# Supplementary Materials

## Assessing B-Z DNA Transitions in Solutions via Infrared Spectroscopy

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### 1. Spectral analysis of Z-DNA induced by NaCl

#### 1.1 Infrared spectral fitting of Z-DNA induced by NaCl

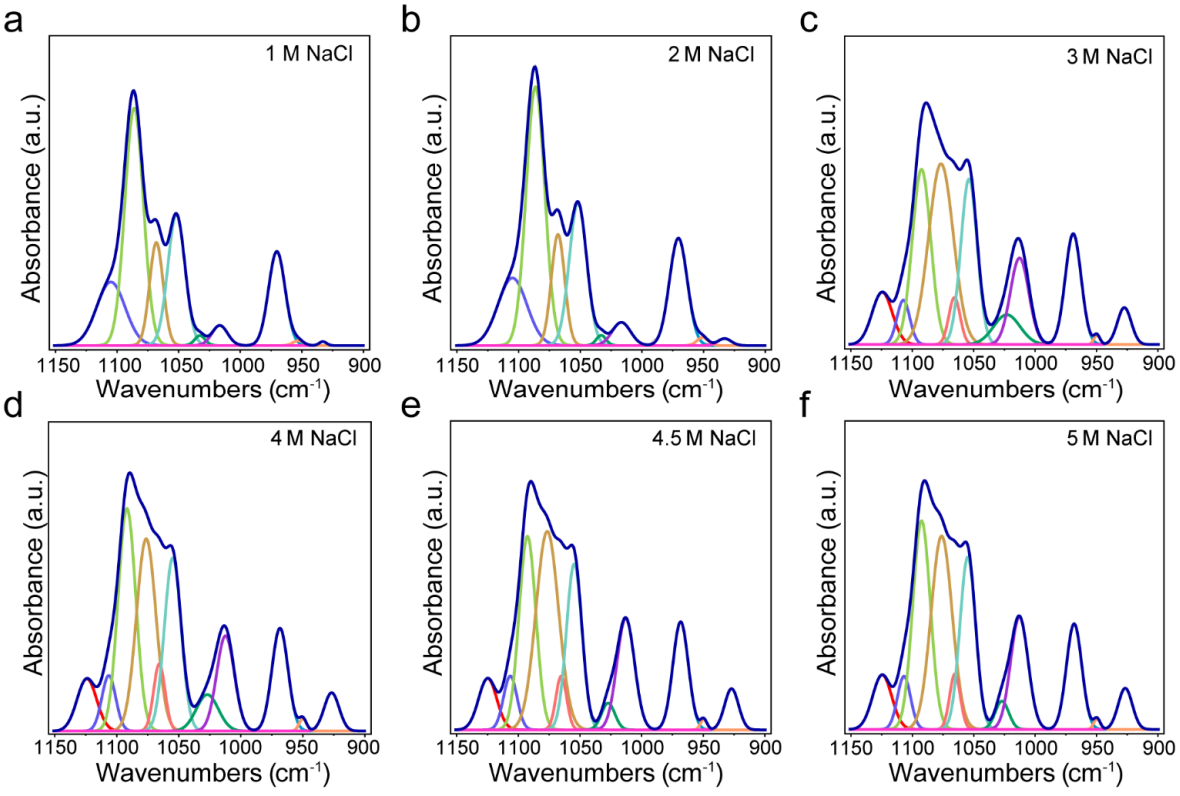



Figure S1. Fitting results of representative infrared spectra of dsDNA induced by different concentrations of NaCl in the range of 1150 $cm^{-1}$–900 $cm^{-1}$.

The second derivative processing of the infrared spectrum was carried out to obtain the peak position for peak division, and Origin software was used for Gaussian fitting of the spectrum.

Table S1. Fitting parameters of infrared spectra of dsDNA treated by NaCl.

| Peak | Fit parameters | NaCl | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 M | 2 M | 3 M | 4 M | 4.5 M | 5 M |
| 1 | $x_c$ ($cm^{-1}$) | - | - | 1124.45 | 1123.316 | 1123.259 | 1122.903 |
| | w ($cm^{-1}$) | - | - | 16.467 | 17.525 | 18.572 | 18.851 |
| | $A/A_{970}$ | - | - | 0.6 | 0.683 | 0.701 | 0.766 |
| 2 | $x_c$ ($cm^{-1}$) | 1105.049 | 1105 | 1107.211 | 1105.913 | 1105.478 | 1105.821 |
| | w ($cm^{-1}$) | 23.051 | 23.497 | 10.464 | 10.402 | 9.434 | 9.151 |
| | $A/A_{970}$ | 1.16 | 1.021 | 0.323 | 0.409 | 0.33 | 0.309 |
| 3 | $x_c$ ($cm^{-1}$) | 1086.131 | 1086.303 | 1092.437 | 1091.818 | 1092.603 | 1092.52 |
| | w ($cm^{-1}$) | 15.018 | 15.047 | 14.963 | 14.193 | 13.618 | 14.231 |
| | $A/A_{970}$ | 2.826 | 2.505 | 1.824 | 2.375 | 1.943 | 2.244 |
| 4 | $x_c$ ($cm^{-1}$) | - | - | 1076.549 | 1076.252 | 1076.527 | 1076.174 |
| | w ($cm^{-1}$) | - | - | 20.093 | 15.75 | 18.407 | 17.121 |
| | $A/A_{970}$ | - | - | 2.527 | 2.277 | 2.693 | 2.503 |
| 5 | $x_c$ ($cm^{-1}$) | 1068.116 | 1068.036 | 1065.616 | 1065.693 | 1065.217 | 1065.34 |
| | w ($cm^{-1}$) | 10.985 | 10.84 | 9.241 | 9.208 | 8.667 | 8.641 |
| | $A/A_{970}$ | 0.897 | 0.775 | 0.303 | 0.465 | 0.343 | 0.363 |
| 6 | $x_c$ ($cm^{-1}$) | 1051.827 | 1051.832 | 1053.77 | 1054.846 | 1054.675 | 1054.879 |
| | w ($cm^{-1}$) | 13.962 | 14.151 | 13.317 | 13.467 | 12.613 | 12.836 |
| | $A/A_{970}$ | 1.447 | 1.299 | 1.534 | 1.753 | 1.542 | 1.671 |
| 7 | $x_c$ ($cm^{-1}$) | 1032.77 | 1033.035 | 1023 | 1027 | 1027.153 | 1027.061 |
| | w ($cm^{-1}$) | 8.408 | 7.842 | 21.491 | 17.734 | 10.826 | 11.039 |
| | $A/A_{970}$ | 0.066 | 0.051 | 0.446 | 0.485 | 0.213 | 0.236 |
| 8 | $x_c$ ($cm^{-1}$) | 1016.647 | 1016.692 | 1012.639 | 1012.342 | 1012.88 | 1012.85 |
| | w ($cm^{-1}$) | 12.657 | 14.848 | 15.03 | 14.27 | 14.223 | 14.289 |
| | $A/A_{970}$ | 0.201 | 0.221 | 0.905 | 1.021 | 1.169 | 1.215 |
| 9 | $x_c$ ($cm^{-1}$) | 970.309 | 970.423 | 968.945 | 968.412 | 968.351 | 968.234 |
| | w ($cm^{-1}$) | 13.414 | 14.484 | 12.961 | 12.934 | 12.555 | 12.526 |
| | $A/A_{970}$ | 1 | 1 | 1 | 1 | 1 | 1 |
| 10 | $x_c$ ($cm^{-1}$) | 952.889 | 950.897 | 949.638 | 950.027 | 949.622 | 949.606 |
| | w ($cm^{-1}$) | 8.445 | 8.867 | 5.007 | 5.964 | 5.304 | 5.329 |
| | $A/A_{970}$ | 0.032 | 0.045 | 0.032 | 0.055 | 0.041 | 0.043 |
| 11 | $x_c$ ($cm^{-1}$) | 933 | 933 | 927.318 | 926.759 | 927.04 | 926.608 |
| | w ($cm^{-1}$) | 5.734 | 10.68 | 11.255 | 12.055 | 11.322 | 11.486 |
| | $A/A_{970}$ | 0.018 | 0.047 | 0.29 | 0.347 | 0.344 | 0.358 |

Note: "-" means there is no peak. $x_c$ represents the wavenumber, w represents the bandwidth, A represents the integrated intensity, and $A_{970}$ represents the internal parameter standard.

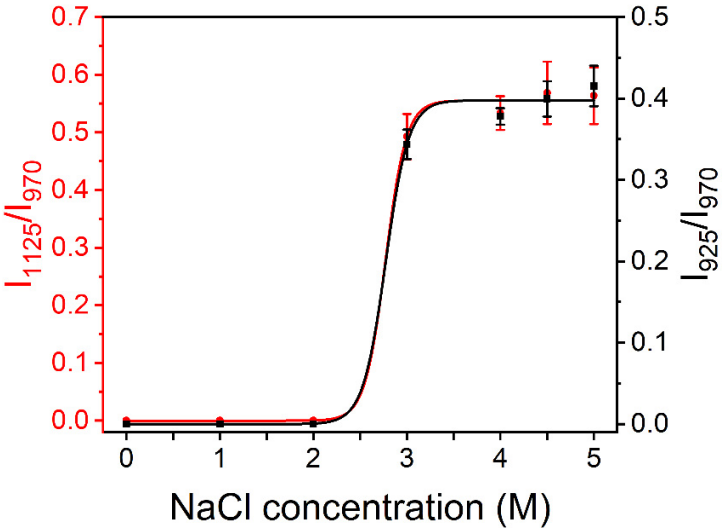



Figure S2. Changes of the ratio of Z-DNA characteristic peaks (925 $cm^{-1}$ and 1125 $cm^{-1}$) intensity to 970 $cm^{-1}$ peak intensity with NaCl treatment.

The fitting function: $y=A_2+(A_1-A_2)/(1+\exp((x-x_0)/dx))$

$I_{1125}/I_{970}$:

Reduced Chi-Sqr 2.41574E-4

Adj. R-Square 0.99712

$I_{925}/I_{970}$:

Reduced Chi-Sqr 2.33505E-4

Adj. R-Square 0.99452

### 1.2 CD spectra of DNA

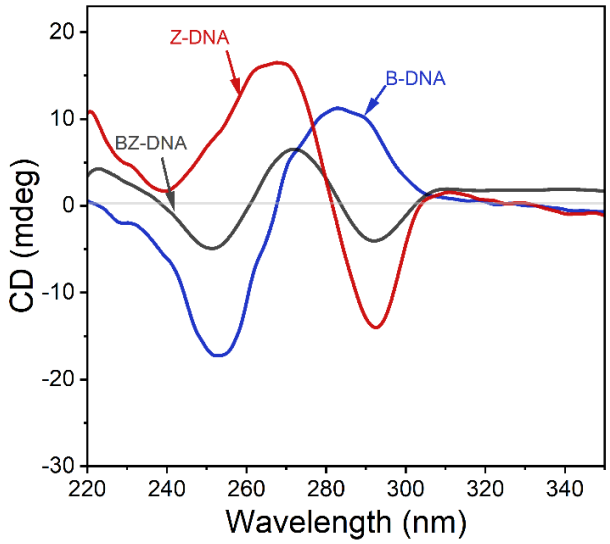



Figure S3. CD spectra for identification of $d(GC)_8$ chiral structures.

### 1.3 Analysis of Z-DNA induced by NaCl through ultraviolet spectroscopy

The instrument used in this work is NanoDrop 2000 ultramicro spectrophotometer (Thermo Scientific, USA). The detection temperature was at room temperature, and ultrapure water was used as a blank control. All nucleic acid samples were kept at the same concentration during detection, and each treatment was set up in triplicate.

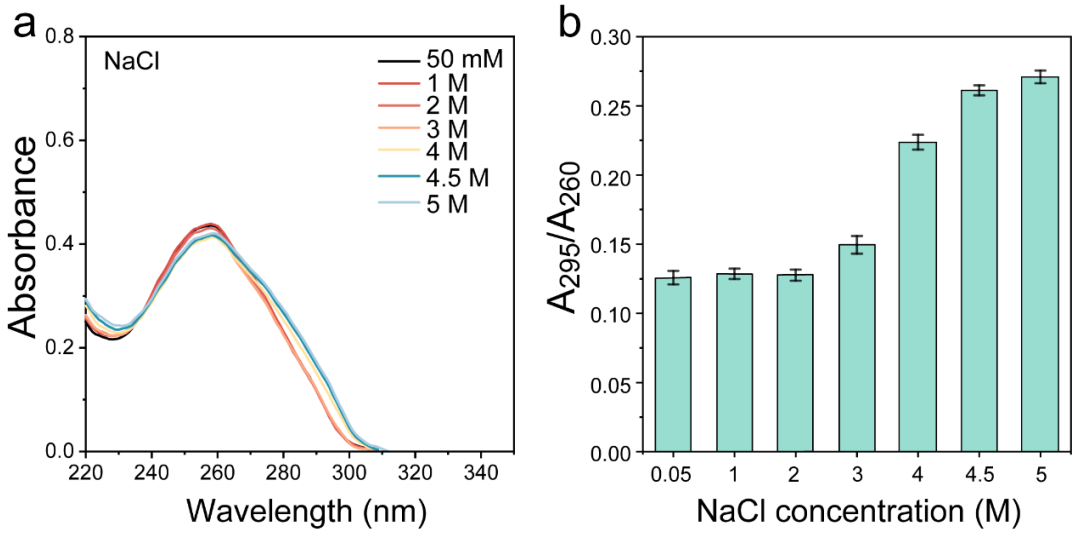



Figure S4. UV analysis of d(GC)$_8$ dsDNA treated with different concentrations of NaCl. a) UV absorption spectra of DNA treated with different concentrations of NaCl; b) Analysis of $A_{295}/A_{260}$ of DNA UV absorption after different NaCl treatments.

## 2. Spectral analysis of Z-DNA induced by $MgCl_2$

### 2.1 Infrared spectral fitting of Z-DNA induced by $MgCl_2$

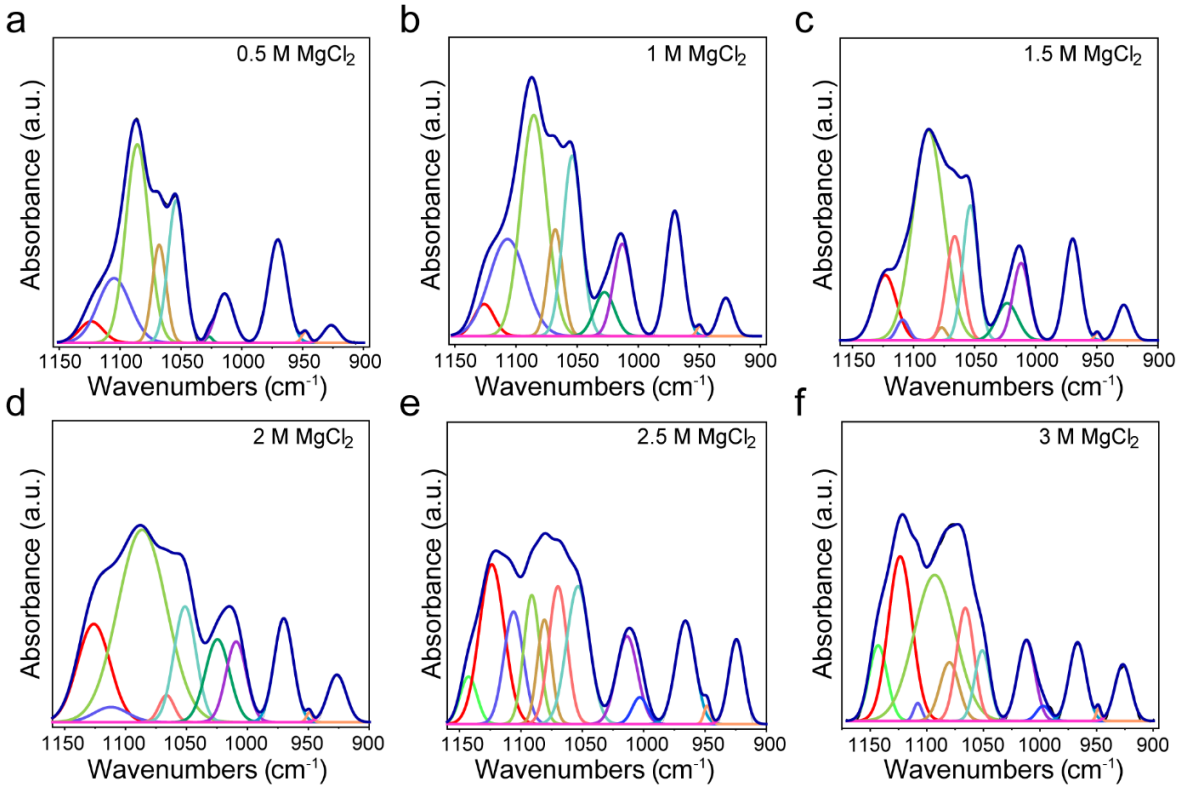



Figure S5. Fitting results of representative infrared spectra of dsDNA induced by different concentrations of $MgCl_2$ in the range of 1150 $cm^{-1}$–900 $cm^{-1}$.

The second derivative processing of the infrared spectrum was carried out to obtain the peak position for peak division, and Origin software was used for Gaussian fitting of the spectrum.

Table S2. Fitting parameters of infrared spectra of dsDNA treated by $MgCl_2$.

| Peak | Fit parameters | $MgCl_2$ | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0.5 M | 1 M | 1.5 M | 2 M | 2.5 M | 3 M |
| 1 | $x_c$ ($cm^{-1}$) | - | - | - | - | 1142.614 | 1143 |
| | w ($cm^{-1}$) | - | - | - | - | 13.75 | 16.027 |
| | $A/A_{970}$ | - | - | - | - | 0.394 | 1.043 |
| 2 | $x_c$ ($cm^{-1}$) | 1123.766 | 1126.134 | 1123.226 | 1126.13 | 1123.653 | 1124.424 |
| | w ($cm^{-1}$) | 19.424 | 16.437 | 18.283 | 26.775 | 20.321 | 19.839 |
| | $A/A_{970}$ | 0.264 | 0.309 | 0.910 | 1.618 | 1.961 | 2.76 |
| 3 | $x_c$ ($cm^{-1}$) | 1104.985 | 1107.029 | 1108.885 | 1112 | 1105.96 | 1109.614 |
| | w ($cm^{-1}$) | 26.459 | 29.754 | 9.82 | 27.679 | 15.853 | 12.196 |
| | $A/A_{970}$ | 1.087 | 1.697 | 0.154 | 0.257 | 1.078 | 0.318 |
| 4 | $x_c$ ($cm^{-1}$) | 1085.858 | 1085.499 | 1087.929 | 1086.381 | 1091.117 | 1093 |
| | w ($cm^{-1}$) | 19.044 | 21.274 | 25.115 | 40.011 | 13.095 | 36.437 |
| | $A/A_{970}$ | 2.404 | 2.765 | 4.028 | 4.739 | 1.021 | 4.579 |
| 5 | $x_c$ ($cm^{-1}$) | - | - | - | - | 1080.84 | 1080 |
| | w ($cm^{-1}$) | - | - | - | - | 11.511 | 16.843 |
| | $A/A_{970}$ | - | - | - | - | 0.726 | 0.851 |
| 6 | $x_c$ ($cm^{-1}$) | - | - | 1077 | - | - | - |
| | w ($cm^{-1}$) | - | - | 7.849 | - | - | - |
| | $A/A_{970}$ | - | - | 0.077 | - | - | - |
| 7 | $x_c$ ($cm^{-1}$) | 1067.9 | 1067.9 | 1066.324 | 1066.374 | 1069.841 | 1066 |
| | w ($cm^{-1}$) | 11.414 | 12.135 | 13.44 | 12.341 | 14.875 | 15.466 |
| | $A/A_{970}$ | 0.711 | 0.761 | 1.068 | 0.205 | 1.239 | 1.505 |
| 8 | $x_c$ ($cm^{-1}$) | 1053.82 | 1053.92 | 1053.438 | 1051.199 | 1053.453 | 1051 |
| | w ($cm^{-1}$) | 13.667 | 15.207 | 12.591 | 18.187 | 18.555 | 13.472 |
| | $A/A_{970}$ | 1.238 | 1.613 | 1.295 | 1.295 | 1.546 | 0.818 |
| 9 | $x_c$ ($cm^{-1}$) | 1026.9 | 1027.608 | 1023.372 | 1024.565 | 1013.615 | 1012.503 |
| | w ($cm^{-1}$) | 5.211 | 16.463 | 17.243 | 19.875 | 17.104 | 14.843 |
| | $A/A_{970}$ | 0.02 | 0.425 | 0.49 | 1.014 | 0.908 | 1.008 |
| 10 | $x_c$ ($cm^{-1}$) | 1014.151 | 1012.907 | 1011.96 | 1009.194 | 1003.179 | 998.663 |
| | w ($cm^{-1}$) | 14.418 | 14.721 | 13.536 | 16.722 | 12.398 | 13.075 |
| | $A/A_{970}$ | 0.451 | 0.799 | 0.798 | 0.83 | 0.2 | 0.201 |
| 11 | $x_c$ ($cm^{-1}$) | 970.383 | 970.119 | 969.745 | 970.141 | 966.02 | 966.812 |
| | w ($cm^{-1}$) | 15.234 | 13.569 | 12.888 | 15.636 | 16.005 | 14.749 |
| | $A/A_{970}$ | 1 | 1 | 1 | 1 | 1 | 1 |
| 12 | $x_c$ ($cm^{-1}$) | 947.6465 | 949.619 | 949.524 | 949.043 | 948.287 | 948.415 |
| | w ($cm^{-1}$) | 6.06902 | 5.107 | 4.547 | 4.846 | 5.4154 | 4.13 |
| | $A/A_{970}$ | 0.042 | 0.03 | 0.028 | 0.029 | 0.06 | 0.043 |
| 13 | $x_c$ ($cm^{-1}$) | 926.462 | 928.282 | 927.961 | 926.458 | 924.507 | 926.764 |
| | w ($cm^{-1}$) | 12.535 | 11.673 | 11.562 | 15.129 | 12.435 | 14.199 |
| | $A/A_{970}$ | 0.14 | 0.262 | 0.314 | 0.442 | 0.638 | 0.693 |

Note: "-" means there is no peak. $x_c$ represents the wavenumber, w represents the bandwidth, A represents the integrated intensity, and $A_{970}$ represents the internal parameter standard.

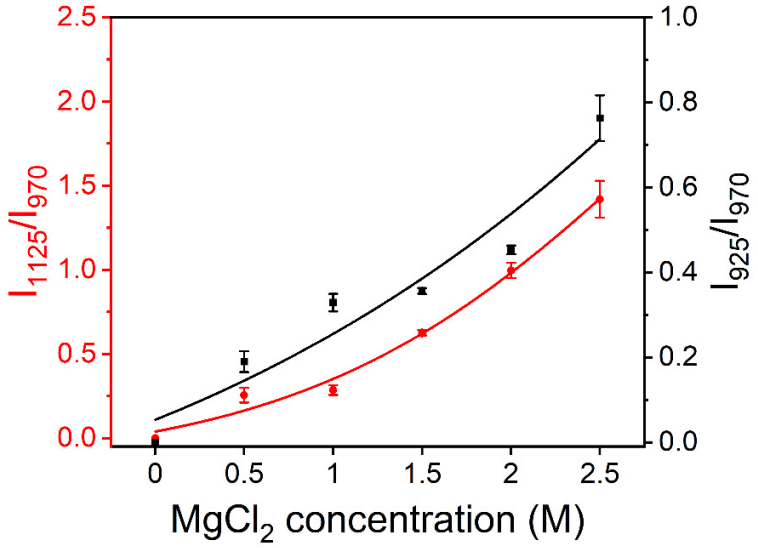



Figure S6. Changes of the ratio of the Z-DNA characteristic peaks (925 $cm^{-1}$ and 1125 $cm^{-1}$) intensity to 970 $cm^{-1}$ peak intensity after $MgCl_2$ treatment.

The fitting function: $y=A_2+(A_1-A_2)/(1+\exp((x-x_0)/dx))$

$I_{1125}/I_{970}$:

Reduced Chi-Sqr 0.00293

Adj. R-Square 0.98956

$I_{925}/I_{970}$:

Reduced Chi-Sqr 0.00413

Adj. R-Square 0.93731

**2.2 Analysis of Z-DNA induced by $MgCl_2$ through ultraviolet spectroscopy**

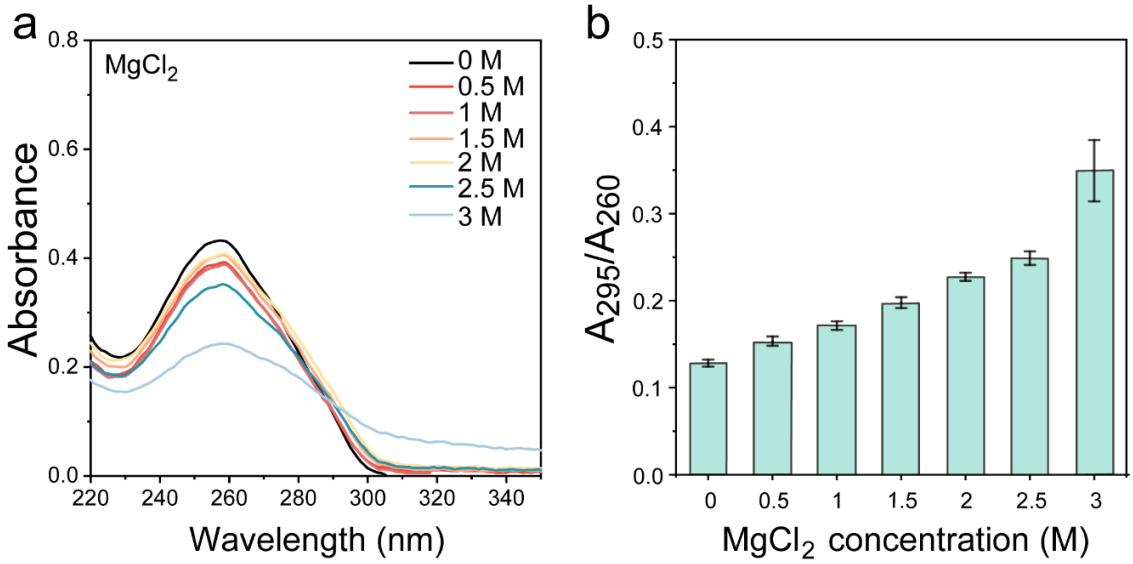



Figure S7. UV analysis of $d(GC)_8$ dsDNA treated with different concentrations of $MgCl_2$. a) The UV absorption spectra of DNA treated with different concentrations of $MgCl_2$; b) The analysis of $A_{295}/A_{260}$ of DNA UV absorption after different concentrations of $MgCl_2$ treatments.

## 3. Spectral analysis of Z-DNA induced by ethanol

### 3.1 Infrared spectral fitting of Z-DNA induced by ethanol

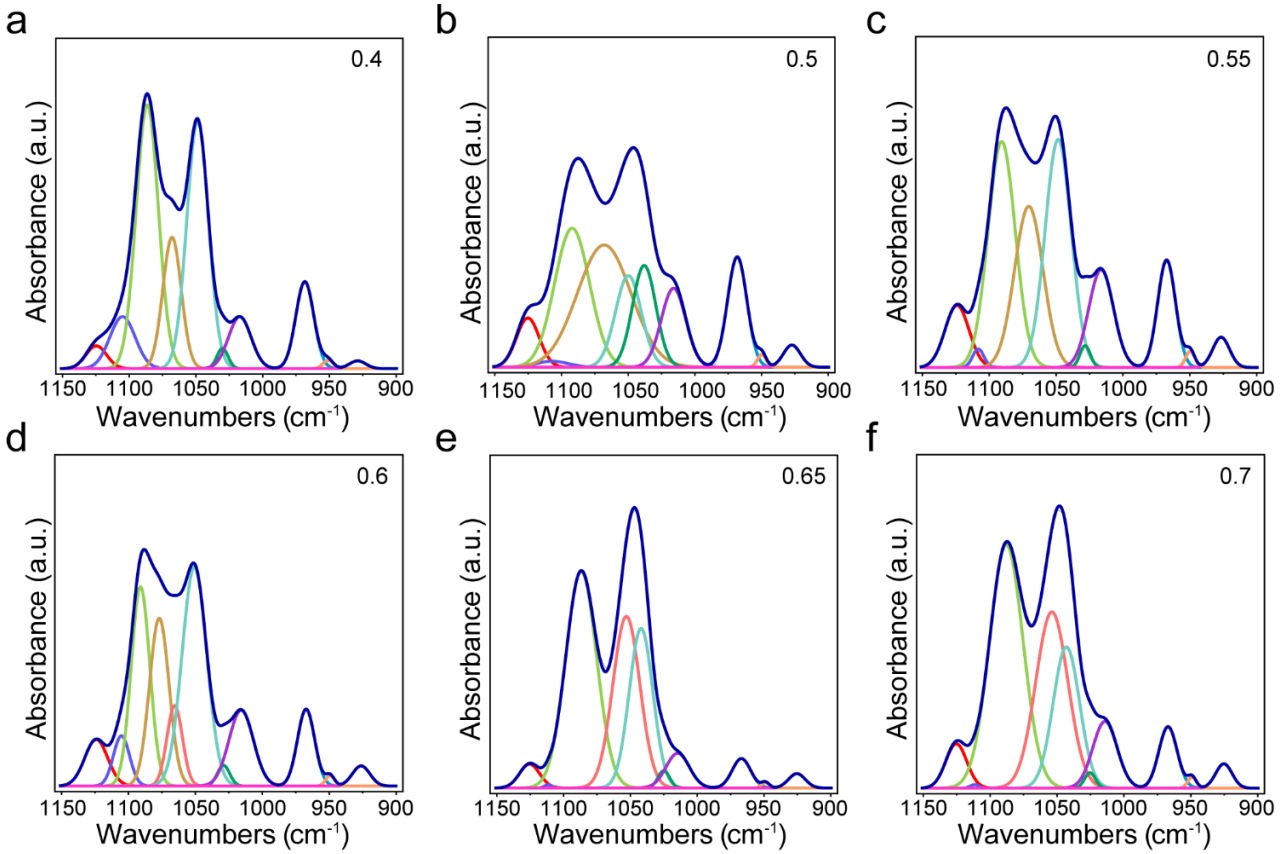



Figure S8. Fitting results of representative infrared spectra of dsDNA induced by ethanol of different volume ratios in the range of 1150 $cm^{-1}$–900 $cm^{-1}$.

The second derivative processing of the infrared spectrum was carried out to obtain the peak position for peak division, and Origin software was used for Gaussian fitting of the spectrum.

Table S3. Fitting parameters of infrared spectra of dsDNA treated by ethanol.

| Peak | Fit parameters | Ethanol (v/v) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0.4 | 0.5 | 0.55 | 0.6 | 0.65 | 0.7 |
| 1 | $x_c$ ($cm^{-1}$) | 1124.66 | 1125.202 | 1124.092 | 1124.079 | 1125.497 | 1125.302 |
| | w ($cm^{-1}$) | 15.449 | 17.054 | 17.969 | 17.121 | 15.064 | 15.749 |
| | $A/A_{970}$ | 0.299 | 0.522 | 0.774 | 0.805 | 0.891 | 0.831 |
| 2 | $x_c$ ($cm^{-1}$) | 1105 | 1107 | 1108.202 | 1105.225 | 1111.61 | 1111.529 |
| | w ($cm^{-1}$) | 18.999 | 24.146 | 8.361 | 12.11 | 5.309 | 6.327 |
| | $A/A_{970}$ | 0.842 | 0.089 | 0.107 | 0.613 | 0.042 | 0.026 |
| 3 | $x_c$ ($cm^{-1}$) | 1086.43 | 1092.057 | 1090.704 | 1090.976 | 1086.835 | 1087.909 |
| | w ($cm^{-1}$) | 16.686 | 26.183 | 20.333 | 13.916 | 23.117 | 25.075 |
| | $A/A_{970}$ | 3.751 | 2.264 | 3.187 | 2.793 | 12.406 | 7.254 |
| 4 | $x_c$ ($cm^{-1}$) | 1067.9 | 1068 | 1070.411 | 1076.965 | - | - |
| | w ($cm^{-1}$) | 13.779 | 40.237 | 21.317 | 14.643 | - | - |
| | $A/A_{970}$ | 1.543 | 3.059 | 2.384 | 2.478 | - | - |
| 5 | $x_c$ ($cm^{-1}$) | - | - | - | 1065.447 | 1053 | 1053.863 |
| | w ($cm^{-1}$) | - | - | - | 11.561 | 20.199 | 24.034 |
| | $A/A_{970}$ | - | - | - | 0.937 | 8.579 | 5.037 |
| 6 | $x_c$ ($cm^{-1}$) | 1048.692 | 1049.844 | 1048.442 | 1050.697 | 1042 | 1043 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | w ($cm^{-1}$) | 16.214 | 19.387 | 19.472 | 18.468 | 17.684 | 19.763 |
| | $A/A_{970}$ | 3.418 | 1.104 | 3.08 | 4.079 | 6.985 | 3.316 |
| 7 | $x_c$ ($cm^{-1}$) | 1025.507 | 1038 | 1028.281 | 1029.015 | 1025.037 | 1025.388 |
| | w ($cm^{-1}$) | 14.116 | 17.543 | 7.74 | 9.235 | 8.747 | 8.046 |
| | $A/A_{970}$ | 0.479 | 1.11 | 0.117 | 0.194 | 0.374 | 0.147 |
| 8 | $x_c$ ($cm^{-1}$) | 1012.857 | 1015.997 | 1016.323 | 1015.766 | 1014.601 | 1014.182 |
| | w ($cm^{-1}$) | 12.858 | 18.366 | 18.476 | 18.267 | 17.438 | 18.205 |
| | $A/A_{970}$ | 0.415 | 0.902 | 1.254 | 1.399 | 1.500 | 1.445 |
| 9 | $x_c$ ($cm^{-1}$) | 968.406 | 968.225 | 967.351 | 967.261 | 966.883 | 967.056 |
| | w ($cm^{-1}$) | 13.476 | 14.566 | 13.402 | 12.901 | 13.73 | 13.675 |
| | $A/A_{970}$ | 1 | 1 | 1 | 1 | 1 | 1 |
| 10 | $x_c$ ($cm^{-1}$) | 950.93 | 949.809 | 949.781 | 949.372 | 948.854 | 949.188 |
| | w ($cm^{-1}$) | 7.774 | 6.54 | 6.878 | 6.57 | 5.74 | 6.371 |
| | $A/A_{970}$ | 0.055 | 0.051 | 0.077 | 0.069 | 0.078 | 0.083 |
| 11 | $x_c$ ($cm^{-1}$) | 928.878 | 927.26 | 926.609 | 926.156 | 925.368 | 925.324 |
| | w ($cm^{-1}$) | 12.269 | 12.771 | 12.565 | 13.089 | 12.531 | 12.236 |
| | $A/A_{970}$ | 0.081 | 0.176 | 0.264 | 0.266 | 0.445 | 0.355 |

Note: "-" means there is no peak. $x_c$ represents the wavenumber, w represents the bandwidth, A represents the integrated intensity, and $A_{970}$ represents the internal parameter standard.

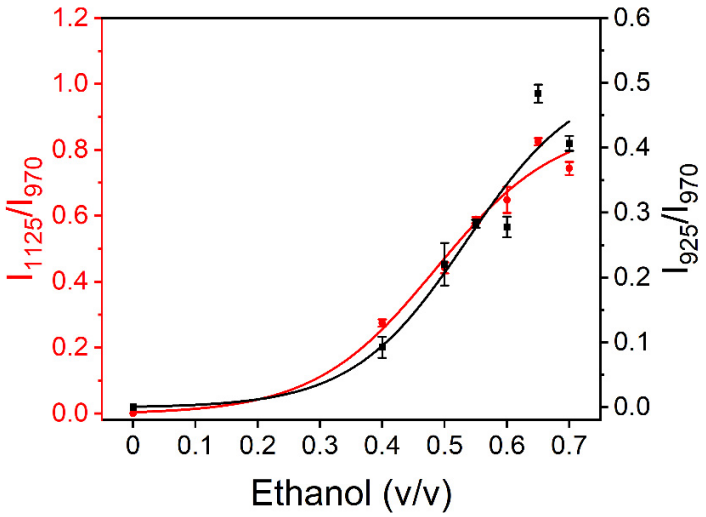



Figure S9. Changes of the ratio of the Z-DNA characteristic peaks (925 $cm^{-1}$ and 1125 $cm^{-1}$) intensity to 970 $cm^{-1}$ peak intensity after ethanol treatment.

The fitting function: $y=A_2+(A_1-A_2)/(1+\exp((x-x_0)/dx))$

$I_{1125}/I_{970}$:

Reduced Chi-Sqr 0.00182

Adj. R-Square 0.97806

$I_{925}/I_{970}$:

Reduced Chi-Sqr 0.00213

Adj. R-Square 0.92453

### 3.2 Analysis of Z-DNA induced by ethanol through ultraviolet spectroscopy

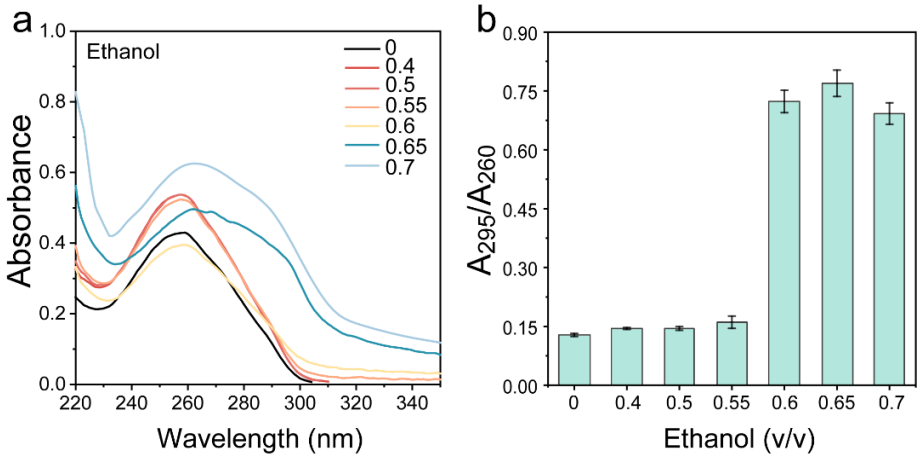



Figure S10. UV analysis of d(GC)$_8$ dsDNA after ethanol treatment at different volume ratios. a) The UV absorption spectra of DNA treated with different volume ratios of ethanol; b) The analysis of $A_{295}/A_{260}$ of DNA UV absorption after different ethanol treatments.

## 4. Changes analysis of 1016 cm$^{-1}$ peak in FTIR spectra

In addition to the Z-DNA characteristic peaks of 1125 cm$^{-1}$and 925 cm$^{-1}$, furanose 1016 cm$^{-1}$ also changed with the concentration changes of inducers.

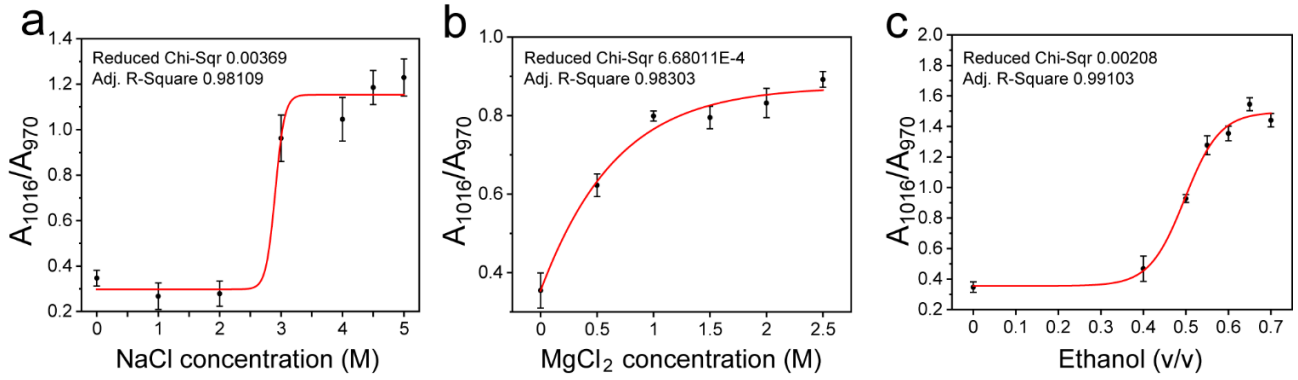



Figure S11. The ratio changes of 1016 cm$^{-1}$ peak area to 970 cm$^{-1}$ peak area with the concentration of inducers (data were supplied in the spectral fitting). a) The changes of $A_{1016}/A_{970}$ of d(GC)$_8$ dsDNA induced by NaCl; b) The changes of $A_{1016}/A_{970}$ of d(GC)$_8$ dsDNA induced by $MgCl_2$; c) The changes of $A_{1016}/A_{970}$ of d(GC)$_8$ dsDNA induced by ethanol. The fitting function: $y=A_2+(A_1-A_2)/(1+\exp((x-x_0)/dx))$

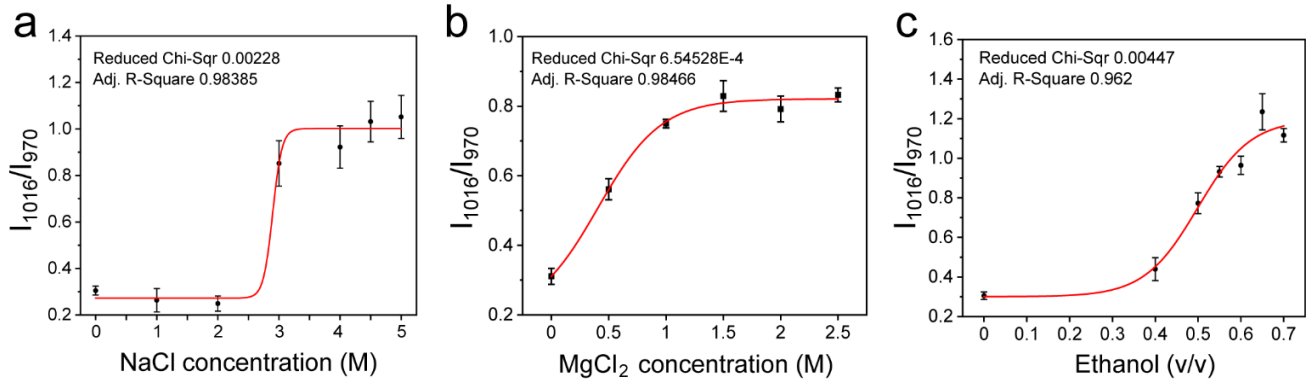



Figure S12. The ratio changes of 1016 cm$^{-1}$ peak intensity to 970 cm$^{-1}$ peak intensity with the concentration of inducers (data were supplied in the spectral fitting). a) The changes of $I_{1016}/I_{970}$ of d(GC)$_8$ dsDNA induced by NaCl; b) The changes of $I_{1016}/I_{970}$ of d(GC)$_8$ dsDNA induced by $MgCl_2$; c) The changes of $I_{1016}/I_{970}$ of d(GC)$_8$ dsDNA induced by ethanol. The fitting function: $y=A_2+(A_1-A_2)/(1+\exp((x-x_0)/dx))$

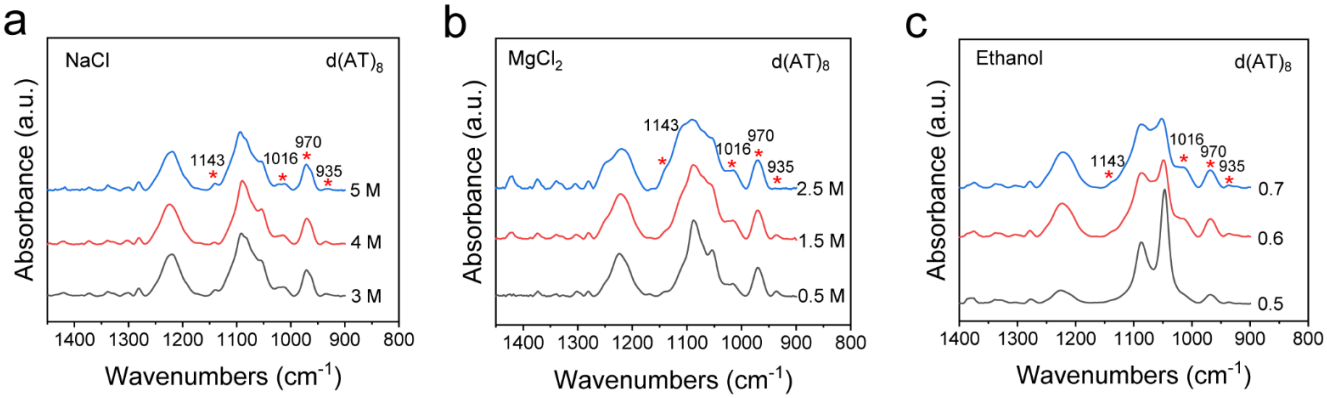



Figure S13. $d(AT)_8$ infrared spectra under different induction conditions. a) FTIR spectra of $d(AT)_8$ dsDNA treated with different concentrations of NaCl; b) FTIR spectra of $d(AT)_8$ dsDNA treated with different concentrations of $MgCl_2$; c) FTIR spectra of $d(AT)_8$ dsDNA treated with different volume ratios of ethanol.