Biology for MX

MSE – 212

Prof. Maartje M.C. Bastings
Programmable Biomaterials Laboratory

Course 2: Proteins and Protein Based Materials

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Course Content

BLOCK 1: Introduction and engineering with cellular components

Lecture 1.	Intro to biology and cells	(February 21)
Lecture 2.	**Proteins and protein based materials**	**(February 28)**
Lecture 3.	DNA and DNA-based materials	(March 6)
Exercise 1.	*Proteins, peptides and DNA*	*(March 13)*

BLOCK 2: Inter- and intracellular action

Lecture 4.	ECM, adhesion and artificial matrices	(March 20)
Lecture 5.	Virus, antibodies and immune engineering	(March 27)
Lecture 6.	Bacteria	(April 10)
Exercise 2.	*Nanoparticles and Scaffolds*	*(April 17)*

BLOCK 3: Physics of biological processes

Lecture 7.	Receptors and targeting	(April 24)
Lecture 8.	Endocytosis	(May 1)
Lecture 9.	Signaling and communication	(May 8)
Exercise 3.	*Engineering functionality*	*(May 15)*
Lecture 10.	Revision and conclusion	(May 22)
Open office.	*Questions, discussion, exam prep*	*(May 29)*

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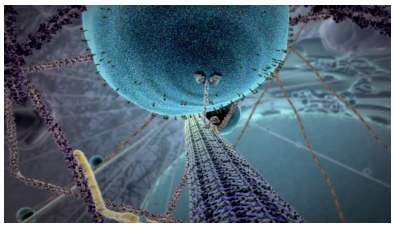
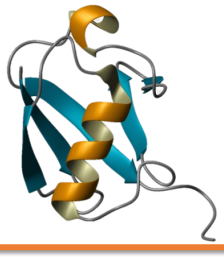
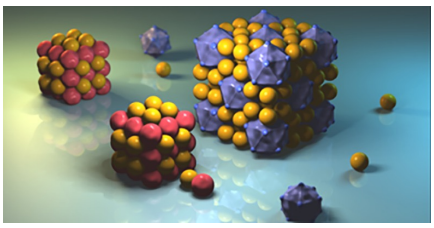
On Today's Menu:

Fundaments of Proteins ***Engineering with Proteins***

Part 1	Part 2	Part 3
What are Proteins? - Structure - Function - Production	**Protein Engineering** - Mutants - Synthesis - Folding Prediction (AI)	**Protein-based Materials** - Particles - Surfaces - Fun stuff

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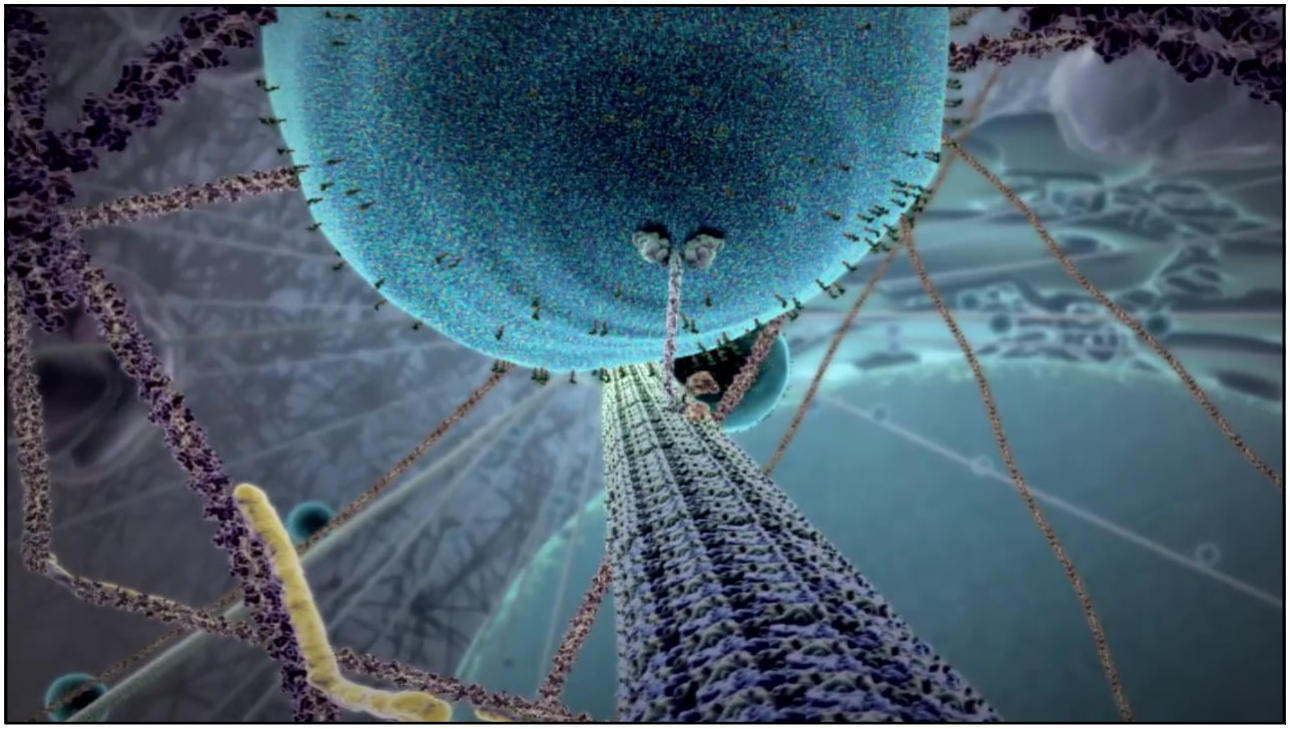

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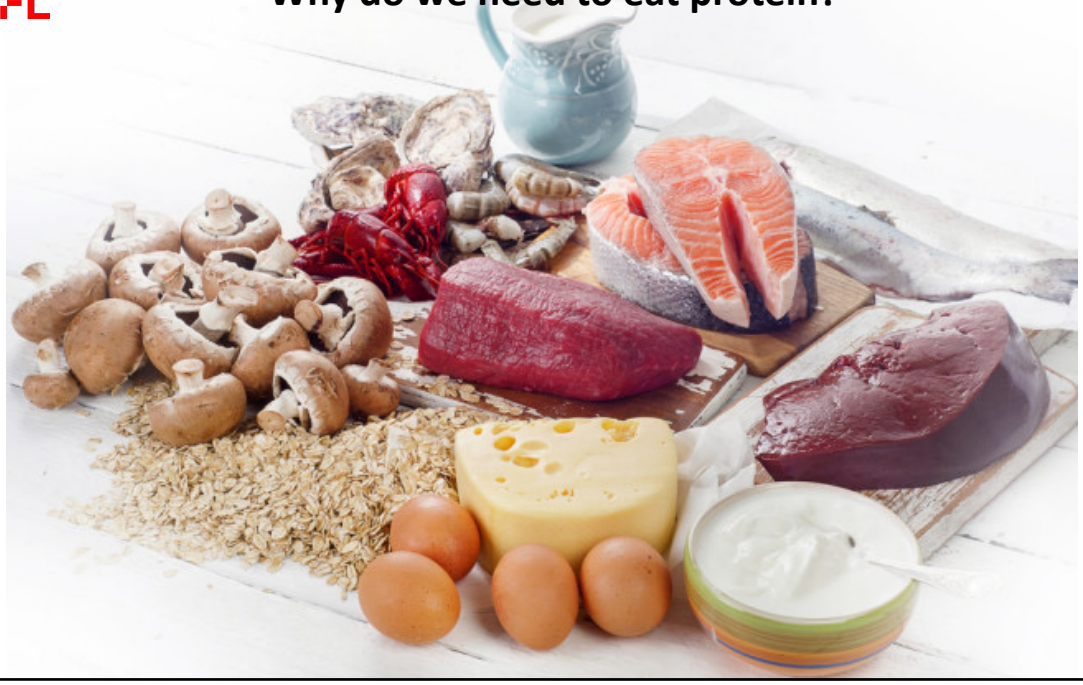
Why do we need to eat protein?



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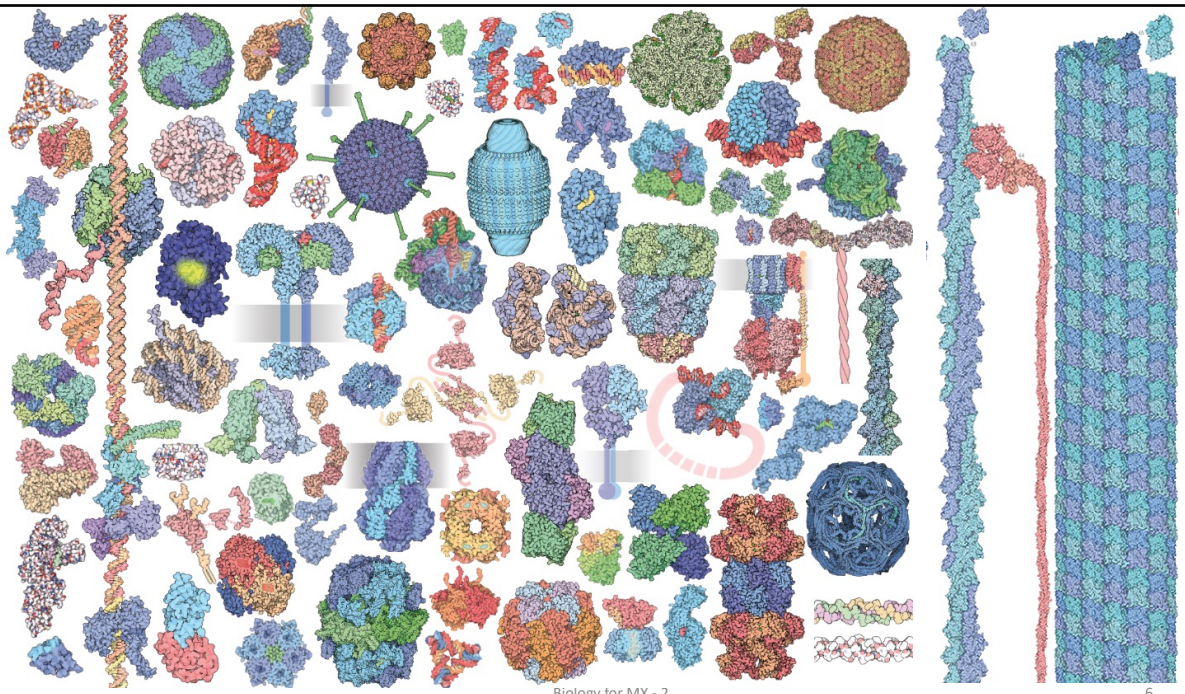

https://pdb101.rcsb.org/motm/motm-about

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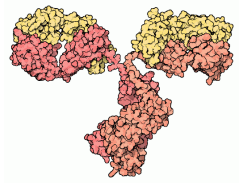
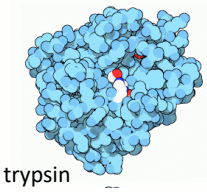
7 Types of Proteins

All figures from David Goodsell

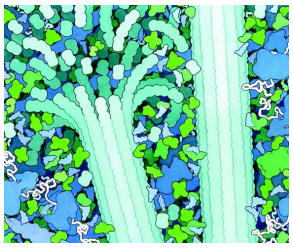
Antibodies:
Protection



Enzymes:
Chemical reactions

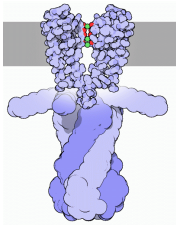


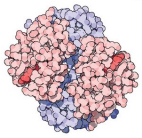
Structural proteins:
Support and Strength

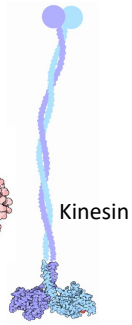


Hormonal proteins:
Regulation, development

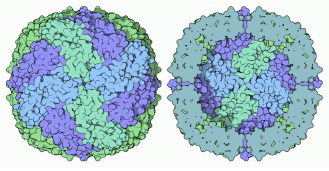
Transport proteins
Communication



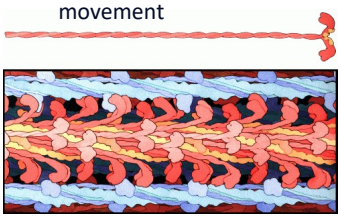




Storage proteins
Reserves



Contractile proteins
movement



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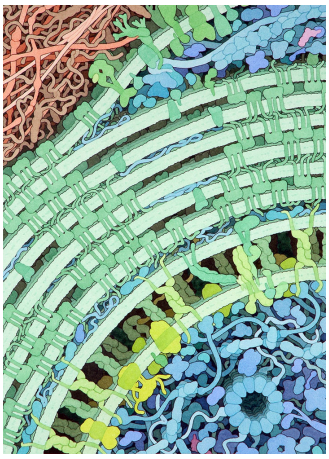
David Goodsell

Professor of Computational Biology at the Scripps Research Institute,
Research Professor at Rutgers University, Scientific Outreach Lead at the RCSB Protein Data Bank.



https://ccsb.scripps.edu/goodsell/

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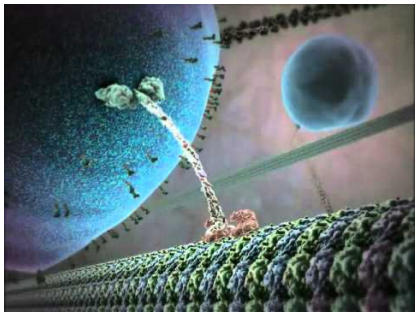
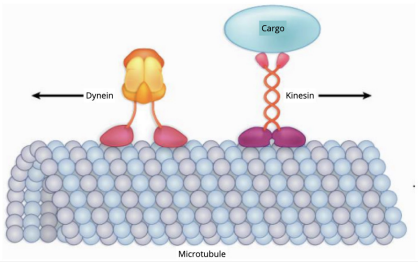
Motor Proteins: Kinesin and Dynein

Nature's robots!

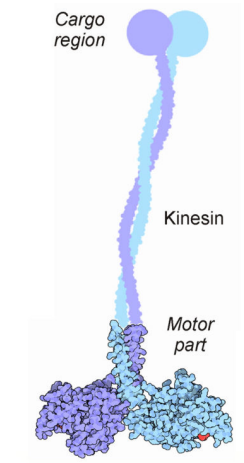


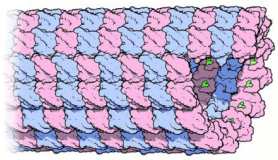


Motor proteins use the **energy of ATP hydrolysis** to move along microtubules or actin filaments. They mediate the sliding of filaments relative to one another and the transport of membrane-enclosed matter along filament tracks.

1.motor proteins on **actin filaments** are members of the **myosin superfamily**.

2.The motor proteins on **microtubules** are members of the **kinesin superfamily or the dynein family**.





Microtubule

iBiology course on Motor Proteins
https://youtu.be/9RUHJhskW00?si=cSsXC4tNnkldJqyp

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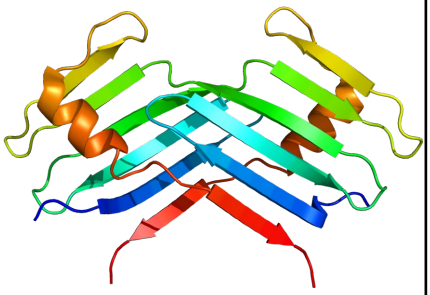
What are Proteins?

Proteins are large biomolecules consisting of one or more **long chains of amino acid residues**.

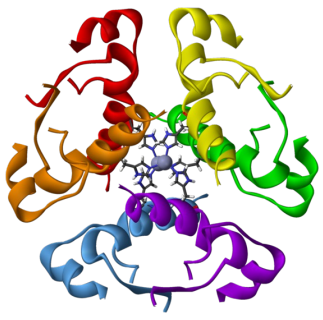
Proteins **differ** from one another primarily in their **sequence of amino acids**, which is dictated by the nucleotide sequence of their genes, and which usually results in protein **folding** into a specific **3D structure that determines its activity.**

7 types of proteins:
antibodies, contractile proteins, enzymes, hormonal proteins, structural proteins, storage proteins, and transport proteins

Serine/Threonine Protein Kinase Plk4



Insulin hexamer



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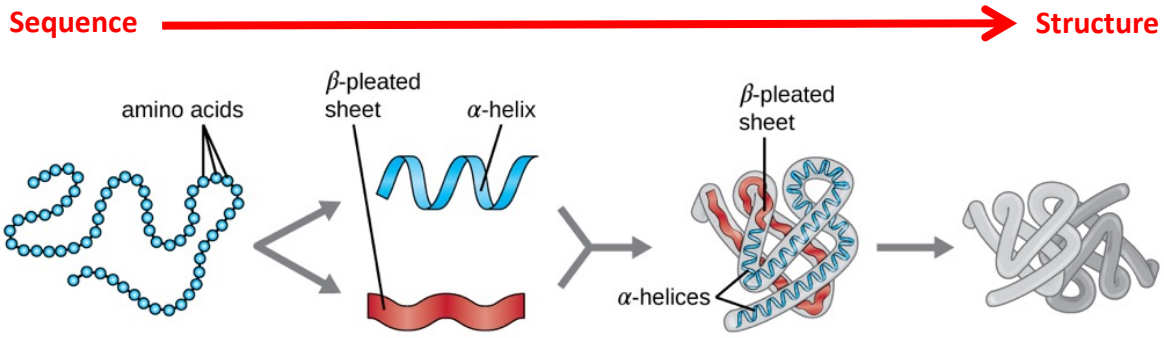
Fundamentals of Proteins



Primary Protein Structure
Sequence of a chain of amino acids

Secondary Protein Structure
Local folding of the polypeptide chain into helices or sheets

Tertiary Protein Structure
three-dimensional folding pattern of a protein due to side chain interactions

Quaternary Protein Structure
protein consisting of more than one amino acid chain

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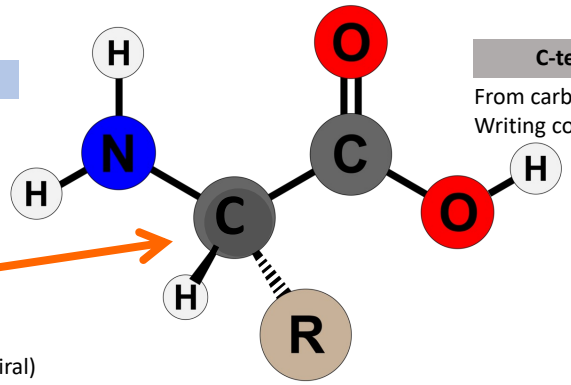
EPFL

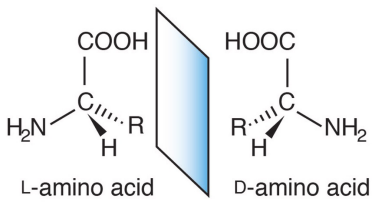
Amino Acids

N-terminus
From amine.



C-terminus
From carboxyl.
Writing convention is always N → C

Chiral central carbon!
Natural AAs are all in **L-form**
(Except Glycine, which is not chiral)



Side-chain
Can be polar (uncharged), apolar (hydrophobic), Basic or Acidic

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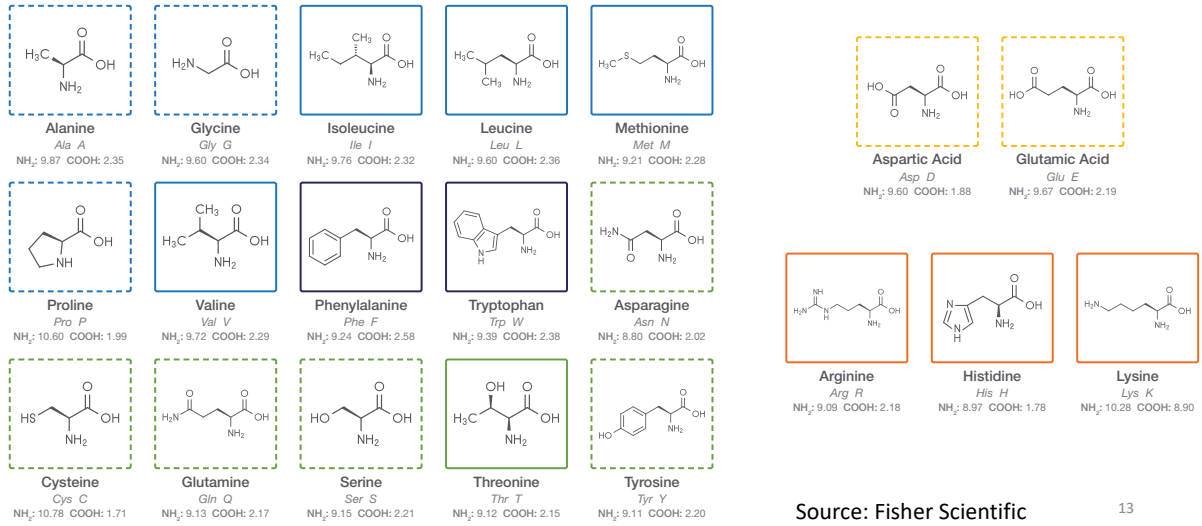
20 Amino Acids

Chart Key

Alkyl · Aromatic · Neutral · Acidic · Basic · Essential · Non-Essential

Note: The NH_2 and COOH values listed below are pK_a values.



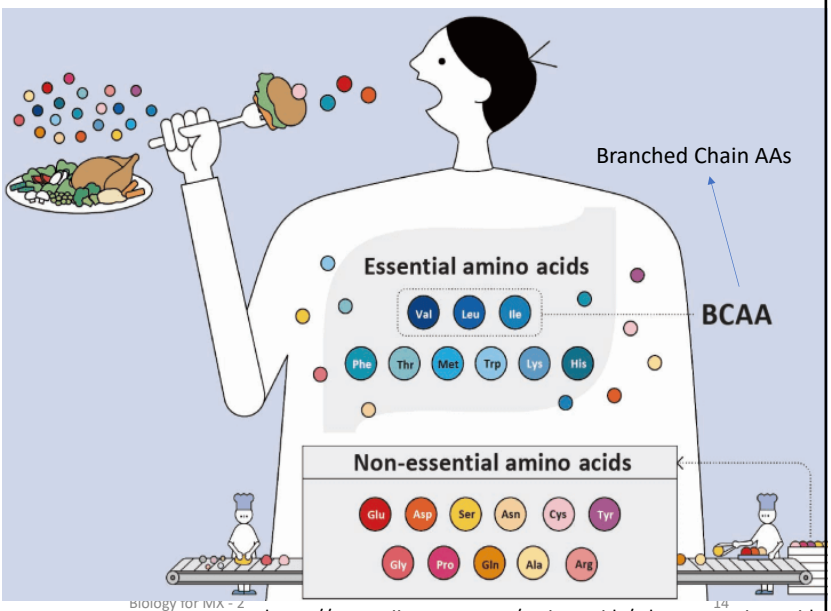
Source: Fisher Scientific

20 Amino Acids

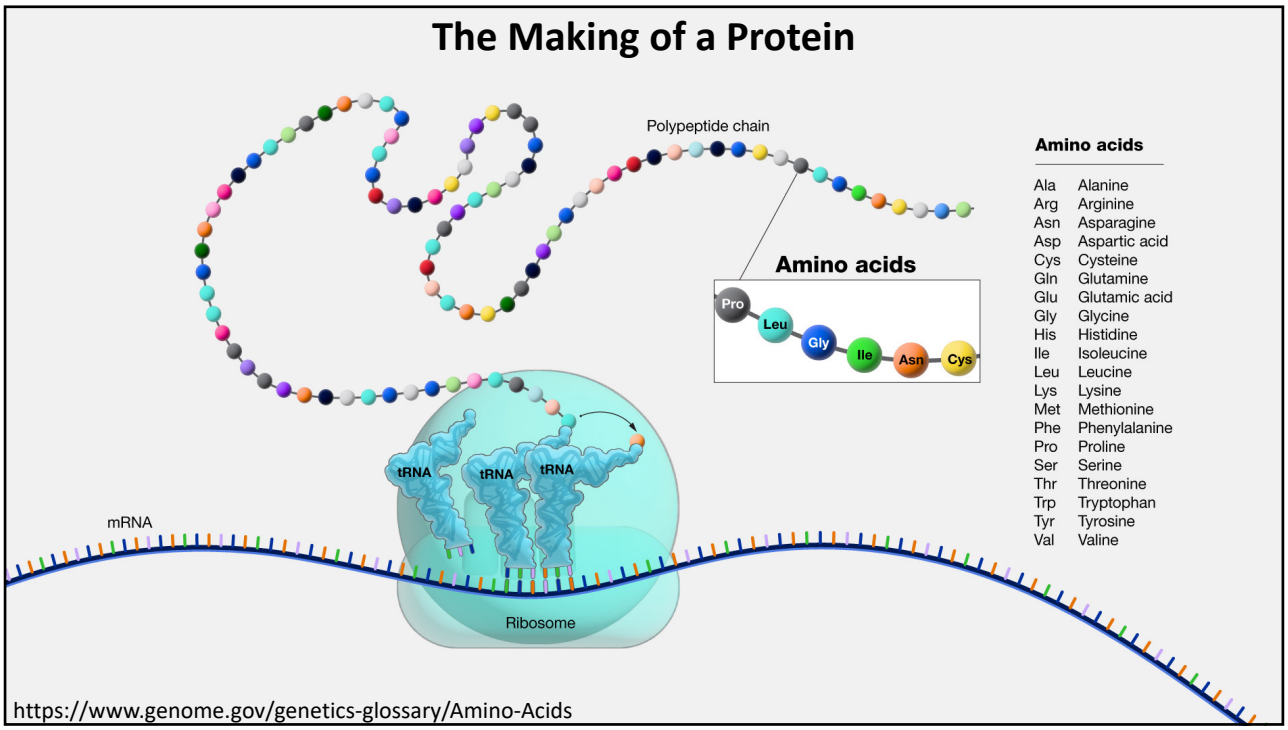
Non Essential: our body can make them
Alanine; Asparagine; Aspartic acid; Glutamic acid; Serine

Conditionally-Essential : healthy bodies can make them under normal physiologic conditions. They become essential under certain conditions like starvation or inborn errors of metabolism.
Arginine; Cysteine ; Glutamine; Glycine; Proline; Tyrosine

Essential : cannot be made by us. Dietary protein provides these amino acids, which are needed to make certain hormones and other important molecules.
Histidine; Isoleucine; Leucine; Lysine; Methionine; Phenylalanine; Threonine; Tryptophan; Valine



https://www.ajinomoto.com/amino-acids/what-are-amino-acids



https://www.genome.gov/genetics-glossary/Amino-Acids

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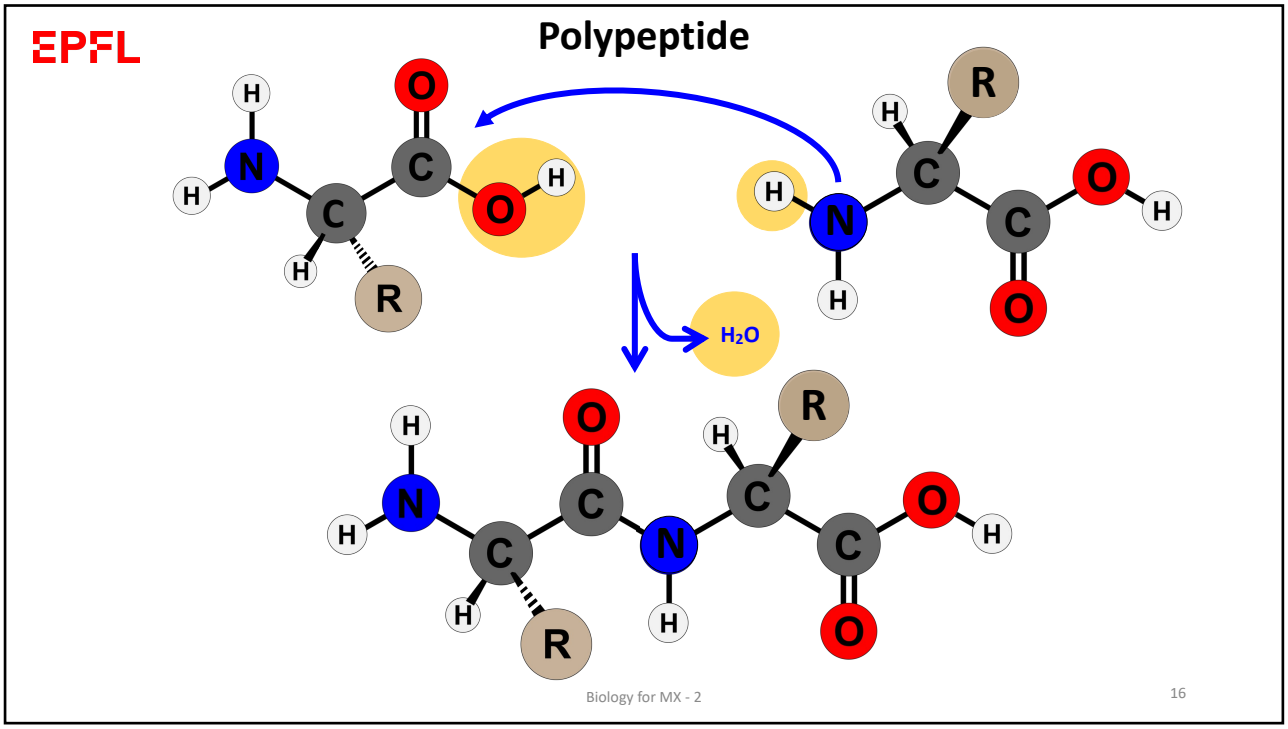

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Polypeptide

Blackboard example

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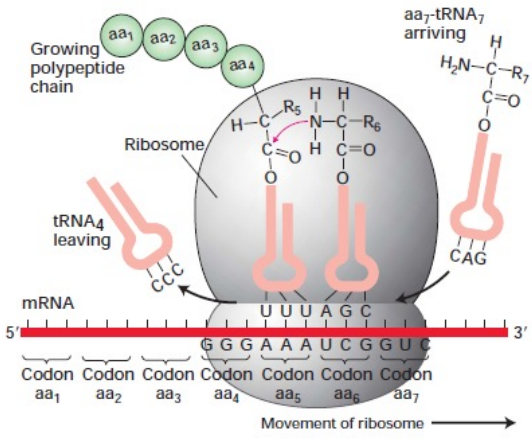
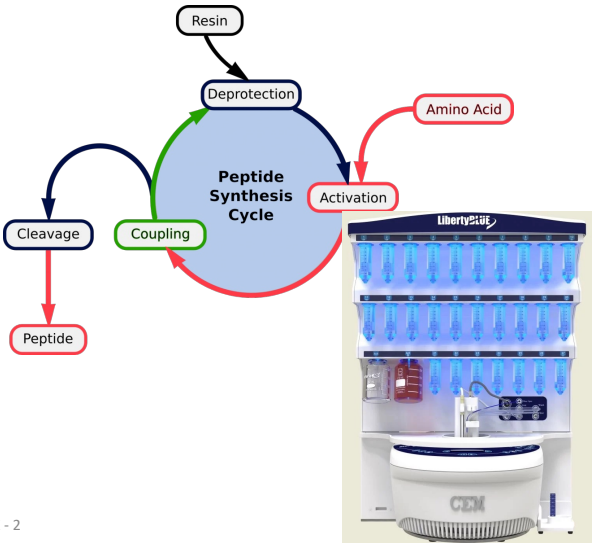
Peptide Production

In the cell by the ribosome: N → C



In the chemistry lab : C → N



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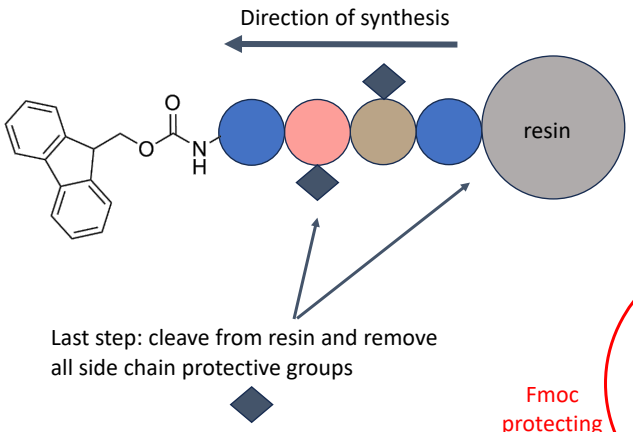
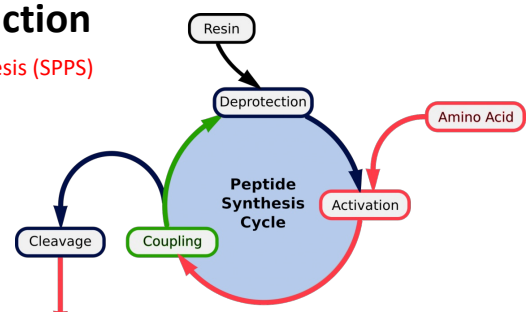
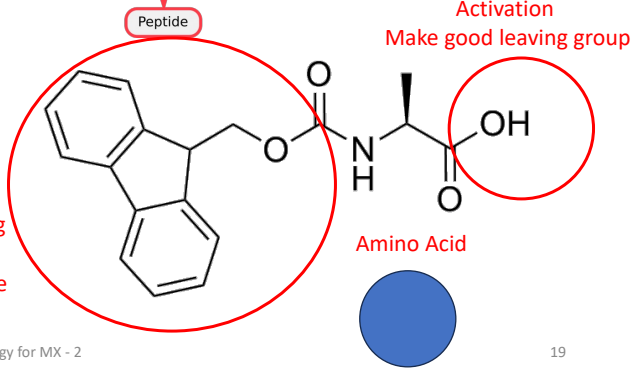
EPFL

Peptide Production

Solid Phase Peptide Synthesis (SPPS)







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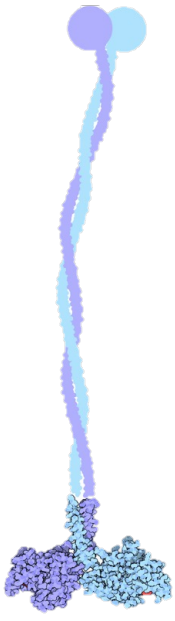
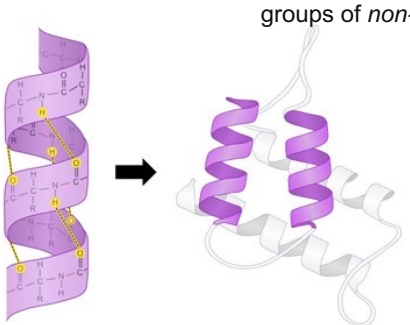
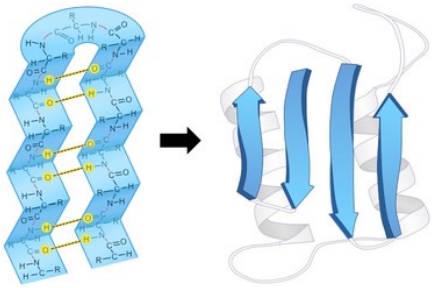
EPFL

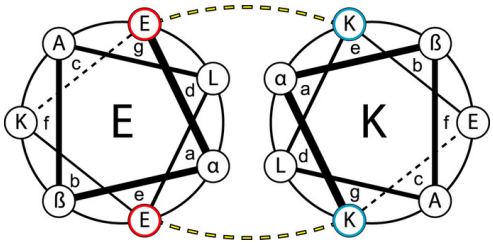
Helix and Sheet

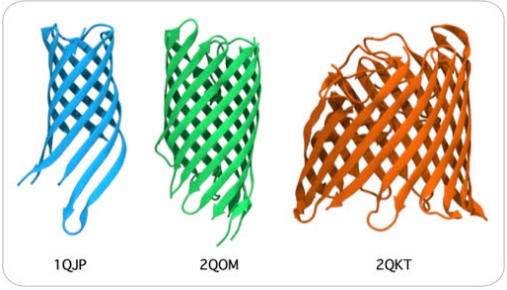
hydrogen bonding between the amine and carboxyl groups of *non-adjacent* amino acids











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Break

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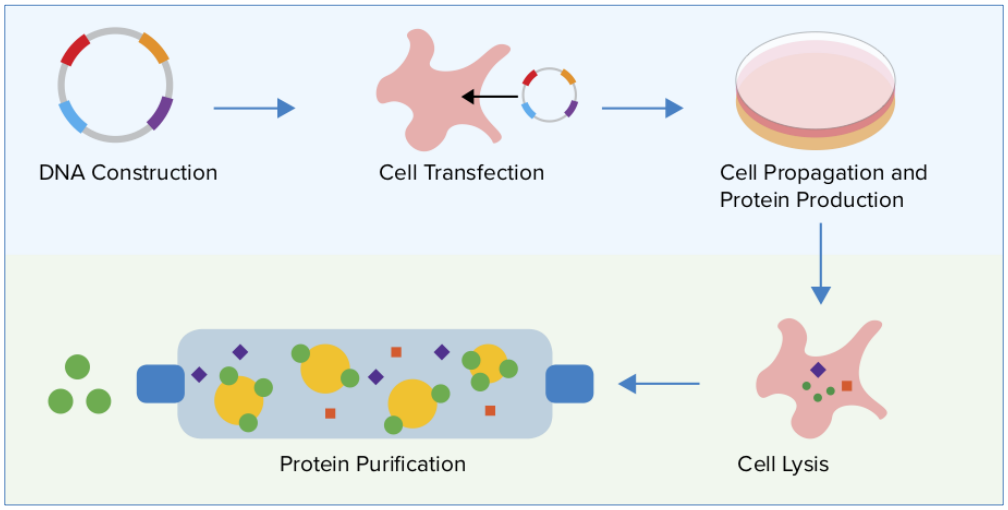
How to make a protein in the lab?

Recombinant proteins are **proteins** encoded by **recombinant** DNA that has been cloned in an expression vector that supports expression of the gene.



https://blog.addgene.org/plasmids-101-protein-expression

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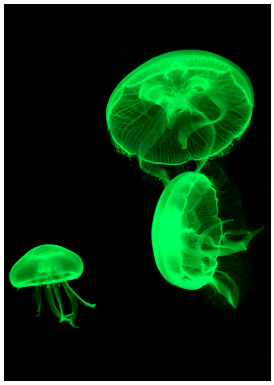
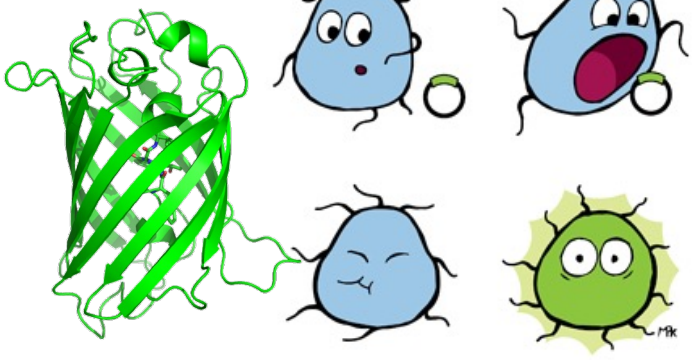
How to make a protein in the lab?

GFP = **Green Fluorescent Protein**
Nobel Prize Chemistry 2008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Protein Engineering – Mutations

Protein engineering is the conception and production of unnatural polypeptides, often through modification of amino acid sequences that are found in nature.

Change the color by mutations of specific amino acids
" point mutations "

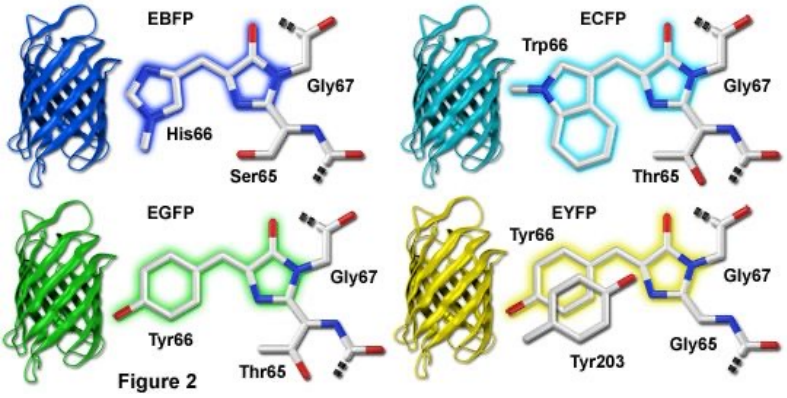

Figure 2

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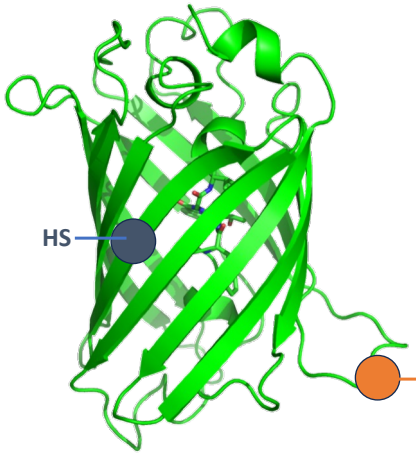
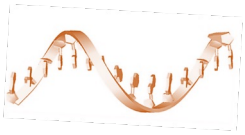
Protein Engineering – Mutations

Chemically interesting mutations:



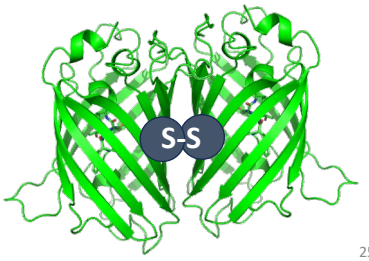


Identify an amino acid on a random section

Change to Lysine → chemical ligations to other molecules

Identify an amino acid on a outward facing surface

Change to Cysteine → Induce sulphor bridge = dimerization



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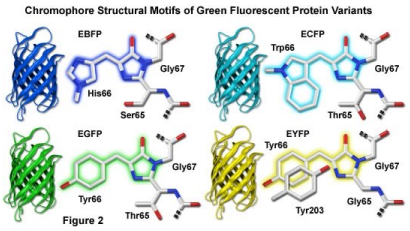
Protein Engineering – What?

Protein engineering is the conception and production of unnatural polypeptides, often through modification of amino acid sequences that are found in nature.

Synthetic **protein** structures and functions can now be **designed** entirely on a **computer** or produced through **directed evolution** in the laboratory.



Directed Evolution

In **directed evolution**, random mutagenesis (by error-prone PCR or sequence saturation mutagenesis) is applied to a protein, and a **selection regime** is used to select variants having **desired traits**.

Further rounds of mutation and selection are then applied which mimics natural evolution and produces superior results (in function) to rational design.

Rational Design

In **rational protein design**, a scientist uses detailed knowledge of the structure and function of a protein to make desired changes.

The drawback is that **detailed structural knowledge** of a protein is often **unavailable**, and it can be difficult to predict the effects of mutations since structural information provides a **static picture** of the structure.

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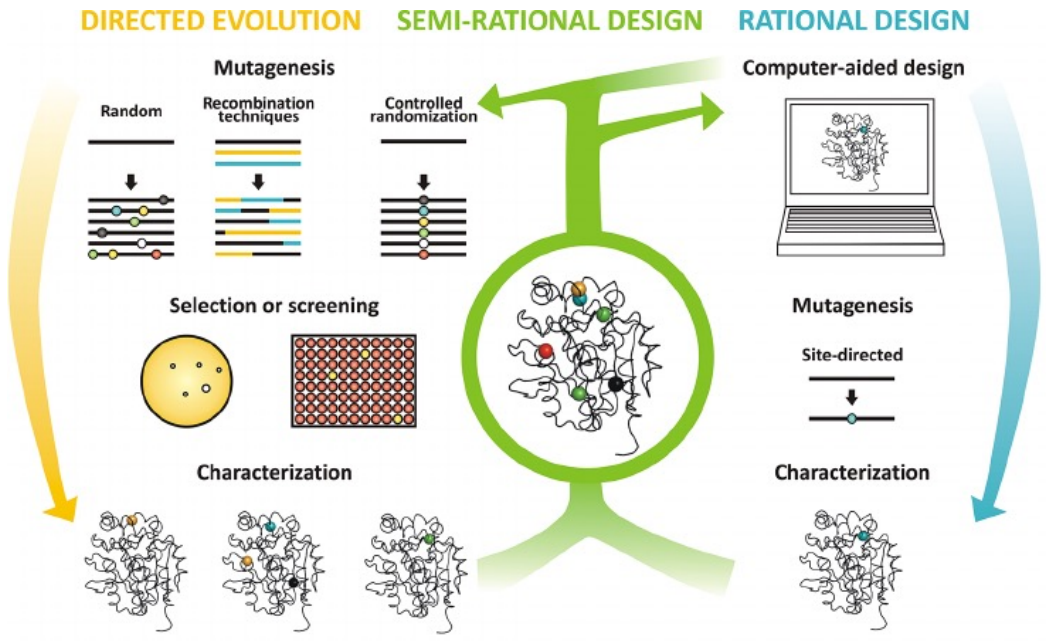

DO - 10.1021/cs400684x

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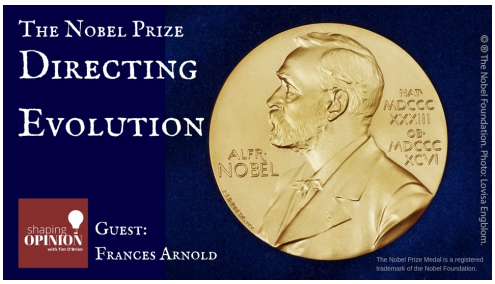
2018 Nobel Prize: Frances Arnold

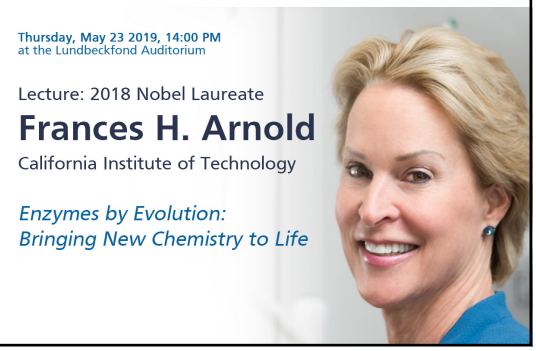
Evolution – the adaption of species to different Environments – has created an enormous diversity of life.

Frances Arnold has used the same principles – genetic change and selection – to **develop proteins that solve humankind's chemical problems.**

In 1993, Arnold conducted the **first directed evolution of enzymes**, which are proteins that catalyze chemical reactions.

The uses of her results include more environmentally friendly manufacturing of chemical substances, such as pharmaceuticals, and the production of renewable fuels.





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Helpful Tools: The Protein Databank

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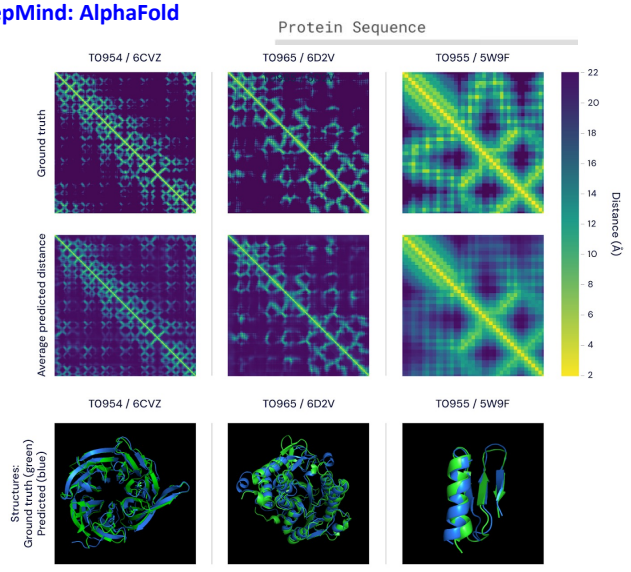
Prediction of Folding: AI in Biology

Google DeepMind: AlphaFold

AlphaFold works in two steps

1) multiple sequence alignments: comparison of protein's sequence with similar ones in a **database** to reveal **pairs** of amino acids that don't lie next to each other in a chain, but that tend to appear in tandem. DeepMind trained a **neural network** to take such pairings and predict the distance between two paired amino acids in the folded protein.

2) Create a physically possible — but nearly random — **folding arrangement** for a sequence. Instead of another neural network, it used an optimization method called **gradient descent** to iteratively refine the structure so it came close to the (not-quite-possible) predictions from the first step.



Nature **588**, 203-204 (2020)
doi: https://doi.org/10.1038/d41586-020-03348-4

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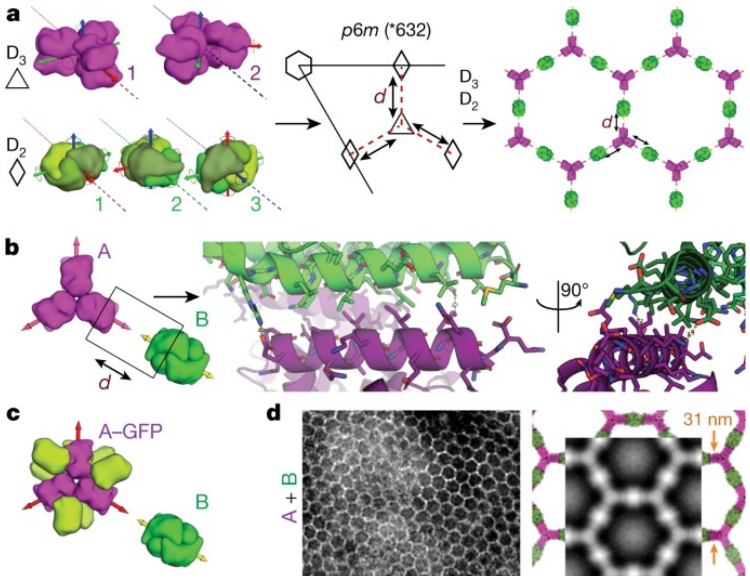
2D Crystalline Protein Surfaces



Crystalline binary layers by designing rigid interfaces between pairs of dihedral protein building blocks, in a *p6m* lattice.

Because the material is designed from the ground up, the components can be readily **functionalized** and their **symmetry** reconfigured, enabling formation of ligand arrays with distinguishable surfaces.

With the rapid developments in de novo design of protein building-blocks and quantitative microscopy techniques, this provides the basis for a future of programmable biomaterials for synthetic and living systems.

Nature 589, 468–473(2021)

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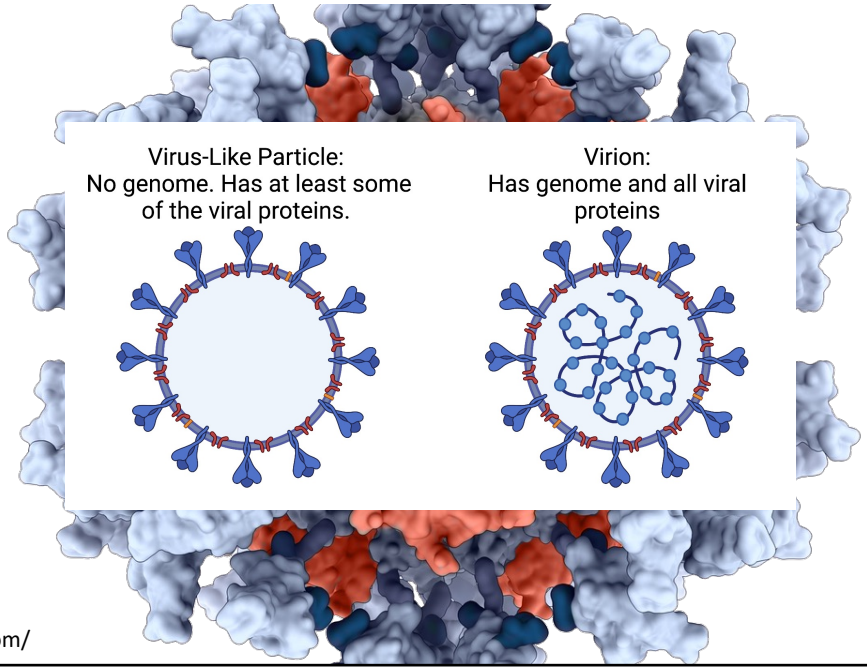
Virus Like Particles



https://icosavax.com/

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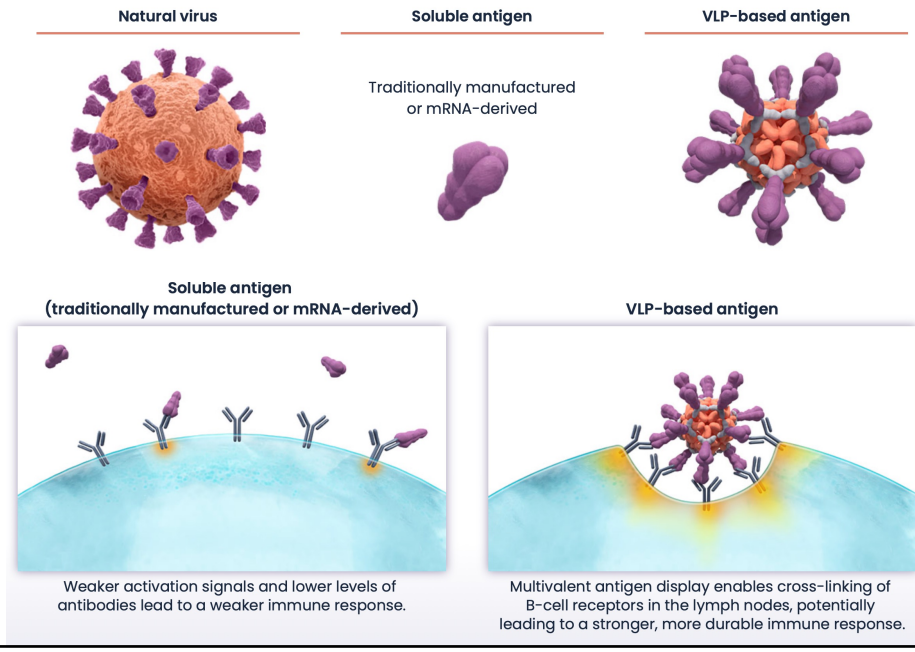
Mimic STRUCTURE of Real Virus



https://icosavax.com/

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In the News

PHARMA, BIOPHARMA

Icosavax lands $100M to take its virus-like particle vaccines into clinical trials

RA Capital led the $100 million Series B round of funding for Icosavax, a startup developing vaccines based on virus-like particles. The biotech is preparing for clinical tests of vaccines for respiratory syncytial virus, human metapneumovirus, and SARS-CoV-2.

By FRANK VINLUAN

Post a comment / Apr 7, 2021 at 8:20 AM

AstraZeneca — AstraZeneca Websites

What science can do · R&D · Our therapy areas · Our company · Careers · Investors · Media · Sustainability · Partnering

Acquisition of Icosavax Completed

PUBLISHED
19 February 2024

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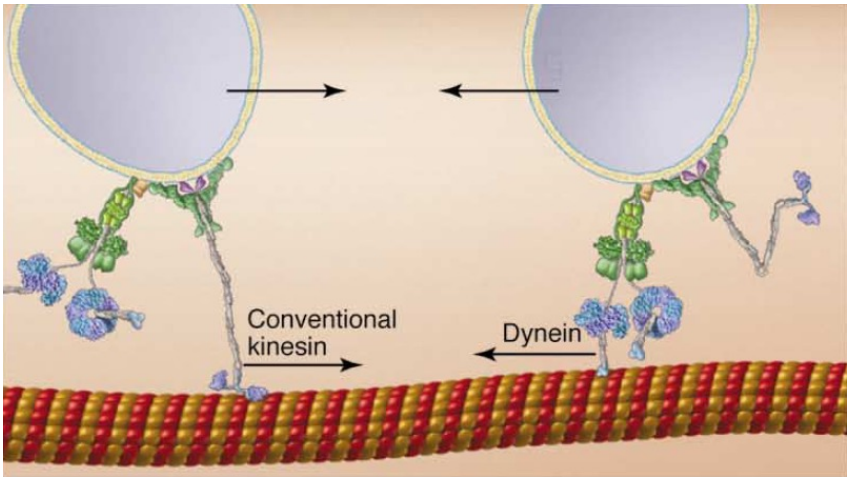
Engineering Fun Stuff



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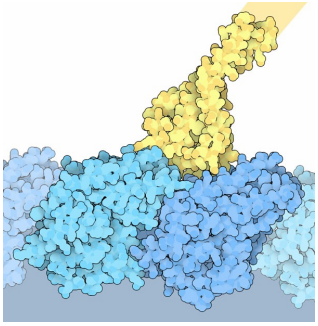
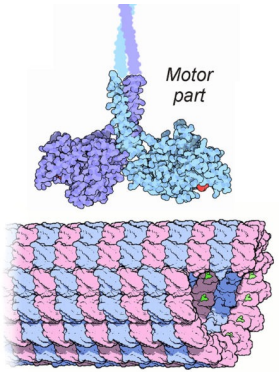
Protein tug of war

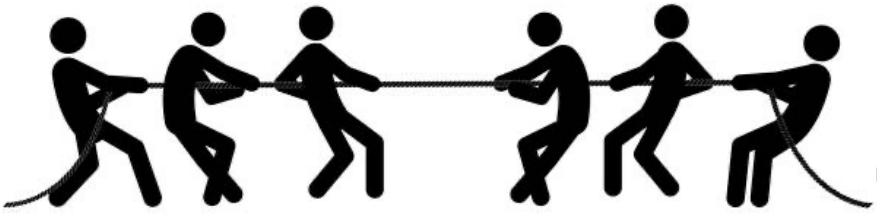


Team Dynein

What to do in the lab?
Make proteins, purify
Add DNA at specific location (point mutations)
Connect to scaffold (characterization)
Add to tubules on microscope



Team Kinesin



Microtubule

Science 2012
10.1126/science.1226734

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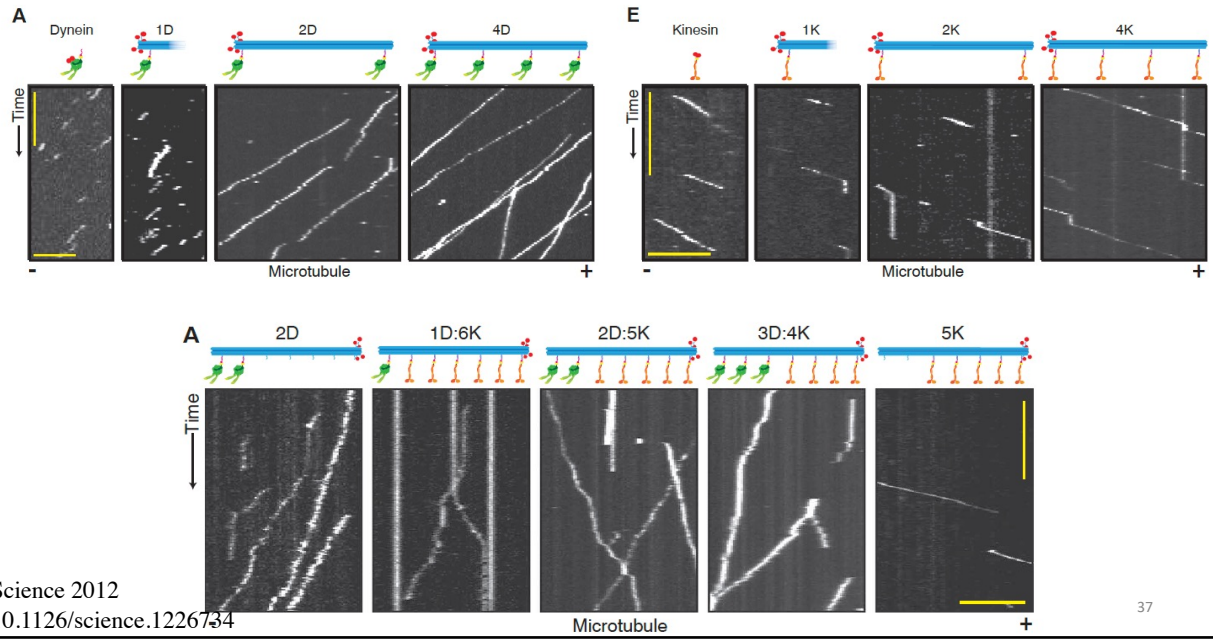
Protein tug of war



Science 2012
10.1126/science.1226734

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Conclusion

Proteins are extremely important macromolecules that enable life and show remarkable mechanical properties thanks to their hierarchical organization.

They can be easily engineered and produced with relative low cost in the lab.

This enables a central use of proteins as material tools to create modular architectures with on-demand function.

With DNA as a template for synthesis, all copies of a protein are identical, providing uniformity in materials building blocks.

Through recent advances in artificial intelligence, de novo protein design will likely experience a huge jump in performance the next year.

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