Mutation-Induced Rigidity in the Fyn SH2 Domain Enhances pY-Binding Affinity at the Cost of Peptide Specificity

Supplementary information

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Table S1. Secondary-structure names and corresponding one-symbol label used in DSSP and this work.

Secondary structure name	Label in DSSP	Label in this work
Alpha-helix	H	H
Residue in an isolated beta-bridge	B	D
Extend strand that participates in beta-ladder	E	E
3_10-helix	G	G
pi-helix	I	I
kappa-helix	P	P
Bend	S	S
Hydrogen-bonded turn	T	T
Loop	~	K

Table S2. Number of sites in different regions with different secondary structures between the crystal structure of the wild-type Fyn SH2 (PDB id: 1AOT) and the predicted structure of its mutant.

Name of regions	Region	Length	Type	Number of sites with different secondary structures
N-terminal	[1-13]	13	Unstructured	8
αA	[14-22]	9	Structured	1
ABloop	[23-30]	8	Unstructured	4
βB	[31-35]	5	Structured	0
BCloop	[36-42]	7	Unstructured	6
βC	[43-51]	9	Structured	3
CDloop	[52-54]	3	Unstructured	0
βD	[55-65]	11	Structured	3
DEloop	[66-70]	5	Unstructured	3
βE	[71-72]	2	Structured	0
EF loop	[73-76]	4	Unstructured	0
βF	[77-79]	3	Structured	0
FBloop	[80-81]	2	Unstructured	0
αB	[82-91]	10	Structured	2
BGloop	[92-101]	10	Unstructured	3
βG	[102-103]	2	Structured	2
C-terminal	[104-106]	3	Unstructured	2
Total number	/	106		37

Note: Different regions are identified by dynamically stable secondary structures.

Table S3. Contact domain residues for 204pY in the crystal structure and the predicted structure.

Fyn_SH2_WT	L12	G13	R14	K15	D16	A17	R34	E35	S36	E37	T38	V39
	Y43	A44	L45	S46	K59	H60	Y61	L62	I63	\	D67	
Fyn_SH2_MT	L12	G13	R14	K15	\	A17	R34	E35	S36	E37	T38	V39
	\	A44	L45	S46	K59	H60	Y61	L62	\	R64	\	

Table S4. Contact domain residues for I207 in the crystal structure and the predicted structure.

Fyn_SH2_WT	Y61	I73	T74	T75	R76	Y89	A93	G95	L96	S97
Fyn_SH2_MT	Y61	I73	T74	T75	R76	Y89	A93	G95	L96	S97

Table S5. Numbers of different positions among the secondary-structure sequences from initial and dynamically stable secondary structures for different systems.

Name of regions	MT vs WT	WT_pY vs WT	MT_pY vs MT	MT_pY vs WT_pY	WT vs WT_initial	MT vs MT_initial
N-terminal	4	6	2	0	7	4
αA	0	0	0	0	0	1
ABloop	6	5	0	3	4	2
βB	0	0	0	0	0	0
BCloop	2	2	0	0	7	0
βC	0	0	0	0	3	0
CDloop	0	0	0	0	0	0
βD	0	0	0	0	3	0
DEloop	0	2	0	0	5	2
βE	0	0	0	0	0	0
EF loop	0	2	0	0	0	0
βF	0	3	0	1	0	2
FBloop	0	0	0	0	0	0
αB	0	0	0	0	2	0
BGloop	4	5	2	5	3	6
βG	2	0	0	2	2	0
C-terminal	0	0	2	1	0	2
Total number	18	25	6	12	36	19

Note: In the table, the dynamically stable secondary structure of Fyn_SH2_WT is referred to by WT, Fyn_SH2_MT by MT, Fyn_SH2_WT+pYEEI by WT_pY, Fyn_SH2_MT+pYEEI by MT_pY, Fyn_SH2_WT by WT_initial, and Fyn_SH2_MT by MT_initial.

Table S6. Hydrogen bonds between the domain and the pY-peptide with occupancy greater than 60%.

Hydrogen bond		Occupancy (%)	
Donor	Acceptor	Fyn_SH2_WT+pYEEI	Fyn_SH2_MT+pYEEI
ARG34-Side	PTR204-Side	91.2	96.2
GLU37-Main	PTR204-Side	66.5	92.1
GLU205-Main	HIS60-Main	35.1	84.9
THR38-Side	PTR204-Side	28.6	76.5
SER36-Side	PTR204-Side	78.6	63.6

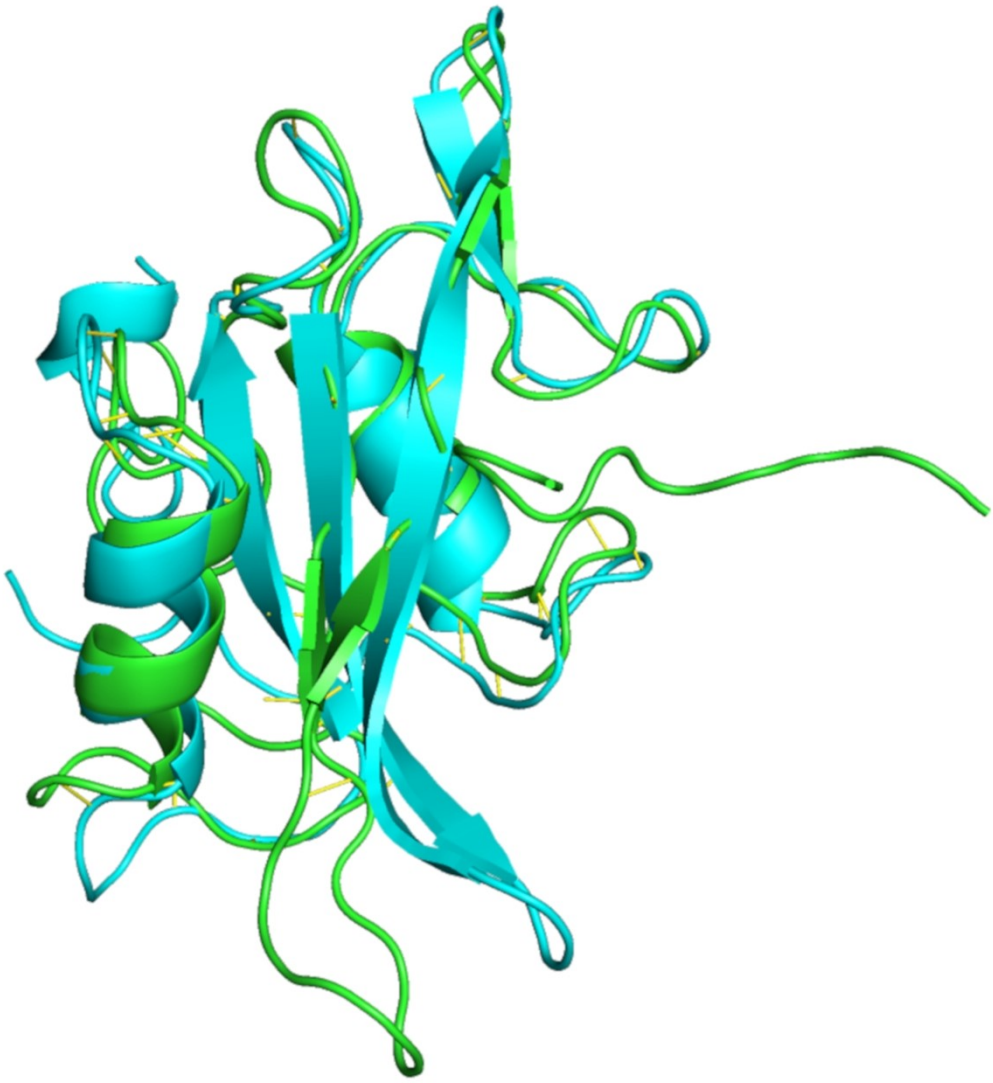

Figure S1. Aligned mutant (colored in blue) and wild-type (colored in green) structures.

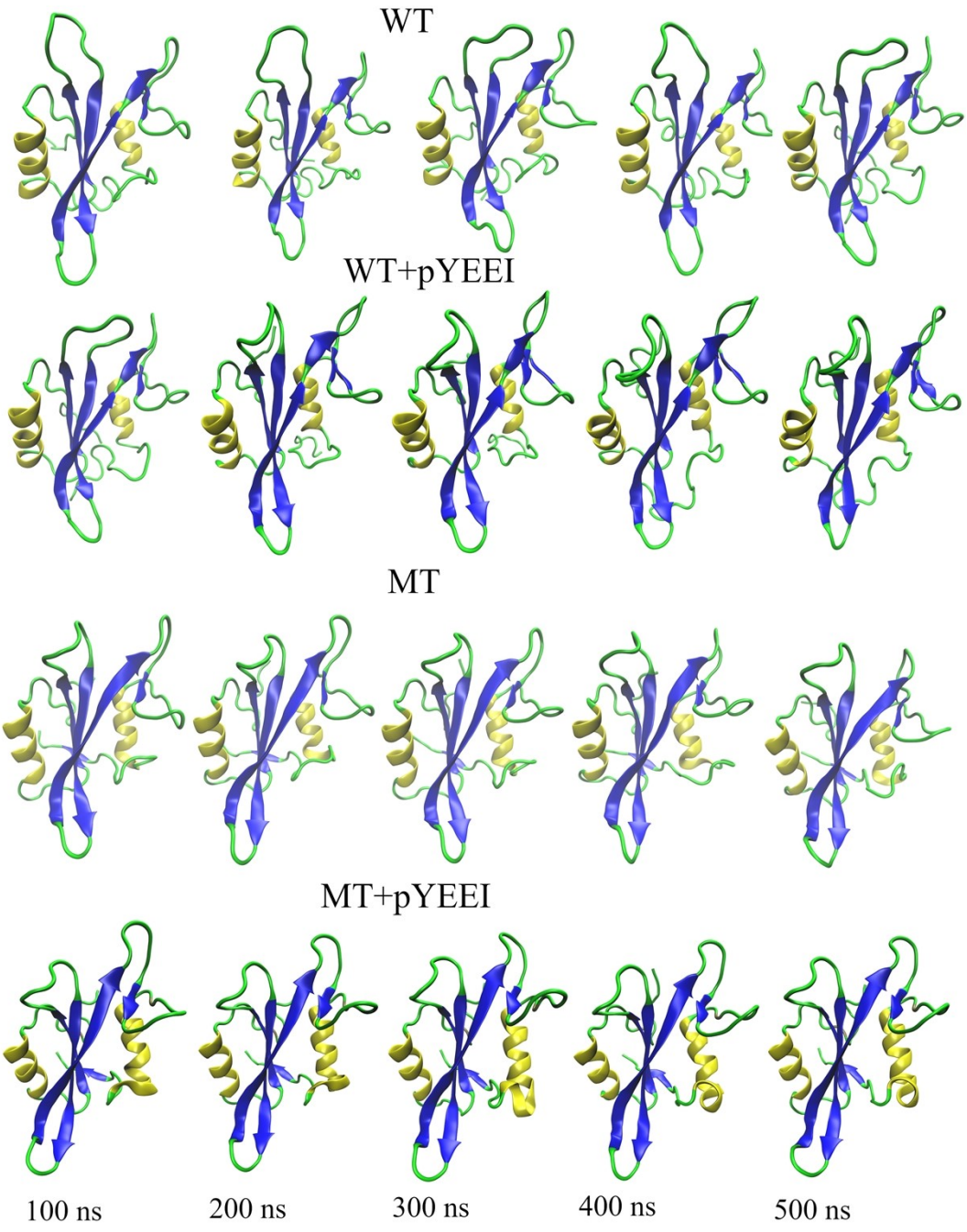

Figure S2. Conformations sampled from different systems with the interval of 100 ns.

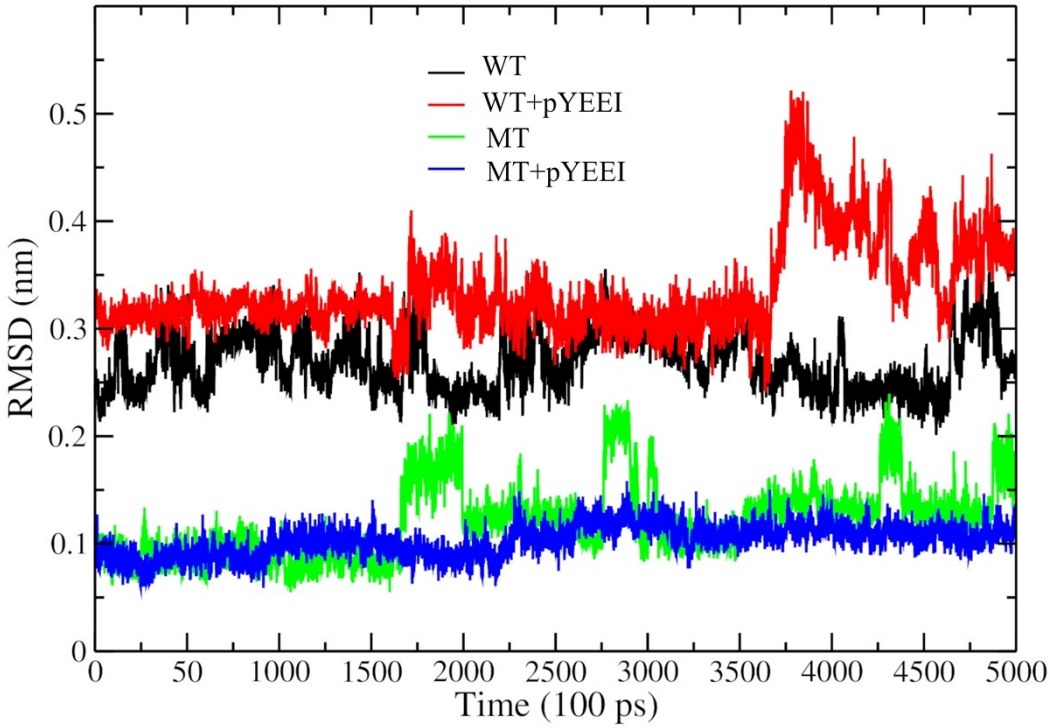

Figure S3. RMSDs of the SH2 domain for different systems.

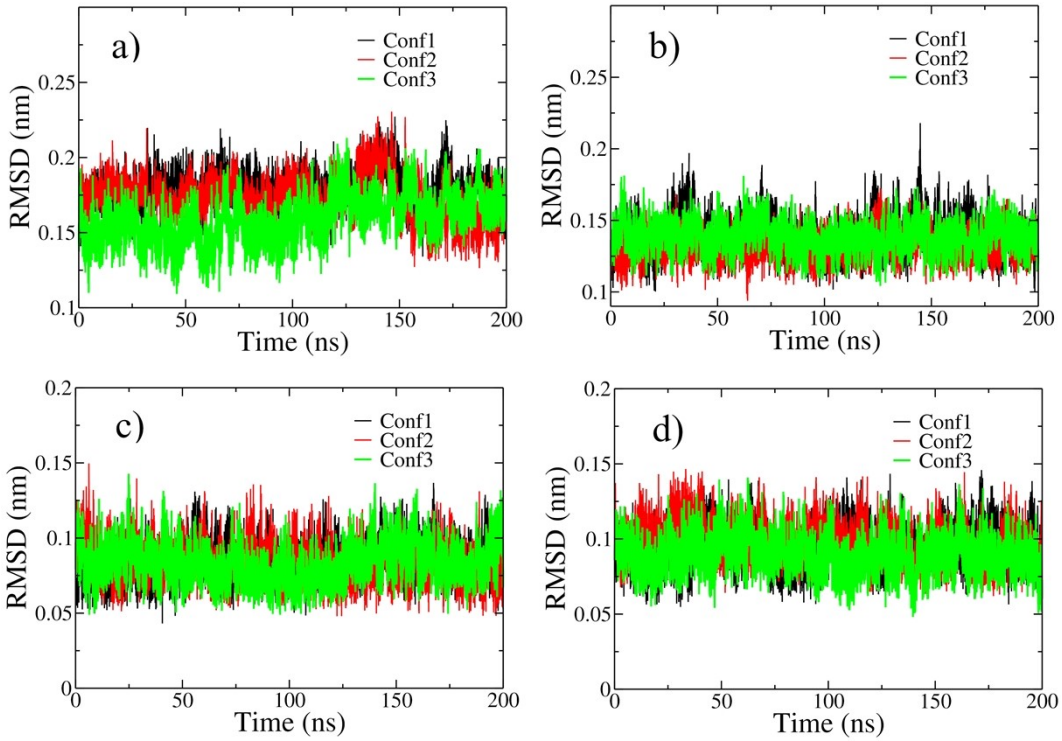

Figure S4. RMSDs of the SH2 domain in 200-ns simulations with different initial structures. a) The wild-type Fyn SH2 monomer. b) The wild-type Fyn SH2 complexed with pYEEI. c) The mutant Fyn SH2 monomer. d) The mutant Fyn SH2 complexed with pYEEI.

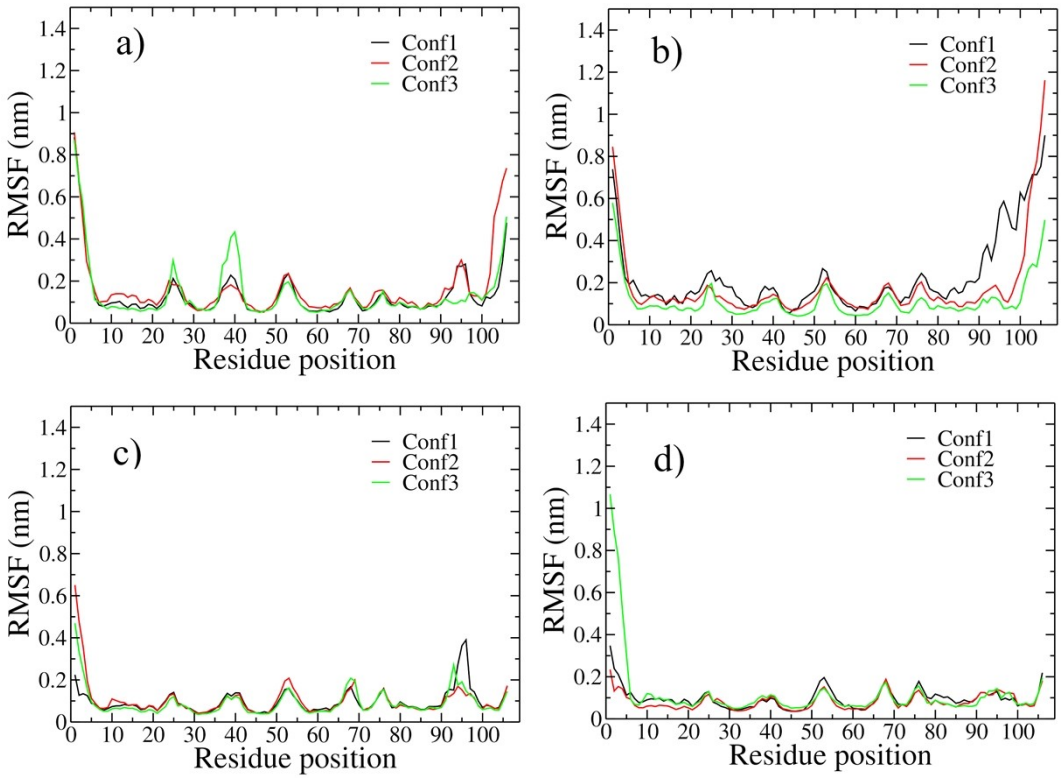

Figure S5. RMSFs of the SH2 domain in 200-ns simulations with different initial structures. a) The wild-type Fyn SH2 monomer. b) The wild-type Fyn SH2 complexed with pYEEI. c) The mutant Fyn SH2 monomer. d) The mutant Fyn SH2 complexed with pYEEI.

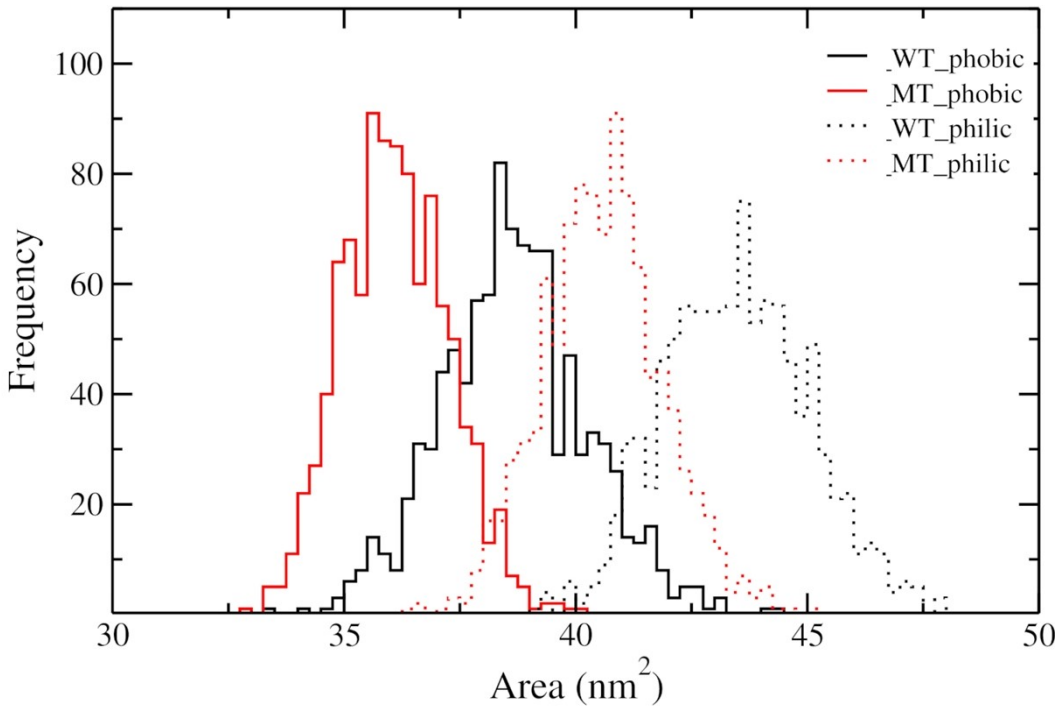

Figure S6. Hydrophobic and hydrophilic SASAs of the wild-type and mutant complexes.

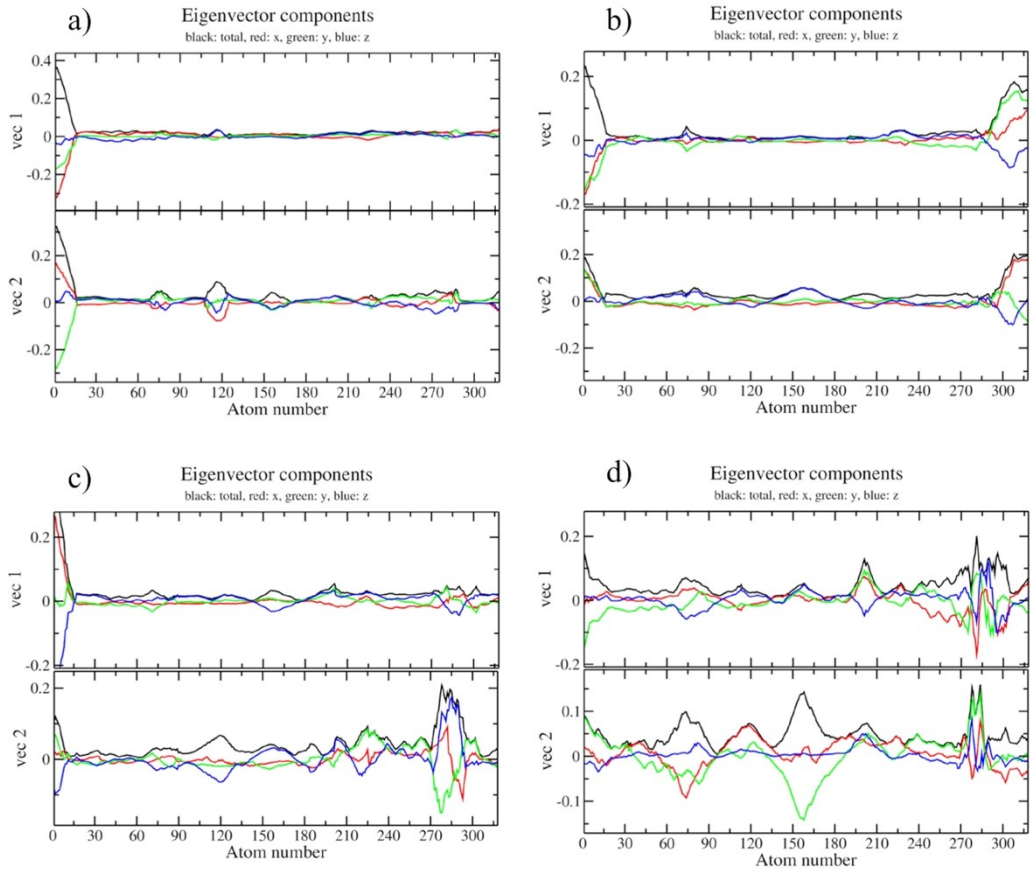

Figure S7. Eigenvector components of PC1 and PC2 for the isolated wild-type Fyn SH2 (a), wild-type Fyn SH2 complex (b), isolated mutant (c), and mutant complex (d).

Figure S8. Secondary-structure sequences obtained by various methods for the wild-type and mutant systems.

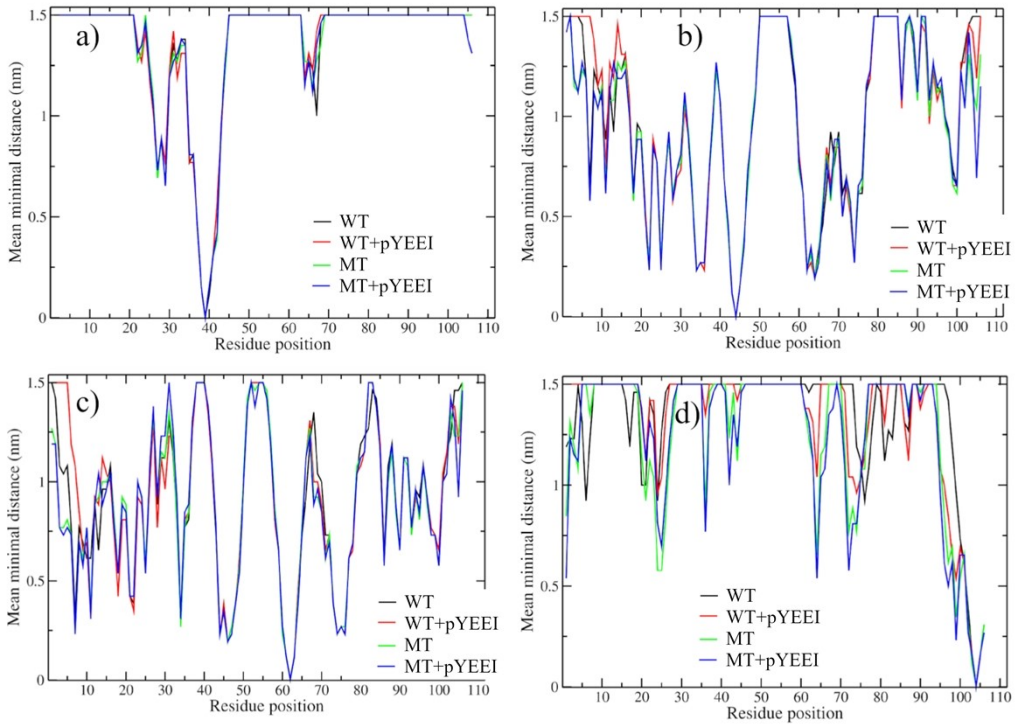

Figure S9. Distances of residues T39/V39 (a), S44/A44 (b), K62/L62 (c), and S104 (d) in the domain from other residues for all the four systems.

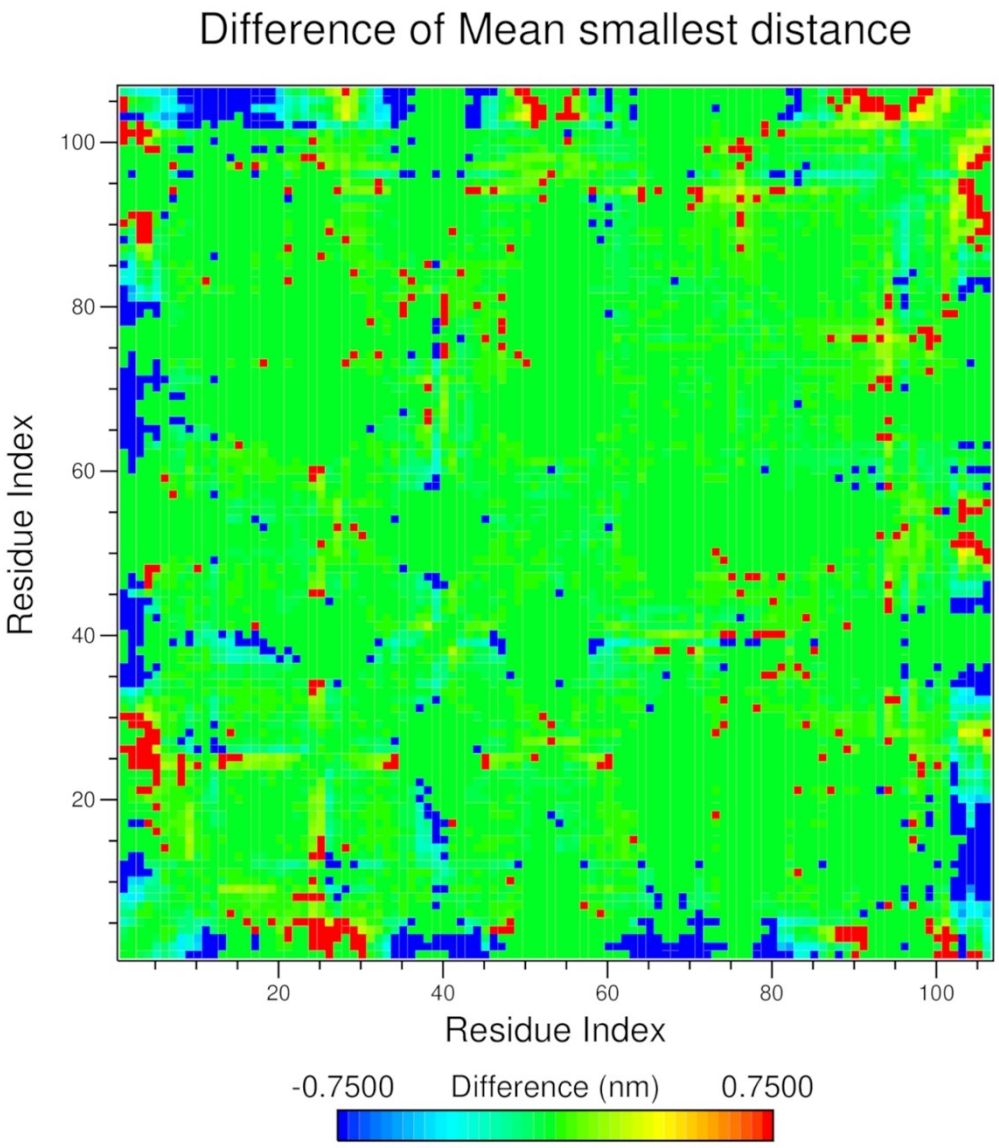

Figure S10. Difference of the contact maps between the isolated wild-type Fyn SH2 and the mutant.

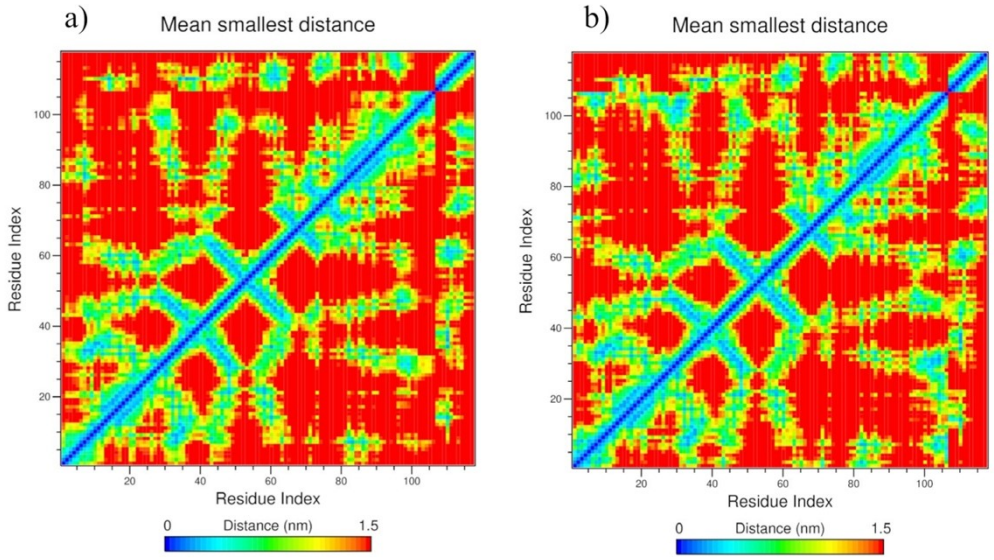

Figure S11. Residue-residue contact maps of the complex for the wild-type Fyn SH2 (a), and the mutant (b).

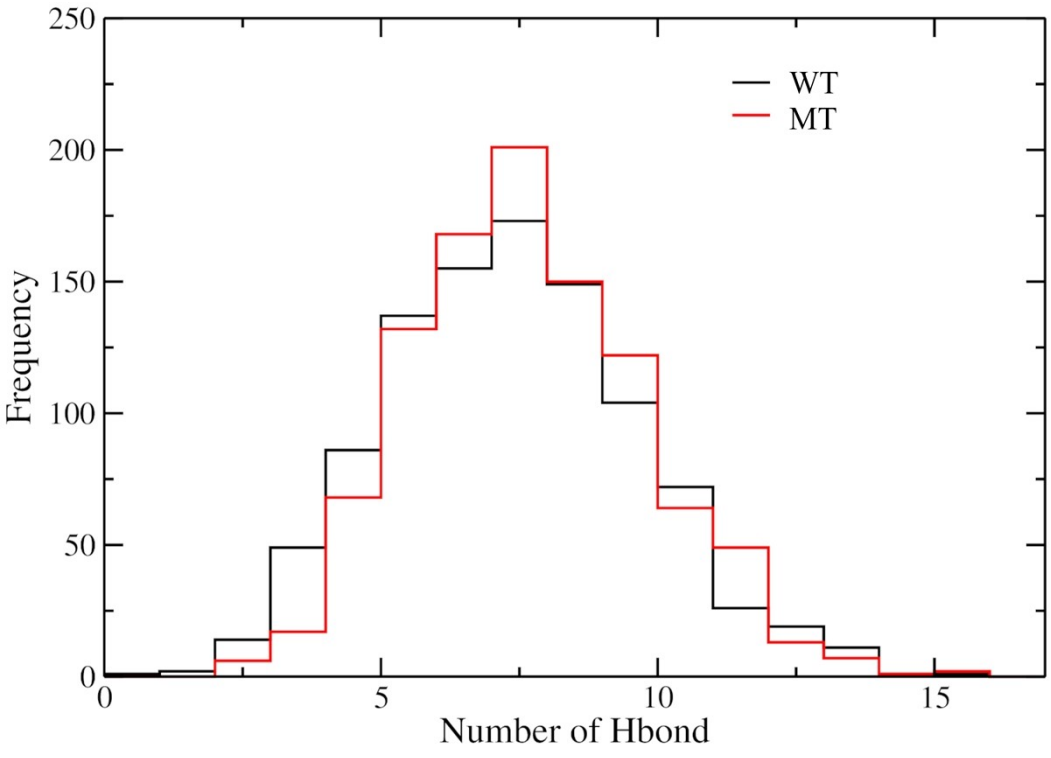

Figure S12. Frequencies of hydrogen bonds between the domain and the pY-peptide for the wild-type and mutant complex systems.

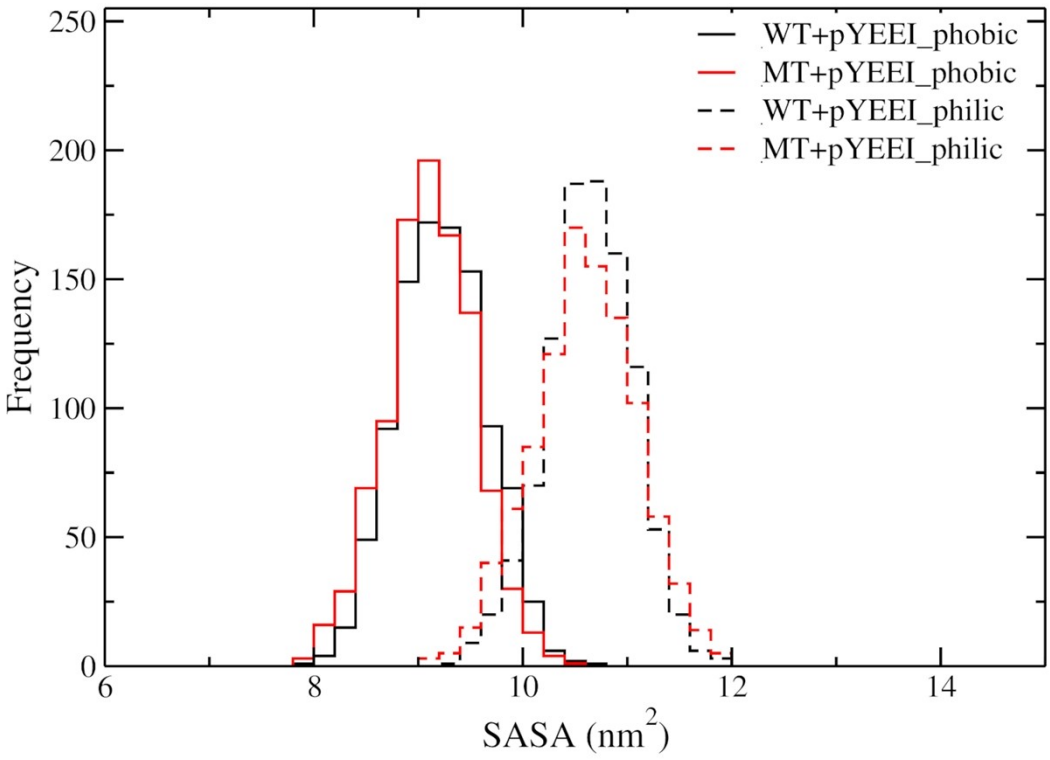

Figure S13. Total hydrophobic and hydrophilic contact surfaces between the domain and the pY-peptide for the wild-type and mutant complex systems.

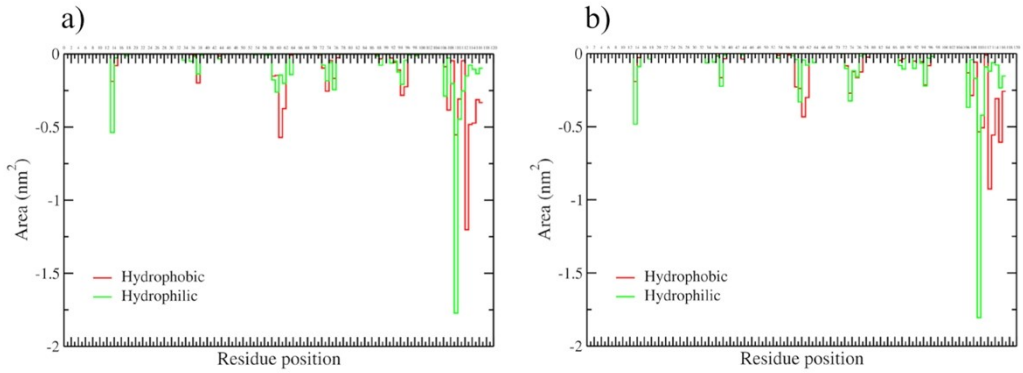

Figure S14. Hydrophobic and hydrophilic contact surfaces for each residue of the domain and the pY-peptide for the wild-type (a) and mutant (b) complex systems.

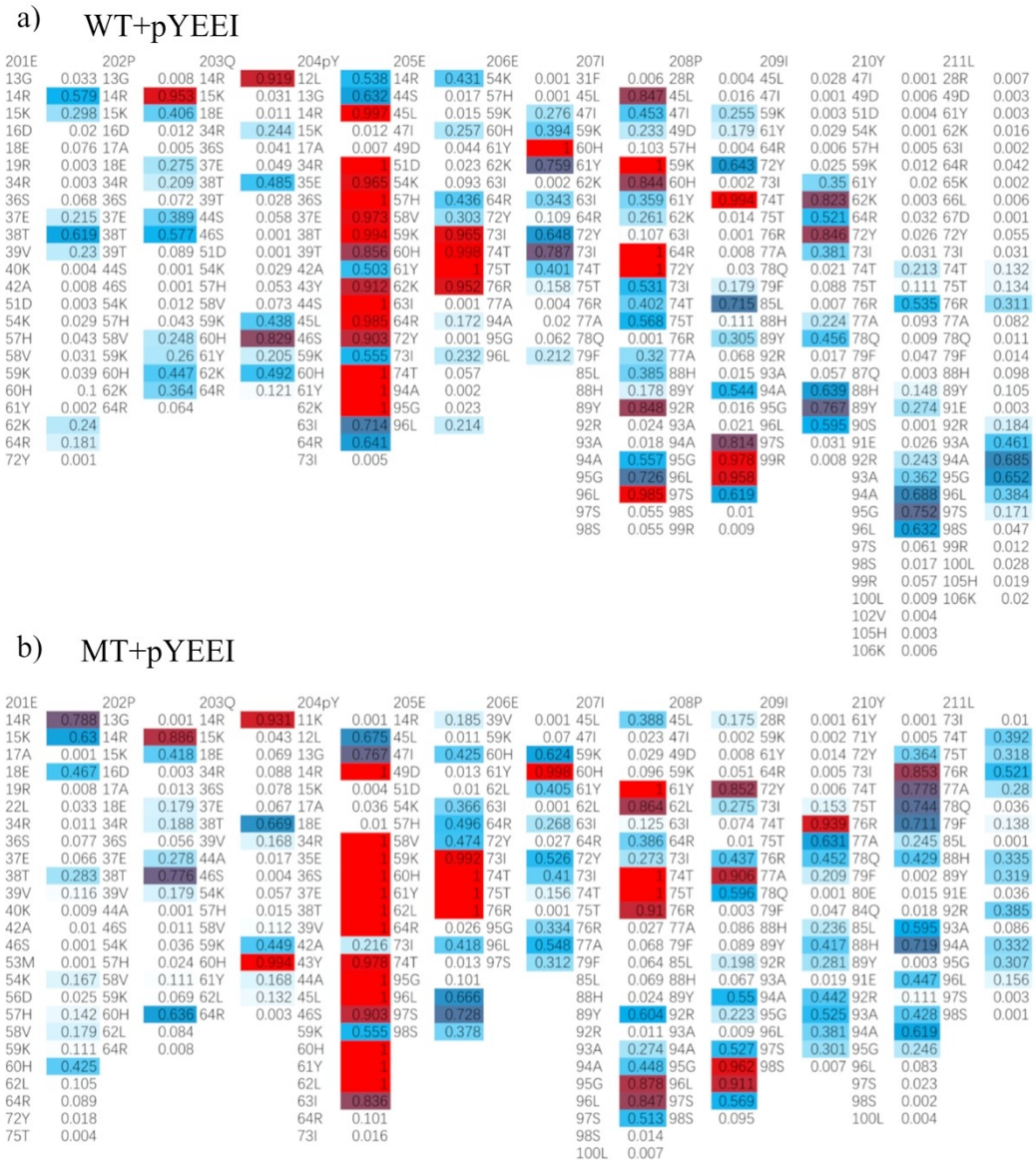

Figure S15. List of the contact protein residues and their occupancies for each pY-peptide residue in the wild-type (a) and the mutant (b) complex systems. To visualize different contact stabilities, the occupancies greater than 0.6 are colored with gradually fading red reflecting the decrease of the value, and blue for those greater than 0.1 and less than 0.6.