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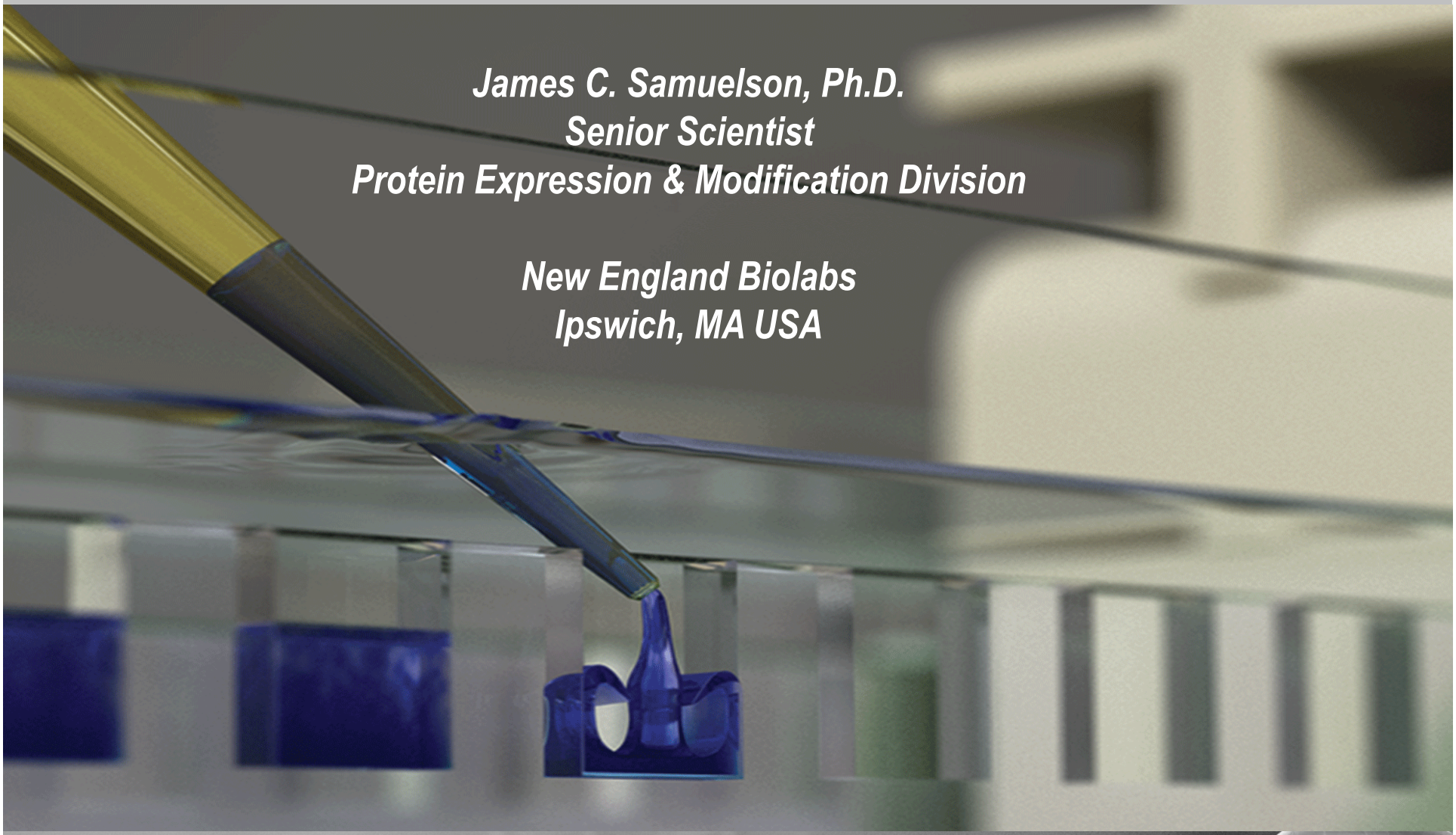
Application of phage display and plasmid display to broaden the specificity of human Fbs1 for capture of N-glycosylated peptides

James C. Samuelson
New England Biolabs, USA

Application of Phage Display and Plasmid Display to Broaden the Specificity of Human Fbs1 for Capture of N-glycosylated Peptides.



James C. Samuelson, Ph.D.
Senior Scientist
Protein Expression & Modification Division

New England Biolabs
Ipswich, MA USA

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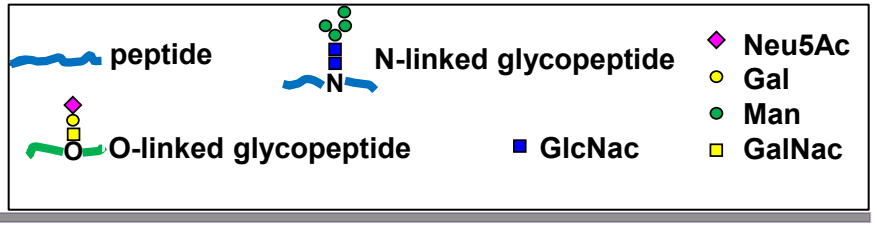
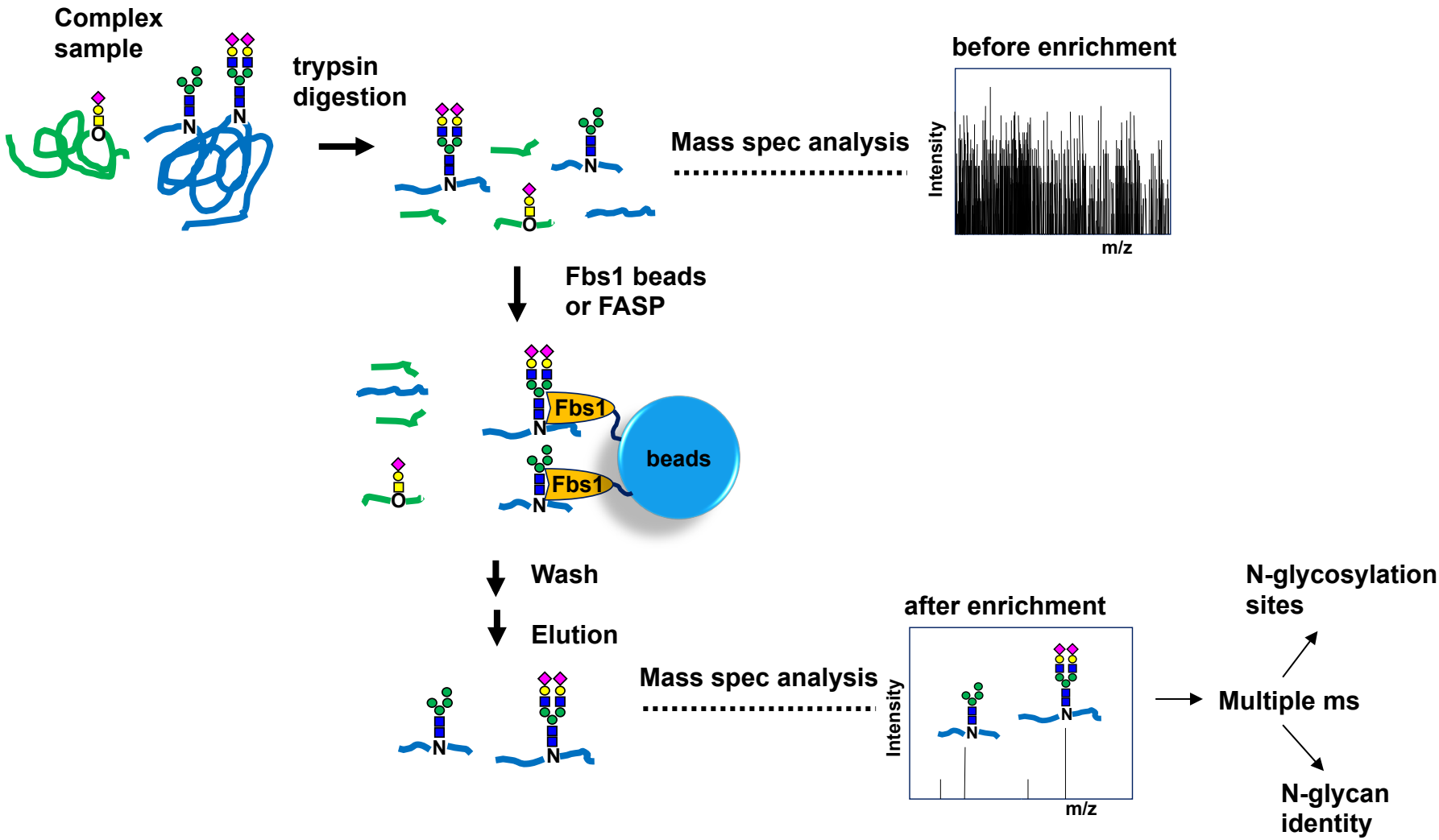
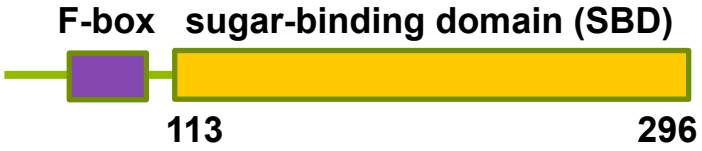
Goal: Enrichment of N-linked Glycopeptides



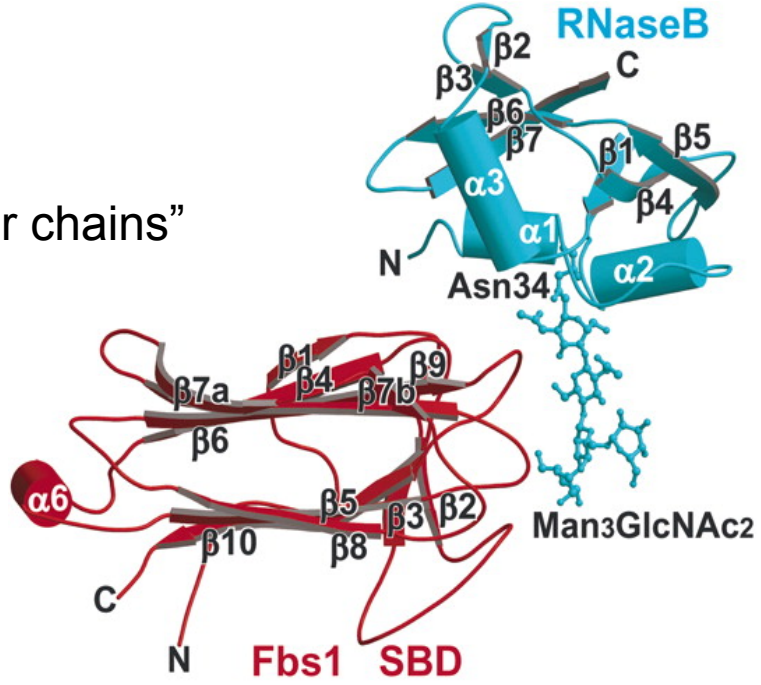


Fbs1 binds the pentasaccharide core motif of N-glycans –



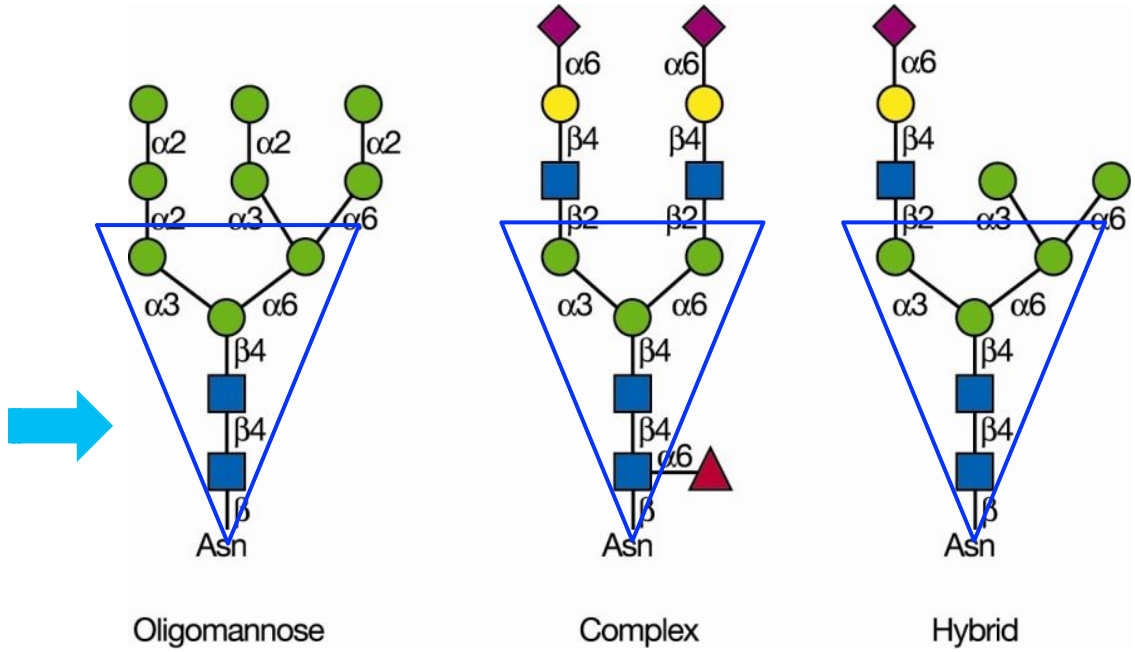
Fbs1 = "F-box protein that recognizes sugar chains"

- **Fbs1 recognizes N-glycans, as part of ubiquitin mediated ER-associated degradation pathway (ERAD).**



Mizushima et al. (2007) *PNAS* vol. 104

Fbs1 recognizes the Man3GlcNAc2 core structure



Trimannosyldiacetylchitobiose (Man3GlcNAc2) is the core structure of mammalian N-linked glycans

Challenges

Fbs1 preferably binds to high mannose glycans

1) low affinity to complex sialic acid containing glycans

Challenges

Fbs1 binds to high mannose *and* complex N-glycans –

1) low affinity to complex sialic acid containing glycans

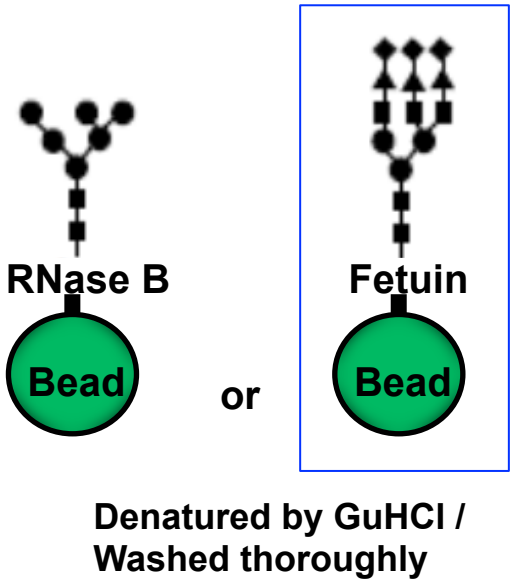
2) Enrichment for mass spec analysis of biological samples requires non-biased capture of all N-glycan types

Methods Applied to Alter Fbs1 glycan binding specificity

1) M13 Phage Display

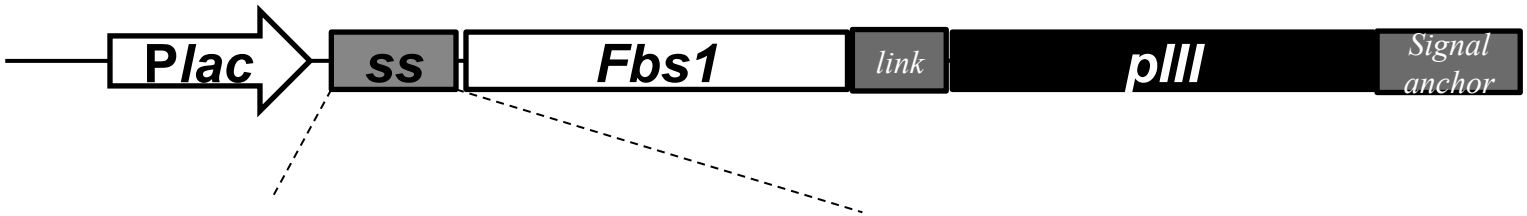
2) Plasmid Display

In both cases, Fbs1 libraries were panned against immobilized glycoprotein



Denatured by GuHCl /
Washed thoroughly

M13 Phage display: **Target protein is translocated across the inner membrane**



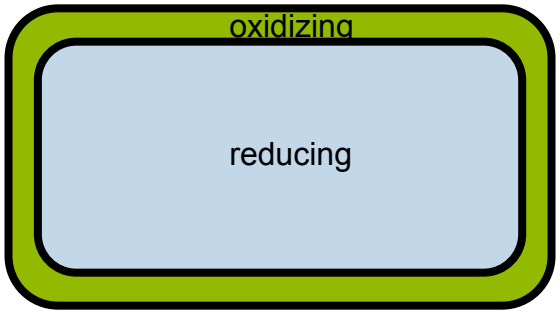
PelB-ss: MKYLLPTAAAGLLLLAAQPAMA

DsbA-ss: MKKIWLALAGLVLAFSASA

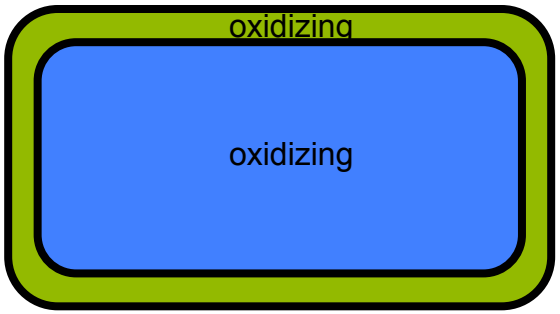
Signal type / mechanism	example	protein type
Sec-dependent / post-translational	PelB-ss	Secretory proteins/ antibodies
SRP-dependent / co-translational	**DsbA-ss**	**Proteins that fold rapidly, non-secretory proteins**

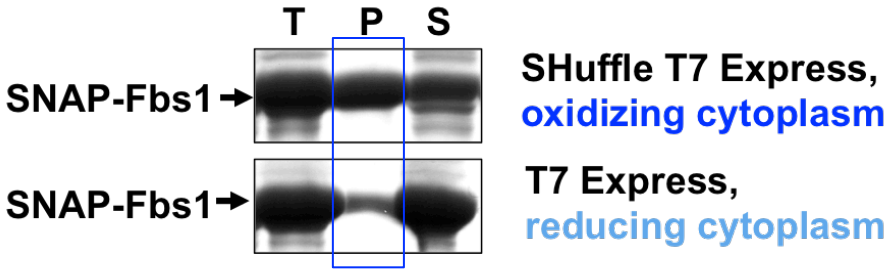
Fbs1 misfolds in an oxidizing environment

wt *E.coli*



SHuffle: *trxB/gor* cytoplasm

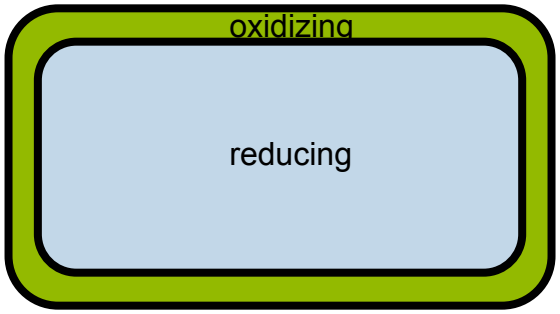




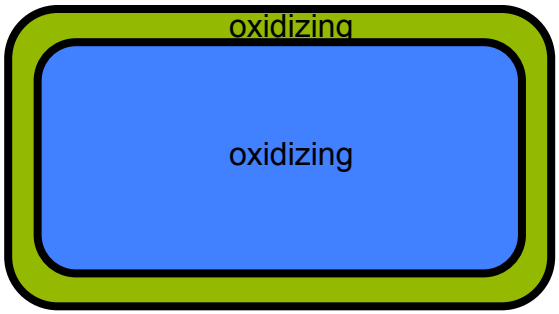
P = pellet of misfolded Fbs1

strains lacking protein disulfide oxidoreductase (*dsbA*) are viable:

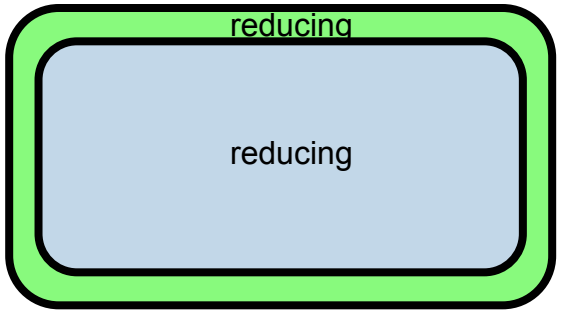
wt *E.coli*



SHuffle: *trxB/gor* cytoplasm

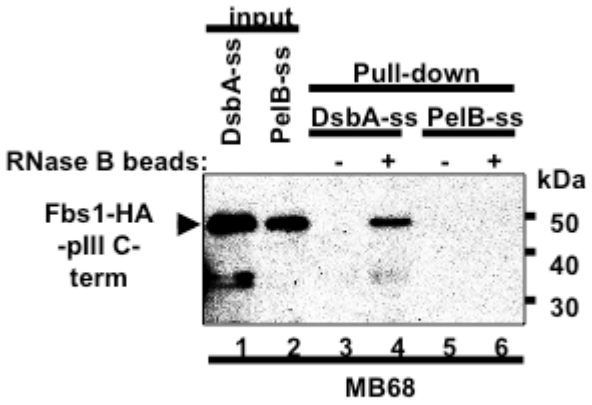


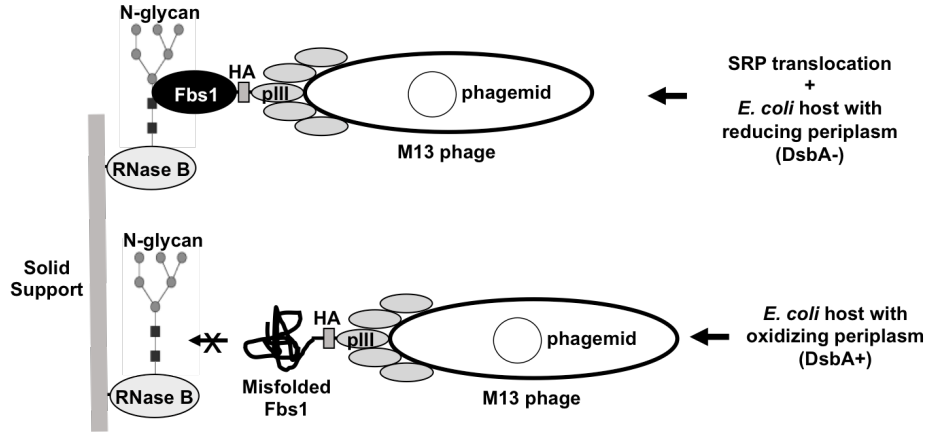
E.coli: dsbA periplasm



M13 display of non-secretory proteins
e.g. Proteins with cysteines that
do not form disulfides

M13 display of functional Fbs1 requires a "reducing" periplasm *and* co-translational export!





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A DsbA-Deficient Periplasm Enables Functional Display of a Protein with Redox-Sensitive Folding on M13 Phage

Minyong Chen and James C. Samuelson*

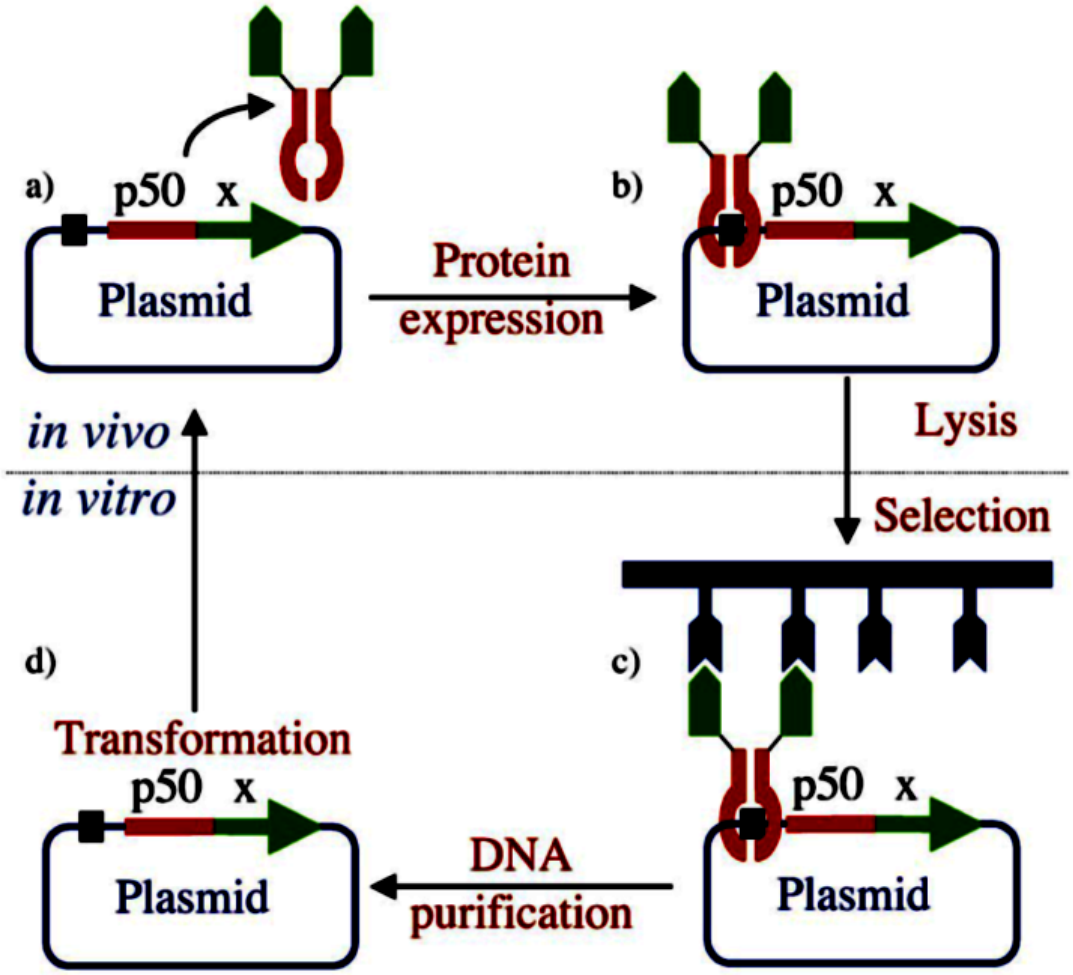
New England Biolabs, Inc., 240 County Road, Ipswich, Massachusetts 01938, United States

May23, 2016

Plasmid Display

Speight RE *et al*, *Chemistry and Biology* 8 (2001) 951-965

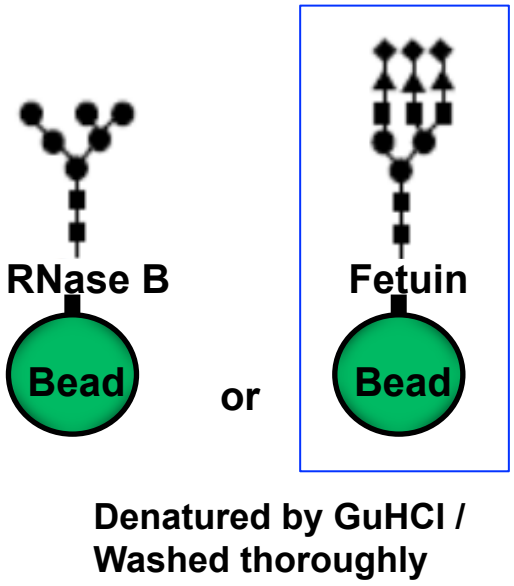
- Protein fused to p50 (nuclear factor κB, NF- κB)
- p50 Binding site: -GGGAATTCCC-
- Kd: ~10 pM
- Half life: ~47 hr (with K-Glutamate)
- Protein secretion not required (as in phage display)
- *In vitro* panning is simple and rapid



Plasmid Display Applied to Alter Fbs1 glycan binding specificity

Fbs1 libraries were panned against immobilized Fetuin

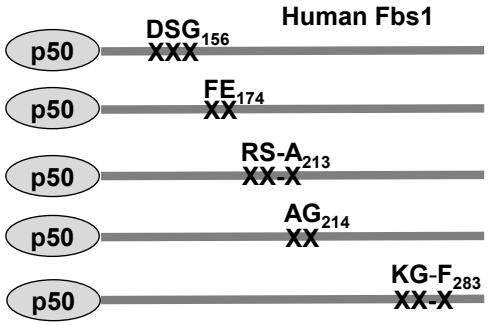
-to select variants with higher affinity to complex, sialylated glycans



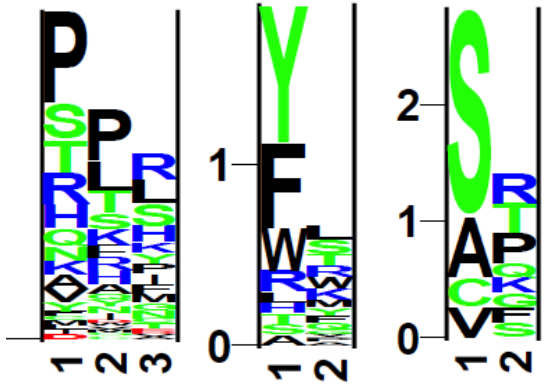
Fbs1 Mutation patterns after 5 cycles of panning against Fetuin beads

Amino acid sequence of human Fbs1 SBD:

C_{92}QQEGLVPEGGVEEERDHWQQFYFLSKRRRNLLRNPCGEEDLEGWCDV
EHGGDGWRVEELPG**D$_{154}$S$_{155}$G$_{156}$**VEFTHDESVKKYFASS**F$_{173}$E$_{174}$**WC
RKAQVIDLQAEGYWEELLDTTQPAIVVKDWYSG**R$_{210}$S$_{211}$**D**A$_{213}$G$_{214}$**CLYE
LTVKLLSEHENVLAEFSSGQVAVPQDSDGGGWMEISHTFTDYGPGVRFVRF
EHGGQDSVYW**K$_{280}$G$_{281}$**W**F$_{283}$**GARVTNSSVWVEP$_{296}$



Sequences after 5x pannings:

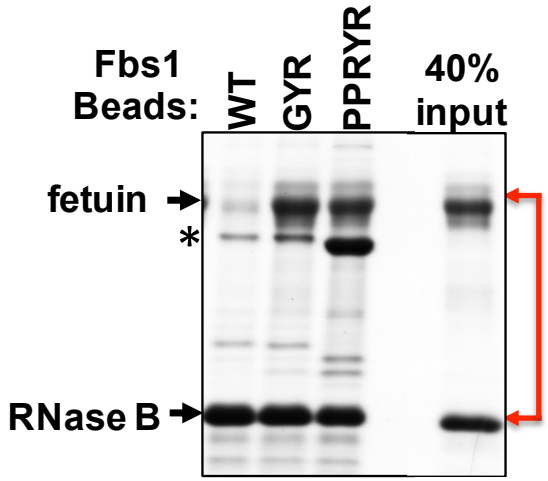


No Mutants Enriched

WT Fbs1 sequence: **D S G$_{156}$ F E$_{174}$ A G$_{214}$ RS-A$_{213}$ KG-F$_{283}$**

Elimination of Bias: GYR and PPRYR variants

Pull-down by Fbs1 beads

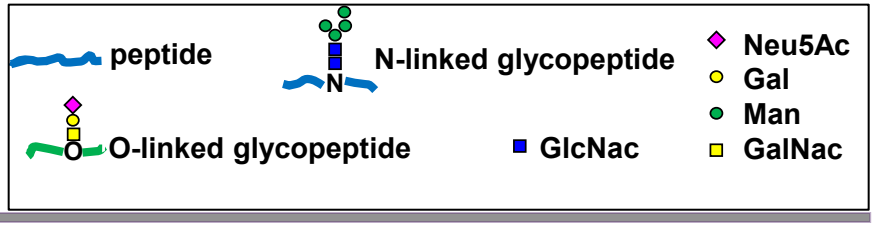


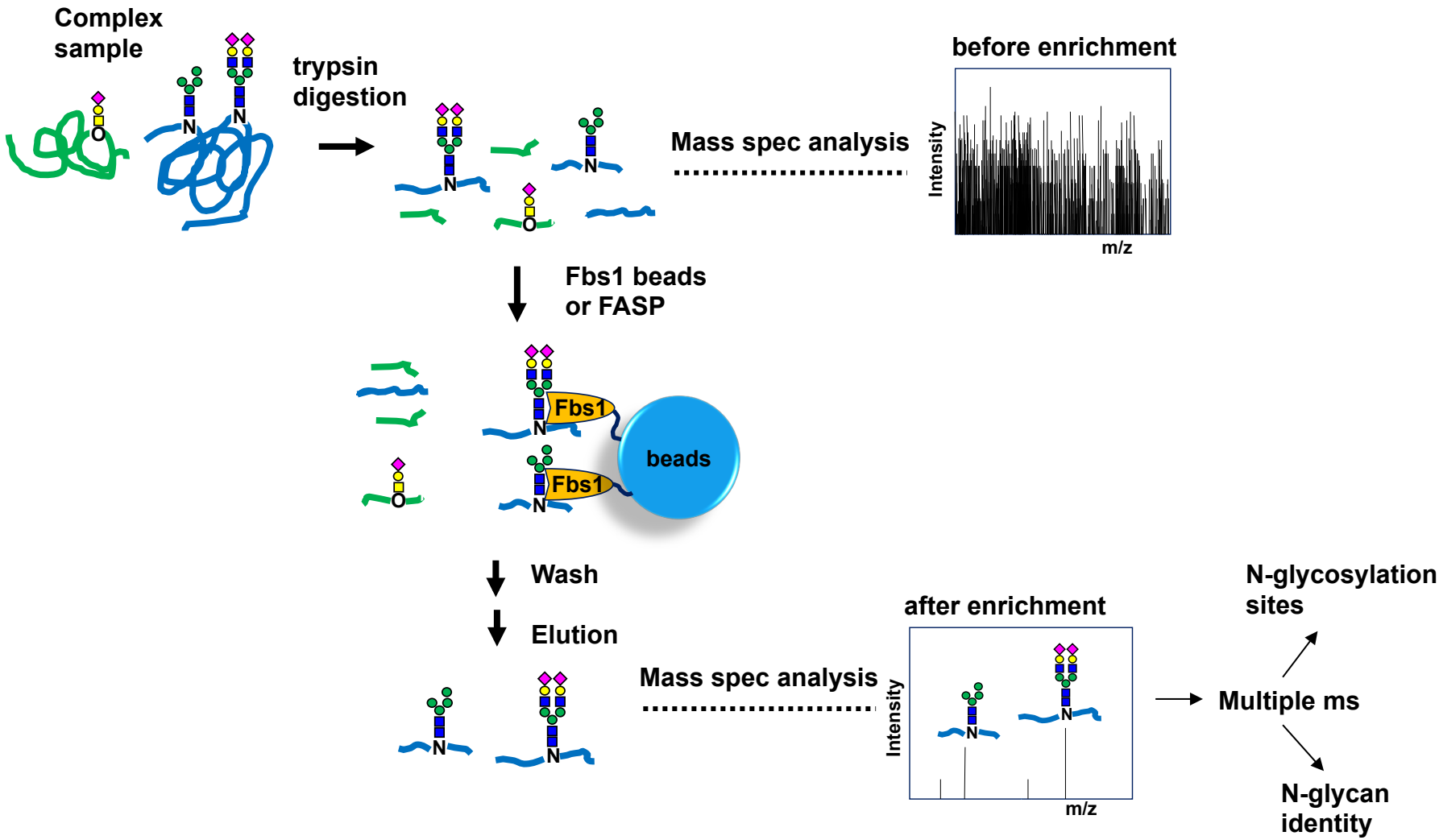
Recovery Ratio

	Fetuin/RNase B
input	1
WT	0.29
GYR	0.98
PPRYR	1.05

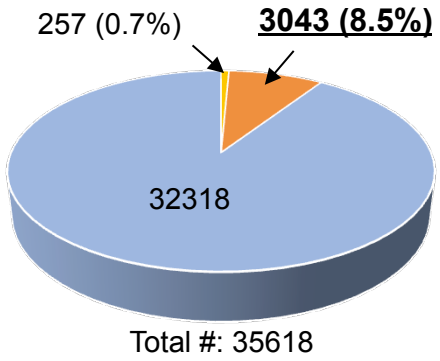
Assay: Denatured RNase B and Fetuin mixture pulled down by wt Fbs1 or Fbs1 mutant beads, analysis by SDS-PAGE.

Goal: Enrichment of N-linked Glycopeptides

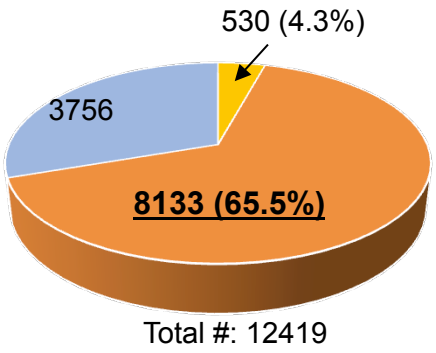




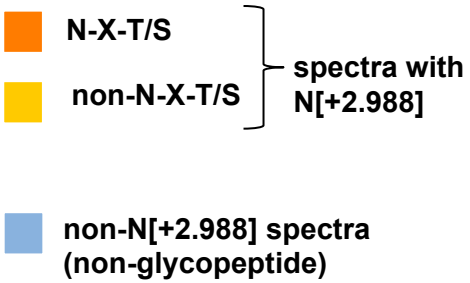
Human serum: N-linked Glycopeptide Enrichment to 66%



pre-enrichment



Fbs1 GYR enrichment



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An engineered high affinity Fbs1 carbohydrate binding protein for selective capture of N-glycans and N-glycopeptides

Minyong Chen[1], Xiaofeng Shi[1], Rebecca M. Duke[1], Cristian I. Ruse[1], Nan Dai[1], Christopher H. Taron[1] & James C. Samuelson[1]

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Acknowledgements: co-authors

Minyong Chen, primary author
Christopher Taron, Scientific Director
Stephen Shi
Rebecca Duke
Cristian Ruse
Nan Dai