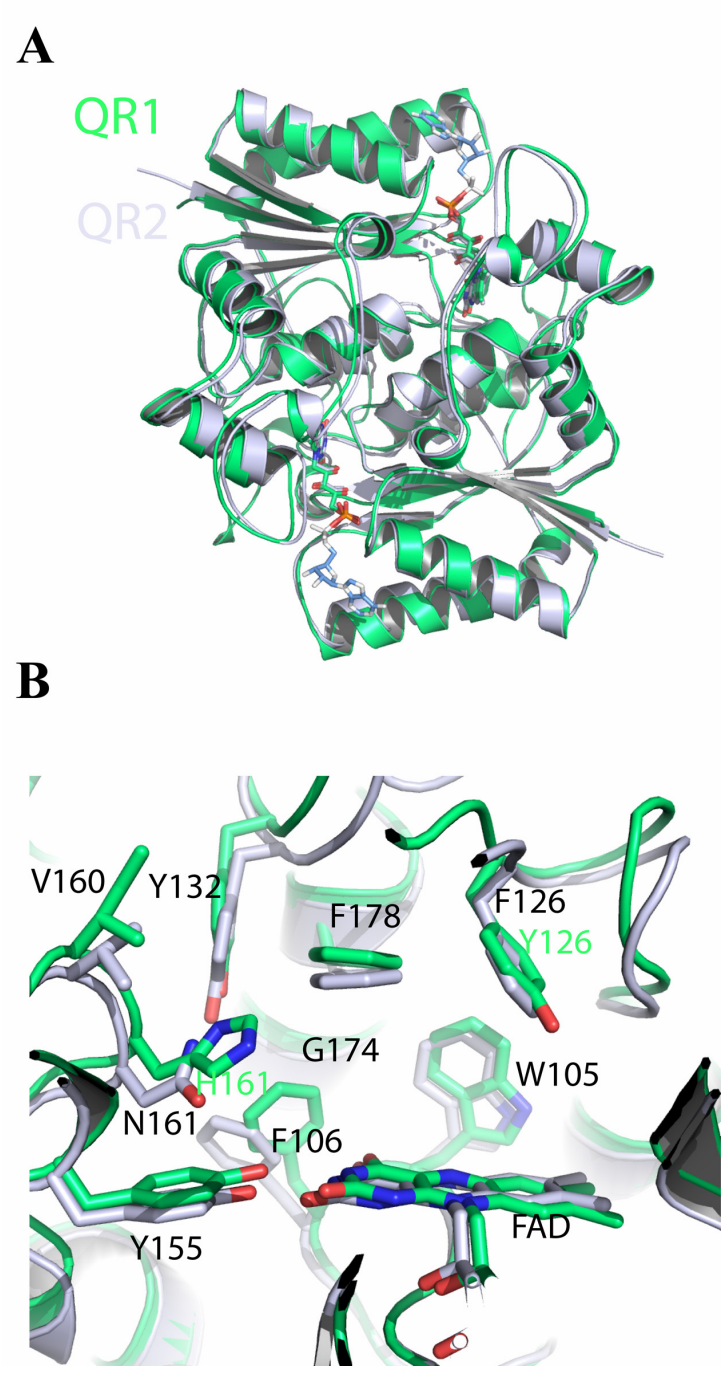
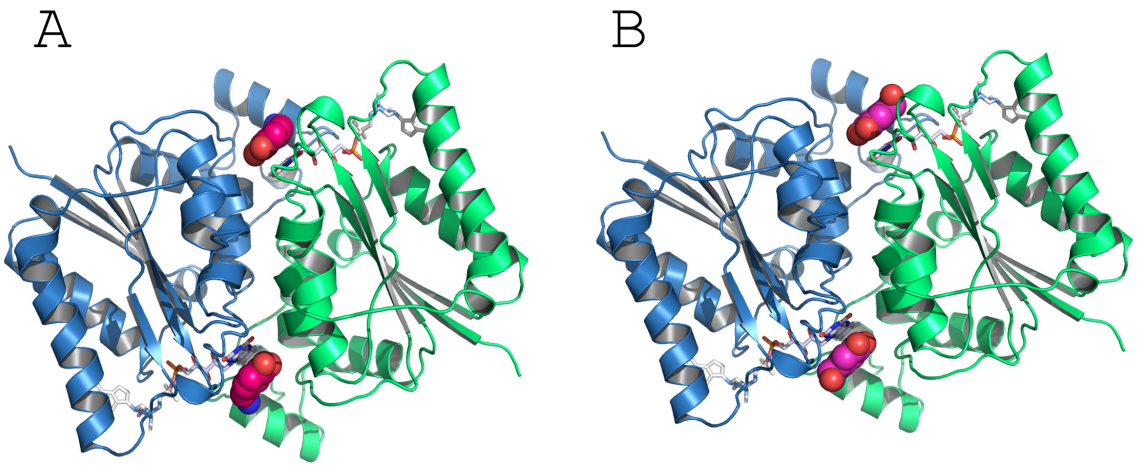
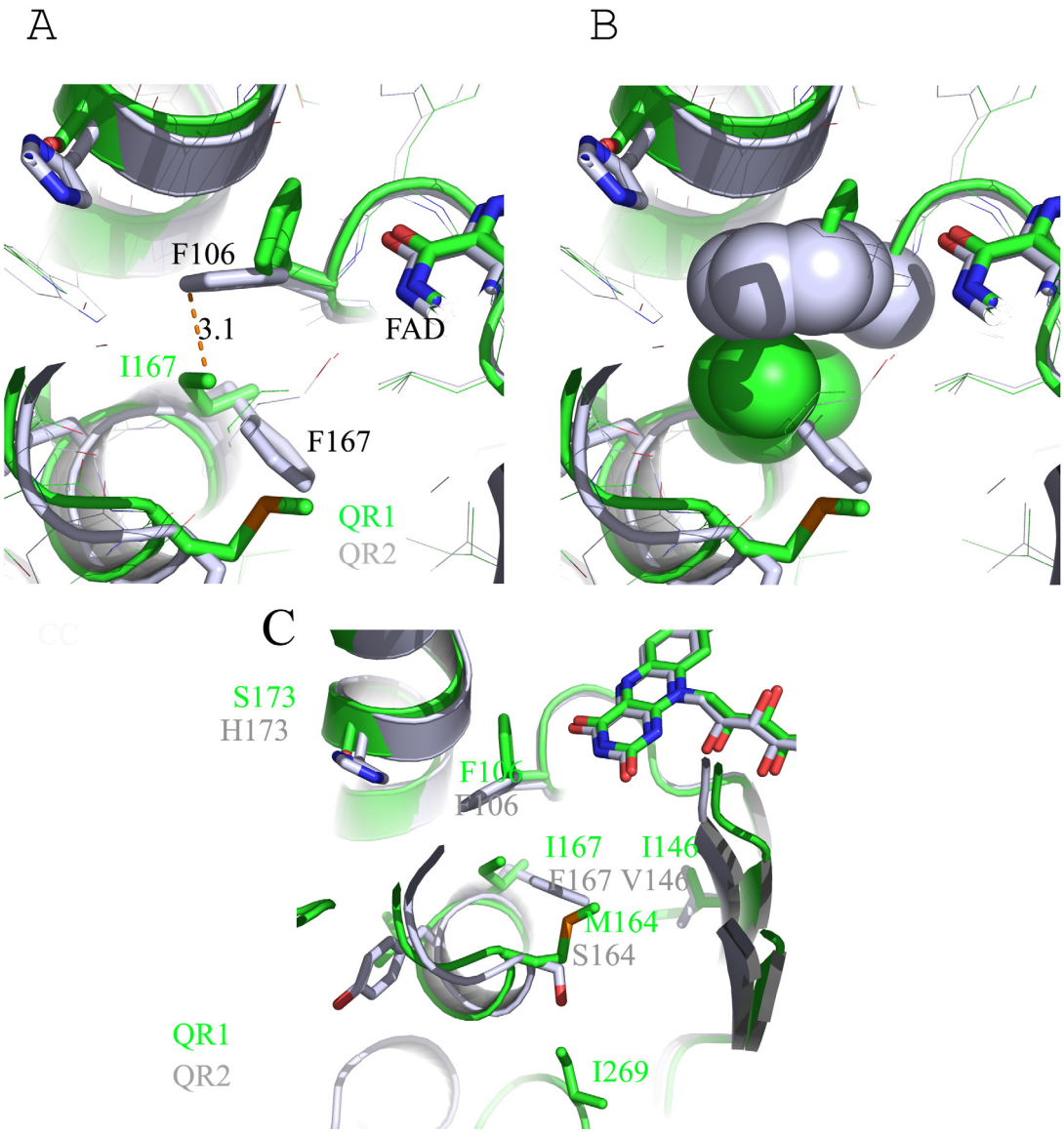
Supplemental Data



Supplemental Figure 1. Overall structural comparisons between QR1 and QR2. The three-dimensional structures of QR1 (green) and QR2 (gray) are aligned and further optimized for the overlap of co-factor FAD molecules. (**A**) The overall three-dimensional structures are nearly overlapping except for a few loop regions. (**B**) The conservation between QR1 and QR2 further extends to the active site residues.



Supplemental Figure 2. (A) Overall structure of the QR2-dopamine complex. Dopamine (space filling model) binds to the active sites of QR2 at the dimer interface. (B) Overall structure of the QR2-adrenochrome complex. Adrenochrome is shown in a space-filling model, and the co-factor FAD is shown in stick model.



Supplemental Figure 3. Residue differences between QR1 and QR2 near F106. (A) The side-chains of F106 adopt different rotations as a result of surrounding residue differences between these two proteins, especially residue 167. In QR2, amino acid 167 is a phenylalanine, whereas in QR1 it is an isoleucine. The steric hindrance of the bulky I167 side-chain forces F106 to adopt a different rotamer in QR1. The distance labeled is in Å. **(B)** The steric hindrance can be further illustrated between I167 and F106 sidechains in a ball and stick model if F106 adopts similar rotation in QR1 as that of QR2. **(C)** Residue 167 is not the sole contributor for sidechain rotational differences of F106 between QR1 and QR2. Amino acid differences at residue 167, 164, and 146 all contribute to the rotational differences of the side-chains of F106 between QR1 and QR2.

Supplemental Table 1
Data collection and refinement statistics (molecular replacement)

	QR2-dopamine	QR2-Adrenochrome
Data collection		
Wavelength (Å)	0.9789	
Space group	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions		
a, *b*, *c* (Å)	83.44, 106.95, 56.53	83.83, 106.46, 56.61
α, β, γ (°)	90, 90, 90	90, 90, 90
*Resolution (Å)	2.1 (2.18-2.10)	2.5 (2.59-2.50)
R_{sym} or R_{merge}	12.8% (41.3%)	15.5% (45.3%)
$I / \sigma I$	15 (3)	11 (3)
Completeness (%)	99.7 (99.7)	99 (99)
Redundancy	6.7 (6.1)	6.4 (5.9)
Refinement		
Resolution (Å)	50-2.1	50-2.5
No. reflections	30172	18100
R_{work} / R_{free} [#]	19.4%/23.7%	19.8%/25.9%
No. atoms		
Protein	3648	3648
Ligand/ion	22 (dopamine)	26 (adrenochrome)
Water	346	293
B-factors		
Protein	22.5	24.54
Ligand/ion	36.9	44.5
Water	31.1	29.3
R.m.s. deviations		
Bond lengths (Å)	0.007	0.008
Bond angles (°)	1.2	1.2

*Values in parentheses are for highest-resolution shell.
[#]R_{free} is the *R*-factor for a selected subset (10%) of the reflections that were not included in prior refinement calculations.