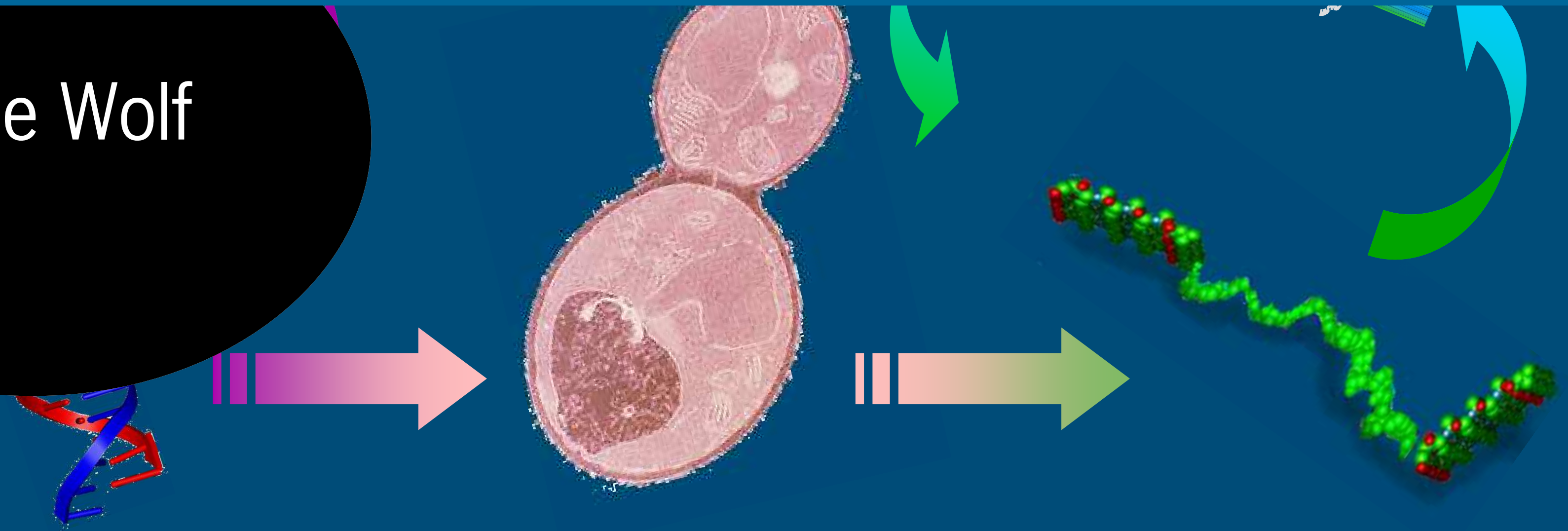
Digital polyelectrolytes

A swindlers pastime or a musical experience?

Frits de Wolf



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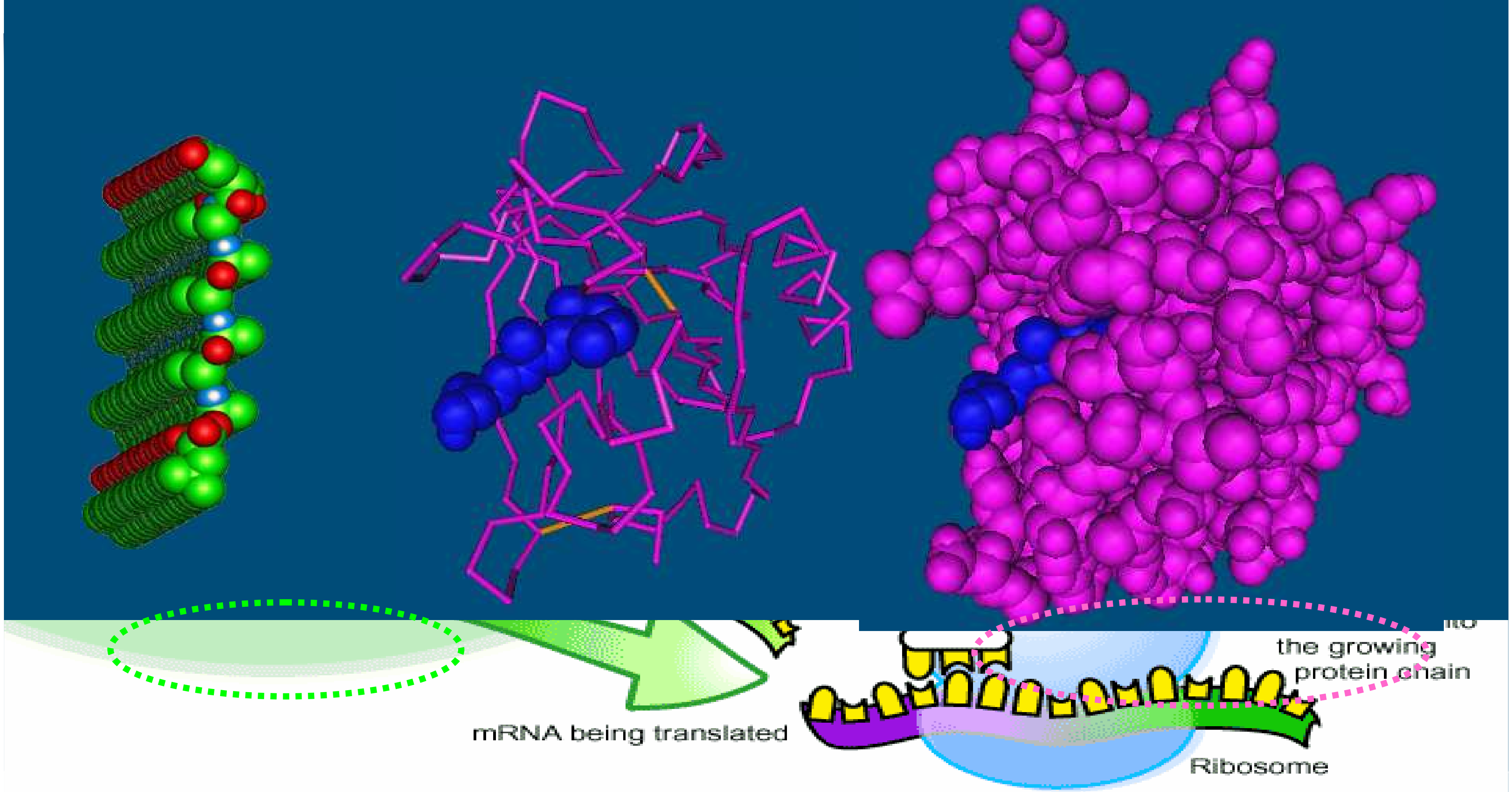
Traditional polymers

- Distribution of molecular weights & block lengths
- Random monomer sequence
- Usually not chiral
- Poor biocompatibility
- Environmental concerns (solvents, chemicals, bio degradability)

Living cells can make sequential polymers



Building blocks from nature

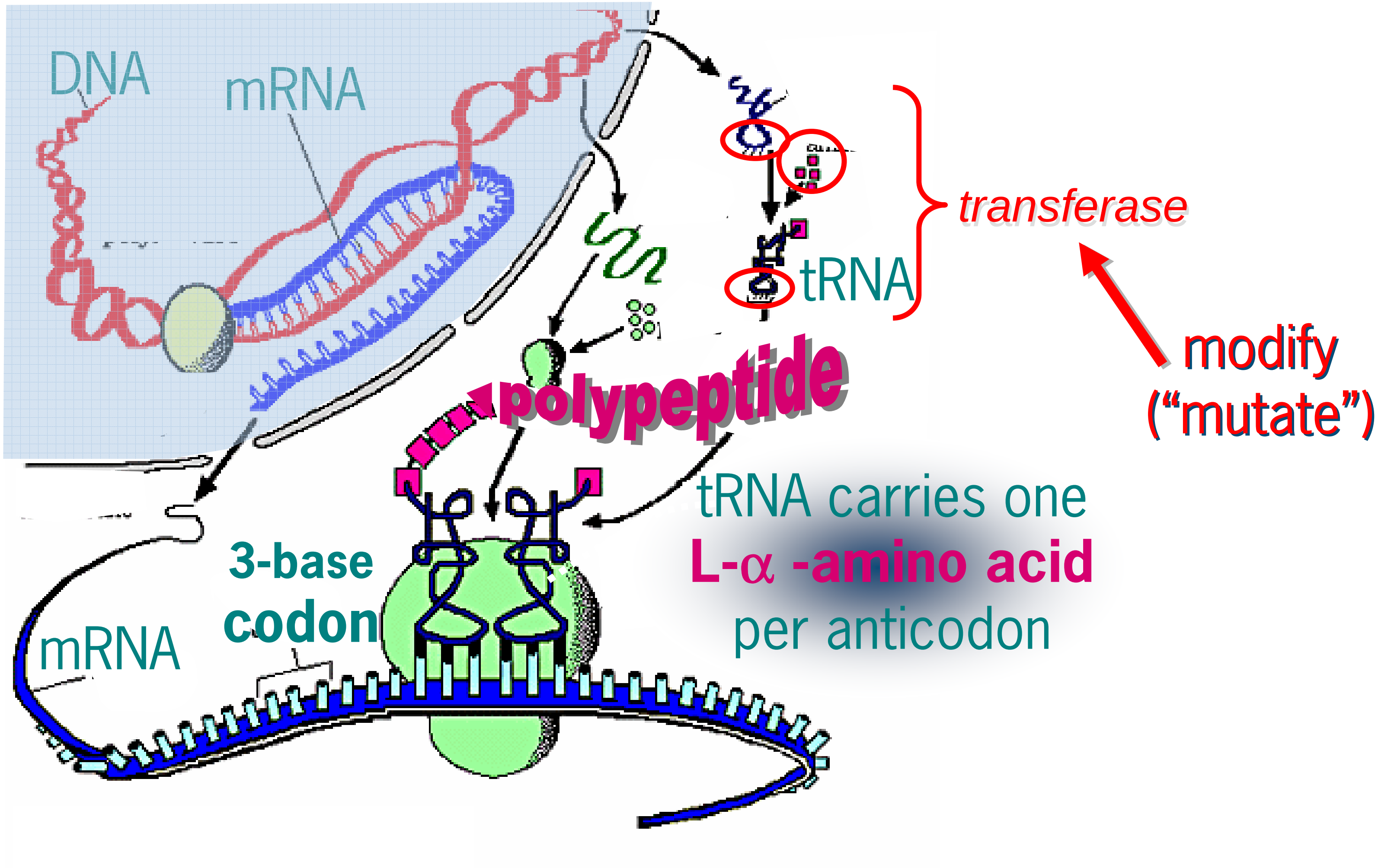
- collagen (gelatin = modified collagen)
- silk fibroin
- elastin
- keratin
- mussel adhesive proteins, fibronectin
- fibrin
- tubulin
- actin, myosin

Monomers for designer blocks

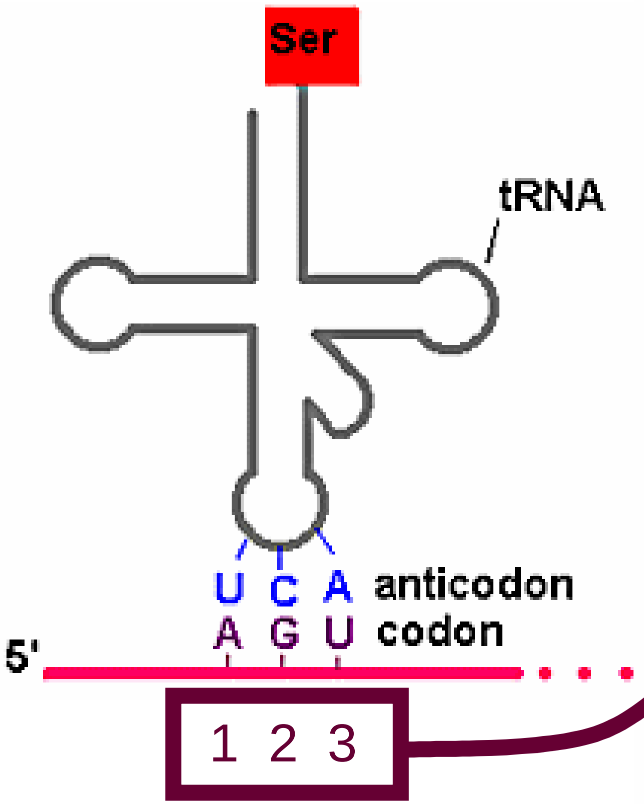
20 Different natural α-amino acids

- size
- polarity
- reactive side groups (–SH)
- hydrogen bond donors and acceptors
- conformational preference in a polypeptide
- aliphatic side chains (branched / not branched)
- aromatic side groups

Insertion of non-natural monomers



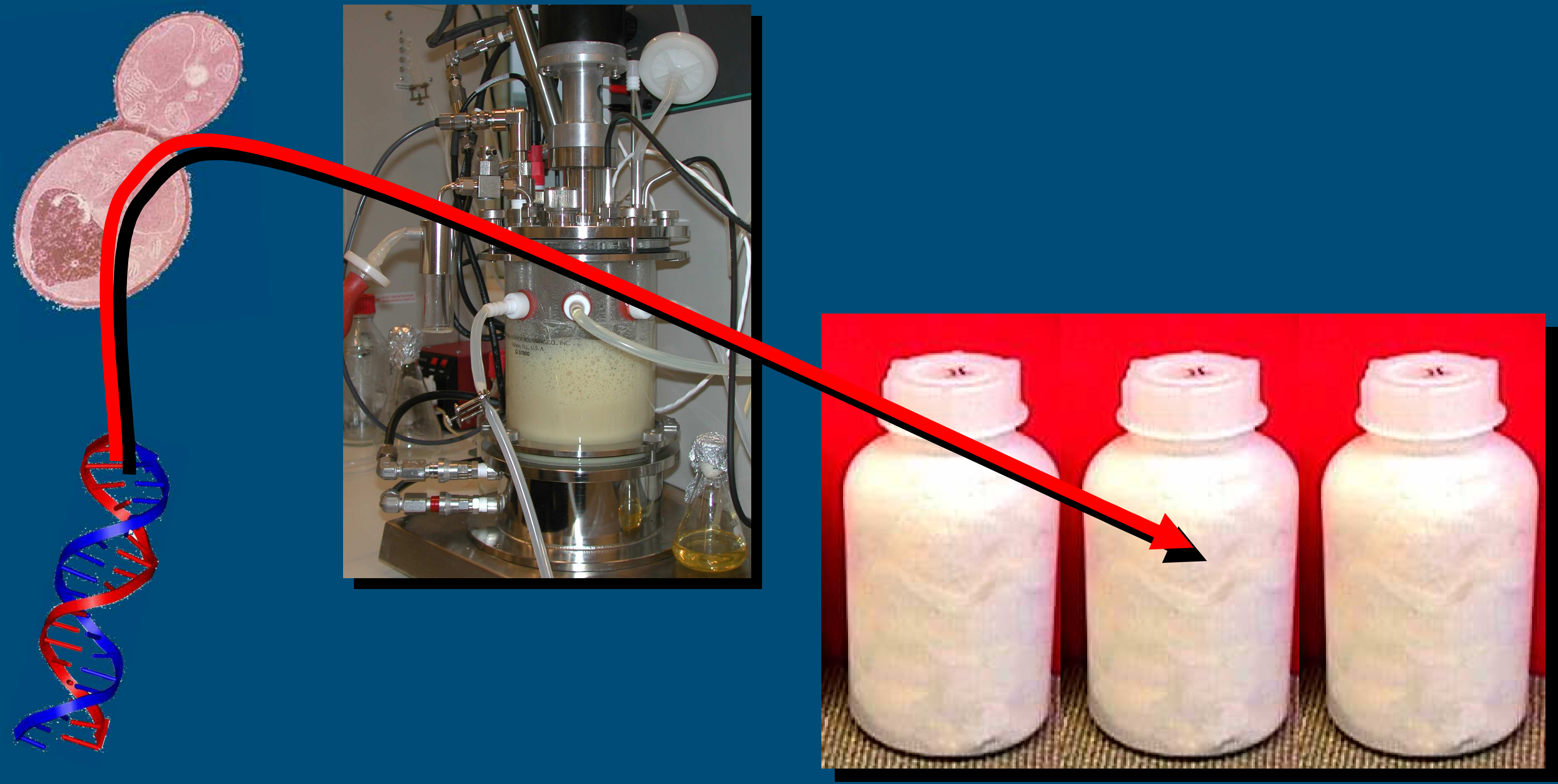
Insertion of non-natural monomers



2nd base in codon

1st base in codon	U	C	A	G	3rd base in codon
U	Phe	Ser	Tyr	Cys	U
U	Phe	Ser	Tyr	Cys	C
U	Leu	Ser	STOP	STOP	A
U	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
C	Leu	Pro	His	Arg	C
C	Leu	Pro	Gln	Arg	A
C	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
A	Ile	Thr	Asn	Ser	C
A	Ile	Thr	Lys	Arg	A
A	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
G	Val	Ala	Asp	Gly	C
G	Val	Ala	Glu	Gly	A
G	Val	Ala	Glu	Gly	G

Biosynthesis of protein polymers



Protein polymers: application areas

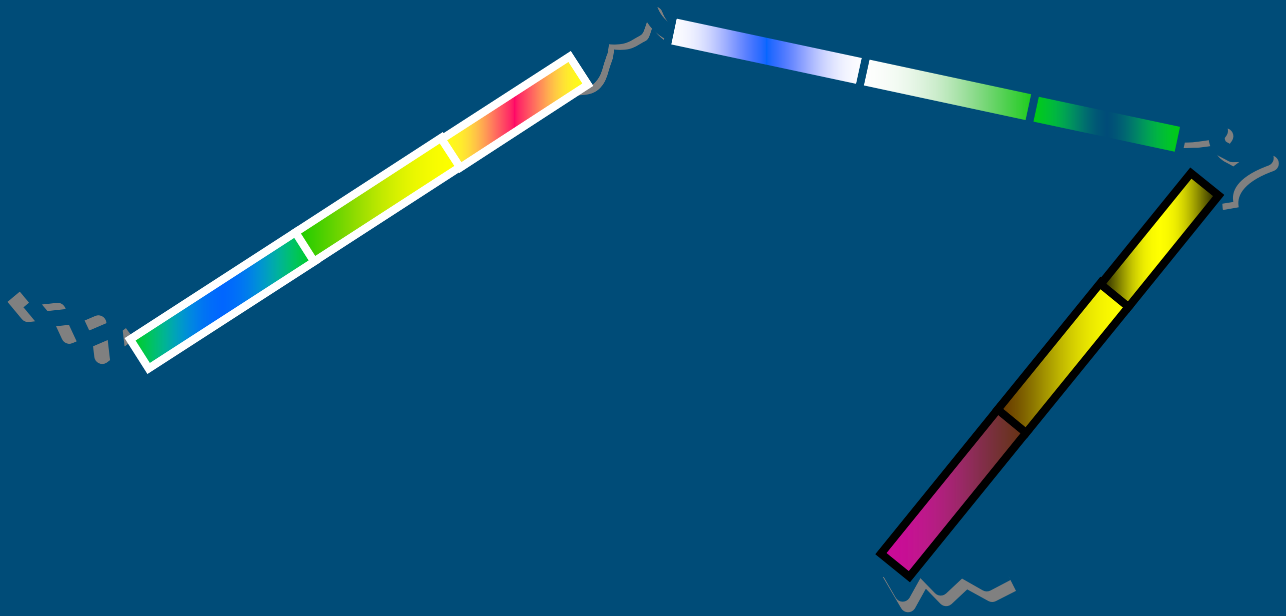
- Surgical materials (yarns, glues, fillers)
- Drug carrier materials
- Tissue engineering scaffolds

- (Bio)separation resins
- Laboratory materials

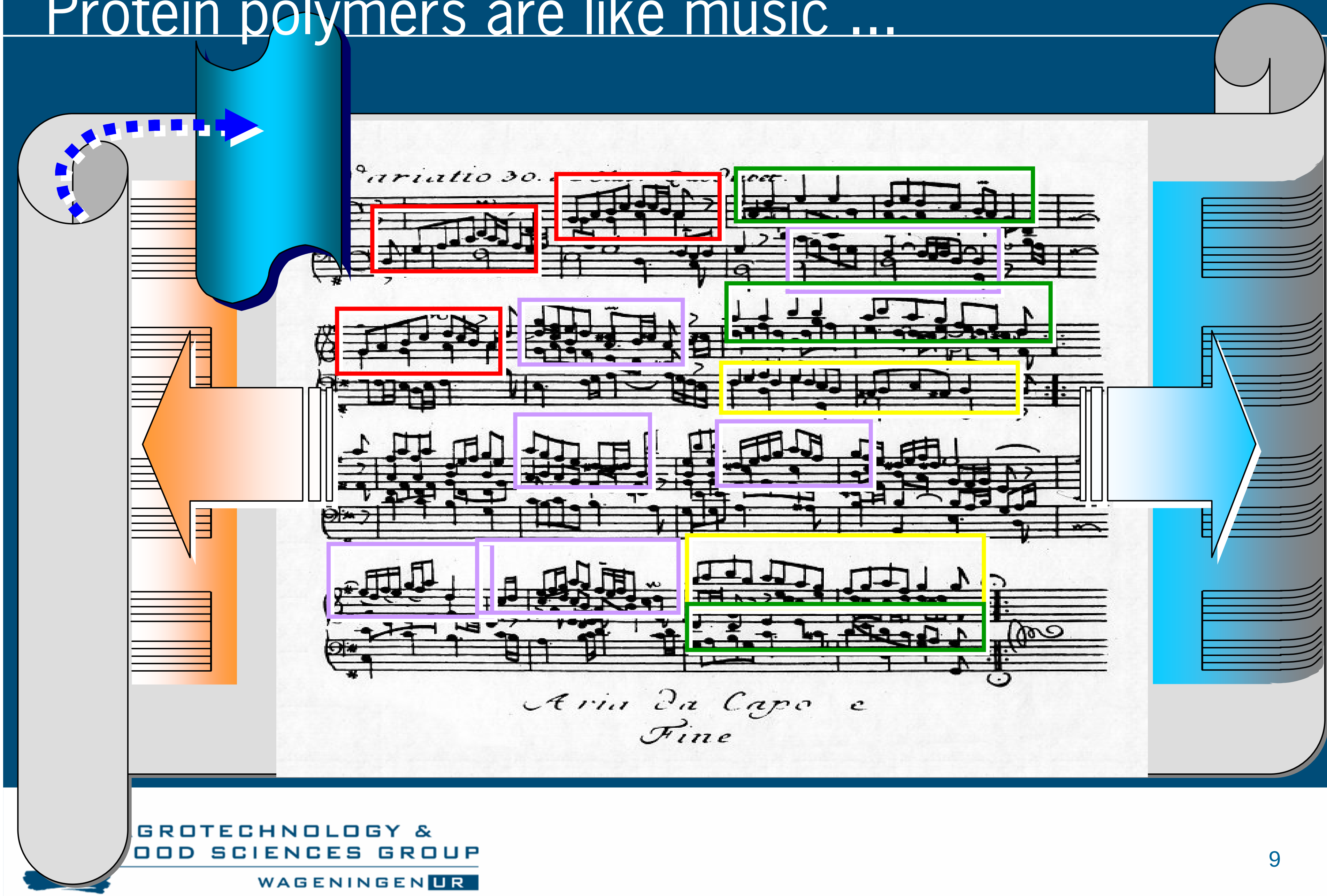
- Smart coatings
- Strong fibers

Protein polymers are like music ...

Protein polymers are like music ...



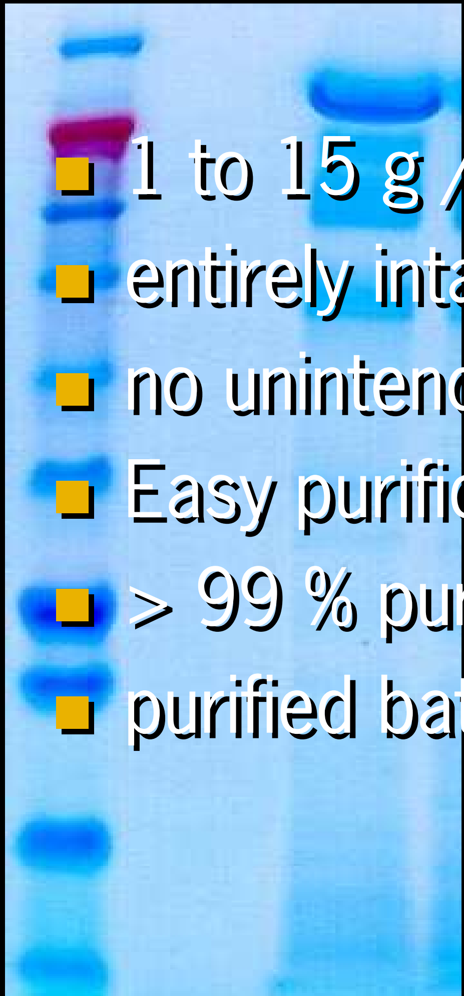
Protein polymers are like music ...

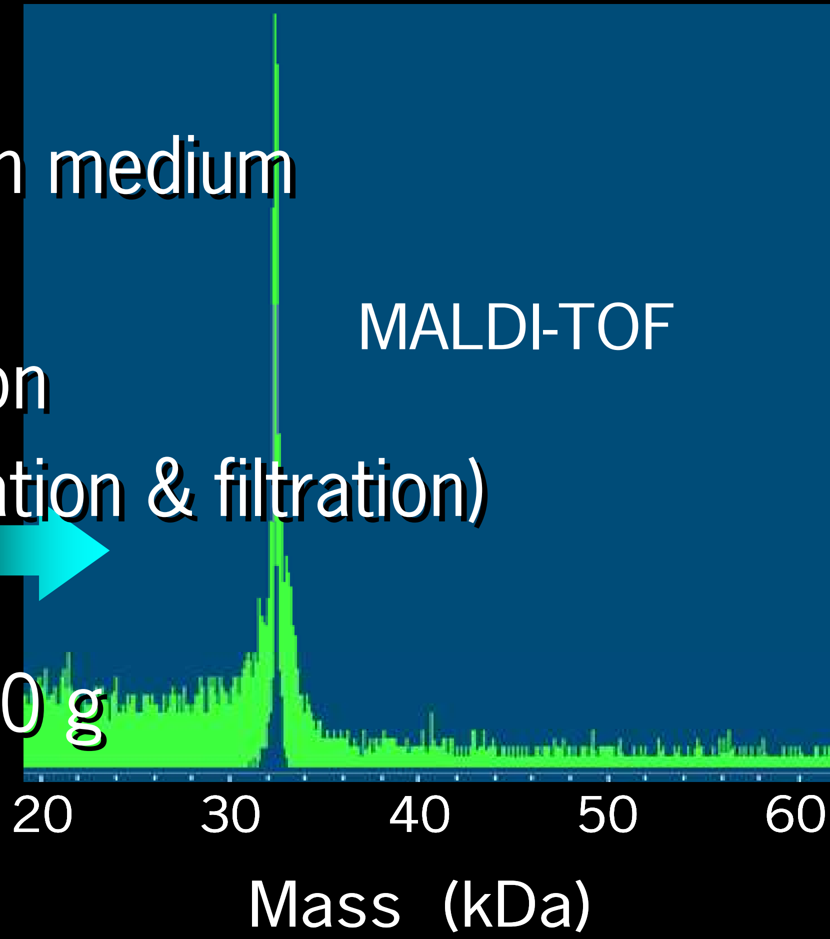


Protein polymers are like music ...

Biosynthesis of protein polymers

- 1 to 15 g / L in production medium
- entirely intact
- no unintended glycosylation
- Easy purification (precipitation & filtration)
- > 99 % pure
- purified batches of 0.1-200 g





DNA templates for protein polymers

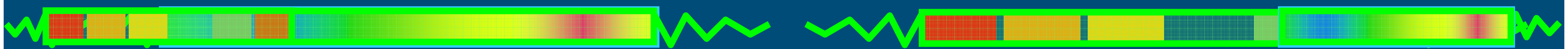
combine into one block copolymer!

isolation of natural DNA template

...resulting in one DNA template...

synthesis of novel designer DNA template

blocks with defined length and designed monomer sequence
(300-6000 DNA base pairs / 100-2000 amino acids)



... encoding the desired polypeptide building blocks

...encoding the entire polypeptide...

combine into one block copolymer!

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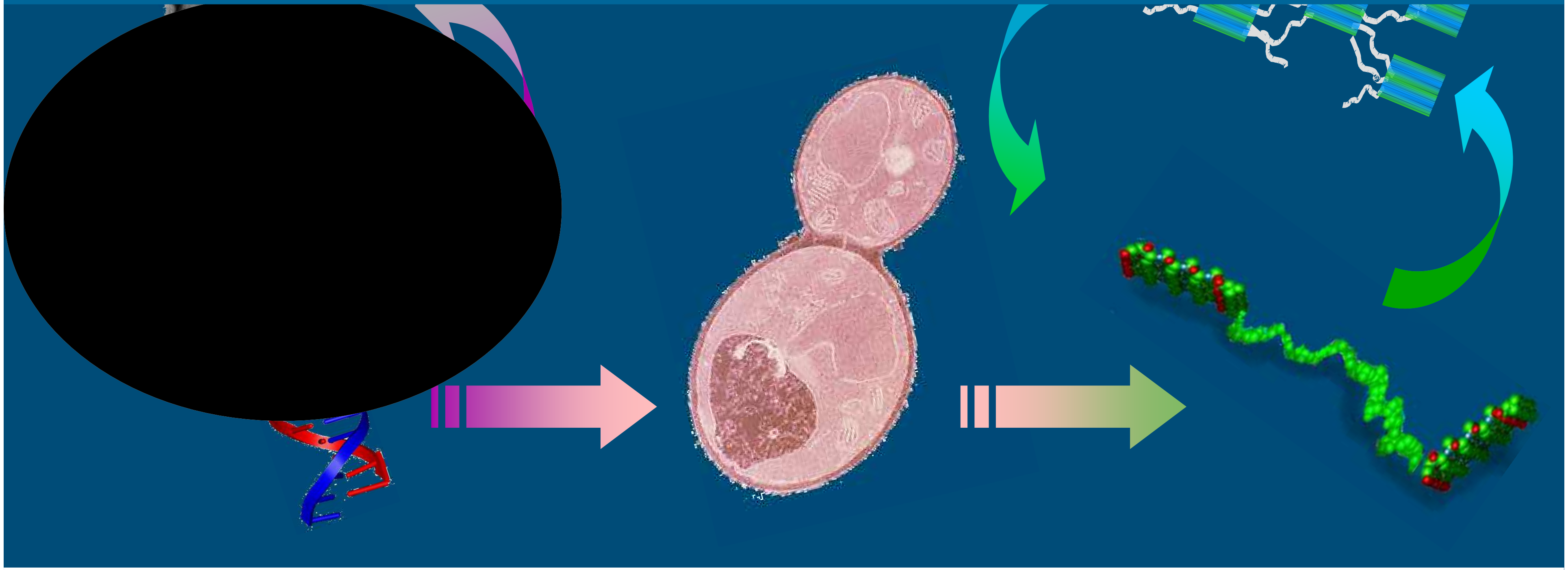
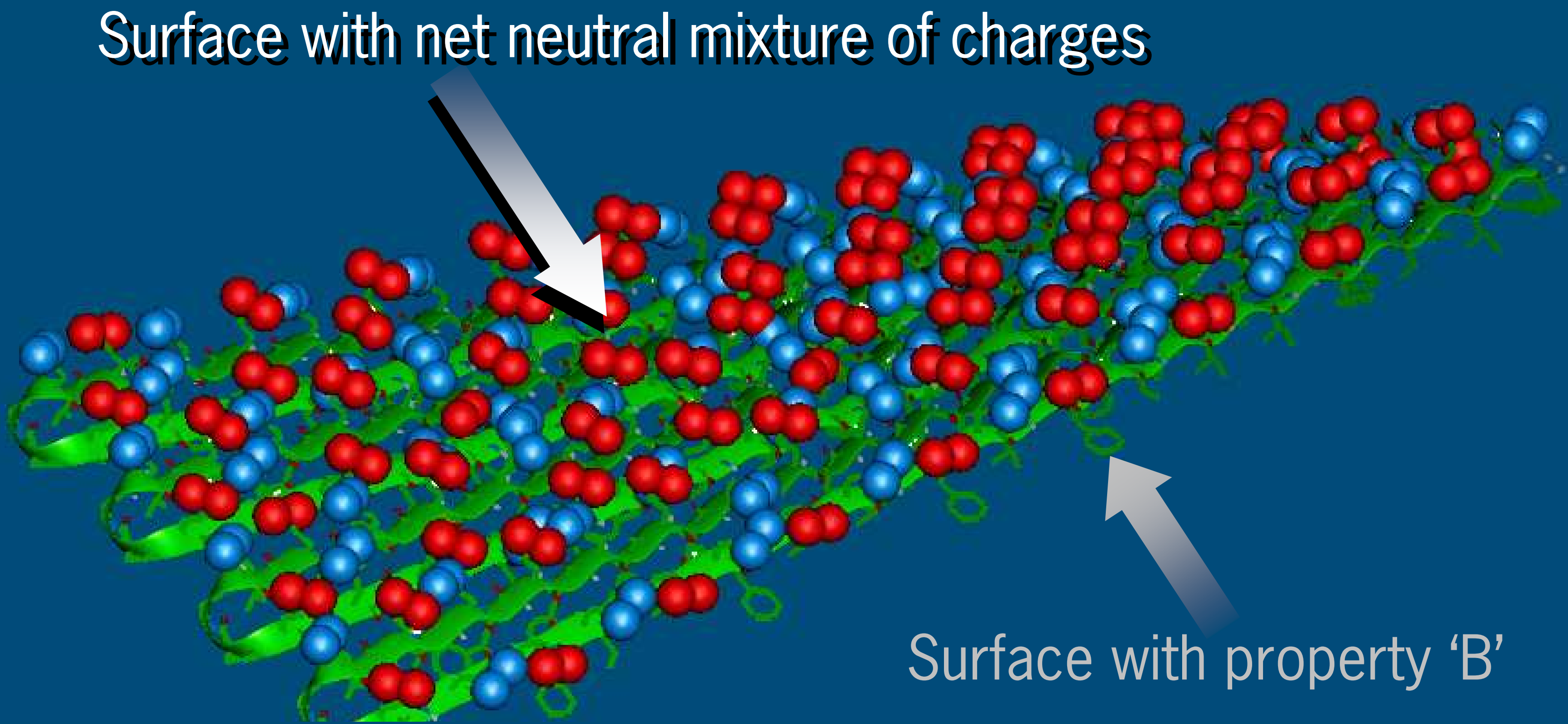
Some structure / function modules used in protein polymers .....

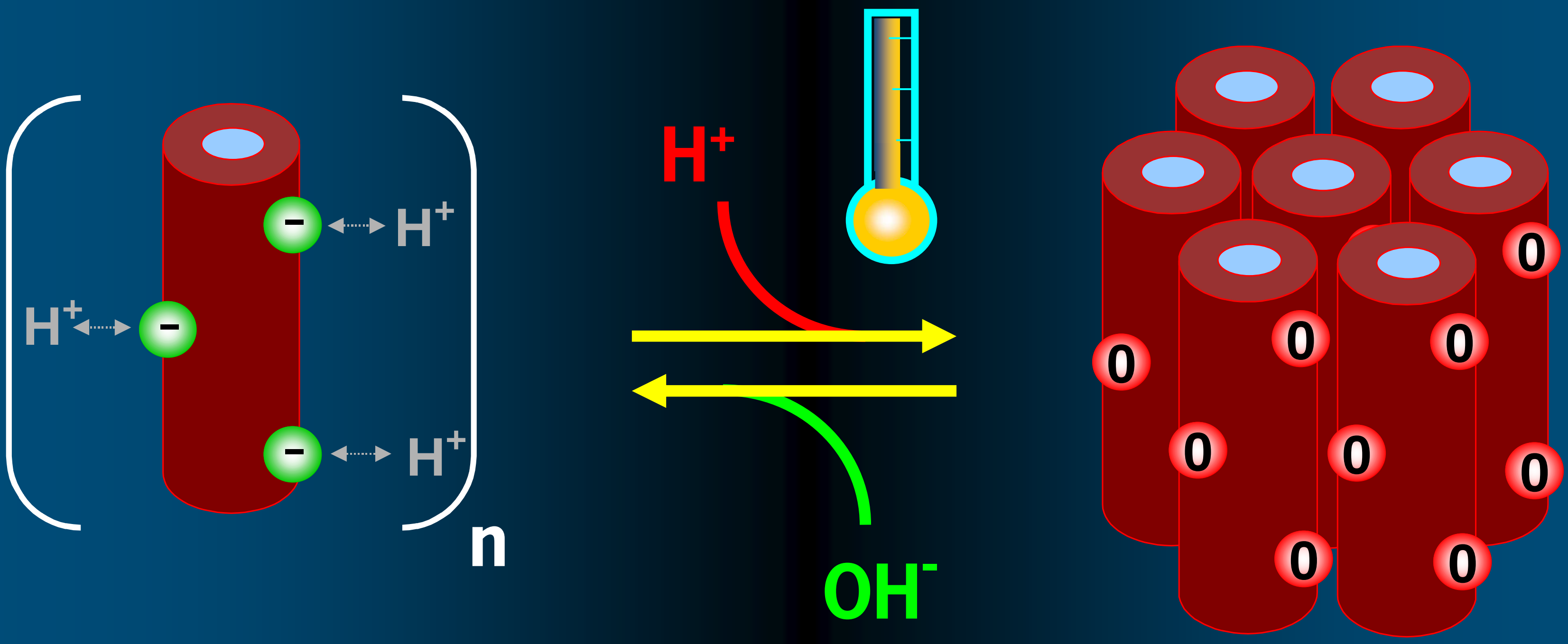


Charged surface-active protein polymers



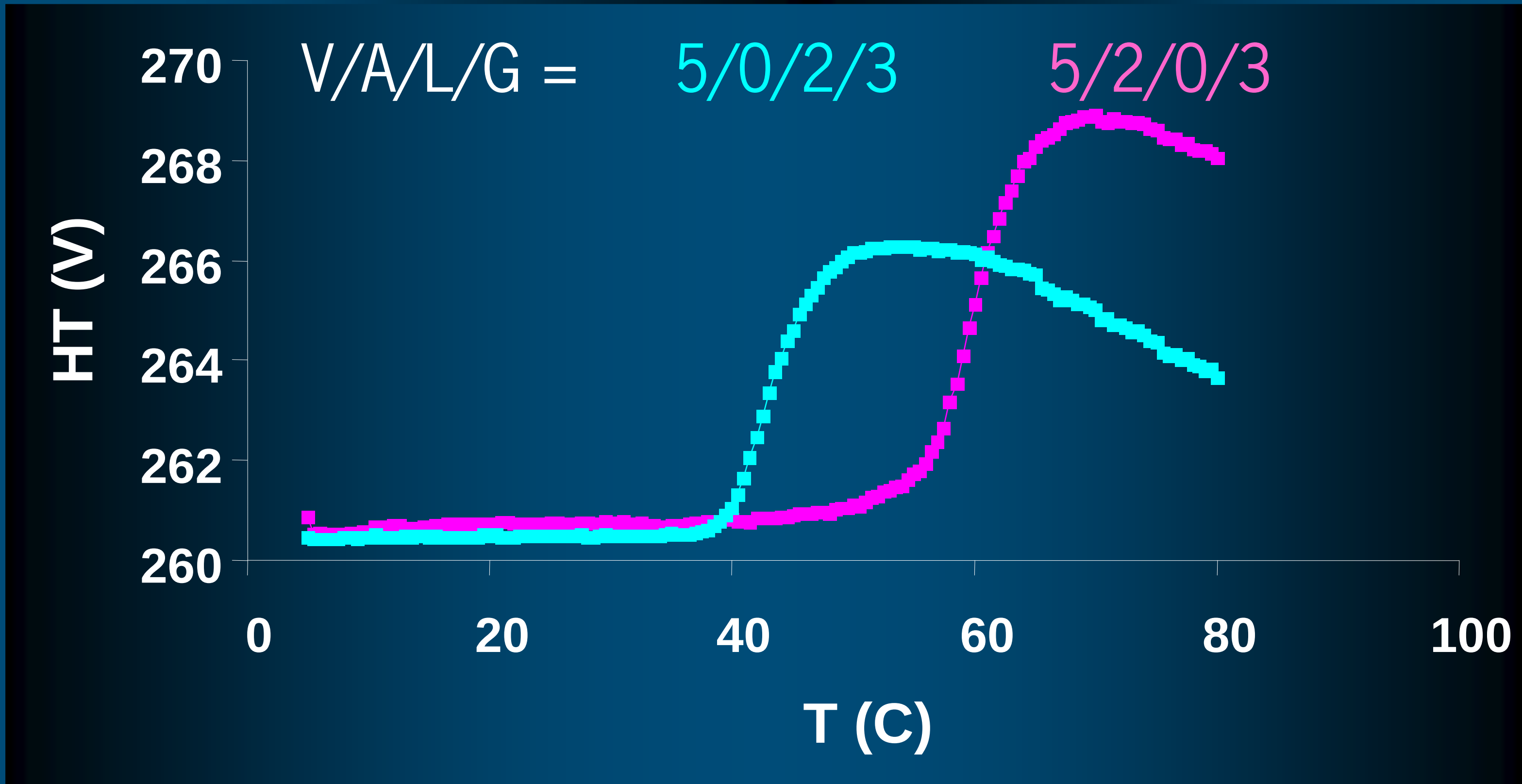
Elastin-like protein polymers: LCST

Val-Pro-Gly-Xaa-Gly repeats, stimulus responsive



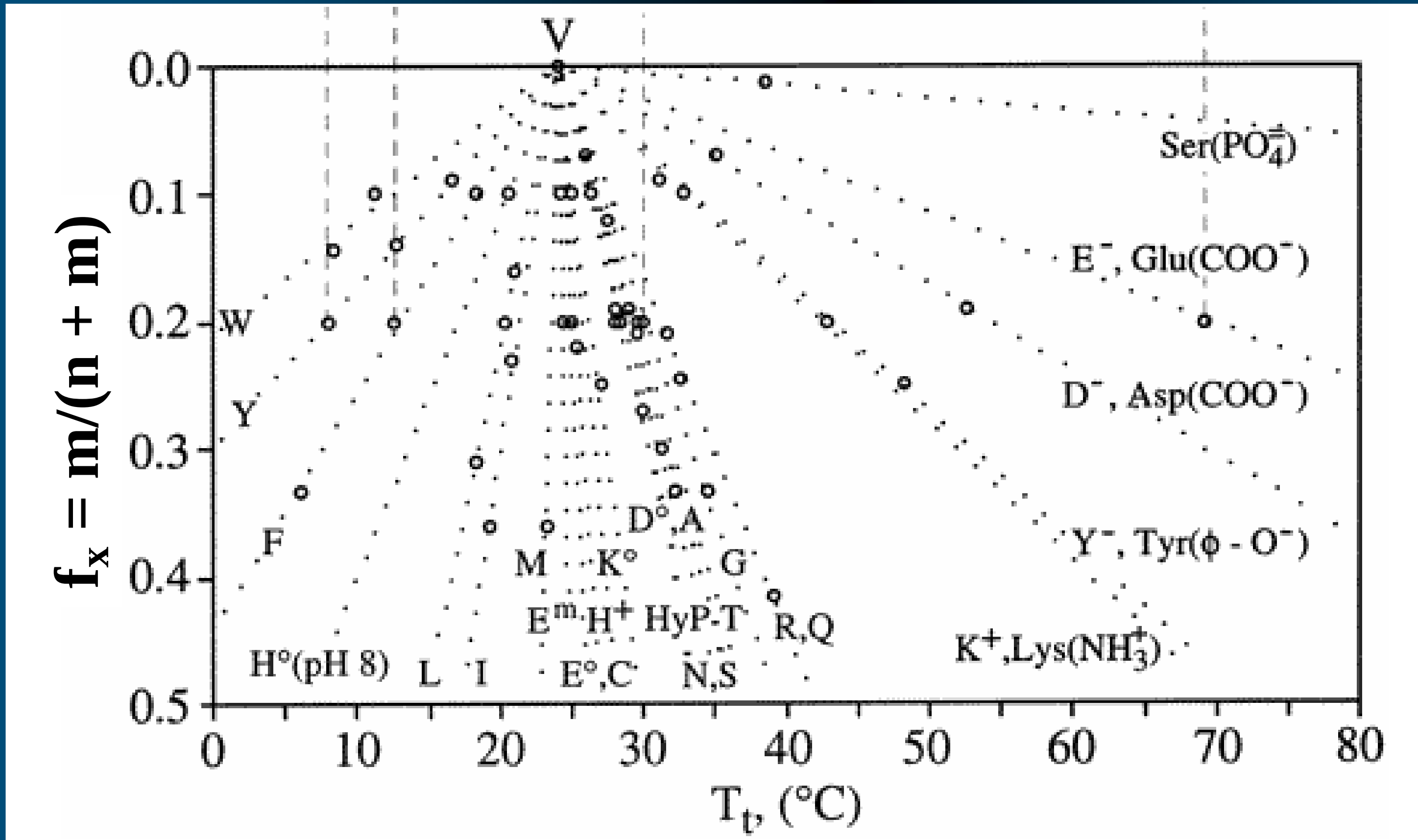
Elastin-like protein polymers: LCST

Turbidity measurements with two different polymers (450 amino acids, i.e. about 35 kDa)



Elastin-like protein polymers: LCST

Many different polymers: [(VPGVG) $_n$ (VPGXG) $_m$]

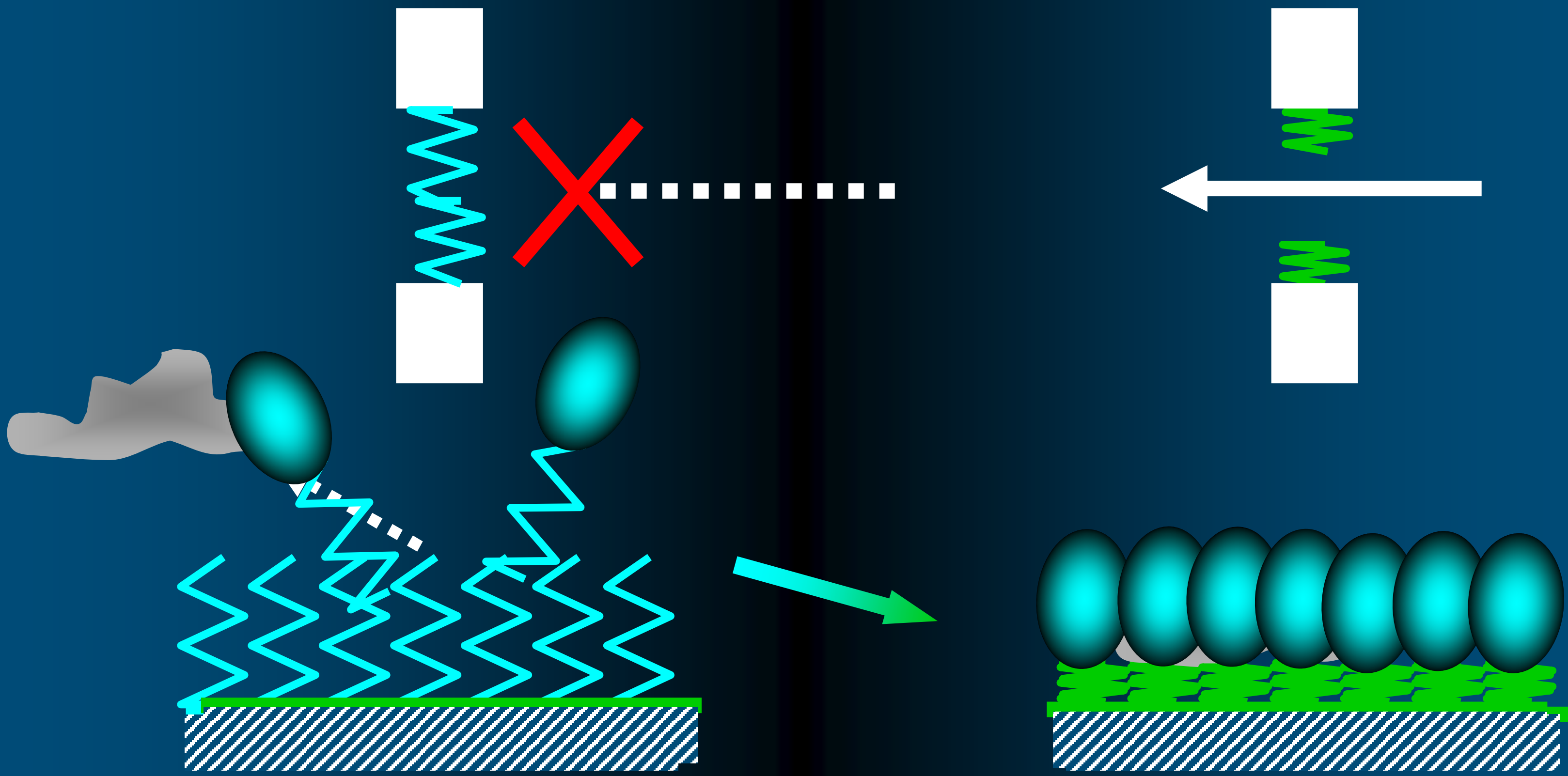


(from Dan W. Urry (1997)

J. Phys. Chem. B, 101 (51): pp. 11007-11028)

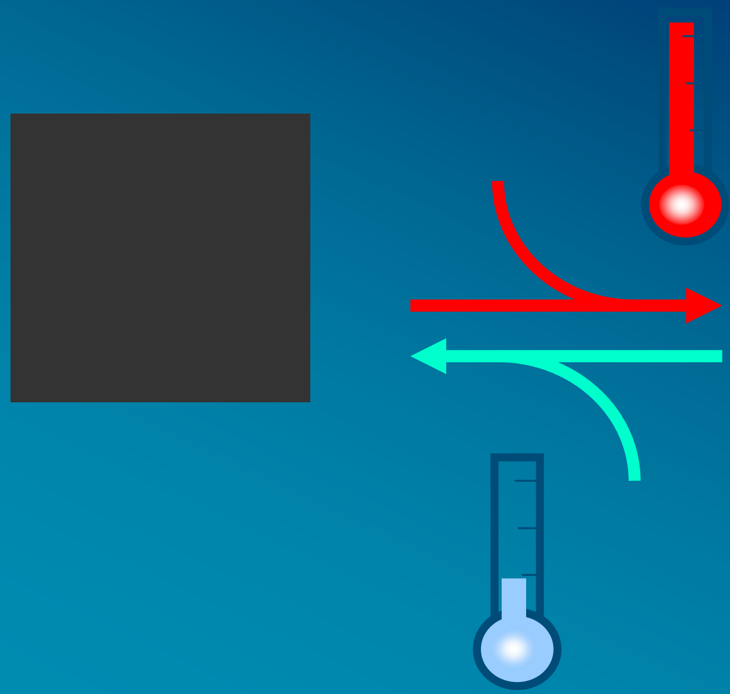
Elastin-like protein polymers: use of LCST

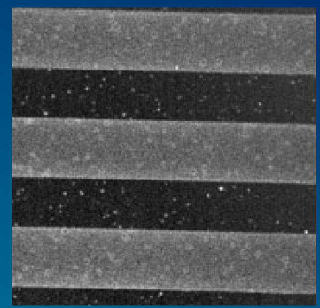
Characteristic 2: stimulus responsive



Elastin-like protein polymers: use of LCST

Characteristic 2: stimulus responsive

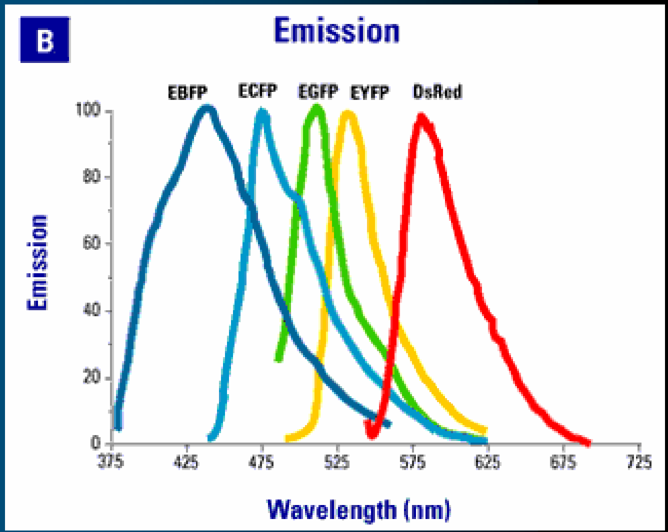


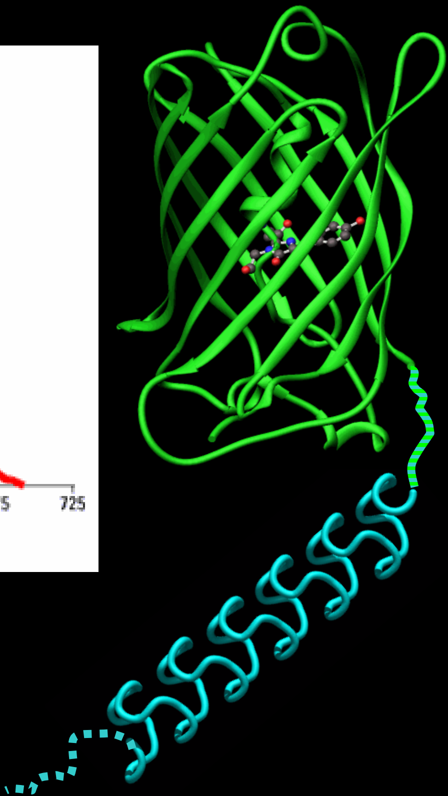


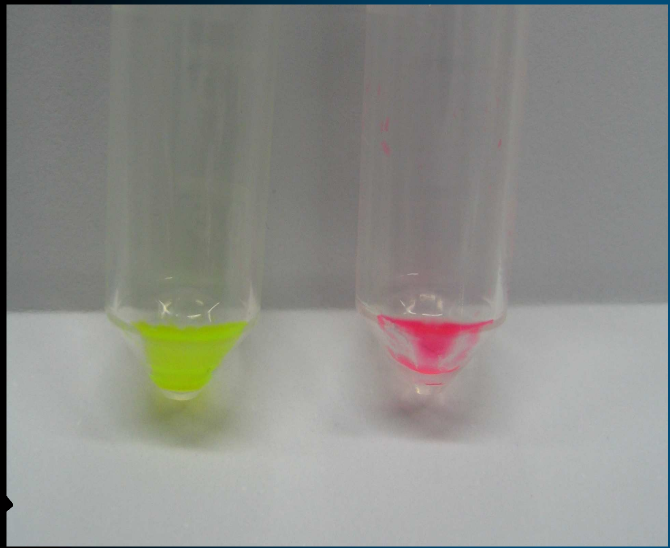
Thioredoxin-ELP fusion
on functionalized gold surface
(Chilkoti et al., Duke Univ. , NC)

Elastin-like protein polymers: use of LCST

Block copolymers: elastin-like + fluorescent protein

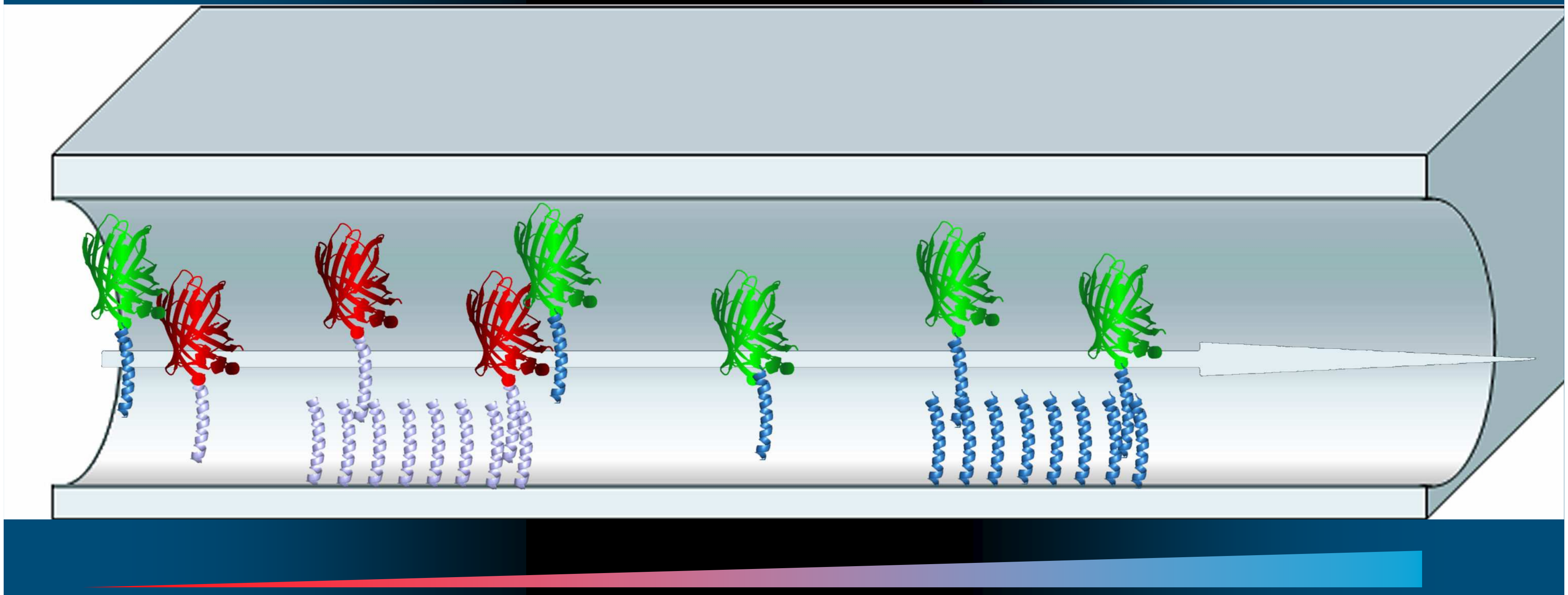






Elastin-like protein polymers: use of LCST

Temperature-controlled positional assembly of enzymes



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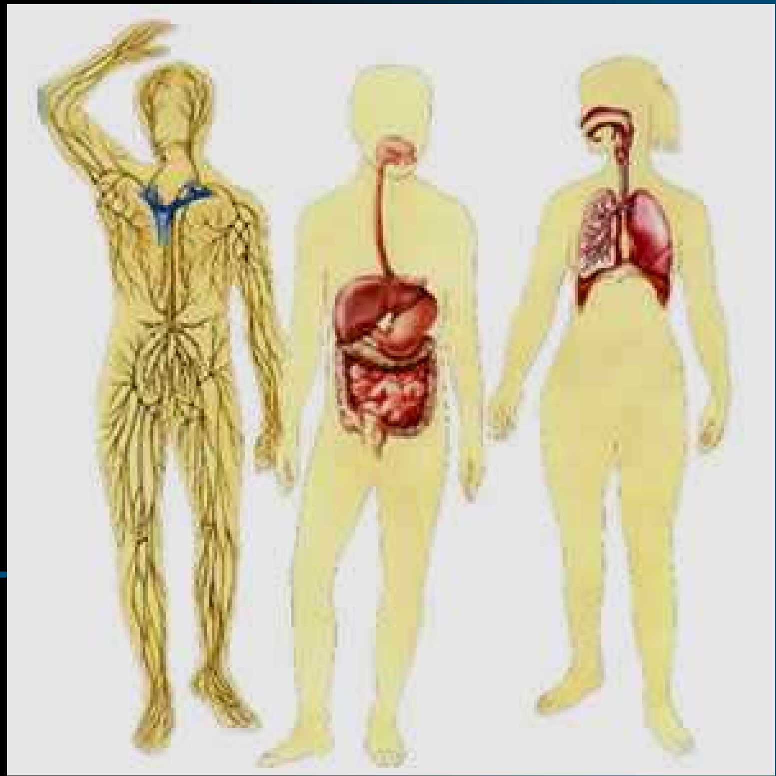
Collagen-like protein polymers

The most abundant protein in the body



Collagen-like protein polymers: use

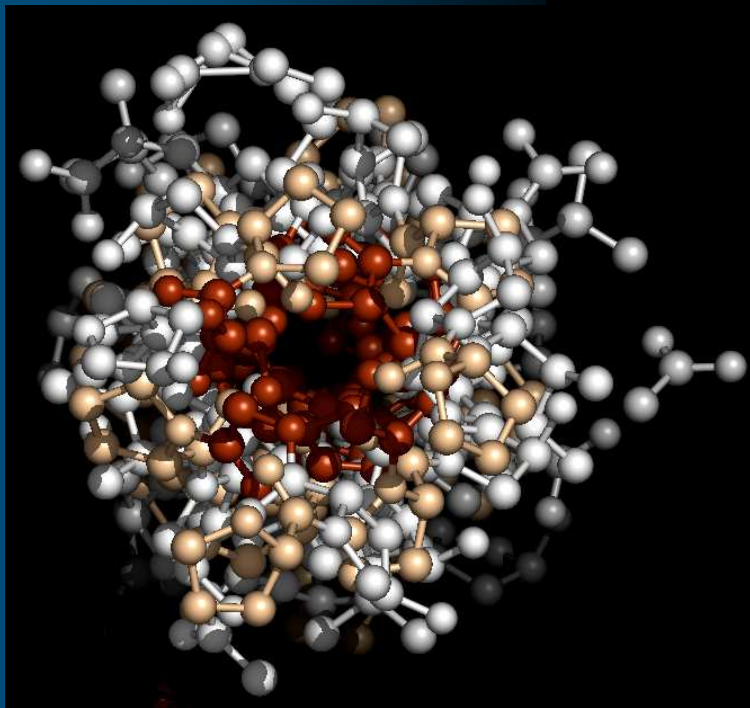
Biomaterials applications & market

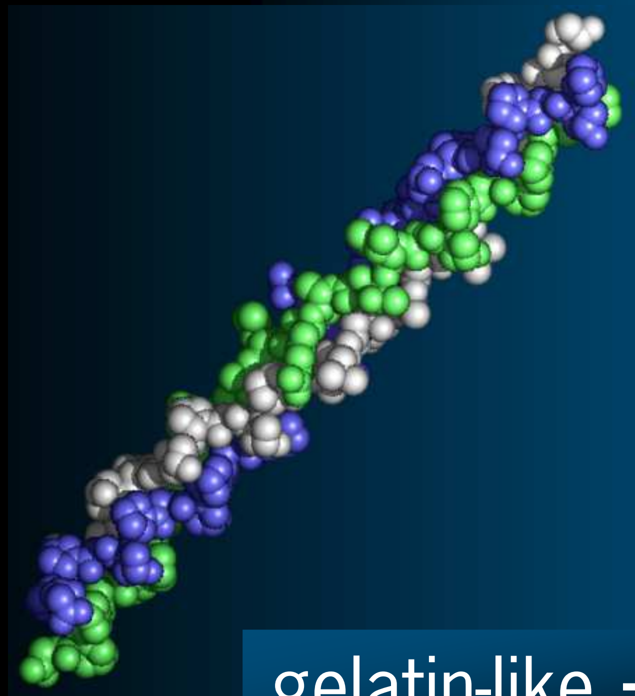
- cosmetic surgery 0.1 G€
- dental surgery 0.65 G€
- cardiovascular surgery 8 G€
- drug delivery 6.3 G€
- surgical sutures 1.7 G€
- tissue augmentation & urology 0.1 G€
- wound care 4 - 5 G€
- tissue engineering 0.02 G€

- total 35 G€ (raw biomaterials > 1 G€)
- 5-30 % growth / year
- *(TE: growth to 80 G€)*

Collagen-like protein polymers: self-assembly

Characteristic: (Gly-X1-X2) repeats, 10 – 67 % Pro
Pro in X2 modified to Pro-OH → helix stabilization

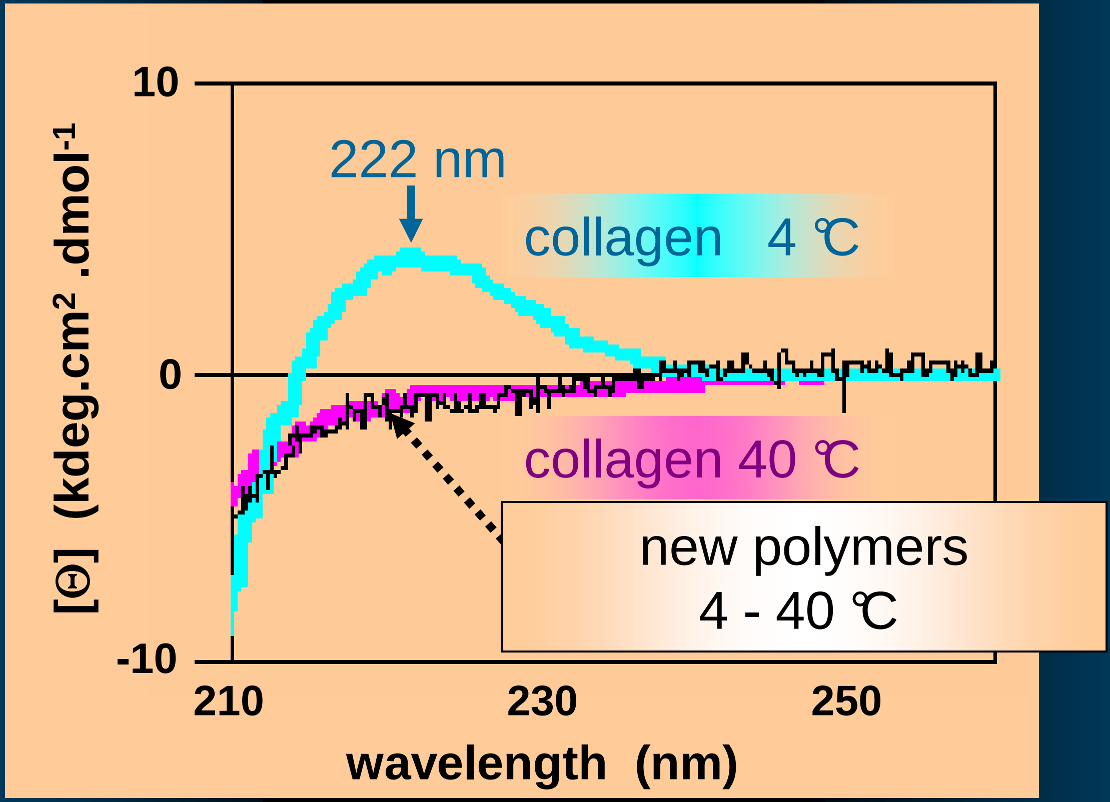




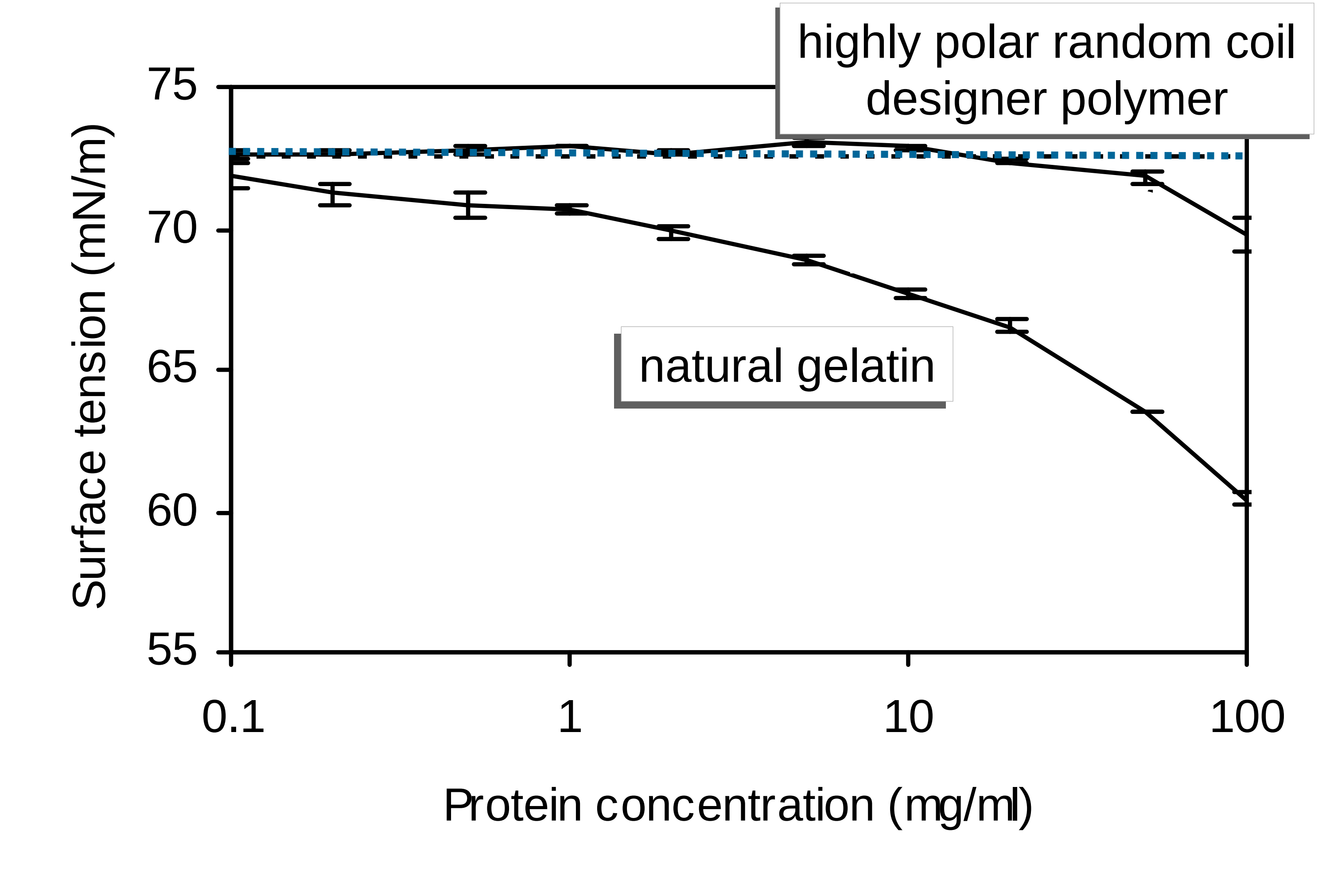
gelatin-like → gels

Collagen-like p.p.: self assembly suppressed:

modified polymers forming no helix



Collagen-like protein polymers: polarity modified



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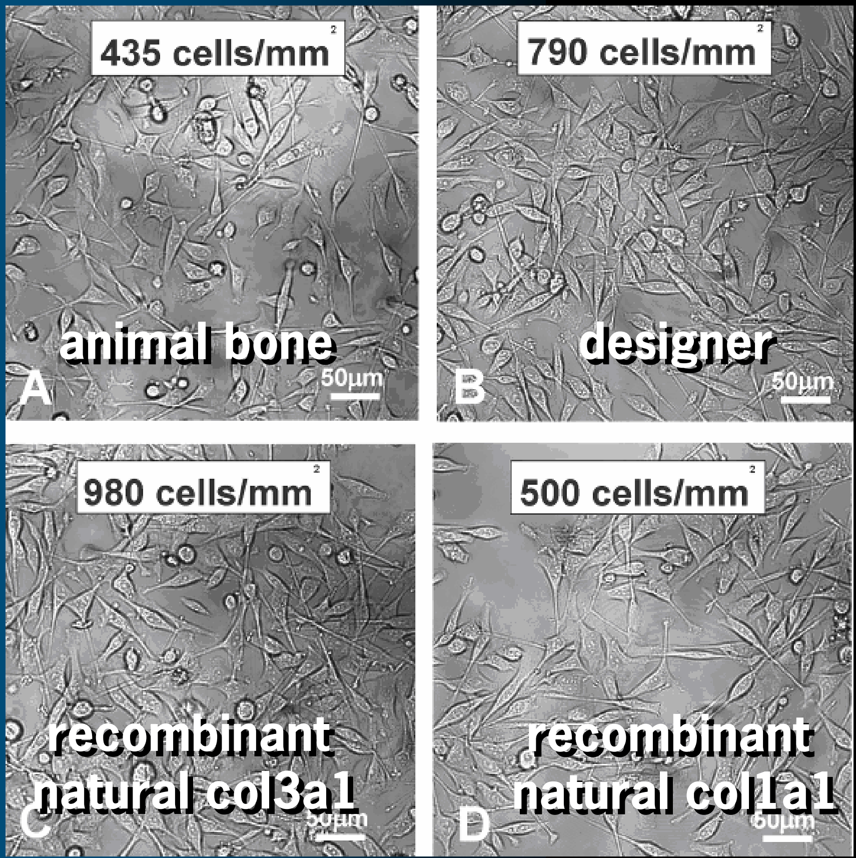
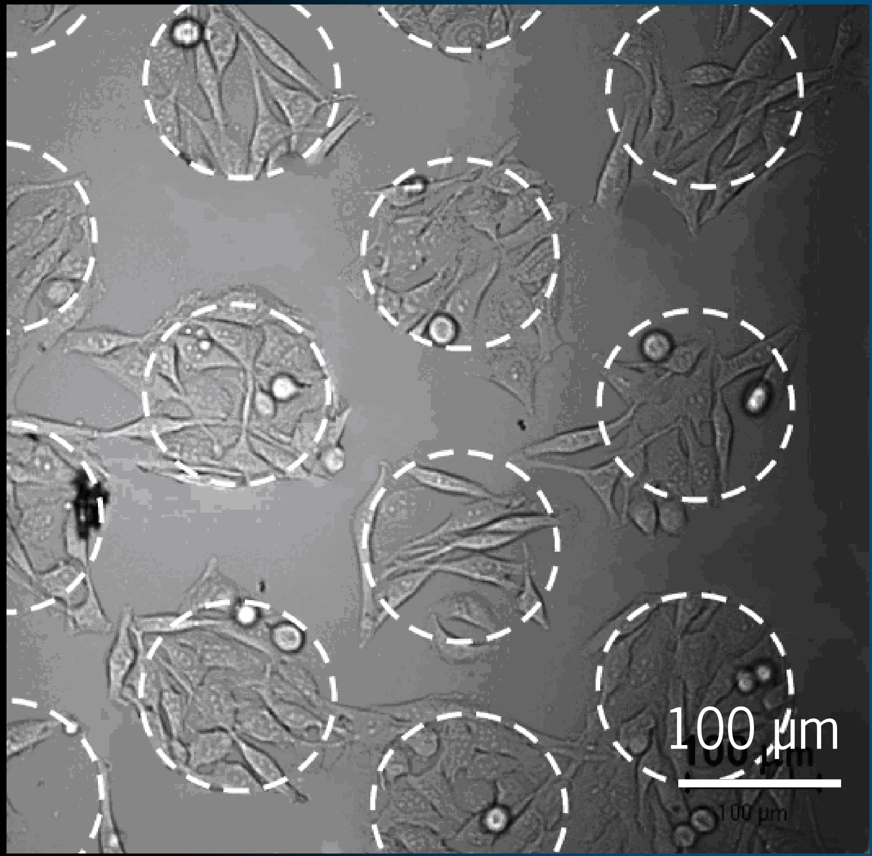
Collagen-like protein polymers: cell-binding coils

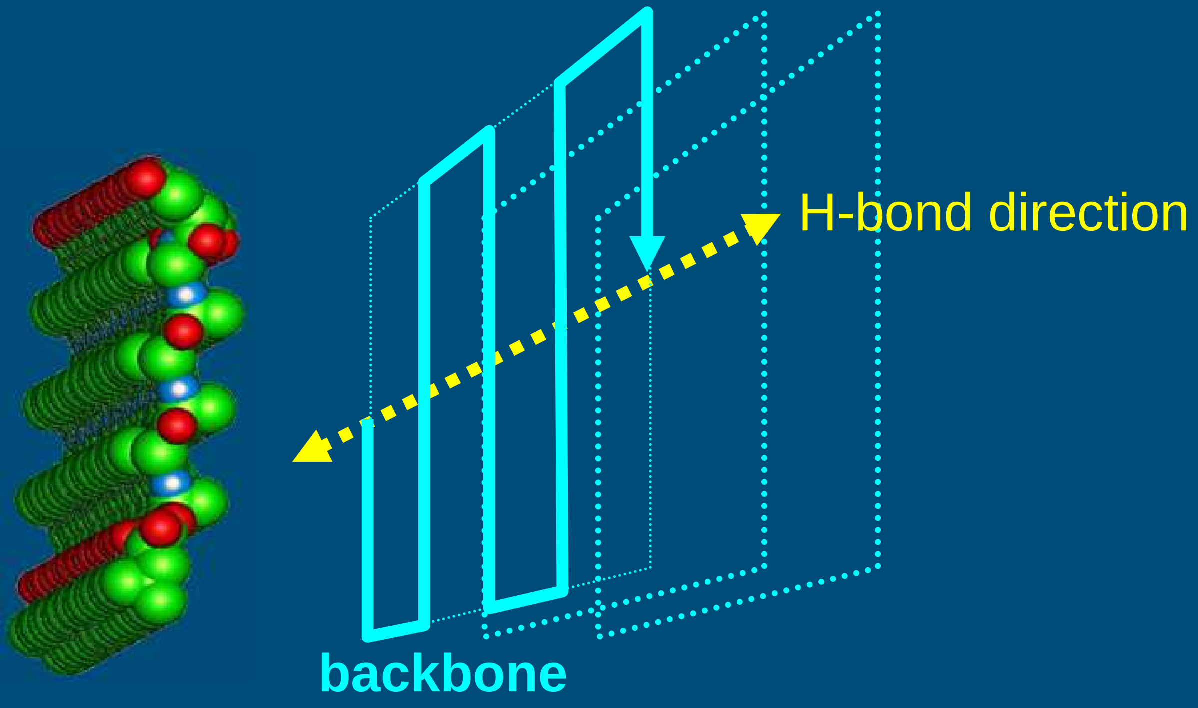
Example: collagen like polymers attracting human cervical carcinoma (HeLa) cells





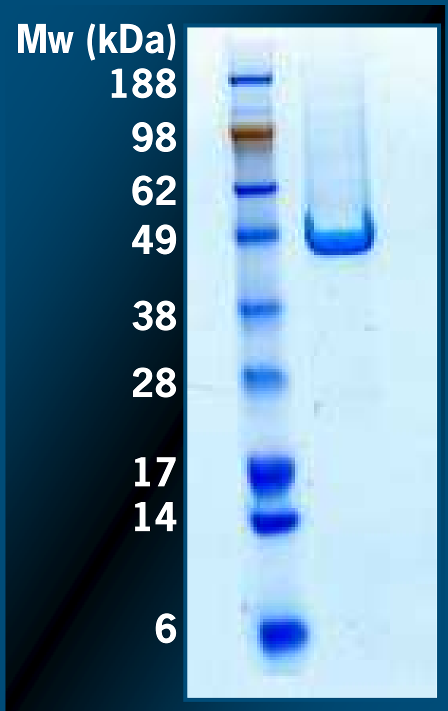
Silk-like protein polymers: self assembly

Gly-Ala repeats → ß-sheet stacks possible (e.g. pH-induced)



Block A: silk-like, polyanionic

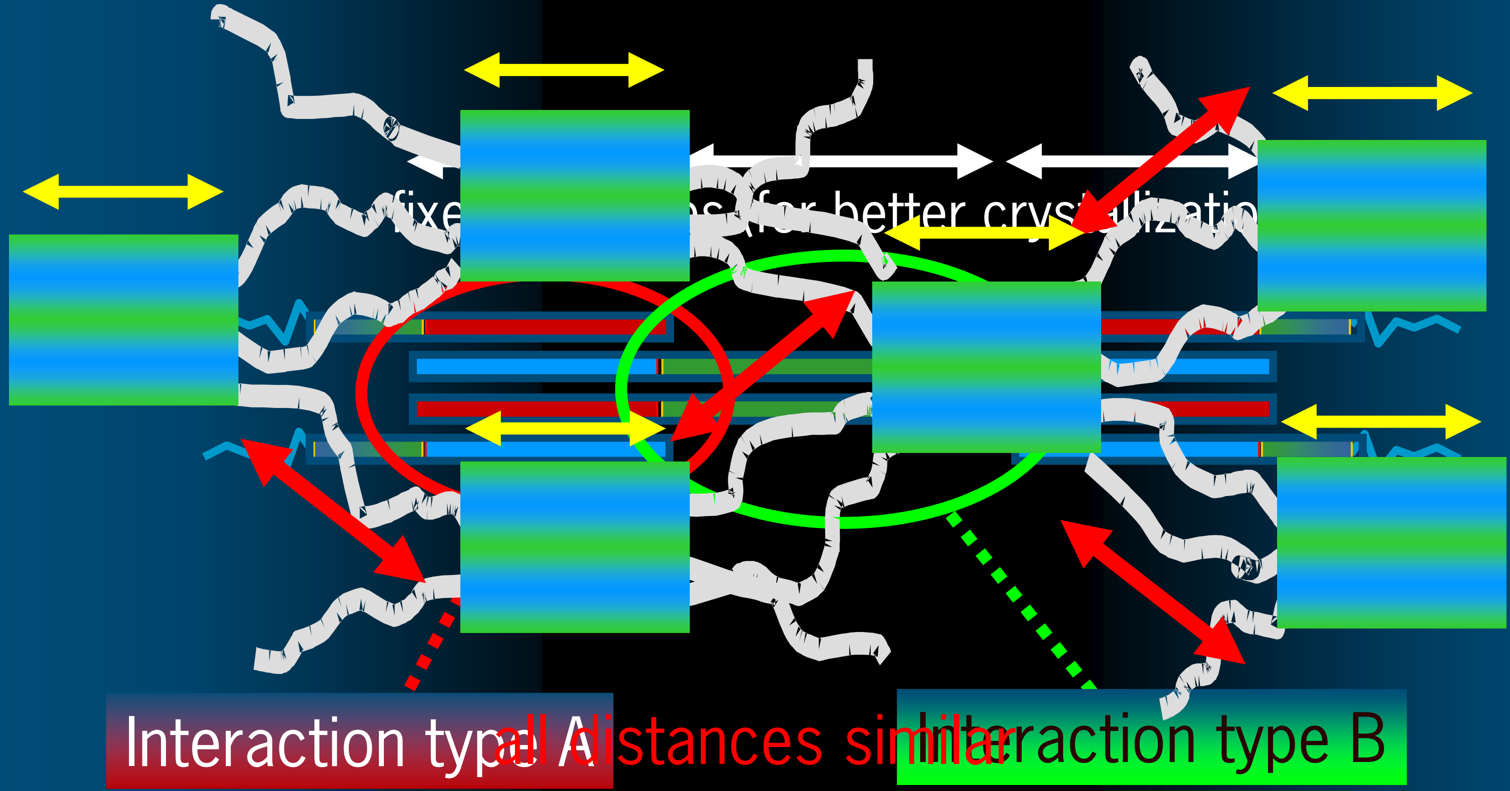
Gly-Ala repeats → ß-sheet stacks possible



- ((Gly-Ala)$_3$-Gly-Glu)$_n$
- random, extended ~ pH > 5 (CD)
- ß-sheet, turn at low pH or methanol (FTIR)

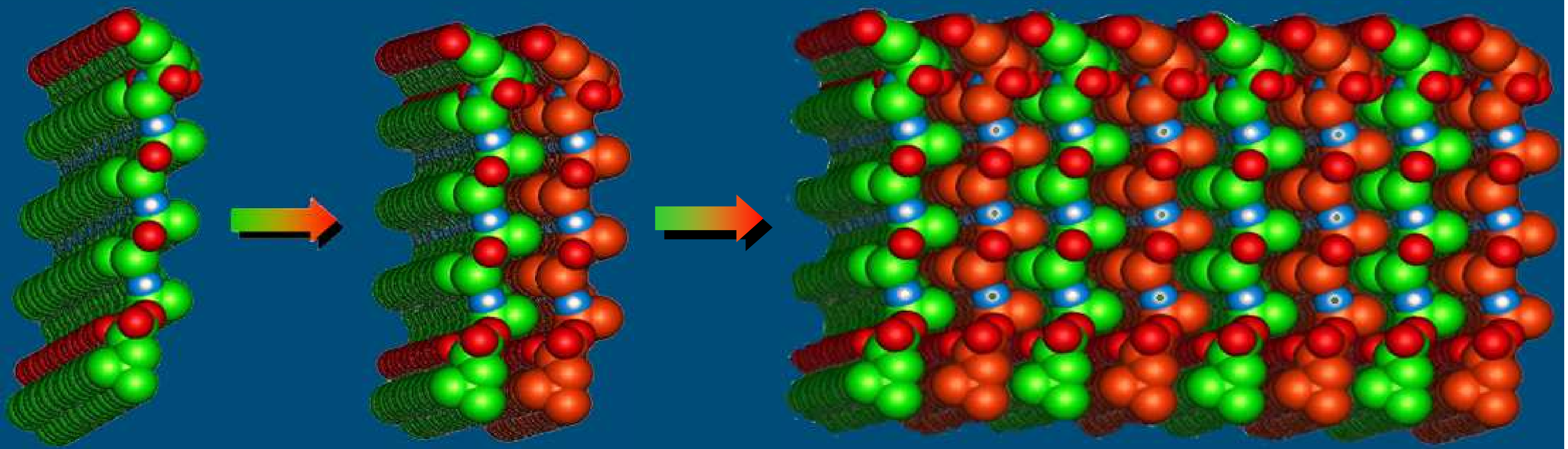
Multiblock protein polymers

Nanostructured materials

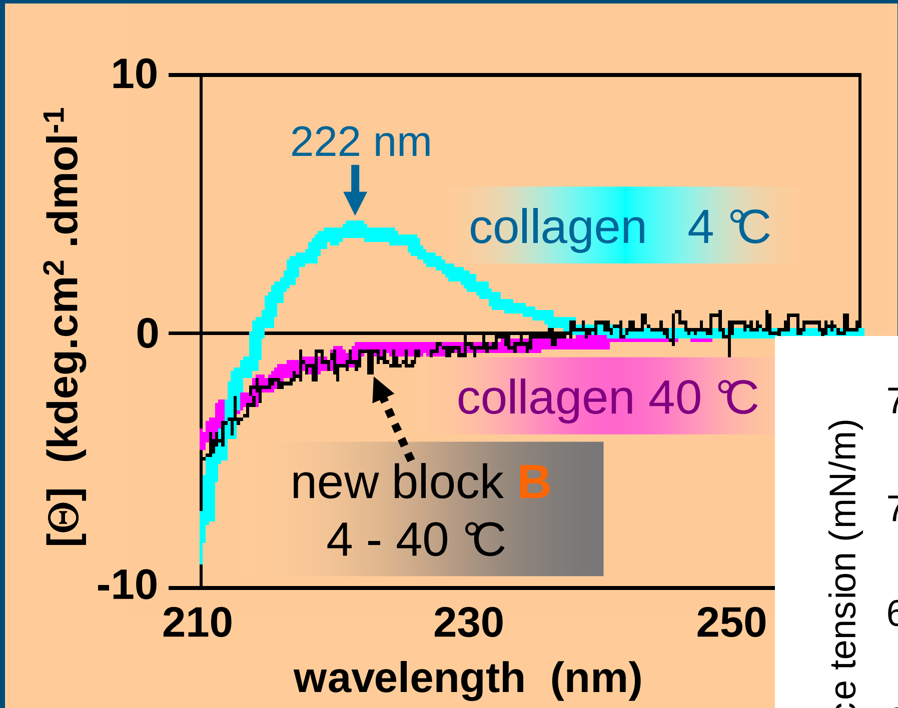


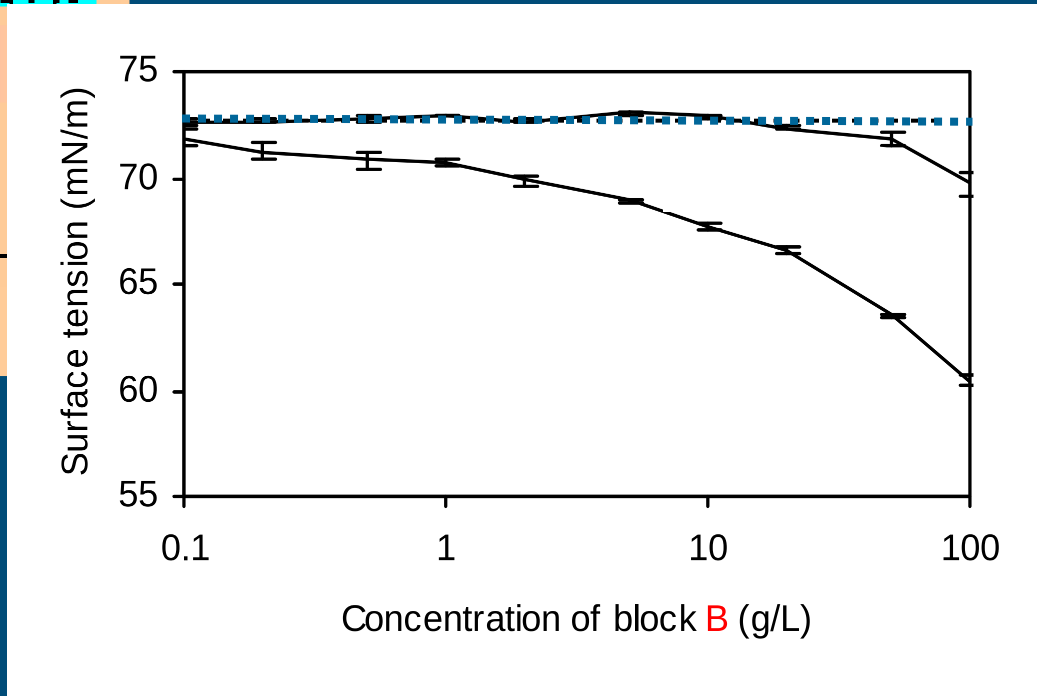
Block A: silk-like

Gly-Ala repeats → ß-sheet stacks possible



Block B: collagen-like, but non-helical, polar



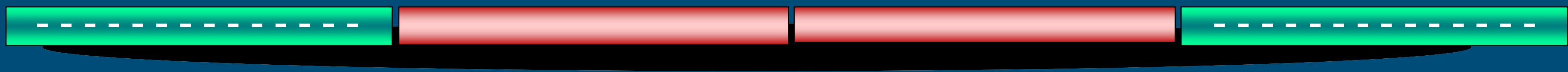


Triblock protein polymers

- ABBA (silk-coll.-coll.-silk)

((Gly-Ala)$_3$-Gly-Glu)$_{24}$ ((Gly-X1-X2)$_{99}$)$_2$

GAGAGAGE---//----GAGE-GAGAPGEPGNP-------------//------------------GNQGPAGE-GAGAGAGE-//-GAGAGE



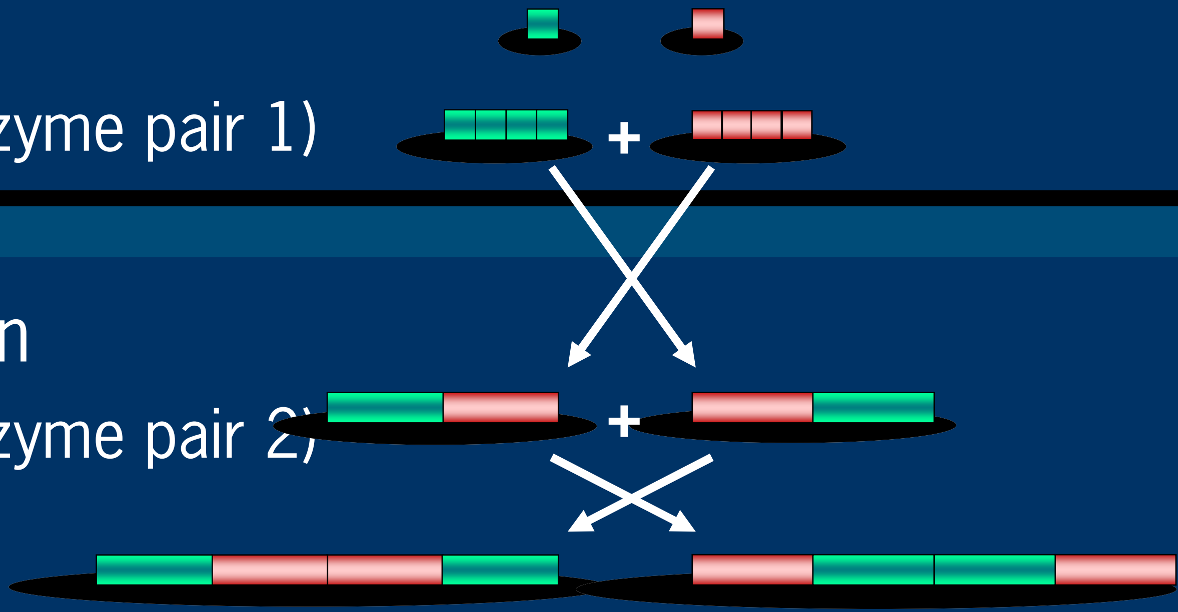
- BAAB (coll.-silk-silk-coll.)

- ~200 amino acids per block:
- total ~800 amino acids, i.e. 66 kDa

Construction of triblock DNA templates

- Block elongation

  (DNA-processing enzyme pair 1)

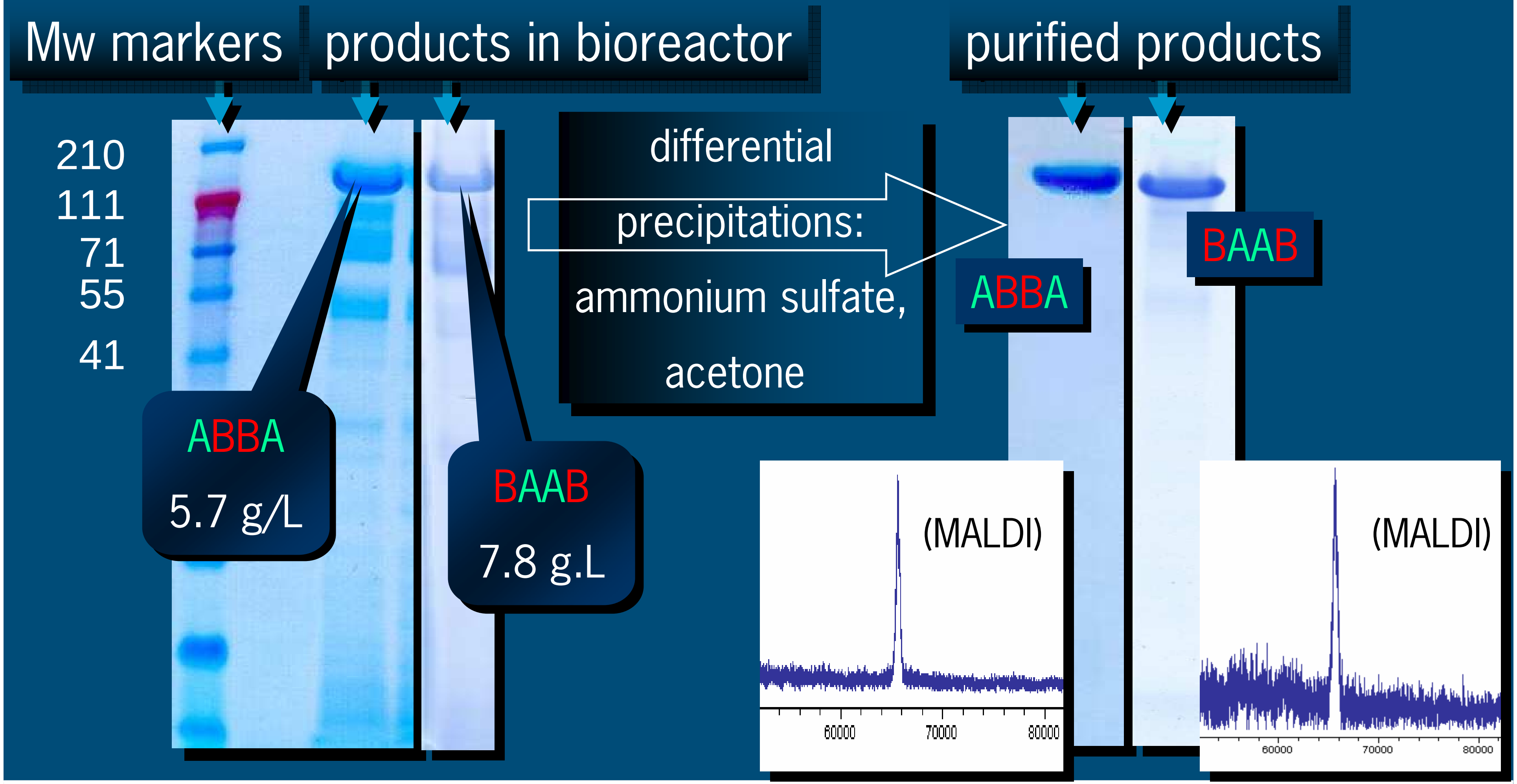


- Block combination

  (DNA-processing enzyme pair 2)

- One DNA template (one gene) → one protein !

Production of triblock protein polymers in yeast



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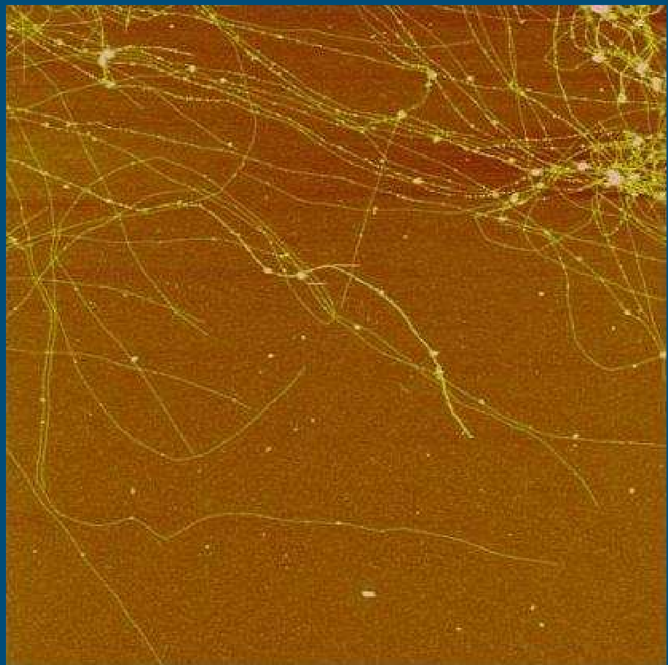
DPI DUTCH POLYMER INSTITUTE

Long fibers formed upon charge neutralization

BAAB

(coll.-silk-silk-coll.)

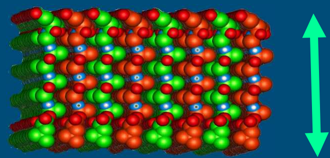


5 µm

AFM

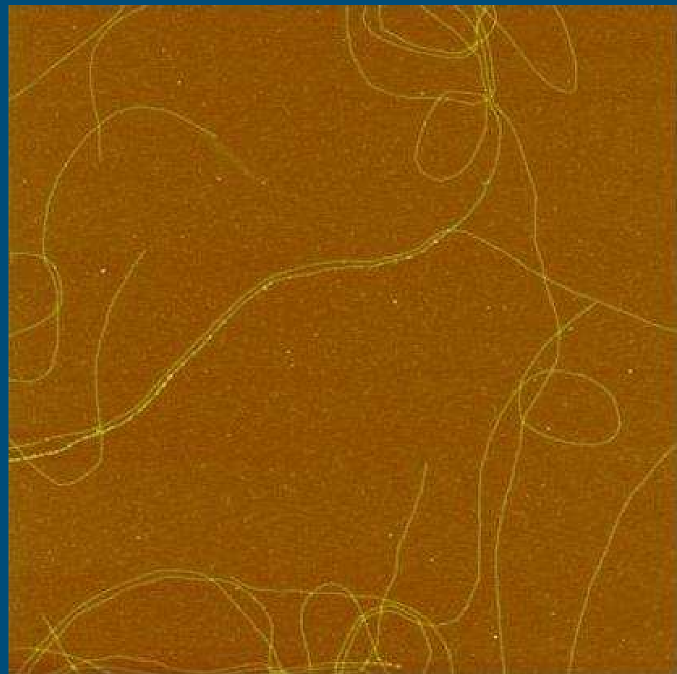
(silica support)

fibril height:
2 nm



ABBA

(silk-coll.-coll.-silk)



5 µm

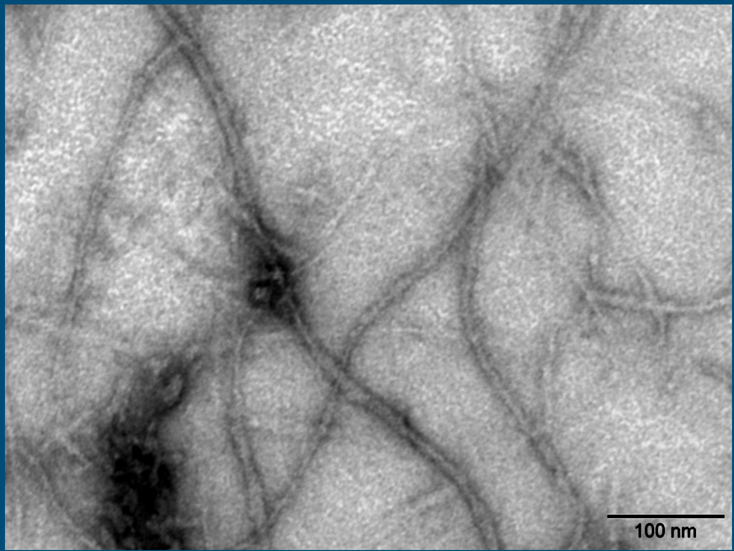
Long fibers formed upon charge neutralization

BAAB

(coll.-silk-silk-coll.)

ABBA

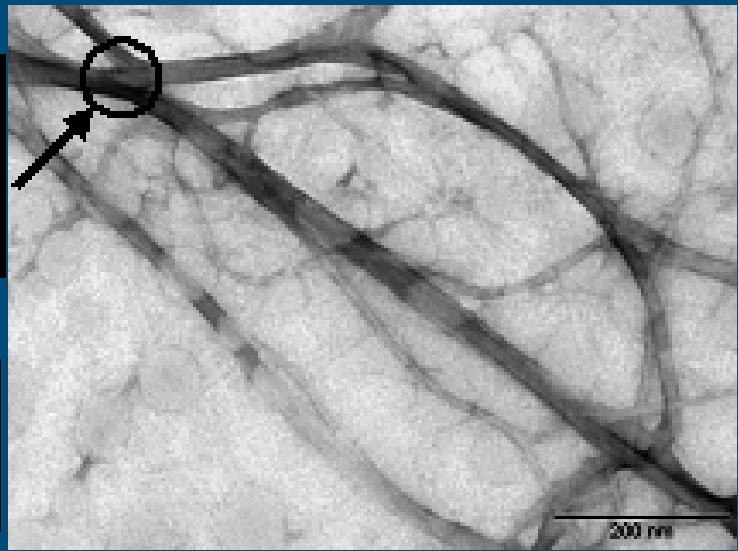
(silk-coll.-coll.-silk)



TEM

(neg. stain)

5 - 10 nm
wide fibrils



100 nm ↔

100 nm ↔

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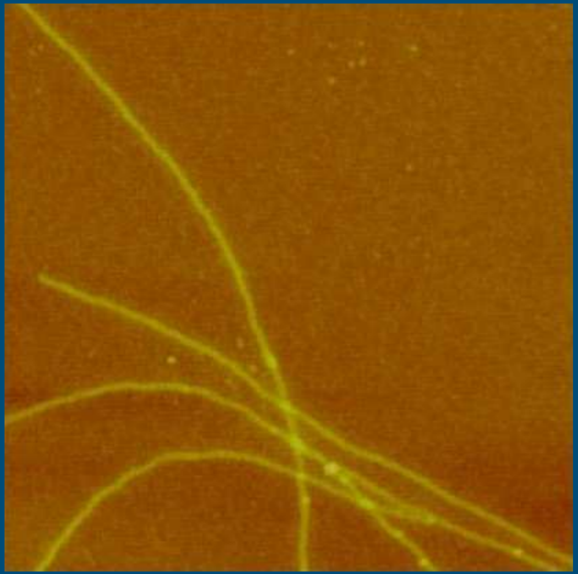
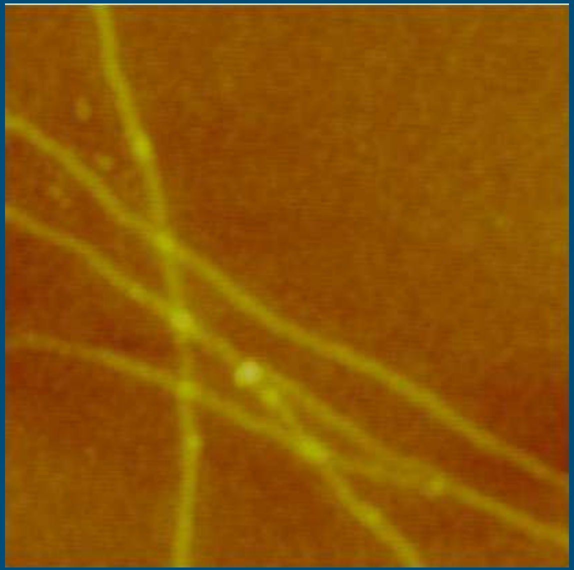
Long fibers formed upon charge neutralization

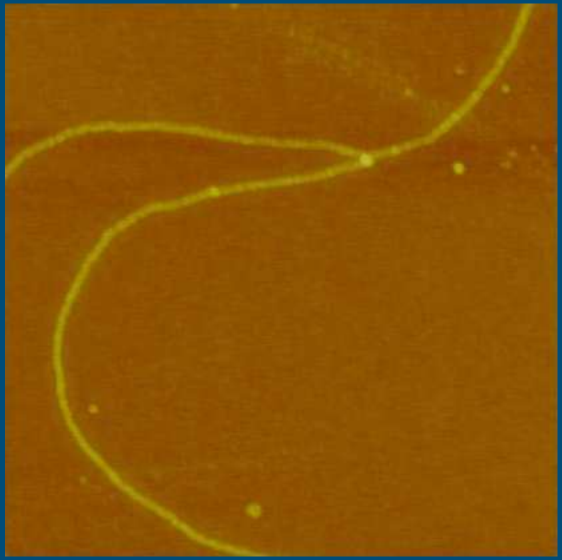
AFM on

BAAB + ABBA mixtures

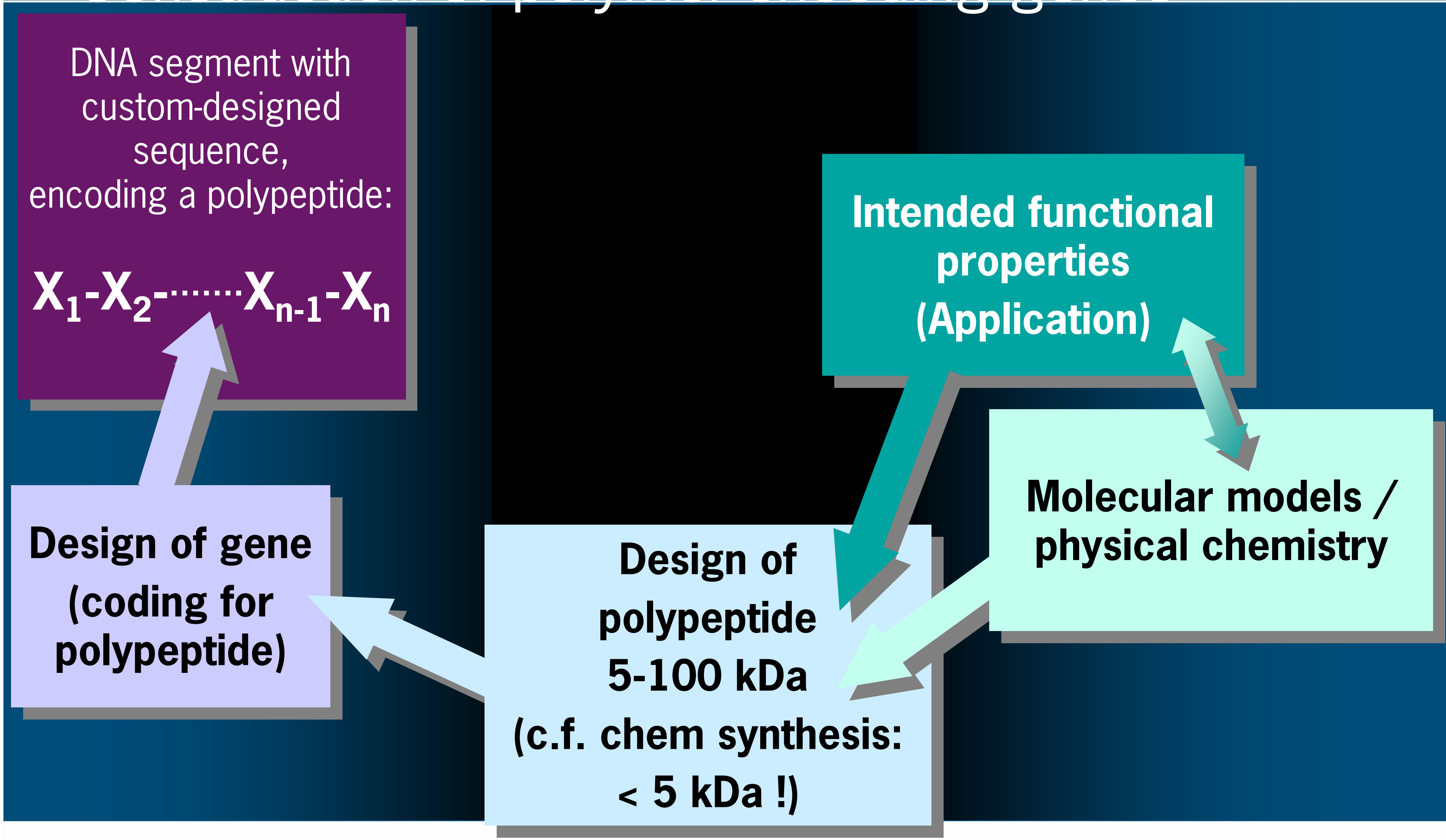
(coll.-silk-silk-coll. + silk-coll.-coll.-silk)







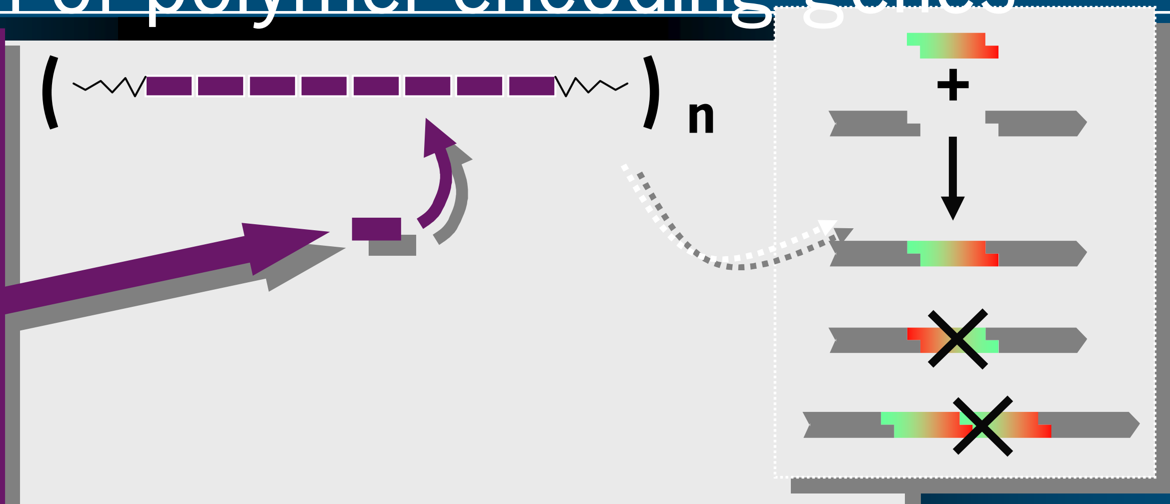
Construction of polymer-encoding genes



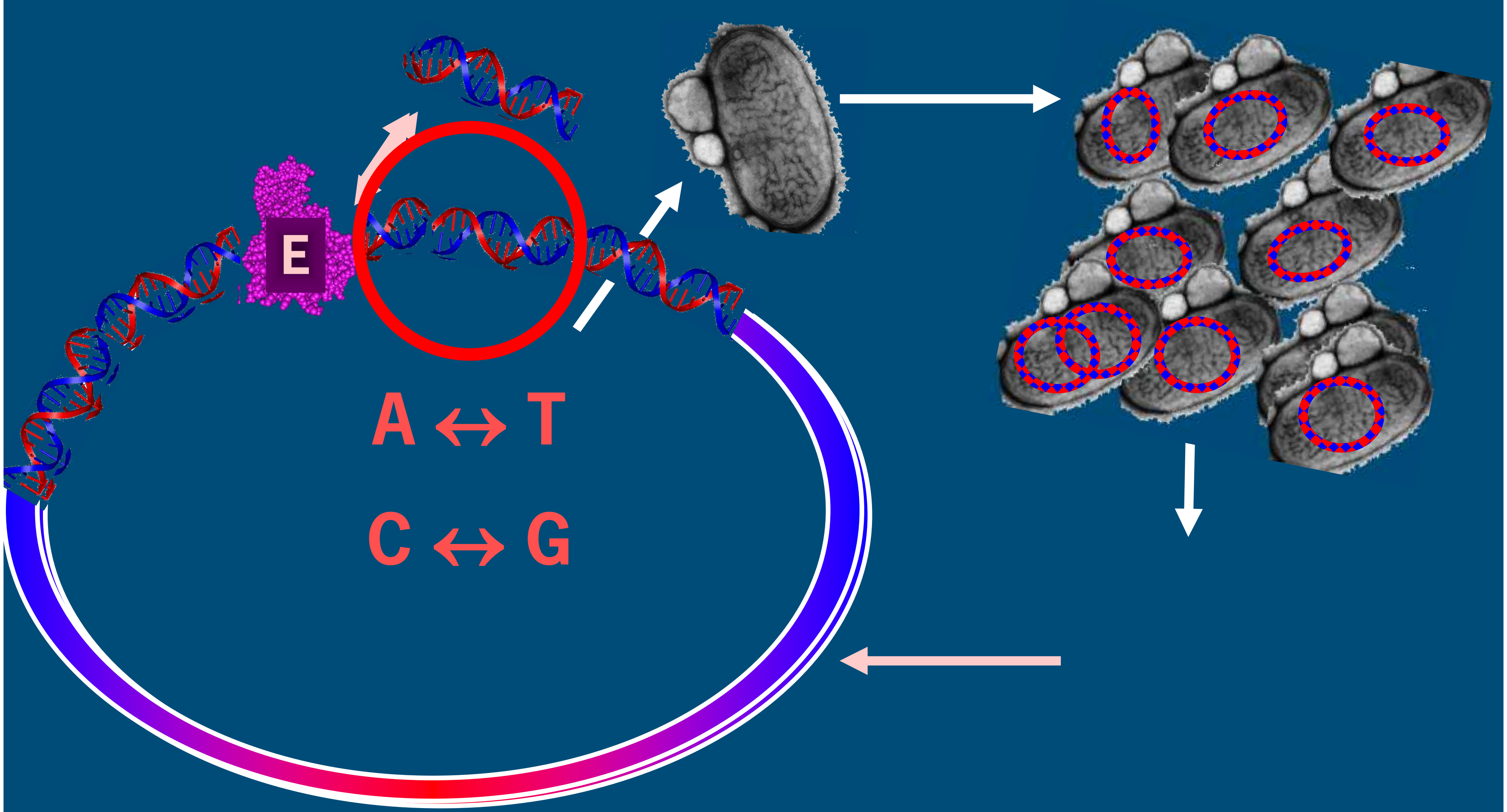
Construction of polymer-encoding genes

DNA segment with
custom-designed
sequence,
encoding a polypeptide:

X_1-X_2-·······X_{n-1}-X_n



DNA templates for protein polymers



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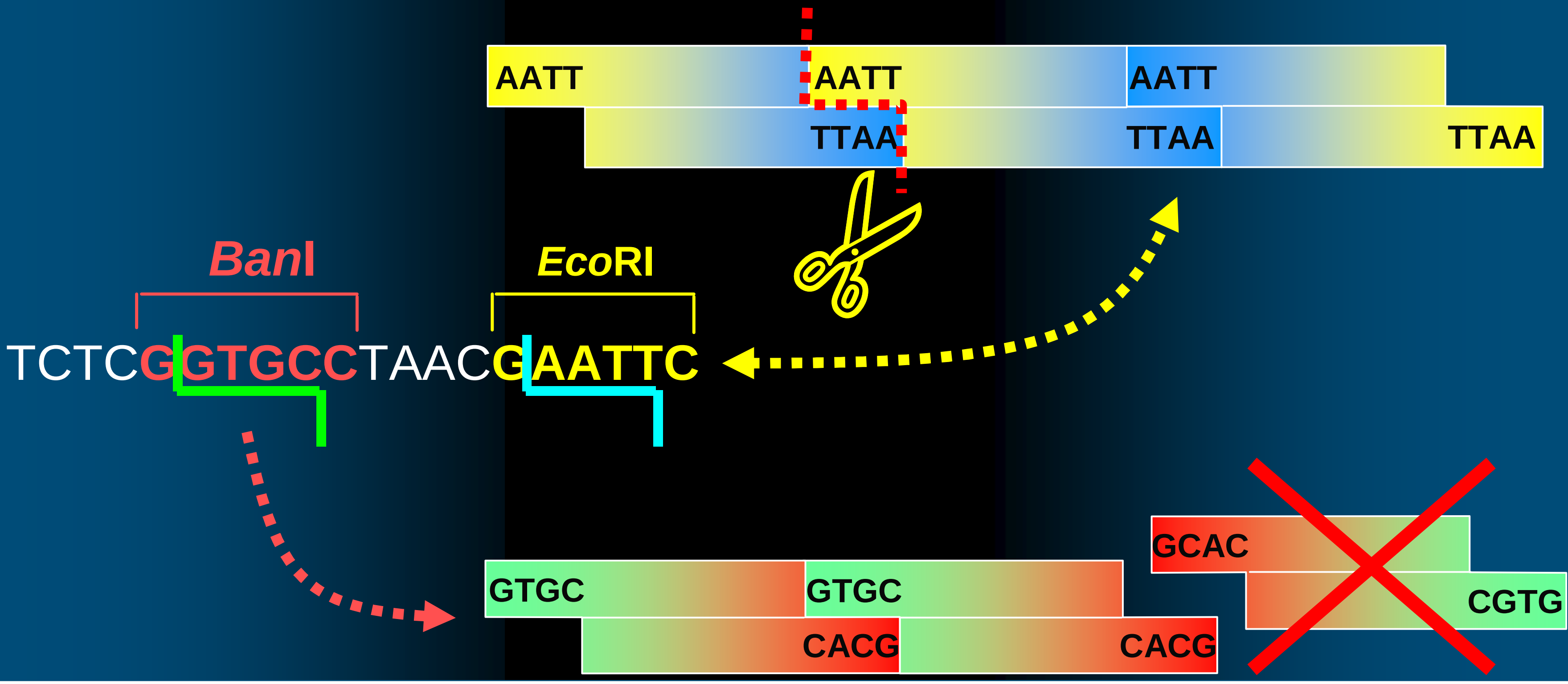
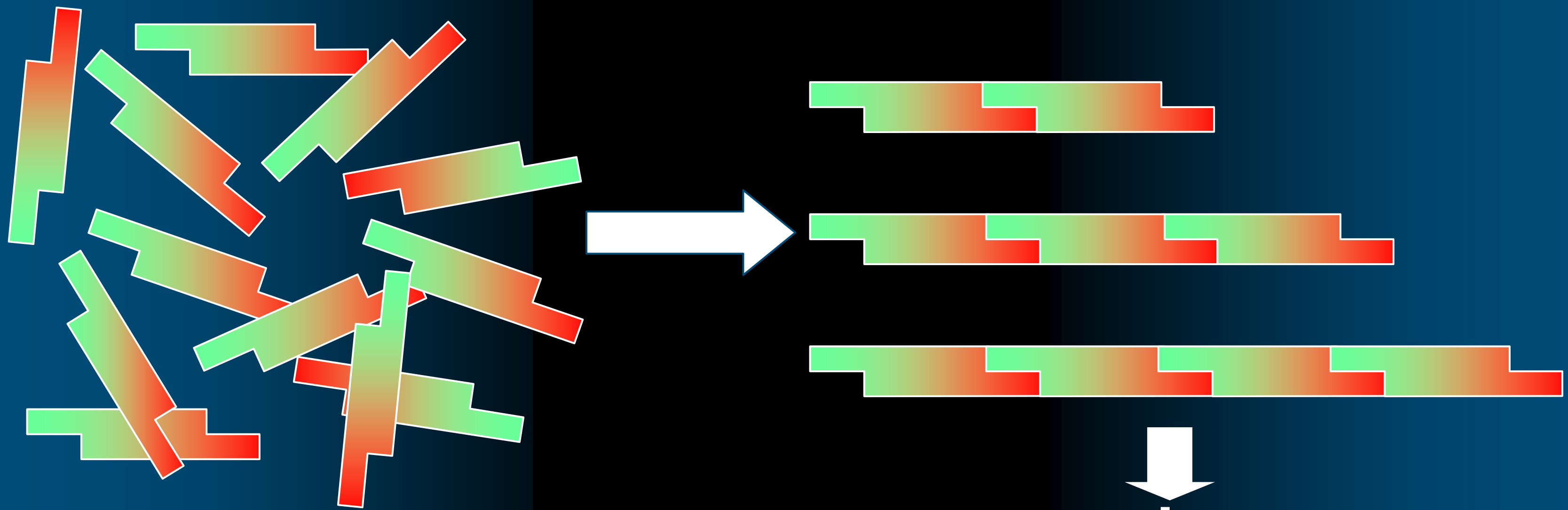
Construction of polymer-encoding genes

Directional polymerization of gene segments



Construction of polymer-encoding genes

Directional polymerization of gene segments

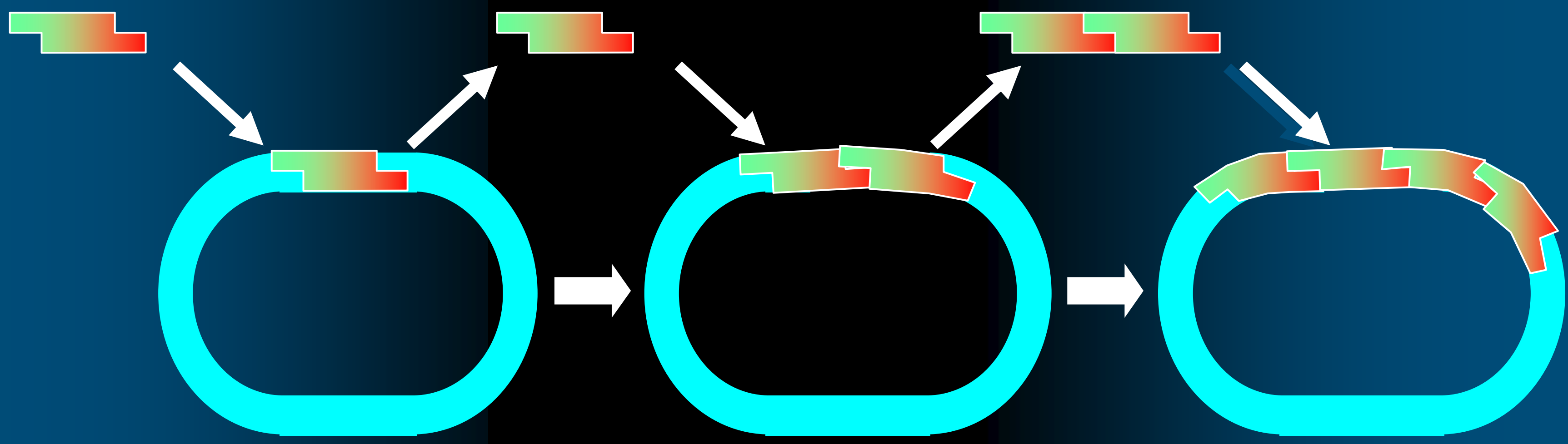


etc.

(separate on gel and select Mw)

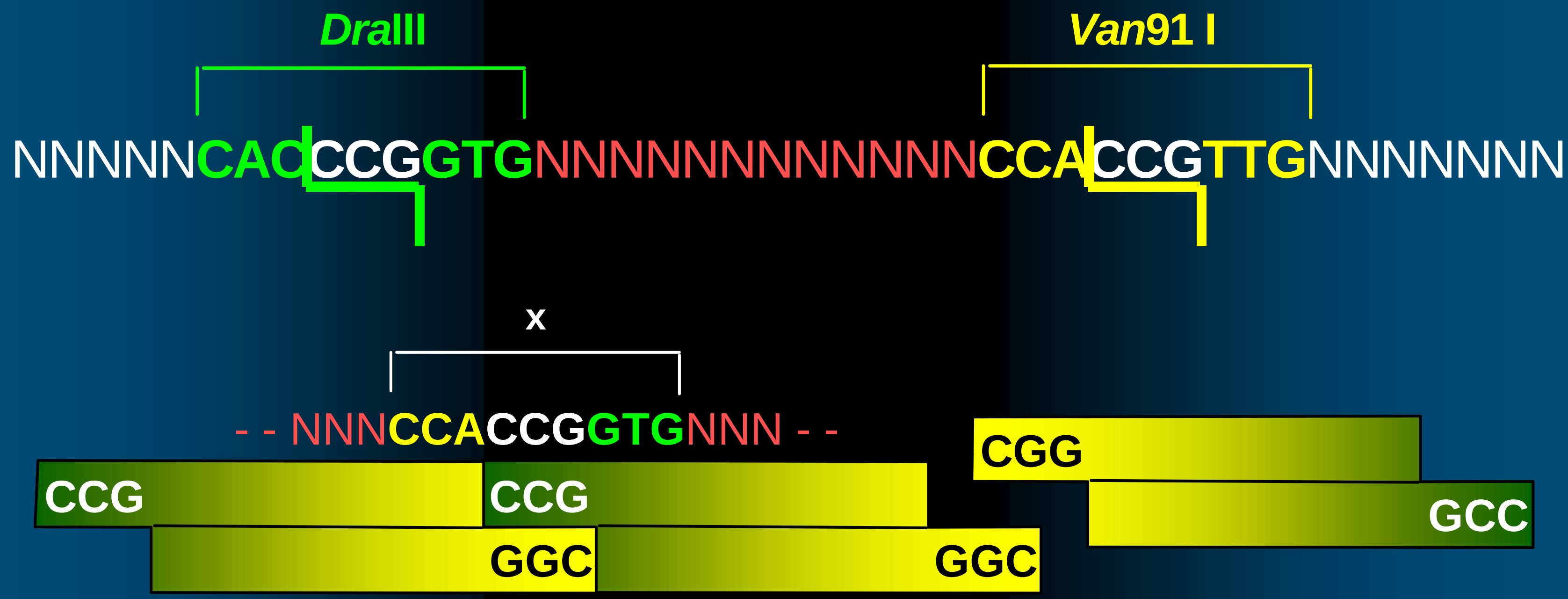
Construction of polymer-encoding genes

Recursive directional ligation



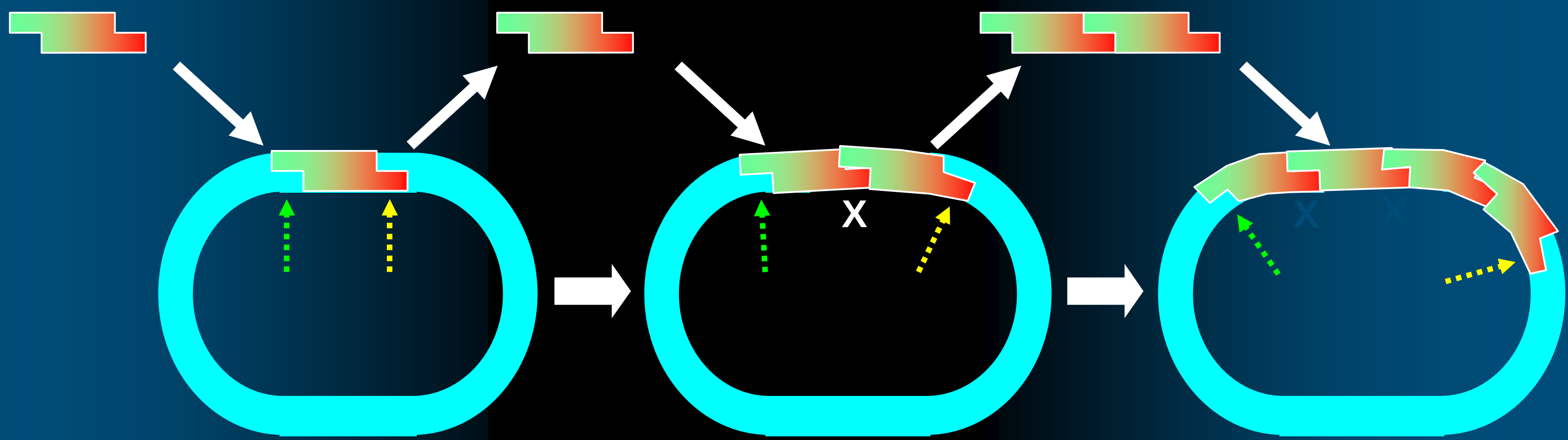
Construction of polymer-encoding genes

Recursive directional ligation



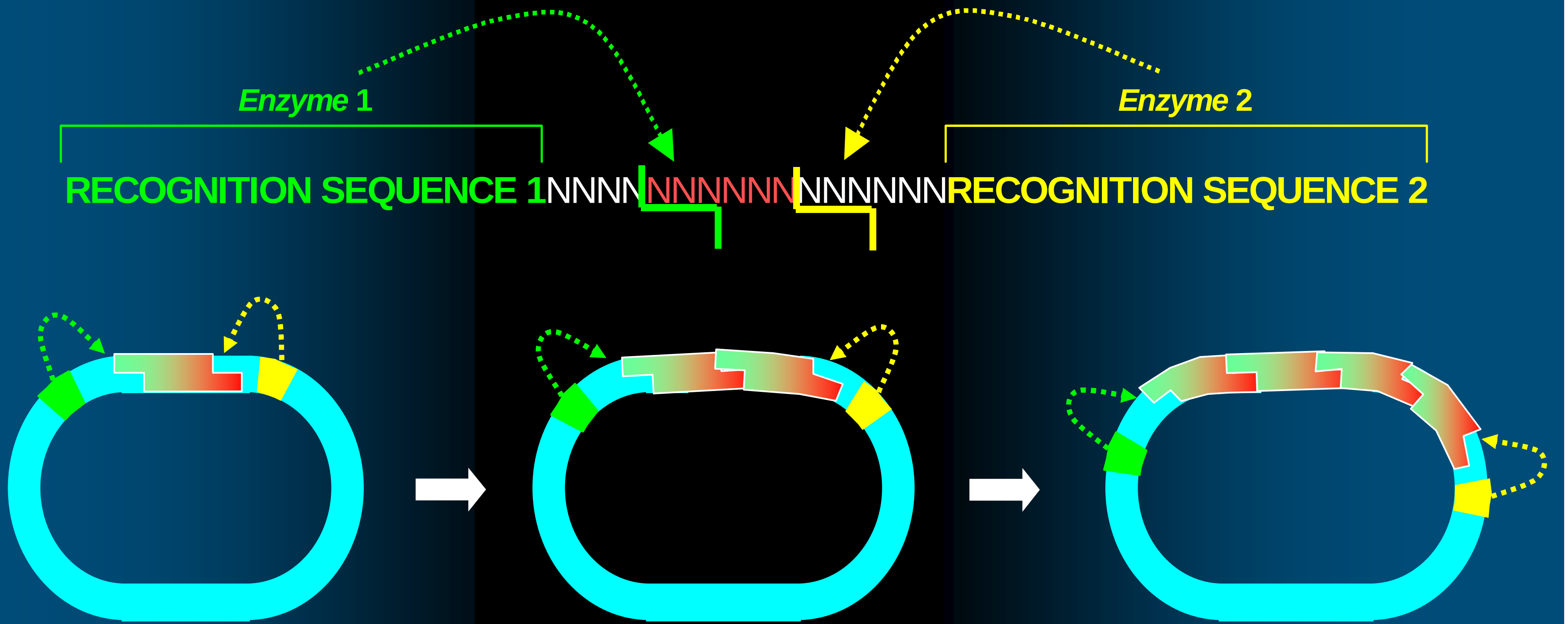
Construction of polymer-encoding genes

Recursive directional ligation



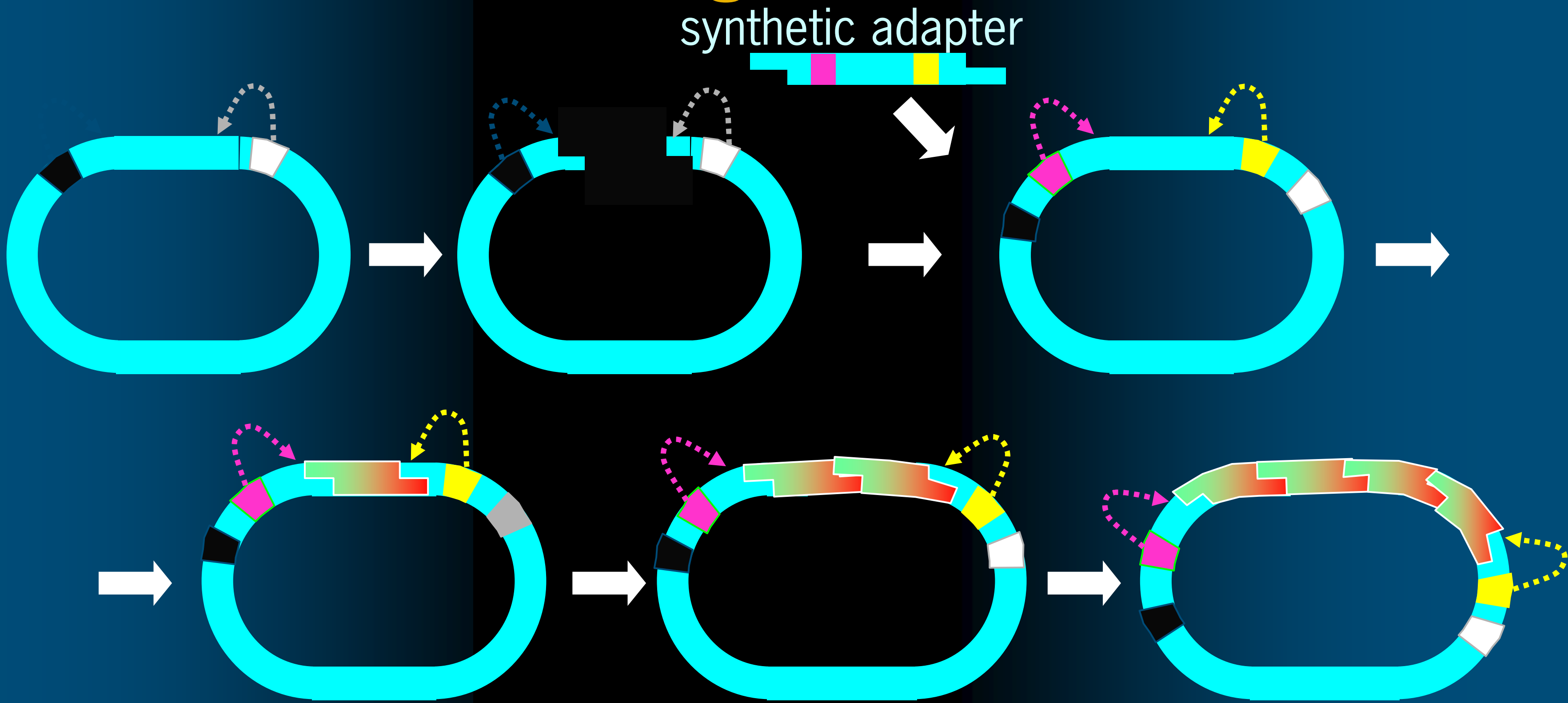
Construction of polymer-encoding genes

Recursive directional ligation



Construction of polymer-encoding genes

Recursive directional ligation



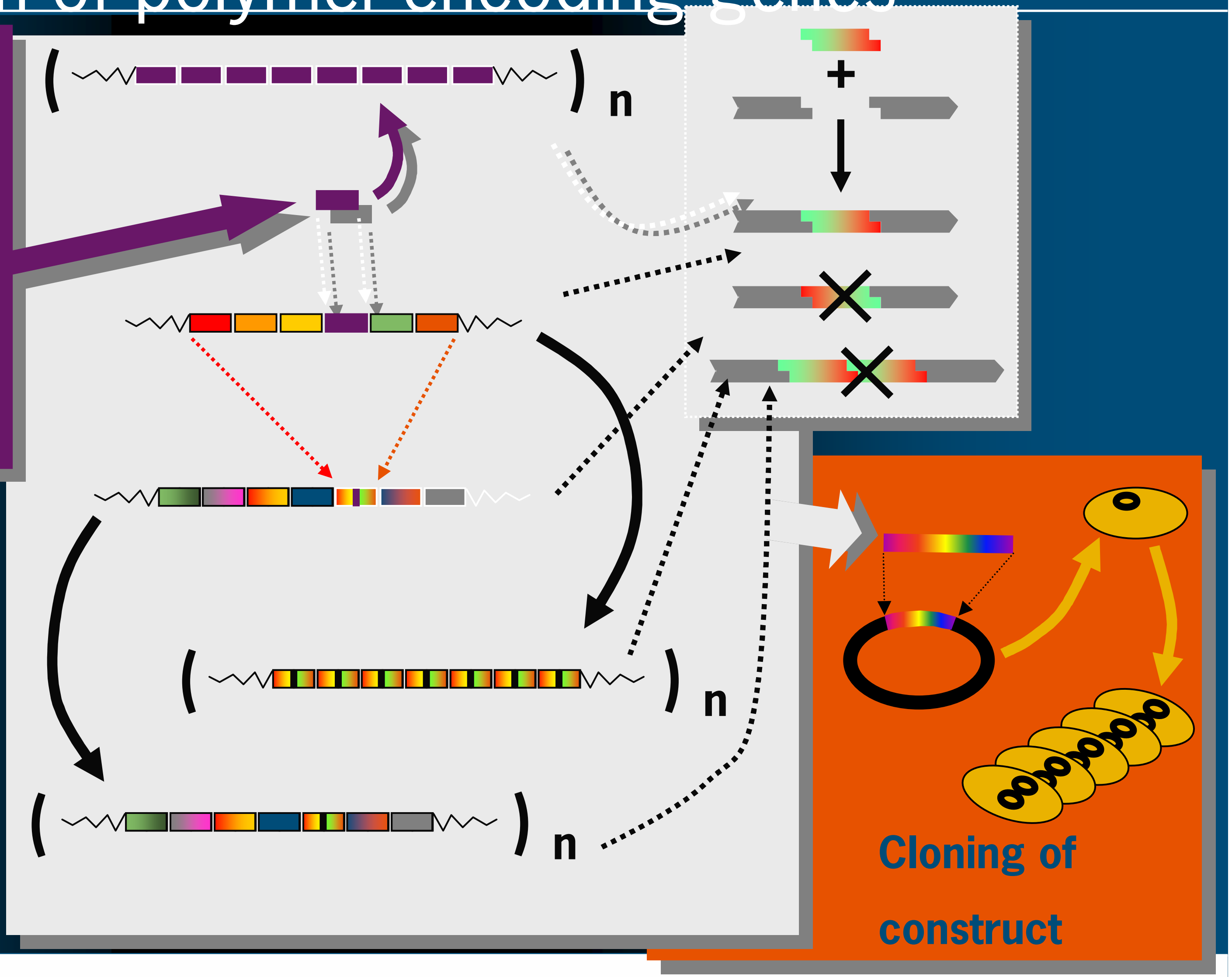
Construction of polymer-encoding genes

Recursive directional ligation

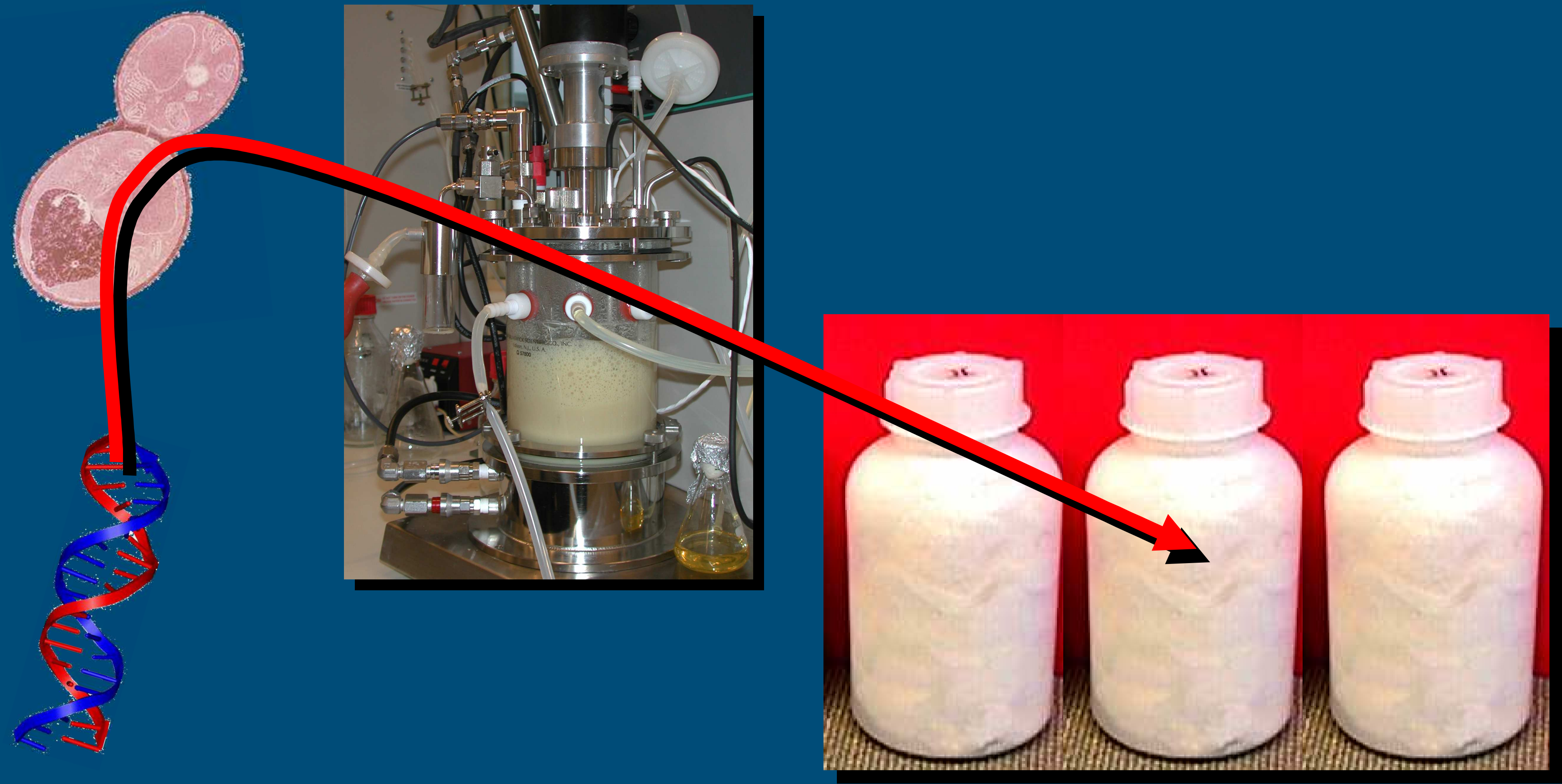
Construction of polymer-encoding genes

DNA segment with custom-designed sequence, encoding a polypeptide:

X_1-X_2-·······X_{n-1}-X_n



Protein polymer biosynthesis



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(Thank you for your attention)

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