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**Ultrasensitive detection of lysozyme in droplet-based microfluidic devices**

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**Abstract**

Lysozyme (LYS) is a bacteriolytic enzyme, available in secretions such as saliva, tears and human milk. LYS is an important defence molecule of the innate immune system, and its overexpression can be a consequence of diseases such as leukaemia, kidney disease and sarcoidosis. This paper reports on a digital microfluidic-based approach that combines the gold nanoparticle-enhanced chemiluminescence with aptamer interaction to detect human lysozyme into droplets 20 nanoliters in volume. The described method allows identifying LYS with a 44.6 femtomolar limit of detection, using sample volume as low as 1  $\mu$ L and detection time in the range of 10 minutes. We used luminol to generate the chemiluminescence and demonstrated that the compartmentalization of LYS in droplets also comprising gold nanoparticles provided enhanced luminescence. We functionalized

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