

**Bioorthogonal non-canonical
amino acid tagging
- BONCAT -**

BONCAT in microbial ecology, as of August 2021, part I

Hatzenpichler R et al. *In situ* visualization of newly synthesized proteins in environmental microbes using amino acid tagging and click chemistry

Environ Microbiol, 16: 2568-2590 (2014)

➤ first application of BONCAT to uncultured microbes; development of BONCAT-FISH; correlation of BONCAT with nanoSIMS

Samo TJ et al. Broad distribution and high proportion of protein synthesis active marine bacteria revealed by click chemistry at the single cell level

Front Microbiol, 1: 48 (2014)

➤ application of BONCAT to seawater; correlation of BONCAT with microautoradiography

Hatzenpichler R and Orphan VJ Detection of protein-synthesizing microorganisms in the environment via bioorthogonal non-canonical amino acid tagging (BONCAT)

Book chapter for Hydrocarbon and Lipid Microbiology Protocols, Springer Protocols Handbooks, doi 10.1007/8623_2015_61 (2015)

➤ description of how to design and protocols for how to perform BONCAT-experiments using AHA and HPG

Hatzenpichler R et al. Visualizing *in situ* translational activity for identifying and sorting slow-growing archaeal-bacterial consortia

Proc Natl Acad Sci USA, 113: E4069-E4078 (2016)

➤ development of activity-based cell-sorting via bioorthogonal labeling (BONCAT-FACS); applied BONCAT-(CARD)FISH and BONCAT-FACS to deep-sea sediment consortia catalyzing the anaerobic oxidation of methane with sulfate

Leizeaga et al. Using Click-Chemistry for Visualizing *in Situ* Changes of Translational Activity in Planktonic Marine Bacteria

Front Microbiol, 8: 2360 (2017)

Pasulka AL et al. Interrogating marine virus-host interactions and elemental transfer with BONCAT and nanoSIMS-based methods

Environ Microbiol, 20: 671-692 (2018)

➤ first application of BONCAT to environmental phages and viruses; estimate of marine viral production rates by BONCAT and nanoSIMS

Couradeau et al. Probing the active fraction of soil microbiomes using BONCAT-FACS

Nat Comm, 10: 2770 (2019)

➤ first application of BONCAT to soil samples; reports that a surprisingly high proportion of soil microbes is translationally active

Sebastian et al. High Growth Potential of Long-Term Starved Deep Ocean Opportunistic Heterotrophic Bacteria

Front Microbiol, 10: 760 (2019)

Steward et al. Metabolic Implications of Using BioOrthogonal Non-Canonical Amino Acid Tagging (BONCAT) for Tracking Protein Synthesis

Front Microbiol, 11:197 (2020)

BONCAT in microbial ecology, as of August 2021, part II

Valentini et al. *Bioorthogonal non-canonical amino acid tagging reveals translationally active subpopulations of the cystic fibrosis lung microbiota*

Nature Comm, 11: 2287 (2020)

- Case study that applies BONCAT and BONCAT-FACS to cystic fibrosis patients' lung microbiomes

Lindivat et al. *Bioorthogonal Non-canonical Amino Acid Tagging Combined With Flow Cytometry for Determination of Activity in Aquatic Microorganisms*

Front Microbiol, 11: 1929 (2020)

Reichart et al. *Activity-based cell sorting reveals responses of uncultured archaea and bacteria to substrate amendment*

The ISME J, 14: 2851-2861 (2020)

- Uses BONCAT-FACS to detect changes in single cell activity of a hot spring microbial community incubated in the presence of various growth substrates or under changing physicochemical conditions

Riva et al. *Conversion of Rutin, a Prevalent Dietary Flavonol, by the Human Gut Microbiota*

Front Microbiol, 11: 585428 (2020)

Taguer et al. *Translational activity is uncoupled from nucleic acid content in bacterial cells of the human gut microbiota*

Gut Microbes, 13: e1903289 (2021)

- First application of BONCAT to human gut microbes (stool samples).

Chen et al. *Isolating and characterizing translationally active fraction of anammox microbiota using *bioorthogonal non-canonical amino acid tagging**

Chem Eng J, 418: 129411 (2021)

Michels et al. *Amino acid analog induces stress response in marine *Synechococcus**

Appl Environm Microbiol, DOI: 10.1128/AEM.00200-21 (2021)

Bergkessel and Delavaine. *Diversity in Starvation Survival Strategies and Outcomes among Heterotrophic Proteobacteria*

Microb Physiol, DOI: 10.1159/000516215 (2021)

Marlow et al. *Spatially resolved correlative microscopy and microbial identification reveal dynamic depth- and mineral- dependent anabolic activity in salt marsh sediment*

Environ Microbiol, doi:10.1111/1462-2920.15667 (2021)

- BONCAT, resin embedding, fluorescence microscopy, electron microscopy, and energy dispersive X-ray spectroscopy are combined to reveal the spatial patterning of translationally active cells around specific mineral particles in heterogenous salt marsh sediment



BONCAT in microbial ecology, as of August 2021, part III

Martinez-Varela et al. Bacterial responses to background organic pollutants in the northeast subarctic Pacific Ocean
Environ Microbiol, doi:10.1111/1462-2920.15646 (2021)

BONCAT is a Next-generation physiology approach

Definition: ...any combination of techniques that analyze the phenotype of an individual cell in a microbiome in a non-destructive way, which enables the physical separation of this cell based solely on its phenotype for subsequent, downstream applications

Specifically, BONCAT is a type of substrate analog probing (SAP)

SAP uses molecules that carry either a fluorescence tag or a side group amenable to azide-alkyne click chemistry to obtain information on the overall biosynthetic activity or specific enzymatic function of the cell.

Hatzenpichler et al., 2020

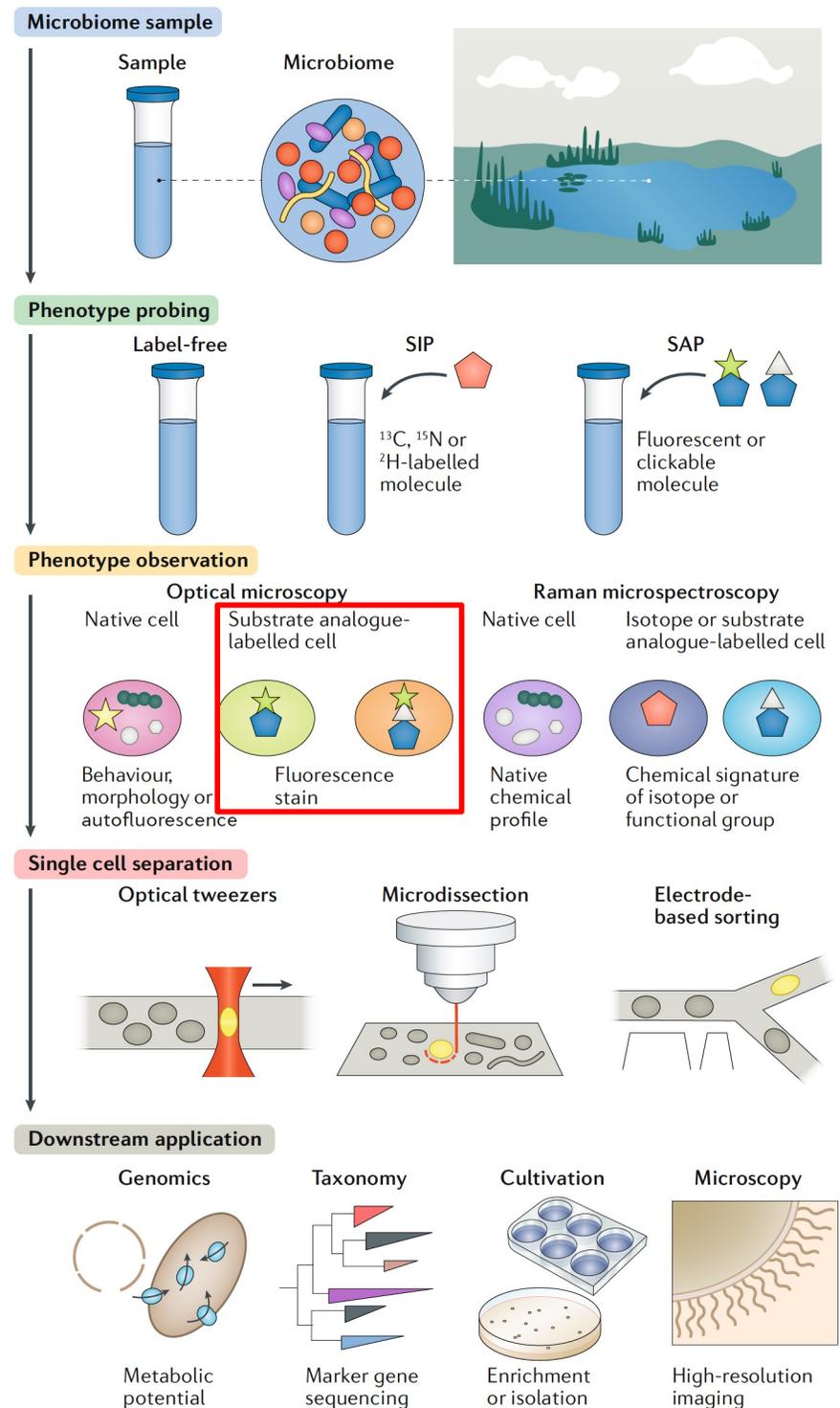


Fig. 2 | Next-generation physiology workflow to study microorganisms.

First, some definitions

bioorthogonal

non-interacting with cellular functionalities

non-canonical

synthetic, not part of biological machinery

Click chemistry

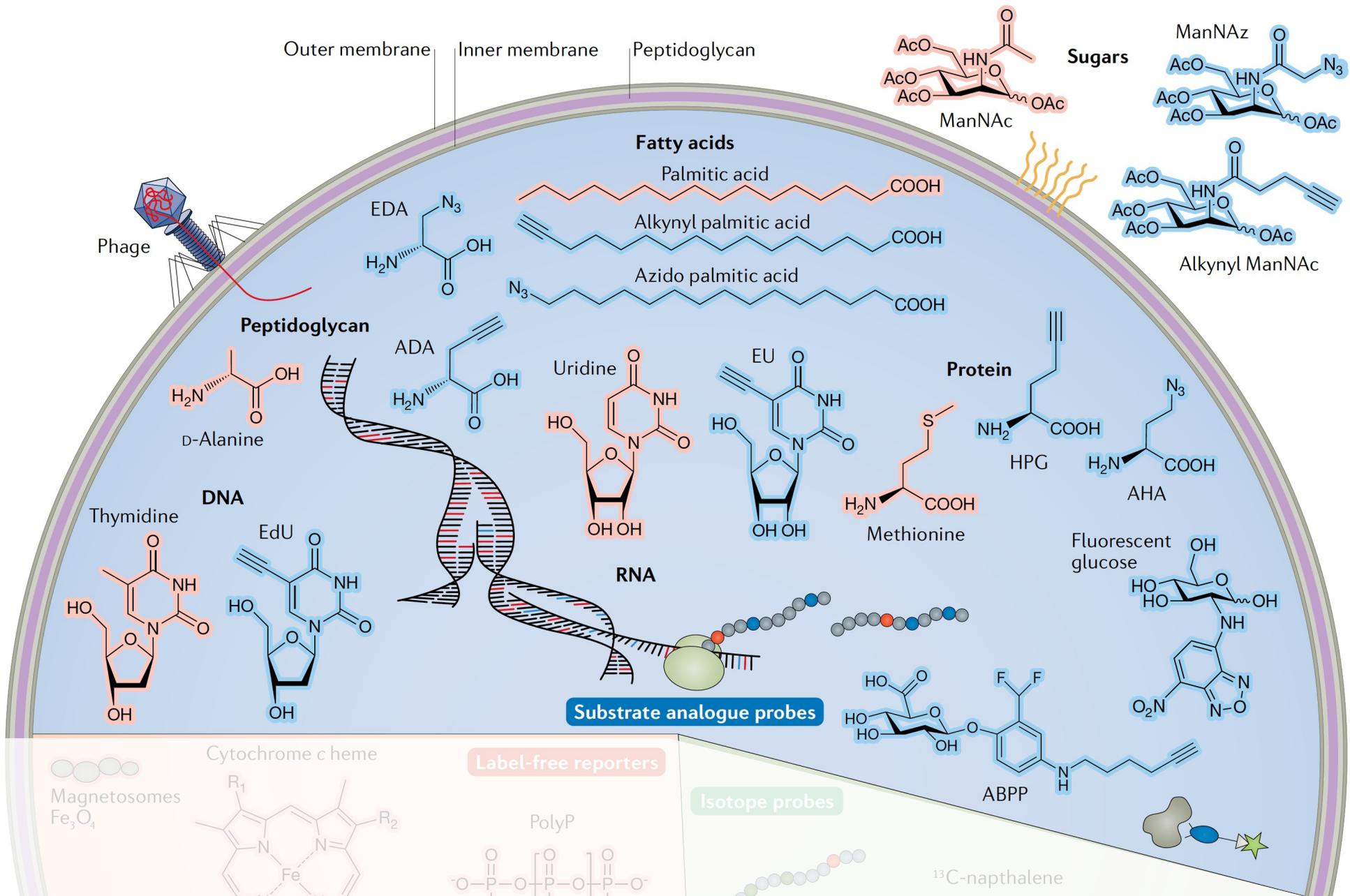
complete conversion of reagents to single product

+ mild conditions

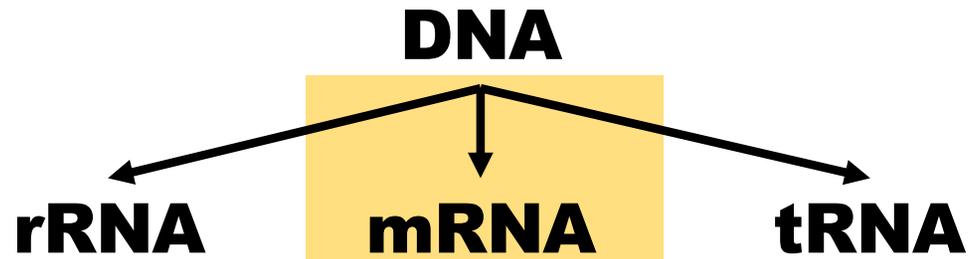
+ very fast

+ in water

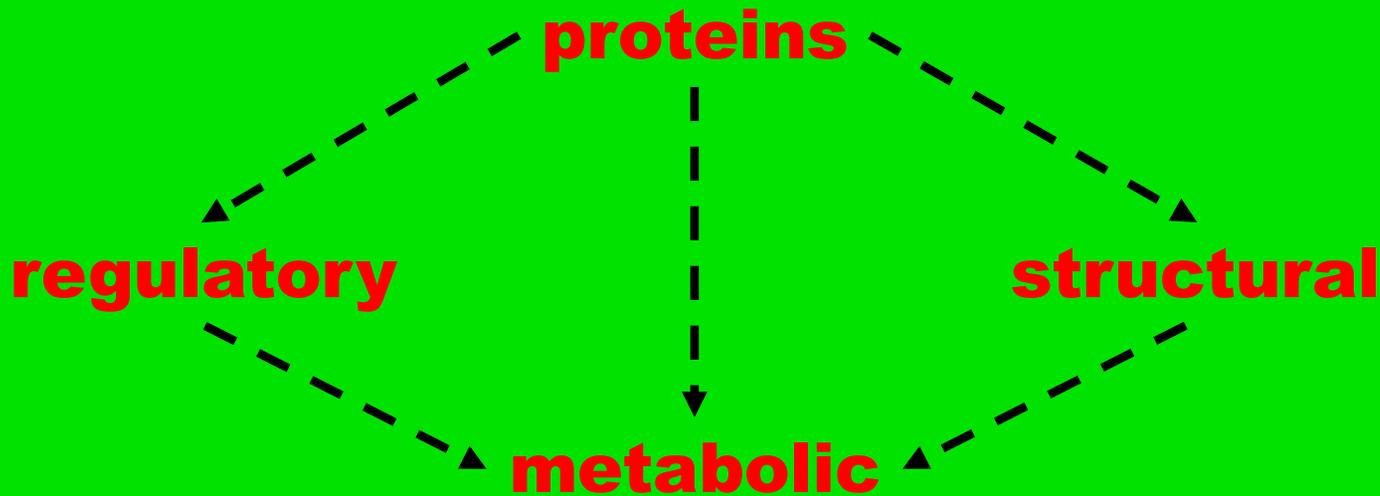
Examples for clickable substrate analogs



Activity assays on individual cell level



mRNA-FISH

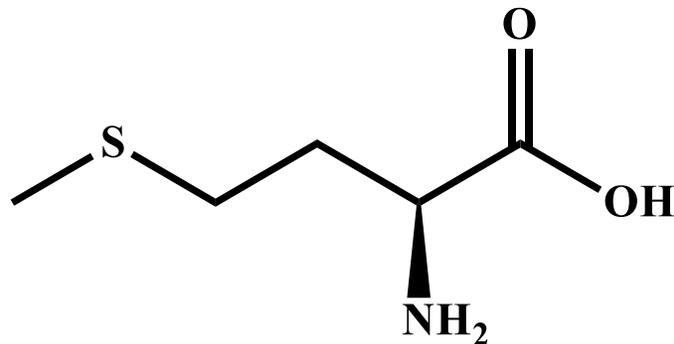


BONCAT

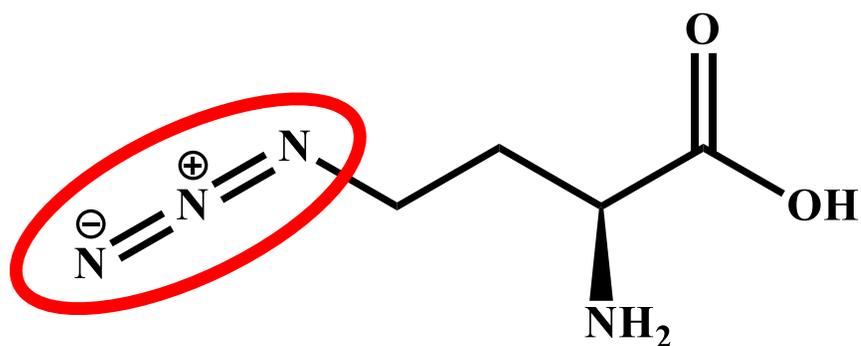
**build-up of biomass
& growth**

isotopic labeling

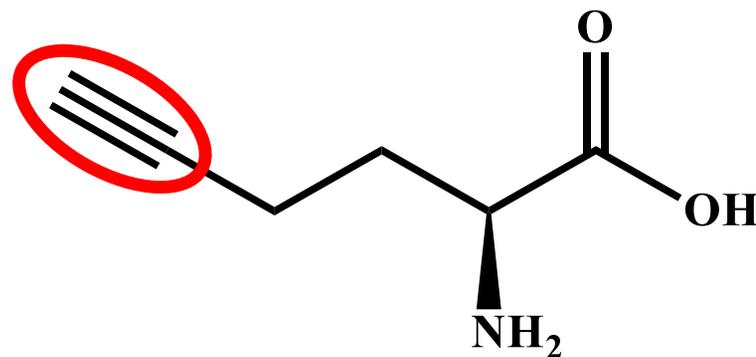
Bioorthogonal non-canonical amino acids



L-Methionine
Met



L-Azidohomoalanine
AHA

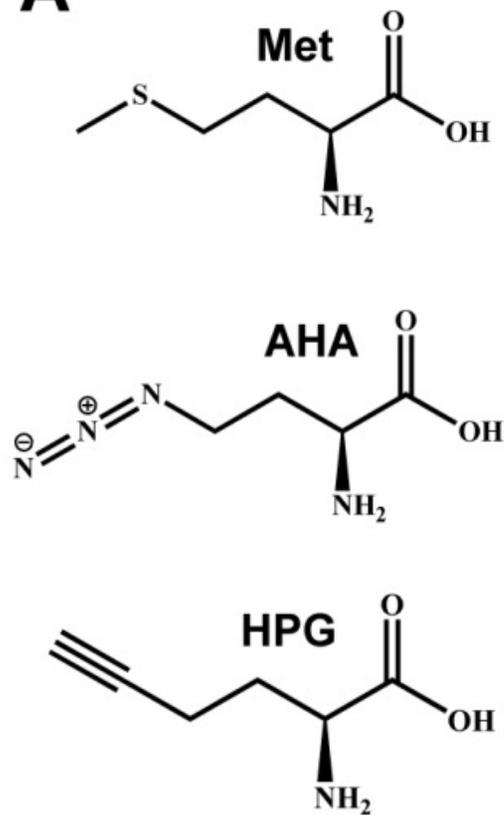


L-Homopropargylglycine
HPG

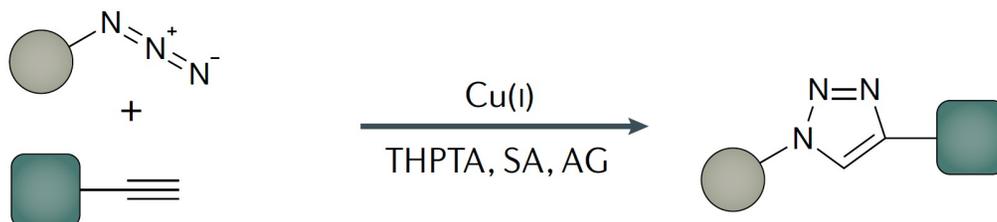
synthetic amino acids
incorporate into new proteins instead of Met

Azide-alkyne click chemistry reactions

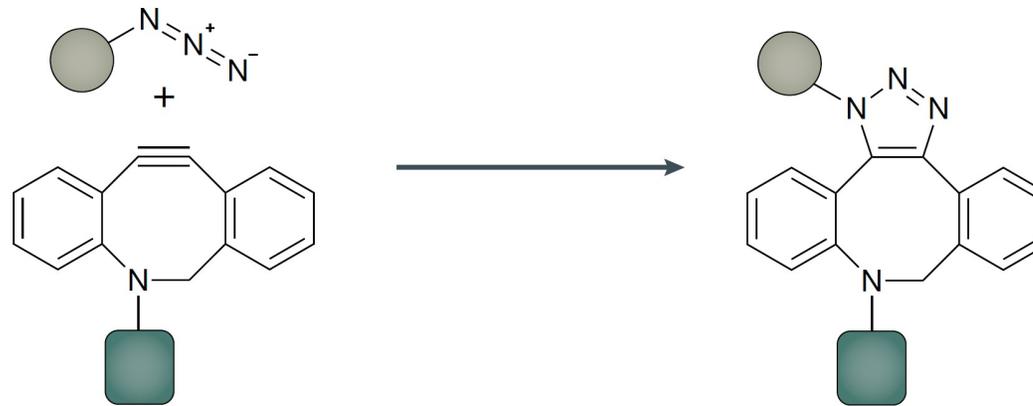
A



b Copper catalysed



c Strain promoted

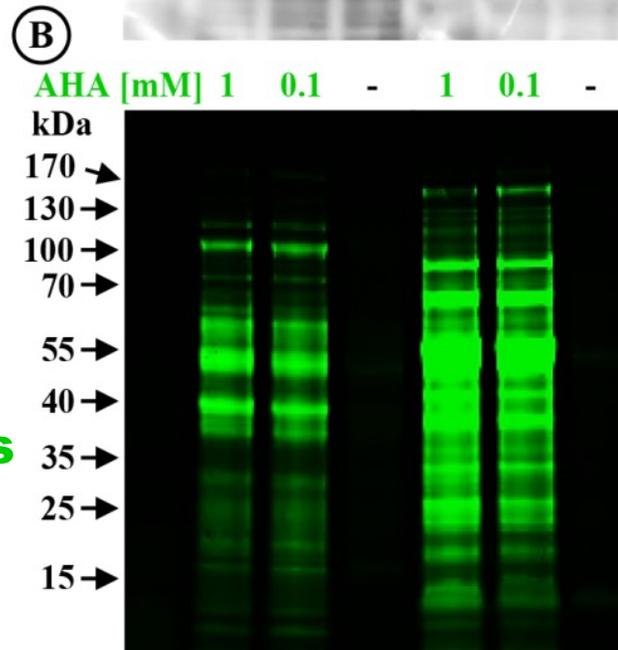
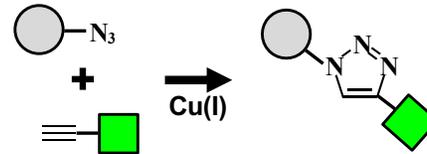
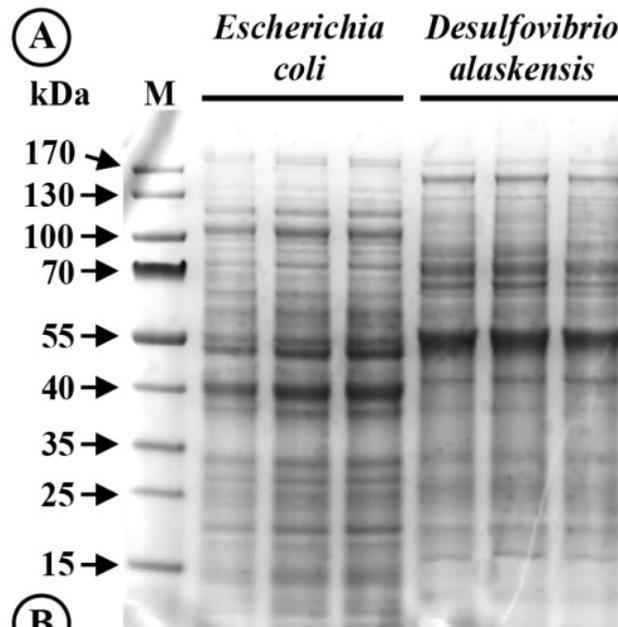


A. Structures of Met and its surrogates AHA and HPG, which compete with Met during translation.

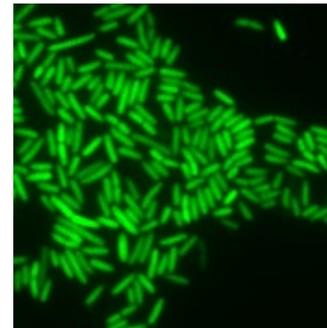
B. In Cu(I)-catalyzed click chemistry an azide group (N_3) is linked to a terminal alkyne residue, yielding a triazole conjugate.

C. Strain-promoted click chemistry allows the copper-less conjugation of an azide group (N_3) with a cyclo-octyne-carrying molecule, yielding a triazole conjugate.

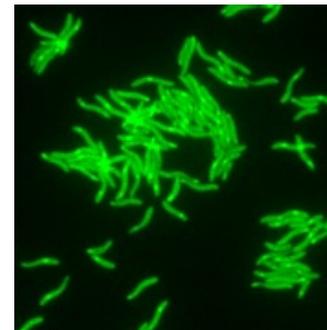
Incorporation into newly made proteins



new proteins

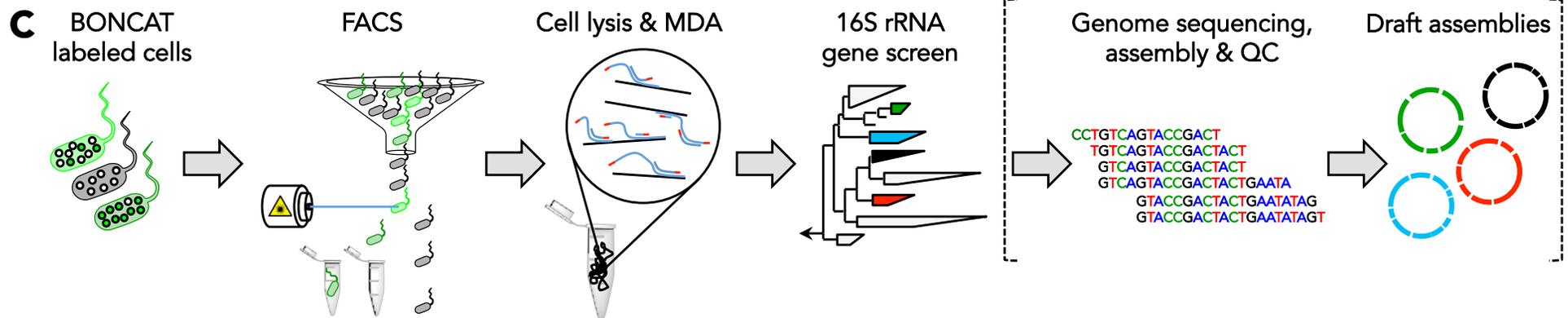
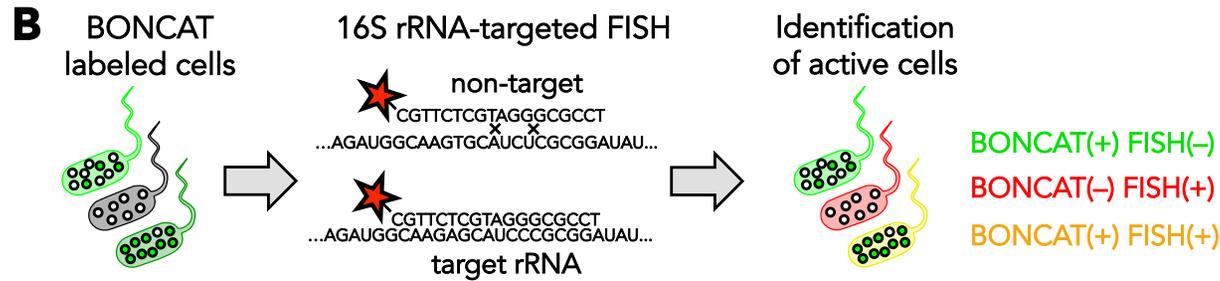
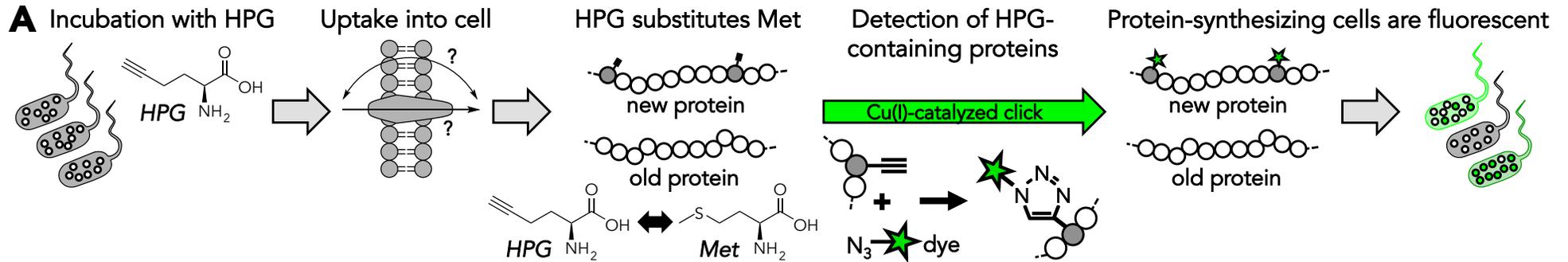


E. coli
respiring glucose

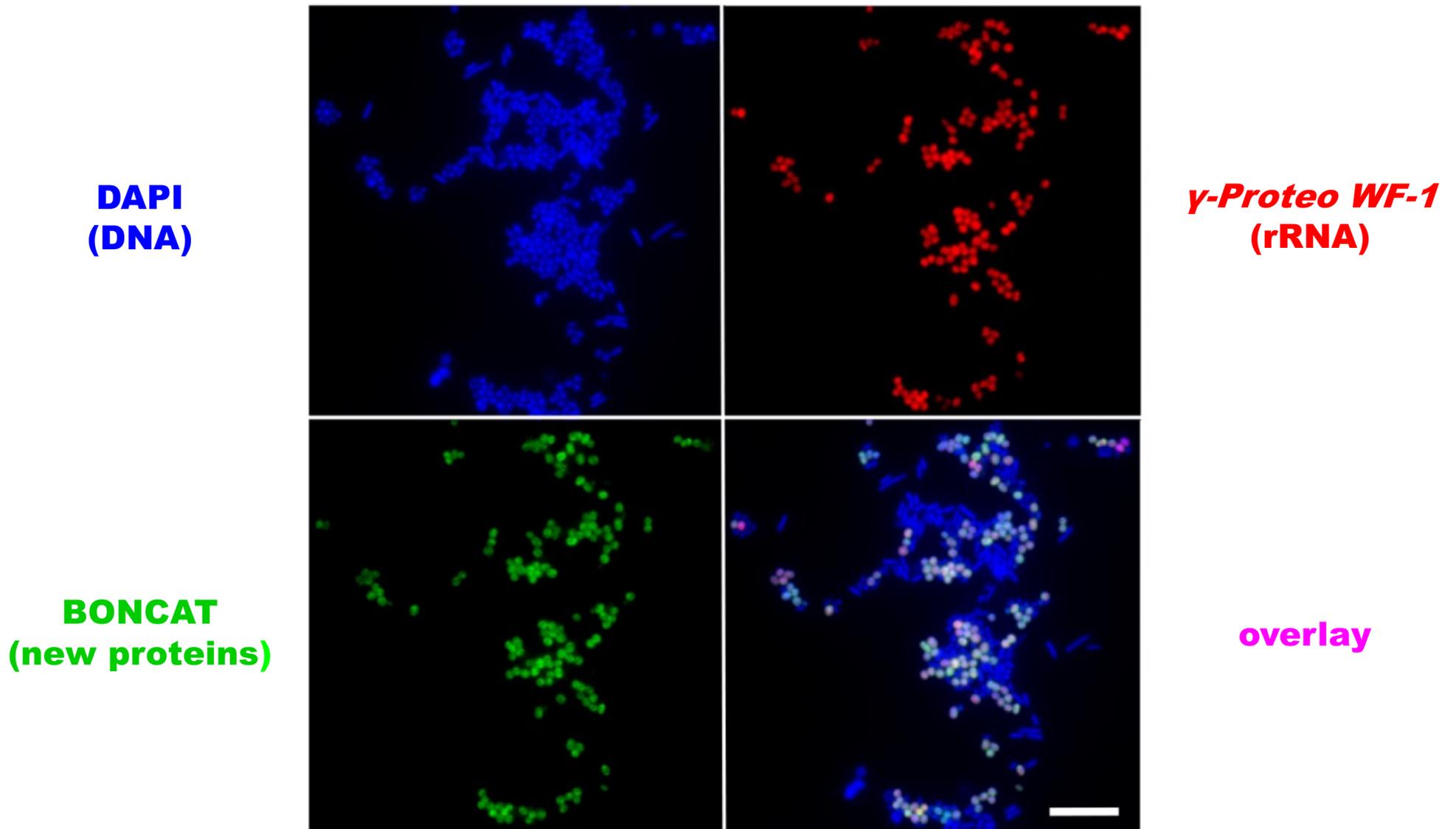


D. alaskensis
sulfate reducer

Visualizing, identifying, and sorting translationally active microbes



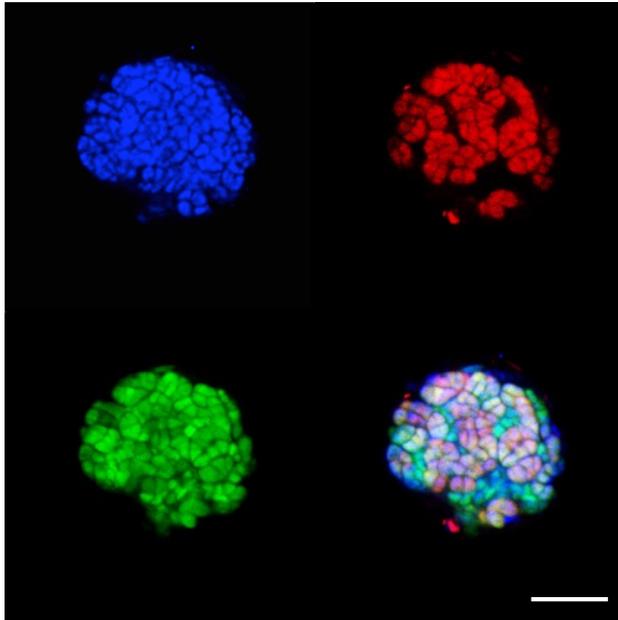
Identification of translationally active cells



Bar = 10 μ m

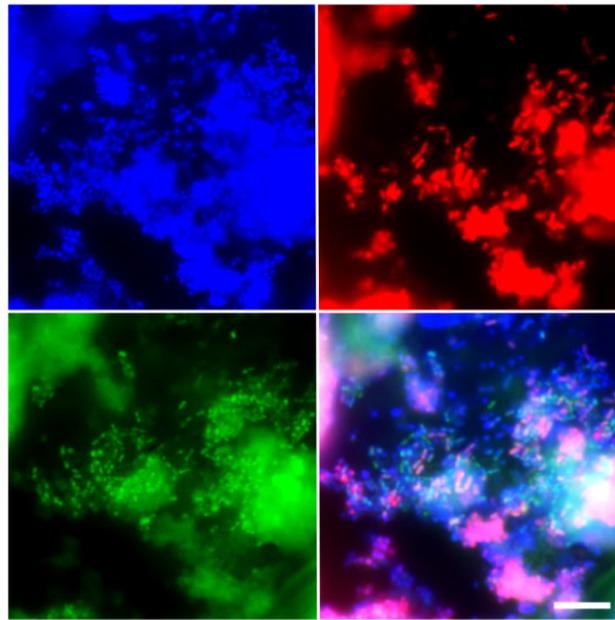
BONCAT-FISH of uncultured microbes

Arch915



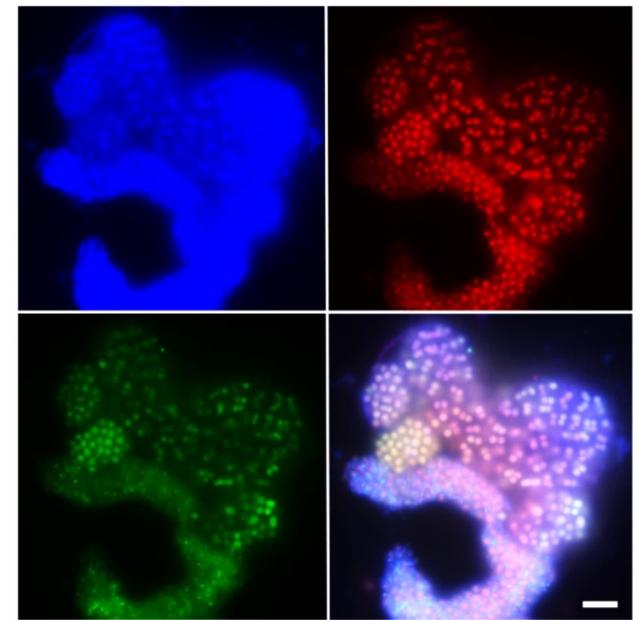
**Methane seep
ANME-SRB consortium**

EUB338 I-III



Tongue biofilm and saliva

Gam42a + competitor



**Freshwater from Lily
pond on Caltech campus**

**DAPI
(DNA)**

**BONCAT
(new proteins)**

**FISH
(rRNA)**

Overlay

Hatzenpichler *et al.*, 2014; Hatzenpichler *et al.*, 2015; Hatzenpichler *et al.*, 2016

Visualizing new proteins *in situ*

**generally applicable
(works for all taxonomies and
physiologies tested so far)**

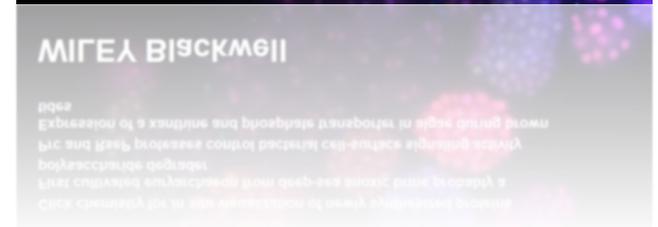
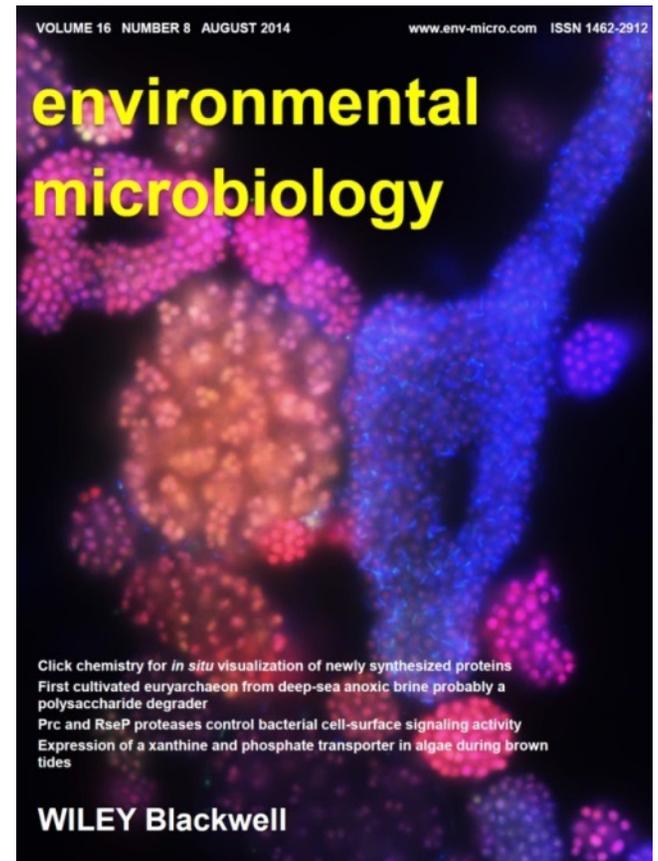
**detectable after 2%
of generation time**

**FISH-BONCAT links function
and identity of a cell**

**BONCAT correlates with
 $^{15}\text{NH}_3$ incorporation (nanoSIMS)**

**no change in protein expression
(Bagert *et al.*, 2014)**

Hatzenpichler lab



Hatzenpichler *et al.*, 2014

Limitations and advantages of BONCAT-FISH

uptake and incorporation

Methionine-rich samples are tough

hard to quantitate amount of new proteins in uncultured cells

potential for cell inactivation or community shifts

links cellular identity and function

fluorescence-based *in situ* activity studies

metabolic screening

activity-based cell-sorting

fast + highly selective + cheap + easily available

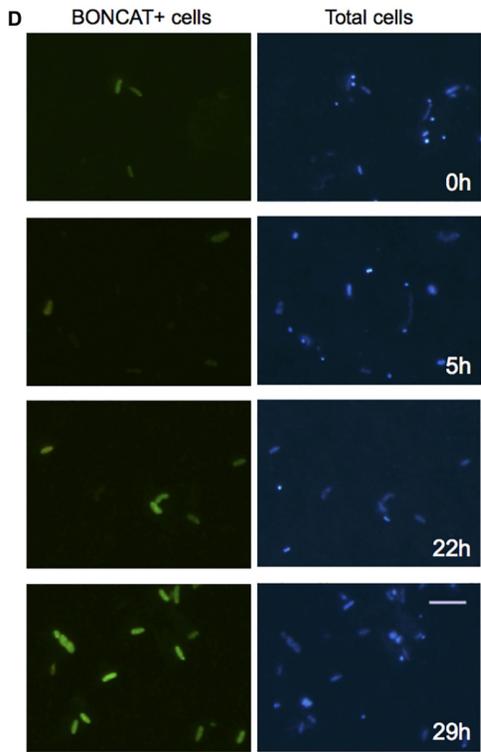
1 h

azide-alkyne

~\$500

epi-scope

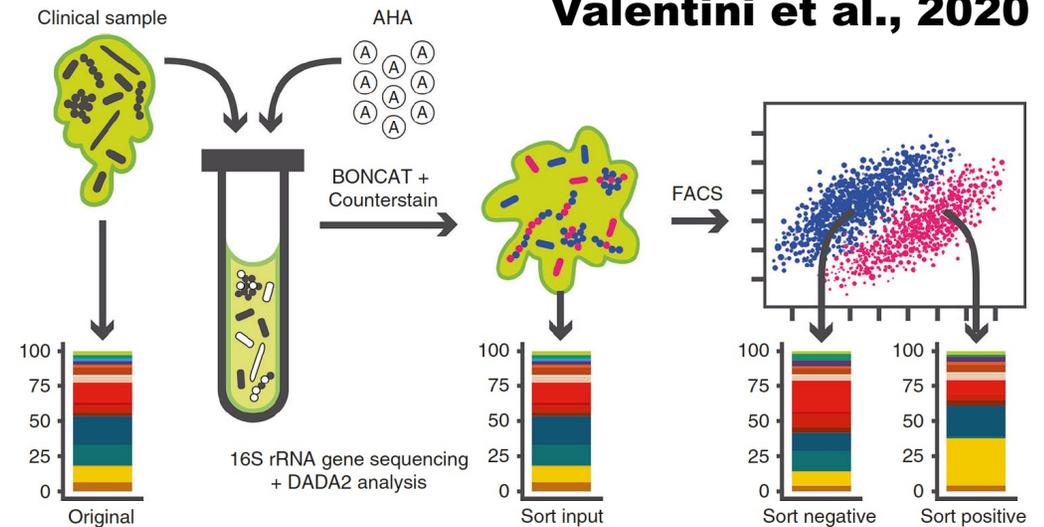
Research examples



Watching cells resuscitate after long-term starvation

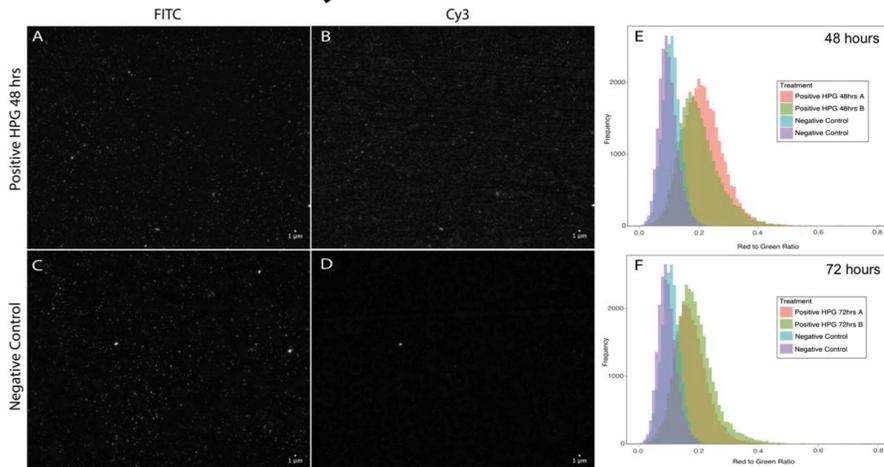
Sebastian et al., 2019

Active lung microbiome of cystic fibrosis patients
Valentini et al., 2020



Studying virus turnover in bacterioplankton

Pasulka et al., 2018



Sorting and identifying the active fraction of cells in soil with BONCAT-FACS

Couradeau et al. 2019

