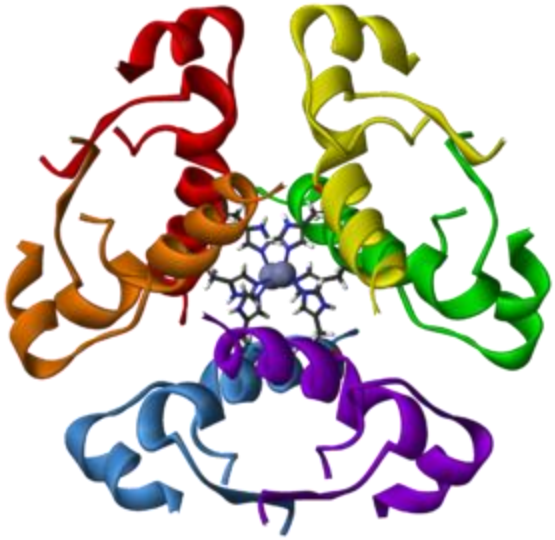
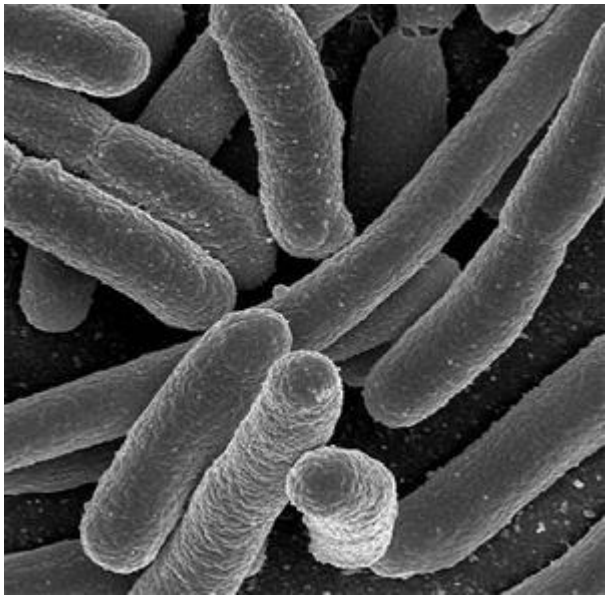
Gen2Co: 2nd generation *E.coli* protein cell factories

Lloyd W. Ruddock

University of Oulu, Finland

30 years ago FDA approved first recombinant biologic





In the early years prokaryotic production dominated the market

- Low cost
- Rapid growth
- High biomass
- Easy cultivation and manipulation
- FDA friendly

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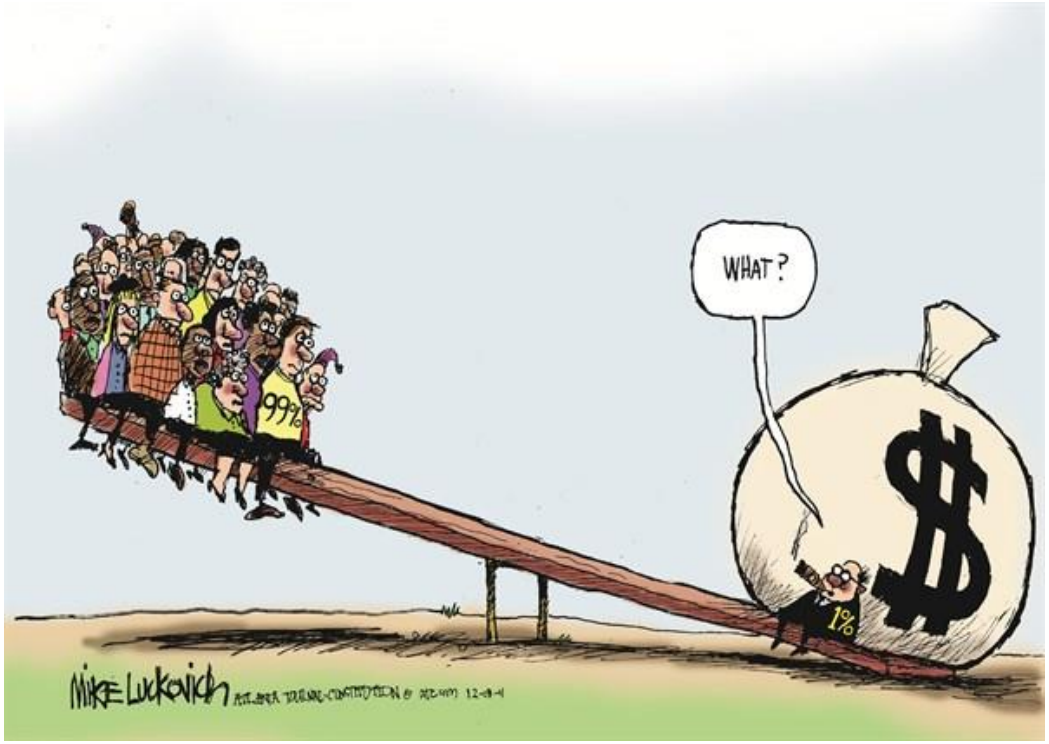

But investment in eukaryotic systems has changed the balance

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Current rule of thumb:

Intracellular proteins	Prokaryotic production
Extracellular proteins	Eukaryotic production

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In reality the issue is post translational modifications.

In particular disulfide bond formation and N-glycosylation

Gen2Co:

Combining advantages of prokaryotic and eukaryotic organisms for recombinant protein production.

i.e. a prokaryote that can make disulfide bonded, N-glycosylated proteins in high yields and secrete them to the media.

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Eukaryotic N-glycan heterogeneity

Eukaryotic proteins are often heterogenous in the number and types of N-glycans added, even on a single protein & often with significant batch-to-batch variation.

This has major implications since N-glycans can modulate:

- Biological activity
- Stability
- Clearance
- Immunogenicity

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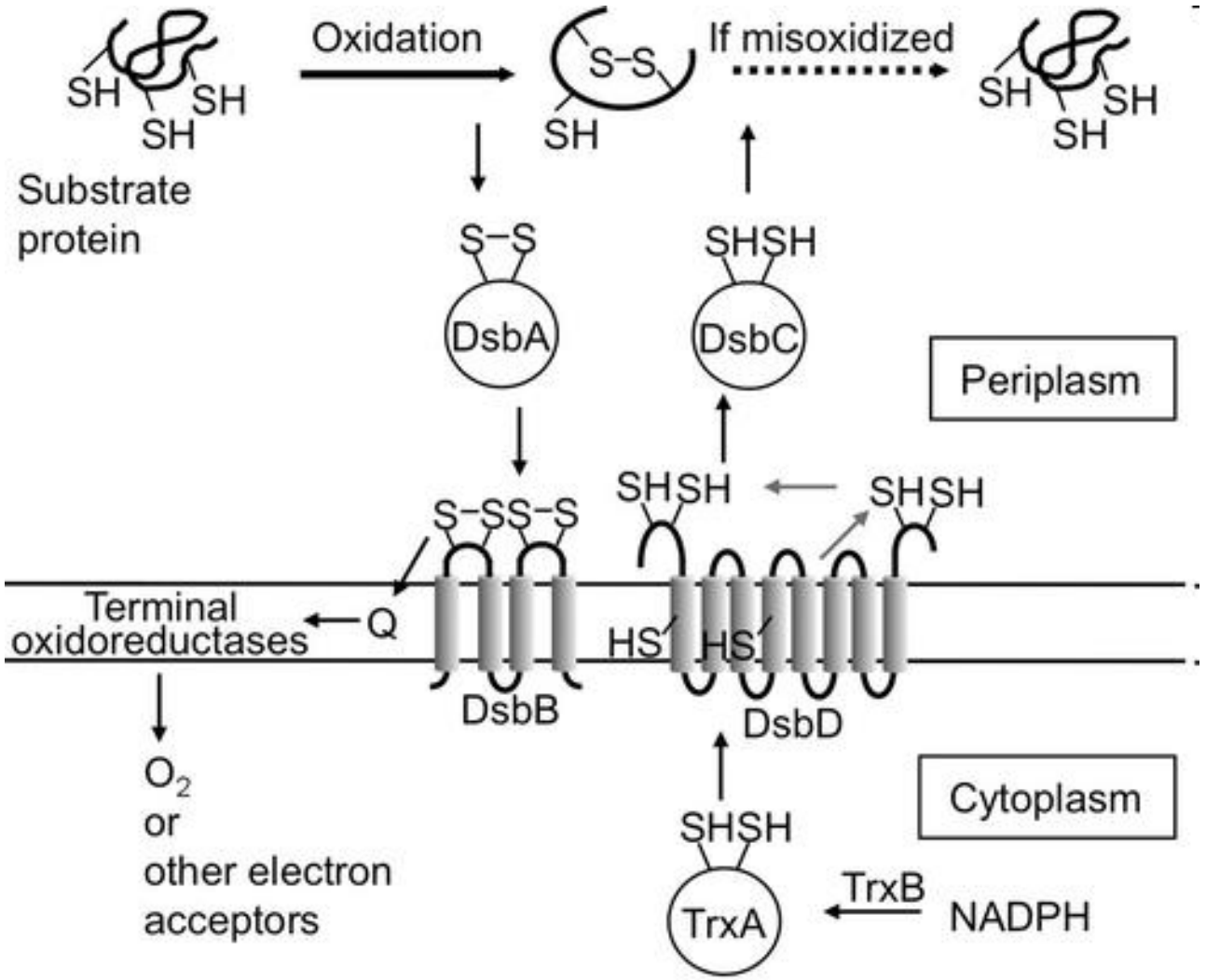
Periplasmic disulfide bond formation in *E.coli*



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In vivo N-glycosylation

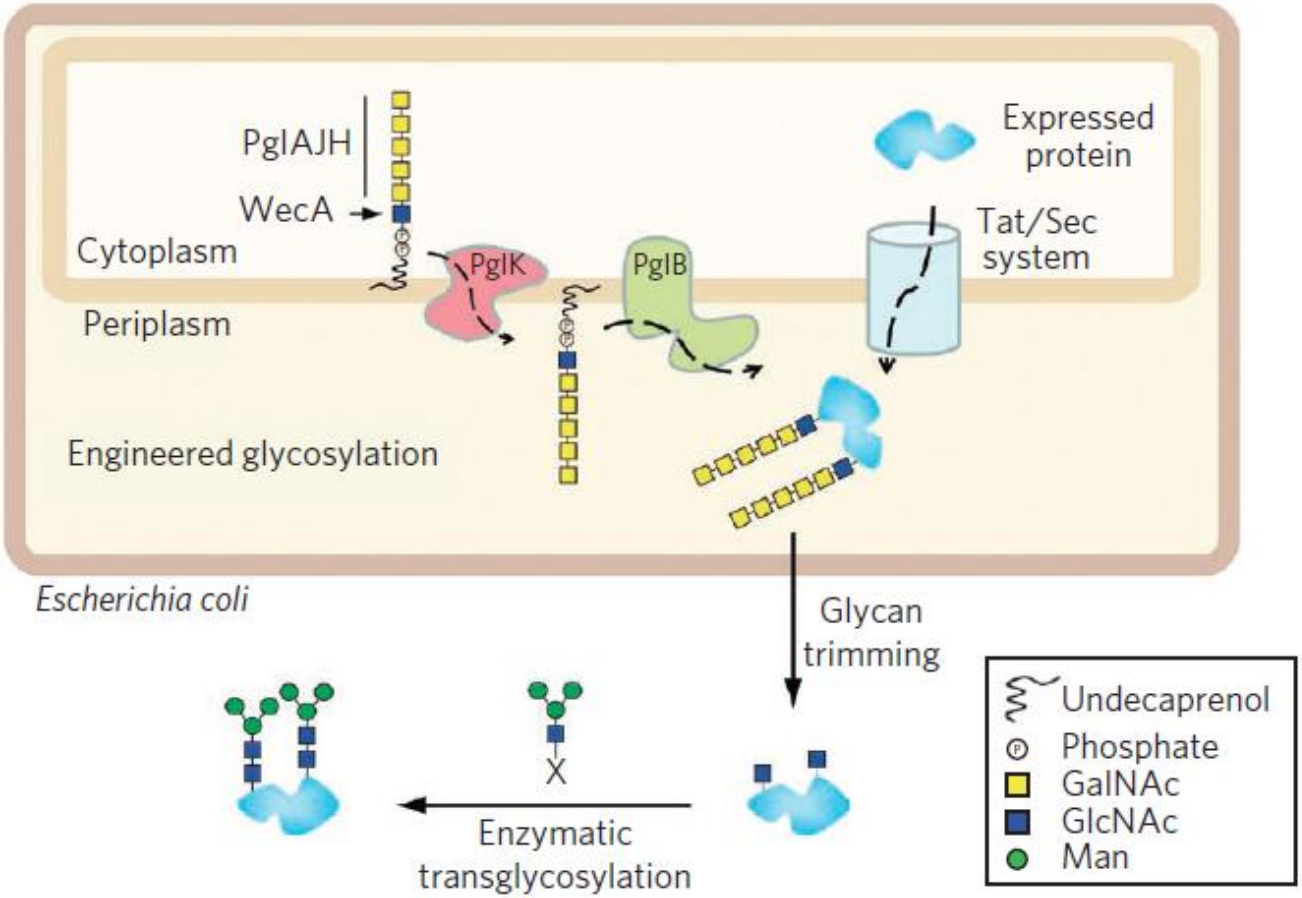
+

Ex vivo transglycosylation

BRIEF COMMUNICATION
PUBLISHED ONLINE: 28 FEBRUARY 2010 | DOI: 10.1038/NCHEMBIO.314
nature chemical biology

A combined method for producing homogeneous glycoproteins with eukaryotic N-glycosylation

Flavio Schwarz[1], Wei Huang[2,3], Cishan Li[2,3], Benjamin L Schulz[1], Christian Lizak[1], Alessandro Palumbo[4], Shin Numao[1], Dario Neri[4], Markus Aebi[1]* & Lai-Xi Wang[2,3]*



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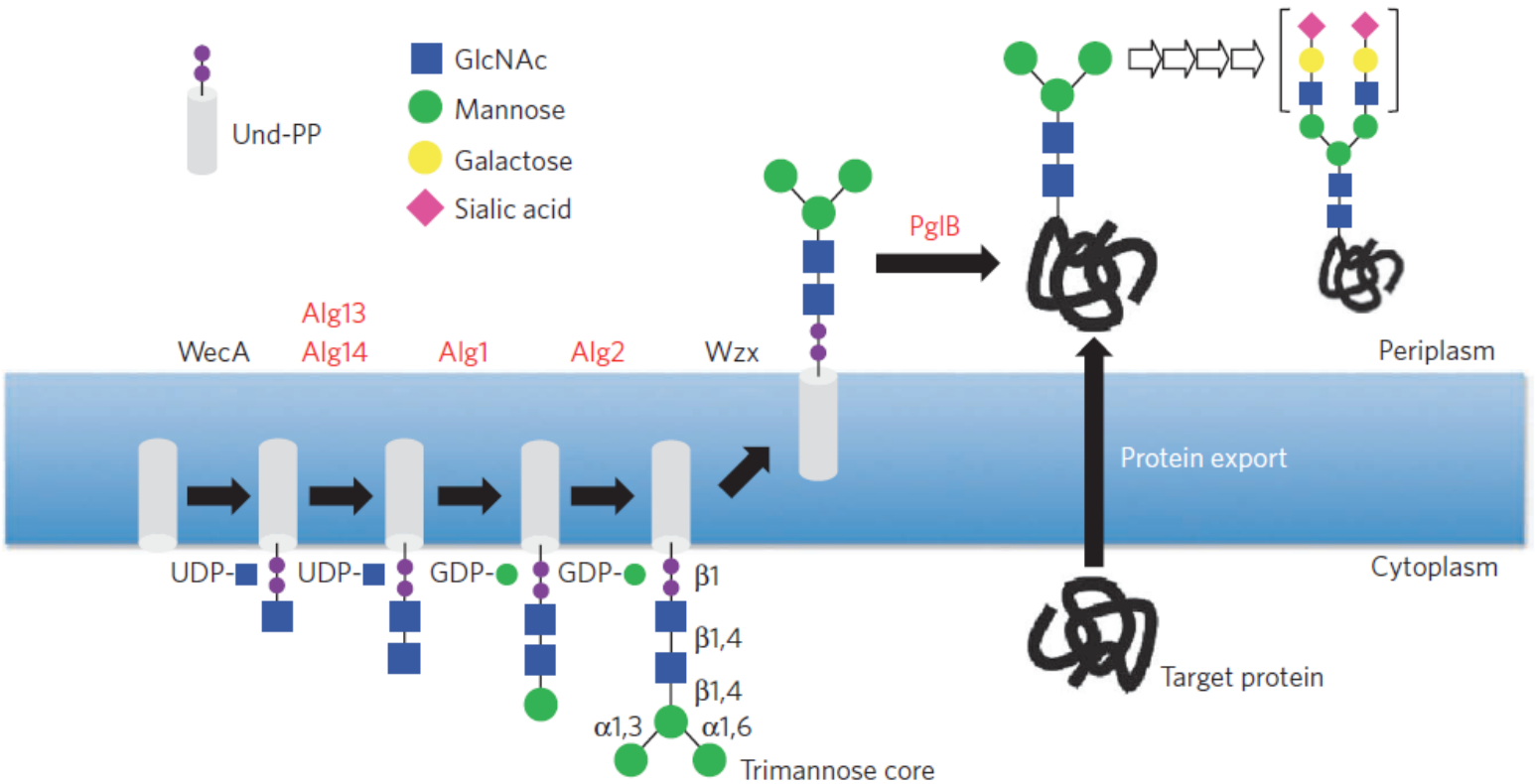
Engineered pathways based on PglB promiscuity

BRIEF COMMUNICATION
PUBLISHED ONLINE: 25 MARCH 2012 | DOI: 10.1038/NCHEMBIO.921
nature chemical biology

An engineered eukaryotic protein glycosylation pathway in *Escherichia coli*

Juan D Valderrama-Rincon[1,6], Adam C Fisher[2,6], Judith H Merritt[2], Yao-Yun Fan[3], Craig A Reading[2], Krishan Chhiba[4], Christian Heiss[5], Parastoo Azadi[5], Markus Aebi[3] & Matthew P DeLisa[1*]



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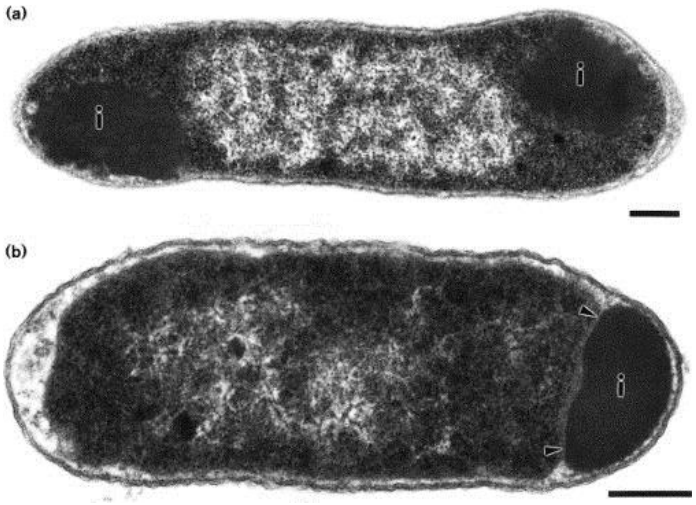
Limitations of the periplasm:

- Low volume / low capacity => low yields (?)

E.coli: Ronald Wetzel (SKB)



- Secretion machinery easily overloaded

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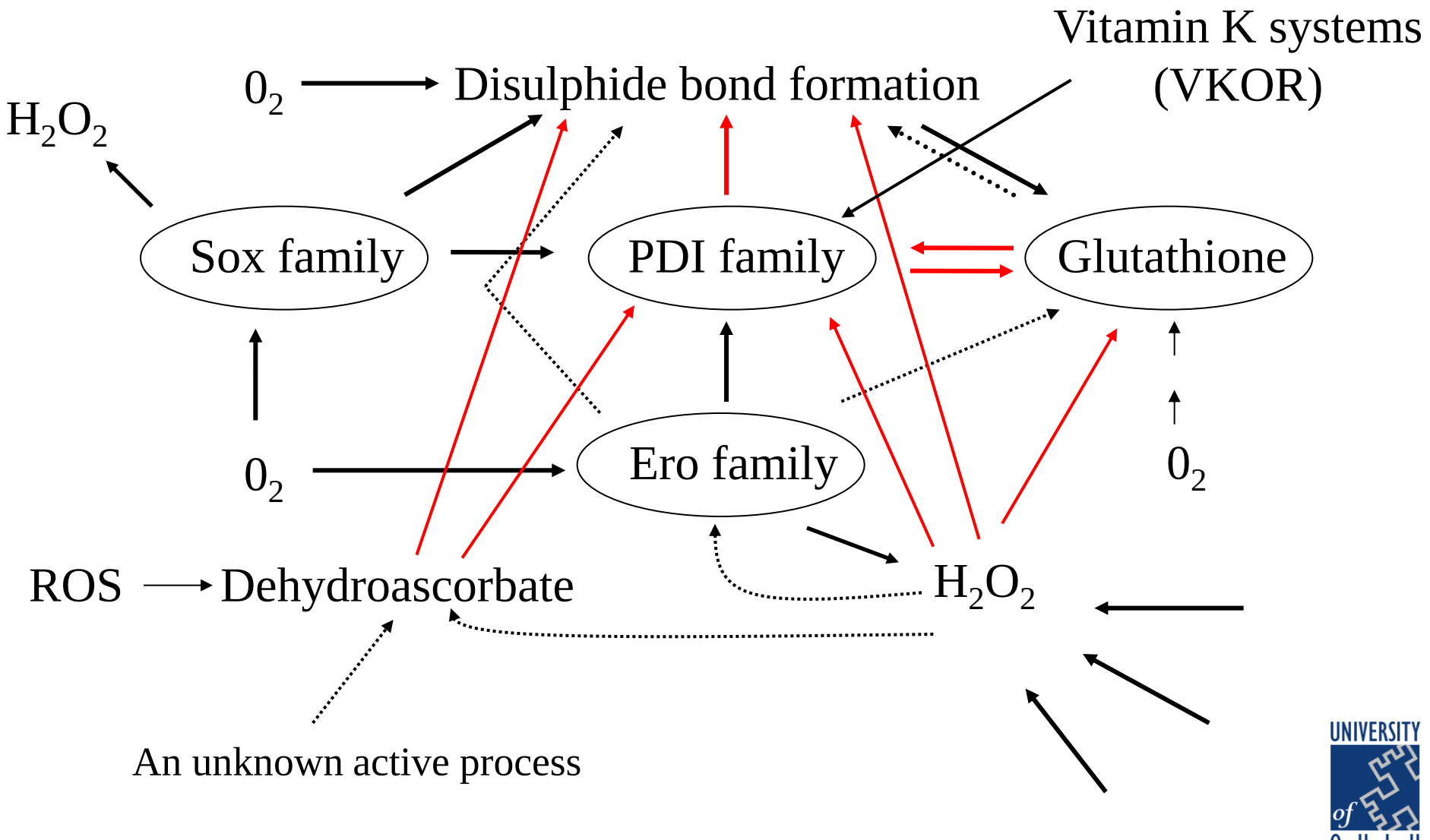
Disulphide bond formation in the ER



Disulphide bonds are annoying

Can we use information from natural systems for disulfide bond formation in the cytoplasm of *E.coli*?

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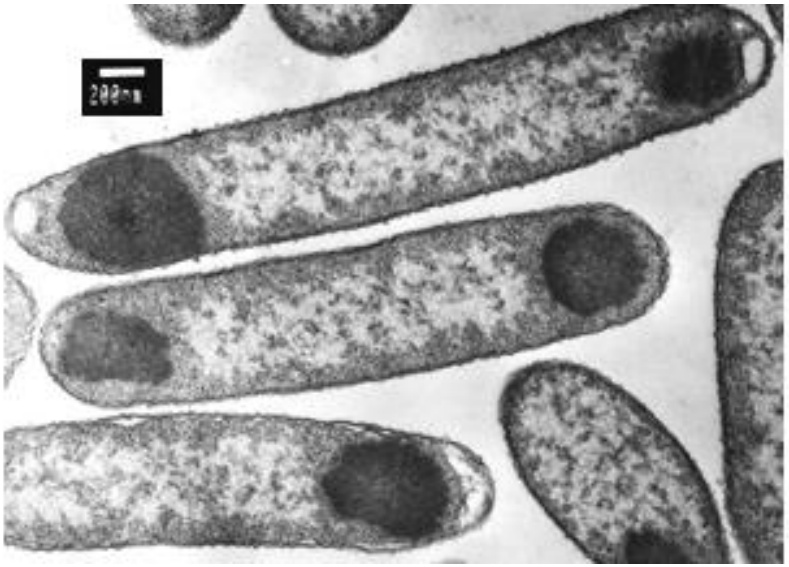
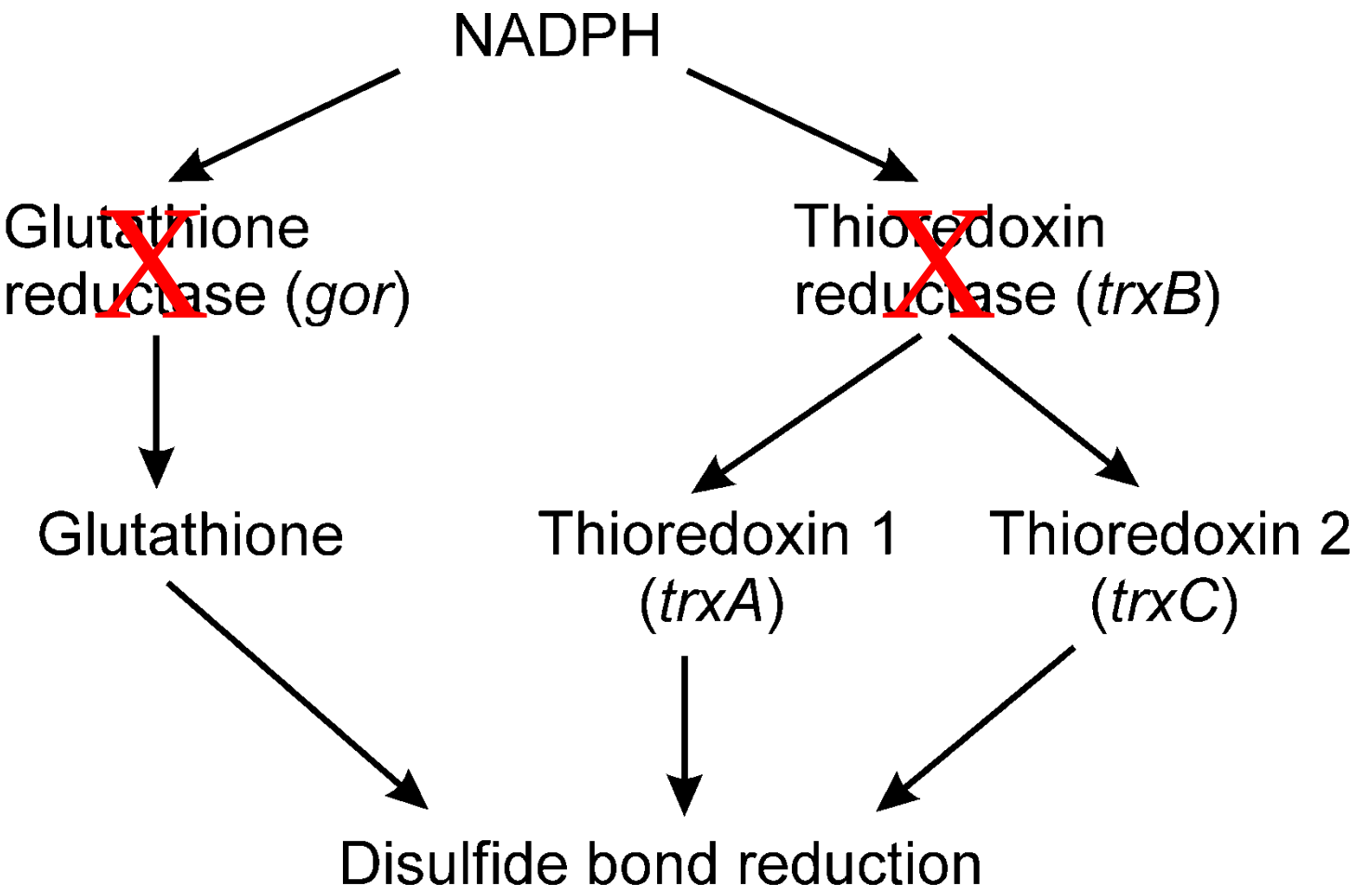
Cytoplasmic expression

Naturally reducing environment => inclusion bodies



Pathways for disulfide bond reduction in *E.coli* cytoplasm



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These strains remove reducing pathways.

They do **not** add a pathway for catalyzing *de novo* disulfide bond formation

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So how about adding core eukaryotic catalysts to the cytoplasm of *E.coli*?

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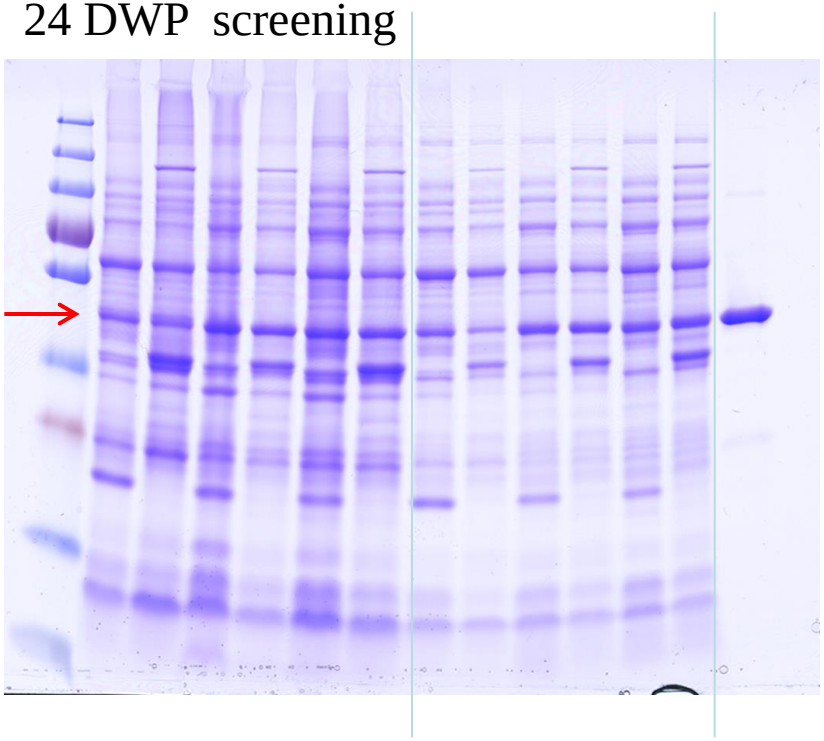
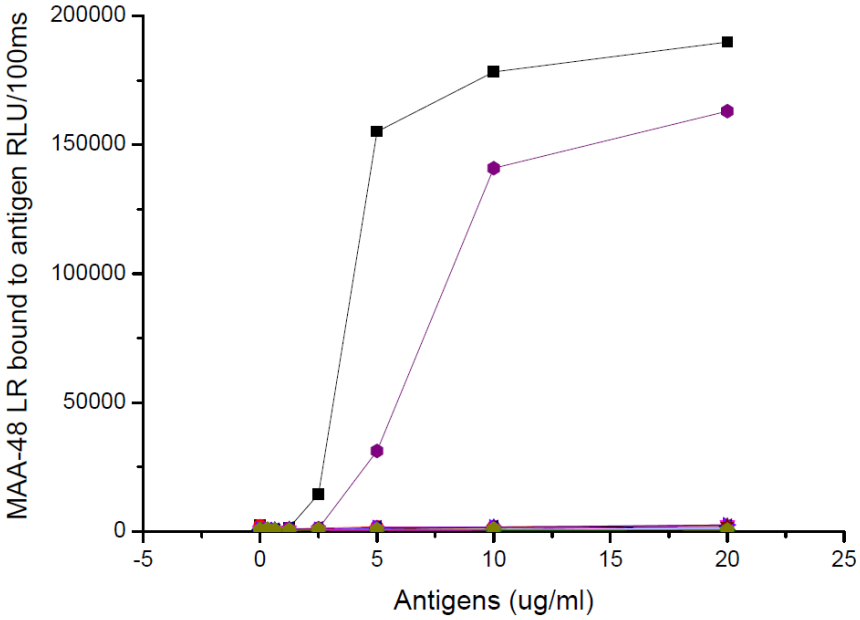
CyDisCo has been successfully used for >90 proteins

24 DWP screening



Total Soluble



In excess of 250mg/L of disulfide linked Fab, from shake-flasks

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CyDisCo works in any *E.coli* strains and in any media

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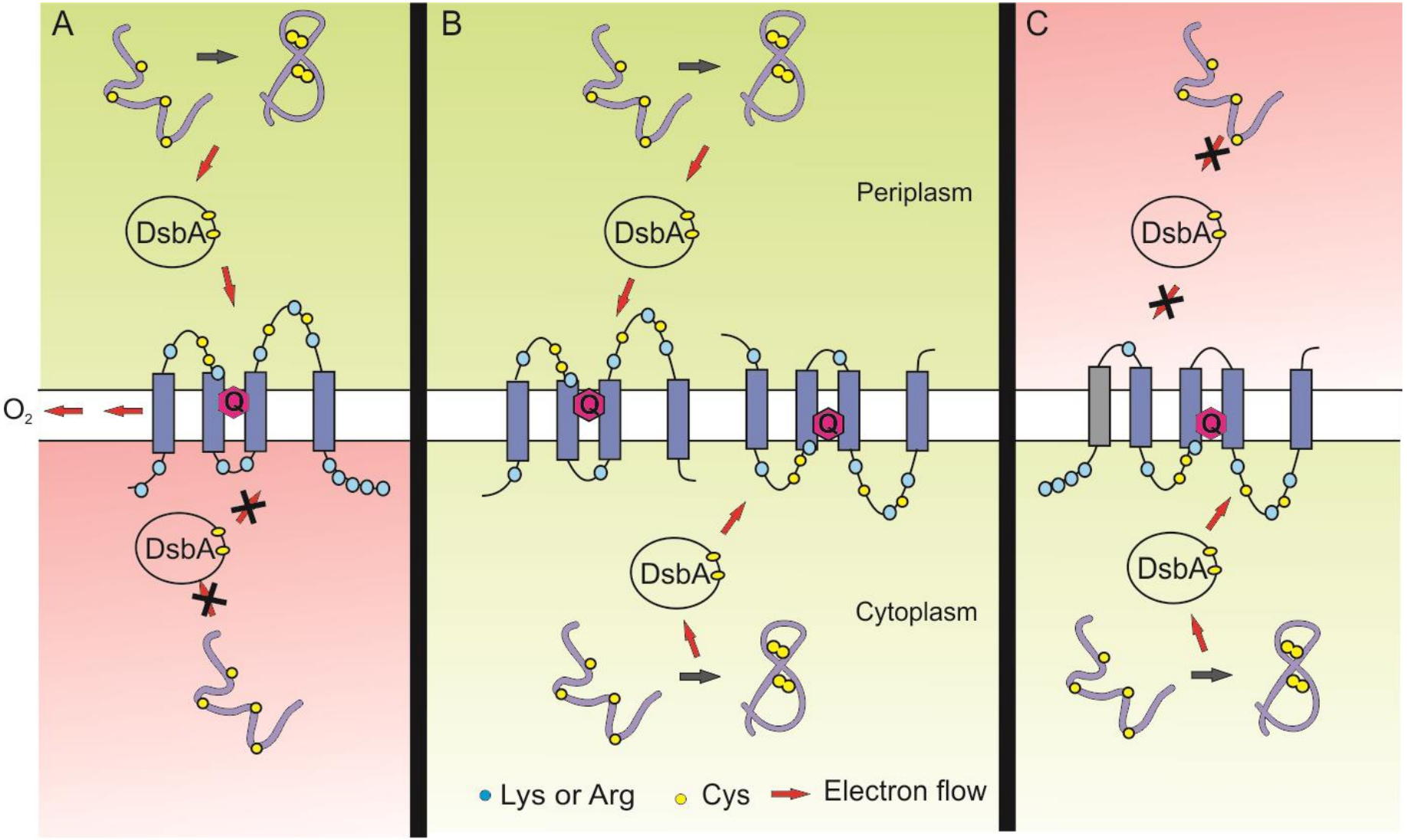
What about adapting other systems?

How about inverting DsbB to get disulphide bond formation in the cytoplasm?



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Three routes for efficient disulfide bond formation in *E.coli* cytoplasm, what about N-glycosylation?

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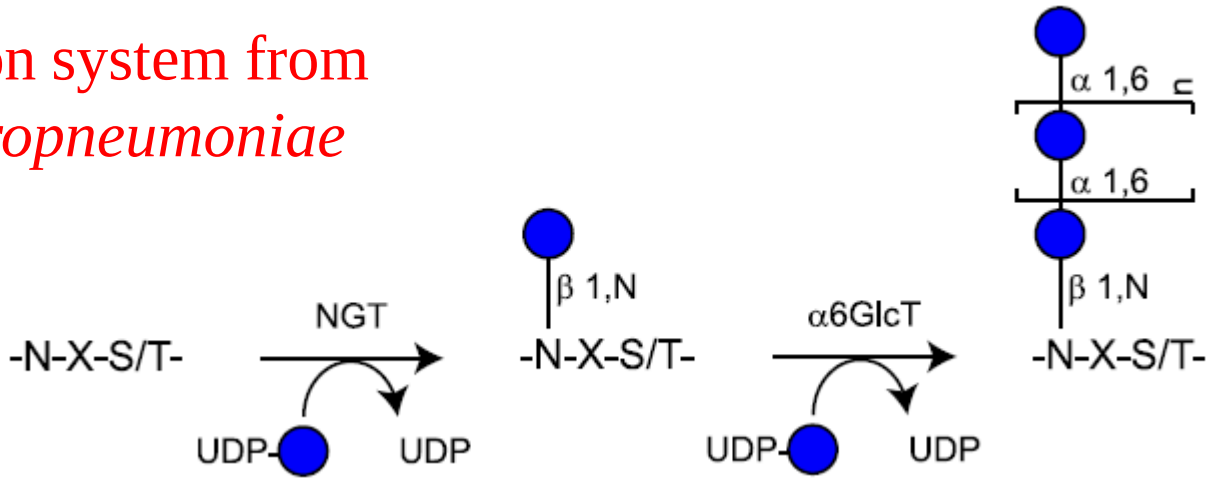
Cytoplasmic N-glycosylation in *E.coli*

Uses N-glycosylation system from *Actinobacillus pleuropneumoniae*



THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 286, NO. 40, pp. 35267–35274, October 7, 2011

Cytoplasmic *N*-Glycosyltransferase of *Actinobacillus pleuropneumoniae* Is an Inverting Enzyme and Recognizes the N*X*(S/T) Consensus Sequence*[S]

Received for publication, June 28, 2011, and in revised form, August 11, 2011 Published, JBC Papers in Press, August 18, 2011, DOI 10.1074/jbc.M111.277160

Flavio Schwarz[‡], **Yao-Yun Fan**[‡1], **Mario Schubert**[§2], **and Markus Aebi**[‡3]

From the Institutes of [‡]*Microbiology and* [§]*Molecular Biology and Biophysics, Department of Biology, ETH Zürich, 8093 Zürich, Switzerland*

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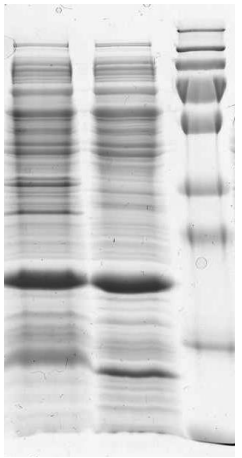
Combining CyDisCo

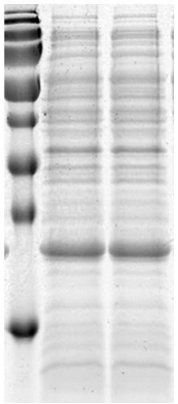
Combining CyDisCo with:

- Large scale production and processing

Human interleukin 6: 1.0 g/L purified product



Human growth hormone 1: 1.1 g/L purified product



scFv: 0.6g/L purified product

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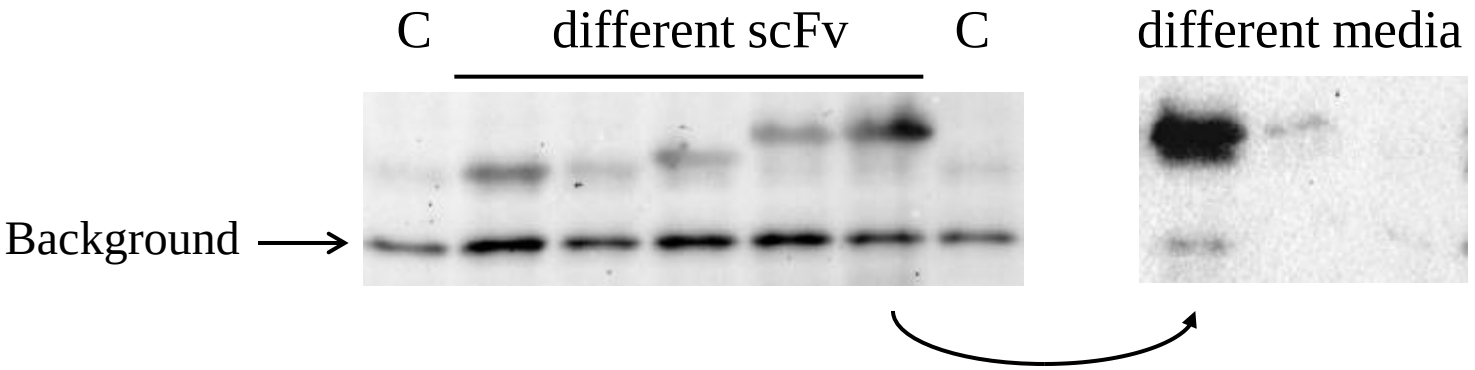
Combining CyDisCo with:

- N-glycosylation in the cytoplasm

In the ER disulfide bond formation goes hand in hand with N-glycoslyation, can we do the same in the *E.coli* cytoplasm?



Anti N-glycosylation WB

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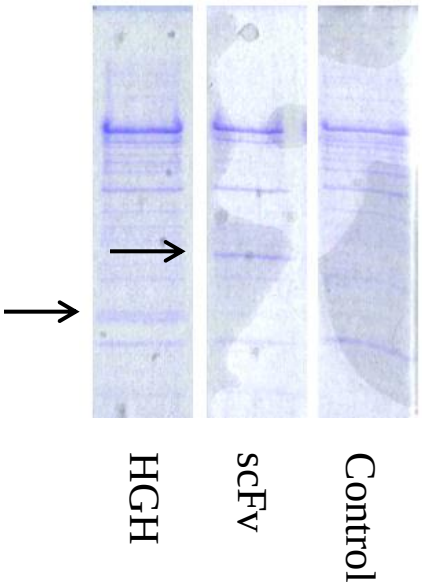
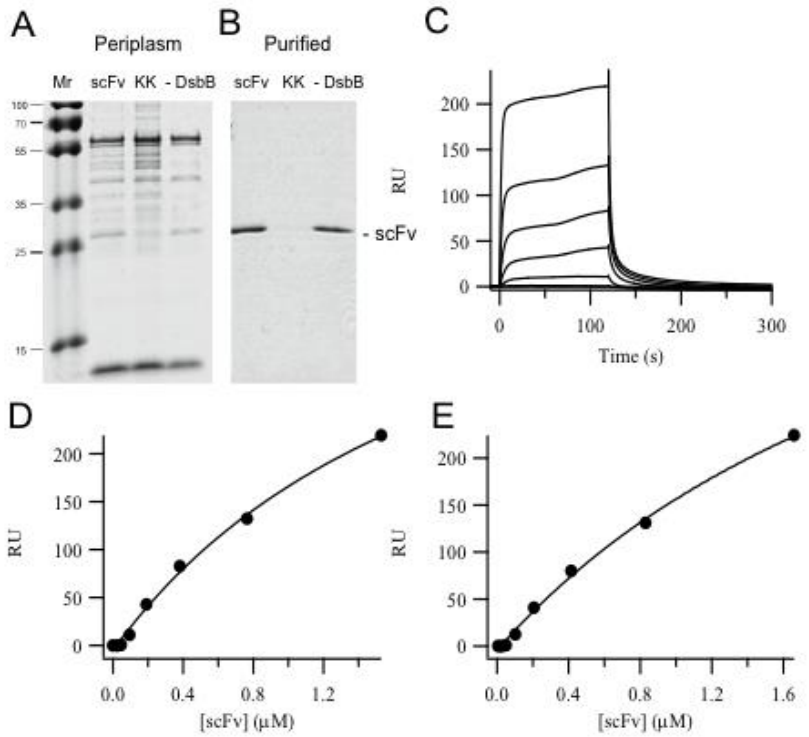
Combining CyDisCo with:

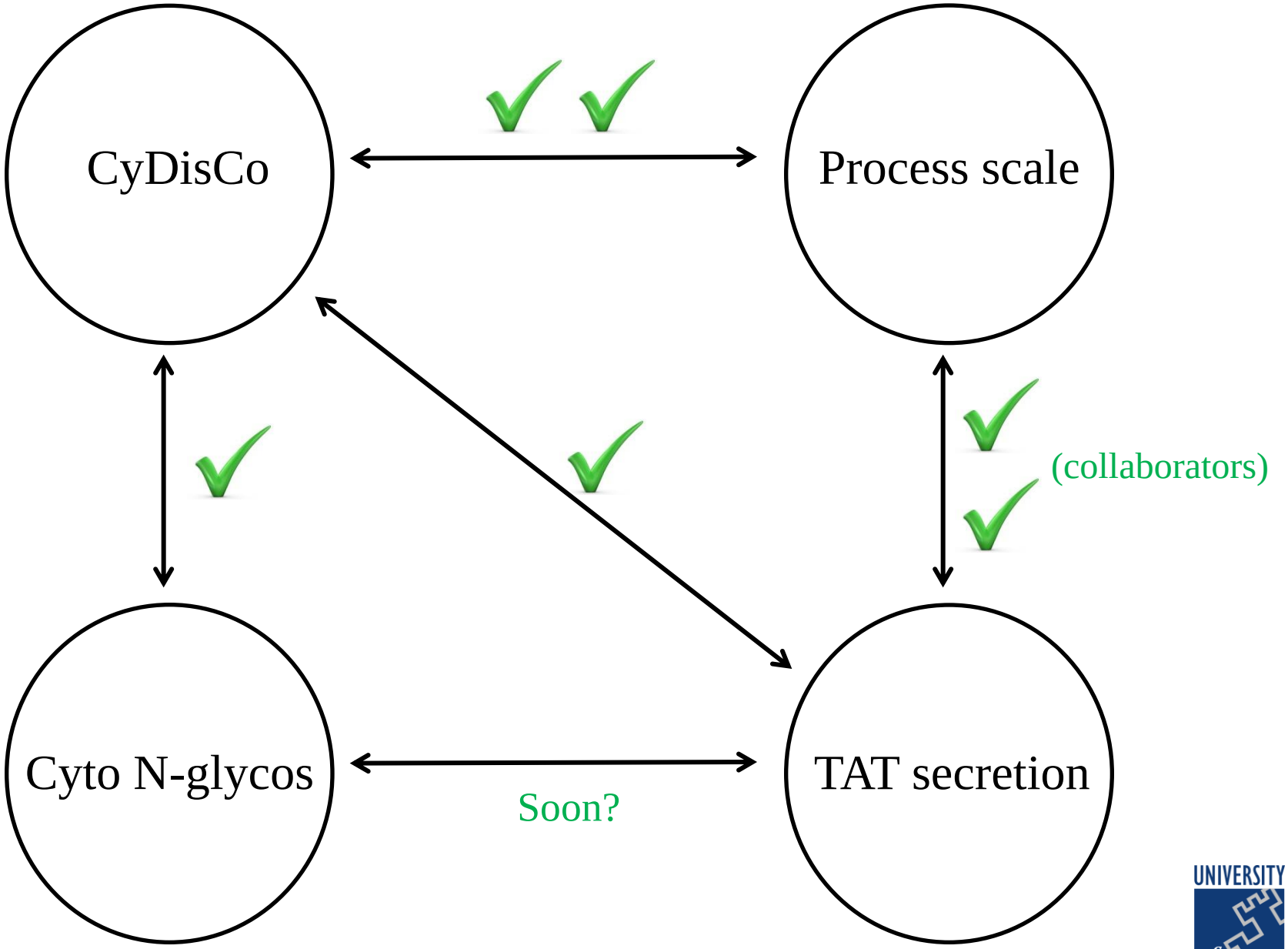
• TAT secretion system

Twin arginine translocation system is a secretion system with in-built quality control mechanism i.e. only correctly folded proteins are secreted. Analogous to the ER-exit site quality control system.

Periplasmic extracts






CyDisCo
Process scale
Cyto N-glycos
TAT secretion
(collaborators)
Soon?

Summary

Efficient disulfide bond formation +/- N-glycosylation in the cytoplasm of *E.coli* offers up new, interesting, possibilities for the industrial production of homogenous proteins in high yields.

It will not replace eukaryotic protein production, but offers an alternative solution that may create an interesting balance between eukaryotic and prokaryotic expression of biologics.

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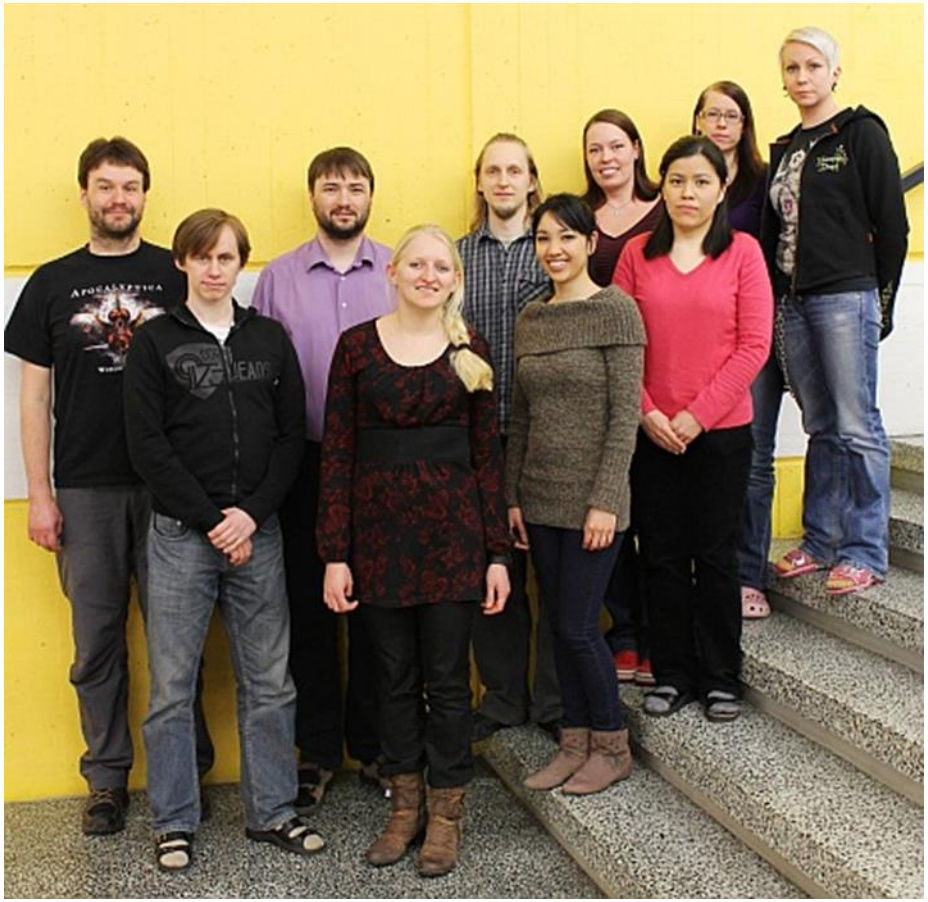
Acknowledgements



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