Bioorthogonal non-canonical amino acid tagging

- BONCAT -

 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

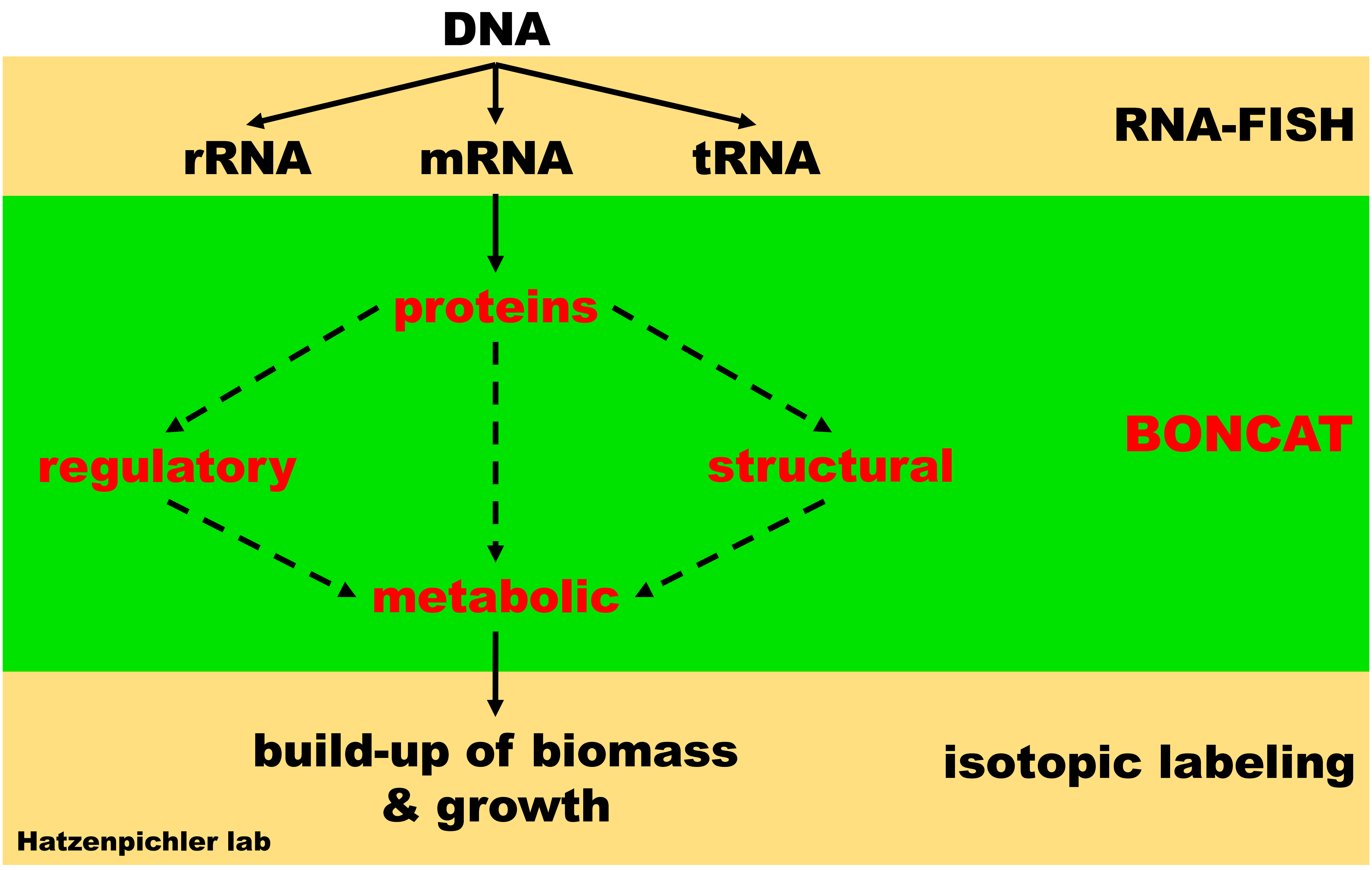
complete conversion of reagents to single product

+ mild conditions

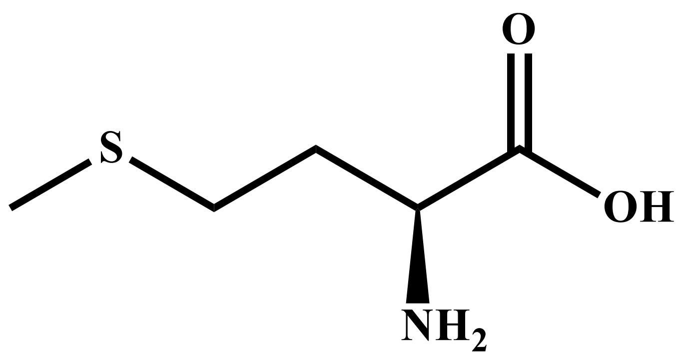
+ very fast

+ in water

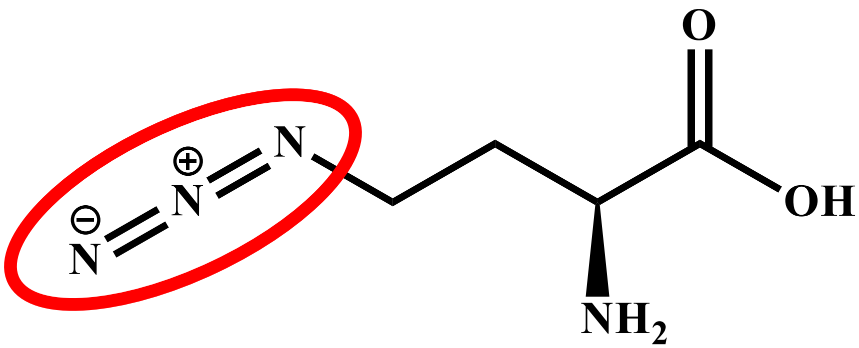
Activity assays on individual cell level

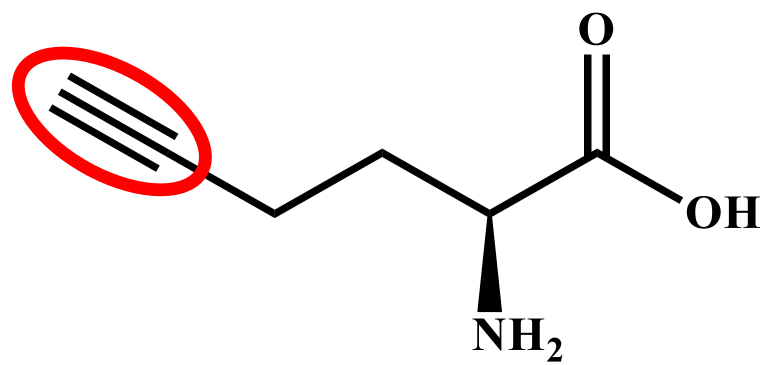


Bioorthogonal non-canonical amino acids



***L*-Methionine**
Met





***L*-Azidohomoalanine**
AHA

***L*-Homopropargylglycine**
HPG

synthetic amino acids
incorporate into new proteins instead of Met

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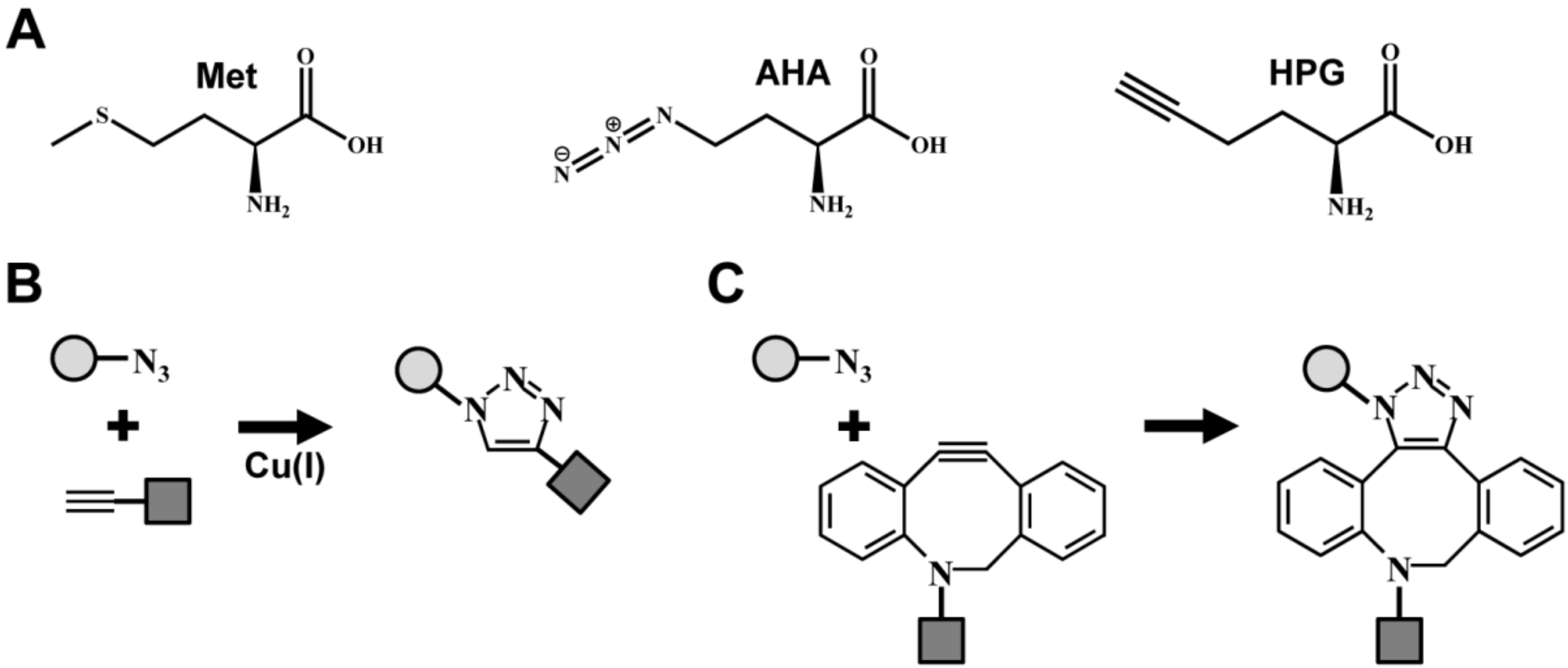
Azide-alkyne click reactions



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

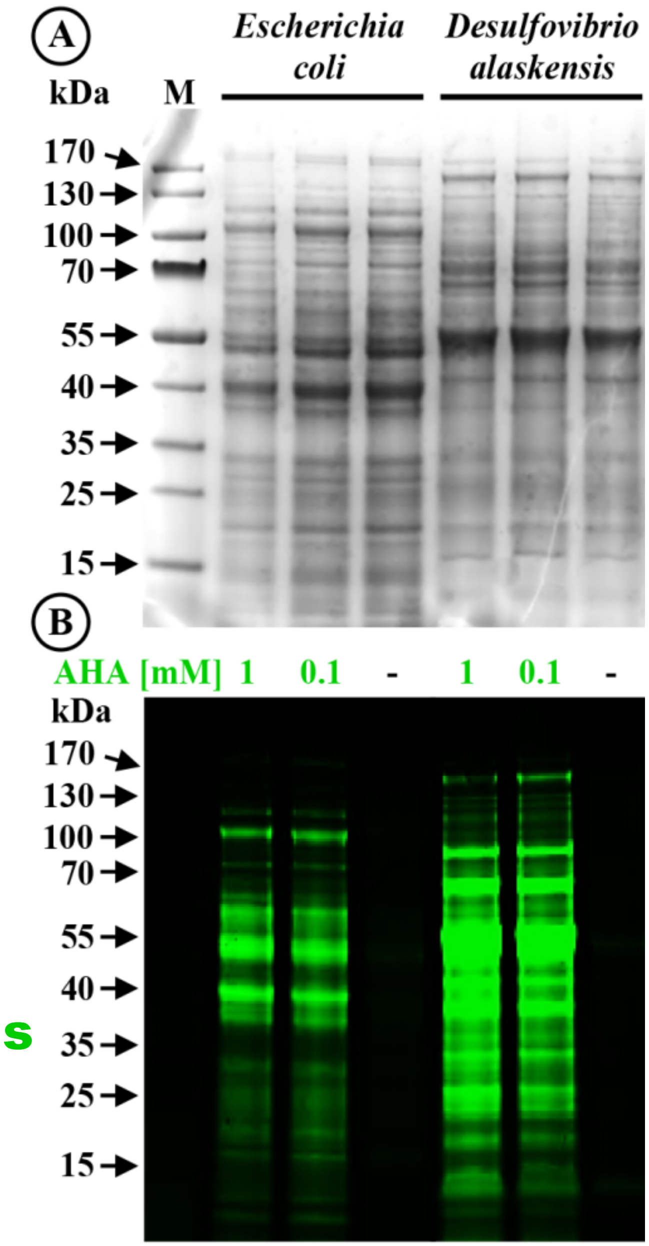
B. In Cu(I)-catalyzed click chemistry an azide group (N_3) is linked to a terminal alkyne residue, yielding a triazole conjugate.

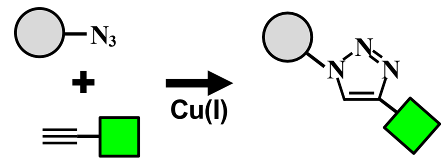
C. Strain-promoted click chemistry allows the copper-less conjugation of an azide group (N_3) with a cyclo-octyne-carrying molecule, yielding a triazole conjugate.

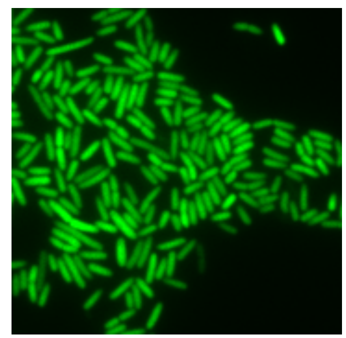
Hatzenpichler *et al.*, 2015

Incorporation into newly made proteins

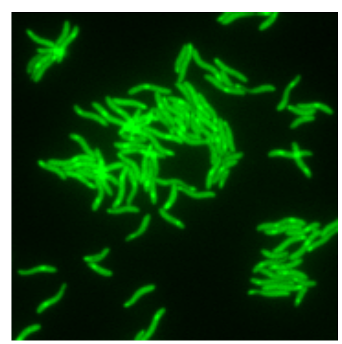
new proteins







E. coli
respiring glucose



D. alaskensis
sulfate reducer

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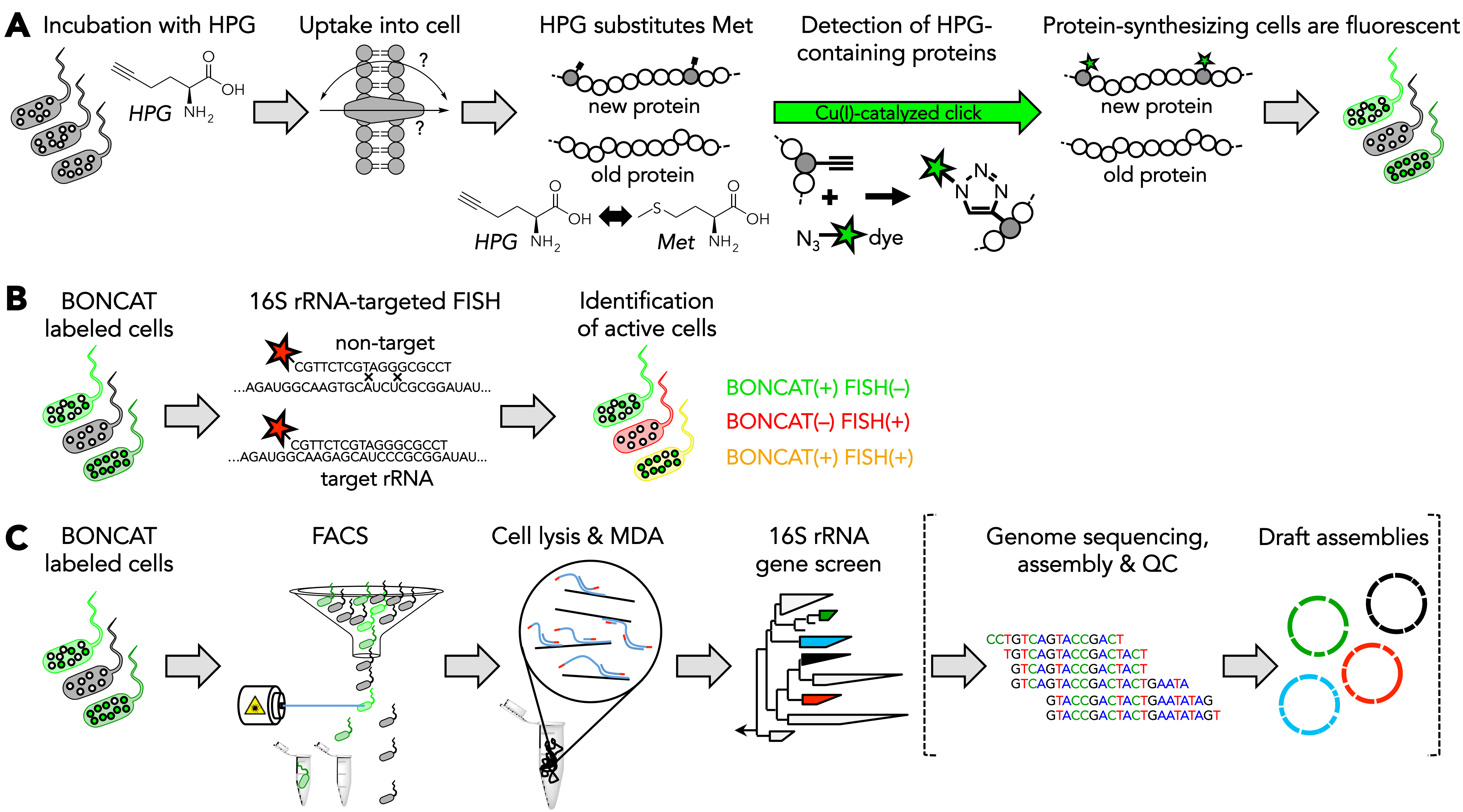
Hatzenpichler *et al.*, 2014

Visualizing, identifying, and sorting translationally active microbes



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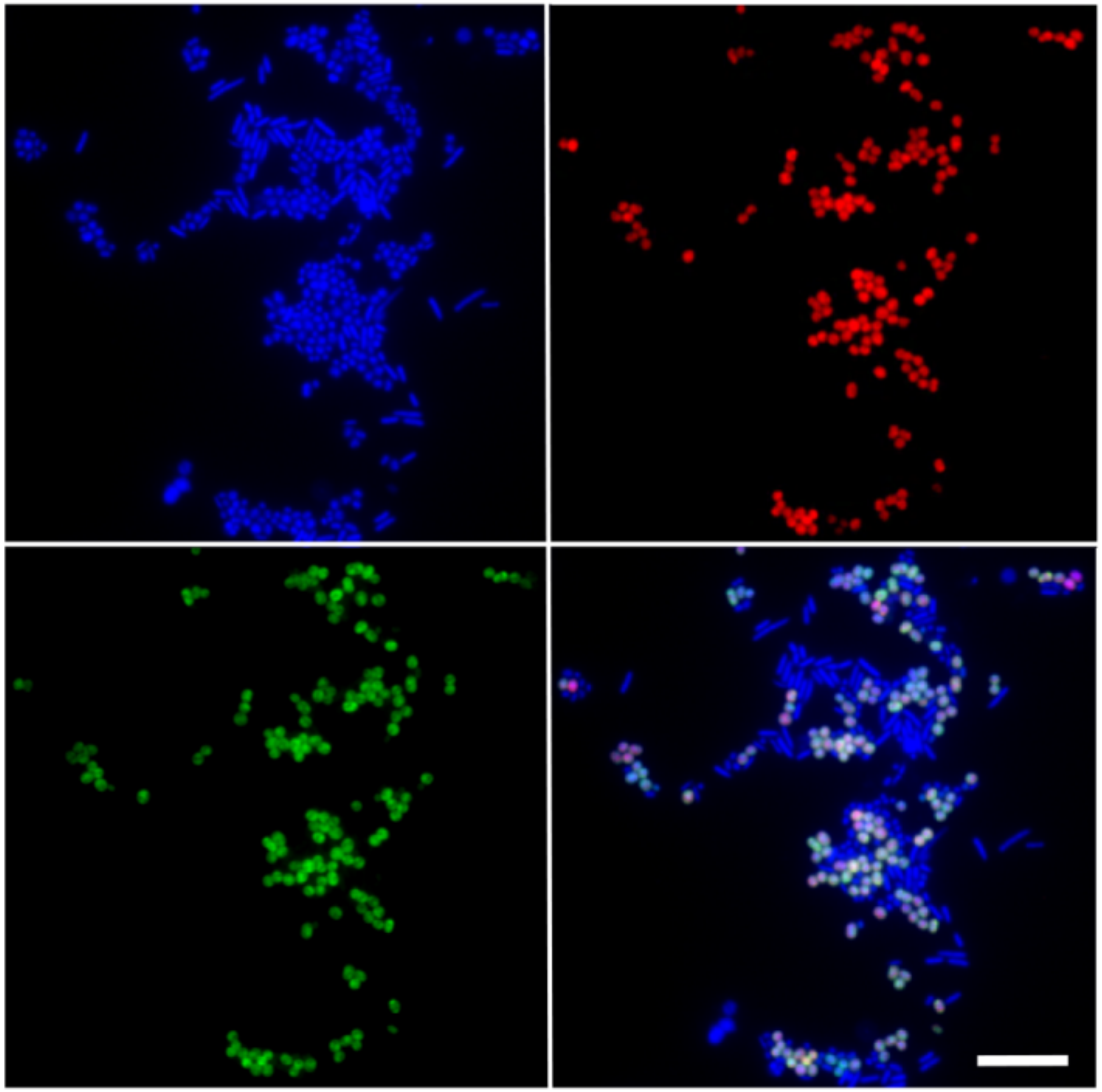
Hatzenpichler *et al.*, 2016

Identification of translationally active cells

DAPI
(DNA)

γ-Proteo WF-1
(rRNA)



BONCAT
(new proteins)

overlay

Bar = 10 µm

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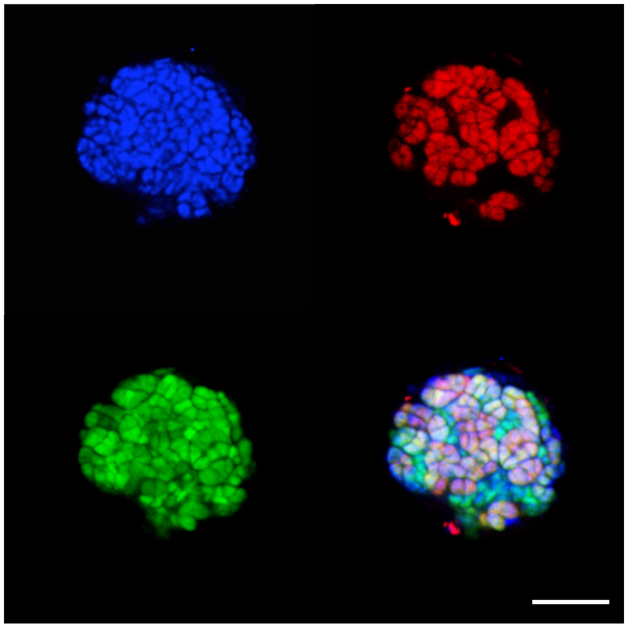
Hatzenpichler *et al.*, 2014

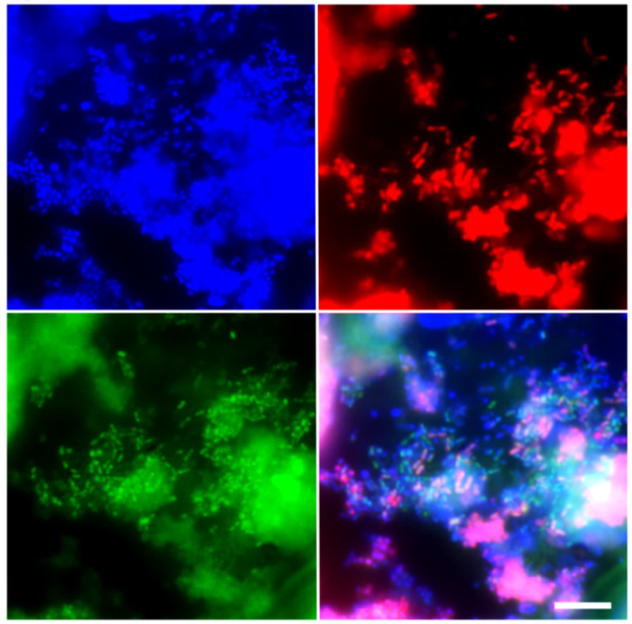
BONCAT-FISH of uncultured microbes

Arch915



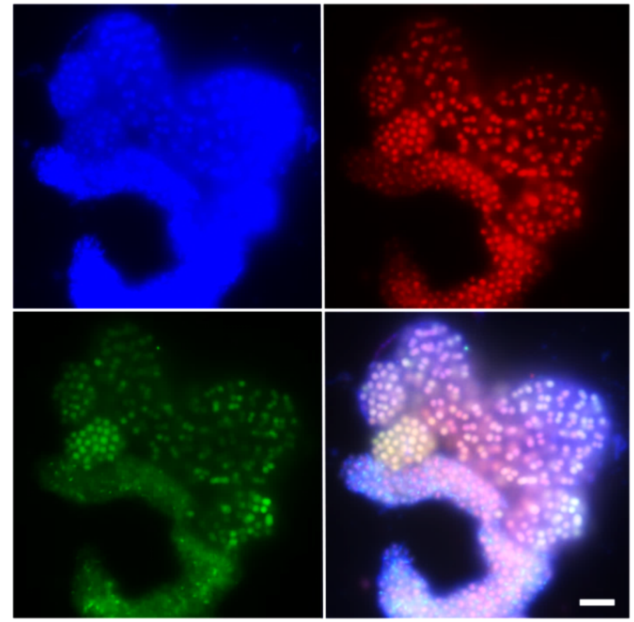
Methane seep
ANME-SRB consortium

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*

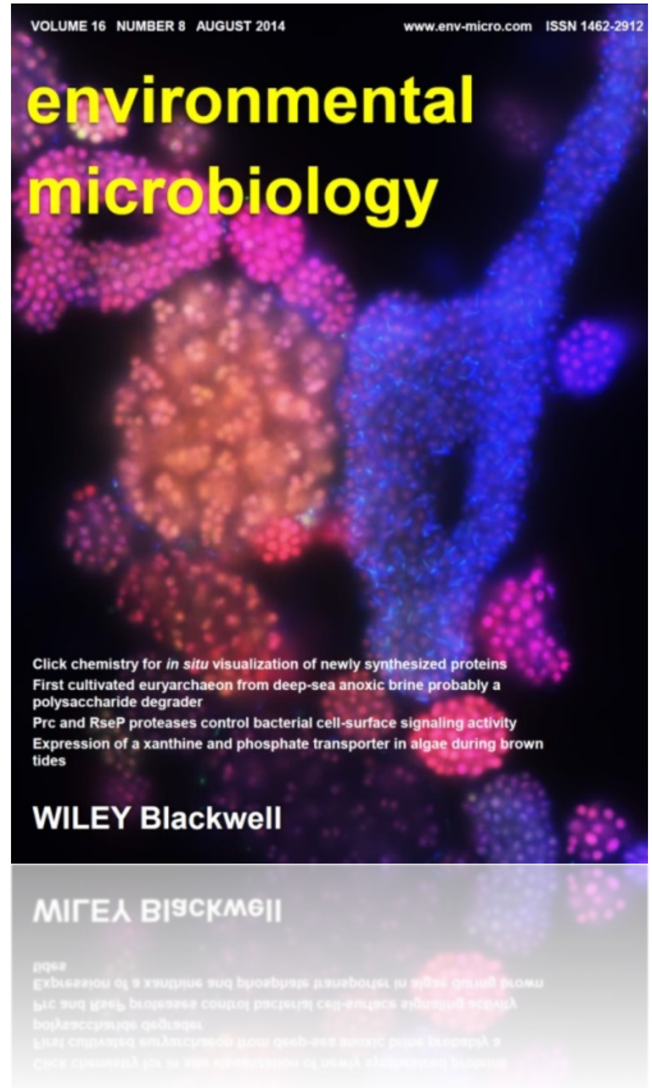
generally applicable
(works for all taxonomies and physiologies tested so far)

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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BONCAT in environmental microbiology (as of Jul 2019)

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 in the environment; 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 Mar Sci, doi: 10.3389/fmars.2014.00048 (2014)
- ➢ *application of BONCAT to seawater; correlation of BONCAT with MAR*

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*

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; applied BONCAT-FISH and BONCAT-FACS to deep-sea sediment consortia

Pasulka AL et al.
Interrogating marine virus-host interactions and elemental transfer with BONCAT and nanoSIMS-based methods
Environ Microbiol, DOI: 10.1111/1462-2920.13996 (accepted) 2017
- ➢ *first application of BONCAT to viruses; estimate of marine viral production rates by BONCAT and nanoSIMS*

Leizeaga et al.
Using Click-Chemistry for Visualizing *in Situ* Changes of Translational Activity in Planktonic Marine Bacteria
Front Aquat Microbiol, doi: 10.3389/fmicb.2017.02360 (2017)

Couradeau et al.
Probing the active fraction of soil microbiomes using BONCAT-FACS
Nat Comm, 10: 2770 (2019)

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