**Supplementary materials**

**Specific recognition between YTHDF3 and m6A-modified RNA:** **an all-atom molecular dynamics simulation study**

Wenxue Zhou, Zhongjie Han, Zhixiang Wu, Weikang Gong, Shuang Yang, Lei Chen, Chunhua Li\*

*Faculty of Environmental and Life Sciences, Beijing University of Technology, Beijing 100124, China.*

\*All correspondence should be addressed to Chunhua Li (Email: [chunhuali@bjut.edu.cn](mailto:chunhuali@bjut.edu.cn)).

Table S1. Non-bonding interaction energies (kcal/mol) of aromatic cage residues with m6A and its CH3 in methylated complex and with adenosine (A) in unmethylated complex.

|  |  |  |  |
| --- | --- | --- | --- |
| **Interaction pairs** | **Electrostatic** | **vdW** | **Total** |
| Methylated complex |  |  |  |
| Trp438- m6A | -1.46±0.72 | -2.88±0.68 | -4.34±0.92 |
| Trp492- m6A | -1.90±0.40 | -1.93±0.38 | -3.83±0.67 |
| Trp497- m6A | -0.27±1.23 | -6.47±1.44 | -6.74±1.56 |
| Trp438- CH3 | -0.61±0.37 | -0.86±0.41 | -1.47±0.43 |
| Trp492- CH3 | -0.89±0.55 | -1.37±0.31 | -2.26±0.66 |
| Trp497- CH3 | 0.39±0.62 | -0.89±0.29 | -0.50±0.74 |
| Unmethylated complex |  |  |  |
| Trp438- A | -0.48±0.90 | -2.27±0.59 | -2.75±1.04 |
| Trp492- A | -0.20±0.65 | -0.24±0.34 | -0.44±0.97 |
| Trp497- A | -0.48±0.99 | -1.97±1.74 | -2.45±2.07 |

Table S2. Comparison of H-bonds for the methylated and unmethylated complexes from the MD trajectories.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Methylated complex** | | | |  | **Unmethylated complex** | | |
| **Protein atom** | | **Methylated RNA atom** | **occupancy** |  | **Protein atom** | **RNA atom** | **occupancy** |
| **TYR424-Main-N** | | **m6A-Side-N3** | **86.86%** |  | **TYR424-Main-N** | **A3-Side-N3** | **76.07%** |
| **CYS439-Main-O** | | **m6A-Side-N6** | **72.30%** |  | **CYS439-Main-O** | **A3-Side-N6** | **70.28%** |
| **ASN468-Side-ND2** | | **m6A-Side-O3'** | **63.55%** |  | **ASN468-Side-ND2** | **A3-Side-O3’** | **58.23%** |
| **LYS496-Side-NZ** | | **m6A-Side-O1P** | **74.17%** |  |  |  |  |
| **ASP534-Side-CG** | | **m6A-Side-O2'** | **56.00%** |  |  |  |  |
| **ASP534-Side-OD2** | | **m6A-Side-O2'** | **55.71%** |  |  |  |  |
| **ASP534-Side-OD1** | **m6A-Side-O2'** | **42.75%** |  |  |  |  |
| ARG533-Side-NH1 | C4-Side-O2P | 78.27% |  | ARG533-Side-NH1 | C4-Side-O2P | 66.40% |
|  |  |  |  | ARG533-Side-NH1 | C4-Side-O4’ | 47.10% |
| GLY469-Main-N | U5-Side-O1P | 77.56% |  | GLY469-Main-N | U5-Side-O1P | 77.83% |
| LYS422-Side-NZ | U5-Side-O1P | 71.95% |  | LYS422-Side-NZ | U5-Side-O1P | 84.39% |
| ASN468-Main-CA | U5-Side-O1P | 60.10% |  | ASN468-Main-CA | U5-Side-O1P | 44.41% |
| ASN531-Side-ND2 | U5-Side-O4' | 41.39% |  |  |  |  |

Only the H-bonds with an occupancy > 40% are considered. The H-bonds associated with adenosine are highlighted in bold.



Figure S1. Time evolutions of RMSDs of YTHDF3 and its complexes with methylated and unmethylated RNA respectively from three independent MD simulations. Cα atoms in protein and C5’ atoms in RNA are considered in RMSD calculation.



Figure S2. First slowest motion modes (depicted with a cone model) from the PCA performed on the three independent trajectories of MD simulations on YTHDF3 in its apo and complexed forms with methylated and unmethylated RNA respectively. The cone’s length is proportional to the motion magnitude, and the cone’s orientation indicates the motion direction.

**Results of PC2 and PC3 from PCA analysis for apo\_pro, m6A\_com and A\_com systems**

We illustrated the structural changes along the second and third principle components (PC2 and PC3) for apo\_pro, m6A\_com and A\_com systems as shown in Figure S3. From Figure S3, m6A-modified RNA binding attenuates the protein motion amplitudes along PC2 and PC3 on the whole, while the unmethylated RNA binding makes protein instable to some extent and in addition the unmethylated RNA shows an escape trend from the binding pocket.



Figure S3. The second (a) and third (b) slowest motion modes (depicted with a cone model) from PCA analysis for apo\_pro, m6A\_com and A\_com systems.



Figure S4. Distance changes over time in apo\_pro (a), m6A\_com (b) and A\_com (c) systems. Dis1 is the distance between the mass centers of residues Ser493, Gln494 in recognition loop and of α1, and Dis2 is the distance between the mass centers of loop4 and of residues Ser532-Glu537 in loop6.



Figure S5. Local structures of the aromatic cage from the low energy conformations of YTHDF3 in the methylated RNA-bound (a) and unmethylated RNA-bound (b) states respectively. π-π stacking interactions are represented by yellow dotted lines.



Figure S6. Changes of buried SASAs between YTHDF3 and methylated/ unmethylated RNA based on the equilibrium trajectories.



Figure S7. Radial distributions of the water molecules within 8 Å from the N6 atom of adenosine in the methylated and unmethylated complex systems (a) with their low energy conformations’ aromatic cage structures shown in (b) and (c) respectively where water molecules are drawn as spheres.



Figure S8. Schematics of the interaction networks of YTHDF3 with methylated RNA (a) and with unmethylated RNA (b) respectively. Yellow circles are for nucleotides, blue circles for residues, green lines for H-bonds, purple lines for van der Waals interactions, and blue lines for π-π stacking interactions.