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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Bioluminescence data acquired on a Synergy Neo2 multi-mode microplate reader (Biotek). Biolayer interferometry data acquired on an Octet RED96 (ForteBio).
Data analysis	Bioluminescence data were analyzed and plotted using GraphPad Prism 8. Target response curves were fitted using a Sigmoidal 4PL fit in GraphPad Prism 8. Limit of detection calculations were performed using Simple Linear regression in GraphPad Prism 8. BLI data was analyzed using ForteBio Data Analysis Software version 9.0.0.10 and plotted using GraphPad Prism 8. The model building and structure refinement were performed by using COOT and PHENIX. The design models and RosettaScripts code used in the manuscript have been deposited to http://files.ipd.uw.edu/pub/de_novo_design_of_tunable_biosensors_2021/designcode_and_models.zip. The code for the numerical simulations shown in this manuscript are available at http://files.ipd.uw.edu/pub/de_novo_design_of_tunable_biosensors_2021/model_simulation.py. All protein structure images were generated using PyMOL 2.0.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The atomic coordinates of sCageHA_267-1S have been deposited in the Protein Data Bank (http://www.rcsb.org) under an accession code 7CBC. The design models and RosettaScripts code used in the manuscript have been deposited to http://files.ipd.uw.edu/pub/de_novo_design_of_tunable_biosensors_2021/designcode_and_models.zip. The code for the numerical simulations shown in this manuscript are available at http://files.ipd.uw.edu/pub/de_novo_design_of_tunable_biosensors_2021/model_simulation.py. The original experimental data that support the findings of this work are available from the corresponding authors upon request. Plasmids encoding the biosensor proteins described in this article are available from the corresponding authors upon request.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to pre-determine the sample size. in vitro experiments were done in triplicate.
Data exclusions	No sample was excluded from data analysis
Replication	The results were successfully replicated using different batches of pure proteins on different days.
Randomization	De-identified clinical serum samples were randomly used for the detection of spiked target proteins.
Blinding	no blinding was employed since all experiments are in vitro.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	1. SARS-CoV Matrix Antibody (ProSci, Cat. No.: 3527) 2. SARS Nucleocapsid Protein Antibody (18F629.1) (NovusBio Cat. No. NBP2-24745) 3. HzKR127-3.2
Validation	1. SARS-CoV Matrix Antibody (ProSci, Cat. No.: 3527) is a rabbit IgG polyclonal antibody raised against a peptide corresponding to 13 amino acids near the amino-terminus of human SARS-CoV Matrix protein. The antibody is proven to bind the immunogen by ELISA. by the manufacturer. This antibody has predicted crossreactivity with SARS-CoV-2 Matrix protein based on immunogen sequence: human SARS-CoV2 Matrix protein: (identity 77%, homology 93%) by the manufacturer, which is confirmed in this work. 2. SARS Nucleocapsid Protein Antibody (18F629.1) is validated by Western Blot by the manufacturer. The antibody was developed by immunizing mice with a synthetic peptide corresponding to amino acids 354-385 from the N (SARS Nucleocapsid) for the Human

SARS coronavirus. Immunogen Percent Identity to SARS-CoV-2 Nucleocapsid Protein predicted to be 100% by the manufacturer and cross-reactivity confirmed in this work.
3. Validation of the antibody function is thoroughly described here: Kim, J. H. et al. Enhanced humanization and affinity maturation of neutralizing anti-hepatitis B virus preS1 antibody based on antigen-antibody complex structure. FEBS Lett. 589, 193–200 (2015). The antibody was produced by Wi and Hong (Department of Systems Immunology, College of Biomedical Science, Kangwon National University, Chuncheon 200-701, Republic of Korea).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK293F (Invitrogen; No. K9000-01)
Authentication	Cells were not further authenticated in the laboratory.
Mycoplasma contamination	HEK293F cells were tested negative for Mycoplasma by the provider, and it was not further confirmed in the laboratory
Commonly misidentified lines (See ICLAC register)	*Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*

Human research participants

Policy information about studies involving human research participants

Population characteristics	Serum specimens were derived from excess plasma or sera from adults (>18 yo) of both genders kindly provided by the Director of the Clinical Chemistry Division, the hospital of University Washington. All anonymized donor specimens were provided de-identified.
Recruitment	the donors consented to have their excess specimens be used for other experimental studies, they could be transferred to our study without additional consent.
Ethics oversight	the Clinical Chemistry Division, the hospital of University Washington.

Note that full information on the approval of the study protocol must also be provided in the manuscript.