Supporting Information

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SI Material and Methods

Protein Preparation, Crystallization, and Data Collection. The 1E9 $Leu^{H47}Trp/Arg^{H100}Trp$ Fab (1E9dm) was produced as described in ref. 4 and stored at 4°C in 20 mM Tris·HCl, 100 mM NaCl, 0.5 mM EDTA, pH 7.2. Crystals were obtained by vapor diffusion at 22°C with a protein concentration of 6.5 mg/ml, using 1.5–1.75 M $(NH_4)_2SO_4$, 0.15 M Na-citrate, 0.01% PEG 20,000, pH 5.5–6.5 as precipitant.

The 1E9dm progesterone complex was prepared by adding 1 μl of saturated progesterone solution (dissolved in EtOH) to 25 μl of a 6 mg/ml protein solution and incubating for 4.5 h at room temperature. Crystals were obtained from 1.5 M $(NH_4)_2SO_4$, 0.15 M Na-citrate, and 0.01% PEG 20,000 at pH 7.5. The 1E9dm 5β-androstane-3,17-dione complex was prepared by adding steroid (dissolved in DMSO) at a final concentration of 20 mM with 10% DMSO, to a 10 mg/ml protein solution and subsequent incubation overnight at 4°C. Crystals grew from 2.0 M $(NH_4)_2SO_4$, 0.15 M Na-citrate, and 0.01% PEG 20,000 at pH 5.5.

Solid Li_2SO_4 was added as a cryoprotectant to the drops containing the Fab crystals, and to the reservoir, and was allowed to dissolve for at least a couple of hours. Crystals were directly flash-cooled from this solution in liquid nitrogen. Data were collected at beamlines 8.2.1 and 8.2.2 at the Advanced Light Source, Lawrence Berkeley National Laboratory, Berkeley, CA, and reduced and scaled with HKL2000 (1) (Table 1).

Structure Determination and Refinement. The 1E9dm apo structure was determined by molecular replacement (MR), using the coordinates of wt 1E9 Fab (PDB entry 1C1E). Solutions for the individual variable and constant domains for one Fab/ASU were found by using PHASER (2). The MR model was subjected to rigid body refinement and restrained all-atom refinement with simulated annealing, using CNS (3). The two engineered mutations in the combining site, several mutations in the constant domains [due to the chimeric nature of the recombinant 1E9 Fab (4)], and two stabilizing mutations that were introduced to increase the expression levels (4) could unambiguously be built into difference electron density maps. The model was further refined by alternating cycles of model building with COOT (5) and refinement with REFMAC5 (6). Waters, sulfates, and a Tris molecule that bound in the combining site were added to the model (Table 1).

The steroid complex structures were determined by rigid body refinement of the final 1E9dm apo structure followed by restrained all atom refinement with simulated annealing. At this stage, pronounced Fo-Fc difference electron density at the 3σ level was clearly defined for each steroid ligand. The models were iteratively refined with COOT and REFMAC5. Waters, sulfates, and the steroid ligands were built into the models in the last stages of refinement. The quality of the structures was checked with WHATCHECK (7) and MOLPROBITY (8) (Table 1).

Structure Analysis. rmsds were calculated with LSQ (9), hydrogen bonds were calculated with HBPLUS (10), van der Waals contacts were calculated with CONTACTSYM (11), and buried molecular surface areas were calculated with MS (12) with a 1.7 Å probe radius and standard van der Waals radii (13). Graphics were produced with PYMOL (14) and MOE (Chemical Computing Group).

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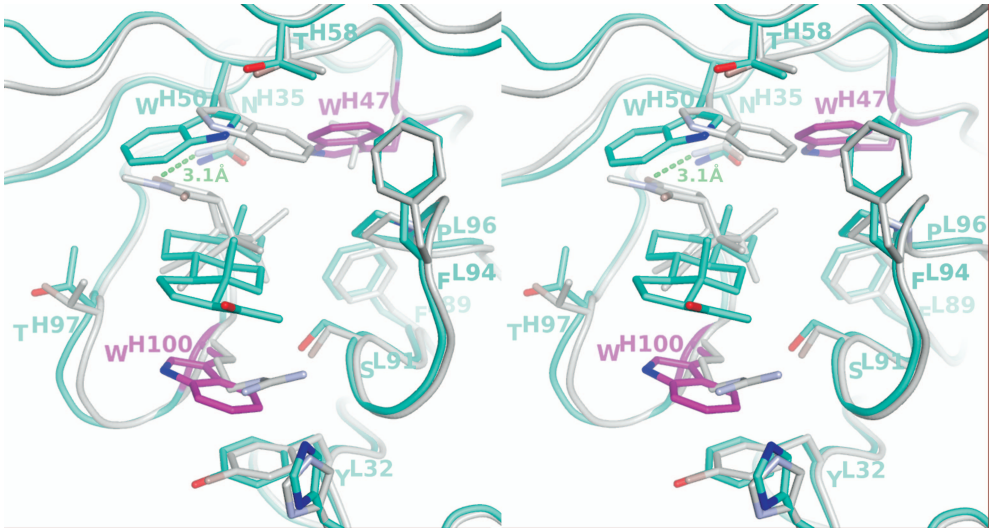

Fig. S1. Wall-eyed stereo representation of the combining site of 1E9dm (Leu^{H47}Trp/Arg^{H100}Trp) bound to progesterone (cyan) superimposed with 1E9 bound to its TSA (gray). This view is approx. 90° rotated around the *z* axis as compared with Figs. S2, S3, and S4 to demonstrate the movement of the Trp^{H50} side chain in 1E9dm caused by the Leu^{H47}Trp mutation.

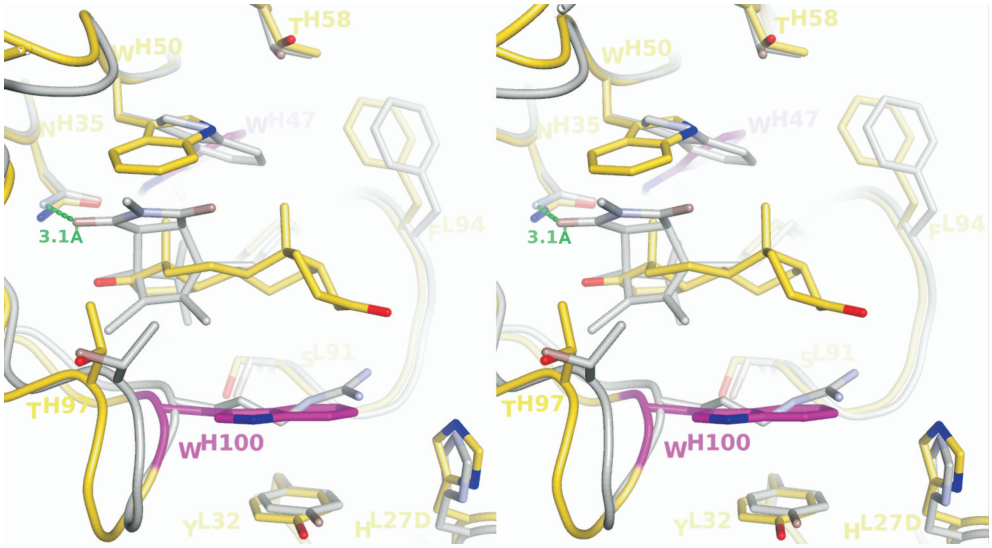

Fig. S2. Wall-eyed stereo representation of the combining site of 1E9dm (Leu^{H47}Trp/Arg^{H100}Trp) bound to 5β-androstane-3,17-dione (orange) superimposed with 1E9 bound to its TSA (gray).

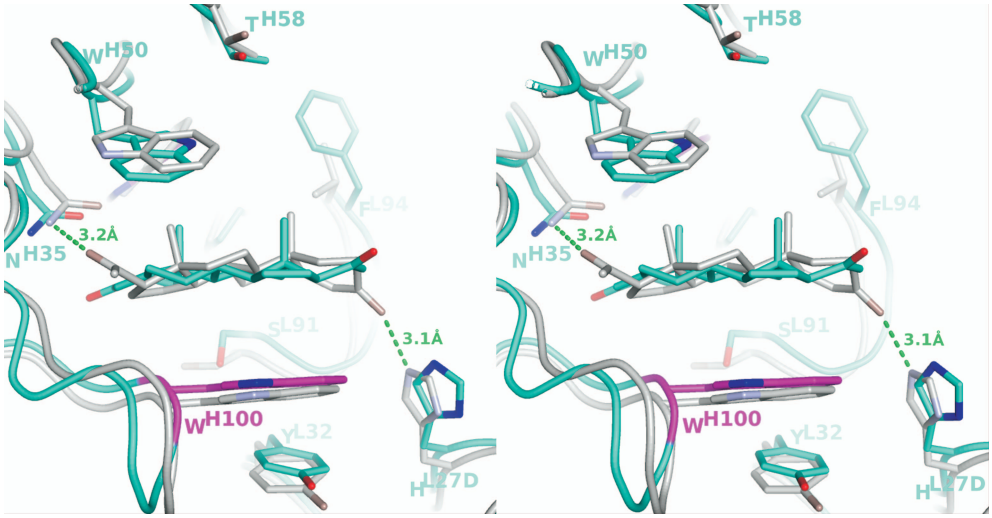

Fig. S3. Wall-eyed stereo representation of the superimposed 1E9dm (Leu^{H47}Trp/Arg^{H100}Trp) (cyan) and DB3 (gray) combining sites with bound progesterone. 1E9dm binds the steroid in an inversed head-to-tail *syn* binding mode with a buried A ring, whereas it is bound in a *syn* orientation with a buried D ring by DB3.

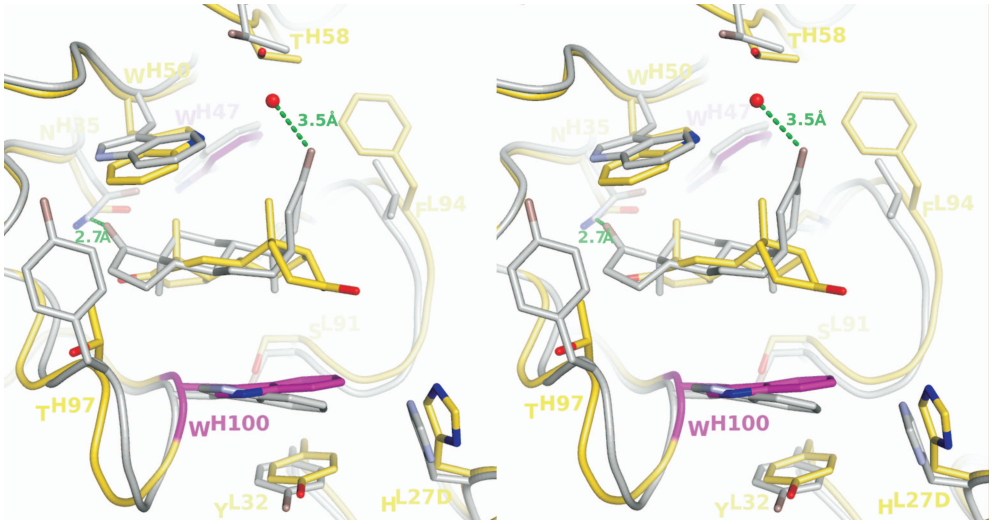

Fig. S4. Wall-eyed stereo representation of the superimposed 1E9dm ($Leu^{H47}Trp/Arg^{H100}Trp$) (orange) and DB3 (gray) combining sites with bound 5β-androstane-3,17-dione. Although 1E9dm and DB3 both bind the steroid with a buried steroid D ring, 1E9dm utilizes a *syn* binding mode and DB3 an *anti* binding mode.