# Proteins with genetically encoded tailor made functionalities

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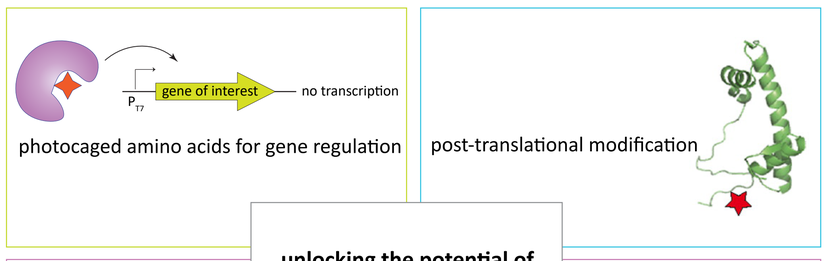
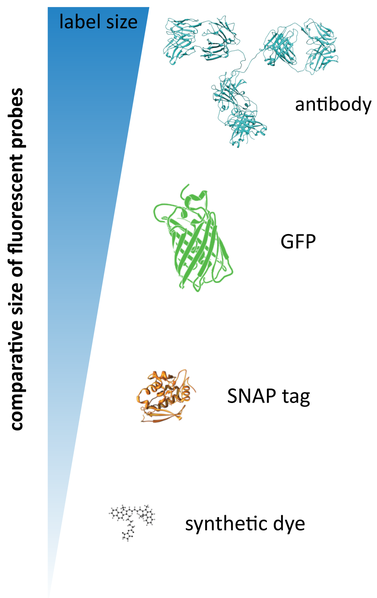
## Introduction

The ability to site-specifically modify a protein can be a powerful tool in elucidating consequences of a variety of post translational modifications or visualizing proteins *in-vivo*.
Genetic code expansion is unique in its ability to control protein function with single-residue precision in a variety of organisms. It is widely used to perform labeling for microscopy, photocontrol cellular function, protein crosslinking or introduce post-translational modifications.

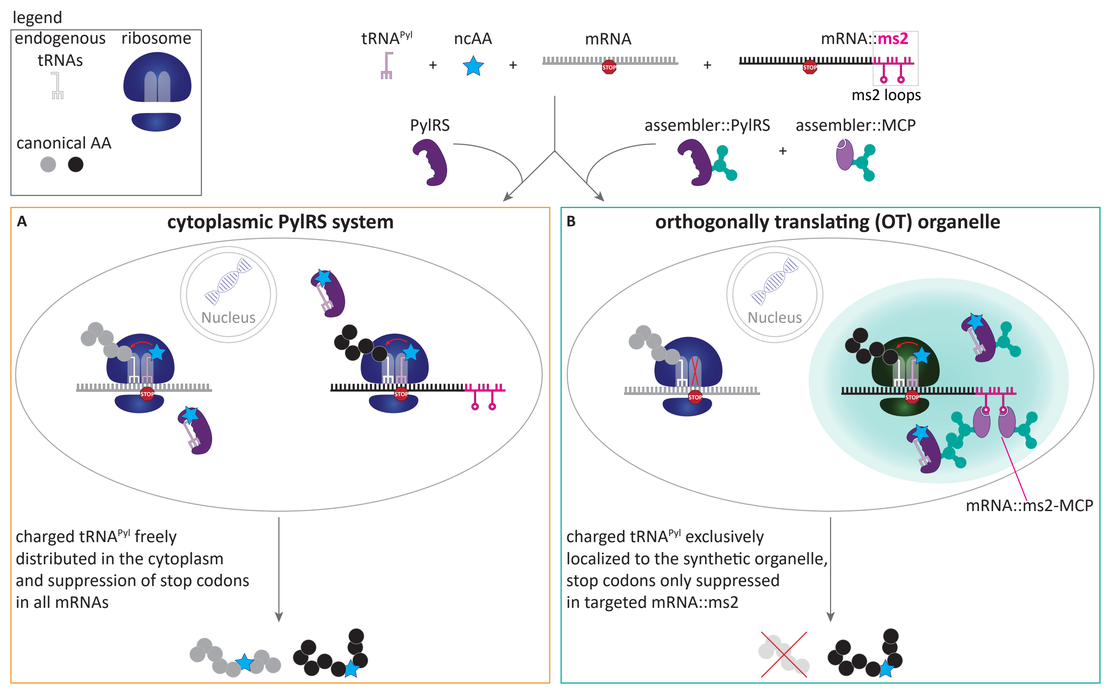
Particularly relevant for high resolution fluorescence microscopy is its strength to bring down the the size of a fluorescent probe to a minimum. This is achieved by introducing an orthogonal tRNA/synthetase suppressor pair into the living host which reassigns a stop codon to incorporate a noncanonical amino acid (ncAA) harbouring a unique chemical manipulation handle. To this handle a small fluorophore can be coupled in a biocomaptible fashion.
Despite the potential, this technology suffers from lack of mRNA selectivity which imapcts cellular physiology. Furthermore, the method is limited by the number of availabe codons to reassign.



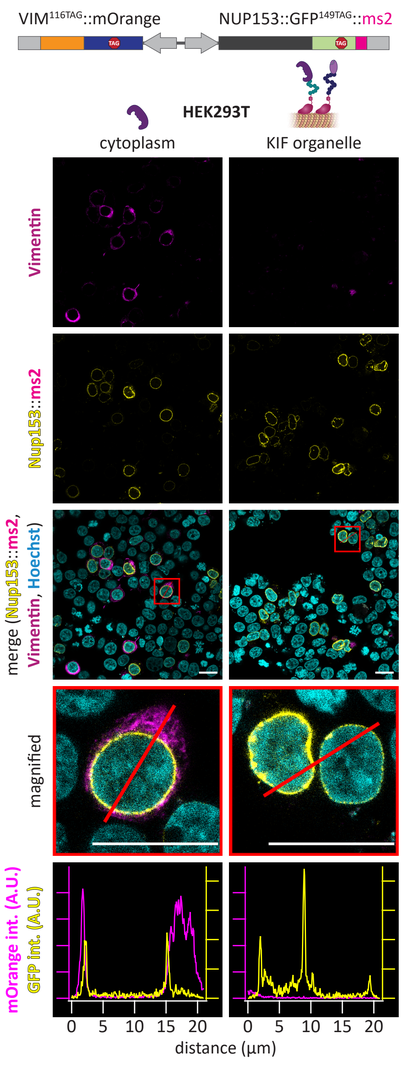




## Designer organelle to synthesize multi-functional proteins

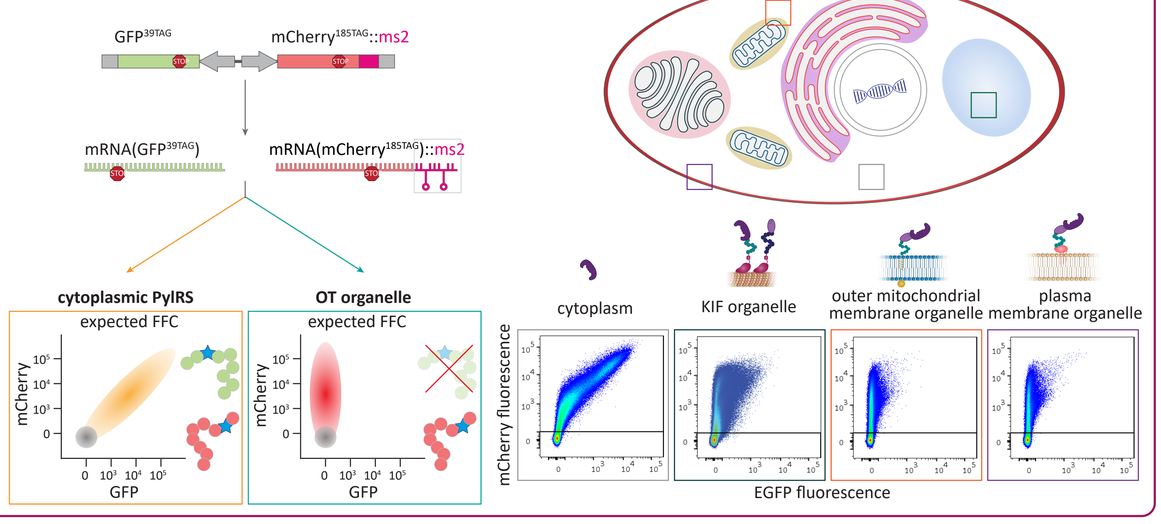
To solve the selectivity issue we hypothesized that it is possible to create an orthogonal translation system by spatially enriching specific components of the GCE machinery in an artificial orthogonally translating (OT) organelle.
(**A**) The cytoplasmic pyrrolysyl aminoacyl-tRNA- synthetase (PylRS) aminoacylates its cognate stop codon suppressor $tRNA^{Pyl}$ with an ncAA. This leads to cotranslational ncAA incorporation when the respective stop codon occurs in the mRNA of the POI. However, many endogenous mRNAs terminate on the same stop codon, potentially leading to ncAA misincorporation.
(**B**) Spatially enriching components for GCE, i.e. the mRNA of the POI, PylRS, $tRNA^{Pyl}$, and ribosomes through the use of phase-separating "assemblers" to an OT organelle should enable selective translation of only the recruited mRNAs.
Whereas, in (A) GCE is stop codon specific, in (B), it is stop codon- and mRNA-specific.




## Selective translation by OT organelle

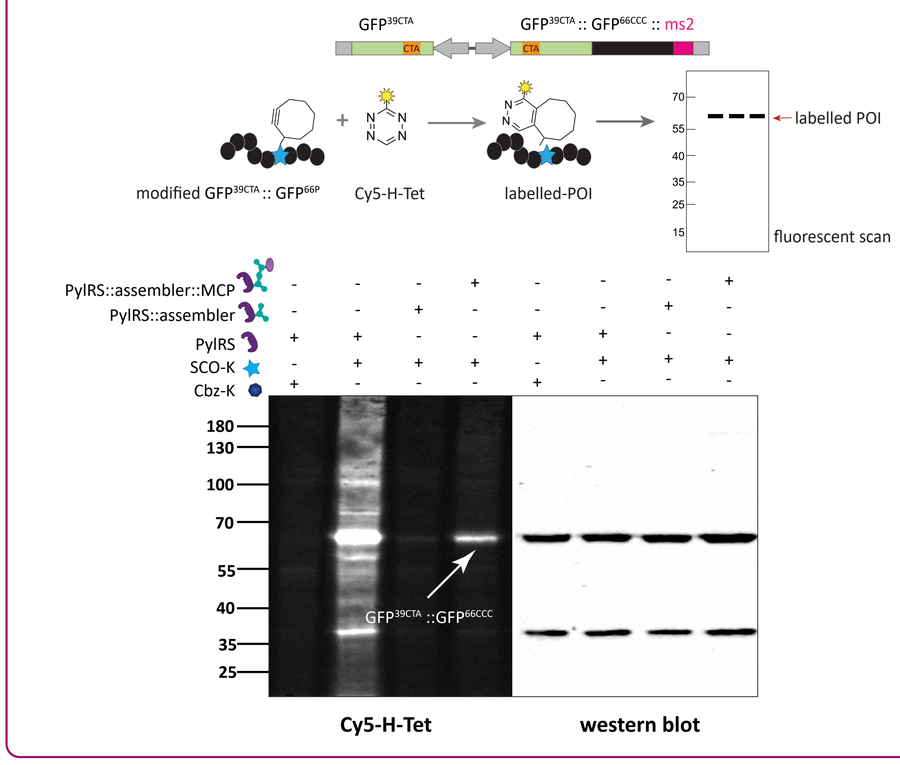



The ability of the synthetic OT organelle to render GCE protein specific was validated by confocal microscopy and flow-cytometry. In both cases a pair of reporter proteins were expressed simultaneously from a bicistronic promoter. However mRNA of only one of them was equipped with an organelle targeting domain (ms2 loop). Since the GCE machinery is confined to the organelle, only the targetted mRNA should get translated.
Confocal images of HEK293T shows the organelle selectively translating NUP153::$GFP^{149TAG}$ whereas in the absence of the same both $vimentin^{114TAG}$::mOrange and NUP153::$GFP^{149TAG}$ are expressed.
In agreement with our hypothesis flow-cytometry plots for the various types of organelles show a dominant signal for the POI $mCherry^{185TAG}$ as opposed to the cytoplasmic system that shows signal for both $mCherry^{185TAG}$ and $GFP^{39TAG}$



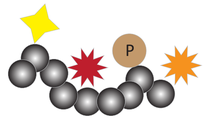

## GCE beyond stop codons

Sense codon reassignment is the key to unlock the plethora of possibilities that is limited by restricting GCE to only stop codons. It has the potential to ultimately synthesize an entire bio-polymer with any desired functionality. However, it is challenging to design a reporter to assess success of this approach since unlike amber suppression the full length POI is translated even in case of failure. Hence we have used unique click-labelling properties of the ncAA to distinguish the modified POI.




## Summary & outlook

We have successfully been able to build a synthetic membraneless organelle that is capable of selectively translating mRNA of choice and thus have made amber suppression protein specific. The aim now is to expand GCE to all the available sense codons and towards that effect we already have promising results for the reassignment of CTA codon. The success of this approach will provide us with the flexibility to introduce multiple ncAAs of desired functionality. It could for e.g be useful for studying disease models by the introduction of associated post-translational modifications to a POI simultaneously with multiple labels to elucidate the change in behaviour of the same. The organelle itself can be developed further to facilitate other biological reactions or mimic different naturally occuring condensed organelles and thereby provide a simpler model for studying the same.




A hypothetical protein with a caging group to regulate its activity and two site specifically incorporated dyes whose FRET measurements can elucidate conformational changes induced by a post-translational modification.

### References

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