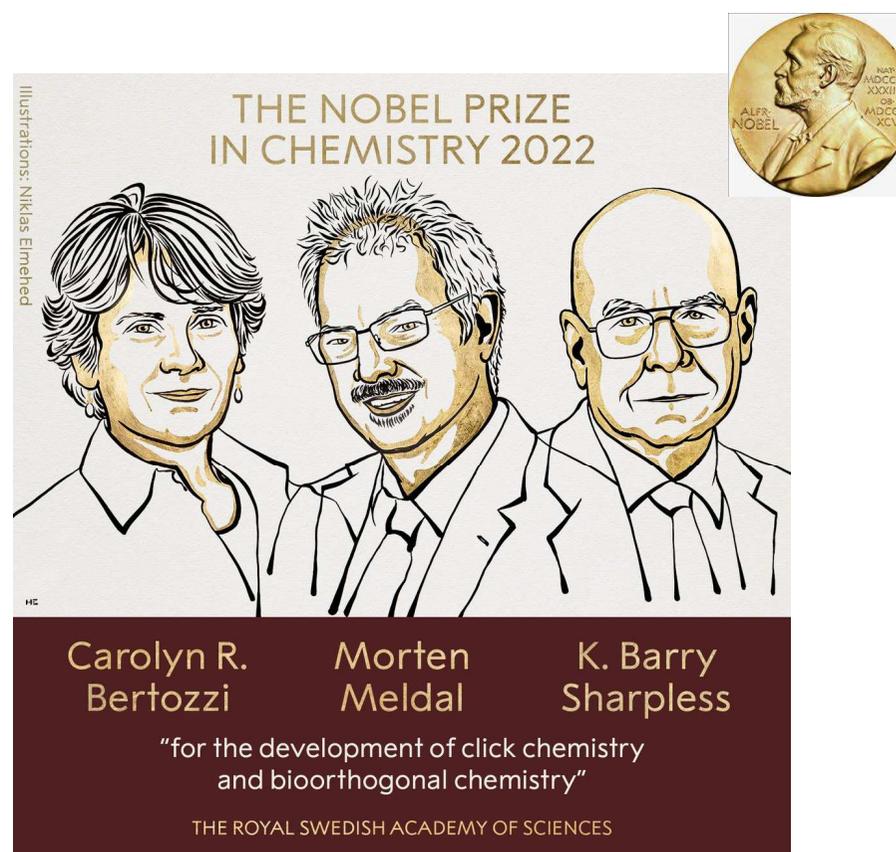
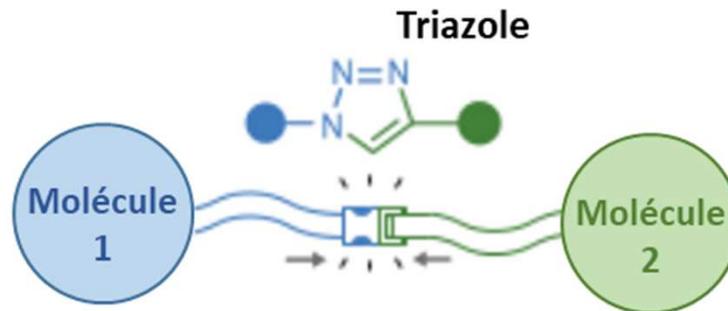
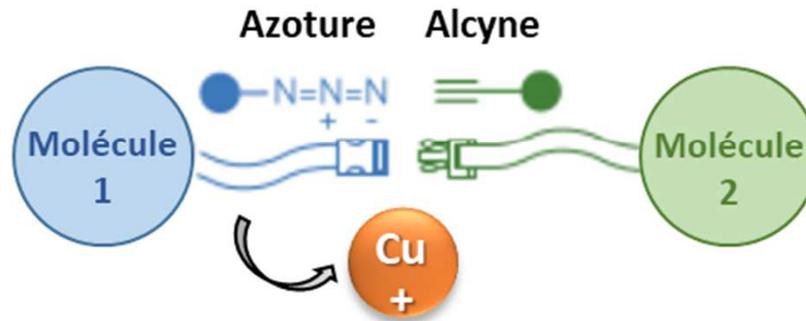
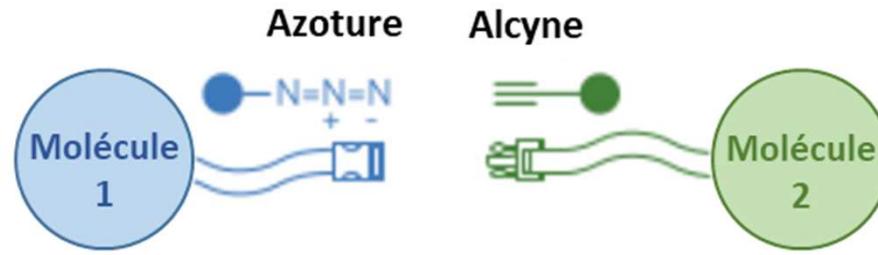


GEPHYX 2023

La chimie click et la chimie bioorthogonale une voie d'accès vers de nouveaux outils thérapeutiques



Chimie click et Chimie bio-orthogonale



Adaptable à de nombreux
domaines

Solvants non toxiques
idéalement : eau

Rendement élevés

Conditions réactionnelles simples
(facile à mettre en œuvre)



Réactifs facilement
disponibles

Réaction rapide

Produit facilement isolable

« Économisation des atomes »

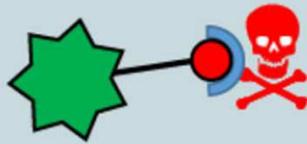
Réaction régio- et
stéréosélective

Peu de sous-produits
(non-réactifs, non toxiques, inoffensifs
pour l'environnement, facilement
éliminables)

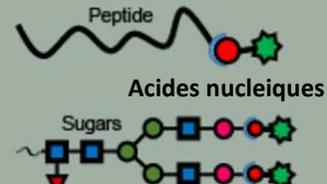
Anti-cancéreux, anti-microbien, anti-viral



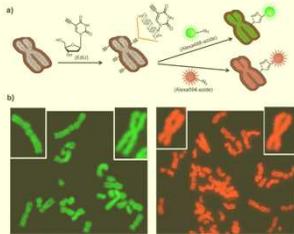
Drugs



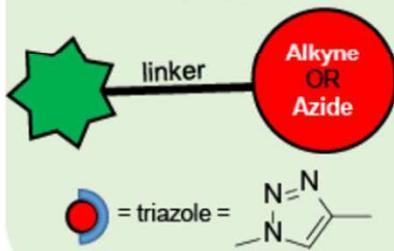
Biomolécules



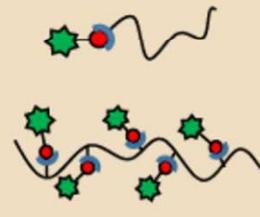
Imagerie



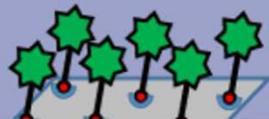
Azide-Alkyne Click Chemistry Applications



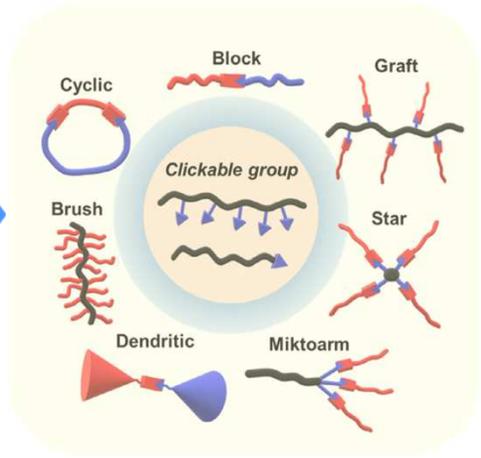
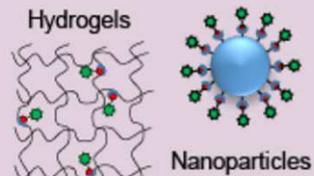
Polymères



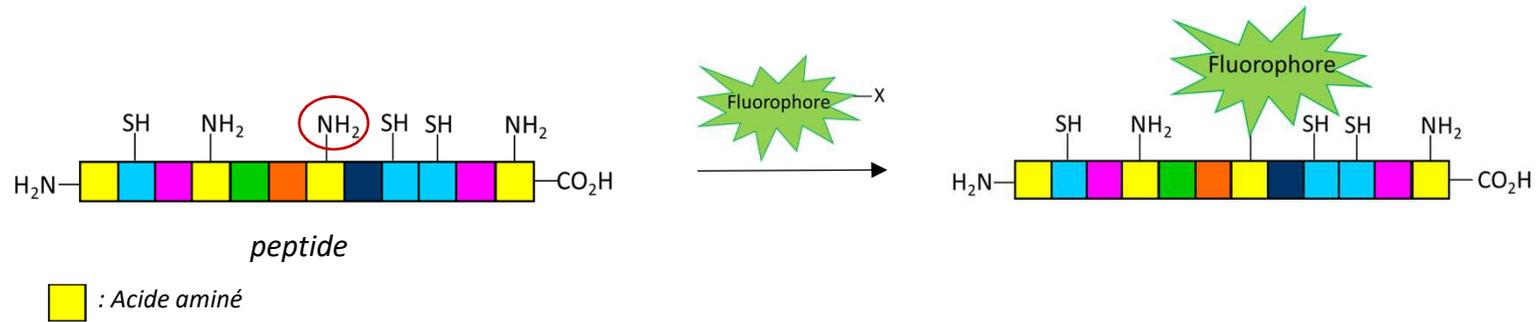
Surface Functionalization



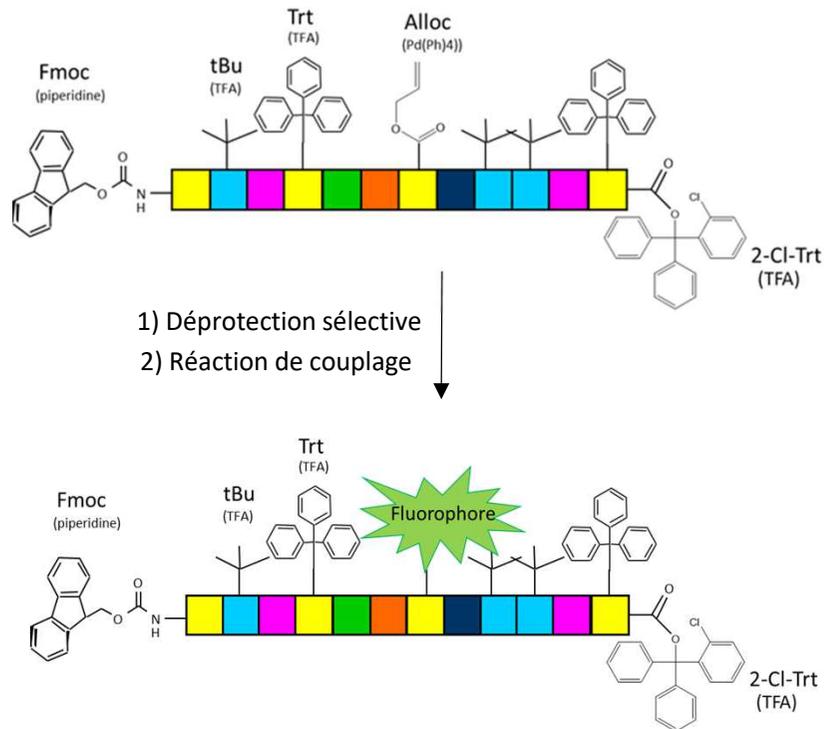
Advanced Biomaterials



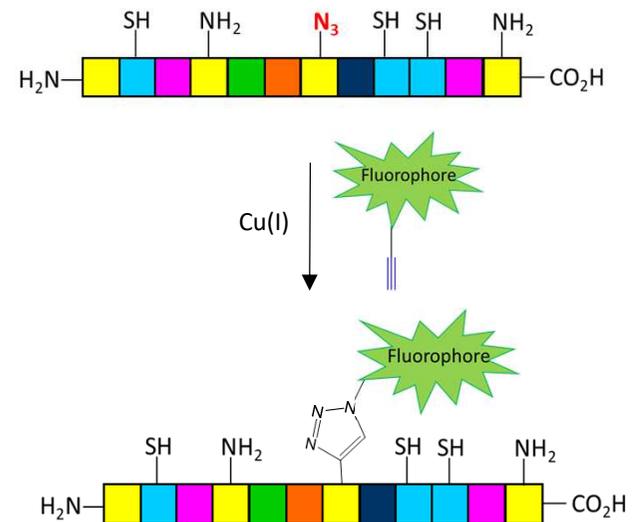
Chimie « classique » / Chimie Click

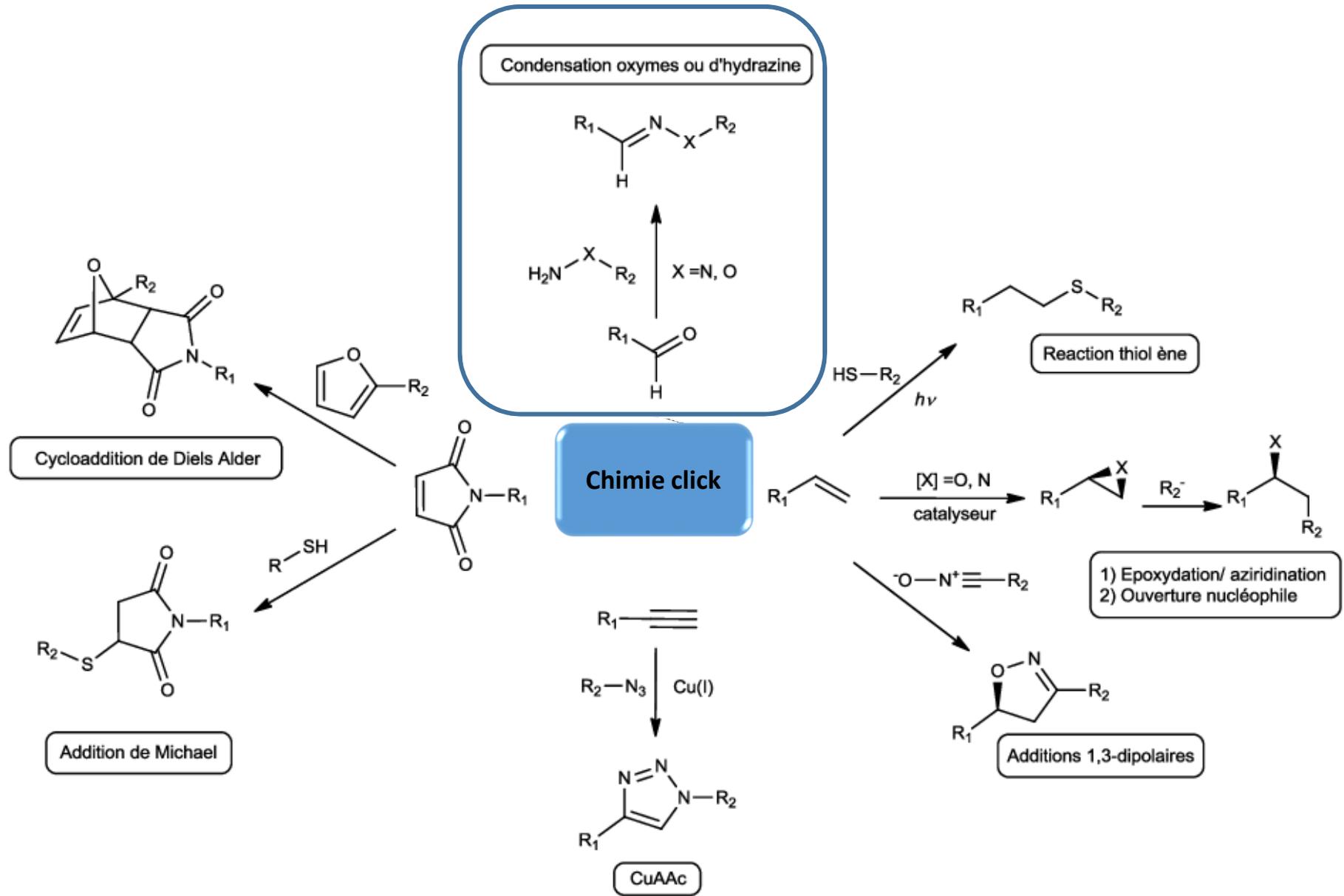


Chimie classique :

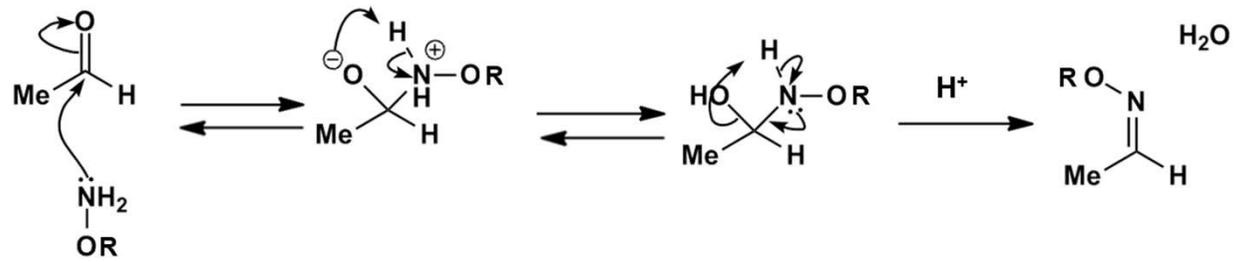


Chimie Click :





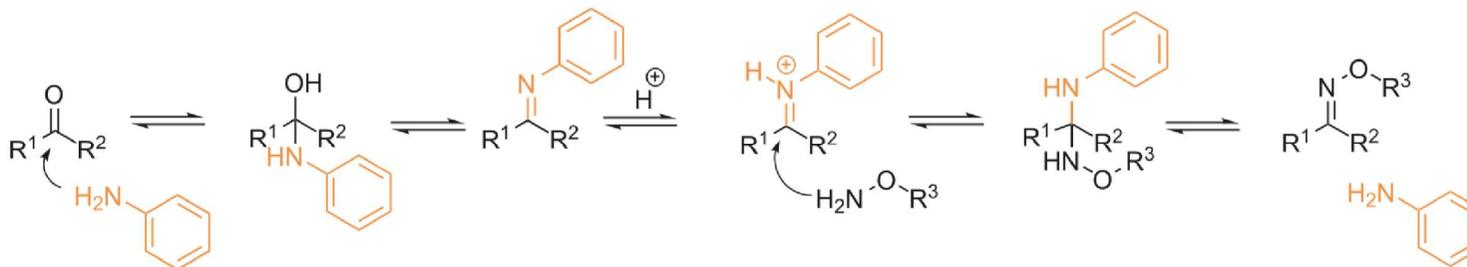
Conjugaison par lien Oxyne :

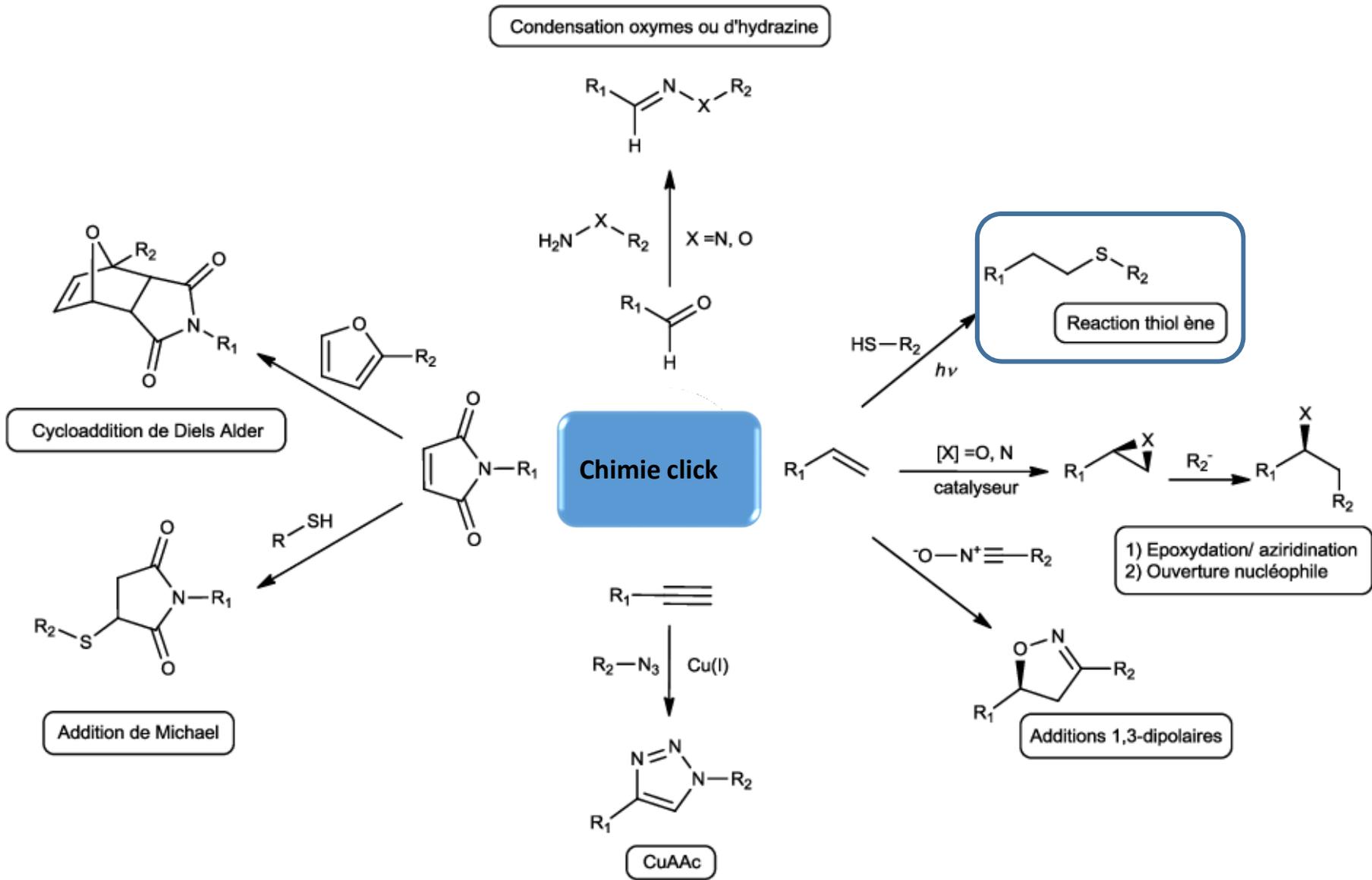


- Cinétique lente
- Réaction réalisable à pH 6-7 -> pH 4.5 optimal



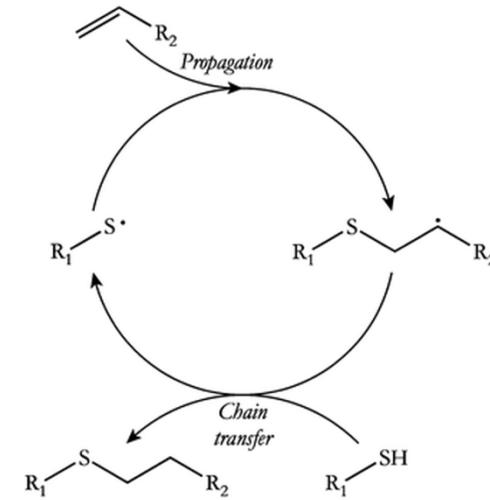
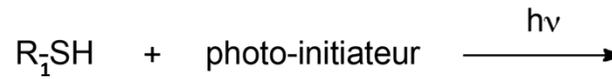
Utilisation de catalyseur (aniline) :



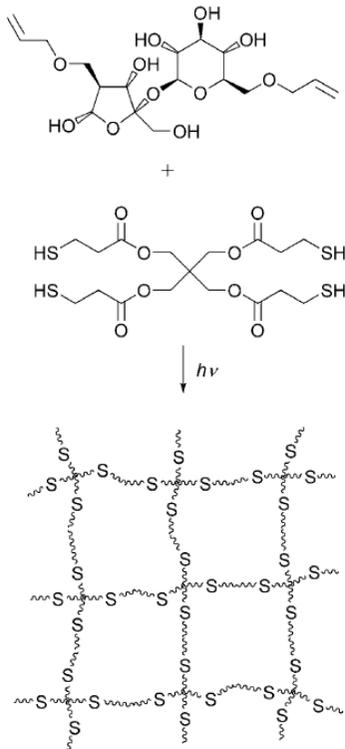


Réaction Thiol-ène :

TEC (Thiol-ene Coupling)



Application : préparation d'un polymère de saccharose

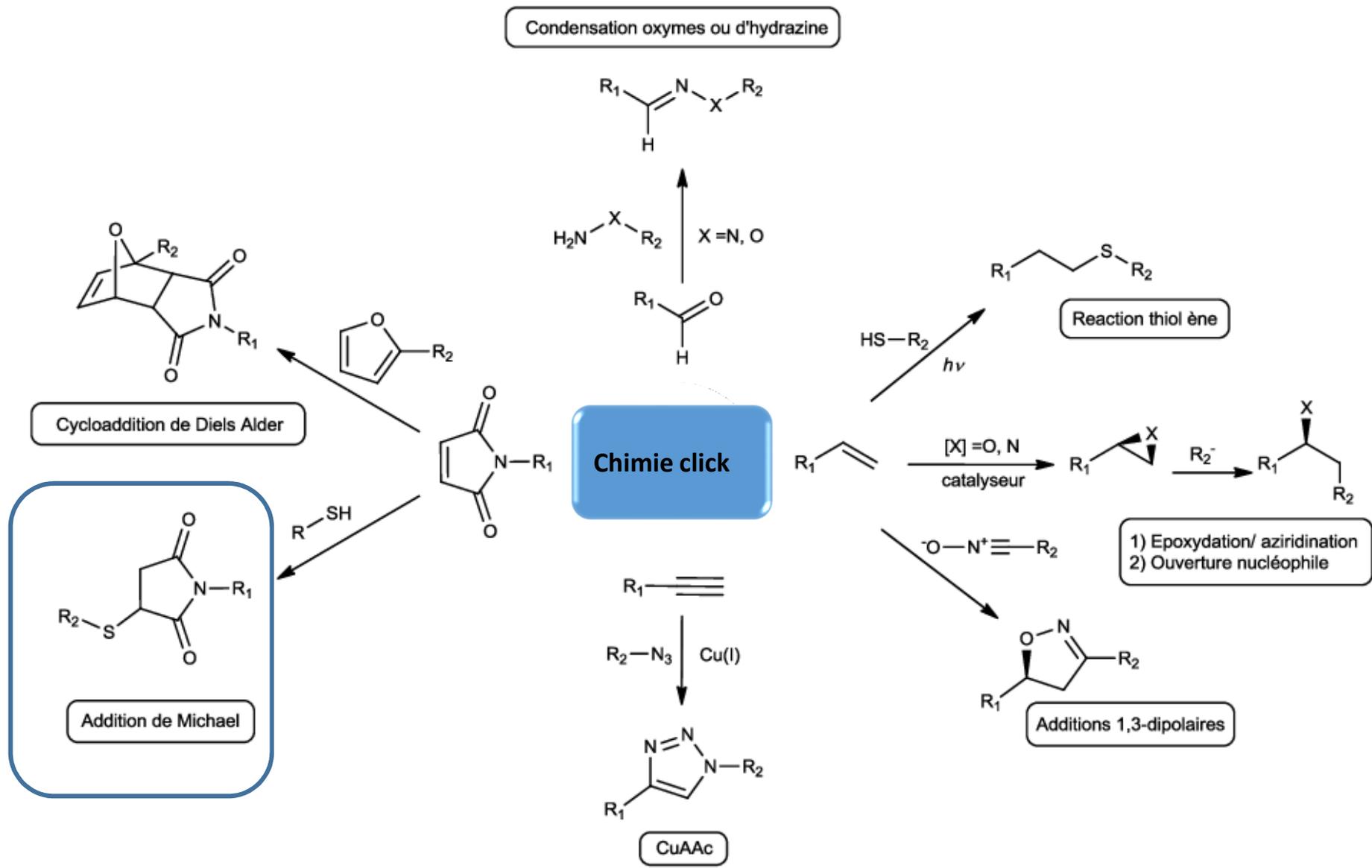


Saccharose fonctionnalisé

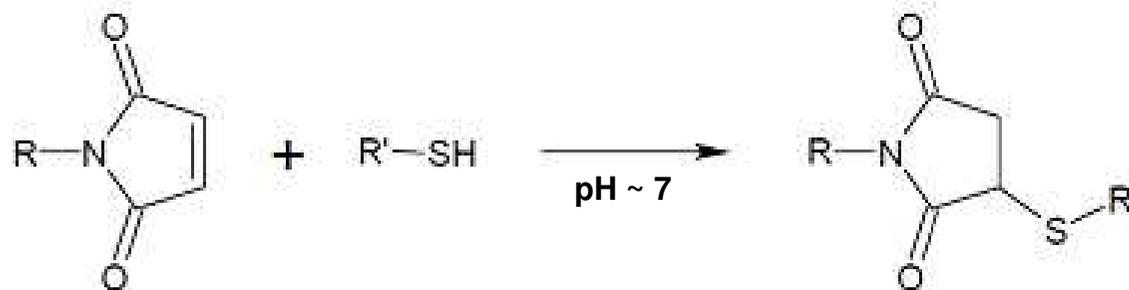
Matrice contenant les fonctions thioles

La réaction de polymérisation a été réalisée en moins de 20s avec près de 90 % de conversion des groupes fonctionnels

Ce type de polymère peut présenter des applications potentielles comme biomatériaux.



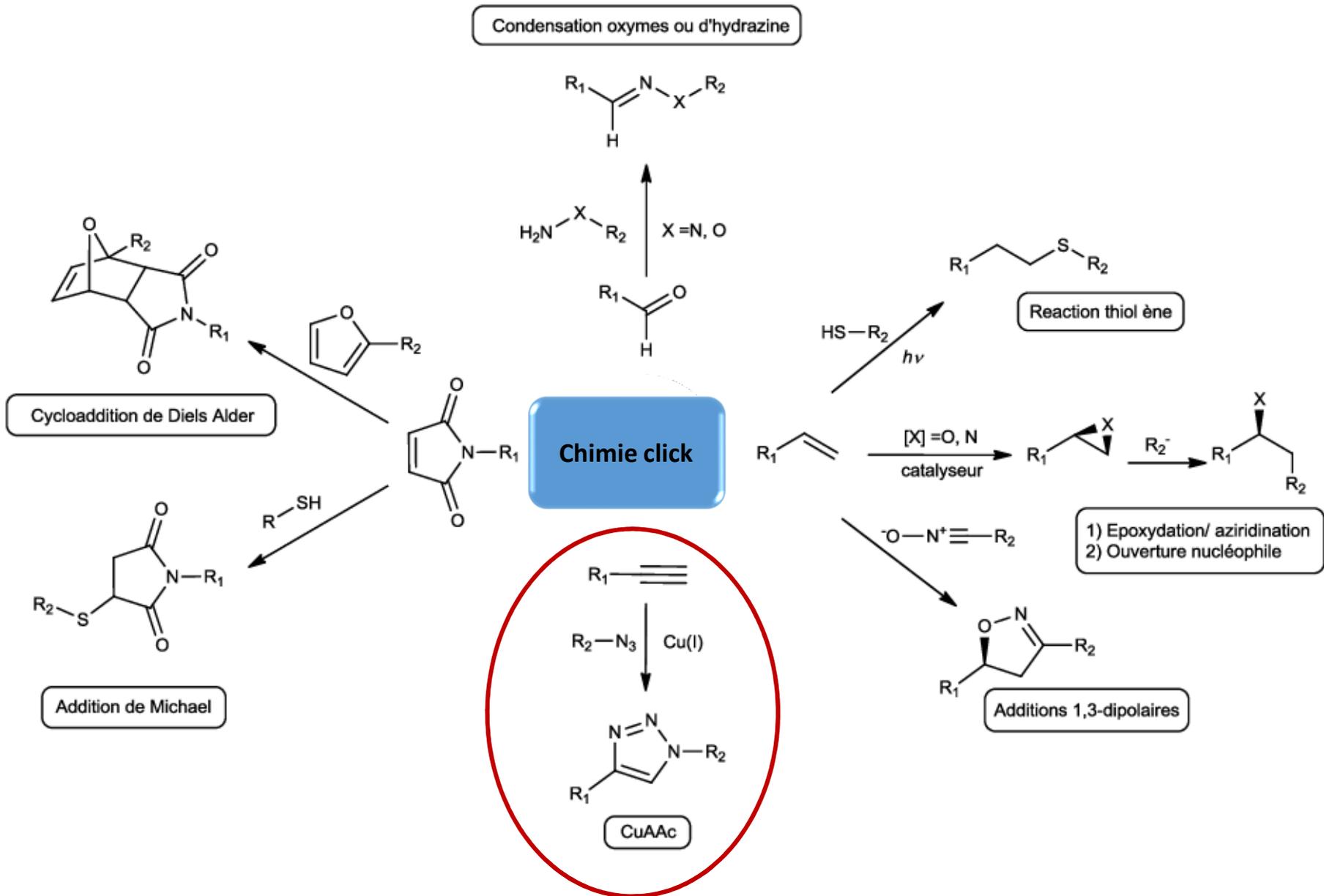
Addition de Michael - Thiol :



Le maléimide est couramment utilisé pour la bioconjugaison avec des drogues

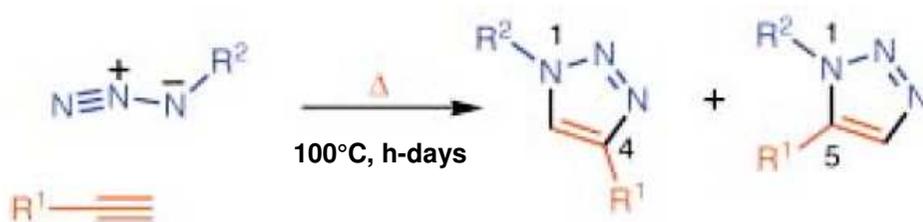


La FDA (Food and Drug Administration) a approuvé l'utilisation d'anticorps conjugué à une drogue



Cycloaddition 1,3-dipolaire :
CuAAC (copper(I)-catalyzed azide-alkyne cycloaddition) :

Version originale: cycloaddition azoture-alcyne de Huisgen (1963)

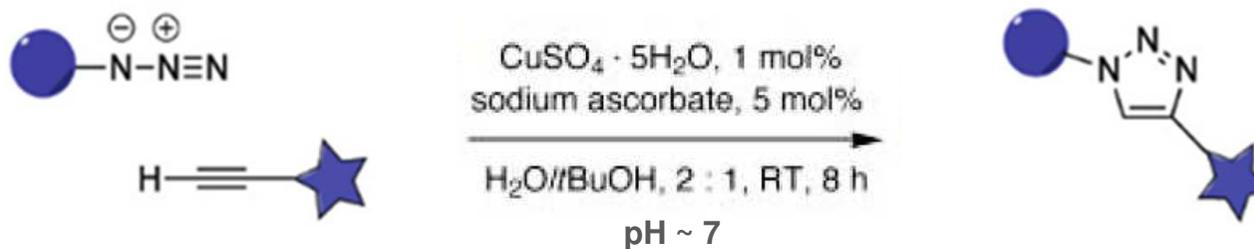


Rolf Huisgen

Catalyse au Cuivre (I) CuAAC



Morten Meldal Barry Sharpless



Morten Meldal, *J. Org. Chem.* **2002**, 67, 3057-3064

K. Barry Sharpless, *Angew. Chem. Int. Ed.* **2002**, 41, 2596-2599

De la chimie click à la chimie bioorthogonale

Le catalyseur Cuivre (I), utilisé pour CuAAC est cytotoxique, limitant son utilisation pour les systèmes vivants.



Carolyn Bertozzi
2003

SPAAC (strain-promoted alkyne-azide cycloaddition) :

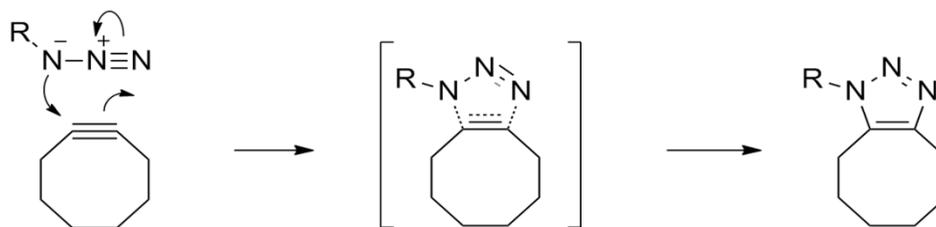


Cycloaddition azoture - cyclooctyne **SPAAC**

Réaction :

- quantitative
- Rapide
- Très sélective
- Sans sous-produit
- Réalisée dans l'eau
- Temp. Ambiante ou 37°C

Mécanisme :

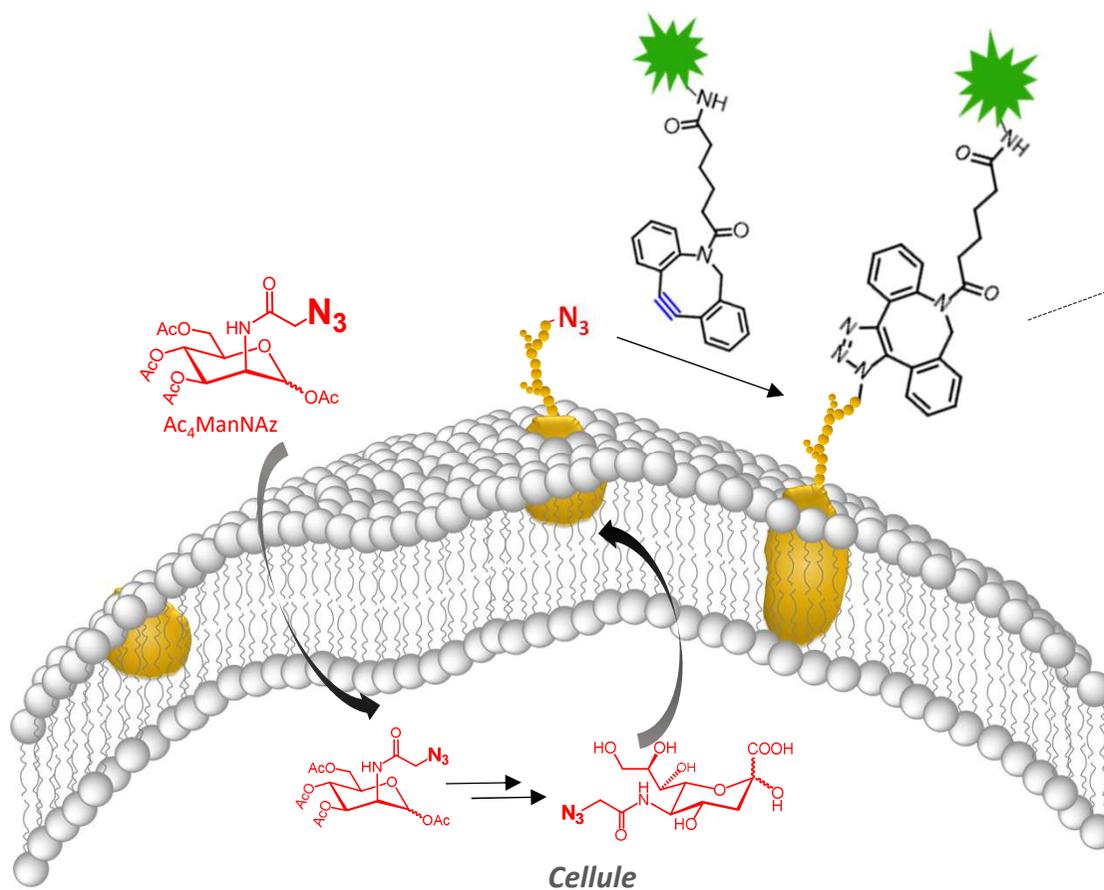


Chimie bioorthogonale

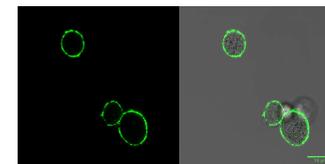


Carolyn Bertozzi
2003

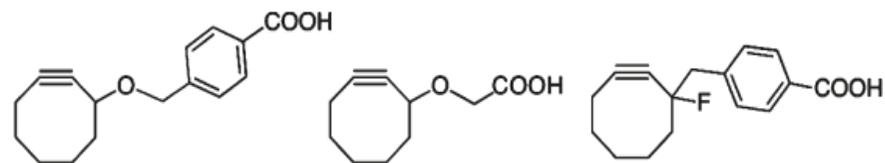
SPAAC : Azoture + Alcynes cycliques
strain-promoted alkyne-azide cycloaddition



Imagerie cellulaire



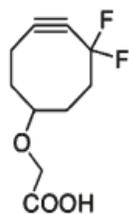
Cyclooctynes synthétisés pour la chimie click SPAAC dans les systèmes vivants :



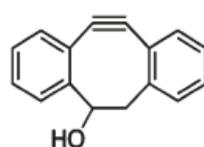
OCT
 $k = 0.0024 \text{ M}^{-1}\text{s}^{-1}$

ALO
 $k = 0.0013 \text{ M}^{-1}\text{s}^{-1}$

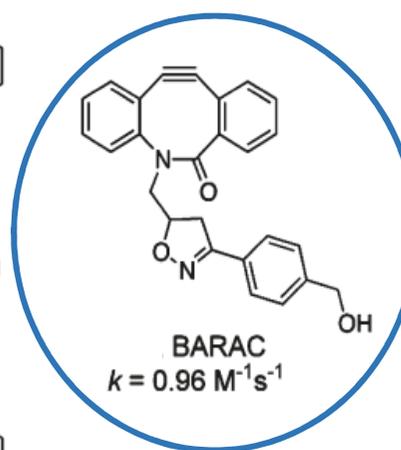
MOFO
 $k = 0.0043 \text{ M}^{-1}\text{s}^{-1}$



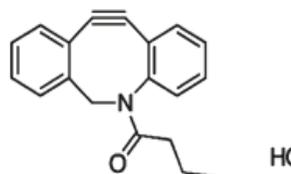
DIFO
 $k = 0.076 \text{ M}^{-1}\text{s}^{-1}$



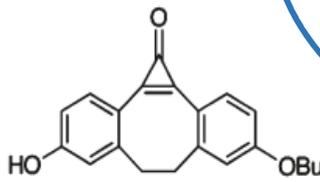
DIBO
 $k = 0.057 \text{ M}^{-1}\text{s}^{-1}$



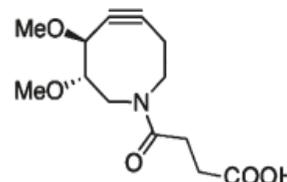
BARAC
 $k = 0.96 \text{ M}^{-1}\text{s}^{-1}$



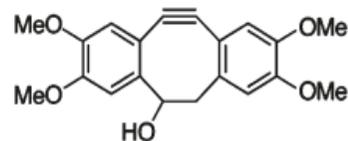
DIBAC (ADIBO)
 $k = 0.31 \text{ M}^{-1}\text{s}^{-1}$



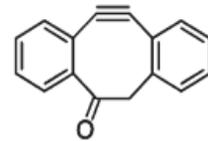
$k = 0.076 \text{ M}^{-1}\text{s}^{-1}$



DIMAC
 $k = 0.0030 \text{ M}^{-1}\text{s}^{-1}$

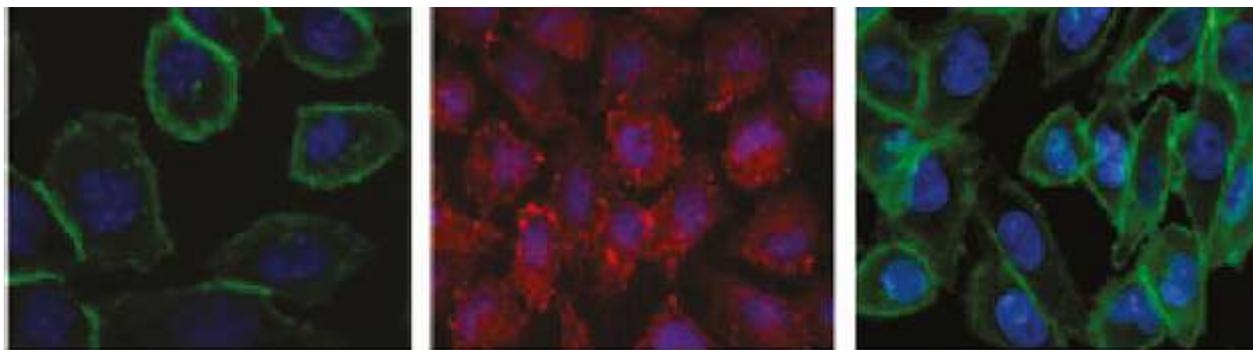
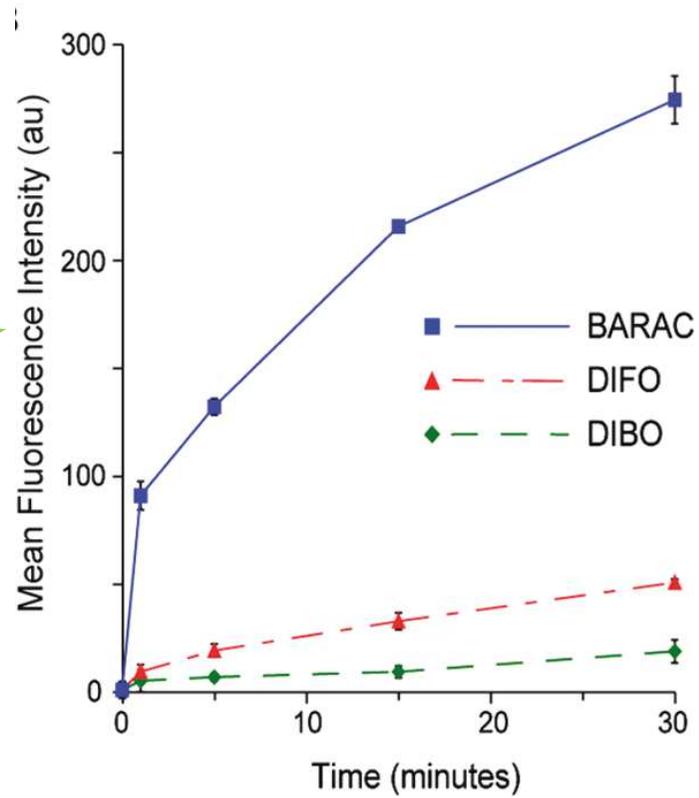
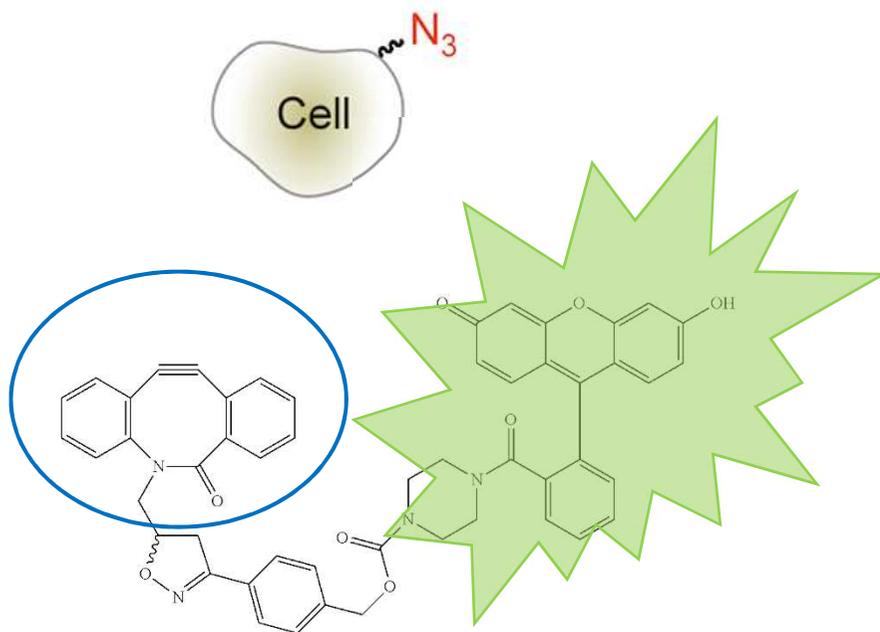


TMDIBO
 $k = 0.094 \text{ M}^{-1}\text{s}^{-1}$



keto-DIBO
 $k = 0.26 \text{ M}^{-1}\text{s}^{-1}$

Cyclooctynes synthétisés pour la chimie click SPAAC dans les systèmes vivants :

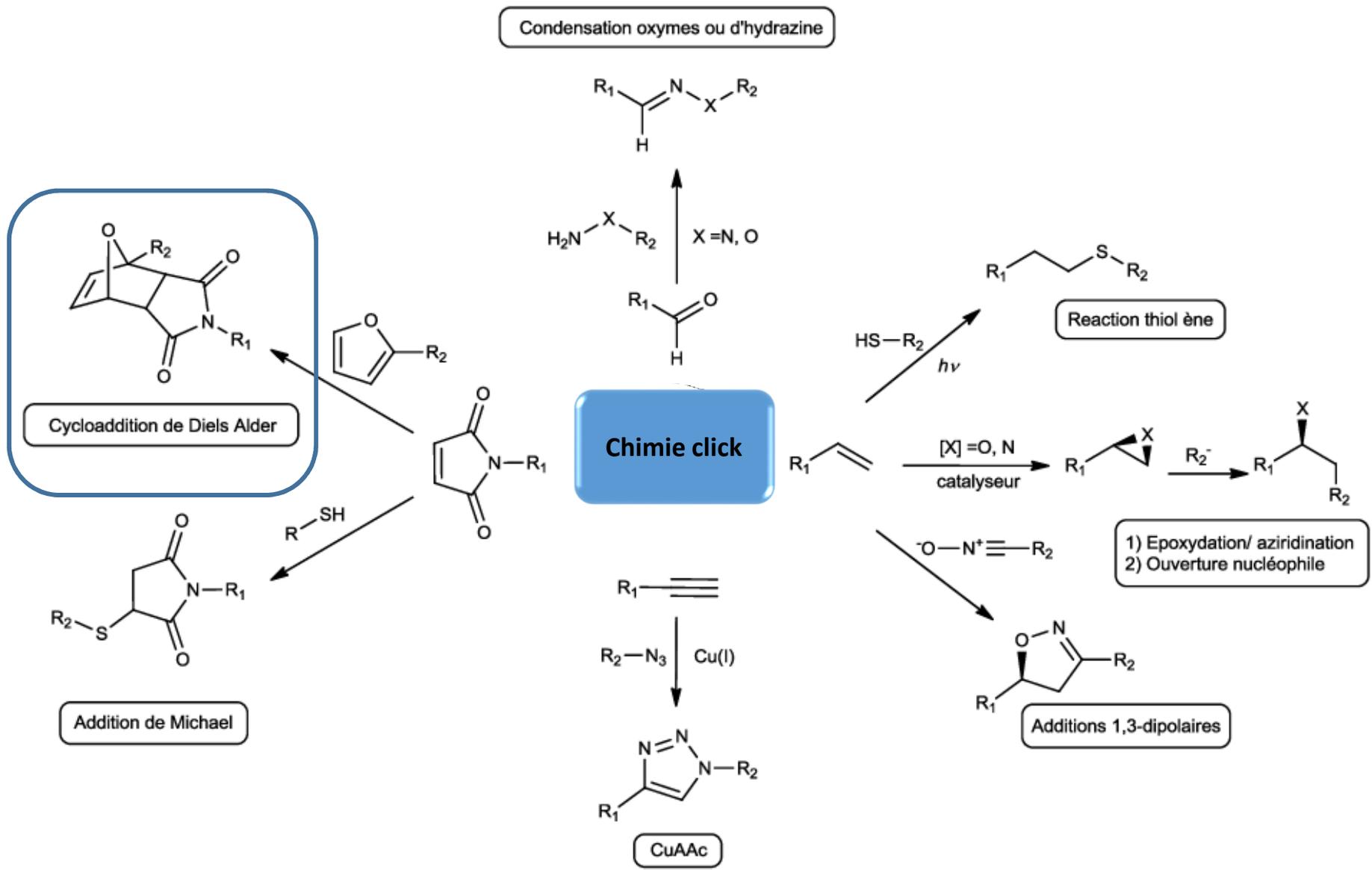


SPAAC using :

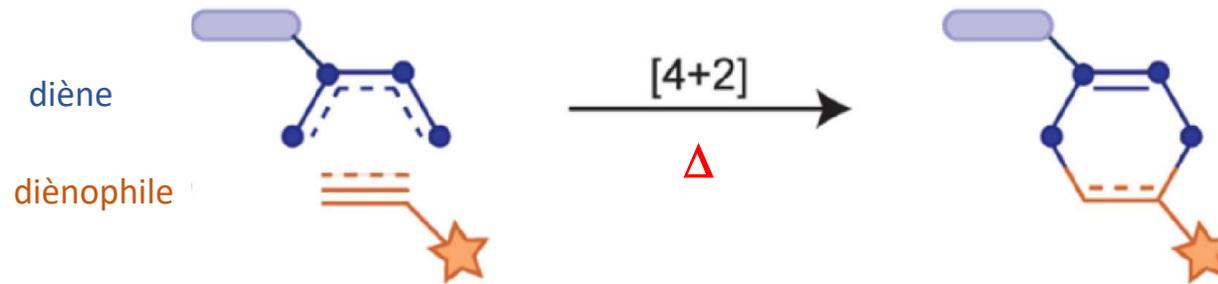
DIBO

DIFO

BARAC

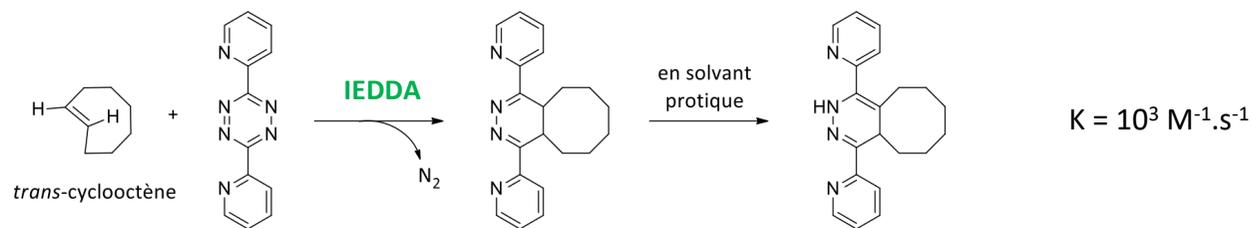
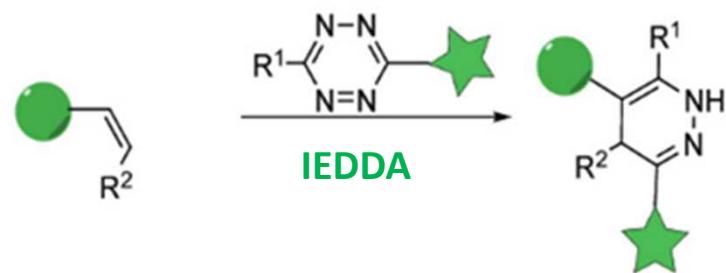


Cycloaddition dipolaire [4+2] : Réaction de Diels-Alder



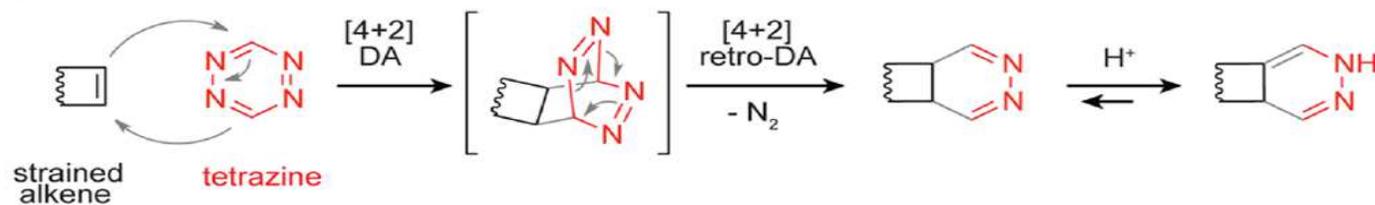
- Nécessite un chauffage : peu compatible avec des conditions biologiques.
- Cinétique lente (réaction limitée en milieu biologique).

Réaction de Diels-Alder à demande inverse d'électron (IEDDA) :

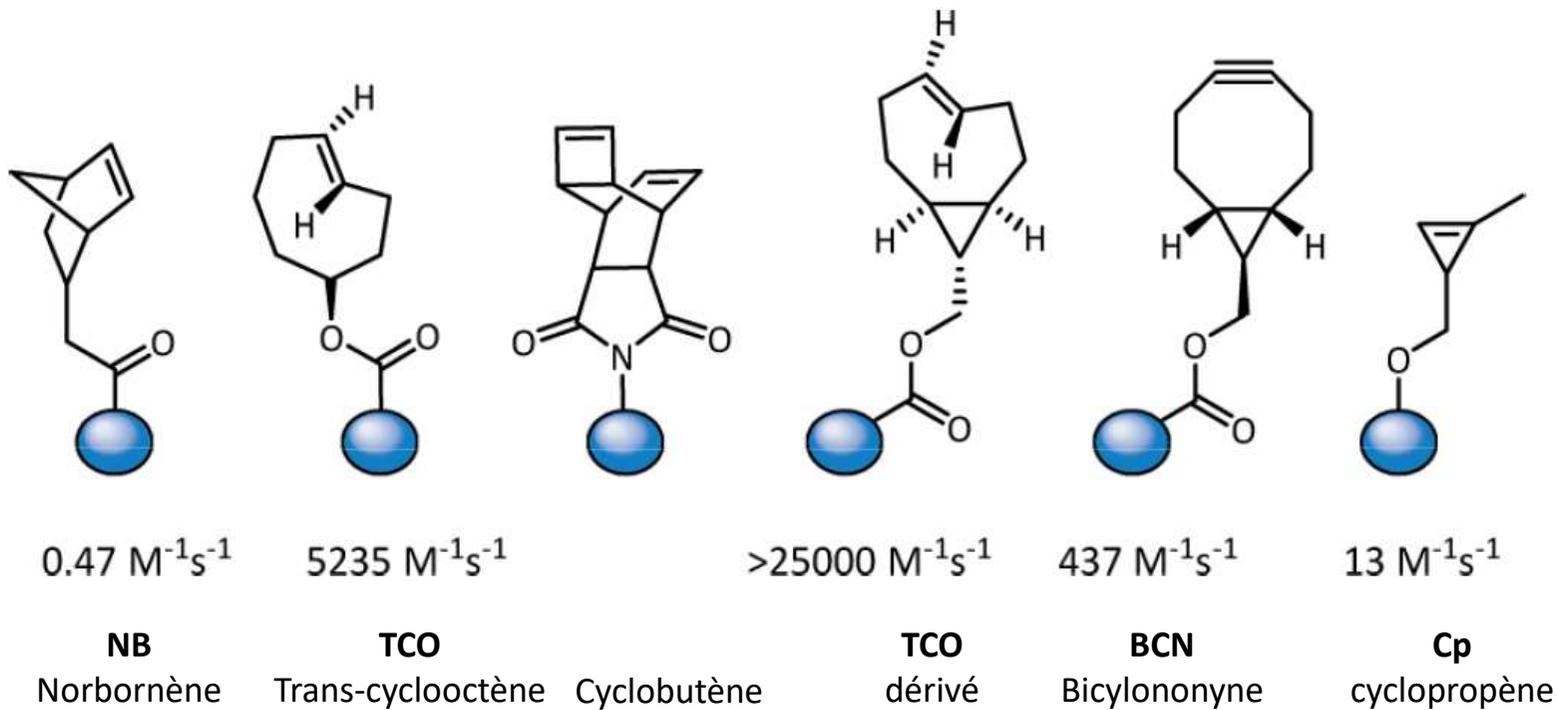


Fox et coll. (2008)

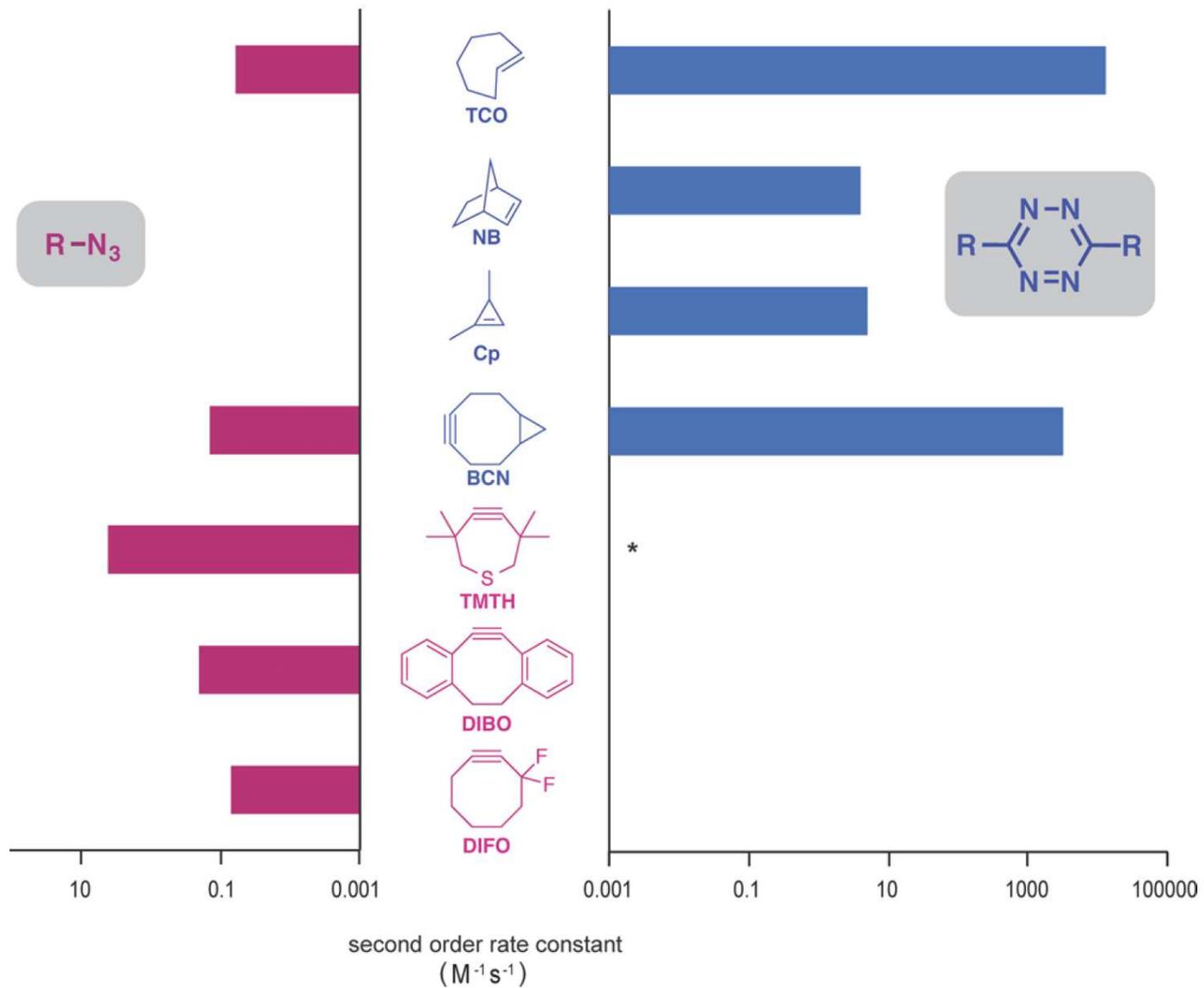
Mécanisme :



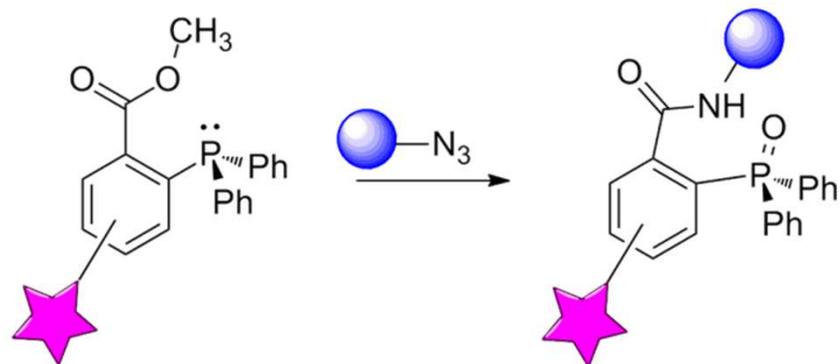
Diénofiles décrits pour leur réactivité avec les tétrazines :



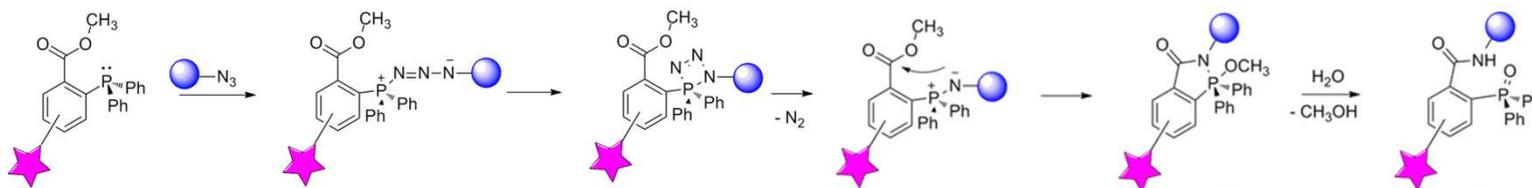
Réactivité vis-à-vis d'un azoture ou d'un tétrazine



Réaction de Staudinger :

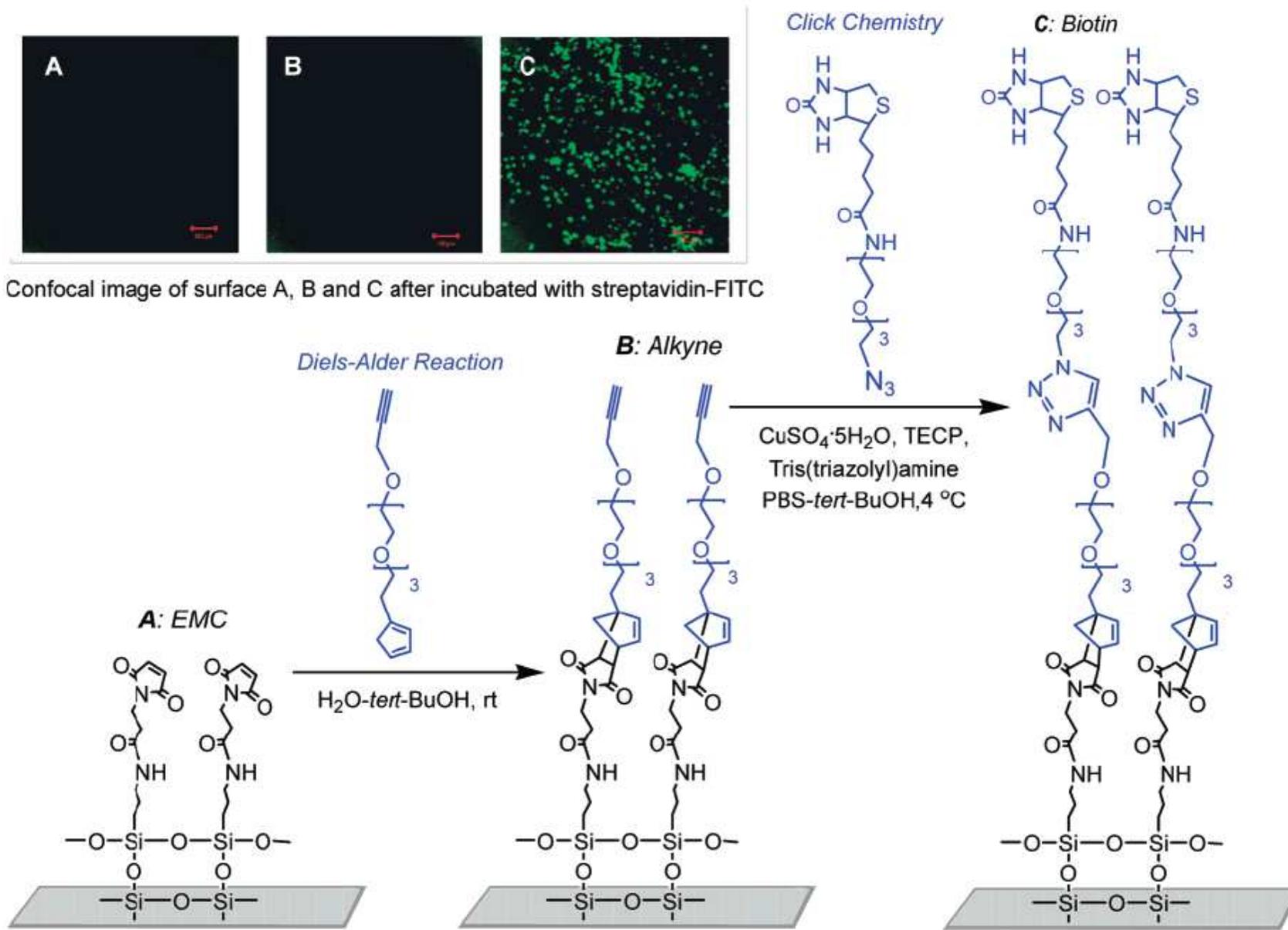


Mécanisme :



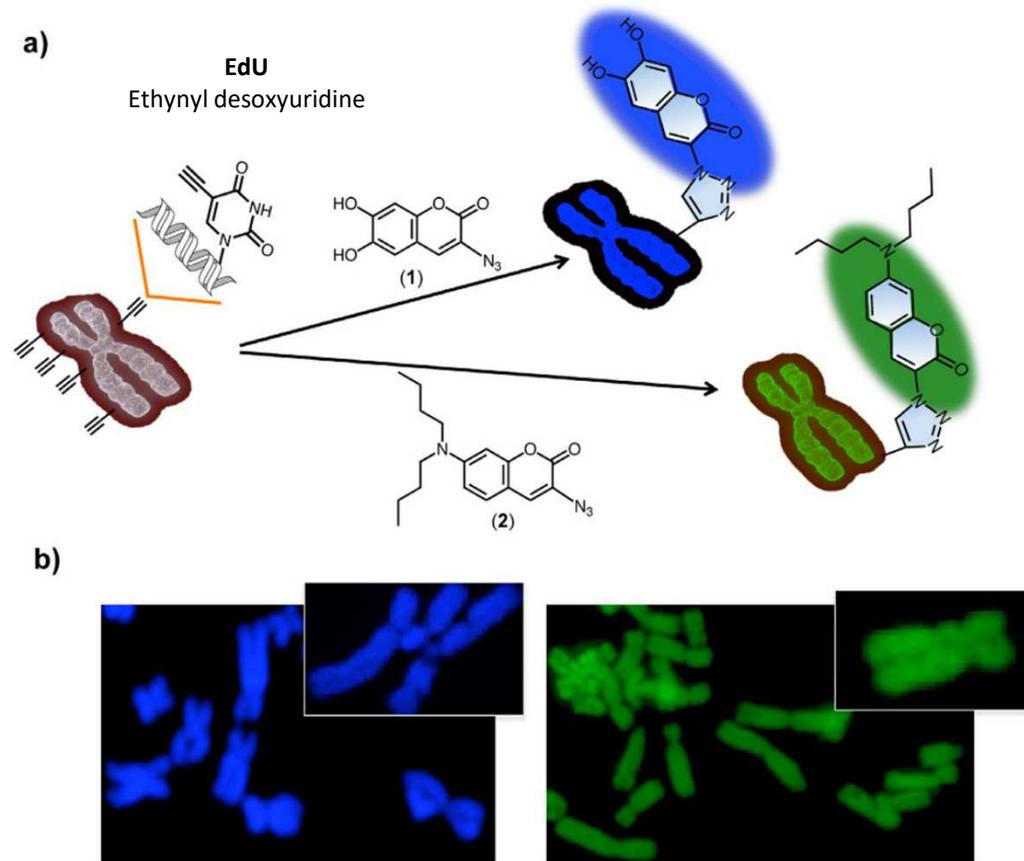
Quelques exemples d'applications ...

Diels-Alder and azide-alkyne cycloadditions for surface functionalization



Marquage fluorescent de chromosomes :

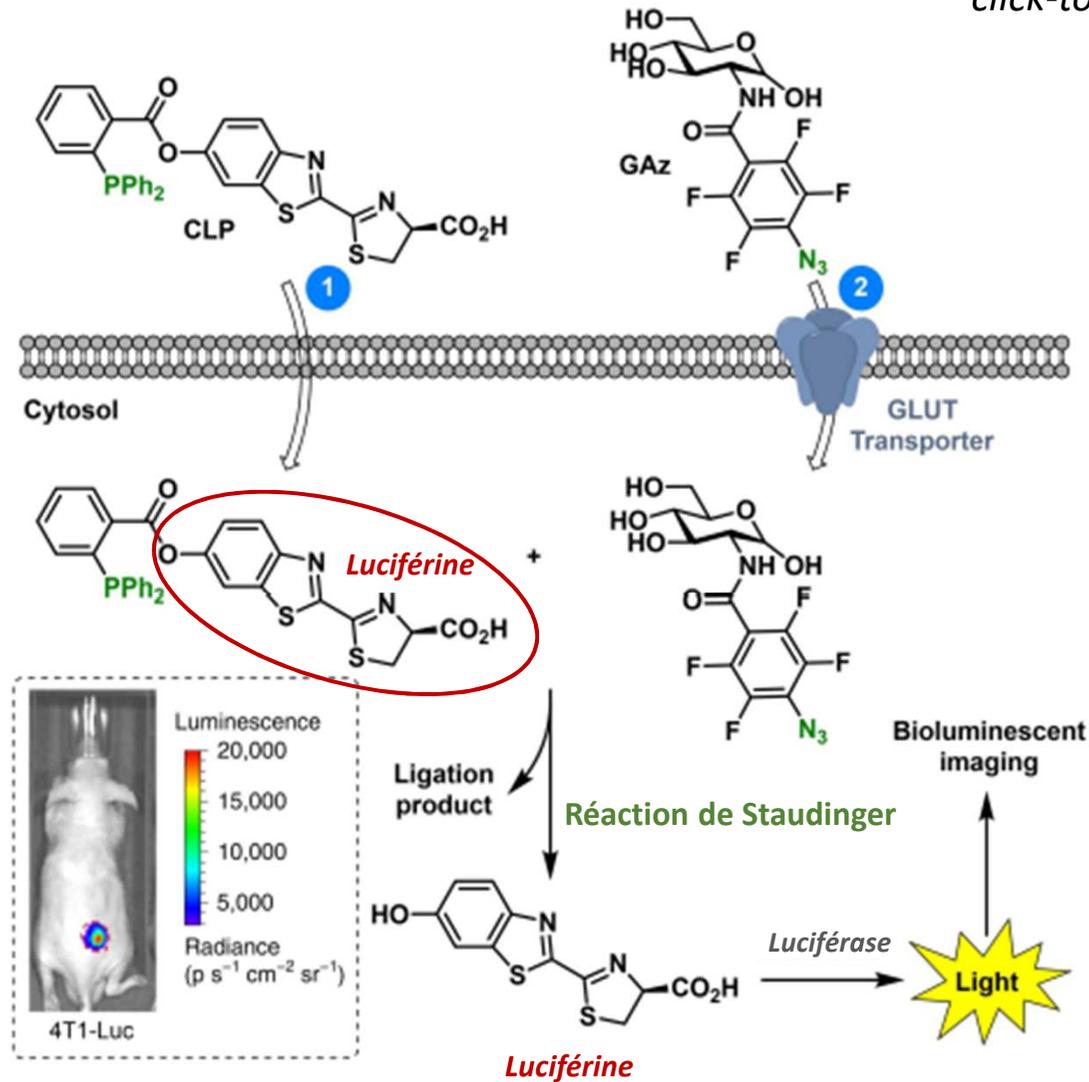
CuAAC



Chromosome imaging by using 1 and 2. (a) Schematic of a light-up (turn on) reporter strategy to stain chromosomal DNA. The pro-fluorophores 1 or 2 reacted with EdU-labeled chromosome to induce a strong fluorescence response for staining chromosome in blue (1) or green (2) color. (b) Chromosomes were stained with 1 (blue) or 2 (green). The inset panel is at higher magnification. Observed by fluorescence microscopy.

Approche d'imagerie des tumeurs sur modèle murin

click-to-release reactions



Imaging and quantification of D-glucose uptake in 4T1-Luc-bearing Swiss nu/nu mice using the GAz and CLP. GAz was injected 24 h after CLP.

Voir une tumeur en imagerie TEP par chimie « click » in vivo.



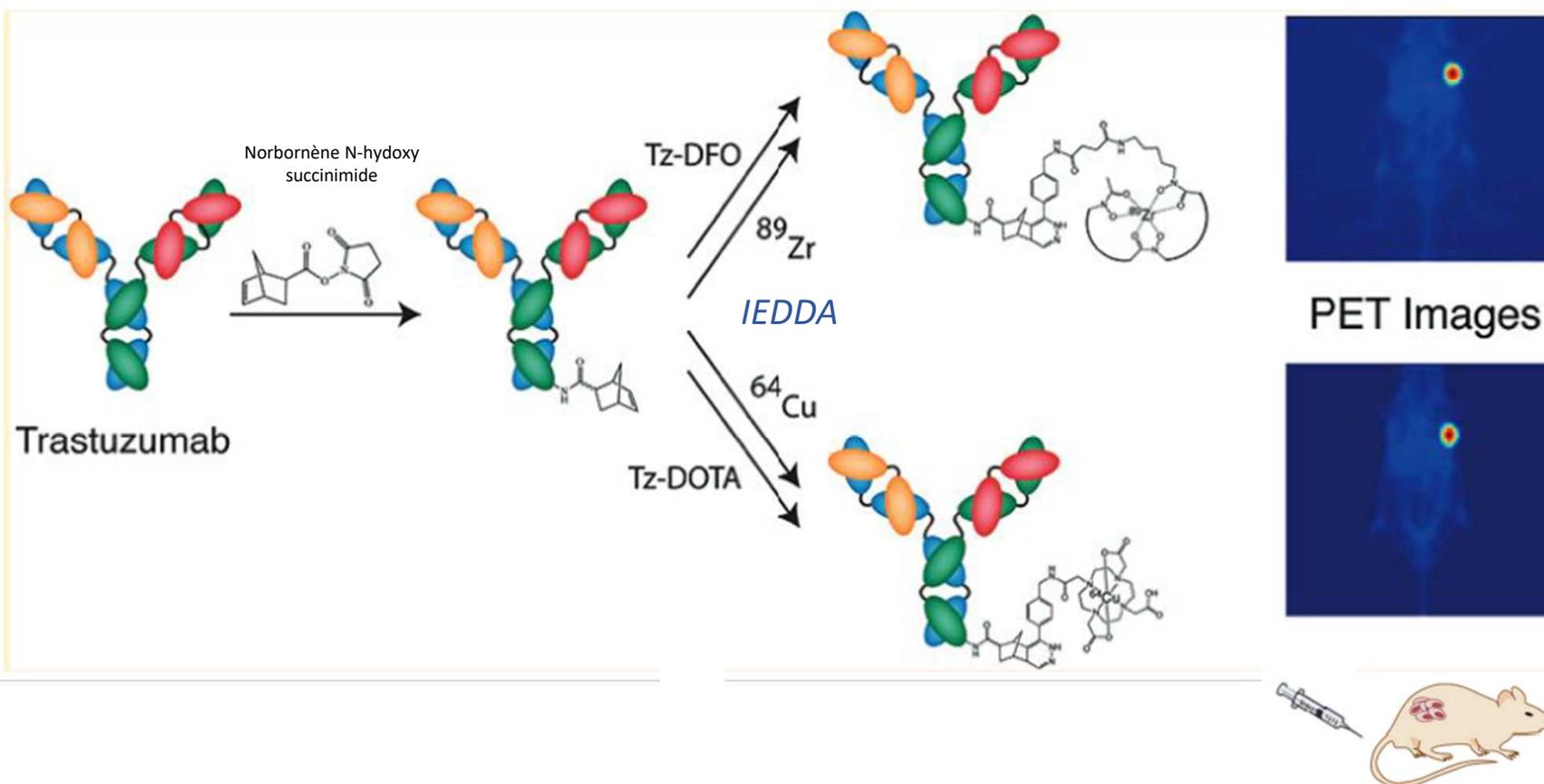
Bioconjugate Chemistry

ARTICLE

pubs.acs.org/bc

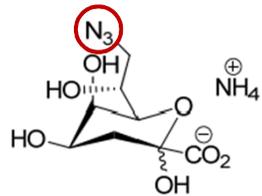
Modular Strategy for the Construction of Radiometalated Antibodies for Positron Emission Tomography Based on Inverse Electron Demand Diels–Alder Click Chemistry

Brian M. Zeglis,[†] Priya Mohindra,[†] Gabriel I. Weissmann,[†] Vadim Divilov,[†] Scott A. Hilderbrand,[‡] Ralph Weissleder,[‡] and Jason S. Lewis^{*†}

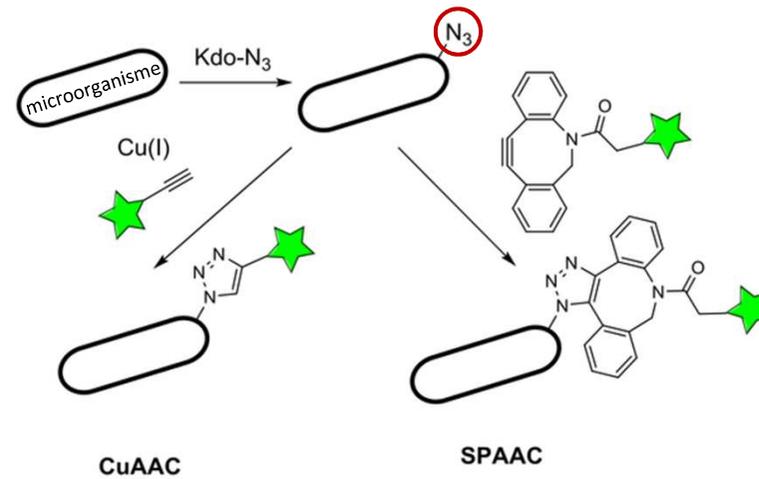
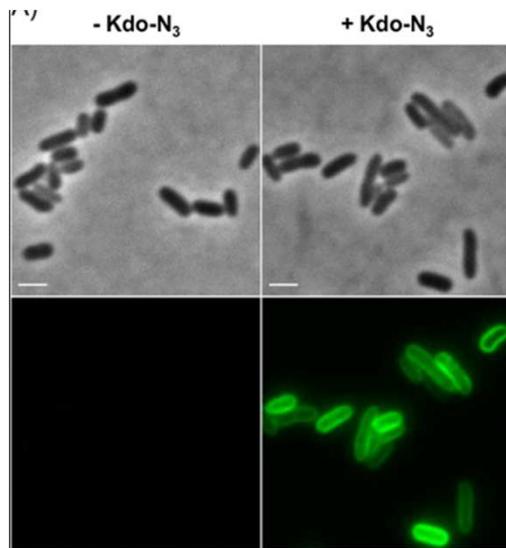


Tests de diagnostic médical (légionellose, infections urinaires)

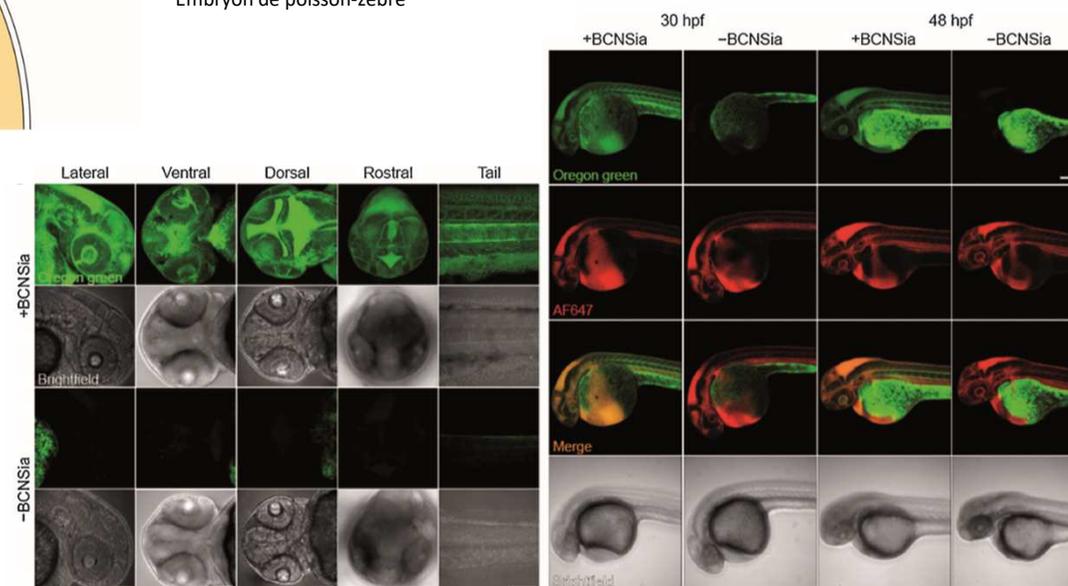
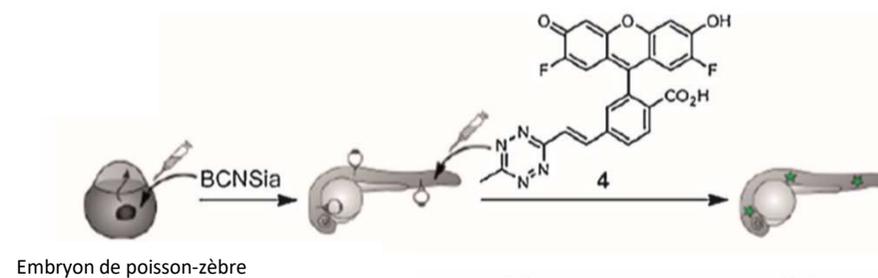
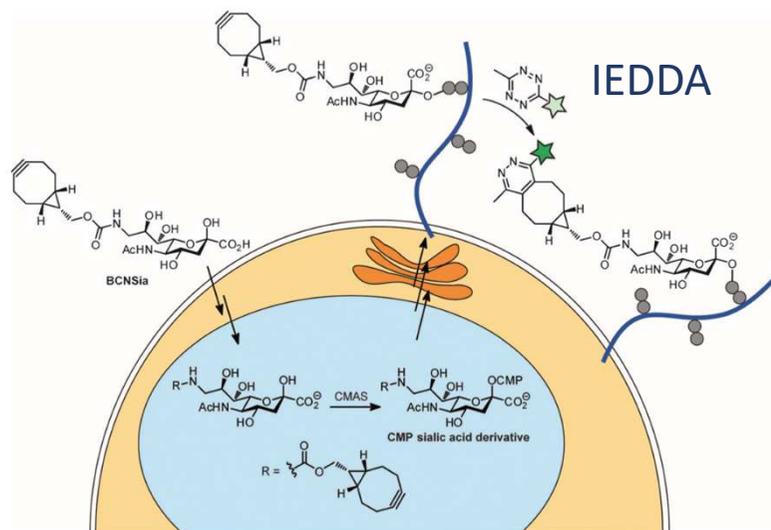
Ex. : test qualitatif de détection des sérogroupes de *Legionella pneumophila* (Lp) dans les lavages broncho-alvéolaires et dans les aspirations bronchiques



Kdo-N3
Ammonium 8-azido-3,8-dideoxy-D-manno-octulosonate



Imagerie par fluorescence de glycanes de poisson-zèbre par chimie bioorthogonale



Etude réalisée au laboratoire

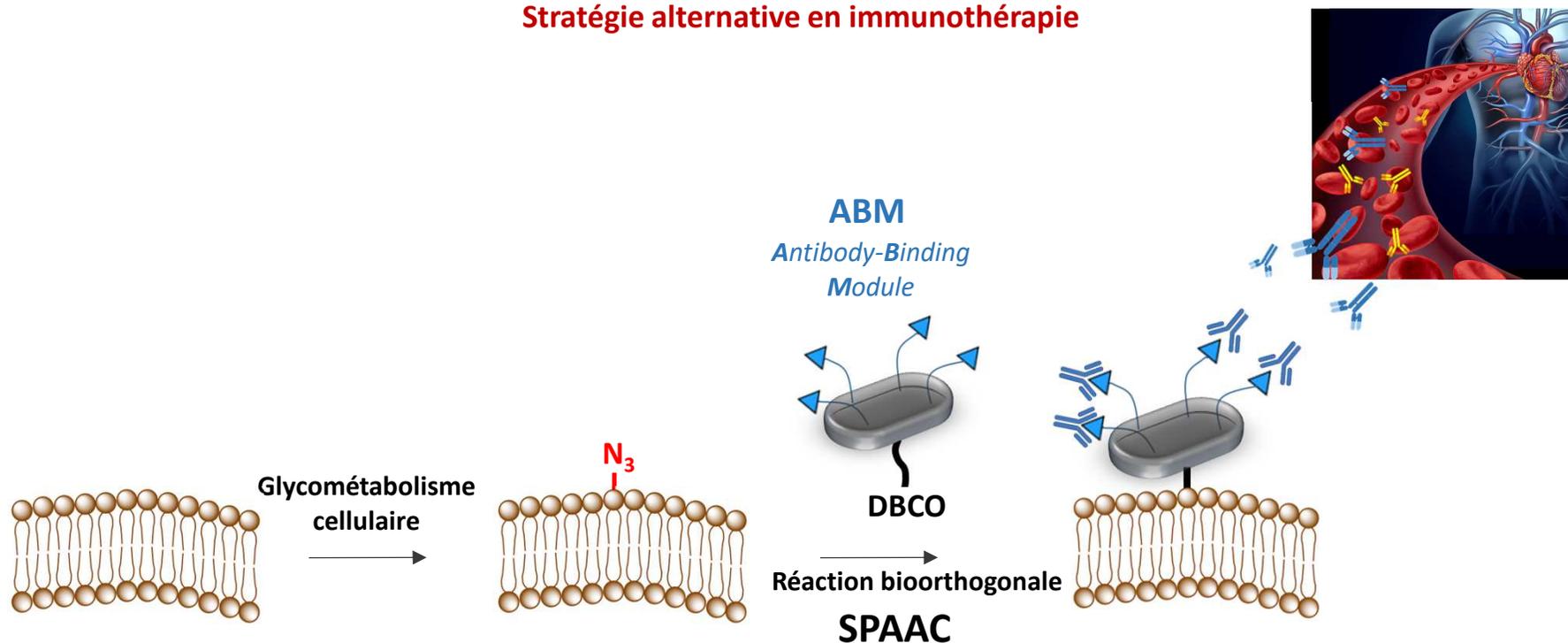
N. Berthet



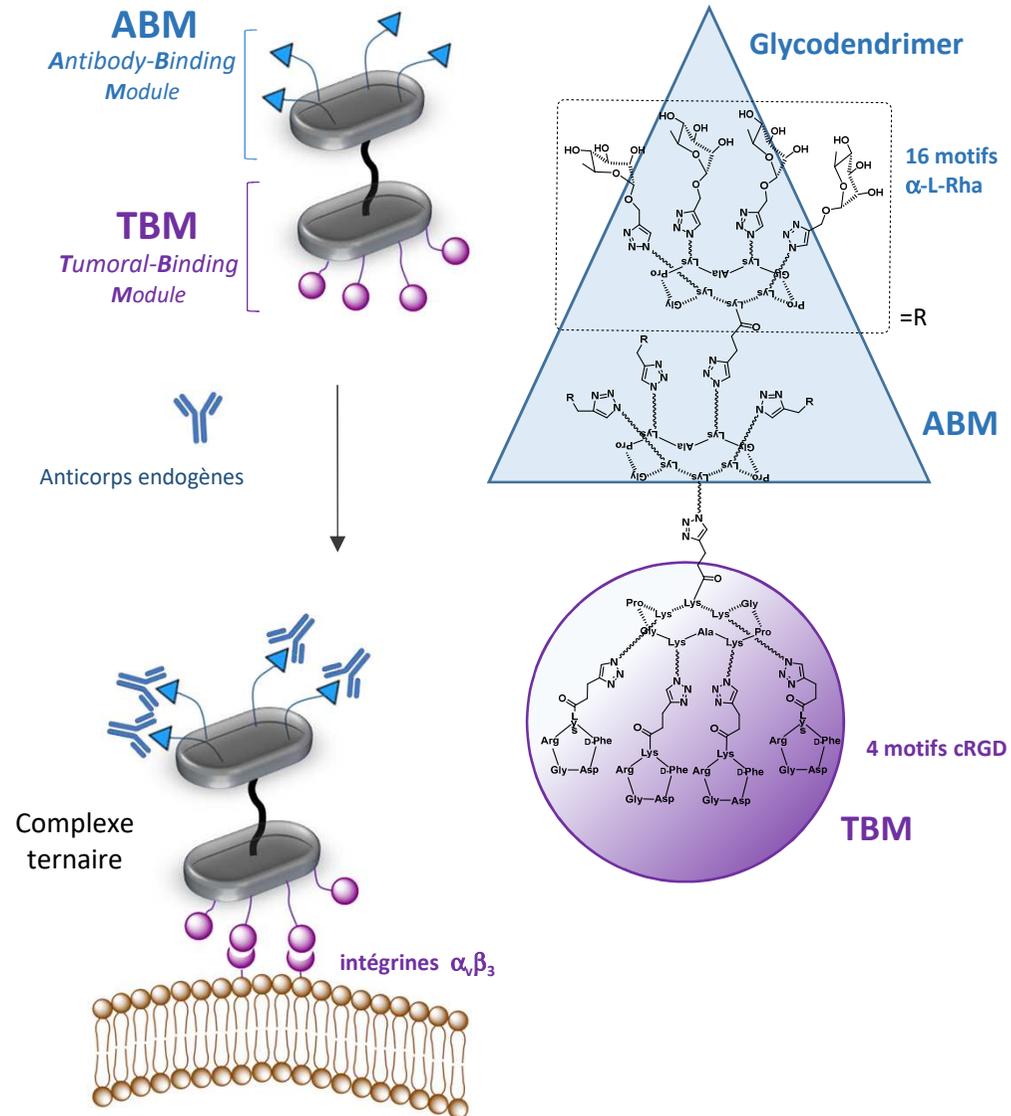
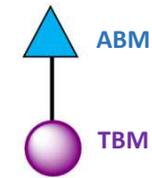
Etiquetage métabolique des cellules cancéreuses à l'aide de glycodendrimères pour stimuler une réponse immunitaire conduisant à la destruction cellulaire



Stratégie alternative en immunothérapie



Stratégie ARM « Bi-modulaire » (Antibody Recruiting Molecules)



Points faibles ...

- Formation du complexe ternaire

Interactions non covalentes :

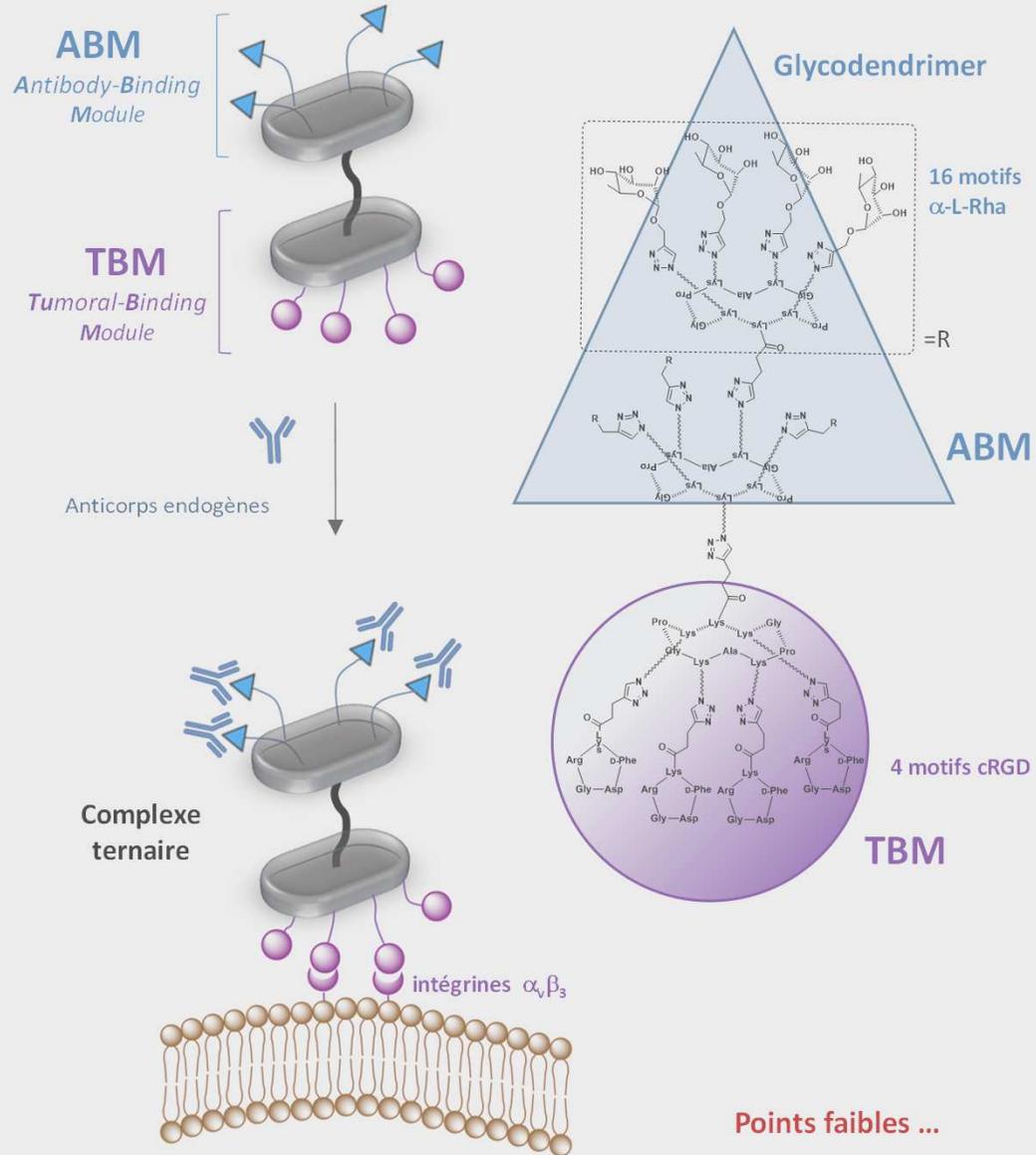
- TBM / récepteurs cellulaires

- ABM / anticorps

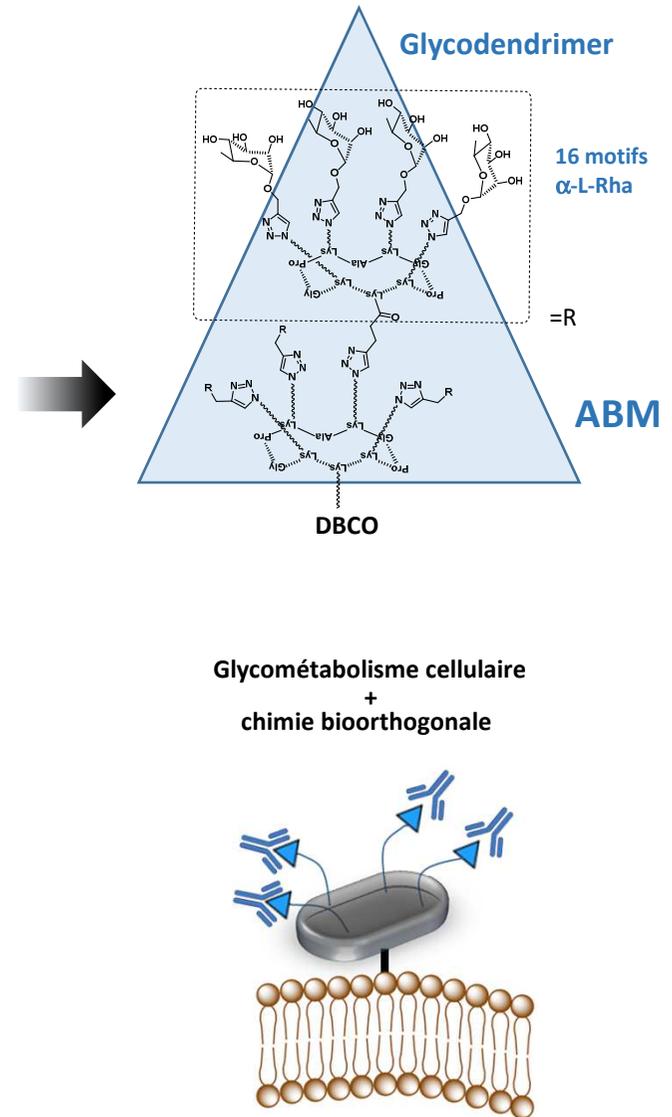
- Processus d'internalisation de l'ARM



Stratégie ARM « Bi-modulaire » « Antibody Recruiting Molecules »

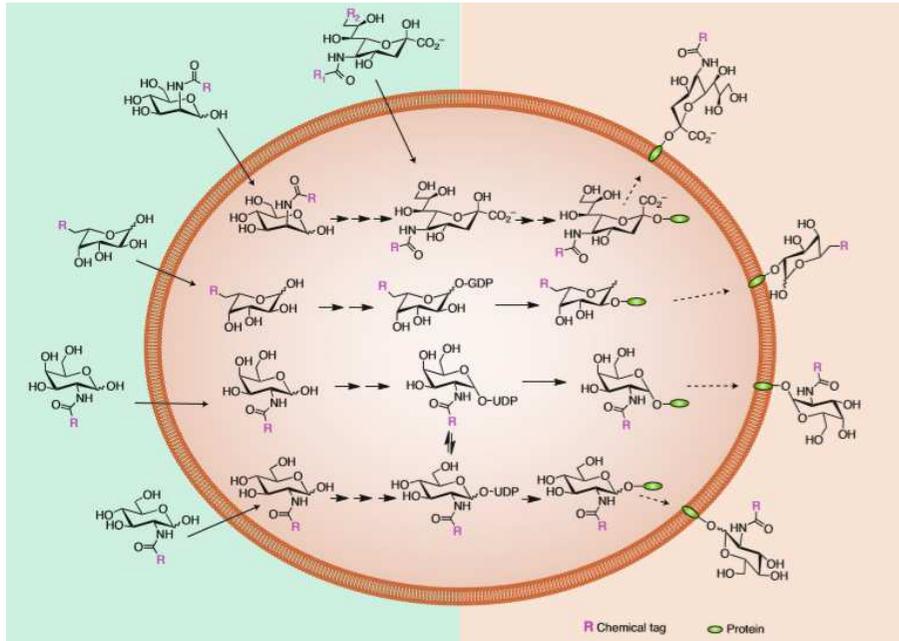


Fixation covalente de l'ABM à la surface de la cellule

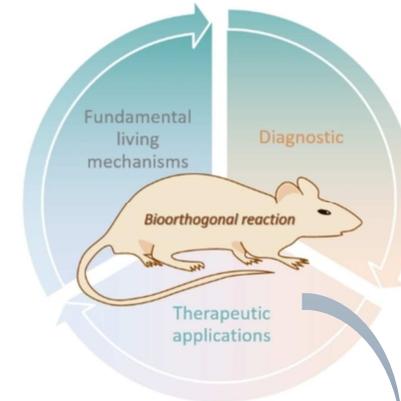


Marquage cellulaire par glycoingénierie métabolique et chimie bioorthogonale

Métabolisation de sucres modifiés

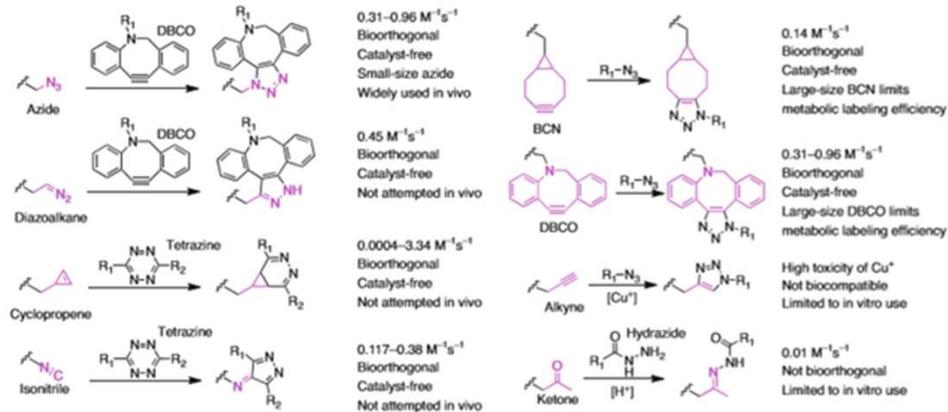


La chimie bioorthogonale
Les applications *in vivo*



K. Porte, M. Riberaud, R. Châtre, D. Audisio, S. Papot, F. Taran. Bioorthogonal reactions in animals. *ChemBiochem.* **2021**, *22*, 100-113.

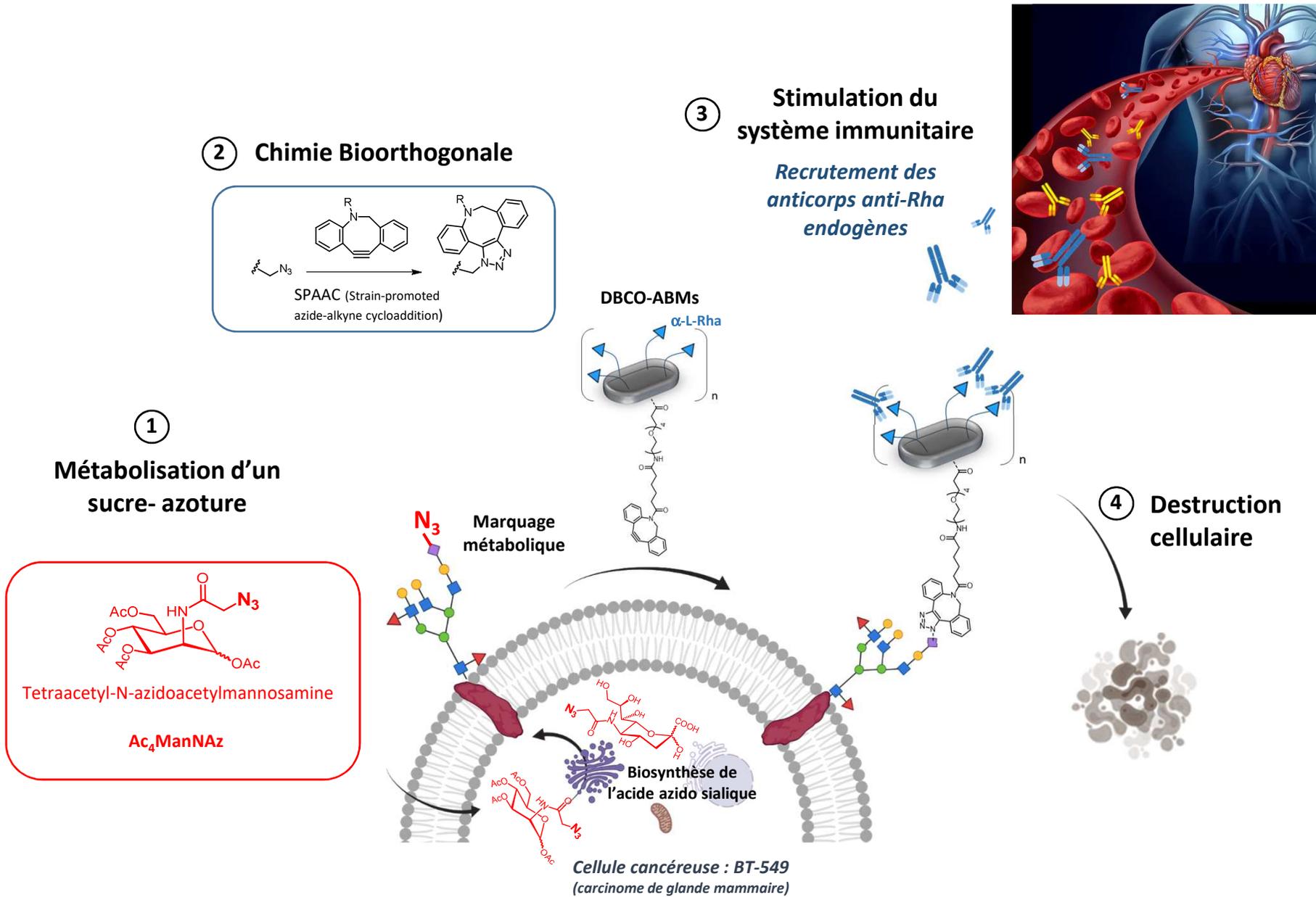
chimie bioorthogonale



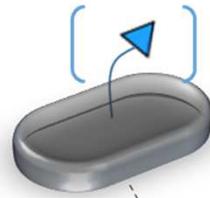
Applications antitumorale

- délivrance :
- Agents chimiothérapeutiques
 - Radioisotopes
 - Photosensibilisateurs
 - Agents photoacoustiques (guidage pour thérapies photothermiques)
 - Agents stimulant le système immunitaire

Marquage cellulaire par des glycodendrimers antigéniques : ABMs (*Antibody-Binding Module*)

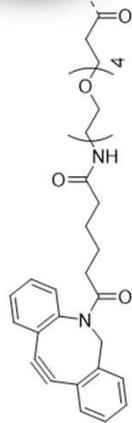


ABM
Antibody-Binding
Module

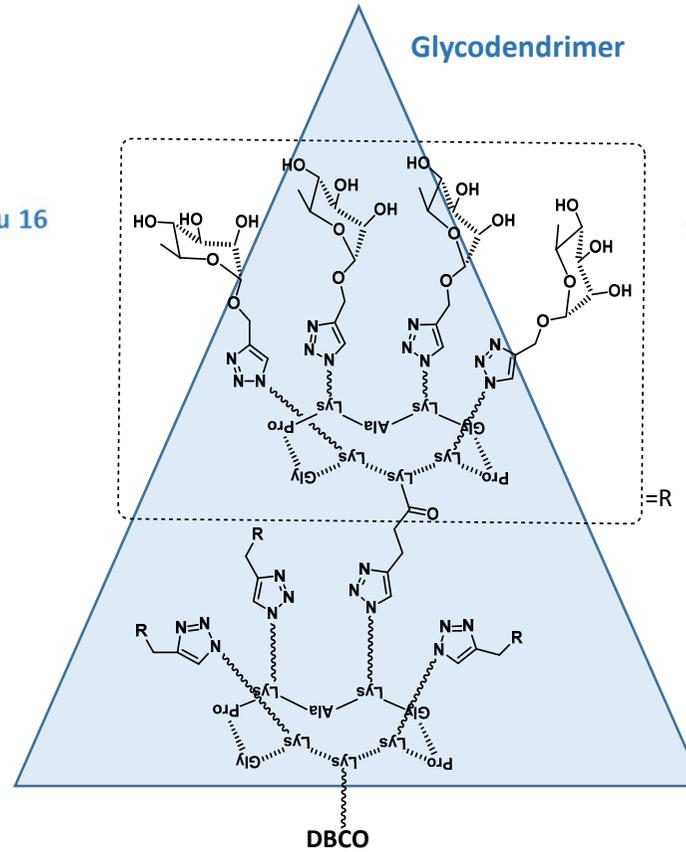


$n = 1, 4 \text{ ou } 16$

DBCO :



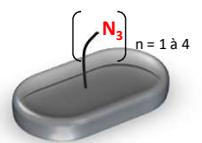
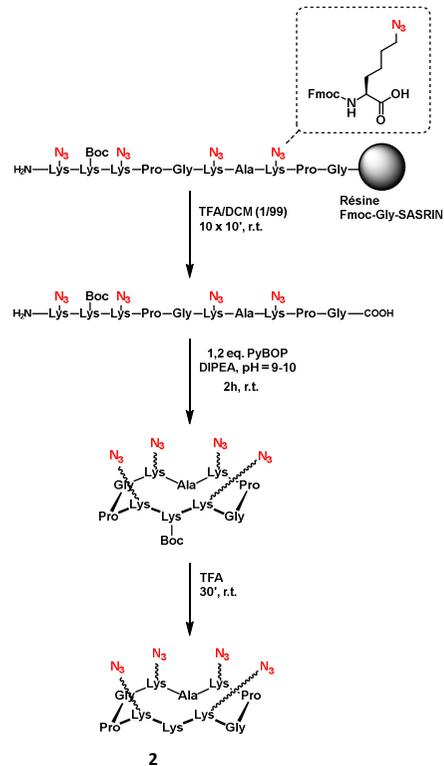
ABM
Glycodendrimer



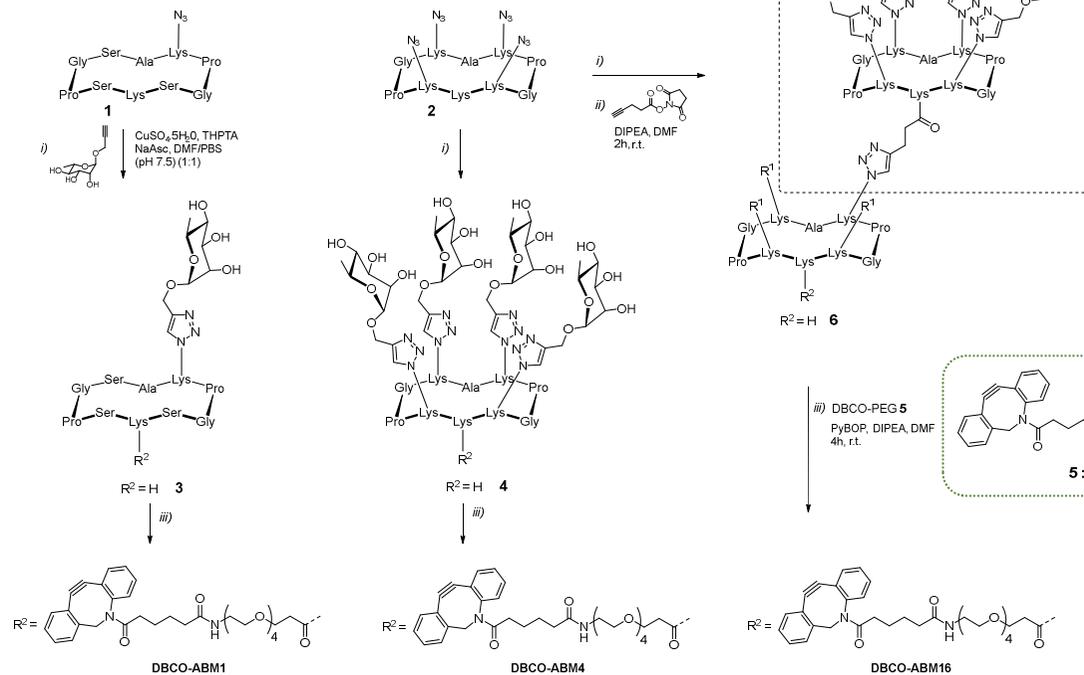
Anticorps
16 motifs α -L-Rha
Antigène



Synthèse du châssis moléculaire peptidique

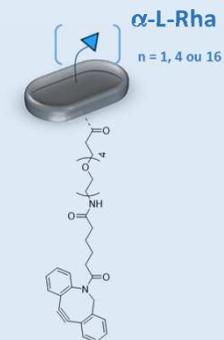
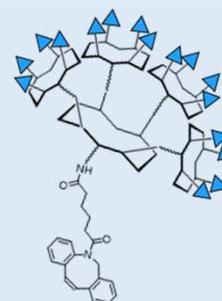
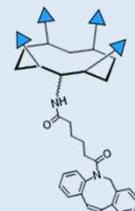
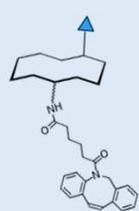


Synthèse des conjugués DBCO-ABMs



Dr. David Goyard

DBCO-ABMs



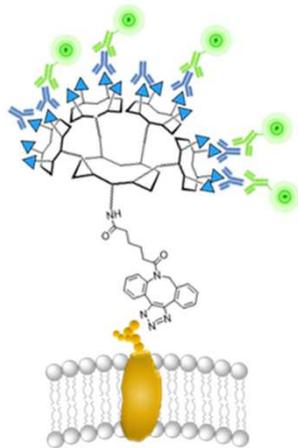
Evaluations Biologiques

Cellules BT-549

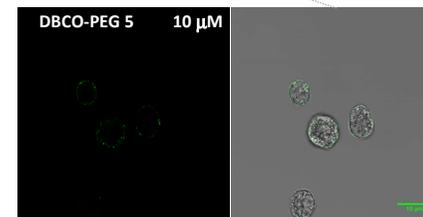
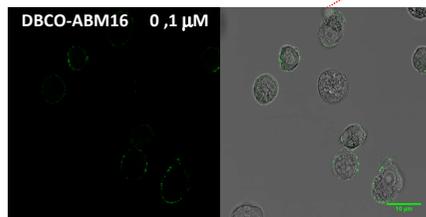
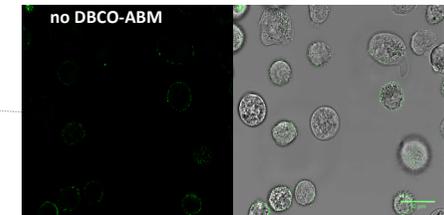
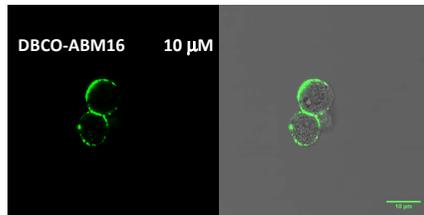
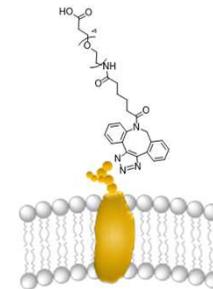
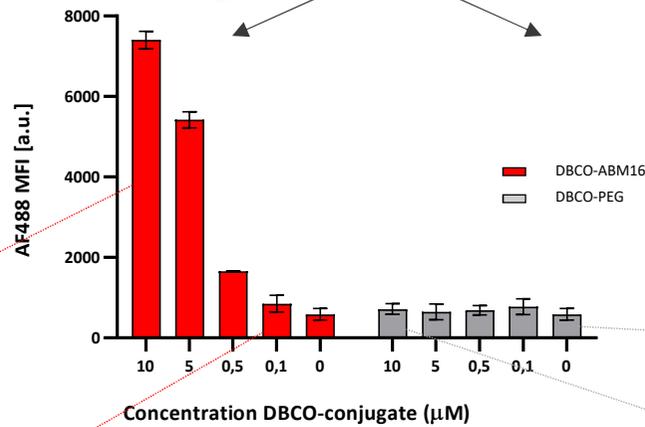
Couplage DBCO-ABM/ Azido surface cellulaire (SPAAC)



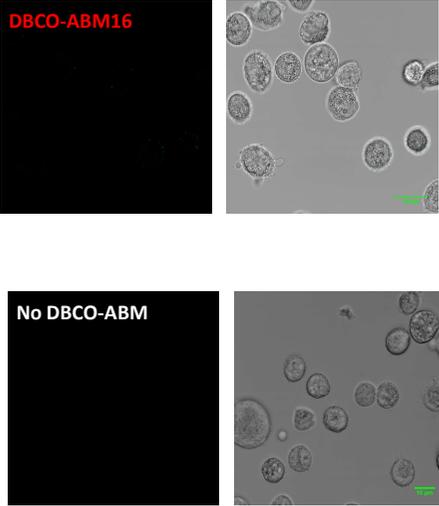
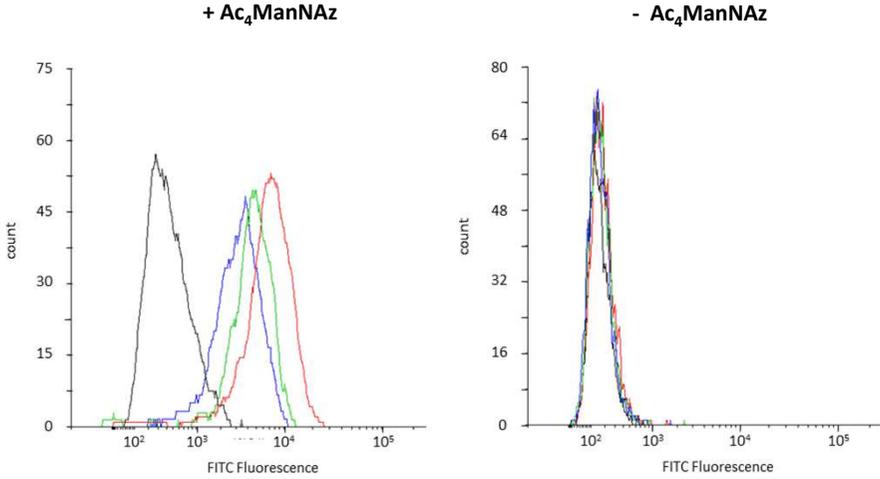
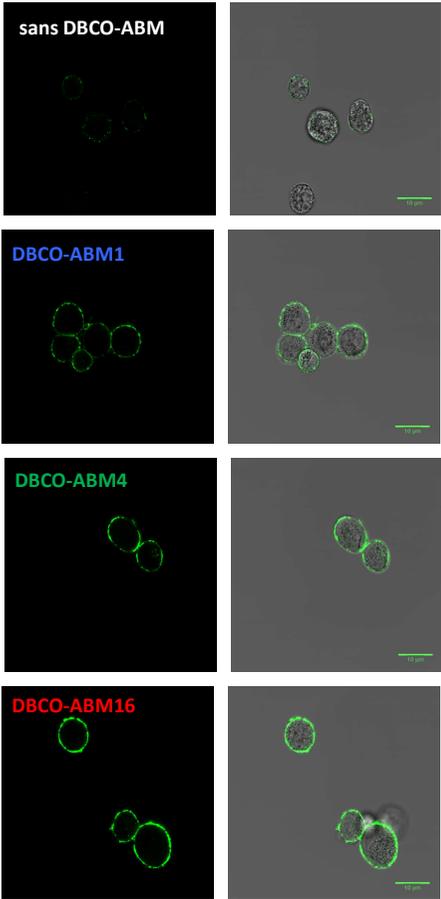
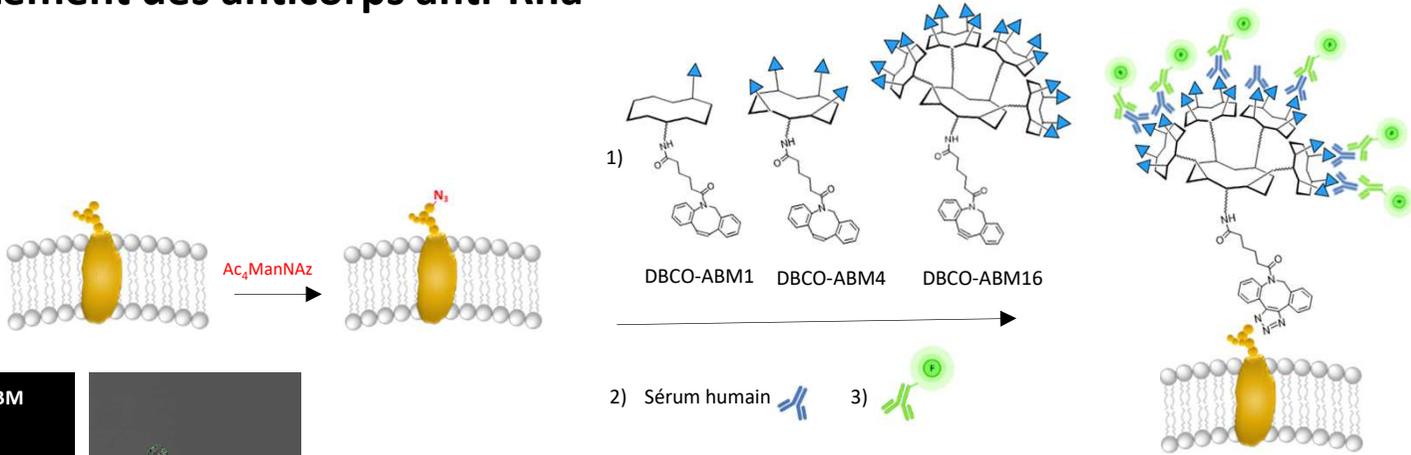
Cellules tumorales : BT-549
(carcinome de glande mammaire)

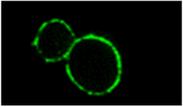


- 1) DBCO-ABM16
- 2) Atc anti-Rha (HS)
- 3)

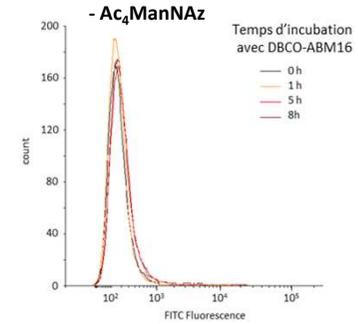
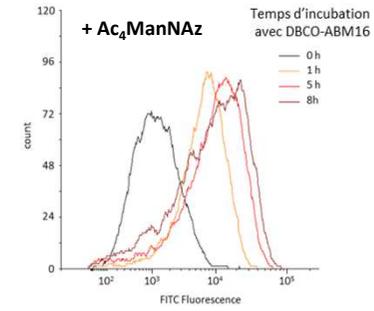
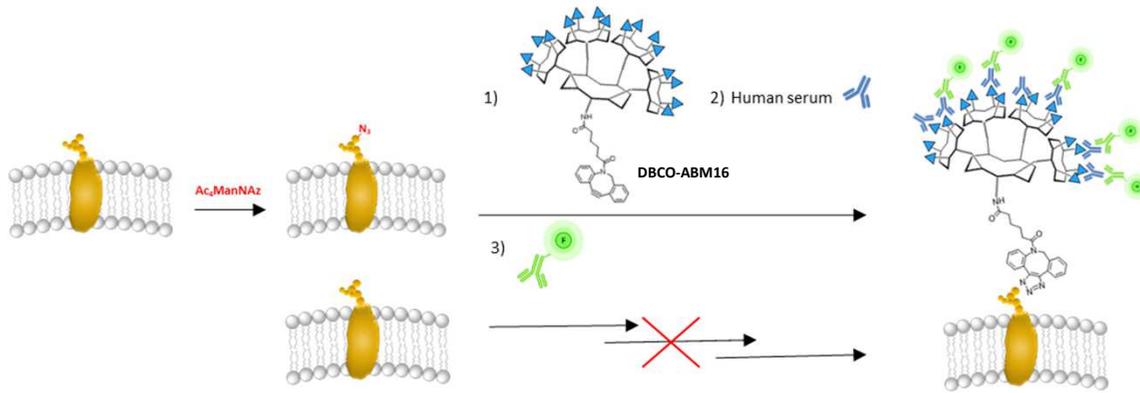


Influence de la multiplicité de présentation du motif α -L-Rha de l'ABM sur le recrutement des anticorps anti-Rha

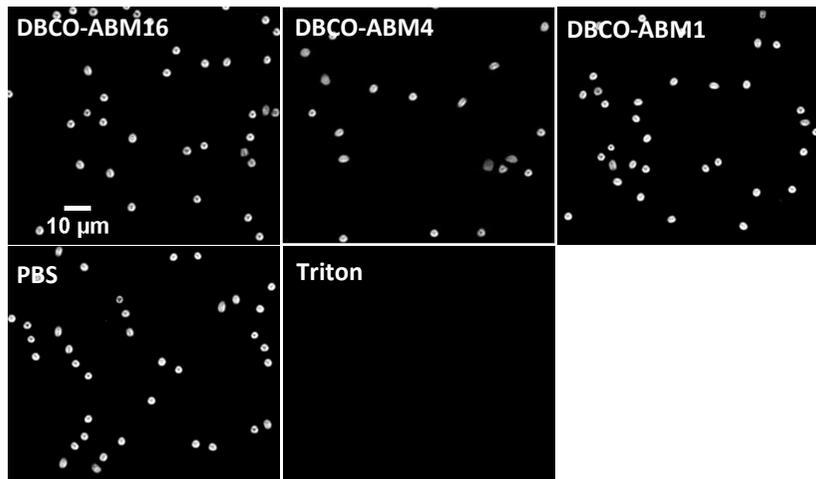




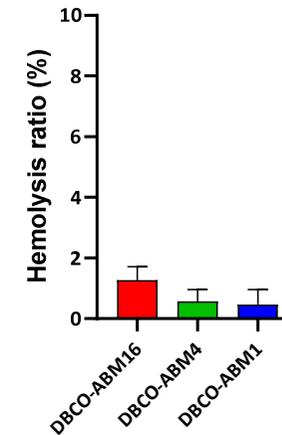
Stabilité de marquage cellulaire par l'ABM16

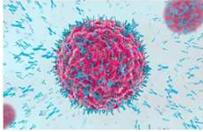


Compatibilité *in vitro* des conjugués DBCO-ABMs / cellules sanguines humaines (globules rouges)

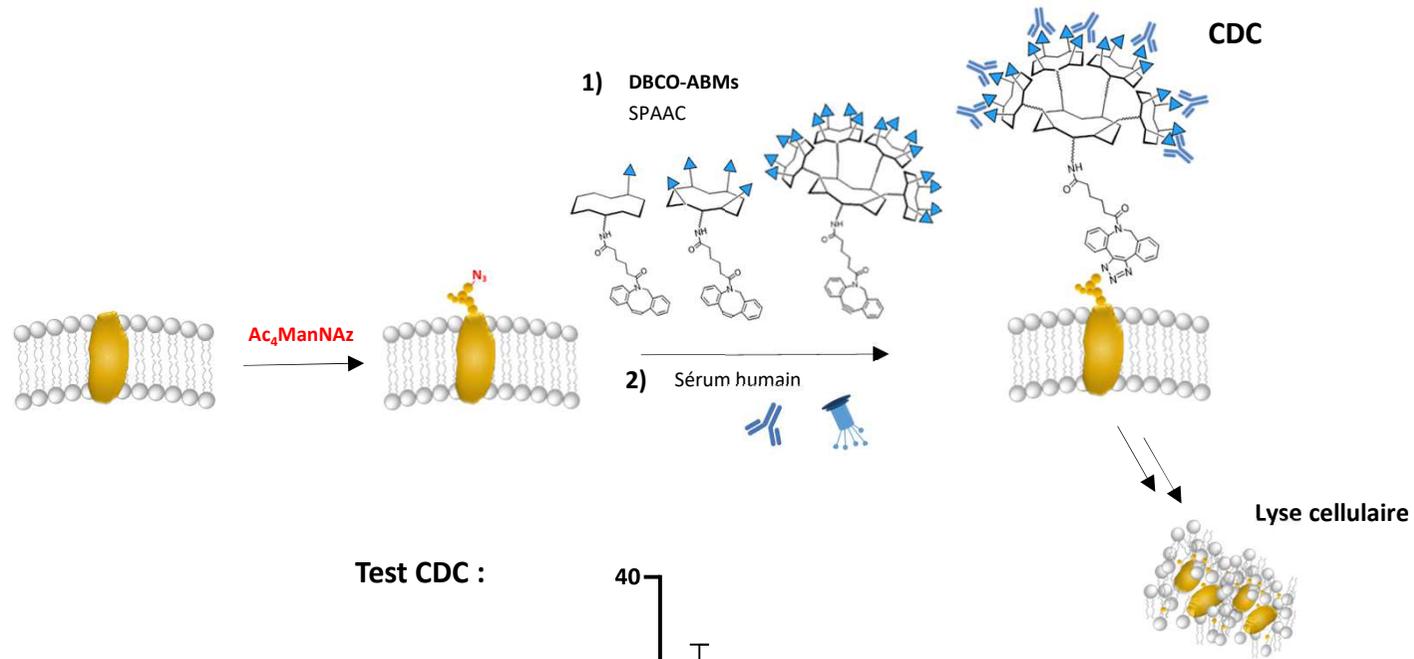


Test d'hémolyse

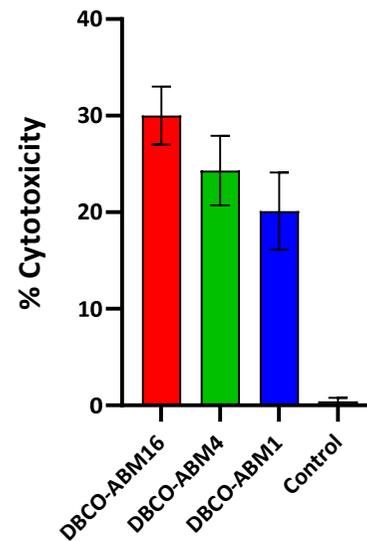




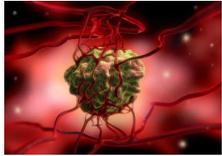
Cytotoxicité (CDC) induite par les ABMs exposés à la surface des cellules cancéreuses en présence de sérum humain.



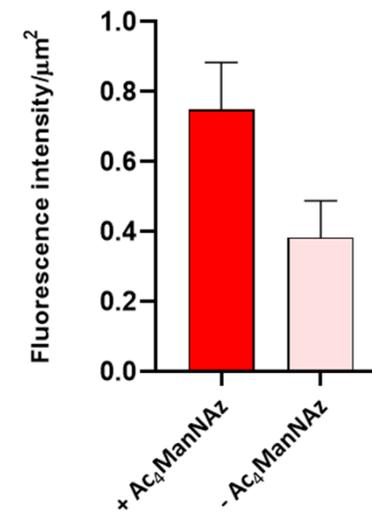
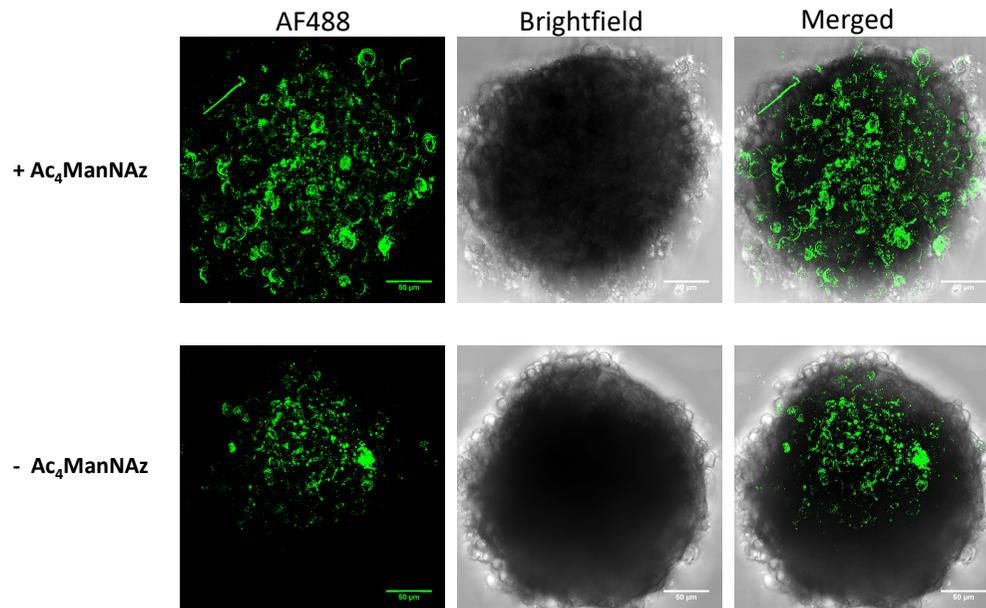
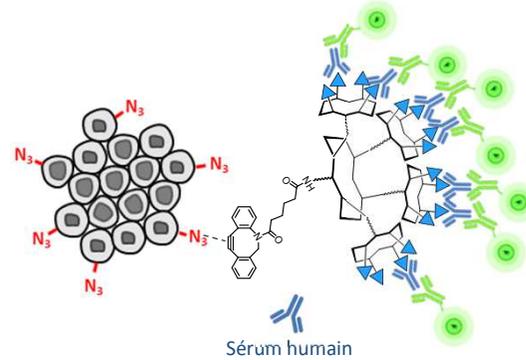
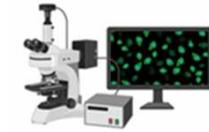
Test CDC :



Résultats en accord avec un meilleur recrutement des anticorps anti-Rha par l'ABM16.



Recrutement des anticorps à la surface de sphéroïdes de cellules cancéreuses (modèle de tumeurs solides).



- Efficacité de la combinaison «glycométabollisme cellulaire » + Chimie click bioorthogonale

➔ Décoration du glycocalyx cellulaire par des ABMs glycodendrimériques

- Efficacité sur modèle de tumeur solide

- Le greffage d'ABM à haute densité en Rhamnose conduit à une stimulation d'une réponse immunitaire cytotoxique

