Welcome to BCII lesson 13

Chimie Biologique II

Biological Chemistry II

BIO-213

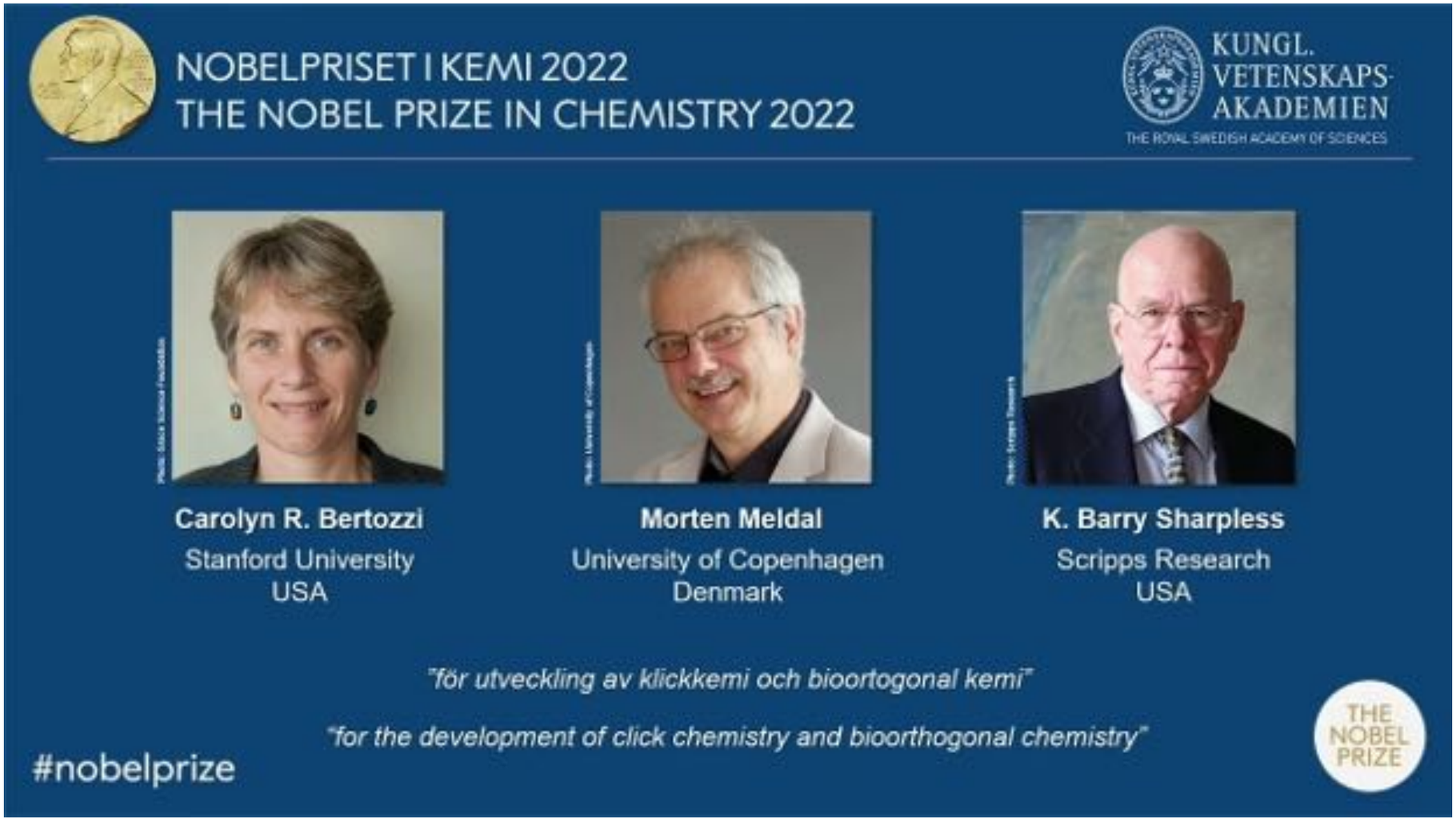
Teacher

Giovanni D’Angelo, IBI

Lecture 13

Chemical biology approaches to study biomolecules

A new way of thinking chemical reactions – Nobel price for chemistry in 2022



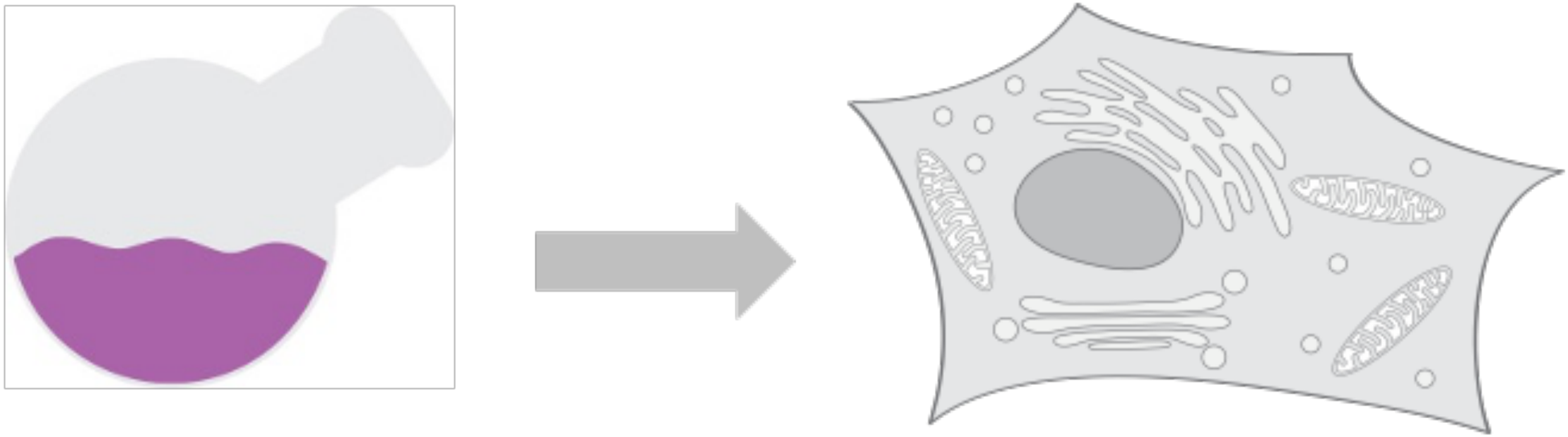
Why do we need bioorthogonal chemistry?

Most biological questions involve:	**Approaches to address these questions:**
• **Localisation** of a biomolecule or changes in localisation upon definded cues	• Genetically encoded or self-labeling tags (proteins), antibodies, dyes (DNA)
• **Identification** of biomolecules	• Mass spectrometry (proteomics, lipidomics, metabolomics) or sequencing (DNA, RNA)
• **Perturbations, modulation** of biomolecules (synthesis, degradation, modifications)	• Drugs/inhibitors (proteins), overexpression/knockout (proteins, DNA)

There are a lot of tools for proteins and DNA, however very little is available to study RNA, sugars, lipids and other metabolites.

Chemical biology can help here!

Chemistry in living systems - Bioorthogonal chemistry



Reaction flask

- Controll over all reaction parameters (temperature, concentration of educts, mixing…)
- Liberty to chose reaction conditions

Cell

- Little to no control over reaction parameters
- Presence of all sorts of functional groups
- Aqueous environment…

Chemistry in living systems - Bioorthogonal chemistry

In 2003, Carolyn R. Bertozzi coined the term **Bioorthogonal Chemistry:**

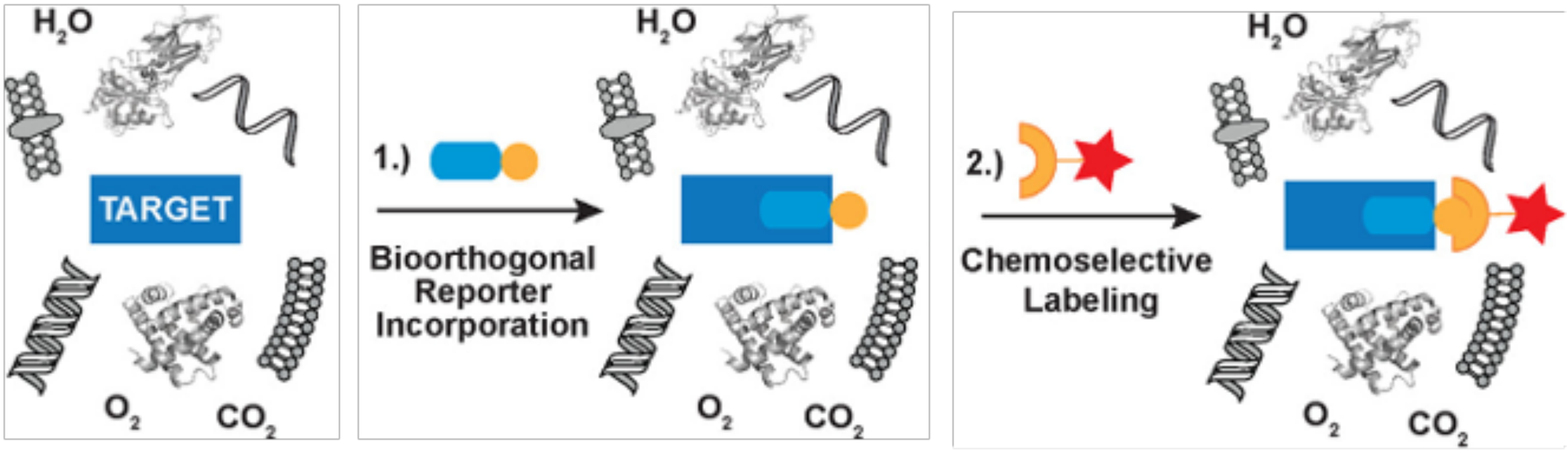
"Any chemical reaction that can occur inside of living systems without interfering with native biochemical processes"

IL Niklas Elmehed © Nobel Prize Outreach

- occurs in physiological environments
- high yields
- not affected by water, endogenous nucleophiles, electrophiles, reductants, or oxidants found in complex biological environments
- fast and forms stable reaction products
- involves functional groups not naturally present in biological systems

Chemistry in living systems - Bioorthogonal chemistry

The idea: A **defined target molecule** in a complex environment reacts selectively with a **reporter**, that can then be used for **visualisation** or **purification**.



This requires the introduction of a **bioorthogonal handle** into the target molecule and the **successful coupling** of the target molecule and the reporter.

The click-chemistry principle

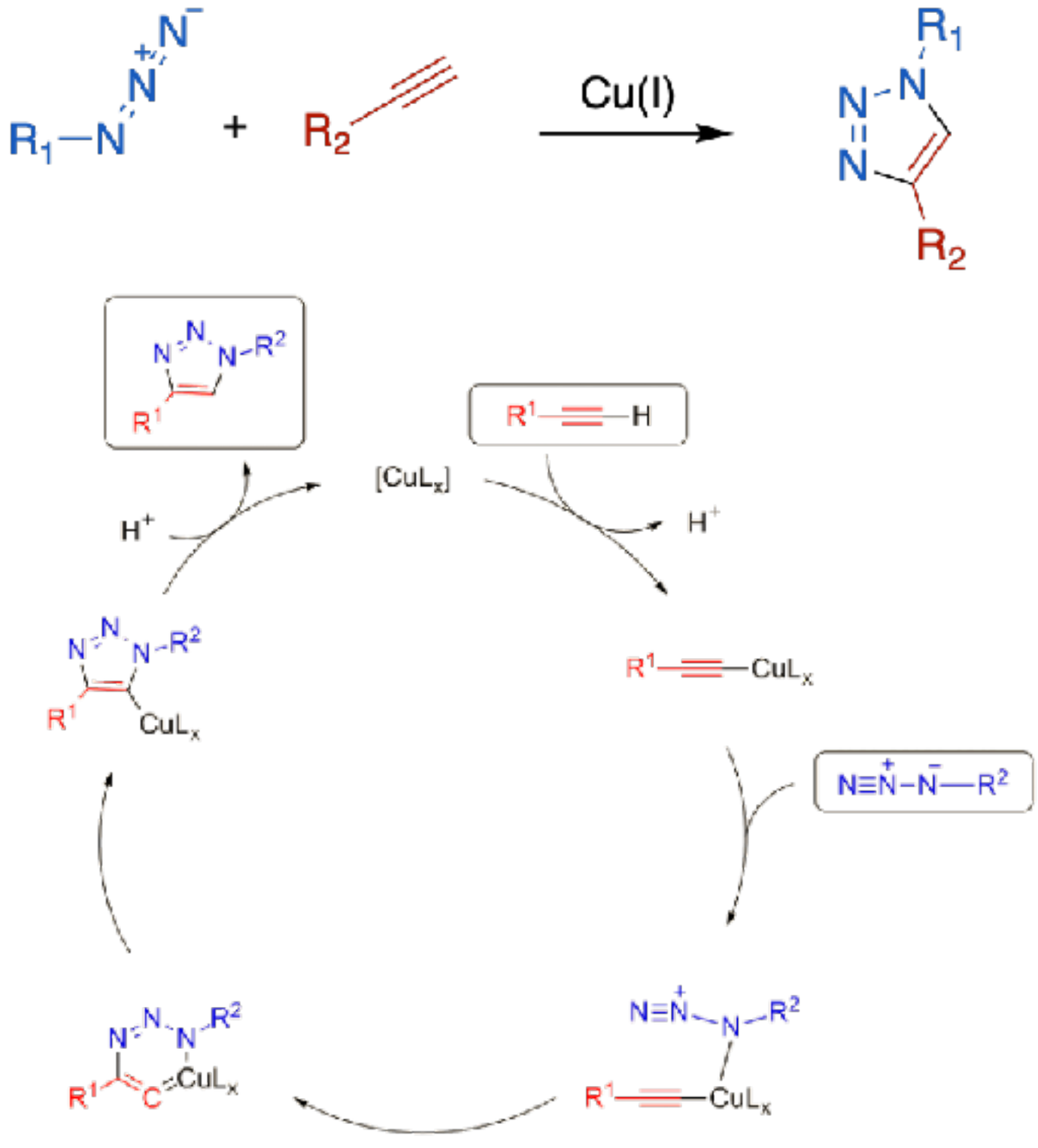
In 2001, inspired by nature, K. Barry Sharpless coined the term **Click Chemistry**

The concept includes chemical reactions with the following **criteria**:

- high yields
- wide in scope
- use readily available starting materials and reagents
- create only byproducts that can be removed without chromatography
- are stereospecific
- simple to perform
- can be conducted in easily removable or benign solvents such as water
- have high atom economy

Ill. Niklas Elmehed © Nobel Prize Outreach

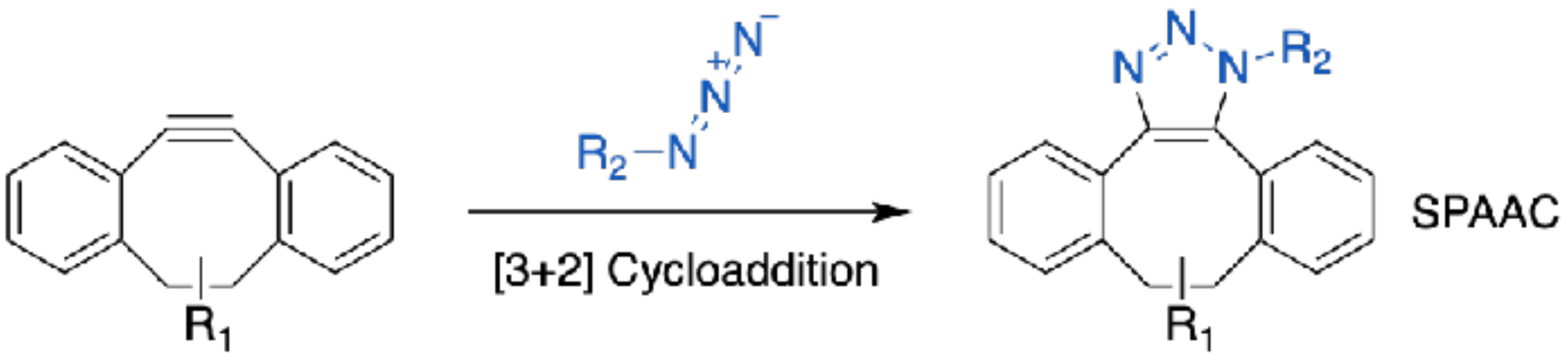
Copper(I)-catalysed azide-alkyne cycloaddition (CuAAC)



Ill. Niklas Elmehed © Nobel Prize Outreach

Strain-promoted azide-alkyne cycloaddition (SPAAC)

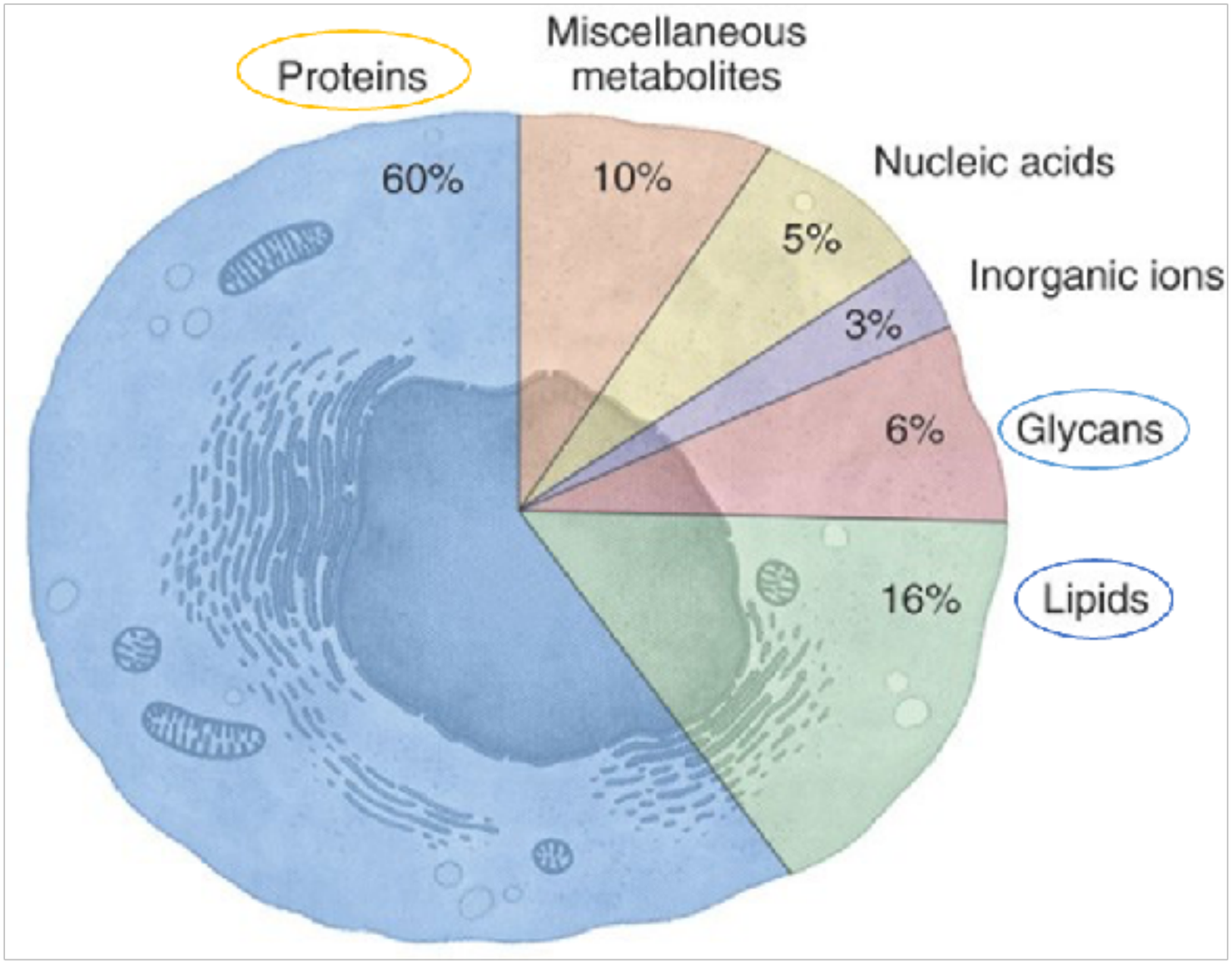
… SPAAC was developed by Bertozzi to remove the copper catalysts and to improve kinetics of copper-free azide-alkyne cycloaddition reactions



Ill. Niklas Elmehed © Nobel Prize Outreach

How to get the bioothogonal handle into living systems?

For a **bioorthogonal reaction *in vivo***, one of the reaction partners must be integrated into the target biomolecule → **The biorthogonal handle**

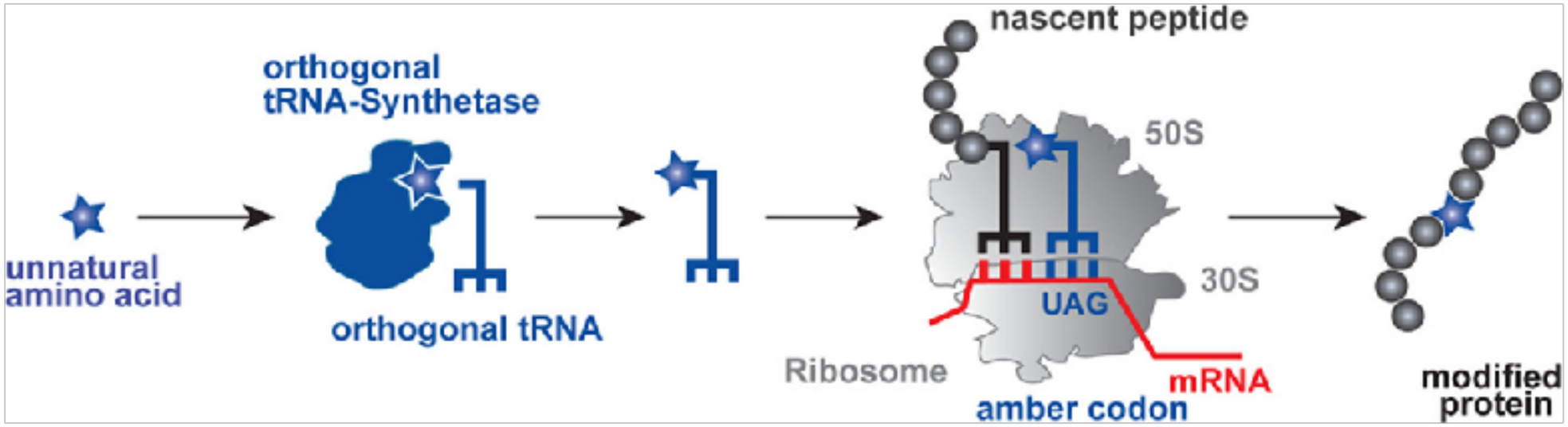


Bioorthogonal handles on proteins

- **Noncanonical amino acids (ncAA)** are amino acids not normally found in proteins
- ncAA bear functional groups that form one reactant in the bioorthogonal reaction pair
- Two approaches for the incorporation of ncAA: **residue-specific** and **site-specific**
- Residue-specific incorporation is the **substitution of a natural amino acid** with an unnatural one (**can replace every occurrence** of the natural amino acid in the protein)
- Site-specific incorporation **targets a ncAA to a specific location** in the protein (expanded genetic code)

Bioorthogonal handles on proteins: Site-specific

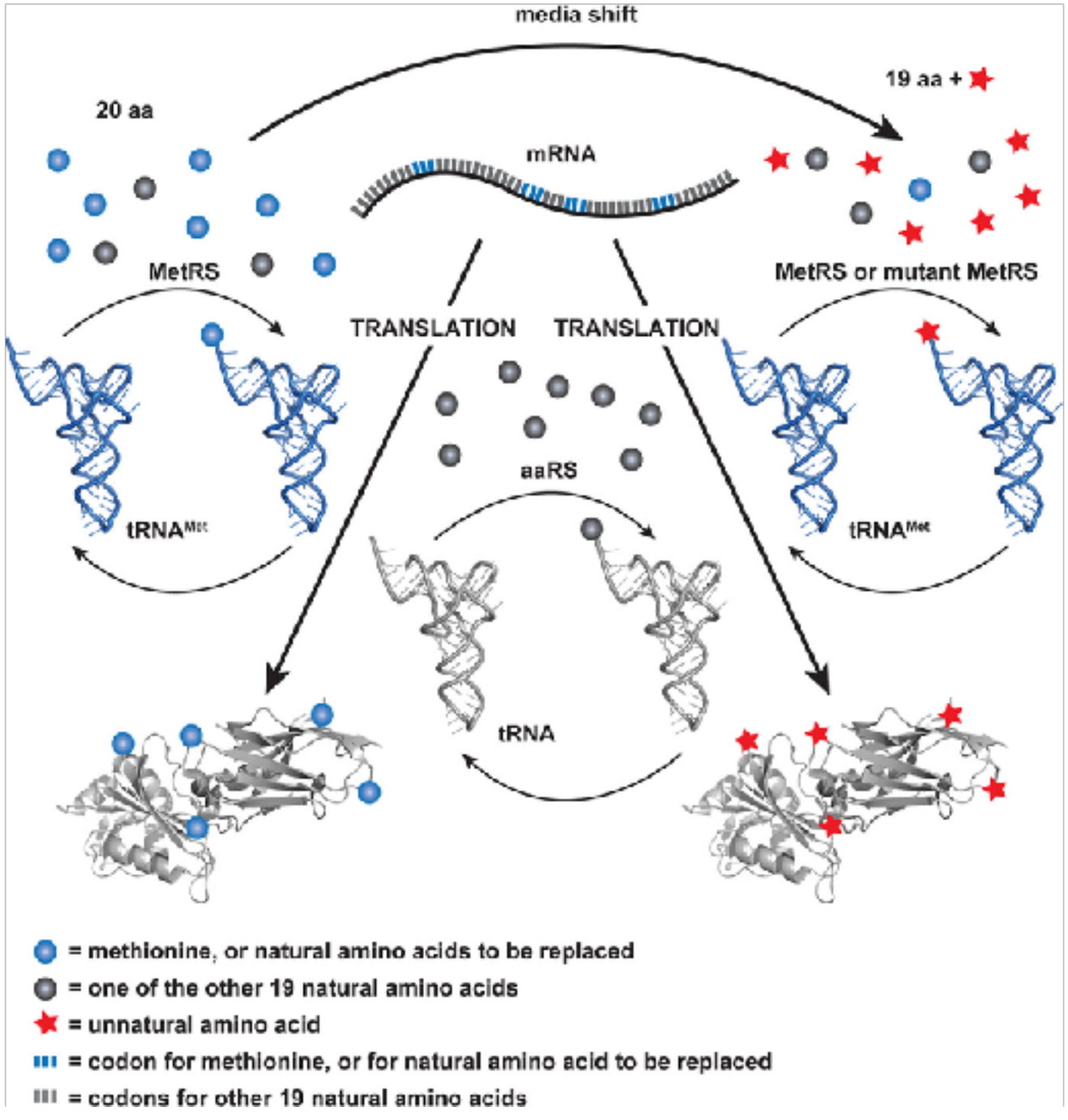
An aminoacyl-tRNA synthetase/tRNA pair is used to insert the unnatural amino acid into the protein using an **amber stop codon (UAG)** on the mRNA → **genetic code expansion**



Genetic code expansion requires:

- an **orthogonal aminoacyl-tRNA synthetase/tRNA pair**,
- a **blank codon (amber stop codon)** to encode unnatural amino acid incorporation
- methods to **convert the specificity of the aminoacyl-tRNA synthetase active site** to transfer the unnatural amino acid, but no natural amino acids

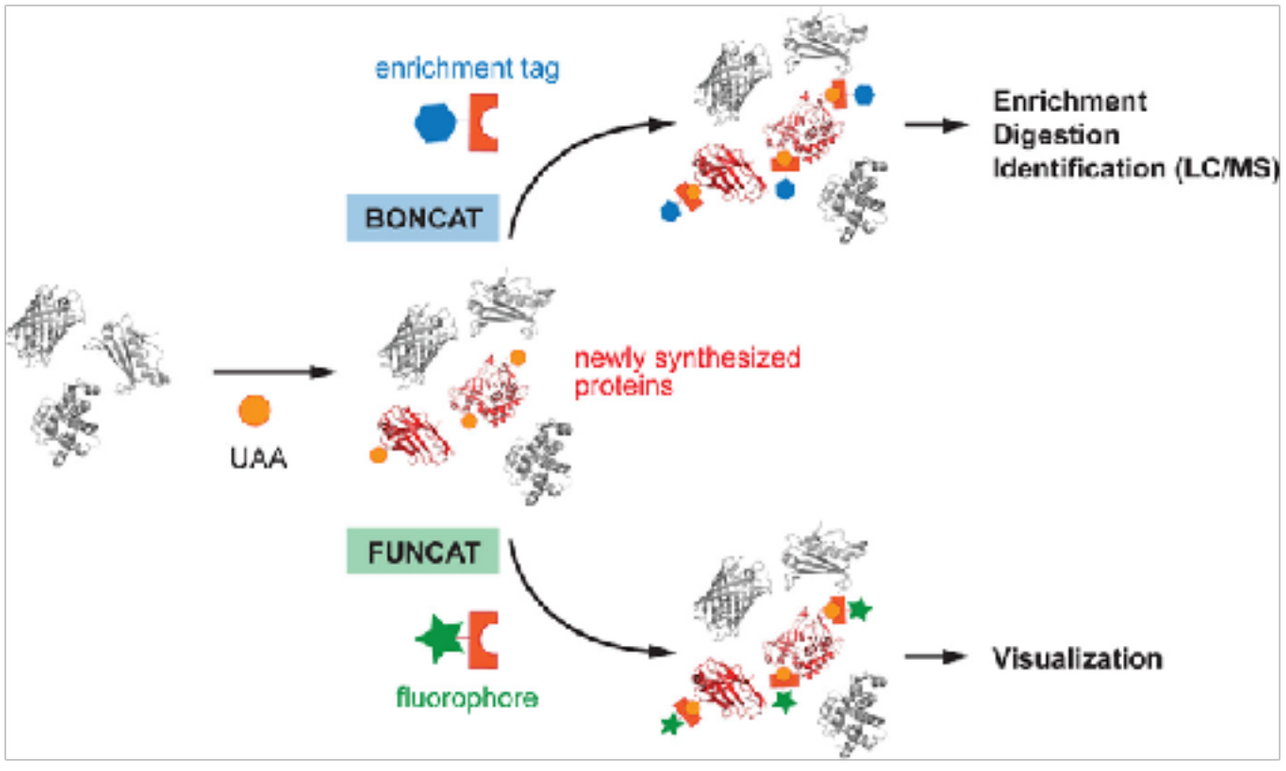
Bioorthogonal handles on proteins: Residue-specific



- natural mRNA contains codons for the 20 natural amino acids (gray).
- Methionine (blue sphere, corresponding blue codons on mRNA) is replaced with an unnatural amino acid (red star).
- medium shift removes methionine, in an auxotrophic strain, and introduces the unnatural amino acid (red star) together with the other 19 natural amino acids (gray spheres).
- the unnatural amino acid is charged to $tRNA^{Met}$, and the unnatural amino acid is incorporated into proteins in place of methionine.

Profiling time-resolved protein synthesis: BONCAT and FUNCAT

These methods uses residue-specific incorporation of unnatural AA to enrich **and identify newly synthesized proteins** by LC/MS or visualization

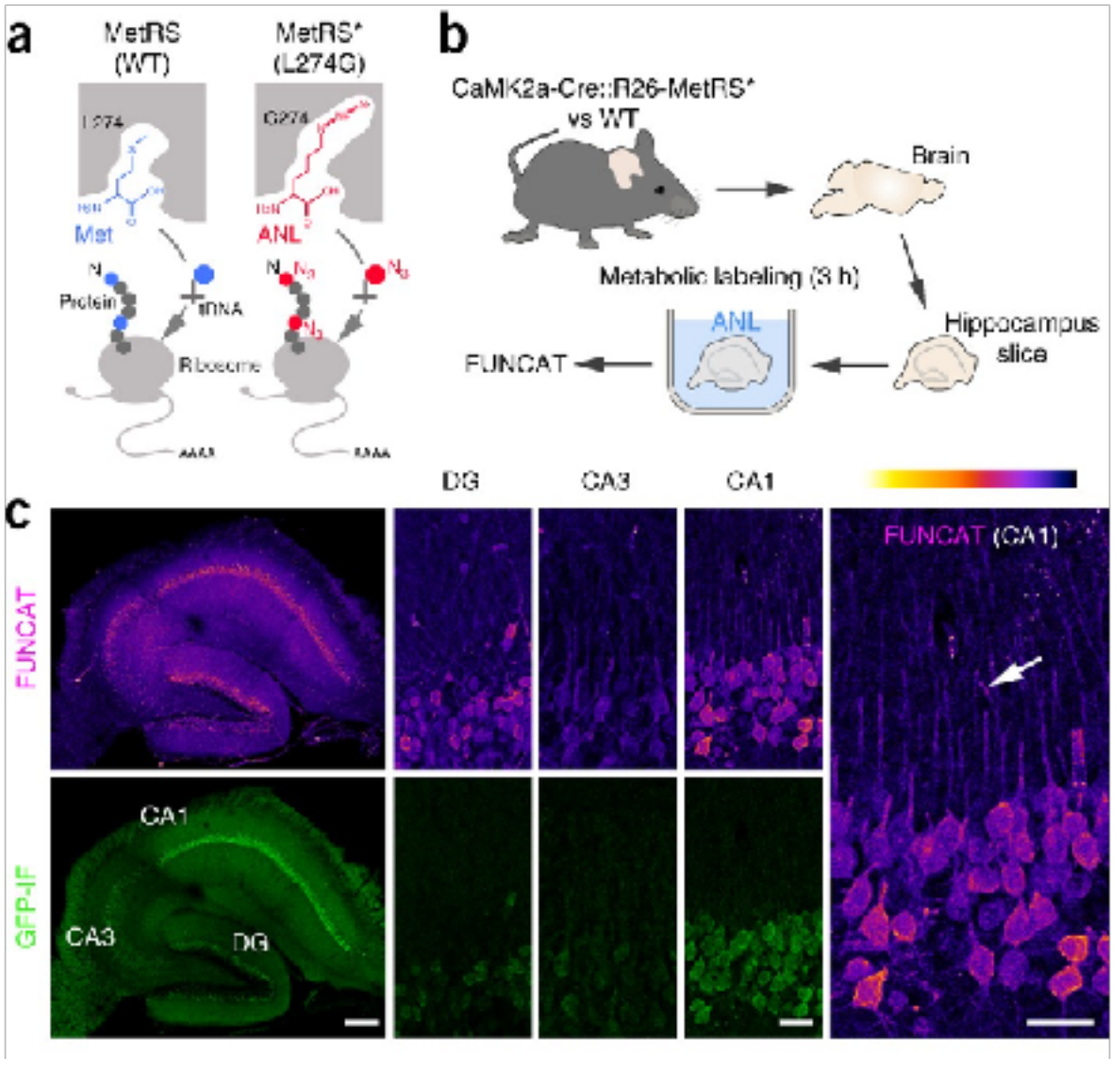


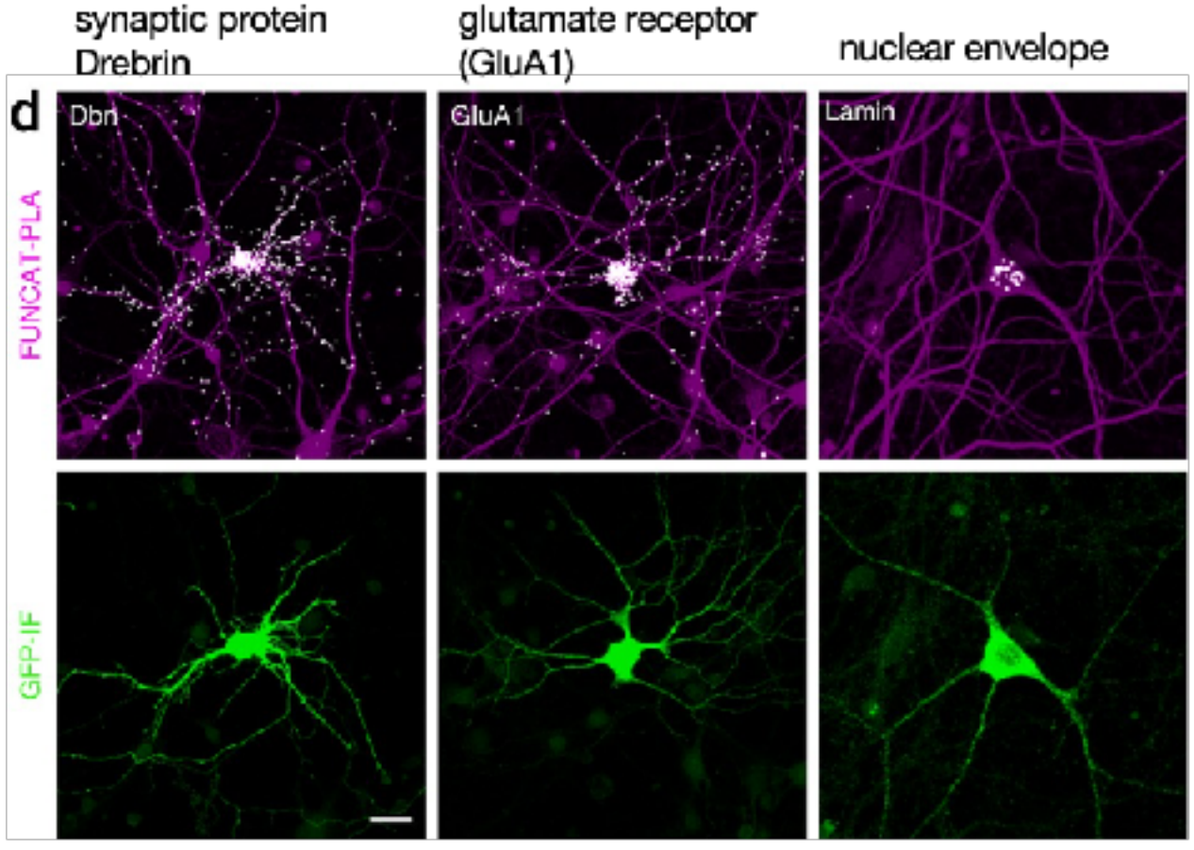
BONCAT allows to identify newly synthesized proteins

FUNCAT allows labelled proteins to be visualized by fluorescence microscopy or gel-band imaging methods

BONCAT (bioorthogonal noncanonical amino acid tagging), FUNCAT (fluorescent noncanonical amino acid tagging)

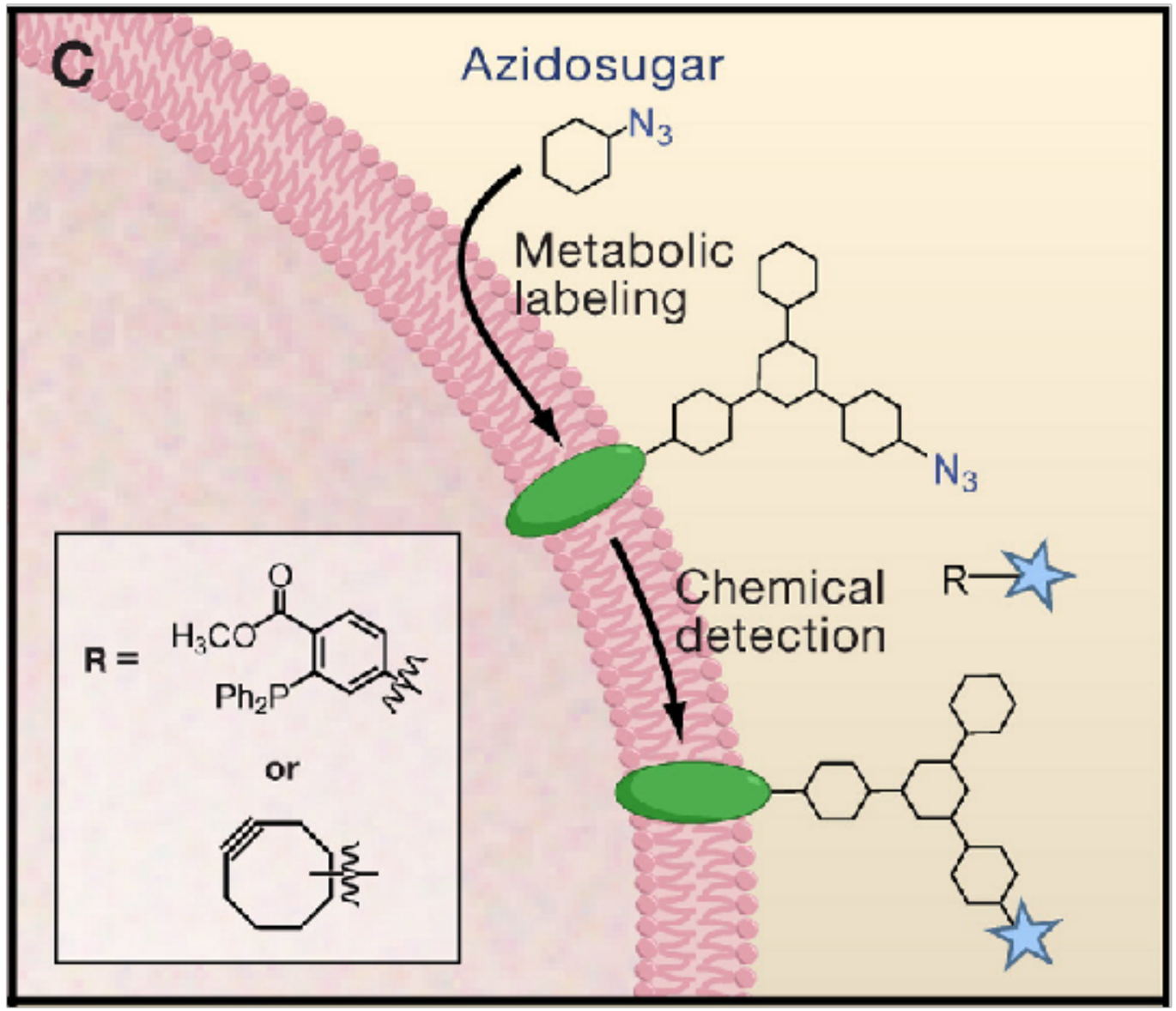
Cell-type-specific metabolic labelling of nascent proteomes *in vivo*





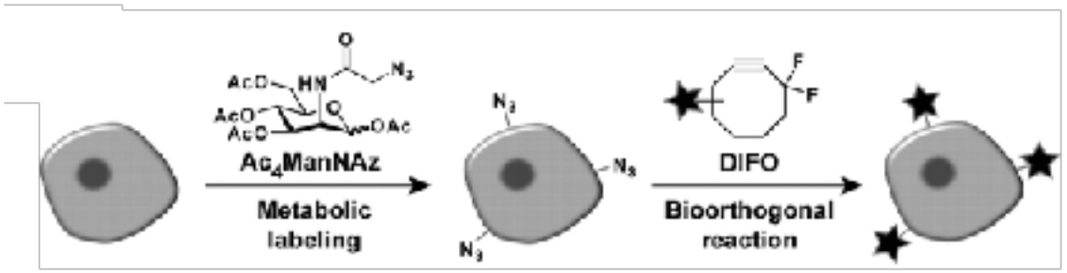
… more than 200 proteins are differentially regulated in hippocampal excitatory neurons depending on exposing mice to an environment with enriched sensory cues…

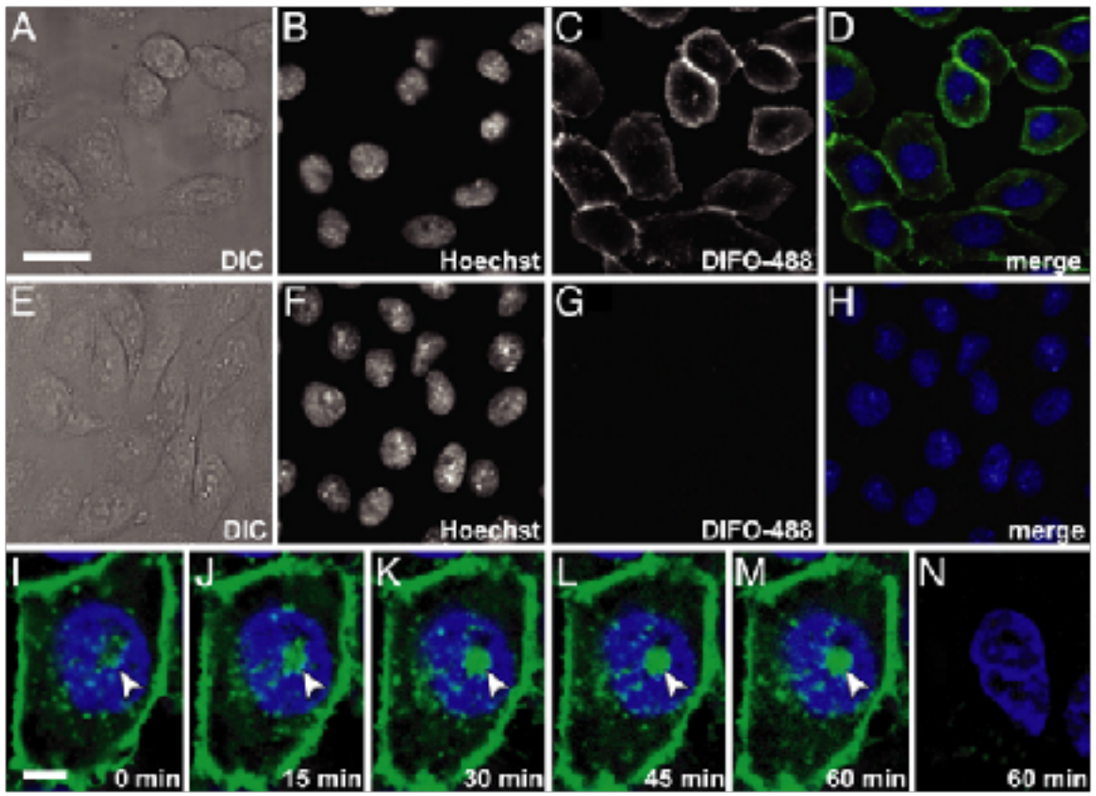
Bioorthogonal chemistry for sugar and glycan imaging



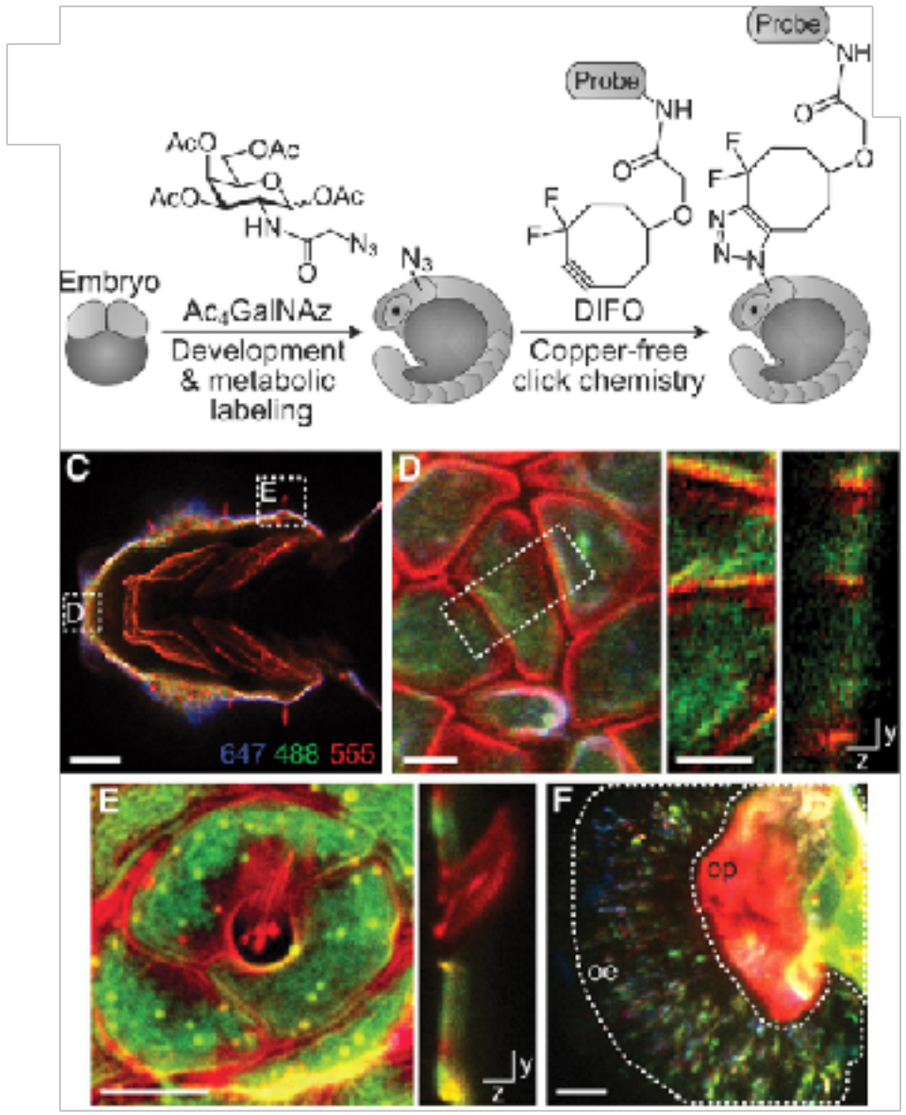
- **Synthetic azidosugars** are fed to the cell and get incorporated into cellular glycans by the cell's metabolic machinery
- Once on the cell surface, the azidosugars are detected
- Depending on the probe (e.g., fluorophore or affinity tag), this can be used to **image** or **enrich glycoconjugate subtypes**

Visualizing glycans in cells and developing zebrafish





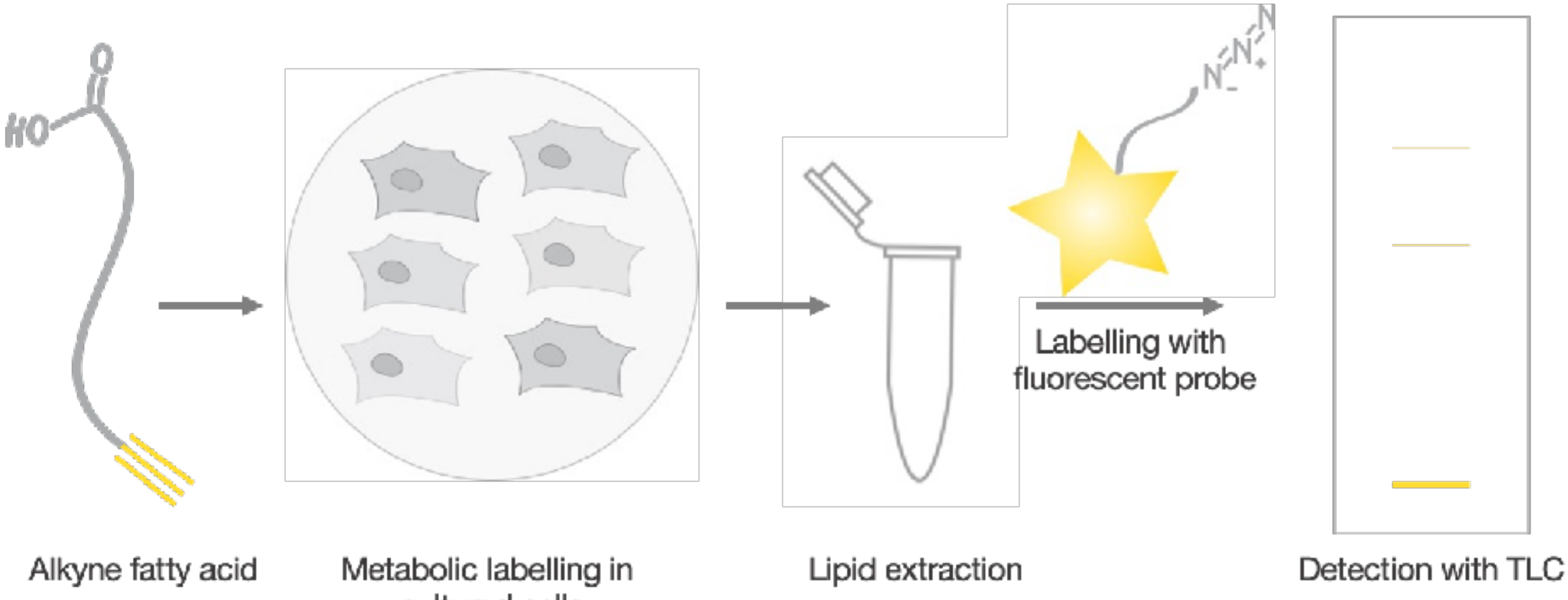
… the synthetic azidosugar is incorporated into the cellular glycans on the cell surface and can there be reacted with a dye using click chemistry (SPAAC)…



… **sequential pulses** of the same azidosugar and subsequent labelling reveals **differences in the cell-surface expression, intracellular trafficking, and tissue distribution of glycans** throughout zebrafish embryogenesis…

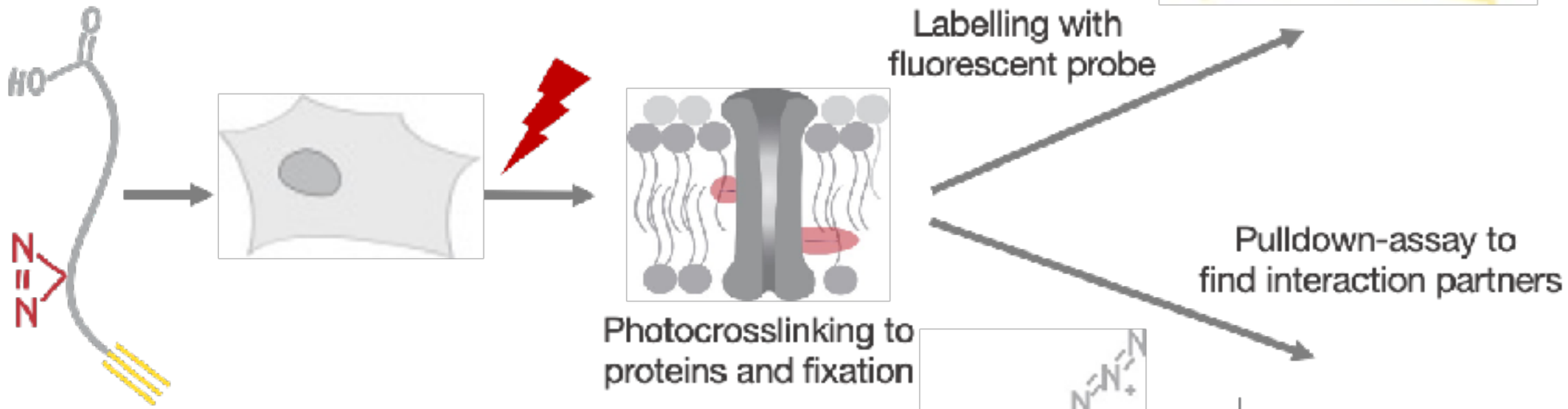
Bioorthogonal chemistry for lipids: Tracing lipid metabolism

- A click handle can be intrduced by using synthetic lipids that are fed to the cells
- Extracting the lipids from the cells at different time points can provide snapshots of lipid metabolism by visualizing the lipids on TLCs with a clickable dye
- Can also be used to study metablolizing enyme activities in *in vitro* systems

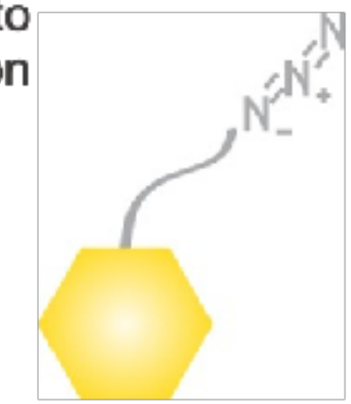


Bioorthogonal chemistry for lipid imaging

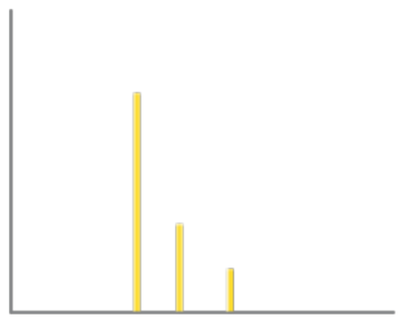
- The addition of a photoactivatabel diazarine allows to fix the lipid to the protein scaffold
- subsequent visualization of the lipid by clicking a fluorophore to the alkyne



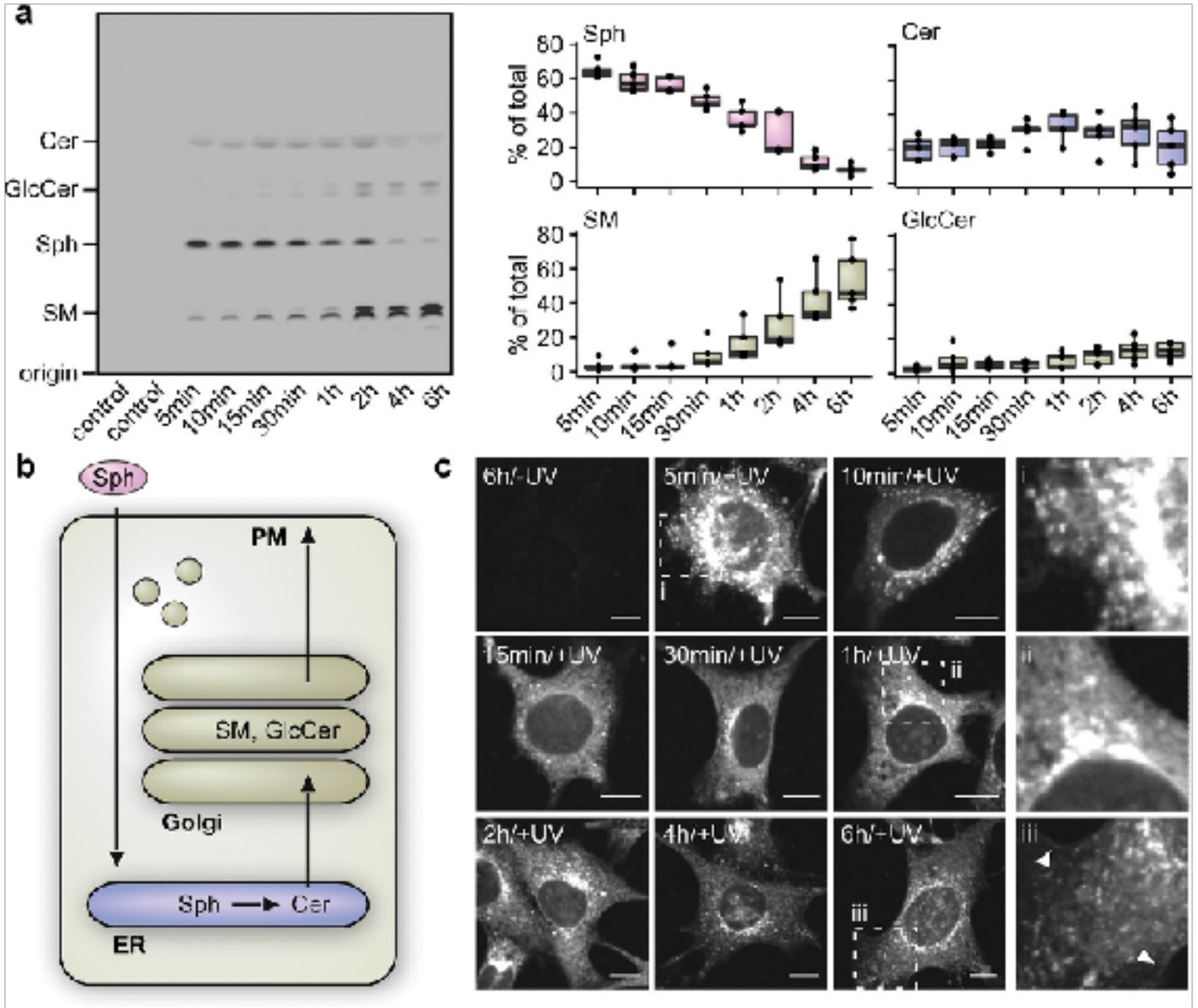
- The probes can also be used to study the interactome of lipids by analysing the lipid-protein conjugates
- For this the lipid-protein conjugates are enriched using an affinity tag such as biotin



Affinity tag (biotin)



Metabolic fate of sphingosine (pacSph)

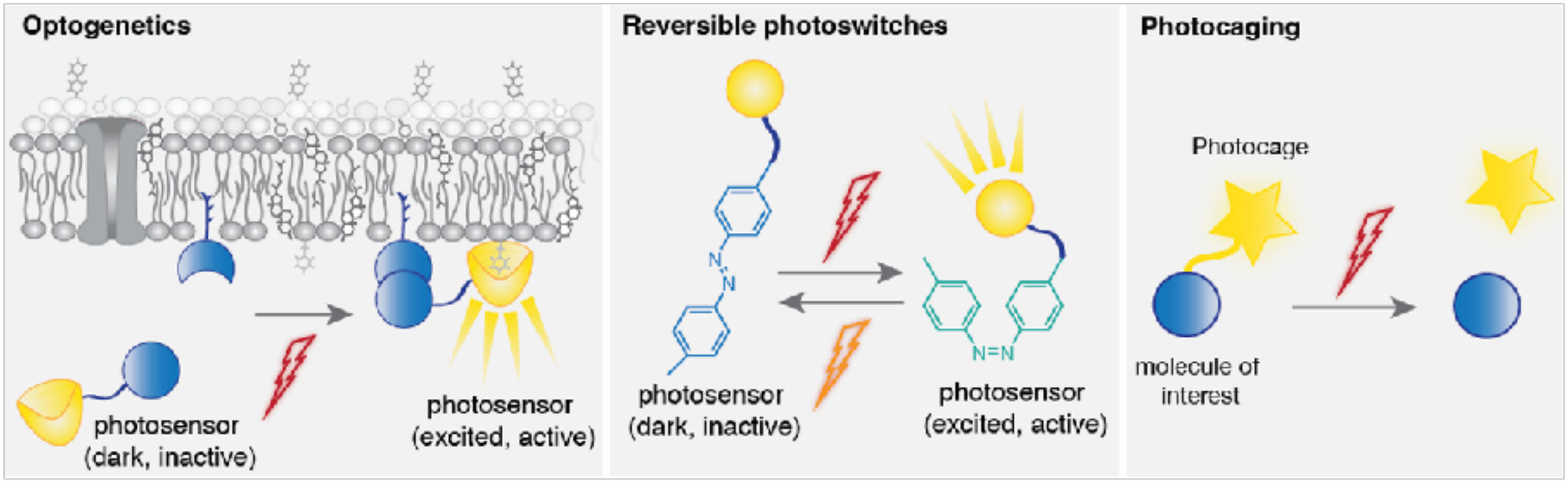


- a quantitative analysis of *de novo* synthesized sphingolipids by visualization and proteomic analysis of their interacting proteins.
- metabolic flux of *de novo* synthesized sphingolipids can be followed in time and space
- new insights into the transport and metabolism of sphingolipids

Break

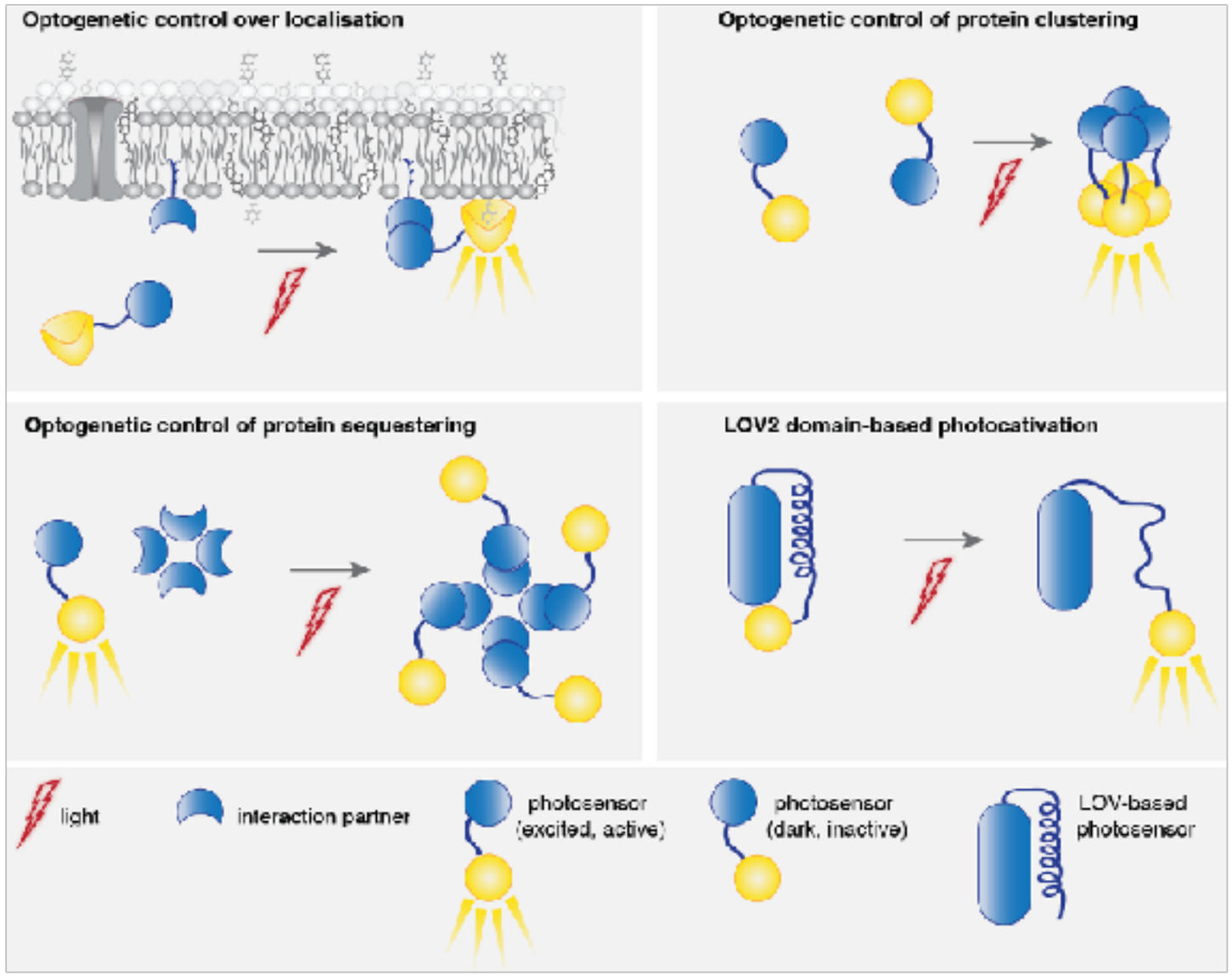
Light-triggered tools: Overview

- Irreversible photocleavage, reversible photoswitching, and genetically encoded systems are all methods to manipulate biomolecules using light as a trigger
- Systems which can be regulated by using light as a trigger can be used for very sophisticated experiments to study a system with regard to space, time, or extent of activation.



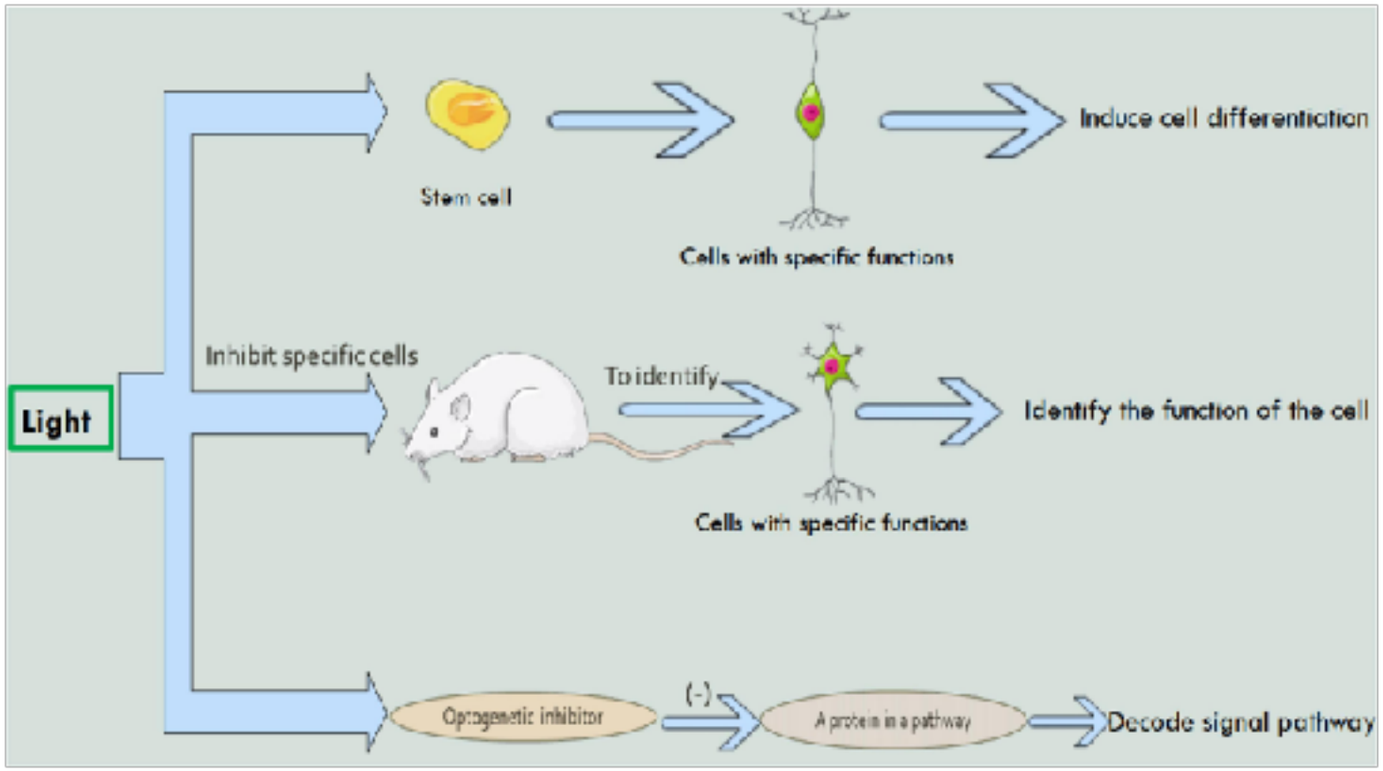
Optogenetics - Concept

In optogenetics, **exogenous genes coding for light-sensitive proteins** are expressed in cells, and **illumination is used to alter cellular behaviour**. Optogenetics involves the development of light-sensitive proteins, strategies for delivering their genes to specific cells, targeted illumination and finally, compatible readouts for reporting on changes in cell, tissue and animal behaviour.

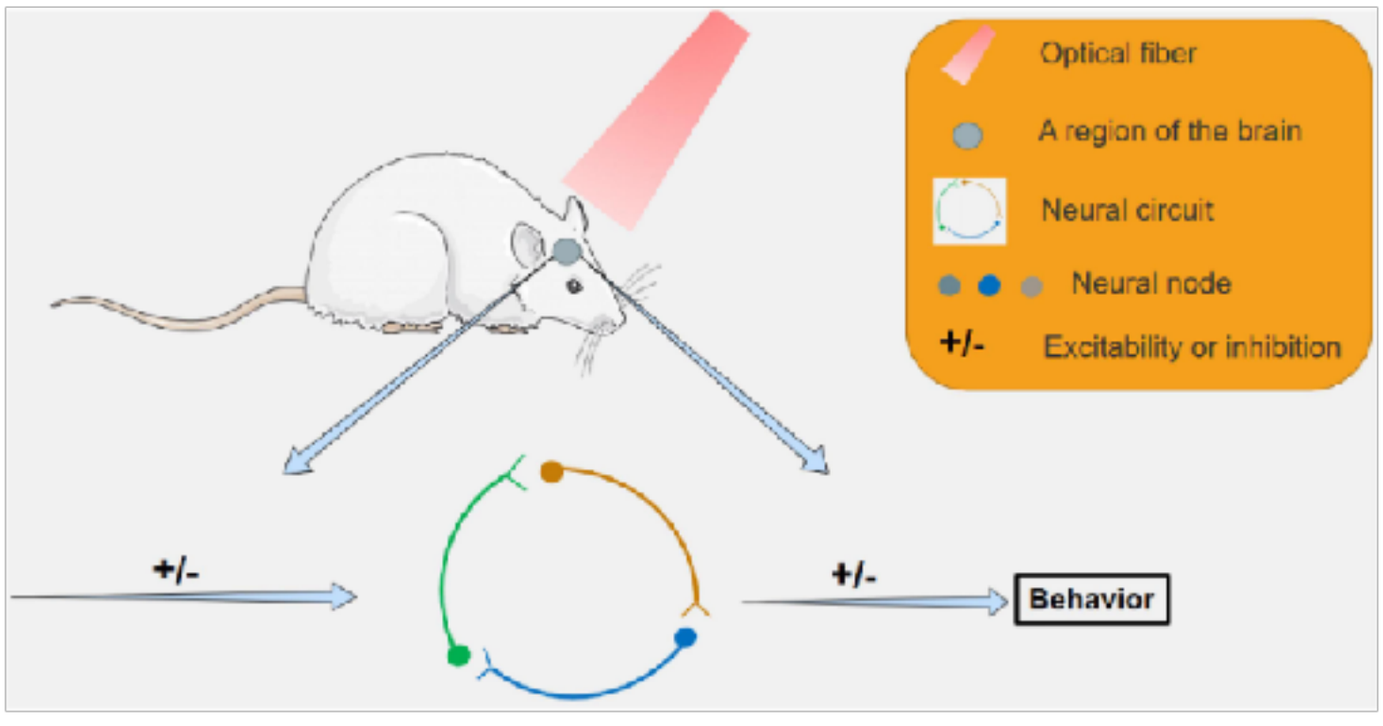


- Light-induced protein dimerization can be used to recruit a protein of interest to a specific intracellular location, where it can pursue its function
- Light-dependent oligomerization (clustering) can induce active functional signaling hubs or inhibit protein function
- Light-induced dimerization can also be adopted to sequester a protein of interest away from its site of action
- Photo-uncaging based on LOV domains can be used to directly control protein activity with light

Optogenetics in neurobiology



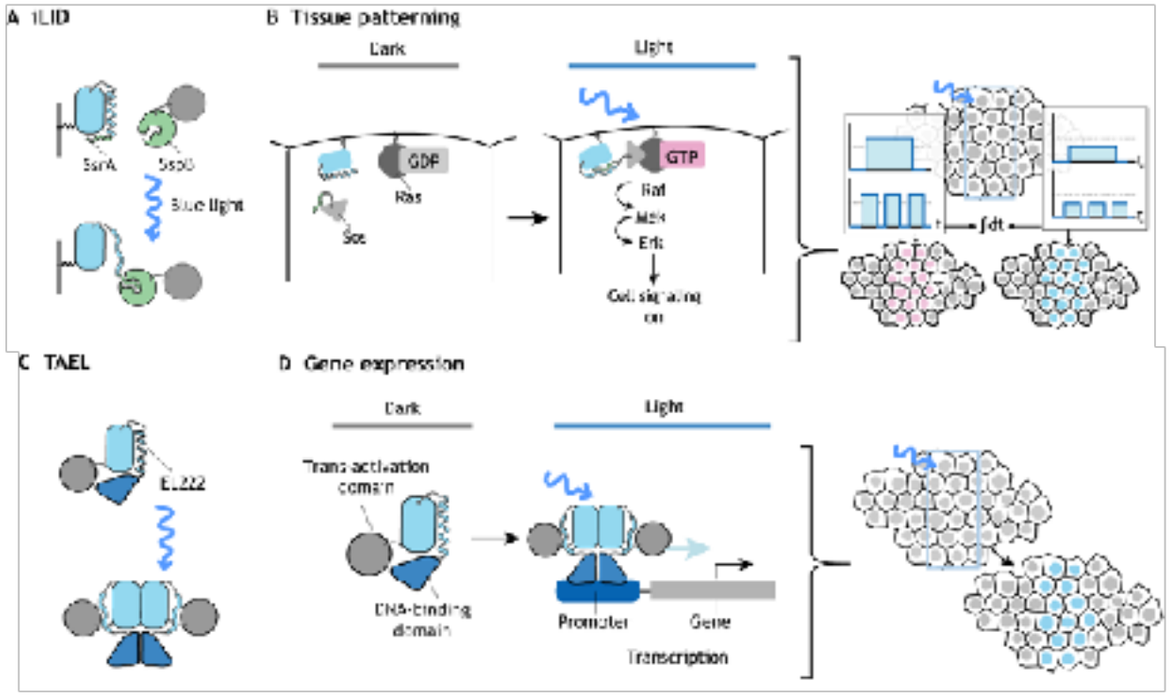
Using optogenetics, it is possible to induce stem cell differentiation, identify cell function, and decode intercellular signaling pathways.



Studying neural circuits by optogenetic methods:

By targeting specific areas with light, specific neural circuits can be inhibited or activated, leading to behavioural changes in mice, and related neural circuits can be studied.

LOV domain-based optogenetic manipulation of animal development



LOV domains (blue), heteromeric interacting partners (green, protein of interests (gray) engineered recognition motif (yellow)

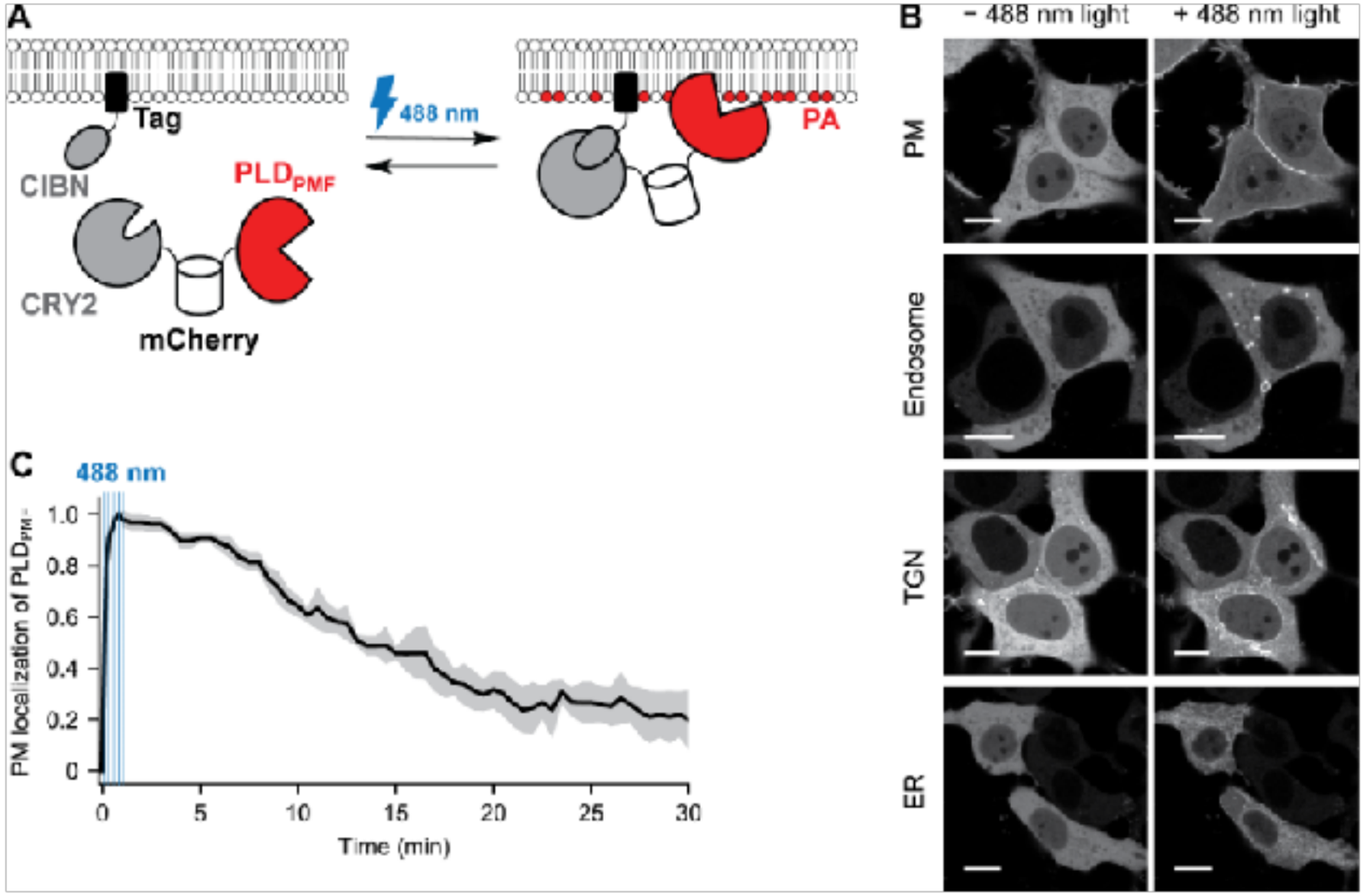
(A,B) The iLID heterodimerization system is used to recruit the Ras-GEF Sos to the plasma membrane and activate Erk signaling upon blue-light illumination during early Drosophila embryogenesis. By varying the temporal pattern and intensity of light activation, cell signaling and tissue patterning can be controlled.

(C,D) The TAEL homodimerization system has been applied in zebrafish embryos to induce gene expression upon light activation. Light-dependent conformational changes in TAEL cause homodimerization and DNA binding of the dimer to a specific promoter region (dark blue) triggering gene expression.

(E,F) A Jα of AsLOV2 engineered to contain a nuclear localization signal (NLS) that is exposed only upon light-induced Jα unfolding causes target proteins to shuttle into nuclei. Similarly, the LANS system has been used in C. elegans to induce nuclear shuttling of the transcription factor Lin1.

(G,H) The small GTPase Rac1 can be photo-caged using the AsLOV2 domain (PA-Rac1). Upon light-induced unfolding of the LOV domain, PA-Rac1 becomes active inducing remodeling of the actomyosin network (red) and lamellipodia formation (pink). PA-Rac1 has been used in Drosophila oocytes to guide the movement of border cells using light.

Optogenetics to modify lipid headgroups



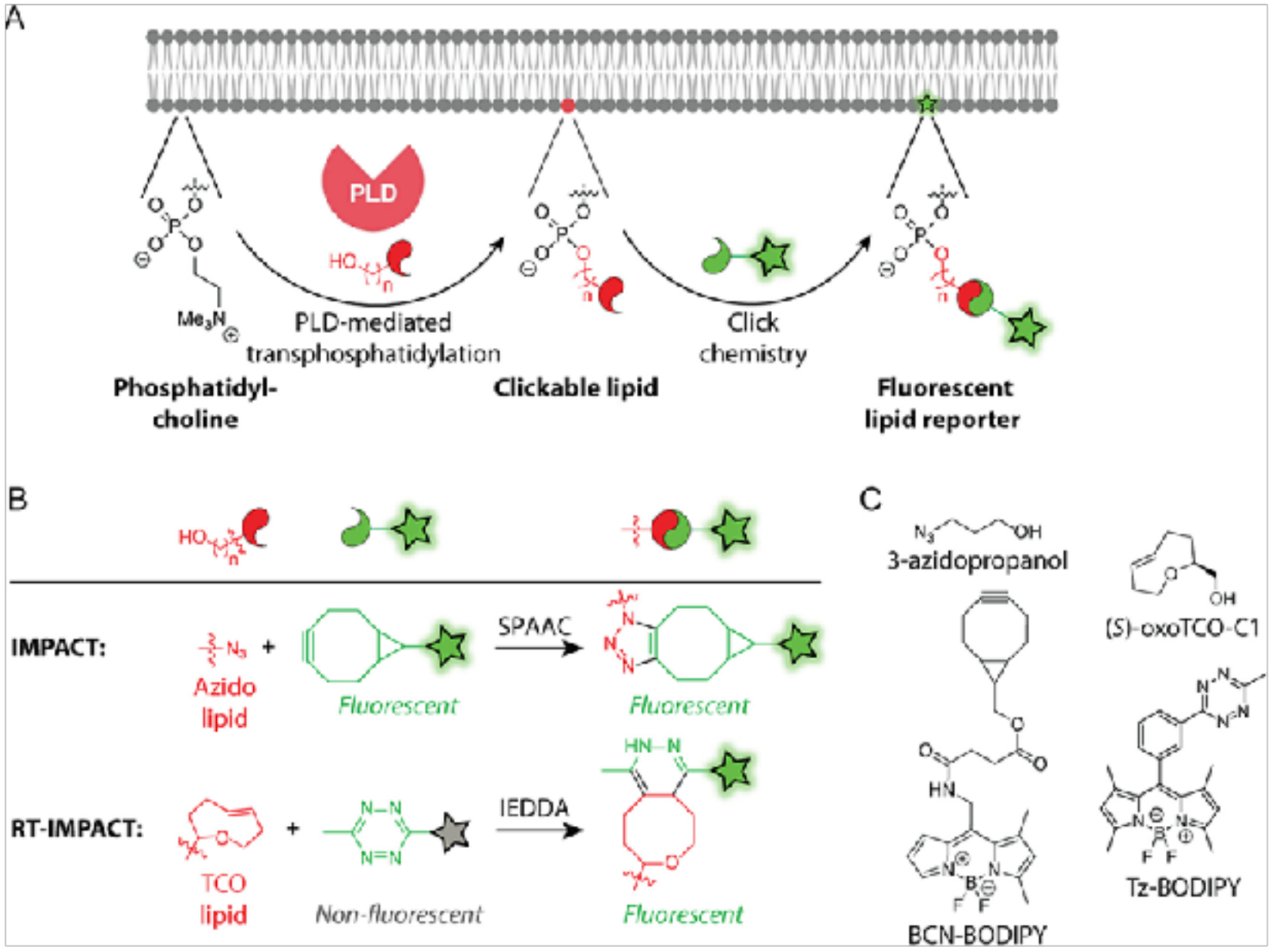
optoPLD for the spatiotemporal control of PA production:

(A) Schematic depicting the design of optoPLD. A single plasmid encodes (1) a fusion of CRY2, mCherry, and PLD_{PMF}; and (2) a fusion of CIBN to an organelle targeting tag. CRY2–CIBN heterodimerization induced by blue light (488 nm) causes recruitment of PLD_{PMF} to the desired membrane.

(B) Confocal images showing recruitment of optoPLD to different organelle membranes. Shown is the CRY2-mCherry-PLD_{PMF} localization before and after illumination with 488-nm light. Scale bars: 10 μm.

(C) Reversibility of optoPLD recruitment.

Combining optogenetics and bioorthogonal chemistry

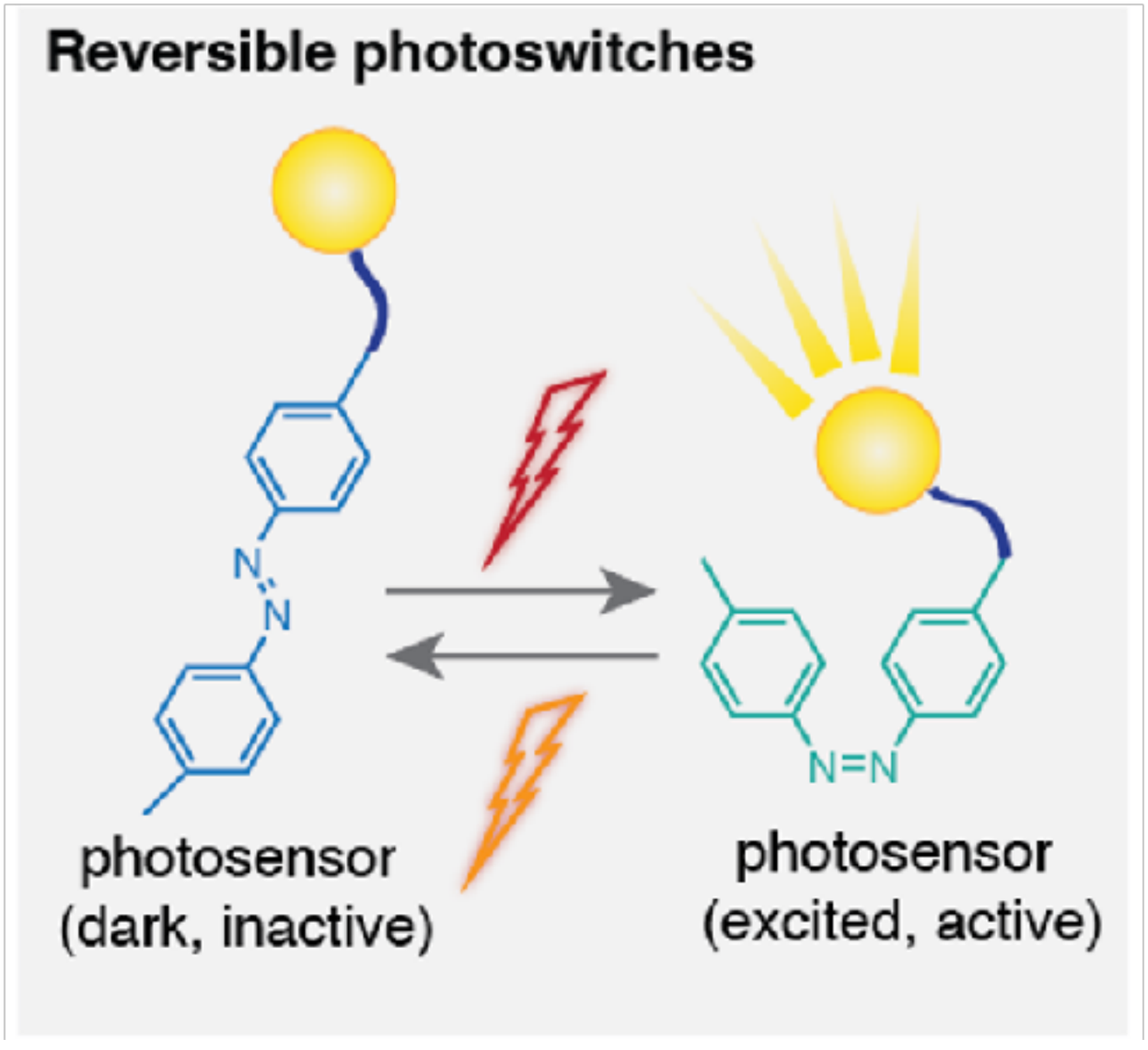


- This approach uses an engineered phosphor lipase D that modifies the headgroup by introducing a biorthogonal handle
- The biorthogonal handle can then be reacted with a dye to visualize the localisation of the lipid

Imaging phospholipase D activity with clickable alcohols via transphosphatidylation (IMPACT)

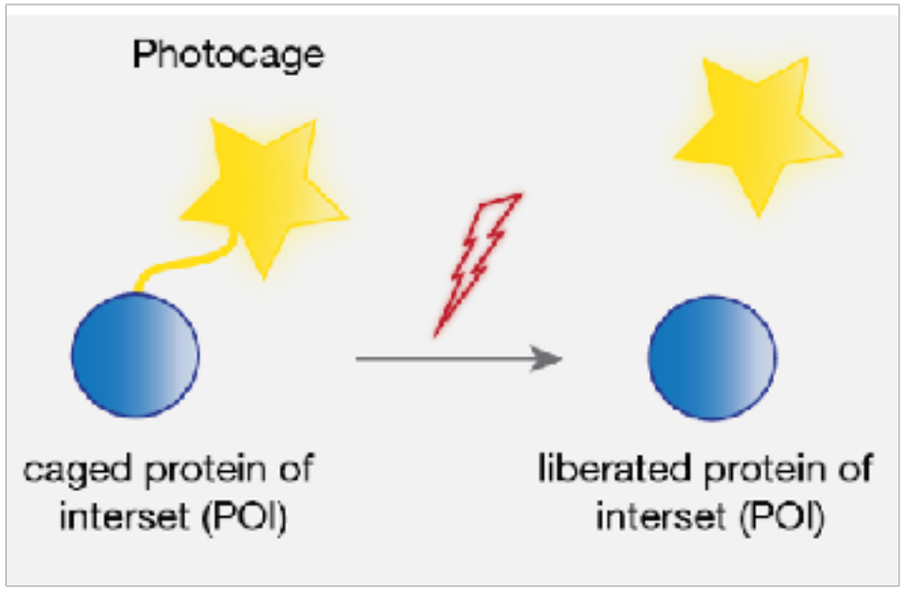
Photoswitchable tools

A photoswitch is a type of molecule that can change its structural geometry and chemical properties upon irradiation with electromagnetic radiation.



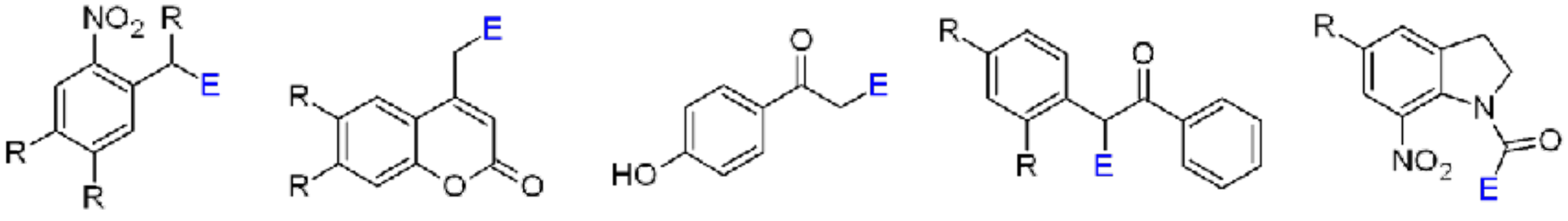
Photocages - Concept

Concept: A photolabile group (photocage) is covalently attached to a metabolite or other small molecule. The photocage can be removed using light of a specific wavetlenth.



- Spatio-temporal control over the release of the molecule of interest.
- Efficiency of the release can be tuned by light intensity

Common photocaging groups:



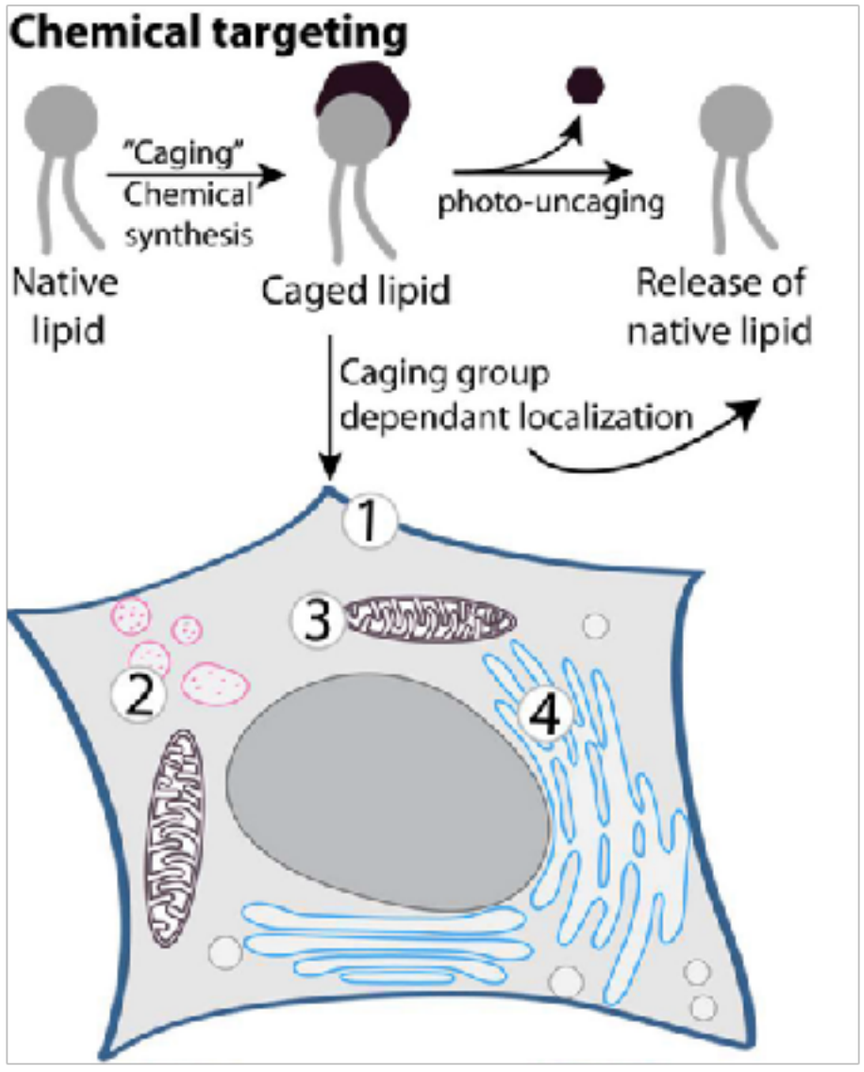
E: Effector

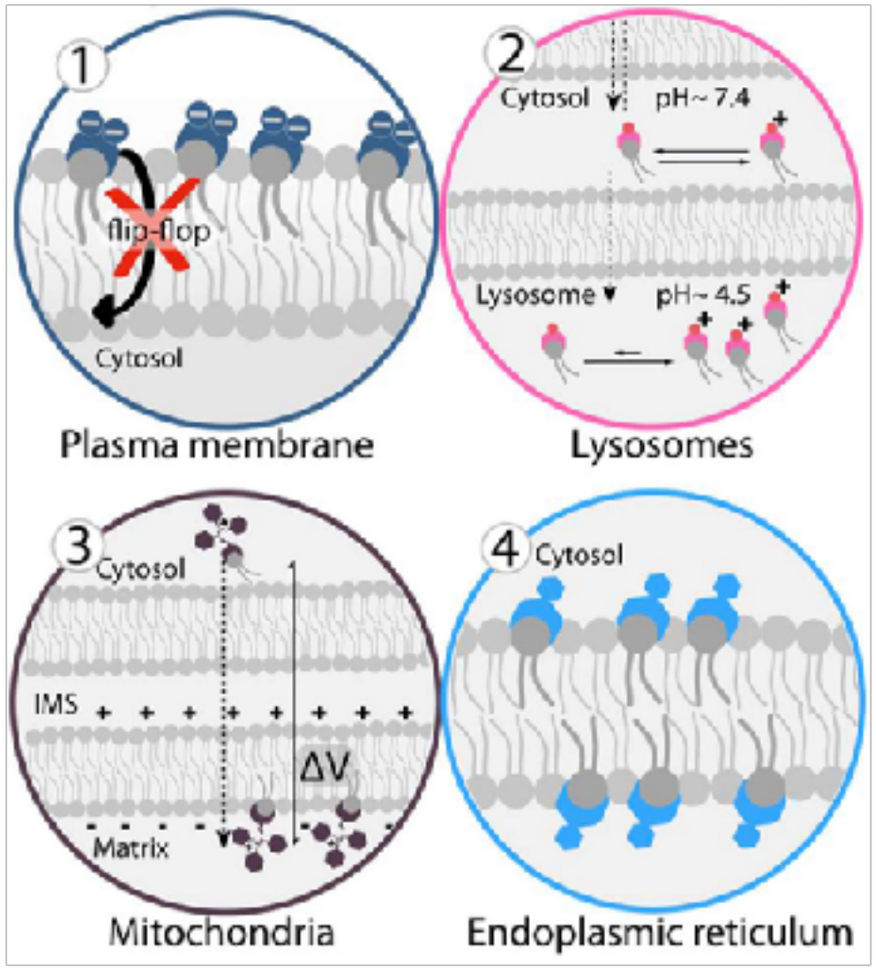
Properties of caged compounds

- **Stability:** The unexposed substance should be stable. If a caged compound slowly decomposes during storage or in solution even without exposure to light, the actually inactive caged compound is often contaminated with the active effector molecule. This can greatly distort the interpretation of experimental results.
- **Biochemical inactivity:** The biochemical function of the effector molecule must be suppressed as completely as possible by linking it to the photolysable group.
- **Appropriate photolysis properties:** Three parameters are important here:
    - Speed: Photolytic release of the effector from the caged compound should be much faster than the process triggered by the effector molecule in the system under study. Only then can a statement be made about the speed of the subsequent process.
    - Specificity: After exposure, if possible, only one photochemical reaction of the caged compound should take place, so that no by-products are formed.
    - Efficiency: The cleavage of the caged compound should take place with as little light as possible.
- **Light absorption** at wavelengths longer than 300 nm. Many biomolecules, including proteins and nucleic acids, absorb UV light at wavelengths around 260-280 nm. If the caged compound also has to be excited in this range, then other sample components already filter part of the for radiation required for photolysis and can themselves suffer photodamage as a result.
- **Low reactivity and toxicity** of the reaction products. Photolytic cleavage produces not only the desired effector molecule, but also a compound derived from the photolysable group itself. If possible, this should not undergo any further reactions and be non-toxic.
- **Good solubility** of the caged compound and the reaction products.

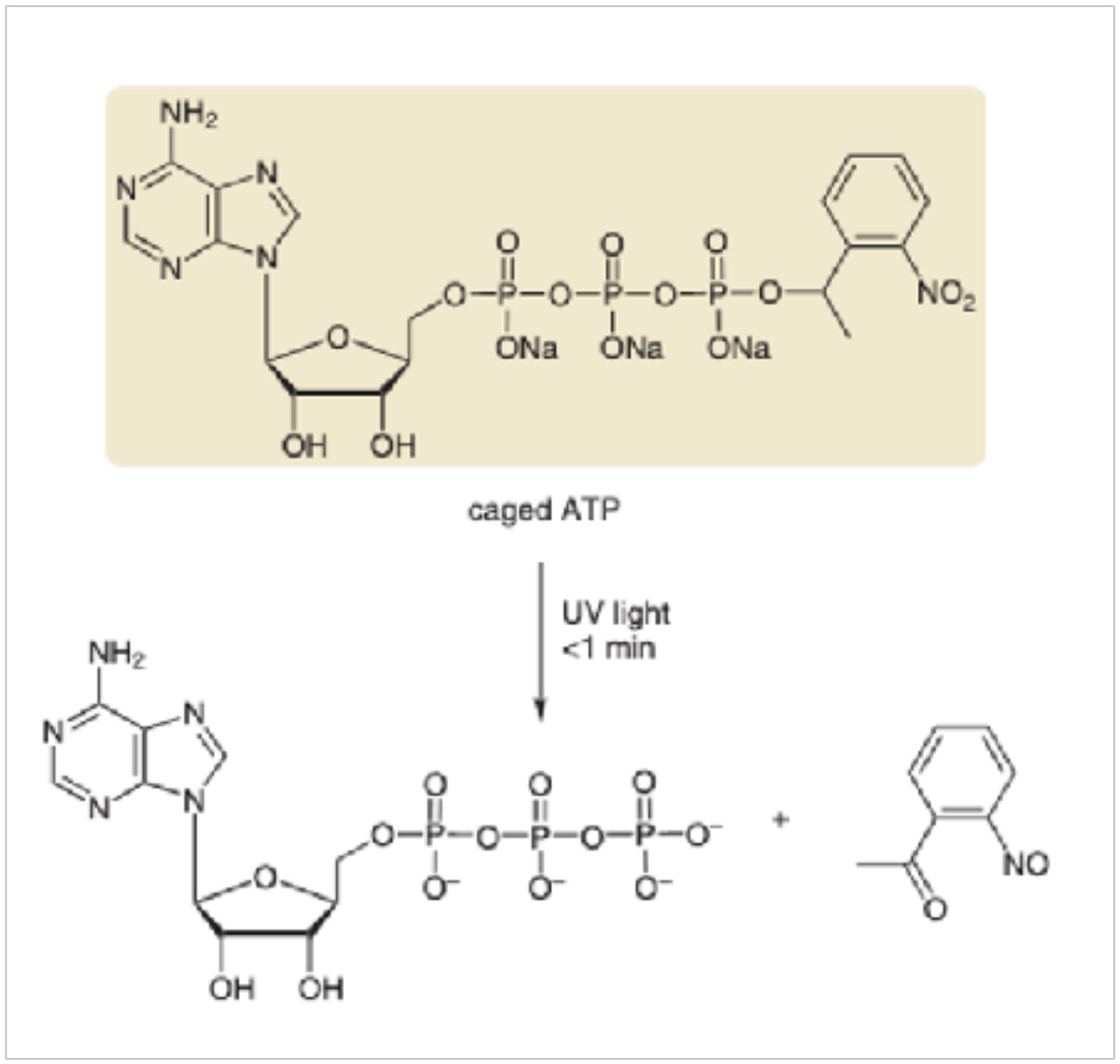
Special photocages

The photocage can also be used to target themolecule of interest to specific subcellular organelles or structures by exploiting the specific physico-chemical properties of the target structure.





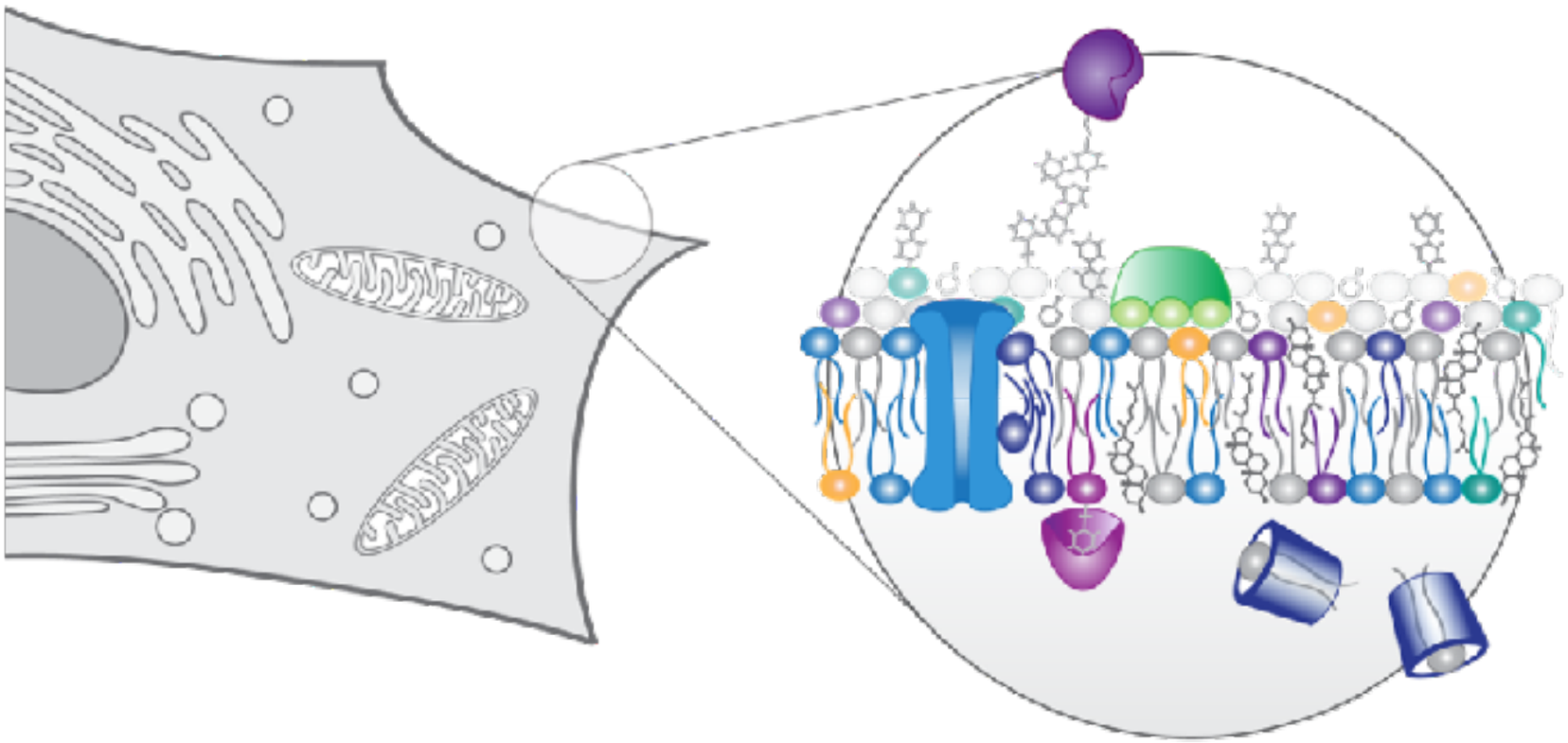
Caged ATP and GTP:



- The release of inorganic phosphate from ATP is crucial to numerous life processes from cell movement to cell signaling
- In 1978, Kaplan et al. expanded the concept of photolabile protecting groups to ATP and demonstrated the controlled release of ATP to activate Na^+ /K^+ pumps in resealed red blood cell ghosts

- The photolabile 'caged phosphate' was coupled to ADP through a DCC mediated phosphorylation
- Upon irradiation with UV light, the caged ATP is photolyzed, releasing ATP and a reactive nitrosoketone
- The ATPase inhibition of the Na^+ /K^+ pump caused by the nitrosoketone byproduct can be reversed by incorporating glutathione into the assay

Biological membranes and lipid diversity

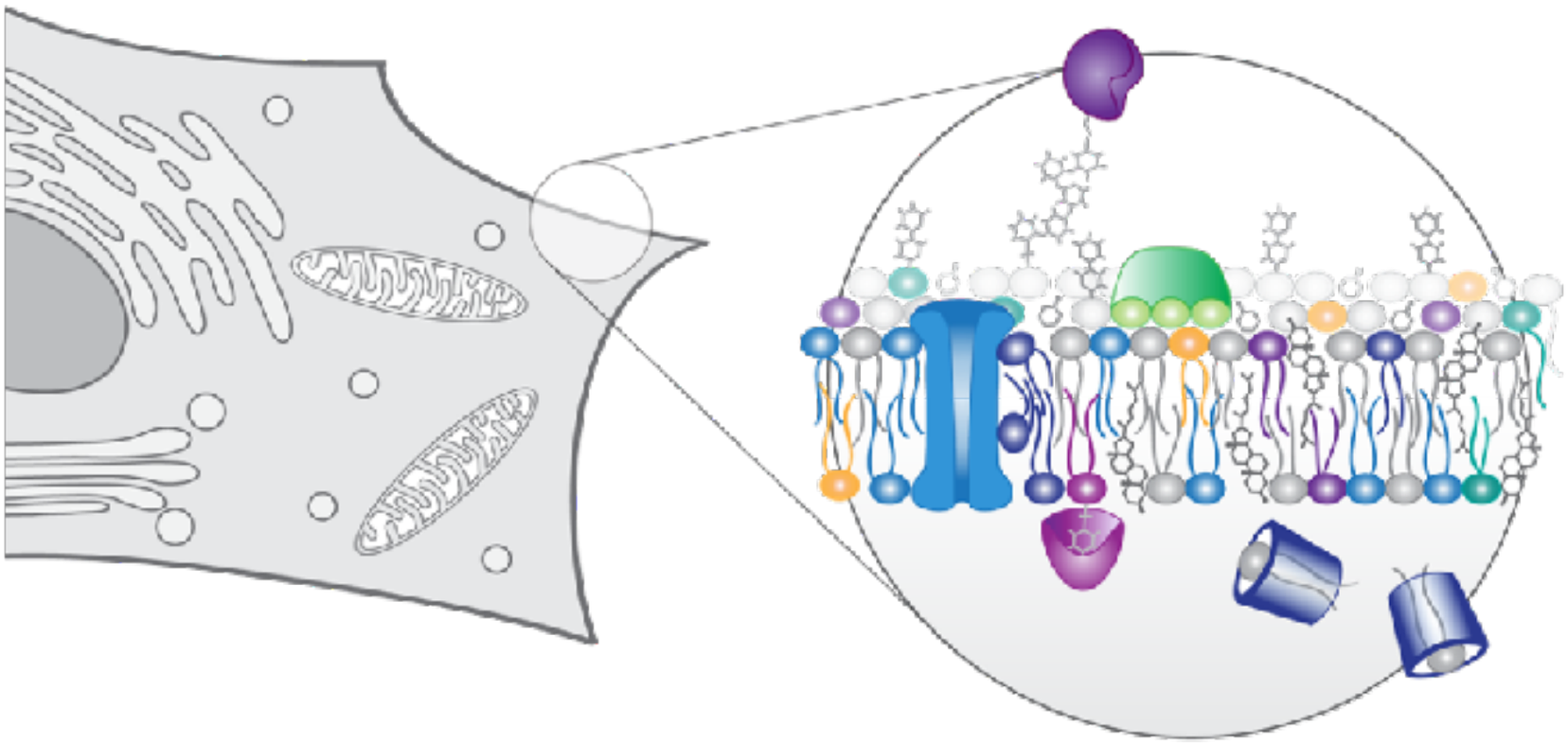


> 10 major lipid classes

> 40 000 lipid species

What is the biological function of lipid diversity?

Biological membranes and lipid diversity

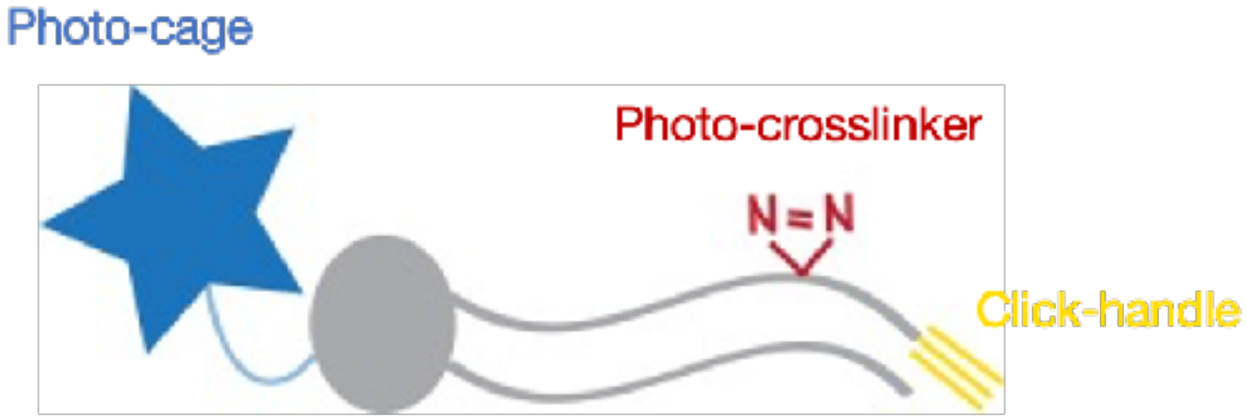


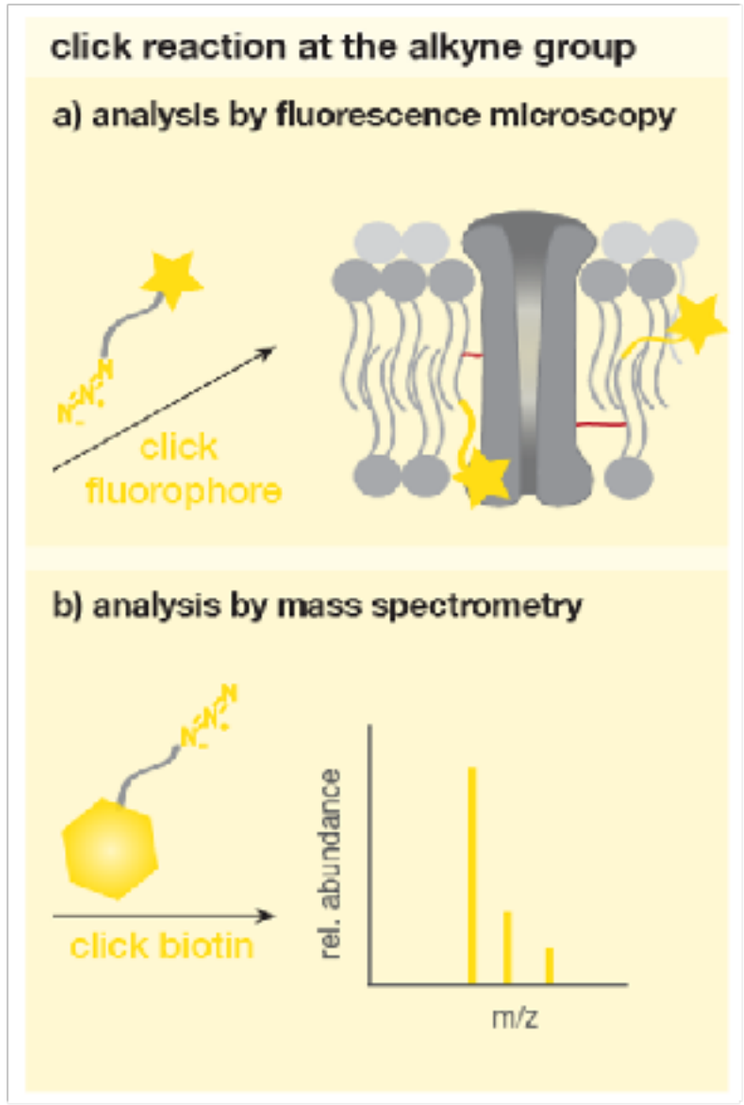
> 10 major lipid classes

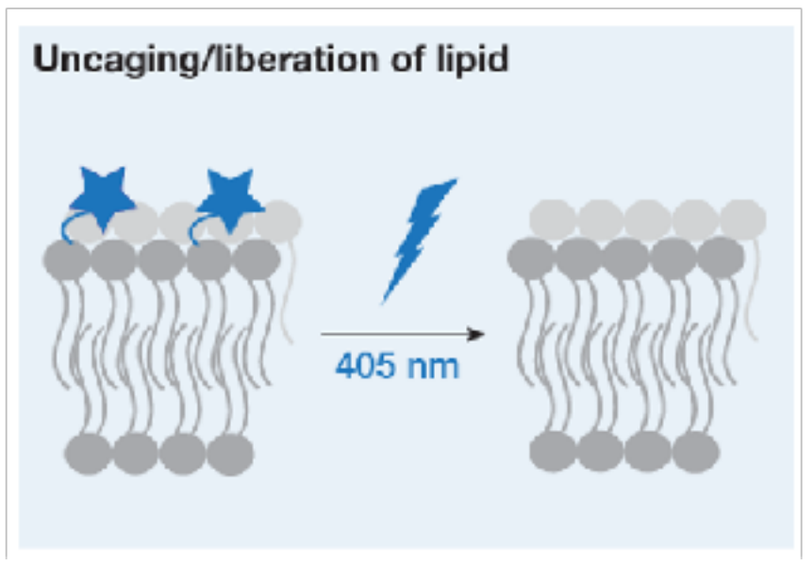
> 40 000 lipid species

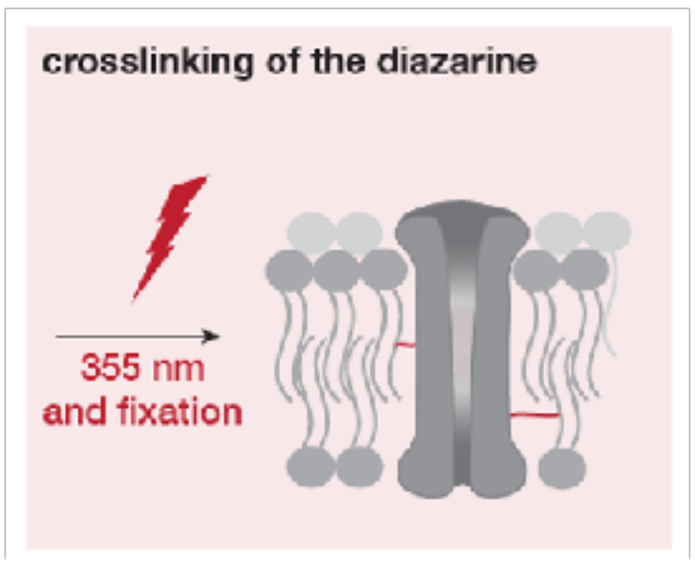
What is the biological function of lipid diversity?

Bioorthogonal chemistry for lipids: Trifunctional lipids









Take home messages for this lecture…

Bioorthogonal chemistry

"Any chemical reaction that can occur inside of living systems without interfering with native biochemical processes"

- It allows both fluorescent labeling and affinity-tagging
- It is especially powerful to study metabolites and small molecules which can not be addressed on a genetic level

Light-triggered tools

"Tools which can be regulated by using light as a trigger to study a system with regard to space, time, or extent of activation"

- Optogenetic tools can control protein activity and localisation by light
- Photoswitches are molecules that undergo a light-triggered conformational change
- Photocages allow to liberate small molecules, metabolites and second messengers
- Specific photocages prelocalize the molecule of interest in an organelle specific manner

→ **Chemical biology and biorthogonal chemistry offer powerful tools to liberate molecules, control their activity, localize and study the interaction network of metabolites**