

**Title:** Intrinsic tenase blood biomarker imprinted polymer-aptasensor with carbon nanohorn and gold nano-urchin construct for primitive-phase diagnosis of haemophilia B

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**Abstract:** The selective biomimetic aptasensor for blood coagulation factor IX protein (FIX) detection was developed using an interdigitated electrode with an Archimedean spiral pattern. In contrast to conventional molecularly imprinted polymer (MIP) techniques, aptamer was employed as a macromonomer to accelerate double binding affinity. To preserve the aptamer profile in its protein-binding orientation, FIX protein and thiol-modified RNA aptamers were complexed prior to MIP fabrication. The immobilized aptamer-FIX complex was surrounded by a polymer generated by the electropolymerization of 3-thiophene acetic acid (3TAA). Subsequent to FIX protein removal, leaves imprinted cavities facilitate selective FIX protein detection in conjunction with the affinity of embedded aptamer affinity. The Archimedean IDE surface was functionalized with carbon nanohorn (CNH) and gold nanourchin (GNU) to increase the imprinting ratios and sensor sensitivity. The developed FIX-aptasensor shows a detection limit of 0.06 fM, which is 660-fold higher than aptamer-embedded MIP nanoparticles. Moreover, the sensor exhibited greater selectivity for FIX, discriminating IgG and thrombin. As a preliminary study for clinical use, the sensor was used to analyze human serum without target spiking and detected FIX-protein with a relative standard deviation of 9.18%. It was ascertained that the sensor maintained 85% sustained performance for a duration of five weeks.

**Keywords:** Aptasensor, Blood coagulation factor IX (FIX), Molecularly imprinted polymer (MIP), RNA aptamer, Electropolymerization, Carbon nanohorn (CNH), Gold nanourchin (GNU)

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