

# Leucine Zipper as a Fine Tuner for the DNA Binding; Revisited with Molecular Dynamics Simulation of the Fos-Jun bZIP Complex

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Leucine zipper dynamically tunes the degree of bifurcation of the DNA binding segments in the basic region of the Fos-Jun bZIP complex. Molecular dynamics simulation indicated that site-specific mutagenesis of conserved leucine residues inside the leucine zipper domain caused the change of dynamic behavior of the basic region, and efficient DNA binding occurs only within a certain range of distance between the two DNA binding segments in the basic region. Distribution of  $\alpha$ -helices in the hinge region is also suggested to influence the bifurcation of the DNA binding segments.

## Introduction

One decade has passed since the first discovery of the leucine zipper domain in DNA binding proteins.<sup>1,2</sup> This structural motif has been detected in several transcription factors, such as the CCAAT box binding protein C/EBP, the yeast factor GCN4 and oncogene products Myc, Fos and Jun.<sup>3</sup> In addition to its direct role in protein-protein dimerization, the leucine zipper is known to be essential for DNA binding.<sup>4,5</sup> The leucine zipper is generally believed to serve an indirect structural role in DNA binding by leading to correct positioning of the two basic DNA-binding segments in the dimer.<sup>6,7</sup> As DNA should approach to a proper position to interact with basic domains of transcription factors, juxtaposition of the two basic domains for DNA must be important for gene transcription.<sup>8</sup> If the role of leucine zipper is simply to connect the two basic regions, normal dimerization ability must induce normal DNA binding affinity for a certain bZIP protein. However, the existence of some mutant bZIP proteins with normal dimerization ability but decreased DNA binding affinity,<sup>2,9</sup> may indicate that the leucine zipper has additional important function that has been overlooked. We suggest a new insight on this hidden function of the leucine zipper by means of molecular dynamics simulation.

## Experimental Section

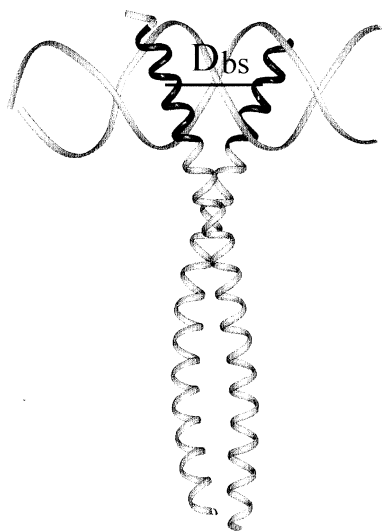
The initial structure was derived from the crystal structure of Fos-Jun-DNA complex<sup>10</sup> (PDB entry code: 1FOS) by separating the DNA from the dimer and with explicit hydrogens added. From the two models in the Fos-Jun-DNA crystal structure, complex I was chosen as the model structure for our simulation. The residues with no electronic density for the side chains and the mutated serines were replaced by the original amino acids. Mutant models were constructed referring to two independent experiments done by Kouzarides *et al.*<sup>2</sup> and Neuberger *et al.*<sup>11</sup> L2, L3, L4 correspond to E172, E179, E186 in the crystal structure. Hydrogen atoms were

re-adjusted to represent pH 7; glutamic acids and aspartic acids were negatively charged, lysine and arginine were positively charged, and all other side chains, C- and N-terminal were set to be neutral. A preliminary optimization of the rotational degrees of the reverted or mutated side chains was performed, and the entire protein was energy-minimized to eliminate any unfavorable side chain interactions.

Molecular dynamics calculation on each of the resulting system was performed with Insight/Discover program (version 97.0) using consistent valence force field.<sup>12</sup> The calculations were conducted with a non-bonded cutoff of 9.5 Å imposed over a switching distance of 1 Å on an atom-by-atom basis. The non-bond list is automatically updated whenever any atom moves more than one-half the buffer width of 0.5 Å. MD calculation was performed using the Velocity Verlet algorithm to integrate the equations of motions with a 1 fs time-step at constant volume.<sup>13</sup> The MD simulation was divided into two phases, 100ps of equilibration phase and 1000ps of production phase. The initial atomic velocities were assigned from a Gaussian distribution corresponding to a temperature of 300 K. As the potential energies appeared to stabilize in less than 100ps for all the systems, the equilibration phase of each trajectory was discarded. A constant temperature of 300 K was maintained using the Berendsen algorithm and a coupling constant of 0.15ps.<sup>14</sup> Bulk solvent was treated implicitly by using a distance-dependent dielectric constant in which electrostatic interactions between protein atoms were reduced by a sigmoidal screening factor.<sup>15</sup> Intermediate structures were saved every 1ps for the analysis.

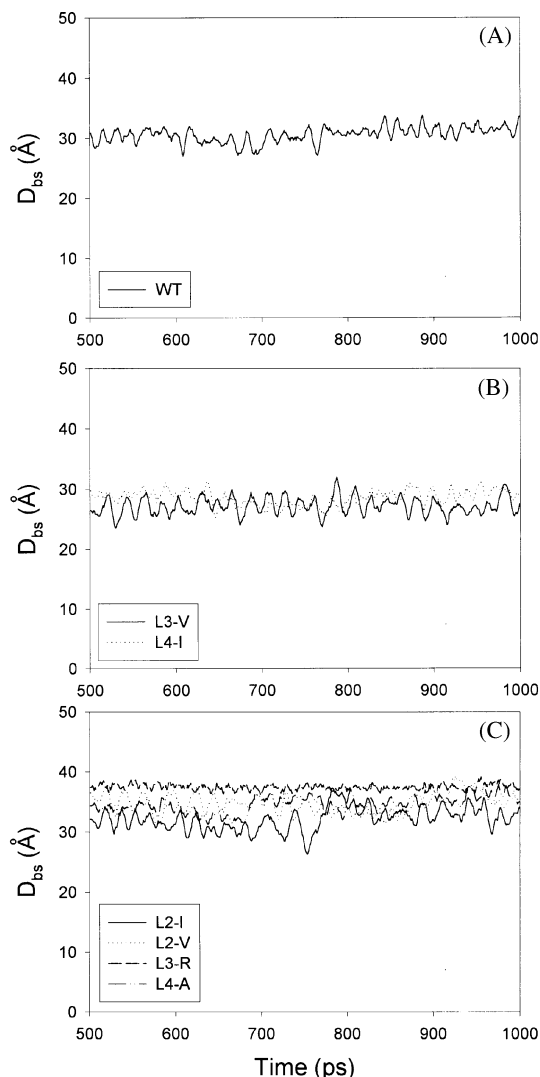
## Results

Due to the availability of large amount of experimental data related to the site-specific mutagenesis and the X-ray crystal structure coordinates,<sup>10</sup> we selected the Fos-Jun bZIP heterodimer as a model system. Various mutant models were constructed according to the two independent experiments done by Kouzarides *et al.*<sup>2</sup> and Neuberger *et al.*<sup>11</sup> Referring to



**Figure 1.** Definition of the distance between two DNA binding segments. Fourteen amino acids passing through the major groove of the cognate DNA in each basic region were defined as DNA binding segments. These amino acids correspond to residue E142-E155 of Fos and F266-F279 of Jun in the crystal structure of the Fos-Jun-DNA complex.<sup>10</sup> The crystal structure is represented as a ribbon and the DNA binding segments are marked dark. The line connecting the center of masses of each binding segment approximately cut the DNA into halves, and the distance between two binding segments in the MD trajectories was defined as  $D_{bs}$ . The time-averaged  $D_{bs}$  is defined as  $D_{avg}$ .

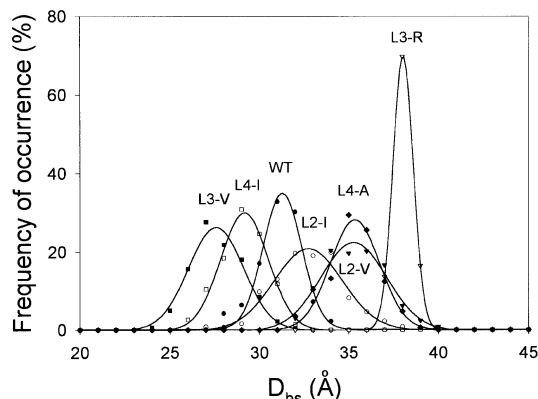
the experiment by Kouzarides *et al.*, of the five leucines in Fos (designated L1 to L5 from the N to C terminus) inside the zipper domain, L2, L3, and L4 were changed to isoleucine, arginine, and isoleucine to generate mutants L2-I, L3-R, and L4-I, respectively. Referring to the experiment by Neuberg *et al.*, L-2, L-3, L-4 in Fos zipper were changed to valine, valine, and alanine to generate mutants L2-V, L3-V, and L4-A, respectively. Each of all mutants formed Fos-Jun complex normally, but its DNA binding ability was either decreased (L2-I, L3-R, L2-V, L4-V) or retained (L4-I, L3-V) comparing with wild type (WT). For convenience, each of these two groups was named as 'D (decreased)' and 'R (Retained)' respectively. The resulting models were subjected to the MD simulation. Among the various dynamic properties in the MD trajectories, we found out that the time series of the distance fluctuation between the two DNA binding segments showed interesting relationship with the experimental results. Distance between the two DNA binding segments ( $D_{bs}$ ) in the basic region is shown in Figure 1.  $D_{bs}$ s of wild type and mutants converged on equilibrium distances within 500ps and were stable until 1ns. During the last 500ps, we have used for all of the analyses reported below. The distribution of  $D_{bs}$  of each model system shows almost perfect Gaussian type during 500-1000 ps (more than 99%). Compared with WT, group R (L3-V, L4-I) and group D (L2-I, L3-R, L2-V, L4-V) showed interesting relationship with the degree of bifurcation at its equilibrium as shown in Figure 2. There is a clear difference in time-averaged  $D_{bs}$  ( $D_{avg}$ ) between group D ( $34.8 \pm 2.4 \text{ \AA}$ ) and group R ( $27.9 \pm 1.5 \text{ \AA}$ ). All the



**Figure 2.** Time series from the MD trajectories for the distance between DNA binding segments ( $D_{bs}$ ).  $D_{bs}$  for each system showed a tendency to stabilize around a characteristic value in less than 500ps. (A) WT, (B) L3-V and L4-I (group R), (C) L2-I, L2-V, L3-R and L4-A (group D). Group D showed wider bifurcation of basic arms than group R and WT.

mutants with weaker DNA binding potentials reached out a wider bifurcation comparing with WT ( $30.6 \pm 1.3 \text{ \AA}$ ). This result may suggest that the degree of bifurcation is determined by sequence integrity of the zipper domain and affects DNA binding ability even with normal dimerization. Figure 3 displays the histogram of distribution of  $D_{bs}$ s of WT and mutants. A difference of distributed  $D_{bs}$  of two groups were clearly observed, which confirms that each group reached a different bifurcation. The distribution showed that the group R, which exhibited similar DNA binding affinity to WT, has narrower bifurcation distance than the group D of decreased DNA binding affinities. These results might suggest that a certain distance would exist within which complex formation with DNA occurs efficiently. Outside the appropriate range of  $D_{bs}$ , the DNA binding potential would decrease even though dimerization of Fos-Jun occurred normally.

It has been known that the secondary structure of the basic

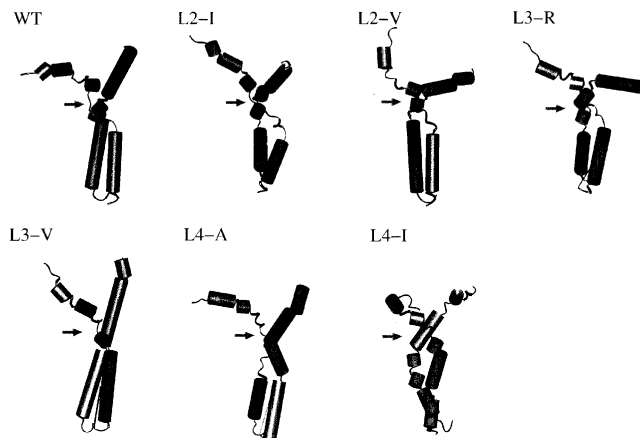


**Figure 3.** Histogram showing the distribution of  $D_{bs}$  during 500-1000 ps in the production phase of each MD simulation. The frequency of occurrence was counted at  $1^\circ$  interval and regressed to a normal distribution. (●: WT, ○: L2-I, ▽: L3-R, □: L4-I, ▼: L2-V, ■: L3-V, ◆: L4-A)

region in a bZIP protein is disordered in the absence of DNA, and adopts  $\alpha$ -helical conformation upon DNA binding.<sup>16,17</sup> The disordered secondary structure in the basic region may assist to overcome kinetic energy barrier on the DNA binding process. To examine the  $\alpha$ -helix content, the *intra*-helical hydrogen bonds between  $O_i-NH_{i+4}$  (where  $i$  represents the residue number) were measured from the MD trajectories. The criteria used for a hydrogen bond ( $A \cdots H-D$ ) was that the distance between the acceptor (A) atom and the donor atom should be less than  $3.5 \text{ \AA}$  and the angle  $A \cdots H-D$  should be larger than  $120^\circ$ .<sup>18</sup> During the production period,  $\alpha$ -helix content was  $\sim 35\%$  in the basic region (N-terminus to L1) and  $\sim 80\%$  in the zipper region (L1 to L5) for each model system. Although no apparent differences were found for overall  $\alpha$ -helix contents among WT and mutants, each time-averaged structure shows a characteristic tendency. Figure 4 shows the cartoon of the time-averaged structures of various Fos-Jun bZIP complexes during 500-1000ps of MD simulation. Specific substitution of leucine in the leucine zipper domain induced the change of the distribution of  $\alpha$ -helices in the average structure. Wider range of unfolding around the first leucine of the zipper domain, *i.e.* wider hinge region was observed in mutant Fos-Jun bZIP complexes of group *D*, which have weak DNA binding potential. The perturbation in the leucine zipper sequence seems to influence the bifurcation of the DNA binding segments by changing the range of the hinge region, which may possibly be determined by the strength of molecular interactions inside the leucine zipper sequence during the molecular dynamics simulation. As the molecules in solution are always in a certain dynamic state depending on inter- and intra-molecular interactions, the integrity of leucine zipper sequence determining the strength of coiled-coiled interactions would be very important to induce accessible geometrical conformations.

### Discussion

Based on the MD simulation, we suggest that the leucine zipper functions as a fine tuner to control the dynamic mode



**Figure 4.** The time-averaged structure of each model system taken from 500 to 1000 ps in the MD simulation. Secondary structure classification was performed to each average structure by the method of Kabsch and Sander and displayed as a cartoon.<sup>21</sup> The  $\alpha$ -helices are displayed as cylinders, and the turns and random coils are shown as thin threads. Note the secondary structure around the first leucine of the leucine zipper. In WT,  $\alpha$ -helices were lost across 4 residues in Fos and 3 residues in Jun. In group *D*,  $\alpha$ -helices were broken in the wider range around the first leucine; L2-I (16 residues in Fos and 3 residues in Jun), L3-R (16 residues in Fos and 3 residues in Jun), L2-V (12 residues in Fos and 2 residues in Jun), and L4-A (10 residues in Fos and 11 residues in Jun). In group *R*, the region with broken  $\alpha$ -helix is comparable to that of WT; L4-I (6 residues in Fos and 1 residue in Jun) and L3-V (6 residues in Fos and 1 residue in Jun). The first leucines were indicated by arrows.

of the basic region for the efficient gene transcription. It has been reported that replacement of leucine zipper by an intermolecular disulfide bond in the GCN4 basic regions not only mediated protein dimerization but also displayed DNA binding ability with nanomolar affinity.<sup>19</sup> However, this DNA binding occurred only at  $4^\circ\text{C}$ , not at higher temperature.<sup>9</sup> Although not claimed by authors, their experiments may suggest dynamic tuning mode of leucine zipper that is possibly temperature dependent. The temperature-dependent dynamic motions of bifurcation of binding region could explain this. Dynamic tuning of degree of bifurcation of binding segment may not be exclusive to leucine zipper. Interesting resemblance was found for the segmental flexibility of antibody on the antigen binding affinity.<sup>20</sup> Each isotypes of various antibodies showed specific degree of bifurcation, without which effective antigen binding did not occur.

The action mechanism of the bZIP transcription factor was generally believed to occur by scissors grip<sup>3</sup> or induced helical fork model.<sup>7</sup> Scissors grip model put emphasis on the degree of bifurcation of DNA binding region, whereas an induced helical fork model focused on the  $\alpha$ -helical contents of the DNA binding region. If we consider these two parameters - degree of bifurcation and  $\alpha$ -helix content of the basic region - to be critical for the DNA binding efficiency, can we predict two groups (*D* and *R*) from various Jun-Fos mutants? We tried to quantify the relative deviations of the mutants from wild-type in the MD trajectories as a function of these two parameters. The equilibrium conformation of wild-type (WT) after 1 ns MD simulation was used as a control for the

**Table 1.** A total relative deviation of  $\alpha$ -helical contents and bifurcation distance between wild-type (WT) and each mutant

$\lambda$	RD( $\lambda$ ) <sup>a</sup>					
	L2-I	L2-V	L3-R	L3-V	L4-A	L4-I
0.0	3.5	29.7	40.0	7.6	12.7	6.4
0.1	6.4	27.3	36.5	7.3	14.2	8.3
0.2	9.3	24.9	33.0	6.9	15.6	10.3
0.3	12.3	22.5	29.4	6.5	17.0	12.3
<b>0.4</b>	<b>15.2</b>	<b>20.1</b>	<b>25.9</b>	<b>6.2</b>	<b>18.4</b>	<b>14.2</b>
<b>0.5</b>	<b>18.2</b>	<b>17.7</b>	<b>22.4</b>	<b>5.8</b>	<b>19.8</b>	<b>16.2</b>
0.6	21.1	15.2	18.8	5.4	21.2	18.1
0.7	24.1	12.8	15.3	5.0	22.7	20.1
0.8	27.0	10.4	11.8	4.7	24.1	22.0
0.9	30.0	8.0	8.3	4.3	25.5	24.0
1.0	32.9	5.6	4.7	3.9	26.9	25.9

<sup>a</sup>RD( $\lambda$ ) =  $\lambda\Phi(h_\alpha) + (1-\lambda)\psi(D_{bs})$ , where RD( $\lambda$ ) represents the total relative deviation from WT,  $\Phi(h_\alpha)$  for relative deviation of  $\alpha$ -helical content and  $\psi(D_{bs})$  for that of bifurcation distance ( $0 \leq \lambda \leq 1$ ). The values of RD( $\lambda$ ) at  $\lambda = 0.4$  or  $0.5$  were written in bold letters. In this case, the values of RD( $\lambda$ ) of group R (L-3V and L4-I) were smaller than those of group D.

comparison of the relative deviations of the mutants because there is no known solution structure of Fos-Jun bZIP complex. The relative deviation of the  $\alpha$ -helix content of the basic region was represented as  $\Phi(h_\alpha)$  and that of the bifurcation distance was represented as  $\psi(D_{bs})$ . Hydrogen bonding between  $i$ -th and  $i+4$ -th amino acids of binding region was considered for  $\alpha$ -helices.<sup>22</sup> If these two parameters contribute to the DNA binding efficiency independently, the relative deviation (RD) of the MD trajectory of a mutant bZIP protein can be represented as equation (1),

$$RD(\lambda) = \lambda \Phi(h_\alpha) + (1-\lambda)\psi(D_{bs}) \quad (1)$$

, where  $\lambda$  represents the weighting factor of the  $\alpha$ -helix content and  $(1-\lambda)$  represents that of the bifurcation degree of the basic region ( $0 \leq \lambda \leq 1$ ).  $\Phi(h_\alpha)$  and  $\psi(D_{bs})$  were determined as follows; (a) determine the absolute value of the relative percent deviation between each mutant and wild-type by subtraction, (b) divide this value by the sum of absolute value of relative percent deviation to normalize it. Depending on the value of  $\lambda$ , each mutant showed its characteristic value of RD( $\lambda$ ). In equation (1), the smaller the value of RD( $\lambda$ ), the closer the pattern of the MD simulation of a mutant reaches to that of WT. By varying the value of  $\lambda$  from 0 to 1 at 0.1 intervals, we calculated the value of RD( $\lambda$ ) of each mutant (Table 1). When  $\lambda$  was 0.4 or 0.5, the values

of RD( $\lambda$ ) of the R group (L-3V and L4I) which showed normal DNA binding ability were smaller than those of D group. This implies that the bifurcation distance and  $\alpha$ -helical content of the basic region have approximately equal contribution to the DNA binding ability. According to our model system, both  $\alpha$ -helical content and bifurcation distance may contribute to the efficient DNA binding of the Fos-Jun bZIP complex. Further investigations are needed in this regard.

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