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# Electrochemical detection of cortisol using carbon nanotubes (CNT) modified with a pyrene derivative

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**Summary:**
Constant high cortisol levels resulting from chronic stress impair mental health and performance, yet real-time monitoring remains challenging. For non-invasive analysis, an electrochemical cortisol sensor was developed using carbon nanotubes (CNTs) non-covalently functionalized with pyrene derivatives (PY). Functionalized CNTs enabled cortisol interaction, with sensitivity and dynamic range evaluated through differential pulse voltammetry (DPV). Molecular dynamics (MD) simulations further clarified cortisol–pyrene interactions. This approach lays the groundwork for simple, non-invasive stress monitoring.

**Keywords:** Carbon nanotubes, Pyrene derivatives, Electrochemical sensing, Cortisol detection, Molecular dynamics

## Introduction

Mental health has become a major concern in recent decades, with chronic workplace stress recognized as a significant issue worldwide. This condition adversely affects work performance and overall well-being. Continuous monitoring of stress biomarkers—particularly cortisol, a well-established indicator of chronic stress—provides a promising approach to mitigating these effects. Consequently, there is a growing demand for accessible, non-invasive cortisol monitoring biofluids, such as saliva and sweat [1].

Electrochemical biosensors employing antibodies, aptamers, or molecularly imprinted polymers (MIPs) have been extensively studied [2,3]. For antibody immobilization, materials such as ZnO, molybdenum disulfide ($MoS_2$), gold nanoparticles (AuNP), silver oxide (Ag/AgO), and reduced graphene oxide (rGO) have been explored. Aptamer-based systems have used platinum/graphene and screen-printed graphene electrodes (SPGE), magnetic beads (MBs), and graphene quantum dots (GQD). MIP-based sensors offer a lower cost alternative, with materials such as AuNP-doped poly-o-PD, indium oxide ($In_2O_3$), and polydimethylsiloxane (PDMS)/carbon nanotube-cellulose nanocrystal (CNT-CNC). Although they offer varying sensitivity and detection ranges, the limitations persist: antibody instability and use of animal hosts, aptamer biodegradation, costly and time-consuming production, complex MIP optimization, and secondary probes. While more straightforward approaches using CNT showed significant potential in biosensing applications (e.g., metalloporphyrin-CNT electrodes), they often require complex chemistry and high costs.

Here, we demonstrate a CNT-based cortisol sensor exploiting the intrinsic properties of pyrene derivatives [4] for selective detection. This approach eliminates expensive biorecognition molecules and complex chemical modifications, with the potential for real-time stress monitoring in wearable devices.

## Results

Commercial screen-printed carbon electrodes (SPCEs) were modified with carbon nanotubes non-covalently functionalized with pyrene derivatives (CNT-PY) via drop-casting. The sensor response to cortisol solutions (µM range) was evaluated by differential pulse voltammetry (DPV) (Fig. 1). The pyrene derivative (PY) exhibited characteristic electrochemical behavior with a distinct reduction peak at -1.1 V in cortisol-free solution. Increasing cortisol concentrations caused partial inhibition of this peak's intensity (Fig. 1). A calibration curve for the CNT-PY sensor (Fig. 2) was established across a cortisol concentration range of 10-760 µM. The sensor demonstrated good sensitivity to cortisol (3.85

μA/log[CORT] μM) with a determination coefficient ($R^2$) of 0.948.

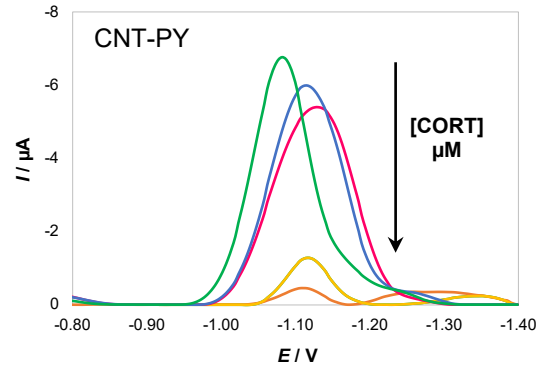


*Fig. 1. DPV obtained for CNT-PY sensor in response to a range of μM cortisol concentration solutions.*

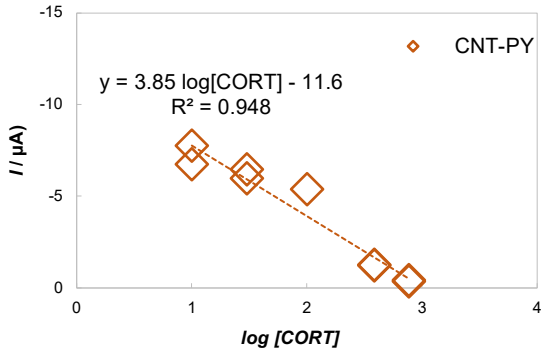


*Fig. 2. CNT-PY sensor calibration curve for different cortisol concentrations using DPV.*

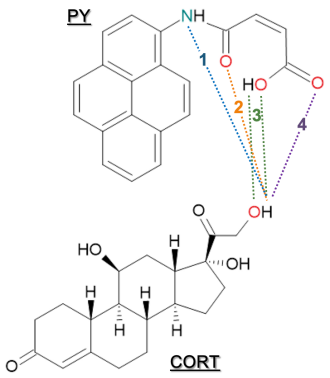


*Fig. 3. Schematics of most frequent PY and cortisol interactions.*

*Tab. 1: Hydrogen bond analysis results of MD simulations.*

| HBond Type | HBond/ CORT | % CORT | **Occupancy max %** |
|---|---|---|---|
| 1 | 0.8 | 40 | 0.17 |
| 2 | 1.1 | 80 | 0.28 |
| 3 | 1.8 | 70 | 0.09 |
| 4 | 1.8 | 80 | 0.11 |

To clarify the interaction mechanism between PY and cortisol, molecular dynamics (MD) simulations were performed using LAMMPS software. The modeled systems contained 4000 ethanol molecules, 20 PY molecules, and 10 cortisol molecules. Ethanol was modeled using the OPLS-AA force field, while the rest of the system followed the GROMOS54A7 force field, with parameters obtained from the ATB database. For the NPT ensemble, the temperature was maintained at 300 K using a Nosé-Hoover thermostat. Visual Molecular Dynamics (VMD) software was used to perform hydrogen bond (HBond) analysis between specific PY and cortisol functional groups (Fig. 3).

Table 1 summarizes the HBond analysis results. HBond type 4 (see Figure 3) showed the highest HBond frequency per cortisol molecule (HBond/CORT) and involved the highest percentage of cortisol molecules (% CORT) in PY interactions. Overall, the maximum occupancy (HBond persistence over time) remained low, consistent with the system's high dynamics. These results suggest that PY-cortisol interactions occur primarily through the chemical groups involved in HBond type 4. This non-covalent interaction between the two molecules could explain the partial passivation of PY´s electroactive site due to cortisol presence, which accounts for the observed inhibition of its reduction peak at −1.1 V.

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