Title is a full sentence which tells the story of what was done

Development of a general defined media for *Pichia pastoris* protein expression

Catie Bartlett

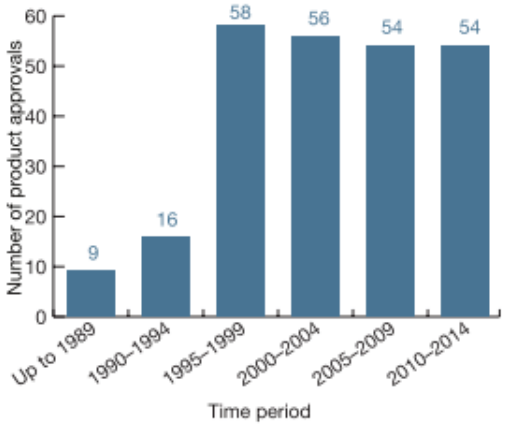
Course 10 – 3rd Year Talk

May 1, 2017

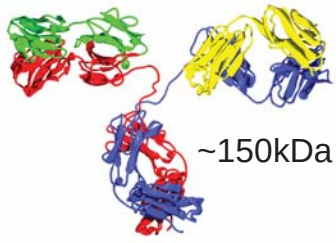
Slide title is a sentence that tells the main point, images support

An increasing fraction of new medicines are recombinant protein therapeutics

Over 200 products approved in past 20 years



Monoclonal antibodies



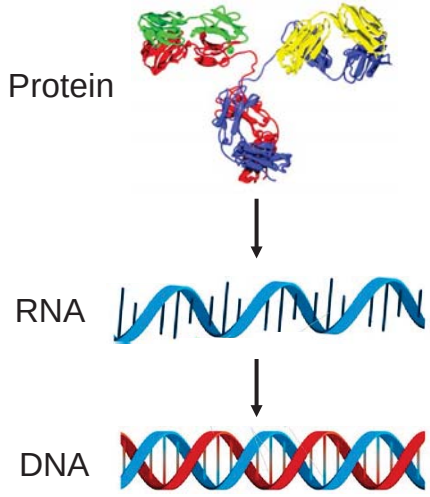
~150kDa

Enzymes
20-50kDa

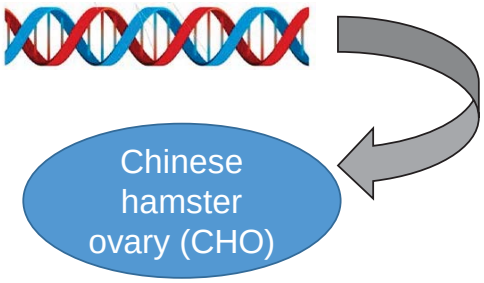
Walsh, *Nature Biotechnology* (2014)
Protein Data Bank

These proteins are made using cells

Design gene from protein



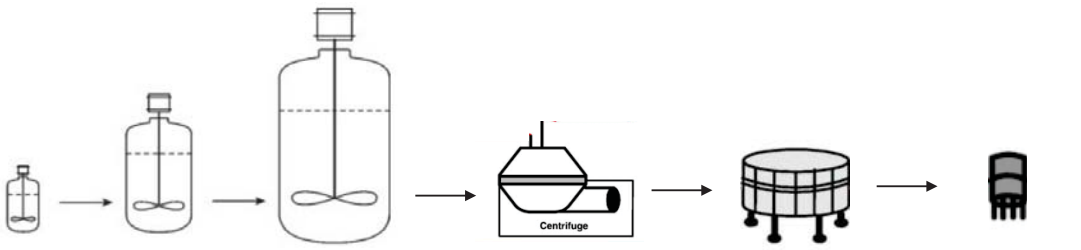
Incorporate into cell's genome



Cells express the foreign protein and secrete it into the culture broth

Image sequences are being used to convey a process rather than lists of text

Current CHO-based production process has a few challenges



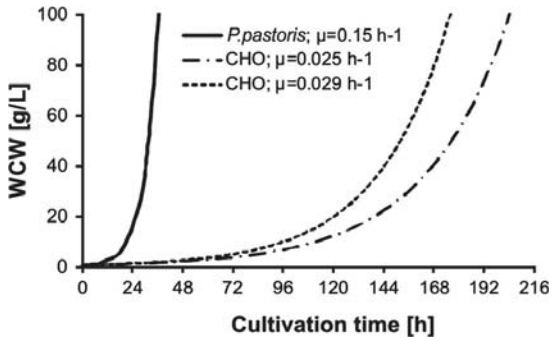
- Raw materials are expensive
- Production runs are long (2 weeks+)
- Foreign gene integration is complex

We might have suggested that the bullets be left out here–the list is already separated by lines!

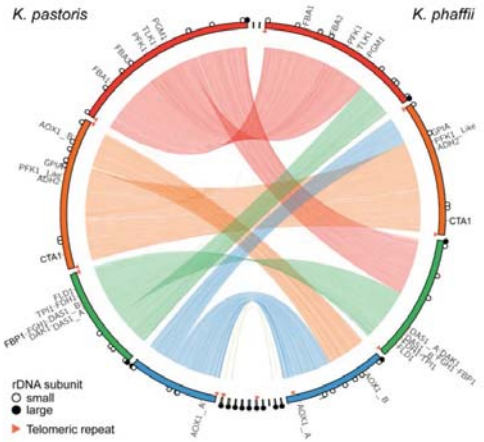
Adapted from Birch and Racher, *Advanced Drug Delivery Reviews* (2006)

Pichia pastoris holds great potential for manufacturing of biologic drugs

Fast growth to high cell density



Small genome – 4 chromosomes



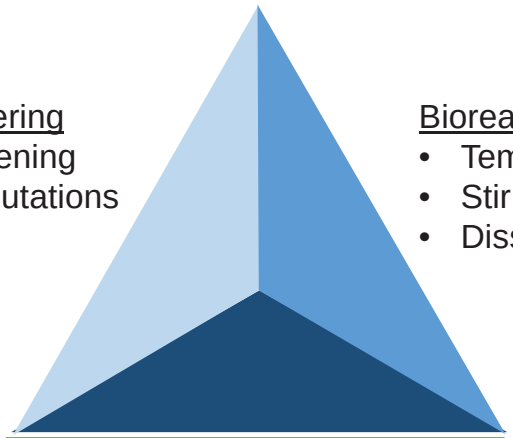
Color and an offset box are used to highlight an important take-away

However, rates of production (titers) are typically lower than those achieved with CHO cells

Kunert and Reinhart, *Appl Microbiol Biotechnol* (2016)

Love et al., *BMC Genomics* (2016)

Upstream process development toolbox has 3 main components



Strain engineering
- Clone screening
- Targeted mutations

Bioreactor parameters
- Temperature
- Stir rate
- Dissolved O_2

Easy to understand visual that separates a list spatially

Media formulation
- Nutrients (C, N, P, S)
- Feeding strategy

Media for *Pichia* has not been studied as extensively as for CHO

Media type	CHO	*Pichia*
Complex Nutrient-rich but difficult to characterize	Fetal bovine serum	Buffered complex medium (BMGY)
Defined All components are known, favored by regulators	Carbon source, salts, proteins, hormones, vitamins, amino acids, lipids, etc. Examples: Ham's F-12, proprietary formulations	Carbon source, salts, trace elements Examples: BSM, FM22, d'Anjou

Here it would be a little neater if the table entries were top aligned with each other!

Approach and methods

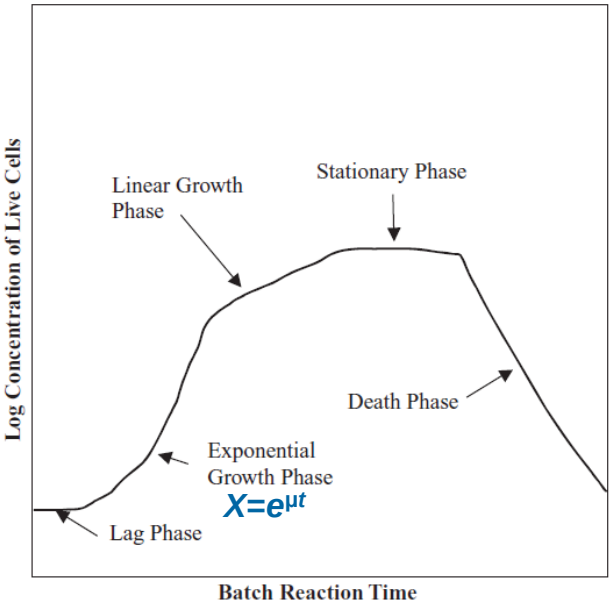
Our goal was to design a defined media that reduced the metabolic burden on the organism, evaluated by growth rate

Slide offsets the main goal (top) from less critical information (below)

We integrated three strategies

- Systematic screening to understand limitations of current media and identify nutrient supplements
- Analytical methods to identify nutrients and tune concentrations
- Transcriptomics for deeper view of biological processes

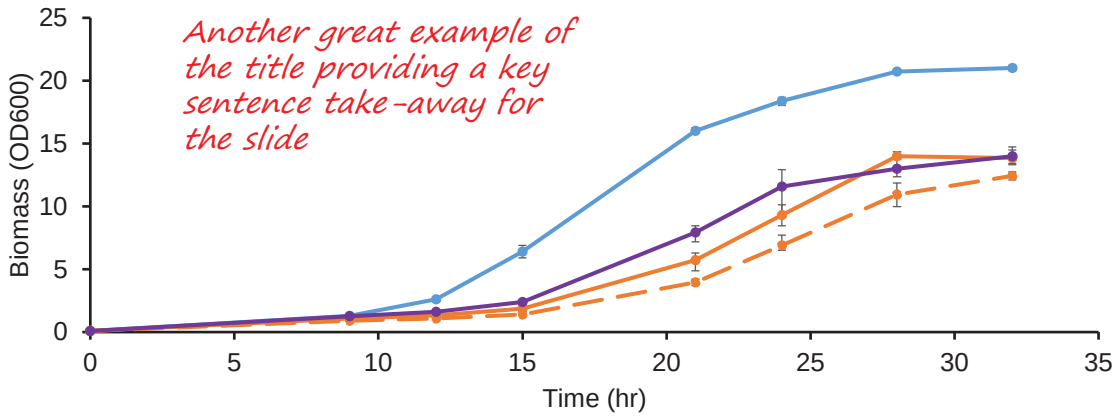
Reminder: idealized growth phases for batch fermentation



The author uses a cartoon of data to describe to the audience what their type of science looks like

E. B. Nauman, *Chemical Reactor Design, Optimization, and Scaleup,* Second Edition. (2008)

For *Pichia*, growth in basal salts media is significantly slower than in complex media



Another great example of the title providing a key sentence take-away for the slide

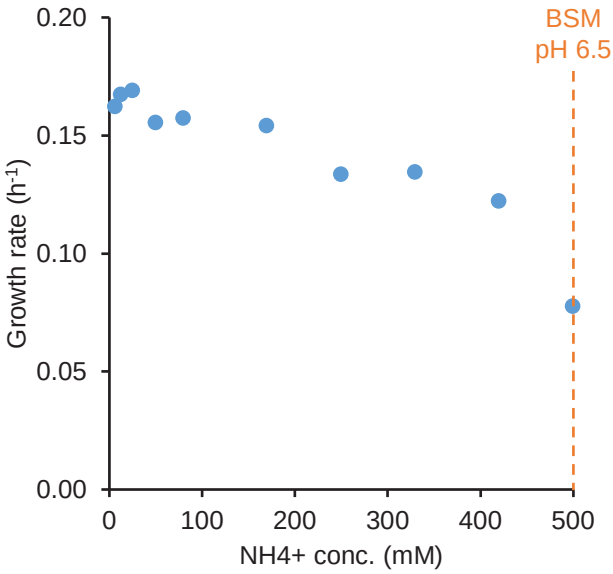
	BMGY	BSM, pH 5.0
μ (h^{-1})	0.212	0.143

Something in the basal salts medium was inhibiting growth

10mL microtiter plates

Bartlett et al., manuscript in preparation

Reducing ammonium concentration increased exponential growth rate to 0.17h^{-1}



No extra words distract from the plot–the speaker will guide you through understanding the data!

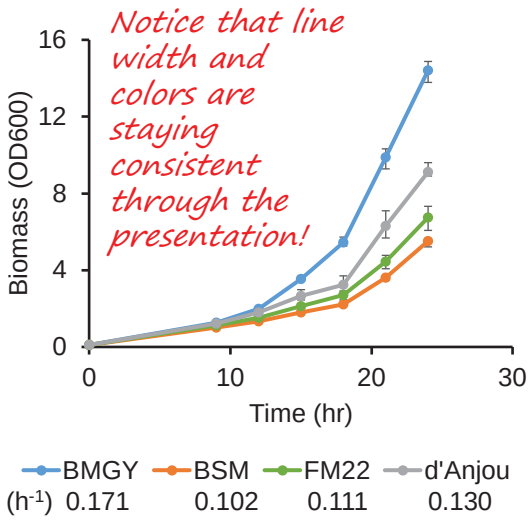
10mL microtiter plates
Bartlett et al., manuscript in preparation

Looking for further improvement, we tried other salts formulations

FM22 and d'Anjou medium have both been used for *Pichia* fermentations

- Lower salt content than BSM

For comparison, set NH_4^+ concentrations of all to 25mM



Used d'Anjou medium with 25mM NH_4^+ as base for further optimization

10mL microtiter plates
Bartlett et al., manuscript in preparation

We used knowledge about complex media to select defined components for screening

From HPLC:

Amino acid	Concentration (mM)
Arginine	4.2
Alanine	4.0
Lysine	3.2
Glycine	2.7
Glutamate	2.4
Leucine	2.3
Phenylalanine	1.7
Isoleucine	1.1
Serine	0.8
Tyrosine	0.3
Total	22.8

Bartlett et al., manuscript in preparation

Stock solutions of some nutrients have previously been tried

- Vitamins
- Nucleosides

Probably another instance where the bullets are just adding noise!

Carbohydrate concentrations in yeast extract have been measured

- Lactate: up to 10mM
- Trehalose: up to 5mM

Verduyn et al., *Yeast* (1992)
Hellenbroich et al., *Appl. Microbiol. Biotechnol.* (1999)
Zhang et al., *Biotechnol. Bioeng.* (2003)

Glutamine, arginine, and vitamins had the greatest impact on growth rate

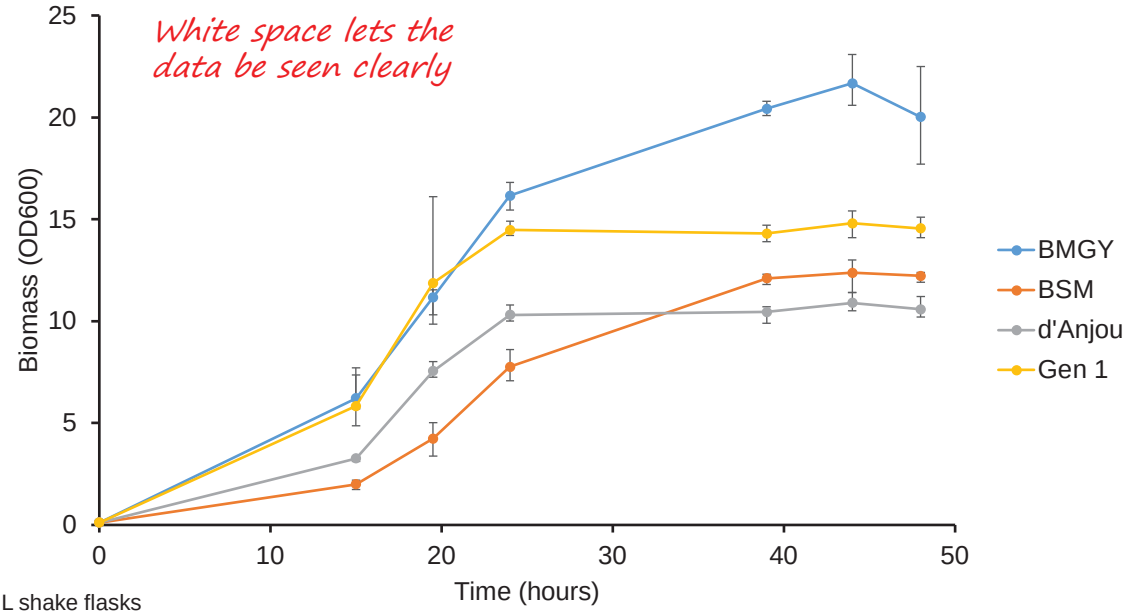
Supplement	Concentration	μ (h^{-1})
Complex media	-	0.248 ± 0.001
None	-	0.196 ± 0.001
Glutamine	5mM	0.217 ± 0.001
Vitamins	1x	0.204 ± 0.001
Arginine	5mM	0.202 ± 0.001
Lysine	5mM	0.196 ± 0.002
Nucleotides	1x	0.196 ± 0.002
Alanine	5mM	0.195 ± 0.002
Trehalose	5mM	0.193 ± 0.001
Lactate	10mM	0.186 ± 0.002

Great use of a box or color change to highlight important details

Generation 1 medium included these nutrients in a low-ammonium d'Anjou base

Bartlett et al., manuscript in preparation

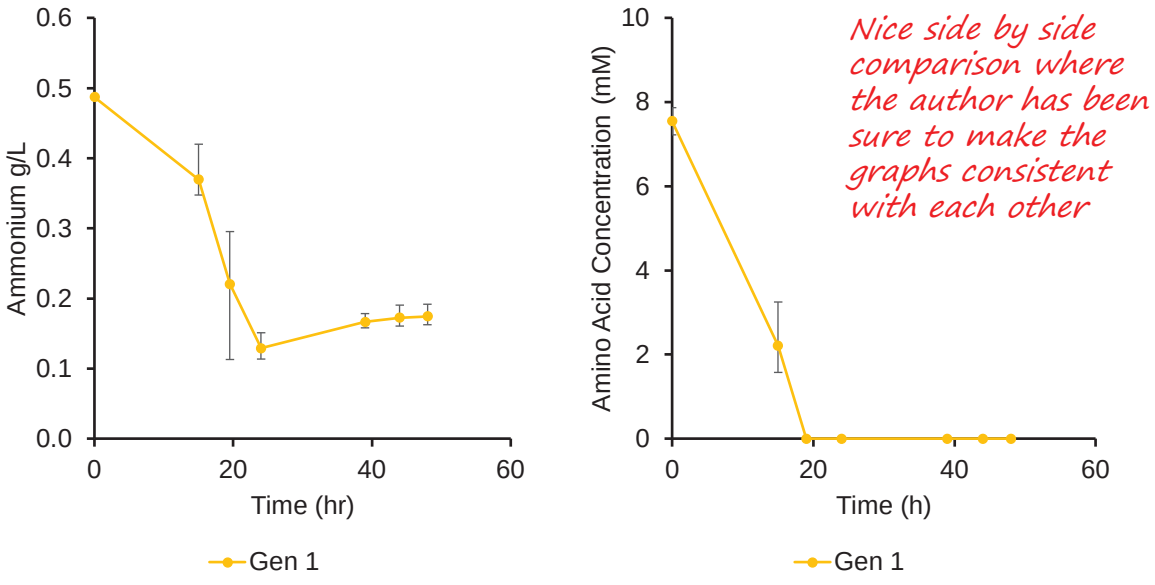
Growth in Generation 1 medium was comparable to BMGY during exponential phase, then leveled off



1L shake flasks

Bartlett et al., manuscript in preparation

In Gen 1 medium, NH_4^+ was sufficient but amino acids were fully consumed



Bartlett et al., manuscript in preparation

To further characterize metabolic differences, we performed RNA-Seq

Prepares the audience for the type of data they will see

Output:

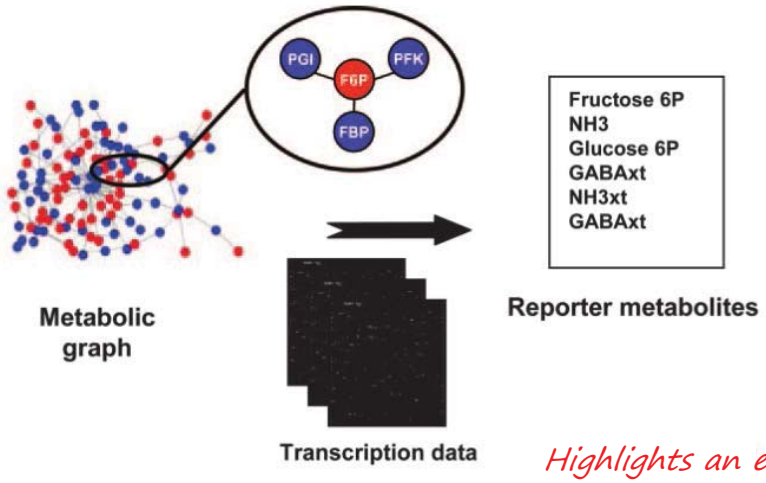
Gene	BMGY	d'Anjou	Gen 1
ARO10	#	#	#
POX1	#	#	#
CAR1	#	#	#
POT1	#	#	#
GDH3	#	#	#
CAR2	#	#	#
COX15	#	#	#
SPS4	#	#	#
FLO9	#	#	#
PUT1	#	#	#
…	#	#	#

Data is analyzed by comparing gene expression between conditions

Computational methods have been developed for different levels of comparison:

- Individual genes
- Pathways or gene sets

Reporter metabolites method was used to identify expression differences at pathway level

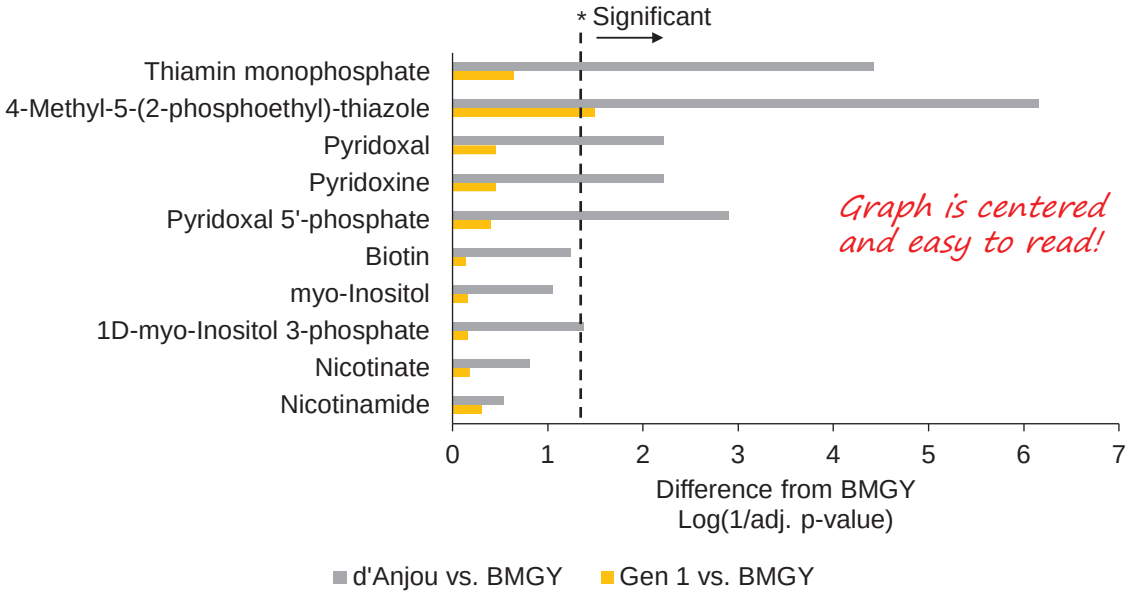


Highlights an exciting detail!!

This method has not previously been used for media design

Patil and Nielsen, *PNAS* (2005)
Tomas-Gamisans et al., *PLoS One* (2016)

Known difference in vitamin metabolism was visible in the transcriptome

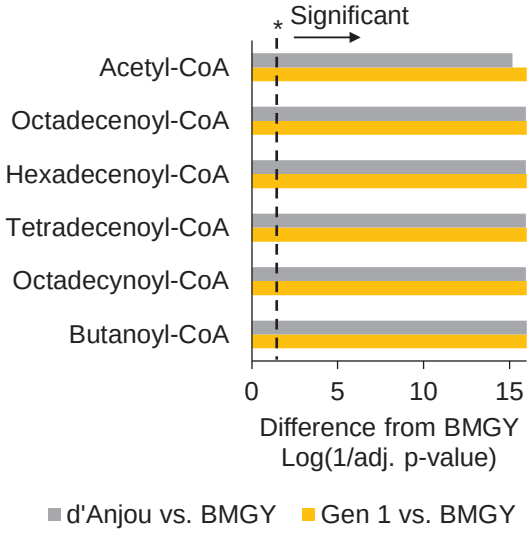


Graph is centered and easy to read!

Bartlett et al., manuscript in preparation

Slide title provides a description of experiments and the implication is highlighted below!

We used the same approach to identify other areas with significantly different metabolism



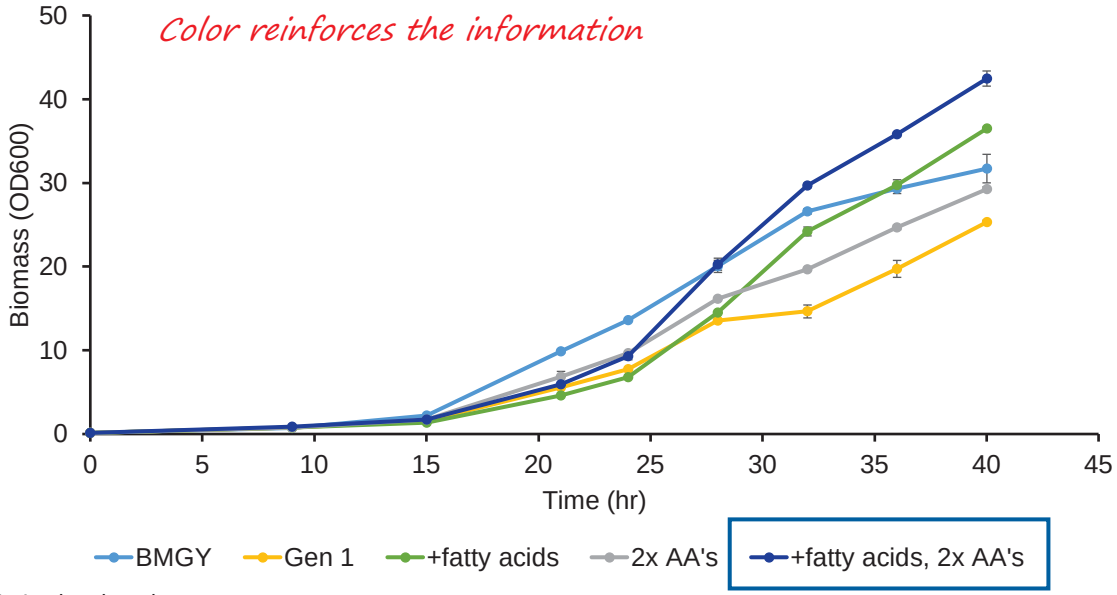
The most significantly different metabolites for both defined formulations are involved in fatty acid oxidation

Fatty acids are present in BMGY but not in either defined formulation

Implication: try adding fatty acids

Bartlett et al., manuscript in preparation

Fatty acids and increases to amino acid concentrations improved performance



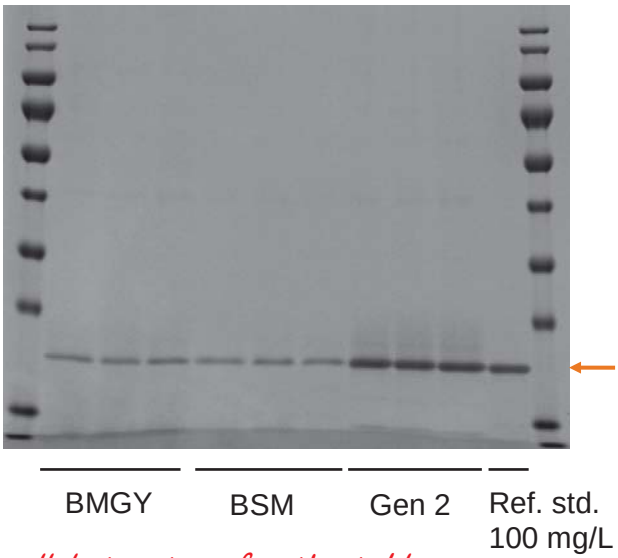
Gen 2 media

10mL microtiter plates

Bartlett et al., manuscript in preparation

hGH productivity was ~10x higher in Gen 2 medium than BMGY or BSM

Media	BMGY	BSM	Gen 2
Biomass after outgrowth (OD600)	14.3	9.03	17.9
Biomass after induction (OD600)	23.5	15.4	23.1
Titer by GX (mg/L)	22.1	<LOD	201

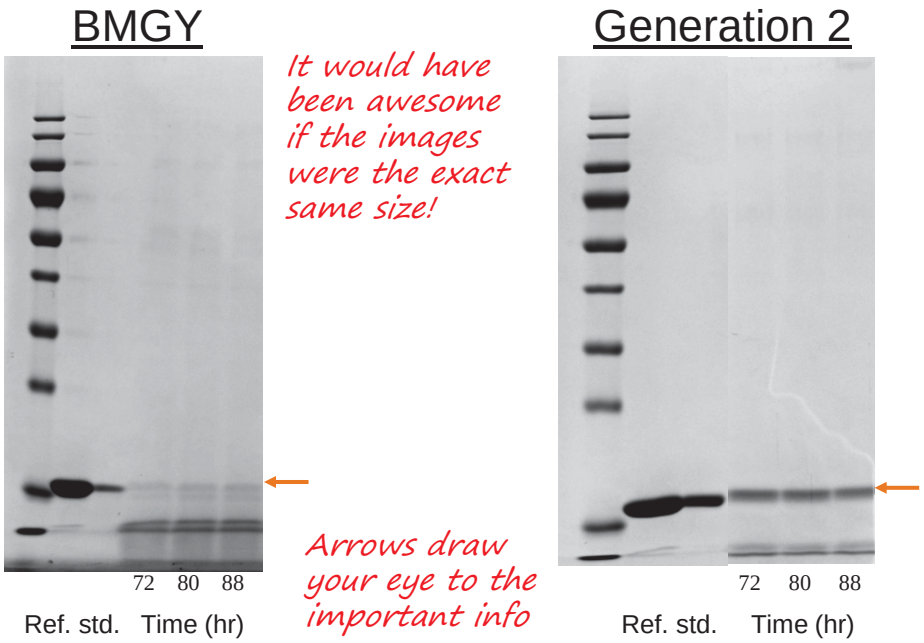


We see parallel structure for the table and raw data which is intuitive for us to understand

200mL shake flasks

Bartlett et al., manuscript in preparation

G-CSF productivity in bioreactors was also higher in Gen 2 medium than BMGY



It would have been awesome if the images were the exact same size!

Arrows draw your eye to the important info

Bartlett et al., manuscript in preparation

Great job not using bullets where they weren't needed, the list separates it automatically!

Summary of results

We developed a defined media for *Pichia pastoris* that supported cell growth at the same rate as in BMGY and led to higher protein productivity

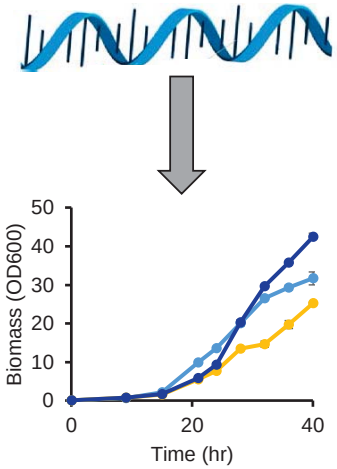
We identified metabolic gaps and addressed them through transcriptomics, analytical methods, and systematic screening

Future work will focus on optimizing Generation 2 media specifically for productivity

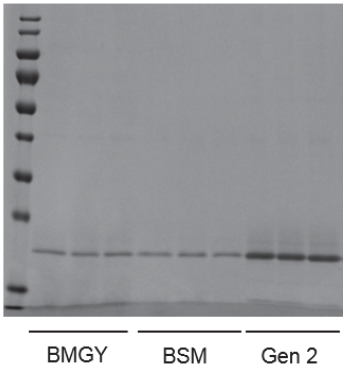
It's a great idea to tie back experimental conclusions to the motivation you worked so hard to build at the beginning!

Implications

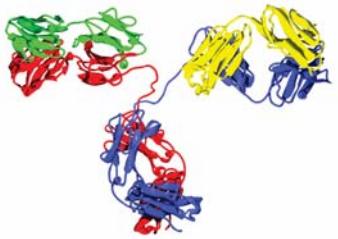
Transcriptomic analysis is powerful



Productivity in *Pichia* will increase



Biologic manufacturing costs are addressable



Acknowledgments

Thesis committee

J. Christopher Love (adviser)

Richard Braatz

Kristala Prather

Love Lab and InSCyT Team

Kerry Love

Joe Brady, Laura Crowell, Neil Dalvie, Amos Lu, Nick Mozdzierz

Alex Bonnyman, John Clark, Bill Doherty, Di Liu

Sierra Brooks, Angel Kuo, Trey Roberts

Danielle Camp

Sequencing support

Charlie Whittaker (KI)

Jon Penterman (BMC)

Fangming Zheng (BMC)

Funding

This material is based upon work supported by the Defense Advanced Research Projects Agency (DARPA) and SPAWAR Systems Center Pacific (SSC Pacific) under Contract No. N66001-13-C-4025

This work was supported in part by the Koch Institute Support (core) Grant P30-CA14051 from the National Cancer Institute.

Any opinions, findings and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the Defense Advanced Research Projects Agency (DARPA) and SPAWAR Systems Center Pacific (SSC Pacific).

Questions?