

Cell Therapy 2014 (2014-8-30, Las Vegas)

Bacteria-mediated expression of cargo drugs and reporter gene for cancer theranostics

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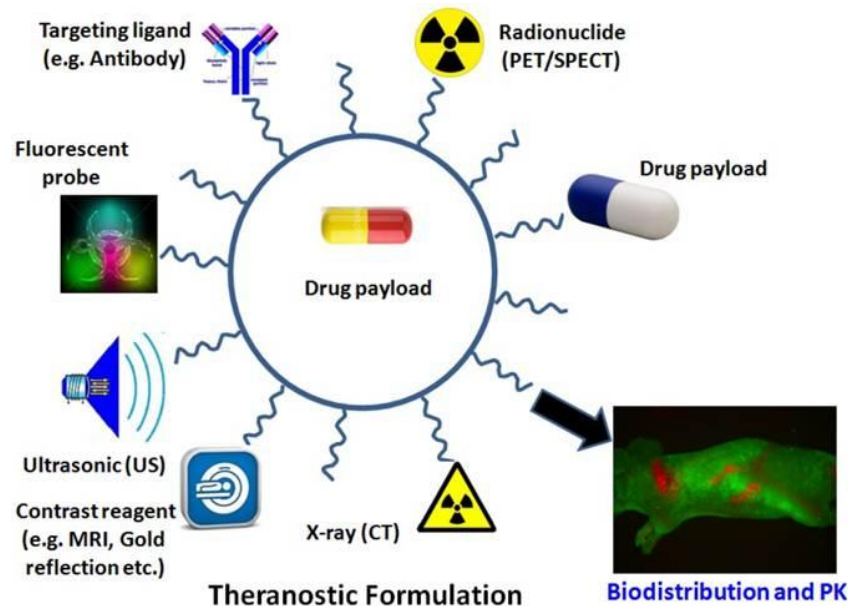
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What Is Theranostics (Theragnosis)?

Theranostics (Therapy + Diagnosis)

- Integrating Therapeutic Function in Imaging Agent
- Prediction or Monitoring of Response to Therapy



Outline

- **Microbes:** Finding and curing cancers with bugs
- **Repebody:** Artificial antibody targeting EGFR
- **Peptides:** Novel peptide for cancer targeting



The First Practice for Bacteriolytic Therapy a Century Ago



William B. Coley (1862-1936)
Coley's toxin (*S. pyogenes*)



“I had found one case of very malignant **round-celled sarcoma of the neck, four times recurrent, in which an attack of erysipelas** had accidentally occurred shortly after the last operation by Dr. Bull. **During these attacks of erysipelas, the tumour of the neck entirely disappeared and the patient left the hospital in good health.** After great effort I finally succeeded in tracking the after history of this patient and found him alive and well, 7 years later.” (*Clin Orthop* 1893)

- Coley deliberately infected one of his own sarcoma patients with *Streptococcus pyogenes*, and within weeks his patient made a dramatic recovery.
- Coley began using a heat-killed bacteria to make the treatment safer.



Great Expectations in Cancer Therapy

(SARAH DEWEERDT. Nature 2013; 504: S4-S5)

- **Coley's cases** (897) vs. **Matched controls** (1,675) from NCI-SEER (NCI's Surveillance Epidemiology End Result database)
- Controls: treated with modern Tx.
- *“What Coley did for his sarcoma patients back then was better than what we're doing for these patients today”*



Sarcoma	Coley's case (n=128)	NCI-SEER database (n=1,675)
Median survival	8.9 y	7.0 y
10-y-survival rate	50%	38%

Overall 5-year cancer survival

1950-54: 35%
1992-98: 63.8%

10-y survival rate	Coley's case (n=128)	NCI-SEER database (n=1,675)
Kidney cancer	33%	23%
Ovarian cancer	55%	29%



Why Coley's vaccine pushed out of favor?

- Hard to standardize: requires **careful calibration** and **bed-side care** for each patient
- Scientists didn't understand the **underlying mechanisms**
- Some doctors couldn't replicate the results



Bacteria Returned!

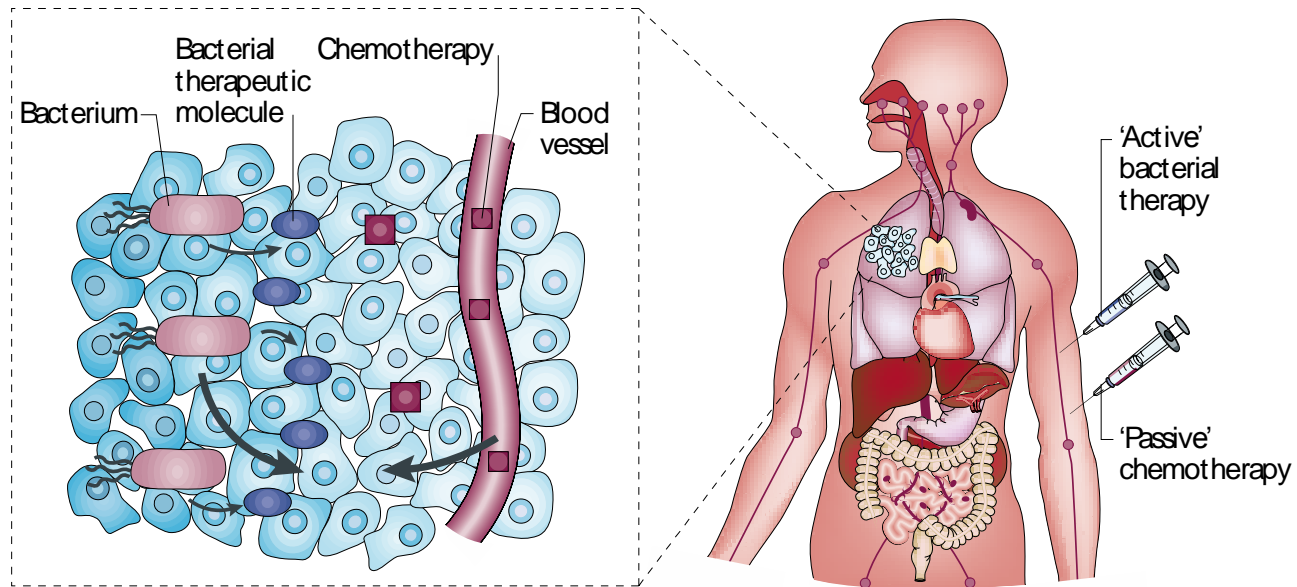
Strengths over other therapies

- Attenuated strain available: 1/100,000 ~ 1/1000,000-fold
- Targeting & Proliferation: T/N ratio > 1,000
- Self propelling: entropically unlimited
- Toxic to cells
- Modern technology to genetically engineer bacteria

	20 min	4 h	Day 1	Day 2	Day 4	Day 6	Day 8
Tumor	9.52x10 ² ±3.18	1.54x10 ³ ±2.21	5.11x10 ⁶ ±2.83	8.96x10 ⁷ ±2.54	3.53x10 ⁸ ±3.57	5.62x10 ⁸ ±4.61	6.85x10 ⁸ ±2.86
Liver	7.10x10 ⁶ ±1.68	2.24 x10 ⁶ ±0.78	5.48 x10 ³ ±0.45	1.40 x10 ² ±0.60	0	0	0
Spleen	1.60 x10 ⁷ ±0.24	1.52 x10 ⁶ ±0.79	1.12 x10 ³ ±0.62	8.15 x10 ¹ ±3.84	0	0	0
Lung	2.00 x10 ⁴ ±0.23	1.13 x10 ³ ±0.93	0	0	0	0	0



Advantages of BC over other therapies

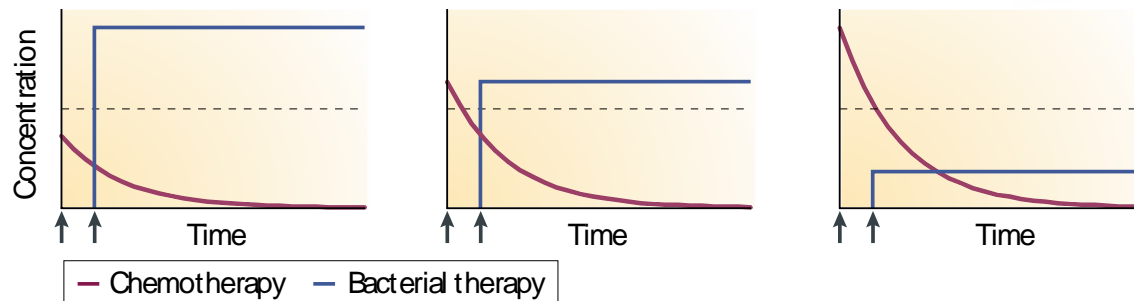


Tumour

System

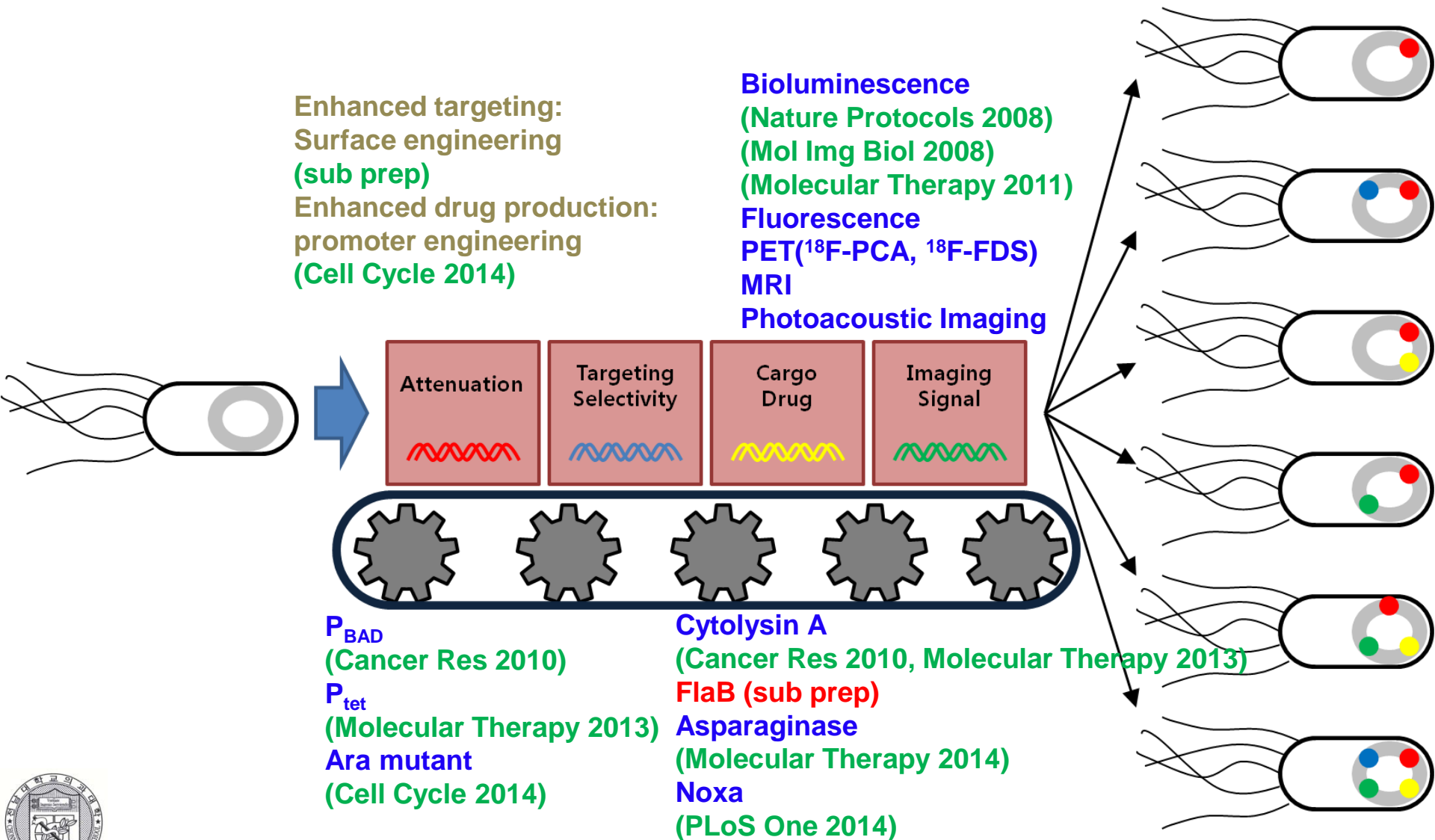
Distal

Proximal



Programming for Theranostics

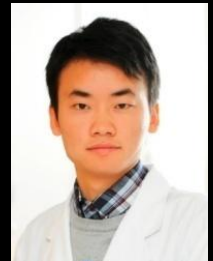
Combined Imaging & Therapy



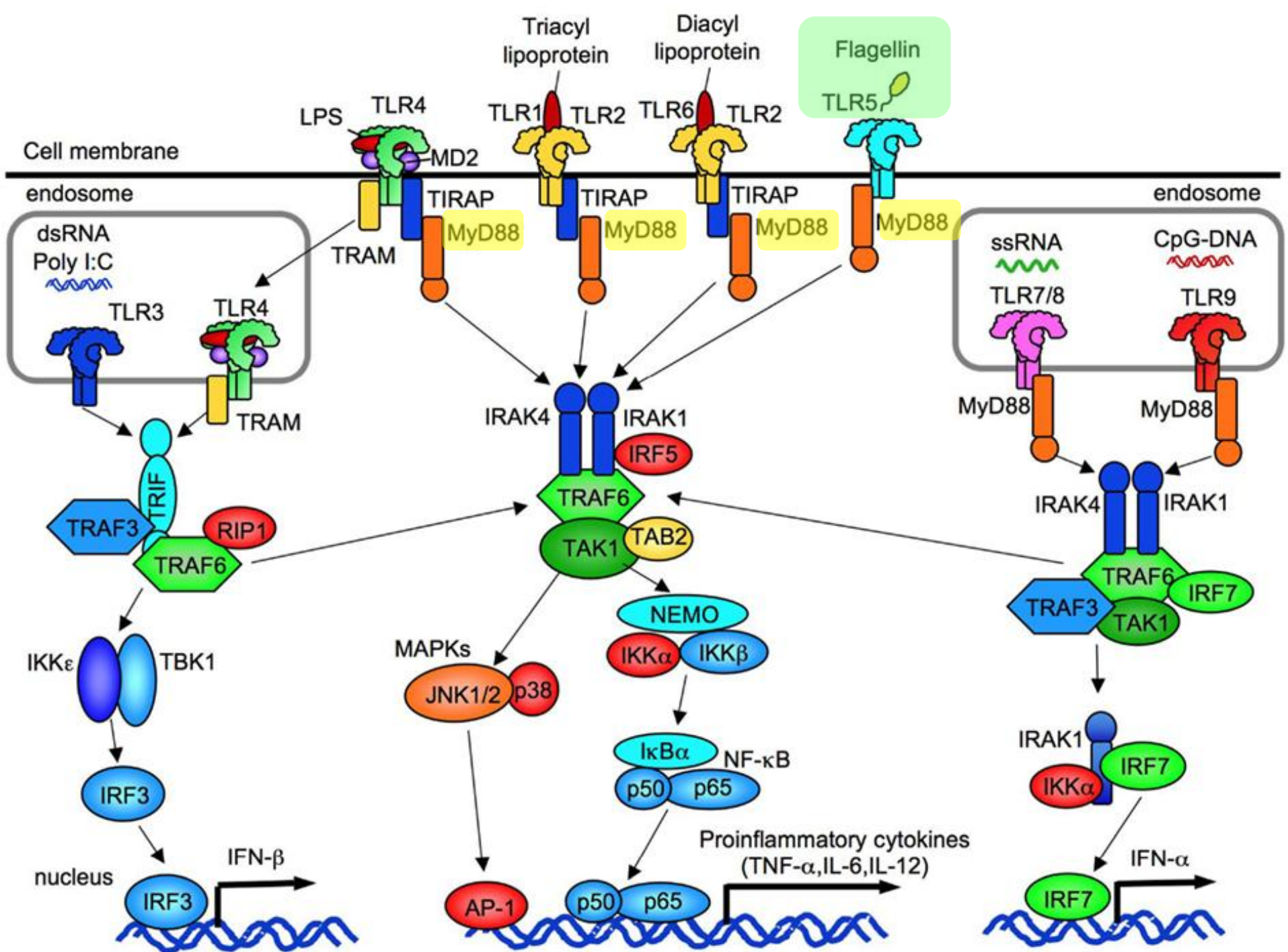
Triggering Drug Production in Bacteria

FlaB: Flagellin B of *Vibrio Vulnificus*

P_{BAD} promoter: inducible by L-arabinose

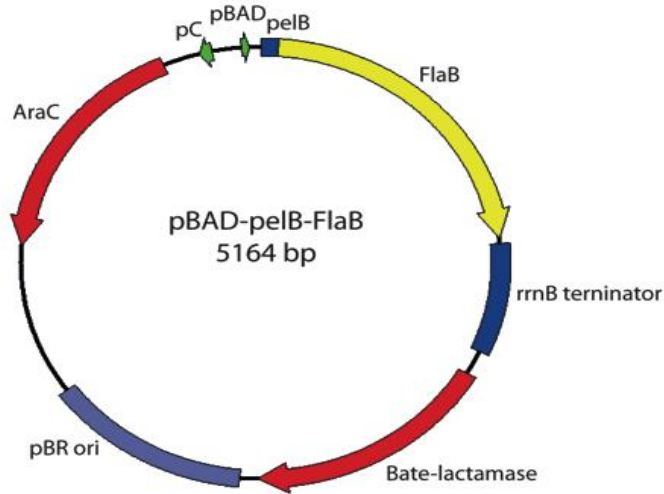


Jinhai Zheng

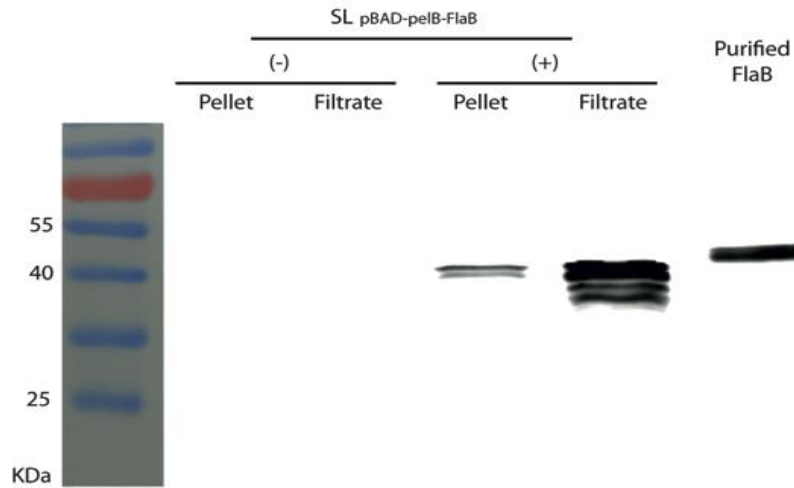


TLR5 expression: Not tumor surface, But macrophage

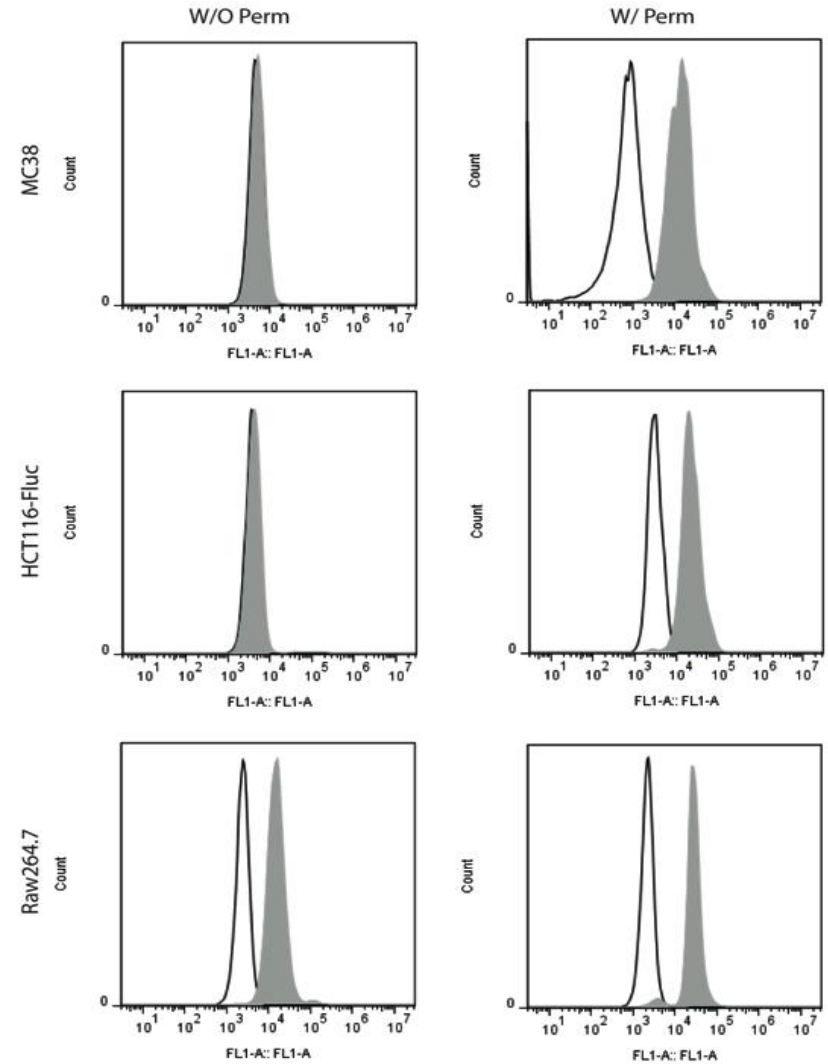
A



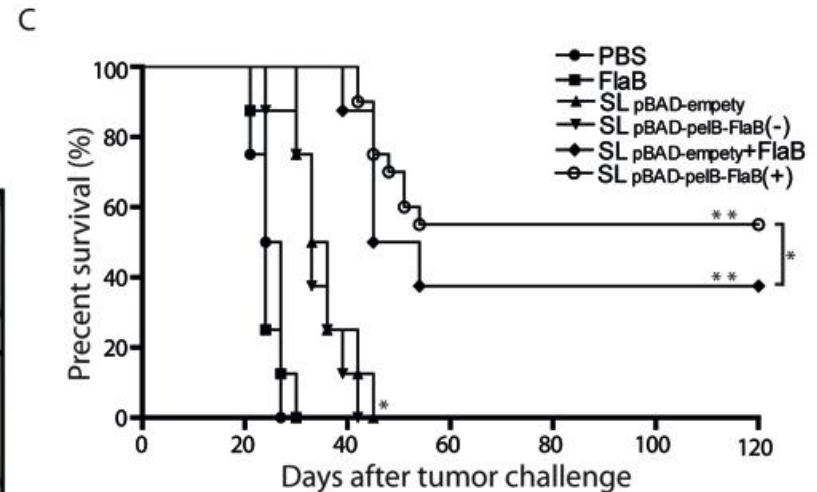
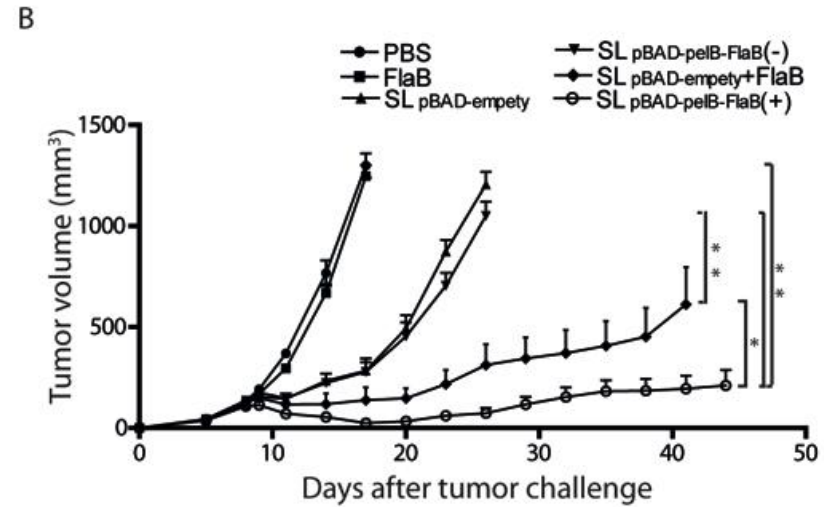
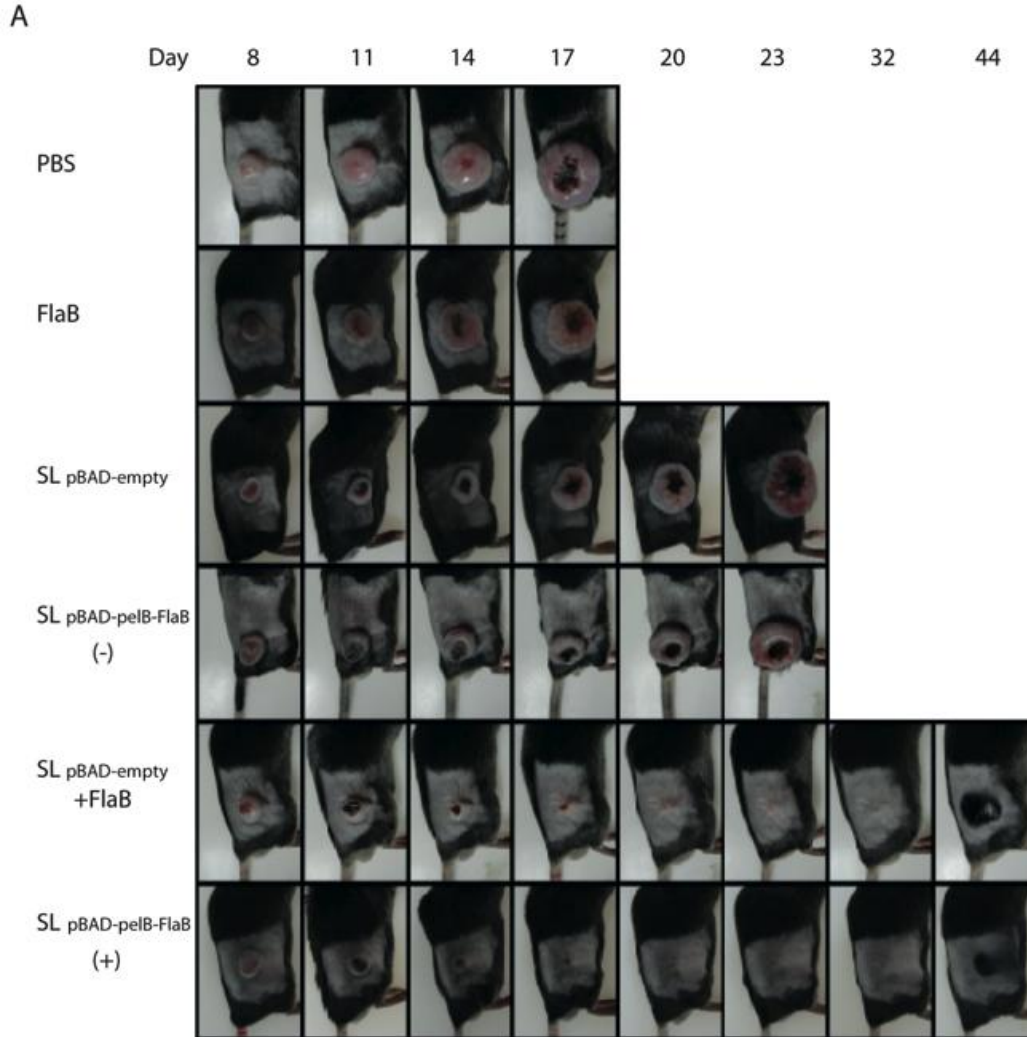
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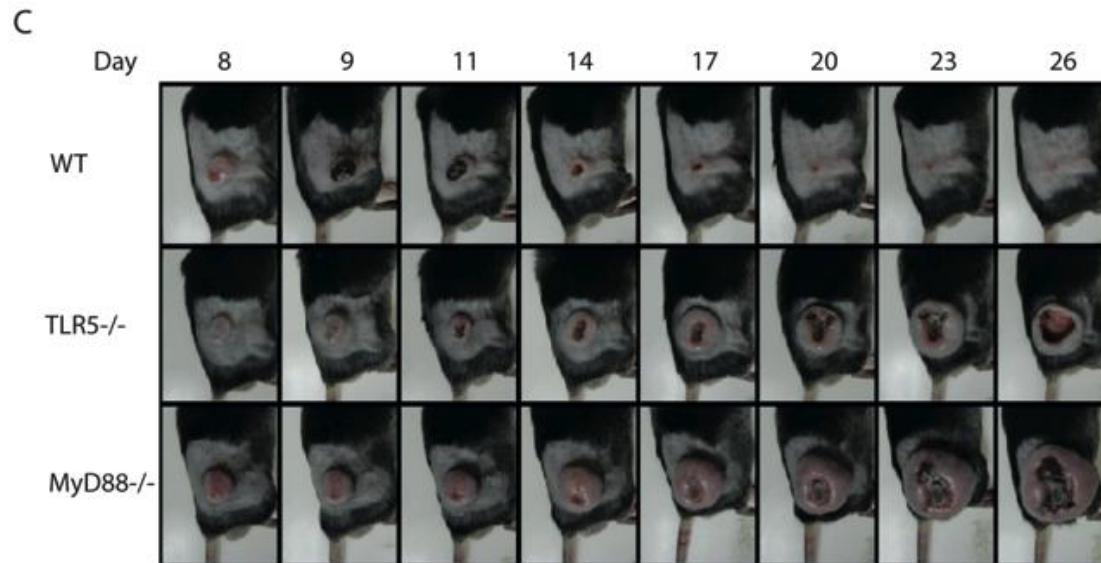
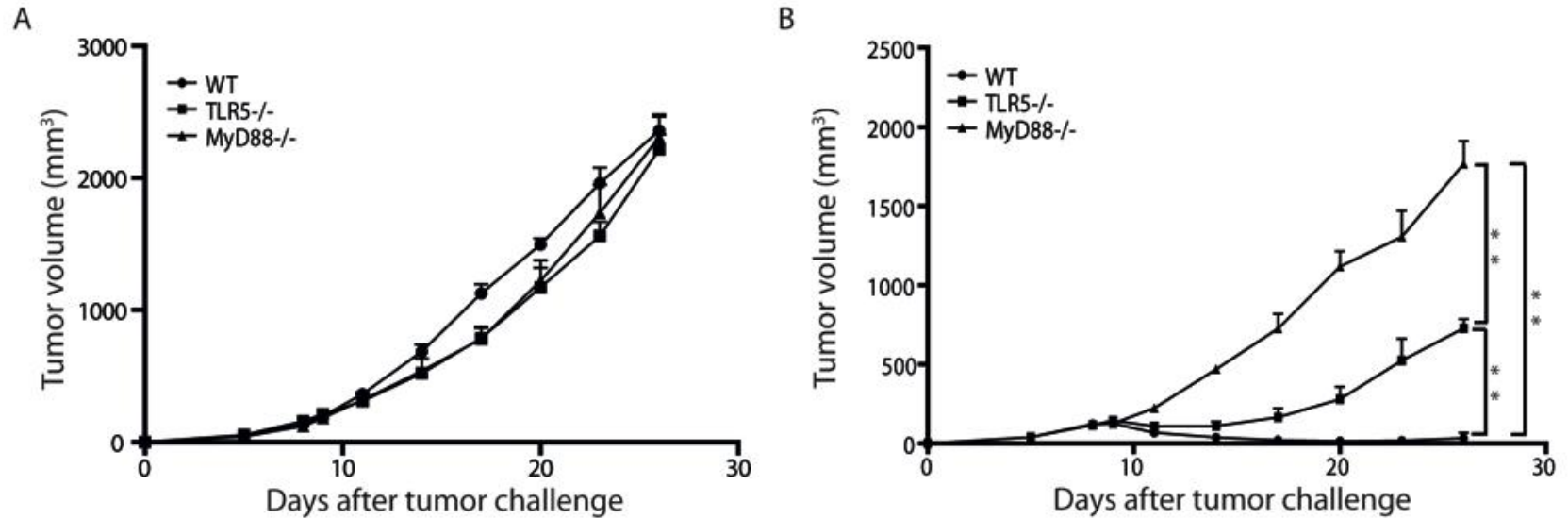
C



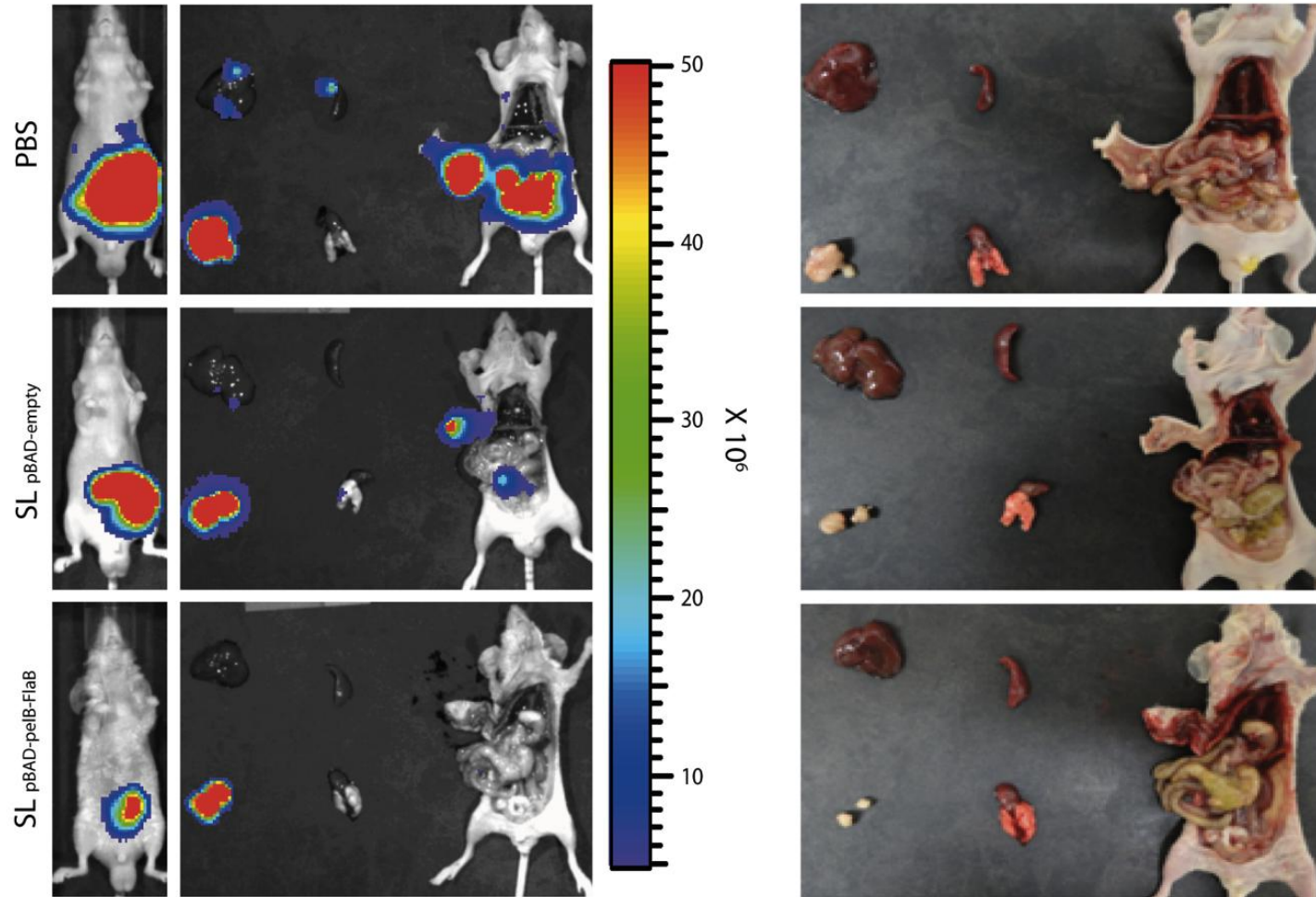
Effect of FlaB expressing *Salmonellae* on MC38 cancer model



FlaB effect on TLR5 signaling knock-out mice



Effect of FlaB expressing *Salmonellae* on Orthotopic Colon Cancer and Metastasis

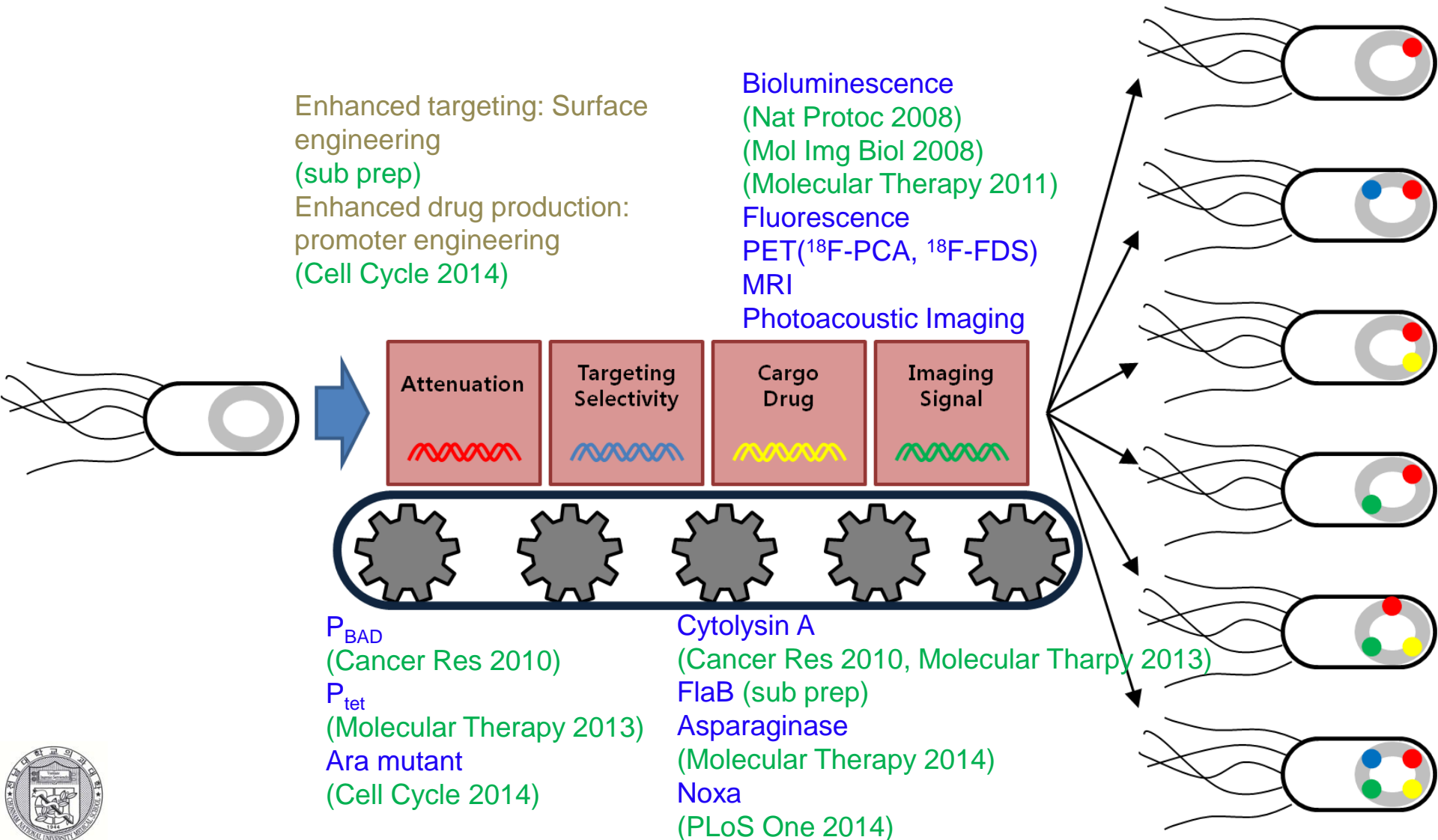


	PBS (n=5)	SLpBAD-empty (n=5)	SLpBAD-FlaB (n=5)
Liver	9	3	0
Spleen	3	0	0
Lung	4	1	0
Mesentery/Intestine	32	7	0
Peritoneal wall	8	6	1
Total	56	17	1

?

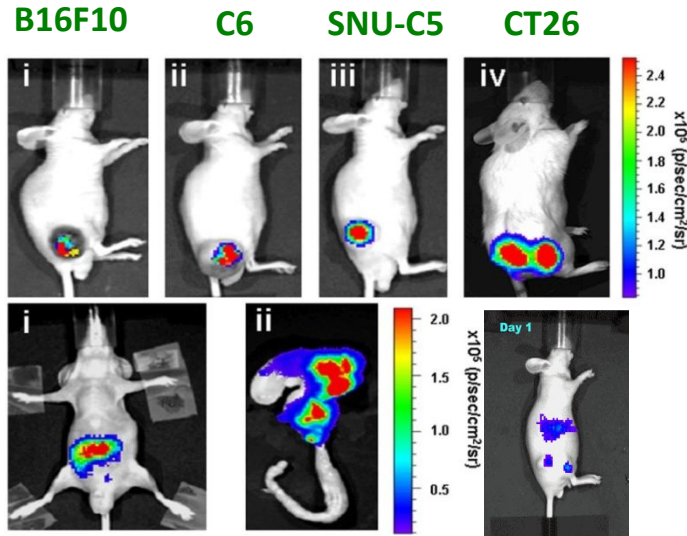
Programming for Theranostics

Combined Imaging & Therapy

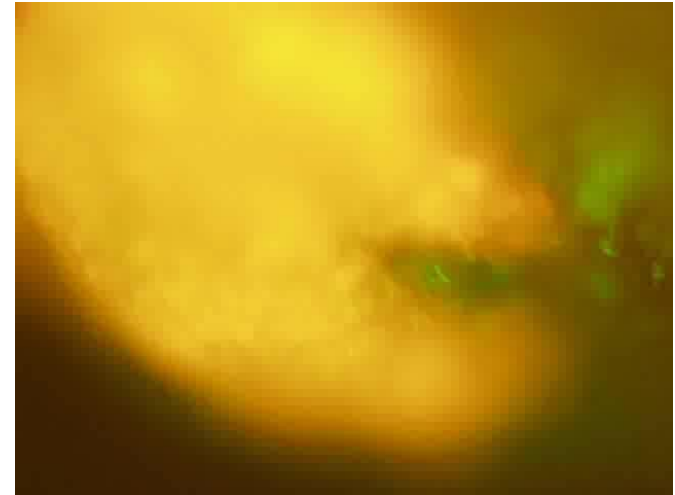


Optical Imaging of Tumor Targeting

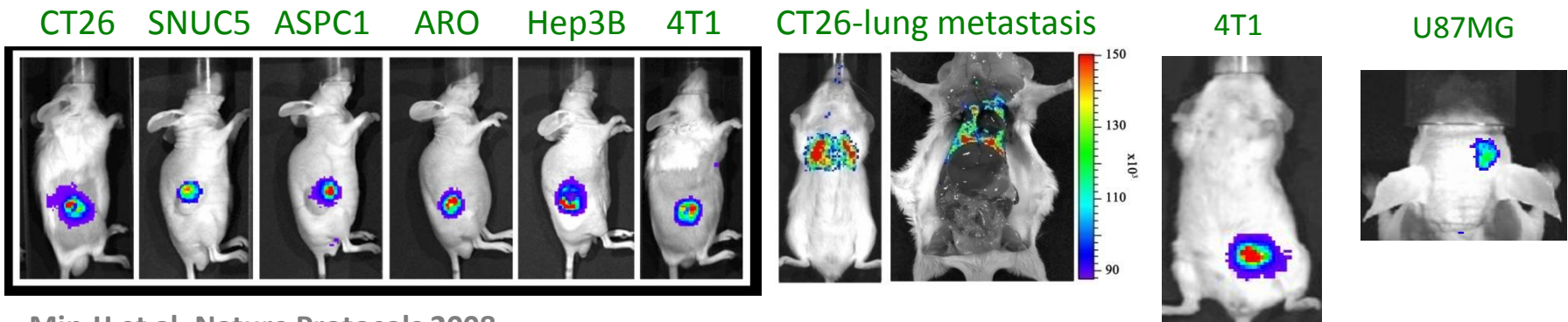
E.coli K-12 MG1655



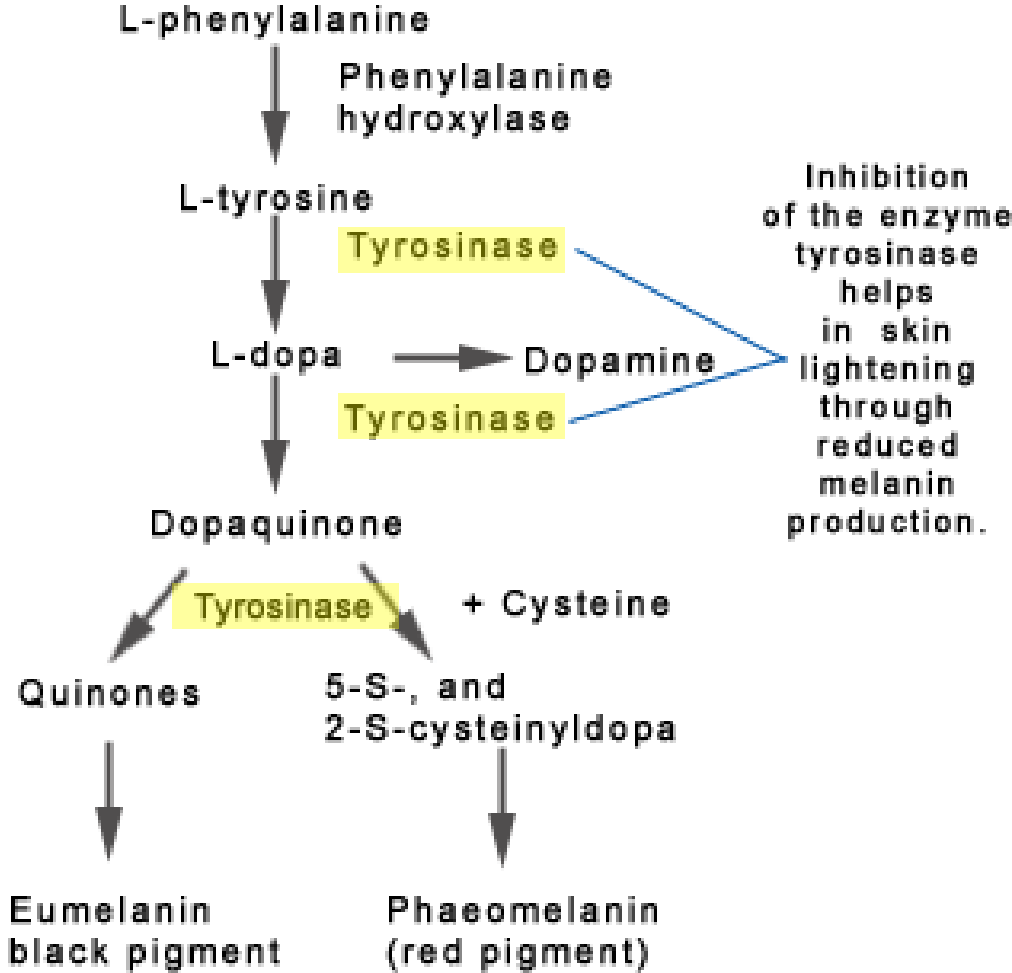
Metastatic LN of ASPC1-RFP (pancreatic cancer)



Salmonella typhimurium defective in ppGpp synthesis (SLΔppGpp)



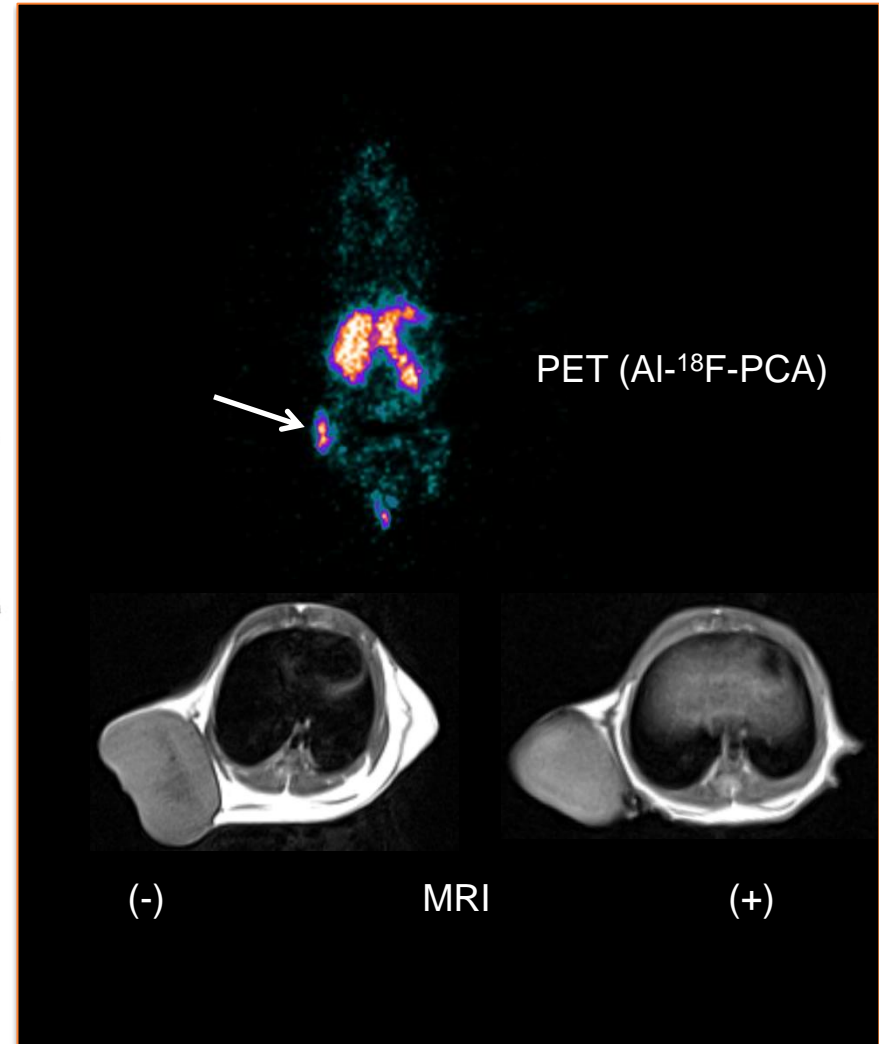
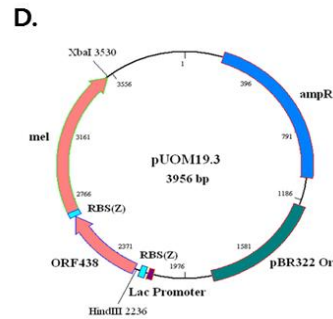
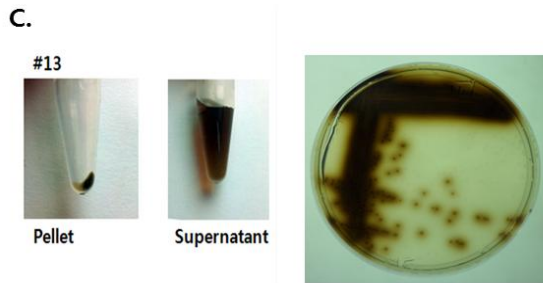
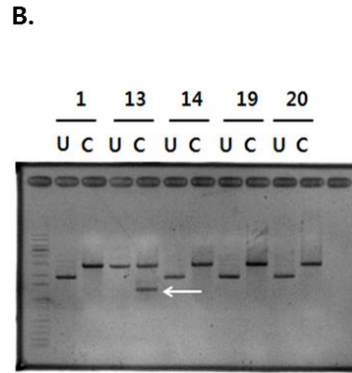
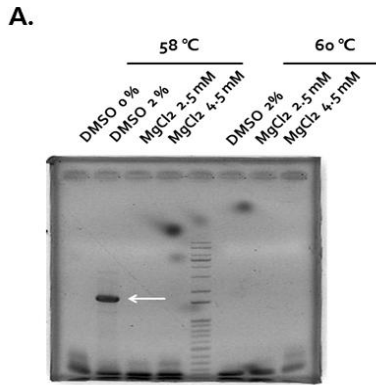
Melanin Biosynthesis



Tyrosinase, melanin-producing enzyme; As a reporter gene for bacteria



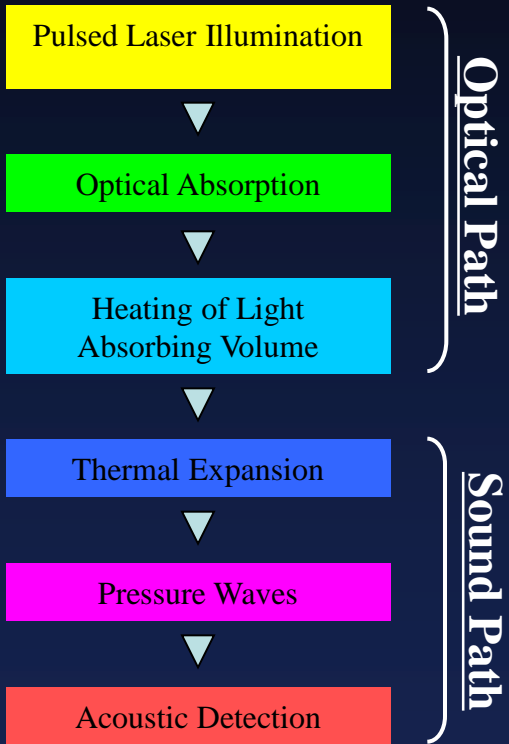
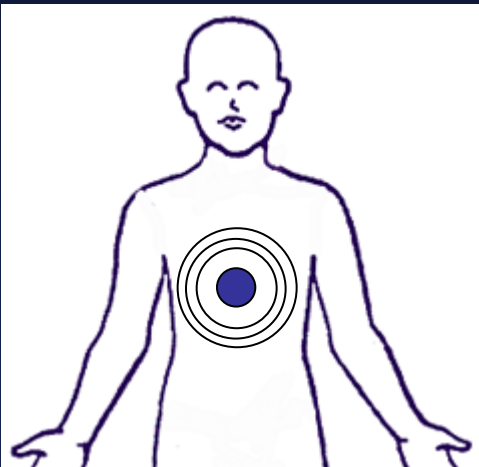
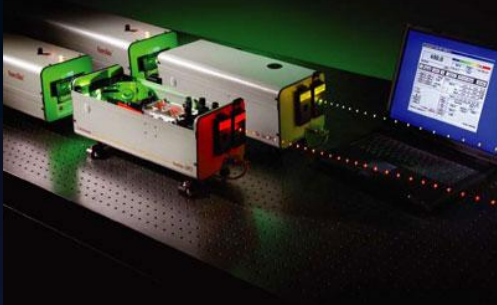
Hong, Hyun



Groups	A	B	C
	LB media	LB, L-Tyr	LB, L-Tyr, CuSO4
1. SLΔppGpp/pUC19 (empty)			
2. SLΔppGpp/pUOM19.3			
3. Only media			



Photo(opto)acoustic Imaging



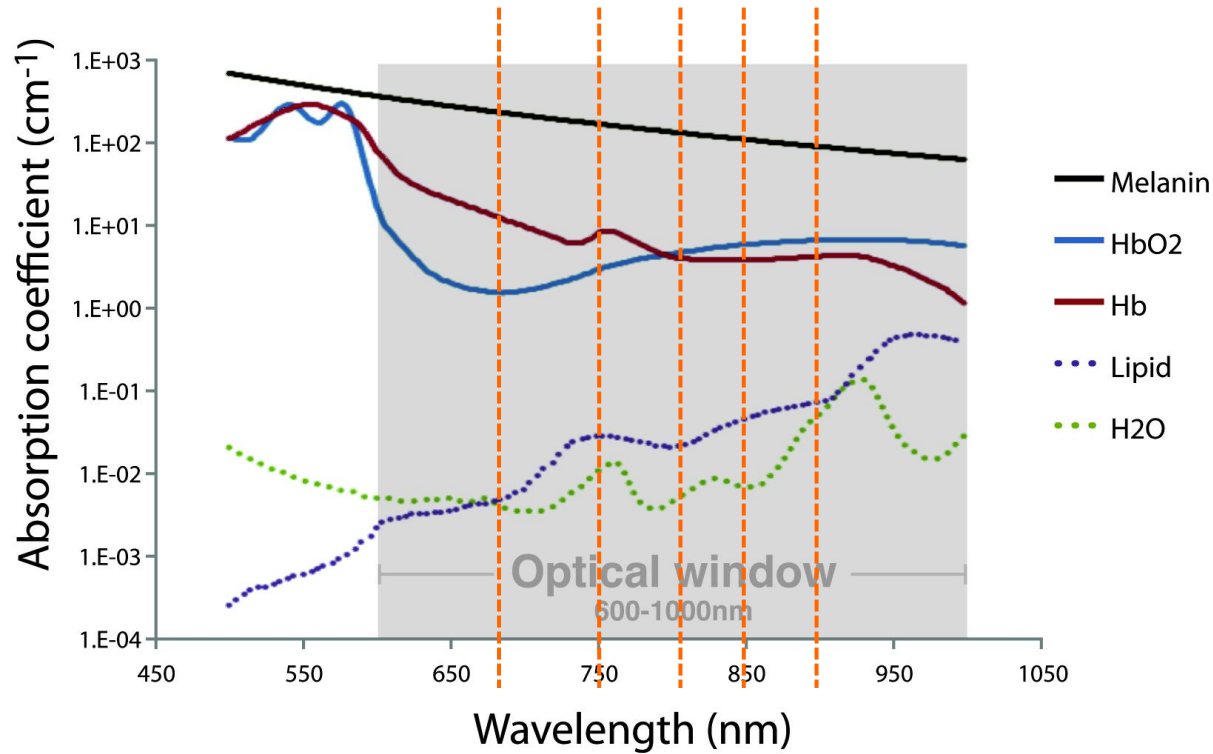
Advantages

1. Deep Penetration
2. High Resolution
3. Multiplexing
4. Specificity

Limitations

- No bones / air
- Technology is not mature yet

Photoabsorption



$$\mu_a(\lambda_i) = \varepsilon_{\text{HbR}}(\lambda_i)[\text{HbR}] + \varepsilon_{\text{HbO}_2}(\lambda_i)[\text{HbO}_2], \quad (1)$$

$$\begin{bmatrix} [\text{HbR}] \\ [\text{HbO}_2] \end{bmatrix}_{(x,y,z)} = (M^T M)^{-1} M^T \Phi(x,y,z) K, \quad (2)$$

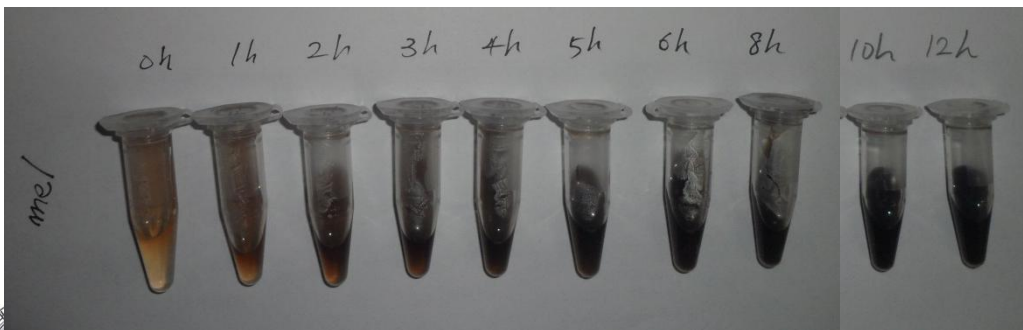
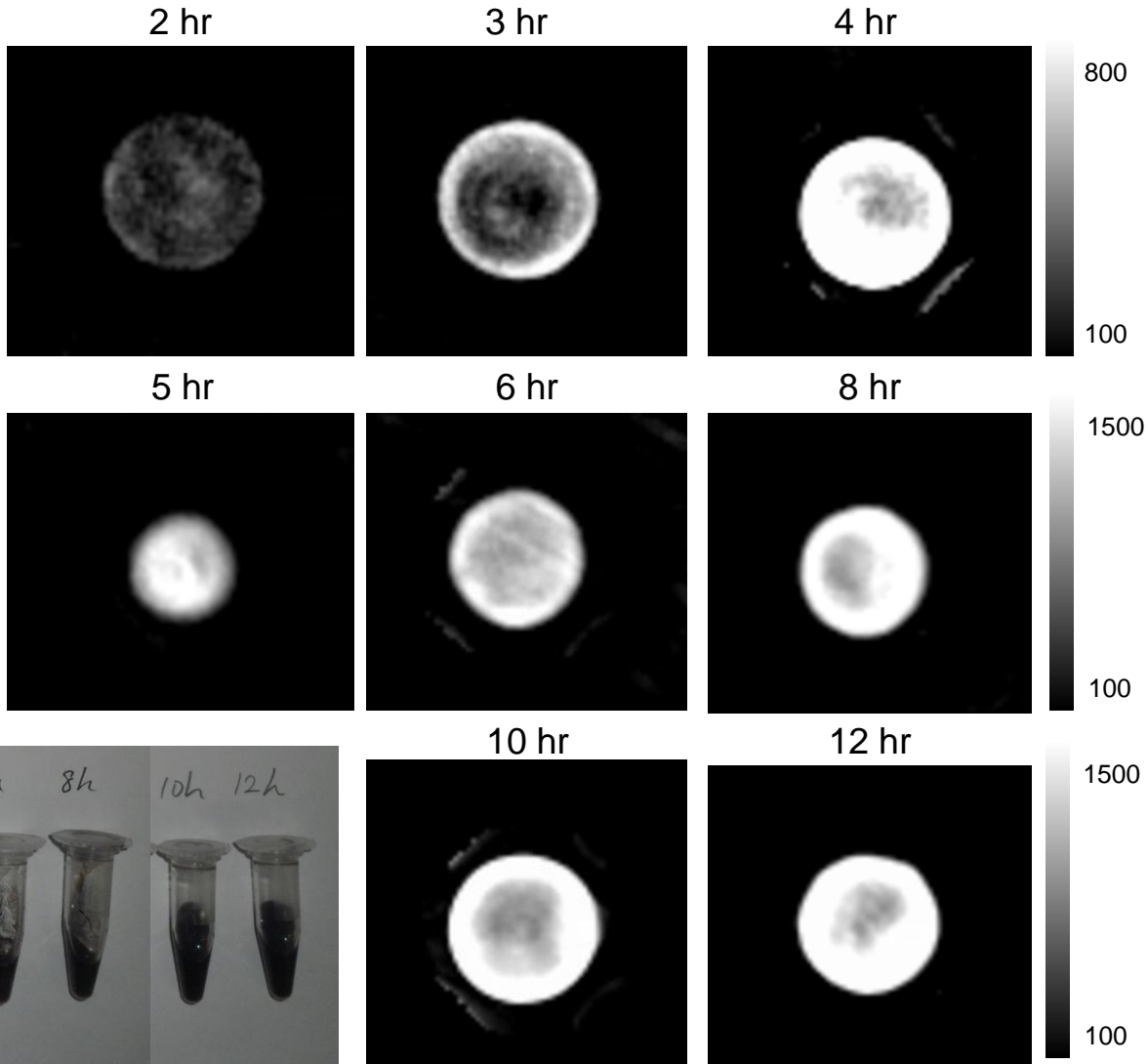
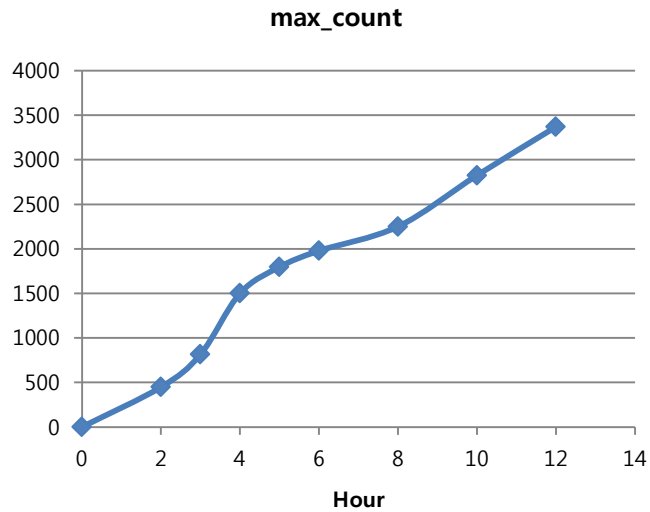
where

$$M = \begin{bmatrix} \varepsilon_{\text{HbR}}(\lambda_1) & \varepsilon_{\text{HbO}_2}(\lambda_1) \\ \vdots & \vdots \\ \varepsilon_{\text{HbR}}(\lambda_n) & \varepsilon_{\text{HbO}_2}(\lambda_n) \end{bmatrix}, \quad \Phi(x,y,z) = \begin{bmatrix} \phi(\lambda_1, x, y, z) \\ \vdots \\ \phi(\lambda_n, x, y, z) \end{bmatrix},$$

and K is the proportionality coefficient that is related to the ultrasonic parameters and the wavelength-dependent change of the local optical fluence as light passes through the skin.^{15,16} Thus, the SO_2 image is calculated as

$$\text{SO}_{2(x,y,z)} = \frac{[\text{HbO}_2]_{(x,y,z)}}{[\text{HbO}_2]_{(x,y,z)} + [\text{HbR}]_{(x,y,z)}}. \quad (3)$$

PAI of Melan-Producing *Salmonella* (*in vitro*)



Photoacoustic Tomography (PAT) in Tumor

Before injection of Melanin

OxyHemoglobin

DeoxyHemoglobin

Melanin

Merge image

After injection of Melanin

OxyHemoglobin

DeoxyHemoglobin

Melanin

Merge image



Photoacoustic Tomography (PAT) in Tumor Melanin peak

Before



After



Acknowledgments

Min's Lab (LOVMI)

Park, Seung Hwan, PhD

Kim, Dong-Yeon, PhD

Nguyen, Hong Vu, PhD

Yun, Misun

Phan, Thuy Xuan

Zheng, Jinhai

Hong, Hyun

Choo, Yunjin

Duong, Thi Quynh Mai

Park, Yong-Dae

Pyo, A-young

Kim, Hyun Sik

Park, Suk Ho

Yoo, Saerom

Moon, Yunsun

Nuclear Medicine

Bom, Hee-Seung, MD, PhD

Song, Ho-Cheon, MD, PhD

Kwon, Seong Young, MD

Microbiology

Choy, Hyon Eel, PhD

Rhee, Joon Haeng, MD, PhD

Hong, Yeongjin, PhD

KAIST

Kim, Hak-Sung, PhD

Fraunhofer IZI, Leipzig, Germany

Michael Szardenings, PhD

Funding Agencies

의학-첨단과학 융합기술개발사업 (미래부)

미래유망융합기술개발사업 (미래부)

해외우수연구기관 유치사업 (미래부)

질병중심중개연구 (복지부)

Knowing is not enough; we must apply.
Willing is not enough; we must do. **-Goethe-**

감사합니다



Toxicity of FlaB expressing *Salmonellae*

