GR chaperone cycle mechanism revealed by cryo-EM: the GR-maturation complex

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GR chaperone cycle mechanism revealed by cryo-EM: the GR-maturation complex

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Abstract

Hsp90 is a conserved and essential molecular chaperone responsible for the folding and activation of hundreds of ‘client’ proteins¹⁻³. The glucocorticoid receptor (GR) is a model client that constantly depends on Hsp90 for activity⁴⁻⁹. Previously, we revealed GR ligand binding is inhibited by Hsp70 and restored by Hsp90, aided by the cochaperone p23¹⁰. However, a molecular understanding of the chaperone-induced transformations that occur between the inactive Hsp70:Hsp90 ‘client-loading complex’ and an activated Hsp90:p23 ‘client-maturation complex’ is lacking for GR, or for any client. Here, we present a 2.56Å cryo-EM structure of the GR-maturation complex (GR:Hsp90:p23), revealing that the GR ligand binding domain is, surprisingly, restored to a folded, ligand-bound conformation, while simultaneously threaded through the Hsp90 lumen. Also, unexpectedly, p23 directly stabilizes native GR using a previously uncharacterized C-terminal helix, resulting in enhanced ligand-binding. This is the highest resolution Hsp90 structure to date and the first atomic resolution structure of a client bound to Hsp90 in a native conformation, sharply contrasting with the unfolded kinase:Hsp90 structure¹¹. Thus, aided by direct cochaperone:client interactions, Hsp90 dictates client-specific folding outcomes. Together with the GR-loading complex structure (Wang et al. 2020), we present

the molecular mechanism of chaperone-mediated GR remodeling, establishing the first complete chaperone cycle for any client.

Introduction

Hsp90 is required for the functional maturation of 10% of the eukaryotic proteome, including signaling proteins, such as kinases, and steroid hormone receptors (SHRs), such as GR^{1,3,12}. We previously uncovered the biochemical basis for GR's Hsp90 dependence using *in vitro* reconstitution starting with an active GR ligand binding domain (hereafter GR, for simplicity)¹⁰. We demonstrated that GR ligand binding is regulated by a cycle of GR:chaperone complexes (**Fig. 3d**). In this chaperone cycle, GR is first inhibited by Hsp70, then loaded onto Hsp90:cochaperone Hop (Hsp70/Hsp90 organizing protein) forming an inactive GR:Hsp90:Hsp70:Hop loading complex (Wang et al. 2020). Upon ATP hydrolysis on Hsp90, Hsp70 and Hop are released, and p23 is incorporated to form an active GR:Hsp90:p23 maturation complex, restoring GR ligand binding with enhanced affinity. Progression through this cycle is coordinated by the ATPase activities of both Hsp70 and Hsp90, which dictate large conformational rearrangements^{2,13}. Particularly, Hsp90 functions as a constitutive dimer that undergoes an open-to-closed transition upon ATP binding and this conformational cycle is further regulated by cochaperones¹⁴. The cochaperone p23 specifically binds and stabilizes the closed Hsp90 conformation¹⁵ and p23 is required for full reactivation of GR ligand binding *in vitro*¹⁰ and proper function *in vivo*¹⁶. Altogether, the coordinated actions of Hsp70, Hsp90, and cochaperones remodel the conformation of GR to control access to the buried, hydrophobic ligand binding pocket.

While the Hsp90/Hsp70 chaperone systems are fundamental in maintaining protein homeostasis, the absence of client:chaperone structures has precluded a mechanistic understanding of the remodeling process for any client. The kinase:Hsp90 structure¹¹ first revealed how Hsp90 can stabilize an inactive client, but provided no insights to explain how Hsp90 can reactivate a client, such as GR. Here, we report a high-resolution cryo-EM structure of the GR-maturation complex, providing a long-awaited molecular mechanism for chaperone-mediated client remodeling and activation.

Results

Sample preparation and structure determination

The maturation complex sample was prepared through *in vitro* reconstitution of the GR chaperone cycle, where the MBP (maltose binding protein)-GR ligand binding domain was incubated with Hsp70, Hsp40, Hop, Hsp90, and p23, allowing GR to progress through the chaperone cycle to reach the maturation complex (**Materials and Methods, Extended Data Fig. 1a-d**). A 2.56Å cryo-EM reconstruction of the maturation complex was obtained (**Fig. 1a; Extended Data Fig. 2a,b; Extended Data Table 1**) using RELION¹⁷ and atomic models were built in Rosetta starting from previously published atomic structures. The structure reveals a fully closed, nucleotide-bound Hsp90 dimer (Hsp90A and B) complexed with a single GR and a single p23, which occupy the same side of Hsp90 (**Fig. 1a,b; Extended Data Fig. 3a**).

Hsp90 stabilizes GR in a native, active conformation

GR and Hsp90 have three major interfaces (**Fig. 1c-e**): (1) the Hsp90 lumen:GR_{pre-Helix1}; (2) the Hsp90_{MD/CTD}:GR_{Helix1}, and (3) the Hsp90_{MD/CTD}:GR Helices 3, 4, and 9. In the first

interface, the N-terminal GR_{pre-Helix1} (GR⁵²³⁻⁵³¹) is threaded through the closed Hsp90 lumen (~735Å² buried surface area (BSA))(Fig. 1c). The Hsp90 lumen provides a mostly hydrophobic ‘tunnel’ that captures GR_{pre-Helix1} (Extended Data Fig. 4a). Specifically, two residues (GR^{L525,L528}) occupy hydrophobic binding pockets within the Hsp90 lumen. The interaction is further stabilized by multiple hydrogen bonds from Hsp90 to the backbone and side chains of GR_{pre-Helix1} (Extended Data Fig. 4b). Interface 2 is mainly comprised of the short GR_{Helix1} (GR⁵³²⁻⁵³⁹) packing up against the amphipathic helical hairpin (Hsp90_{amphi-α}) in the C-terminal domain of Hsp90B (Hsp90B_{CTD}), which shifts slightly to accommodate GR_{Helix1}, while GR helices 8 and 9 also interact with Hsp90B (~488Å² BSA)(Fig. 1d, Extended Data Fig. 4c). In interface 3, the Hsp90B_{amphi-α} packs against GR Helix 3 and the conserved, solvent exposed hydrophobic residues Hsp90^{F349,W320}, located in the middle domain of Hsp90A (Hsp90A_{MD}), make contact with Helices 4 and 9 on GR (~467Å² BSA)(Fig. 1e, Extended Data Fig. 4d). Notably, Hsp90^{F349,W320} also make contact with GR in the loading complex (Wang et al. 2020).

Surprisingly, in the maturation complex, despite being bound to Hsp90, GR adopts an active, folded conformation (Cα RMSD of 1.24Å to crystal structure 1M2Z¹⁸). Specifically, GR_{Helix12}, a dynamic motif responsive to ligand binding, is in the agonist-bound position, as in the crystal structure (1M2Z) (Extended Data Fig. 5a). However, unlike the crystal structure, GR_{Helix12} is not stabilized by a co-activator peptide. Unexpectedly, the density also revealed that GR is ligand-bound (Extended Data Fig. 5b). The only ligand source was the initial GR purification with agonists and GR was extensively dialyzed, which removes the vast majority of ligand¹⁰. During preparation of the maturation complex, GR likely rebound residual ligand and despite multiple washes, the ligand remained bound, suggesting a slow ligand off-rate from the

maturation complex. Based on the ligand density and positions of GR^{Y735}, the bound ligand is likely dexamethasone (**Extended Data Fig. 5b**).

p23 stabilizes Hsp90 while also interacting directly with GR

The Hsp90:p23 interface is comparable to the crystal structure of the yeast Hsp90:p23 complex (2CG9)¹⁵, where p23 makes extensive contacts with the N-terminal domains of Hsp90 (Hsp90_{NTDS}) to stabilize the Hsp90 closed state (~1274Å² BSA)(**Extended Data Fig. 6a-d**). Only one p23 is bound to the Hsp90 dimer, although two p23 molecules are in the yeast Hsp90:p23 structure and a 2-fold excess of p23 to Hsp90 was added during complex preparation. Consistent with a previous report, GR binding on Hsp90 may favor incorporation of a single p23⁹. The slight asymmetry observed here between the Hsp90_{NTD} dimer interfaces (**Extended Data Fig. 3b**) combined with the avidity afforded by simultaneous interactions with Hsp90 and GR likely provides a molecular explanation.

Unexpectedly, p23 also makes direct and extensive contacts with GR (~702Å² BSA) through the early part (p23¹¹²⁻¹³³) of its ~57 residue C-terminal tail (p23¹⁰⁴⁻¹⁶⁰), while the following 27 tail residues (p23¹³⁴⁻¹⁶⁰) were not visible. As seen in the yeast Hsp90:p23 crystal structure (2CG9), the beginning of the p23 tail (p23^{F103,N104,W106}) interacts with the Hsp90B_{NTD} (**Extended Data Fig. 6c**). The following loop (p23¹⁰⁸⁻¹¹⁸) forms multiple hydrogen bonds and salt bridges with both GR and Hsp90 (**Fig. 2a,b**). Although the tail was previously thought to be unstructured¹⁹, we found a 13-residue helix (p23¹¹⁹⁻¹³¹, p23_{tail-helix}) bound to GR (**Fig. 2a,b; Extended Data Fig. 7a,b**). This newly identified p23_{tail-helix} was also predicted by multiple state-of-the-art secondary structure prediction algorithms (**Extended Data Fig. 7e**). The hydrophobic surface of the p23_{tail-helix} packs against an exposed hydrophobic patch on the GR surface made by

helices 9 and 10 (**Extended Data Fig. 7b**). The p23_{tail-helix} also contacts the C-terminal strand of GR (GR^{H775}), potentially allosterically stabilizing the dynamic GR_{Helix12} (**Fig. 2b, Extended Data Fig. 5c**).

Notably, in both p23 and GR this novel p23:GR interface is conserved across vertebrates (**Fig. 2c,d**). The GR hydrophobic patch is also conserved across SHRs, which are thought to undergo similar regulation by Hsp90/Hsp70 (**Extended Data Fig. 7c**). Attesting to its importance beyond SHRs, the p23_{tail-helix} motif is conserved in yeast, which lack SHRs; however, there are two additional predicted helices in the much longer yeast p23 tail (**Extended Data Fig. 7d**). Due to the high level of conservation of the p23_{tail-helix} and the hydrophobic patch on SHRs, we reasoned that other proteins may utilize a p23_{tail-helix}-like motif to bind SHRs. Using ScanProsite²⁰ to search the human proteome for a p23_{tail-helix}-like motif ("FXXMMN"), remarkably, Nuclear Coactivator 3 (NCoA3/SRC-3), a canonical co-activator protein for SHRs, was among the 10 hits. The identified NCoA3 motif (**FNSMMNQM**) aligns with the p23_{tail-helix} sequence and contains the key conserved hydrophobic residues that interact with GR (**Fig. 2c, Extended Data Fig. 7d**). This suggests NCoA3 may use this newly identified motif to bind GR at the novel interface, in addition to using its LXXLL motif to bind SHRs at Helix 12 (Activation Function 2 (AF-2) Helix)²¹.

The p23_{tail-helix} is necessary for enhanced GR ligand binding in the chaperone cycle

To quantitatively assess the importance of the p23:GR interface for the enhanced ligand binding in the chaperone cycle, we compared full length p23 to one which lacks both the p23_{tail-helix} and the last 27 C-terminal residues (p23_{Δhelix-tail}), and one which only maintains the p23 helix (p23_{Δtail})(**Extended Data Fig. 8a**). In both cases, the loop between the p23 core and the helix is

preserved as this makes critical Hsp90 contacts. While p23_{Δtail} had no significant effect on the chaperone-mediated enhancement of ligand binding, p23_{Δhelix-tail} abolished the enhancement, reducing binding almost to GR alone levels (**Fig. 2e; Extended Data Fig. 8b**). Thus, the observed chaperone-mediated ligand binding enhancement is dependent upon the presence of the p23_{tail-helix}. Importantly, p23_{Δhelix-tail} did not reduce the GR ligand binding activity to the same extent as omitting p23, indicating that the p23 core plays a distinct and critical role in stabilizing the closed Hsp90 conformation in the maturation complex. Interestingly, p23 also had an effect on GR ligand binding independent of Hsp90, with addition of p23 to GR modestly increasing ligand binding (**Extended Data Fig. 8c**).

Hsp90 contains lumen density in non-GR containing reconstructions

From the same GR:Hsp90:p23 dataset, we also obtained reconstructions of Hsp90:p23 (2.66Å resolution) (**Extended Data Fig. 9a-c**) and MBP:Hsp90:p23 (3.63Å resolution) (**Extended Data Fig. 10a-c**). In both complexes, the Hsp90 lumen contains density (**Extended Data Fig. 9d, 10e**). The Hsp90:p23 complexes comprise ~25% of all Hsp90-containing particles and the MBP:Hsp90:p23 complexes comprise ~5.5%, compared with GR:Hsp90:p23 complexes, which comprise ~17%. In the MBP:Hsp90:p23 complex, one p23 with low occupancy is bound to Hsp90 on the opposite side of MBP. The MBP is in a partially unfolded state, as density for the two C-terminal helices is missing. The MBP C-terminal region likely threads through Hsp90, accounting for the lumen density. The MBP is also in an apo state, consistent with the unfolding of the last two helices which form part of its binding pocket (**Extended Data Fig. 10d**).

Discussion

We present the first atomic resolution structure of a client bound to Hsp90 in a native folded conformation, as well as the highest resolution structure of full-length Hsp90 to date. In the maturation complex, GR simultaneously threads through the closed Hsp90 lumen and adopts a native, ligand-bound conformation that is extensively stabilized by both Hsp90 and the p23_{tail}-helix. No GR^{apo} complexes were identified during image analysis, suggesting GR^{apo} is either too dynamic or quickly released from the complex. The native GR conformation in the maturation complex is in striking contrast with the loading complex, in which GR is partially unfolded and unable to bind ligand (Wang et al. 2020). In both complexes, GR threads through the Hsp90 lumen and also interacts on the surface with Hsp90^{F349,W320}, although different GR segments are involved. Supporting a general role in client recognition, Hsp90^{W320} is critical for client activation *in vivo*²²⁻²⁴. The active, native GR in our complex also starkly contrasts with the only other structure of a closed Hsp90:client complex, which stabilizes an unfolded kinase client¹¹. The Hsp90 conformation is nearly identical in both structures and both clients are threaded through the Hsp90 lumen in a similar manner, suggesting a universal binding mode for Hsp90 clients (**Extended Data Fig. 11a,b**). Although the overall Hsp90:client interactions are similar, the outcomes for folding and function of these two clients are opposing, demonstrating evolutionarily determined, client-specific conformational remodeling by Hsp90 (**Extended Data Fig. 11c**).

While previously thought to be a general cochaperone whose primary function is to stabilize a closed Hsp90, our structure reveals that p23 also makes extensive contacts with GR through a previously uncharacterized helix in the p23 tail. This p23_{tail}-helix is necessary for the observed enhanced GR ligand binding activity *in vitro* and may act by stabilizing ligand-bound GR, securing GR within the complex to indirectly stabilize GR_{Helix1}, and/or by allosterically

positioning the dynamic GR_{Helix12}. Thus, p23 not only serves as a cochaperone to stabilize the closure of Hsp90, but also directly contributes to client maturation. In support of this essential p23:GR interaction, the p23_{tail-helix} and GR hydrophobic groove are well conserved. In fact, the hydrophobic groove is conserved across SHRs, indicating the p23_{tail-helix} may contribute to the Hsp90-dependent chaperoning of all SHRs. Indeed, the activity of all SHRs is dependent on p23¹⁶ and the progesterone receptor (PR) requires the p23 tail for enhanced ligand binding activity²⁵. Intriguingly, NCoA3 contains a p23_{tail-helix}-like motif, suggesting other GR coregulators may utilize this novel helix motif to bind the hydrophobic groove on GR and compete with p23, potentially facilitating GR release. Surprisingly, the p23_{tail-helix} is conserved among eukaryotes that lack SHRs; however, previous studies have demonstrated that the p23 tail has general chaperoning activities^{19,25}. To this point, we observed p23 alone could modestly enhance the ligand binding activity of GR independent of Hsp90. Along with the discovery that the Hop cochaperone interacts with the client in the loading complex (Wang et al. 2020), these findings support an emerging paradigm in which Hsp90 cochaperones make specific, direct contact with Hsp90 clients to aid in client recognition and function¹¹.

Together with the structure of the GR-loading complex (Wang et al. 2020), we provide for the first time, a complete picture of the chaperone cycle for any client (**Fig. 3d**). These two structures reveal that GR transitions from a partially unfolded conformation in the loading complex to an active, folded conformation in the maturation complex. In the loading complex, GR_{pre-Helix1} is captured by Hsp70, GR_{Helix1} is stabilized by Hop, and GR_{post-Helix1} is threaded through the semi-closed Hsp90 lumen. First, Hsp70 releases and then Hop releases, which lets GR_{pre-Helix1} slide into the Hsp90 lumen, allowing GR_{Helix1} to refold onto the GR core, thereby generating a ligand binding capable, native GR, stabilized by the p23_{tail-helix} (**Fig. 3b**). During

206 this transition, Hsp90 twists to the fully closed conformation, which likely facilitates client
207 sliding and helps rearrange the client binding site to fully enclose GR_{pre-Helix1} (**Fig. 3a**). Perhaps
208 most critical for GR function, GR becomes protected from Hsp70 rebinding and inhibition once
209 it is in the maturation complex. GR_{Helix1} has been proposed to function as a lid over the GR
210 ligand binding pocket²⁶, thus ligand likely binds during the transition from the loading complex
211 to the maturation complex, just as GR_{Helix1} slides through the Hsp90 lumen to seal the ligand
212 binding pocket (**Fig. 3c**). In line with previous studies, our findings suggest that ligand-bound
213 GR may be translocated to the nucleus in the maturation complex^{27,28}, perhaps with the aid of
214 FKBP52²⁹, where it would be protected by Hsp90 from re-inhibition by Hsp70. Supporting this,
215 Hsp90 and p23 have been found in the nucleus colocalized with GR³⁰, suggesting the maturation
216 complex may persist even after translocation.

217 The proposed sliding mechanism may be a general theme for Hsp90's client remodeling
218 mechanism. Our two other reconstructions, Hsp90:p23 and MBP:Hsp90:p23, have density in the
219 Hsp90 lumen, suggesting Hsp90 has bound regions in our construct other than GR. Given that
220 GR_{Helix1} slides through the Hsp90 lumen from the loading complex to the maturation complex, it
221 is possible that Hsp90 can act processively during open-to-closed transitions to remodel other
222 client domains beyond the one initially engaged. This would explain these other structural
223 classes, although this remains to be tested. Nevertheless, the mechanism of GR_{Helix1} sliding
224 explains how Hsp90 can provide protected refolding of client domains as they exit the lumen to
225 become directly stabilized by cochaperones. Our results suggest Hsp90 may use this mechanism
226 to allow domains to fold independently on either side of the lumen or uncouple annealed
227 misfolded regions to ensure folding fidelity.

228

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Materials and Methods

Data analysis and figure preparation

Figures were created using UCSF Chimera v.1.14¹ and UCSF ChimeraX v0.94². GR ligand binding data was analyzed using Prism v.8.4.3 (GraphPad).

Protein expression and purification

Human Hsp90 α , Hsp70 (Hsp70A1A), Hop, p23, p23 Δ tail (1-133), p23 Δ helix-tail (1-112), and yeast Ydj1 were expressed in the pET151 bacterial expression plasmid with a cleavable N-terminal, 6x-His tag. Human Bag-1 isoform 4 (116-345) was expressed in a pET28a vector with a cleavable N-terminal, 6x-His tag. Proteins were expressed and purified by the following procedure. Proteins were expressed in bacterial BL21 star (DE3) strain. Cells were grown in either LB or TB at 37°C until OD₆₀₀ reached 0.6-0.8 and then induced with 0.5 mM IPTG overnight at 16°C. Cells were harvested and lysed in 50 mM Potassium Phosphate pH 8, 500 mM KCl, 10 mM imidazole pH 8, 10% glycerol, 6 mM β ME, and Roche cOmplete, mini protease inhibitor cocktail using an EmulsiFlex-C3 (Avestin). Lysate was centrifuged and the soluble fraction was affinity purified by gravity column with Ni-NTA affinity resin (QIAGEN). The protein was eluted with 30 mM Tris pH8, 50 mM KCl, 250 mM imidazole pH 8, and 6 mM β ME. For Hsp90, Hsp70, and Ydj1, an extra wash step with 0.1% Tween20 and 2 mM ATP/MgCl₂ was added to the Ni-NTA resin before eluting. The 6x-His tag was removed with TEV protease during the following overnight dialysis in 30 mM Tris pH 8, 50 mM KCl, and 6 mM β ME. Cleaved protein was then loaded onto an ion exchange column, MonoQ 10/100 GL (GE Healthcare), with 30 mM Tris pH 8, 50 mM KCl, and 6 mM β ME and eluted with a linear gradient of 50-500 mM KCl. Protein was further purified by size exclusion in 30 mM HEPES pH

7.5, 50 mM KCl, 10% glycerol, 1 mM DTT using a HiLoad 16/60 Superdex 200 (GE Healthcare) or Hi Load 16/60 Superdex 75 (GE Healthcare). For Hsp70, each peak from ion exchange was collected separately and purified by size exclusion in 30 mM HEPES pH 7.5, 100 mM KCl, 10% glycerol, 4 mM DTT, where only the monomeric peak was then collected. Protein was concentrated, flash frozen, and stored at -80°C.

GR-LBD expression and purification

For GR, the ligand binding domain (LBD) (F602S) (521-777) was codon optimized and expressed in the pMAL-c3X derivative with an N-terminal cleavable 6x-His-MBP tag. GR-LBD was expressed and purified as previously described³.

GR-maturation complex sample preparation

The GR chaperone cycle was reconstituted *in vitro* with purified components as previously described³. Buffer conditions were 30 mM HEPES pH 8, 50 mM KCl, 0.05% Tween20, and 2 mM TCEP. Proteins and reagents were added at the following concentration: 5 μM MBP-GR LBD, 2 μM Hsp40, 5 μM Hsp70, 5 μM Hop, 15 μM Hsp90, 15 μM p23, 5 mM ATP/MgCl₂. This reaction was incubated at room temperature for 60 minutes, then 15 μM p23, 15 μM Bag-1, and 20 mM sodium molybdate (used to stabilize the closed conformation of Hsp90^{4,5}) were added, and the reaction was incubated at room temperature for another 30 minutes. Following incubation, amylose resin (New England Biolabs) was added to the reactions in a 1:1 ratio and incubated at 4°C with nutation. Resin was then washed 4 times with wash buffer (30 mM HEPES pH 8, 50 mM KCl, 5 mM ATP/MgCl₂, 0.05% Tween20, 2 mM TCEP, 20 mM sodium molybdate) and eluted with 50 mM maltose. The elution was analyzed by

SDS-PAGE (**Extended Data Fig.1a**). The elution was concentrated and purified by size exclusion using a Shodex KW-804 on an Ettan LC (GE Healthcare)(**Extended Data Fig.1b,c**). Fractions containing the full complex were concentrated to ~2 μM . 2.5 μL of sample was applied to glow-discharged QUANTIFOIL R1.2/1.3, 400-mesh, copper holey carbon grid (Quantifoil Micro Tools GmbH) and plunge-frozen in liquid ethane using a Vitrobot Mark IV (FEI) with a blotting time of 15 seconds, at 10°C, and with 100% humidity.

Cryo-EM data acquisition

The images were collected on a FEI Titan Krios electron microscope (Thermo Fisher Scientific) operating at 300kV using a K3 direct electron camera (Gatan) and equipped with a Bioquantum energy filter (Gatan) set to a slit width of 20 eV (example micrograph **Extended Data Fig. 1d**). Images were recorded at a nominal magnification of 105,000 $\times$, corresponding to a physical pixel size of 0.835Å. A nominal defocus range of 0.8 μm –2.0 μm underfocus was used. A total exposure of 5.9 seconds was used with 0.05 second subframes (117 total frames). The total accumulated electron dose was 60 electrons/Å² and 0.5128 electrons/Å²/frame. Data was acquired using SerialEM software v.3.8-beta⁶.

A small dataset on the GR-maturation complex was collected before the larger dataset described above. The smaller dataset was collected from the same GR-maturation complex sample preparation concentrated to 1.2 μM with grids prepared in a similar manner. Images were collected on a FEI Titan Krios electron microscope (Thermo Fisher Scientific) operating at 300kV using a K3 direct electron camera (Gatan). Images were recorded at a nominal magnification of 105,000 $\times$, corresponding to a physical pixel size of 0.835Å. A nominal defocus

range of 0.8 μm –2.0 μm underfocus was used. A total exposure of 3.0 seconds was used with 0.0255 second subframes (118 total frames). Data was acquired using SerialEM software.

Cryo-EM data processing

The smaller dataset consisted of ~1500 dose-fractionated image stacks, which were motion corrected using UCSF MotionCor2⁷ and analyzed with RELION v.3.0.8⁸. Motion corrected images without dose weighting were used for contrast transfer function (CTF) estimation using CTFFIND v.4.1⁹ and template-based particle picking was done with Gautomatch v.0.53 (<http://www.mrc-lmb.cam.ac.uk/kzhang/>) with the Hsp90:p23 crystal structure (2CG9) as a reference to select a total of 718,080 particles. Multiple rounds of 3D classification were performed with 2CG9 as a low pass filtered (40 Å) initial model until a medium-resolution (~8 Å) GR:Hsp90:p23 reconstruction was obtained from 13,570 particles. This reconstruction was used as a reference for the larger dataset.

The larger dataset consisted of 5,608 dose-fractionated image stacks, which were motion corrected using UCSF MotionCor2 and analyzed with RELION v.3.0.8. Motion corrected images with dose weighting were used for contrast transfer function (CTF) estimation using CTFFIND v.4.1 and reference-free particle picking was done with RELION v.3.0.8 Laplacian-of-Gaussian auto-picking to select a total of 6,062,152 particles. The processing scheme is depicted in **Extended Data Fig. 2a**. An initial round of three-dimensional (3D) classification was performed without symmetry using a reference model from a previously collected smaller dataset (see above). The class with clearly recognizable Hsp90 density was used for a second round of 3D classification. In this second round, a class with only Hsp90:p23 density was obtained (454,385 particles). This class was refined after per-particle CTF and beam-tilt correction in RELION to a nominal resolution of 2.66 Å. Particles from two other classes, which

contained GR density, were combined (~1 million particles) for a third round of 3D classification. After the third round of 3D classification, particles from classes with the best GR density were then combined (~340,000 particles) and refined. To improve the resolution of GR and the p23 tail helix, these regions were further refined using focused classification with a mask including GR and the p23 tail helix. The best focused classes were combined (140,217 particles) and refined to a nominal resolution of 2.56 Å. Using the 2.56 Å reconstruction, per-particle CTF and beam-tilt were refined using RELION. Although the FSC showed slightly improvement over the pre-refined reconstruction at medium resolution range (5–10 Å), the nominal resolution at 0.143 FSC remained unchanged. Nevertheless, we used the CTF/beam-tilt refined particles for the following focused refinement on GR:p23 tail helix and for the resulting reconstructions used for model building. To further improve the resolution of GR and the p23 tail helix for model building, these regions were refined using focused refinement with a mask including GR and the p23 tail helix.

From the third round of 3D classification, particles from 3D classes with MBP density were combined (~650,000 particles) and refined. To improve the resolution of MBP, the MBP region was further refined using focused classification with a mask on MBP. The best focused 3D classes were combined (31,556 particles) and refined to a nominal resolution of 3.63 Å after per-particle CTF and beam-tilt correction in RELION.

All final reconstructions were post-processed in RELION in which the nominal resolution was determined by the gold standard Fourier shell correlation (FSC) using the 0.143 criterion (**Extended Data Fig. 2b**). Maps were sharpened and filtered automatically determined by RELION according to an estimated overall map B-factor and filtered to their estimated resolution. RELION was used to estimate the local resolution of each map (**Extended Data Fig.**

2a). For GR:Hsp90:p23, a composite map was generated by combining the overall refinement map with the GR:p23 tail focused refinement map using vop maximum in Chimera.

Model building and refinement

For the GR-maturation complex atomic model, the dexamethasone-bound human GR crystal structure (1M2Z) and the human p23 crystal structure (1EJF) were used as a starting model for model building. A homolog model of human Hsp90 α was derived from human Hsp90 β from the Hsp90:Cdk4:Cdc37 cryo-EM structure (5FWK) with the sequence alignment (86% sequence identity) obtained from HHpred server¹⁰ and this was also used as a starting model for model building (**Extended Data Table 1**). Models were refined using Rosetta v.3.11 throughout. Following the split map approach¹¹ to prevent and monitor overfitting, the Rosetta iterative backbone rebuilding procedure was used to refine models against one of the half maps obtained from RELION, with the other half map only used for validations. The structurally uncharacterized p23_{tail-helix} was first *de novo* built into the density using RosettaCM¹² and then was further refined using the same Rosetta iterative backbone rebuilding procedure. With a proper density weight obtained using the half maps, the final model of the GR:Hsp90:p23 complex was refined against the full reconstruction allowing only sidechain and small-scale backbone refinement. The final refinement statistics are provided (**Extended Data Table 1**). For the Hsp90:p23 and MBP:Hsp90:p23 map densities (**Extended Data Fig. 9a** and **Extended Data Fig. 10a**), the Hsp90:p23 atomic model from the GR-maturation complex was docked into the map densities. For MBP:Hsp90:p23, the apo MBP crystal structure¹³ (1OMP) was docked into the map density. In **Extended Data Fig. 10d**, the maltose-bound MBP crystal structure¹⁴ (1ANF) is docked into the map density for comparison.

Fluorescence polarization assays

Fluorescence polarization of fluorescent dexamethasone (F-dex)(Life Technologies) was measured on a SpectraMax M5 plate reader (Molecular Devices) with excitation/emission wavelengths of 485/538 nm, temperature control set at 25°C. Buffer conditions were 50 mM HEPES pH 8, 100 mM KCl, 2 mM DTT. For equilibrium ligand binding in **Fig. 2e** and **Extended Data Fig. 8b**, proteins were pre-equilibrated together at room temperature for 60 minutes prior to F-dex addition. Proteins and reagents were added at the following concentration: 20 nM F-dex, 250 nM GR, 2 μ M Hsp40, 15 μ M Hsp70, 15 μ M Hsp90, 15 μ M Hop, 15 μ M p23 or p23 tail mutants, and 5 mM ATP/MgCl₂. Ligand binding was initiated with 20nM F-dex and association was measured until reaching saturation. For the GR control sample, 3 experiments were done with a 1-hour room temperature preincubation of GR in the reaction buffer and 3 experiments were done without a preincubation of GR to account for small effects on equilibrium ligand binding from the preincubation. The plotted equilibrium values represent the mean of 3 separate experiments (except the GR control reaction, which represents the mean of 6 separate experiments), with error bars representing the standard deviation. For equilibrium ligand binding in **Extended Data Fig. 8c**, proteins were pre-equilibrated together at room temperature for 60 minutes prior to F-dex addition. Proteins and reagents were added at the following concentration: 20 nM F-dex, 250 nM GR and 15 μ M p23 or p23 tail mutants. Ligand binding was initiated with 20nM F-dex and association was measured until reaching saturation. The plotted equilibrium values represent the mean of 7 separate experiments (6 separate experiments for the GR + p23 Δ helix-tail reaction) and polarization values were normalized to the equilibrium ligand binding of 250nM GR and 20nM F-dex. GR ligand binding behavior was affected by

buffer conditions; therefore, reactions were always normalized such that each reaction had equivalent amounts of buffer reagents.

Sequence alignments and p23_{tail-helix} motif search

For the p23 sequence alignments in **Fig. 2c** and **Extended Data Fig. 7d**, sequences were aligned in Clustal Omega¹⁵ and visualized in JalView 2.11.1.0¹⁶. Sequences in the alignment are: *H. sapiens* p23, *M. musculus* p23, *R. norvegicus* p23, *G. gallus* p23, *X. troicalis* p23, *D. melanogaster* p23, *A. thaliana* p23, and *S. cerevisiae* p23 (Uniprot accession numbers: Q15185, Q9R0Q7, P83868, Q90955, Q5U4Z0, Q7SZQ8, A0A0B4K6D2, Q8L7U4, P28707, respectively). For Fig. 2d, the ConSurf server^{17,18} was used to select and align 87 GR sequences. The human GR crystal structure¹⁹ (4P6X) was used to select sequences from UNIREF90 with maximal percent ID at 95% and minimal percent ID at 65%. Conservation scores were calculated and provided by the server. The conservation scores calculated by ConSurf were mapped onto GR from the maturation complex atomic model using Chimera.

For **Extended Data Fig. 7c**, the sequences were aligned in Clustal Omega and mapped onto GR from the maturation complex using Chimera. Sequences in the alignment are the human steroid hormone receptors: glucocorticoid receptor, mineralocorticoid receptor, androgen receptor, progesterone receptor, estrogen receptor α and β (Uniprot accession numbers: P04150, P08235, P10275, P06401, E3WH19, Q92731, respectively). Conservation was calculated using AL2CO²⁰ parameters (unweighted frequency estimation and entropy-based conservation measurement). Relating to **Fig. 2c** and **Extended Data Fig. 7d**, the p23_{tail-helix} motif search was performed using ScanProsite²¹. The motif “FXXMMN” was used to search the UniProtKB

sequence database with taxonomy restricted to *Homo Sapiens*. There were 10 total hits on the motif, which included p23 and NCoA3/SRC-3.

Data availability

The cryo-EM maps generated in this study have been deposited in the Electron Microscopy Data Bank (EMDB) under the accession numbers EMD-23004 (GR:Hsp90:p23), EMD-23006 (Hsp90:p23), EMD-23005 (MBP:Hsp90:p23). The atomic coordinates have been deposited in the PDB under the accession number 7KRJ (GR:Hsp90:p23).

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Author Contributions

C.M.N. and R.Y.-R.W. designed and executed biochemical experiments, cryo-EM sample preparation, data collection, data processing, and model building. C.M.N., R.Y.-R.W., and D.A.A. conceived the project, interpreted the results, and wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional Information

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Figure 1: Architecture of the GR-maturation complex

a, Composite cryo-EM map of the GR-maturation complex. Hsp90A (dark blue), Hsp90B (light blue), GR (yellow), p23 (green). This color scheme is maintained in all figures that show the structure. **b**, Atomic model built into the cryo-EM map. **c**, Interface 1 of the Hsp90:GR interaction depicting the GR_{pre-Helix 1} region (GR⁵²³⁻⁵³¹) threading through the Hsp90 lumen. Hsp90A/B are in surface representation. Hydrophobic residues on Hsp90A/B are colored in pink. **d**, Interface 2 of the Hsp90:GR interaction depicting GR_{Helix 1} (GR⁵³²⁻⁵³⁹) packing against the entrance to the Hsp90 lumen. Hsp90A/B are in surface representation. Hydrophobic residues on Hsp90A/B are colored in pink. **e**, Interface 3 of the Hsp90:GR interaction depicting residues on the Hsp90_{AMD} loops (Hsp90A^{N318, W320, R346, F349}) and Hsp90B_{amphi-α} (Hsp90B^{T624, Y627, M628}) packing against GR, which is in surface representation.

Figure 2: p23_{tail-helix} interactions and effect on GR ligand binding

a, Interface between the p23_{tail-helix} (green), GR (yellow, surface representation), and Hsp90 (Hsp90A-dark blue, Hsp90B-light blue). The p23_{tail-helix} (p23¹¹⁹⁻¹³¹) binds the top of GR, while the preceding p23 loop (p23¹⁰⁸⁻¹¹⁹) interacts with GR and Hsp90. **b**, Interface between the p23_{tail-helix} (green), GR (yellow), and Hsp90 (Hsp90A-dark blue, Hsp90B-light blue) showing interacting side chains and hydrogen bonds (dashed pink lines). **c**, Sequence alignment of eukaryotic p23 showing conservation of the p23_{tail-helix} sequence. The top green line indicates the position of the p23_{tail-helix} in the maturation complex atomic model. Slashes indicate insertions in the sequence not shown (longer sequences shown in **Extended Data Fig. 7d**). The alignment is colored according to the ClustalW convention. **d**, Sequence conservation mapped onto GR in surface representation using ConSurf, colored from most variable (white) to most conserved residues (maroon). 87 GR sequences were used for the calculation. The p23_{tail-helix} (light green) is overlaid to indicate the p23:GR interface. **e**, Equilibrium binding of 20nM fluorescent dexamethasone to 250nM GR with chaperone components and p23 tail mutants measured by fluorescence polarization (±SD). Assay conditions: 5mM ATP, 2uM Hsp40, 15uM Hsp70, Hsp90, Hop, and p23 or p23 tail mutants. Individual data points shown in **Extended Data Fig. 8b**.

Figure 3: Mechanism of GR activation by Hsp90

a, Surface representation of Hsp90 in the GR-loading complex (Wang et al. 2020) versus the GR-maturation complex, showing the change in Hsp90 conformation. Hsp90A (dark blue), Hsp90B (light blue). **b**, Top view of the loading complex (top), where GR_{Helix 1} is extended through the Hsp90 lumen, versus a top view of the maturation complex (bottom), where GR_{Helix 1} is docked back onto GR. Hsp70 (orange, partially transparent surface), Hop (pink, partially transparent surface), Hsp90A (dark blue, surface representation), Hsp90B (light blue, surface representation), GR (yellow), p23 (green, partially transparent surface). **c**, Schematic showing the conformational change of the GR_{Helix 1} region from the loading complex to the maturation complex. Boxes represent chaperone and co-chaperone binding sites along the GR_{Helix 1} region (H=hydrophobic interface, P=polar interface). The GR_{pre-Helix 1} strand is highlighted (tan). Color scheme is maintained from (b). **d**, Schematic of the GR chaperone-dependent activation cycle. Ligand-bound, active GR (left) is aggregation prone under physiological conditions. GR ligand binding is inhibited upon binding to Hsp70. Inactive GR is then loaded onto Hsp90, with Hop, to form the loading complex, where GR_{Helix 1} is extended through the Hsp90 lumen. Hsp70 and Hop release and p23 binds to form the maturation complex, where GR_{Helix 1} is redocked back onto the body of GR and ligand binding is restored.

Extended Data Fig. 1: Sample Preparation

a, Coomassie-stained SDS-PAGE with elution from the MBP-GR pulldown from the *in vitro* reconstituted GR chaperone cycle. Assay conditions are as follows- Lane 1: 5uM MBP-GR; Lane 2: 5uM MBP-GR, 2uM Hsp40, 5uM Hsp70, 5uM Hop, 15uM Hsp90, 15uM Bag-1, 30uM p23, 5mM ATP, 20mM molybdate. **b**, Shodex KW-804 size exclusion chromatography profile of the GR maturation complex purified by MBP-GR pulldown from the reconstituted GR chaperone cycle. **c**, Coomassie-stained SDS-PAGE of the fractions from size exclusion chromatography. Colors indicate which gel lanes correspond to specific regions of the size exclusion chromatography profile. Sample fractions from the region highlighted in purple were collected and used for cryo-EM analysis. **d**, Representative electron micrograph for the cryo-EM dataset.

Extended Data Fig. 2: Cryo-EM Data Analysis

a

b

a, Cryo-EM data processing procedure with 3D reconstructions colored by local resolution. **b**, Gold-standard Fourier shell correlation (FSC) curves of the 3D reconstructions. The black dashed lines intercept the y axis at an FSC value of 0.143. For GR:Hsp90:p23, the map-model FSC is plotted in blue, with the blue dashed line intercepting the y axis at an FSC value of 0.5.

Extended Data Fig. 3: Hsp90 Conformation and Nucleotide-Bound State

a

b

a, GR maturation complex map density with atomic model showing ATP-magnesium density in both Hsp90 protomers (Hsp90A/B). Bottom images show increased contour level on the map density to indicate that the ATP gamma phosphate position has relatively strong density, likely corresponding to molybdate (see Methods). **b**, Atomic model of a symmetric Hsp90 dimer (orange) compared with Hsp90 from the maturation complex atomic model, indicating a slight asymmetry in the Hsp90 dimer interface in the maturation complex. Hsp90A (dark blue), Hsp90B (light blue), p23 (green).

Extended Data Fig. 4: Hsp90-GR Interfaces

a

b

c

d

Atomic model of the maturation complex with Hsp90A (dark blue), Hsp90B (light blue), GR (yellow). **a**, View of the GR_{pre-helix 1} strand threaded through the Hsp90 lumen and GR helices 1 and 3 packing against the entrance to the Hsp90 lumen. Side chains on GR in contact with Hsp90 are shown. Hsp90A/B are in surface representation. Hydrophobic residues on Hsp90 are colored in pink. **b**, Interface 1 of the Hsp90:GR interaction depicting the GR_{pre-Helix 1} region (GR⁵²³⁻⁵³¹) threading through the Hsp90 lumen. Side chains in contact between GR and Hsp90 are shown, along with hydrogen bonds (dashed pink lines). **c**, Interface 2 of the Hsp90:GR interaction depicting GR_{Helix 1} (GR⁵³²⁻⁵³⁹) packing against Hsp90. Side chains in contact between GR and Hsp90 are shown, along with hydrogen bonds (dashed pink lines). **d**, Interface 3 of the Hsp90:GR interaction depicting residues on the Hsp90A_{MD} loops (Hsp90A^{N318, W320, F349, R346}) and Hsp90B_{amphi-α} (Hsp90B^{T624, Y627, M628}) packing against GR. Side chains in contact between GR and Hsp90 are shown, along with hydrogen bonds (dashed pink lines).

Extended Data Fig. 5: GR is in a Native, Ligand-Bound Conformation

a

b

Dexamethasone-bound GR

Cortisol-bound GR

c

a, Atomic model of GR from the maturation complex (yellow) compared with GR from the crystal structure (1M2Z) (pink) with co-activator peptide TIF2 (purple), and ligand (orange). GR_{Helix 12} is indicated. **b**, GR maturation complex map density (sharpened with B factor -40) with atomic models. In the top images, the ligand density is shown with either the agonist dexamethasone docked (left) or the agonist cortisol docked (right). Arrow indicates the extra carbon atom in dexamethasone compared to cortisol. In the bottom images, density for GR^{Y735} is shown with either the dexamethasone-bound crystal structure docked (left image, 1M2Z) or the cortisol-bound crystal structure docked (right image, 4P6X). **c**, Atomic model of GR (yellow) and p23 (green) from the maturation complex highlighting the interaction between the p23_{tail-helix} and the GR C-terminus, which connects to GR_{Helix 12}.

Extended Data Fig. 6: Hsp90-p23 Interfaces

a

b

c

d

Atomic model of the maturation complex with Hsp90A (dark blue), Hsp90B (light blue), GR (yellow), p23 (green). **b**, Interface 1 of the Hsp90:p23 interaction depicting Hsp90B interacting with one side of the p23 core. Side chains in contact between p23 and Hsp90B are shown, along with hydrogen bonds (dashed pink lines). **c**, Interface 2 of the Hsp90:p23 interaction depicting Hsp90B interacting with the base of the p23 core. Side chains in contact between p23 and Hsp90B are shown, along with hydrogen bonds (dashed pink lines). **d**, Interface 3 of the Hsp90:p23 interaction depicting Hsp90A interacting with the side of the p23 core. Side chains in contact between p23 and Hsp90 are shown, along with hydrogen bonds (dashed pink lines).

Extended Data Fig. 7: The p23_{tail}-helix-GR Interface

a, Maturation complex composite density map showing density for the p23 tail with the atomic model built in. GR (yellow), p23 (green). **b**, Interface between the p23_{tail-helix} (green) and GR (colored by hydrophobicity, surface representation) showing the p23_{tail-helix} binds to a hydrophobic patch on GR. p23 side chains interacting with GR are shown. **c**, Sequence identity across human steroid hormone receptors (GR, mineralocorticoid receptor, androgen receptor, progesterone receptor, estrogen receptor α and β) plotted onto the GR structure. The p23_{tail-helix} (light green) was overlaid to indicate the p23:GR interface. **d**, Sequence alignment of eukaryotic p23 showing conservation of the p23_{tail-helix} sequence. The bottom aligned sequence is the p23_{tail-helix}-like motif identified in NCoA3 using the ScanProsite server. Red boxes on the *S. cerevisiae* p23 sequence indicate predicted helices from the PsiPred server. The alignment is colored according to the ClustalW convention. **e**, Secondary structure predictions for human p23 from three different servers. Porter 4.0 (orange), RaptorX (blue), Psipred (purple). The p23_{tail-helix} from the maturation complex atomic model is shown with the top green lines.

Extended Data Fig. 8: Effect of p23 Tail Mutants on GR Ligand Binding

a, Depiction of the two p23 tail mutants (p23 Δ tail, residues 1-133; p23 Δ helix-tail, residues 1-112) used in the GR ligand binding assays. **b**, Individual data points corresponding to **Fig. 3e**. Equilibrium binding of 20nM fluorescent dexamethasone to 250nM GR with chaperone components and p23 tail mutants measured by fluorescence polarization ($\pm$ SD). Assay conditions: 5mM ATP, 2uM Hsp40, 15uM Hsp70, Hsp90, Hop, and p23 or p23 tail mutants. **c**, Equilibrium binding of 20nM fluorescent dexamethasone to 250nM GR with addition of 15uM p23 or p23 tail mutants measured by fluorescence polarization ($\pm$ SD). Polarization values were normalized to the equilibrium binding of 20nM fluorescent dexamethasone to 250nM GR without p23/p23 tail mutants.

Extended Data Fig. 9: Hsp90-p23 Complex

a, Cryo-EM density map of the Hsp90:p23 complex. Hsp90A (dark blue), Hsp90B (light blue), p23 (green). This color scheme is maintained in all figures that show the structure. **b**, Cartoon representation of the Hsp90:p23 complex illustrating the MBP and GR LBD are not present in the map density (represented by a gray box). **c**, Atomic model of Hsp90 and p23 from the GR-maturation complex docked into the Hsp90:p23 map density. **d**, Top view of the Hsp90:p23 complex density map with clipping plane to show unidentified density (gray) through the Hsp90 lumen.

Extended Data Fig. 10: MBP-Hsp90-p23 Complex

a, Cryo-EM density map of the MBP:Hsp90:p23 complex. Far right image shows the 8Å lowpass-filtered density map. Hsp90A (dark blue), Hsp90B (light blue), p23 (green), MBP (orange). This color scheme is maintained in all figures that show the structure. **b**, Cartoon representation of the MBP:Hsp90:p23 complex illustrating the GR LBD is not present in the map density (represented by a gray box). **c**, Apo MBP crystal structure (1OMP) and atomic model of Hsp90 and p23 from the GR-maturation complex docked into the MBP:Hsp90:p23 map density. Far right image shows the density map lowpass-filtered to 8Å. **d**, Maltose-bound MBP crystal structure (1ANF) docked into the MBP:Hsp90:p23 map density. MBP (orange), maltose (pink). **e**, Top view of the MBP:Hsp90:p23 complex density map with clipping plane to show unidentified density (gray) through the Hsp90 lumen.

Extended Data Fig. 11: Comparison of the GR-Maturation Complex with the Hsp90-Kinase Complex

a

b

c Hsp90B Hsp90A

active, folded GR

Hsp90B Hsp90A

inactive, partially unfolded kinase

a, Structure of Hsp90 bound to an unfolded kinase client (5FWK) with a strand of the kinase client threaded through the Hsp90 lumen. The two hydrophobic residues on the kinase (Cdk4^{V89,V92}) that occupy the Hsp90 hydrophobic pockets are displayed. Hsp90A (dark blue, surface representation), Hsp90B (light blue, surface representation), Cdk4 kinase (purple). Hydrophobic residues on Hsp90 are colored in pink. **b**, Structure of Hsp90 bound to an unfolded kinase client (5FWK) depicting Hsp90A^{F341} (Hsp90A^{F349} in the GR-maturation complex) and Hsp90B_{amphi-α} packing against the kinase. Hsp90A (dark blue), Hsp90B (light blue), Cdk4 kinase (purple, surface representation). **c**, Top images are atomic models of the GR-maturation complex and Hsp90:kinase complex showing both clients thread through the closed Hsp90 lumen. Hsp90A (dark blue, surface representation), Hsp90B (light blue, surface representation), GR (yellow), Cdk4 kinase (purple). Bottom images are schematics demonstrating that both clients thread through the mostly hydrophobic Hsp90 lumen, but have different folding outcomes (H=hydrophobic interface).

Extended Data Table 1: Cryo-EM Data Collection, Refinement, and Validation Statistics

	GR:Hsp90:p23 (EMD-23004) (PDB 7KRJ)	Hsp90:p23 (EMDB-23006)	MBP:Hsp90:p23 (EMDB-23005)
Data Collection and Processing			
Magnification	105,000	105,000	105,000
Voltage (kV)	300	300	300
Electron Exposure (e-/Å²)	60	60	60
Defocus range (um)	0.8-2.0	0.8-2.0	0.8-2.0
Pixel size (Å)	0.835	0.835	0.835
Symmetry imposed	C1	C1	C1
Initial particles (no.)	~6,000,000	~6,000,000	~6,000,000
Final particles (no.)	140,217	454,385	31,566
Map resolution (Å)	2.56	2.66	3.63
FSC threshold	0.143	0.143	0.143
Map resolution range	2.4-3.6	2.5-3.5	3.4-6.9
Model Refinement			
Initial models used (PDB code)	5FWK, 1M2Z, 1EJF		
Model resolution (Å)	2.98		
FSC threshold	0.5		
Map sharpening <i>B</i> factor (Å²)	-32		
Model composition			
Non-hydrogen atoms	13460		
Protein residues	13368		
Ligands	92		
<i>B</i> factors (Å²)			
Protein (average, min - max)	76.2, 38.3 - 238.7		
Ligand (average, min - max)	57.6, 26.5 - 119.7		
R.m.s deviations			
Bond length (Å)	0.0261		
Bond angle (°)	1.81		
Validation			
Molprobity score	0.67		
Clashscore	0.48		
Poor rotamers (%)	0.07%		
Ramachandran plot			
Favored (%)	97.98		
Allowed (%)	1.90%		
Disallowed (%)	0.12%		

Figures

Figure 1

Architecture of the GR-maturation complex

Figure 2

p23tail-helix interactions and effect on GR ligand binding

Figure 3

Mechanism of GR activation by Hsp90