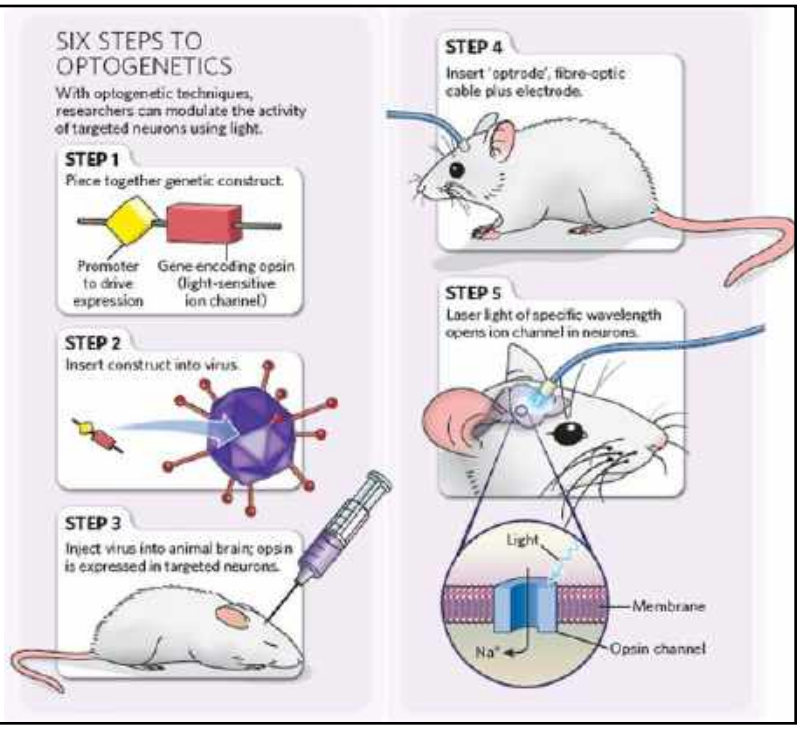
What is Optogenetics?

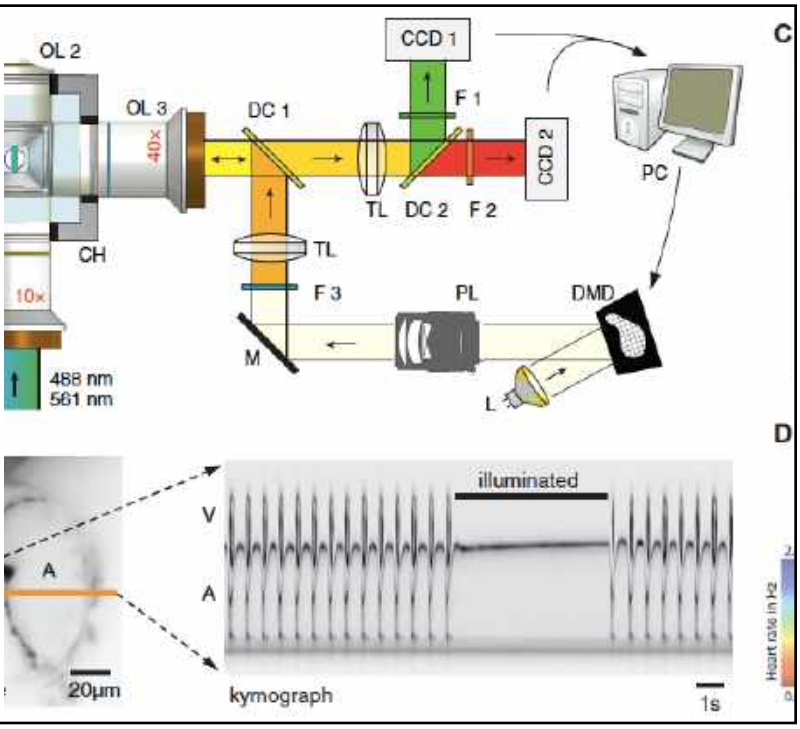
- Optogenetics is a technique that uses light to control neurons of ion channels that have been genetically modified to be light-sensitive.
- Optogenetics has various applications that allow for the identification of specific neurons and networks, cell signaling pathways, and control of behaviour.
- Optogenetics has been used to control cell behaviours in both prokaryotic and eukaryotic cells, but has been mostly focused on eukaryotic cells, but trials in bacterial cells have been emerging in the past few years.



1

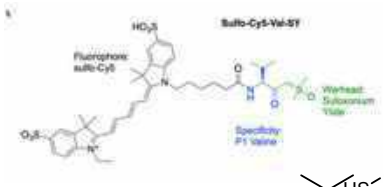
Optogenetic Control of Cardiac Function

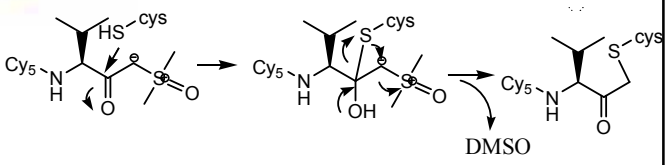
- Arrenberg, A., Stainier, D., Baier, H., Huisken, J. used optical tools and transgenic expression of light-gated ion channels in embryonic zebrafish to locate and control cardiac pacemaker cells.
- Concept of the experiment was based on similar properties in mammalian and nonmammalian vertebrate's cardiac conduction systems (CCS)



2

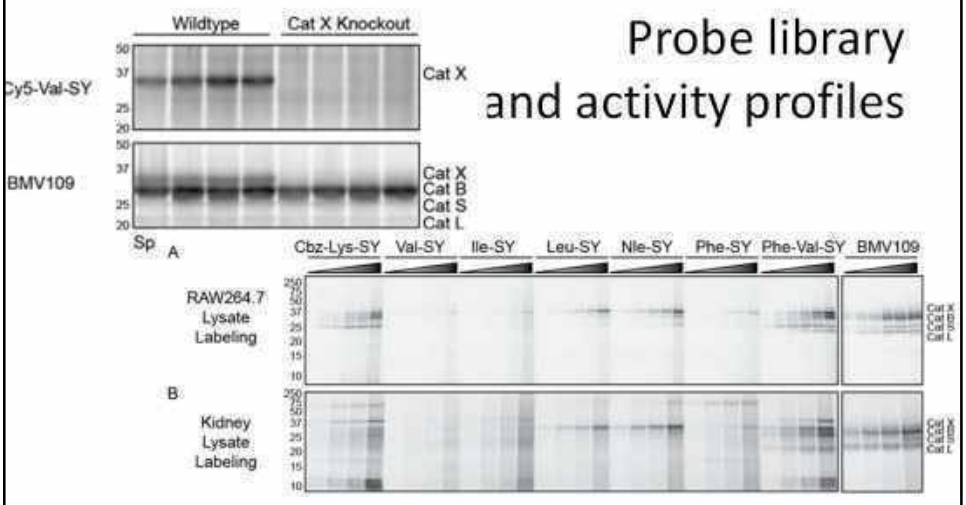
Mechanism of action





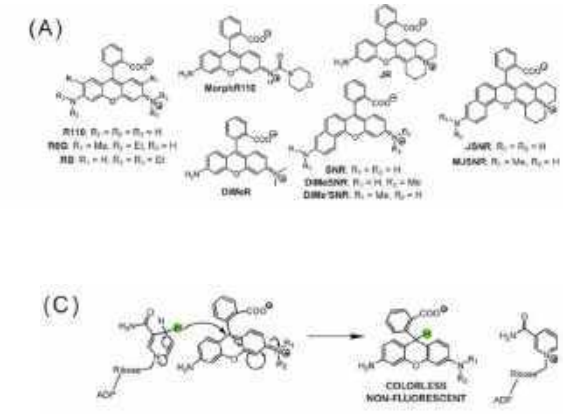
Cathepsin X is a cysteine protease
Probe is an irreversible inhibitor using the sulfoxonium warhead
Probe is specific to nucleophilicity of sulfur because it is soft

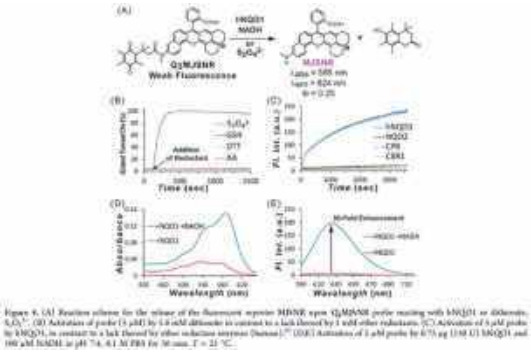
Probe library and activity profiles



Cathepsin type specificity was shown with different amino acids at P1 recognition site
Val-SY showed most specificity towards Cath X compared to BMV109
Probe can be used in vivo to show specific cathepsin x activity

Environmentally Robust Rhodamine Reporters for Probe-based Cellular Detection of the Cancer-linked Oxidoreductase hNQO1





1

Environmentally Robust Rhodamine Reporters for Probe-based Cellular Detection of the Cancer-linked Oxidoreductase hNQO1

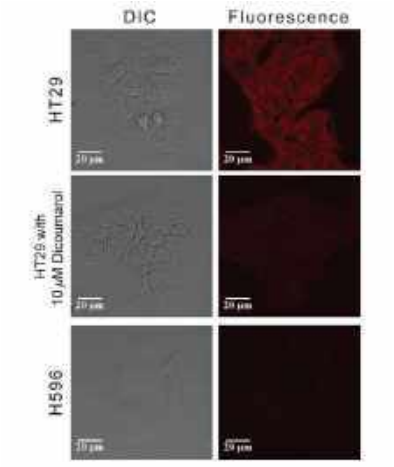


Figure 6. Confocal microscopy images of HT29 (positive), HT29 pretreated with 10 μM dicoumarol, and H596 (negative) cells incubated with 5 μM Q1MJSNR for 10 min at 37 °C. Fluorescence images are in the right column and differential interference contrast (DIC) images in the left. The probe was excited with a 543 nm HeNe laser line (laser intensity = 48%).

<u>Summary and Applications of the probe</u>

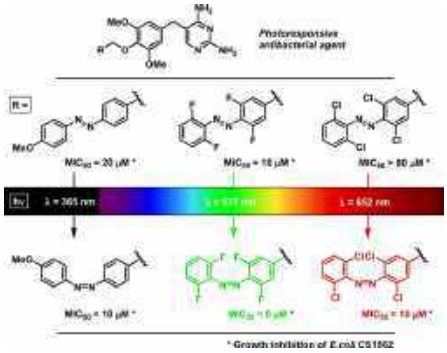
- Successfully determined that alkylation on either the nitrogen's or the xanthene core decreases reactivity of reduction from NADH via a hydride transfer mechanism.
- Synthesised a NADH-stable seminaphthorhodamine fluorescent probe for detection of the cancer-linked oxidoreductase enzyme hNQO1 that is highly expressed in many human tumour cells.
- Can be used in cell-based drug screening assays that are targeting the hNQO1 enzyme.
- Possible derivatives of the probe could be used for discriminating between healthy and cancerous cells in fluorescence guided surgical imaging and resection of cancer tissue via a topical spray-on application.

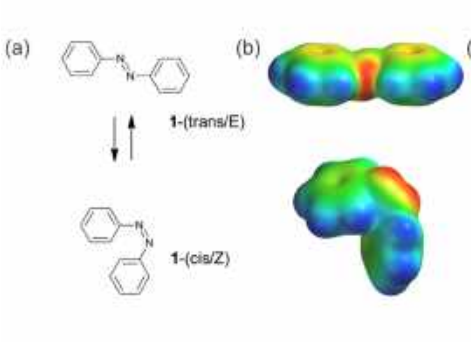
2

a place of mind

Photoswitching of Azobenzenes

Structurally diverse analogues of trimethoprim created and probed for antibiotic activity in both cis and trans confirmations. Ortho-positions modified to change wavelength of switching.





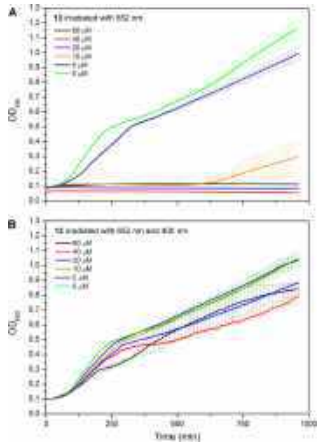
Physical Mechanism of photoswitching under controversy. Involves activation of the N-N pi bond to the associated pi* bond

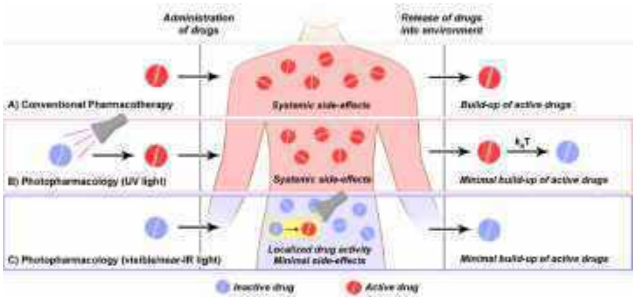
Planar trans/E confirmation roughly 50kJ/mol more stable than cis/S confirmation.

a place of mind

Reversibility

Antibiotic build-up in systems leads to antibiotic resistance and nefarious side-effects.
Controlled photoswitching allows drugs to be turned on in a very specific location with minimal side effects.
Red-shift needed as UV light is damaging, has low penetration, and wavelength need to be distinct for control of confirmation





652nm switches compound to trans confirmation, preventing E. coli from multiplying. 400nm switches it back, allowing cell division.

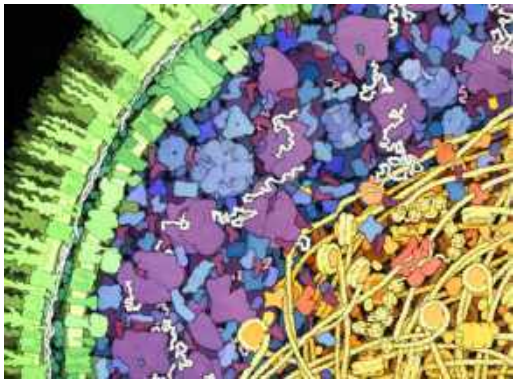
David Bakker

FRET Sensor of Molecular Crowding

ACS Sens. 2019, 4, 1835−1843

Molecular Crowding

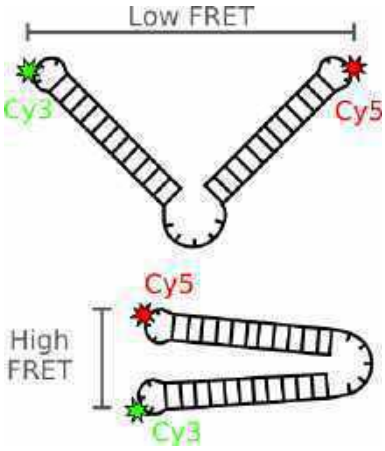
- It is Squishy Inside the Cell
- Macromolecules Exclude Volume



https://doi.org/10.1016/S0968-0004(01)01938-7

FRET Sensor Design

- dsDNA is Fairly Rigid, ssDNA is not



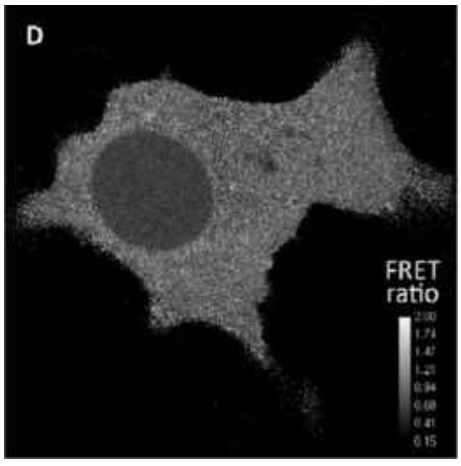
https://doi.org/10.1021/acssensors.9b00569

David Bakker

Application of FRET Sensor

Cytoplasm vs Nucleoplasm

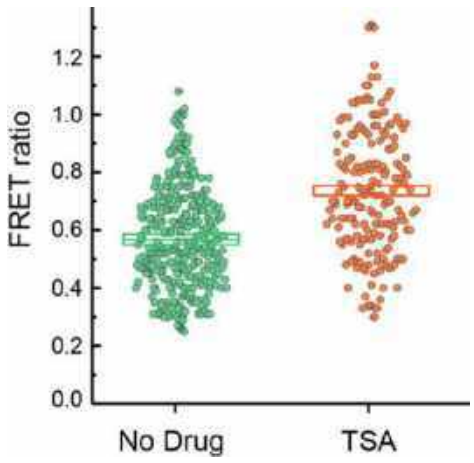
- Cells Transfected With Sensor
- Cytoplasm has Higher FRET & is More Crowded



https://doi.org/10.1021/acssensors.9b00569

Chromatin Unraveling

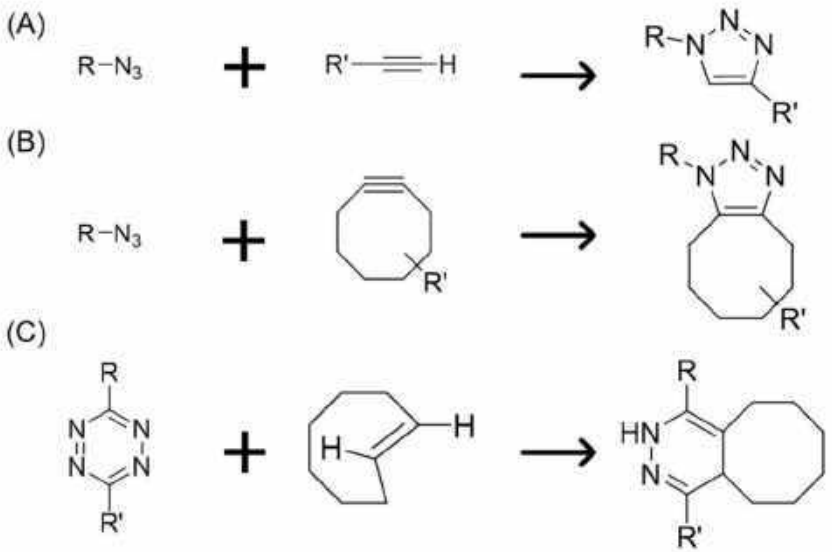
- Trichostatin A (TSA) Disrupts Nucleosomes & Unravels Chromatin - Increases FRET Ratio

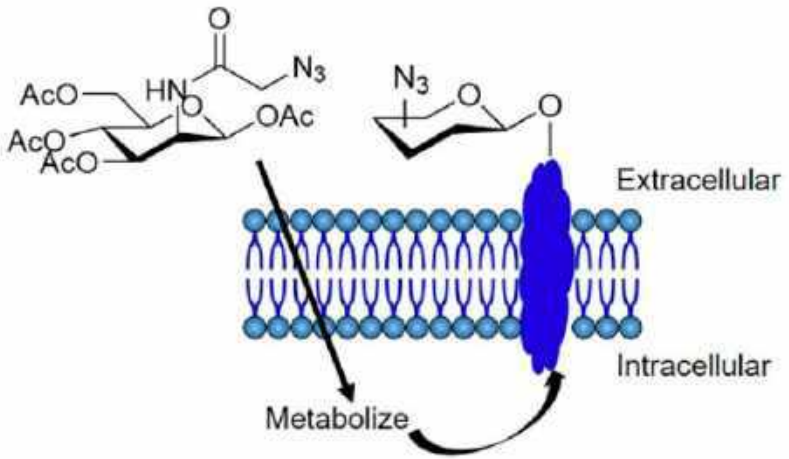


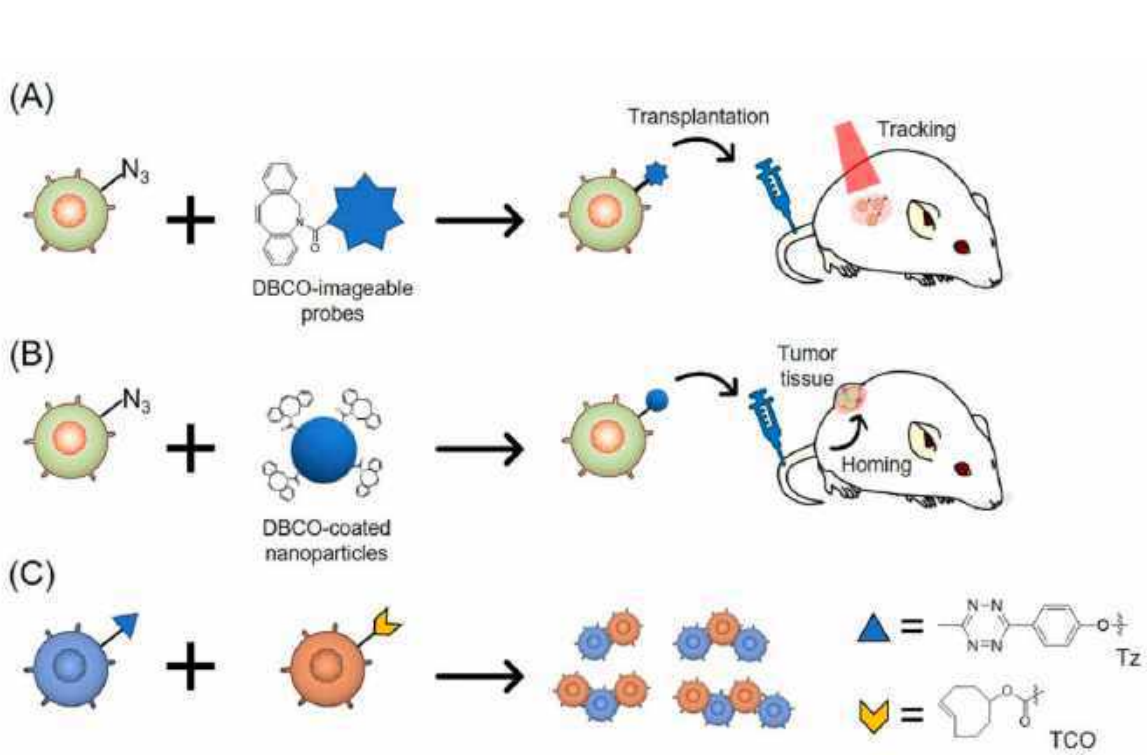
https://doi.org/10.1021/acssensors.9b00569

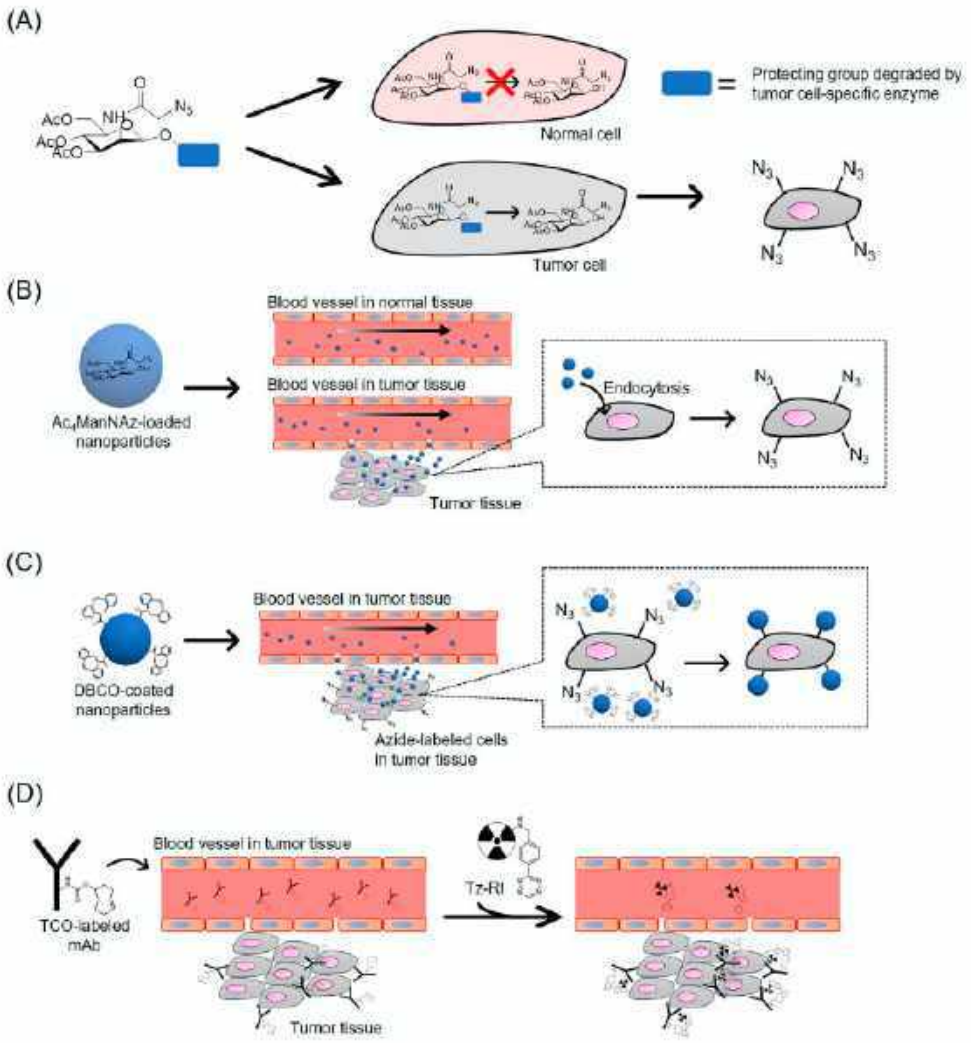
Click Chemistry as a Tool for Cell Engineering and Drug Delivery[1]

By: Cristian W. Kwasnek









[1] Takayama, Y., Kusamori, K., & Nishikawa, M. (2019, January 4). Click Chemistry as a Tool for Cell Engineering and Drug Delivery. Retrieved March 30, 2020, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6337375/

Optogenetics

How can we modulate the function of select neural circuits *in vivo*?

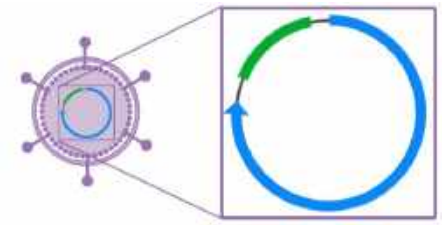
Activate or inhibit neural circuitry

1) Selectively (eg. one type of neuron in one small area)
2) Acutely
3) With minimal invasion

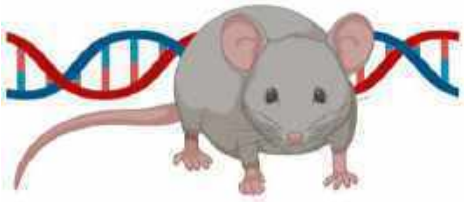
Engineer neurons to express a light-sensitive ion channel (an opsin) if they express a specific transcription factor

Gene delivery:

Viral vectors

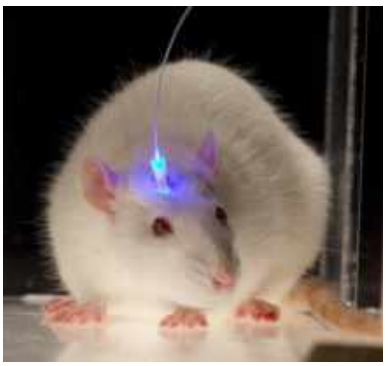


Transgenic organisms



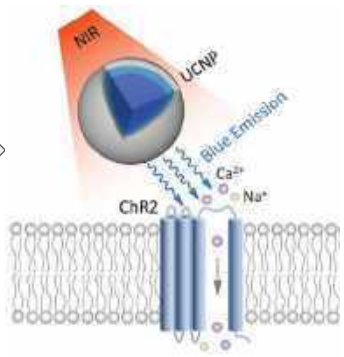
Upconversion Nanoparticles

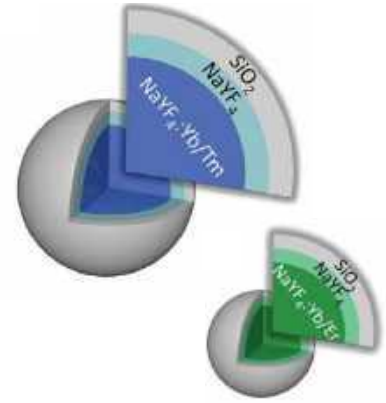
-Optogenetics usually requires the use of a laser inserted into the brain



-invasive, unpractical

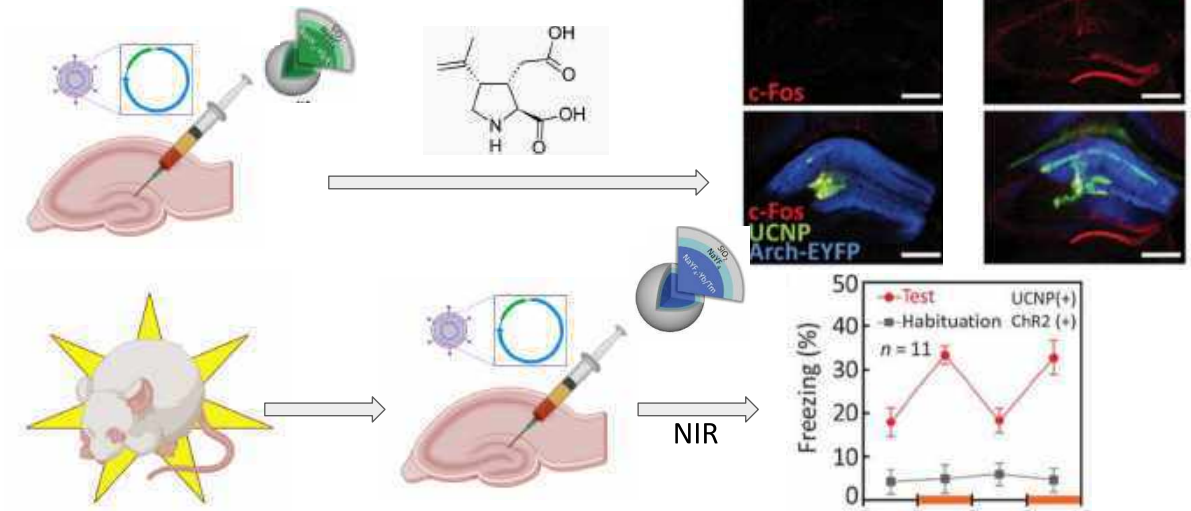
-Upconversion nanoparticles exhibit anti-stokes shifts.





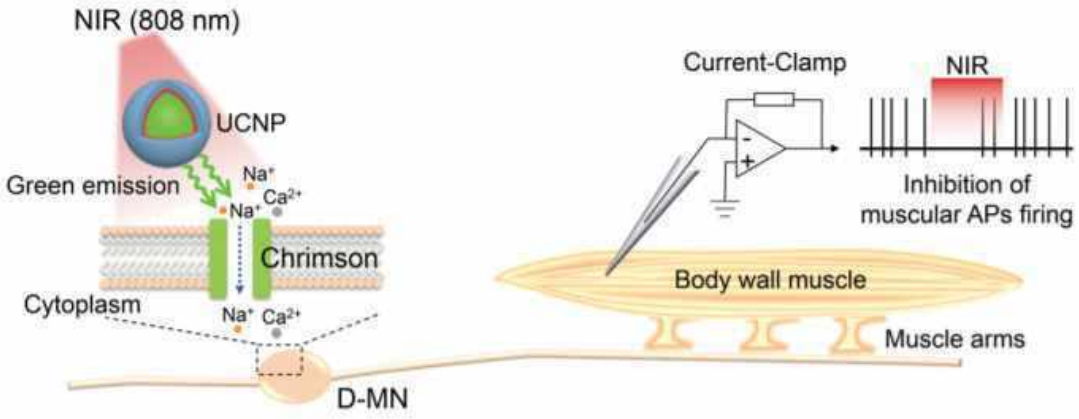
-lanthanide doped: multiple 4f excitation levels
-consist of sensitizer/activator pairs (Yb/Tm, Yb/Er)
-upconversion nanoparticles are enhanced by core-shell designing

Application: Silencing seizures and triggering fear responses in mice



Chen, S.; Weitemier, A. Z.; Zeng, X.; He, L.; Wang, X.; Tao, Y.; Huang, A. J. Y.; Hashimotodani, Y.; Kano, M.; Iwasaki, H.; et al. Near-Infrared Deep Brain Stimulation via Upconversion Nanoparticle–Mediated Optogenetics. *Science (80-.).* **2018**, *359* (6376), 679–684. https://doi.org/10.1126/science.aaq1144.

Application: Controlling the behaviour of *C. elegans*



Ao, Y.; Zeng, K.; Yu, B.; Miao, Y.; Hung, W.; Yu, Z.; Xue, Y.; Tan, T. T. Y.; Xu, T.; Zhen, M.; et al. An Upconversion Nanoparticle Enables Near Infrared-Optogenetic Manipulation of the Caenorhabditis Elegans Motor Circuit. *ACS Nano* **2019**, *13* (3), 3373–3386. https://doi.org/10.1021/acsnano.8b09270.

Metabolic labeling with bioorthogonal chemical reporters

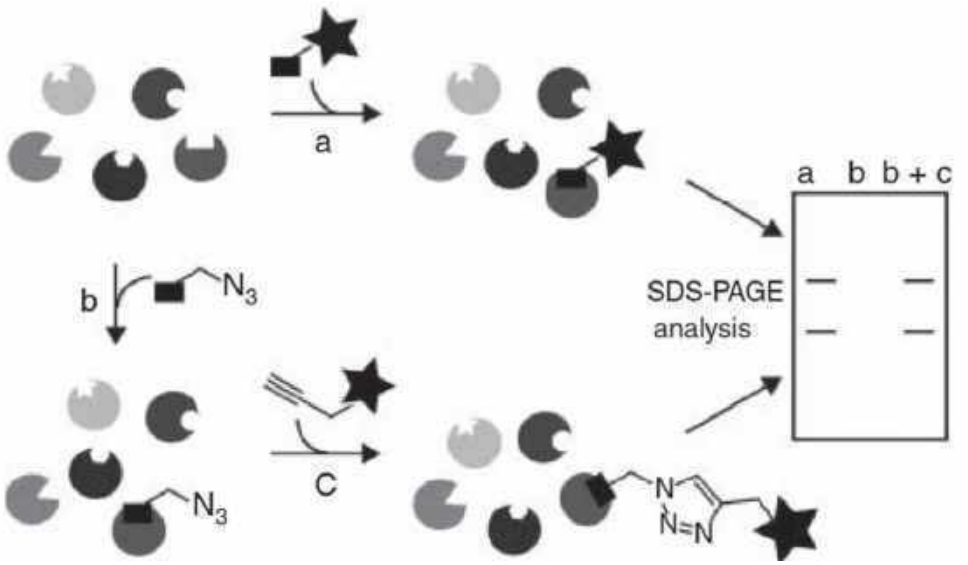
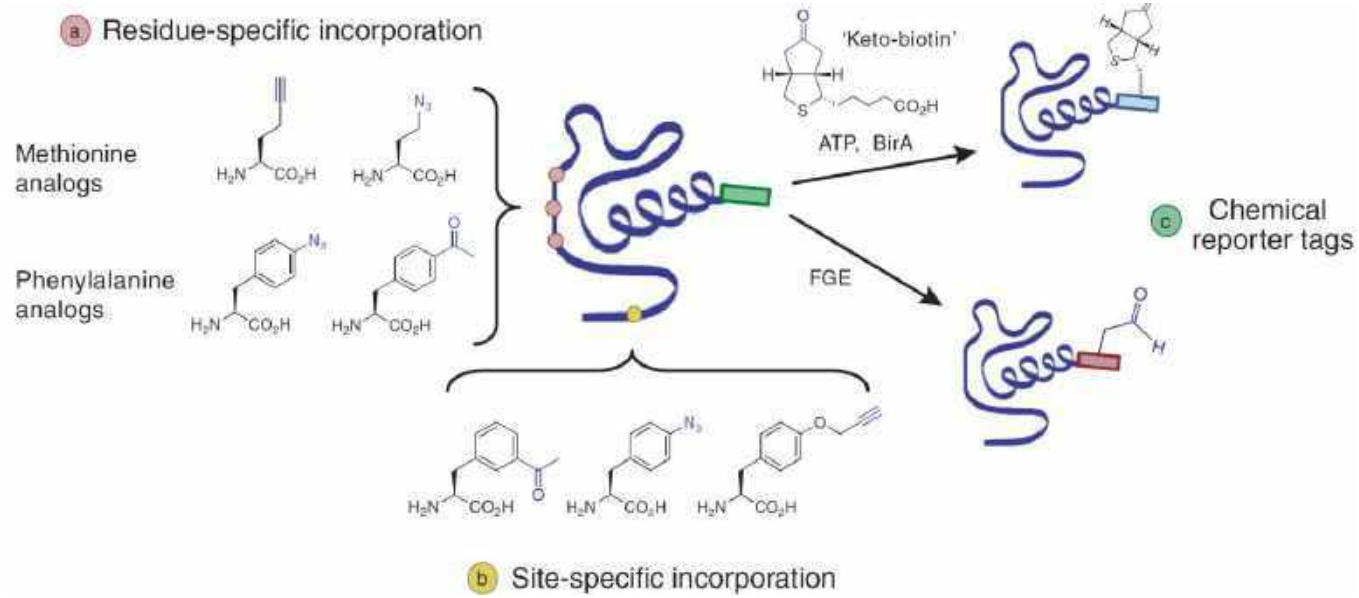
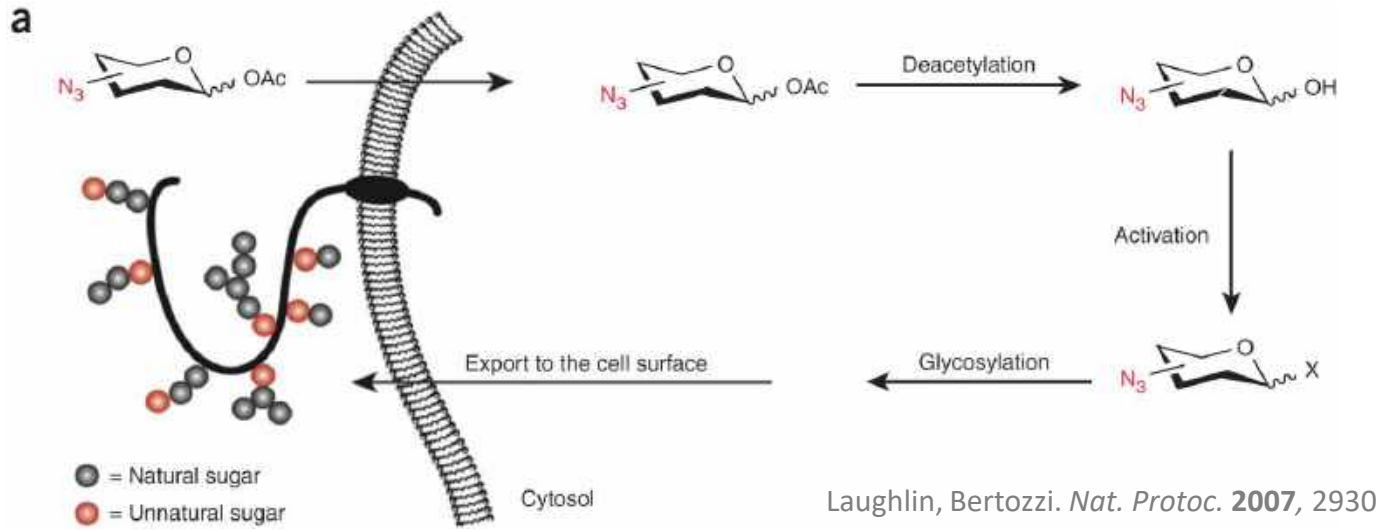


Figure 13.4a. *CCSCB ch. 13.3.1.* **2014**, 198

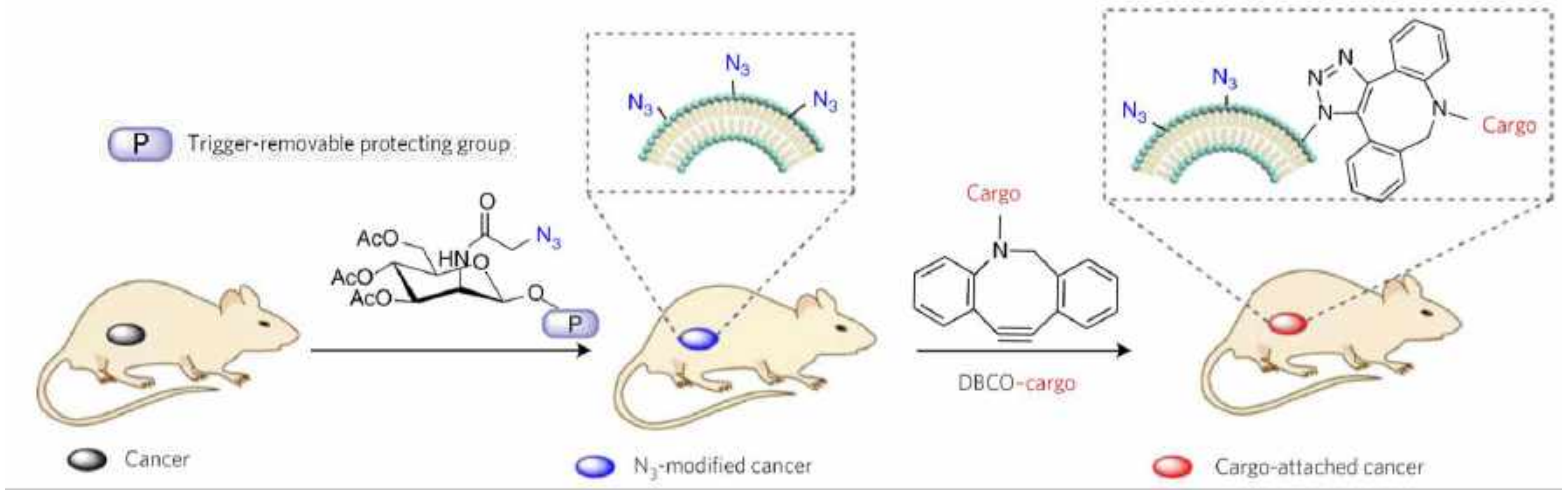


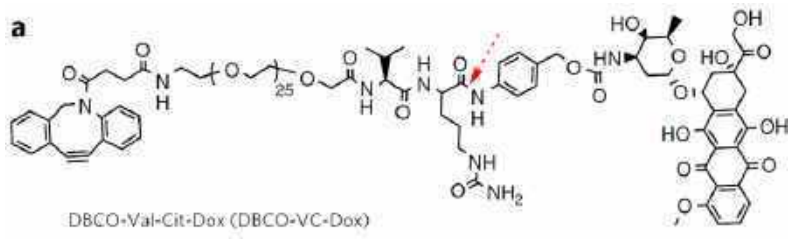
Prescher, Bertozzi. *Nat. Chem. Bio.* **2005**, 13

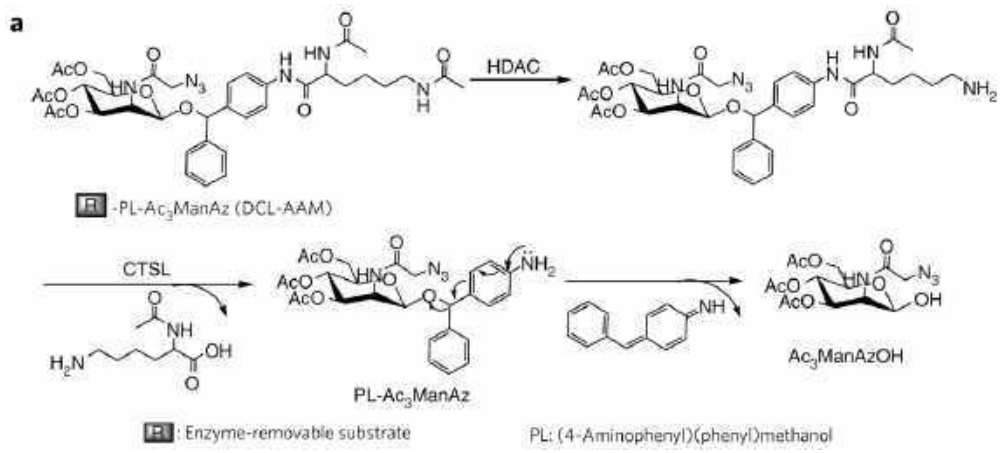


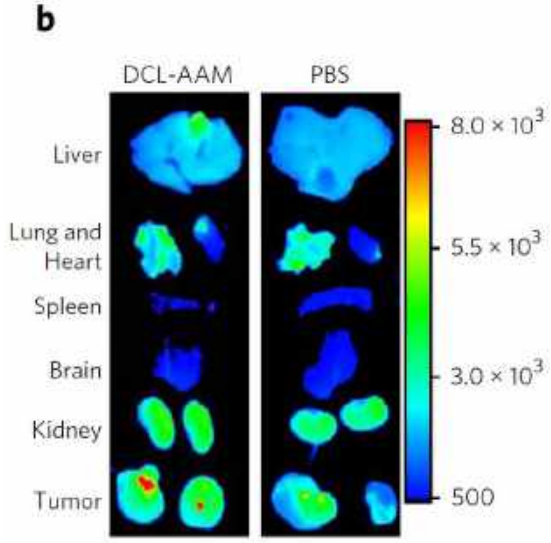
Laughlin, Bertozzi. *Nat. Protoc.* **2007**, 2930

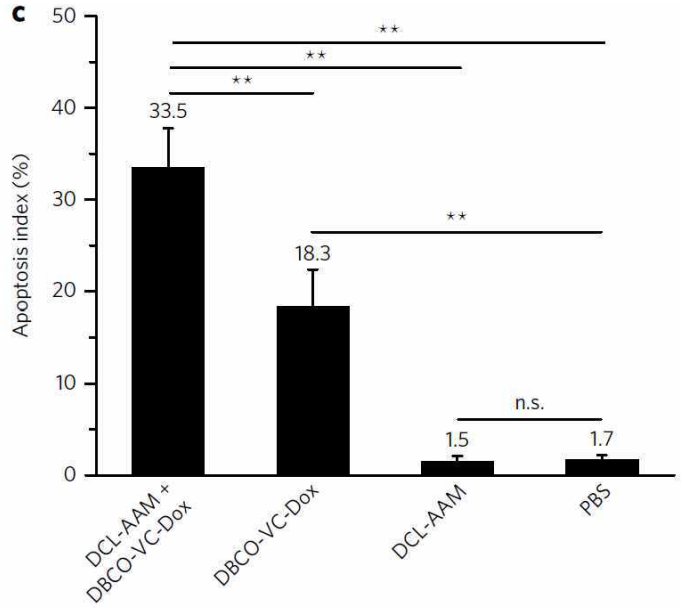
Active tissue targeting *via* anchored click chemistry (ATTACK)





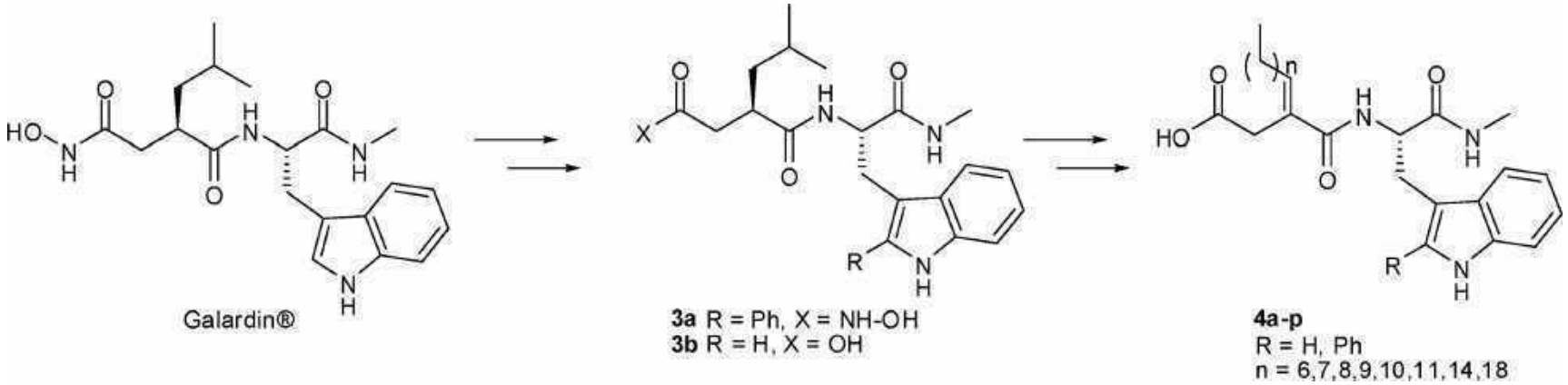


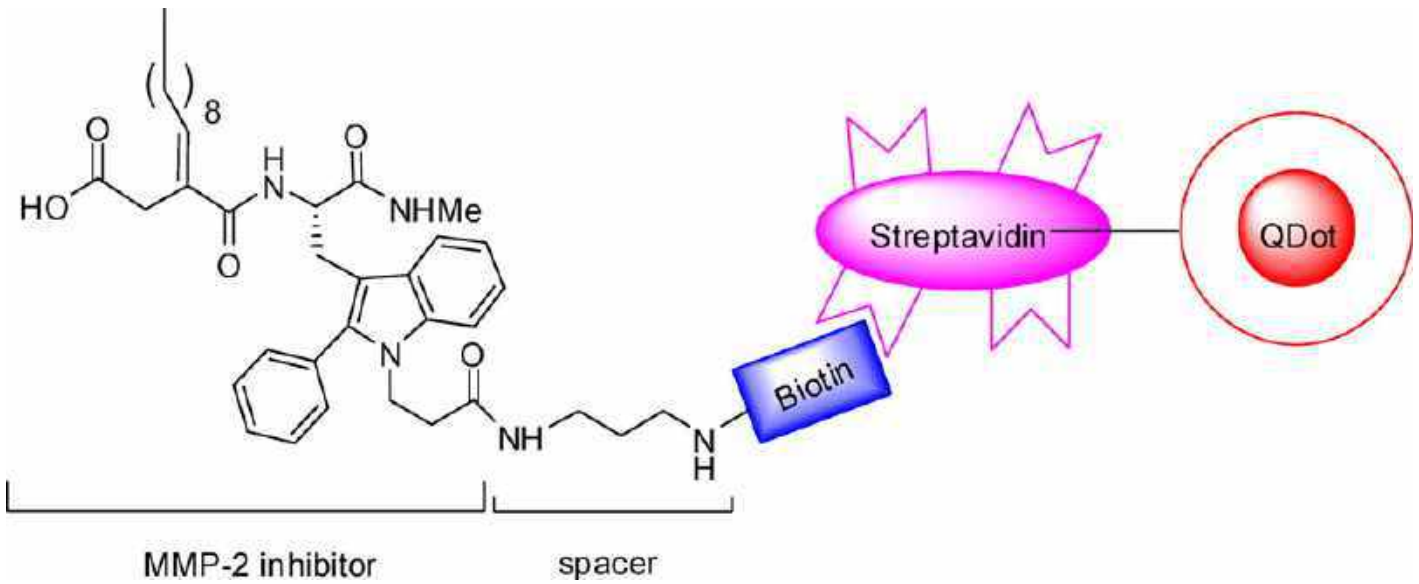


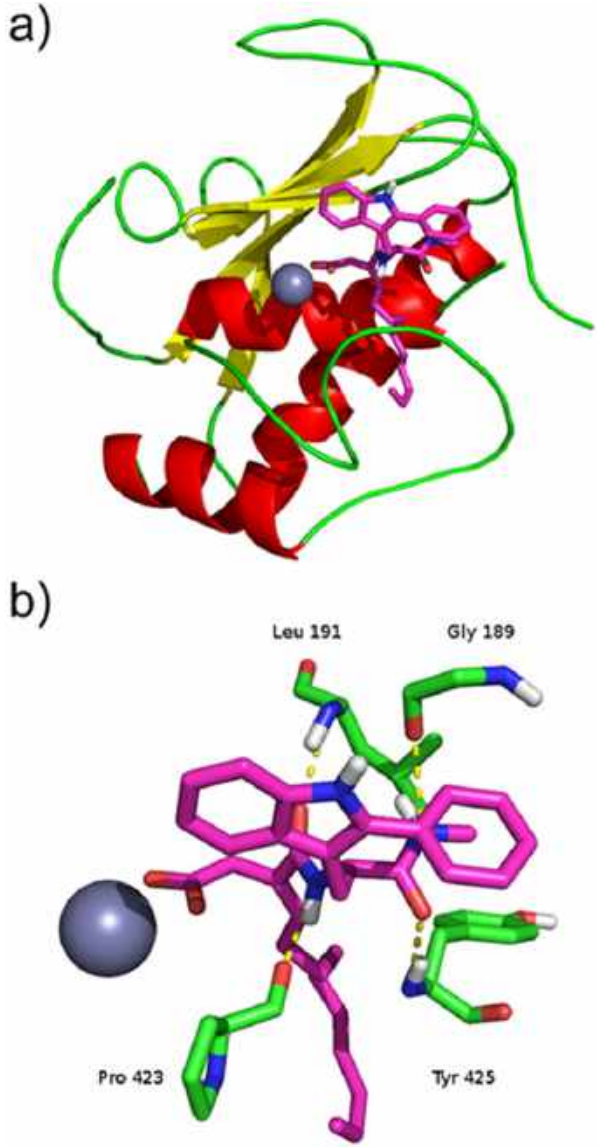


Wang, H., *et al*. Selective *in vivo* metabolic cell-labeling-mediated cancer targeting. *Nat. Chem. Biol.* **2017**, *13* (4), 415–424. https://doi.org/10.1038/nchembio.2297.

Design, Synthesis, and Use of MMP-2 Inhibitor-Conjugated Quantum Dots in Functional Biochemical Assays







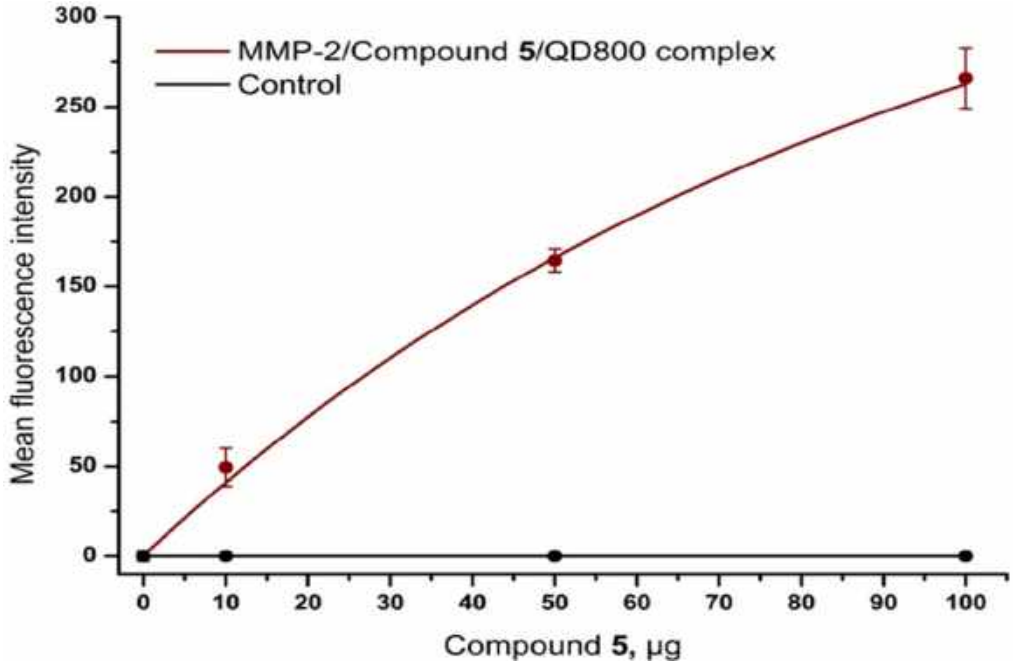
Bourguet et al. *Bioconjugate Chem* **2016.**

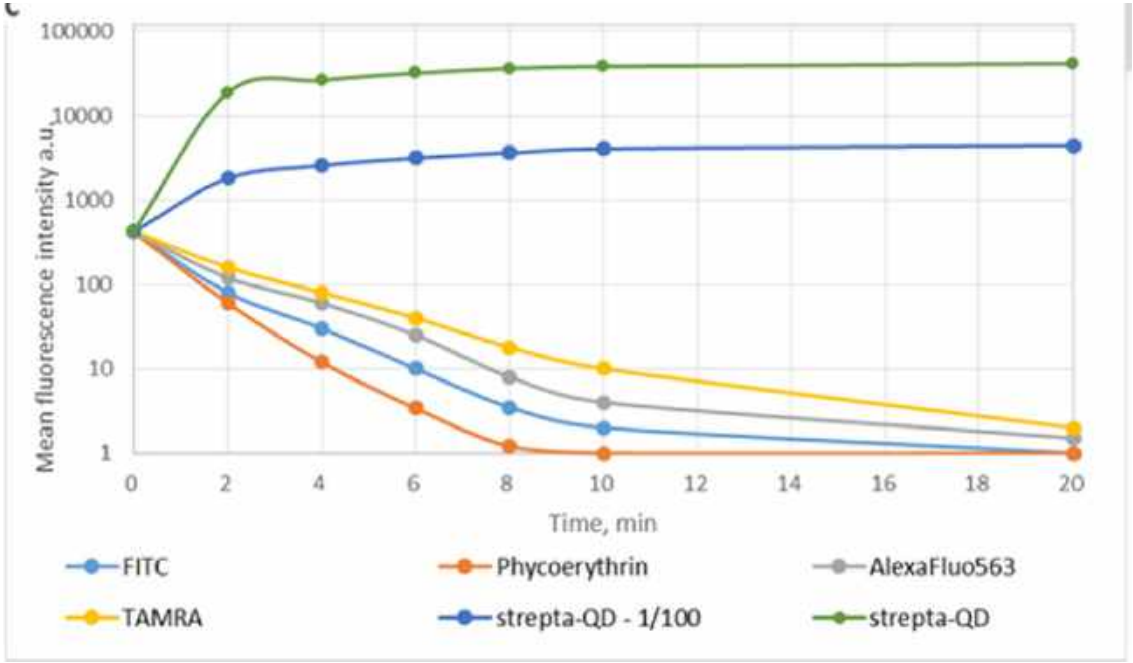
Design, Synthesis, and Use of MMP-2 Inhibitor-Conjugated Quantum Dots in Functional Biochemical Assays

Quantum Dots (QDs):

- used for many biomedical applications
- characterized by narrow composition and size dependent emission wavelength, extreme brightness, high photostability, and chemical robustness
- provides possibility for multiplexing
- used in this study to evaluate MMP-2 inhibition

QD's have an advantage over organic dyes as they allow the detection signal level to be increased by more than two orders of magnitude





Bourguet et al. *Bioconjugate Chem* **2016.**

Problem and Contextual Significance

GOAL: Obtain more information on cellular RNA activities to further amplify our understanding of RNAi.

RNA Interference (RNAi)

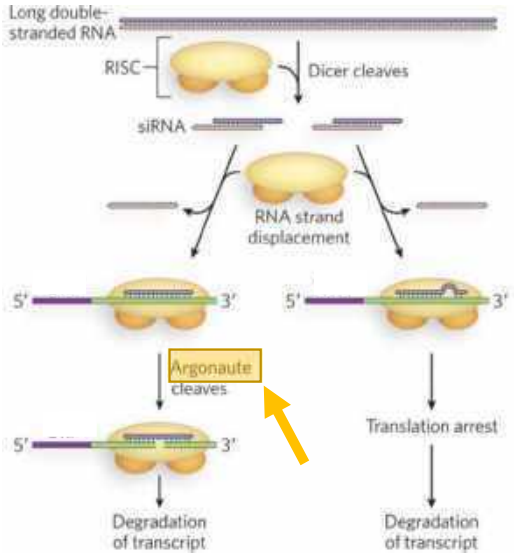


Fig. 22-17. Alternative fates of siRNA-target mRNAs.

Problem

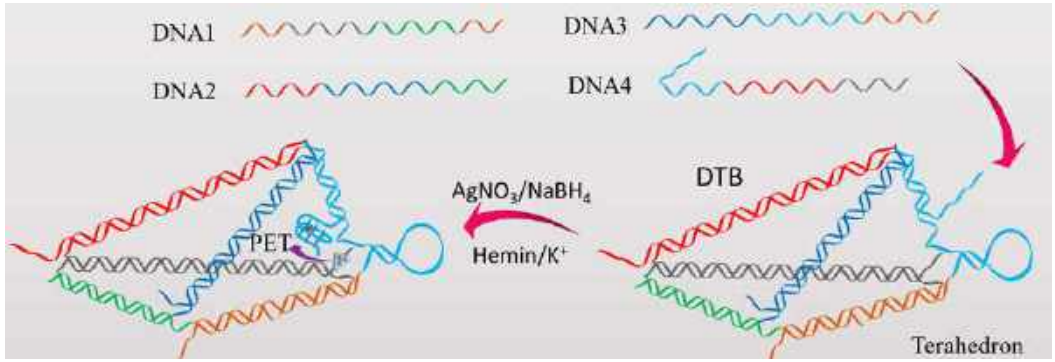
RNA is a transient species

Solution

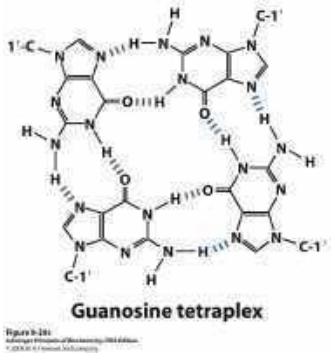
Designing a new probe by manipulating biomacromolecules

Concept

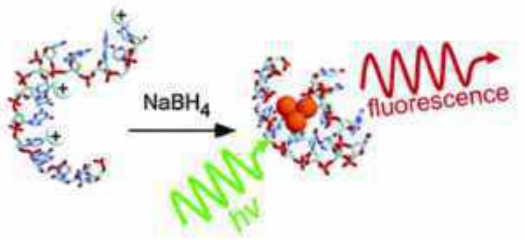
DNA Origami → DNA Tetrahedron Based Biosensor for Argnaute2 Assay



G-quadraplex (G4)



Ag Nanocluster (AgNC)

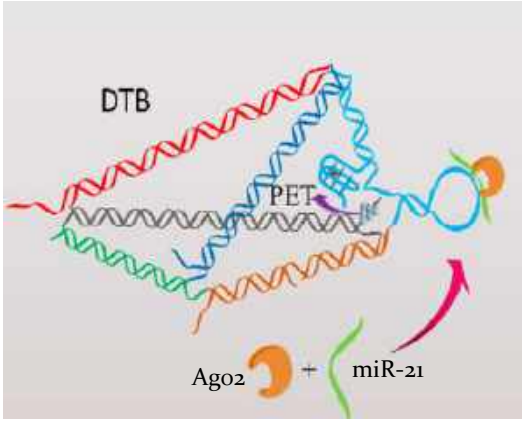


Cox, M., Douda, J., O'Donnel, M., *Molecular Biology: Principles and Practice.* US: W. H. Freeman, 2nd edition, **2015.**

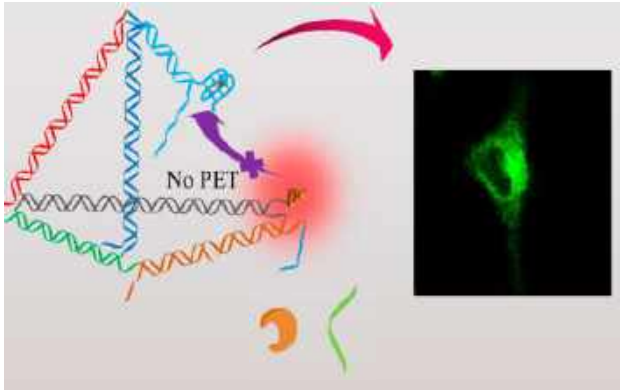
Zhang, K., Huang, W., Huang Y., Li, H., Wang, K., Zhu, X., Xie, M. *Anal. Chem.* **2019,** 91, 7086-7096.
Yuan, Z., Chen, Y., Li, H., Chang, H., *Chem. Commun.* **2014,** 69.
Lehninger, A. L., Nelson, D. L., Cox, M. M. *Lehninger Principles of Biochemistry.* US: W. H. Freeman, 5th edition.

Mechanism

Pair in proximity

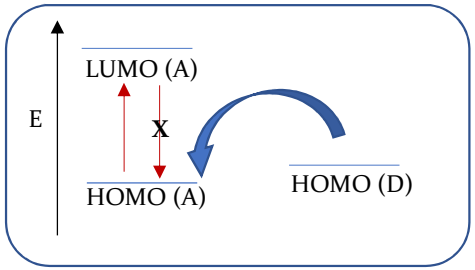


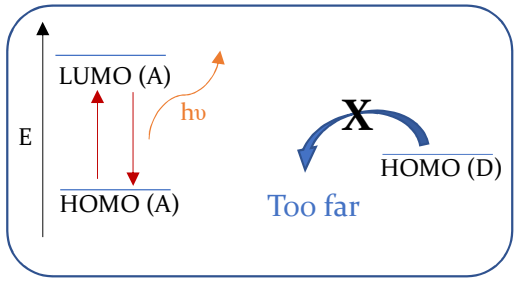
Cleavage of harpin structure and separation of pair



PET (Photoinduced Electron Transfer) PAIR

Donor (D): G4 Acceptor (A): AgNC





Fluorescence Detection
Excitation at 560nm and
Emission at 620nm

Application

Ago2/miR-21 Complex Assay in Single cells

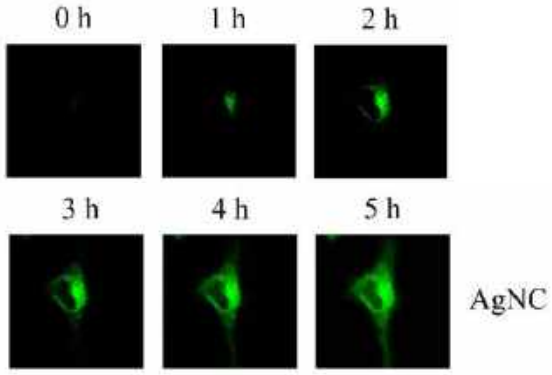


Fig. 3 – Time course of images of HeLa cells incubated with 25μL of DTB.

Ribonuclease H Assay in single cells

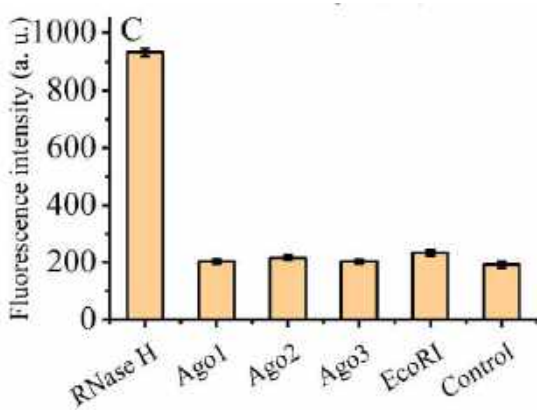
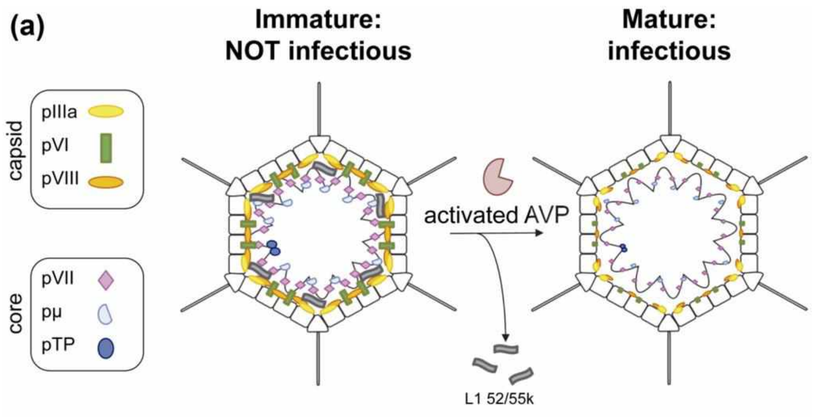


Fig. 6(c) – Specificity by using the DTB for the rest of Rnase H and other proteins.

Zhang, K., Huang, W., Huang Y., Li, H., Wang, K., Zhu, X., Xie, M. *Anal. Chem.* **2019,** 91, 7086-7096.

Concept and Technique



Adenoviral protease (AVP):

- Virus maturation and infectivity
- Cysteine protease
- Cleaves seven viral proteins

Mangel, W. & San Martín, C. Structure, Function and Dynamics in Adenovirus Maturation. *Viruses* **6**, 4536–4570 (2014).

Problem:
Detection methods and quantification assays for viruses are time-consuming

Solution:
A genetically encoded switch-on fluorescent biosensor with a viral protease site as a switch (cVisensor)

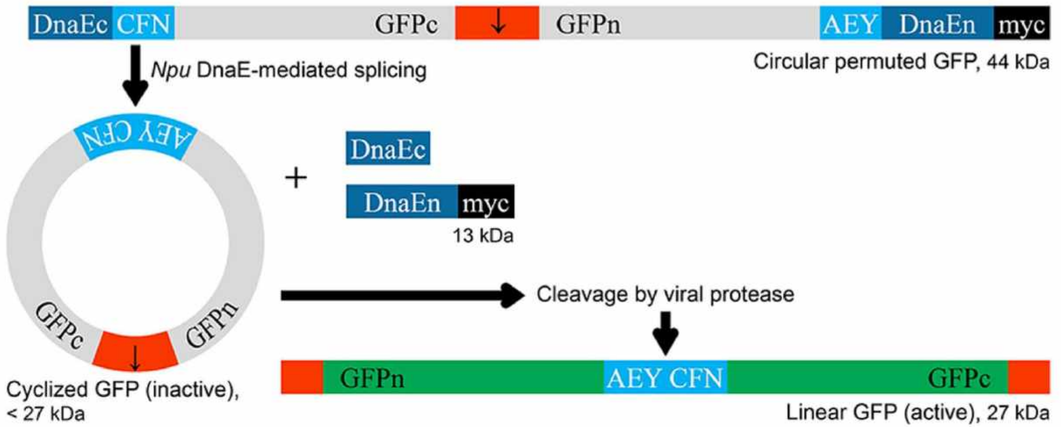
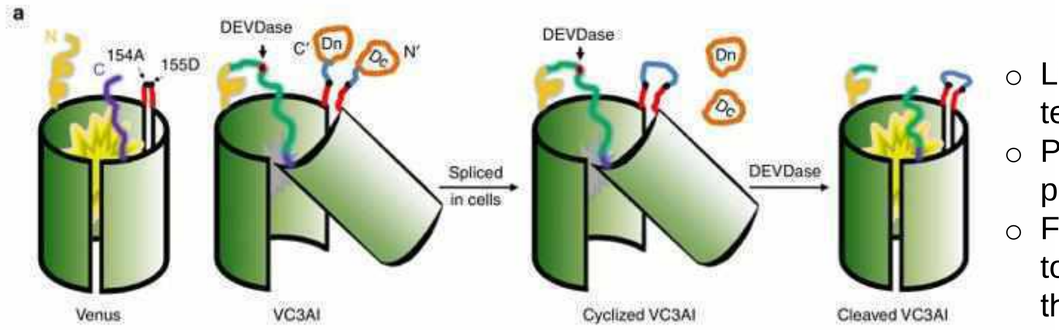


Figure 1. Design of the switch-on fluorescent biosensor.

- Structural distortion of GFP by *Npu* DNaE
- Addition of AVP cleavable sequence relieves the distortion
- cGFP emits fluorescence once proteolytic cleavage takes place

Protein cyclization:



- Linked the N- and C-termini with polypeptide
- Polypeptide cleaved by protease
- Fused *Npu* DnaE intein to the two ends to ligate the N' and C' ends

Zhang, J. *et al.* Visualization of caspase-3-like activity in cells using a genetically encoded fluorescent biosensor activated by protein cleavage. *Nat. Commun.* **4**, 2157 (2013).

Application

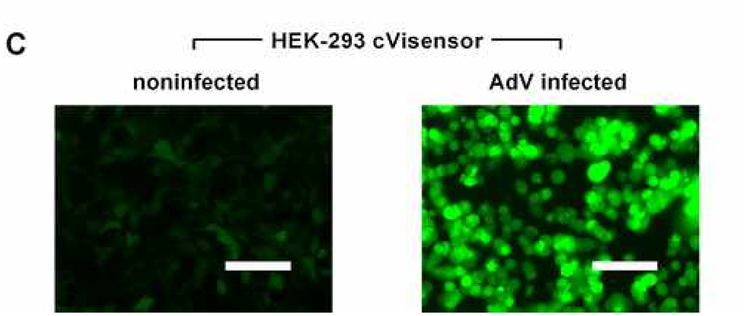


Figure 4. Fluorescence microscopy of HEK-293 cVisensor cell clone 48 h postinfection.

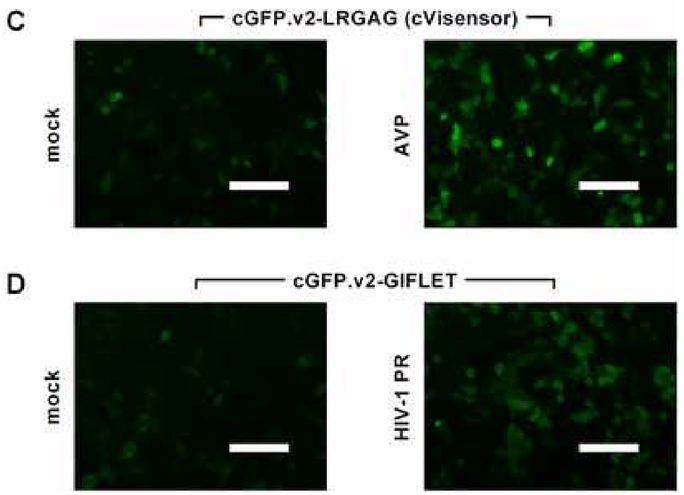
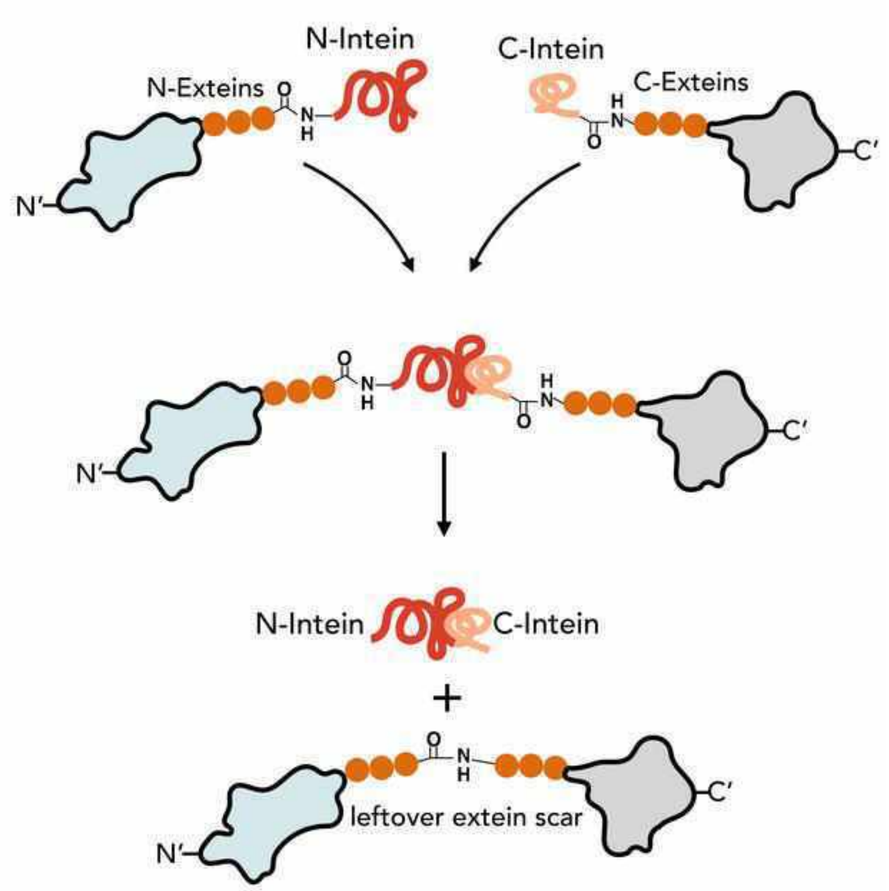


Figure 2. Fluorescence microscopy of cGFP 48 h after cotransfection with plasmids coding the viral proteases (AVP or HIV-1).

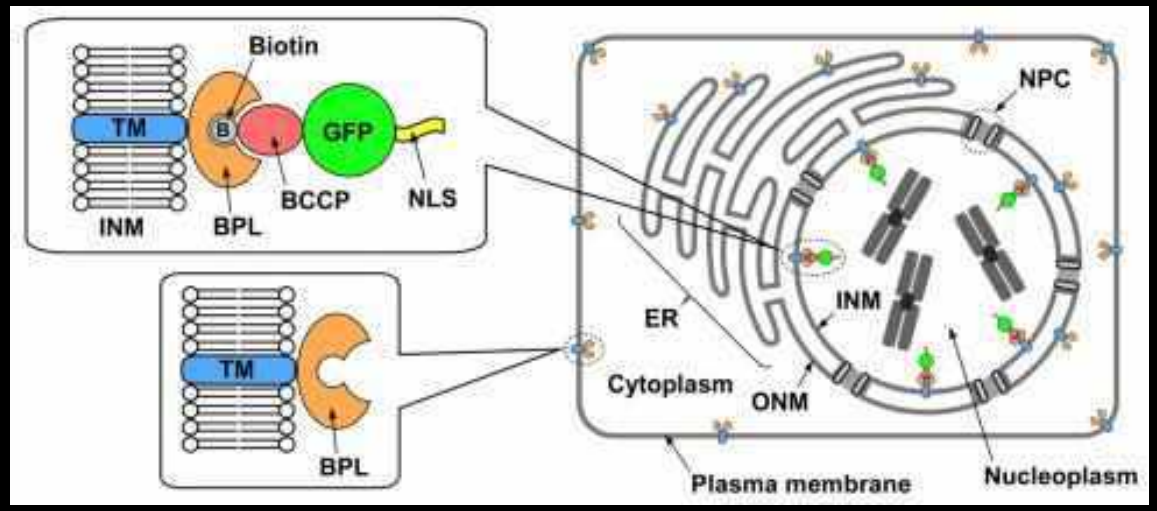
Mechanism



Trans-splicing mechanism reaction by split inteins.
http://2014.igem.org/Team:Heidelberg/Project/Background

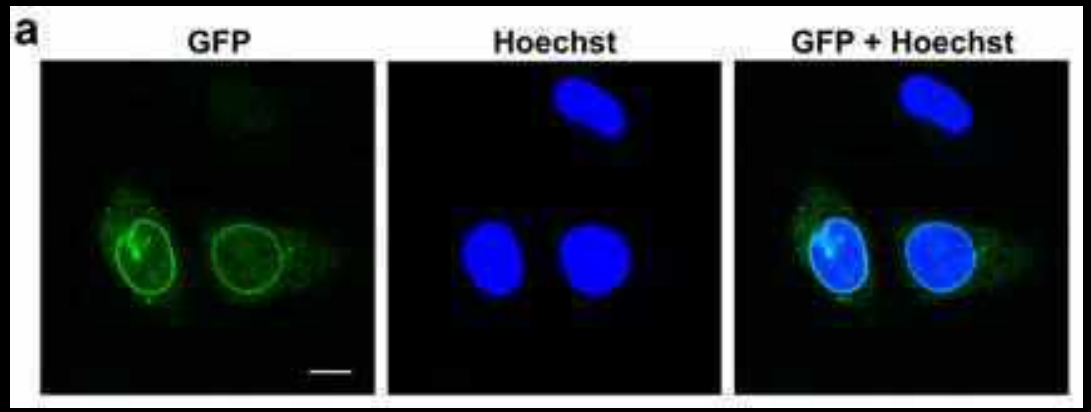
Methods

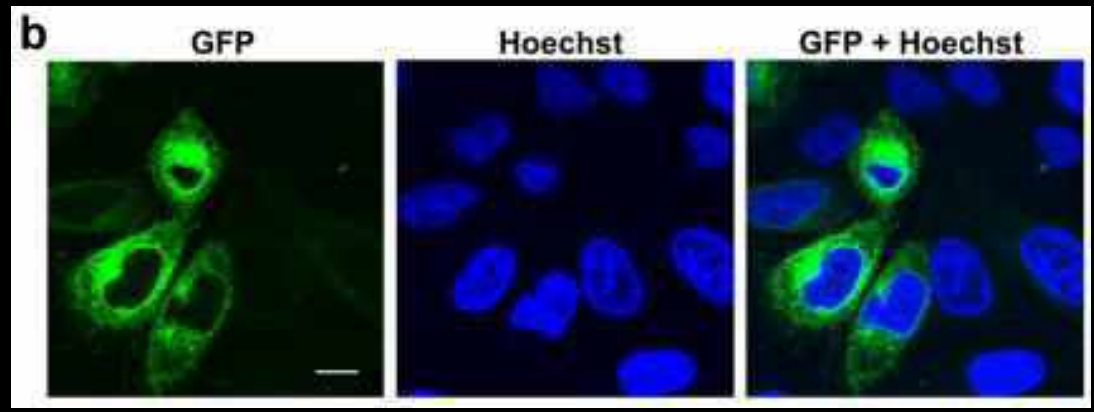
Goal:
*O*btain real time imaging of cellular division to gain insight on the dynamics of the process.



Important Features:
- Biotinylation reaction from *S. tokodaii*
- Restriction of fluorescent complex to inner membrane
- Red fluorescent protein (mApple)

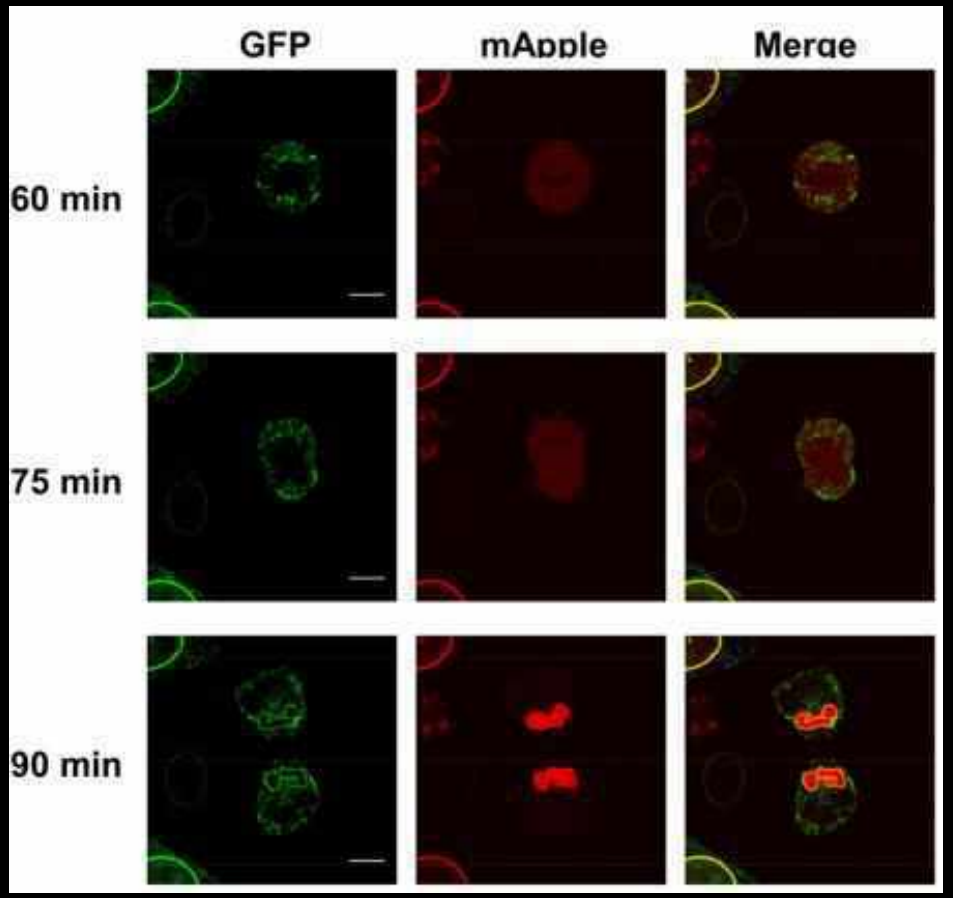
Feasibility:

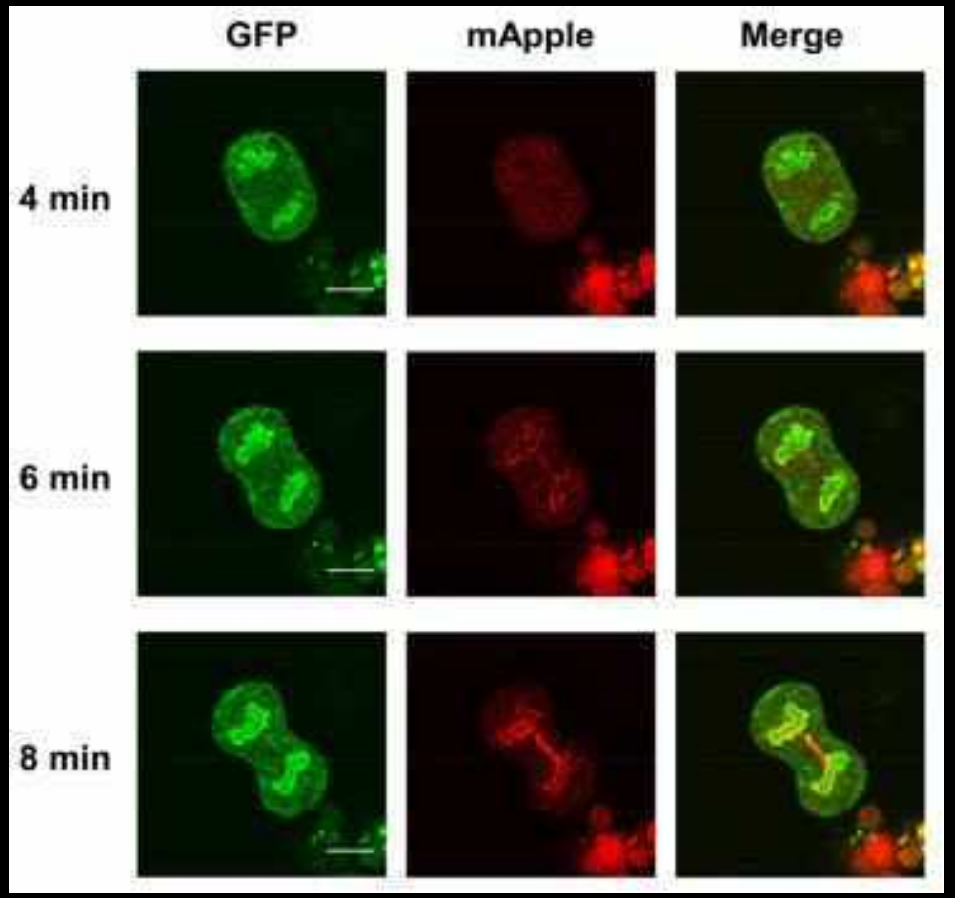


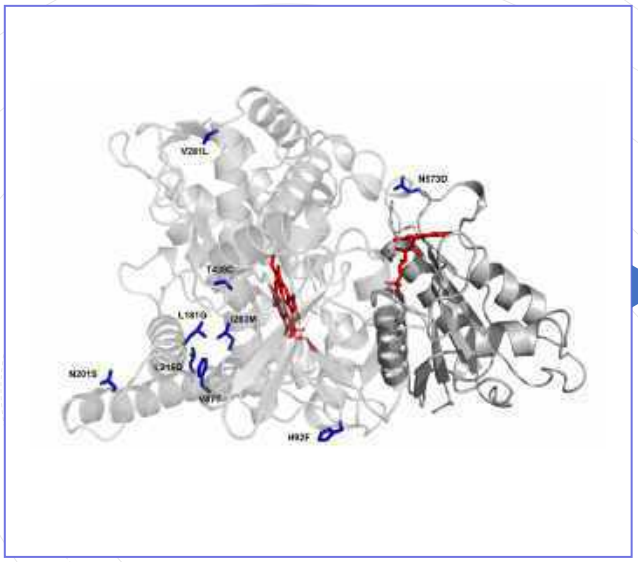


Results

- Used mApple red fluorescent protein to label Lamina A
- Time lapse of HeLa cells obtained every 2 min from beginning of anaphase until cytokinesis
- Differences in timing of formation of nuclear envelope and lamina







Directed Evolution of a Cytochrome P450 Carbene Transferase for Selective Functionalization of Cyclic Compounds

Matthew McConnachie

Brandenberg, O. F.; Chen, K.; Arnold, F. H. Directed Evolution of a Cytochrome P450 Carbene Transferase for Selective Functionalization of Cyclic Compounds. *J. Am. Chem. Soc.* **2019**, *141* (22), 8989–8995. https://doi.org/10.1021/jacs.9b02931.

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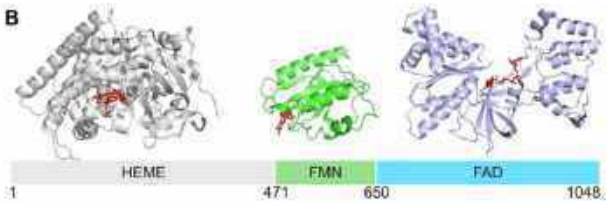
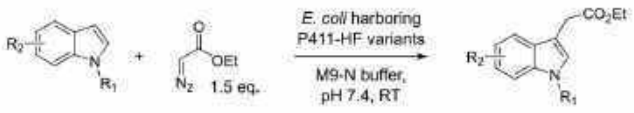
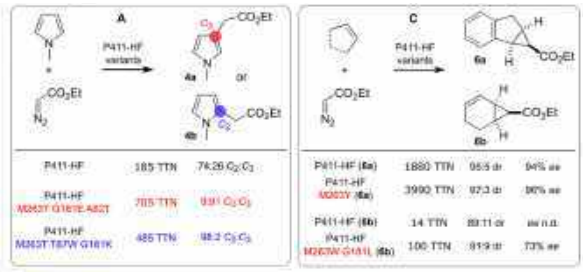
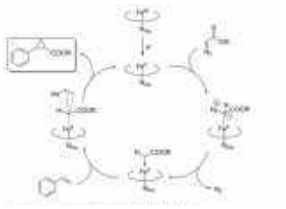


- Evolution was started from a p411-CIS which was previously evolved for styrene cyclopropanation.
- 6 rounds of directed evolution were performed using using both random mutagenesis with error prone PCR and site saturation mutagenesis resulting in a library of over 7000 plasmid
- It was found that a mutation with a stop codon at the end of the FMN region and 11 mutations resulted in a highly active alkylation enzyme named p411-HF.





- Further directed evolution from p411-HF yielded both regio selective carbene addition between C2 and C3 a well as Enantiomerically selective cycloproponation of cyclic internal alkenes



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