SUMMARY

[0008] In a first aspect, there is provided a method of reducing oxidation of a polypeptide in an aqueous formulation comprising adding an amount of N-acetyltryptophan to the formulation that prevents oxidation of the polypeptide, wherein the polypeptide comprises at least one tryptophan residue with a solvent-accessible surface area (SASA) of greater than about 80Å^2. In a second aspect, there is provided a method of reducing oxidation of a polypeptide in an aqueous formulation comprising adding an amount of N-acetyltryptophan to the formulation that prevents oxidation of the polypeptide, wherein the polypeptide comprises at least one tryptophan residue with a solvent-accessible surface area (SASA) of greater than about 30%. In a third aspect, there is provided a method of reducing oxidation of a polypeptide in an aqueous formulation comprising determining the SASA values of tryptophan residues in the polypeptide and adding an amount of N-acetyltryptophan to the formulation that prevents oxidation of the polypeptide if at least one tryptophan residue has a solvent-accessible surface area (SASA) of greater than about 80Å^2. In some embodiments, the SASA value of the tryptophan residues in calculated by molecular dynamic simulation.

[0009] In some embodiments, the N-acetyltryptophan is added to the formulation to a concentration of about 0.1 mM to about 5 mM. In some embodiments, the N-acetyltryptophan is added to the formulation to a concentration of about 0.1 mM to about 1 mM. In some embodiments, the N-acetyltryptophan is added to the formulation to a concentration about 0.3 mM.

[0010] In some embodiments, the oxidation of the polypeptide is reduced by about 50%, 75%, 80%, 85%, 90%, 95% or 99%. In some embodiments, the formulation is stable at about 2 °C to about 8 °C for about 1095 days.

[0011] In some embodiments, the protein concentration in the formulation is about 1 mg/mL to about 250 mg/mL. In some embodiments, the formulation has a pH of about 4.5 to about 7.0. In some embodiments, the formulation further comprises one or more excipients selected from the group consisting of a stabilizer, a buffer, a surfactant, and a tonicity agent.

[0012] In some embodiments, the formulation is a pharmaceutical formulation suitable for administration to a subject. In some embodiments, the protein is an antibody. In some embodiments, the antibody is a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric antibody, a multispecific antibody, or an antibody fragment.

[0013] In a fourth aspect, there is provided a liquid formulation comprising a polypeptide and an amount of N-acetyltryptophan to prevent oxidation of the polypeptide,

wherein the polypeptide has at least one tryptophan residue with a SASA of greater than about 80Å^2. In some embodiments, the N-acetyltryptophan is added to the formulation to a concentration of about 0.1 mM to about 5 mM. In some embodiments, the N-acetyltryptophan is added to the formulation to a concentration of about 0.1 mM to about 1 mM. In some embodiments, the N-acetyltryptophan is added to the formulation to a concentration about 0.3 mM. In some embodiments, the oxidation of the polypeptide is reduced by about 50%, 75%, 80%, 85%, 90%, 95% or 99%. In some embodiments, the formulation is stable at about 2 °C to about 8 °C for about 1065 days.

[0014] In some embodiments, the protein concentration in the formulation is about 1 mg/mL to about 250 mg/mL. In some embodiments, the formulation has a pH of about 4.5 to about 7.0. In some embodiments, the formulation further comprises one or more excipients selected from the group consisting of a stabilizer, a buffer, a surfactant, and a tonicity agent.

[0015] In some embodiments, the formulation is a pharmaceutical formulation suitable for administration to a subject. In some embodiments, the protein is an antibody. In some embodiments, the antibody is a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric antibody, a multispecific antibody, or an antibody fragment.

[0016] In a fifth aspect, there is provided a method for screening a formulation for reduced oxidation of a polypeptide wherein the polypeptide comprises at least one tryptophan residue with a SASA of greater than about 80Å^2, the method comprising adding an amount of N-acetyltryptophan to an aqueous composition comprising the polypeptide, adding 2,2'-azobis (2-aminopropane) dihydrochloride (AAPH) to the composition, incubating the composition comprising the polypeptide, N-acetyltryptophan and AAPH for about 14 hours at about 40 °C, measuring the polypeptide for oxidation of tryptophan residues in the polypeptide, wherein a formulation comprising an amount of N-acetyltryptophan that results in no more than about 20% oxidation of tryptophan residues of the polypeptide is a suitable formulation for reduced oxidation of the polypeptide. In some embodiments, the N-acetyltryptophan and AAPH are incubated for less than about any of 10 hours, 11 hours, 12 hours, 14 hours, 16 hours, 20 hours, or 24 hours. In some embodiments, no more than about any of 15%, 20% 25%, 30%, or 35%, oxidation of tryptophan residues of the polypeptide is a suitable formulation for reduced oxidation of the polypeptide.

[0017] In a sixth aspect, there is provided a method for screening a formulation for reduced oxidation of a polypeptide comprising determining the SASA values of tryptophan residues in the polypeptide, wherein a tryptophan residue with a SASA of greater than about

80Å2, is subject to oxidation, adding an amount of N-acetyltryptophan to an aqueous composition comprising the polypeptide, adding 2,2'-azobis (2-aminopropane) dihydrochloride (AAPH) to the composition, incubating the composition comprising the polypeptide, N-acetyltryptophan and AAPH for about 14 hours at about 40 °C, measuring the polypeptide for oxidation of tryptophan residues in the polypeptide, wherein a formulation comprising an amount of N-acetyltryptophan that results in no more than about 20% oxidation of tryptophan residues of the polypeptide is a suitable formulation for reduced oxidation of the polypeptide.

[0018] In some embodiments of the above aspects, the SASA value of the tryptophan residues in calculated by molecular dynamic simulation.

[0019] In a seventh aspect, there is provided a kit comprising the liquid formulation of the fourth aspect. In an eighth aspect, there is provided an article of manufacture comprising the liquid formulation of the fourth aspect.

[0020] Provided herein are formulations comprising a protein and N-acetyl-tryptophan (NAT), and methods of making and using the formulations.

[0021] In some embodiments, the liquid formulation is a pharmaceutical formulation suitable for administration to a subject. In some embodiments, the formulation is aqueous.

[0022] In some embodiments, the NAT prevents oxidation of tryptophan in the protein.

[0023] In some embodiments, the protein in the formulation is susceptible to oxidation. In some embodiments, tryptophan in the protein is susceptible to oxidation. In some embodiments, the protein is an antibody (e.g., a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric antibody, or antibody fragment). In some embodiments, the protein concentration in the formulation is about 1 mg/mL to about 250 mg/mL.

[0024] In some embodiments, the formulation further comprises one or more excipients selected from the group consisting of a stabilizer, a buffer, a surfactant, and a tonicity agent. In some embodiments, the formulation has a pH of about 4.5 to about 7.0.

[0025] In a ninth aspect, there is provided a method to determine if a polypeptide in a liquid formulation comprises a tryptophan residue susceptible to oxidation, the method comprising calculating one or more molecule descriptors based on the amino acid sequence of the polypeptide for each tryptophan residue in the polypeptide and applying the one or more molecule descriptors to a machine learning algorithm trained on the one or more molecule descriptors to predict tryptophan oxidation, wherein the molecule descriptors include one or

more of the following: a) number of aspartic acid sidechain oxygens within 7Å of tryptophan delta carbon, b) sidechain solvent accessible surface area (SASA), c) delta carbon SASA, d) total positive charge within 7Å of tryptophan delta carbon, e) backbone SASA, f) tryptophan sidechain angles, g) packing density within 7Å of tryptophan delta carbon, h) tryptophan backbone angles, i) SASA of pseudo-pi orbitals, j) backbone flexibility, or k) total negative charge within 7Å of tryptophan delta carbon. In some embodiments, two, three, four, five, six, seven, eight, nine, ten or eleven molecule descriptors are used. In some embodiments, the molecule descriptors comprise the following: a) number of aspartic acid sidechain oxygens within 7Å of tryptophan delta carbon, b) sidechain solvent accessible surface area (SASA), c) delta carbon SASA, d) total positive charge within 7Å of tryptophan delta carbon, e) backbone SASA, f) tryptophan sidechain angles, and g) packing density within 7Å of tryptophan delta carbon. In some embodiments, oxidation of greater than 35% of tryptophan residues at a particular site indicates susceptibility to oxidation. In some embodiments, the protein is an antibody. In some embodiments, the antibody is a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric antibody, a multispecific antibody, or an antibody fragment.

[0026] In some embodiments, the machine learning algorithm was trained by matching molecule descriptors from molecular dynamic simulations of polypeptides based on amino acid sequence of the polypeptide with experimental data for each tryptophan residue in the polypeptide. In some embodiments, the one or more molecule descriptors are calculated using a computer.

[0027] In a tenth aspect, there is provided a method to reduce oxidation of a polypeptide, comprising identifying tryptophan residues susceptible to oxidation according to the method as described herein and introducing an amino acid substitution in the polypeptide to replace one or more tryptophan residues susceptible to oxidation with amino acid residues that are not subject to oxidation. In an eleventh aspect, there is provided a method to reduce oxidation of a polypeptide, comprising introducing an amino acid substitution in the polypeptide to replace one or more tryptophan residues susceptible to oxidation, wherein the one or more tryptophan residues susceptible to oxidation was identified by the method as described herein. In some embodiments, the tryptophan residue is replaced by an amino acid residue selected from the group consisting of tyrosine, phenylalanine, leucine, isoleucine, alanine, and valine.

[0028] In a twelfth aspect, there is provided a method to reduce oxidation of a polypeptide in an aqueous formulation, comprising determining the presence of one or more tryptophan residues in the polypeptide susceptible to oxidation according to the method as described herein, and adding an effective amount of an anti-oxidation agent to the aqueous formulation comprising a polypeptide having a one or more tryptophan residues susceptible to oxidation. In a thirteenth aspect, there is provided a method to reduce oxidation of a polypeptide in an aqueous formulation, comprising adding an amount of an anti-oxidation agent to the aqueous formulation to prevent oxidation, wherein polypeptide comprises one or more tryptophan residues susceptible to oxidation identified by the method as described herein. In some embodiments, the anti-oxidation agent is N-acetyltryptophan. In some embodiments, the N-acetyltryptophan is added to the formulation to a concentration of about 0.1 mM to about 5 mM. In some embodiments, the N-acetyltryptophan is added to the formulation to a concentration of about 0.1 mM to about 1 mM. In some embodiments, the N-acetyltryptophan is added to the formulation to a concentration about 0.3 mM. In some embodiments, the oxidation of the polypeptide is reduced by about 50%, 75%, 80%, 85%, 90%, 95% or 99%. In some embodiments, the formulation is stable at about 2 °C to about 8 °C for about 1095 days. In some embodiments, the protein concentration in the formulation is about 1 mg/mL to about 250 mg/mL. In some embodiments, the formulation has a pH of about 4.5 to about 7.0. In some embodiments, the formulation further comprises one or more excipients selected from the group consisting of a stabilizer, a buffer, a surfactant, and a tonicity agent. In some embodiments, the formulation is a pharmaceutical formulation suitable for administration to a subject. In some embodiments, the protein is an antibody. In some embodiments, the antibody is a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric antibody, a multispecific antibody, or an antibody fragment.

[0029] In a fourteenth aspect, there is provided a liquid formulation comprising a polypeptide and an amount of N-acetyltryptophan to prevent oxidation of the polypeptide, wherein the polypeptide has at least one tryptophan residue susceptible to oxidation as measured by the method as described herein. In some embodiments, the N-acetyltryptophan is added to the formulation to a concentration of about 0.1 mM to about 5 mM. In some embodiments, the N-acetyltryptophan is added to the formulation to a concentration of about 0.1 mM to about 1 mM. In some embodiments, the N-acetyltryptophan is added to the formulation to a

concentration about 0.3 mM. In some embodiments, the oxidation of the polypeptide is reduced by about 50%, 75%, 80%, 85%, 90%, 95% or 99%. In some embodiments, the formulation is stable at about 2 °C to about 8 °C for about 1065 days. In some embodiments, the protein concentration in the formulation is about 1 mg/mL to about 250 mg/mL. In some embodiments, the formulation has a pH of about 4.5 to about 7.0. In some embodiments, the formulation further comprises one or more excipients selected from the group consisting of a stabilizer, a buffer, a surfactant, and a tonicity agent. In some embodiments, the formulation is a pharmaceutical formulation suitable for administration to a subject. In some embodiments, the protein is an antibody. In some embodiments, the antibody is a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric antibody, a multispecific antibody, or an antibody fragment. In some embodiments, there is provided a kit comprising the liquid formulation. In some embodiments, there is provided an article of manufacture comprising the liquid formulation.

[0030] In a fifteenth aspect, there is provided a method for screening a formulation for reduced oxidation of a polypeptide wherein the polypeptide comprises at least one tryptophan susceptible to oxidation identified by the method as described herein, the method comprising adding an amount of N-acetyltryptophan to an aqueous composition comprising the polypeptide, adding 2,2'-azobis (2-aminopropane) dihydrochloride (AAPH) to the composition, incubating the composition comprising the polypeptide, N-acetyltryptophan and AAPH for about 14 hours at about 40 °C, measuring the polypeptide for oxidation of tryptophan residues in the polypeptide, wherein a formulation comprising an amount of N-acetyltryptophan that results in no more than about 20% oxidation of tryptophan residues of the polypeptide is a suitable formulation for reduced oxidation of the polypeptide. In a sixteenth aspect, there is provided a method for screening a formulation for reduced oxidation of a polypeptide comprising a) identifying a polypeptide comprising one or more tryptophan residues susceptible to oxidation by the method as described herein, b) adding an amount of N-acetyltryptophan to an aqueous composition comprising the polypeptide identified in step a), c) adding 2,2'-azobis (2-aminopropane) dihydrochloride (AAPH) to the composition, d) incubating the composition comprising the polypeptide, N-acetyltryptophan and AAPH for about 14 hours at about 40 °C, e) measuring the polypeptide for oxidation of tryptophan residues in the polypeptide, wherein a formulation comprising an amount of N-acetyltryptophan that results

in no more than about 20% oxidation of tryptophan residues of the polypeptide is a suitable formulation for reduced oxidation of the polypeptide. In a seventeenth aspect, there is provided a kit comprising the liquid formulation of the fourteenth aspect. In an eighteenth aspect, there is provided an article of manufacture comprising the liquid formulation of the fourteenth aspect.

[0031] In a nineteenth aspect, there is provided amethod for measuring N-acetyl tryptophan (NAT) degradation in a composition comprising N-acetyl tryptophan, the method comprising a) applying the composition to a reverse phase chromatography material, wherein the composition is loaded onto the chromatography material equilibrated in a solution comprising a mobile phase A and a mobile phase B, wherein mobile phase A comprises acid in water and mobile phase B comprises acid in acetonitrile, b) eluting the composition from the reverse phase chromatography material with a solution comprising mobile phase A and mobile phase B wherein the ratio of mobile phase B to mobile phase A is increased compared to step a), wherein NAT degradants elute from the chromatography separately from intact NAT, c) quantifying the NAT degradants and the intact NAT. In some embodiments, the ratio of mobile phase B to mobile phase A in step a) is about 2:98. In some embodiments, the ratio of mobile phase B to mobile phase A in step b) increases linearly. In some embodiments, the ratio of mobile phase B to mobile phase A in step b) increases stepwise. In some embodiments, the flow rate of the chromatography is about 1.0 mL/minute. In some embodiments, the ratio of mobile phase B to mobile phase A is increased to about 30:70. In some embodiments, the ratio of mobile phase B to mobile phase A is increased to about 30:70 in about 16 minutes. In some embodiments, the ratio of mobile phase B to mobile phase A is further increased to about 90:70. In some embodiments, the ratio of mobile phase B to mobile phase A is further increased to about 90:70 in about 18.1 minutes. In some embodiments, the ratio of mobile phase B to mobile phase A is increased to about 26:74. In some embodiments, the ratio of mobile phase B to mobile phase A is increased to about 26:74 in about 14 minutes. In some embodiments, the ratio of mobile phase B to mobile phase A is further increased to about 90:70. In some embodiments, the ratio of mobile phase B to mobile phase A is further increased to about 90:70 in about 16.5 minutes. In some embodiments, mobile phase A comprises about 0.1% acid in water. In some embodiments, mobile phase B comprises about 0.1% acid in acetonitrile. In some embodiments, the acid is formic acid. In some embodiments, the reverse phase chromatography material comprises a C18 moiety. In some embodiments, the reverse phase chromatography material comprises a solid support. In some embodiments, the solid support comprises silica. In some embodiments, the reverse phase chromatography material is contained in a column. In some embodiments, the reverse phase chromatography material is a high performance liquid chromatography (HPLC) material or an ultra-high performance liquid chromatography

(UPLC) material. In some embodiments, NAT and NAT degradation products are detected by absorbance at 240 nm. In some embodiments, NAT degradation products are identified by mass spectrometry. In some embodiments, the concentration of NAT in the composition is about 10 nM to about 1 mM. In some embodiments, NAT degradation products include one or more of N-Ac-(H, 1,2,3,3a,8,8a-hexahydro- 3a-hydroxypyrrolo [2,3-b]-indole 2-carboxylic acid) (N-Ac-PIC), N-Ac- oxyindolylalanine (N-Ac-Oia), N-Ac- N-formyl-kynurenine (N-Ac-NFK), N-Ac- kynurenine (N-Ac-Kyn) and N-Ac-2a,8a-dihydroxy-PIC.

[0032] In a twentieth aspect, there is provided a method for measuring N-acetyl tryptophan (NAT) degradation in a composition comprising N-acetyl tryptophan and a polypeptide, the method comprising a) diluting the composition with about 8 M guanidine, b) removing the polypeptide from the composition, c) applying the composition to a reverse phase chromatography material, wherein the composition is loaded onto the chromatography material equilibrated in a solution comprising a mobile phase A and a mobile phase B, wherein mobile phase A comprises acid in water and mobile phase B comprises acid in acetonitrile, d) eluting the composition from the reverse phase chromatography material with a solution comprising mobile phase A and mobile phase B wherein the ratio of mobile phase B to mobile phase A is increased compared to step a), wherein NAT degradants elute from the chromatography separately from intact NAT, e) quantifying the NAT degradants and the intact NAT. In some embodiments, the composition is diluted in about 8M guanidine such that the final concentration of NAT in the composition ranges from about 0.05 mM to about 0.2 mM. In some embodiments, the composition is diluted in about 8M guanidine such that the final concentration of polypeptide in the composition is less than or equal to about 25 mg/mL. In some embodiments, the polypeptide is removed from the composition by filtration. In some embodiments, the filtration uses a filtration membrane with a molecular weight cut-off of about 30 kDal. In some embodiments, the ratio of mobile phase B to mobile phase A in step a) is about 2:98. In some embodiments, the ratio of mobile phase B to mobile phase A in step b) increases linearly. In some embodiments, the ratio of mobile phase B to mobile phase A in step b) increases stepwise. In some embodiments, the flow rate of the chromatography is about 1.0 mL/minute. In some embodiments, the ratio of mobile phase B to mobile phase A is increased to about 30:70. In some embodiments, the ratio of mobile phase B to mobile phase A is increased to about 30:70 in about 16 minutes. In some embodiments, the ratio of mobile phase B to mobile phase A is further increased to about 90:70. In some embodiments, the ratio of mobile phase B to mobile phase A is further increased to about 90:70 in about 18.1 minutes. In some embodiments, the ratio of mobile

phase B to mobile phase A is increased to about 26:74. In some embodiments, the ratio of mobile phase B to mobile phase A is increased to about 26:74 in about 14 minutes. In some embodiments, the ratio of mobile phase B to mobile phase A is further increased to about 90:70. In some embodiments, the ratio of mobile phase B to mobile phase A is further increased to about 90:70 in about 16.5 minutes. In some embodiments, mobile phase A comprises about 0.1% acid in water. In some embodiments, mobile phase B comprises about 0.1% acid in acetonitrile. In some embodiments, the acid is formic acid. In some embodiments, the reverse phase chromatography material comprises a C18 moiety. In some embodiments, the reverse phase chromatography material comprises a solid support. In some embodiments, the solid support comprises silica. In some embodiments, the reverse phase chromatography material is contained in a column. In some embodiments, the reverse phase chromatography material is a high performance liquid chromatography (HPLC) material or an ultra-high performance liquid chromatography (UPLC) material. In some embodiments, NAT and NAT degradation products are detected by absorbance at 240 nm. In some embodiments, NAT degradation products are identified by mass spectrometry. In some embodiments, the concentration of NAT in the composition is about 0.1 mM to about 5 mM. In some embodiments, the concentration of NAT in the composition is about 0.3 mM. In some embodiments, NAT degradation products include one or more of N-Ac-PIC, N-Ac-Oia, N-Ac-NFK, N-Ac-Kyn and N-Ac-2a,8a-dihydroxy-PIC.

[0033] In some embodiments of the above aspects, the protein concentration in the formulation is about 1 mg/mL to about 250 mg/mL. In some embodiments, the formulation has a pH of about 4.5 to about 7.0. In some embodiments, the formulation further comprises one or more excipients selected from the group consisting of a stabilizer, a buffer, a surfactant, and a tonicity agent. In some embodiments, the formulation is a pharmaceutical formulation suitable for administration to a subject. In some embodiments, the polypeptide is an antibody. In some embodiments, the antibody is a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric antibody, a multispecific antibody or antibody fragment.

[0034] In a twenty-first aspect, there is provided a method to monitor degradation of NAT in a composition comprising measuring the degradation of NAT in a sample of the composition according to the methods as described herein, wherein the method is repeated one or more times. In some embodiments, the method is repeated every month, every two months, every four months or every six months.

[0035] In a twenty-second aspect, there is provided a quality assay for a pharmaceutical composition, the quality assay comprising measuring degradation of NAT in a sample of the pharmaceutical composition according to the methods as described herein, wherein the amount of NAT degradants measured in the composition determines if the pharmaceutical composition is suitable for administration to an animal. In some embodiments, an amount of NAT degradants in the pharmaceutical composition of less than about 10 ppm indicates that the pharmaceutical composition is suitable for administration to the animal.

[0036] It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the present disclosure. These and other aspects of the disclosure will become apparent to one of skill in the art. These and other embodiments of the disclosure are further described by the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0037] **FIG. 1** shows the protection from AAPH stress-induced oxidation of various tryptophan residues by NAT in proteins Mab2, Mab4, Mab1, and Mab6. Both graphs present the same data, differing in x-axis scale. The legend includes the *in silico*-calculated solvent-accessible surface area for each residue tested.

[0038] **FIGS. 2**A and **2**B show the relationship between tryptophan oxidation by AAPH and % sidechain SASA. **FIG. 2**A shows results from a data set including 38 IgG1 mAbs. **FIG. 2**B shows results from a data set including 121 mAbs across diverse frameworks including IgG1, IgG2, IgG4, and murine.

[0039] **FIG. 3** shows random decision forest accuracy as a function of the number of estimators used during training.

[0040] **FIG. 4** shows random decision forest accuracy as a function of the number of features considered during training.

[0041] **FIG. 5** shows random decision forest accuracy as a function of the tree depth used during training.

[0042] **FIG. 6** shows the feature importance (gini importance) of the 14 most relevant simulation-based molecule descriptors for an optimized random decision forest. Training parameters included: 5000 estimators, 3 features considered per node, and a tree depth of 10.

[0043] **FIG. 7** shows potential degradants of NAT (b series), along with the corresponding Trp degradants (a series).

[0044] **FIG. 8A** shows reverse phase chromatograms 0.2 mM NAT after subjection to different stress conditions. Starred peaks represent peaks only observed under ICH light stress. **FIG. 8B** shows

CLAIMS

What is claimed is:

1. A method of reducing oxidation of a polypeptide in an aqueous formulation comprising adding an amount of N-acetyltryptophan to the formulation that prevents oxidation of the polypeptide, wherein the polypeptide comprises at least one tryptophan residue with a solvent-accessible surface area (SASA) of greater than about 80Å^2.

2. A method of reducing oxidation of a polypeptide in an aqueous formulation comprising adding an amount of N-acetyltryptophan to the formulation that prevents oxidation of the polypeptide, wherein the polypeptide comprises at least one tryptophan residue with a solvent-accessible surface area (SASA) of greater than about 30%.

3. A method of reducing oxidation of a polypeptide in an aqueous formulation comprising determining the SASA values of tryptophan residues in the polypeptide and adding an amount of N-acetyltryptophan to the formulation that prevents oxidation of the polypeptide if at least one tryptophan residue has a solvent-accessible surface area (SASA) of greater than about 80Å^2.

4. The method of claim 3, wherein the SASA value of the tryptophan residues in calculated by molecular dynamic simulation.

5. The method of any one of claims 1-4, wherein the N-acetyltryptophan is added to the formulation to a concentration of about 0.1 mM to about 5 mM;

wherein optionally the N-acetyltryptophan is added to the formulation to a concentration of about 0.1 mM to about 1 mM;

wherein optionally the N-acetyltryptophan is added to the formulation to a concentration about 0.3 mM;

wherein optionally the oxidation of the polypeptide is reduced by about 50%, 75%, 80%, 85%, 90%, 95% or 99%;

wherein optionally the formulation is stable at about 2 °C to about 8 °C for about 1095 days;

wherein optionally the protein concentration in the formulation is about 1 mg/mL to about 250 mg/mL;

wherein optionally the formulation has a pH of about 4.5 to about 7.0;

wherein optionally the formulation further comprises one or more excipients selected from the group consisting of a stabilizer, a buffer, a surfactant, and a tonicity agent;

wherein optionally the formulation is a pharmaceutical formulation suitable for administration to a subject;

wherein optionally the protein is an antibody, and

wherein optionally the antibody is a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric antibody, a multispecific antibody, or an antibody fragment.

6. A liquid formulation comprising a polypeptide and an amount of N-acetyltryptophan to prevent oxidation of the polypeptide, wherein the polypeptide has at least one tryptophan residue with a SASA of greater than about 80$Å^2$.

7. The liquid formulation of claim 6, wherein the N-acetyltryptophan is added to the formulation to a concentration of about 0.1 mM to about 5 mM;

wherein optionally the N-acetyltryptophan is added to the formulation to a concentration of about 0.1 mM to about 1 mM;

wherein optionally the N-acetyltryptophan is added to the formulation to a concentration about 0.3 mM;

wherein optionally the oxidation of the polypeptide is reduced by about 50%, 75%, 80%, 85%, 90%, 95% or 99%;

wherein optionally the formulation is stable at about 2 °C to about 8 °C for about 1065 days;

wherein optionally the protein concentration in the formulation is about 1 mg/mL to about 250 mg/mL;

wherein optionally the formulation has a pH of about 4.5 to about 7.0;

wherein optionally the formulation further comprises one or more excipients selected from the group consisting of a stabilizer, a buffer, a surfactant, and a tonicity agent;

wherein optionally the formulation is a pharmaceutical formulation suitable for administration to a subject;

wherein optionally the protein is an antibody, and

wherein optionally the antibody is a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric antibody, a multispecific antibody, or an antibody fragment.

8. A method for screening a formulation for reduced oxidation of a polypeptide wherein the polypeptide comprises at least one tryptophan residue with a SASA of greater than about 80Å^2, the method comprising

adding an amount of N-acetyltryptophan to an aqueous composition comprising the polypeptide,

adding 2,2'-azobis (2-aminopropane) dihydrochloride (AAPH) to the composition,

incubating the composition comprising the polypeptide, N-acetyltryptophan and AAPH for about 14 hours at about 40 °C,

measuring the polypeptide for oxidation of tryptophan residues in the polypeptide,

wherein a formulation comprising an amount of N-acetyltryptophan that results in no more than about 20% oxidation of tryptophan residues of the polypeptide is a suitable formulation for reduced oxidation of the polypeptide.

9. A method for screening a formulation for reduced oxidation of a polypeptide comprising

determining the SASA values of tryptophan residues in the polypeptide, wherein a tryptophan residue with a SASA of greater than about 80Å^2, is subject to oxidation,

adding an amount of N-acetyltryptophan to an aqueous composition comprising the polypeptide,

adding 2,2'-azobis (2-aminopropane) dihydrochloride (AAPH) to the composition,

incubating the composition comprising the polypeptide, N-acetyltryptophan and AAPH for about 14 hours at about 40 °C,

measuring the polypeptide for oxidation of tryptophan residues in the polypeptide,

wherein a formulation comprising an amount of N-acetyltryptophan that results in no more than about 20% oxidation of tryptophan residues of the polypeptide is a suitable formulation for reduced oxidation of the polypeptide.

10. The method of claim 9, wherein the SASA value of the tryptophan residues in calculated by molecular dynamic simulation.

11. A kit comprising the liquid formulation of claim 6 or 7.

12. An article of manufacture comprising the liquid formulation of claim 6 or 7.

13. A method to determine if a polypeptide in a liquid formulation comprises a tryptophan residue susceptible to oxidation, the method comprising calculating one or more molecule descriptors based on the amino acid sequence of the polypeptide for each tryptophan residue in the polypeptide and applying the one or more molecule descriptors to a machine learning algorithm trained on the one or more molecule descriptors to predict tryptophan oxidation, wherein the molecule descriptors include one or more of the following:

a) number of aspartic acid sidechain oxygens within 7Å of tryptophan delta carbon,

b) sidechain solvent accessible surface area (SASA),

c) delta carbon SASA,

d) total positive charge within 7Å of tryptophan delta carbon,

e) backbone SASA,

f) tryptophan sidechain angles,

g) packing density within 7Å of tryptophan delta carbon,

h) tryptophan backbone angles,

i) SASA of pseudo-pi orbitals,

j) backbone flexibility, or

k) total negative charge within 7Å of tryptophan delta carbon.

14. The method of claim 13, wherein two, three, four, five, six, seven, eight, nine, ten or eleven of the molecule descriptors are used in the molecular simulation; or

wherein optionally the molecule descriptors comprise the following:

a) number of aspartic acid sidechain oxygens within 7Å of tryptophan delta carbon,

b) sidechain solvent accessible surface area (SASA),

c) delta carbon SASA,

d) total positive charge within 7Å of tryptophan delta carbon,

e) backbone SASA,

f) tryptophan sidechain angles, and

g) packing density within 7Å of tryptophan delta carbon;

wherein optionally the machine learning algorithm was trained by matching molecule descriptors from molecular dynamic simulations of polypeptides based on amino acid sequence of the polypeptide with experimental data for each tryptophan residue in the polypeptide;

wherein optionally oxidation of greater than 35% of tryptophan residues at a particular site indicates susceptibility to oxidation;

wherein optionally the one or more molecule descriptors are calculated using a computer;

wherein optionally the protein is an antibody, and
wherein optionally the antibody is a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric antibody, a multispecific antibody, or an antibody fragment.

15. A method to reduce oxidation of a polypeptide, comprising identifying tryptophan residues susceptible to oxidation according to the method of claim 13 or 14 and introducing

an amino acid substitution in the polypeptide to replace one or more tryptophan residues susceptible to oxidation with amino acid residues that are not subject to oxidation.

16. A method to reduce oxidation of a polypeptide, comprising introducing an amino acid substitution in the polypeptide to replace one or more tryptophan residues susceptible to oxidation, wherein the one or more tryptophan residues susceptible to oxidation was identified by the method of claim 13 or 14.

17. The method of claim 15 or 16, wherein the tryptophan residue is replaced by an amino acid residue selected from the group consisting of tyrosine, phenylalanine, leucine, isoleucine, alanine, and valine.

18. A method to reduce oxidation of a polypeptide in an aqueous formulation, comprising determining the presence of one or more tryptophan residues in the polypeptide susceptible to oxidation according to the method of claim 13 or 14, and adding an effective amount of an anti-oxidation agent to the aqueous formulation comprising a polypeptide having a one or more tryptophan residues susceptible to oxidation.

19. A method to reduce oxidation of a polypeptide in an aqueous formulation, comprising adding an amount of an anti-oxidation agent to the aqueous formulation to prevent oxidation, wherein polypeptide comprises one or more tryptophan residues susceptible to oxidation identified by the method of claim 13 or 14.

20. The method of claim 19, wherein the anti-oxidation agent is N-acetyltryptophan;

wherein optionally the N-acetyltryptophan is added to the formulation to a concentration of about 0.1 mM to about 5 mM;

wherein optionally the N-acetyltryptophan is added to the formulation to a concentration of about 0.1 mM to about 1 mM; and

wherein optionally the N-acetyltryptophan is added to the formulation to a concentration about 0.3 mM.

21. The method of any one of claims 18-20, wherein the oxidation of the polypeptide is reduced by about 50%, 75%, 80%, 85%, 90%, 95% or 99%;

wherein optionally the formulation is stable at about 2 °C to about 8 °C for about 1095 days;

wherein optionally the protein concentration in the formulation is about 1 mg/mL to about 250 mg/mL;

wherein optionally the formulation has a pH of about 4.5 to about 7.0.;

wherein optionally the formulation further comprises one or more excipients selected from the group consisting of a stabilizer, a buffer, a surfactant, and a tonicity agent;

wherein optionally the formulation is a pharmaceutical formulation suitable for administration to a subject;

wherein optionally the protein is an antibody, and

wherein optionally the antibody is a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric antibody, a multispecific antibody, or an antibody fragment.

22. A liquid formulation comprising a polypeptide and an amount of N-acetyltryptophan to prevent oxidation of the polypeptide, wherein the polypeptide has at least one tryptophan residue susceptible to oxidation as measured by the method of claim 13 or 14.

23. The liquid formulation of claim 22, wherein the N-acetyltryptophan is added to the formulation to a concentration of about 0.1 mM to about 5 mM;

wherein optionally the N-acetyltryptophan is added to the formulation to a concentration of about 0.1 mM to about 1 mM;

wherein optionally the N-acetyltryptophan is added to the formulation to a concentration about 0.3 mM;

wherein optionally the oxidation of the polypeptide is reduced by about 50%, 75%, 80%, 85%, 90%, 95% or 99%;

wherein optionally the formulation is stable at about 2 °C to about 8 °C for about 1065 days;

wherein optionally the protein concentration in the formulation is about 1 mg/mL to about 250 mg/mL;

wherein optionally the formulation has a pH of about 4.5 to about 7.0;

wherein optionally the formulation further comprises one or more excipients selected from the group consisting of a stabilizer, a buffer, a surfactant, and a tonicity agent;

wherein optionally the formulation is a pharmaceutical formulation suitable for administration to a subject;

wherein optionally the protein is an antibody, and

wherein optionally the antibody is a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric antibody, a multispecific antibody, or an antibody fragment.

24. A method for screening a formulation for reduced oxidation of a polypeptide wherein the polypeptide comprises at least one tryptophan susceptible to oxidation identified by the method of claim 13 or 14, the method comprising

adding an amount of N-acetyltryptophan to an aqueous composition comprising the polypeptide,

adding 2,2'-azobis (2-aminopropane) dihydrochloride (AAPH) to the composition,

incubating the composition comprising the polypeptide, N-acetyltryptophan and AAPH for about 14 hours at about 40 °C,

measuring the polypeptide for oxidation of tryptophan residues in the polypeptide,

wherein a formulation comprising an amount of N-acetyltryptophan that results in no more than about 20% oxidation of tryptophan residues of the polypeptide is a suitable formulation for reduced oxidation of the polypeptide.

25. A method for screening a formulation for reduced oxidation of a polypeptide comprising

a) identifying a polypeptide comprising one or more tryptophan residues susceptible to oxidation by the method of claim 13 or 14,

b) adding an amount of N-acetyltryptophan to an aqueous composition comprising the polypeptide identified in step a),

c) adding 2,2'-azobis (2-aminopropane) dihydrochloride (AAPH) to the composition,

d) incubating the composition comprising the polypeptide, N-acetyltryptophan and AAPH for about 14 hours at about 40 °C,

e) measuring the polypeptide for oxidation of tryptophan residues in the polypeptide,

wherein a formulation comprising an amount of N-acetyltryptophan that results in no more than about 20% oxidation of tryptophan residues of the polypeptide is a suitable formulation for reduced oxidation of the polypeptide.

26. A kit comprising the liquid formulation of claim 22 or 23.

27. An article of manufacture comprising the liquid formulation of claim 22 or 23.

28. A method for measuring N-acetyl tryptophan (NAT) degradation in a composition comprising N-acetyl tryptophan, the method comprising

a) applying the composition to a reverse phase chromatography material, wherein the composition is loaded onto the chromatography material equilibrated in a solution comprising a mobile phase A and a mobile phase B, wherein mobile phase A comprises acid in water and mobile phase B comprises acid in acetonitrile,

b) eluting the composition from the reverse phase chromatography material with a solution comprising mobile phase A and mobile phase B wherein the ratio of mobile phase B to mobile phase A is increased compared to step a), wherein NAT degradants elute from the chromatography separately from intact NAT,

c) quantifying the NAT degradants and the intact NAT.

29. The method of claim 28, wherein the ratio of mobile phase B to mobile phase A in step a) is about 2:98;

wherein optionally the ratio of mobile phase B to mobile phase A in step b) increases linearly;

wherein optionally the ratio of mobile phase B to mobile phase A in step b) increases stepwise;

wherein optionally the flow rate of the chromatography is about 1.0 mL/minute,

wherein optionally the ratio of mobile phase B to mobile phase A is increased to about 30:70,

wherein optionally the ratio of mobile phase B to mobile phase A is increased to about 30:70 in about 16 minutes;

wherein optionally the ratio of mobile phase B to mobile phase A is further increased to about 90:70,

wherein optionally the ratio of mobile phase B to mobile phase A is further increased to about 90:70 in about 18.1 minutes;

wherein optionally the ratio of mobile phase B to mobile phase A is increased to about 26:74,

wherein optionally the ratio of mobile phase B to mobile phase A is increased to about 26:74 in about 14 minutes;

wherein optionally the ratio of mobile phase B to mobile phase A is further increased to about 90:70,

wherein optionally the ratio of mobile phase B to mobile phase A is further increased to about 90:70 in about 16.5 minutes;

wherein optionally mobile phase A comprises about 0.1% acid in water;

wherein optionally mobile phase B comprises about 0.1% acid in acetonitrile;

wherein optionally the acid is formic acid;

wherein optionally the reverse phase chromatography material comprises a C18 moiety;

wherein optionally the reverse phase chromatography material comprises a solid support,

wherein optionally the solid support comprises silica;

wherein optionally the reverse phase chromatography material is contained in a column;

wherein optionally the reverse phase chromatography material is a high performance liquid chromatography (HPLC) material or an ultra-high performance liquid chromatography (UPLC) material;

wherein optionally NAT and NAT degradation products are detected by absorbance at 240 nm;

wherein optionally NAT degradation products are identified by mass spectrometry;

wherein optionally the concentration of NAT in the composition is about 10 nM to about 1 mM; and

wherein optionally NAT degradation products include one or more of N-Ac-(H, 1,2,3,3a,8,8a-hexahydro- 3a-hydroxypyrrolo [2,3-b]-indole 2-carboxylic acid) (N-Ac-PIC), N-Ac- oxyindolylalanine (N-Ac-Oia), N-Ac- N-formyl-kynurenine (N-Ac-NFK), N-Ac-kynurenine (N-Ac-Kyn) and N-Ac-2a,8a-dihydroxy-PIC.

30. A method for measuring N-acetyl tryptophan (NAT) degradation in a composition comprising N-acetyl tryptophan and a polypeptide, the method comprising

a) diluting the composition with about 8 M guanidine,

b) removing the polypeptide from the composition,

c) applying the composition to a reverse phase chromatography material, wherein the composition is loaded onto the chromatography material equilibrated in a solution comprising a mobile phase A and a mobile phase B, wherein mobile phase A comprises acid in water and mobile phase B comprises acid in acetonitrile,

d) eluting the composition from the reverse phase chromatography material with a solution comprising mobile phase A and mobile phase B wherein the ratio of mobile phase B to mobile phase A is increased compared to step a), wherein NAT degradants elute from the chromatography separately from intact NAT,

e) quantifying the NAT degradants and the intact NAT.

31. The method of claim 30, wherein the composition is diluted in about 8M guanidine such that the final concentration of NAT in the composition ranges from about 0.05 mM to about 0.2 mM;

wherein optionally the composition is diluted in about 8M guanidine such that the final concentration of polypeptide in the composition is less than or equal to about 25 mg/mL;

wherein optionally the polypeptide is removed from the composition by filtration,

wherein optionally the filtation uses a filtration membrane with a molecular weight cut-off of about 30 kDal;

wherein optionally the ratio of mobile phase B to mobile phase A in step a) is about 2:98;

wherein optionally the ratio of mobile phase B to mobile phase A in step b) increases linearly;

wherein optionally the ratio of mobile phase B to mobile phase A in step b) increases stepwise;

wherein optionally the flow rate of the chromatography is about 1.0 mL/minute,

wherein optionally the ratio of mobile phase B to mobile phase A is increased to about 30:70,

wherein optionally the ratio of mobile phase B to mobile phase A is increased to about 30:70 in about 16 minutes;

wherein optionally the ratio of mobile phase B to mobile phase A is further increased to about 90:70,

wherein optionally the ratio of mobile phase B to mobile phase A is further increased to about 90:70 in about 18.1 minutes,

wherein optionally the ratio of mobile phase B to mobile phase A is increased to about 26:74,

wherein optionally the ratio of mobile phase B to mobile phase A is increased to about 26:74 in about 14 minutes;

wherein optionally the ratio of mobile phase B to mobile phase A is further increased to about 90:70,

wherein optionally the ratio of mobile phase B to mobile phase A is further increased to about 90:70 in about 16.5 minutes;

wherein optionally mobile phase A comprises about 0.1% acid in water;

wherein optionally mobile phase B comprises about 0.1% acid in acetonitrile;

wherein optionally the acid is formic acid;

wherein optionally the reverse phase chromatography material comprises a C18 moiety;

wherein optionally the reverse phase chromatography material comprises a solid support,

wherein optionally the solid support comprises silica;

wherein optionally the reverse phase chromatography material is contained in a column;

wherein optionally the reverse phase chromatography material is a high performance liquid chromatography (HPLC) material or an ultra-high performance liquid chromatography (UPLC) material;

wherein optionally NAT and NAT degradation products are detected by absorbance at 240 nm;

wherein optionally NAT degradation products are identified by mass spectrometry;

wherein optionally the concentration of NAT in the composition is about 0.1 mM to about 5 mM;

wherein optionally the concentration of NAT in the composition is about 0.3 mM;

wherein optionally NAT degradation products include one or more of N-Ac-PIC, N-Ac-Oia, N-Ac-NFK, N-Ac-Kyn and N-Ac-2a,8a-dihydroxy-PIC;

wherein optionally the protein concentration in the formulation is about 1 mg/mL to about 250 mg/mL;

wherein optionally the formulation has a pH of about 4.5 to about 7.0;

wherein optionally the formulation further comprises one or more excipients selected from the group consisting of a stabilizer, a buffer, a surfactant, and a tonicity agent;

wherein optionally the formulation is a pharmaceutical formulation suitable for administration to a subject;

wherein optionally the polypeptide is an antibody, and

wherein optionally the antibody is a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric antibody, a multispecific antibody or antibody fragment.

32. A method to monitor degradation of NAT in a composition comprising measuring the degradation of NAT in a sample of the composition according to the methods of any one of claims 28-31, wherein the method is repeated one or more times.

33. The method of claim 32, wherein the method is repeated every month, every two months, every four months or every six months.

34. A quality assay for a pharmaceutical composition, the quality assay comprising measuring degradation of NAT in a sample of the pharmaceutical composition according to the methods of any one of claims 28-31, wherein the amount of NAT degradants measured in the composition determines if the pharmaceutical composition is suitable for administration to an animal.