5A). In the DEX-bound GR LBD structure, Q642 is perpendicular to the axis of helix 7 and forms hydrogen bonds with the C-17α hydroxyl group of DEX. Upon binding of MF, the C-17α furoate group pushes Q642 away, bending it nearly 90° into a position parallel to the axis of helix 7 (Figure 5A).

[0196] Since the Q642 orientation is the only large change in the ligand binding pocket upon binding of MF, the inventors mutated Q642 to make it smaller (Q642A), larger (Q642F), hydrophobic (Q642L), or charged (Q642E, Q642K), or to make only a slight change (Q642N). We tested those mutations using either MF or DEX at sub-saturated concentration (MF, 1 nM; DEX, 10 nM). Interestingly, for one mutation (Q642N), DEX activity was nearly abolished, while MF activity remained maximal (Figure 5B). Thus, a single mutation could completely separate the activity of MF from that of DEX. Other mutations caused loss of most of the activity for both DEX and MF; the exception, Q642L, had half the activity with MF but no or very low activity with DEX. The 17α furoate of MF also slightly changed the conformation around M560 and M639, but mutations in those residues did not have same effect as the Q642N mutation (FIGS. 12A-12B).

[0197] To analyze the prominent role of Q642 on recognizing ligands of different potency, the inventors determined full dose-response curves for MF, DEX, and cortisol—representing high, medium, and low potency, respectively—in binding to Q642N in a GR transactivation assay (Figure 5C). For MF, the dose response curve of Q642N was indistinguishable from that of the wild type. For DEX relative to wild type, Q642N caused a large rightward shift of the curve, with the EC50 changing from 7.5 nM to 40 nM, a 5-fold decrease in potency. For cortisol, the Q642N receptor variant was inactive, even at saturation concentration. Thus, the single mutation Q642N has the ability to completely separate ligands of high, medium, and low potency, suggesting that Q642 serves as a sensor that recognizes ligands of different potency. When binding a medium- or low-potency glucocorticoid (e.g., DEX or cortisol), Q642 forms a hydrogen bond with the 17α hydroxyl group to tether the bound ligand in position within the ligand binding pocket. When binding to a highly potent ligand such as MF, Q642 is pushed away by the 17α lipophilic group. This change, coordinated with other small changes caused by ligand binding, perturbs helices 6, 6, and 7, leading to the expansion of the loop between helix 5 and helix 6 and changing the orientation of the C-terminus of the AF2 helix (Figure 1B), resulting in the characteristics of high potency.

[0198] To investigate the exact role of Q642 in binding of different ligands, we tested the ligand binding ability of GR Q642A, for which DEX has almost no transactivation activity at a single unsaturated concentration (Figure 5B). In an *in vitro* binding assay using the cytosol from AD293 cells that expressed either wild-type GR or GR Q642A, the Q642A mutant