Bioorthogonal non-canonical amino acid tagging

- BONCAT -

 doi: 10.13140/RG.2.1.3698.7040/1

BONCAT in environmental microbiology (as of February 2020)

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 Aquat 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 Aquat 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 Aquat Microbiol, 10: 760 (2019)

Steward et al. Metabolic Implications of Using BioOrthogonal Non-Canonical Amino Acid Tagging (BONCAT) for Tracking Protein Synthesis
Front Micriol, 11:197 (2020)

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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

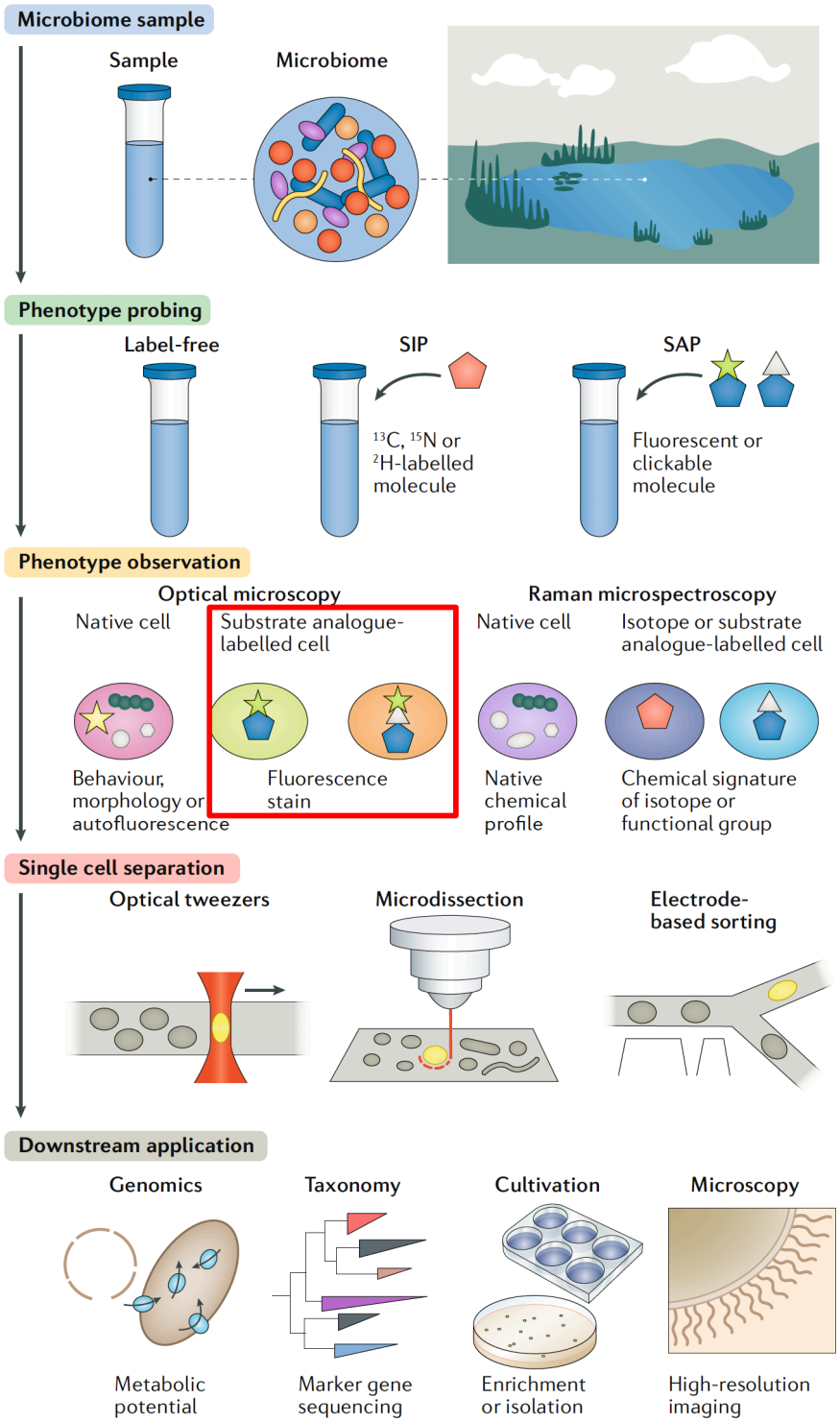


Fig. 2 | **Next-generation physiology workflow to study microorganisms.**

First, some definitions

bioorthogonal

non-interacting with cellular functionalities

non-canonical

synthetic, not part of biological machinery

Click chemistry

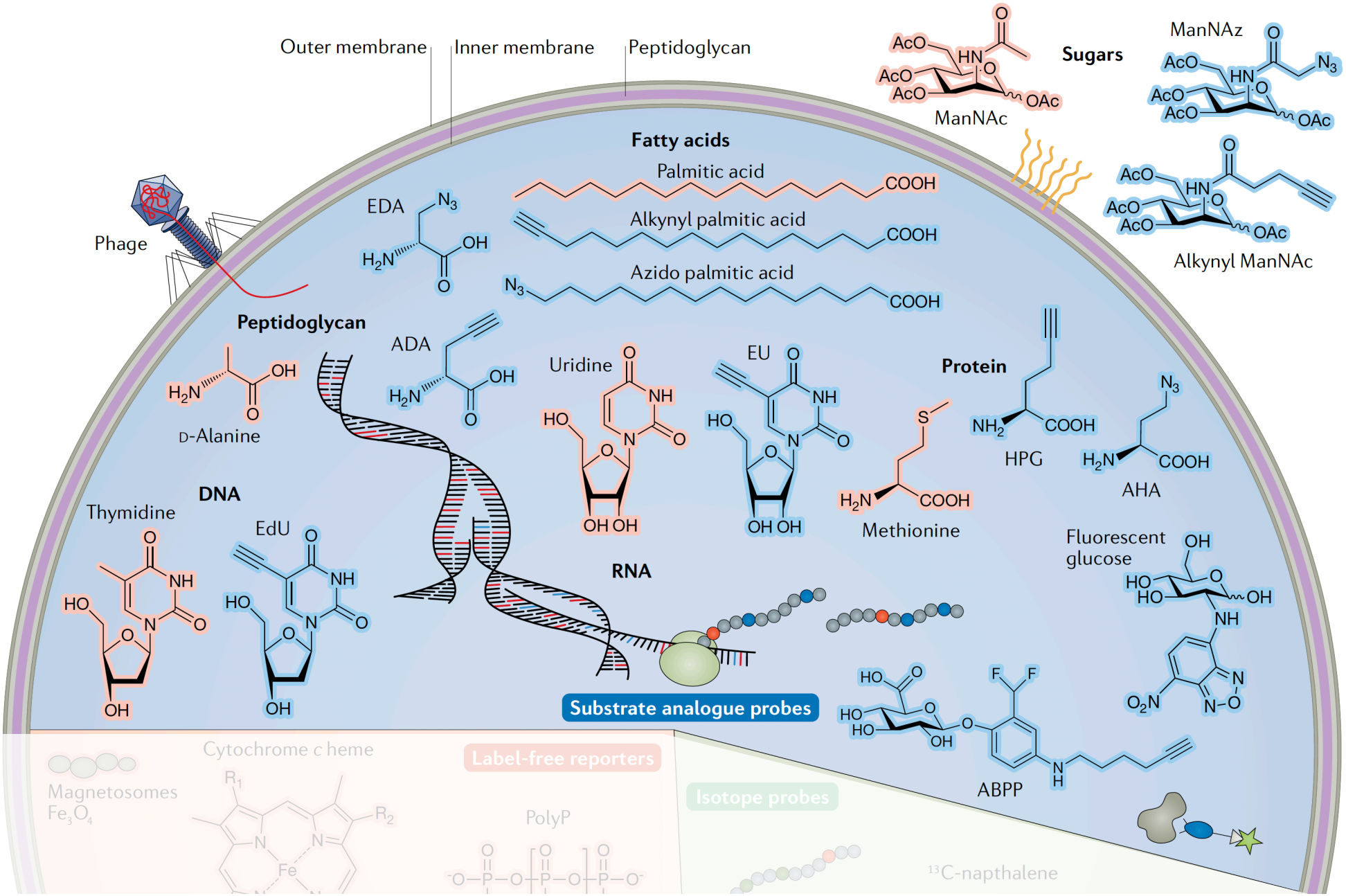
complete conversion of reagents to single product

+ mild conditions

+ very fast

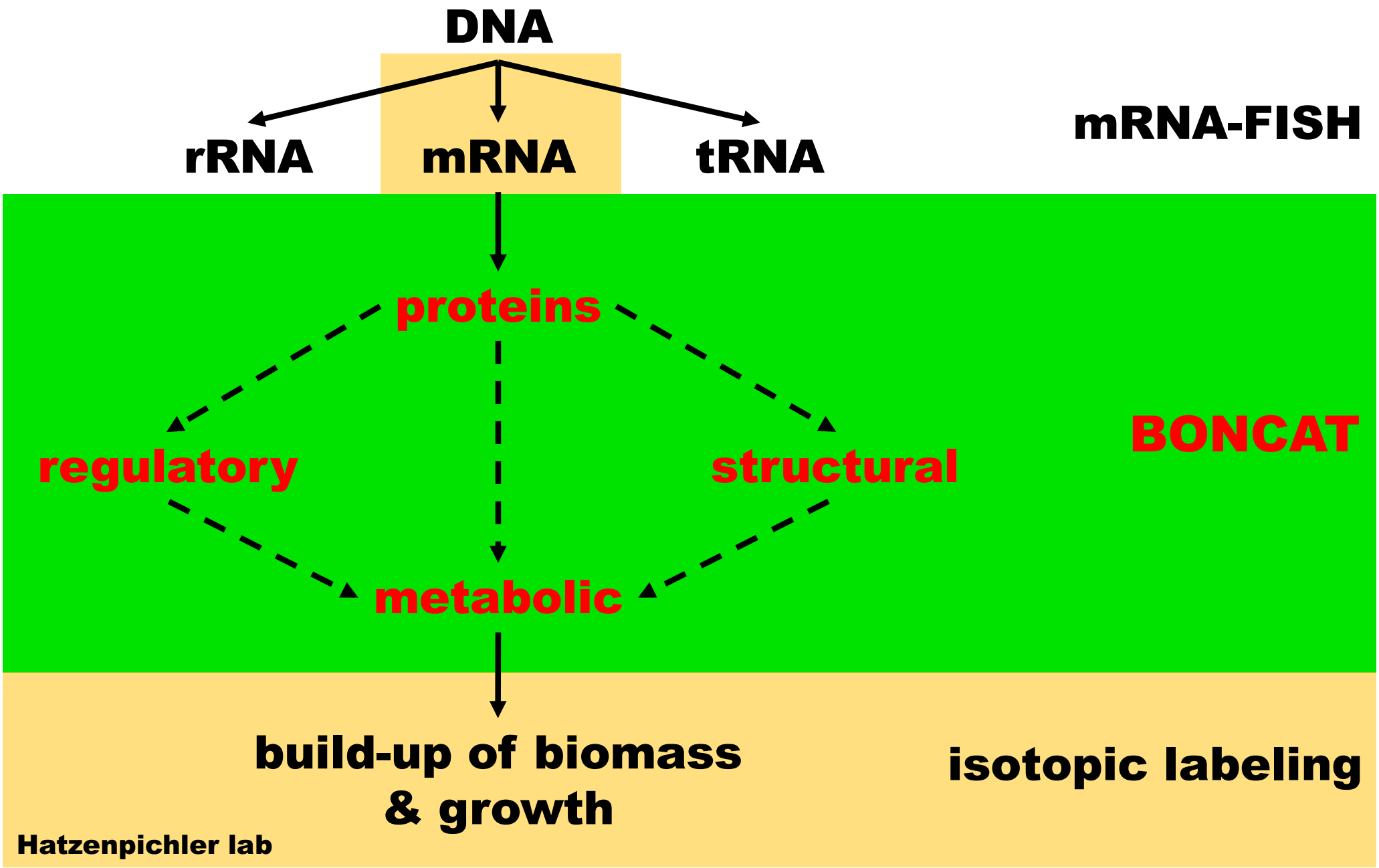
+ in water

Examples for clickable substrate analogs

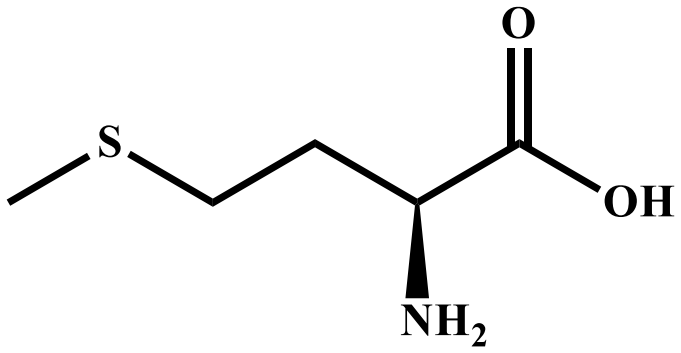


Hatzenpichler *et al.*, 2020

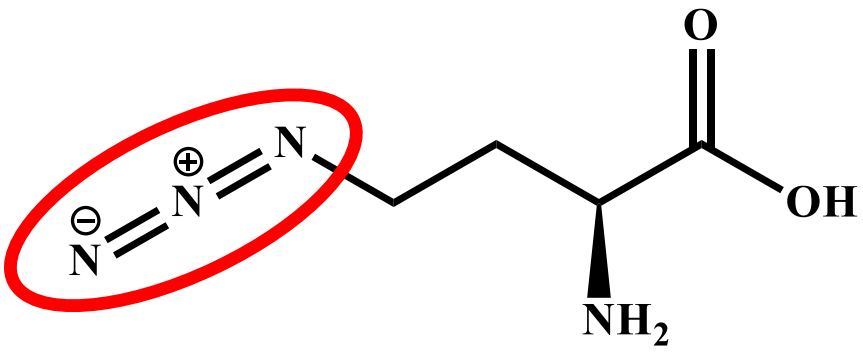
Activity assays on individual cell level



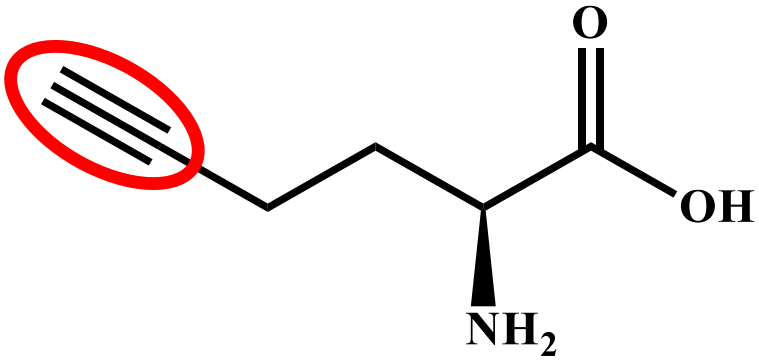
Bioorthogonal non-canonical amino acids



***L*-Methionine**
Met



***L*-Azidohomoalanine**
AHA



***L*-Homopropargylglycine**
HPG

synthetic amino acids
incorporate into new proteins instead of Met

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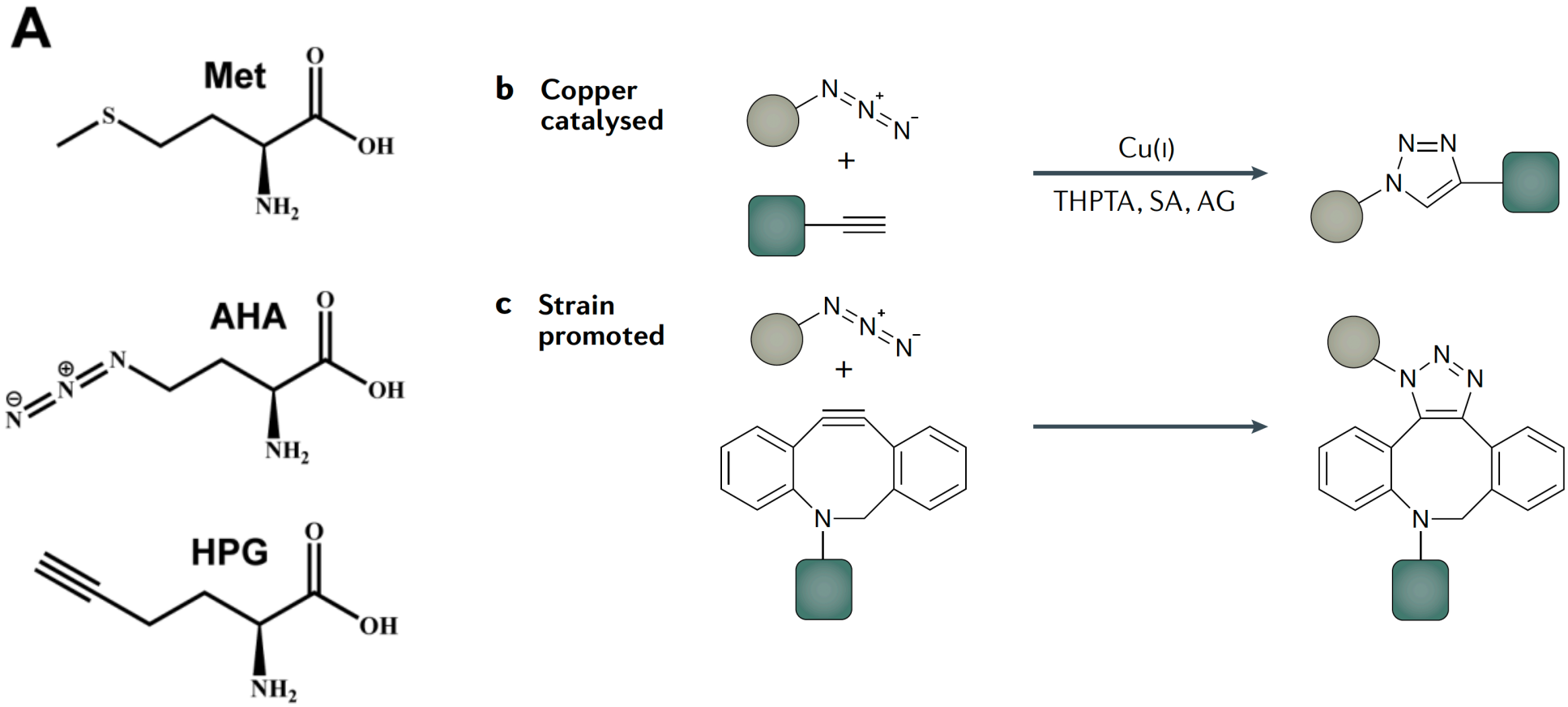
Azide-alkyne click chemistry reactions



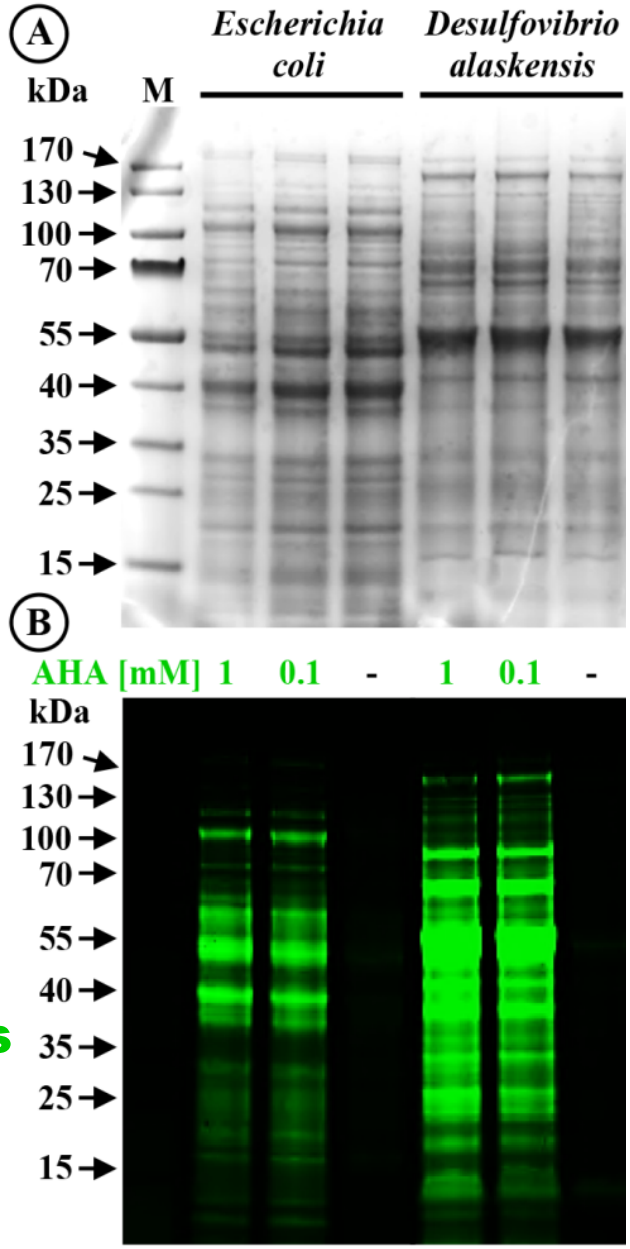
A. Structures of Met and its surrogates AHA and HPG, which compete with Met during translation.

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

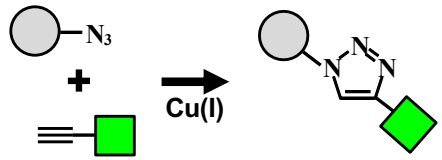
C. Strain-promoted click chemistry allows the copper-less conjugation of an azide group (N_3) with a cyclo-octyne-carrying molecule, yielding a triazole conjugate.

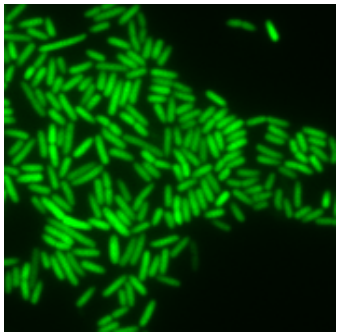
Hatzenpichler lab **Hatzenpichler *et al.*, 2015** **Hatzenpichler *et al.*, 2020**

Incorporation into newly made proteins

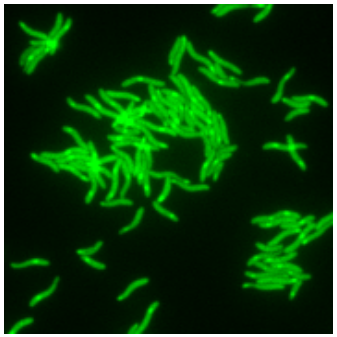


new proteins





E. coli
respiring glucose



D. alaskensis
sulfate reducer

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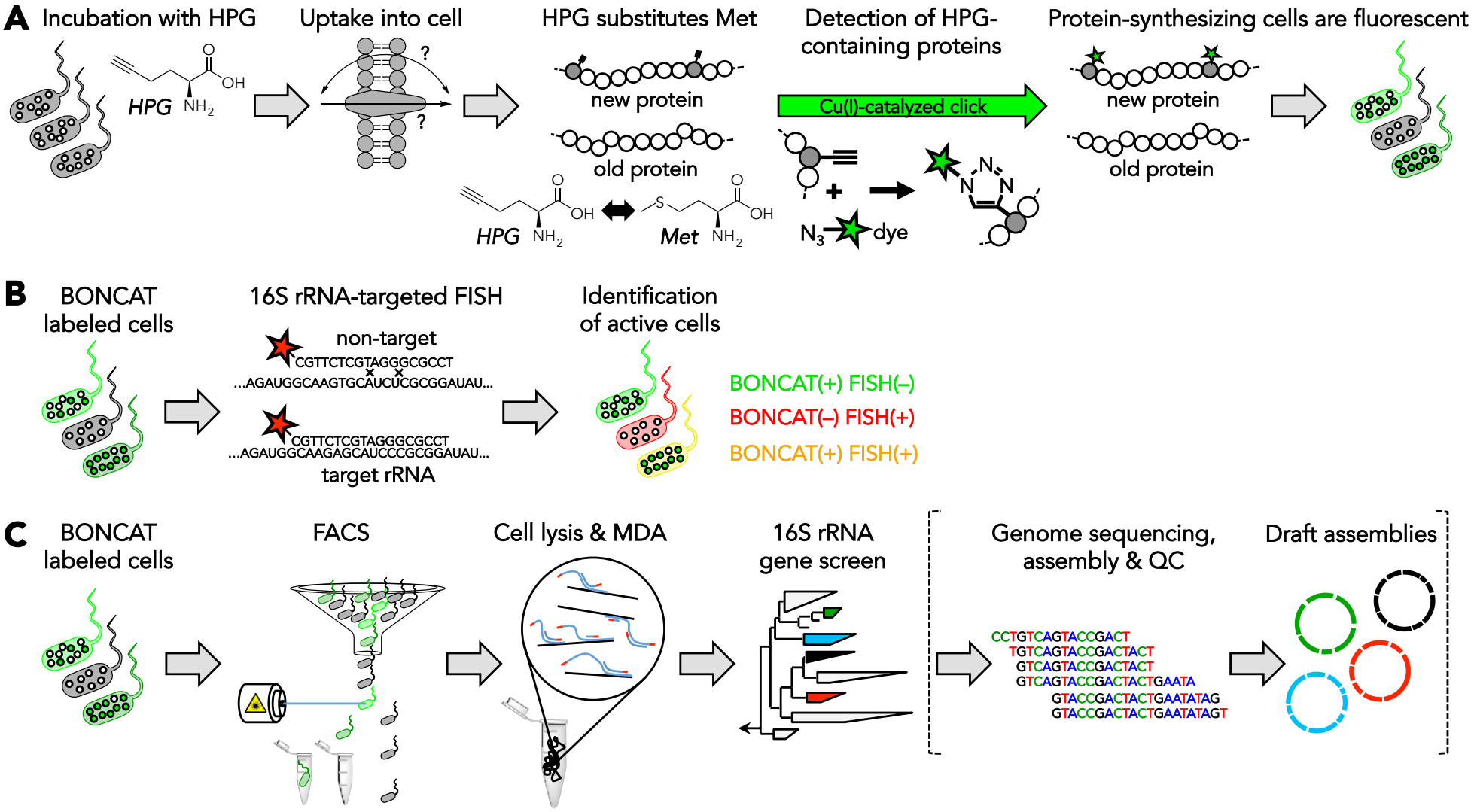
Hatzenpichler *et al.*, 2014

Visualizing, identifying, and sorting translationally active microbes



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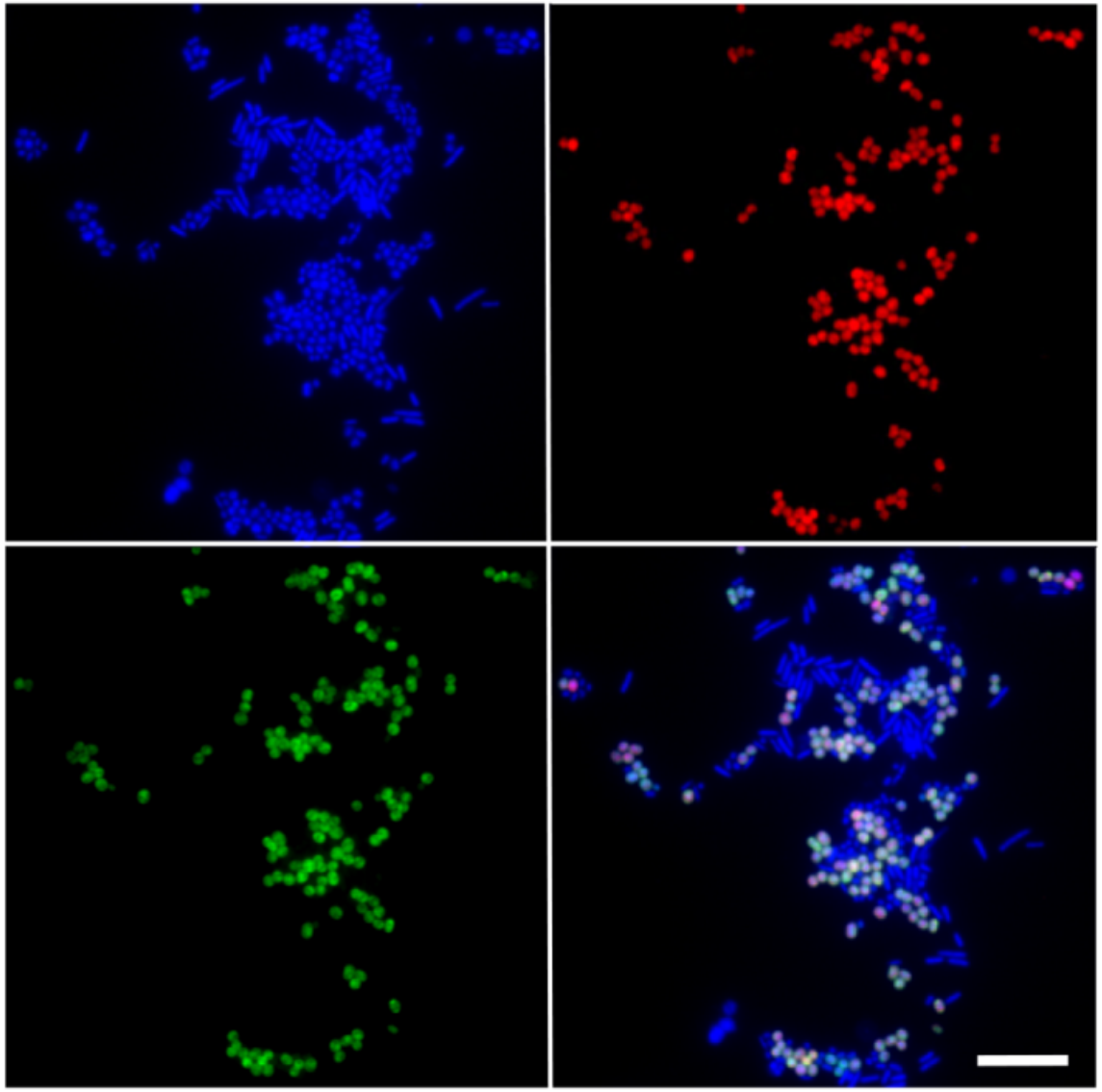
Hatzenpichler *et al.*, 2016

Identification of translationally active cells

DAPI
(DNA)



γ-Proteo WF-1
(rRNA)

BONCAT
(new proteins)

overlay

Bar = 10 µm

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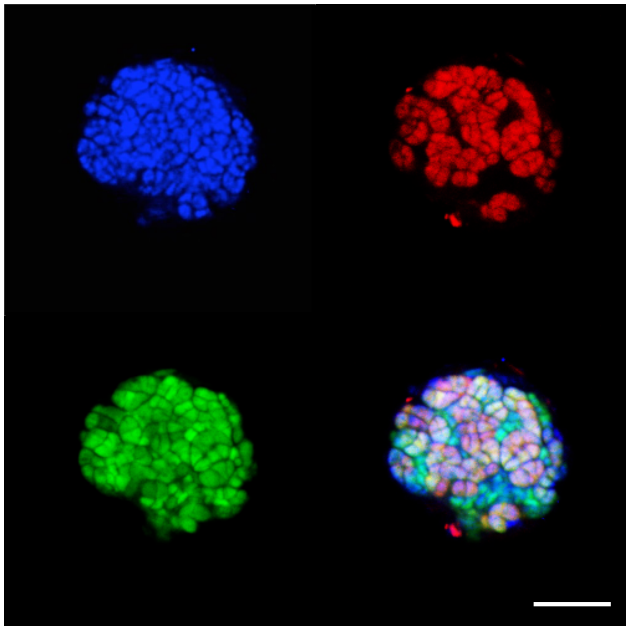
Hatzenpichler *et al.*, 2014

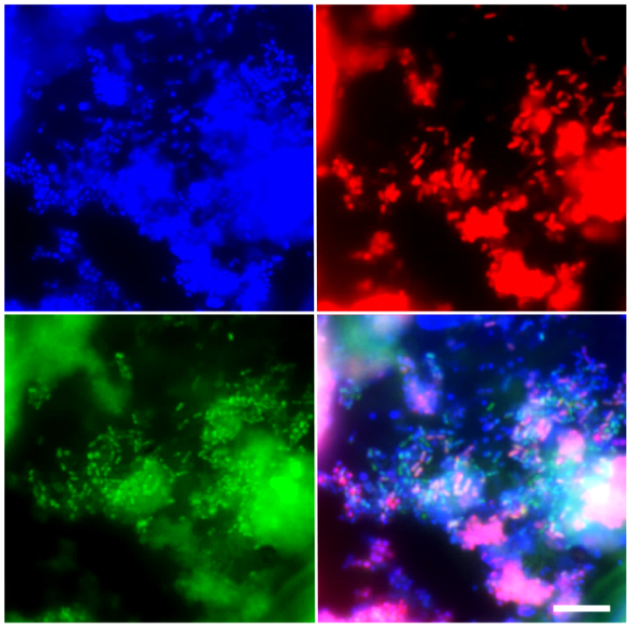
BONCAT-FISH of uncultured microbes

Arch915



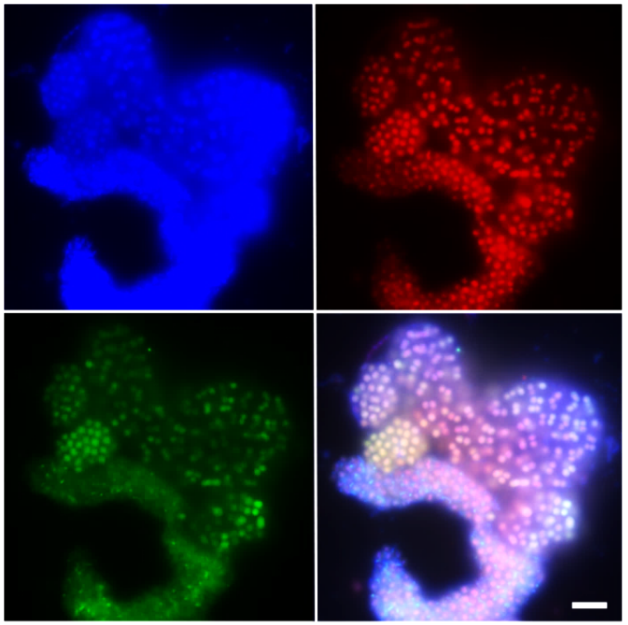
Methane seep
ANME-SRB consortium

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

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Visualizing new proteins *in situ*

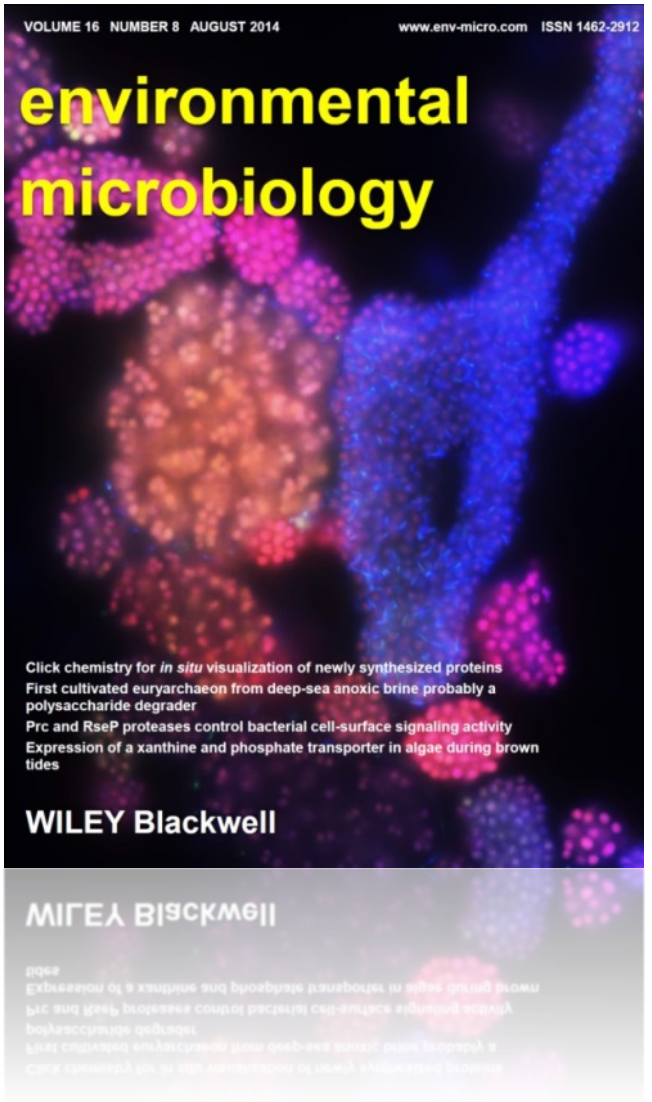
generally applicable
(works for all taxonomies and physiologies tested so far)

detectable after 2%
of generation time

FISH-BONCAT links function and identity of a cell

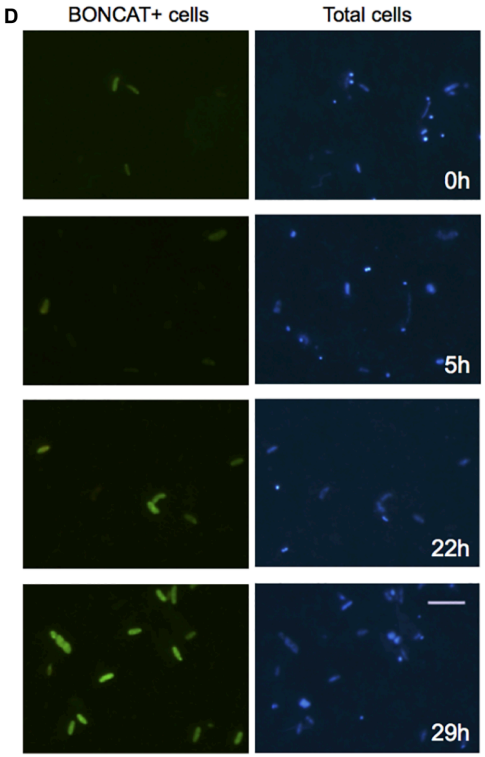
BONCAT correlates with $^{15}NH_3$ incorporation (nanoSIMS)

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



Hatzenpichler *et al.*, 2014

Research examples

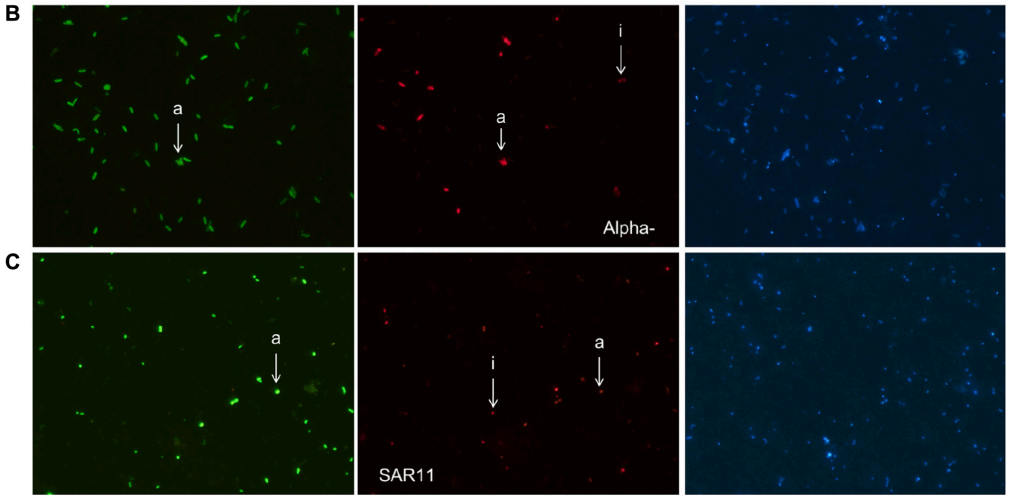


Watching cells resuscitate after long-term starvation

Sebastian et al., 2019

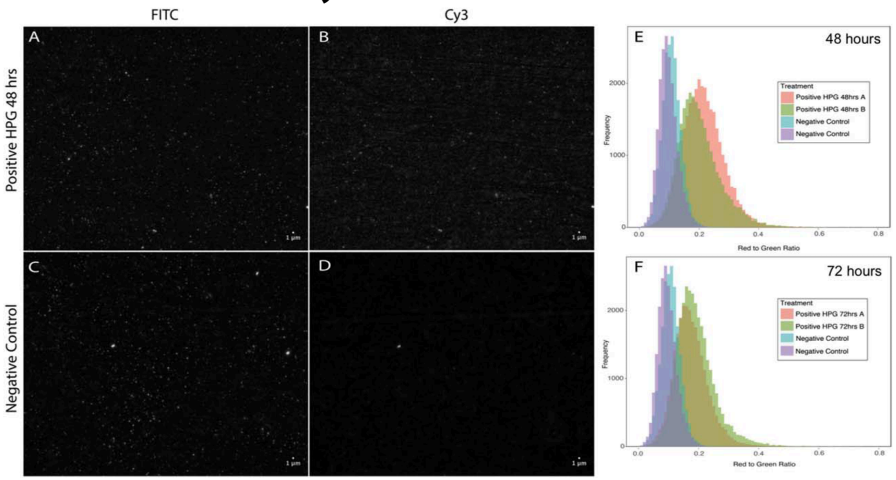
Detecting activity of environmentally relevant bacteria in ocean water

Leizeaga et al., 2017



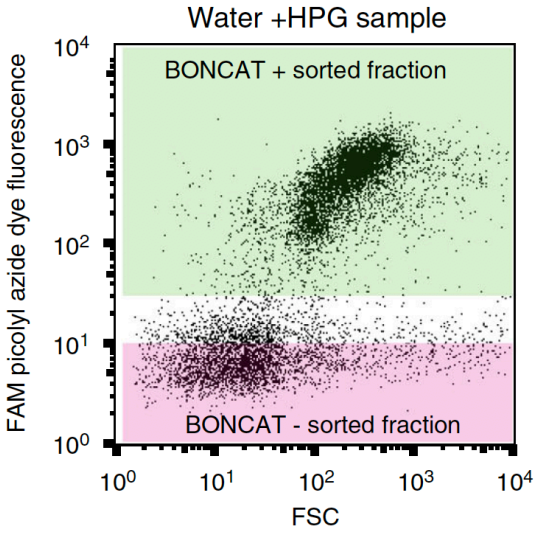
Studying virus turnover in bacterioplankton

Pasulka et al., 2018



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

Couradeau et al. 2019



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