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Acidification of the Oxygen Scavenging System in Single-Molecule Fluorescence Studies: In Situ Sensing with a Ratiometric Dual-Emission Probe

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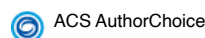
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Abstract

For most of the single-molecule fluorescence studies to date, biomolecules of interest are labeled with small organic dyes which suffer from their limited photostability evidenced by blinking and photobleaching. An enzymatic oxygen scavenging system of glucose oxidase and catalase is widely used to improve the dye photostability but with the unfavorable side effect of producing gluconic acid. It is known that accumulation of this byproduct in solution can lead to considerable acidification, but the uncertainty in its severity under experimentally relevant conditions has been a long-standing area of concern due to the lack of a suitable assay. In this paper we report a fluorescence-based analytical assay for quantitatively assessing the acidification of oxygen scavenging systems in situ. By using a ratiometric, dual-emission dye, SNARF-1, we observed the presence and, for the first time, measured the severity of solution acidification due to the oxygen scavenging system for a number of conditions relevant to single-molecule studies. On the basis of the quantitative analysis of the acidification profile under these conditions, practical guidelines for optimizing the oxygen scavenging system are provided. This in situ assay should be applicable to a large variety of future single-molecule fluorescence studies.

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