Supplemental Data

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Residue Ala^{213} of methylated His_6-PR and its nearby region. The residues Pro^{206} and Ile^{207} (stick presentation, in green) are most closely positioned to Ala^{213} (stick presentation, in purple) in the neighboring symmetry molecule. The shortest distances between the Ala^{213} side chain carbon and Pro^{206} and Ile^{207} are 3.47 Å and 3.57 Å, respectively.

Supplemental Figure 2. Size exclusion chromatogram of methylated and un-methylated His_6-PR. *Aspergillus niger* glucose oxidase (150 kDa), BSA (67 kDa), ovalbumin (45 kDa) and α-Chymotrypsin (25 kDa), were used to create the calibration curve (dark blue). Un-methylated His_6-PR (5 mg/ml) shows two peaks in the chromatograph (blue). The major peak at 65 ml elution volume represents the monomeric form of PR in solution. The corresponding molecular weight of ~38 kDa is consistent with findings from SDS-PAGE (data not shown). The second peak at 56 ml elution volume (~72 kDa) indicates the dimeric form of PR in solution. According to the peak area, the ratio between monomer and dimer is around 4:1. Methylated His_6-PR (5 mg/ml) shows a single peak (pink), indicating that methylated PR exists only as monomer in solution, and the molecular weight is ~48 kDa.

Supplemental Figure 3. NADPH binding site. All the surrounding residues (Ser^{205}, Gly^{208}, Gly^{279}, Thr^{280}, Thr^{281}, Lys^{282}, Asn^{285}, Asn^{288}, and Asn^{289}) that were observed to form hydrogen bonds with the cofactor NADPH (stick presentation in green) are shown in white stick presentation. The hydrogen bonds are represented by dashed black lines.

Supplemental Figure 4. Two symmetric holo His_6-PR-A213W molecules in the crystal packing. Active sites were occupied by the neighbour symmetry molecule. The catalytic tetrad (Asp^{52}, Tyr^{57}, Lys^{84} and His^{126}) is in red. NADPH is in green stick presentation, and the modeled nicotinamide riboside part of NADPH is shown in black.

Supplemental Figure 5. Structural comparison of PR and a "generic" AKR to display the diversity in the C-terminus. *A.* The orientation of PR model to depict the decoration of the five additional β-strands (OOB1-5, purple) at C-terminus. OOB = out of barrel. *B.* A "generic" AKR (3α-Hydroxysteroid dehydrogenase, PDB 1WLI) (38) with the same orientation as *A.* β1 and β8 which are missing in PR are in blue. The missing residues in Loop B are represented by dashed black lines. Loop A-C are in green. N- and C-terminus are marked with N and C.

Supplemental TABLE 1A

MAD data collection and phasing statistics of methylated His_6-PR

	Peak	Inflection	High remote
Wavelength (Å)	1.0723	1.0726	0.9537
R_{merge} (%)[a]	6.7 (59.9)	7.8 (83.2)	8.1 (62.5)
Completeness (%)	96.8 (90.4)	97.7 (95.7)	98.3 (95.2)
Multiplicity	2.13 (2.05)	2.14 (2.09)	4.18 (3.76)
$I/\sigma(I)$	11.13 (1.77)	10.32 (1.38)	14.87 (2.66)
Resolution (Å)		20-2.50 (2.50-2.63)	
Unit cell (Å)		a=60.52, b=91.83, c=143.81	
Space Group		$C222_1$	
SHELXD			
Highest resolution for sub-structure solution (Å)		3.7	
CC all[b]		47.71	
No. of Pt sites		2	
SHELXE			
Solvent content		0.55	
Contrast		0.634	
Connectivity		0.750	
Pseudo-Free CC (%)[c]		65.26	

[a] $R_{merge} = |I_i - < I_i >|I_i$, I_i is the average intensity value of the equivalent reflections.

Supplemental TABLE 1B

X-ray data collection and refinement statistics of methylated apo His_6-PR-A213W and un-methylated His_6-PR-A213W complex with NADPH

Structure	mPR-A213W[a]	PR-A213W+NADPH[b]
Data collection		
Wavelength (Å)	1.0	1.0
Total reflections	58206	132770
Unique reflections	20584	14294
Mosaicity	0.641	0.241
Resolution (Å)	40-2.20 (2.32-2.20)	20-2.33 (2.41-2.33)
Completeness (%)	95.4 (84.0)	99.6 (96.0)
$I/\sigma(I)$	27.8 (3.1)	45 (2.5)
R_{merge} (%)[c]	5.8 (17.9)	7.9 (61.4)
Space Group	$C222_1$	$P3_221$
Unit cell (Å)	a = 58.09, b = 93.6 c = 142.98	a = b= 54.5, c = 200.4
Refinement statistics		
R_{work}[d] /R_{free} (%)[e]	21.2/24.7	21.5/25.4
No. of protein atoms	2188	2227
No. of water molecules	69	26
No. of $NADP^+$ atoms		
R.M.S.D.[f] Bond length (Å)	0.008	0.008
R.M.S.D.[f] Bond angle (°)	1.093	1.299
Average B-factors (Å^2)		
Protein	54.2	45.4
Water	51.1	45.1
$NADP^+$		44.4
Ramachandran analysis (%)		
Most favored region	94.2	95.3
Allowed	4.7	4.3
Disallowed	1.1	0.4
PDB code	3V0U	3V0T

[a]mPR-A213W: methylated His_6-PR-A213W mutant;

[b]PR A213W +NADPH: unmethylated His_6-PR-A213W complexed with NADPH;

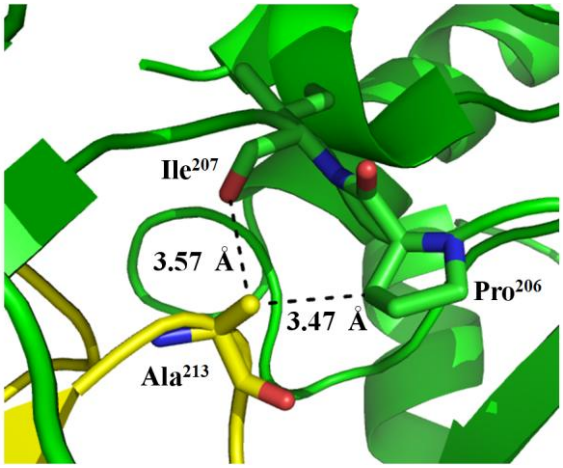
[c]$R_{merge} = |I_i - < I_i >|I_i$, I_i is the average intensity value of the equivalent reflections;

[d]$R_{work} = \Sigma(|F_o - F_c|)/ \Sigma|F_o|$, F_o and F_c are observed and calculated structure factors, respectively.

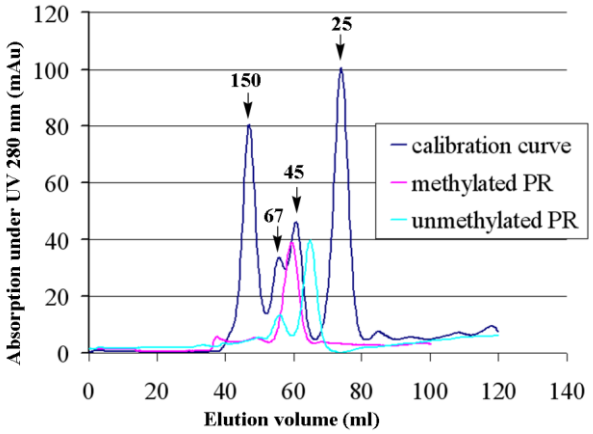
[e]R_{free} was calculated using 5 % randomly excluded data from refinement.

[f]R.M.S.D.: root mean square deviation.

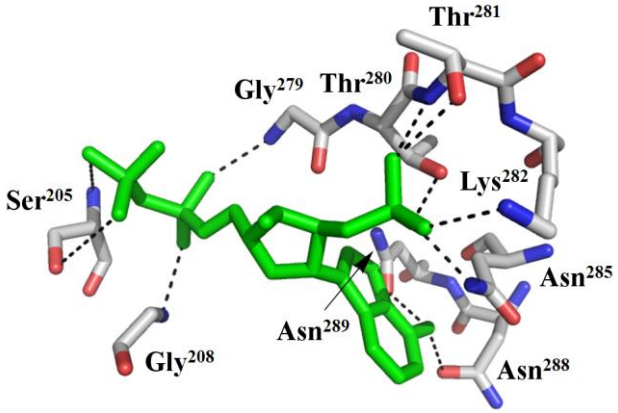
Supplemental Figure 1



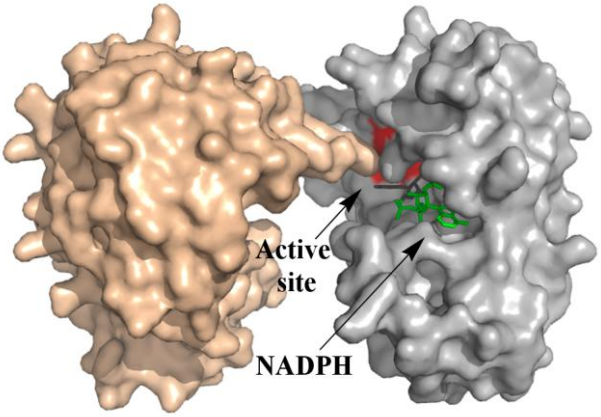
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5