The effect of Asp-His-Ser/Thr-Trp tetrad on the thermostability of WD40-repeat Proteins[†]

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Table S1. The list PDB_ID of non-WD40 β-propeller. None of Asp-His-Ser/Thr-Trp tetrad has been found in the 65 non-WD40 β-propeller proteins.

The list of β-propeller proteins except WD40-repeat proteins	
No. of blade	**PDB_Id**
3	1N7V
4	1CK7, 1FBL, 1GEN, 1HXN, 1ITV, 1PEX, 1QHU, 1SU3, 2JXY, 3C7X
5	1GYH, 1TL2, 1UV4, 3C2U
6	1CRU, 1EUR, 1F8E, 1IJQ, 1K32, 1N7D, 1NMB, 1NPE, 1NSC, 1OFZ, 1PJX, 1Q7F, 1RWI, 1USR, 1V0E, 1V04, 1W0P, 1W8O, 1ZGK, 2AH2, 2GHS, 2HQS, 2JKB, 2OJH, 2P4O, 2QE8, 2SLI, 2VPJ, 3B7E, 3SIL
7	1A12, 1JOF, 1JTD, 1K3I, 1K32, 1L0Q, 1OLZ, 1PBY, 1QFM, 1RI6, 1UTC, 1XIP, 1XKS, 2BBK, 2IWK, 2VDR, 3B7F
8	1XFD, 2BGR

Table S2. The hydrogen bond parameters of the five tetrads in the WDR5 wild type and three mutants based on the crystal structures.

	The hydrogen-bond parameters of five tetrads														
	D66-H44-S62-W72			**D108-H86-S104-W114**			**D150-H128-S146-W156**			**D192-H170-S188-W198**			**D324-H300-S318-W330**		
	D66-H44	**H44-S62**	**S62-W72**	**D108-H86**	**H86-S104**	**S104-W114**	**D150-H128**	**H128-S146**	**S146-W156**	**D192-H170**	**H170-S188**	**S188-W198**	**D324-H300**	**H300-S318**	**S318-W330**
WT	**1.83/154.4**	**2.09/135.6**	**1.94/168.3**	**1.91/164.7**	**1.71/155.2**	**1.84/172.4**	**2.02/150.8**	**2.23/117.6**	**1.93/167.5**	**2.36/125.0**	**2.24/118.0**	**1.86/163.7**	**1.90/156.2**	**2.37/134.8**	**1.85/169.6**
S62A	**1.78/154.8**			**1.80/160.3**	**1.94/160.7**	**1.77/171.7**	**1.84/152.8**	**1.97/132.5**	**1.77/170.6**	**2.37/139.7**	**2.14/122.4**	**1.98/161.4**	**2.16/143.0**	**1.85/132.2**	**1.89/177.2**
W330F	**2.09/150.1**	**1.87/128.4**	**2.06/170.4**	**1.82/157.4**	**2.01/171.6**	**1.71/172.5**	**1.67/163.2**	**2.11/130.6**	**1.72/164.7**	**2.26/128.7**	**2.23/116.6**	**2.05/151.3**	**2.21/147.7**	**1.93/127.1**	
W330Y	**1.99/150.5**	**1.95/137.4**	**1.87/170.8**	**2.02/149.1**	**2.12/171.8**	**1.69/161.8**	**1.66/164.9**	**2.33/137.4**	**1.89/148.9**	**2.27/155.2**	**2.25/118.8**	**2.03/157.7**	**2.18/149.4**	**2.42/130.3**	

Table S3. The error approximation of CD measurement at 230nm in the different concentration of GdmHCl.

WDR5 WT																	
GdmHCl	0.0	0.5	1.0	1.5	2.0	2.5	3.0	3.3	3.6	4.0	4.2	4.4	4.6	4.8	5.0	5.5	6.0
Folded Fraction	100	104±12	106±14	110±17	113±11	99±4	105±6	97±4	97±2	85±8	65±4	41±8	26±7	14±5	7±3	2±5	0

WDR5 S62A																	
GdmHCl	0.0	0.5	1.0	1.5	2.0	2.2	2.4	2.6	3.0	3.2	3.4	3.6	3.8	4.0	5.0	5.5	6.0
Folded Fraction	100	103±9	93±11	91±6	85±7	81±5	73±11	66±2	50±3	43±2	30±5	20±3	8±4	1±3	3±5	5±2	0

WDR5 S146A									
GdmHCl	0.0	0.5	0.75	1.25	1.5	1.75	2.0	2.5	3.0
Folded Fraction	100	103±3	93±8	58±2	20±4	6±4	1±4	3±7	0

WDR5 S188A

GdmHCl	0.0	0.5	1.0	1.5	1.75	2.0	2.5	3.0
Folded Fraction	100	101±4	92±10	83±15	58±5	5.9±3	2.7±2	0

WDR5 W330F																	
GdmHCl	0.0	0.5	1.0	1.5	2.0	2.5	3.0	3.3	3.6	3.8	4.0	4.2	4.4	4.6	5.0	5.5	6.0
Folded Fraction	100	100±16	108±12	104±13	98±4	102±8	96±6	82±10	71±2	55±9	43±11	27±3	22±4	12±2	4±3	0±6	0

WDR5 W330Y																	
GdmHCl	0.0	0.5	1.0	1.5	2.0	2.5	3.0	3.4	3.6	3.8	4.0	4.2	4.4	4.6	5.0	5.5	6.0
Folded Fraction	100	103±5	106±13	105±5	108±10	100±4	95.7±4	83±2	71±2	58±8	43±2	27±3	22±8	11.8±5	4±4	1±3	0

WDR5 D192E											
GdmHCl	0.0	0.5	1.0	1.5	2.0	2.5	3.0	3.3	3.6	4.0	4.4
Folded Fraction	100	103±6	104±10	90±11	83±14	66±8	38±11	20±5	12±3	5.6±2	0

WDR5 W324E												
GdmHCl	0.0	0.5	1.0	1.5	2.0	2.5	3.0	3.2	3.4	3.6	3.8	4.0
Folded Fraction	100	105±10	106±15	98±7	98±12	90±7	71±10	46±5	31±5	10±3	4.5±3	0

Table S4. The solvent-accessible surface Area (SASA) of hydrogen bonds acceptors and donors in five Asp-His-Ser/Thr-Trp tetrads of S62A, W330F and W330Y mutants. The unit of SASA is $Å^2$.

WDR5 (S62A/W330F/W330Y)										
	Residues in tetrad				**D**		**H**		**S/T**	**W/Y**
No.	**D**	**H**	**S/T**	**W/Y**	**OD1**	**OD2**	**ND1**	**NE2**	**OG**	**NE1**
1	**D66**	**H44**	**S62**	**W72**	**0/0/0**	**0.1/0.1/0.4**	**0/0/0**	**0/0/0**	**0/0/0**	**5.8/0/0**
2	**D108**	**H86**	**S104**	**W114**	**0/0/0**	**0.2/0.2/1.7**	**0/0/0**	**0/0/0**	**0/0/0**	**0/0/0**
3	**D150**	**H128**	**S146**	**W156**	**0/0/0**	**2.8/2.3/2.3**	**0/0/0**	**0/0/0**	**0/0/0**	**0/0/0**
4	**D192**	**H170**	**S188**	**W198**	**0/0/0**	**0.9/0.5/0.9**	**0/0/0**	**0/0/0**	**0/0/0**	**0/0/0**
5	**D324**	**H300**	**S318**	**W330**	**1.0/1.0/0.1**	**1.0/1.0/3.3**	**0/0/0**	**0/0/0**	**0/0/0**	**0/0/0**

Table S5. The solvent-accessible surface area of polar atoms of residues involved in the tetrads belonging to 25 crystal structures. The unit is Å^2.

WDR5 (2G99)										
	Residues in tetrad				D		H		S/T	W/Y
No.	D	H	S/T	W/Y	OD1	OD2	ND1	NE2	OG	NE1
1	D66	H44	S62	W72	0	0.1	0	0	0	0
2	D108	H86	S104	W114	0	2.7	0	0	0	0
3	D150	H128	S146	W156	1.7	0	0	0	0	0
4	D192	H170	S188	W198	0	1.8	0	0	0	0
5	D324	H300	S318	W330	1.2	1.9	0	0	0	0

SIF2 (1R5M)										
	Residues in tetrad				D		H		S/T	W/Y
No.	D	H	S/T	W/Y	OD1	OD2	ND1	NE2	OG	NE1
1	E243	H208	T237	W247	*0*	*14.4*	0	0	0	0
2	D280	H260	S278	W288	0	1.3	0	0	0	0
3	D380	H358	S376	W386	0	0.6	0	0	0	0
4	D421	H400	S417	W427	0	0	0	0	0	0

TLE1 (1GXR)										
	Residues in tetrad				D		H		S/T	W/Y
No.	D	H	S/T	W/Y	OD1	OD2	ND1	NE2	OG	NE1
1	*E485*	H483	T501	W510	*12.9*	*38.3*	0	0	0	0
2	D637	H615	T633	W643	0	0.9	0	0	0	0
3	D719	H697	S715	W725	0	0.2	0	0	0	0

TUP1 (1ERJ)										
	Residues in tetrad				D		H		S/T	W/Y
No.	D	H	S/T	W/Y	OD1	OD2	ND1	NE2	OG	NE1
1	D506	H484	S502	W512	0	0	0	0	0	0
2	D597	H575	S593	W603	0	2.4	0	0	0	0
3	D651	H629	S647	W657	0	0.9	0	0	0	0
4	D699	H671	S695	W705	0	4.5	0	0	0	0

SKI8P (1SQ9)										
	Residues in tetrad				D		H		S/T	W/Y
No.	D	H	S/T	W/Y	OD1	OD2	ND1	NE2	OG	NE1
1	D35	H15	S31	W41	0	4.2	0.5	0	0	0
2	D312	H290	S308	W318	0	0.1	0	0	0	0

Ciao1 (3FM0)										
	Residues in tetrad				D		H		S/T	W/Y
No.	D	H	S/T	W/Y	OD1	OD2	ND1	NE2	OG	NE1/OH
1	D37	H14	S33	W43	0	4	0	0	0	0
2	D82	H60	S78	W88	0	4.5	0	0	0	0
3	D126	H104	T122	W132	0	3.2	0	0	0.3	0
4	D171	H149	S167	*Y177*	0	3.8	0	0	0	0
5	D215	H193	S211	W221	0	3.3	0	0	0	0
6	D325	H302	S321	W331	0	4.5	0.1	0	0	0

Asc1 (3FRX)										
	Residues in tetrad				D		H		S/T	W/Y
No.	D	H	S/T	W/Y	OD1	OD2	ND1	NE2	OG	NE1/OH
1	D39	H16	S35	W45	0	3.7	0	0	0	0
2	D86	H64	S82	W92	0	5.5	0	0	0	0
3	D128	H106	S124	W134	0	2.8	0	0	0	0
4	D175	H147	S171	W181	0	1.1	0	0	0	0
5	D217	H195	S213	W223	0	10.6	1.2	0	0	0

Cytosolic Iron-sulphur Assembly Protein-1 (2HES)										
	Residues in tetrad				D		H		S/T	W/Y
No.	D	H	S/T	W/Y	OD1	OD2	ND1	NE2	OG	NE1/OH
1	D174	H152	S170	W180	0	3.4	0	0	0	0
2	D220	H196	S216	W226	0	4	0	0	0	0
3	D270	H249	S266	*Y276*	0	3.2	0	0	0	1.8
4	D318	H293	T314	W324	0	7.3	0	0	0	0

DDB2(3EI3)										
	Residues in tetrad				D		H		S/T	W/Y
No.	D	H	S/T	W/Y	OD1	OD2	ND1	NE2	OG	NE1/OH
1	D300	H277	T296	W306	0	3.7	0	0	0	0

MBP RACK1A (3DM0)										
	Residues in tetrad				D		H		S/T	W/Y
No.	D	H	S/T	W/Y	OD1	OD2	ND1	NE2	OG	NE1/OH
1	D1037	H1014	S1033	W1043	0	0	0	0	0	0
2	D1084	H1062	S1080	W1090	0	1.4	0	0	0	0
3	D1126	H1104	S1122	W1132	0	4.3	0	0	0	0
4	D1173	H1149	S1169	W1179	2.5	0	0	0	0	0
5	D1215	H1193	S1211	W1221	0.7	0.2	0.2	0	0	0

PHOSPHATAS (3DW8)

	Residues in tetrad				D		H		S/T	W/Y
No.	D	H	S/T	W/Y	OD1	OD2	ND1	NE2	OG	NE1/OH
1	D196	H176	S194	W203	0	0.1	0	0	0	0
2	*E83*	H80	S113	W122	*20.4*	*50.4*	0	0	0	0

BUB3P (1YFQ)

	Residues in tetrad				D		H		S/T	W/Y
No.	D	H	S/T	W/Y	OD1	OD2	ND1	NE2	OG	NE1/OH
1	D272	H241	T268	W278	5.2	0.4	0	1.1	1.8	0

Coronin-1 (2AQ5)

	Residues in tetrad				D		H		S/T	W/Y
No.	D	H	S/T	W/Y	OD1	OD2	ND1	NE2	OG	NE1/OH
1	D103	H80	S99	W109	0	2.4	0	0	0	0
2	D153	H130	S149	W159	0	2.3	0	0	0	0
3	*E244*	H218	T236	W250	26	17	0	0	0	0.1

AIP1 (1NR0)

	Residues in tetrad				D		H		S/T	W/Y
No.	D	H	S/T	W/Y	OD1	OD2	ND1	NE2	OG	NE1/OH
1	D78	H58	S76	W86	0	1.5	0.2	0	0	0
2	D211	H189	S207	*Y217*	0	0.9	0	0	0	0
3	D260	H238	S256	W266	0	3.1	0	0	0	0
4	D343	H323	S341	W351	0	1	0	0	0	0
5	D557	H535	T553	W563	0	3.1	0	0	0	0
6	D601	H579	S597	W607	0	3.7	0	0	0	0

Gβ (1GP2)

	Residues in tetrad				D		H		S/T	W/Y
No.	D	H	S/T	W/Y	OD1	OD2	ND1	NE2	OG	NE1/OH
1	D76	H54	S72	W82	0	1.3	0	0	0	0
2	D163	H142	T159	W169	0	0.9	2.3	0	0.1	0.1
3	D205	H183	S201	W211	0	1.1	0	0	0	0
4	D247	H225	T243	*F241*	0	0	0	0	0	
5	D333	H311	T329	W339	0	0.7	0	0	0	0

FBW7 (2OVP)

	Residues in tetrad				D		H		S/T	W/Y
No.	D	H	S/T	W/Y	OD1	OD2	ND1	NE2	OG	NE1/OH
1	D400	H379	S396	W406	0	14.4	0	0	0	0
2	D440	H420	S436	W446	0	0.8	0	0	0	0

3	D480	H460	S476	W486	0	0	0	0	0	0
4	D520	H500	S516	W526	0	0.4	0	0	0	0
5	D560	H540	S556	W566	0	2.7	0	0	0	0
6	D600	H580	S596	W606	0	2.6	0	0	0	0
7	D643	H623	T639	W649	1.7	0	0	0	0	0

P55 (3C99)

	Residues in tetrad				D		H		S/T	W/Y
No.	D	H	S/T	W/Y	OD1	OD2	ND1	NE2	OG	NE1/OH
1	D203	H180	S199	W209	0	0	0	0.1	0	0
2	D253	H230	S249	W259	1.4	0	0	0	0	0
3	D299	H276	T295	W305	0	4.8	0	0	0	0
4	D343	H320	S339	W349	0	1	0	0	0	0
5	D400	H377	S396	W406	0	1.2	0	0	0	0

EED (2QXV)

	Residues in tetrad				D		H		S/T	W/Y
No.	D	H	S/T	W/Y	OD1	OD2	ND1	NE2	OG	NE1/OH
1	*E317*	H297	S313	W323	44.1	27.8	7.7	0	0	0
2	D256	H234	S252	W262	0	3.1	0	0	0	0
3	D211	H188	S207	W217	0	6.4	0	0	0	0

SRO7 (2OAJ)

	Residues in tetrad				D		H		S/T	W/Y
No.	D	H	S/T	W/Y	OD1	OD2	ND1	NE2	OG	NE1/OH
1	D292	H290	T288	W298	0	0	0	0	0.3	0

β-TrCP1 (1P22)

	Residues in tetrad				D		H		S/T	W/Y
No.	D	H	S/T	W/Y	OD1	OD2	ND1	NE2	OG	NE1/OH
1	D326	H306	T322	W332	0	0.8	0	0	0	0
2	D366	H346	T362	W372	0	8.4	0	0	0	0
3	D409	H389	S405	W415	2.6	0	0	0	0	0
4	D449	H429	S445	W455	0	2	0	0	0	0
5	D489	H469	S485	W495	5.3	0.7	1.1	0	0	0
6	D538	H518	S534	W544	1.8	0	0	0	0	0

P40 (1K8K)

	Residues in tetrad				D		H		S/T	W/Y
No.	D	H	S/T	W/Y	OD1	OD2	ND1	NE2	OG	NE1/OH
1	D73	H51	T69	W79	0	0	0	0	0	0

2	D351	H325	T347	W357	0	0	0	0	0	0

ALFA2(1VYH)										
	Residues in tetrad				D		H		S/T	W/Y
No.	D	H	S/T	W/Y	OD1	OD2	ND1	NE2	OG	NE1/OH
1	D129	H107	S125	W135	2.8	1.5	0	0	0	0
2	D171	H149	S167	W177	0	2.1	0	0	0	0
3	D213	H191	S209	W219	4.1	0	0	0	0	0
4	D255	H233	S251	W261	0	4.5	0	0	0	0
5	D317	H275	S313	W323	0	3.9	0	0	0	0
6	D359	H337	S355	W365	0	0	12.4	0.8	0	0
7	D401	H379	T397	W407	0	3.1	0	0	0	0

CDC4(1NEX)										
	Residues in tetrad				D		H		S/T	W/Y
No.	D	H	S/T	W/Y	OD1	OD2	ND1	NE2	OG	NE1/OH
1	D401	H380	T397	*Y407*	0	1.8	0	0	0	0.9
2	D442	H421	S438	W448	9.3	0	0	0	0	0
3	D486	H462	T482	W492	0	2.8	0	0	0	0
4	D549	H529	S545	W555	0	3.3	0	0	0	0
5	D591	H569	S587	W597	0	2.5	0	0	0	0
6	D651	H631	S647	W657	0	3.3	0	0	0	0
7		H670	S688	*Y697*			0	0	0	4.9

Sec13(3BG1)										
	Residues in tetrad				D		H		S/T	W/Y
No.	D	H	S/T	W/Y	OD1	OD2	ND1	NE2	OG	NE1/OH
1	D80	H56	S76	W86	0	0.7	0	0	0	0
2	D126	H102	S130	W140	3.4	0	0	0	1.5	1.9
3	D188	H149	S184	W194	0	0	0	0	0	0
4	D237	H211	S233	W243	0	2.8	0	0	0	0

Seh1 (3EWE)										
	Residues in tetrad				D		H		S/T	W/Y
No.	D	H	S/T	W/Y	OD1	OD2	ND1	NE2	OG	NE1/OH
1	D78	H54	S74	W84	15.7	0	1.2	0	0	0

Trm82 (2VDU)										
	Residues in tetrad				D		H		S/T	W/Y
No.	D	H	S/T	W/Y	OD1	OD2	ND1	NE2	OG	NE1/OH
1	D261	H240	S257	W267	0	4.6	0	0	0	0

Rpn14 (3ACP)										
	Residues in tetrad				D		H		S/T	W/Y
No.	D	H	S/T	W/Y	OD1	OD2	ND1	NE2	OG	NE1/OH
1	D157	H135	S153	W163	0	0	0	0	0	0
2	D199	H177	T195	W205	0	0	0	0	0	0

RbBP4 (3GFC)										
	Residues in tetrad				D		H		S/T	W/Y
No.	D	H	S/T	W/Y	OD1	OD2	ND1	NE2	OG	NE1/OH
1	D199	H176	S195	W205	0	2.4	0	0.1	0	0
2	D249	H226	S245	W255	0	1	0	0	0	0
3	D295	H272	T291	W301	0	0	0	0	0	0
4	D339	H316	S335	W345	0	7.5	0	0	0	0
5	D396	H373	S392	W402	0	9.7	0.2	0	0	0

RbBP7 (3CFS)										
	Residues in tetrad				D		H		S/T	W/Y
No.	D	H	S/T	W/Y	OD1	OD2	ND1	NE2	OG	NE1/OH
1	D198	H175	S194	W204	0	1.7	0	0	0	0
2	D248	H225	S244	W254	0	2.1	2.1	0	0	0
3	D294	H271	T290	W300	0	6.4	0	0	0	0
4	D338	H315	S334	W344	0	0	0.5	0	0	0
5	D395	H372	S391	W401	0	11.3	0	0	0	0

WDR92 (3I2N)										
	Residues in tetrad				D		H		S/T	W/Y
No.	D	H	S/T	W/Y	OD1	OD2	ND1	NE2	OG	NE1/OH
1	D138	H110	T134	W144	0	2.8	0	0	0	0

Table S6. The total SASA of WDR5 wild type, S62A, W330F and W330Y mutants. Apparently, the difference of SASA is quite small as we consider the variation of the structures and errors in the SASA calculations.

	WT	S62A	W330F	W330Y	WT-S62A	WT-W330F	WT-W330Y
SASA(Å^2)	11398.7	11273.2	11321.9	11397.4	125.5	76.8	1.3

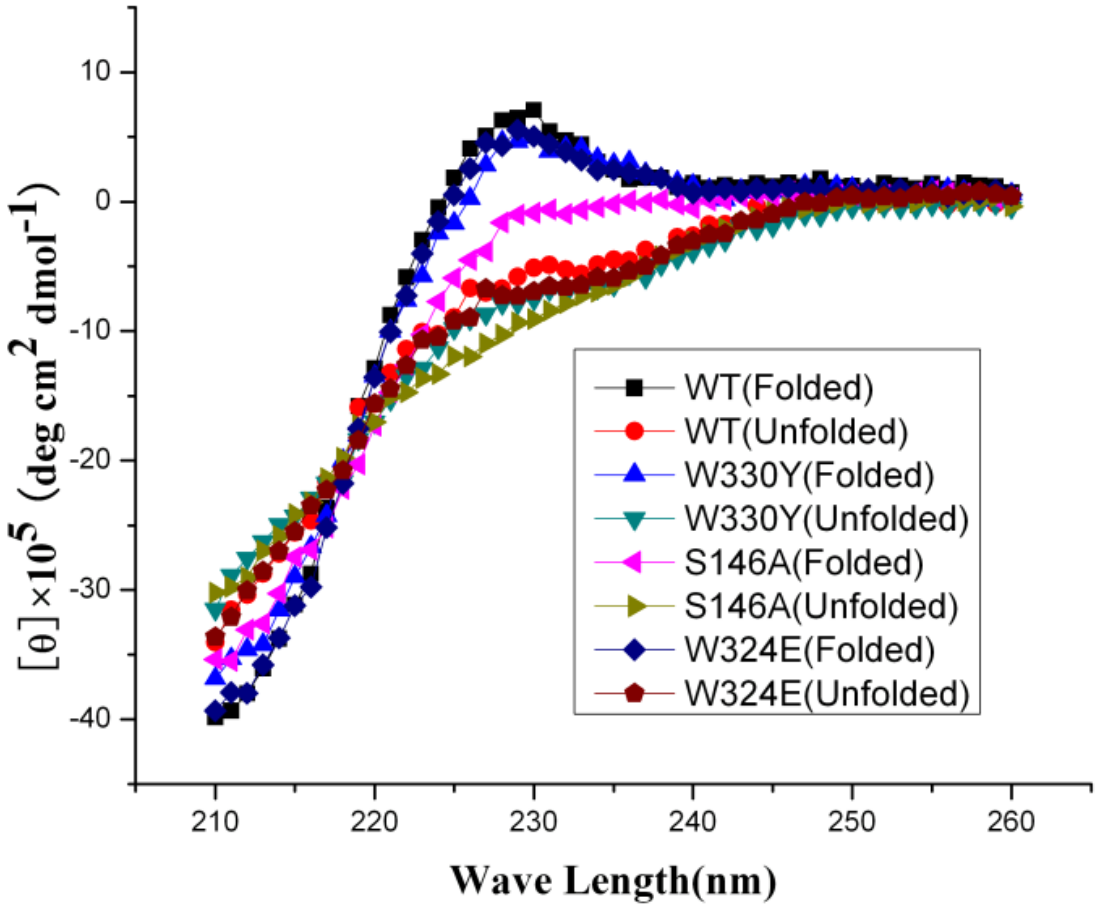

Figure S1. The different intensities of fully folded and unfolded WDR5 wild type and some mutants at ~230nm and pH 7.5 in 1×PBS.

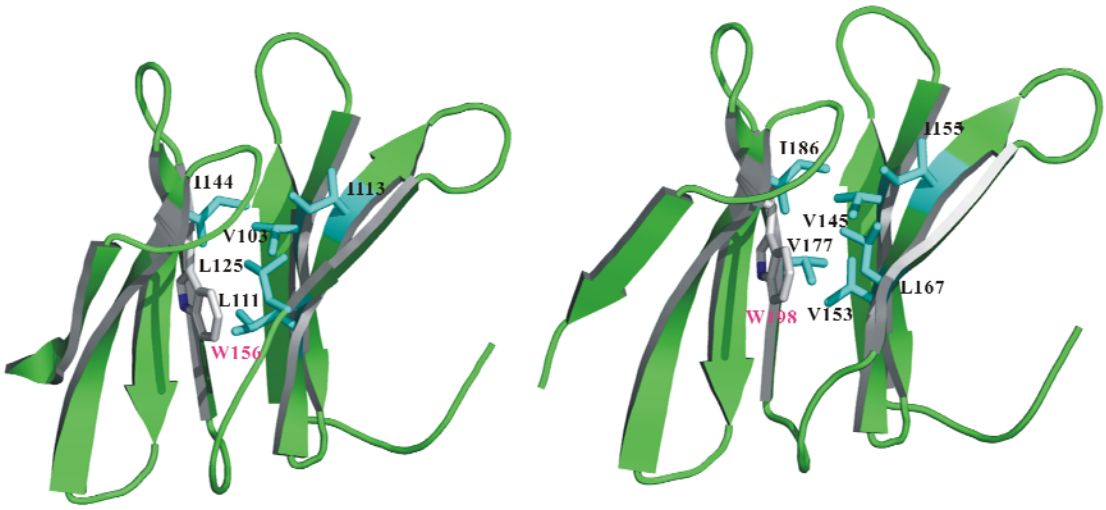

Figure S2. The hydrophobic environments of Trp156 and Trp198 indole rings.

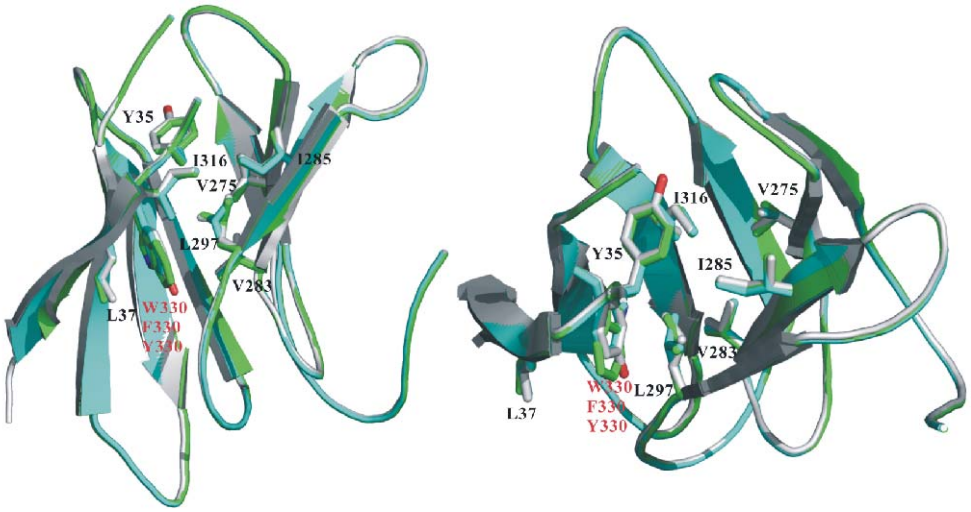

Figure S3. The hydrophobic environment of Trp330, Phe330 and Tyr330.

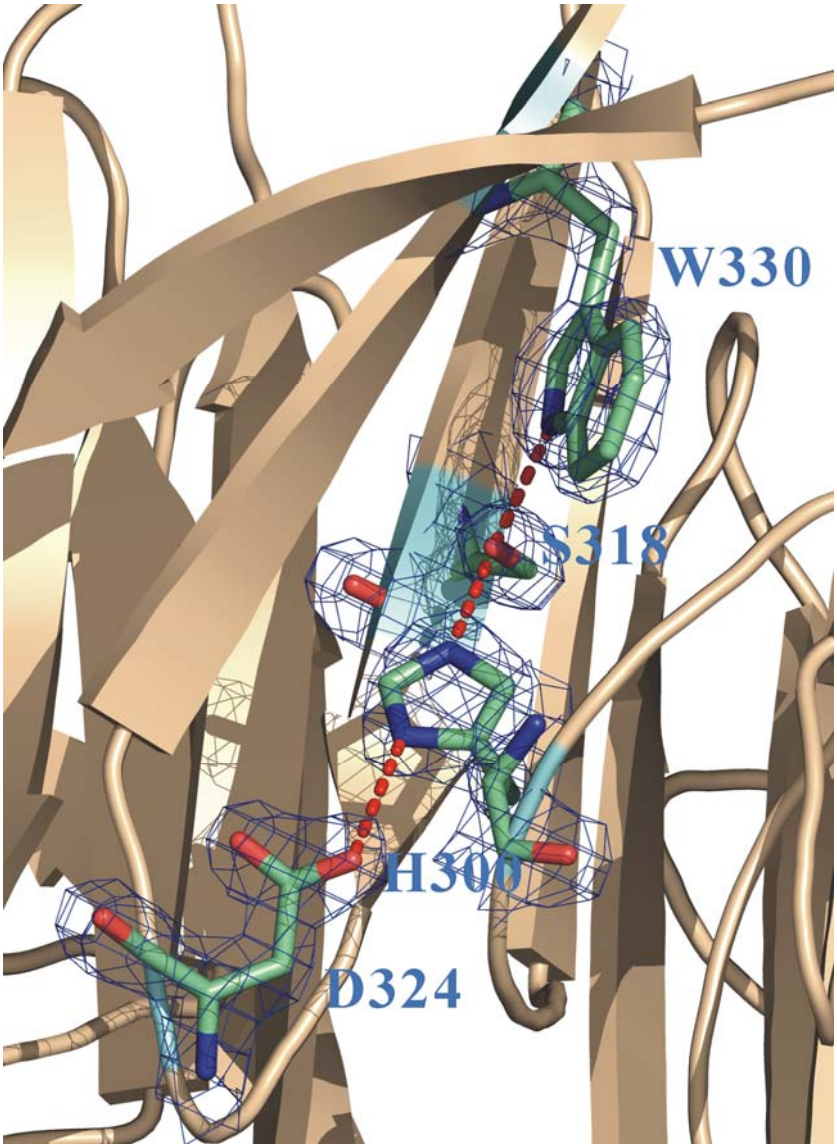

Figure S4. The electron density map of Asp324-His300-Ser318-Trp330.