Supplementary Materials for

Correlation of Local Effects of DNA Sequence and Position of Beta-Alanine Inserts with Polyamide-DNA Complex Binding Affinities and Kinetics

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Materials and Methods

Chemical Characterization of KA1055, dImImPyPyγPyImβPyβDp

HPLC/Mass Spectrometry (HRMS) (MS^n ESI^+) calculated for $C_{54}H_{70}N_{21}O_{10}$ MH^+ 1172.56145; found 1172.55845. Elemental analysis calculated for KA1055·3TFA·7H_2O, $C_{60}H_{86}N_{21}O_{23}F_9$: C, 43.93%; H, 5.28%; F, 10.42; N, 17.93%; found: C, 44.20%; H, 5.21%; F, 10.00; N, 17.92%.

NMR conditions and descriptions: 500 or 600 MHz referenced to residual solvent, DMSO-d^6 or MeOH-d^4, temp (500 MHz = 27 °C, 600 MHz = 25°C), ex. indicate protons exchangeable in MeOH-d^4 or D_2O.

^{1}H NMR (600 MHz, DMSO-d^6) 10.33 (s, 1H, ex.), 10.32 (s, 1H, ex.), 9.93 (s, 1H, ex.), 9.88 (s, 1H, ex.), 9.83 (s, 1H, ex.), 9.75 (s, 1H, ex.), 9.23 (brs, 1H, ex.), 8.03 (m, 3H, ex.), 7.92 (t, J=5.9Hz, 1H, ex), 7.58 (s, 1H), 7.50 (s, 1H), 7.47 (d, J=0.5Hz, 1H), 7.282 (d, J=1.8Hz, 1H), 2.276 (d, J=1.8Hz, 1H), 7.18 (d, J=1.7Hz, 1H), 7.15 (d, J=1.8Hz, 1H), 7.10 (d, J=1.7Hz, 1H), 7.09 (d, J=1.2Hz, 1H), 6.91 (d, J=1.8Hz, 2H), 6.90 (d, J=1.8Hz, 2H), 6.67 (d, J=2.3Hz, 1H**),** 4.016 (s, 3H)**,** 4.014 (s, 3H), 3.94 (s, 3H), 3.85 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H), 3.78 (s, 3H), 3.52 (m, 2H), 3.36 (m, 2H), 3.21 (m, 2H), 3.10 (m, 2H), 2.99 (m, 2H), 2.73 (d, J=4.7Hz, 6H), 2.52 (t, J=6.4Hz, 2H), 2.33 (t, J=7.0Hz, 2H), 2.28 (t, J=7.1Hz, 2H), 1.79 (m, 2H), 1.73 (m, 2H).

^{1}H NMR (500 MHz, DMSO-d^6) 10.35 (s, 1H, ex.), 10.33 (s, 1H, ex.), 9.94 (s, 1H, ex.), 9.89 (s, 1H, ex.), 9.84 (s, 1H, ex.), 9.74 (s, 1H, ex.), 9.23 (brs, 1H, ex.), 8.04 (m, 3H, ex.), 7.92 (t, J=6.0Hz, 1H, ex.), 7.58 (s, 1H), 7.50 (s, 1H), 7.47 (d, J=1.0Hz, 1H), 7.284 (d, J=1.9H, 1H), 7.281 (d, J=1.6Hz, 1H), 7.19 (d, J=1.9Hz, 1H), 7.16 (d, J=1.9Hz, 1H), 7.11 (d, J=1.9Hz, 1H), 7.09 (d,

J=1.0Hz, 1H), 6.91 (d, J=1.9Hz, 2H), 6.90 (d, J=1.9Hz, 2H), 6.67 (d, J=1.9Hz, 1H**),** 4.014 (s, 3H)**,** 4.012 (s, 3H), 3.94 (s, 3H), 3.85 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H), 3.78 (s, 3H), 3.52 (m, 2H), 3.36 (m, 2H), 3.21 (m, 2H), 3.10 (m, 2H), 2.99 (m, 2H), 2.73 (d, J=4.7Hz, 6H), 2.33 (t, J=7.3Hz, 2H), 2.28 (t, J=7.3Hz, 2H), 1.78 (m, 2H), 1.72 (m, 2H).

^{1}H NMR (500 MHz, DMSO-d^6 plus D_2O) 7.57 (s, 1H), 7.48 (s, 1H), 7.45 (d, J=0.7Hz, 1H), 7.27 (d, J=1.6Hz, 1H), 7.26 (d, J=1.9Hz, 1H), 7.17 (d, J=1.9Hz, 1H), 7.13 (d, J=1.9Hz, 1H), 7.10 (d, J=1.0Hz, 1H), 7.09 (d, J=1.9Hz, 1H), 6.90 (d, J=1.9Hz, 2H), 6.89 (d, J=1.9Hz, 2H), 6.66 (d, J=1.9Hz, 1H**),** 4.003 (s, 3H)**,** 3.999 (s, 3H), 3.93 (s, 3H), 3.84 (s, 3H), 3.81 (s, 3H), 3.80 (s, 3H), 3.76 (s, 3H), 3.35 (m, 2H), 3.20 (m, 2H), 3.09 (m, 2H), 2.98 (m, 2H), 2.72 (s, 6H), 2.33 (t, J=7.0Hz, 2H), 2.28 (t, J=7.3Hz, 2H), 1.78 (m, 2H), 1.72 (m, 2H).

^{1}H NMR (500 MHz, MeOH-d^4) 7.53 (s, 1H), 7.36 (s, 1H), 7.31 (d, J=1.0Hz, 1H), 7.27 (d, J=1.9Hz, 1H), 7.15 (d, J=1.6Hz, 1H), 7.12 (d, J=1.0Hz, 1H), 7.08 (d, J=1.9Hz, 1H), 7.04 (d, J=1.9Hz, 1H), 6.92 (d, J=1.9Hz, 1H), 6.87 (d, J=1.9Hz, 1H), 6.79 (d, J=1.9Hz, 1H), 6.72 (d, J=1.9Hz, 1H), 4.08 (s, 6H), 3.97 (s, 3H), 3.91 (s, 3H), 3.87 (s, 3H), 3.85 (s, 3H), 3.82 (s, 3H), 3.62 (t, J=6.6 Hz, 2H), 3.55 (t, J=6.6Hz, 2H), 3.40 (t, J=6.6Hz, 2H), 3.25 (t, J=6.6Hz, 2H), 3.06 (m, 2H), 2.82 (s, 6H), 2.59 (t, J=6.5Hz, 2H), 2.47 (t, J=6.6Hz, 2H), 2.41 (t, J=7.0Hz, 2H), 1.98 (m, 2H), 1.86 (m, 2H).

Reverse Phase HPLC/mass spectrometry and NMR data are shown for KA1055 (Figures S1-9): Agilent 1100 HPLC; MSD ESI^+ and diode array detection; column: 70 × 4.6 mm Macherey-

Nagel Nucleodur C18 column; 5µ particles; gradient: 5-60% MeCN/water with 0.1% formic acid at 0.5 mL/min; retention time 11.3 min (300 nm).

Figure Legends

Figure S1. HPLC/MS of KA1055: UV/Vis traces at 254, 300 and 220 nm, Total Ion Current (TIC).

Figure S2. HPLC/MS of KA1055: Area % at 300 nm

Figure S3. Mass spectrum of KA1055 from ESI^+ HPLC/MS showing mono- and di-protonated species, $[M+H]^+$ and $[M+2H]^{2+}$.

Figure S4. Expansion of the mass spectrum of KA1055 for $[M+H]^+$.

Figure S5. Further expansion of the mass spectrum of KA1055 for $[M+H]^+$.

Figure S6. 1H NMR (600 MHz, DMSO-d^6) of KA1055.

Figure S7. 1H NMR (500 MHz, DMSO-d^6) of KA1055.

Figure S8. 1H NMR (500 MHz, DMSO-d^6 plus D_20) of KA1055.

Figure S9. 1H NMR (500 MHz, MeOH-d^4) of KA1055.

Figure S10. Thermal melting curves of the DNA hairpin duplex containing the TGGCTT site in the absence and presence of KA1002, KA1007, KA1055 and KA1039 at 0.5:1, 1:1, 1.5:1 and 2:1 molar ratios of compound to DNA.

Figure S11. Mutant DNA sequences of TGGCTT with the hairpin loop underlined.

Figure S12. Models of PA binding induced DNA bending. (A) X-ray crystal structure of cyclic eight-ring PA bound to 5'-CCAGTACTGG-3' (PDB 3OMJ, ref. 39.) (B) Docking models of B-

DNA, Glucocorticoid receptor DNA-binding domain (GRDBD)+ DNA, PA+ DNA and PA+ DNA+ GRDBD based on the X-ray crystal structures PDB 3G6P and PDB 3OMJ (ref. 41).

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Different Inj Volume from Sequence !     Actual Inj Volume : 5.000 µl
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Sample Info     : lyophilized combined lots of PA#3


Additional Info : Peak(s) manually integrated
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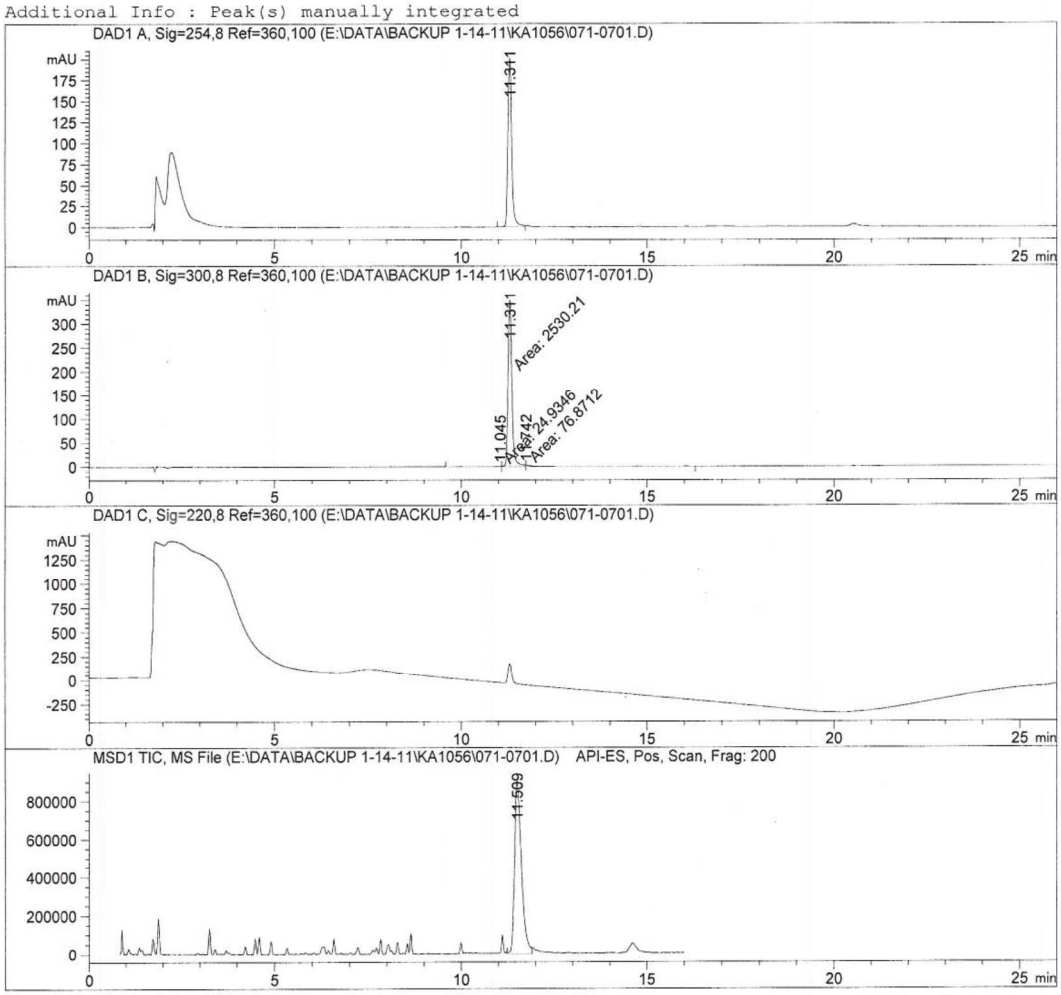


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Figure S1

Area Percent Report

Sorted By : Signal
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Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=254,8 Ref=360,100

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Totals :				1426.93359	200.44853	

Signal 2: DAD1 B, Sig=300,8 Ref=360,100

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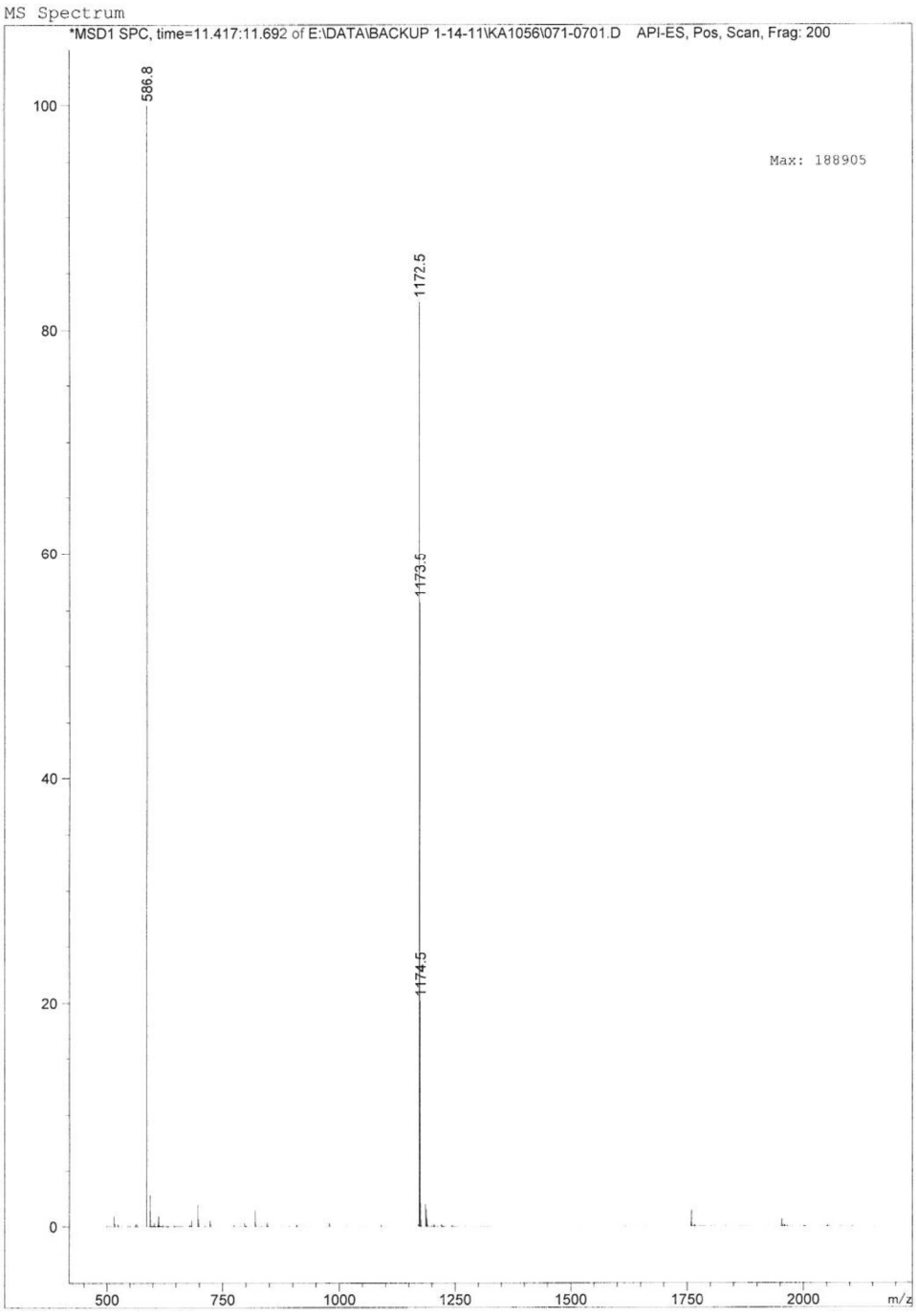
Totals :				2632.01349	354.39186	

Figure S2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1172.5
1173.5
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20
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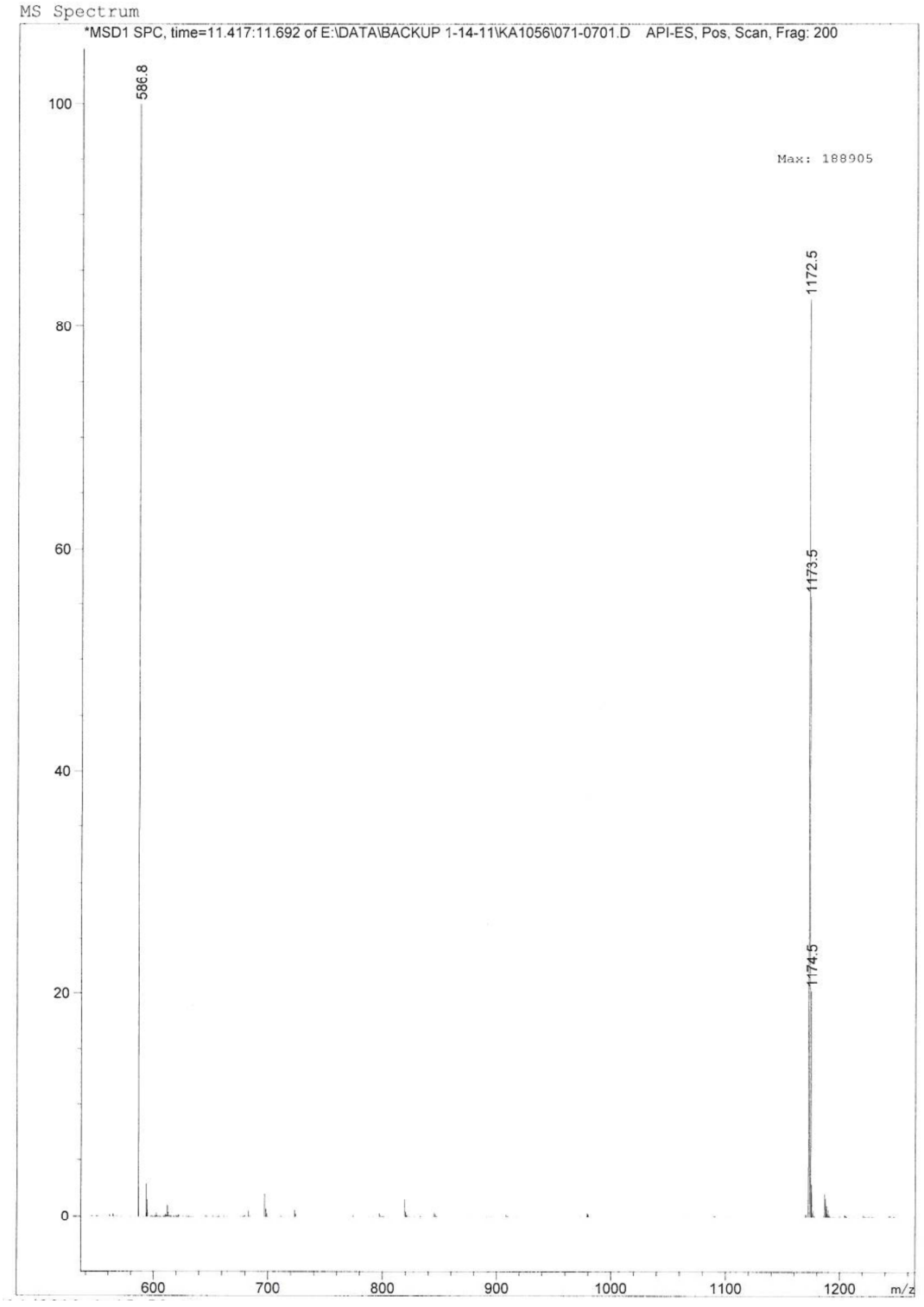
1750
2000
m/z

Figure S3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Max: 188905
1172.5
1173.5
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20
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800
900
1000
1100
1200
m/z

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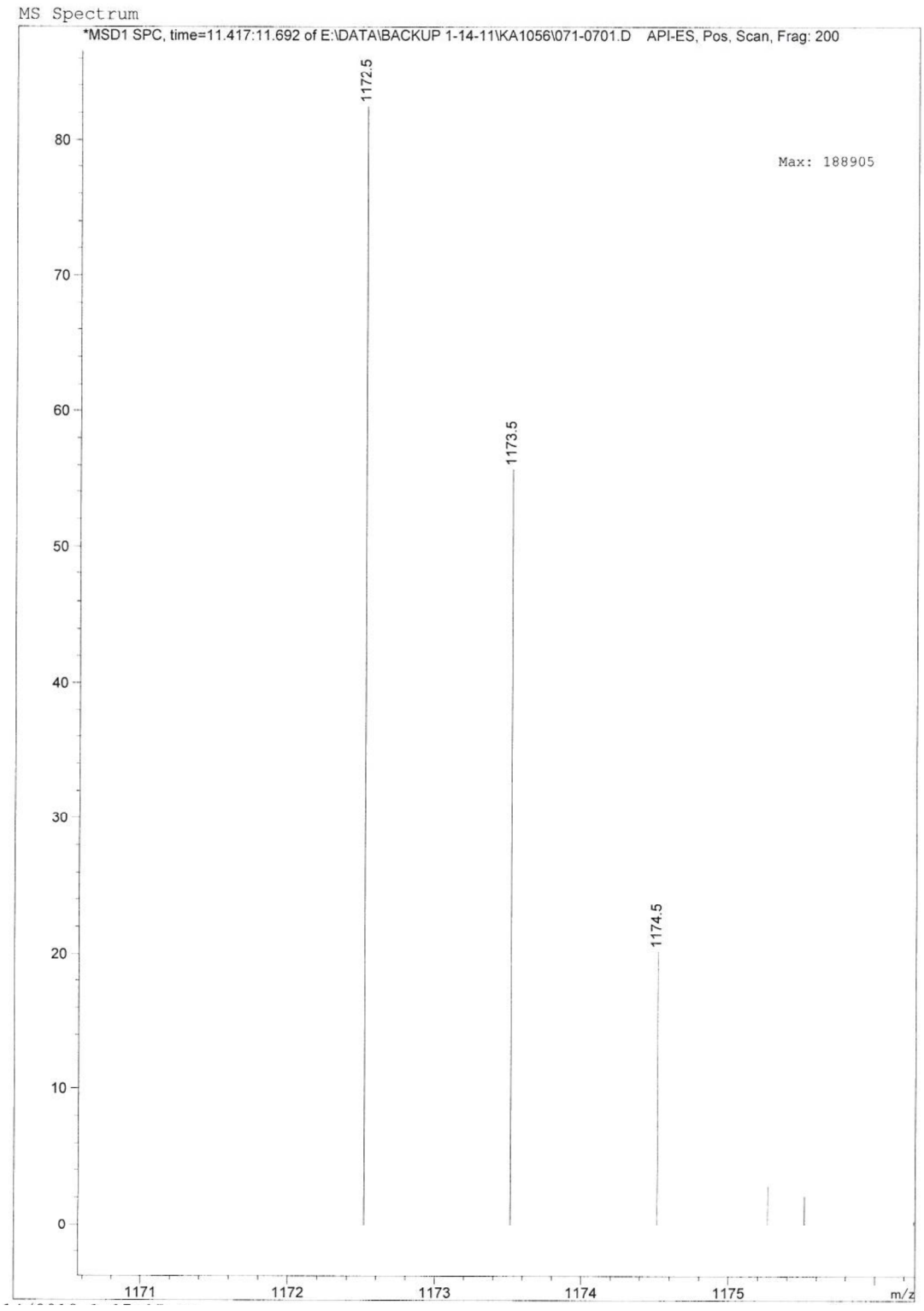
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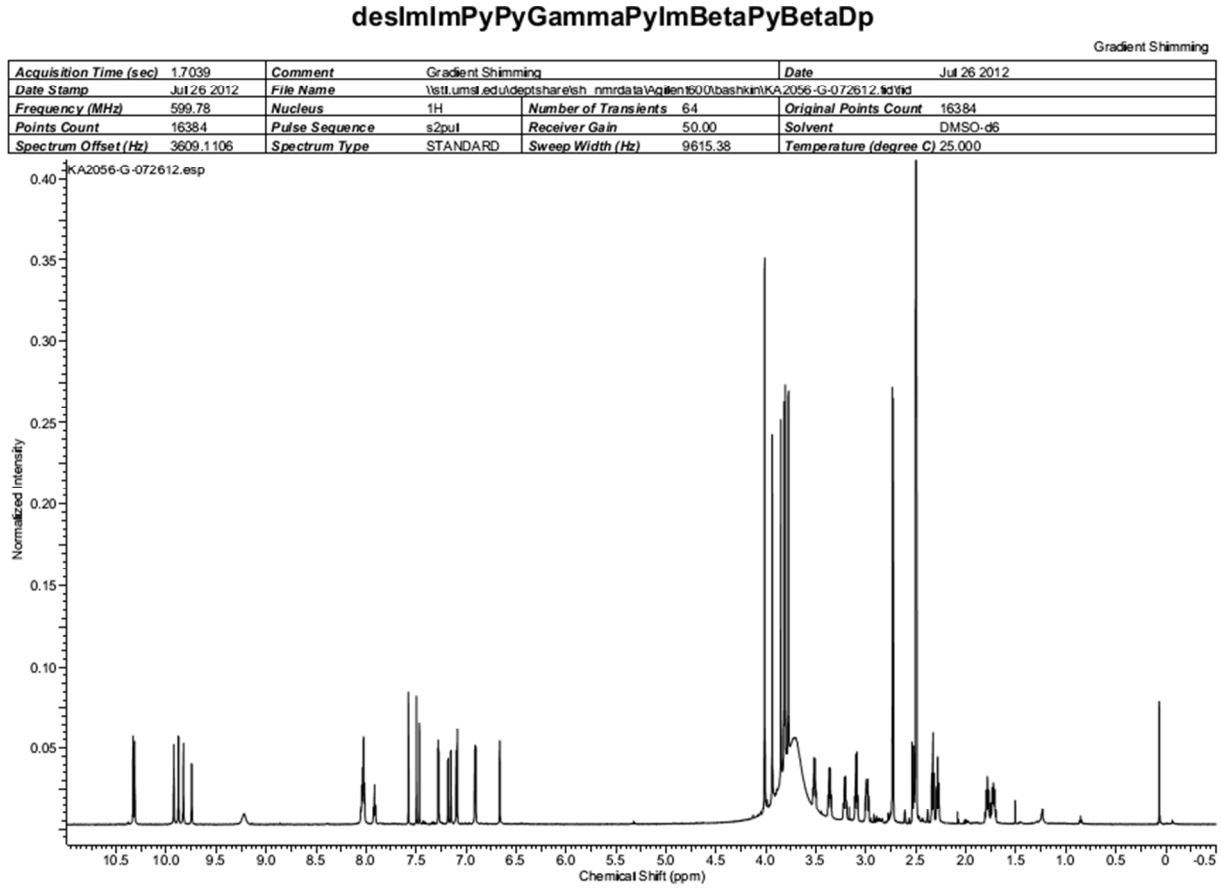
1175
m/z

Figure S5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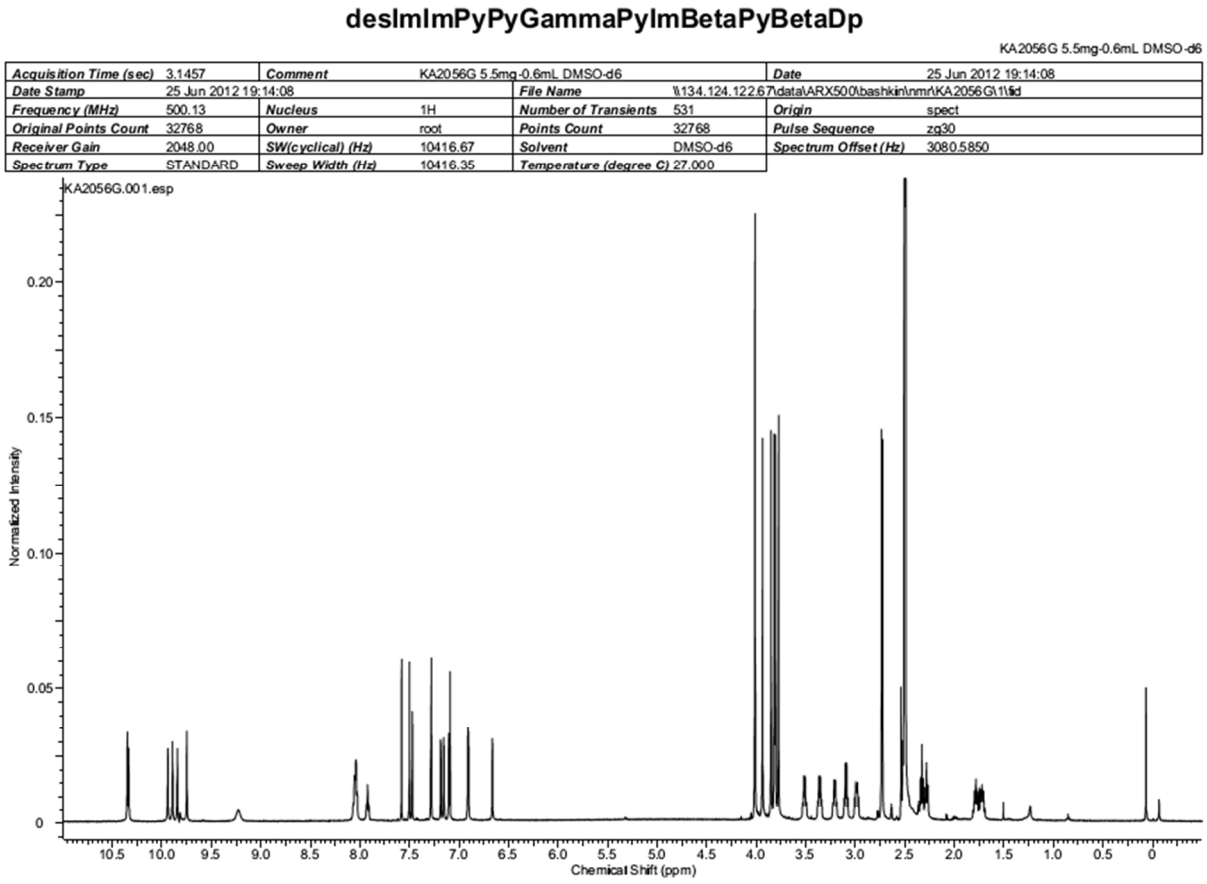
Normalized Intensity
Chemical Shift (ppm)

Figure S6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Comment KA2056G 5.5mg-0.6mL DMSO-d6
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0.05
0
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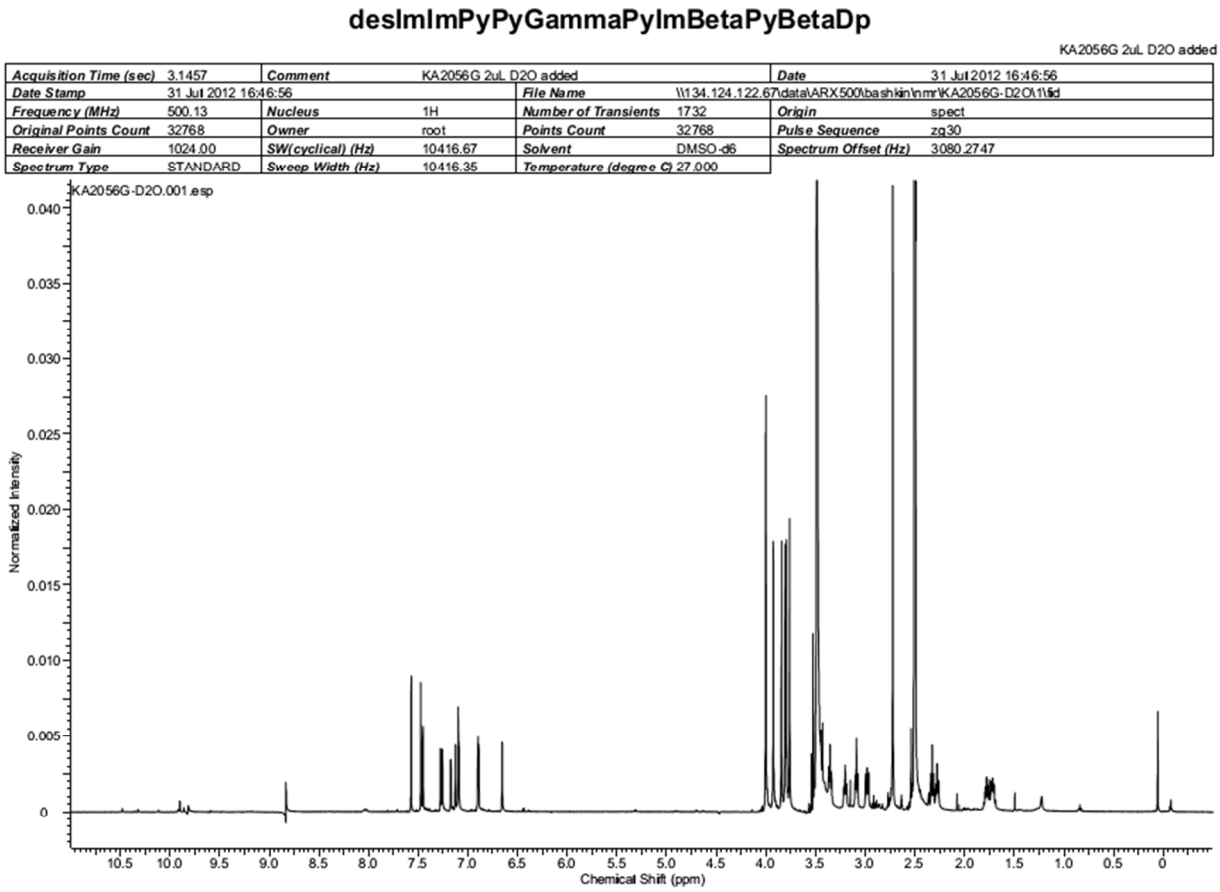
Chemical Shift (ppm)

Figure S7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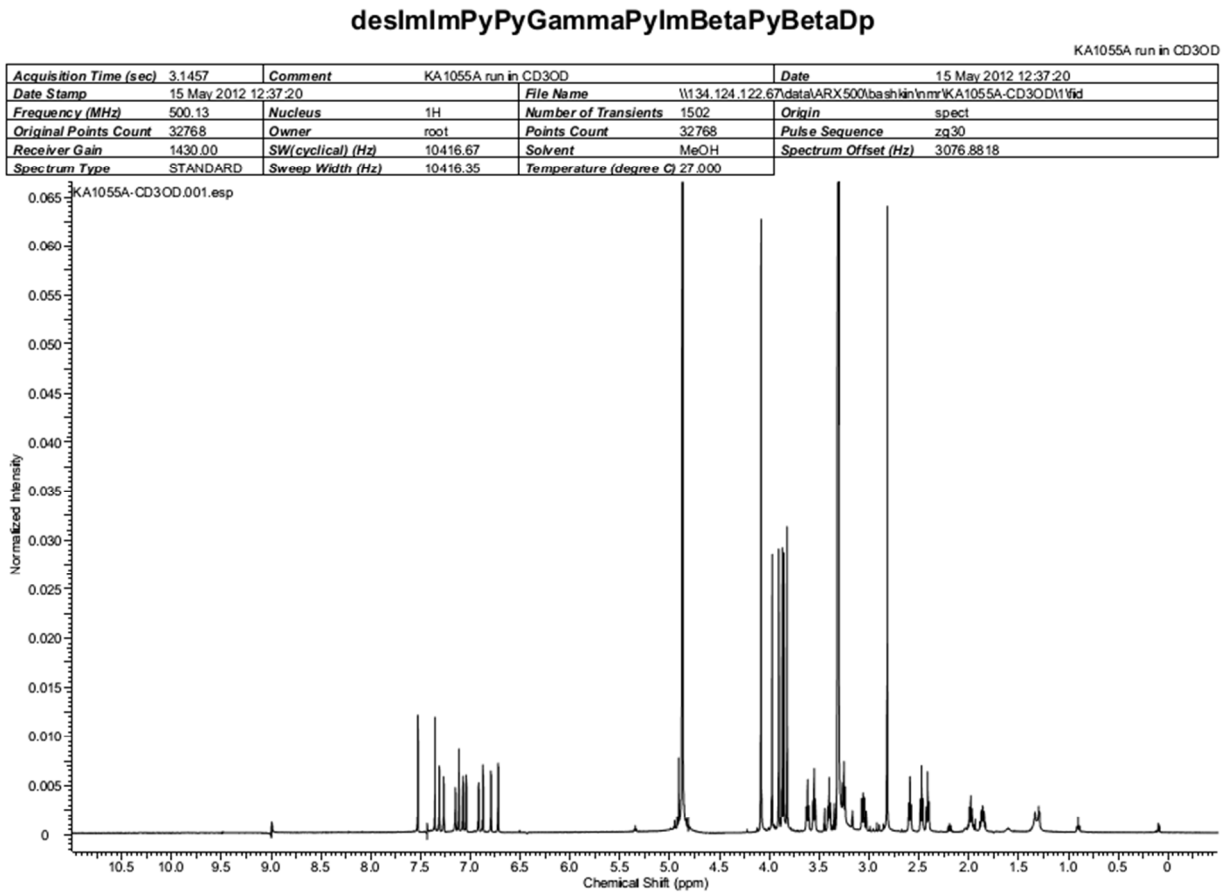
Chemical Shift (ppm)

Figure S8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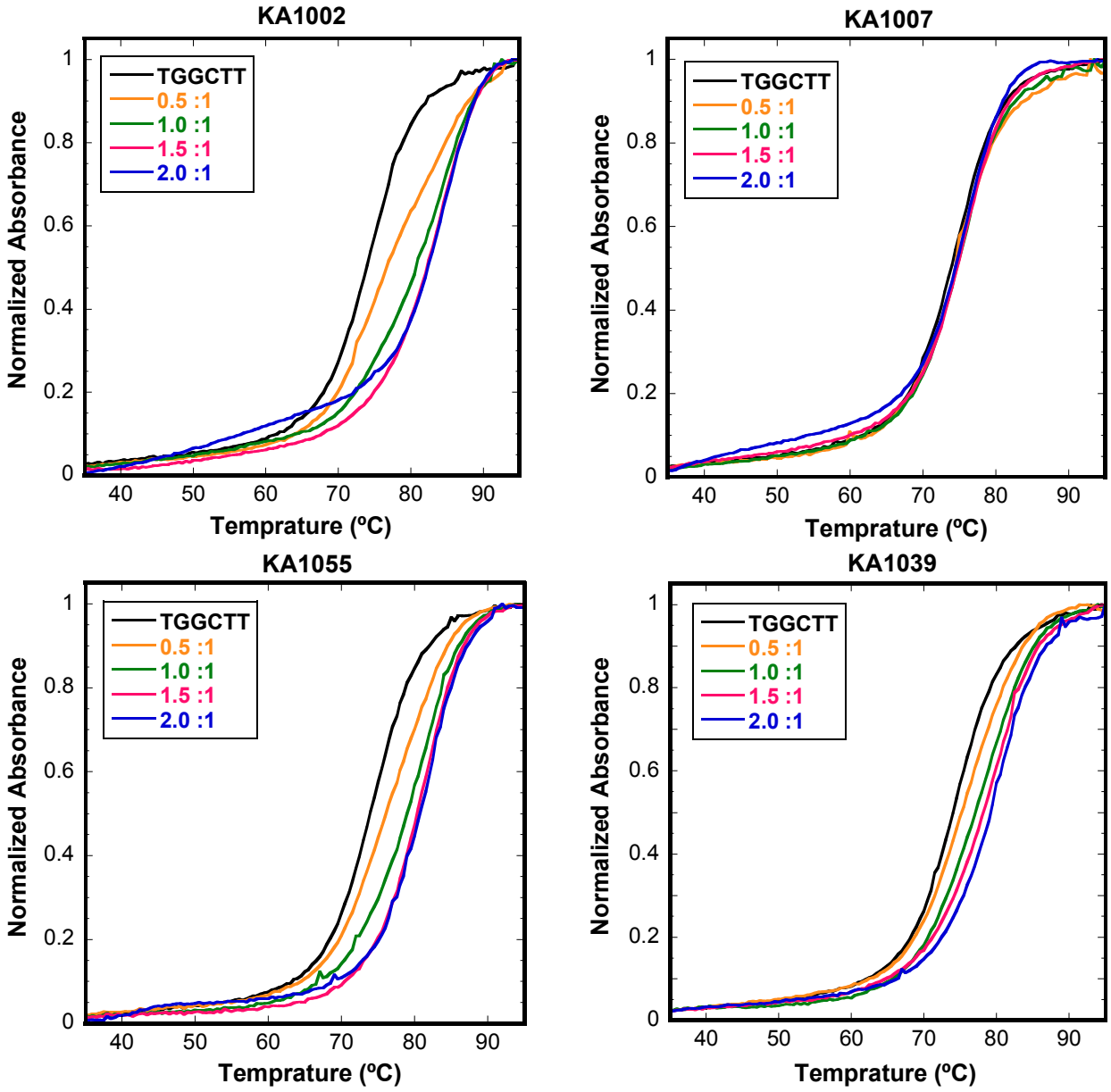
Normalized Intensity
Chemical Shift (ppm)

Figure S9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1.0 :1
1.5 :1
2.0 :1
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40
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70
80
90

Figure S10

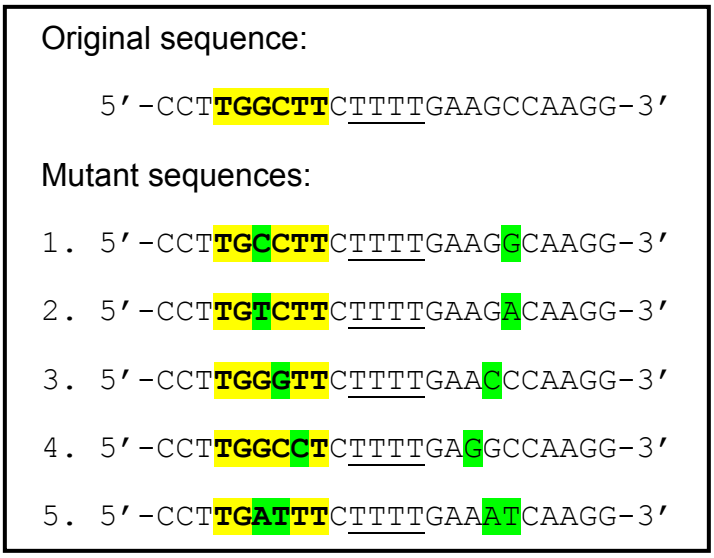
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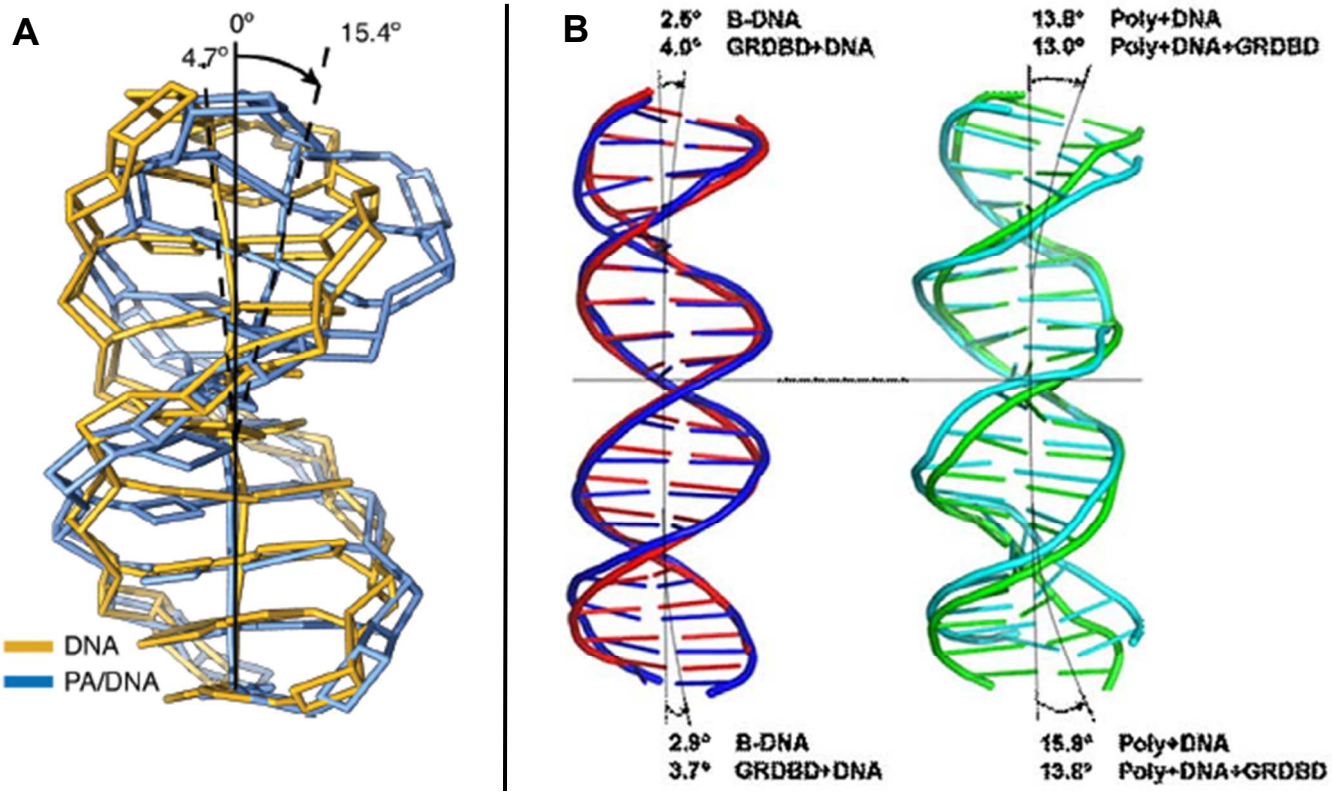
Figure S11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4.7°
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B
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2.9° B-DNA
3.7° GRDBD+DNA
15.8° Poly+DNA
13.8° Poly+DNA+GRDBD

Figure S12