

Finding drugs in the garden: Harnessing plant metabolic diversity

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Humans have used plants as medicines for thousands of years



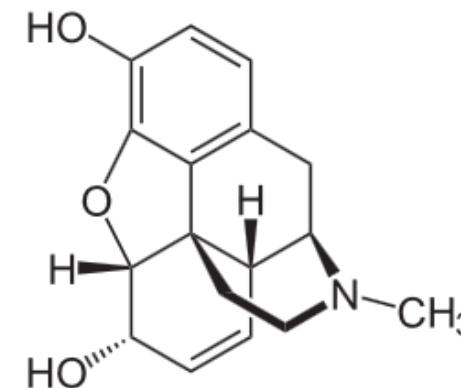
Chinese traditional medicine

The 19th century: the beginning of the pharmaceutical industry



H. E. Merck laboratory, Darmstadt, Germany, 1826

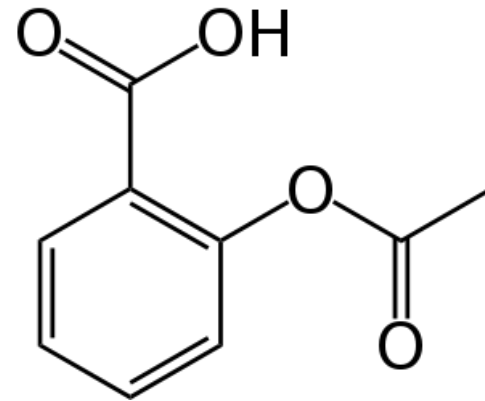
The first naturally derived pure medicine to be commercialized was morphine (by Merck, 1826)



Morphine

In 2013 ~ 523,000 kilograms of morphine were produced

The first natural product to be chemically synthesized on a commercial scale was salicylic acid by Bayer (1899)

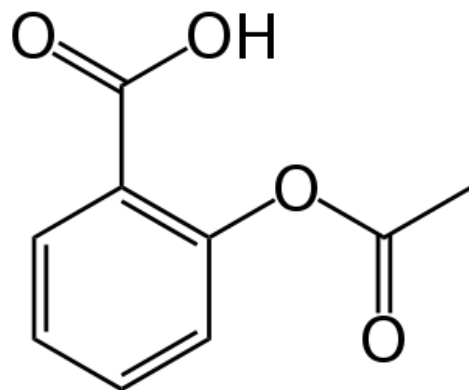
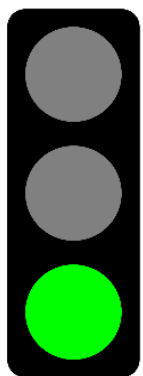


Salicylic
acid

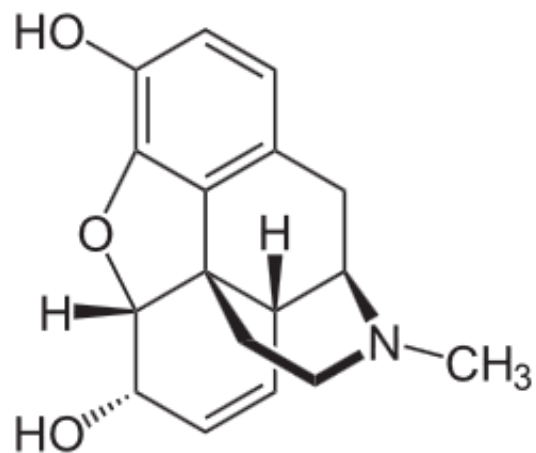


Salicylic acid, in the form of leaves from the willow tree, has been used for its health effects for at least 2,400 years

Chemical synthesis of useful molecules from plants is often difficult/impossible

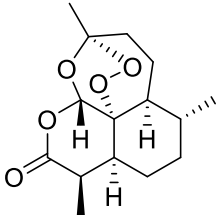


Salicylic acid - 2 steps



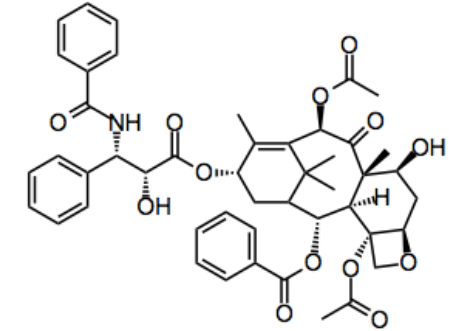
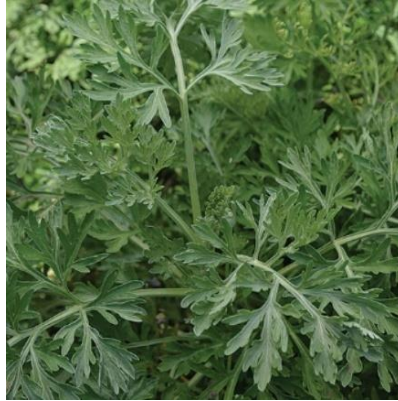
Morphine - 19 steps

Plants are very talented chemists



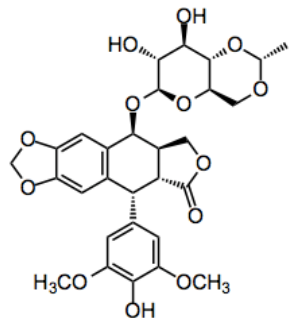
Artemisinin
Wormwood
(*Artemisia annua*)

ANTI-MALARIAL



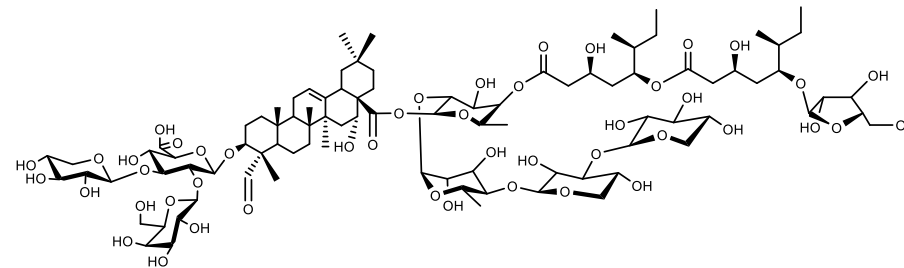
Taxol
Yew (*Taxus baccata*)

ANTI-CANCER



Etoposide
Mayapple (*Podophyllum*)

ANTI-CANCER



QS-21
Soapbark (*Quillaja saponaria*)

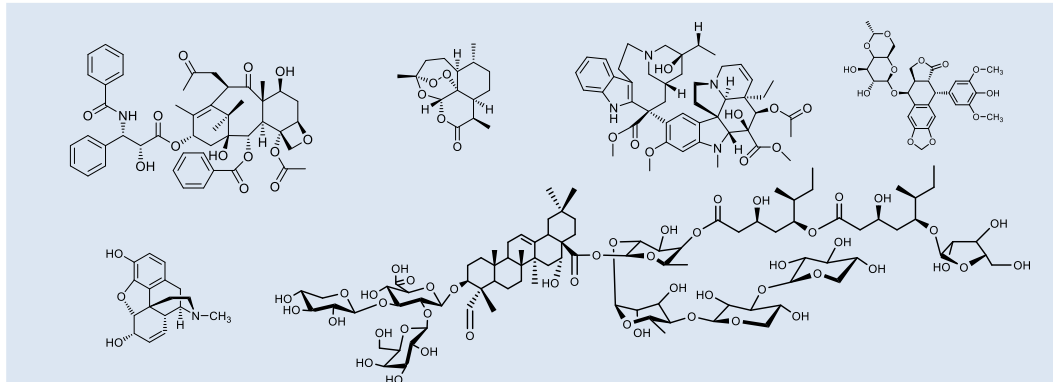
VACCINE ADJUVANT



Around 50% of drugs in current use are natural products or natural product-derived

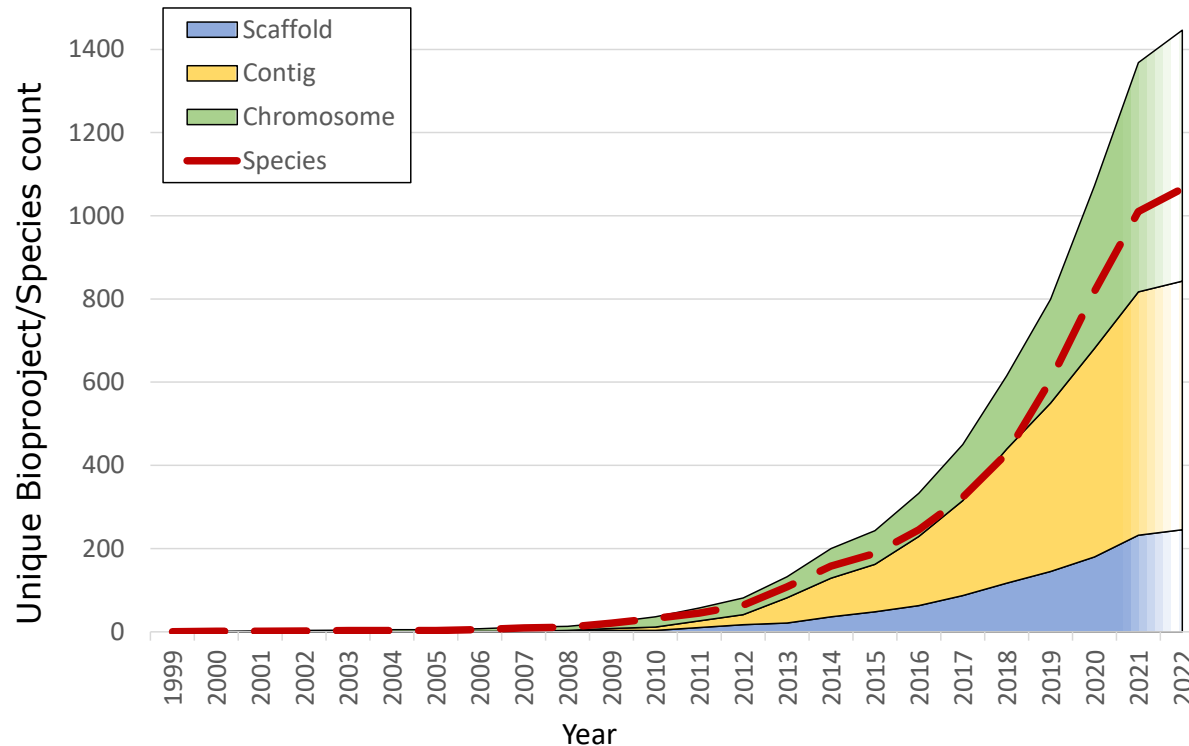


- Plants produce a wealth of bioactive natural products
- There are over 400,000 species of higher plants on the planet
- The vast majority of potentially useful molecules cannot be accessed at scale
- The known compounds are only the tip of the iceberg



There has been rapid expansion in plant genome sequence data in recent years and this is about to explode

Submissions of unique plant genomes and species to NCBI

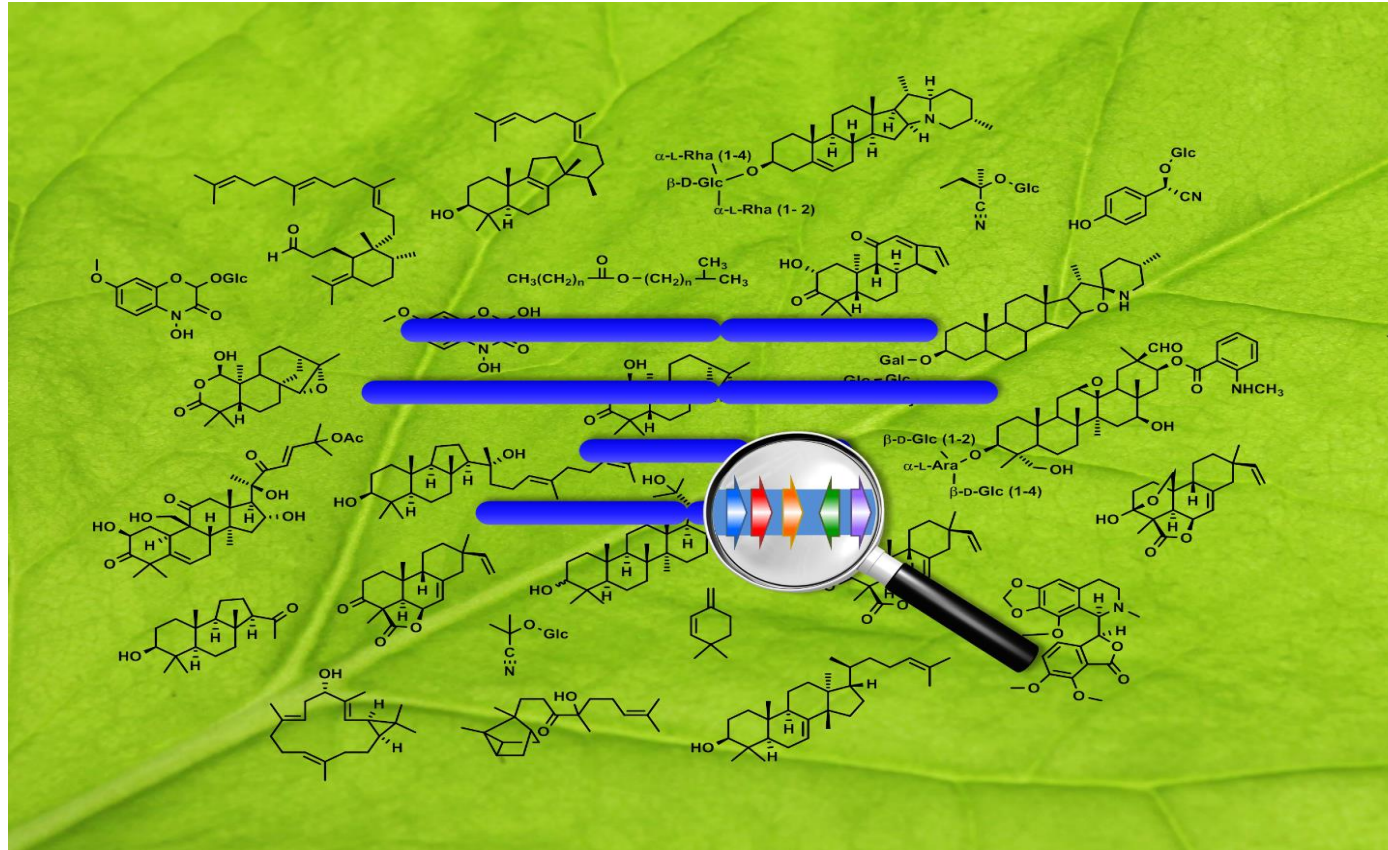


Major new large-scale sequencing initiatives

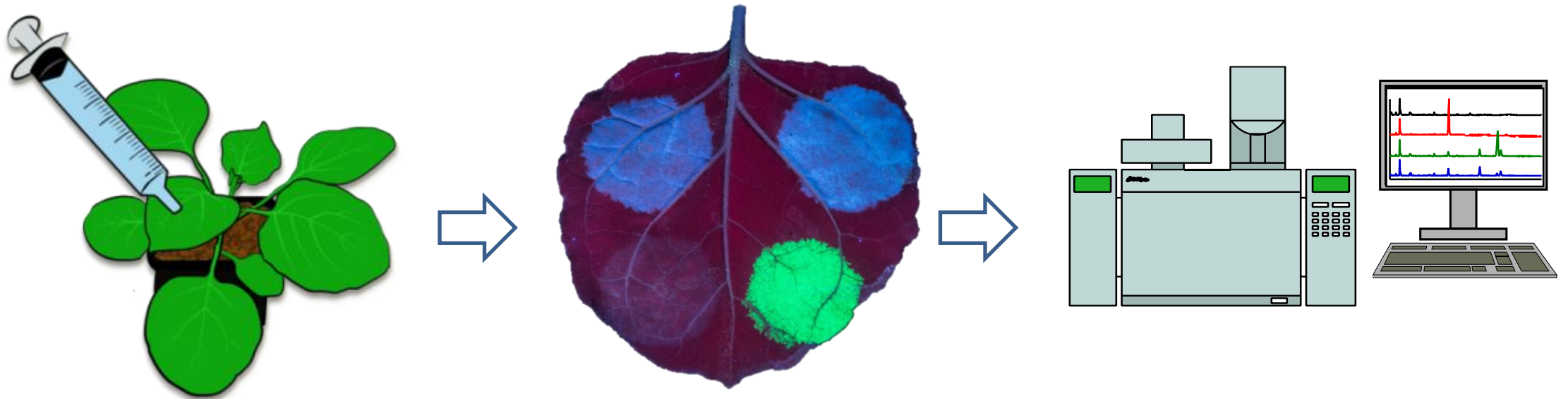


Plant genomes contain the instruction manual to make an enormous array of as yet undiscovered chemical diversity, including new drugs

Translating DNA into chemistry: (1) Rapid identification of pathway genes in plant genomes using new computational methods



Translating DNA into chemistry: (2) Prototype enzymes and pathways using powerful rapid transient plant expression technology



5 days from leaf infiltration to metabolite analysis

Translating DNA into chemistry: (3) Preparative-scale purification of chemicals and analogues



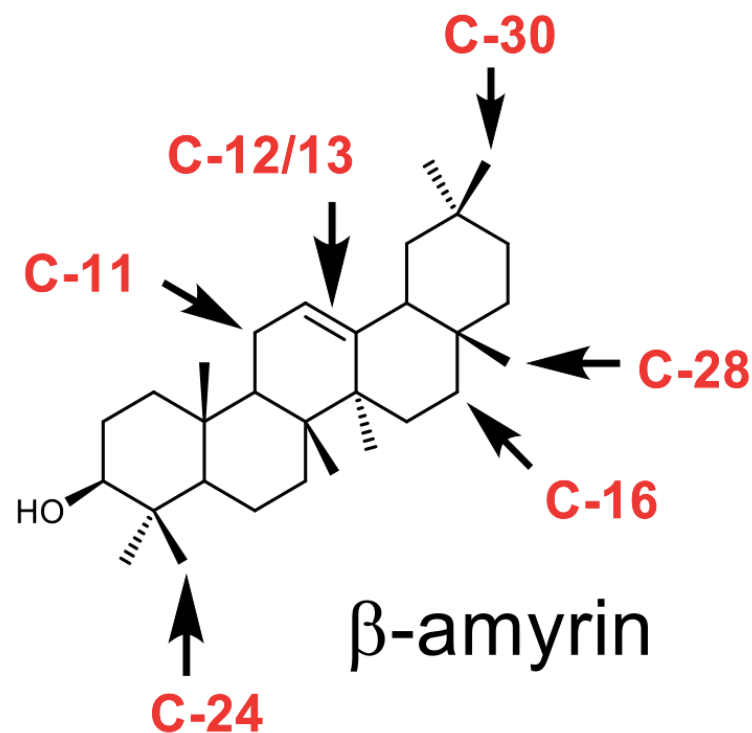
~1 g of 98% pure target compound

Production in plants is scalable



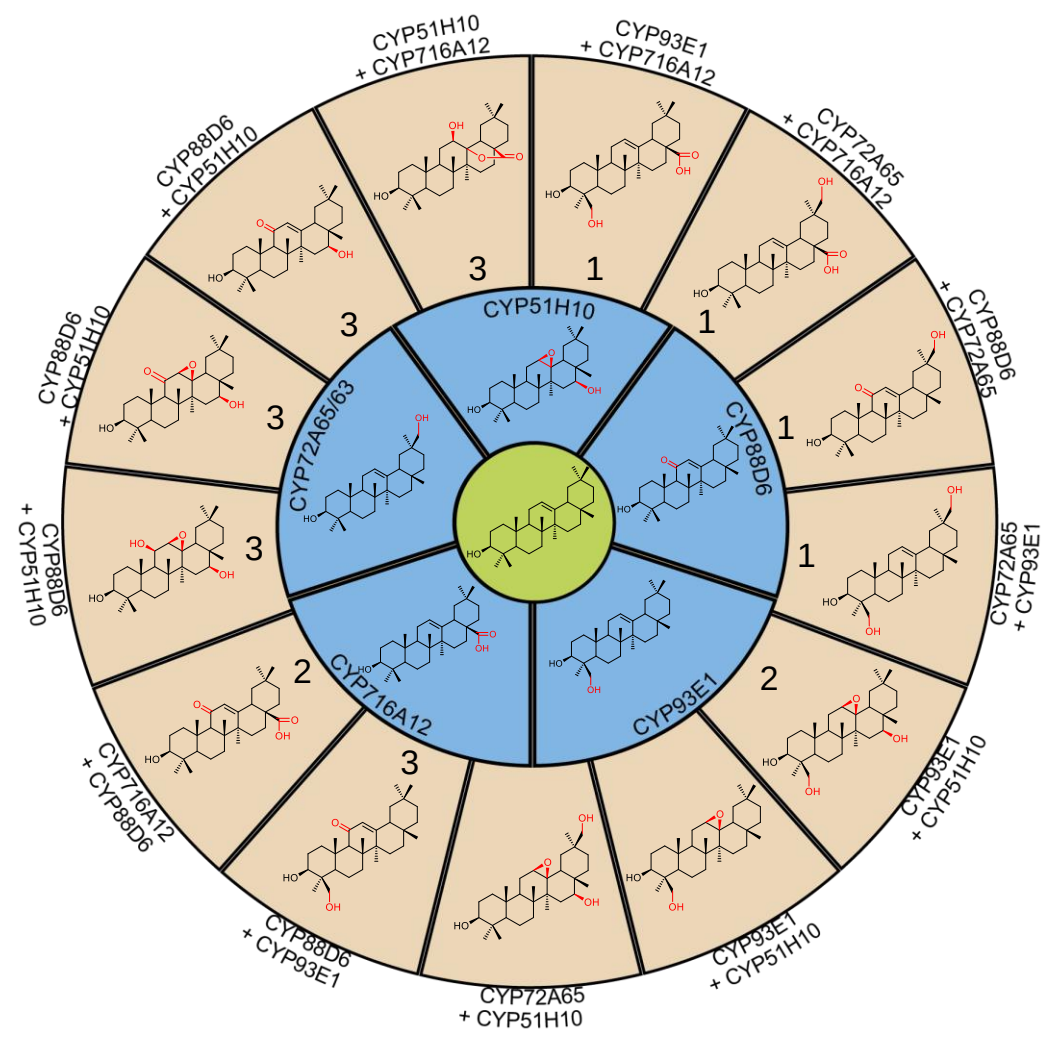
YouTube: [Medicago/vaccine](https://www.youtube.com/watch?v=Medicago/vaccine) – from nucleic acid sequence to 10 million doses of flu vaccine in under a month

The challenge of natural products and medicinal chemistry

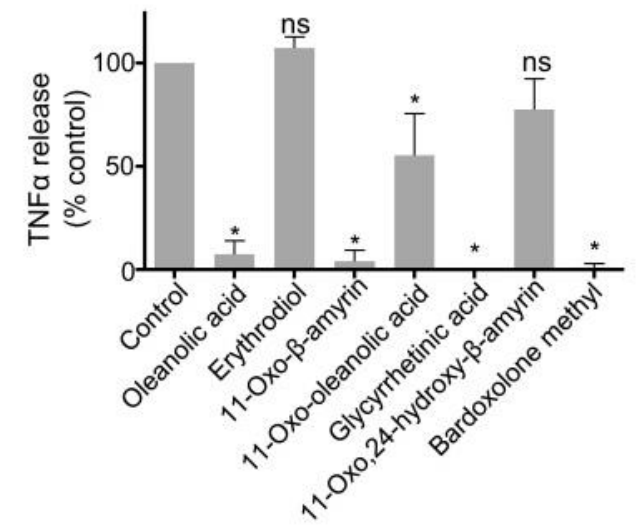


- Selective modification of complex hydrocarbon scaffolds is problematic using synthetic chemistry
- Enzymes have regio- and stereo- selectivity and so overcome this block

Functionalisation of a complex hydrocarbon scaffold by combinatorial biosynthesis using a prototype enzyme toolkit

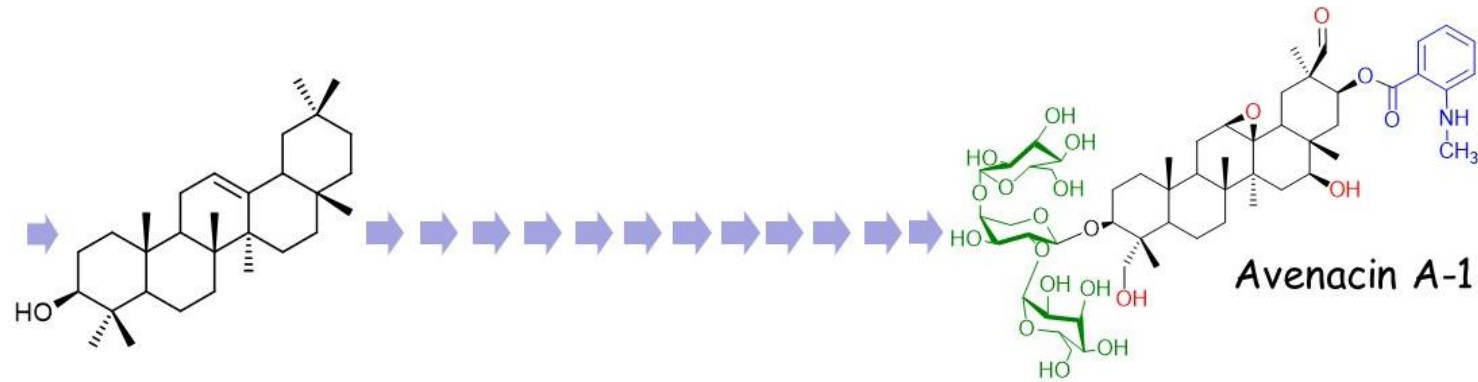


Compound	IC ₅₀ (μM)
β-Amyrin	>200
EpHβA	>200
11α-Hydroxy-β-amyrin	>200
11-Oxo-β-amyrin	26.3 ± 1.1
24-Hydroxy-β-amyrin	>200
Erythrodiol	23.7 ± 4.9
Oleanolic acid	26.5 ± 6.7
30-Hydroxy-β-amyrin	>200
11-Oxo-oleanolic acid	30.6 ± 4.2
Glycyrrhetic acid	25.7 ± 2.9
11-Oxo,24-hydroxy-β-amyrin	>200
Bardoxolone methyl	0.7 ± 0.1



Bioassaying suites of purified analogues

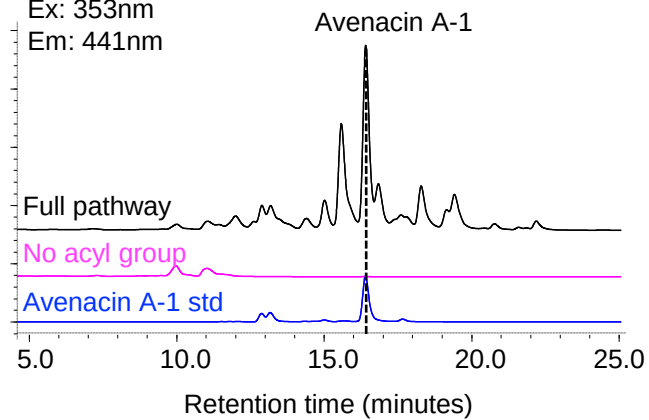
Reconstitution of the 12-step avenacin pathway in *Nicotiana benthamiana*



Fluorescence spectra

Ex: 353nm

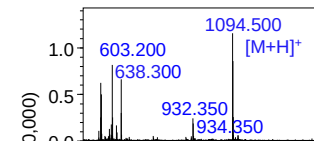
Em: 441nm



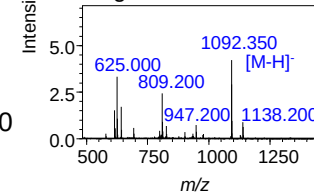
Full pathway MS

(15.6-15.9 min)

Positive mode



Negative mode



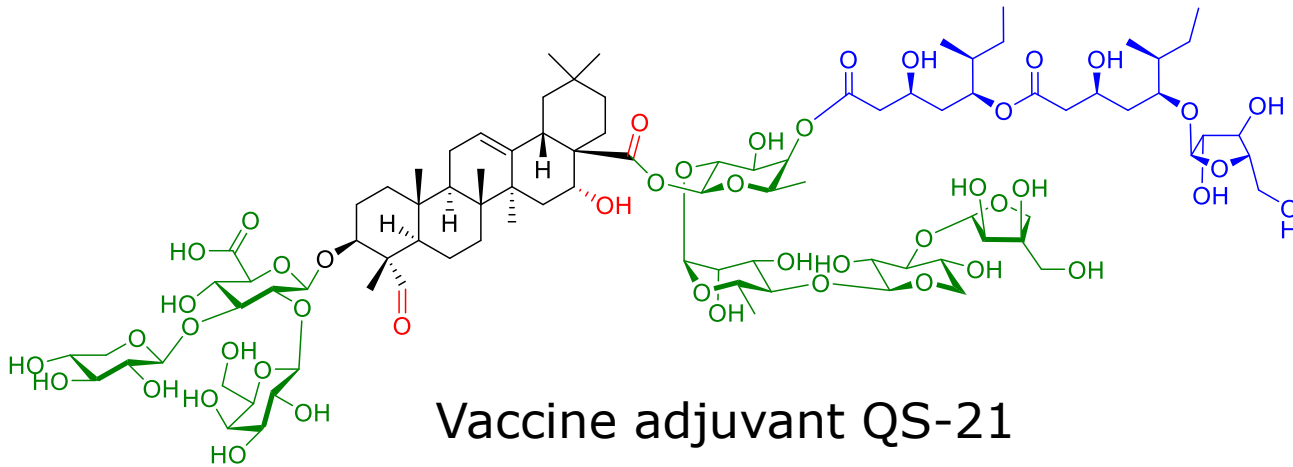
Control leaf



All pathway genes



Elucidation and reconstitution of a complex biosynthetic pathway of major medicinal importance

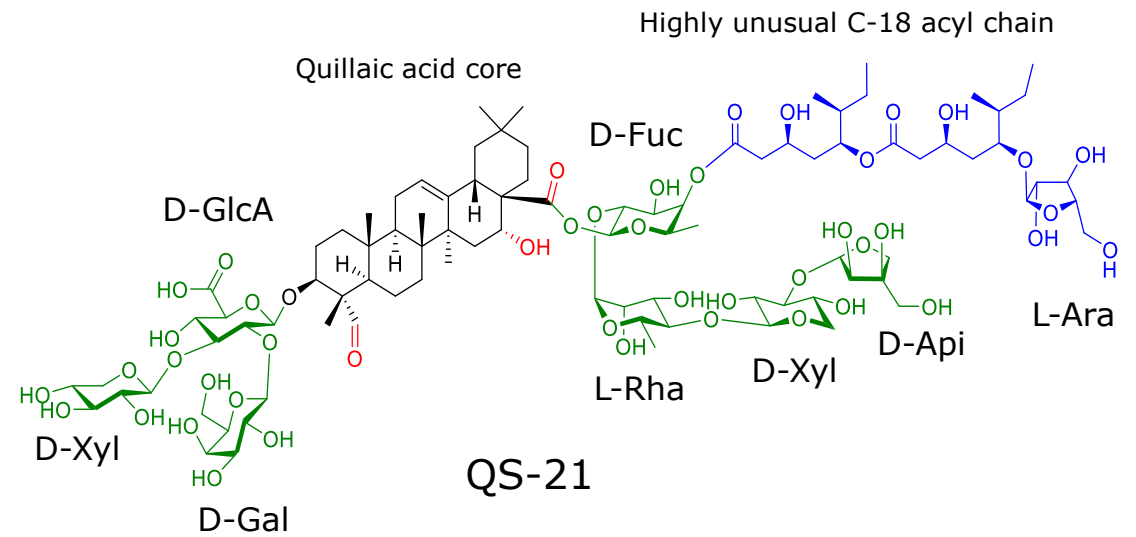
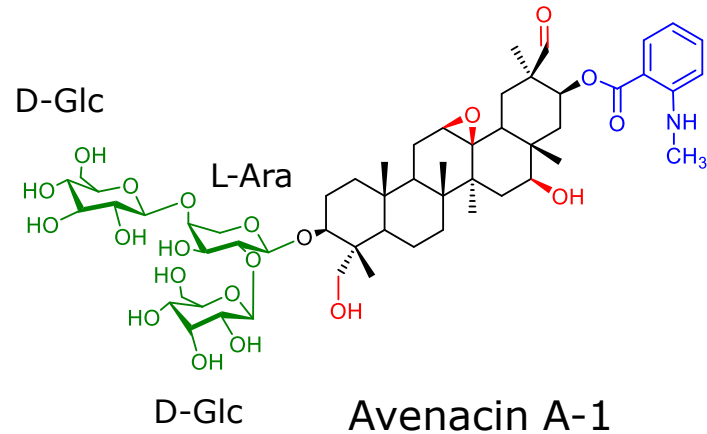


The Chilean soapbark tree
(*Quillaja saponaria*)

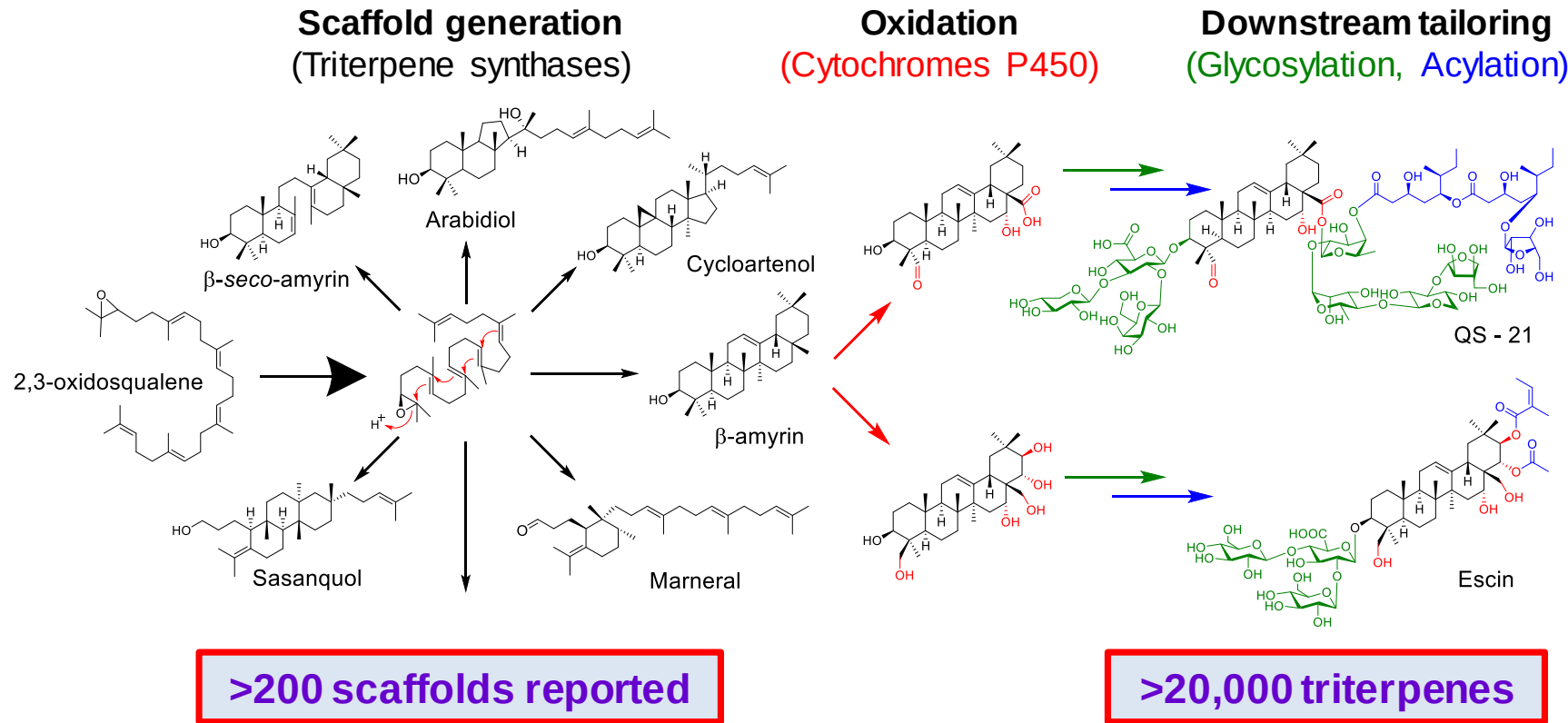


Licensed for use as an
adjuvant in vaccines for
shingles, malaria and
COVID-19

Examples of structurally diverse triterpenes

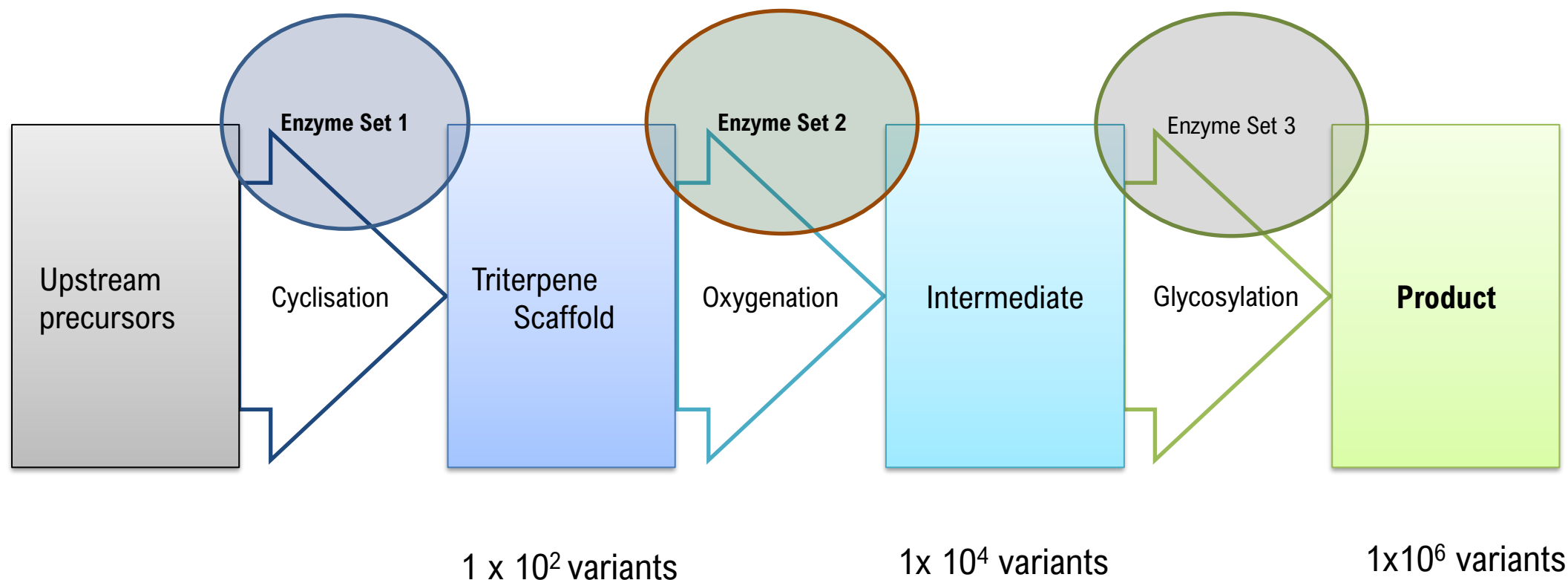


The triterpenes: enormous structural diversity generated from a single simple precursor



- A wide range of potential medicine, agriculture and industrial applications

New chemical space: Diversity potential



With multiple enzymes in each enzyme set, each producing a different molecular transformation, and each set additive to the previous set, many thousands of different molecular forms can be generated by the random selection of one or more enzymes from each set into a pathway assembly. Unique biological activities can be traced back to a specific pathway, for further production of test material

We work on diverse organisms



Wheat – Polturak et al. (2022)



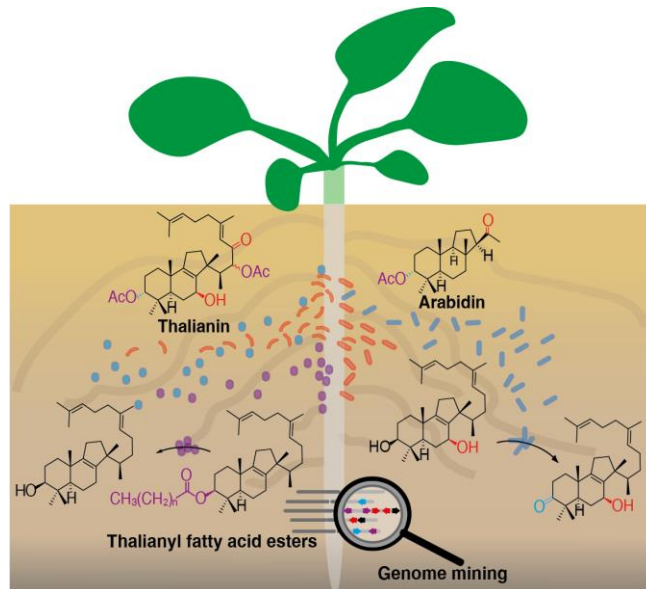
Pink shepherd's purse – Liu et al. (2019)



Soapwort – Jo et al., in prep.



Chinaberry tree – Hodgson et al. (2019);
Pena Rodriguez, Hodgson et al., submitted



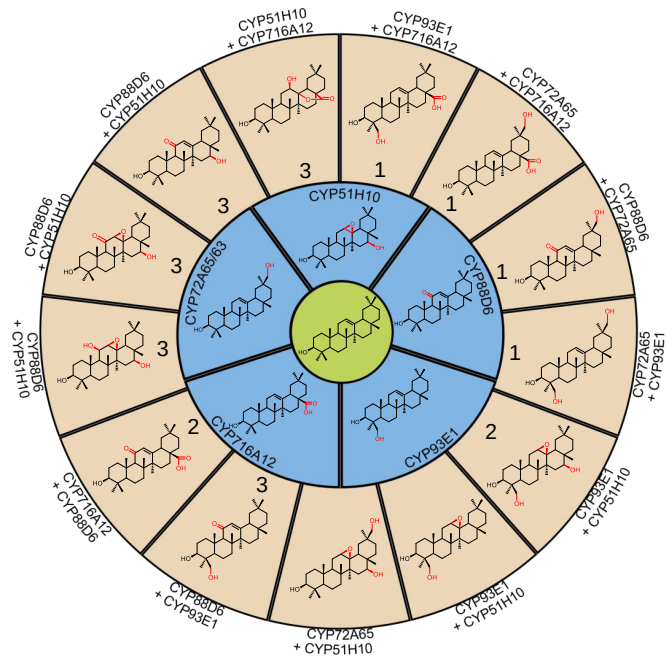
Arabidopsis - Huang et al. (2019)



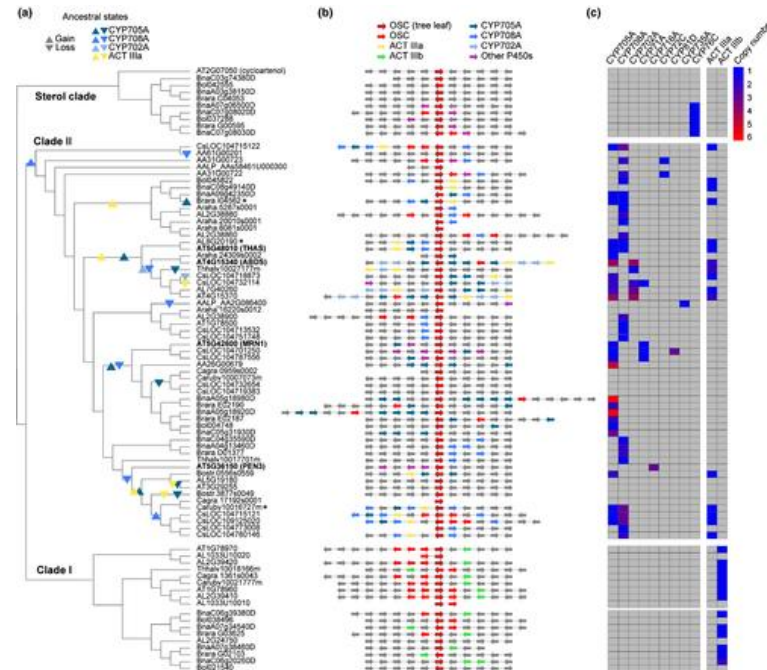
Sea cucumber – Thimmappa et al. (2022)



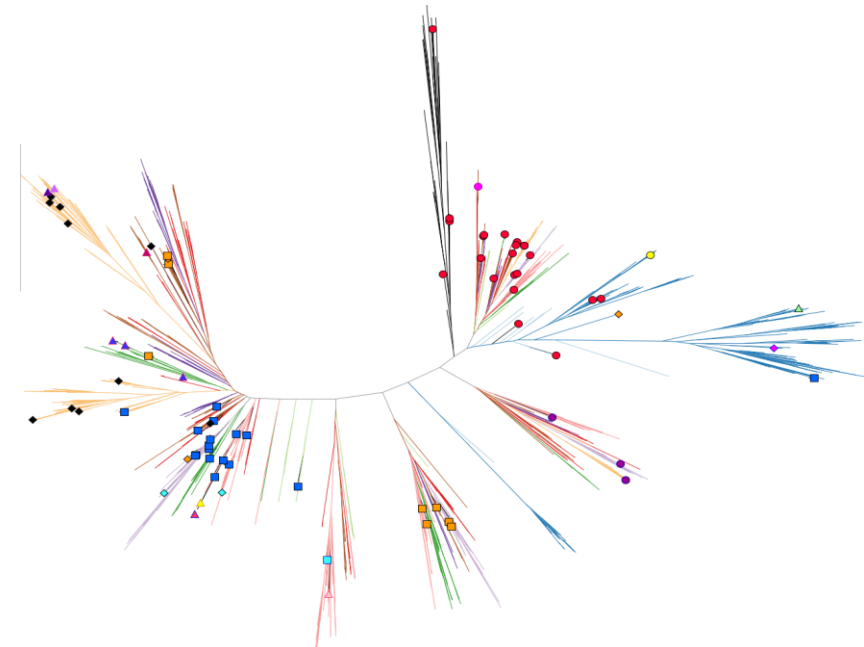
We harness the chemical engineering instruction manual of the Plant Kingdom



Combinatorial biosynthesis



Pan-genome mining

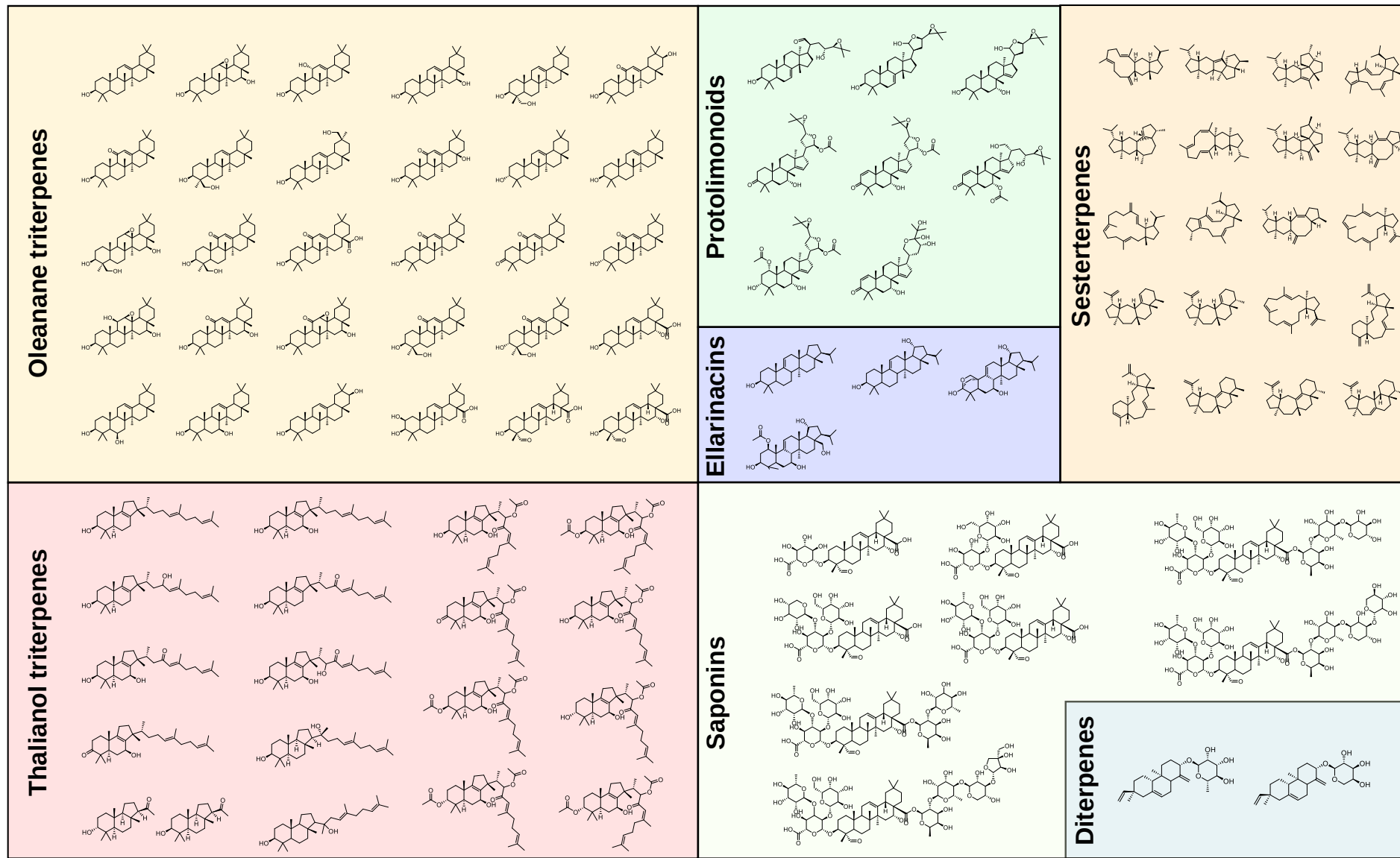


Enzyme superfamilies

We have made over 100 compounds at preparative scale in *Nicotiana benthamiana*

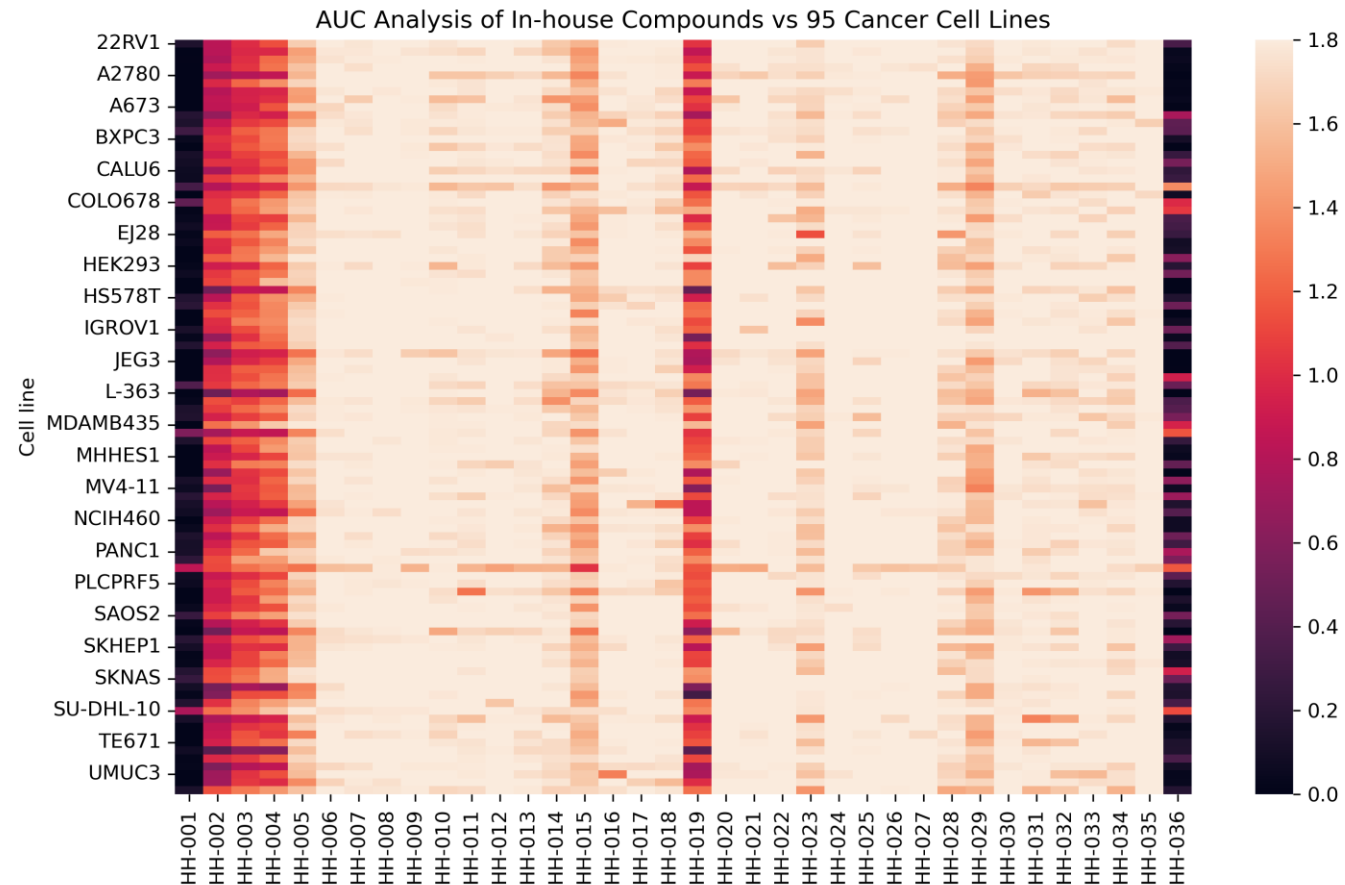
	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	A
1													Summary stats												
2	Identity	No of plants	Dry weight (g)	Amount purified (mg)	Yield (mg/g dw)	# plants /mg	# mg /plant	In stock?	Amount	Person responsible	SMILES string		Total compounds purified	Average dry leaf weight per plant	Largest single yield (mg)	Smallest single yield (mg)	Max yield (mg/g DW)	Min yield (mg/g DW)	Average yield (mg/g DW)						
3	β-amyirin	459	244.0	809.00	3.318	0.57	1.78	Yes			<chem>O[C@H]1CC[C@@H]2[C@@H](O)[C@H](O)[C@@H](O)[C@@H]2[C@H]1O</chem>		89	0.69	809.00	0.80	3.87	0.00	0.49						
4	12,13β-epoxy,16β-hydroxy-β-amyirin	70	20.4	79.00	3.873	0.89	1.13				<chem>O[C@H]1CC[C@@H]2[C@@H](O)[C@H](O)[C@@H](O)[C@@H]2[C@H]1O</chem>														
5	11α-hydroxy-β-amyirin	23	10.4	7.00	0.673	3.29	0.30				<chem>O[C@H]1CC[C@@H]2[C@@H](O)[C@H](O)[C@@H](O)[C@@H]2[C@H]1O</chem>														
6	11-oxo-β-amyirin	23	10.4	10.40	1.000	2.21	0.45	Yes			<chem>O[C@H]1CC[C@@H]2[C@@H](O)[C@H](O)[C@@H](O)[C@@H]2[C@H]1O</chem>														
7	24-hydroxy-β-amyirin	17	3.0	4.70	1.567	3.62	0.28	Yes			<chem>O[C@H]1CC[C@@H]2[C@@H](O)[C@H](O)[C@@H](O)[C@@H]2[C@H]1O</chem>														
8	30-hydroxy-β-amyirin	30	19.0	6.50	0.342	4.62	0.22				<chem>O[C@H]1CC[C@@H]2[C@@H](O)[C@H](O)[C@@H](O)[C@@H]2[C@H]1O</chem>														
9	12,13β-epoxy,16β,24-dihydroxy-β-amyirin	98	30.2	17.00	0.563	5.65	0.18				<chem>O[C@H]1CC[C@@H]2[C@@H](O)[C@H](O)[C@@H](O)[C@@H]2[C@H]1O</chem>														
10	11-oxo, 24-hydroxy-β-amyirin	112	18.5	41.00	2.216	2.73	0.37				<chem>O=C1[C@H]2CC[C@@H]3[C@@H](O)[C@H](O)[C@@H]3[C@H]2O1</chem>														
11	11-oxo-oleanolic acid	141	70.0	48.00	0.657	3.07	0.33				<chem>O=C1[C@H]2CC[C@@H]3[C@@H](O)[C@H](O)[C@@H]3[C@H]2O1</chem>														
12	11β,16β-dihydroxy, 12,13β-epoxy-β-amyirin	112	30.1	6.00	0.199	18.67	0.05				<chem>O[C@H]1CC[C@@H]2[C@@H](O)[C@H](O)[C@@H](O)[C@@H]2[C@H]1O</chem>														
13	11-oxo,16β-hydroxy-β-amyirin	112	30.1	3.50	0.118	32.00	0.03				<chem>O[C@H]1CC[C@@H]2[C@@H](O)[C@H](O)[C@@H](O)[C@@H]2[C@H]1O</chem>														
14	11-oxo,12,13β-epoxy,16β-hydroxy-β-amyirin	112	30.1	14.00	0.465	8.00	0.13				<chem>O[C@H]1CC[C@@H]2[C@@H](O)[C@H](O)[C@@H](O)[C@@H]2[C@H]1O</chem>														
15	12β-hydroxyoleanane-13β,28-olide	98	29.0	5.10	0.176	18.82	0.05																		
16	6β-hydroxy-β-amyirin	100	53.4	18.26	0.304	8.15	0.16	Yes			<chem>O[C@H]1CC[C@@H]2[C@@H](O)[C@H](O)[C@@H](O)[C@@H]2[C@H]1O</chem>														
17	7β-hydroxy-β-amyirin	100	50.4	29.63	0.590	3.37	0.30	Yes			<chem>O[C@H]1CC[C@@H]2[C@@H](O)[C@H](O)[C@@H](O)[C@@H]2[C@H]1O</chem>														
18	21β-hydroxy-β-amyirin	100	151.5	5.66	0.037	17.67	0.06				<chem>O[C@H]1CC[C@@H]2[C@@H](O)[C@H](O)[C@@H](O)[C@@H]2[C@H]1O</chem>														
19	16β-hydroxy-β-amyirin	100	117.2	203.69	1.738	0.49	2.04	Yes			<chem>O[C@H]1CC[C@@H]2[C@@H](O)[C@H](O)[C@@H](O)[C@@H]2[C@H]1O</chem>														
20	23-hydroxy-β-amyirin	100	91.2	30.00	0.329	3.33	0.30	Yes			<chem>O[C@H]1CC[C@@H]2[C@@H](O)[C@H](O)[C@@H](O)[C@@H]2[C@H]1O</chem>														
21	21β-hydroxy-11-oxo-β-amyirin	100	81.0	4.49	0.055	22.27	0.04	Yes			<chem>O[C@H]1CC[C@@H]2[C@@H](O)[C@H](O)[C@@H](O)[C@@H]2[C@H]1O</chem>														
22	11-oxo-erythrodilol	100	81.0	4.26	0.052	23.47	0.04	Yes			<chem>O[C@H]1CC[C@@H]2[C@@H](O)[C@H](O)[C@@H](O)[C@@H]2[C@H]1O</chem>														
23	epi-β-amyirin	100	49.9	187.20	3.351	0.80	1.87	Yes			<chem>O[C@H]1CC[C@@H]2[C@@H](O)[C@H](O)[C@@H](O)[C@@H]2[C@H]1O</chem>														
24	β-amyirin	100	49.9	10.29	0.206	9.72	0.10				<chem>O[C@H]1CC[C@@H]2[C@@H](O)[C@H](O)[C@@H](O)[C@@H]2[C@H]1O</chem>														
25	11-oxo-β-amyirin	100	54.2	5.65	0.104	17.70	0.06				<chem>O[C@H]1CC[C@@H]2[C@@H](O)[C@H](O)[C@@H](O)[C@@H]2[C@H]1O</chem>														
26	11-oxo-β-amyrone	100	54.2	32.11	0.592	3.11	0.32	Yes			<chem>O=C1[C@H]2CC[C@@H]3[C@@H](O)[C@H](O)[C@@H]3[C@H]2O1</chem>														
27	11-oxo-epi-β-amyirin	100	54.2	13.62	0.251	7.34	0.14				<chem>O[C@H]1CC[C@@H]2[C@@H](O)[C@H](O)[C@@H](O)[C@@H]2[C@H]1O</chem>														
28	11-oxo-24-hydroxy-β-amyirin	100	74.1	20.72	0.280	4.83	0.21	Yes			<chem>O[C@H]1CC[C@@H]2[C@@H](O)[C@H](O)[C@@H](O)[C@@H]2[C@H]1O</chem>														
29	11-oxo-24-hydroxy-epi-β-amyirin	100	74.1	59.66	0.805	1.68	0.60	Yes			<chem>O[C@H]1CC[C@@H]2[C@@H](O)[C@H](O)[C@@H](O)[C@@H]2[C@H]1O</chem>														
30	Echinocystic acid	100	40.8	12.70	0.310	7.87	0.13	Yes			<chem>O[C@H]1CC[C@@H]2[C@@H](O)[C@H](O)[C@@H](O)[C@@H]2[C@H]1O</chem>														
31	Augustic acid	100	69.5	35.93	0.517	2.78	0.36	Yes			<chem>O[C@H]1CC[C@@H]2[C@@H](O)[C@H](O)[C@@H](O)[C@@H]2[C@H]1O</chem>														
32	dihydroniloticin	144	88.6	88.00	1.253	1.67	0.60	Yes	10 mg	Hannah	<chem>O=C1[C@H]2CC[C@@H]3[C@@H](O)[C@H](O)[C@@H]3[C@H]2O1</chem>														
33	melianol	160	198.5	6.00	0.030	26.67	0.04	Yes	1 mg	Hannah	<chem>O=C1[C@H]2CC[C@@H]3[C@@H](O)[C@H](O)[C@@H]3[C@H]2O1</chem>														
34	apo-melianol	115	159.9	13.10	0.082	8.78	0.11	Yes	2 mg	Hannah	<chem>O=C1[C@H]2CC[C@@H]3[C@@H](O)[C@H](O)[C@@H]3[C@H]2O1</chem>														
35	21-acetyloxy-apo-melianone	121	150.1	77.20	0.514	1.57	0.84	Yes	20 mg	Hannah	<chem>O=C1[C@H]2CC[C@@H]3[C@@H](O)[C@H](O)[C@@H]3[C@H]2O1</chem>														
36	epi-neemfruitin B	113	112.5	2.00	0.018	56.50	0.02	No		Hannah	<chem>O=C1[C@H]2CC[C@@H]3[C@@H](O)[C@H](O)[C@@H]3[C@H]2O1</chem>														
37	MaL7AT & MaAKR product of epi-neemfruitin B	110	140.1	20.00	0.143	5.50	0.18	No		Hannah	<chem>CC(O)[C@H]1C[C@H]2[C@@H]3[C@@H](O)[C@H](O)[C@@H]3[C@H]2O1</chem>														
38	MaL3AKR product of epi-neemfruitin B	108	114.7	2.00	0.017	54.00	0.02	No		Hannah	<chem>CC(O)[C@H]1C[C@H]2[C@@H]3[C@@H](O)[C@H](O)[C@@H]3[C@H]2O1</chem>														
39	MaAKR & MaCYP716AD4 product of epi-neemfruitin B	120	123.1	0.60	0.005	200.00	0.01	No		Hannah	<chem>O[C@H]1C[C@H]2[C@@H]3[C@@H](O)[C@H](O)[C@@H]3[C@H]2O1</chem>														
40	Gypsogenin	209	150.3	28.00	0.188	7.46	0.13	Yes	1.6 mg	Amr	<chem>O=C1[C@H]2CC[C@@H]3[C@@H](O)[C@H](O)[C@@H]3[C@H]2O1</chem>														
41	Quilicic acid	209	150.3	30.00	0.200	6.97	0.14	Yes	2 mg	Amr	<chem>O=C1[C@H]2CC[C@@H]3[C@@H](O)[C@H](O)[C@@H]3[C@H]2O1</chem>														

We have made over 100 compounds at preparative scale in *Nicotiana benthamiana*



We can harness the chemical engineering ability of the Plant Kingdom and use it to engineer new bioactives

HTS results of 36 compounds against 95 cancer cell lines - 3,420 data points (AUC of IC50 data)



The 19th century: the beginning of the pharmaceutical industry



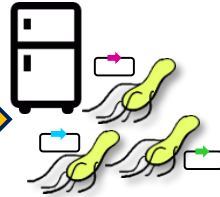
H. E. Merck laboratory, Darmstadt, Germany, 1826

The 21st century: the Biorevolution

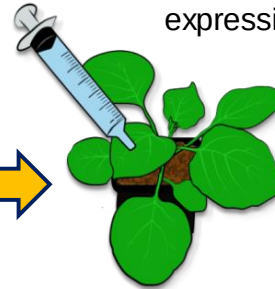
INPUT Instructions from plants



DNA-encoded
enzyme toolkit

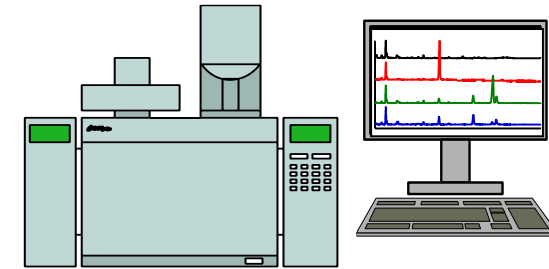


Transient plant
expression



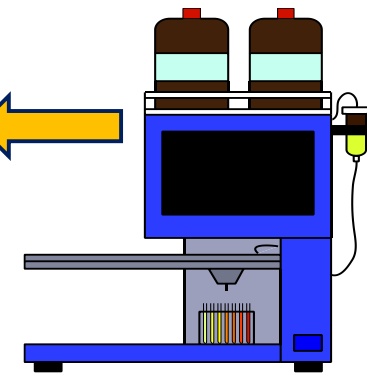
Screen chemical libraries

Metabolite analysis

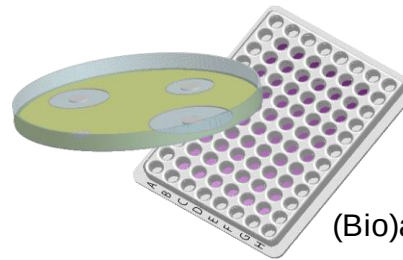


*Deep data platform,
iterative learning*

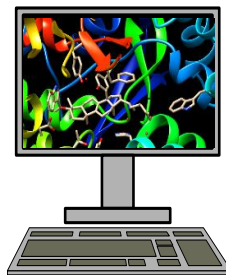
Scale-up



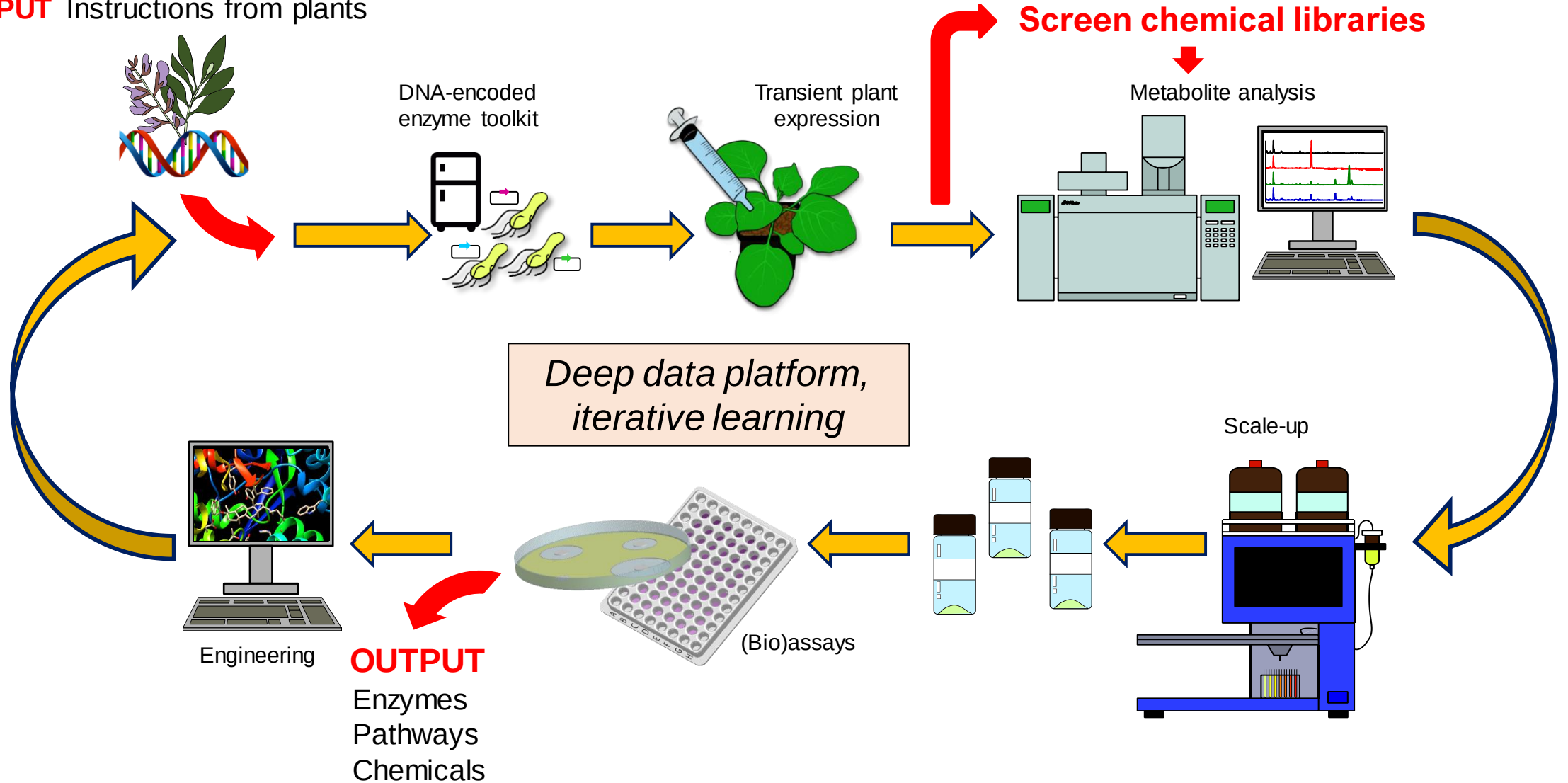
(Bio)assays



Engineering



OUTPUT
Enzymes
Pathways
Chemicals



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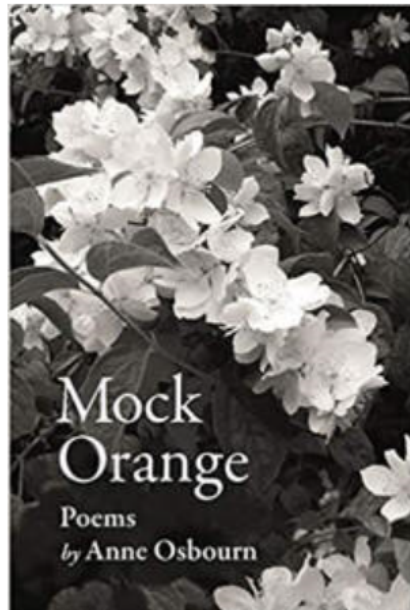


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