[0024] It should be understood that this invention is not limited to the particular methodology, protocols, material, reagents, and substances, etc., described herein. The terminologies used herein are for the purpose of describing particular embodiments only and are not intended to limit the scope of the present invention, which is defined solely by the claims/items.

[0025] All publications and patents cited throughout the text of this specification (including all patents, patent applications, scientific publications, manufacturer's specifications, instructions, etc.), whether supra or infra, are hereby incorporated by reference in their entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention. To the extent the material incorporated by reference contradicts or is inconsistent with this specification, the specification will supersede any such material.

Summary

[0025a] Aspects of the invention are defined in the independent claims. Some optional features of the invention are defined in the dependent claims.

[0026] The present invention is partly based on the surprising findings of polynucleotide sequences ("polynucleotides of the present invention") whose expression, preferably overexpression led to an increase in the yield of protein of interest (POI). This disclosure provides methods and materials useful for improving the yield of POI by engineering host cells such that they are capable of overexpressing one or more of the newly identified polynucleotide sequences to encode one or more helper proteins.

[0027] The term ""yield" refers to the amount of POI or model protein(s) as described herein, in particular SDZ-Fab (SEQ ID NO: 25 and 26) and HyHEL-Fab (SEQ ID NO: 29 and 30), respectively, which is, for example, harvested from the engineered host cell, and increased yields can be due to increased amounts of production or secretion of the POI by the host cell. Yield may be presented by mg POI/g biomass (measured as dry cell weight or wet cell weight) of a host cell. The term "titer" when used herein refers similarly to the amount of produced POI or model protein, presented as mg POI/L culture supernatant. An increase in yield can be determined when the yield obtained from an engineered host cell is compared to the yield obtained from a host cell prior to engineering, i.e., from a non-engineered host cell.

Preferably, “yield” when used herein in the context of a model protein as described herein, is determined as described in Example 5c. Accordingly, the “yield” when used herein in the context of a model protein as described herein is also referred to as “Fab yield” or “Fab titer”. A Fab titer is given as mg/L and a Fab yield as mg/g biomass (measured as dry cell weight or wet cell weight).

Briefly, *P. pastoris* strains CBS7435mut^S pPM2d_pAOX HyHEL and/or CBS7435mut^S pPM2d_pAOX SDZ (see Example 1 for their generation) which express the model protein HyHEL-Fab and SDZ-Fab, respectively, are engineered with a polynucleotide encoding a helper protein or functional homologue thereof as described herein. For co-overexpression, the gene encoding a helper protein is cloned under control of the *P. pastoris* GAP promoter and transformed into the Fab producing strains as described in example 4. For underexpression the gene encoding a KO target or its functional homologue is knocked out from the genome of the Fab producing strain (see example 6). Engineered cells are grown in YP-medium containing 10 g/L glycerol and 50 µg/mL Zeocin overnight at 25°C (see Example 5a). Aliquots of such a culture (corresponding to a final OD_{600} of 2.0) are transferred to synthetic medium M2 containing 20 g/L glucose and a glucose feed tablet (described in Example 5a) and incubated for 25 h at 25°C. Cultures are washed and resuspended in synthetic medium M2 and aliquots (corresponding to a final OD_{600} of 4.0) are transferred into synthetic medium M2 supplemented with 5 g/L methanol. Methanol (5g/L) is added 3 more times every 12 hours. After 48 h, cells are harvested by centrifugation. Biomass is determined by measuring the weight of the cell pellet derived from 1 mL cell suspension. The supernatant is used for quantification of SDZ-Fab or HyHEL-Fab, respectively, by ELISA (described in Example 5c). Specifically, an anti-human IgG antibody (e.g. ab7497, Abcam) is used as coating antibody and a e.g. goat anti-human anti-human IgG (Fab specific) antibody (e.g. Sigma A8542, alkaline phosphatase conjugated) is used as detection antibody. Commercial Human Fab/Kappa, IgG fragment is used as standard with a starting concentration of 100 ng/mL, supernatant samples are diluted accordingly. An increase in the yield may be determined based on a comparison of POI yield before and after the cell is engineered to overexpress the polypeptide. A standard test involving model proteins SDZ-Fab and/or HyHEL-Fab as shown in the example may be used to determine the yield difference.

[0028] In a first aspect, the present disclosure relates to one or more of newly discovered helper proteins and its or their use to increase POI yield. The present disclosure is based on, but not limited to, the helper protein HP1, HP2, HP3, HP4, HP5, HP6, HP7, HP8, HP9, or HP10 or functional homologues thereof. The meaning of functional homologue is defined in the latter part of the application. The amino acid sequences of the helper proteins

HP1 to HP9 are listed respectively in SEQ ID NO: 1-9 and 162, respectively. As used herein, such proteins are referred to in the present invention interchangeably in plural or singular forms, which however should be understood as in singular form unless expressly stated otherwise.

[0029] The present disclosure additionally relates to the polynucleotides encoding the helper proteins (hereinafter referred to as “polynucleotides of the present invention” or “a polynucleotide of the present invention”) and their individual or combined use to increase POI yield. The polynucleotide(s) can be introduced into a host cell or, if already existing in the cell, manipulated in a way such that they are overexpressed. The polynucleotide of the present disclosure encodes any one of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, or 162 or functional homologues thereof. Examples of the polynucleotide sequences are as set forth in SEQ ID NO: 13, 14, 15, 16, 17, 18, 19, 20, 21, or 163.

[0030] The present disclosure provides an isolated polynucleotide sequence comprising, consisting essentially of, or consisting of SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, or SEQ ID NO: 163 and optionally may be operably linked to a promoter which may be heterologous to said polynucleotide sequence. Furthermore, the present disclosure provides an isolated polynucleotide sequence which encodes a polypeptide sequence comprising any one of SEQ ID NO: 1-9 or 162, or functional homologues thereof. Preferably, the present disclosure provides an isolated polynucleotide sequence which encodes a polypeptide sequence comprising SEQ ID NO: 1 or functional homologues thereof. Preferably, the present disclosure provides an isolated polynucleotide sequence which encodes a polypeptide sequence comprising SEQ ID NO: 2 or functional homologues thereof. Preferably, the present disclosure provides an isolated polynucleotide sequence which encodes a polypeptide sequence comprising SEQ ID NO: 3 or functional homologues thereof. Preferably, the present disclosure provides an isolated polynucleotide sequence which encodes a polypeptide sequence comprising SEQ ID NO: 4 or functional homologues thereof. Preferably, the present disclosure provides an isolated polynucleotide sequence which encodes a polypeptide sequence comprising SEQ ID NO: 5 or functional homologues thereof. Preferably, the present disclosure provides an isolated polynucleotide sequence which encodes a polypeptide sequence comprising SEQ ID NO: 6 or functional homologues thereof. Preferably, the present disclosure provides an isolated polynucleotide sequence which encodes a polypeptide sequence comprising SEQ ID NO: 7 or functional homologues thereof. Preferably, the present disclosure provides an isolated polynucleotide sequence which encodes a polypeptide sequence comprising SEQ ID NO: 8 or functional homologues thereof. Preferably, the present disclosure provides an isolated polynucleotide sequence which

encodes a polypeptide sequence comprising SEQ ID NO: 9 or functional homologues thereof. Preferably, the present disclosure provides an isolated polynucleotide sequence which encodes a polypeptide sequence comprising SEQ ID NO: 162 or functional homologues thereof. In some embodiments, the present disclosure provides an isolated polynucleotide sequence having at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO: 13, 14, 15, 16, 17, 18, 19, 20, 21, or 163. More preferably, the isolated polynucleotide sequence has 100% sequence identity with SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, or SEQ ID NO: 163.

[0031] The present disclosure provides the use of a polynucleotide sequence according to the invention for integration into a host cell in its chromosome or in a plasmid, mini-plasmid, YAC, BAC, cosmid, or any other vector or the like. The polynucleotide sequence according to the disclosure may be introduced into a host cell, e.g., via transformation or transfection. Additionally, the disclosure provides the use of such polynucleotide sequence in the manufacturing of a protein of interest in a host cell.

[0032] Furthermore, the present disclosure provides an isolated polypeptide comprising the polypeptide sequence of any one of SEQ ID NO: 1-9 or 162, or functional homologues thereof. Preferably, the present disclosure provides an isolated polypeptide comprising the polypeptide sequence of SEQ ID NO: 1 or functional homologues thereof. Preferably, the present disclosure provides an isolated polypeptide comprising the polypeptide sequence of SEQ ID NO: 2 or functional homologues thereof. Preferably, the present disclosure provides an isolated polypeptide comprising the polypeptide sequence of SEQ ID NO: 3 or functional homologues thereof. Preferably, the present disclosure provides an isolated polypeptide comprising the polypeptide sequence of SEQ ID NO: 4 or functional homologues thereof. Preferably, the present disclosure provides an isolated polypeptide comprising the polypeptide sequence of SEQ ID NO: 5 or functional homologues thereof. Preferably, the present disclosure provides an isolated polypeptide comprising the polypeptide sequence of SEQ ID NO: 6 or functional homologues thereof. Preferably, the present disclosure provides an isolated polypeptide comprising the polypeptide sequence of SEQ ID NO: 7 or functional homologues thereof. Preferably, the present disclosure provides an isolated polypeptide comprising the polypeptide sequence of SEQ ID NO: 8 or functional homologues thereof. Preferably, the present disclosure provides an isolated polypeptide comprising the polypeptide sequence of SEQ ID NO: 9 or functional homologues thereof. Preferably, the

present disclosure provides an isolated polypeptide comprising the polypeptide sequence of SEQ ID NO: 162 or functional homologues thereof. In a further aspect, the present disclosure provides an isolated polypeptide with a polypeptide sequence having at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, or 162.

[0033] In a second aspect, the present disclosure provides a recombinant host cell for manufacturing a protein of interest, wherein the host cell is engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, or 162 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% sequence identity to an amino acid sequence as shown in SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, or 162.

[0034] Preferably, the present disclosure provides a recombinant host cell for manufacturing a protein of interest, wherein the host cell is engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 1 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100% sequence identity to an amino acid sequence as shown in SEQ ID NO: 1.

[0035] Preferably, the present disclosure provides a recombinant host cell for manufacturing a protein of interest, wherein the host cell is engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 2 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% sequence identity to an amino acid sequence as shown in SEQ ID NO: 2.

[0036] Preferably, the present disclosure provides a recombinant host cell for manufacturing a protein of interest, wherein the host cell is engineered to overexpress a

polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 3 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% sequence identity to an amino acid sequence as shown in SEQ ID NO: 3.

[0037] Preferably, the present disclosure provides a recombinant host cell for manufacturing a protein of interest, wherein the host cell is engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 4 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% sequence identity to an amino acid sequence as shown in SEQ ID NO: 4.

[0038] Preferably, the present disclosure provides a recombinant host cell for manufacturing a protein of interest, wherein the host cell is engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 5 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% sequence identity to an amino acid sequence as shown in SEQ ID NO: 5.

[0039] Preferably, the present disclosure provides a recombinant host cell for manufacturing a protein of interest, wherein the host cell is engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 6 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% sequence identity to an amino acid sequence as shown in SEQ ID NO: 6.

[0040] Preferably, the present disclosure provides a recombinant host cell for manufacturing a protein of interest, wherein the host cell is engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 7 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49,

50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% sequence identity to an amino acid sequence as shown in SEQ ID NO: 7.

[0041] Preferably, the present disclosure provides a recombinant host cell for manufacturing a protein of interest, wherein the host cell is engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 8 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% sequence identity to an amino acid sequence as shown in SEQ ID NO: 8.

[0042] Preferably, the present disclosure provides a recombinant host cell for manufacturing a protein of interest, wherein the host cell is engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 9 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% sequence identity to an amino acid sequence as shown in SEQ ID NO: 9.

[0043] Preferably, the present disclosure provides a recombinant host cell for manufacturing a protein of interest, wherein the host cell is engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 162 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% sequence identity to an amino acid sequence as shown in SEQ ID NO: 162.

[0044] In a preferred embodiment, the helper protein, preferably when overexpressed, may increase the yield of the model protein SDZ-Fab (SEQ ID NO: 25 for heavy chain and SEQ ID NO: 26 for light chain; Fig. 2) or HyHEL-Fab (SEQ ID NO: 29 for heavy chain and SEQ ID NO: 30 for light chain; Fig. 2) in the host cell by at least 1%, such as at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190 or at least 200%, compared to the host cell prior to being engineered to overexpress said helper protein. A host cell prior to engineering does not overexpress the helper protein of the present invention

and after engineering is able to overexpress the helper protein under suitable culturing conditions. It has been surprisingly found that exemplary recombinant cells described in the Examples were all able to increase the yield of the model protein SDZ-Fab or HyHEL-Fab by at least 20% (1.2 fold change). In some instances, the yield increased by 140%, as shown in Example 7.

[0045] Preferably, the present disclosure provides a recombinant host cell for manufacturing a protein of interest, wherein the host cell is engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 1 or a functional homologue thereof, wherein the functional homologue has at least 30% sequence identity to SEQ ID NO: 1, wherein the helper protein, preferably when overexpressed, may increase the production of the model protein SDZ-Fab or HyHEL-Fab in the host cell by at least 1%, such as at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190 or at least 200%, compared to the host cell prior to engineering.

[0046] Preferably, the present disclosure provides a recombinant host cell for manufacturing a protein of interest, wherein the host cell is engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 2 or a functional homologue thereof, wherein the functional homologue has at least 30% sequence identity to SEQ ID NO: 2, wherein the helper protein, preferably when overexpressed, may increase the production of the model protein SDZ-Fab or HyHEL-Fab in the host cell by at least 1%, such as at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190 or at least 200 or more %, compared to the host cell prior to engineering.

[0047] Preferably, the present disclosure provides a recombinant host cell for manufacturing a protein of interest, wherein the host cell is engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 3 or a functional homologue thereof, wherein the functional homologue has at least 30% sequence identity to SEQ ID NO: 3, wherein the helper protein, preferably when overexpressed, may increase the production of the model protein SDZ-Fab or HyHEL-Fab in the host cell by at least 1%, such as at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190 or at least 200% or more, compared to the host cell prior to engineering.

[0048] Preferably, the present disclosure provides a recombinant host cell for manufacturing a protein of interest, wherein the host cell is engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 4 or a functional homologue thereof, wherein the functional homologue has at least 30% sequence identity to SEQ ID NO: 4, wherein the helper protein, preferably when overexpressed, may increase the production of the model protein SDZ-Fab or HyHEL-Fab in the host cell by at least 1%, such as at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190 or at least 200% or more, compared to the host cell prior to engineering.

[0049] Preferably, the present disclosure provides a recombinant host cell for manufacturing a protein of interest, wherein the host cell is engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 5 or a functional homologue thereof, wherein the functional homologue has at least 30% sequence identity to SEQ ID NO: 5, wherein the helper protein, preferably when overexpressed, may increase the production of the model protein SDZ-Fab or HyHEL-Fab in the host cell by at least 1%, such as at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190 or at least 200% or more, compared to the host cell prior to engineering.

[0050] Preferably, the present disclosure provides a recombinant host cell for manufacturing a protein of interest, wherein the host cell is engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 6 or a functional homologue thereof, wherein the functional homologue has at least 30% sequence identity to SEQ ID NO: 6, wherein the helper protein, preferably when overexpressed, may increase the production of the model protein SDZ-Fab or HyHEL-Fab in the host cell by at least 1%, such as at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190 or at least 200% or more, compared to the host cell prior to engineering.

[0051] Preferably, the present disclosure provides a recombinant host cell for manufacturing a protein of interest, wherein the host cell is engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 7 or a functional homologue thereof, wherein the functional homologue has at least 30% sequence identity to SEQ ID NO: 7, wherein the helper protein, preferably when overexpressed, may increase the production of the model protein SDZ-Fab or HyHEL-Fab in

the host cell by at least 1%, such as at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190 or at least 200% or more, compared to the host cell prior to engineering.

[0052] Preferably, the present disclosure provides a recombinant host cell for manufacturing a protein of interest, wherein the host cell is engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 8 or a functional homologue thereof, wherein the functional homologue has at least 30% sequence identity to SEQ ID NO: 8, wherein the helper protein, preferably when overexpressed, may increase the production of the model protein SDZ-Fab or HyHEL-Fab in the host cell by at least 1%, such as at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190 or at least 200% or more, compared to the host cell prior to engineering.

[0053] Preferably, the present disclosure provides a recombinant host cell for manufacturing a protein of interest, wherein the host cell is engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 9 or a functional homologue thereof, wherein the functional homologue has at least 30% sequence identity to SEQ ID NO: 9, wherein the helper protein, preferably when overexpressed, may increase the production of the model protein SDZ-Fab or HyHEL-Fab in the host cell by at least 1%, such as at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190 or at least 200% or more, compared to the host cell prior to engineering.

[0054] Preferably, the present disclosure provides a recombinant host cell for manufacturing a protein of interest, wherein the host cell is engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 162 or a functional homologue thereof, wherein the functional homologue has at least 30% sequence identity to SEQ ID NO: 162, wherein the helper protein, preferably when overexpressed, may increase the production of the model protein SDZ-Fab or HyHEL-Fab in the host cell by at least 1%, such as at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190 or at least 200%, compared to the host cell prior to engineering.

[0055] In a third aspect, the present disclosure provides the use of the engineered host cells for manufacturing a protein of interest. The host cells can be advantageously used for introducing polynucleotides encoding one or more POI(s), and thereafter can be cultured under suitable condition to express the POI.

[0056] In a fourth aspect, the present disclosure provides a method of increasing the yield of a protein of interest in a host cell, comprising overexpressing a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, or 162 or a functional homologue thereof, wherein the functional homologue has at least 30% sequence identity to an amino acid sequence as shownin SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, or 162.

[0057] Preferably, the method comprises overexpressing a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 1 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% sequence identity to SEQ ID NO: 1.

[0058] Preferably, the method comprises overexpressing a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 2 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% sequence identity to SEQ ID NO: 2.

[0059] Preferably, the method comprises overexpressing a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 3 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% sequence identity to SEQ ID NO: 3.

[0060] Preferably, the method comprises overexpressing a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 4 or a functional

homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% sequence identity to SEQ ID NO: 4.

[0061] Preferably, the method comprises overexpressing a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 5 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% sequence identity to SEQ ID NO: 5.

[0062] Preferably, the method comprises overexpressing a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 6 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% sequence identity to SEQ ID NO: 6.

[0063] Preferably, the method comprises overexpressing a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 7 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% sequence identity to SEQ ID NO: 7.

[0064] Preferably, the method comprises overexpressing a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 8 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% sequence identity to SEQ ID NO: 8.

[0065] Preferably, the method comprises overexpressing a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 9 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% sequence identity to SEQ ID NO: 9.

[0066] Preferably, the method comprises overexpressing a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 162 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% sequence identity to SEQ ID NO: 162.

[0067] In a fifth aspect, the present disclosure provides a method of increasing the yield of a protein of interest in a host cell, comprising:

- engineering the host cell to overexpress a helper protein encoded by a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, or 162 or a functional homologue thereof, wherein the functional homologue has at least 30% sequence identity to an amino acid sequence as shown in SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, or 162,
- recombining in said host cell a heterologous polynucleotide encoding the protein of interest, and
- culturing said host cell under suitable conditions to overexpress the helper protein or a functional homologue and the protein of interest or proteins of interest.

[0068] In the context of methods for increasing the yield of a protein the order of the “engineering step” and “recombining step” can alternatively be reversed such that the “recombining step” precedes the “engineering step”. Notably, as described herein, the yield of a protein of interest is increased when a helper protein is overexpressed and/or a KO protein is underexpressed.

[0069] Preferably, the present disclosure provides a method of increasing the yield of a protein of interest in a host cell, comprising:

- engineering the host cell to overexpress a helper protein encoded by a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 1 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% sequence identity to an amino acid sequence as shown in SEQ ID NO: 1,
- recombining in said host cell a heterologous polynucleotide encoding a protein of interest, and
- culturing said host cell under suitable conditions to overexpress the helper protein or a functional homologue and the protein of interest or proteins of interest.

[0070] Preferably, the present disclosure provides a method of increasing the yield of a protein of interest in a host cell, comprising:

- engineering the host cell to overexpress a helper protein encoded by a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 2, or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100%, sequence identity to an amino acid sequence as shown SEQ ID NO: 2,
- recombining in said host cell a heterologous polynucleotide encoding a protein of interest, and
- culturing said host cell under suitable conditions to overexpress the helper protein or a functional homologue and the protein of interest or proteins of interest.

[0071] Preferably, the present disclosure provides a method of increasing the yield of a protein of interest in a host cell, comprising:

- engineering the host cell to overexpress a helper protein encoded by a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 3 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93,

94, 95, 96, 97, 98, 99 or 100%, sequence identity to an amino acid sequence as shown in SEQ ID NO: 3,

- recombining in said host cell a heterologous polynucleotide encoding a protein of interest, and
- culturing said host cell under suitable conditions to overexpress the helper protein or a functional homologue and the protein of interest or proteins of interest.

[0072] Preferably, the present disclosure provides a method of increasing the yield of a protein of interest in a host cell, comprising:

- engineering the host cell to overexpress a helper protein encoded by a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 4 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100%, sequence identity to an amino acid sequence as shown in SEQ ID NO: 4,
- recombining in said host cell a heterologous polynucleotide encoding a protein of interest, and
- culturing said host cell under suitable conditions to overexpress the helper protein or a functional homologue and the protein of interest or proteins of interest.

[0073] Preferably, the present disclosure provides a method of increasing the yield of a protein of interest in a host cell, comprising:

- engineering the host cell to overexpress a helper protein encoded by a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 5 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100%, sequence identity to an amino acid sequence as shown in SEQ ID NO: 5,
- recombining in said host cell a heterologous polynucleotide encoding a protein of interest, and
- culturing said host cell under suitable conditions to overexpress the helper protein or a functional homologue and the protein of interest or proteins of interest.

[0074] Preferably, the present disclosure provides a method of increasing the yield of a protein of interest in a host cell, comprising:

- engineering the host cell to overexpress a helper protein encoded by a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 6 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100%, sequence identity to an amino acid sequence as shown in SEQ ID NO: 6,
- recombining in said host cell a heterologous polynucleotide encoding a protein of interest, and
- culturing said host cell under suitable conditions to overexpress the helper protein or a functional homologue and the protein of interest or proteins of interest.

[0075] Preferably, the present disclosure provides a method of increasing the yield of a protein of interest in a host cell, comprising:

- engineering the host cell to overexpress a helper protein encoded by a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 7 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100%, sequence identity to an amino acid sequence as shown in SEQ ID NO: 7,
- recombining in said host cell a heterologous polynucleotide encoding a protein of interest, and
- culturing said host cell under suitable conditions to overexpress the helper protein or a functional homologue and the protein of interest or proteins of interest.

[0076] Preferably, the present disclosure provides a method of increasing the yield of a protein of interest in a host cell, comprising:

- engineering the host cell to overexpress a helper protein encoded by a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 8 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47,

48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100%, sequence identity to an amino acid sequence as shown in SEQ ID NO: 8,

- recombining in said host cell a heterologous polynucleotide encoding a protein of interest, and
- culturing said host cell under suitable conditions to overexpress the helper protein or a functional homologue and the protein of interest or proteins of interest.

[0077] Preferably, the present disclosure provides a method of increasing the yield of a protein of interest in a host cell, comprising:

- engineering the host cell to overexpress a helper protein encoded by a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 9 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100%, sequence identity to an amino acid sequence as shown in SEQ ID NO: 9,
- recombining in said host cell a heterologous polynucleotide encoding a protein of interest, and
- culturing said host cell under suitable conditions to overexpress the helper protein or a functional homologue and the protein of interest or proteins of interest.

[0078] Preferably, the present disclosure provides a method of increasing the yield of a protein of interest in a host cell, comprising:

- engineering the host cell to overexpress a helper protein encoded by a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 162 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100%, sequence identity to an amino acid sequence as shown in SEQ ID NO: 162,
- recombining in said host cell a heterologous polynucleotide encoding a protein of interest, and

- culturing said host cell under suitable conditions to overexpress the helper protein or a functional homologue and the protein of interest or proteins of interest.

[0079] In a sixth aspect, the present disclosure provides a method of manufacturing a protein of interest in a host cell comprising:

- providing the host cell engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, or 162 or a functional homologue thereof, wherein the functional homologue has at least 30% sequence identity to an amino acid sequence as shown in SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, or 162, wherein said host cell comprises a heterologous polynucleotide encoding a protein of interest;
- culturing the host cell under suitable conditions to overexpress the helper protein and express protein of interest.

[0080] Preferably, the present disclosure provides a method of manufacturing a protein of interest in a host cell comprising:

- providing the host cell engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 1 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100%, sequence identity to an amino acid sequence as shown in SEQ ID NO: 1, wherein said host cell comprises a heterologous polynucleotide encoding a protein of interest;
- culturing the host cell under suitable conditions to overexpress the helper protein and express protein of interest.

[0081] Preferably, the present disclosure provides a method of manufacturing a protein of interest in a host cell comprising:

- providing the host cell engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 2 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100%, sequence identity to an amino acid sequence as shown in SEQ ID

NO: 2, wherein said host cell comprises a heterologous polynucleotide encoding a protein of interest;

- culturing the host cell under suitable conditions to overexpress the helper protein and express protein of interest.

[0082] Preferably, the present disclosure provides a method of manufacturing a protein of interest in a host cell comprising:

- providing the host cell engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 3 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100%, sequence identity to an amino acid sequence as shown in SEQ ID NO: 3, wherein said host cell comprises a heterologous polynucleotide encoding a protein of interest;
- culturing the host cell under suitable conditions to overexpress the helper protein and express protein of interest.

[0083] Preferably, the present disclosure provides a method of manufacturing a protein of interest in a host cell comprising:

- providing the host cell engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 4 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100%, sequence identity to an amino acid sequence as shown in SEQ ID NO: 4, wherein said host cell comprises a heterologous polynucleotide encoding a protein of interest;
- culturing the host cell under suitable conditions to overexpress the helper protein and express protein of interest.

[0084] Preferably, the present disclosure provides a method of manufacturing a protein of interest in a host cell comprising:

- providing the host cell engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 5 or a functional

homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100%, sequence identity to an amino acid sequence as shown in SEQ ID NO: 5, wherein said host cell comprises a heterologous polynucleotide encoding a protein of interest;

- culturing the host cell under suitable conditions to overexpress the helper protein and express protein of interest.

[0085] Preferably, the present disclosure provides a method of manufacturing a protein of interest in a host cell comprising:

- providing the host cell engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 6 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100%, sequence identity to an amino acid sequence as shown in SEQ ID NO: 6, wherein said host cell comprises a heterologous polynucleotide encoding a protein of interest;
- culturing the host cell under suitable conditions to overexpress the helper protein and express protein of interest.

[0086] Preferably, the present disclosure provides a method of manufacturing a protein of interest in a host cell comprising:

- providing the host cell engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 7 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100%, sequence identity to an amino acid sequence as shown in SEQ ID NO: 7, wherein said host cell comprises a heterologous polynucleotide encoding a protein of interest;
- culturing the host cell under suitable conditions to overexpress the helper protein and express protein of interest.

[0087] Preferably, the present disclosure provides a method of manufacturing a protein of interest in a host cell comprising:

- providing the host cell engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 8 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100%, sequence identity to an amino acid sequence as shown in SEQ ID NO: 8, wherein said host cell comprises a heterologous polynucleotide encoding a protein of interest;
- culturing the host cell under suitable conditions to overexpress the helper protein and express protein of interest.

[0088] Preferably, the present disclosure provides a method of manufacturing a protein of interest in a host cell comprising:

- providing the host cell engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 9 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100%, sequence identity to an amino acid sequence as shown in SEQ ID NO: 9, wherein said host cell comprises a heterologous polynucleotide encoding a protein of interest;
- culturing the host cell under suitable conditions to overexpress the helper protein and express protein of interest.

[0089] Preferably, the present disclosure provides a method of manufacturing a protein of interest in a host cell comprising:

- providing the host cell engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 162 or a functional homologue thereof, wherein the functional homologue has at least 30%, such as at least 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97,

The current application discloses the following concepts:

1. A recombinant host cell for manufacturing a protein of interest, wherein the host cell is engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 4, 1, 2, 3, 5, 6, 7, 8, 9, or 162 or a functional homologue thereof, wherein the functional homologue has at least 30% sequence identity to an amino acid sequence as shown in SEQ ID NO: 4, 1, 2, 3, 5, 6, 7, 8, 9, or 162.

2. The host cell of concept 1, wherein said helper protein or said functional homologue thereof increases the yield of the model protein SDZ-Fab (SEQ ID NO: 25 and 26) and/or HyHEL-Fab (SEQ ID NO: 29 and 30) compared to the host cell prior to engineering.

3. The host cell of any one of the preceding concepts, wherein said polynucleotide is integrated in the genome of said host cell or contained in a vector or plasmid.

4. The host cell of any one of the preceding concepts, wherein the overexpression of the polynucleotide is achieved by (i) using a recombinant promoter which drives expression of said polynucleotide or by (ii) modifying a regulatory sequence operably linked to said polynucleotide.

5. The host cell of any one of the preceding concepts, wherein the host cell is *Pichia pastoris, Hansenula polymorpha, Trichoderma reesei, Saccharomyces cerevisiae, Kluyveromyces lactis, Yarrowia lipolytica, Pichia methanolica*, *Candida boidinii and Komagataella, and Schizosaccharomyces pombe*.

6. The host cell of any one of the preceding concepts, wherein 2, 3, 4, 5, 6, 7, 8 or more of helper proteins selected from SEQ ID NO: 4, 1, 2, 3, 5, 6, 7, 8, 9, or 162 or functional homologues thereof are overexpressed.

7. The host cell of any one of the preceding concepts, wherein the host cell is engineered to underexpress at least one polynucleotide encoding a protein having an amino acid having at least 50% sequence identity to an amino acid sequence as shown in SEQ ID NO: 10, 11 and/or 12.

8. The host cell of any one of the preceding concepts, comprising a heterologous polynucleotide sequence encoding the protein of interest.

9. The host cell of concept 8, wherein the protein of interest is an enzyme, a therapeutic protein, a food additive or feed additive, or antibody or antibody fragment.

10. The host cell of any one of the preceding concepts, wherein the host cell is engineered to

    (i) overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 4 or a functional homologue thereof and SEQ ID NO: 2 or a functional homologue thereof,

    (ii) overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 162 or a functional homologue thereof and SEQ ID NO: 2 or a functional homologue thereof,

    (iii) overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 1 or a functional homologue thereof and SEQ ID NO: 2 or a functional homologue thereof and is further engineered to underexpress a polynucleotide encoding a protein having an amino acid as shown in SEQ ID NO: 10 or a functional homologue thereof, or

    (iv) overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 4 or a functional homologue thereof and SEQ ID NO: 1 or a functional homologue thereof and is further engineered to underexpress a polynucleotide encoding a protein having an amino acid as shown in SEQ ID NO: 10 or a functional homologue thereof.

11. Use of the host cell of any one of the preceding concepts for manufacturing a protein of interest.

12. A method of increasing the yield of a protein of interest in a host cell, comprising overexpressing a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 4, 1, 2, 3, 5, 6, 7, 8, 9, or 162 or a functional homologue thereof, wherein the functional homologue has at least 30% sequence identity to an amino acid sequence as shown in SEQ ID NO: 4, 1, 2, 3, 5, 6, 7, 8, 9, or 162.

13. A method of increasing the yield of a protein of interest in a host cell comprising:

    - engineering the host cell to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 4, 1, 2, 3, 5, 6, 7, 8, 9, or 162 or a functional homologue thereof, wherein the functional

homologue has at least 30% sequence identity to an amino acid sequence as shown in SEQ ID NO: 4, 1, 2, 3, 5, 6, 7, 8, 9, or 162,

- recombining in said host cell a heterologous polynucleotide encoding the protein of interest,

- culturing said host cell under suitable conditions to overexpress the helper protein or the functional homologue and the protein of interest, and optionally

- isolating the protein of interest from the cell culture.

14. A method of manufacturing a protein of interest in a host cell comprising:

- providing the host cell engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 4, 1, 2, 3, 5, 6, 7, 8, 9, or 162 or a functional homologue thereof, wherein the functional homologue has at least 30% sequence identity to an amino acid sequence as shown in SEQ ID NO: 4, 1, 2, 3, 5, 6, 7, 8, 9, or 162, wherein said host cell comprises a heterologous polynucleotide encoding a protein of interest;

- culturing the host cell under suitable conditions to overexpress the helper protein or the functional homologue and express the protein of interest, and optionally

- isolating the protein of interest from the cell culture.

15. The method in any one of the preceding concepts, wherein the host cell is engineered to underexpress at least one polynucleotide encoding a protein having an amino acid having at least 50% sequence identity to an amino acid sequence as shown in SEQ ID NO: 10, 11 and/or 12.

Claims

1. A recombinant host cell for manufacturing a heterologous protein of interest, wherein the host cell comprises a heterologous polynucleotide sequence encoding the protein of interest and is engineered to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 4 or a functional homologue thereof, wherein the functional homologue has at least 70% sequence identity to an amino acid sequence as shown in SEQ ID NO: 4.

2. The host cell of claim 1, wherein said helper protein or said functional homologue thereof increases the yield of the model protein SDZ-Fab (SEQ ID NO: 25 and 26) and/or HyHEL-Fab (SEQ ID NO: 29 and 30) compared to the host cell prior to engineering.

3. The host cell of any one of the preceding claims, wherein said polynucleotide is integrated in the genome of said host cell or contained in a vector or plasmid.

4. The host cell of any one of the preceding claims, wherein the overexpression of the polynucleotide is achieved by (i) using a recombinant promoter which drives expression of said polynucleotide or by (ii) modifying a regulatory sequence operably linked to said polynucleotide.

5. The host cell of any one of the preceding claims, wherein the host cell is *Pichia pastoris, Hansenula polymorpha, Trichoderma reesei, Saccharomyces cerevisiae, Kluyveromyces lactis, Yarrowia lipolytica, Pichia methanolica*, *Candida boidinii Komagataella, or Schizosaccharomyces pombe*.

6. The host cell of any one of the preceding claims, wherein 1, 2, 3, 4, 5, 6, 7, 8 or more further helper proteins selected from SEQ ID NO: 1, 2, 3, 5, 6, 7, 8, 9, or 162 or functional homologues thereof are also overexpressed, wherein the functional homologue has at least 70% sequence identity to an amino acid sequence as shown in SEQ ID NO: 1, 2, 3, 5, 6, 7, 8, 9, or 162.

7. The host cell of any one of the preceding claims, wherein the host cell is engineered to underexpress at least one polynucleotide encoding a KO protein having an amino acid sequence having at least 70% sequence identity to an amino acid sequence as shown in SEQ ID NO: 10, 11 and/or 12, wherein underexpression is achieved by knocking-out the polynucleotide encoding the KO protein or a functional homologue thereof from the

genome of said host cell, disrupting the polynucleotide encoding the KO protein or a functional homologue thereof in the host cell, disrupting a promoter which is operably linked with said polypeptide encoding the KO protein or a functional homologue thereof, replacing the promoter which is operably linked with said polypeptide encoding the KO protein or a functional homologue thereof with another promoter which has lower promoter activity, or disrupting expression control sequences of the KO protein or a functional homologue thereof, such as ribosomal binding sites, transcriptional start and stop sequences, translational start and stop sequences, and enhancer or activator sequences, or post-transcriptional gene silencing.

8. The host cell of any one of the preceding claims, wherein the protein of interest is an enzyme, a therapeutic protein, a food additive or feed additive, or antibody or antibody fragment.

9. The host cell of claim 6, wherein the host cell is engineered to

    (i) overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 4 or a functional homologue thereof, wherein the functional homologue has at least 70% sequence identity to an amino acid sequence as shown in SEQ ID NO: 4 and SEQ ID NO: 2 or a functional homologue thereof, wherein the functional homologue has at least 70% sequence identity to an amino acid sequence as shown in SEQ ID NO: 2, or

    (ii) overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 4, wherein the functional homologue has at least 70% sequence identity to an amino acid sequence as shown in SEQ ID NO: 4 or a functional homologue thereof and SEQ ID NO: 1 or a functional homologue thereof, wherein the functional homologue has at least 70% sequence identity to an amino acid sequence as shown in SEQ ID NO: 1 and is further engineered to underexpress a polynucleotide encoding a KO protein having an amino acid as shown in SEQ ID NO: 10 or a functional homologue thereof having at least 70% sequence identity to an amino acid sequence as shown in SEQ ID NO: 10,

    wherein underexpression is achieved by knocking-out the polynucleotide encoding the KO protein or a functional homologue thereof from the genome of said host cell, disrupting the polynucleotide encoding the KO protein or a functional homologue thereof in the host cell, disrupting a promoter which is operably linked with said

polypeptide encoding the KO protein or a functional homologue thereof, replacing the promoter which is operably linked with said polypeptide encoding the KO protein or a functional homologue thereof with another promoter which has lower promoter activity, or disrupting expression control sequences of the KO protein or a functional homologue thereof, such as ribosomal binding sites, transcriptional start and stop sequences, translational start and stop sequences, and enhancer or activator sequences, or post-transcriptional gene silencing.

10. Use of the host cell of any one of the preceding claims for manufacturing a protein of interest.

11. A method of increasing the yield of a protein of interest in a host cell, comprising overexpressing a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 4 or a functional homologue thereof, wherein the functional homologue has at least 70% sequence identity to an amino acid sequence as shown in SEQ ID NO: 4.

12. A method of increasing the yield of a protein of interest in a host cell comprising:

    - engineering a host cell to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 4 or a functional homologue thereof, wherein the functional homologue has at least 70% sequence identity to an amino acid sequence as shown in SEQ ID NO: 4,

    - engineering said host cell to comprise a heterologous polynucleotide encoding the protein of interest,

    - culturing said host cell under suitable conditions to overexpress the helper protein or the functional homologue and the protein of interest, and optionally

    - isolating the protein of interest from the cell culture.

13. A method of manufacturing a protein of interest in a host cell comprising:

    - engineering a host cell to overexpress a polynucleotide encoding a helper protein having an amino acid sequence as shown in SEQ ID NO: 4 or a functional homologue thereof, wherein the functional homologue has at least 70% sequence identity to an amino acid sequence as shown in SEQ ID NO: 4, wherein said host cell comprises a heterologous polynucleotide encoding a protein of interest;

- culturing the host cell under suitable conditions to overexpress the helper protein or the functional homologue and express the protein of interest, and optionally

- isolating the protein of interest from the cell culture.

14. The method of any one of claims 11-13, wherein the host cell is engineered to underexpress at least one polynucleotide encoding a KO protein having an amino acid having at least 70% sequence identity to an amino acid sequence as shown in SEQ ID NO: 10, 11 and/or 12, wherein underexpression is achieved by knocking-out the polynucleotide encoding the KO protein or a functional homologue thereof from the genome of said host cell, disrupting the polynucleotide encoding the KO protein or a functional homologue thereof in the host cell, disrupting a promoter which is operably linked with said polypeptide encoding the KO protein or a functional homologue thereof, replacing the promoter which is operably linked with said polypeptide encoding the KO protein or a functional homologue thereof with another promoter which has lower promoter activity, or disrupting expression control sequences of the KO protein or a functional homologue thereof, such as ribosomal binding sites, transcriptional start and stop sequences, translational start and stop sequences, and enhancer or activator sequences, or post-transcriptional gene silencing.