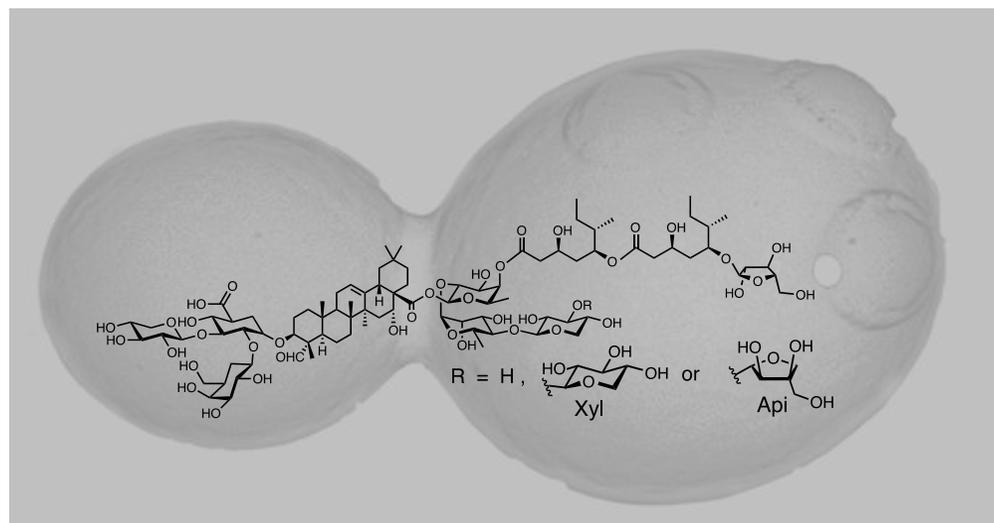


The sweet side of drug discovery: *How sugars can help shape the future of medicine*

Yuzhong Liu, PhD

Assistant Professor

Department of Chemistry



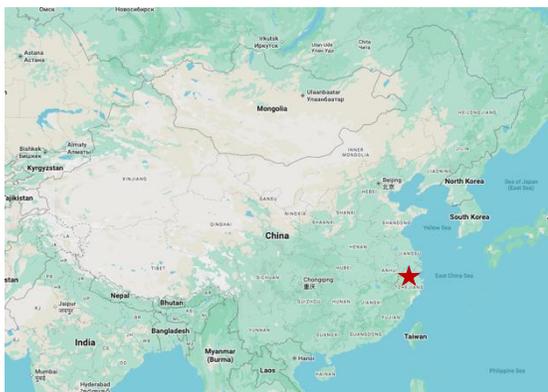
**Scripps
Research**



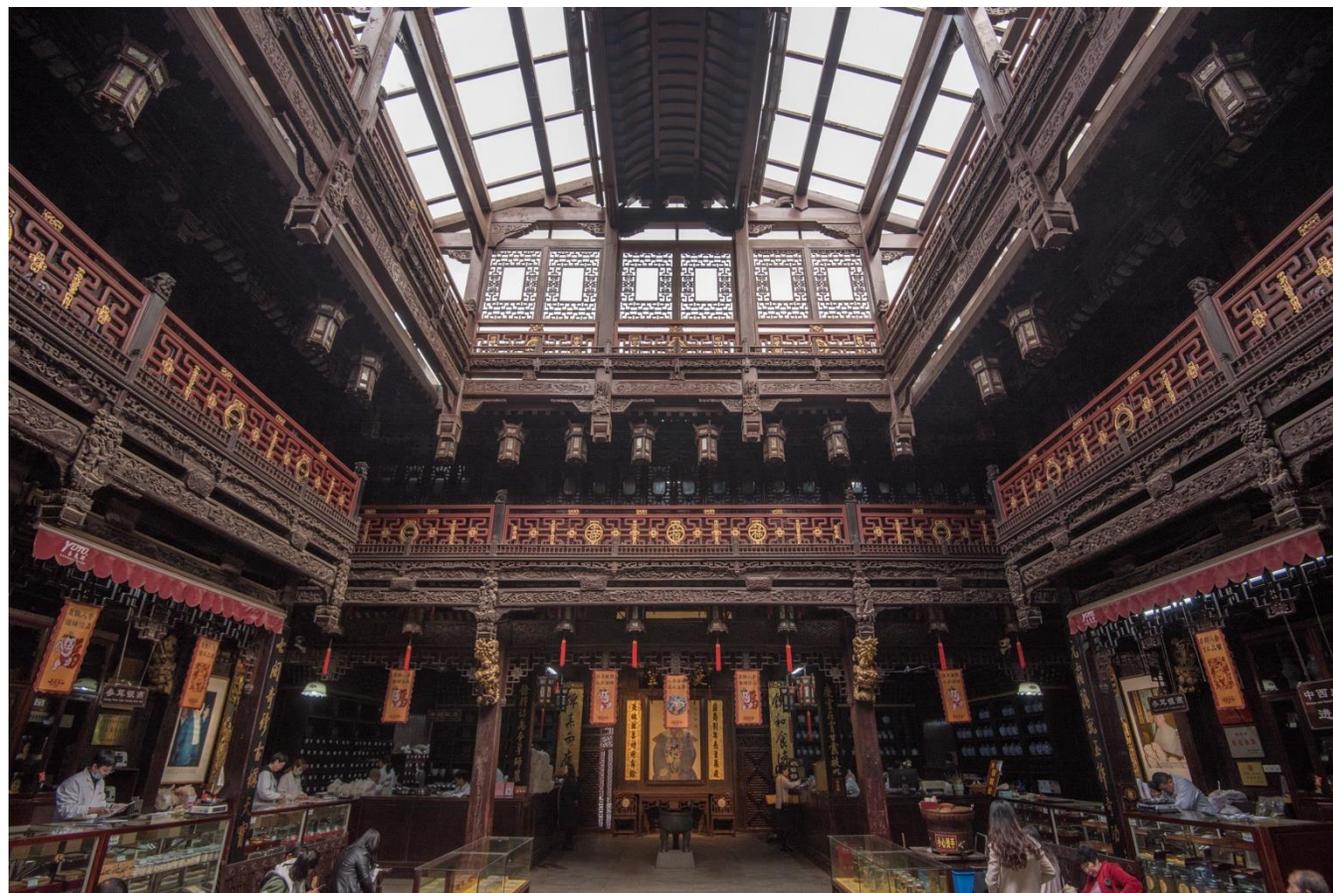
**THE
FRONT
ROW**
at Scripps Research

- How are molecular medicines made today?
- Why supply becomes a bottleneck?
- Sugars as key drivers to improve pharmacological outcomes
- Synthetic biology as a discovery and manufacturing platform
 - Engineering microbes to make essential medicines

My first encounter of natural products



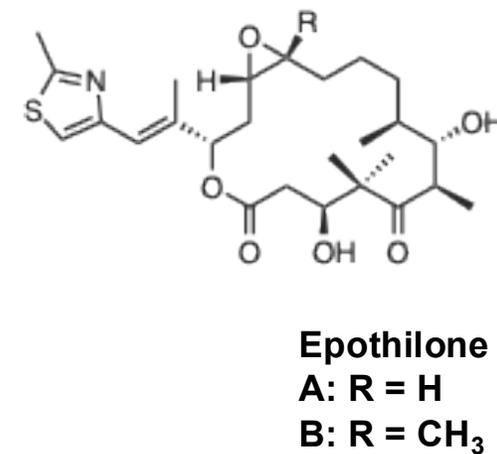
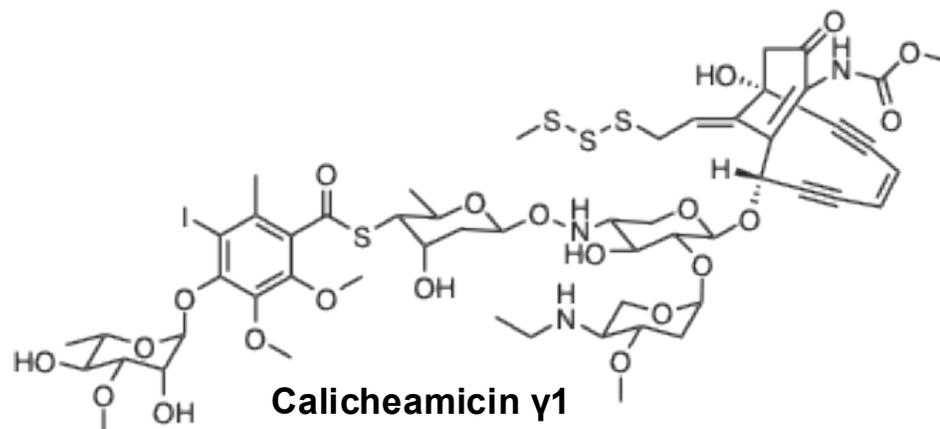
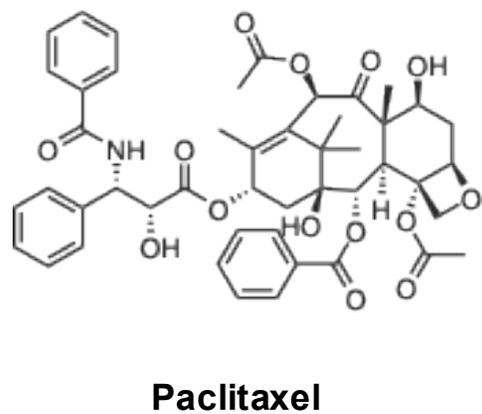
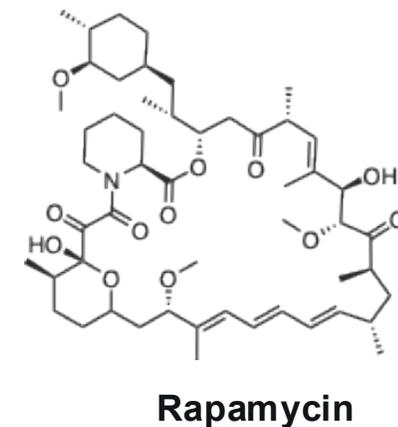
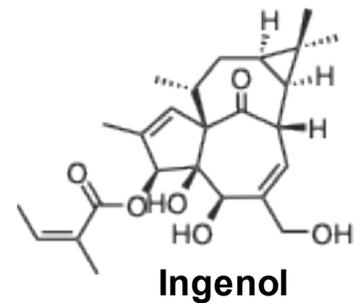
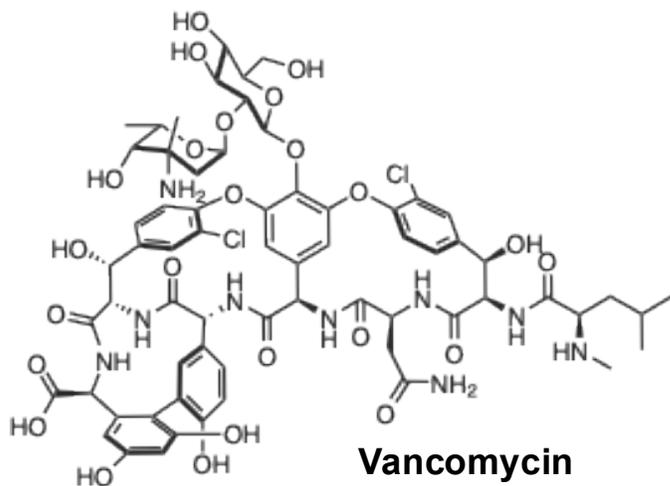
Hangzhou, China



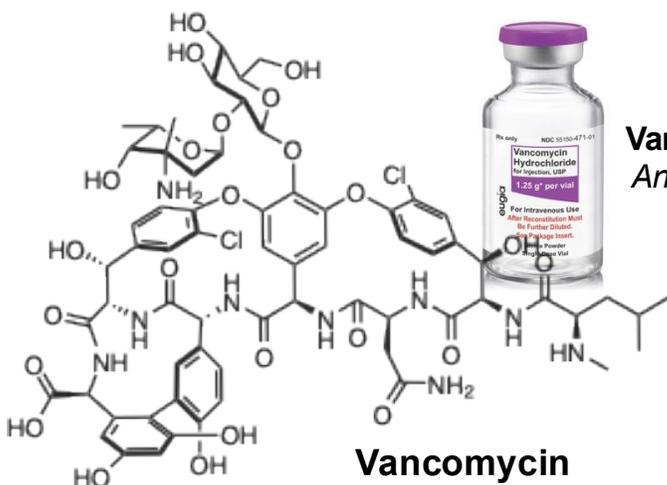
胡慶余堂 (Hu Qing Yu Tang)

**“是乃仁術，真不二價”
(Art of benevolence, genuine goods at fair prices)**

Molecules at the center of modern medicine



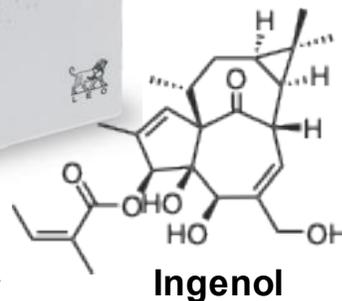
Molecules at the center of modern medicine



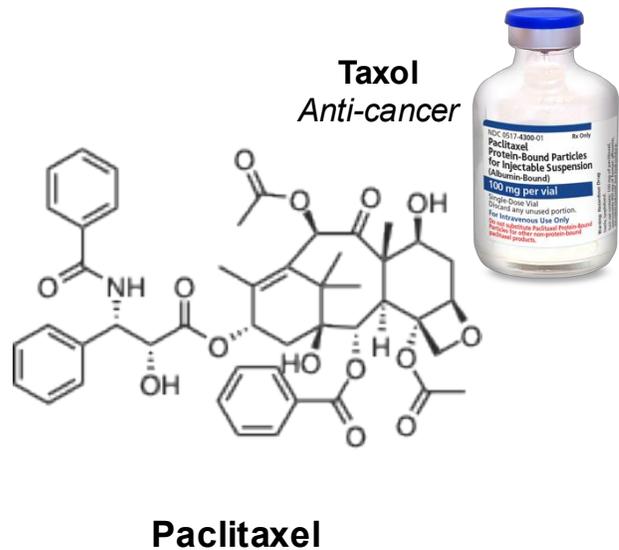
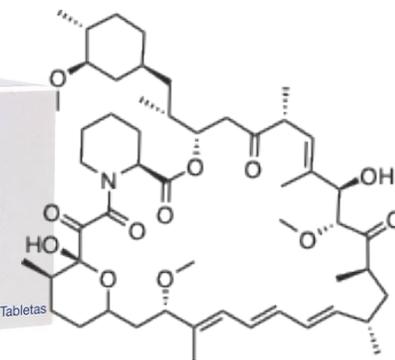
Vancocin
Antibiotic



Picato
Anti-actinic keratosis



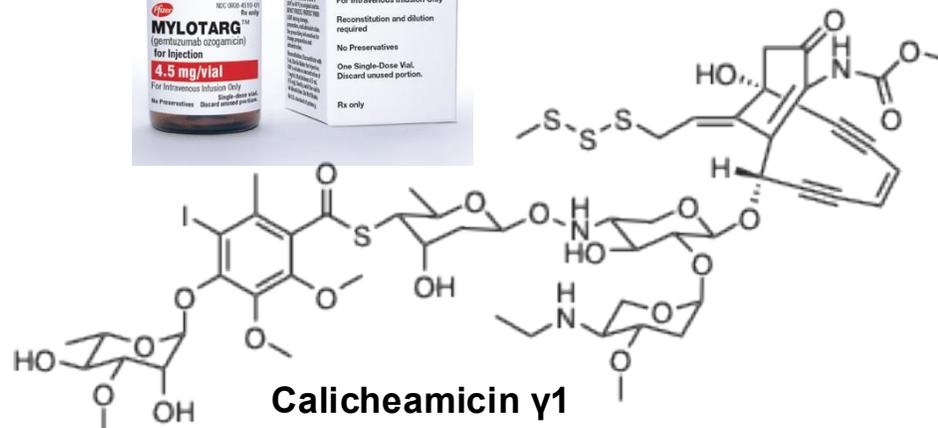
Rapamune
Immunosuppressant



Taxol
Anti-cancer



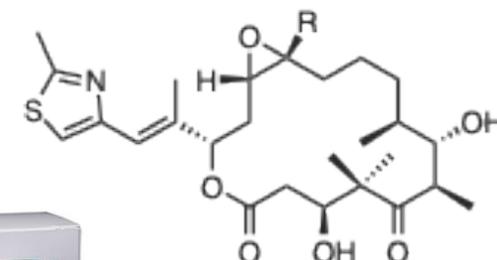
Mylotarg
Anti-cancer



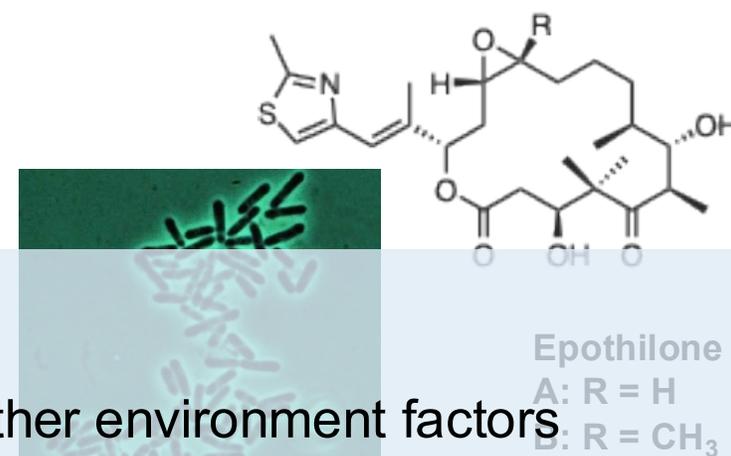
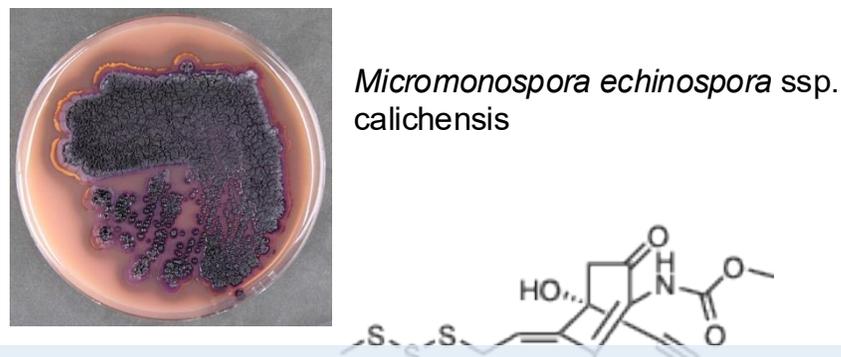
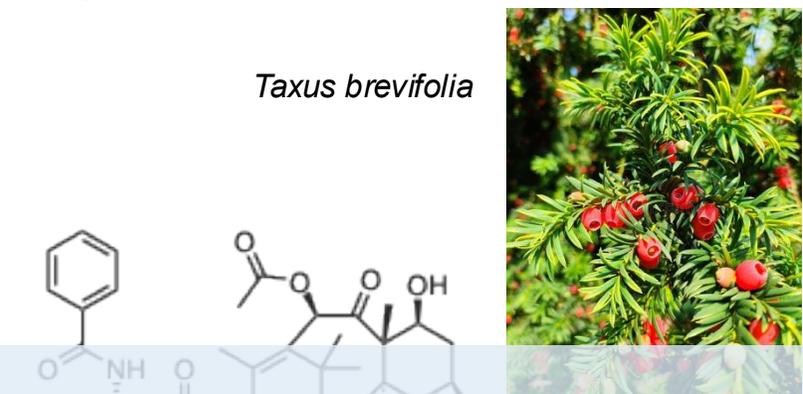
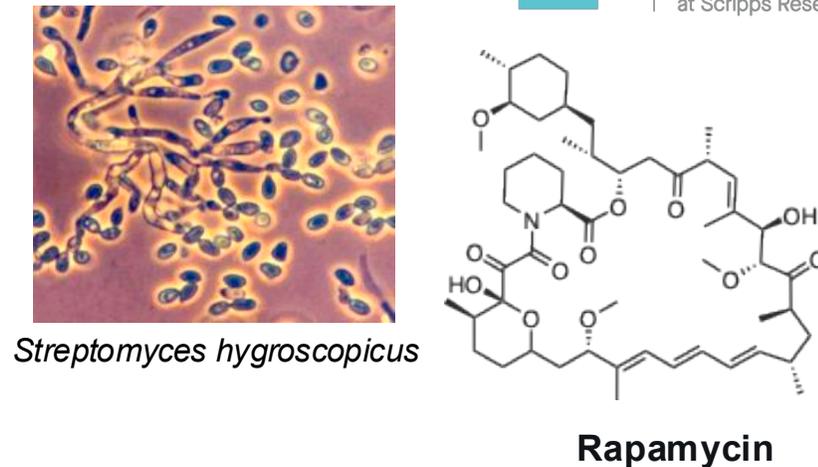
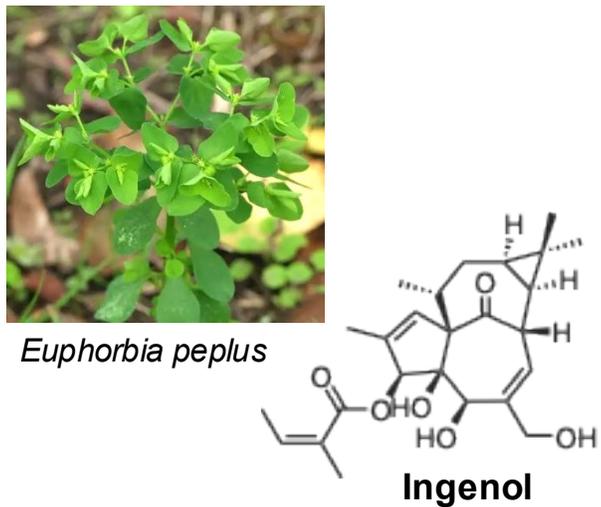
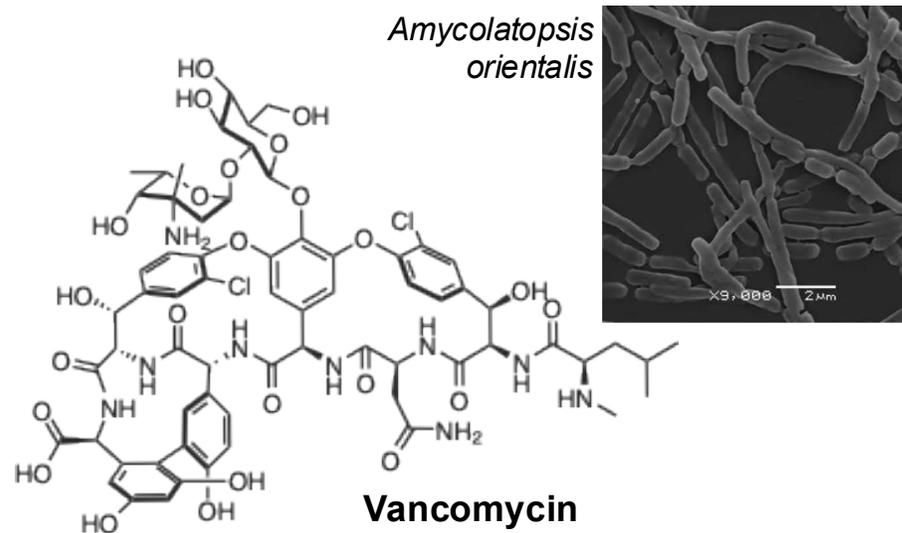
Ixempra
Anti-cancer



Epothilone
A: R = H
B: R = CH₃



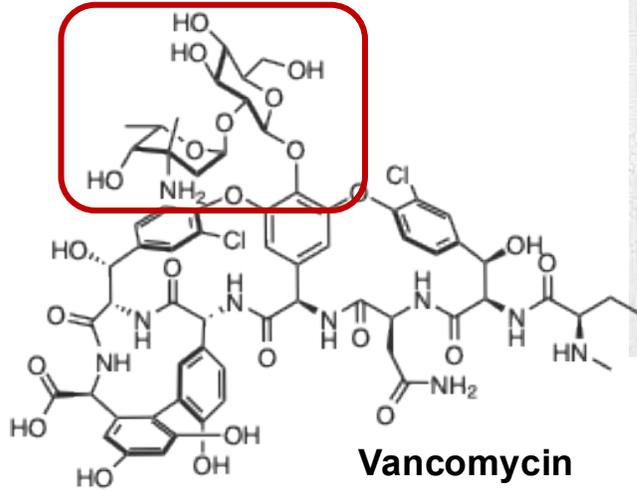
Molecules at the center of modern medicine



Limitations in relying on nature

- Supply risks: long growth period, climate change, pest, and other environment factors
- Drug shortages: essential medicines, supply chain, geo- and socio-political factors
- Costly and inefficient extraction: small mass percentage, similar structures, cost

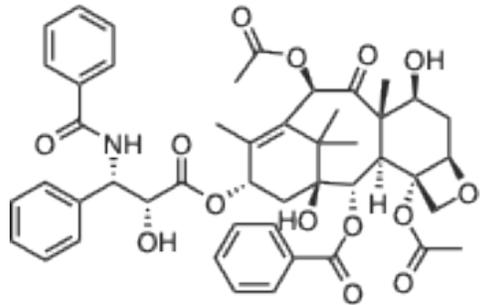
Molecules at the center of modern medicine



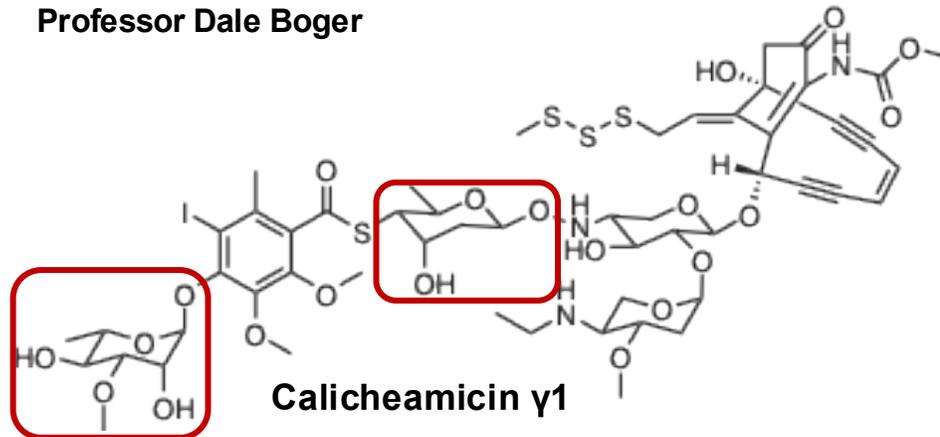
J. Am. Chem. Soc. **1998**, *120*, 8920–8926.
J. Am. Chem. Soc. **1999**, *121*, 43, 10004–10011.



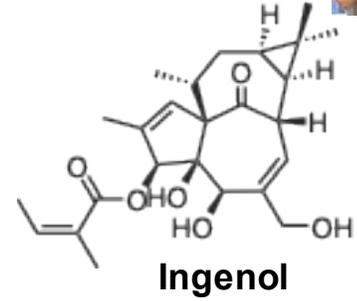
Professor Dale Boger



Nature **1994**, *367*, 630–634



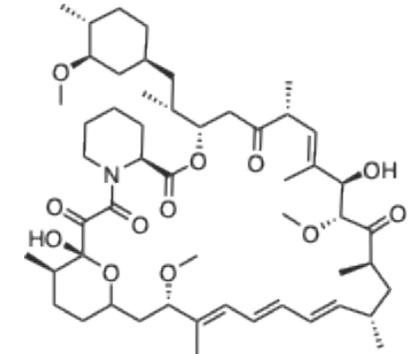
J. Am. Chem. Soc. **1993**, *115*, 7625–7635



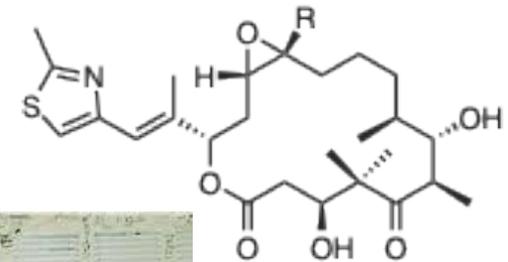
Science **2013**, *341*, 878–882



Professor Phil Baran



J. Am. Chem. Soc. **1993**, *115*, 4419–4420

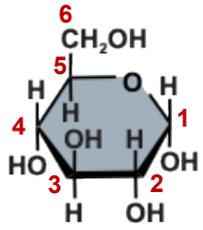


Nature **1997**, *387*, 268–272

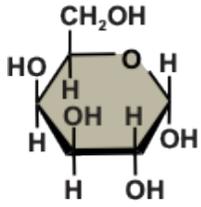


Professor K.C. Nicolaou

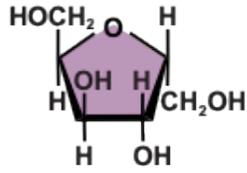
Sugars / Carbohydrates / Glycosylation



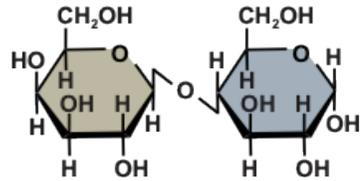
Glucose



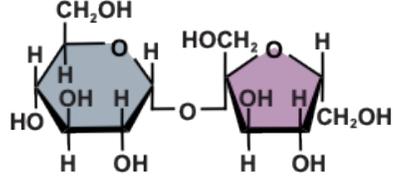
Galactose



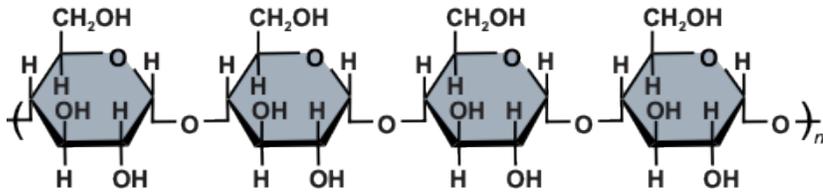
Fructose



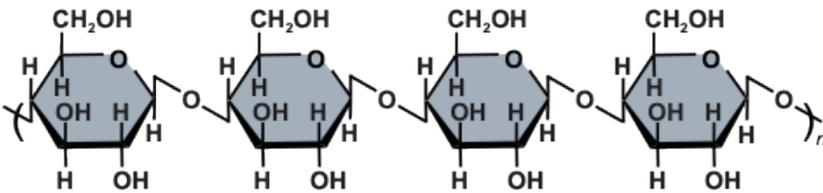
Lactose



Sucrose



Starch



Cellulose



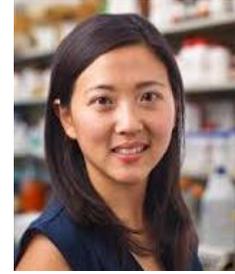
Prof. Carolyn Bertozzi



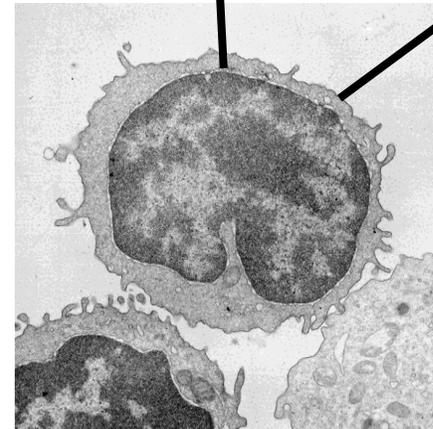
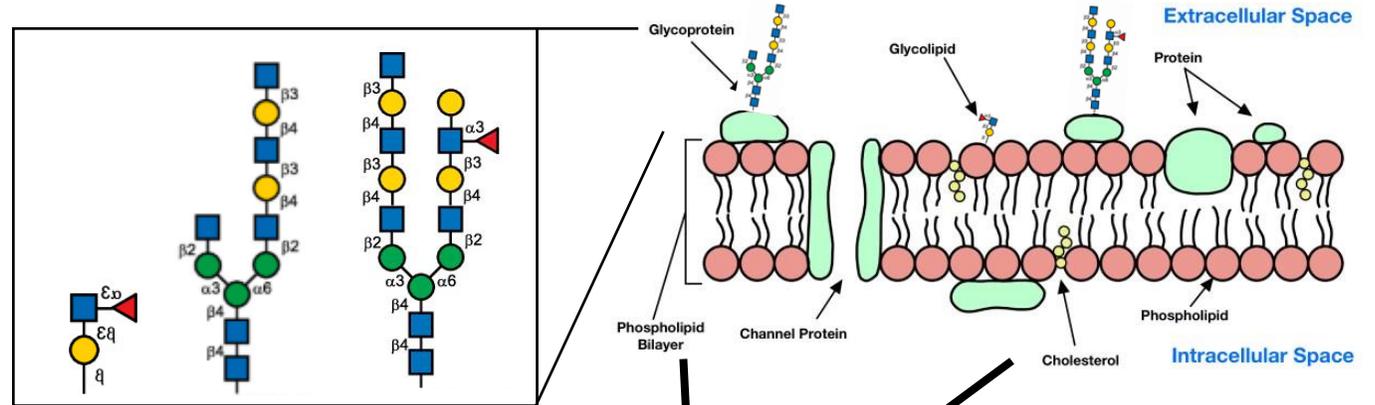
Prof. Laura Kiessling



Prof. James Paulson



Prof. Mia Huang

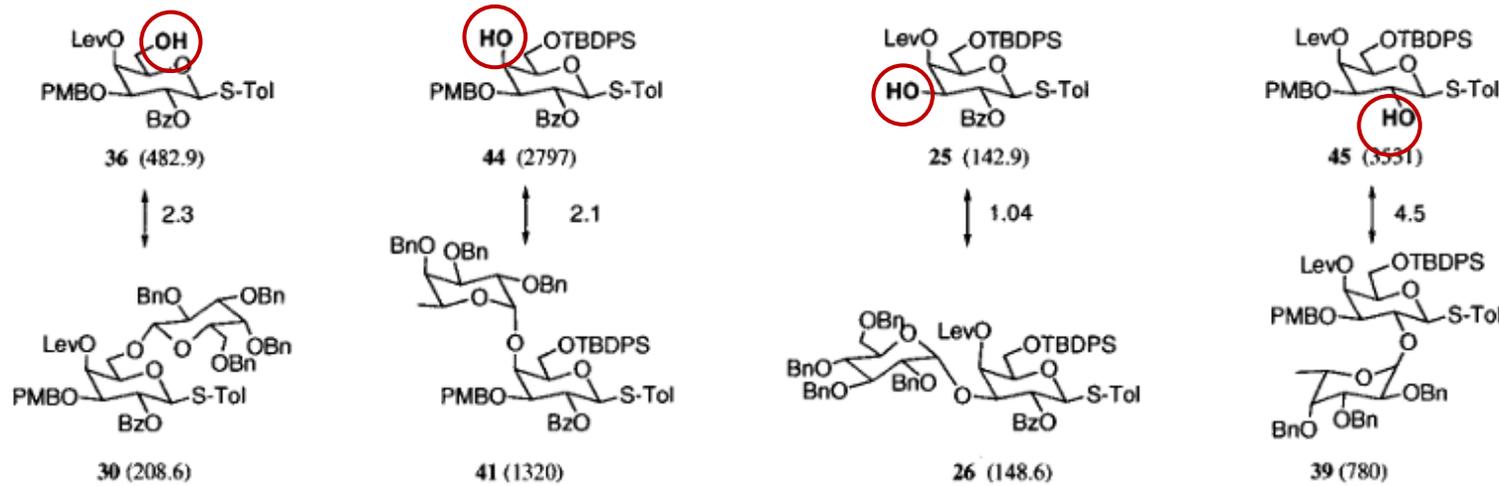
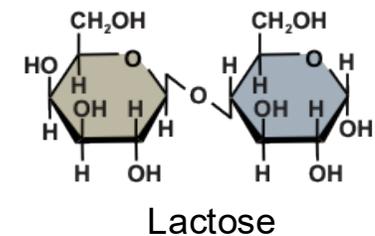
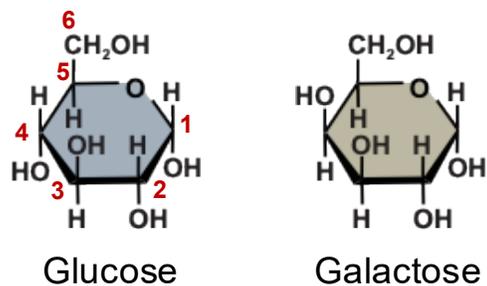


cell-cell
antibody
immune
bacterium
virus

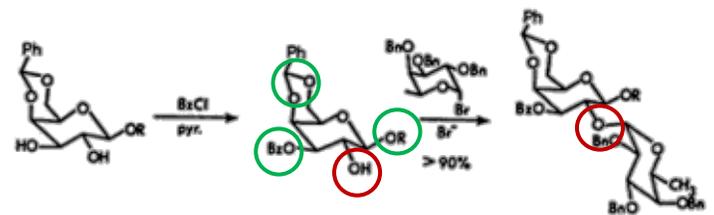


Chemical glycosylation

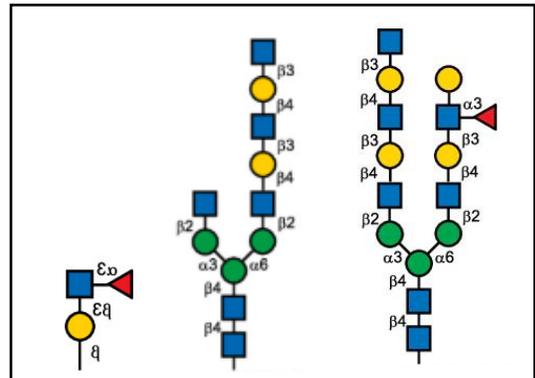
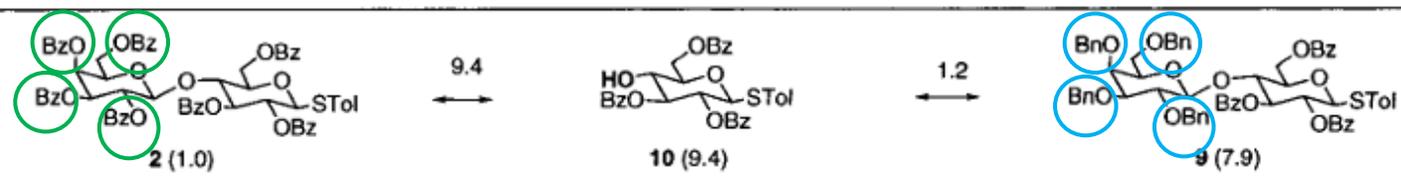
One-pot glycan synthesis



Chemical glycan synthesis



Ber. Dtsch. Chem. Ges. **1893**, 26, 1643–1649.
Tetrahedron **1968**, 24, 5855–5871.
Canad. J. Chem., **1973**, 51, 42.
Tetrahedron Lett. **1980**, 21, 1421–1424.



Professor Chi-Huey Wong
J. Am. Chem. Soc., **1999**, 121, 734–753.

QS-21 as a potent vaccine adjuvant

- Adjuvants: molecules that boost the immune response of a vaccine.
- The addition of an adjuvant to a vaccine stimulates the human body to produce more antibodies, reducing the amount of vaccine needed and potentially increasing the efficacy of the vaccine.



Shingles (91.3% efficacy)
Shingles vaccine efficacy increased from 37.6% to 91.3% with adjuvant



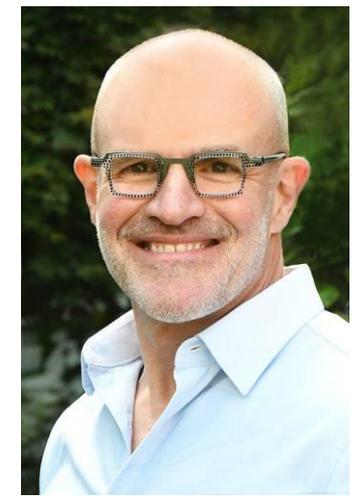
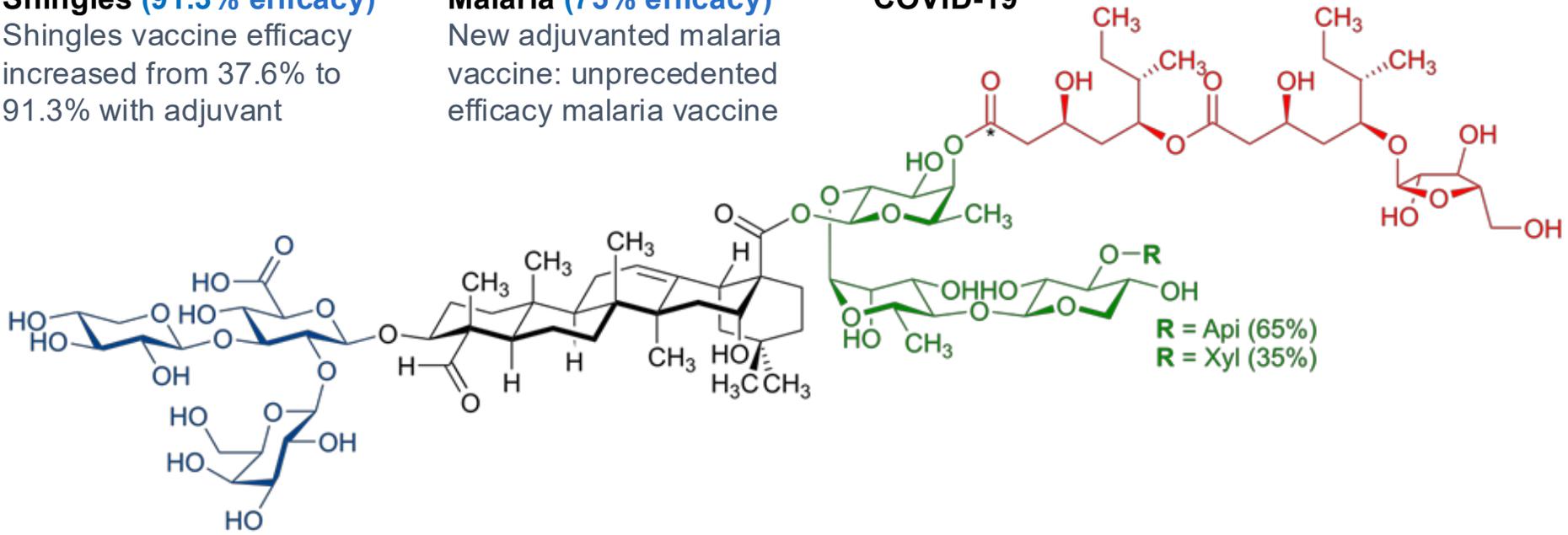
Malaria (75% efficacy)
New adjuvanted malaria vaccine: unprecedented efficacy malaria vaccine



COVID-19



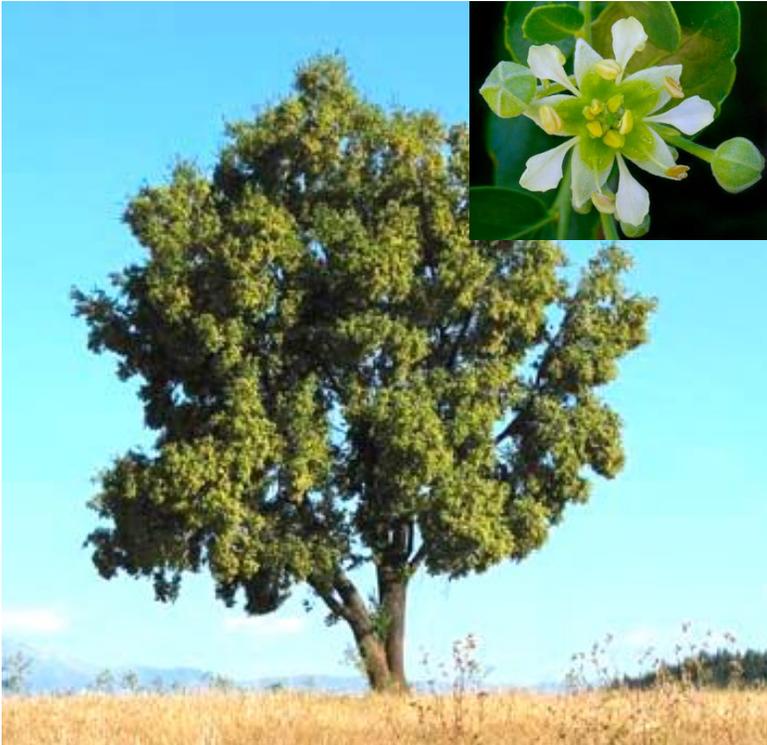
RSV (94.1% efficacy)
RSV vaccine with adjuvant
non-adjuvanted candidates failed trials



Professor Jay Keasling

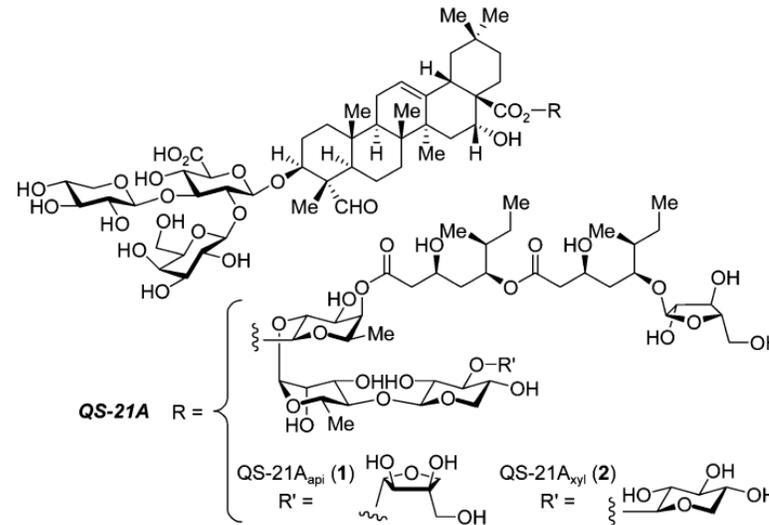
Current QS-21 production strategies

Extraction and purification from *Quillaja Saponaria*

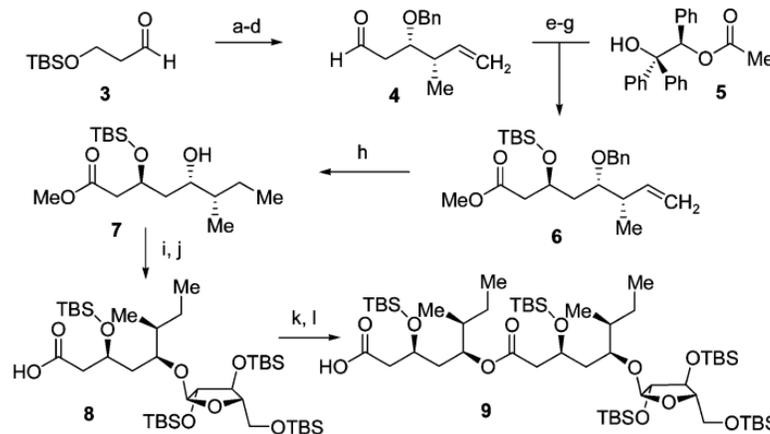


- Specific areas in Chile
- Laborious extraction, low yield
- Non-sustainable production

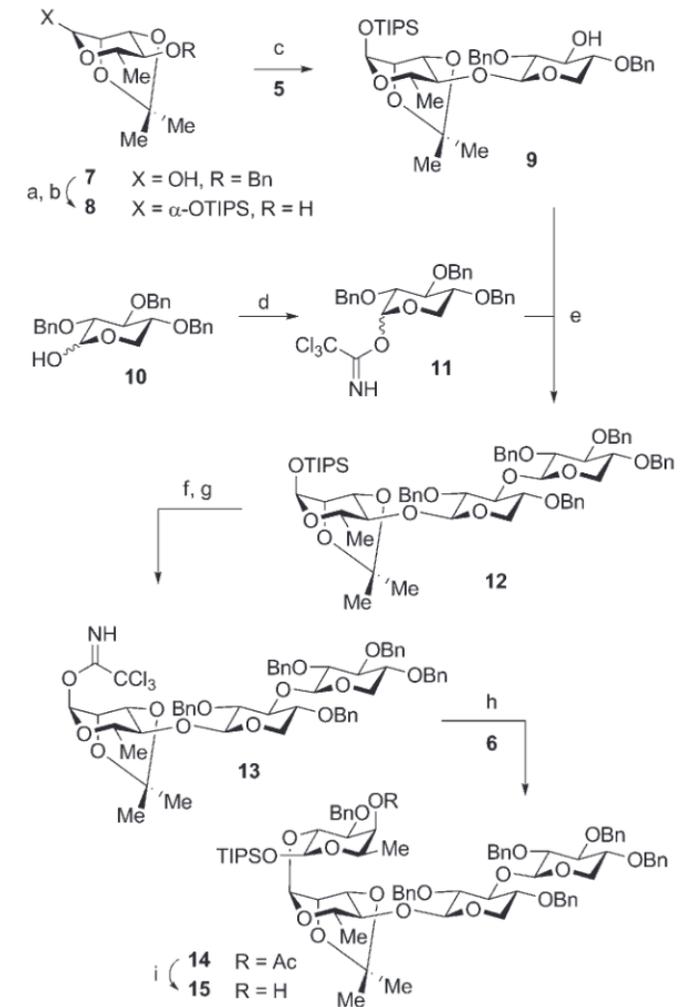
Total synthesis of QS-21 and derivatives



Acyl chain synthesis

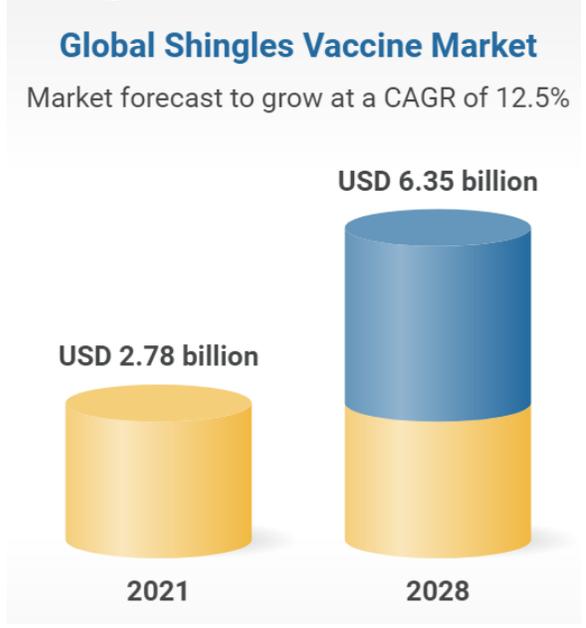


Tetrasacchride synthesis

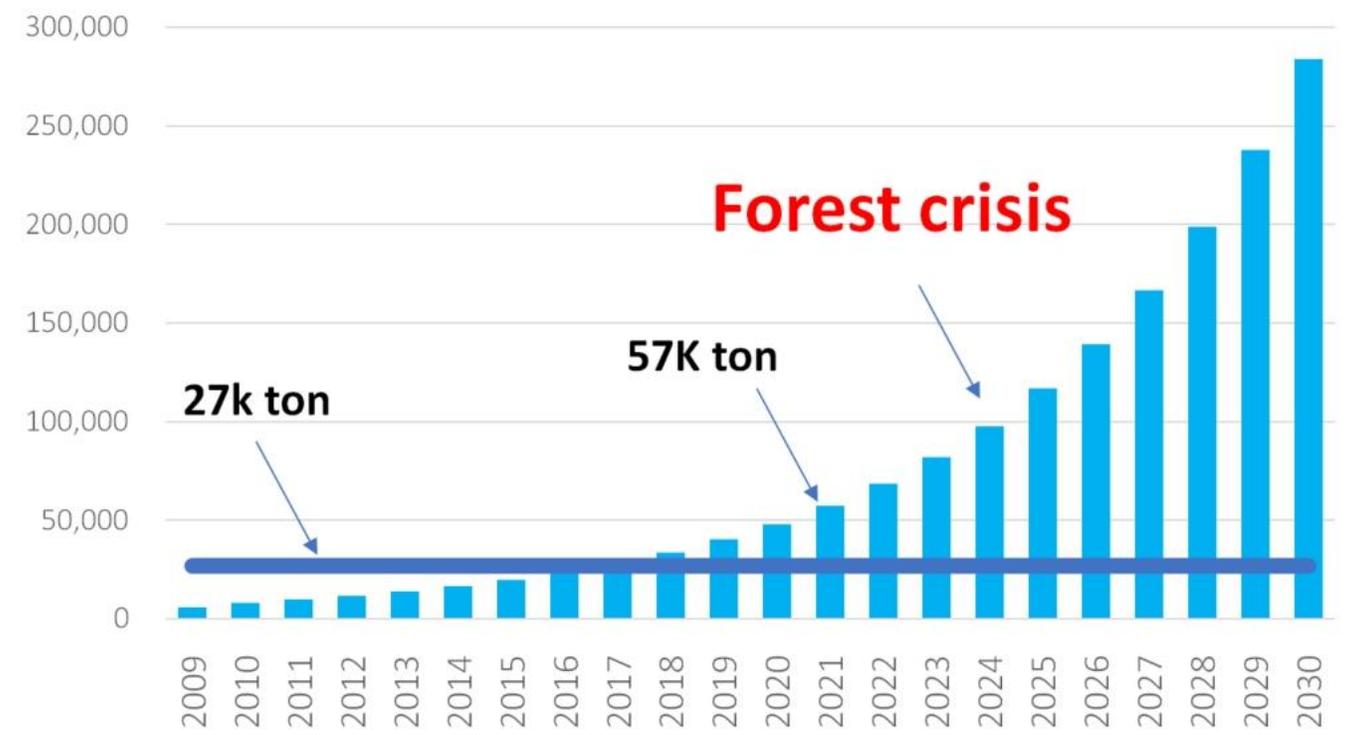
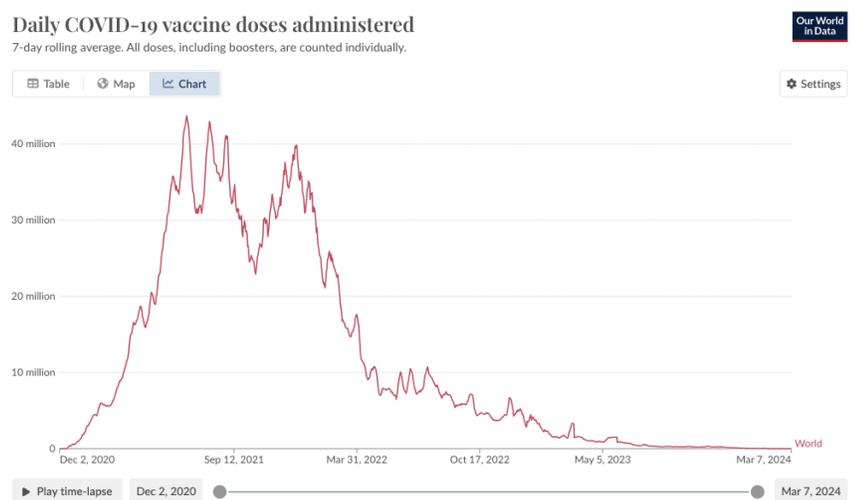


Current challenges associated with QS-21 production

Increasing demand for vaccines



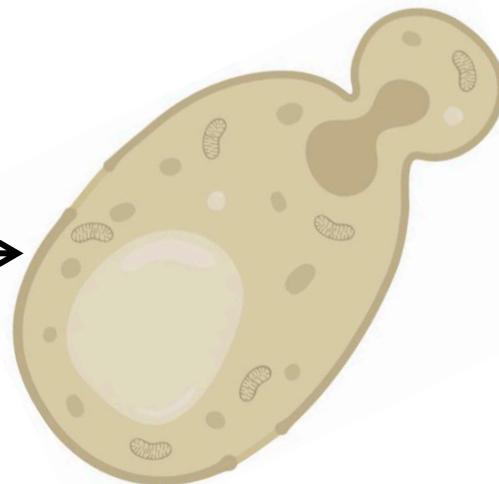
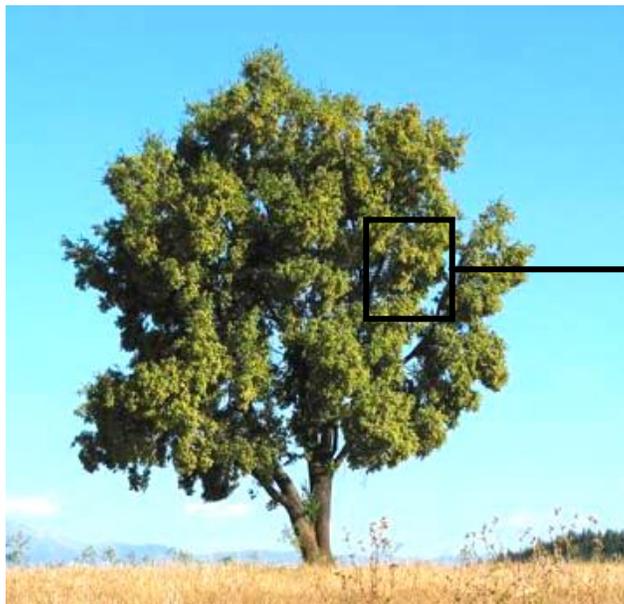
Pandemic preparedness



■ Q. saponaria biomass harvest in Chile (ton)
 ■ Sustainable production of Q. saponaria biomass in Chile (ton)

- Overharvesting has led to a forest crisis in Chile

Synthetic biology to make complex natural products



Saccharomyces cerevisiae
(baker's yeast)

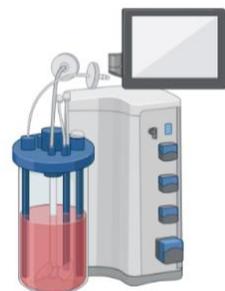
Opportunity in microbial hosts

- Microorganisms can be engineered to produce the exact natural product
- The pathways can be altered to produce unnatural products
- The production is easily scalable

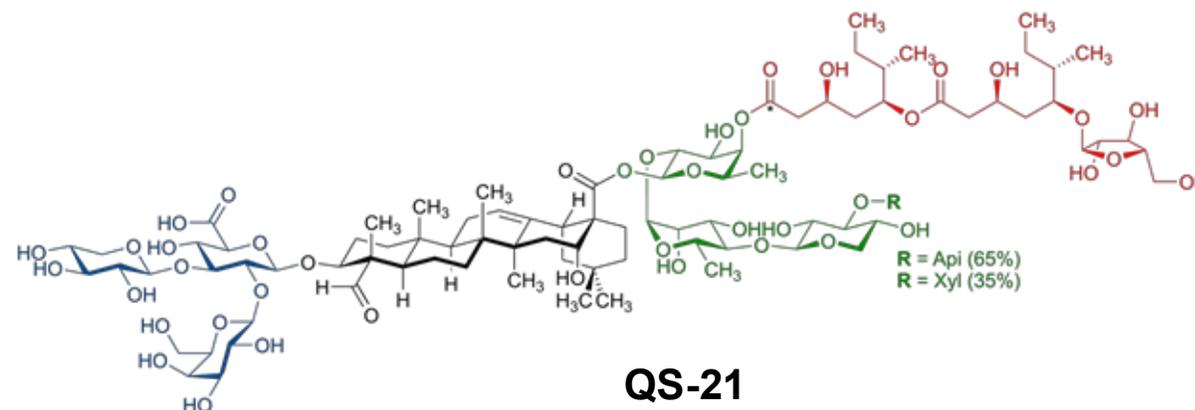
Production scheme



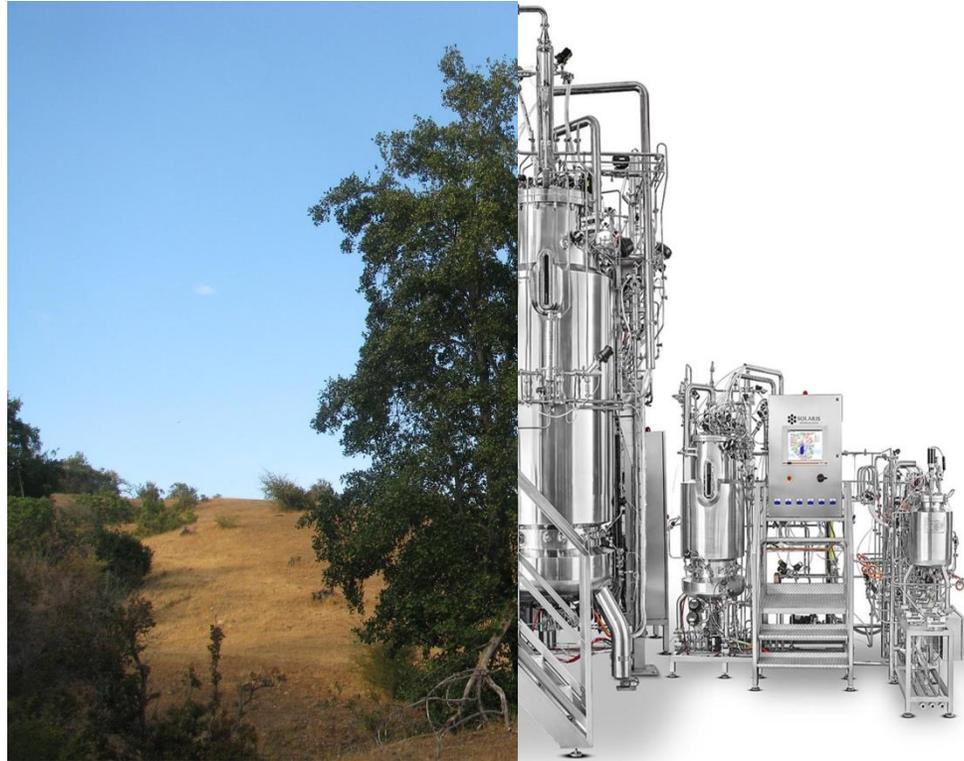
Simple sugars



Yeast fermentation



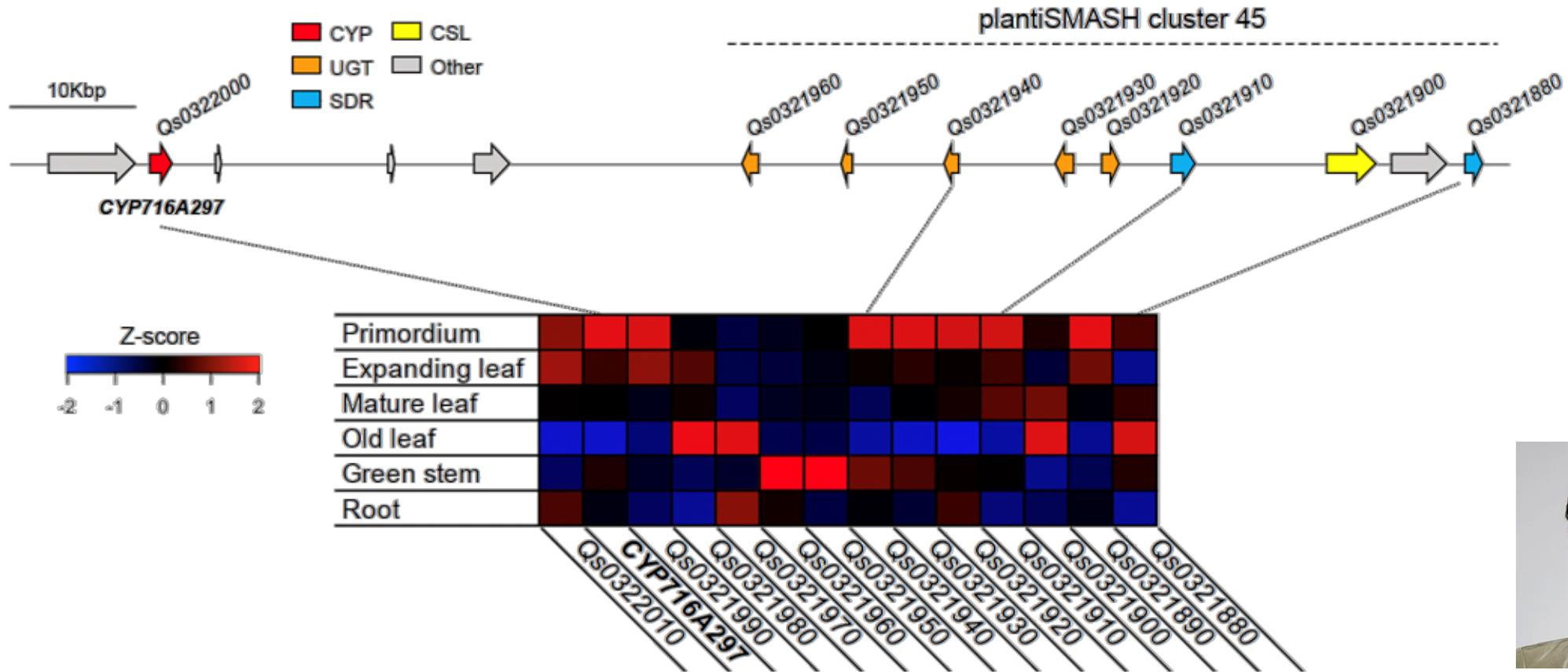
Synthetic biology to make complex natural products



Decades vs Days

- **1000-times** higher space-time yield leading to 1000x higher accessibility
- **Immediate** on-demand scalability
- **Decreased purification cost** as molecules of interest can be selectively synthesized
- **Sustainable** and portable production scheme

Annotated QS-21 biosynthetic pathway



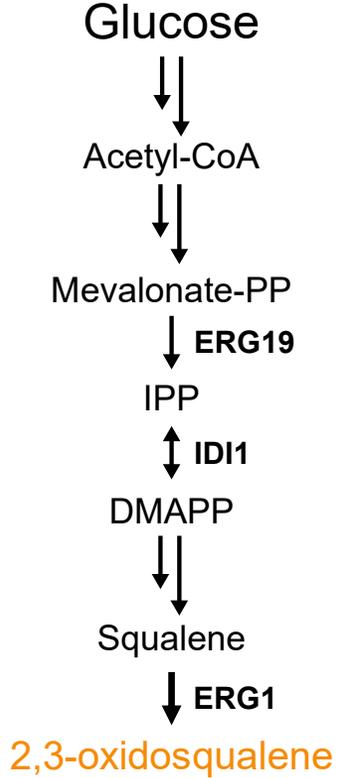
Professor Anne Osbourn



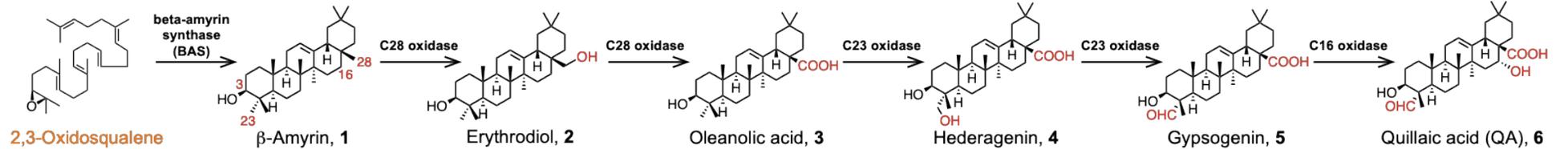
John Innes Centre

QS-21 biosynthetic pathway

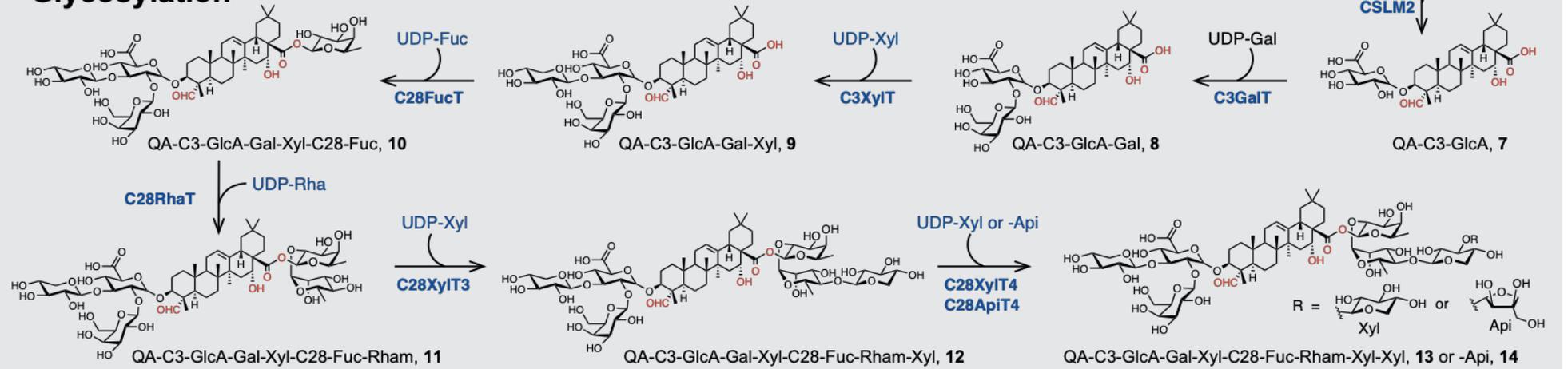
Mev Pathway



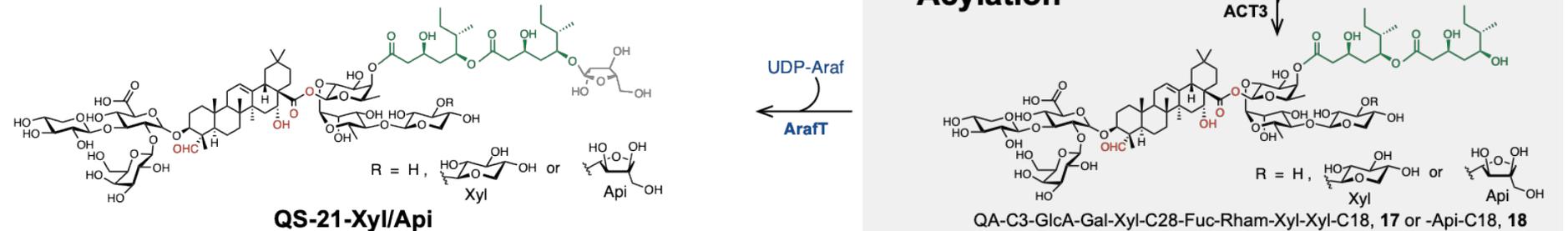
Triterpenoid synthesis



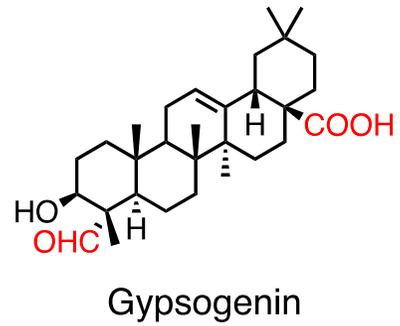
Glycosylation



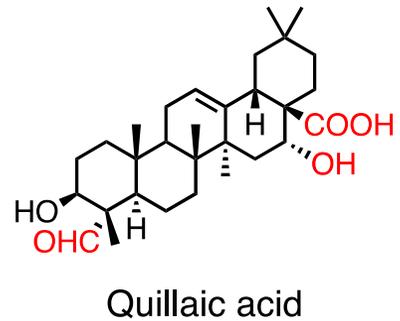
Acylation



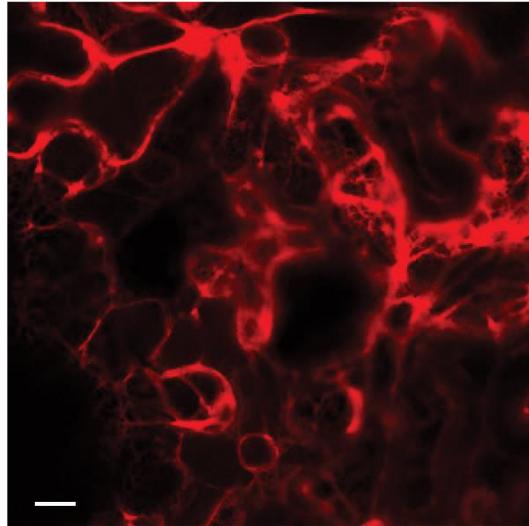
Lost in translation: Subcellular localization study of C16 oxidase



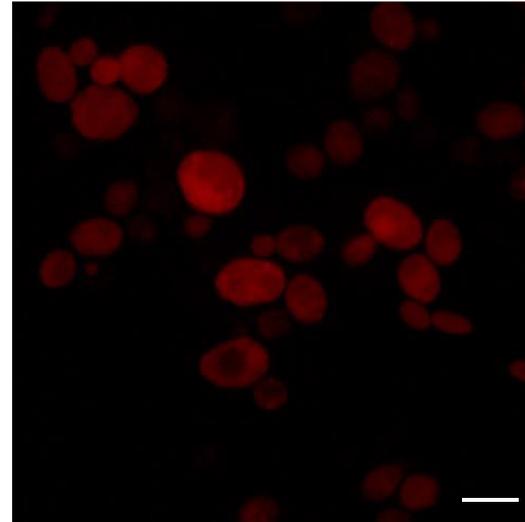
C16 oxidation



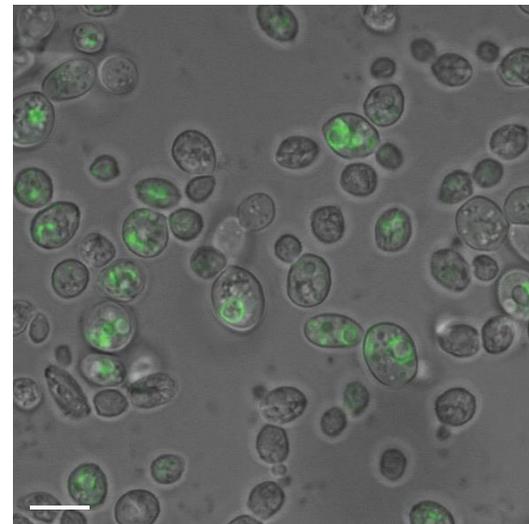
C16 oxidase-mCherry



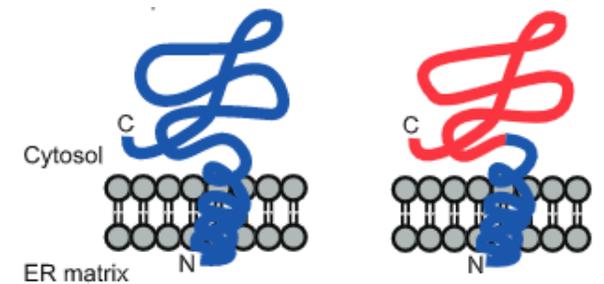
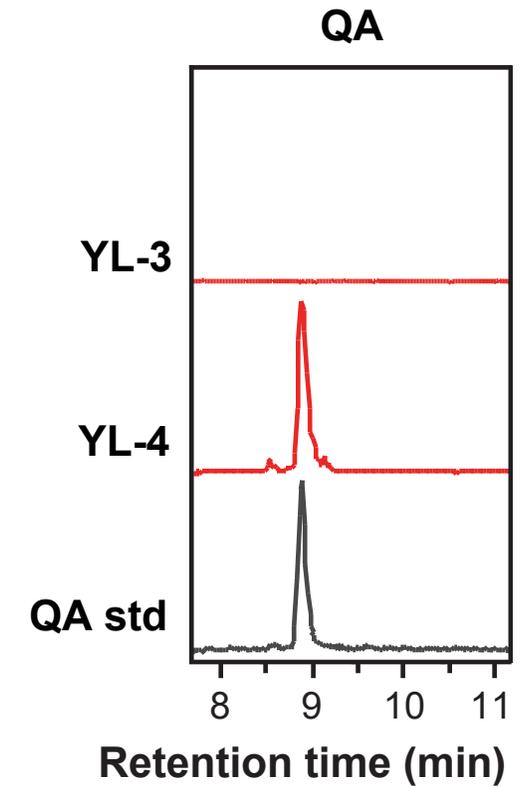
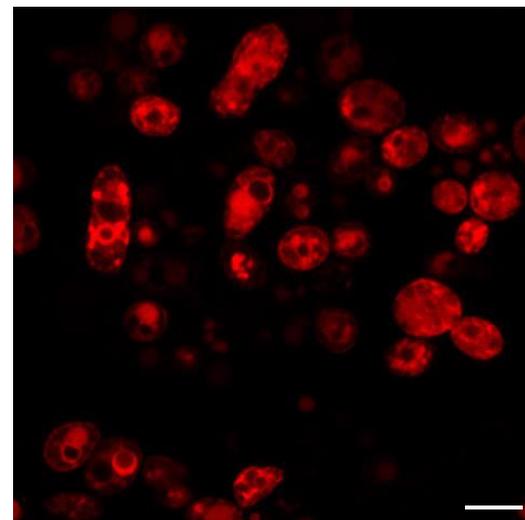
C16 oxidase-mCherry



C28 oxidase-GFP



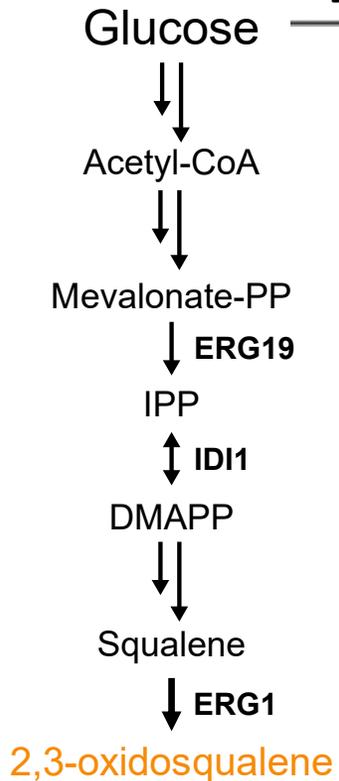
C28TMD-C16 oxidase-mCherry



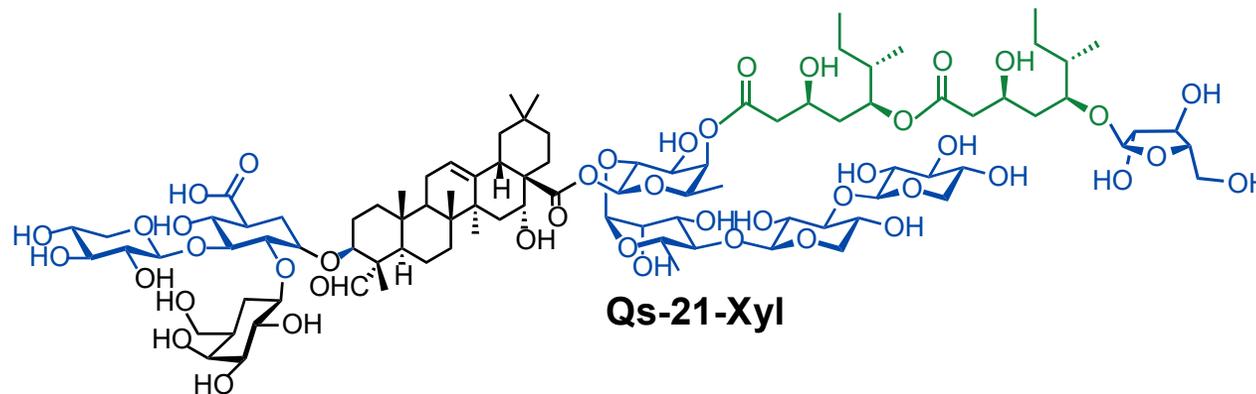
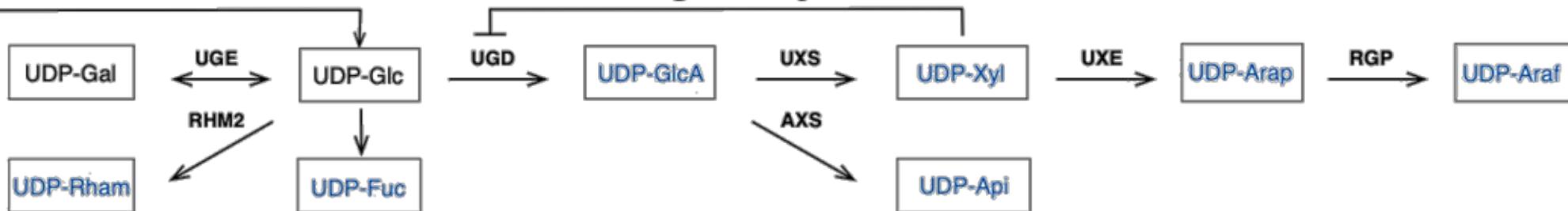
Necessary modules for QS-21 biosynthesis

- The lack of nucleotide sugar variety in yeast

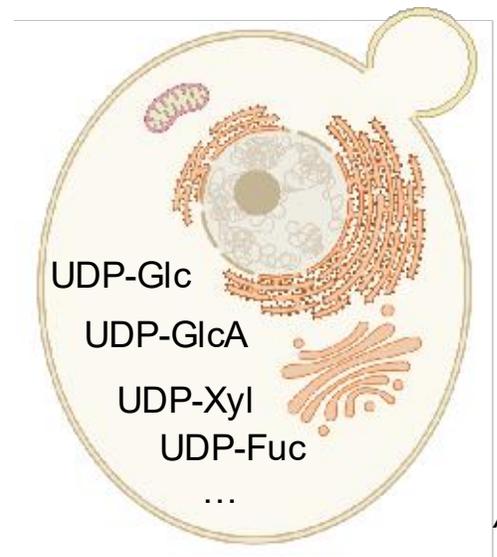
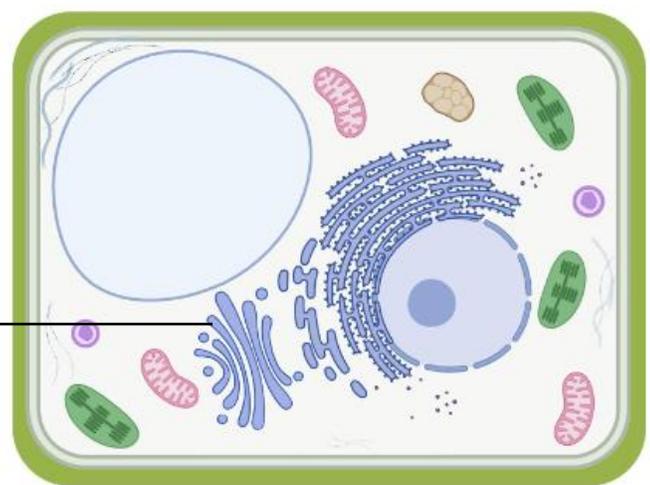
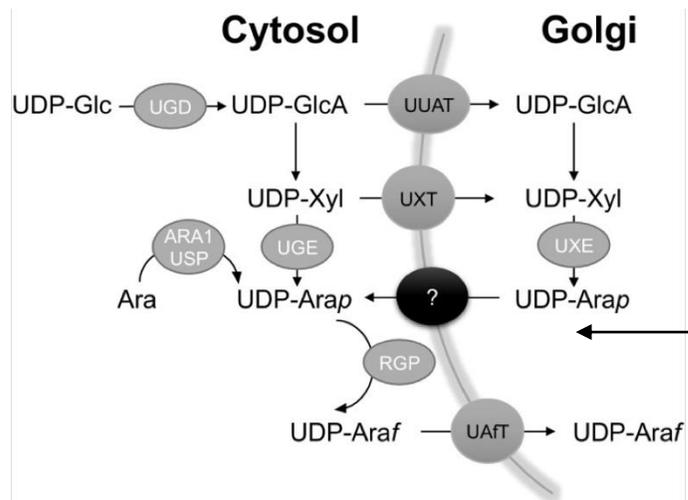
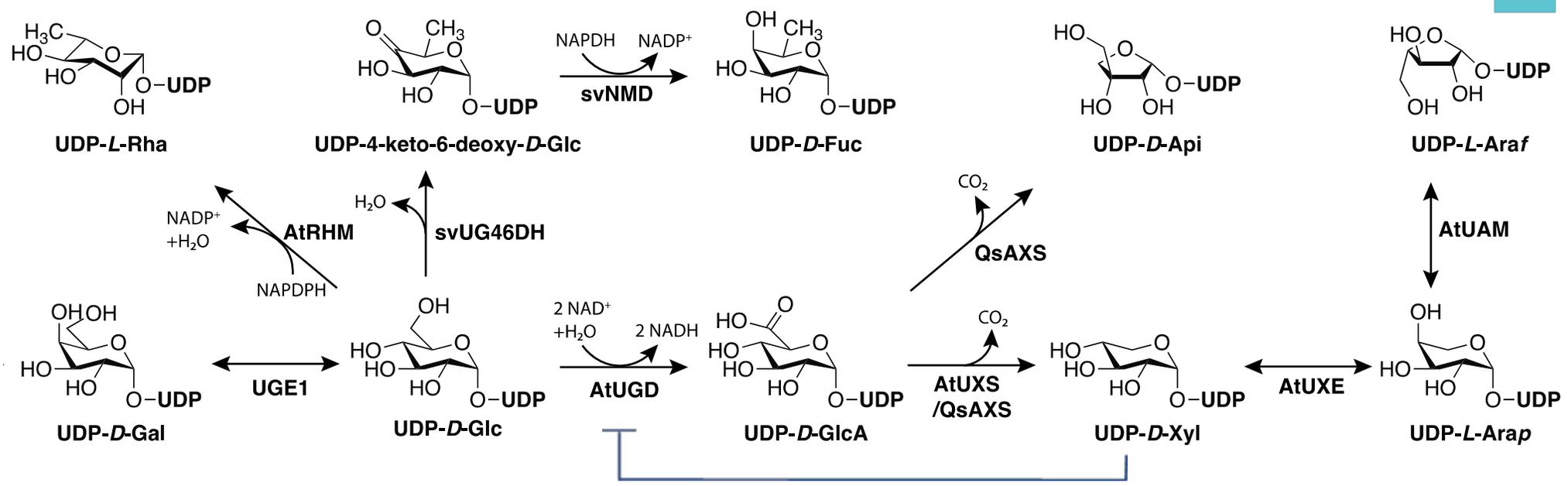
Mev Pathway



Nucleotide sugar biosynthesis



Nucleotide UDP-sugar biosynthesis in yeast



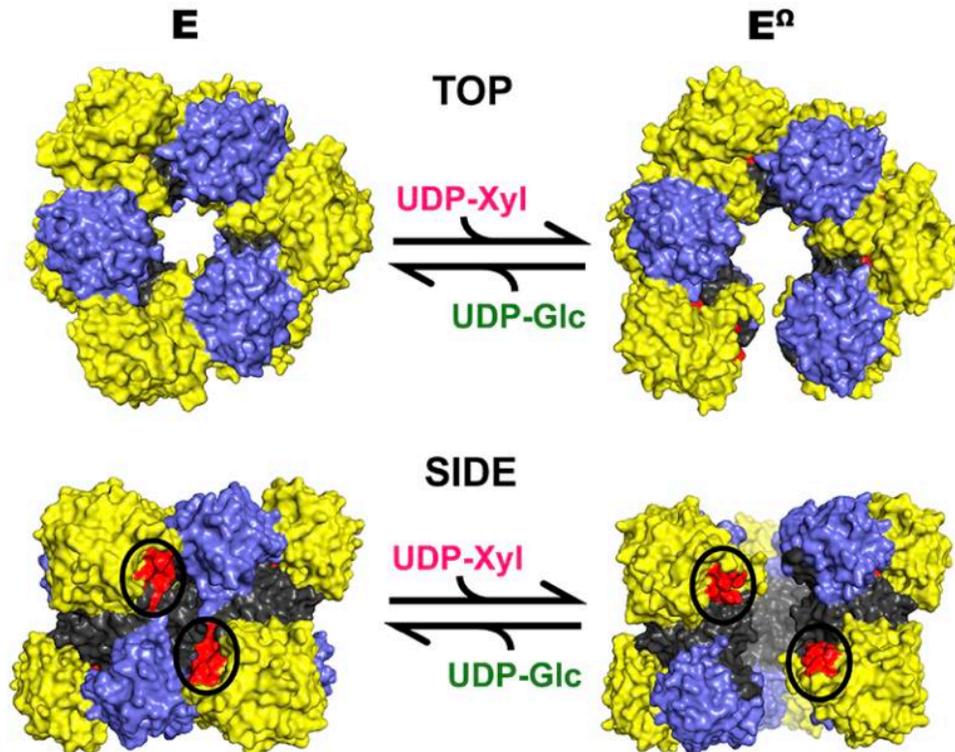
Sam Crowe

ACS Syn. Biol., 2024, 13, 1215–1224.
ACS Syn. Biol., 2024, 13, 1589–1599.

Strategies to alleviate the UDP-Xyl inhibition

- HsUGD mutation

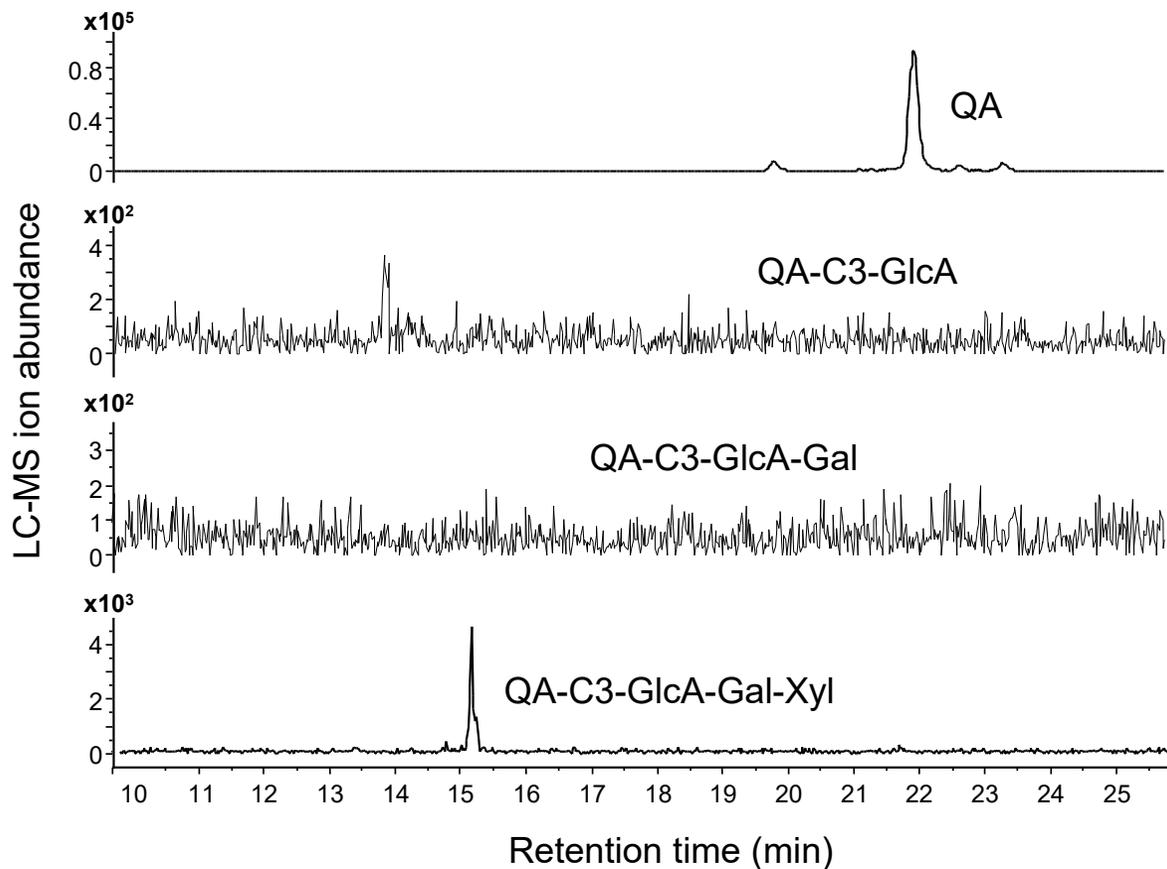
HsUGD AtUGD alignment to identify target residue



Score	Expect	Method	Identities	Positives	Gaps
588 bits(1517)	0.0	Compositional matrix adjust.	289/473(61%)	357/473(75%)	13/473(2%)
Query 6	KICCGAGYVGGPTCSVIAHMCPEIRVTVVVDVNESRINAWNSPTLPIYEPGLKEVVESCR				65
Sbjct 3	KICCGAGYVGGPT +V+A CPEI V VVD++E RINAWNS LPIYEPGL++VV+ CR				62
Query 66	GKNLFFSTNIDDAIKEADLVFISVNTPTKTYGMGKGRADLKYIEACARRIVQNSNGYKI				125
Sbjct 63	GKNLFFST+++ + E+D+VF+SVNTPTKT G+G G+AADL Y E+ AR I S KI				122
Query 126	VTEKSTVPVRAAESIRRIFDANTKPNLNLQVLSNPEFLAEGTAIKDLKNPDRVLIGGDET				185
Sbjct 123	V EKSTVPVR AE+I +I N+K + Q+LSNPEFLAEGTAIKDL NPDRLVIGG +T				181
Query 186	PEGQRAVQALCAVYEHWPREKILTTNTWSSELSKLAANAFLAQRISINSISALCEATG				245
Sbjct 182	GQ+A++AL VY HWVP E+I+ TN WS+ELSKLAANAFLAQRIS+N++SALCEATG				241
Query 246	ADVEEVATAIGMDQRIGNKFLKASVGFSGSCFQKDVNLNLVYLCEALNLPVARYWQOVID				305
Sbjct 242	ADV +VA A+G D RIG KFL ASVGFSGSCFQKD+LNL+Y+CE LPE A YW+QV+				301
Query 306	MNDYQRRRFASRIIDSLFNTVTDKKAAILGFAPKKTGDTRESSSIYISKYLMDEGAHLH				365
Sbjct 302	+NDYQ+ RFA+R++ S+FNTV+ KKAAILGFAPKKTGDTRE+ +I + L+ + A L				361
Query 366	IYDPKVPREQIVVDLSHPGVSED-----DQVSRVLTISKDPYEACDGAHAVVICT				415
Sbjct 362	IYDP+V EQI DLS D +S V + D YEA AH + + T				421
Query 416	EWDMFKELDYERIHKKMLKPAFIFDGRVLDGLHNLQTIQFQIETIGKKVSS				468
Sbjct 422	EWD FK LD+++I M KPAF+FDGR V+D + +L+ IGF + +IGK + S				472

- AtUGDA101L is constructed

Strategies to alleviate the UDP-Xyl inhibition

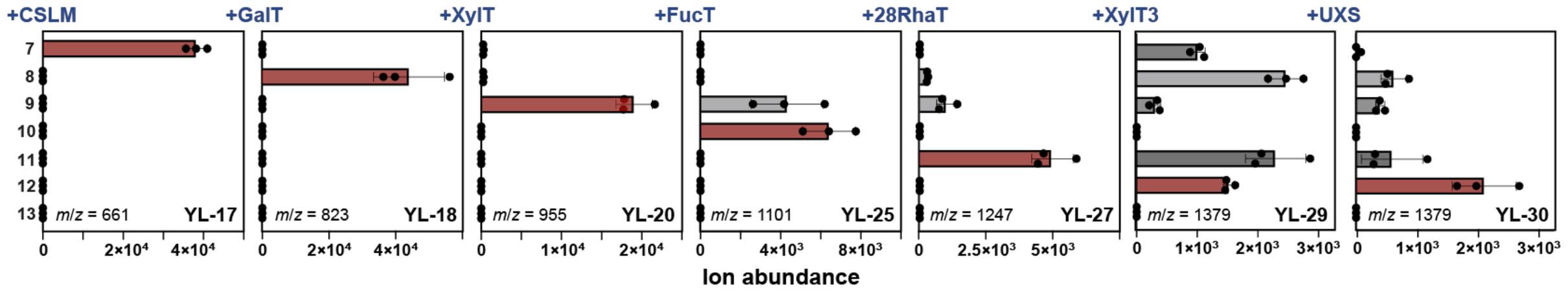
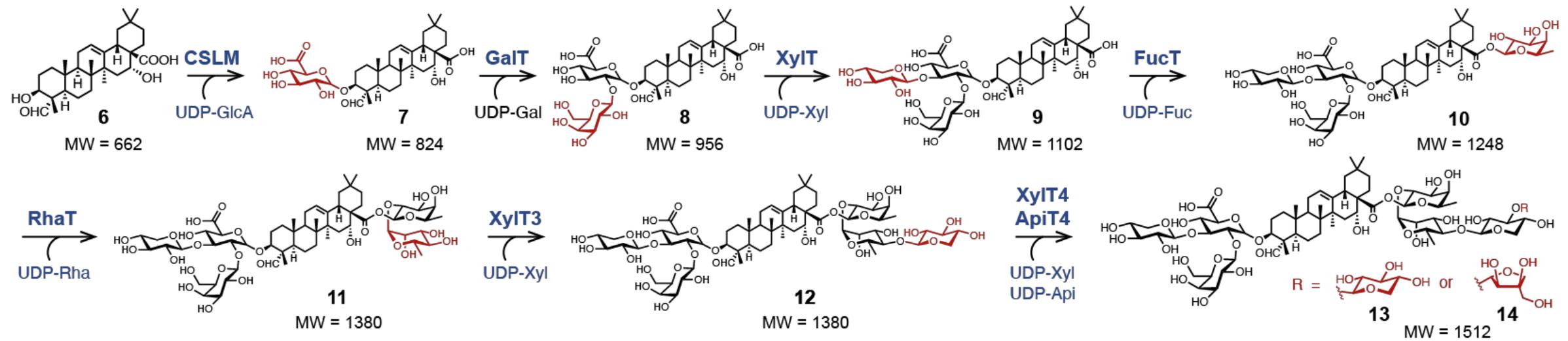


HsUGD AtUGD alignment to identify target residue

Score	Expect	Method	Identities	Positives	Gaps
588 bits(1517)	0.0	Compositional matrix adjust.	289/473(61%)	357/473(75%)	13/473(2%)
Query 6	KICCGAGYVGGPTCSVIAHMCPEIRVTVVVDVNESRINAWNSPTLPIYEPGLKEVVEVESC				65
Sbjct 3	KICCGAGYVGGPT +V+A CPEI V VVD++E RINAWNS LPIYEPGL++VV+ CR				62
Query 66	GKNLFFSTNIDDAIKEADLVFISVNTPTKTYGMGKGRADLKYIEACARRIVQNSNGYKI				125
Sbjct 63	GKNLFFST+++ + E+D+VF+SVNTPTKT G+G G+AADL Y E+ AR I S KI				122
Query 126	VTEKSTVPVRAAESIRRIFDANTKPNLNLQVLSNPEFLAEGTAIKDLKPNDRVLIIGGDET				185
Sbjct 123	VVEKSTVPVRTAEAEIKILTHNSK-GIEFQILSNPEFLAEGTAIKDLYNPNDRVLIIGGRDT				181
Query 186	PEGQRAVQALCAVYEHVWPREKILTTNTWSSELSKLAANAFLAQRISINSISALCEATG				245
Sbjct 182	GQ+A++AL VY HWVP E+I+ TN WS+ELSKLAANAFLAQRIS+N++SALCEATG				241
Query 246	ADVEEVATAIGMDQRIGNKFLKASVGFSGFQKDVNLVYLCEALNLPVARYWQOVID				305
Sbjct 242	ADV +VA A+G D RIG KFL ASVGFSGFQKD+LNL+Y+CE LPE A YW+QV+				301
Query 306	MNDYQRRRFASRIIDSLFNTVTDKKAAILGFAPKKTGDTRESSSIYISKYLMDEGAHLH				365
Sbjct 302	+NDYQ+ RFA+R++ S+FNTV+ KKAAILGFAPKKTGDTRE+ +I + L+ + A L				361
Query 366	IYDPKVPREQIVVDLSHPGVSED-----DQVSRVLTISKDPYEACDGAHAVVICT				415
Sbjct 362	IYDP+V EQI DLS D +S V + D YEA AH + + T				421
Query 416	EWDMFKELDYERIHKKMLKPAFIFDGRVLDGLHNLQITIGFQIETIGKVVSS				468
Sbjct 422	EWD FK LD+++I M KPAF+FDGR V+D + +L+ IGF + +IGK + S				472

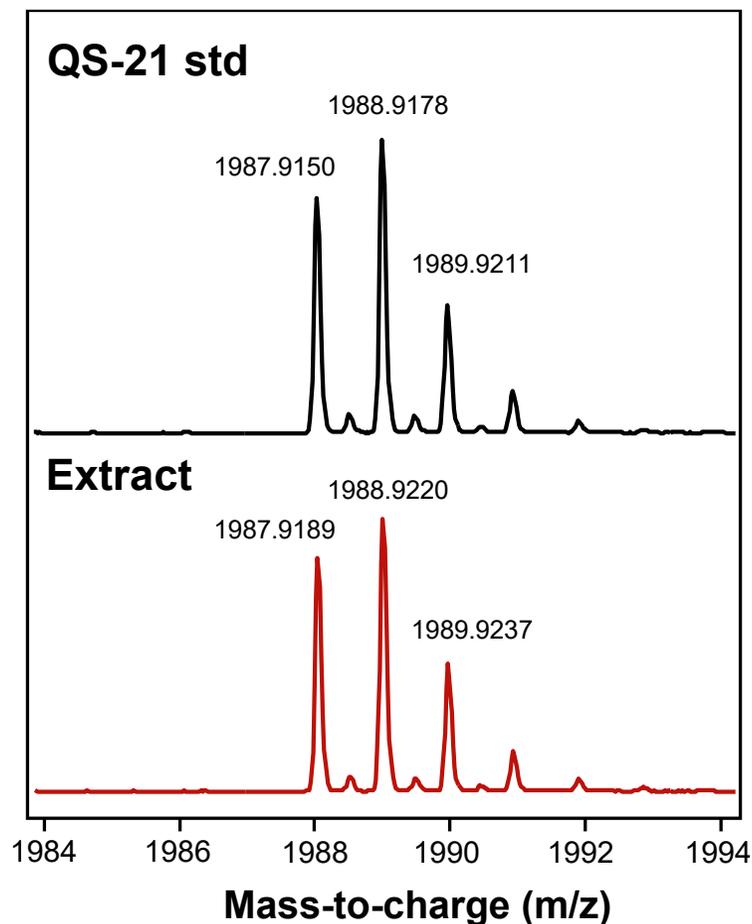
- AtUGDA101L is constructed

QS-21 glycosylation

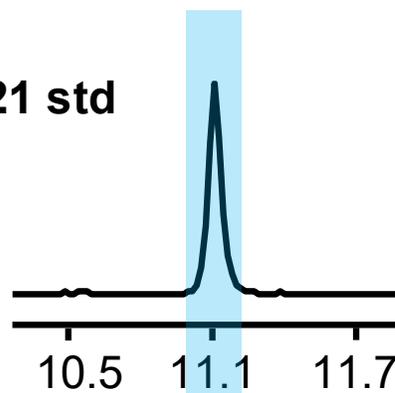


- Functional expression of nucleotide sugar synthases to make 6 heterologous sugars, as well as their corresponding sugar transferases

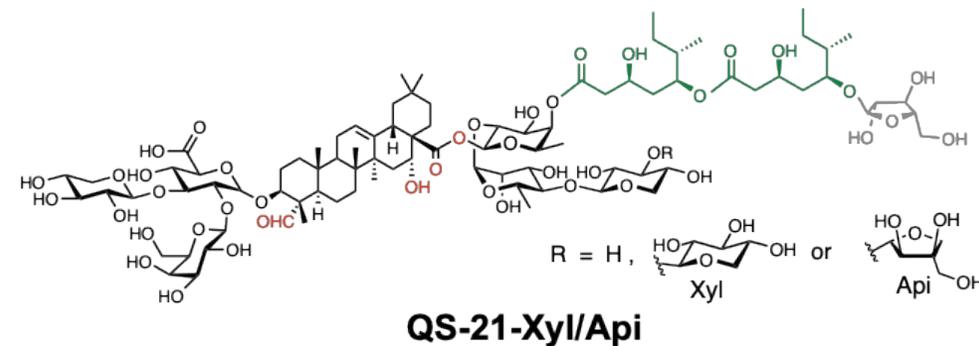
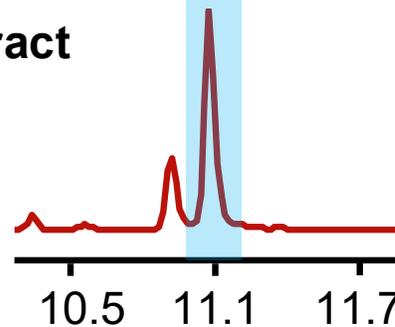
Terminal Araf biosynthesis and addition to make QS-21



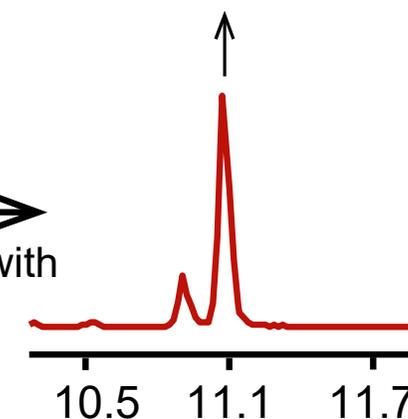
QS-21 std



Extract

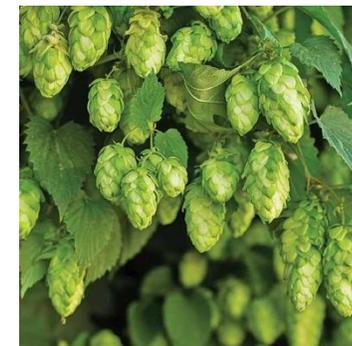
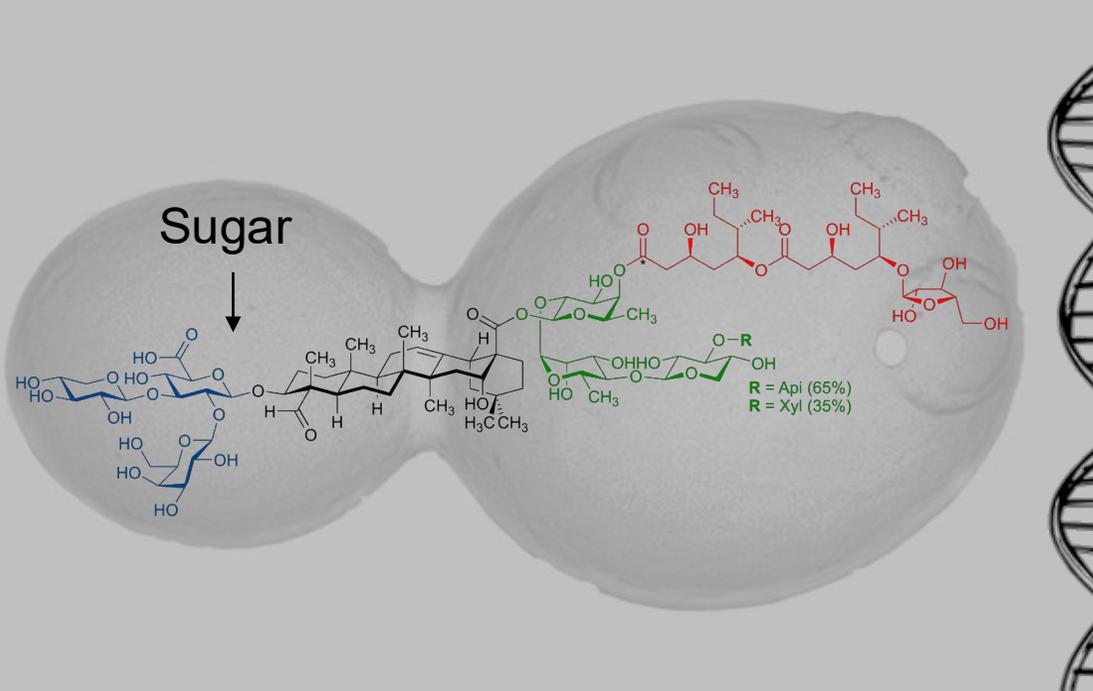


Spiked with
QS-21



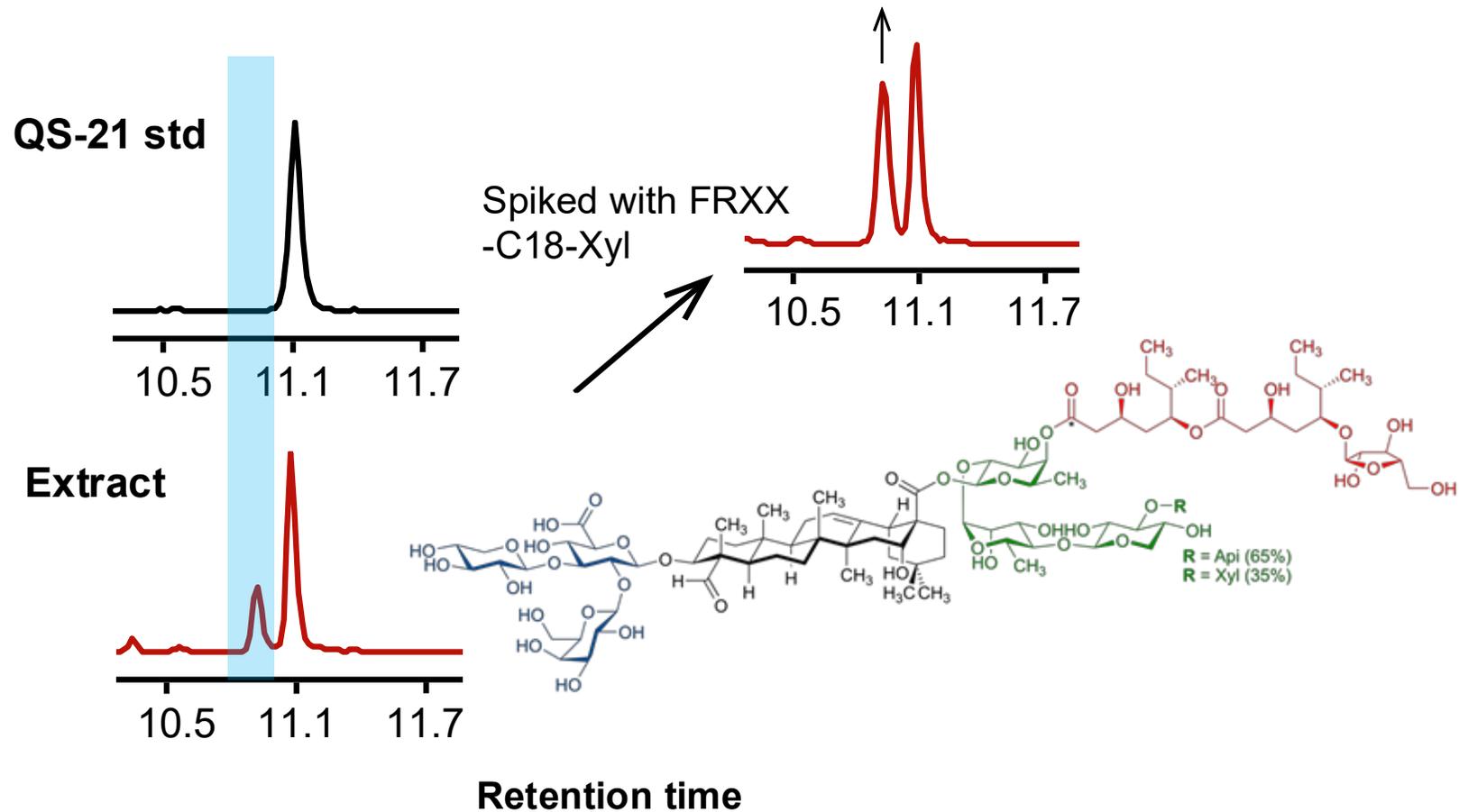
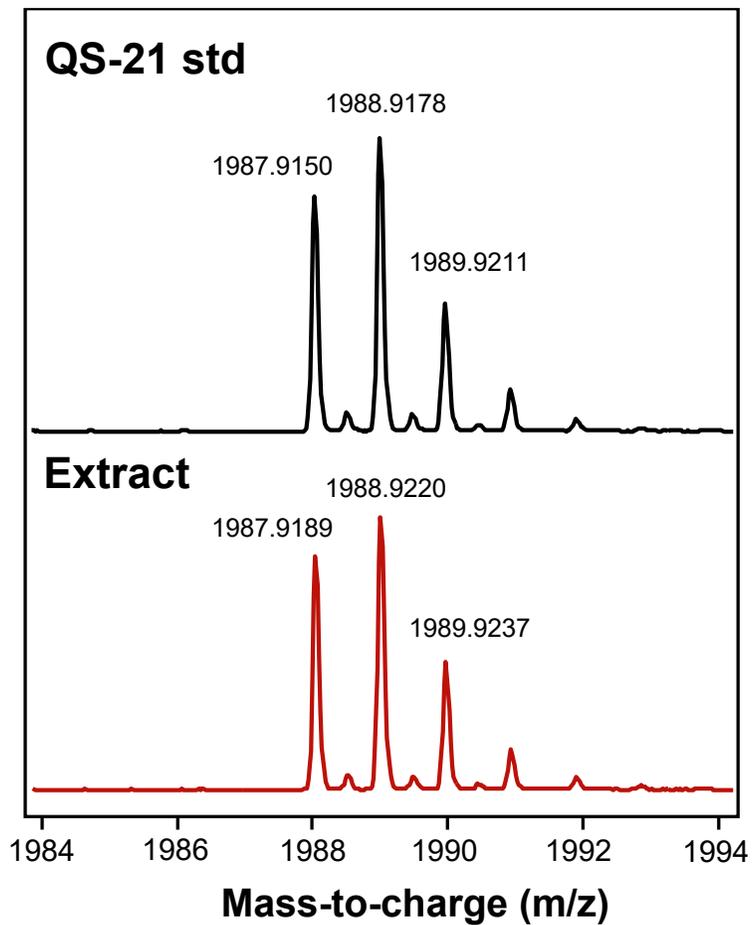
- Confirmed by analytical team, “cleanest extract ever” compared to *Q. saponaria*

Complete biosynthesis of QS-21 in yeast



- Complete biosynthesis of QS-21-Xyl and QS-21-Api in yeast
- 37 enzymatic steps
- 38 heterologous genes
 - Terpene synthase, various homologues
 - P450s and their redox partners (CPR, cytb5, various homologues)
 - Nucleotide sugar synthases
 - Glycosyltransferases
 - Type I, type III PKS, KR
- 8 yeast genes over expressed

Terminal Araf biosynthesis and addition to make QS-21



A microbial biosynthetic platform for complex carbohydrates

IN THE PIPELINE | BIOLOGICAL NEWS

Good News for the Chilean Soapbark Tree - And For Us

20 FEB 2024 · 10:36 AM ET · BY DEREK LOWE · 3 MIN READ · COMMENTS

SHARE:



I've mentioned a peculiar vaccine additive here several times over the years. Vaccines often contain **adjuvants**, compounds that stimulate the immune system on injection and potentiate the effects of the vaccine antigens themselves. One that's become very important in recent years is **AS01**, a mixture of bacterial lipopolysaccharides (always something that sets off immune responses) and a complex natural product (**QS-21**) that's isolated from the **Chilean soapbark tree**. Now, LPS is something that you can generally get in quantity by culturing bacteria, but culturing a huge stand of Chilean soapbark trees is something else again. **This 2020 article** at *The Atlantic* is a good look at the situation: QS-21 is crucial to the success of a shingles vaccine and a malaria vaccine, and it was a key component of several coronavirus vaccine efforts (and ended up being used in the Novavax one). As it turns out, it's not in the mRNA coronavirus vaccines, because the presence of the mRNA and the lipid nanoparticles by themselves seems to be enough of an immune stimulator, but it's proving to be a very valuable substance in a lot of other cases.

Which is a problem. There have been several situations over the years where a natural product is so useful and its chemical synthesis is so much of an undertaking that the original source looks like it might become an endangered species. That happened with **taxol/docetaxel/Taxotere**, which was originally isolated from the bark of the Pacific yew tree. The demand for the compound looked fit to make the Pacific yew extinct, until great research efforts first established a semi-synthetic route from an intermediate in the tree's needles (which could be harvested more more sustainably than the bark, as you'd imagine), and then established that it's **actually a fungus** in the yew tree tissue that is making the compound in the first place - which led to it being produced by culturing the fungus. Cell culture is often the final most desirable answer in these situations, but it's not always easy to realize.

"There is no particular reason why QS-21 itself should be the best adjuvant of the series (in fact, it still has some residual cellular toxicity), and this will be just the way to study its structure-activity relationships to see if there's something even better."

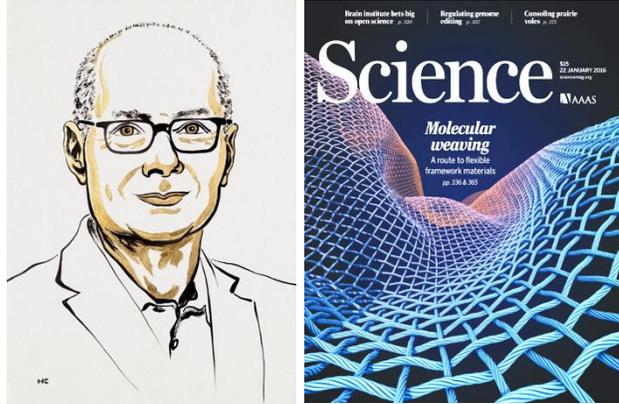
Can we evolve Nature's medicines ourselves?



- Sugars impart biological functions, important for drug performance
- Synthetic biology can accelerate drug discovery, lower cost, and be more sustainable
- Safer, more potent therapies that are affordable for everyone

Acknowledgements

PhD Advisor
Dr. Omar Yaghi

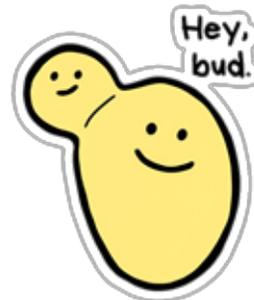


Science, 2016, 351, 365–369.

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Mentors and collaborators



Thank you all for your attention!

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