Expression of Selectively Labeled P450_{cam}

1. Selective labeling with $^{13}$C-Proline ($^{13}$C-Pro, U-$^{15}$N, $^2$H-CYP101):
   Strain: *E. coli* NCM533 (proline auxotrophic and Kan$^R$)

*Growth medium*

To 1 kg of D$_2$O (903 ml, Cambridge Isotope Lab (CIL) #DLM-4-1000), add

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$_2$HPO$_4$</td>
<td>6.14 g</td>
</tr>
<tr>
<td>KH$_2$PO$_4$</td>
<td>2.71 g</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.47 g</td>
</tr>
<tr>
<td>$^{15}$NH$_4$Cl</td>
<td>1 g</td>
</tr>
<tr>
<td>MgSO$_4$</td>
<td>0.22 g</td>
</tr>
<tr>
<td>CaCl$_2$</td>
<td>8 mg</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>25 mg</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>45 mg</td>
</tr>
<tr>
<td>D$_8$-glycerol</td>
<td>5 g</td>
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<tr>
<td>$^{13}$C-proline</td>
<td>0.1 g</td>
</tr>
</tbody>
</table>

Filter to sterilize, and then add the following sterile additives:

- 0.1 M FeCl$_3$ 90 µl
- Perdeuterated P1 metal mix* 1.8 ml
- 2% thiamine 11 µl

*: made by drying the water containing P1 metal mix and redissolve the salts into D$_2$O.

*Growth procedure*

**Fresh transformants:** the plasmid pDNC334A was electroporated into *E. coli* NCM533; the transformation plate is incubated at 37°C overnight.

**Starter culture:** inoculate a single colony from above transformation plate into 5 ml LB containing appropriate antibiotics and incubate at 37°C till OD$_{600}$ reaches 0.6.

**Scale up:** transfer the starter culture to 120 ml fresh LB medium with antibiotics and incubate at 37°C with shaking till OD$_{600}$ reaches 0.6.

**Transfer to H$_2$O-M9+ medium:** spin down the cell with centrifugation at 6k rpm in a sterile centrifuge bottle, resuspend the cell pellet to 700 ml M9+ medium, incubate at 37°C with shaking till OD reaches 1.0.

**Transfer to D$_2$O-based isotopic M9+ medium:** spin down the cell with centrifugation at 6k rpm in sterile centrifuge bottles (carry-over of H$_2$O solutions should be avoided as much as possible). Resuspend cell pellets in the D$_2$O-based isotopic M9+ medium (~900 ml), continue incubation at 37°C with shaking till OD reaches 1.0.

**IPTG Induction:** add 70 mg δ-aminolevulinic acid to the culture and incubate for 30 min; add 0.214 g solid IPTG (final concentration: 1 mM) and 0.7 mg solid camphor (final concentration: 5 µM) to induce protein expression in the presence of camphor. Continue to incubate at 37°C with shaking for 12 hrs to 24 hrs till the culture exhibits orange color.

**Storage:** Spin down the cell, measure the weight of cell pellet (normally 1 liter of cell culture will resulted in ~5 g of cell paste), and store at −70°C till ready to purify.
2. Selective labeling with $^{15}$N-Val (same procedure for $^{15}$N-Ile, Leu, Phe labeling)
Strain: *E. coli* BL21(DE3)

*Growth medium (per liter)*
- 5× M9 salts: 200 ml
- P1 metal mix: 2 ml
- 1 M MgSO$_4$: 2 ml
- 1 M CaCl$_2$: 2 ml
- 50 mg/ml Kanamycin: 1 ml
- 0.1 M FeCl$_3$: 0.1 ml
- 2% thiamine: 12.5 µl
- 20% glucose: 15 ml
- Amino acid mix (containing 0.1 mg for each amino acid except Val): 150 ml

*Growth procedure*

**Fresh transformants:** the plasmid pET-P450$_{cam}$ was electroporated into BL21(DE3); the transformation plate is incubated at 37°C overnight.

**Starter culture:** inoculate a single colony from above transformation plate into 5 ml LB containing appropriate antibiotics and incubate at 37°C till OD$_{600}$ reaches 0.6.

**Scale up:** transfer the starter culture to 50 ml fresh LB medium with antibiotics and incubate at 37°C with shaking till OD$_{600}$ reaches 0.6.

**Transfer to M9+ medium:** spin down the cell with centrifugation at 6k rpm in a sterile centrifuge bottle, resuspend the cell pellet in 50 ml M9+ medium, and incubate at 37°C with shaking for 2 hrs.

**Scale up in M9+ medium:** transfer the 50 ml culture in M9 into 1 liter M9+ medium with above recipe, continue incubation at 37°C with shaking till OD reaches 0.8.

**Add heme precursor, labeled amino acid, and IPTG Induction:** add 70 mg δ-aminolevulinic acid and 0.1 g $^{15}$N-Val to the culture and incubate for 30 min; add 1 ml IPTG (1 M) and 1 ml camphor (5 mM) to induce protein expression in the presence of camphor. Continue to incubate at 37°C with shaking for 12 hrs. The cell color should be orange indicating well-expressed P450$_{cam}$.

**Storage:** Spin down the cell, measure the weight of cell pellet (normally 1 liter of cell culture will resulted in ~5 g of cell paste), and store at −70°C till ready to purify.