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Structural basis of glucocorticoid receptor signaling bias

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Dissociation between the healthy and toxic effects of cortisol, a major stress-responding hormone has been a widely used strategy to develop anti-inflammatory glucocorticoids with fewer side effects. Such strategy falls short when treating brain disorders as timing and activity state within large-scale neuronal networks determine the physiological and behavioral specificity of cortisol response. Advances in structural molecular dynamics posit the bases for engineering glucocorticoids with precision bias for select downstream signaling pathways. Design of allosteric and/or cooperative control for the glucocorticoid receptor could help promote the beneficial and reduce the deleterious effects of cortisol on brain and behavior in disease conditions.

Introduction

Cortisol is a human steroid hormone (corticosterone in rodents) released from the adrenal gland that uses nuclear receptors, firmly characterized molecularly, as well as hypothetical membrane receptors to adjust demands of the internal and external milieu. The receptors use an arsenal of rapid (seconds-to-minutes) non-genomic and slow (hours-to-days) acting genomic actions within cell ensembles in the brain and body affecting development, growth, metabolism and behavior (1). The low affinity nuclear glucocorticoid receptor (GR) is the

most widespread in the body while the high affinity mineralocorticoid receptor (MR) is more cell-type specific and often co-expressed with GR (2). Both receptor types are well established soluble and diffusible signaling entities unlike their membrane-bound counterparts supposed to transduce the rapid activities (3). Even if rapid steroid effects are unequivocal, the nature, or even existence of membrane-associated GR and MR is still debated due to the indirect pharmacological and genetic evidence (4-8).

Glucocorticoids are mainstay of treatment for many inflammatory and immune conditions, which however comes with side effects, particularly with chronic administration and high doses. Adverse and dissuasive effects of treatment include a diabetic-like state, peptic ulcer, gastrointestinal bleeding, increased blood pressure, iatrogenic Cushing's syndrome, osteoporosis, skin atrophy, delayed wound healing, glaucoma, cataract, cognitive impairment and other neurological dysfunctions (9, 10). Alternate treatments with fewer side effects targeting cortisol release, inflammation or immunosuppression may however display inferior therapeutic efficacy. Glucocorticoids are the most powerful treatment for a wide range of diseases, remaining indispensable, despite the seriousness of the side effects associated with chronic administration. Therefore, alleviating the adverse effects of glucocorticoids while retaining the desired property among a large array of functions (e.g. anti-inflammatory, immunosuppressant, cognitive enhancer, neuroprotective, proapoptotic...) remains a major focus for innovation. Topical administration for the skin, the eyes and aerosol delivery for the lungs are well tolerated but inadequate for most targeted diseases of the brain and inner body. Moreover, inhalation steroids have – epidemiologically – been associated with side effects (11, 12). Finally, the risks of using glucocorticoids in neurological disorders despite the benefits currently precludes clinical applications beyond the experimental.

The unravelling of the molecular bases of ligands recognition in the cortisol binding pocket prompted the synthesis of more potent glucocorticoids but side effects remain in spite of improved selectivity (13, 14). Development of new activity-modifying ligands outside of the cortisol binding domain could promote the graded or biased responses that are compatible with lesser side effects for treating conditions of resistance and hypersensitivity to glucocorticoids (15). A major question remains how to screen for such selective and dissociative activities. Here, we apply to GR the concepts of allosteric

cooperativity and biased signaling (see [box 1](#) for definitions) as developed originally for G-protein coupled receptors, to promote the design of glucocorticoids dissociating the beneficial from the detrimental effects (16).

GR structural allostery

Allostery is an activity-modifying process defined as the structural changes propagated from one site to another, often distal, functional site like the ligand-binding pocket ([Figure 1A](#)). Crystallography and nuclear magnetic resonance (NMR) revealed mechanisms common to most ligands (agonists and antagonists) with a similar pharmacophore (i.e. acting at the classical binding pocket) that transduces conformational surface changes of the activation function-2 (AF-2) coregulator-binding surface. This is conserved across most species and in many nuclear receptors (13, 17-19). For example, molecular dynamics of helix-12 in the ligand binding domain predict docking of either corepressors or coactivators (20), serving as structural basis for screening ligands that would stabilize conformations desired for agonistic or antagonistic effects (21, 22). Mutations in or surrounding the helix-12 often cause GR signalling defects and glucocorticoid resistance (23, 24). A recent study indicates how ligand-driven conformational shifts of helix-12 permit signal transmission from the ligand binding-domain to the transactivation domain and beyond to downstream GR-docking effectors (25). Conformational flexibility can explain how ligands with distinct chemical scaffolds and pharmacophores could elicit graded responses (full/partial agonism) and biased signaling (pathway specificity). Screening of GR ligands that bias the equilibrium toward the antagonist conformational state produced dissociative responses with anti-inflammatory effects devoid of transactivation responses (26). However, dissociative compounds have been disappointing in clinical situations because transactivation of anti-inflammatory compounds is also needed for full efficacy (27). Synthetic ligands were also designed to favor the transactivation of target genes over transrepression (28). Such ligands also have intrinsic limitations as the desired response often consists of a mixture of activated and suppressed genes in target tissues (29). Additionally, high-affinity GR modulators were characterized as partial agonists in some tissues and antagonists in others, permitting the dissociation between beneficial and deleterious effects of excessive and sustained levels of endogenous corticosterone in a

model of chronic stress (30). These modulators were identified based on differential GR-coregulator interactions.

Combining NMR with surface plasmon resonance revealed that different coregulators with distinct amino acid sequence motifs vary in their ability to propagate ligand-induced allostery to downstream protein interaction networks. For example, direct binding of GR to either the Nuclear Receptor Coactivator-2 (NCOA2) or the Peroxisome Proliferative Activated Receptor, Gamma, Coactivator 1 (PRGC1) depends on subtle but distinct conformational changes activable with selective ligands (25). Mutational analyses previously revealed the importance of AF-2 binding to proteins like the Heat Shock Protein-90 (HSP90) and 14-3-3 for controlling the equilibrium between alternative GR conformational states adopted upon ligand binding (31, 32). GR may adopt a high or low affinity state for effector substrates depending on mutations, chemical scaffolds of ligands, protons and cofactors (33). Concentrations of salts, pH and titrations of ligands may also affect allosteric transmission between GR functional domains. Taken together, allosteric coupling of the ligand-binding pocket with the coregulator-binding sites opens the possibility to design drugs and modulators that can exert desired effects via specific downstream pathways (34).

GR structural cooperativity

Cooperativity is defined by the structural changes propagated between two or more protein sequences that act dependently on each other ([Figure 1B](#)). In particular, the folding of intrinsically disordered protein sequences abundant in the N-terminal domain harboring the activation domain-1 (AF-1) sets the basis for cooperative coupling between distinct functional domains in GR (35). Direct proof that ligand-induced binding and folding of the intrinsically disordered parts of AF-1 can cooperatively influence the binding to a natively structured domain came from studies of the AF-1 binding with coregulators like the Tata-Box Binding Protein (TBP) and the Tumor Susceptibility-101 (TSG101) that increase the affinity of the DNA-binding domain for palindromic responsive elements (36, 37). Some allosteric ligands act as agonists under certain conditions and as antagonists in others for a given function which contrasts with the orthosteric ligands that typically stabilize one or the other conformation. Switching between positive and negative allosteric modulation of a

given function does not necessarily require that the interaction between cortisol and its pharmacophore differs (33). Instead, the cooperativity between structural domains prior to ligand stimulation could set the evoked response. Adopting conformational surfaces prone to functional cooperativity between domains has an energetic cost that varies between the agonistic and antagonistic folding states (33). Remarkably, the energy threshold needed to fold the AF-1 can be reduced by cortisol-independent mechanisms.

Post-translational modifications influence the energy requirement for structural cooperativity (38). For example, phosphorylation sites are abundant in the intrinsically disordered parts of GR and have the competence to switch between dynamic conformations or to stabilize a transition state cued to the biochemical environment (39). The chemical, steric and electrostatic attributes of the phosphoryl moiety can modify intra-molecular interactions like those between Ser211 and Arg214. Folding of the AF-1 is more pronounced when three well-established cortisol-dependent sites (Ser203, Ser211, Ser226) are phosphorylated simultaneously rather than independently corresponding to greater loss-of-function in the triple mutant compared to single mutants (40). Indeed, Ser211 also makes hydrogen bond to Trp213 when Ser203 and Ser226 are also phosphorylated, forming docking sites for direct binding with coregulators (41).

Isoforms of GR that differ in length in the intrinsically disordered parts of the N-terminal domain due to alternative translational start codons also exhibit differential activities (42), DNA-binding affinities (35), transcriptomes and tissue expressions (43, 44). Moreover, shedding parts of the intrinsically disordered sequences harboring multiple GR phosphorylation sites in the N-terminal domain via a Caspase-1 cleavage site blunts the transcriptional response to glucocorticoid therapy, which is associated with disease progression and poor prognosis in patients with acute lymphoblastic leukemia (45). Often, a proline next to the phosphorylated residue can adopt cis or trans conformations in the amino acid chain to direct protein-protein interactions. This is the case for Pro526 when Thr524 and Ser617 are phosphorylated in the AF-2 domain. The proline isomer then allows the carbonyl oxygen of Leu525 to form a hydrogen bond with amino groups Lys120 and Asn173, stimulating subsequent intermolecular interaction with the cytosolic scaffold protein 14-3-3, a negative regulator of GR transcriptional activity (32). Mutation of both Thr524 and Ser617 phosphosites in the AF-2 domain impairs GR binding to 14-3-3 but

incompletely (by 35%) given the dependency on Ser134 in the AF-1 domain (46). The phosphorylation of Ser134 is not dependent on glucocorticoid binding, suggesting that cooperativity between the AF-1 and AF-2 domains must be guided by the biochemical context of the cells. Of note, the cortisol-independent phosphorylation sites locate next to predicted α -helices of the N-terminal domain (Figure 2A), unlike cortisol-dependent sites that reside in its most disordered parts (Figure 2B). Therefore, it is paramount to consider how phosphorylation events could promote structural order from entropy for screening new generations of glucocorticoid modulators.

GR signaling bias

Signaling bias is defined as the dynamic structural cooperativity that propagates parts of the full spectrum allosteric transmission between functional domains evoked by cortisol (Figure 3A). Biased signaling of GR results from a large repertoire of folded transitional structures that dynamically switch, via allostery and cooperativity, from a native state to stimulated states. Here, the native state is predetermined at any given time by intracellular biochemical circumstances prior to ligand exposure while the stimulated states are glucocorticoid-bound. A dynamic equilibrium between alternative conformations permits differential binding to downstream effectors (47). Cooperativity between cortisol's pharmacophore and effector's sites sets the basis for signaling pathway specificity expected to vary between different tissues and cell types (48). GR relies on dimerization and palindromic DNA ligands -the glucocorticoid-responsive elements (GRE) and rare non-palindromic negative regulatory elements (nGRE)- to drive transcriptional activation and repression (49, 50). Specific interactors affect the direction of the response as for example, AP-1 and NF κ B for transrepression and CREB1, SRC1-3 and CBP for transactivation (2, 51, 52).

Timing is also an important factor as the biochemical state of cell activity guides GR access to its coregulators through reversible post-translational modifications and subcellular dynamic distributions (Figure 3B, (53)). For example, GR phosphorylation status has been linked to a GR interaction network with specific coregulators that signal via differential pathways in various cell types (1, 54). GR is phosphorylated in absence of cortisol binding, albeit it is enhanced by agonists but not by antagonists (55). This means

that dephosphorylation of GR is more than an off-switch. Dephosphorylation was previously showed to be a necessary step for other transcription factors to traffic into the nucleus and to bind to DNA (56). The stoichiometry of phosphorylation at cortisol-dependent and -independent sites reflects a balance between kinases and phosphatases activities available for GR at a given time and space (2). For instance, in neurons, unliganded GR binds to its preferred phosphatase, PP5 ensuring low levels of constitutive phosphorylation, whereas corticosterone binding triggers the release of PP5 from GR as a permissive act for any kinases to take the stage (57). AKT, CDK, ERK, P38, JNK are all phosphorylating GR at particular sites, which means that cortisol binding concurrent with (one or more) pathways that activate specific kinases may help present GR with multiple conformations in different parts of the cell (Figure 3C). One good example is neurotrophic signaling that transforms cortisol response in neuronal cells (58) and in neuronal networks (59) given that the release of Brain-Derived Neurotrophic factor (BDNF) is restricted by neural activity and behavioral experience (Figure 3D). Yet, such context-dependence is not limited to BDNF as cytokines, neurotransmitters and peptide hormones that can concur with cortisol binding may also transform the glucocorticoid output response (60).

Engineering of designer drugs for biasing cortisol response

To customize the next generation of GR ligands to a desirable effect, one must not only integrate the concepts of allostery, cooperativity and biased signaling into drug discovery programs but also take into consideration the outcome measures for screening that effect. Innovation shall be directed toward the unmet needs, that is not only the tissue or cellular specificity but also the dissociation between the non-genomic and the genomic glucocorticoid effects (3). Better assays are needed for screening the non-genomic effects because they might have better therapeutic potential than the genomic effects in particular settings. In the brain, rapid non-genomic effects alter neurotransmission with an arsenal of trans-synaptic messengers (retrograde and anterograde) that must impinge on the expression of the slow genomic effects through homeostatic scaling of neuronal networks (61), coincidence of signaling pathways (60) and epigenomic priming (62). A possible direction to overcome prior bottlenecks and pitfalls of drug discovery would be to focus on protein-protein interactions between GR and a synaptic (rapid) and/or a nuclear (slow)

effector to screen for dissociative modulators. New structural studies of GR interaction with select coactivators better defines the cavities to target in future high throughput screening for dissociative modulators (63-65).

The conformational states adopted by select cooperative residues can tailor coregulator identity and bias the signaling response between the desired therapeutic and the unwanted side effects. GR interactomes provides a resource for future clinical and biological discovery, and drug design based on ligand/coregulator dependencies (66, 67). With cortisol on board all the time at naturally occurring oscillating levels (68, 69), the allosteric/cooperative approach to new biased glucocorticoid drugs appears like an added value compared to molecules with constitutive agonistic or antagonistic activities (70). Bivalent ligand strategy (see [Box 1](#) for definition) is also possible to integrate the allostery of the pharmacophore with the cooperativity of coregulator docking, allowing to bypass unfavorable cellular environments ([Figure 4A](#)). This type of approach opens the possibility of using structure-based drug optimization strategies to customize therapeutic effects while minimizing adverse effects (47). Separating GR transrepression from transactivation activities resulted in glucocorticoids with selective response at pro-inflammatory gene targets but in vivo applications are still limited by some opposing effects of post-translational modifications and cell type specificities (10).

Engineering of an allosteric control of protein function is often used to make sensors (e.g. metabolites, neurotransmitters, ions or pH) and modifiers (e.g. chemogenetics and optogenetics) (71). Mutational analysis of a protein prototype revealed that only 5% of residues participate in its core allosteric transmission while a far larger amount of residues enriched at the protein surface and targeted by post-translational modifications act as allosteric modifiers (72). This suggests more potential for screening drugs targeting the allosteric sites (allo-drug) compared to orthosteric compounds (ortho-drugs). Given that allosteric sites are less conserved than cortisol's pharmacophore, allo-drugs would potentially be more specific relative to other nuclear receptors and offer lesser side effects than ortho-drugs. Validated algorithms can now integrate protein entropy with physical dynamics and perturbation propagation to predict allosteric transmission pathways (73). Future research will determine if these tools can be applied to GR for developing dissociative ligands. Drugs engineered with a photo-sensitive cage (e.g. photoswitch or

photolytic, [Figure 4C](#)) have proven superior to parent molecules in terms of spatiotemporal precision and dosage to reach desired effects (74). Light-switchable nanobodies (camelid single chain antibodies) whose binding to the target surface of interest in a protein could be enhanced or suppressed by light illumination is also an area for future glucocorticoid research (75). Additionally, phosphorylation within the epitope binding of nanobodies offers the possibility to design phosphorylation-state locked drugs for cooperative modulation of glucocorticoids ([Figure 4B](#)). Prototypical light-sensitive therapeutics are molecules targeting membrane receptors like G protein-coupled receptors (76) but photo-switchable ligands for nuclear receptors are emerging (77, 78). This increases prospects for the design of new glucocorticoids with better benefit/risk ratio. Prospective applications of phototherapeutics derived from animal studies include metastatic cancer, rheumatoid arthritis, microbial infections, stroke, neurological and psychiatric diseases (74).

Perspectives for therapeutic manipulation of GR

There are 3 major disturbances of GR signaling in diseases: (i) ligand availability, (ii) receptor/effector levels, and (iii) signal transduction. A major difficulty in targeting GR is to respect the diversity of responses across contexts, cells and tissues (62). In most therapeutic contexts, only a limited number of tissues or cell types would be the actual target, and even then, it may be beneficial to only modulate a part of the cellular effects of glucocorticoids (e.g. leaving effects on mitochondrial function intact). Improving the benefit/risk ratio of glucocorticoids would require targeting diseased cells, tissues and organs while preserving the healthy ones that also express GR.

Congenital or acquired glucocorticoid resistance is a common feature of cortisol secretion defects, loss-of-function mutations, GR haploinsufficiency or ectopic levels of the decoy GR- β isoform (lacking cortisol-binding) and feedforward MAP-Kinases signaling (9, 79). One difficult issue with acquired glucocorticoid resistance is that it is uneven throughout the cells and tissues of the organism. Correction of glucocorticoid resistance in patients with acute lymphoblastic leukemia is possible with Caspase inhibitors because the ectopic activation of Caspase-1 in leukocytes sheds the intrinsically disordered parts of GR that promote cooperativity between domains (45). Inhibitors of P38 kinase restore cortisol sensitivity by acting on GR phosphorylation in airways smooth muscle cells that could

benefit patients with asthma and chronic obstructive pulmonary dysfunctions without side effects of chronic glucocorticoid therapy (80). Activators of the dual-specificity phosphatase DUSP1, a GR-inducible immediate early gene poorly expressed in conditions of glucocorticoid-resistance (e.g. inflammatory bone disorders, atherosclerosis, pulmonary disease (81), major depression, Alzheimer's disease and others (82)) suppress MAPK activity, reduce GR phosphorylation and accelerate the resolution of inflammation without side effects of chronic glucocorticoid therapy. Moreover, a dissociative compound modifying the phosphorylation status of GR (lack of Ser211 phosphorylation) displays anti-inflammatory properties in mice without inducing hyperglycemia as a side effect (83). Therefore, structural cavities involved in allosteric modulation of a given GR function via cell-type specific coregulators provide an entry point to design glucocorticoids with lesser pleiotropic effects (70).

Conclusion

GR presents itself in multiple conformations throughout the cells of our bodies depending on ligand titration, receptor/effector forms and levels, and feedback/feedforward signaling at the time of treatment. Existing strategies for biased signaling rely on the presence of either interacting (pro-inflammatory) transcription factors, or on the presence of specific coregulators molecules. An alternative strategy is to make use of allosteric modulation of GR to change functions, perhaps even at a subcellular resolution (84). Such a next generation of glucocorticoids should take advantage of GR signaling bias for tailoring safety and efficacy.

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CReDiT

FJ: Conceptualization, Writing – original draft ; OCM: Writing – review & editing, MPM: Writing – review & editing.

REFERENCES

1. Garabedian MJ, Harris CA, Jeanneteau F. Glucocorticoid receptor action in metabolic and neuronal function. *F1000Res*. 2017; **6**:1208.
2. Arango-Lievano M, Lambert WM, Jeanneteau F. Molecular Biology of Glucocorticoid Signaling. *Adv Exp Med Biol*. 2015; **872**:33-57.
3. Groeneweg FL, Karst H, de Kloet ER, Joels M. Rapid non-genomic effects of corticosteroids and their role in the central stress response. *J Endocrinol*. 2011; **209**(2): 153-67.
4. Liston C, Cichon JM, Jeanneteau F, J. Z, Chao MV, Gan WB. Circadian glucocorticoid oscillations promote learning-dependent synapse formation and maintenance. *Nature Neuroscience*. 2013; **16**(6): 698-705.
5. Hu W, Zhang M, Czéh B, Flügge G, Zhang W. Stress impairs GABAergic network function in the hippocampus by activating nongenomic glucocorticoid receptors and affecting the integrity of the parvalbumin-expressing neuronal network. *Neuropsychopharmacology*. 2010; **35**(8): 1693-707.
6. Di S, Maxson MM, Franco A, Tasker JG. Glucocorticoids regulate glutamate and GABA synapse-specific retrograde transmission via divergent nongenomic signaling pathways. *J Neurosci*. 2009; **29**(2): 393-401.
7. Karst H, Berger S, Turiault M, Tronche F, Schutz G, Joels M. Mineralocorticoid receptors are indispensable for nongenomic modulation of hippocampal glutamate transmission by corticosterone. *Proceedings of the National Academy of Sciences of the United States of America*. 2005; **102**(52): 19204-7.
8. Karst H, den Boon FS, Vervoort N, Adrian M, Kapitein LC, Joëls M. Non-genomic steroid signaling through the mineralocorticoid receptor: Involvement of a membrane-associated receptor? . *Mol Cell Endocrinol*. 2022; **541**.
9. Sevilla LM, Jiménez-Panizo A, Alegre-Martí A, Estébanez-Perpiñá E, Caelles C, Pérez P. Glucocorticoid Resistance: Interference between the Glucocorticoid Receptor and the MAPK Signalling Pathways. *Int J Mol Sci* 2021; **22**(18): 10049.
10. Clark AR, Belvisi MG. Maps and legends: the quest for dissociated ligands of the glucocorticoid receptor. *Pharmacol Ther*. 2012; **134**(1): 54-67.
11. Savas M, Vinkers CH, Rosmalen JGM, Hartman CA, Wester VL, van den Akker ELT, Iyer AM, McEwen BS, van Rossum EFC. Systemic and Local Corticosteroid Use Is Associated with Reduced Executive Cognition, and Mood and Anxiety Disorders. *Neuroendocrinology*. 2020; **110**(3-4): 282-91.
12. van der Meulen M, Amaya JM, Dekkers OM, Meijer OC. Association between use of systemic and inhaled glucocorticoids and changes in brain volume and white matter microstructure: a cross-sectional study using data from the UK Biobank. *BMJ Open*. 2022; **12**(8): e062446.
13. Bledsoe RK, Montana VG, Stanley TB, Delves CJ, Apolito CJ, McKee DD, Consler TG, Parks DJ, Stewart EL, Willson TM, Lambert MH, Moore JT, Pearce KH, Xu HE. Crystal structure of the glucocorticoid receptor ligand binding domain reveals a novel mode of receptor dimerization and coactivator recognition. *Cell*. 2002; **110**(1): 93-105.
14. Weikum ER, Okafor CD, D'Agostino EH, Colucci JK, E.A. O. Structural Analysis of the Glucocorticoid ReceptorLigand-Binding Domain in Complex with TriamcinoloneAcetonide

and a Fragment of the Atypical Coregulator, SmallHeterodimer Partner. *Mol Pharmacol.* 2017; **92**:12-21.

15. Weikum ER, Knuesel MT, Ortlund EA, Yamamoto KR. Glucocorticoid receptor control of transcription: precision and plasticity via allostery. *Nat Rev Mol Cell Biol.* 2017; **18**(3): 159-74.

16. Kenakin T. Theoretical Aspects of GPCR-Ligand Complex Pharmacology. *Chem Rev.* 2017; **117**(1): 4-20.

17. Shiau AK, Barstad D, Loria PM, Cheng L, Kushner PJ, Agard DA, Greene GL. The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. *Cell.* 1998; **95**(7): 927-37.

18. Baker ME, Chandsawangbhuwana C, Ollikainen N. Structural analysis of the evolution of steroid specificity in the mineralocorticoid and glucocorticoid receptors. *BMC Evol Biol.* 2007; **7**:24.

19. Edman K, Hosseini A, Bjursell MK, Aagaard A, Wissler L, Gunnarsson A, Kaminski T, Köhler C, Bäckström S, Jensen TJ, Cavallin A, Karlsson U, Nilsson E, Lecina D, Takahashi R, Grebner C, Geschwindner S, Lepistö M, Hogner AC, Guallar V. Ligand Binding Mechanism in Steroid Receptors: From Conserved Plasticity to Differential Evolutionary Constraints. *Structure.* 2015; **23**(12): 2280-90.

20. Schoch GA, D'Arcy B, Stihle M, Burger D, Bär D, Benz J, Thoma R, Ruf A. Molecular switch in the glucocorticoid receptor: active and passive antagonist conformations. *J Mol Biol.* 2010; **395**(3): 568-77.

21. Hu X, Du S, Tunca C, Braden T, Long KR, Lee J, Webb EG, Dietz JD, Hummert S, Rouw S, Hegde SG, Webber RK, Obukowicz MG. The antagonists but not partial agonists of glucocorticoid receptor ligands show substantial side effect dissociation. *Endocrinology.* 2011; **152**(8): 3123-34.

22. Kroon J, Viho EMG, Gentenaar M, Koorneef LL, van Kooten C, Rensen PCN, Kooijman S, Hunt H, Meijer OC. The development of novel glucocorticoid receptor antagonists: From rational chemical design to therapeutic efficacy in metabolic disease models. *Pharmacological research.* 2021; **168**:105588.

23. McMahon SK, Pretorius CJ, Ungerer JP, Salmon NJ, Conwell LS, Pearen MA, Batch JA. Neonatal complete generalized glucocorticoid resistance and growth hormone deficiency caused by a novel homozygous mutation in Helix 12 of the ligand binding domain of the glucocorticoid receptor gene (NR3C1). *J Clin Endocrinol Metab.* 2010; **95**(1): 297-302.

24. Nicolaidis NC, Galata Z, Kino T, Chrousos GP, Charmandari E. The human glucocorticoid receptor: molecular basis of biologic function. *Steroids.* 2010; **75**(1): 1-12.

25. Köhler C, Carlström G, Gunnarsson A, Weininger U, Tångefjord S, Ullah V, Lepistö M, Karlsson U, Papavoine T, Edman K, Akke M. Dynamic allosteric communication pathway directing differential activation of the glucocorticoid receptor. *Sci Adv.* 2020; **6**(29): eabb5277.

26. Hu X, Pang J, Zhang J, Shen C, Chai X, Wang E, Chen H, Wang X, Duan M, Fu W, Xu L, Kang Y, Li D, Xia H, Hou T. Discovery of Novel GR Ligands toward Druggable GR Antagonist Conformations Identified by MD Simulations and Markov State Model Analysis. *Adv Sci (Weinh).* 2022; **9**(3): e2102435.

27. Vandevyver S, Dejager L, Tuckermann J, Libert C. New insights into the anti-inflammatory mechanisms of glucocorticoids: an emerging role for glucocorticoid-receptor-mediated transactivation. *Endocrinology.* 2013; **154**(3): 993-1007.

28. De Bosscher K, Beck IM, Ratman D, Berghe WV, Libert C. Activation of the Glucocorticoid Receptor in Acute Inflammation: the SEDIGRAM Concept. *Trends Pharmacol Sci.* 2016; **37**(1): 4-16.
29. De Bosscher K, Desmet SJ, Clarisse D, Estébanez-Perpiña E, Brunsveld L. Nuclear receptor crosstalk - defining the mechanisms for therapeutic innovation. *Nature reviews Endocrinology.* 2020; **16**(7): 363-77.
30. Zalachoras I, Houtman R, Atucha E, Devos R, Tijssen AM, Hu P, Lockey PM, Datson NA, Belanoff JK, Lucassen PJ, Joëls M, de Kloet ER, Roozendaal B, Hunt H, Meijer OC. Differential targeting of brain stress circuits with a selective glucocorticoid receptor modulator. *Proc Natl Acad Sci U S A.* 2013; **110**(19).
31. Ricketson D, Hostick U, Fang L, Yamamoto KR, Darimont BD. A conformational switch in the ligand-binding domain regulates the dependence of the glucocorticoid receptor on Hsp90. *J Mol Biol.* 2007; **368**(3): 729-41.
32. Munier CC, De Maria L, Edman K, Gunnarsson A, Longo M, MacKintosh C, Patel S, Snijder A, Wissler L, Brunsveld L, Ottmann C, Perry MWD. Glucocorticoid receptor Thr524 phosphorylation by MINK1 induces interactions with 14-3-3 protein regulators. *J Biol Chem.* 2021; **296**100551.
33. Motlagh HN, Hilser VJ. Agonism/antagonism switching in allosteric ensembles. *Proc Natl Acad Sci U S A.* 2012; **109**(11): 4134-9.
34. Meijer FA, Leijten-van de Gevel IA, de Vries RMJM, Brunsveld L. Allosteric small molecule modulators of nuclear receptors. *Molecular and cellular endocrinology.* 2019; **485**20-34.
35. Li J, White JT, Saavedra H, Wrabl JO, Motlagh HN, Liu K, Sowers J, Schroer TA, Thompson EB, Hilser VJ. Genetically tunable frustration controls allostery in an intrinsically disordered transcription factor. *eLife.* 20176:e30688.
36. White JT, Rives J, Tharp ME, Wrabl JO, Thompson EB, Hilser VJ. Tumor Susceptibility Gene 101 Regulates the Glucocorticoid Receptor through Disorder-Mediated Allostery. *Biochemistry.* 2021; **61**(21): 1647-57.
37. Kumar R, Volk DE, Li J, Lee JC, Gorenstein DG, Thompson EB. TATA box binding protein induces structure in the recombinant glucocorticoid receptor AF1 domain. *Proc Natl Acad Sci U S A.* 2004; **101**(47): 16425-30.
38. Liberman AC, Antunica-Noguerol M, Arzt E. Modulation of the Glucocorticoid Receptor Activity by Post-Translational Modifications. *Nuclear Receptor Research.* 2014; **11**-15.
39. Garza AM, Khan SH, Kumar R. Site-specific phosphorylation induces functionally active conformation in the intrinsically disordered N-terminal activation function (AF1) domain of the glucocorticoid receptor. *Mol Cell Biol.* 2010; **30**(1): 220-30.
40. Khan SH, McLaughlin WA, Kumar R. Site-specific phosphorylation regulates the structure and function of an intrinsically disordered domain of the glucocorticoid receptor. *7.* 2017; **1**(15440).
41. Kumar R, Thompson EB. Role of Phosphorylation in the Modulation of the Glucocorticoid Receptor's Intrinsically Disordered Domain. *Biomolecules.* 2019; **9**(3): 95.
42. Lu NZ, Cidlowski JA. Translational regulatory mechanisms generate N-terminal glucocorticoid receptor isoforms with unique transcriptional target genes. *Mol Cell.* 2005; **18**(3): 331-42.

43. Oakley RH, Ramamoorthy S, Foley JF, Busada JT, Lu NZ, Cidlowski JA. Glucocorticoid receptor isoform-specific regulation of development, circadian rhythm, and inflammation in mice. *Faseb J*. 2018; **32**(10): 5258-71.
44. Cao Y, Bender IK, Konstantinidis AK, Shin SC, Jewell CM, Cidlowski JA, Schleimer RP, Lu NZ. Glucocorticoid receptor translational isoforms underlie maturational stage-specific glucocorticoid sensitivities of dendritic cells in mice and humans. *Blood*. 2013; **121**(9): 1553-62.
45. Paugh SW, Bonten EJ, Savic D, Ramsey LB, Thierfelder WE, Gurung P, Malireddi RK, Actis M, Mayasundari A, Min J, Coss DR, Laudermilk LT, Panetta JC, McCorkle JR, Fan Y, Crews KR, Stocco G, Wilkinson MR, Ferreira AM, Cheng C, Yang W, Karol SE, Fernandez CA, Diouf B, Smith C, Hicks JK, Zanut A, Giordanengo A, Crona D, Bianchi JJ, Holmfeldt L, Mullighan CG, den Boer ML, Pieters R, Jeha S, Dunwell TL, Latif F, Bhojwani D, Carroll WL, Pui CH, Myers RM, Guy RK, Kanneganti TD, Relling MV, Evans WE. NALP3 inflammasome upregulation and CASP1 cleavage of the glucocorticoid receptor cause glucocorticoid resistance in leukemia cells. *Nat Genet*. 2015; **47**(6): 607-14.
46. Galliher-Beckley AJ, Williams JG, Cidlowski JA. Ligand-independent phosphorylation of the glucocorticoid receptor integrates cellular stress pathways with nuclear receptor signaling. *Mol Cell Biol*. 2011; **31**(23): 4663-75.
47. de Vink PJ, Koops AA, D'Arrigo G, Cruciani G, Spyrakis F, Brunsveld L. Cooperativity as quantification and optimization paradigm for nuclear receptor modulators. *Chem Sci*. 2022; **13**(9): 2744-52.
48. Hilser VJ, Thompson EB. Structural dynamics, intrinsic disorder, and allostery in nuclear receptors as transcription factors. *J Biol Chem*. 2011; **286**(46): 39675-82.
49. Johnson TA, Paakinaho V, Kim S, Hager GL, Presman DM. Genome-wide binding potential and regulatory activity of the glucocorticoid receptor's monomeric and dimeric forms. *Nature communications*. 2021; **12**(1): 1987.
50. Hudson WH, Youn C, Ortlund EA. The structural basis of direct glucocorticoid-mediated transrepression. *Nat Struct Mol Biol*. 2013; **20**(1): 53-8.
51. Newton R, Holden NS. Separating transrepression and transactivation: a distressing divorce for the glucocorticoid receptor? *Mol Pharmacol*. 2007; **72**(4): 799-809.
52. Hudson WH, Vera IMS, Nwachukwu JC, Weikum ER, Herbst AG, Yang Q, Bain DL, Nettles KW, Kojetin DJ, Ortlund EA. Cryptic glucocorticoid receptor-binding sites pervade genomic NF- κ B response elements. *Nature communications*. 2018; **9**(1): 1337.
53. Vanderhaeghen T, Timmermans S, Watts D, Paakinaho V, Eggermont M, Vandewalle J, Wallaeyns C, Van Wyngene L, Van Looveren K, Nuyttens L, Dewaele S, Vanden Berghe J, Lemeire K, De Backer J, Dirkx L, Vanden Berghe W, Caljon G, Ghesquière B, De Bosscher K, Wielockx B, Palvimo JJ, Beyaert R, Libert C. Reprogramming of glucocorticoid receptor function by hypoxia. *EMBO Rep*. 2022; **23**(1): e53083.
54. Keenan CR, Lew MJ, Stewart AG. Biased signalling from the glucocorticoid receptor: Renewed opportunity for tailoring glucocorticoid activity. *Biochem Pharmacol*. 2016; **112**: 6-12.
55. Wang Z, Frederick J, Garabedian MJ. Deciphering the phosphorylation "code" of the glucocorticoid receptor in vivo. *J Biol Chem*. 2002; **277**(29): 26573-80.
56. Gardner KH, Montminy M. Can You Hear Me Now? Regulating Transcriptional Activators by Phosphorylation. *Science Signaling*. 2005; **301**: pe44.

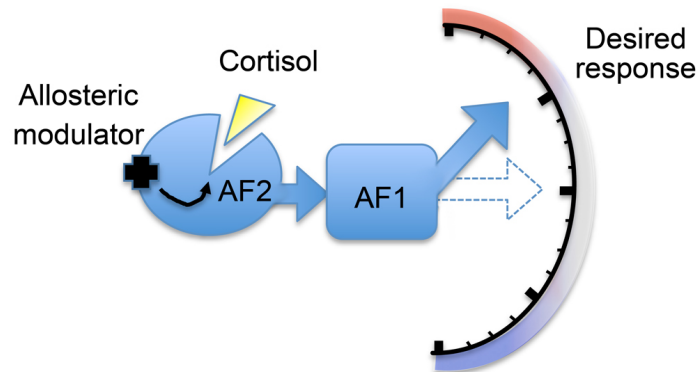
57. Arango-Lievano M, Lambert WM, Bath KG, Garabedian MJ, Chao MV, Jeanneteau F. Neurotrophic-priming of glucocorticoid receptor signaling is essential for neuronal plasticity to stress and antidepressant treatment. *Proc Natl Acad Sci U S A*. 2015; **112**(51): 15737-42.
58. Lambert WM, Xu C-F, Neubert TA, Chao MV, Garabedian MJ, Jeanneteau F. BDNF-signaling rewrites the glucocorticoid transcriptome via glucocorticoid receptor phosphorylation. *Molecular and Cellular Biology*. 2013; **33**(18): 3700-14.
59. Jeanneteau F, Borie A, Chao M, Garabedian M. Bridging the gap between BDNF and glucocorticoid effects on brain networks. *Neuroendocrinology*. 2019.
60. Arango-Lievano M, Jeanneteau F. Timing and crosstalk of glucocorticoid signaling with cytokines, neurotransmitters and growth factors. *Pharmacological research*. 2016; **113**(Pt A): 1-17.
61. Jeanneteau F, Coutellier L. The glucocorticoid footprint on the memory engram. *Current Opinion in Endocrine and Metabolic Research*. 2022100378.
62. Huzard D, Rappeneau V, Meijer OC, Touma C, Arango-Lievano M, Garabedian MJ, Jeanneteau F. Experience and activity-dependent control of glucocorticoid receptors during the stress response in large-scale brain networks. *Stress*. 20201-24.
63. Biggadike K, Bledsoe RK, Coe DM, Cooper TW, House D, Iannone MA, Macdonald SJ, Madauss KP, McLay IM, Shipley TJ, Taylor SJ, Tran TB, Uings IJ, Weller V, Williams SP. Design and x-ray crystal structures of high-potency nonsteroidal glucocorticoid agonists exploiting a novel binding site on the receptor. *Proc Natl Acad Sci U S A*. 2009; **106**(43): 18114-9.
64. Jin Z, Lin H, Srinivasan S, Nwachukwu JC, Bruno N, Griffin PR, Nettles KW, Kamenecka TM. Synthesis of novel steroidal agonists, partial agonists, and antagonists for the glucocorticoid receptor. *Bioorg Med Chem Lett*. 2017; **27**(2): 347-53.
65. Wang RY, Noddings CM, Kirschke E, Myasnikov AG, Johnson JL, Agard DA. Structure of Hsp90-Hsp70-Hop-GR reveals the Hsp90 client-loading mechanism. *Nature*. 2022; **601**(7893): 460-4.
66. Broekema MF, Hollman DAA, Koppen A, van den Ham HJ, Melchers D, Pijnenburg D, Ruijtenbeek R, van Mil SWC, Houtman R, Kalkhoven E. Profiling of 3696 Nuclear Receptor-Coregulator Interactions: A Resource for Biological and Clinical Discovery. *Endocrinology*. 2018; **159**(6): 2397-407.
67. Bakker E, Tian K, Mutti L, Demonacos C, Schwartz JM, Krstic-Demonacos M. Insight into glucocorticoid receptor signalling through interactome model analysis. *PLoS Comput Biol*. 2017; **13**(11): e1005825.
68. Kalafatakis K, Russell GM, Harmer CJ, Munafo MR, Marchant N, Wilson A, Brooks JC, Durant C, Thakrar J, Murphy P, Thai NJ, Lightman SL. Ultradian rhythmicity of plasma cortisol is necessary for normal emotional and cognitive responses in man. *Proc Natl Acad Sci U S A*. 2018; **115**(17): E4091-E100.
69. Sarabdjitsingh RA, Spiga F, Oitzl MS, Kershaw Y, Meijer OC, Lightman SL, de Kloet ER. Recovery from disrupted ultradian glucocorticoid rhythmicity reveals a dissociation between hormonal and behavioural stress responsiveness. *J Neuroendocrinol*. 2010; **22**(8): 862-71.
70. Meijer OC, Koorneef LL, Kroon J. Glucocorticoid receptor modulators. *Endocrinol (Paris)*. 2018; **79**(3): 107-11.
71. Vishweshwaraiah YL, Chen J, Dokholyan NV. Engineering an Allosteric Control of Protein Function. *J Phys Chem B*. 2021; **125**(7): 1806-14.

72. McCormick JW, Russo MA, Thompson S, Blevins A, Reynolds KA. Structurally distributed surface sites tune allosteric regulation. *eLife*. 2021; **10**e68346.
73. Wang J, Jain A, McDonald LR, Gambogi C, Lee AL, Dokholyan NV. Mapping allosteric communications within individual proteins. *Nature communications*. 2020; **11**(1): 3862.
74. Vickerman BM, Zywoot EM, Tarrant TK, Lawrence DS. Taking phototherapeutics from concept to clinical launch. *Nat Rev Chem*. 2021; **5**(11): 816-34.
75. Gil AA, Carrasco-López C, Zhu L, Zhao EM, Ravindran PT, Wilson MZ, Goglia AG, Avalos JL, Toettcher JE. Optogenetic control of protein binding using light-switchable nanobodies. *Nature communications*. 2020; **11**(1): 4044.
76. Paoletti P, Ellis-Davies GCR, Mourou A. Optical control of neuronal ion channels and receptors. *Nat Rev Neurosci*. 2019; **20**(9): 514-32.
77. Morstein J, Trads JB, Hinnah K, Willems S, Barber DM, Trauner M, Merk D, Trauner D. Optical control of the nuclear bile acid receptor FXR with a photohormone. *Chem Sci*. 2019; **11**(2): 429-34.
78. Hinnah K, Willems S, Morstein J, Heering J, Hartrampf FWW, Broichhagen J, Leippe P, Merk D, Trauner D. Photohormones Enable Optical Control of the Peroxisome Proliferator-Activated Receptor γ (PPAR γ). *J Med Chem*. 2020; **63**(19): 10908-20.
79. Barnes PJ, Adcock IM. Glucocorticoid resistance in inflammatory diseases. *Lancet*. 2009; **373**(9678): 1905-17.
80. Bouazza B, Debba-Pavard M, Amrani Y, Isaacs L, O'Connell D, Ahamed S, Formella D, Tliba O. Basal p38 mitogen-activated protein kinase regulates unliganded glucocorticoid receptor function in airway smooth muscle cells. *American journal of respiratory cell and molecular biology*. 2014; **50**(2): 301-15.
81. Hoppstädter J, Ammit AJ. Role of Dual-Specificity Phosphatase 1 in Glucocorticoid-Driven Anti-inflammatory Responses. *Front Immunol*. 2019; **10**: 1446.
82. Arango-Lievano M, Peguet C, Catteau M, Parmentier ML, Wu S, Chao MV, Ginsberg SD, Jeanneteau F. Deletion of Neurotrophin Signaling through the Glucocorticoid Receptor Pathway Causes Tau Neuropathology. *Scientific reports*. 2016; **6**: 37231.
83. De Bosscher K, Vanden Berghe W, Beck IM, Van Molle W, Hennuyer N, Hapgood J, Libert C, Staels B, Louw A, Haegeman G. A fully dissociated compound of plant origin for inflammatory gene repression. *Proc Natl Acad Sci U S A*. 2005; **102**(44): 15827-32.
84. Van Moortel L, Thommis J, Maertens B, Staes A, Clarisse D, De Sutter D, Libert C, Meijer OC, Eyckerman S, Gevaert K, De Bosscher K. Novel assays monitoring direct glucocorticoid receptor protein activity exhibit high predictive power for ligand activity on endogenous gene targets. *Biomed Pharmacother*. 2022; **152**: 113218.
85. Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, Tunyasuvunakool K, Bates R, Žídek A, Potapenko A, Bridgland A, Meyer C, Kohl SAA, Ballard AJ, Cowie A, Romera-Paredes B, Nikolov S, Jain R, Adler J, Back T, Petersen S, Reiman D, Clancy E, Zielinski M, Steinegger M, Pacholska M, Berghammer T, Bodenstein S, Silver D, Vinyals O, Senior AW, Kavukcuoglu K, Kohli P, Hassabis D. Highly accurate protein structure prediction with AlphaFold. *Nature*. 2021; **596**(7873): 583-9.
86. Varadi M, Anyango S, Deshpande M, Nair S, Natassia C, Yordanova G, Yuan D, Stroe O, Wood G, Laydon A, Žídek A, Green T, Tunyasuvunakool K, Petersen S, Jumper J, Clancy E, Green R, Vora A, Lutfi M, Figurnov M, Cowie A, Hobbs N, Kohli P, Kleywegt G, Birney E, Hassabis D, Velankar S. AlphaFold Protein Structure Database: massively expanding the

structural coverage of protein-sequence space with high-accuracy models. *Nucleic Acids Research*. 2021; **50**(D1): D439-D44.

FIGURES

A



B

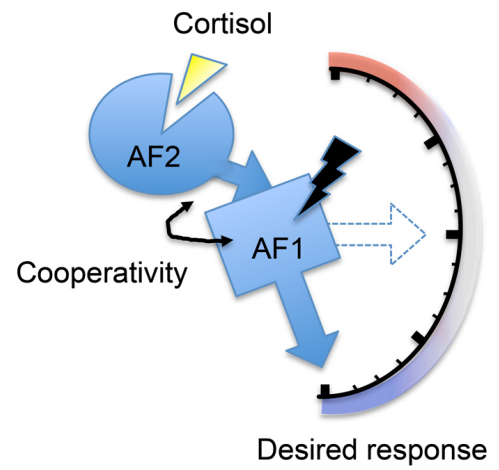


Figure 1. Modulation of cortisol response

A. Allosteric modulation transforms cortisol response.

B. Cooperativity between functional domains synergize with cortisol response.

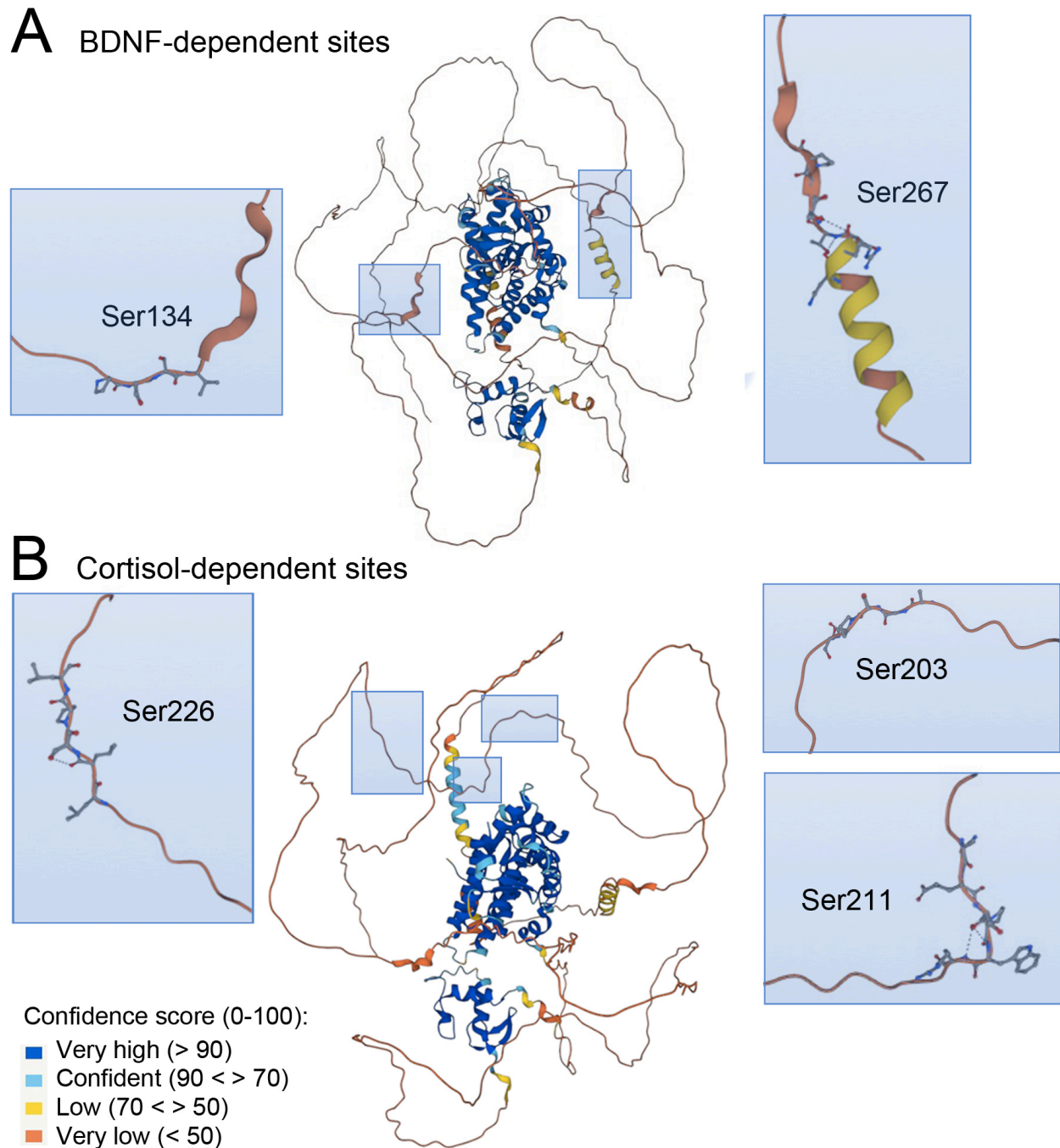
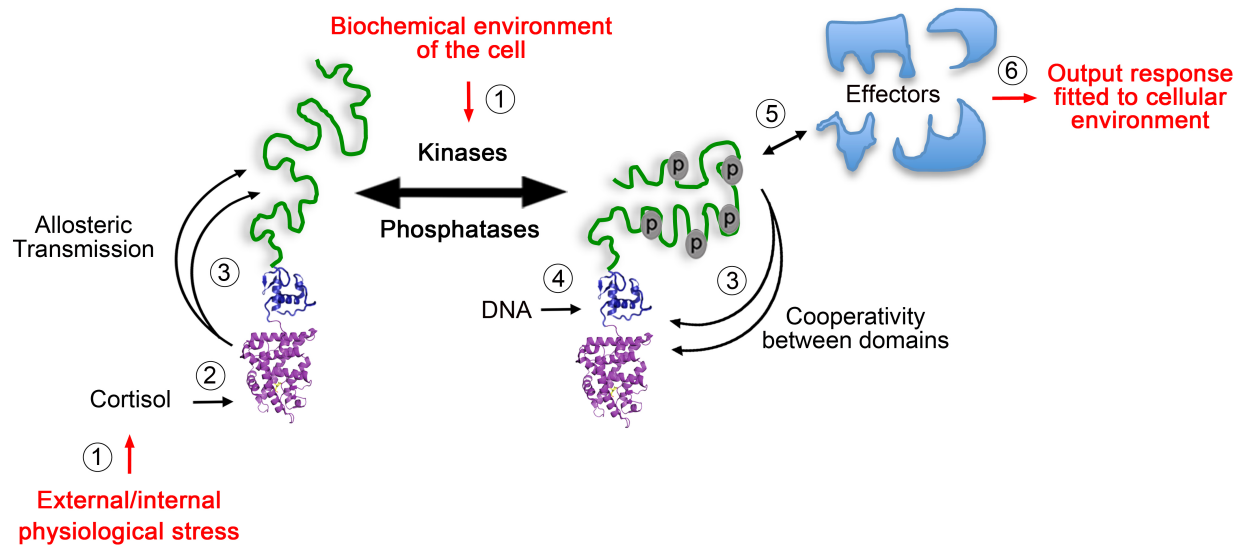


Figure 2. Predicted structure of the glucocorticoid receptor distinguishes the cortisol-dependent from the -independent phosphorylation sites

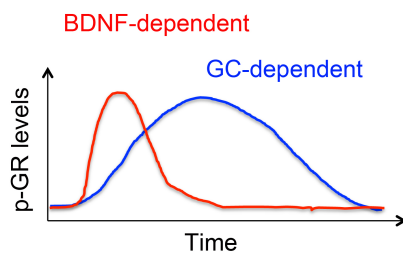
A. BDNF-dependent sites are adjacent to predicted α -helices in the N-terminal domain.

B. Cortisol-dependent sites locate in the highly disordered unfolded parts of the N-terminal domain. Structure confidence is determined with the AlphaFold Protein Structure Database (<https://alphafold.ebi.ac.uk>) (85, 86).

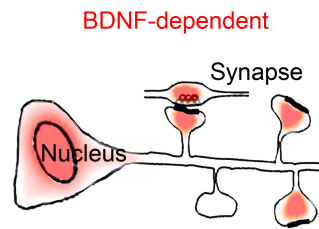
A. Structure



B. Timing



C. Subcellular



D. Network

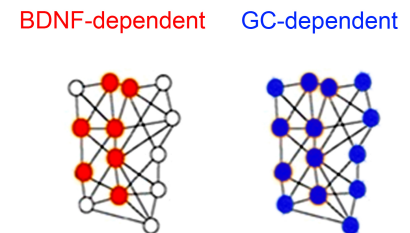


Figure 3. Signaling bias of the glucocorticoid receptor on multiple scales

A. Molecular dynamics in GR domains are influenced by the concentrations of salts, pH and ligands available in the cellular environment (1). The state of cell activity alters GR post-translational modifications mostly in its N-terminal domain made of intrinsically disordered sequences contrary to the natively folded ligand-binding and DNA-binding domains. Reversible phosphorylation sets the range of cooperativity between domains induced by the allosteric transmission of cortisol binding (2) through the shifting of the α -helices (3). Consensus DNA sequences up to 15 base pairs binds to GR zinc fingers (4). Through allostery and cooperativity, GR adopts docking sites for specific effectors within the available range (5). The structural bias determined by phosphorylation prior to cortisol stimulation scales the range of output responses fitted to the cellular environment (6).

B. Distinct temporal domains of GR phosphorylation at BDNF-dependent and GC-dependent sites, rapid (minutes) and slow (minutes to hours), respectively.

C. GR phosphorylation at BDNF-dependent sites distributes within the cytoplasm and synapses, unless cortisol binding forces GR into the nucleus.

D. GR phosphorylation at BDNF-dependent sites is specific of cells responding to BDNF, a neurotrophic factor released at the synapse in an activity-dependent fashion. It is detected in task-allocated cell ensembles expressing c-fos. In contrast, GR phosphorylation at GC-dependent sites is more widespread between cell types and tissues.

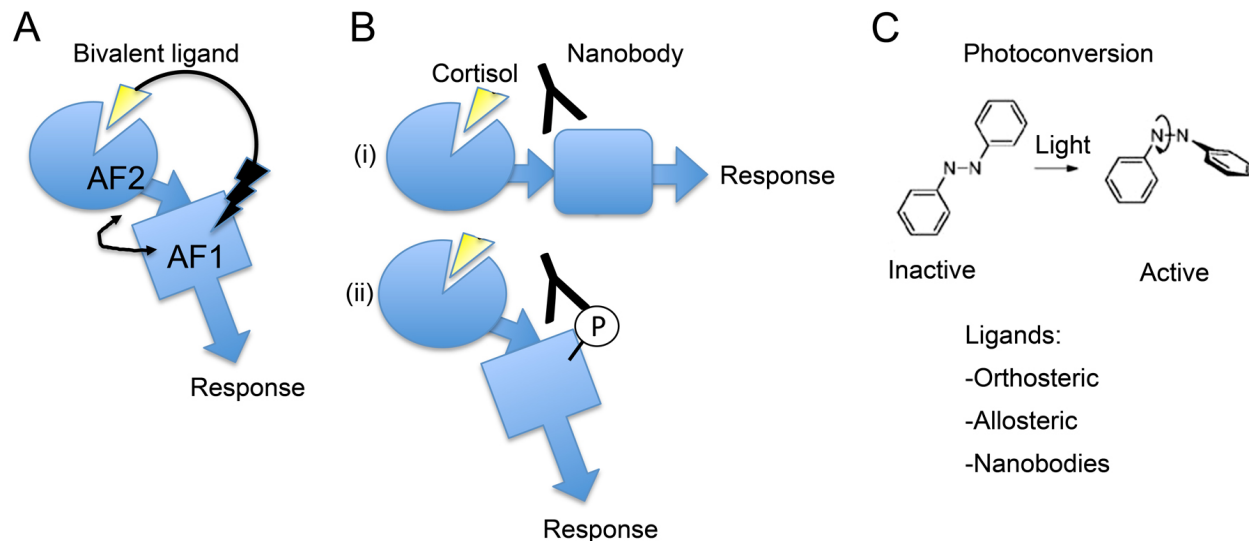


Figure 4. Next generation of glucocorticoid modulators

A. Bivalent ligands could target both the orthosteric and allosteric sites to promote biased signaling.

B. Nanobodies can be selected to promote the activation or suppression of the protein target function, acting at the orthosteric or allosteric sites. Nanobody can be selected to recognize either the (i) non-phosphorylated or (ii) phosphorylated form of the epitope.

C. GR ligands (orthosteric or allosteric) and nanobodies could be conjugated with a photoconvertible scaffold known as photoswitch or photolytic (chemical or protein) to gain temporal and spatial precision of action thereby limiting side effects. Here presented is the red-shifted fast-relaxed azobenzene scaffold but many others exist with distinct attributes.

BOX 1. Key terms

Orthosteric ligands: interact with the pharmacophore of the endogenous ligand.

Allosteric ligands: bind to a site other than the pharmacophore of the natural ligand.

Bivalent ligands: consist of 2 molecules connected by a linker and binding to distinct sites in the same protein or partners proximal in structure.

Cooperativity: occurs when binding to a site influences binding to another.

Biased signaling: is a differential transduction cascade assigned to peculiar receptor conformations