Effects of polymorphic variation on the thermostability of heterogenous populations of CYP3A4 and CYP2C9 enzymes in solution

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SUPLEMENTARY INFORMATION

Table S1 Primers used to introduce single point mutations into CYP3A4 and CYP2C9 genes within the pBJW102.2 vector by inverse PCR

Mutation	Change	Forward Primer (5'-3')	Reverse Primer (5'-3')	T_A (°C)
3A4				
G56D	224 G>A	ACTTTTGTATGTTTGACATGGAATG	CCTTATGGTAGGACAAAATATTTCC	51
K96E	343 A>G	GAAGAATGTTATTCTGTCTTCAC	CACTAGCACTGTTTTGATCATGTC	54
P218R	710 C >G	GATTCTTTCTCTCAATAACAGTCT	GATCCAAAAAATCAAATCTTAAAAG	47
S222P	721 T>C	CCAATAACAGTCTTTCCATTCCTC	GAGAAAGAATGGATCCAAAAAATC	51
I223R	725 T>G	GAACAGTCTTTCCATTCCTCATC	TTGAGAGAAAGAATGGATCCAAA	54
L293P	935 T>C	CGGAGCTCGTGGCCCAATCAATTA	GATCGGACAGAGCTTTGTGGGACT	62
L373F	1174 C>T	TTTGAGAGGGTCTGCAAAAAAGATG	TCTCATAGCAATTGGGAATAATCTG	55
M445T	1391 T>C	CGAGGTTTGCTCTCATGAACATG	TGCCAATGCAGTTTCTGGGTCCAC	70
2C9				
V76M	271 G>A	ATGCTGCATGGATATGAAGCAGTG	CACTATGGGTTTCAGGCCAAAATA	57
R132Q	440 G>A	AGAATTTTGGGATGGGGAAGAGG	GCAGCGTCATGAGGGAGAAACG	62
R150H	494 G>A	ACTGCCTTGTGGAGGAGTTGAGA	GGGCTTCCTCTTGAACACGGTC	62
Q214L	686 A>T	TGATCTGCAATAATTTTTCTCCTA	GGATCCAGGGGCTGCTCAAAAT	57
P279T	880 C>A	ACATCTGAATTTACTATTGAAAGC	TTGGTTGTGCTTTTCCTTCTCCA	57
I359L	1120 A>C	CTTGACCTTCTCCCCACCAGCCT	GTATCTCTGGACCTCGTGCACCA	64
I359T	1121 T>C	CTGACCTTCTCCCCACCAGCCT	TGTATCTCTGGACCTCGTGCACC	64

All primers were 5'phosphorylated. Annealing temperatures (T_A) used for each set of primers are shown in the last column.

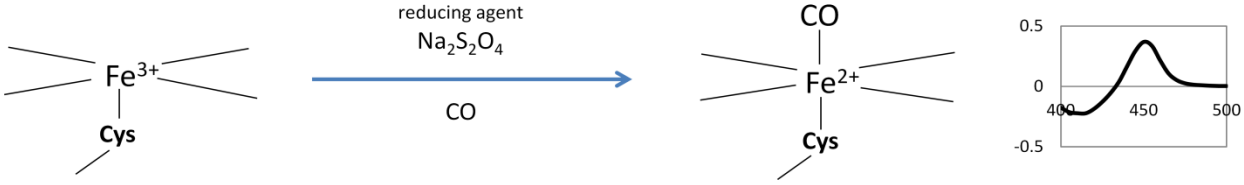

Figure S1. Schematic of CO P450 spectral assay. The ferric haem ion is converted to a ferrous ion in the presence of a reducing agent, followed by the co-ordination of CO at the axial position leading to an absorption peak at 450 nm.

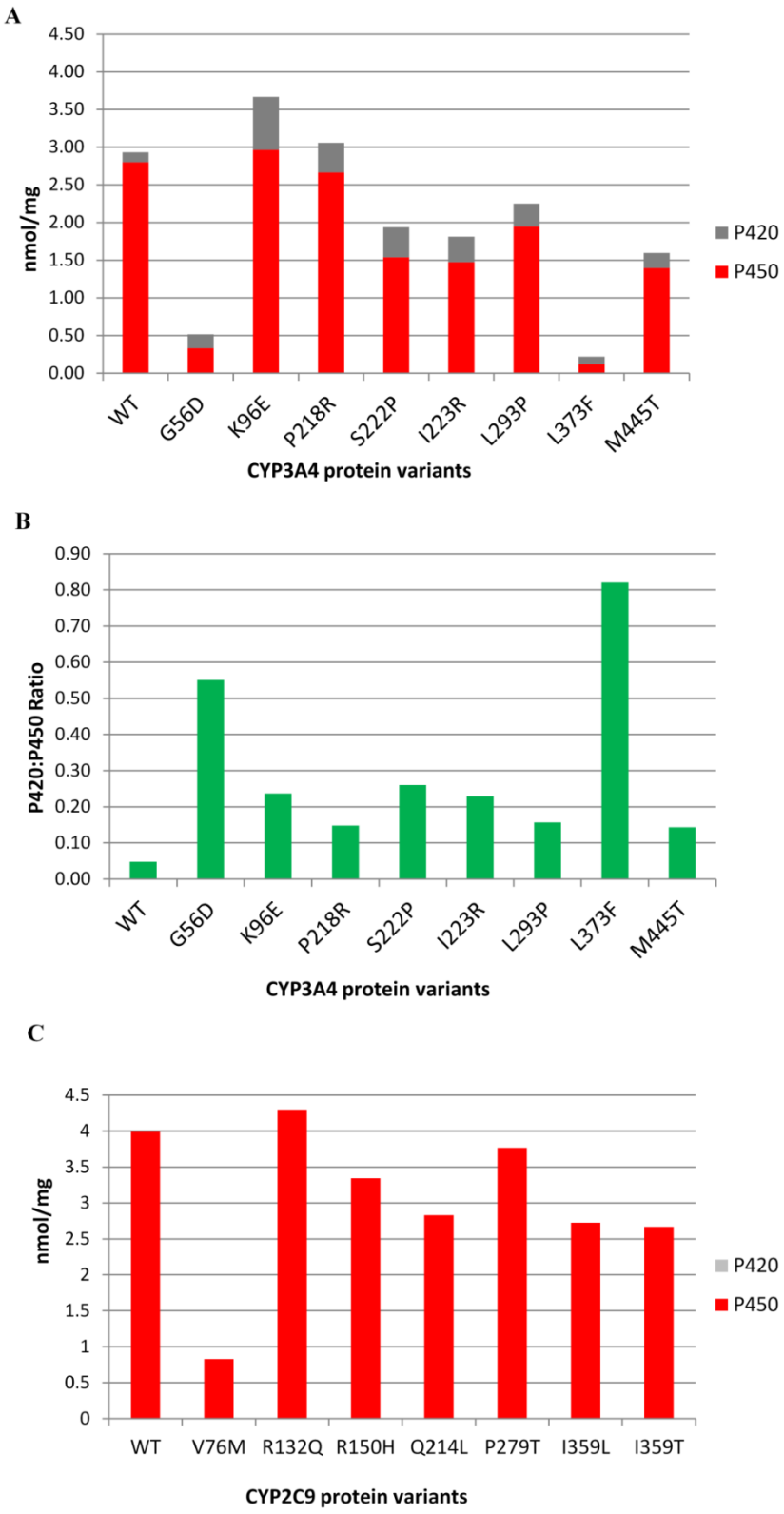

Figure S2. P450 and P420 protein content in CYP3A4 and CYP2C9 protein samples measured by CO P450 spectral assays. (A) nmols P450 and P420 per mg of total protein for CYP3A4 wild-type and variant samples. (B) P420:P450 ratios for CYP3A4 wild-type and variant samples. (C) nmols P450 per mg of total protein for CYP2C9 wild-type and variant samples. Note: P420 protein was not detected in any of the CYP2C9 protein preparations.

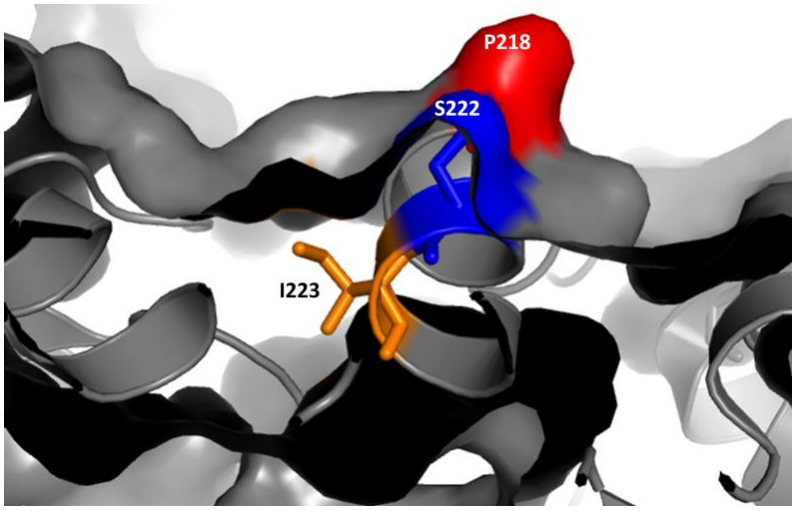

Figure S3. Surface accessibility of CYP3A4 residues P218, S222 and I223. PDB structure 1TQN was rendered using PyMOL, illustrating the surface accessibility of residues P218 (red), S222 (blue) and I223 (yellow) within the F' helix. P218 and S222 side chains form part of the surface of the molecule whereas I223 is buried beneath the surface.