

bond to form cystine (SCH₂CH(NH₂)CO₂H)₂. Cystine has low solubility in water and readily precipitates out of solution. As a result, it has not been possible to produce a shelf-stable, liquid cell culture medium containing concentrations of tyrosine and cysteine required for maximal cell growth and/or protein production.

Applicants have also tried to address this problem by using less than the desired concentration of tyrosine or cysteine in the aqueous cell culture medium. In so doing, it is possible to achieve an acceptable aqueous shelf life. However, this is accomplished at the expense of optimal cell growth, protein production or viral production. In other words, cell culture medium with lower concentrations of tyrosine and cysteine supports reduced cell growth and/or protein production as compared to cell culture medium with the optimal concentrations of tyrosine and cysteine.

Thus, there exists a current need for a medium, concentrated medium or concentrated feed supplement containing amounts of tyrosine and cysteine sufficient to support maximal cell growth and/or protein or viral production while avoiding the problems caused by the limited solubility of tyrosine or the limited stability of cysteine, including the tendency of cysteine to precipitate out of solution over time. In addition, there exists a need for such a feed supplement to be in a concentrated form, so that its addition would not greatly add volume to the final cell culture system. There also exists a need that when such nutrient feeds are added, the pH and osmolality of the resulting system be automatically balanced, and that the medium or concentrated feed be available in a liquid or a dry format. Lastly, there exists a need for a single-regimen, concentrated feed supplement comprising amounts of cysteine and tyrosine sufficient to support maximal cell growth and/or protein production, and/or expressed protein of high quality.

SUMMARY

The compositions of the disclosure are directed, in part, to cell culture media, concentrated media, or concentrated feed supplements comprising concentrations of cysteine and tyrosine that support maximal cell growth and/or protein, or viral production while avoiding the problems caused by their limited solubility and stability. In one aspect, the present disclosure refers to a supplement comprising alanine-tyrosine and alanine-cysteine dimer. In one example, the media, concentrated feed supplements, or concentrated media may be serum-free compositions. In one example, the compositions may comprise human serum components, like human serum albumin. In a further example, the human serum albumin may be recombinant, (r- human serum albumin), derived from recombinant sources, in

some preferred cases, from plant sources like rice, corn, wheat, potato, or from fungal sources, or from yeast or other equivalent microorganisms well-known to be used in the art (xeno-free culture). In another example, the media, concentrated feed supplements, or concentrated media may be protein-free compositions. In yet another example, the media, concentrated feed supplements, or concentrated media may be protein-hydrolysate free compositions, and further, may be free of fractions of protein-hydrolysates. In a certain example, the media, concentrated feed supplements, or concentrated media may be serum-free, protein-free, and protein hydrolysate-free compositions. In a preferred example, these compositions may comprise components that are chemically-defined, serum-free, protein-free and free of any protein hydrolysates, or fractions thereof. In certain examples, the cell culture medium, concentrated medium or cell culture feed supplement having one or more small peptides, or one or more dipeptides, further does not contain one or more of the following: lipids, hydrolysates or a fraction thereof, or growth factors.

The disclosure is also directed, in part, to methods for analyzing the compositions described above for the presence or absence of a short peptide comprising cysteine or tyrosine. The analysis of the media is performed by any known method in the art, for instance, by mass spectrometry (LCMS), capillary electrophoresis or HPLC.

In particular, the present disclosure is directed to a cell culture medium, a concentrated feed, or a cell culture supplement comprising one or more small peptides having two to six amino acids, as defined later in the application. Therefore, the present disclosure provides a cell culture medium, a concentrated feed, or a cell culture supplement, wherein the medium, feed or supplement comprises one or more small peptides selected from X₁₋₅-tyrosine, X₁₋₅-cysteine, tyrosine- X₁₋₅, and cysteine- X₁₋₅, or any small peptide where cysteine or tyrosine is anywhere within the short peptide of 1 to 6 amino acids (for e.g., X-cysteine- X₁₋₄, X-tyrosine- X₁₋₄, etc.) or a salt thereof, wherein X is any amino acid, and wherein the N-terminal amino acid of the short peptide has a free amino group. In one example, X is alanine or glycine. In another example X is serine, valine, proline, aspartic acid, or glutamic acid. In a particular example, the present disclosure provides a cell culture medium, a concentrated feed, or a cell culture supplement, wherein the medium, feed or supplement comprises one or more dipeptides selected from X-tyrosine, X-cysteine, tyrosine-X, and cysteine-X, or a salt thereof, wherein X is any amino acid, and wherein the N-terminal amino acid of the dipeptide has a free amino group. In one example, X is alanine or glycine. In

another example X is serine, valine, proline, aspartic acid, or glutamic acid. In yet another example, the one or more dipeptides are alanyl tyrosine and/or alanyl cysteine. The cell culture medium, concentrated feed, or cell culture supplement may be a liquid solution or a dry powder, such as a dry powder media (DPM) or an agglomerated powder (AGT™). In one example, the liquid solution is stored at 2-8°C and remains free of precipitate for over 12 months.

In one example of the disclosure, the concentration of tyrosine (as comprised within the small peptide), in solution, in a medium, feed or supplement, will be greater than the concentration of tyrosine that would remain soluble in an identical solution, if the tyrosine were present as a monomer (that is, tyrosine in a small peptide may make the solution "supersaturated for tyrosine"). In one example, the solution will have between about 1 and at least about 100 times, or about 1 and at least about 25 times, the soluble tyrosine concentration as would be capable with the monomer. For example, the concentration of small peptides or dipeptides in some cell culture media, concentrated feeds or supplements may be about 1 to at least about 5 times, about 1 to at least about 10 times, about 1 to at least about 15 times, about 1 to at least about 20 times, about 1 to at least about 30 times, about 1 to at least about 40 times, about 1 to at least about 50 times, about 1 to at least about 60 times, about 1 to at least about 70 times, about 1 to at least about 80 times, about 1 to at least about 90 times, about 1 to at least about 100 times, about 10 to at least about 20 times, about 10 to at least about 30 times, about 10 to at least about 40 times, about 10 to at least about 50 times, about 10 to at least about 60 times, about 10 to at least about 70 times, and so on, the soluble tyrosine concentration as would be capable with the monomer.

In one example of the disclosure, the concentration of cysteine (as comprised within the small peptide), in solution, in a medium, feed or supplement, will be greater in the solution than the concentration of cysteine that would remain soluble in an identical solution if the cysteine were present as a monomer (that is, cysteine in a small peptide may make the solution "supersaturated for cysteine"). In one example, the solubility of cysteine (or lack thereof) would include, for example, loss of solubility caused by conversion of cysteine to cystine. In one example, the solution will comprise at least, between about 1 and about 25 times, the soluble cysteine concentration capable with the monomer. For example, the concentration of small peptides or dipeptides in some cell culture media, concentrated feeds or supplements may be at about 1 to at least about 5 times, about 1 to at least about 10 times, about 1 to

at least about 15 times, about 1 to at least about 20 times, about 1 to at least about 30 times, about 1 to at least about 40 times, about 1 to at least about 50 times, about 1 to at least about 60 times, about 1 to at least about 70 times, about 1 to at least about 80 times, about 1 to at least about 90 times, about 1 to at least about 100 times, about 1 to at least about 110 times, about 10 to at least about 20 times, about 10 to at least about 30 times, about 10 to at least about 40 times, about 10 to at least about 50 times, about 10 to at least about 60 times, about 10 to at least about 70 times, about 10 to at least about 80 times, about 10 to at least about 90 times, about 10 to at least about 100 times, about 10 to at least about 110 times, and so on, the soluble cysteine concentration as would be capable with the monomer. In one example, the solution is supersaturated for both tyrosine and cysteine.

In one example, the solution will be supersaturated for both tyrosine and cysteine in, for example, the ranges described above for each of tyrosine or cysteine alone.

In one example, the addition of the concentrated feeds, media or supplements described above could reduce the volume of supplementation to a culture system, for e.g., a fed-batch culture, which may already be in progress. The culture may be in progress for a few hours to a few days. In another example, increasing the solubility of the cysteine and tyrosine comprised within small peptides allows for the design of single part concentrated feeds, which may also be pH neutral, which is part of the disclosure. The disclosure is directed in part to preparation of single regimen concentrated feeds, concentrated media and concentrated supplements comprising cysteine and tyrosine comprised within a small peptide, to increase their solubility and stability within the composition.

The cell culture medium, concentrated feed or cell culture supplement having one or more small peptides, or one or more dipeptides optionally comprises one or more of the following: a carbohydrate, a vitamin, a salt, an inorganic element, a buffering agent, and an amino acid, or a salt thereof. In one example the carbohydrate is a hexose sugar. Alternately, pentose, hexose, or their derivatives or other equivalents may be used. In some instances, oligosaccharides or their derivatives may be used; or, the hexose may be selected from the group consisting of glucose, galactose, fructose and maltose. In a particular example, the carbohydrate is glucose.

In one example, the amino acid, or salt thereof, is one or more of arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, histidine, isoleucine,

leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

In one example, the cell culture medium, concentrated feed or cell culture supplement comprises a 1) first dipeptide, X-tyrosine, or a salt thereof, and a second dipeptide, X-cysteine, or a salt thereof, wherein X is alanine or glycine and wherein the alanine or glycine has a free amino group, 2) a carbohydrate, such as glucose, and 3) an amino acid, or a salt thereof. The amino acid, or a salt thereof, may comprise one or more of arginine, asparagine, aspartic acid, cysteine, glutamic acid, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

In one example, the cell culture medium, concentrated feed or cell culture supplement has one or more small peptides, or one or more dipeptides, suitable for culturing a bacterial cell, a yeast cell, a plant cell, or an animal cell, such as an insect cell (e.g., *Drosophila* cells, *Spodoptera* cells or *Trichoplusia* cells), a nematode cell (e.g., *C. elegans* cells) or a mammalian cell (e.g., CHO cells, COS cells, VERO cells, BHK cells, AE-1 cells, SP2/0 cells, L5.1 cells, PerC6, hybridoma cells, HEK 293, including human cells).

In certain examples, the cell culture medium having one or more small peptides or one or more dipeptides is a 1X formulation. In other example, the cell culture medium is concentrated as a 2X or greater formulation, as discussed elsewhere in this application. In certain examples, the one or more small peptides or dipeptides, such as X-tyrosine, X-cysteine, tyrosine-X, or cysteine-X, are present in the cell culture medium at a concentration of about 1 g/L to about 16 g/L, about 1 g/L to about 10 g/L, about 1 g/L to about 5 g/L, about 2.5 g/L to about 16 g/L, about 2.5 g/L to about 10 g/L, about 2.5 g/L to about 5 g/L, or about 2.5 g/L to about 8.5 g/L.

In another example, the disclosure provides a method of culturing a cell, comprising contacting the cell with the small peptide- or dipeptide-containing cell culture medium described herein under conditions supporting the cultivation of the cell. Any cell may be cultured according to the present methods, particularly bacterial cells, yeast cells, plant cells or animal cells. In one example, the animal cell for culturing according to the present methods is an insect cell (e.g., *Drosophila* cells, *Spodoptera* cells or *Trichoplusia* cells), a nematode cell (e.g., *C. elegans* cells) or a mammalian cell (e.g., CHO cells, COS cells, VERO cells, BHK cells, AE-1 cells, SP2/0 cells, L5.1 cells, PerC6, hybridoma cells, HEK 293 or other human cells).

In another example, the method of culturing a cell comprises contacting the cell with a basal cell culture medium under conditions supporting the cultivation of the cell and supplementing the basal cell culture medium with a cell culture medium, a cell culture supplement, or a cell culture feed, having one or more small peptides or one or more dipeptides, as described herein. In one example, the one or more dipeptides selected from X-tyrosine, X-cysteine, tyrosine-X, or cysteine-X, or a salt thereof, wherein X is selected from alanine, glycine, serine, valine, proline, aspartic acid, or glutamic acid and wherein the N-terminal amino acid of the one or dipeptides has a free amino group. In another example, the one or more dipeptides are alanyl tyrosine and/or alanyl cysteine.

In one example, the basal cell culture medium is supplemented with a solution, comprising cysteine and tyrosine in a small peptide, on more than one day, wherein the solution may be a concentrated medium, or a concentrated feed supplement. In certain examples, applicants may refer to the use of media, feed, supplements, or other solutions which comprise small peptides (including, for example, solutions comprising dipeptides which comprise at least one tyrosine or cysteine residue). Indeed, one example of applicants' disclosure is the use of such solutions in the culturing of cells. In that respect, one of skill in the art will appreciate that in certain recitations herein, Applicants may refer to a specific supplement, media(um), feed or solution merely as an example of an example that could equally apply to any of the types of solutions described herein. For example, one of skill in the art would understand that a recitation of a "supplement" with a specific amino acid composition can equally describe a "feed" with a like composition and vice versa.

The basal cell culture medium, concentrated medium, concentrated feed or supplement may comprise a small peptide- or dipeptide-containing medium or feed on day 0, day 1, day 2, day 3, day 4 or 5 days after starting the cell culture and is supplemented daily thereafter through day 13 or 14 or until the viability of the culture drops below a pre-determined level (eg. 50%). The basal cell culture medium is optionally supplemented with the small peptide- or dipeptide-containing cell culture medium at about 2% of the total volume of the basal cell culture medium. In one example, the cell produces a protein, a peptide, a small RNA (for e.g., miRNA, siRNA, etc). By protein is meant, a recombinant protein or a naturally occurring protein. By protein, whether recombinant or natural, is also meant a full-length protein or a part thereof (like a domain, a motif, a polypeptide chain or polypeptide fragment).

By protein, whether recombinant or natural, is also meant an intracellular protein, an extracellular protein, a secreted protein, hormone, cytokine, receptor, extracellular matrix protein, an immunoglobulin or a part of the immunoglobulin, or a fragment thereof. Preferably, the protein yield may be higher with the cell culture medium, feed or supplement comprising the short peptides, or dipeptides of the disclosure. In a specific example, for instance, the protein yield for an immunoglobulin may be greater than 3000 mg/L of immunoglobulin, after at least 14 days in culture. In another example, the cell can produce a virus or a VLP (virus like particle). In yet another example, the cell may produce a desired cell product like a vitamin, a metabolite, a glycoprotein, a carbohydrate, a lipid, or a lipoprotein. In another example, the cell itself is grown to be harvested. The media and feed supplement compositions of the disclosure comprise concentrations of cysteine and tyrosine that support maximal cell growth for viral or VLP production, or maximal vitamin production, or glycoprotein, or vaccine production, etc., while avoiding the problems caused by their limited solubility.

Any cell may be cultured according to the present methods, for instance bacterial cells, yeast cells, plant cells, insect cells or animal cells, including mammalian cells. In one example, the animal cell for culturing according to the present methods is an insect cell (e.g., *Drosophila* cells, *Spodoptera* cells or *Trichoplusia* cells), a nematode cell (e.g., *C. elegans* cells) or a mammalian cell (e.g., CHO cells, COS cells, VERO cells, BHK cells, AE-1 cells, SP2/0 cells, L5.1 cells, PerC6, hybridoma cells, or other human cells). In one example, the cell is a CHO cell.

Another example provides a method of preparing a cell culture medium, comprising combining one or more small peptides or dipeptides, as described herein, with a carbohydrate, such as glucose, and at least one amino acid or salt thereof, such as arginine, asparagine, aspartic acid, cysteine, glutamic acid, histidine, isoleucine, lysine, methionine, phenylalanine, proline, hydroxyproline, serine, threonine, tryptophan, tyrosine, glutamine, and valine. In one example, the one or more dipeptides are selected from X-tyrosine, X-cysteine, tyrosine-X, or cysteine-X, or a salt thereof, wherein X is selected from alanine, glycine, serine, valine, proline, aspartic acid, or glutamic acid and wherein the N-terminal amino acid of the one or dipeptides has a free amino group. In another example, the one or more dipeptides are alanyl tyrosine and/or alanyl cysteine.

In other examples, the cell culture medium prepared according to this method is concentrated as a 2X or greater formulation, as discussed elsewhere in this

application. In certain examples, the one or more small peptides or dipeptides, such as alanyl tyrosine and/or alanyl cysteine, are present in the cell culture medium prepared according to this method.

For example, the concentration of small peptides or dipeptides in some cell culture media or the concentrated media or feeds may be about 0.5 g/L to about 30 g/L, about 0.5 g/L to about 25 g/L, about 0.5 g/L to about 20 g/L, about 0.5 g/L to about 16 g/L, about 0.5 g/L to about 10 g/L, about 0.5 g/L to about 5 g/L, about 0.5 g/L to about 4 g/L, about 1 g/L to about 30 g/L, about 1 g/L to about 20 g/L, about 1 g/L to about 16 g/L, about 1 g/L to about 10 g/L, about 1 g/L to about 5 g/L, about 2.5 g/L to about 30 g/L, about 2.5 g/L to about 20 g/L, about 2.5 g/L to about 16 g/L, about 2.5 g/L to about 10 g/L, about 2.5 g/L to about 5 g/L, or about 2.5 g/L to about 4.5 g/L, about 5 g/L to about 30 g/L, about 5 g/L to about 25 g/L, about 5 g/L to about 20 g/L, about 5 g/L to about 16 g/L, about 5 g/L to about 10 g/L, about 5 g/L to about 5 g/L, about 5 g/L to about 4 g/L, and so on.

Another example provides a composition comprising the small peptide- or dipeptide-containing cell culture medium described herein and at least one cell, as described above. In one example, the at least one cell is a CHO cell.

Yet another example is directed to kits for use in the cultivation of a cell. The kit may comprise one or more containers containing the small peptide- or dipeptide-containing cell culture medium described herein. The kit optionally comprises at least one additional component selected from at least one growth factor, at least one animal tissue extract, at least one animal organ extract, at least one animal gland extract, at least one enzyme, at least one protein, at least one vitamin, at least one cytokine, at least one lipid, at least one trace element, at least one extracellular matrix component, at least one buffer, at least one antibiotic, and at least one viral inhibitor. The kit may also comprise one or more cells or cell types.

Another example is directed to methods of producing a virus or a VLP using the small peptide- or dipeptide-containing cell culture media described herein. Specifically, the method comprises (a) contacting a cell (e.g., a mammalian cell) with a virus under conditions suitable to promote the infection of the cell by the virus; and (b) cultivating the cell in the culture media described herein under conditions suitable to promote the production of virus by the cell. In one example, the cell that produces the virus or VLP is a mammalian cell, such as a CHO cell, or an insect cell, or a plant cell, or a

fungal cell. In another example, a non-mammalian virus or VLP is engineered such that it can infect a mammalian host cell, for *e.g.*, a human cell.

In yet another example, the disclosure provides methods of producing a polypeptide, such as an immunoglobulin or a fragment thereof, using the small peptide- or dipeptide-containing cell culture media described herein. Specifically, the method comprises cultivating a cell that has been genetically engineered to produce a polypeptide in the dipeptide-containing culture media under conditions suitable for expression of the polypeptide by the cell. In one example, the cell is a mammalian cell, such as a CHO cell.

Therefore, one example of the disclosure is directed to a cell culture medium, concentrated feed, or concentrated feed supplement comprising at least one small peptide, said peptide comprising at least two amino acids, wherein at least one of the amino acids is a cysteine or a tyrosine. Another example of the disclosure is directed to a cell culture medium, concentrated feed, or concentrated feed supplement described above, wherein at least one of the remaining amino acids of the small peptide are selected from the group consisting of alanine, glycine, serine, valine, proline, aspartic acid, and glutamic acid. Yet another example of the disclosure, at least one of the remaining amino acids of the small peptide is alanine or glycine. Another example of the disclosure is directed to a cell culture medium, concentrated feed, or concentrated feed supplement medium described above, wherein at least one of the small peptides is a dipeptide selected from the group consisting of X-tyrosine, X-cysteine, tyrosine-X, and cysteine-X, or a salt thereof, and wherein X is selected from the group consisting of alanine, glycine, serine, valine, proline, aspartic acid, and glutamic acid, and/or, and wherein the N-terminal amino acid of the one or small peptides has a free amino group. In one example the X is alanine or glycine.

In some examples of this disclosure, the small peptide may be either a dipeptide, a tripeptide, a tetrapeptide, a pentapeptide, a hexapeptide, a heptapeptide, an octapeptide, a nonapeptide, or a decapeptide; or, the small peptide may be at least about two to ten amino acids in length; or in some examples, the small peptide is at least about ten amino acids in length. In some examples, the short peptide may comprise two, three, four, five or six amino acids.

In other examples, the cell culture medium is a liquid solution.

In any one of the cell culture medium described above, the cell culture medium, concentrated feed, or concentrated feed supplement is a dry powder or a granulated dry powder.

In a further example, any one of the cell culture medium described above may comprise a carbohydrate and an amino acid, or a salt thereof. In one example, the carbohydrate is a hexose. In other examples, the amino acid, or salt thereof, is any one or more of arginine, asparagine, aspartic acid, cysteine, glutamic acid, histidine, isoleucine, lysine, methionine, phenylalanine, proline, hydroxyproline, serine, threonine, tryptophan, tyrosine, and valine. In a further example, the media further comprises a vitamin, a salt, a buffering agent, or an inorganic element.

In some examples described above, the cell culture medium, concentrated feed, or concentrated feed supplement does not contain a lipid, a hydrolysate or a fraction thereof, or a growth factor. In other examples described above, the cell culture medium, concentrated feed, or concentrated feed supplement does not contain a protein. Thus in some preferred examples, the cell culture medium, concentrated feed, or reconstituted medium comprising a small peptide may be serum-free, protein-free and/or hydrolysate-free.

In particular examples, the cell culture medium, concentrated feed, or concentrated feed supplement is concentrated as a 2X or greater formulation. In a preferred example, the cell culture medium, concentrated feed, or concentrated feed supplement described above comprises one or more dipeptides, which may be either alanyl tyrosine and/or alanyl cysteine and/or alanyl cystine dimer. In a preferred example, the one or more dipeptides may be present at a concentration of about 1 g/L to about 16 g/L; or the one or more dipeptides may be present at a concentration of about 2.5 g/L to about 8.5 g/L. In one example, the liquid solution stored at 2-8°C remains free of precipitate for over 12 months.

The present disclosure is also directed to a method of culturing a cell, comprising contacting the cell with a basal cell culture medium under conditions supporting the cultivation of the cell and supplementing the basal cell culture medium with a concentrated feed or medium described above. The supplementation may be done during a fed-batch culture; or performed in addition to an existing feeding schedule; or, the supplementation may be performed continuously as opposed to incrementally with bolus additions. In some cases, the basal cell culture medium may be supplemented with the concentrated feed or medium on more than one day; or, the

supplementation of the basal cell culture medium may be done with a single concentrated feed, or with multiple concentrated feeds; or, the supplementation may occur either from day 0, day 1, day 2, day 3, day 4 or day 5 after starting the cell culture, and is supplemented daily thereafter through day 13 or 14; or, each
5 supplementation may be about 1-10%, or about 5-20%, of the total starting volume of the basal cell culture medium.

In all examples of the methods used herein, the cell may be an engineered cell; or the cell may be a recombinant cell; or the cell may be a plant cell, or the cell may be any one of an animal, a plant, an insect, an avian, yeast, algal or a fish cell. The animal
10 cell may be mammalian, insect, bovine, primate, or a pluripotent stem cell. In one example, the animal cell may be a mammalian cell; and the mammalian cell may be keratinocytes, cervical epithelial cells, bronchial epithelial cells, tracheal epithelial cells, kidney epithelial cells and retinal epithelial cells) and established cell lines and their strains (e.g., 293 embryonic kidney cells, BHK cells, HeLa cervical epithelial cells and
15 PER-C6 retinal cells, MDBK (NBL-1) cells, 911 cells, CRFK cells, MDCK cells, CHO cells, BeWo cells, Chang cells, Detroit 562 cells, HeLa 229 cells, HeLa S3 cells, Hep-2 cells, KB cells, LS180 cells, LS174T cells, NCI-H-548 cells, RPMI 2650 cells, SW-13 cells, T24 cells, WI-28 VA13, 2RA cells, WISH cells, BS-C-I cells, LLC-MK₂ cells, Clone M-3 cells, 1-10 cells, RAG cells, TCMK-1 cells, Y-1 cells, LLC-PK₁ cells,
20 PK(15) cells, GH₁ cells, GH₃ cells, L2 cells, LLC-RC 256 cells, MH₁C₁ cells, XC cells, MDOK cells, VSW cells, and TH-I, B1 cells, or derivatives thereof), fibroblast cells from any tissue or organ (including but not limited to heart, liver, kidney, colon, intestines, esophagus, stomach, neural tissue (brain, spinal cord), lung, vascular tissue (artery, vein, capillary), lymphoid tissue (lymph gland, adenoid, tonsil, bone marrow,
25 and blood), spleen, and fibroblast and fibroblast-like cell lines (e.g., CHO cells, TRG-2 cells, IMR-33 cells, Don cells, GHK-21 cells, citrullinemia cells, Dempsey cells, Detroit 551 cells, Detroit 510 cells, Detroit 525 cells, Detroit 529 cells, Detroit 532 cells, Detroit 539 cells, Detroit 548 cells, Detroit 573 cells, HEL 299 cells, IMR-90 cells, MRC-5 cells, WI-38 cells, WI-26 cells, MiCl₁ cells, CHO cells, CV-1 cells, COS-
30 1 cells, COS-3 cells, COS-7 cells, Vero cells, DBS-FrhL-2 cells, BALB/3T3 cells, F9 cells, SV-T2 cells, M-MSV-BALB/3T3 cells, K-BALB cells, BLO-11 cells, NOR-10 cells, C₃H/IOTI/2 cells, HSDM₁C₃ cells, KLN₂O₅ cells, McCoy cells, Mouse L cells, Strain 2071 (Mouse L) cells, L-M strain (Mouse L) cells, L-MTK⁻ (Mouse L) cells, NCTC clones 2472 and 2555, SCC-PSA1 cells, Swiss/3T3 cells, Indian muntjac cells,

SIRC cells, C_{II} cells, and Jensen cells, Sp2/0, NS0, NS1 cells or engineered cells thereof. In one example, the mammalian cell may be a CHO cell.

In all examples of the methods used herein, the cells may produce an immunoglobulin or a fragment thereof; and the cell may produce greater than 3000 mg/L of immunoglobulin or a fragment thereof.

Alternately, in all examples of the methods used herein, the cells may produce a virus or a virus-like particle (VLP); and the virus may be a recombinant virus. The virus may be but is not limited to an adenovirus, lentivirus, baculovirus, sendai virus, vaccinia virus, or an engineered viral derivative thereof. In the examples described herein, the VLP may carry a nucleic acid, or it may carry a RNA.

In yet another example, the present disclosure is also directed to a method of preparing a cell culture medium, concentrated feed or cell culture supplement, comprising combining one or more small peptides with a carbohydrate and at least one amino acid or salt thereof, wherein each of the one or more small peptides comprises a cysteine or a tyrosine. In one example, the amino acids of the one or more small peptides, which are other than cysteine or tyrosine, (or referred to as the remaining amino acids) may be selected from the group consisting of alanine, glycine, serine, valine, proline, aspartic acid, arginine, glutamine or glutamic acid. In one preferred example, the amino acids other than cysteine or tyrosine, are alanine or glycine. In another set, the remaining amino acids may also be one or more of arginine, asparagine, aspartic acid, cysteine, glutamic acid, histidine, isoleucine, lysine, methionine, phenylalanine, proline, hydroxyproline, serine, threonine, tryptophan, tyrosine, and valine. In another preferred example of the above method, one or more dipeptides may be selected from X-tyrosine, X-cysteine, tyrosine-X, or cysteine-X, or a salt thereof, wherein X is selected from alanine, glycine, serine, valine, proline, aspartic acid, arginine or glutamic acid, and wherein the N-terminal amino acid of the one or more dipeptide has a free amino group. In a most preferred example, X is alanine or glycine. In a most preferred example, the carbohydrate may be glucose. Additionally, the cell culture medium may further comprise a vitamin, a salt, a buffering agent, or an inorganic element; or, may not contain a lipid, a hydrolysate or a fraction thereof, or a growth factor; or may not contain a protein; or, the cell culture medium may be concentrated as a 2X or greater formulation; or, the one or more dipeptides may be alanyl tyrosine and/or alanyl cysteine; or, the one or more dipeptides are present in the cell culture medium at a concentration of about 1 g/L to about 16 g/L; or preferably, the one or more dipeptides

are present at a concentration of about 2.5 g/L to about 8.5 g/L. In most examples, the cell culture medium stored at 2-8°C remains free of precipitate for over 12 months.

The present disclosure is also directed to a method for analyzing a cell culture medium, concentrated feed, or reconstituted medium, the method comprising
5 determining the presence or absence of a short peptide comprising cysteine or tyrosine in the medium. In one example, the cell culture medium, concentrated feed, or reconstituted medium may be serum-free, protein-free, hydrolysate-free, and the analyzing may be performed by, but may not be limited to, mass spectrometry (LCMS); capillary electrophoresis and HPLC.

10 The disclosure is also directed to a composition comprising the cell culture medium, concentrated feed, or reconstituted medium described above and a cell.

The disclosure is also directed to a method of making a recombinant protein in a cell culture medium comprising: contacting a cell with the cell culture medium, concentrated feed, or reconstituted medium described above; and cultivating said cell
15 under conditions suitable for the growth of said cell and/or the expression of said recombinant protein.

The disclosure is also directed to a kit for the cultivation of a cell *in vitro*, said kit comprising one or more containers, wherein a first container contains a medium comprising at least one dipeptide described above, and wherein said medium supports
20 the growth of the cell in culture and/or the expression of recombinant protein. The culturing of the cell may be in suspension or in adherent culture. The growth may be high-density growth.

The disclosure is also directed to a method of making a virus or a viral particle in a cell culture medium, concentrated feed, or reconstituted medium described above comprising: culturing a recombinant cell in the cell culture medium described above,
25 wherein the medium supports the growth of said cell under conditions suitable for expression of said virus or viral particle.

The disclosure is also directed to a recombinant protein produced by the methods described above; or, the virus or viral particle produced by the methods
30 described above; or, the use of a cell culture medium, concentrated feed, or reconstituted medium described above, to produce a virus or a recombinant protein; or, a culture medium, concentrated feed, or reconstituted medium produced by the methods described above.

What is claimed:

1. A supplement comprising alanine-tyrosine and alanine-cysteine dimer.
- 5 2. The supplement of claim 1, which is a liquid solution.
3. The supplement of claim 1, which is a dry powder.
4. The supplement of claim 1, wherein the alanine-tyrosine and alanine-cysteine dimer each
10 are present at a concentration of 1 g/L to 16 g/L.
5. The supplement of claim 4, wherein the alanine-tyrosine and alanine-cysteine dimer each
are present at a concentration of 2.5 g/L to 8.5 g/L.
- 15 6. The supplement of claim 1, wherein the alanine-cysteine dimer is L-alanyl-L-cysteine
disulfide.
7. The supplement of claim 6, wherein the supplement is used for culturing a cell.
- 20 8. The supplement of claim 1, wherein the supplement increases the titer of a
recombinant protein produced in a cell, and wherein the cell is a genetically engineered
mammalian cell.
9. The supplement of claim 8, wherein the supplement is a cell culture supplement.