Supporting Information

Intelligent Design of 14-3-3 Docking Proteins Utilizing Synthetic Evolution Artificial Intelligence (SYN-AI)

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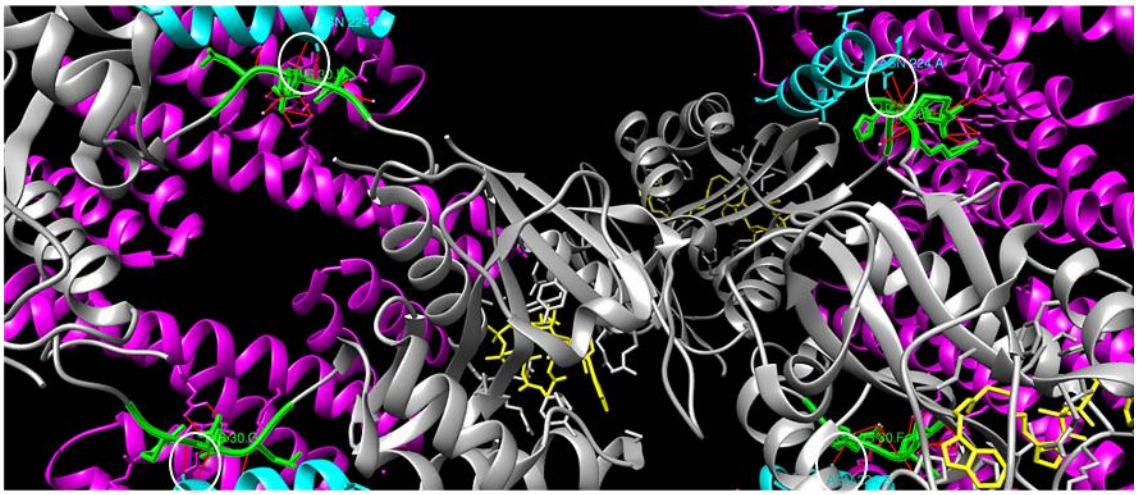
A.



B.

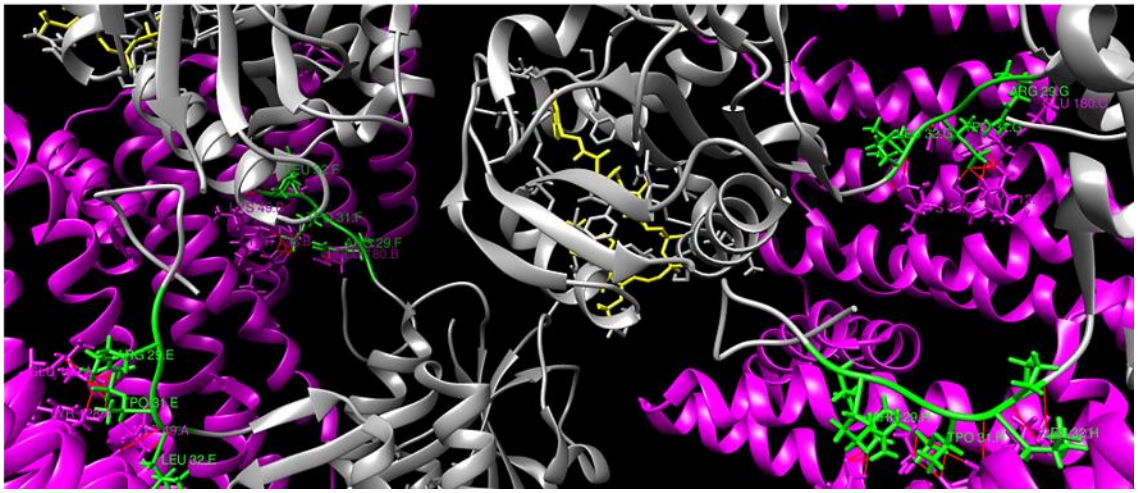


Figure S1. 14-3-3 ζ serotonin N-acetyl transferase hydrogen bonding. Native 14-3-3 serotonin N-acetyl transferase structure in reported in [75], **(A)**. The SYN-AI-1 serotonin N-acetyl transferase complex was built utilizing UCSF Chimera. Native rotamers were mutated to those characterizing SYN-AI-1 and residues 215 – 228 of chains A – D deleted. Corrected structures were obtained by performing energy minimization utilizing AMBER. Wherein, 100 steepest decent steps were performed on each chain of the octamer followed by 10 conjugate gradient steps. The process was repeated until a good structure was obtained. Energy minimization was then performed on the entire complex utilizing 200 steepest decent steps followed by 10 conjugate gradient steps, **(B)**.

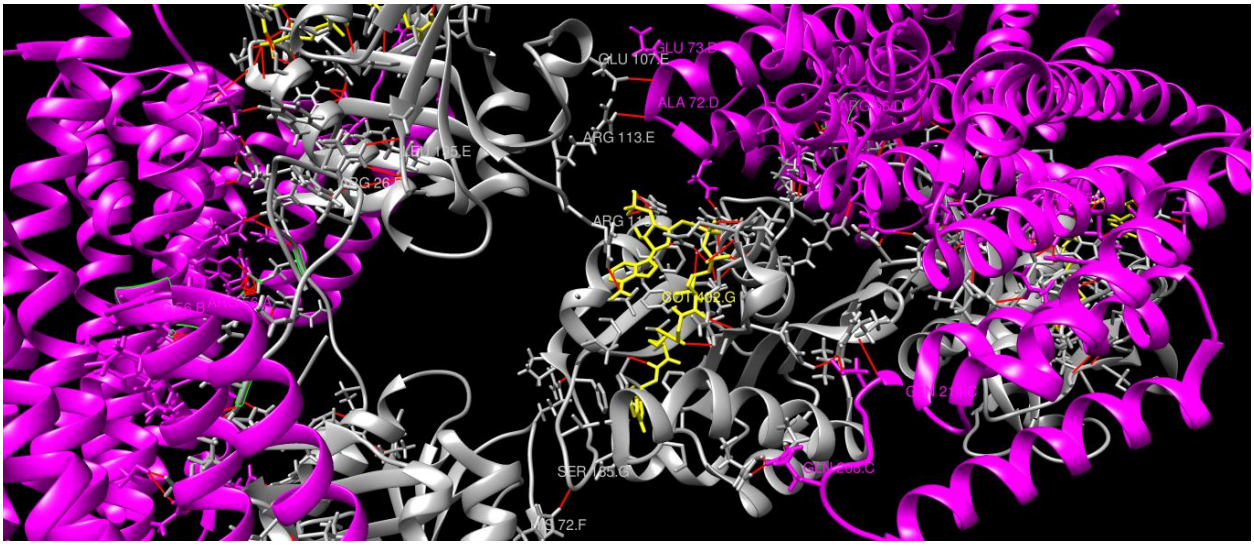


Figure S2. Serotonin N-acetyl Transferase Hydrogen Bond Formation. USCF Chimera was utilized to build the SYN-AI-1 AANAT complex from the native crystal structure reported in 75. Rotamers of the native structure were mutated to those characterizing SYN-AI-1 and C' terminal residues 215-228 deleted. Energy minimization was performed utilizing Amber until. Hydrogen bond formation was analyzed at a Van der Waals overlap distance of >= 0.6 Å with a correction of 0.5 Å subtracted for potential hydrogen bonding pairs. Contacts or clashes of less then four bonds apart were exclude.

Table S.1 Hydrogen bonding between the serotonin N-acetyl transferase conserved [RRHTLP] motif and the native 14-3-3 ζ tetramer were analyzed utilizing UCSF Chimera.

WAT	6328 O	ARG	28.E	O	WAT	6328 H2	3.276	2.505
LYS	49.A NZ	LEU	32.E	O	LYS	49.A HZ1	2.758	1.919
LYS	49.A NZ	PRO	33.E	O	LYS	49.A HZ3	2.788	2.132
ARG	56.A NH1	TPO	31.E	O2P	ARG	56.A HH12	2.851	2.005
ARG	56.A NH1	TPO	31.E	O3P	ARG	56.A HH12	2.767	1.85
ARG	56.A NH2	TPO	31.E	O1P	ARG	56.A HH22	2.744	1.762
ARG	56.A NH2	TPO	31.E	O2P	ARG	56.A HH22	2.938	2.16
ARG	56.A NH2	TPO	31.E	O3P	ARG	56.A HH22	3.322	2.519
ARG	127.A NH2	TPO	31.E	O1P	ARG	127.A HH21	2.862	1.847
ARG	127.A NH2	TPO	31.E	O3P	ARG	127.A HH21	3.051	2.344
TYR	128.A OH	TPO	31.E	O3P	TYR	128.A HH	2.643	1.666
LYS	49.B NZ	LEU	32.F	O	LYS	49.B HZ1	2.773	1.973
LYS	49.B NZ	PRO	33.F	O	LYS	49.B HZ2	2.745	2.028
ARG	56.B NH1	TPO	31.F	O2P	ARG	56.B HH12	2.862	2.034
ARG	56.B NH1	TPO	31.F	O3P	ARG	56.B HH12	2.759	1.834
ARG	56.B NH2	TPO	31.F	O1P	ARG	56.B HH22	2.788	1.814
ARG	56.B NH2	TPO	31.F	O2P	ARG	56.B HH22	2.865	2.081
ARG	56.B NH2	TPO	31.F	O3P	ARG	56.B HH22	3.289	2.481
ARG	127.B NE	TPO	31.F	O1P	ARG	127.B HE	3.267	2.411
ARG	127.B NH2	TPO	31.F	O1P	ARG	127.B HH21	2.878	1.894
ARG	127.B NH2	TPO	31.F	O3P	ARG	127.B HH21	2.98	2.194
TYR	128.B OH	TPO	31.F	O3P	TYR	128.B HH	2.644	1.667

LYS	49.C NZ	LEU	32.G	O	LYS	49.C HZ1	2.724	1.869
LYS	49.C NZ	PRO	33.G	O	LYS	49.C HZ3	2.767	2.037
ARG	56.C NH1	TPO	31.G	O2P	ARG	56.C HH12	2.823	1.959
ARG	56.C NH1	TPO	31.G	O3P	ARG	56.C HH12	2.786	1.882
ARG	56.C NH2	TPO	31.G	O1P	ARG	56.C HH22	2.723	1.739
ARG	56.C NH2	TPO	31.G	O2P	ARG	56.C HH22	3.008	2.234
ARG	56.C NH2	TPO	31.G	O3P	ARG	56.C HH22	3.302	2.496
ARG	127.C NH2	TPO	31.G	O1P	ARG	127.C HH21	2.846	1.819
TYR	128.C OH	TPO	31.G	O3P	TYR	128.C HH	2.649	1.67
LYS	49.D NZ	LEU	32.H	O	LYS	49.D HZ1	2.806	2.05
LYS	49.D NZ	PRO	33.H	O	LYS	49.D HZ3	2.737	2.002
ARG	56.D NH1	TPO	31.H	O2P	ARG	56.D HH12	2.865	2.048
ARG	56.D NH1	TPO	31.H	O3P	ARG	56.D HH12	2.749	1.825
ARG	56.D NH2	TPO	31.H	O1P	ARG	56.D HH22	2.818	1.844
ARG	56.D NH2	TPO	31.H	O2P	ARG	56.D HH22	2.866	2.097
ARG	56.D NH2	TPO	31.H	O3P	ARG	56.D HH22	3.223	2.406
ARG	127.D NE	TPO	31.H	O1P	ARG	127.D HE	3.145	2.222
ARG	127.D NH2	TPO	31.H	O1P	ARG	127.D HH21	3.024	2.088
ARG	127.D NH2	TPO	31.H	O3P	ARG	127.D HH21	2.92	2.059
TYR	128.D OH	TPO	31.H	O3P	TYR	128.D HH	2.681	1.71
ARG	29.E NH1	GLU	180	A OE2	ARG	29.E HH11	2.726	1.766
LEU	32.E N	ASN	173	A OD1	LEU	32.E H	2.849	1.841
ARG	28.F NH2	GLU	180	B OE1	ARG	28.F HH21	3.003	2.142
ARG	28.F NH2	GLU	180	B OE2	ARG	28.F HH21	3.245	2.288
ARG	29.F NH1	GLU	180	B OE2	ARG	29.F HH11	2.748	1.804
LEU	32.F N	ASN	173	B OD1	LEU	32.F H	2.858	1.853
ARG	29.G NH1	GLU	180	C OE2	ARG	29.G HH11	2.694	1.746
LEU	32.G N	ASN	173	C OD1	LEU	32.G H	2.873	1.868
ARG	28.H NE	GLU	180	D OE2	ARG	28.H HE	3.08	2.16
ARG	28.H NH2	GLU	180	D OE1	ARG	28.H HH21	2.724	1.713
ARG	28.H NH2	GLU	180	D OE2	ARG	28.H HH21	3.267	2.466
ARG	29.H NH1	GLU	180	D OE2	ARG	29.H HH11	2.783	1.876
LEU	32.H N	ASN	173	D OD1	LEU	32.H H	2.856	1.855

Table S.2. Hydrogen bonding between the Serotonin N-acetyl transferase conserved [RRHTLP] motif and the native 14-3-3 ζ tetramer were analyzed utilizing UCSF Chimera.

LYS	49.A NZ	LEU	32.E	O	LYS	49.A HZ1	3.387	2.654
ARG	56.A NH1	TPO	31.E	O2P	ARG	56.A HH12	2.87	1.879
ARG	56.A NH2	TPO	31.E	O1P	ARG	56.A HH22	3.124	2.218
ARG	56.A NH2	TPO	31.E	O2P	ARG	56.A HH22	3.565	2.83
ARG	127.A NH2	TPO	31.E	O3P	ARG	127.A HH21	3.039	2.048
TYR	128.A OH	TPO	31.E	O3P	TYR	128.A HH	2.949	2.049
ASN	224.A ND2	HIS	30.E	O	ASN	224.A HD22	2.92	1.921
LYS	49.B NZ	LEU	32.F	O	LYS	49.B HZ3	3.317	2.614
ARG	56.B NH1	TPO	31.F	O2P	ARG	56.B HH12	2.777	1.78
ARG	56.B NH2	TPO	31.F	O1P	ARG	56.B HH22	3.008	2.132
ARG	56.B NH2	TPO	31.F	O2P	ARG	56.B HH22	3.528	2.817
ARG	127.B NH2	TPO	31.F	O3P	ARG	127.B HH22	3.133	2.125
TYR	128.B OH	TPO	31.F	O3P	TYR	128.B HH	2.805	1.911
ASN	224.B ND2	HIS	30.F	O	ASN	224.B HD22	3.031	2.033
LYS	49.C NZ	LEU	32.G	O	LYS	49.C HZ2	3.358	2.654
ARG	56.C NH1	TPO	31.G	O2P	ARG	56.C HH12	2.808	1.809
ARG	56.C NH2	TPO	31.G	O1P	ARG	56.C HH22	3.023	2.111
ARG	127.C NH2	TPO	31.G	O3P	ARG	127.C HH22	3.159	2.156
TYR	128.C OH	TPO	31.G	O3P	TYR	128.C HH	2.88	1.986
ASN	224.C ND2	HIS	30.G	O	ASN	224.C HD22	3.055	2.056
LYS	49.D NZ	LEU	32.H	O	LYS	49.D HZ1	3.247	2.564
ARG	56.D NH1	TPO	31.H	O2P	ARG	56.D HH12	2.77	1.787
ARG	56.D NH2	TPO	31.H	O1P	ARG	56.D HH22	3.013	2.137
ARG	56.D NH2	TPO	31.H	O2P	ARG	56.D HH22	3.54	2.836
ARG	127.D NH2	TPO	31.H	O3P	ARG	127.D HH22	3.106	2.1
TYR	128.D OH	TPO	31.H	O3P	TYR	128.D HH	2.744	1.849
ASN	224.D ND2	HIS	30.H	O	ASN	224.D HD22	3.027	2.043
ARG	29.E NH1	GLU	180	A OE1	ARG	29.E HH11	3.091	2.391
HIS	30.E N	ASN	224	A OD1	HIS	30.E H	2.831	1.836
LEU	32.E N	ASN	173	A OD1	LEU	32.E H	2.797	1.791
ARG	29.F NH1	GLU	180	B OE1	ARG	29.F HH11	2.991	2.261
HIS	30.F N	ASN	224	B OD1	HIS	30.F H	2.922	1.935
LEU	32.F N	ASN	173	B OD1	LEU	32.F H	2.774	1.768
ARG	29.G NH1	GLU	180	C OE1	ARG	29.G HH11	3.092	2.36
HIS	30.G N	ASN	224	C OD1	HIS	30.G H	2.876	1.88
LEU	32.G N	ASN	173	C OD1	LEU	32.G H	2.833	1.829
ARG	28.H NH1	WAT	6328	O	ARG	28.H HH11	3.406	2.445
ARG	29.H NH1	GLU	180	D OE1	ARG	29.H HH11	2.938	2.187
HIS	30.H N	ASN	224	D OD1	HIS	30.H H	2.898	1.918
LEU	32.H N	ASN	173	D OD1	LEU	32.H H	2.696	1.703