Bioorthogonal non-canonical amino acid tagging

- BONCAT -

Roland Hatzenpichler www.environmental-microbiology.com doi: 10.13140/RG.2.1.3698.7040/1

First, some definitions

bioorthogonal

non-interacting with cellular functionalities

non-canonical

synthetic, not part of biological machinery

Click chemistry

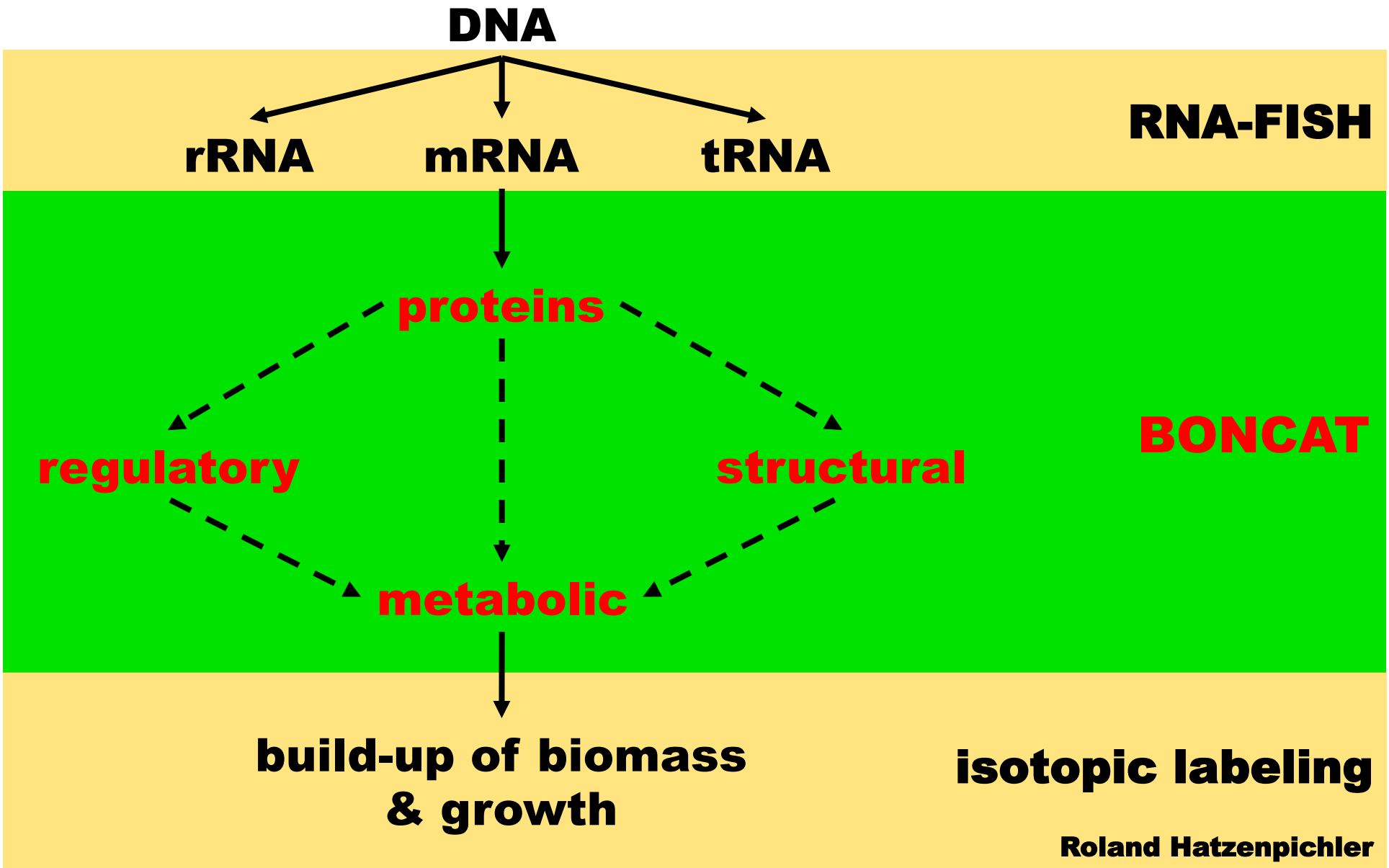
complete conversion of reagents to single product

+ mild conditions

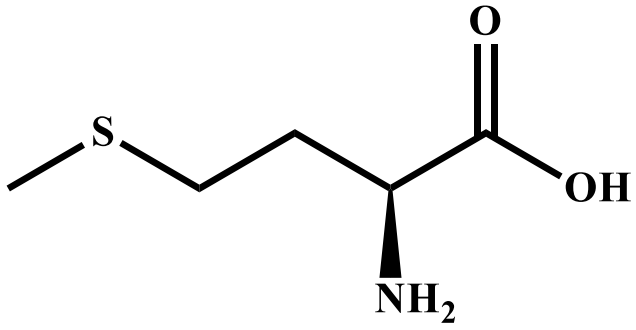
+ very fast

+ in water

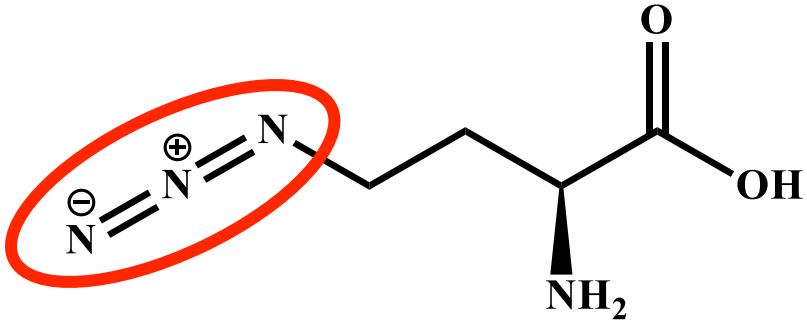
Activity assays on individual cell level



Bioorthogonal non-canonical amino acids

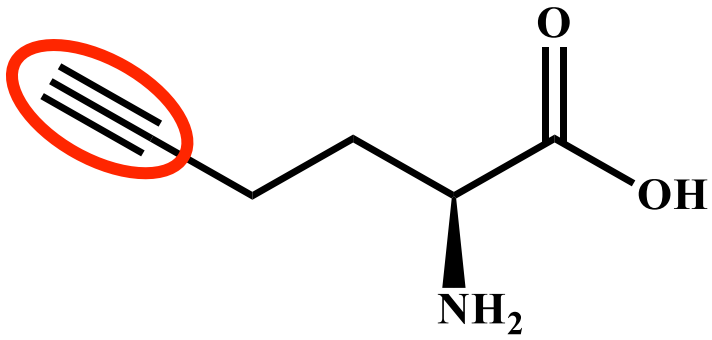


***L*-Methionine**
Met



***L*-Azidohomoalanine**

AHA



***L*-Homopropargylglycine**

HPG

synthetic amino acids
incorporate into new proteins instead of Met

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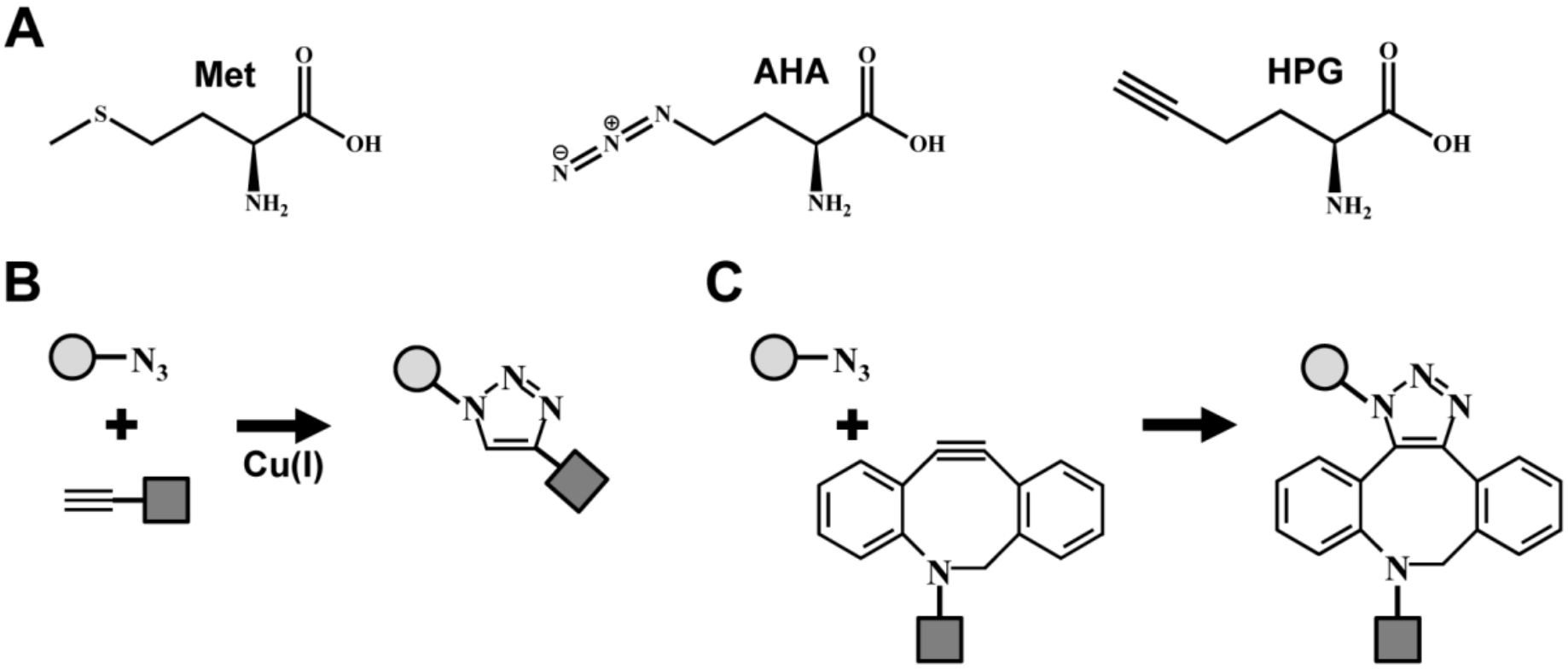
Azide-alkyne click reactions



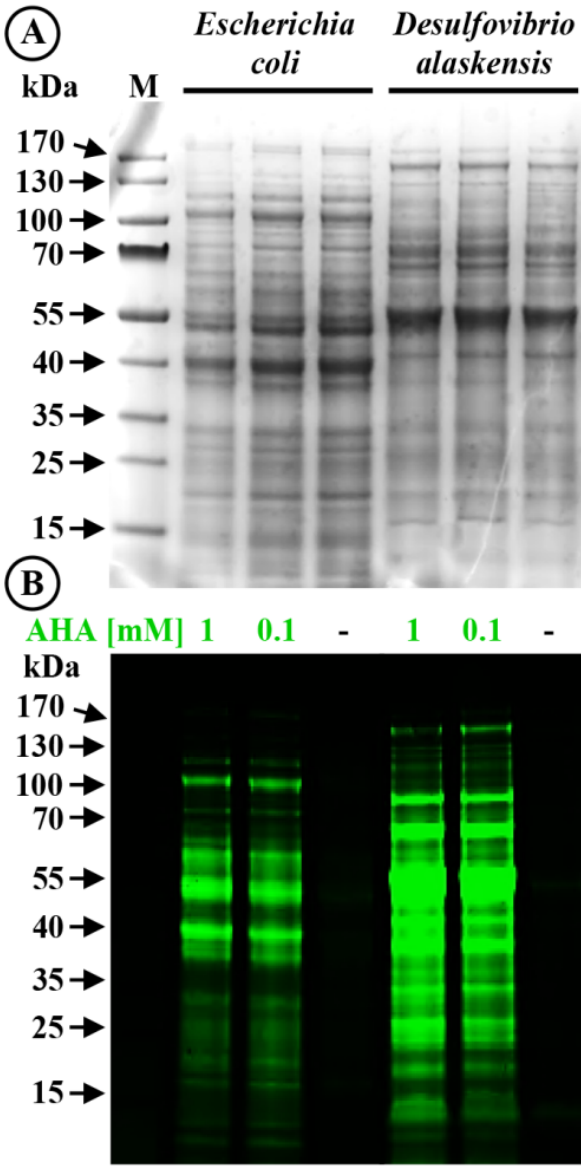
A. Structures of Met and its surrogates AHA and HPG, which compete with Met during translation.

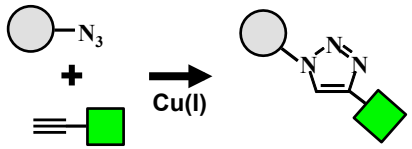
B. In Cu(I)-catalyzed click chemistry an azide group (N_3) is linked to a terminal alkyne residue, yielding a triazole conjugate.

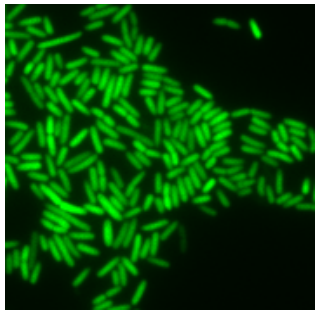
C. Strain-promoted click chemistry allows the copper-less conjugation of an azide group (N_3) with a cyclo-octyne-carrying molecule, yielding a triazole conjugate.

Hatzenpichler *et al.*, 2015

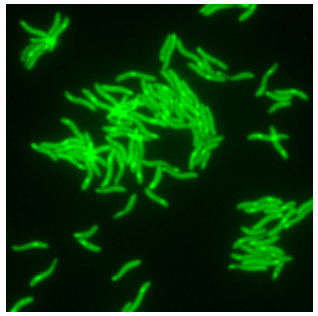
Incorporation into newly made proteins







E. coli
respiring glucose

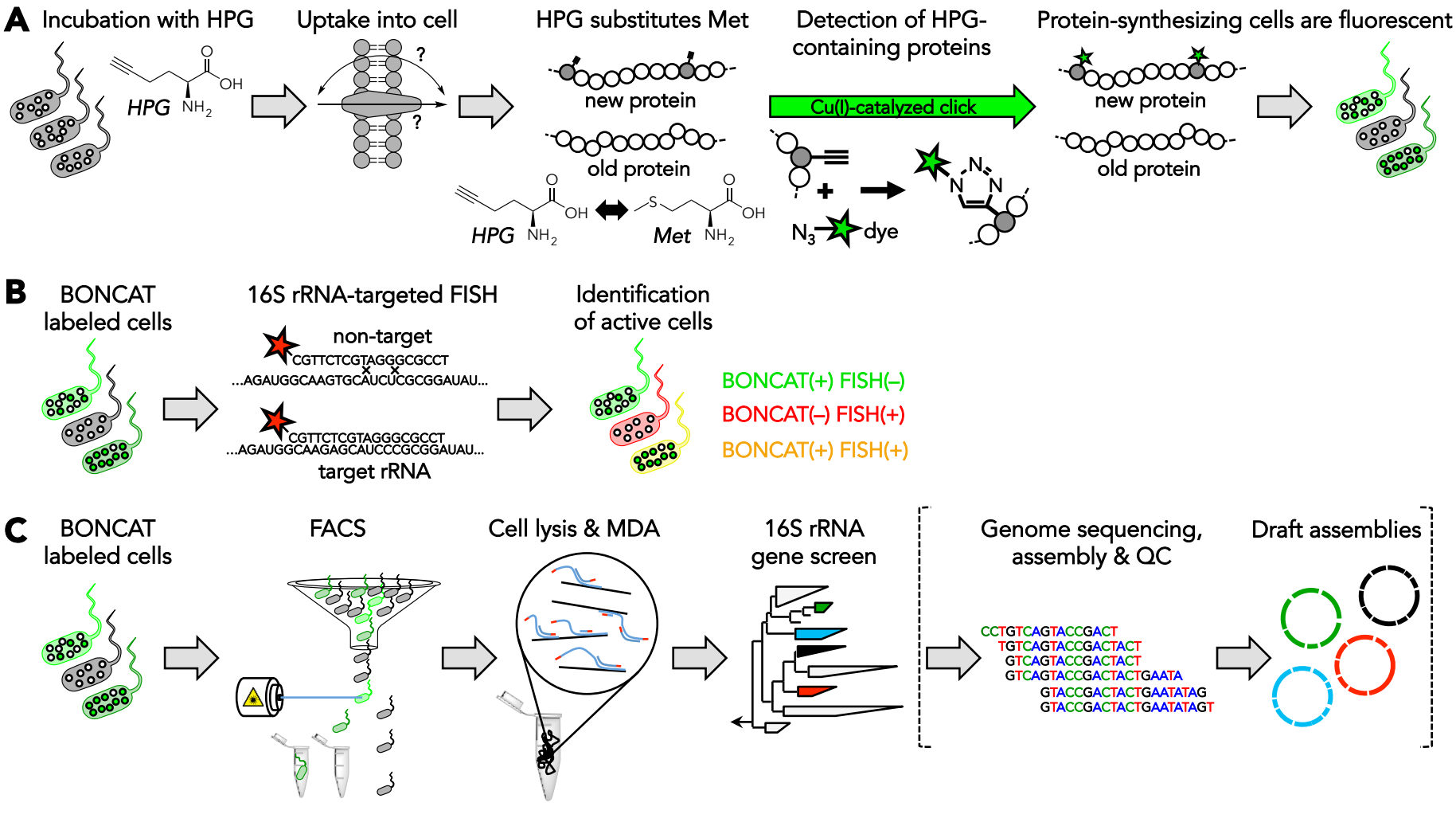


D. alaskensis
sulfate reducer

new proteins

Hatzenpichler *et al.*, 2014

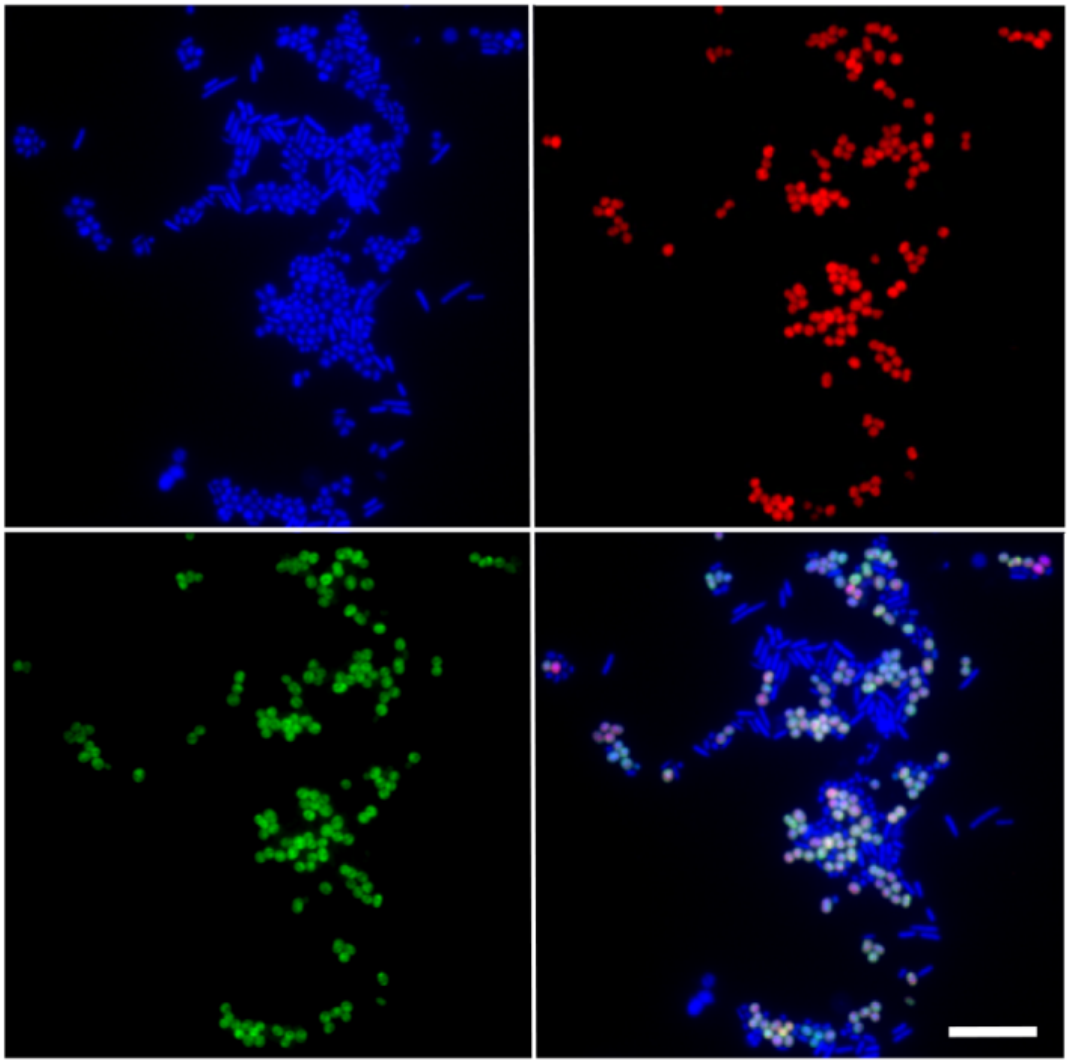
Visualizing, identifying, and sorting translationally active microbes



Hatzenpichler *et al.*, 2016

Identification of translationally active cells

DAPI
(DNA)



γ-Proteo WF-1
(rRNA)

BONCAT
(new proteins)

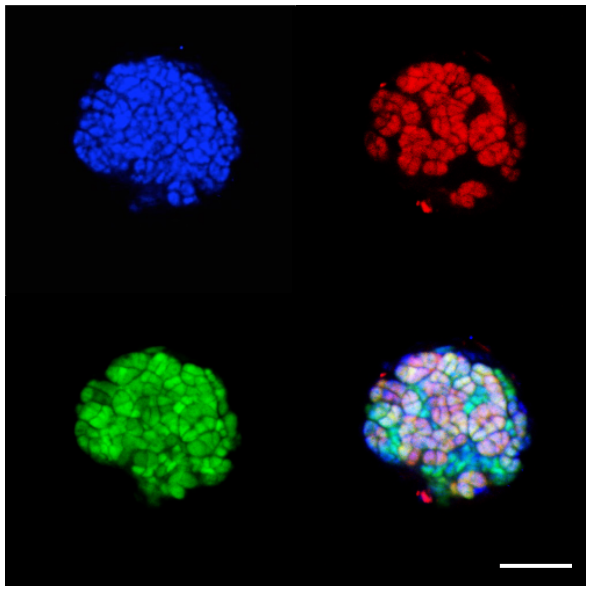
overlay

Bar = 10 µm

Hatzenpichler *et al.*, 2014

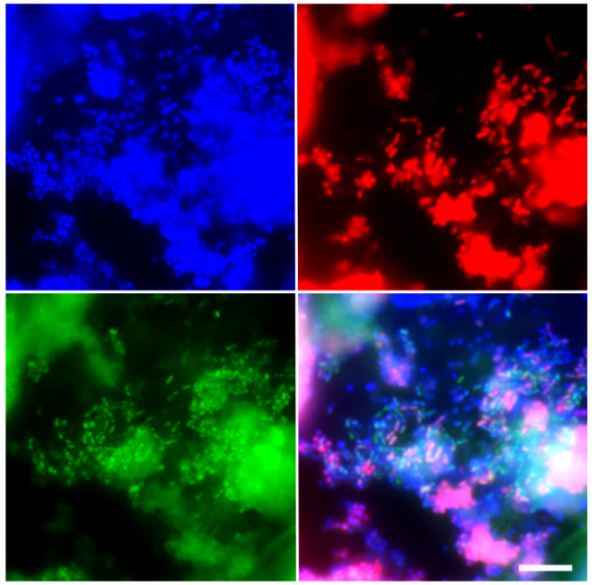
BONCAT-FISH of uncultured microbes

Arch915



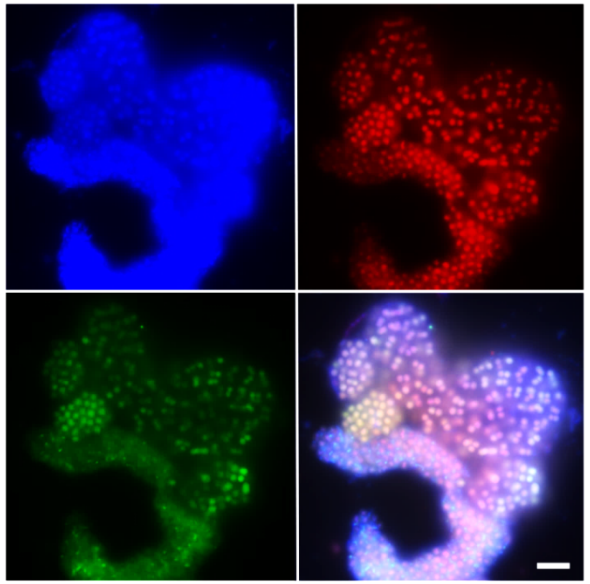
Methane seep
ANME-SRB consortium

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

Visualizing new proteins *in situ*

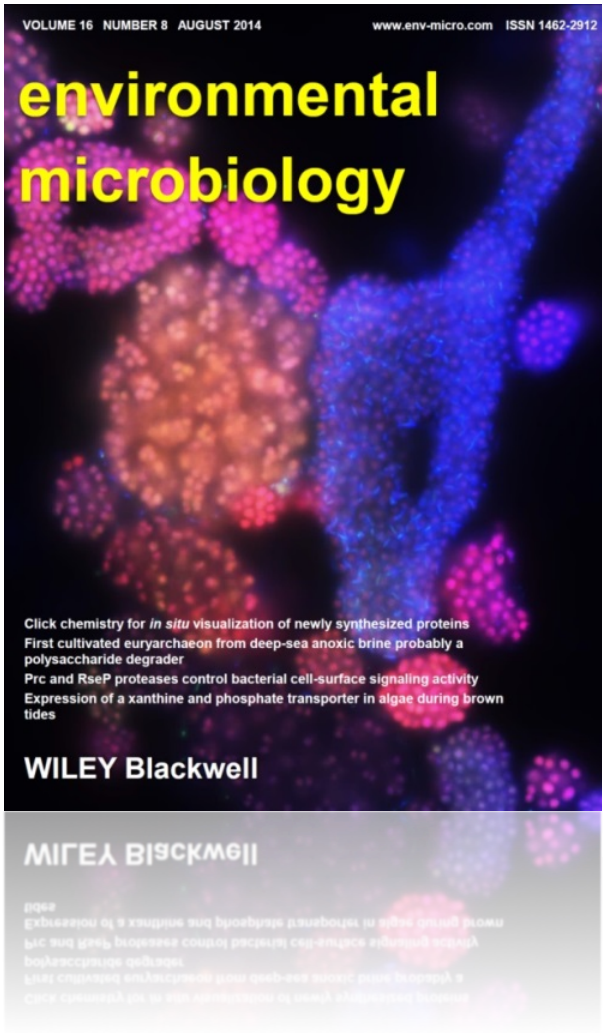
generally applicable
(works for all taxonomies and physiologies tested so far)

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

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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BONCAT in environmental microbiology
(as of May 2016)

Hatzenpichler R, Connon SA, Goudeau D, Malmstrom RR, Woyke T, Orphan VJ
Visualizing *in situ* translational activity for identifying and sorting slow-growing archaeal-bacterial consortia
Proc Natl Acad Sci USA, in press (2016)
- **application of BONCAT-FISH and BONCAT-FACS to ANME-SRB consortia from three methane seep sediments; development of activity-based cell-sorting via bioorthogonal labeling**

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, Vol. 7: Single-cell and single-molecule methods
Springer Protocols Handbooks, doi 10.1007/8623_2015_61 (2015)
- ***description of how to design and perform BONCAT-experiments using AHA and HPG***

Samo TJ, Smriga S, Malfatti F, Sherwood B, and Azam F
Broad distribution and high proportion of protein synthesis active marine bacteria revealed by click chemistry at the single cell level
Front Mar Sci, doi: 10.3389/fmars.2014.00048 (2014)
- ***application of BONCAT to seawater; correlation of BONCAT with MAR***

Hatzenpichler R, Scheller S, Tavormina PL, Babin B, Tirrell D, and Orphan VJ
***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 in the environment; development of BONCAT-FISH; correlation of BONCAT with nanoSIMS***