Electronic Supplementary Material (ESI) for Molecular Systems Design & Engineering.

Supplementary Information

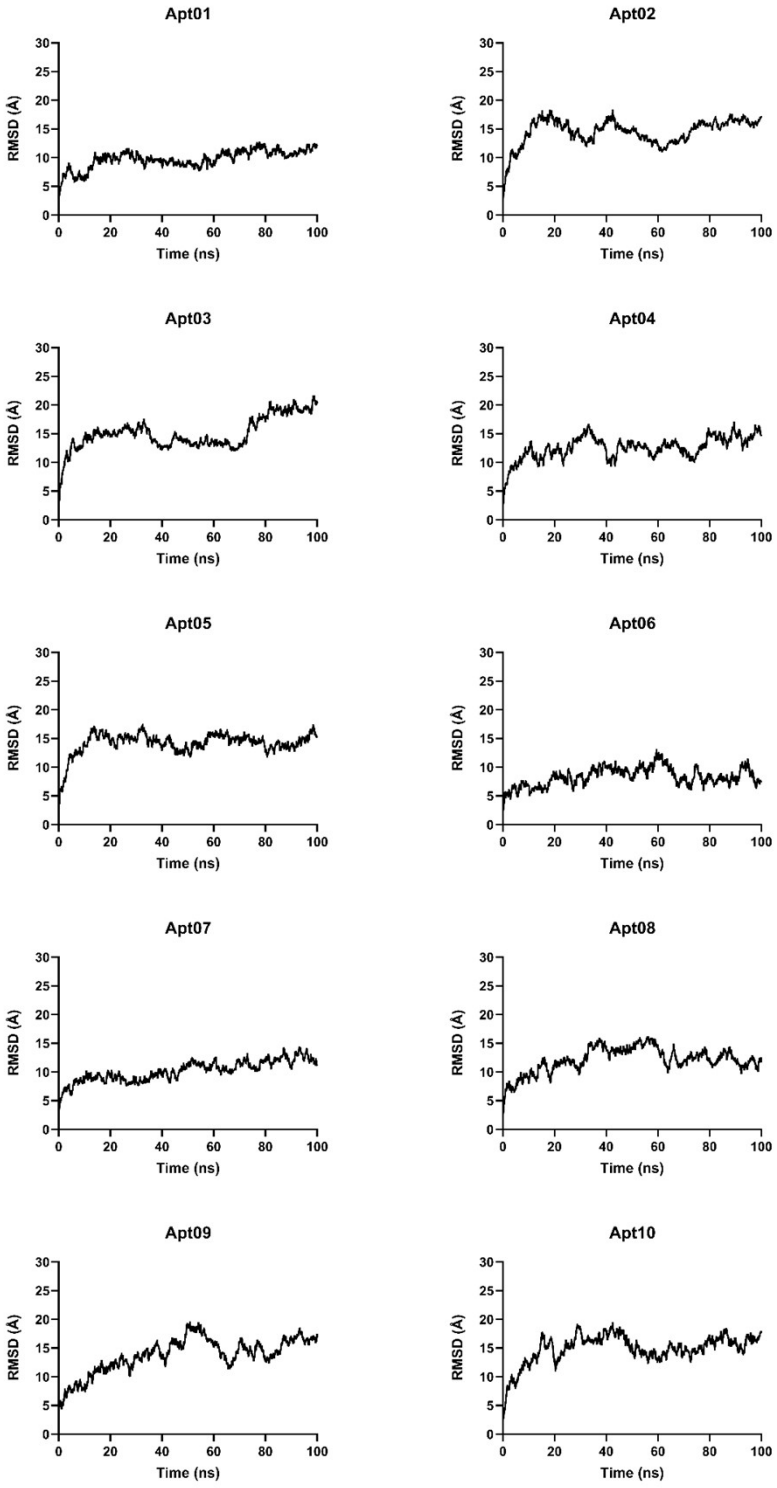

Figure S1. Root Mean Square Deviation (RMSD) values of all non-hydrogen atoms as a function of time for the 100 ns of the Molecular Dynamic (MD) simulation of aptamers Apt01 to Apt10. The equilibrated structure was used as the reference point for the RMSD calculation.

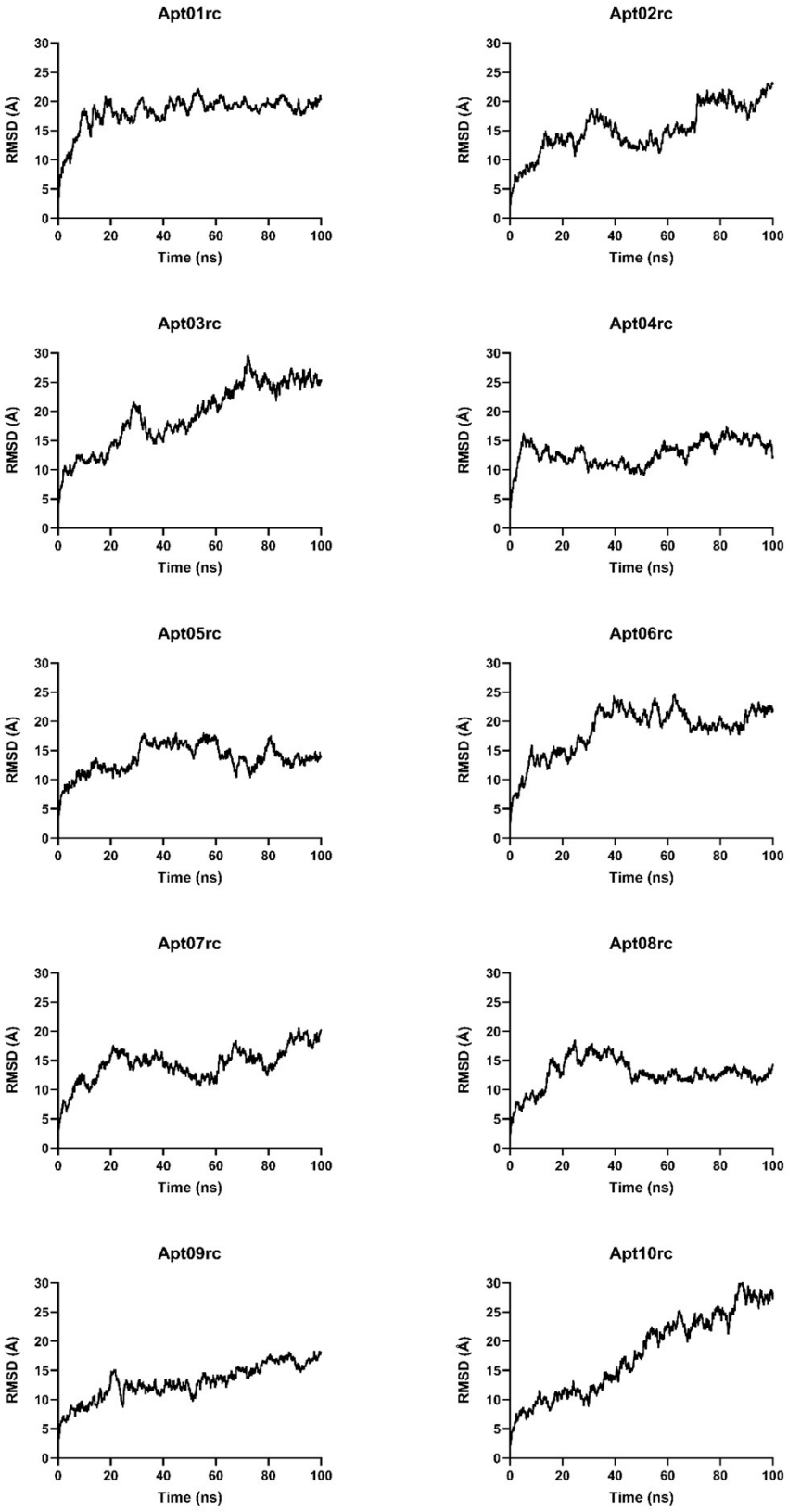

Figure S2. Root Mean Square Deviation (RMSD) values of all non-hydrogen atoms as a function of time for the 100 ns of the Molecular Dynamic (MD) simulation of aptamers Apt01rc to Apt10rc. The equilibrated structure was used as the reference point for the RMSD calculation.

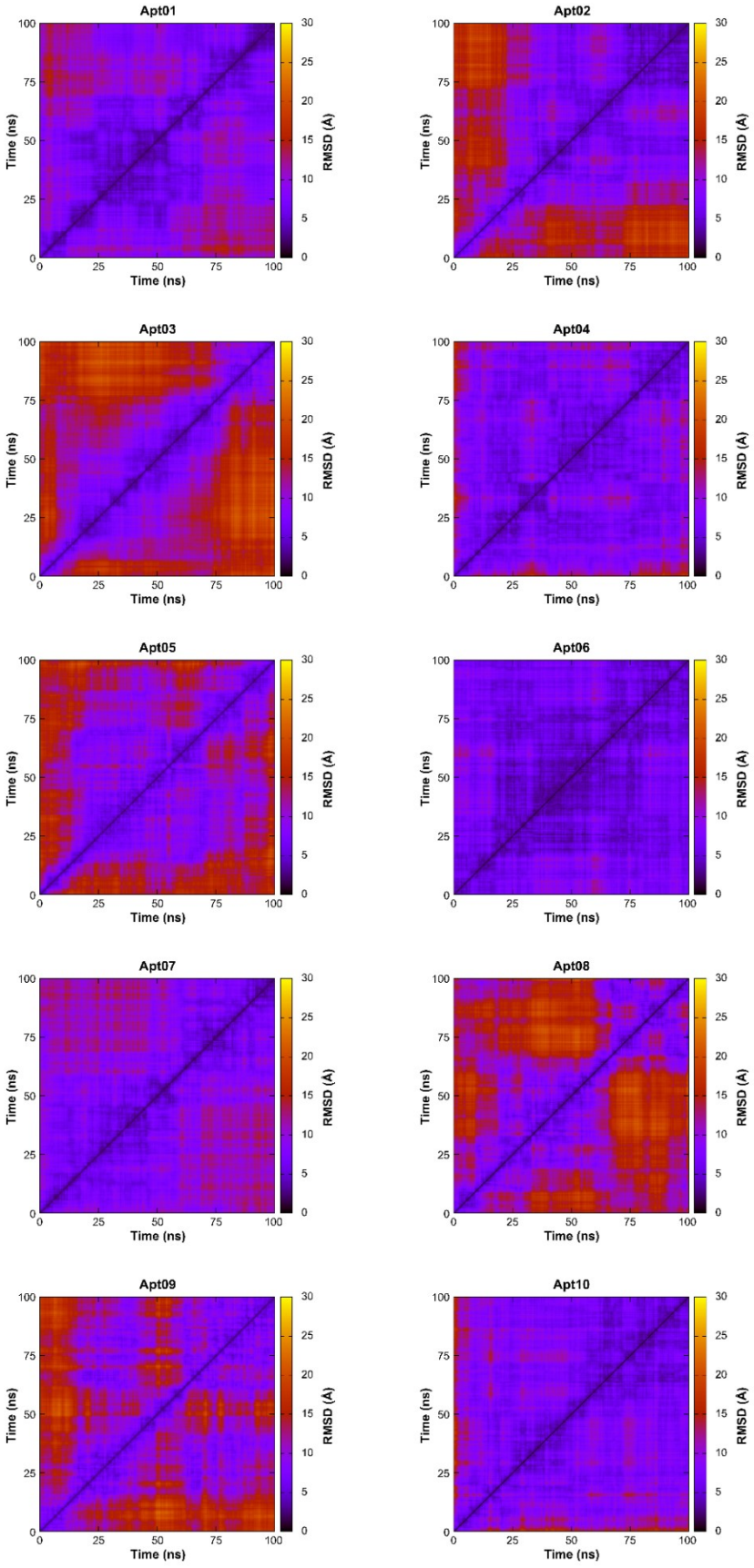

Figure S3. Pairwise Root Mean Square Deviation (RMSD) of all non-hydrogen atoms of aptamers Apt01 to Apt10.

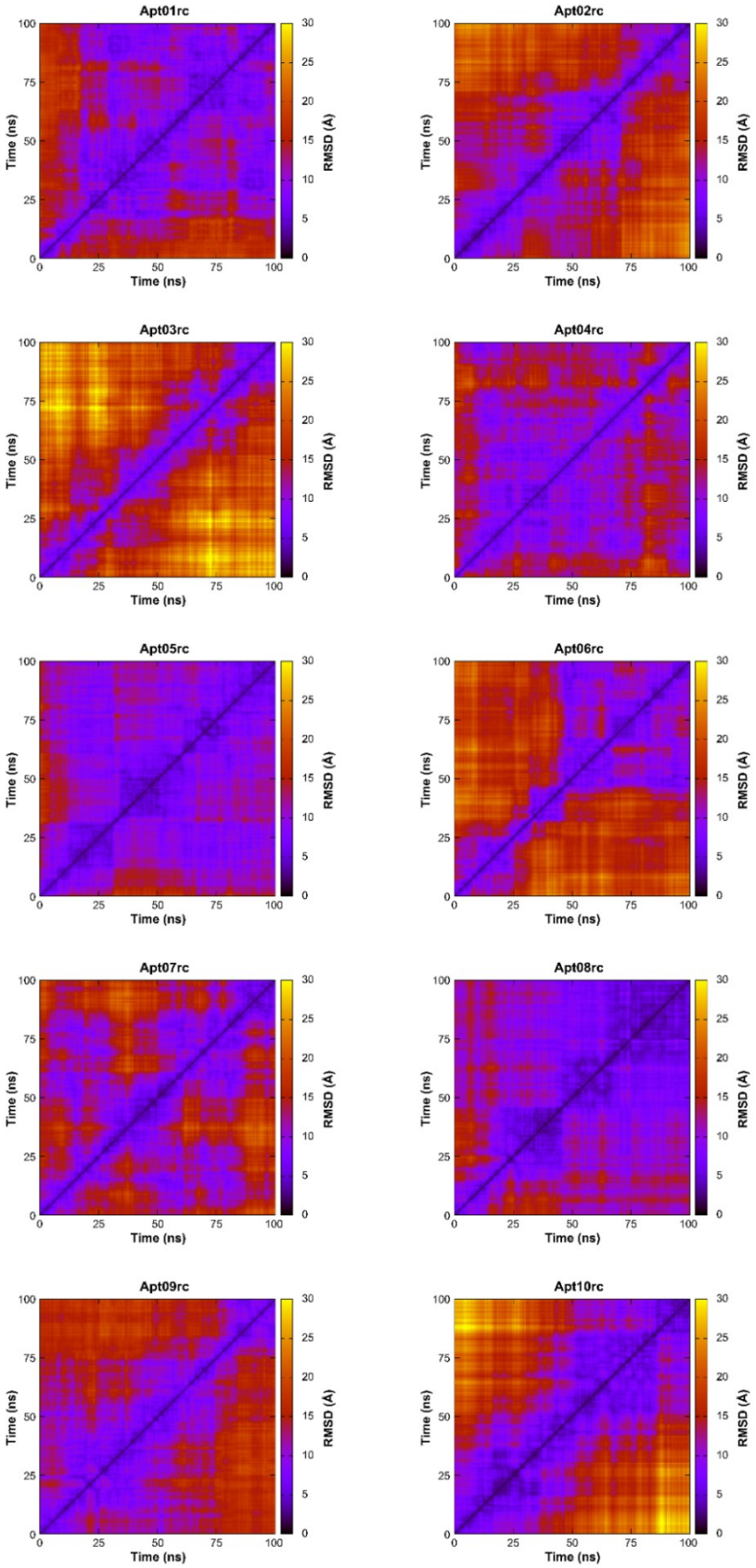

Figure S4. Pairwise Root Mean Square Deviation (RMSD) of all non-hydrogen atoms of aptamers Apt01rc to Apt10rc.

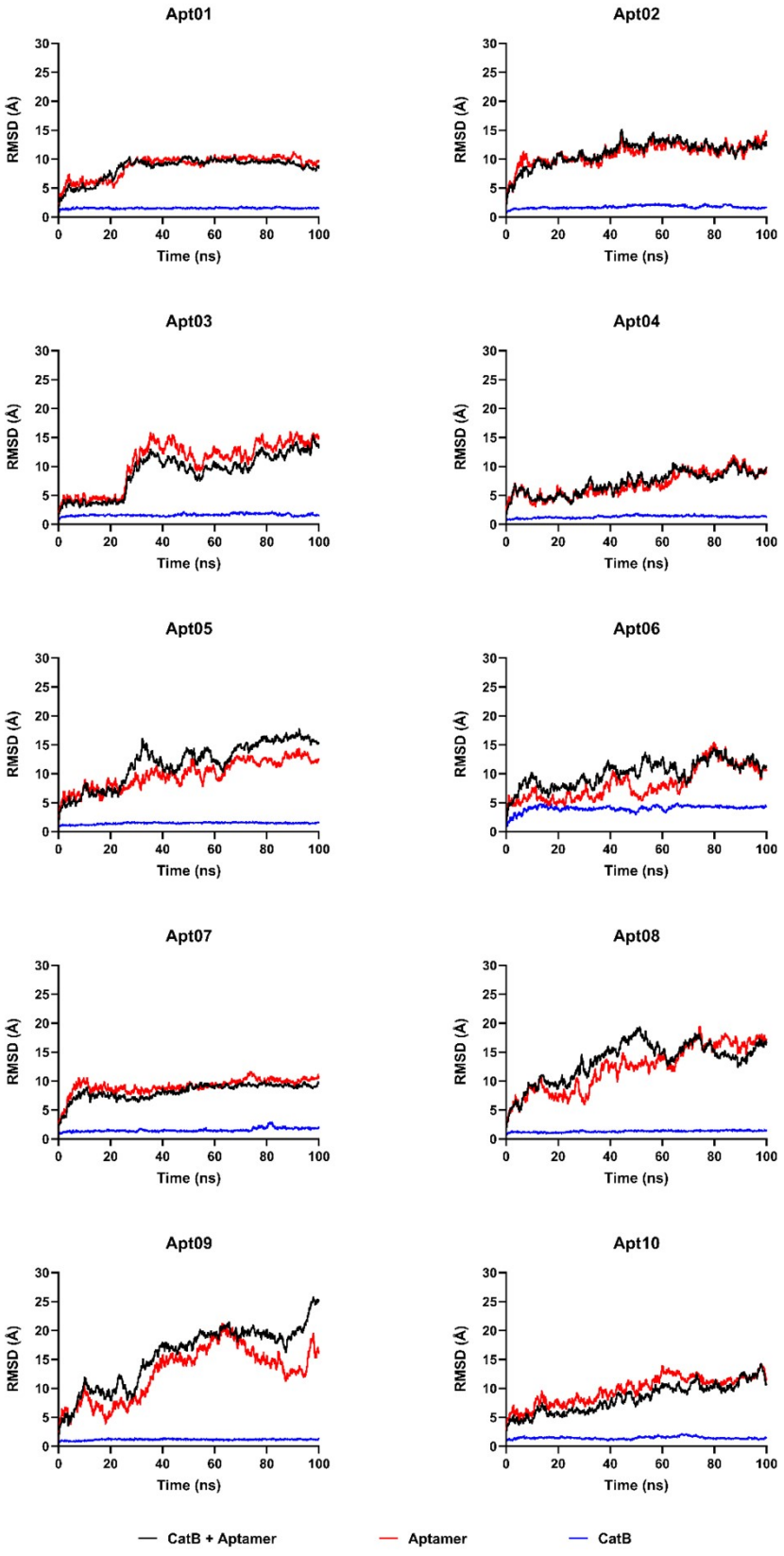

Figure S5. Root Mean Square Deviation (RMSD) values as a function of time for the 100 ns of the Molecular Dynamic (MD) simulation of the complexes between aptamers Apt01 to Apt10 and Cathepsin B (Cat B). The equilibrated structure was used as the reference point for the RMSD calculation. In black is represented the RMSD of all non-hydrogen atoms of the complex CatB–Apt; red represents the RMSD of all non-hydrogen atoms of the aptamer; and in blue is represented the RMSD of Cα atoms of Cat B backbone.

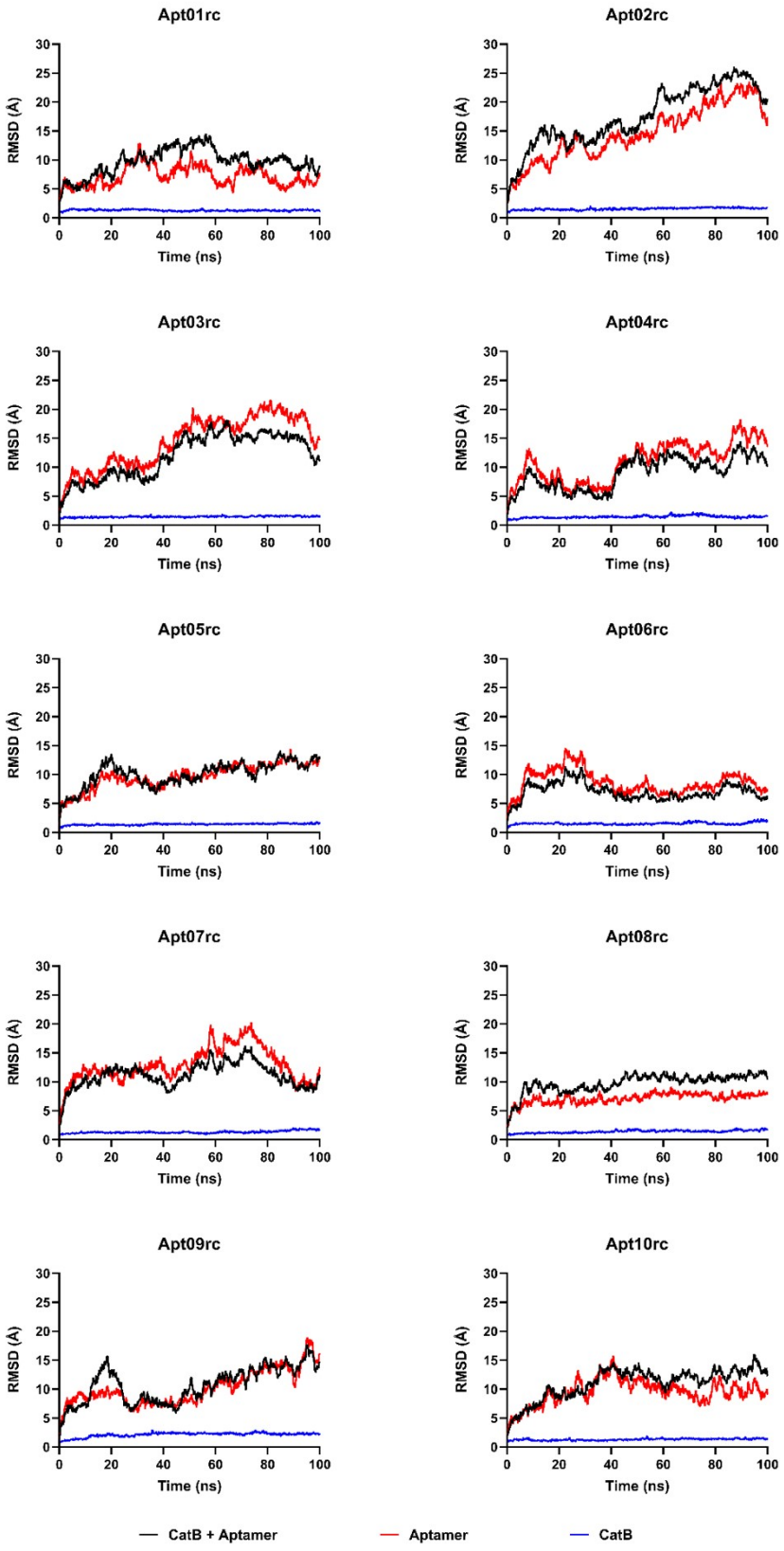

Figure S6. Root Mean Square Deviation (RMSD) values as a function of time for the 100 ns of the Molecular Dynamic (MD) simulation of the complexes between aptamers Apt01rc to Apt10rc and Cathepsin B (Cat B). The equilibrated structure was used as the reference point for the RMSD calculation. The RMSD of all non-hydrogen atoms of the complex CatB–Apt is represented in black; the RMSD of all non-hydrogen atoms of the aptamer is shown in red; and the RMSD of Cα atoms of Cat B backbone is shown in blue.

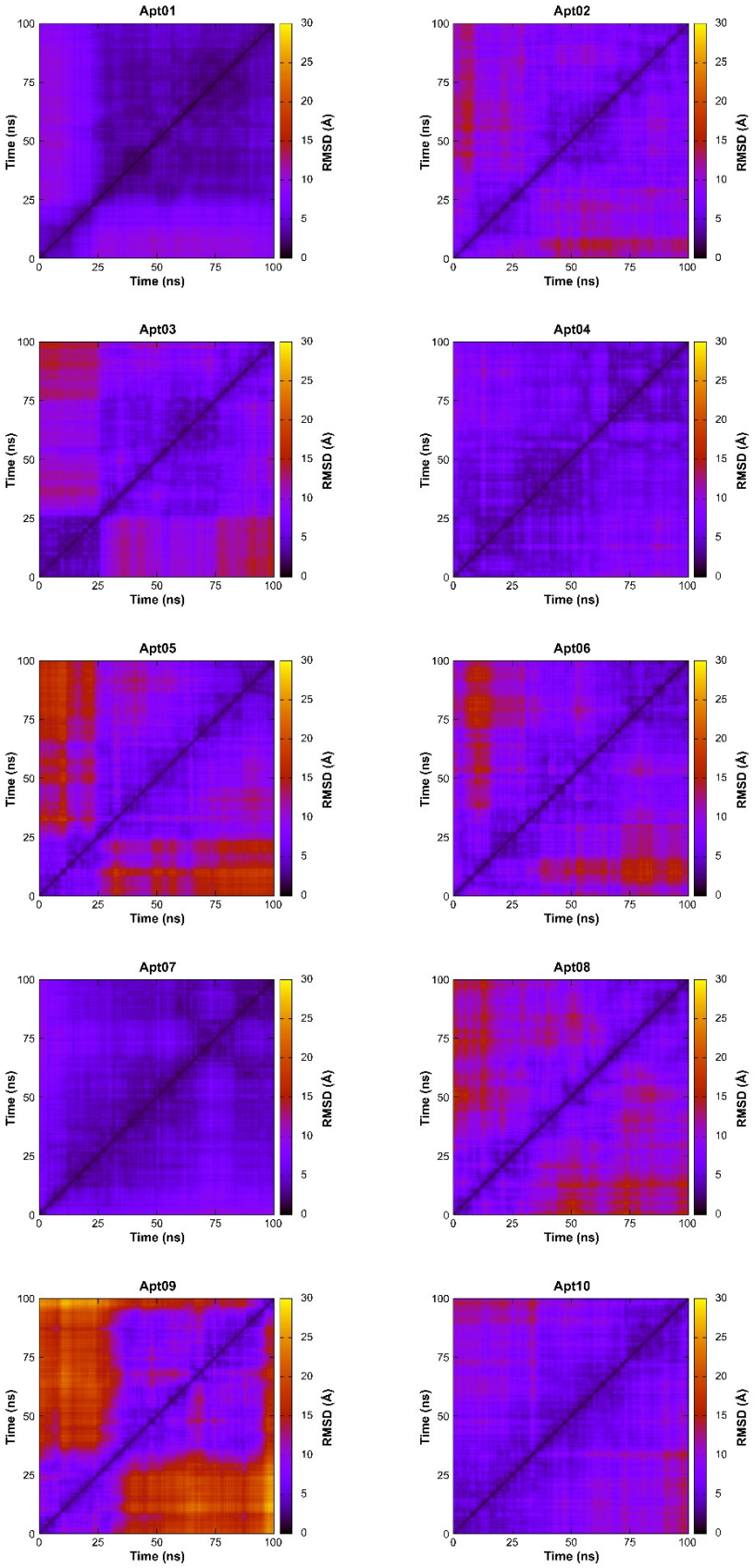

Figure S7. Pairwise Root Mean Square Deviation (RMSD) of all non-hydrogen atoms of the complex between the protein Cathepsin B and aptamers Apt01 to Apt10.

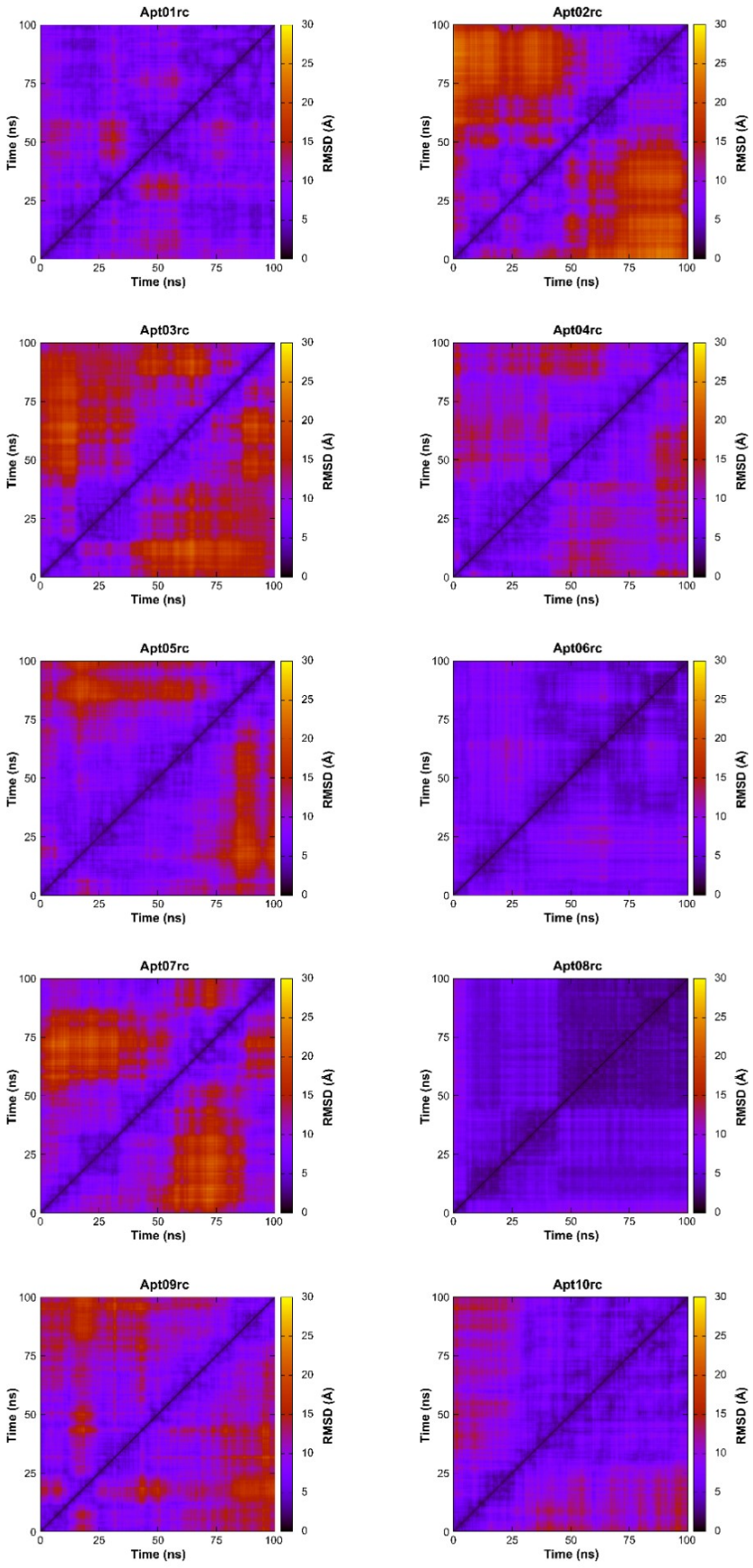

Figure S8. Pairwise Root Mean Square Deviation (RMSD) of all non-hydrogen atoms of the complex between the protein Cathepsin B and aptamers Apt01rc to Apt10rc.

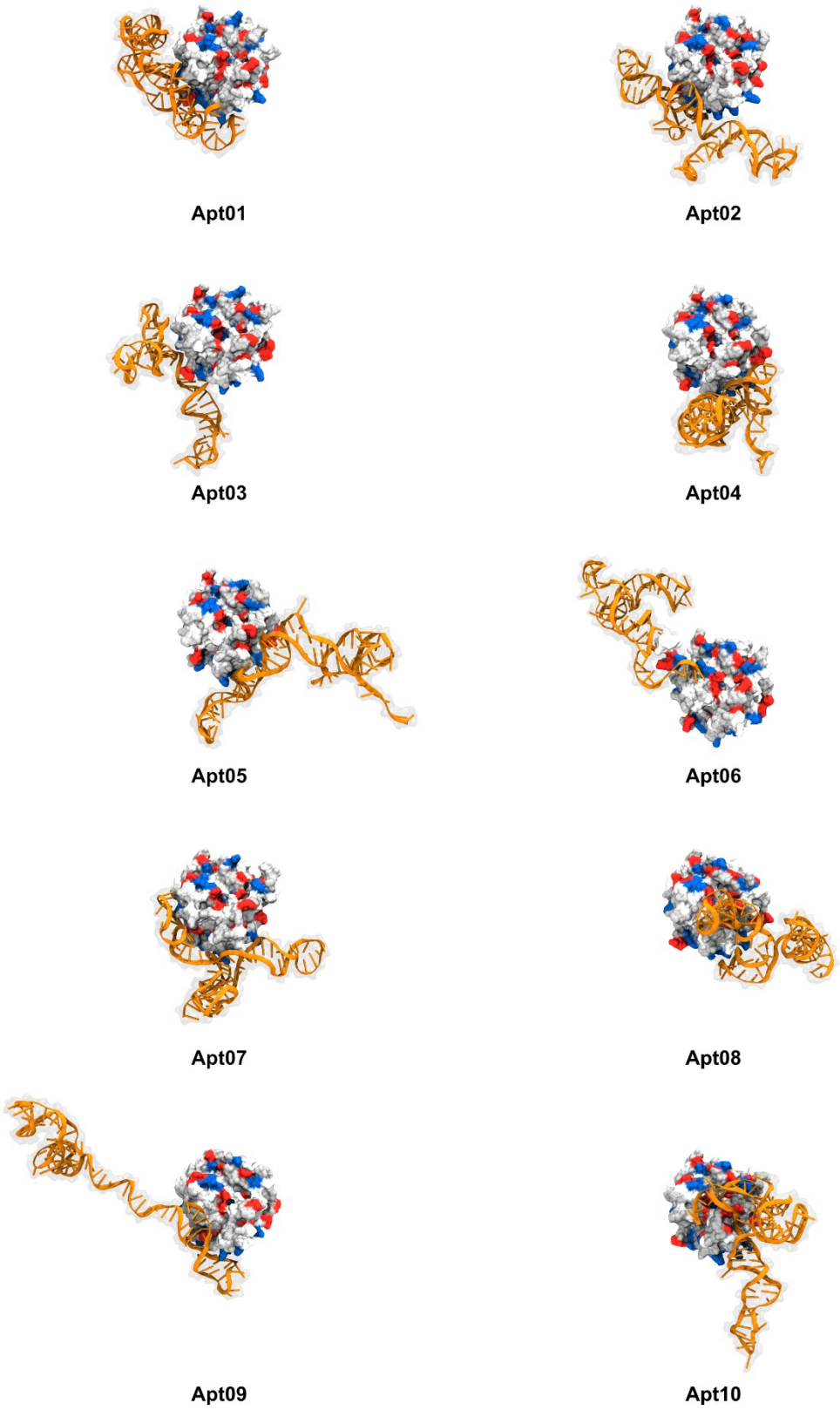

Figure S9. Representative structures of the complexes between the protein Cathepsin B (CatB) and aptamers Apt01 to Apt10 obtained by K-means clustering of the molecular dynamics simulation. CatB is represented in Surface and colored according to its residue type: Blue, positively charged side groups; Red, negatively charged side groups; White, non-polar with aliphatic or aromatic side groups; Grey, polar with uncharged side groups. The aptamers are represented in NewCartoon and are colored in Orange. All complexes are aligned by CatB.

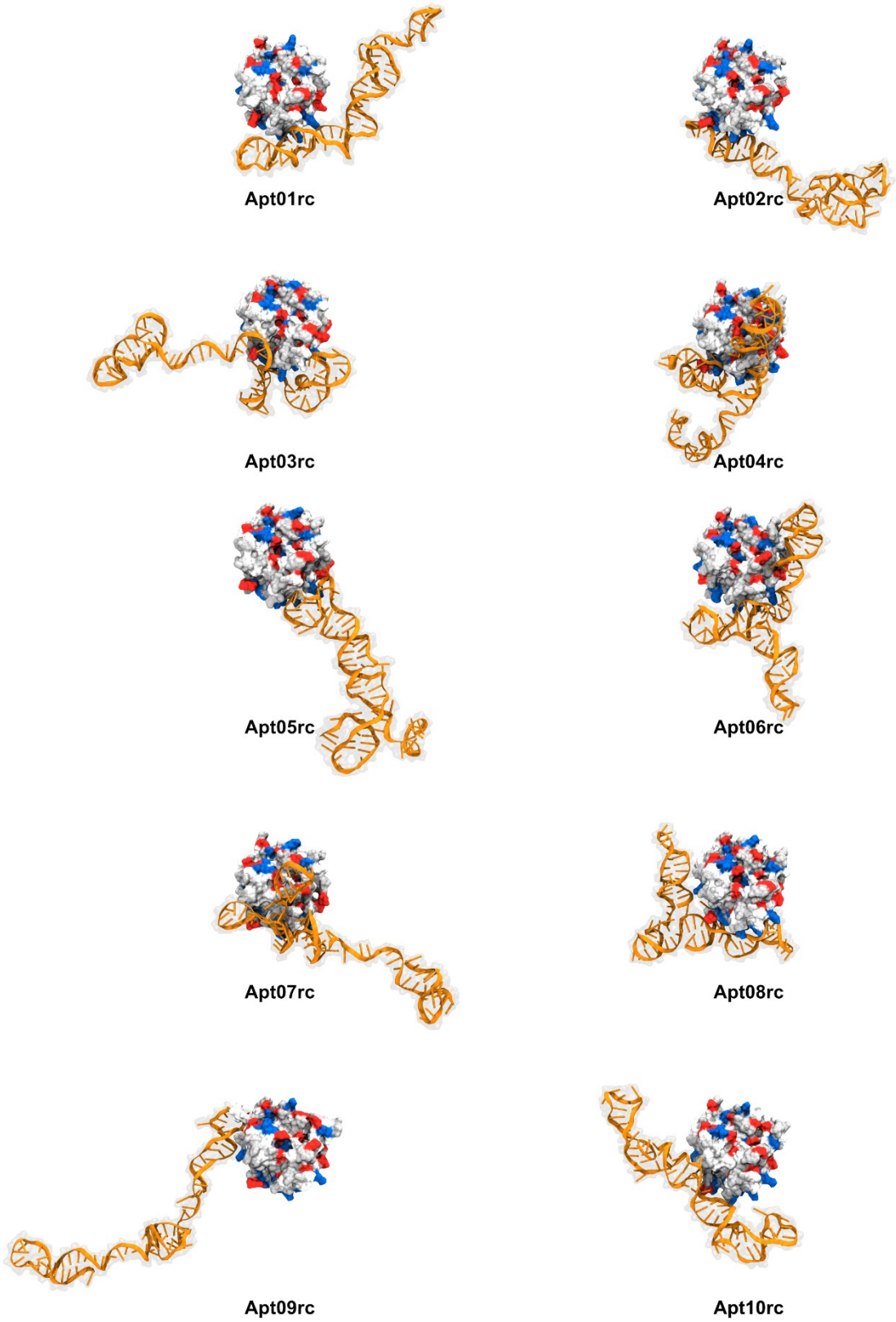

Figure S10. Representative structures of the complexes between the protein Cathepsin B (CatB) and aptamers Apt01rc to Apt10rc obtained by K-means clustering of the molecular dynamics simulation. CatB is represented in Surface and colored according to its residue type: Blue, positively charged side groups; Red, negatively charged side groups; White, non-polar with aliphatic or aromatic side groups; Grey, polar with uncharged side groups. The aptamers are represented in NewCartoon and are colored in Orange. All complexes are aligned by CatB.

Table S1. Amino acid residues involved in the top five longest hydrogen bonds established between CatB and each aptamer. The amino acid of CatB involved in the hydrogen bond, the percentage of simulation time where the hydrogen bond is formed, and the maximum lifetime of such hydrogen bond were determined for the 100 ns of the MD simulations of the CatB-Apt docked complexes. The aptamer chosen for experimental validation is highlighted in bold and grey.

	Top 1			Top 2			Top 3			Top 4			Top 5		
Aptamer	**Amino acid**	**%Time**	**Maximum Lifetime**	**Amino acid**	**%Time**	**Maximum Lifetime**	**Amino acid**	**%Time**	**Maximum Lifetime**	**Amino acid**	**%Time**	**Maximum Lifetime**	**Amino acid**	**%Time**	**Maximum Lifetime**
Apt01	Asp254	28%	0.9 ns	Asn149	24%	1.7 ns	Asn47	23%	0.8 ns	Tyr148	21%	1.4 ns	Arg252	20%	1.5 ns
Apt02	Asp252	43%	2.2 ns	Tyr148	37%	1.1 ns	Ser137	27%	1.9 ns	Tyr148	25%	0.6 ns	Asp254	23%	0.8 ns
Apt03	Arg85	66%	1.3 ns	Asn167	65%	2.0 ns	Ser150	60%	1.1 ns	Tyr148	50%	1.4 ns	Ser150	41%	0.8 ns
Apt04	Gly135	69%	0.9 ns	Thr139	55%	2.2 ns	Tyr140	49%	0.6 ns	Cys128	31%	1.8 ns	Lys141	29%	0.4 ns
Apt05	Thr139	92%	9.2 ns	Lys141	81%	2.8 ns	Cys132	51%	1.5 ns	Lys144	18%	0.8 ns	Lys130	17%	1.1 ns
Apt06	Arg8	29%	3.2 ns	Cys14	26%	0.4 ns	Arg235	21%	3.3 ns	Gln10	18%	1.1 ns	Arg8	18%	4.6 ns
Apt07	Lys130	67%	1.6 ns	Thr253	52%	0.9 ns	Tyr146	50%	4.1 ns	Asn81	41%	1.0 ns	Ser129	33%	1.2 ns
Apt08	Thr139	53%	17.6 ns	Glu133	21%	3.7 ns	Glu133	19%	2.9 ns	Lys141	13%	0.7 ns	Lys141	13%	0.3 ns
Apt09	Arg252	69%	2.5 ns	Tyr146	50%	1.2 ns	Thr253	49%	0.9 ns	Arg252	48%	1.0 ns	Asp254	30%	1.7 ns
Apt10	Glu95	50%	2.2 ns	Tyr94	32%	2.0 ns	Ser129	29%	1.7 ns	Tyr94	24%	0.5 ns	Lys127	21%	1.2 ns
Apt01rc	Thr139	50%	1.1 ns	Tyr140	41%	0.9 ns	Cys132	26%	0.4 ns	Lys141	22%	0.7 ns	Lys141	19%	0.7 ns
Apt02rc	Thr253	90%	5.5 ns	Thr84	64%	13.9 ns	Thr253	47%	1.0 ns	Asp254	41%	0.7 ns	Ser150	40%	1.3 ns
Apt03rc	Arg252	67%	2.0 ns	Gln142	43%	0.6 ns	Arg85	43%	2.5 ns	Tyr148	40%	2.8 ns	Arg85	38%	1.6 ns
Apt04rc	Thr139	87%	8.9 ns	Arg252	65%	2.2 ns	Cys132	61%	0.7 ns	Arg252	54%	4.1 ns	Gly135	49%	1.7 ns
Apt05rc	Lys127	53%	0.5 ns	Cys132	46%	0.6 ns	Tyr140	32%	0.5 ns	Thr139	24%	0.6 ns	Lys127	19%	0.4 ns
Apt06rc	Ser137	86%	12.7 ns	Ser129	65%	4.3 ns	Gly135	58%	1.4 ns	Lys127	56%	1.1 ns	Thr139	51%	1.2 ns
Apt07rc	Gly135	63%	0.8 ns	Ser129	54%	3.4 ns	Ser137	43%	0.7 ns	Lys130	29%	0.6 ns	Tyr136	28%	0.4 ns
Apt08rc	**Ser65**	**64%**	**31.0 ns**	**Ser65**	**45%**	**1.0 ns**	**Tyr148**	**33%**	**0.5 ns**	**Cys128**	**32%**	**0.8 ns**	**Arg85**	**30%**	**1.3 ns**
Apt09rc	Arg235	67%	1.7 ns	Arg235	63%	2.8 ns	Ser4	31%	1.4 ns	Trp206	25%	0.7 ns	Tyr165	24%	1.9 ns
Apt10rc	Asn47	21%	0.6 ns	His45	13%	0.2 ns	Tyr165	12%	1.5 ns	Asn47	5%	0.2 ns	Gln142	5%	0.7 ns

Table S2. Amino acid residues of CatB that establish at least one hydrogen bond with an aptamer for more than 50% of the simulation time. The number of hydrogen bonds corresponds to the sum of all hydrogen bonds established between that amino acid residue and any aptamer.

Amino acid	# H-Bonds
Thr139	6
Arg252	4
Gly135	3
Lys127	2
Ser129	2
Lys130	2
Cys132	2
Tyr146	2
Arg235	2
Thr253	2
Ser65	1
Thr84	1
Arg85	1
Glu95	1
Ser137	1
Lys141	1
Tyr148	1
Ser150	1
Asn167	1