extending the duration of the cell culture, but this is restrictive and costly, and could affect product quality. The present invention addresses this and other problems.

SUMMARY

[0004] In one aspect, there is provided a method for removing a cysteine cap from a protein molecule, the method comprising: culturing a host cell comprising a protein molecule having at least one capped cysteine residue, and contacting the host cell culture with cystine, whereby the cysteine cap is removed from the protein molecule. In an embodiment, the host cell culture is further contacted with dissolved oxygen (DO). In a further embodiment, simultaneously or subsequent to contacting the host cell culture with cystine, the host cell culture is contacted with a first manipulation of DO at a set point of 0%-50% DO, and subsequent to contacting the host cell culture with the first manipulation of DO, the host cell culture is contacted with a second manipulation of DO at a set point of 20%-100% DO. In an embodiment, the host cell culture is contacted with the first manipulation of DO for 0.5-8 hours. In an embodiment, the host cell culture is contacted with the second manipulation of DO for 0.5-8 hours. In an embodiment, the first manipulation of DO is at a set point of 0% DO. In an embodiment, the second manipulation of DO is at a set point of 100% DO.

[0005] In an embodiment, the protein described above is an antibody. In a further embodiment, the antibody is combined with a drug-linker compound under conditions sufficient to form an antibody-drug conjugate. In a further embodiment, the antibody has at least two engineered cysteine residues. In an embodiment, the engineered cysteine residues are present in the heavy constant region of the antibody molecule. In another embodiment, the engineered cysteine residues are present in the heavy chain or light chain variable region of the antibody molecule. In an embodiment, the cysteine cap is an engineered cysteine cap (EC-cap).

[0006] In an embodiment, the host cell culture is contacted with cystine on day 10 of the host cell culture. In another embodiment, the host cell culture is contacted with cystine daily throughout the host cell culture duration. In another embodiment, the host cell culture is contacted with cystine on the final day of the host cell culture duration. In an embodiment, the cystine is added at a concentration of between 0.1 mM and 5 M. In a further embodiment, the cystine is added at a concentration of 4 mM.

[0006a] In another aspect, there is provided a method for removing and re-forming a cysteine cap from a protein molecule, the method comprising:

culturing a host cell comprising a protein molecule having at least one capped cysteine residue; and

contacting the host cell culture with cystine;

simultaneously or subsequent to contacting the host cell culture with cystine, contacting the host cell culture with a first manipulation of DO at a set point of 0%-50%;

subsequent to contacting the host cell culture with DO at a set point of 0%-50%, contacting the host cell culture with a second manipulation of DO at a set point of 20%-100%; whereby the cysteine cap is removed from the protein molecule and the protein molecule is re-capped with a cysteine molecule.

[0006b] In another aspect, there is provided a method for removing a cysteine cap from a protein molecule, the method comprising:

culturing a host cell comprising a protein molecule having at least one capped cysteine residue; and

contacting the host cell culture with a symmetrical disulfide;

whereby the cysteine cap is removed from the protein molecule.

WHAT IS CLAIMED IS:

1. A method for removing a cysteine cap from a protein molecule, the method comprising:

culturing a host cell comprising a protein molecule having at least one capped cysteine residue; and

contacting the host cell culture with cystine;

whereby the cysteine cap is removed from the protein molecule.

2. The method of claim 1, further comprising contacting the host cell culture with dissolved oxygen (DO).

3. The method of claim 1, further comprising:

simultaneously or subsequent to contacting the host cell culture with cystine, contacting the host cell culture with a first manipulation of DO at a set point of 0%-50% DO;

subsequent to contacting the host cell culture with the first manipulation of DO, contacting the host cell culture with a second manipulation of DO at a set point of 20%-100% DO.

4. The method of claim 3, wherein the contacting the host cell culture with the first manipulation occurs for a duration of 0.5-8 hours.

5. The method of claim 4, wherein the contacting the host cell culture with the first manipulation occurs for a duration of 2 hours.

6. The method of claim 3, wherein the contacting the host cell culture with the second manipulation occurs for a duration of 0.5-8 hours.

7. The method of claim 6, wherein the contacting the host cell culture with the second manipulation occurs for a duration of 2 hours.

8. The method of claim 3, wherein the first manipulation of DO is at a set point of 0% DO.

9. The method of claim 3, wherein the second manipulation of DO is at a set point of 100% DO.

10. The method of any of claims 1-3, wherein the protein is an antibody.

11. The method of claim 10, further comprising combining the antibody with a drug-linker compound under conditions sufficient to form an antibody-drug conjugate.

12. The method of claim 10, wherein the antibody has at least two engineered cysteine residues.

13. The method of claim 12, wherein the engineered cysteine residues are present in the heavy constant region of the antibody molecule.

14. The method of claim 12, wherein the engineered cysteine residues are present in the heavy chain or light chain variable region of the antibody molecule.

15. The method of any of claims 1-3, whereby the protein molecule is re-capped with a cysteine molecule.

16. The method of any of claims 1-3, wherein the cysteine cap is an engineered cysteine cap (EC-cap).

17. The method of any of claims 1-3, wherein the host cell culture is contacted with cystine on day 10 of the host cell culture.

18. The method of any of claims 1-3, wherein the host cell culture is contacted with cystine daily throughout the host cell culture duration.

19. The method of any of claims 1-3, wherein the host cell culture is contacted with cystine on the final day of the host cell culture duration.

20. The method of any of claims 1-3, wherein the cystine is added at a concentration of between 0.1 mM and 5 M.

21. The method of any of claims 1-3, wherein the cystine is added at a concentration of 4 mM.

22. A method for removing and re-forming a cysteine cap from a protein molecule, the method comprising:

culturing a host cell comprising a protein molecule having at least one capped cysteine residue; and

contacting the host cell culture with cystine;

simultaneously or subsequent to contacting the host cell culture with cystine, contacting the host cell culture with a first manipulation of DO at a set point of 0%-50%;

subsequent to contacting the host cell culture with DO at a set point of 0%-50%, contacting the host cell culture with a second manipulation of DO at a set point of 20%-100%;

whereby the cysteine cap is removed from the protein molecule and the protein molecule is re-capped with a cysteine molecule.

23. A method for removing a cysteine cap from a protein molecule, the method comprising:

culturing a host cell comprising a protein molecule having at least one capped cysteine residue; and

contacting the host cell culture with a symmetrical disulfide;

whereby the cysteine cap is removed from the protein molecule.