infinite signal, photoactivation or inactivation

Requirements for photophysics: brightness, photostability,

Detection of low copy numbers. We can detect single molecules. While shining very low light.

There are efforts in developing chemical evolution to get specificity, and to develop efficient intracellular delivery

What chemists should develop is an equivalent of GFP in terms of specific targeting, but 100x brighter.

QDots are no better than 20 rhodamines and bleach ultimately ???

Develop collaborations between chemists and biologists, same for physicists. Difficult because biologists are not always available.

We need to incorporate the whole spectrum in a single team/lab

Use the biosensor list to propose new probes and technologies.