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Capacitance-derived dielectric constants demonstrate differential preinitiation complexes in TBP-independent and TBP-dependent transcription

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Abstract

The electronic properties of proteins and DNA may change dramatically upon complex formation, yet there are not many experimental methods which can be used to measure these properties. It has been previously shown that measuring the capacitance of a solution containing interacting DNA and protein species can yield information about changing dipole moments. The measured dielectric constant relates directly to the dipole moment of the complexes in solution. Here, we apply this method to partial transcription initiation complexes in order to investigate the changing electronic properties in the transcriptional preinitiation complex. These experiments are the first reported observations relating to the overall dipole moment and its changes in preinitiation complex formation. Comparing results from TBP-independent and TBP-dependent transcriptional systems shows a divergence in the electronic properties of built-up transcription complexes, suggesting that they initiate transcription by significantly different electronic and structural pathways.

Keywords: Capacitance measurement of protein interactions; Dielectric constant; Specific protein–DNA interaction; Transcription initiation; TBP-independent transcription; TBP-dependent transcription

1. Introduction

The electrostatic properties of proteins and their complexes are critical to their selectivity for interaction and function. Changes in local molecular electronics may affect function in many cellular processes, including gene transcription and regulation. In transcription initiation, the formation of a preinitiation complex (PIC) brings many polar proteins together in a specific structure [1], the potential energy of which may be important for the subsequent steps of transcription. It is known that transcription factors which bind DNA can cause large shifts in the local electronic environment of the DNA and may play a role in preparing DNA for transcription initiation [2]. The additional binding of other proteins in the PIC may also contribute to this change in electronic behavior. Some of these observed changes may be structural in nature, while others are not. The contribution of dynamic electronic environments due to complex formation in the regulation of transcription is unclear and remains to be determined.

It has been demonstrated previously that capacitance measurements on protein and DNA solutions can yield qualitative data about the dipole moment of interacting protein and DNA [2]. No modifications to the proteins or DNA are required in this method, so the observed interactions are entirely without impediment or interference. Because these interactions between species can be observed dynamically, this analysis can be extended to ternary complexes simply by adding another interacting protein to a preexisting protein–DNA complex. Here, we compare data on a TATA box-binding protein (TBP)-dependent transcriptional system, using the adenovirus major late promoter (AdMLP), TBP and transcription factor IIB (TFIIB) [3], to a TBP-independent transcription-

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al system, using the adeno-associated virus P5 promoter (P5), Yin Yang 1 (YY1) initiator protein and TFIIB [4]. We applied capacitance analysis to extract a quantitative value of the dielectric constant of the bound complex in solution (ε), which correlates with the overall dipole moment. This total dipole moment is indicative of the geometry and orientation in which the proteins complex together. We demonstrate that the binding of TFIIB to the TBP-TATA box complex only slightly changes the dielectric environment in solution, while TFIIB binding to the YY1-P5 promoter complex induces a much larger change. This differential behavior suggests that these two transcriptionally active systems appear to function by dissimilar electronic and structural mechanisms, though they share similar components.

2. Experimental

2.1. Capacitance measurements

The capacitance of solution samples was measured using a revised capacitor-on-a-chip device as shown in Fig. 1. A gold micro-strip was deposited on a 1-mm alumina substrate (Al_2O_3, ε=10). Ion milling was used to etch a 225-μm discontinuity in the gold strip, thus creating a series linked capacitor. To measure capacitance, 0.8 μL of protein, DNA or complex in solution was placed on the capacitor. The device was connected to a Hewlett-Packard 8516A S-parameter Test Box and a Hewlett-Packard 8510C Network Analyzer to measure the scattering parameters. Scattering parameters were measured across a range of frequencies from 45 MHz to 2.4 GHz in increments of 32.4 MHz using a Hewlett-Packard 83623A Series Synthesized Sweeper. All measurements were performed at ambient pressure and temperature.

A number of advances have been made in our capacitance-measuring apparatus. First, the design of the capacitor cell has been simplified from many parallel lines to one transmission line, eliminating ambiguity about droplet placement as well as line resonance noise, as determined by field simulations (not shown). The alumina surface and gold transmission line define a simple system which behaves predictably, even at high frequencies. Taken in combination with the higher quality of measurement equipment, we are able to examine a much broader frequency range and measure the dielectric response more generally.

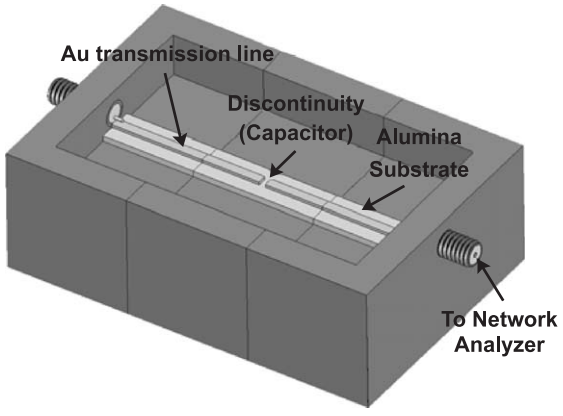

Fig. 1. Diagram of the capacitor device.

Fig. 2. Purity of the recombinant proteins. Recombinant TFIIB (1), TBP (2), and YY1 (3) were expressed in *E. coli* and purified. Each protein sample was run using SDS-PAGE and visualized by silver staining.

2.2. Transcriptional components

Gel-purified dsDNA promoter fragments (Oligos, etc.) and highly purified recombinant TFIIB [5] were used to model the transcriptional systems. Highly purified recombinant YY1 [6] was used in the TBP-independent system, and highly purified recombinant TBP was used in the TBP-dependent system. All proteins were expressed in *Escherichia coli*, isolated and purified near homogeneity (>99% by HPLC) using chromatographic methods. Gel electrophoresis separation with silver staining was used to demonstrate protein purity (Fig. 2). The buffer system consisted of 50 mM NaCl and 5 mM HEPES at pH 7.6.

2.3. Gel-shift assays

The YY1-P5 and YY1-P5-TFIIB complex formation under our buffer conditions was verified by gel shift using [^{32}P]-labeled DNA oligonucleotides, as previously described [4]. Likewise, the TBP-TATA and TBP-TATA-TFIIB complex formation was also verified using the same means (Fig. 3). The gel-shift reactions were visualized by scanning the gels on a Molecular Dynamics PhosphorImager 400-B. Capacitance measurements were done on ter-

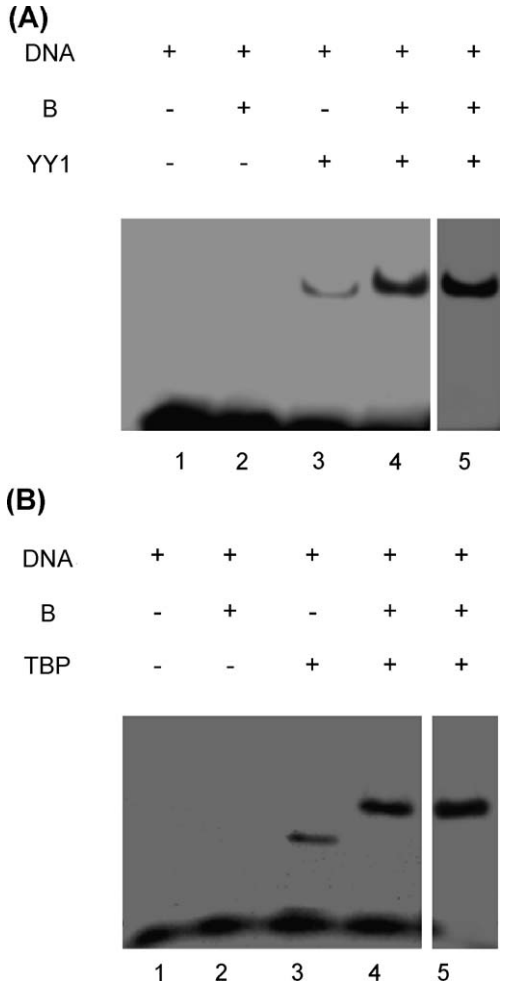

Fig. 3. Formation of ternary complexes. Gel-shift reactions were used to demonstrate the binding of P5 promoter DNA by TFIIB and YY1 (A), and binding of the AdMLP DNA by TFIIB and TBP (B). Lanes 1, DNA alone; lanes 2, DNA with TFIIB; lanes 3, DNA with YY1/TBP; lanes 4, DNA with TFIIB and YY1/TBP; lanes 5, DNA with TFIIB and YY1/TBP (no excess DNA).

nary complex solutions without any excess DNA (Fig. 3a, lane 5, and b, lane 5).

Protein concentrations were estimated by applying the Bradford method. The DNA concentration was estimated by UV absorption at 260 nm.

2.4. Extraction of dielectric constant (ε)

Our measurement cell can be considered as an ideal transmission line with a series discontinuity (Fig. 4). Y_L is defined as the admittance of the discontinuity, normalized to the line admittance. For a general unknown Y_L, we have

$$Y_L = \frac{S_{meas}/S_{thru}}{2(1 - S_{meas}/S_{thru})} \quad (1)$$

where S_{meas} values are measured scattering parameters of the solution samples, and S_{thru} values are the base-scattering parameters of the system, when the discontinuity is shorted.

In a capacitor that is electrically small, we may assume that

$$Y_L = i\omega C Z_0 \quad (2)$$

where Z_0 is the ideal line impedance and ù is the angular frequency of the driving potential.

Therefore

$$C = \frac{S_{meas}/S_{thru}}{i\omega C Z_0(1 - S_{meas}/S_{thru})} \quad (3)$$

For our capacitor geometry, the capacitance of the dry discontinuity (C_{dry}) is the parallel sum of the capacitance of the space between the plates (C_{air}) and the substrate capacitance (C_{sub}).

$$C_{dry} = C_{air} + C_{sub} \quad (4)$$

This was verified with computer simulations of the capacitor field pattern. When a solution sample with a dielectric constant ε is in the capacitor, C_{air} must be scaled by that constant. Therefore, the capacitance of the cell with the sample present is represented as

$$C_{sample} = \varepsilon C_{air} + C_{sub} \quad (5)$$

and

$$C_{sample} - C_{dry} = (\varepsilon - 1)C_{air}. \quad (6)$$

C_{dry} is the capacitance of the empty cell and was measured to be 24 fF. In order to isolate C_{air}, where the solution samples are placed, we performed a field simulation and determined a scaling factor α such that

$$C_{air} = \alpha C_{dry} \approx 5 \text{ fF}. \quad (7)$$

For an alumina substrate (ε_{sub}=10), α is calculated to be 0.208. The dielectric constant of the solution placed in the capacitor is therefore

$$\varepsilon = 1 - \frac{1}{\alpha} + \frac{1}{\alpha}\left(\frac{C_{sample}}{C_{dry}}\right). \quad (8)$$

We have reported previously that the measured capacitance is related to the dipole moment of the solvated DNA,

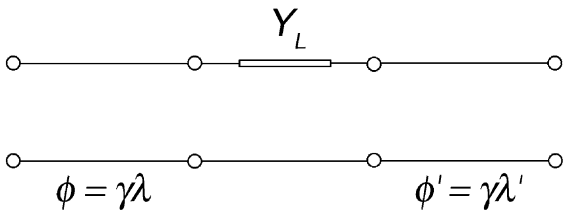

Fig. 4. Diagrammatic representation of the impedance of a discontinuous electrical transmission line.

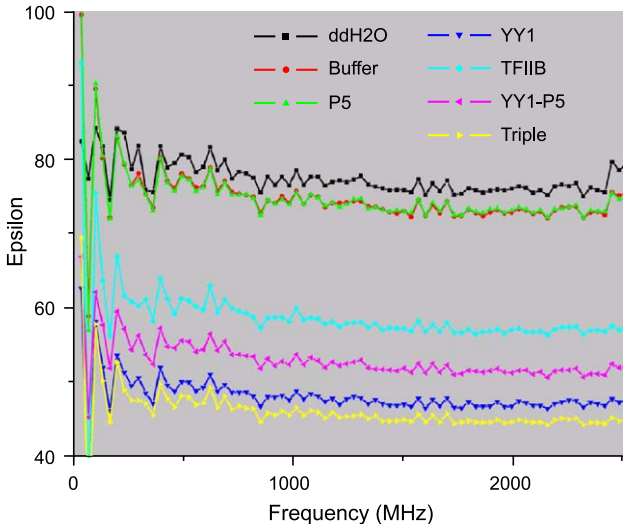

Fig. 5. Derived dielectric constant (ε) values for TBP-independent solutes in buffer.

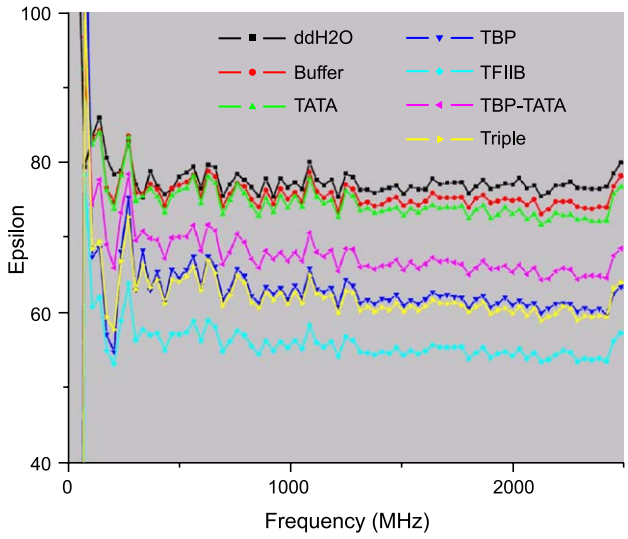

Fig. 7. Derived dielectric constant (ε) values for TBP-dependent solutes in buffer.

protein or complex. In this paper, we take our analysis further and extrapolate an effective dielectric constant ε using the Clausius–Mossotti equation. We also examine the polarizability of the solute and how it changes as a result of complexation.

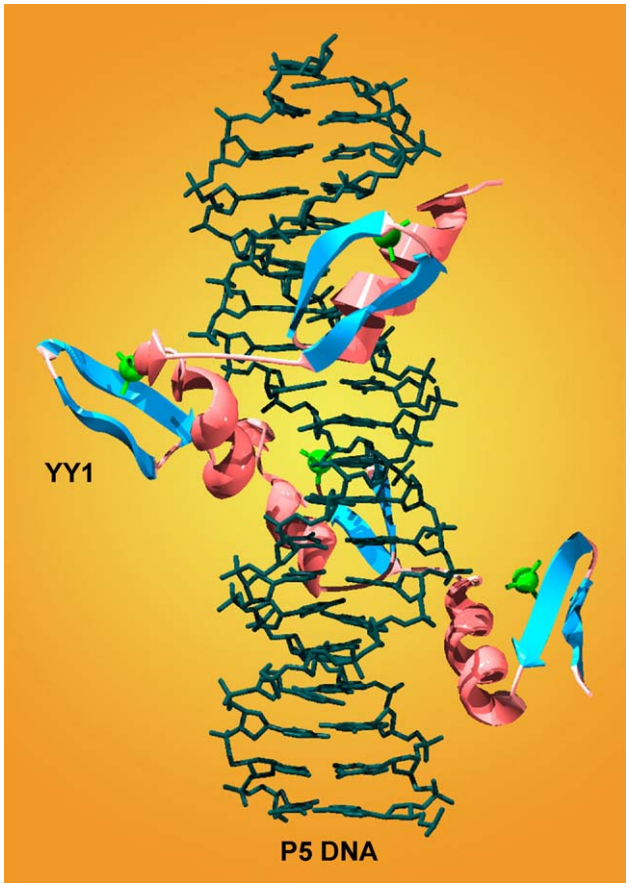

Fig. 6. Crystal structure of YY1 core domain complexed with P5 promoter DNA (PDB ID:1UBD).

3. Results and discussion

3.1. Capacitance in a TBP-independent transcriptional system

Capacitance measurements were taken for components of a TBP-independent transcriptional system, including a portion of the adeno-associated viral P5 promoter (P5), Yin Yang 1 (YY1) and transcription factor IIB (TFIIB). Extracted ε values are graphed for each component and combinations of the components (Fig. 5). Analogous with previously reported capacitance curves, the ε values varied

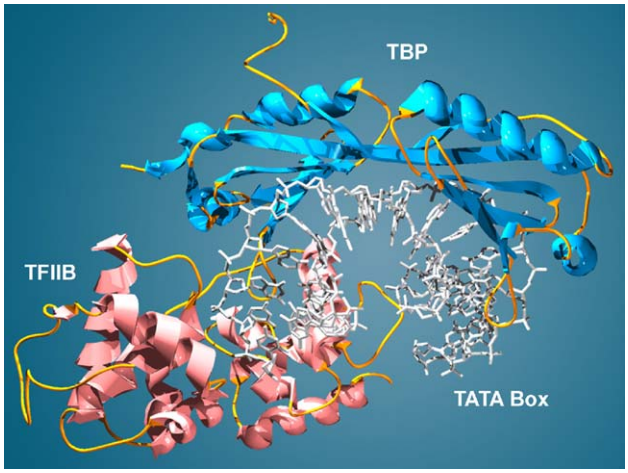

Fig. 8. Co-crystal structure of TATA box-TBP-TFIIB ternary complex (PDB ID: 1VOL).

Table 1
Averaged dielectric constant (ε) values and standard deviations for TBP-independent solution samples

Values	ddH_2O	Buffer	P5	YY1	TFIIB	YY1-P5	Ternary
(A) TBP-independent system (50 MHz–2.5 GHz range)							
ε	77.5	76.0	75.0	63.6	56.2	68.2	62.7
SDε	2.1	16.9	18.6	10.7	24.9	10.5	17.3
(B) TBP-independent system (500 MHz–2.5 GHz range)							
ε	80.1	76.9	76.4	48.9	59.6	54.0	46.7
SDε	0.8	1.8	1.9	1.8	2.6	2.5	2.3

for solutions containing interacting moieties. The averaged dielectric values for the whole spectrum are given in Table 1A. Notably, there is a great deal of noise in the system at frequencies below 500 MHz, most likely stemming from dominant ionic contributions to capacitance at lower frequencies. Frequencies ranging from 500 MHz to 2.5 GHz give the most consistent data and perhaps offer the best working zone for our study. Table 1B includes averaged values excluding the results from 50–500 MHz, which gives ε values with a standard deviation not greater than 2.6 (dimensionless units).

As expected, ε is 80.1±0.8 for doubly distilled water. This is an indication that both the measurement and the calculation work correctly. The working buffer slightly decreases the measured capacitance of water, as the ions form solvation spheres, which are polarizable and weakly dipolar. When we add the linear P5 DNA fragment to the solution, the capacitance does not change very much. The presence of DNA oligomers does not lower the measured capacitance because short, linear templates are not polar. Solvation in water results in a net dipole moment which is close to zero for DNA. YY1 in solution has an ε of 48.9±1.8, but when it complexes with the P5 DNA, it is raised slightly to 54.0±2.5. The fact that the measured ε of this complex is greater than the protein alone demonstrates that we are not simply "diluting" water to change the effective capacitance of solution. In fact, at our experimental concentrations, the excess of water ensures that this is not a factor. The small difference in dielectric constant between free YY1 and bound YY1-P5 can be understood from the crystal structure of the YY1-P5 complex [7], which shows little deformation of DNA upon YY1 binding (Fig. 6).

YY1, the P5 promoter and TFIIB have been shown to interact in a transcription preinitiation complex [6]. Gel-shift reactions demonstrate that YY1, but not TFIIB, binds the P5 promoter fragment, and that TFIIB binds the YY1-P5 duplex, apparently stabilizing the binding of DNA (Fig. 6). Upon the addition of a third interacting element to the system, we can derive the effective dielectric constant ε of the ternary complex in solution, measured to be 46.7±2.3, slightly less than the YY1-DNA complex. As might be expected, interacting dipolar species will generally associate in a manner such that their dipole moments tend to cancel out. Peculiar to this ternary system, the addition of a 38-kDa protein such as TFIIB shifts the migration position of the ternary complex only slightly relative to the YY1-P5 duplex [6]. This may be explained in terms of the overall shape of the complex in a non-denaturing assay such as this gel shift. If TFIIB associates so as to make the shape of the complex more globular, it should migrate more easily in the gel. This seems to support the ε values, which indicate that TFIIB most likely interacts to minimize the dipole moment.

3.2. Capacitance in a TBP-dependent transcriptional system

In TBP-dependent transcriptional components, we observed consistent ε values for distilled water, our buffer system, DNA fragments and TFIIB (Fig. 7). The results of the complexation, however, yield decidedly different results. TBP, a saddle-shaped protein, is more polar than YY1 in solution, as demonstrated by its larger ε of 63.9±1.1 (Table 2). When it binds the TATA box in DNA, it is known to induce a severe kink in the DNA double helix [8]. This change may be reflected by the increase in ε of TBP-TATA over TBP alone. When DNA is bent upon TBP binding, an additional, rather large dipole moment contribution is created. Note that YY1-P5 has a much lower ε value than TBP-TATA. This is due to the fact that YY1 does not distort the DNA double helix appreciably upon binding.

When TFIIB is added to the solution, the dielectric constant of the complex decreases, but is still 64.2±0.1, and higher than TFIIB alone (57.1±0.2). The crystal structure of this ternary complex reveals that TFIIB binds on one side of TBP [9], resulting in a highly polar complex (Fig. 8). Note that in comparison to the YY1 ternary complex, the migration position of the TBP ternary complex is significantly higher in the gel-shift experiment (Fig. 3B).

It is notable that in the TBP-dependent transcriptional system, as the complex builds up, the dipole moment of the complex remains high. The TBP-dependent ternary complex appears to be significantly more polar than the ternary complex in the TBP-independent case. Perhaps complex formation in the TBP-dependent system serves to store up potential energy in the form of electrostatic potential for use in transcription initiation. This is in striking contrast to the case for the TBP-independent system above. For YY1-dependent transcription from the AAV P5 promoter, the

Table 2
Averaged dielectric constant (ε) values and standard deviations for TBP-dependent solution samples

Values	ddH_2O	Buffer	TATA	TBP	TFIIB	TBP-TATA	Ternary
(A) TBP-dependent system (50 MHz–2.5 GHz range)							
ε	78.0	73.9	72.4	53.5	52.3	63.7	55.8
SDε	0.3	8.9	11.3	39.8	15.1	19.9	26.6
(B) TBP-dependent system (500 MHz–2.5 GHz range)							
ε	79.0	77.5	76.5	63.9	57.1	69.3	64.2
SDε	1.2	0.7	0.3	1.1	0.2	1.1	0.1

proteins seem to associate in such a way that the dipole moment is minimized.

4. Conclusion

Our capacitance measurements and dielectric constant calculations lead us to believe that building up the YY1-dependent, TBP-independent transcriptional complex minimizes the dipole moment of the complex, while complexation in the TBP-dependent transcriptional system tends to increase the dipole moment of the complex. If true, it suggests that these transcriptional complexes associate differently and may function in a completely divergent manner from one another. For the dipole moment to be drastically different in two ternary systems which share DNA and TFIIB as components, major structural dissimilarities are necessary. TBP-independent and TBP-dependent transcription, it appears, act through completely different modes of transcription, from differing preinitiation complexes.

Acknowledgements

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References

[1] G. Orphanides, T. Lagrange, D. Reinberg, The general transcription factors of RNA polymerase II, Genes Dev. 10 (1996) 2657–2683.
[2] C.H. Choi, J. Moser, N. Sobourin, K. Blagoev, M. Naughton, A. Usheva, YY1-DNA interaction results in a significant change of electronic context as measured by capacitance, Biophys. Chem. 103 (2003) 109–115.
[3] J.D. Parvin, H.T. Timmers, P.A. Sharp, Promoter specificity of basal transcription factors, Cell 68 (1992) 1135–1144.
[4] A. Usheva, T. Shenk, TATA-binding protein-independent initiation: YY1, TFIIB, and RNA polymerase II direct basal transcription on supercoiled template DNA, Cell 76 (1994) 1115–1121.
[5] C.H. Choi, M. Hiromura, A. Usheva, Transcription factor IIB acetylates itself to regulate transcription, Nature 424 (2003) 965–969.
[6] A. Usheva, T. Shenk, YY1 transcriptional initiator: protein interactions and association with a DNA site containing unpaired strands, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 13571–13576.
[7] H.B. Houbaviy, A. Usheva, T. Shenk, S.K. Burley, Cocrystal structure of YY1 bound to the adeno-associated virus P5 initiator, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 13577–13582.
[8] M. Horikoshi, C. Bertuccioli, R. Takada, J. Wang, T. Yamamoto, R.G. Roeder, Transcription factor TFIID induces DNA bending upon binding to the TATA element, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 1060–1064.
[9] D.B. Nikolov, H. Chen, E.D. Halay, A.A. Usheva, K. Hisatake, D.K. Lee, R.G. Roeder, S.K. Burley, Crystal structure of a TFIIB-TBP-TATA-element ternary complex, Nature 377 (1995) 119–128.