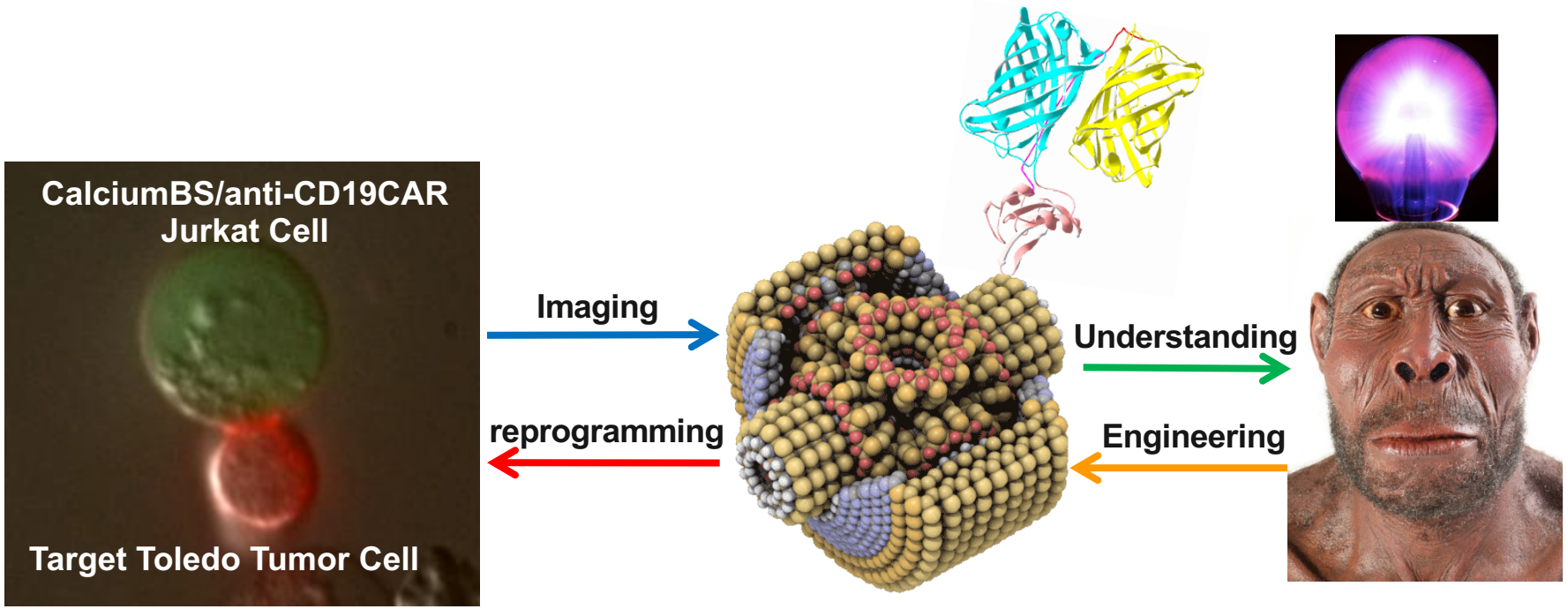
Cellular Engineering for Cancer Immunotherapy

Peter Yingxiao Wang, Professor
Department of Bioengineering, Institute of Engineering in Medicine, University of California at San Diego

Molecular Imaging and Reprogramming of Cells



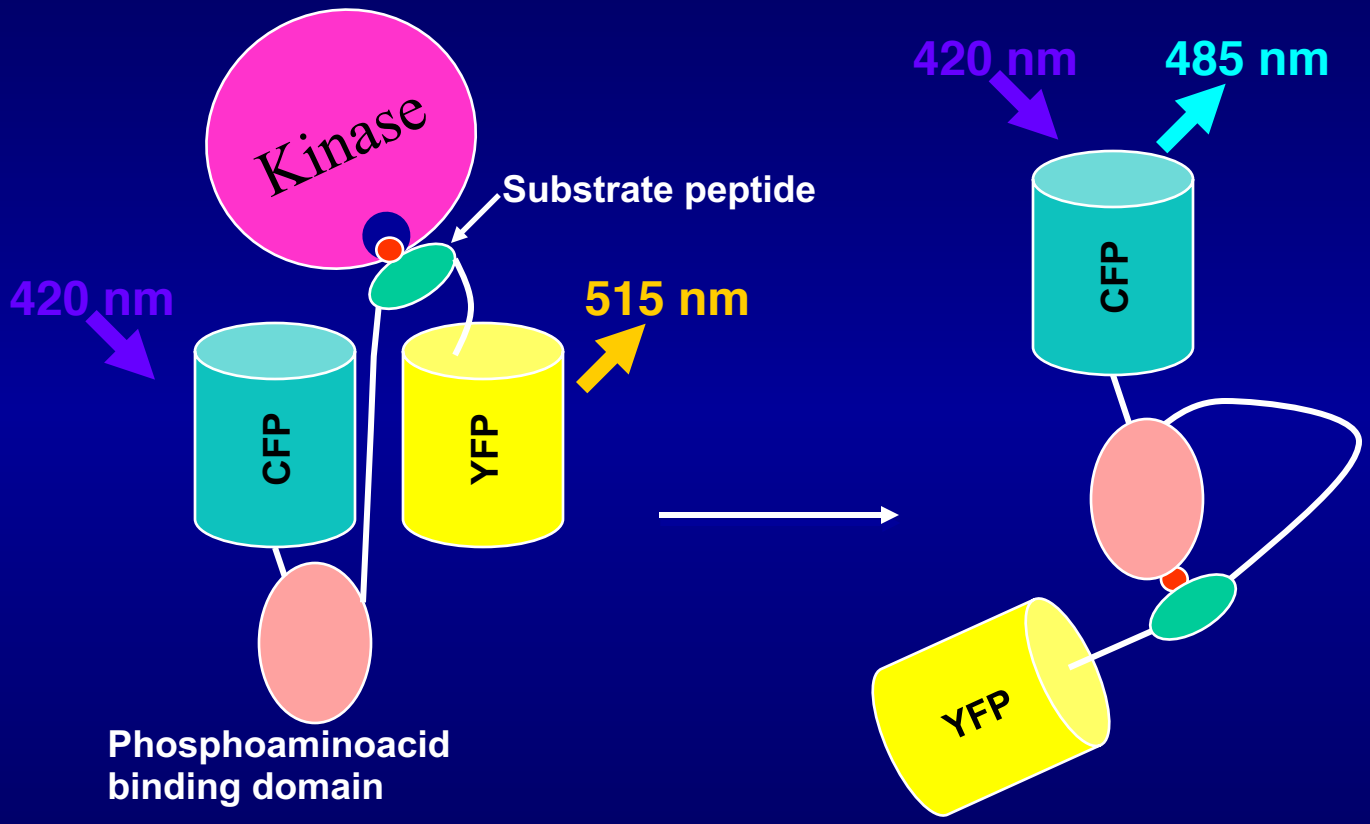
Outline

1. **The engineering and application of FRET biosensors for molecular imaging in live cells**
2. **The engineering of a machinery molecule for sensing and actuating to re-engineer macrophages for tumor eradication**
3. **The engineering of remote controllable immuno-cells for therapeutics via mechanical stimulation**

Outline

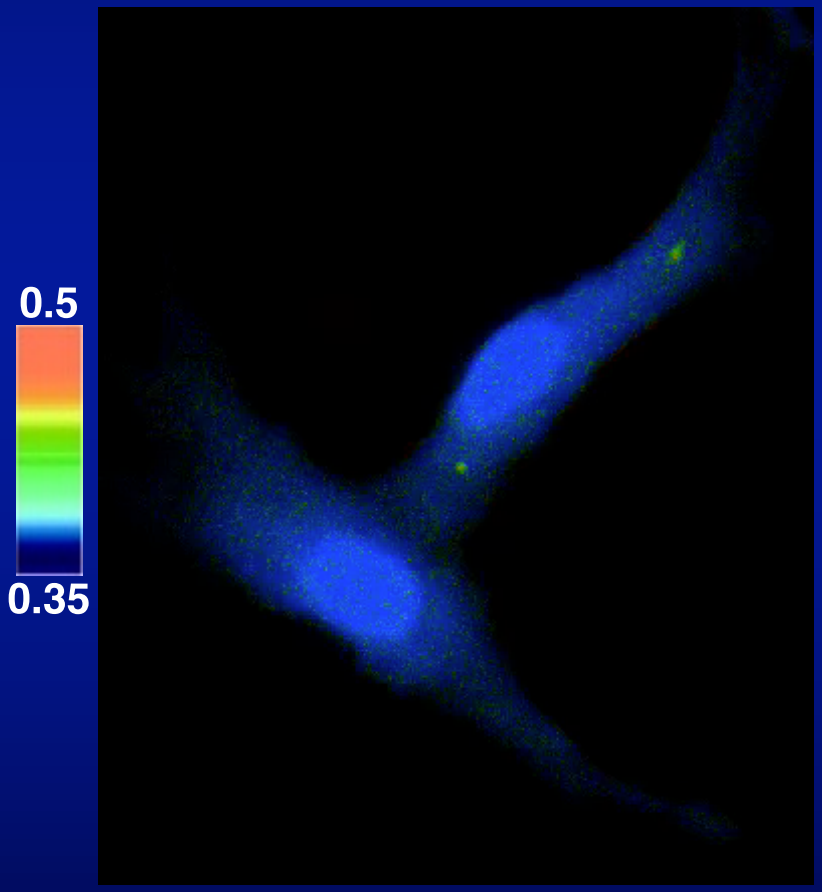
1. **The engineering and application of FRET biosensors for molecular imaging in live cells**
2. **The engineering of a machinery molecule for sensing and actuating to re-engineer macrophages for tumor eradication**
3. **The engineering of remote controllable immuno-cells for therapeutics via mechanical stimulation**

A General Design for Imaging Kinase Activities



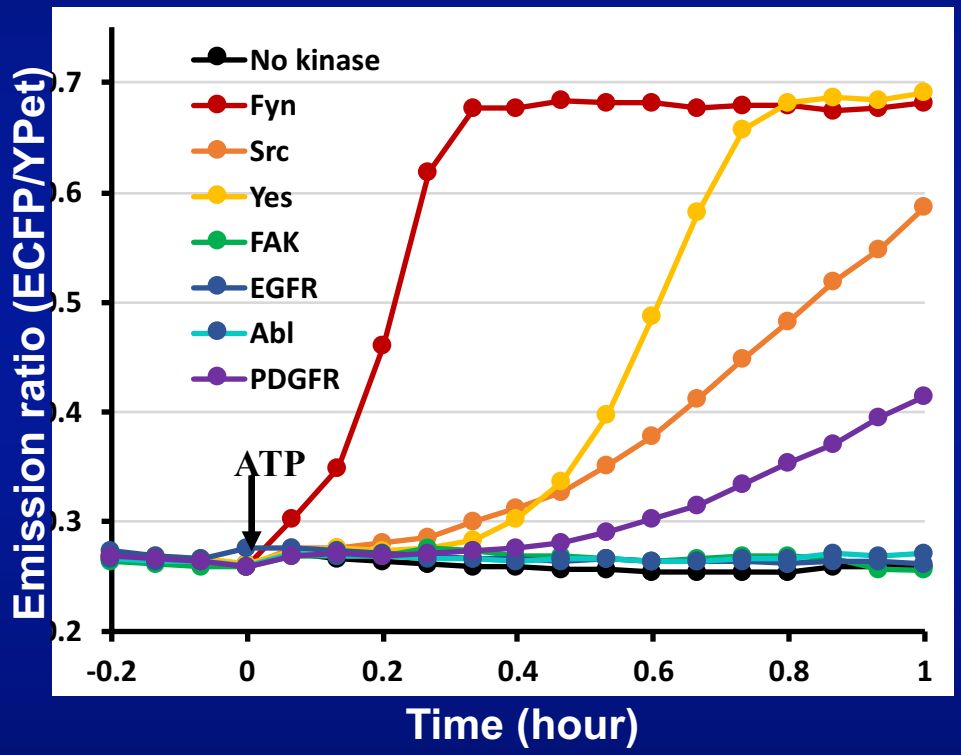
The FRET Biosensors and Their Applications

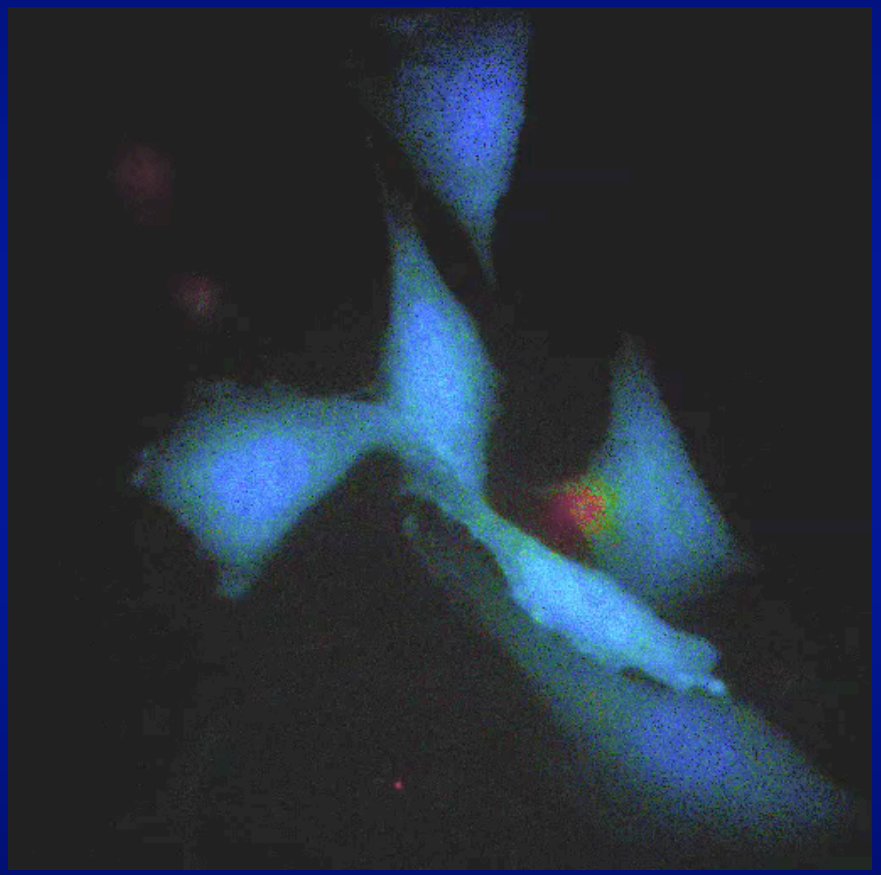
(FRET: fluorescence resonance energy transfer)

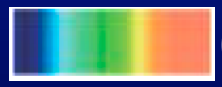


Wang, et al. Nature, 2005
Ouyang, et al. PNAS, 2008
Seong et al, Nature Communications, 2011
Sun et al, Nature Communications, 2013

PDGF-stimulated FRET response of Fyn biosensor in MEF cells

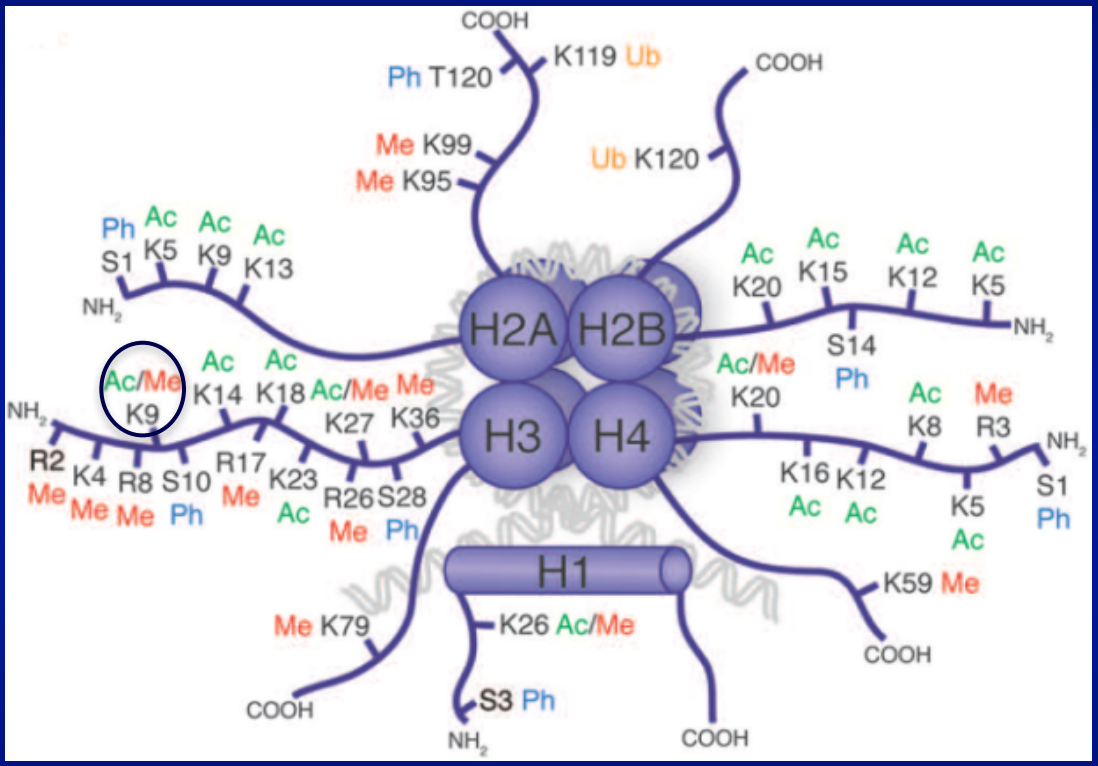




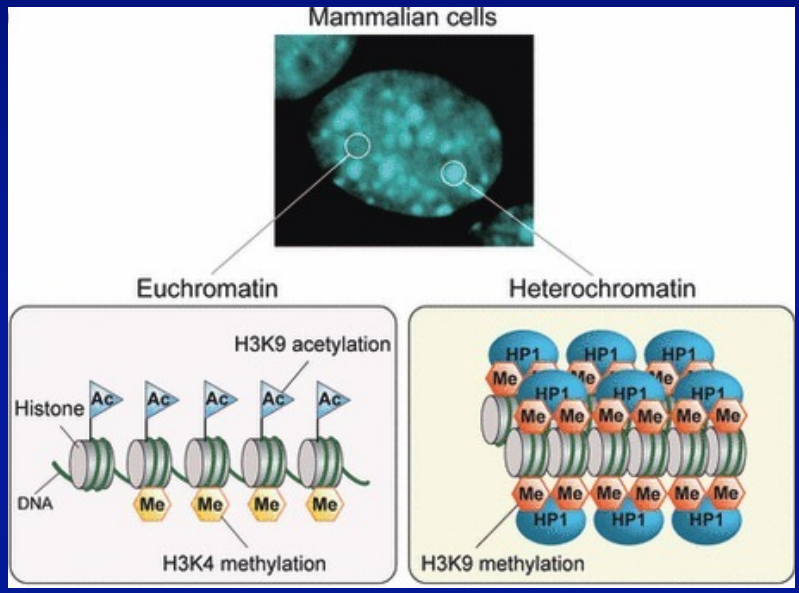


Ouyang et al ACS Sensors 2019

Epigenetics and Histone Code



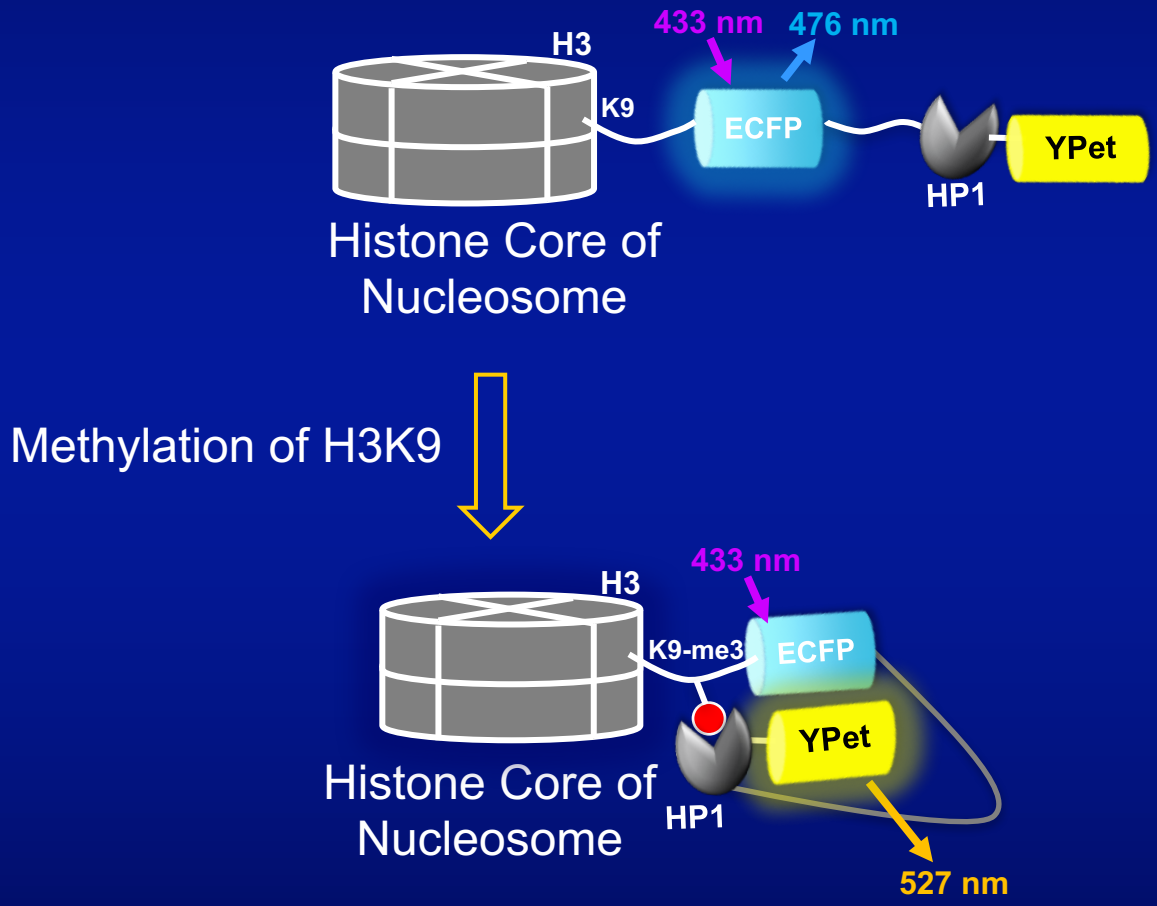
Cota, et al. Stem Cells and Epigenetic Reprogramming. 2013. DOI: 10.5772/55983.



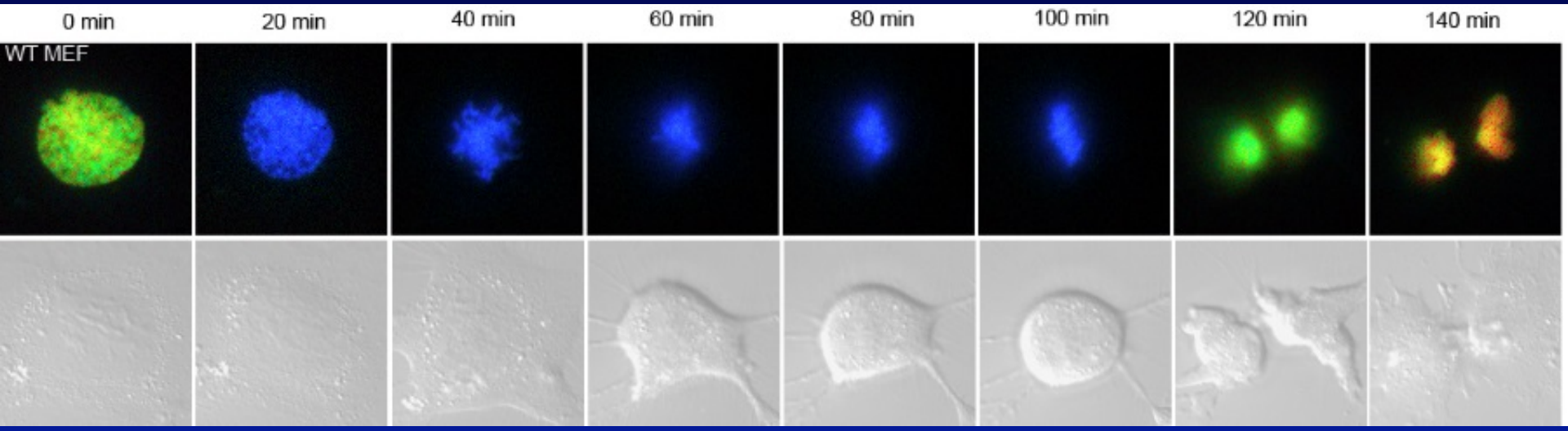
HP1: Heterochromatin-1

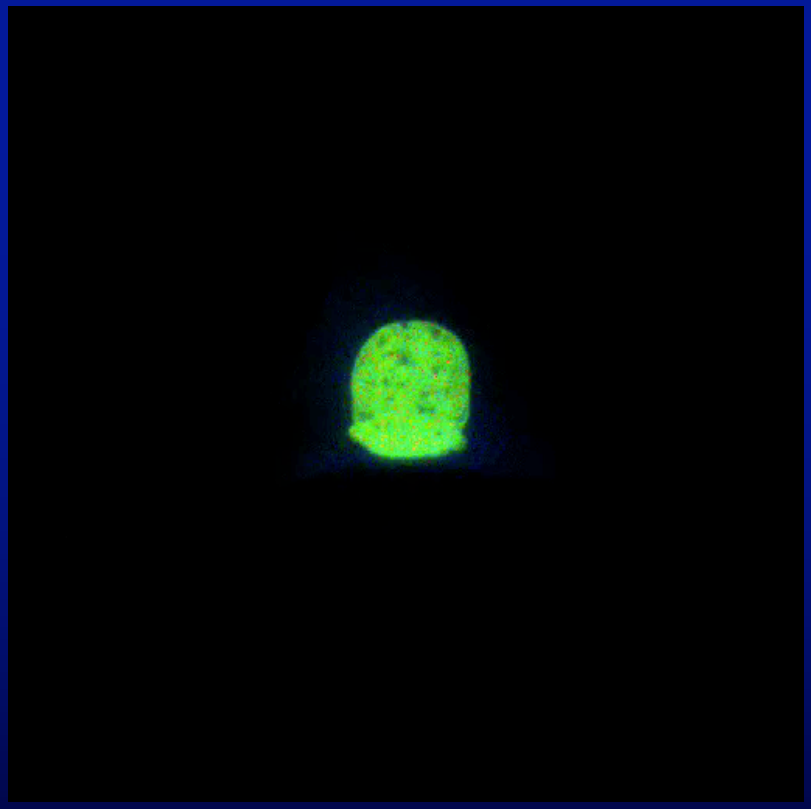
Goto DB, Nakayama J - Dev. Growth Differ. (2011)

A FRET Biosensor for Histone H3K9 Methylation



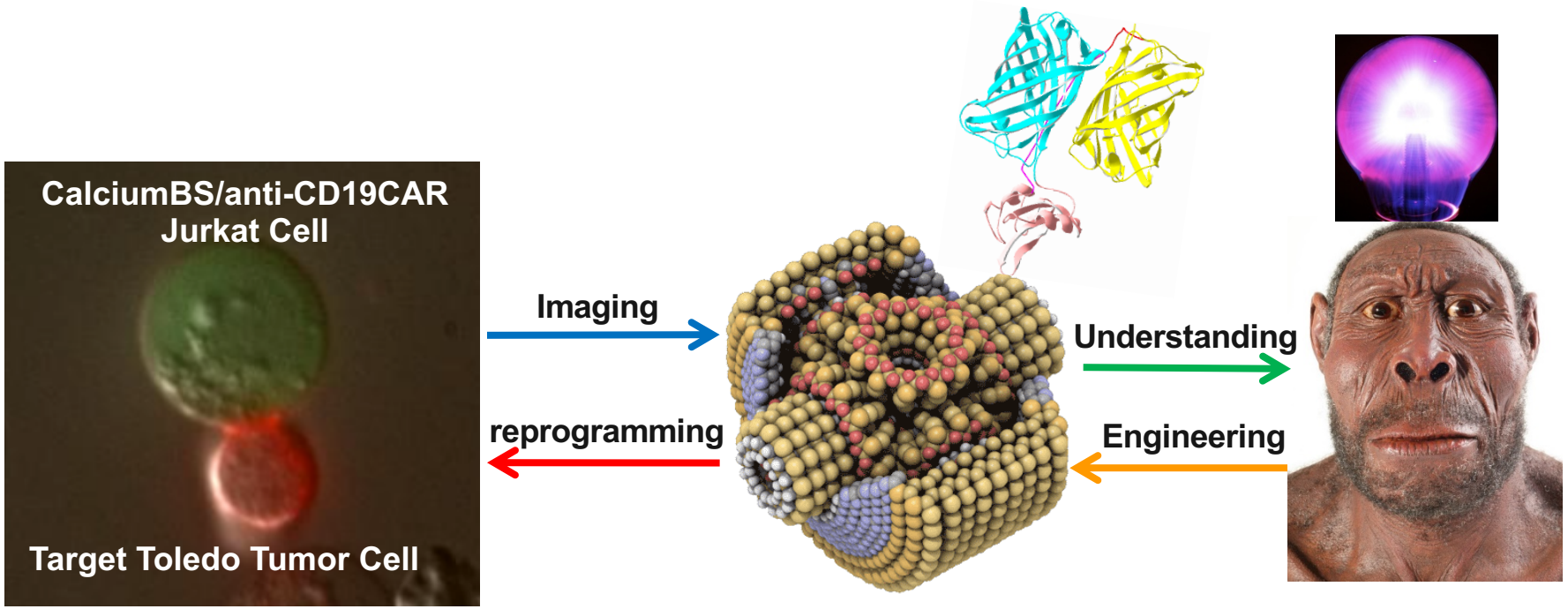
The Dynamic Nature of H3K9 Methylation in a MEF Cycle





Peng Q et al PNAS 2018

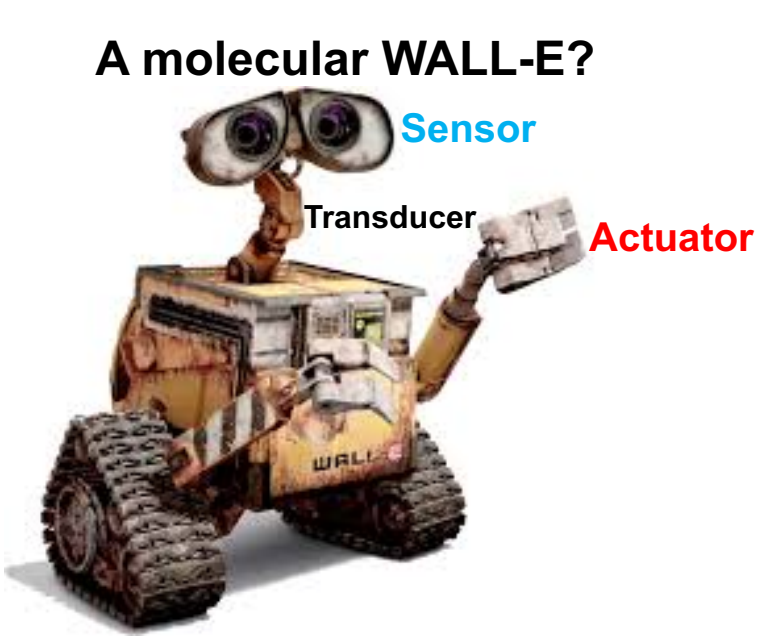
Molecular Imaging and Reprogramming of Cells

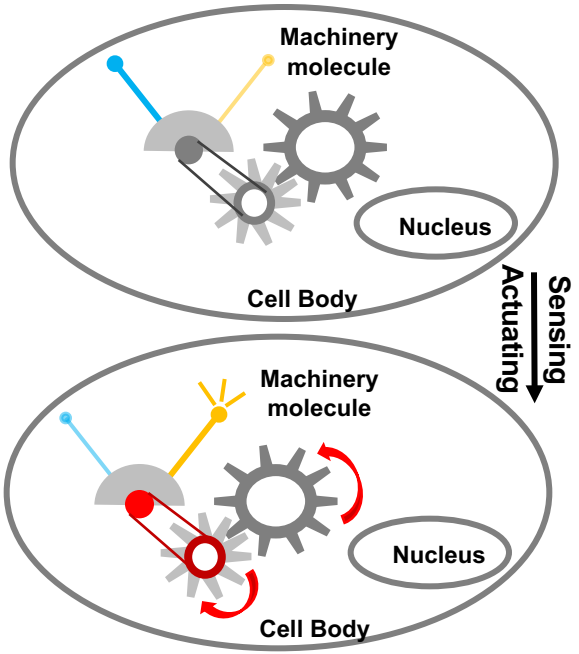


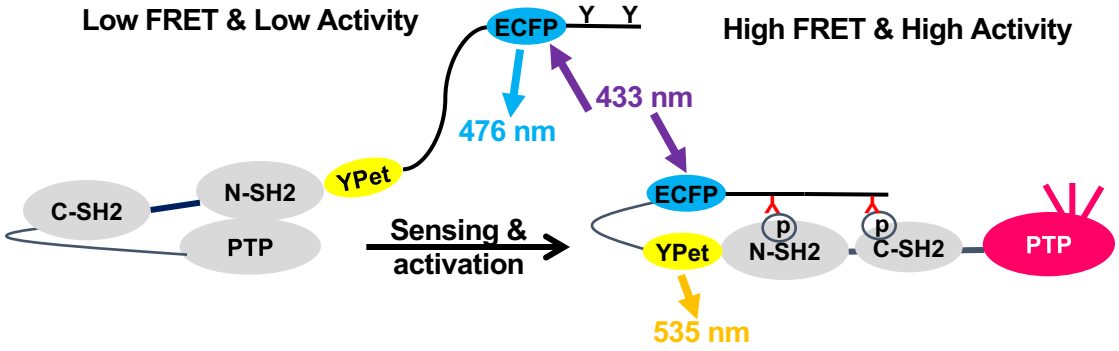
Outline

1. **The engineering and application of FRET biosensors for molecular imaging in live cells**
2. **The engineering of a machinery molecule for sensing and actuating to re-engineer macrophages for tumor eradication**
3. **The engineering of controllable immuno-cells**

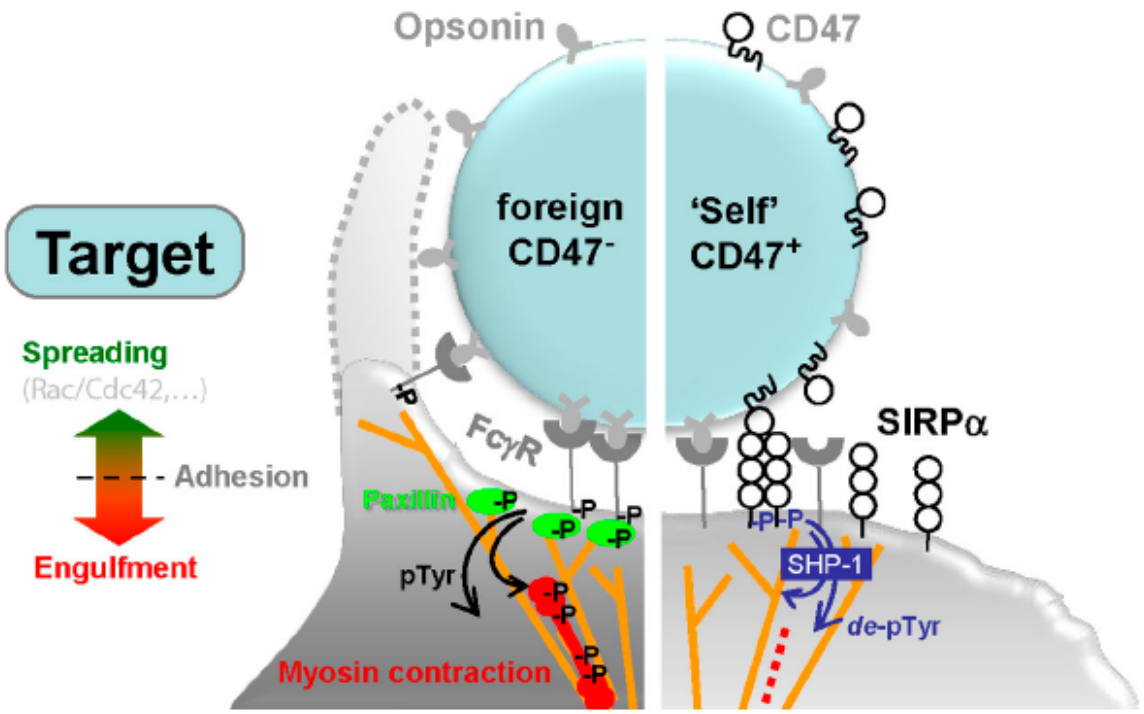
A machinery molecule for imaging and actuating Shp2, a protein tyrosine phosphotase





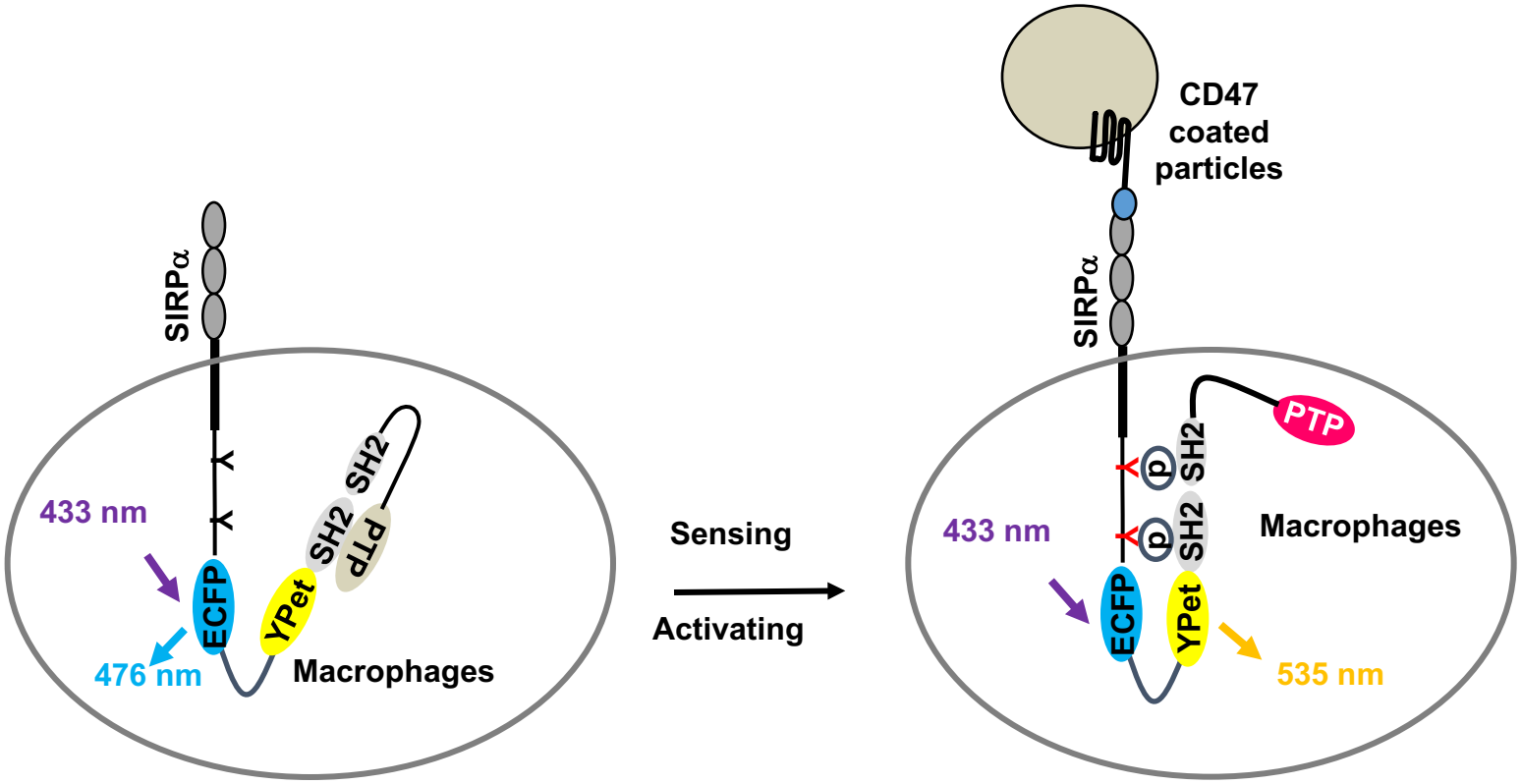


The role of Shp2 machinery molecule in manipulating macrophage phagocytosis

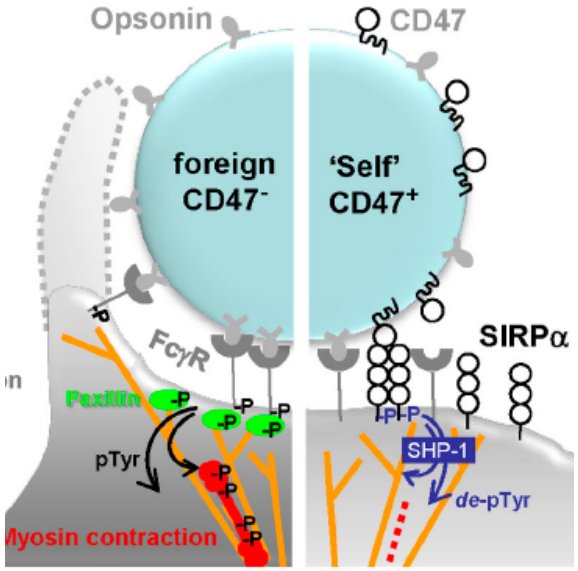


The Journal of Cell Biology, Vol 180, 989–1003

Strategy to improve sensitivity of machinery molecule

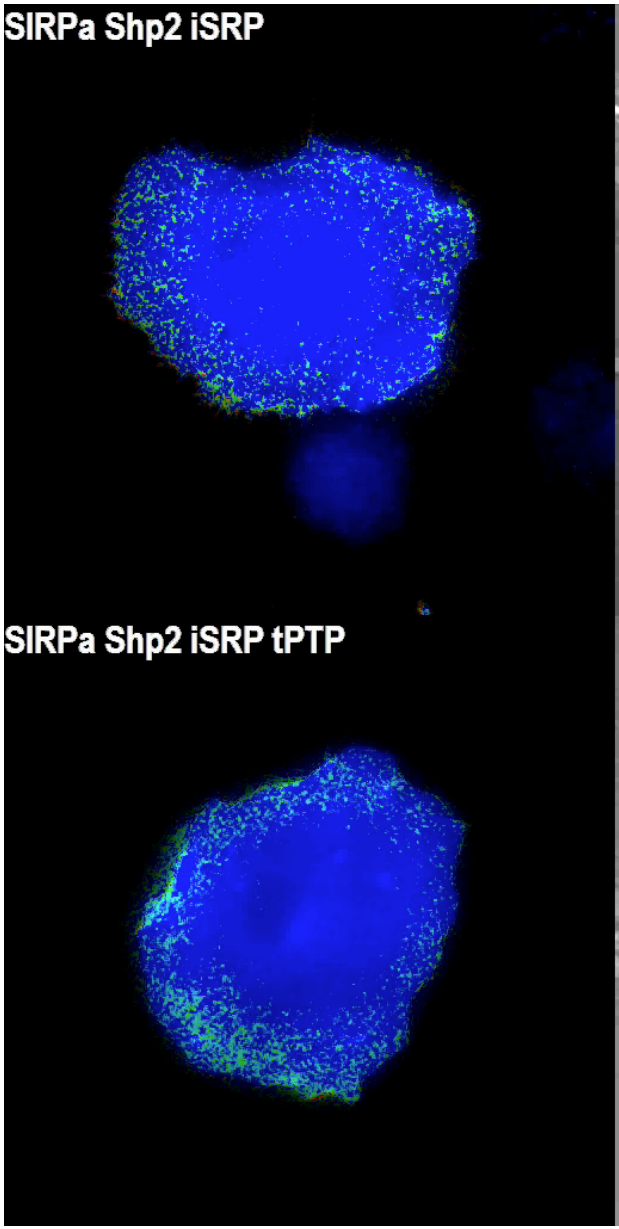


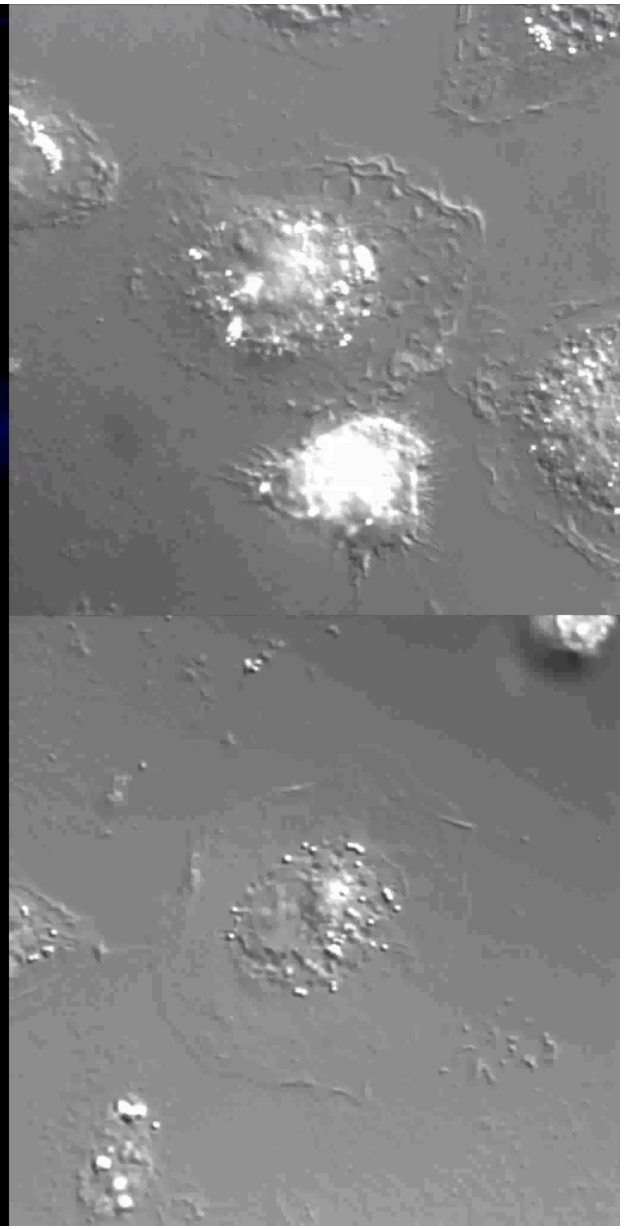
The engineering of iSNAP for sensing and activating in live macrophage cells (Inspiration from Roger)



The Journal of Cell Biology, Vol 180, 989–1003

Sun J. et al Nat Comm 2017





Outline

1. **The engineering and application of FRET biosensors for molecular imaging in live cells**
2. **The engineering of a machinery molecule for sensing and actuating to re-engineer macrophages for tumor eradication**
3. **The engineering of controllable immuno-cells for therapeutics**

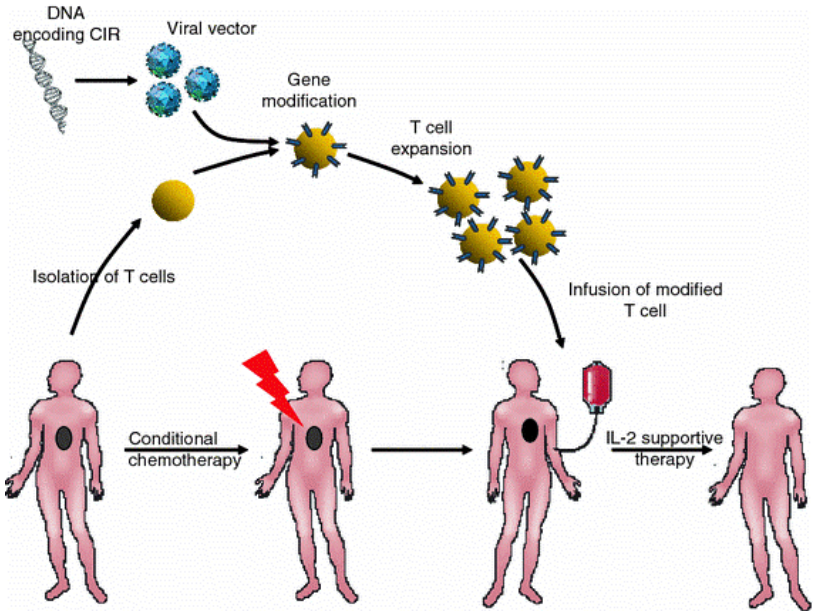
Background

- Immunotherapy is revolutionary for cancer therapies
- The #1 breakthrough of 2013 ranked by Science
- Activate immuno-system in the body
- CAR (chimeric antigen receptor) T immunotherapy is becoming a prominent and dominant approach.
- Memory T cells induced by CAR T approach can last for years in suppressing tumor relapse.

Background

The clinical flow chart of CAR T cancer Immunotherapy:

- immuno cells from patients can be isolated and genetically engineered. At the same time, patients will undergo chemotherapy to reduce host response to engineered immuno cells.
- Engineered immuno cells will be perfused back into the patients to recognize and kill tumor cells.



Background

CAR T therapies for blood tumors:

Table 1 | **CD19-specific-CAR T-cell therapy outcomes in patients with B-ALL**

Institution	CAR design	Patient population	Outcome	Toxicities	Reference
MSKCC	CD28, CD3ζ	• *n* = 32 adults • R/R B-ALL	91% CR	• B-cell aplasia • CRS	NCT01044069 (REF. 13)
UPenn/ CHOP	4-1BB, CD3ζ	• *n* = 30 children and young adults • B-ALL	90% CR	• B-cell aplasia • CRS	NCT01626495 (REF. 15)
NCI	CD28, CD3ζ	• *n* = 20 children and young adults • B-ALL	70% CR	• B-cell aplasia • CRS	NCT01593696 (REF. 17)
Fred Hutchinson	4-1BB, CD3ζ	• *n* = 20 adults • B-ALL	83% CR	CRS	NCT01865617 (REF. 18)

Preconditioning chemotherapy was used in all the trials shown in this table. B-ALL, B-cell acute lymphoblastic leukaemia; chemo, chemotherapy; CHOP, Children's Hospital of Philadelphia; CR, complete response; CRS, cytokine-release syndrome; Fred Hutchinson, Fred Hutchinson Cancer Research Center; MSKCC, Memorial Sloan Kettering Cancer Center; NCI, National Cancer Institute; R/R, relapsed and/or refractory; UPenn, The University of Pennsylvania.

Background

In 2013, the global market of cancer drugs was about $65 billions. It is expected that this market will reach $100 billions in 2018. With an estimated growth of $25-45 billion per year, it will reach $150 billions in 2020. CAR T is becoming a prevalent technology for cancer therapy and hence is expected to share a large portion of this market. There are indeed a growing list of rising-star companies in this direction, with some of the products aligning along the pipeline for markets.

Company	Country	Focus and Lead Product Candidate	Clinical Trial Status	Year Founded	IPO Date	Market Cap
Cellectis S.A.	France	CAR-T based cancer immunotherapies UCART19: chronic lymphocytic leukemia	Phase 1	2000	Mar 2015	$675M
Bellicum Pharma	USA	CAR-T cellular immunotherapies BPX-601: hematological cancers	Phase 2	2004	Dec 2014	$620M
Kite Pharma	USA	CAR-based cancer immunotherapies KTE-C19: large B cell lymphoma	Phase 2	2009	Jun 2014	$2.7B
Juno Therapeutics	USA	CAR-T based cancer immunotherapies JCAR015B: acute lymphoblastic leukemia	Phase 2	2013	Dec 2014	$3.3B

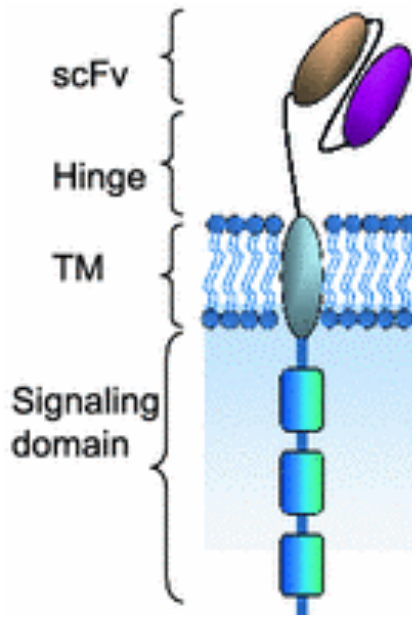
Acquired by Gilead in 2017 with 11.9 billion

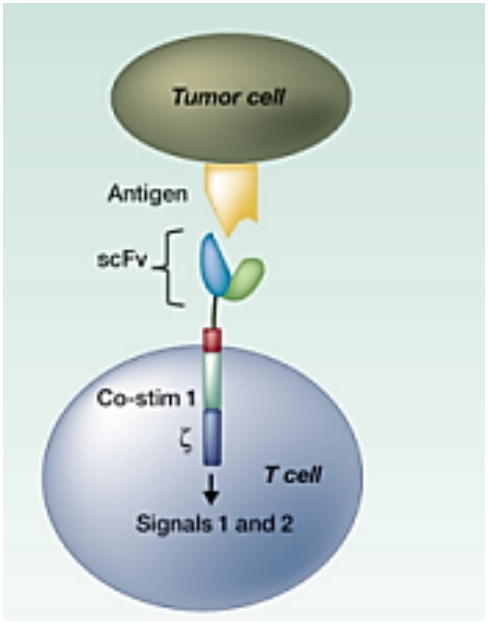
Acquired by Celgene in 2018 with 9 billion

Cell Design Labs founded in **2015**, and **acquired by Gilead in 2018 with 567 million**

Background

Chimeric Antigen Receptor T Cell Therapy (CAR-T)

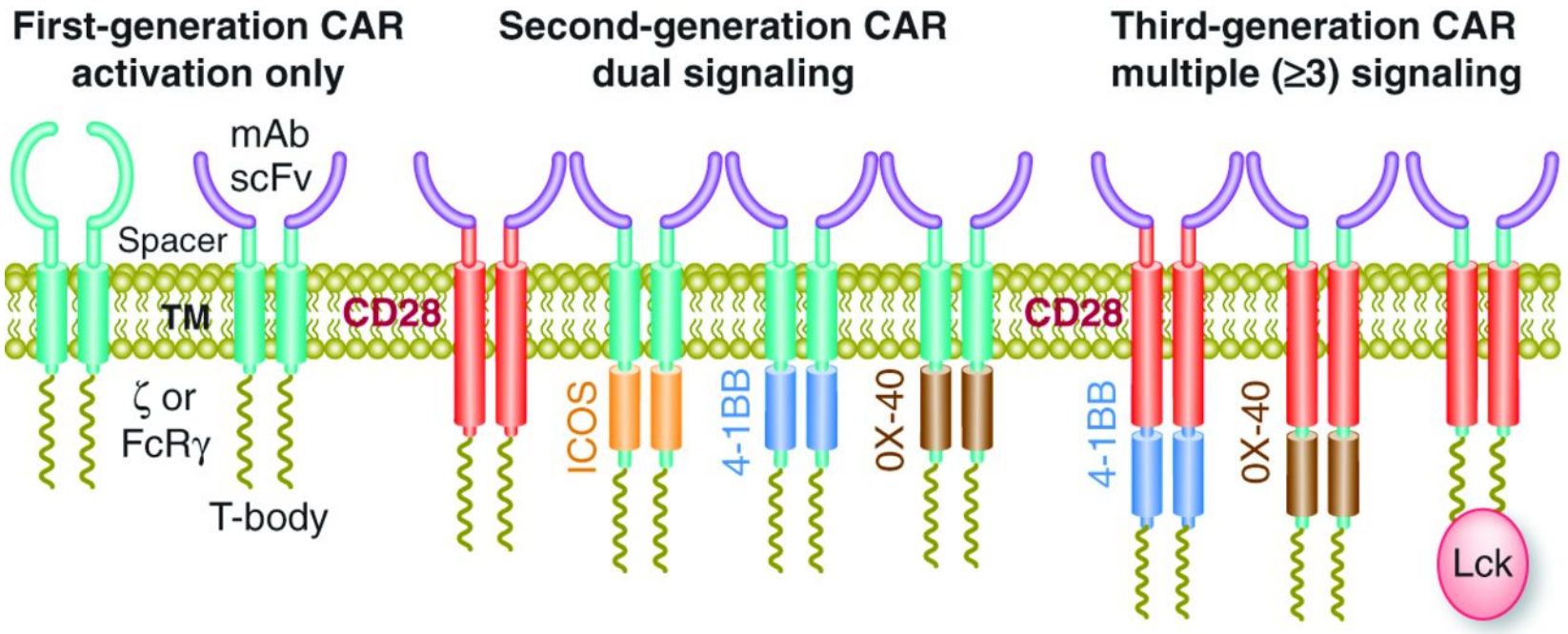




CAR can be genetically engineered to express in immuno cells. These engineered immuno cells can recognize antigens on tumor cells and trigger immuno response to kill tumor cells.

Background

The Developmental History of CAR T Cancer Immunotherapy



First Generation of CAR: **with one activator**
Second Generation of CAR: **with two activators**
Third Generation of CAR: **with three or more activators**

Challenges

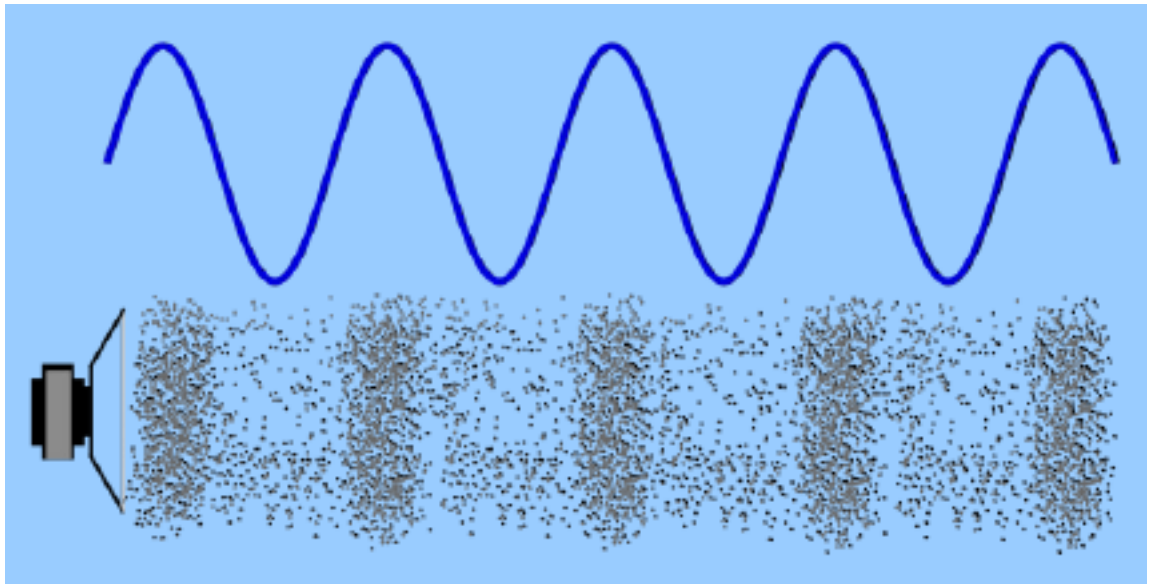
CAR T cell therapy is becoming a paradigm-shifting therapeutic approach for cancer treatment. However, major challenges, particularly safety control, remain before CAR-based immunotherapy can become widely adopted.

1. Cytokine storm: CAR T cells can rapidly grow and expand upon the engagement of target tumor cells. The consequent release of cytokines can lead to cytokine storm which can be life-threatening.

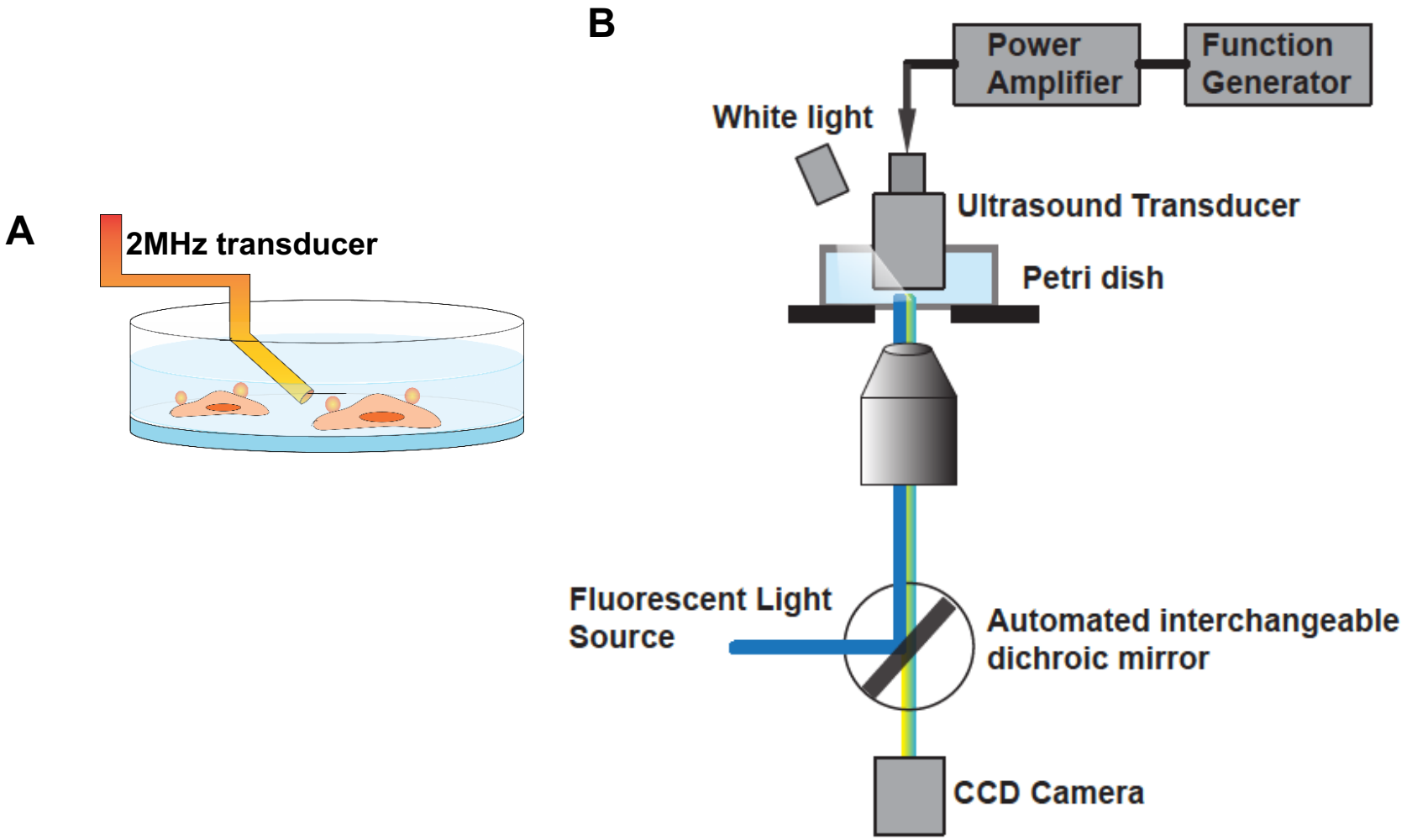
2. On-target but off-tumor toxicities: The non-specific targeting of the CAR T cells against normal/nonmalignant tissues have caused patient deaths.

Remote and Non-invasive Control of Cells with ultrasound

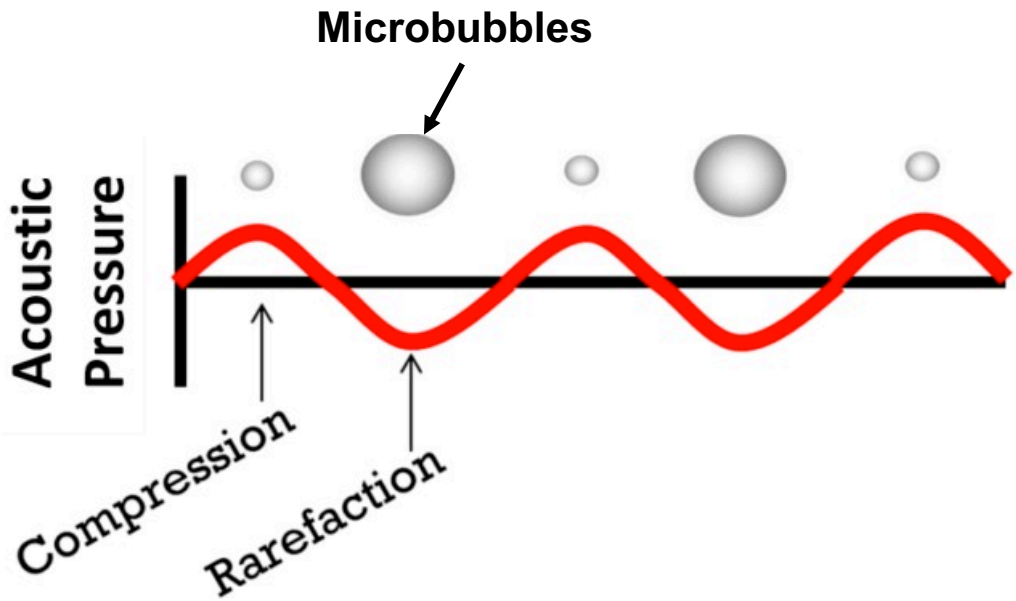
Ultrasound signals are Mechanical and Longitudinal waves that can transfer a distance.

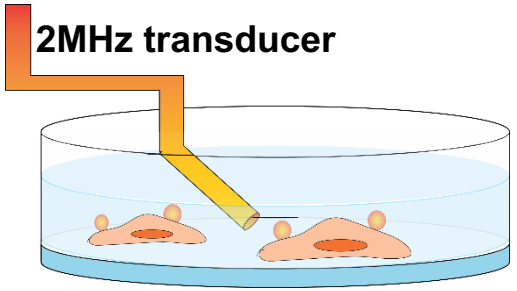


Remote and Non-invasive Control of Cells with ultrasound

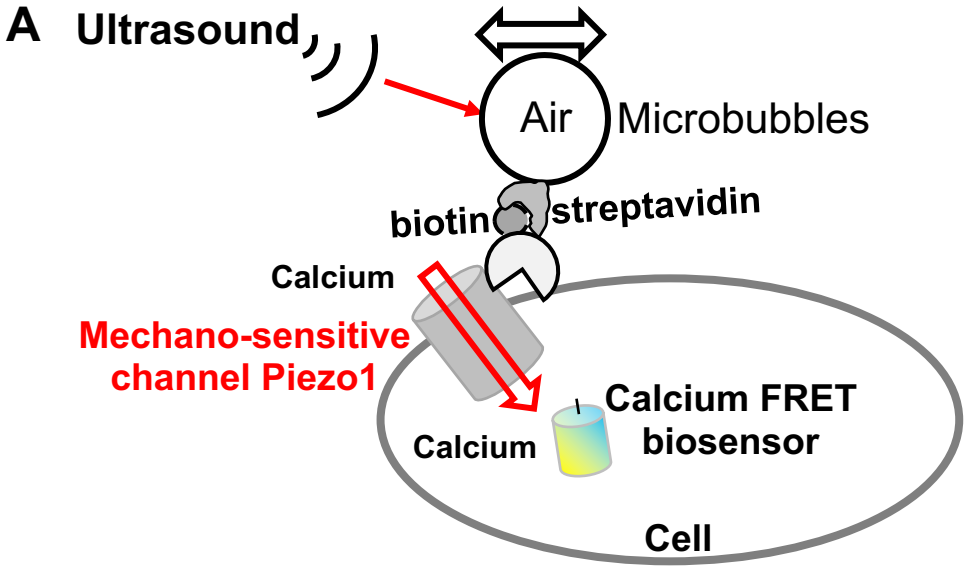


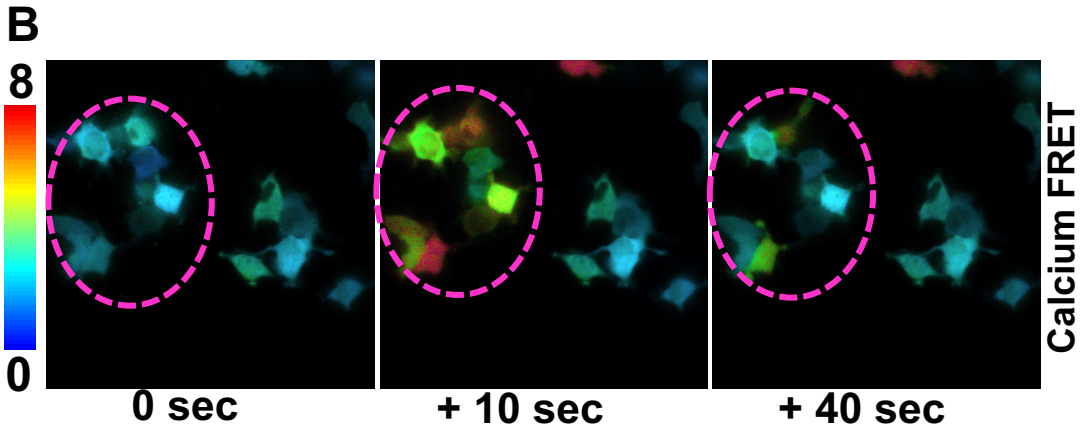
Microbubbles can serve as a mechano amplifer for sensing ultrasound signals

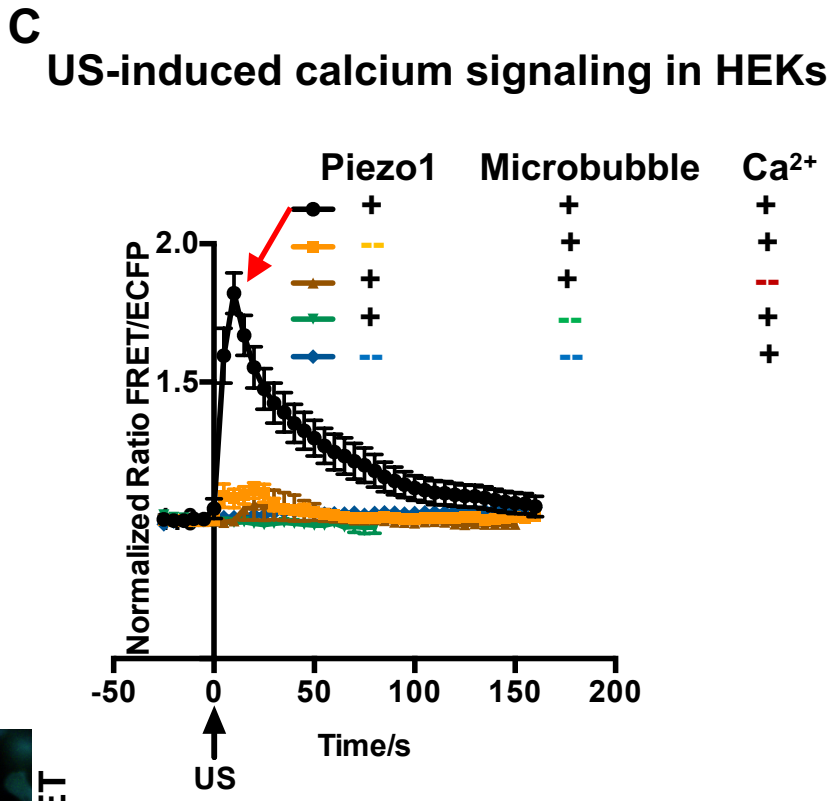




Ultrasound-controllable *Mechano-sensors*

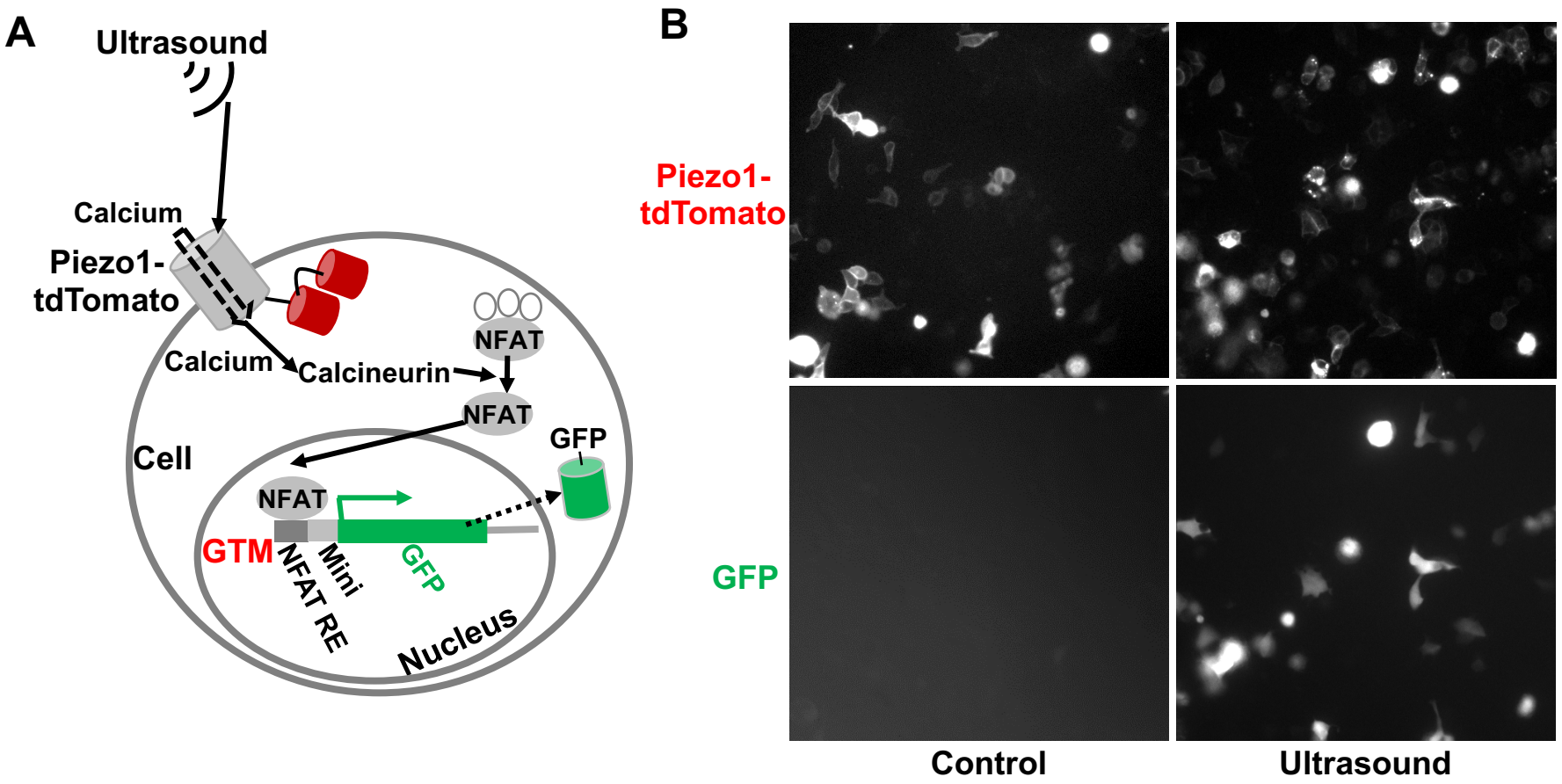






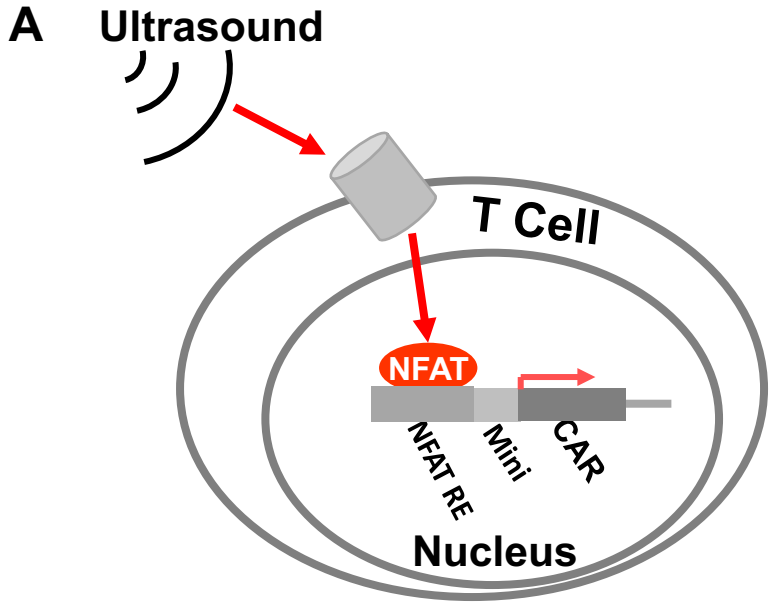
Piezo1 can serve as a **mechanical sensor** remotely controllable by Ultrasound.

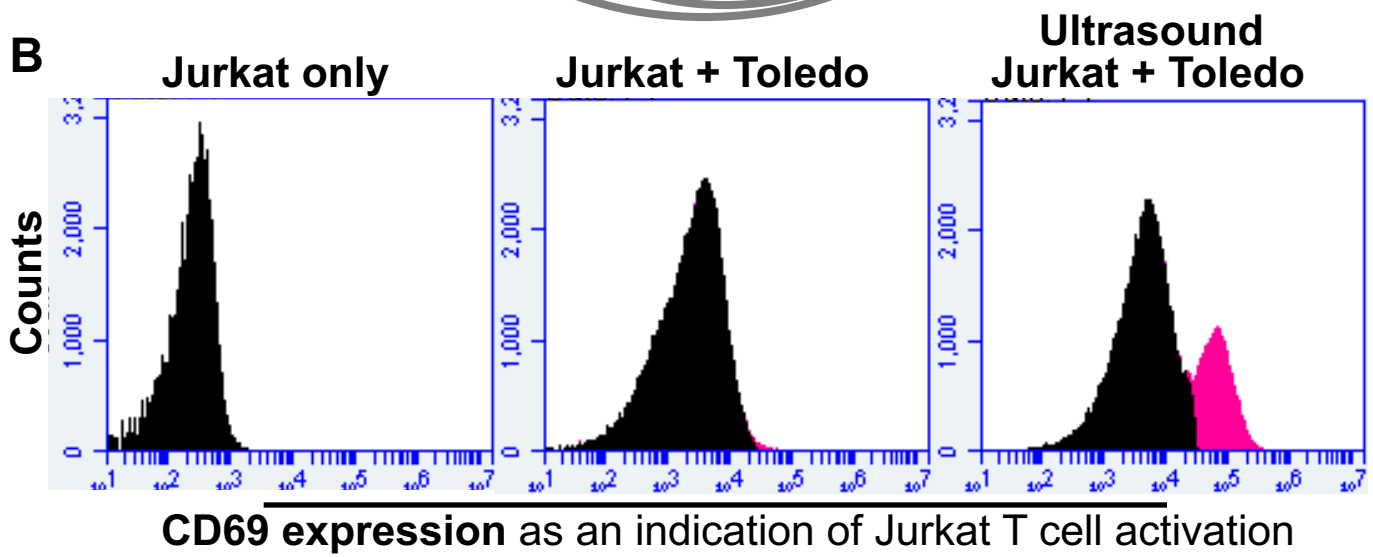
Ultrasound-controllable genetic transducing modules (*GTMs)*



NFAT RE can serve as a **GTM** for Piezo1, remotely controllable by ultrasound.

Ultrasound-Controllable *CAR production and activation* in Jurkat T cells

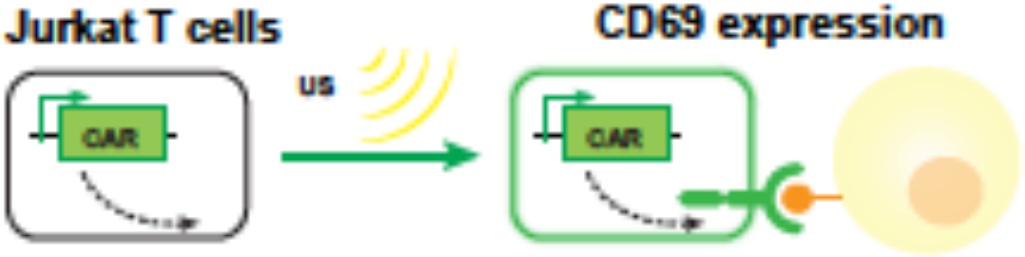


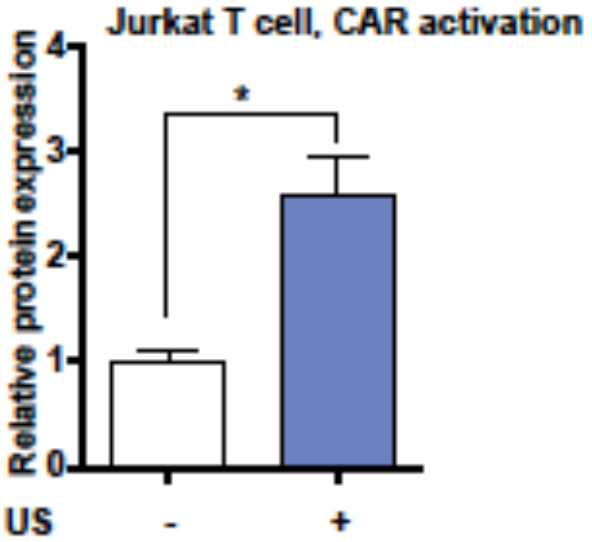


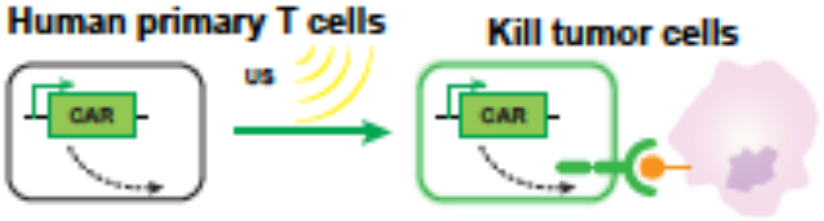
CD69 expression as an indication of Jurkat T cell activation

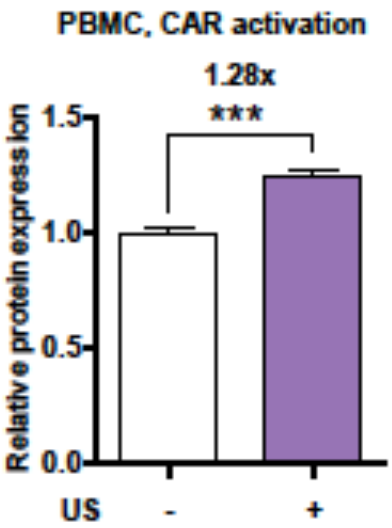
Ultrasound-controllable CAR T cells will be examined in animals targeting tumors.

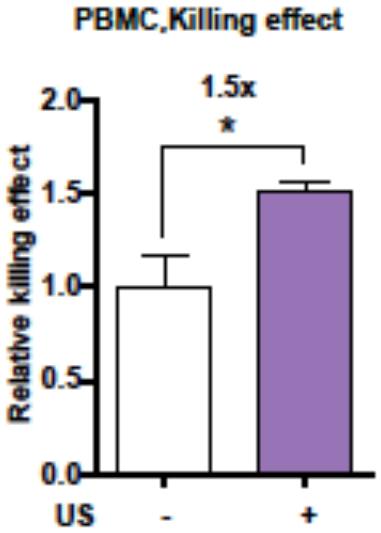
Ultrasound-Controllable *CAR production and activation* in Jurkat and Primary T cells











Pan Y. et al PNAS 2018

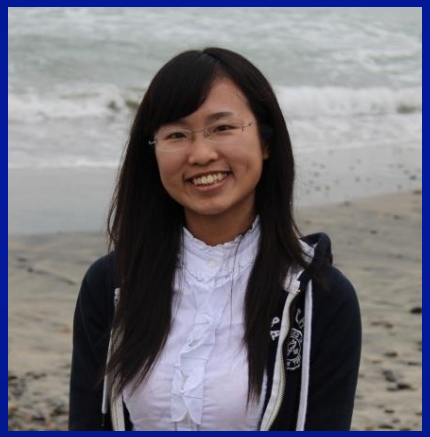
Summary

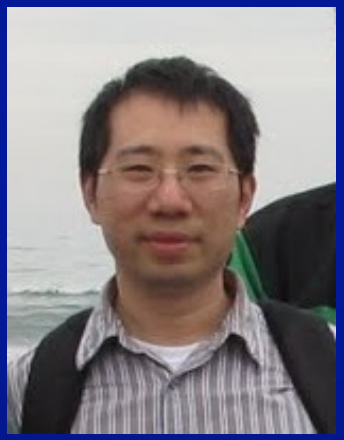
1. **The FRET biosensors can provide powerful tools for the live-cell imaging of various signaling events at single cell levels, including epigenetic dynamics during cell division.**
2. **Molecular engineering can allow us to design systems for the precise control of immunocells and guide their functions in engaging with tumor cells.**

Acknowledgments

Lab and Former Members:

Drs. Mingxing Ouyang, Qin Peng, Jie Sun, Lei Lei, Ziliang Huang, Shaoying Lu, Yijia Pan, Pengzhi Wang, Molly Allen, Mint Praopim Limsakul, Shirley Wu, Eddie Chang, Ya Gong





NIH R01GM125379, R01HL121365, R01GM126016, R33CA204704, R21CA209629

NSF CBET1360341

UC San Diego