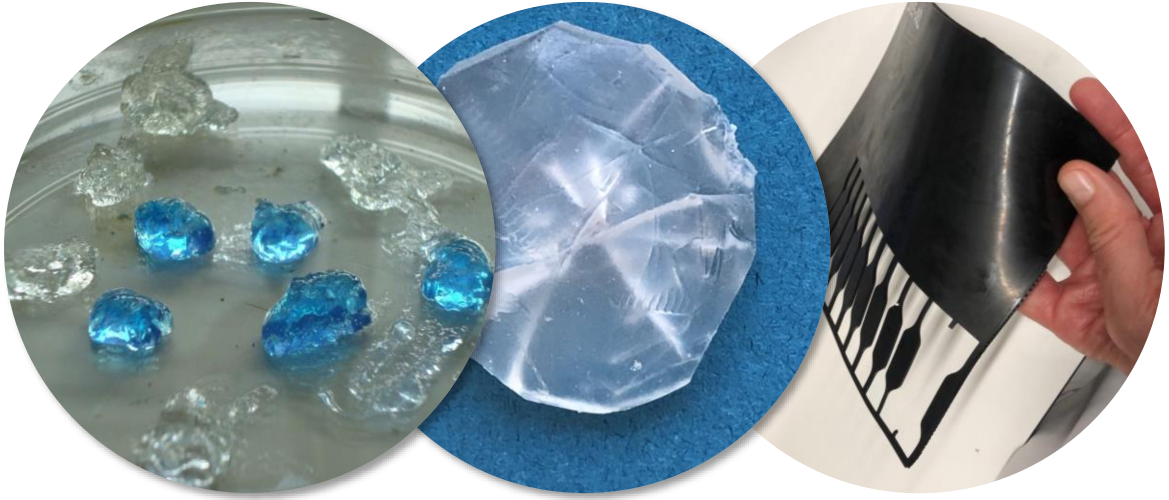
Versatile polymers from microbes

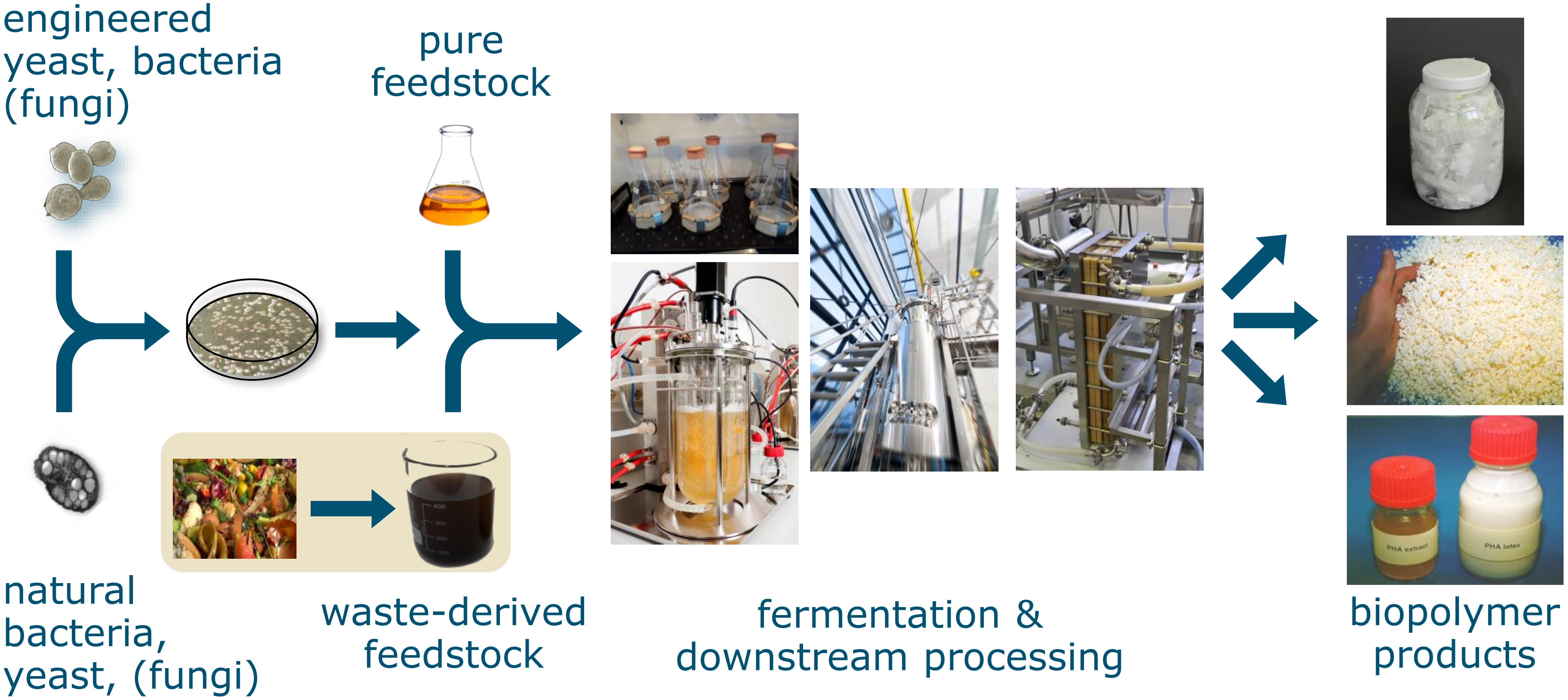
Circular and Biobased Products Symposium

June 22nd, 2023, Frits de Wolf, Wageningen Food & Biobased Research

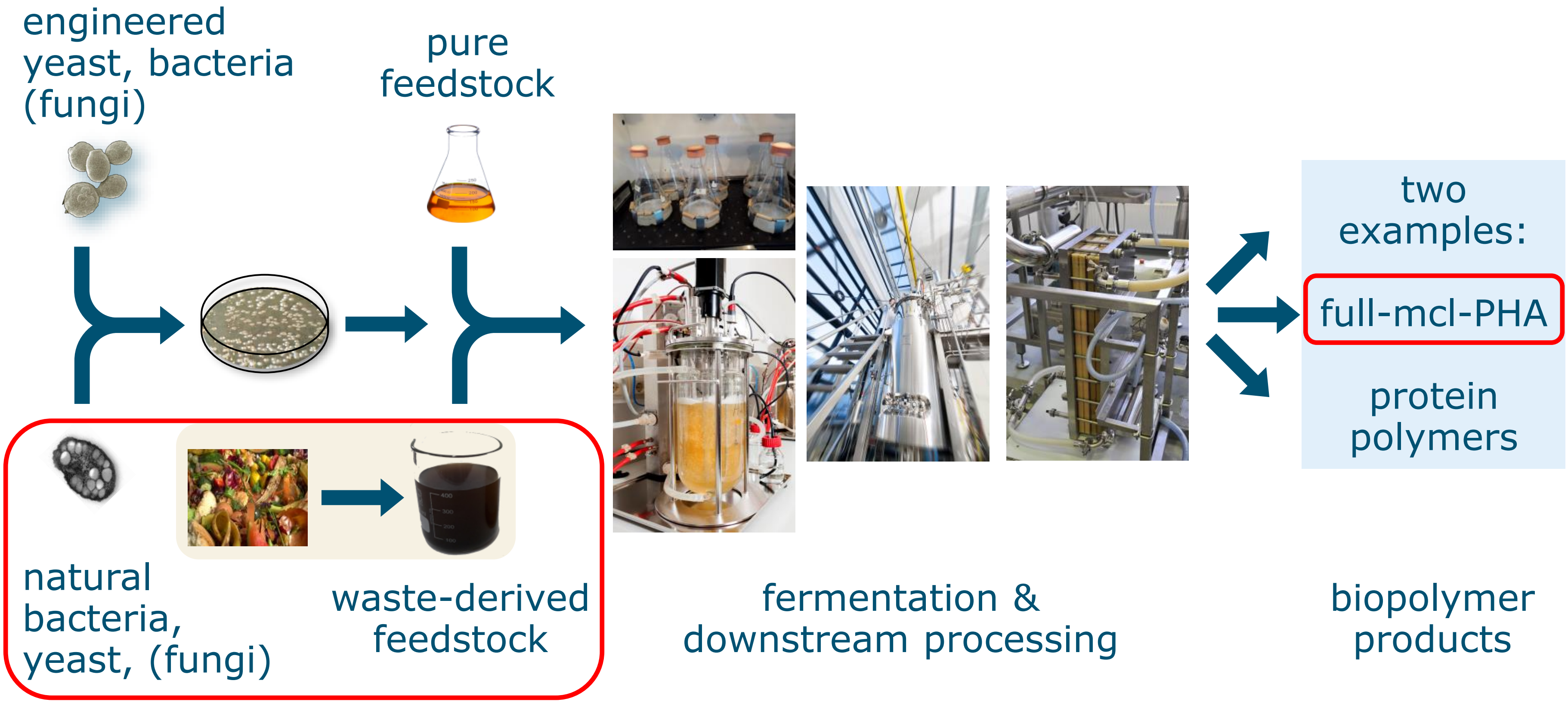


WAGENINGEN
UNIVERSITY & RESEARCH

Microbial (bio)polymer production

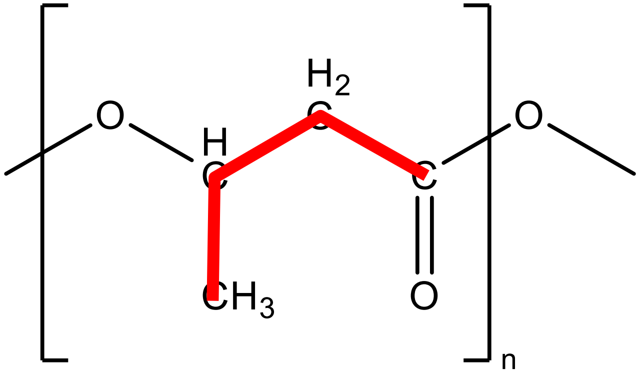


Microbial (bio)polymer production



Full-mcl-PHA: 100% C6-C14 monomer units

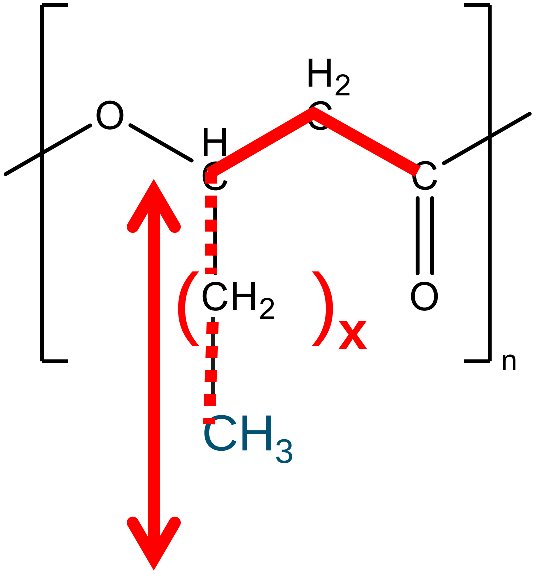
Well known: scl-PHA (PHB/PHBV)



exclusively **C4** / C5 monomer units

contrast with
more commonly available “mcl”-PHA
(= PHB with low % C6/C8/C10)

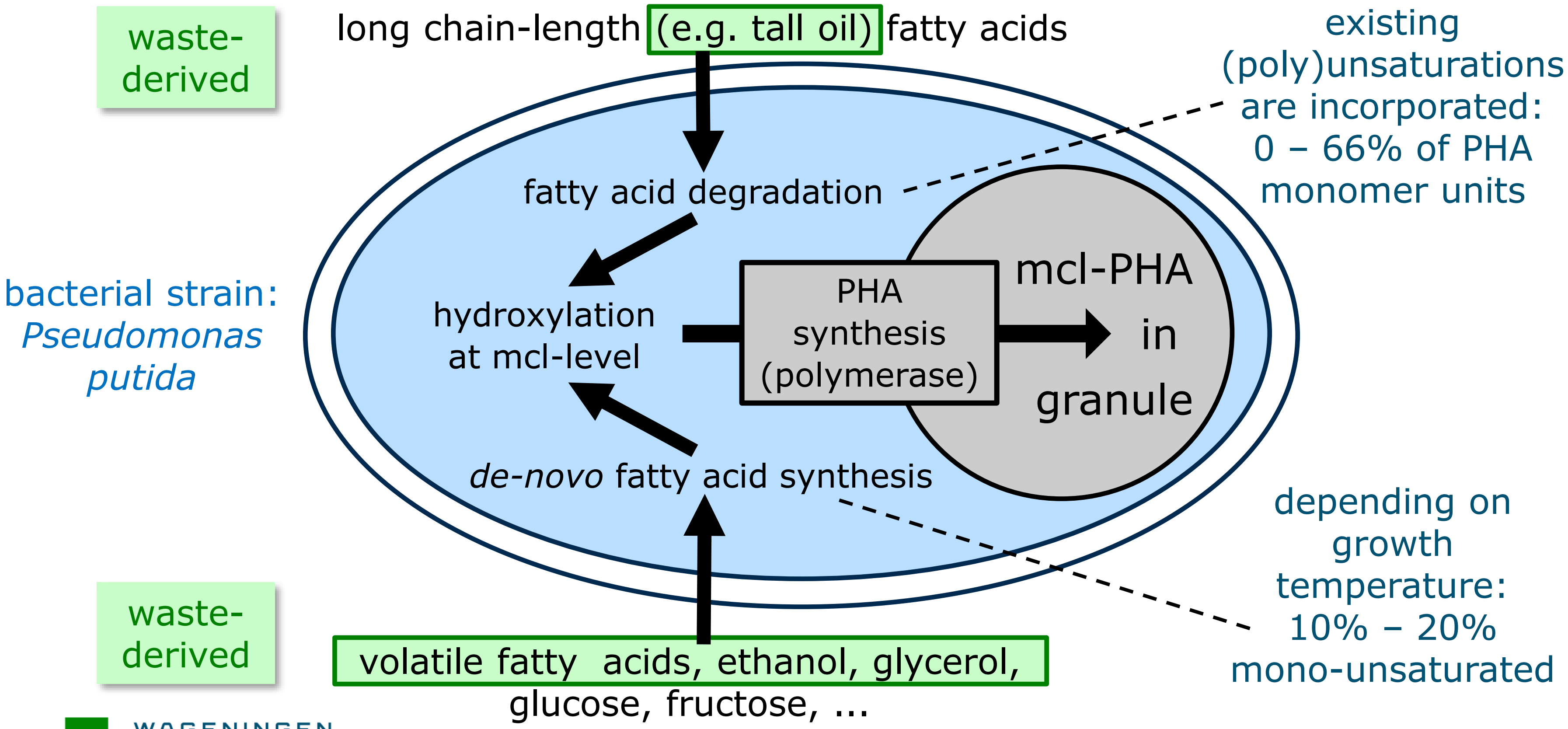
More special: mcl-PHA



exclusively **C6 – C14** monomer units

presence of **<u>un</u>**saturated side chains with **C=C** bonds

Feedstock-dependent full-mcl-PHA synthesis



Full-mcl-PHA isolation from bacterial cells

By solvent extraction:

water-washed, wet cells (no drying energy)

↓

ethanol wash (lipid removal)

↓

extraction, e.g. by ethyl acetate

↓

solid/liquid separation

↓

evaporation & solvent recycling

By water-borne processing:

water-washed, wet cells

↓

enzyme/alkaline/heat/surfactant treatment

↓

washing full-mcl-PHA particles

↓

precipitation by pH shift

↓

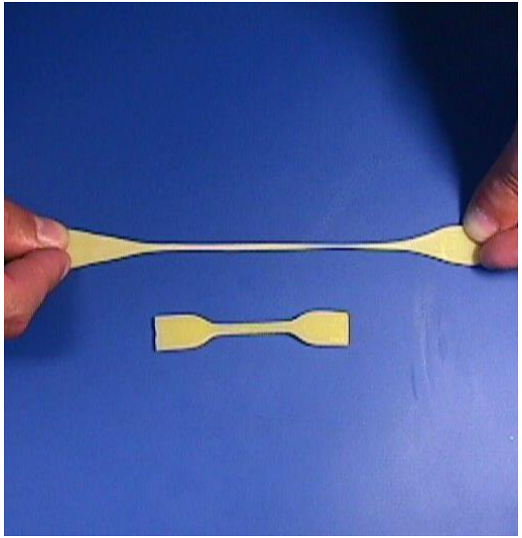
drying

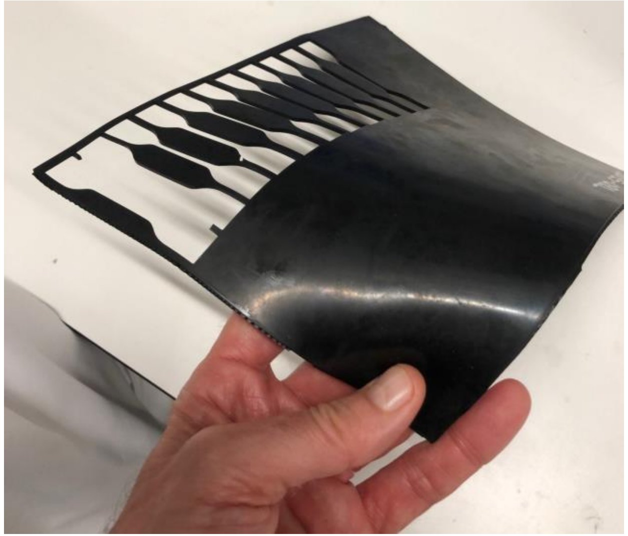
Full-mcl-PHA properties tailored by feedstock

from VFA, ethanol, glycerol, or glucose:
T_g -50 °C
stiff at room temperature, crystallinity ~13%
T_m +60 °C

from tall oil fatty acid or linseed oil:
T_g -60 °C
fluid at room temperature
T_m & crystallinity not detectable

Full-mcl-PHA properties modified post-synthesis





crosslinking
of C=C
double bonds

Versatile full-mcl-PHA post synthesis processing

polymeric mcl-PHA
from green solvent / latex
→ partial / full hydrolysis (bio-catalytically / at high pH)

↓

coatings, paints,
glues, rubbers

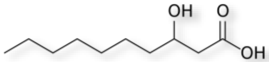
partial / full hydrolysis
(bio-catalytically / at high pH)

↓

oligo-esters → compatibilizers

↓

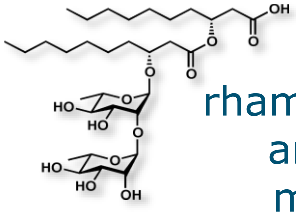
free, monomeric
hydroxy fatty acids → anti-microbials, wetting agents



↓

bio-catalysis

↓



rhamnolipid-like
and other
molecules → anti-microbials, bio-surfactants

WAGENINGEN
UNIVERSITY & RESEARCH

Versatile full-mcl-PHA post synthesis processing

polymeric mcl-PHA
from green solvent / latex

→ partial / full hydrolysis
(bio-catalytically / at high pH)

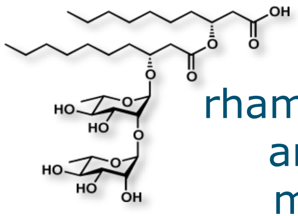
- Toxic to cells used for production
- Low titers / continuous removal required
- Isolation is difficult: surfactants bind to interfaces, protein, cells, etc.

oligo-esters → compatibilizers

free, monomeric
hydroxy fatty acids → anti-microbials, wetting agents

hard to produce directly by fermentation

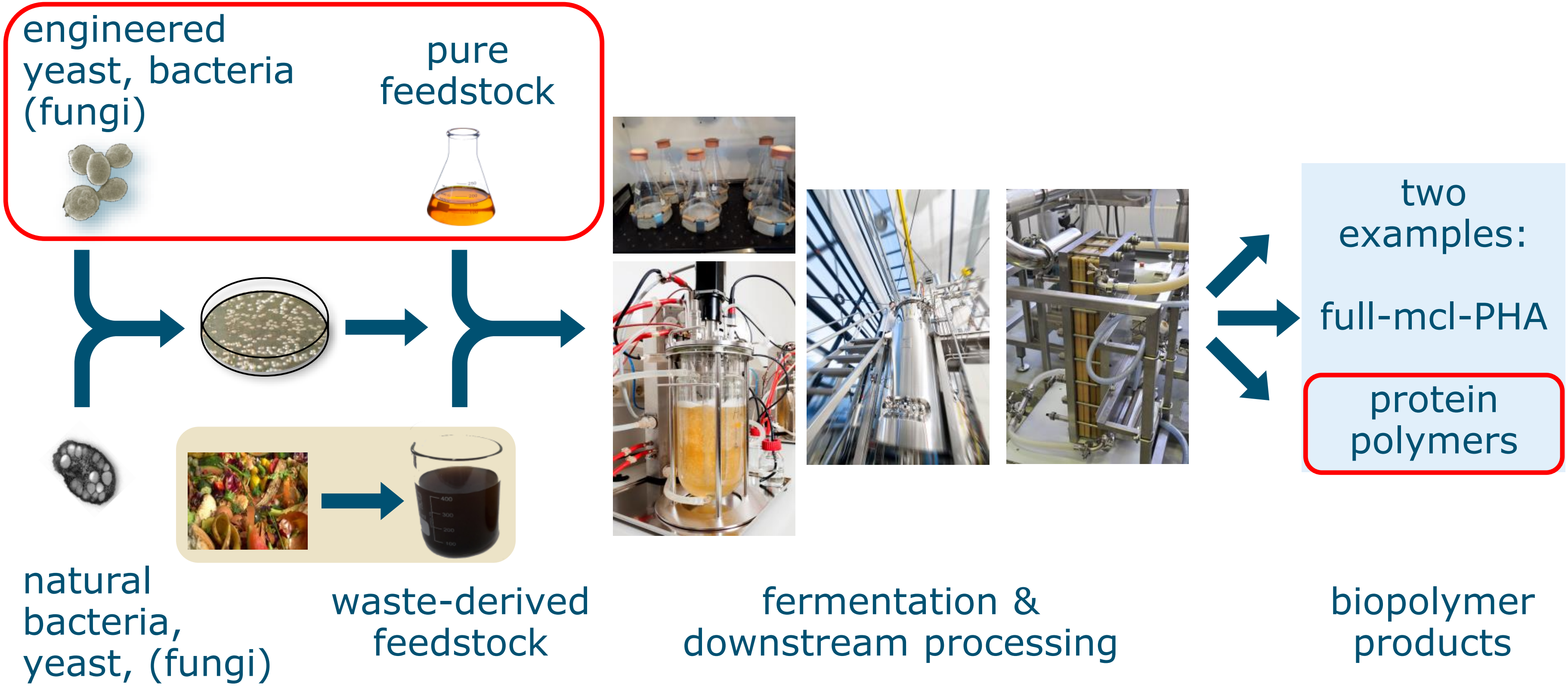
bio-catalysis



rhamnolipid-like
and other
molecules → anti-microbials, bio-surfactants

WAGENINGEN
UNIVERSITY & RESEARCH

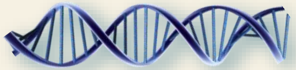
Microbial (bio)polymer production



Protein polymer materials are not new, but still in development

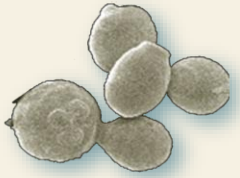
- Silk (from silk worms): ~ 4000 BC
- Collagen / leather / glue / gelatin: probably even older
- Casein (for glue, buttons, ties: 1st half of 20th century)
- End 20th century – present: protein polymers produced by microbes
    - Spider silk: for example Spiber (Japan) / the North Face; Amsilk (Germany) / Adidas, Bold Threads (USA)
    - Collagen: for example Modern Meadow (USA)
    - Various protein polymers for biomedicine/cosmetics/...

Modular protein polymers from Wageningen FBR



synthetic designer gene

yeast or bacterial cells



fermentation

product isolation:

cell removal by microfiltration

↓

bulk precipitation

↓

washing

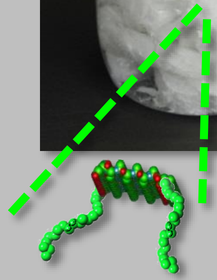
↓

desalting

↓

drying

designer protein polymer:



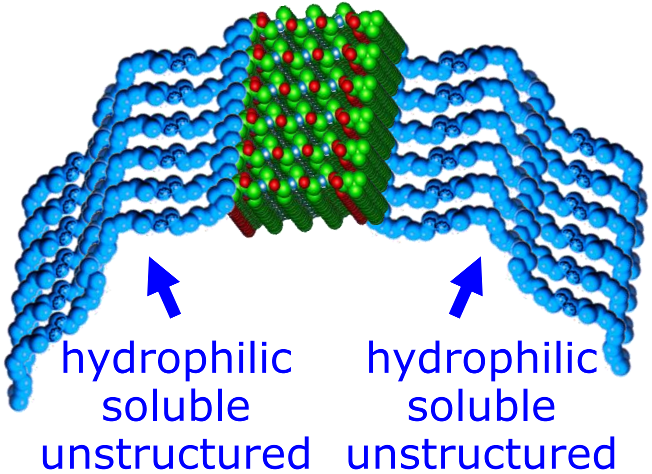
Example: tri-modular designer protein polymer

pH-responsive silk:

soluble unstructured

↓ ↑

crystal

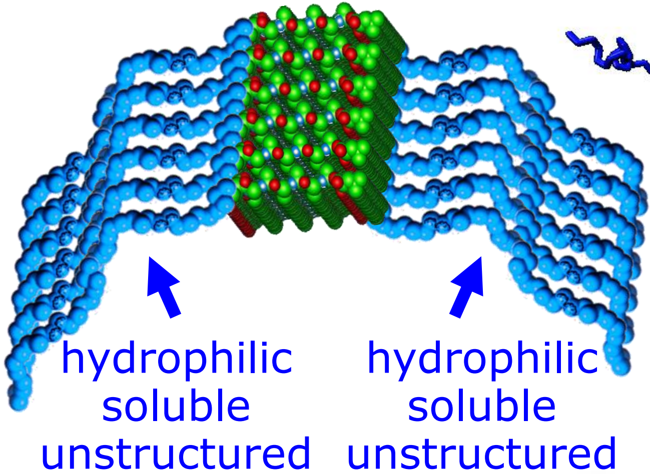


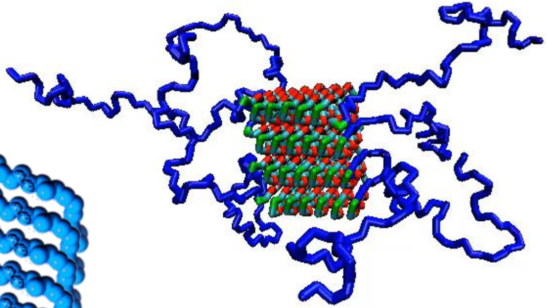
Example: tri-modular designer protein polymer

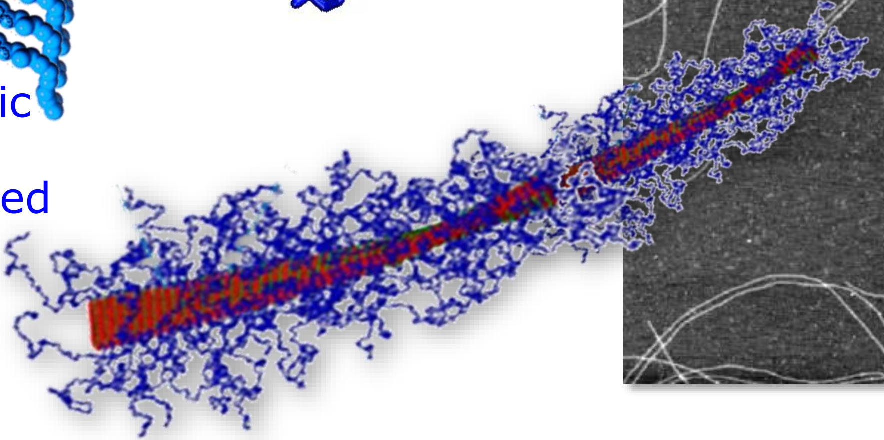
pH-responsive silk:

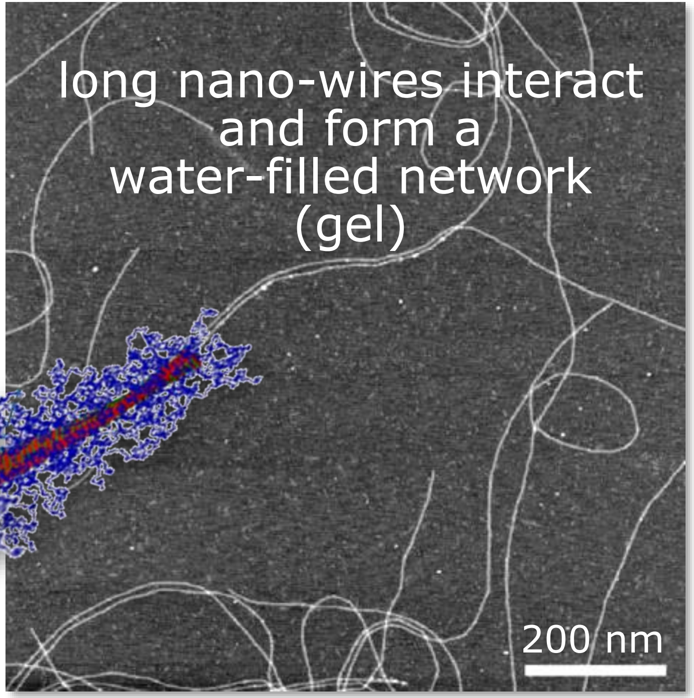
soluble unstructured
↓ ↑
crystal

choice by design: { acidic gel ←→ neutral fluid
neutral gel ←→ acidic fluid }

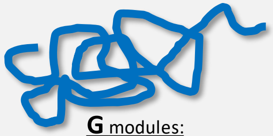




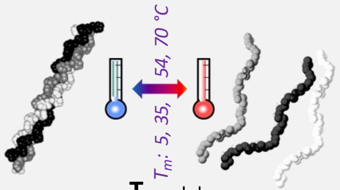




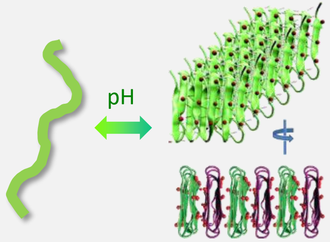
Modular protein polymers: examples of modules developed at WFBR



G modules:
natural col1a1 or col3a1
gelatin (i.e. Gly-Xxx-Yyy triplet) sequence
non-hydroxylated, non-gelling random coil
up to very high production levels: 15 g/L

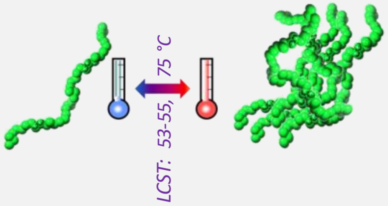


T modules:
short collagen modules forming triple helical trimeric knots below the melting temperature (T_m), which is well tunable from 5 to 70 °C

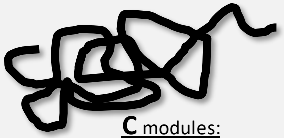


long beta sheet stacks
at pH > 5-6,
or at pH < 4-5
with different polymer modules

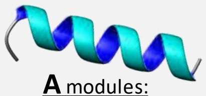
S modules:
silk-inspired pH-responsive
$(GAGAGAGX)_n$ modules, X being pH-responsive
(X = His for stacks at neutral pH, in ‘ S^H ’)



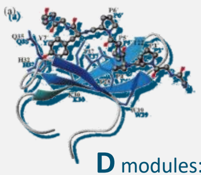
E modules:
elastin-inspired $(VPGXG)_n$ modules:
soluble random coils below,
and in condensed phase above the ‘LCST’ temperature



C modules:
very hydrophilic,
gelatin-like (i.e. Gly-Xxx-Yyy triplet) designer sequence:
99 residues, ~22 % Pro, pI ~4.9
non-hydroxylated, non-gelling random coil

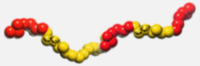


A modules:
short antibacterial modules



D modules:
heterodimer-forming modules

B^{H6} / B^{K12} / ... module series:
cationic poly(Lys) / poly(His) modules
binding to DNA or negatively-charged surfaces,
or with His-tag function. Possibly anti-bacterial.
Natural B^{Ssod7} binds specifically to DNA without preference for a sequence



B^{RGD} / B^{KRSR} modules:
short glycine-rich random coil module with
either two GRGDSP cell-binding,
or three KRSR osteoblast-stimulating sequences

WAGENINGEN UNIVERSITY & RESEARCH

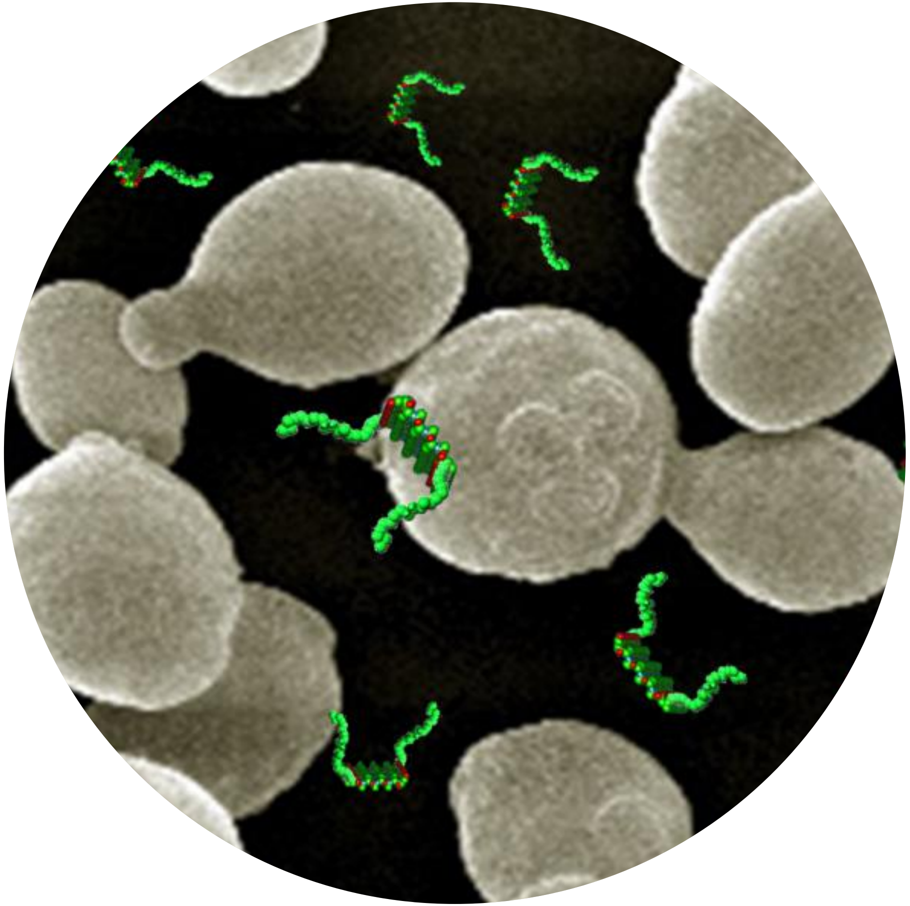
Microbial protein polymers: ‘unlimited’ versatility

- Replacement of more traditional materials: silk, collagen, etc.
- Food colloids,
- Edible coatings
- Personal care: responsive / bioactive / selectively binding / anti-microbial gels
- Biomedicine:
  - cell / tissue culture scaffolds
  - Slow release agents
  - Protective / anti-microbial / anti-freeze materials
  - ……

Different polymers: different 'worlds'

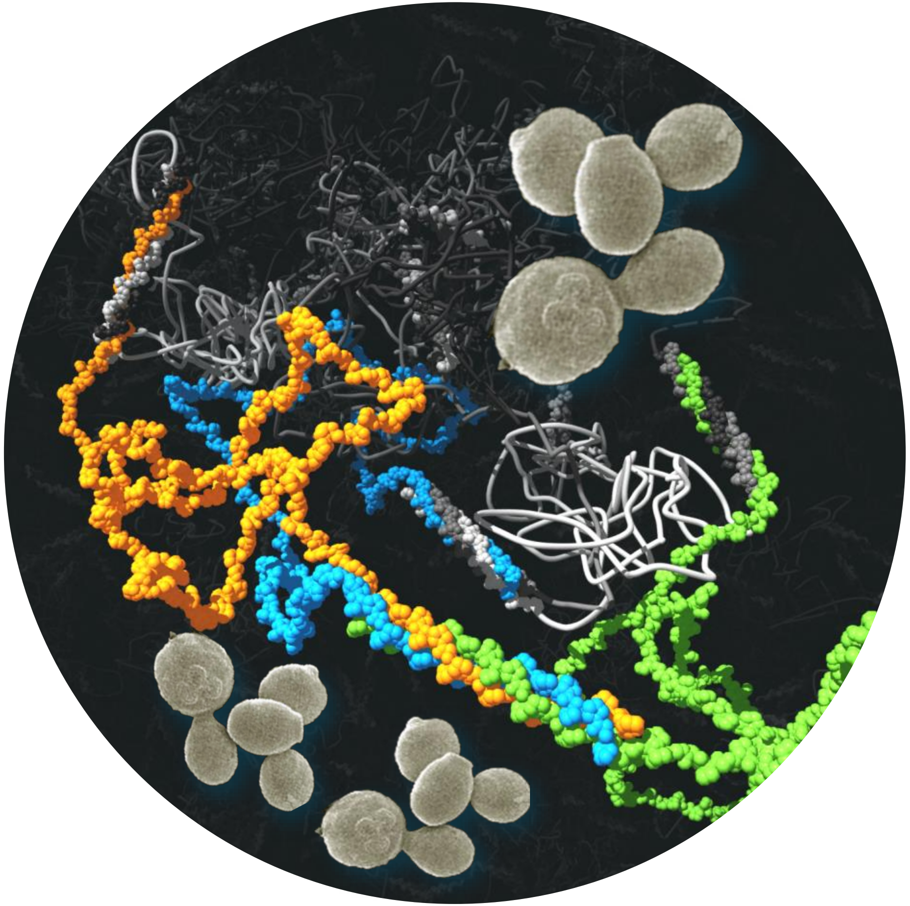
- PE, PP, etc. (non-food): ≤2 €/kg, many million tons/yr
- Animal gelatin (food & non-food): 3-30 €/kg; ~600,000 tons/yr
- Animal collagen (food & non-food): 20-50 €/kg; ~300,000 tons/yr
- Silk worm silk (non-food): 30-40 €/kg; ~150,000 tons/yr
- Scl-PHA (non-food): 10-20 €/kg; as yet ~10,000–100,000 tons/yr
- Full-mcl-PHA (non-food): ~20 €/kg?; as yet low volume
- Microbial designer protein polymers (exactly defined):
    - Silk, gelatin, etc.: (biomedicine, cosmetics, (specialty) food ingredients, *materials?*): 50(?)-500 €/kg; as yet low volume
    - Special functional designer protein polymers (biomedicine): 500-5000 €/kg; low volume

Thank you for your attention



WAGENINGEN
UNIVERSITY & RESEARCH

Thank you for your attention



WAGENINGEN UNIVERSITY & RESEARCH

Versatile full-mcl-PHA post synthesis processing

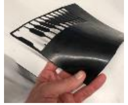
polymeric mcl-PHA
from green solvent / latex

↓

coatings, paints,
glues, rubbers







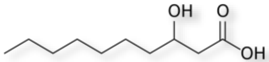
→ partial / full hydrolysis
(bio-catalytically / at high pH)

↓

oligo-esters → compatibilizers

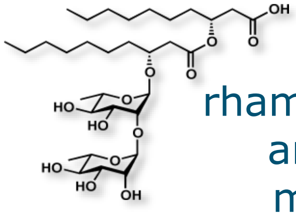
↓

free, monomeric
hydroxy fatty acids → anti-microbials,
wetting agents



↓

bio-catalysis



↓

rhamnolipid-like
and other
molecules → anti-microbials,
bio-surfactants

hard to produce
directly
by fermentation

Full-mcl-PHA composition* tailored by feedstock

Substrate	$C_{6:0}$	$C_{8:0}$	$C_{8:1}$	$C_{10:0}$	$C_{10:1}$	$C_{12:0}$	$C_{12:1}$	$C_{12:2}$	$C_{14:0}$	$C_{14:1}$	$C_{14:2}$	$C_{14:3}$	$C_{16:3}$
Glucose	tr[b]	6.9	–	74.3	–	7.7	8.8	–	tr[b]	1.6	–	–	–
Fructose	0.5	12.6	–	70.8	–	5.7	8.5	–	0.3	1.6	–	–	–
Decanoic acid	5.3	52.3	–	42.3	–	–	–	–	–	–	–	–	–
Glycerol	1.7	21.4	–	63.6	–	3.8	8.6	–	0.1	0.8	–	–	–
Coconut fatty acids	3.8	38.1	–	37.8	–	18.1	1.0	–	1.1	–	–	–	–

*of *Pseudomonas putida*

From Huijberts et al. (1992) Appl. Eviron. Microbiol. 58 (2) 536-44

Full-mcl-PHA composition* tailored by feedstock

Substrate	$C_{6:0}$	$C_{8:0}$	$C_{8:1}$	$C_{10:0}$	$C_{10:1}$	$C_{12:0}$	$C_{12:1}$	$C_{12:2}$	$C_{14:0}$	$C_{14:1}$	$C_{14:2}$	$C_{14:3}$	$C_{16:3}$
Glucose	tr[b]	6.9	–	74.3	–	7.7	8.8	–	tr[b]	1.6	–	–	–
Fructose	0.5	12.6	–	70.8	–	5.7	8.5	–	0.3	1.6	–	–	–
Decanoic acid	5.3	52.3	–	42.3	–	–	–	–	–	–	–	–	–
Linoleic acid	5.6	38.9	–	22.7	–	–	15.9	–	–	–	16.9	–	–
Glycerol	1.7	21.4	–	63.6	–	3.8	8.6	–	0.1	0.8	–	–	–
Coconut fatty acids	3.8	38.1	–	37.8	–	18.1	1.0	–	1.1	–	–	–	–
Oleic acid	4.4	33.5	–	32.2	–	14.4	tr[b]	–	–	15.5	tr[b]	–	–
Tall oil fatty acids	4.1	22.9	5.2	26.6	–	5.9	11.4	–	–	5.5	17.7	–	–
Linseed oil fatty acids	2.5	11.6	7.9	13.3	7.8	5.6	3.5	9.1	1.1	5.0	6.8	19.1	6.7

*from *Pseudomonas putida*: % fatty acid

From Huijberts et al. (1992) Appl. Eviron. Microbiol. 58 (2) 536-44

WAGENINGEN UNIVERSITY & RESEARCH

Full-mcl-PHA composition* tailored by feedstock

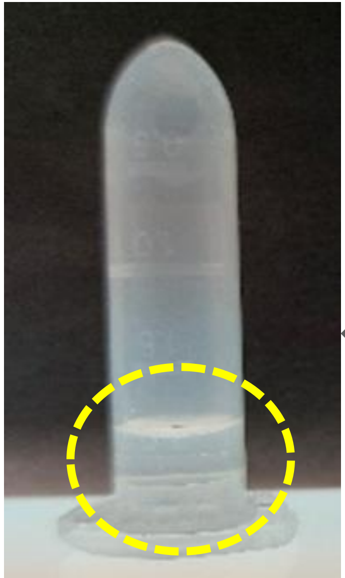
Substrate	% unsaturated monomers
Glucose	10.4
Fructose	10.1
Decanoic acid	0
Linoleic acid	32.8
Glycerol	9.4
Coconut fatty acids	1.0
Oleic acid	15.5
Tall oil fatty acids	39.8
Linseed oil fatty acids	65.9

*from *Pseudomonas putida*: % fatty acid

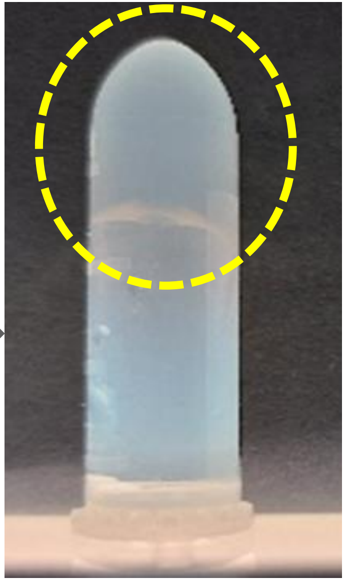
From Huijberts et al. (1992) Appl. Eviron. Microbiol. 58 (2) 536-44

Protein polymer materials: collagen-silk-collagen tri-modular polymers

fluid:

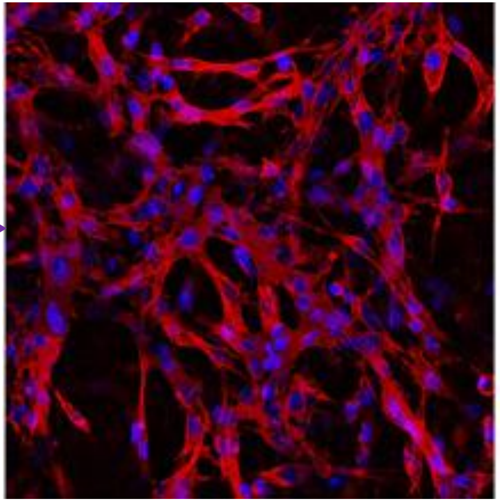


gel:
(1-10 kPa at 1-2% w/v):

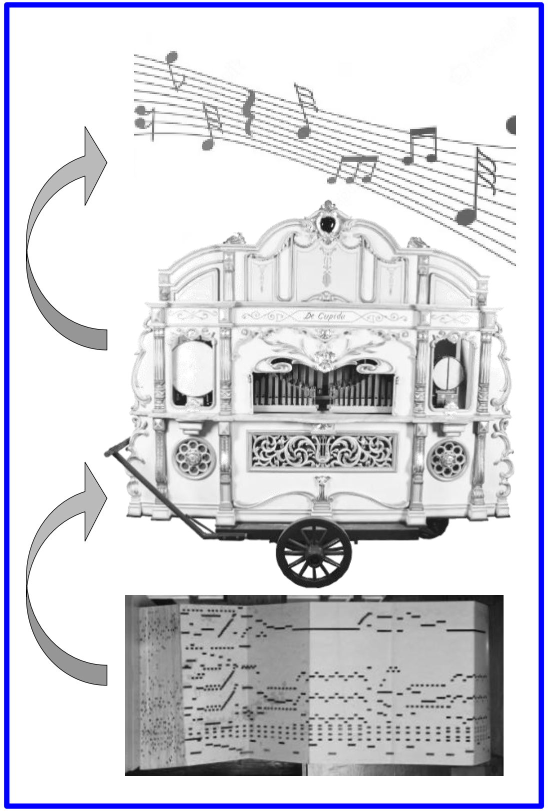


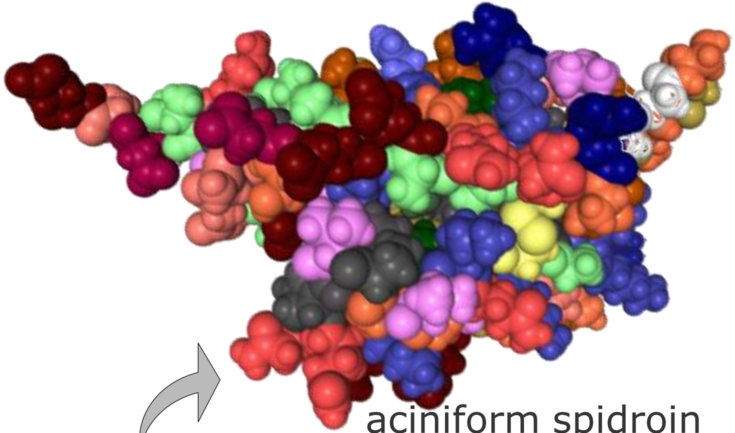
Choice by design:
- fluid when in acid
  ↑↓
- gel at pH >5-6 →
- gel when in acid
  ↑↓
- fluid at pH >5-6

osteoblast cells cultured in gel with additional cell-binding module:

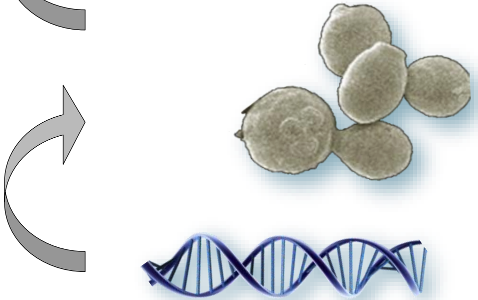


Proteins are monodisperse, sequential amino acid polymers (poly-amide), encoded by DNA



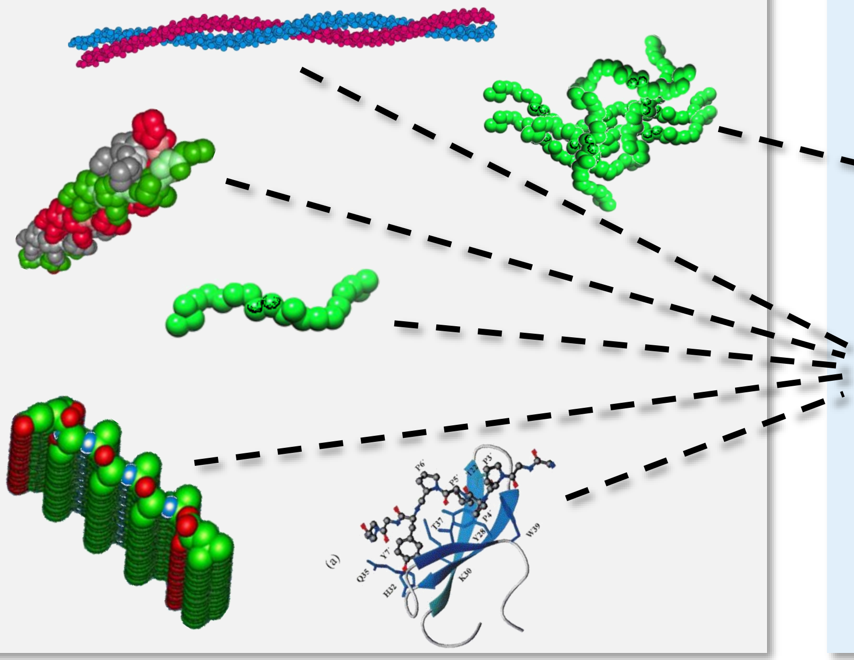


aciniform spidroin
(*Nephila antipodiana*)

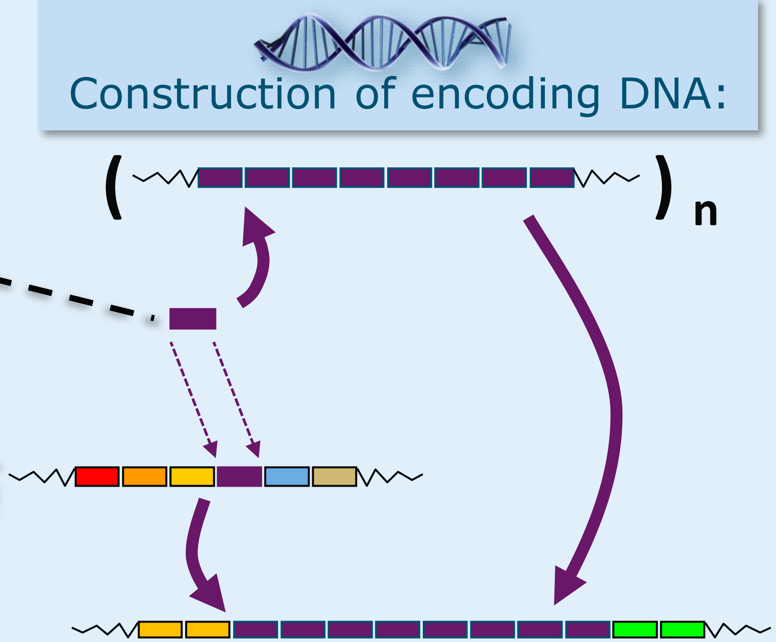


Modular protein polymers (WFBR, PPTI pioneers)

design inspired by protein modules:

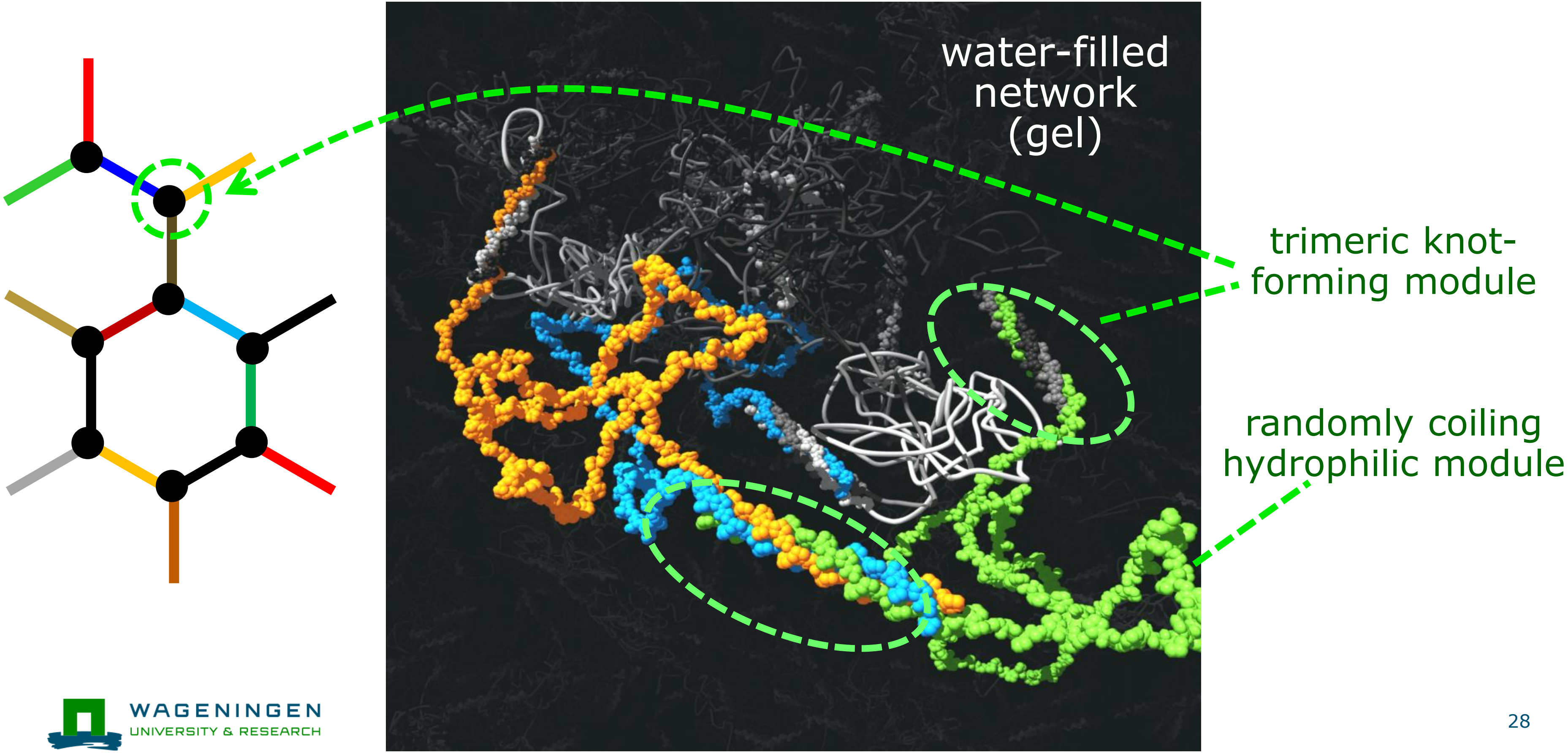


Construction of encoding DNA:

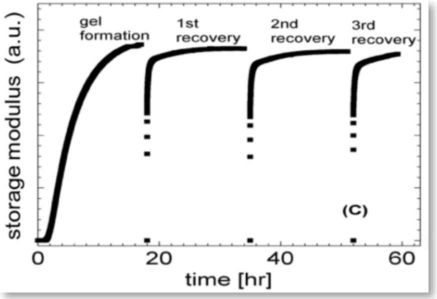


in-vitro "seamless" gene construction

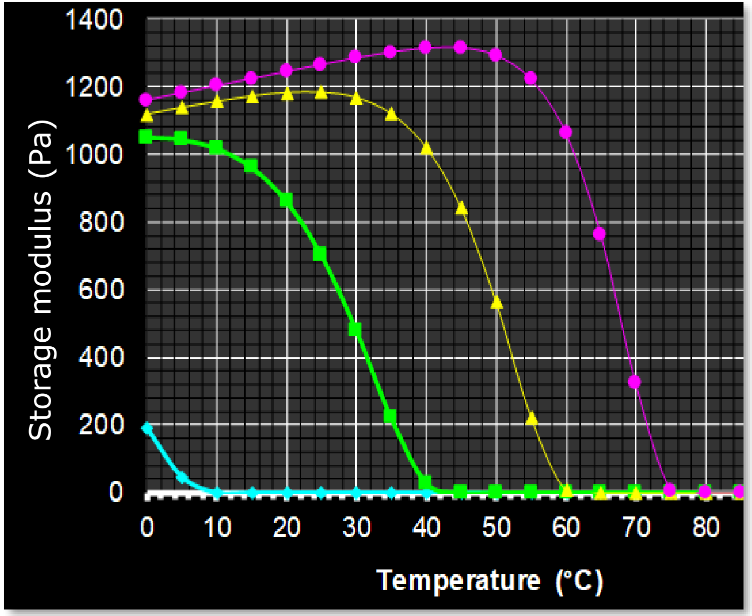
Protein polymer materials: tri-modular gelatin



Protein polymer materials: tri-modular gelatin



Self-healing



Tailored melting temperature

- Suitable for slow release (hrs – weeks)
- Option: built-in activity (anti-microbial, anti-freeze, specific binding)