bond to form cystine ($SCH_2CH(NH_2)CO_2H)_2$. 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 invention 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, there is provided a serum-free, protein-free concentrated feed comprising small peptides comprising cysteine and tyrosine, each of said peptides comprising six or fewer amino acids, wherein for each said peptide at least one of the amino acids is a cysteine and/or a tyrosine, and at least one of the remaining amino

acids is selected from the group consisting of alanine, serine, threonine, glycine, valine, proline, arginine, asparagine, glutamine, histidine, lysine, aspartic acid, and glutamic acid, preferably alanine or glycine, and wherein said eukaryotic concentrated feed is hydrolysate-free.

In one aspect, there is provided a method of preparing a serum-free, protein-free concentrated feed, comprising combining small peptides with a carbohydrate, preferably glucose, and at least one amino acid or salt thereof, wherein the small peptides are peptides as disclosed herein.

In one aspect, there is provided a kit for the cultivation of a cell in vitro, said kit comprising one or more containers, wherein a first container contains a serum-free, protein-free concentrated feed as disclosed herein, and wherein said serum-free, protein-free concentrated feed supports the growth of the cell in culture and/or the expression of recombinant protein or a virus and/or a viral particle.

In one aspect, there is provided a use of a serum-free, protein-free concentrated feed as disclosed herein for culturing cells and/or to produce a virus or a recombinant protein, wherein said cells are selected from insect cells, nematode cells, CHO cells, COS cells, VERO cells, BHK cells, AE-1 cells, SP2/0 cells, L5.1 cells, PerC6, and hybridoma cells.

In one aspect, there is provided a hydrolysate-free cell culture medium comprising peptides, wherein each peptide comprises six or fewer amino acids, wherein for each of said peptide comprises at least a cysteine and/or a tyrosine, wherein at least one of the remaining amino acids is selected from the group consisting of alanine, serine, threonine, glycine, valine, proline, arginine, asparagine, glutamine, histidine, lysine, aspartic acid, and glutamic acid, and wherein the medium supports increased eukaryotic cell growth and/or increased protein production or increased virus production.

In one aspect, there is provided a eukaryotic cell culture system in suspension, comprising a serum-free and protein-free cell culture medium, at least one peptide and a cell, wherein the at least one peptide comprises six or fewer amino acids; wherein each of said peptides comprises at least a cysteine and/or a tyrosine; wherein the peptide is not hydrolysate-derived; wherein at least one of the remaining amino acids is selected from the group consisting of alanine, serine, threonine, glycine, valine, proline, arginine, asparagine, glutamine, histidine, lysine, aspartic acid, and glutamic acid; and

wherein the culture supports increased cell growth, or produces increased protein or virus.

In one ~~aspect~~embodiment, the media, concentrated feed supplements, or concentrated media may be serum-free compositions. In one embodiment~~aspect~~, the compositions may comprise human serum components, like human serum albumin. In a further aspect, the human serum albumin may be recombinant, (r- human serum albumin), derived from recombinant sources, in

some preferred cases, form 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 embodiment~~aspect~~, the media, concentrated feed supplements, or concentrated media may be protein-free compositions. In yet another embodiment~~aspect~~, 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 embodiment~~aspect~~, the media, concentrated feed supplements, or concentrated media may be serum-free, protein-free, and protein hydrolysate-free compositions. In a preferred embodiment~~aspect~~, these compositions may comprise components that are chemically-defined, serum-free, protein-free and free of any protein hydrolysates, or fractions thereof. In certain embodiments, 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 ~~invention~~ 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 X_{1-5}-tyrosine, X_{1-5}-cysteine, tyrosine- X_{1-5}, and cysteine- X_{1-5}, 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- X_{1-4}, X-tyrosine- X_{1-4}, 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 embodiment, X is alanine or glycine. In another embodiment X is serine, valine, proline, aspartic acid, or glutamic acid. In a particular embodiment, 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 embodiment, X is alanine or glycine. In

another embodiment X is serine, valine, proline, aspartic acid, or glutamic acid. In yet another embodiment, 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 embodiment, the liquid solution is stored at 2-8°C and remains free of precipitate for over 12 months.

In one embodiment of the ~~invention~~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 embodiment, 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 embodiment of the ~~invention~~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 embodiment, the solubility of cysteine (or lack thereof) would include, for example, loss of solubility caused by conversion of cysteine to cystine. In one embodiment, 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 embodiment, the solution is supersaturated for both tyrosine and cysteine.

In one embodiment, 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 embodiment, 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 embodiment, 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 invention. The ~~invention~~ 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 embodiment 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 embodiment, the carbohydrate is glucose.

In one embodiment, 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 embodiment, 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 embodiment, 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 embodiments, the cell culture medium having one or more small peptides or one or more dipeptides is a 1X formulation. In other embodiment, the cell culture medium is concentrated as a 2X or greater formulation, as discussed elsewhere in this application. In certain embodiments, 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 ~~aspect~~embodiment, 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 embodiment, 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).

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 invention. In a specific ~~aspect~~ embodiment, 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 embodiment, the cell can produce a virus or a VLP (virus like particle). In yet another embodiment, the cell may produce a desired cell product like a vitamin, a metabolite, a glycoprotein, a carbohydrate, a lipid, or a lipoprotein. In another embodiment, the cell itself is grown to be harvested. The media and feed supplement compositions of the invention 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 embodiment, 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 embodiment, the cell is a CHO cell.

Another ~~aspect~~ embodiment 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 embodiment, 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 embodiment, the one or more dipeptides are alanyl tyrosine and/or alanyl cysteine.

In other embodiments, 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 embodiments, 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 ~~aspect~~ embodiment 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 embodiment, the at least one cell is a CHO cell.

Yet another ~~aspect~~ embodiment 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 ~~aspect~~ embodiment 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 embodiment, 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 embodiment, 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 embodiment~~aspect~~, 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 embodiment, the cell is a mammalian cell, such as a CHO cell.

Therefore, one embodiment ~~aspect~~ of the ~~invention~~ 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 embodiment ~~aspect~~ of the ~~invention~~ 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 embodiment ~~aspect~~ of the ~~invention~~disclosure, at least one of the remaining amino acids of the small peptide is alanine or glycine. Another embodiment ~~aspect~~ of the ~~invention~~ 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 embodiment ~~aspect~~ the X is alanine or glycine.

In some embodiments ~~aspects~~ of this ~~invention~~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 embodiments~~aspects~~, the small peptide is at least about ten amino acids in length. In some embodiments~~aspects~~, the short peptide may comprise two, three, four, five or six amino acids.

In other embodiments~~aspects~~, 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 embodimentaspect, any one of the cell culture medium described above may comprise a carbohydrate and an amino acid, or a salt thereof. In one embodiment, the carbohydrate is a hexose. In other embodiments, 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 embodiment, the media further comprises a vitamin, a salt, a buffering agent, or an inorganic element.

In some embodiments aspects 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 embodiments aspects described above, the cell culture medium, concentrated feed, or concentrated feed supplement does not contain a protein. Thus in some preferred aspects, 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 embodimentsaspects, the cell culture medium, concentrated feed, or concentrated feed supplement is concentrated as a 2X or greater formulation. In a preferred embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, the liquid solution stored at 2-8°C remains free of precipitate for over 12 months.

The present invention 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 supplementation may be about 1-10%, or about 5-20%, of the total starting volume of the basal cell culture medium.

In all embodiments ~~aspects~~ 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 cell may be mammalian, insect, bovine, primate, or a pluripotent stem cell. In one embodiment~~aspect~~, 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 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-MK_2 cells, Clone M-3 cells, 1-10 cells, RAG cells, TCMK-1 cells, Y-1 cells, LLC-PK_1 cells, PK(15) cells, GH_1 cells, GH_3 cells, L2 cells, LLC-RC 256 cells, MH_1C_1 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, 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, $MiCl_1$ cells, CHO cells, CV-1 cells, COS-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, C_3H/IOTI/2 cells, $HSDM_1C_3$ cells, KLN_2O_5 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 embodimentaspect, the mammalian cell may be a CHO cell.

In all embodiments aspects 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 embodiments aspects 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 embodiments aspects described herein, the VLP may carry a nucleic acid, or it may carry a RNA.

In yet another embodimentaspect, the present invention 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 embodiment, 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 aspect, 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 embodiment aspect 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 embodimentaspect, X is alanine or glycine. In a most preferred embodimentaspect, 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 aspects, the cell culture medium stored at 2-8°C remains free of precipitate for over 12 months.

The present ~~invention~~ disclosure is also directed to a method for analyzing a cell culture medium, concentrated feed, or reconstituted medium, the method comprising determining the presence or absence of a short peptide comprising cysteine or tyrosine in the medium. In one embodiment~~aspect~~, 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.

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

The ~~invention~~ 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 under conditions suitable for the growth of said cell and/or the expression of said recombinant protein.

The ~~invention~~ 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 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 ~~invention~~ 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, wherein the medium supports the growth of said cell under conditions suitable for expression of said virus or viral particle.

The ~~invention~~ disclosure is also directed to a recombinant protein produced by the methods described above; or, the virus or viral particle produced by the methods 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 cell culture medium 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.

2. The cell culture medium of claim 1, 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.

3. The cell culture medium of claim 2, wherein at least one of the remaining amino acids of the small peptide is alanine or glycine.

4. The cell culture medium of claim 1, 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.

5. The cell culture medium of claim 1, wherein X is alanine or glycine.

6. The cell culture medium of any one of claims 1-5, wherein the cell culture medium is a liquid solution.

7. The cell culture medium of any one of claims 1-5, wherein the cell culture medium is a dry powder or a granulated dry powder.

8. The cell culture medium of any one of claims 1-7, further comprising a carbohydrate and an amino acid, or a salt thereof.

9. The cell culture medium of claim 8, wherein the carbohydrate is a hexose.

10. The cell culture medium of claim 8 or 9, wherein the amino acid, or salt thereof, is one or more of arginine, asparagine, aspartic acid, cysteine, glutamic acid, histidine, isoleucine, lysine, methionine, phenylalanine, proline, hydroxyproline, serine, threonine, tryptophan, tyrosine, and valine.

11. The cell culture medium of any one of claims 1-10, further comprising a vitamin, a salt, a buffering agent, or an inorganic element.

12. The cell culture medium of any one of claims 1-11, wherein the cell culture medium does not contain a lipid, a hydrolysate or a fraction thereof, or a growth factor.

13. The cell culture medium of any one of claims 1-12, wherein the cell culture medium does not contain a protein.

14. The cell culture medium of any one of claims 1-13, wherein the cell culture medium is concentrated as a 2X or greater formulation.

15. The cell culture medium of any one of claims 4-14, wherein the one or more dipeptides are alanyl tyrosine, and/or alanyl cysteine, and/or alanyl cystine dimer.

16. The cell culture medium of any one of claims 4-6 or 8-14, wherein the one or more dipeptides are present at a concentration of about 1 g/L to about 16 g/L.

17. The cell culture medium of any one of claims 4-6 or 8-14, wherein the one or more dipeptides are present at a concentration of about 2.5 g/L to about 8.5 g/L.

18. The cell culture medium of any one of claims 6 or 8-17, wherein the liquid solution stored at 2-8°C remains free of precipitate for over 12 months.

19. 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 according to any one of claims 1-18.

20. The method according to claim 19, wherein the basal cell culture medium is supplemented with the concentrated feed or medium on more than one day.

21. The method of claim 20, wherein the supplementation of the basal cell culture medium with the concentrated feed or medium occurs 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.

22. The method of any one of claims 19-21, wherein the supplementation of the basal cell culture medium is at about 1-10%, or about 5-20%, of the total starting volume of the basal cell culture medium.

23. The method of any one of claims 19-22, wherein the cell is an animal, a plant, an insect, an avian, yeast, algal, or a fish cell.

24. The method of claim 22, wherein the cell produces an immunoglobulin or a fragment thereof.

25. The method of claim 24, wherein the cell produces greater than 3000 mg/L of immunoglobulin or a fragment thereof.

26. A method of preparing a cell culture medium, 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.

27. The method of claim 26, wherein the amino acids of the one or more small peptides, other than cysteine or tyrosine, are selected from alanine, glycine, serine, valine, proline, aspartic acid, arginine, glutamine or glutamic acid.

28. The method of claim 27, wherein the amino acids of the one or more small peptides, other than cysteine or tyrosine, are alanine or glycine.

29. The method of claim 26, wherein the one or more small peptides are 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, arginine or glutamic acid and wherein the N-terminal amino acid of the one or dipeptides has a free amino group.

30. The method of claim 29, wherein X is alanine or glycine.

31. The method of any one of claims 26-30, wherein the carbohydrate is glucose.

32. The method of any one of claims 26-31, wherein at least one the amino acid, or salt thereof, is one or more of arginine, asparagine, aspartic acid, cysteine, glutamic acid,

histidine, isoleucine, lysine, methionine, phenylalanine, proline, hydroxyproline, serine, threonine, tryptophan, tyrosine, and valine.

33. The method of any one of claims 26-32, wherein the cell culture medium further comprises a vitamin, a salt, a buffering agent, or an inorganic element.

34. The method of any one of claims 26-33, wherein the cell culture medium does not contain a lipid, a hydrolysate or a fraction thereof, or a growth factor.

35. The method of any one of claims 26-34, wherein the cell culture medium does not contain a protein.

36. The method of any one of claims 26-35, wherein the cell culture medium is concentrated as a 2X or greater formulation.

37. The method of any one of claims 29-36, wherein the one or more dipeptides are alanyl tyrosine and/or alanyl cysteine.

38. The method of any one of claims 29-37, wherein 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.

39. The method of any one of claims 29-37, wherein the one or more dipeptides are present at a concentration of about 2.5 g/L to about 8.5 g/L.

40. The method of any one of claims 29-39, wherein the cell culture medium stored at 2-8°C remains free of precipitate for over 12 months.

41. A method for analyzing a cell culture medium, concentrated feed, or reconstituted medium, the method comprising determining the presence or absence of a short peptide comprising cysteine or tyrosine in the medium.

42. The method for analyzing a serum-free, protein-free, hydrolysate-free, cell culture medium, concentrated feed, or reconstituted medium of claim 41 wherein the analyzing is performed by a method selected from the group comprising: mass spectrometry (LCMS); capillary electrophoresis and HPLC.

43. The method for analyzing a serum-free, protein-free, hydrolysate-free, cell culture medium, concentrated feed, or reconstituted medium of claim 41 wherein the short peptide comprises two, three, four, five or six amino acids.

44. The method for analyzing a medium of claim 41 wherein the short peptide is a dipeptide.

45. The method of claim 23, wherein the CHO cell produces a virus.

46. The method of claim 45, wherein the virus is a recombinant virus or a virus-like particle (VLP).

47. The method of claim 46, wherein the virus is an adenovirus, lentivirus, baculovirus, sendai virus, vaccinia virus, or an engineered viral derivative thereof.

48. The method of claim 46, wherein the VLP carries a nucleic acid.

49. The method of claim 48, wherein the nucleic acid is RNA.

50. The cell culture medium of claim 9, wherein the hexose is selected from the group consisting of glucose, galactose, fructose and maltose.

51. A cell culture medium, concentrated feed, or reconstituted medium comprising a small peptide comprising cysteine or tyrosine, wherein said cell culture medium, concentrated feed, or reconstituted medium is serum-free, protein-free and/or hydrolysate-free.

52. The cell culture medium, concentrated feed, or reconstituted medium of claim 51 wherein said small peptide comprises two, three, four, five or six amino acids.

53. A cell culture medium, concentrated feed, or reconstituted medium of claim 1 that supports the production of a recombinant protein.

54. The cell culture medium, concentrated feed, or reconstituted medium of claim 53 wherein the recombinant protein is an immunoglobulin or a fragment thereof.

55. A composition comprising the cell culture medium, concentrated feed, or reconstituted medium of claim 1 and a cell.

56. 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 of Claim 1; and cultivating said cell under conditions suitable for the growth of said cell and/or the expression of said recombinant protein.

57. 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 according to claim 1, and wherein said medium supports the growth of the cell in culture and/or the expression of recombinant protein.

58. The kit of claim 57 wherein said culture is in suspension or is adherent.

59. The kit of claim 57 wherein said growth is high-density growth.

60. A method of making a virus or a viral particle in a cell culture medium, concentrated feed, or reconstituted medium of claim 1 comprising: culturing a recombinant cell in the cell culture medium of claim 1, wherein the medium supports the growth of said cell under conditions suitable for expression of said virus or viral particle.

61. A recombinant protein produced by the method of claim 56.

62. A virus or viral particle produced by the method of claim 60.

63. Use of a cell culture medium, concentrated feed, or reconstituted medium of claim 1 to produce a virus or a recombinant protein.

64. A culture medium, concentrated feed, or reconstituted medium produced by the method of claim 26.

65. The cell culture medium of claim 1 or 64, wherein the small peptide is either a dipeptide, a tripeptide, a tetrapeptide, a pentapeptide, a hexapeptide, a heptapeptide, an octapeptide, a nonapeptide, or a decapeptide.

66. The cell culture medium of claim 1 or 64, wherein the small peptide is at least about two to ten amino acids in length.

67. The cell culture medium of claim 1 or 64, wherein the small peptide is at least about ten amino acids in length.

68. The method of claim 22, wherein said supplementation is done during a fed-batch culture.

CLAIMS

1. A serum-free, protein-free concentrated feed comprising small peptides comprising cysteine and tyrosine,
each of said peptides comprising six or fewer amino acids,
wherein for each said peptide at least one of the amino acids is a cysteine and/or a tyrosine, and at least one of the remaining amino acids is selected from the group consisting of alanine, serine, threonine, glycine, valine, proline, arginine, asparagine, glutamine, histidine, lysine, aspartic acid, and glutamic acid, preferably alanine or glycine,
and wherein said eukaryotic concentrated feed is hydrolysate-free.

2. The serum-free, protein-free concentrated feed of claim 1, wherein the concentrated feed is at a neutral pH.

3. The serum-free, protein-free concentrated feed of any of the claims 1 or 2, which increases the titer of a recombinant protein produced in the cell culture system.

4. The serum-free, protein-free concentrated feed of any of the claims 1 to 3, which increases the viable cell density of the cell culture system.

5. The serum-free, protein-free concentrated feed of claim 1 to 4, 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, valine, proline, aspartic acid, and glutamic acid, preferably wherein X is alanine or glycine and/or, and wherein the N-terminal amino acid of the one of the small peptides has a free amino group.

6. The serum-free, protein-free concentrated feed of any one of claims 1-5, wherein the cell culture medium or concentrated feed is a liquid solution or a dry powder or a granulated dry powder.

7. The serum-free, protein-free concentrated feed of any one of the preceding claims, wherein the small peptides or dipeptides are present at a concentration of 1 g/L to 16 g/L, preferably 2.5 g/L to 8.5 g/L.

8. The serum-free, protein-free concentrated feed of any one of the preceding claims which is a liquid solution and remains free of precipitate for over 12 months when stored at 2-8°C.

9. A method of preparing a serum-free, protein-free concentrated feed, comprising combining small peptides with a carbohydrate, preferably glucose, and at least one amino acid or salt thereof, wherein the small peptides are peptides as defined in any one of the preceding claims.

10. The method of claim 9, wherein the serum-free, protein-free concentrated feed further comprises a vitamin, a salt, a buffering agent, or an inorganic element and optionally an animal-origin free peptide carrier protein which is animal origin free, and/or wherein it does not contain a lipid, a hydrolysate or a fraction thereof, or a growth factor .

11. The method of claim 10 wherein the concentrated feed is added during fed-batch culture or added continuously, to a bioreactor or a fermenter.

12. A kit for the cultivation of a cell in vitro, said kit comprising one or more containers, wherein a first container contains a serum-free, protein-free concentrated feed according to any one of claims 1 to 11, and wherein said serum-free, protein-free

concentrated feed supports the growth of the cell in culture and/or the expression of recombinant protein or a virus and/or a viral particle.

13. Use of a serum-free, protein-free concentrated feed, according to any one of claims 1 to 11 or the kit of claim 12 for culturing cells and/or to produce a virus or a recombinant protein, wherein said cells are selected from insect cells, nematode cells, CHO cells, COS cells, VERO cells, BHK cells, AE-1 cells, SP2/0 cells, L5.1 cells, PerC6, and hybridoma cells.

14. A hydrolysate-free cell culture medium comprising peptides, wherein each peptide comprises six or fewer amino acids, wherein for each of said peptide comprises at least a cysteine and/or a tyrosine, wherein at least one of the remaining amino acids is selected from the group consisting of alanine, serine, threonine, glycine, valine, proline, arginine, asparagine, glutamine, histidine, lysine, aspartic acid, and glutamic acid, and wherein the medium supports increased eukaryotic cell growth and/or increased protein production or increased virus production.

15. A eukaryotic cell culture system in suspension, comprising a serum-free and protein-free cell culture medium, at least one peptide and a cell,
wherein the at least one peptide comprises six or fewer amino acids; wherein each of said peptides comprises at least a cysteine and/or a tyrosine; wherein the peptide is not hydrolysate-derived;
wherein at least one of the remaining amino acids is selected from the group consisting of alanine, serine, threonine, glycine, valine, proline, arginine, asparagine, glutamine, histidine, lysine, aspartic acid, and glutamic acid; and wherein the culture supports increased cell growth, or produces increased protein or virus.