Therefore, there exists not only a need for improved delivery systems, but also means for producing such systems which find compatibility with molecules (payloads) having a range of sizes and molecular properties.

The present invention solves one or more of the above-mentioned problems.

**SUMMARY**

In one aspect, the present disclosure refers to the use of a leader sequence of a *Photorhabdus* Virulence Cassettes (PVC) effector, for packaging a payload into a PVC Needle Complex; wherein the payload is one or more selected from a polypeptide, a nucleic acid, or a combination thereof; and wherein the leader peptide and the payload form an effector fusion that is distinct from a wild-type PVC effector protein. In another aspect, the present disclosure refers to a method for manufacturing a PVC Needle Complex comprising a payload, the method comprising: a. contacting a PVC Needle Complex with an effector fusion comprising a leader peptide of a PVC effector fused to a payload; b. wherein said payload is one or more selected from a polypeptide, a nucleic acid or a combination thereof; and c. wherein the effector fusion is distinct from a wild-type PVC effector protein.

In another aspect, the present disclosure refers to an *in vitro* and/or *ex vivo* method for delivering a payload into a cell, the method comprising: a. contacting a cell with a PVC Needle Complex comprising an effector fusion; b. wherein the effector fusion comprises a leader peptide of a PVC effector fused to a payload; c. wherein said payload is one or more selected from a polypeptide, a nucleic acid or a combination thereof; and d. wherein the effector fusion is distinct from a wild-type PVC effector protein. In another aspect, the present disclosure refers to a method for suppressing a pest, the method comprising: a. contacting a pest, or a target area comprising a pest, with a PVC Needle Complex comprising an effector fusion; b. wherein the effector fusion comprises a leader peptide of a PVC effector fused to a payload; c. wherein said payload is one or more selected from a polypeptide, a nucleic acid or a combination thereof; and d. wherein the effector fusion is distinct from a wild-type PVC effector protein.

In another aspect, the present disclosure refers to a PVC Needle Complex, for use in therapy; a. wherein the PVC Needle Complex comprises an effector fusion which comprises a leader peptide of a PVC effector fused to a payload; b. wherein said payload is one or more selected from a polypeptide, a nucleic acid, or a combination thereof; and c. wherein the effector fusion is distinct from a wild-type PVC effector protein. In another aspect, the present disclosure refers to a PVC Needle Complex comprising an effector fusion; a. wherein said effector fusion comprises a PVC effector leader sequence fused to a payload; b. wherein said payload is one or more selected from a polypeptide, a nucleic acid or a combination thereof; and c. wherein the effector fusion is distinct from a wild-type PVC effector protein. In another aspect, the present disclosure refers to an effector fusion, comprising a leader peptide of a PVC effector fused to a payload; a. wherein said payload is one or more selected from a polypeptide, a nucleic acid or a combination thereof; and b. wherein the effector fusion is distinct from a wild-type PVC effector protein; wherein the leader peptide comprises amino acid residues 1-50 or 2-50 of a PVC effector. In another aspect, the present disclosure refers to an isolated leader peptide a PVC effector.

In another aspect, the present disclosure refers to an expression vector comprising an isolated nucleic acid molecule which encodes an isolated leader peptide of a PVC effector, wherein the leader peptide comprises amino acid residues 1-50 or 2-50 of a PVC effector. In yet another aspect, the present disclosure refers to a host cell comprising an isolated nucleic acid molecule as disclosed herein, or an expression vector as disclosed herein.

**DETAILED DESCRIPTION**

The present invention is predicated on the surprising finding that toxigenic *Photorhabdus Virulence Cassettes* (PVC) effector proteins of *Photorhabdus* bacteria comprise a previously unknown "leader sequence" (or "leader peptide"), which functions to package (or "load") PVC effectors into a so called PVC Needle Complex(e.g. "nanosyringe"), which subsequently delivers the PVC effector to a target cell where it exerts its toxigenic effect(s) (the PVC effectors representing a payload of such nanosyringes). Moreover, the inventors have found that such leader sequences can be practically utilized to direct a payload linked thereto to be packaged into a PVC Needle Complex (and related/ homologous complexes), a well characterized molecular delivery system of *Photorhabdus*. Thus, the newly discovered leader sequence surprisingly functions to load the PVC Needle Complex with a molecular payload (or "warhead").

Further to this finding, the inventors have developed an advantageous, practical utility for such leader sequence for packaging/ loading 'heterologous' payloads (including non-*Photorhabdus* molecules) into PVC Needle Complexes, independent of the size, molecular properties or provenance of the heterologous payload.

In a first aspect the invention provides use of a *Photorhabdus Virulence Cassettes* (PVC) effector leader sequence, for packaging a payload into a PVC Needle Complex;

wherein the payload is one or more selected from a polypeptide, a nucleic acid, or a combination thereof (preferably a polypeptide); and

wherein the leader sequence and the payload form an effector fusion that is distinct from a wild-type PVC effector protein.

In one aspect, an aspect of the invention provides use of a PVC effector leader sequence, for packaging a payload into a PVC Needle Complex;

wherein the payload is one or more selected from a polypeptide, a nucleic acid, or a combination thereof (preferably a polypeptide); and

wherein the leader sequence and the payload form a fusion that is distinct from a PVC effector protein (e.g. wild-type PVC effector protein).

In other words, the invention provides in one aspect a method for packaging a payload into a PVC Needle Complex with a PVC effector leader sequence, comprising contacting an (effector) fusion with a PVC Needle Complex, wherein the payload is one or more selected from a polypeptide, a nucleic acid, or a combination thereof (preferably a polypeptide); and wherein the leader sequence and the payload form the (effector) fusion, that is distinct from a PVC effector protein (e.g. wild-type PVC effector protein).

The terms "fusion" and "effector fusion", in the context of a (effector) fusion formed by the leader sequence and the payload (and that is distinct from a wild-type PVC effector protein) are used interchangeably herein.

## CLAIMS

1. Use of a leader peptide of a *Photorhabdus* Virulence Cassettes (PVC) effector, for packaging a payload into a PVC Needle Complex;

wherein the payload is one or more selected from a polypeptide, a nucleic acid, or a combination thereof; and

wherein the leader peptide and the payload form an effector fusion that is distinct from a wild-type PVC effector protein.

2. The use according to claim 1, wherein the payload (of the effector fusion) is a therapeutic polypeptide, a cytotoxic polypeptide, or a nucleic acid-editing polypeptide.

3. The use according to claim 1 or claim 2, wherein the PVC Needle Complex and the effector fusion are expressed in a heterologous bacterial expression system, a yeast cell, an insect cell and/or a mammalian cell.

4. The use according to any one of the preceding claims, wherein:
   a. the leader peptide comprises amino acid residues 1-50 or 2-50 of a PVC effector;
   b. the leader peptide comprises an amino acid sequence having at least 60% sequence identity to one or more sequence selected sequence from SEQ ID NO.: 47 - SEQ ID NO.: 92;
   c. the PVC effector comprises an amino acid sequence of one or more sequence selected from SEQ ID NO.: 1 - SEQ ID NO.: 46;
   d. the PVC effector comprises a sequence selected from SEQ ID NO: 4, SEQ ID NO: 22, SEQ ID NO: 25, SEQ ID NO: 30, SEQ ID NO: 32 and SEQ ID NO: 46; and/or
   e. the leader peptide is covalently fused to the payload, preferably at an N-terminus of the payload.

5. A method for manufacturing a PVC Needle Complex comprising a payload, the method comprising:
   a. contacting a PVC Needle Complex with an effector fusion comprising a leader peptide of a PVC effector fused to a payload;
   b. wherein said payload is one or more selected from a polypeptide, a nucleic acid or a combination thereof; and
   c. wherein the effector fusion is distinct from a wild-type PVC effector protein.

6. The method according to claim 5, wherein said contacting occurs within a cell, in a cell lysate, or in a purified cell lysate.

7. An *in vitro* and/or *ex vivo* method for delivering a payload into a cell, the method comprising:
   a. contacting a cell with a PVC Needle Complex comprising an effector fusion;

b. wherein the effector fusion comprises a leader peptide of a PVC effector fused to a payload;
c. wherein said payload is one or more selected from a polypeptide, a nucleic acid or a combination thereof; and
d. wherein the effector fusion is distinct from a wild-type PVC effector protein.

8. A method for suppressing a pest, the method comprising:
a. contacting a pest, or a target area comprising a pest, with a PVC Needle Complex comprising an effector fusion;
b. wherein the effector fusion comprises a leader peptide of a PVC effector fused to a payload;
c. wherein said payload is one or more selected from a polypeptide, a nucleic acid or a combination thereof; and
d. wherein the effector fusion is distinct from a wild-type PVC effector protein.

9. A PVC Needle Complex, for use in therapy;
a. wherein the PVC Needle Complex comprises an effector fusion which comprises a leader peptide of a PVC effector fused to a payload;
b. wherein said payload is one or more selected from a polypeptide, a nucleic acid, or a combination thereof; and
c. wherein the effector fusion is distinct from a wild-type PVC effector protein.

10. A PVC Needle Complex comprising an effector fusion;
a. wherein said effector fusion comprises a leader peptide of a PVC effector fused to a payload;
b. wherein said payload is one or more selected from a polypeptide, a nucleic acid or a combination thereof; and
c. wherein the effector fusion is distinct from a wild-type PVC effector protein.

11. An effector fusion, comprising a leader peptide of a PVC effector fused to a payload;
a. wherein said payload is one or more selected from a polypeptide, a nucleic acid or a combination thereof; and
b. wherein the effector fusion is distinct from a wild-type PVC effector protein;
wherein the leader peptide comprises amino acid residues 1-50 or 2-50 of a PVC effector.

12. The method of any one of claims 5-8, the PVC Needle Complex of claim 10, or the effector fusion of claim 11, wherein the payload is a therapeutic polypeptide, a cytotoxic polypeptide, or a nucleic acid-editing polypeptide.

13. The method of any one of claims 5-8 or 12, the PVC Needle Complex of claim 10 or 12, or the effector fusion of claim 11 or 12, wherein the PVC Needle Complex and the effector fusion are expressed in a heterologous bacterial expression system, a yeast cell, an insect cell and/or a mammalian cell.

14. An isolated leader peptide of a PVC effector.

15. The method of any one of claims 5-8 or 12-13, the PVC Needle Complex for use of claim 9, the PVC Needle Complex of claim any one of claims 10, 12 or 13, the effector fusion of any one of claims 11-13, or the isolated leader peptide of a PVC effector of claim 14, wherein:

a. the leader peptide comprises amino acid residues 1-50 or 2-50 of a PVC effector;

b. the leader peptide comprises an amino acid sequence having at least 60% sequence identity to one or more sequence selected from SEQ ID NO.: 47 - SEQ ID NO.: 92;

c. the PVC effector comprises an amino acid sequence of one or more sequence selected from SEQ ID NO.: 1 - SEQ ID NO.: 46;

d. the PVC effector comprises a sequence selected from SEQ ID NO: 4, SEQ ID NO: 22, SEQ ID NO: 25, SEQ ID NO: 30, SEQ ID NO: 32 and SEQ ID NO: 46; and/or

e. the leader sequence is covalently fused to a payload.

16. An expression vector comprising an isolated nucleic acid molecule which encodes an isolated leader peptide of a PVC effector, wherein the leader peptide comprises amino acid residues 1-50 or 2-50 of a PVC effector.

17. A host cell comprising an expression vector of claim 16.

18. The host cell of claim 17, wherein said host cell is one or more a selected from a mammalian cell, an insect cell, a yeast cell, a bacterial cell, and/or a plant cell; preferably wherein said bacterial cell is an *E. coli* cell; or

wherein said host cell is a *Photorhabdus* cell; preferably

wherein said *Photorhabdus* cell comprises a *Photorhabdus* PVC operon operably linked to an inducible promoter.