CLAIMS

What is claimed is:

1. A method of screening an enzyme for one or more characteristics, the method comprising the steps of:

(a) generating a plurality of droplets, wherein at least one droplet comprises: an enzyme, a reaction substrate; and one or more redox cofactors, or one or more redox cofactors and one or more detection reagents, wherein the enzyme converts the reaction substrate to a target product;

(b) detecting a signal emitted from the droplet, wherein the signal is a fluorescence signal, a bioluminescence signal or a chemiluminescence signal, (i) wherein the signal is emitted when the redox cofactor is oxidized or reduced when the reaction substrate is converted to the target product by the enzyme, or (ii) wherein the signal is emitted when the redox cofactor is oxidized or reduced when the target product is further converted to a subsequent product by the one or more detection reagents in the droplet.

2. A method of screening an enzyme for one or more characteristics, comprising:

in a fluidic device comprising an incubation region comprising a plurality of incubation chambers including a first incubation chamber and a second incubation chamber, wherein the plurality of incubation chambers are configured to allow continuous flow of a plurality of droplets, performing the steps of:

culturing a cell in the droplet to form a plurality of cells while flowing the cell from the first incubation chamber to the second incubation chamber;

continuously flowing the droplet in the incubation region, wherein the droplet comprises, an enzyme, a reaction substrate, and one or more redox cofactors, or one or more redox cofactors and one or more detection reagents, wherein the enzyme converts the reaction substrate to a target product; and

generating a signal from the droplet, wherein the signal is a fluorescence signal, a bioluminescence signal or a chemiluminescence signal.

~~3. A fluidic system, comprising:~~

a plurality of droplets including a first droplet comprising a redox cofactor, wherein a concentration of the redox cofactor in the droplet is greater than or equal to 5 μM;

a microfluidic channel containing the droplet; and

a droplet-sorting region in fluidic communication with the microfluidic channel, wherein the droplet-sorting region comprises a set of electrodes adjacent to the microfluidic channel.

4. A fluidic system, comprising:

a microfluidic channel containing a plurality of droplets,

wherein at least one droplet comprises a redox cofactor and a detection reagent; and

wherein the detection reagent is configured to react with a redox couple of the redox cofactor to produce a luminescent signal.

5. A fluidic system, comprising:

a first reagent chamber configured to contain a first liquid and a plurality of cells, at least one of the cells comprising an enzyme;

a second reagent chamber, wherein the second chamber is configured to contain a reaction substrate;

a carrier fluid chamber configured to contain a second liquid immiscible with the first fluid, and in fluidic communication with at least the first reagent chamber;

a merging region configured to allow merging of the first and second liquids;

an incubation region in fluidic communication with the merging region, wherein the incubation region comprises:

a plurality of incubation chambers including a first incubation chamber and a second incubation chamber in fluidic wherein the first and second incubation chambers are configured to allow continuous flow of droplets between the chambers;

a droplet-sorting region in fluidic communication with the incubation region, wherein the droplet-sorting region comprises:

a detection area,

a set of electrodes downstream from the detection area configured to sort the plurality of droplets based on detection of a component in the droplet,

~~a collection channel,~~

~~and a waste channel; and~~

~~a bubble trap positioned between the incubation region and the droplet-sorting region.~~

~~6. The fluidic system of the preceding claim, further comprising a second carrier fluid chamber within the droplet-sorting region.~~

~~7. A fluidic system, comprising:~~

~~a first reagent chamber configured to contain a first liquid and a plurality of cells, at least one of the cells comprising an enzyme;~~

~~a second reagent chamber, wherein the second chamber is configured to contain a reaction substrate;~~

~~a carrier fluid chamber configured to contain a second liquid immiscible with the first fluid, and in fluidic communication with at least the first reagent chamber;~~

~~a merging region configured to allow merging of the first and second liquids;~~

~~an incubation region in fluidic communication with the merging region, wherein the incubation region comprises a plurality of incubation chambers including a first incubation chamber and a second incubation chamber;~~

~~a detection area comprising a first detector, wherein the first detector is configured to determine a first luminescent signal of a first wavelength and a second luminescent signal of a second wavelength, wherein the first wavelength and the second wavelength are different;~~

~~a droplet-sorting region in fluidic communication with the incubation region;~~

~~a set of electrodes downstream from the detection area configured to sort the plurality of droplets based on detection of a component or components in the droplet;~~

~~a collection channel; and~~

~~a waste channel.~~

~~8. The fluidic system of the preceding claim, further comprising a second detector positioned upstream the first detector, optionally wherein the second detector is configured to receive a first luminescent signal of a first wavelength and a second luminescent signal of a second wavelength, wherein the first wavelength and the second wavelength are different.~~

39. The method of any one of the preceding claims, further comprising screening the enzyme for the one or more characteristics based on the level of emitted signal detected compared to a reference signal, wherein a change in the level of emitted signal compared to the reference signal indicates that the enzyme has the one or more characteristics.

410. The method of any one of the preceding claims, further comprising isolating the droplets based on the level of emitted signal detected compared to a reference signal.

~~11. The method of any one of the preceding claims, further comprising sorting the droplets based on the level of emitted signal detected compared to a reference signal.~~

512. The method of any one of the preceding claims, further comprising culturing cells within the plurality of droplets.

~~13. The method of the preceding claim, wherein culturing comprises proliferating cells within the plurality of droplets.~~

614. The method of any one of the preceding claims, further comprising lysing one or more cells.

715. The method of any one of the preceding claims, wherein detecting comprises detecting intracellular or cell surface-displayed enzymes.

816. The method of any one of the preceding claims, wherein detecting comprises detecting extracellular enzymes.

~~17. The method of any one of the preceding claims, wherein a lower signal compared to a reference signal is detected in the absence of NADH.~~

~~18. The method of any one of the preceding claims, wherein a fluorescence signal with an excitation wavelength of 590 nm and an emission wavelength of 620 nm is emitted in the presence of NADH.~~

9~~19~~. The method of any one of the preceding claims, wherein the enzyme is a redox cofactor-dependent enzyme and the signal is emitted when the redox cofactor is oxidized or reduced when the reaction substrate is converted to the target product by the enzyme.

10~~20~~. The method of any one of the preceding claims, wherein the enzyme is a redox cofactor-independent enzyme and the signal is emitted when the redox cofactor is oxidized or reduced when the target product is further converted to a subsequent product by the one or more detection reagents.

11~~21~~. The method of any one of the preceding claims, wherein generation of the plurality of droplets in step a) further comprises incubating the host cell in each droplet with culture media in the droplet to increase the number of host cells prior to adding the reaction substrate, detection reagents, one or more detection reagents, lysis buffer, redox cofactor or combinations thereof.

12~~22~~. The method of ~~claim~~ any one of the preceding claims, wherein the droplets are isolated by using a method selected from the group consisting of fluorescence-activated droplet sorting (FADS), fluorescence-activated cell sorting (FACS) and magnetic-activated cell sorting (MACS), acoustic control techniques, magnetic control techniques, pneumatic control techniques, thermal control techniques, electric control techniques and combinations thereof.

13~~23~~. The method of any one of the preceding claims, wherein the one or more characteristics of the enzyme is selected from the group consisting of enzyme activity, stability, specificity, stereoselectivity, ability to convert a non-natural substrate and combinations thereof.

14~~24~~. The method of any one of the preceding claims, further comprising the step of sequencing the amino acid sequence, the polynucleotide sequence, or both, of the enzyme in the isolated droplets, optionally wherein the sequenced enzyme is used to generate a library of enzyme variants.

15~~25~~. The method of any one of the preceding claims, wherein the enzyme is expressed in a host cell, and wherein the plurality of droplets further comprises the host cell or fragments thereof.

~~26. The method of any one of the preceding claims, further comprising detecting a luminescent signal upstream the sorting region, optionally comparing the first luminescent signal with the signal emitted from the droplet.~~

~~27. The method of any one of the preceding claims, further comprising detecting a first and second luminescent signal, optionally comprising comparing the first and second luminescent signal or a derived signal of the first and second luminescent signal to the signal emitted from the droplet.~~

~~28. The method of any one of the preceding claims, wherein a signal generated upstream a sorting region is transmitted to the sorting region.~~

~~29. The method of any one of the preceding claims, further comprising applying a voltage to a set of sorting electrodes.~~

16~~30~~. Use of the method of any one of the preceding claims for screening an enzyme for increasing conversion of its natural substrate, increasing conversion of its non-natural substrate, increasing catalytic activity, increasing stereoselectivity, increasing thermal stability, increasing stability in a predetermined pH range, or a combination thereof.

17~~31~~. The ~~system or~~ method of any one of the preceding claims, wherein the one or more redox cofactors is selected from the group consisting of NAD^+, NADH, $NADP^+$, NADPH, NAD^+ and $NADP^+$, and NADH and NADPH.

18~~32~~. The ~~system or~~ method of any one of the preceding claims, wherein each droplet of the plurality of droplets further comprises one or more detection reagents that react with the oxidized or reduced redox cofactor to produce the emitted signal.

~~33. The system or method of claim 32, wherein the redox cofactor is NADH or NADPH or both, and wherein the one or more detection reagents is selected from the group consisting of:~~

~~i) resazurin and diaphorase;~~

~~ii) proluciferin, luciferase, a reductase, ATP and Mg^{2+}; and~~

~~iii) a quantum dot.~~

~~34. The system or method of claim 32, wherein the redox cofactor is NAD^+ or $NADP^+$ or both, and wherein the one or more detection reagents is selected from the group consisting of:~~

~~i) a quantum dot; and~~

~~ii) a protein biosensor.~~

~~35. The system or method of any one of claims 33-34, wherein the quantum dot is a CdSe/CdS/CdZnS/ZnS quantum dot.~~

~~36. The system or method of any one of claims 33-35, wherein the quantum dot is a CdSe/CdS/CdZnS/ZnS quantum dot coated with carboxylic acid and bovine serum albumin and Nile blue dye.~~

~~37. The system or method of any one of claims 33-36 wherein the quantum dot is water soluble.~~

~~38. The system or method of any one of claims 33-37, wherein the quantum dot is a CdSe/CdS/CdZnS/ZnS quantum dot coated with 3-mercaptopropionic acid and linked to a NAD+ binding domain.~~

~~39. The system or method of claim 38 wherein the NAD^+ binding domain is a thiolated DBC1 protein sensor.~~

~~40. The system or method of any one of claims 38-39, wherein compared to a reference signal, a lower fluorescence signal with excitation wavelength of 538 nm and emission wavelength of 595 nm is emitted in the presence of NAD^+.~~

~~41. The system or method of any one of the preceding claims, wherein the enzyme is a redox cofactor-dependent enzyme or a redox cofactor-independent enzyme.~~

1942. The ~~system or~~ method of any one of the preceding claims, wherein the host cell is selected from the group consisting of a mammalian cell, a plant cell, a bacterial cell, a fungal cell, a yeast cell, a protozoan cell, an algal cell and an archaeal cell.

~~43. The system or method of any one of the preceding claims, wherein the at least one or more of the plurality of droplets further comprises a lysis buffer.~~

~~44. The system or method of any one of the preceding claims, wherein the plurality of droplets are selected from the group consisting of water-in-oil droplets, water-in-oil-in-water droplets, oil-in-water droplets and hydrogel droplets.~~

~~45. The system or method of any one of the preceding claims, wherein the plurality of droplets have a diameter of between about 10 µm and 100 µm.~~

~~46. The system or method of any one of the preceding claims, wherein the plurality of droplets have a diameter of about 30 µm.~~

~~47. The system or method of any one of the preceding claims, wherein the enzyme is selected from the group consisting of an intracellular enzyme, a surface displayed enzyme, or an extracellular enzyme.~~

~~48. The system or method of any one of the preceding claims, wherein the enzyme is selected from the group consisting of secondary alcohol dehydrogenase from *Candida parapsilosis* (CpSADH), epoxide hydrolase from *Sphingomonas sp*. HXN-200 (SpEH), styrene oxide isomerase (SOI), P450 monooxygenase (P450pyr), amine dehydrogenase from *Rodococcus* (AmDH), and butanediol dehydrogenase (BDHA).~~

2049. The ~~system or~~ method of any one of the preceding claims, wherein the enzyme is an enzyme variant selected from a library of enzyme variants of a single enzyme, or wherein the enzyme is selected from a group of different enzymes.

~~50. The system or method of any one of the preceding claims, wherein the cofactor is an exogenous cofactor.~~

~~51. The system or method of any one of the preceding claims, further comprising one or more droplets each containing at least one cell comprising an endogenous cofactor concentration, and wherein an exogenous cofactor concentration is greater than an endogenous cofactor concentration.~~

~~52. The system or method of any one of the preceding claims, further comprising a detector positioned upstream of a sorting region.~~

~~53. The system or method of any one of the preceding claims, further comprising a bubble trap, the bubble trap optionally comprising a vacuum port.~~

21. A fluidic system, comprising:

a first reagent chamber configured to contain a first liquid and a plurality of cells, at least one of the cells comprising an enzyme;

a second reagent chamber, wherein the second chamber is configured to contain a reaction substrate;

a carrier fluid chamber configured to contain a second liquid immiscible with the first fluid, and in fluidic communication with at least the first reagent chamber;

a merging region configured to allow merging of the first and second liquids;

an incubation region in fluidic communication with the merging region, wherein the incubation region comprises:

a plurality of incubation chambers including a first incubation chamber and a second incubation chamber in fluidic wherein the first and second incubation chambers are configured to allow continuous flow of droplets between the chambers;

a droplet-sorting region in fluidic communication with the incubation region, wherein the droplet-sorting region comprises:

a detection area,

a set of electrodes downstream from the detection area configured to sort the plurality of droplets based on detection of a component in the droplet,

a collection channel,

and a waste channel; and

a bubble trap positioned between the incubation region and the droplet-sorting region.