subsequent screening of enzyme variants, have been used to fine-tune enzymes for practical applications.

The traditional microtiter plate-based enzyme screening approach suffers from low throughput and prolonged development times. The use of microfluidic-based platforms has enabled high-throughput enzyme screening to speed up enzyme development via directed evolution. However, the majority of current state-of-the-art microfluidics-based technologies rely on labeled reaction substrates, for example fluorophore-labeled substrates, for the generation of the fluorescence signal in the enzyme screening process. Accordingly, improved systems and methods are needed.

SUMMARY

Systems and methods for screening enzymes are generally described. The enzymatic activity may depend on the presence of a cofactor or may be independent of the presence of the cofactor. The subject matter of the present disclosure involves, in some cases, interrelated products, alternative solutions to a particular problem, and/or a plurality of different uses of one or more systems and/or articles.

In one example, a method of screening an enzyme for one or more characteristics is described, 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.

In another example, a method of screening an enzyme for one or more characteristics is described, the method 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.

In another example, a fluidic system, comprising, the 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.

In another example, a fluidic system is described, the 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.

In yet another example, a fluidic system is described, the 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. In some examples, the fluidic

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

In another example still, a fluidic system is described, the 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.

In another example, there is provided 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 or more of the plurality of droplets comprises: an enzyme, a reaction substrate; and one or more redox cofactors, or one or more redox cofactors and one or more detection enzymes, wherein the enzyme converts the reaction substrate to a target product; (b) detecting a signal emitted from each droplet, wherein the signal is a fluorescence signal, a bioluminescence signal or a chemiluminescence signal, and (i) wherein the signal is emitted when the one or more redox cofactors 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 one or more redox cofactors is oxidized or reduced when the target product is further converted to a subsequent product by the one or more detection enzymes in the droplet; (c) 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; and (d) isolating the droplets based on the level of emitted signal detected compared to the reference signal.

In another example, provided herein is a 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.

In one aspect, the present disclosure provides a method of screening a target enzyme for one or more characteristics, the method comprising the steps of:

(a) generating a plurality of droplets, wherein at least one droplet comprises: the target enzyme, a reaction substrate, one or more redox cofactors, and one or more detection reagents comprising a detection enzyme, wherein the target enzyme is configured to convert the reaction substrate to a target product, and wherein the target enzyme is a redox cofactor-independent enzyme; and

(b) detecting a signal emitted from the droplet, wherein the signal is a fluorescence signal, a bioluminescence signal or a chemiluminescence signal, wherein the signal is emitted when the target product is further converted to a subsequent product by the one or more redox cofactors and the one or more detection reagents in the droplet.

In another aspect, the present disclosure provides 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 a target enzyme, a reaction substrate, one or more redox cofactors and one or more detection reagents comprising a detection enzyme, wherein the target enzyme converts the reaction substrate to a target product, and wherein the target enzyme is a redox cofactor-independent enzyme;

reacting the target enzyme and the reaction substrate; and

generating a signal from the droplet when the target product is further converted to a subsequent product by the one or more redox cofactors and the one or more detection reagents, wherein the signal is a fluorescence signal, a bioluminescence signal or a chemiluminescence signal.

Other advantages and novel features of the present disclosure will become apparent from the following detailed description of various non-limiting embodiments of the invention when considered in conjunction with the accompanying figures. In cases where the present specification and a document incorporated by reference include conflicting and/or inconsistent disclosure, the present specification shall control.

BRIEF DESCRIPTION OF THE DRAWINGS

Non-limiting embodiments of the present invention will be described by way of example with reference to the accompanying figures, which are schematic and are not intended to be drawn to scale. In the figures, each identical or nearly identical component illustrated is typically represented by a single numeral. For purposes of clarity, not every component is labeled in every figure, nor is every component of each embodiment of the invention shown where illustration is not necessary to allow those of ordinary skill in the art to understand the invention. In the figures:

FIGS. 1A-1B show cross-sectional schematic side views of a droplet containing a cell containing a target enzyme merging with reagents, according to some embodiments;

FIGS. 1C-1E show cross-sectional schematic side views illustrating the generation of a luminescent signal in a screening using a detection reagent, according to some embodiments;

FIG. 1F shows a cross-sectional schematic side view illustrating the generation of a luminescent signal in a droplet screening without the use of a detection reagent, according to some embodiments;

FIG. 2A shows a schematic illustration of a microfluidic device for screening enzymes, according to some embodiments;

CLAIMS

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

(a) generating a plurality of droplets, wherein at least one droplet comprises:

the target enzyme, a reaction substrate, one or more redox cofactors, and one or more detection reagents comprising a detection enzyme, wherein the target enzyme is configured to convert the reaction substrate to a target product, and wherein the target enzyme is a redox cofactor-independent enzyme; and

(b) detecting a signal emitted from the droplet, wherein the signal is a fluorescence signal, a bioluminescence signal or a chemiluminescence signal, wherein the signal is emitted when the target product is further converted to a subsequent product by the one or more redox cofactors and 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 a target enzyme, a reaction substrate, one or more redox cofactors and one or more detection reagents comprising a detection enzyme, wherein the target enzyme converts the reaction substrate to a target product, and wherein the target enzyme is a redox cofactor-independent enzyme;

reacting the target enzyme and the reaction substrate; and

generating a signal from the droplet when the target product is further converted to a subsequent product by the one or more redox cofactors and the one or more detection reagents, wherein the signal is a fluorescence signal, a bioluminescence signal or a chemiluminescence signal.

3. The method of claim 1 or 2, further comprising screening the target 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.

4. The method of claim 1 or 2, further comprising isolating the droplets based on the level of emitted signal detected compared to a reference signal.

5. The method of claim 1, further comprising culturing cells within the plurality of droplets.

6. The method of claim 2 or 5, further comprising lysing one or more cells.

7. The method of claim 2 or 5, wherein detecting comprises detecting intracellular or cell surface-displayed enzymes.

8. The method of claim 2 or 5, wherein detecting comprises detecting extracellular enzymes.

9. The method of claim 1, 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, redox cofactor or combinations thereof.

10. The method of claim 1 or 2, 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 combinations thereof.

11. The method of claim 1 or 2, 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.

12. The method of claim 1 or 2, wherein the target enzyme is a protein molecule, a nucleic acid molecule or a polynucleotide molecule, 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.

13. The method of claim 1 or 2, wherein the enzyme is expressed in a host cell, and wherein the plurality of droplets further comprises the host cell.

14. Use of the method of claim 1 or 2 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.

15. The method of claim 1 or 2, 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.

16. The method of claim 1 or 2, 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.

17. The method of claim 1 or 2, 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.

18. The method of claim 1 or 2, 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.