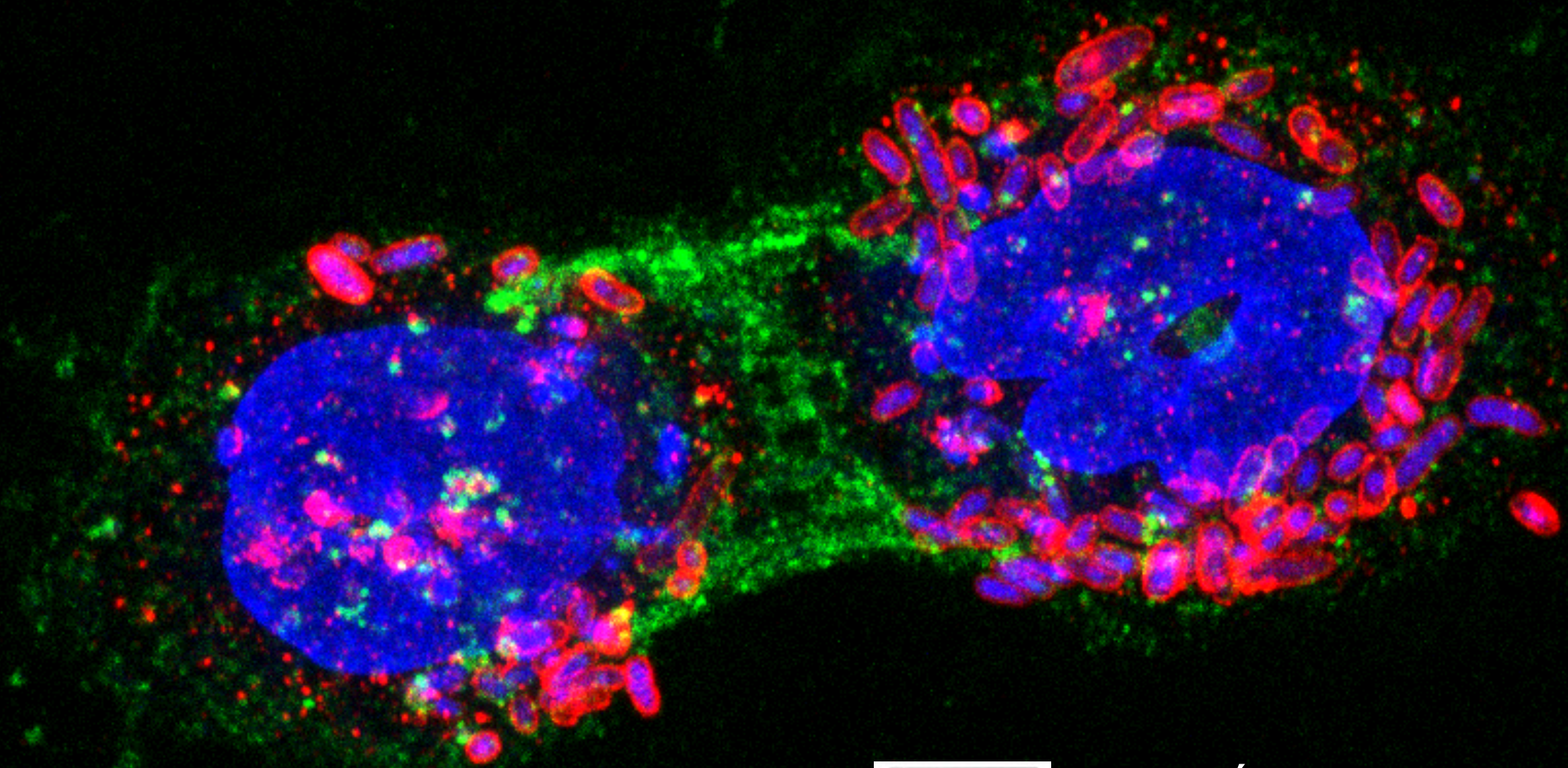


# Overview on the use of engineered bacteria as therapeutic agents



**Dr. Luis Ángel Fernández**

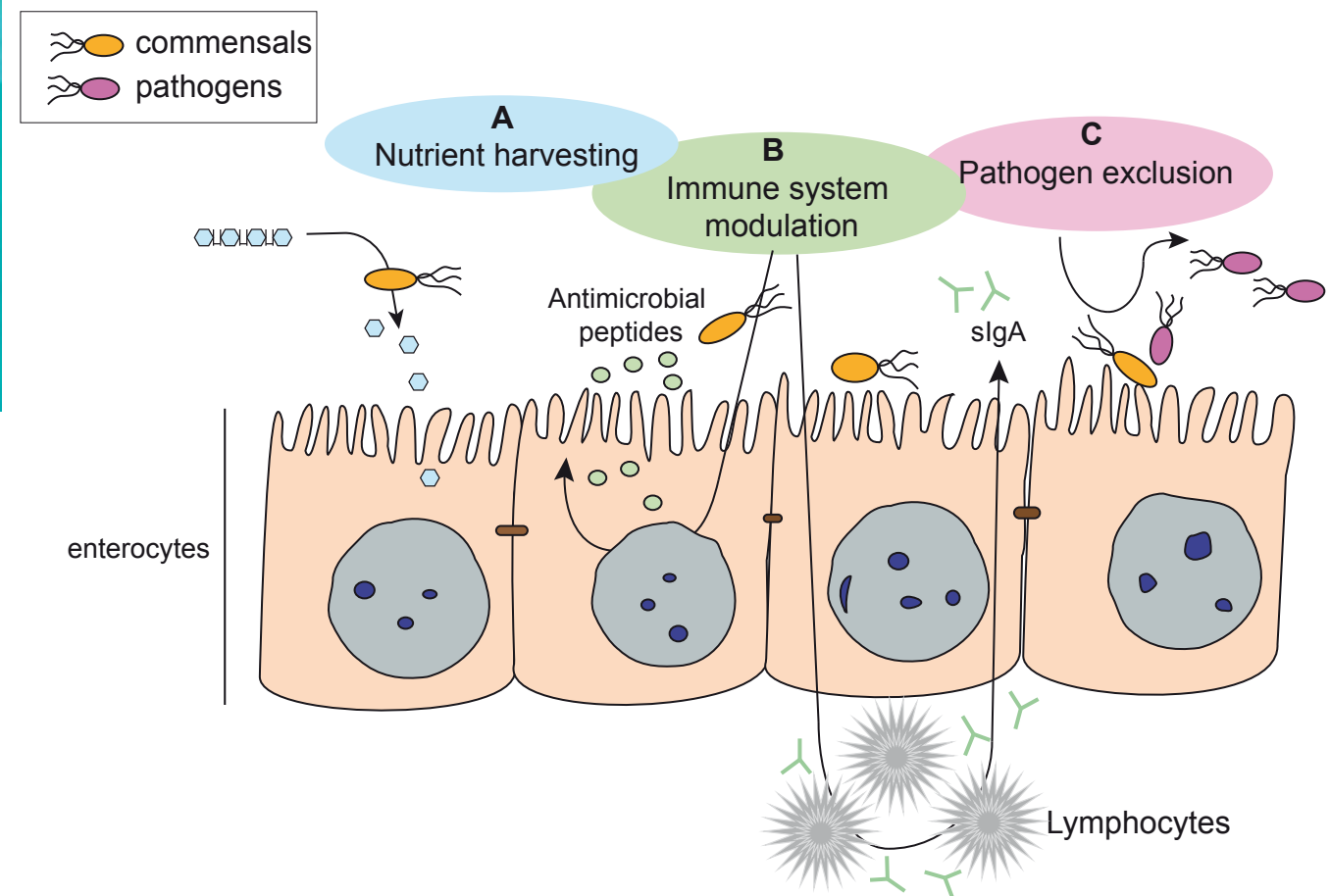
*Department of Microbial Biotechnology  
Centro Nacional de Biotecnología-CSIC  
Madrid, Spain*



# Microbiota and health



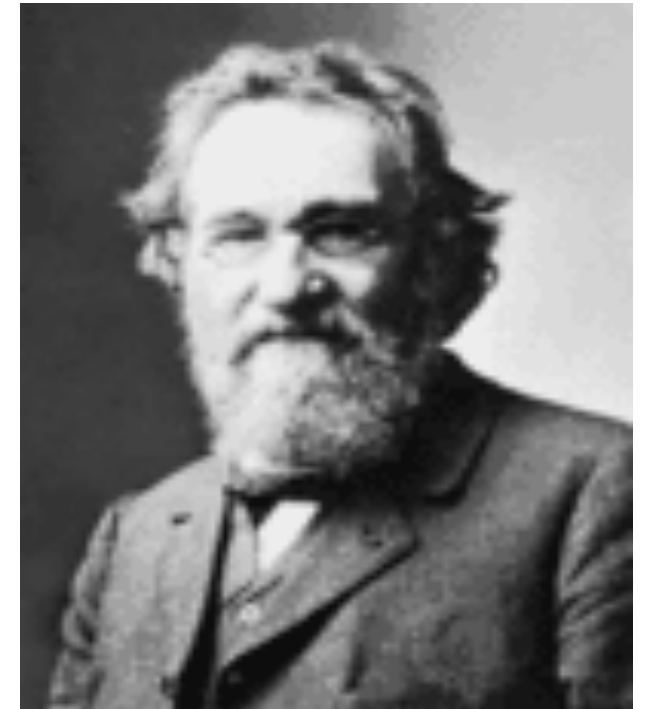
- Metabolism of nutrients and vitamins
- Protection against pathogens
- Immune system development



# First concept of human intervention on the microbiota

Elie Metchnikoff (Pasteur Institute, 1907) suggested the possibility of colonizing the gut with beneficial flora:

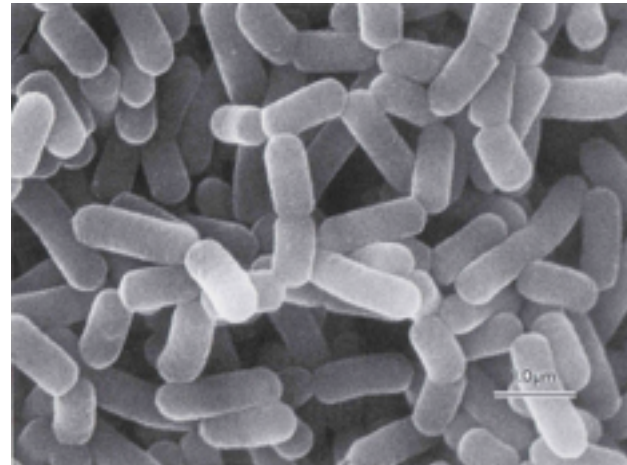
"the dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes"



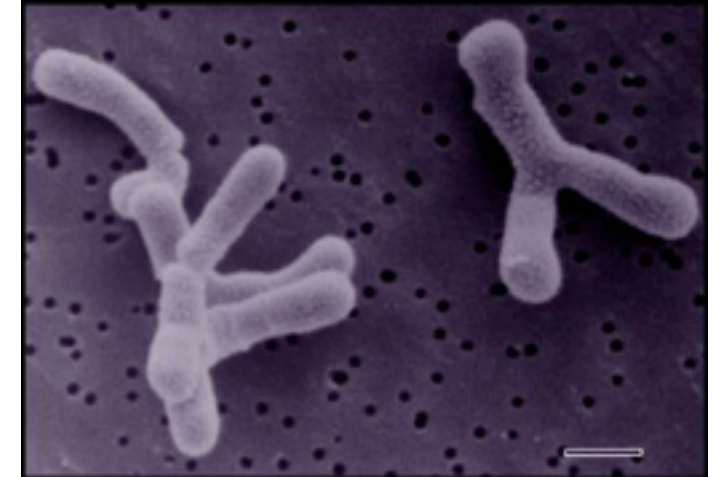
**Elie Metchnikoff**

# Classical application of bacteria for human health

## I- Probiotics



*Lactobacillus casei*



*Bifidobacterium.*

## II- Vaccines



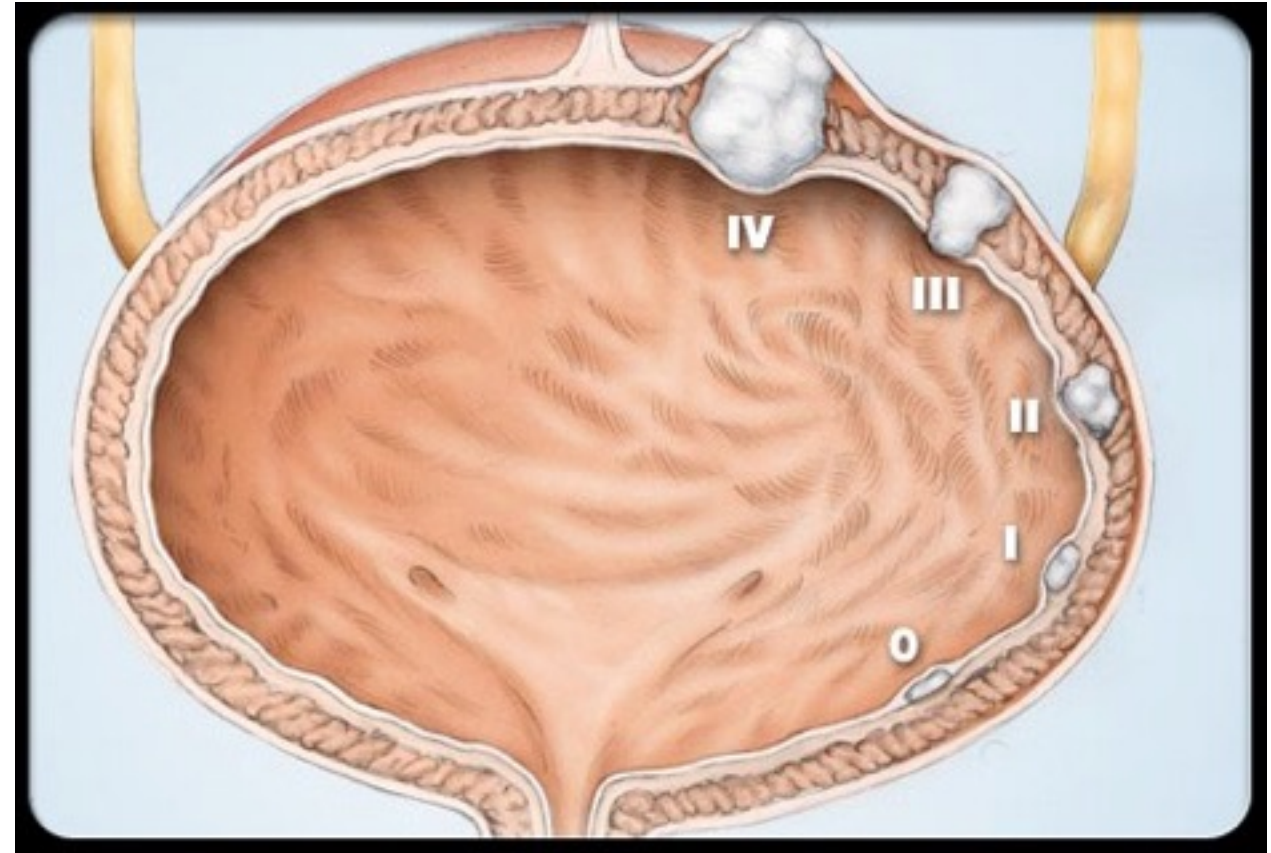
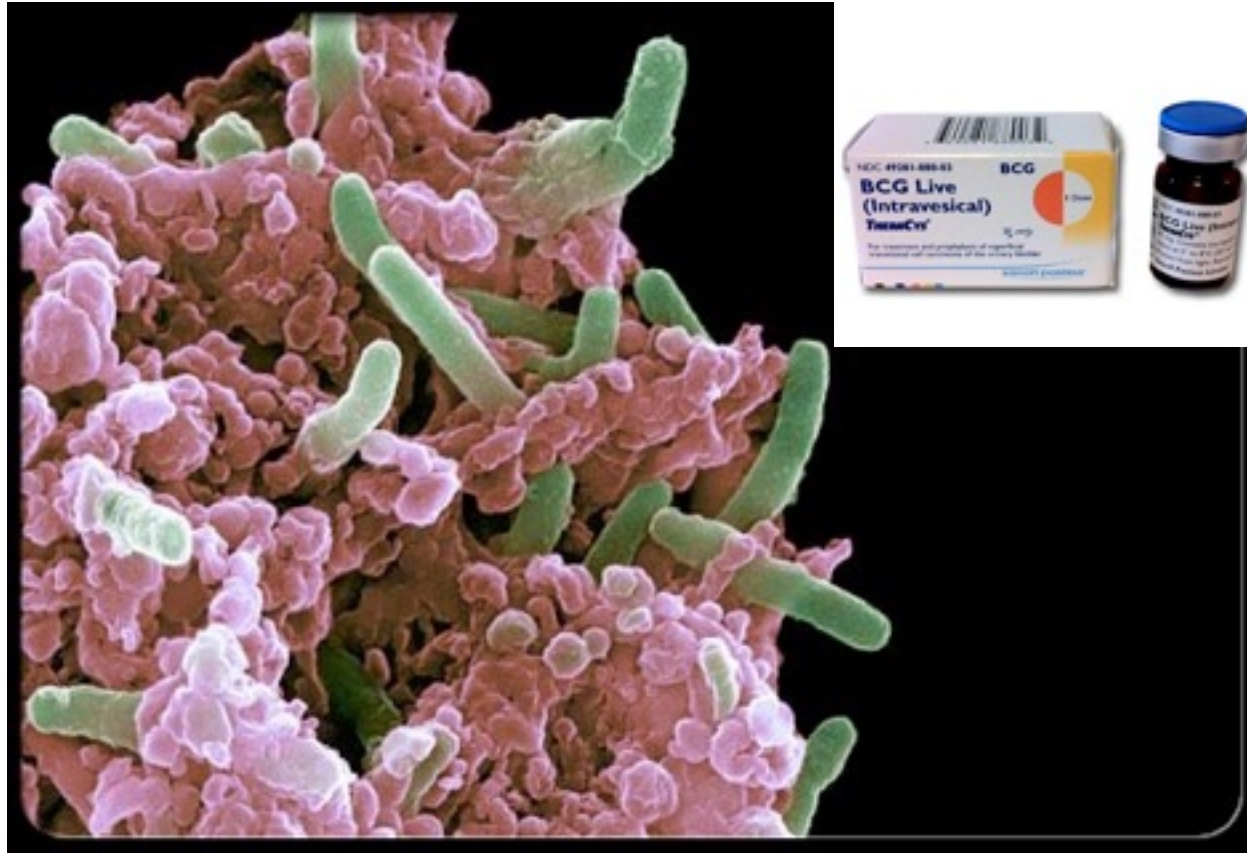
*Mycobacterium tuberculosis*  
vaccine



*Bacillus Calmette-Guérin (BCG)*



# BCG immunotherapy of bladder cancer



📌 Bacillus Calmette–Guérin (BCG), an attenuated strain of *Mycobacterium bovis*, has been used in the intravesical treatment of non-muscle invasive bladder cancer (NMIBC) for over 30 years

# Probiotic *E. coli* strains of medical use

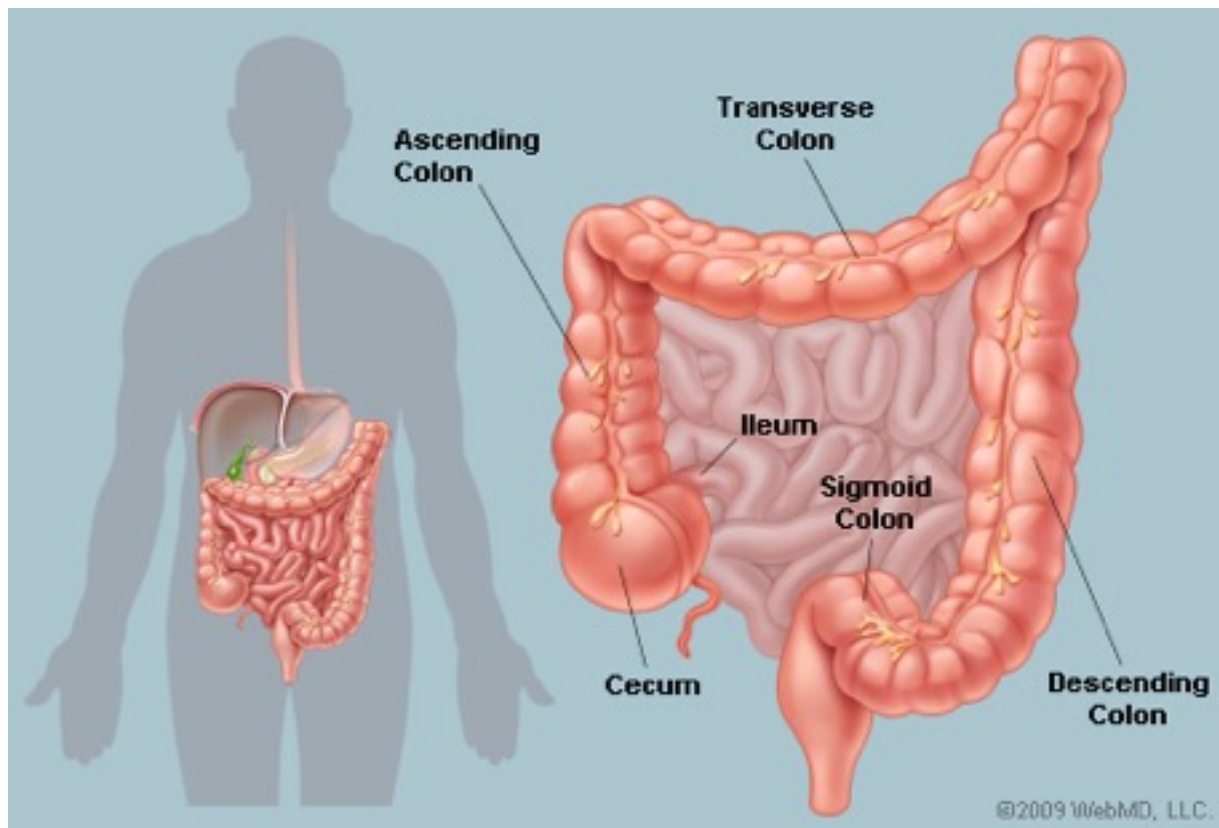
## *E. coli* Nissle 1917



- Colonizer of the gastrointestinal tract.
- Treatment of bowel inflammation and competition with gastrointestinal pathogens

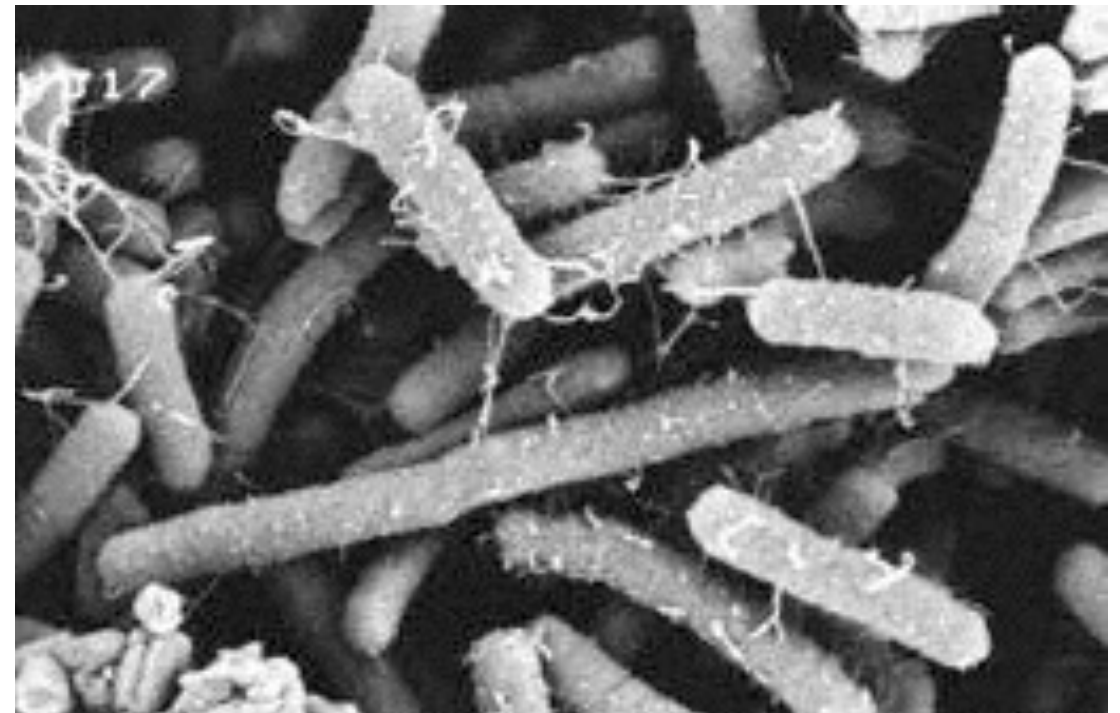
## *E. coli* ABU 83972

- Colonizer of the bladder in the urinary tract (asymptomatic bacteriuria).
- Used for the treatment of recurrent urinary tract infections caused by uropathogenic *E. coli*



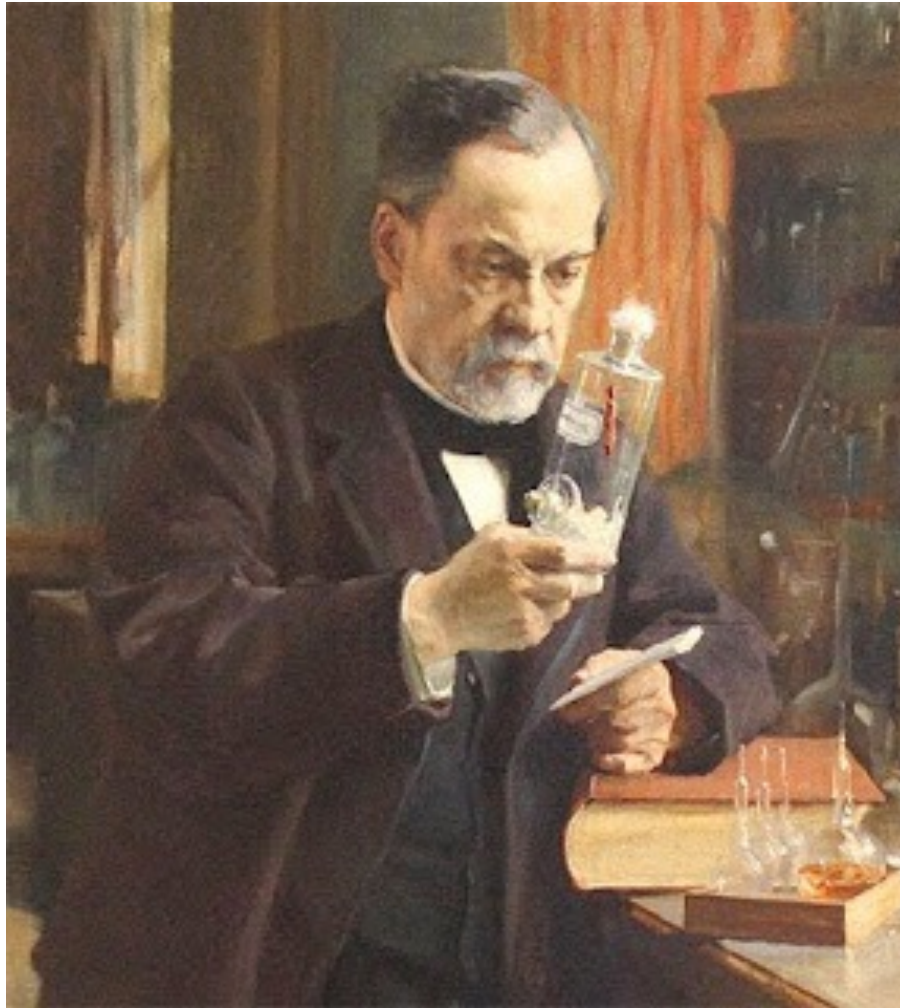


# Probiotic *E. coli* Nissle 1917

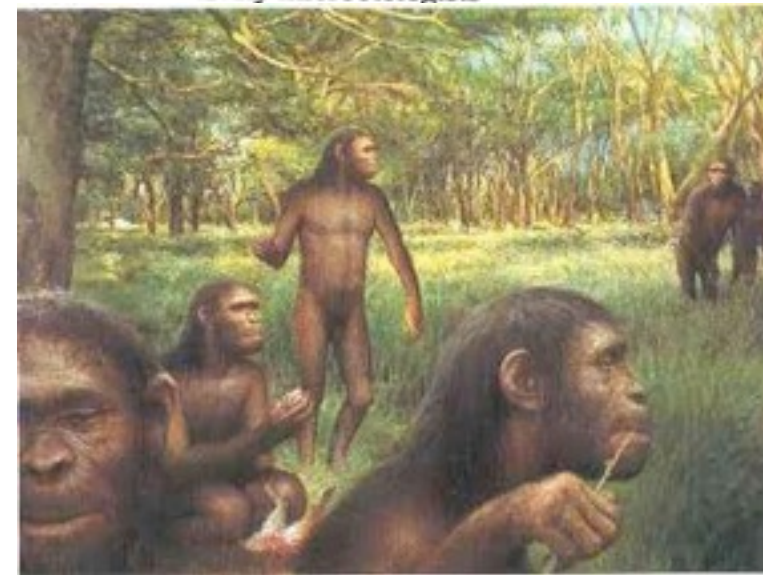




## Still at the “microbe hunters” age



Early microbiologists

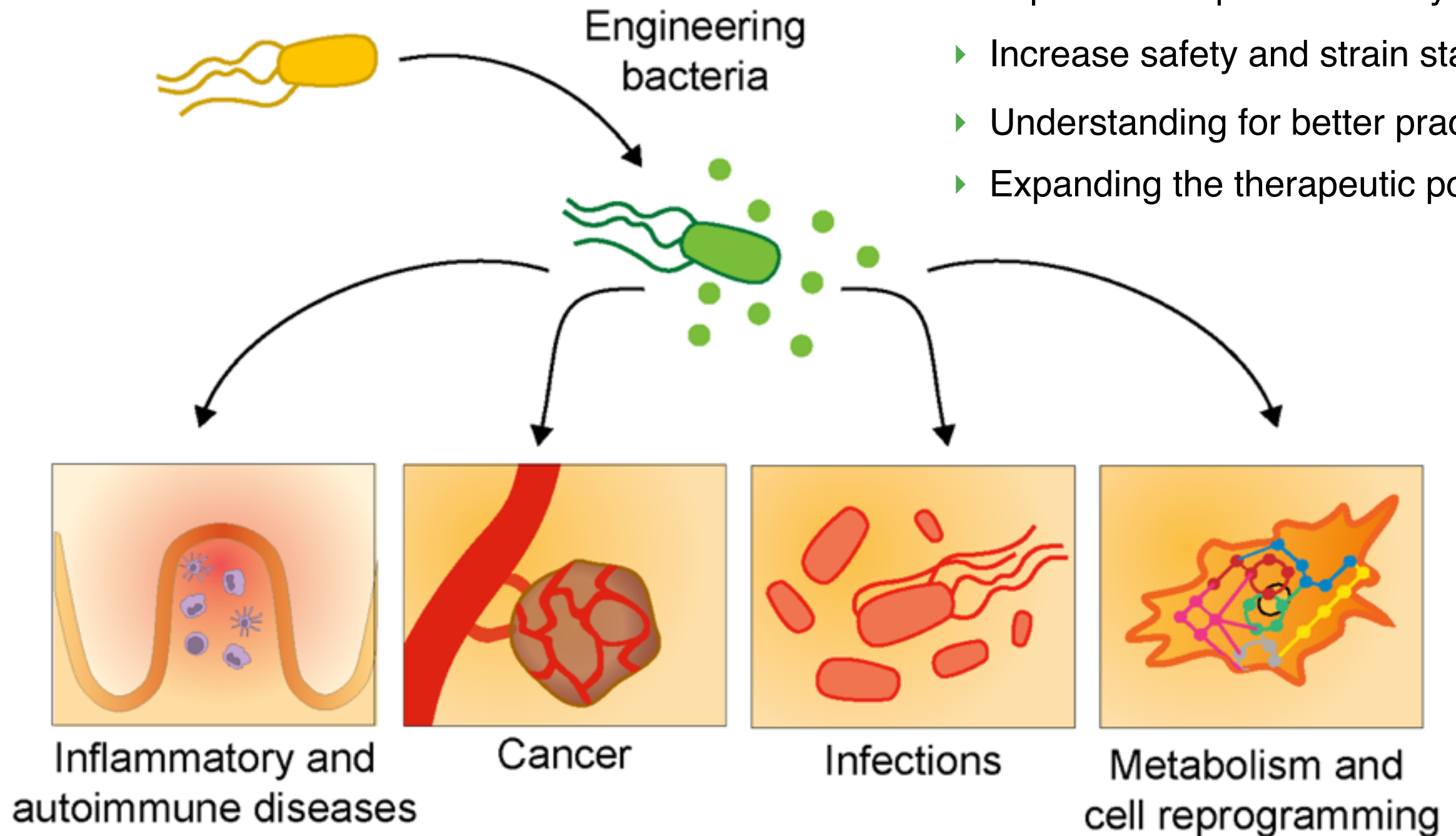




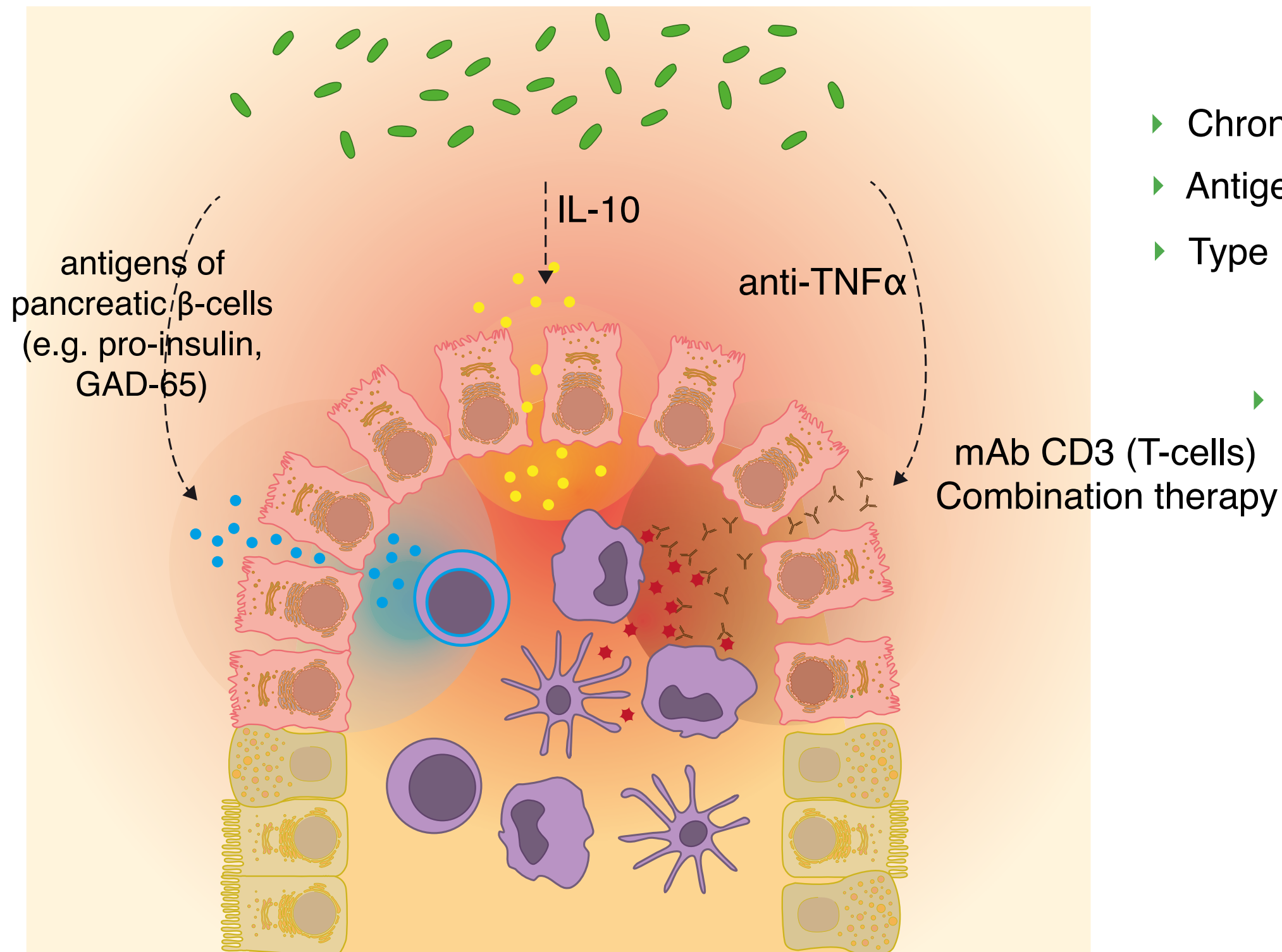
# Engineering bacteria for biomedical applications

## Why should we engineer bacteria?

- ▶ Improve therapeutic efficacy
- ▶ Increase safety and strain stability
- ▶ Understanding for better practice
- ▶ Expanding the therapeutic potential



# Engineering bacteria against inflammatory and autoimmune disorders



- ▶ Chronic inflammation
- ▶ Antigen tolerance
- ▶ Type 1 diabetes

Braat *et al.*, 2006

Vandenbroucke *et al.*, 2010

Huibregtse *et al.*, 2012

Takiisi *et al.*, 2012

Robert *et al.*, 2014



# Engineering bacteria against metabolic disorders

## ► Hyperglycemia in diabetes

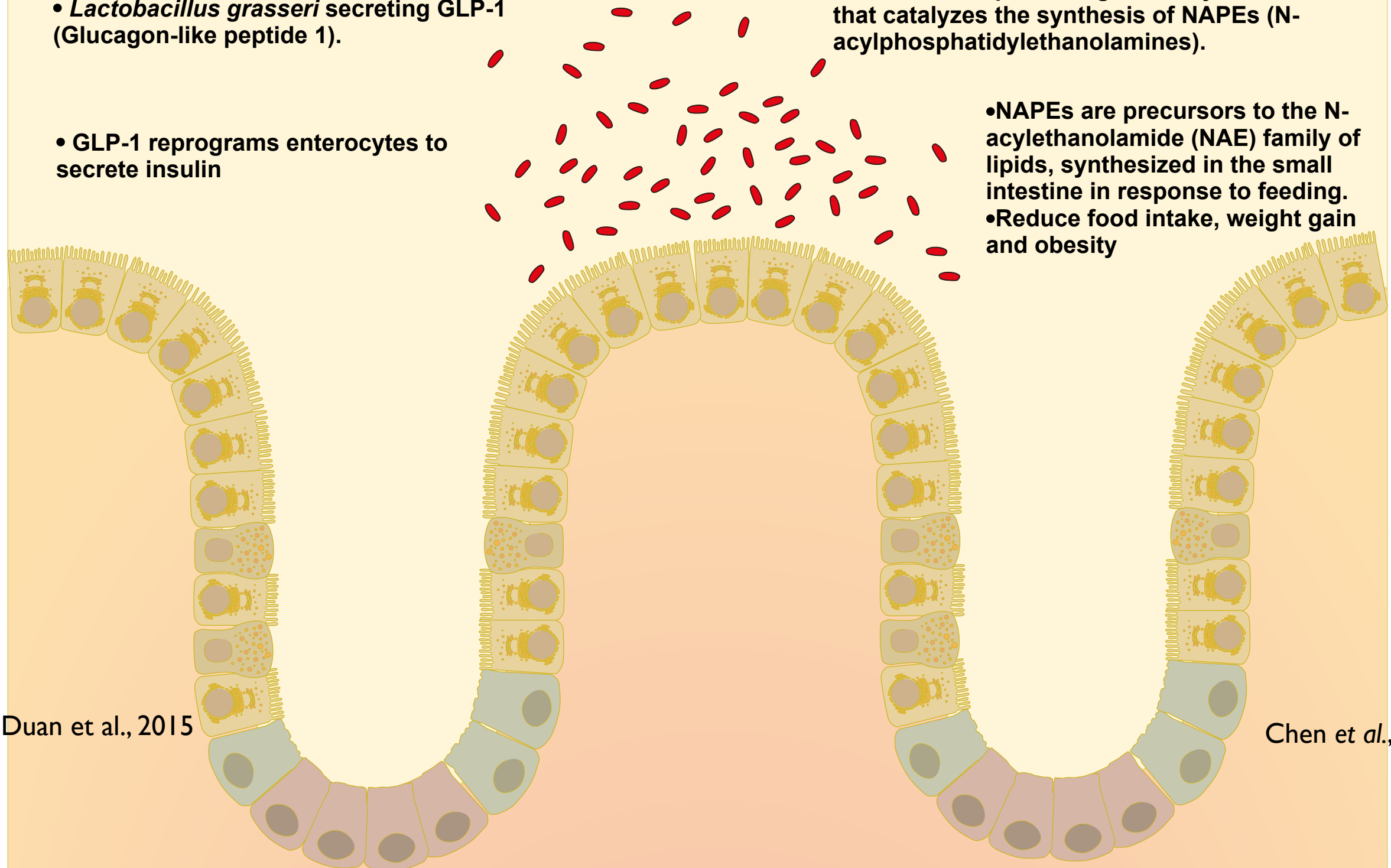
- *Lactobacillus grasseri* secreting GLP-1 (Glucagon-like peptide 1).
- GLP-1 reprograms enterocytes to secrete insulin

## ► Obesity

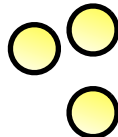
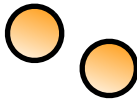
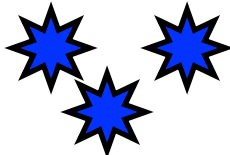
- *E coli* Nissle producing an N-acyltransferase that catalyzes the synthesis of N-acylphosphatidylethanolamines).
- N-acylphosphatidylethanolamines (NAPEs) are precursors to the N-acyl ethanolamide (NAE) family of lipids, synthesized in the small intestine in response to feeding.
- Reduce food intake, weight gain and obesity

Duan et al., 2015

Chen et al., 2014



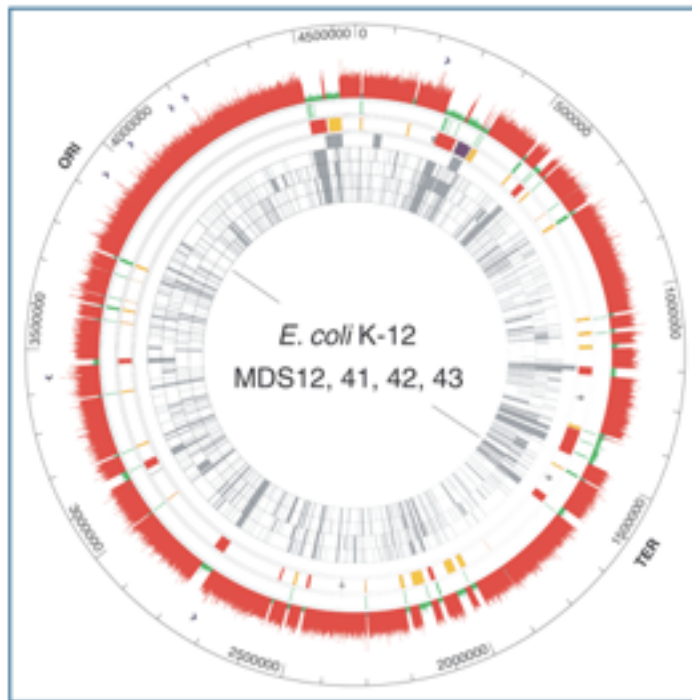
# Some examples of engineered probiotics

| Product                                                                                                | Model                                                                                                                                                                      | Application                | Example                                                                                                                                            |
|--------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------|
| DNA                   |                                                                                          | Vaccines                   | <i>Lactobacillus acidophilus</i> delivering FMDV VP1 gene of foot-and-mouth disease virus (FMDV) (Li et al., 2007, <i>Vaccine</i> , 25:902)        |
| Peptide               |                                                                                          | HIV-1                      | <i>E. coli</i> Nissle 1917 secreting anti-HIV peptide (Rao et al., 2005, <i>PNAS</i> , 102: 11993)                                                 |
| Antibody fragments  |                                                                                        | Immunotherapy              | <i>Lactobacillus rhamnosus</i> GG expressing anti-rotavirus antibody fragment (Álvarez et al., 2015, <i>Appl Environment Microbiol</i> , 81: 5784) |
| Antigens            |                                                                                        | Vaccines                   | <i>Lactobacillus plantarum</i> expressing the fragment C of tetanus toxin (Grangette et al., 2001, <i>Infect Immun</i> , 69:1547)                  |
| Cytokines           |   | Inflammatory bowel disease | <i>Lactococcus lactis</i> expressing IL-10 (Braat et al., 2006, <i>Clin. Gastroenterol. Hepatol.</i> , 4:754)                                      |
| Enzymes             |                                                                                        | Obesity, colitis           | <i>E. coli</i> Nissle 1917 expressing an N-acyltransferase for production of NAPEs (Chen et al., 2014, <i>J Clin Invest</i> 124:3391)              |



# Synthetic biology and the design of bacteria

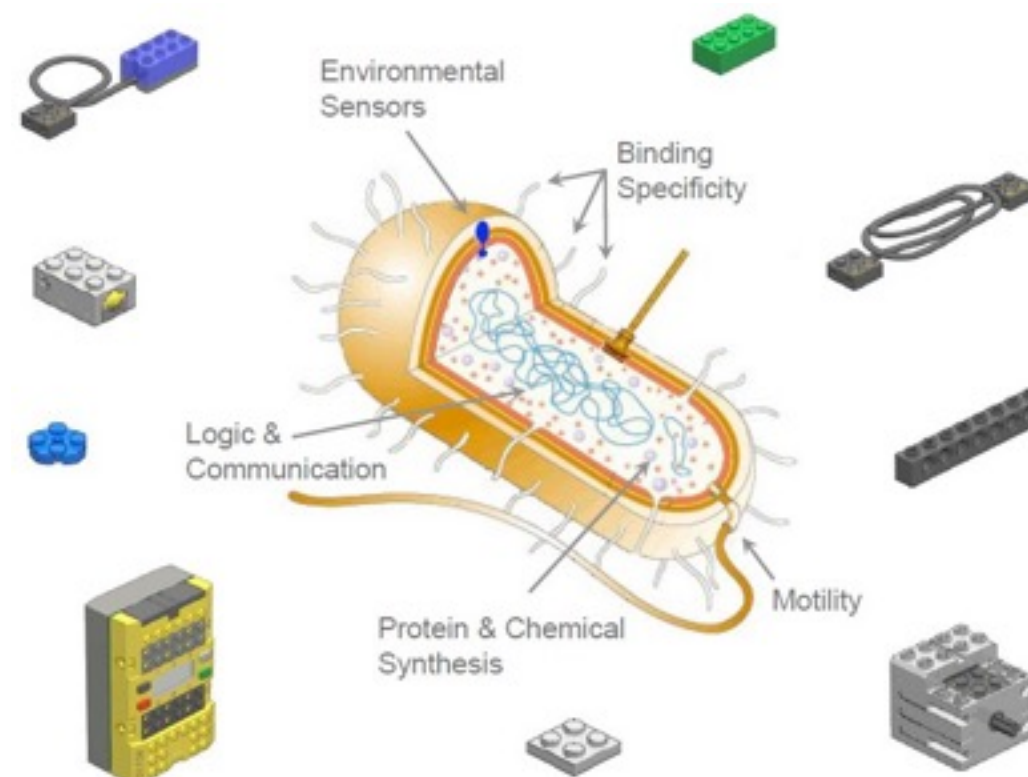
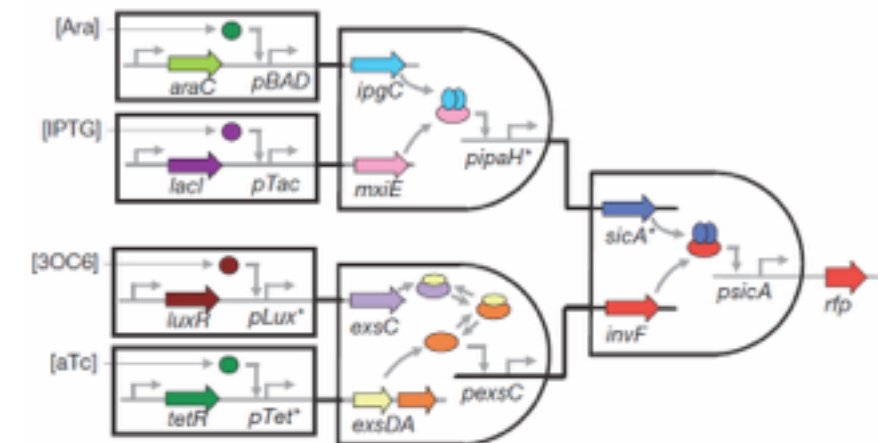
genome manipulation



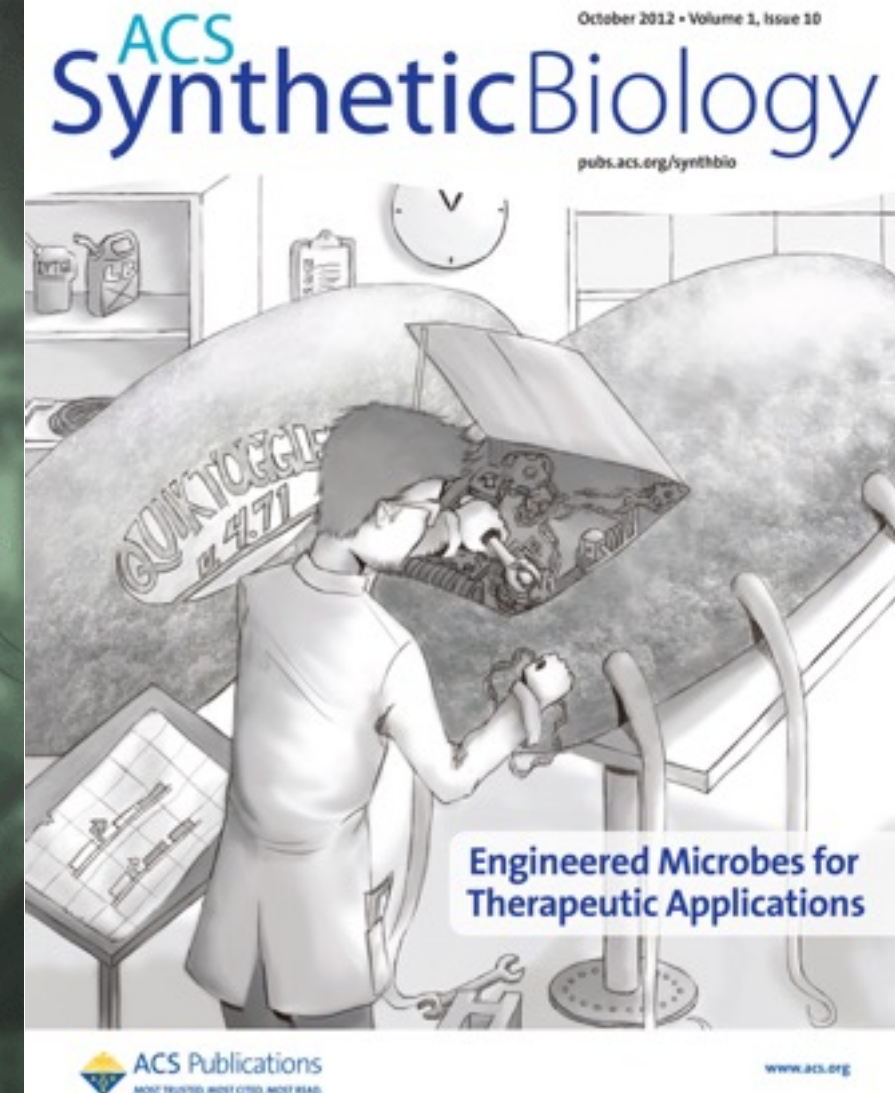
biological building blocks



genetic circuits

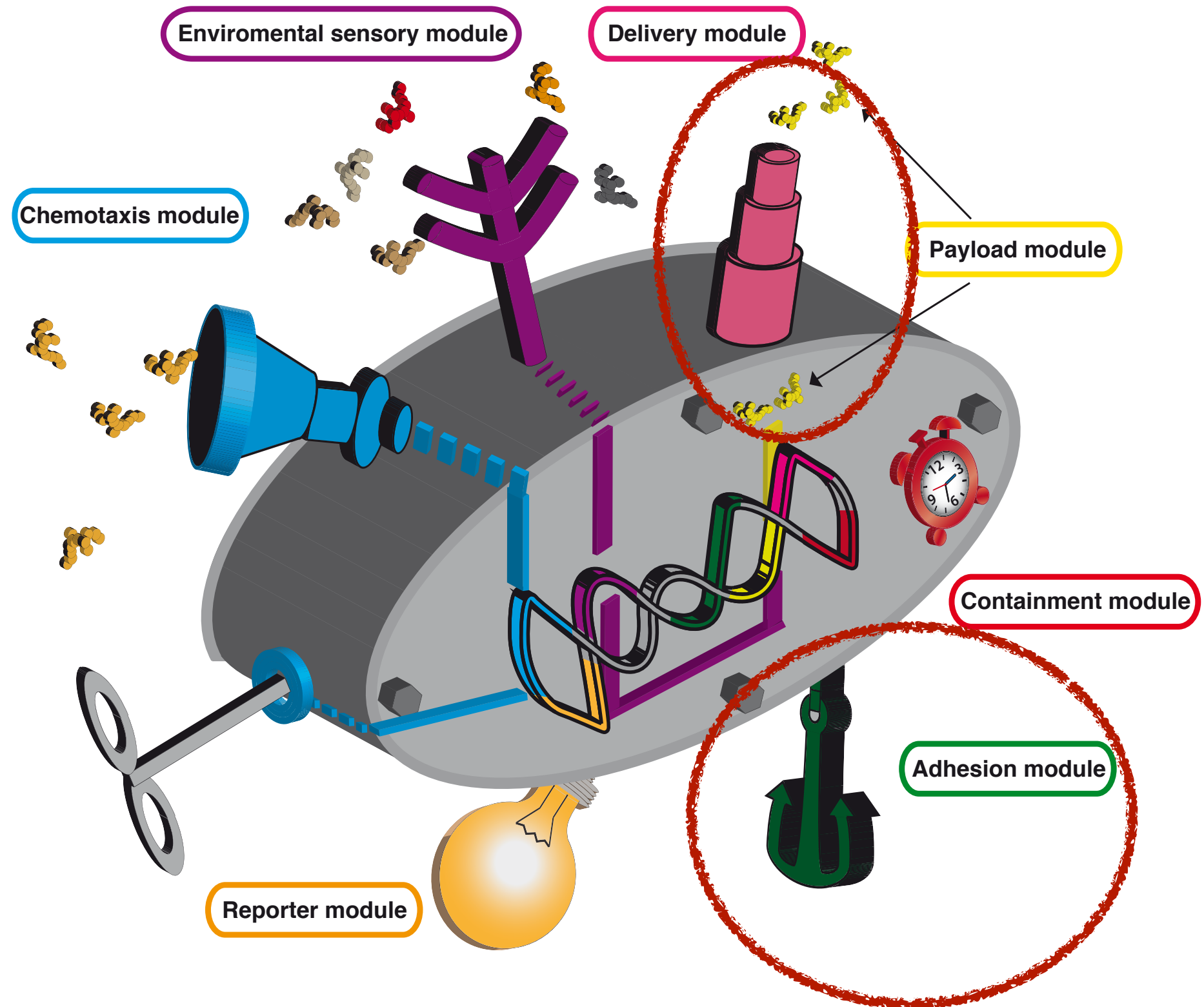


# “Bacterial robots” to monitor our bodies and combat diseases

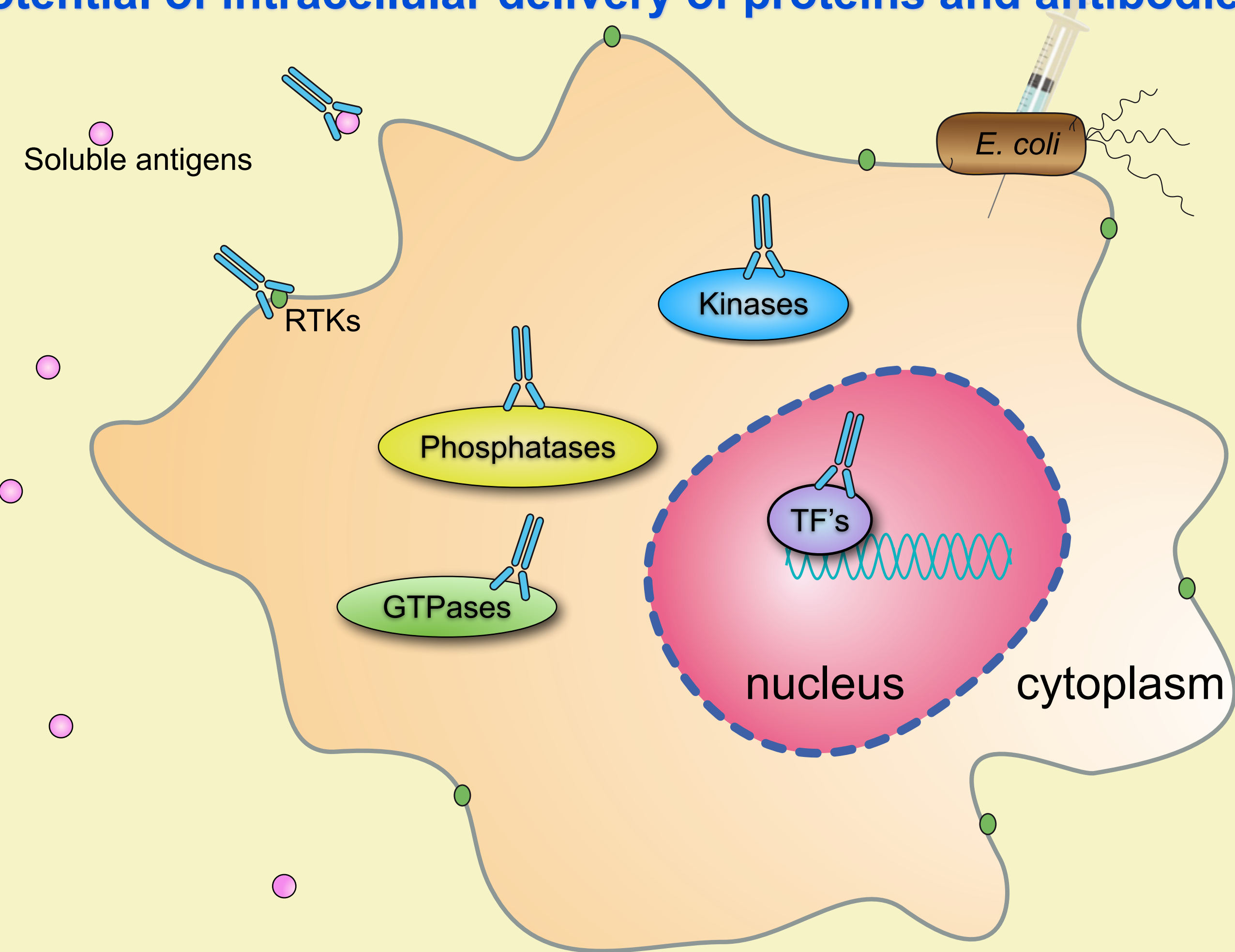




# A modular synthetic bacterium

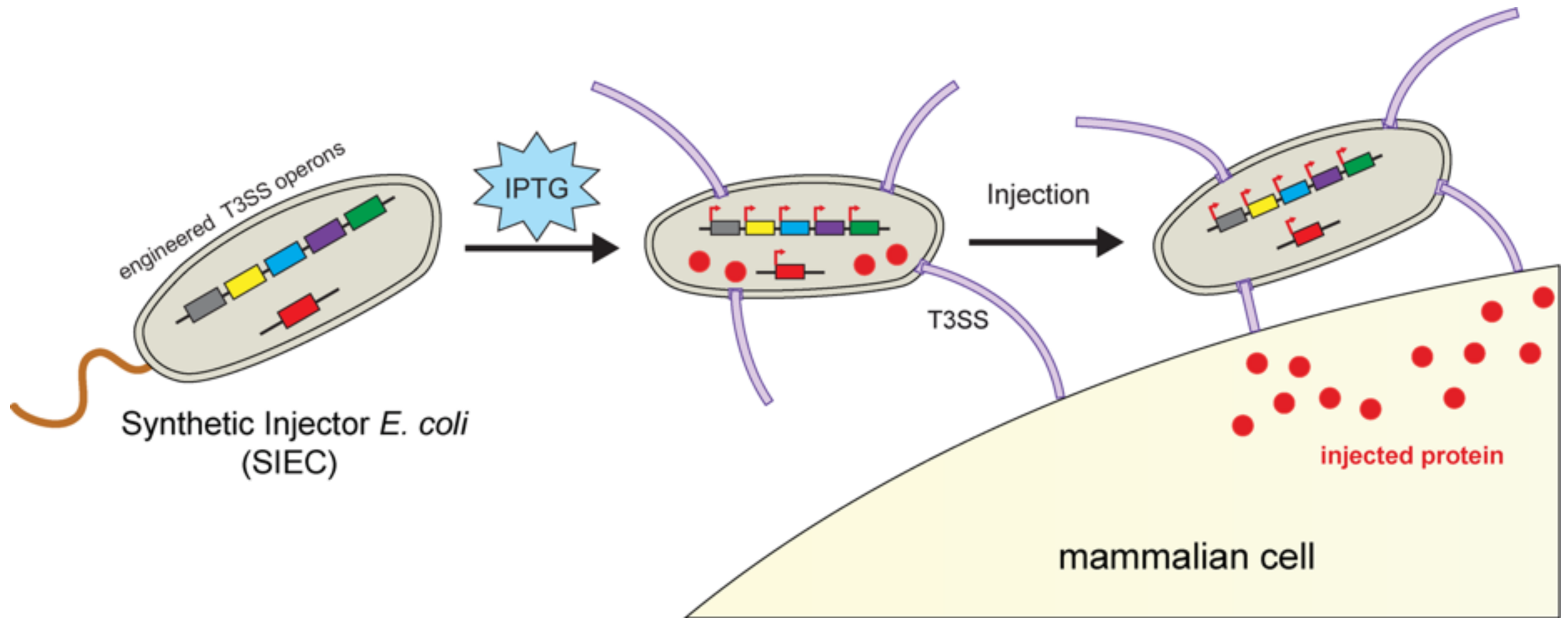


# Potential of intracellular delivery of proteins and antibodies

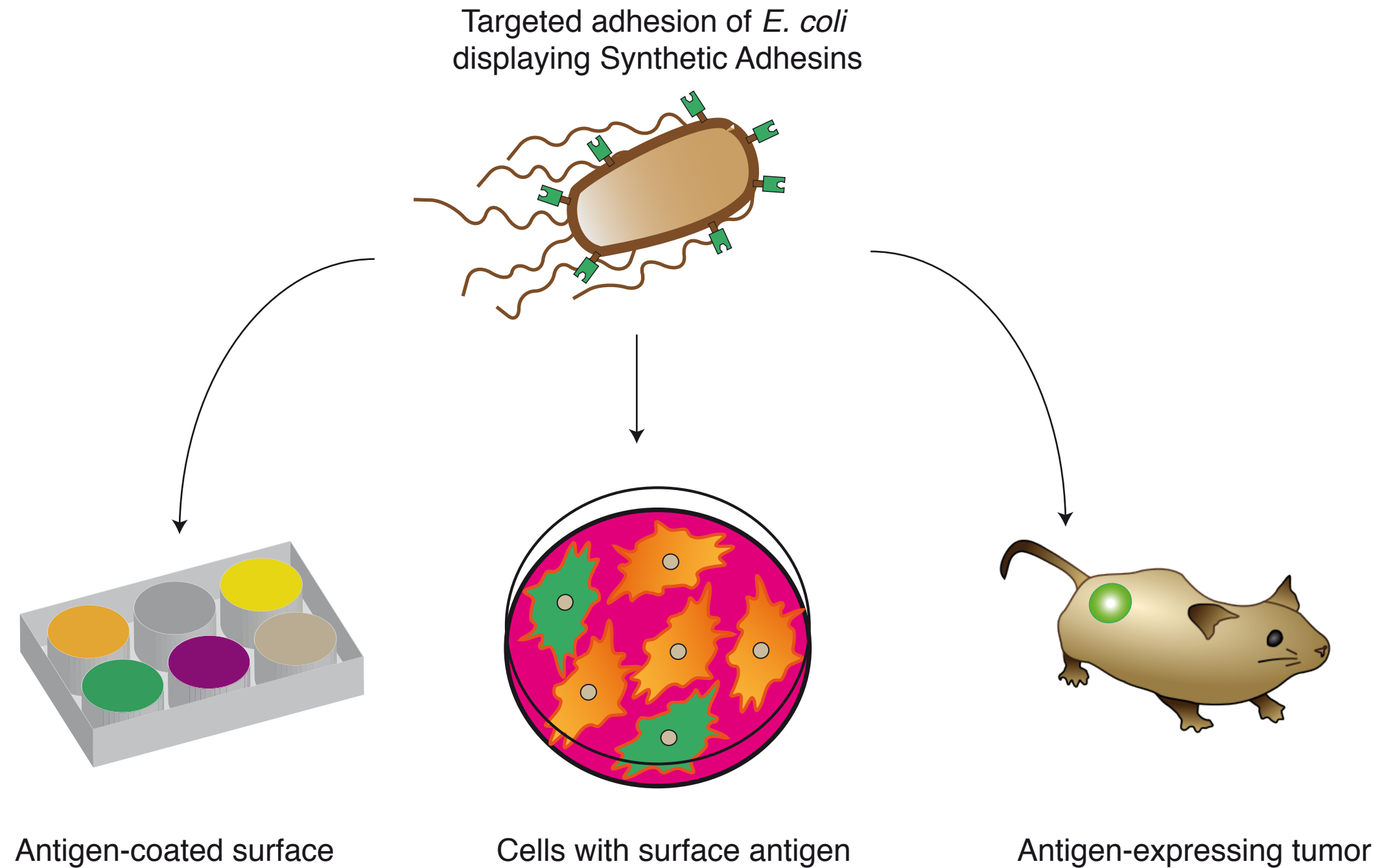




# Synthetic Injector *E. coli*



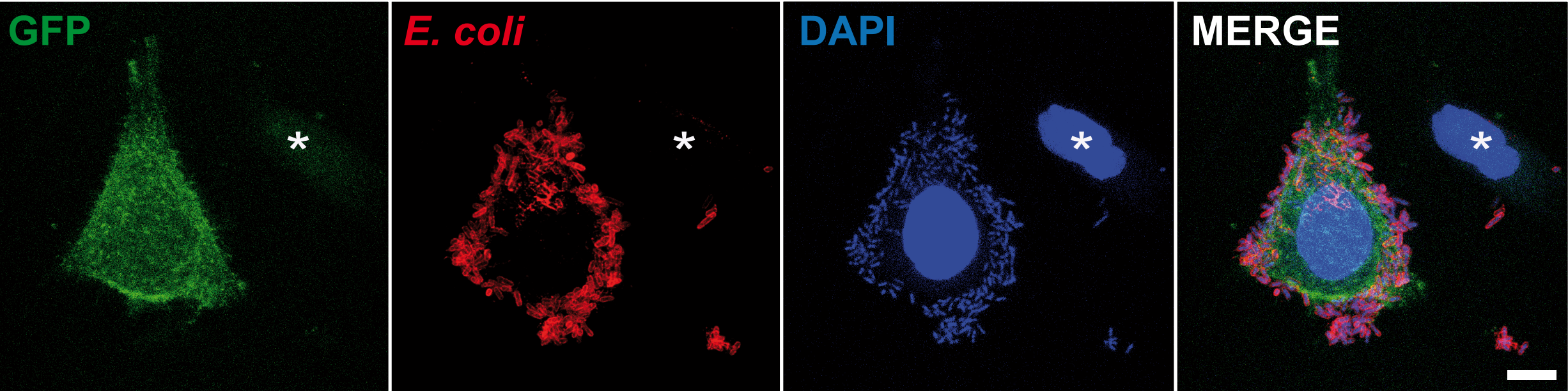
# Synthetic adhesins to target *E. coli* to specific cells



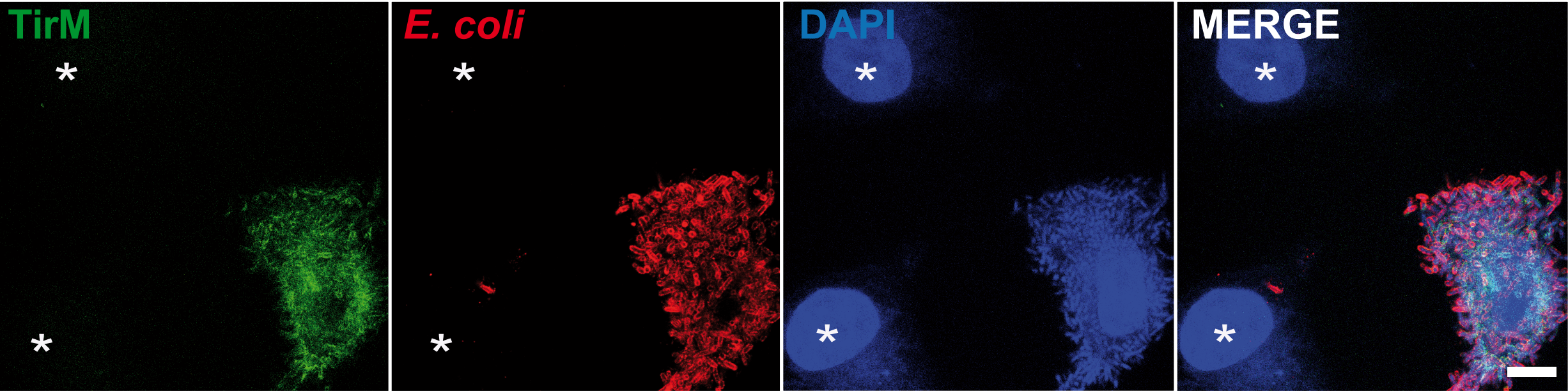


# Adhesion of *E. coli* to target cells with surface antigen

EcM1/uxSagfp



EcM1/uxSAtir





# From “microbe hunters” to “microbe designers”





# Acknowledgements

Carlos Piñero  
David Ruano  
Beatriz Álvarez  
Valencio Salema  
Massiel Cepeda  
Carmen Mañas  
*Gustavo Bodelón*  
*Elvira Marín*  
*Ana Blanco-Toribio*

*Funding:*

