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(G*) Harnessing Electrochemistry with Surface-Enhanced Raman Spectroscopy for the Analysis of Hemoglobin Defects on Plasmonic Nanostructured Films

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Hemoglobin (Hb), the cornerstone of oxygen transport in the body, holds crucial diagnostic significance for disorders like β -Thalassemia and sickle cell anemia. Conventional blood assays often grapple with issues of delays, cost, and accessibility. In this study, we unveil an innovative nano-biosensor leveraging surface-enhanced Raman spectroscopy (SERS), offering swift and real-time detection of iron-containing molecules, with a primary focus on Hb, the predominant iron-containing compound in blood. This detection could be used with minimal samples and great sensitivity.

Our sensor's foundation involves gold and silver thin film substrates, crafted through pulsed laser ablation and electrochemical deposition techniques, precisely tuned to resonate with 633 and 532 nm Raman lasers. Functionalization with a novel heteroaromatic ligand L, a derivative of alpha-lipoic acid and 2-(2-pyridine)imidazo[4,5,f]-1,10-phenanthroline, enables the creation of a highly selective Hb sensor. The sensing mechanism hinges on the coordination bonds formed between the phenanthroline unit of L and the iron center in the heme unit of the Hb protein.

Our sensor chip exhibits stability over a week, maintaining high sensitivity to Hb. Leveraging the characteristic SERS band of L observed at 1390 cm^{-1} , associated with the porphyrin methine bridge, we discern fluctuations in intensity corresponding to varying concentrations of normal Hb. This dynamic information is harnessed to assess iron content, facilitating the diagnosis of iron excess or deficiency indicative of various diseases. Furthermore, the SERS spectra distinguish $\text{Fe}^{2+}/\text{Fe}^{3+}$ redox species, providing insights into the oxygen-carrying capacity of Hb. Validation through electrochemical SERS, utilizing silver nanofilm on ITO, scrutinizes changes in $\text{Fe}^{2+}/\text{Fe}^{3+}$, potentially enabling early diagnosis of health conditions manifesting alterations in the oxidative states of iron in Hb.

Distinctive SERS bands in the "fingerprints region" allow discrimination between normal Hb and abnormal Hb variants. Density Functional Theory –Molecular Dynamic (DFT-MD) calculations correlate the experimental vibrational peaks enhancing the robustness of our findings. This study lays a pioneering foundation for extending our approach towards developing a lateral flow assay, promising a rapid and accurate diagnosis of Hb disorders. Our nano-biosensor holds transformative potential, heralding a new era in hemoglobin analysis and associated disorder diagnostics.

Keyword-1

Hemoglobin

Keyword-2

Surface-enhanced Raman spectra

Keyword-3

Plasmonic nanofilms

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