

Supporting Information

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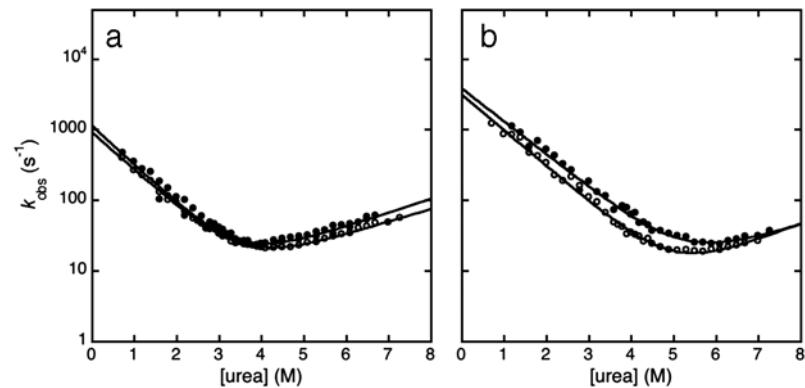


Fig. S1. Chevron plot of Cter-NPM1 with (Closed Circles) and without (Open Circles) the His6-tag. Kinetics measured in the absence (A) and in the presence (B) of 0.5 M NaCl. Calculated folding parameters revealed that folding was essentially unaffected by the presence of the tag.

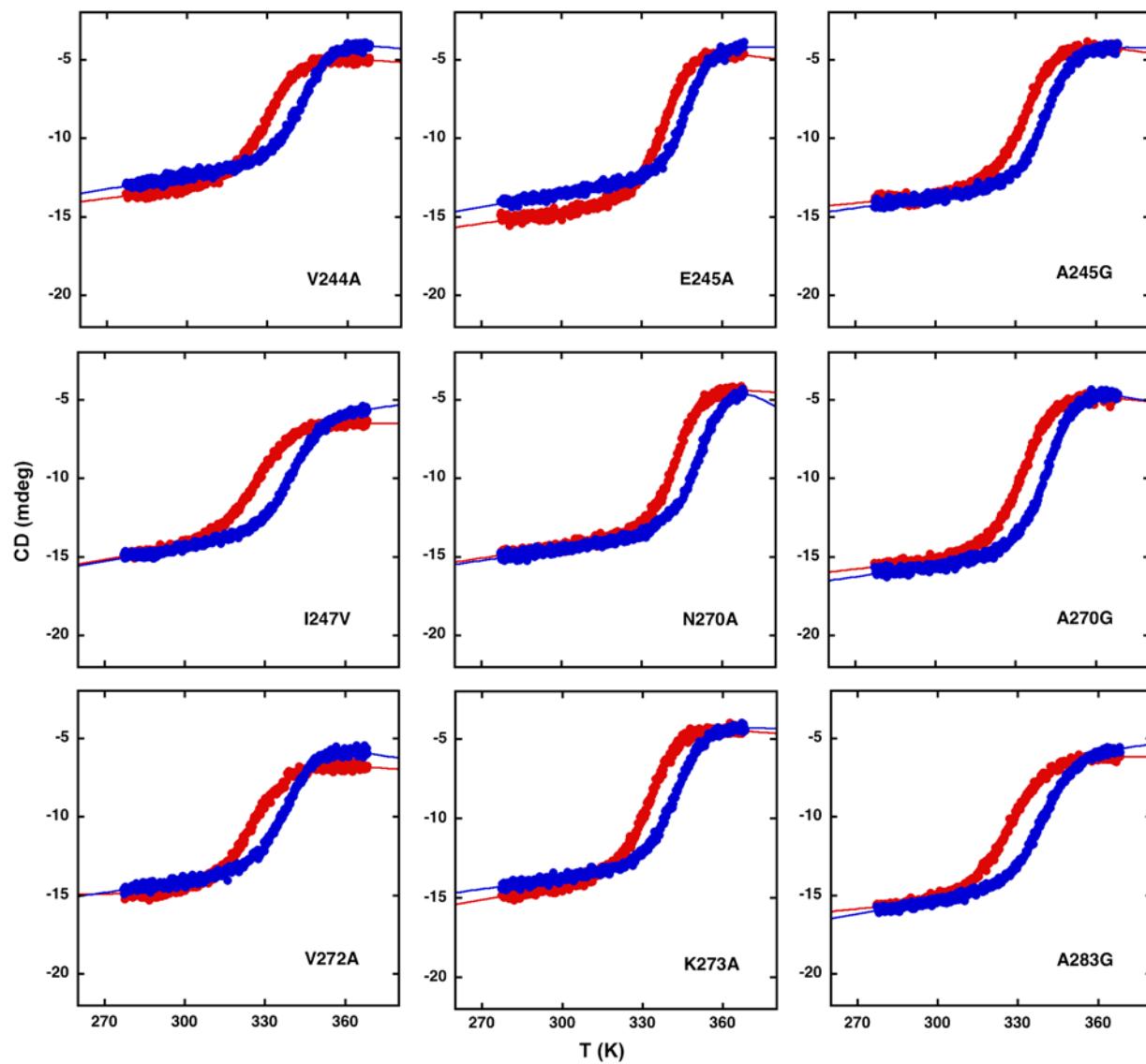


Fig. S2. Thermal melt unfolding of selected mutants of Cter-NPM1 in the absence (Red) and in the presence (Blue) of 0.5 M NaCl. Lines are the best fit to a two-state model.

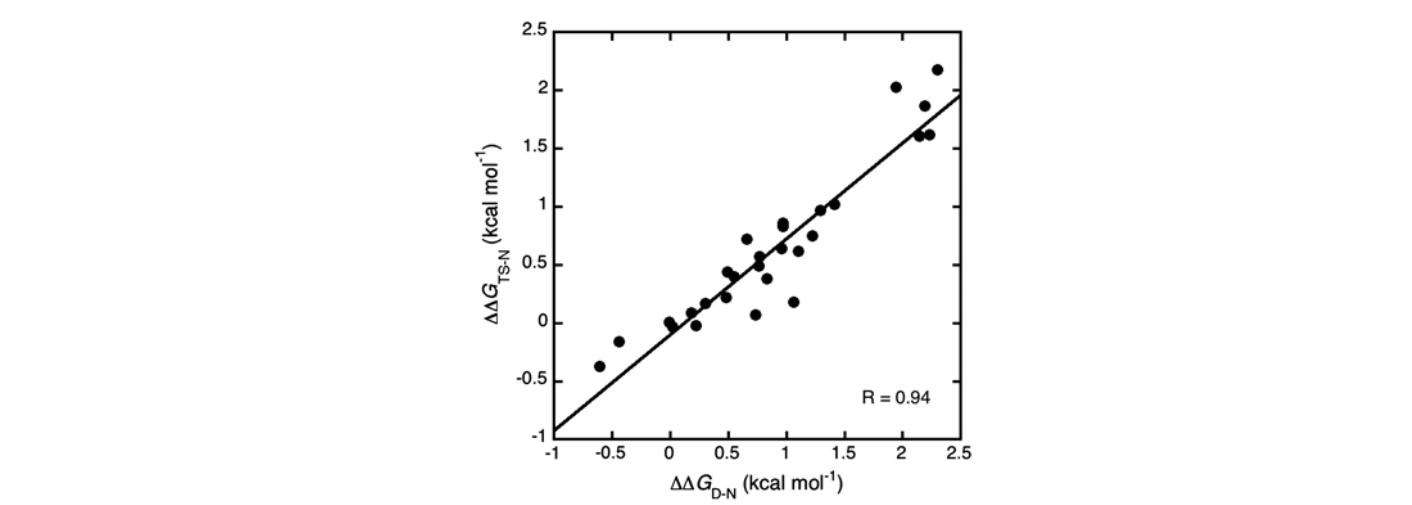


Fig. S3. Relationship between the activation free energy and unfolding free energy in Cter-NPM1. The line is the best fit to a linear equation ($R = 0.94$). A linear Brønsted plot suggests that the folding transition state is a distorted version of the native state, being stabilized by a weak extended nucleus. This behavior is a fingerprint of the nucleation-condensation model.

Table S1. Folding parameters of Cter-NPM1 and site directed mutants, measured at pH 7.0 and 283 K in the presence of 0.5 M NaCl.

| | k_F (s ⁻¹) | m_F (kcal mol ⁻¹ M ⁻¹) | k_u (s ⁻¹) | m_u (kcal mol ⁻¹ M ⁻¹) | T_m (K) |
|-----------|--------------------------|---|--------------------------|---|-----------------|
| Wild-type | 3900 ± 300 | 0.60 ± 0.02 | 1.4 ± 0.8 | 0.25 ± 0.03 | 346.1 ± 0.6 |
| V244A | 6500 ± 1100 | 0.60 ± 0.03 | 7.9 ± 1.4 | 0.19 ± 0.04 | 343.7 ± 0.3 |
| E245A | 3000 ± 300 | 0.50 ± 0.02 | 1.3 ± 0.3 | 0.22 ± 0.03 | 346.8 ± 0.2 |
| A245G | 2500 ± 200 | 0.50 ± 0.02 | 3.8 ± 0.4 | 0.23 ± 0.03 | 341.4 ± 0.2 |
| D246A | 9400 ± 1600 | 0.60 ± 0.03 | 2.4 ± 0.2 | 0.15 ± 0.02 | 351.7 ± 0.5 |
| A246G | 4000 ± 500 | 0.50 ± 0.04 | 1.2 ± 0.3 | 0.28 ± 0.04 | 338.7 ± 0.2 |
| I247V | 3300 ± 400 | 0.45 ± 0.02 | 1.4 ± 0.3 | 0.25 ± 0.04 | 340.1 ± 0.2 |
| K248A * | - | - | - | - | - |
| A248G * | - | - | - | - | - |
| A249G | 3300 ± 300 | 0.55 ± 0.02 | 19 ± 1.0 | 0.1 ± 0.01 | 335.7 ± 0.2 |
| K250A | 5300 ± 600 | 0.55 ± 0.01 | 0.5 ± 0.1 | 0.25 ± 0.02 | 350.2 ± 0.2 |
| A250G | 4800 ± 700 | 0.47 ± 0.04 | 3.0 ± 1.0 | 0.28 ± 0.04 | 338.8 ± 0.2 |
| M251A * | - | - | - | - | - |
| A253G | 2200 ± 300 | 0.40 ± 0.03 | 7.3 ± 2.0 | 0.18 ± 0.04 | 335.1 ± 0.3 |
| I255V | 2700 ± 300 | 0.45 ± 0.02 | 1.8 ± 0.5 | 0.21 ± 0.05 | 339.1 ± 0.2 |
| L261A | 5900 ± 100 | 0.65 ± 0.02 | 14 ± 1 | 0.3 ± 0.02 | 333.7 ± 0.2 |
| A266G | 6700 ± 900 | 0.65 ± 0.03 | 1.8 ± 0.5 | 0.19 ± 0.05 | 348.3 ± 0.3 |
| F268A * | - | - | - | - | - |
| I269V | 4300 ± 600 | 0.60 ± 0.03 | 0.6 ± 0.04 | 0.26 ± 0.05 | 349.4 ± 0.3 |
| N270A | 5100 ± 500 | 0.53 ± 0.01 | 0.9 ± 0.07 | 0.20 ± 0.02 | 353.1 ± 0.5 |
| A270G | 1500 ± 120 | 0.53 ± 0.03 | 2.9 ± 0.4 | 0.21 ± 0.03 | 342.4 ± 0.2 |
| V272A | 2500 ± 300 | 0.63 ± 0.03 | 3.2 ± 0.6 | 0.23 ± 0.03 | 338.5 ± 0.2 |
| K273A | 2200 ± 200 | 0.55 ± 0.02 | 3.3 ± 0.3 | 0.22 ± 0.04 | 342.3 ± 0.2 |
| A273G | 800 ± 80 | 0.57 ± 0.03 | 5.0 ± 0.4 | 0.21 ± 0.02 | 333.9 ± 0.2 |
| F276A * | - | - | - | - | - |
| A283G | 3500 ± 500 | 0.60 ± 0.03 | 0.7 ± 0.1 | 0.26 ± 0.04 | 338.9 ± 0.2 |
| I284V | 2200 ± 200 | 0.54 ± 0.02 | 1.6 ± 0.5 | 0.20 ± 0.02 | 329.5 ± 0.2 |
| L287A | 1300 ± 150 | 0.53 ± 0.05 | 50 ± 10 | 0.14 ± 0.02 | 333.6 ± 0.8 |
| W288F | 8800 ± 1000 | 0.72 ± 0.05 | 30 ± 5 | 0.11 ± 0.02 | 328.7 ± 0.1 |
| W290F | 2000 ± 720 | 0.73 ± 0.09 | 9.0 ± 2.0 | 0.12 ± 0.02 | 334.7 ± 0.2 |
| K292A | 7200 ± 1000 | 0.57 ± 0.03 | 6.3 ± 1.2 | 0.15 ± 0.03 | 331.3 ± 0.2 |
| A292G | 4500 ± 650 | 0.60 ± 0.06 | 30 ± 9.0 | 0.07 ± 0.02 | 319.5 ± 0.1 |
| L294A | 8000 ± 1000 | 0.76 ± 0.09 | 16 ± 4.0 | 0.19 ± 0.04 | 338.2 ± 0.2 |

*These mutants expressed poorly and could not be characterized

Table S2. Folding parameters of Cter-NPM1 and site directed mutants, measured at pH 7.0 and 283 K.

| | k_F (s^{-1}) | m_F (kcal mol $^{-1}$ M $^{-1}$) | k_u (s^{-1}) | m_u (kcal mol $^{-1}$ M $^{-1}$) | T_m (K) |
|-----------|--------------------|-------------------------------------|--------------------|-------------------------------------|-------------|
| Wild-type | 1100 ± 100 | 0.75 ± 0.03 | 2.9 ± 0.6 | 0.27 ± 0.02 | 336.5 ± 0.2 |
| V244A | 900 ± 100 | 0.74 ± 0.05 | 13 ± 2.0 | 0.29 ± 0.02 | 330.4 ± 0.2 |
| E245A | 1000 ± 90 | 0.66 ± 0.03 | 2.7 ± 0.6 | 0.25 ± 0.02 | 338.5 ± 0.1 |
| A245G | 750 ± 100 | 0.66 ± 0.04 | 5.4 ± 1.1 | 0.26 ± 0.02 | 333.7 ± 0.2 |
| D246A | 950 ± 90 | 0.68 ± 0.03 | 3.4 ± 0.7 | 0.26 ± 0.02 | 339.0 ± 0.2 |
| A246G | 700 ± 70 | 0.67 ± 0.03 | 4.5 ± 0.7 | 0.30 ± 0.02 | 324.1 ± 0.2 |
| I247V | 1000 ± 60 | 0.75 ± 0.03 | 6.3 ± 0.8 | 0.25 ± 0.02 | 327.4 ± 0.2 |
| K248A * | - | - | - | - | - |
| A248G * | - | - | - | - | - |
| A249G | 550 ± 80 | 0.69 ± 0.06 | 18 ± 2 | 0.19 ± 0.01 | 322.5 ± 0.2 |
| K250A | 1700 ± 150 | 0.64 ± 0.02 | 1.5 ± 0.6 | 0.21 ± 0.04 | 341.1 ± 0.2 |
| A250G | 700 ± 60 | 0.36 ± 0.02 | 4.4 ± 0.1 | 0.22 ± 0.08 | 329.9 ± 0.1 |
| M251A * | - | - | - | - | - |
| A253G | 600 ± 90 | 0.74 ± 0.07 | 16 ± 2 | 0.25 ± 0.01 | 321.0 ± 0.2 |
| I255V | 700 ± 70 | 0.61 ± 0.04 | 6.8 ± 1.6 | 0.20 ± 0.02 | 326.1 ± 0.2 |
| L261A | 1300 ± 300 | 0.69 ± 0.2 | 110 ± 50 | 0.26 ± 0.08 | 318.3 ± 0.4 |
| A266G | 700 ± 100 | 0.77 ± 0.05 | 4.2 ± 0.6 | 0.24 ± 0.01 | 333.0 ± 0.1 |
| F268A * | - | - | - | - | - |
| I269V | 700 ± 60 | 0.74 ± 0.03 | 2.8 ± 0.5 | 0.26 ± 0.02 | 335.4 ± 0.1 |
| N270A | 1800 ± 150 | 0.62 ± 0.02 | 2.2 ± 0.8 | 0.22 ± 0.03 | 342.9 ± 0.2 |
| A270G | 400 ± 40 | 0.70 ± 0.04 | 2.9 ± 0.5 | 0.23 ± 0.02 | 328.8 ± 0.1 |
| V272A | 500 ± 70 | 0.93 ± 0.08 | 11 ± 1 | 0.29 ± 0.01 | 324.6 ± 0.2 |
| K273A | 800 ± 80 | 0.73 ± 0.04 | 7.9 ± 1.3 | 0.19 ± 0.02 | 332.9 ± 0.1 |
| A273G | 200 ± 30 | 0.75 ± 0.07 | 8.8 ± 1.1 | 0.19 ± 0.01 | 324.2 ± 0.2 |
| F276A * | - | - | - | - | - |
| A283G | 500 ± 50 | 0.68 ± 0.04 | 5.7 ± 1.1 | 0.20 ± 0.02 | 326.7 ± 0.2 |
| I284V | 900 ± 130 | 0.94 ± 0.06 | 13.3 ± 1.4 | 0.18 ± 0.01 | 322.8 ± 0.1 |
| L287A | 900 ± 180 | 1.25 ± 0.5 | 140 ± 20 | 0.33 ± 0.15 | 318.0 ± 0.1 |
| W288F | 600 ± 240 | 1.0 ± 0.3 | 80 ± 9 | 0.14 ± 0.01 | 310.5 ± 0.2 |
| W290F | 360 ± 40 | 0.75 ± 0.1 | 50 ± 4 | 0.16 ± 0.01 | 321.6 ± 0.2 |
| K292A | 1200 ± 100 | 0.71 ± 0.04 | 10 ± 1.6 | 0.23 ± 0.01 | 321.9 ± 0.8 |
| A292G | 650 ± 100 | 0.76 ± 0.09 | 32 ± 5 | 0.18 ± 0.02 | 312.4 ± 0.8 |
| L294A | 400 ± 80 | 0.50 ± 0.13 | 50 ± 14 | 0.17 ± 0.03 | 317.1 ± 0.8 |

*These mutants expressed poorly and could not be characterized