High Performance De Novo Cortisol Biosensors

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BACKGROUND

Cortisol is an essential steroid hormone that is involved in numerous physiological processes such as the stress response, regulation of blood pressure, immune modulation, and regulation of the sleep cycle. Cortisol levels can vary based on several factors. Cortisol imbalances can indicate adrenal disorders, such as Cushing's Syndrome and Addison's Disease. Cortisol imbalance can also lead to disruption in the sleep cycle, increased stress, and metabolic disorders. Given these facts, accurate and accessible cortisol monitoring is crucial for diagnostics and overall health.

Standard methods for monitoring cortisol levels involve enzyme-linked immunosorbent assays or liquid chromatography-tandem mass spectrometry. These methods, while reliable are performed in laboratory settings using expensive equipment and take significant time to produce results. Reliable on-site or at-home detection methods of cor are unavailable, but would be important tools

TECHNOLOGY DESCRIPTION

The technology is a biosensor for cortisol that involves two components: a recognition domain that binds cortisol and undergoes significant conformational changes; and a reporter system, such as split luciferase, that generates a detectable signal upon reconstitution. Naturally occurring cortisol-binding proteins, such as corticosteroid-binding globulin and the glucocorticoid receptor lack the ability to undergo substantial conformational changes upon cortisol binding or possess complex folding patterns that hinder biosensor development.

To overcome these limitations, Andy Yeh's team at UC Santa Cruz designed cortisol-responsive protein dimerization systems from scratch using computational de novo protein design. These de novo proteins dimerize in the presence of cortisol, bringing split luciferase domains into proximity to form reconstituted and active luciferases, which produce luminescence.

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OTHER INFORMATION

KEYWORDS

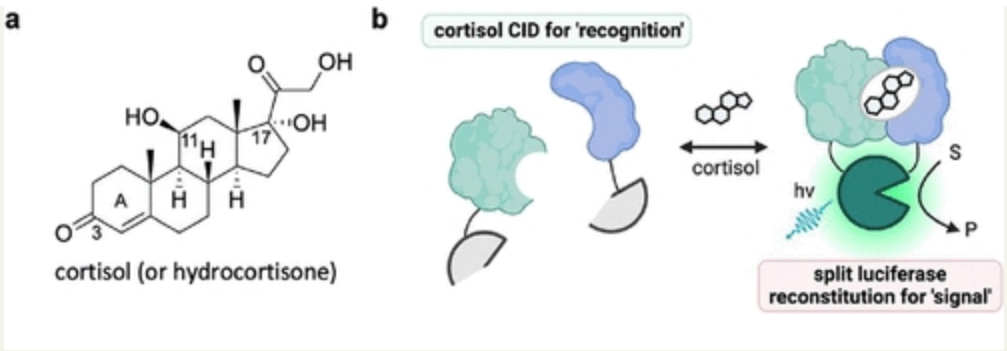
cortisol, biosensor, de novo protein, luciferase, chemically induced dimerization, AI protein design

CATEGORIZED AS

- Biotechnology
  - Health

RELATED CASES

2025-951-0



Specifically: in previous work a number of de novo designed nuclear transfer factor 2 (NTF2) scaffolds were developed that bound small molecules. One of these, hcy129.1, binds cortisol with nanomolar affinity, within the concentration range of cortisol in the body. Strucutral studies showed that hcy129.1 binds the core steroid structure of cortisol but leaves the 3-carbonyl group and adjacent carbon atoms of steroid ring A exposed to the solvent.

This work developed another protein that specifically binds to the hcy129.1-cortisol complex without binding cortisol alone. Three mutations - R43I, R95Q, and Q128L were introduced into hcy129.1 because these modifications removed charged and polar residues that could obstruct binding to the hcy129.1-cortisol complex. The mutant form of hcy129.1 is referred to as mhcy129. It has similar folding profile to hcy129.1 and also has nanomolar binding affinity to cortisol.

To design cortisol-dependent chemically induced dimerization (CID) systems, the team used RIFdock to place ‘lid’ protein scaffolds against the open mhcy129-cortisol complex interface. A library of previously described helical bundles was docked explicitly to form contacts with the exposed cortisol interface. This docking strategy generated numerous ternary complexes by making contacts with the cortisol ligand, where mhcy129 and the lid proteins interact only in the presence of cortisol to minimize ligand-independent dimerization. Subsequently, the team performed sequence design on the docked complexes using a combination of Rosetta FastDesign and ProteinMPNN. The designed ternary complex structures were prefiltered *in silico* to ensure that the lid proteins formed enough physical interactions with both cortisol and mhcy129.

A library of designed lid proteins was screened for binding to mhcy129-cortisol experimentally by expression on yeast cells.

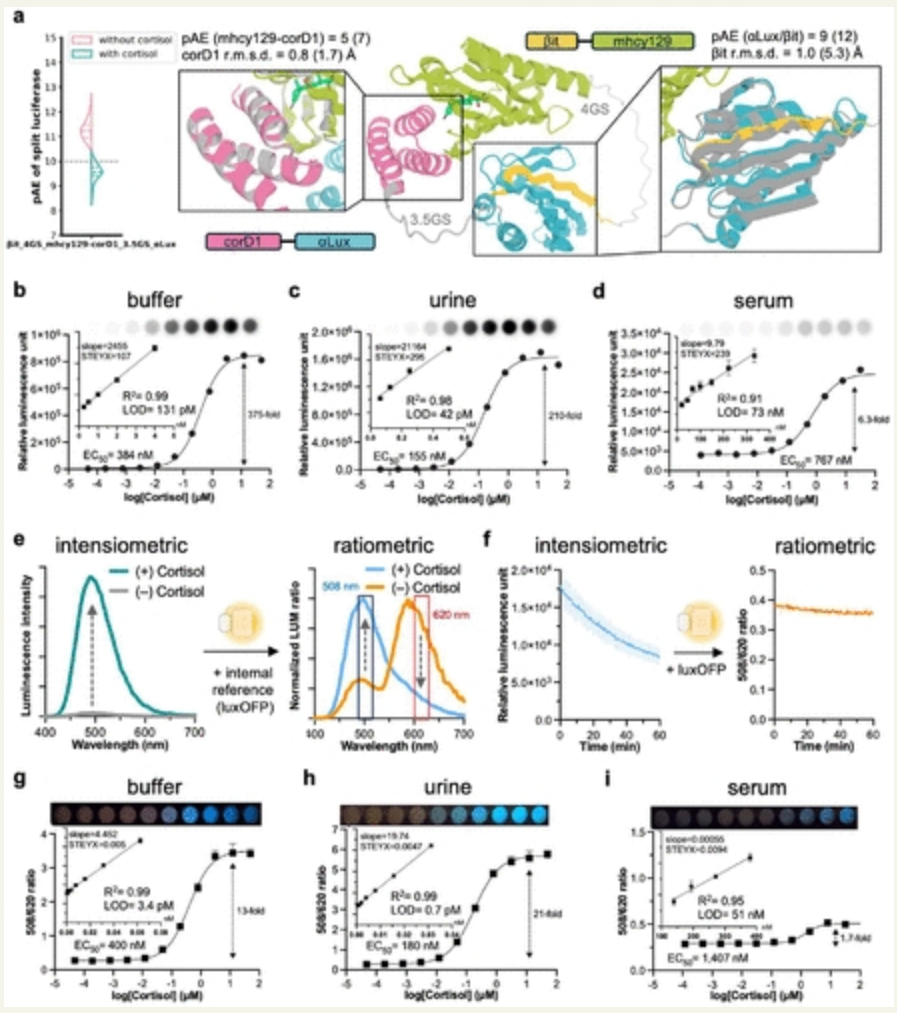
Reporter modules used in this study were two different split luciferase systems: the NanoBiT(R) split luciferase system from Promega and the LuxSit sPro(R) system from Monod Biosciences. The Yeh lab is in the process of developing new split luciferase systems that perform better in the cortisol sensor.

The combination of fusing cortisol recognition modules with split luciferase reporter modules presents a vast design space, which requires the sampling of a large number of possible configurations to satisfy important factors such as compatible domain arrangement, suitable linker length, and matched geometric orientation. So AlphaFold3 was used to sample the designable space.

A lid protein called corD1 consistently showed superior predictions from computational analysis, so it was selected for more computational sampling across a number of linker lengths.

Ultimately the following monomers were expressed corD1-3.5GS-αLux, mhcy129-4GS-βitsPro, and βitsPro-4GS-mhcy129. The fusion proteins based on the αLux/βit binary system showed monomeric and monodispersed protein folding profiles. Additionally, SEC trace analysis showed a clear shift in retention time, suggesting that corD1-3.5GS-αLux and βitsPro-4GS-mhcy129 form a heterodimer triggered by the addition of cortisol.

To assess the performance of the de novo biosensors for cortisol detection, the team measured the luminescence signals of these biosensors with varying cortisol concentrations. The corD1-3.5GS-αLux/βitsPro-4GS-mhcy129 luminescent biosensor exhibited a 375-fold increase in emission with a limit of detection (LOD) of 131 pM while the corD1-3.5GS-αLux/mhcy129-4GS-βitsPro pair showed higher luminescence intensity at saturated cortisol concentrations along with a 153-fold change with a LOD of 82 pM. Both biosensor pairs displayed a detection regime spanning over 4 orders of magnitude and significantly outperformed that of the previous NanoBiT-based design, which showed a 28-fold change with a LOD of 63 nM in HBS buffer. Moreover, the designed corD1-3.5GS-αLux/βitsPro-4GS-mhcy129 biosensor maintained a robust 210-fold signal change with a LOD of 42 pM in synthetic urine and a 6.3-fold signal change with a LOD of 73 nM in human serum. In contrast, the NanoBiT system showed a 21-fold change (LOD = 46 nM) in urine and a 2.8-fold change (LOD = 398 nM) in serum under identical conditions. Although serum components attenuated the sensor performance, the broad dynamic range and high luminescence intensity of our designed biosensors still allowed for straightforward signal detection using a standard camera.



APPLICATIONS

On-site cortisol diagnostics

At home cortisol monitoring

Cortisol level monitoring

Chemical induced dimerization protein design

Therapeutic monitoring

De novo protein design

ADVANTAGES

Wide dynamic range

Visible light signal

Highly sensitive

Able to be used for on-site care

Low-cost

User-friendly and straightforward

Signal detectable with phone camera

RELATED MATERIALS

- De Novo Design of High-Performance Cortisol Luminescent Biosensors - 07/28/2025
- Artificial biosensor can better measure the body’s main stress hormone - 07/28/2025

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