Supporting Materials and Methods

LG335 (1) Synthesis. 3-(1-Carbonyl)propyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthylene 2,5-dimethyl-2,5-hexanediol (5.0 g, 34 mmol) was dissolved in anhydrous benzene (150 ml). $AlCl_3$ (5.0 g, 38 mmol) was added slowly while the mixture was stirred in an ice bath, followed by stirring at room temperature for 1 h. Another portion of $AlCl_3$ (5.0 g, 38 mmol) was then added, and the reaction was heated to 50°C and stirred overnight. The brown solution was poured over iced 0.4 M HCl (50 ml) and extracted with ether (3 × 50 ml). The organic layer was then sequentially washed with water, saturated aqueous $NaHCO_3$, and brine (80 ml each) and dried ($MgSO_4$). The solvent was removed *in vacuo* to afford 6.2 g of a yellow liquid (2).

The crude product was then mixed with propionyl chloride (3.2 ml, 37 mmol), and the resulting solution added dropwise to a mixture of $AlCl_3$ (5.0g, 38 mmol) in dichloroethane (20 ml) while maintaining the temperature between 20°C and 25°C. The mixture was stirred for 2 h at room temperature, at which point it was quenched by pouring carefully over ice. The reaction mixture was then extracted methylene chloride (3 × 10 ml). The organics layers were then combined and washed with water and saturated aqueous $NaHCO_3$, and the volatiles were removed by rotary evaporation. The product was purified by silica gel column chromatography eluting with a hexanes/chloroform ratio of 4:1, then 1:1, to yield 6.9 g (28 mmol, 73%) of product as a yellow oil (3, 4).

3-Propyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthylene. 3-(1-Carbonyl)propyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthylene (1.0 g, 4.1 mmol) in MeOH (10 ml), H_2O (1 ml), and concentrated HCl (3 drops) was treated with 10% Pd/C (144 mg) and subjected to catalytic hydrogenation conditions at 60 psi (1 psi = 6.89 kPa) while heating gently overnight. When the reaction was considered complete (R_f = 0.76, 5% EtOAc in hexanes), it was filtered through a Celite pad and rinsed with MeOH (10 ml) and hexane (50 ml). Water (1 ml) was then added to the filtrate, and the organic phase was separated and washed with brine (2 × 20 ml). The aqueous layer was washed with hexanes (2 × 20

ml). The organic layers were dried (Na_2SO_4) and filtered, and the volatiles were removed by rotary evaporation to produce 510 mg (2.2 mmol, 54%) of a colorless oil (5).

4-[(3-Propyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic Acid (LG335). 3-Propyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthylene (2.2 g, 9.5 mmol) and chloromethyl terephthalate (2.0g, 10 mmol) were dissolved in dichloroethane (20 ml), and $FeCl_3$ (80 mg, 490 μmol) was added. The reaction mixture was stirred at 75°C for 24 h. The reaction was then cooled, and MeOH (20 ml) was added. The resulting slurry was stirred for 7 h at room temperature, filtered, and rinsed with cold MeOH (20 ml) to result in 2.1 g (5.5 mmol, 58%) of white crystals (6).

The crystals (107 mg, 280 μmol) were stirred in MeOH (2 ml), to which 5 M KOH (0.5 ml) was added. This mixture was refluxed for 30 min, cooled to room temperature, and acidified with 20% aqueous HCl (0.5 ml). The MeOH was evaporated, and the residue was extracted with EtOAc (2 × 5 ml). The organic layers were combined, dried ($MgSO_4$), and filtered. The filtrate was treated with hexane (10 ml) and reduced in volume to 2 ml. After standing overnight the resulting crystals were collected to provide 39 mg (103 μmol, 37%) as a white powder (1). mp, 250–252°C; H^1 NMR ($CDCl_3$) δ 0.88 (t, 3H, –$CH_2CH_2CH_3$), 1.20 (s, 6H, CH_3), 1.32 (s, 6H, CH_3), 1.55 (dt, 2H, –$CH_2CH_2CH_3$), 1.69 (s, 4H, CH_2), 2.65 (t, 2H, –$CH_2CH_2CH_3$), 7.20 (s, 1H, Ar–CH), 7.23 (s, 1H, Ar–CH), 7.89 (d, 2H, Ar–CH), 8.18 (d, 2H, Ar–CH); MS electron impact (EI) positive (POS) *m/z* for $C_{25}H_{30}O_3$: Calculated (Calc.) 378.2189, Found 378.2195; Analysis (Anal.) for $C_{25}H_{30}O_3$: Calc. C:79.33, H:7.99, Found C:79.10, H:7.96.

Expression Plasmids. pGBDRXRα was cut with *Sma*I and *Nco*I, filled in, and blunt-end ligated to eliminate 153 aa of the human retinoic acid receptor α (RXR) domain-binding domain (DBD). A *Hin*dIII site in the Trp selectable marker was silently deleted, and the sole remaining *Hin*dIII site was cut, filled in, and blunt-end ligated to remove the restriction site. Unique *Hin*dIII and *Sac*I sites were inserted into the RXR ligand-binding domain (LBD) gene, and *Mfe*I and *Eco*RI sites were removed from the plasmid by using QuikChange Site-Directed Mutagenesis (Stratagene) to create pGBDRXRαL-SH-ME.

pLuc_CRBPII_MCS was made by site-directed mutagenesis from pLucMCS (Stratagene). Site-directed primers were designed to incorporate a cellular retinol-binding protein (CRBP)-II response element in the multiple cloning site (MCS), controlling transcription of the firefly luciferase gene

Plasmid Construction. The zero background plasmid, pGBDRXR:3Stop, was constructed by using QuikChange Site-Directed Mutagenesis with pGBDRXRαL-SH-ME as the template and the 3Stop insertion cassette (described below) as primers.

The 3Stop insertion cassette was synthesized by using PCR from eight oligonucleotides (Fig. 7*a*). All PCRs were done by using 2.5 units of Pfu Polymerase (Stratagene), 1× Pfu buffer (Stratagene), 0.8 mM dNTPs, 50 ng of pGBDRXRαL-SH-ME as a template, 125 ng of primers, and sterile water to make 50 μl. First, four small cassettes were synthesized in reactions containing the following primers: Cassette 1, F (5'-CGGAATTTCC CATGGGC-3'), BP f (5'-CTCGCCGAAC GACCCGGTCA CCGCATGCCA CTAGTGG-3'), and BPr (5'-CCGCTTGGCC CACTCCACTA GTGGCATGCG GTGACC-3'); Cassette 2, BPf, BPr, SEf (5'-CGGGCAGGCT GGAATGAGCT CCTCGACGGA ATTCTCC-3'), and SEr (5'-CAGCCCGGTG GCCAGGAGAA TTCCGTCGAG GAGCTC-3'); Cassette 3, SEf, SEr, AMf (5'-CTCTGCGCTC CATCGGGCTT AAGTGCCCAC CAATTGACAC-3'), and AMr (5'-CTCCAGCATC TCCATAAGGA AGGTGTCAAT TGGTGGGCAC TTAAGC-3'); Cassette 4, AMf, AMr, and R (5'-CAAAGGATGG GCCGCAG-3'). The cassettes were cleaned with either the DNA Clean and Concentrator 5 or the Zymoclean Gel DNA Recovery kit (both from Zymo Research, Orange, CA), depending on product purity. The four cassettes were used to make the final 3Stop insertion cassette in a PCR that contained each cassette, primers F and R, dNTPs, Pfu Polymerase (Stratagene), and sterile water to a final volume of 50 μl. The 3Stop cassette was cleaned by using the Zymoclean Gel DNA Recovery kit.

Insertion Cassette Library Construction. The library of insertion cassettes with randomized codons was constructed in a similar manner to that above. The four cassettes, FBP, BPSE, SEAM and AMR, were made in the following ways (Fig. 7*b*).

For the FBP cassette, oligos BP1 (5'-GGCAAACATG GGGCTGAACC CCAGCTCGCC GAACGACCCG GTCACC-3'), BP2 [5'-GCCCACTCCA CTAGTGTGAA AAGCTGTTTG TC(A, C, or T)(A or G)(C or G)(A, C, or T)(A or G)(C or G)TT GGCA(A, C, or T)(A or G)(C or G)GTT GGTGACCGGG TCGTTCG-3'], BP3 (5'-CTTTTCACAC TAGTGGAGTG GGCCAAGCGG ATCCCACACT TCTCAGAG-3'), and BP4 (5'-GGGGCAGCTC TGAGAAGTGT GGGATCCG-3') were mixed with TE (10 mM Tris•HCl/1 mM EDTA, pH 8.0) containing 100 mM NaCl to bring the total volume to 50 μl. The mixture was heated to 95°C for 1 min, then slowly cooled to 10°C. The annealed mixture was combined with EcoPol buffer (NEB, Beverly, MA), dNTPs, ATP, Klenow fragment of DNA polymerase (NEB), T4 DNA ligase (NEB) and sterile water to 200 μl and kept at 25°C for 45 min before heat inactivation at 75°C for 20 min. The product was cleaned with DNA Clean and Concentrator 5 to make the BP cassette. Next, the BP cassette was combined with Pfu buffer (Stratagene), pGBDRXR:3Stop, oligo F, dNTPs, Pfu Polymerase, and sterile water to make 50 μl for a PCR. The final FBP product (300 bp) was purified by using the Zymoclean Gel DNA Recovery Kit.

The BPSE cassette was made in two consecutive PCRs. First, SE1 (5'-GCAGGCTGGA ATGAGCTCCT C(A, G, or T)(C or T)(G or C)GCCTCC (A, G, or T)(C or T)(G or C)TCCCACC GCTCCATC-3') and SE2 (5'-CCGGTGGCCA GGAGAATTCC GTCCTTCACG GCGATGGAGC GGTGGG-3') were combined with Pfu buffer, dNTPs, Pfu Polymerase, and sterile water to make 50 μl. After five PCR cycles, pGBDRXR:3Stop and BP cassette were added to the reaction, and the PCR was continued for 30 cycles. The product (240 bp) was purified by using the Zymoclean Gel DNA Recovery kit.

The SEAM cassette was constructed in a similar way to the BPSE cassette. SE1 and SE2 were mixed with Pfu buffer (Stratagene), dNTPs, Pfu Polymerase, and sterile water to 25

μl. Simultaneously, AM1 (5'-GGCTCTGCGC TCCATCGGGC TTAAGTGCCT GGAACAT(A, G, or T)(C or T)(G or C)TTSCTTCTTC AAGCTCATCG GGG-3') and AM2 (5'-GCATCTCAAT AAGGAAGGTG TCAATTGTGT GTCCCCGATG AGCTTGAAGA A-3') were combined with Pfu buffer, dNTPs, Pfu Polymerase, and sterile water to 25 μl. After five cycles, these two reactions were mixed, and pGBDRXR:3Stop was added. The PCR was continued for 30 cycles. The PCR product (460 bp) was purified by using the Zymoclean Gel DNA Recovery kit.

The AMR cassette was made similarly to the FBP cassette. AM1 and AM2 were mixed with TE containing 100 mM NaCl to make 50 μl, heated to 95°C for 1 min, then slowly cooled to 10°C. The annealed mixture was combined with EcoPol buffer, dNTPs, Klenow fragment, and sterile water to 200 μl and kept at 25°C for 45 min before heat inactivation at 75°C for 20 min. The product (AM) was precipitated with isopropanol. Next, AM cassette and primer R were combined with Pfu buffer, pGBDRXR:3Stop, dNTPs, Pfu Polymerase, and sterile water to make 50 μl for a PCR. The product (140 bp) was purified by using the Zymoclean Gel DNA Recovery kit.

The four cassettes (FBP, BPSE, SEAM, and AMR) were combined in a PCR to make the library of randomized insertion cassettes (6mutIC). The library was cleaned by using Bio-Spin 30 columns (Bio-Rad).

Yeast Selection Plates and Transformation. Synthetic complete (SC) media and plates were made as previously described (7). Selective plates were made without Trp and Leu (–Trp –Leu) or without Ade, Trp, and Leu (–Ade –Trp –Leu). Ligands were added to the media after cooling to 50°C.

The randomized cassette library was homologously recombined into the pGBDRXR:3Stop plasmid using the following method. pGBDRXR:3Stop was first digested with *Bss*HII and *Eag*I (NEB) and then treated with calf intestinal phosphatase (NEB) to make a vector cassette. This vector cassette (1 μg) and 6mutIC (9 μg) were transformed according to the transformation protocol of Geitz and Woods (8) on a 10×

scale into the PJ69-4A yeast strain, which had previously been transformed with a plasmid (pGAD10BAACTR) (A.B., E. I. Chang, L.J.S., and D.F.D., unpublished data) expressing the nuclear receptor coactivator ACTR fused to the yeast Gal4 activation domain. Homologous regions between the vector cassette and the insertion cassette allow the yeast to homologously recombine the insertion cassette with the vector cassette, forming a circular plasmid with a complete RXR LBD gene. The transformation mixture (1 ml) was spread on each of 10 large plates of SC –Ade –Trp –Leu medium containing 10 μM LG335. The transformation mixture (2 and 20 μl) was also spread on SC –Trp –Leu medium. These plates were grown for 4 days at 30°C.

Molecular Modeling. Docking of LG335 to modified binding pockets was done by using the INSIGHTII module AFFINITY (Accelrys, San Diego). The wild-type RXR with 9-*cis* retinoic acid (9cRA) crystal structure (9) was modified by using the BIOPOLYMER module (Accelrys) "residue replace" tool to make mutations in the binding pocket that corresponded to the mutations in variants I268;I130A;F313A;L436F, I268V;A272V;I310L;F313M, and I268A;I310S;F313A;L436F. The ligand was placed in the binding pocket by superimposing the carboxylate carbon and two carbons in the tetrahydronaphthalene ring of LG335 onto corresponding carbons of 9cRA in the crystal structure. A Monte Carlo simulation was performed first, followed by simulated annealing of the best docked conformations.

Library Evaluation. To evaluate the efficiency of library creation and selection, we take a binary approach: either the sequence is or is not a designed sequence. Eq. **1** is the relevant binomial distribution for statistical evaluation of the libraries.

$$P = \frac{(N-1)!}{(k-1)!(N-k)!} p^k (1-p)^{N-k} \qquad [\mathbf{1}]$$

In Eq. **1**, N is the number of sequenced plasmids, k is the number of background or designed plasmids, p is the frequency of the occurrence of either background or designed plasmid, and P is the measure of certainty. Applying Eq. **1** to the libraries, we conclude

with 95% certainty that the unselected library is at least 72% background and the selected library is at least 78% designed sequences.

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