Laboratory of Functional Chemo-Systems

Breaking new ground in the fields of *in cell* and *in vivo* synthetic chemistry

UNIVERSITÉ DE STRASBOURG

FRC International Center for Frontier Research in Chemistry

medalis

cnrs

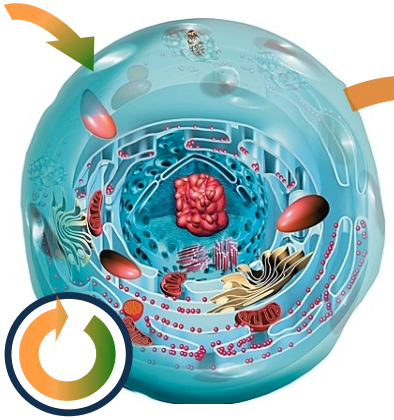
For decades, chemists have considered chemistry as a mean to obtain molecules. At LFCS we view chemistry and chemical reactions more as a way to directly interact with biological systems and living organisms.

To what extent is the wide repertoire of chemical reactions usable in biological media ?

- Define reaction's utility profile
- Define the bio-compatibility criteria

Bioconjugation
Native ligation
Edman degradation
DNA templated reactions
O-mesitylenesulfonylhydroxylamine (MSH)
Cys ➔dehydro-Ala

Bio-selective Reactions



Biothiol turn-on FRET probes
Acido-labile functions
ROS sensitive probes

Bio-responsive reactions

Bio-orthogonal reactions

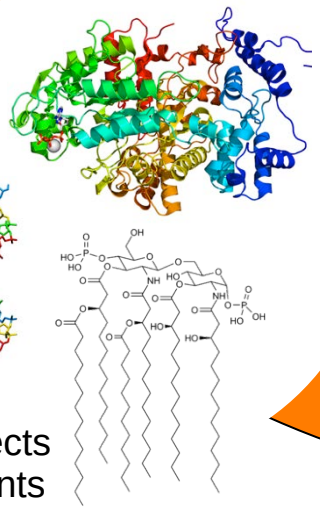
CuCAAC – SPAAC
Pd Catalyzed C-C coupling
Photo-release
Dithionite-triggered azo cleavage

➢ Define the bio-compatibility criteria

Stratifying the Chemical Biology Complexity

Amino acids
Nucleosides
Metabolites
37°C buffer

2900 endogenous metabolites
> 10.000 dynamic range
78 organs
600 muscles
Infinity of pathologic states



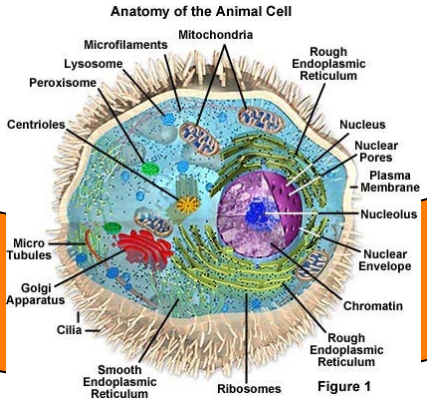
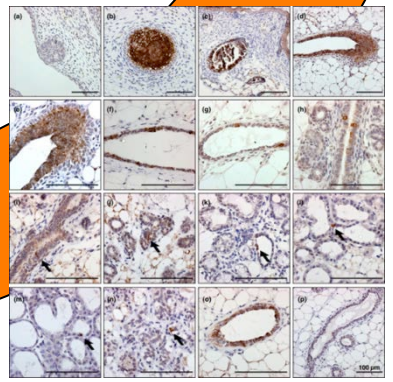


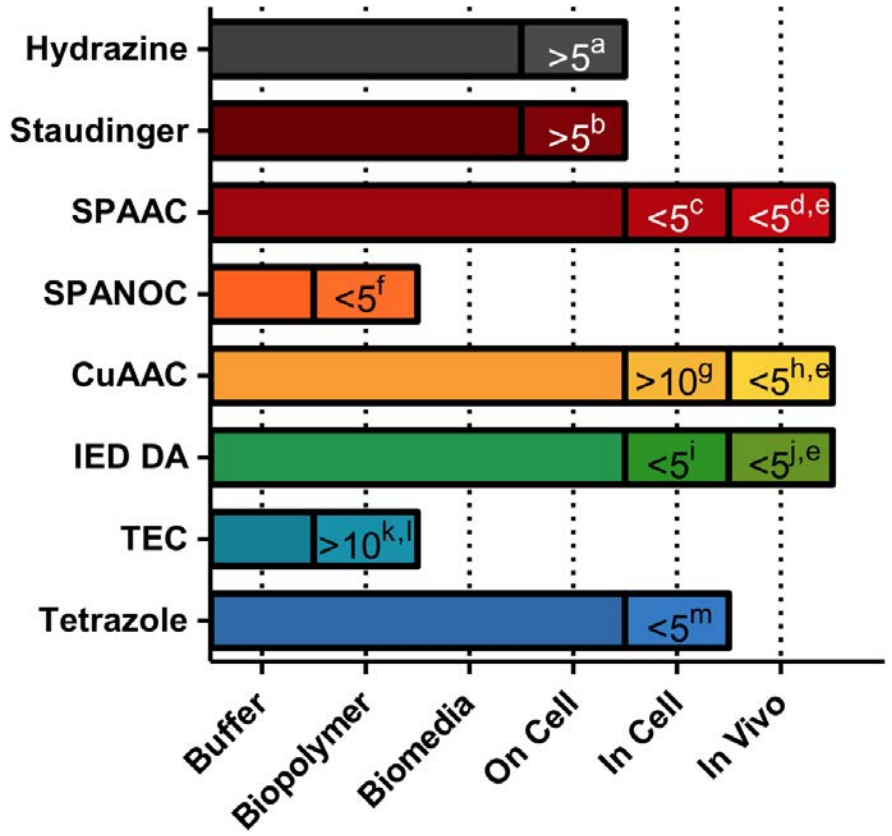
Figure 1



Cooperative effects
Microenvironments
Diffusion and kinetic
Recognition

Evolving media
Compartmentalization, Coexistence
Transport
200 distinct tissues; 10 development states
20.000 genes; 170.000 gene interactions
6.000 chemical reactions, 1.500 signaling pathways

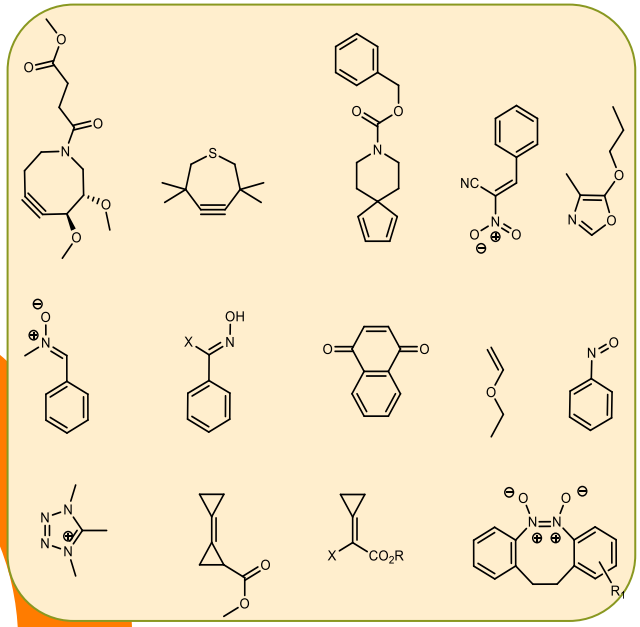
Bibliographic survey on bio-orthogonal bond-forming "click" reactions uses



Bio-orthogonal reaction development strategy

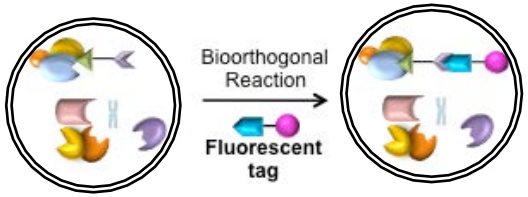
Primary screening

HPLC Assay			Fluorescence assay	
SpAAC			CuAAC	
< 40%	41-70%	>70%	< 50%	> 50%
Conversion at 1h			Conversion at 10min	



Reactivity survey

Prospective applications



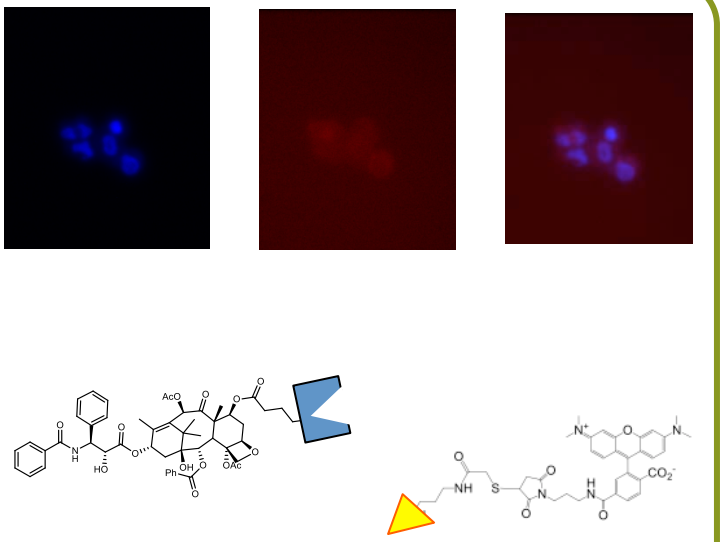
Target fishing

Drug tracking

In vivo chemistry

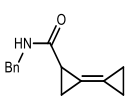
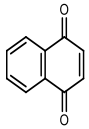
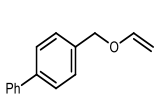
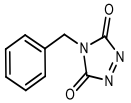
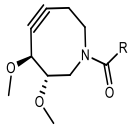
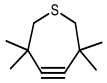
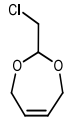
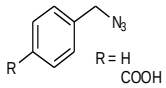
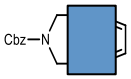
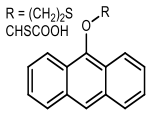
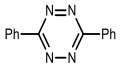
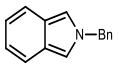
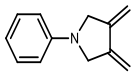
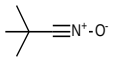
Biosynthetic macromolecules

Material sciences



Living cell validation

catalysis, reagents, ...bond breaking

Reagent							
	< 1 %	2 %	10 %		51 %	> 70%	2 %
	5%	17 %	< 1 %	13 %	10 %	> 99 %	1 %
	< 1%	11%	< 1%	5 %	2 %	< 1 %	28 %
	10 %	4 %	22 %		< 1 %	17 %	23 %
	64 %	69 %	18 %		15 %	< 1 %	1 %
	< 1%	7 %	8 %		21 %	20 %	14 %
	16 %	16 %	8 %		< 1 %	> 99 %	7 %
	5 %	73 %	< 1%		32 %	42 %	18 %

> 80 %

71-80%

61-70%

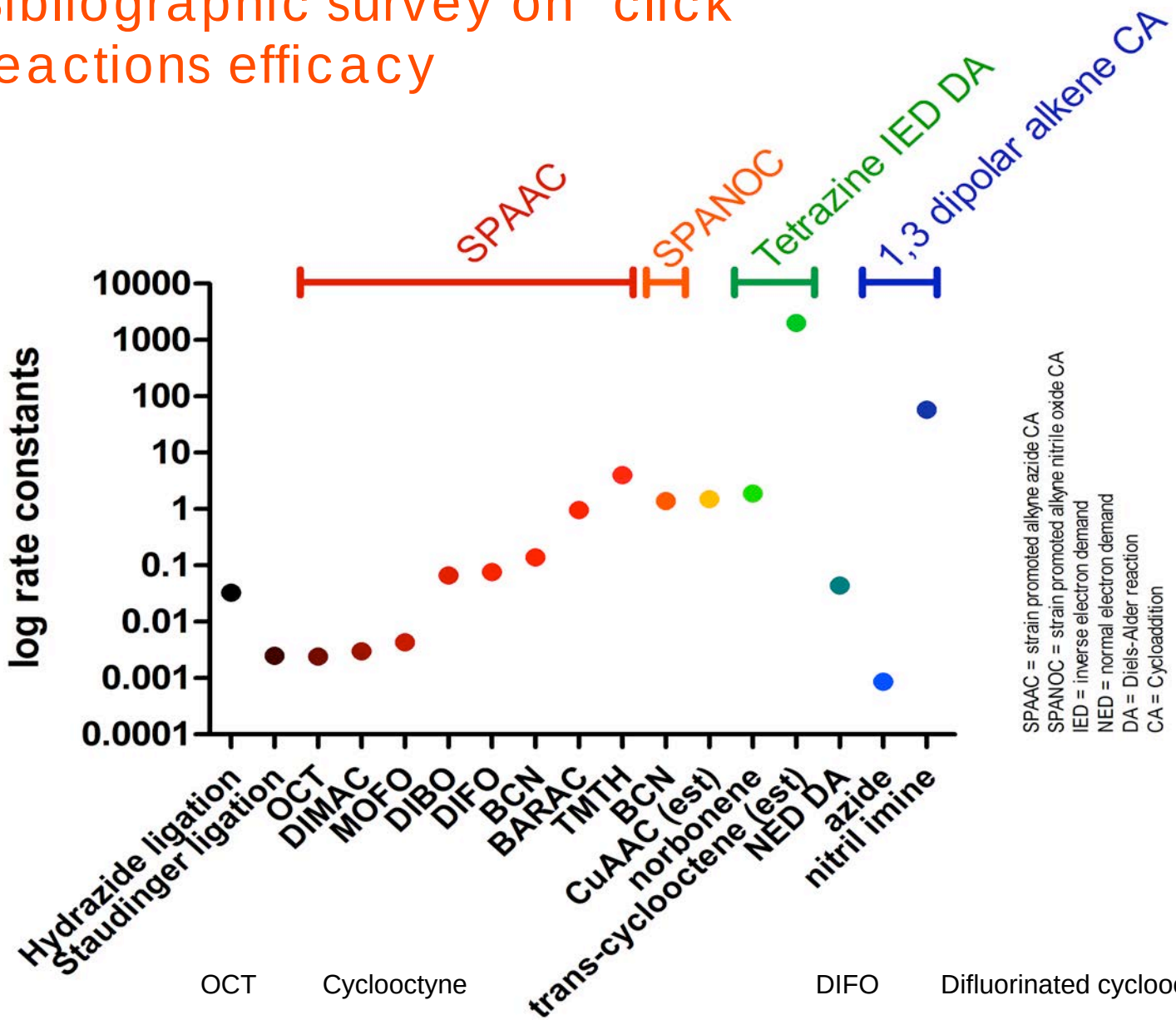
31-60%

0-30%

Penging more investigation

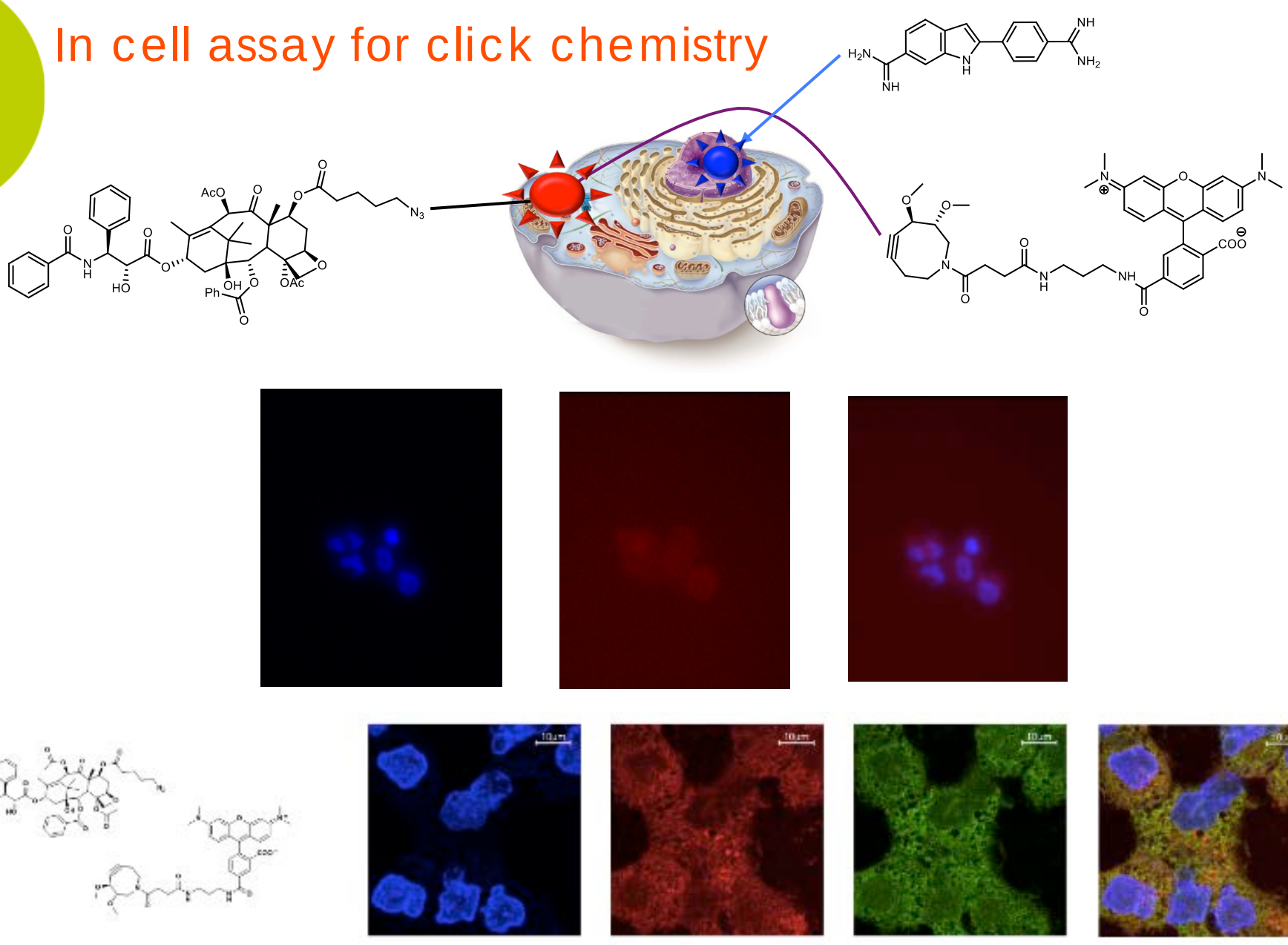
6.25 mM; 25°C; t = 1h; PBS/DMSO 6:4;

Bibliographic survey on "click" reactions efficacy

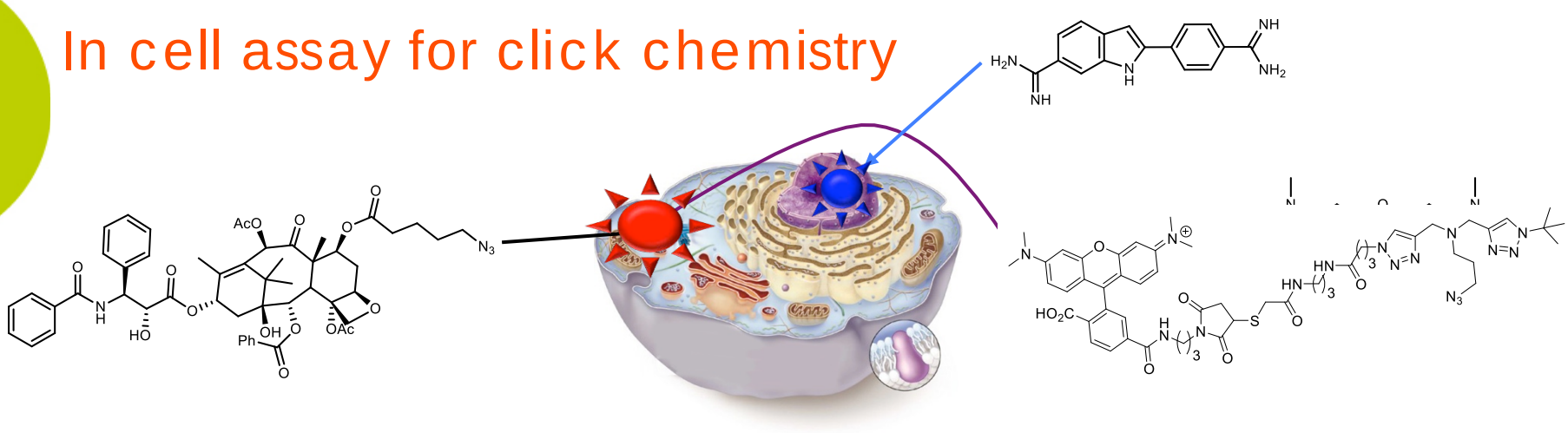


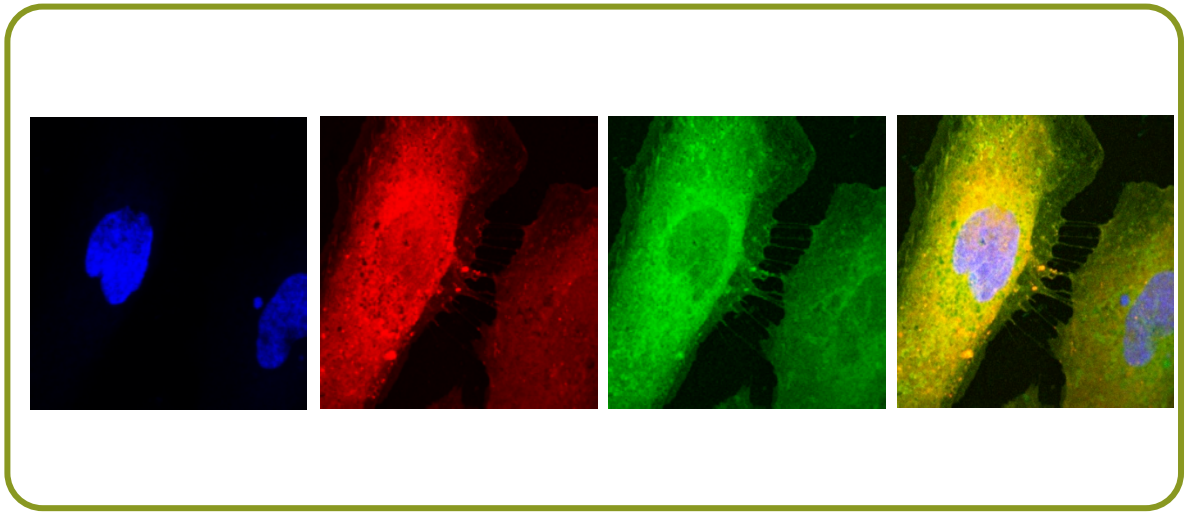
OCT	Cyclooctyne	DIFO	Difluorinated cyclooctyne
DIMAC	6,7-dimethoxyazacyclooct-4-yne	BCN	Bicyclononyne
MOFO	Monofluorinated cyclooctyne	BARAC	Biarylazacyclooctynone
DIBO	Dibenzocyclooctyne	TMTH	Tetramethylthiaheptyne

In cell assay for click chemistry



In cell assay for click chemistry

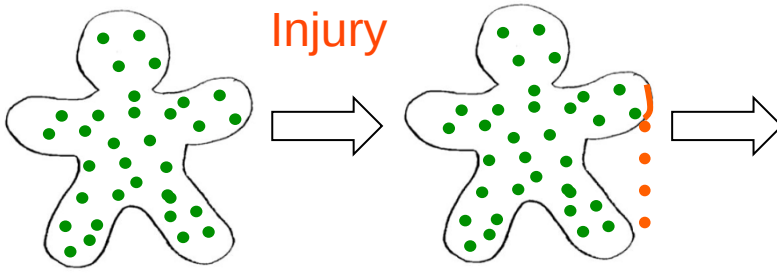




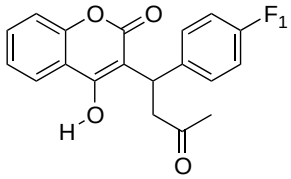
Living cell validation

Collaboration Dr. Frédéric Taran / CEA Saclay

Switch-off drug's bioactivity *in vivo* or trigger fast drug clearance

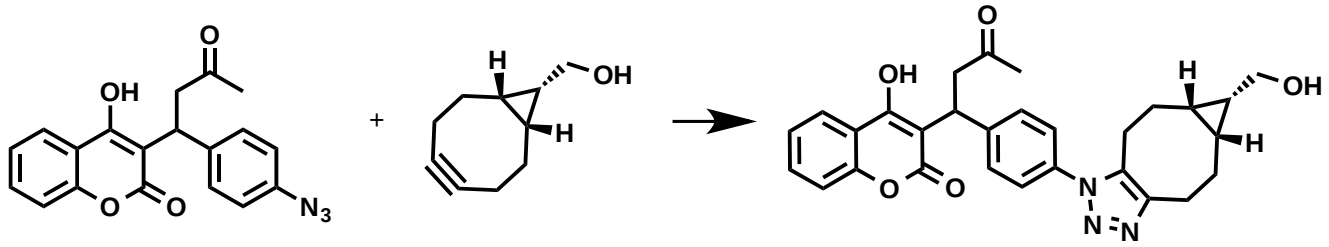


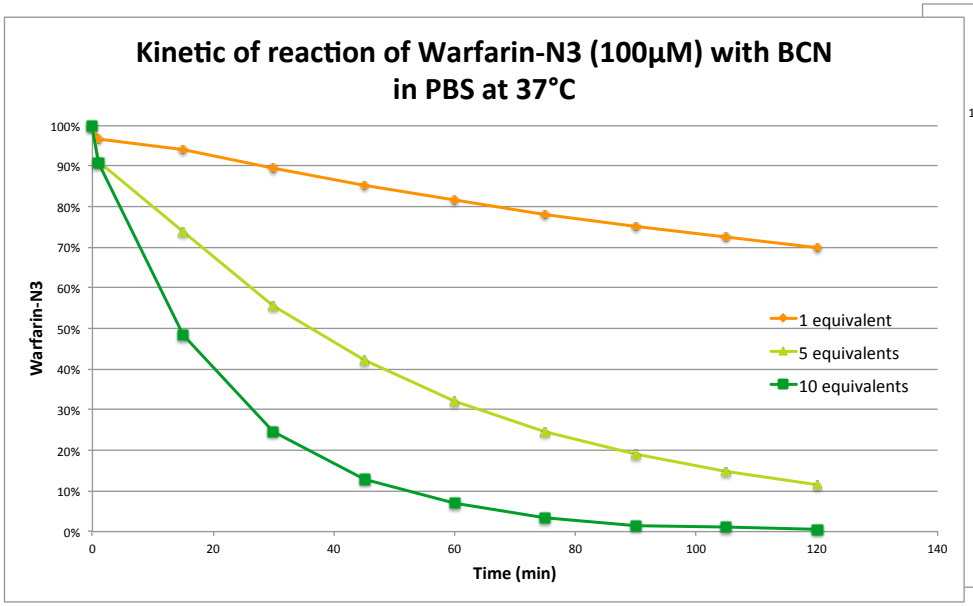
Risks of hemorrhagic accidents caused by anticoagulants of the class of the antivitamin K (AVK), who in France is among the main cause of iatrogenic accidents causing hospitalization.

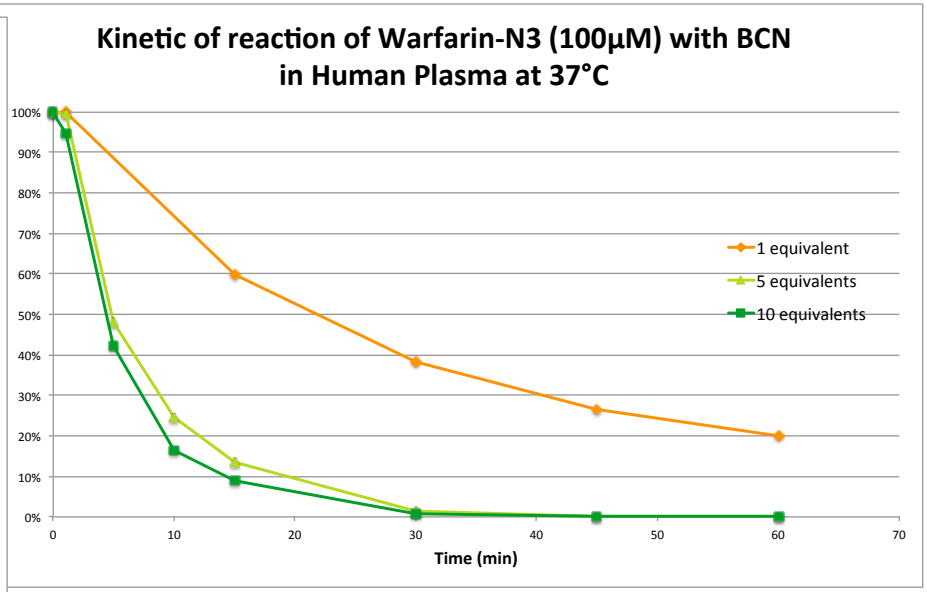


Coumaphène
Warfarine

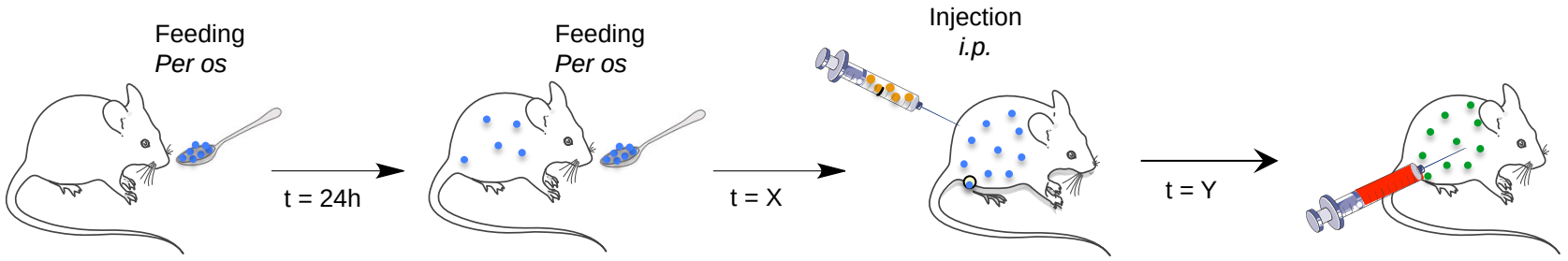
Kinetics of Warfarin/BCN click

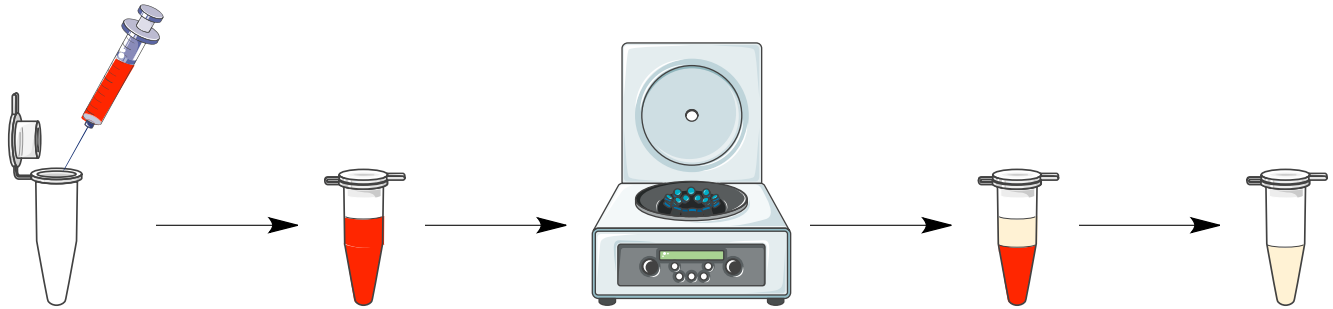


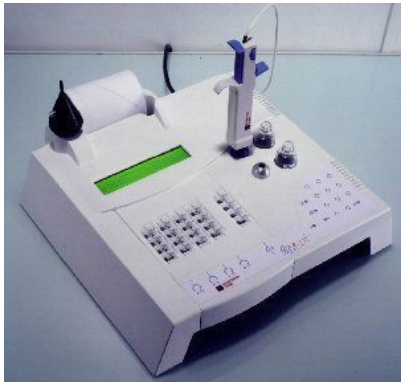




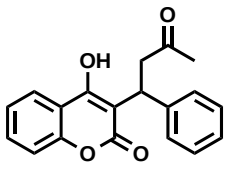
In-vivo assay of the activity of Warfarin derivatives

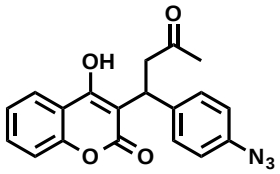


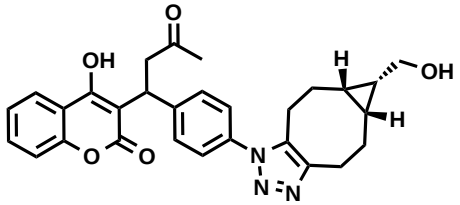


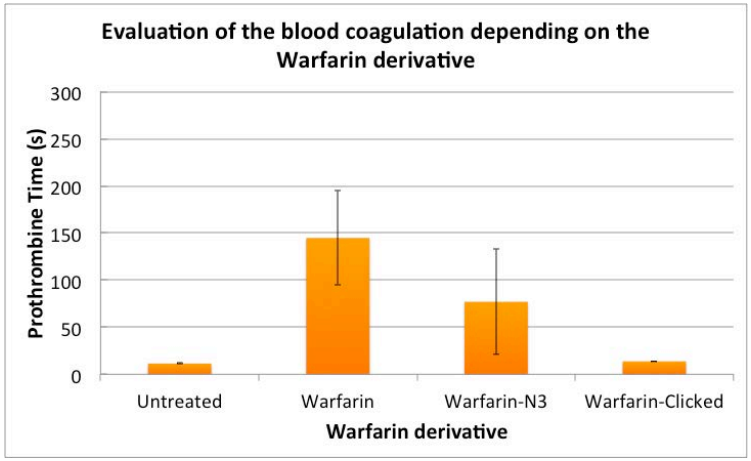


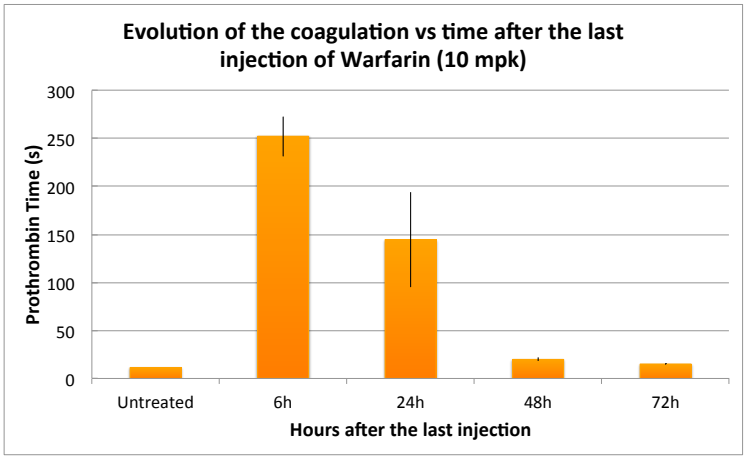
Activity of Warfarin derivatives and *in vivo* click

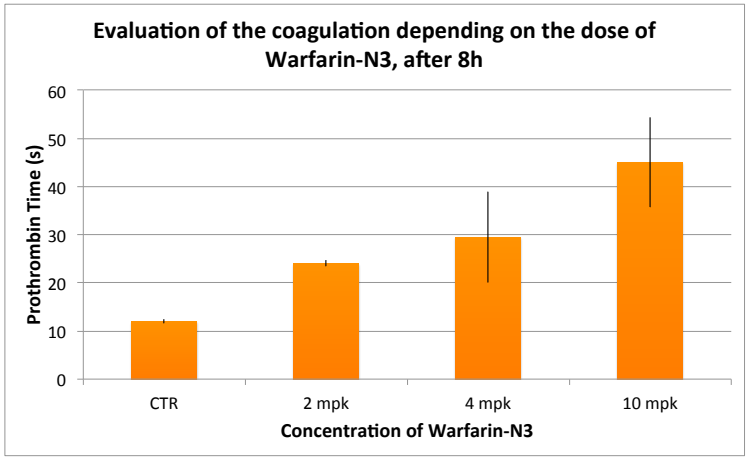


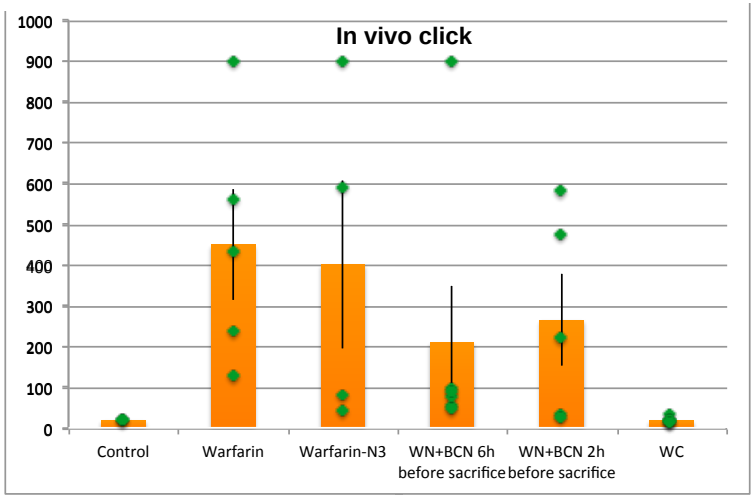












Metabolomic HRMS analysis

Protocols

Day 1, 9 am : Mice fed with their respective warfarin derivative (2 mpk)

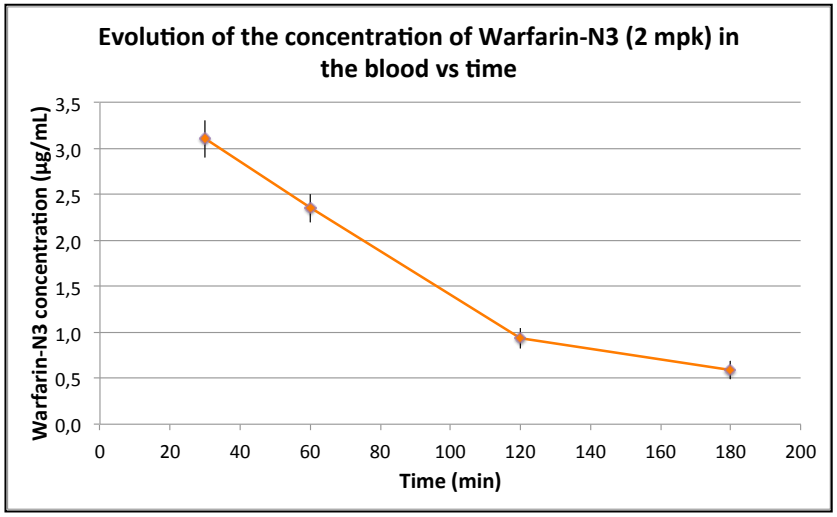
Day 2, 8:45 am : First collection of blood

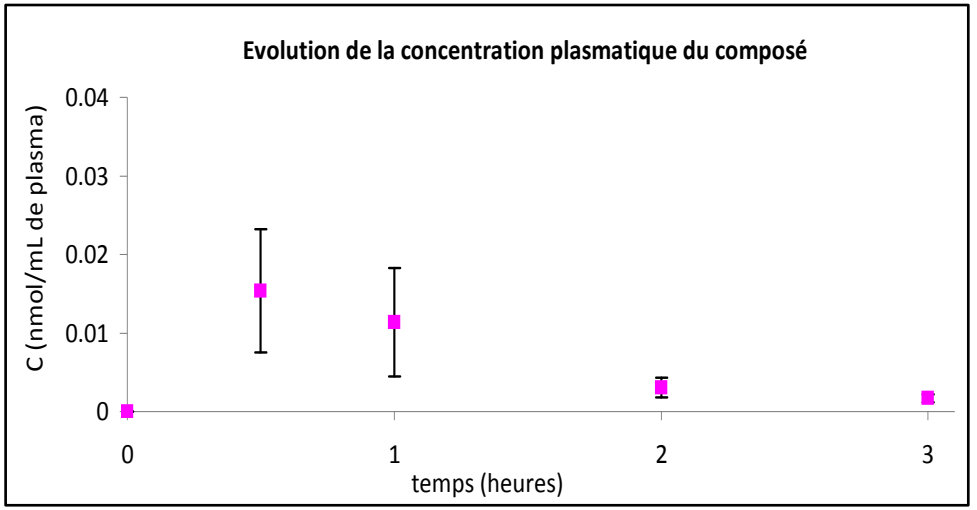
Day 2, 9 am : Mice fed with their respective warfarin derivative (2 mpk)

Day 2, 9:30 : Collection of blood 30 min, 1h, 2h and 3h after the injection

No detection of BCN in blood (MS ionization suppression)

PK of Warfarin-N3 and Clicked Warfarin (calibration curve: R^2=0,997)

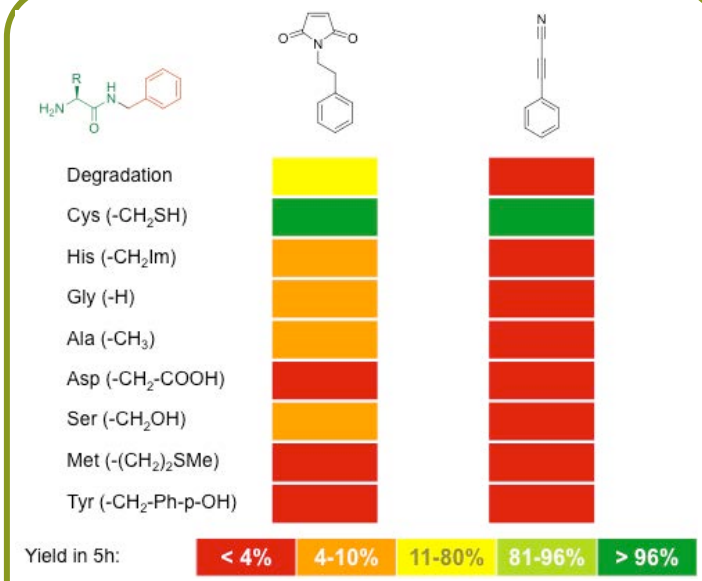




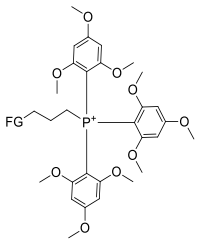
No warfarin-N3 before the second injection

Bio-selectivity profiling

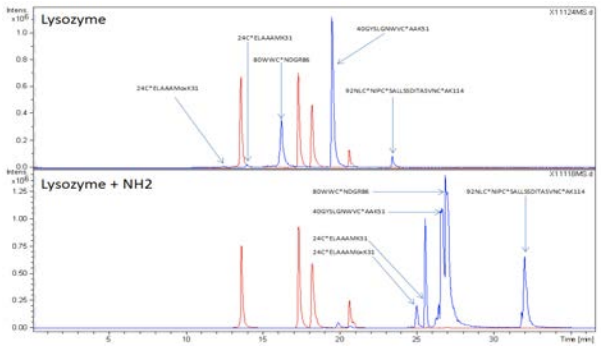
Primary screening



Functional validation

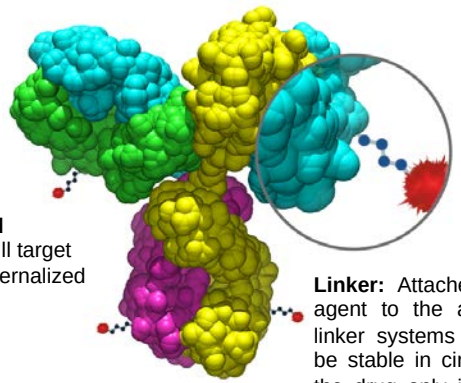


1 MRSLLILVLC FLPLAALGKV FG**R**CELAAAM **KR**HGLDNY**R**G YSLGNWVCAA
51 **K**FESNFNTQA TN**R**NTDGSTD YGILQINS**R**W WCNDG**R**TPGS **R**NLCNIPCSA
101 LLSSDITASV NCA**KK**IVSDG NGMNAWVAW**R** N**RCK**GTDVQA WI**R**GC**R**L



Prospective applications

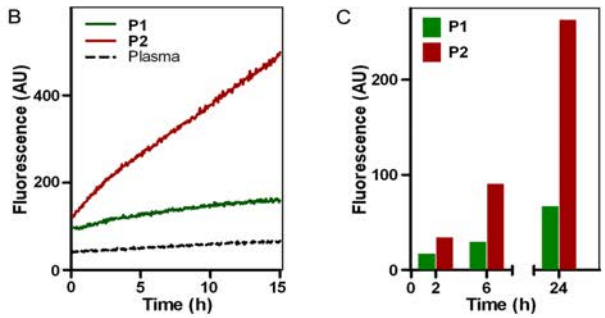
Antibody: Specific for a tumor-associated antigen that has restricted expression on normal cells.



Drug Payload
Designed to kill target Cells when internalized and released.

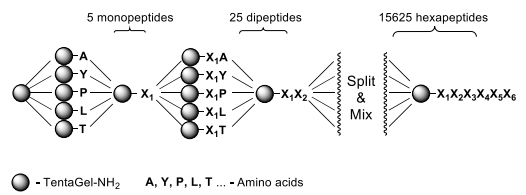
Linker: Attaches the cytotoxic agent to the antibody. Newer linker systems are required to be stable in circulation release the drug only inside targeted cells.

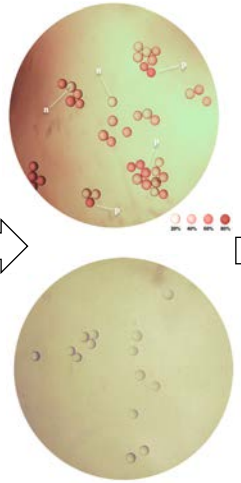
Bio-stability assays



Bio-selectivity profiling / 2

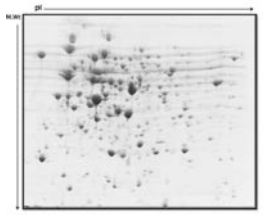
Combinatorial screening



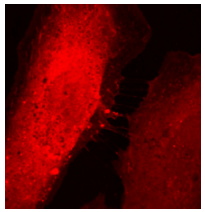


#	Amino acid present	Sequence	
1	Asp, Gln, Gln, Ser, Phe, Lys	FDQSQK	Colourless beads (n)
2	Gln, Gly, Thr, Pro, Tyr, Asp	GTQPDY / PGQTDY	
3	Gln, Ser, Gly, Ala, Pro, Gly	GGQPAS	
4	Gln, Gly, Ala, Tyr, Ile, Leu	GLIAQY / GLQIAY	
5	Gln, Gly, His, Ile, Leu, Phe	FGLIQH / GLFIQH	
6	Gly, His, Ala, Ile, Leu, Leu	GLLIAH	
7	Gln, His, Thr, Pro, Pro, Ile	PTIPQH	
8	Asp, Gly, His, His, Thr, Ala	GDHTAH / GTHADH	
9	Asp, His, Thr, Ala, Pro, Leu	PDLTAH / PTLADH	
10	Gly, Arg, Thr, Tyr, Leu, Cys	RGLTCY / GYLTRC	Coloured beads (p)
11	Pro, Cys, Leu, Leu, Lys, Lys	KLLPCK	
12	Gln, Ser, Arg, Thr, Cys, Lys	RTQSCK / KTQSRC	
13	Phe, Cys, Tyr, Arg, Ser, Tyr	RYFSCY	
14	Ala, Cys, Ile, Leu, Lys, Lys	KLIACK	
15	Asp, Ser, Arg, Cys, Leu, Lys	RDLSCK / KDLSRC	
16	Asp, Arg, Pro, Cys, Phe, Arg	RDFPRC	
17	Asp, Gly, His, Tyr, Ile, Cys	GDHICY / GYHIDC	

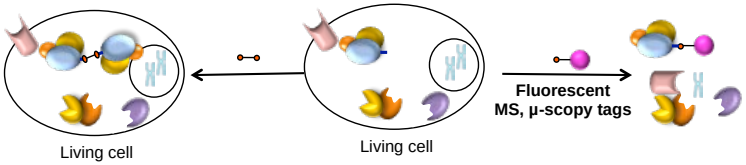
Prospective applications



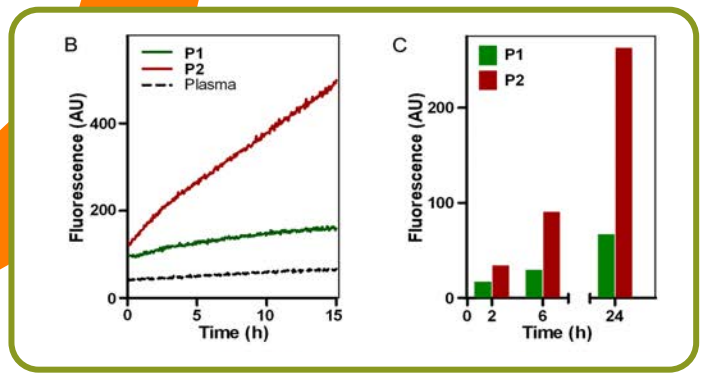
Native protein expression



Label native proteins



Stabilize protein complex



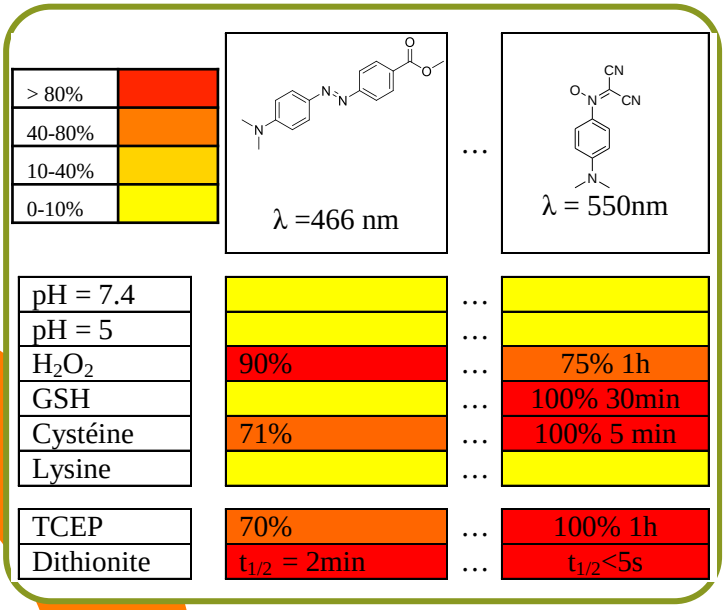
Bio-stability assays

Library of 3D protein motifs

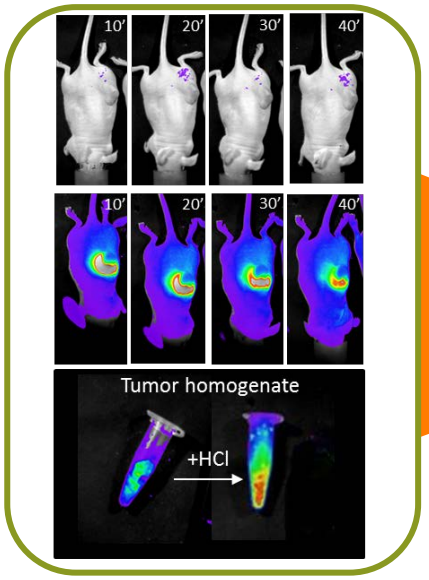
Searching for bio-responsive chemical function

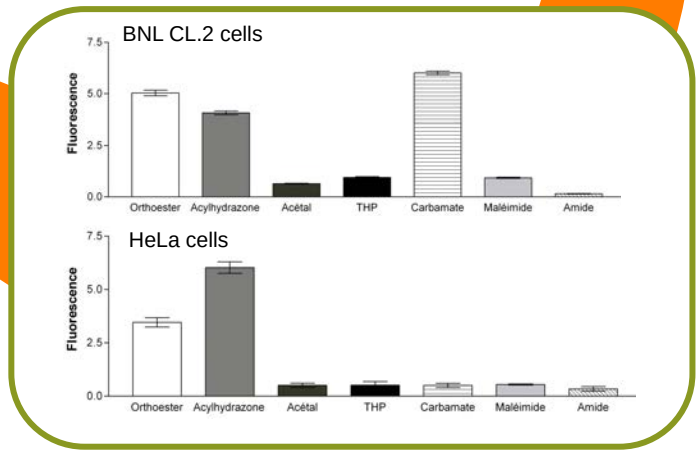
Endogenous chemically reactive bio-modulator

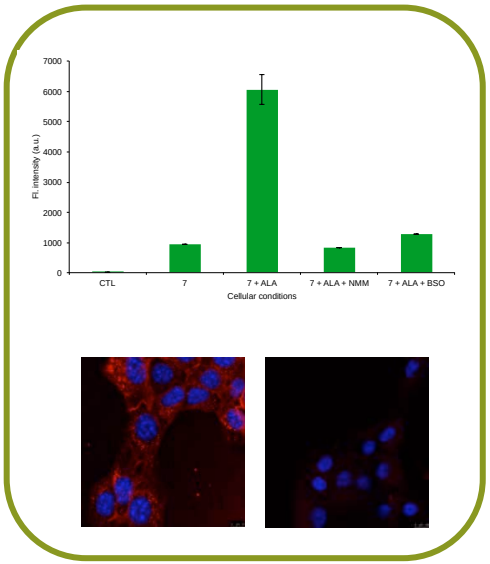
Small molecule biomodulator	Pathologies
pH Alteration	Brain disease (Parkinson)
Glutathione (GSH)	Viral disease
Nitric oxide (NO)	Aging
Hydroperoxides (H_2O_2)	Glomerular disease
Reactive Oxygen Species (ROS)	Oncology
Carbon monoxide (CO)	Alcoholism
Hydrogen sukfude (H_2S)	Ischemia–reperfusion injury,
Free metal (Fe, Mn, Cu)	Cardiac conditions
Malondialdehyde (MDA)	Premature birth
4 Hydroxynonenal	Erectile dysfunction
Methylglyoxal	Cigarette smoke effect
3-Acetyl-2,5-hexanedione	Cornea disease
Phosphocholine	Cystic fibrosis
Lactates, Créatine, Myoinositol	Viral infection
Thioredoxin	



Primary screening







In cell dyshomeostasis model

Cytomic approach for bio-responsive cleavage

Biospecificity / bioorthogonality / bioresponsivity biocompatibility are promising field of investigation for chemists and biologists to….

- Search fro bond breaking reactions
- Study reagents biocompatibility and compartmentalization
- Develop pathology-relevant models
- Investigate the meaning of bio-orthogonality
- Integrate bio and synthetic processes
- Develop time resolved reactivity monitoring systems
- Invent new applications

… bring original insight and alternative thinking.

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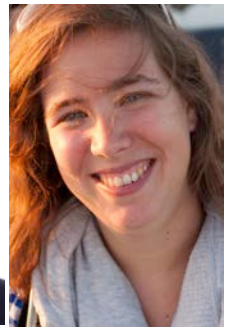

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Merci à vous....