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Expanding the building bricks of life

Genetic code expansion, non-standard amino acids, photocaging and optogenetics

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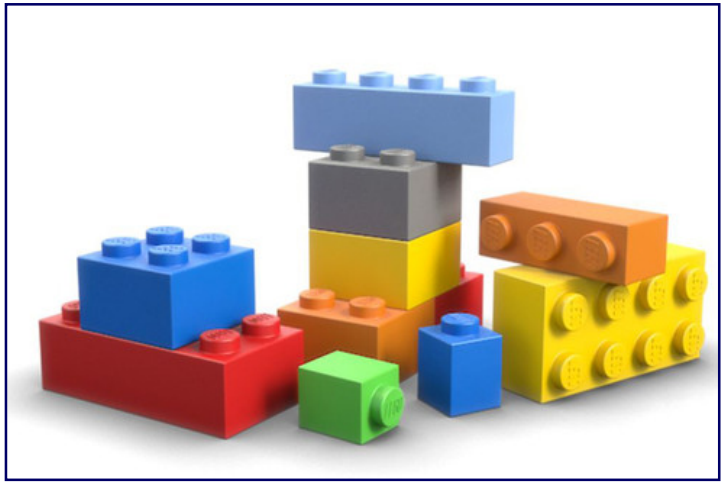
What will we talk about

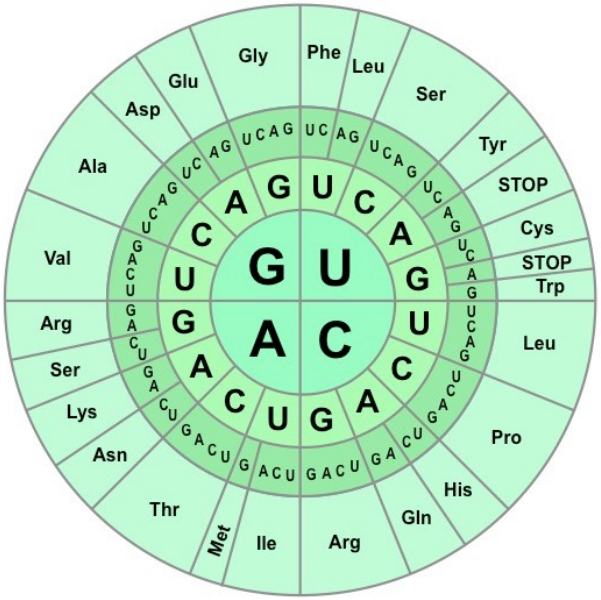
- Genetic code expansion
  - Goals and applications
- Non-standard amino acids
  - Concepts, methodology, success stories
- Cell-free protein expression
  - Strategies, methodology, disadvantages, success stories
- Optogenetics and photocaging in synthetic biology
  - Concepts, methodology, success stories

Building bricks: the LEGO system

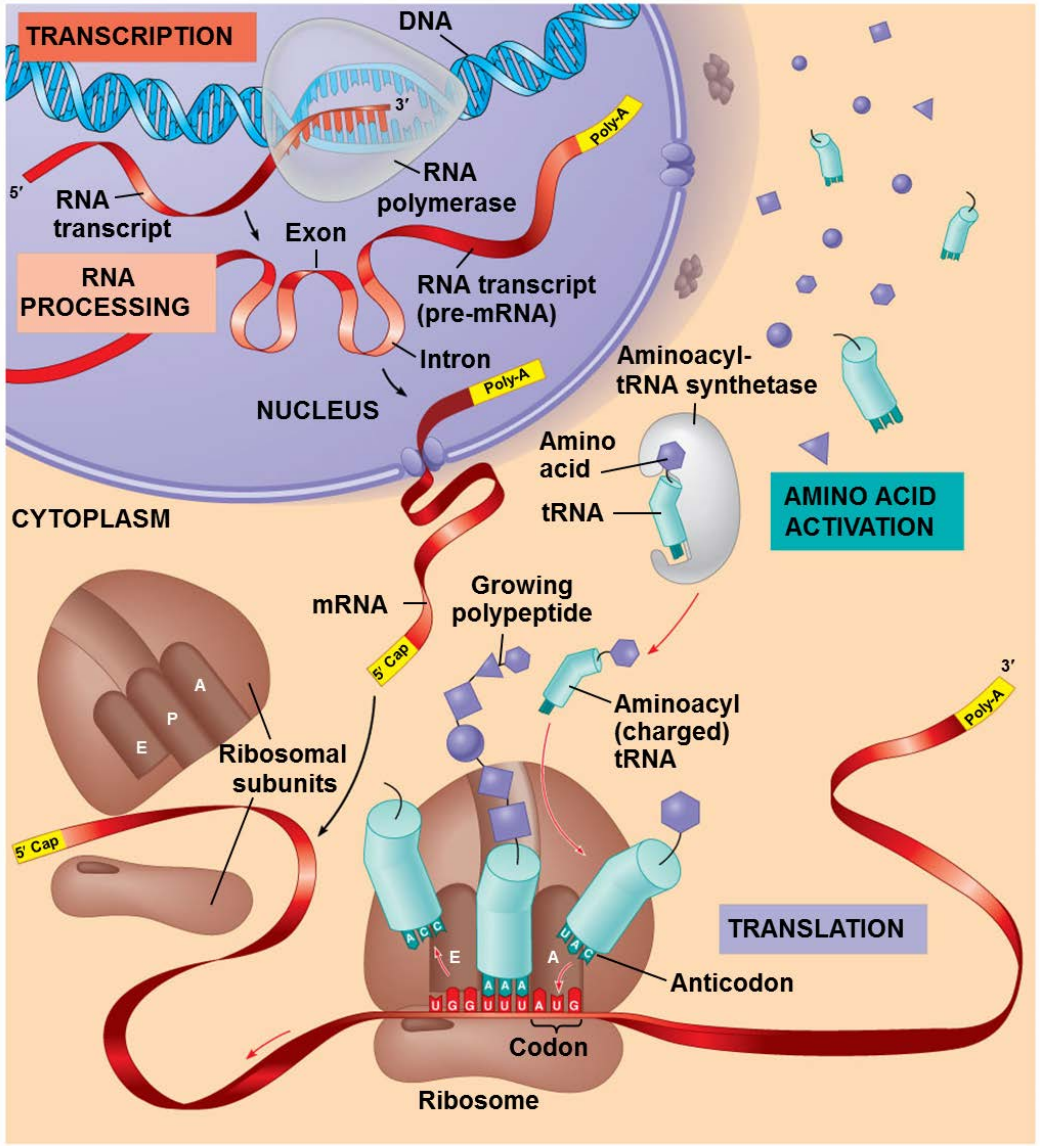


Genetic code expansion (GCE)

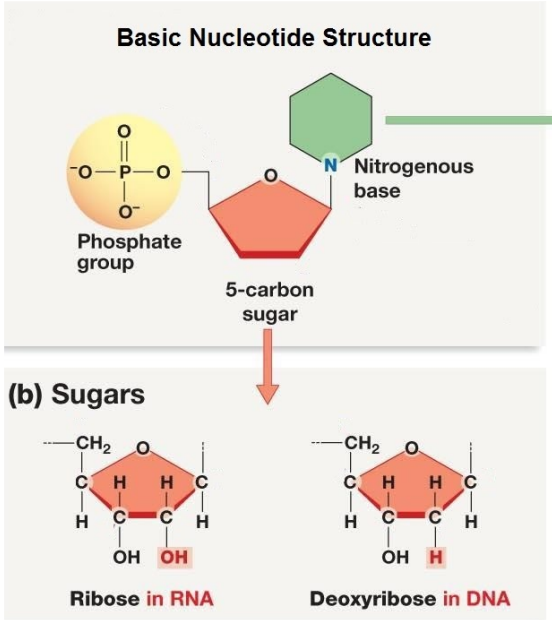
Concepts
Methods
Applications

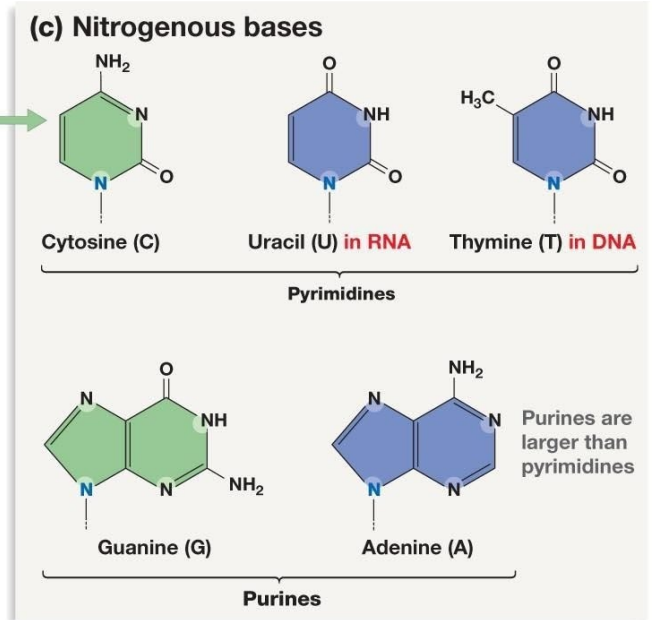


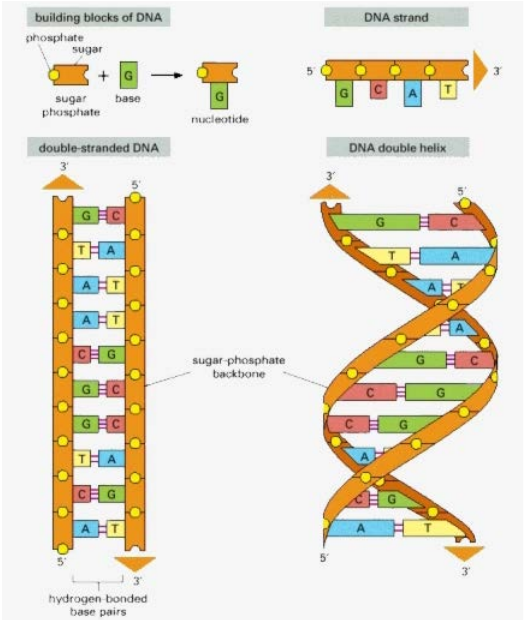
How DNA directs protein synthesis



Standard building blocks of DNA and RNA







Non-standard building blocks of DNA and RNA

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REPORT

Hachimoji DNA and RNA: A genetic system with eight building blocks

Shuichi Hoshika[1,2,*], Nicole A. Leal[1,2,*], Myong-Jung Kim[1,2], Myong-Sang Kim[1], Nilesh B. Karalkar[1,2], Hyo-Joong Kim[1], Ali...
+ See all authors and affiliations

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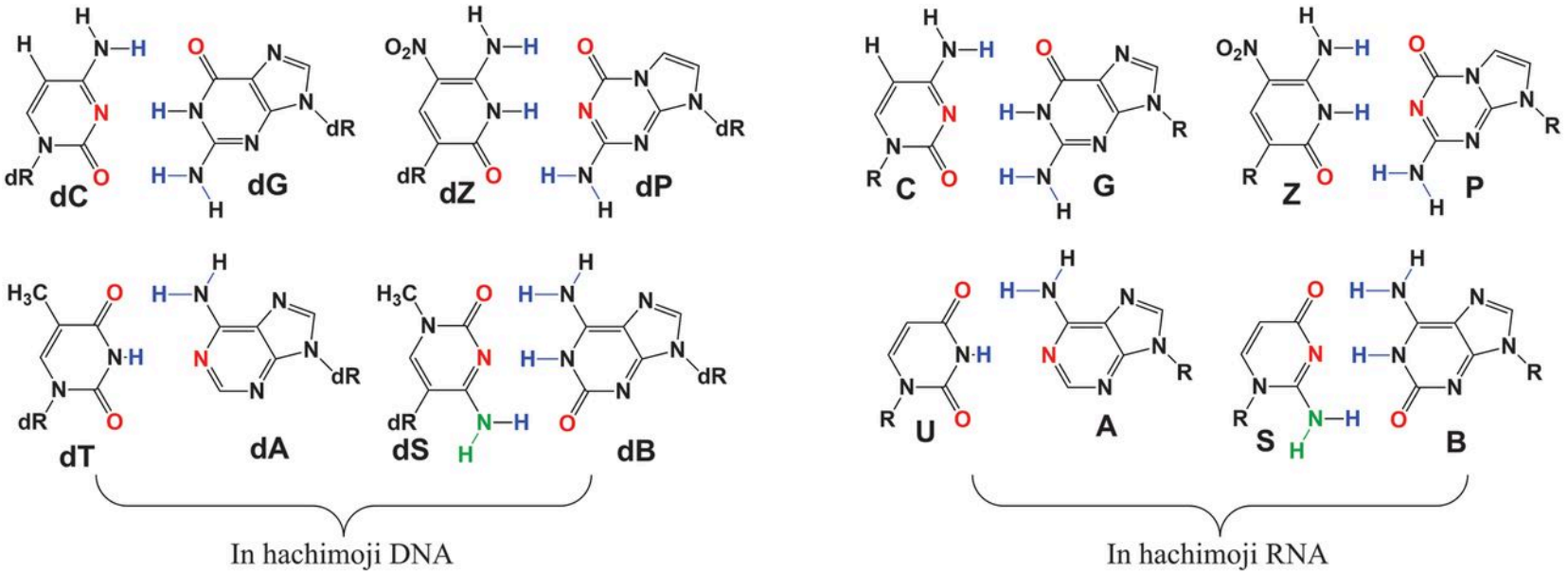
Article Figures & Data Info & Metrics eLetters PDF

Expanding the genetic code

DNA and RNA are naturally composed of four nucleotide bases that form hydrogen bonds in order to pair. Hoshika *et al.* added an additional four synthetic nucleotides to produce an eight-letter genetic code and generate so-called hachimoji DNA. Coupled with an engineered T7 RNA polymerase, this expanded DNA alphabet could be transcribed into RNA. Thus, new forms of DNA that add information density to genetic biopolymers can be generated that may be useful for future synthetic biological applications.

The eight nucleotides of hachimoji DNA and hachimoji RNA are designed to form eight-letter genetic code

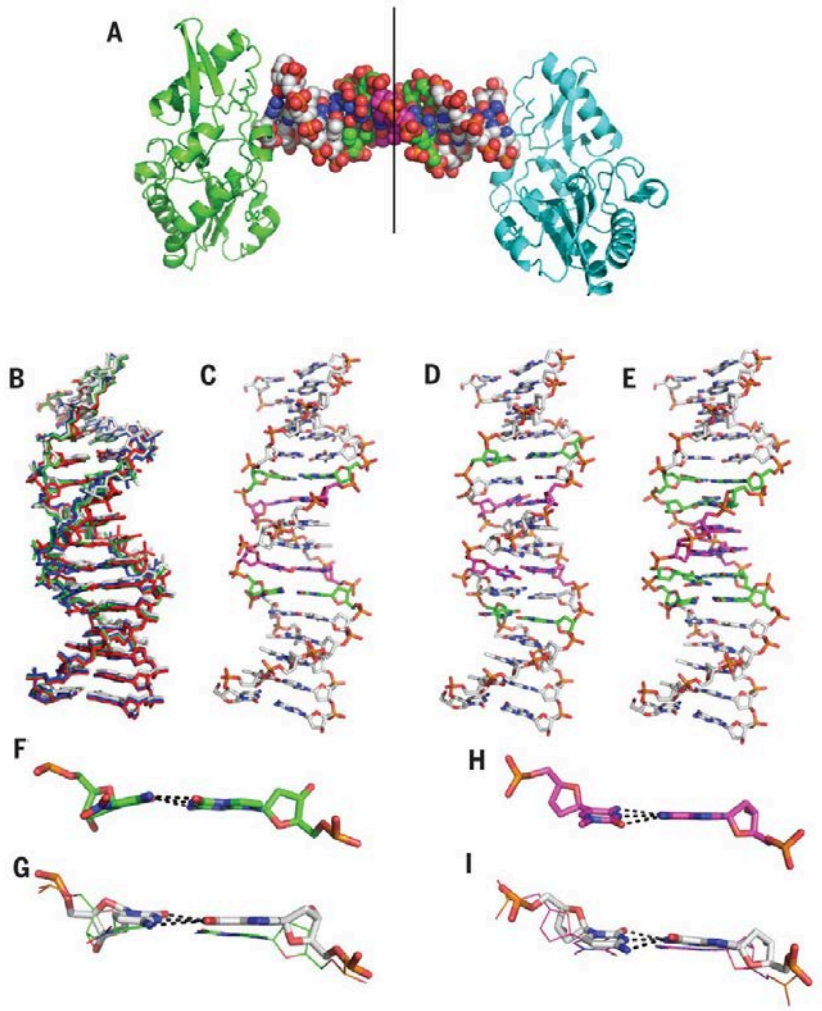
Genetic system from eight (“**hachi**”) building block “letters” (“**moji**”)



Hydrogen bond donor atoms involved in pairing are blue; hydrogen bond acceptor atoms are red. The left two pairs in each set are formed from the four standard nucleotides (note missing hydrogen-bonding group in the A:T pair, a peculiarity of standard terran DNA and RNA). The right two pairs in each set are formed from the four new nonstandard nucleotides.

https://science.sciencemag.org/content/363/6429/884

Crystal structures of PB, PC, and PP hachimoji DNA



(**A**)The host-guest complex with two N-terminal fragments from Moloney murine leukemia virus reverse transcriptase (in green and cyan) bound to a 16-mer PP hachimoji DNA; in the duplex sphere model, Z:P pairs are green and S:B pairs are magenta

(**B**) Hachimoji DNA structures PB (green), PC (red), and PP (blue) are superimposed with GC DNA (gray)

(**C**) Structure of hachimoji DNA with self-complementary duplex 5′-CTTATPBTASZATAAG (“PB”)

(**D**) Structure of hachimoji DNA with self-complementary duplex 5′-CTTAPCBTASGZTAAG (“PC”)

(**E**) Structure of hachimoji DNA with self-complementary duplex with six consecutive nonstandard 5′-CTTATPPSBZZATAAG (PP) components. DNA structures are shown as stick models with P:Z pairs (carbon atoms, green), B:S pairs (carbon atoms, magenta), and natural pairs (carbon atoms, gray)

(**F** to **I**) Examples of largest differences in detailed structures. The Z:P pair from the PB structure (F) is more buckled than the corresponding G:C pair (G). The S:B pair from the PB structure (H) exhibits a propeller angle similar to that in the corresponding G:C pair (I).

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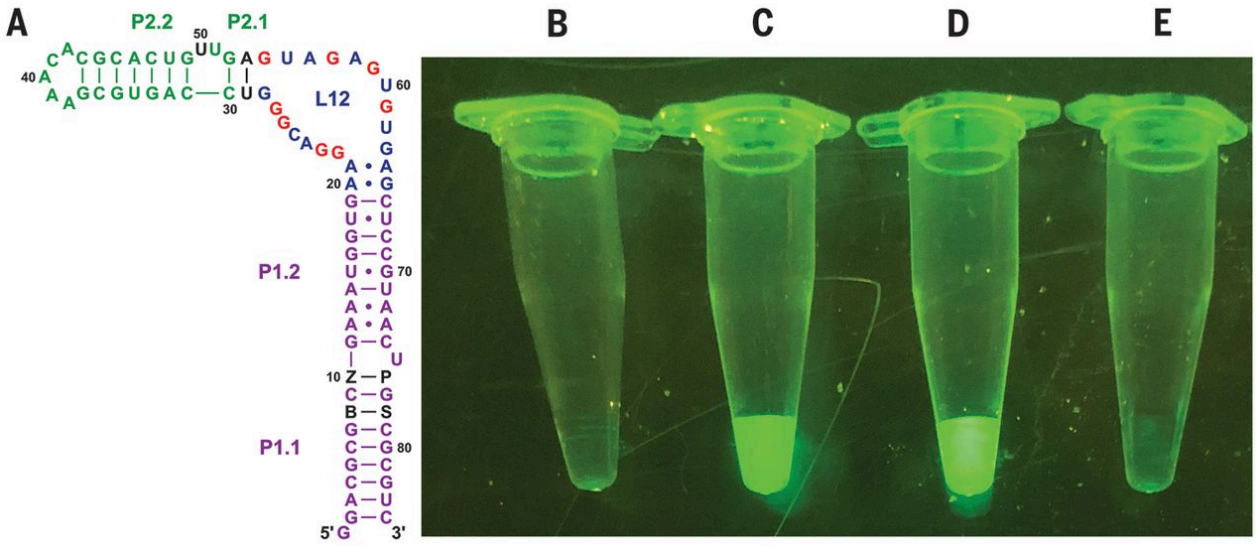
Structure and fluorescent properties of hachimoji RNA molecules



(A) Schematic showing the full hachimoji spinach variant aptamer; additional nucleotide components of the hachimoji system are shown as black letters at positions 8, 10, 76, and 78 (B, Z, P, and S, respectively). The fluor binds in loop L12. Fluorescence of various species in equal amounts as determined by UV. Fluorescence was visualized under a blue light (470 nm) with an amber (580 nm) filter.
(B) Control with fluor only, lacking RNA
(C) Hachimoji spinach with the sequence shown in (A)
(D) Native spinach aptamer with fluor
(E) Fluor and spinach aptamer containing Z at position 50, replacing the A:U pair at positions 53:29 with G:C to restore the triple observed in the crystal structure. This places the quenching Z chromophore near the fluor; CD spectra suggest that this variant had the same fold as native spinach

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Hachimoji DNA- and RNA-like molecules: summary

- DNA- and RNA-like systems built from eight nucleotide “letters” (hence the name “hachimoji”) that form four orthogonal pairs
- These synthetic systems meet the structural requirements needed to support Darwinian evolution, including a polyelectrolyte backbone, predictable thermodynamic stability, and stereoregular building blocks that fit a Schrödinger aperiodic crystal
- Measured thermodynamic parameters predict the stability of hachimoji duplexes, allowing hachimoji DNA to increase the information density of natural terran DNA
- Three crystal structures show that the synthetic building blocks do not perturb the aperiodic crystal seen in the DNA double helix
- Hachimoji DNA was then transcribed to give hachimoji RNA in the form of a functioning fluorescent hachimoji aptamer. These results expand the scope of molecular structures that might support life, including life throughout the cosmos.

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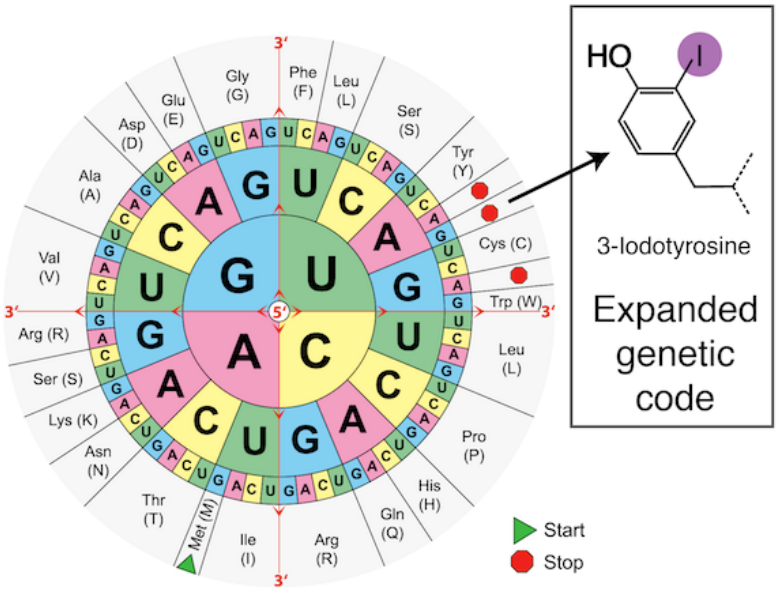
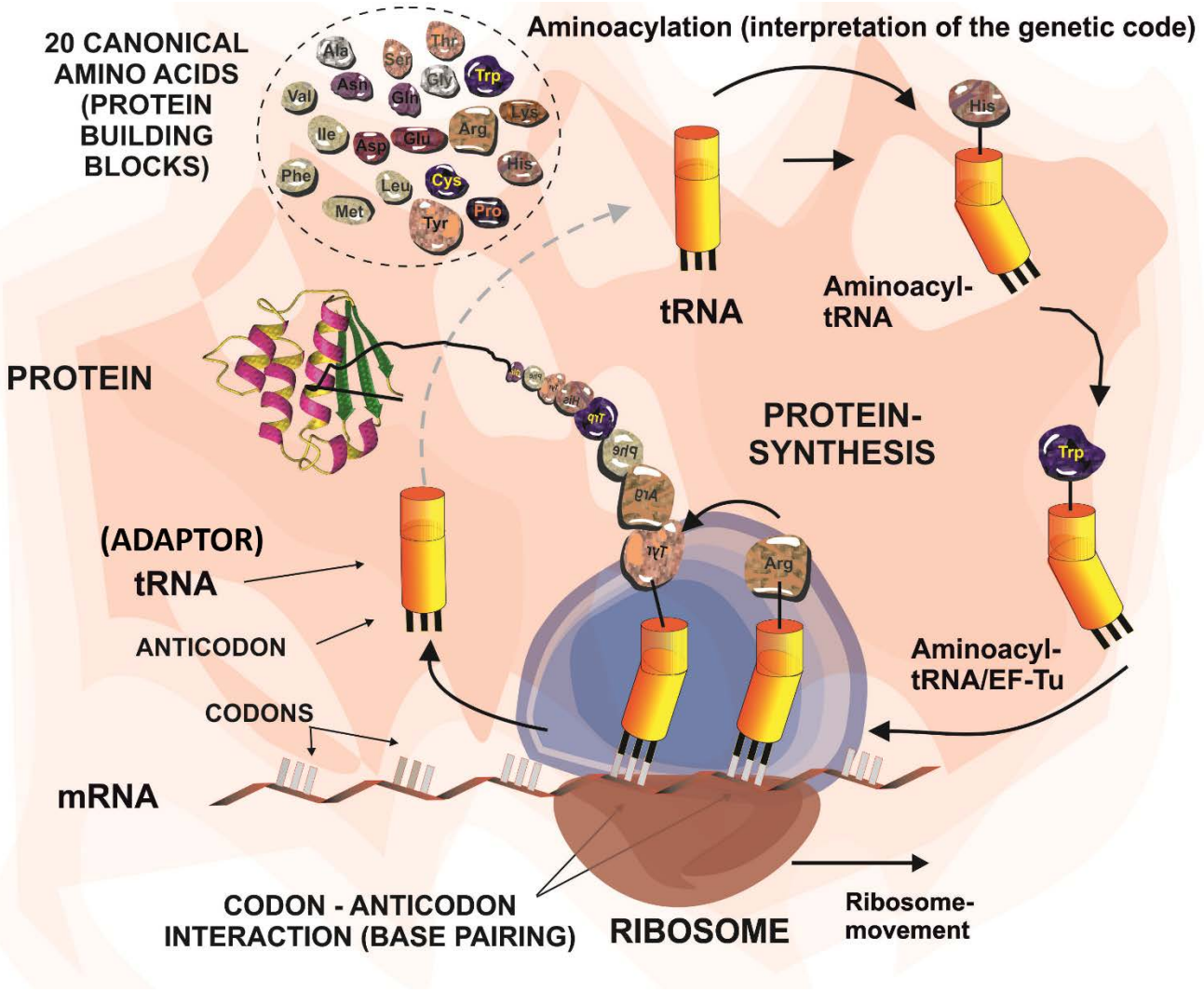
Non-standard amino acids

Concepts
Methods
Applications



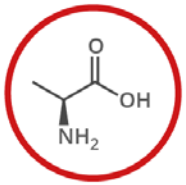
The ribosome, a protein-RNA nanomachine for protein synthesis



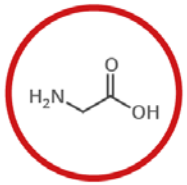
A GUIDE TO THE TWENTY COMMON AMINO ACIDS

AMINO ACIDS ARE THE BUILDING BLOCKS OF PROTEINS IN LIVING ORGANISMS. THERE ARE OVER 500 AMINO ACIDS FOUND IN NATURE - HOWEVER, THE HUMAN GENETIC CODE ONLY DIRECTLY ENCODES 20. 'ESSENTIAL' AMINO ACIDS MUST BE OBTAINED FROM THE DIET, WHILST NON-ESSENTIAL AMINO ACIDS CAN BE SYNTHESISED IN THE BODY.

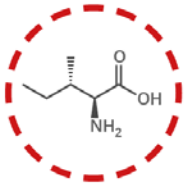
Chart Key: ALIPHATIC · AROMATIC · ACIDIC · BASIC · HYDROXYLIC · SULFUR-CONTAINING · AMIDIC · NON-ESSENTIAL · ESSENTIAL



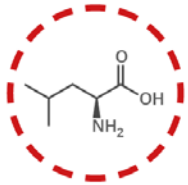
ALANINE A
Ala
GCT, GCC, GCA, GCG



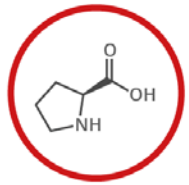
GLYCINE G
Gly
GGT, GGC, GGA, GGG



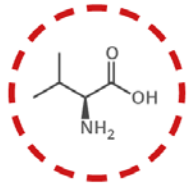
ISOLEUCINE I
Ile
ATT, ATC, ATA



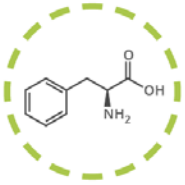
LEUCINE L
Leu
CTT, CTC, CTA, CTG, TTA, TTG



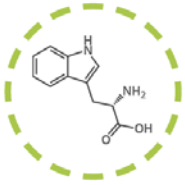
PROLINE P
Pro
CCT, CCC, CCA, CCG



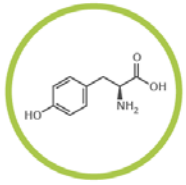
VALINE V
Val
GTT, GTC, GTA, GTG



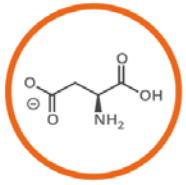
PHENYLALANINE F
Phe
TTT, TTC



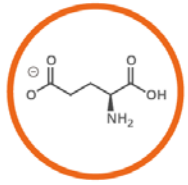
TRYPTOPHAN W
Trp
TGG



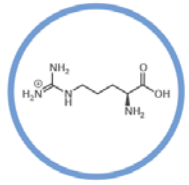
TYROSINE Y
Tyr
TAT, TAC



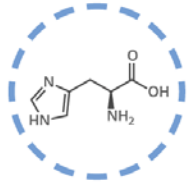
ASPARTIC ACID D
Asp
GAT, GAC



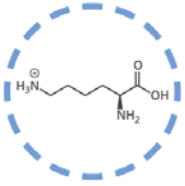
GLUTAMIC ACID E
Glu
GAA, GAG



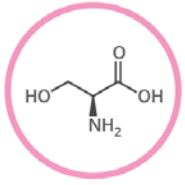
ARGININE R
Arg
CGT, CGC, CGA, CGG, AGA, AGG



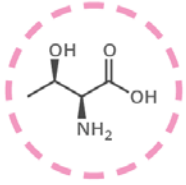
HISTIDINE H
His
CAT, CAC



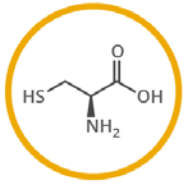
LYSINE K
Lys
AAA, AAG



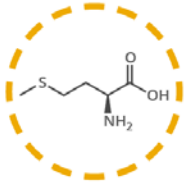
SERINE S
Ser
TCT, TCC, TCA, TCG, AGT, AGC



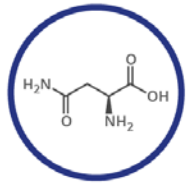
THREONINE T
Thr
ACT, ACC, ACA, ACG



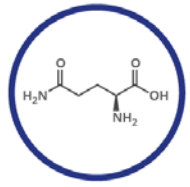
CYSTEINE C
Cys
TGT, TGC



METHIONINE M
Met
ATG



ASPARAGINE N
Asn
AAT, AAC



GLUTAMINE Q
Gln
CAA, CAG

Note: This chart only shows those amino acids for which the human genetic code directly codes for. Selenocysteine is often referred to as the 21st amino acid, but is encoded in a special manner. In some cases, distinguishing between asparagine/aspartic acid and glutamine/glutamic acid is difficult. In these cases, the codes asx (B) and glx (Z) are respectively used.

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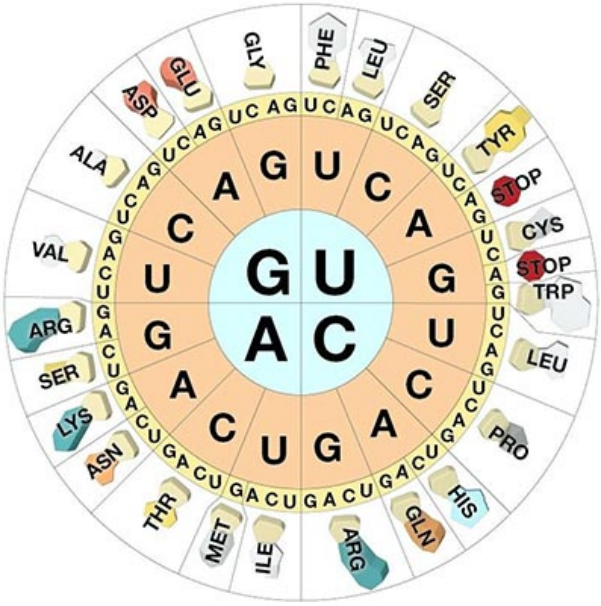
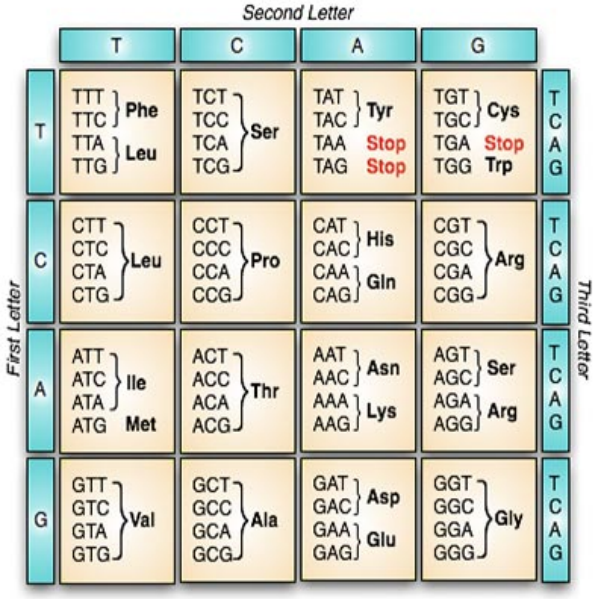
Redundancy of the genetic code

- Degeneracy of codons is the redundancy of the genetic code, exhibited as the multiplicity of three-base pair codon combinations that specify an amino acid
- The genetic code is degenerate mainly at the third codon position
- The genetic code consists of 64 triplet codons specifying 20 canonical amino acids and 3 stop signals

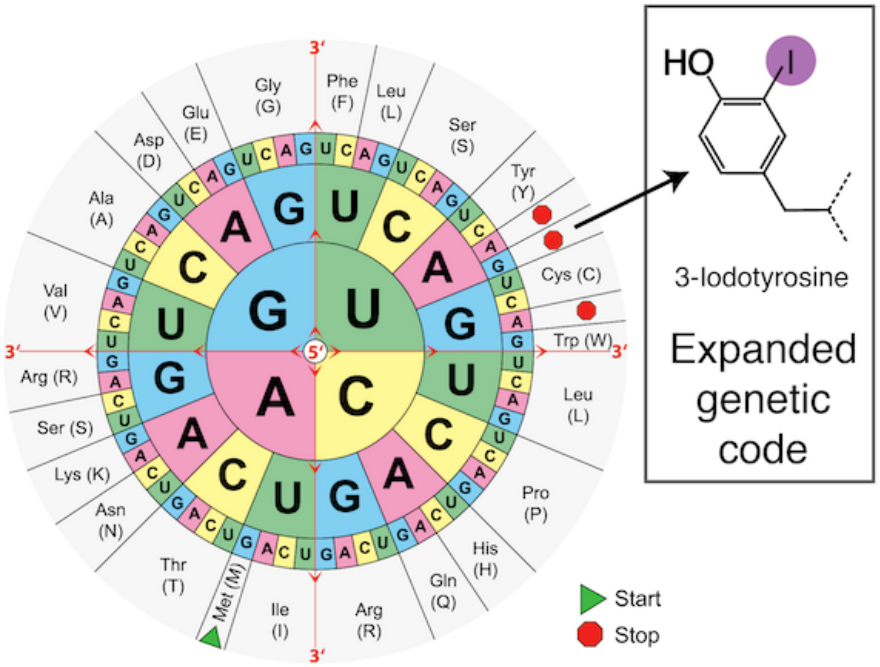




Is it possible to expand genetic code for new amino acids?

Expanded genetic code

An **expanded genetic code** is an artificially modified genetic code in which one or more codons have been re-allocated to encode an amino acid that is not among the twenty naturally-encoded (canonical) amino acids

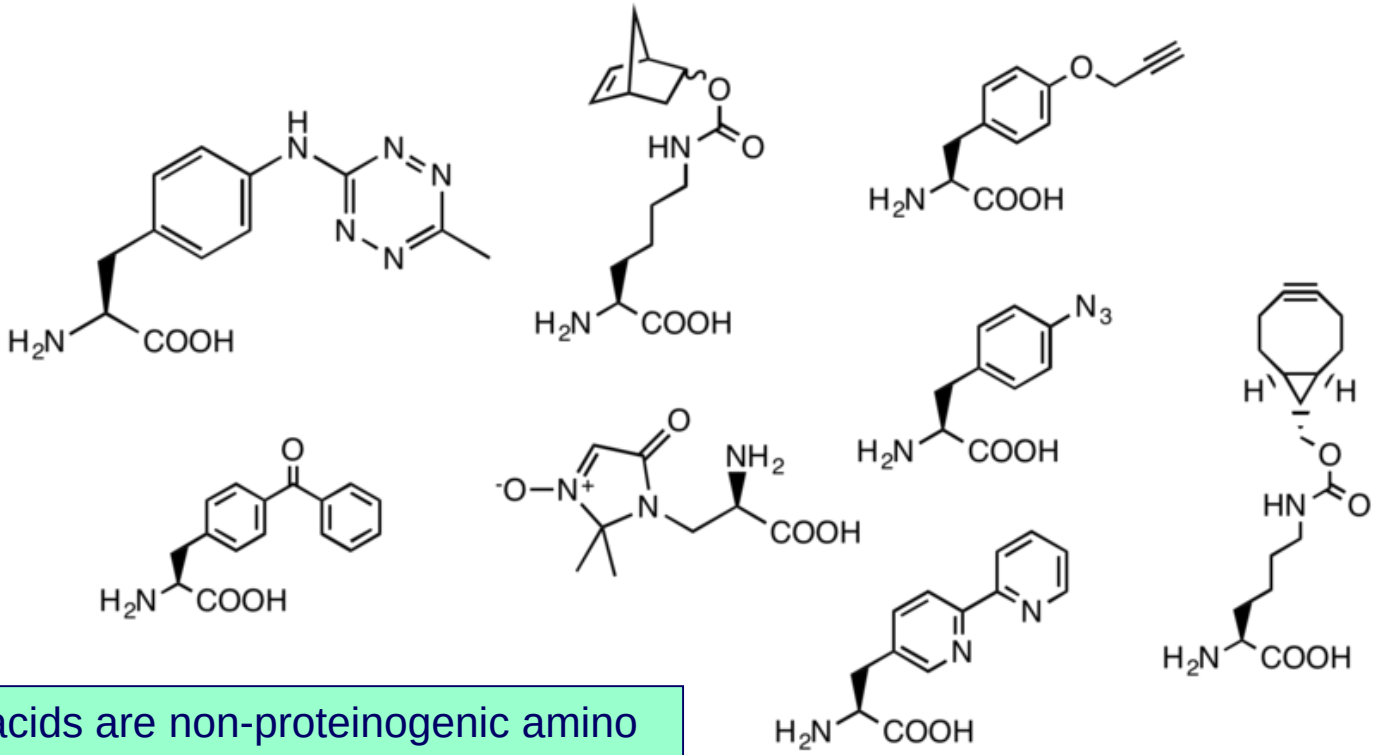


The key prerequisites to expand the genetic code are:

- the non-standard amino acid to encode
- an unused codon to adopt
- a tRNA that recognises this codon
- a tRNA synthetase that recognises only that tRNA and only the non-standard amino acid

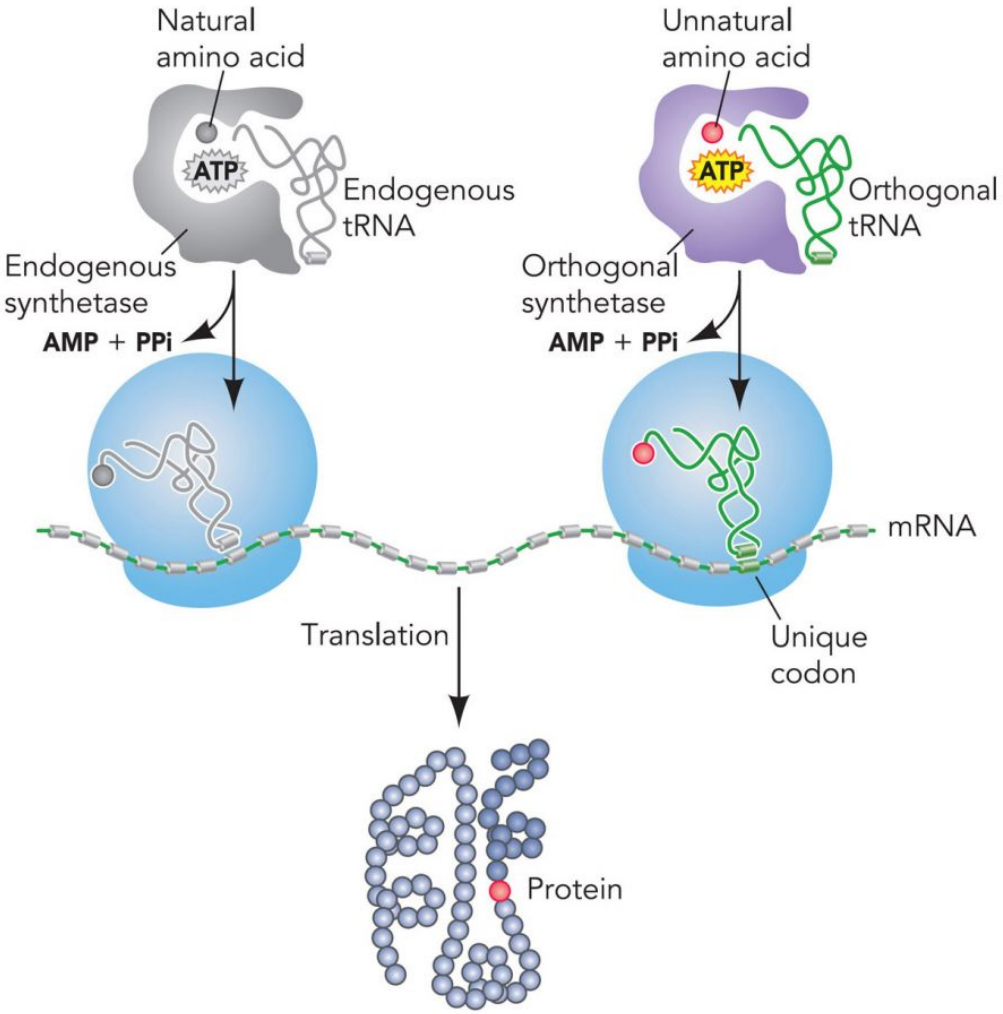
https://en.wikipedia.org/wiki/Expanded_genetic_code

Unnatural amino acids: examples



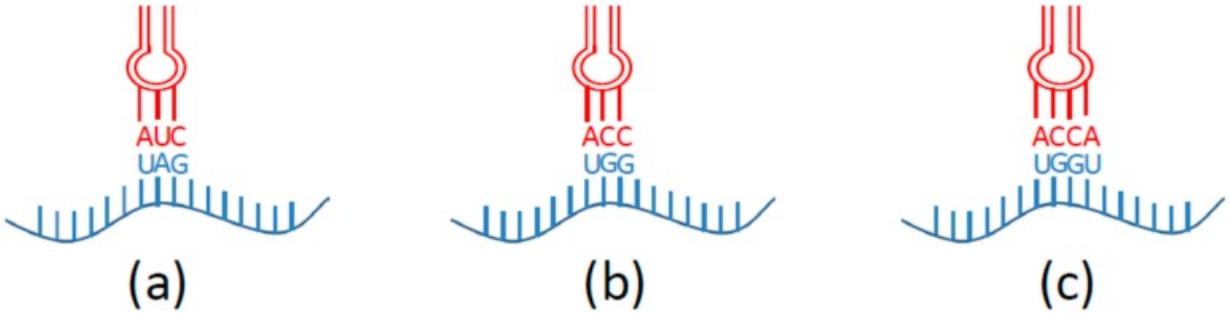
Unnatural amino acids are non-proteinogenic amino acids that either occur naturally or are chemically synthesized. Unnatural amino acids represent a nearly infinite array of **diverse structural elements** for the development of new synthetic proteins via the **expanded genetic code** approach.

Incorporation of unnatural amino acid into proteins: a basic concept



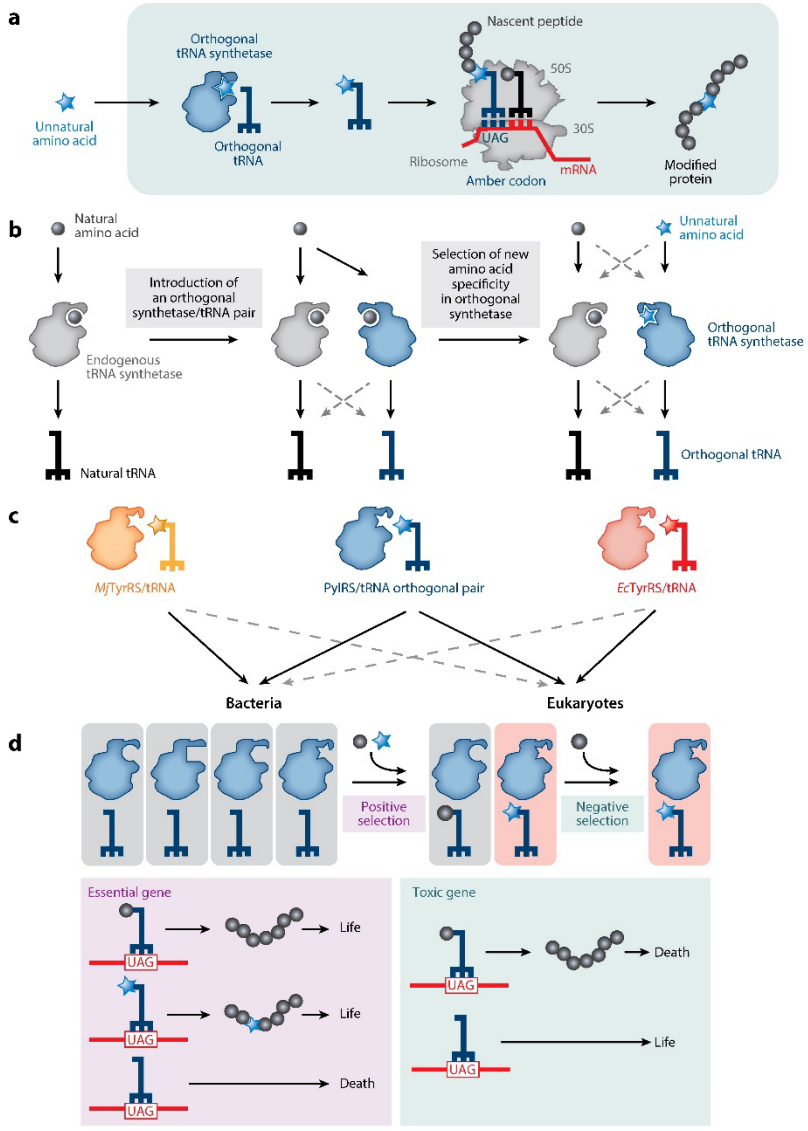
- The method involves the generation of an orthogonal tRNA/synthetase pair, which does not crosstalk with endogenous tRNA/synthetase pairs but is functionally compatible with the protein translational machinery
- The orthogonal synthetase is engineered to charge the desired unnatural amino acid (Uaa) onto the orthogonal tRNA, and the tRNA decodes a unique codon (such as the amber stop codon UAG) to incorporate the Uaa into proteins through translation

Site-specific incorporation of NAAs into proteins



- (**a**) Initially, site-specific incorporation of NAA into a protein was based on **nonsense suppression**, where the term “nonsense suppression” refers to the use of nonsense codons and suppressor tRNAs, which recognize stop codons. The amber UAG stop codon has been most frequently used for the incorporation of NAAs, but the use of the ochre (UAA) stop and the opal (UGA) stop codons have been applied, especially for the incorporation of diverse NAAs into one recombinant protein. The major advantage of the nonsense suppression technique lies in its simplicity. Nevertheless, even amber stop codon terminates about 320 genes in the model laboratory strain of *E. coli* K-12, meaning that the NAA would be incorporated not only in the protein of interest but also in response to TAG stop codons of the genes present in the genome
- (**b**) **Rarely used sense codons**, such as arginine (AGG) or proline (CCC) codons of *E. coli*, can be used for direct NAA incorporation in proteins
- (**c**) Finally, design of **extended codons and frameshift suppression**. In this approach, an mRNA containing an extended codon consisting of four or five bases is read by a modified aa-tRNA containing the corresponding extended anticodon, and a full-length protein containing NAA at the specific site is obtained

Site-specific incorporation of NAAs into proteins



(a) Genetic code expansion enables the site-specific incorporation of an unnatural amino acid into a protein via cellular translation

(b) The process of discovering orthogonal aminoacyl-tRNA (transfer RNA) synthetases for unnatural amino acids

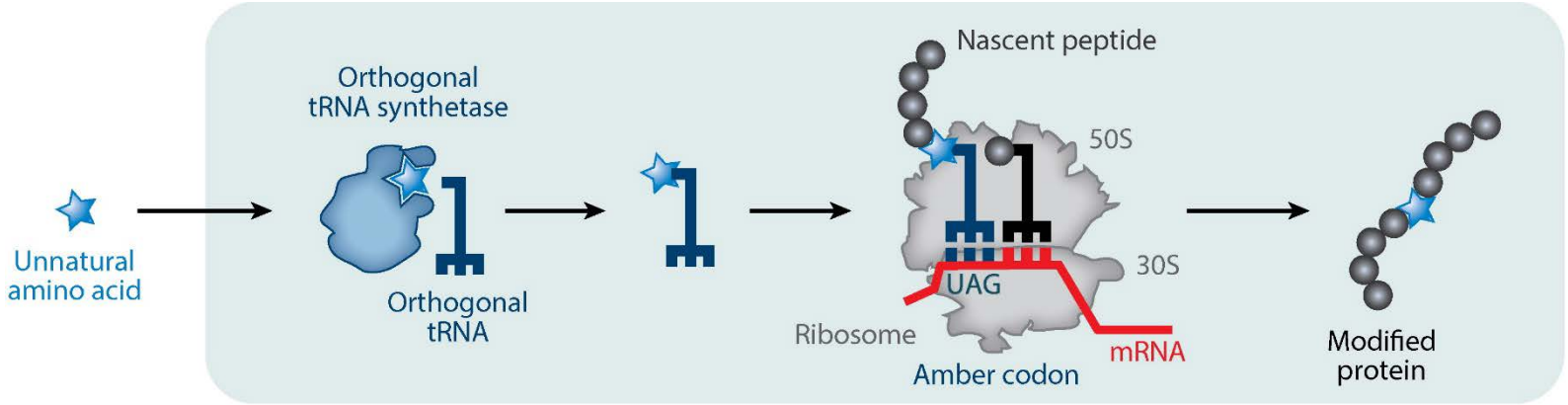
(c) Orthogonality of synthetase/tRNA pairs in different hosts. The solid lines indicate that a pair is orthogonal in a host

(d) Sequential positive and negative selections enable the discovery of synthetase/tRNA pairs that direct the incorporation of unnatural amino acids

Abbreviations: Ec TyrRS,Escherichia coli tyrosyl-tRNA synthetase; Mj TyrRS, Methanococcus janaschii tyrosyl-tRNA synthetase; mRNA, messenger RNA; PylRS, pyrrolysyl-tRNA synthetase

Unnatural acid incorporation into proteins

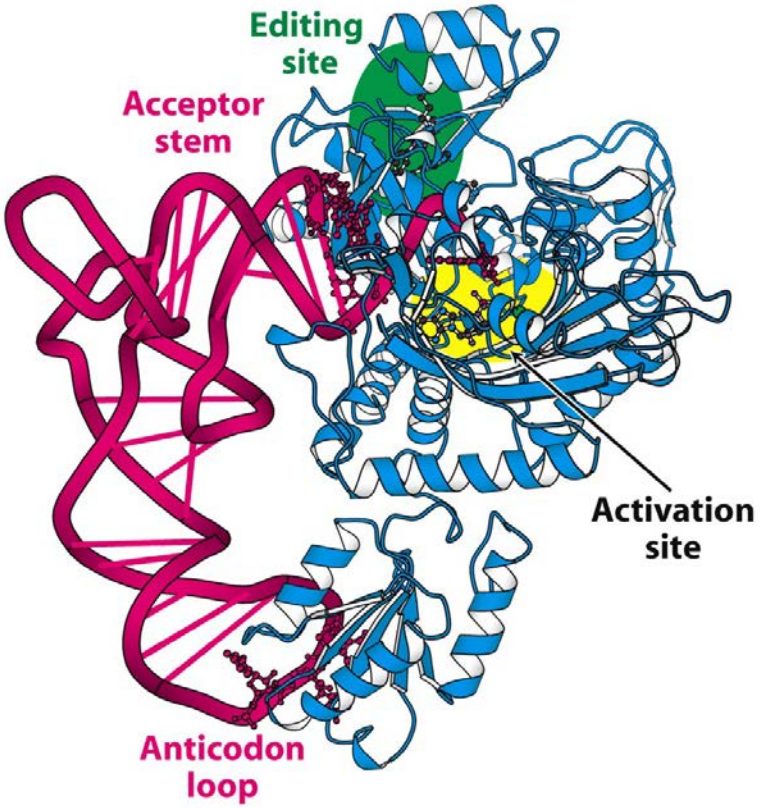
In a typical unnatural amino acid incorporation experiment, the unnatural amino acid is added to the cell, recognized by the altered-specificity orthogonal synthetase, and used to aminoacylate the orthogonal tRNA. The orthogonal tRNA is decoded by the ribosome in response to a blank codon (which has been inserted into a gene of interest) on the messenger RNA (mRNA), leading to unnatural amino acid incorporation at a genetically defined position in the encoded polypeptide chain.



Genetic code expansion enables the site-specific incorporation of an unnatural amino acid into a protein via cellular translation

Orthogonal aminoacyl-tRNA synthetases

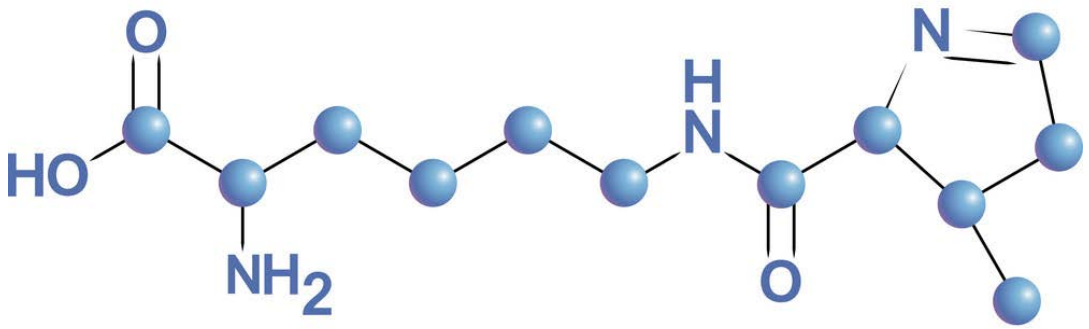
To use an orthogonal pair for genetic code expansion, the active site of the synthetase must specifically bind an unnatural amino acid, and no natural amino acids, and must transfer the unnatural amino acid onto its cognate orthogonal tRNA.



- For orthogonal synthetases that bind one of the 20 common amino acids (*Mj*TyrRS, EcTyrRS, Ec LeuRS), this process requires evolution of the enzyme active site to destroy natural amino acid binding and facilitate specific unnatural amino acid binding

- PylRS naturally binds pyrrolysine but none of the common amino acids, so it is not necessary to mutate the active site to remove natural amino acid binding. Moreover, while the substrate scope of PylRS has been substantially expanded by directed evolution, some useful unnatural amino acids can also be incorporated using the wild-type PylRS, thereby facilitating unnatural amino acid incorporation

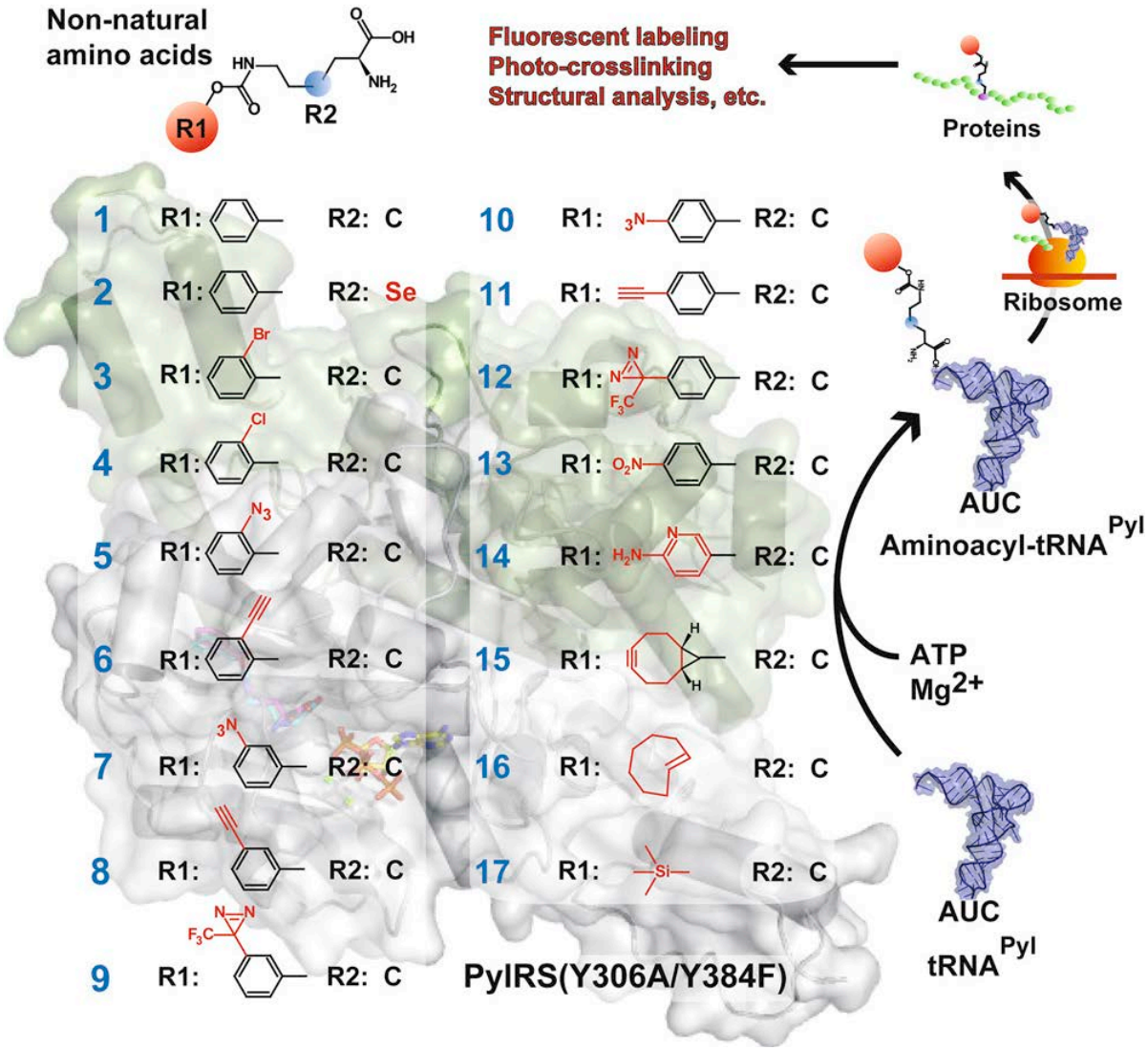
Pyrrolysine



- **Pyrrolysine** is encoded by the 'amber' stop codon (**UAG**) is an amino acid that is used in the biosynthesis of proteins in some methonogenic archeae and bacteria.
- It is not present in eukaryotes.

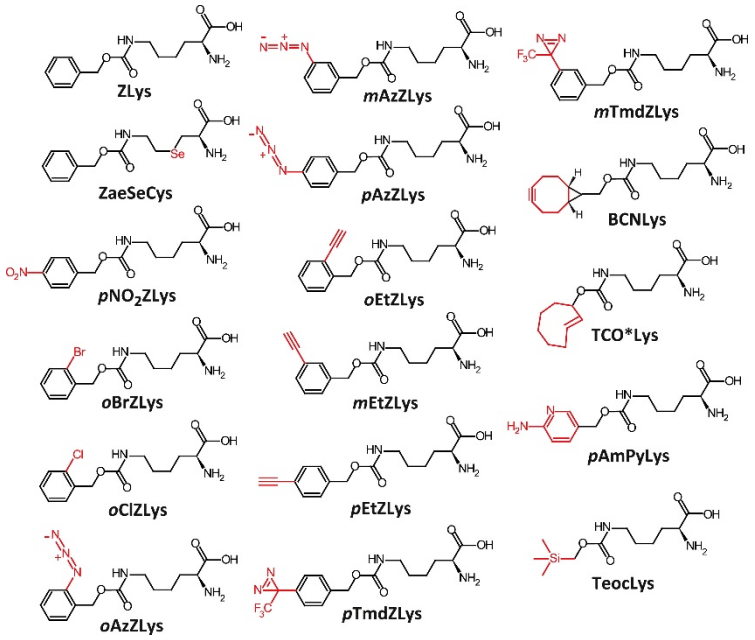
https://www.sciencedirect.com/science/article/pii/S2451945619301047

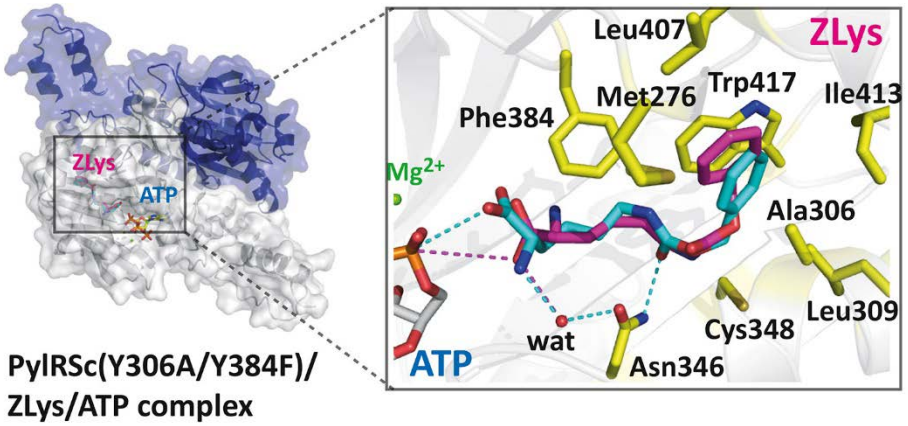
Pyrrolysyl-tRNA synthetase (PylRS) and tRNA^{Pyl}



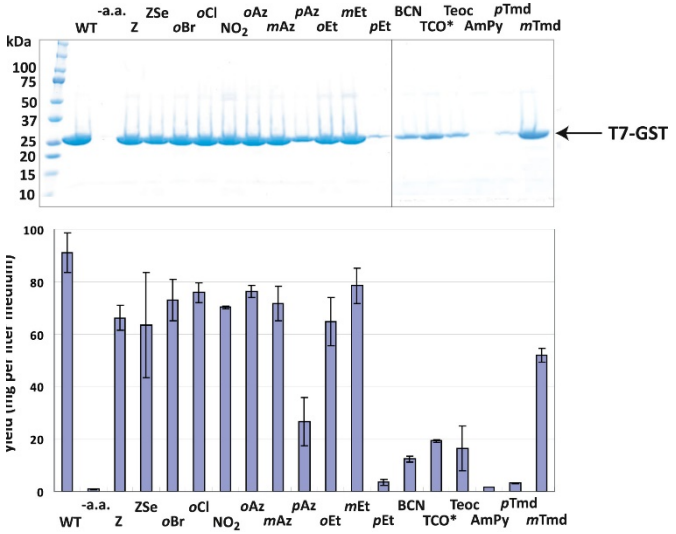
https://www.sciencedirect.com/science/article/pii/S2451945619301047

Incorporation of the non-natural lysine derivatives





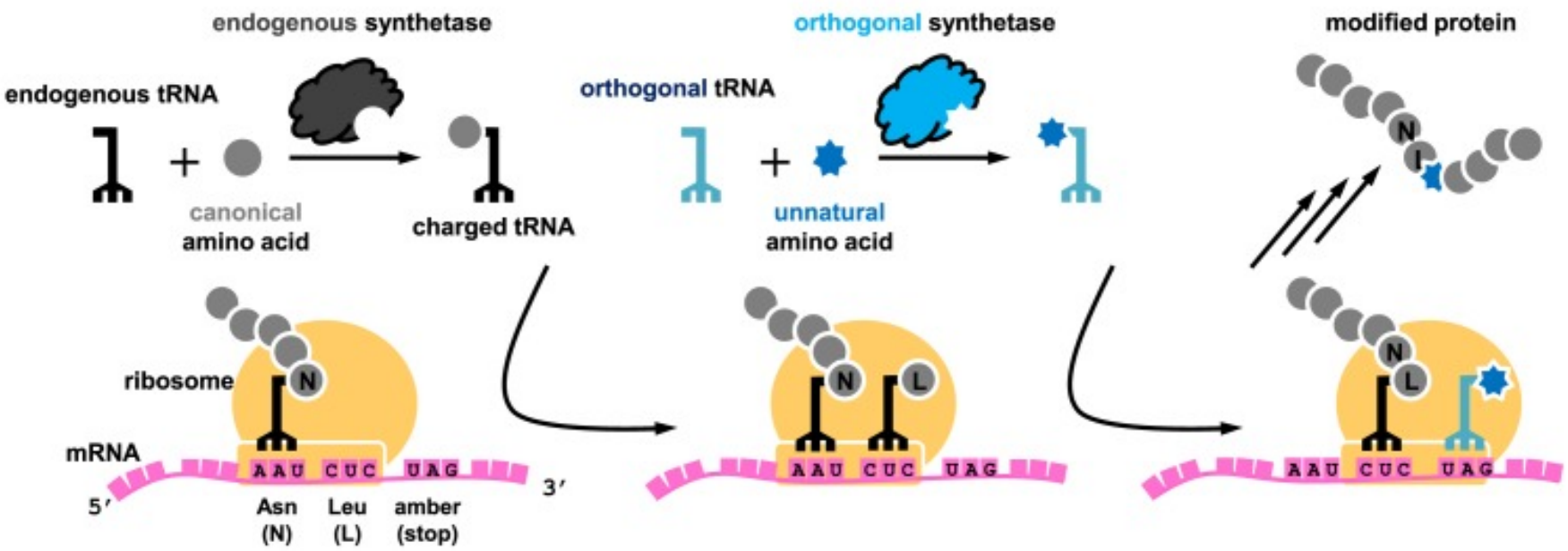
PylRSc(Y306A/Y384F)/
ZLys/ATP complex



- A mutant pyrrolysyl-tRNA synthetase, PylRS(Y306A/Y384), acts on diverse amino acids
- The PylRS mutant and $tRNA^{Pyl}$ incorporated 17 non-natural amino acids into proteins
- Crystal structures of the PylRS mutant bound with 14 of the amino acids were solved
- This information will facilitate the structure-based design of novel amino acids

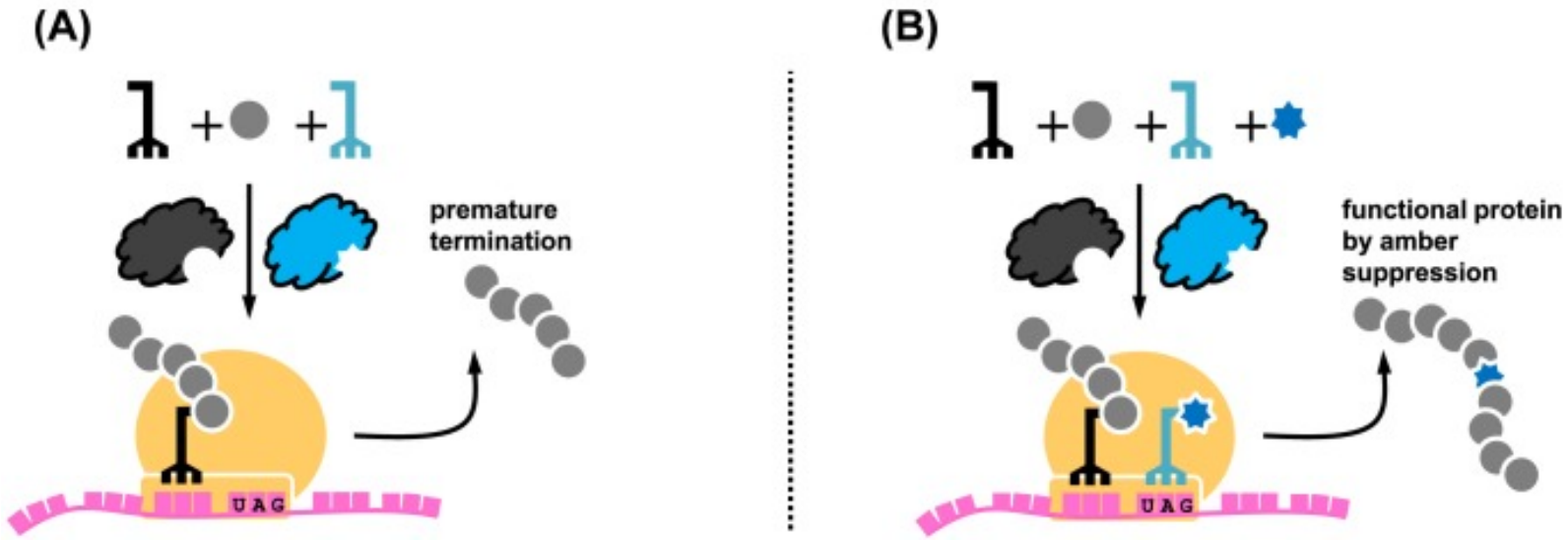
https://www.sciencedirect.com/science/article/pii/S2451945619301047

Mechanism of genetic code expansion for incorporation of an unnatural amino acid by amber suppression



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6610526/

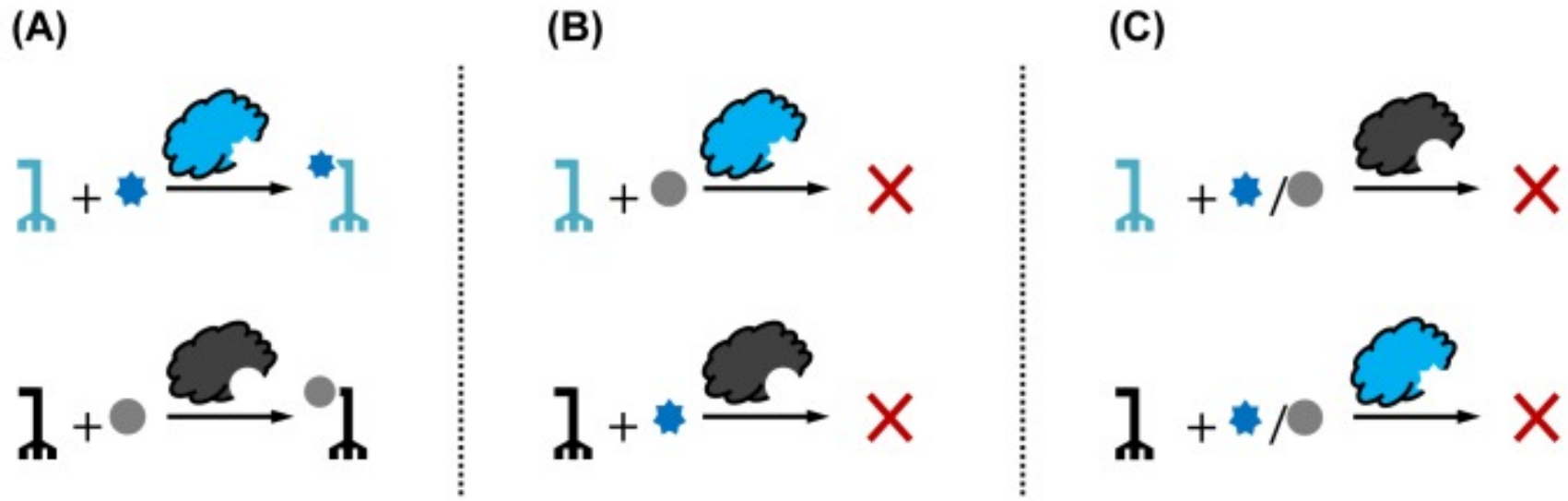
Use of amber suppression to switch on protein production



(**A**) Absence of the unnatural amino acid leads to recognition of the UAG codon for translation termination. (**B**) Addition of the unnatural amino acid leads to amber suppression and successful production of the full-length and functional protein.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6610526/

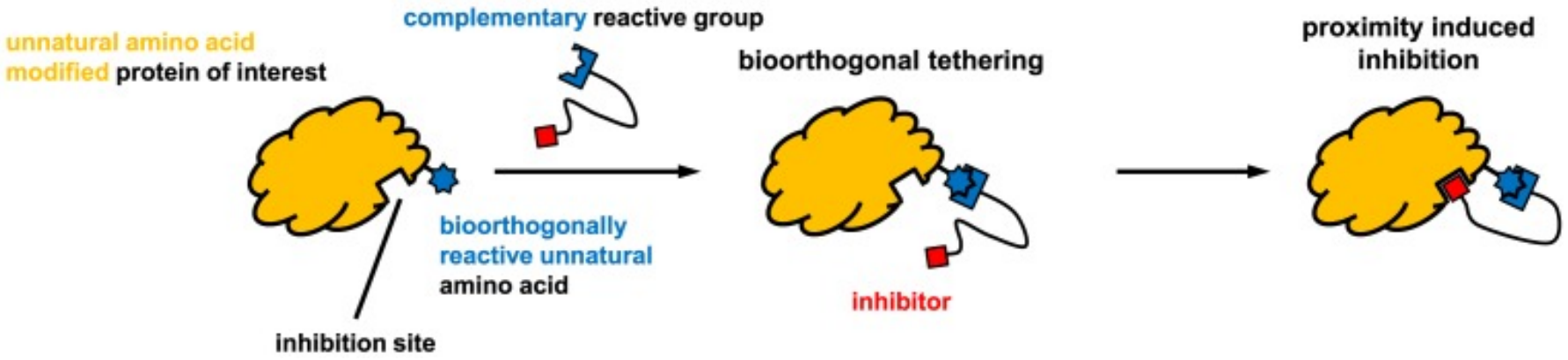
Allowed and not allowed reactivities between the orthogonal and endogenous aaRS/tRNA pairs



(**A**) Matching amino acid and aaRS/tRNA pairs; (**B**) mismatched amino acids; (**C**) mismatched aaRS/tRNA pairs.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6610526/

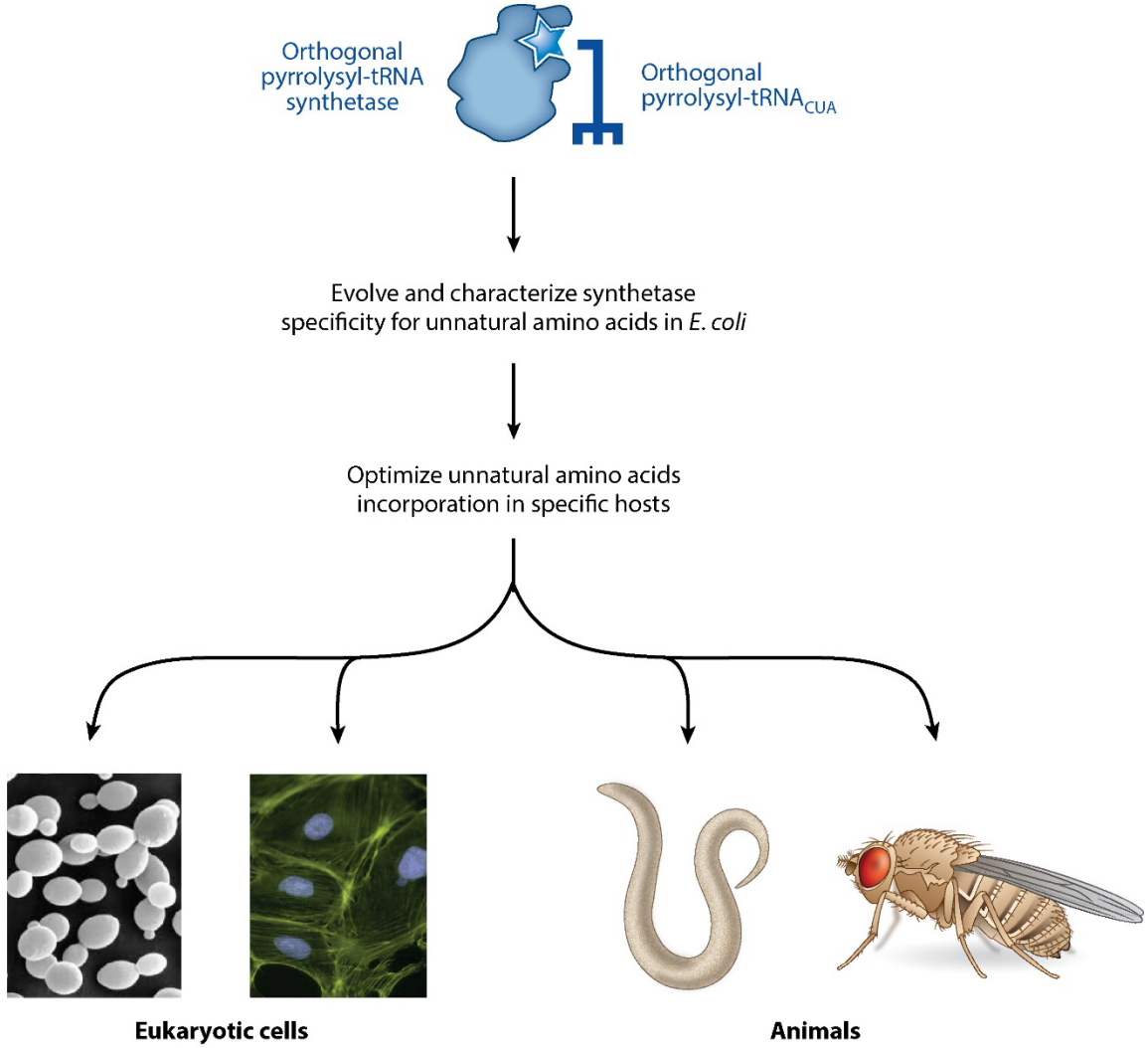
Example of the small-molecule approach to protein inhibition



- Use of unnatural amino acid incorporation for selective inhibition of protein function by bioorthogonal tethering
- In comparison with light-induced activation or inhibition, small molecules can be used to activate or inhibit the target protein in deep animal tissue or intact animals which are not easily accessible by light

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6610526/

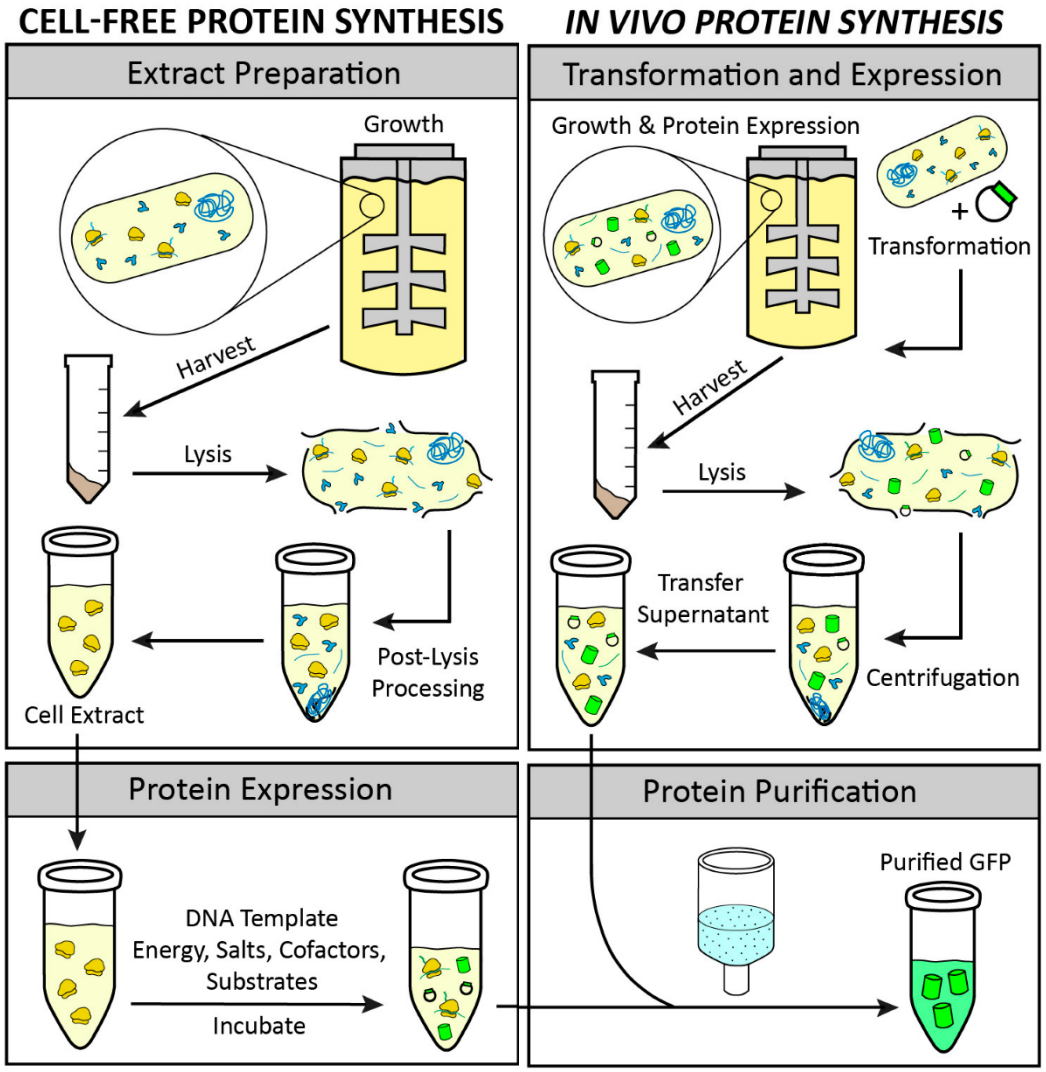
Site-specific incorporation of NAAs into proteins



Cell-free protein synthesis (CFPS)

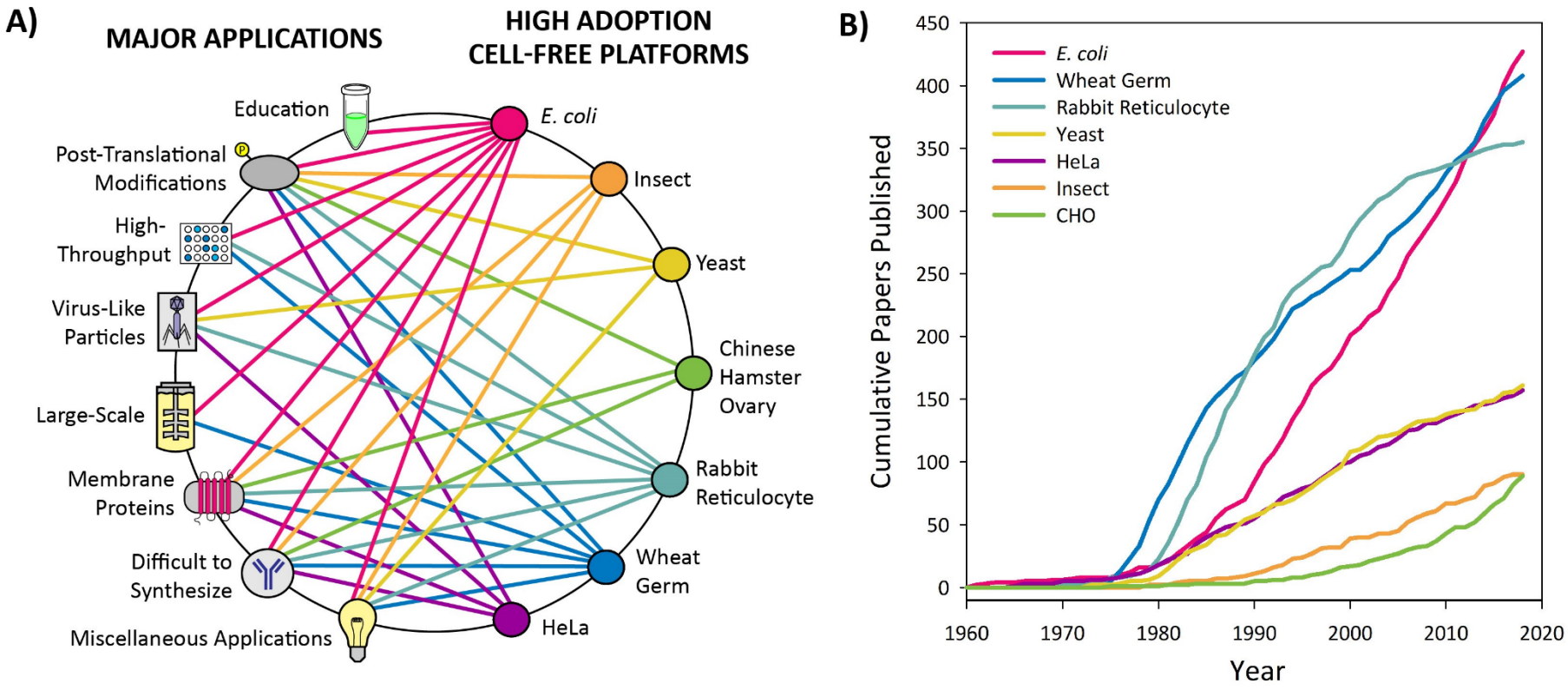
Concepts
Methods
Applications

Cell-Free Protein Synthesis (CFPS)



- Cell-free protein synthesis (CFPS) is a platform technology that provides new opportunities for protein expression
- The advantages of CFPS over in vivo protein expression include its open system, the elimination of reliance on living cells, and the ability to focus all system energy on production of the protein of interest

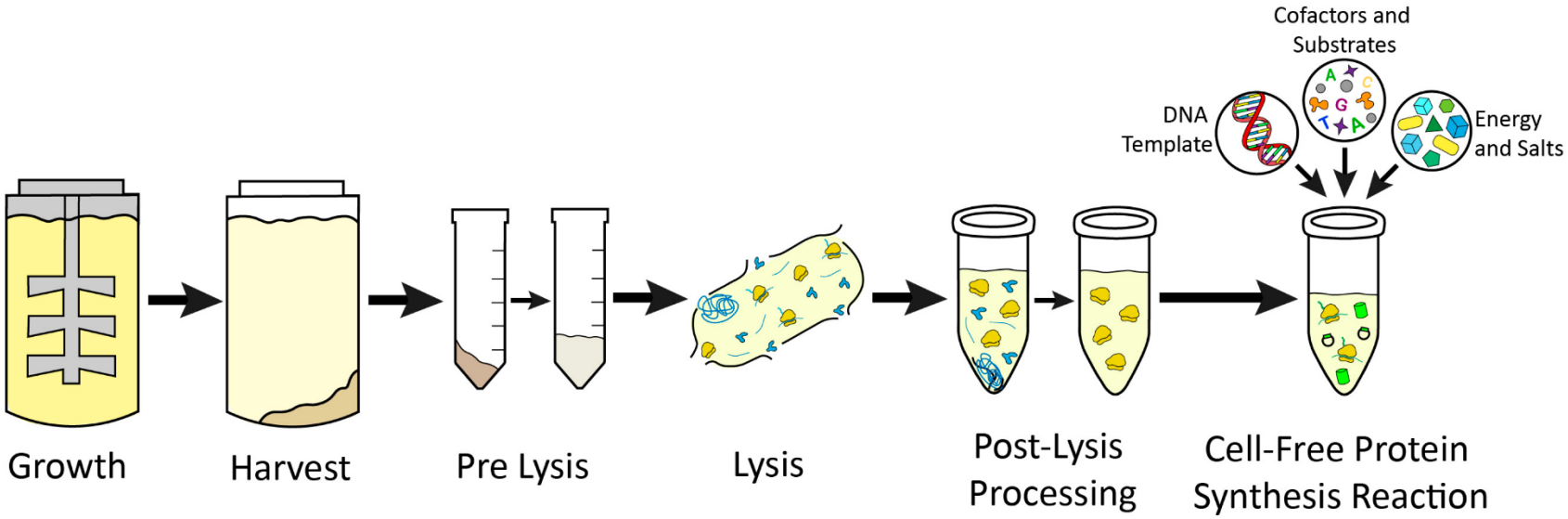
Cell-free platforms and their applications



(A) Web of the applications enabled by low adoption cell-free platforms. Connections shown are based on applications that have been published or that have been proposed in publications.

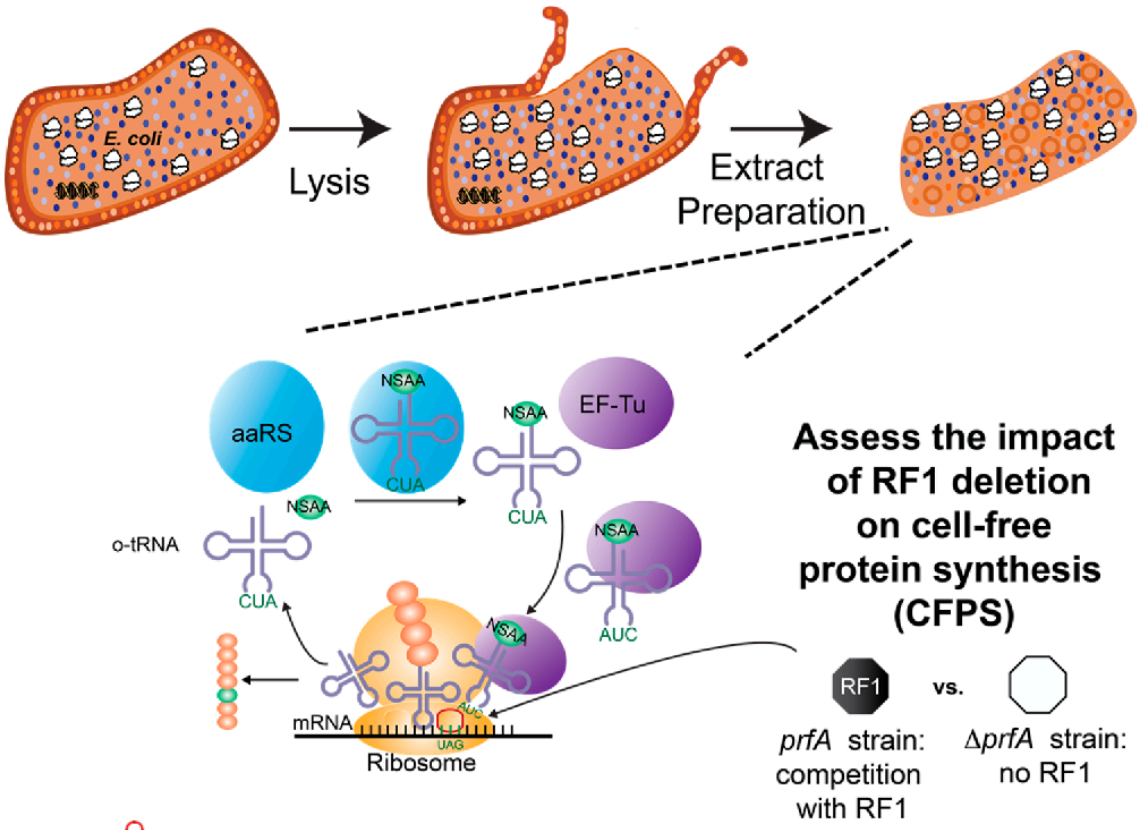
(B) Cumulative number of peer-reviewed publications over the last 60 years for cell-free platforms.

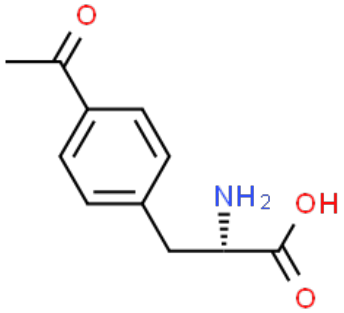
Preparation of cell-free extract and set-up of CFPS reactions



General workflow for preparation of cell-free extract and set up of CFPS reactions. A visualization from cell growth to the CFPS reaction is depicted above for a new user, highlighting the main steps involved.

CFPS reaction incorporating nonstandard amino acids





p-acetyl-L-phenylalanine

- Scheme of cell-free protein synthesis reaction incorporating nonstandard amino acid to investigate the effect of RF1
- Cell extracts containing transcription and translation machinery are prepared from rEc.E13 or rEc.E13.ΔprfA strains
- Plasmid DNA template of sfGFP containing single or multiple **amber codon** sites, **orthogonal tRNA/aaRS**, **NSAA**, T7 RNA polymerase, and other cofactors are added as necessary to activate the cell-free protein synthesis (CFPS) reaction

CFPS from extracts of a genomically recoded organism

nature COMMUNICATIONS

Rey W. Martin, Benjamin J. Des Soye, Yong-Chan Kwon, Jennifer Kay, Roderick G. Davis, Paul M. Thomas, Natalia I. Majewska, Cindy X. Chen, Ryan D. Marcum, Mary Grace Weiss, Ashleigh E. Stoddart, Miriam Amiram, Arnaz K. Ranji Charna, Jaymin R. Patel, Farren J. Isaacs, Neil L. Kelleher, Seok Hoon Hong & Michael C. Jewett

Nature Communications 9, Article number: 1203 (2018) | Download Citation
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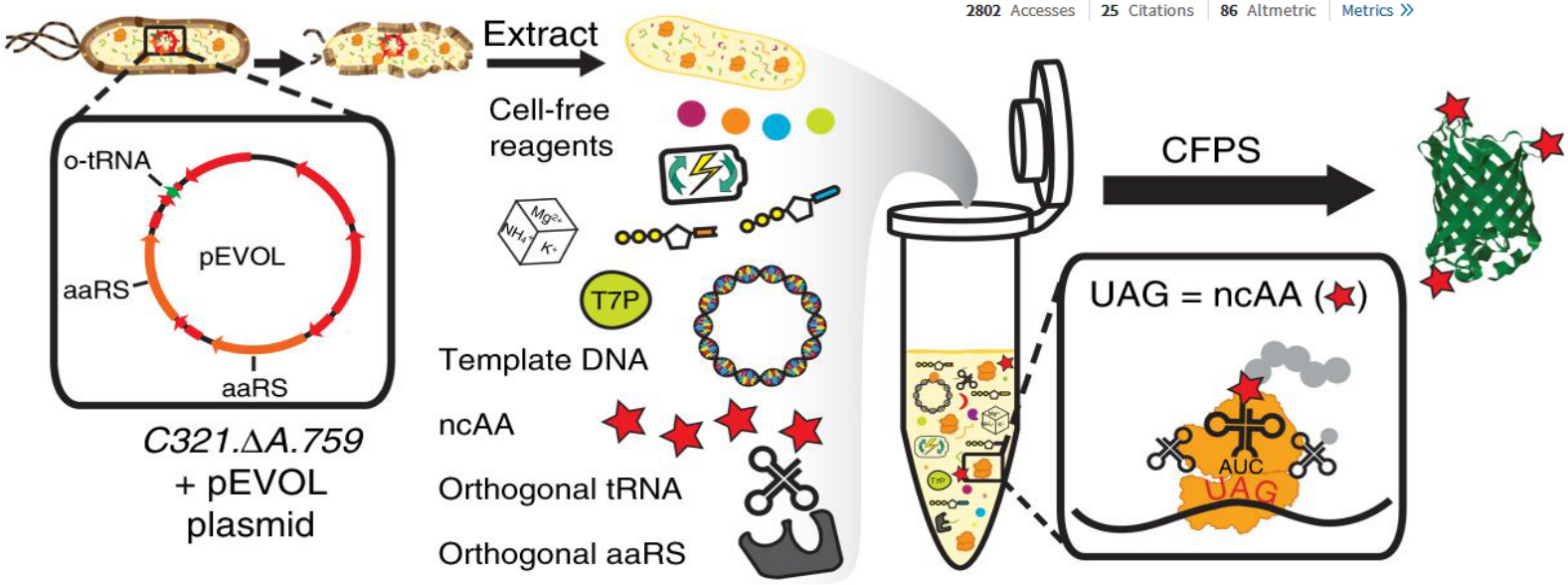
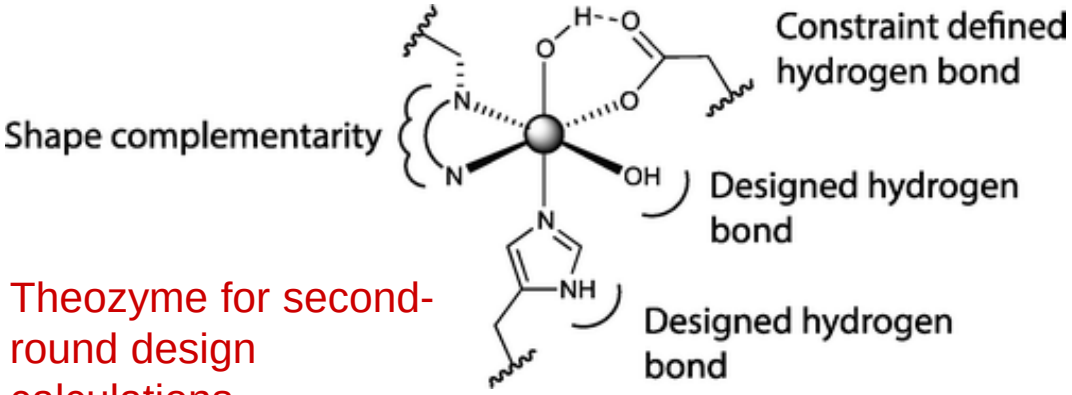


- Schematic of the production and utilization of crude extract from genomically recoded organisms with plasmid overexpression of orthogonal translation components for cell-free protein synthesis
- CFPS reactions are supplemented with the necessary substrates (e.g., amino acids, NTPs, etc.) required for in vitro transcription and translation as well as purified orthogonal translation system (OTS) components to help increase the ncAA incorporation efficiency
- aaRS, aminoacyl tRNA synthetase; ncAA, non-canonical amino acid; T7P, T7 RNA polymerase; UAG, amber codon

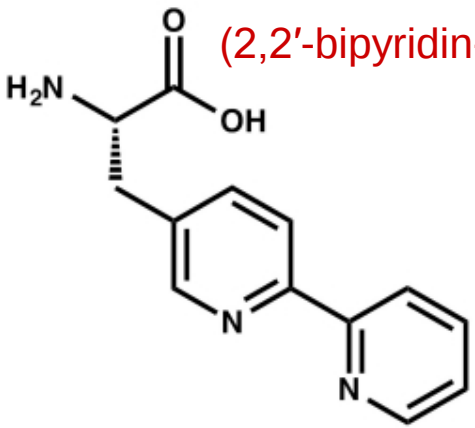
A designed metalloprotein using an unnatural amino acid

An example of computational protein design II.

- Genetically encoded unnatural amino acids could facilitate the design of proteins and enzymes of novel function
- The Rosetta design was used to design metalloproteins in which the amino acid **(2,2′-bipyridin-5yl)alanine** (Bpy-Ala) is a primary ligand of a bound metal ion
- A buried metal binding site with octahedral coordination geometry consisting of Bpy-Ala, two protein-based metal ligands, and two metal-bound water molecules
- Experimental characterization revealed a Bpy-Ala-mediated metalloprotein with the ability to bind divalent cations including Co^{2+}, Zn^{2+}, Fe^{2+}, and Ni^{2+}, with a *K*d for Zn^{2+} of ~40 pM



Theozyme for second-round design calculations



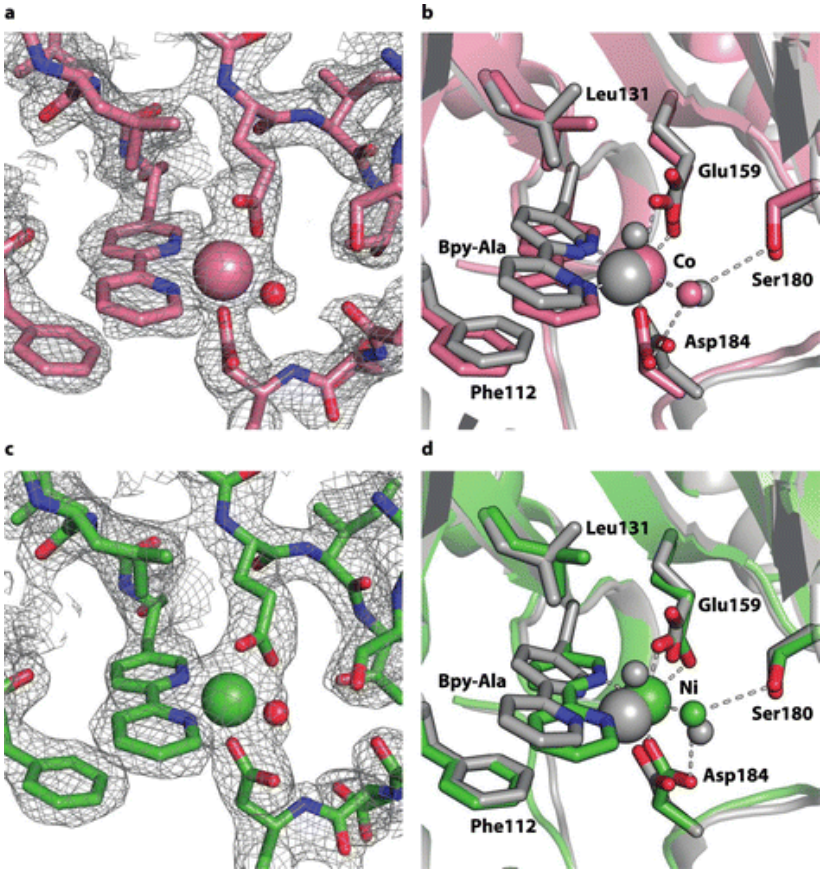
(2,2′-bipyridin-5yl)alanine

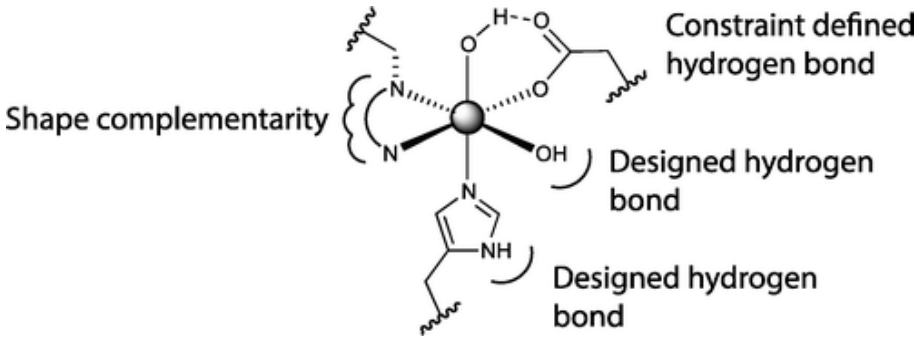
Mills *et al*., J. Am. Chem. Soc., 135: 13393-13399 (2013)

A designed metalloprotein using an unnatural amino acid

An example of computational protein design II.

- X-ray crystal structures of the designed protein bound to Co^{2+} and Ni^{2+} have RMSDs to the design model of 0.9 and 1.0 Å respectively over all atoms in the binding site.





X-ray crystallographic analysis of MB_07 bound to Co^{2+} and Ni^{2+}. Electron density from a 2*F*o–*F*c map in the vicinity of the Bpy-Ala bound to Co^{2+} (a) and Ni^{2+} (c) contoured at 1.0 σ. Density for Bpy-Ala, Co^{2+} or Ni^{2+} (pink and green spheres, respectively), D184, E159, and a metal-bound water molecule (red spheres) is visible.

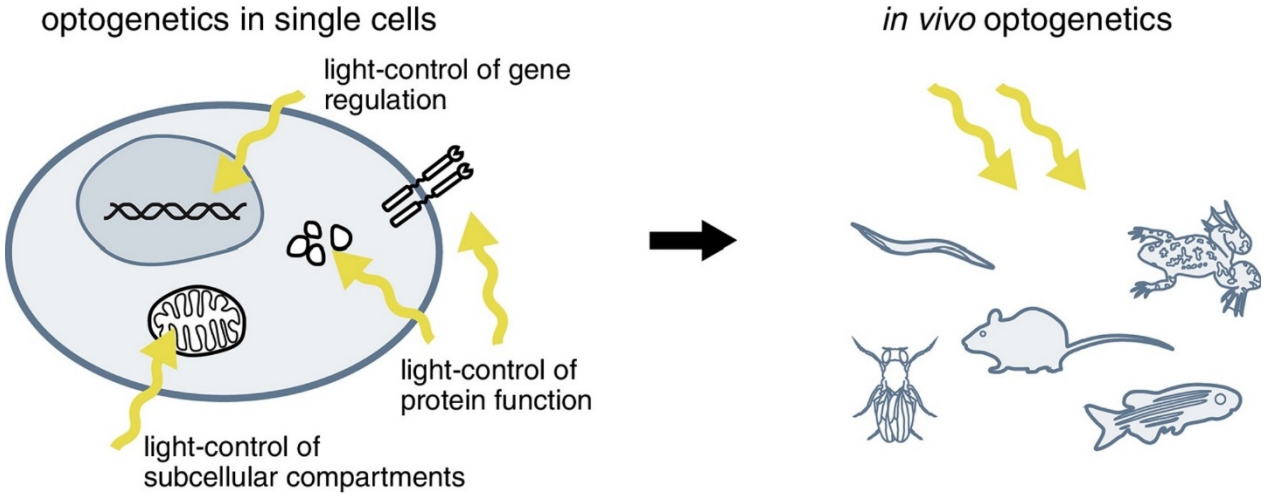
Mills *et al*., J. Am. Chem. Soc., 135: 13393-13399 (2013)

Optogenetics

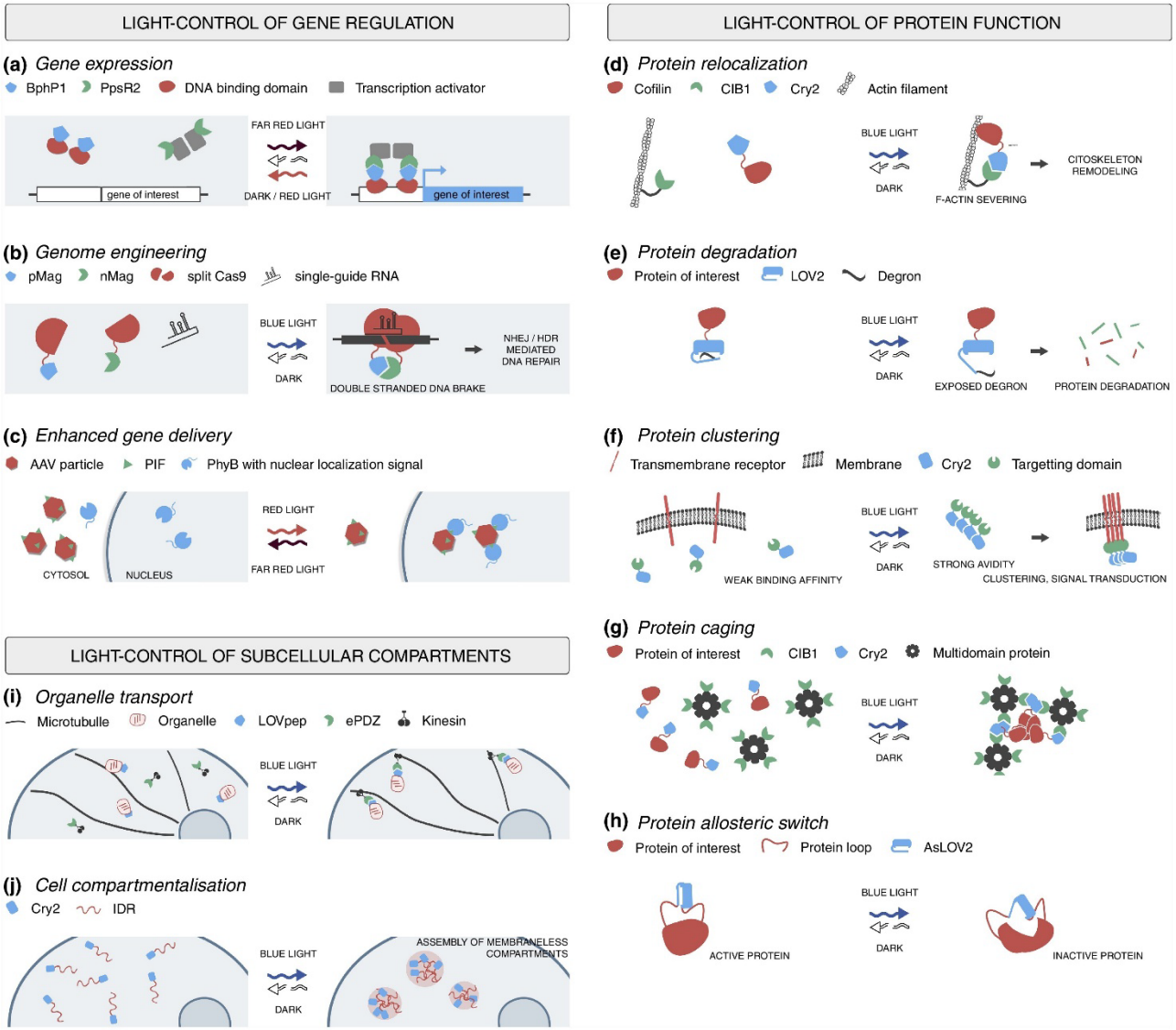
Concepts
Methods
Applications

What is optogenetics?

- Optogenetics provides the ability to gain **light-control over genetically encoded cell components**, and thereby ultimately over signalling pathways or biological processes
- Contrary to chemically inducible systems, where spatiotemporal capabilities are limited by the diffusion and the half-life of inducing molecules, light provides an exquisite **precision of control** and thus opens unique possibilities to disentangle the principles that govern nature
- By delivering optical control at the speed (millisecond-scale) and with the precision (cell type–specific) required for biological processing, optogenetic approaches have opened **new landscapes for the study and engineering of biological systems**

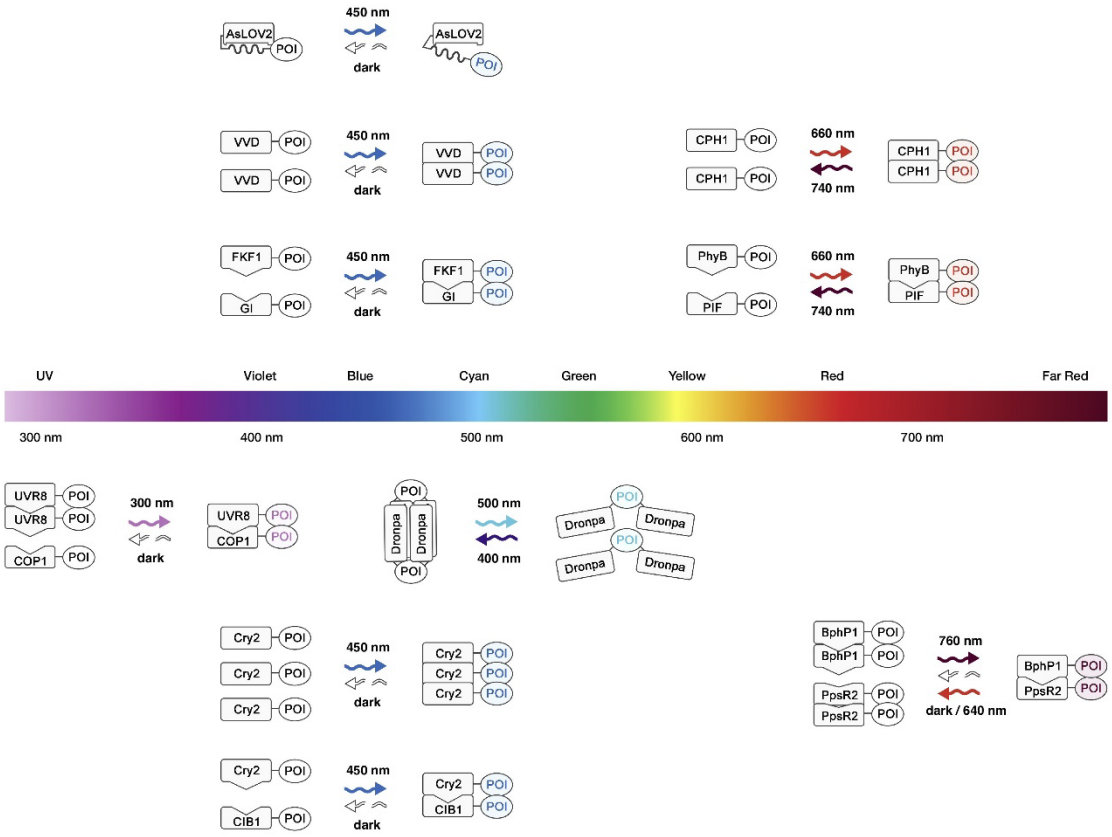


Optogenetic approaches to control gene regulation, protein function and subcellular compartments



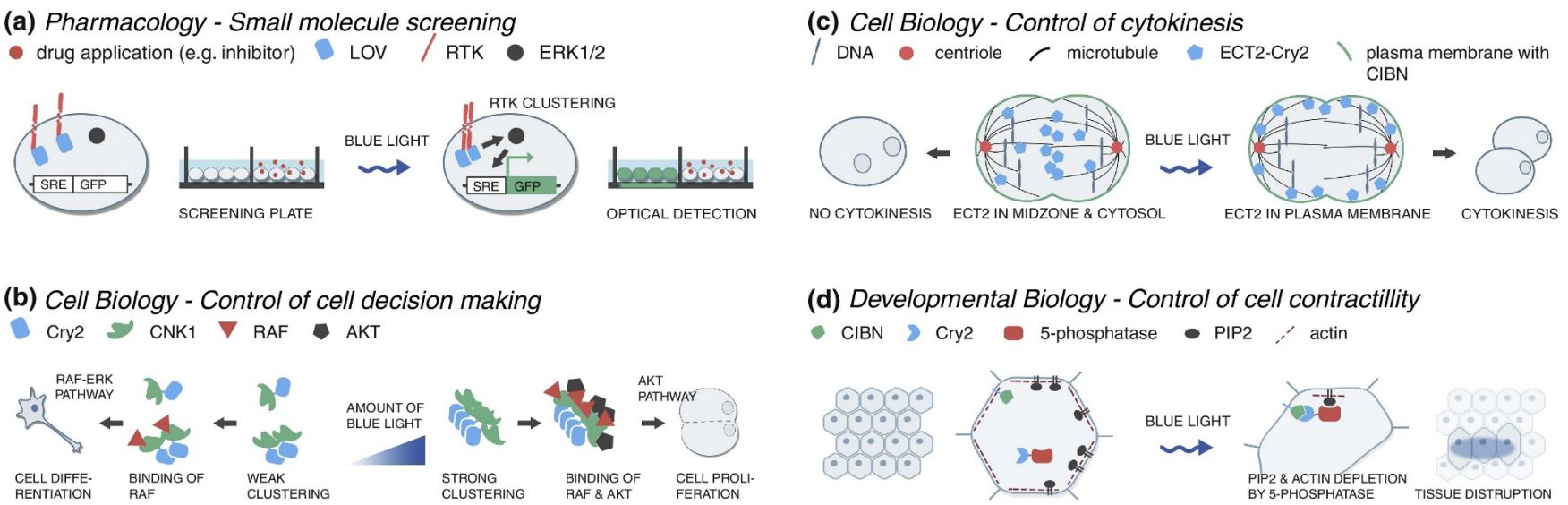
https://doi.org/10.1016/j.copbio.2017.06.010

Toolbox of light-responsive modules



Optogenetic switch modules. AsLOV2, *Avena sativa* light-oxygen-voltage domain 2; BphP1, bacterial bathy phytochrome RpBphP1; CIB1, cryptochrome-interacting basic helix-loop-helix 1; COP1, constitutive photomorphogenesis protein 1; CPH1, cyanobacterial phytochrome 1; CRY2, cryptochrome 2; FKF1, Kelch repeat, F-box protein; GI, GIGANTEA; PHYB, phytochrome B; PIF, phytochrome interacting factor; POI, protein of interest; PpsR2, oxygen-responsive transcription regulator; UVR8, ultraviolet B receptor; VVD, vivid.

Examples of optogenetic applications



(a) Light-controlled activation of a receptor tyrosine kinase (RTK) signaling pathway as a platform for optogenetics-assisted small-molecule screening

(b) Light-controlled CNK1 clustering reveals the protein's role as a molecular platform for cellular decision-making

(c) Light-controlled localization of Rho guanine exchange factor ECT2 as an optogenetic system to show that the protein's plasma membrane association during anaphase is necessary and sufficient for cytokinesis

(d) Light-controlled localization of 5-phosphatase OCRL as an optogenetic tool to control cell contractility during tissue morphogenesis

Legend: AKT, protein kinase B; CNK1, connector enhancer of KSR 1; ERK1/2, extracellular signal-regulated kinase; GFP, green fluorescent protein; RAF, rapidly accelerated fibrosarcoma kinase; SRE, serum response element

Optogenetics: conclusions

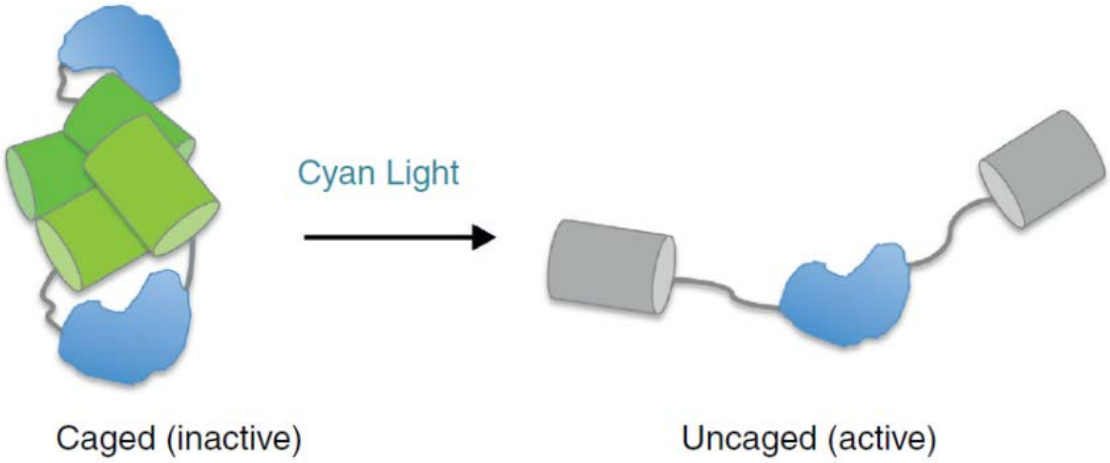
- Optogenetics uses light to control genetically encoded cell components
- It is a precise tool for systematically addressing complex biological questions
- It has a potential for applications in synthetic biology, medicine, pharmacology and elsewhere
- The latest optogenetic systems move from single cells to *in vivo* applications

Photocaging in synthetic biology

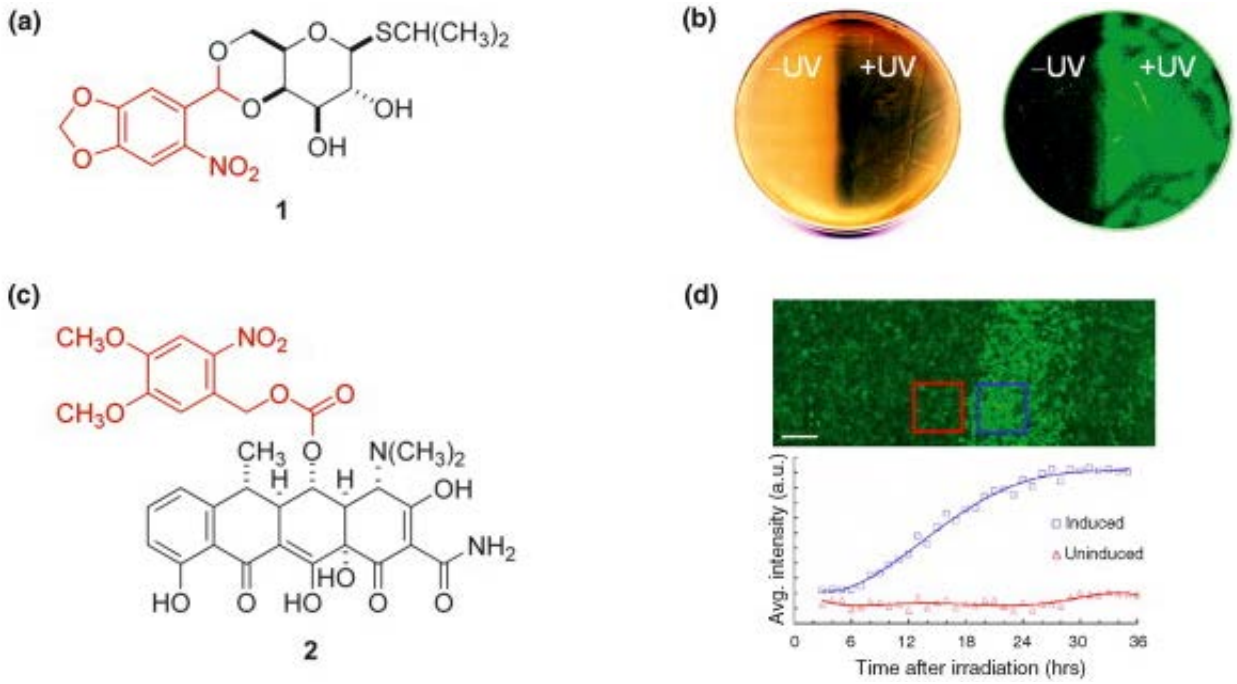
Concepts
Methods
Applications

Photochemical control of protein function

- Biological processes are regulated with a high level of spatial and temporal resolution
- To understand and manipulate these processes, we need to be able to regulate them with Nature's level of precision
- In this context, light is a unique regulatory element because it can be precisely controlled in terms of location, timing and amplitude
- Moreover, most biological laboratories have a wide range of light sources as standard equipment



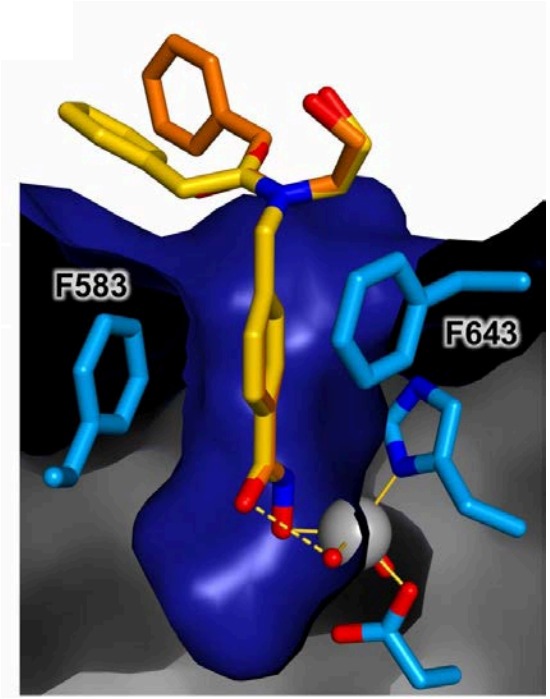
Photochemical regulation of gene expression

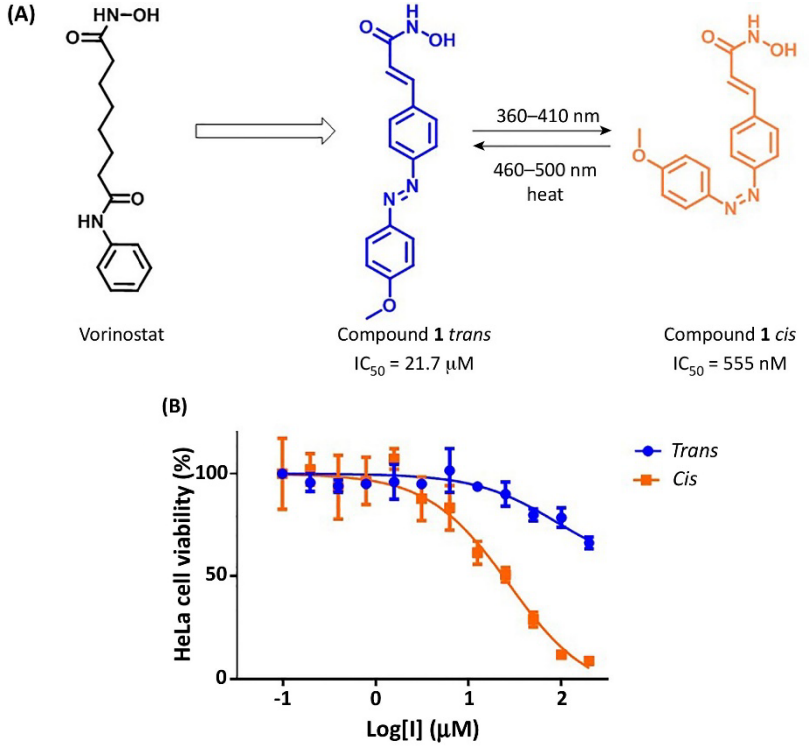


(a) Light-activatable small-molecule inhibitors of gene expression repressor proteins. Caged IPTG **1**
(b) Bacterial lithography experiment with **1** showing light-controlled induction and spatial control of β-galactosidase (left) and GFP (right) in a bacterial lawn (ø 10 cm)
(c) Caged doxycycline **2**
(d) Spatial control of GFP expression using **2**, with irradiation through a 344-μm photomask (scale bar 250 μm); irradiated (blue) and non-irradiated (red) cells were quantified and fluorescence intensity is shown as a function of time. The light-removable caging groups are shown in red

https://doi.org/10.1016/j.tibtech.2010.06.001

Photochemical control over enzyme activity

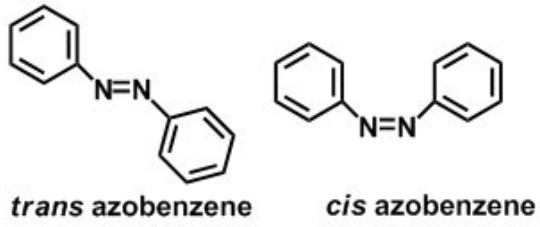




(a) Photo-control over the activity of histone deacetylase 2 (HDAC2). Based on known HDAC2 inhibitor vorinostat, compound **1** was designed. Upon irradiation, compound **1** switches from *trans* to *cis* form, becoming 39-fold more active as an HDAC2 inhibitor.
(b) Dose–response curve for compound **1** in *trans* (blue) and *cis* (orange) form on cell viability of HeLa cells.

https://doi.org/10.1016/j.tibs.2018.05.004

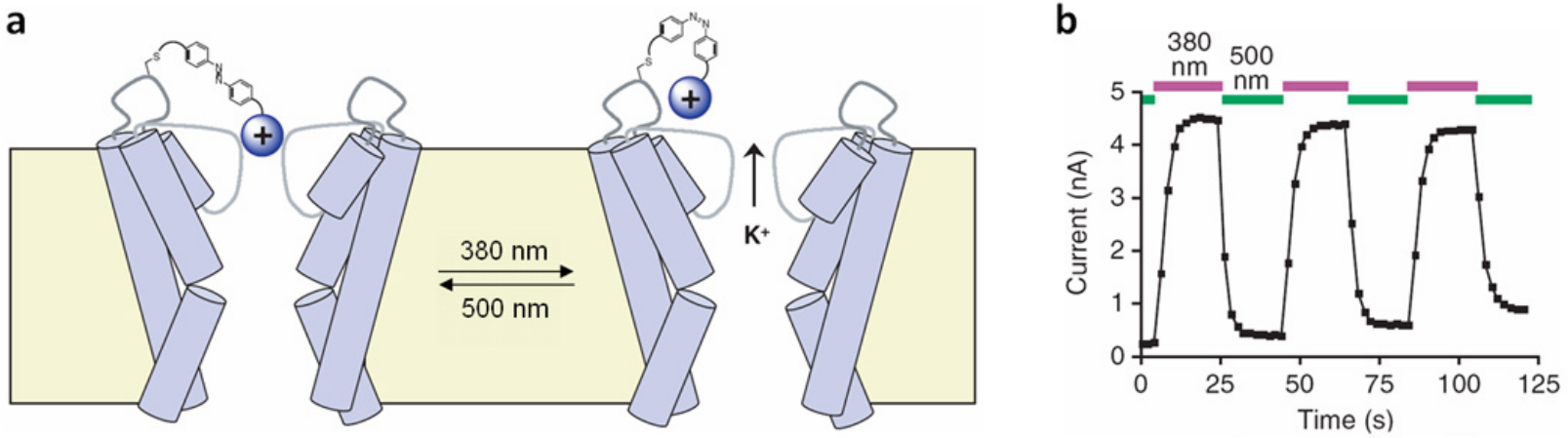
Reversible photochemical activation of K^+ ion channel



trans azobenzene *cis* azobenzene

Three distinct features:

- a quaternary ammonium group
- a photo-isomerizable azobenzene group
- an electrophilic cysteine-reactive group (e.g. acrylamide, chloroacetamide, epoxide or maleimide)



(a) Schematic of PAL-gated K^+ channel: The sphere labelled with a plus sign represents a quaternary ammonium group, which blocks the channel when the diazobenzene is in the *trans* conformation. Irradiation at 380 nm switches the diazobenzene from *trans* to *cis*, thus enabling K^+ flow. Irradiation with 500 nm light reverses the switching event, thus blocking the ion channel.

(b) Light-controlled membrane potential of a PAL-gated K^+ channel measured in dependence of light irradiation.

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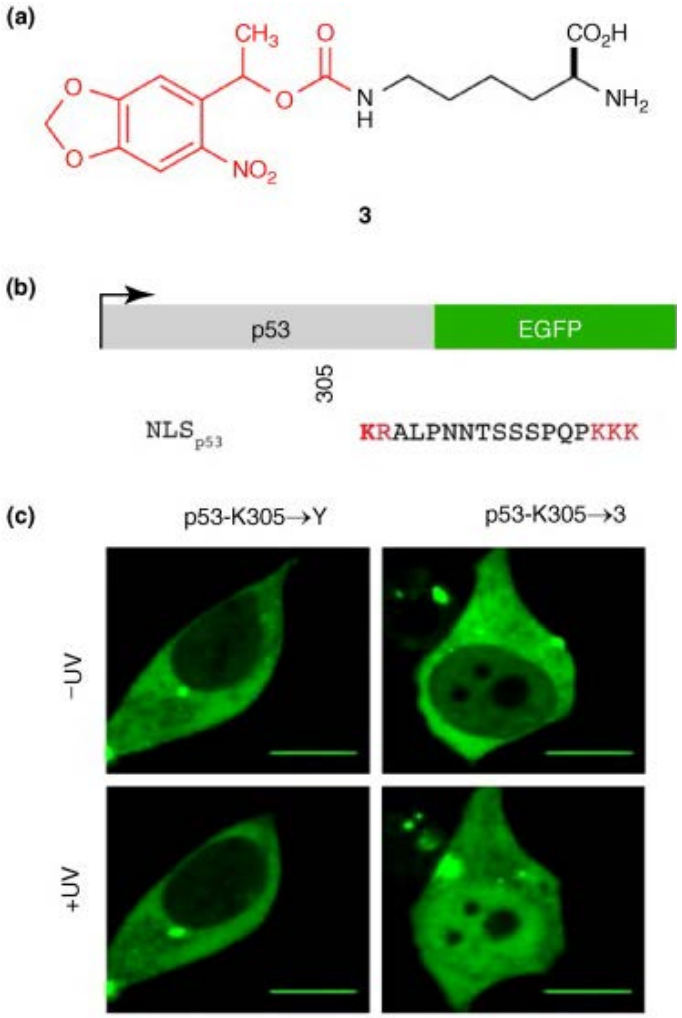
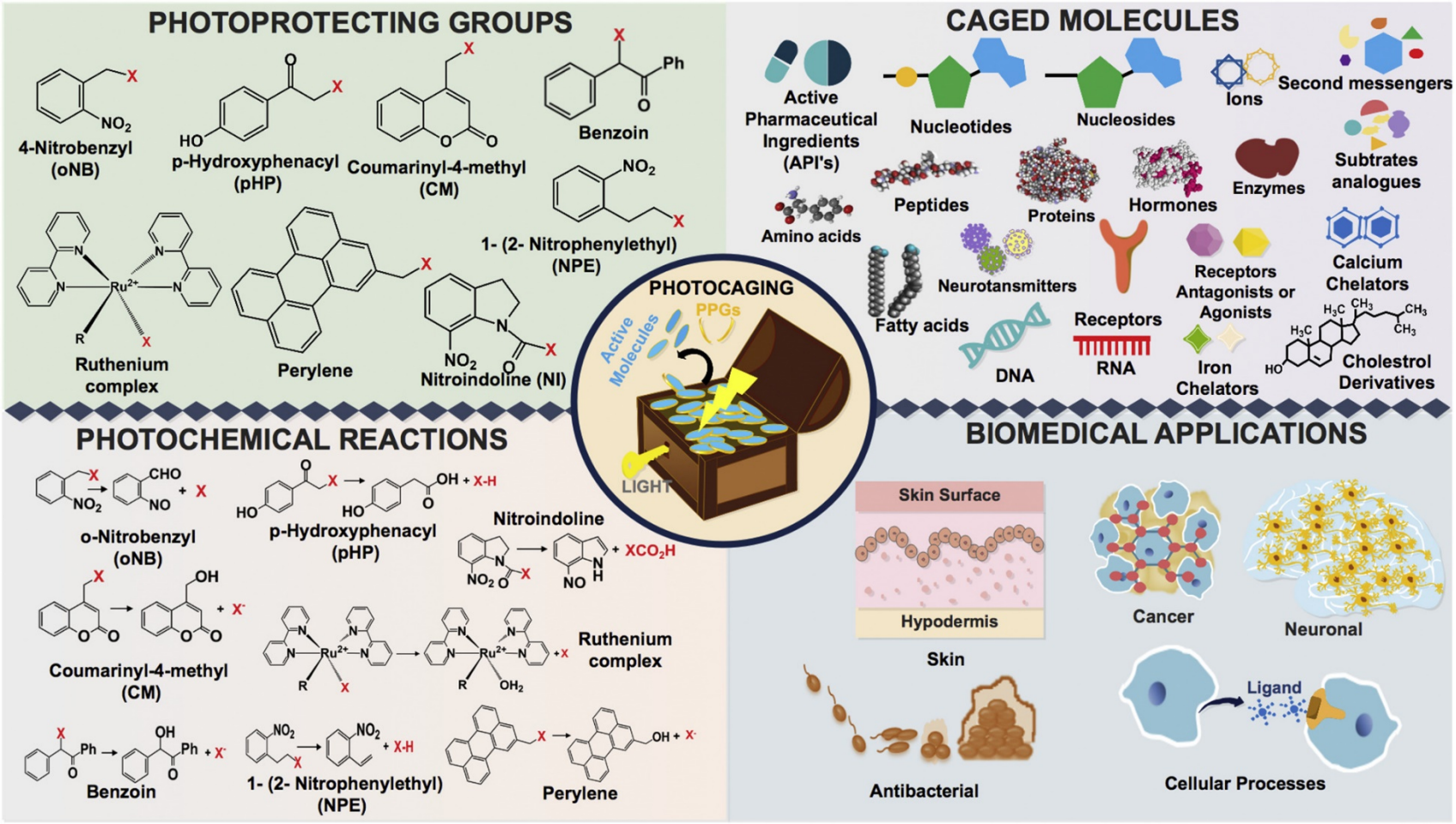
Photochemical protein localization in cells



(a) Genetically encoded caged lysine (light-removable caging group shown in red)
(b) Gene diagram of *p53–egfp* with the crucial lysine K305 in bold
(c) Nuclear import of EGFP in cells after introduction and photolysis of the caged lysine **3** introduced into position 305 in NLSp53. A mutation of K305 to tyrosine (K305→Y) blocks transport into the nucleus, regardless of light irradiation (left). Introduction of the caged lysine **3** at position 305 (K305→**3**) blocks transport of p53–EGFP, but facilitates translocation into the nucleus after brief light irradiation (365 nm, 5 s) (right). Scale bar, 10 μm.

https://doi.org/10.1016/j.tibtech.2010.06.001

Overview of photocaging applications in biology



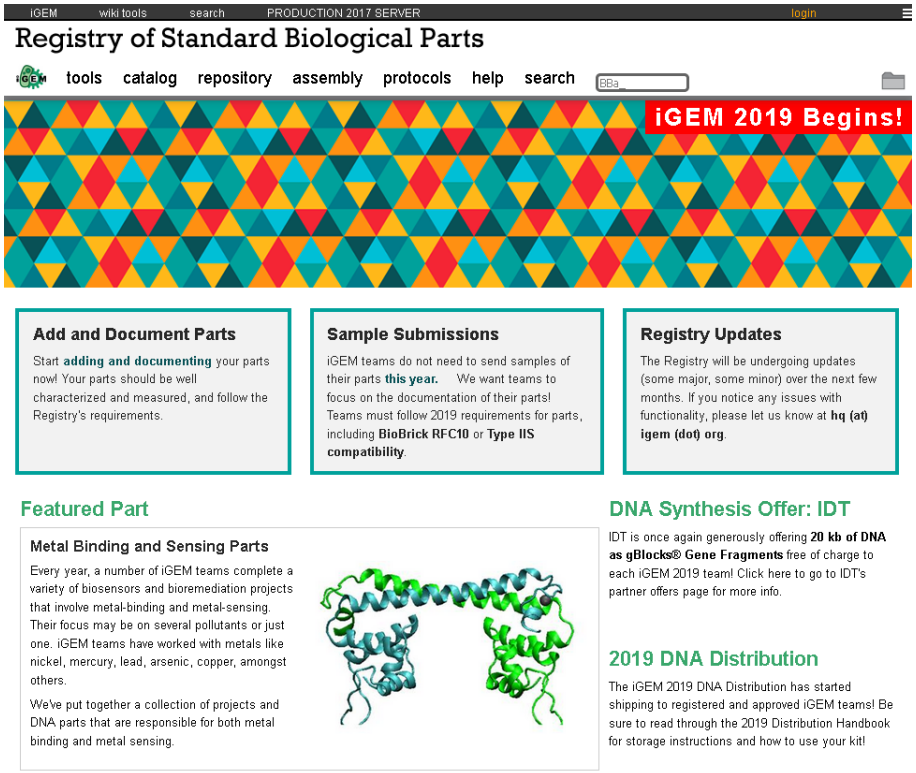
Representation of the photoprotecting groups, caged molecules, photochemical reactions behind the use of photocaging approaches in different biomedical applications.

Questions

Supplementary materials

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4090895/
https://pubs.acs.org/doi/10.1021/sb300094q

Structure-based engineering and design of parts



- Until now, structural biology has been mainly used in synthetic biology approaches to design new parts/components and tools
- Many of the characterized components can be found in the Registry of Standard Biological Parts (http://partsregistry.org/)
- Of special importance is the recently developed “SYNZIP protein toolbox” because it contains a complete biophysical quantitative description (i.e., affinities) of synthetic domains

Genetic circuits for signal processing

Genetic circuits are commonly built by synthetic biologists to enable cells to take some input and compute what to do. Beyond a cell being activated by a single input, cells could be engineered to respond to multiple signals and to make decisions based on both its neighbouring cells and environment.

One published example of mammalian genetic circuits is ‘Boolean logic and arithmetic through DNA excision’ (BLADE) and it was able to build 113 different circuits in embryonic kidney and Jurkat T cells. The BLADE system uses recombinases to create logic gates but can also interface with CRISPR–Cas9 to regulate host cell genes. Large circuits like the ones demonstrated with BLADE could be combined with synthetic receptors so cells can make decisions based on what combination of signals they see.

Receptor engineering

New types of receptors can be engineered to detect different types of molecules and turn on some cellular output function. The more receptors that can be readily engineered the more molecules that can be used as inputs to a therapeutic cell. It has been shown that synthetic notch receptors can be made for use with different recognition domains as well as different transcription factors as activation domains. That means that a researcher can decide both what the receptor responds to and what it turns on after it binds its target. Typical CAR-Ts only change the input of the receptor, but this kind of receptor can swap out either the input or output or both. Having several independent receptors would allow much more complex programming of therapeutic cells to respond to signals within the human body.

Safety kill switch

Since toxicity can be an issue, it would be good to have an off switch in the event of a problem. One form of toxicity that can happen with CAR-T is called “Cytokine Release Syndrome” or “Cytokine Storm” for especially severe cases. This can happen when the immune system has such a strong response that many inflammatory cytokines are released triggering mild to severe symptoms including fever, headache, rash, rapid heartbeat, low blood pressure, and trouble breathing.

Synthetic biologists have already worked on a number of “kill switches” in different organisms. A kill switch could be applied to CAR-T by having a drug-inducible kill-switch in the the T-cells that would give the medical team a way to kill off the modified cells as soon as a bad side effect starts to show up. Researchers and companies are also seeking multiple ways to either switch off or kill the T-cells when they’re not needed. These variations on a kill switch can help to make sure CAR-T cell therapy has extra safety measures in place.

There are many types of cancer and many ways in which a cancer can avoid successful treatment. Hopefully mammalian synthetic biology can add something to the immunotherapy toolkit by improving CAR-T or other approaches to enlist the immune against cancer.

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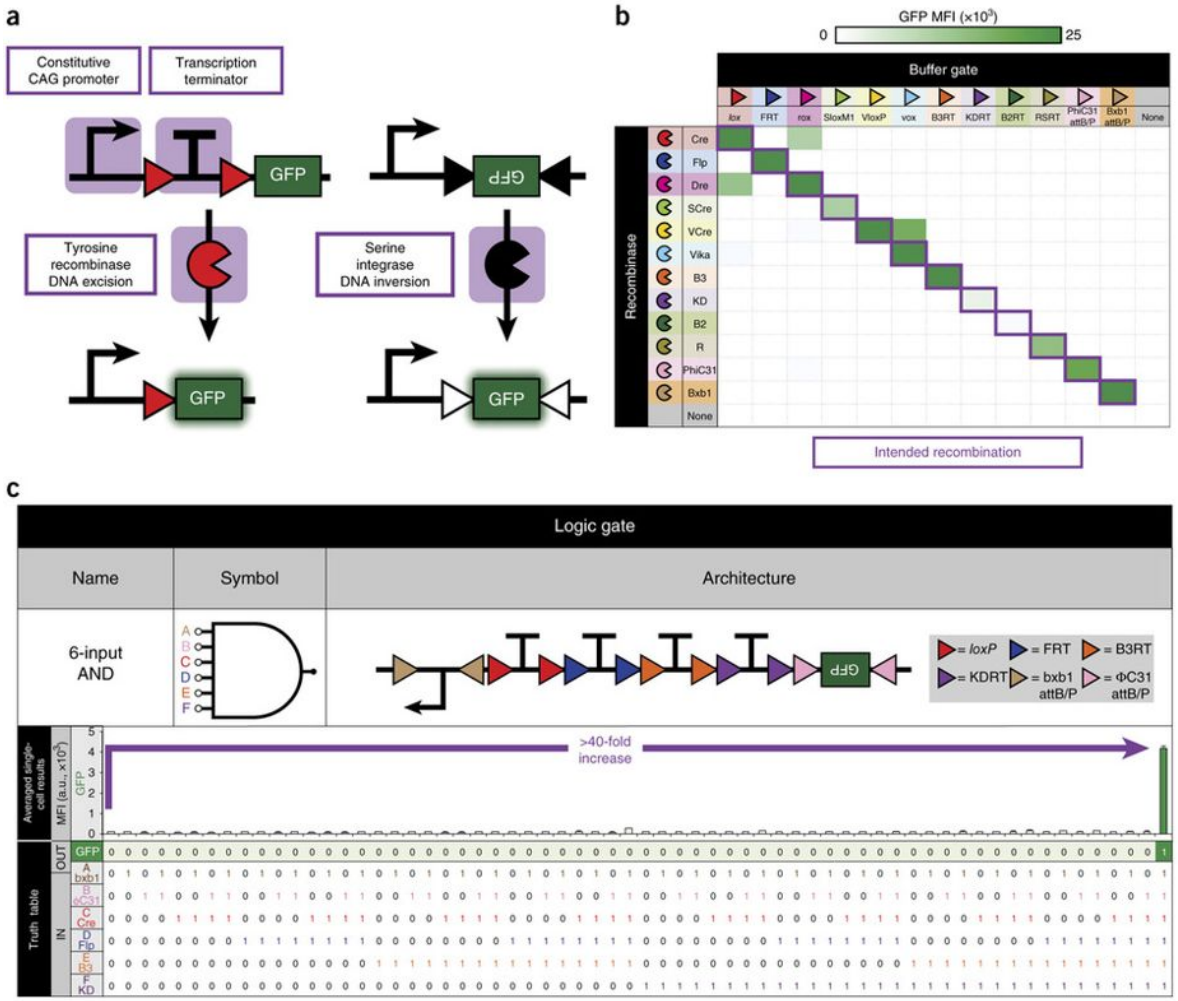
Article | Published: 15 May 2019

Total synthesis of *Escherichia coli* with a recoded genome

Julius Fredens, Kaihang Wang, Daniel de la Torre, Louise F. H. Funke, Wesley E. Robertson, Yonka Christova, Tiongsun Chia, Wolfgang H. Schmied, Daniel L. Dunkelmann, Václav Beránek, Chayasith Uttamapinant, Andres Gonzalez Llamazares, Thomas S. Elliott & Jason W. Chin

Nature **569**, 514–518 (2019) | Download Citation

Implementation of multi-input gates in mammalian cells



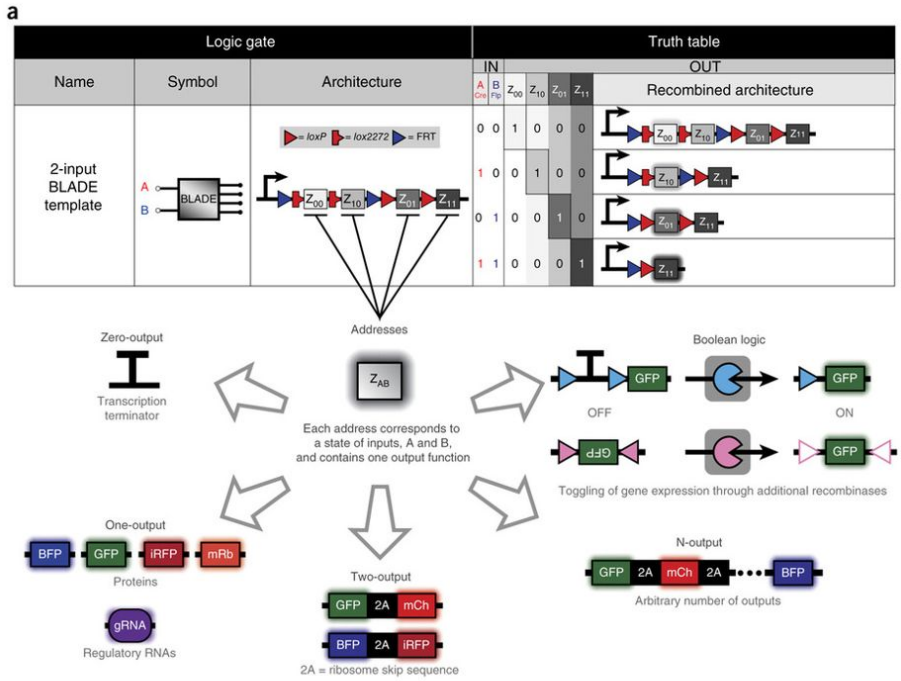
(a) Recombinases can perform simple BUF logic operations, either by tyrosine-recombinase-mediated excision (left) or serine-integrase-mediated inversion (right).

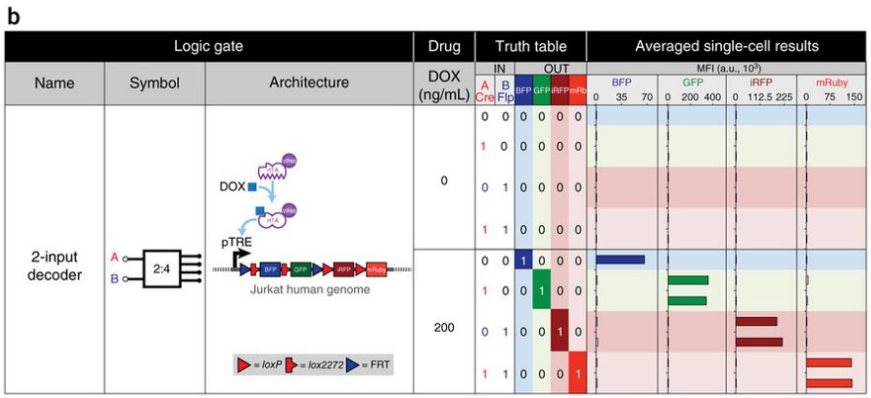
(b) Recombinases are tested for their recombination efficiency and orthogonality on all BUF logic reporters.

(c) A 6-input AND-gate that produces GFP when all inputs are present. MFI, mean fluorescence intensity from *n* = 3 transfected cell cultures; a.u., arbitrary units. Error bars, s.e.m.

Weinberg *et al.*, Nature Biotechnology 35: 453-462 (2017)

Four distinct output functions based on two inputs

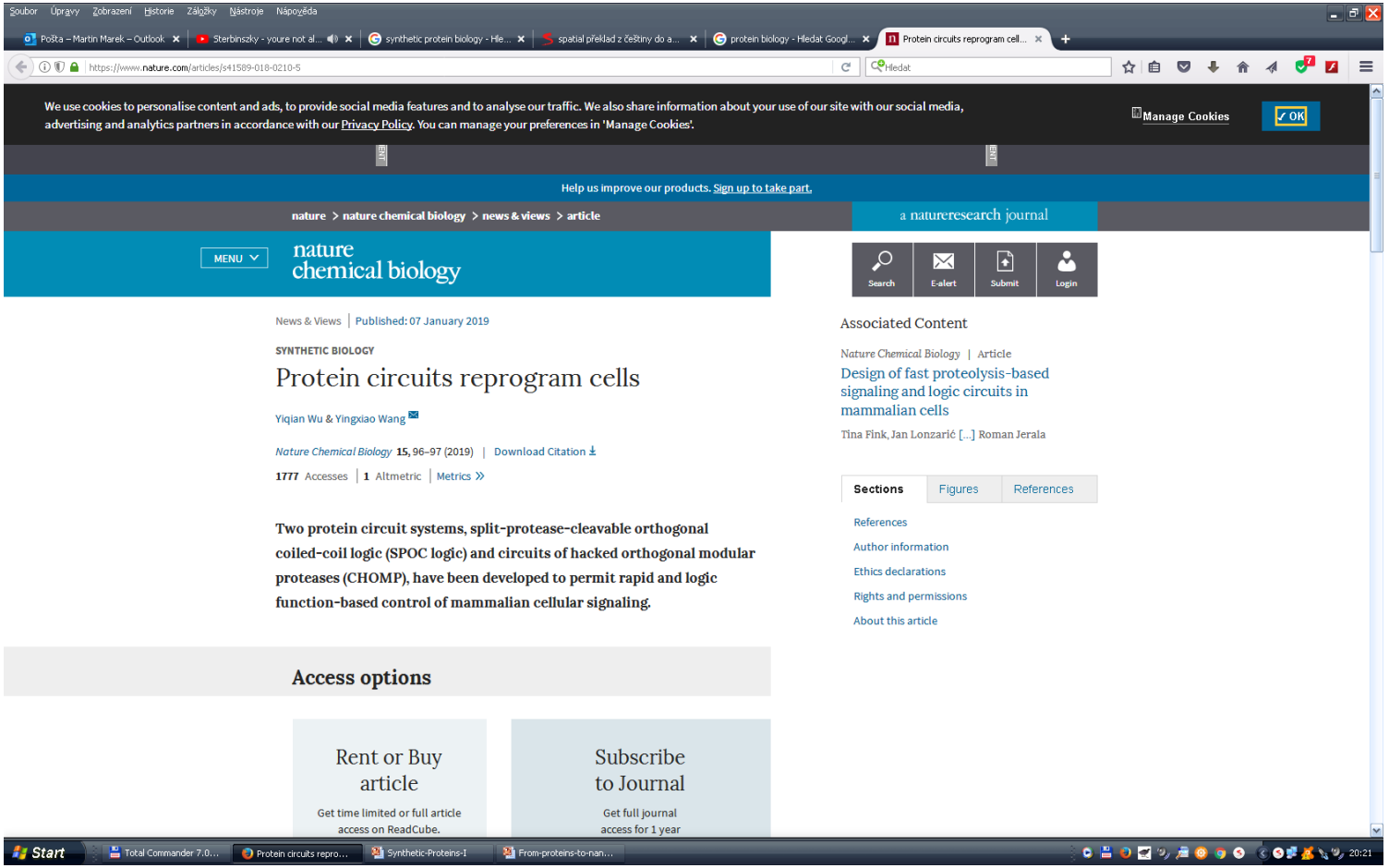




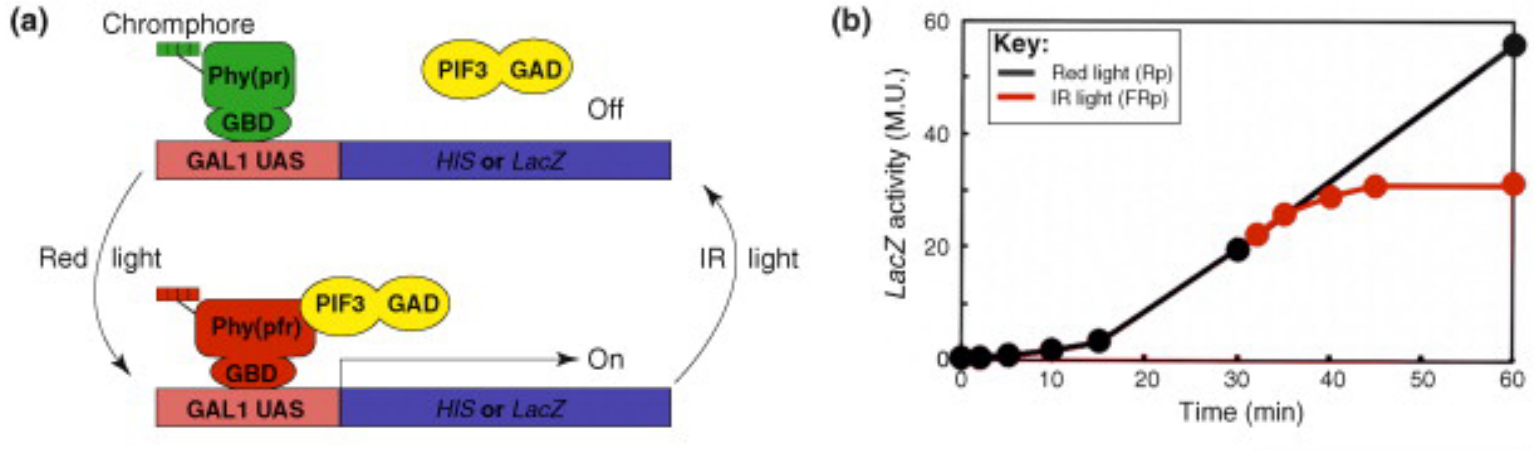
(a) 2-input BLADE template on one plasmid with a single transcriptional unit. This template contains four distinct regions of DNA (addresses) downstream of a promoter. Each address corresponds to an output function and is accessed or deleted via site-specific DNA recombination. Each address can be programmed from different configurations ranging from zero-inputs to Boolean functions. The first address (Z00), which is the closest to the promoter, corresponds to a state where no recombinase is expressed (A = 0, B = 0). If the Z00 address contains a protein coding sequence, then that gene will be expressed. Gene expression from the other addresses downstream of Z00 will be blocked by the presence of the Z00 protein coding region. In the presence of recombinase A, which corresponds to state (A = 1, B = 0), addresses Z00 and Z01 will be removed, thus moving address Z10 directly downstream of the promoter and allowing gene expression of address Z10 only to occur. Similarly, when only recombinase B is present (A = 0, B = 1), addresses Z00 and Z10 are excised, allowing Z01 to be moved directly downstream of the promoter. Finally, when both recombinases are expressed (A = 1, B = 1), addresses Z00, Z01, Z10 are all excised, thus placing Z11 downstream of the promoter unobstructed by the other addresses.

(a) Integrated 2-input BLADE decoder with tagBFP, EGFP, iRFP720, and mRuby2 as addresses Z00, Z10, Z01, and Z11 respectively. Plasmids constitutively expressing Cre and/or Flp are then stably integrated. Three days of doxycycline (DOX) treatment is used to permit the rtTA-VP48 protein to bind to the tetracycline response elements promoter (pTRE) to activate gene expression. Mean fluorescence intensity (MFI) is plotted of either *n* = 1 or *n* = 2 independent integrations. a.u., arbitrary units.

- Koukni na toto



Light-regulated protein-protein interactions

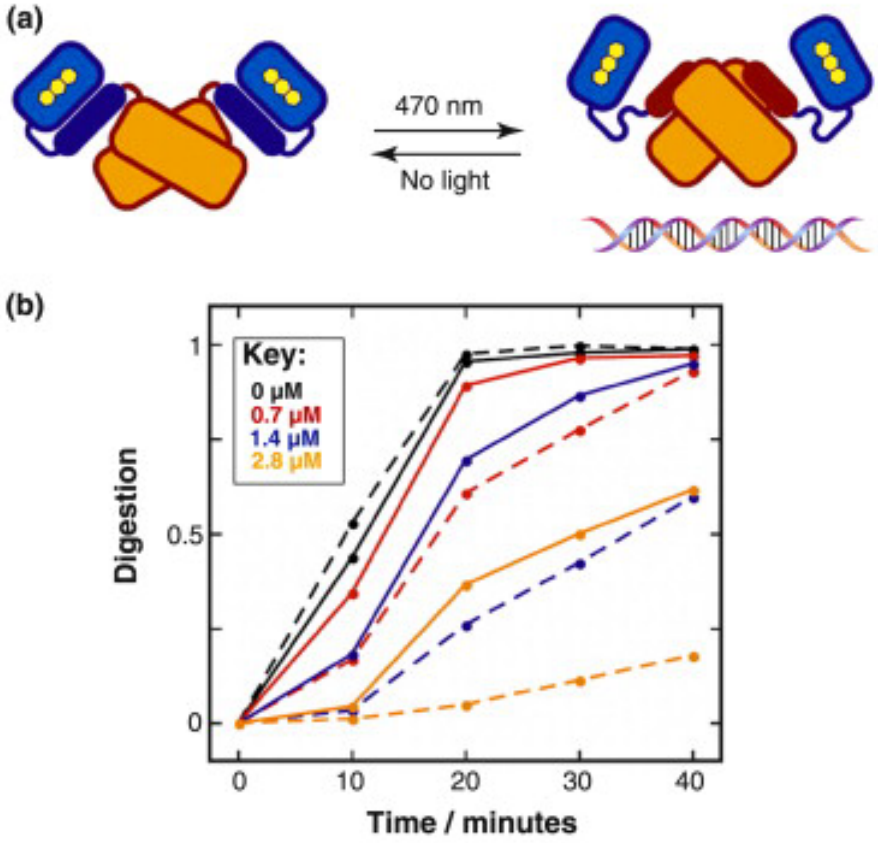


(a) Light-controlled expression of *lacZ* via PhyB–PIF protein interaction. Schematic of the light-controlled transcriptional activator. Phytochrome (Phy), which is fused to the DNA-binding region of GAL4 (GBD), reversibly binds to PIF. PIF is fused to the transcriptional activating domain of GAL4 (GAD), thus activating gene expression on exposure to red light and deactivating it by dissociation on exposure to IR light.

(b) LacZ (β-galactosidase) activity induced with a pulse of red light (Rp, black line), and arrested with pulses of far-red light (FRp, red line), thus showing that the system is reversible and can be used to control *lacZ* expression.

https://doi.org/10.1016/j.tibtech.2010.06.001

Genetically encoded photoresponsive protein domains



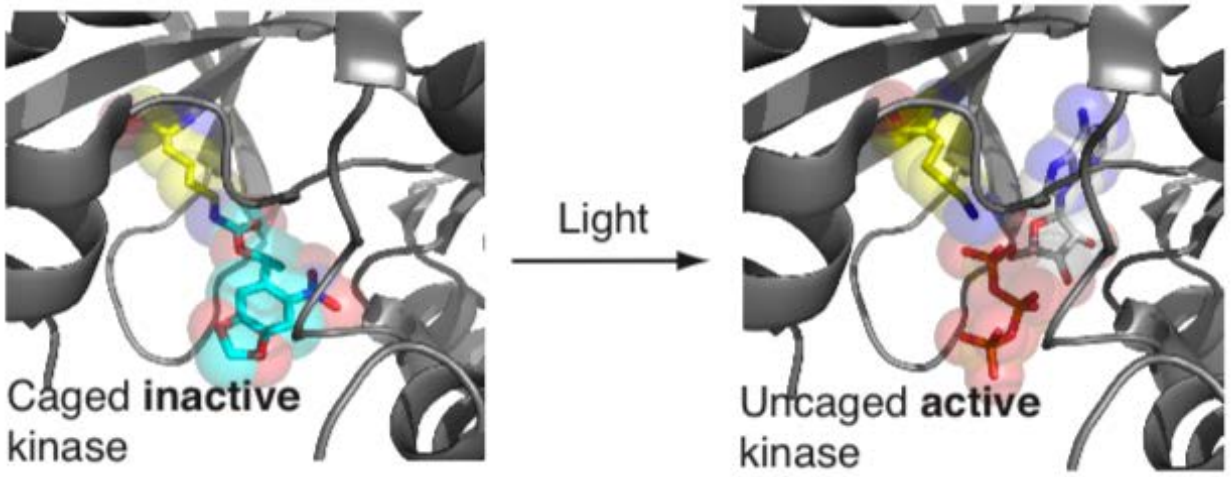
(a) Mechanism of LovTAP, a reversibly switchable DNA-binding protein. Before exposure to blue light, Jα (dark blue) is associated with the LOV domain (light blue), which renders the Trp repressor region (light orange) inactive. After irradiation with blue light (470 nm) a conformational change occurs and Jα (now dark orange) dissociates from the LOV domain, in turn activating the Trp repressor. The active protein binds to DNA at a *lac* operator region. The original conformation of the protein is slowly (tens of seconds) reassumed after incubation in the dark and the Trp repressor region dissociates from the DNA, thus making the activity of LovTAP reversible.

(b) LovTAP protects DNA against RsaI digestion at the *lac* operator site. An increase in the concentration of LovTAP decreases digestion, as does irradiation with blue light (dashed lines, irradiated; solid lines, non-irradiated).

https://doi.org/10.1016/j.tibtech.2010.06.001

Light-induced activation or inhibition (photocaging)

- Use of light to unmask or modify unnatural amino acids and subsequently regulate protein function
- Depending on the nature of the light-responsive group, it is possible to either activate, inhibit, or reversibly switch on/off protein function
- Unnatural amino acids containing a **photocage** (i.e. a photolabile protecting group) have been widely used for protein activation
- When replacing a functionally critical amino acid residue with the corresponding photocaged amino acid, the target protein becomes inactive; upon light irradiation, the photocage is removed, thereby restoring the protein's function



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6610526/

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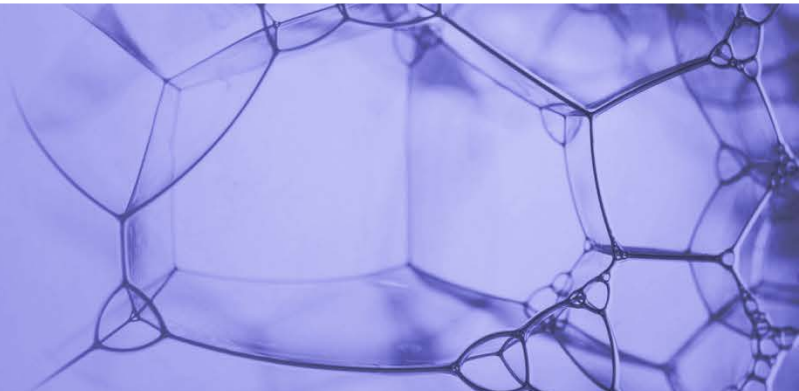

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