

When human cytochrome P450s were first expressed in *E. coli*, the N terminal amino acid sequence was modified to enable the bacteria to express the protein [Richardson, TH *et al. Arch. Biochem. Biophys.* **323** (1995): 87-96, Gillam, EMJ *et al. Arch. Biochem. Biophys.* **305** (1993): 123-131].

For example, original method:

CYP3A4	Native	MALIPDLAMETWLLLAVSL
	Expressed	MA-----LLLAVFL
		Ten amino acids deleted and one changed

Instead of altering the amino acid sequence, which may well be involved in interacting with NADPH P450 reductase, Cypex adds a cleavable bacterial leader sequence (ompA) to the protein to enable expression in *E. coli*. [Pritchard, MP *et al Arch. Biochem. Biophys.* **345** (1997): 342-354, Pritchard, MP *et al Pharmacogenetics* **8** (1998): 33-42]

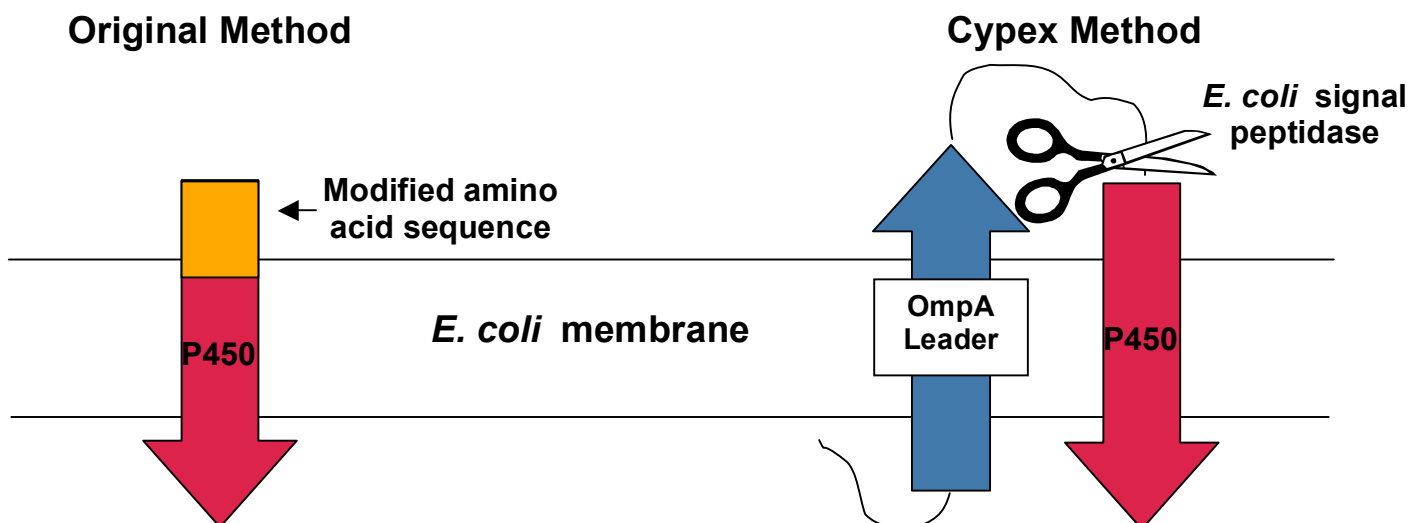
For example, Cypex's patented method:

CYP3A4 **[ompA] APMALIPDLAMETWLLLAVSL**

↑

The leader sequence is cleaved (at the position indicated) from the protein by an endogenous signal peptidase, releasing the P450 into the *E. coli* membrane. In some cases, we add two extra amino acids to the N terminal end of the protein (in red above) to ensure that the leader sequence is removed by the signal peptidase. These two amino acids are retained on the cytochrome P450.

In summary:



Does it make a difference? Yes...

Testosterone turnover by human CYP3A4
(nmol/min/nmol P450)

Modified (original method)	Cypex method
24 ± 2	42 ± 2

Bufuralol turnover by human CYP2D6
(nmol/min/nmol P450)

Modified (original method)	Cypex method
1.7 ± 0.1	5.7 ± 0.2