Supporting Information

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Fig. S1. HSQC spectra of S6^{wt} and P^{54–55}, before and after 60 h of exchange in D₂O. (*A*) S6^{wt} in H₂O. (*B*) P^{54–55} in H₂O. (*C*) S6^{wt} after 60-h incubation in D₂O. (*D*) P^{54–55} after 60-h incubation in D₂O.



Fig. S2. Representative time courses for HD exchange of S6. (A) Under the experimental conditions exchange from the denatured ensemble (global exchange) yields no appreciable loss of H during 60 h. (B) Intermediate exchange. (C) For the most rapidly exchanging positions the H are lost within the 20-min dead time of the experiment.



Fig. S3. Plot of HD protection factors (Table 1) vs. local stability ($\Delta\Delta G_{U/F}$, Table S2) as estimated from the stability loss upon point mutation to Ala. The correlation R = 0.68 is obtained by fitting to the entire dataset, including the points at the thresholds for global (arrow up) and dead-time exchange (arrow down).





S6^{wt}

P⁵⁴⁻⁵⁵

Movie S1. Video showing the HD-exchange patterns of S6^{wt} and P^{54–55} (Fig. 4 in main text). The protection factors of S6^{wt} and P^{54–55} are overall very similar and do not respond to the changes in folding pathway induced by circular permutation. The backbone positions are color-coded according to the protection factors (PF) in Fig. S3.

Movie S1 (AVI)

Table S1. NOE factors for $S6^{wt}$ and the permutant P^{54-55}



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S6 ^{wt}			P ^{54–55}		
Amino acid	NOE	Error	Amino acid	NOE	Error
			NA1		
	0.50	0.04			
	0.59	0.04	RZ44	0.72	0.01
K5	0.55	0.02	K545	0.75	0.01
14 OF	0.83	0.03	14 ₄₆	0.80	0.02
Q5	0.77	0.02	Q5 ₄₇	0.80	0.02
V6	0.67	0.03	V6 ₄₈	0.76	0.02
N7	0.77	0.02	N/49	0.82	0.02
18	0.94	0.02	1850	0.78	0.02
V9	0.85	0.03	V9 ₅₁	0.79	0.02
L10	0.77	0.03	L10 ₅₂	0.81	0.02
N11			N11 ₅₃		
P12			P12 ₅₄		
N13			N13 ₅₅		
L14	0.66	0.02	L14 ₅₆	0.68	0.01
D15	0.73	0.02	D15 ₅₇	0.75	0.01
Q16	0.76	0.02	Q16 ₅₈	0.76	0.01
S17	0.78	0.01	S17 ₅₉	0.78	0.01
Q18	0.65	0.02	Q18 ₆₀	0.80	0.01
L19	0.81	0.02	L19 ₆₁	0.79	0.01
A20	0.83	0.02	A20 ₆₂	0.80	0.01
L21	0.83	0.01	L2162	0.83	0.01
F22	0.71	0.02	E2264	0.79	0.01
K23	0.95	0.02	K23cr	0.82	0.01
F2/	0.55	0.02	F24	0.02	0.01
125	0.97	0.02		0.25	0.01
125	0.87	0.02	12567	0.04	0.01
0.027	0.73	0.02	007	0.02	0.02
Q27			Q27 ₆₉	0.84	0.01
R28	0.74	0.04	R2870	0.82	0.01
A29	0.74	0.01	A29 ₇₁	0.81	0.01
L30	0.86	0.02	L30 ₇₂	0.80	0.02
Q31	0.77	0.02	Q31 ₇₃	0.82	0.01
N32	0.59	0.01	N32 ₇₄	0.76	0.01
Y33	0.77	0.02	Y33 ₇₅	0.78	0.02
G34	0.76	0.02	G34 ₇₆	0.79	0.02
A35	0.76	0.01	A35 ₇₇	0.74	0.01
R36	0.73	0.02	R36 ₇₈	0.71	0.02
V37	0.79	0.02	V37 ₇₉		
E38	0.78	0.03	E38 ₈₀	0.79	0.02
K39	0.76	0.02	K39 ₈₁	0.74	0.02
V40	0.52	0.02	V40 ₈₂	0.55	0.02
E41	0.84	0.03	E41 ₈₃	0.80	0.02
E42	0.71	0.02	E42 ₈₄		
L43	0.63	0.03	L4385	0.77	0.03
G44	0.64	0.03	G4486	0.71	0.02
145			145.27		
B/16	0.74	0.03	B/600	0.78	0.03
P/17	0.69	0.05	P/7	0.70	0.05
1.47	0.03	0.02	1.49	0.72	0.02
L40	0.37	0.05	L40 ₉₀	0.57	0.02
A49	0.04	0.02	A49 ₉₁	0.16	0.01
¥50			¥50 ₉₂		
P51			P51 ₉₃		
152			152 ₉₄		
A53	-0.50	0.04	A53 ₉₅	-6.47	0.08
L54	-0.40	0.04	L54 ₉₆	-2.22	0.02
D55			D552	-0.27	0.00
P56			P56 ₃		
Q57	0.35	0.02	Q574	0.62	0.03
G58	0.77	0.03	G58₅	0.72	0.03
Y59	0.78	0.03	Y59 ₆	0.82	0.03
F60	0.85	0.03	F607	0.79	0.03
L61	0.72	0.03	L61 ₈	0.79	0.03
W62	0.72	0.02	W62。	0.75	0.02
·	···-				0.02

S6^{wt}

Amino acid NOE Error Amino acid NOE Error Y63 Y63₁₀ 0.79 0.02 Q64 Q6411 0.02 V65 0.84 0.02 V65₁₂ 0.79 0.76 0.02 0.02 E66 E66₁₃ 0.85 M67 0.78 0.02 M67₁₄ 0.81 0.02 P68 P6815 E69 0.75 0.03 $E69_{16}$ 0.73 0.02 D70 0.83 0.01 D7017 0.75 0.01 R71 0.70 0.02 $R71_{18}$ 0.71 0.02 V72₁₉ 0.01 V72 0.74 0.02 0.82 N73 0.81 0.02 N7320 0.81 0.02 D74 D74₂₁ 0.15 0.00 L75 0.76 0.02 L75₂₂ 0.84 0.01 A76 0.95 0.02 A7623 0.76 0.01 R77 0.77 0.01 R77₂₄ 0.81 0.01 E78 0.86 0.02 0.80 0.02 E78₂₅ L79 0.81 0.02 L79₂₆ 0.77 0.02 R80 0.77 0.02 R80₂₇ 0.78 0.01 181 181₂₈ 0.78 0.02 0.73 0.02 R82 R82₂₉ 0.76 0.02 D83₃₀ D83 0.75 0.02 0.83 0.01 N84 0.77 0.02 0.82 0.02 N8431 V85 0.79 0.02 0.79 0.02 V8532 R86 0.78 0.03 R8633 0.82 0.03 R87 0.70 0.02 R87₃₄ 0.81 0.02 V88 0.84 0.02 V8835 0.80 0.02 M89 0.76 0.02 M89₃₆ V90 0.79 0.02 V90₃₇ 0.73 0.03 V91 0.75 0.03 V9138 K92 0.76 0.02 K92_{A39} 0.73 0.02 S93 0.80 0.02 S93₄₀ 0.71 0.02 Q94_{T41} Q94 0.56 0.02 0.66 0.01 E95 0.19 0.02 E95_{T42} 0.71 0.01 P96₄₃ P96 -0.06 0.02 F97 0.01 F97 0.72 L98 -0.660.01 L98 A99 A99 N100 N100 A101 A101

P^{54–55}

The residues are labeled according to the S6^{wt} sequence and subscripts refer to P^{54–55} numbering.

Table S2.	ϕ va	lues and	free-e	energy	changes	upon	point
mutation	(Δ Δ	GU/F) for	r S6 ^{wt}	and P ⁵	54–55		

Mutation	${ m S6^{wt}} \phi$	$\rm S6^{wt}\Delta\DeltaG_{U/F}$	P ^{54–55} ϕ	$P^{54-55 \Delta \Delta} G_{U/F}$
V6A ₄₈	0.51 ¹ *	3.64	0.57*	3.38
18A ₅₀	0.45*	4.17	0.40*	3.44
L10A ₅₂	0.32	4.62	0.18	4.20
L19A ₆₁	0.25	2.61	0.05	1.90
126A ₆₈	0.40*	3.03	0.23	1.33
L30A72	0.34	3.66	0.14	2.87
V37A ₇₉	0.27	2.66	0.07	1.17
V40A ₈₂	—	0.74	_	0.16
L48A ₉₀	—	0.65	_	0.30
F60A7		1.23	_	0.69
L61A ₈	0.25	3.42	0.08	2.97
Y63A ₁₀	0.21	3.91	0.10	2.69
V65A ₁₂	0.38	3.33	0.17	2.74
V72A ₁₉	0.17	1.42	0.00	1.14
L75A ₂₂	0.41*	2.08	0.51*	1.83
L79A ₂₆	0.17	4.78	0.23	4.03
V85A ₃₂	0.08	3.46	0.15	2.74
V88A35	0.15	2.09	0.61*	1.59
V90A ₃₇	0.16	2.86	0.56*	2.33

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Adapted from data in ref. 1. Subscripts refer to P^{54–55} numbering. *The positions with f > 0.4 are shown in Fig. 1.

 Haglund E, Lindberg MO, Oliveberg M (2008) Changes of protein folding pathways by circular permutation. Overlapping nuclei promote global cooperativity J Biol Chem 283:27904–27915.

Table S3. The groups of overlapping cross-peaks referred to in Table 1

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Group	Amino acid	$\log k_{ex}^{obs}$	Structural location	Comment
1	E31 ₇₃	-5.55	α 1, hydrogen bonds with Q27 ₆₉ .	Overlapping region where the cross-peaks of Y3375
	Y33 ₇₅	-2.9	End of α 1/loop 1, possible hydrogen bond with L30 ₇₂ .	and $K39_{81}$ are lost at $t = 20$ min.
	K39 ₈₁	-2.9	β 2, solvent-exposed amide.	
2	\$17 ₅₉	-2.9	α 1, no apparent hydrogen bond partner.	Overlapping region where the individual cross-peaks
	K2365	Global	α 1, forms backbone hydrogen bond with L19 ₆₁ .	are either lost at $t = 20$ min or still persist at $t =$
	L3072	Global	α 1, forms backbone hydrogen bond with 12668.	60 h.
	N100	-2.9	Next to the N terminus, no apparent hydrogen bond partner.	
3	A3577	-2.9	Loop 2, no apparent hydrogen bond partner.	Overlapping region where assignments of R47 and K54 are unclear; can be swapped. A35 and one of
	R47 ₈₉	-2.9/-4.28	End of β 3, no apparent hydrogen bond partner.	R47/K54 are lost at $t = 20$ min, whereas the other persists after $t = 60$ h. The high protection factor
	K54 ₉₆	-2.9/-4.28	Loop 3, no apparent hydrogen bond partner.	of the remaining group seems inconsistent with the solvent-exposed amides of both R47 and K54.
4	N11 ₅₃	-5.04	β 1, forms backbone hydrogen bond with N84 ₃₁ .	Overlapping region where the individual cross-peaks
	N1355	-2.9	Loop 1, no apparent hydrogen bond partner.	are either lost at $t = 20$ min or exchange slowly.
	Q574	-2.9	H ^N points towards solvent from β 3, no apparent hydrogen bond partner.	
5	12668	Global	α 1, forms backbone hydrogen bond with E22 ₆₄ .	Overlapping region where the individual cross-peaks
	15294	-2.9	Loop 3, no apparent hydrogen bond partner.	are either lost at $t = 20$ min or still persist at $t =$
	D552	-2.9	Loop 3, no apparent hydrogen bond partner.	60 h.
6	L45 ₈₇	-2.9	β 2, amide points towards solvent.	Overlapping region where the individual cross-peaks
	Y50 ₉₂	-2.9	Loop 3, no apparent hydrogen bond partner.	are either lost at $t = 20$ min or exchange slowly.
	Q6411	-4.31	β 3, forms backbone hydrogen bond with K39 ₈₁ .	
7	R46 ₈₈	-2.9	End of β 2, forms backbone hydrogen bond with G58 ₅ .	Overlapping region where all cross-peaks are lost at $t = 20$ min.
	Y59 ₆	-2.9	Beginning of β 3, forms backbone hydrogen bond with L10 ₅₂ .	
	V8835	-2.9	β 4, amide points into solvent.	
8	V37 ₇₉	-2.9	β 2, amide points towards solvent.	Overlapping region where the individual cross-peaks
	E43 ₈₅	-2.9	β 2, forms backbone hydrogen bond with F60 ₇ .	are either lost at $t = 20$ min or exchange slowly.
	M89 ₃₆	-4.60	β 4, forms backbone hydrogen bond with N7 ₄₉ .	
9	V9 ₅₁	Global	β 1, forms backbone hydrogen bond with R87 ₃₄ .	Overlapping region where the individual cross-peaks
	Q18 ₆₀	-2.9	Beginning of α 1, no apparent hydrogen bond partner.	are either lost at $t = 20$ min or still persist at $t = 60$ h.
	W62 ₉	-2.9	β 3, forms backbone hydrogen bond with E41 ₈₃ .	
10	E24 ₆₆	-4.69	α 1, forms backbone hydrogen bond with A20 ₆₂ .	Overlapping region where the individual cross-peaks
	D74 ₂₁	-2.9	 α 2, hydrogen bonding implicated from crystal structure ambiguous. 	are either lost at $t = 20$ min or exchange slowly.
11	Y59 ₆	-2.9	Flexible end of β 3, close to the P ^{54–55} N terminus.	Overlapping region where the individual cross-peaks
	E66 ₁₃	-3.55	β 3, forms backbone hydrogen bond with R36 ₇₈ .	are either lost at $t = 20$ min or exchange very fast.
	V8835	-2.9	β 4, amide points towards solvent.	
	R46 ₈₈	-2.9	Flexible end of β 2 close to the P ^{54–55} C terminus.	
12	L45 ₈₇	-2.9	β 2, amide points towards solvent.	Overlapping region where L45 ₈₇ is lost at $t = 20$ min
	Q6411	-3.64	β 3, forms backbone hydrogen bond with K39 ₈₁ .	and Q64 ₁₁ exchanges by intermediate rate.
13	152 ₉₄	-2.9	Loop 3, no apparent hydrogen bond partner.	Overlapping region where 152_{94} is lost at $t = 20$ min
	I26 ₆₈	Global	α 1, forms backbone hydrogen bond with E22 ₆₄ .	and I26 ₆₈ persists at $t = 60$ h.
14	N11 ₅₃	-4.64	β 1, forms backbone hydrogen bond with N84 ₃₁ .	Overlapping region where N13 ₅₅ is lost at $t = 20$ min
	N1355	-2.9	Loop 3, no apparent hydrogen bond partner.	and N11 ₅₃ exchanges relatively slowly.
15	D74 ₂₁	-2.9	 α 2, hydrogen bonding implicated from crystal structure ambiguous. 	Overlapping region where $D74_{21}$ is lost at $t = 20$ min and $E24_{66}$ exchanges by intermediate rate.
	E24 ₆₆	-3.80	α 1, forms backbone hydrogen bond with A20 ₆₂ .	

As seen from the descriptions, the spectral overlap has little bearing on the HD-exchange rate constants because the disappearance of the involved cross-peaks in most cases takes place on well-separated time scales.