The experimental chemical hardness in the interaction between \(\beta\)-tubulin and epothilone B

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Abstract. We showed the use of the experimental chemical hardness in the analysis of the interaction between \(\beta\)-tubulin and epothilone B. The interaction is analyzed by exposing the Epothilone B to a sensor based on an organic self-assembled monolayer functionalized with Beta tubulin. The proposed methodology is nondestructive and label-free. It allows us to compare the interaction between Beta-tubulin and different concentrations of the pharmacological molecule, i.e., epothilone B. The interaction is analyzed by the experimental hardness obtained according to the Miranda-Bueno methodology. We observed a linear behavior between the logarithm of the chemical hardness and the logarithm of epothilone B concentration. The results suggest the experimental chemical hardness can be used to compare the interaction of \(\beta\)-tubulin with epothilone B and with other pharmacological molecules.

1. Introduction

Cancer is one of the biggest problems faced by humanity. Its high incidence, morbidity, and mortality added to its ability to mutate and develop therapy resistance make it a challenge to find new anticancer agents as well as design novel therapeutics [1,2]. Identifying molecules with potential anticancer activity is a complicated and time-consuming task that evidences the need to improve molecule screening methodologies. Furthermore, drug-target binding affinity plays a critical role in treatment success. A high affinity is related to greater selectivity and fewer side effects. Then, a way to search for new anticancer agents is by comparing unknown molecules with known targets [3].

For instance, taxanes are a group of chemotherapeutic drugs whose target is the \(\beta\)-tubulin, a monomer that along with the \(\alpha\)-tubulin conforms the microtubules; the microtubules are the basic unit of the cytoskeleton and are responsible of move and organize the chromosome to the mitosis. The binding of a taxane molecule to \(\beta\)-tubulin hyper-stabilizes the microtubules and block the cellular mitosis [4,5].

Taxol and docetaxel are taxanes widely used for the treatment of many human tumors like non-small cell lung cancer, ovarian, and breast cancer; however, in many cases, cancer develops resistance to this kind of drug [6]. Epothilone B (EpoB) is a molecule with a different structure which shares the same action mechanism and similar effects of taxol [5]. This molecule has shown to be the most effective microtubule stabilizer causing fewer side effects than taxol, especially in the case of taxane resistant tissues [7]. Then, we choose EpoB to study its affinity interaction
with β-tubulin based on the assumption that the affinity interaction among molecules can be described in terms of the reactivity indexes.

The reactivity indexes can be appropriately defined in the framework of the conceptual density functional theory (DFT), e.g., the chemical potential and chemical hardness, as functional derivatives of the total energy \[8\]. Due to our interest of studying the affinity interaction of a known system with β-tubulin, the chemical hardness could be selected as an reactivity index to measure this interaction. The chemical hardness \((η)\) is a DFT concept defined as the second derivative of energy \((E)\) as a function of the system particles number \((N)\), Equation (1).

\[
η = \frac{\delta^2 E}{\delta N^2}
\]  
(1)

From Equation (1), \((η)\) can be intended as the resistance of the chemical system to change its electronic structure. An important issue is that \((η)\) can be measured directly by electrochemical experiments as shown Miranda-Bueno \[9\], it can be specifically obtained in terms of the experimentally measured electrochemical capacitance \((C_µ)\). For a mesoscopic system with more than \(10^{14}\) electronic states \((η)\) can be calculated with a good approximation as Equation (2).

\[
η = \frac{γ}{C_µ}
\]  
(2)

In Equation (2), \((γ)\) is a proportional constant.

Accordingly, we obtained the chemical hardness of a system consisting of β-tubulin immobilized on a self-assembled monolayer and interacting with EpoB electrochemical capacitance spectroscopy (ECS). This is, measuring the complex impedance spectra to obtain the complex capacitance using the Equation (3).

\[
C^* = \frac{1}{jωAZ^*} = \frac{Z''}{ωA|Z|^2} - j \frac{Z'}{ωA|Z|^2},
\]  
(3)

where \(ω\) is the angular frequency of the excitation signal and \(A\) the studied area.

We obtained the experimental chemical hardness of β-tubulin exposed to epothilone B and used it to quantify the interaction between the two molecules.

2. Methods and materials
A label-free sensor was fabricated with human β-tubulin immobilized on a mixed self-assembled monolayer (SAM). The SAM was prepared by immersion of a 2 mm diameter gold electrode into an ethanolic solution containing 11-ferrocenyl-1-undecanethiol and 16-mercaptoundecanoic acid in a ratio (9:1) at -10 °C. The gold electrode was pretreated by mechanical polishing and electrochemical cleaning, as described by Santos, et al. \[10\]. After 16 h, the gold electrode was rinsed with MilliQ water and dried with nitrogen gas, and the carboxylic groups were activated with EDC-NHS, followed by incubation with a 20 µL aliquot of β-tubulin during 1 h. Electrochemical measurements were used to characterize the sensor, and all measurements were carried out by triplicate, in a TBAClO\(_4\) solution at -10 °C with a PGSTAT104 potentiostat/galvanostat from AUTOLAB.

The obtained modified surface was characterized by cyclic voltammetry (CV) and ECS to ensure the correct attachment of the species. Finally the electrode was incubated with 20 µL aliquots of 0 µM, 5 µM, 15 µM, 40 µM, and 1 mM of EpoB dissolved in DMSO, each one during half an hour and followed by CV (0.0 V to 0.7 V at a scan rate of 100 mVs\(^{-1}\)) and ECS (frequency ranged from 1 MHz to 10 mHz with an amplitude of 104 V). ECS measures were carried on around the half-wave potential.

Python algorithms were developed to process the data. The chemical hardness \((η)\) was calculated as the inverse of the diameter of the semicircle formed by plotting the capacitance in
the complex plane (the imaginary part \( C'' \) Vs. the real part \( C' \)). Inverse modeling was applied directly to experimental data to parametrize and extract the semicircle diameter. An analytical curve was constructed by plotting \( \eta \) as a function of the Epo B concentration. The low limit of detection (LOD), and quantification (LOQ) were obtained following the IUPAC standard [11].

3. Results and discussion
We measured the complex capacitance of the sensor, described above, exposed to different concentrations of EpoB, and plotted the data in a complex plane to obtain Figure 1. With a Python algorithm, we extracted the distance between the real axis cutting-off points of the semicircle formed in this diagram, i.e., the electrochemical capacitance \( C_\mu \). Using \( C_\mu \), and since its inverse is proportional to the chemical hardness (\( \eta \)), [9], we constructed Figure 2. Experimental results, Figure 1 and Figure 2, showed differences due to the EpoB concentration and suggests the sensor can analyze the interaction between \( \beta \)-tubulin and epothilone B.

The diameter of the circles in Figure 1 decreases as the concentration of EpoB increases. It is important to note that the circles in Figure 1 were obtained by the application of an inverse modeling to raw data in a similar way to each of them. The application of the same algorithm to all data guarantees not to introduce biases due to data processing. Similarly, Figure 2 was obtained by applying the same algorithm to all experimental data.

In this study, we use a label-free sensor exposed to a molecule in different concentrations, to analyze the interaction between them. The label-free sensor was exposed to an environment of TBAClO\(_4\) with EpoB; then, the exposure to different concentrations of EpoB changes the environment and the interaction of the EpoB with the sensor. It is known the chemical hardness is a reactivity index that is highly sensitive to environmental changes [9,12,13], in consequence, the variations observed in the Figure 2 evidence the capability of the experimental hardness to evaluate the interaction among the studied molecules.
Previous studies have shown the inverse of electrochemical capacitance can be used to construct analytical curves [14]. In this work, an analytical curve, Figure 2, was constructed by plotting the logarithm of the difference between the chemical hardness of each concentration used and the one for the blank $\log(\eta - \eta_B)$ in terms of the logarithm of the epothilone B concentration (EpoB). The possibility to construct the analytical curve in terms of the experimental chemical hardness lets to increase our understanding of the physical-chemical processes that occur in the sensor, e.g., to describe possible interaction mechanisms.

The analytical curve, Figure 2, exhibits a linear correlation with a correlation factor near to one, LOD = 48 nM, and LOQ = 159 nM. These results show that the chemical hardness of the proposed sensor increases with the Epothilone concentration, this suggests both that the proposed methodology allows sensing the $\beta$-tubulin - EpoB interaction and that the experimental chemical hardness allows studying the binding affinity between molecules and its targets.

4. Conclusions
The experimental chemical hardness of a system containing $\beta$-Tubulin and interacting with Epothilone B, was obtained from experimental data, and let to analyze their interaction by the construction of an analytical curve. The results allowed inferring that the interaction between $\beta$-tubulin and epothilone B can be sensed and quantified with a LOD = 48 nM, and LOQ = 159 nM. These results show that the Miranda-Bueno model is appropriate to study molecular reactivity and could be useful to propose a methodology to search for new molecules with therapeutical applicability to cancer.

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