|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Protein Complex**  **(Alp14-monomer concentration 4.43 uM)** | **µM tubulin\* bound** | | **µM tubulin**  **free** | | **TOG1-TOG2**  **:αβ-tubulin** | | |
| **wt-Alp14-monomer (2μM)** | |  | |  | |  |
| 1 Alp14-monomer: 1 Tub 100 mM KCl | | 4.57 ± 0.08 | | 0.43 ± 0.08 | | 1.04 |
| 1 Alp14-monomer: 2 Tub 100 mM KCl | | 8.82 ± 0.16 | | 1.18 ± 0.16 | | 2 |
| 1 Alp14-monomer: 1 Tub 200 mM KCl | | 3.37 ± 0.27 | | 1.63 ± 0.27 | | 0.76 |
| 1 Alp14-monomer: 2 Tub 200 mM KCl | | 5.97 ± 0.12 | | 4.03 ± 0.12 | | 1.35 |
| 1 Alp14-monomer:1 Tub:1 DRP 100 mM KCl | | 4.33 ± 0.13 | | 0.67 ± 0.13 | | 0.98 |
| 1 Alp14-monomer:2 Tub:2 DRP 100 mM KCl | | 7.23 ± 0.01 | | 2.77 ± 0.01 | | 1.64 |
| 1 Alp14-monomer:1 Tub:1 DRP 200 mM KCl | | 4.13 ± 0.09 | | 0.87 ± 0.09 | | 0.94 |
| 1 Alp14-monomer: 2 Tub 2 DRP 200 mM KCl | | 5.67 ± 0.03 | | 4.34 ± 0.03 | | 1.28 |
| **wt-Alp14-dimer (1μM)** | |  | |  | |  |
| 1 Alp14-dimer: 1 Tub 100 mM KCl | | 4.31 ± 0.07 | | 0.69 ± 0.07 | | 0.98 |
| 1 Alp14-dimer: 2 Tub 100 mM KCl | | 7.11 ± 0.14 | | 2.89 ± 0.14 | | 1.61 |
| 1 Alp14-dimer: 1 Tub 200 mM KCl | | 3.44 ± 0.37 | | 1.56 ± 0.37 | | 0.78 |
| 1 Alp14-dimer: 2 Tub 200 mM KCl | | 5.46 ± 0.34 | | 4.55 ± 0.34 | | 1.24 |
| 1 Alp14-dimer: 1 Tub: 2 DRP 100 mM KCl | | 4.23 ± 0.02 | | 0.78 ± 0.02 | | 0.96 |
| 1 Alp14-dimer: 1 Tub: 2 DRP 100 mM KCl | | 8.33 ± 0.09 | | 1.67 ± 0.09 | | 1.89 |
| 1 Alp14-dimer: 1 Tub: 1 DRP 200 mM KCl | | 4.17 ± 0.29 | | 0.83 ± 0.29 | | 0.95 |
| 1 Alp14-dimer: 2 Tub: 2 DRP 200 mM KCl | | 6.02 ± 0.17 | | 3.99 ± 0.17 | | 1.36 |
|  | |  | |  | |  |
| **TOG square Interface mutants (1μM)** | |  | |  | |  |
| 1 INT1: 2 Tub 100mM KCl | | 8.34 ± 0.07 | | 1.66 ± 0.07 | | 1.74 |
| 1 INT1: 2 Tub 200mM KCl | | 5.67 ± 0.04 | | 4.33 ± 0.04 | | 1.18 |
| 1 INT2: 2 Tub 100mM KCl | | 9.52 ± 0.3 | | 0.48 ± 0.2 | | 2.02 |
| 1 INT2: 2 Tub 200mM KCl | | 6.82 ± 0.2 | | 3.1 ±0.1 | | 1.45 |
| 1 INT1+2: 2 Tub 100mM KCl | | 8.44 ± 0.05 | | 1.56 ± 0.05 | | 1.86 |
| 1 INT1+2: 2 Tub 200mM KCl | | 6.85 ± 0.04 | | 3.15 ± 0.04 | | 1.46 |
|  | |  | |  | |  |
| **Inactivated TOG mutants (1μM)** | |  | |  | |  |
| 1 TOG1M: 1 Tub 100 mM KCl | | 4.04 ± 0.07 | | 0.97 ± 0.07 | | 0.92 |
| 1 TOG1M: 2 Tub 100 mM KCl | | 4.94 ± 0.06 | | 5.06 ± 0.06 | | 1.12 |
| 1 TOG1M: 1 Tub 200 mM KCl | | 1.66 ± 0.25 | | 3.34 ± 0.25 | | 0.38 |
| 1 TOG1M: 2 Tub 200 mM KCl | | 2.23 ± 0.1 | | 7.77 ± 0.1 | | 0.51 |
|  | |  | |  | |  |
| 1 TOG2M: 1 Tub 100 mM KCl | | 3.53 ± 0.04 | | 1.48 ± 0.04 | | 0.8 |
| 1 TOG2M: 2 Tub 100 mM KCl | | 6.24 ± 0.10 | | 3.76 ± 0.1 | | 1.41 |
| 1 TOG2M: 1 Tub 200 mM KCl | | 3.15 ± 0.24 | | 1.85 ± 0.24 | | 0.72 |
| 1 TOG2M: 2 Tub 200 mM KCl | | 4.28 ± 0.18 | | 5.72 ± 0.18 | | 0.97 |
| \*Standard error is defined based on combined data from duplicated SEC runs | | | | | | |
|  | | | | | | |

**Supplementary File 2:** Capacities of MT polymerase TOG1-TOG2 to bind αβ-tubulin, influenced by ionic strength