

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.

Bovine	MVLAGLLLLLLTLLAGAHLLWGRWKLRLNLHLPPLVPGFLHLLQPNLPIHLLSLTQKLGVPVY	<u>A</u>	<u>β1-1</u>	60	
Human	MLLLG-LLLLPLLAGARLLWNWVKLRSLHLPPLAPGFLHLLQPDLPPIYLLGLTQKFGPIY			59	
Bovine	RLRLGLQE β 1-2	<u>B</u>	<u>B'</u>	120	
Human	RLHLGLQDVVVLNSKRTIEEAMVKKWADFAGRPEPLTYKLVSKNYPDLSLGDYSLLWKAH			119	
Bovine	KKLTRSALLLGRSSMEPWVDQLTQEFCEMRVQAGAPVTIQKEFSLLTCSIICYLTFGN	<u>C</u>	<u>D</u>	<u>E</u>	180
Human	KKLTRSALLLGIRDSMEPVVEQLTQEFCEMRMQPGTPVAIEEFSLLTCSIICYLTFGD			179	
Bovine	--KEDTLVHAFHDCVQDLMKTWDHWSIQILDMPFLRFFPNPGLWRLKQAIENRDHMVEK	<u>F</u>	<u>F'</u>	<u>G</u>	238
Human	KIKDDNLMPAYYKCIQEV β 1-2			239	
Bovine	QLTRHKE β 1-2	<u>H</u>	<u>I</u>	298	
Human	QLRQHKE β 1-2			299	
Bovine	LSWAVAFLLHHPEIQRR β 1-2	<u>J</u>	<u>K</u>	358	
Human	LSWAVVFLHHPEIQRR β 1-2			359	
Bovine	PLALPHRTTRPSSIFGYDIPEGMVVIPNLQGAHLDET β 1-2	<u>B1-3</u>	<u>β1-4</u>	<u>K'</u>	418
Human	PLALPHRTTRPSSISGYDIPEGTVIIPNLQGAHLDET β 1-2			419	
Bovine	AFGCGARVCLGESLARLELFVVLRLRLQAFTLLPPPVGALPSLQDPYCGVNLKVQPFQV	<u>L</u>	<u>β2-1</u>	<u>β2-2</u>	478
Human	AFGCGAPVCLGEPLARLDLFFVLRLLQAFTLLPS-GDALPSLQPLPHCSVILKMQPFQV			478	
Bovine	RLQPRGVEAGAWESASAQ			496	
Human	RLQPRG--MGAHSPGQNG			494	

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  $\mu\text{M}$ 
;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 dissoci.
ES + S <==> ESS     :      K2 dissoci.
```

[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]

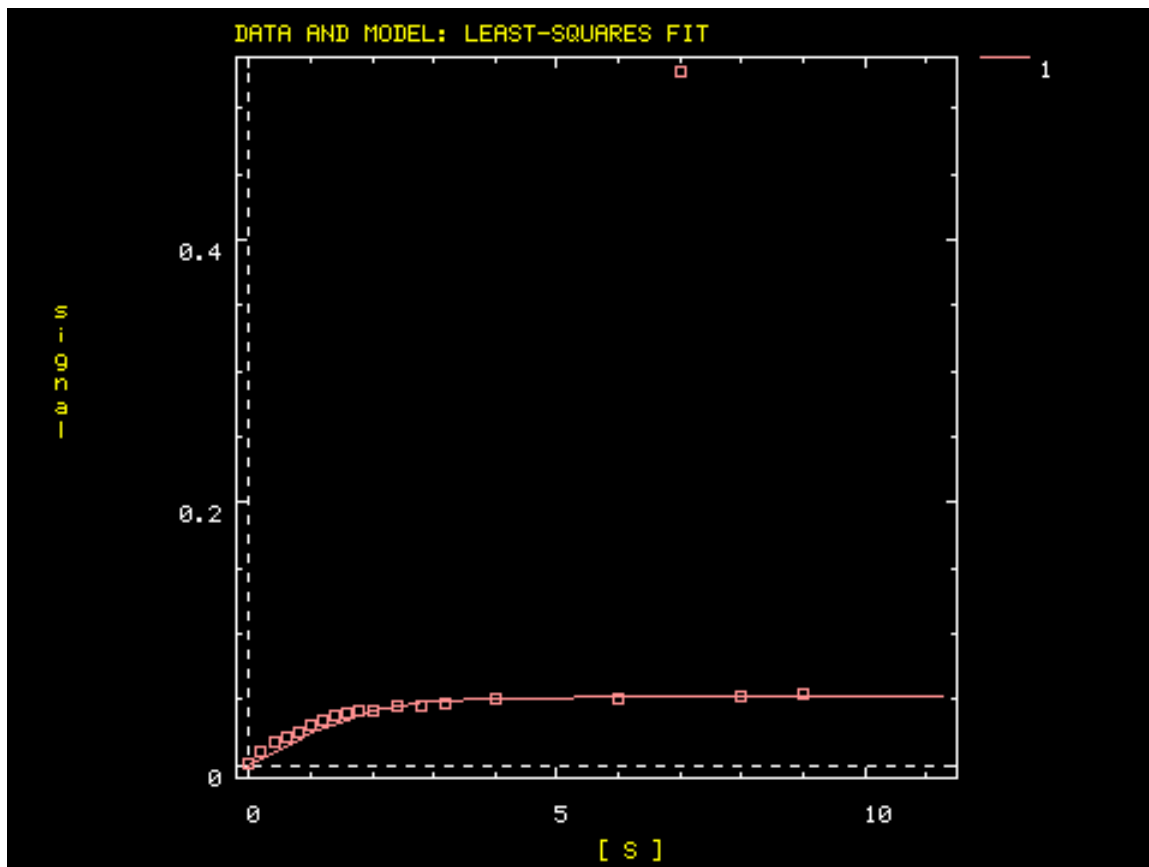
```
directory ./projects/21A217OHP/run45
```

[end]

Data:

```
;All units in  $\mu\text{M}$ ,  $\Delta\text{A}390\text{-A}420$   
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 dissociation.

ESS <====> S + ES : K2 dissociation.

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

```

      c o m p l e x
      [c] [d]
element + --- ---
      [a] | 1 1
      [b] | 1 2

```

Stability constant matrix

```

      c o m p l e x
      [c] [d]
constant + --- ---
      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.

