of the hydrazide reagent for quantifying the carbonyl content which leads to an overestimation of the carbonylation content.

[005] US 2006/0216756 A1 discloses a method for two-dimensional evaluation of the nature of oxidized protein, the method comprises the steps of specific fluorescent labeling of the carbonyl groups of the oxidized protein in a horny layer specimen taken from skin. US 2007/0110670 A1 discloses methods comprising the labeling of carbonyl groups of an oxidized protein with a fluorescence dye for evaluating hair damage. WO2012175519 A1 describes a method for detecting and/or quantifying protein carbonyl groups in at least one protein-containing sample comprising the steps of labeling protein carbonyl groups with hydrazide followed by an analysis of the sample using one-dimensional or two-dimensional gel electrophoresis.

[006] In the present description, a simplified, more robust and more accurate protein **C**arbonylation **A**ssay using **L**ucifer **Y**ellow carbohydrazide (CALY) is described which overcomes the problems of the prior art as described above.

SUMMARY

[007] The description relates to a method and kits for determining the total carbonylation level on a polypeptide.

[008] In one example, the description relates to a method for determining the total carbonylation level on a polypeptide wherein the method comprises the steps of a) contacting the polypeptide in a solution with a hydrazide dye under conditions such that the hydrazide dye can react with the carbonyl groups present on the amino acid residues of the polypeptide to form a polypeptide-dye-complex, b) removing unbound hydrazide dye from the resultant solution from step a), c) determining in the resultant solution from step b) the concentration of the hydrazide dye bound in the polypeptide-dye-complex and the concentration of the polypeptide bound in the polypeptide-dye-complex, and d) determining the total carbonylation level of the polypeptide based on the ratio of the concentration of the hydrazide

dye bound in the polypeptide-dye-complex to the concentration of the polypeptide bound in the polypeptide-dye-complex.

[008a] In one aspect, the present disclosure refers to a method for determining the total carbonylation level on amino acid residues of a metal-catalyzed oxidized polypeptide wherein the method comprises the steps of a) Contacting the polypeptide in a solution with a hydrazide dye under conditions such that the hydrazide dye can react with the carbonyl groups present on the amino acid residues of the polypeptide to form a polypeptide-dye-complex, b) Removing unbound hydrazide dye from the resultant solution from step a), c) Determining in the resultant solution from step b) the concentration of the hydrazide dye bound in the polypeptide-dye-complex and the concentration of the polypeptide bound in the polypeptide-dye-complex, and d) Determining the total carbonylation level on amino acid residues of the metal-catalyzed oxidized polypeptide based on the ratio of the concentration of the hydrazide dye bound in the polypeptide-dye-complex to the concentration of the polypeptide bound in the polypeptide-dye-complex.

[009] In one example, the hydrazide dye is LY-CH. In one example, step a) is performed in the presence of alkali metal ions. In one example, the amino acid residues of the polypeptide are selected from the group consisting of arginine, lysine, proline and threonine. In one example, the polypeptide is an antibody. In one example, the antibody is a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric antibody, a multi-specific antibody, an antibody fragment, an antibody drug conjugate, a THIOMAB™ or a THIOMAB™ drug conjugate. In one example, the polypeptide and the hydrazide dye is contacted in step a) in a final molar ratio of 6,000 – 10,000. In one example, step a) is performed in the presence of a non-ionic surfactant. In one example, the non-ionic surfactant is reduced Triton X-100. In one example, step a) is performed at a temperature of 35ºC – 39ºC. In one example, step a) is performed for 10 – 20 h. In one example, step b) is performed by a method selected from the group consisting of filtration, gel filtration and dialysis. In one example, step b) is performed by filtration. In one example, filtration is carried out in the presence of 100 – 300 mM potassium phosphate and in the presence of 200 – 300 mM potassium chloride.

[0010] In another example, the description relates to a kit for carrying out the method as described herein, wherein the kit comprises hydrazide dye and a buffer. In one aspect, the present disclosure refers to a kit comprising a hydrazide dye and a buffer as a combined product for use in the method as disclosed herein. In one example, the hydrazide dye is LY-CH. In one example, the buffer is lithium MES buffer. In one example, the kit further comprises a non-ionic surfactant, a standard for the polypeptide and a standard for hydrazide dye. In one example, the non-ionic surfactant is reduced Triton X-100.

BRIEF DESCRIPTION OF THE FIGURES

[0011] **Figure 1** shows the major carbonylation products from metal-catalyzed oxidation.

[0012] **Figure 2A** shows the derivatization reaction between LY-CH and the carbonyl group.

WHAT IS CLAIMED IS:

1. A method for determining the total carbonylation level on amino acid residues of a metal-catalyzed oxidized polypeptide wherein the method comprises the steps of

a) Contacting the polypeptide in a solution with a hydrazide dye under conditions such that the hydrazide dye can react with the carbonyl groups present on the amino acid residues of the polypeptide to form a polypeptide-dye-complex,

b) Removing unbound hydrazide dye from the resultant solution from step a),

c) Determining in the resultant solution from step b) the concentration of the hydrazide dye bound in the polypeptide-dye-complex and the concentration of the polypeptide bound in the polypeptide-dye-complex, and

d) Determining the total carbonylation level on amino acid residues of the metal-catalyzed oxidized polypeptide based on the ratio of the concentration of the hydrazide dye bound in the polypeptide-dye-complex to the concentration of the polypeptide bound in the polypeptide-dye-complex.

2. The method according to claim 1, wherein the hydrazide dye is LY-CH.

3. The method according to claim 1, wherein step a) is performed in the presence of alkali metal ions.

4. The method according to claim 3, wherein the alkali metal ions are lithium ions.

5. The method according to claim 1, wherein the amino acid residues of the polypeptide are selected from the group consisting of arginine, lysine, proline and threonine.

6. The method according to claim 1, wherein the polypeptide is an antibody.

7. The method according to claim 6, wherein the antibody is a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric

antibody, a multi-specific antibody, an antibody fragment, an antibody drug conjugate, a THIOMAB™ or a THIOMAB™ drug conjugate.

8. The method according to claim 1, wherein the polypeptide and the hydrazide dye is contacted in step a) in a final molar ratio of 6,000 – 10,000.

9. The method according to claim 1, wherein step a) is performed in the presence of a non-ionic surfactant.

10. The method according to claim 9, wherein the non-ionic surfactant is reduced Triton X-100.

11. The method according to claim 1, wherein step a) is performed at a temperature of 35ºC – 39ºC.

12. The method according to claim 1, wherein step a) is performed for 10 – 20 h.

13. The method according to claim 1, wherein step b) is performed by a method selected from the group consisting of filtration, gel filtration and dialysis.

14. The method according to claim 1, wherein step b) is performed by filtration.

15. The method according to claim 14, wherein filtration is carried out in the presence of 100 – 300 mM potassium phosphate and in the presence of 200 – 300 mM potassium chloride.

16. A kit comprising a hydrazide dye and a buffer as a combined product for use in the method according to claim 1.

17. The kit according to claim 16, wherein the hydrazide dye is LY-CH.

18. The kit according to claim 16, wherein the buffer is lithium MES buffer.

19. The kit according to claim 16, wherein the kit further comprises a non-ionic surfactant, a standard for the polypeptide and a standard for the hydrazide dye.

20. The kit according to claim 19, wherein the non-ionic surfactant is reduced Triton X-100.