**SUPPLEMENTARY MATERIAL**

# Structural Determinants for Protein Unfolding and Translocation by the Hsp104 Protein Disaggregase

Jungsoon Lee[1], Nuri Sung[1], Lythou Yeo[1], Changsoo Chang[4],

Sukyeong Lee[1*] and Francis T.F. Tsai[1,2,3*]

[1]Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030, USA; [2]Department of Molecular and Cellular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030, USA; [3]Department of Molecular Virology and Microbiology, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030, USA and [4]Structural Biology Center, Biosciences Division, Argonne National Laboratory, 9700 Cass Avenue, Argonne, IL 60439, USA.

## SUPPLEMENTARY METHOD

### Analytical size-exclusion chromatography

Purified Hsp104 and $Hsp104_{4D}$ (15 μM monomer) were incubated with 5 mM ATPγS in refolding buffer (25 mM HEPES-KOH pH 7.5, 150 mM potassium acetate, 10 mM magnesium acetate, and 10 mM DTT) for 1 h on ice to allow self-assembly. Hsp104 and $Hsp104_{4D}$ oligomers were analyzed on a Superdex PC 3.2/30 column preequilibrated in 25 mM HEPES-KOH pH 7.5, 150 mM potassium acetate, 10 mM magnesium acetate, 1 mM DTT and 50 μM ATPγS. Molecular weight standards (Bio-Rad) are shown.

**SUPPLEMENTARY TABLE**

**Supplementary Table S1.** Data collection and refinement statistics

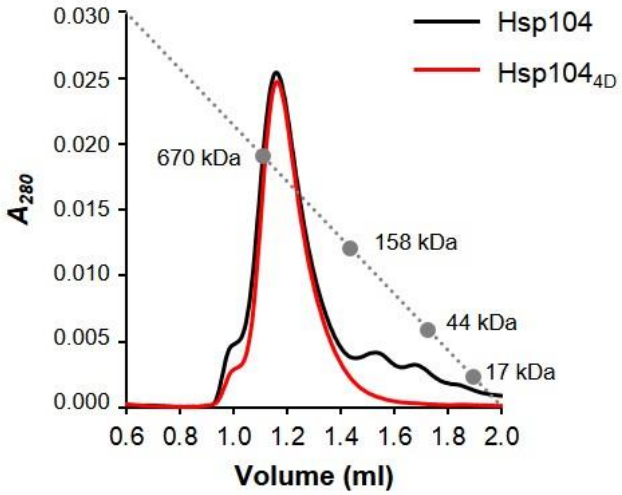
Values in parenthesis are for the highest resolution shell.

| | |
|---|---|
| Data collection | |
| Wavelength (Å) | 1.0396 |
| Temperature (K) | 93 |
| Space group | *P* $2_12_12_1$ |
| *a*, *b*, *c* (Å) | 66.56, 75.82, 235.65 |
| α, β, γ (°) | 90.0, 90.0, 90.0 |
| Resolution range (Å) | 49.04 - 2.59 (2.68 - 2.59) |
| Total No. of reflections | 238,759 |
| No. of unique reflections | 36,873 |
| Completeness (%) | 97.3 (80.3) |
| Multiplicity | 6.5 (3.9) |
| <I/σ(I)> | 16.5 |
| $R_{sym}$ § | 0.058 |
| Overall *B* factor from Wilson plot ($Å^2$) | 64.54 |
| Structure refinement | |
| Resolution range (Å) | 46.04 - 2.60 (2.65 - 2.60) |
| Completeness (%) | 97.76 |
| No. of reflections, working/test set | 36,797 / 2,587 |
| Final $R_{cryst}$ ¶ | 0.2408 (0.3391) |
| Final $R_{free}$ ¶ | 0.2859 (0.4437) |

| | |
|---|---|
| No. of molecules in asymmetric unit | 3 |
| No. of non-H atoms | |
| Protein | 7,864 |
| Water | 24 |
| Total | 7,888 |
| R.m.s. deviations | |
| Bonds (Å) | 0.002 |
| Angles (°) | 0.558 |
| Average B factors (Å$^2$) | |
| Protein | 97.85 |
| Water | 71.89 |
| All atoms | 97.77 |
| Ramachandran plot | |
| Most favoured (%) | 96.48 |
| Allowed (%) | 3.12 |
| Outliers (%) | 0.39 |

[§] $R_{sym} = \Sigma_{hkl} \Sigma_i |I_i(hkl) - \langle I(hkl) \rangle| / \Sigma_{hkl} \Sigma_i I_i(hkl)$, where $\langle I(hkl) \rangle$ is the mean of $i$ observations $I_i(hkl)$ of reflection $hkl$.

[¶] $R_{cryst}$ and $R_{free} = \Sigma_{hkl} ||F_{obs}| - |F_{calc}|| / \Sigma_{hkl} |F_{obs}|$, where $F_{obs}$ and $F_{calc}$ are the observed and calculated structure factors, respectively, calculated for recorded data ($R_{cryst}$) and for 7.03 % of the data omitted in refinement ($R_{free}$).




**Supplementary Figure S1**. Analytical size-exclusion chromatograms of Hsp104 and Hsp104$_{4D}$ in the presence of nucleotide. Both Hsp104 (black) and Hsp104$_{4D}$ (red) form hexamer assembles with ATPγS. Molecular weight standards are shown.