Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

for

NAMI-A Preferentially Reacts with Sp1 protein: Understanding Anti-metastasis Effect of the Drug

Contents

Experimental details

Protein Expression and Purification: Proteins used in this work were obtained according to the literature[1] and were characterized by electrophoresis and electrospray ionization mass spectrometry (ESI-MS).

ESI-MS Analysis of the Reaction of NAMI-A with Sp1: The mass spectrometric analysis was carried out on an Exactive Plusmass spectrometer (Thermo Fisher Scientific). 10 μM Sp1-zf2 was incubated with 2 molar equivalents of NAMI-A in 100 mM ammonium acetate at 37 °C for 12 h. Then samples were directly infused. Data were processed using XCalibur software (version2.0, Thermo Finnigan).

Fluorescence Measurements: Fluorescence spectra were recorded on a Hitachi F-4600 fluorescence spectrophotometer using a quartz cuvette with the path length of 1 cm. The reactions were conducted on 10 μM proteins with NAMI-A in 10 mM HEPES buffer.

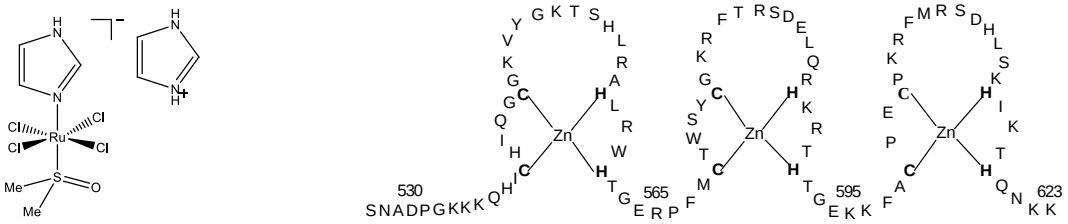
Electrophoretic Mobility Shift Assay: 20 μM of Sp1(530-623) was incubated with 10 μM *surviving* promoter in gel-shift buffer (20 mM HEPES 100 mM NaCl and 500 μM GSH at pH 7.40) for 0.5 h. Then the complexes were incubated with different concentration of NAMI-A at room temperature for 4 h. Samples were loaded on a native 15% polyacrylamide gel and run in 0.5×Tris-borate buffer. The gel was stained with Ethidium Bromide and visualized under UV.

Circular Dichroism (CD): CD measurements were performed on a Jasco J-810 CD spectrometer using a 1.0 cm path length quartz cuvette. 30 μM Proteins were incubated with different molar equivalents of NAMI-A in 10 mM phosphate buffer (pH 7.0) at 37 °C for 24 h. Spectra were scanned from 280 nm to 190 nm with a speed of 100 nm·min^{-1}; each spectrum was repeated three times.

NMR Spectroscopy: NMR spectra were recorded on a Bruker Avance 600 MHz spectrometer equipped with TCI CryoProbe. The samples were performed on 0.3 mM ^{15}N-labeled Sp1-zf2 with 2 molar equivalents of NAMI-A in 20 mM HEPES buffer containing 100 mM NaCl. Data were processed and analyzed using NMRPipe.

References

1. S. Chen, D. Xu, H. Jiang, Z. Xi, P. Zhu and Y. Liu, *Angew. Chem. Int. Ed.*, 2012, **51**, 12258-12262.



Scheme S1. The structure of NAMI-A and the sequence of zinc-finger domain of Sp1.

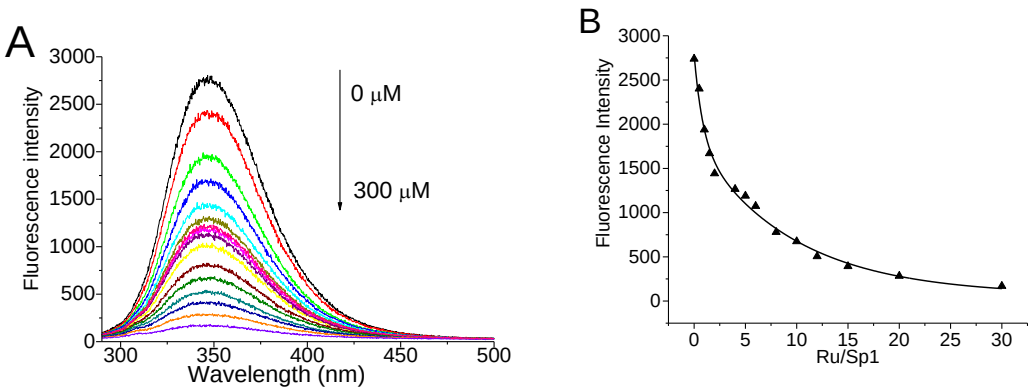


Figure S1. Fluorescence Characterization of NAMI-A binding to Sp1. (A) The fluorescence spectra of Sp1 after reaction with NAMI-A. (B) Plot of the fluorescence intensity of Sp1 at 350 nm in the reaction of NAMI-A. 10 μM protein was incubated with NAMI-A at 37 °C for 24 h in 10 mM HEPES, pH 6.80.

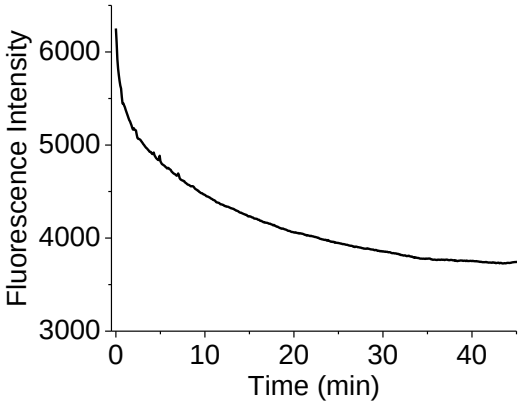


Figure S2. Kinetic process of the reaction of Sp1-zf2 with NAMI-A. Time-dependent measurement of the fluorescence intensity of Sp1-zf2 during the reaction with NAMI-A. The reaction was performed on 10 μM Sp1-zf2 with 2 molar equivalents of NAMI-A at 37 °C.

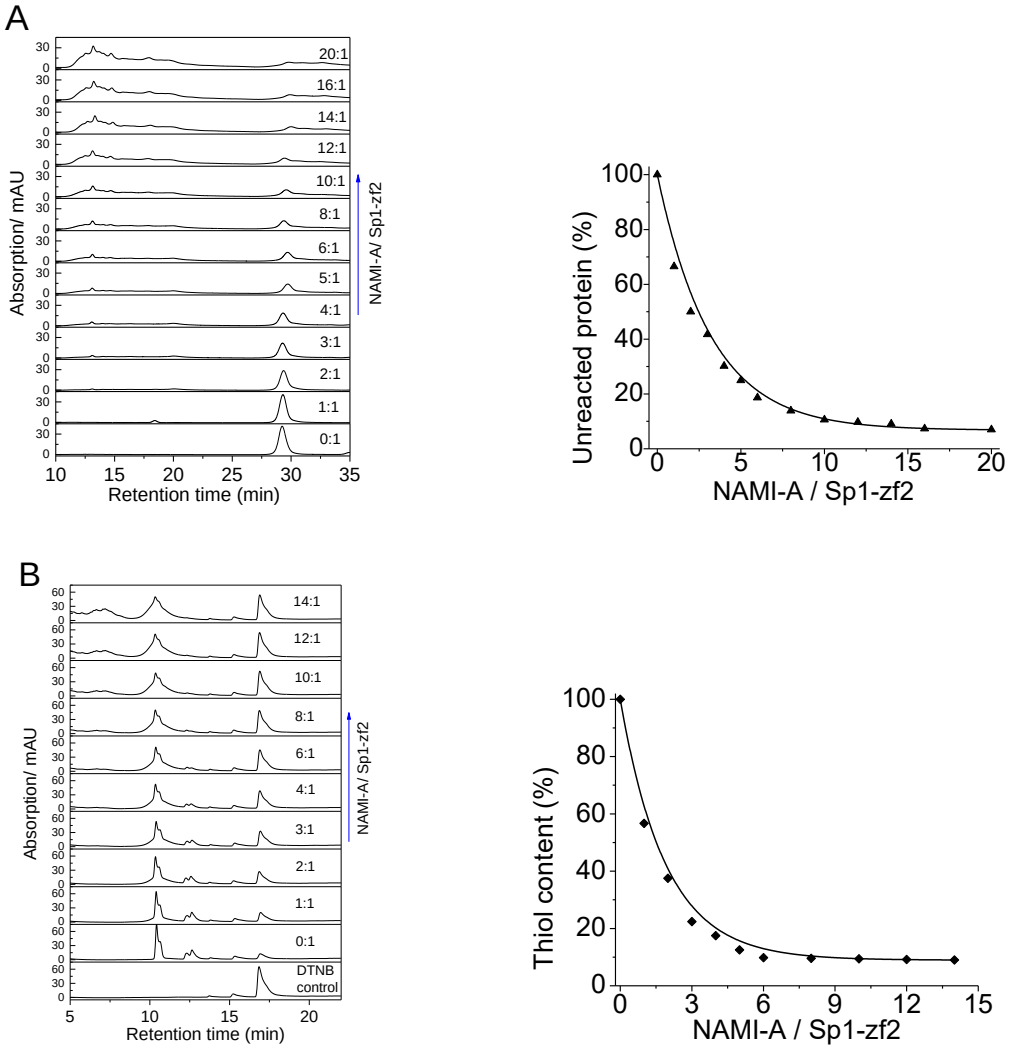


Figure S3. Characterization the reaction of Sp1-zf2 with NAMI-A. (A) Cationic ion exchange chromatography analysis the reaction of NAMI-A with Sp1-zf2. Sp1-zf2 (46 µM) was incubated with NAMI-A at 37 °C for 24h in 50 mM HEPES, pH 6.8. (B) Thiol content in Sp1-zf2 measured by DTNB after the reaction with NAMI-A. Reactions were carried out on Sp1-zf2 (100 µM) with different amount of NAMI-A in 100 mM HEPES buffer (pH 6.8) at 37 °C for 24 h. A 100 µM DTNB was added to the reaction mixture. The HPLC profiles were recorded on C18 reversed-phase column.

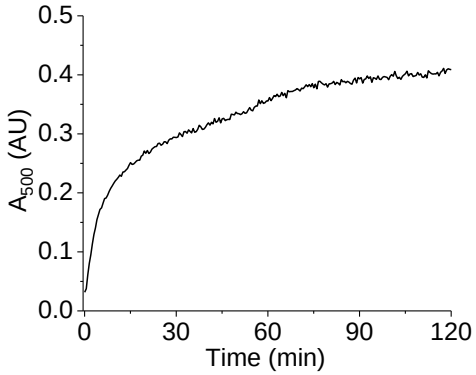


Figure S4. Zinc release from Sp1-zf2 upon the reaction of NAMI-A. Reactions were carried out on Sp1-zf2 (30 µM) with 2 molar equivalents of NAMI-A at 37 °C.

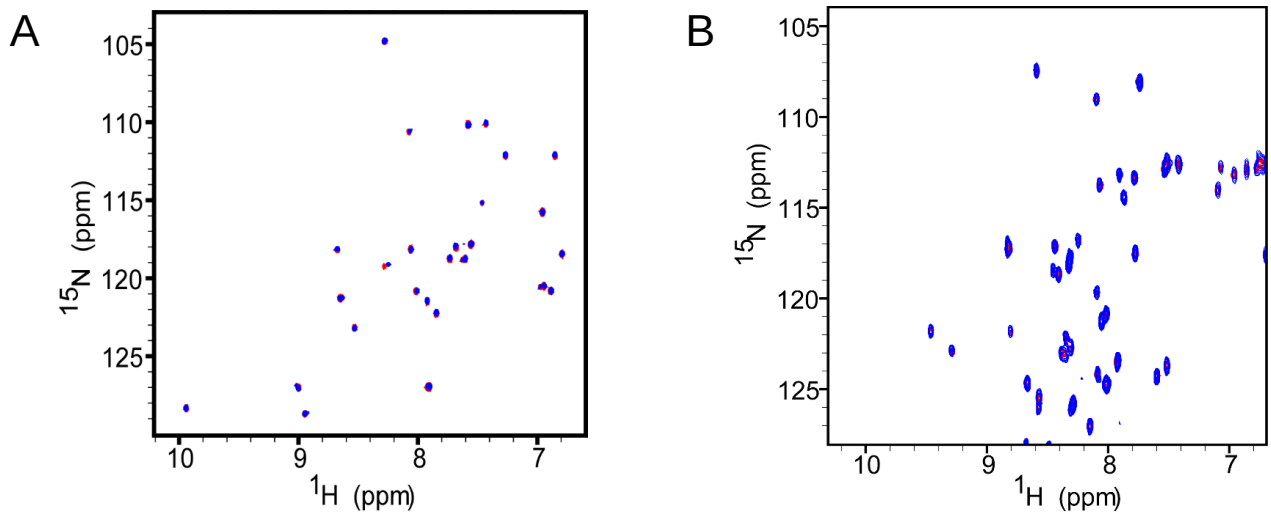


Figure S5. (A) Overlay of NMR spectra of ^{15}N-labeled Sp1-zf2 before (blue) and after (red) incubation with 2 molar equivalents of natural isotopic abundance Ru-NCp7. Ru-NCp7 was prepared by the NCp7 reaction with NAMI-A. NMR spectra were recorded at 298 K in 20 mM HEPES buffer (pH 6.8) containing 100 mM NaCl. (B). Overlay of NMR spectra of ^{15}N-labeled NCp7 before (blue) and after (red) incubation with 2 molar equivalents of natural isotopic abundance Ru- Sp1-zf2. Ru- Sp1-zf2 was prepared by the Sp1-zf2 reaction with NAMI-A. NMR spectra were recorded at 298 K in 20 mM HEPES buffer (pH 6.8) containing 100 mM NaCl.

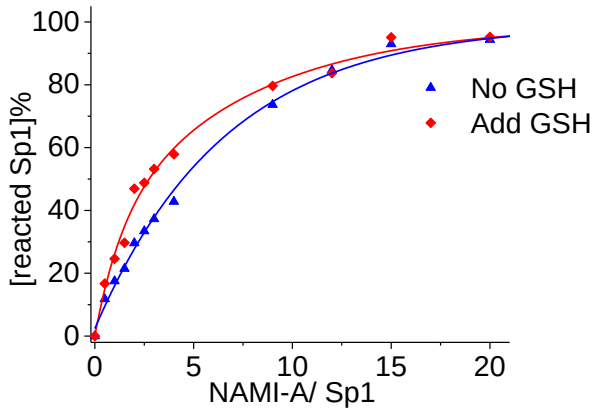


Figure S6. Characterization the effect of GSH on the reaction of NAMI-A with Sp1. Fluorescence spectra of Sp1 in the reaction with different amount of NAMI-A in the absence or presence of GSH. 10 µM protein was incubated with NAMI-A in the absence or presence of 1 mM GSH at 37 °C for 24 h in 10 mM HEPES, pH 6.80.

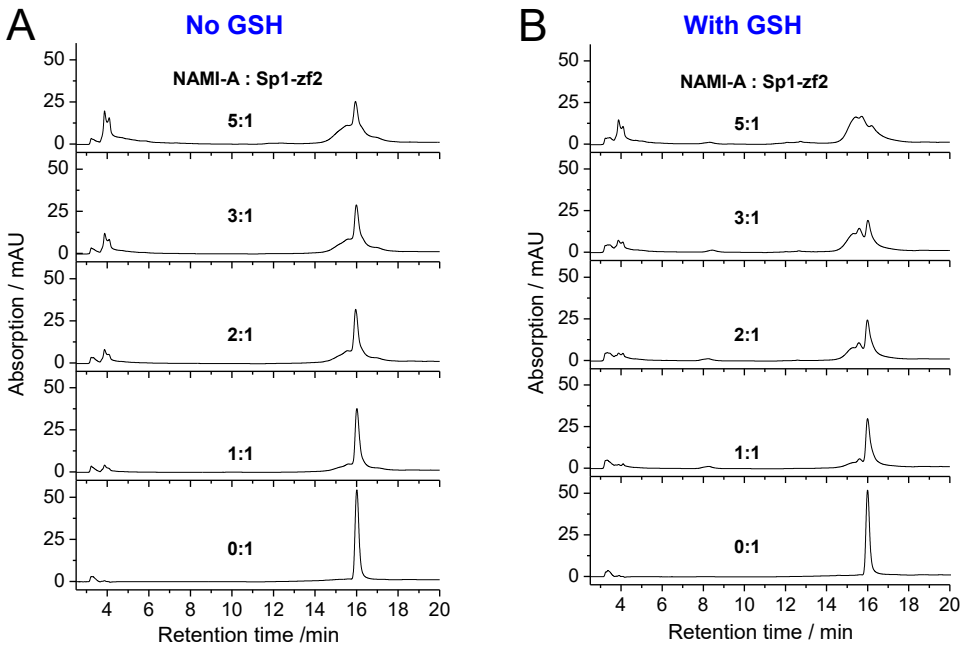


Figure S7. HPLC profiles of Sp1-zf2 in the reaction with NAMI-A. HPLC profiles of Sp1-zf2 in the reaction with NAMI-A In the absence (A) or presence (B) of 1 mM GSH. Sp1-zf2 (100 µM) was incubated with NAMI-A at 37 °C for 48h in 100 mM HEPES, pH 6.8. HPLC analyses were performed with an Agilent 1200 system equipped with a Kromasil C18 reverse phase column and recorded by UV detection at 280 nm.

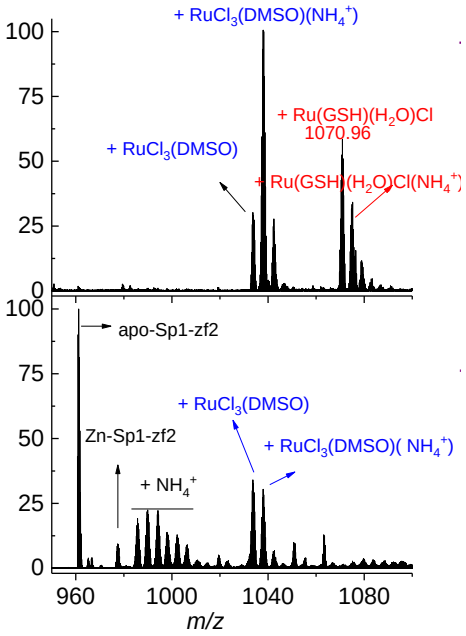


Figure S8. ESI-MS spectra of Sp1-zf2 reacted with 2 molar equivalents of NAMI-A in the absence or presence of 5 molar equivalents of GSH at 37 °C for 12h. 10 µM Sp1-zf2 was incubated with 2 molar equivalents of NAMI-A at 37 °C for 12h in 100 mM NH_4OAc buffer.

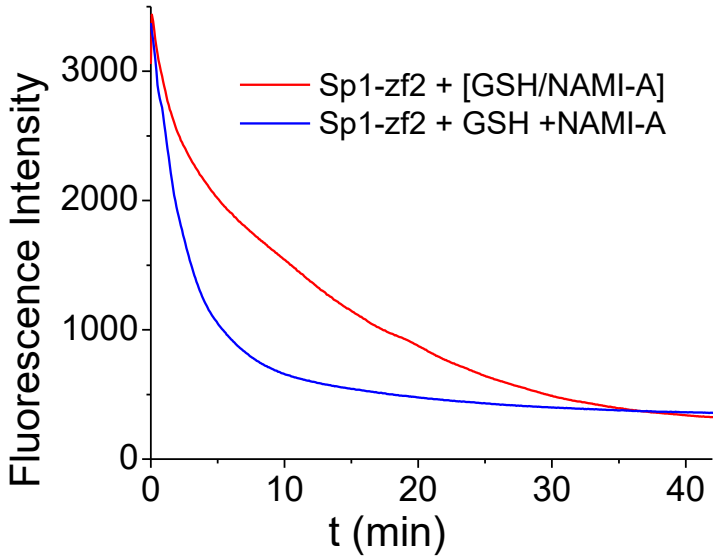


Figure S9. Time-dependent measurement of the fluorescence intensity of Sp1-zf2 during the reaction with NAMI-A in the presence of GSH (blue) or with GSH/NAMI-A adducts (red). The reaction was performed on 10 µM Sp1-zf2 with 4 molar equivalents of NAMI-A at 37 °C.

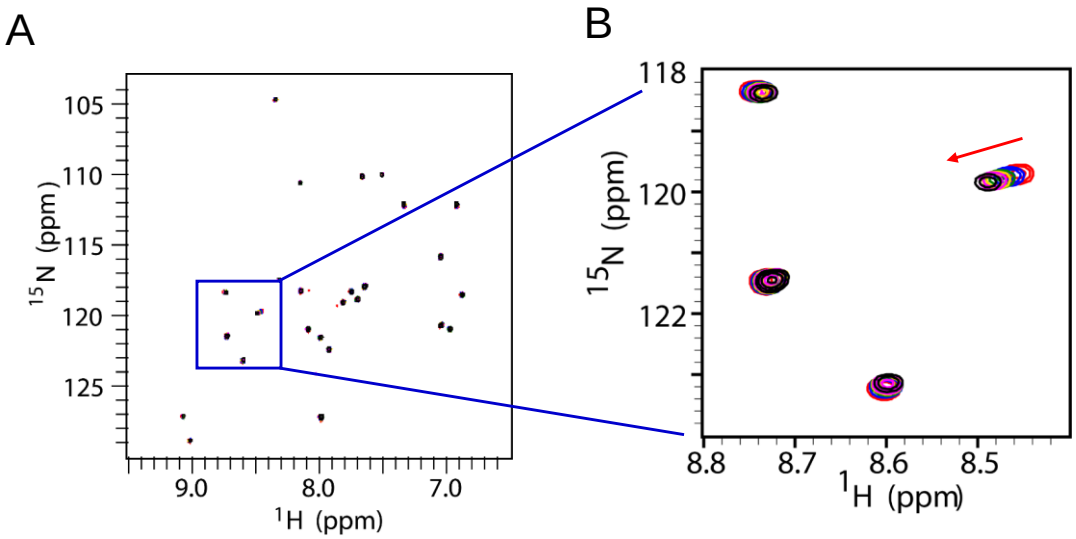


Figure S10. Time-dependent NMR spectra of Sp1-zf2 in the reaction with NAMI-A. (A) Overlay of NMR spectra of Sp1-zf2 before (red) and after incubation with 2 molar equivalents of NAMI-A at 25 °C for 2 h (blue), 4 h (green), 6 h (gold), 8 h (magenta) and 10 h (black) in 20 mM HEPES buffer containing 100 mM NaCl (pH 6.8). (B) the partial enlarged enlargement of A.

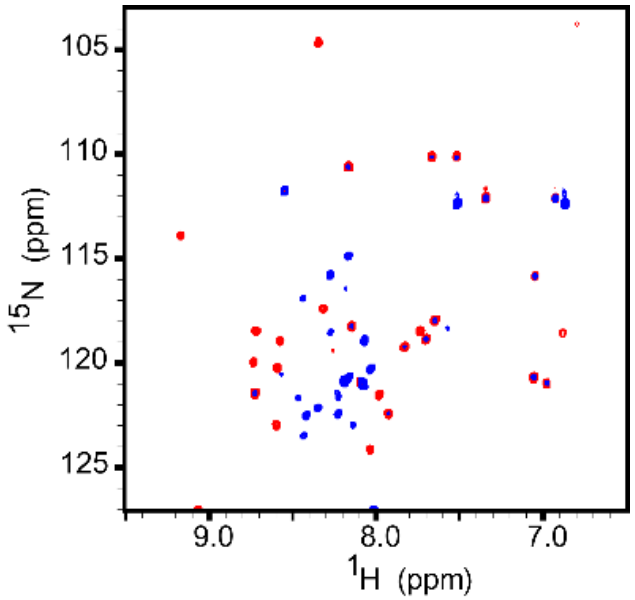


Figure S11. Overlay of NMR spectra of Sp1-zf2 before (red) and after (red) incubation with 2 molar equivalents of NAMI-A at 25 °C for 10 h in 20 mM HEPES buffer containing 100 mM NaCl (pH 6.8) in the presence of 2 mM GSH.

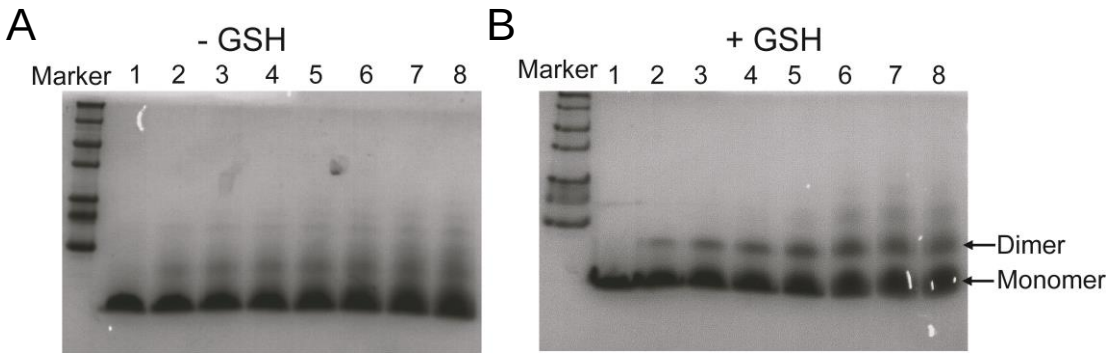


Figure S12. Tricine SDS-PAGE analyses the effect of GSH on the aggregation of Sp1-zf2 induced by NAMI-A. Lanes 1-8: Sp1-zf2 (80 µM) was incubated with different molar equivalents of NAMI-A (0, 0.5, 2, 3, 4, 6, 8 and 10, respectively) at 37 °C for 12h in 100 mM HEPES, pH 6.8. Reactions were performed without (A) or with (B) 1 mM GSH addition.

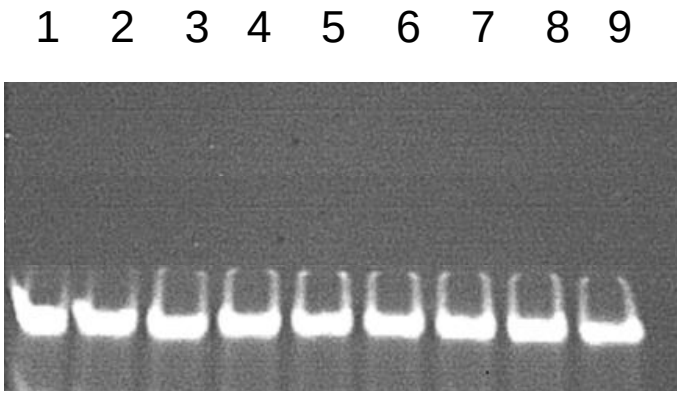


Figure S13. Characterization the reaction of DNA with NAMI-A. Lane 1: *survivin* promoter; Lanes 2-9: DNA was incubated with different molar equivalents of NAMI-A (2, 4, 6, 8, 10, 15, 20 and 30, respectively).