Structural insights into the mechanism of human soluble guanylate cyclase

Yunlu Kang1,4, Rui Liu1,4, Jing-Xiang Wu1 & Lei Chen1,2,3*

Soluble guanylate cyclase (sGC) is the primary sensor of nitric oxide. It has a central role in nitric oxide signalling and has been implicated in many essential physiological processes and disease conditions. The binding of nitric oxide boosts the enzymatic activity of sGC. However, the mechanism by which nitric oxide activates the enzyme is unclear. Here we report the cryo-electron microscopy structures of the human sGCα1β1 heterodimer in different functional states. These structures revealed that the transducer module bridges the nitric oxide sensor module and the catalytic module. Binding of nitric oxide to the β1 haem–nitric oxide and oxygen binding (H–NOX) domain triggers the structural rearrangement of the sensor module and a conformational switch of the transducer module from bending to straightening. The resulting movement of the N termini of the catalytic domains drives structural changes within the catalytic module, which in turn boost the enzymatic activity of sGC.

Nitric oxide (NO) is a gaseous signalling molecule that is involved in many important physiological processes, such as vasodilatation, neurotransmission, platelet aggregation, immunity, cell proliferation, and mitochondrial respiration12,13. The dysregulation of NO signalling has been linked to cardiovascular disease, sepsis, acute lung injury, and multiple organ failure13,14. NO signalling is initiated by the activation of NO synthase (NOS), which generates NO in response to physiological stimuli3. NO readily permeates target cell membranes, and after diffusing across the membrane, it binds and activates soluble guanylate cyclase (sGC), the primary NO acceptor5. sGC catalyses the cyclization reaction of guanosine triphosphate (GTP) to generate inorganic polyphosphate and the secondary messenger cyclic guanosine monophosphate (cGMP)5. cGMP then acts on downstream effectors, including cGMP-regulated protein kinases, phosphodiesterases, and ion channels, to regulate physiological processes in the cell2. Genetic mutations of sGC in humans are associated with coronary artery disease6, moyamoya disease, achalasia, and hypertension7,8, and it is a validated drug target for the treatment of pulmonary hypertension and chronic heart failure4. Drugs that activate or stimulate sGC also have therapeutic potential in fibrotic diseases, systemic sclerosis, chronic kidney diseases, neuroprotection, dementia, and sickle cell disease9.

sGC is a heterodimeric protein complex composed of one α-subunit and one β-subunit. In humans, the α1 and β1 subunits are widely expressed in many tissues, while the expression of α2 and β2 subunits is tissue-specific10. The α- and β-subunits have the same sequence homology and are similarly organized into modular domains, including an N-terminal H-NOX domain, a Per/Arnt/Sim (PAS) domain, a coiled-coil (CC) domain, and a C-terminal catalytic domain. The PAS and CC domains mediate protein–protein interactions, and the catalytic domain is responsible for enzymatic activity5,11. The H-NOX domain of the β-subunit contains a ferrous b-type haem prosthetic group that facilitates the high-affinity binding of NO5,11. Under pathological conditions or oxidative stress, the ferrous haem can be oxidized to ferric haem12, and haem-oxidized sGC has low activity even in the presence of NO13.

Several structures of isolated sGC domains have been solved by X-ray crystallography or NMR. These structures include the human β1 H-NOX domain (PDB ID: 5MNW), the Manduca sexta α-PAS domain14, the human β1 CC domain15, and the human α1β1 catalytic domain heterodimer16,17. Recent negative stain electron microscope studies18 have revealed the general shape of the full-length mammalian sGC at a resolution of 25–40 Å, and hydrogen–deuterium exchange experiments mapped NO-induced structural changes onto the primary sequence of the full-length sGC19. Despite these pioneering structural efforts, the allosteric mechanism that underlies the activation of the distal catalytic domain in response to binding of NO to the β-subunit H-NOX domain remains unclear at the atomic level, mainly owing to the lack of high-resolution structural information on intact sGC in different functional states. Here, we have used cryo-electron microscopy (cryo-EM) to determine the structure of the human α1β1 sGC holoenzyme in both the inactive and NO-activated states at a resolution of 3.9 Å and 3.8 Å, respectively. We also obtained a 6.8 Å resolution cryo-EM map of the constitutively active β1(H105C) mutant. These structures uncover not only the detailed domain–domain interfaces, but also the activation mechanism of human sGC.

Structure determination
We purified sGC composed of human α1 and β1 subunits to apparent homogeneity (Fig. 1a, Extended Data Fig. 1a, b), and the protein showed the characteristic ultraviolet–visual light (UV–vis) spectrum of sGC with ferrous haem bound (Extended Data Fig. 1c) and robust NO-activated GTP cyclase activity (Fig. 1b). By contrast, the β1(H105C) mutant, in which the haem group is unable to bind, showed constitutively high basal activity and was insensitive to NO activation20 (Fig. 1b). We prepared a haem-unliganded state sample, a haem-oxidized state sample (Extended Data Fig. 1c) and an NO-activated state sample using the wild-type sGC protein (see Methods). These samples were subjected to single-particle cryo-EM analysis (Extended Data Figs. 1–4) and we obtained overall resolutions of 4 Å, 3.9 Å, and 3.8 Å, respectively. Map qualities were further improved by dividing the whole molecule into two bodies, the larger ‘N lobe’ and the smaller ‘C lobe’, and subsequent multibody refinements21 (Supplementary
Structure of sGC in the inactive state

Both the haem-unliganded and the haem-oxidized sGC were in a ‘bent’ conformation (Fig. 1c–e, Supplementary Video 3). In our cryo-EM reconstructions, we found that the overall structure of sGC in the haem-unliganded state (4 Å) is essentially the same as that in the haem-oxidized state (3.9 Å), with a root mean square deviation (r.m.s.d.) of only 0.28 Å (Extended Data Fig. 9a), in accordance with the functional data, which showed that the haem-unliganded and haem-oxidized states have low activity (Fig. 1b). Therefore, both of the structures were considered as the inactive state, and the 3.9 Å haem-oxidized state is used in further discussion of the inactive state. The structure of the inactive sGC occupies a 3D space of 140 Å × 75 Å × 75 Å (Fig. 1c–e). The large N lobe is composed of α1 H-NOX, α1 PAS, β1 PAS, and β1 H-NOX domains. These domains are arranged in a pseudo-two-fold symmetric manner, with the scaffolding PAS domains at the centre and the H-NOX domains at the periphery (Fig. 1c). The H-NOX domains and PAS domains are essential for NO sensing and form the N-terminal sensor module of sGC (Fig. 1c). The CC domains of both subunits form the transducer module that bridges the N-terminal sensor module and the C-terminal catalytic module (Fig. 1d).

A haem molecule binds inside the β1 H-NOX domain, and its five-coordinated Fe ion is tightly bound to H105 of αF, as evidenced by the strong connecting density between them (Fig. 1f). By contrast, the αE H-NOX domain does not bind haem owing to a steric clash (Extended Data Fig. 9b). The structure of each PAS domain resembles that of the M. sexta sGC α-subunit (PDB ID: 4GJ4; Extended Data Fig. 9c, d). Extensive domain–domain interactions stabilize the structure of sGC in the inactive state (Extended Data Fig. 7, Supplementary Notes 1–3). The β1 H-NOX domain, especially the εE and εF helices, interacts with both the neighbouring PAS heterodimers and the transducer module (Extended Data Figs. 2g, 7a–e, Supplementary Note 1). These interactions further stabilize the transducer module in the bent conformation, in which both the α1 and β1 CC domains are broken into two short helices (αM and αN) connected by a near 90° turn (Extended Data Fig. 7f). The two αN helices pack in a ‘leucine zippers’ manner and interact extensively with the catalytic module (Extended Data Fig. 7f–h, Supplementary Note 2). In the catalytic module, the two subunits are organized in a pseudo-symmetric manner as well, but the angle between domains is different from that of the isolated catalytic module (Extended Data Fig. 9e, f, Supplementary Note 3). Compared with the adenylate cyclase in the active state (PDB ID: 1CJU), the structure of the catalytic module shows steric clashes between the substrate and the protein residues (Extended Data Fig. 9g). This suggests that the structure of sGC in the inactive state is incompatible with substrate binding, consistent with previous studies that showed that inactive sGC has a high Michaelis constant (Kₘ) (Supplementary Note 3). The domain–domain interactions observed in the inactive state were further validated by cysteine cross-linking under oxidative conditions (Extended Data Fig. 7i–l, Supplementary Note 4).

Structure of sGC in NO-activated state

The NO-activated sGC has a dumbbell-shaped extended structure (Fig. 2), in which the sensor module moves away from the catalytic module (Fig. 2, Supplementary Video 4). This is markedly different from the bent conformation of the inactive state. In addition, the overall structure of the constitutively active β1(H105C) mutant, in the absence of NO donors, shows a similar extended conformation (Extended Data Fig. 4g). This structural agreement suggests that this large overall conformational change is associated with enhanced enzymatic activity and the full activation of sGC, but probably does not result from the S-nitrosylation of sGC by NO, which is a covalent modification of cysteine residues that can lead to desensitization of sGC under certain conditions (Extended Data Fig. 4j, Supplementary Note 5).

Despite the large overall conformational change, the general domain arrangement within each module in the NO-activated state is...
The structure of sGC in the NO-activated state (coloured as in Fig. 1a) is superimposed onto the structure of sGC in the inactive module. The structure of sGC in the NO-activated state result in completely new interfaces between the adjacent domains of the inactive state (Extended Data Fig. 8a). This not only permits the binding of the substrate analogue GMPCPP (Fig. 3f), which was added during the NO-activation process, but also alters the local chemical environment of the pocket to make it possible for small stimulators to plug in and activate the enzyme (Supplementary Note 9). In the map of the NO-activated state, we observe a strong density corresponding to the substrate analogue GMPCPP (Fig. 3f), which was added during the NO-activation process. This suggests that the current conformation is likely to correspond to an NO-bound state, because excess NO donor DEA NONOate was added to the sample and NO binds sGC with picomolar-range high affinity.

To determine the functional importance of this bending–straightening conformational change, we mutated residues in the αM–αN linker to either prolines or alanines. Prolines generate kinks in helical structures because they cannot form hydrogen bonds on the main chain. Therefore, proline mutations should destabilize the helical structures of αMNs in the NO-activated state, and these proline mutants may favour the inactive conformation. Indeed, proline mutations of D423 in the α1CC domain or G356 in the α1 subunit rendered sGC unresponsive to NO activation, although these mutants could incorporate haem normally (Fig. 3a, b, Extended Data Fig. 8d). By contrast, mutations of the same set of residues into alanines had no such effect (Fig. 3a, b), indicating that the continuous helical structures of the αMNs are essential for activation of sGC by NO.

In the NO-activated conformation, the interface between the α1 and β1 CC domains is markedly different from that observed in the inactive state (Fig. 3a, Extended Data Fig. 7f, Supplementary Note 8). Besides the overall bending–straightening movements of each CC domain, the αN helix of the α1 subunit rotates approximately 70° around the αN helix of the β1 subunit (Fig. 3c). The separation of the C termini of the transducer modules also decreases. The distance between the Co atoms of P459 of the α1 subunit and P399 of the β1 subunit shrinks from 26 Å to 20 Å (Fig. 3d). This drives the structural reorganization of the connecting catalytic module, in which the catalytic domain of the α1 subunit rotates 17° relative to the β1 catalytic domain (Fig. 3e, Extended Data Figs. 8f, 9i, j). These movements increase the volume of the central pocket from 1,375 Å³ to 1,549 Å³ and reorganize the catalytic centre (Extended Data Fig. 9k). This not only permits the binding of the substrate GTP and the cofactor Mg²⁺ ions but also alters the local chemical environment of the pocket to make it possible for small stimulators to plug in and activate the enzyme (Supplementary Note 9). In the map of the NO-activated state, we observe a strong density corresponding to the substrate analogue GMPCPP (Fig. 3f), which was added during cryo-EM sample preparation. By comparing the current structure with the active adenylate cyclase structure (PDB ID: 1CJU) (Extended Data Fig. 9l), we found that the residues responsible for substrate binding

maintained (Figs. 1, 2). In the electron density map of the NO-activated state, the H105–Fe bond of β1 H-NOX is cleaved, as evidenced by the clear separation between each density (Fig. 2c). This suggests that the current conformation is likely to correspond to an NO-bound state, because excess NO donor DEA NONOate was added to the sample and NO binds sGC with picomolar-range high affinity. However, we could not explicitly model the NO molecules or the haem deformation owing to the limited resolution. The binding of NO induces a conformational change in β1 H-NOX in which the C-terminal subdomain rotates relative to the N-terminal subdomain (Extended Data Fig. 9h). When αF (residues 96–107) of the β1 subunit was used as the reference to superimpose the structure of the NO-bound β1 H-NOX domain onto the structure of the inactive state, the Co atom of N62 in the N-terminal subdomain was displaced by 4.6 Å (Extended Data Fig. 8a) and, more importantly, the NO-bound β1 H-NOX domain sterically clashed with the adjacent domains of the inactive state (Extended Data Fig. 8a). This indicates that the inactive state structure is incompatible with the NO-bound β1 H-NOX domain and, therefore, a structural rearrangement is required to accommodate the conformational change of the β1 H-NOX domain upon NO binding. Indeed, we observed structural changes within the sensor module in which α1 H-NOX underwent a small downward movement while β1 H-NOX underwent a large rotational and translational movement (Fig. 2d).

These conformational changes of the sensor module in the NO-activated state result in completely new interfaces between the NO-bound β1 H-NOX domain and its adjacent domains (Extended Data Figs. 4e, 8b, c, Supplementary Notes 6, 7). Many residues contribute to this new interface; among them, D106 on αF of the β1 H-NOX domain forms important polar interactions with other residues (Fig. 2e). We found that sGC with the β1(D106A) mutation had normal haem incorporation but impaired activation by NO (Fig. 2f, Extended Data Fig. 8d). To determine the functional importance of this bending–straightening conformational change, we mutated residues in the αM–αN linker to either prolines or alanines. Prolines generate kinks in helical structures because they cannot form hydrogen bonds on the main chain. Therefore, proline mutations should destabilize the helical structures of αMNs in the NO-activated state, and these proline mutants may favour the inactive conformation. Indeed, proline mutations of D423 in the α1CC domain or G356 in the α1 subunit rendered sGC unresponsive to NO activation, although these mutants could incorporate haem normally (Fig. 3a, b, Extended Data Fig. 8d). By contrast, mutations of the same set of residues into alanines had no such effect (Fig. 3a, b), indicating that the continuous helical structures of the αMNs are essential for activation of sGC by NO.

In the NO-activated conformation, the interface between the α1 and β1 CC domains is markedly different from that observed in the inactive state (Fig. 3a, Extended Data Fig. 7f, Supplementary Note 8). Besides the overall bending–straightening movements of each CC domain, the αN helix of the α1 subunit rotates approximately 70° around the αN helix of the β1 subunit (Fig. 3c). The separation of the C termini of the transducer modules also decreases. The distance between the Co atoms of P459 of the α1 subunit and P399 of the β1 subunit shrinks from 26 Å to 20 Å (Fig. 3d). This drives the structural reorganization of the connecting catalytic module, in which the catalytic domain of the α1 subunit rotates 17° relative to the β1 catalytic domain (Fig. 3e, Extended Data Figs. 8f, 9i, j). These movements increase the volume of the central pocket from 1,375 Å³ to 1,549 Å³ and reorganize the catalytic centre (Extended Data Fig. 9k). This not only permits the binding of the substrate GTP and the cofactor Mg²⁺ ions but also alters the local chemical environment of the pocket to make it possible for small stimulators to plug in and activate the enzyme (Supplementary Note 9). In the map of the NO-activated state, we observe a strong density corresponding to the substrate analogue GMPCPP (Fig. 3f), which was added during cryo-EM sample preparation. By comparing the current structure with the active adenylate cyclase structure (PDB ID: 1CJU) (Extended Data Fig. 9l), we found that the residues responsible for substrate binding
and catalysis are in similar positions, indicating that the current sGC structure represents a catalytically competent conformer.

**Structural mechanism of sGC activation**

By analysing the structures of individual sGC domains in both inactive and NO-activated states, we found that the structures of the scaffolding PAS dimer remain relatively unchanged among the different states, with a r.m.s.d. of 0.91 Å (Extended Data Fig. 9m). Therefore, we used the structures of the PAS dimer as a reference point to align and compare the two full-length structures (Fig. 4a). During activation, the α1 H-NOX domain makes a small concomitant downward movement, while the interfaces between α1 H-NOX and its adjacent domains are largely maintained (Fig. 2d). This suggests that the α1 H-NOX domain may play a role that is mainly sensor-specific instead of being involved in NO signal transduction (Supplementary Video 5). This is in agreement with the finding from an activity assay that the H-NOX domain of the α1 subunit is dispensable for NO activation (Fig. 4b). By contrast, the local conformational change of the β1 H-NOX domain upon NO binding drives the structural rearrangement of the sensor module (Fig. 2d), which, along with previous functional data, suggests that the H-NOX domain of the β1 subunit has an essential role in NO sensing. Indeed, complete removal of the β1 H-NOX domain rendered the sGC enzyme trapped in a relatively low activity state and unresponsive to NO activation (Fig. 4b). This suggests that the β1 H-NOX domain in the NO-bound state is necessary to stabilize the sGC enzyme in an active conformation. Further supporting this conclusion, disruption of the interactions between β1 H-NOX and adjacent domains by mutation also diminished activation by NO (Fig. 2f). The structural changes in the sensor module upon binding of NO trigger the bending–straightening conformational switch of the transducer module. As a result, the distal catalytic module rotates 86° in a swing-like manner and the centre of mass of the catalytic module is spatially displaced by 101 Å (Fig. 4a). Inhibition of the straightening by proline mutations abolishes activation by NO (Fig. 3b), which suggests that the conformational
change in the transducer module is essential for activation of the catalytic module. Furthermore, we found that the catalytic module changes from a conformation that cannot bind substrate to a catalytically competent conformation (Fig. 3e, f, Extended Data Figs. 8f, 9g, k), which explained how the binding of NO decreases the $k_{\text{on}}$ of sGC-GTP and increases the catalytic constant ($k_{\text{cat}}$) of sGC-GTP (Fig. 4e). It has been previously proposed that the activation of sGC by NO involves two steps, and our structure observations are compatible with this two-step hypothetic model (Supplementary Note 10). Notably, information flow in the reverse direction, from the catalytic module to the sensor module, has been suggested by published functional data.[26][28][30]. Therefore, the transducer module acts as an allosteric structural coupler between the sensor module and the catalytic module to allow the bi-directional flow of information within the sGC molecule (Supplementary Note 11).

Online content
Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-019-1584-6.

Received: 22 May 2019; Accepted: 3 September 2019; Published online 12 September 2019.
Methods

Cell culture. HEK293F (Thermo Fisher Scientific) suspension cells were cultured in Freestyle 293 medium (Thermo Fisher Scientific) or SIMM 293-TI medium (Sino Biological) supplemented with 1% FBS at 37 °C with 6% CO2 and 70% humidity. It is reported that HEK293F is a female cell line. S9 insect cells (Thermo Fisher Scientific) were cultured in SIM SF (Sino Biological) at 27 °C. The cell lines were routinely checked to be negative for mycoplasma contamination but have not been authenticated.

Protein expression and purification. cDNA of Drosophila melanogasterα1, mouse, and human sGC were cloned into a modified BacMam expression vector32,33 and transfected into HEK293F cells for screening by fluorescence-detection size exclusion chromatography (FSEC)34 on a Superox 6 increase 5/150 GL. The combination of C-terminal GFP-tagged human α1 and non-tagged β1 subunits yielded a stable heterodimer. sGC protein composed of an α1 and β1 subunit is the most predominant isomorph, and it has been widely used as a model protein to elucidate the biochemical, biophysical, and structural properties of mammalian sGC.25 The coding sequences of human α1 and β1 subunits were transformed into the pFastbac dual vector and expression was driven by p10 or polyh ylimin promoters. The corresponding baculovirus was generated using the Bac-to-Bac system.

S9 insect cells at a density of 4 × 10^6/mL in SIM SF medium were infected with the baculovirus and cultured at 27 °C in a shaker for 72 h before harvesting and storage at −80 °C. Cells corresponding to 500 ml culture were thawed and resuspended with 20 ml lysis buffer (50 mM Tris pH 8.0 at 4 °C, 150 mM NaCl) containing 1 μg/ml aprotinin, 1 μg/ml pepstatin, 1 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride (PMSF), 2 μM diithiothreitol (DTT), and 1 μM ethylenediaminetetraacetic acid (EDTA). Cells were broken by sonication in 5 s intervals followed by a 5 s pause at 50% output for 20 min. Unbroken cells, cell debris, and membranes were removed by ultracentrifugation at 40,000 r.p.m. for 1 h at 4 °C using a Ti70 rotor (Beckman). An excess amount of purified glutathione S-transferase-tagged GFP-nanobody35 was added to the supernatant and incubated at 4 °C for 10 min with rotation. Samples were then loaded onto 4 ml Glutathione Sepharose 4B columns (GE Healthcare) and washed with TBS buffer (20 mM Tris, pH 8.0, 150 mM NaCl) containing 1 mM DTT at 4 °C. Protein was eluted from glutathione-Sepharose 4B beads (GE Healthcare) using 20 mM Tris, pH 8.0, 150 mM NaCl and 1 mM DTT at 4 °C. The eluate was diluted with buffer A (20 mM Tris, pH 8.0 at 4 °C) to a conductivity lower than 5 mS/cm and loaded onto a 1-l HiTrap Q HP (GE Healthcare). The protein was eluted with buffer B (20 mM Tris, pH 8.0, 500 mM NaCl) at 4 °C in a linear gradient using the AKTA pure system (GE Healthcare). The peak fractions containing sGC were pooled and incubated with precipitate protease overnight to cleave the tag from the protein. The digested protein was further purified by Superdex 200 increase 200 (GE Healthcare) running in buffer containing 20 mM HEPES (pH 7.4), 50 mM NaCl and 2 mM tris (2-carboxyethyl) phosphine (TCEP). The peak fractions containing the sGC protein were pooled and concentrated. The UV–vis spectrum was measured using a spectrometer (Pultton) in the cuvette mode.

Activity assay. The protein used for cryo-EM sample preparation was diluted with 20 mM triethanolamine (TEA, pH 7.6), 300 mM NaCl 1 mM DTT and subjected to activity assay as described below. For the haem-oxidized sample, the protein was diluted with 20 mM TEA (pH 7.6), 300 mM NaCl and preincubated with 20 μM NS2028 at 25 °C for 30 min and then added DTT to the final concentration of 1 mM for activity assay. To generate the sGC mutant protein for activity assay, the coding sequences of the α1 subunit with a C-terminal GFP-tag and the β1 subunit were cloned into pFastBac expression vectors, respectively. To generate the α1κ (ΔNNOX) and the β1κ (ΔNNOX) constructs, the N-terminal 273 amino acids of α1 subunit were removed. Additional mutations of C176A, C239A, C669S, C453Y, and C460G were created in the α1 subunit and C292N in the β1 subunit. The coding sequences of α1κ and a C-terminal GFP-tag and β1κ without tags were cloned into modified BacMam expression vectors32,33. Then specific amino acids were mutated into cysteines using the Quick Change method. Cysteine mutants were transfected into HEK293F cells with polyethylenimine (PEI) (PolySciences) at a density of 2.0 × 10^6/ml. Cells were harvested 72 h after transfection and broken by passing through a syringe filter insert in inner chamber ten times. Unwanted debris were removed by centrifugation at 14,800 r.p.m. for 10 min at 4 °C. sGC proteins were purified from supernatants using Streptactin Beads 4FF resin (Smart-Lifesciences, China). Protein samples were cross-linked on ice for 30 min by adding Cu(m)(1,10-phenanthroline) to a final concentration of 30 μM to promote disulfide bond formation. Protein samples were subjected to 4–15% gradient SDS–PAGE (Beyotime Biotechnology, China) for separation either in non-reducing condition or reducing condition (in the presence of 100 mM DTT). The fluorescence was detected using a ChemiDoc MP (Bio-Rad) fluorescence imaging system.

Cryo-EM data acquisition. Cryo-grids were screened on a Talos Arctica electron microscope (Thermo Fisher Scientific) operating at 200 kV using a Ceta 16M camera (Thermo Fisher Scientific). The screened grids were transferred to a Titan Krios electron microscope (Thermo Fisher Scientific) operating at 300 kV with an energy filter set to a slit width of 20 eV. Images were recorded using a K2 Summit direct electron camera (Thermo Fisher Scientific) in super-resolution mode at a nominal magnification of 130,000 ×, corresponding to a calibrated super-resolution pixel size of 0.522 Å. The defocus range was set from −1.5 μm to −2 μm. Each image was acquired as a 7.68-s movie stack (32 frames) with a dose rate of 0.25 e− Å^-2 s^-1, resulting in a total dose of about 48 e− Å^-2. All data acquisition was done using SerialEM.

Cryo-EM data processing. The data processing workflows are illustrated in Extended Data Figs. 1–4 and Extended Data Table 1. Super-resolution movie stacks were motion-corrected, mag-distortion corrected, dose-weighted, and binned to a pixel size of 1.045 Å by MotionCor2 1.1.0 using 5 × 5 patches39. Contrast transfer function (CTF) parameters were estimated from non-dose-weighted micrographs using Gctf v1.0640. Micrographs with ice or ethane contamination, empty carbon, and poor CTF fit (≤5 Å) were manually removed. All classification and 3D reconstructions performed iteratively at least twice. The highest resolution was estimated using cryoSPARC42 using the selected particles from 2D classification. The particles
were further subjected to 3D classification to remove bad particles using the initial model, which was low-pass filtered to 30 Å as the reference. The particles selected from good 3D classes were re-centred and re-extracted, and their local CTF parameters were individually determined using Gctf v1.06.40. These particles were imported into cisTEM43 and subjected to 3D classification with auto-masking. The particles from the best 3D classes calculated by cisTEM were exported into Relion 3.0 and subjected to 3D auto-refinement to generate the consensus map. However, the two large lobes of sGC in the consensus maps showed blurry features, which were indicative of continuous conformational heterogeneities. Therefore, we divided the whole molecule into two bodies—the larger N lobe and the smaller C lobe—for further multibody refinement (Extended Data Figs. 1–4) in Relion 3.0. The subsequent local map qualities were greatly improved (Extended Data Figs. 2a, b, c, e). In detail, two soft masks that cover the N lobe and C lobe were generated from the consensus map, which was edited manually in UCSF Chimera using the volume eraser tool44. 3D multi-body refinements45 were performed using the two soft masks of the lobes and the parameters determined from previous 3D auto-refinement. The motions of the bodies were analysed by relion_flex_analyse in Relion 3.0. The two half-maps of each lobe generated by 3D multi-body refinement were subjected to post-processing in Relion 3.0. The masked and sharpened maps of each lobe were aligned to the consensus map using UCSF Chimera and summed to generate the composite map for visualization and interpretation. All of the resolution estimations were based on a Fourier shell correlation (FSC) of 0.143 cutoff after correction of the masking effect42. B-factors used for map sharpening were automatically estimated by the post-processing procedure in Relion 3.0.

Model building: The position of the β1 H-NOX domain was first identified according to its distinguishable haem group density. Other domains were assigned by the domain–domain linkers that are visible in the post-processed map (Extended Data Fig. 2h). The homology models of individual H-NOX, PAS and catalytic domains were generated by the Phyre2 server46 based on the structures of the human β1 H-NOX domain (PDB: 5MNW), M. sexta α PAS domain (PDB: 4G4J)48 and human α1-β1 catalytic domain heterodimer (PDB: 3UVI)49. The models were placed into the corresponding composite maps using UCSF chimeraw40 and manually rebuilt in Coot50. The composite maps were then converted into mtz files and the models were further refined by Phenix in reciprocal space40 and Coot in real space. During model building, we found that the structures of the catalytic module in the haem-oxidized state and the haem-unliganded state were essentially the same, but we observed a positive difference density around the α1κ–α1 loop of the α1 catalytic domain in the haem-oxidized state sample (Extended Data Fig. 2i). During the preparation of the haem-oxidized sample, we supplemented oxidizing reagent NS5208, substrate GTP-γS and cofactor Mg2+ ions into the sGC protein. Therefore, based on the local chemical environment, this positive density might represent Mg2+ ions together with highly negatively charged phosphate groups that possibly came from the decomposition of the GTP-γS molecule. However, these two large lobes of sGC were not modelled. Volumes of the catalytic pocket were calculated using Caver49 with the large probe radius 5 Å and the small probe radius 2.4 Å.

Quantification and statistical analysis. Global resolution estimations of cryo-EM density maps are based on the 0.143 FSC criterion49. The local resolution was estimated using Relion 3.041. The number of technical replicates (n) and the relevant statistical parameters for each experiment (such as mean or standard deviation) are described in the figure legends. No statistical methods were used to pre-determine sample sizes.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability
Cryo-EM maps of the haem-unliganded, haem-oxidized, NO-activated and β1(H105C) mutant sGC structures have been deposited in the EMDB under accession numbers EMD-9883, EMD-9884, EMD-9885 and EMD-9886, respectively. Atomic coordinates of the haem-unliganded, haem-oxidized and NO-activated sGC structures have been deposited in the PDB under accession numbers 6T0J, 6T1J and 6T2J, respectively.

31. Vermehren-Schmaedick, A., Ainsley, J. A., Johnson, W. A., Davies, S. A. & Morton, D. B. Behavioral responses to hypoxia in Drosophila larvae are mediated by atypical soluble guanylyl cyclases. Genetics 186, 183–196 (2010).
32. Goehring, A. et al. Screening and large-scale expression of membrane proteins in mammalian cells for structural studies. Nat. Protoc. 9, 2574–2585 (2014).
33. Li, N. et al. Structure of a pancreatic ATP-sensitive potassium channel. Cell 168, 110–111 (2017).
34. Kawate, I. & Gouaux, E. Fluorescence-detection size-exclusion chromatography for precryocrystallographic screening of integral membrane proteins. Structure 14, 673–681 (2006).
35. Tang, Q. et al. Structure of the receptor-activated human TRPC6 and TRPC3 ion channels. Cell Res. 28, 746–755 (2018).
36. Olesen, S. P. et al. Characterization of NS 2028 as a specific inhibitor of soluble guanylyl cyclase. Br. J. Pharmacol. 123, 299–309 (1998).
37. Brandwein, H. J., Lewicki, J. A., Waldman, S. A. & Murad, F. Effect of GTP analogues on purified soluble guanylyl cyclase. J. Biol. Chem. 257, 1309–1311 (1982).
38. Artz, J. D., Toader, V., Zavorin, S. I., Bennett, B. M. & Thatcher, G. R. In vitro activation of soluble guanylyl cyclase and nitric oxide release: a comparison of NO donors and NO mimetics. Biochemistry 40, 9256–9264 (2001).
39. Zheng, S. Q. et al. MotionCor2: anisotropic correction of beam-induced motion for improved cryo-electron microscopy. Nat. Methods 14, 331–332 (2017).
40. Zhang, K. Gctf: Real-time CTF determination and correction. J. Struct. Biol. 193, 1–12 (2016).
41. Zivanov, J. et al. New tools for automated high-resolution cryo-EM structure determination in RELION-3. elife 7, e42166 (2018).
42. Punjabi, A., Rubinstein, J. L., Fleet, D. J. & Brubaker, M. A. cryoSPARC: algorithms for rapid unsupervised cryo-EM structure determination. Nat. Methods 14, 290–296 (2017).
43. Grant, T., Rohou, A. & Grigorieff, N. cisTEM, user-friendly software for single-particle image processing. elife 7, e55383 (2018).
44. Pettersen, E. F. et al. UCSF Chimera—a visualization system for exploratory research and analysis. J. Comput. Mol. 25, 1605–1612 (2004).
45. Chen, S. et al. High-resolution noise substitution to measure overfitting and validate resolution in 3D structure determination by single particle electron cryomicroscopy. Ultramicroscopy 135, 24–35 (2013).
46. Kelley, L. A., Mezulis, S., Yates, C. M., Wass, M. N. & Sternberg, M. J. The Phyre2 web portal for protein modeling, prediction and analysis. Nat. Protoc. 10, 845–858 (2015).
47. Emmsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. Features and development of Coot. Acta Crystallogr. D 66, 486–501 (2010).
48. Adams, P. D. et al. PHENIX: a comprehensive Python-based system for macromolecular structure solution. Acta Crystallogr. D 66, 213–221 (2010).
49. Jurcik, A. et al. CAVER Analyst 2.0: analysis and visualization of channels and tunnels in protein structures and molecular dynamics trajectories. Bioinformatics 34, 3586–3588 (2018).
50. Rosenthal, F. B. & Henderson, R. Optimal determination of particle orientation, absolute hand, and contrast loss in single-particle electron cryomicroscopy. J. Mol. Biol. 333, 721–745 (2003).

Acknowledgements We thank all members of the Chen laboratory for their help; J. Han for sharing the human sGC cDNA; and D. B. Morton for sharing Drosophila and mouse sGC cDNAs. Cryo-EM data collection was supported by the Electron Microscopy Laboratory and Cryo-EM Platform of Peking University with the assistance of X. Li, Z. Guo, B. Shao, X. Pei and G. Wang. Part of the structural computation was also performed on the Computing Platform of the Center for Life Science and High-performance Computing Platform of Peking University. The work was supported by grants from the Ministry of Science and Technology of China (National Key R&D Program of China, 2016YFA0502004 to the National Natural Science Foundation of China (91857001, 31622021, 31821091 and 31870833 to L.C.), Beijing Natural Science Foundation (7192097 to L.C.), Young Thousand Talents Program of China (to L.C.) and the China Postdoctoral Science Foundation (2016M600856, 2017T100014 and 2019M650324, and 2019T100014 to J.-X.W.). J.-X.W. is supported by the Boya Postdoctoral Fellowship of Peking University.

Author contributions L.C. initiated the project and screened expression constructs. R.L. purified protein and prepared the cryo-EM sample. Y.K. and R.L. collected the cryo-EM data with the help of J.-X.W. Y.K. processed the cryo-EM data with the help of L.C. L.C. built and refined the atomic model. Y.K. did the enzymatic activity assay. R.L. did the disulfide bond cross-linking experiment. All authors contributed to manuscript preparation.

Competing interests The authors declare no competing interests.

Additional information Supplementary information is available for this paper at https://doi.org/10.1038/s41586-019-1584-6.

Correspondence and requests for materials should be addressed to L.C.

Peer review information Nature thanks Mark Gladwin, Jesus Tejero, Focco van den Akker and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Reprints and permissions information is available at http://www.nature.com/reprints.
Extended Data Fig. 1 | Biochemical characterization of the human α1β1 sGC heterodimer protein and single-particle cryo-EM data processing procedure for sGC in the inactive (haem-oxidized) state. a, Size-exclusion chromatography of sGC on a superdex 200 column. The fractions indicated by dashed lines were pooled for cryo-EM sample preparation. b, SDS–PAGE of the size-exclusion chromatography fractions labelled in a. Arrows show the positions of the α1 and β1 subunits. For gel source data, see Supplementary Fig. 1. c, UV-vis spectra of purified sGC before (red) and after (black) treatment with the haem oxidant NS2028. a–c, The experiments were repeated independently three times with similar results. d, A representative raw micrograph of sGC in the inactive (haem-oxidized) state. e, Representative 2D class averages of sGC in the inactive (haem-oxidized) state. f, The angular distribution for the consensus refinement of the inactive (haem-oxidized) state is indicated by the sizes of spheres. g, The cryo-EM data processing workflow for sGC in the inactive (haem-oxidized) state. The positions of the Soret peaks are indicated by arrowheads.
Extended Data Fig. 2 | Conformational heterogeneity and local density quality of sGC in the inactive (haem-oxidized) state. a, Gold-standard FSC curves of haem-oxidized sGC after correction for masking effects. Resolution estimations were based on the criterion of the FSC 0.143 cutoff. b, Local resolution distribution of the composite map of sGC in the inactive (haem-oxidized) state. c, Histogram of the eigenvectors that contribute to the variance. The top eigenvector is highlighted in grey. d, Histogram of the amplitudes along the top eigenvectors shows monomodal distribution. Particle populations with amplitudes less than −3 or greater than 3 are indicated as red and blue arrows, respectively. e, A 4 Å low-pass filtered map reconstructed from particles indicated as red and blue arrows in d. N-lobes were used for alignment. f, Representative cryo-EM densities of fragments from each individual domain. g, Representative cryo-EM densities of several key residues involving the interactions between β1 H-NOX and adjacent domains in the inactive (haem-oxidized) state. h, The cryo-EM map of the sGC in the haem-oxidized state. The putative linkers between the H-NOX and PAS domains are shown in grey. The B-factor of the map was adjusted to −100 Å² during the post-processing procedure to visualize features with high flexibility. i, Cryo-EM maps of the catalytic module in the haem-oxidized state (cyan) and the haem-unliganded state (purple). The density of the putative phosphate groups is shown in grey.
Extended Data Fig. 3 | Single particle cryo-EM data processing of the sGC sample in the NO-activated state. **a**, Representative raw micrograph of sGC in the NO-activated state. **b**, Representative 2D class averages of sGC in the NO-activated state. **c**, The angular distribution for the consensus refinement of the NO-activated state is indicated by the sizes of the spheres. **d**, The cryo-EM data processing workflow for sGC in the NO-activated state. **e**, Gold-standard FSC curves (after correction for the masking effects) of NO-activated sGC. Resolution estimations are based on the criterion of FSC 0.143 cutoff. **f**, Local resolution distribution of the composite map of sGC in the NO-activated state.
Extended Data Fig. 4 | Conformational heterogeneity and local density quality of sGC in the NO-activated state. a, Histogram of the eigenvectors that contribute to the variance. The top eigenvector is highlighted in grey. b, Histogram of the amplitudes along the top eigenvectors shows monomodal distribution. Particle populations with amplitudes less than −2 or greater than 2 are indicated with red and blue arrows, respectively. c, 4 Å low-pass filtered maps reconstructed from particles indicated as red and blue arrows in b. N-lobes were used for alignment. d, Representative cryo-EM densities of fragments from each individual domain. e, Representative cryo-EM densities of several key residues involving the interactions between β1 H-NOX and adjacent domains in the NO-activated state. f, Cryo-EM map of the catalytic module in the NO-activated state (yellow). The putative density of the α1 C terminus is shown in grey, αQ of the α1 subunit is shown in pink, and the αO–βK fragment of the β1 subunit is shown in cyan. The B-factor of the map was adjusted to −100 Å² in post-processing to visualize features with high flexibility. g, The side view of the cryo-EM map of the β1(H105C) mutant sGC. h, Gold-standard FSC curves (after correction for masking effects) of the β1(H105C) mutant sGC. Resolution estimations were based on the criterion of FSC 0.143 cutoff. i, Cryo-EM map of β1(H105C) mutant sGC (pink) and NO-activated sGC (cyan). The haem density is shown in yellow. The map of NO-activated sGC was low-pass filtered to 6.8 Å. j, The locations of cysteine residues that are involved in sGC desensitization (α1 C244, β1 C78 and β1 C122) are indicated with their Cα atoms shown as red spheres. Because the loop containing α1 C244 is disordered in the NO-activated state, only the termini of the loop (α1 L235 and α1 Y252) containing α1 C244 are labelled.
Extended Data Fig. 5 | Sequence alignment of the sGC $\alpha$-subunit. The sequences of the Homo sapiens $\alpha_1$ subunit, H. sapiens $\alpha_2$ subunit, Danio rerio $\alpha_1$ subunit, D. rerio $\alpha_2$ subunit, and M. sexta $\alpha_1$ subunit are aligned. Conserved residues are coloured in grey. Residues that are mutated to cysteines for oxidative cross-linking are indicated with a black box. Mutations for activity assay are indicated with a red box. The residues corresponding to H105 in the $\beta_1$ subunit are indicated with a yellow box. Secondary structural elements are indicated as follows: arrows, $\beta$-sheets; cylinders, $\alpha$-helices; lines, loops. Unmodelled residues are shown as dashed lines. The colours of arrows and cylinders are as in Fig. 1a.
Extended Data Fig. 6 | Sequence alignment of the sGC β1 subunit. The sequences of the *H. sapiens* β1 subunit, *D. rerio* β1 subunit, and *M. sexta* β1 subunit are aligned. Conserved residues are coloured in grey. Residues that are mutated to cysteines for oxidative cross-linking are indicated with a black box. Mutations for activity assay are indicated with a red box. Secondary structural elements are indicated as follows: arrows, β-sheets; cylinders, α-helices; lines, loops. Unmodelled residues are shown as dashed lines. The colours of arrows and cylinders are as in Fig. 1a.
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | Domain–domain interfaces of sGC in the inactive state. a, Side view of soluble guanylate cyclase in the inactive state, highlighting key interfaces (grey rectangles). Each domain is coloured as in Fig. 1a. The surface of sGC is shown in transparency. b, The interface between the α1 H-NOX domain and the PAS domains boxed in a. c, The interface between the PAS domains boxed in a. d, The interface between β1 H-NOX and adjacent domains boxed in a. e, A 180° rotated view compared to d. f, The structure of the transducer module boxed in a. The side chains of α1 L425 and β1 L365 that are in close proximity are shown as spheres. g, The interface between the transducer module and the catalytic module boxed in a. h, A 90° rotated top view compared to g. i, End-point activity of the less-Cys construct (sGCα1LC, α1LC + β1LC) compared to the wild-type sGC with CGFP. Mean ± s.d., n = 3 biologically independent samples. j, SDS–PAGE of the in vitro disulfide bond cross-linking experiment of α1LC(L275C) with β1LC(L365C) under reducing and non-reducing conditions. Oxidative cross-linking happened only when the cysteine mutants, α1(L275C) and β1(L365C), were present in both subunits simultaneously. The experiments were repeated independently three times with similar results. For gel source data, see Supplementary Fig. 1. k, SDS–PAGE of the in vitro disulfide bond cross-linking experiment of α1LC(L425C) with β1LC(L365C) under reducing and non-reducing conditions. Oxidative cross-linking happened only when the cysteine mutants, α1(L425C) and β1(L365C), were present in both subunit simultaneously. For gel source data, see Supplementary Fig. 1. The experiments were repeated independently three times with similar results. l, SDS–PAGE of the in vitro disulfide bond cross-linking experiment of α1LC(L275C) with β1LC(L365C) and α1LC(L275C) with β1LC(A316C) under reducing and non-reducing conditions. In contrast to α1(L275C) with β1(A316C) and α1(L425C) with β1(L365C), α1(L275C) did not crosslink with β1(L365C), and α1(L425C) did not crosslink with β1(A316C), owing to their long spatial distance. For gel source data, see Supplementary Fig. 1. The experiments were repeated independently twice with similar results.
Extended Data Fig. 8 | Domain–domain interfaces of sGC in the NO-activated state. a, Superposition of the NO-bound β1 H-NOX domain structure (purple) onto the inactive state structure (grey) by alignment of the αF helices. The steric clashes between the side chains of the NO-bound β1 H-NOX domain (purple sphere) and the side chains of the PAS and CC domains of the inactive state (grey sphere) are marked by red circles if their atom-to-atom distances are smaller than 2.2 Å. The arrow indicates the positional change of the Cα atoms of β1 N62 induced by NO binding. b, The interface between β1 H-NOX and adjacent domains of sGC in the NO-activated state. c, A 180° rotated view compared to b, d, Soret peaks of the sGC mutants show markedly decreased NO activation. The experiments were repeated independently twice with similar results. e, The transducer module in the NO-activated state, coloured as in Fig. 1a. f, Top view of the structural comparison of the catalytic module between the inactive state (grey) and the NO-activated state (coloured). The GMPCPP molecule is shown as sticks. The Cα atoms of α1 P459 and β1 P399 are shown as spheres.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | Structural comparisons of each domain.

a, Structural comparison of the full-length human sGC between the haem-oxidized state (coloured) and the haem-unliganded state (grey).
b, Structural comparison between the α1 H-NOX domain (pink) and the β1 H-NOX domain (blue) in the inactive state. Both α1 H-NOX and β1 H-NOX share common structural features with prokaryotic H-NOX domains, which are composed of both N-terminal and C-terminal subdomains. The N-terminal αA helix of the α1 subunit that occupies the haem binding pocket is shown in red. The haem molecule of the β1 H-NOX domain is shown as a yellow stick. The approximate boundaries of N-terminal and C-terminal subdomains are indicated by dashed lines.
c, Structural comparison between the human α1 PAS domain (red) and the M. sexta Ms α PAS domain (grey, PDB ID:4GJ4). d, Structural comparison between the human β1 PAS domain (blue) and the M. sexta Ms α PAS domain (grey, PDB ID:4GJ4). e, Structural comparison between the catalytic module of the full-length sGC in the inactive state (coloured) and the isolated catalytic domain heterodimer (grey, PDB ID: 4NI2). The β1 subunit was used for structural alignment. f, 90° rotated view compared to e. g, Structural comparison between the catalytic module of the full-length sGC in the inactive state (coloured) and the catalytic domain of the active adenylate cyclase (grey, PDB ID: 1CJU, chain A&B). The β1 subunit was used for structural alignment. The residues of sGC that are within 2.2 Å of the substrate are considered as steric clashes and shown as red spheres.
h, Structural comparison between the NO-activated state (purple) and the inactive state (grey) of the human β1 H-NOX domain. The N-terminal subdomain was used for alignment and the movements are indicated as red arrows.
i, Structural comparison between the catalytic module of the full-length sGC in the NO-activated (colored) and the isolated catalytic domain heterodimer (grey, PDB ID: 4NI2). The β1 subunit was used for structural alignment. A inter-domain rotational conformational change is observed. j, A 90° rotated view compared to i, k, Cutaway views of the sGC catalytic module in the inactive state and the NO-activated state. The catalytic module is shown in surface representation colored by electrostatic potential calculated in Pymol. The pockets inside the catalytic module are indicated by arrows. GMPCPP molecule is shown as sticks.
l, Structural comparison of the catalytic core between the active adenylate cyclase (grey, PDB ID: 1CJU, chain A and B) and sGC in the NO-activated state (coloured). m, Structural comparison between the NO-activated state (coloured) and the inactive state (grey) of the human α1 and β1 PAS heterodimer.
Extended Data Table 1 | Cryo-EM data collection, refinement and validation statistics

|                        | Haem-unliganded state | Haem-oxidized state | NO-activated state | β1(H105C)                  |
|------------------------|------------------------|---------------------|--------------------|-----------------------------|
|                        | 6JT0 EMD-9883           | 6JT1 EMD-9884       | 6JT2 EMD-9885       | EMD-9886                    |
| **Data collection and processing** |                        |                     |                    |                             |
| Magnification          | 130,000 ×              | 130,000 ×           | 130,000 ×          | 130,000 ×                   |
| Voltage (kV)           | 300                    | 300                 | 300                | 300                         |
| Electron exposure (e⁻/Å²) | 48                    | 48                  | 48                 | 48                          |
| Defocus range (µm)     | -1.5 to -2.0           | -1.5 to -2.0        | -1.5 to -2.0       | -1.5 to -2.0                |
| Pixel size (Å)         | 1.045                  | 1.045               | 1.045              | 1.045                       |
| Symmetry imposed       | C1                     | C1                  | C1                 | C1                          |
| Initial particle images (no.) | 1,043,262            | 1,873,492           | 5,110,358          | 581,868                     |
| Final particle images (no.) | 229,111               | 379,909             | 497,307            | 41,710                      |
| Map resolution (Å)     | 4.0 (3.9/4.4)*         | 3.9 (3.7/4.0)*      | 3.8 (3.6/3.9)*     | 7.6 (6.8/6.8)*              |
| FSC threshold          | 0.143                  | 0.143               | 0.143              | 0.143                       |
| Map resolution range (Å) | 250.0-3.9             | 250.0-3.7           | 250.0-3.6          | 250.0-6.8                   |
| **Refinement**         |                        |                     |                    |                             |
| Initial model used (PDB code) | 5MNW, 4GJ4, 3UVJ     | 5MNW, 4GJ4, 3UVJ    | 5MNW, 4GJ4, 3UVJ   |                             |
| Model resolution (Å)   | 4.0                    | 3.8                 | 3.8                |                             |
| FSC threshold          | 0.5                    | 0.5                 | 0.5                |                             |
| Model resolution range (Å) | 250.0-4.0            | 250.0-3.8           | 250.0-3.8          |                             |
| Map sharpening B factor (Å²) | (-145/-219)*        | (-167/-223)*        | (-169/-210)*       |                             |
| Model composition      |                        |                     |                    |                             |
| Non-hydrogen atoms     | 8,602                  | 8,602               | 8,192              |                             |
| Protein residues       | 1,118                  | 1,118               | 1,075              |                             |
| Ligands                | 1                      | 1                   | 4                  |                             |
| B factors (Å²)         | 172.24                 | 160.29              | 133.90             |                             |
| Protein               | 172.68                 | 160.66              | 134.40             |                             |
| Ligand                | 84.88                  | 84.74               | 81.19              |                             |
| R.m.s. deviations      |                        |                     |                    |                             |
| Bond lengths (Å)       | 0.004                  | 0.004               | 0.003              |                             |
| Bond angles (°)        | 0.699                  | 0.760               | 0.663              |                             |
| **Validation**         |                        |                     |                    |                             |
| MolProbity score       | 2.47                   | 2.45                | 2.24               |                             |
| Clashscore             | 10.91                  | 10.27               | 10.08              |                             |
| Poor rotamers (%)      | 4.73                   | 4.62                | 3.54               |                             |
| Ramachandran plot      |                        |                     |                    |                             |
| Favored (%)            | 93.91                  | 93.82               | 95.71              |                             |
| Allowed (%)            | 5.73                   | 5.82                | 4.09               |                             |
| Disallowed (%)         | 0.36                   | 0.36                | 0.29               |                             |

*The numbers outside the brackets are from the consensus refinement. Numbers inside brackets are from the multibody refinement (N-lobe/C-lobe).
Corresponding author(s): Lei Chen
Last updated by author(s): 2019/08/20

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 Authors & Referees 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.

- 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 | SerialEM 3.6.11 |
|-----------------|----------------|
| Data analysis   | MotionCor2, GCTF, Gautomatic, RELION 3.0, cisTEM, cryoSPARC, PHENIX, Coot, UCSF Chimera, Pymol, GraphPad Prism 6, Microsoft Excel, caver |

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

Cryo-EM maps and atomic coordinates of the heme-unliganded, heme-oxidized, NO-activated and β1 H105C mutant sGC have been deposited in the EMDB and PDB under the ID codes EMDB: EMD-9883, EMD-9884, EMD-9885, EMD-9886 and PDB: 6JT0, 6JT1, 6JT2, respectively.
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 predetermination of sample size was performed. Sufficient cryo-EM data were collected to achieve adequate map resolutions for model building. The enzymatic activity assay experiments were performed with three biological replicates. The sample size was based on previous studies in the field and clearly indicated in the legends. |
| Data exclusions | Cryo-EM micrographs with ice or ethane contamination, empty carbon, and poor CTF fit (> 5 Å) were excluded manually. Particles belonging to bad classes were discarded and the data processing flowchart were summarized in Extended Data Fig. 1f, 3c. These criteria were pre-established and the procedure is a common practise in cryo-EM image analysis. No data was excluded in functional studies. |
| Replication | All attempts at replication were successful according to the detailed protocol described in the methods section. The numbers of replication were described in figure legends. |
| Randomization | For cryo-EM 3D refinement, all particles were randomly split into two groups. |
| Blinding | The investigators were blinded to group allocation during cryo-EM data collection and analysis. |

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 |
| - | - |
| - | - |
| - | - |
| - | - |
| - | - |

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s) | FreeStyle 293-F and Sf9 were from Thermo Fisher Scientific. |
Authentication | None of the cell line used was authenticated. |
Mycoplasma contamination | All cell lines were tested negative for mycoplasma contamination. |
Commonly misidentified lines (See ICLAC register) | No commonly misidentified cell lines were used. |