

Supporting Information

The role of KRSIK motif of human angiogenin in heparin and DNA binding

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Expression, refolding and purification

DNA encoding the catalytic domain of hAng (Q1-P123, numbered from the catalytic domain of hAng) was cloned into pET28a. The plasmid was transformed into the *E. coli* BL21(DE3) strain. The cells were grown to an OD₆₀₀ of 0.6-0.8 at 37 °C and the protein including His₆-tagged at the N-terminus was induced with the addition of 1 mM isopropyl β-D-thiogalactopyranoside (IPTG). Following 4 h of incubation, the cells were harvested. The cells were suspended in a lysis buffer (20 mM Tris-HCl, 300 mM NaCl, 5 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride (PMSF), pH 8.0) and then were sonicated on ice and centrifuged at 50,000 g for 30 min. The pellets were washed with a washing buffer (20 mM Tris-HCl, 300 mM NaCl, 0.5 mM EDTA, pH 8.0) using a homogenizer and centrifuged at 50,000 g for 20 min. Following three washings, the pellets were solubilized in 10 ml (for 1 L culture) of denaturation buffer (7 M guanidine hydrochloride, 50 mM Tris-HCl, 20 mM DTT, pH 8.0) and ultracentrifuged at 200,000 g for 1 h. The supernatant was stored at -80 °C. The protein was refolded by rapid dilution in a refolding buffer (20 mM Tris-HCl, 300 mM NaCl, 20 % glycerol, 5 mM β-mercaptoethanol, pH 7.5) at 4 °C with a final concentration of 0.05

mg/ml and left to stand for 24 h without stirring. Ni-NTA resin was added to the solution containing the refolded protein and stirred for 1 h at 4 °C. Following the collection of Ni-NTA resin in a gravity column, the resin was washed with a washing buffer (20 mM Tris-HCl, 300 mM NaCl, 5 mM β -mercaptoethanol, 20 mM imidazole, pH 7.5). The protein was eluted with an elution buffer (20 mM Tris-HCl, 300 mM NaCl, 5 mM β -mercaptoethanol, 500 mM imidazole, pH 7.5). To remove the N-terminal His₆-tag, the protein was mixed with thrombin and dialyzed in a buffer (20 mM Tris-HCl, 300 mM NaCl, pH 7.5) at 4 °C overnight. The final purification step was performed by size-exclusion chromatography (HiLoad 16/60 Superdex 75 column, GE Healthcare) in a buffer (20 mM Tris-HCl, 300 mM NaCl, pH 7.5).

Multi-angle light scattering (MALS)

For calculation of the molecular weights of apo hAng and the hAng-heparin complex, high-performance liquid chromatography (HPLC, Shimadzu in KBSI) combined MALS (Wyatt Technology Corporation in KBSI) spectra were obtained in a buffer containing 20 mM Bis-tris and 100 mM NaCl, at pH 6.0 and 30 °C.

NMR spectroscopy

NMR measurements were performed with Avance II 800 MHz and 900 MHz spectrometers (Bruker in KBSI). A total of 1.1 mM ¹⁵N and ¹³C-labeled hAng was used for 3D structure determination in a buffer containing 20 mM sodium phosphate, 50 mM NaCl, 5 mM sodium acetate, and 10% D₂O, at pH 6.0 and 30 °C. The backbone signals were assigned by analyzing 2D ¹H-¹⁵N HSQC and 3D heteronuclear correlation spectra (HNCA, HN(CO)CA, HNCO, HN(CA)CO, NHCACB, and CBCA(CO)NH). All NMR spectra were processed with Bruker

Topspin 3.0 and analyzed with Sparky 3.113 (Goddard T.G. and Kellner D.G., University of California, San Francisco). To measure the binding affinity of tetrasaccharide heparin to hAng, binding curves were obtained from a two-dimensional ^1H - ^{15}N HSQC titration of ^{15}N -labeled hAng with heparin. Unfortunately, the binding affinity for the complex of hAng-(CT/GA)₄ could not be measured by the NMR method due to the precipitation of hAng upon increasing the concentration of DNA. It seems that the neutralization of positively charged hAng complex by increasing the concentration of negatively charged DNA facilitates the precipitation of the protein like longer (CT/GA)-repeat oligomers. Moreover, the longer (CT/GA)-repeat double strand oligomers (> 8-mer) induced high precipitation of the hAng, thus, it was not possible to characterize the binding properties for longer DNA oligomers using NMR.

Isothermal Titration Calorimetry (ITC) experiment

ITC measurements were performed at 30 °C using an Auto-iTC200 (Malvern) with a reference power of 10 cal/s and a stirring speed of 1000 rpm. Protein samples were dialyzed against ITC buffer containing 50 mM Bis-Tris pH 6.0 and 100 mM NaCl overnight at 4 °C. Ligand samples were dissolved in the dialysis buffer. For measurements of K_d values, the 200- μL sample cell was filled with protein (60 μM) and the 40- μL injection syringe was filled with ligand (600 μM). Each titration typically involved 19 injections (volume, 2 μL ; duration, 4 s) in 150 s intervals. Data fitting and analysis were carried out using Origin v. 7.0 software (MicroCal).

Electrophoretic mobility shift assay (EMSA)

Polyacrylamide gel (12 %) was prepared in TBE buffer (0.5 X) and used for the EMSA experiment. 0.25 mM (CT/GA)₄ DNA (8-mer) in the absence or presence of hAng was loaded on

the gel. Gel electrophoresis was performed in TBE buffer (0.5X) at room temperature. For the detection of the DNA fragment, the gel was stained with high-sensitive DNA staining solution (Fast Blast DNA stain, Bio-Rad) for 3 min at room temperature.

Nuclear translocation

HeLa cells were maintained in RPMI 1640 (Invitrogen, USA) with 10% FBS (invitrogen, USA) in the presence of penicillin (100 units/ml). Cell cultures were maintained at 37 °C under a humidified atmosphere of 5% CO₂. HeLa cells were incubated with hAng wild type and mutant types (0.5 µg/ml) at 37 °C for 30 min. The cells were washed with PBS and fixed with paraformaldehyde at 25 °C for 15 min. Then, the cells were washed with PBS and 0.1% TritonX-100 at 25 °C for 10 min and washed 3 times with PBS. Cells were blocked with 1% BSA (bovine serum albumin) and incubated at 4 °C overnight with the 1st hAng antibody (2mg/ml, abcam). Then, the cells were washed with PBS and incubated with the 2nd antibody (mouse IgG) for 40 min, and stained with DAPI at 25 °C for 2 min. The cells were imaged at original 60X magnification using a fluorescence microscope (Olympus, USA). The images were analyzed with Image J (NIH, USA).

Heparin-Sepharose chromatography

To examine hAng binding to heparin, the proteins were loaded on Heparin-Sepharose HP column (1ml, GE Healthcare) equilibrated with 10 mM Tris-HCl and 10 mM NaCl at pH 7.5 and eluted with a linear gradient of NaCl up to 1 M.

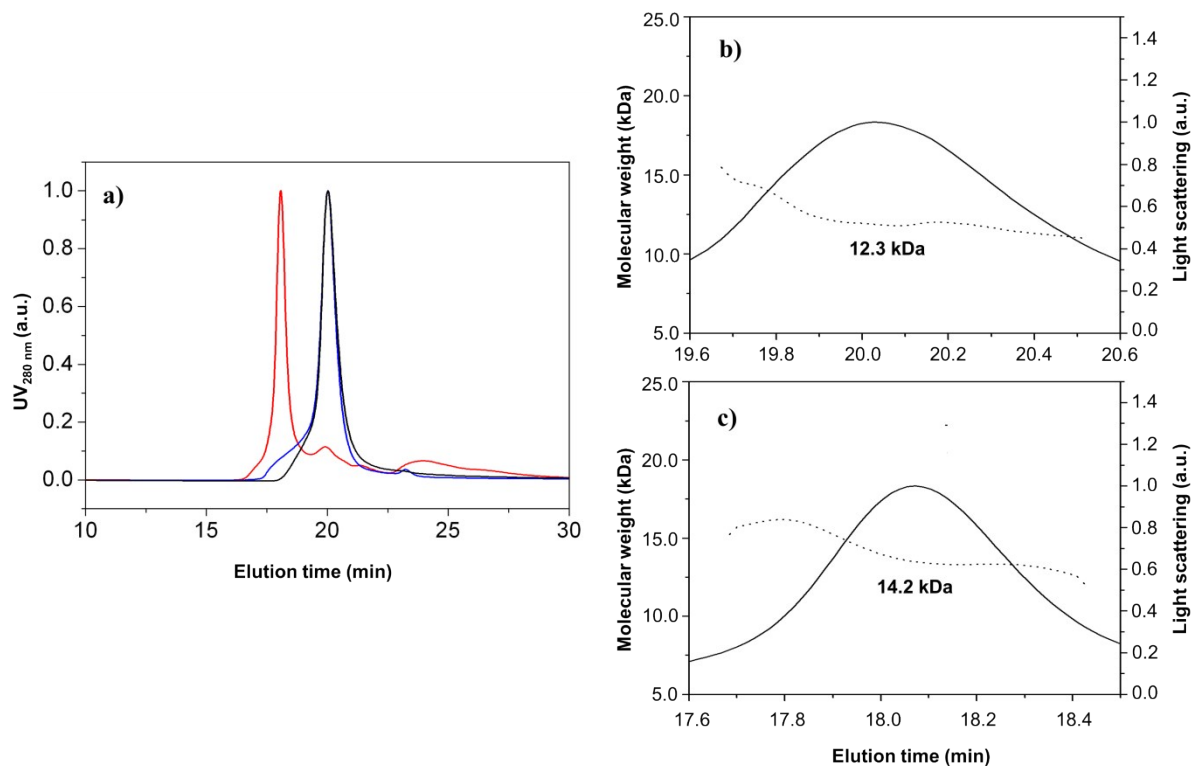


Figure S1. Measurement of molecular weight of apo hAng and hAng-heparin (tetrasaccharide) complex using HPLC (a) combined MALS spectra (b and c). In panel a), hAng : heparin ratios are 1 : 0 (black), 1 : 0.5 (blue), and 1 : 10 (red), respectively. In panel b) and c), hAng : heparin ratios are 1 : 0 and 1 : 10 , respectively. The molecular weights and the light scatterings are represented by dotted lines and straight lines, respectively. Theoretically, hAng = 14.5 kDa, tetrasaccharide heparin = 1.2 kDa.

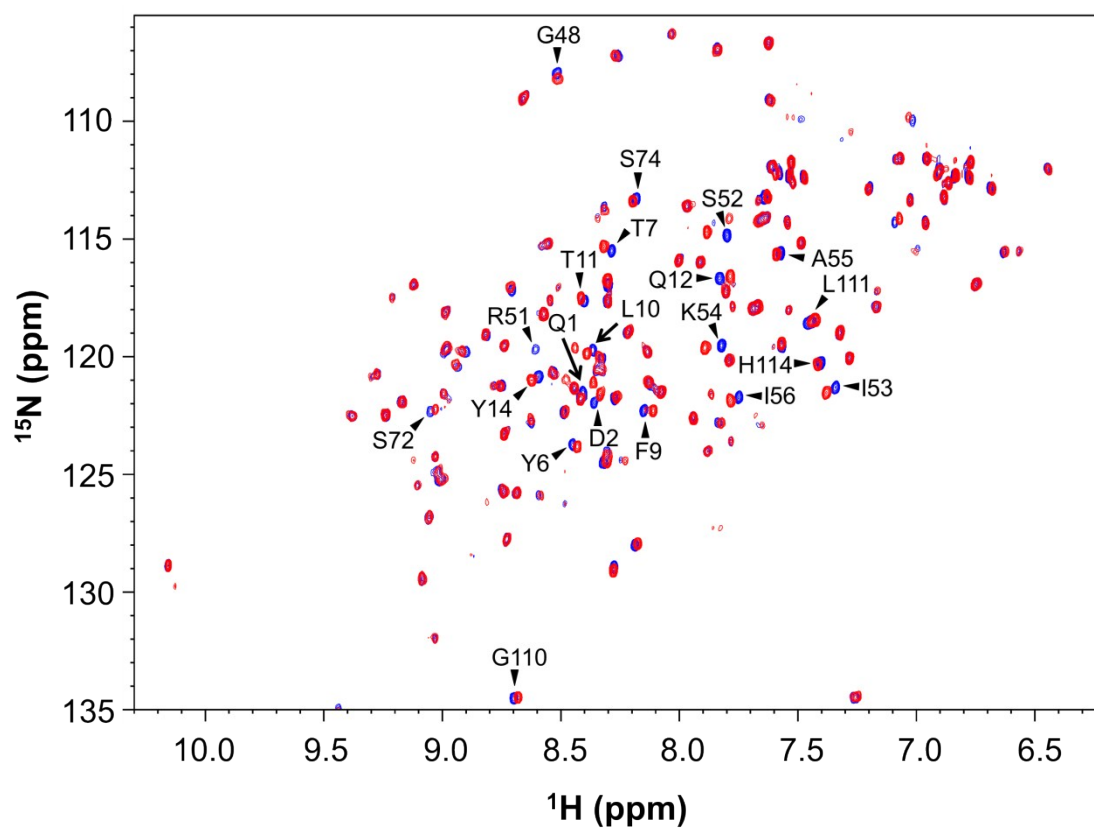


Figure S4. ^1H - ^{15}N HSQC NMR spectra of hAng in the absence (blue) or presence (red) of tetrasaccharide heparin at 30 °C. 0.1 mM protein and 0.1mM heparin were used in 20 mM sodium phosphate, 5mM sodium acetate, 50 mM NaCl, pH 6.0.

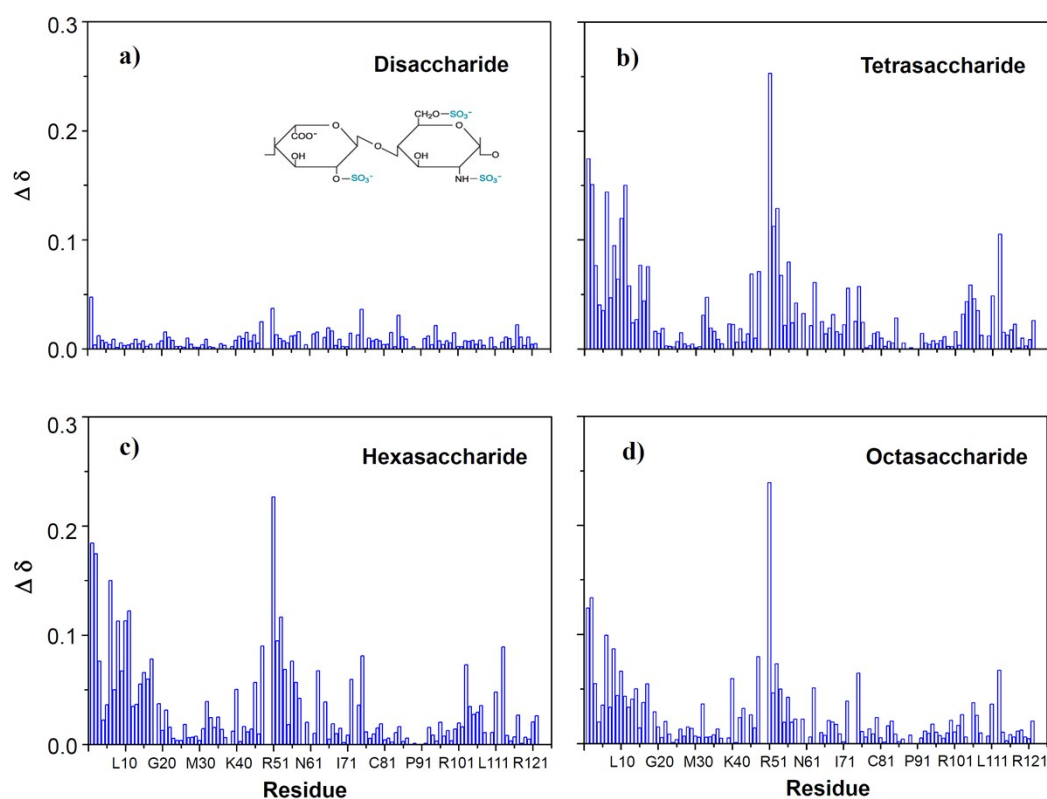


Figure S5. Weighted average of ^1H - ^{15}N chemical shift perturbations of the hAng residues upon binding to the heparin fragments (**a-d**). Chemical shift perturbation data were calculated at 1 to 0.5 molar ratio of hAng to heparin. The chemical structure of the disaccharide unit of heparin is shown in panel **a**).

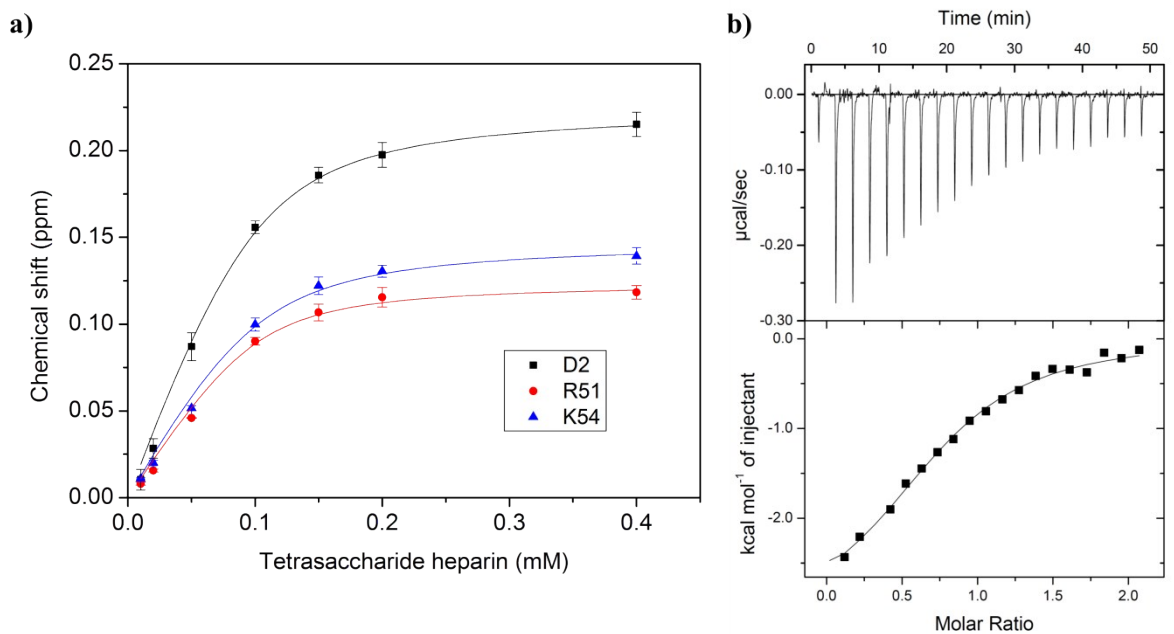


Figure S6. Measurement of the binding affinity of hAng wild type with tetrasaccharide heparin.

a) NMR titration curves of hAng (100 μ M) with the tetrasaccharide heparin in 20 mM Bis-tris 100 mM NaCl pH 6.0 at 30 $^{\circ}$ C. The curves were fitted to a single site binding model using the nonlinear least squares method. Chemical shift values were calculated by the weighted individual chemical shifts of the three residues (D2, R51, and K54.). **b)** ITC data of a titration of hAng wild type (60 μ M) with tetrasaccharide heparin to measure the binding affinity in 50 mM Bis-Tris pH 6.0 and 100 mM NaCl at 30 $^{\circ}$ C.

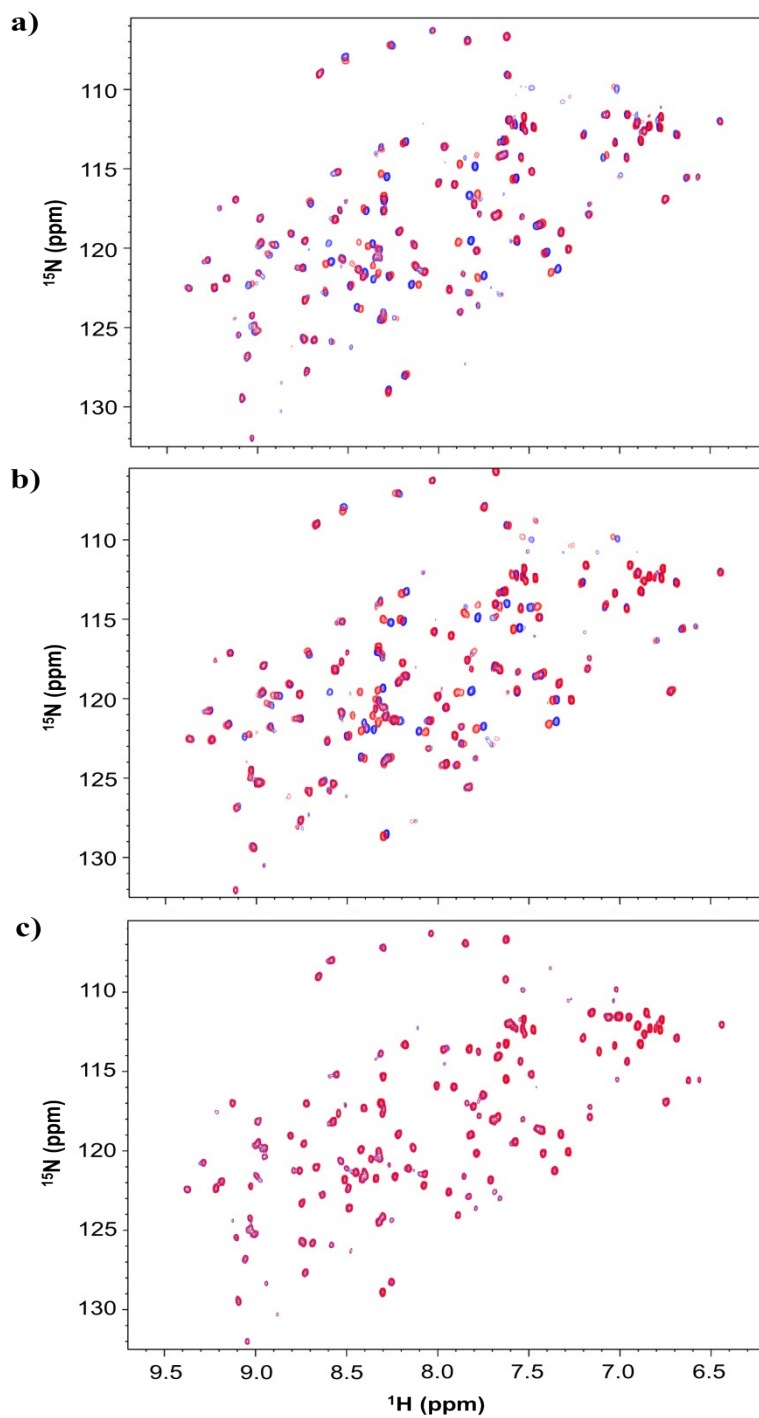


Figure S7. ^1H - ^{15}N HSQC spectra for the hAng wild type (a), the hAng (R31A/R32A/R33A) mutant (b), and the hAng (K50Q/R51A/K54Q) mutant (c) in 20 sodium phosphate, 50 mM NaCl, 5mM sodium acetate, pH 6.0 at 30 °C (blue color, 0.1 mM protein only; red color, in the

presence of 0.1 mM tetrasaccharide heparin).

Table S1. K_d values for the wild type and hAng (R31A/R32A/R33A) and hAng (K50Q/R51A/K54Q) mutants using NMR titration and ITC experiment as described in the Figure S6. In the case of NMR titration, chemical shift values were calculated by the weighted individual chemical shifts of the three residues (D2, R51, and K54 residues for the wild type and the hAng (R31A/R32A/R33A) mutant; D2, Y6, and H8 residues (located on the $\alpha 1$ helix) for the hAng (K50Q/R51A/K54Q) mutant because the ^1H - ^{15}N cross peaks of Q51 and Q54 residues corresponding to R51 and K54 residues, respectively, were not assigned).

Method	Wild type (μM)	hAng (R31A/R32A/R33A) mutant (μM)	hAng (K50Q/R51A/K54Q) mutant (μM)
NMR	14.4 (± 2.2)	107.5 (± 7.7)	753.0 (± 42.4)
ITC	13.1 (± 1.17)	38.9 (± 2.1)	ND*

*, K_d value for the hAng (K50Q/R51A/K54Q) mutant was not determined by ITC experiment because enthalpy changes were very low and the titration curve was not available for curve fitting.

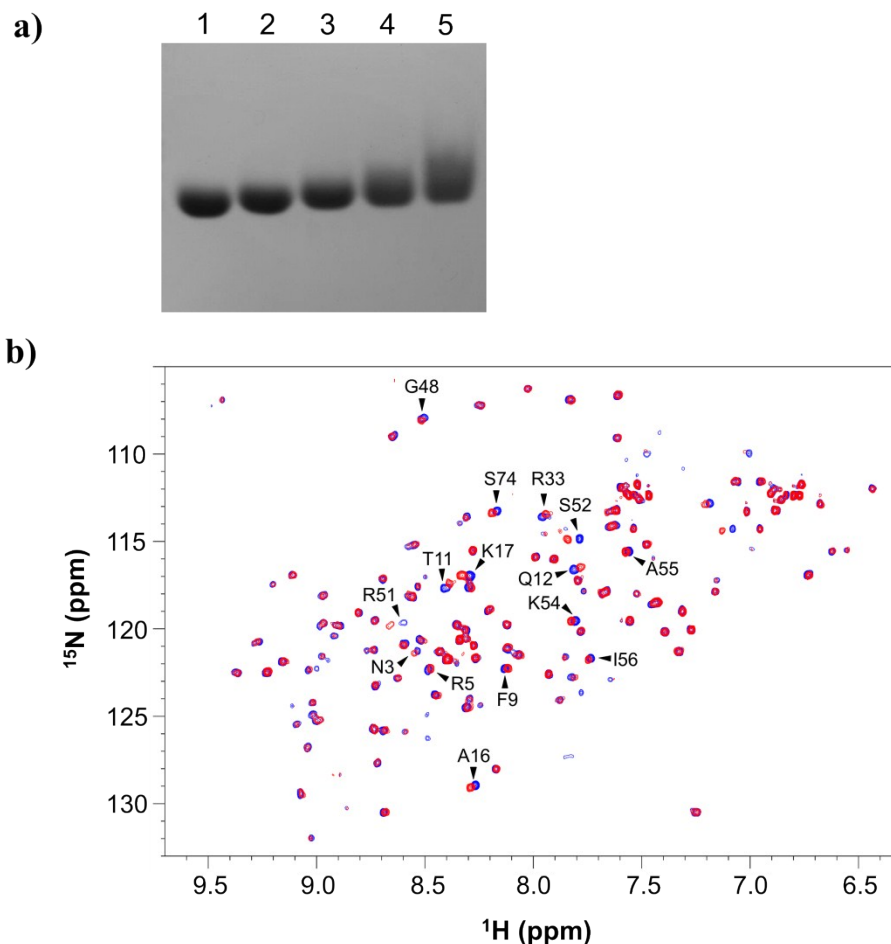


Figure S8. a) EMSA for the (CT/GA)₄ double strand DNA (0.25 mM) in the absence or presence of hAng. The molar ratios of DNA : hAng are 1 : 0 (lane 1), 1 : 1 (lane 2), 1 : 2 (lane 3), 1 : 4 (lane 4), and 1 : 8 (lane 5). b) ¹H-¹⁵N HSQC spectra of hAng in the absence (blue) or presence (red) of (CT/GA)₄ double strand DNA at 30 °C. 0.1 mM protein and 0.1mM DNA were used in 20 mM sodium phosphate, 5mM sodium acetate, 50 mM NaCl, pH 6.0.

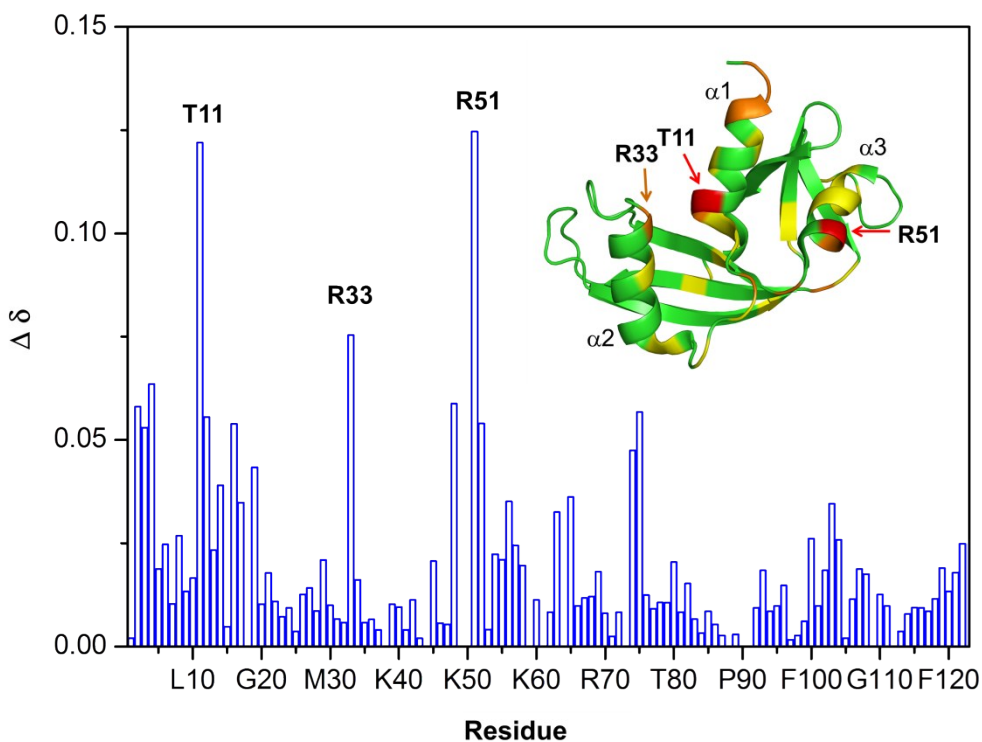


Figure S9. Weighted average of ^1H - ^{15}N chemical shift perturbations of hAng residues upon binding to DNA. Inset: binding site mapping of the DNA on the hAng structure (PDB: 1ANG). Amino acids showing chemical shift perturbations with the addition of DNA are displayed in yellow, orange, and red: yellow, $0.02 < \Delta\delta$; orange, $0.05 < \Delta\delta < 0.1$; red, $\Delta\delta > 0.1$. The arrows indicate the main residues perturbed by DNA binding.

The binding affinity for the complex of hAng-(CT/GA)₄ could not be determined due to the precipitation of hAng upon increasing the concentration of DNA (ESI, NMR spectroscopy)

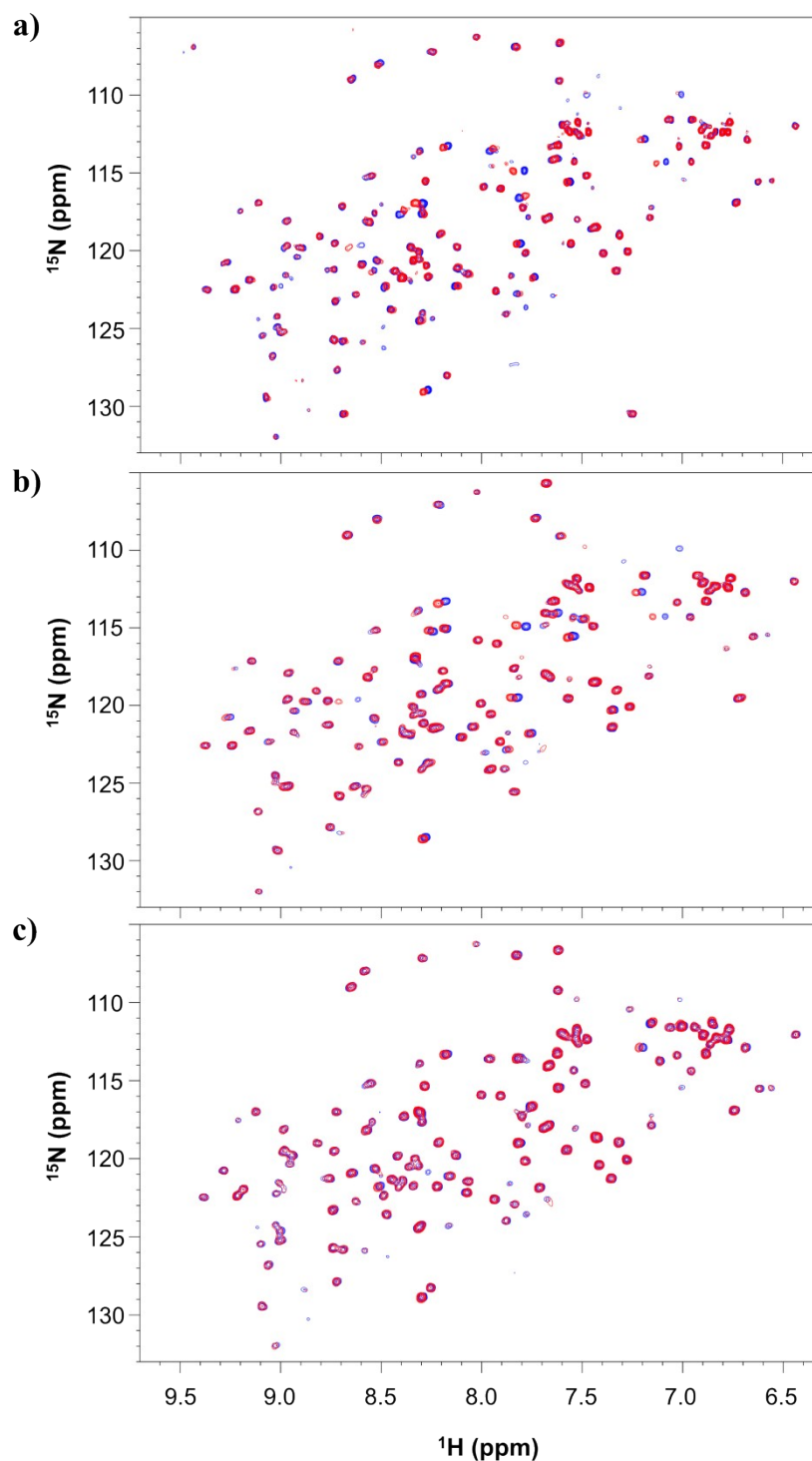


Figure S10. ^1H - ^{15}N HSQC spectra at 30 °C for the hAng wild type (a), the hAng (R31A/R32A/R33A) mutant (b), and the hAng (K50Q/R51A/K54Q) mutant (c) in 20 mM sodium phosphate, 50 mM NaCl, 5 mM sodium acetate, pH 6.0 (blue color, 0.1 mM protein only; red

color, in the presence of 0.1 mM (CT/GA)₄ DNA).

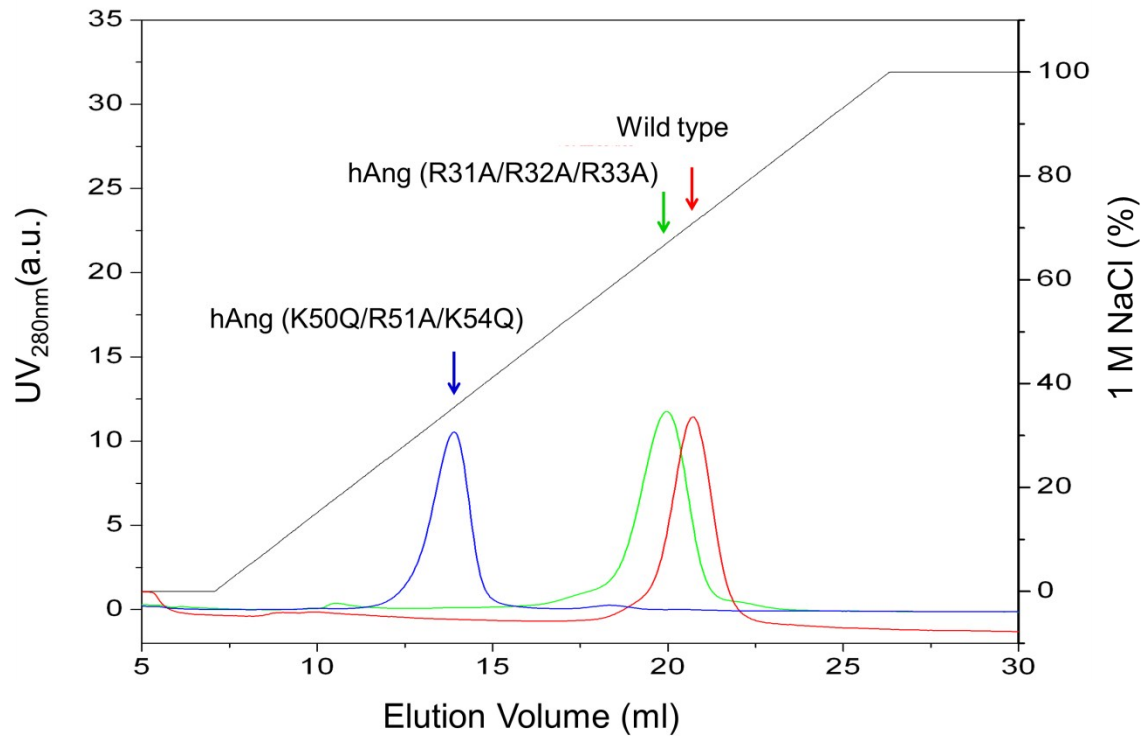


Figure S11. Heparin-Sepharose chromatography for the hAng wild type and mutants. The proteins were eluted by increasing the concentration of NaCl (red color for the hAng wild type; green color for the hAng (R31A/R32A/R33A) mutant; blue color for the hAng (K50Q/R51A/K54Q) mutant). The hAng wild type and the hAng (R31A/R32A/R33A) mutant were eluted at ~710 mM and ~670 mM NaCl, respectively. The elution peak of the hAng (K50Q/R51A/K54Q) mutant was observed at ~410 mM NaCl.