environmental parameters such as ambient humidity, light intensity and temperature in the vicinity of the working electrode.

In more detail, the technique developed by the inventors makes use of a working electrode that is functionalised with sensing elements that are electronically coupled to the underlying electrode. The distribution of electrons between the sensing elements and the electrode has an associated capacitive (and charge dynamic) fingerprint which changes sensitively as the local environment changes.

In the method of the invention, electrochemical impedance measurements are obtained across a range of different applied potentials (rather then the single underlying potential that is applied in a conventional EIS method). From the plurality of measurements obtained at different potentials are obtained measurements of the real and/or imaginary component of the complex capacitance, $C'$ and/or $C''$, as a function of potential at a fixed frequency ($\omega$). It has been found that integration of the measured $C'$ and $C''$ values over potential gives an integrated measurement value that intimately reflects, and therefore senses the nature of, the local environment of the working electrode. Specifically, the present invention provides:

[1] A sensing method comprising:

(A) obtaining, by electrochemical impedance spectroscopy conducted across a range of applied potentials, a plurality of measurements of the complex impedance, $Z^*$, of a system that has a working electrode that comprises an electrode substrate functionalised with sensing elements whose electrochemical response to the applied potentials is sensitive to a change in the local environment of the electrode, the sensing elements having a dimension of from 0.5 to 10 nm;

(B) converting said plurality of measurements of $Z^*$ into a plurality of measurements of the real component of the complex capacitance, $C'$ at a selected frequency $\omega$ and/or the imaginary component of the complex capacitance, $C''$, at a selected frequency $\omega$;

(C) integrating the measurements of (a) $C'$, (b) $C''$, or (c) any combination of $C'$ and $C''$, at the selected frequency $\omega$ as a function of applied potential to obtain an integrated measurement value; and

(D) evaluating the local environment of the electrode from said integrated measurement value.

The sensing method of the invention is suitable for electrochemical sensing by probing the quantum capacitance of a functionalised electrode. It may therefore be referred to as a "quantum capacitance sensing method".

The invention also therefore provides a quantum capacitance sensing method comprising steps (A), (B), (C) and (D) as defined above for the sensing method of the invention.

[2] An apparatus for use in a sensing method, which apparatus comprises:

- an electrochemical spectrometer that comprises a working electrode, a counter electrode and a potentiostat, wherein said working electrode comprises an electrode substrate functionalised with sensing elements whose electrochemical response to applied potentials is sensitive to a change in the local environment of the electrode, the sensing elements having a dimension of from 0.5 to 10 nm;
- a receiver configured to receive, from said electrochemical spectrometer, input data comprising a plurality of measurements of complex impedance, Z*, across a range of applied potentials; and
- a processor configured to (i) convert said plurality of measurements of Z* into a plurality of measurements of the real component of the complex capacitance, $C'$, at a selected frequency $\omega$ and/or the imaginary component of the complex capacitance, $C''$, at a selected frequency $\omega$, and (ii) integrate said measurements of (a) $C'$, (b) $C''$, or (c) any combination of $C'$ and $C''$, at the selected frequency $\omega$ as a function of applied potential to obtain an integrated measurement value. The sensing method may be a quantum capacitance sensing method, i.e. the apparatus may be for use in a quantum capacitance sensing method.

[3] A storage medium storing computer readable code for implementation by a computer or network of computers, the code, when implemented, causing the computer or network of computers to implement the steps of:

- obtaining, from an electrochemical spectrometer, input data comprising a plurality of measurements of complex impedance, Z*, across a range of applied potentials;
- converting said plurality of measurements of Z* into a plurality of measurements of the real component of the complex capacitance, C′, at a selected frequency ω and/or the imaginary component of the complex capacitance, C″, at a selected frequency ω; and
- integrating said measurements of (a) C′, (b) C″, or (c) any combination of C′ and C″, at the selected frequency ω as a function of applied potential to obtain an integrated measurement value.

Further preferred features and embodiments are described in the accompanying description and the appended claims.

**Brief description of the Figures**

Figure 1 depicts analytical curves constructed from the electronic density and its associated DOS variations, obtained as described in the Example. Box (b) shows $C_q=e^2$ *g(E)* variation (obtained from CS measurements) due to the interaction between anti-CRP and CRP in an electroactive molecular layer at varying CRP concentrations (the trend of increasing CRP concentration is indicated in the Figure). Absolute energies are shown in the top x-axis. The lines are an adjustment of experimental data (represented by dots) to Gaussian expected shape. Box (a) shows the linear relationship (analytical curve) between the natural logarithm of CRP concentration and the system's electron density.

Figure 2 depicts conductance changes and an associated analytical curve, obtained as described in the Example. Box (a) shows measurements of the conductivity due to a variation on electron density at various CRP concentrations (the trend of increasing CRP concentration is indicated in the Figure). Box (b) shows an analytical curve obtained from the variation on electron conductance on the layer due to the interaction between anti-CRP and CRP.

Figure 3 is a schematic representation of a working electrode (gold) functionalised with sensing elements that are electronically coupled to the underlying electrode. In this example, a sheet of graphene is mounted on the electrode and a protein serving as a

When conducting an EIS measurement, the bias potential (or direct current potential) may be set at any desired value. This DC or bias potential is known herein as the applied potential. The method of the present invention involves obtaining a plurality of measurements of the complex impedance across a range of applied potentials (which allows for the subsequent integration over applied potential), i.e. a number of EIS measurements are obtained each at different selected potentials. Typically the plurality of measurements of the complex impedance obtained by EIS is at least three measurements, preferably at least five measurements, such as at least ten or even at least twenty measurements, i.e. the range of applied potentials typically comprises at least three different applied potentials, preferably at least five different applied potentials, such as at least ten or even at least twenty different applied potentials.

In the step of converting the plurality of measurements of Z* into a plurality of measurements of the real component of the complex capacitance, C′, measurements of C′ at a (fixed/single) selected frequency ω are used. As would be well known to a skilled person C′ typically varies as ω changes (i.e. C′ is a function of ω). The appropriate selected frequency ω will of course depend on the construction of a particular electrode and on the nature of the sensing method being undertaken. However, determination of a suitable selected frequency ω is routine. The skilled person could easily, for example, identify a value of ω where the obtained values of C′ are satisfactorily high (e.g. at or close to the maximum value of C′ across the frequency range applied in a routine EIS scan) and/or responsive to the particular characteristic of the electrode's local environment that one is seeking to probe. Analogous principles apply when the plurality of measurements of Z*are converted into a plurality of measurements of the imaginary component of the complex capacitance, C″.

Conversion of Z* at the selected frequency ω into C′ and/or C″ is routine and well known in the art. In particular, in a standard practical EIS analysis, the complex impedance function $Z^*(\omega)$ at a particular potential can be converted phasorially into complex capacitance $C^*(\omega)$ with its real and imaginary components, using the equation $C^*(\omega) = 1/i\omega Z^*(\omega)$.

Integration of the measurements of C′ and/or C″ as a function of applied potential can also be routinely performed, for example using "area under the graph methods" when

C′, C″ or any combination of C′ and C″ is plotted against applied potential and/or by way of well known and routine computerised algorithms for integrating empirically derived data.

It has been found that integration of either C′ and C″ at the selected frequency ω as a function of applied potential provides an "integrated measurement value" that is suitable for sensing, i.e. that can be used to report on the local environment of the electrode when the EIS measurements were conducted. Specifically, an integrated measurement value derived from the integration of C′ is related to the density of states (DOS) of the system, i.e. it reflects the quantum capacitance (as exemplified in Figure 1 of the Example). An integrated measurement value derived from the integration of C″ is related to the conductance of the system (as exemplified in Figure 2 of the Example).

In practice, it may sometimes be preferable (for pure simplicity of operation) to obtain the integrated measurement value by integration of only one of C′ and C″ at the selected frequency ω as a function of applied potential. In a first preferred embodiment, therefore, the plurality of measurements of Z* is converted into a plurality of measurements of the real component of the complex capacitance, C′ at the selected frequency ω and these measurements are converted as a function of applied potential to obtain the integrated measurement value. Further, in a second preferred embodiment, the plurality of measurements of Z* is converted into a plurality of measurements of the imaginary component of the complex capacitance, C″ at the selected frequency ω and these measurements are converted as a function of applied potential to obtain the integrated measurement value.

However, since both C′ and C″ can be used, it will also be apparent to the skilled person that an integrated measurement value can be obtained by integrating any combination of C′ and C″, at the selected frequency ω as a function of applied potential. For example, any sum of the values of C′ and C″ (where C′ and/or C″ are possibly weighted with any negative or positive constants) or any multiple or quotient of the values of C′ and C″ can be used.

It is therefore to be understood that the term "quantum capacitance sensing method", as used herein, embraces methods in which the integrated measurement value is obtained by integration of only C″ at the selected frequency ω as a function of applied potential, such that the integrated measurement value reflects the conductance of the system rather than quantum capacitance, as well as methods in which the integrated measurement

the known reference drug (and therefore being worthy of further study). By contrast, failure to bind may lead to rejection of the drug candidate.

It will appreciated that in this sensing method the working electrode may form part of an array comprising a plurality of working electrode each functionalised with said receptor moieties, said array thereby being suitable for use the simultaneous screening of a plurality of drug candidates. This array set-up enables high throughput screening of many drug candidates at a time.

*Sensing of environmental parameters*

In a still further embodiment, the method is a method for sensing a change in an environmental parameter in the local environment of the electrode. Examples of such environmental parameters include the temperature of the local environment, the light intensity in the local environment (e.g., intensity of visible light, or alternatively or additionally the intensity of UV light) and the humidity in the local environment.

In such methods, the interaction of the light, temperature or ambient/surface water affects the measured DOS or electron density (obtained from the integrated DOS) at the sensing elements. The associated change in electrochemical response is measurable and can be readily calibrated for subsequent application.

**Apparatus**

The present invention also provides an apparatus for use in a sensing method, typically a sensing method of the present invention. This apparatus comprises an electrochemical spectrometer whose working electrode is functionalised with sensing elements, i.e. an electrochemical spectrometer that is specially adapted for performing the method of the present invention. The working electrode is as described herein.

The apparatus further comprises (a) a receiver configured to receive, from said electrochemical spectrometer, input data comprising a plurality of measurements of complex impedance, Z*, across a range of applied potentials; and (b) a processor configured to (i) convert said plurality of measurements of Z* into a plurality of measurements of the real and/or imaginary component of the complex capacitance, $C'$ and/or $C''$, at a selected frequency $\omega$, and (ii) integrate said measurements of $C'$, $C''$ or combination of $C'$ and $C''$, at the selected frequency $\omega$ as a function of applied potential to obtain an integrated measurement value. The receiver and processor can be part of a computer. The functionality of the receiver and processor can be achieved by

## Claims

1. A sensing method comprising:

(A) obtaining, by electrochemical impedance spectroscopy conducted across a range of applied potentials, a plurality of measurements of the complex impedance, Z*, of a system that has a working electrode that comprises an electrode substrate functionalised with sensing elements whose electrochemical response to the applied potentials is sensitive to a change in the local environment of the electrode, the sensing elements having a dimension of from 0.5 to 10 nm;

(B) converting said plurality of measurements of Z* into a plurality of measurements of the real component of the complex capacitance, C′, at a selected frequency ω and/or the imaginary component of the complex capacitance, C″, at a selected frequency ω;

(C) integrating the measurements of (a) C′, (b) C″, or (c) any combination of C′ and C″, at the selected frequency ω as a function of applied potential to obtain an integrated measurement value; and

(D) evaluating the local environment of the electrode from said integrated measurement value.

2. A sensing method according to claim 1, wherein in step (A) said obtaining, by electrochemical impedance spectroscopy conducted across a range of applied potentials, a plurality of measurements of the complex impedance, comprises obtaining at least five measurements of the complex impedance at different applied potentials.

3. A sensing method according to claim 1 or 2, wherein said evaluating in step (D) is performed by comparing said integrated measurement value with one or more reference values obtained by performing the steps (A), (B) and (C) under conditions where the local environment of the electrode is known.

comprises an electrode substrate functionalised with sensing elements whose electrochemical response to applied potentials is sensitive to a change in the local environment of the electrode, the sensing elements having a dimension of from 0.5 to 10 nm;

- a receiver configured to receive, from said electrochemical spectrometer, input data comprising a plurality of measurements of complex impedance, Z*, across a range of applied potentials; and
- a processor configured to (i) convert said plurality of measurements of Z* into a plurality of measurements of the real component of the complex capacitance, C′, at a selected frequency ω and/or the imaginary component of the complex capacitance, C″, at a selected frequency ω, and (ii) integrate said measurements of (a) C′, (b) C″, or (c) any combination of C′ and C″, at the selected frequency ω as a function of applied potential to obtain an integrated measurement value.

18. An apparatus according to claim 17, further comprising an output unit configured to output data generated from said integrated measurement value.

19. A sensing method according to any one of claims 1 to 16, or an apparatus according to claim 17 or claim 18, wherein said sensing method is a quantum capacitance sensing method.

20. A storage medium storing computer readable code for implementation by a computer or network of computers, the code, when implemented, causing the computer or network of computers to implement the steps of:

- obtaining, from an electrochemical spectrometer, input data comprising a plurality of measurements of complex impedance, Z*, across a range of applied potentials;
- converting said plurality of measurements of Z* into a plurality of measurements of the real component of the complex capacitance, C′, at a selected frequency ω and/or the imaginary component of the complex capacitance, C″, at a selected frequency ω; and

- integrating said measurements of (a) C′, (b) C″, or (c) any combination of C′ and C″, at the selected frequency ω as a function of applied potential to obtain an integrated measurement value.