

Supporting Information for:

Allosteric Modulation of Cytochrome P450 Enzymes by NADPH Cytochrome P450 Reductase
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*Running title: *Reductase Allosteric Modulation of P450 Enzymes*

Supporting Figure 1. Sequences for FMN domain, P450 catalytic domains, and the corresponding FMND/CYP450 proteins.

Supporting Figure 2. Aligned sequences for the reductase FMN domain, the isolated P450 enzymes, and the artificial fusion proteins thereof.

Supporting Figure 3. Generation and characterization of FMND/P450 fusion enzymes compared to the corresponding isolated P450 enzyme.

Supporting Figure 4. Spectral titrations for drug-metabolizing P450 enzymes CYP3A4, CYP2A6, and CYP2D6, and the corresponding FMND/CYP3A4, FMND/CYP2A6m and FMND/CYP2D6 fusion proteins with their respective substrates and inhibitors.

Supporting Figure 5. Spectral titrations for steroidogenic CYP17A1 and CYP21A2, and the corresponding FMND/CYP17A1 and FMND/CYP21A2 fusion proteins with their respective substrates and inhibitors.

Supporting Figure 1. Sequences for FMN domain, P450 catalytic domains, and the corresponding FMND/CYP450 proteins. The human NADPH-cytochrome P450 FMN domain protein sequence (blue) was identical to a previously-reported, folded domain, containing FMN (44). The TDGTS linker (black, bold) is a synthetic database-informed sequence (54) successfully used to generate similar fusions of mitochondrial P450 fusion to its redox partner adrenodoxin (39). The P450 sequences (red) are those of the catalytic domain used to determine structures. The C-terminal His-tags (green) were added to facilitate purification using metal-affinity chromatography.

FMN Domain

MGTLTSSVRESSFVEKMKKTGRNIIVFYGSQTGTAEFANRLSKDAHRYGMRGMSADPEEYDLADLSSLP
EIDNALVVFCMATYEGDPTDNAQDFYDWLQETDVDLSGVKFAVFGNGKTYEHFNAMGKYVDKRLEQLG
AQRIFELGLGDDDDGNLEEDFITWREQFWPAVCEHFGVEATGEESSHHHHHH

CYP3A4

MALYGTHSHGLFKKLGIPGPTPLPFLGNILSYHKGFCMFDMECHKKYGKVVWGFYDGQQP
VLAITDPMIKTVLVKECYSVFTNRRPFGPVGFMKSAISIAEDEEWKRLRSLLSPTFTSG
KLKEMVPIIAQYGDVLRNLRREAETGKPVTLKDVFGAYSMDVITSTSTSGVNIIDSLNNPQ
DPFVENTKLLRFDFLDPFFLSITVFPFLIPILEVLNICVFPREVTNFLRKSVKRMKESR
LEDTQKHRVDFLQLMIDSQNSKETESHKALSDLELVAQSIIFIFAGYETTSSVLSFIMYE
LATHPDVQQKLQEEIDAVLPNKAPPTYDTVLQMEYLDMVVNETLRLFPPIAMRLERVCKKD
VEINGMFI PKGVVMI PSYALHRDPKYWTEPEKFLPERFSKKNKDNIDPYIYTPFGSGPR
NCIGMRFALMNMKLALIRVLQNF SFKPKKETQIPLKLSLGGLLQPEKPVVLKVESRDGTV
SGAHHHH

FMND/CYP3A4

MGTLTSSVRESSFVEKMKKTGRNIIVFYGSQTGTAEFANRLSKDAHRYGMRGMSADPEEYDLADLSSLP
EIDNALVVFCMATYEGDPTDNAQDFYDWLQETDVDLSGVKFAVFGNGKTYEHFNAMGKYVDKRLEQLG
AQRIFELGLGDDDDGNLEEDFITWREQFWPAVCEHFGVEATGEESS**TDGTS**MALYGTHSHGLFKKLGIPG
PTPLPFLGNILSYHKGFCMFDMECHKKYGKVVWGFYDGQQPVLAITDPMIKTVLVKECYSVFTNRRPFGP
VGFMKSAISIAEDEEWKRLRSLLSPTFTSGKLKEMVPIIAQYGDVLRNLRREAETGKPVTLKDVFGAYS
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KSVKRMKESRLEDTQKHRVDFLQLMIDSQNSKETESHKALSDLELVAQSIIFIFAGYETTSSVLSFIMYEL
ATHPDVQQKLQEEIDAVLPNKAPPTYDTVLQMEYLDMVVNETLRLFPPIAMRLERVCKKDVEINGMFI PKG
VVMI PSYALHRDPKYWTEPEKFLPERFSKKNKDNIDPYIYTPFGSGPRNCIGMRFALMNMKLALIRVLQ
NF SFKPKKETQIPLKLSLGGLLQPEKPVVLKVESRDGTVSGAHHHHHH

CYP2D6

MAKKTSSKGKLPPLPLPGLGNLLHVDFQNTPYCFDQLRRRFGDVFSLQLAWTPVVVLN
GLAAVREALVTHGEDTADRPPVPIITQILGFGPRSQGVFLARYGPAWREQRRFSVSTLRNL
GLGKKSLEQWVTEEAACLCAAFANHSGRPFRPNGLLDKAVSNVIASLTCGRRFEYDDPRF
LRLDLAQEGLKEESGFLREVLNAVPLLHI PALAGKVLRFQKAFALTQLDELLEHRMTW
DPAQPPRDLEAFLAEMEKAKGNPESSFNDENLRIVVADLFSAGMVTSTTTLAWGLLLMI
LHPDVQRRVQQEIDDVIGQVRRPEMGDQAHMPYTTAVIHEVQRFQGDIVPLGVTHMTSRDI
EVQGFRI PKGTTLITNLSSVLKDEAVWEKPFRRFHPEHFLDAQGHFVKPEAFLPFSAGRA
CLGEPLARMELFLFFTSLQHFSFSVPTGQPRPSHHGVFAFLVSPSPYELCAVPRHHHH

FMND/CYP2D6

MGTLTSSVRESSFVEKMKKTGRNIIVFYGSQTGTAEFANRLSKDAHRYGMRGMSADPEEYDLADLSSLP
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AQRIFELGLGDDDDGNLEEDFITWREQFWPAVCEHFGVEATGEESS**TDGTS**AKKTSSKGKLPPLPLPGL

GNNLHVDFQNTPYCFDQLRRRFGDVFSLQLAWTPVVVLNGLAAVREALVTHGEDTADRPPVPITQILGFG
PRSQGVFLARYGPAWREQRRFSVSTLRNLGLGKKSLEQWVTEEAACLCAAFANHSGRPFRPNGLLDKAVS
NVIASLTCGRREFEYDDPRFLRLDLAQEGLKEESGFLREVLNAVVPVLLHIPALAGKVLRFQKAFQTQDE
LLTEHRMTWDPAQPPRDLTEAFLAEMEKAKGNPESSFNNDENLRIVVADLFSAGMVTSTTLLAWGLLLMIL
HPDVQRRVQQEIDDVIGQVRRPEMGDQAHMPYTTAVIHEVQRFQGDIVPLGVTHMTSRDIEVQGFRI PKGT
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FSFSVPTGQPRPSHHGVFAFLVSPSPYELCAVPRHHHHH

CYP2A6

MAKKTSSKGKLP PGPTPLPFIGNYLQLNTEQMYNSLMKISERYGPVFTIHLGPRRVVVL CGHDA
VREALVDQAEFFSGRGEQATFDWVFKGYGVVFSNGERAKQLRRFSIATLRDFGVGKRGIEERI Q
EEAGFLIDLALRGTTGGANIDPTFFLSRTVSNVISSIVFGDRFDYKDKEFLSLLRMMLGIFQFTST
STGQLYEMFSSVMKHLPGPQQQAFQLLQGLEDFIAKKVEHNQRTLDPNSPRDFIDSF LIRMQEE
EKNPNTEFYLNKLVMTTLNLFIGGTETVSTTLRYGFLLLMKHPEVEAKVHEEIDRVIGKNRQPK
FEDRAKMPYMEAVIHEIQRFQGDVIPMSLARRVKKDTKFRDFFLPKGTEVYPMLGSVLRDPSFFS
NPQDFNPQHFLNEKGQFKSDAFVPPFSIGKRNCFGEGGLARMELFLFFTTVMQNFRLKSSQSPKD
IDVSPKHVGFATIPRNYTMSFLPRHHHH

FMND/CYP2A6

MGTLTSSVRESSFVEKMKKTGRNIIVFYGSQTGTAEFANRLSKDAHRYGMRGMSADPEEYDLADLSSLP
EIDNALVVFCMATYGECDPTDNAQDFYDWLQETDVDLSGVKFAVFGGLGNKTYEHFNAMGKYVDKRLEQLG
AQRIFELGLGDDDDGNLEEDFITWREQFWPAVCEHFGVEATGEESSTDGTSMAKKTSSKGKLP PGPTPLP
FIGNYLQLNTEQMYNSLMKISERYGPVFTIHLGPRRVVVL CGHDAVREALVDQAEFFSGRGEQA
TFDWVFKGYGVVFSNGERAKQLRRFSIATLRDFGVGKRGIEERI QEEAGFLIDLALRGTTGGANID
PTFFLSRTVSNVISSIVFGDRFDYKDKEFLSLLRMMLGIFQFTSTSTGQLYEMFSSVMKHLPGP
QQQAFQLLQGLEDFIAKKVEHNQRTLDPNSPRDFIDSF LIRMQEEEKNPNTEFYLNKLVMTTLN
LFIGGTETVSTTLRYGFLLLMKHPEVEAKVHEEIDRVIGKNRQPKFEDRAKMPYMEAVIHEIQRF
QGDVIPMSLARRVKKDTKFRDFFLPKGTEVYPMLGSVLRDPSFFSNPQDFNPQHFLNEKGQFKK
SDAFVPPFSIGKRNCFGEGGLARMELFLFFTTVMQNFRLKSSQSPKIDVSPKHVGFATIPRNYTM
SFLPRHHHHHHHHHH

CYP17A1

MAKKTGAKYPKSLLSLPLVGSPLPFLPRHGHMHNFFKLQKKYGPIYSVRMGTKTTVIVGHHQLA
KEVLIKKGKDFSGRPQMATLDIASNNRKGIAFADSGAHWQLHRRLAMATFALFKDGDQKLEKII
CQEISTLCDMLATHNGQSIDISFPVFAVAVTNVISLICFNNTSYKNGDPELNVIQNYNEGIIDNLS
KDSLVDLVPWLKIFPNKTLKLSHVKIRNDLLNKILENYKEKFRSDSITNMLDTLMQAKMNSD
NGNAGPDQDSELLSDNHILTTIGDIFGAGVETTSVVKWTLAFLHNPQVKKLYEEIDQNVGF
SRTPTISDRNRLLEATIREVLRRLRPVAPMLIPKANVDSSIGEFVAVDKGTEVIINLWALHHN
EKEWHQPDQFMPERFLNPAGTQLISPSVSYLPFGAGPRSCIGEILARQELFLIMAWLLQRFDLE
VPDDGQLPSLEGIPKVVFLIDSFVKVIKVRQAWREAQAEGSTHHHH

FMND/CYP17A1

MGTLTSSVRESSFVEKMKKTGRNIIVFYGSQTGTAEFANRLSKDAHRYGMRGMSADPEEYDLADLSSLP
EIDNALVVFCMATYGECDPTDNAQDFYDWLQETDVDLSGVKFAVFGGLGNKTYEHFNAMGKYVDKRLEQLG
AQRIFELGLGDDDDGNLEEDFITWREQFWPAVCEHFGVEATGEESSTDGTSMAKKTGAKYPKSLLSLPLV
GSLPFLPRHGHMHNFFKLQKKYGPIYSVRMGTKTTVIVGHHQLAKEVLIKKGKDFSGRPQMAT
LDIASNNRKGIAFADSGAHWQLHRRLAMATFALFKDGDQKLEKII CQEISTLCDMLATHNGQSI
DISFPVFAVAVTNVISLICFNNTSYKNGDPELNVIQNYNEGIIDNLSKDSLVDLVPWLKIFPNKTL

EKLSHVKIRNDLLNKILENYKEKFRSDSITNMLDTLMQAKMNSDNGNAGPDQDSELLSDNHIL
TTIGDIFGAGVETTTSVVKWTLAFLLLHNPQVKKKLYEEIDQNVGFSRTPTISDRNRLLEATI
REVLRLRPVAPMLIPHKANVDSSIGFAVDKGTEVIINLWALHHNEKEWHQPDQFMPERFLNPA
GTQLISPSVSYLPFGAGPRSCIGEILARQELFLIMAWLLQRFDLEVPDDGQLPSLEGIPKVVFL
IDSFVKIKVRQAWREAQAEGSTHHHH

CYP21A1

MAKKTSSKGKLPPLAPGFLHLLQPDLPIYLLGLTQKFGPIYRLHLGLQDVVVLNSKRTIEEAMV
KKWADFAGRPEPLTYKLVSKNYPDLSLGDYSLWKAHKKLTRSALLLGIRDSMEPVVEQLTQEF
CERMRAQPGTPVAIEEEFSLTCSIICYLTFGDKIKDDNLMPAYYKCIQEVLTWWSHQIIVD
VIPFLRFFPNPGLRRLKQAIEKRDIHIVEMQLRQHKEVSLVAGQWRDMDYMLQGVQPSMEEGSG
QLLEGHVHMAAVDLLIGGTETTANTLSWAVVFLHHPEIQORLQEELDELGPGASSSRVPYKD
RARLPLLNATIAEVLRLRPVVPLALPHRTTRPSSISGYDIPEGTVIIPNLQGAHLDETVERPH
EFPDRFLEPGKNSRALAFGCGARVCLGEPLARLELFVVLTRLLQAFTLLPSGDALPSLQPLPH
CSVILKMQPFQVRLQPRGMGAHSPGQNQHSHHHH

FMND/CYP21A2

MGTLTSSVRESSFVEKMKKTGRNIVFYGSQTGTAEFANRLSKDAHRYGMRGMSADPEEYDLADLSSLP
EIDNALVVFCMATYEGDPTDNAQDFYDWLQETDVDLSGVKFAVFGNGKTYEHFNAMKYVDKRLEQLG
AQRIFELGLGDDDGLEEDFITWREQFWPAVCEHFGVEATGEESS**TDGTS**AKKTSSKGKLPPLAPGFLHL
LQPDLPYLLGLTQKFGPIYRLHLGLQDVVVLNSKRTIEEAMVKKWADFAGRPEPLTYKLVSKNYPDLSL
GDYSLWKAHKKLTRSALLLGIRDSMEPVVEQLTQEFCEMRAQPGTPVAIEEEFSLTCSIICYLTFG
KIKDDNLMPAYYKCIQEVLTWWSHQIIVDVIPFLRFFPNPGLRRLKQAIEKRDIHIVEMQLRQHKEV
AGQWRDMDYMLQGVQPSMEEGSGQLLEGHVHMAAVDLLIGGTETTANTLSWAVVFLHHPEIQORLQE
ELDDELGPGASSSRVPYKDRARLPLLNATIAEVLRLRPVVPLALPHRTTRPSSISGYDIPEGTVIIPNLQ
GAHLDETVERPHEFPDRFLEPGKNSRALAFGCGARVCLGEPLARLELFVVLTRLLQAFTLLPSGDALP
SLQPLPHCSVILKMQPFQVRLQPRGMGAHSPGQNQHSHHHH

Supporting Figure 2. Aligned sequences for the reductase FMN domain, the isolated P450 enzymes, and the artificial fusion proteins thereof. The TDGTS linker (black, bold) is a synthetic database-informed sequence (54) successfully used to generate similar fusions of mitochondrial P450 fusion to its redox partner adrenodoxin (39). The P450 sequences (red) are those of the catalytic domain used to determine structures. The C-terminal His-tags (green) were added to facilitate purification using metal-affinity chromatography.

FMND	MGTLTSSVRESSFVEKMKKTGRNIIVFYGSQTGTAEEFANRLSKDAHRYGMRGMSADPEE
FMND/CYP3A4	MGTLTSSVRESSFVEKMKKTGRNIIVFYGSQTGTAEEFANRLSKDAHRYGMRGMSADPEE
CYP3A4	-----
FMND	YDLADLSSLPEIDNALVVFCMATYEGEDPTDNAQDFYDWLQETDVDLSGVKFAVFGLGNK
FMND/CYP3A4	YDLADLSSLPEIDNALVVFCMATYEGEDPTDNAQDFYDWLQETDVDLSGVKFAVFGLGNK
CYP3A4	-----
FMND	TYEHFNAMGKYVDKRLEQLGAQRIFELGLGDDDDGNLEEDFITWREQFWPAVCEHFVGEAT
FMND/CYP3A4	TYEHFNAMGKYVDKRLEQLGAQRIFELGLGDDDDGNLEEDFITWREQFWPAVCEHFVGEAT
CYP3A4	-----
FMND	GEES
FMND/CYP3A4	GEESTDGTS MALYGTHSHGLFKKLGIPGPTPLPFLGNILSYHKGFCMFDECHKKYGKV
CYP3A4	----- MALYGTHSHGLFKKLGIPGPTPLPFLGNILSYHKGFCMFDECHKKYGKV -----
FMND	-----
FMND/CYP3A4	WGFYDQQQPVLAITDPDMIKTVLVKECYSVFTNRRPFGPVGFMKSAISIAEDEEWKRLRS
CYP3A4	WGFYDQQQPVLAITDPDMIKTVLVKECYSVFTNRRPFGPVGFMKSAISIAEDEEWKRLRS
FMND	-----
FMND/CYP3A4	LLSPTFTSGKLEKEMVPIIAQYGDVLRNLRREAETGKPVTLKDVFGAYSMDVITSTSTFGV
CYP3A4	LLSPTFTSGKLEKEMVPIIAQYGDVLRNLRREAETGKPVTLKDVFGAYSMDVITSTSTFGV
FMND	-----
FMND/CYP3A4	NIDSLNNPQDPFVENTKKLLRFDFLDPFFLSITVFPFLIPILEVLNICVFPREVTNFLRK
CYP3A4	NIDSLNNPQDPFVENTKKLLRFDFLDPFFLSITVFPFLIPILEVLNICVFPREVTNFLRK
FMND	-----
FMND/CYP3A4	SVKRMKESRLEDTQKHRVDFLQLMIDSQNSKETESHKALSDELVAQSIIFIFAGYETTS
CYP3A4	SVKRMKESRLEDTQKHRVDFLQLMIDSQNSKETESHKALSDELVAQSIIFIFAGYETTS
FMND	-----
FMND/CYP3A4	SVLSFIMYELATHPDVQKQLQEEIDAVLPNKAPPTYDTVLQMEYLDMVVNETLRLFPIAM
CYP3A4	SVLSFIMYELATHPDVQKQLQEEIDAVLPNKAPPTYDTVLQMEYLDMVVNETLRLFPIAM
FMND	-----
FMND/CYP3A4	RLERVCKKDVINGMFI PKGVVMI PSYALHRDPKYWTEPEKFLPERFSKKNKDNIDPYI
CYP3A4	RLERVCKKDVINGMFI PKGVVMI PSYALHRDPKYWTEPEKFLPERFSKKNKDNIDPYI
FMND	-----
FMND/CYP3A4	YTPFGSGPRNCIGMRFALMNMKLALIRVLQNF'SFKPKKETQIPLKLSLGGLLQPEKPVVL
CYP3A4	YTPFGSGPRNCIGMRFALMNMKLALIRVLQNF'SFKPKKETQIPLKLSLGGLLQPEKPVVL
FMND	----- HHHHHH -----
FMND/CYP3A4	KVESRDGTVSGAHHHHHH
CYP3A4	KVESRDGTVSGAHHHH -----

FMND
FMND/CYP2D6
CYP2D6

MGTLTSSVRESSFVEKMKKTGRNIIVFYGSQTGTAEFFANRLSKDAHRYGMRGMSADPEE
MGTLTSSVRESSFVEKMKKTGRNIIVFYGSQTGTAEFFANRLSKDAHRYGMRGMSADPEE

FMND
FMND/CYP2D6
CYP2D6

YDLADLSSLPEIDNALVVFCMATYGECDPTDNAQDFYDWLQETDVLDSGVKFAVFGLNK
YDLADLSSLPEIDNALVVFCMATYGECDPTDNAQDFYDWLQETDVLDSGVKFAVFGLNK

FMND
FMND/CYP2D6
CYP2D6

TYEHFNAMGKYVDKRLEQLGAQRIFELGLGDDDNLEEDFITWREQFWPAVCEHFGVEAT
TYEHFNAMGKYVDKRLEQLGAQRIFELGLGDDDNLEEDFITWREQFWPAVCEHFGVEAT

FMND
FMND/CYP2D6
CYP2D6

GEES-----
GEES**TDGTS**AKKTSSKGKLP PGPLPLPGLGNLLHVDFQNTPYCFDQLRRRFGDVFSLQL
-----**MAKKTSSKGKLP PGPLPLPGLGNLLHVDFQNTPYCFDQLRRRFGDVFSLQL**

FMND
FMND/CYP2D6
CYP2D6

AWTPVVVNLGLAAVREALVTHGEDTADRPPVPITQILGFGRSQGVFLARYGPAWREQRR
AWTPVVVNLGLAAVREALVTHGEDTADRPPVPITQILGFGRSQGVFLARYGPAWREQRR

FMND
FMND/CYP2D6
CYP2D6

FSVSTLRNLGLGKKSLEQWVTEEAACLCAAFANHSGRPFPPNGLLDKAVSNVIASLTCGR
FSVSTLRNLGLGKKSLEQWVTEEAACLCAAFANHSGRPFPPNGLLDKAVSNVIASLTCGR

FMND
FMND/CYP2D6
CYP2D6

RFEYDDPRFLRLLDLAQEGLKEESGFLREVLNAV PVL LHI PALAGKVLRFQKAF L T Q L D E
RFEYDDPRFLRLLDLAQEGLKEESGFLREVLNAV PVL LHI PALAGKVLRFQKAF L T Q L D E

FMND
FMND/CYP2D6
CYP2D6

LLTEHRMTWDP AQ P P R D L T E A F L A E M E K A K G N P E S S F N D E N L R I V V A D L F S A G M V T T S T T
LLTEHRMTWDP AQ P P R D L T E A F L A E M E K A K G N P E S S F N D E N L R I V V A D L F S A G M V T T S T T

FMND
FMND/CYP2D6
CYP2D6

LAWGLLLMILHPDVQRRVQQEIDDVIGQVRRPEMGDQAHPYTTAVIHEVQRFGDIVPLG
LAWGLLLMILHPDVQRRVQQEIDDVIGQVRRPEMGDQAHPYTTAVIHEVQRFGDIVPLG

FMND
FMND/CYP2D6
CYP2D6

VTHMTRSRIEVQGFRI PKGTTLITNLSSVLKDEAVWEKPF RFHPEHFLDAQGHFVKPEAF
VTHMTRSRIEVQGFRI PKGTTLITNLSSVLKDEAVWEKPF RFHPEHFLDAQGHFVKPEAF

FMND
FMND/CYP2D6
CYP2D6

LPFSAGRRACLGEPLARMEFLFFTSLLQHFSFSVPTGQPRPSHHGVFAFLVSPSPYELC
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FMND
FMND/CYP2D6
CYP2D6

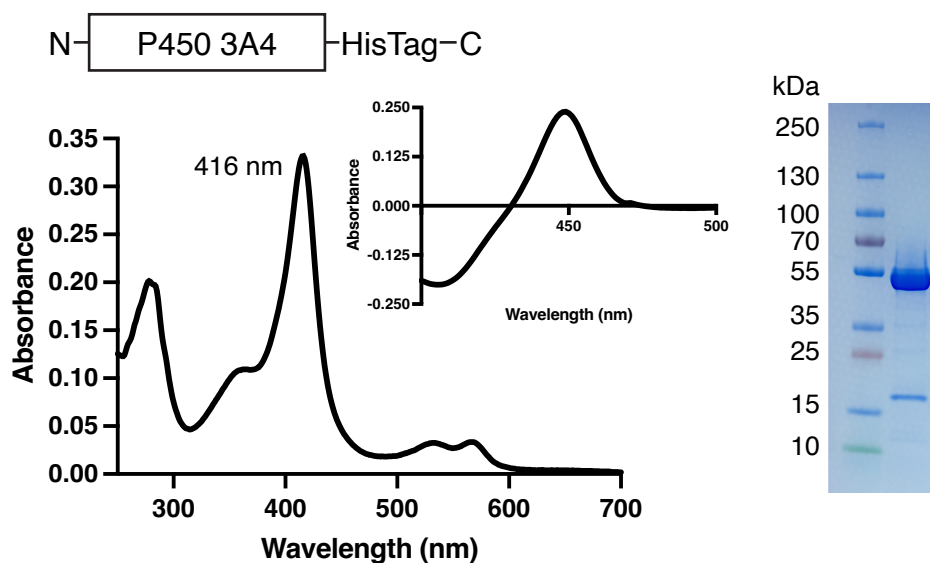
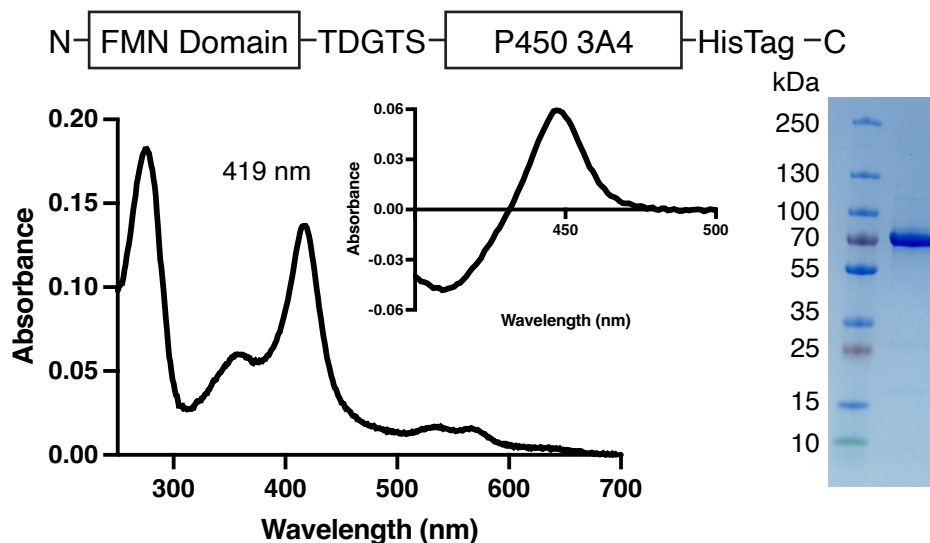
----**HHHHHH**
AVPRHHHHHH
AVPRHHHH--

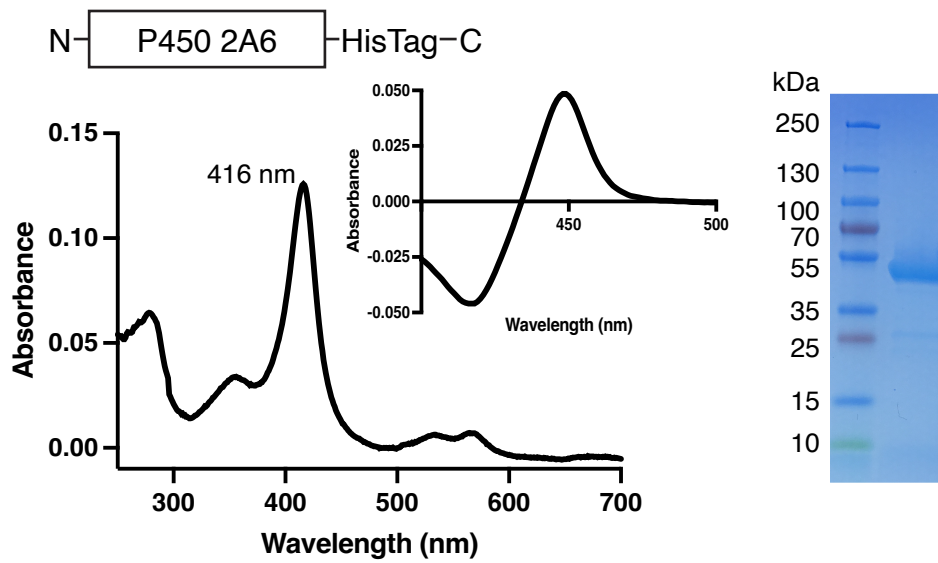
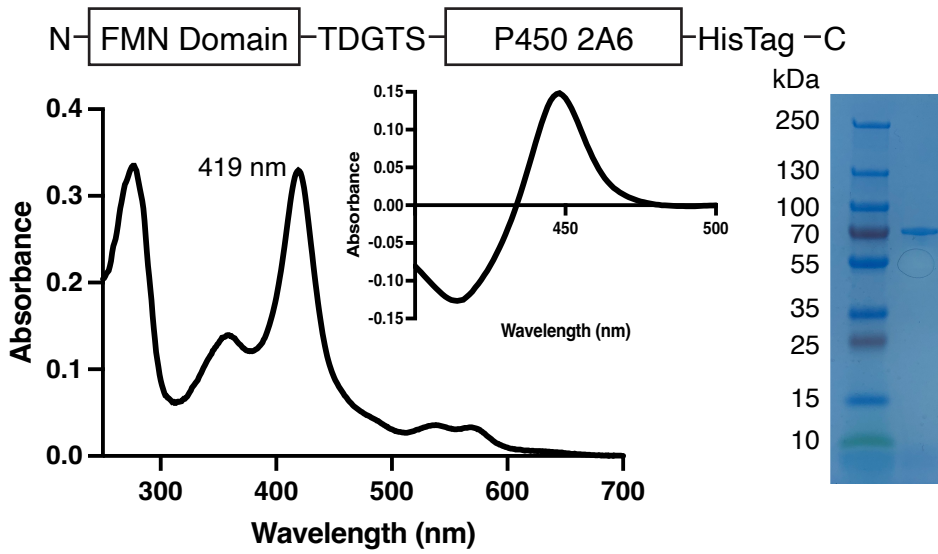
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FMND FMND/CYP2A6 CYP2A6	YDLADLSSLPEIDNALVVFCMATYEGDPTDNAQDFYDWLQETDVDLSGVKFAVFGLGNK YDLADLSSLPEIDNALVVFCMATYEGDPTDNAQDFYDWLQETDVDLSGVKFAVFGLGNK -----
FMND FMND/CYP2A6 CYP2A6	TYEHFNAMGKYVDKRLEQLGAQRIFELGLGDDDDGNLEEDFITWREQFWPAVCEHFVGEAT TYEHFNAMGKYVDKRLEQLGAQRIFELGLGDDDDGNLEEDFITWREQFWPAVCEHFVGEAT -----
FMND FMND/CYP2A6 CYP2A6	GEES----- GEES TDGTS MAKKTSSKGLPPGPTPLPFIGNYLQLNTEQMYNSLMKISERYGPVFTIH -----MAKKTSSKGLPPGPTPLPFIGNYLQLNTEQMYNSLMKISERYGPVFTIH
FMND FMND/CYP2A6 CYP2A6	----- LGPRRVVLCGHDAVREALVDQAEFSGRGEQATFDWVFKGYGVVFSNGERAKQLRRFSI LGPRRVVLCGHDAVREALVDQAEFSGRGEQATFDWVFKGYGVVFSNGERAKQLRRFSI
FMND FMND/CYP2A6 CYP2A6	----- ATLRDFGVGKRGIEERIQQEAGFLIDALRGTTGGANIDPTFFLSRTVSNVISSIVFGDRFD ATLRDFGVGKRGIEERIQQEAGFLIDALRGTTGGANIDPTFFLSRTVSNVISSIVFGDRFD
FMND FMND/CYP2A6 CYP2A6	----- YKDKEFLSLLRMMLGIFQFTSTSTGQLYEMFSSVMKHLPGPQQQAFQLLQGLEDFIAKKV YKDKEFLSLLRMMLGIFQFTSTSTGQLYEMFSSVMKHLPGPQQQAFQLLQGLEDFIAKKV
FMND FMND/CYP2A6 CYP2A6	----- EHNQRTLDPNSPRDFIDSFLIRMQEEKPNTEFYLNLMVTTLNLFIGGTETVSTTLRY EHNQRTLDPNSPRDFIDSFLIRMQEEKPNTEFYLNLMVTTLNLFIGGTETVSTTLRY
FMND FMND/CYP2A6 CYP2A6	----- GFLLLMKHPEVEAKVHEEIDRVI GKNRQPKFEDRAKMPYMEAVIHEIQRF GDVIPMSLAR GFLLLMKHPEVEAKVHEEIDRVI GKNRQPKFEDRAKMPYMEAVIHEIQRF GDVIPMSLAR
FMND FMND/CYP2A6 CYP2A6	----- RVKKDKTKFRDFFLPKGTEVYPMLGSVLRDPSFFSNPQDFNPQHFLNEKGQFKKSDAFVFP RVKKDKTKFRDFFLPKGTEVYPMLGSVLRDPSFFSNPQDFNPQHFLNEKGQFKKSDAFVFP
FMND FMND/CYP2A6 CYP2A6	----- SIGKRNCFGEGLARMELFLFFTVMQNFRLKSSQSPKIDIVSPKHVGFATIPRNYTMSFL SIGKRNCFGEGLARMELFLFFTVMQNFRLKSSQSPKIDIVSPKHVGFATIPRNYTMSFL
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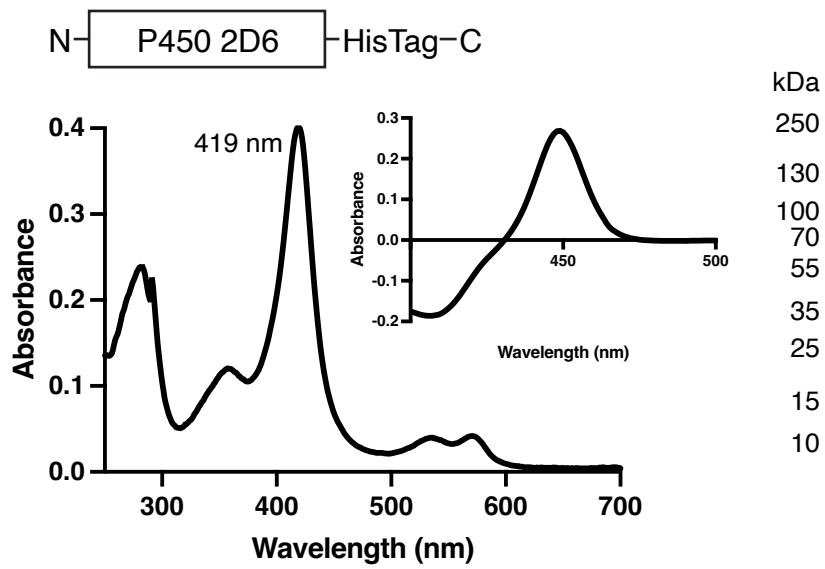
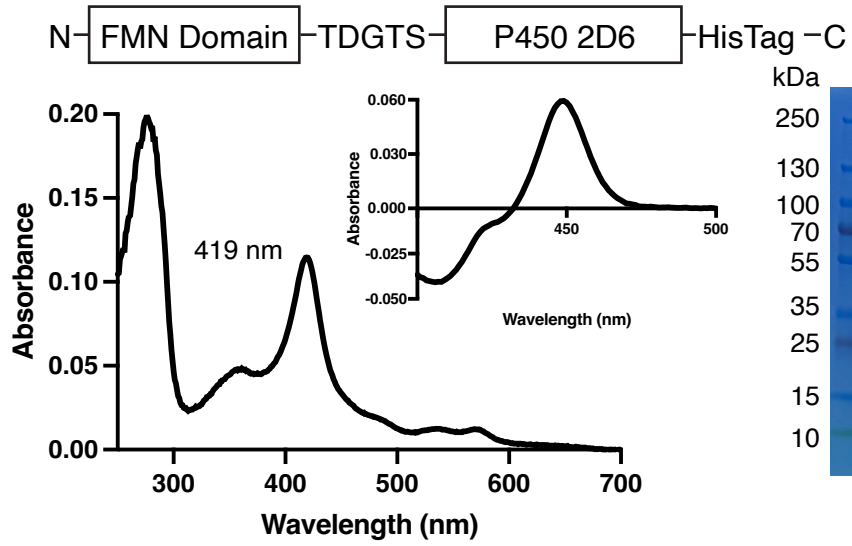
FMND FMND/CYP17A1 CYP17A1	MGTLTSSVRESSFVEKMKKTGRNIIVFYGSQTGTAEFFANRLSKDAHRYGMRGMSADPEE MGTLTSSVRESSFVEKMKKTGRNIIVFYGSQTGTAEFFANRLSKDAHRYGMRGMSADPEE -----
FMND FMND/CYP17A1 CYP17A1	YDLADLSSLPEIDNALVVFCMATYGECDPTDNAQDFYDWLQETDVLDSGVKFAVFLGNK YDLADLSSLPEIDNALVVFCMATYGECDPTDNAQDFYDWLQETDVLDSGVKFAVFLGNK -----
FMND FMND/CYP17A1 CYP17A1	TYEHFNAMGKYVDKRLEQLGAQRIFELGLGDDGNLEEDFITWREQFWPAVCEHFGVEAT TYEHFNAMGKYVDKRLEQLGAQRIFELGLGDDGNLEEDFITWREQFWPAVCEHFGVEAT -----
FMND FMND/CYP17A1 CYP17A1	GEES----- GEES TDGTS MAKKTGAKYPKSLLSLPLVGSPLPFLPRHGHMHNFFKLQKKGPIYSVRM -----MAKKTGAKYPKSLLSLPLVGSPLPFLPRHGHMHNFFKLQKKGPIYSVRM
FMND FMND/CYP17A1 CYP17A1	----- GKTTTIVGHHQLAKEVLIKKGKDFSGRPQMATLDIASNNRKGIAFADSGAHWQLHRRLA GKTTTIVGHHQLAKEVLIKKGKDFSGRPQMATLDIASNNRKGIAFADSGAHWQLHRRLA
FMND FMND/CYP17A1 CYP17A1	----- MATFALFKDGDQKLEKIICQEISTLCDMLATHNGQSIDISFPVFAVTVNISLICFNNTSY MATFALFKDGDQKLEKIICQEISTLCDMLATHNGQSIDISFPVFAVTVNISLICFNNTSY
FMND FMND/CYP17A1 CYP17A1	----- KNGDPELNVIQYNEGIIDNLSKDSLVDLVPWLKIFPNKLEKLSHVKIRNDLLNKILE KNGDPELNVIQYNEGIIDNLSKDSLVDLVPWLKIFPNKLEKLSHVKIRNDLLNKILE
FMND FMND/CYP17A1 CYP17A1	----- NYKEKFRSDSITNMLDTLMQAKMNSDNGNAGPDQDSELSDNHILTTIGDIFGAGVETTT NYKEKFRSDSITNMLDTLMQAKMNSDNGNAGPDQDSELSDNHILTTIGDIFGAGVETTT
FMND FMND/CYP17A1 CYP17A1	----- SVVKWTLAFLHNPQVKKKLYEEIDQNVGFSRTPTISDRNRLLEATIREVLRRLRPVAP SVVKWTLAFLHNPQVKKKLYEEIDQNVGFSRTPTISDRNRLLEATIREVLRRLRPVAP
FMND FMND/CYP17A1 CYP17A1	----- MLIPHKANVDSSIGFAVDKGTEVIINLWALHHNEKEWHQPDQFMPERFLNPAGTQLISP MLIPHKANVDSSIGFAVDKGTEVIINLWALHHNEKEWHQPDQFMPERFLNPAGTQLISP
FMND FMND/CYP17A1 CYP17A1	----- SVSYLPFGAGPRSCIGEILARQELFLIMAWLLQRFDLEVPDDGQLPSLEGIPKVVFLLIDS SVSYLPFGAGPRSCIGEILARQELFLIMAWLLQRFDLEVPDDGQLPSLEGIPKVVFLLIDS
FMND FMND/CYP17A1 CYP17A1	-----HHHHH FKVKIKVRQAWREAQEGSTHHHH FKVKIKVRQAWREAQEGSTHHHH

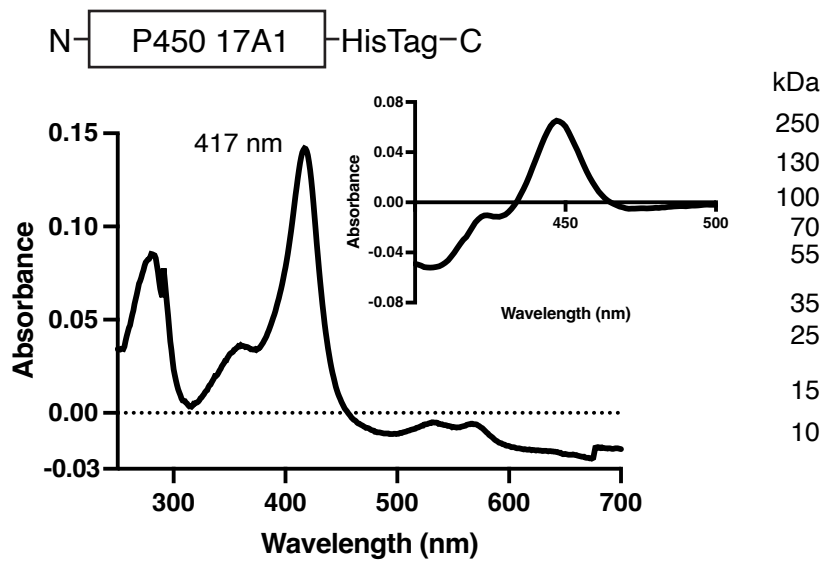
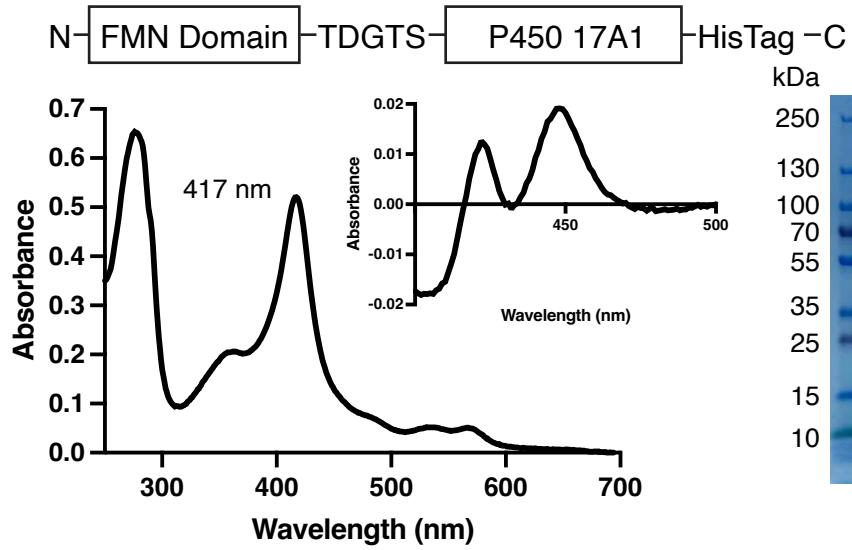
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FMND FMND/CYP21A2 CYP21A2	YDLADLSSLPEIDNALVVFCMATYEGGDPTDNAQDFYDWLQETDVLDSGVKFAVFLGNK YDLADLSSLPEIDNALVVFCMATYEGGDPTDNAQDFYDWLQETDVLDSGVKFAVFLGNK -----
FMND FMND/CYP21A2 CYP21A2	TYEHFNAMGKYVDKRLEQLGAQRIFELGLGDDDNLEEDFITWREQFWPAVCEHFGVEAT TYEHFNAMGKYVDKRLEQLGAQRIFELGLGDDDNLEEDFITWREQFWPAVCEHFGVEAT -----
FMND FMND/CYP21A2 CYP21A2	GEES----- GEES TDGTS AKKTSKGLPPLAPGFLHLLQPDLPITYLLGLTQKFGPIYRLHLGLQDVV -----MAKTSKGLPPLAPGFLHLLQPDLPITYLLGLTQKFGPIYRLHLGLQDVV
FMND FMND/CYP21A2 CYP21A2	----- VLNSKRTEEAMVKKWADFAGRPEPLTYKLVSKNYPDLSLGDYSLLWKAHKKLTRSALLL VLNSKRTEEAMVKKWADFAGRPEPLTYKLVSKNYPDLSLGDYSLLWKAHKKLTRSALLL
FMND FMND/CYP21A2 CYP21A2	----- GIRDSMEPVVEQLTQEFCEMRAQPGTPVAIEEEFSLTCSIICYLTFGDKIKDDNLMPA GIRDSMEPVVEQLTQEFCEMRAQPGTPVAIEEEFSLTCSIICYLTFGDKIKDDNLMPA
FMND FMND/CYP21A2 CYP21A2	----- YYKCIQEVLTWWSHSIQIVDVI PFLRFFPNPGLRRLKQAI EKRDHIVEMQLRQHESLV YYKCIQEVLTWWSHSIQIVDVI PFLRFFPNPGLRRLKQAI EKRDHIVEMQLRQHESLV
FMND FMND/CYP21A2 CYP21A2	----- AGQWRDMDYMLQGVAQPSMEEGSGQLLEGHVHMAAVDLLIGGTETTANTLSWAVVFLH AGQWRDMDYMLQGVAQPSMEEGSGQLLEGHVHMAAVDLLIGGTETTANTLSWAVVFLH
FMND FMND/CYP21A2 CYP21A2	----- HPEIQQLQEELDHELPGASSSRVPYKDRARLPLLNATIAEVLRLRPVVPLALPHRTR HPEIQQLQEELDHELPGASSSRVPYKDRARLPLLNATIAEVLRLRPVVPLALPHRTR
FMND FMND/CYP21A2 CYP21A2	----- PSSISGYDIPEGTVIIPNLQGAHLDET VWERPHEFWPDRFLEPGKNSRALAFGCGARVCL PSSISGYDIPEGTVIIPNLQGAHLDET VWERPHEFWPDRFLEPGKNSRALAFGCGARVCL
FMND FMND/CYP21A2 CYP21A2	----- GEPLARLELFVVLTRLLQAFTLLPSGDALPSLQPLPHCSVILKMQPFQVRLQPRGMGAHS GEPLARLELFVVLTRLLQAFTLLPSGDALPSLQPLPHCSVILKMQPFQVRLQPRGMGAHS
FMND FMND/CYP21A2 CYP21A2	----- HHHHHH PGQNQH HHHHHH PGQNQH HHHHHH

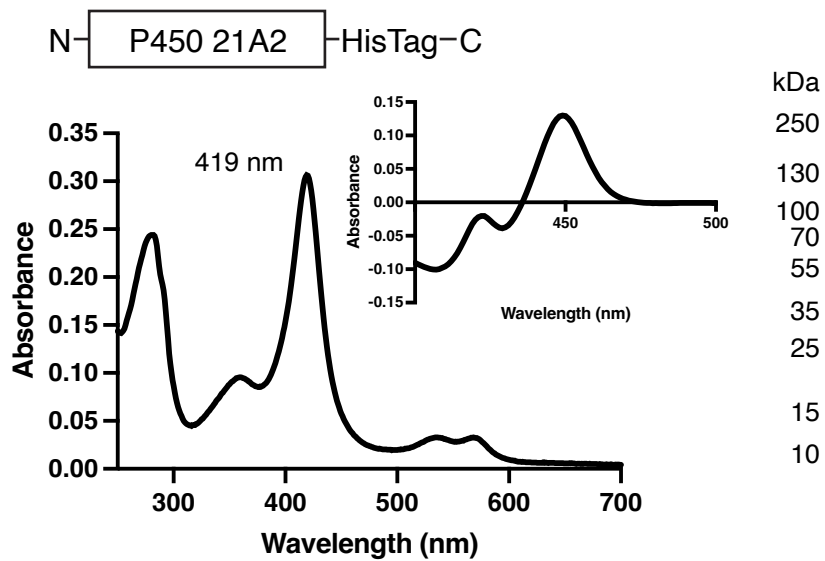
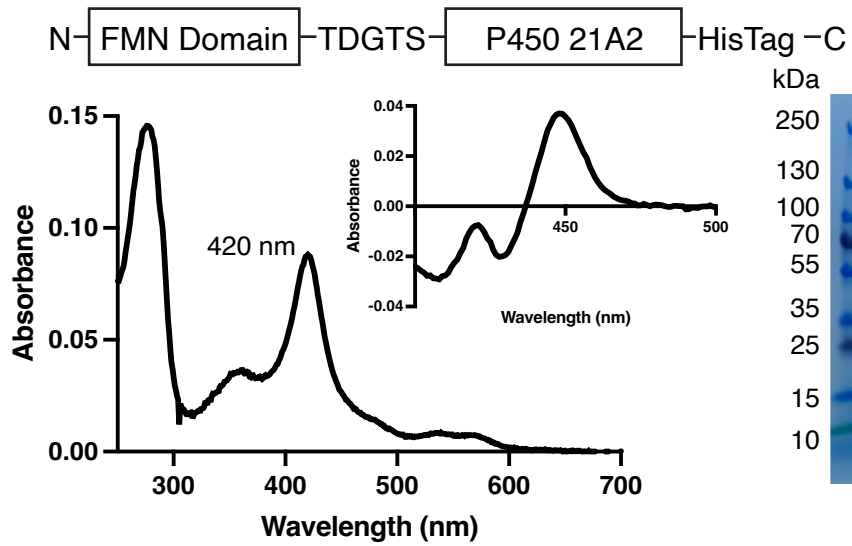
Supporting Figure 3. Generation and characterization of FMND/P450 fusion enzymes compared to the corresponding isolated P450 enzyme. Fusion enzymes consisted of the reductase FMN domain plus a 5-amino acid linker plus the catalytic P450 domain with a histidine tag, while the isolated P450 enzymes had only a C-terminal histidine tag (top graphics). These constructs yielded purified protein with a water-bound Soret peak in the absolute spectrum (main image) and a typical reduced-carbon monoxide difference spectrum (inset). The purified protein runs on an SDS-PAGE gel at the expected molecular weight of 75-77 kDa for the fusions and 54-56 kDa for the isolated P450 enzymes (right). Panels for the FMND/CYP2A6 and CYP2A6 from Figure 1 are presented again here to facilitate comparisons between enzymes.





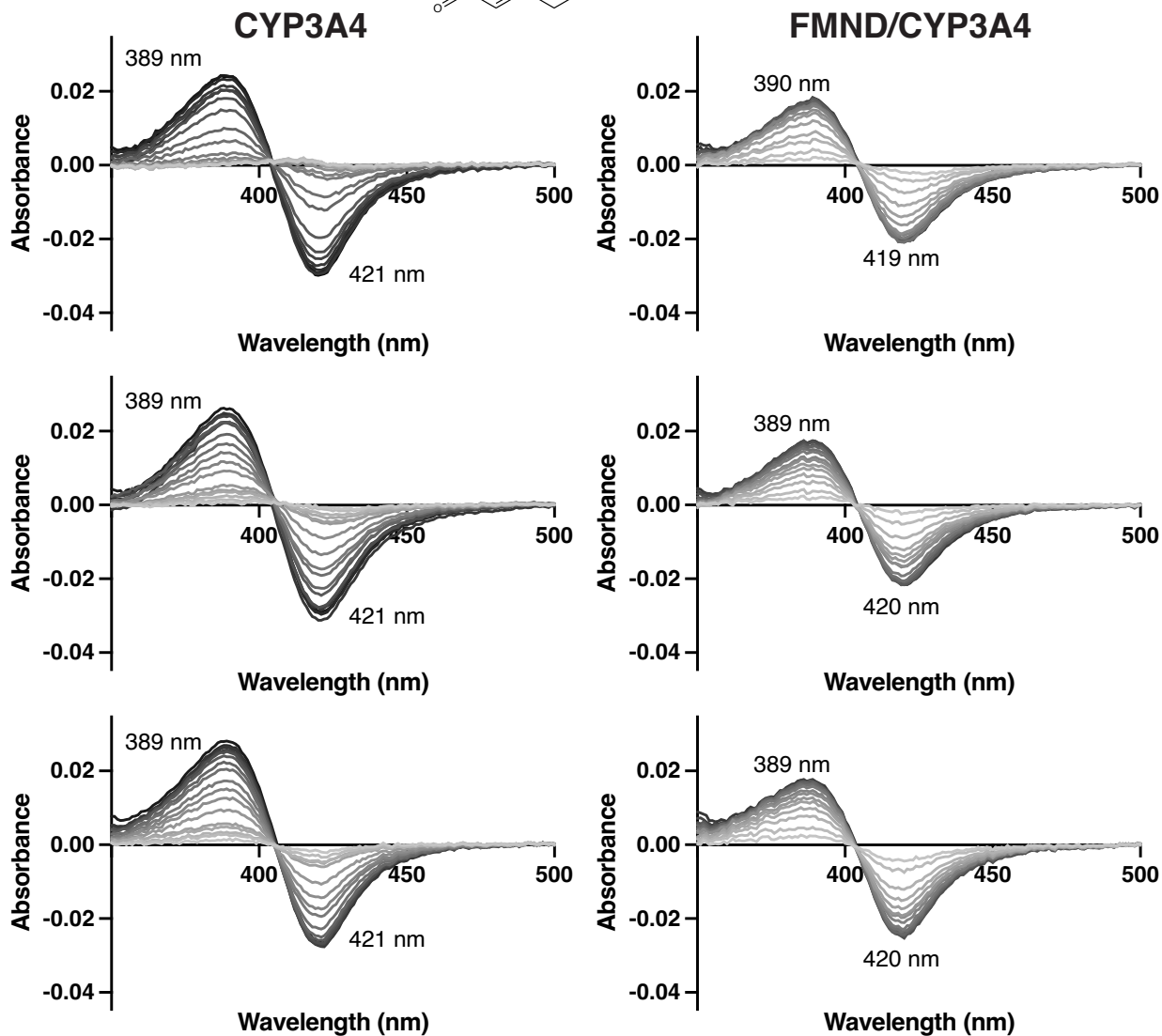
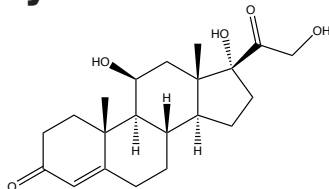




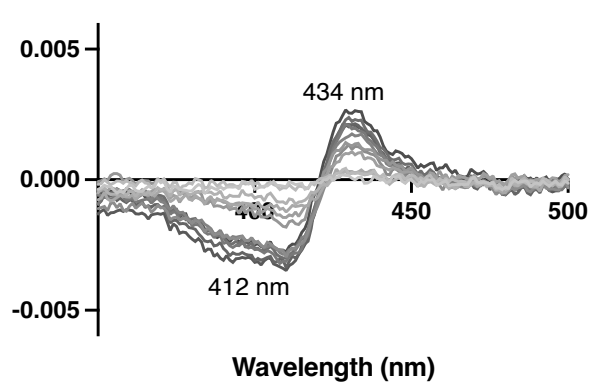
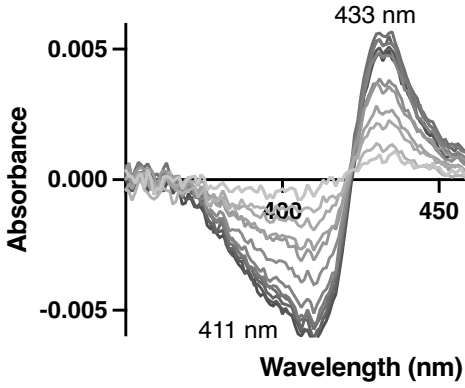
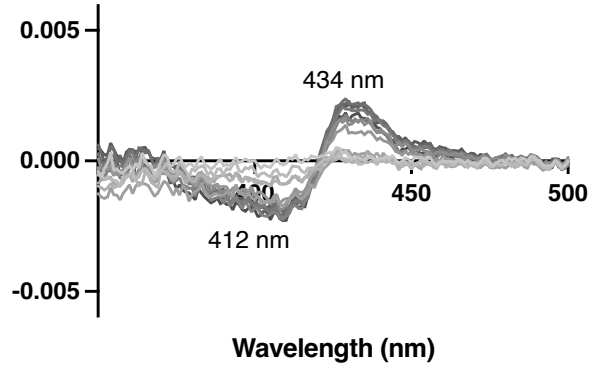
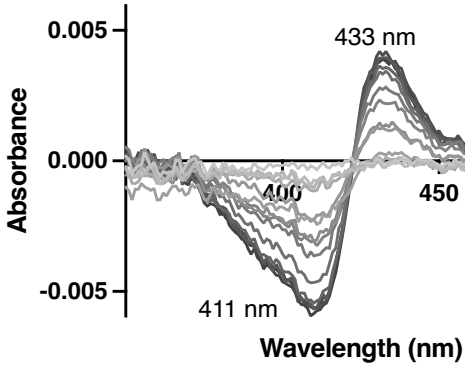
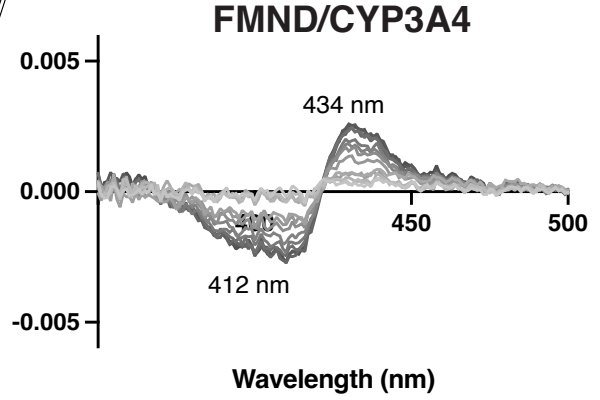
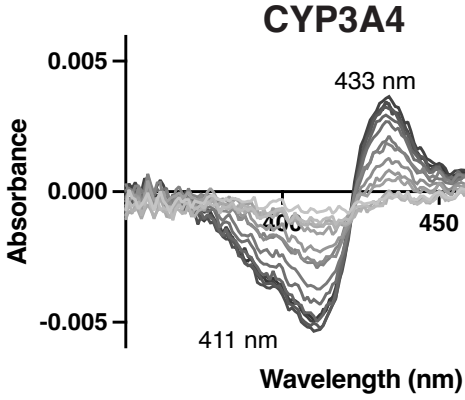
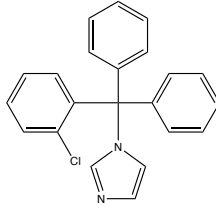


Supporting Figure 4. Spectral titrations for drug-metabolizing P450 enzymes CYP3A4, CYP2A6, and CYP2D6. Low ligand concentrations are shown in light grey and higher concentrations in darker grey. For CYP3A4 and FMND/CYP3A4 the ligands and their concentrations are hydrocortisone (10 μ M-1.5 mM) and clotrimazole (0.9-67 nM). For CYP2D6 and FMND/CYP2D6 the ligands and their concentrations are thioridazine (1-450 μ M) and prinomastat (0.2-84 μ M). For CYP2A6 and FMND/CYP2A6 the ligands and their concentrations are coumarin (0.5-699 μ M) and pilocarpine (0.5-439 μ M).

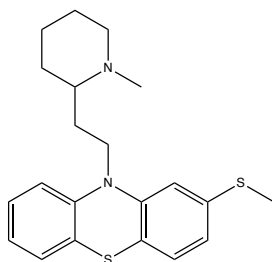
Hydrocortisone



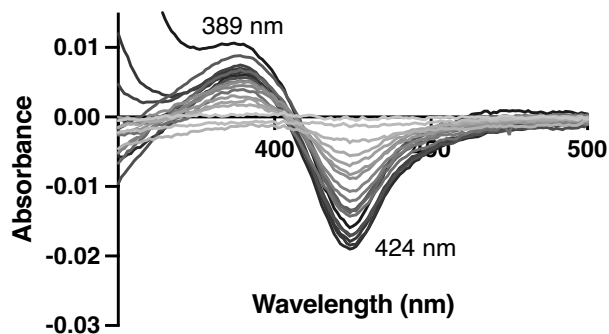
Clotrimazole



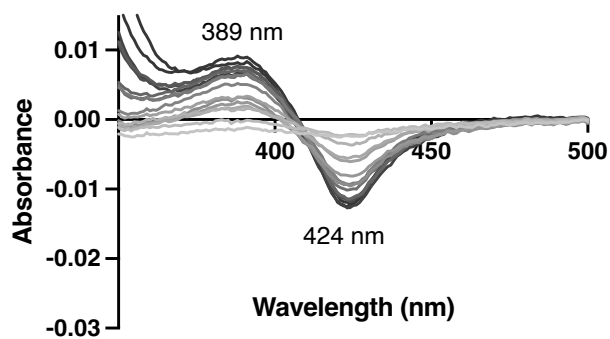
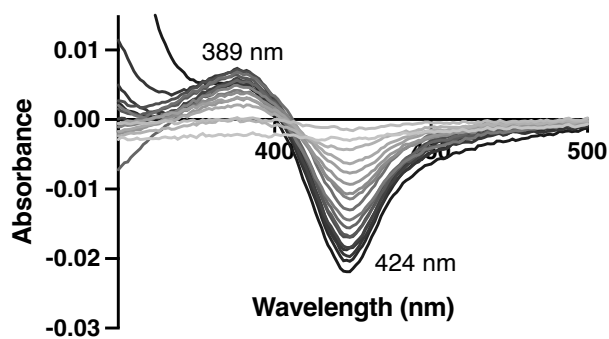
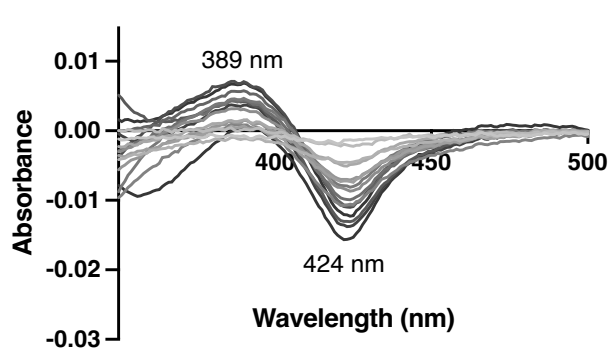
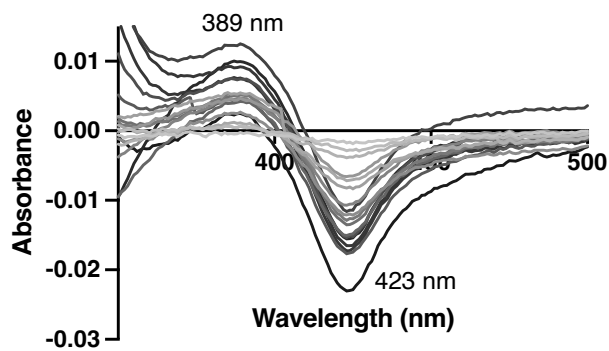
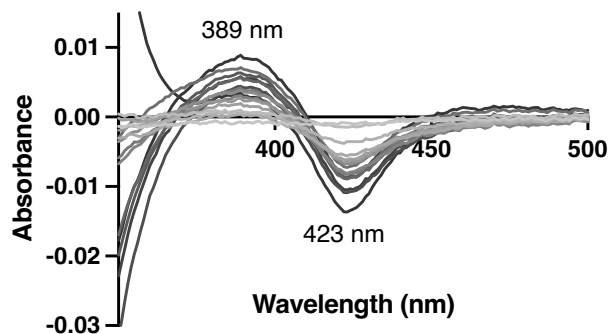
Thioridazine



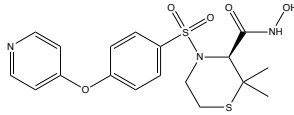
CYP2D6



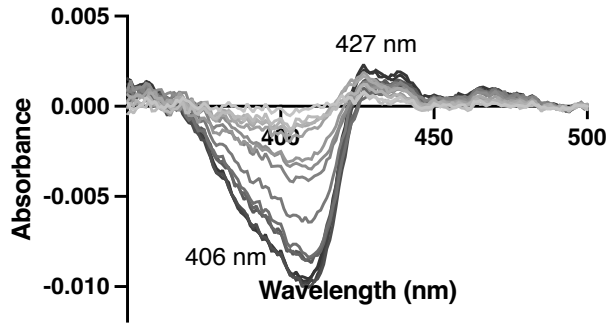
FMND/CYP2D6



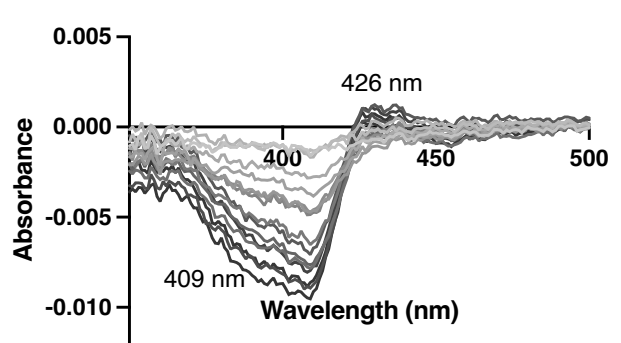
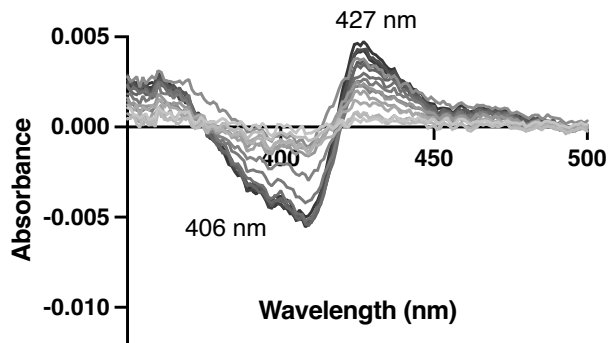
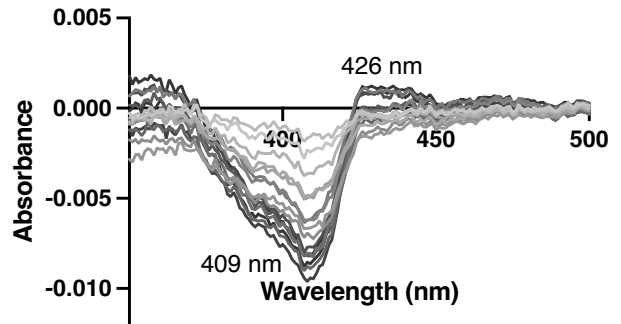
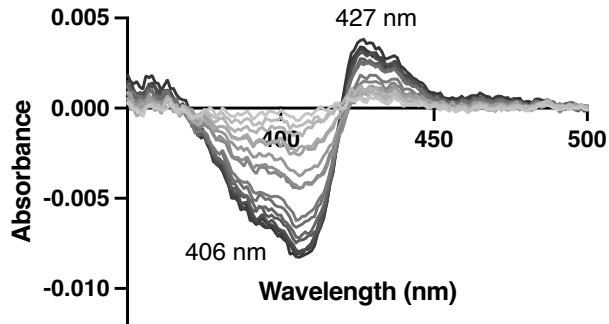
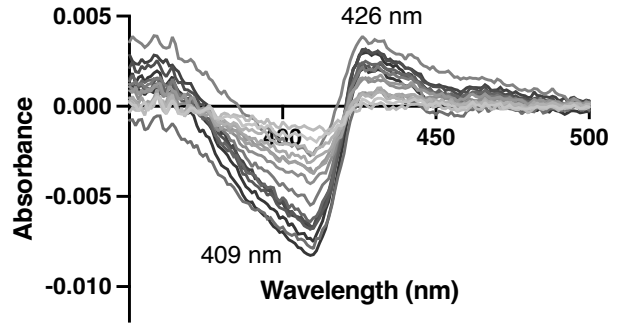
Prinomastat



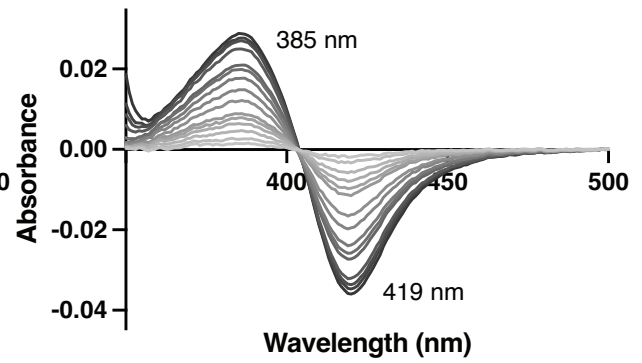
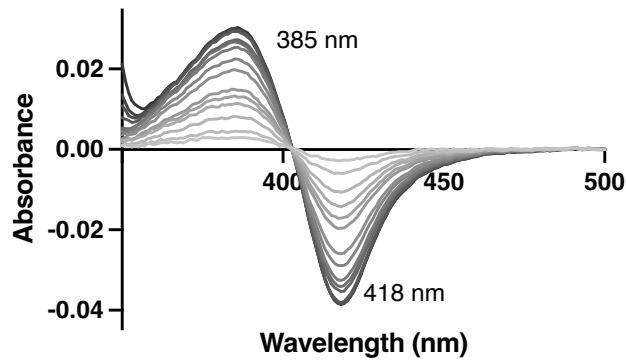
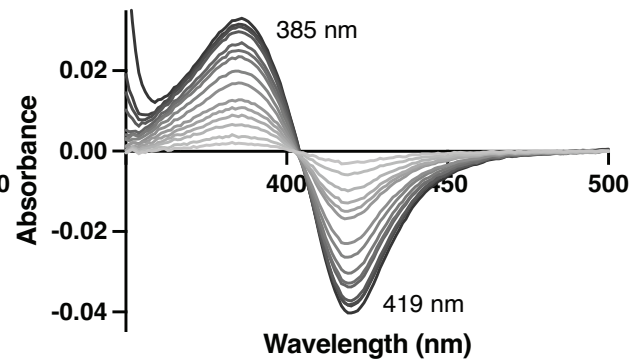
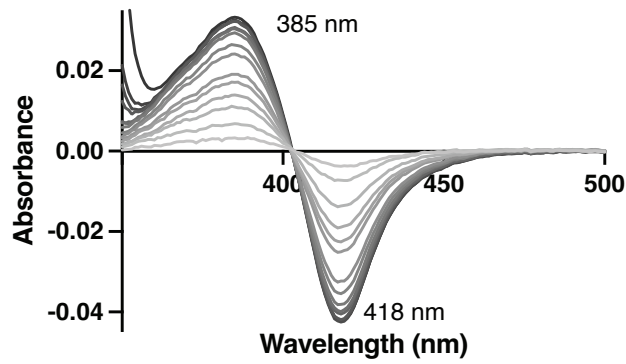
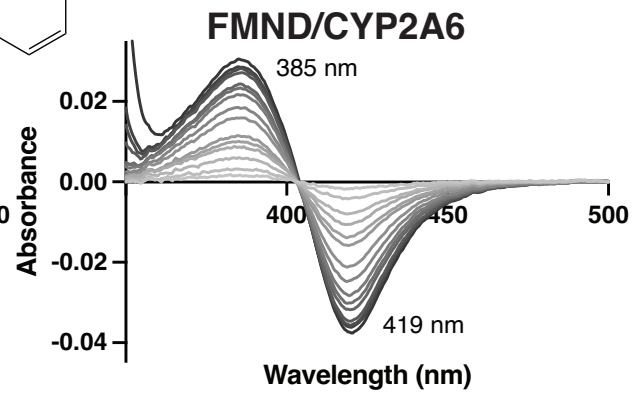
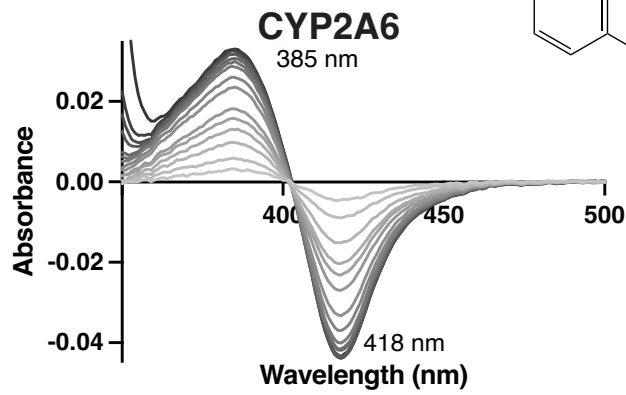
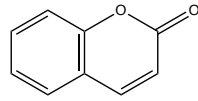
CYP2D6



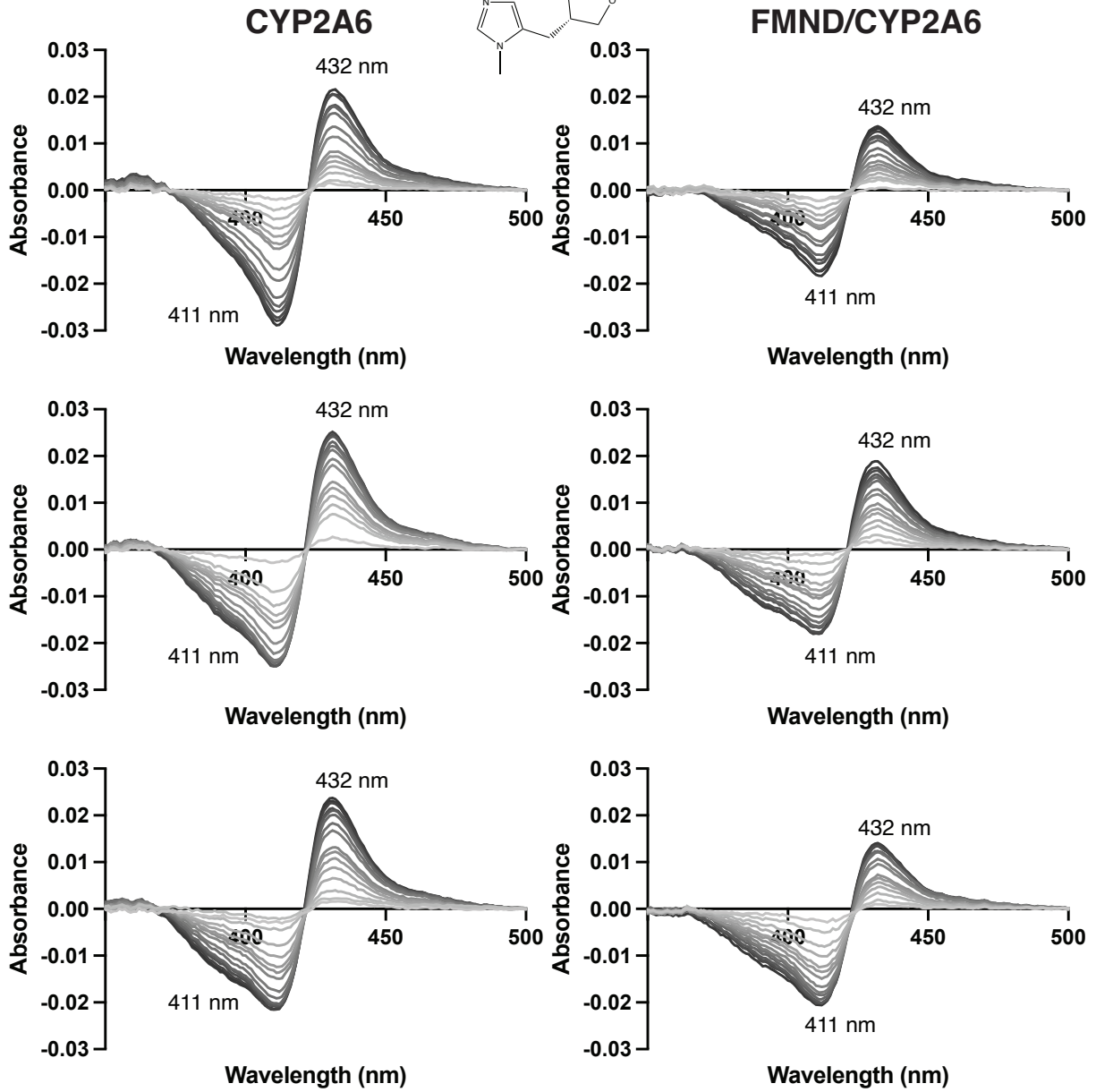
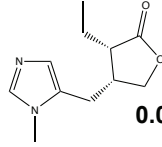
FMND/CYP2D6



Coumarin

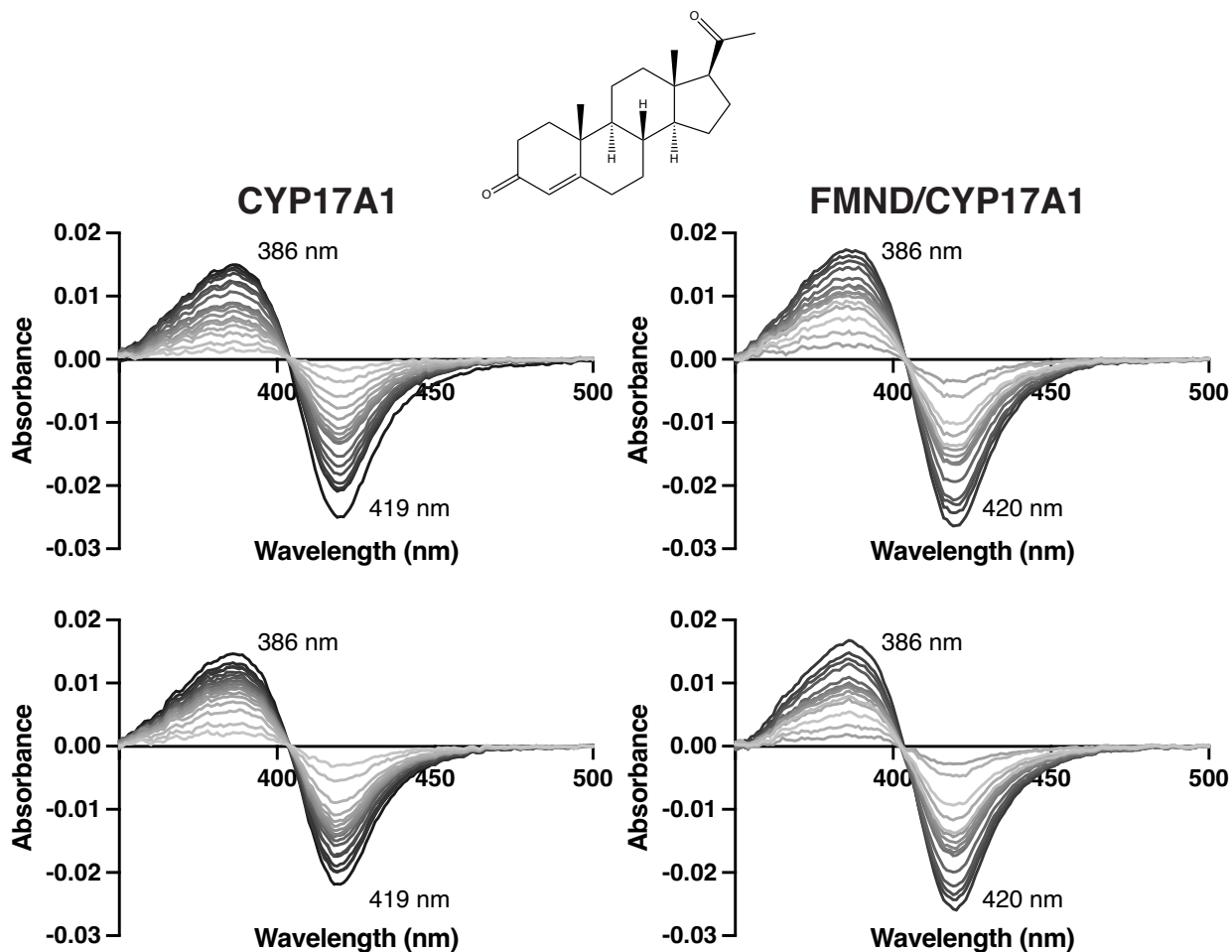


Pilocarpine

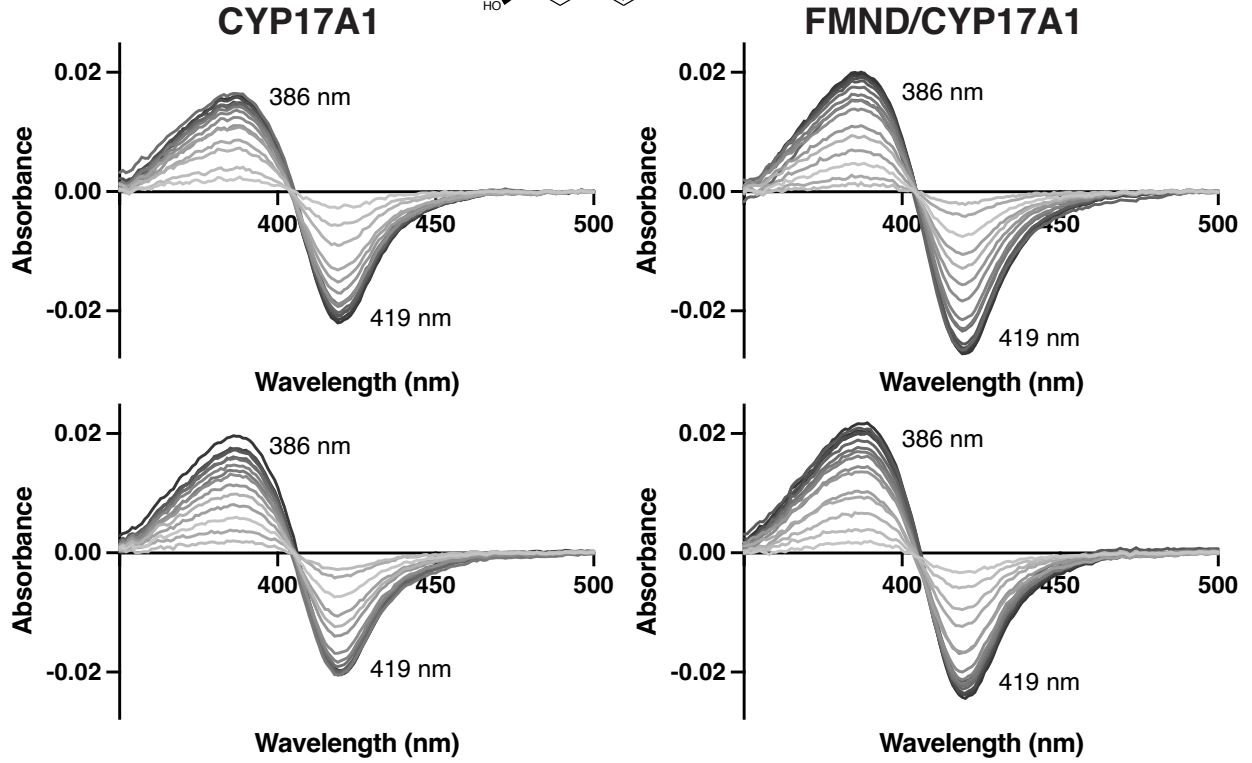
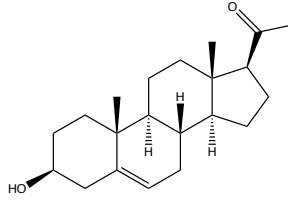


Supporting Figure 5. Spectral titrations for steroidogenic CYP17A1 and CYP21A2 and their corresponding FMND/CYP450 fusion proteins. Low ligand concentrations are shown in light grey and higher concentrations in darker grey. For CYP17A1 the ligands and their concentration ranges are progesterone (20 nM-12.2 μ M), pregnenolone (20 nM-5 μ M), 17 α -hydroxyprogesterone (20 nM-23 μ M), 17 α -hydroxypregnenolone (20 nM-5 μ M), and ketoconazole (20 nM-2.6 μ M). For CYP21A2 the ligands and their concentration ranges are progesterone (50 nM-33 μ M), 17 α -hydroxyprogesterone (100 nM-30 μ M), and ketoconazole (20 nM-20 μ M).

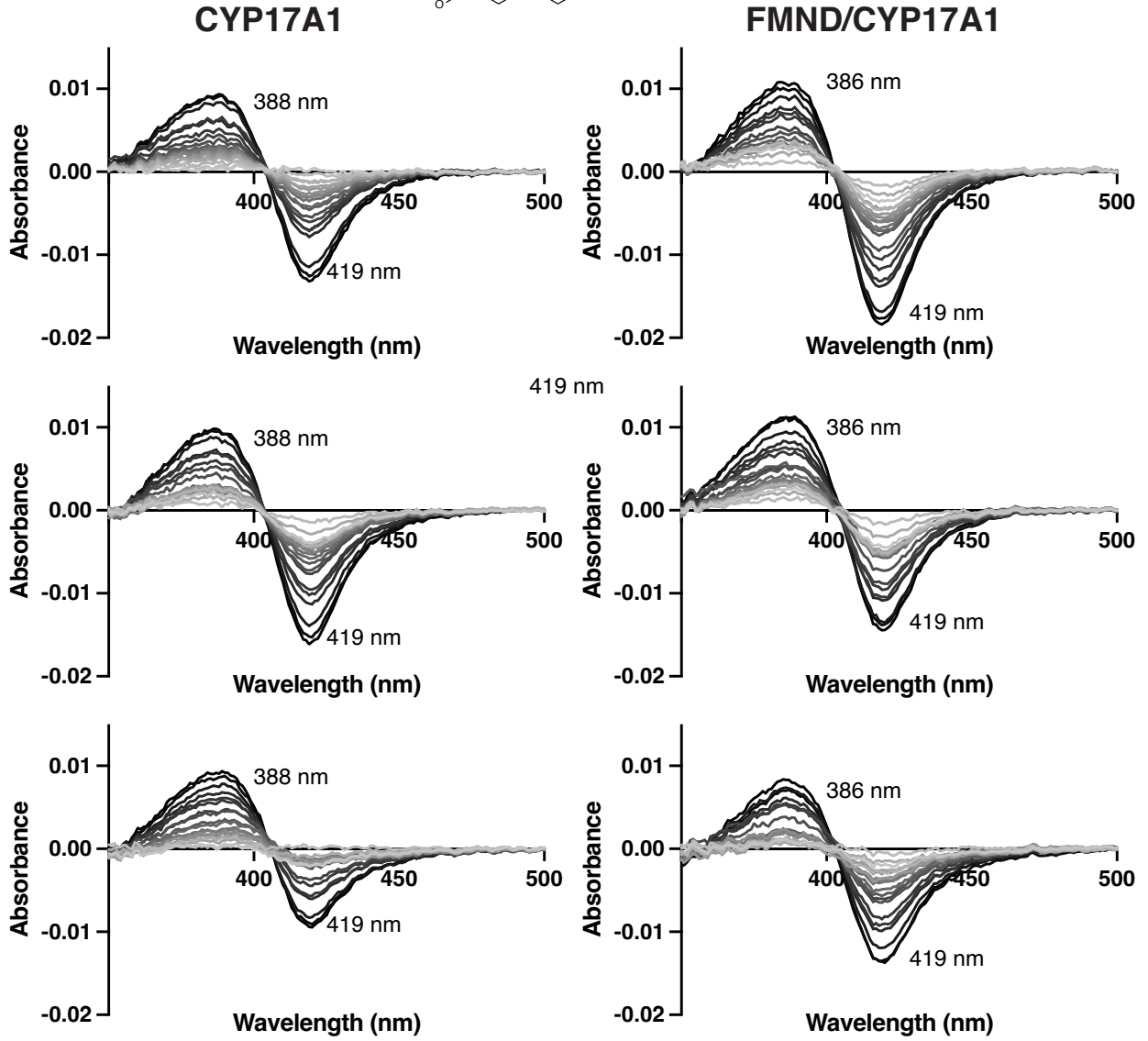
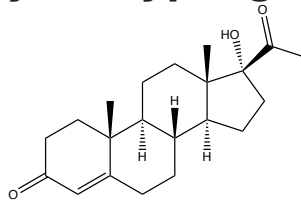
Progesterone



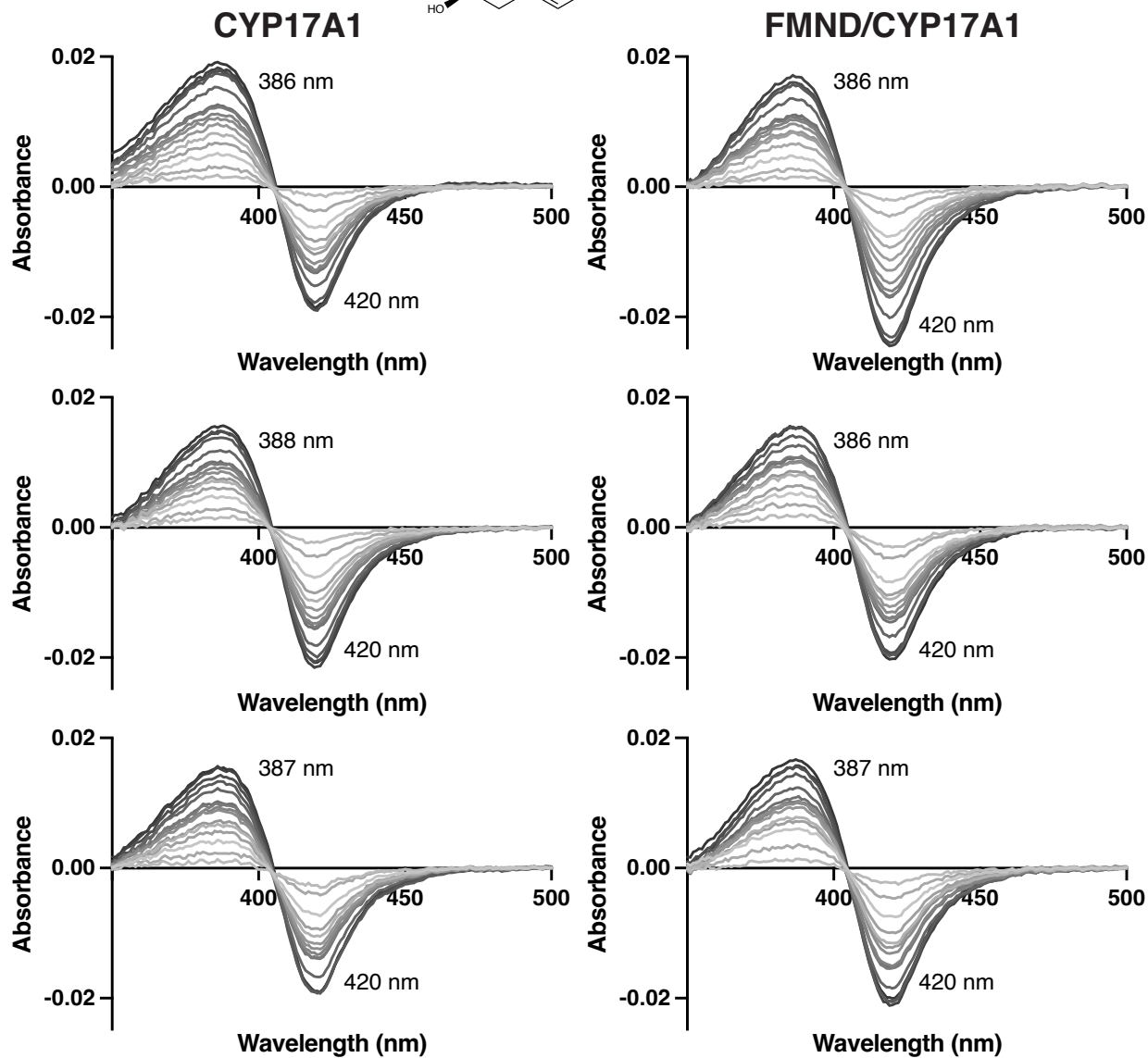
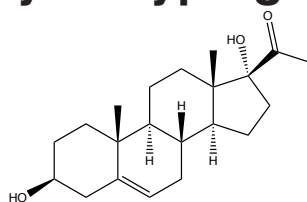
Pregnenolone



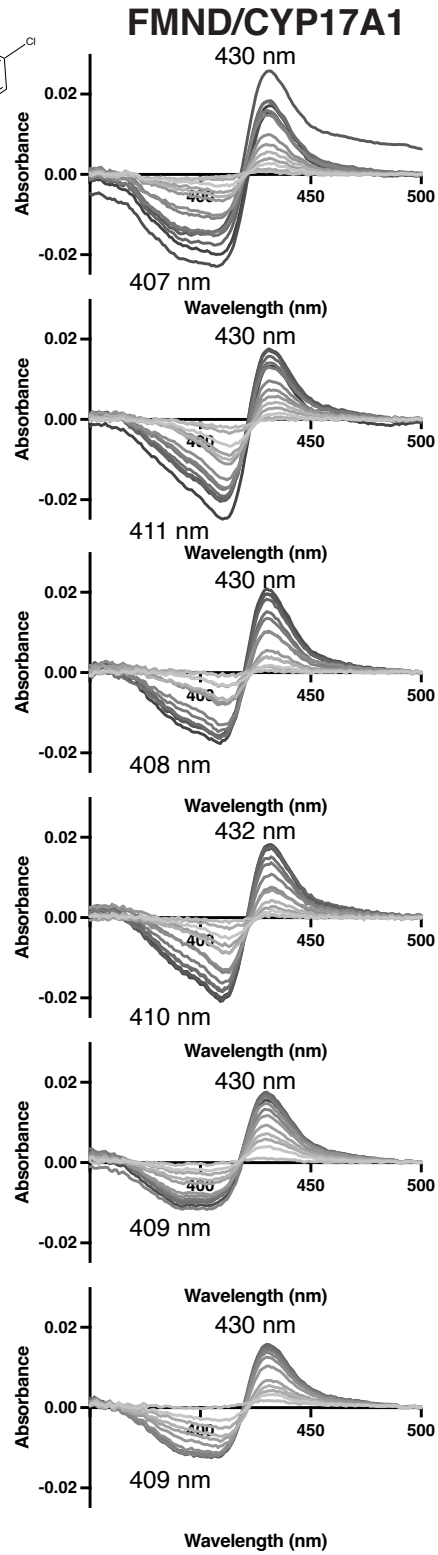
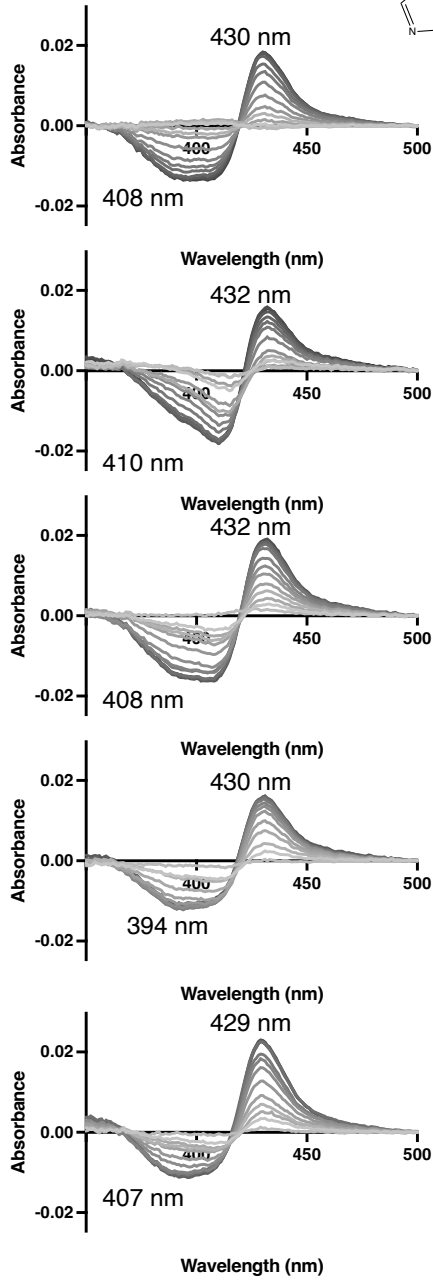
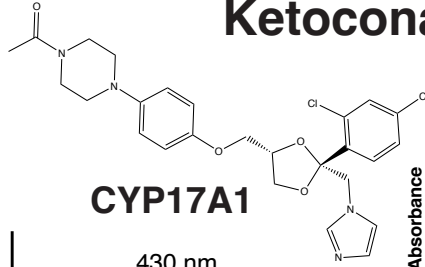
17 α -Hydroxyprogesterone



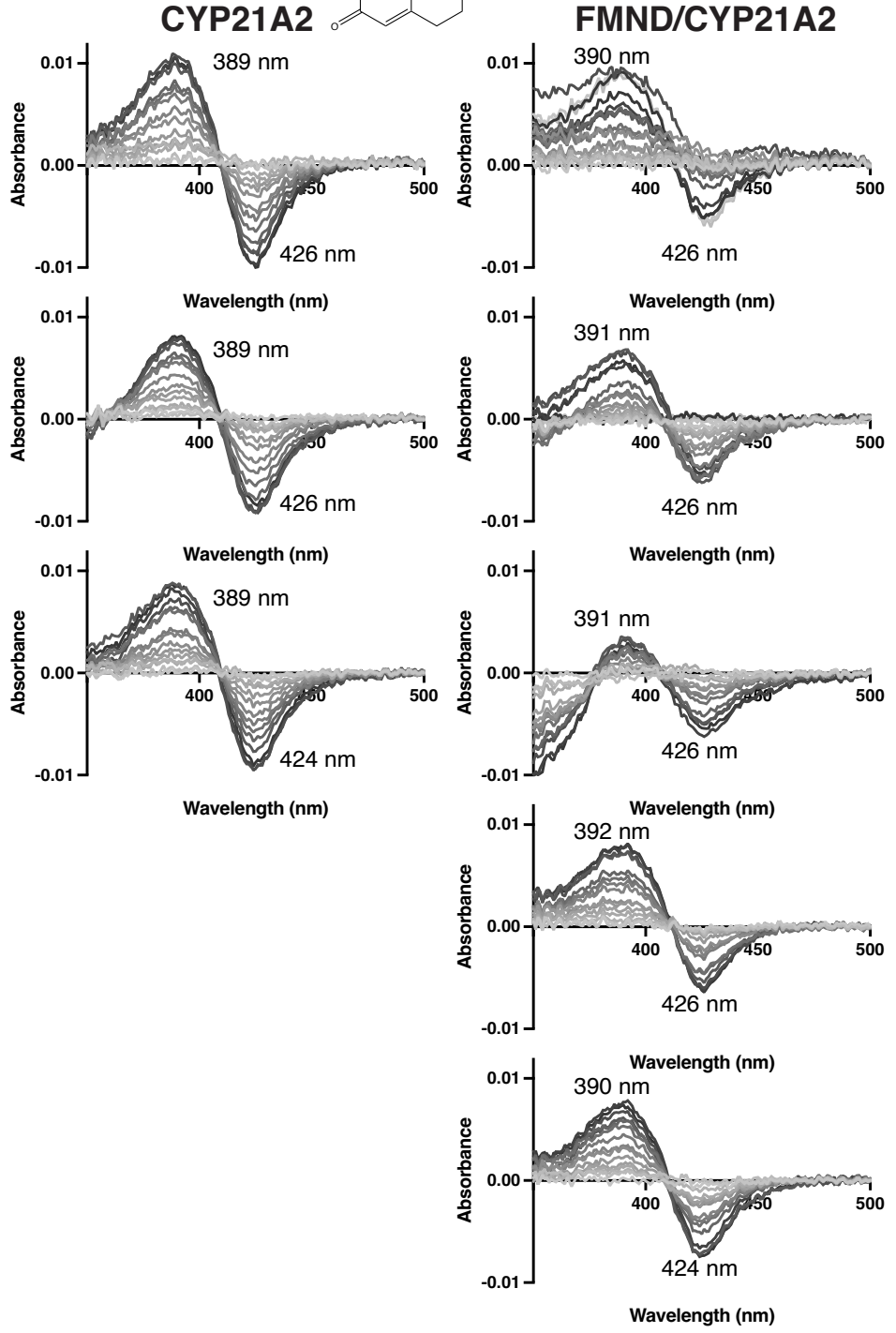
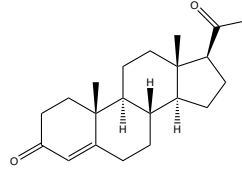
17 α -Hydroxypregnenolone



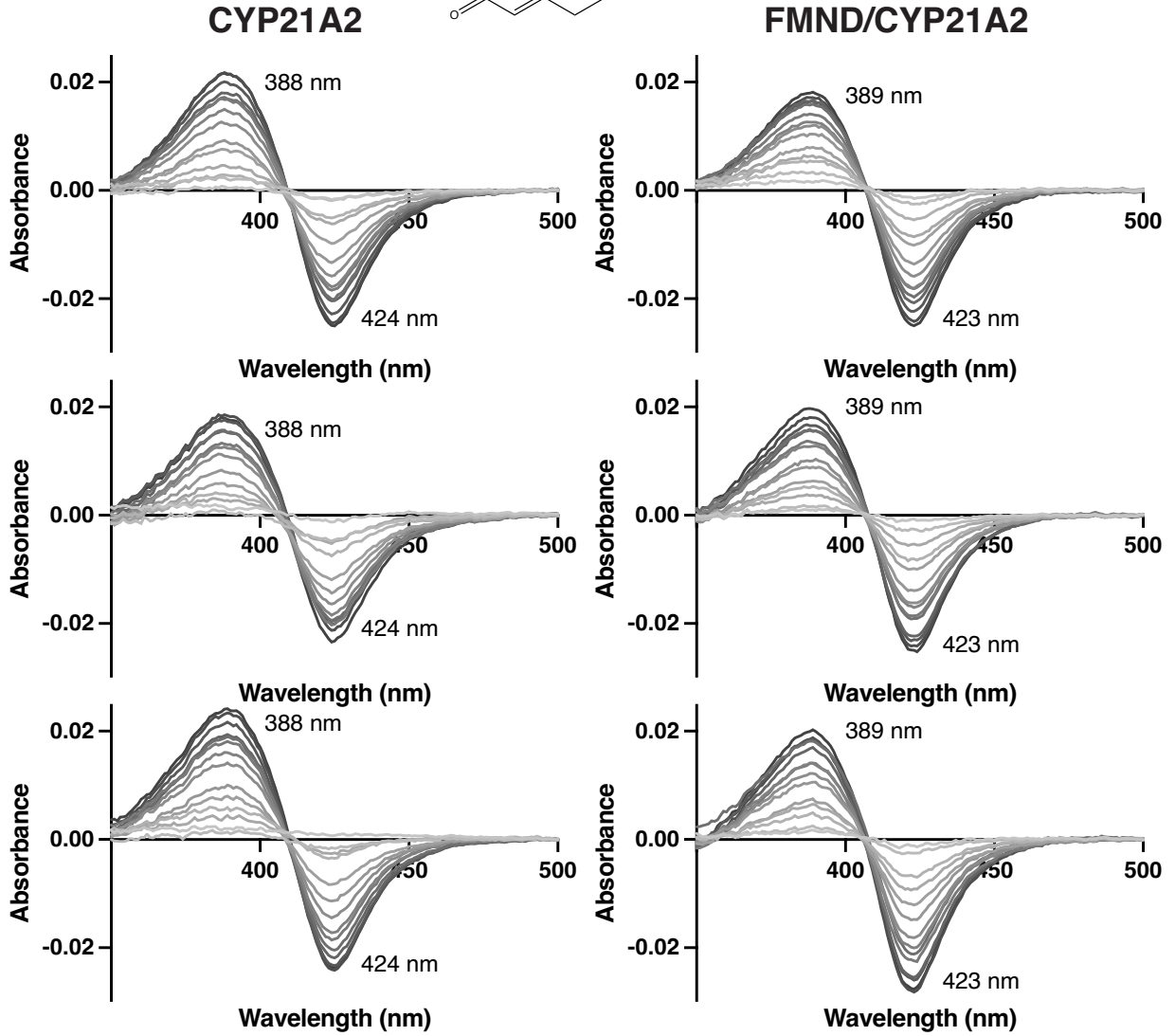
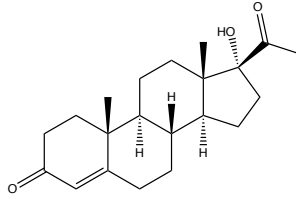
Ketoconazole



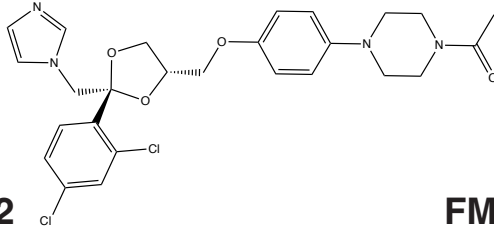
Progesterone



17 α -Hydroxyprogesterone



Ketoconazole



CYP21A2

FMND/CYP21A2

