

## Supplementary Information

### **Dynamic nucleotide flipping drives super-resolution fluorescence activation in the RhoBAST RNA aptamer**

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## Contents

### **Supplementary Tables**

|                            |   |
|----------------------------|---|
| Supplementary Table 1..... | 3 |
| Supplementary Table 2..... | 4 |
| Supplementary Table 3..... | 5 |

### **Supplementary Figure**

|                             |   |
|-----------------------------|---|
| Supplementary Figure 1..... | 6 |
|-----------------------------|---|

**Supplementary Table 1 | Crystallographic statistics of bound and free-form RhoBAST-WT**

| RhoBAST                                   | WT/TMR-DN-bound        | WT/Free-form           |
|-------------------------------------------|------------------------|------------------------|
| <b>Data Collection</b>                    |                        |                        |
| Space group                               | P 6 <sub>5</sub> 2 2   | P 6 <sub>5</sub>       |
| a, b, c (Å)                               | 83.3, 83.3, 95.1       | 83.3, 83.3, 102.74     |
| $\alpha, \beta, \gamma$ (°)               | 90, 90, 120            | 90, 90, 120            |
| Wavelength (Å)                            | 0.97946                | 1.10067                |
| Resolution (Å)                            | 2.80-50.00 (2.80-2.85) | 2.53-50.00 (2.53-2.57) |
| R <sub>pim</sub>                          | 0.024 (1.026)          | 0.023 (0.159)          |
| I / $\sigma$ I                            | 32.5 (0.6)             | 56.7 (4.1)             |
| Completeness (%)                          | 100.0 (100.0)          | 100.0 (100.0)          |
| Redundancy                                | 34.5 (30.9)            | 19.9 (20.3)            |
| CC <sub>1/2</sub>                         | 1.013 (0.50)           | 0.981 (0.944)          |
| <b>Refinement</b>                         |                        |                        |
| Resolution (Å)                            | 2.80-25.22 (2.80-2.90) | 2.53-41.84 (2.53-2.62) |
| No. reflections                           | 5126 (480)             | 13450 (1286)           |
| R <sub>work</sub> / R <sub>free</sub> (%) | 19.61/24.20            | 20.56/24.17            |
| No. of atoms                              |                        |                        |
| RNA                                       | 1233                   | 2466                   |
| Ligand                                    | 53                     |                        |
| Mg <sup>2+</sup>                          | 3                      | 15                     |
| Water                                     | 8                      | 55                     |
| B-factors (Å <sup>2</sup> )               |                        |                        |
| RNA                                       | 100.35                 | 61.23                  |
| Ligand                                    | 105.67                 |                        |
| Mg <sup>2+</sup>                          | 99.27                  | 66.49                  |
| Water                                     | 100.09                 | 61.07                  |
| R.M.S deviations                          |                        |                        |
| Bond length (Å)                           | 0.007                  | 0.005                  |
| Bond angles (°)                           | 1.390                  | 1.078                  |

\* Values for the highest-resolution shell are in parentheses.

**Supplementary Table 2 | Crystallographic statistics of bound and free-form RhoBAST-G38 mutants**

| <b>RhoBAST</b>                            | <b>G38A Mutant<br/>Free-form</b> | <b>G38A Mutant<br/>TMR-DN-bound</b> | <b>G38U Mutant<br/>Free-form</b> |
|-------------------------------------------|----------------------------------|-------------------------------------|----------------------------------|
| <b>Data Collection</b>                    |                                  |                                     |                                  |
| Space group                               | P 6 <sub>5</sub> 2 2             | P 6 <sub>1</sub> 2 2                | P 6 <sub>5</sub> 2 2             |
| a, b, c (Å)                               | 83.1, 83.1, 102.0                | 84.0, 83.0, 106.7                   | 81.4, 81.41, 98.7                |
| $\alpha, \beta, \gamma$ (°)               | 90, 90, 120                      | 90, 90, 120                         | 90, 90, 120                      |
| Wavelength (Å)                            | 0.9785                           | 0.9785                              | 0.9785                           |
| Resolution (Å)                            | 2.44-41.60<br>(2.44-2.54)        | 2.75-30.00<br>(2.75-2.80)           | 2.50-30.00<br>(2.50-2.54)        |
| R <sub>pim</sub>                          | 0.021 (0.336)                    | 0.047 (0.518)                       | 0.033 (0.245)                    |
| I / $\sigma$ I                            | 42.3 (3.6)                       | 34.3 (1.7)                          | 45.5 (1.33)                      |
| Completeness (%)                          | 99.9 (99.4)                      | 99.4 (88.5)                         | 99.8 (96.9)                      |
| Redundancy                                | 36.9 (39.0)                      | 36.1 (32.4)                         | 35.2 (20.6)                      |
| CC <sub>1/2</sub>                         | 0.999 (0.744)                    | 0.98 (0.64)                         | 1.083 (0.764)                    |
| <b>Refinement</b>                         |                                  |                                     |                                  |
| Resolution (Å)                            | 2.44-41.60<br>(2.44-2.53)        | 2.75-27.49<br>(2.75-2.85)           | 2.50-29.82<br>(2.50-2.59)        |
| No. reflections                           | 8132 (774)                       | 6152 (586)                          | 7067 (664)                       |
| R <sub>work</sub> / R <sub>free</sub> (%) | 21.74/25.73                      | 21.47/26.70                         | 20.28/24.74                      |
| No. of atoms                              |                                  |                                     |                                  |
| RNA                                       | 1236                             | 1227                                | 1234                             |
| Ligand                                    |                                  | 53                                  |                                  |
| Mg <sup>2+</sup>                          | 1                                | 1                                   | 1                                |
| Water                                     | 13                               | 12                                  | 1                                |
| B-factors (Å <sup>2</sup> )               |                                  |                                     |                                  |
| RNA                                       | 62.38                            | 83.62                               | 68.33                            |
| Ligand                                    |                                  | 89.52                               |                                  |
| Mg <sup>2+</sup>                          | 83.24                            | 87.71                               | 70.47                            |
| Water                                     | 67.08                            | 85.28                               | 63.03                            |
| R.M.S deviations                          |                                  |                                     |                                  |
| Bond length (Å)                           | 0.005                            | 0.004                               | 0.005                            |
| Bond angles (°)                           | 1.151                            | 0.868                               | 1.134                            |

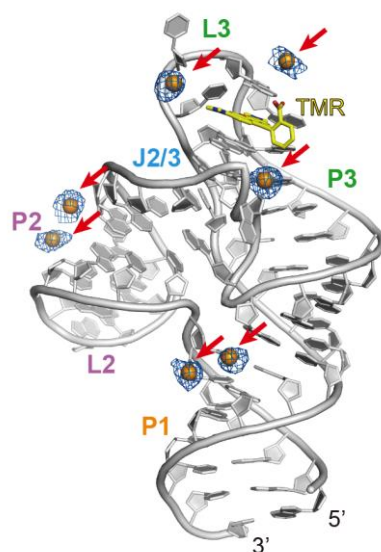
\* Values for the highest-resolution shell are in parentheses.

**Supplementary Table 3 | Crystallographic statistics of RhoBAST-WT in complex with TMR and 5-TAMRA**

| <b>RhoBAST</b>                            | <b>TMR-bound Se-soaked</b> | <b>5-TAMRA-bound</b>   |
|-------------------------------------------|----------------------------|------------------------|
| <b>Data Collection</b>                    |                            |                        |
| Space group                               | P 6 <sub>5</sub> 2 2       | P 6 <sub>5</sub> 2 2   |
| a, b, c (Å)                               | 84.5, 84.5, 107.3          | 82.5, 82.5, 105.9      |
| $\alpha, \beta, \gamma$ (°)               | 90, 90, 120                | 90, 90, 120            |
| Wavelength (Å)                            | 0.9792                     | 0.97946                |
| Resolution (Å)                            | 2.51-43.27 (2.51-2.65)     | 2.97-35.80 (2.97-3.15) |
| R <sub>pim</sub>                          | 0.013 (0.312)              | 0.075 (0.366)          |
| I / $\sigma$ I                            | 41.1 (2.8)                 | 10.6 (2.5)             |
| Completeness (%)                          | 94.5 (100.0)               | 99.9 (100.0)           |
| Redundancy                                | 35.5 (37.3)                | 35.7 (38.3)            |
| CC <sub>1/2</sub>                         | 1 (0.82)                   | 0.994 (0.285)          |
| <b>Refinement</b>                         |                            |                        |
| Resolution (Å)                            | 2.51-30.22 (2.51-2.65)     | 2.97-35.81 (2.97-3.08) |
| No. reflections                           | 7764 (801)                 | 4801 (467)             |
| R <sub>work</sub> / R <sub>free</sub> (%) | 22.84/25.66                | 22.48/26.52            |
| No. of atoms                              |                            |                        |
| RNA                                       | 1233                       | 1237                   |
| Ligand                                    | 29                         | 32                     |
| Mg <sup>2+</sup>                          | 3                          | 1                      |
| Water                                     | 10                         | 6                      |
| Se                                        | 7                          |                        |
| B-factors (Å <sup>2</sup> )               |                            |                        |
| RNA                                       | 65.2                       | 80.94                  |
| Ligand                                    | 59                         | 83.64                  |
| Mg <sup>2+</sup>                          | 73                         | 76.10                  |
| Water                                     | 62.5                       | 82.46                  |
| Se                                        | 127.7                      |                        |
| R.M.S deviations                          |                            |                        |
| Bond length (Å)                           | 0.006                      | 0.003                  |
| Bond angles (°)                           | 1.224                      | 0.806                  |

\* Values for the highest-resolution shell are in parentheses.

## Supplementary Figure



**Supplementary Figure 1 | Anomalous difference electron density map for selenourea-soaked TMR-bound RhoBAST crystals.** Anomalous electron density map contoured at level 3.0  $\sigma$  for Se sites (orange balls, labelled with red arrow) in one asymmetric unit of TMR-bound RhoBAST, which were used to solve the phase problem of the structure.