CLAIMS

What is claimed is:

1. A method of refolding a complex multimeric protein expressed in a non-mammalian expression system and present in a volume at a concentration of 2.0 g/L or greater comprising:

   (a) contacting the protein with a refold buffer comprising a redox component comprising a final thiol-pair ratio having a range of 0.001 to 100 and a ~~redox buffer strength~~thiol-pair buffer strength of 2 mM or greater and one or more of:

      (i) a denaturant;

      (ii) an aggregation suppressor; and

      (iii) a protein stabilizer;

   to form a refold mixture; wherein the thiol-pair ratio is calculated according to Equation 1:

$$\frac{[\text{reductant}]^2}{[\text{oxidant}]};$$

   wherein the thiol-pair buffer strength is calculated according to Equation 2:

$$2[\text{oxidant}]+[\text{reductant}];$$

   wherein said thiol-pair ratio and said thiol-pair buffer strength are varied in a full or partial factorial matrix; and

   wherein the thiol-pair ratio value and thiol-pair buffer strength value used in said refold buffer are those respective values resulting in a higher yield of the desired form of said complex multimeric protein than is obtained prior to said varying of said thiol-pair ratio and said thiol-pair buffer strength;

   (b) incubating the refold mixture; and

   (c) isolating the protein from the refold mixture.

2. The method of claim 1, wherein the final thiol-pair ratio is selected from the group consisting of 0.05 to 50, 0.1 to 50, 0.25 to 50, 0.5 to 50, 0.75 to 40, 1.0 to 50 or 1.5 to 50, 2 to 50, 5 to 50, 10 to 50, 15 to 50, 20 to 50, 30 to 50 or 40 to 50.

3. The method of claim 1, wherein the thiol-pair buffer strength is selected from the group consisting of greater than or equal to 2.25 mM, 2.5 mM, 2.75 mM, 3 mM, 5 mM, 7.5 mM, 10 mM or 15 mM.

~~3.~~4. The method of claim 1, wherein the protein concentration present in the volume is selected from a 2.0 g/L to 40.0 g/L.

~~4.~~5. The method of claim 1, wherein the protein is present in the volume in a non-native limited solubility form.

~~5.~~6. The method of claim 4, wherein the non-native limited solubility form is an inclusion body.

~~6.~~7. The method of claim 1, wherein the protein is present in the volume in a soluble form.

~~7.~~8. The method of claim 1, wherein the protein is recombinant.

~~8.~~9. The method of claim 1, wherein the protein is an endogenous protein.

~~9.~~10. The method of claim 1, wherein the protein is an antibody.

~~10.~~11. The method of claim 1, wherein the protein is a complex protein.

~~11.~~12. The method of claim 1, wherein the protein is a multimeric protein.

~~12.~~13. The method of claim 1, wherein the protein is an Fc-protein conjugate.

~~13.~~14. The method of claim 1, wherein the non-mammalian expression system is one of a bacterial expression system and a yeast expression system.

~~14.~~15. The method of claim 1, wherein the denaturant is selected from the group consisting of urea, guanidinium salts, dimethyl urea, methylurea and ethylurea.

~~15.~~16. The method of claim 1, wherein the protein stabilizer is selected from the group consisting of arginine, proline, polyethylene glycols, non-ionic surfactants, ionic surfactants, polyhydric alcohols, glycerol, sucrose, sorbitol, glucose, Tris, sodium sulfate, potassium sulfate and osmolytes.

~~16.~~17. The method of claim 1, wherein the aggregation suppressor is selected from the group consisting of arginine, proline, polyethylene glycols, non-ionic surfactants, ionic surfactants, polyhydric alcohols, glycerol, sucrose, sorbitol, glucose, Tris, sodium sulfate, potassium sulfate and osmolytes.

~~17.~~18. The method of claim 1, wherein the thiol-pairs comprise at least one component selected from the group consisting of glutathione-reduced, glutathione-oxidized, cysteine, cystine, cysteamine, cystamine and beta-mercaptoethanol.

~~18.~~19. The method of claim 1, wherein the incubation is performed under non-aerobic conditions.

~~19.~~20. The method of claim 1, wherein the isolation comprises contacting the mixture with an affinity separation matrix.

~~20.~~21. The method of claim 19, wherein the affinity separation matrix is a Protein A resin.

~~21.~~22. The method of claim 19, wherein the affinity resin is a mixed mode separation matrix.

~~22.~~23. The method of claim 1, wherein the isolating comprises contacting the mixture with an ion exchange separation matrix.

~~23.~~24. The method of claim 1, wherein the isolating further comprises a filtration step.

~~24.~~25. The method of claim 23, wherein the filtration step comprises depth filtration.

26. The method of claim 1, wherein said variation further comprises systematic variation of incubation-time, concentration of said complex multimeric protein and pH.