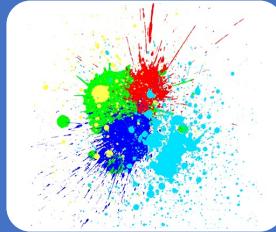


Expedite metabolic engineering of anaerobes with FAST of The Twinkle Factory

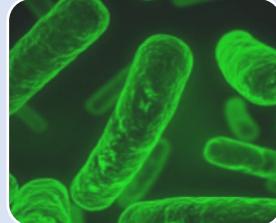
CLOSTRIDIUM XVI – Toulouse, Sept. 14-17th, 2022

Luc Lenglet

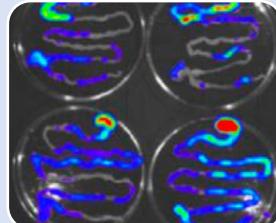
Expedite metabolic engineering of anaerobes with FAST of The Twinkle Factory



FAST technology

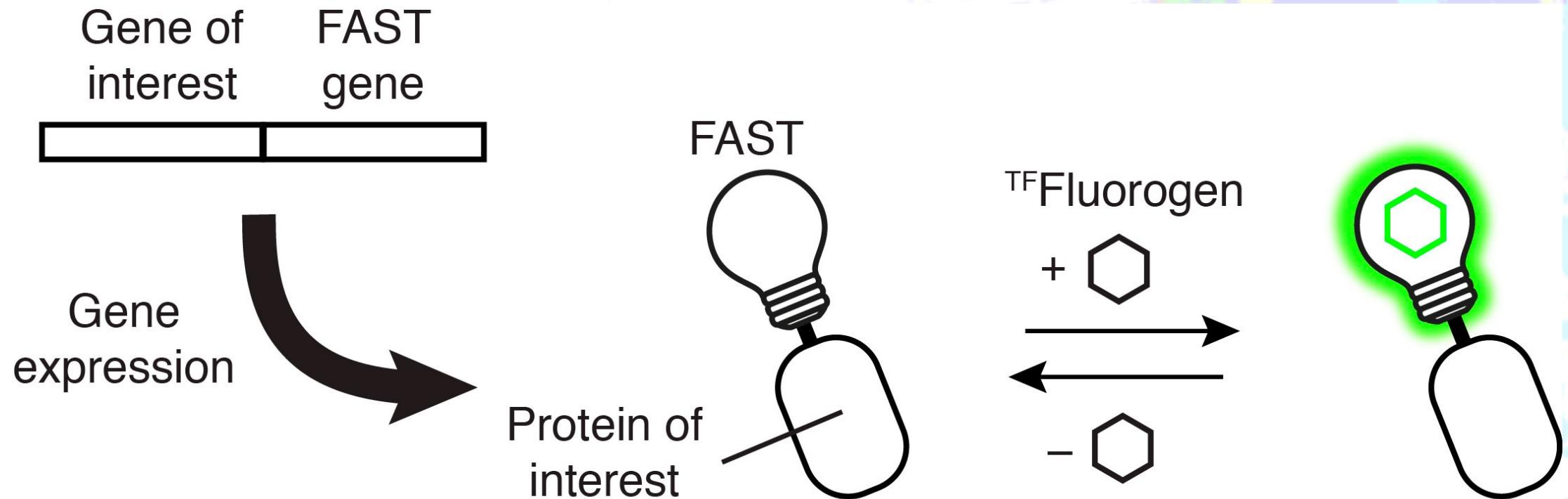


FAST in anaerobes



Beyond metabolic engineering

FAST is a genetically-encoded tool for the specific fluorescent labeling of proteins



*FAST: *Fluorescence-Activating and absorption-Shifting Tag*

Unique features



2

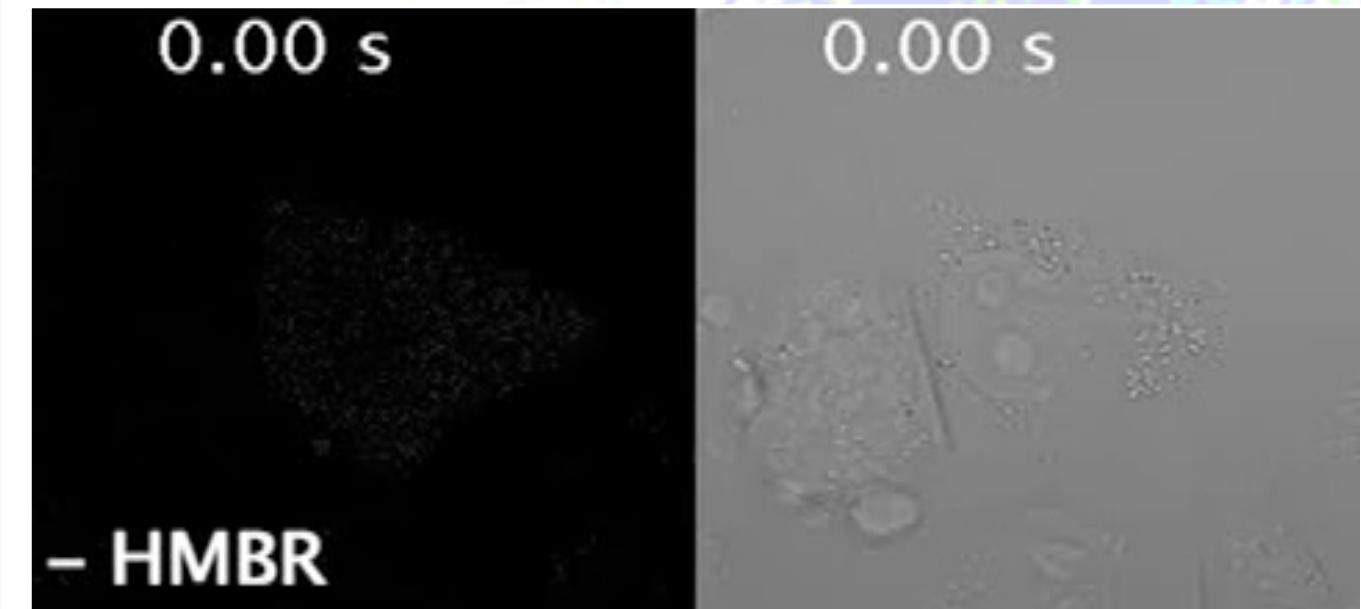
instantaneous



no O₂

reversible

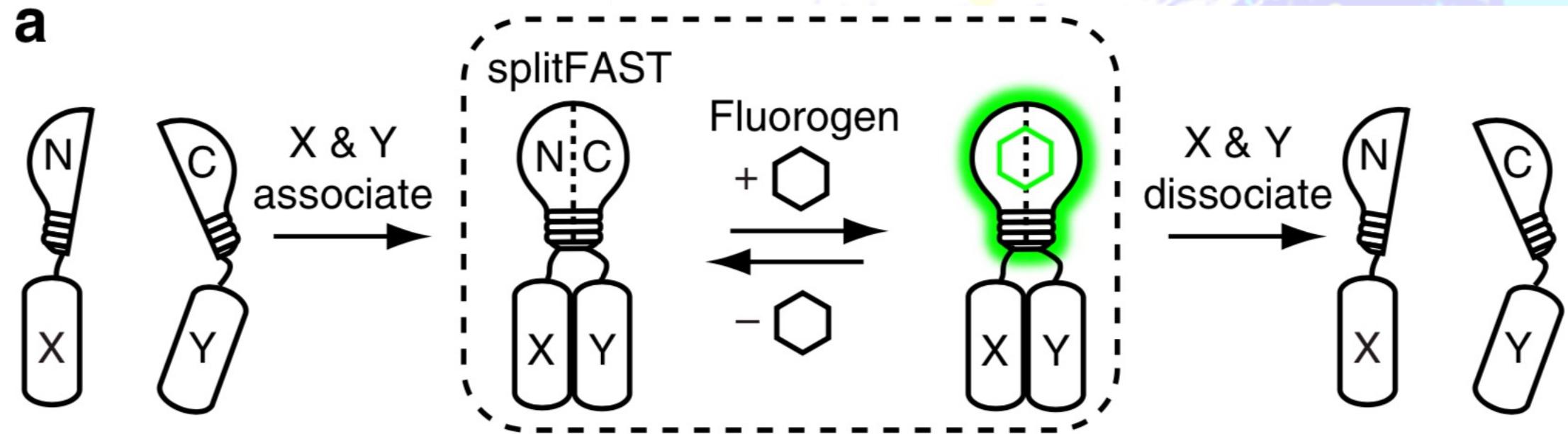
3



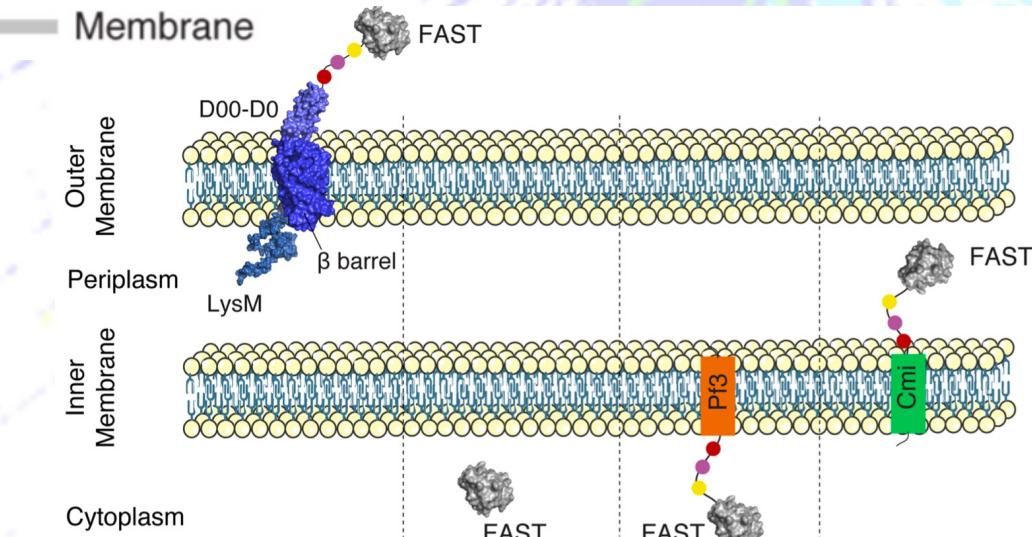
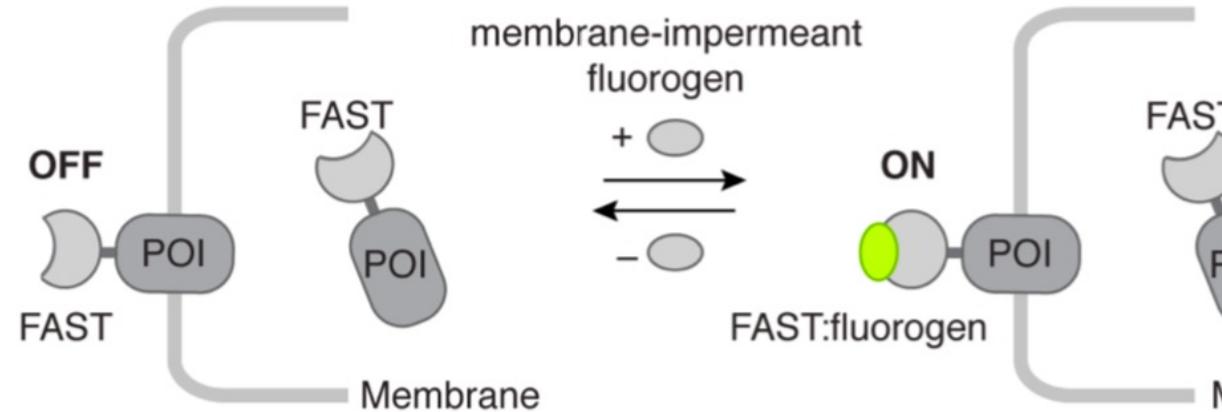
Unprecedented options to label proteins in living cells and organisms

	Fluorescent proteins	Self-labeling tags	FAST
Size	25-30 kDa	20-35 kDa	14 kDa
Tendency to oligomerize	Yes	No	No
Requirement for O ₂	Yes	No	No
Fluorescence maturation	10 min – 2 h	Few minutes	Instantaneous
N- and C-termini fusion	Yes	Yes	Yes
Insertion	Yes	No	Yes
Labeling step	No	Yes	Yes
Washing step	No	Yes	No
Reversible labeling	No	No	Yes
Color change	No	Yes	Yes
Selective cell-surface labeling	No	Yes	Yes

splitFAST allows protein-protein interactions investigations

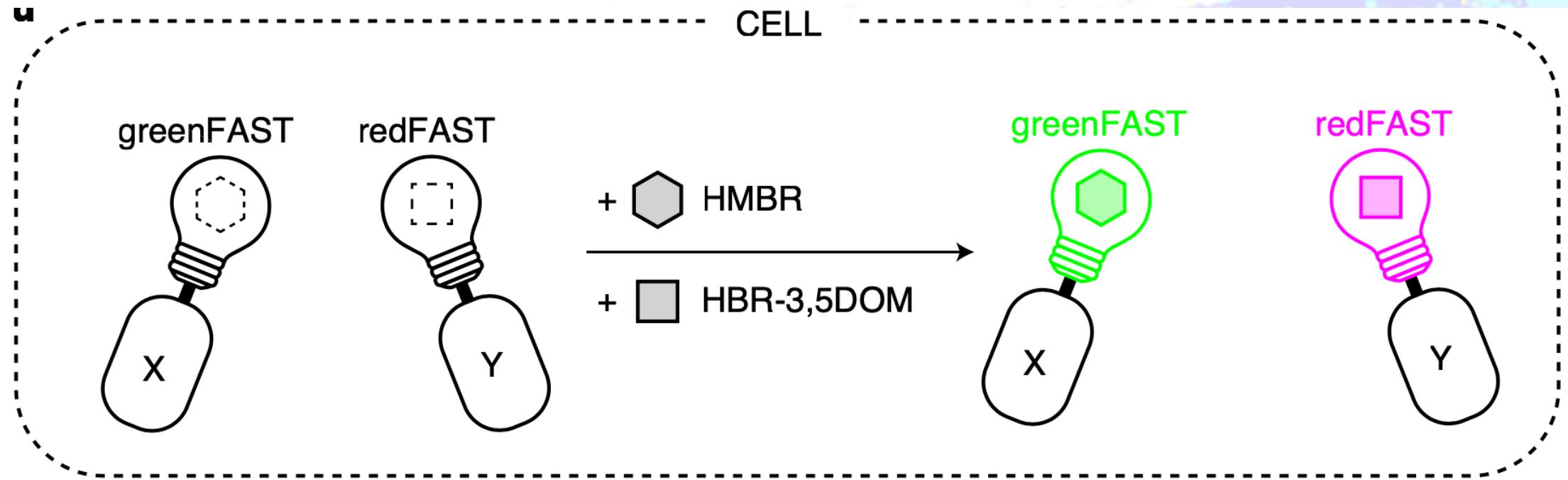


Combining together non-permeant and permeant fluorogens allows to monitor protein trafficking

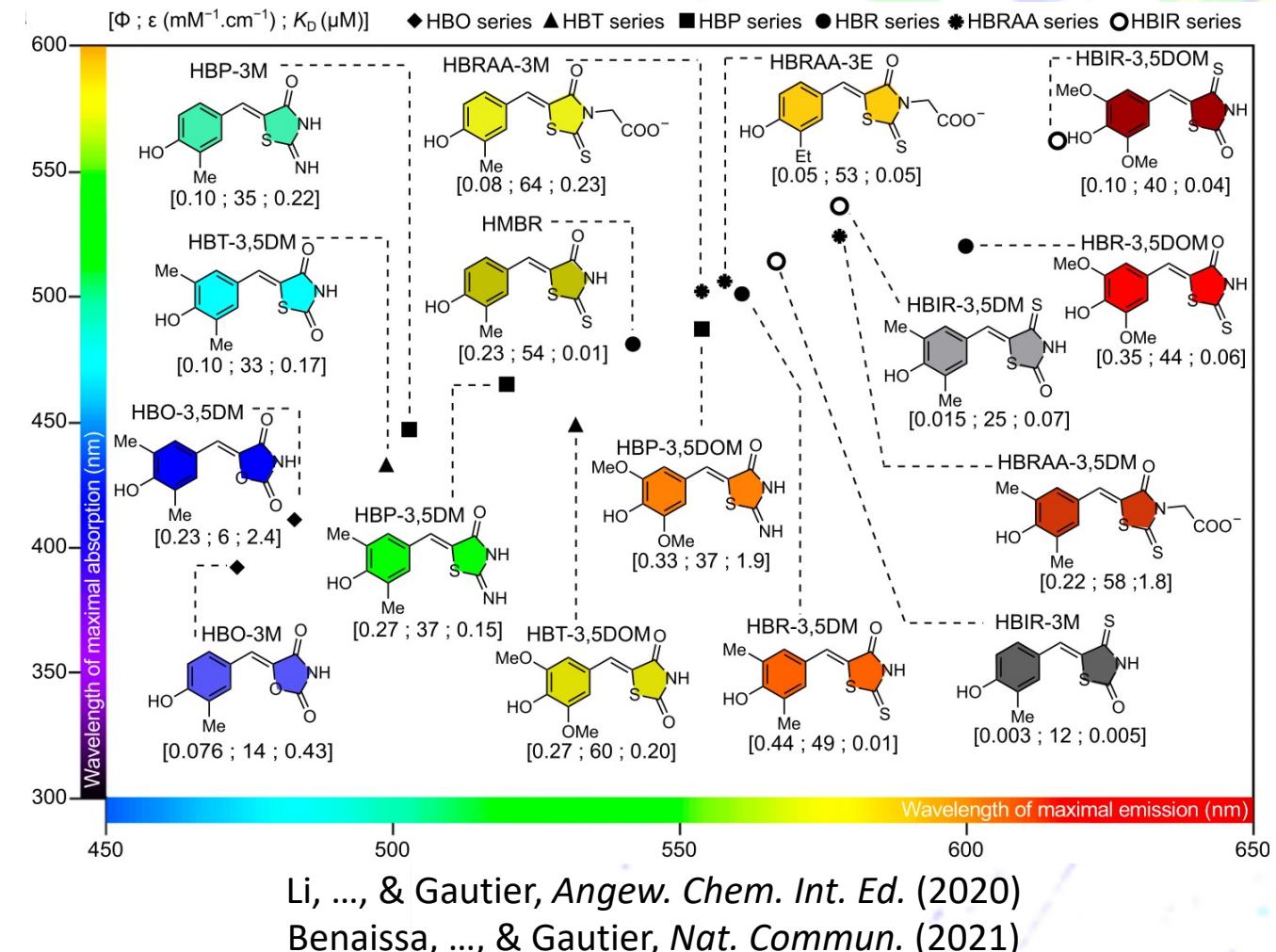


Chekli, ..., & Beloin, *Sci. Rep.* (2020)
Li, ..., & Gautier, *Bioconjugate Chem.* (2018)

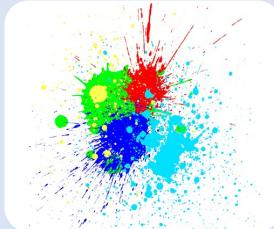
greenFAST & redFAST allow orthogonal multicolor labeling



Promiscuous pFAST has allowed widening further the range of fluorogens



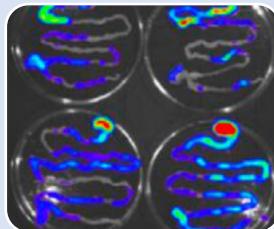
Expedite metabolic engineering of anaerobes with FAST of The Twinkle Factory



FAST technology



FAST in anaerobes



Beyond metabolic engineering

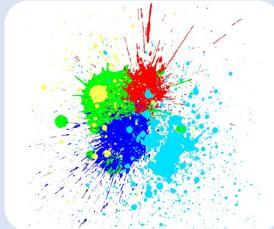
Prof. Papoutsakis first identified the potential of FAST for *Clostridium* research

- [Mbio 2020](#) – Charubin, K., Modla, S., Caplan, J. L., & Papoutsakis, E. T. Interspecies Microbial Fusion and Large-Scale Exchange of Cytoplasmic Proteins and RNA in a Syntrophic *Clostridium* Coculture.
- [Appl. Environ. Microbiol. 2020](#) – Charubin, K., Streett, H., & Papoutsakis, E. T. Development of Strong Anaerobic Fluorescent Reporters for *Clostridium acetobutylicum* and *Clostridium ljungdahlii* Using HaloTag and SNAP-tag Proteins.
- [Appl. Environ. Microbiol. 2019](#) – Streett, H. E., Kalis, K. M., & Papoutsakis, E. T. A strongly fluorescing anaerobic reporter and protein-tagging system for *Clostridium* organisms based on the fluorescence-activating and absorption-shifting tag protein (FAST).
- [Metab. Eng. 2018](#) – Charubin, K., Bennett, R. K., Fast, A. G., & Papoutsakis, E. T. Engineering *Clostridium* organisms as microbial cell-factories: challenges & opportunities.

Since then, implementation of FAST has boomed across anaerobes

- [bioRxiv 2022](#) – Adlung, N., & Scheller, S. Application of the fluorescence-activating and absorption-shifting tag (FAST) for flow cytometry in methanogenic archaea.
- [J. Bacteriol. 2022](#) – Hernandez, E., & Costa, K. C. The fluorescence-activating and absorption-shifting tag (FAST) enables live-cell fluorescence imaging of *Methanococcus maripaludis*.
- [Appl. Microbiol. Biotechnol. 2022](#) – Mook, A., Beck, M., Baker, J., Minton, N., Dürre, P., Bengelsdorf, F. Autotrophic lactate production from H₂ + CO₂ using recombinant and fluorescent FAST-tagged *Acetobacterium woodii* strains.
- [ACS Synth. Biol. 2022](#) – Flaiz, M., Baur, T., Gaibler, J., Kröly, C., & Dürre, P. Establishment of Green- and Red-Fluorescent Reporter Proteins Based on the Fluorescence-Activating and Absorption-Shifting Tag for Use in Acetogenic and Solventogenic Anaerobes.
- [Curr. Opin. Biotechnol. 2021](#) – Streett, H., Charubin, K., & Papoutsakis, E. T. Anaerobic fluorescent reporters for cell identification, microbial cell biology and high-throughput screening of microbiota and genomic libraries.
- [Biotechnol. Biofuels 2021](#) – Flaiz, M., Ludwig, G., Bengelsdorf, F. R., & Dürre, P. Production of the Biocommodities Butanol and Acetone from Methanol with Fluorescent FAST-tagged Proteins using Metabolically Engineered Strains of *Eubacterium Limosum*.

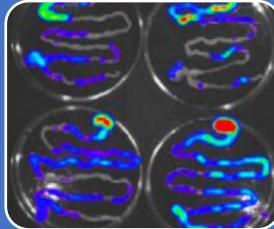
Expedite metabolic engineering of anaerobes with FAST of The Twinkle Factory



FAST technology



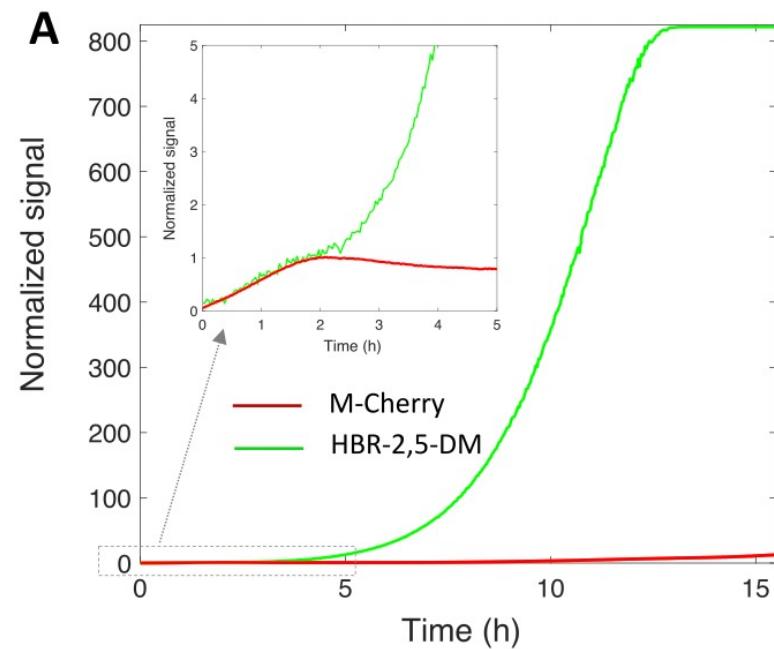
FAST in anaerobes



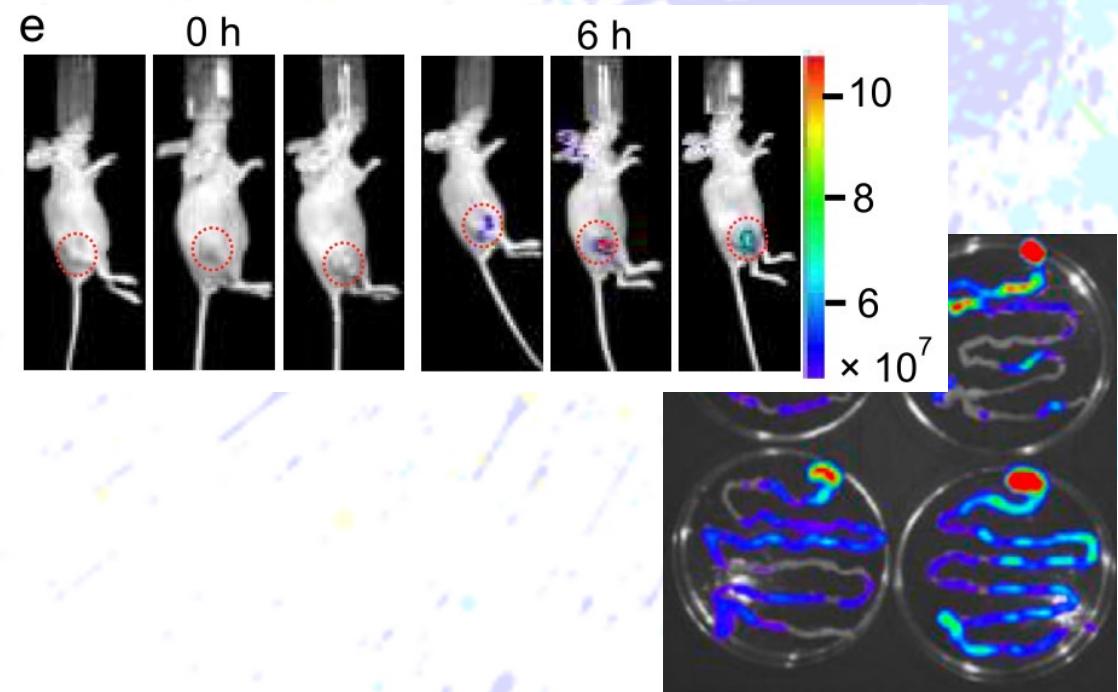
Beyond metabolic engineering

Food for thought

monitoring mature biofilms dynamics



mouse microbiome imaging (gut/tumor)



Monmeyran, ..., & Henry, *Sci. Rep.* (2018)
Cao, ..., & Liu, *Mater. Today Bio* (2022)

Looking forward to expanding The Twinkle Factory community

www.the-twinkle-factory.com

www.linkedin.com/company/the-twinkle-factory

www.twitter.com/twinklefactory1

45 rue d'Ulm, Paris, France

