

# **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**

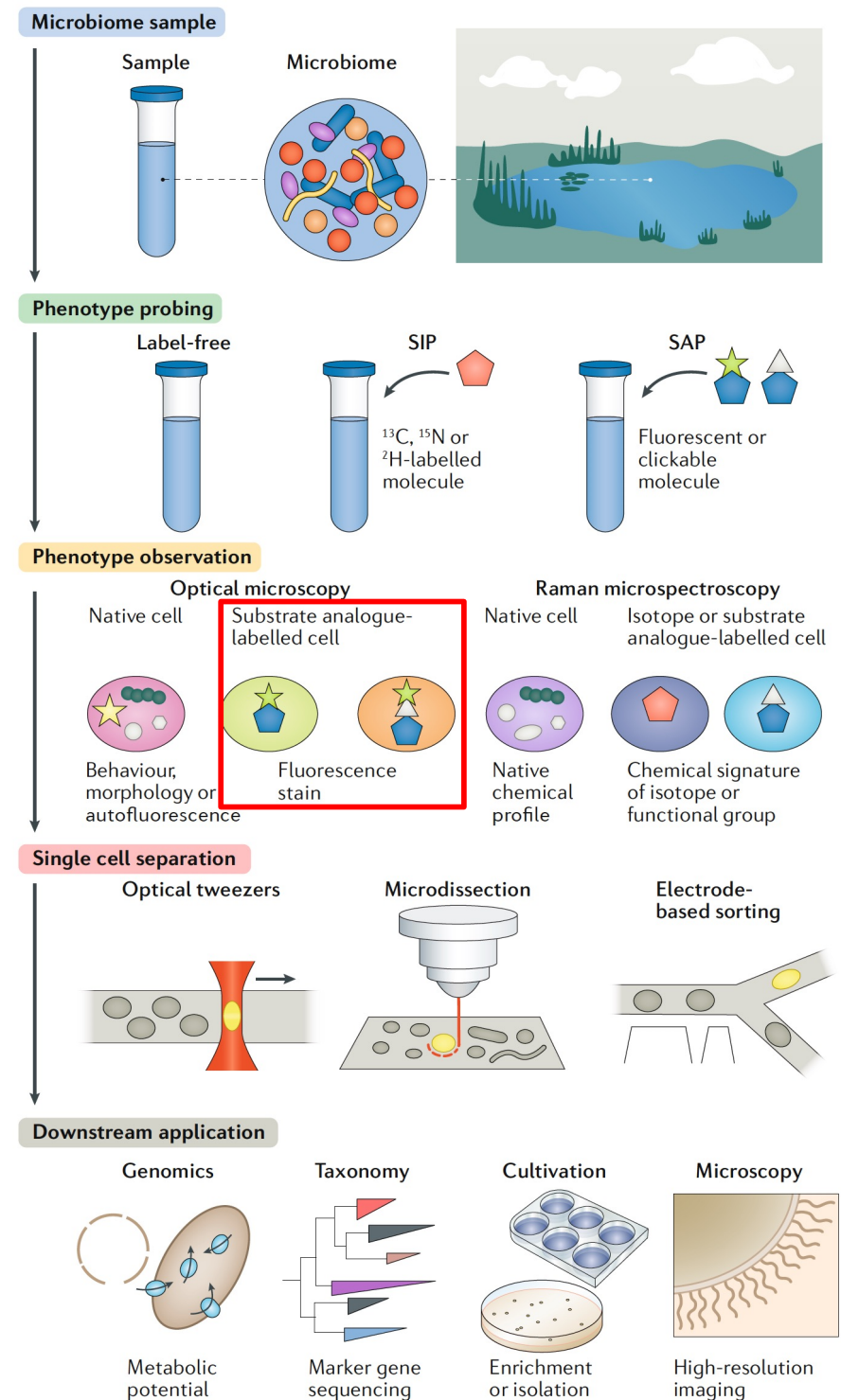


Fig. 2 | Next-generation physiology workflow to study microorganisms.

# **First, some definitions**

**bioorthogonal**

**non-interacting with cellular functionalities**

**non-canonical**

**synthetic, not part of biological machinery**

**Click chemistry**

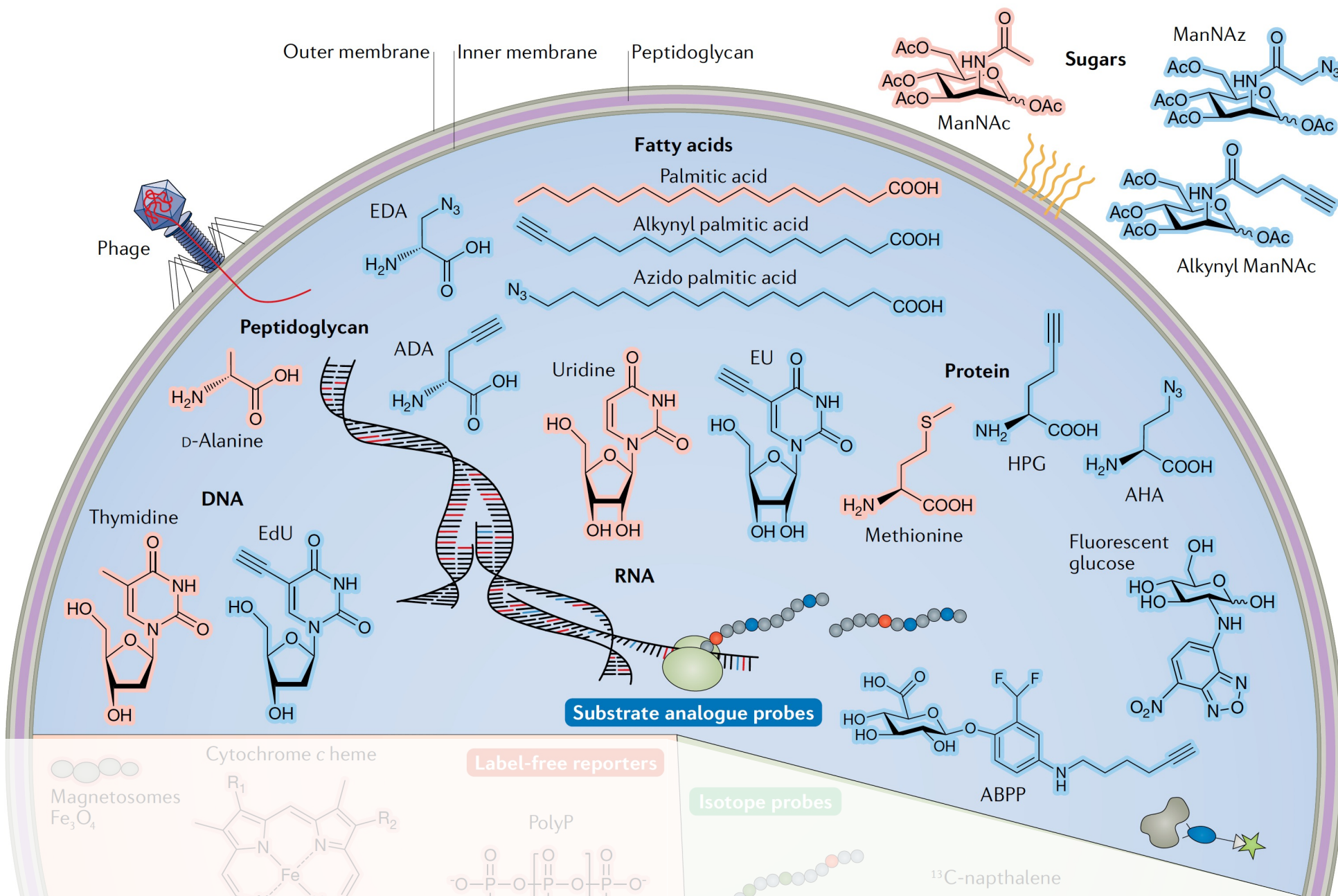
**complete conversion of reagents to single product**

**+ mild conditions**

**+ very fast**

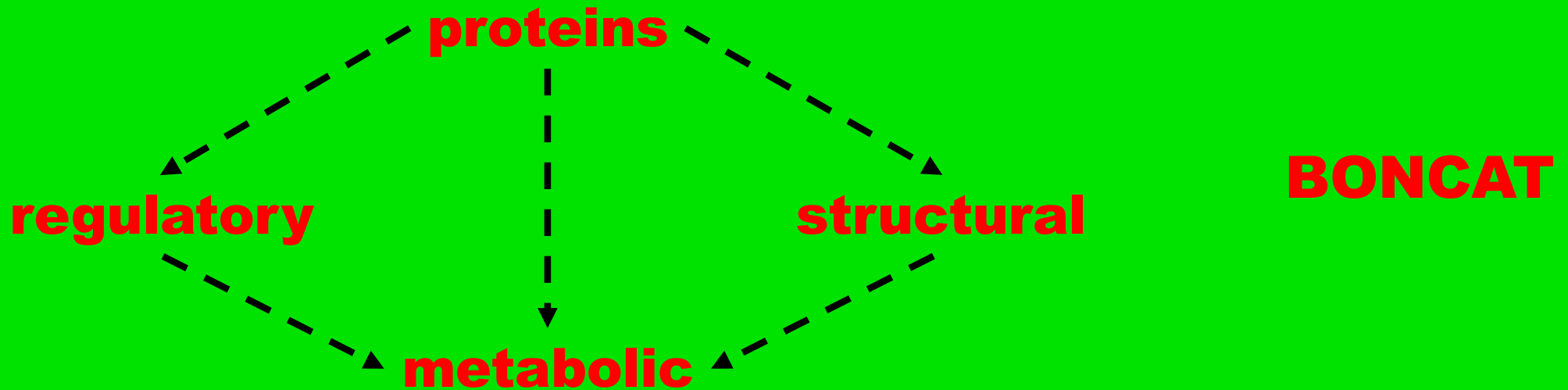
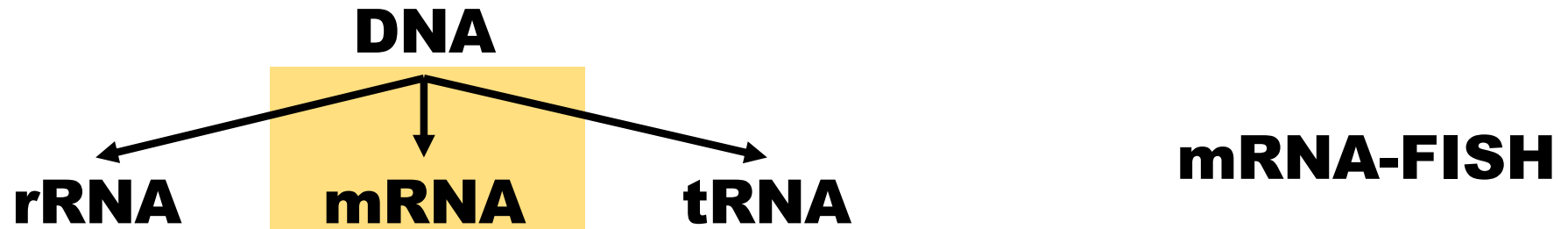
**+ in water**

# Examples for clickable substrate analogs





# Activity assays on individual cell level

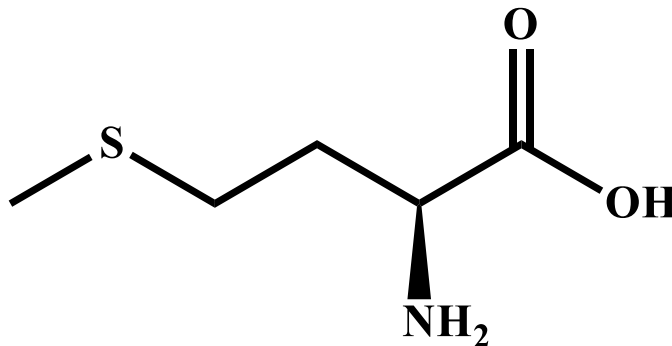


**build-up of biomass  
& growth**

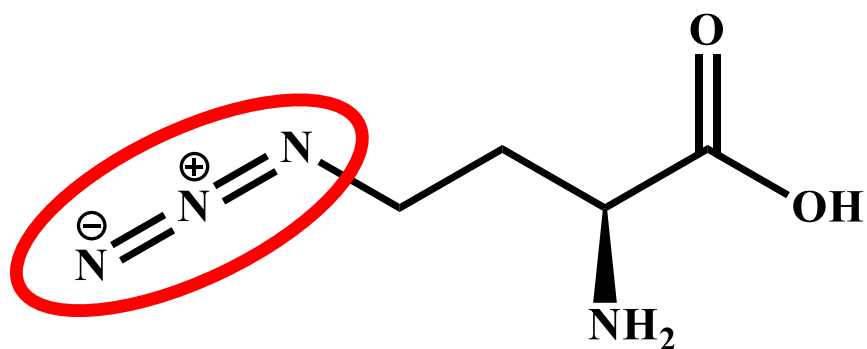
**isotopic labeling**



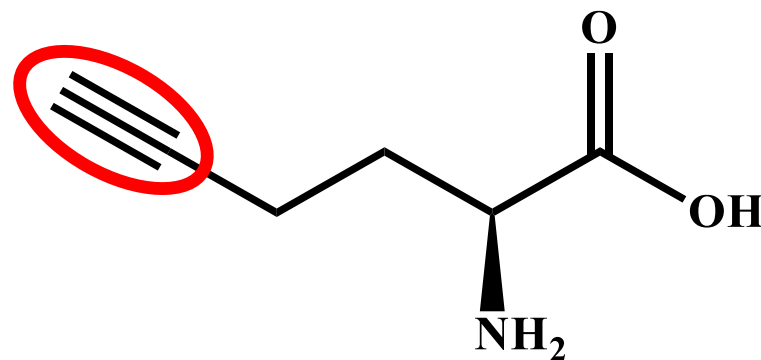
# Bioorthogonal non-canonical amino acids



**L-Methionine**  
**Met**



**L-Azidohomoalanine**  
**AHA**

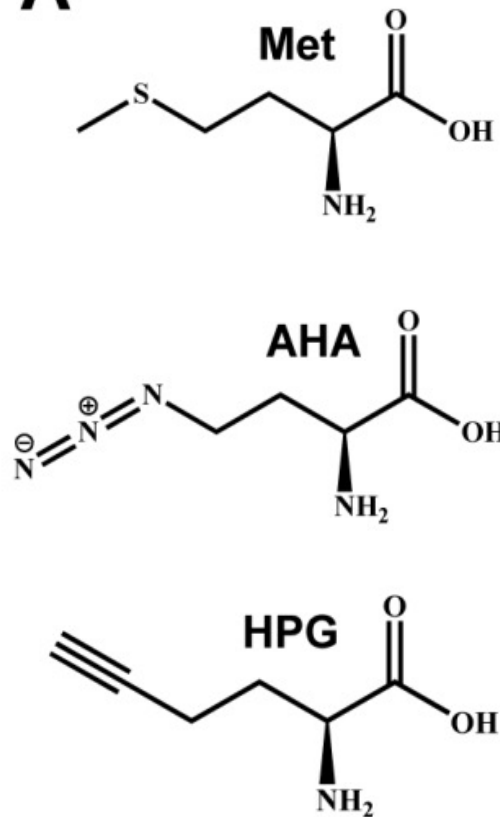


**L-Homopropargylglycine**  
**HPG**

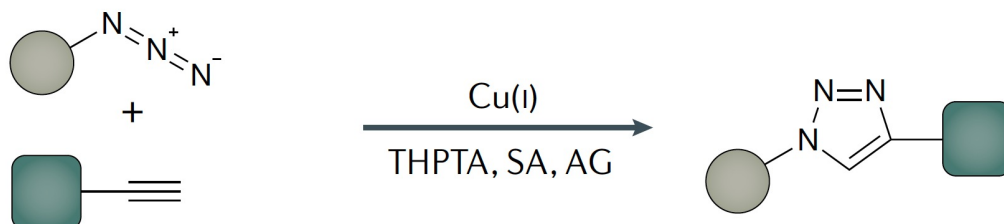
**synthetic amino acids**  
**incorporate into new proteins instead of Met**

# Azide-alkyne click chemistry reactions

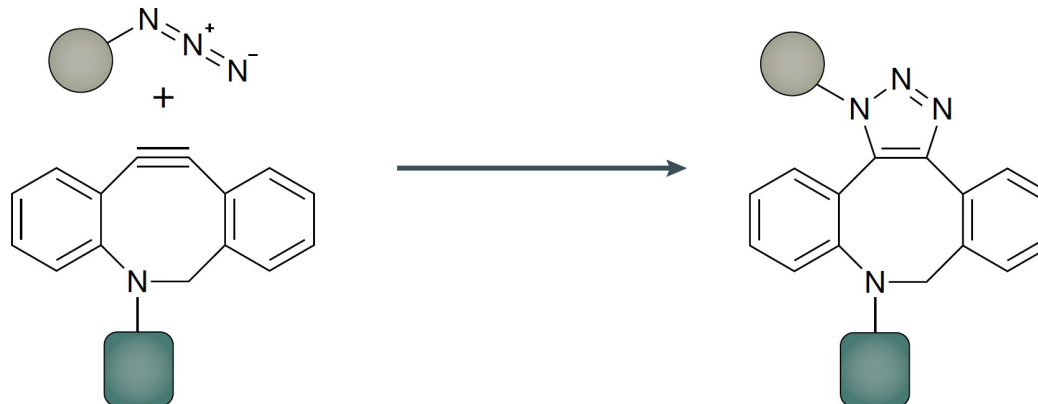
**A**



**b** Copper catalysed



**c** Strain promoted

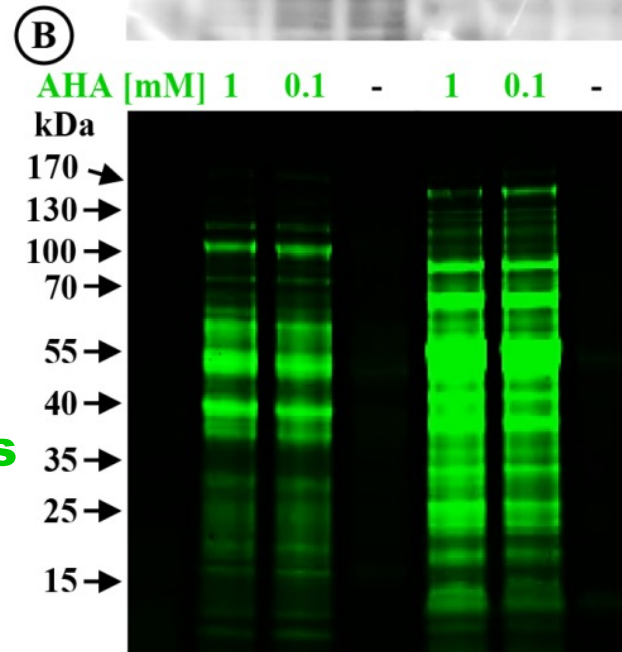
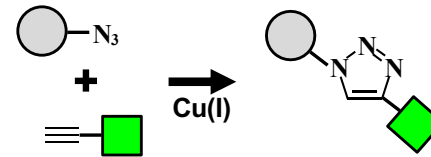
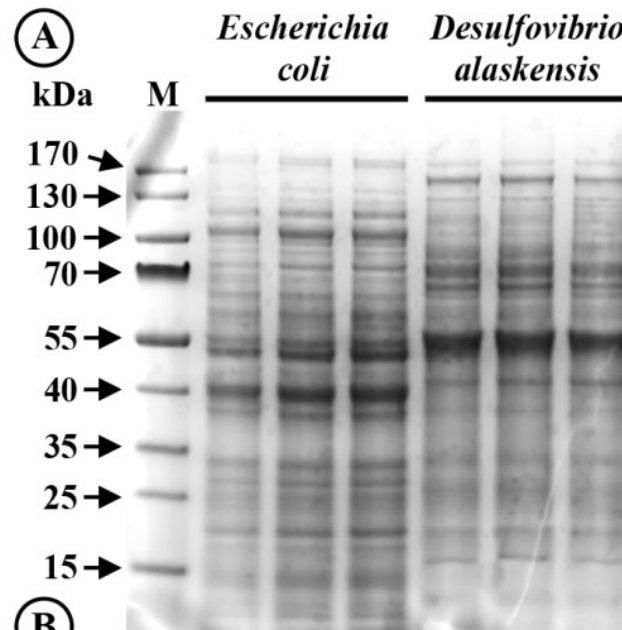


**A. Structures of Met and its surrogates AHA and HPG, which compete with Met during translation.**

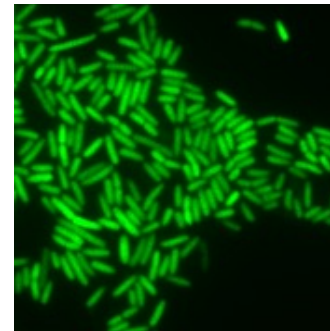
**B. In Cu(I)-catalyzed click chemistry an azide group (N<sub>3</sub>) is linked to a terminal alkyne residue, yielding a triazole conjugate.**

**C. Strain-promoted click chemistry allows the copper-less conjugation of an azide group (N<sub>3</sub>) with a cyclo-octyne-carrying molecule, yielding a triazole conjugate.**

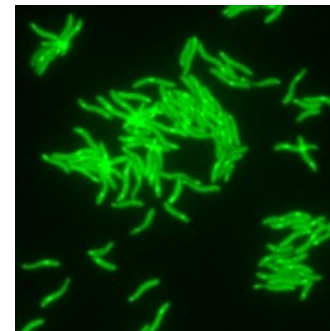
# Incorporation into newly made proteins



new proteins

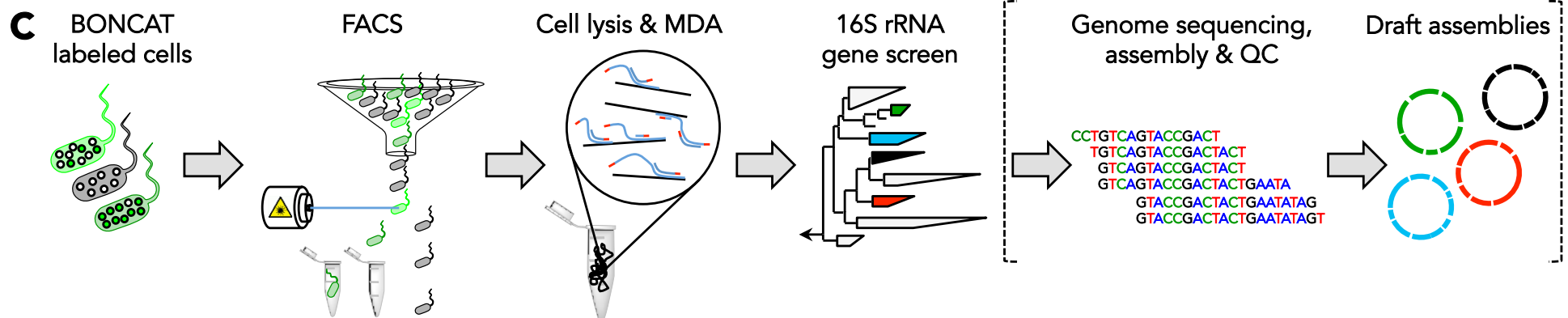
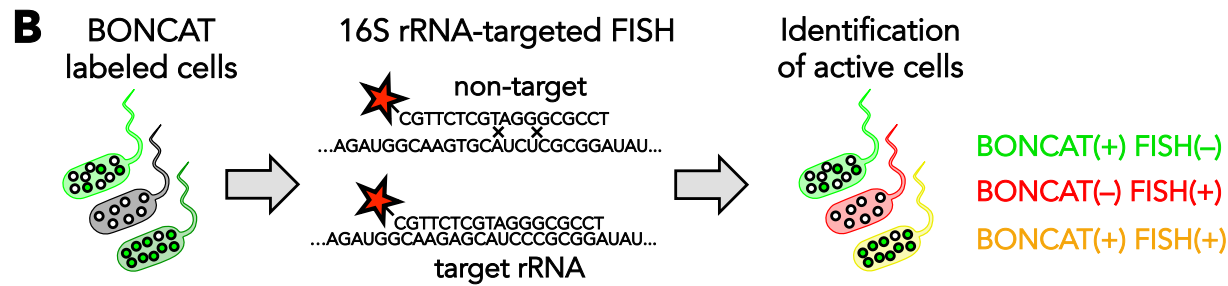
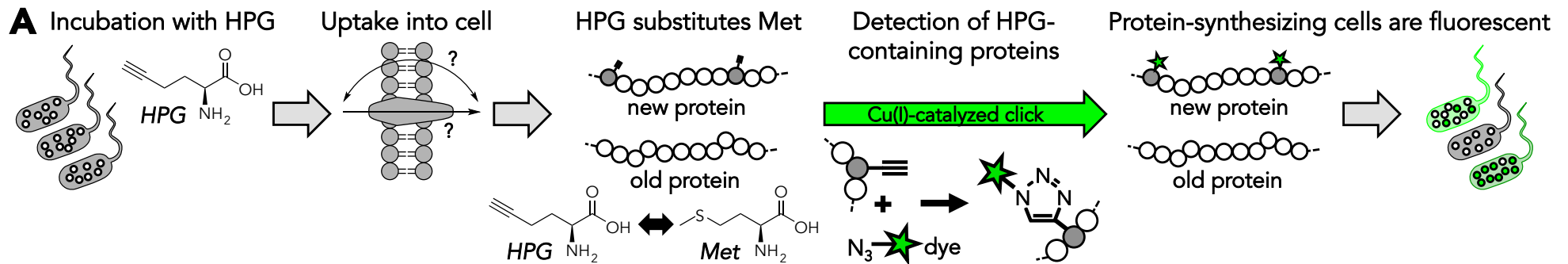


*E. coli*  
respiring glucose

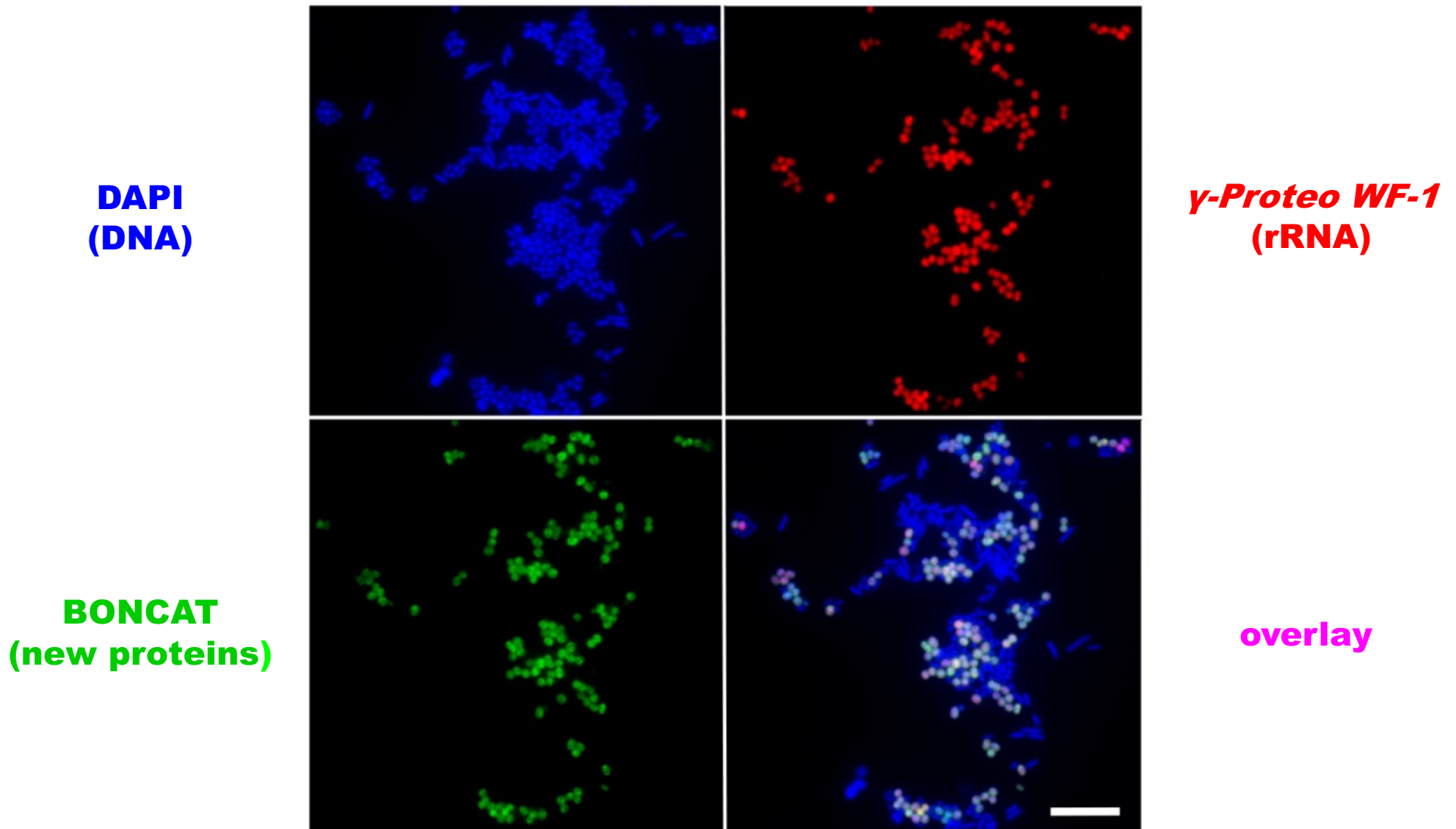


*D. alaskensis*  
sulfate reducer

# Visualizing, identifying, and sorting translationally active microbes



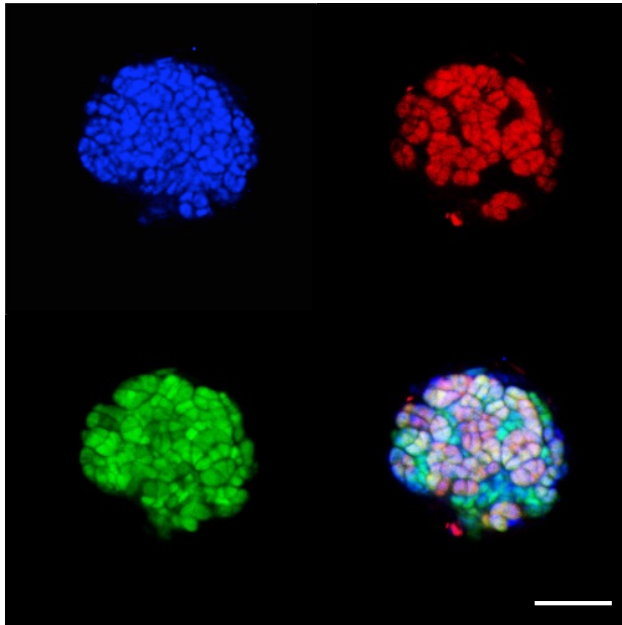
# Identification of translationally active cells



Bar = 10  $\mu$ m

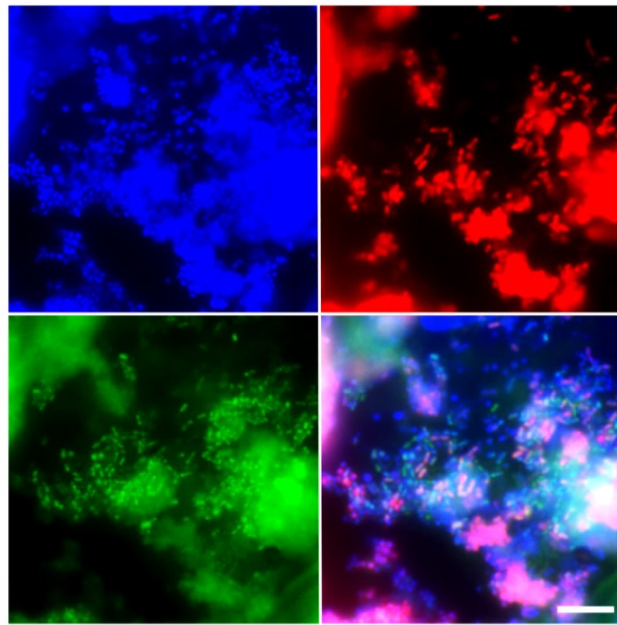
# BONCAT-FISH of uncultured microbes

**Arch915**



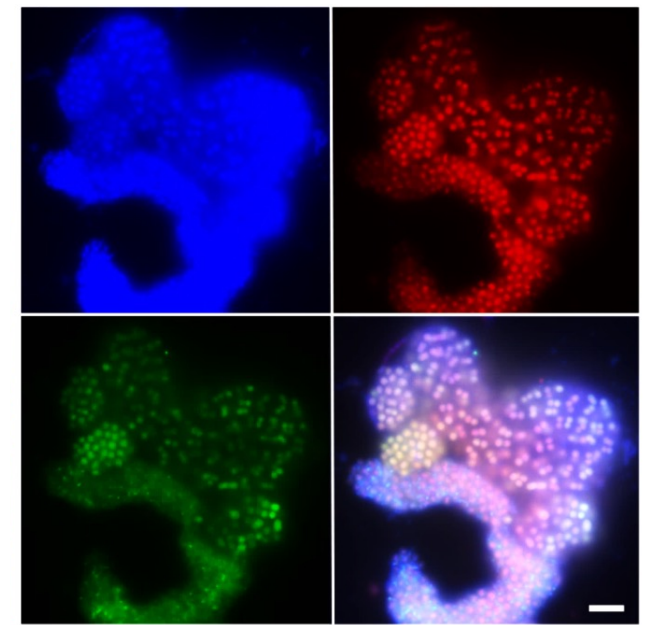
**Methane seep  
ANME-SRB consortium**

**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**

**Hatzenpichler lab**



# 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**

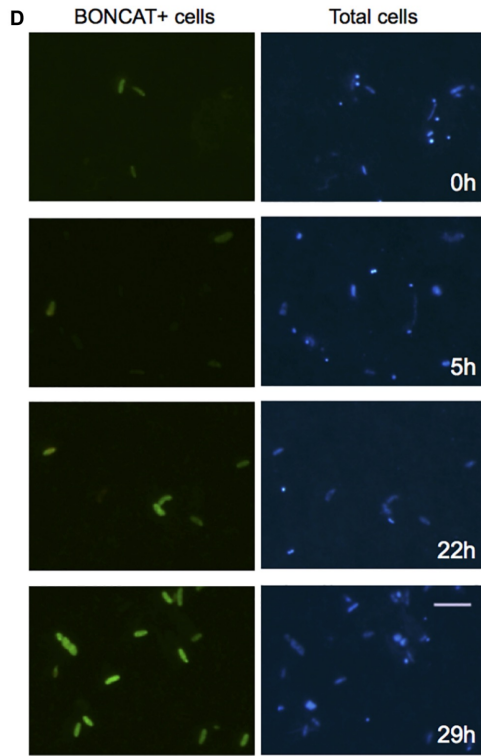
**metabolic screening**

**activity-based cell-sorting**

**fast + highly selective + cheap + easily available**

**1 h      azide-alkyne      ~\$500      epi-scope**

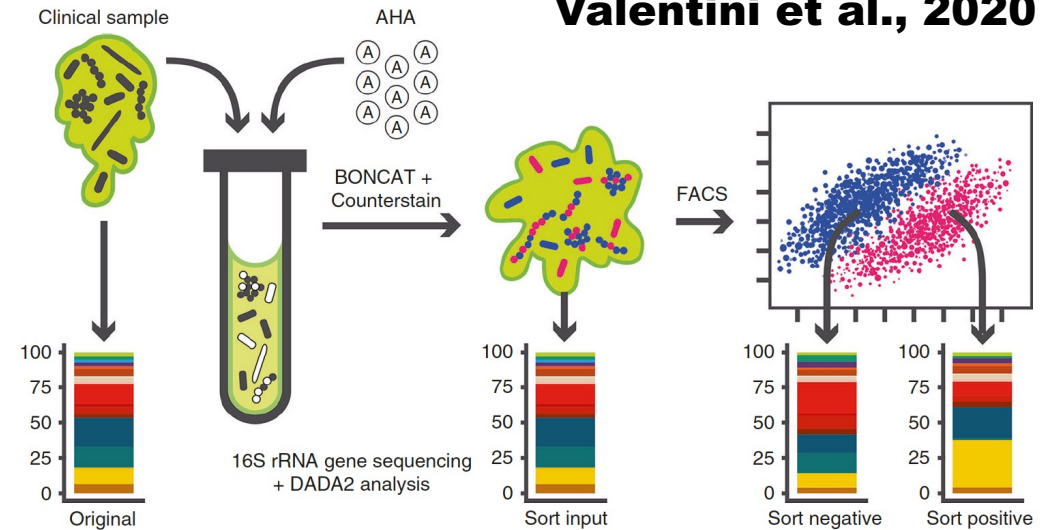
# Research examples



**Watching cells  
resuscitate after  
long-term  
starvation**

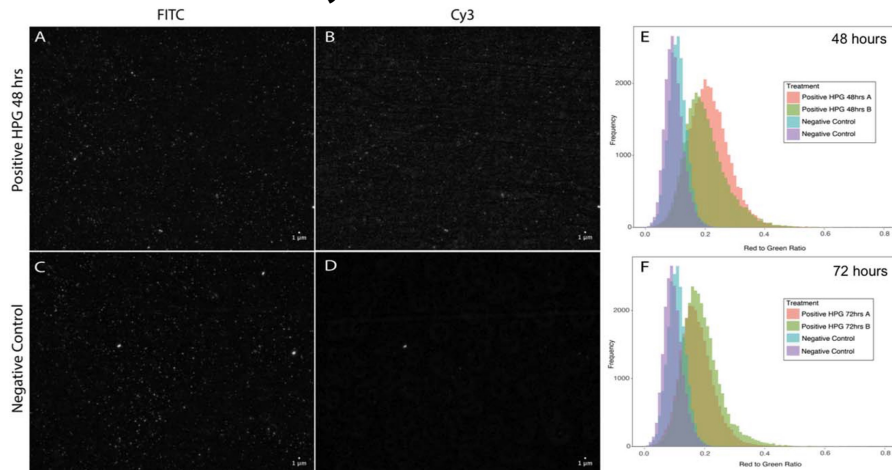
**Sebastian et al.,  
2019**

**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 o  
cells in soil with  
BONCAT-FACS**

**Couradeau et al.  
2019**

