Corrections

Mapping Protein Domains Involved in Macromolecular Interactions: A Novel Protein Footprinting Approach, by Ewa Heyduk and Tomasz Heyduk*, Volume 33, Number 32, August 16, 1994, pages 9643−9650.

It was brought to our attention by Dr. Claude F. Meares that the following sentence in the Materials and Methods section describing footprinting reactions (p 9644, column 2, lines 7−10), "Typically, 50 μL of 5 μM CRP was subjected to nonspecific cleavage by adding freshly prepared $FeSO_4$, ascorbate, EDTA, and H_2O_2 to a final concentration of 1, 20, 2, and 1 mM, respectively", could be interpreted to indicate that the reagents were added sequentially in that order. Since this is not how these experiments were done and since sequential addition of reagents is not appropriate, a detailed description of the manner in which the reagents should be added is given below.

Cleavage reactions were performed in 10−100 μL volumes. Protein solutions (5 μM CRP) were placed in Eppendorf tubes, and 1−2 μL of stock solutions of Fe^{2+}−EDTA, ascorbate, and H_2O_2 was spotted on the wall of each Eppendorf tube. The cleavage reactions were initiated by simultaneously adding Fe^{2+}−EDTA, ascorbate, and H_2O_2 through brief centrifugation in an Eppendorf microcentrifuge. Concentrations of stock solutions of Fe^{2+}, EDTA, ascorbate and H_2O_2 were adjusted depending on the total volume of the reaction such that their concentrations in the reaction mixture were 1, 2, 20, and 1 mM, respectively. For example, for cleavage reactions in a total volume of 10 μL concentrations of stock solutions used were 40 mM Fe^{2+}, 80 mM EDTA, 0.2 M ascorbate, and 10 mM H_2O_2, and 1 μL of Fe^{2+}−EDTA, ascorbate, and H_2O_2 was used. The stock solution of Fe^{2+}−EDTA was prepared prior to use by mixing stocks of Fe^{2+}, EDTA, and water in 1:1:2 v/v ratio.

We thank Dr. Meares for bringing this issue to our attention.

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