

exploiting genetically encoded unnatural amino acids

technical journal club
16/02/2016

Manuela Pfammatter

protein biosynthesis

incorporation of unnatural amino acids – expanding the genetic code

expanding the genetic code of *E. coli*

Wang et al., Science, 2001

expanding the genetic code of an animal

Greiss et al., JACS, 2011

recent applications of unnatural amino acids

studying conformational changes by FRET

Wang et al., Nat Chem, 2014

studying the regulation of gene expression

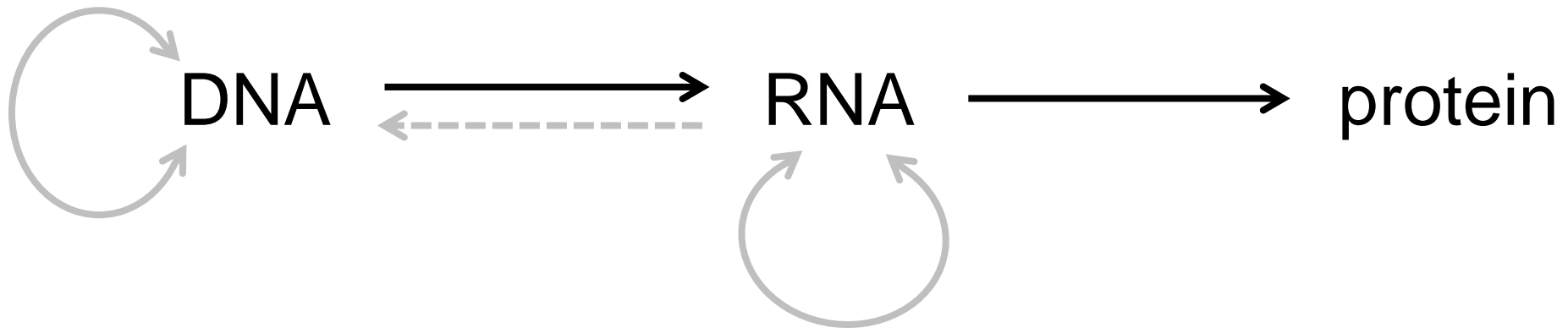
Elsässer et al., Nat Methods, 2016

conclusion & outlook

central dogma of molecular biology

heritable information

functional unit



unnatural polymers
new functions?

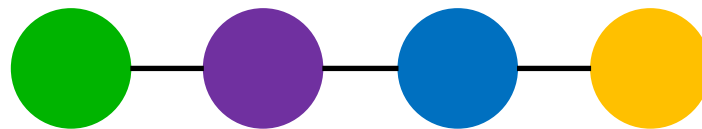
unnatural
amino acids

20 naturally
occurring
amino acids

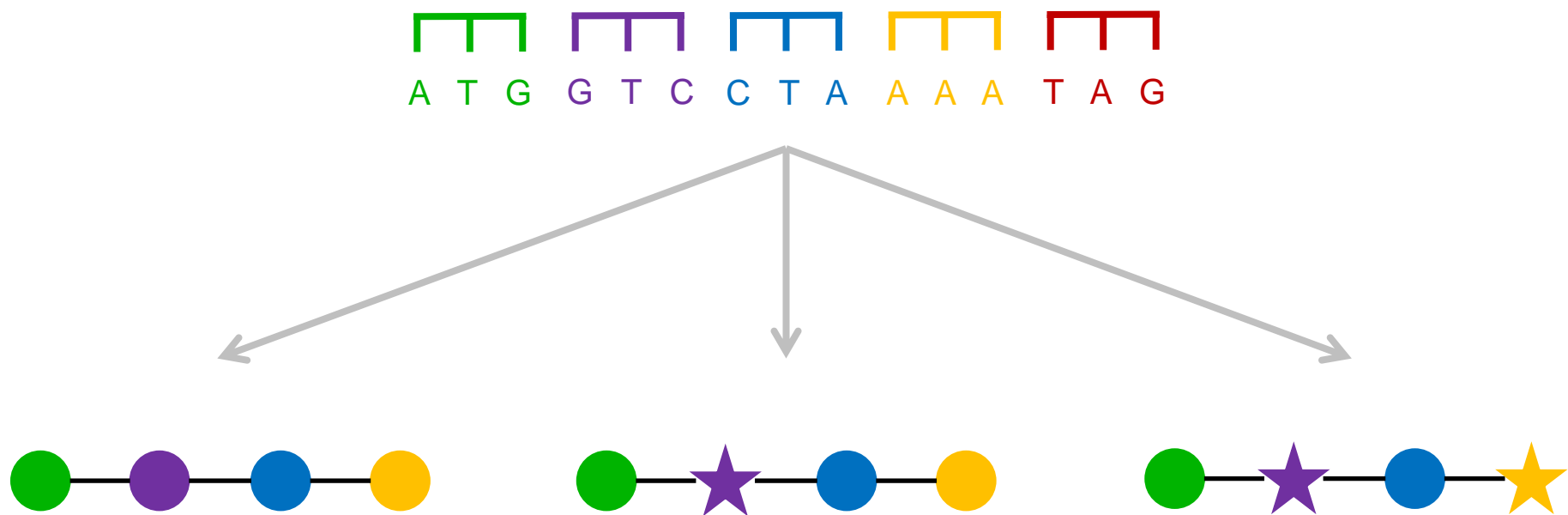
mechanism of translation



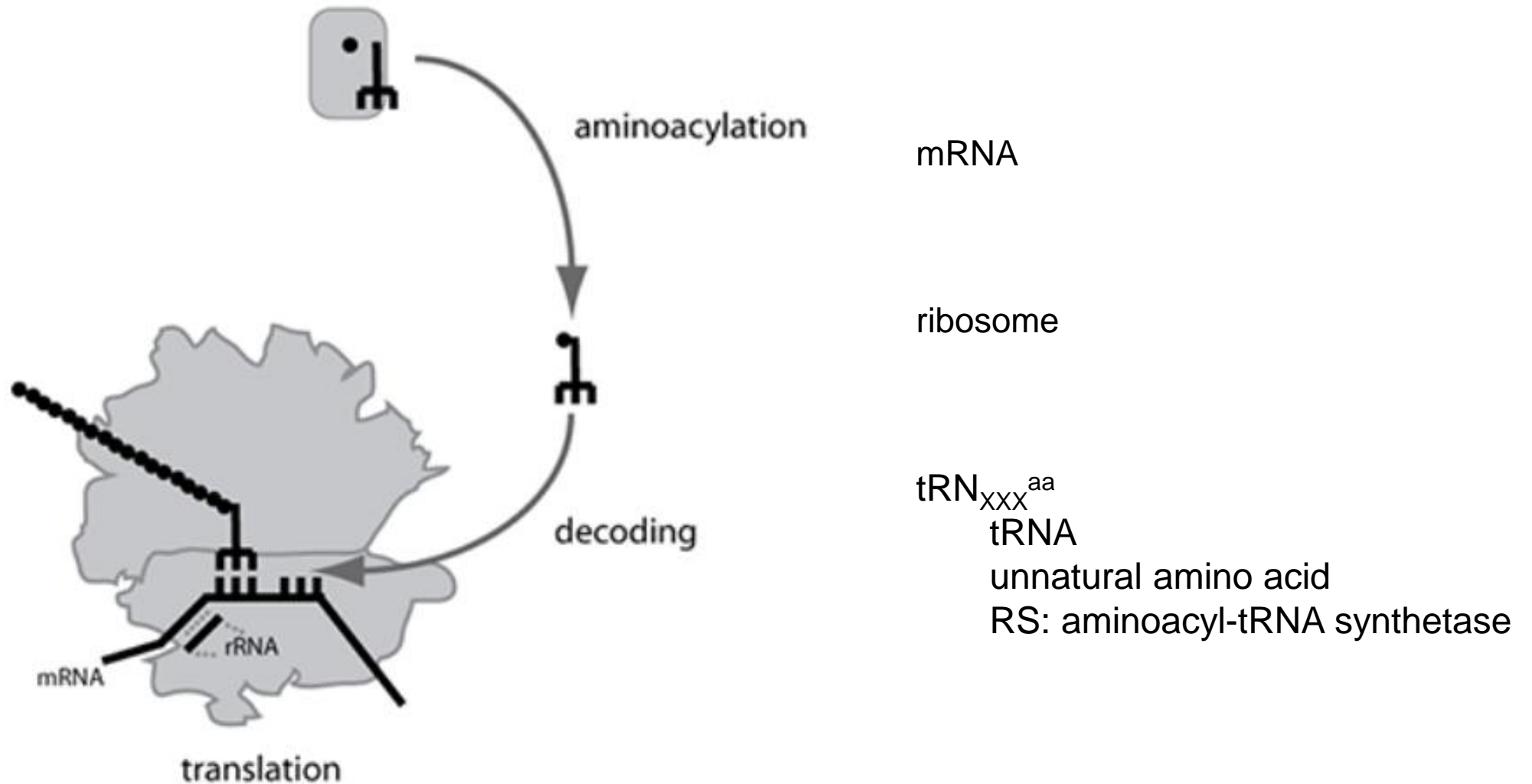
translation



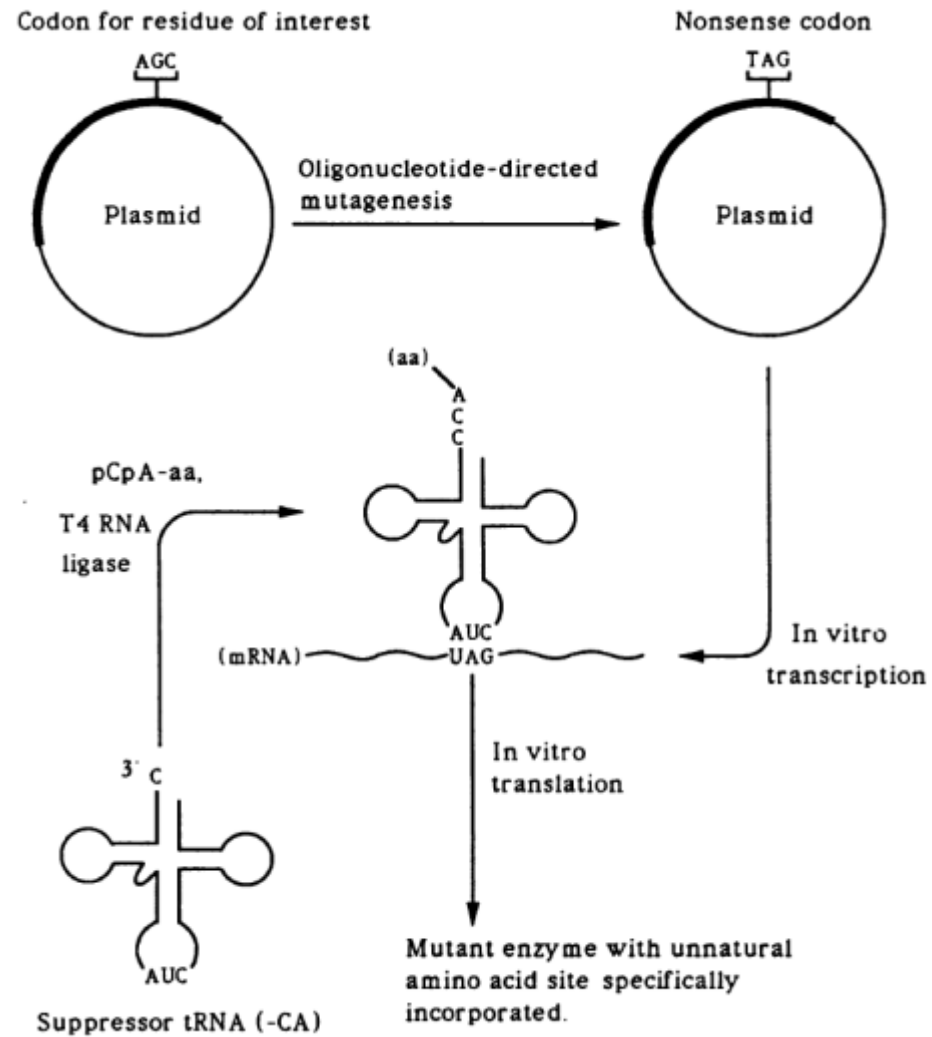
incorporation of unnatural amino acids – expanding the genetic code



protein biosynthesis and the translational machinery



site-specific incorporation of unnatural amino acids into protein



expanding the genetic code of an organism



E. coli

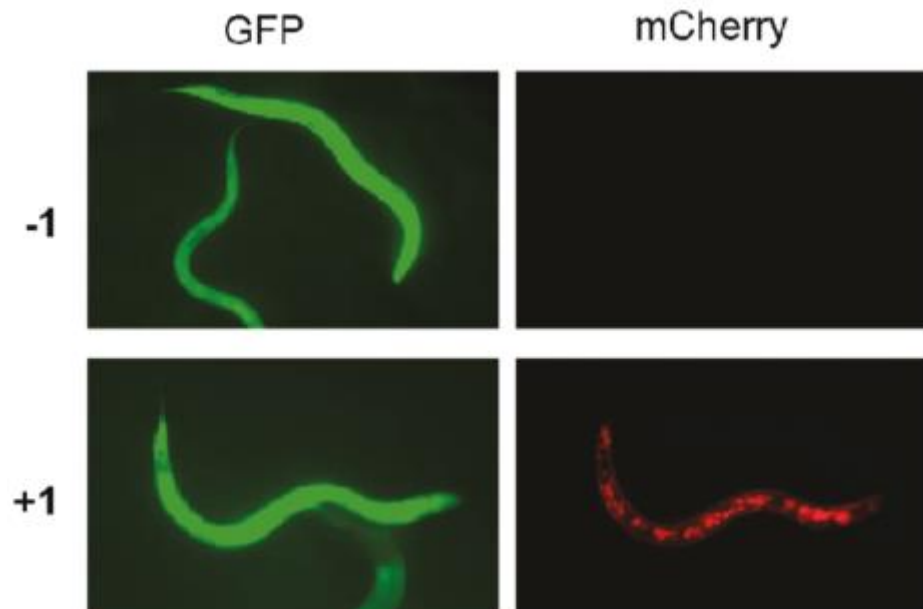
Wang and Schultz, 2001

C. elegans

Greiss and Chin, 2011

mammalian cells

Schmied and Chin, 2014



Optimized orthogonal translation of unnatural amino acids enables spontaneous protein double-labelling and FRET

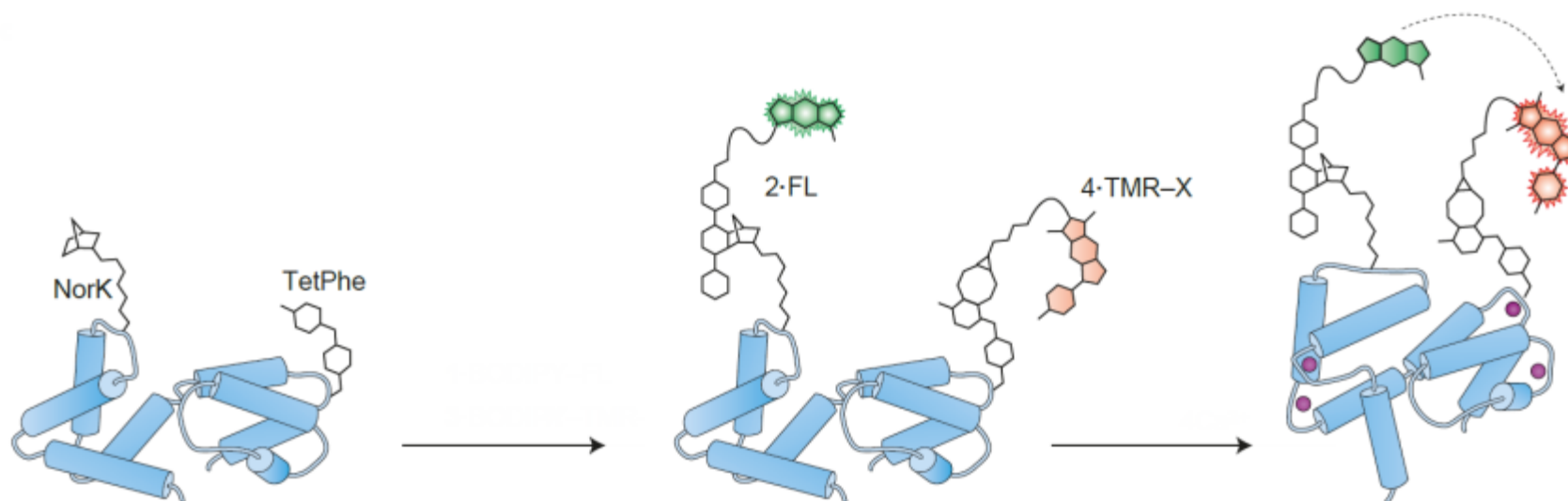
Kaihang Wang^{1,†}, Amit Sachdeva^{1,†}, Daniel J. Cox¹, Nabil M. Wilf[†], Kathrin Lang¹, Stephen Wallace¹, Ryan A. Mehl² and Jason W. Chin^{1*}

site-specific incorporation of multiple distinct unnatural amino acids

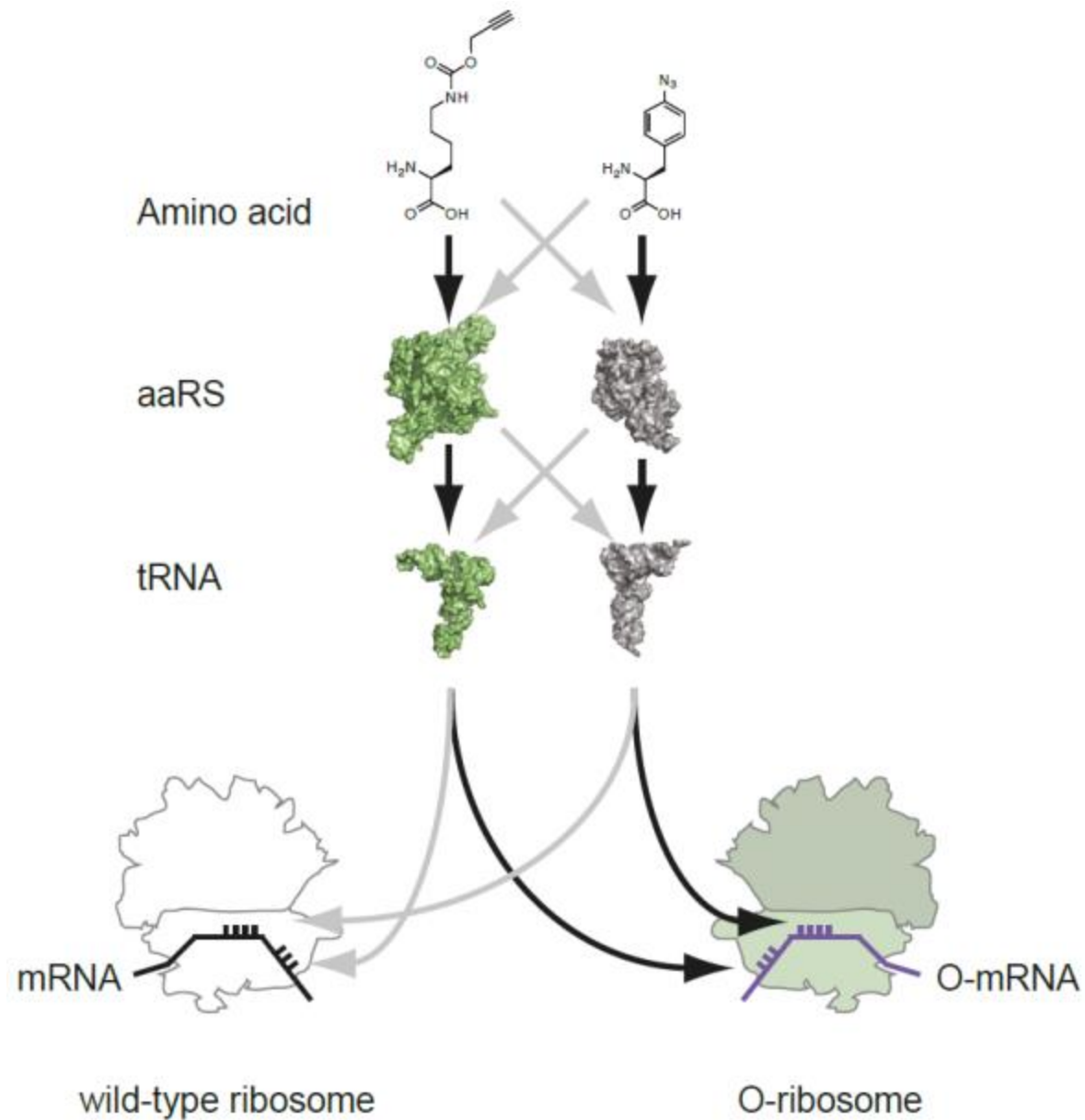
labelling pairs of unnatural amino acids

→ intraprotein FRET

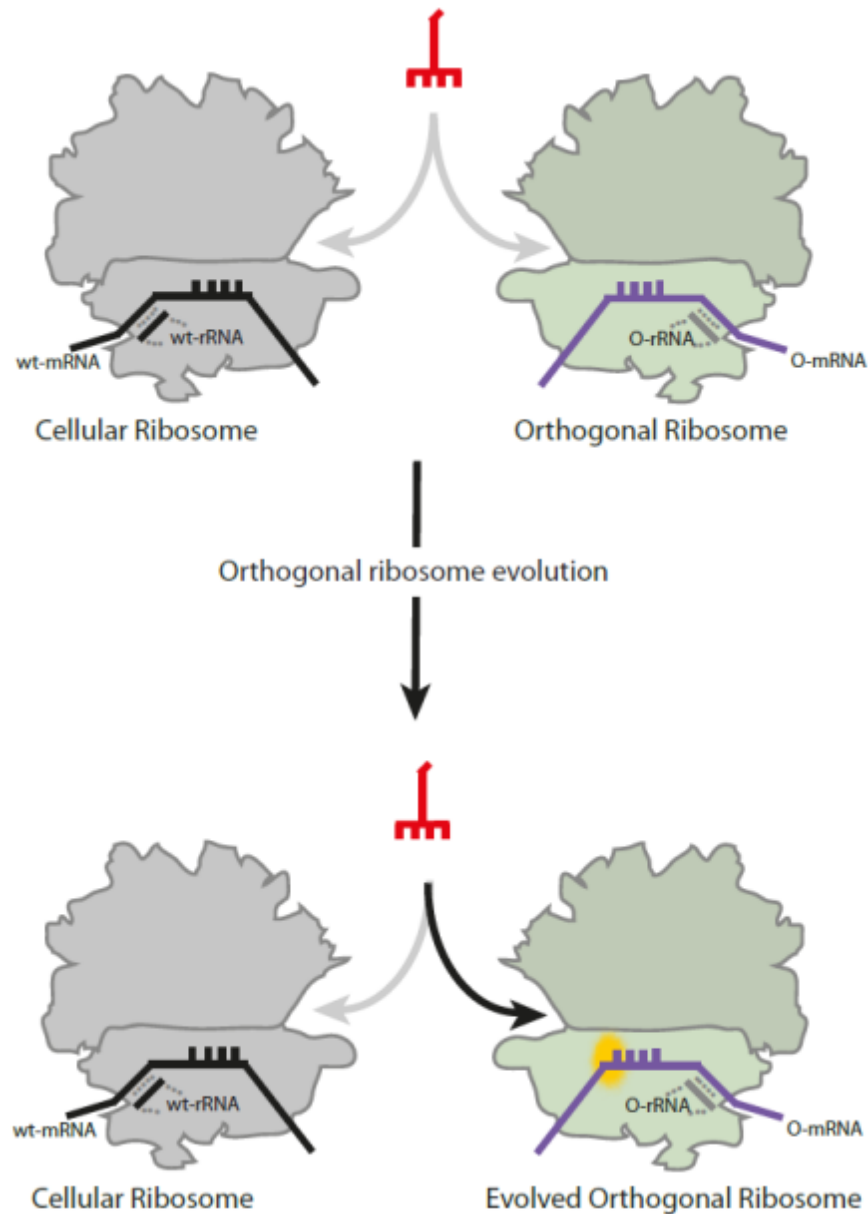
=> probing protein structure and dynamics



site-specific incorporation of multiple unnatural amino acids

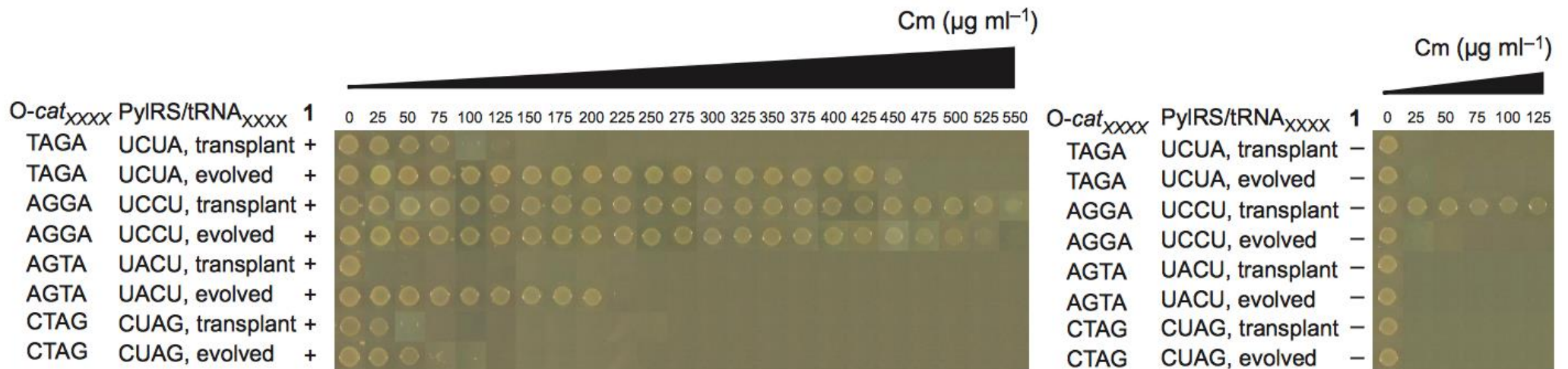
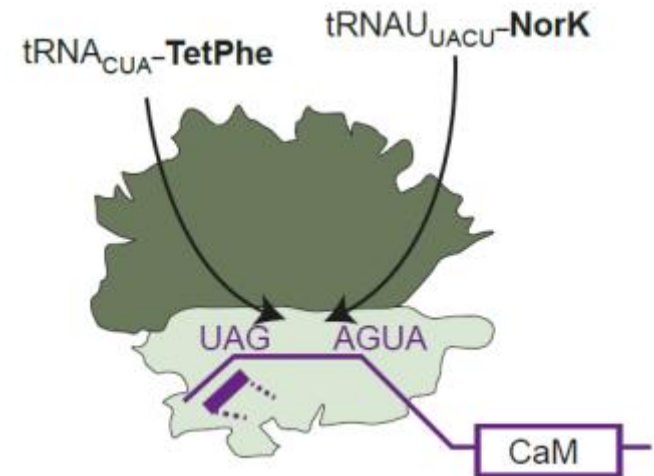
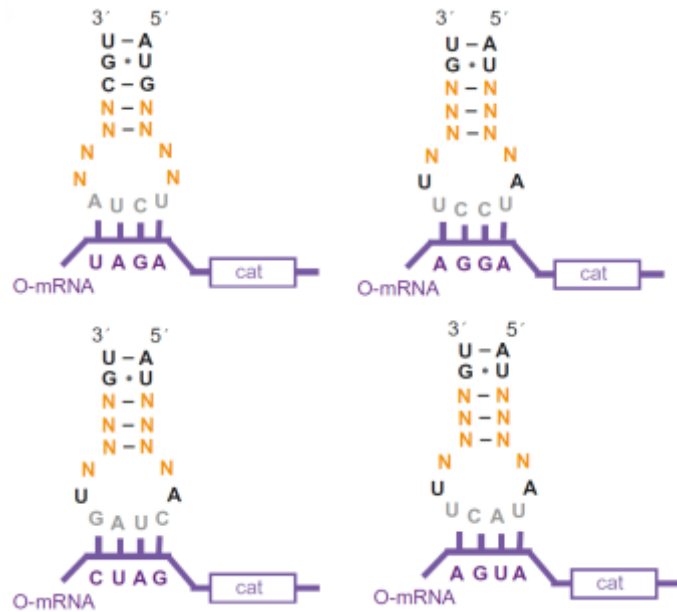


evolution of an orthogonal quadruplet decoding ribosome

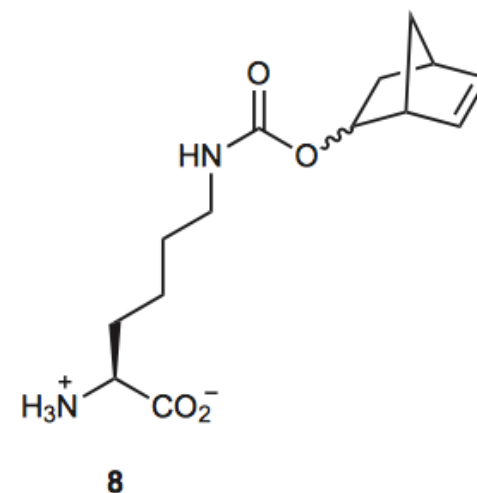
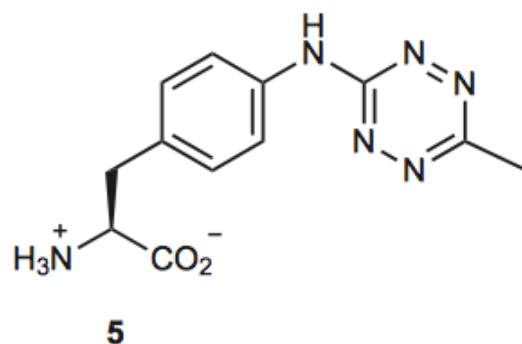
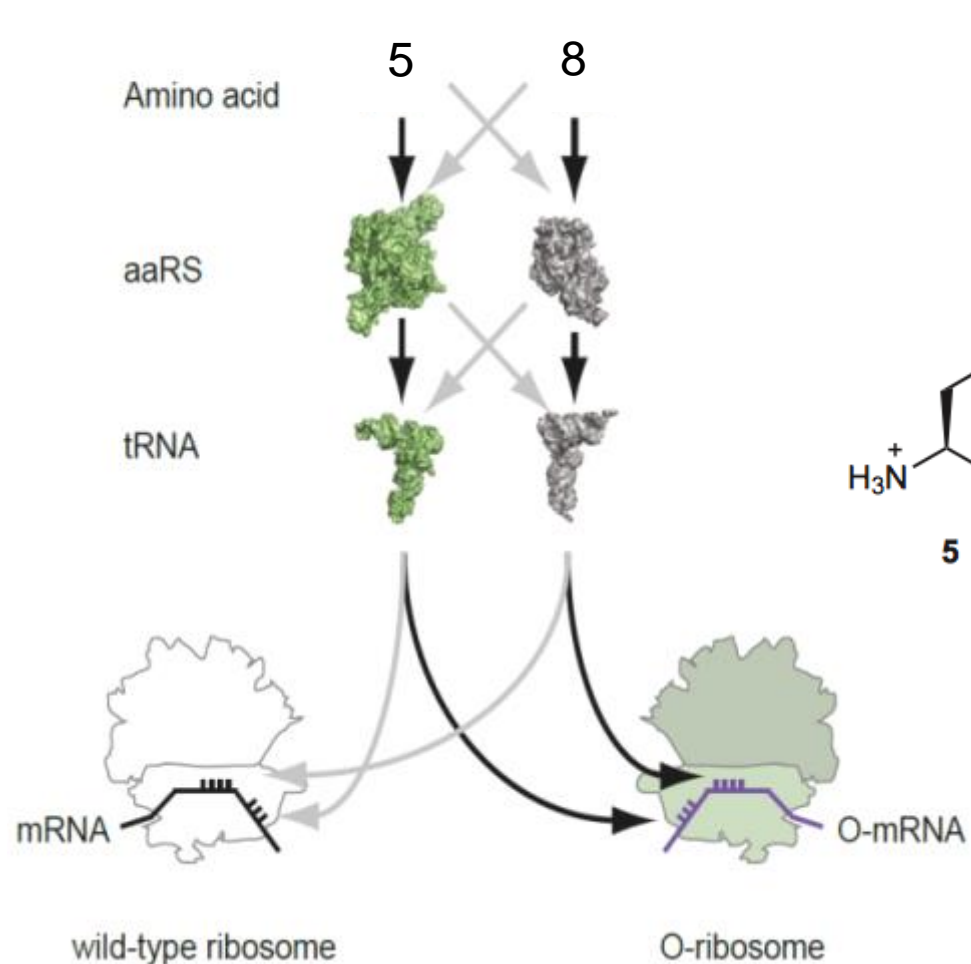


- mutagenesis in 16S rRNA (decoding centre)
- orthogonal aaRS/tRNA pairs
- mutant mRNA
 - decoding of quadruplet codons
 - orthogonal ribosome

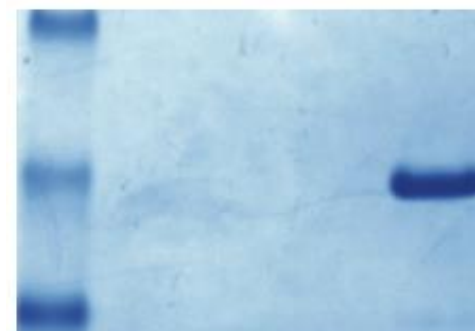
tRNA optimisation for incorporation unnatural amino acids



incorporation of unnatural amino acid pairs



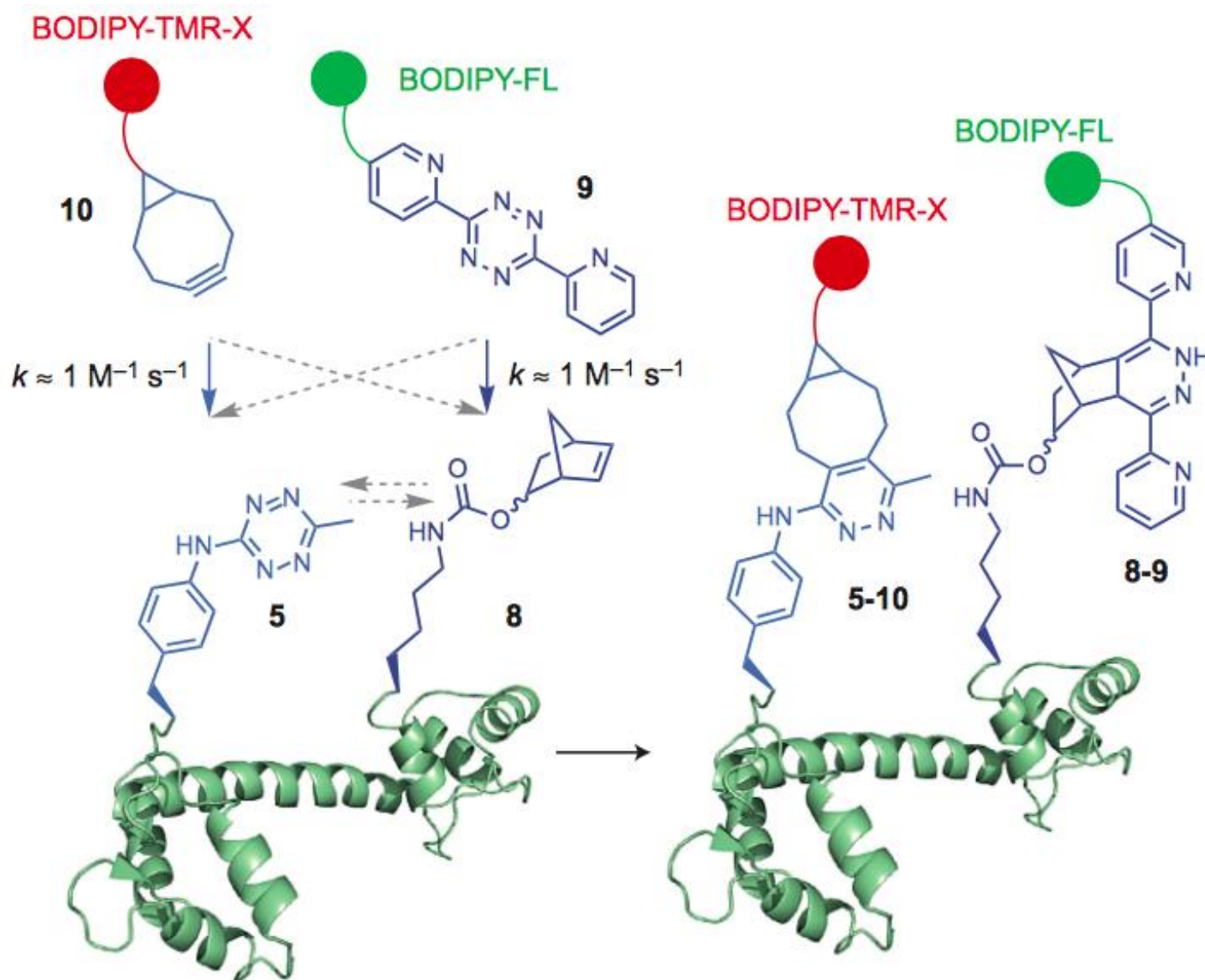
5:	-	+	-	+
8:	-	-	+	+



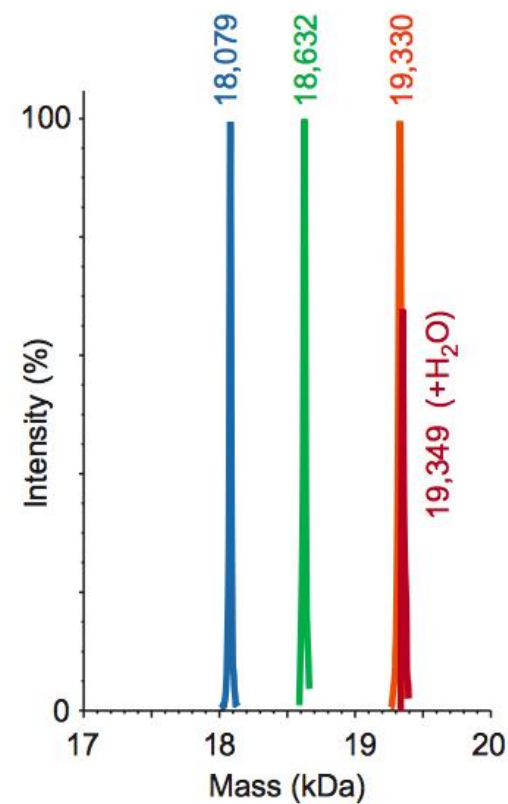
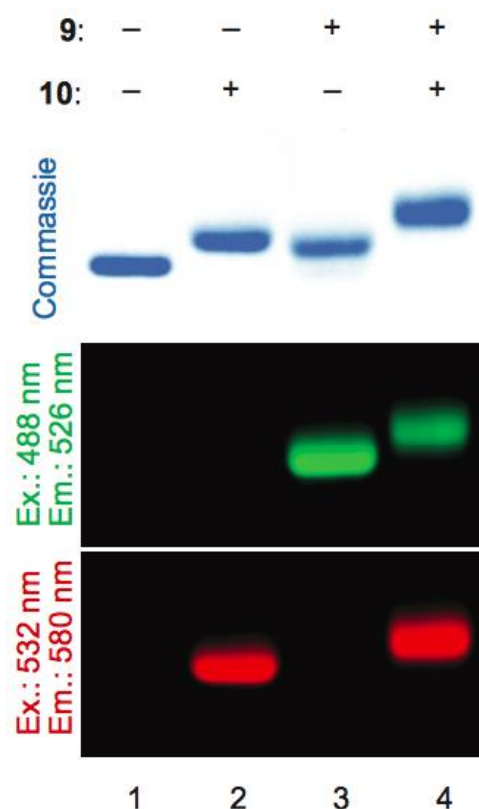
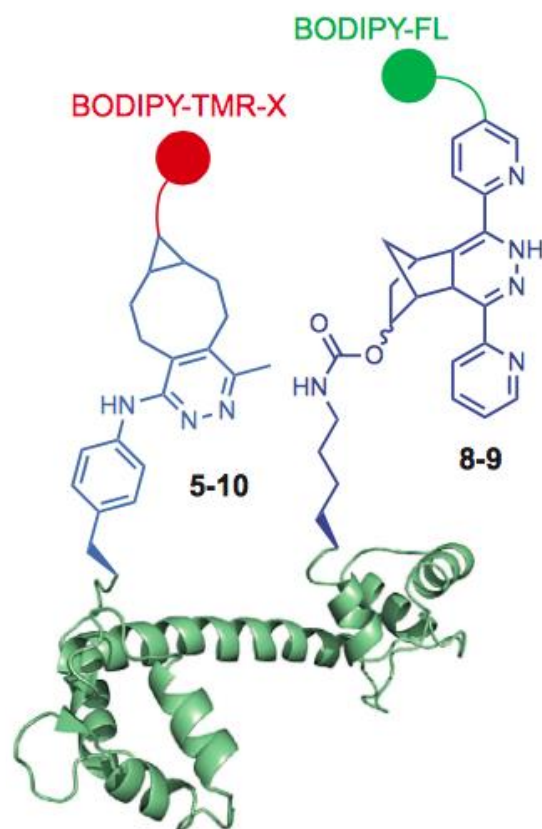
CaM

pRSF ribo-Q1 O-*gst-cam*_{1TAG+149AGTA}
 pSUP aa(5)RS/tRNA_{CUA}
 pCDF aa(8)RS/tRNA_{UACU}

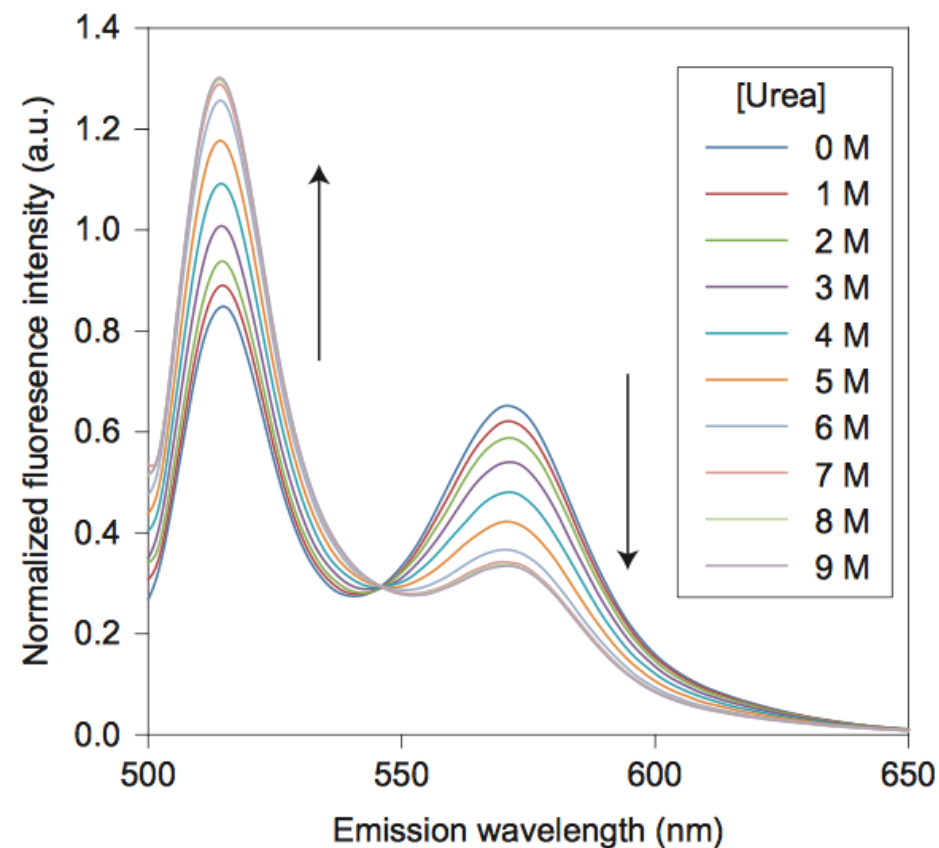
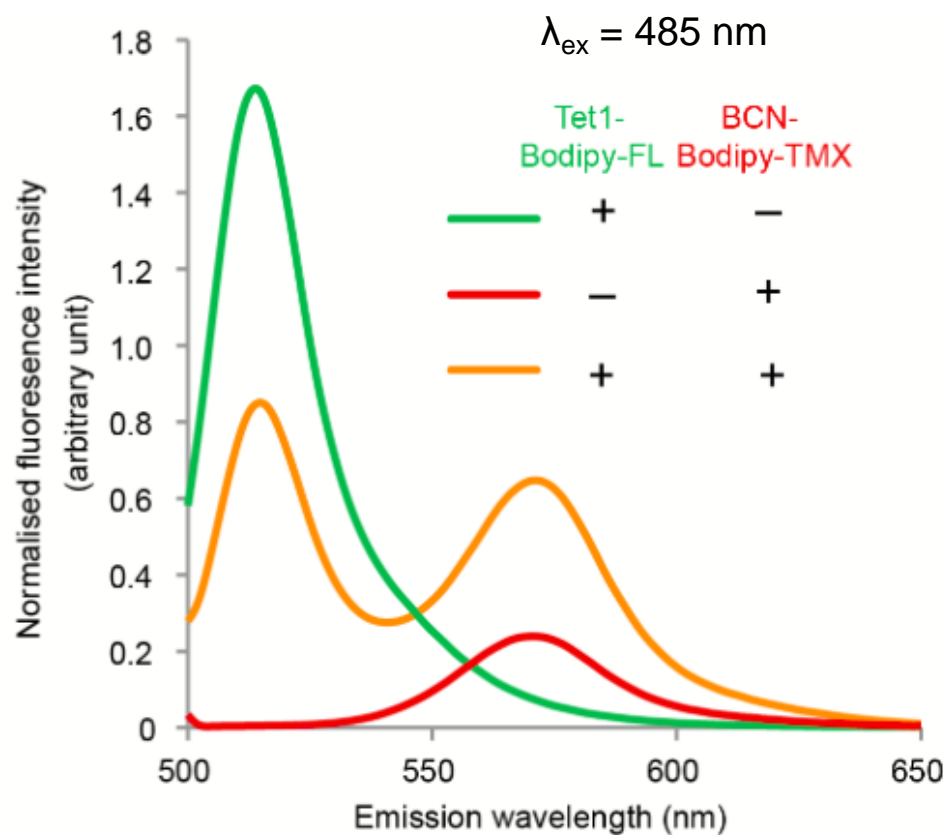
labelling unnatural amino acid with FRET fluorophores



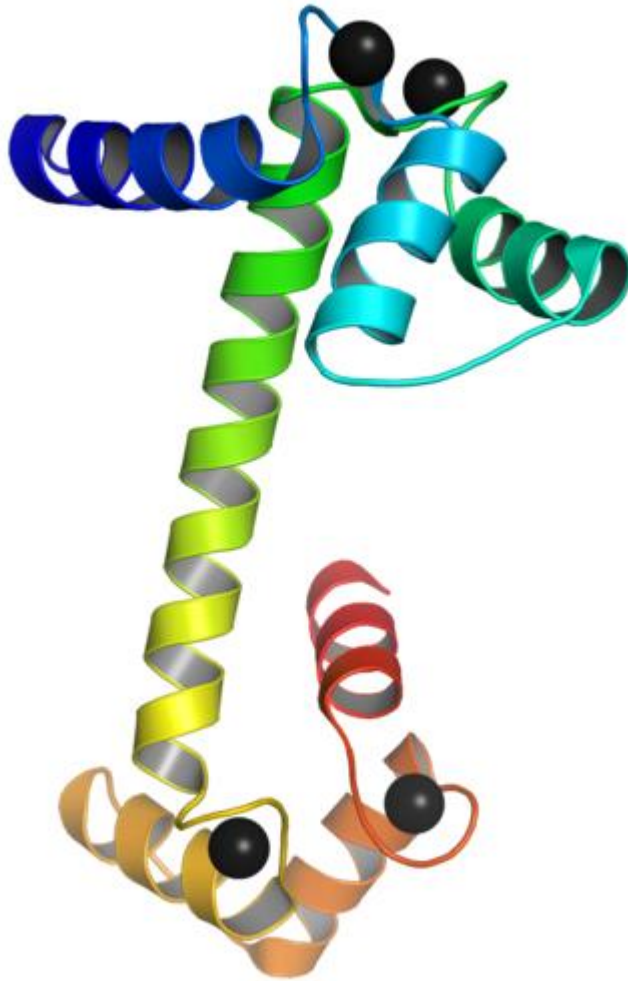
labelling unnatural amino acid with FRET fluorophores



using FRET to follow protein conformational change



studying calcium binding of CaM

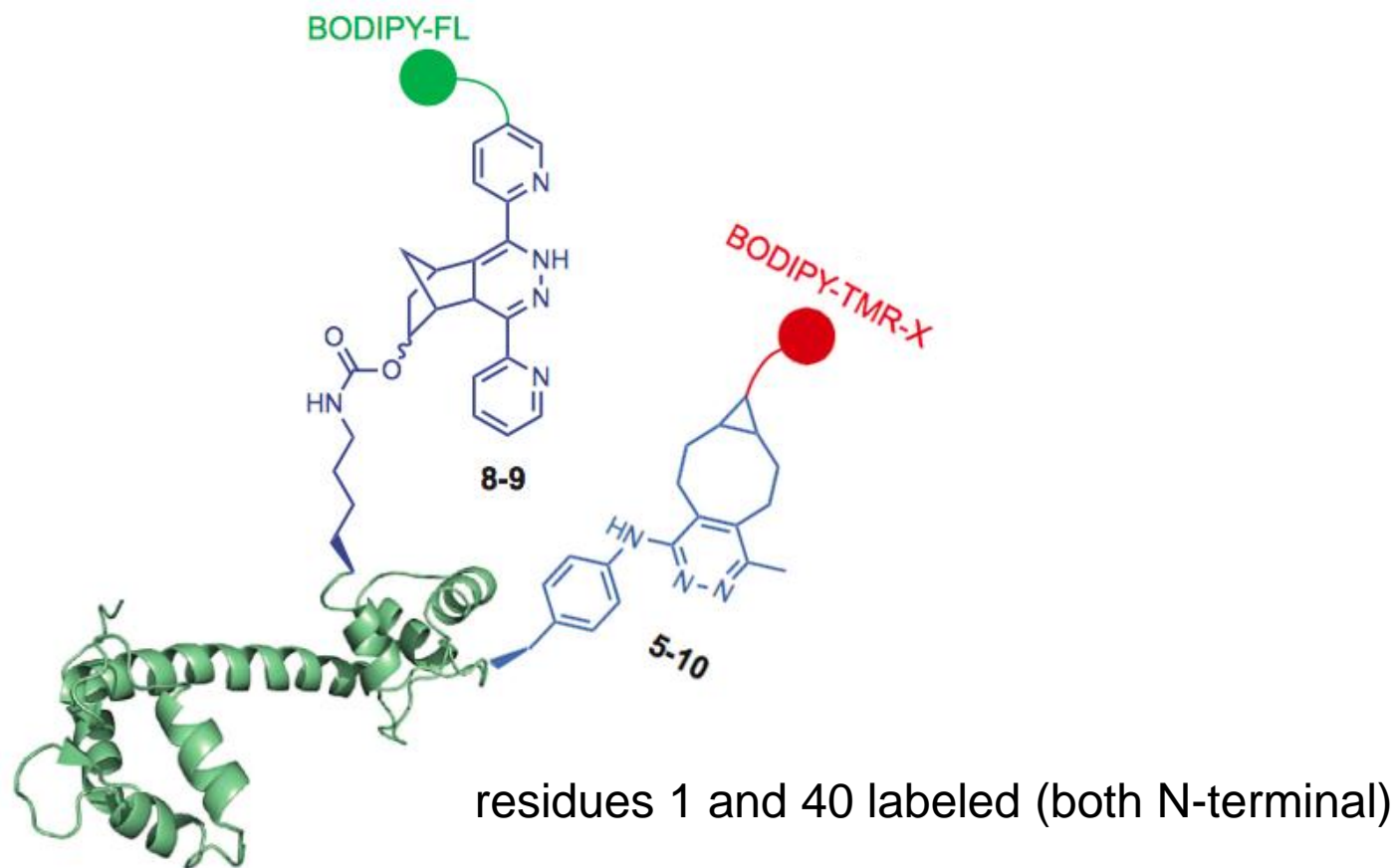


4EF-hand motifs

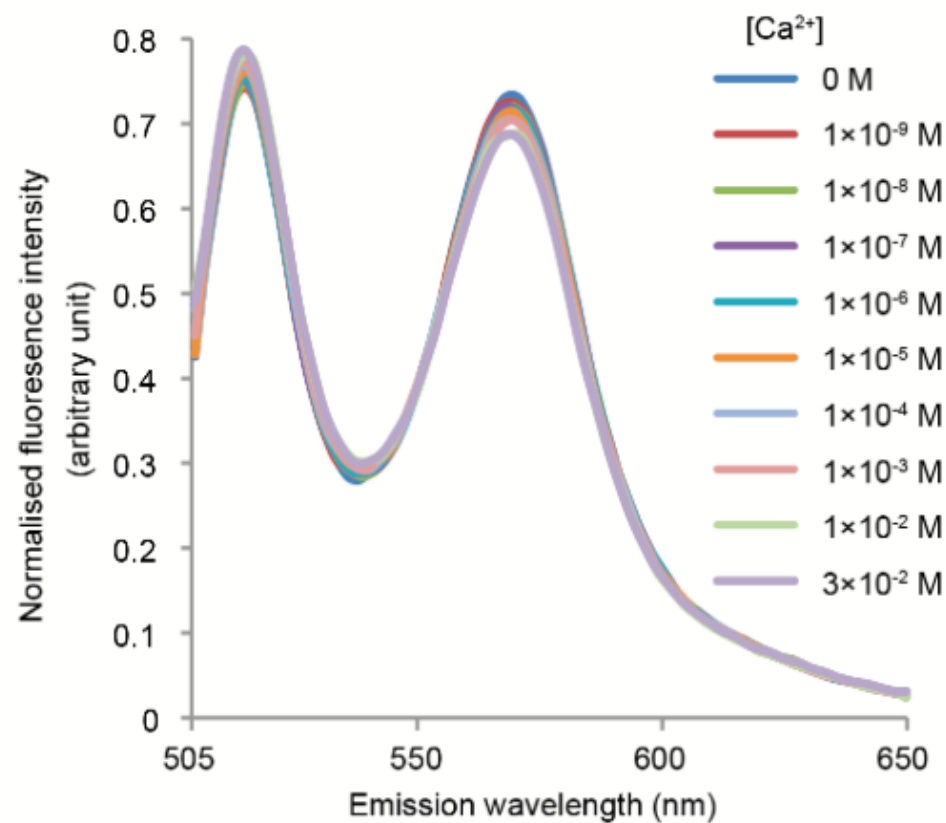
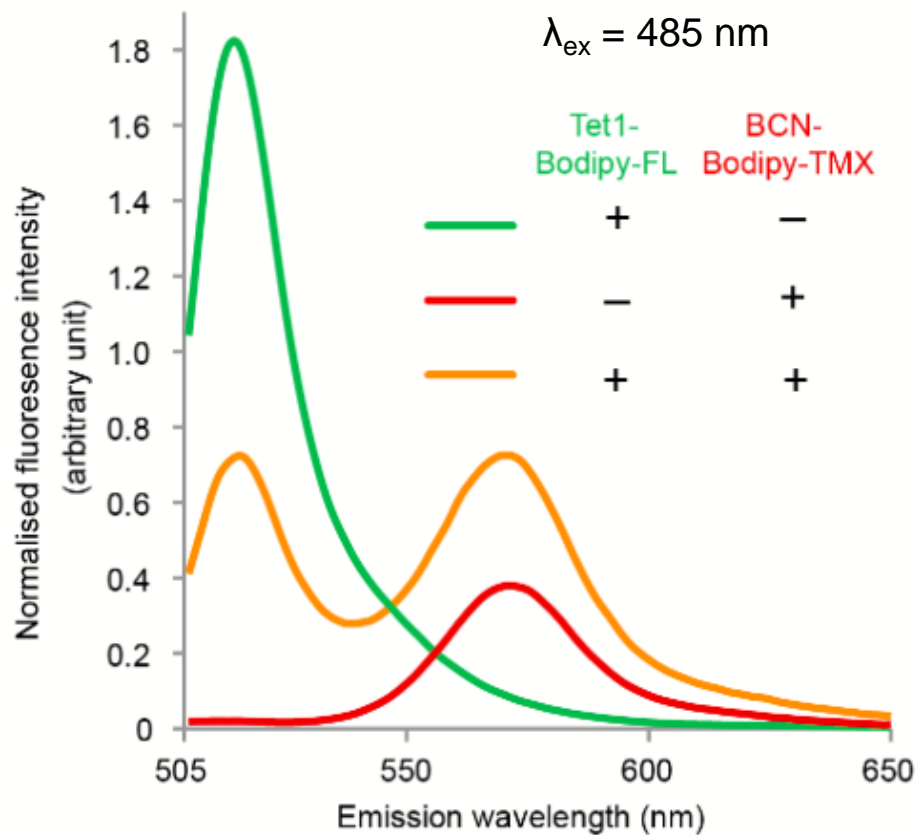
binding sites for calcium ions

PDB: 1EXR

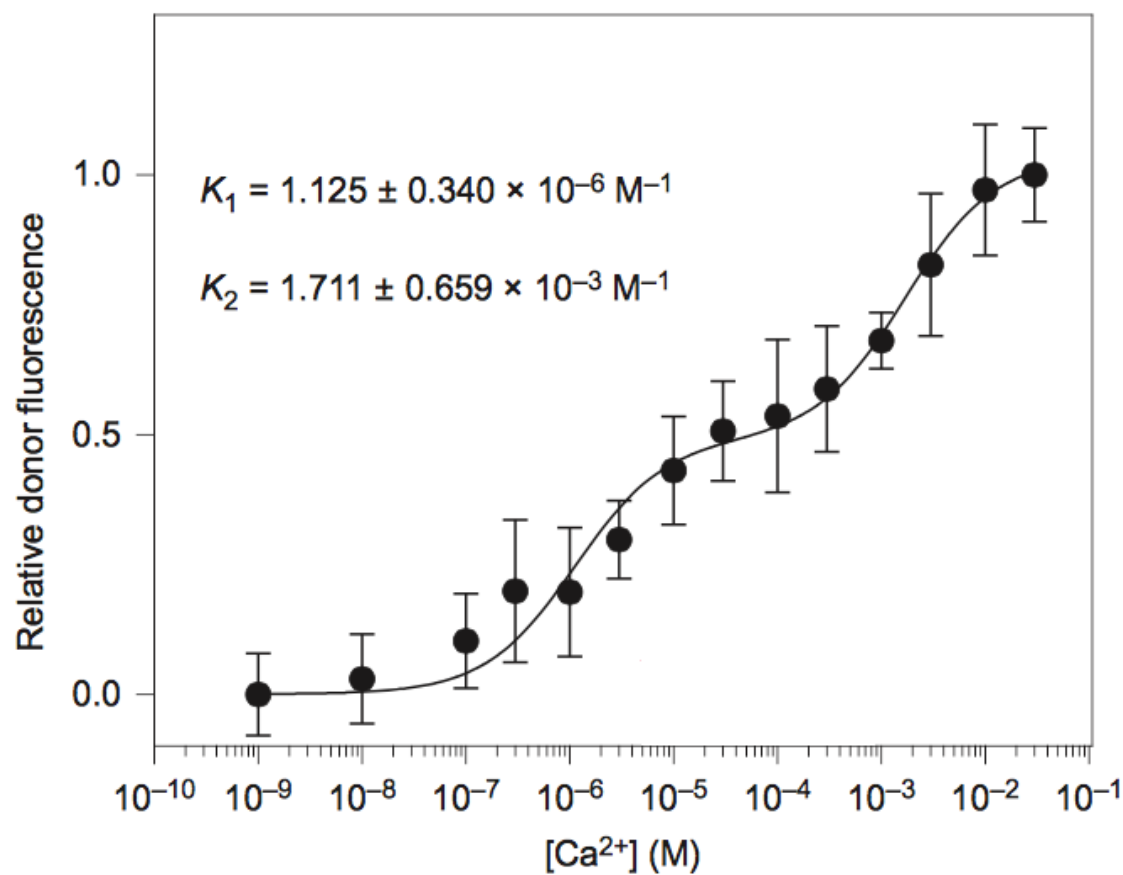
labelling unnatural amino acid with FRET fluorophores



using FRET to follow protein conformational change



using FRET to follow protein conformational change



conclusions

site-specific incorporation of multiple unnatural amino acids

orthogonal translation system

decoding amber stop codon and quadruplet codons

labelling of unnatural amino acid pairs with FRET fluorophores

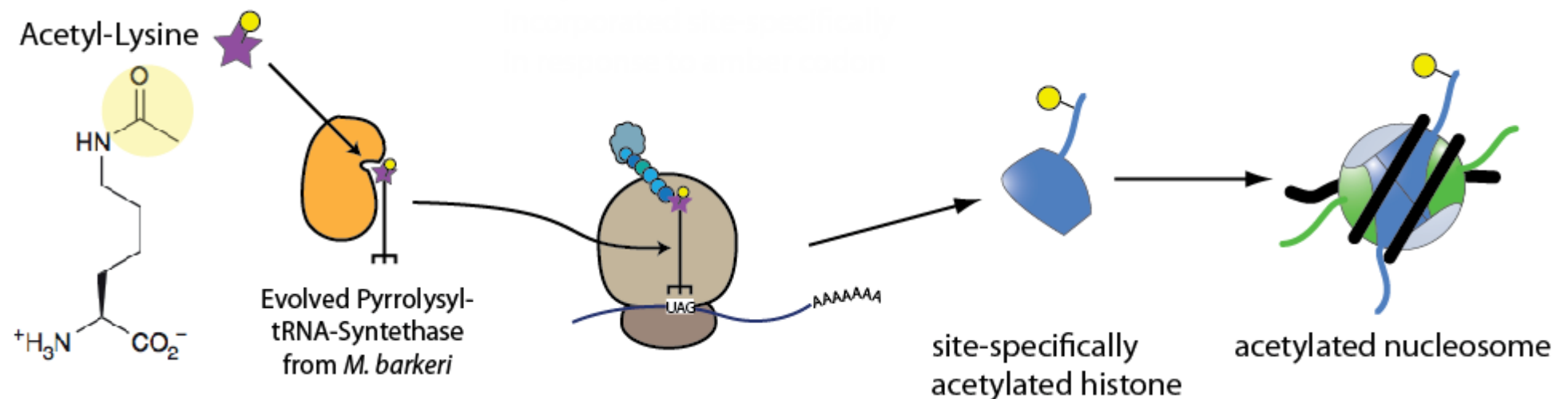
> probing protein structure and conformational changes

Genetic code expansion in stable cell lines enables encoded chromatin modification

Simon J Elsässer¹⁻³, Russell J Ernst¹, Olivia S Walker^{1,2} & Jason W Chin^{1,2}

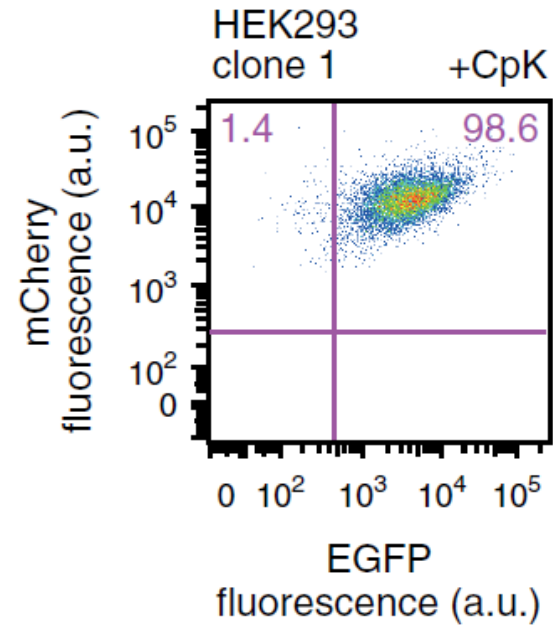
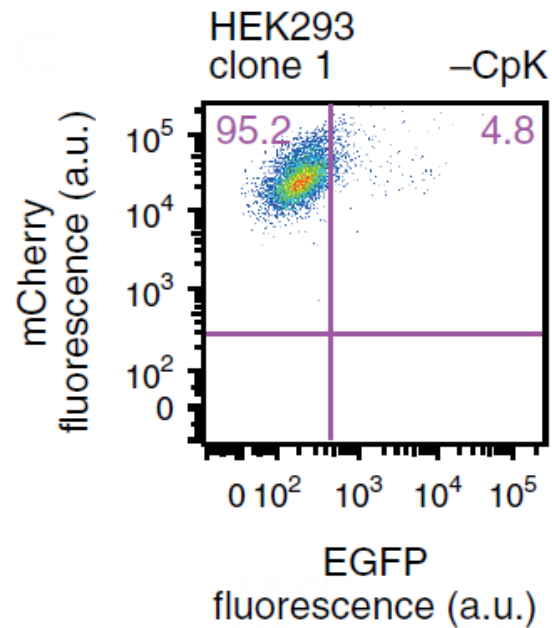
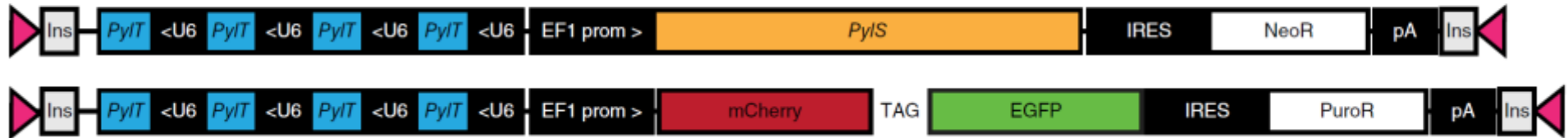
genetically encode histone acetylation

- study the function of acetylation in chromatin



stable cell lines for unnatural amino acid incorporation

PiggyBac targeting cassettes

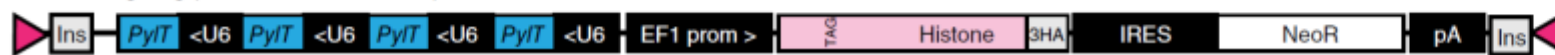


site-specific incorporation of acetyl-lysine into histone H3 in ESCs

First targeting (parent)



Second targeting (H3.2 and H3.3 cell lines)



Targeting strategy

1st targeting:
4xPylT/AcKS-Dendra2

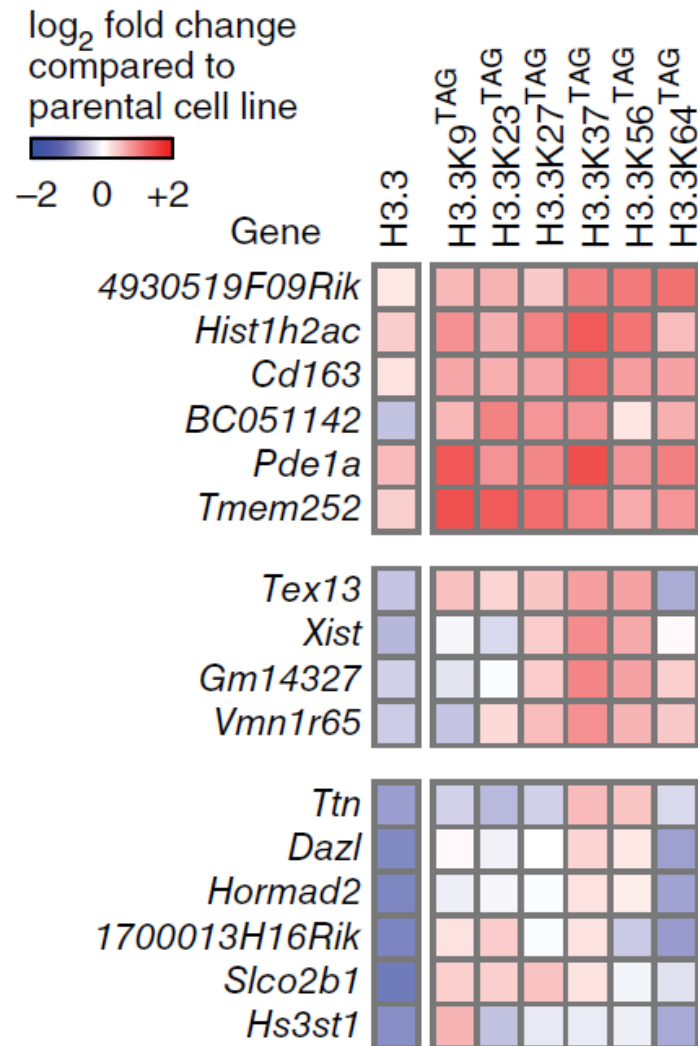


parental cell lines

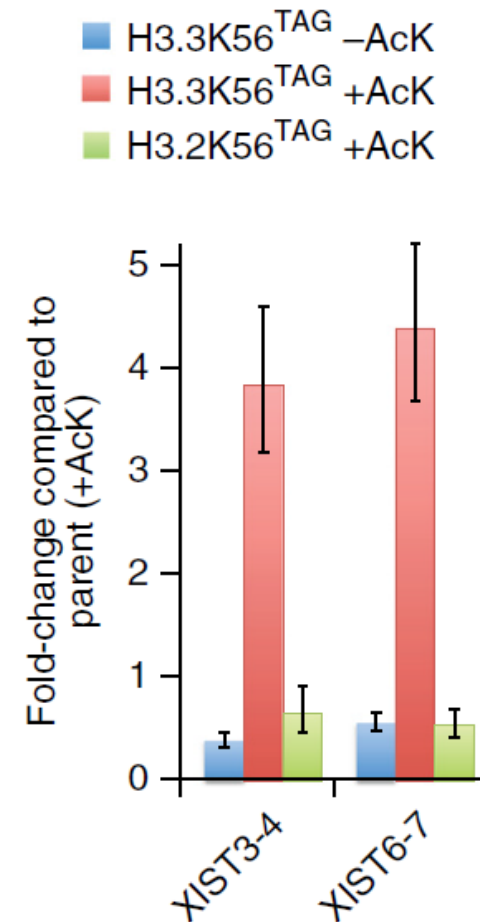
2nd targeting:
4xPylT/Histone



site-specific incorporation of acetyl-lysine into histone H3 in ESCs



RT-qPCR of *Xist* transcript



conclusions

stable cell lines for unnatural amino acid incorporation

HEK293, mouse ESC

homogeneous incorporation of unnatural amino acids into target proteins

N- ϵ -acetyl-lysine incorporation in histone H3

> changes in gene expression

sythetic epigenetics

conclusions & outlook

multiple site-directed incorporation of unnatural amino acids

generalisation of the approach?

various applications (post-translational modifications, epigenetics, artificial polymers, imaging,...)

