Specifically, a cp caspase-2 comprising one or more of said amino acid substitutions, also referred to as “cp caspase-2 variant”, comprises improved P1’ tolerance compared to a cp caspase-2 not comprising said substitutions. Specifically, the cp caspase-2 variants provided herein comprise improved P1’ tolerance for at least one amino acid other than glycine.

Thus, in one aspect, the present disclosure provides a single-chain circular permuted caspase-2 (cp caspase-2) comprising the following structure from N- to C-terminus:

i. a small subunit of a caspase-2 comprising a sequence selected from the group consisting of SEQ ID No. 3, SEQ ID No. 91, SEQ ID No. 94, SEQ ID No. 97, SEQ ID No. 100, SEQ ID No. 103, SEQ ID No. 106, SEQ ID No. 109, SEQ ID No. 112, SEQ ID No. 115, SEQ ID No. 118, or a functionally active variant thereof comprising a sequence with at least 70% sequence identity to SEQ ID No. 3, SEQ ID No. 91, SEQ ID No. 94, SEQ ID No. 97, SEQ ID No. 100, SEQ ID No. 103, SEQ ID No. 106, SEQ ID No. 109, SEQ ID No. 112, SEQ ID No. 115, or SEQ ID No. 118; covalently linked, either via a linker or directly, to

ii. a large subunit of a caspase-2 comprising a sequence selected from the group consisting of SEQ ID No. 4, SEQ ID No. 90, SEQ ID No. 93, SEQ ID No. 96, SEQ ID No. 99, SEQ ID No. 102, SEQ ID No. 105, SEQ ID No. 108, SEQ ID No. 111, SEQ ID No. 114, SEQ ID No. 117, or a functionally active variant thereof comprising a sequence with at least 70% sequence identity to SEQ ID No. 4, SEQ ID No. 90, SEQ ID No. 93, SEQ ID No. 96, SEQ ID No. 99, SEQ ID No. 102, SEQ ID No. 105, SEQ ID No. 108, SEQ ID No. 111, SEQ ID No. 114, or SEQ ID No. 117,

wherein said cp caspase-2 is a protease and with the proviso that in case of a selection of SEQ ID No. 3 for the small subunit and SEQ ID No. 4 for the large subunit, then the cp caspase 2 comprises one or more amino acid substitutions, selected from

i. Gly171, substituted with D, or an amino acid selected from the group consisting of R, K, E, Q, N, A, S, T, P, H, Y

ii. Glu105, substituted with V, or an amino acid selected from the group consisting of C, L, I, M, F, W, R, K, D, Q, N

iii. Glu172, substituted with V, or an amino acid selected from the group consisting of C, L, I, M, F, W, R, K, D, Q, N

iv. Asp282, substituted with E, or T, or an amino acid selected from the group consisting of R, K, Q, N, G, A, S, P, H, Y

v. Val225, substituted with G, or an amino acid selected from the group consisting of A, S, T, P, H, Y, C, L, I, M, F, W

vi. Lys83, substituted with E, or an amino acid selected from the group consisting of R, D, Q, N,

vii. His185, substituted with A, or an amino acid selected from the group consisting of G, S, T, P, Y,

viii. Val255, substituted with M, or an amino acid selected from the group consisting of C, L, I, F, W, and/or

ix. Asp285, substituted with E, or Y, or an amino acid selected from the group consisting of R, K, Q, N, G, A, S, T, P, H,

with reference to the positions of SEQ ID No. 6, or positions functionally equivalent to positions of SEQ ID No. 6.

According to a further specific example, the cp caspase-2 provided herein comprises a propeptide of a small caspase-2 subunit (SS propeptide), fused to the N-terminus of the small subunit. Specifically, the SS propeptide comprises one or more amino acid substitutions at the C-terminus of the SS propeptide. Specifically, the SS propeptide of the cp caspase-2 described herein is modified to prevent cleavage at its C-terminus. Specifically, the SS propeptide comprises an amino acid substitution at position Asp^{14} of SEQ ID No. 2 or at a position functionally equivalent to Asp^{347} of SEQ ID No. 11, specifically Asp is substituted to Ala.

According to a specific example, the SS propeptide described herein comprises the amino acid sequence of SEQ ID No. 2, wherein X can be any amino acid except D or E, specifically it is A, or a variant thereof having 1, 2, 3, 4, or 5 point mutations or deletions. Specifically, said variant is a functionally active variant. Preferably, the SS propeptide sequence comprises the amino acid sequence of SEQ ID No. 2, wherein X is not D or E.

According to a further specific example, the cp caspase-2 provided herein comprises one or more linker sequences, specifically consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 or even more amino acid residues. Specifically, the linker can comprise more than 20 or 30 or even more amino acids, as long as the caspase retains its functional activity as described herein. Specifically, the linker sequence comprises glycine, alanine and/or serine residues. Specifically, the linker comprises at least one glycine and serine residue, more specifically the linker is GS, GSG, GGSGG, GSGSGSGS and/or GSAGSAAGSG.

Specifically, the cp caspase-2 comprises a subunit-linker sequence, which is a linker sequence between the small subunit and the large subunit of the cp caspase-2 described herein.

According to a further specific example, the cp caspase-2 provided herein comprises one or more C-terminal or N-terminal tags, specifically selected from the

Specifically, the caspase-2 variant provided herein comprises improved P1' tolerance for at least one amino acid other than glycine compared to a caspase-2 not comprising the respective amino acid substitution.

Specifically, the caspase-2 variant described herein comprises at least 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97 or 98 % sequence identity to SEQ ID No. 11, SEQ ID No. 89, SEQ ID No. 92, SEQ ID No. 95, SEQ ID No. 98, SEQ ID No. 101, SEQ ID No. 104, SEQ ID No. 107, SEQ ID No. 110, SEQ ID No. 113 or SEQ ID No. 116 and comprises one or more amino acid substitutions at positions 409, 431, 212, 213, 266, 296, 226, 323 or 326 of SEQ ID No. 11 or at a position functionally equivalent to any of positions 409, 431, 212, 213, 266, 296, 323 or 326 of SEQ ID No. 11 or a combination thereof.

Specifically, the caspase-2 described herein comprises at least a small caspase-2 subunit and a large caspase-2 subunit.

Specifically, the small subunit of the caspase-2 described herein comprises the amino acid sequence of SEQ ID No. 3, SEQ ID No. 91, SEQ ID No. 94, SEQ ID No. 97, SEQ ID No. 100, SEQ ID No. 103, SEQ ID No. 106, SEQ ID No. 109, SEQ ID No. 112, SEQ ID No. 115, SEQ ID No. 118, or a functionally active variant thereof comprising at least at least 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97 or 98 % sequence identity. Specifically, the large subunit of the caspase-2 described herein comprises the amino acid sequence of SEQ ID No. 4, SEQ ID No. 90, SEQ ID No. 93, SEQ ID No. 96, SEQ

Thus, in another aspect, the present disclosure provides a method of producing a circular permuted caspase-2 (cp caspase-2) as described herein comprising the steps of

i. cloning a nucleotide sequence encoding a cp caspase-2 under the control of a promoter into an expression vector,
ii. transforming a host cell with said vector,
iii. culturing the transformed host cell under conditions wherein the cp caspase-2 is expressed,
iv. optionally isolating the cp caspase-2 from the host cell culture, optionally by disintegrating the host cells, and
v. optionally purifying the cp caspase-2.

Specifically, the nucleic acid sequence encoding the caspase-2 described herein is operably linked to a promoter. Specifically, the promoter is an inducible or a constitutive promoter. Specifically, the promoter is selected from the group consisting of

ii. an expression vector, optionally comprising an affinity tag, preferably a 6His tag, a linker sequence, and/or a nucleotide sequence coding for a recognition site, preferably VDVAD.

The kit may optionally further comprise chromatography material for affinity chromatography, preferably an IMAC (immobilized metal affinity chromatography) material, preferably Ni-NTA (Ni- Nitrilotriacetic acid) chromatography material, preferably pre-packed in a chromatography column.

Further optionally comprised in the kit is a plasmid comprising the nucleotide sequence from 5´ to 3´ encoding an affinity tag, preferably a 6His tag, optionally a linker sequence and a nucleotide sequence coding for the recognition site, preferably VDVAD. Via a multiple cloning site, a DNA sequence encoding a POI can be inserted into the plasmid directly fused to the nucleotide sequence encoding the recognition site.

Further described herein is a pharmaceutical composition comprising the caspase-2 or cp caspase-2 provided herein and optionally one or more excipients.

Further described herein is use of the caspase-2 or cp caspase-2 provided herein for preparing a pharmaceutical composition.

Specifically, the caspase-2 or cp caspase-2 described herein is provided for use in the treatment of a disease. Specifically, the caspase-2 or cp caspase-2 described herein is provided for use in the treatment of cancer, osteoporosis, Alzheimer’s disease, Parkinson’s disease, inflammatory disease, or auto-immune diseases, specifically via proteolytically attacking respective disease relevant proteins.

Specifically, the caspase-2 or cp caspase-2 described herein is provided for the manufacture of a medicament for the treatment of cancer, Alzheimer’s disease, Parkinson’s disease or inflammatory disease.Further provided herein is a protein tag for enhanced expression of a POI, comprising a solubility enhancement tag and the amino acid sequence VDVAD (SEQ ID NO:45). Specifically, the sequence VDVAD is at the C-terminus of the protein tag described herein, specifically directly linked to the N-terminus of the POI.

CLAIMS

1. A single-chain circular permuted caspase-2 (cp caspase-2) comprising the following structure from N- to C-terminus:
    i. a small subunit of a caspase-2 comprising a sequence selected from the group consisting of SEQ ID No. 3, SEQ ID No. 91, SEQ ID No. 94, SEQ ID No. 97, SEQ ID No. 100, SEQ ID No. 103, SEQ ID No. 106, SEQ ID No. 109, SEQ ID No. 112, SEQ ID No. 115, SEQ ID No. 118, or a functionally active variant thereof comprising a sequence with at least 70% sequence identity to SEQ ID No. 3, SEQ ID No. 91, SEQ ID No. 94, SEQ ID No. 97, SEQ ID No. 100, SEQ ID No. 103, SEQ ID No. 106, SEQ ID No. 109, SEQ ID No. 112, SEQ ID No. 115, or SEQ ID No. 118; covalently linked, either via a linker or directly, to
    ii. a large subunit of a caspase-2 comprising a sequence selected from the group consisting of SEQ ID No. 4, SEQ ID No. 90, SEQ ID No. 93, SEQ ID No. 96, SEQ ID No. 99, SEQ ID No. 102, SEQ ID No. 105, SEQ ID No. 108, SEQ ID No. 111, SEQ ID No. 114, SEQ ID No. 117, or a functionally active variant thereof comprising a sequence with at least 70% sequence identity to SEQ ID No. 4, SEQ ID No. 90, SEQ ID No. 93, SEQ ID No. 96, SEQ ID No. 99, SEQ ID No. 102, SEQ ID No. 105, SEQ ID No. 108, SEQ ID No. 111, SEQ ID No. 114, or SEQ ID No. 117,

wherein said cp caspase-2 is a protease and with the proviso that in case of a selection of SEQ ID No. 3 for the small subunit and SEQ ID No. 4 for the large subunit, then the cp caspase 2 comprises one or more amino acid substitutions, selected from

i. Gly171, substituted with D, or an amino acid selected from the group consisting of R, K, E, Q, N, A, S, T, P, H, Y

ii. Glu105, substituted with V, or an amino acid selected from the group consisting of C, L, I, M, F, W, R, K, D, Q, N

iii. Glu172, substituted with V, or an amino acid selected from the group consisting of C, L, I, M, F, W, R, K, D, Q, N

iv. Asp282, substituted with E, or T, or an amino acid selected from the group consisting of R, K, Q, N, G, A, S, P, H, Y

v. Val225, substituted with G, or an amino acid selected from the group consisting of A, S, T, P, H, Y, C, L, I, M, F, W

vi. Lys83, substituted with E, or an amino acid selected from the group consisting of R, D, Q, N,

vii. His185, substituted with A, or an amino acid selected from the group consisting of G, S, T, P, Y,

viii. Val255, substituted with M, or an amino acid selected from the group consisting of C, L, I, F, W, and/or

ix. Asp285, substituted with E, or Y, or an amino acid selected from the group consisting of R, K, Q, N, G, A, S, T, P, H,

with reference to the positions of SEQ ID No. 6, or positions functionally equivalent to positions of SEQ ID No. 6.

2. The cp caspase-2 of claim 1, comprising a propeptide of a small caspase-2 subunit (SS propeptide), fused to the N-terminus of the small subunit, preferably wherein the SS propeptide comprises an amino acid substitution at position Asp^{14} of SEQ ID No. 2 or at a position functionally equivalent to Asp^{347} of SEQ ID No. 11, especially preferred wherein Asp is substituted to Ala.

3. The cp caspase-2 of any one of claims 1 or 2, wherein the linker consists of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 amino acid residues, preferably wherein the linker sequence comprises glycine and/or serine residues, more preferred the linker is GS, GGSGG, GSAGSAAGSG, $(GS)_n$, GSG or G_4S.

4. The cp caspase-2 of any one of claims 1 to 3, comprising one or more C-terminal or N-terminal tags selected from the group consisting of affinity tags, solubility enhancement tags and monitoring tags, preferably wherein the affinity tag is selected from the group consisting of poly-histidine tag, poly-arginine tag, peptide substrate for antibodies, chitin binding domain, RNAse S peptide, protein A, ß-galactosidase, FLAG tag, Strep II tag, streptavidin-binding peptide (SBP) tag, calmodulin-binding peptide (CBP), glutathione S-transferase (GST), maltose-binding protein (MBP), S-tag, HA tag, c-Myc tag, SUMO tag, E.coli thioredoxin, NusA, chitin binding domain CBD, chloramphenicol acetyl transferase CAT, LysRS, ubiquitin, calmodulin, and lambda gpV, preferably the tag is a His tag comprising one or more His, more preferably it is a hexahistidine tag, preferably wherein the solubility enhancement tag is selected from the group consisting of T7C, T7B, T7B1, T7B2, T7B3, T7B3, T7B4, T7B5, T7B6, T7B7,

T7B8, T7B9, T7B10, T7B11, T7B12, T7B13, T7A, T7A1, T7A2, T7A3, T7A4, T7A5, T3, N1, N2, N3, N4, N5, N6, N7, T7AC, calmodulin-binding peptide (CBP), DsbA, DsbC, poly Arg, poly Lys, G B1 domain, protein D, Z domain of Staphylococcal protein A, and thioredoxin, preferably wherein the monitoring tag is selected from the group consisting of m-Cherry, GFP and f-Actin.

5. The cp caspase-2 of claim 4, comprising more than one tags, preferably comprising an affinity tag and a solubility enhancement tag, optionally comprising a tag linker sequence linking two tags or linking a tag and the small subunit, the large subunit or the SS propeptide of the cp caspase-2.

6. The cp caspase-2 of claim 5, wherein the affinity tag is a hexahistidine tag and the solubility enhancement tag is a T7AC or a T7A3 tag.

7. The cp caspase-2 of any one of claims 1 to 6, wherein

i. the small subunit of a caspase-2 comprises

a) a first conserved region of the active center with at least 37.5 % amino acid sequence identity to SEQ ID No. 177 (1st consensus: AAMRNTKR) or 100% sequence identity to XXXRNTXX (SEQ ID No. 200), wherein X is any amino acid,

b) a second conserved region of the active center with at least 61.5 % amino acid sequence identity to SEQ ID No. 178 (2nd consensus: EGYAPGTEFHRCK) or 100% sequence identity to EGXXPGXXXHRCK (SEQ ID No. 194), wherein X is any amino acid, and

ii. the large subunit of a caspase-2 comprises

a) a third conserved region of the active center with at least 25.0 % amino acid sequence identity to SEQ ID No. 174 (3rd consensus: G-EKDLEFRSGGDVDH) or 100% sequence identity to X-XXXLXXRXGXXXDX (SEQ ID No. 195), wherein X is any amino acid,

b) a fourth conserved region of the active center with at least 53.3 % amino acid sequence identity to SEQ ID No. 175 (4th consensus: LLSHGVEGGXYGVDG) or 100% sequence identity to XXSHGXXGXXYGXDG (SEQ ID No. 196), wherein X is any amino acid, and

c) a fifth conserved region of the active center with at least 50.0 % amino acid sequence identity to SEQ ID No. 176 (5th consensus: QACRGDET) or 100% sequence identity to QACXGXXX (SEQ ID No. 197), wherein X is any amino acid.

8. The cp caspase-2 of any one of claims 1 to 7, comprising an amino acid sequence comprising at least 70% sequence identity to SEQ ID No. 9, 6, 14, 15, 16, 80, 88, 25, 26, 27, 28, 29, 30, 35, 39, 41, 64, 66, 68, 73, 74, 75, 76, 77, 81, 82, 83, 84, or 85.

9. The cp caspase-2 of any one of claims 1 to 8 comprising amino acid substitutions at positions of SEQ ID No. 6, or at positions functionally equivalent to positions of SEQ ID No. 6, selected from the group consisting of

i. His^{185} and Asp^{282}, preferably comprising H185A and D282T substitutions;
ii. Glu^{105} and Asp^{285}, preferably comprising E105V and D285E substitutions;
iii. Glu^{105}, Gly^{171}, Val^{225} and Asp^{282}, preferably comprising E105V, G171D, V225G and D282E substitutions;
iv. Glu^{105}, Gly^{171}, Val^{225}, Asp^{282} and Asp^{285}, preferably comprising E105V, G171D, V225G, D282E and D285E substitutions;
v. Lys^{83}, Glu^{105}, Glu^{172}, Val^{255} and Asp^{285}, preferably comprising K83E, E105V, E172V, V255M and D285Y substitutions;
vi. Glu^{105} and Gly^{171}, preferably comprising E105V and G171D substitutions;
vii. Glu^{105} and Glu^{172}, preferably comprising E105V and E172V substitutions; and
viii. Gly^{171} and Glu^{172}, preferably comprising G171D and E172V substitutions,

preferably wherein said cp caspase-2 comprises an SS propeptide comprising an amino acid substitution to Ala at position Asp^{14} of SEQ ID No. 2 or at a position functionally equivalent to position Asp^{347} of SEQ ID No. 11.

10. The cp caspase-2 of any one of claims 1 to 9, comprising SEQ ID No. 6 with one or more amino acid substitutions at position 171, 105, 172, 282, 225, 83, 185, or 255 of SEQ ID No. 6; or comprising SEQ ID No. 64, 66 or 68 with one or more amino

acid substitutions at a position functionally equivalent to position 171, 105, 172, 282, 225, 83, 185, or 255 of SEQ ID No. 6, or any combination thereof, preferably comprising any one or more of amino acid substitutions G171D, E105V, E172V, D282E, D282T, V225G, K83E, H185A, V255M and D285Y.

11. The cp caspase-2 of any one of claims 1 to 10, comprising an amino acid sequence selected from the group consisting of SEQ ID No. 1, 13, 17, 18, 23, 24, 51, 52, 54, 70, 71, 72, 78, 79, 86, 87, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191 and 192, or an amino acid sequence having at least 70% sequence identity with any one of SEQ ID No. 1, 13, 17, 18, 23, 24, 51, 52, 54, 70, 71, 72, 78, 79, 86, 87, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191 and 192.

12. The cp caspase-2 of any one of claims 1 to 11, comprising a C-terminal tag and an amino acid substitution at positions 285 and 292 of SEQ ID No. 6 or at a position functionally equivalent to positions 285 and 292 of SEQ ID No. 6, specifically comprising substitutions to Glu and Ser (D285E and D292S).

13. The cp caspase-2 of any one of claims 1 to 12, wherein said cp caspase-2 is recruited by a recognition site for proteolytic cleavage, comprising 5 amino acids of the sequence P5 P4 P3 P2 P1, wherein

P1 can be any amino acid, preferably it is D or E,
P2 can be any amino acid, preferably it is A,
P3 can be any amino acid, preferably it is V,
P4 can be any amino acid, preferably it is D, and
P5 can be any amino acid, preferably it is V.

14. A method of producing a circular permuted caspase-2 (cp caspase-2) of any one of claims 1 to 13 comprising the steps of

i. cloning a nucleotide sequence encoding a cp caspase-2 under the control of a promoter into an expression vector,
ii. transforming a host cell with said vector,
iii. culturing the transformed host cell under conditions wherein the cp caspase-2 is expressed,
iv. optionally isolating the cp caspase-2 from the host cell culture, optionally by disintegrating the host cells, and

v. optionally purifying the cp caspase-2.

15. The method of claim 14, wherein the promoter is selected from the group consisting of T7 promoter/operator, XylS/Pm regulator/promoter or variants of the Pm promoter, araBAD promoter/operator, T5, T7A1, T7A2, T7A3 promoter/operator, phoA promoter/regulator and the trp promoter/operator system.

16. The method of any one of claims 14 or 15, wherein the cp caspase-2 comprises a solubility enhancement tag, preferably it comprises a T7AC or a T7A3 tag.

17. The method of any one of claims 14 to 16, wherein the cp caspase-2 comprises an affinity tag, preferably a His tag, and even more preferably a 6-His tag.

18. The method of any one of claims 14 to 17, wherein the host cell is an *E. coli* cell.

19. The method of any one of claims 14 to 18, wherein culturing of step (iii) comprises a fed-batch phase for expression of the cp-caspase-2, said fed batch phase specifically cromprising a growth rate μ of about 0,01-0,1 h^{-1}, and induction of expression of the cp caspase-2 by addition of IPTG at a concentration of about 0,01 – 1,5 μmol/g actual CDM (cell dry mass).

20. The method of claim 19, wherein the growth rate μ is about 0,03-0,07 h^{-1}, preferably it is about 0,05-0,07 h^{-1} or 0,03-0,05 h^{-1}, preferably it is any of about 0,03, 0,05 or 0,07 h^{-1}.

21. The method of claim 19 or 20, wherein the IPTG concentration is about 0,5-1,3 μmol/g CDM, preferably it is about 0,5-0,9 μmol/g CDM or about 0,9-1,3 μmol/g CDM, preferably it is about 0,5, 0,9 or about 1,3 μmol/g CDM.

22. The method of any one of claims 19 to 21, wherein culturing of step (iii) further comprises a first fed-batch phase for the production of biomass, prior to the fed-batch phase for the expression of the cp caspase-2, said first fed-batch phase comprising a growth rate, μ of about 0,07-0,3 h^{-1}.

23. The method of claim 22, wherein the growth rate μ is about 0,1-0,2 h^{-1}, preferably about 0,13-0,21 h^{-1}, even more preferably about 0,16-0,18 h^{-1} and most preferably it is about 0,17 h^{-1}.

24. A method of producing a protein of interest (POI), comprising the steps of:

i. providing a fusion protein comprising from N- to C-terminus one or more tags, optionally one or more tag-linker sequences and a caspase recognition site N-terminally fused to the POI, wherein said caspase recognition site is specifically recognized by the cp caspase-2 of any one of claims 1 to 13,

ii. contacting said fusion protein with said cp caspase-2 for a period of time sufficient for said cp caspase-2 to cleave the fusion protein, and

iii. optionally purifying the POI.

25. The method of claim 24, comprising the steps of:

i. expressing the fusion protein comprising from N- to C-terminus optionally one or more tags, optionally one or more tag-linker sequences and a caspase recognition site N-terminally fused to the POI, wherein said caspase recognition site is specifically recognized by the cp caspase-2 of any one of claims 1 to 13; and the cp caspase-2 of any one of claims 1 to 13 specifically recognizing the recognition site of the fusion protein, in the same host cell,

ii. optionally, wherein said fusion protein and cp caspase-2 are under the same promoter,

iii. cultivating the host cell, wherein said cp caspase-2 cleaves the fusion protein in vivo in the cell, and

iv. optionally isolating the POI from the cell and optionally purifying the POI.

26. The method of claim 24 or 25, wherein the fusion protein comprises a caspase recognition site comprising 5 amino acids of the sequence P5 P4 P3 P2 P1, and a cleavage site P1/P1', wherein P1' is the N-terminal amino acid of the POI.

27. The method of claim 24 or 25, wherein the fusion protein and the cp caspase-2 are under transcriptional control of different promoters and wherein the expression of the cp caspase-2 is induced after expression of the fusion protein.

28. The method of any one of claims 24 to 27, wherein the fusion protein comprises the cp caspase-2 of any one of claims 1 to 13, specifically wherein the fusion

protein comprises said cp caspase-2 at its N- or C-terminus, and wherein the fusion protein comprises the following structure from N- to C-terminus:

i. one or more N-terminal tags,
ii. optionally one or more tag-linker sequences and
iii. a caspase recognition site comprising 5 amino acids of the sequence P5 P4 P3 P2 P1,
iv. a cleavage site P1/P1',
v. a POI, and

wherein P1' is the N-terminal amino acid of said POI and said cp caspase-2 specifically recognizes said recognition site.

29. The method of any one of claims 24 to 28, comprising the steps of:

i. expressing a fusion protein in a host cell comprising the following structure from N- to C-terminus:
   a. an N-terminal affinity tag,
   b. optionally a linker sequence,
   c. a caspase recognition site,
   d. a cleavage site P1/P1', and
   e. a POI,

   wherein P1' is the N-terminal amino acid of the POI, and wherein said recognition site is specifically recognized by the cp caspase-2 of any one of claims 1 to 13,
ii. isolating said fusion protein
iii. purifying said fusion protein using the N-terminal affinity tag,
iv. providing a cp caspase-2 of any one of claims 1 to 13, specifically recognizing the recognition site of the fusion protein,
v. contacting said fusion protein with said cp caspase-2 for a period of time sufficient for said cp caspase-2 to cleave the fusion protein,
vi. optionally removing the cleaved affinity tag, and optionally the non-cleaved fusion protein using the affinity tag and the cp caspase-2, and
vii. optionally further purifying the POI.

30. The method of claim 29, wherein the cp caspase-2 comprises at its N- or C-terminus an affinity tag identical to the affinity tag of the fusion protein and wherein the cp caspase-2 is removed in step vi. using said affinity tag.

31. The method of claim 29 or 30, comprising the steps of

i. expressing a fusion protein comprising one or more N-terminal affinity tags, optionally one or more tag-linker sequences, a caspase recognition site and a cleavage site P1/P1', wherein P1' is the N-terminal amino acid of the POI, and a POI, wherein said recognition site is specifically recognized by the cp caspase-2 of any one of claims 1 to 13, in a host cell, and

ii. isolating the fusion protein and binding / capturing the fusion protein on a solid support using the affinity tag,

iii. providing a cp caspase-2 of any one of claims 1 to 13, specifically recognizing the recognition site of the fusion protein,

iv. contacting said cp caspase-2 with the bound / captured fusion protein for a period of time sufficient for said cp caspase-2 to cleave the fusion protein,

v. releasing the POI from the solid support, and

vi. isolating and optionally further purifying the POI.

32. An isolated nucleotide sequence, preferably comprised in a vector or in an expression cassette, encoding the cp caspase-2 of any one of claims 1 to 13.

33. A host cell or a host cell line expressing the cp caspase-2 of any one of claims 1 to 13, wherein the host cells are selected from the group consisting of bacterial cells, yeast cells, insect cells, mammalian cells and plant cells, preferably the host cells are bacterial or yeast cells selected from the group consisting of *E. coli*, *Pseudomonas sp.*, *Bacillus sp., Streptomyces sp., Saccharomyces sp., Schizosaccharomyces sp.*, *Pichia sp., Kluyveromyces sp.* and *Hansenula sp..*

34. Use of the cp caspase-2 of any one of claims 1 to 13 for the *in vitro* cleavage of a substrate in a non-human organism, preferably wherein the non-human organism is a prokaryotic organism, especially preferred *E. coli*.

35. Use of the cp caspase-2 of any one of claims 1 to 13 for the production of a protein of interest (POI).