

CLAIMS

- 1) A method for manufacturing a protein, which protein is an antibody or antigen-binding fragment thereof and which protein comprises a cysteine hinge residue comprising:
 - a) culturing an *E. coli* host cell under conditions such that they express the protein in the periplasm,
 - b) adding an extraction buffer to the host cells and subjecting the host cells to a heat treatment step,
 - c) purifying said protein from a mixture containing the host cells and other contaminants, wherein said purification comprises at least one chromatography step and wherein a reducing agent is added to said mixture and the protein thereof is maintained in the presence of said reducing agent from the first chromatography step until the last chromatography step,
 - d) adding polyethylene glycol (PEG) to the protein,
 - e) coupling the protein to the PEG via a covalent binding to the cysteine hinge residue of the protein and
 - f) recovering said protein coupled to a PEG,wherein the reducing agent is 0.5 mM to 2 mM glutathione.
- 2) The method according to any preceding claim, wherein the reducing agent is removed from the recovered antibody or antigen-binding fragment in step c).
- 3) The method according to any preceding claim, wherein said antigen binding fragment is a Fab'.
- 4) The method according to any preceding claim, wherein said PEG molecule is 40,000 PEG-maleimide.
- 5) The method according to any preceding claim wherein the purification of the antibody or antibody-binding fragment thereof of step c) comprises a first chromatography step that is a cation exchange chromatography from where a first eluate containing the antibody or antibody-binding fragment thereof is eluted, and a second chromatography step that is an anion exchange chromatography to produce a flow-through containing the antibody or antibody-binding fragment thereof.