Supplementary Information

CW EPR Parameters Reveal Cytochrome P450 Binding Modes

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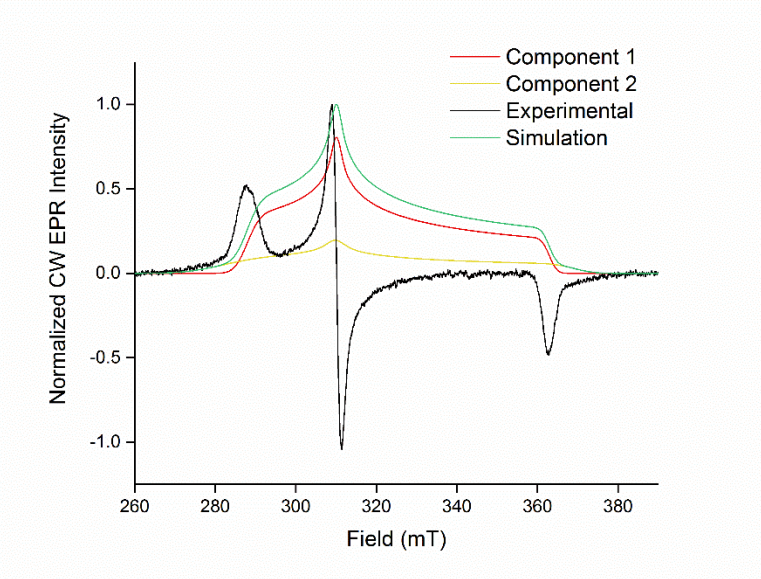
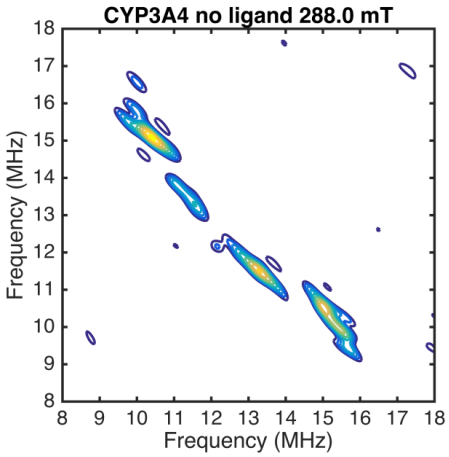
SI Section 1: CW and HYSCORE spectra

CW spectra (plotted as absorbance) and corresponding HYSCORE spectra. Assignments are listed and justified, where WB and DC stand for water-bridged and directly-coordinated components, respectively. Resting state components and components that were left as unknown assignments are also listed.

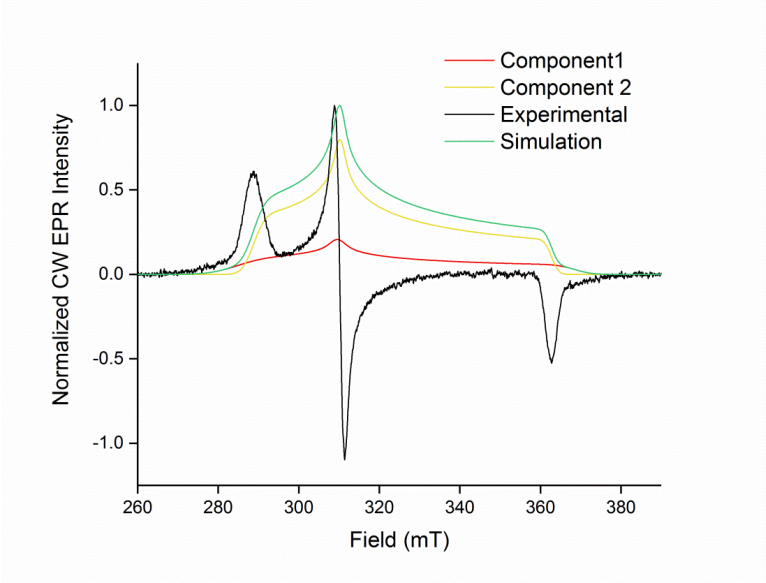
CYP3A4 no ligand

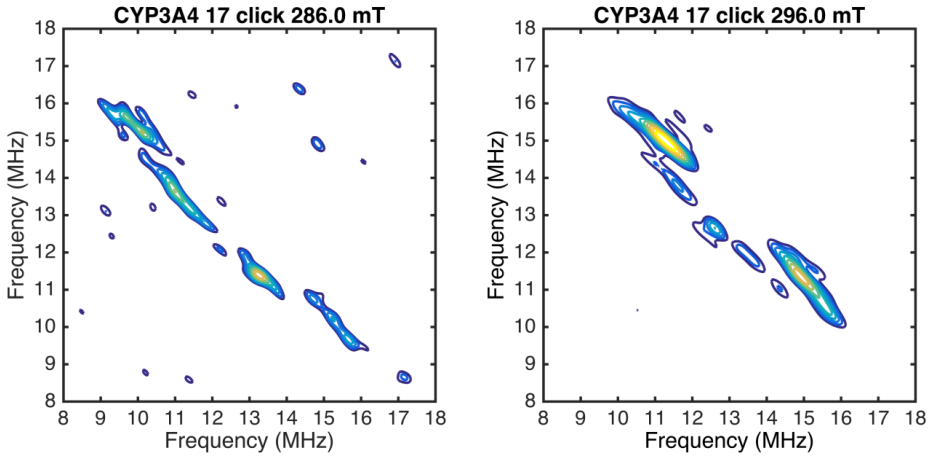




CYP3A4 no ligand component 1: resting state enzyme
CYP3A4 no ligand component 2: no HYSCORE data available; left as unknown.

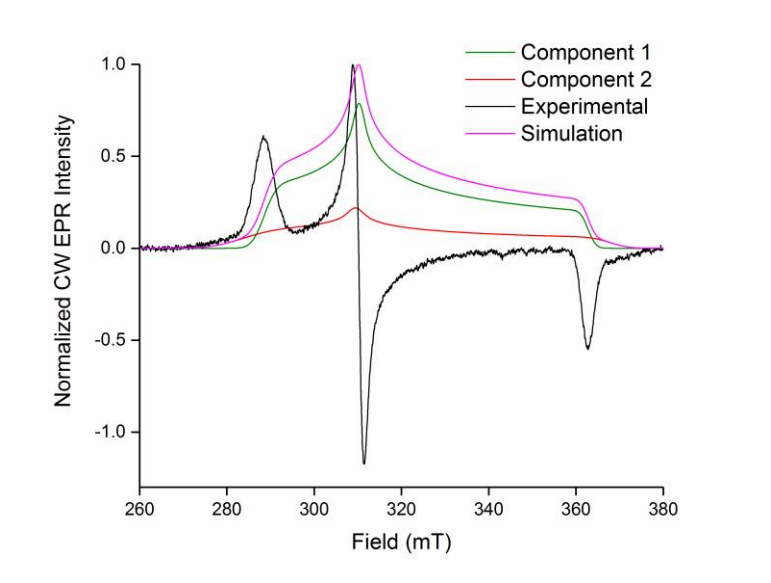
CYP3A4 17-click

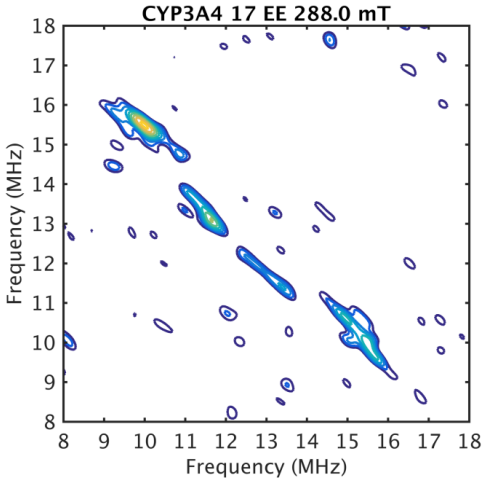




CYP3A4 17-click component 1: WB
CYP3A4 17-click component 2: Unknown, no HYSCORE data available for a lower field where component 1 does not contribute. Water signal significantly decreases relative to cysteine, so DC is likely.

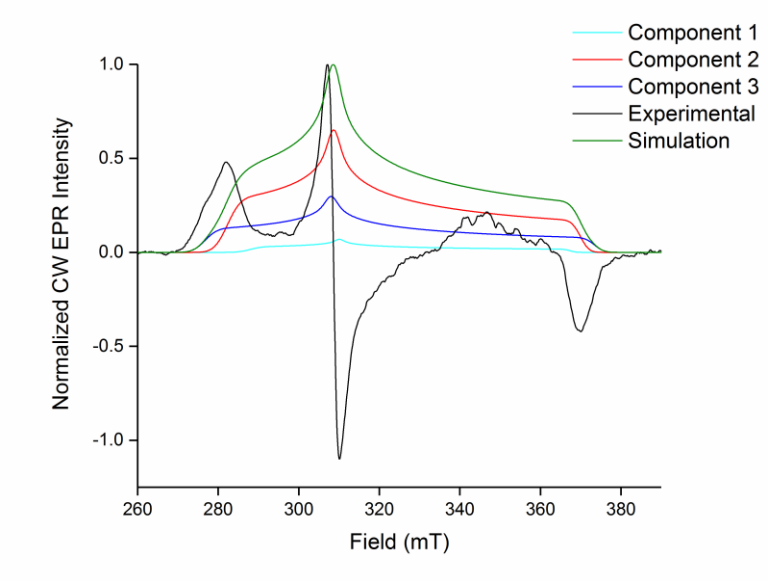
CYP3A4 17-EE

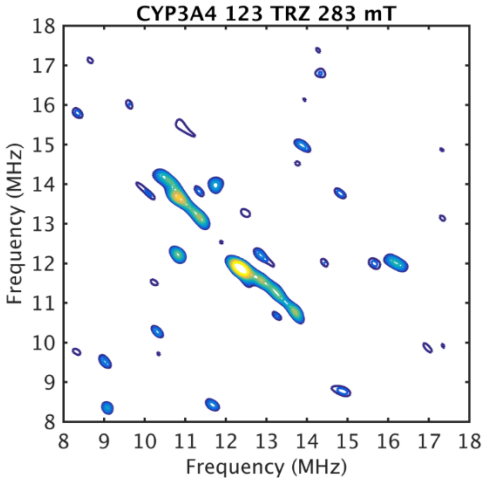




CYP3A4 17-EE component 1: WB
CYP3A4 17-EE component 2: No HYSCORE data available; left as unknown.

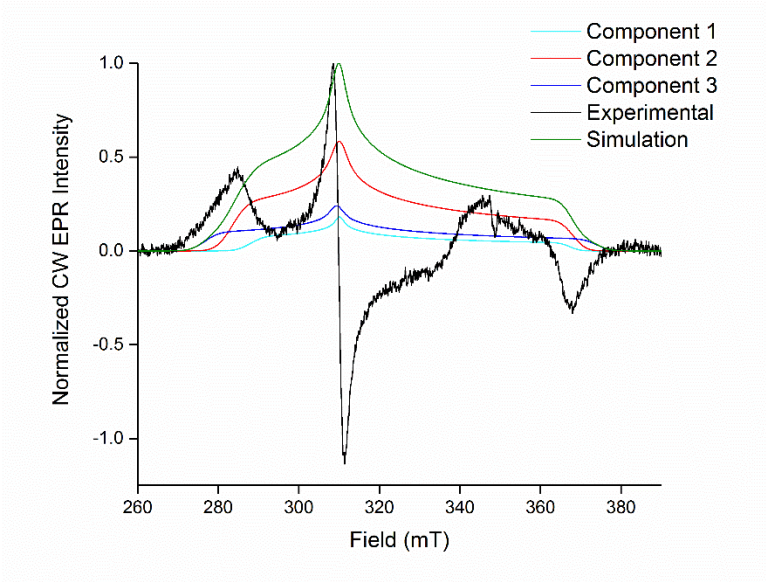
CYP3A4 123-TRZ

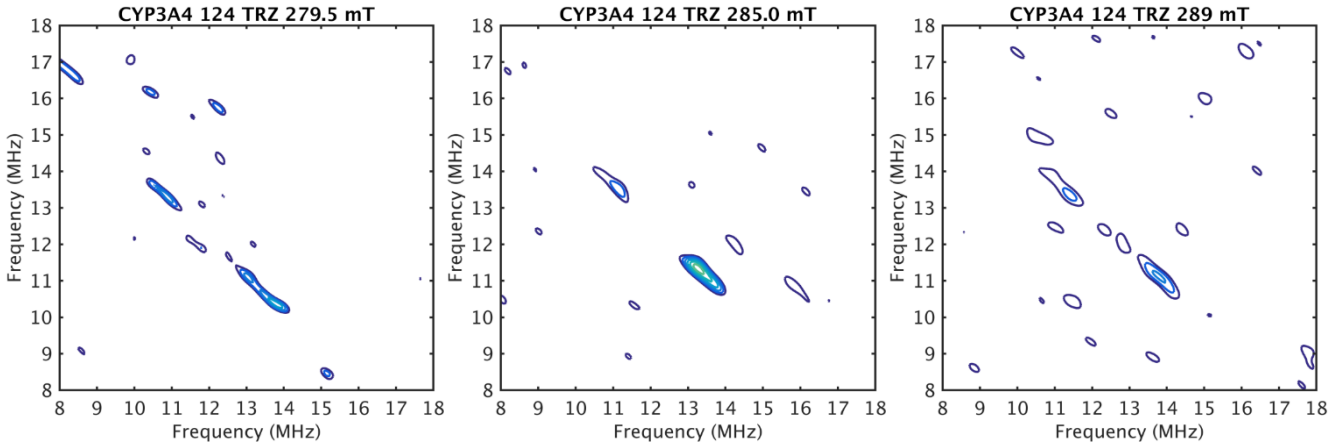




CYP3A4 123-TRZ component 1: No HYSCORE data that distinguishes it from other components; left as unknown.
CYP3A4 123-TRZ component 2: DC; makes up significant amount of signal at 283.0 mT
CYP3A4 123-TRZ component 3: DC; makes up significant amount of signal at 283.0 mT

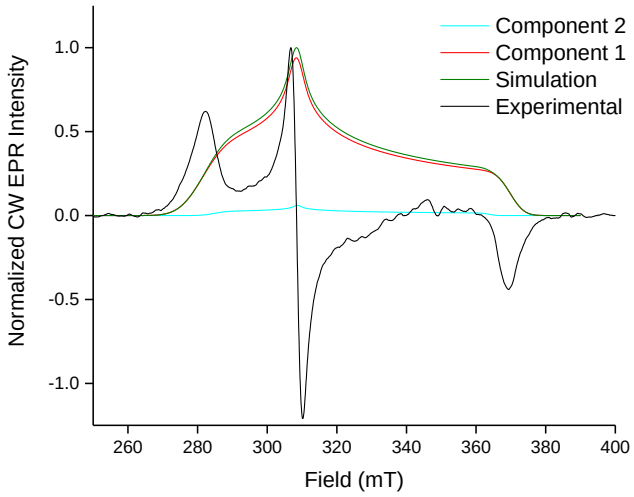
CYP3A4 124-TRZ

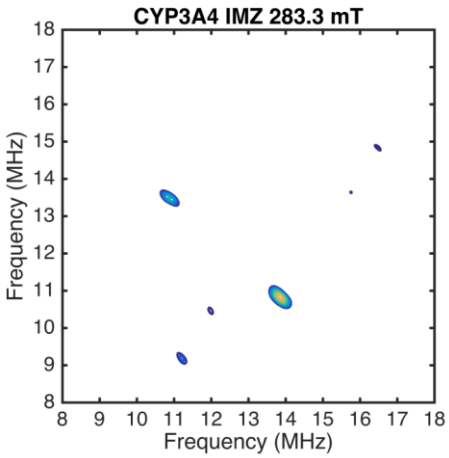




CYP3A4 124-TRZ component 1: Potential water protons not above noise level; left as unknown.
CYP3A4 124-TRZ component 2: DC
CYP3A4 124-TRZ component 3: DC

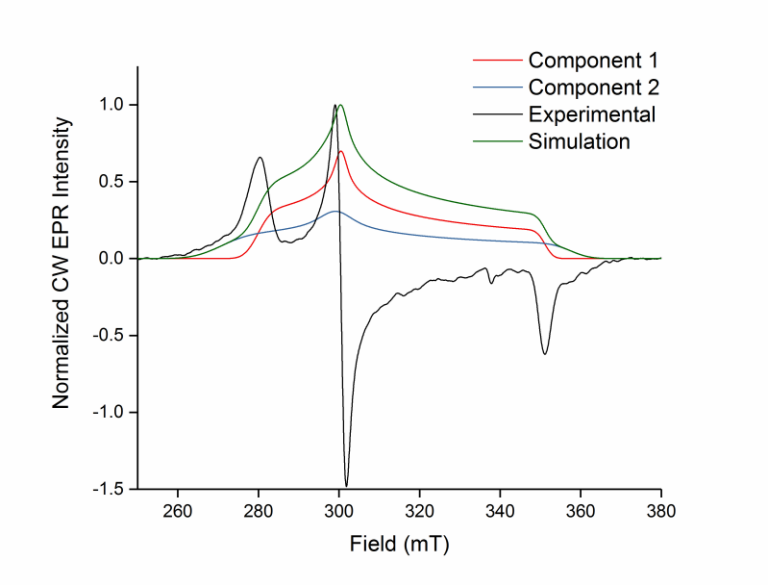
CYP3A4 IMZ

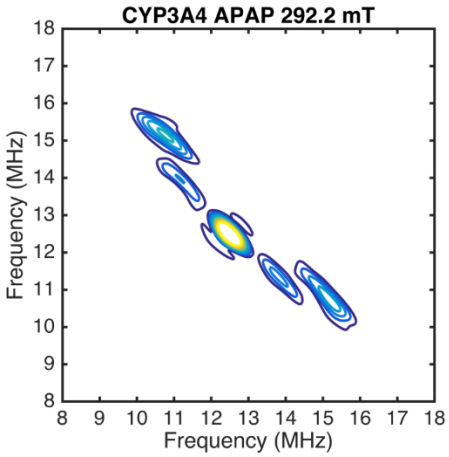




CYP3A4 IMZ component 1: DC; no water protons at a field where component 1 contributes significantly to overall signal.
CYP3A4 IMZ component 2: UKN; overlaps with component 1. No HYSCORE data available that will sufficiently separate it from component 1.

CYP3A4 APAP

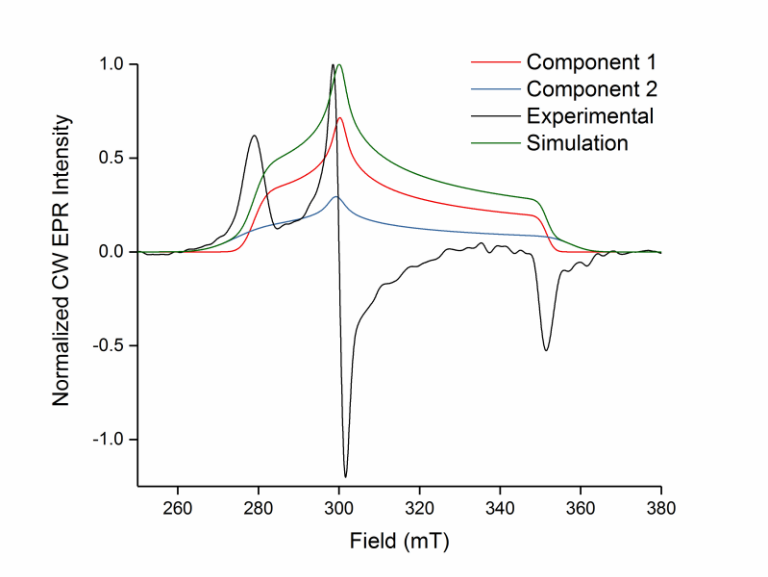


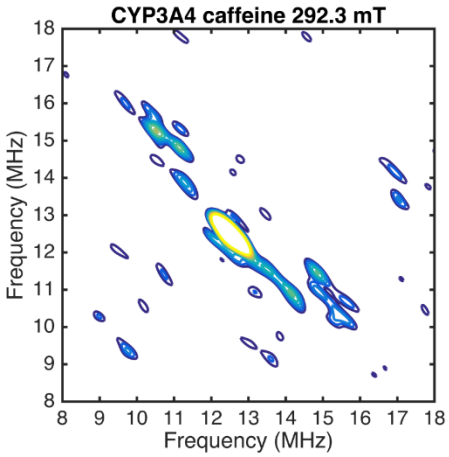


CYP3A4 APAP component 1: WB; intense water proton peaks at a field where component 1 contributes significantly to overall signal.

CYP3A4 APAP component 2: UKN; overlaps with component 1. No HYSCORE data available that will sufficiently separate it from component 1.

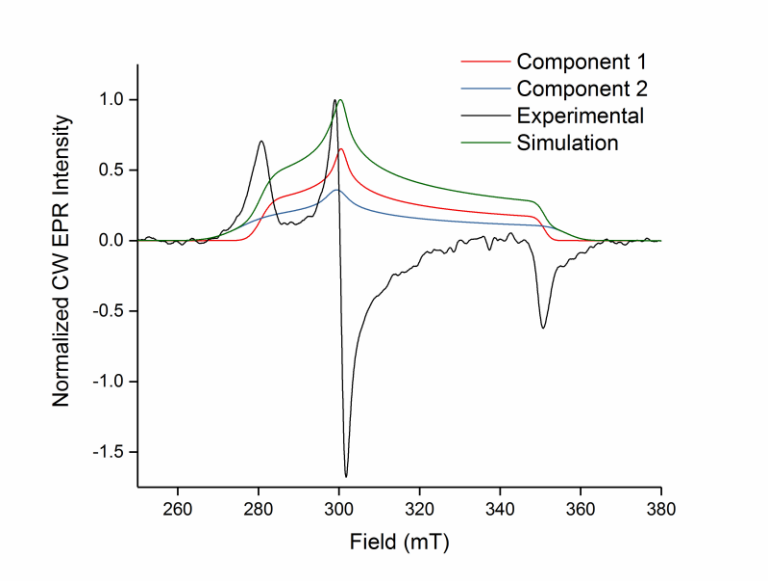
CYP3A4 caffeine

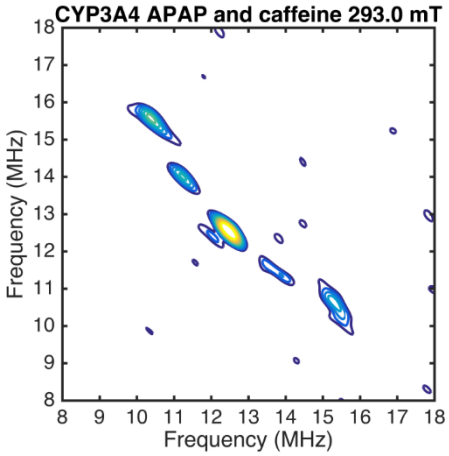




CYP3A4 caffeine component 1: WB; intense water proton peaks at a field where component 1 contributes significantly to overall signal.
CYP3A4 caffeine component 2: UKN; overlaps with component 1. No HYSCORE data available that will sufficiently separate it from component 1.

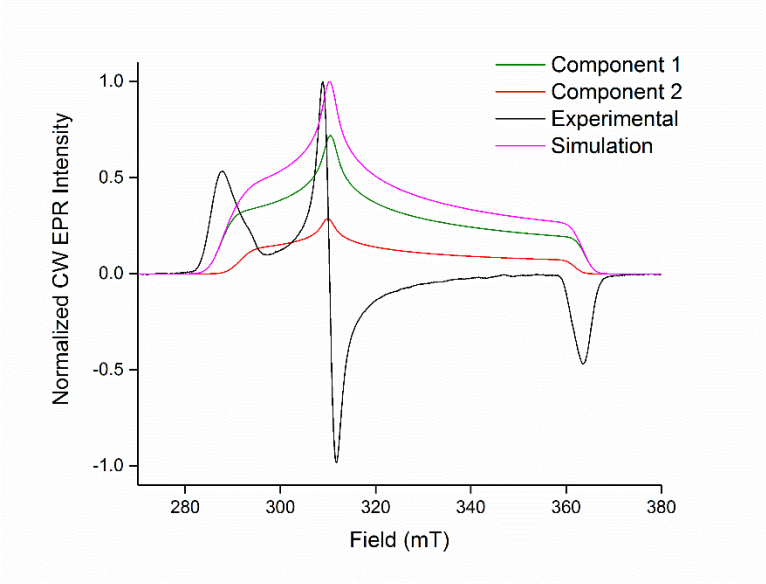
CYP3A4 APAP/caffeine

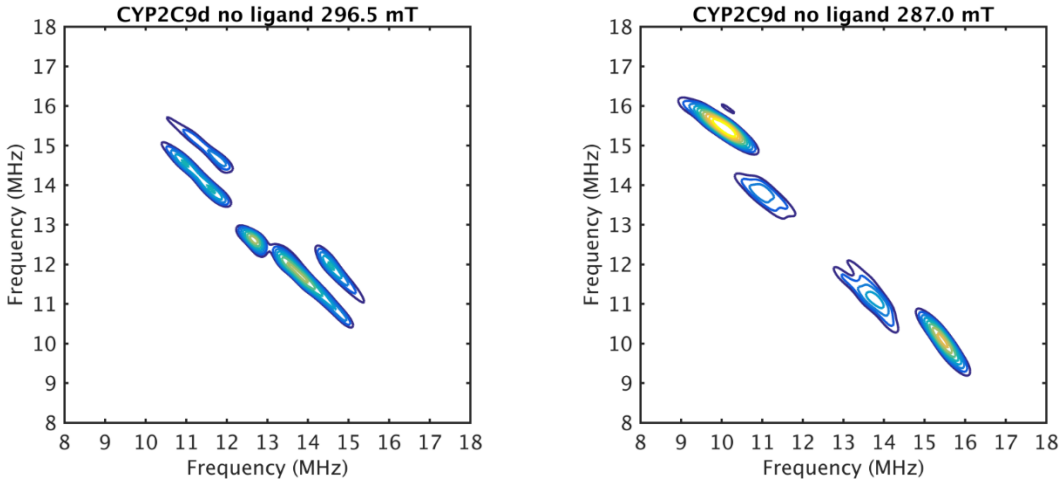




CYP3A4 APAP/caffeine component 1: WB; intense water proton peaks at a field where component 1 contributes significantly to overall signal.
CYP3A4 APAP/caffeine component 2: UKN; overlaps with component 1. No HYSCORE data available that will sufficiently separate it from component 1.

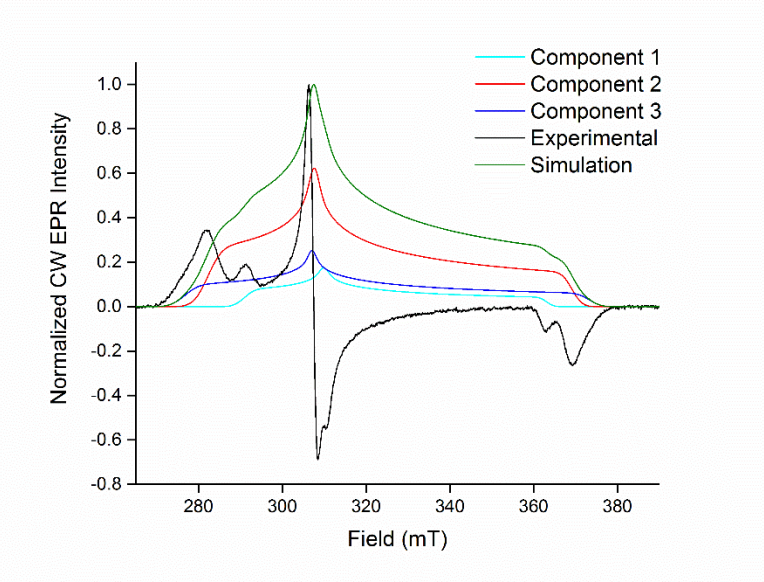
CYP2C9d No Ligand

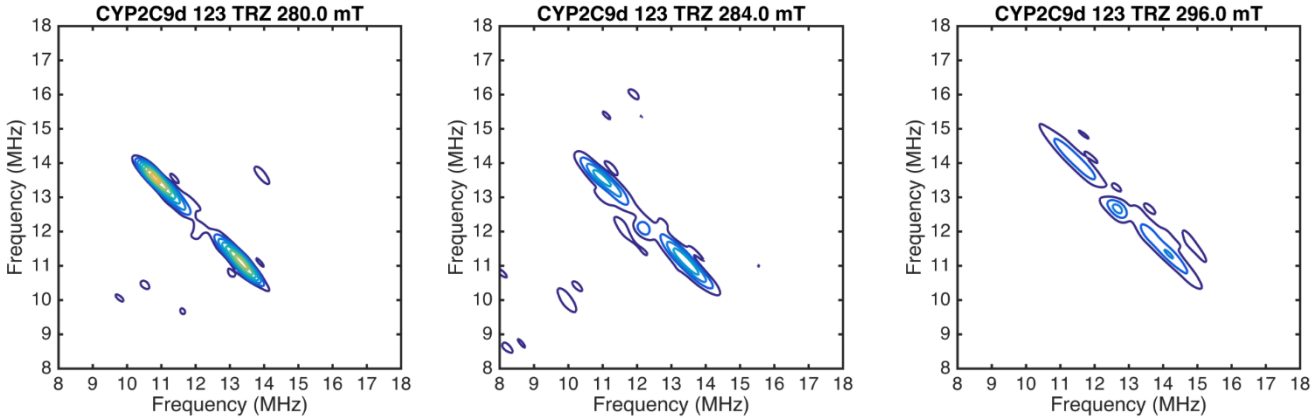




CYP2C9d no ligand component 1: resting state enzyme
CYP2C9d no ligand component 2: resting state enzyme

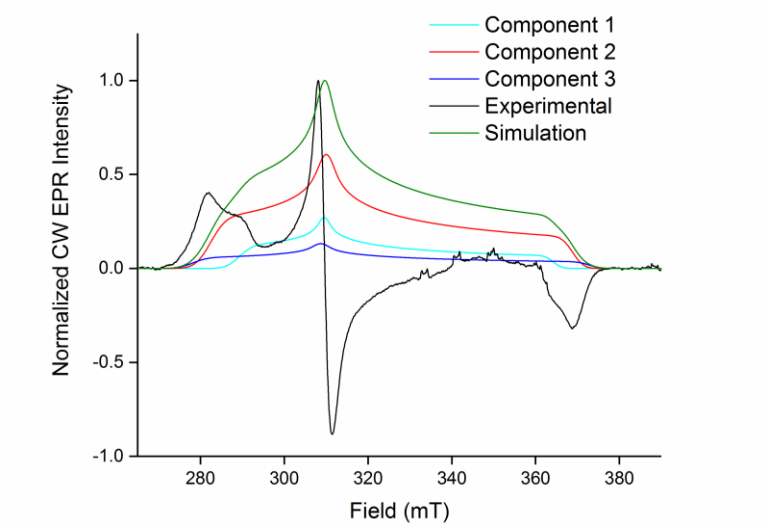
CYP2C9d 123-TRZ

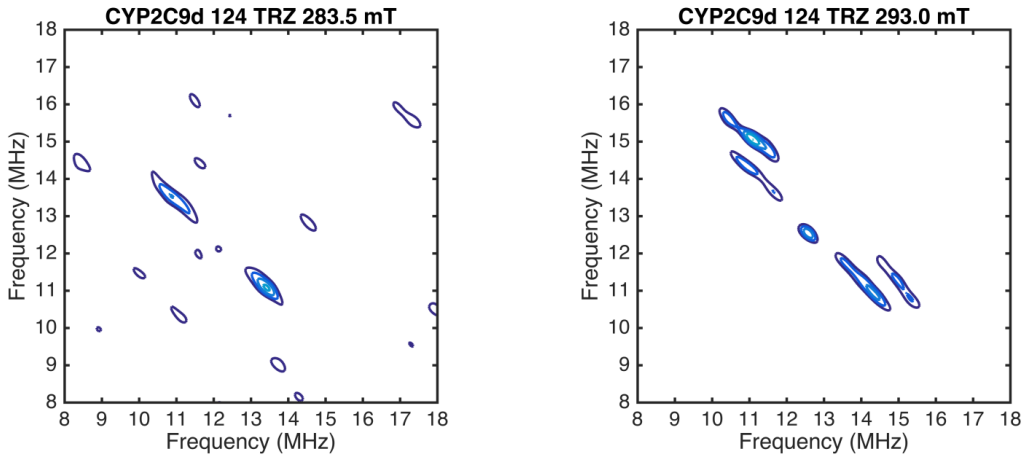




CYP2C9d 123-TRZ component 1: WB. Proton signal is weak, but it is not present at all at lower fields where only components 2 and 3 are present. Component 1 does not make up the majority of the signal at 296.0 mT, so the water proton peaks are expected to be weak if they are present.
CYP2C9d 123-TRZ component 2: DC
CYP2C9d 123-TRZ component 3: DC

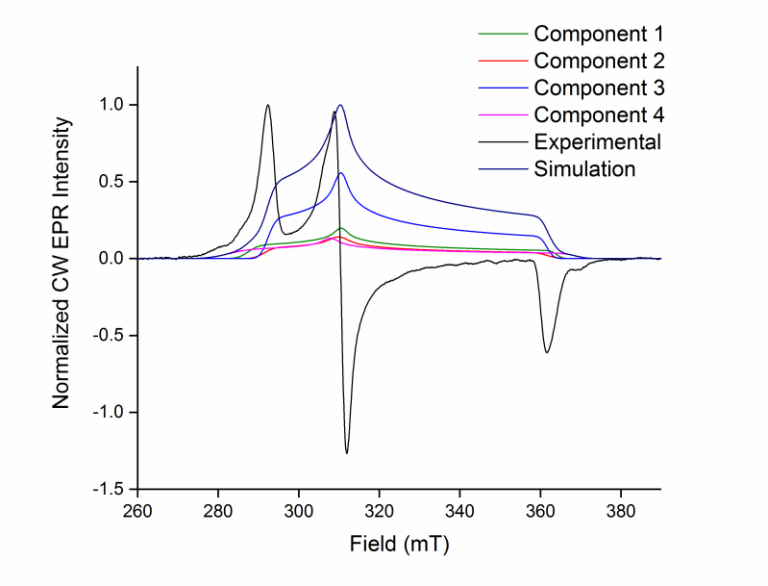
CYP2C9d 124-TRZ

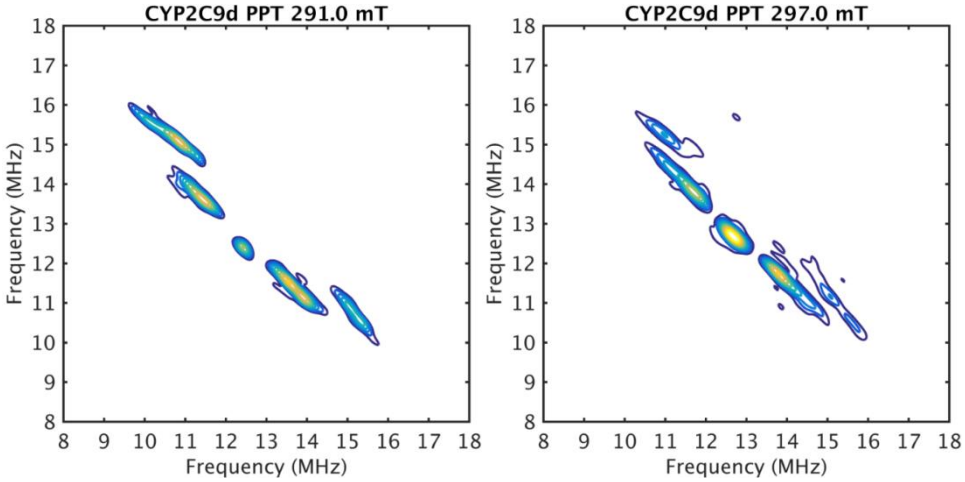




CYP2C9d 124-TRZ component 1: WB. The water proton peaks are not present at 283.5 mT, where only components 2 and 3 are present. They are relatively intense at 293.0 mT where component 1 starts to contribute to the overall signal.
CYP2C9d 124-TRZ component 2: DC
CYP2C9d 124-TRZ component 3: DC

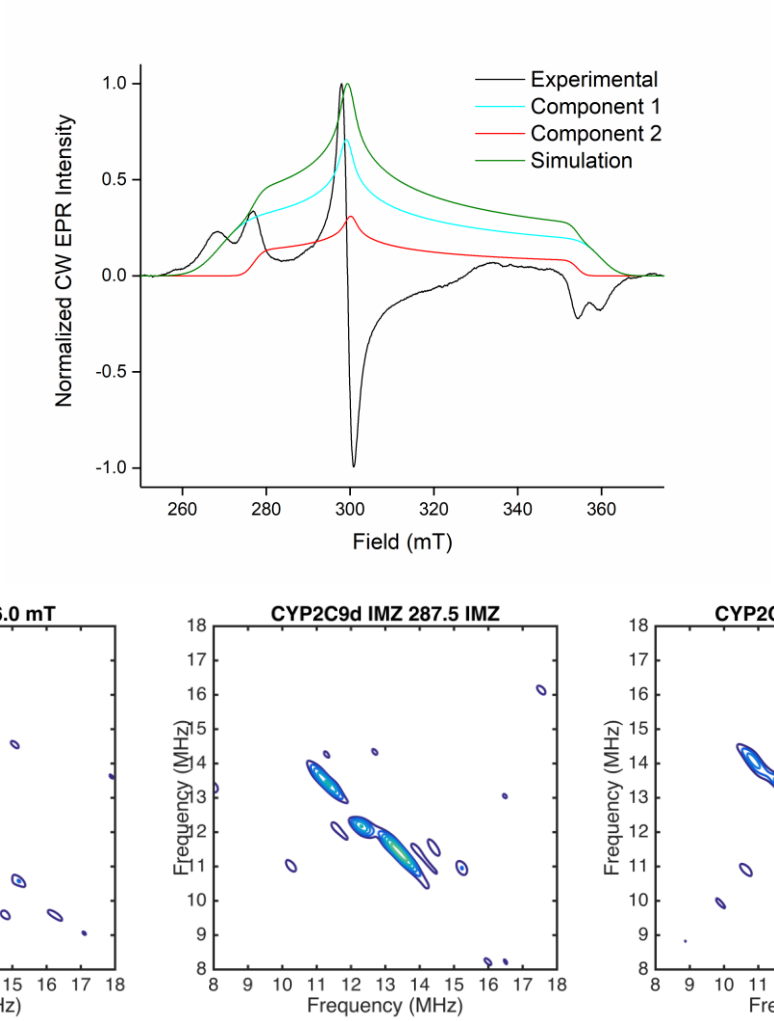
CYP2C9d PPT





CYP2C9d PPT component 1: Residual resting state enzyme
CYP2C9d PPT component 2: Residual resting state enzyme
CYP2C9d PPT component 3: DC. It is the only signal that is dramatically increasing from 291.0 mT to 297.0 mT. The only other signals that increases in that range are components 1 and 2, but those represent residual resting state enzyme and therefore should increase the intensity of the water proton peaks in that range. The intensity decreases, suggesting that component 3 is DC.
CYP2C9d PPT component 4: No HYSCORE data available at a field that distinguishes it from other components; left as unknown.

CYP2C9d IMZ



CYP2C9d IMZ component 1: DC. No water peaks present at a field where it contributes significantly to the overall spectrum.
CYP2C9d IMZ component 2: DC. Still no water peaks present at fields where it is contributing to overall spectrum.

SI Section 2: Tables of drug concentrations and all CW parameters

Sample	Concentration
CYP3A4 with 123-TRZ	50 mM
CYP3A4 with 124-TRZ	40 µM
CYP3A4 with 17-click	225 µM
CYP3A4 with 17-EE	160 µM
CYP3A4 with acetaminophen	1 mM
CYP3A4 with caffeine	1 mM
CYP3A4 with imidazole	4 mM
CYP2C9d with 123-TRZ	350 µM
CYP2C9d with 124-TRZ	200 µM
CYP2C9d with PPT	350 µM
CYP2C9d with imidazole	500 µM

Table S1: Concentrations of all drugs used.

Sample Component	g_x	g_y	g_z	g_x strain	g_y strain	g_z strain	weight	weight (%)	tetragonal field	rhombicity	binding mode
CYP3A4 resting	1.923	2.251	2.425	0.019	0.015	0.050	1.740	76.30	5.357	0.914	resting
CYP3A4 resting	1.891	2.255	2.486	0.046	0.025	0.152	0.540	23.70	5.129	0.816	resting
CYP3A4 with 17click	1.924	2.250	2.418	0.017	0.015	0.045	0.915	76.30	5.342	0.930	WB
CYP3A4 with 17click	1.894	2.256	2.457	0.041	0.023	0.109	0.284	23.60	4.986	0.884	UKN
CYP3A4 with 17EE	1.924	2.250	2.420	0.018	0.015	0.044	0.919	75.10	5.365	0.922	WB
CYP3A4 with 17EE	1.897	2.257	2.467	0.041	0.021	0.115	0.304	24.90	5.076	0.859	UKN
CYP3A4 with 123 Triazole	1.900	2.251	2.425	0.014	0.013	0.045	0.120	5.80	4.968	0.945	UKN
CYP3A4 with 123 Triazole	1.887	2.261	2.474	0.021	0.019	0.053	1.300	62.80	4.879	0.871	DC
CYP3A4 with 123 Triazole	1.869	2.266	2.523	0.022	0.020	0.051	0.650	31.40	4.820	0.800	DC
CYP3A4 with 124 Triazole	1.900	2.251	2.425	0.024	0.012	0.053	0.064	15.30	4.968	0.945	UKN
CYP3A4 with 124 Triazole	1.896	2.251	2.464	0.031	0.022	0.063	0.244	58.40	5.152	0.848	DC
CYP3A4 with 124 Triazole	1.866	2.256	2.524	0.032	0.019	0.061	0.110	26.30	4.954	0.771	DC
CYP3A4 with IMZ	1.919	2.261	2.452	0.019	0.012	0.057	0.089	78.40	4.911	0.868	DC
CYP3A4 with IMZ	1.887	2.265	2.485	0.038	0.024	0.110	1.693	21.60	4.593	0.817	UKN
CYP3A4 with APAP	1.924	2.248	2.414	0.021	0.017	0.051	2.312	57.90	5.351	0.934	WB
CYP3A4 with APAP	1.891	2.255	2.486	0.053	0.043	0.115	1.679	42.10	5.129	0.816	UKN
CYP3A4 with APAP and 1mM caffeine	1.925	2.247	2.408	0.019	0.016	0.048	2.094	58.30	5.345	0.948	WB
CYP3A4 with APAP and 1mM caffeine	1.896	2.257	2.458	0.042	0.030	0.121	1.496	41.70	5.013	0.880	UKN
CYP3A4 with 1 mM caffeine	1.920	2.249	2.422	0.020	0.018	0.050	2.149	67.30	5.336	0.917	WB
CYP3A4 with 1 mM caffeine	1.888	2.257	2.470	0.046	0.023	0.123	1.044	32.70	4.944	0.865	UKN

Table S2: All CYP3A4 sample components with their corresponding g-values, g-strains, weights, tetragonal field, rhombicity, and assigned binding mode. Components labeled in blue as “resting” are resting state enzyme, “DC” are directly-coordinated, “WB” are water-bridged, and “UKN” are unknown components where a binding mode could not be assigned.

Sample Component	g_x	g_y	g_z	g_x strain	g_y strain	g_z strain	weight	weight (%)	tetragonal field	rhombicity	binding mode
CYP2C9D resting	1.917	2.248	2.429	0.019	0.016	0.042	0.797	74.00	5.352	0.899	resting
CYP2C9D resting	1.929	2.252	2.396	0.018	0.015	0.042	0.287	26.00	5.214	1.004	resting
CYP2C9D with 123 Triazole	1.922	2.252	2.400	0.017	0.015	0.038	0.240	14.20	5.121	1.001	WB
CYP2C9D with 123 Triazole	1.891	2.269	2.474	0.023	0.015	0.055	1.018	53.70	4.791	0.894	DC
CYP2C9D with 123 Triazole	1.870	2.273	2.523	0.026	0.012	0.057	0.436	23.00	4.711	0.821	DC
CYP2C9D with 124 Triazole	1.916	2.255	2.414	0.020	0.016	0.049	0.304	22.80	5.062	0.976	WB
CYP2C9D with 124 Triazole	1.892	2.251	2.473	0.025	0.024	0.059	0.838	62.70	5.144	0.832	DC
CYP2C9D with 124 Triazole	1.872	2.261	2.510	0.026	0.024	0.059	0.194	14.50	4.881	0.807	DC
CYP2C9D with PPT	1.917	2.248	2.429	0.015	0.018	0.041	0.200	20.00	5.352	0.899	resting
CYP2C9D with PPT	1.929	2.252	2.396	0.026	0.028	0.037	0.140	14.00	5.214	1.004	resting
CYP2C9D with PPT	1.930	2.248	2.388	0.016	0.019	0.030	0.510	51.00	5.263	1.012	DC
CYP2C9D with PPT	1.893	2.268	2.474	0.028	0.021	0.081	0.150	15.00	4.845	0.888	UKN
CYP2C9d with IMZ	1.875	2.257	2.512	0.042	0.016	0.106	1.290	72.60	5.010	0.786	DC
CYP2C9d with IMZ	1.905	2.248	2.437	0.019	0.017	0.038	0.488	27.40	5.188	0.893	DC

Table S3: All CYP2C9d sample components with their corresponding g-values, g-strains, weights, tetragonal field, rhombicity, and assigned binding mode. Components labeled in blue as “resting” are resting state enzyme, “DC” are directly-coordinated, “WB” are water-bridged, and “UKN” are unknown components where a binding mode could not be assigned.

SI Section 3: Annotated figures

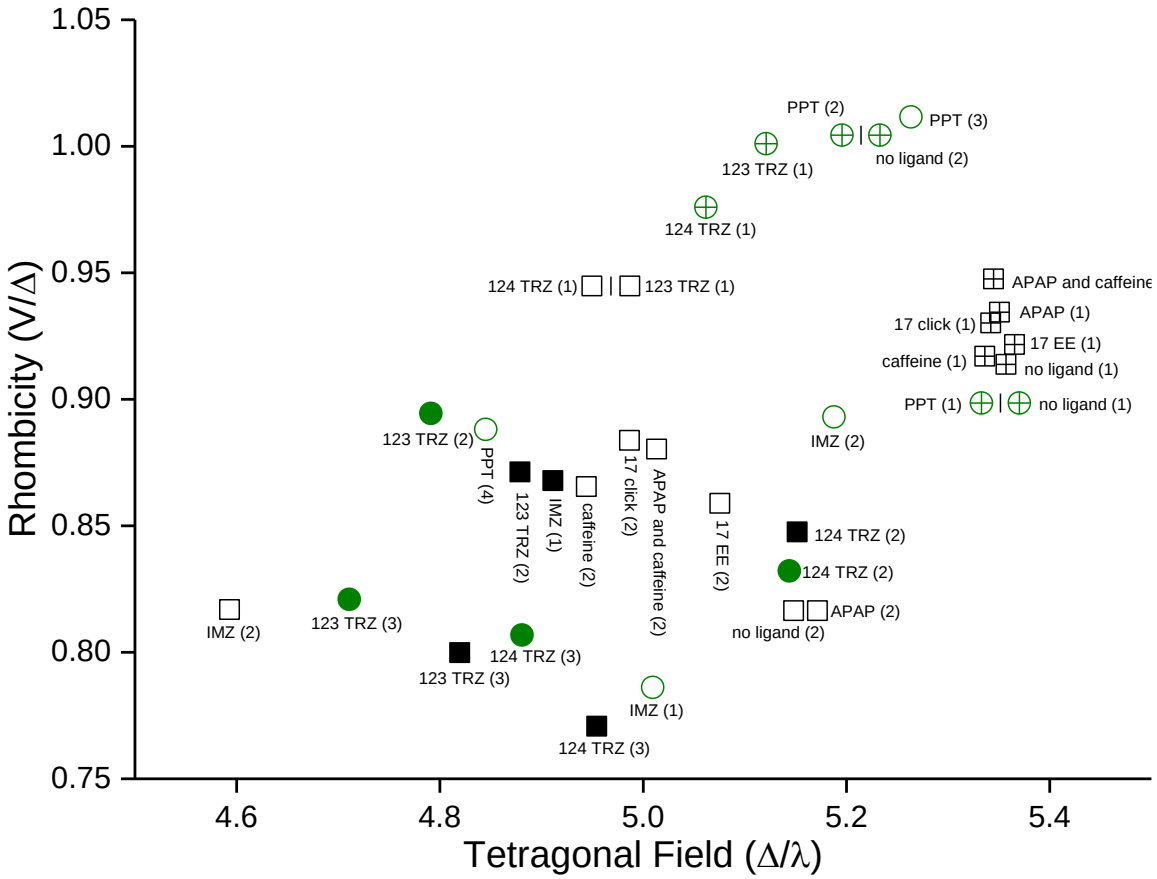


Figure S1: Correlation of binding mode with ligand-field parameters. Points are colored by isoform; green circles are CYP2C9d components and black squares are CYP3A4 components. Solid symbols represent directly-coordinated components, symbols filled with a + represent water-bridged components, and open symbols represent components for which the binding mode could not be assigned. Points have been labeled with their corresponding component.

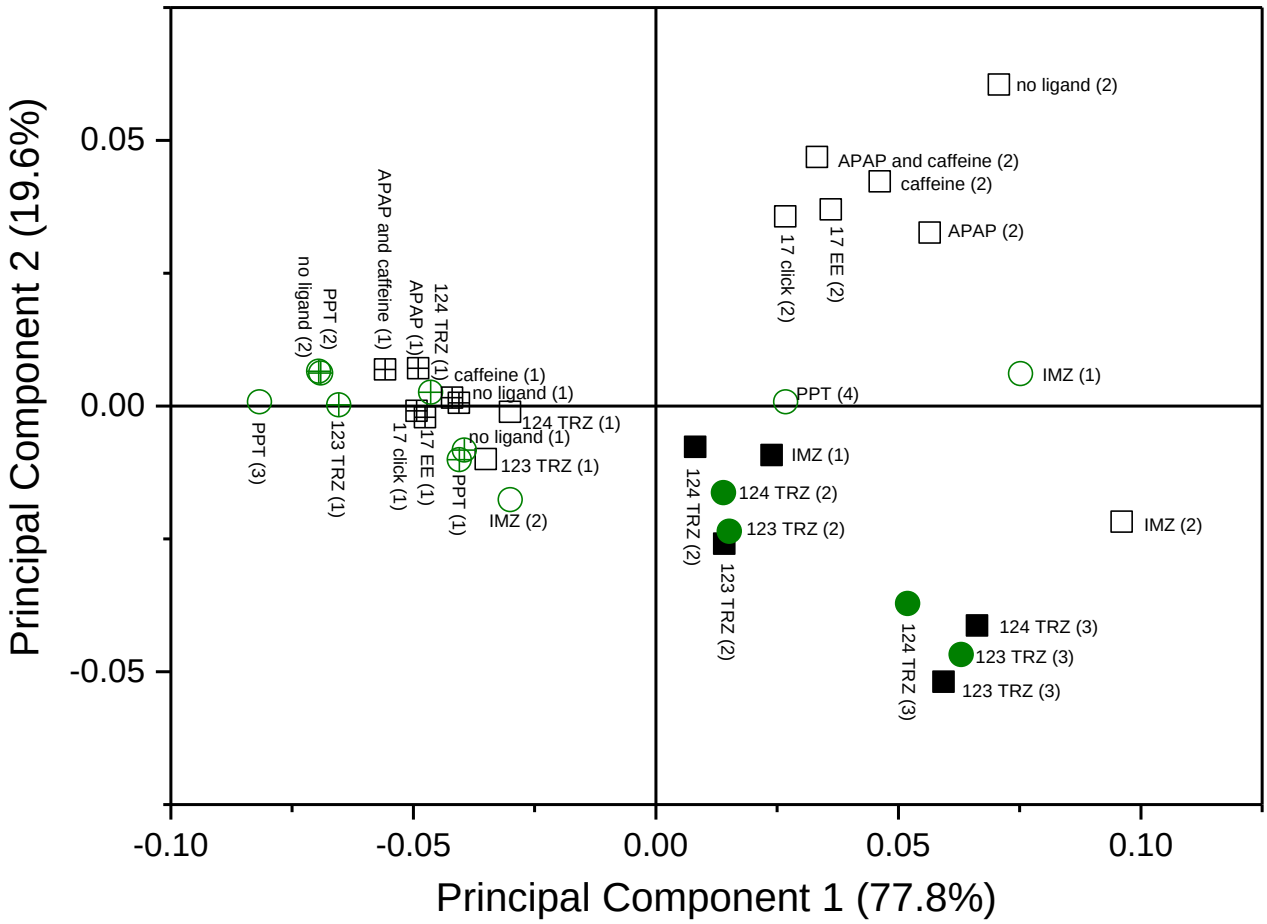


Figure S2: A biplot of the scores with respect to the first and second principal components. Points are colored by isoform; green circles are CYP2C9d components and black squares are CYP3A4 components. Solid symbols represent directly-coordinated components, symbols filled with a + represent water-bridged components, and open symbols represent components for which the binding mode could not be assigned. Points have been labeled with their corresponding component.