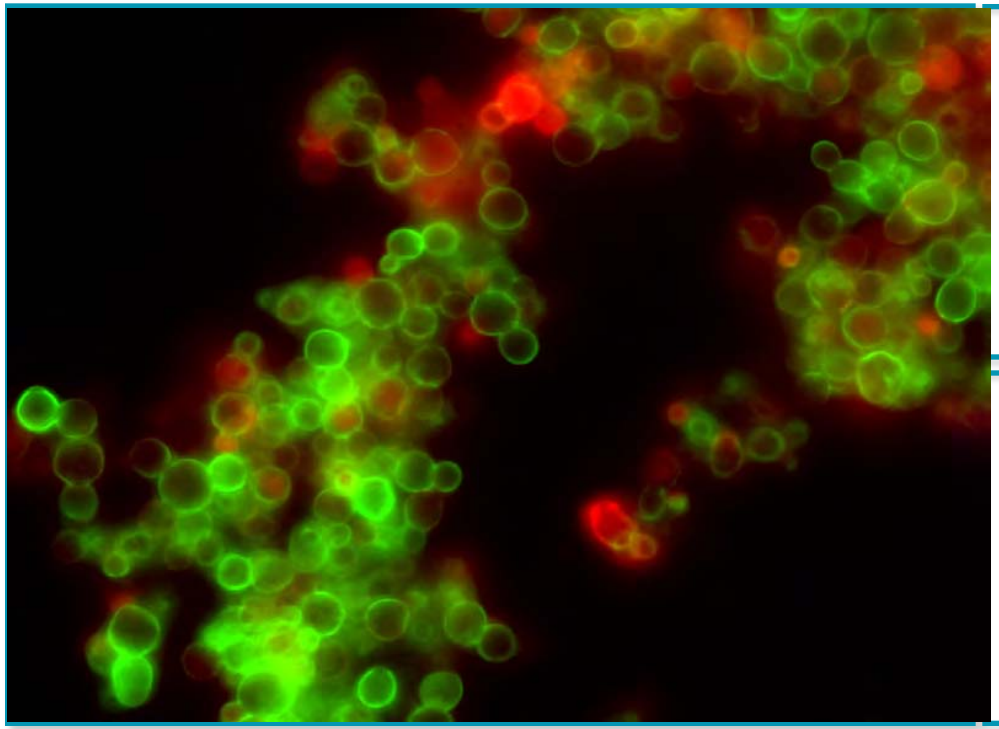

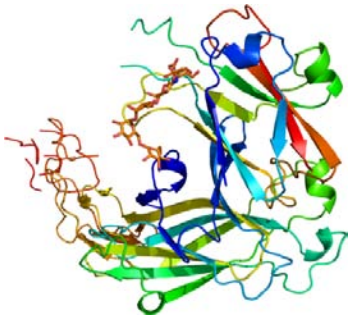

Pichia
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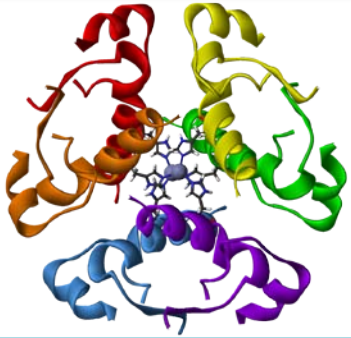


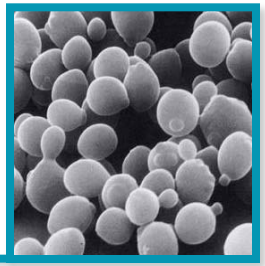
Yeast Display System

Pichia SuperMan5 + Vector System

Research Corporation Technologies (RCT)

www.pichia.com
www.rctech.com

Common Recombinant Protein Expression Systems



Prokaryotic

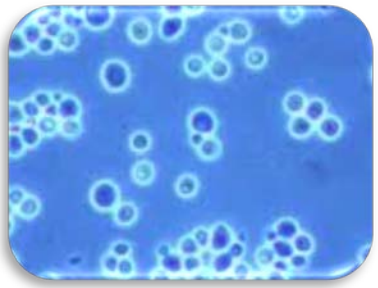
Bacteria

E. Coli
Pseudomonas

Eukaryotic

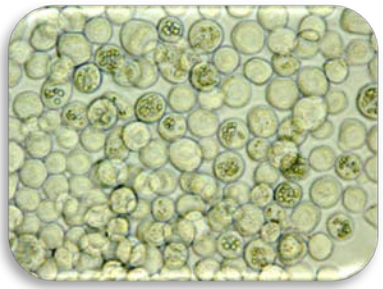
Yeast

Pichia pastoris
S. cerivisae



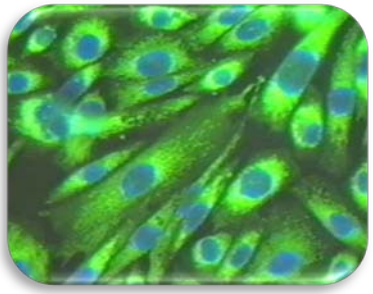
Insect

Baculovirus
Sf cells



Mammalian

CHO
HEK293



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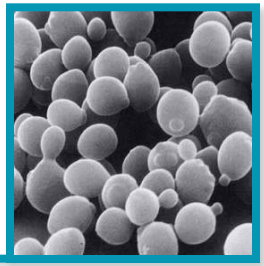
Protein Display Systems



Prokaryotic/Cell-free	Eukaryotic
• Bacterial – Cell surface display – Phage display • Cell-Free systems – DNA display – Ribosomal display	• Yeast Display – S. Cerivisae – Pichia Pastoris – Pichia GlycoSwitch • Mammalian Cell Display – CHO

Pichia
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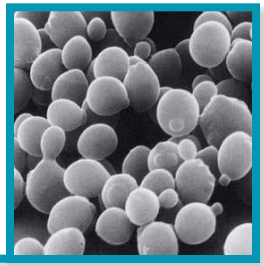
Choosing a Display System



Simple Proteins

- No complex folding
- No complex post-translational modifications (e.g. glycosylation)
- Examples Include: cytokines, single domains, receptor fragments, mAb fragments

✓ **Yeast**

✓ E. coli

✓ Cell free systems

Complex Proteins

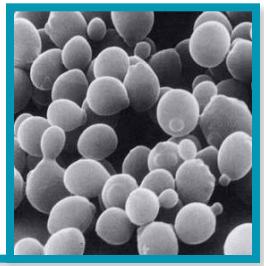
- Multiple disulfide bonds
- Post-translational modifications
- Examples include: receptors, GPCR, ion channels, mABs, mAb fragments, antigens

✓ **Yeast**

✓ Mammalian

Pichia
Technology From RCT

Why Yeast Display?



Displays simple and complex proteins on cell surface

- Highly folded proteins with multiple disulfide bonds are easily expressed and displayed
- Preferred system for antibody fragments and binding domains

Proteins are secreted in base expression system

- Natural system for display
- Same system can be used to produce protein of interest

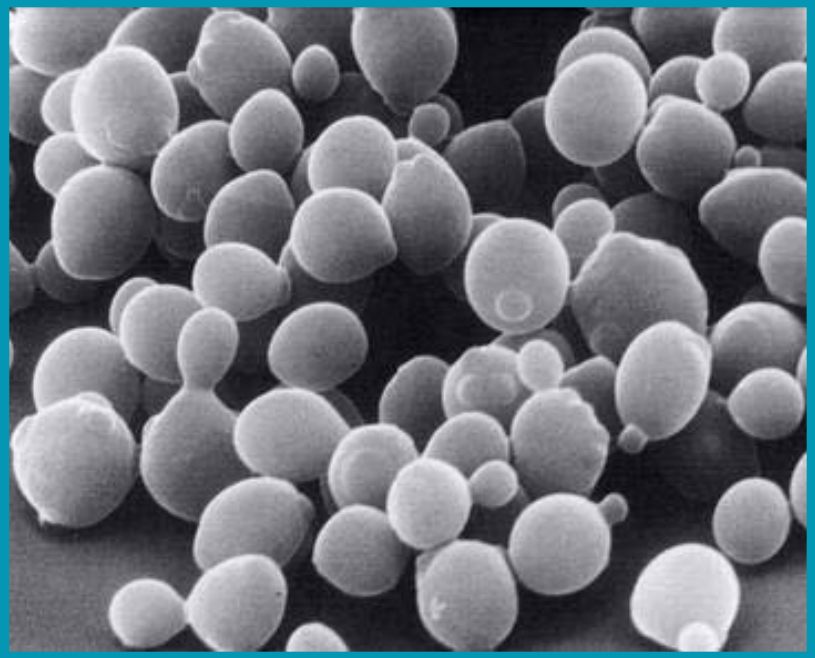
Higher expression levels

- Increased surface expression, low cost, easy culture conditions
- No endotoxin issues if performing cell-based screens, safe system

Easy selection and provides more data/screen

- FACS sorting can isolate positive binders and determine binding affinities in same experiment
- Ideal for smaller affinity maturation libraries and binder optimization work

Pichia
Technology From RCT

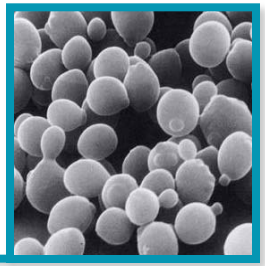


PICHIA GLYCOSWITCH® EXPRESSION SYSTEM

Human-like Glycosylation in Yeast

Pichia
Technology From RCT

Pichia GlycoSwitch® System



N-linked glycosylation

Hyper-mannosylation is not desirable !

N-acetylglucosamine

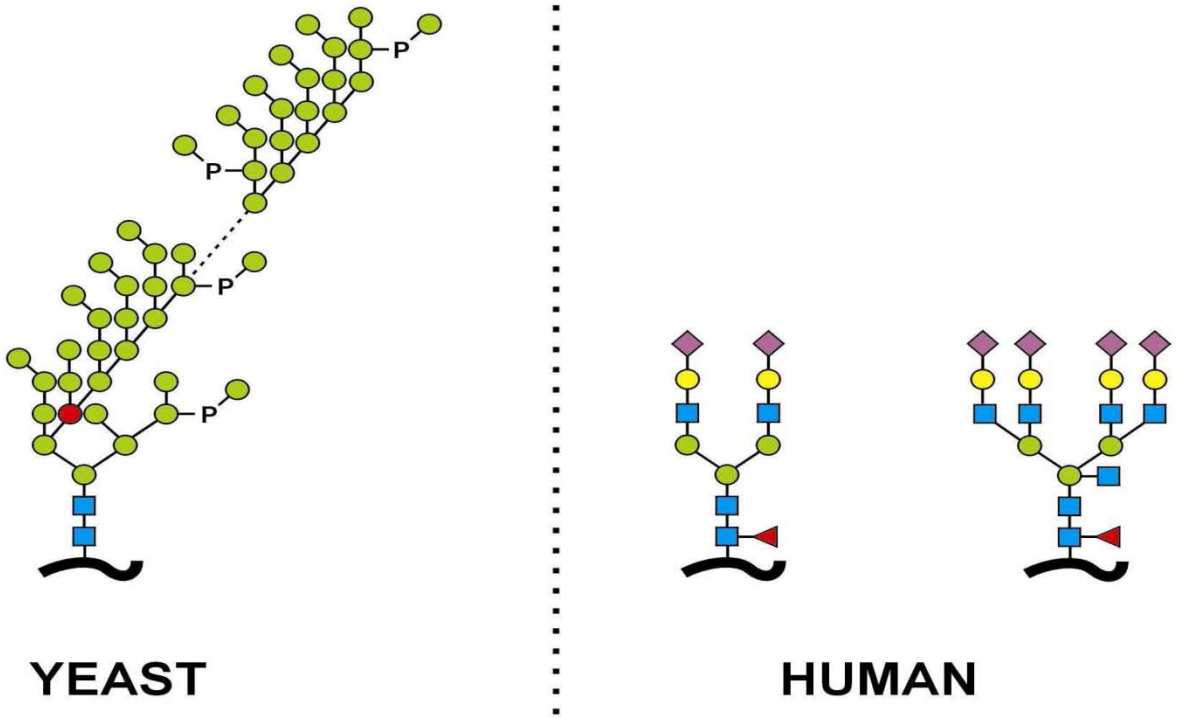
sialic acid

fucose

galactose

mannose

N-linked site



Pichia
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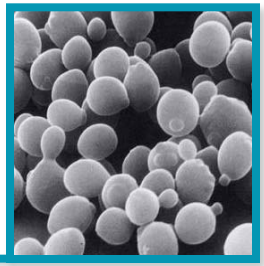
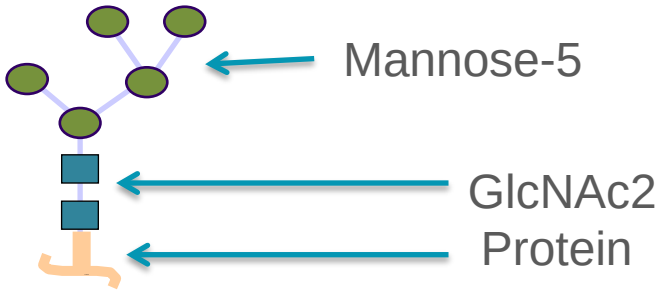
SuperMan5 Strains



- Series of Pichia GlycoSwitch strains that have been engineered to incorporate only mannose-5 sugars on the *Pichia* host proteins and on the exogenous proteins to be displayed
- A suite of SuperMan5 strains are available
  - Including ready-made electrocompetent cells



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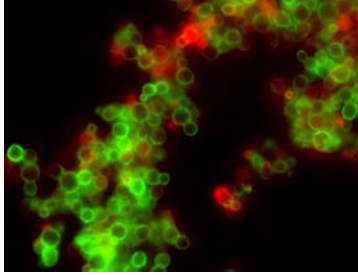
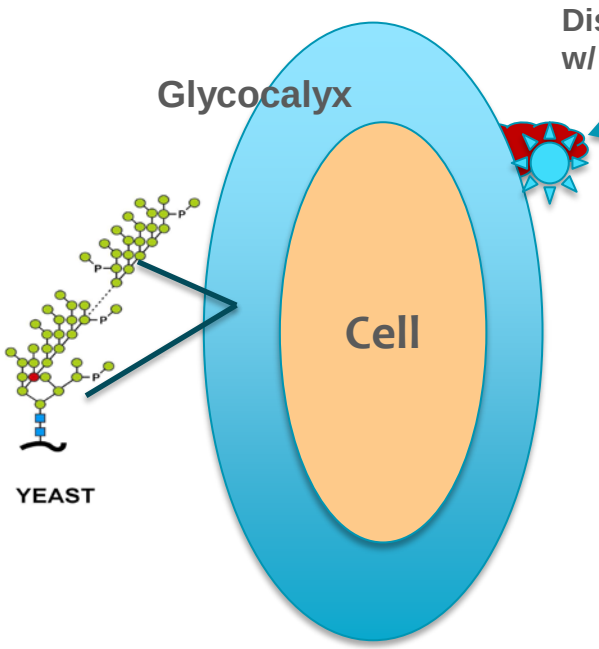
Why SuperMan5 for Display?

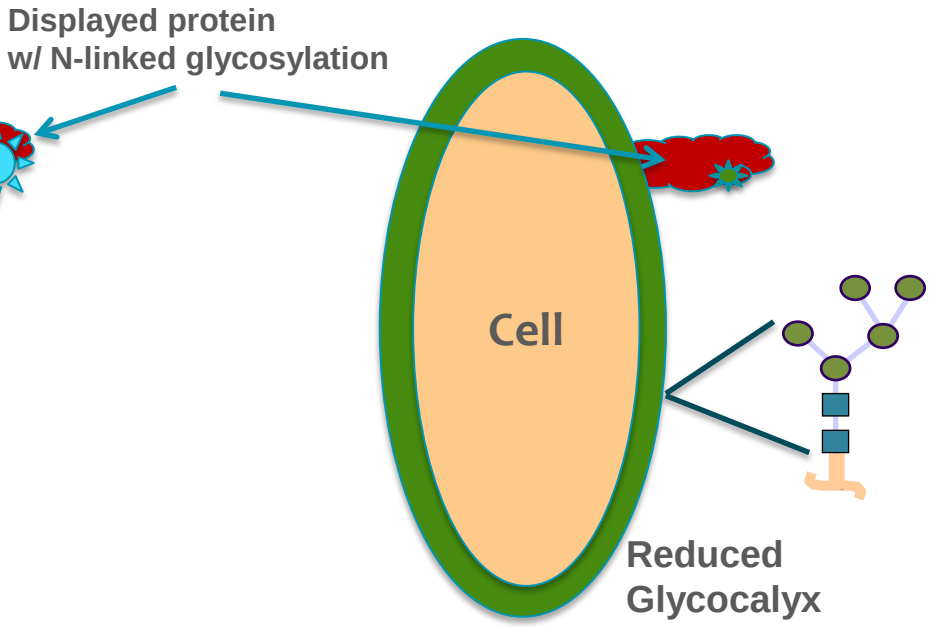


Current System in S. cerivisae



S. cerivisae – thick glycocalyx can affect expression and access to displayed protein. Protein may also have non-natural and excessive glycosylation which can mask key epitopes.

SuperMan5 Display System



SuperMan5 – uniform mannose-5 glycocalyx will allow more protein exposure and not mask important epitopes. May also improve protein folding and expression.

Pichia
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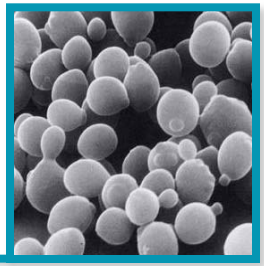
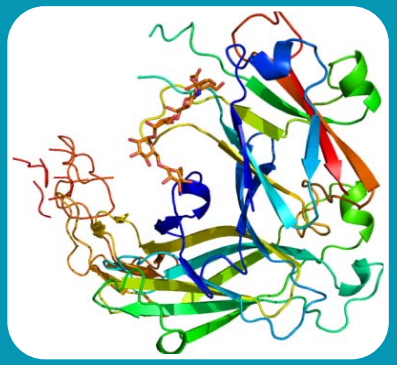
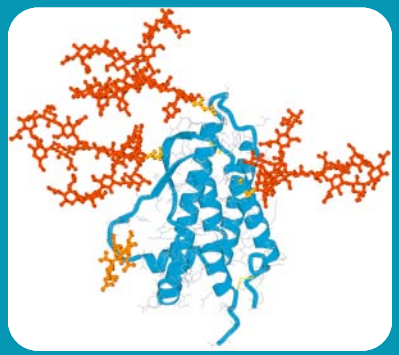
Advantages for SuperMan5 Display







- Homogenous Mannose-5 on host proteins as well, i.e., small glycocalyx on cell surface
- Preserves natural epitopes on protein of interest
- Reduces hidden epitopes buried in glycocalyx or blocked by non-natural glycosylation
- Proteins are displayed as close to the native form as possible
- May enable the display of complex proteins that require glycosylation for proper folding and processing
- Expresses glycoproteins at the same high levels seen with Pichia Classic
- Can use basic SuperMan5 strains or the SuperMan5 protease deficient strains for more complex and labile proteins

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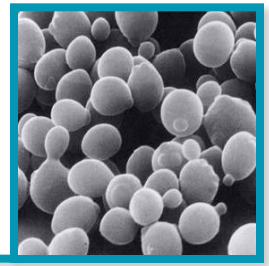
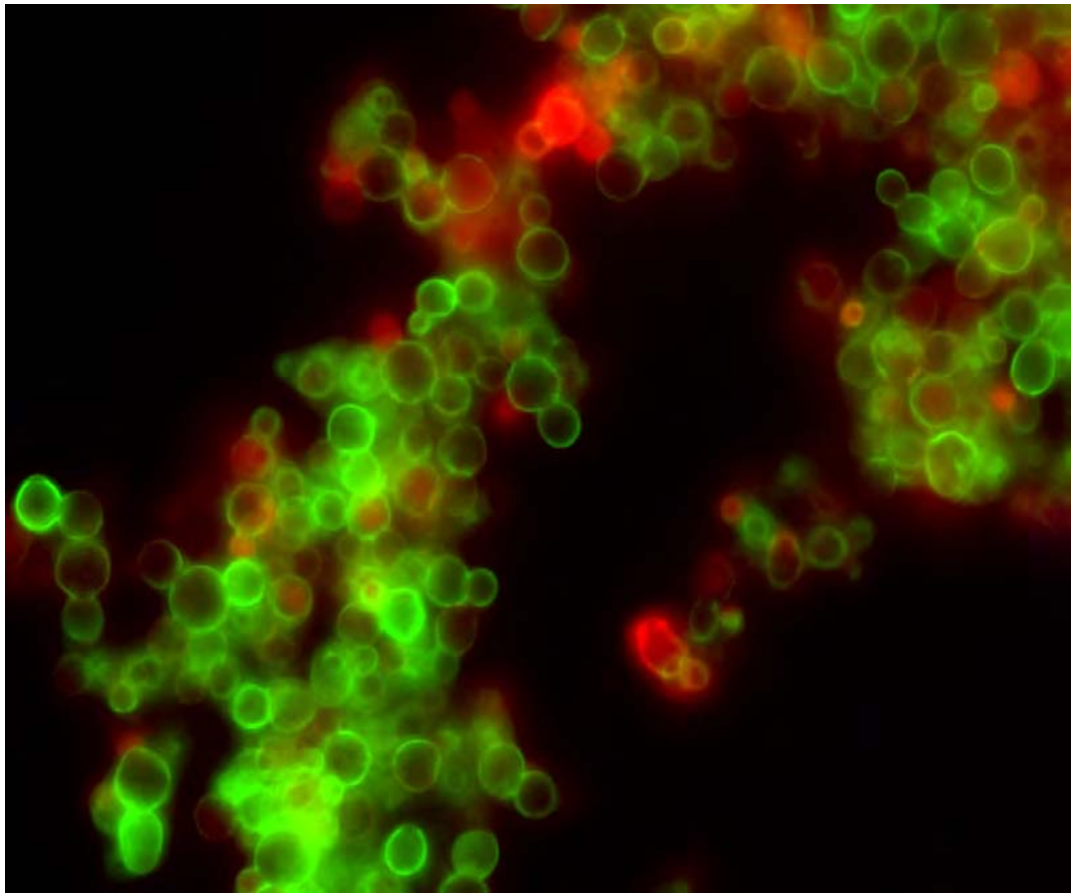
Surface Expression of V5 (green)





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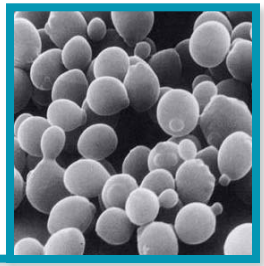
V5 test protein (green); ConA (red)

SuperMan5 Display Products

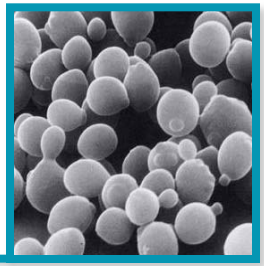


Catalog Number	Product Name	Description
PS006-01	SuperMan5*	1 stab culture ($HIS4^+$)
PS005-01	SuperMan5H ($HIS4^-$)*	1 stab culture ($HIS4^-$)
NA	pJAGs1-SAG1 (AOX1 promoter, G418 resistance, Pme1 linearization site)	2 micrograms
NA	pJANs1-SAG1 (AOX1 promoter, nourseeorthricin resistance, Pme1)	2micrograms
NA	pJAZs1-SAG1 (AOX1, Pme1, zeocin resistance)	2micrograms
NA	pGGs1-SAG1 (GAP promoter, AvrII linearization site, G418)	2micrograms
NA	pJGZs1-SAG1 (GAP promoter, AvrII, zeocin)	2micrograms

Also can ship ready-made competent cells

*Both strains will express your target protein with a mannose-5 structure at N-linked sites. Can include the other SuperMan5 protease deficient strains for complex and labile proteins. See biogrammatics.com for more information.

Purchasing and Licensing



Contact Research Corporation Technologies (RCT) to discuss your particular needs.

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