CLAIMS

What is claimed is:

1. A cortisol sensor comprising or consisting of:

i) a metal nanoparticle;

ii) a plurality of cortisol binding ligands conjugated to the surface of the metal nanoparticle; and

iii) a plurality of peptides conjugated to the surface of the metal nanoparticle by means of a Cysteine (C) residue for reducing non-specific binding of the cortisol binding ligands and/or improving stability of the metal nanoparticle in solution.

2. The cortisol sensor of claim 1, wherein the metal nanoparticle comprises or consists of a noble metal, preferably gold, more preferably the metal nanoparticle is a citrate-coated gold nanoparticle, and wherein the metal nanoparticle has a diameter in the range of 5-50 nm, more preferably a diameter in the range of 10-20 nm, even more preferably a diameter of about 13 nm, so long as the metal nanoparticle has desirable plasmonic properties and/or sensitivity to UV-vis shift in response to differential aggregation.

3. The cortisol sensor of claim 1 or 2, wherein the cortisol binding ligand is a cortisol aptamer, preferably a cortisol DNA aptamer, optionally the cortisol aptamer is conjugated directly to the surface of the metal nanoparticle or indirectly through an intervening linker.

4. The cortisol sensor of claim 3, wherein the cortisol aptamer comprises or consists of a nucleotide sequence set forth in **SEQ ID NO:1, 2** or **3**, or derivatives thereof, preferably the cortisol aptamer comprises or consists of a nucleotide sequence set forth in **SEQ ID NO:2**, or derivatives thereof.

5. The cortisol sensor of claim 3 or 4, wherein the cortisol aptamer is:

(i) labelled with a thiol group, and the thiol group is covalently bound to the surface of the metal nanoparticle, such that the cortisol binding ligand is conjugated to the surface of the metal nanoparticle, preferably the cortisol aptamer is thiol-terminated at the 5' end; and/or

(ii) terminated with an amine group (NH_2), and the amine group is covalently bound to the surface of the metal nanoparticle, such that the cortisol binding ligand is conjugated to the metal nanoparticle, wherein the surface of the metal nanoparticle is functionalized with carboxylic acid groups (COOH); and/or

(iii) biotinylated with a biotin group, optionally introduced via a linker molecule or by incorporating a biotinylated nucleotide during the aptamer synthesis, and the biotin group is bound to the surface of the metal nanoparticle, such that the cortisol binding ligand is conjugated to the metal nanoparticle, wherein the surface of the metal nanoparticle is functionalized with streptavidin.

6. The cortisol sensor of any one of claims 1-5, wherein the plurality of peptides is a plurality of short peptides, optionally each short peptide comprises or consists of an amino acid sequence CXn, CAXn, or CALXn, wherein X denotes any amino acid residue and n is an integer of 1 or more, and/or optionally each short peptide is labelled with a thiol group, preferably the short peptide comprises or consists of an amino acid sequence set forth in **SEQ ID NO: 4** or variants thereof.

7. The cortisol sensor of any one of claims 1-6, wherein:

i) the metal nanoparticle is a gold nanoparticle;

ii) the cortisol binding ligand is a cortisol DNA aptamer comprising or consisting of a nucleotide sequence set forth in **SEQ ID NO: 1, 2** or **3**, or derivatives thereof, and is labelled with a thiol group at the 5' end, wherein the thiol group is covalently bound to the surface of the gold nanoparticle; and

iii) the peptide comprises or consists of an amino acid sequence set forth in **SEQ ID NO: 4** or variants thereof.

8. A method of preparing a cortisol sensor of claim 1, comprising or consisting the steps of:

a) preparing a first solution comprising a plurality of metal nanoparticles;

b) preparing a second solution comprising a plurality of cortisol-binding ligands;

c) adding the first and second solutions together to produce a first mixture;

d) incubating the first mixture under conditions suitable to conjugate the cortisol-binding ligands onto the surface of the metal nanoparticles; and

e) adding a third solution comprising a plurality of peptides to the incubated first mixture under conditions suitable to conjugate the peptides onto the surface of the metal nanoparticles to produce a second mixture comprising one or more of the cortisol sensor, optionally wherein the second mixture is used to prepare a colloidal solution or composition comprising a plurality of the cortisol sensors.

9. The method of claim 8,

wherein the metal nanoparticle is a gold nanoparticle, and step a) comprises: adding a sodium citrate dihydrate solution, preferably about 5mL at a concentration of 40 mM, to boiling gold (III) chloride solution, preferably about 50mL at a concentration of 1mM, to provide a mixture, refluxing the mixture for about 20-40 minutes, preferably about 30min, to provide the first solution comprising a plurality of gold nanoparticles; and cooling the first solution to room temperature, and

wherein the cortisol binding ligand is a cortisol aptamer, and step b) comprises: adding the cortisol aptamer in a buffer solution, optionally shaking the buffer solution comprising the cortisol aptamer; centrifuging the buffer solution to produce a supernatant, preferably the buffer solution is transferred to a 3kDa centrifugal filter; and adding water to the supernatant to provide the second solution, and

wherein step c) further comprises adding an acidic solution, preferably hydrochloric acid, and wherein the second solution is added to the first solution and subsequently the acidic solution is

added thereafter, optionally the acidic solution is in an amount of about 0.5-50µl, preferably about 10 µl, optionally the concentration of the acidic solution is about 0.01M-1M, preferably 0.1M, and

wherein step e) further comprises adding an alkaline solution, preferably sodium hydroxide, optionally the alkaline solution is in an amount of about 0.5-50µl, preferably about 10 µl, optionally the concentration of the alkaline solution is about 0.01M-1M, preferably 0.1M.

10. Use of the cortisol sensor according to any one of claims 1-7 for detecting the presence or absence, and optionally the quantity, of cortisol in a sample.

11. A method to detect the presence or absence, and optionally the quantity, of cortisol in a sample, comprising or consisting the steps of:

a) providing a cortisol sensor according to any one of claims 1-7;

b) contacting the cortisol sensor to the sample in a saline environment that is suitable for binding the cortisol binding ligand to cortisol, to produce a sample mixture; and

c) detecting the presence or absence, and optionally the quantity, of cortisol in the sample mixture by determining the degree of aggregation of the cortisol sensor in the sample mixture, wherein the degree of aggregation of the sample mixture is compared with the degree of aggregation of a control sample and a differential degree of aggregation is indicative of the presence or absence, and optionally the quantity, of cortisol in the sample.

12. The method of claim 11, wherein a bivalent salt ion is added to the sample mixture in step b), wherein the bivalent salt ion is selected from the group consisting of a calcium chloride ($CaCl_2$), calcium carbonate ($CaCO_3$), calcium sulfate ($CaSO_4$), magnesium chloride ($MgCl_2$), magnesium sulfate ($MgSO_4$), magnesium carbonate ($MgCO_3$), barium chloride ($BaCl_2$), barium sulfate ($BaSO_4$), lead acetate ($Pb(CH_3COO)_2$), lead nitrate ($Pb(NO_3)_2$), ferrous sulfate ($FeSO_4$), ferrous chloride ($FeCl_2$) and combinations thereof, preferably the bivalent salt ion is $MgCl_2$.

13. The method of claim 11 or 12, wherein the degree of aggregation is measured by UV-Visible Spectroscopy, microscopy techniques, dynamic light scattering techniques, visual observation of colour, or surface plasmon resonance techniques, preferably the degree of aggregation is measured by UV-vis spectroscopy to obtain a UV-vis spectrum of the sample mixture, wherein the UV-vis spectrum of the sample mixture is compared with the UV-vis spectrum of a control sample and a differential UV-vis spectrum is indicative of the presence or absence, and optionally the quantity, of cortisol in the sample,

optionally, further comprising quantifying the cortisol in the sample mixture based on the absorbance ratio of A_{520}/A_{650}, wherein the absorbance ratio of the sample mixture is compared with the absorbance ratio of a control sample, wherein a differential absorbance ratio between the sample and control sample is indicative of the presence, and quantity, of cortisol in the sample mixture, and the absorbance ratio of the control sample is subtracted from the absorbance ratio of the sample

mixture, preferably the differential absorbance ratio is in the range of 0.2-1.2 and corresponds to cortisol concentrations ranging from 0.1-1000 nM.

14. The method of any one of claims 11-13, wherein the degree of aggregation is determined by measuring the absorbance ratio of the sample mixture, wherein the absorbance ratio of the sample mixture is compared with the absorbance ratio of the control sample, and a differential absorbance ratio is indicative of the presence or absence, and optionally the quantity, of cortisol, preferably an absorbance ratio of A_{520}/A_{650} is measured for the sample mixture and control sample, wherein a differential absorbance ratio of A_{520}/A_{650} between the sample and control sample is indicative of the presence or absence of cortisol in the sample.

15. The method of any one of claims 11-14, wherein the sample is a water sample obtained from an aquaculture tank, preferably a fish tank, or the sample is a biological sample, preferably a biological tissue or fluid sample that has been obtained from an animal, even more preferably a fish tissue sample, optionally the fish tissue sample is derived from the fin, scales, mucus, or plasma of the fish and may be in a fluid form or a solid form, optionally wherein the solid form is processed and extracted into a liquid form.

16. A method of measuring a stress level in a subject, comprising:

a) contacting a biological sample that has been obtained from the subject with a cortisol sensor according to any one of claims 1-7 in a saline environment that is suitable for binding the cortisol binding ligand to cortisol, to produce a biological sample mixture; and

b) detecting the quantity of cortisol in the biological sample mixture by determining the degree of aggregation, wherein the degree of aggregation of the biological sample mixture is compared with the degree of aggregation of a control sample and a differential degree of aggregation is indicative of the quantity of cortisol in the biological sample mixture and the stress level of the subject.

17. A method for assisting in determining a subject's risk for developing an autoimmune disease, an inflammatory disease, and/or an allergic disease through measuring the subject's cortisol level with the method of claim 11, wherein the sample has been obtained from the subject.

18. A method for predicting a subject' s response to a steroid treatment for treating a disorder through measuring the subject's cortisol level with the method of claim 11, wherein the sample has been obtained from the subject.

19. A method for assisting in determining the presence or absence of a mental disorder in a subject through measuring the subject's cortisol level with the method of claim 11, wherein the sample has been obtained from the subject, optionally wherein the mental disorder is one or more of an anxiety disorder, a major depressive disorder (MDD), a post-traumatic stress disorder (PTSD), a traumatic brain injury (TBI), a psychotic disorder, and/or a personality disorder.

20. A method for developing or identifying therapeutic agents capable of modifying the stress level of a subject, the method comprising:

a) measuring the subject's stress level with the method of claim 16,

b) administering a candidate therapeutic agent to the subject,

c) measuring the subject's stress level with the method of claim 16 following the administration of the candidate therapeutic agent,

d) characterizing the candidate therapeutic agent as capable of modifying the stress level of the subject, if the subject's measured cortisol level increases or decreases following administration of the candidate therapeutic agent.

21. A colloidal solution or composition comprising a plurality of cortisol sensors according to any one of claims 1-7.

22. A substrate comprising a plurality of cortisol sensors according to any one of claims 1-7, for use in detecting the presence or absence, and optionally the quantity, of cortisol in a sample.