Evolution as a chemical tool: protein engineering through genetic selection

DF Doyle, B Azizi, LJ Schwimmer, P Rohatgi
School of Chemistry and Biochemistry
Georgia Institute of Technology

Outline

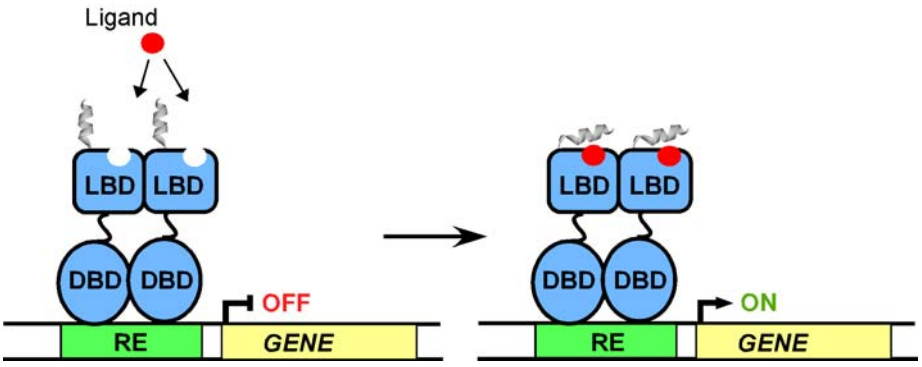
- Principles of evolution
- Nuclear receptors
- Application to gene therapy
- Protein engineering through site-directed mutagenesis
- Protein engineering through genetic selection- “chemical complementation”

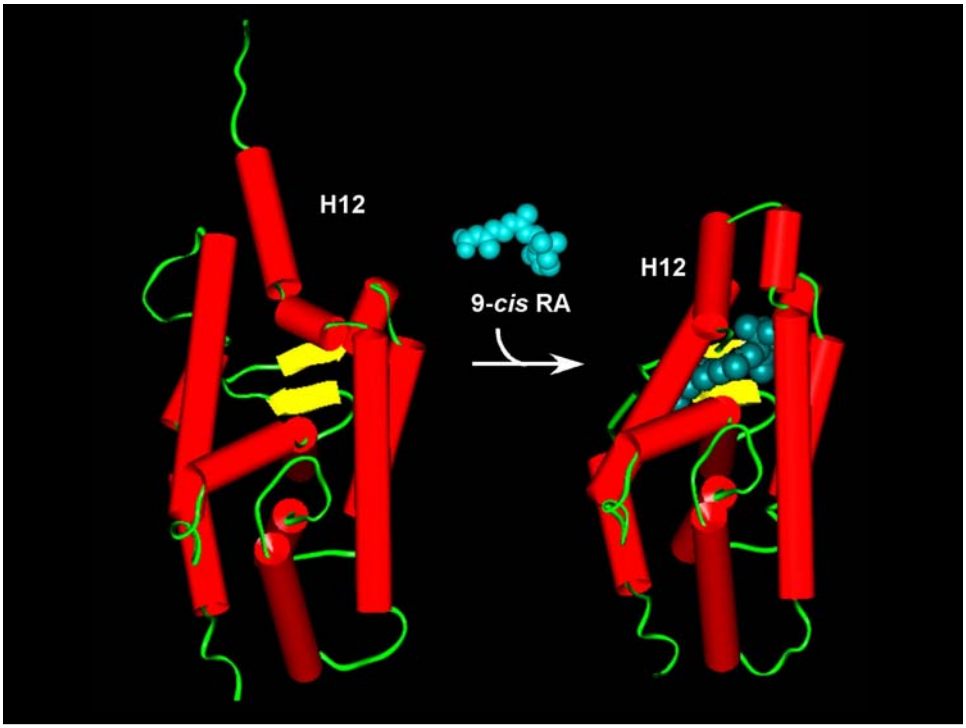
Evolution via Natural Selection

Based on Three Principles:

- Variation (in genes)
- Reproduction
- Competition
  - genetic selection

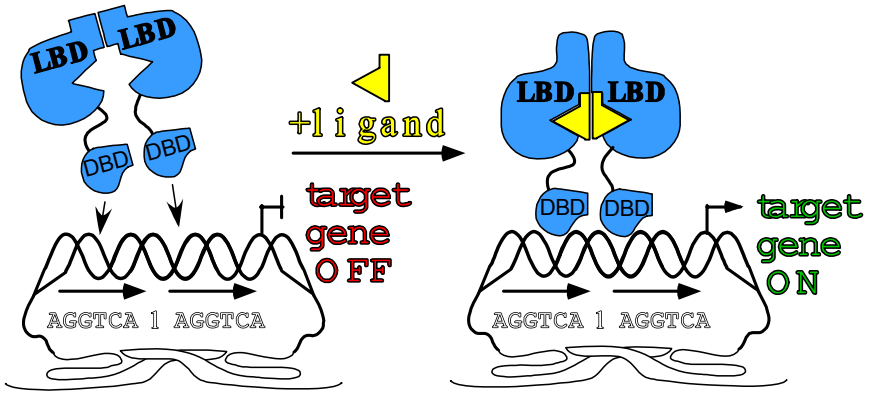
Nuclear receptors are ligand-activated transcription factors





Gene Therapy Application:

Control the expression of a gene at its natural location in the genome

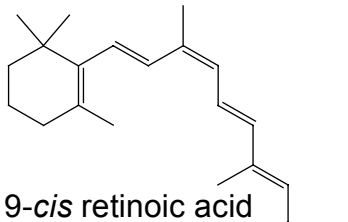


Long-term goal

The ability to engineer a receptor to activate transcription in response to ***any*** small molecule.

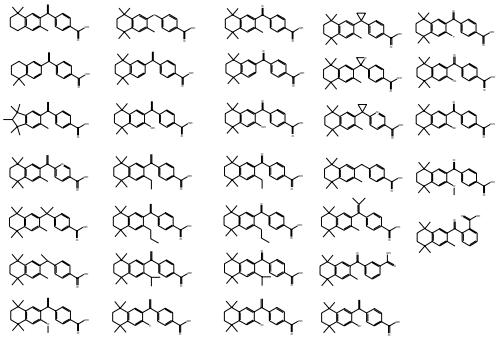
RXR is a nuclear receptor

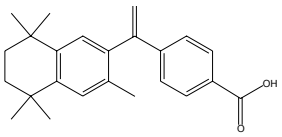
- 9cRA activates RXR



9-*cis* retinoic acid
(9cRA)

Many compounds are synthesized and tested during drug development

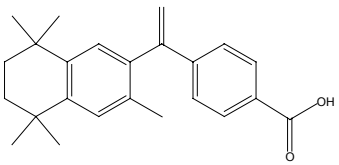


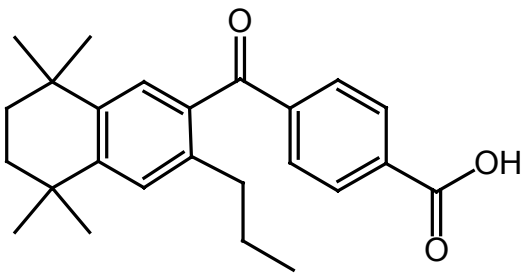


Targretin® (bexarotene)

Many “near-drugs” ⟶ one approved drug compound

The Ligand: LG335



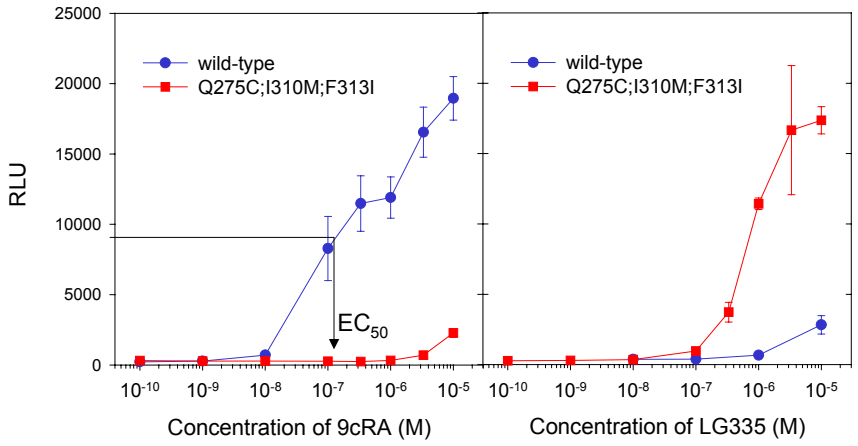


4-[(3-Propyl-5,5,8,8-tetramethyl –5,6,7,8-tetrahydro-2-naphtyl)carbonyl]benzoic Acid (LG335). MS (EI POS) m/z mass for $C_{25}H_{30}O_3$: calc. 378.2195, found 378.2189; Anal. for $C_{25}H_{30}O_3$: calc. C:79.33, H:7.99, found C:79.10, H:7.96

Looking for receptors: the "traditional" approach

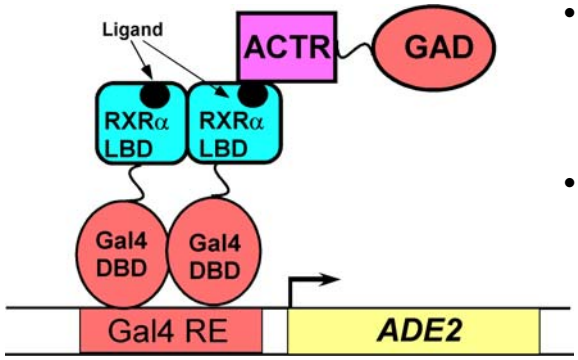
- make mutants (site-directed mutagenesis)
- screen for function

Dose Response Curves: A New Ligand-Receptor Pair



Peet, *et al.*, *Chem Biol* (1998)
Doyle, *et al.*, *JACS* (2001).

Chemical Complementation



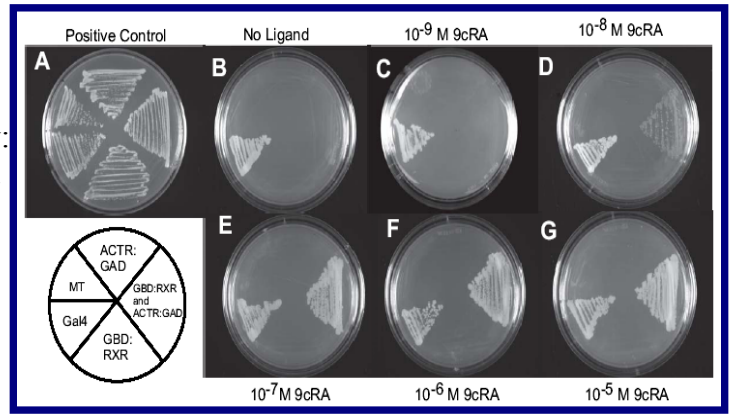
- human RXR and ACTR associate only in the presence of ligand
- expression of *ADE2* allows the yeast to grow on media lacking adenine

Chemical Complementation with wt/RXR + 9cRA

•ZERO background

•High sensitivity: growth occurs at 10 nM

•Large dynamic range: growth comparable to Gal4



Azizi et al., *BBRC,* 2003; submitted.

Protein Engineering

Engineering nuclear receptors for any small molecule

- Transform mutant libraries of nuclear receptors into yeast
- Plate onto media containing small molecules of interest
- Chemical Complementation: only yeast harboring a receptor that activates transcription in response to the small molecule survive (and form a colony)

Protein random mutagenesis perspective

- To completely explore amino acid space in a 100 residue protein requires 20^{100} proteins.
- $20^{100} = 1.3 * 10^{130}$

Protein random mutagenesis perspective

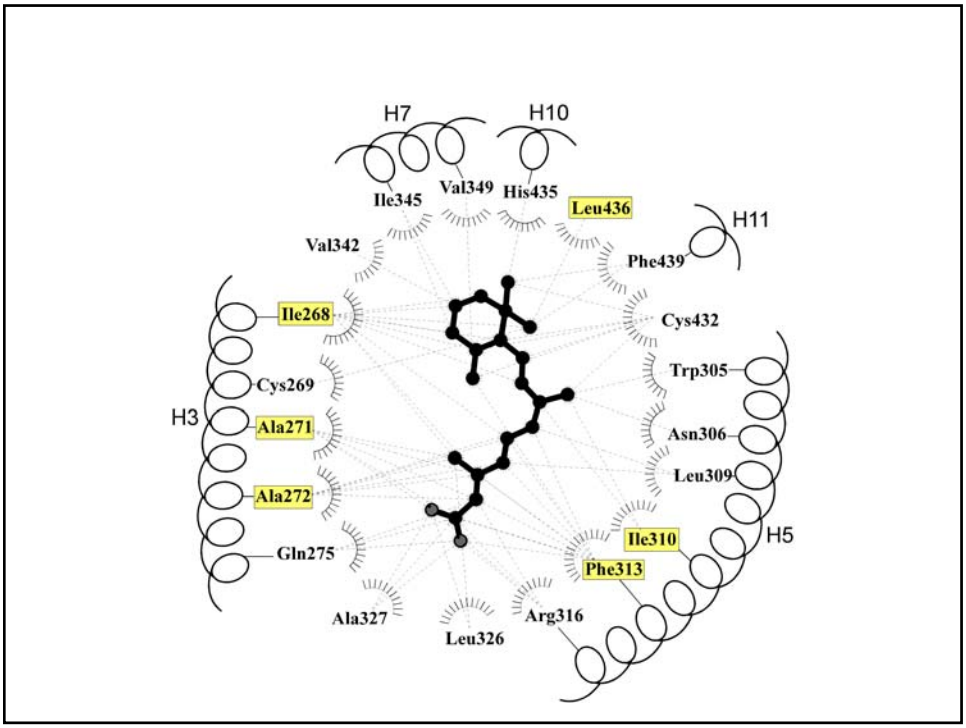
- To completely explore amino acid space in a 100 residue protein requires 20^{100} proteins.
- $20^{100} = 1.3 * 10^{130}$
- There are (only) ~$1 * 10^{57}$ protons in the sun

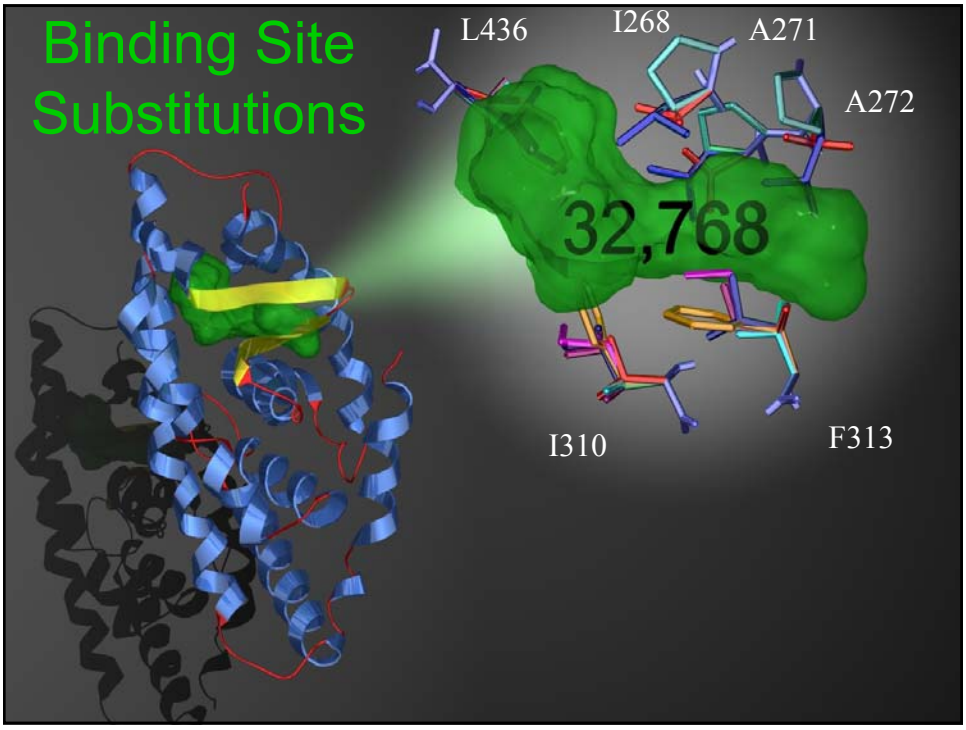
Mutant library design

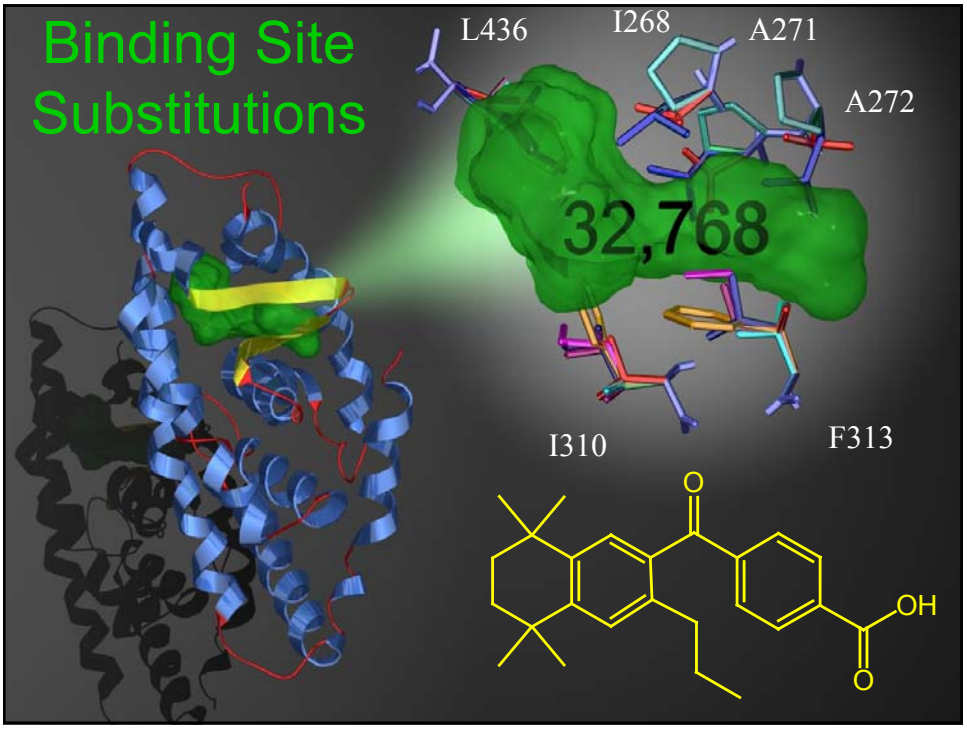
- Hypotheses behind library design:
  - Binding affinity arises from hydrophobic contact
  - Specificity arises from binding pocket size, shape, and hydrogen bonds
- Structure-guided codons to randomize:

I268 → LVAP
A271 → LVAP
A272 → LVAP
I310 → LIVFMAST
F313 → LIVFMAST
L436 → LIVFMAST

- 32,768 possible amino acid combinations


H7
H10
Ile345
Val349
His435
Leu436
H11
Val342
Phe439
Ile268
Cys432
Trp305
Cys269
H3
Ala271
Asn306
Leu309
Ala272
H5
Ile310
Gln275
Phe313
Ala327
Arg316
Leu326


Binding Site
Substitutions
L436
I268
A271
A272
32,768
I310
F313



Results

- ~380,000 member library subjected to chemical complementation with LG335
- ~300 colonies grew

Variant amino acid sequences

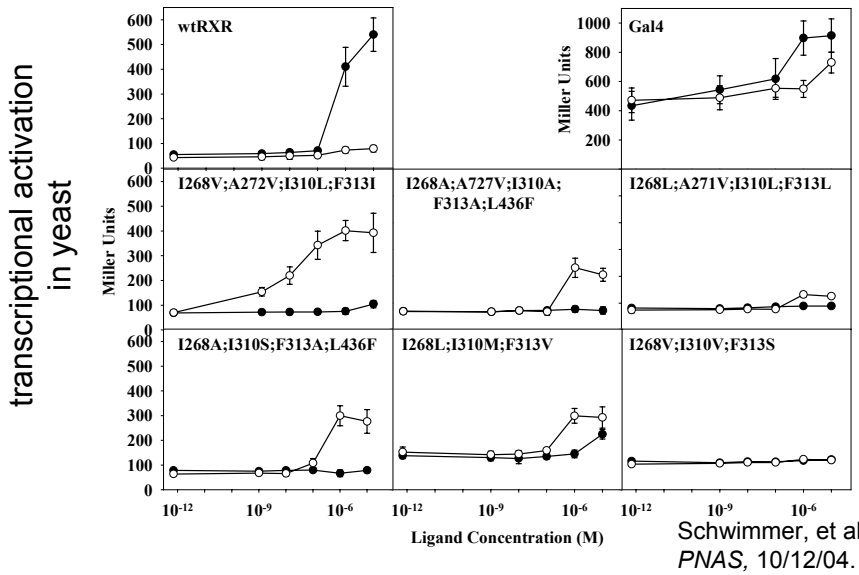
Unselected Library

	I268	A271	A272	I310	F313	L436
1		deleted			deleted	deleted
2		deleted			deleted	deleted
3	V	P	P		S	deleted
4		deleted			deleted	deleted
5		deleted			deleted	A
6		deleted			deleted	deleted
7		deleted			deleted	deleted
8		deleted			deleted	deleted
9		deleted			deleted	F

Selected Library

	I268	A271	A272	I310	F313	L436
1	V		V	L	M	
2	V			V	S	
3	L			M	V	
4	A			S	V	F
5	A			A	A	F
6	A		V	A	A	F
7	L			V	I	
8	L	V		L	L	
9	V	V		L	V	
10	V		V	M	S	M
11	A			M	A	T
12	A			S	A	F
	L V A P			LIVFMSAT		LIVFMSAT

Variants show diverse dose responses



Schwimmer, et al., *PNAS*, 10/12/04.

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Fundamental and Applied
Molecular Evolution Group
www.famecenter.gatech.edu