P450 CYCLE

All P450s follow the same catalytic cycle of;

1. Initial substrate binding
2. First electron reduction
3. Oxygen binding
4. Second electron transfer
5 and 6. Proton transfer/dioxygen cleavage

- P450s contain a range of conserved structural elements needed for the common processes of enzyme reduction and dioxygen binding and activation.

- Substrate selectivity is the result of numerous hypervariable regions distributed throughout the protein that make up the substrate binding cavity.
1. Microsomal P450s – typically metabolize drugs and other xenobiotics
2. Cytosolic P450s – soluble bacterial forms with tight substrate specificities
3. Mitochondrial P450s – typically metabolize steroids (endogenous compounds)
Structural Features of Cytochrome P450s – COMMON FOLD

- Typical ‘heart shaped’ P450 protein
- β-sheet-rich (copper arrows) N-terminus; α-helix-rich (blue coils) C-terminus
- Active site area above the heme bounded by I-helix and F-G domain
- Substrate binding cavity shown in green.
- Heme center (red) buried in lipophilic core between I- and L-helices

- **F-G helix domain** lies perpendicular to I-helix and forms a ‘lid’ to the active site which lies distal to the heme.
**HEME BINDING POCKET**

- **P450 signature sequence** - FxxGxxxCxG

- **Propionate groups** neutralized by hydrogen bonding to Arg, His, Tyr residues
- **Methyl and vinyl side-chains** intercalated between hydrophobic residues
- Several CYP4 enzymes have the unusual property of a covalently-bound heme that is attached to the protein backbone by an ester link between an I-helix glutamate and the C-5 methyl group of the heme
• Formation of P450 Compound I (Nature’s blowtorch) requires ordered proton transfer.

• The input of protons is controlled by the proton transfer groove, a highly conserved area of the I-helix immediately adjacent to the heme iron.

• Unified motif for proton transfer groove is; {ASTG}{ASTG}{DEQN}T{ASTG}

• Of special importance is the invariant Thr residue and an adjacent Acid/Amide residue (sometime referred to as the alcohol-acid pair, that together funnel protons to the external oxygen of the O-O bond to catalyze heterolytic scission and formation of P450 Compound I.
Although this has been well worked out for the soluble bacterial P450, P450 cam, it is less clearly established in mammalian P450s.

Nonetheless, a common device used by researchers working with animal and human forms of P450 is to mutate the conserved Thr to an Ala, e.g. T302A in CYP2B4, making the assumption that the P450 cycle will be backed up in the mutant so that at any time there will be a lower concentration of the high valent iron-oxo species and increased levels of the hydroperoxo-iron precursor(s).

Often this is used to make conclusions about what types of reactions are catalyzed by the different forms of activated P450 species.

Davydov et al., JACS 123:1403-1415 (2001)
Vaz et al., PNAS 93:4644-8 (1996); PNAS 95:3555-60 (1998)
P450 REDUCTION

- Two electrons are provided by the cofactor NADPH.

- Electrons are transferred to the enzyme, **one at a time**, via the co-enzyme cytochrome P450 reductase.

- In some cases, cytochrome b5 inputs the second electron.

- Enzymes all have a largely cytosolic topology.

- Both co-enzymes appear to interact with P450 via electrostatic interactions with basic residues on the proximal face of the P450; the C-D helices and the K-helix, primarily.
Membrane Orientation, Substrate Access and Product Release

• As noted earlier, nearly all P450s sit on top of ER lipid bilayer, facing the cytoplasm.

• Only the hydrophobic N-terminus is ‘buried’ in the membrane.

• The F-G domain also contacts the membrane.

• The F-G domain together with the B-C loop appear to be the most likely ‘gates’ to substrate access from the membrane.

• However, multiple ingres/egress channels into the cytosol may exist.

• N.B. CYP2W1 – a glycosylated, colon tumor-specific displays the opposite (lumenal) orientation! [Gomez et al., Mol. Pharmacol (2010).]
How do P450s discriminate between different ligands?

- Clearly, physiochemical features of the ligand play an important role in determining P450 isoform specificity.

- A ligand will show high selectivity for a particular P450 isoform when the ligand’s size, shape and charge are complementary to the active site features of that particular P450.

- All important human drug metabolizing P450s have now been crystallized; i.e. CYP3A4, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP1A2 and CYP2A6 providing some insights into their ligand selectivities.