Production of Recombinant Spider Silk Proteins for Biomedical Material Applications

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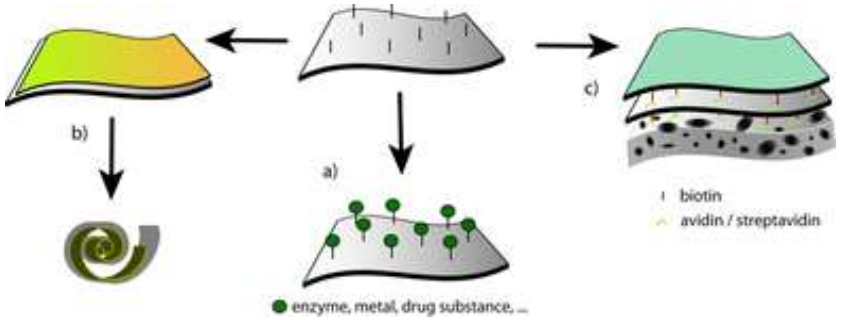
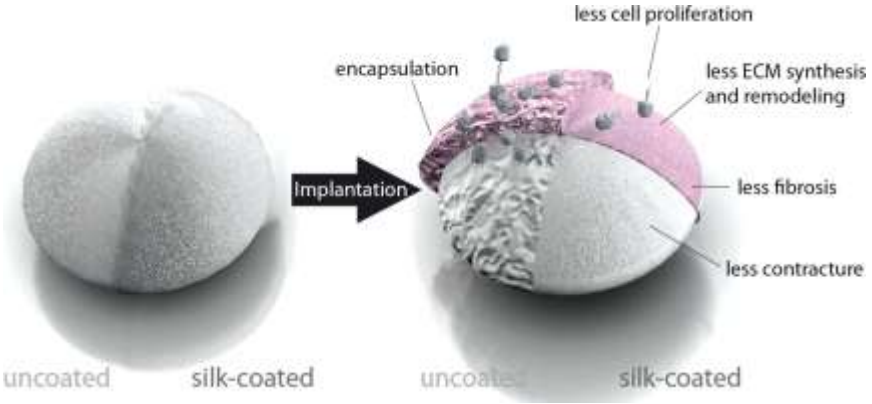
Applications and Uses of SSLPs

Films



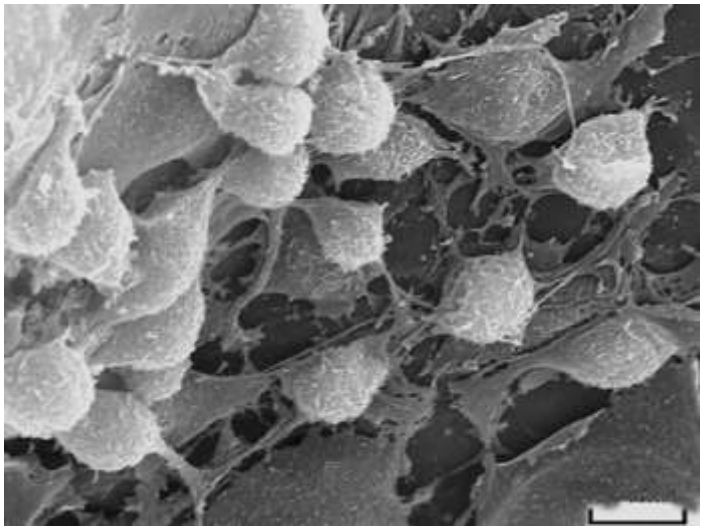
Spieß, K., Wohlrab, S., & Scheibel, T. (2010). *Soft Matter, 6*, 4168-4174.

Coating for implants



Zeplin, P. et. al. (2014). *Adv. Funct. Mater. 24*, 2658-2666

Scaffold for tissue engineering



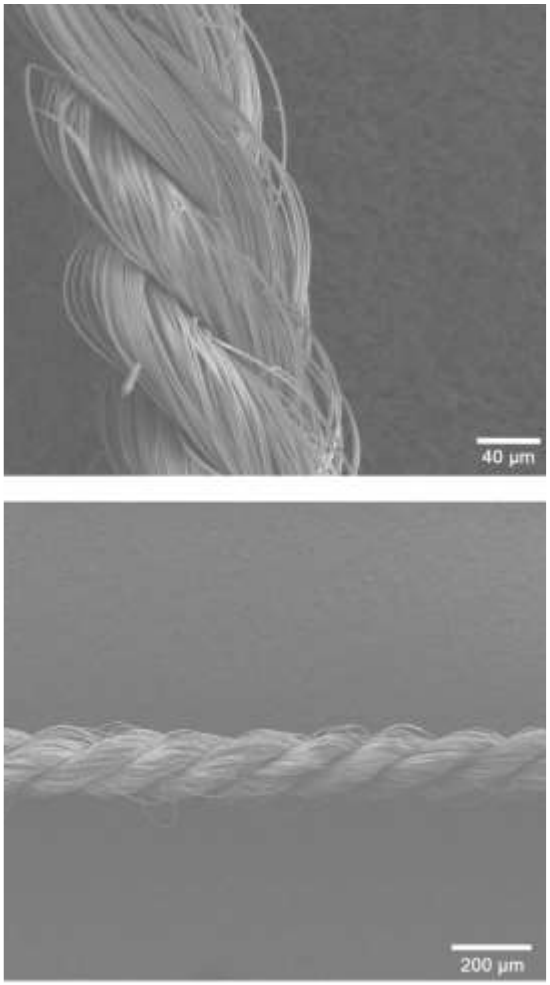
Agapov, I.I., et. al. (2009). *Doklady Biochemistry and Biophysics*, *426*, 127-130.

Applications and Uses of SSLPs

Tendons and Ligaments

MedApparatus. (2013). Anterior Cruciate Ligament.

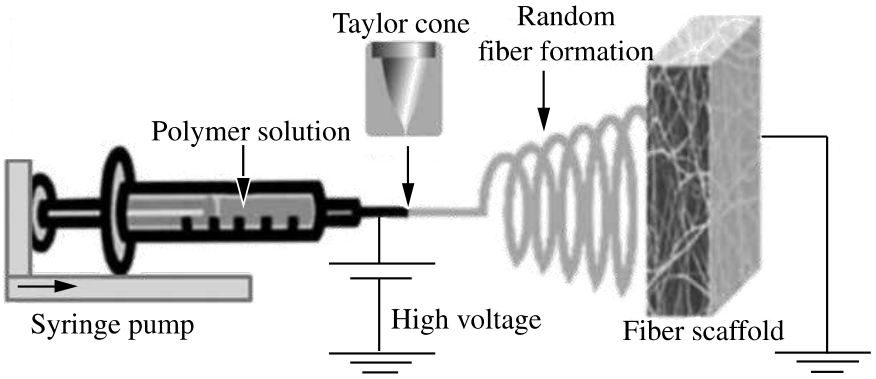
Sutures



Hennecke, K. et. al. (2013). *PLOS ONE, 8*(4), 1-10.

Applications and Uses of SSLPs

Wound dressings



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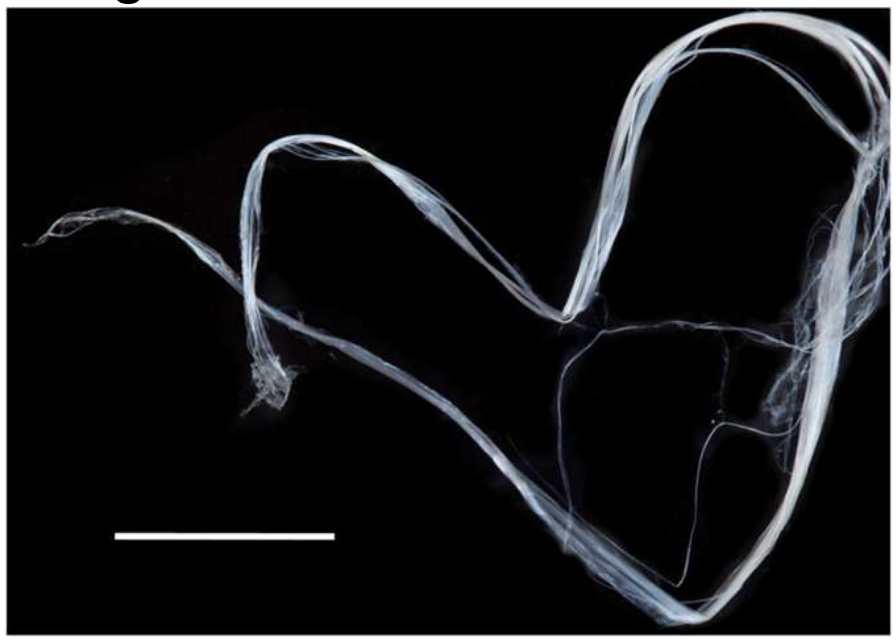
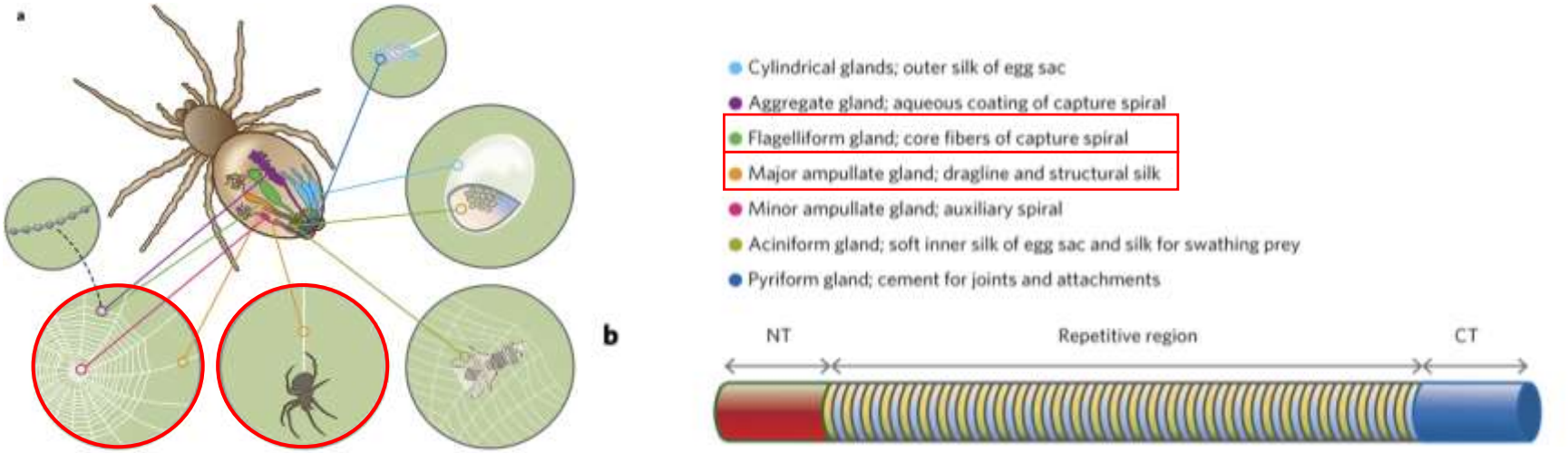


Rising, A., & Johansson, J. (2015). *Nature Chemical Biology, 11*(5), 309-315.

Native Spider Silk Proteins



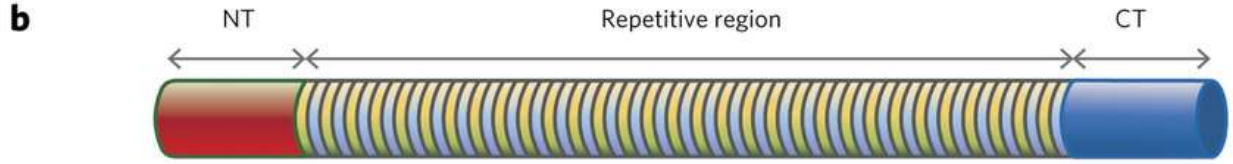
Rising, A., & Johansson, J. (2015). *Nature Chemical Biology, 11*(5), 309-315.

Material	Composition	Extensibility ε_{max} (%)	Modulus $E_{initial}$ (GPa)	Strength σ_{max} (GPa)	Toughness (MJ/m^3)
Dragline silk	MaSp 1 & MaSp 2 proteins	30-35	10	1-4	**160**
Flagelliform silk	Flag protein	200-270	0.003	0.5	150
Collagen tendons (mammalian)	Several proteins	13	1.2	0.12	6
Elastin ligaments (bovine)	Several proteins	150	0.0011	0.002	1.6
Mussel byssus tendons *Distal* *Proximal*	Pre-Col-P, Pre-Col-D & Pre-Col-NG proteins	109 200	0.87 0.016	0.075 0.035	45 35
Kevlar	Para-aramid	2.7	130	3.6	50
Carbon fiber	graphite	1.3	300	4	25
High-tensile steel	Metal alloy	0.8	200	1.5	6

Teulé, F. Fiber Society Meeting (2011, Charleston SC)

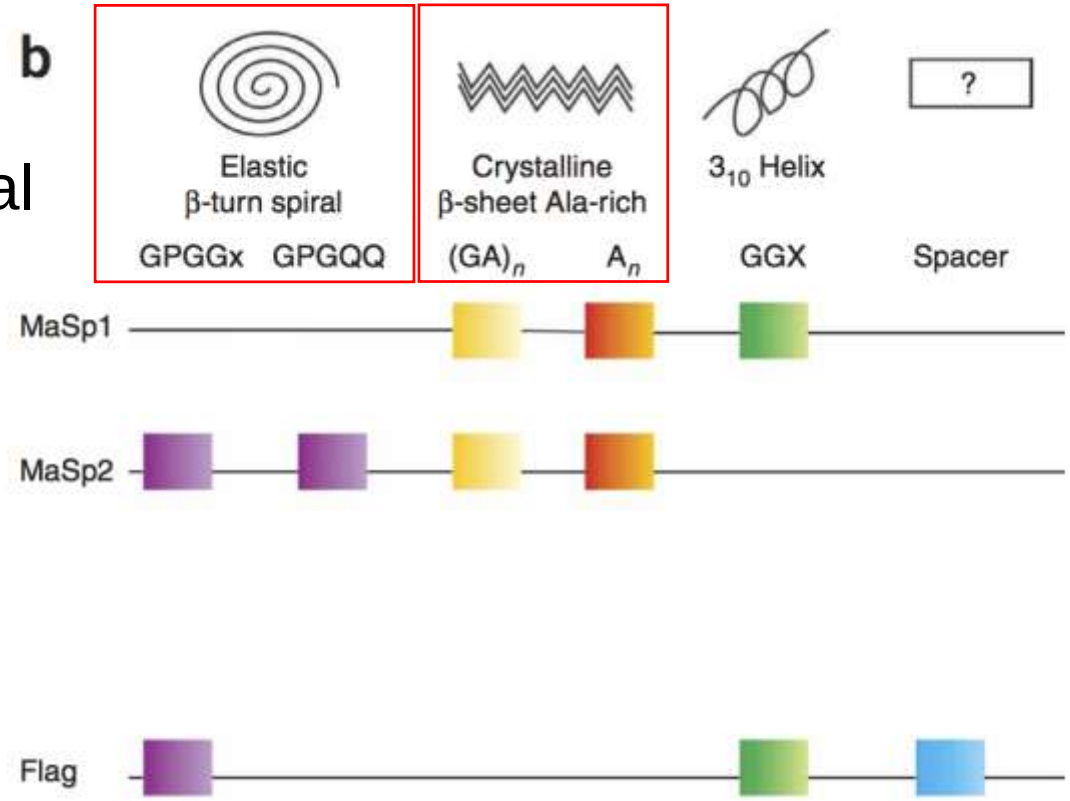
Native Spider Silk Proteins

Modular fibrous proteins with highly repetitive sequences



Rising, A., & Johansson, J. (2015). *Nature Chemical Biology, 11*(5), 309-315.

Amino acid structural motifs



Dragline Silks

Flagelliform Silk

Teulé, F., et. al. (2009). *Nature Protocols, 4*(3), 341-355.

Recombinant Spider Silk Proteins

Silk basic repeat: $(Y1S8)_{20}$
$[(GGYGPGGSGPGGYGPGGSGP)_1\ (GGPSGPGSAAAAAAAAGP)]$

Silk basic repeat: $(A1S8)_{20}$
$[(GGAGPGGAGPGGAGPGGAGP)_1\ (GGPSGPGSAAAAAAAAGP)]$

Y1/A1 = Flag-like elastic forming motif
S8 = MaSp2-like (dragline) crystalline forming motif

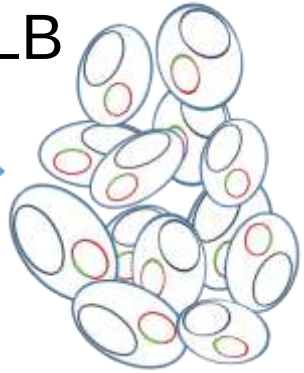
MG$(H)_{10}$SSGHIDDDDKHMLEDPP-[silk repeat]$_{20}$-EISGSGC

MW_{SSLP}: ~60 kDa

Methods – Protein Production

Recombinant bacteria with plasmid

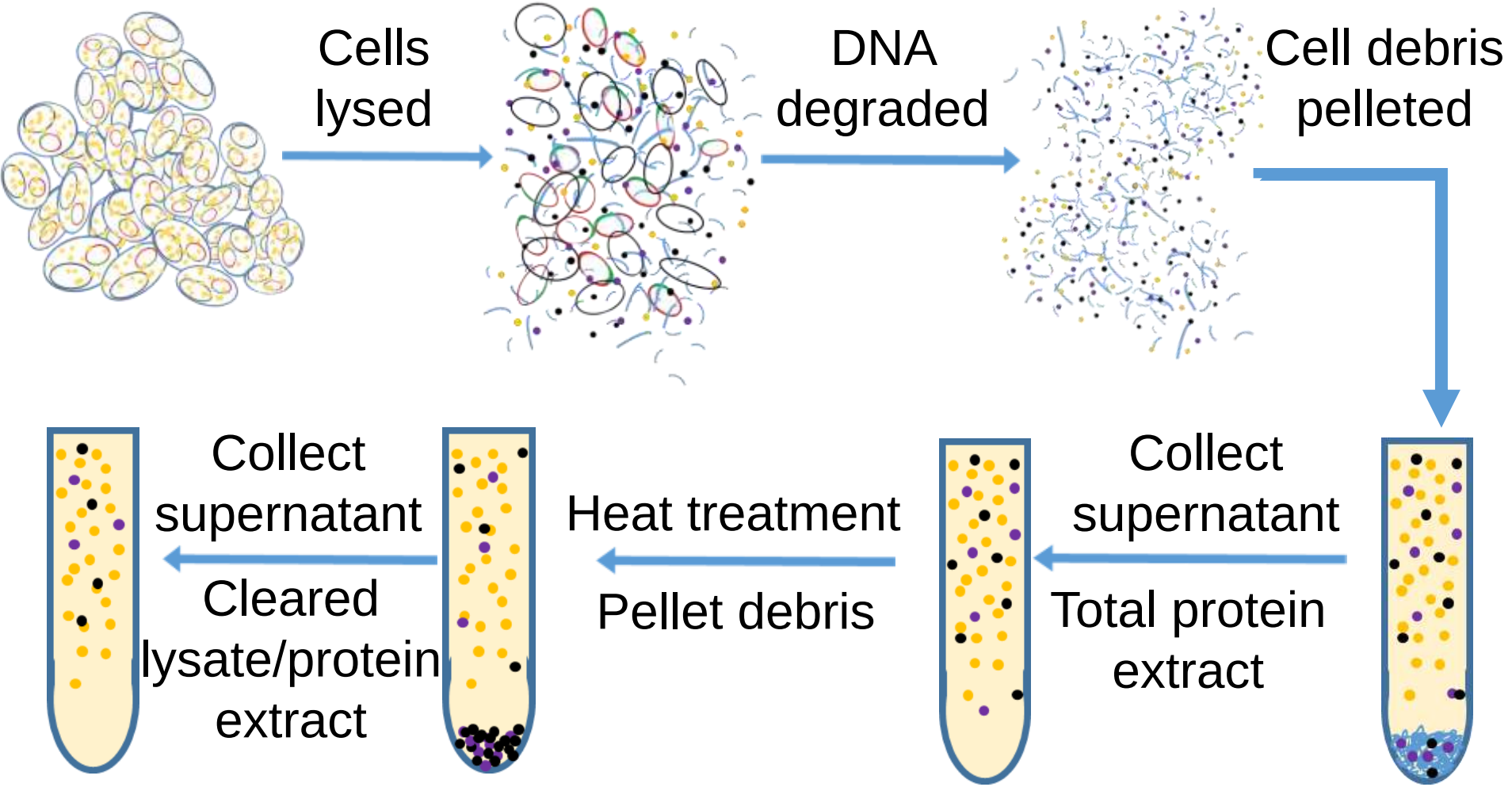
Selectively grown in LB media



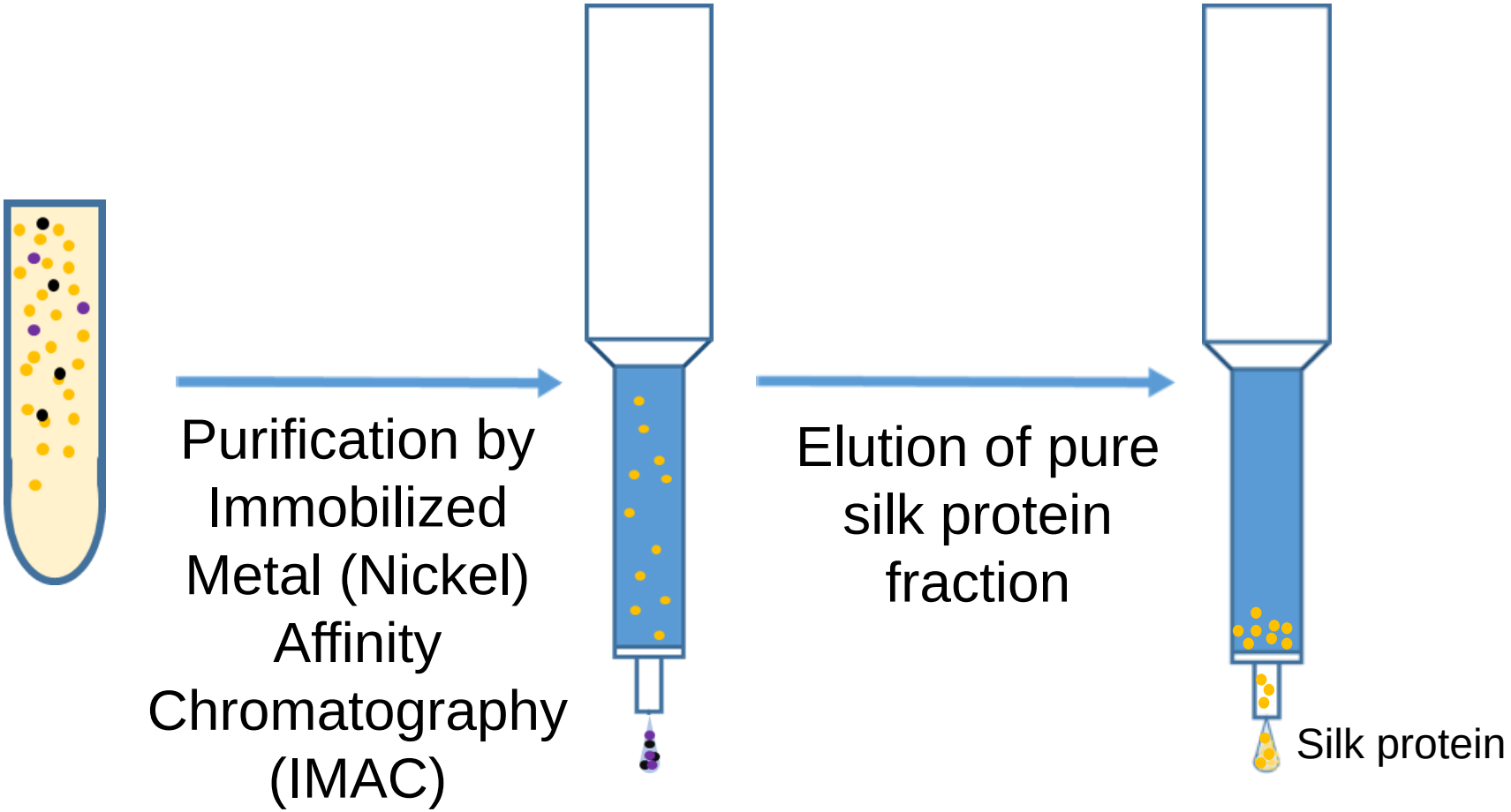
Spider silk gene expression induced with

Isopropyl β-D-1-thiogalactopyranoside (IPTG)

Methods – Protein Extraction

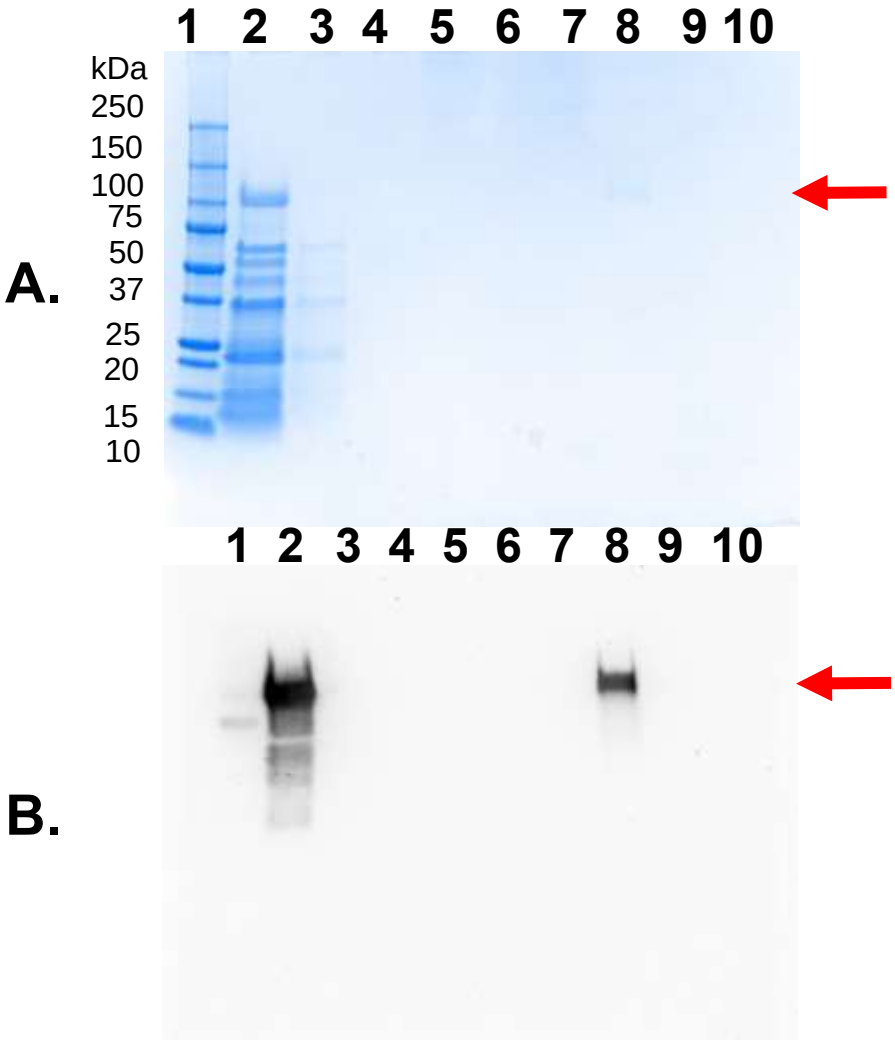


Methods – Protein Purification



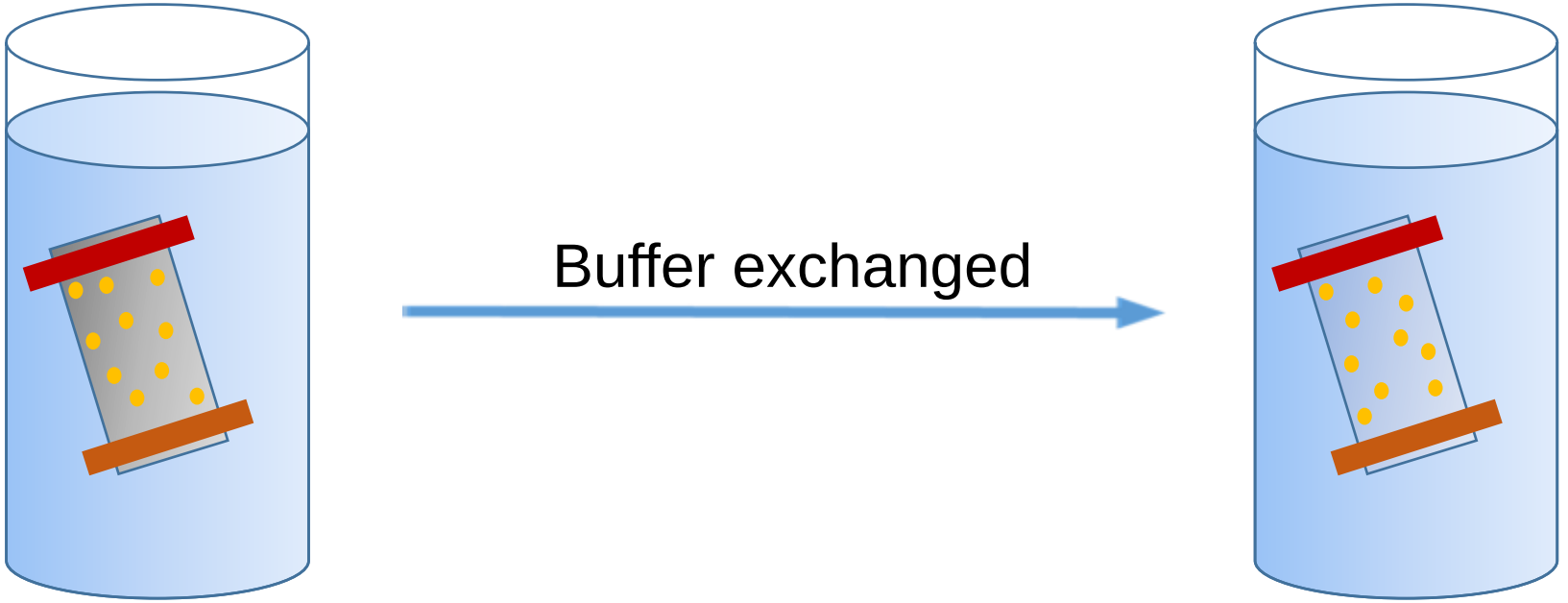
Methods – Quality Control

Coomassie-stained SDS-PAGE and Western blot

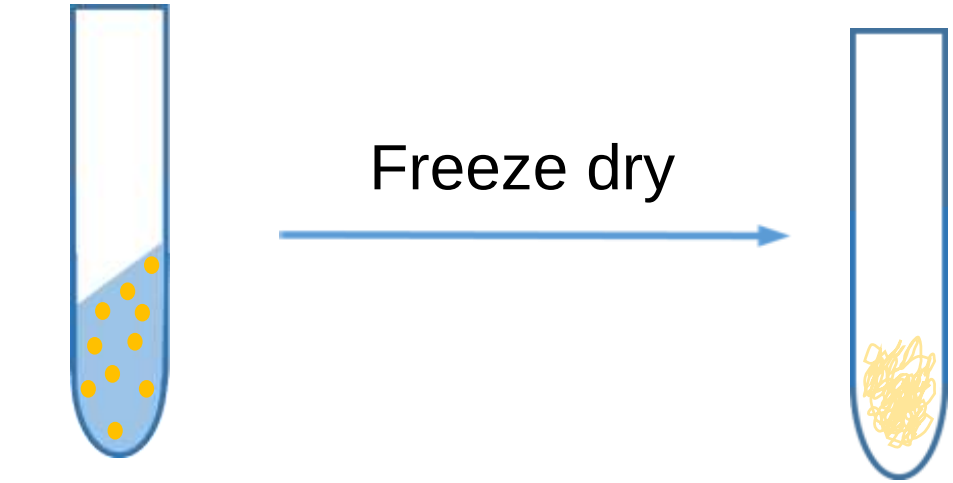


1. *Marker*
2. Heat-treated total protein extract
3. Unbound proteins
4. 20 mM Imidazole wash
5. 40 mM Imidazole wash
6. 60 mM Imidazole wash
7. 80 mM Imidazole wash
8. 250mM Imidazole elution
9. 1X strip EDTA elution

Methods – Dialysis



Methods – Lyophilization



Frozen protein solution

Dry silk proteins

Results

Target production:

~200 mg of each recSSLP

Yield: ~3 mg $Y1S8_{20}$/g silk clone cells

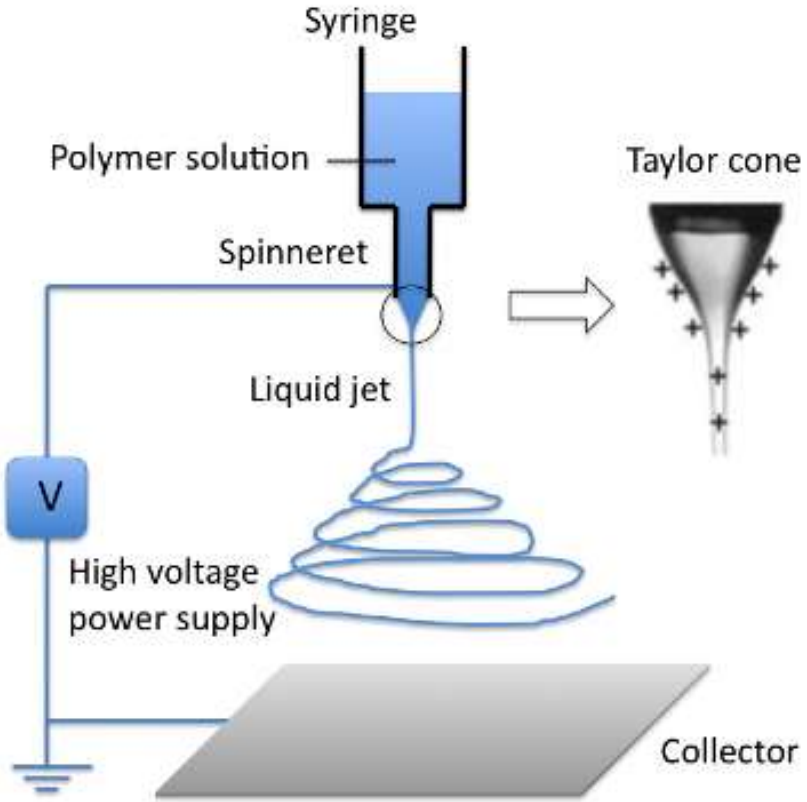
TOTAL: ~370 mg $Y1S8_{20}$

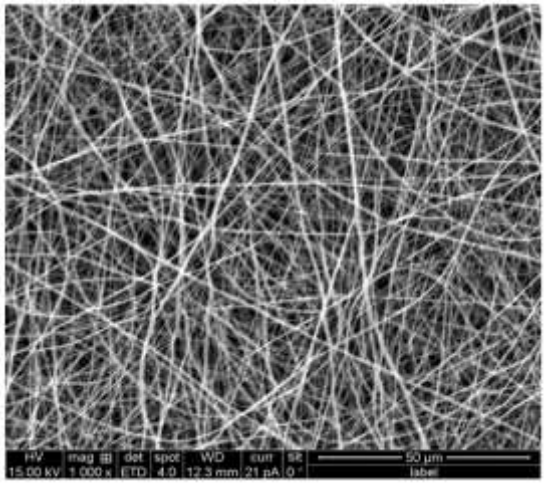
Yield: ~4 mg $A1S8_{20}$/g silk clone cells

TOTAL: ~160 mg $A1S8_{20}$

Future Plans

- Continue producing more silk proteins
- Electrospin nanofiber mats
- Mechanical testing of nanofiber mats
- Mammalian cell biocompatibility tests





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Collaborator: Dr. Patrick Johnson and research team

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Ms. Angela Reddick

Questions?