Supporting Information to Thermodynamic and Kinetic Stability of the Josephin Domain Closed Arrangement: Evidences from Replica Exchange Molecular Dynamics

**Supporting Information**

Gianvito Grasso¹, Jack A. Tuszyński², Umberto Morbiducci³, Ginevra Licandro¹, Andrea Danani¹, and Marco A. Deriu¹
d* Gianvito Grasso and Marco A. Deriu equally contributed to the work.

¹ Istituto Dalle Molle di studi sull'Intelligenza Artificiale (IDSIA), Scuola universitaria professionale della Svizzera italiana (SUPSI), Università della Svizzera italiana (USI), Centro Galleria 2, Manno, CH-6928, Switzerland.

² Department of Physics, University of Alberta, Edmonton AB, Canada.

³ Department of Mechanical and Aerospace Engineering, Politecnico di Torino, CorsoDucadegli Abruzzi 24, IT-10128, Torino, Italy.

corresponding author: Marco A. Deriu: marco.deriu@idsia.ch
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Section 1

S1.1 Replica Exchange Molecular Dynamics

The 1YZB model [1,2] of JD was selected as starting point for the present work. The 1YZB model was solvated in a dodecahedron box where the minimum distance between the protein and the edge of the box was 1 nm, resulting in a molecular system of about 40000 interacting particles. 128 replicas were simulated for temperatures ranging from 300 K to 602 K in NVT ensemble, as done in several works in literature [3–5]. Temperatures are distributed following the exponential spacing law suggested by a number of paper [6,7], keeping the overlap of the potential energy distributions constant across the temperature space (Figure S2). The resulting exchange probability was 0.35. The exchange attempt time interval was set to 2 ps. Each replica was simulated for 50 ns, obtaining a cumulative simulation time of 6.4 µs. It is worth noticing that JD secondary structure is highly conserved along the REMD trajectory at 310K (Figure S2), with the exception of α2 and (partially) α3, in agreement with data coming from literature [8,9]

![Figure S1. Potential energy distribution among REMD simulations. Temperatures were distributed across the replicas in a geometric progression, i.e. with the same ratio used to scale each temperature from the one below it, keeping the overlap of the potential energy distributions constant across the temperature space.](image)
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Figure S2. Secondary structure probability calculated in case of a) Josephin Domain 1YZB model [1,2] compared with b) REMD trajectory snapshots at 310 K. JD secondary structure is highly conserved along the REMD trajectory at 310K, with the exception of α2 and partially α3.
S1.2 Thermodynamic and Kinetic Estimation

In order to obtain a reliable estimate of the JD folding rates, the kinetic description developed by van der Spoel et al [10] was applied to the REMD trajectories, as described in the main text of this paper. The fraction of closed JD sampled along the REMD trajectories is reported in Figure S3a as function of simulation time. The red curve of Figure S3a is optimized according to Equation 5 reported in the main text of the present paper. This function depends on the mix of simulations and temperatures used in the analysis, and the results show that the red curve represents a good fit of the computational data (Figure S3a). From these data, together with the estimation of the forward and backward rate constants for folding, a theoretical melting curve was obtained (Figure S3b).

Figure S3. a) Fraction of closed JD as a function of time (black curve), averaged over the first 30 ns of all 128 REMD simulations, compared with the fit (red curve) obtained by minimizing $X^2$ (Equation 5 of the main text). b) The represented melting curve reports the fraction of closed JD as a function of temperature.
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S1.3 Kinetics Estimation of the Josephin Domain Open-to-Closed Transition by Unbiased Molecular Dynamics

The 1YZB[1,2] model was solvated in a dodecahedron box where the minimum distance between the protein and the edge of the box was 1 nm, resulting in a molecular system of about 40000 interacting particles. The net charge of the system was neutralized by the addition of Cl⁻ and Na⁺ ions. The system was first minimized by 1000 steps of the Steepest Descent energy minimization algorithm and then equilibrated at a temperature of 310K[11] and 1 atm[12]. Then, 100 replicas of the system were created by associating to the system atoms initial random velocities from a Maxwell-Boltzmann distribution at 310K. For each system a 20 ns unbiased MD was carried out for a total sampling time of 2 µs.

The analysis of the scatter plot reporting each unbiased MD trajectory snapshot (Figure S4a) show the JD configuration sampled by unbiased MD in term of Radius of Gyration and α3-α5 distance. The kinetic description developed by Van Der Spoel and coworkers[10] was then applied to the MD trajectories for evaluating the folding kinetic constants. In detail, the red curve of Figure S4b was optimized according to Equation 5 reported in the main manuscript.

Throughout the simulated time (20 ns) by unbiased MD simulations, only transitions from open to intermediate (O-I) and from intermediate to closed (I-C) arrangement were sampled. The fraction of JD closed arrangements reported as function of simulated time (Figure S4b).

In agreement with kinetics reported in Figure 3 of the main manuscript and unbiased MD of our previous work[9], C-I and I-O transitions have not been detected throughout the 20 ns of the 100 MD. For this reason, in case of unbiased simulations, we were able to estimate only the forward rate constant (O-I and I-C).

The above mentioned kinetic data, reported in Figure S5 (τ_{O-I}=88.4 ns and τ_{I-C}=5.8 ns), are in close agreement with the kinetic rate constants calculated by REMD simulations.

Agreement between biased and unbiased simulations can be considered as a proof of the reliability of the kinetic estimation provided in the present work.
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Figure S4. a) Each snapshot, taken from Classical MD trajectory at 310 K, is reported in figure in term of Radius of Gyration and α3-α5 distance. The NMR snapshots derived from the JD models available in literature are colored in red (2AGA), blue (2DOS), black (1YZB) and green (2JRI). b) Fraction of closed JD as a function of time (black curve), averaged over 20 ns of all 100 MD simulations, compared with the fit (red curve) obtained by minimizing $X^2$ (Equation 5 of the main text).

Figure S5. Forward rate constants for folding together with the activation energies at 310 K. Error estimates are given in parentheses. The error estimation was done by varying the cutoff for defining the protein folded state as done in a previous work[10]. In detail, we performed our kinetic analysis by changing of 0.02 nm the Radius of Gyration threshold to discern between open/intermediate, and intermediate/closed state.

|                | Activation energies (kJ/mol) | Rate constant (ns) |
|----------------|-------------------------------|-------------------|
| O-I            | 10.6 (0.2)                    | 88.4 (13)         |
| I-C            | 7.2 (0.1)                     | 5.8 (0.4)         |
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