Supporting Material

MOLECULAR SIMULATION UNCOVERS THE CONFORMATIONAL SPACE OF THE λ CRO DIMER IN SOLUTION

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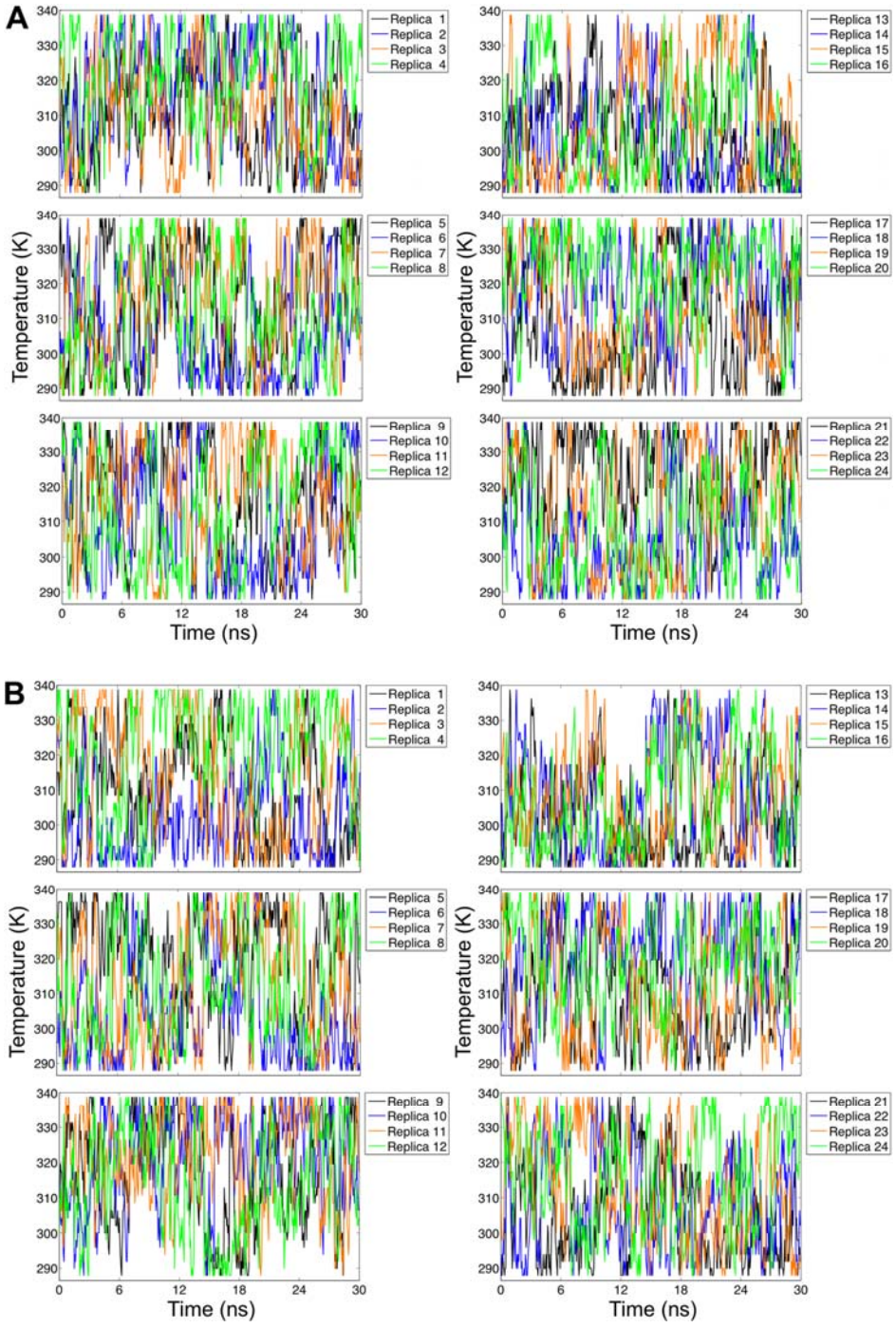

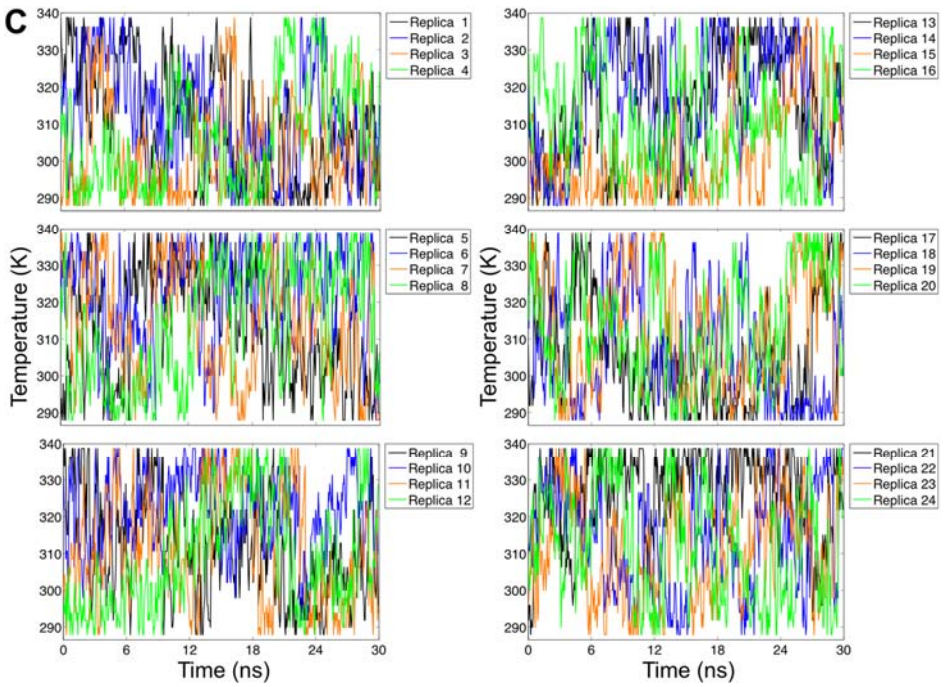

Figure S1. Temperature evolution of the 24 replicas of the REMD simulations started from the *(A)* apo ("closed") Cro X-ray structure, *(B)* bound ("open") X-ray structure, and *(C)* the 7th reported NMR model. The replicas visit a broad range of temperatures.

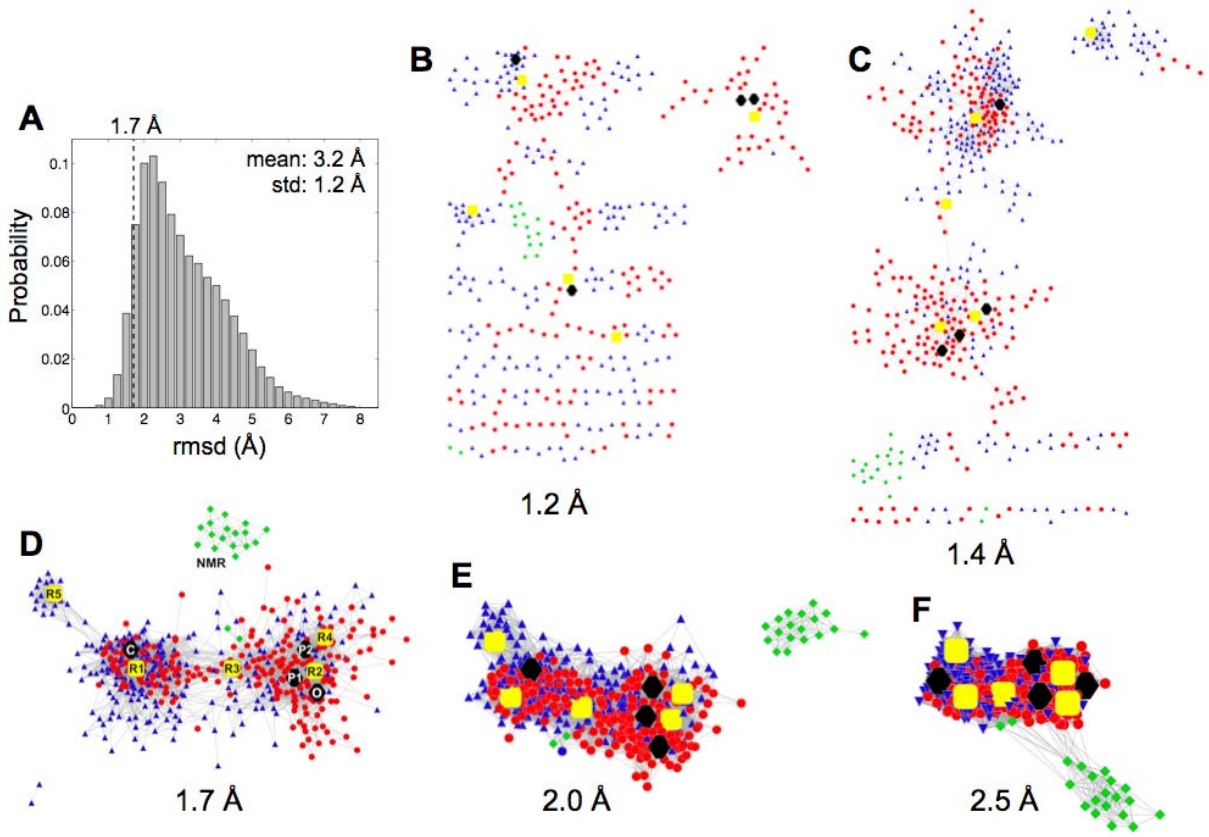

Figure S2. *(A)* The frequency distribution of pairwise rmsd values calculated for the 500 frame combined REMD trajectory that was used to construct the network presented in Fig. 3 of the main text. The vertical dashed line indicates the 1.7 Å Cα rmsd cutoff used for this layout, which is again shown directly below the histogram in *(D)*. In the networks, structures from the simulation started from the closed and open wild-type Cro dimer crystal structures are represented by blue triangles and red circles, respectively. The X-ray structures, NMR models, and representative structures are shown with the same designations as in the main text. *(B)* and *(C)* display networks constructed with smaller cutoffs (1.2 Å and 1.4 Å, respectively). The nodes become increasingly disconnected from one another as the cutoff is lowered, and information about the relationship between clusters is lost. This holds true even if a larger number of frames is used (data not shown). *(E)* and *(F)* show networks made with larger cutoff values (2.0 Å and 2.5 Å, respectively). Clusters merge together as the cutoff is increased, and individual conformational substates can no longer be distinguished. A detailed discussion of the considerations for network construction is the focus of a current study in our lab (Baker, J. et al., *in preparation*).

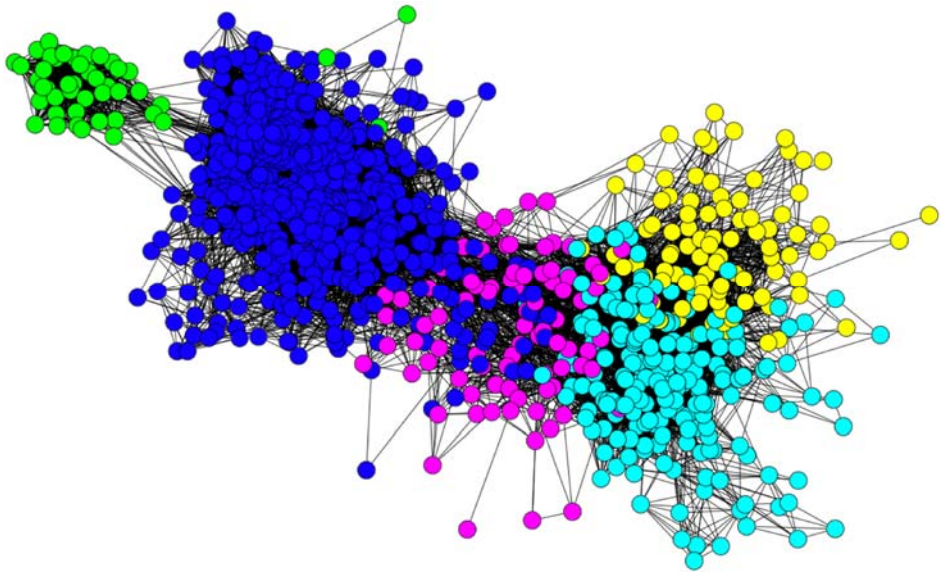

Figure S3. Comparison of the network layout and clustering. The network layout was created using the force-directed algorithm with 1000 frames and a 1.7 Å cutoff. Clusters were calculated with the average linkage algorithm. Following the same cluster designations as in the main text, the blue nodes correspond to cluster 1, cyan to cluster 2, magenta to cluster 3, yellow to cluster 4, and green to cluster 5. In order to directly compare the layout with the clustering results in this manner, 1000 frames had to be used for both algorithms. This is in contrast to the 3,750 frames used to calculate the representative structures discussed in the main text. As a result, seven clusters had to be specified for the average linkage algorithm in order to clearly delineate the five clusters shown in the layout. Two of these clusters were excluded since they represented just 1.2 % and 0.01 % of the nodes. Although node colors are mixed near the center of the layout, qualitatively the clusters determined from the average linkage algorithm agree well with the network layout.

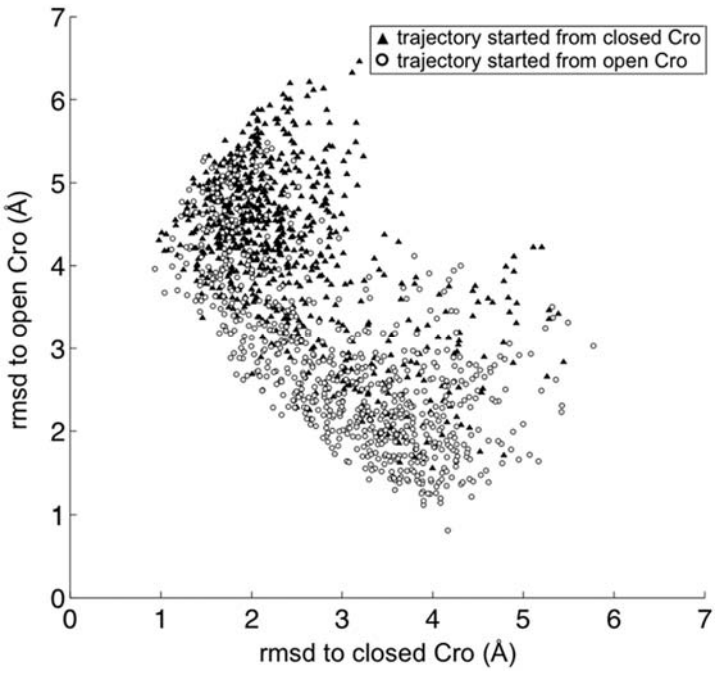

Figure S4. Projection of REMD trajectories onto rmsd space. The rmsd's of the simulations started from the closed Cro (filled triangles) and open Cro (open circles) crystal structures were calculated with respect to both sets of initial coordinates. Both trajectories access open and closed states and sample a similar extent of conformational space.

Comparison of closed and open representative structures

In addition to calculating representative structures for a combined set of coordinates, such conformations were also calculated for the last half (15-30 ns) of the individual REMD trajectories started from the crystal structures using the average linkage algorithm. For the individual trajectories the highest-ranked centroids ($R1_C$ and $R1_O$ for the trajectories started from the *C*losed and *O*pen dimers, respectively) display closed-like conformations similar to R1 for the combined trajectory (1.2 Å rmsd for $R1_C$ and 1.5 Å for $R1_O$) as well as a 1.4 Å rmsd with respect to one another (Table S1 and Fig. S5 *A*). Similarly, the second-ranked centroids ($R2_C$ and $R2_O$) each display an open conformation. $R2_O$ shows a higher degree of structural similarity to the open X-ray structure than does $R2_C$ (1.5 Å and 2.3 Å rmsd, respectively) (Table S1 and Fig. S5 *B*). This is likely because the trajectory started from open Cro sampled open-like dimer conformations more often than did the simulation run from closed Cro.

Table S1. Structural Comparison of Representative Closed and Open Cro Dimers from the Combined and Individual REMD Trajectories

	R1	R1c	R1o	R2	R2c	R2o
R1	N.A.	1.2	1.5	4.2	2.9	3.3
R1c		N.A.	1.4	3.8	2.5	3.0
R1o			N.A.	3.8	2.6	2.8
R2				N.A.	2.3	1.5
R2c					N.A.	1.9
R2o						N.A.

As in Table 2, all rmsd values are reported in Angstroms and were calculated for the Cα atoms of residues 4–56 and 4'–56' of the dimer. R1 and R2 are the top two ranked representative structures as calculated by the average linkage algorithm for the combined REMD trajectory. $R1_C$ and $R2_C$ are the top-ranked structures from the simulation from Closed Cro. Likewise, $R1_O$ and $R2_O$ signify these conformations for the trajectory from Open Cro.

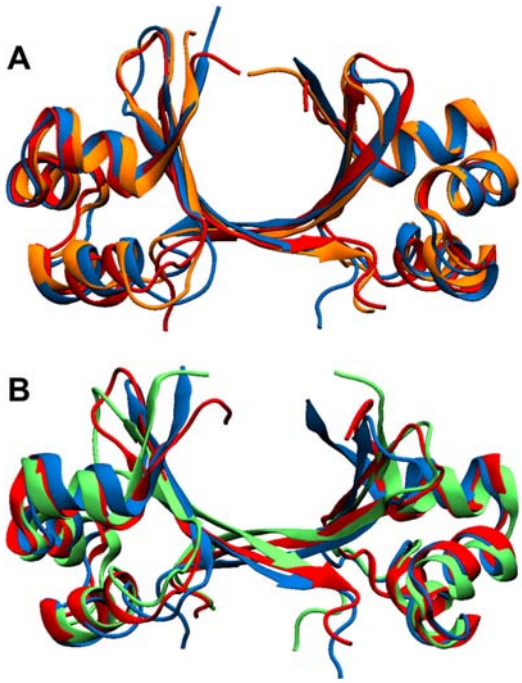

Figure S5. Comparison of representative structures. *(A)* Structural comparison of the top-ranked (closed) representative structures from the combined (R1, orange) and individual (R1c in blue, R1o in red) trajectories. (As in Table S1, "C" and "O" denote the structures calculated for the trajectories started from Closed and Open Cro crystal structures, respectively). *(B)* Similarly, alignment of the second-ranked (open) representative structures R2 (green), R2c (blue), and R2o (red). Rmsd values comparing the global conformation of these structures are shown in Table S1.

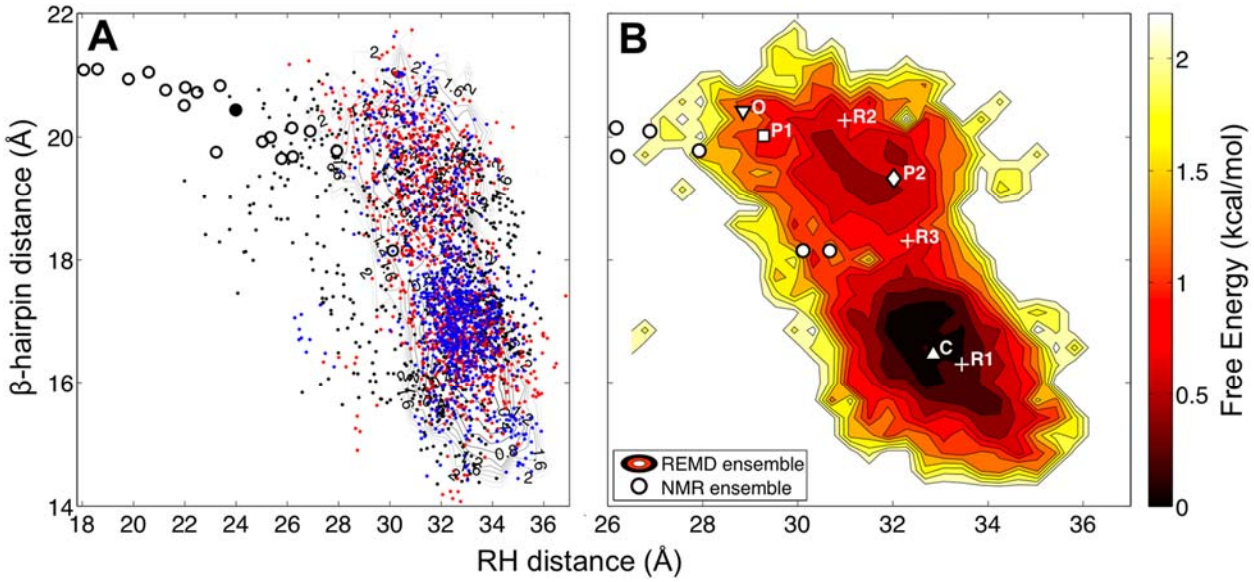

Figure S6. *(A)* Two-dimensional free energy surface from Fig. 5 of the main text showing data from the REMD trajectory started from an NMR model. Free energy values are labeled on the contour map in increments of 0.4 kcal/mol. The RH distance axis is extended to 18 Å in order to show the coordinates of all NMR models, which are represented by circles. The filled circle depicts the 7th reported NMR model, from which simulation was started. Black, red, and blue points represent coordinates sampled by the first third, second third, and last third of this trajectory (30 ns total). Within 10 ns, the trajectory started from the NMR model converges into the conformational space that was sampled by the simulations started from the X-ray structures. This result indicates that the NMR models are not stable conformations in solution. *(B)* Free energy surface calculated using coordinates sampled during all three REMD trajectories – from "closed" X-ray, from "open" X-ray, and from the NMR model. The landscape was constructed as described in the Materials and Methods section of the main text. Even with the additional trajectory from the NMR model, we obtained an essentially identical free energy profile.