Bioorthogonal non-canonical amino acid tagging
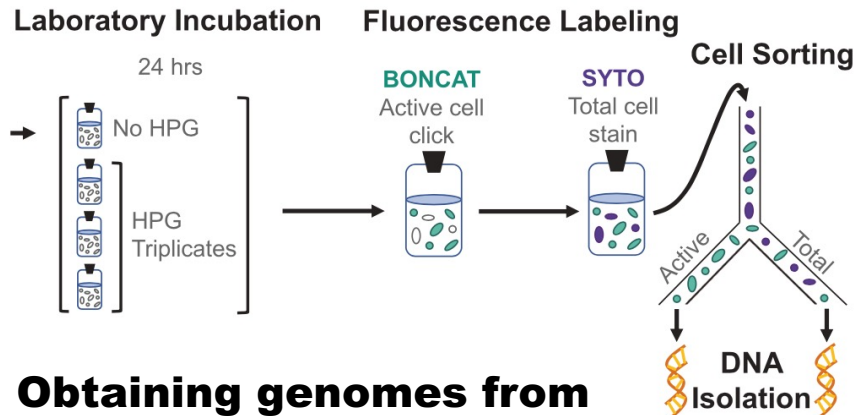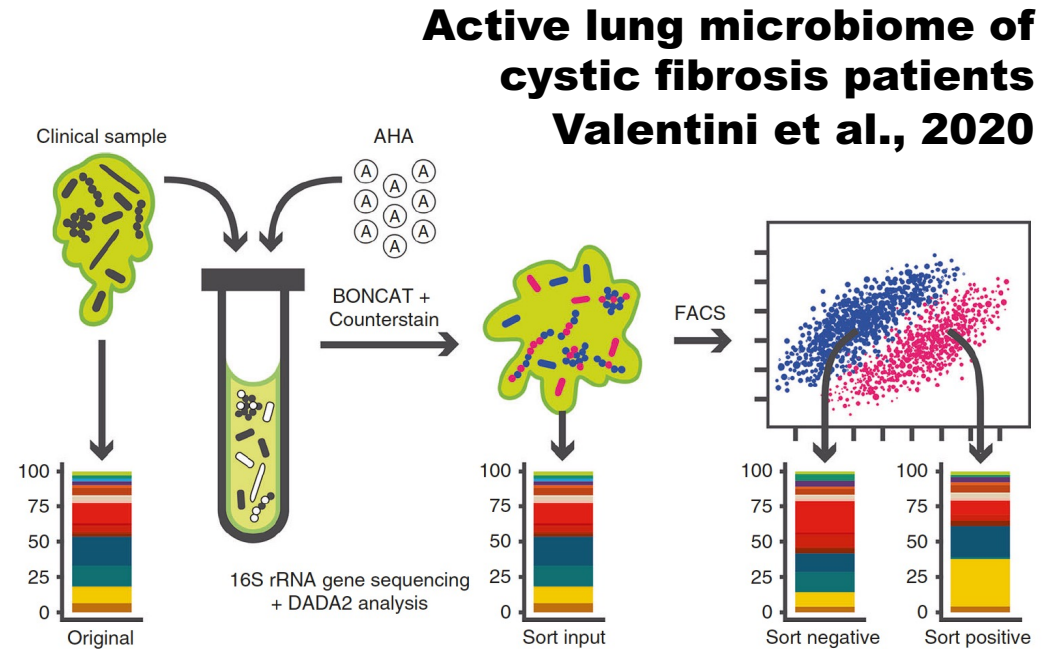# - BONCAT -

Research examples
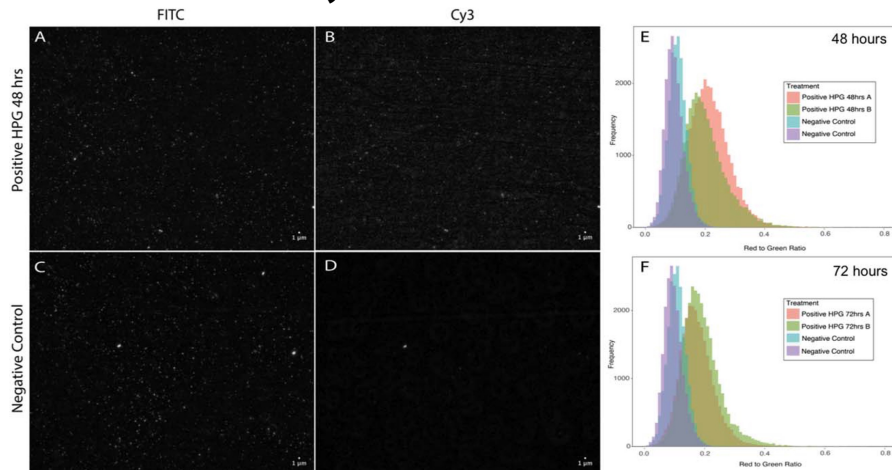

Obtaining genomes from active cells to link phenotype and genotype
McKay & Smith et al., 2021

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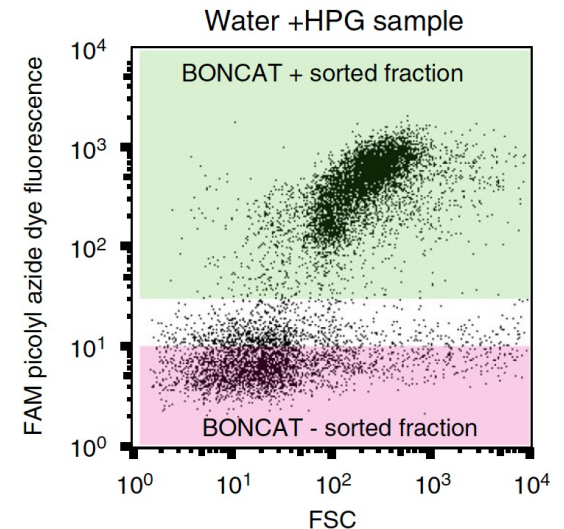**Valentini et al., 2020**

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

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.
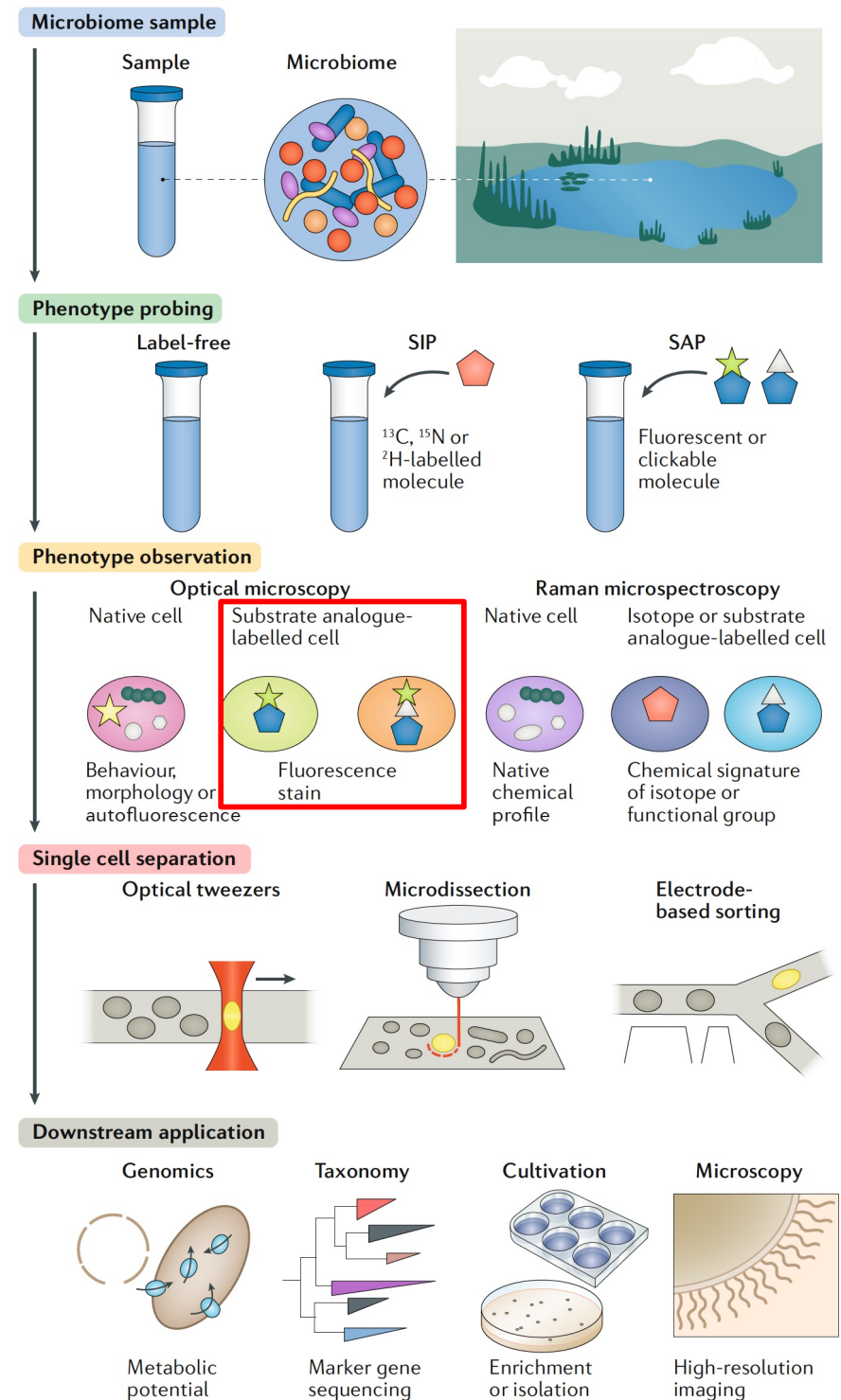
Hatzenpichler et al., 2020

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
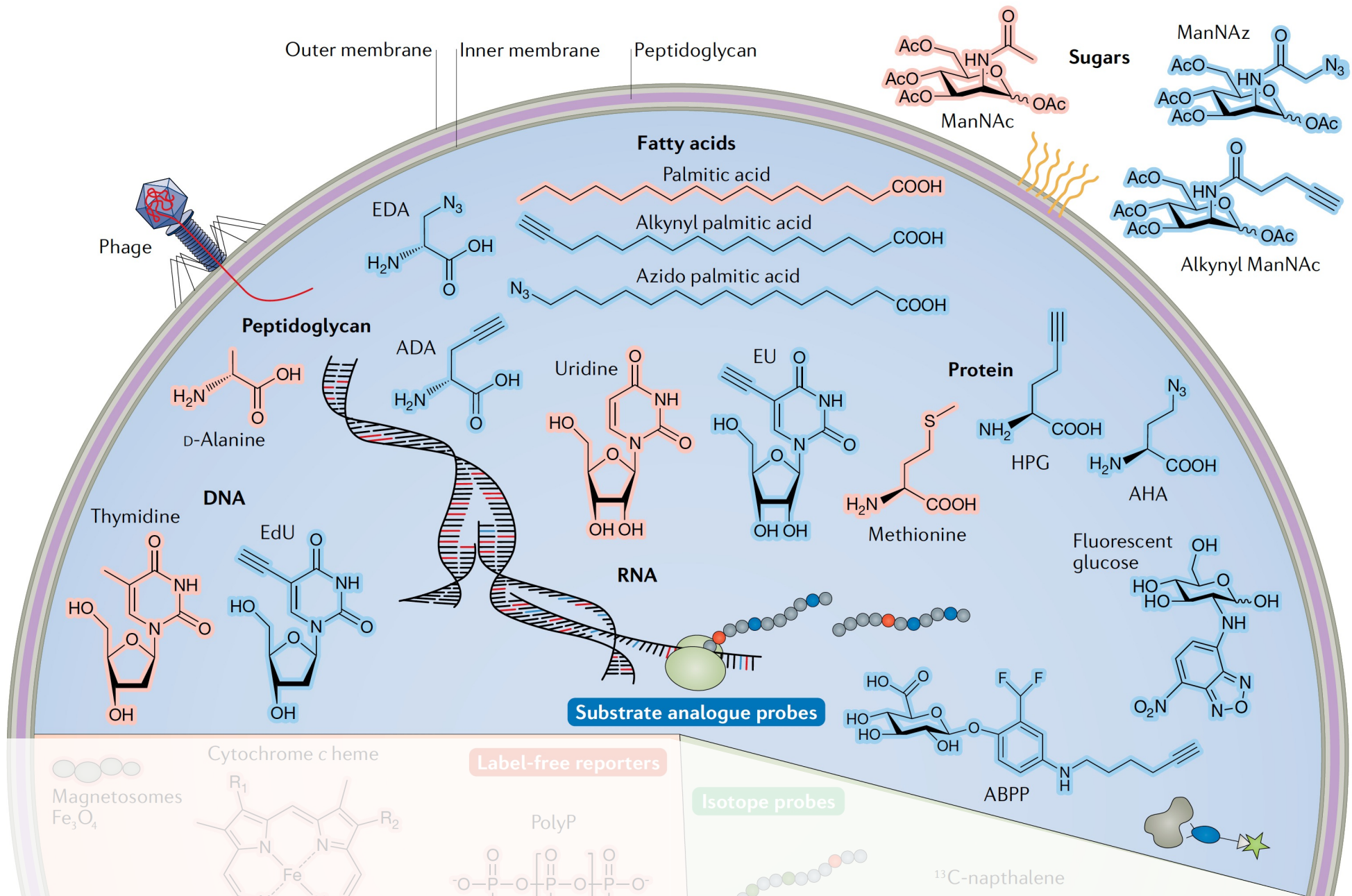
Click chemistry
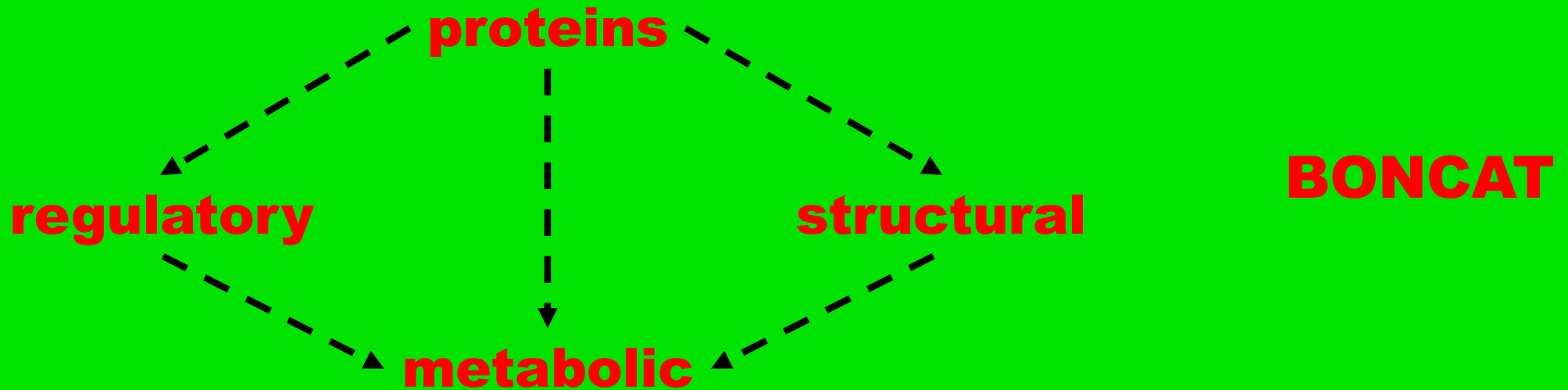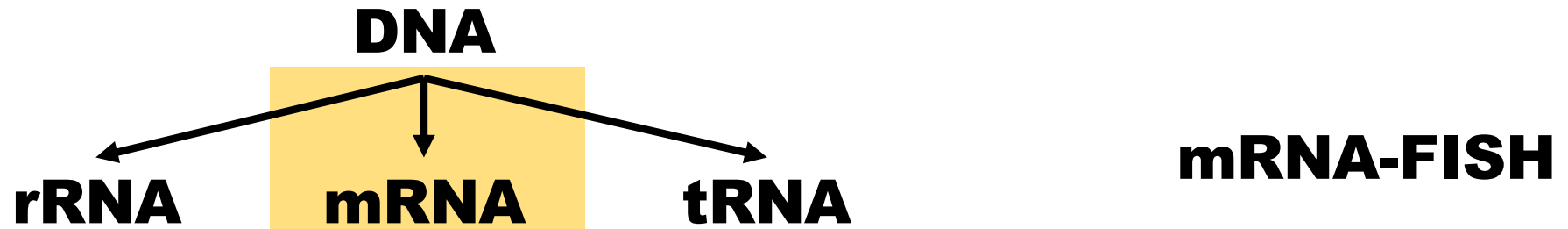**complete conversion of reagents to single product**

+ mild conditions

+ very fast

+ in water

Hatzenpichler lab

Examples for clickable substrate analogs

Hatzenpichler *et al.*, 2020

Limitations and advantages of BONCAT

uptake and incorporation differ between species

Methionine-rich samples are tough (need mM concentrations)

hard to quantitate amount of new proteins in uncultured cells

potential for cell inactivation or community shifts (can be controlled!)

links cellular identity and function (Hatzenpichler et al. 2014)

fluorescence-based *in situ* activity studies (Hatzenpichler et al. 2014)

metabolic screening (Reichart et al. 2020)
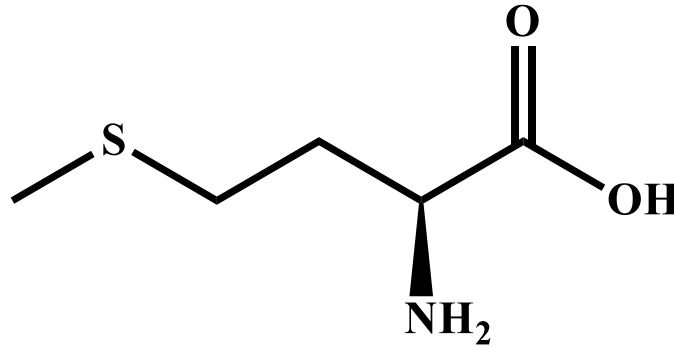
activity-based cell-sorting (Hatzenpichler et al. 2016)
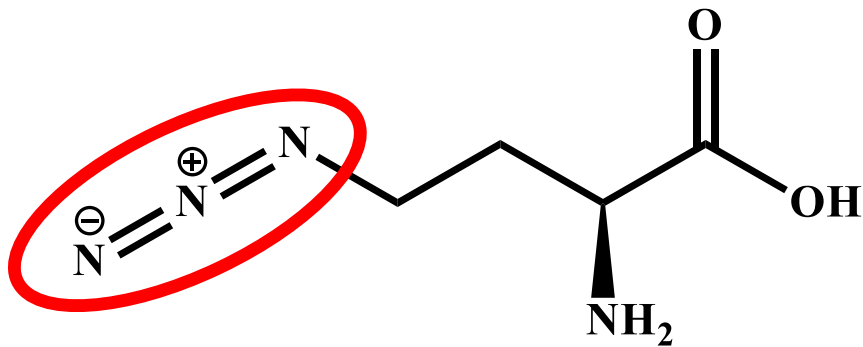
fast + highly selective + cheap + easily available

1 h   azide-alkyne   ~$500   epifluorescence-scope

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Non-canonical amino acids

L-Methionine
Met

L-Azidohomoalanine
AHA
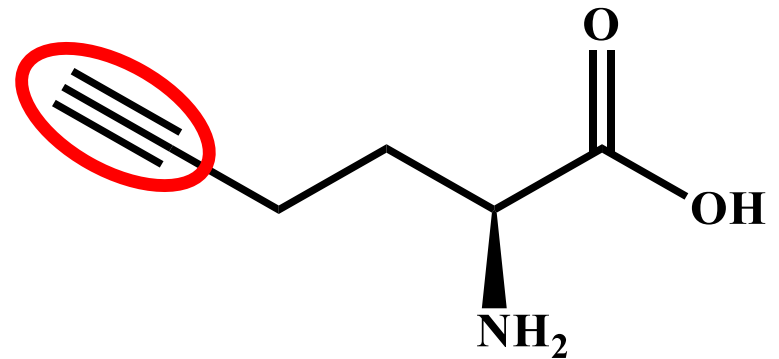
L-Homopropargylglycine
HPG

synthetic amino acids
incorporate into new proteins instead of Met

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Azide-alkyne click chemistry reactions
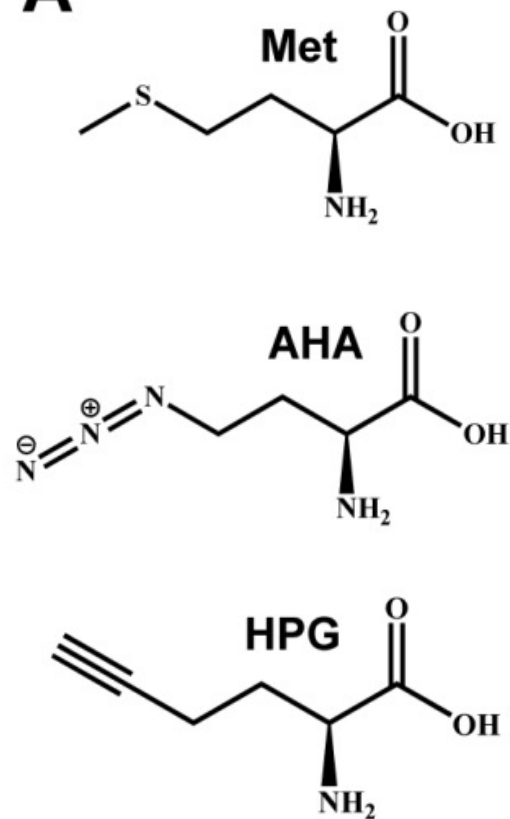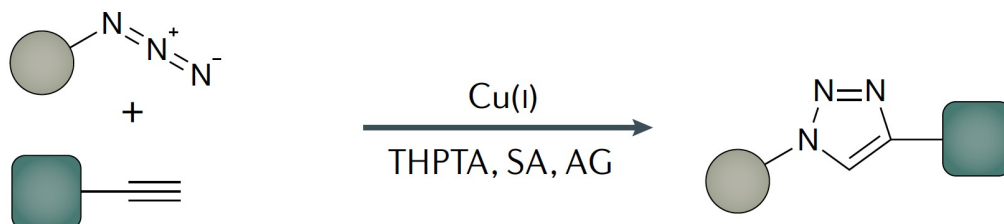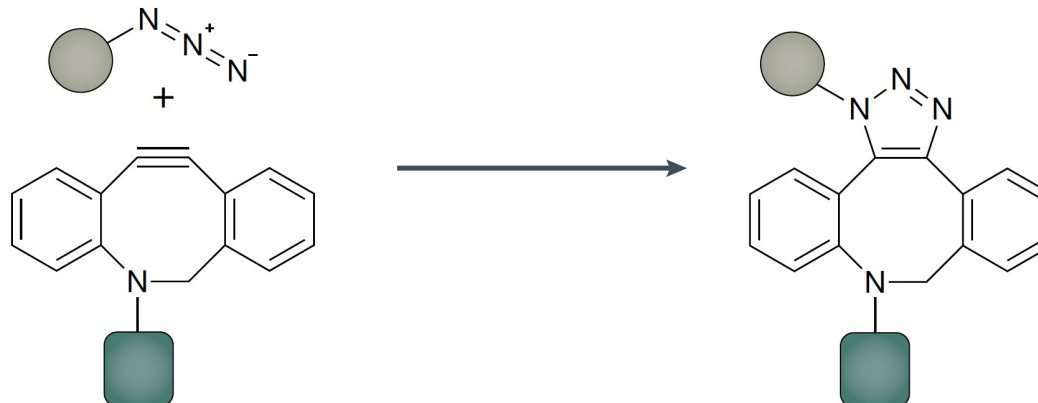
A. Structures of Met and its surrogates AHA and HPG, which compete with Met during translation.

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.

Hatzenpichler lab   Hatzenpichler *et al.*, 2015   Hatzenpichler *et al.*, 2020

Incorporation into newly made proteins
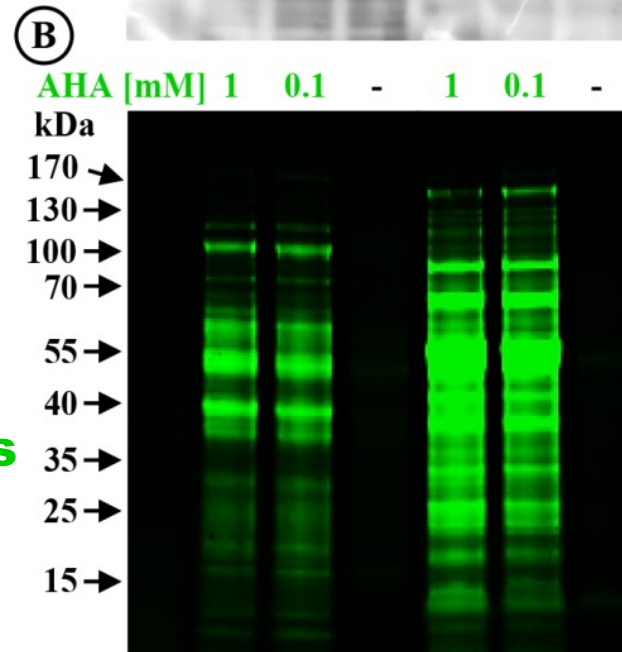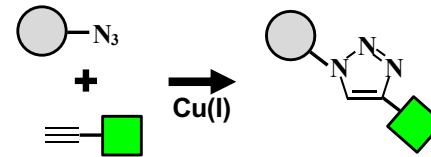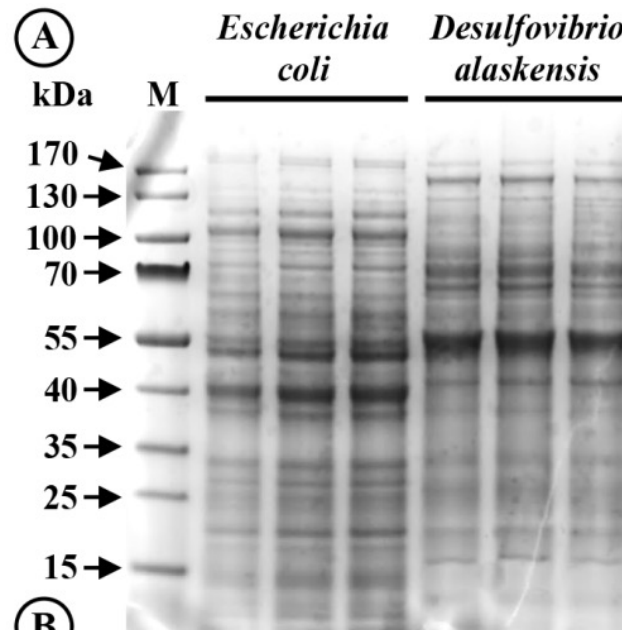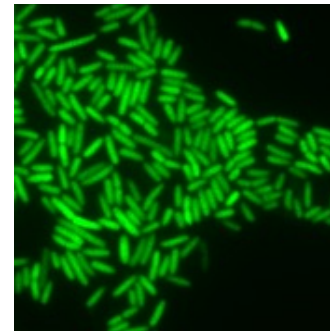
E. coli
respiring glucose
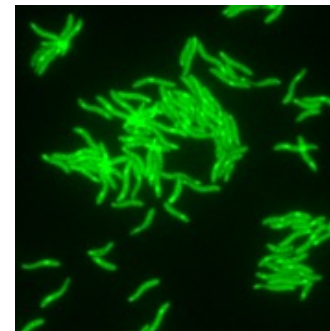
D. alaskensis
sulfate reducer

new proteins

Hatzenpichler lab

Hatzenpichler *et al.*, 2014

Visualizing, identifying, and sorting translationally active microbes

A Incubation with HPG | Uptake into cell | HPG substitutes Met | Detection of HPG-containing proteins | Protein-synthesizing cells are fluorescent

HPG

new protein

old protein

HPG ⟷ Met

Cu(I)-catalyzed click

N₃ dye

new protein

old protein

B BONCAT labeled cells | 16S rRNA-targeted FISH | Identification of active cells

non-target
CGTTCTCGTAGGGCGCCT
...AGAUGGCAAGTGCAUCUCGCGGAUAU...

CGTTCTCGTAGGGCGCCT
...AGAUGGCAAGAGCAUCCCGCGGAUAU...
target rRNA

BONCAT(+) FISH(–)
BONCAT(–) FISH(+)
BONCAT(+) FISH(+)

C BONCAT labeled cells | FACS | Cell lysis & MDA | 16S rRNA gene screen | Genome sequencing, assembly & QC | Draft assemblies

CCTGTCAGTACCGACT
TGTCAGTACCGACTACT
GTCAGTACCGACTACT
GTCAGTACCGACTACTGAATA
GTACCGACTACTGAATATAG
GTACCGACTACTGAATATAGT

Hatzenpichler lab   **Hatzenpichler _et al._, 2016**

Identification of translationally active cells

DAPI
(DNA)

γ-proteo-
bacterium WF-1
(16S rRNA)

BONCAT
(new proteins)

overlay

Bar = 10 μm

Hatzenpichler lab   Hatzenpichler *et al.*, 2014
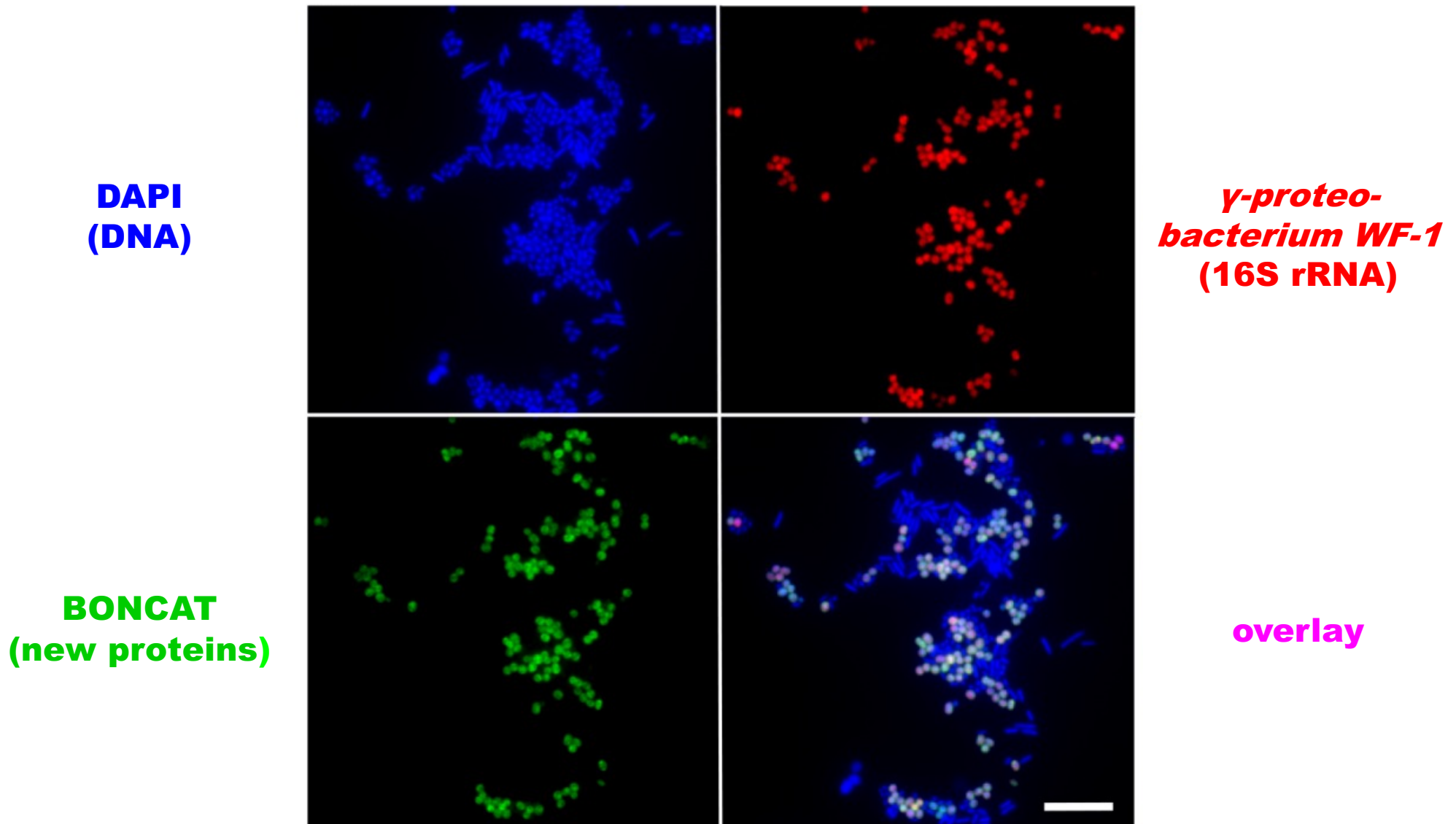
BONCAT-FISH of uncultured microbes

Arch915

EUB338 I-III
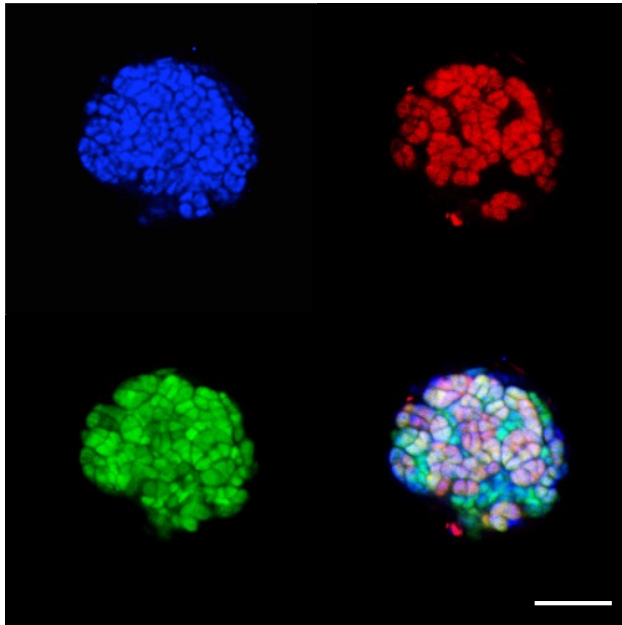
Gam42a + competitor

**Methane seep
ANME-SRB consortium**
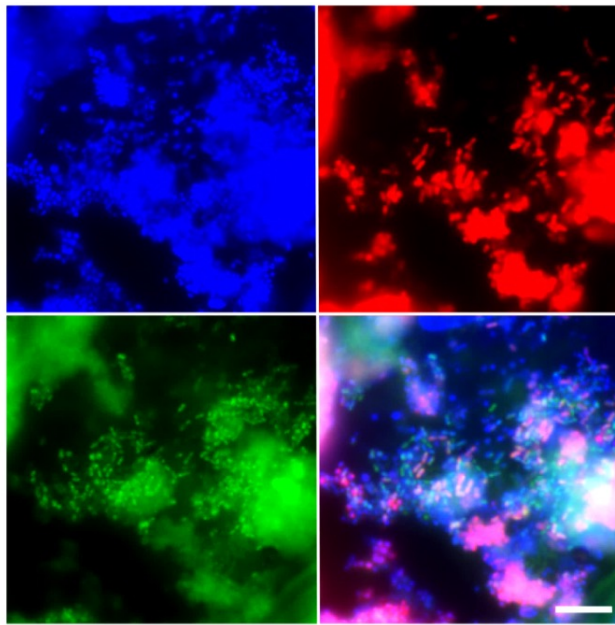
Tongue biofilm and saliva
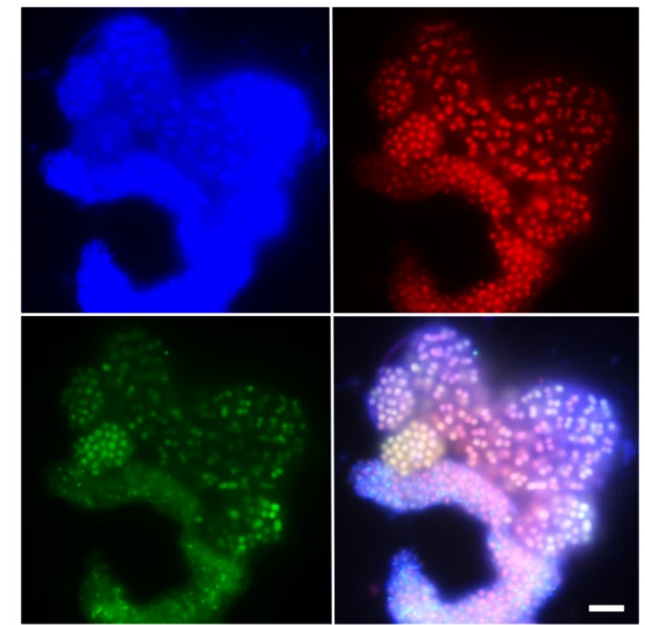
**Freshwater from Lily
pond on Caltech campus**

**DAPI
(DNA)**   **BONCAT
(new proteins)**   **FISH
(rRNA)**   **Overlay**

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

Hatzenpichler lab

Visualizing new proteins *in situ*

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

in *E. coli*, detectable after 2%
of generation time

FISH-BONCAT links function
and identity of a cell

BONCAT correlates with other growth
Proxies (e.g., SIP-nanoSIMS, MAR)

VOLUME 16   NUMBER 8   AUGUST 2014   www.env-micro.com   ISSN 1462-2912

environmental microbiology

Click chemistry for *in situ* visualization of newly synthesized proteins
First cultivated euryarchaeon from deep-sea anoxic brine probably a
polysaccharide degrader
Prc and RseP proteases control bacterial cell-surface signaling activity
Expression of a xanthine and phosphate transporter in algae during brown
tides

WILEY Blackwell

Hatzenpichler *et al.*, 2014

Hatzenpichler lab

BONCAT in microbial ecology, as of April 2022

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)

Kjeldsen et al., On the evolution and physiology of cable bacteria
Proc Natl Acad Sci USA, 116 (38) 19116-19125 (2019)
➢ Uses BONCAT to study the activity of individual cells along cable bacteria filaments

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BONCAT in microbial ecology, as of April 2022

Steward et al. Metabolic Implications of Using BioOrthogonal Non-Canonical Amino Acid Tagging (BONCAT) for Tracking Protein Synthesis
Front Microbiol, 11:197 (2020)
➤ shows that growing E. coli with AHA or HPG alters ~15 % of global mass features (LC-MS). 7 % change if cells are grown on methionine.

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 (2000)

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)

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BONCAT in microbial ecology, as of April 2022

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

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)

Du and Behrens. **Tracking de novo protein synthesis in the activated sludge microbiome using BONCAT-FACS**
Water Research, 205: 117696 (2021)

McKay, Smith et al. **Activity-based, genome-resolved metagenomics uncovers key populations and pathways involved in subsurface conversions of coal to methane**
The ISME J, https://doi.org/10.1038/s41396-021-01139-x (2021)
➢ First study to combine BONCAT-FACS with metagenomics of sorted cells, thus directly linking phenotype and genotype of uncultured cells.

Madill et al., **Activity-Based Cell Sorting Reveals Resistance of Functionally Degenerate *Nitrospira* during a Press Disturbance in Nitrifying Activated Sludge**
mSystems, https://doi.org/10.1128/mSystems.00712-21 (2021)

Taguer et al., **Changes in Gut Bacterial Translation Occur before Symptom Onset and Dysbiosis in Dextran Sodium Sulfate-Induced Murine Colitis**
mSystems, 6: e00507-21 (2021)

Schweitzer et al., **Subsurface hydrocarbon degradation strategies in low- and high-sulfate coal seam communities identified with activity-based metagenomics**
Biofilms and Microbiomes, 8:7 https://doi.org/10.1038/s41522-022-00267-2 (2022)

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