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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<u>Authors & Referees</u> and the<u>Editorial Policy Checklist</u>.

Statistics

For	all st	atistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a	Cor	firmed
	×	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
	x	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
	x	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.
X		A description of all covariates tested
X		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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted Give <i>P</i> values as exact values whenever suitable.
x		For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
X		For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
x		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	com	puter	code
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Data collection	AFM MultimodeVIII (Veeco, USA) built-in software ; tapping mode HS-AFM (RIBM, Japan) built-in software; N-storm (Nikon) built-in software
Data analysis	NanoScope Analysis Ver.1.4 ; Kodec Ver.4. 4. 7. 39 ; Image J Ver.1.8.0; Origin software Ver.9.0; python Ver.3.6
For manuscripts utilizing custo	m algorithms or software that are central to the research but not vet described in published literature. software must be made available to editors/reviewers

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/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 datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable 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

Life sciences study design

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

Sample size	In this paper, we aim to study the transient conformation of antibodies at the single molecular level. Due to the flexibility of the structure of the antibody, the instantaneously binding of antigens to antibodies, and the complexity of instrument AFM and HS-AFM operations, obtaining a high-resolution conformation is not easy. Here, simple random sampling is used to determine the statistics of the sample and the required sample size is estimated by the standard of error limit of 0.05 and confidence of 95%.
Data exclusions	The statistical analysis of all the data in this article did not exclude the data.
Replication	In order to ensure the reproducibility of the data, the reagents used in the experiment were uniformly made and stored separately to ensure the consistency of the concentration used each time, and professional pipette was assigned for single-molecule experiments. In addition, the instrument is stabilized for a period of time before each experiment to reduce instrument error and the same operator reduces human error.
Randomization	The experiment requires us to construct antigens at different distances on DNA origami as samples. Due to the successful technology of DNA origami, the obtained samples are basically the same, showing uniform distribution in solution. Due to the need of AFM and TIRF instruments, we adsorbed DNA origami onto mica and glass pieces through magnesium ions and phospholipid membranes, respectively. The adsorption is random, the only thing we can control is the concentration of origami.
Blinding	Because the DNA origami has addressable characteristics, so we can know clearly that what is the position of antibodies bind to the DNA origami, and analyze the data obtained by AFM with high resolution in combined with the end of Fab is fixed on DNA origami. The each domain of antibody can accurately identify, therefore in the process of data collection and analysis is not blind.

Behavioural & social sciences study design

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

Study description	Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).
Research sample	State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.
Sampling strategy	Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.
Data collection	Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.
Timing	Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.
Data exclusions	If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.
Non-participation	State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.
Randomization	If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.

Ecological, evolutionary & environmental sciences study design

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

Study description

Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.

Research sample	Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.
Sampling strategy	Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.
Data collection	Describe the data collection procedure, including who recorded the data and how.
Timing and spatial scale	Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken
Data exclusions	If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.
Reproducibility	Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.
Randomization	Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.
Blinding	Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.
Did the study involve fiel	d work? Yes No

Field work, collection and transport

Field conditions	Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).
Location	State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).
Access and import/export	Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).
Disturbance	Describe any disturbance caused by the study and how it was minimized.

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	Methods	
n/a Involved in the study	n/a Involved in the study	
Antibodies	ChIP-seq	
🗶 🗌 Eukaryotic cell lines	Flow cytometry	
🗶 🗌 Palaeontology	MRI-based neuroimaging	
🗶 🗌 Animals and other organisms		
🗶 🗌 Human research participants		
🗶 🗌 Clinical data		
Antibodies		

Antibodies used	Monoclonal Anti-Digoxin (Catalog Number D8156, Sigma lot no: 029M4896V) is derived from the hybridoma DI-22. It was obtained from Sigma Aldrich and used in various immunochemical techniques including ELISA, dot blot. Monoclonal Anti-Biotin IgG (Catalog Number B7653, Sigma lot no: 109M4785V) is derived from the BN-34 hybridoma. It was obtained from Sigma Aldrich and used in a wide range of applications including blotting, immunocytochemistry and electron microscopy. Cholesterol antibody (Catalog Number LS-C295824, lot no:133978) is an unconjugated rabbit polyclonal antibody to all species Cholesterol. It was brought from Lifespan Bioscience and various of applications such as ELISA.
Validation	Anti digoxin antibody may be used to detect digoxin-labeled compounds such as oligonucleotides, antibodies, or peptides.

Labeled compounds and corresponding conjugated antibodies can be used for the detection of viruses and bacterial infections in human diagnostics, oncogenes as tumor markers, histocompatibility antigens in transplantation analytics causative research (e.g., in autoimmune diseases), characterization of lymphoid cell subpopulations (e.g., during treatment of lymphomas), determination of genetic defects or genetic defect predispositions (e.g., Alzheimer's disease), and nucleic acid diagnostics. It

was validation by Biuret, RID, ELISA Indirect.

Monoclonal Anti-Biotin recognizes the free biotin molecule and biotin conjugated to various immunoglobulins in ELISA and immunohistochemical techniques. Specificity was verified by using biotinylated goat antibodies reactive against human and rabbit antigens coated on microtiter plates. This antibody can be used in many other applications where biotin can be introduced as a target label. For instance, it has been used in detection of low copy human papilloma virus DNA and mRNA in routine paraffin sections of cervix by sensitive non-isotopic in-situ hybridization. It has also been used successfully for the detection of microinjected biotin-haptenized cytoskeletal proteins to examine directly the pattern of incorporation and turnover of cytoskeletal proteins in living cells.

Anti-cholesterol antibody can be reactivity all species and may be used in a wide range of applications such as ELISA.

Eukaryotic cell lines

Policy information about cell lines	
Cell line source(s)	State the source of each cell line used.
Authentication	Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.
Mycoplasma contamination	Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.
Commonly misidentified lines (See <u>ICLAC</u> register)	Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

Palaeontology

Specimen provenance	Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information).
Specimen deposition	Indicate where the specimens have been deposited to permit free access by other researchers.
Dating methods	If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.

Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Animals and other organisms

Policy information about <u>stu</u>	dies involving animals; ARRIVE guidelines recommended for reporting animal research
Laboratory animals	For laboratory animals, report species, strain, sex and age OR state that the study did not involve laboratory animals.
Wild animals	Provide details on animals observed in or captured in the field; report species, sex and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.
Field-collected samples	For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.
Ethics oversight	Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	Describe the covariate-relevant population characteristics of the human research participants (e.g. age, gender, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."
Recruitment	Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.

Ethics oversight

Identify the organization(s) that approved the study protocol.

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

Clinical data

Policy information about <u>clinical studies</u> All manuscripts should comply with the ICMJEguidelines for publication of clinical research and a completed <u>CONSORT checklist</u> must be included with all submissions.				
Clinical trial registration	Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.			
Study protocol	Note where the full trial protocol can be accessed OR if not available, explain why.			
Data collection	Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.			
Outcomes	Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.			

ChIP-seq

Data deposition

Confirm that both raw and final processed data have been deposited in a public database such as GEO.

Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links May remain private before publication.	For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.
Files in database submission	Provide a list of all files available in the database submission.
Genome browser session (e.g. <u>UCSC</u>)	Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.
Methodology	
Replicates	Describe the experimental replicates, specifying number, type and replicate agreement.
Sequencing depth	Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.
Antibodies	Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.
Peak calling parameters	Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.
Data quality	Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.
Software	Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.

Flow Cytometry

Plots

Confirm that:

The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

All plots are contour plots with outliers or pseudocolor plots.

A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.	
Instrument	Identify the instrument used for data collection, specifying make and model number	
Instrument	Identify the instrument used for data collection, specifying make and model number.	

 Describe the software used to collec community repository, provide acces

Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.

Gating strategy

Cell population abundance

Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.

Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design Indicate task or resting state; event-related or block design. Design type Design specifications Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials. State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used Behavioral performance measures to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects). Acquisition Specify: functional, structural, diffusion, perfusion. Imaging type(s) Specify in Tesla Field strength Sequence & imaging parameters Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle. Area of acquisition State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined. **Diffusion MRI** Used Not used Preprocessing Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, Preprocessing software segmentation, smoothing kernel size, etc.). Normalization If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization. Normalization template Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized. Noise and artifact removal Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration). Define your software and/or method and criteria for volume censoring, and state the extent of such censoring. Volume censoring Statistical modeling & inference Model type and settings Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation). Effect(s) tested Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used. Specify type of analysis: ROI-based Whole brain Both Statistic type for inference Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods. (See Eklund et al. 2016) Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Correction

Models & analysis

n/a Involved in the study Image: Provide the study	
Functional and/or effective connectivity	Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).
Graph analysis	Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).
Multivariate modeling and predictive analysis	Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.