



SDI Review Form 1.6

Journal Name:	Annual Research & Review in Biology
Manuscript Number:	2013_ARRB_8763
Title of the Manuscript:	Current Methods Used in the Protein Carbonyl Assay
Type of the Article	Minireview Article

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
<u>Compulsory</u> REVISION comments		
<u>Minor</u> REVISION comments		
<u>Optional/General</u> comments	This minireview article briefly summarizes methods used for detection and quantification of protein carbonyls. However, there are several deficiencies as listed below: 1) The goal of this article is to review “up-to-date” methods, but several recent developments in this field were not included, e.g. Wehr’s dot blot method (2012 Anal Biochem 423:241-5), Tamarit’s Bodipy-hydrazide based carbonyl detection after 2D gel (2012 J Proteomics 75: 3778-88), and Hollins’ use of oxalyldihydrazide as a crosslinker for enrichment of carbonylated proteins within a microfluidic chip (2012 Lab Chip 12: 2526-32)... 2) As the authors pointed out, mass spectrometry-based proteomic methods are “the most promising techniques” in identification of carbonylated proteins and the carbonylation sites. However, these methods were not reviewed. In stead, the authors simply cited a recently published review paper on the topic at the end of the section 4 (lines 289-90). 3) Line 305-7, the authors concluded that capillary electrophoresis (CE) with laser induced fluorescence is one of the most promising techniques in identifying proteins and residues that susceptible to	1) Endorsed. We updated the mini-review with several recent developments in this field, like dot blot method, microfluidic chip, Bodipy-hydrazide derivatization, iTRAQ techniques. Accordingly, we introduced extra-references: 39, 42, 44, 48, 49, 50, 51 All suggested references indicated were added in the article. 2) Endorsed. Two paragraphs on LC-MS were added, and the correspondent references (48-51). However, the LC-MS proteomic methods for the characterization of changes in protein structure (including oxidative stress induced changes) can be the subject of a minireview by themselves. 3) CE the separation is based on analytes velocity so is possible also to range the carbonyl proteins based on their molecular weight. Derivatization with FTIC and the use of LIF gives the possibility to detect the carbonylation range.



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	carbonylation. However I do not understand how this method can lead to protein identification if no 2D-CE and MS are involved. Additionally, line 264-6 state that this method is capable of detecting proteins with MW ranging from 26 to 30 kDa. If so, how about proteins with MW outside that range? 4) A review paper summarizing the techniques in a field should link a method directly to the most relevant research article(s) that describing the development or improvement of that typical method, so that the readers can quickly evaluate the suitability of the detailed protocol on their research. However, in multiple occasions, e.g. abovementioned deficiency-2, reference-7 (line-182), reference-20 (line-261), reference-6 (line-283), the authors either cited a review paper (ref-7, ref-40, ref-6), or not the original publication describing the method (ref-20, should be Electrophoresis 2008, 29, 475-82). 5) This review focuses on methods used for protein carbonyl assay. But one method described in line 161-5 (ref-30, again a review paper) was actually developed for the detection of carbonyl in small molecules like hexanal, but not for detecting protein carbonyls. Therefore this method should not be included in the review. 6) Regarding the disadvantages of spectrophotometric method, the authors claim in line 140-1 that "(4) the need to use the standard curves" as one of them. I do not think this statement is correct. According to an updated protocol recently published by Levine's lab (Methods Mol Biol. 2013; 965: 265-81. Again, this is also an important update in the field, but not reviewed in current manuscript), carbonyl content can be	As stated in the literature, proteins with MW outside that range, especially those with MW higher will stuck in the capillary, so is not possible to be detected using this technique. 4)Endorsed. We updated the references, Ref 6- was changed with Banan A, Zhang Y, Losurdo J, Keshavarzian A. Gut 2000;46:830- 7 Ref 7- was changed with Requena J. R., Chao C-C., Levine R. L. and Stadtman E. R. Proc Natl Acad Sci USA. 2001; 98(1): 69-74 Ref.20- was changed becoming ref. 25 5)Endorsed. This reference was deleted. 6) Endorsed. This disadvantage was deleted.
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	calculated based on the known molar absorptivity of the hydrazone at 370 nm (22000), and the protein concentration can be calculated based on either a known molar absorptivity for a purified protein, or an estimated molar absorptivity at 276nm (50000) for unknown protein(s). Therefore no standard curves are required for this method. 7) Line-338, ref-10. Please confirm that the cited journal name and page numbers are accurate. My search in PubMed, using the article name provided, returned in different result.	7) Endorsed. The reference was checked and changed, accordingly: - new ref 15
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