



SDI Review Form 1.6

Journal Name:	International Journal of Biochemistry Research & Review
Manuscript Number:	2013_IJBcRR_8453
Title of the Manuscript:	Role of the Trp-disulfide triads in the UV light induced degradation of a monoclonal antibody scFv
Type of the 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)
Compulsory REVISION comments	Lines 79-82. Provide a reference for the experimental procedure. Section 2.8, Fluorescence Spectroscopy. Line 132. The authors need to describe how the spectra are measured. For instance, it is not clear to me whether the spectra (figure 4, figure 6a, figure 7) correspond to the raw emission or this has been corrected for the wavelength dependence of the detector. Likewise, it is not stated whether the fluorescence of the medium (solvent plus rest of non-fluorophore materials) has been subtracted. This must be stated. It also needs to be stated that the fluorescence percentage reported in figure 6b refers to the total integrated fluorescence (I suspect) rather than to the maxima. Figure 5b. Change the scale of the y-axis, so it goes from 0% to 100% (or 110%, if you prefer), rather than from 15% to 110%. Figure 6b. The x-axis scale is currently set so that the last data points overlap the graph frame and the error bars cannot be seen. To fix this, I suggest the x-axis scale is changed to cover the range 0 to 2.5 hrs.	Lines 79-82 (now lines 112): the given lit. 17 Section2.8; line 132 (now lines 164-165) - measurement of the spectra: "The fluorescence spectra were corrected for inner filter effect and blanc spectrum (Raman peak of the water) was subtracted". - -The capture of figure 6b was corrected (for total integrated fluorescence) - Figure 5b was corrected as you proposed - Figure 6b was corrected as you proposed
Minor REVISION comments	Line 42-44. It would be useful to provide some ballpark value for a typical distance between Trp and the S-S bridge.	- line 42-44. (now 67.) " typical mid-point distance in the V-domain of the light chain is 4.6 ± 0.3 from Trp-Cη2 to Sγ-Sγ and in heavy



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	Line 121. I would like to see a better explanation than 'thoroughly degassed'. How was it done? For how long? What test was performed to ensure that the samples were fully degassed? Section 3.1.3 Tryptophan Fluorescence. Lines 201-215. The paragraph carries a lot of information and it is difficult to read. One point of confusion to me was the precise meaning of 'substituted residues' in line 208. I believe that it would be much clearer if you use the expression 'monosubstituted residues' instead. Lines 402-403. When discussing the large distance between S-S bond and binding site, it would be useful to provide a value for such distance.	chain 4.7 ± 0.3 from Trp-Cη2 to Sγ-Sγ" - - line 121. (now 151-152.) "To avoid oxidation, all solutions were thoroughly degassed under vacuum at 2.39 kPa for 5 min, prior to use and kept under N₂ atmosphere during illumination. - - section 3.1.3 lines 201-215. (now 226-241.) in line 233. We mentioned the double mutant. - - line 402-403. (now 419.) we wrote the distance more than 13 Å, see the new reference [12]
<u>Optional/General</u> comments	Line 31. Change 'serves' to 'serve' Line 286. Change 'spectras' to 'spectra'	- - line 31. (now line 51.) - we corrected it - - line 286. (now line 304.) – we corrected it