STRUCTURE-ACTIVITY RELATIONSHIPS

1. CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit under 35 U.S.C. § 119(e) to application US Serial No. 60/901,349, filed February 12, 2007 and US application Serial No. 60/909,937, filed April 3, 2007, the disclosures of which are incorporated herein by reference in their entirety.

2. BACKGROUND

[0002] Methods for screening and identifying polypeptides having desired activities are useful in the preparation of new compounds such as enzymes, hormones, receptors and pharmaceutical compounds. Current methods for identifying novel polypeptides are often time-consuming and costly, requiring screening an enormous number of polypeptide variants, and it may take months or years to find a new polypeptide with the desired activity, if one is ever found. These limitations also make the known methods less than ideal for timely development of commercial products and processes. Thus, compositions and methods useful in accelerated identification of novel polypeptide variants having desired activities in a cost effective and time efficient manner would be highly desirable.

3. SUMMARY

[0003] The present disclosure provides compositions and methods involving a plurality of polypeptides having certain activities, which plurality aids in the identification of new polypeptides with desired or enhanced activities.

[0004] In some embodiments of the disclosure, each polypeptide in the plurality reacts with at least one substrate to produce a product, and at least two members of the plurality are related to a parent polypeptide and react with differing levels of activity upon a given substrate. In some embodiments of the disclosure, each polypeptide in the plurality reacts with at least one substrate to produce a product, and at least two members of the plurality are related to a parent polypeptide and react with different substrates. Also contemplated are compositions comprising pluralities of pluralities, in which each plurality acts on a

different class of substrate, as well as methods of generating and screening these pluralities of pluralities.

[0005] In some embodiments, a plurality of enzymes is made by generating variants of at least one parent enzyme wherein at least a portion of each variant is known and wherein each variant comprises at least one mutation known to affect reactivity with a substrate to produce a product, and selecting two or more variants wherein at least two of the selected variants react with differing levels of activity upon a given substrate. In some embodiments, a plurality of enzymes is made by generating variants of at least one parent enzyme wherein at least a portion of each variant is known and wherein each variant comprises at least one mutation known to affect reactivity with a substrate to produce a product, and selecting two or more variants wherein at least two of the selected variants react with different substrates.

[0006] In some embodiments, the methods involve screening a plurality of polypeptide variants against a ligand of interest, wherein each variant reacts with at least one ligand to produce a detectable signal, and wherein at least two members of the plurality are related to a parent polypeptide and react with differing levels of activity upon a given ligand, and identifying at least one variant that produces a signal upon reaction with the ligand of interest. In some embodiments, the methods involve screening a plurality of polypeptide variants against a ligand of interest, wherein each variant reacts with at least one ligand to produce a detectable signal, and wherein at least two members of the plurality are related to a parent polypeptide and react with different ligands, and identifying at least one variant that produces a signal upon reaction with the ligand of interest.

[0007] In some embodiments, the methods involve making a plurality of polypeptide variants by generating a plurality of polypeptide variants related to a parent polypeptide, a portion of each variant having known amino acid sequence, wherein each variant comprises at least one mutation that enhances reactivity with a substrate as compared to the reactivity of the parent polypeptide with said substrate, and wherein at least two members of the plurality react with differing levels of activity upon a given substrate; and assigning an address to each of the variants. In some embodiments, the methods involve making a plurality of polypeptide variants by generating a plurality of polypeptide variants related to a parent polypeptide, a portion of each variant having known amino acid sequence, wherein

each variant comprises at least one mutation that enhances reactivity with a substrate as compared to the reactivity of the parent polypeptide with said substrate, and wherein at least two members of the plurality react with different substrates; and assigning an address to each of the variants.

[0008] In some embodiments, the methods involve making a plurality of pluralities by generating a first and a second addressable plurality of polypeptide variants, wherein the members of the first plurality are related to one parent polypeptide and the members of the second plurality are related to a second parent polypeptide, wherein at least a portion of the amino acid sequence of each variant is known, wherein each member of each plurality reacts with a substrate to produce a product, and wherein at least two members of the first plurality react with differing levels of activity upon a given substrate and at least one member of the first plurality and at least one member of the second plurality react with different substrates . In some embodiments, the methods involve making a plurality of pluralities by generating a first and a second addressable plurality of polypeptide variants, wherein the members of the first plurality are related to one parent polypeptide and the members of the second plurality are related to a second parent polypeptide, wherein at least a portion of the amino acid sequence of each variant is known, wherein each member of each plurality reacts with a substrate to produce a product, and wherein at least one member of the first plurality and at least one member of the second plurality react with different substrates. Also contemplated are pluralities of host cell colonies and kits for identifying at least one polypeptide having a desired activity using the compositions and methods disclosed herein.

[0008a] In one aspect, there is provided a kit for identifying at least one polypeptide having a desired activity for a ligand of interest, consisting: (i) at least one plurality of active addressable polypeptide variants, wherein each member of each plurality of the active polypeptide variants reacts with at least one ligand to produce a detectable signal, and wherein at least two members of each plurality of the active polypeptide variants are related to a parent polypeptide and are capable of reacting with different ligands, wherein the different ligands do not react with the parent polypeptide; (ii) at least one ligand, wherein said ligand is placed within the wells of a microtiter plate; and (iii) instructions for use.

[0008b] In another aspect, there is provided a kit for identifying at least one polypeptide having a desired activity for a ligand of interest, consisting: (i) at least one plurality of active addressable polypeptide variants, wherein each member of each plurality of the active polypeptide variants reacts with at least one ligand to produce a detectable signal, and wherein at least two members of each plurality of the active polypeptide variants are related to a parent polypeptide and are capable of reacting with different ligands, wherein the different ligands do not react with the parent polypeptide wherein each member of said plurality of active polypeptide variants is placed within a well of a microtiter plate; (ii) at least one ligand; and (iii) instructions for use.

[0008c] In another aspect, there is provided a kit for identifying at least one polypeptide having a desired activity for a ligand of interest, consisting: (i) at least one plurality of active addressable polypeptide variants, wherein each member of the plurality of active addressable polypeptide variants reacts with at least one ligand of a plurality of ligands and at least two members of the plurality of the active addressable polypeptide variants are related to a parent polypeptide, wherein one member has been selected for improved activity for one ligand and the other member has been selected for improved activity for a different ligand in the plurality of ligands as compared to the parent polypeptide; (ii) a plurality of ligands; and (ii) instructions for use.

[0009] Virtually any enzyme/substrate or receptor/ligand combination can be used as the basis for making evolvants used in the compositions and methods disclosed. It is also contemplated that a substrate or ligand of unknown identity can be used to screen polypeptide evolvants of an ancestral or parent enzyme or receptor for a desired activity. In some embodiments, the plurality of polypeptides is a plurality of enzyme candidates. In some embodiments, the substrate screened is an unknown substrate. Also contemplated is a plurality, including members that act on different and diverse substrates, for carrying out different chemical conversions. In some embodiments, each member of a plurality acts on a different substrate. In some embodiments, some members