Characterization of interactions among CYP1A2, CYP2B4, and NADPH-cytochrome P450 reductase: Identification of specific protein complexes

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**Drug Metabolism and Disposition (DMD # 78642)**

***Supplemental Table 1 – Information on the GFP, Rluc, and WT P450 System Protein Constructs***

***Supplemental Figure 1 – Dynafit 4 script used to predict both 7-ethoxyresorufin-O-dealkylation (EROD) and 7-pentoxyresorufin-O-dealkylation (PROD) when CYP1A2 and CYP2B4 exist as functional monomers that simply compete for limiting concentrations of NADPH-cytochrome P450 reductase (CPR)***.

***Supplemental Table 1 – Information on the GFP, Rluc, and WT P450 System Protein Constructs***

| Construct | Parent vector | 5' restriction site | 3' restriction site | Sequence identifier | Peptide linker |
|---|---|---|---|---|---|
| CYP1A2-GFP | pGFP$^2$-N1 | EcoRI | BamHI | NM_001171121.1:29-1576 | WIPPVAT |
| CYP1A2-Rluc | pRluc-N2 | EcoRI | BamHI | NM_001171121.1:29-1576 | WIPTGAT |
| Unlabeled CYP1A2* | CYP1A2-GFP | EcoRI | | NM_001171121.1:29-1579 | |
| CYP2B4-GFP | pGFP$^2$-N1 | NheI | EcoRI | NM_001170859.1:1-1473 | EFSRVCRISSLRYRGPGIPPVAT |
| CYP2B4-Rluc | pRluc-N2 | NheI | EcoRI | NM_001170859.1:1-1473 | EFSRVCRISSLRYRGPGIPTGAT |
| Unlabeled CYP2B4* | CYP2B4-GFP | NheI | | NM_001170859.1:1-1476 | |
| POR-GFP | pGFP$^2$-N3 | EcoRI | HindIII | NM_001160290.1:40-2076 | KLAVPRARDPPVAT |
| POR-Rluc | pRluc-N1 | EcoRI | HindIII | NM_001160290.1:40-2076 | KLAVPRARDPTGAT |
| Unlabeled POR* | POR-GFP | EcoRI | | NM_001160290.1:40-2079 | |

Vector information. Vectors for the expression of labeled proteins were generated by ligating full-length cDNA into the empty BRET vectors shown above. The primers used to amplify the DNA of interest included the indicated restriction sites immediately flanking the coding sequence except for CYP1A2-GFP and CYP1A2-Rluc, for which extra thymidine residues were included between the CYP1A2 CDS and the BamHI site to ensure the tag was in frame. The 3' primers did not include a stop codon.

Each tagged construct had DNA between the 3' restriction site and the GFP or Rluc start codon that resulted in the expression of short peptide linker between the protein of interest and its tag.

To generate vectors for the expression of wild type, unlabeled protein, site directed mutagenesis was used to introduce a stop codon to the immediate 3' end of the GFP-tagged protein's CDS, destroying the 3' restriction site. For CYP1A2 TGG was changed to TGA, for CYP2B4 GAA was mutated to TAA, and for POR AAG was replaced with TAG.

***Supplemental Figure 1 – Dynafit 4 script used to predict both 7-ethoxyresorufin-O-dealkylation (EROD) and 7-pentoxyresorufin-O-dealkylation (PROD) when CYP1A2 and CYP2B4 exist as functional monomers that simply compete for limiting concentrations of NADPH-cytochrome P450 reductase (CPR)***. The terms used are defined as: CYP1A2 "A"; CYP2B4 "B"; CPR, "R"; substrate "S". $K_{ar}$ (the dissociation constant for $K_D^{CPR•CYP1A2}$**)** was 0.08 μM [11;28], and $K_{br}$ (the $K_D^{CPR•CYP2B4}$) was 0.01μM [11;23]. $K_{as}$, and $K_{ars}$ are the dissociation constants for substrate binding to CYP1A2, and CPR•CYP1A2, respectively. $K_{bs}$, and $K_{brs}$ are the dissociation constants for substrate binding to CYP2B4, and CPR•CYP2B4, respectively. The rate constants for product formation by the CPR•CYP1A2•S and CPR•CYP2B4•S are "$k_{ars}$ and $k_{brs}$, respectively. The values for $k_{ars}$ and $k_{brs}$ for PROD were 0.38/min and 1.25/min, and were determined from the single-transfection systems. The values for $k_{ars}$ and $k_{brs}$ for EROD were 8.67/min and 0.015/min, and were determined from the single-transfection systems. This script is based on the following assumptions: (a) Both CYP1A2 and CYP2B4 behave as functional monomers that compete for limiting CPR. (b) The binding of substrate does not significantly affect the affinity of CPR•CYP1A2 or CPR•CYP2B4. (c) The binding of CPR to either P450 does not affect the affinity of substrate for either P450. (d) The substrate concentration is saturating.

```
; Simple competition between two P450s for Limiting CPR. (Dynafit 4)
; Predicted activities for CYP1A2 and CYP2B4 when competing for limiting CPR.

[task]
        data = velocities
        task = fit
        approximation = rapid-equilibrium
        model = michaelis menten

[mechanism]
        A + R <===> A.R    :  Kar    dissoc.
        A + S <===> A.S    :  Kas     dissoc.
        B + R <===> B.R          :  Kbr    dissoc.
        B + S <===> B.S    :  Kbs    dissoc.
        A.R + S <===> A.R.S  : Kars   dissoc.
        A.S + R <===> A.R.S  : Kar   dissoc.
        B.R + S <===> B.R.S  : Kbrs   dissoc.
        B.S + R <===> B.R.S  : Kbr   dissoc.
        A.R.S --> P + A.R : kars
        B.R.S --> P + B.R : kbrs

[constants]
        Kar = 0.08
        Kas = 0.2
        Kars = 0.2
        Kbr = 0.01
        Kbs = 0.2
        Kbrs = 0.2
        kars = 8.67
        kbrs = 0.015

[concentrations]
        S = 20

[responses]
```

```
        P = 1.0

[data]
        directory ./interaction
        sheet P45012.txt
        variable        R
        file P45012.txt | concentration A = 0.051, B = 0.0454

[output]
        directory ./interaction/output/models
[end]
```