Supplemental Data

A Three-dimensional Structure of Steroid 21-Hydroxylase (Cytochrome P450 21A2)

with Two Substrates Reveals Locations of Disease-associated Variants

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FIGURE S1. **Sequence alignment of human and bovine CYP21A2.** The amino acid identity is 79%. Note that the bovine residues 7-180 correspond to human residues 6-179; 181-453 in the bovine sequence correspond to 182-454 in the human sequence; 455-484 are the same in bovine and human; residues 487-496 in the bovine sequence correspond to 485-494 in the human sequence.

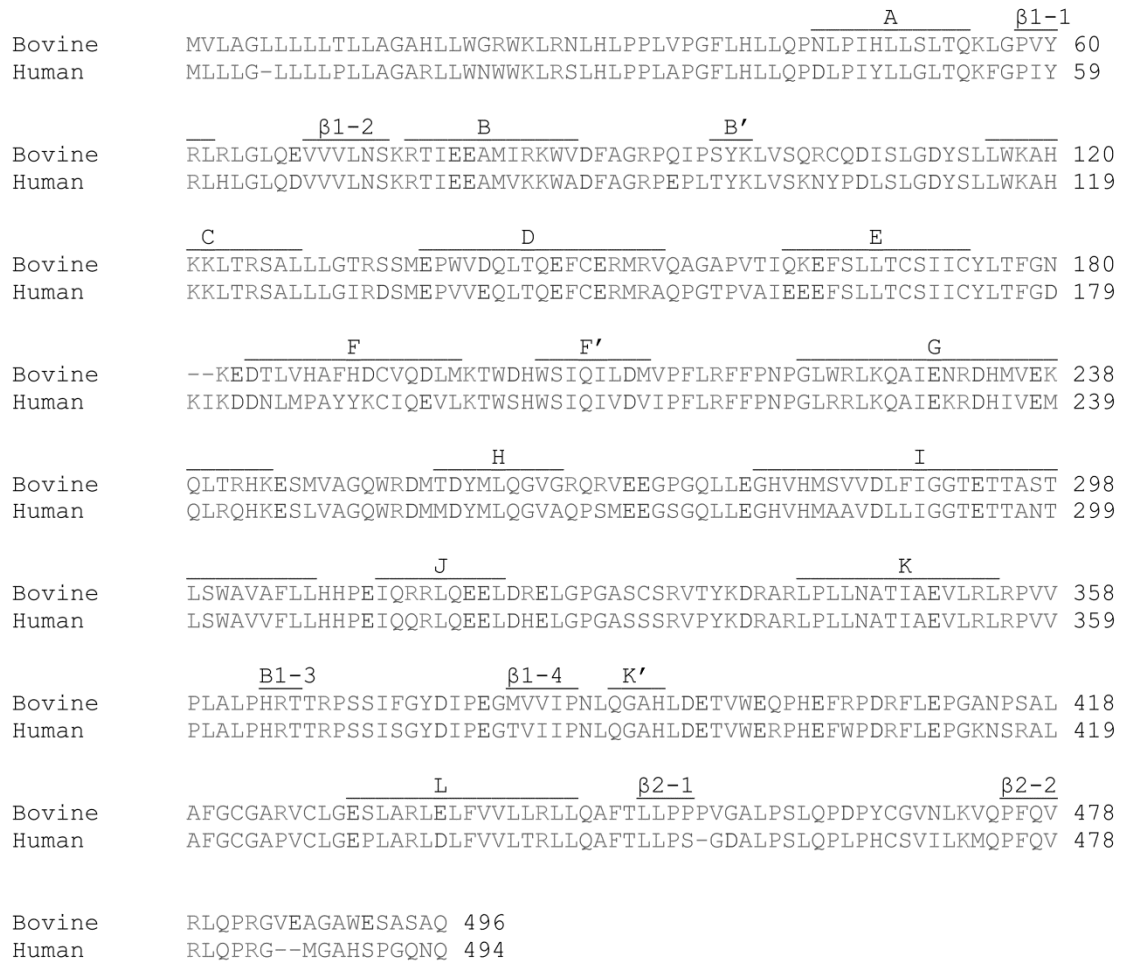


FIGURE S2. **Dynafit iterations for fitting steady-state binding of 17-OH P to CYP21A2.** *B,* Dynafit script for fitting the steady-state titration data (Fig. 6).

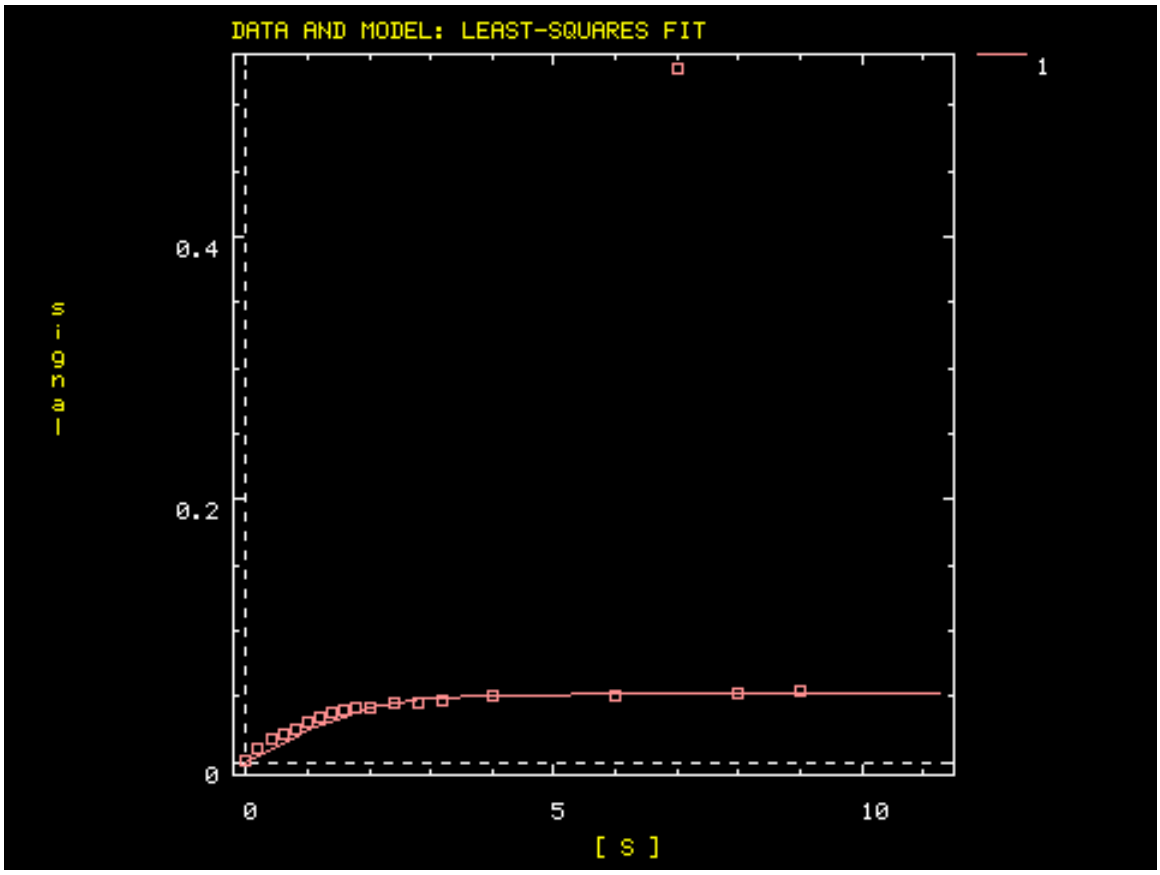
Script as used:

```
; script for plotting Ks results for P450 21A2 binding
;All units in µM
;S = progesterone or 17-OH progesterone, E = P450 21A2
;fit titration data of 21A2 with progesterone or 17-OH ;progesterone using 2-ligand model
; Delta Abs (390-420)vs S
;run 45

[task]

        data = equilibria
        task = fit

[mechanism]

E + S <==> ES       :       K1 dissoc.
ES + S <==> ESS     :       K2 dissoc.

[constants]

K1 = 0.05
K2 = 1?

[concentrations]
E = 2

[responses]
E = 0
S = 0
ES = 0.026
ESS = 0.026

 [sweep]
[equilibria]
variable S = 0,0.2,0.4,0.6,0.8,1.0,1.2,1.4,1.6,1.8,2,2.4,2.8,3.2,4,5,6,7,8,9
file ./scripts/17OHProg.txt

[output]
directory ./projects/21A217OHP/run45

[end]
```

Data:

```
;All units in µM, ΔA390-A420
0.0   0.0015
0.2   0.0101
0.4   0.0178
0.6   0.0217
0.8   0.0259
1.0   0.0299
1.2   0.0335
1.4   0.0374
1.6   0.0397
1.8   0.0420
2.0   0.0418
2.4   0.0445
2.8   0.0460
3.2   0.0475
4.0   0.0505
6.0   0.0510
7.0   0.0527
8.0   0.0533
9.0   0.0535
```

Results:



Program DynaFit version 3.15 [12-21-98]

Execution started Mon Sep 12 13:37:45 2011

SCRIPT FILE

FPG Powerbook:FPG folder:DynaFit:Scripts:P450 21A2 binding45.txt

---

TASK

Fit of complex equilibria

DATA

file :scripts:17OHProg.txt

REACTION MECHANISM

ES <===> E + S : K1 dissoc.
ESS <===> S + ES : K2 dissoc.

THEORETICAL MODEL

Multiple equilibria

Species participating in multiple equilibria

[a] ... E
[b] ... S
[c] ... ES
[d] ... ESS

Stoichiometric matrix

	s p e c i e s			
	[a]	[b]	[c]	[d]
reaction +	---	---	---	---
R1 |	1	1	-1	0
R2 |	0	1	1	-1

Reaction equilibrium constants

K1 ... K1 (dissoc.)
K2 ... K2 (dissoc.)

Formula matrix

element	complex [c]	complex [d]
[a]	1	1
[b]	1	2

Stability constant matrix

constant	complex [c]	complex [d]
K1	-1	-1
K2	0	-1

OUTPUT

LEAST-SQUARES FIT

mean square	0.0118992
standard deviation	0.109083
execution time (min)	0.00723
datapoints	19
parameters	1
iterations	10
subiterations	3
function evaluations	14
error status	1

PARAMETERS & STANDARD ERRORS

Set	Parameter	Initial	Fitted
	K2	1	0.711

COVARIANCE MATRIX

Set	Parameter	Covariances	
	K2	a	a

EIGENVECTORS AND EIGENVALUES

	Eigenvectors
Eigenvalues	1.00
log(C)	0.00

Set Parameter 1
K2 1 100

CONDITION INDICES

Index 0
Set Parameter
K2 100

PLOTS
Best-fit plot: :projects:21A217OHP:run45:tab:fit_01.tab

Execution terminated Mon Sep 12 13:37:59 2011

FIGURE S3. **Stereo view of structure of CYP21A2 with some residues affected by CAH mutations.** The residues in Table 2 are colored cyan. Heme is rendered as a stick model in red. The two substrates (17OHP) are omitted.