Bioorthogonal non-canonical amino acid tagging

- BONCAT -

 doi: 10.13140/RG.2.1.3698.7040/1

BONCAT in microbial ecology, as of April 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 April 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 (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).

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

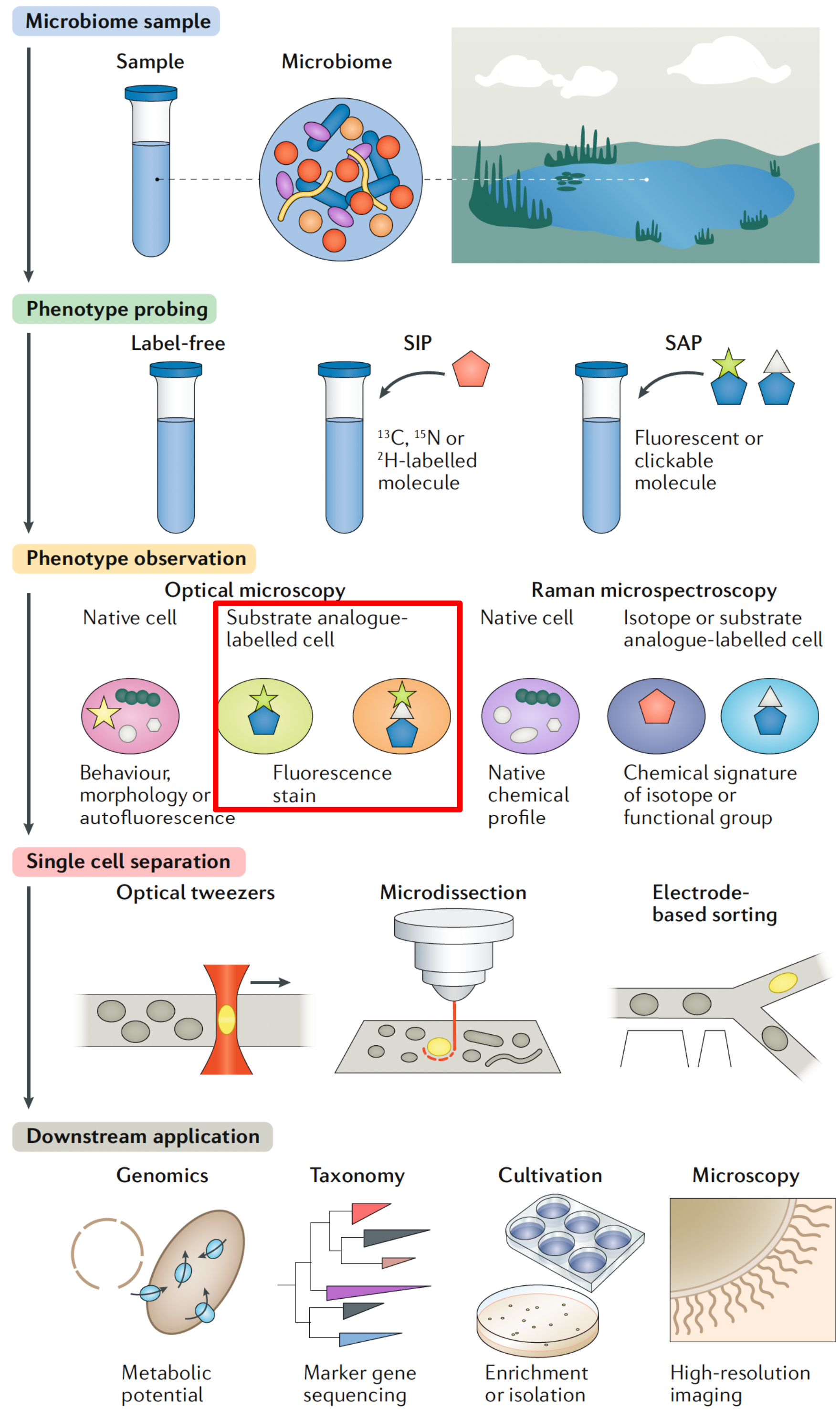


Fig. 2 | **Next-generation physiology workflow to study microorganisms.**

First, some definitions

bioorthogonal

non-interacting with cellular functionalities

non-canonical

synthetic, not part of biological machinery

Click chemistry

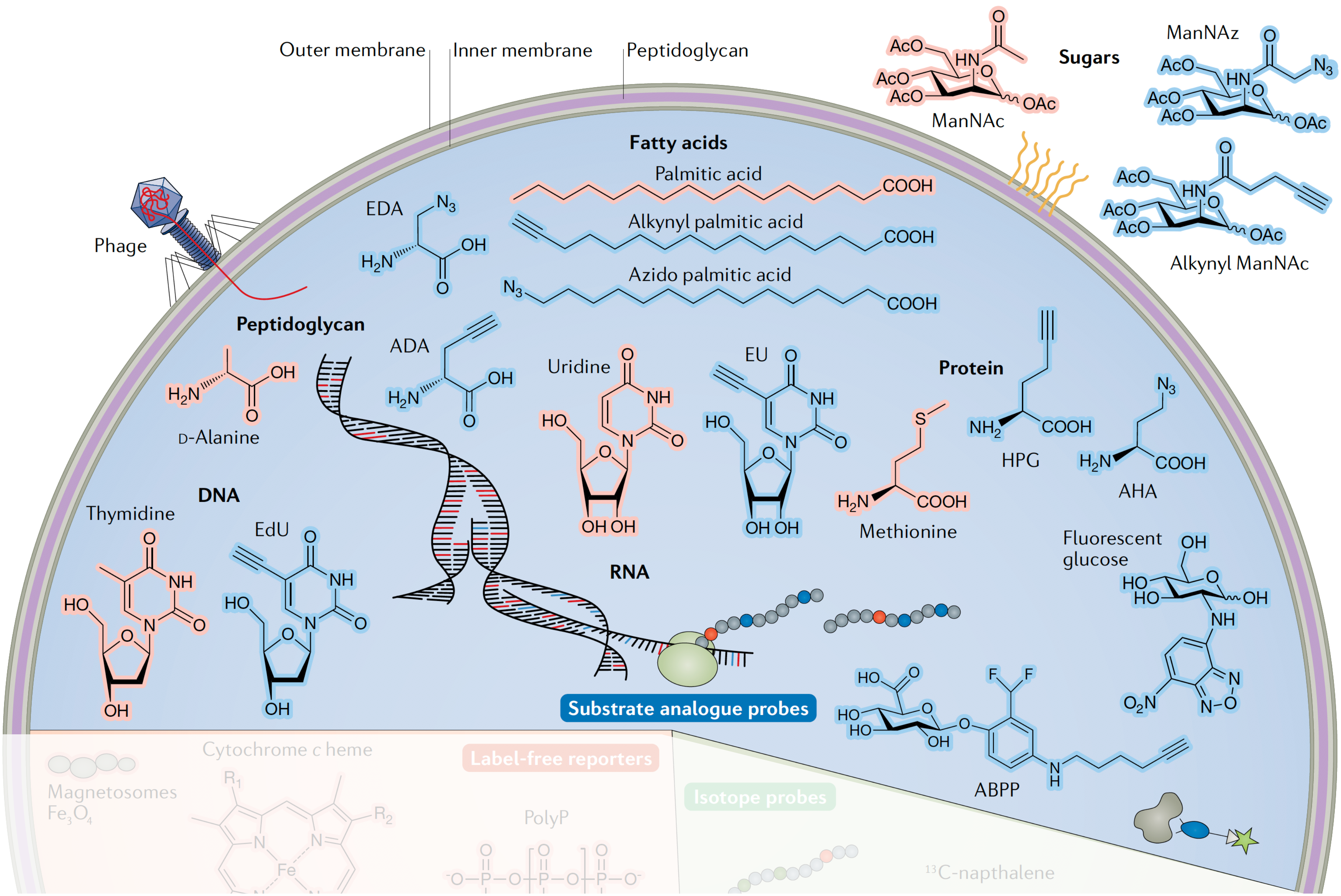
complete conversion of reagents to single product

+ mild conditions

+ very fast

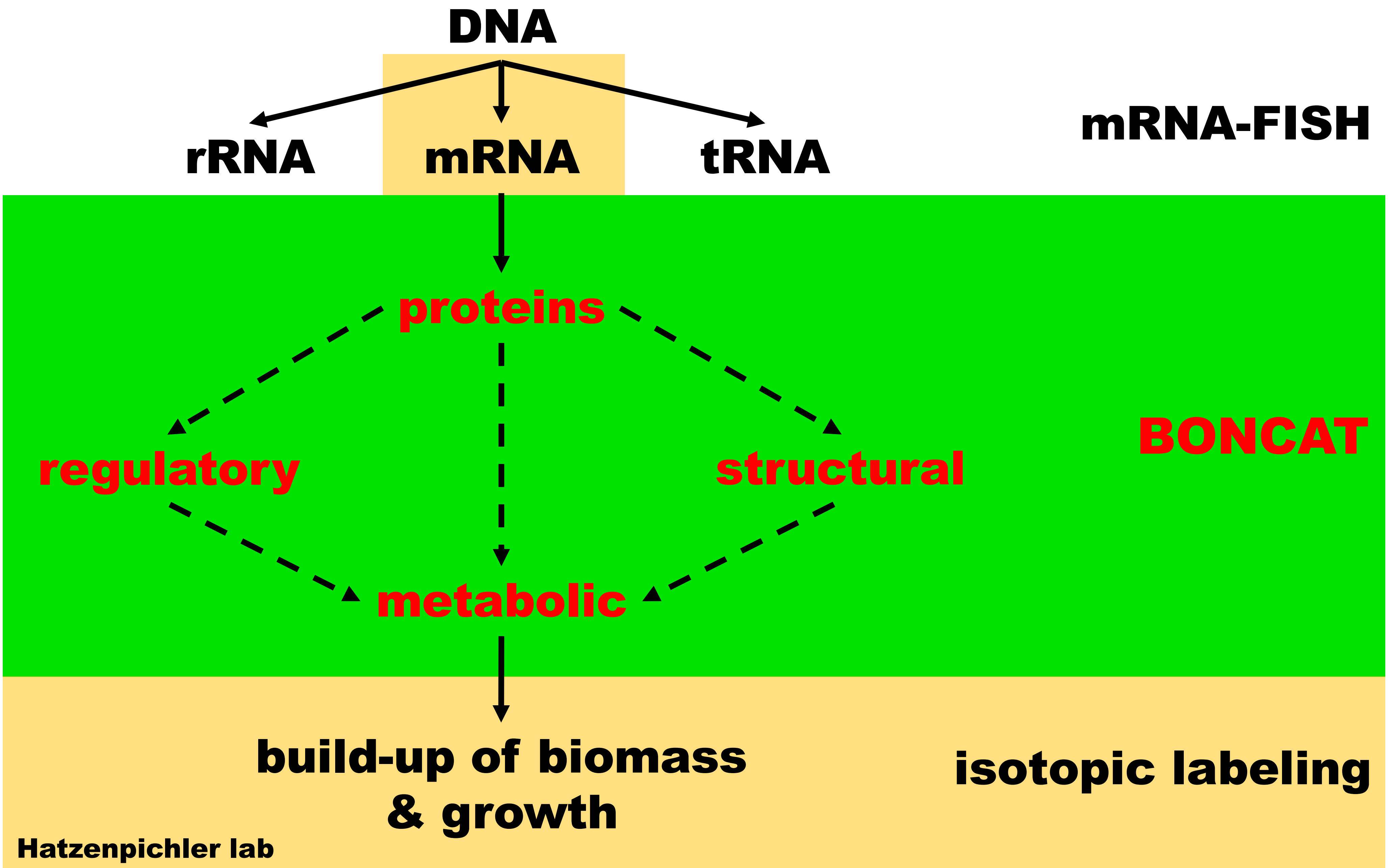
+ in water

Examples for clickable substrate analogs

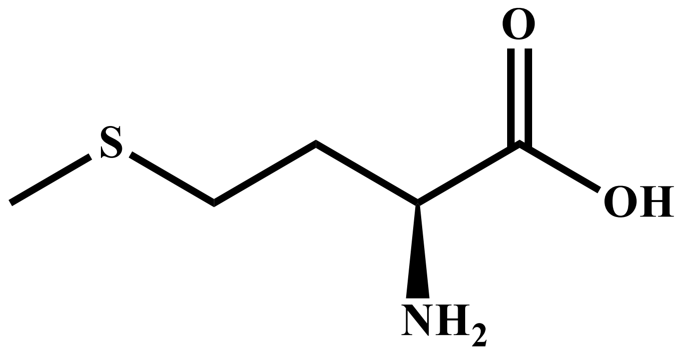


Hatzenpichler *et al.*, 2020

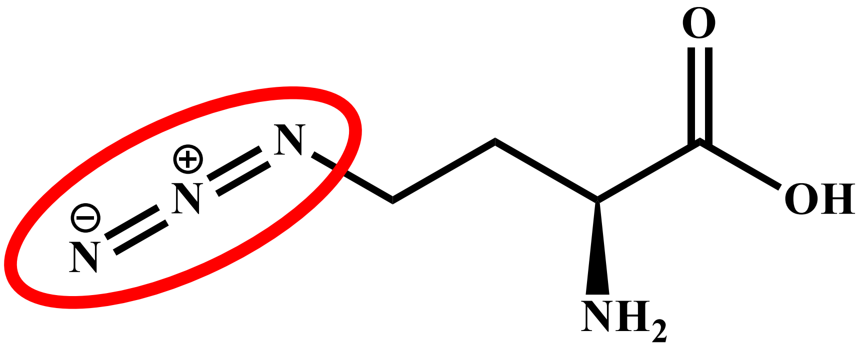
Activity assays on individual cell level



Bioorthogonal non-canonical amino acids

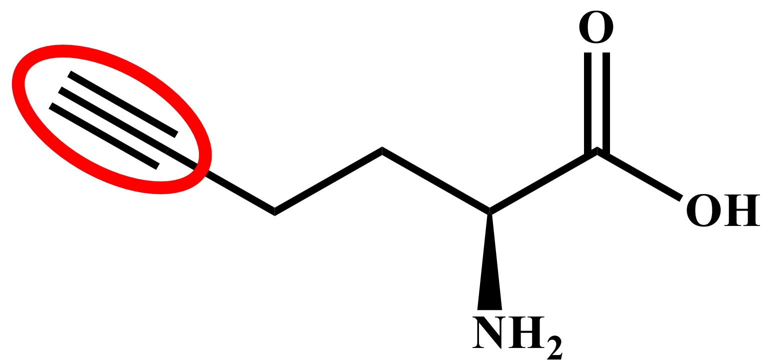


***L*-Methionine**
Met



***L*-Azidohomoalanine**

AHA



***L*-Homopropargylglycine**

HPG

synthetic amino acids
incorporate into new proteins instead of Met

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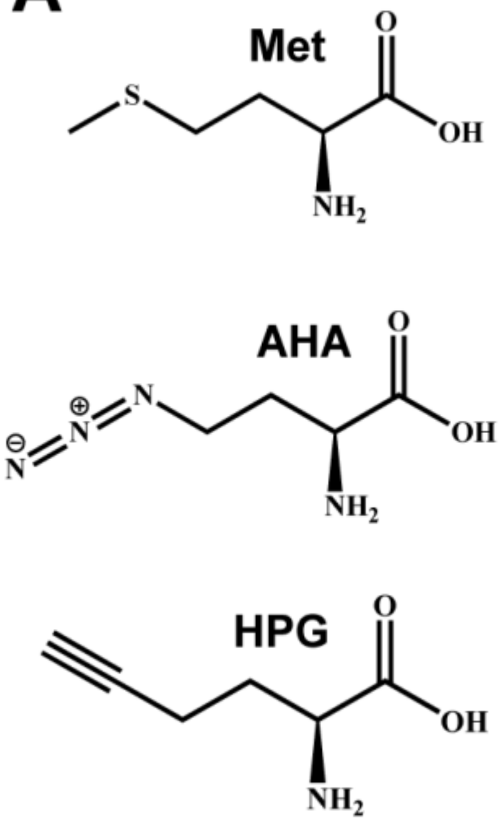
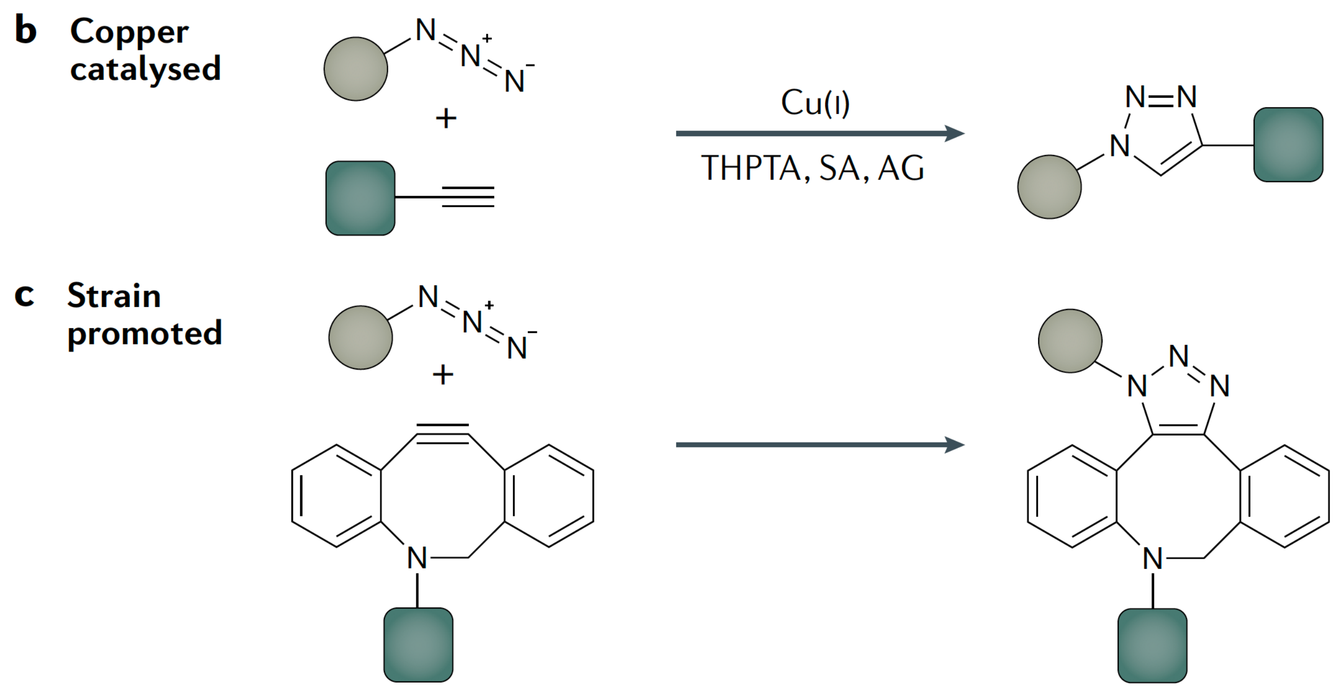
Azide-alkyne click chemistry reactions

A





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.

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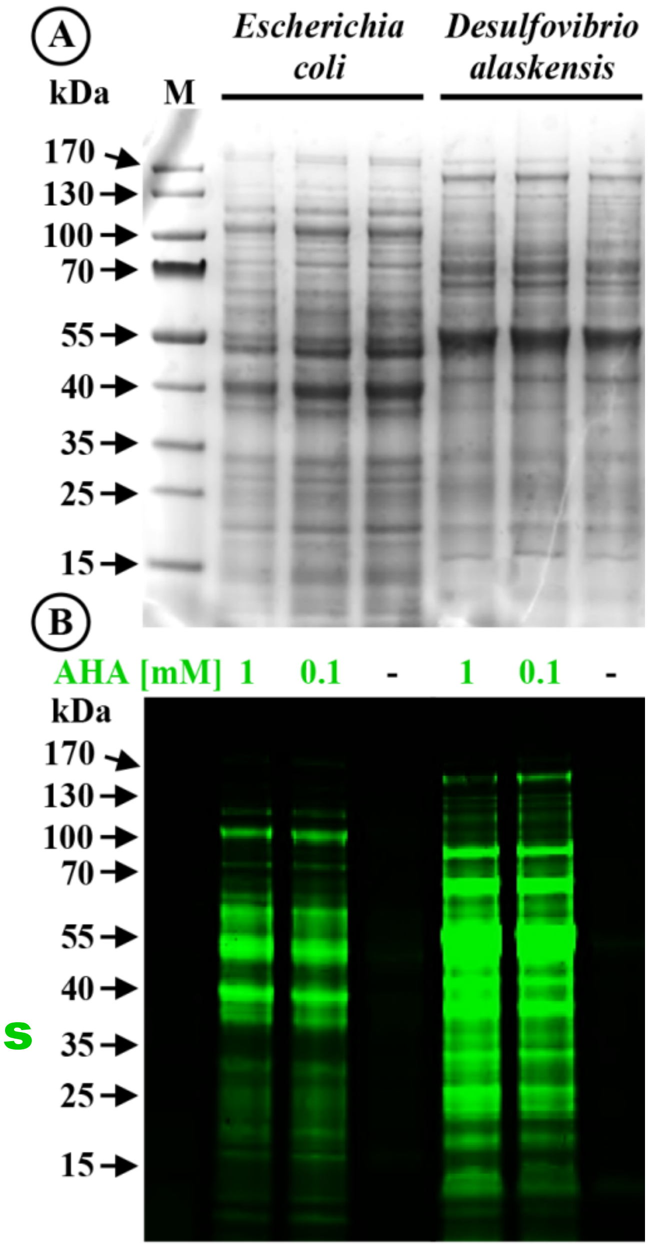
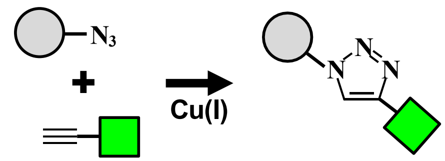
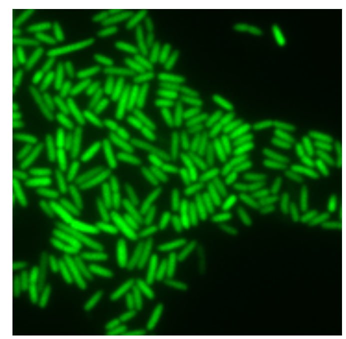
Hatzenpichler *et al.*, 2015 Hatzenpichler *et al.*, 2020

Incorporation into newly made proteins

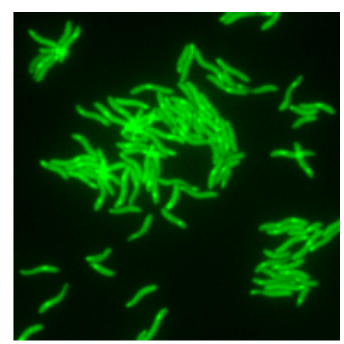
new proteins







E. coli
respiring glucose



D. alaskensis
sulfate reducer

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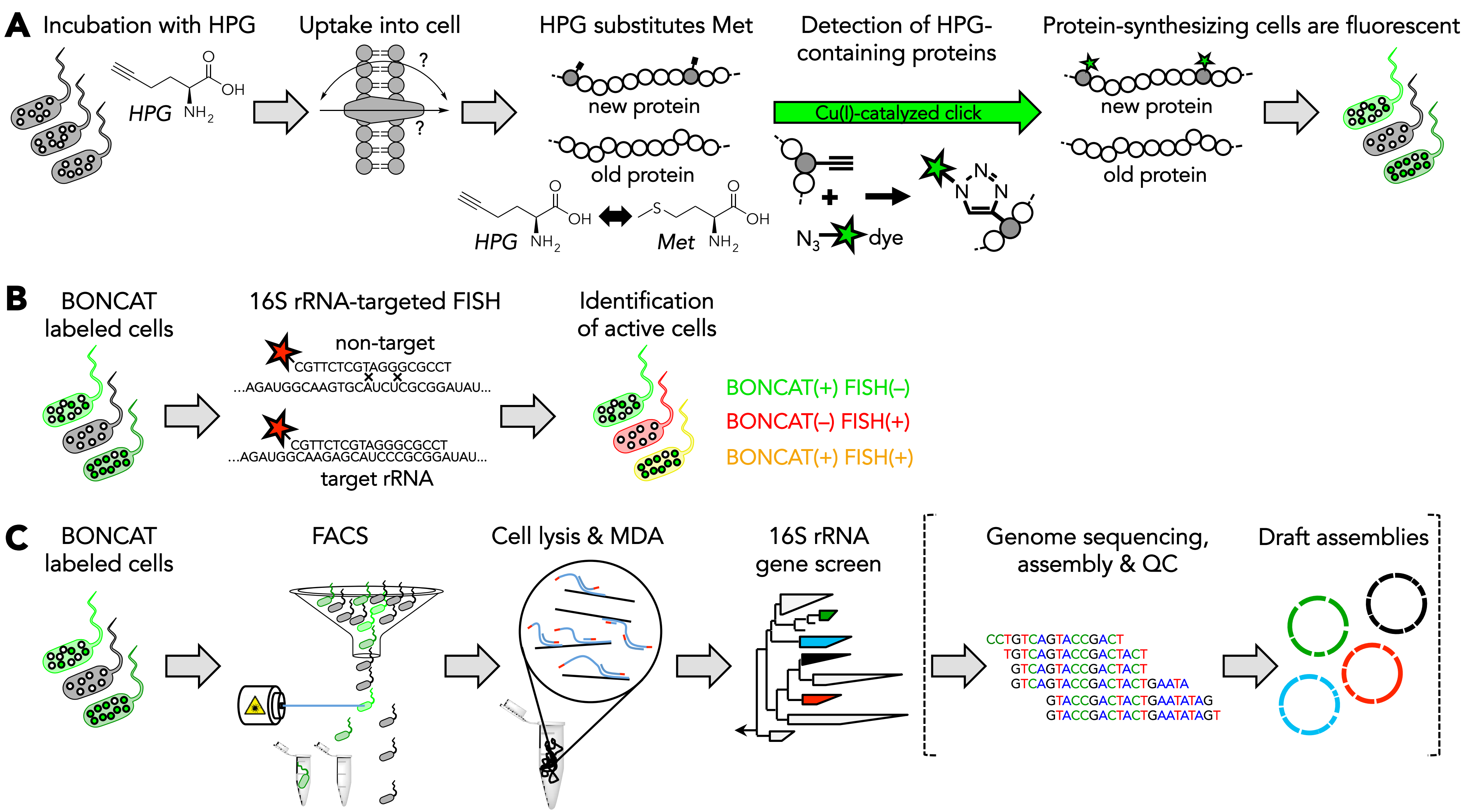
Hatzenpichler *et al.*, 2014

Visualizing, identifying, and sorting translationally active microbes



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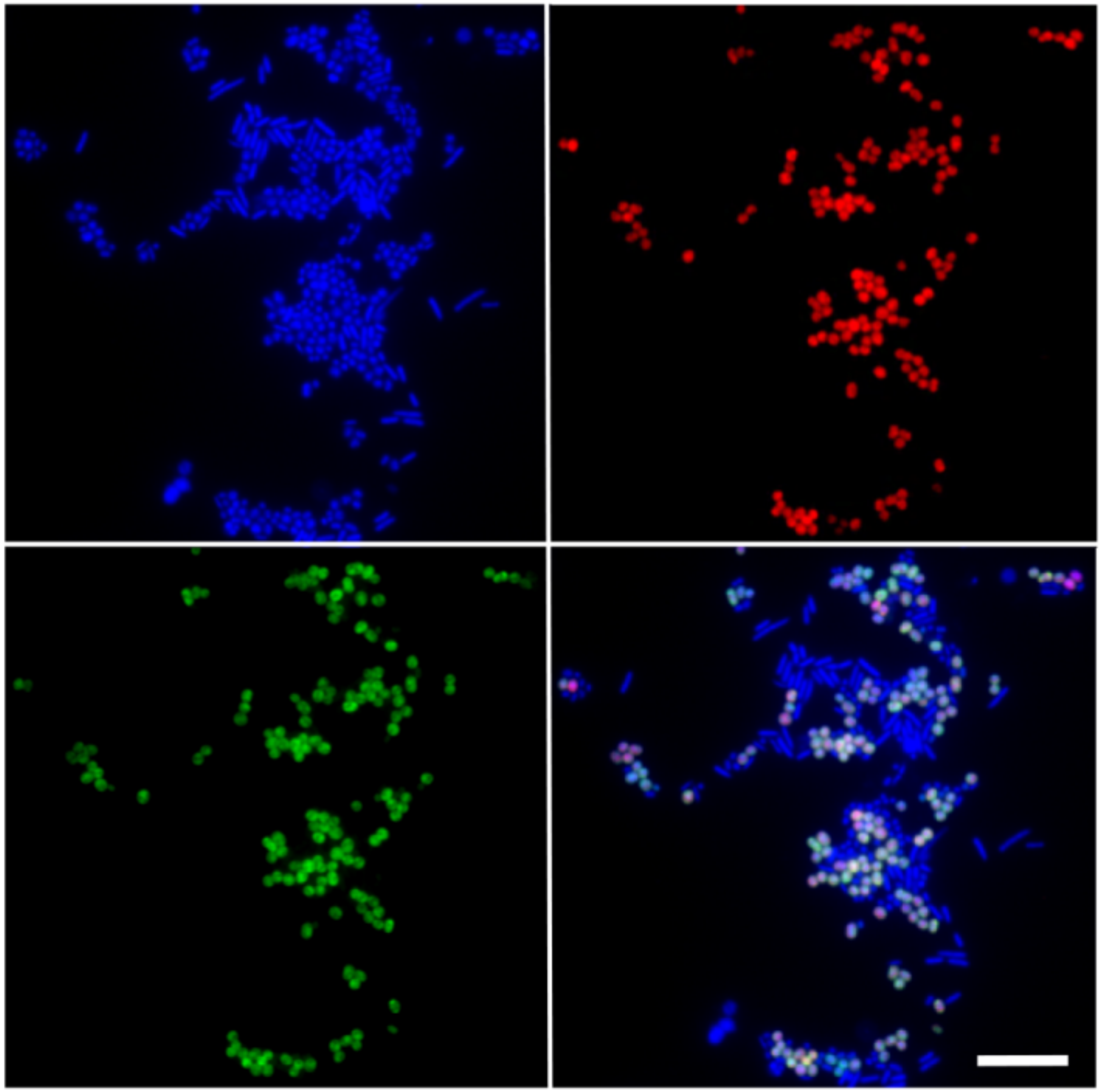
Hatzenpichler *et al.*, 2016

Identification of translationally active cells

DAPI
(DNA)



γ-Proteo WF-1
(rRNA)

BONCAT
(new proteins)

overlay

Bar = 10 µm

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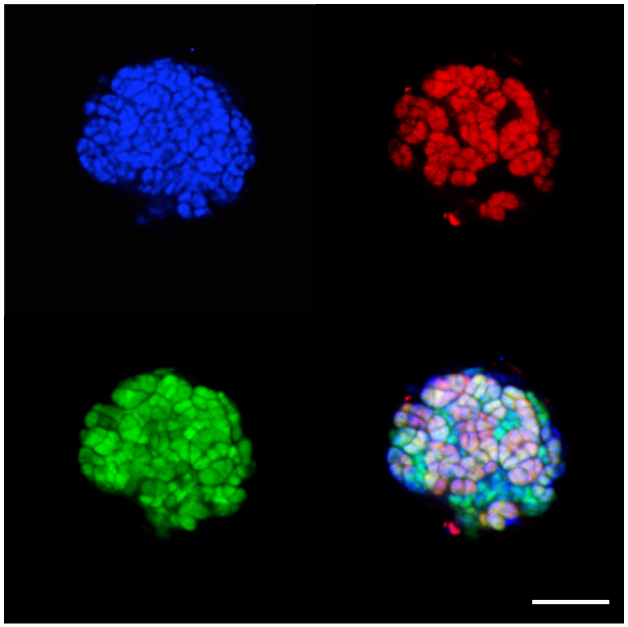
Hatzenpichler *et al.*, 2014

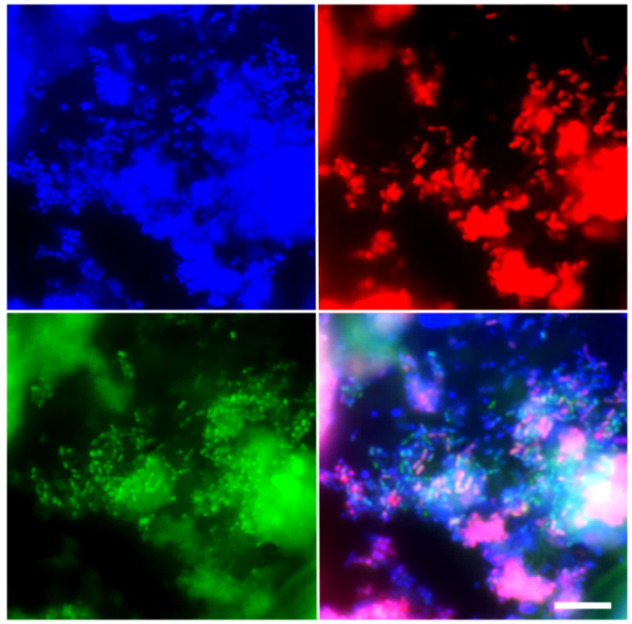
BONCAT-FISH of uncultured microbes

Arch915



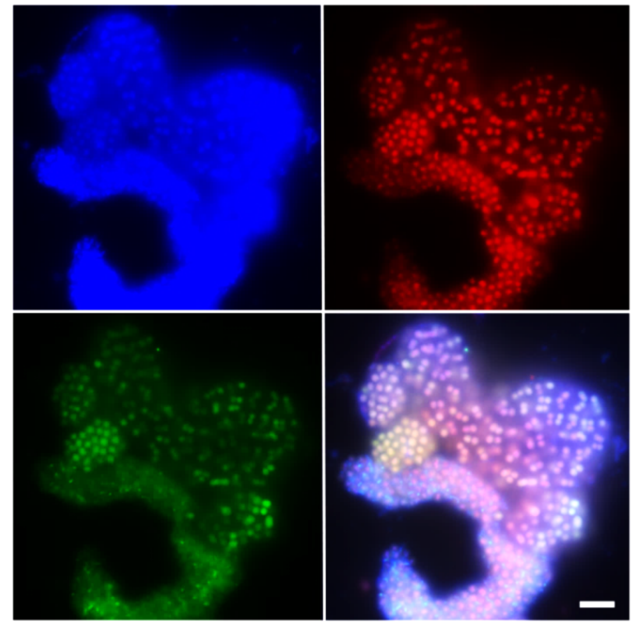
Methane seep
ANME-SRB consortium

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*

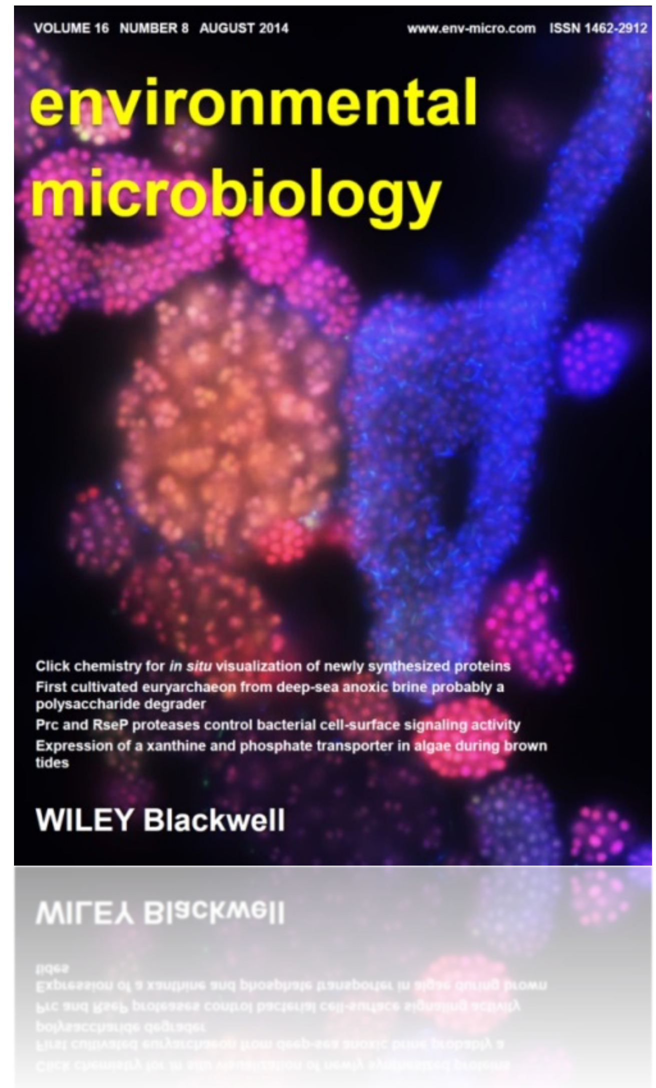
generally applicable
(works for all taxonomies and physiologies tested so far)

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

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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

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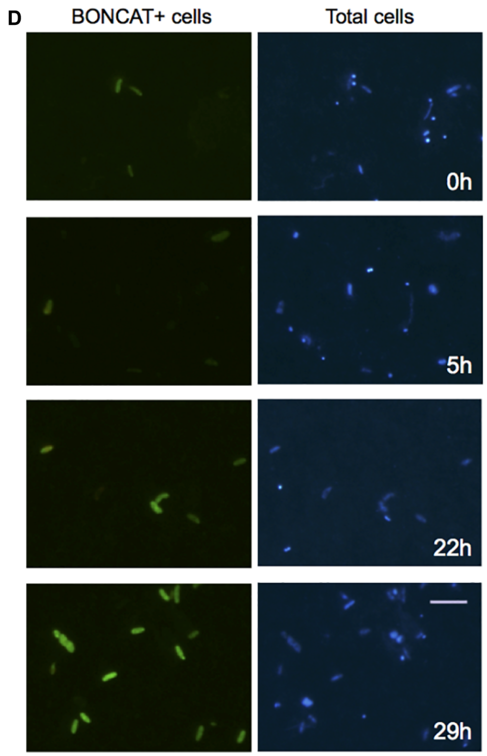
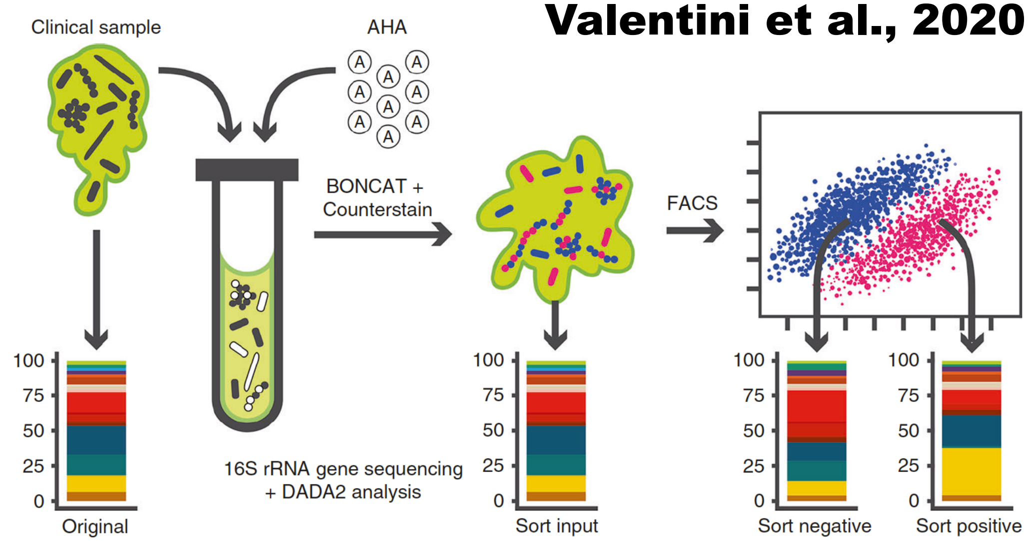
Research examples



Watching cells resuscitate after long-term starvation

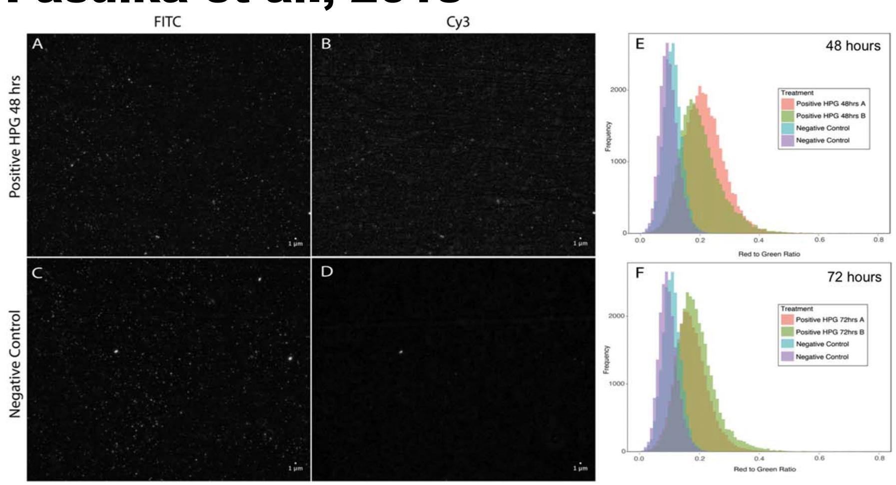
Sebastian et al., 2019

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 o cells in soil with BONCAT-FACS

Couradeau et al. 2019