Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

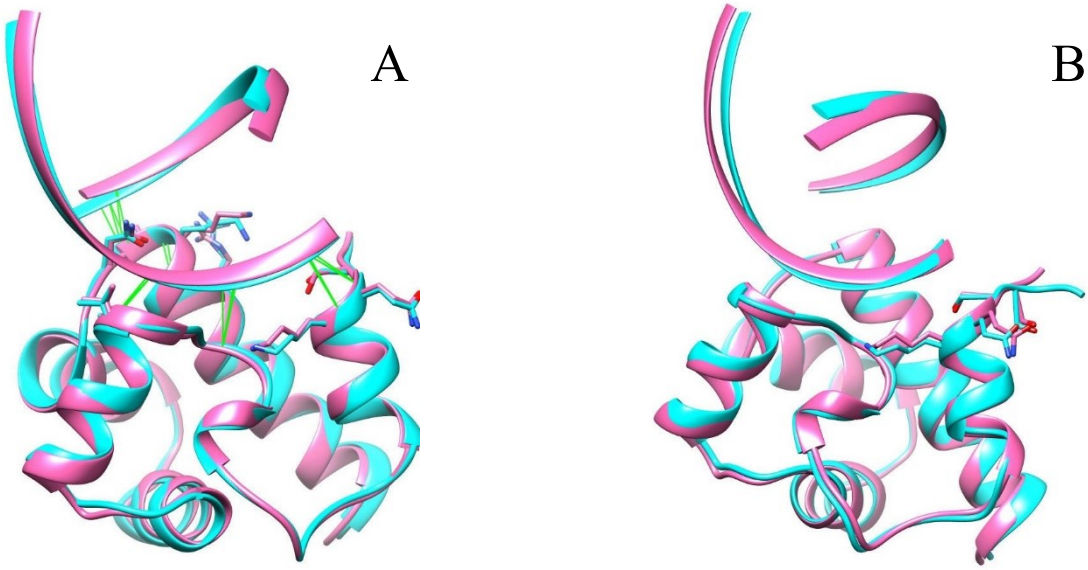


Figure S1. A Overlay of the docked Banf1$_{Thr2}$-DNA complex from HADDOCK 2.4 (cyan) to the co-crystalised complex (PDB ID: 2BZF, hot pink). B Superimposed complex structure of the Banf1$_{Thr2}$-DNA (hot pink) and docked Banf1$_{Met1}$-DNA (cyan). Sidechain of residues 3-6 in Banf1 of both complexes are displayed without hydrogen atoms for the sake of clarity.

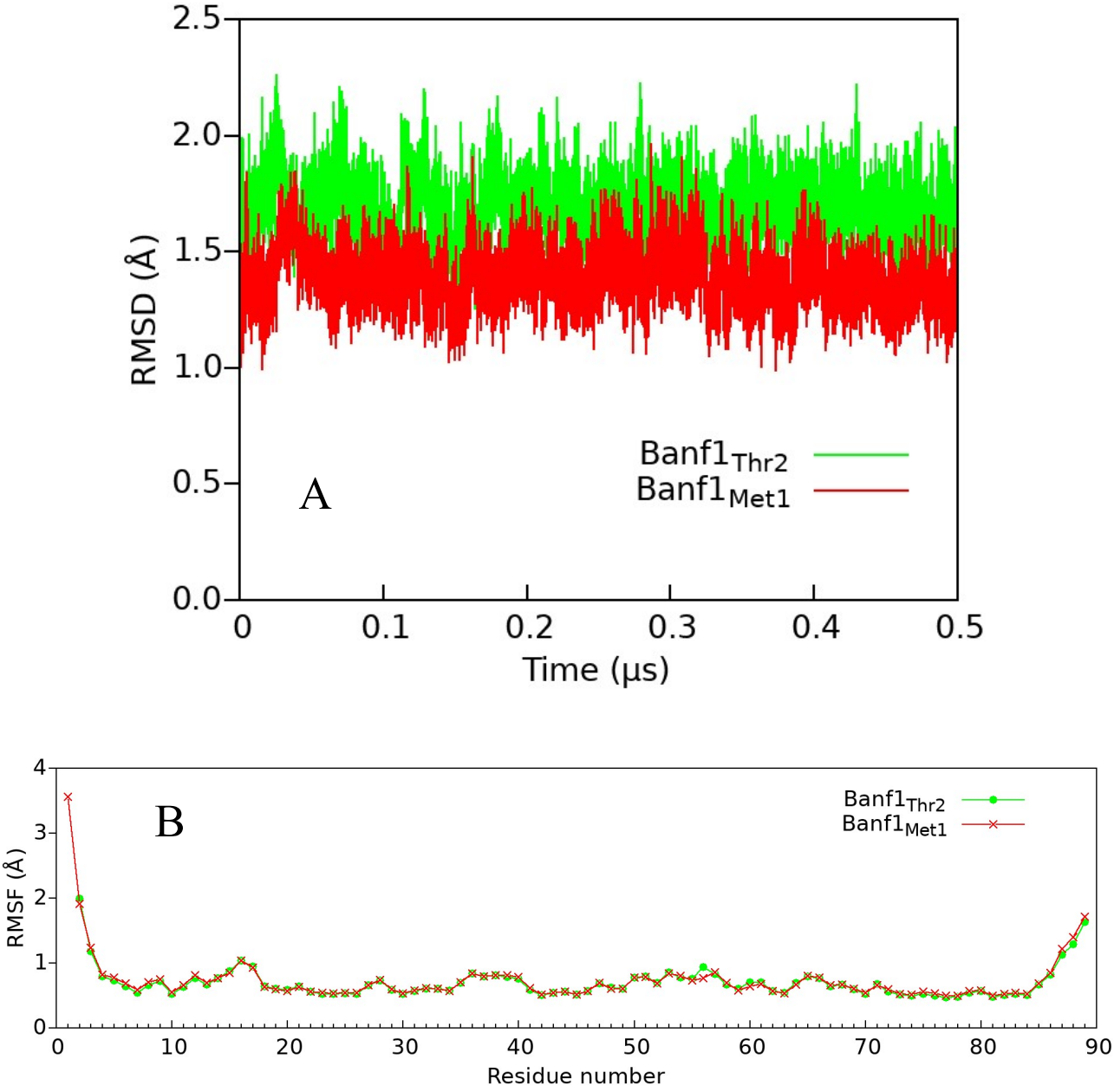


Figure S2. Stability and convergence of MD simulations of Banf1-DNA complexes. A RMSD variations with respect to time of $Banf1_{Thr2}$-DNA (green) and $Banf1_{Met1}$-DNA (red). B RMSF variations with respect to residues of $Banf1_{Thr2}$ (green) in the $Banf1_{Thr2}$-DNA complex and $Banf1_{Met1}$ (red) in the $Banf1_{Met1}$-DNA complex.

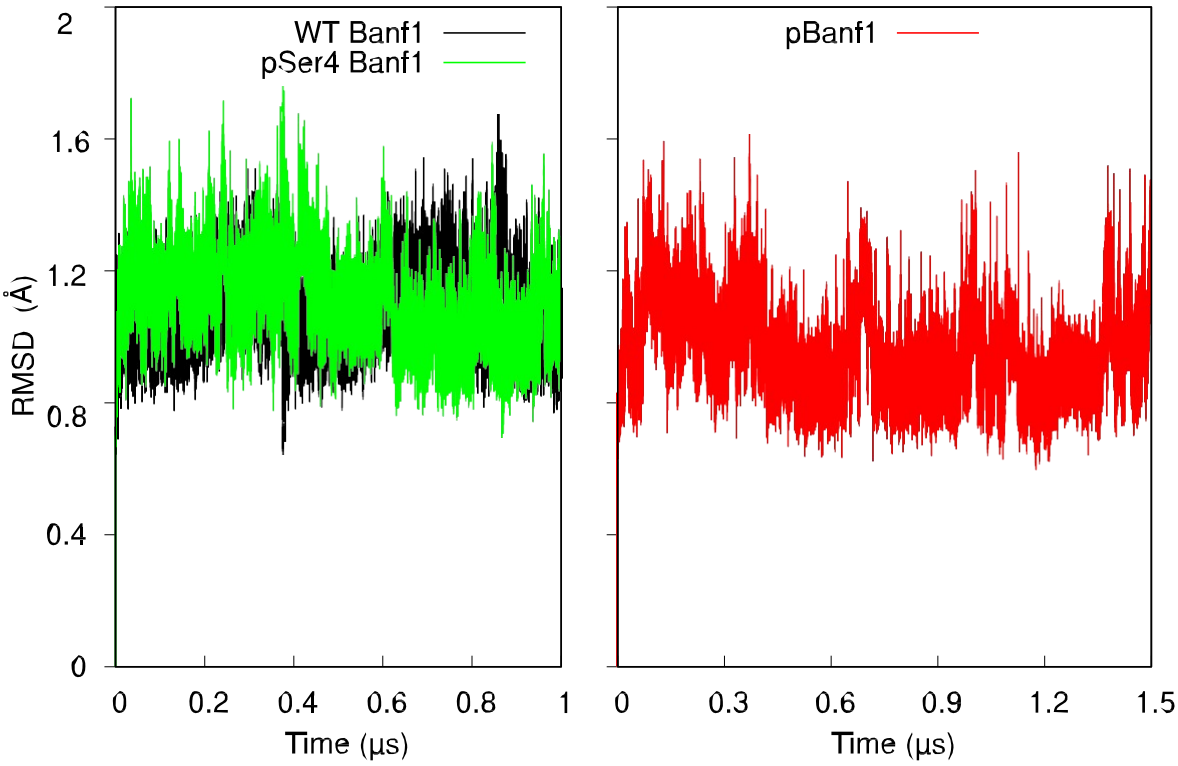


Figure S3. RMSD variations with respect to time of residues 7-89 in $Banf1_{Met1}$, where WT Banf1 is for unphosphorylated $Banf1_{Met1}$, pSer4 Banf1 for mono-phosphorylated $Banf1_{Met1}$, and pBanf1 for di-phosphorylated $Banf1_{Met1}$.

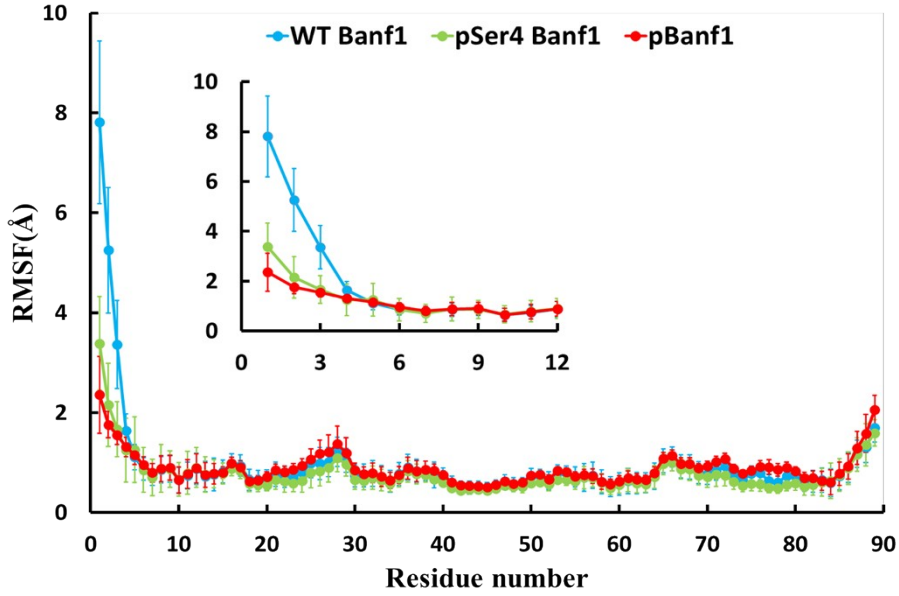


Figure S4. RMSF and its standard deviation of residues 1-89 in $Banf1_{Met1}$ and its zoom in view incorporating the N-terminal residues 1-12 in $Banf1_{Met1}$. The RMSF values were averaged from the RMSF of the two $Banf1_{Met1}$ promoters in the simulation of $Banf1_{Met1}$ dimer and the RMSF of the $Banf1_{Met1}$ monomer in the three simulation replicas of $Banf1_{Met1}$ monomer. Trajectories from the initial 100 ns simulations of unphosphorylated $Banf1_{Met1}$ was discarded and the trajectories of simulations before the mono- and di-phosphorylated $Banf1_{Met1}$ form an additional N-terminal helix were ignored.

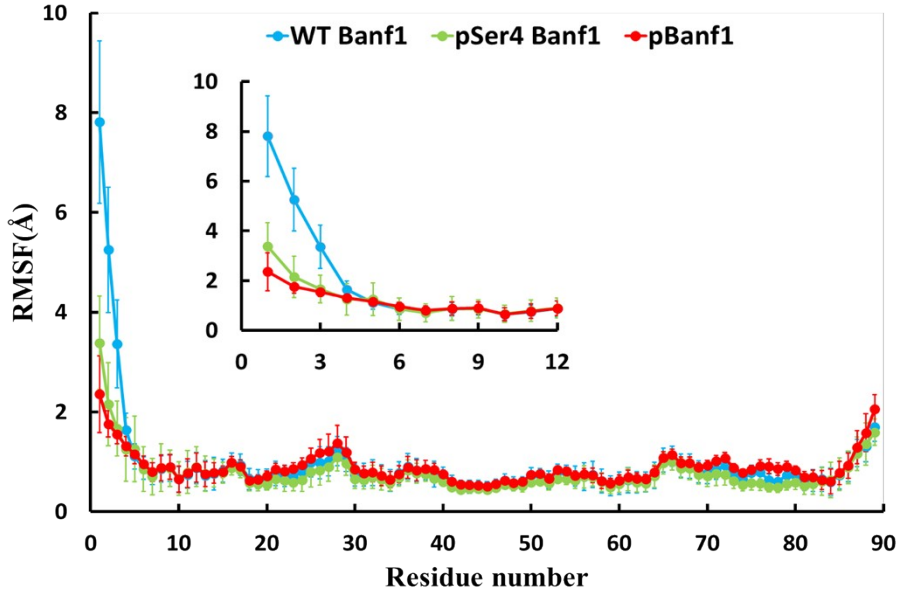


Figure S4. RMSF and its standard deviation of residues 1-89 in $Banf1_{Met1}$ and its zoom in view

incorporating the N-terminal residues 1-12 in $Banf1_{Met1}$. The RMSF values were averaged from the RMSF of the two $Banf1_{Met1}$ promoters in the simulation of $Banf1_{Met1}$ dimer and the RMSF of the $Banf1_{Met1}$ monomer in the three simulation replicas of $Banf1_{Met1}$ monomer. Trajectories from the initial 100 ns simulations of unphosphorylated $Banf1_{Met1}$ was discarded and the trajectories of simulations before the mono- and di-phosphorylated $Banf1_{Met1}$ form an additional N-terminal helix were ignored.

atoms are coloured in red, blue, white and gold respectively.

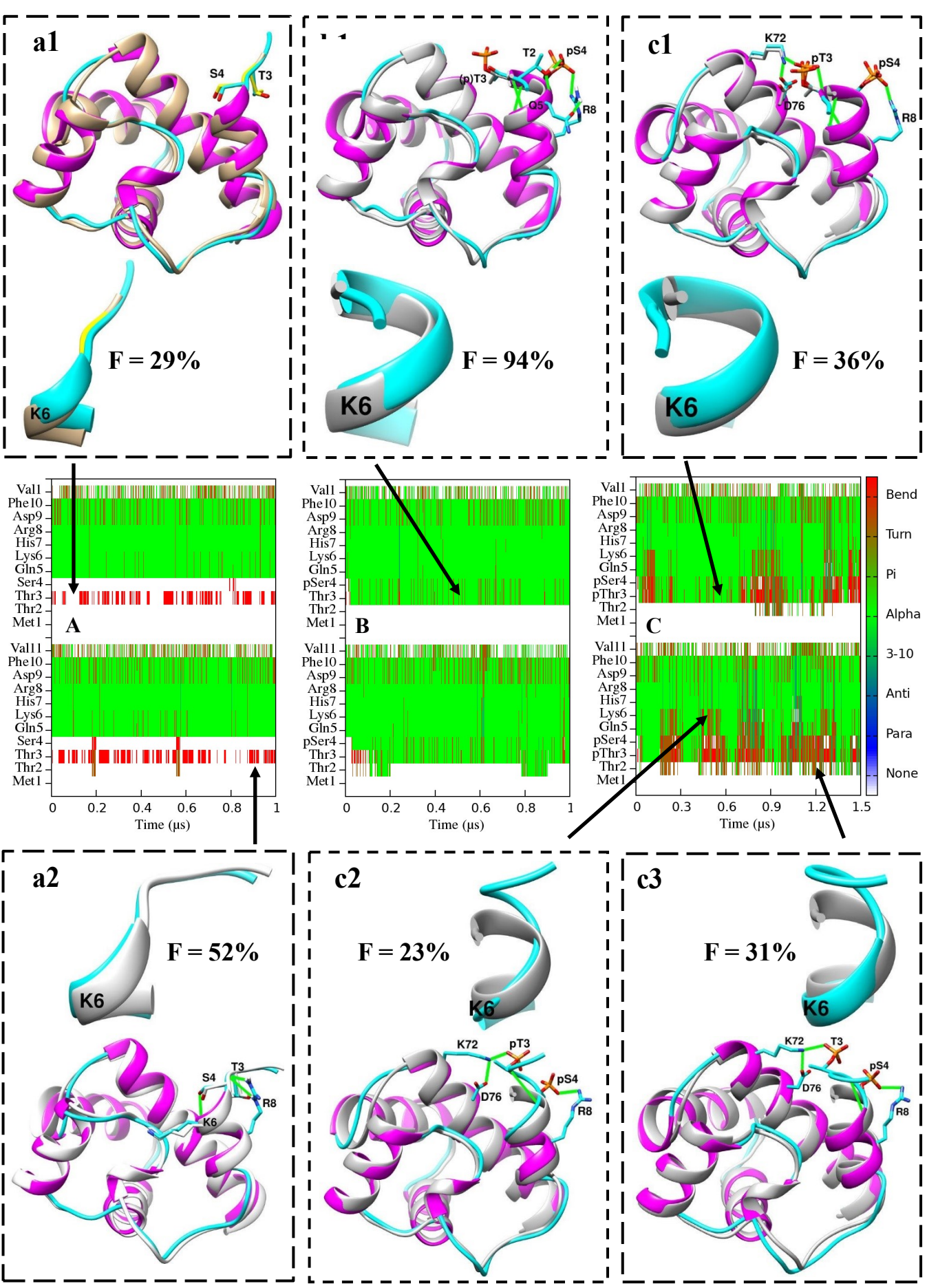

a1
S4
T3
F = 29%
K6
T2
pS4
(p)T3
Q5
R8
F = 94%
K6
c1
K72
pT3
pS4
D76
R8
F = 36%
K6
Val1
Phe10
Asp9
Arg8
His7
Lys6
Gln5
Ser4
Thr3
Thr2
Met1
A
B
C
0
0.2
0.4
0.6
0.8
1
0.3
0.9
1.2
1.5
Time (μs)
Bend
Turn
Pi
Alpha
3-10
Anti
Para
None
a2
F = 52%
K6
S4
T3
R8
K6
c2
F = 23%
K6
K72
pT3
pS4
D76
R8
c3
F = 31%
K6
K72
T3
pS4
D76
R8

Figure S5. Secondary structure maps of the N-terminal residues 1-11 in two protomers of $Banf1_{Met1}$ dimer and the corresponding $Banf1_{Met1}$ representative conformations superimposed with reference structures. A, B, and C are the secondary structure maps of unphosphorylated $Banf1_{Met1}$, mono-phosphorylated $Banf1_{Met1}$, and di-phosphorylated $Banf1_{Met1}$, respectively. a1 and a2 are the two representative conformations of unphosphorylated $Banf1_{Met1}$. b1 is the representative conformation of mono-phosphorylated $Banf1_{Met1}$. c1, c2, and c3 are the representative conformations of di-phosphorylated $Banf1_{Met1}$. Zoom-in view of the N-terminal residues 1-7 of each representative structure is displayed in ribbon. Occupancies (F) of each representative conformation of Banf1 are also displayed accordingly. Conformations from MD are coloured by secondary structure, with helical structure in magenta and coil in cyan. The reference structure in a1 coloured in tan is adopted from chain A of 2BZF, with the phosphorylation sites of Thr3 and Ser4 highlighted in yellow. The reference structure in a2 coloured in white is adopted from chain A of 6UNT. Reference structures used for other conformations coloured in gray are chain A extracted from 7NDY. For clarity, sidechains of pSer4 and pThr3 in c2 and c3 are not displayed. Hydrogen bonds and salt bridges between charged residues are shown as green lines. Sidechains in reference structures are coloured in the same colour as their chain, whereas sidechains in MD representative structures are coloured in cyan. Oxygen, nitrogen, hydrogen and phosphorus atoms are coloured in red, blue, white and gold respectively.

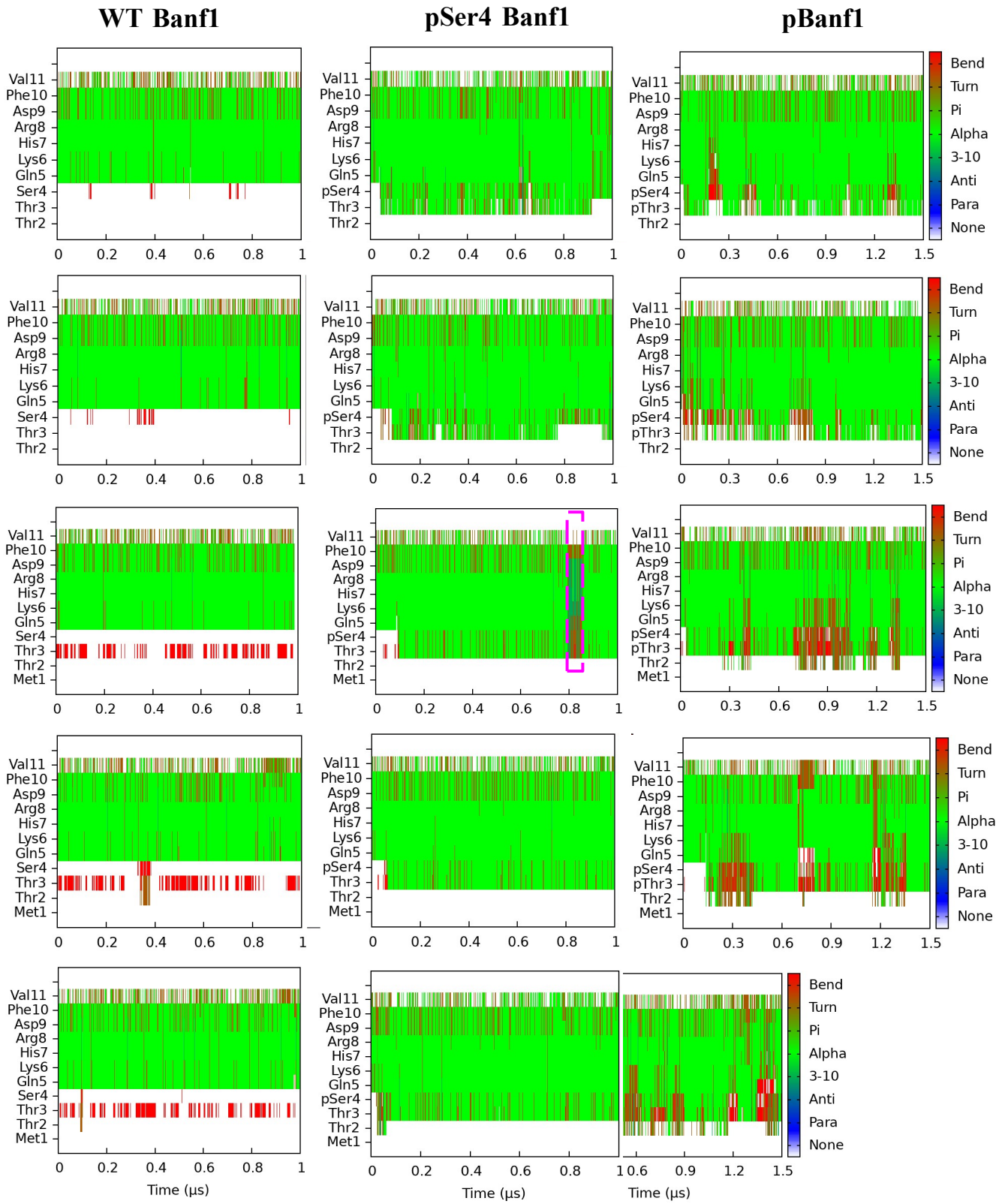


Figure S6. Secondary structure maps with respect to time of the N-terminal residues of Banf1, analysed

from two simulation replicas of $Banf1_{Thr2}$ monomer and three simulation replicas of $Banf1_{Met1}$

monomer starting from different atomic velocities. The three secondary structure maps in the first two rows from left to right are for residues 2-11 of unphosphorylated, mono-phosphorylated and di-phosphorylated $Banf1_{Thr2}$, respectively. The three secondary structure maps in the last three rows from left to right are for residues 1-
11 of unphosphorylated, mono-phosphorylated and di-phosphorylated $Banf1_{Met1}$, respectively. The representative secondary structure of pSer4 $Banf1_{Met1}$ corresponds to the map in the magenta dashed line rectangular is displayed in Figure S7.

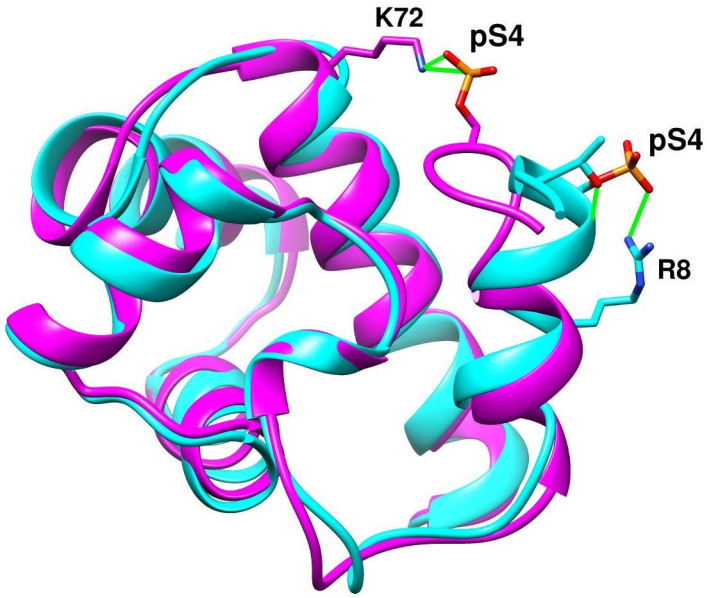
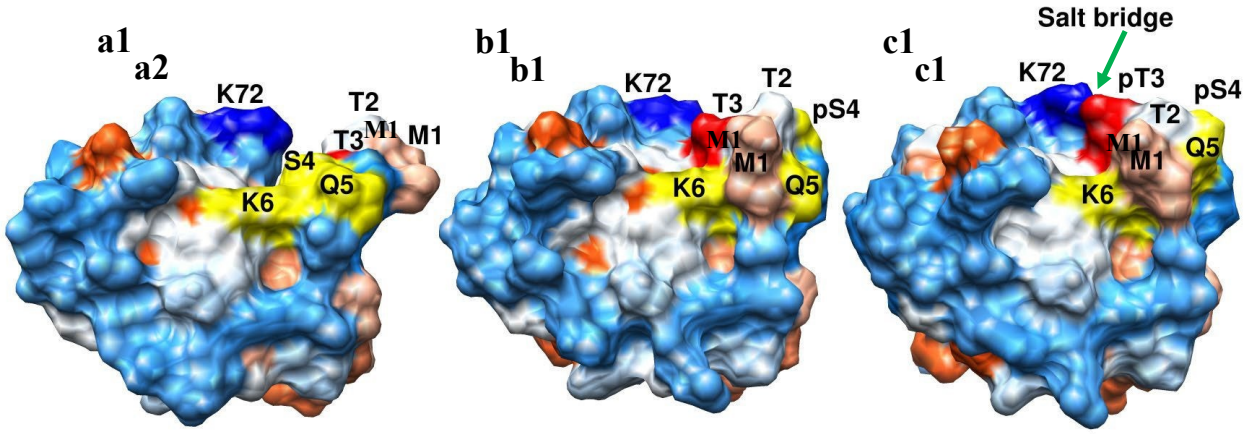


Figure S7. Overlay of the representative structure of pSer4 $Banf1_{Met1}$ (magenta) corresponds to the secondary structure map in the magenta dashed line rectangular in Figure S6 and the representative structure of pSer4 $Banf1_{Met1}$ (cyan) in Figure S5b1.


a1
a2
K72
T2
T3
M1
M1
S4
Q5
K6
b1
b1
K72
T2
T3
pS4
M1
M1
K6
Q5
c1
c1
Salt bridge
K72
pT3
pS4
T2
M1
M1
Q5
K6

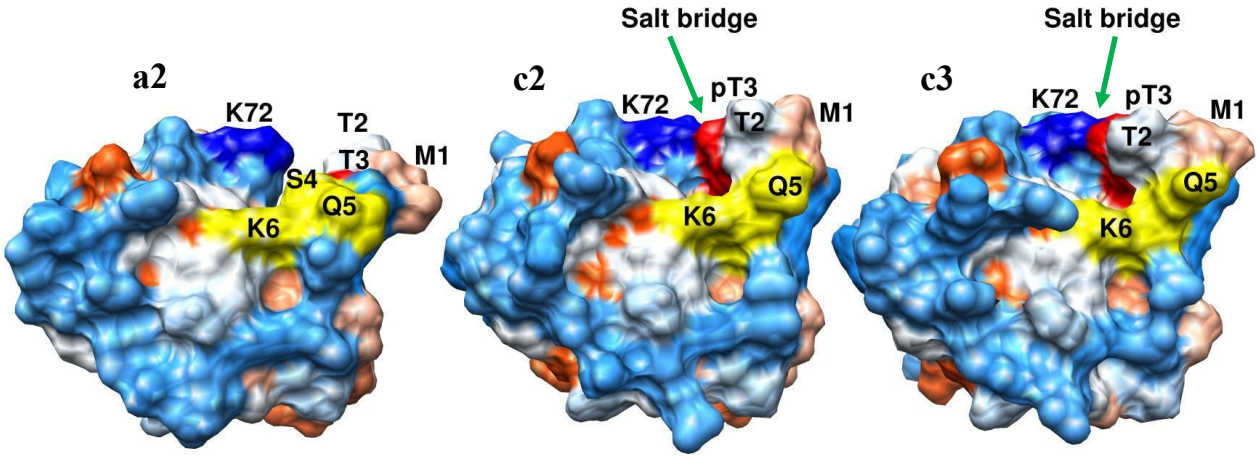


Figure S8. Hydrophobicity surface representation of the representative conformations of $Banf1_{Met1}$ displayed in Figure S5, where blue indicates the surface is hydrophilic and red is for hydrophobic surface. a2 is for one of the representative conformations of WT $Banf1_{Met1}$. b1 is for the representative conformation of pSer4 $Banf1_{Met1}$. c1, c2, and c3 are for the three representative conformations of $pBanf1_{Met1}$. Figure numbers for each representative conformation are consistent with those in Figure S5. N-terminal residues 4-6 involved in DNA binding are highlighted in yellow. Residue Met1, Thr2, (p)Thr3 and Lys72 are coloured in pink, white, red and blue respectively. The locations of salt bridges are directed by green arrows. In c2 and c3, surfaces of pSer4 are behind the surfaces of Met1, Thr2 and Gln5, and are not visible in this perspective.

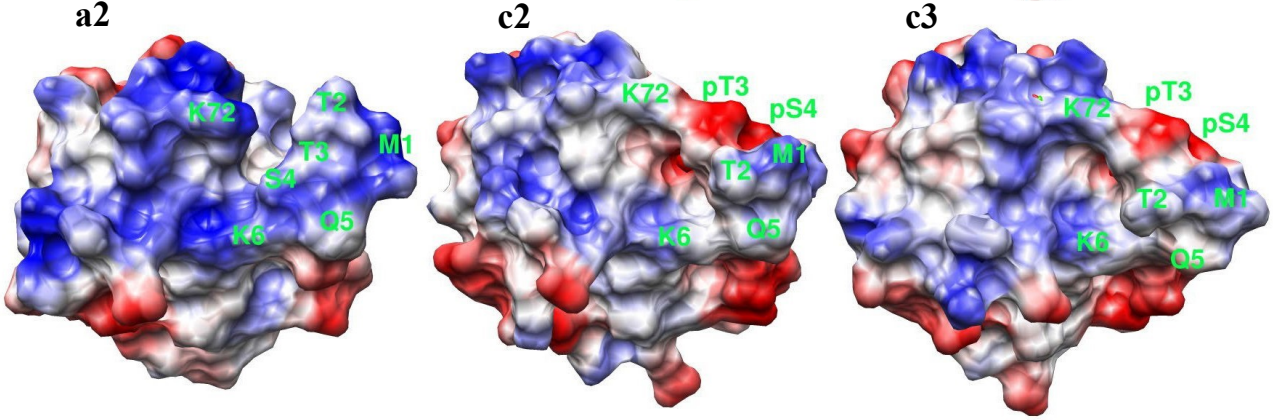


Figure S9. Electrostatic potential surface representation of the representative conformations of Banf1$_{Met1}$ displayed in Figure S5 and S8, where blue, white and red correspond to electropositive, electroneutral, and electronegative surfaces, respectively. a2 is for one of the representative conformations of WT Banf1$_{Met1}$. b1 is for the representative conformation of pSer4 Banf1$_{Met1}$. c1, c2, and c3 are for the three representative conformations of pBanf1$_{Met1}$. Figure numbers for each representative conformation are consistent with those in Figure S5 and S8.

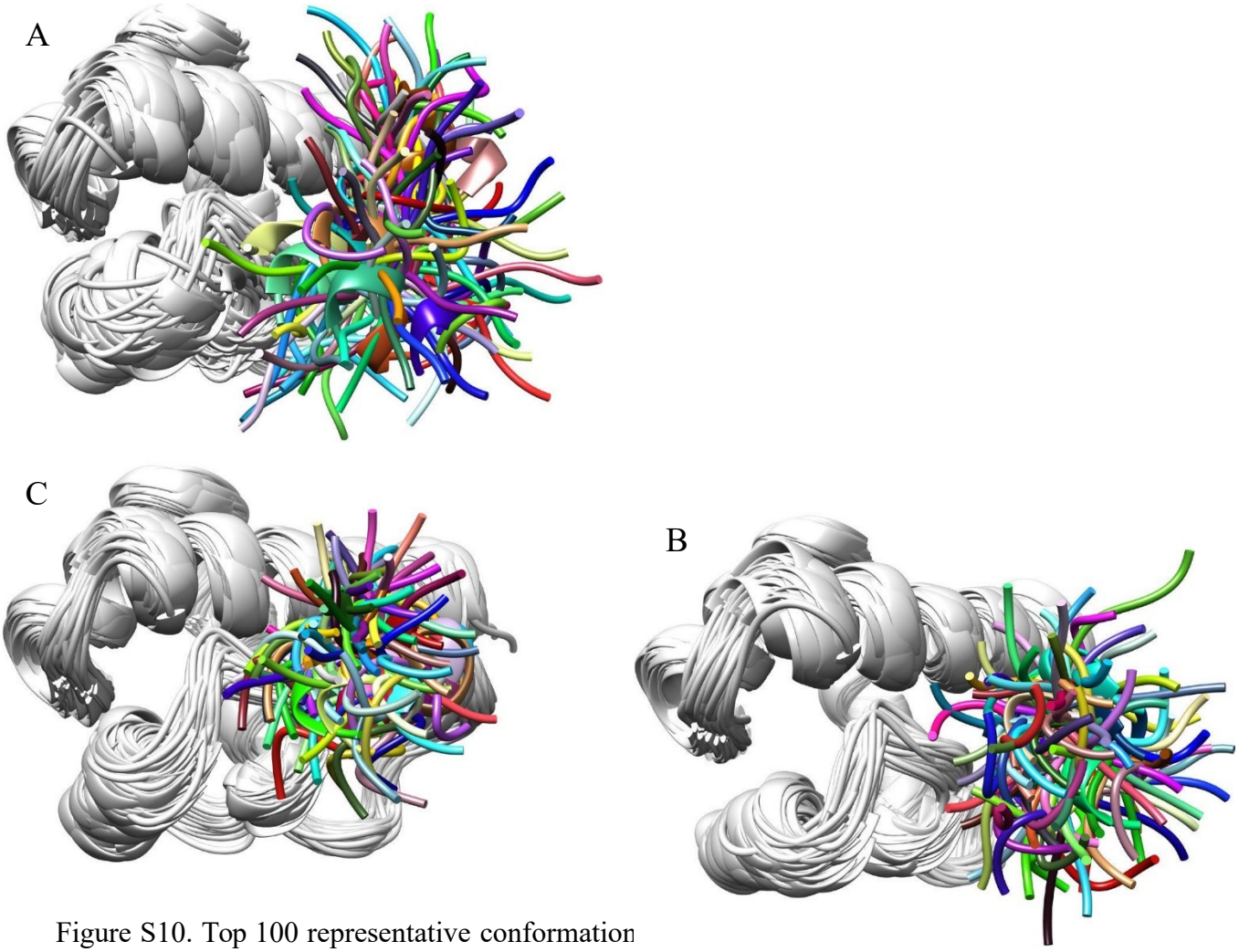


Figure S10. Top 100 representative conformation
mono- phosphorylated (B) and di-phosphorylated (C) Banf1$_{Thr2}$, where residues 8-89

are all coloured in gray and residues 2-7 are coloured differently in different conformations. The ensemble space occupied by Banf1 is restricted by mono-phosphorylation of Ser4. This restriction is more significant when pSer4 Banf1 is further phosphorylated at Thr3 forming pBanf1.