disruption of only the outer membrane and peptidoglycan is still a goal that has not been put into practice on large scale" (page 354).

In WO 2012/013930 purification of recombinant protein from sample or extract of Gram-negative bacterial host cell expressing recombinant protein and disulfide isomerase DsbC, involves adjusting pH of sample or extract to precipitate and separate DsbC is reported. New antibody specific for human tumor necrosis factor (TNF)-alpha, useful for treating TNF-alpha mediated diseases, e.g. congestive heart failure, septic or endotoxic shock, cachexia and adult respiratory distress syndrome is reported in WO 01/94585. In US 2005/048056 preparing a tumor necrosis factor-alpha antibody having a heavy and light chain comprises fermenting a cell mixture, forming a cell pellet and allowing the pellet to stand for a hold time is reported.

In WO 2007/106120 modulating tissue distribution of a peptide molecule comprises administering to the tissue a conjugate molecule comprising serum albumin binding peptide is reported. Peptide ligands with affinity for immunoglobulin (Ig) G, IgM and/or human serum albumin which may be conjugated and used to prolong the elimination halftime of active agents from the circulation are reported in WO 01/45746. In US 2004/001827 modulating tissue distribution of a peptide molecule, for enhancing therapeutic efficacy and reducing side effects, comprises administering to the tissue a conjugate molecule comprising a peptide ligand domain and an active domain is reported.

Summary ~~of the Invention~~

In the current ~~invention~~ disclosure it has been found that a recombinantly produced polypeptide can be isolated from the periplasm of a prokaryotic cell by contacting or incubating the cell with a solution comprising a buffering agent (e.g. Tris) and a chelating agent (e.g. EDTA) at a pH value of about 8 and at room temperature. The method enables the isolation of a recombinantly produced polypeptide in high yields and purity in large scale production processes. The isolated polypeptide shows good feasibility for downstream processes (e.g. purification).

There is provided ~~One aspect as reported herein is~~ a method for producing a polypeptide comprising the step of incubating prokaryotic cells in a solution comprising about 10mM to about 95mM of a buffering agent and about 0.5mM to about 9.5mM of a chelating agent at a pH value of about 7 to about 10 for about 15min to about 6h.

In one embodiment the chelating agent is selected from the group comprising ethylendiamine, nitrilotriacetic acid (NTA), ethylene glycol tetraacetic acid (EGTA), diethylene triamine pentaacetic acid (DTPA), 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA), Ethylenediamine-*N,N'*-disuccinic acid (EDDS), phosphonates (e.g. Ethylenediamine tetra(methylene phosphonic acid) EDTMP or Diethylenetriamine penta(methylene phosphonic acid) DTPMP), citrate, porphyrines (e.g. heme, chlorophyll or vitamin B12). In one embodiment the chelating agent is ethylenediaminetetraacetic acid (EDTA).

There is also provided a ~~One aspect as reported herein is~~ method for producing a polypeptide comprising the step of incubating (resuspended) prokaryotic cells in a solution comprising about 10mM to about 95mM Tris-HCl and about 2mM to about 6mM EDTA at a pH value of about 7 to about 10 for about 15min to about 6h at about 25°C. In one aspect, there is provided a method for producing a polypeptide comprising incubating (resuspended) prokaryotic cells in a solution comprising about 10mM to about 95mM Tris-HCl and about 2mM to about 6mM EDTA at a pH value of about 7 to about 10 for about 15min to about 6h at about 25°C, wherein the solution is substantially free of peptidoglycan hydrolyzing enzymes and sugar.

There is also provided a ~~One aspect as reported herein is~~ method for producing a polypeptide comprising the step of incubating (resuspended) prokaryotic cells in a solution comprising about 10mM to about 95mM Tris-HCl and about 2mM to about 4mM EDTA at a pH value of about 7 to about 10 for about 15min to about 6h at about 25°C.

In one embodiment the concentration of the chelating agent is from about 1mM to about 6mM.In one embodiment the concentration of the chelating agent is from about 1mM to about 4mM. In one embodiment the concentration of the chelating agent is from about 1mM to about 3mM. In one embodiment the concentration of the chelating agent is from about 2mM to about 6mM. In one embodiment the concentration of the chelating agent is from about 2mM to about 4mM. In one embodiment the concentration of the chelating agent is about 2mM. In one embodiment the concentration of the chelating agent is about 4mM. In one embodiment the concentration of the chelating agent is about 6mM.

In one embodiment the concentration of EDTA is from about 0.5mM to 9.5mM. In one embodiment the concentration of EDTA is from about 1mM to about 3mM. In one embodiment the concentration of EDTA is about 2mM. In one embodiment the concentration of EDTA is about 4mM. In one embodiment the concentration of EDTA is about 6mM.

In one embodiment the method as reported herein is characterized in that the EDTA concentration is about 2mM.

In one embodiment the solution used in the method as reported herein is substantially free of sugar. In one embodiment the sugar is sucrose.

In one embodiment the solution used in the method as reported herein is substantially free of sugar and substantially free of peptidoglycan hydrolyzing enzymes. In one embodiment the solution used in the method as reported herein is substantially free of sucrose and substantially free of lysozyme.

In one embodiment the method further comprises after the incubating step the step of isolating the polypeptide.

In one embodiment the method comprises the step of purifying the isolated polypeptide.

In one embodiment the method is characterized in that the EDTA concentration is about 2mM, the pH value is about 8, the incubation time is about 30min, the concentration of Tris-HCl is about 20mM, the incubation temperature is about 25°C and the prokaryotic cell is an E.coli cell.

In one embodiment the method is characterized in that the EDTA concentration is about 4mM, the pH value is about 8, the incubation time is about 30min, the concentration of Tris-HCl is about 40mM, the incubation temperature is about 25°C and the prokaryotic cell is an E.coli cell.

In one embodiment the method is characterized in that the EDTA concentration is about 6mM, the pH value is about 8, the incubation time is about 30min, the concentration of Tris-HCl is about 60mM, the incubation temperature is about 25°C and the prokaryotic cell is an E.coli cell. One aspect as reported herein is the use of a method as reported herein for the isolation of a periplasmic polypeptide from a prokaryotic cell.

In another aspect, there is provided use of a method as defined herein for the isolation of a periplasmic polypeptide from prokaryotic cells.

Description of the Figures

Figure 1: SDS-Page gel of large scale isolation of periplasmic expressed protein (α-Synculein) with the method as reported herein; lanes: LS = length standard, WC = whole cells, Pe = cell pellet, IPP = isolated periplasmic protein.

Figure 2: SDS-Page gel of large scale isolation Pseudomonas exotoxin (Nlys-PE25-LR8M) with the method as reported herein; lanes: