**Supplementary Table 1.** Crystal structures and amino acid sequences used in molecular modeling.

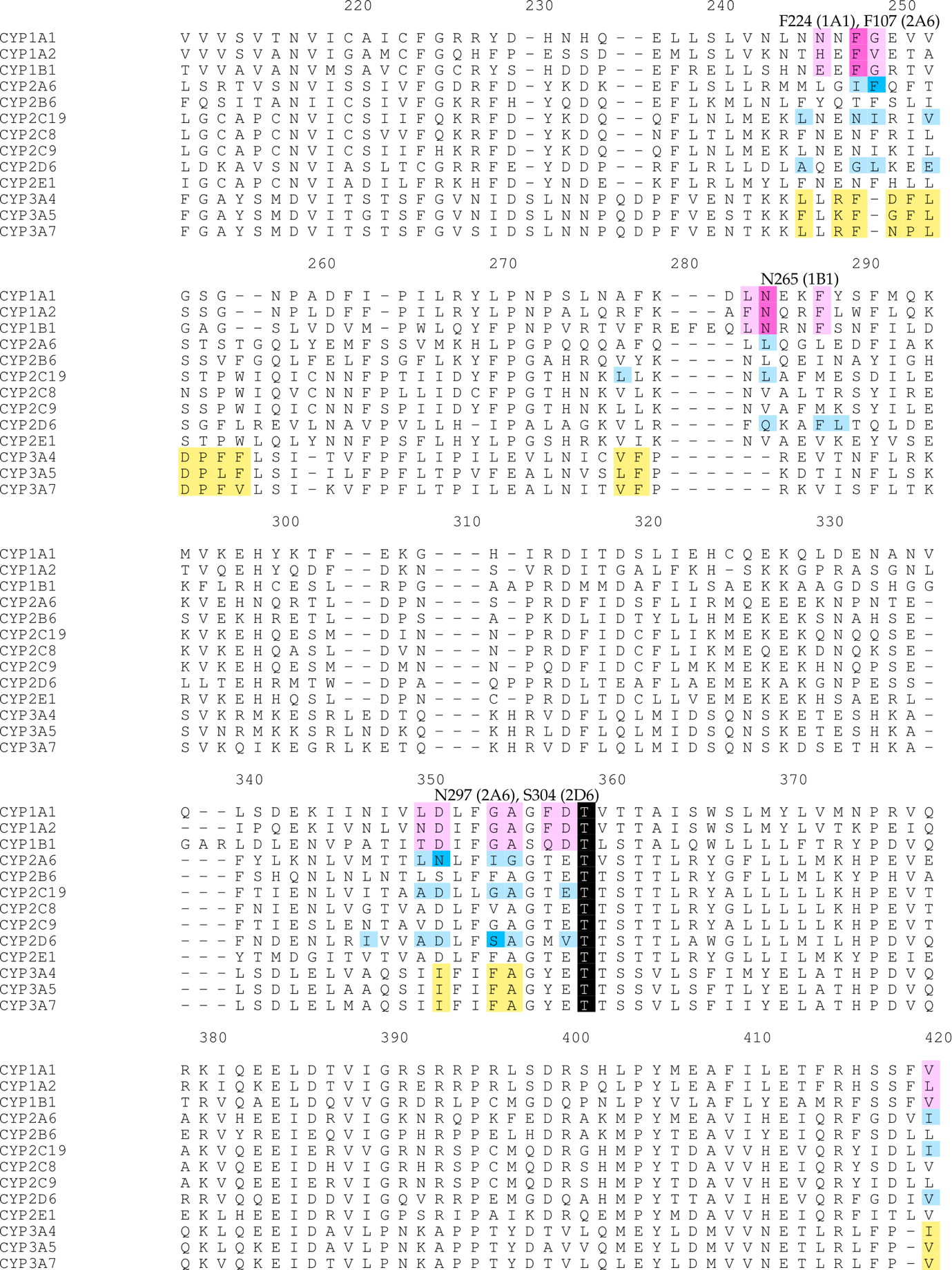
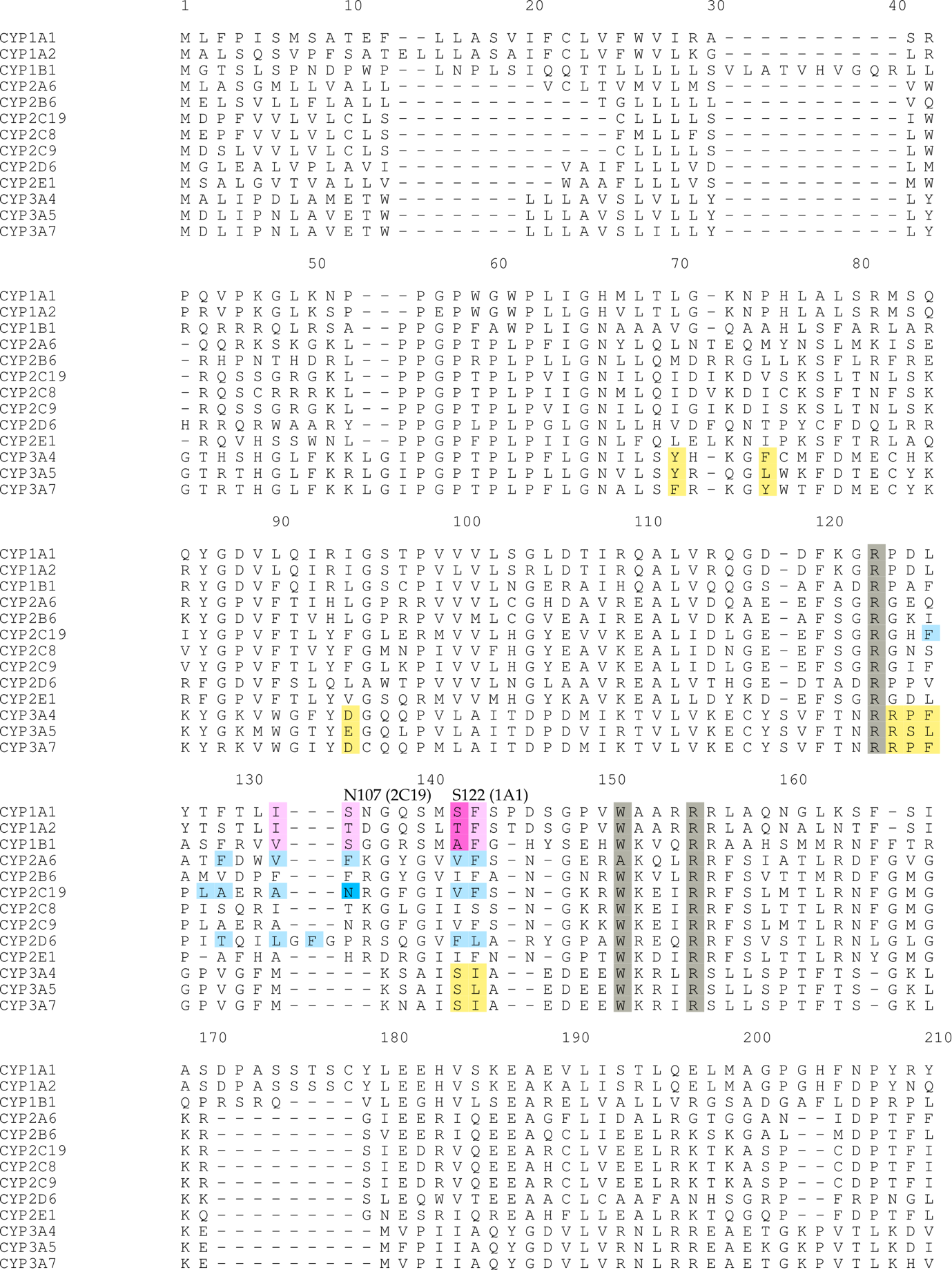
|  |  |  |
| --- | --- | --- |
| CYP form | UniProt entry | PDB ID |
| 1A1 | P04798-1 | 4I8Va,b (Walsh et al. 2013) |
| 1A2 | P05177-1 | 2HI4a (Sansen et al. 2007) |
| 1B1 | Q16678-1 | 3PM0a (Wang et al. 2011) |
| 2A6 | P11509-1 | 1Z10 (Yano et al. 2005)  2FDVa,b (Yano et al. 2006) |
| 2B6 | P20813-1 | 3QOA (Shah et al. 2011)  3UA5 (Shah et al. 2012) |
| 2C8 | P10632-1 | 2VN0 (Schoch et al. 2008) |
| 2C9 | P11712-1 | 1OG5 (Williams et al. 2003)  4NZ2 (Brändén et al. 2014)  1R9O (Wester et al. 2004) |
| 2C19 | P33261-1 | 4GQSa (Reynald et al. 2012) |
| 2D6 | P10635-1 | 3QM4a (Wang et al. 2012)  4WNW (Wang et al. 2015) |
| 2E1 | P05181-1 | 3T3Z (DeVore et al. 2012) |
| 3A4 | P08684-1 | 3UA1 (Sevrioukova & Poulos 2012)  5TE8b (Sevrioukova & Poulos 2017) |
| 3A5 | P20815-1 | 5VEU (Hsu et al. 2018) |
| 3A7 | P24462-1 | - |

1. Structures used in molecular docking
2. Structures used in structure-based sequence pre-alignment

**Supplementary Table 2**. The absorbance properties of the coumarin derivatives.

|  |  |  |
| --- | --- | --- |
| Compound  number | Amax  [nm] | ε abs  [cm-1\*M-1] |
| 1 | 287 | 5 800 |
| 2 | 293 | 6 200 |
| 3 | 293 | 5 700 |
| 4 | 294  333 | 13 100  12 300 |
| 5 | 292 | 17 900 |
| 6 | 340 | 7 200 |
| 7 | 349 | 14 900 |
| 8 | 324 | 14 800 |
| 9 | 340 | 17 400 |
| 10 | 349 | 10 400 |
| 12 | 346 | 19 300 |

**Supplementary Figure 1.** Multiple sequence alignment of the CYP forms. The catalytic sites of the CYP1 family are very conserved (light pink) and have similar amino acids at the positions suggested to be involved in 3-phenylcoumarin binding (pink). ~~CYP2A6 has a unique asparagine (teal) among the CYP2 family in addition to a well-positioned phenylalanine (teal) for possible π­‑ π interactions alongside other hydrophobic amino acids (light blue) in the catalytic site.~~ The catalytic site amino acids (light blue) and the suggested key residues in 3-phenylcoumarin binding (teal) differ among CYP2A6, CYP2D6 and CYP2C19. The amino acids present in either CYP3A4 or CYP3A5 binding sites (yellow) are conserved among the CYP3 forms. The conserved threonine in the helix I (black) and the heme-binding amino acids (grey) are aligned among all sequences. The alignment was built on top of structure-based pre-alignments of CYP2A6 and CYP3A4 to CYP1A1 from Vertaa in Bodil (Lehtonen 2004), and was performed with Malign (Johnson 1993) with structure-based matrix (STRMAT110) (Johnson & Overington 1993) and gap penalty of 30. The structure-based pre-alignments were left out of the figure for clarity. The used structures and sequences are listed in Supplementary Table 1.



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