SUMMARY

[0006] In one aspect, there is provided a cell-based method of identifying one or more enzyme variants that produces an increased level of a ketone as compared to a reference enzyme comprising the steps of:

(a) culturing one or more cells transformed with an expression vector comprising a polynucleotide sequence encoding one or more enzyme variants;

(b) incubating the one or more cells with para-methoxy-2-amino benzamidoxime (PMA);

(c) measuring the level of fluorescence signal emitted by the one or more cells;

(d) comparing the level of fluorescence signal from step c) with a fluorescence signal measured in a cell transformed with an expression vector comprising a polynucleotide sequence encoding the reference enzyme; and

(e) identifying the one or more enzyme variants when the level of fluorescence signal measured in the cell is increased compared to the reference enzyme.

[0006a] In another aspect, provided herein is a cell-based method of identifying one or more enzyme variants that produces an increased level of a ketone as compared to a reference enzyme comprising the steps of:

(a) culturing, in a culture medium, one or more cells transformed with an expression vector comprising a polynucleotide sequence encoding one or more enzyme variants;

(b) incubating the one or more cells with para-methoxy-2-amino benzamidoxime (PMA);

(c) measuring the level of fluorescence signal emitted by the one or more cells;

(d) comparing the level of fluorescence signal from step c) with a fluorescence signal measured in a cell transformed with an expression vector comprising a polynucleotide sequence encoding the reference enzyme; and

(e) identifying the one or more enzyme variants when the level of fluorescence signal measured in the cell is increased compared to the reference enzyme.

[**0006b]** In another aspect, provided herein is a method for identifying one or more enzyme variants that produces an increased level of a ketone as compared to a reference enzyme comprising:

(a) purifying and crystalizing the reference enzyme;

(b) identifying one or more active binding sites and/or one or more sites involved in accessibility to the one or more active binding sites;

(c) introducing one or more amino acid mutations at the active binding sites and/or accessibility sites to generate the one or more enzyme variants; and

(d) subjecting the one or more enzyme variants from step c) to the cell-based method described herein to identify the one or more enzyme variants that produces the increased level of ketone.

[0007] In another aspect, there is provided a system for identifying one or more enzyme variants that produces an increased level of a ketone as compared to a reference enzyme comprising:

(a) purifying and crystalizing the reference enzyme;

(b) identifying one or more active binding sites and/or one or more sites involved in accessibility to the one or more active binding sites;

(c) introducing one or more amino acid mutations at the active binding sites and/or accessibility sites to generate the one or more enzyme variants;

(d) subjecting the one or more enzyme variants from step c) to the cell-based method described herein to identify the one or more enzyme variants that produces the increased level of ketone.

[0008] In yet another aspect, there is provided a genetically engineered enzyme variant identified using the system described herein.

[0009] In yet another aspect, there is provided a genetically engineered CCD1 enzyme comprising one or more mutations, wherein the one or more mutations is a substitution at the one or more amino acid positions selected from the group consisting of A191, A400, C354, E408, F303, F530, H241, I171, V179, L356, L423, N339, N377, Q309, Q415, R405, S189, S220, S240, T173, T242, T411, T412, T421, V227, V401 and V429 of a wild type OfCCD1 (SEQ ID NO: 1).