protease recognition sequence. Prior to *in vitro* cell-free production of a compound, the cognate protease (*i.e.*, a protease that specifically recognizes and cleaves the protease recognition sequence in the recombinant protein) is introduced to, or activated in, the cell-free system such that it can cleave the recombinant enzyme, thereby inactivating it. Also provided herein are methods of producing and screening for recombinant enzymes that can be inactivated selectively and that retain an activity level comparable to that of the wild-type enzymes.

[0004] In one aspect, the present invention refers to recombinant protein comprising a polypeptide derived from SEQ ID NO: 17, said polypeptide comprising a human rhinovirus 3C protease recognition sequence located between amino acids 526 and 527 of SEQ ID NO: 17 wherein, said polypeptide has at least 95% identity with SEQ ID NO: 25 and has phosphoglucose isomerase activity. In some examples, provided herein are recombinant phosphoglucose isomerase (Pgi) proteins with at least one (or one) protease recognition sequence located between two amino acids (*e.g.*, two adjacent amino acids) of the native protein. In other examples, nucleic acids encoding the recombinant phosphoglucose isomerase proteins are provided.

[0005] In some examples, the Pgi protein comprises the sequence of SEQ ID NO:17. In certain examples, the Pgi protein may comprise a sequence that is 90%, 95%, 98%, or 99% homologous to SEQ ID NO:25. In some examples, the nucleic acid encoding the Pgi protein comprises the sequence of SEQ ID NO:1 In certain examples, the nucleic acid encoding the Pgi protein may comprise a sequence that is 90%, 95%, 98%, or 99% homologous to SEQ ID NO:9.

[0006] In some examples, provided herein are recombinant phosphotransacetylase (Pta) proteins with at least one (or one) protease recognition sequence located between two amino acids (*e.g.*, two adjacent amino acids) of the native protein. In other examples, nucleic acids encoding the recombinant phosphotransacetylase proteins are provided.

[0007] In some examples, the Pta protein comprises the sequence of SEQ ID NO:48. In certain examples, the Pta protein may comprise a sequence that is 90%, 95%, 98%, or 99% homologous to SEQ ID NO:48. In some examples, the nucleic acid encoding the Pta protein comprises the sequence of SEQ ID NO:47. In certain examples, the nucleic acid encoding the Pta protein may comprise a sequence that is 90%, 95%, 98%, or 99% homologous to SEQ ID NO:47.

[0008] In some examples, provided herein are recombinant transketolase A (TktA) proteins with at least one (or one) protease recognition sequence located between two amino acids (*e.g.*, two adjacent amino acids) of the native protein. In other examples, nucleic acids encoding the recombinant transketolase A proteins are provided.

CLAIMS

1. A recombinant protein comprising a polypeptide derived from SEQ ID NO: 17, said polypeptide comprising a human rhinovirus 3C protease recognition sequence located between amino acids 526 and 527 of SEQ ID NO: 17 wherein, said polypeptide has at least 95% identity with SEQ ID NO: 25 and has phosphoglucose isomerase activity.

2. The recombinant protein of claim 1, wherein the protease recognition sequence comprises the amino acid sequence of SEQ ID NO:38.

3. The recombinant protein of claim 1, wherein the recombinant protein comprises the amino acid sequence of SEQ ID NO:25.

4. The recombinant protein of claim 1, wherein the protease recognition sequence comprises the amino acid sequence of SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, or SEQ ID NO:45.