

What is claimed is:

CLAIMS

1. A method for the discovery and evolution of metabolic pathways that produce molecules that modulate protein function, comprising:

contacting a population of host cells that each comprise a protein of interest with a population of expression vectors that each comprise ~~ing a different~~ metabolic pathways from a library of different pathways, wherein the host cells are amenable to transfer of the population of expression vectors;

expressing ~~the~~ each metabolic pathways of the population of expression vectors in the population of host cells, wherein a cell or subset of the population of host cells produce a detectable output when ~~the~~ a metabolic pathway comprised in an expression vector of the population of expression vectors produces a product that modulates the protein of interest;

screening the cell or the subset of the population of host cells under conditions that enable measurement of the detectable output in the cell or the subset of the population of host cells;

isolating the cell or the subset of the population of host cells that produce thea detectable output;

isolating the expression vectors ~~s~~ that ~~yield~~ produces the detectable outputs that is higher than a reference ~~the~~ output of a reference vector that harbors a reference pathway, ~~for example, a vector that encodes a~~ wherein the reference pathway ~~that~~ does not produce molecules with concentrations and/or potencies sufficient to modulate the ~~activity of a~~ protein of interest, ~~in the cell or the subset of the population of host cells; and~~

characterizing one or more ~~the~~ products of the metabolic pathways ~~s-encoded~~ comprised in ~~by~~ the expression vectors ~~s~~ that ~~yield~~ produced the detectable outputs ~~s~~ that is ~~are~~ higher than the reference output of ~~said the~~ reference vector in the cell or the subset of the population of host cells.

2. The method of claim 1, wherein the population of host cells further comprise a genetically encoded system in which ~~the~~ activity of thea protein of interest controls ~~the~~ assembly of a protein complex with an activity that is not possessed by either of two or more components of the protein complex and, thus, yields thea detectable output in proportion to the amount of the protein complex formed.

3. The method of claim 1 ~~or claim 2~~, wherein the protein of interest is an enzyme that adds a post-translational modification that causes two proteins, which are initially dissociated, to be covalently linked or to form a noncovalent complex.

4. The method of claim 2 ~~any one of claims 1-3~~, wherein the protein complex is formed by two proteins with a dissociation constant (K_d) less than or equal to the K_d of ~~the~~ complexes formed between SH2 domains and their phosphorylated substrates.

5. The method of ~~any one of claims 1-4~~ claim 1, wherein the metabolic pathways comprised in the population of expression vectors produce phenylpropanoids or nonribosomal peptides.

6. The method of claim 1 ~~any one of claims 1-5~~, wherein the expression vectors that each comprising a different metabolic pathway from the library of different pathways comprise a library of pathways generated by mutating one or more genes within a starting metabolic pathway.

7. The method of ~~any one of claims 1-6~~ claim 1, wherein one or more of the metabolic pathways comprised in the population of expression vectors comprises a set of genes of unknown biosynthetic capability.

8. The method of ~~any one of claims 1-7~~ claim 1, wherein one or more of ~~the~~ metabolic pathways that yield ~~produces a~~ detectable outputs higher than the reference output of the reference vector that harbors the ~~of the~~ reference pathway produces a product that differs from ~~the~~ products of other metabolic pathways of the population of expression vectors.

9. The method of claim 1 ~~any one of claims 1-8~~, wherein one or more of the metabolic pathways that yield ~~produces a~~ detectable outputs higher than the reference output of the reference vector that harbors ~~of the~~ reference pathway produces a larger quantity of a product than ~~the~~ quantities ~~sy~~ of product generated by other metabolic pathways of the population of expression vectors.

10. The method of ~~any one of claims 1-9~~ claim 1, wherein one or more of ~~the~~ metabolic pathways that yield ~~produces a~~ detectable outputs higher than the reference output of the reference vector that harbors the ~~of the~~ reference pathway exhibits a lower cellular toxicity

than cellular toxicities exhibited by other metabolic pathways of the population of expression vectors.

11. The method of ~~any one of claims 1-10~~claim 1, wherein the products of ~~the~~ metabolic pathways encoded by the expression vectors that yield the detectable outputs that are higher than the reference output are characterized by ~~standard analytical methods, preferably by~~ gas chromatography-mass spectrometry (GC/MS), liquid chromatography-mass spectrometry (LC/MS), and/or nuclear magnetic resonance (NMR) spectroscopy.

12. The method of ~~any one of claims 1-11~~claim 1, further comprising isolating the products of the pathways encoded by the expression vectors that yield the detectable outputs that are higher than the reference output.

13. The method of claim 12, further comprising concentrating the products, ~~preferably~~ using a rotary evaporator.

14. The method of claim 12~~-or claim 13~~, further comprising testing the effects of the products on the protein of interest.

15. The method of ~~any one of claims 1-14~~claim 1, wherein the protein of interest is a ubiquitin ligase, a SUMO transferase, a methyltransferase, a demethylase, an acetyltransferase, a glycosyltransferase, a palmitoyltransferase, or a related hydrolase.

16. The method of claim 1, further comprising testing the effects of the products on the protein of interest.

17. The method of claim 2, wherein the protein of interest is an enzyme that adds a post-translational modification that causes two proteins, which are initially dissociated, to be covalently linked or to form a noncovalent complex.

18. The method of claim 6, wherein one or more of the metabolic pathways comprised in the population of expression vectors comprises a set of genes of unknown biosynthetic capability.

19. The method of claim 9, wherein one or more of metabolic pathways that yield detectable outputs higher than the reference output of the reference vector that harbors the reference pathway exhibits a lower cellular toxicity than cellular toxicities exhibited by other metabolic pathways of the population of expression vectors.

20. The method of claim 9, wherein the products of metabolic pathways encoded by the expression vectors that yield the detectable outputs that are higher than the reference output are characterized by gas chromatography-mass spectrometry (GC/MS), liquid chromatography-mass spectrometry (LC/MS), and/or nuclear magnetic resonance (NMR) spectroscopy.

~~16. A composition or system comprising a population of host cells that comprise a protein of interest and a population of expression vectors comprising different metabolic pathways, wherein a cell or subset of the population of host cells produce a detectable output when the metabolic pathway produces a product that modulates the protein of interest, optionally wherein the expression vectors yield detectable outputs higher than the output of a reference vector that harbors a reference pathway, for example, a vector that encodes a pathway that does not produce molecules with concentrations and/or potencies sufficient to modulate the activity of a protein of interest, in the cell or the subset of the population of host cells.~~

~~17. The composition or system of claim 16, wherein the host cells comprise a genetically encoded system in which the activity of a protein of interest controls the assembly of a protein complex with an activity that is not possessed by either of two or more components of the complex and, thus, yields a detectable output in proportion to the amount of complex formed.~~

~~18. The composition or system of claim 16 or claim 17, wherein the protein of interest is an enzyme that adds a post-translational modification that causes two proteins, which are initially dissociated, to be covalently linked or to form a noncovalent complex.~~

~~19. The composition or system of any one of claims 16-18, wherein the complex is formed by two proteins with a dissociation constant (K_d) less than or equal to the K_d of the complexes formed between SH2 domains and their phosphorylated substrates.~~

~~20. The composition or system of any one of claims 16-19, wherein the metabolic pathways produce phenylpropanoids or nonribosomal peptides.~~

~~21. The composition or system of any one of claims 16-20, wherein the expression vectors comprising different metabolic pathways comprise a library of pathways generated by mutating one or more genes within a starting metabolic pathway.~~

~~22. The composition or system of any one of claims 16-21, wherein one or more of the metabolic pathways comprises a set of genes of unknown biosynthetic capability.~~

~~23. The composition or system of any one of claims 16-22, wherein one or more of the metabolic pathways that produces a detectable output higher than the output of the reference pathway produces a product that differs from the products of other metabolic pathways.~~

~~24. The composition or system of any one of claims 16-23, wherein one or more of the metabolic pathways that produces a detectable output higher than the output of the reference pathway produces a larger quantity of a product than the quantity of product generated by other metabolic pathways.~~

~~25. The composition or system of any one of claims 16-24, wherein one or more of the metabolic pathways that produces a detectable output higher than the output of the reference pathway exhibits a lower cellular toxicity than other metabolic pathways.~~

~~26. The composition or system of any one of claims 16-25, wherein the protein of interest is a ubiquitin ligase, a SUMO transferase, a methyl transferase, a demethylase, an acetyltransferase, a glycosyltransferase, a palmitoyltransferase, or a related hydrolase.~~

~~27. A kit comprising a population of expression vectors of any one of claims 16-26.~~

~~28. The kit of claim 27, further comprising the population of host cells of any one of claims 16-26.~~