Synthetic biology meets biomaterials

BioEng458 - lecture 4

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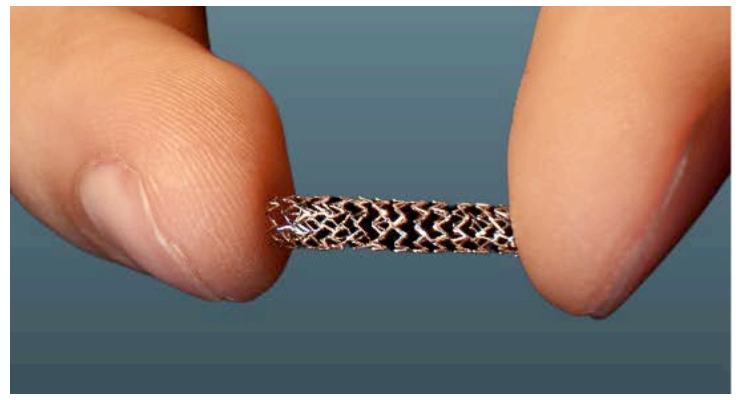
Lecture Outline

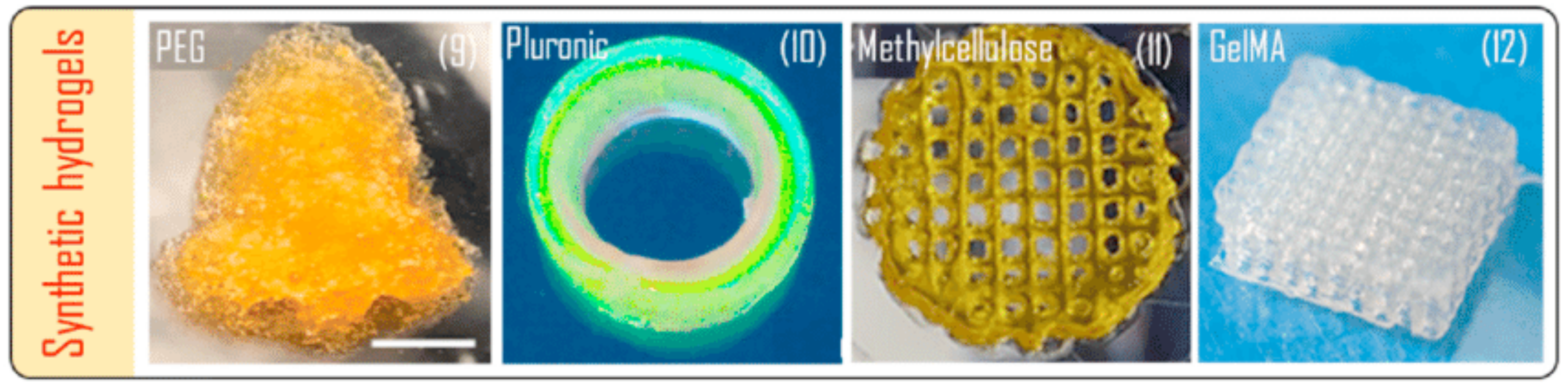
- Biological materials - synthesis
- From genes to materials
- Genetically engineering biological material synthesis
- De novo design of biological biomaterials
- Dinosaur gummy bears

Biomaterials categories

- Synthetic (metals, polymers)

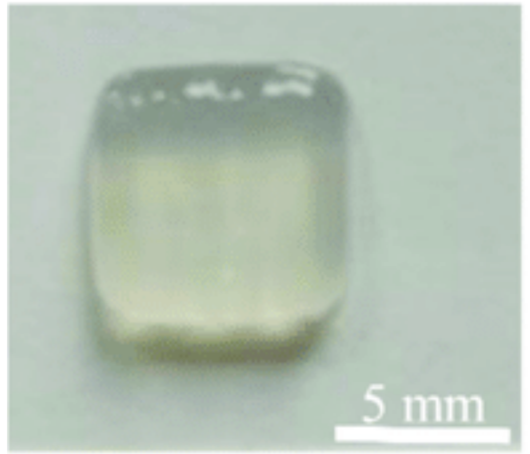
Stent

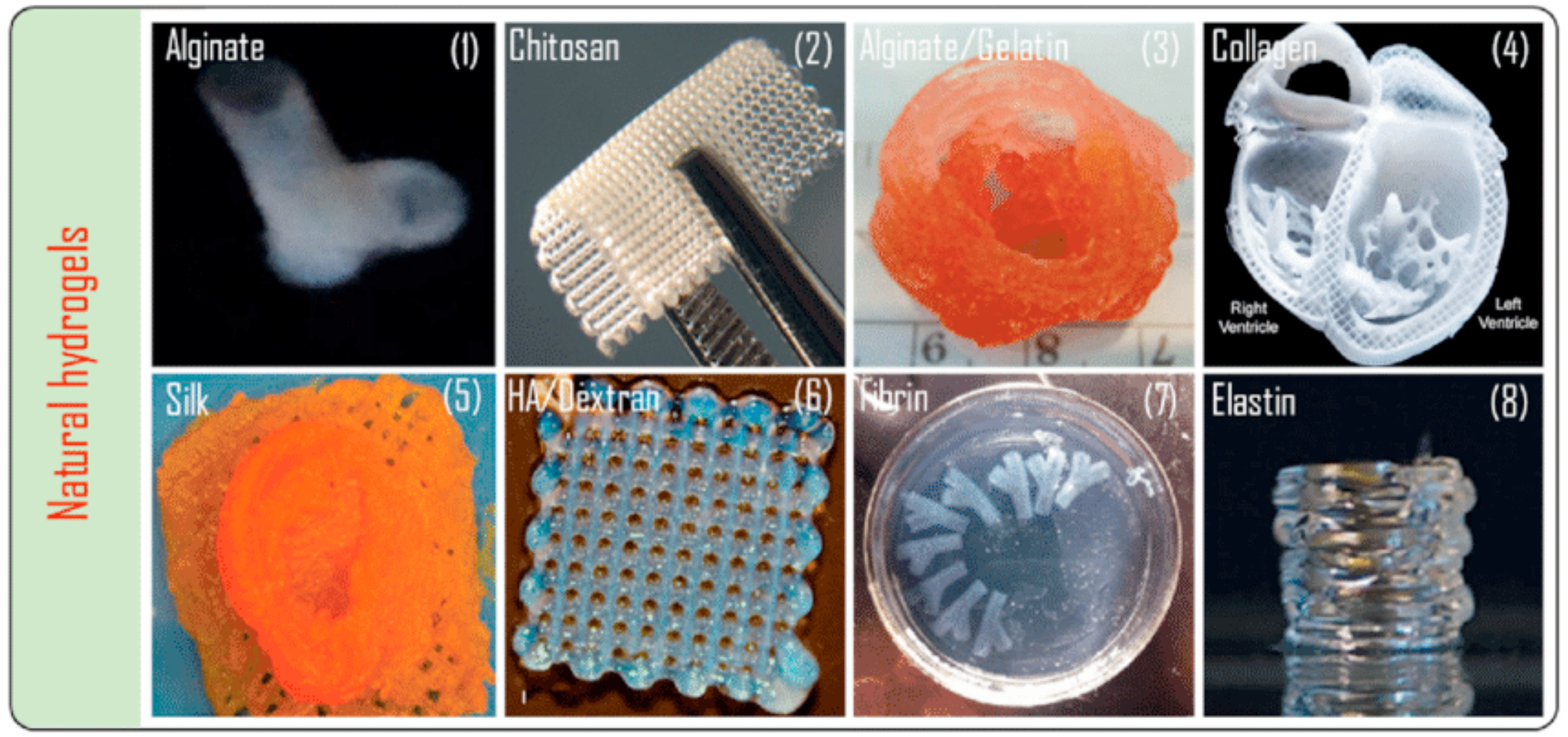




- Natural **-> biological materials**

Alginate scaffold





- Composites

Biological materials

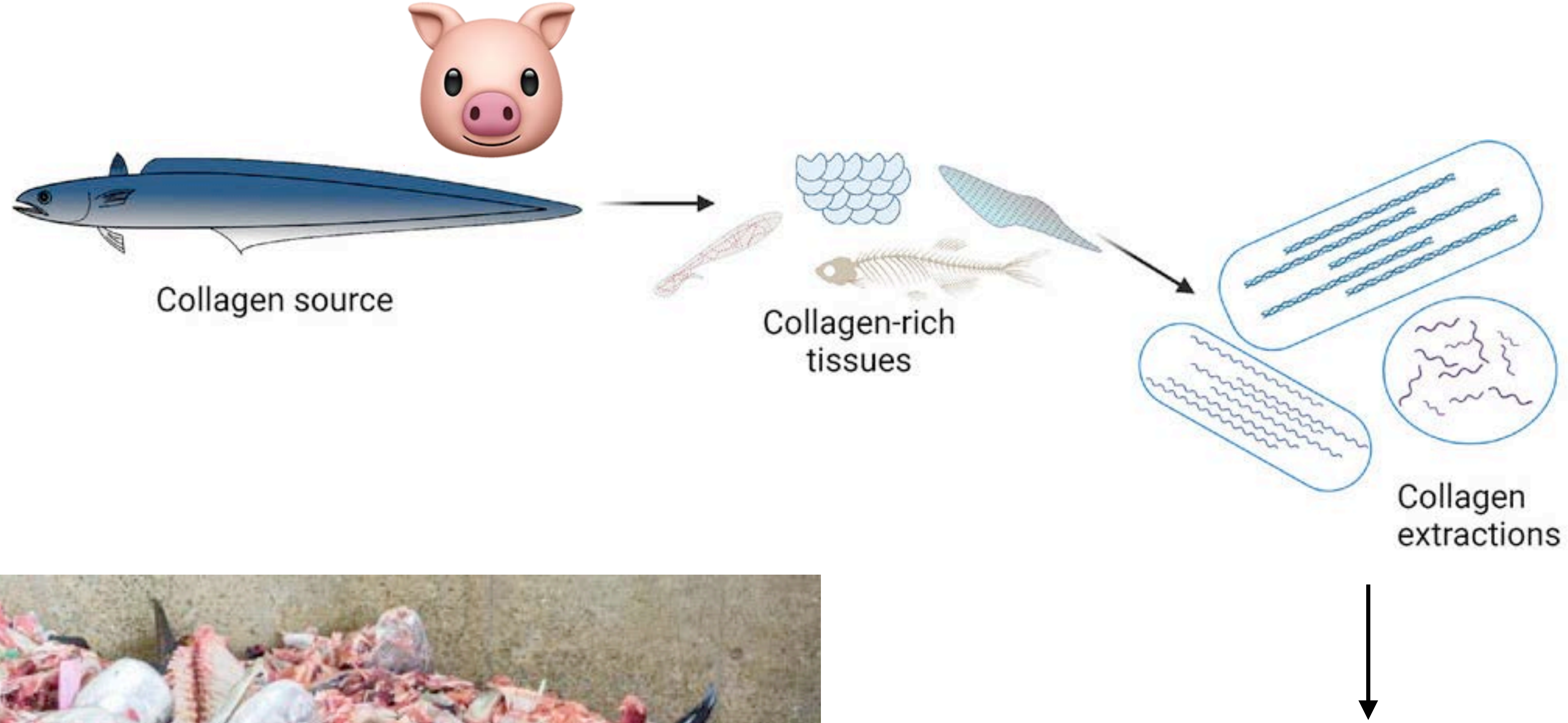
Great candidates for biomaterials

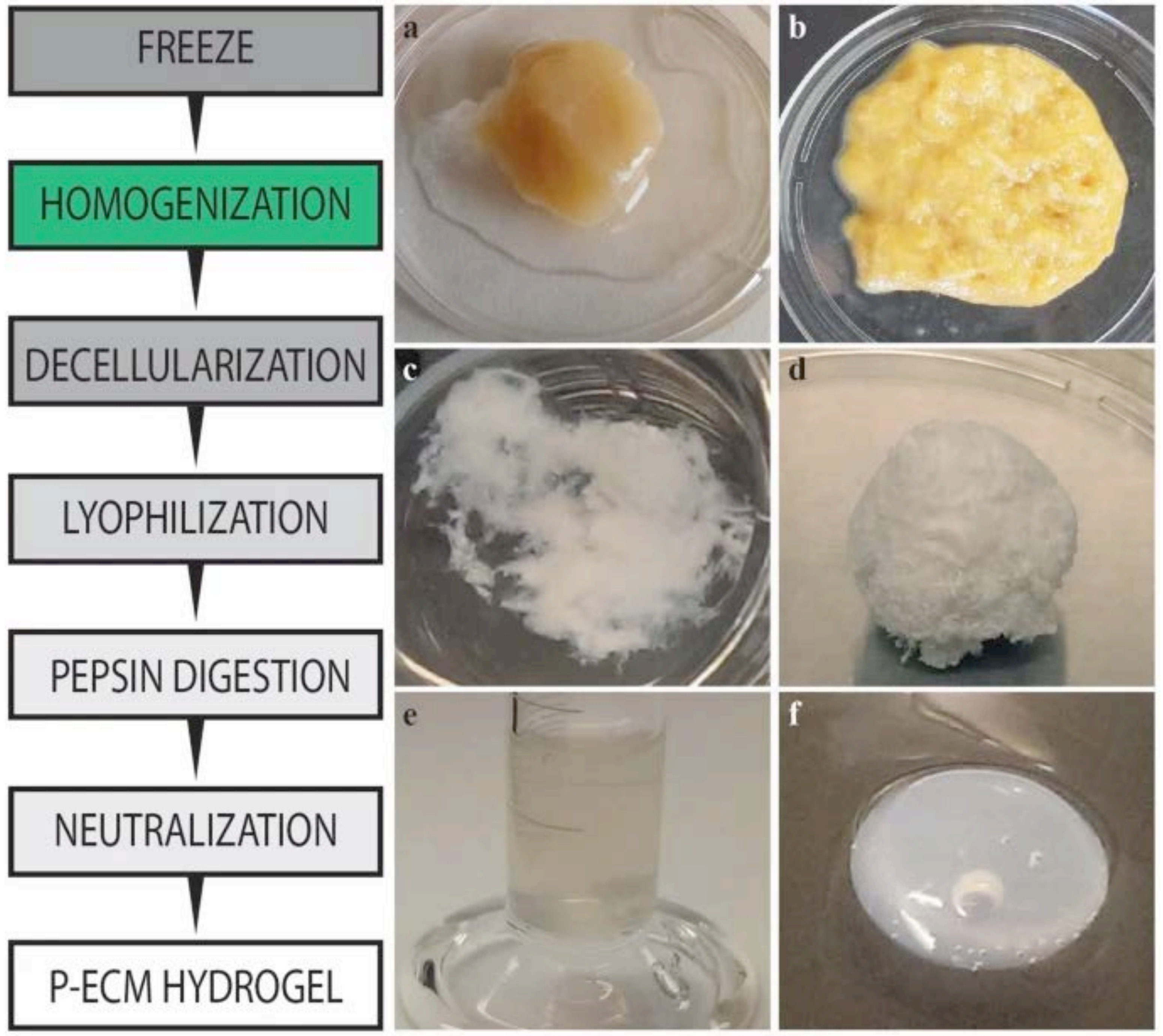
- Biocompatible: well-tolerated by the body
- Bioactive: can already interact functionally with tissue (e.g. collagen)
- Biodegradable: usually replaced by native tissue

Biological materials traditionally used as biomaterials

Biomaterial	Source	Key Properties	Example applications
Gelatin	Hydrolyzed collagen (animal skin/bone)	Biodegradable, biocompatible, hydrophilic	Drug delivery, wound dressings, 3D bioprinting
Hyaluronic Acid	ECM of connective tissues (mammals)	Hydrophilic, viscoelastic, bioactive	Tissue engineering, dermal fillers
Fibrin	Blood plasma	Natural clot-forming, biodegradable	Wound healing, hemostatic agents
Keratin	Wool, feathers, human hair	Strong, biocompatible, bioactive	Wound healing, drug carriers, scaffolds
Pectin	Plant cell walls (fruits)	Gel-forming, biocompatible, biodegradable	Drug delivery, wound dressing, cell encapsulation
Mycelium-based Materials	Fungal mycelium	Biodegradable, tunable mechanical properties	Wound healing, tissue scaffolds
Decellularized ECM	Animal or human tissues	Natural tissue structure, bioactive, supports regeneration	Organ scaffolds, soft tissue engineering
Silk	Silk moth	High tensile strength, biocompatible	Sutures, artificial ligaments
Elastin	ECM of tissues (lungs, arteries, skin)	Elastic, hydrophilic, supports cell adhesion	Skin repair, vascular grafts, soft tissue engineering

Collagen production





247.– 4940.–/1l
Elemis Ultra Smart Pro-Collagen Night Genius
50 ml, Night cream

Ratings
★★★★★

Brand
More from Elemis

Uses of collagen as biomaterial

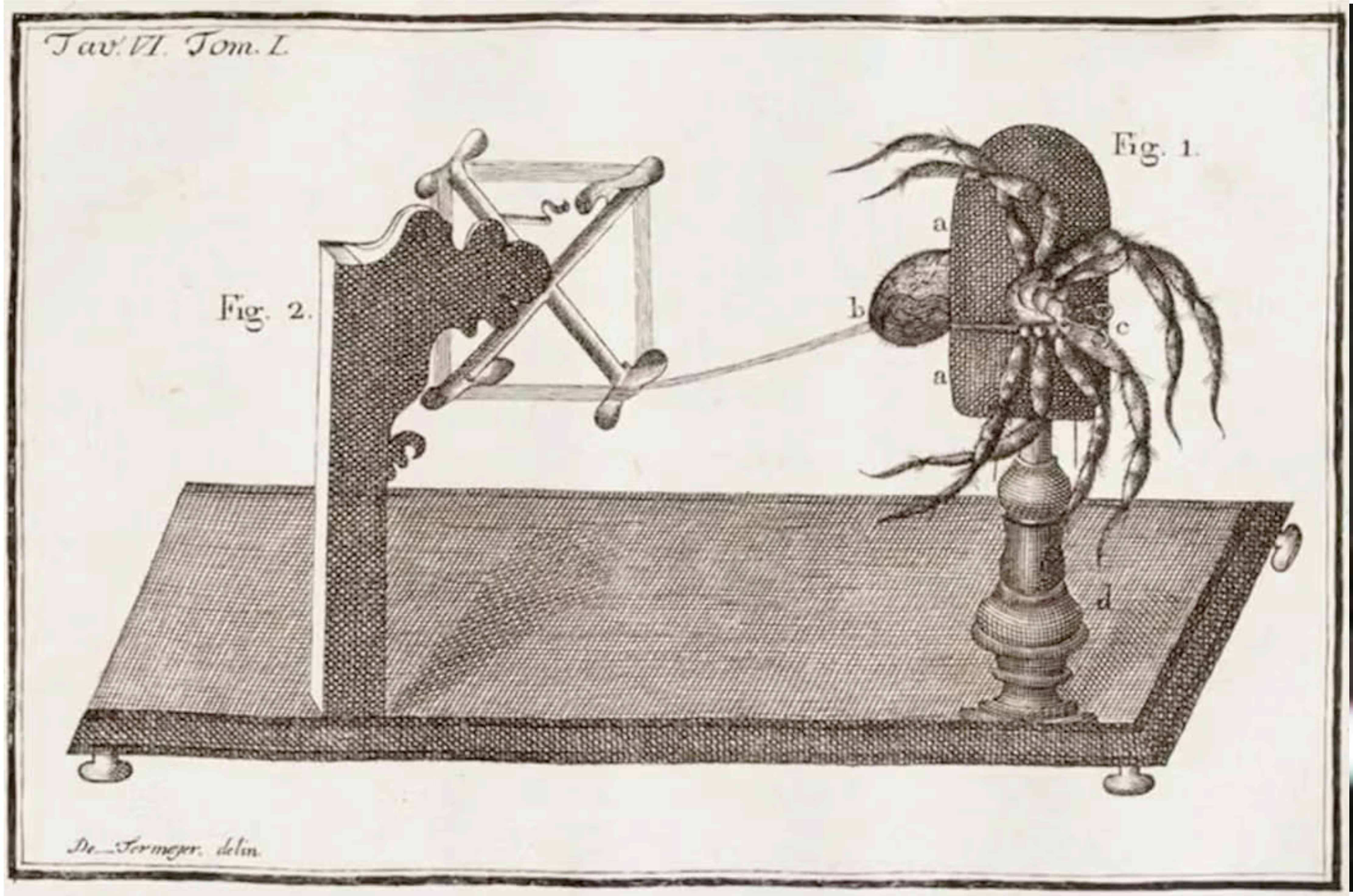
- Tissue engineering/organoids
- Drug delivery
- Wound dressing
- Bioprinting
- Regenerative medicine
- Cosmetics

Collagen sponge

Guided tissue repair membrane

Silk production

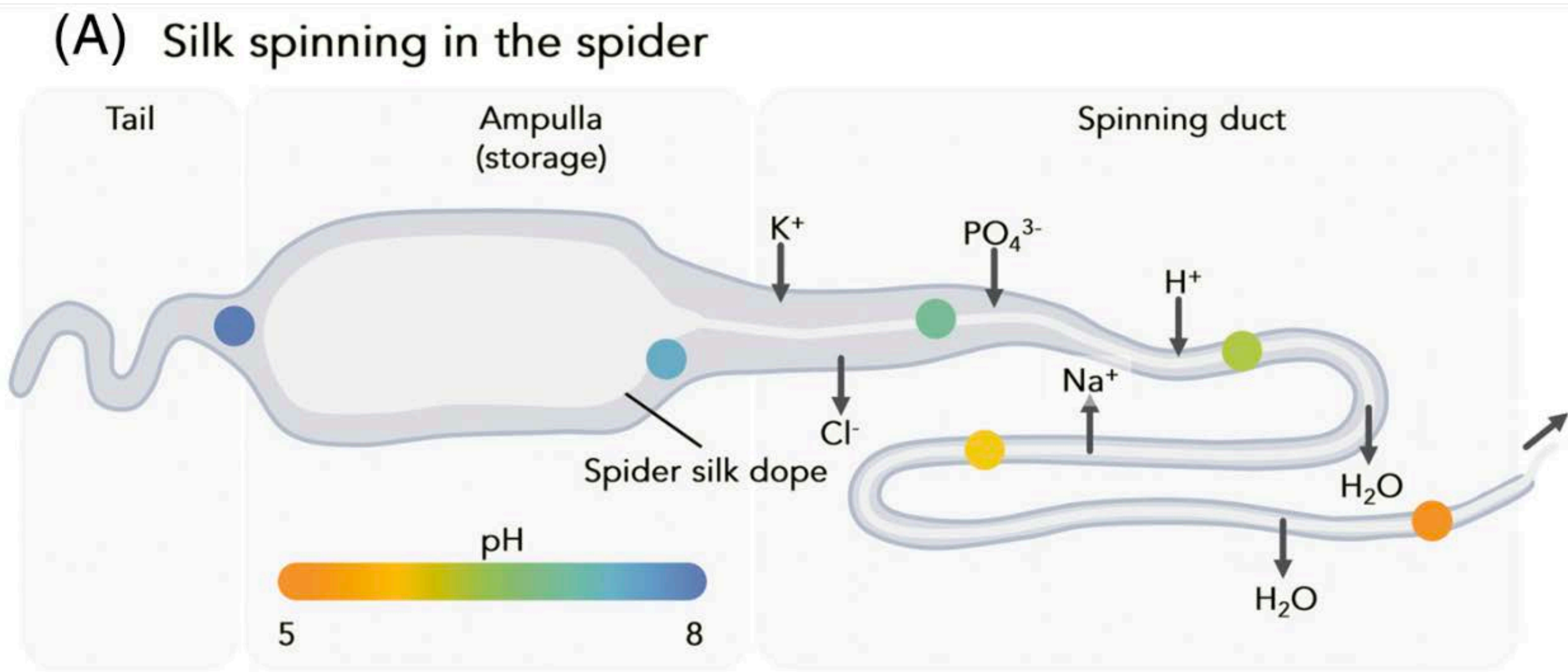
dragline spider silk - high performance in terms of mechanics

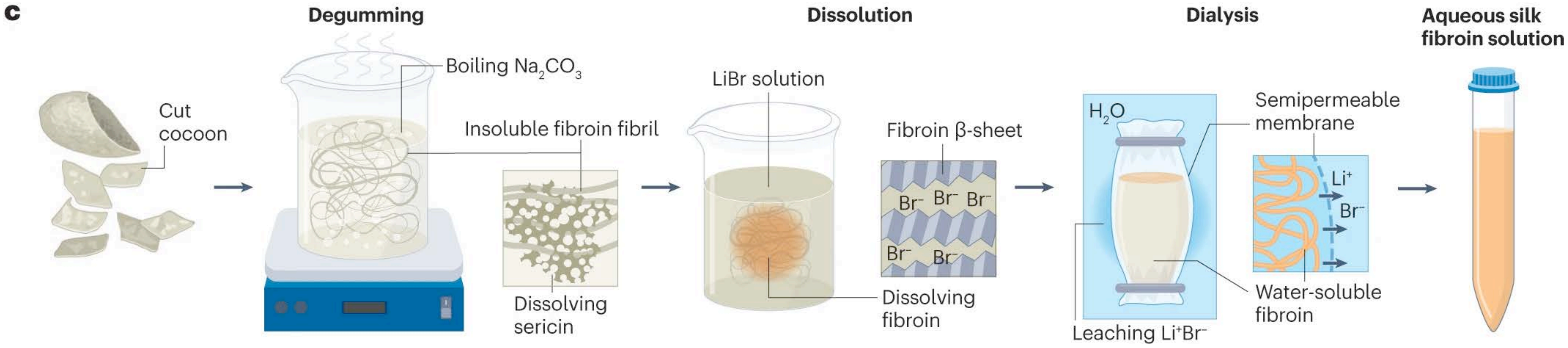


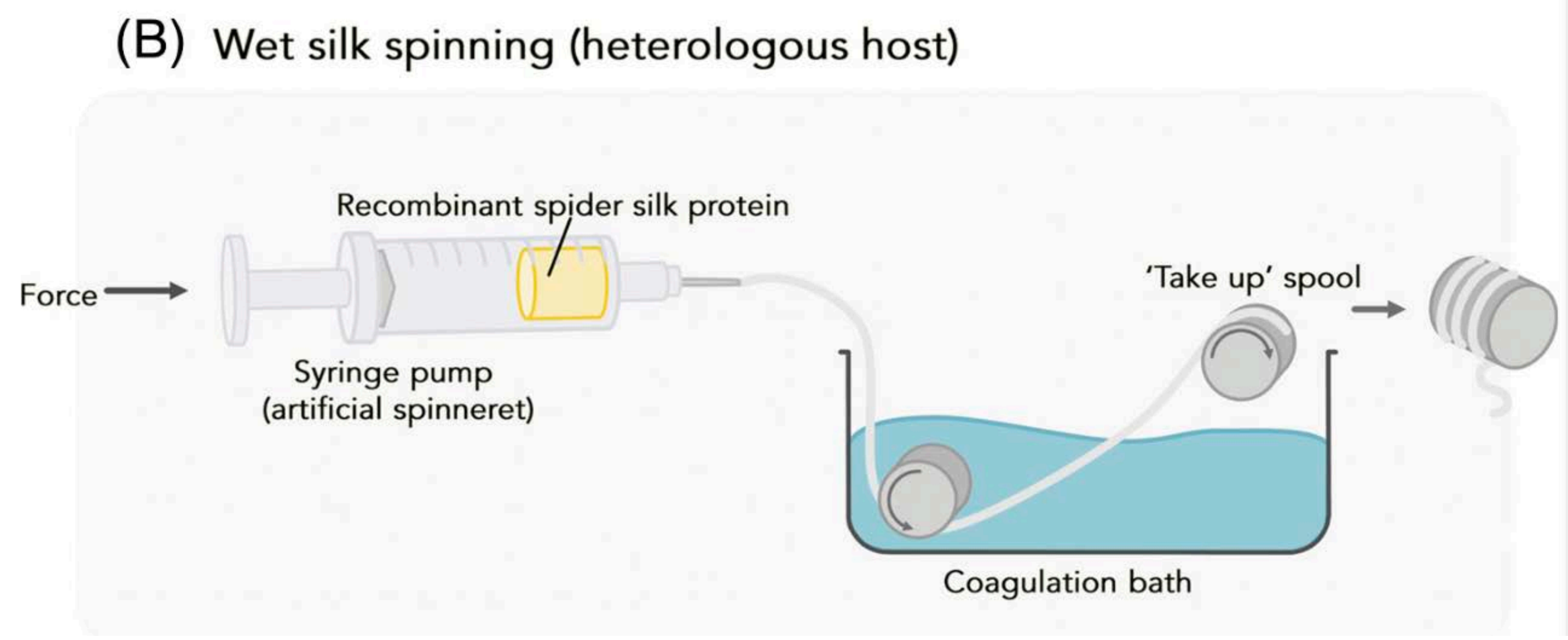
Lower throughput, clearly

Silk production

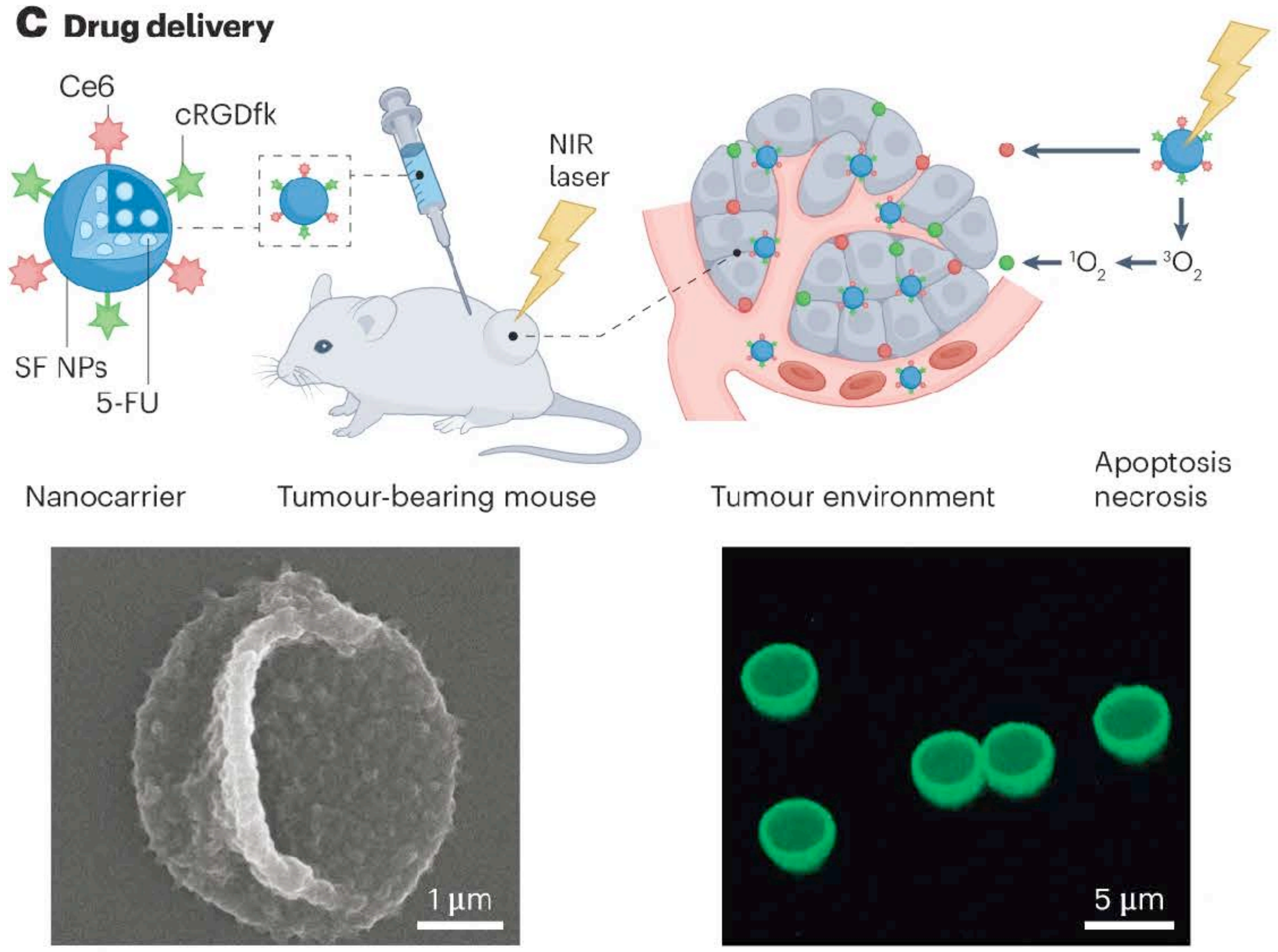
Bombyx mori - fibroin purification

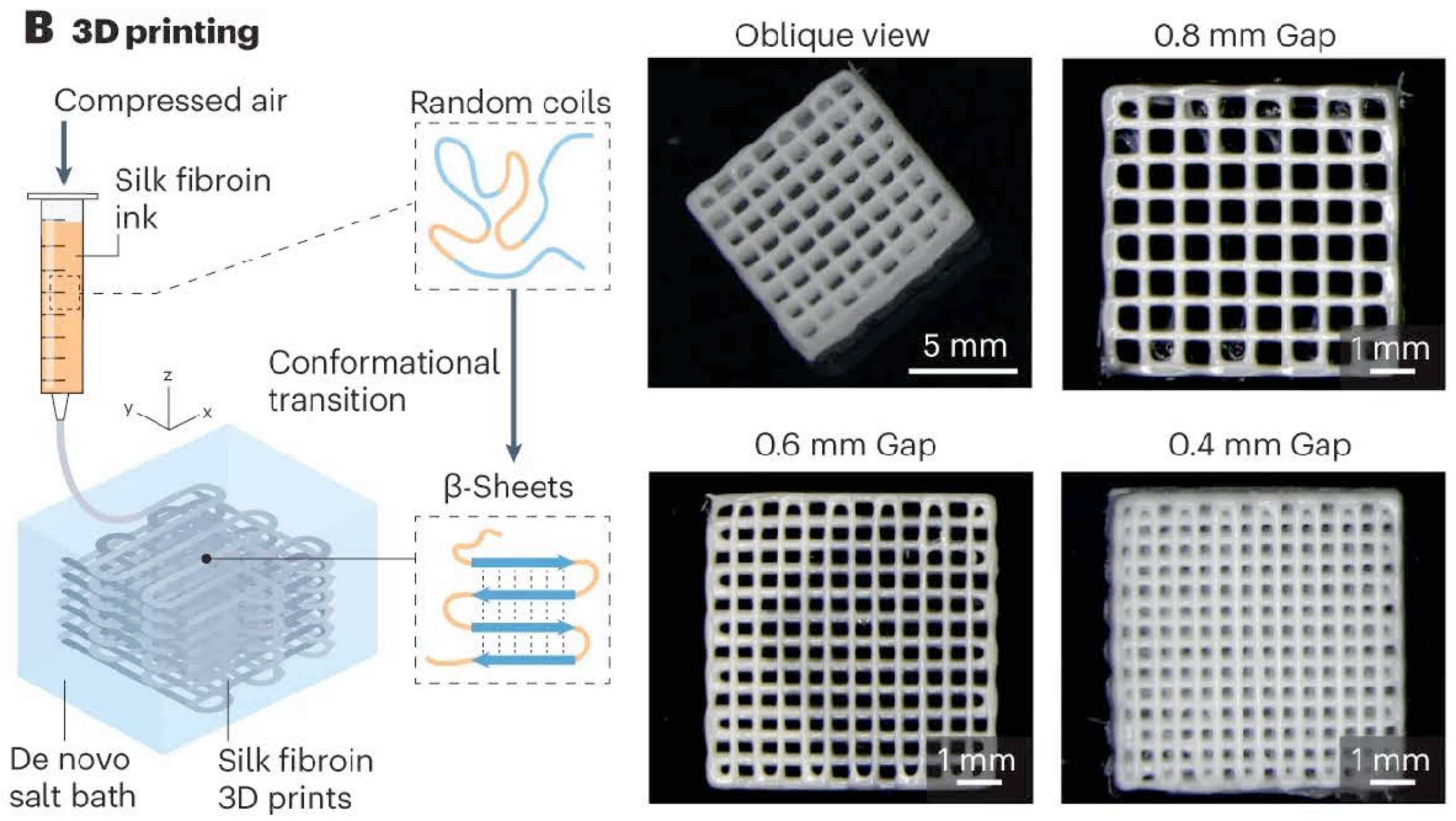


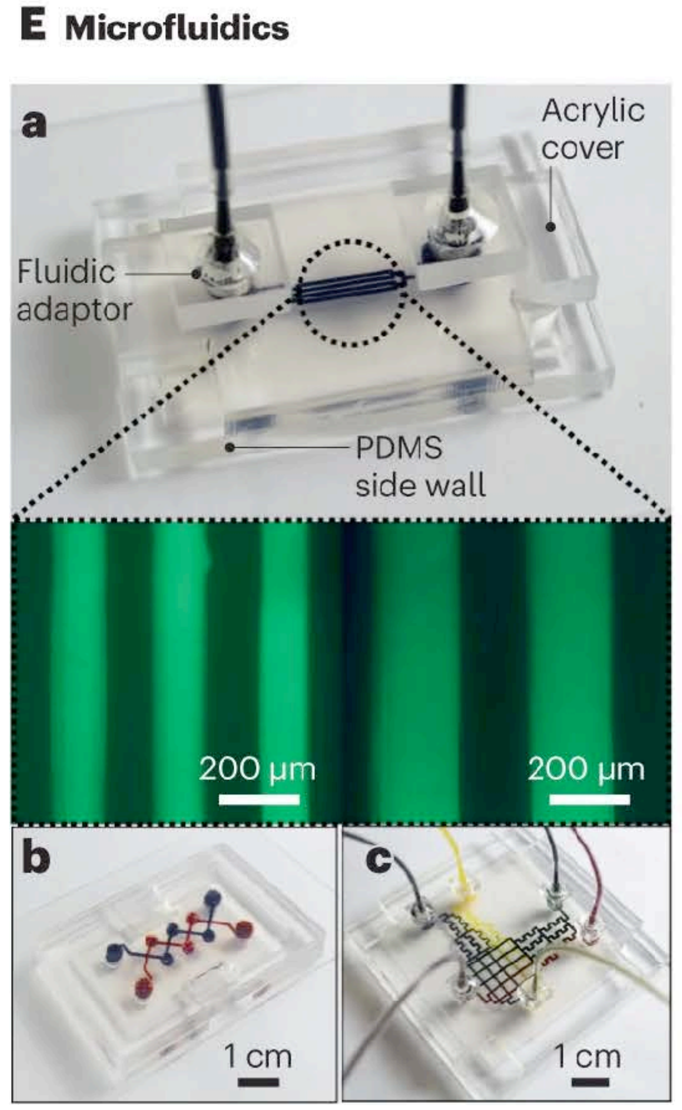




Silk applications

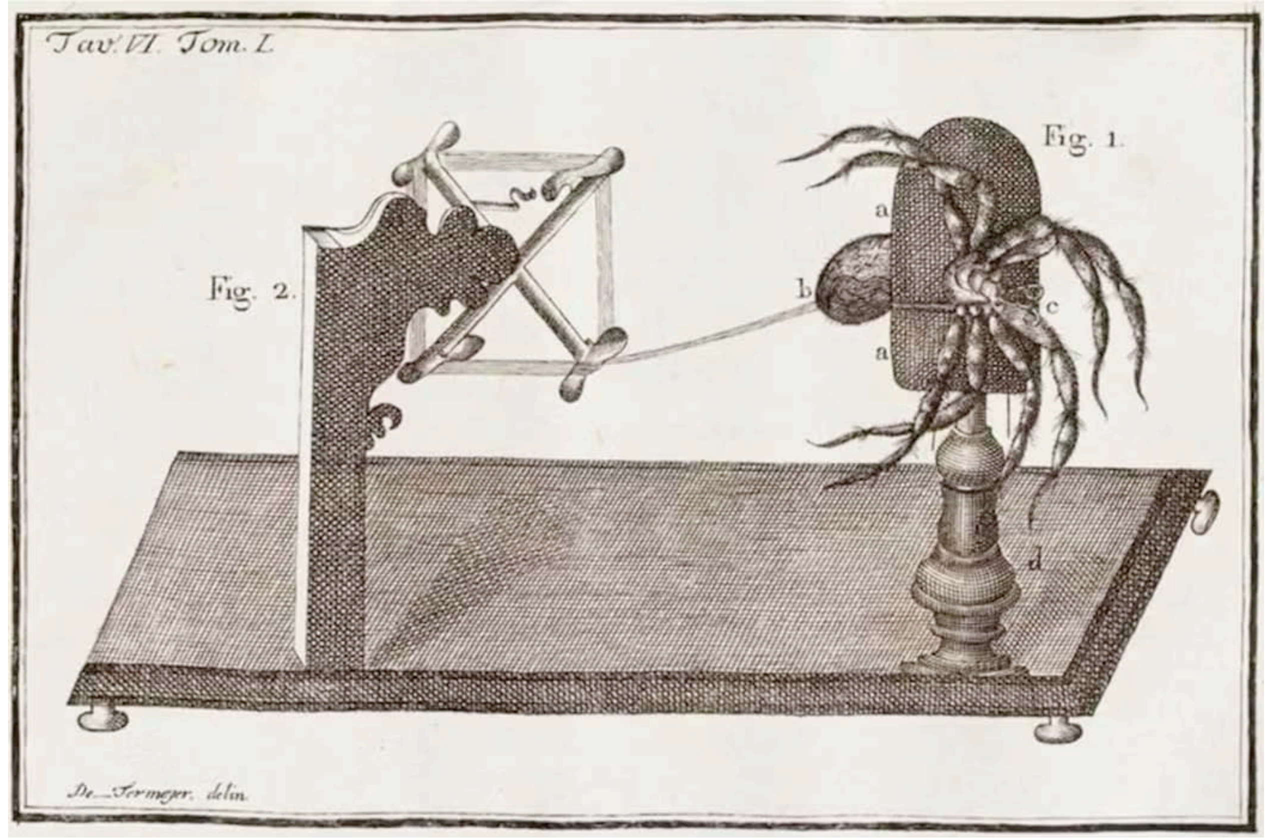






Limitations of biological materials

- Variability, batch to batch inconsistencies
- Residual contaminants
- Limited in control of composition and properties



⟶ **Can we better control their synthesis?**

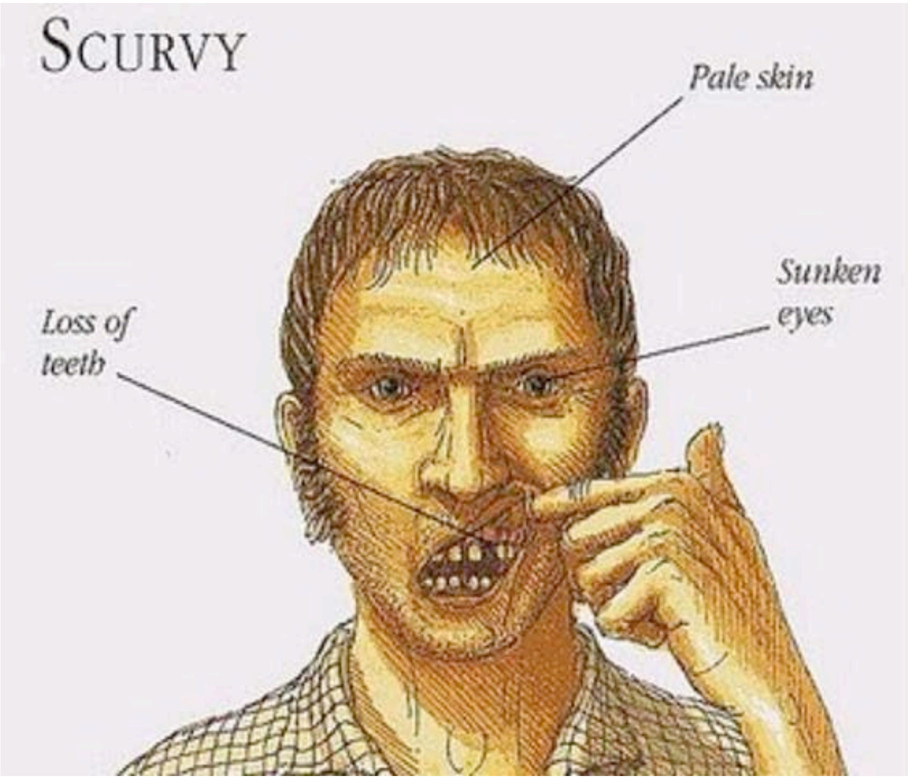
Precision synthesis of biomaterials

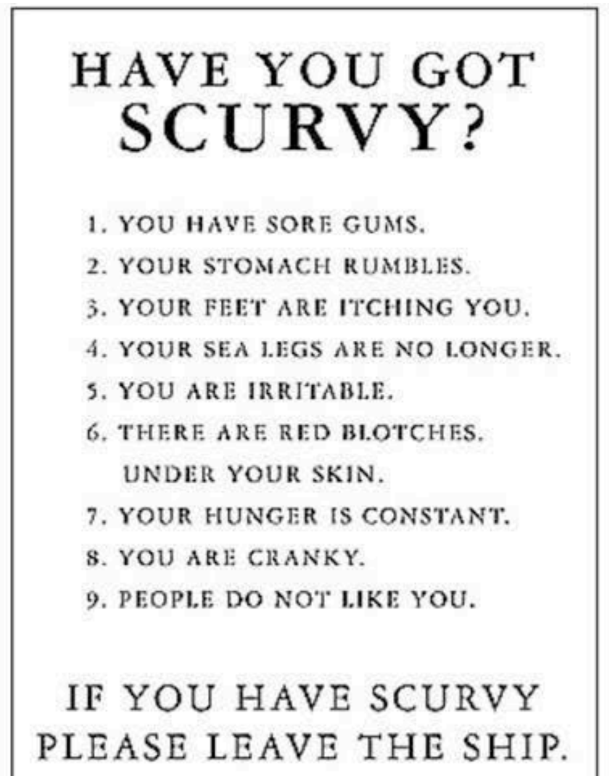
in vivo, biomaterials have specific precise properties enabling function:

- mechanics
- localization → **The organism controls synthesis!**
- timing

Disease associated with aberrant properties

Scleroderma (too much),
Scurvy (abnormal mechanics),
Osteogenesis Imperfecta, a.k.a. brittle bone disease (too little)

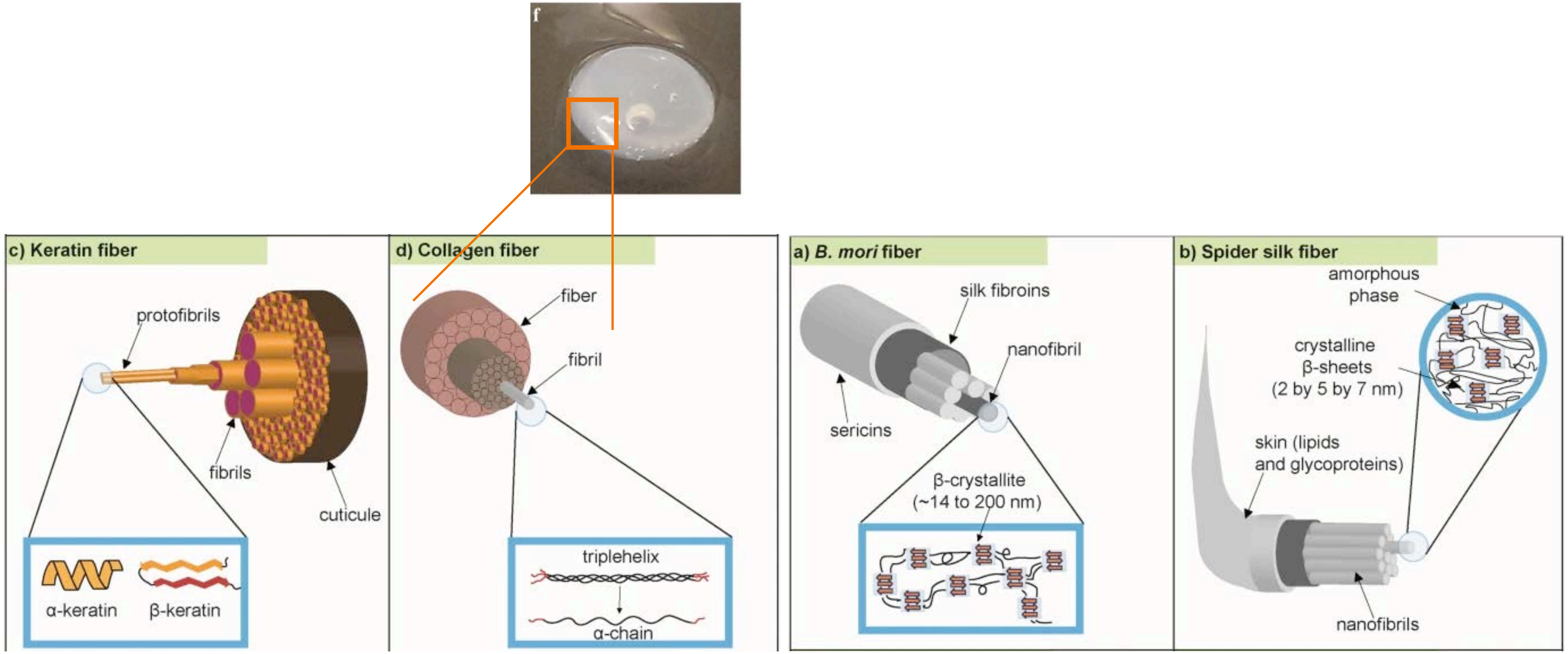




Let’s zoom in:
from the bulk material to its molecules

Biological protein materials form filaments

Protein polymers bundle into higher order structures



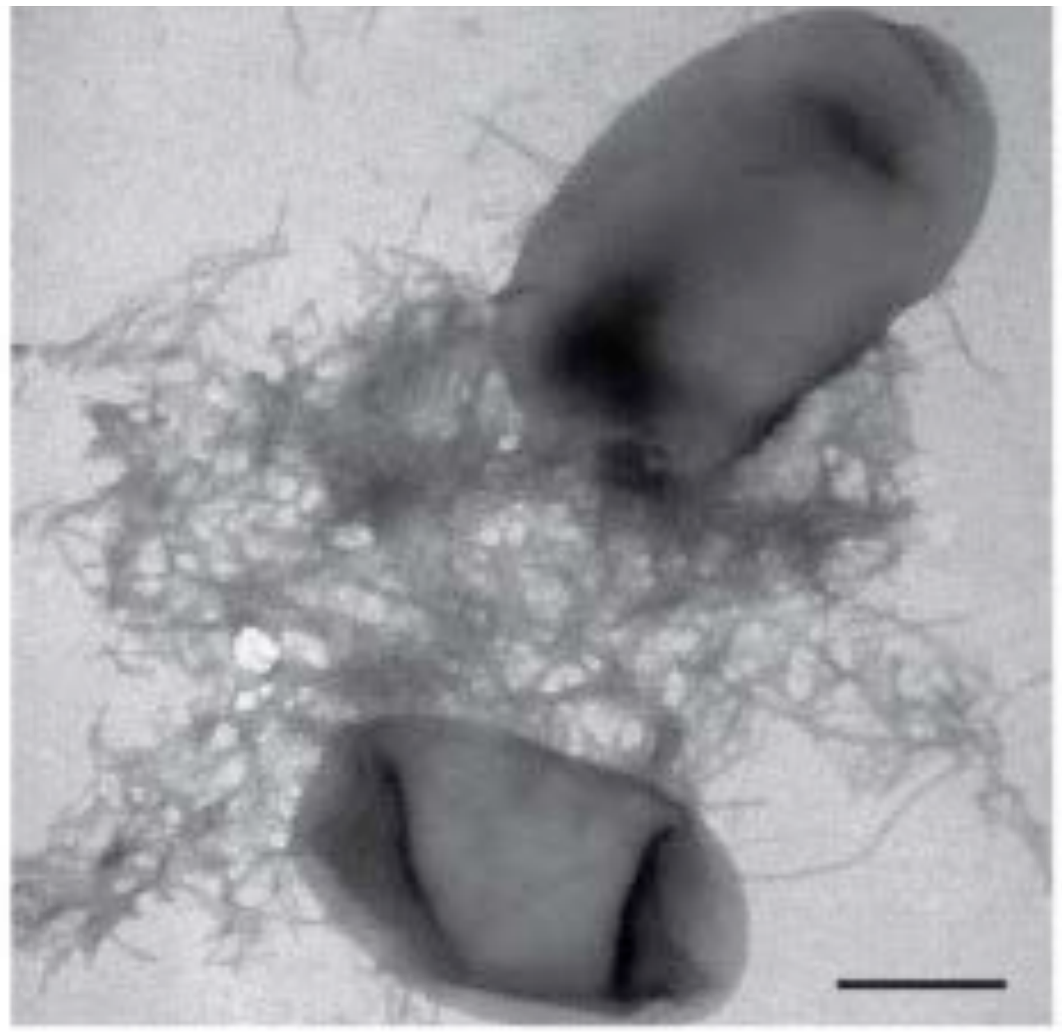
Biological material biosynthetic pathways

	Biomaterial	Organism	Genes Encoding Material or Biosynthesis
proteins	**Collagen**	Animals	COL1A1, COL1A2, COL3A1 (type I & III collagen)
	Elastin	Animals	ELN
	Fibronectin	Animals	FN1
	Keratin	Animals	KRT1, KRT5, KRT14
	Amyloid Proteins	Bacteria (*Escherichia coli*)	csgA, csgB, csgC (Curli fiber formation)
	Silk	Bombyx mori (silkworm), Spiders	FibH, FibL (Silk fibroin genes), MaSP1-2-3
polysaccharides	**Chitosan**	Crustaceans, fungi	CHS1, CHS2 (Chitin synthase genes)
	Bacterial Cellulose	*Komagataeibacter xylinus*	bcsA, bcsB, bcsC, bcsD (Bacterial cellulose synthase)
	Alginate	Brown algae, bacteria	algA, algC, algD (Alginate biosynthesis genes)
	Hyaluronic Acid	Animals, Streptococcus	hasA, hasB, hasC (Hyaluronan synthase)
	Mycelium-based Materials	Fungi (Ganoderma, Pleurotus)	chs1, chs2, fks1 (Chitin and β-glucan synthase)

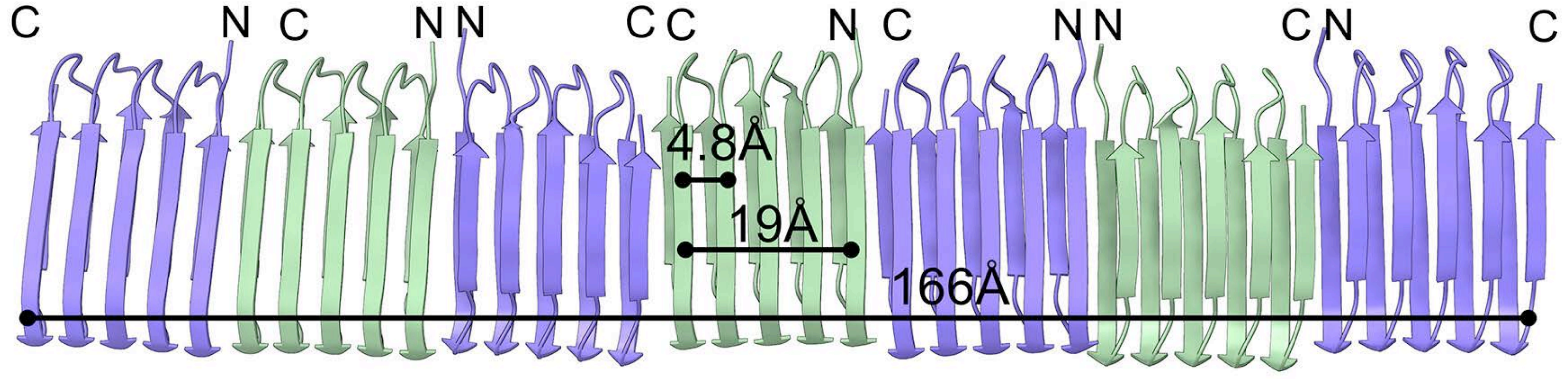
Protein materials are genetically encoded

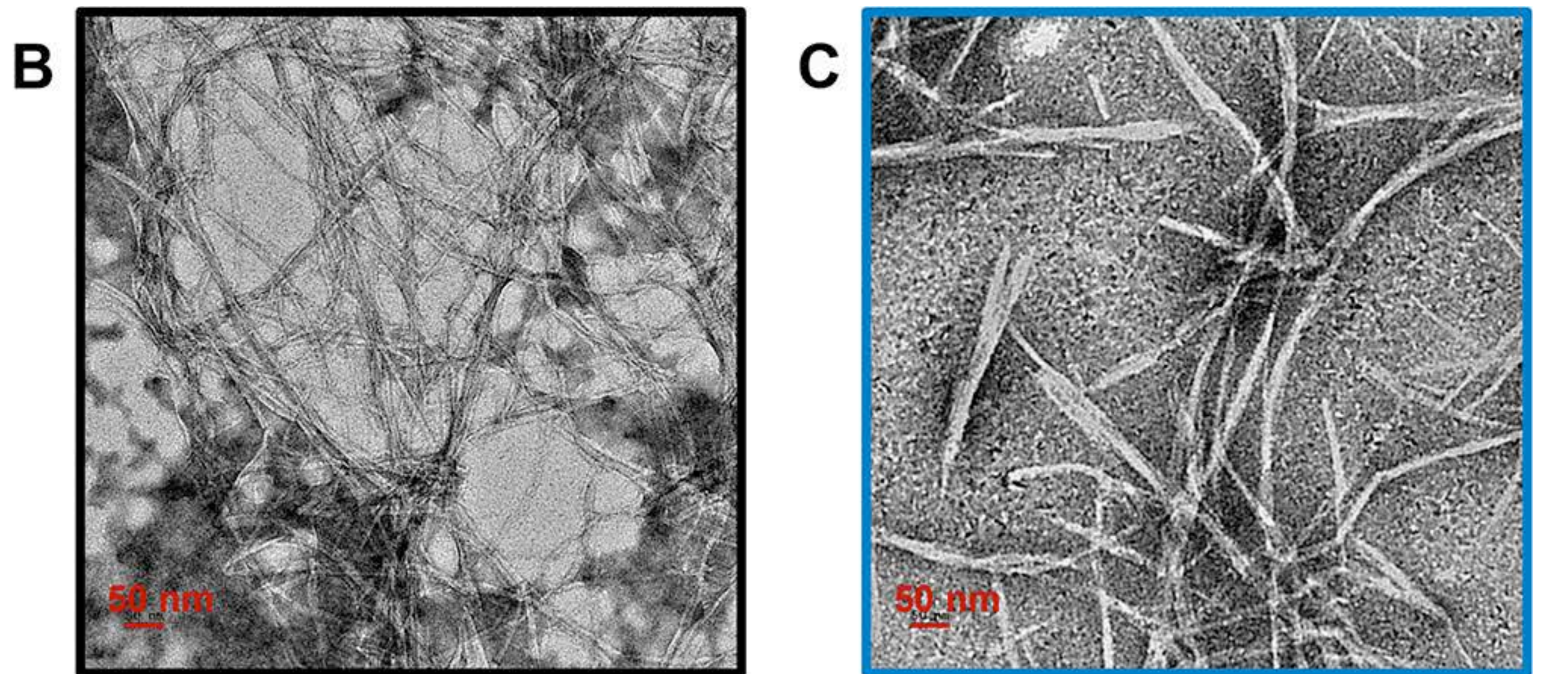
From gene to protein to polymer to filament

CsgA gene



CsgA polymer

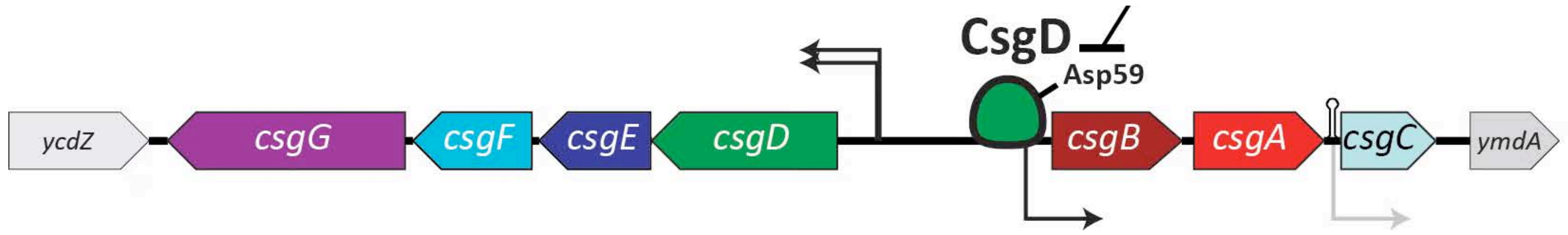




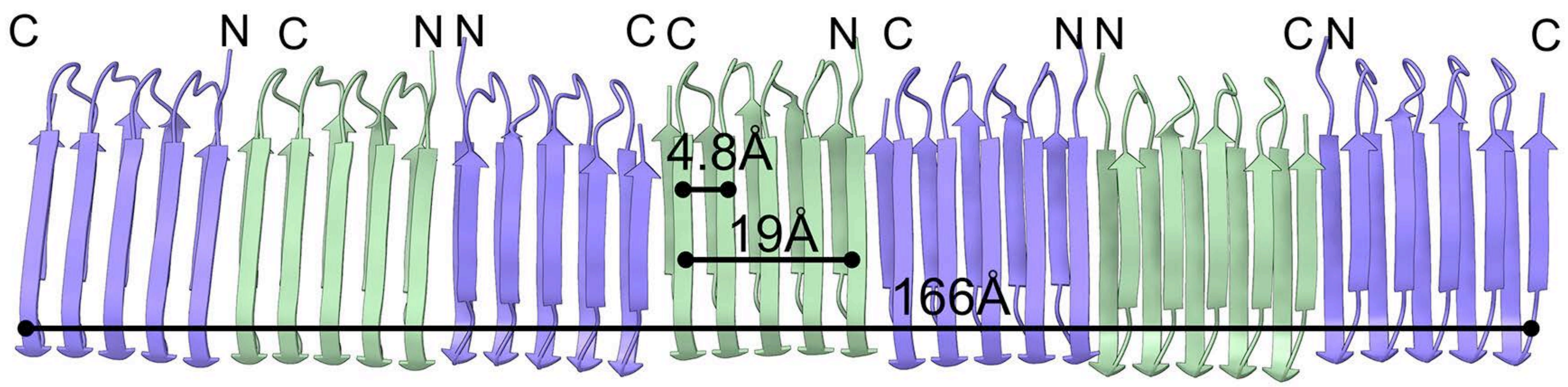
Curli filaments (amyloid)

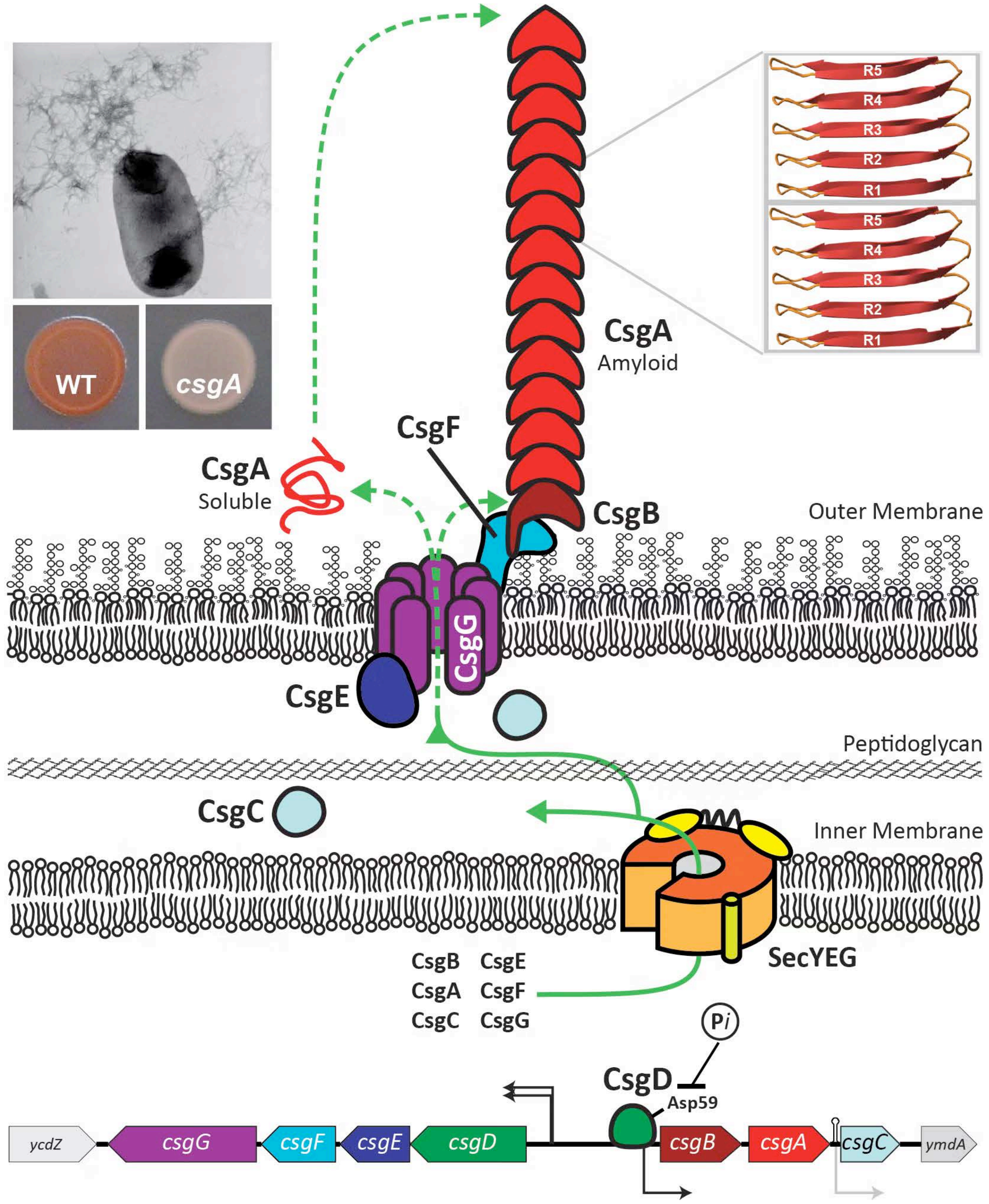
Bacterial filament production

Csg operon



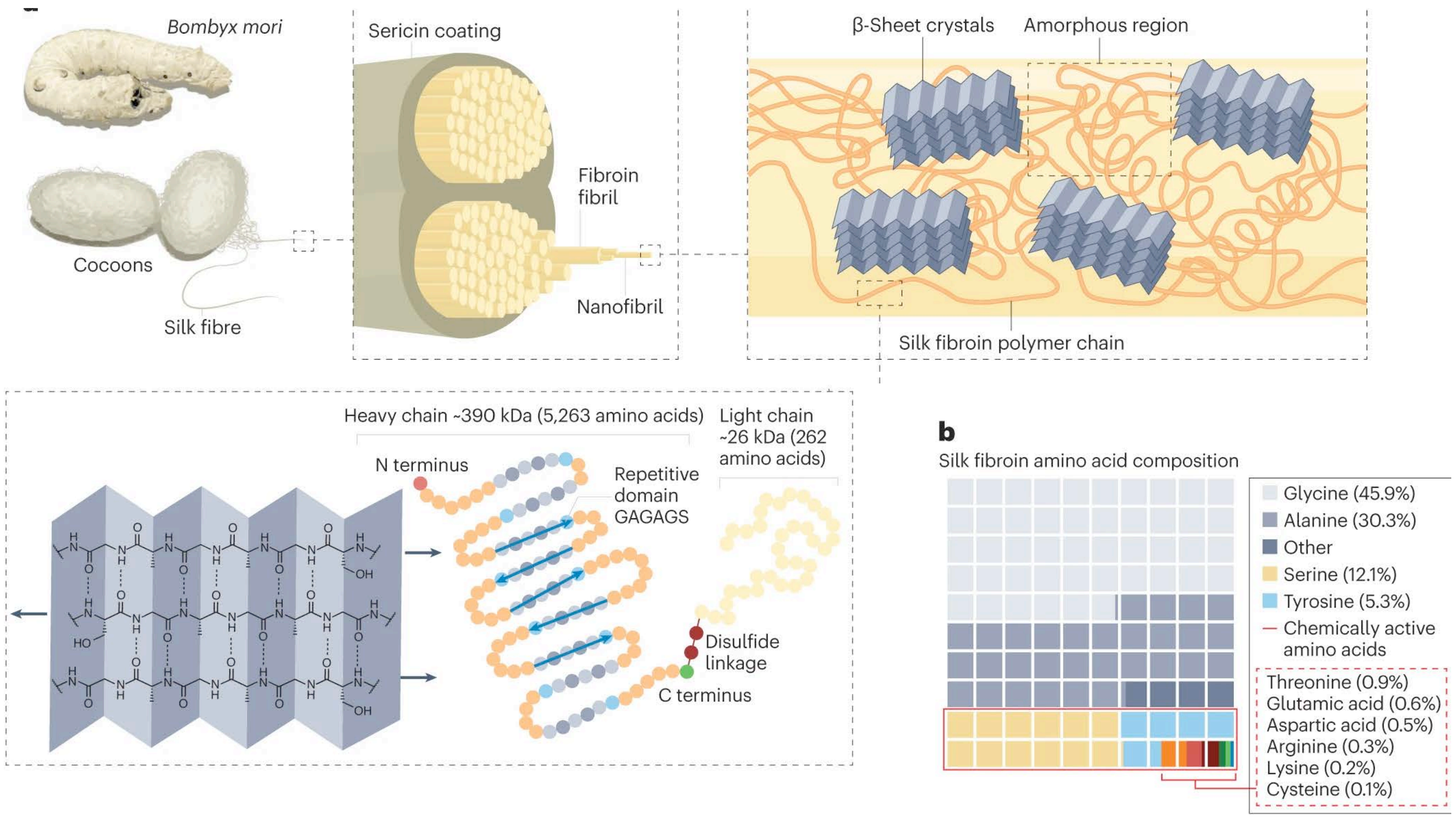
CsgA



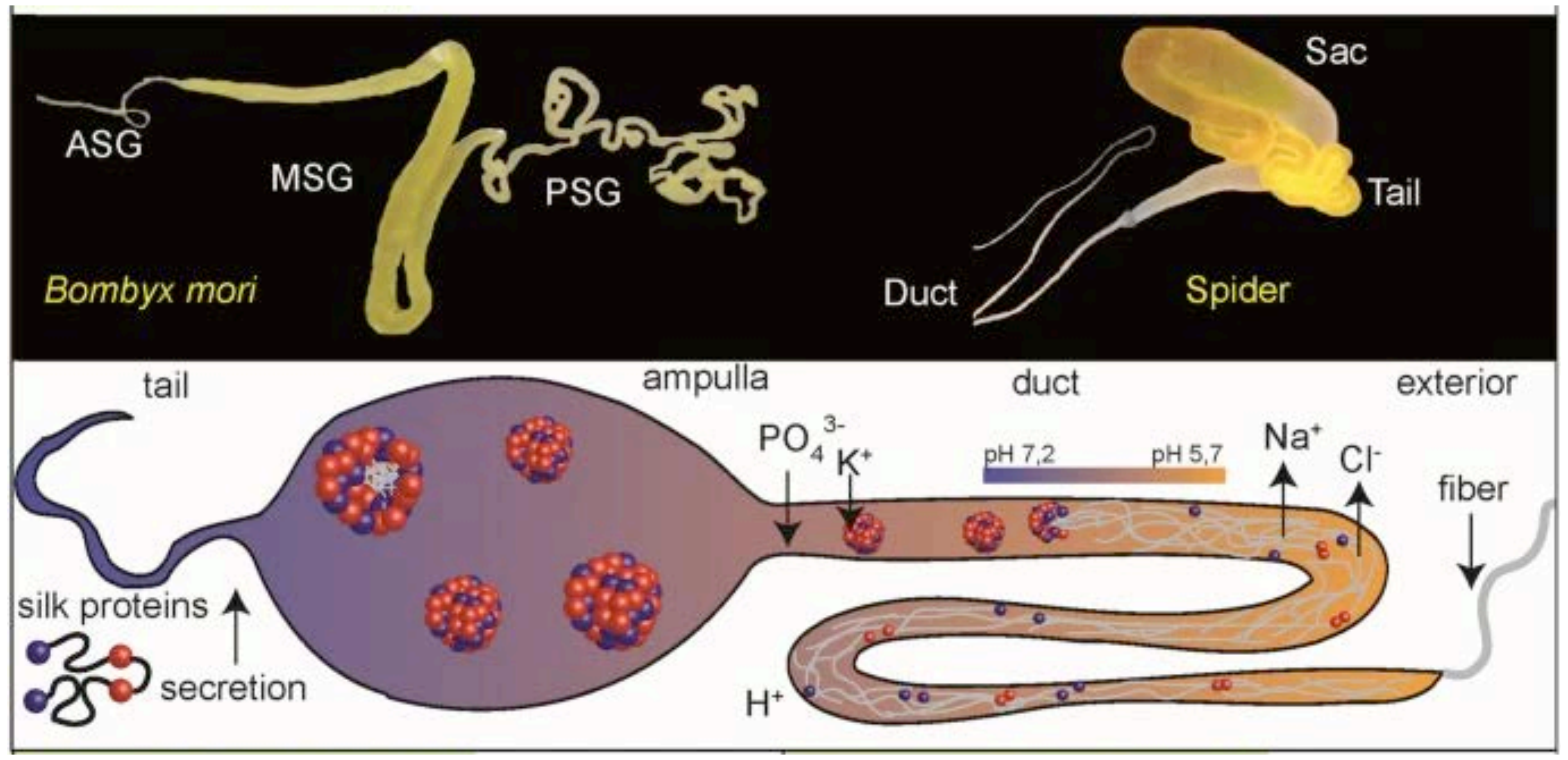


Polymer biogenesis can require additional assembly components

Silk fibroin filament architecture

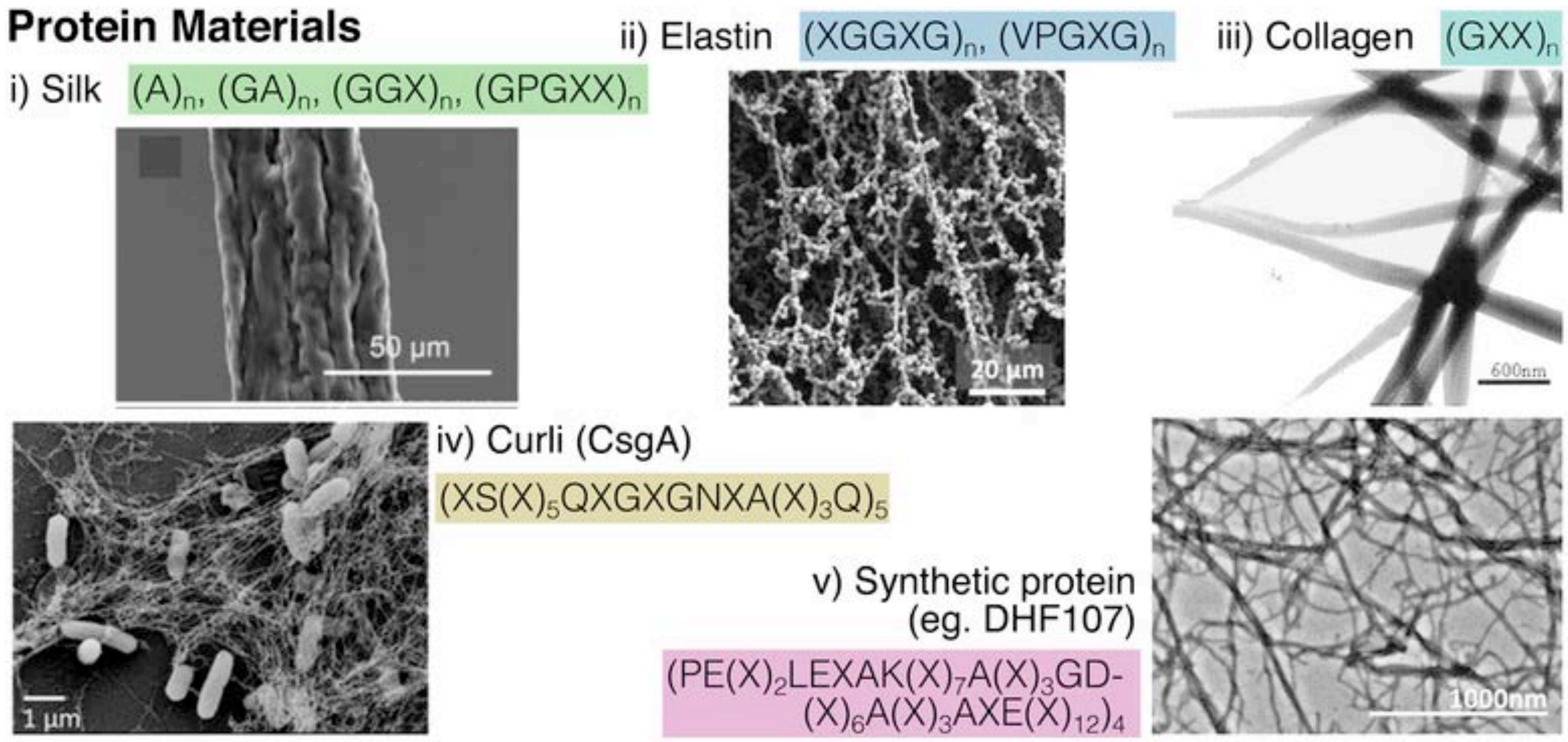


Fiber assembly in vivo

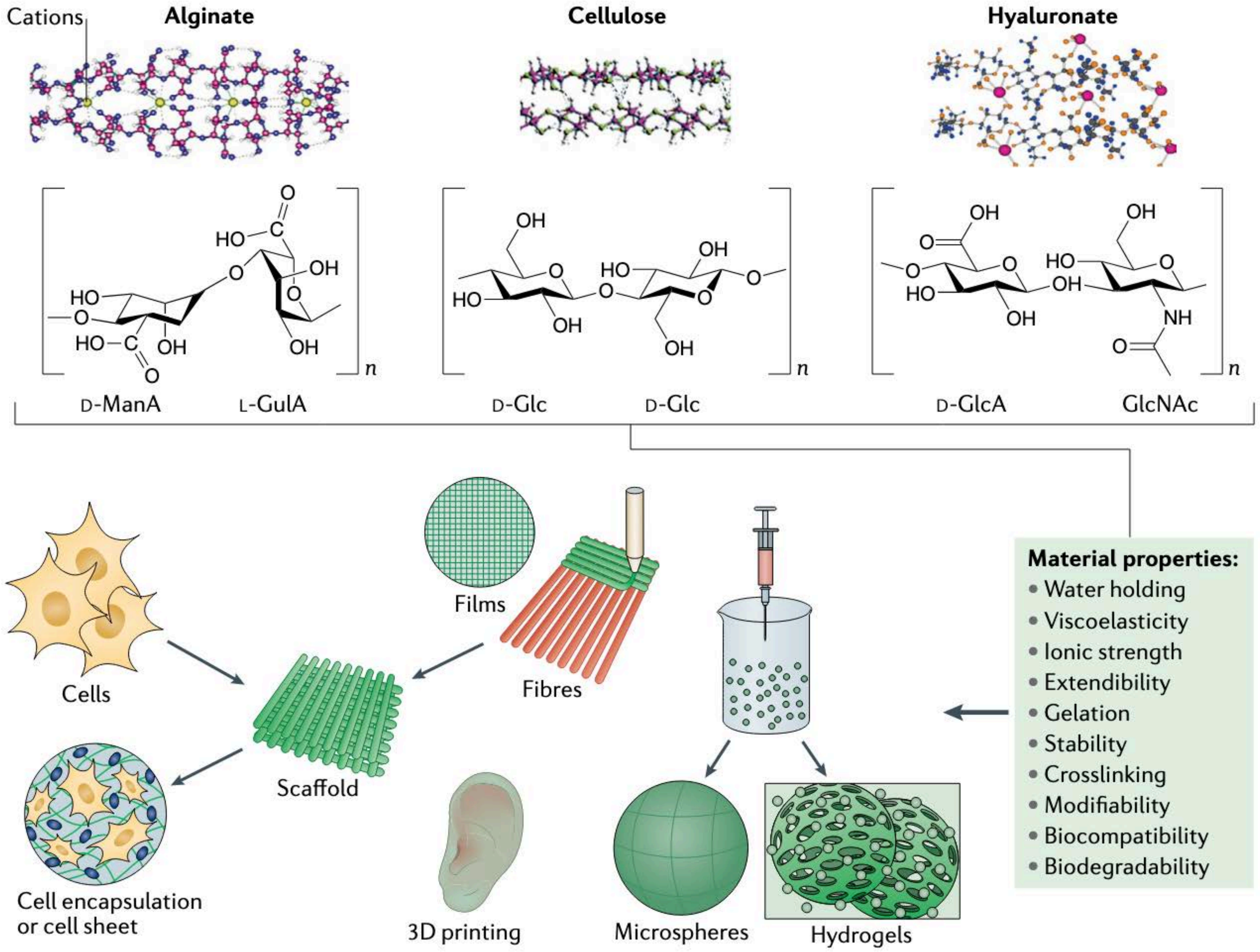


Biological material: protein structural features

Peptide repeats enable material properties



Polysaccharide-based biomaterials



→ Not proteins!!

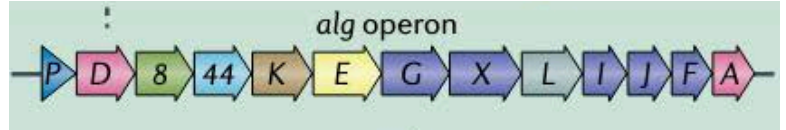
How do cells make sugar polymers?

↓

Enzymes catalyze sugar polymerizations

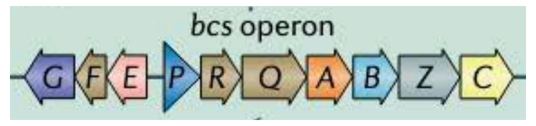
Polysaccharide biosynthesis

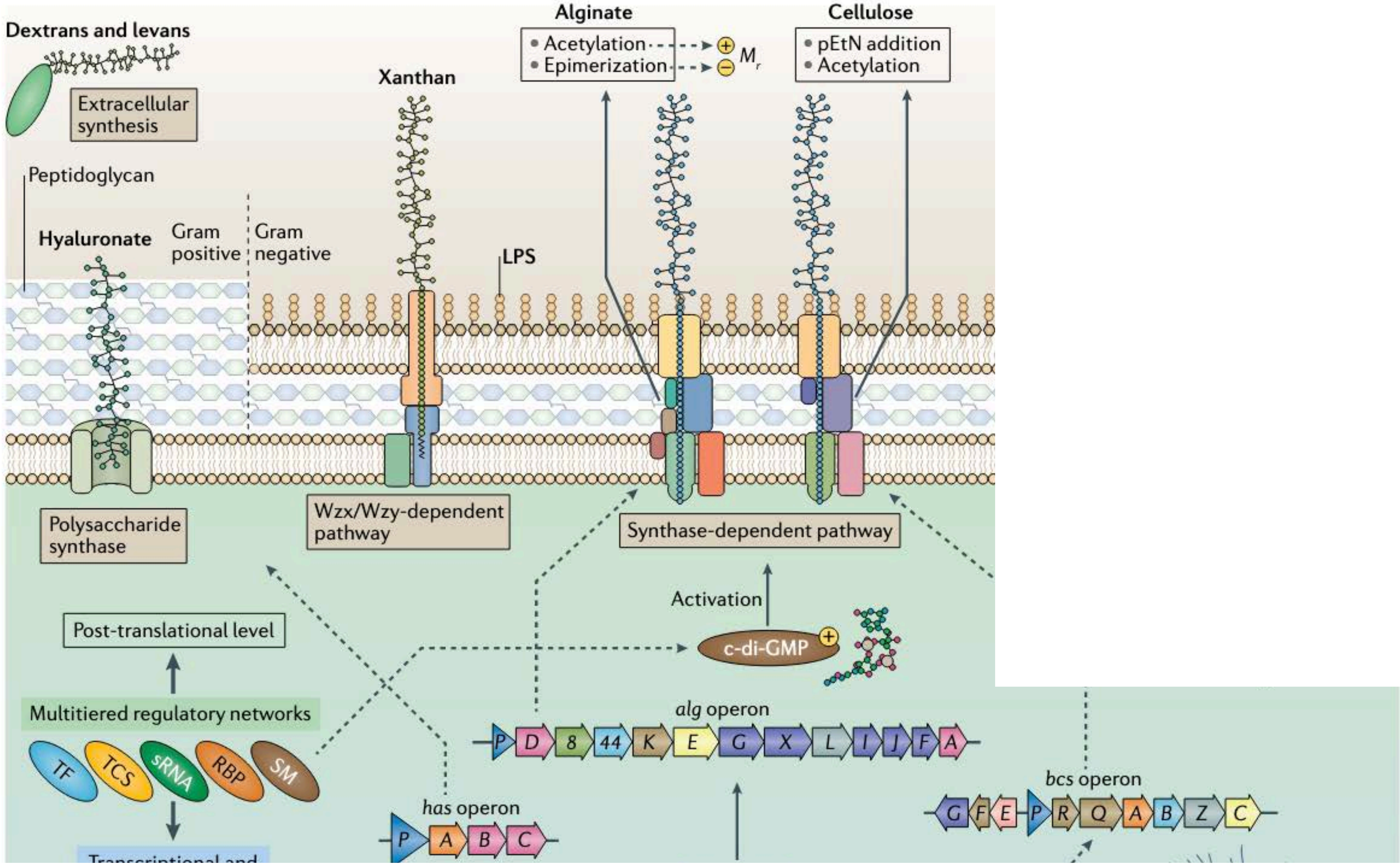
alginate



hyaluronate

cellulose





Complex pathways that are poorly characterized, yet great materials

From genes to (better) biomaterials

We know the genes

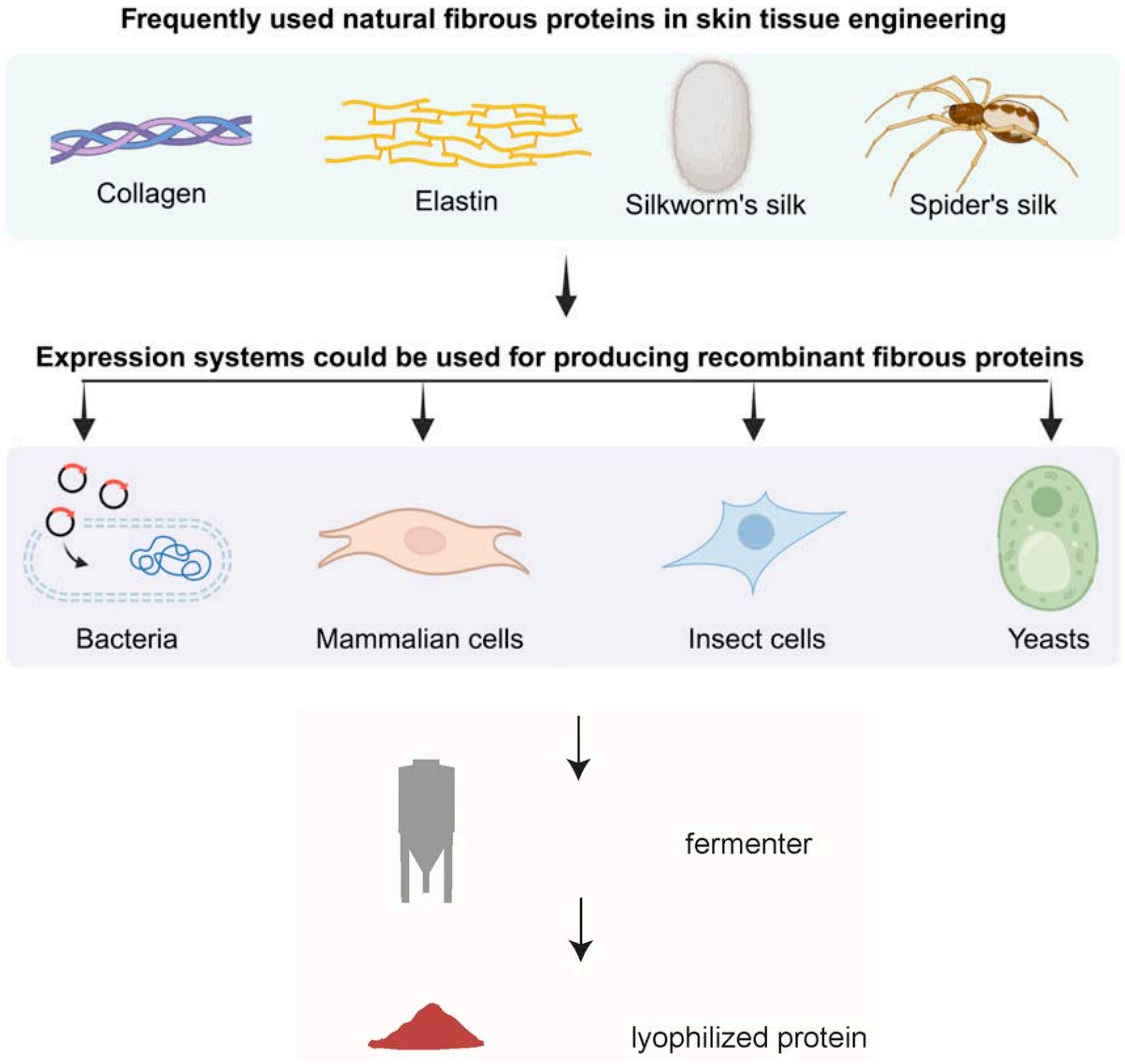
We know the structure-function relationship

Let's produce biological materials recombinantly

↓

Use synthetic biology toolbox to enhance biological materials for biomaterial applications

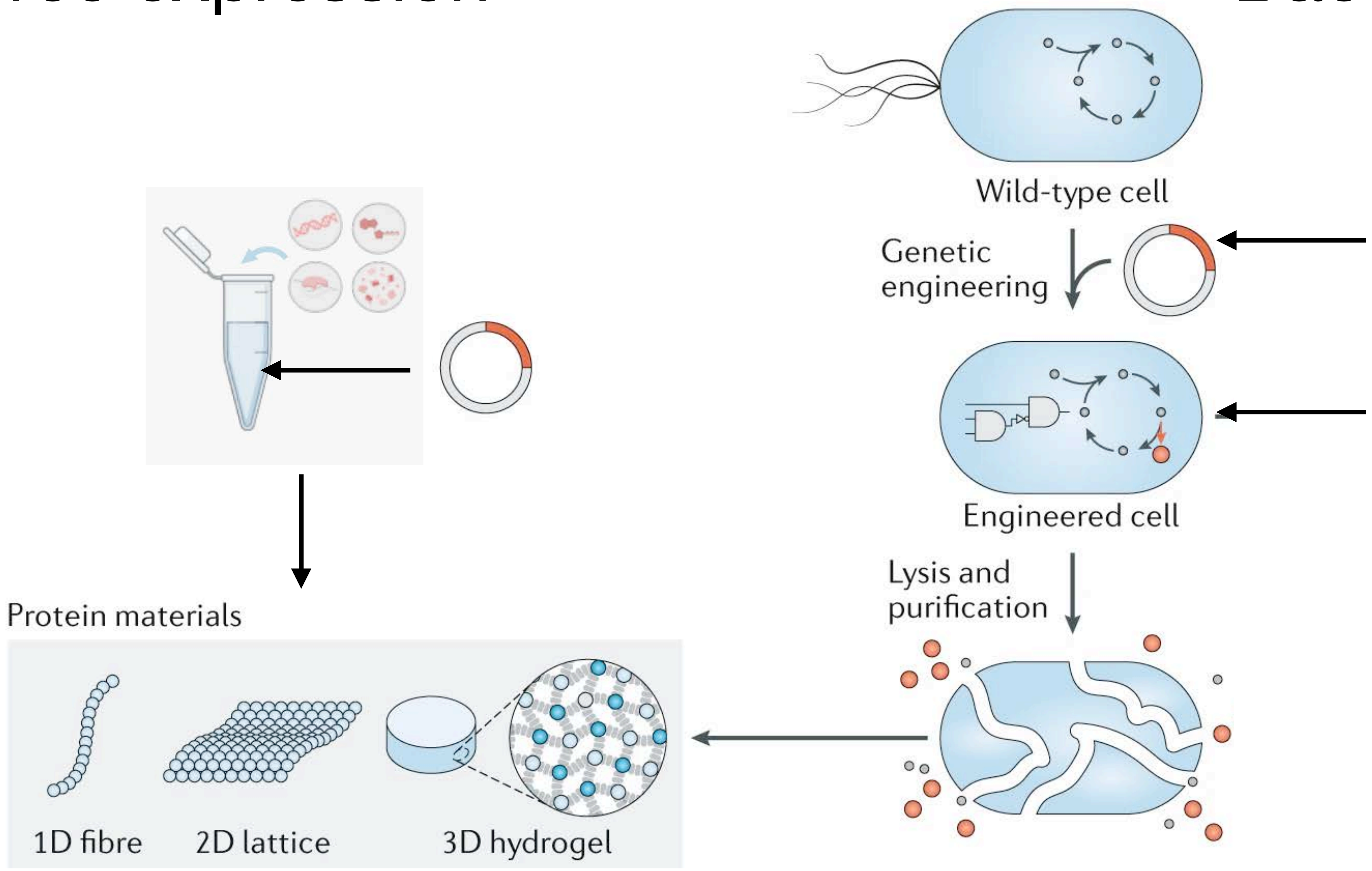
Recombinant biomaterial production



Laying out biomaterial protein production

Cell-free expression

Bacterial based synthesis



Gene of interest

Optimization step

Why synthetic biology?

From passively recovering natural biomaterials (e.g. purification)
to actively engineering natural biomaterials at the molecular level

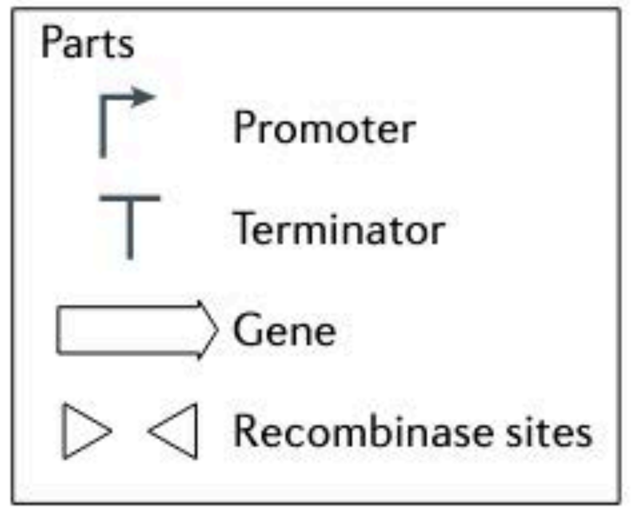
Controlling production with genetic circuits

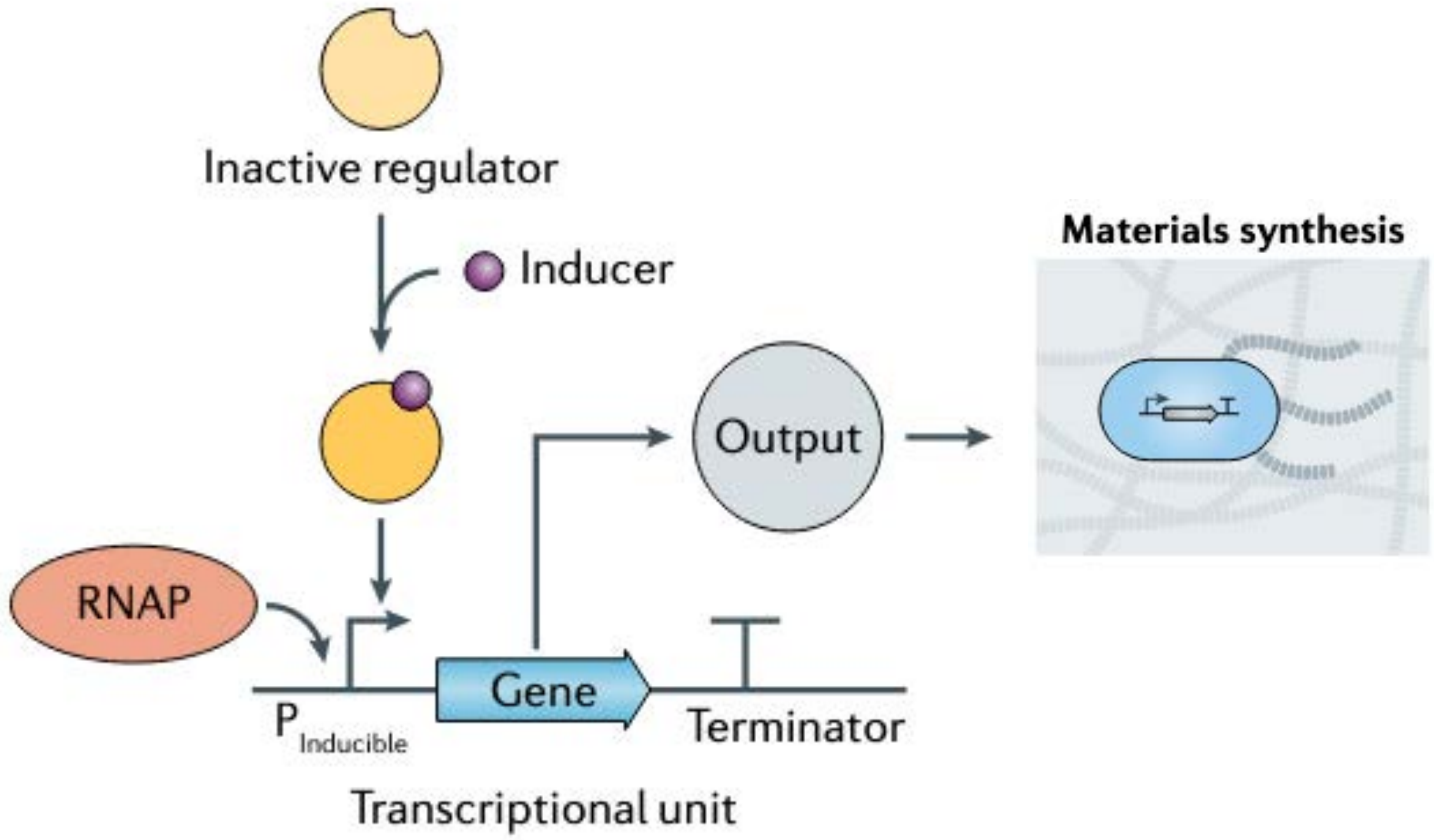
Make use of genetic parts

promoter, RBS, terminators, reporters, plasmids. etc.

example: IPTG inducible promoter

> control of production process, e.g. concentration, stoichiometry





Why synthetic biology for biomaterials?

Programmable: genetic script let us produce material on demand, incorporating logic circuits

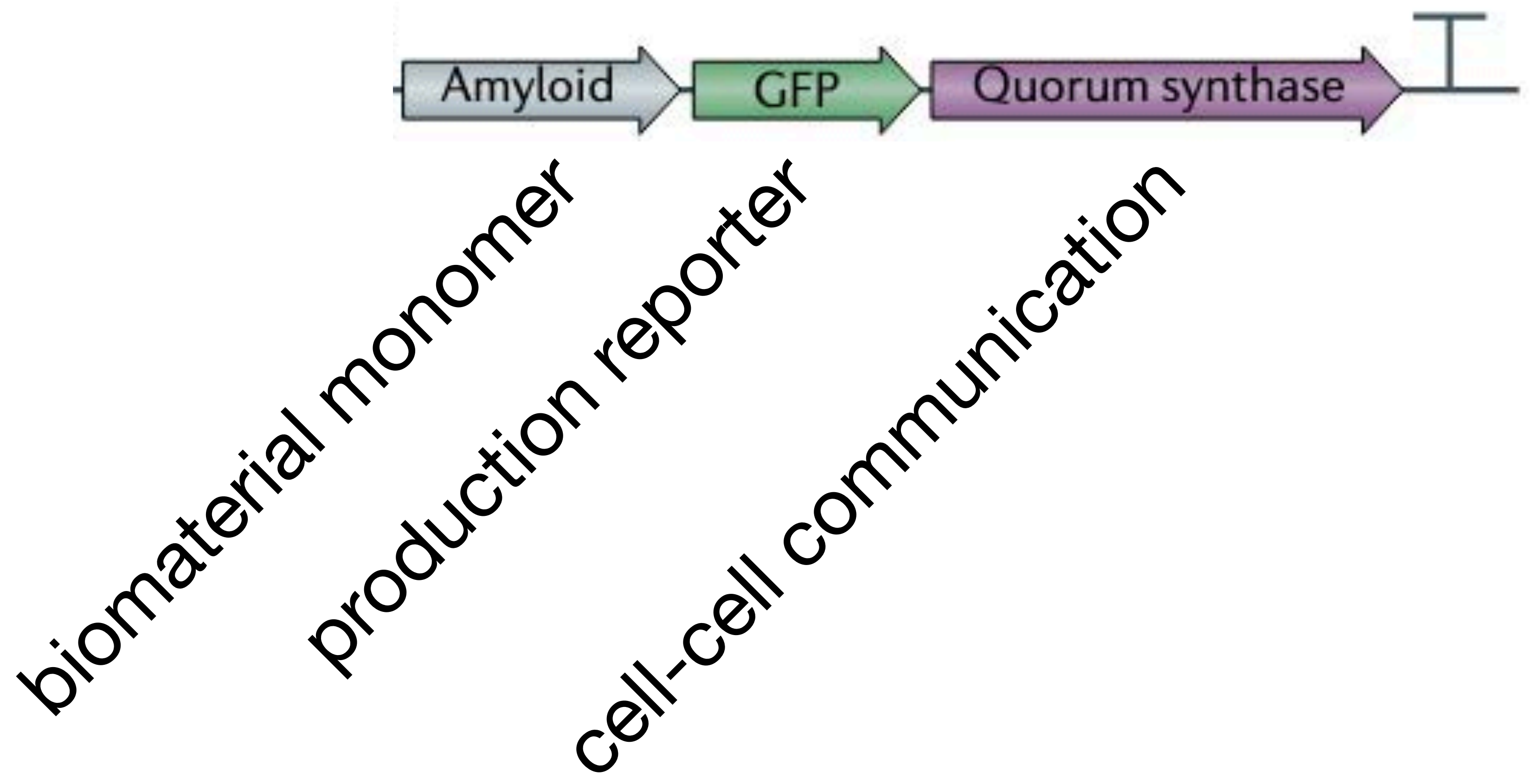
Modular: plug and play by swapping genetic parts

Adaptive and responsive: takes advantage of signaling networks

Improve yield and precision of production process!

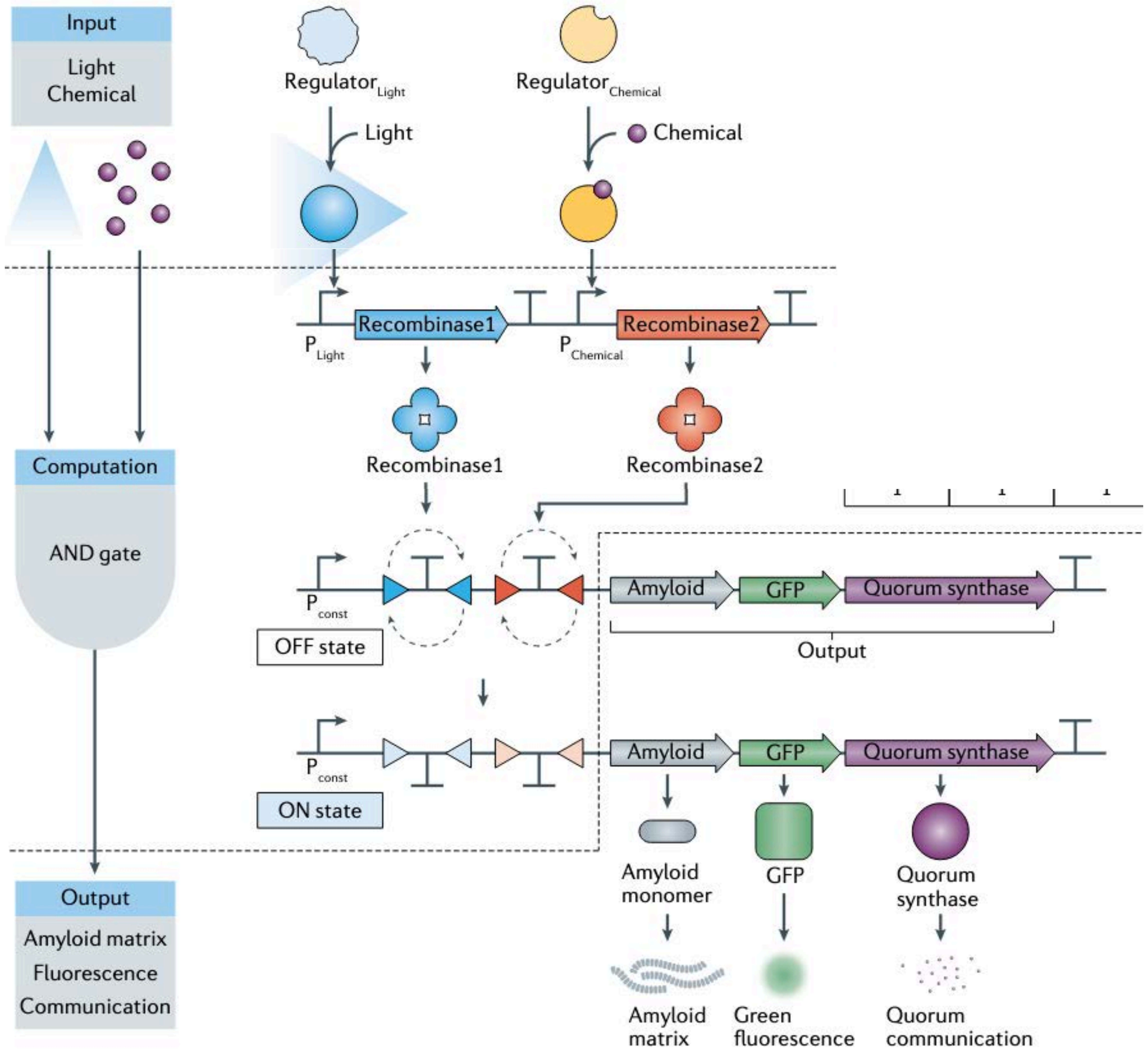
Programming for production

Amyloid production upon light and chemical exposure

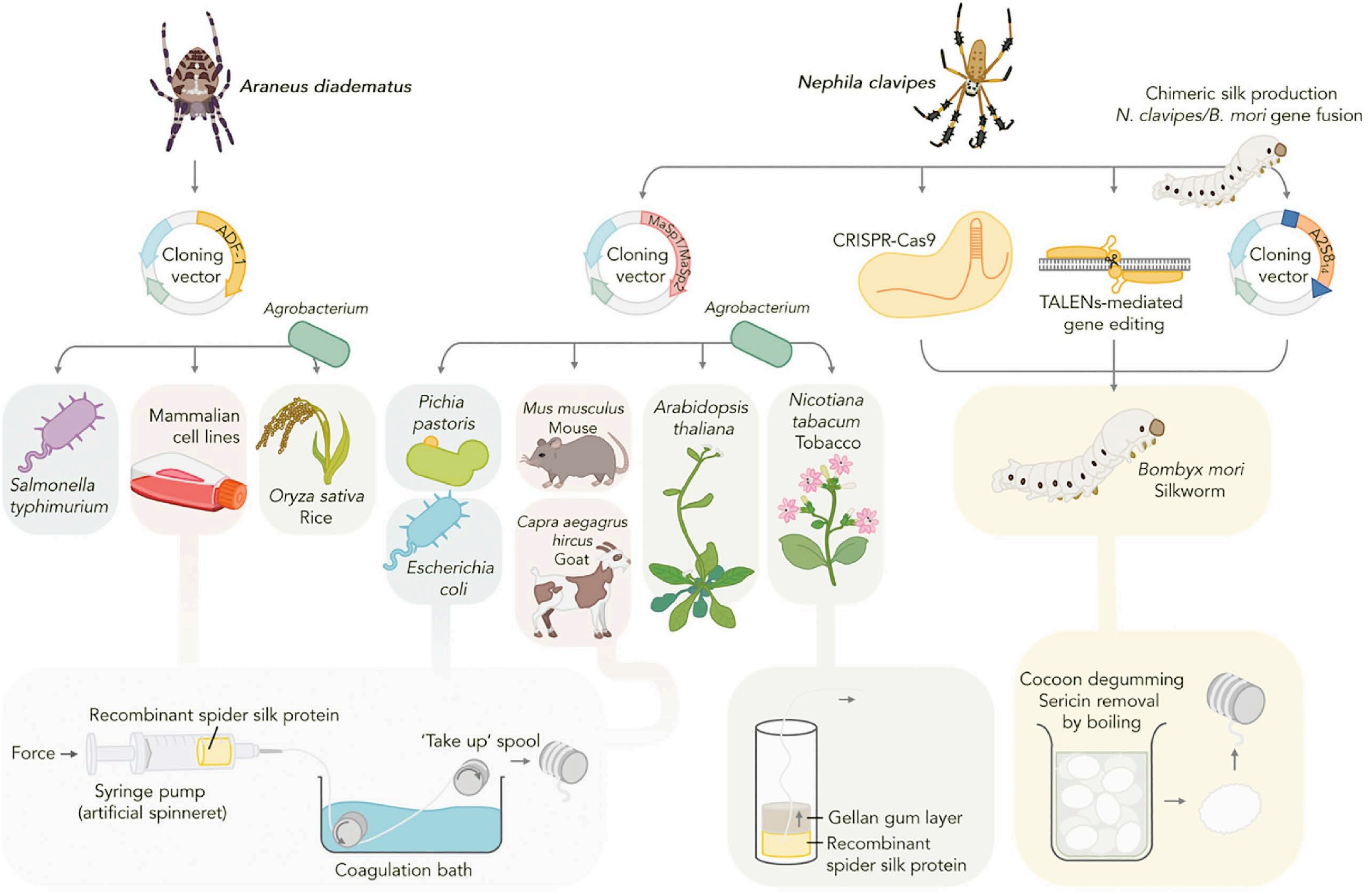


Truth table

Light	Chemical	Output
0	0	0
1	0	0
0	1	0
1	1	1



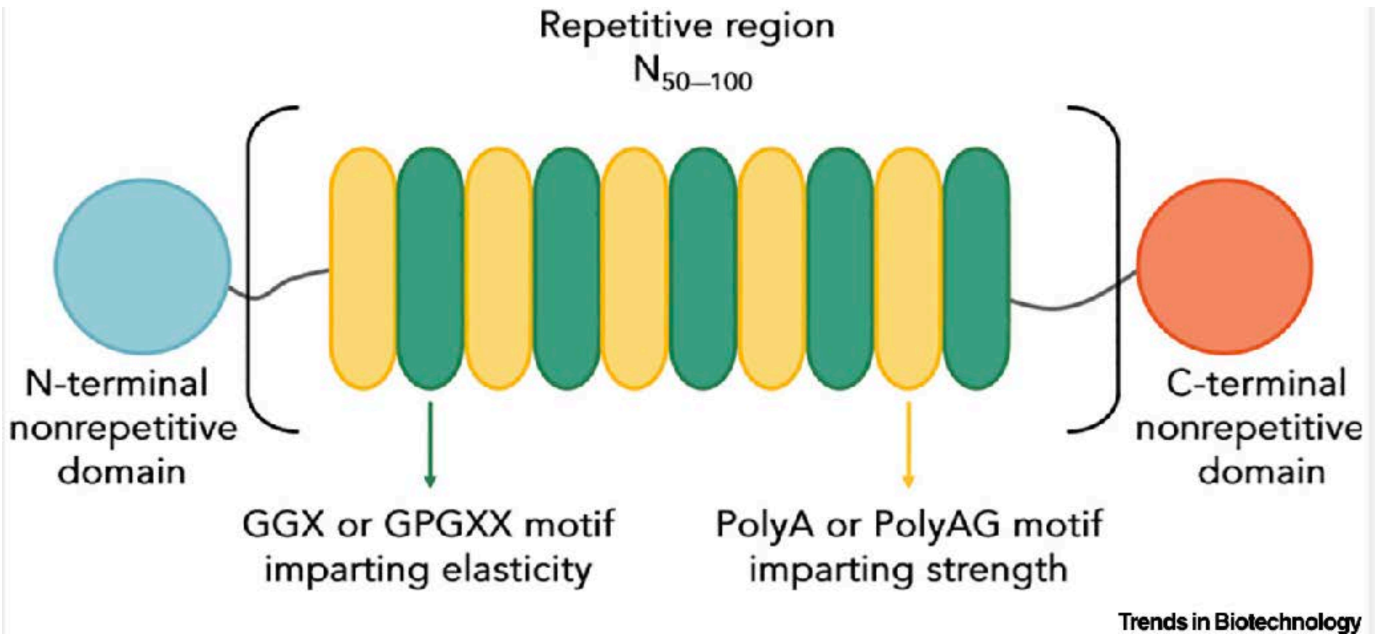
Silk - optimizing efficiency



Trends in Biotechnology

Silk - optimizing efficiency

Spidroin and Fibroin



Many repeats complicate the recombinant approach:

- mRNA secondary structure
- instability
- metabolic burden

Table 1. Selected Examples of Host System Performance in the Production of Recombinant Spidroins

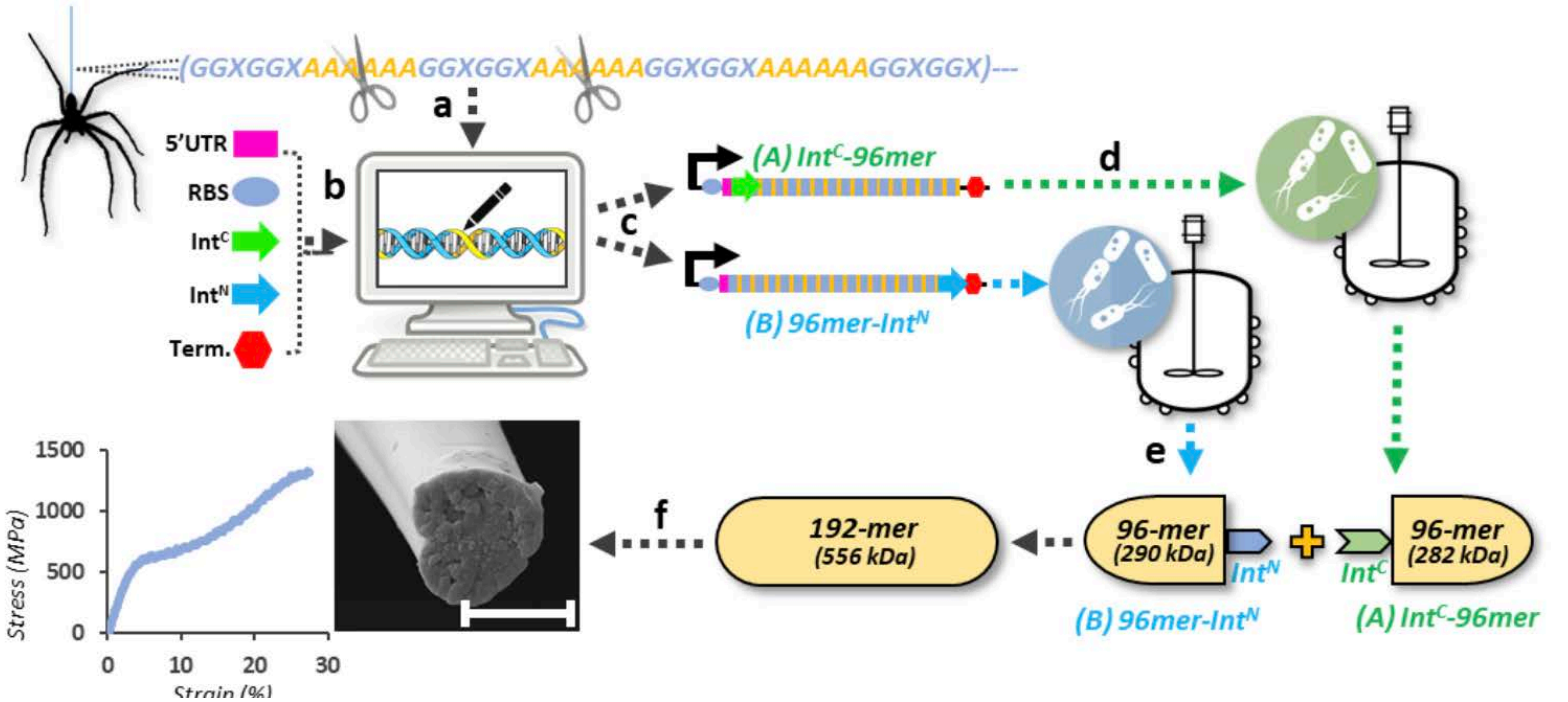
Expression host	Originspecies	Protein homologue	Size (kDa)	Maximum reported yield	Refs
E. coli	*N. clavipes*	MaSp1	100.7 284.9	2700 mg/l 500 mg/l	[22]
E. coli	*N. clavipes*	MaSp1	285 556	2000 mg/l 1240 mg/l	[23]
P. pastoris	*N. clavipes*	MaSp1	65	663 mg/l	[19]
S. typhimurium	*A. diadematus*	ADF-1, -2, and -3	25-56	14 mg/l	[24]
S. cerevisiae	*N. clavipes*	MaSp1	94	450 mg/l	[25]
S. tuberosum	*N. clavipes*	MaSp1, Chimeric	12.9–99.8	0.5% of total proteins	[29]
N. tobacum (leaf)	*N. clavipes*	FLAG	>72 – >250	36 mg/kg	[30]
N. tobacum (seed)	*N. clavipes*	FLAG	>72 – >460	190 mg/kg	[31]
Arabidopsis thaliana	*N. clavipes*	MaSp1	64	18 % of total proteins	[35]
O. sativa	*A. ventricosus*	AvMaSp	22	Not reported	[36]
M. sativa	*N. clavipes*	MaSp2	80	Not reported	Hugie, 2019[a]
B. mori (larvae)	*N. clavipes*	MaSp1–EGFP	70	6 mg per larvae	[46]
B. mori (spun)	*B. mori/N. clavipes*	MaSp1, chimeric	83	Not reported	[47]
B. mori (spun)	*B. mori/N. clavipes*	MaSp2, FLAG, chimeric	78-106	5% of composite fibre protein	[48]
B. mori (spun)	*B. mori/N. clavipes*	MaSp1, chimeric	67	35.2% of composite fibre protein	[50]
B. mori (spun)	*B. mori/N. clavipes*	MaSp1, Chimeric	120–300	Not reported	[44]
S. frugiperda (cell line sf9)	*A. diadematus*	ADF-4	60	50 mg/l	[52]
Baby hamster kidney cells (BHK)	*N. clavipes/ A. diadematus*	ADF-3	60	50 mg/l	[53]
Bovine mammary epithelial alveolar cells (MAC-T)		MaSp1 MaSp2 ADF-3	60–140	Not reported	
Primate cells (COS-1)	*Euprosthenops sp.*	MaSp1	22–25	Not reported	[54]
Transgenic mice	*N. clavipes*	MaSp1, MaSp2	40–55	11.7 mg/L	[56]
Transgenic goats	*N. clavipes*	MaSp1, MaSp2	65	Not reported	[57]
L. tarentolae	*N. clavipes*	MaSp1, MaSp2	73–81	Not reported	[60]

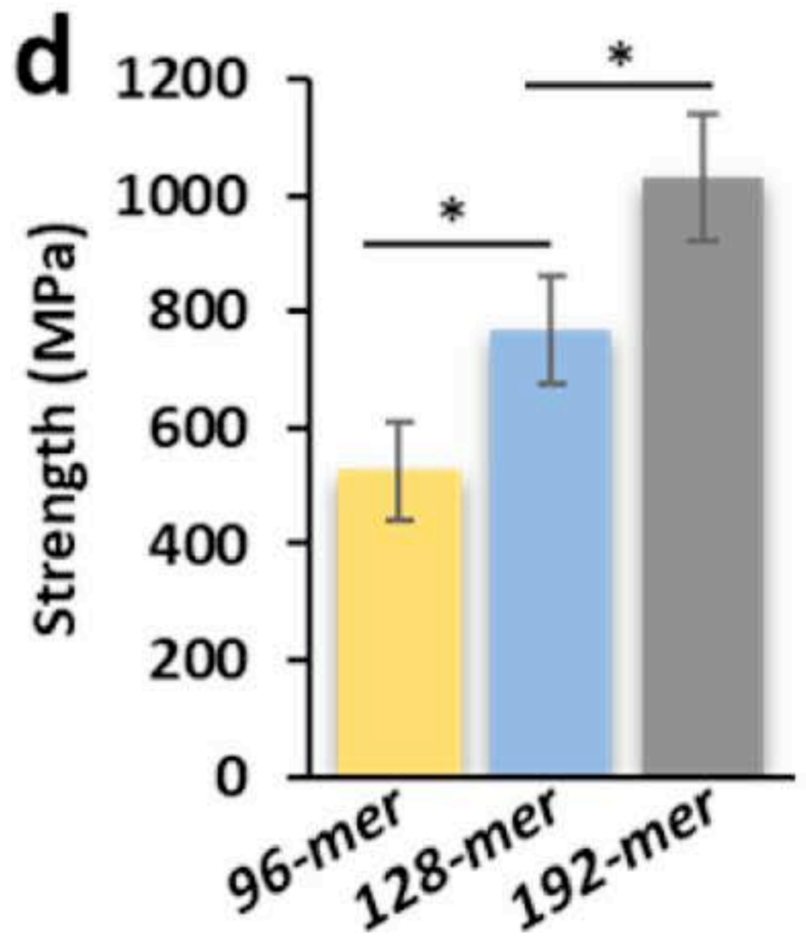
Recombinant production of spider silk

an split approach to yield stronger silk

Larger MW > stronger silk
Large spidroin cannot be recombinantly synthesized

> use a split protein approach (intein-dependent linking)





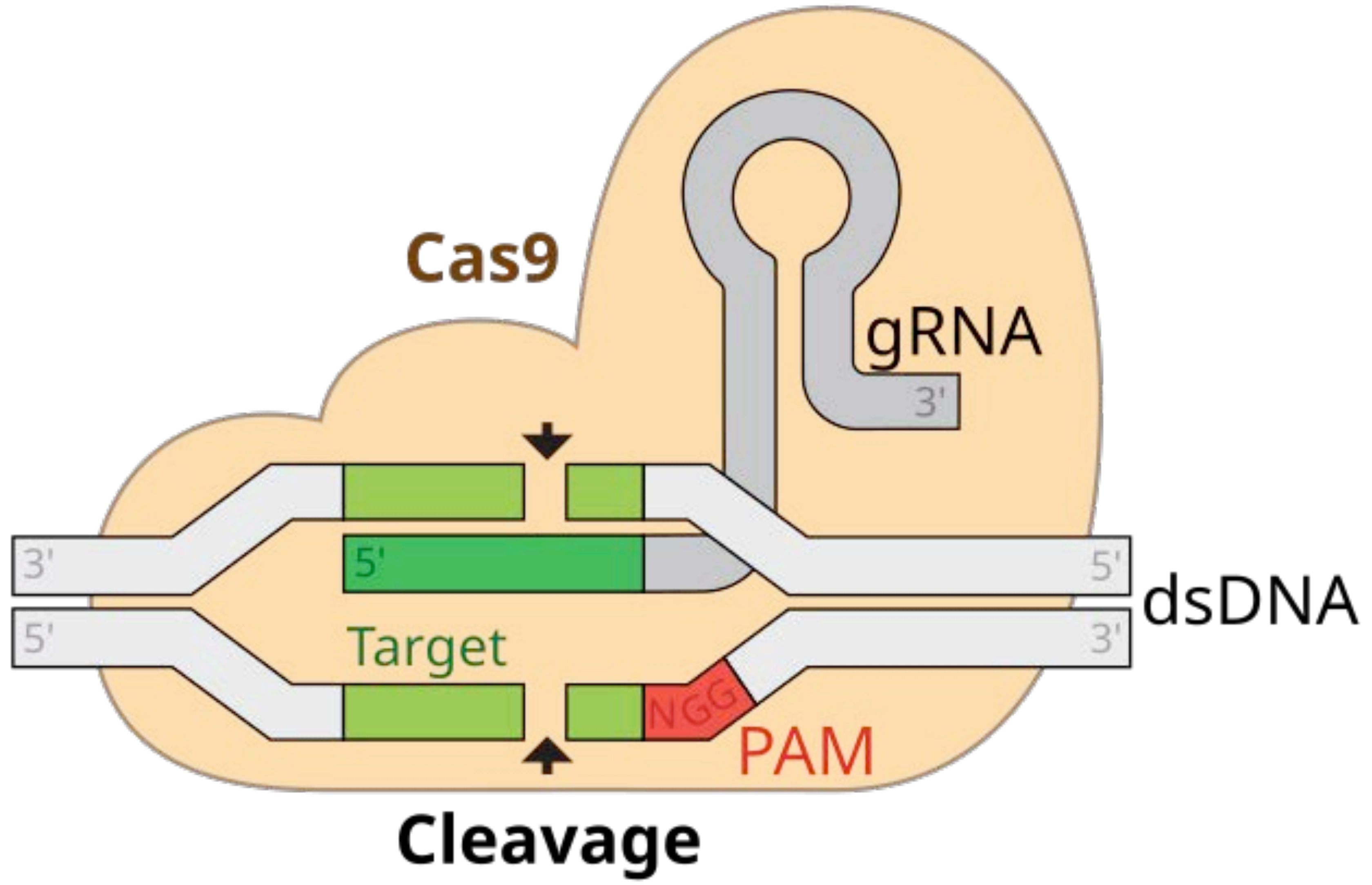
So far…

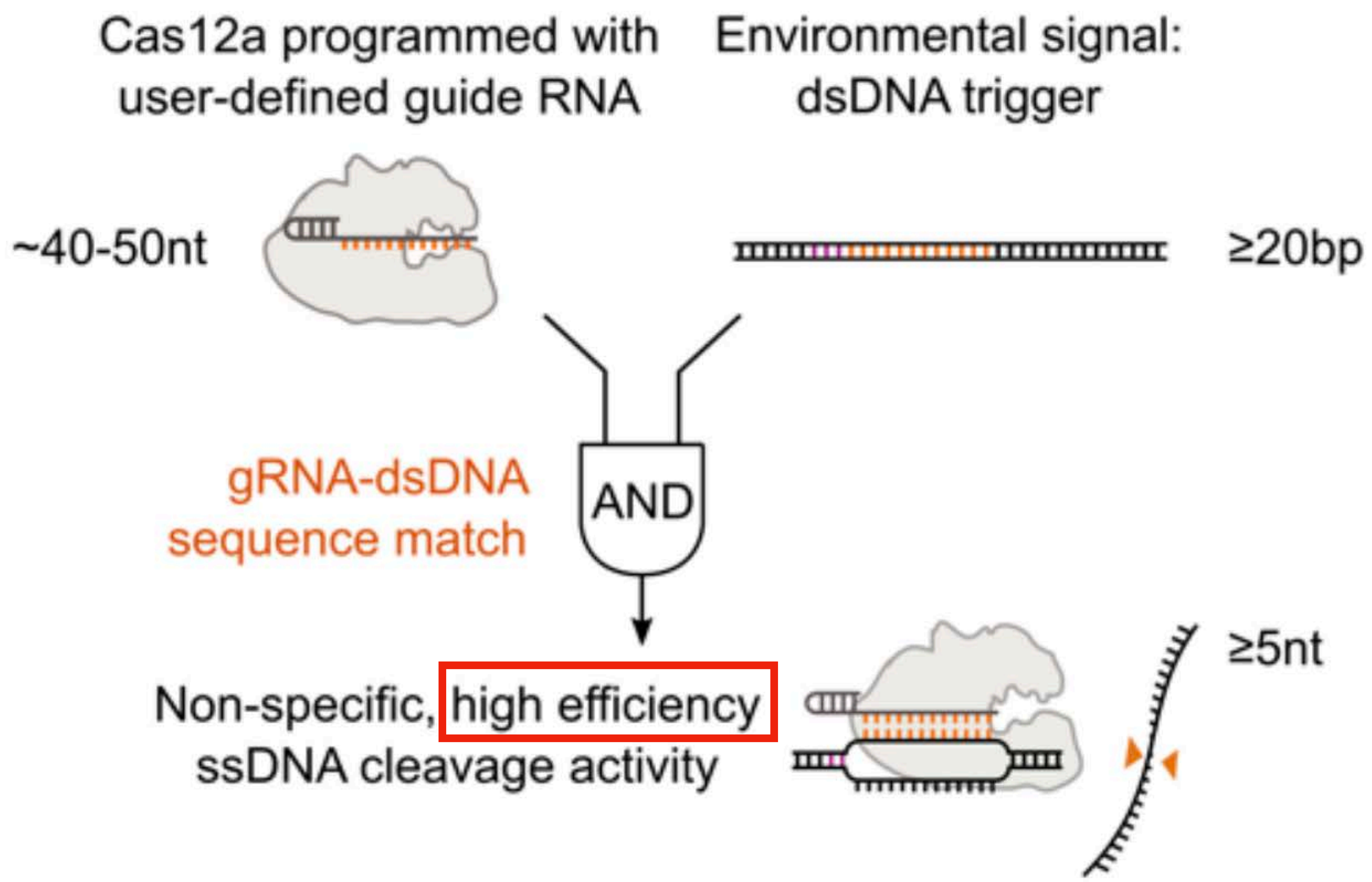
Synthetic biology enhances biomaterial production

How about using synthetic biology to create biomaterials with novel capabilities (e.g. responsive)?

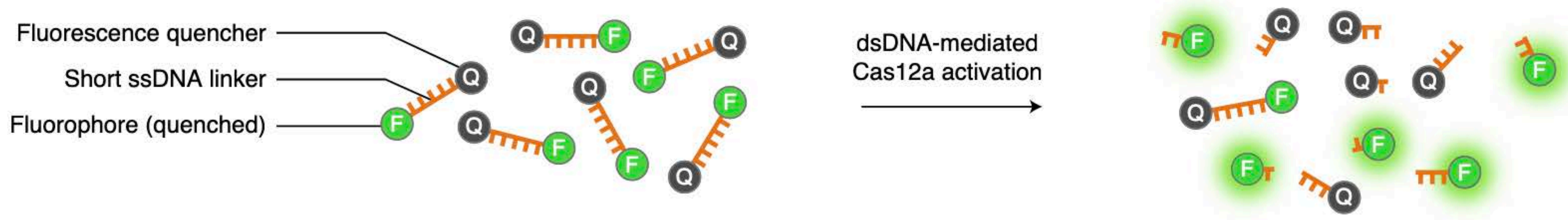
Repurposing CRISPR for material design

when the genetic part becomes the material designer



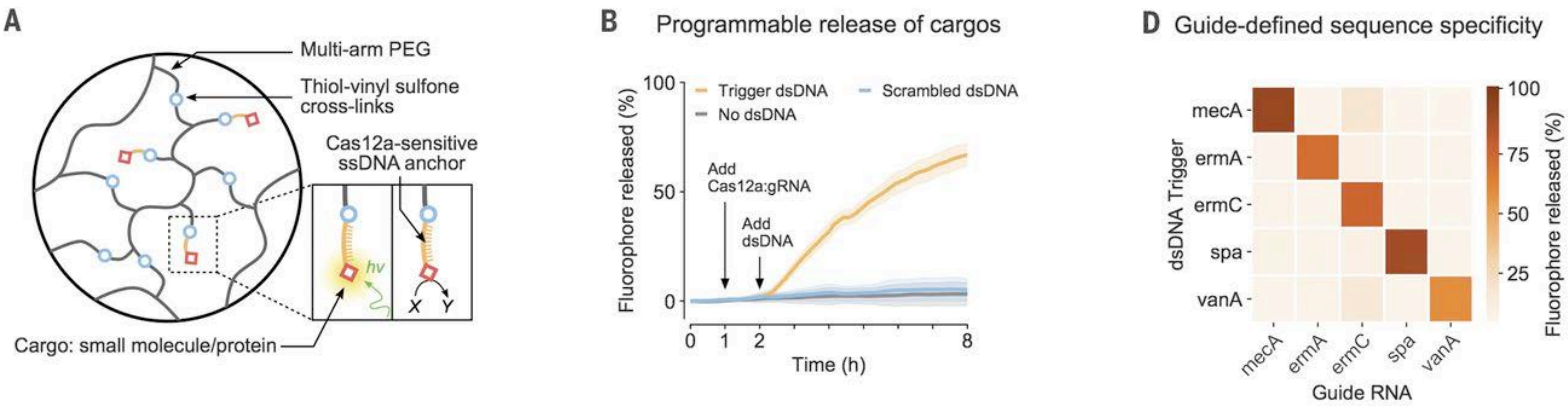


Example: biosensor for dsDNA detection

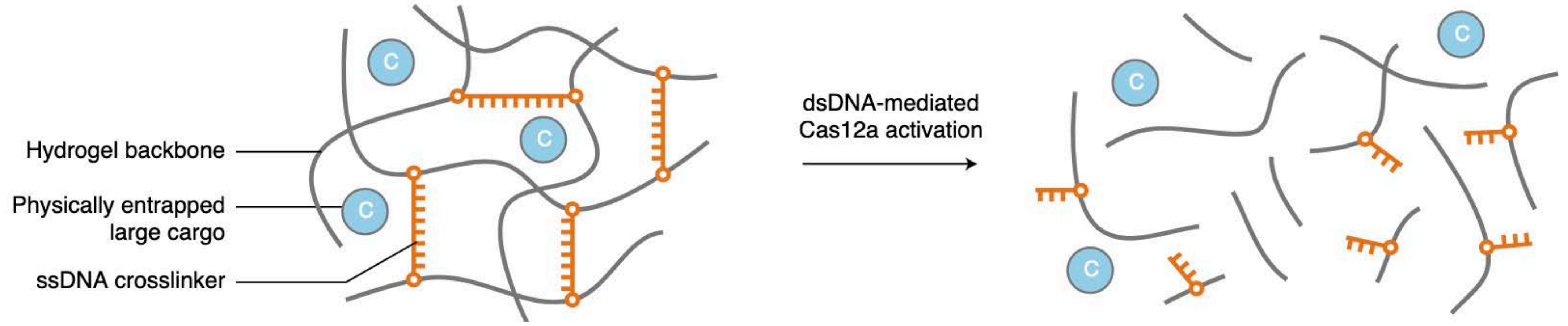


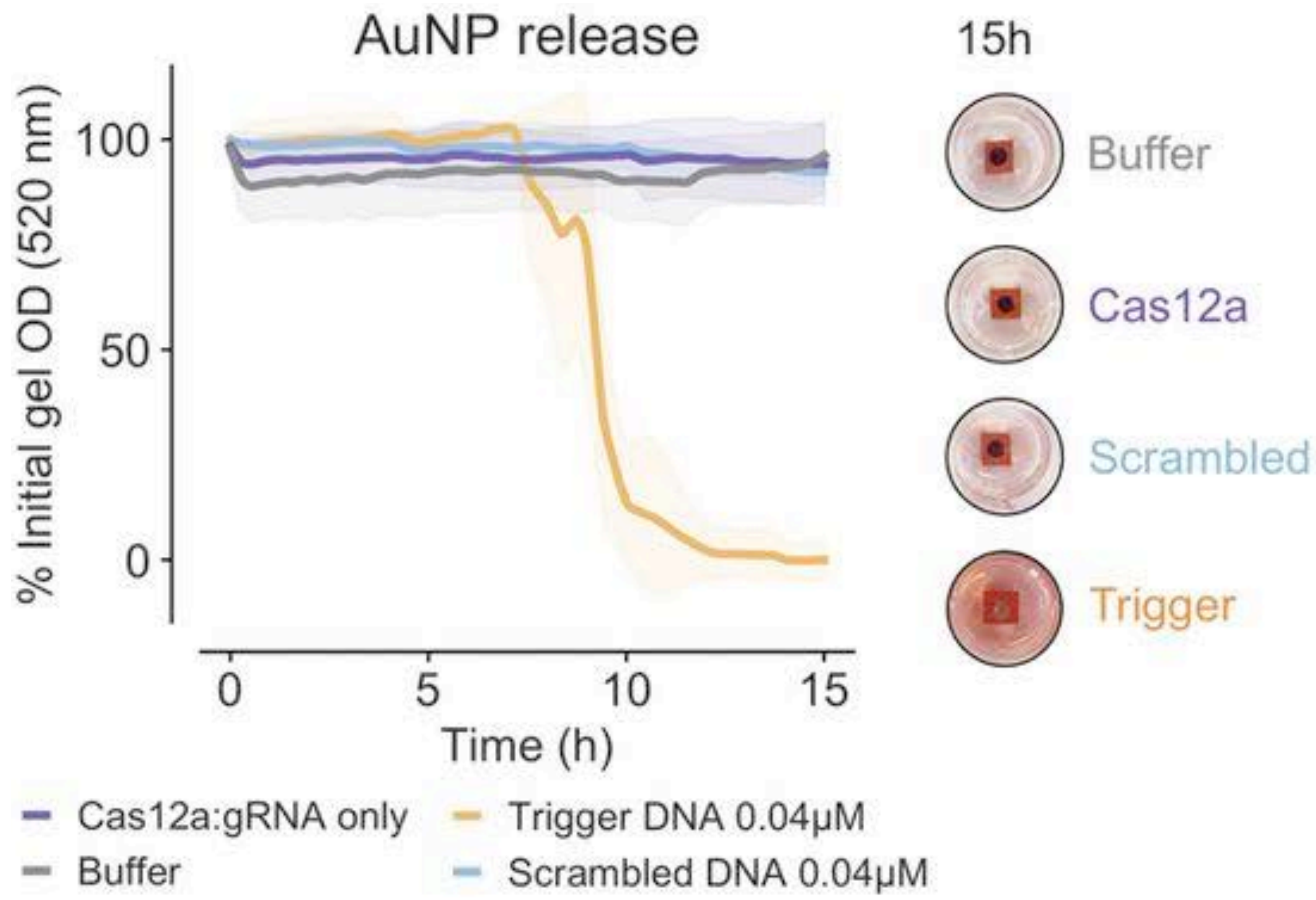
CRISPR-responsive hydrogel with molecular release

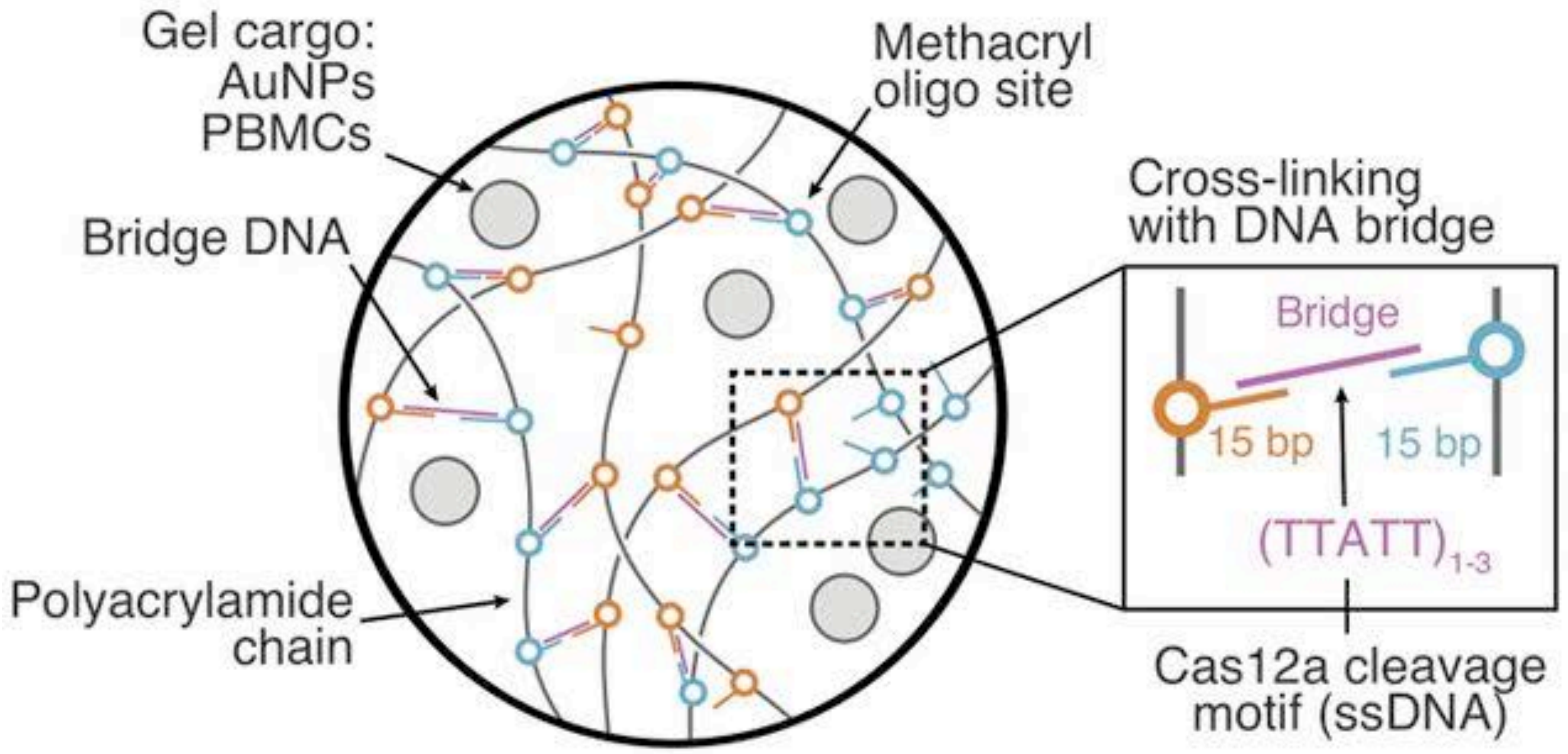
dsDNA input example: gene specific to a virus or bacterium

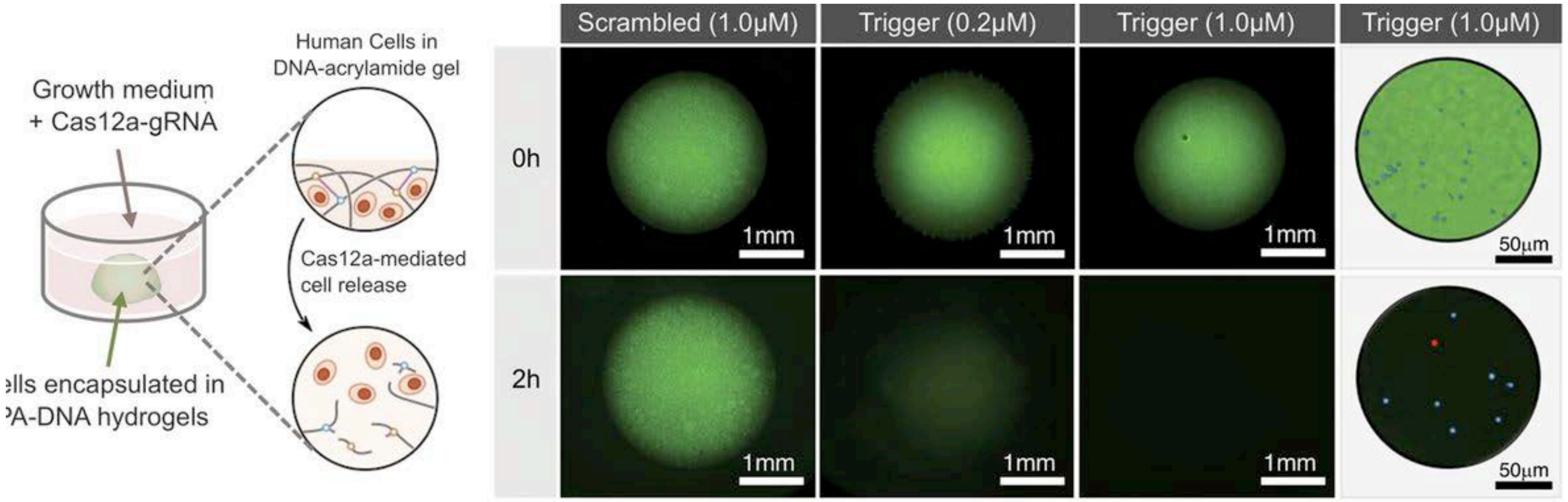


Releasing large cargo





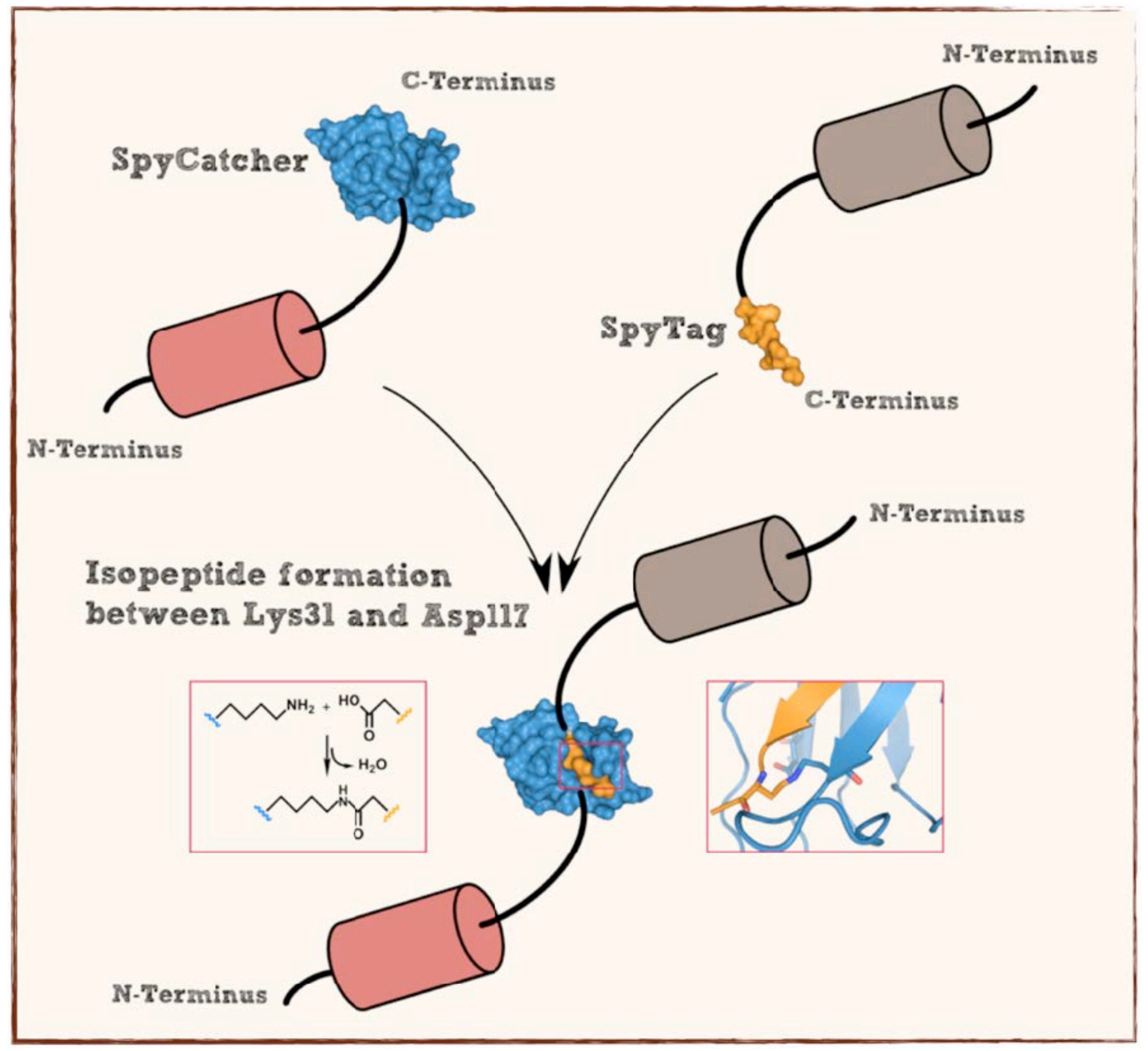


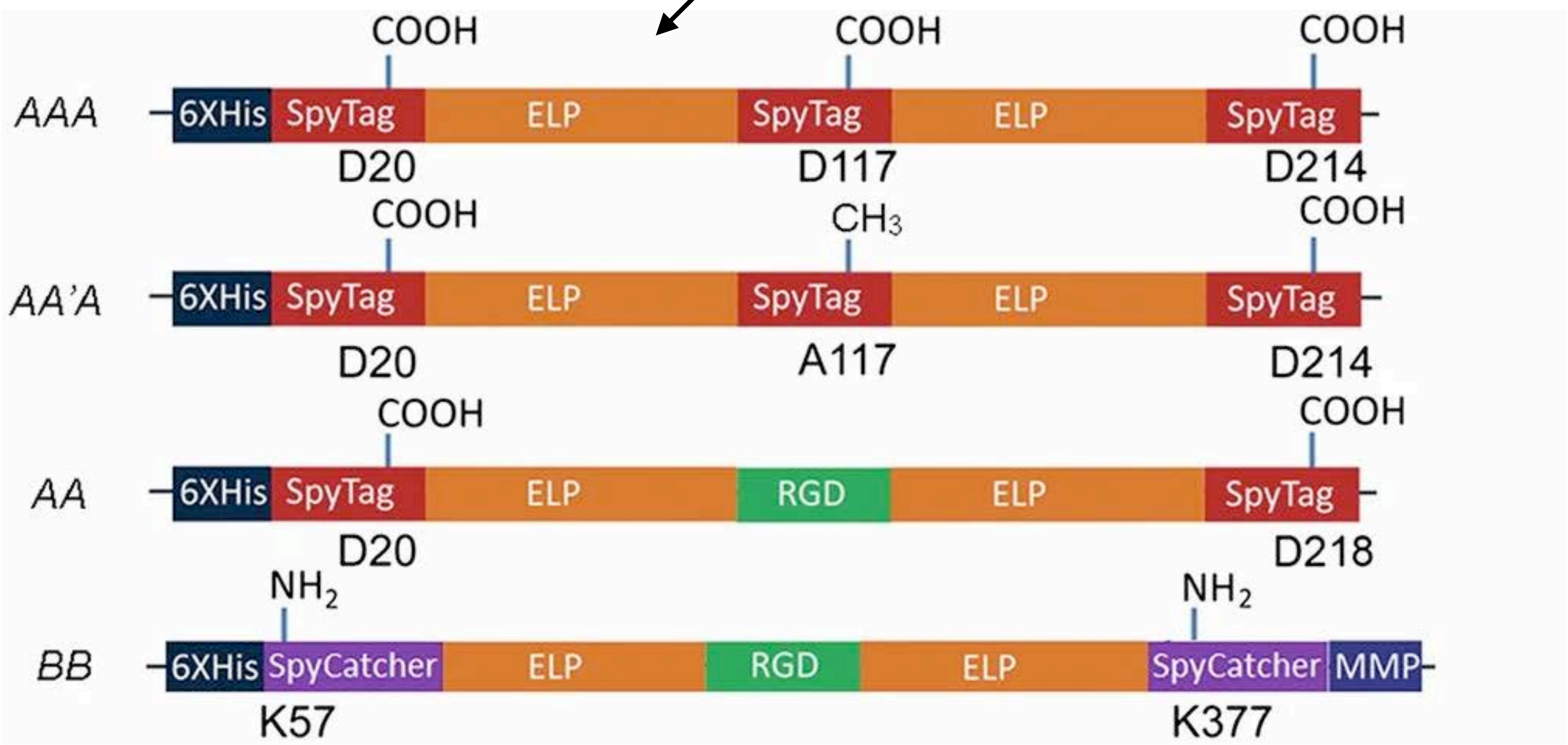


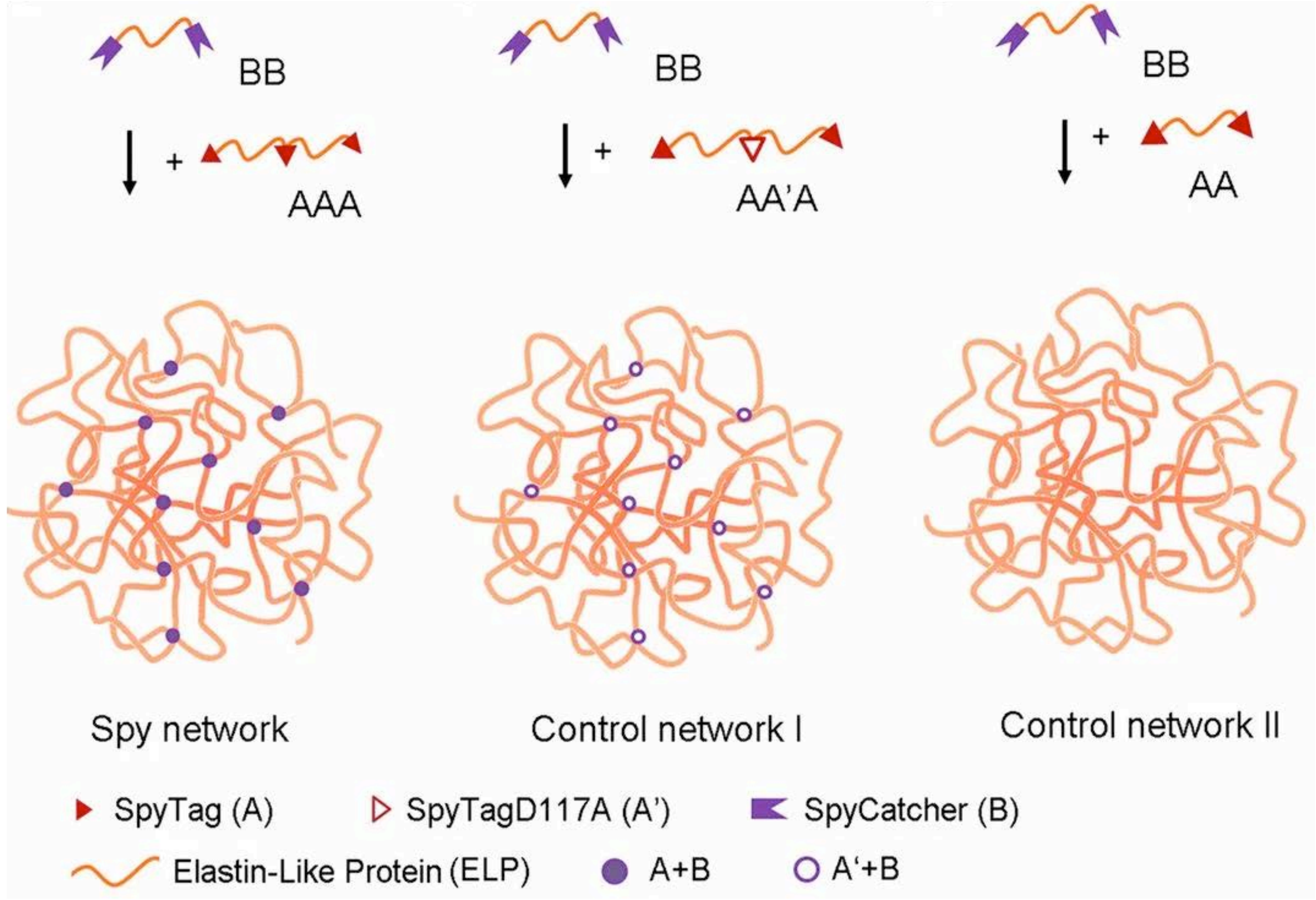
Engineering interactions

Make synthetic polymer hybrids

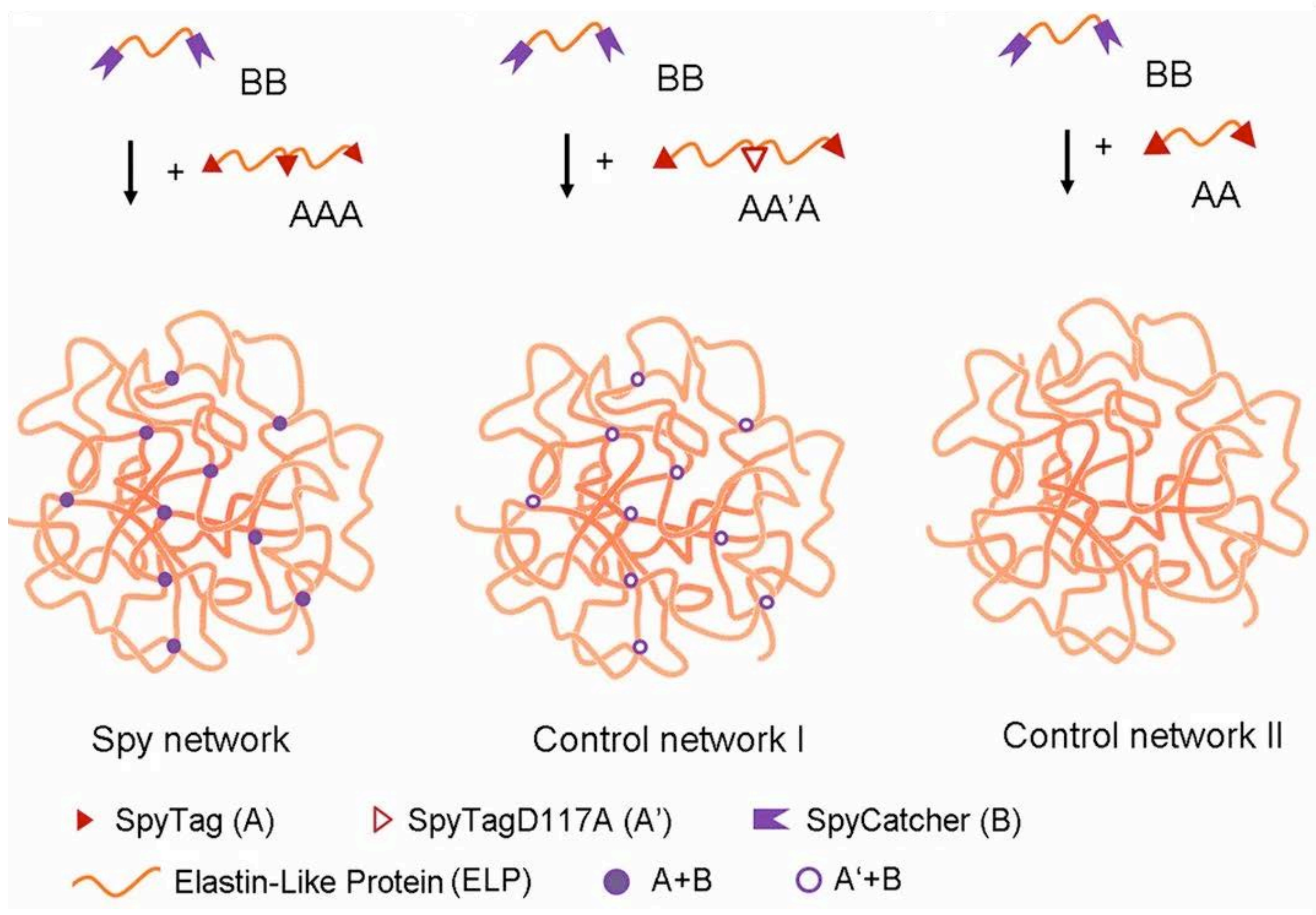
Elastin-like

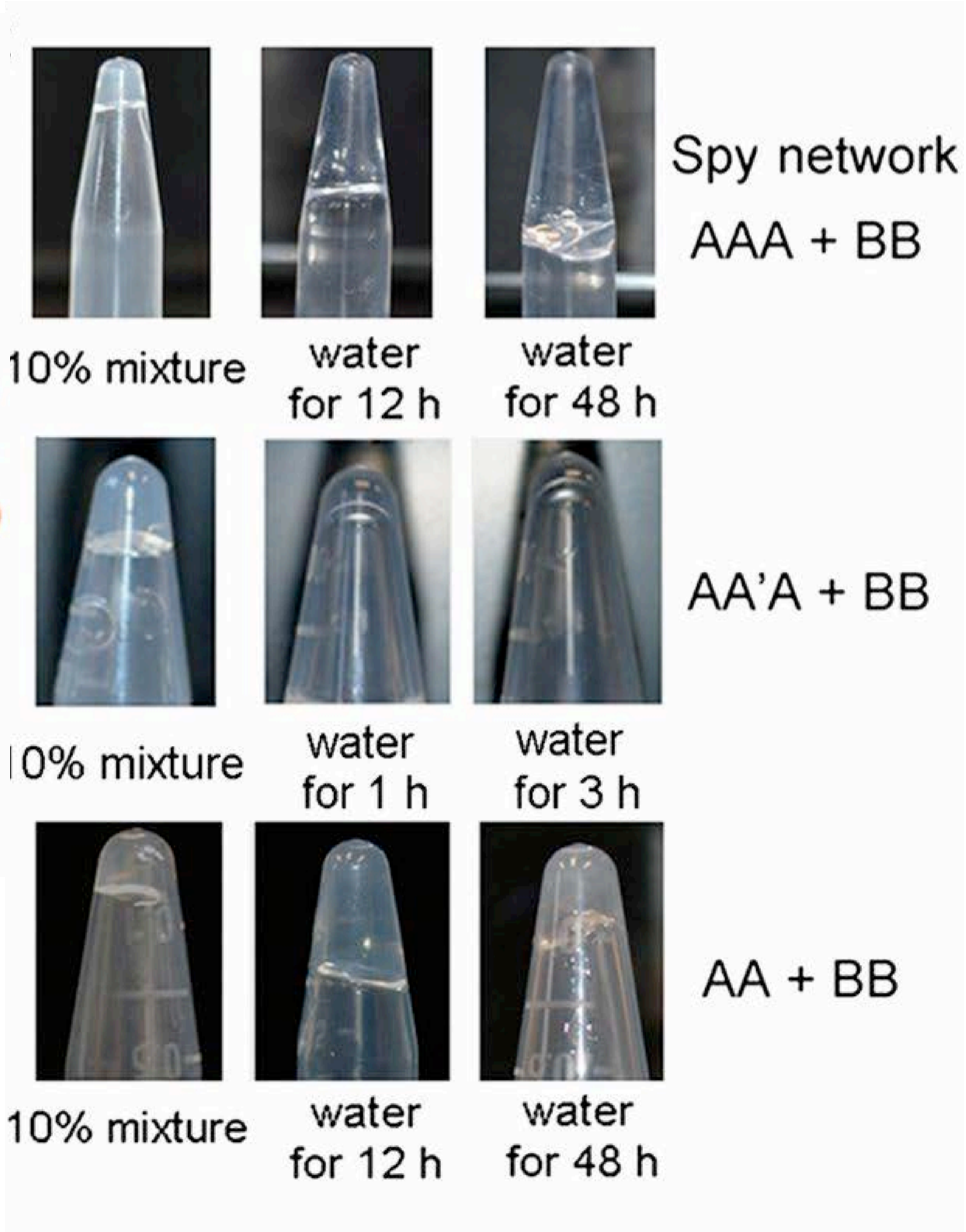


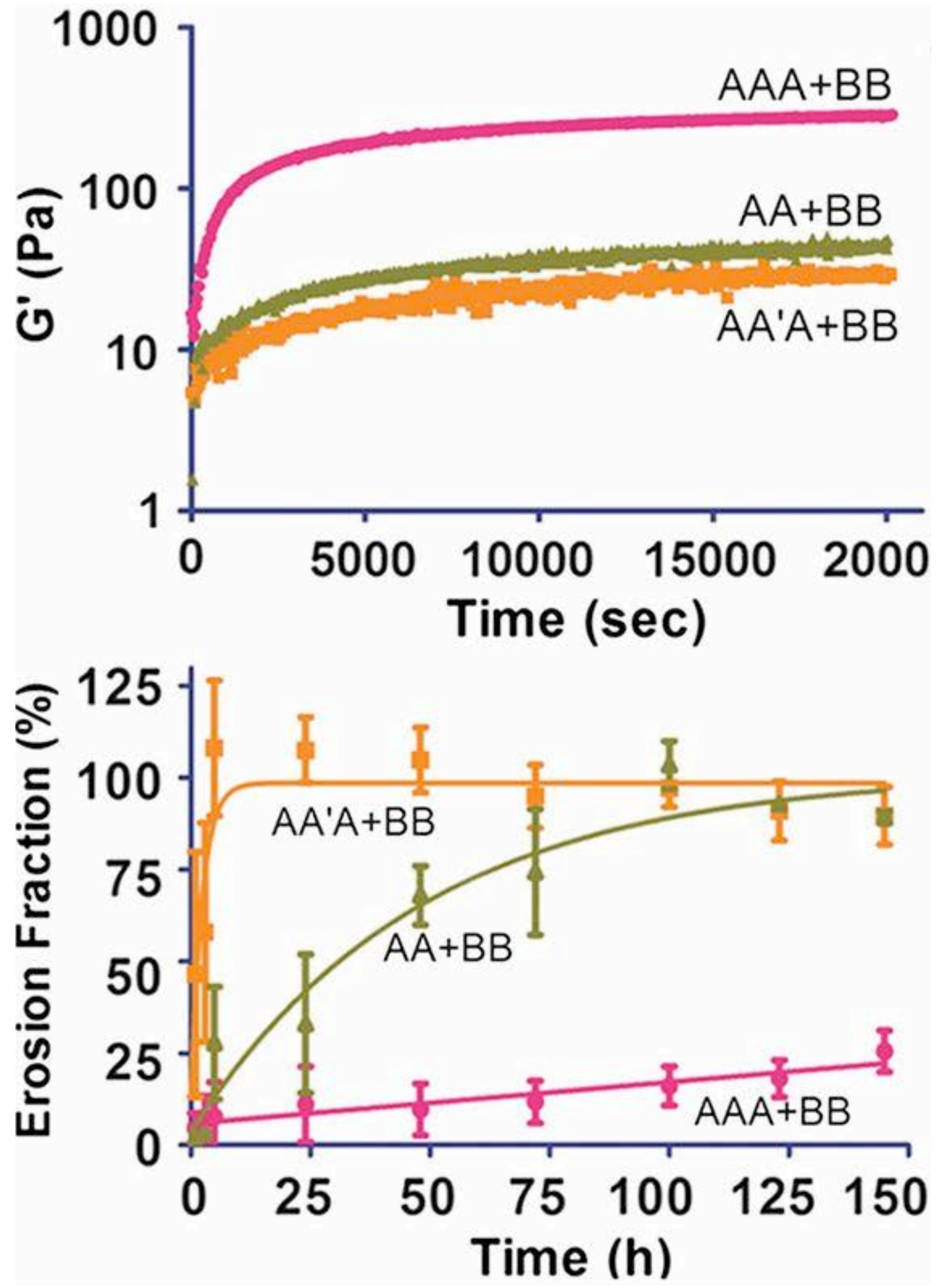




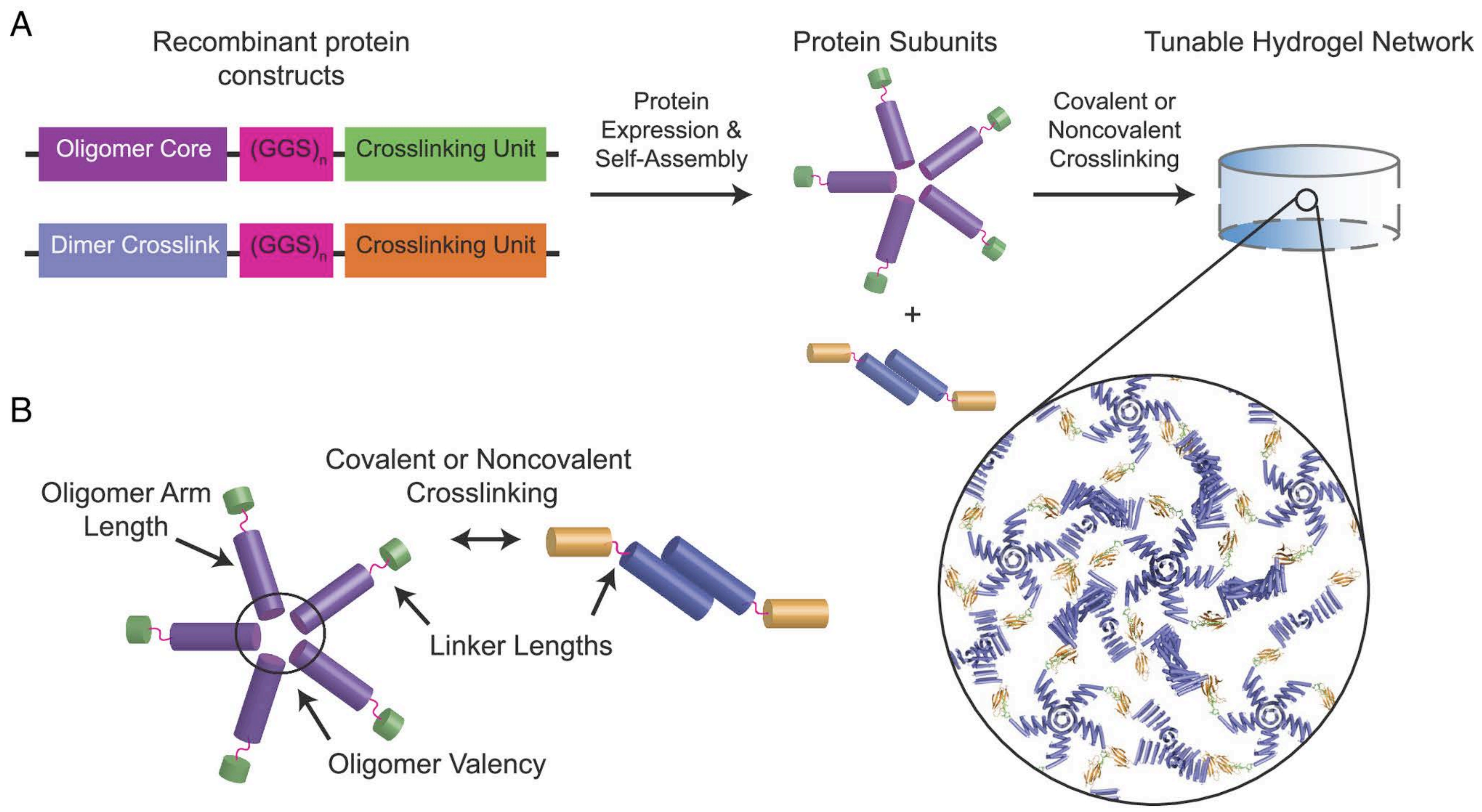
Hydrogel formation



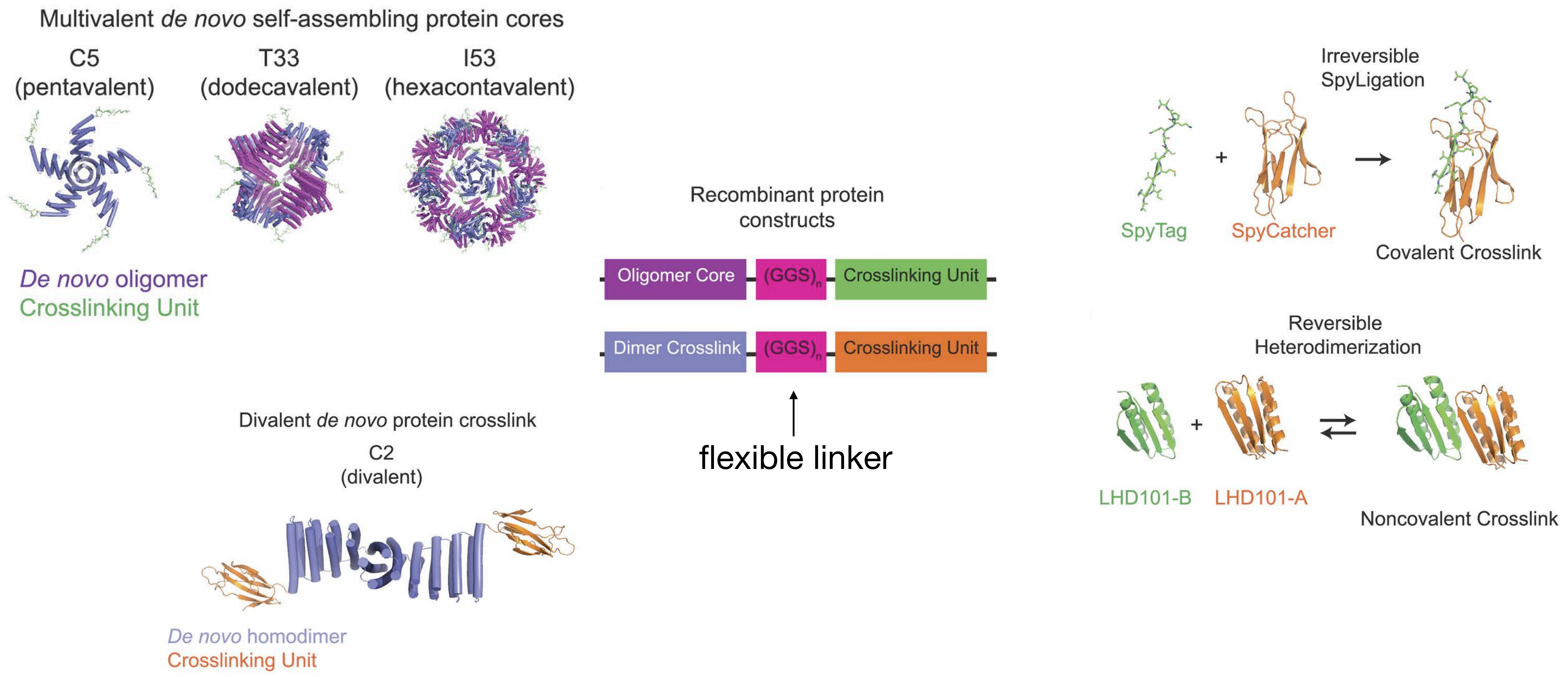




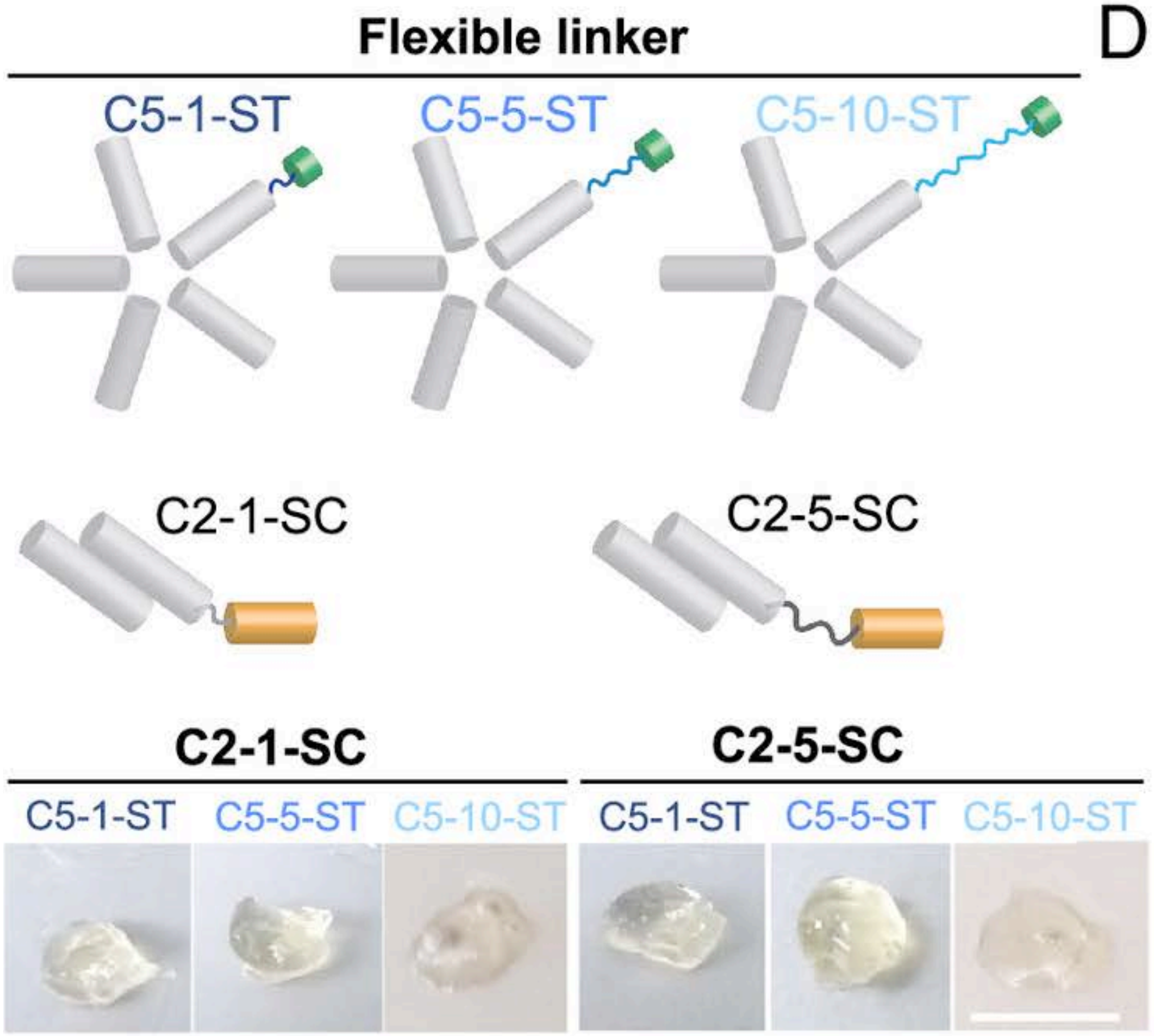
De novo design of protein hydrogels

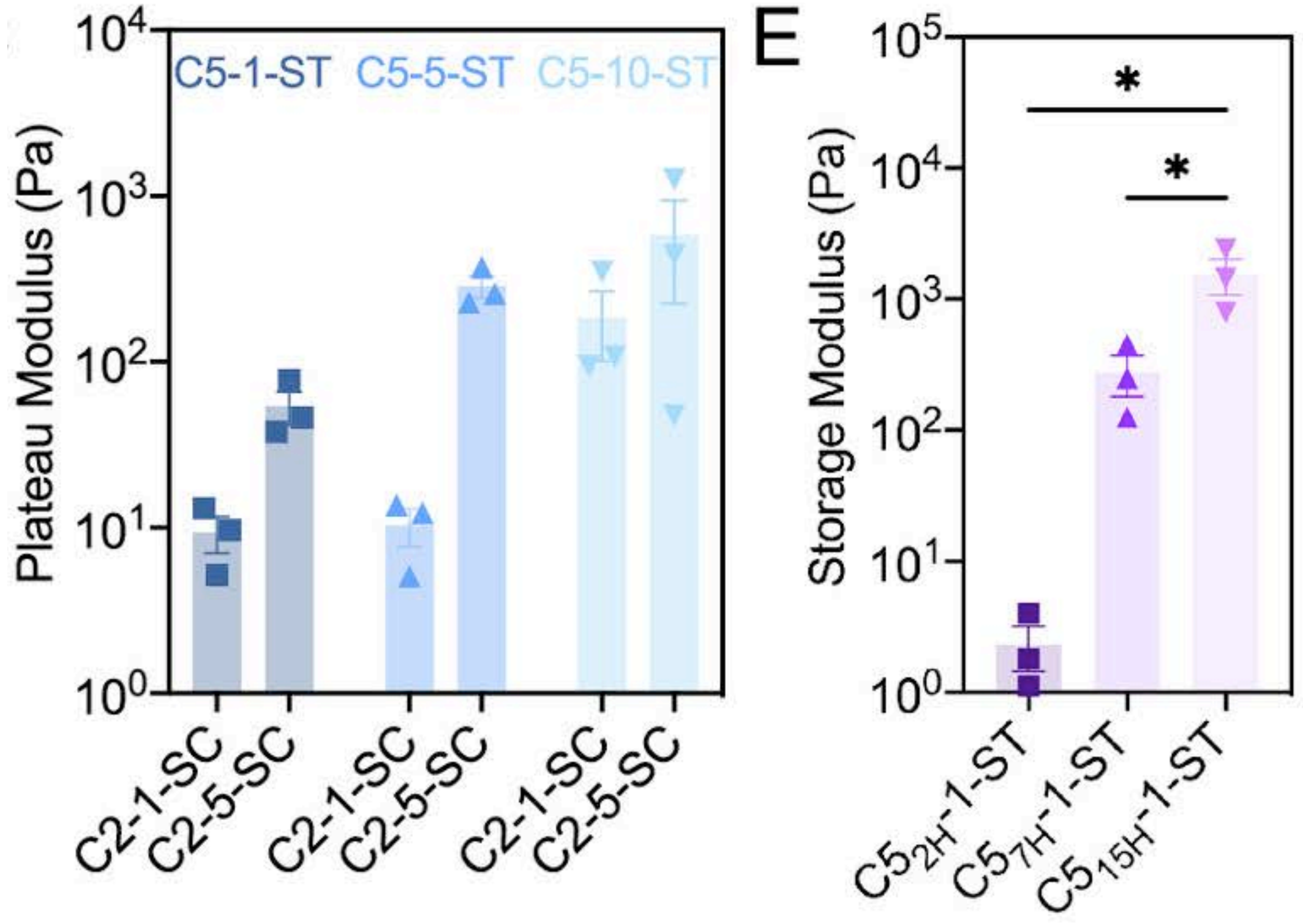


Protein designs for cross linking



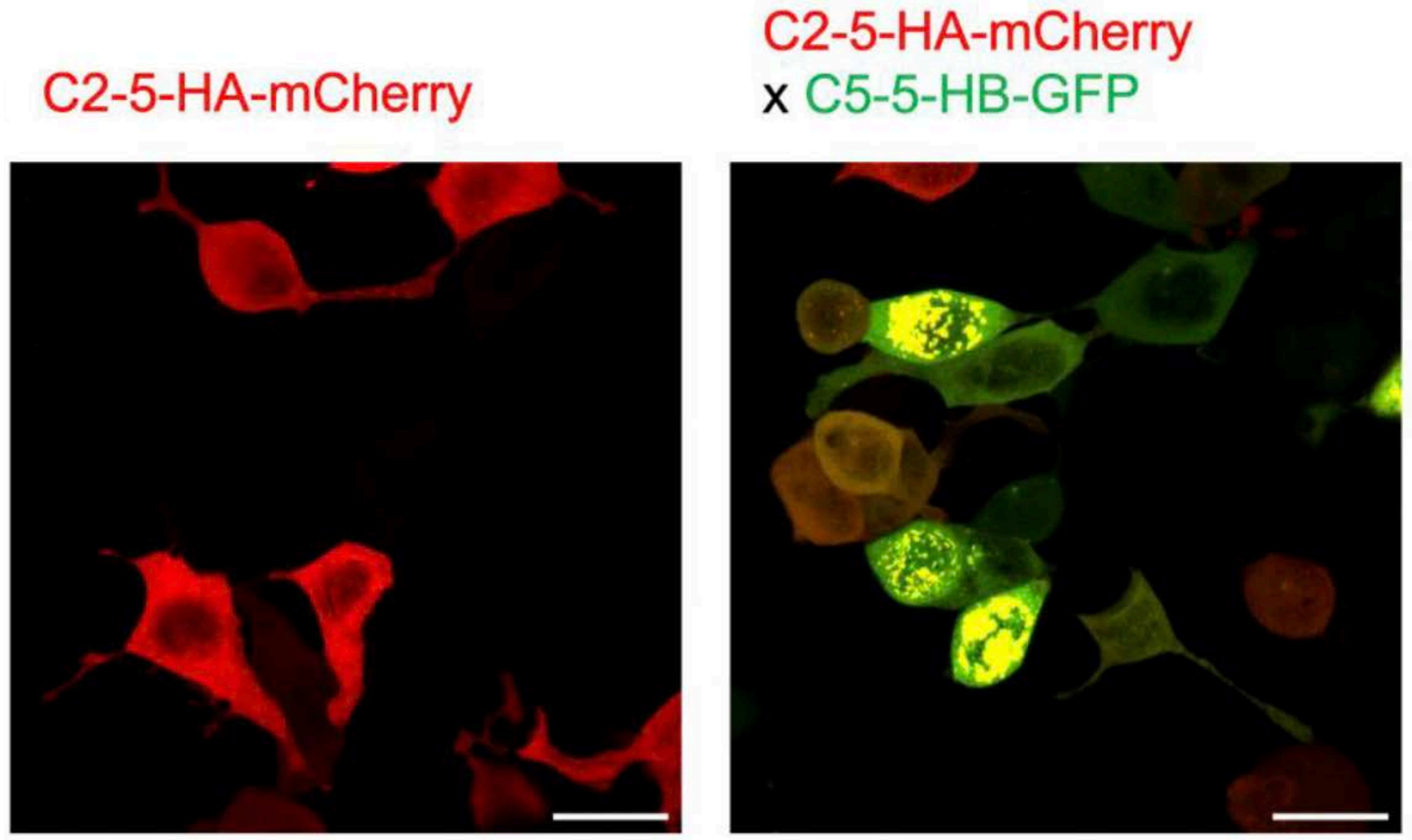
Controlling mechanics with linker length

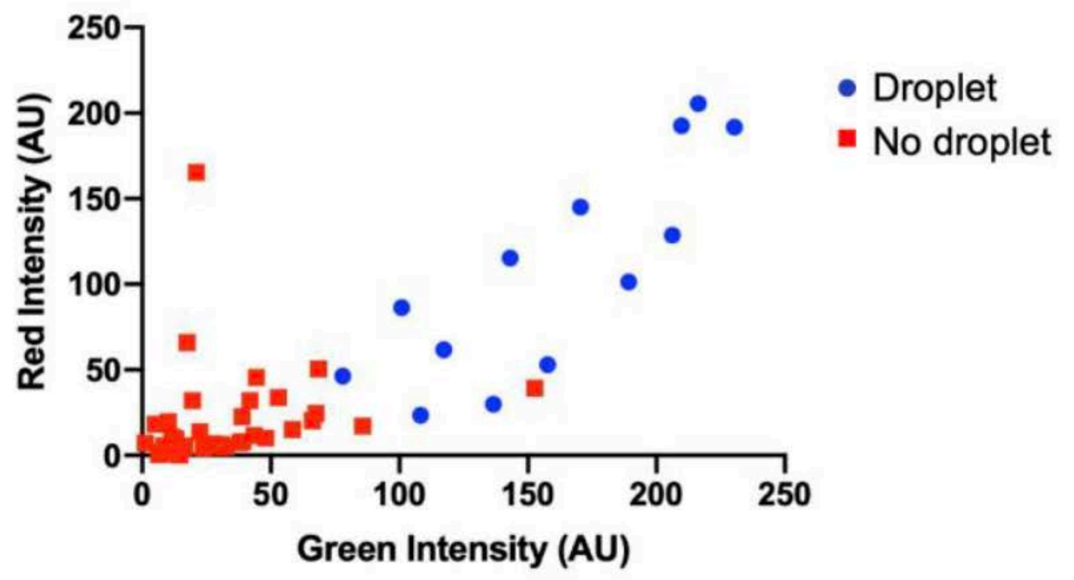




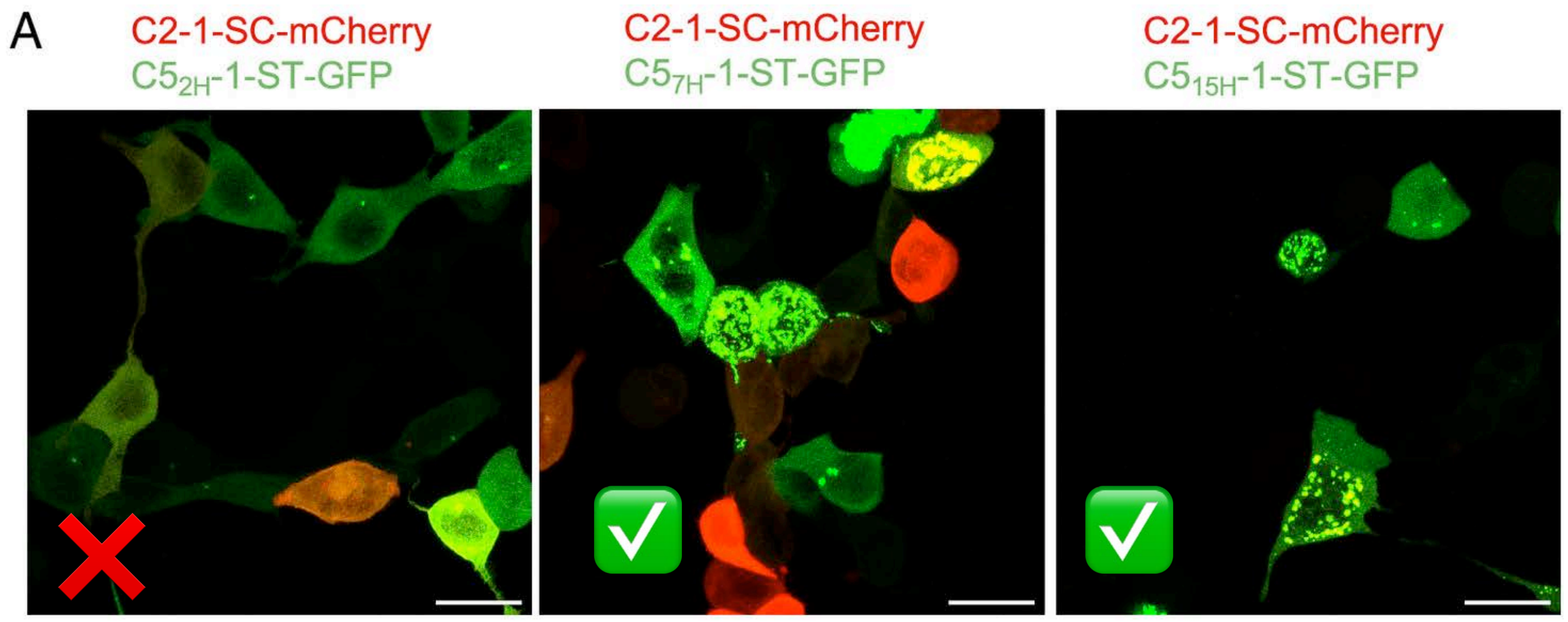
De novo hydrogel formation within cells

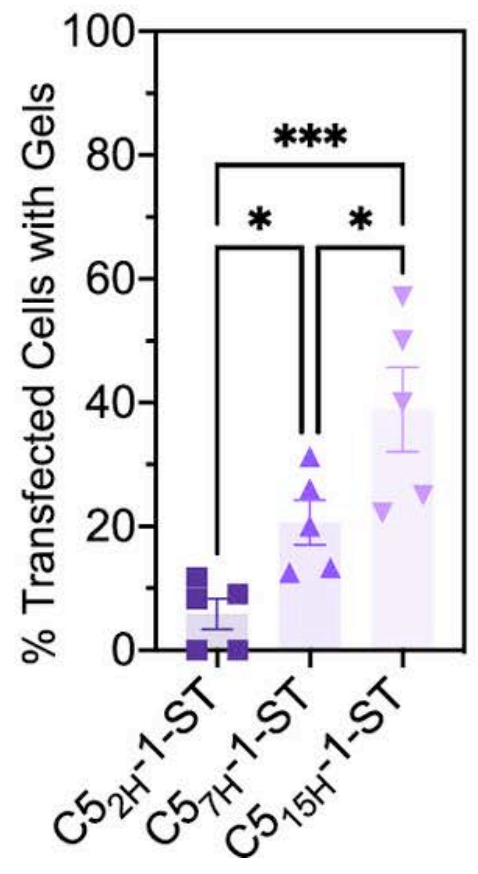
flexible linker





rigid linker





Why is this cool?

A relatively simple way to engineer hydrogels

1. order genes
2. produce proteins recombinantly (*E. coli*)
3. purify
4. crosslink!

>C2-GGS-SpyCatcher (C2-1-SC)

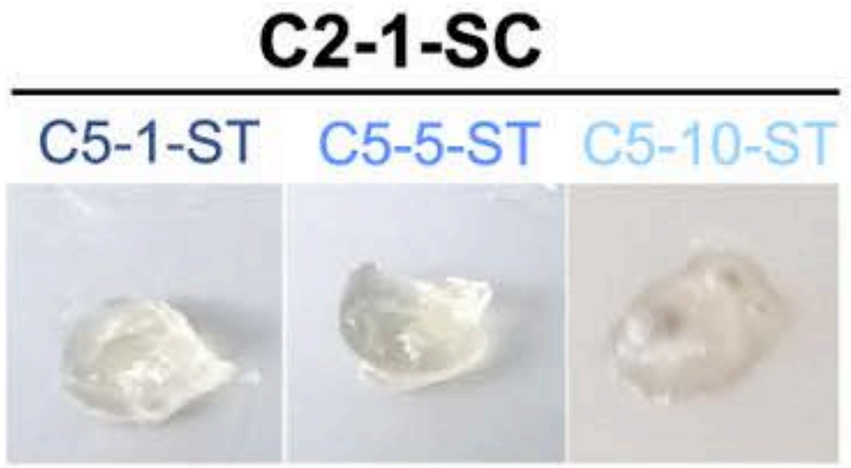
MGTREEIIRELARSLAEQAELTARLERLLRELERLQREGSSDEDVRELLREIKELVREILKLIAEQILLI
AELLLAAIRSEAAELALRAIREAIELCKRSTDEELCQLLLRLALLLMELALLYPDSEAAKLALKAALEAI
ELCKQSTDEELCEELVKLAQKLIELAKRYPDSEAAKLALKAALEAIELCKQSTDEELCEELVKLAQKLIE
LAKRYPDSEEAKRALKEAKELIEQCKESTDEDECRELVKRAEELIREAKEGGSDSATHIKFSKRDIDGKE
LAGATMELRDSSGKTISTWISDGQVKDFYLMPGKYTFVETAAPDGYEIATAITFTVNEQGQVTVNGKATK
GGSWGLEHHHHHH

>C2-(GGS)5-SpyCatcher (C2-5-SC)

MGTREEIIRELARSLAEQAELTARLERLLRELERLQREGSSDEDVRELLREIKELVREILKLIAEQILLI
AELLLAAIRSEAAELALRAIREAIELCKRSTDEELCQLLLRLALLLMELALLYPDSEAAKLALKAALEAI
ELCKQSTDEELCEELVKLAQKLIELAKRYPDSEAAKLALKAALEAIELCKQSTDEELCEELVKLAQKLIE
LAKRYPDSEEAKRALKEAKELIEQCKESTDEDECRELVKRAEELIREAKEGGSGGSGGSGGSGGSDSATH
IKFSKRDIDGKELAGATMELRDSSGKTISTWISDGQVKDFYLMPGKYTFVETAAPDGYEIATAITFTVNE
QGQVTVNGKATKGGSWGLEHHHHHH

>C5-(GGS)1-SpyTag (C5-1-ST)

MGHHHHHHGWSGAHIVMVDAYKPTKGGSNDEKEKLKELLKRAEELAKSPDPEDLKEAVRLAEEVVRERPG
SNLAKKALEIILRAAEELAKLPDPEALKEAVKAAEKVVREQPGSNLAKKAQEIILRAAEELAKLEDEEAL
KEAIKAAEKVIELEPGSELAKEAKRIIEKAAKMLADILRKEMEKIREETEEVKKEIEESKKRPQSESAKN
LILIMQLLINQIRLLALQIRMLVLQLIL



DIY and at home

An automated home-built low-cost fermenter suitable for large-scale bacterial expression of proteins in Escherichia coli

Uwe Riek [1], Roland Tuerk, Theo Wallimann, Uwe Schlattner, Dietbert Neumann

Affiliations + expand

PMID: 18687068 DOI: 10.2144/000112830
Free article

Taking novel biomaterials to the real world

need to scale up!

BIG FERMENTERS

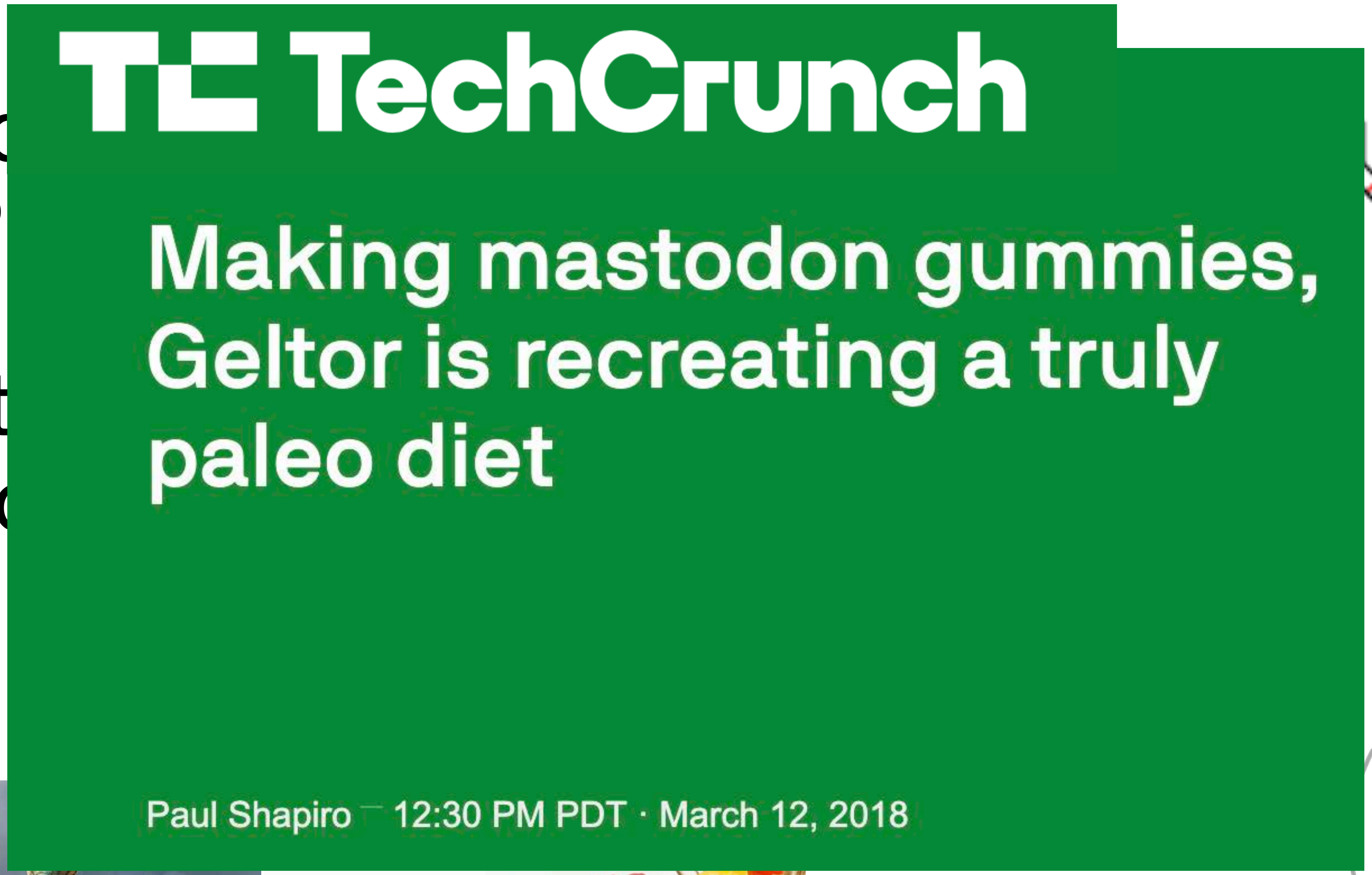
Innovation story

The impossible gummy bear

Nik Ouzounov

Me: what c
after the P

Nik: I want
bears out c



TechCrunch

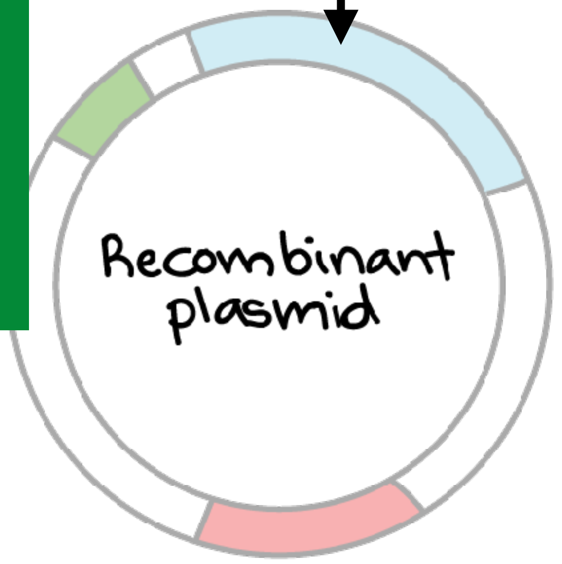
Making mastodon gummies, Geltor is recreating a truly paleo diet

Paul Shapiro 12:30 PM PDT · March 12, 2018

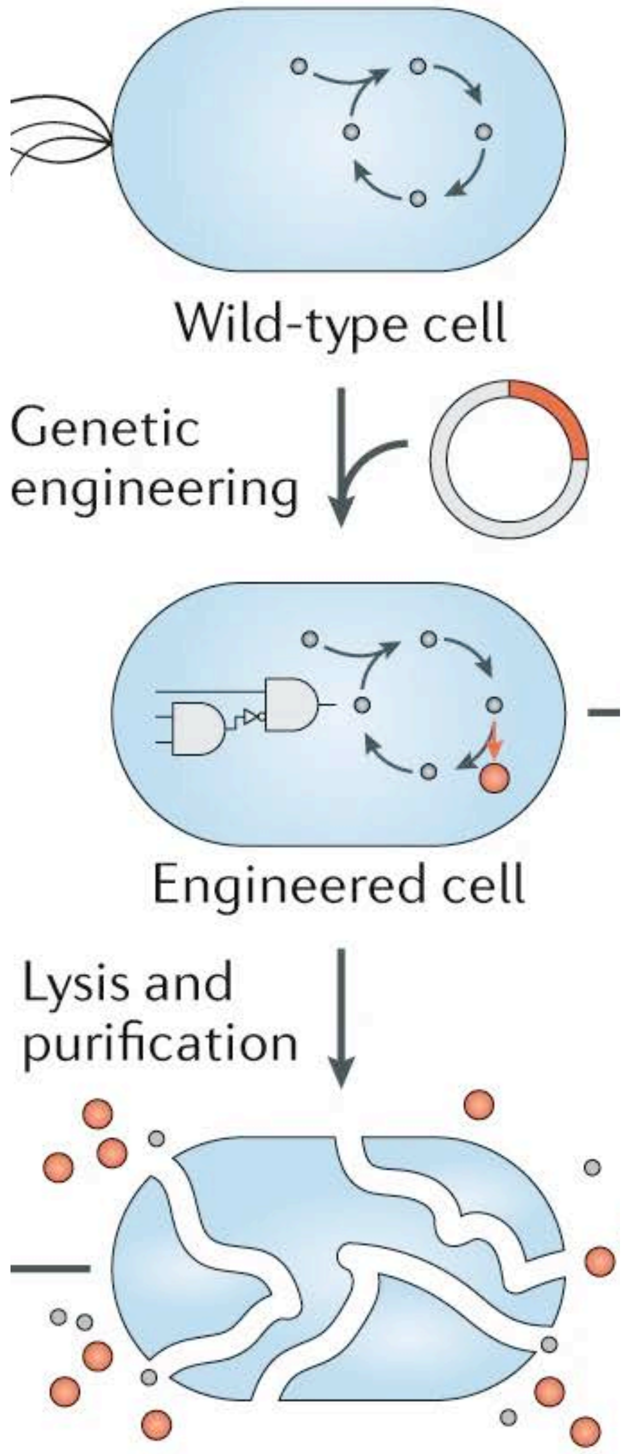
Alex Lorestani

INDIE BIO

Mammoth
Col gene

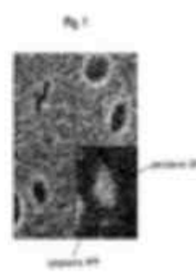


Recombinant collagen: requires synthetic biology



Something special must happen at this step

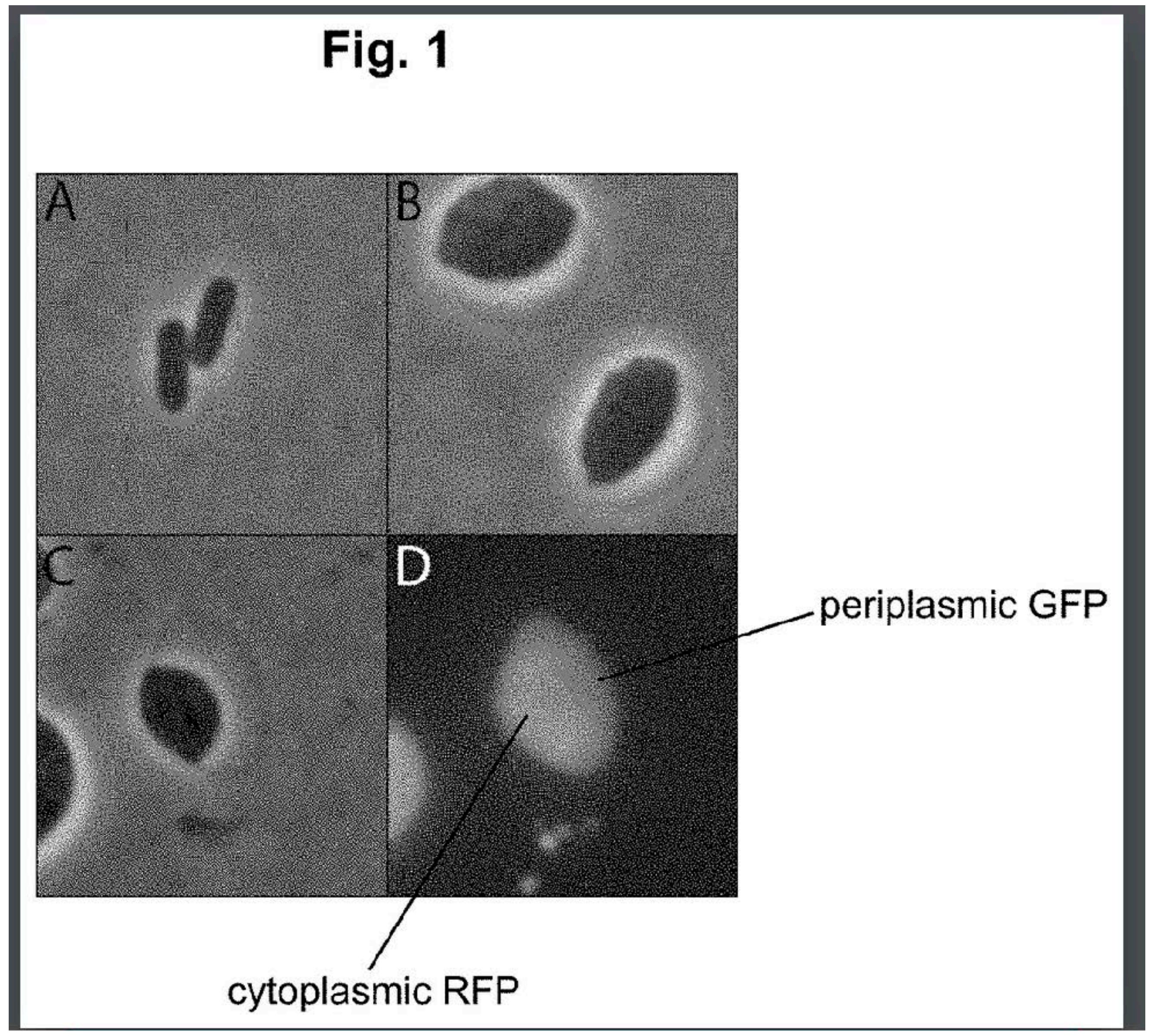
Recombinant collagen and elastin molecules and uses thereof



WO EP US CN JP ~~KR~~ *BR* CA ~~GB~~ *IL SG* • US11180541B2 • Alexander **Lorestani** • Geltor, Inc.

Priority 2017-09-28 • Filed 2018-09-27 • Granted 2021-11-23 • Published 2021-11-23

This disclosure provides non-naturally occurring collagen and elastin molecules. The non-naturally occurring collagens and elastins include truncated collagens, truncated elastins, as well as fusion proteins thereof. The non-naturally occurring collagen and elastin are useful in foods, cosmetics ...



From impossible gummy bears to cosmetics

vegconomist
– the vegan business magazine –

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Startups, Accelerators & Incubators

Start-Up Aims to Replace Animal Gelatine by 2020

The New York Times

Is Bio-Designed Collagen the Next Step in Animal Protein Replacement?

Forbes

BUSINESS > FOOD & DRINK

Geltor Raises $91.3 Million Series B Funding To Accelerate Development Of Animal-Free Ingredients

CNBC | MARKETS | BUSINESS | INVESTING | TECH | POLITICS | VIDEO | INVESTING CLUB | PRO | LIVESTREAM

CNBC UPSTART 100

Lab-grown gelatin is the fake the future, one start-up belie

BUSINESS INSIDER

DOW JONES -0.2% | NASDAQ +1.13% | S&P 500 +0.49% | AAPL -0.75% | NVDA -0.9% | MSFT -0.44% | AM

TECH

See the pitch deck that landed an alternative protein startup $91 million in funding on the promise it could create a pandemic-proof supply chain for ingredients like collagen

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06.23.2021

Episode 185: Alex Lorestani, Co-Founder and CEO of Geltor

When the cell becomes the biomaterial

see you at lecture 6

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