# Supplementary Information

**to the manuscript**

# Interaction of anticancer Ru(III) complexes with single strand and duplex DNA model systems

**by**

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## Content:

**ESI-MS characterization of the ODN systems.**

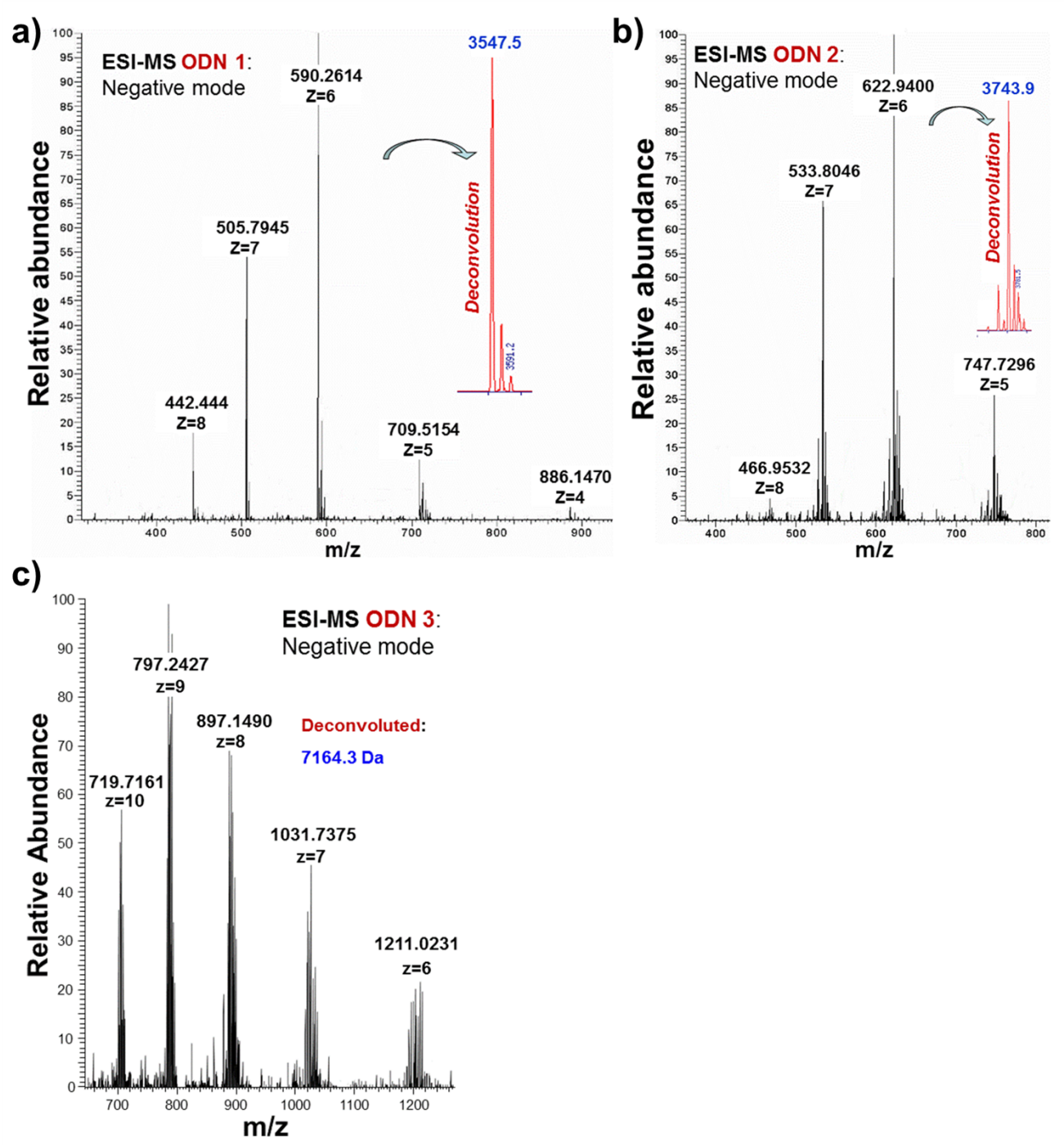



**Figure S1**. ESI-MS spectra of ODNs **1**, **2**, **3** and corresponding molecular ions peaks obtained by deconvolution programs.

## UV and CD analysis of the natural duplex 1/2

Following the variation of the absorbance at 260 nm upon increasing the temperature from 15 to 75 °C, a $T_m$ of 50.3 °C was determined for the duplex **1**/**2** in our working conditions (Figure S2a).

The formation of the duplex structure was monitored also by CD using a tandem cell, *i.e.* a quartz cuvette composed of two distinct compartments, which allows to simultaneously analyse two components in solution, initially separated, by recording first the CD *sum* spectrum and, successively, after mixing, the CD spectrum of the resulting solution (*mix*). In the case of ODNs **1** and **2**, their *sum* spectrum (black line, Figure S2b) was not superimposable to the *mix* (red line), denoting the interaction of the strands and the formation of a B-form DNA duplex structure. The CD signal, after mixing, was found to be stable after ca. 10 min.

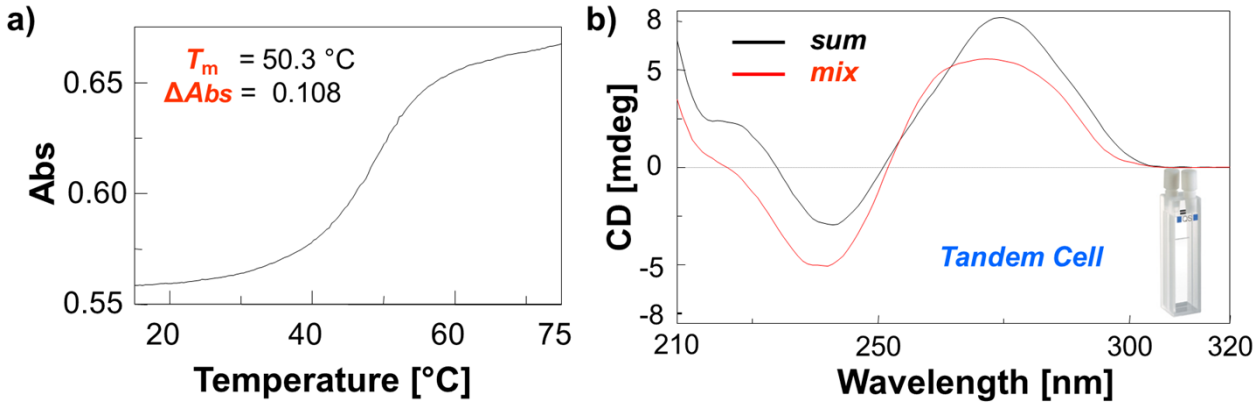
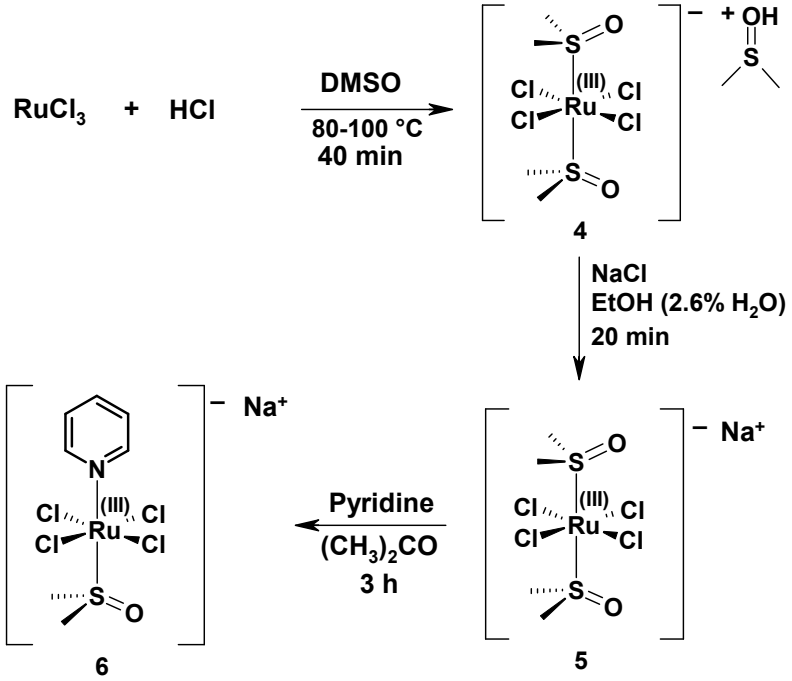



**Figure S2**. a) UV melting curve ($A_{260}$) for duplex **1**/**2** at 2.5 μM concentration in 10 mM phosphate buffer/100 mM NaCl. b) Overlapped CD spectra, recorded in the tandem cell, of ODNs **1** and **2** in separated solutions (*sum* spectrum, black line), and in the solution resulting after mixing of the cuvette and stabilization of the CD signal (*mix* spectrum, red line, 2.5 μM concentration, 10 mM phosphate buffer/100 mM NaCl).

## Synthesis and characterization of the ruthenium complex AziRu

The ruthenium complex AziRu was prepared in our laboratories using reported procedures[1,2] (Scheme S1) starting from ruthenium trichloride. AziRu was identified by: 1) ESI-MS spectrometry (negative mode), which shows the peak of the expected anion, with isotopic pattern almost superimposable to a simulated one (Figure S3a); 2) $^{1}$H-NMR spectroscopy, showing the broad protonic signals at negative ppm (-3.1 and -14.8) of the pyridine and DMSO ligands, diagnostic of a direct binding to the paramagnetic Ru(III) ion (Figure S4), in agreement with literature data,[3] and 3) UV-vis spectroscopy, showing the characteristic band, relative to the ligand-to-metal charge transfer transition (LMCT) for Ru-Cl bond, at ca. 400 nm (Figure S5).




**Scheme S1**. Synthetic scheme for the preparation of AziRu starting from $RuCl_3$.

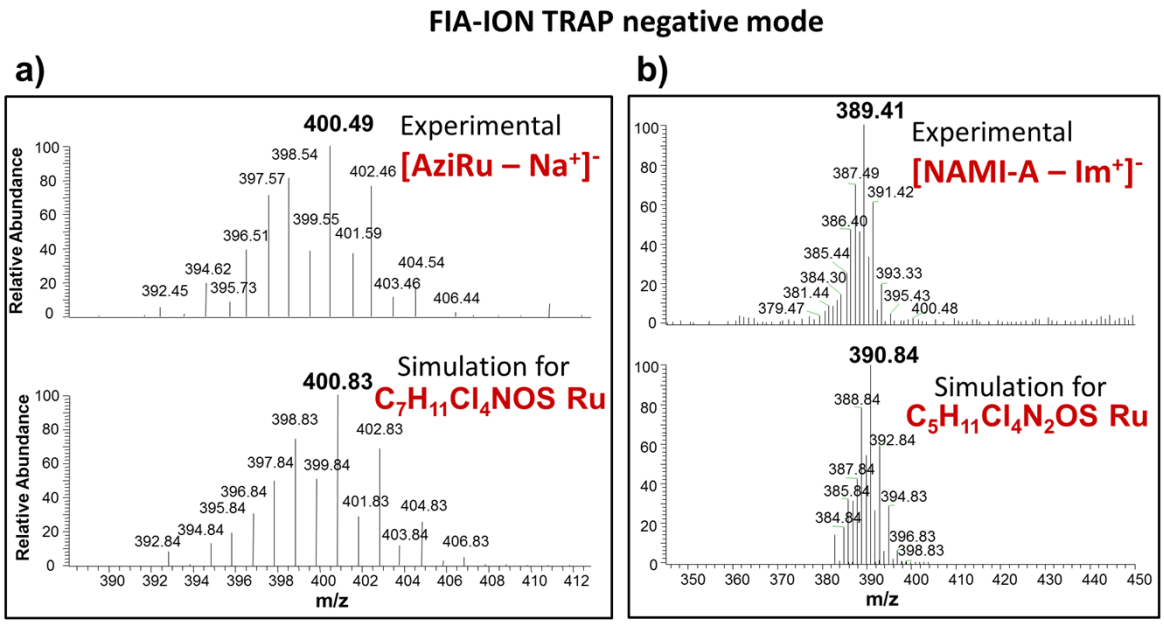



**Figure S3**. Expanded regions of the ESI-MS spectra, recorded in negative ion mode, of AziRu (**a**) and NAMI-A (**b**), freshly dissolved in $CH_3OH/H_2O$, 1:1 (v/v), at a final concentration of 50 μM, compared with the corresponding simulated spectra (bottom part of each panel).

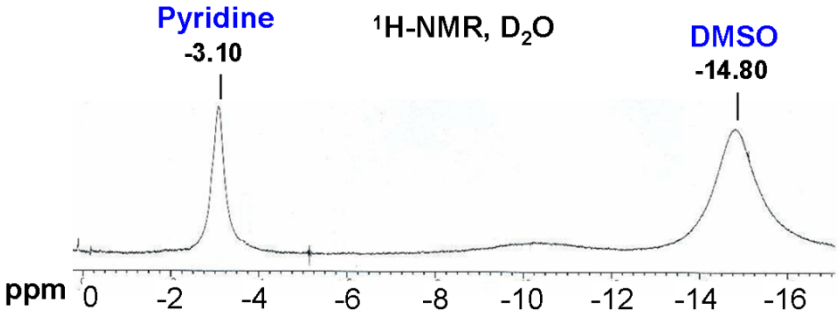



**Figure S4**. $^1$H-NMR spectrum of AziRu (4 mM, 400 MHz) in $D_2O$, registered 5 min after dissolution.

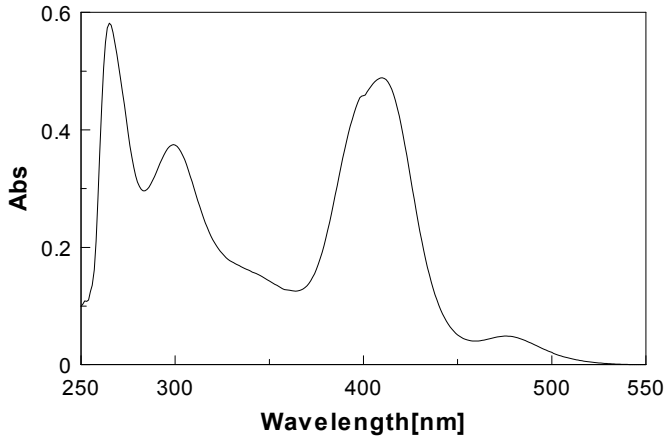



**Figure S5**. UV-vis spectrum of AziRu at 100 μM concentration in DMSO in the region 250-550 nm.

## UV studies

The UV-vis hydrolysis of NAMI-A alone was analysed over time in a saline phosphate buffered solution.

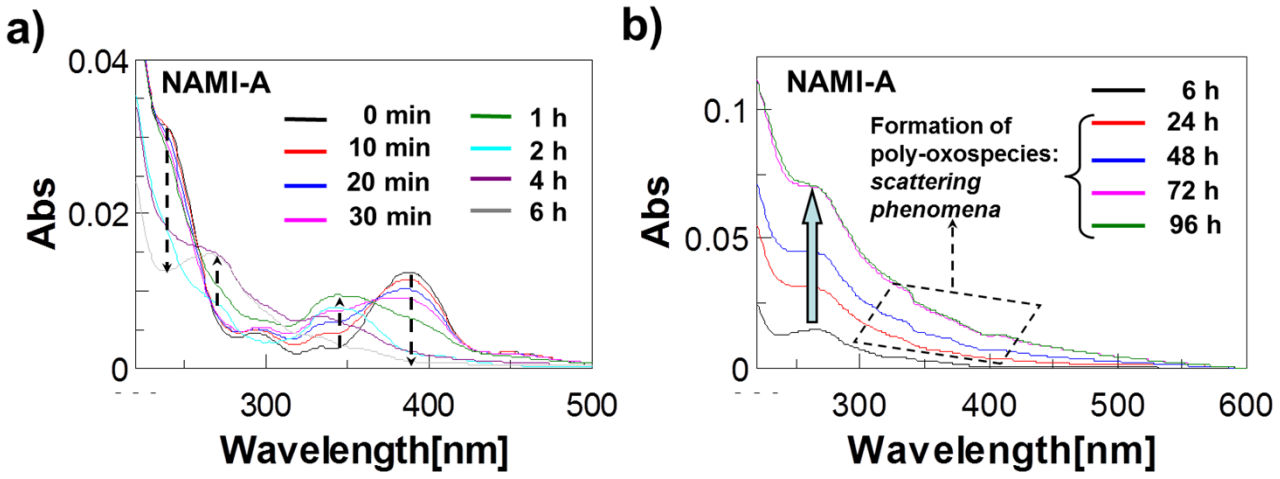



**Figure S6**. UV-vis absorption spectra of NAMI-A (4 μM in 10 mM phosphate buffer/100 mM NaCl, pH = 7.0) registered at different times after dissolution.

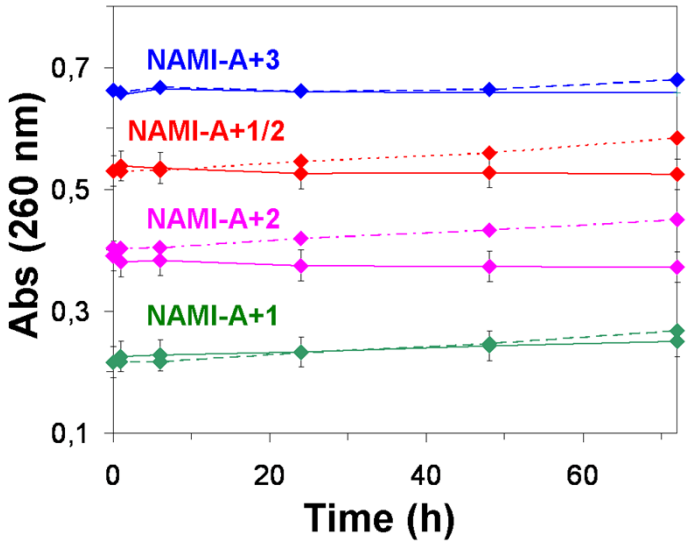



**Figure S7**. Incubation time dependence of the absorbance at 260 nm relative to the solutions containing ODNs **1**, **2** and **3**, and the duplex **1**/**2**, each separately mixed 1:1 with NAMI-A (solid lines); dashed lines are reference curves obtained by adding the absorbance of each oligonucleotidic system with that of NAMI-A alone under the same concentration and incubation time conditions.

The UV-vis hydrolysis of AziRu alone at 50 μM concentration was analysed over time in a saline phosphate buffered solution (Figure S8a) and in $H_2O$ (Fig. S8b).

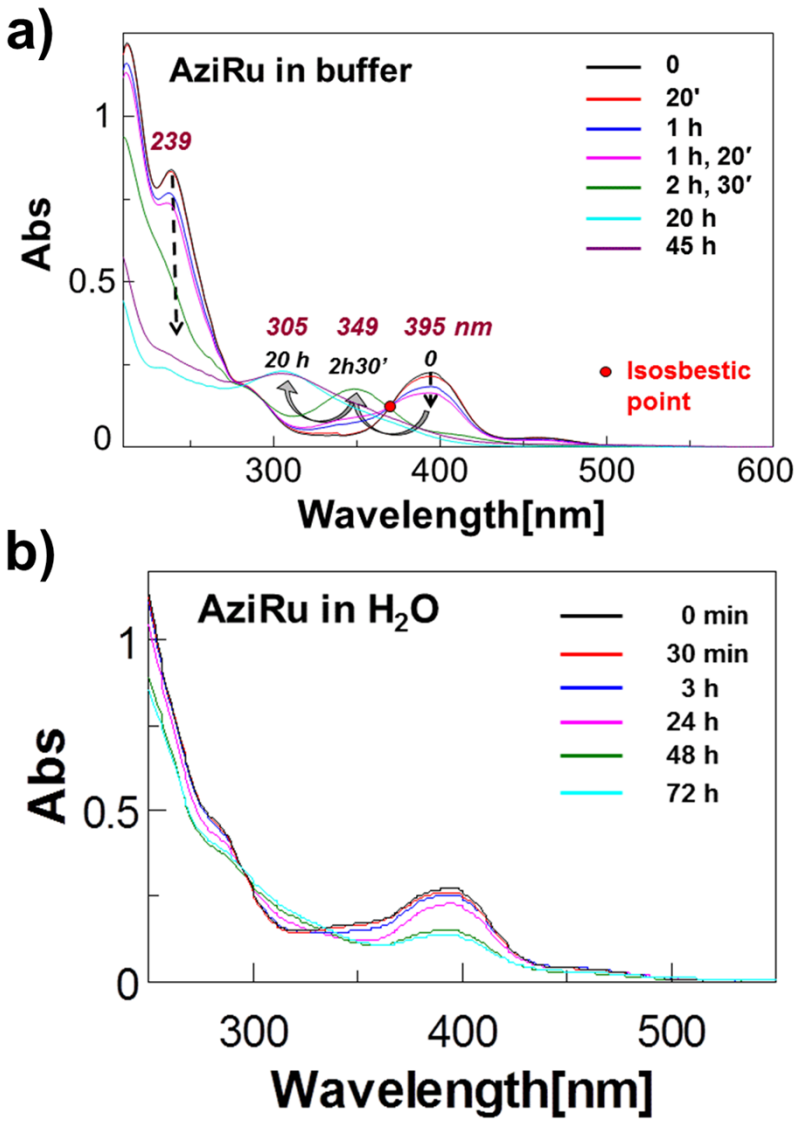



**Figure S8**. UV-vis spectra of AziRu (50 μM) registered at different times after dissolution in 10 mM phosphate buffer/100 mM NaCl, pH = 7.0 (**a**), and pure $H_2O$ (**b**).

When AziRu was dissolved in pure $H_2O$, its hydrolysis resulted slower than in the saline phosphate buffered solution (pH 7.0), and, at least till 72 h, the 395 nm band was still present, while the 349 and 305 nm bands were not detected.

This behaviour reflects the different pH obtained in the two AziRu solutions. In particular, dissolved in pure $H_2O$, AziRu resulted more stable than in solution buffered at neutral or basic pH, due to acidic pH produced in the non-buffered solution, which reached the value of 4.9 at 72 h, as a result of the formation of aqua-complexes (Figure S9).

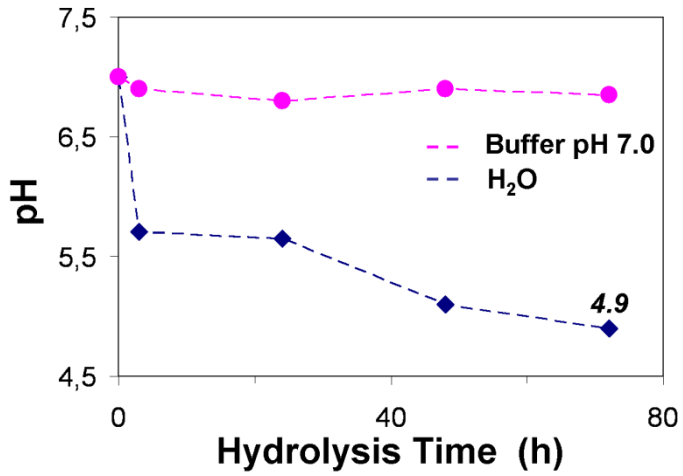



**Figure S9**. pH variation (± 0.1) over time of the solution obtained dissolving AziRu at 50 µM concentration in 10 mM phosphate buffer/100 mM NaCl (magenta circle) and $H_2O$ (blue pyramid).

## CD studies

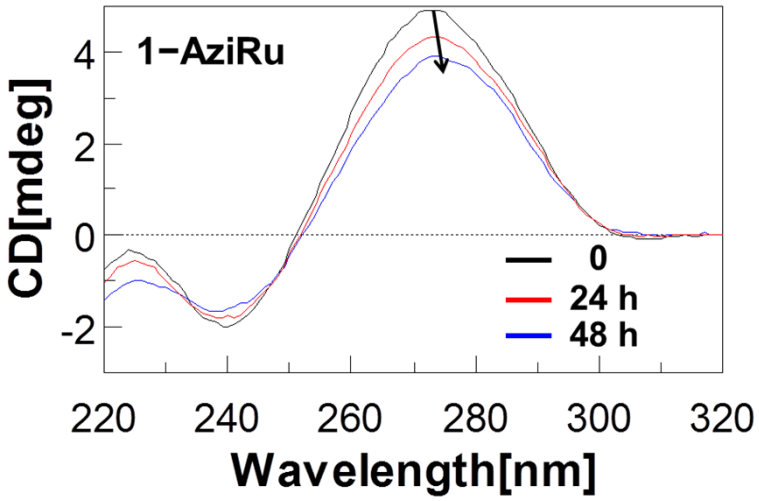



**Figure S10**. CD spectra of ODN **1** (20 μM in 10 mM phosphate buffer/100 mM NaCl), mixed 1:1 with AziRu at different incubation times.

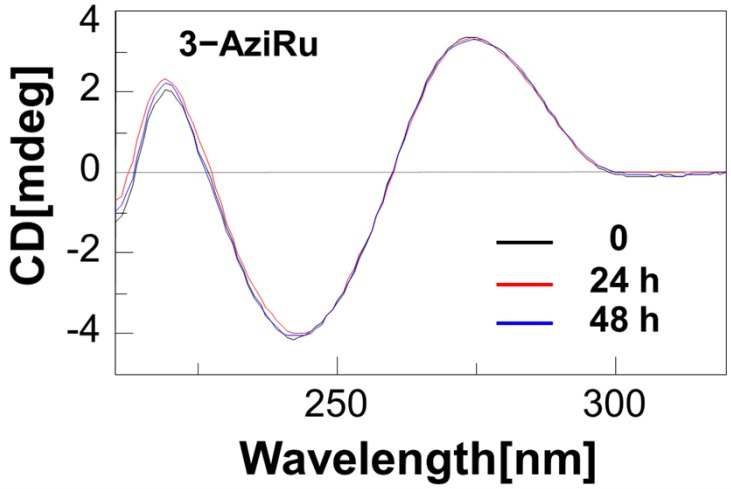



**Figure S11**. CD spectra of ODN **3** (20 μM in 10 mM phosphate buffer/100 mM NaCl), mixed 1:1 with AziRu at different incubation times.

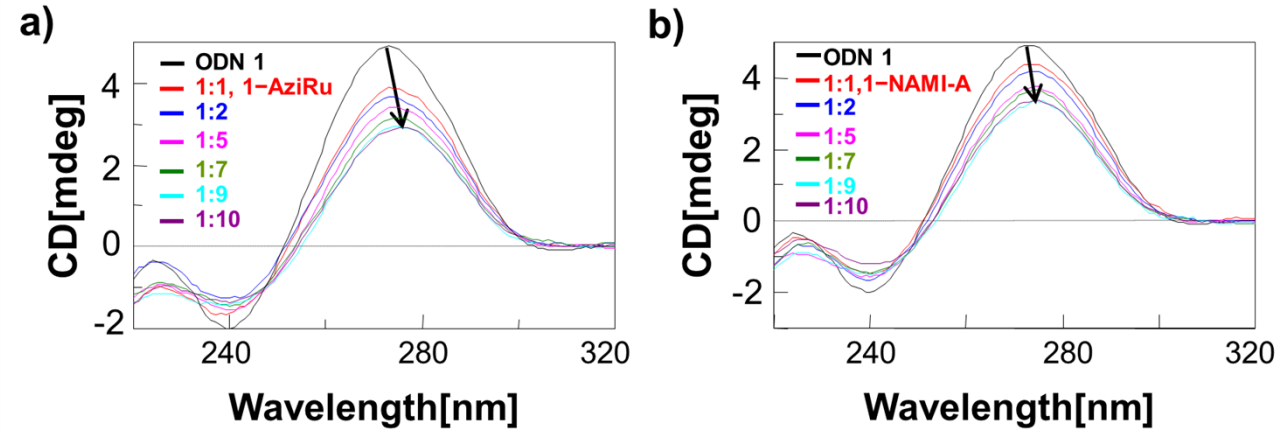



**Figure S12**. CD spectra of ODN **1** (20 μM in 10 mM phosphate buffer/100 mM NaCl) mixed with different amounts (0→10 eq) of AziRu (**a**) and NAMI-A (**b**), registered after 72 h incubation time.

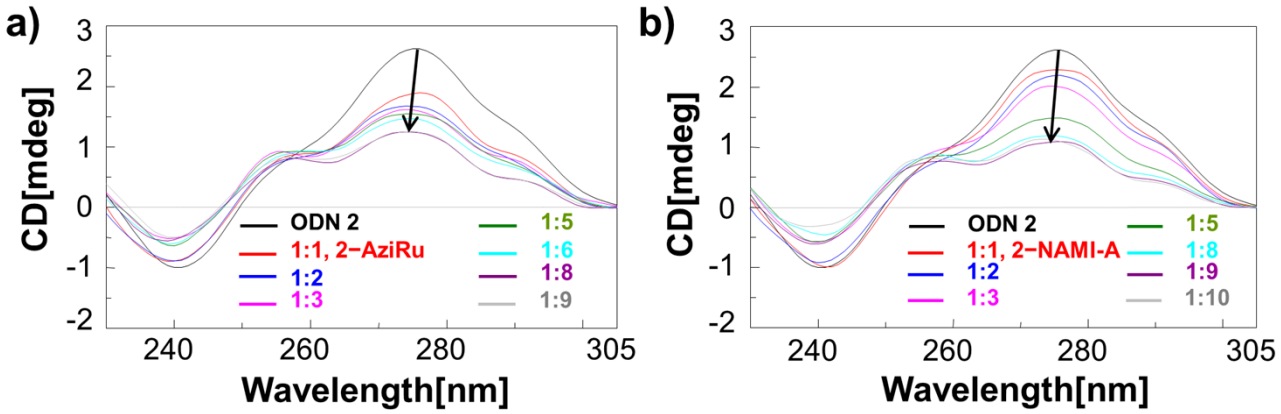



**Figure S13**. CD spectra of ODN **2** (20 μM in 10 mM phosphate buffer/100 mM NaCl) mixed with different amounts (0→10 eq) of AziRu (**a**) and NAMI-A (**b**), registered after 72 h incubation time.

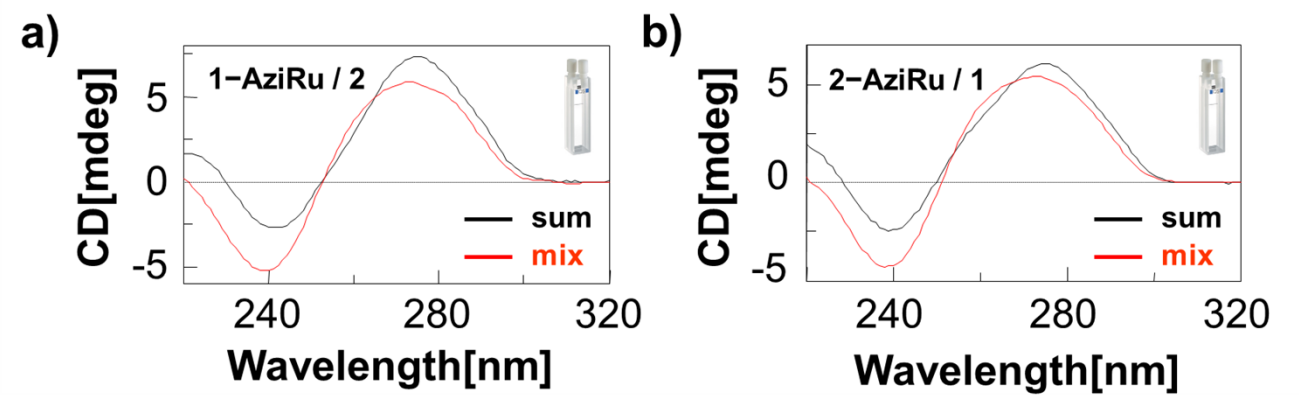



**Figure S14**. CD spectra *sum* (black lines) and *mix* (red lines) of **1**–AziRu/**2** (**a**) and **2**–AziRu/**1** (**b**) at 2.5 μM concentration in 10 mM phosphate buffer/100 mM NaCl registered in a tandem cuvette.

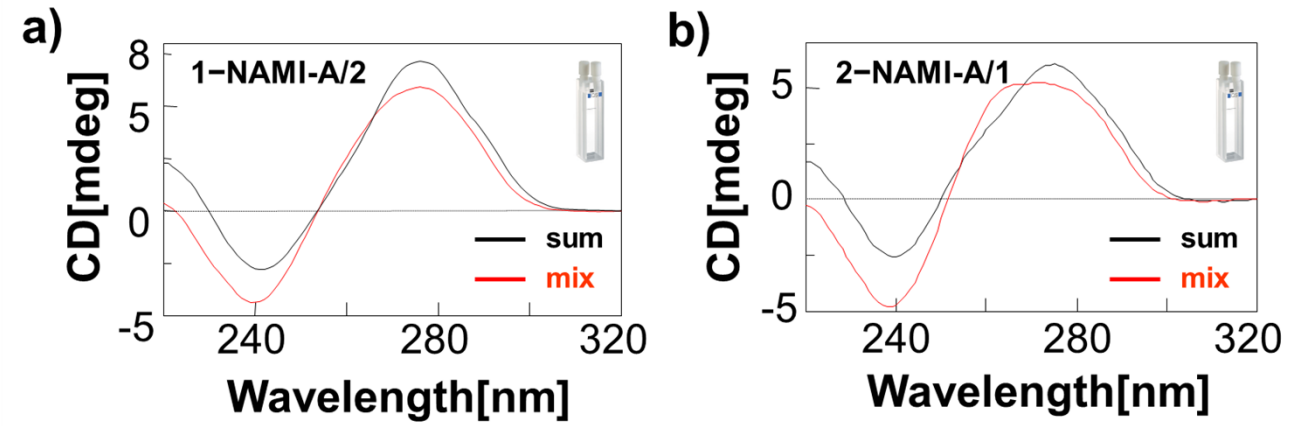



**Figure S15**. CD spectra *sum* (black lines) and *mix* (red lines) of **1**–NAMI-A/**2** (**a**) and **2**–NAMI-A/**1** (**b**) at 2.5 μM concentration in 10 mM phosphate buffer/100 mM NaCl registered in the tandem cuvette.

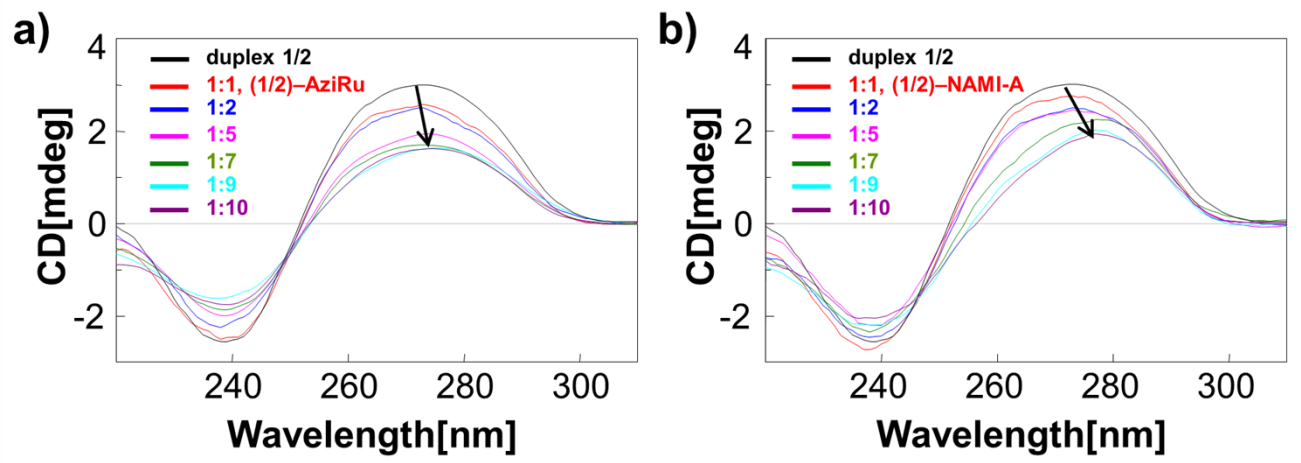



**Figure S16**. CD spectra of the duplex **1**/**2** (10 μM) with different amounts (0→10 eq) of AziRu (**a**) and NAMI-A (**b**), in 10 mM phosphate buffer/100 mM NaCl registered at 72 h incubation time.

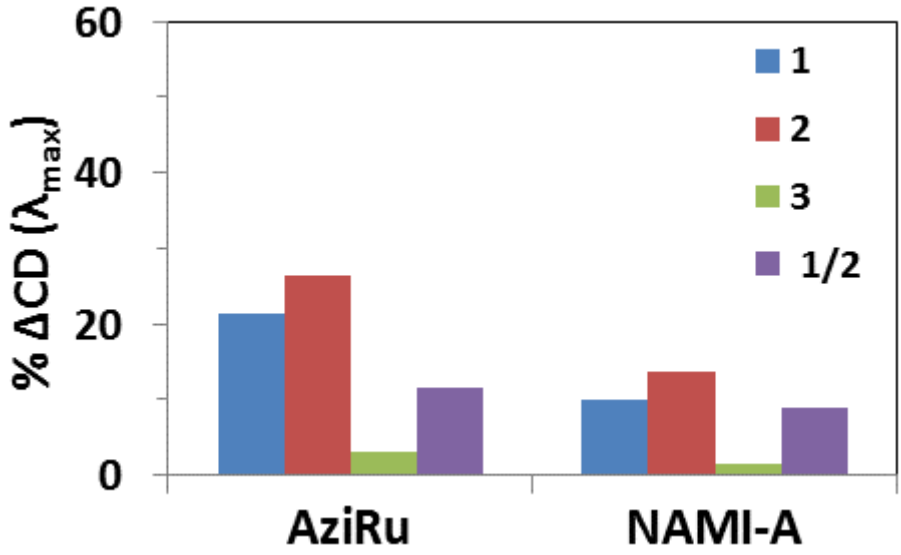



**Figure S17**. Comparison of the % CD signal variation observed in the different ODN systems investigated, upon binding with, respectively, the ruthenium complexes AziRu and NAMI-A. The errors associated with these data are within 3%.

## ESI-MS studies

**Table S1**. Theoretical and deconvoluted peaks relative to the m/z values found in the ESI-MS spectrum of ODN **1** incubated with NAMI-A (see Figure 10 of the main text).

| m/z | Theoretical peak (Da) | Deconvoluted peak (Da) |
|---|---|---|
| 708.51 | 3547.55 | 3547.55 |
| 728.09 | 3648.55 | 3648.55 |
| 741.70 | 3716.70 | 3716.50 |
| 745.50 | 3734.70 | 3735.50 |
| 749.10 | 3752.70 | 3753.50 |

**Table S2**. Theoretical and deconvoluted peaks relative to the m/z values found in the ESI-MS spectrum of ODN **2** incubated with NAMI-A (see Figure 11 of the main text).

| m/z | Theoretical peak (Da) | Deconvoluted peak (Da) |
|---|---|---|
| 747.73 | 3743.65 | 3743.65 |
| 934.91 | 3743.65 | 3743.64 |
| 976.64 | 3912.82 | 3913.56 |
| 980.90 | 3930.82 | 3930.60 |
| 985.65 | 3948.79 | 3949.60 |
| 1246.89 | 3743.65 | 3743.67 |

**Table S3**. Theoretical and deconvoluted peaks relative to the m/z values found in the ESI-MS spectrum of ODN **1** (**a**) and **2** (**b**) incubated with AziRu (see Figure 12 of the main text).

| m/z | Theoretical peak (Da) | Deconvoluted peak (Da) |
|---|---|---|
| **a** | | |
| 606.91 | 3648.56 | 3650.46 |
| 728.09 | 3648.55 | 3648.45 |
| 910.62 | 3648.60 | 3649.48 |
| **b** | | |
| 639.42 | 3844.64 | 3845.52 |
| 767.51 | 3844.65 | 3845.55 |
| 960.13 | 3844.64 | 3847.52 |
| 771.11 | 3862.64 | 3863.55 |
| 774.91 | 3880.64 | 3882.55 |

**References**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3. E. Alessio, G. Balducci, A. Lutman, G. Mestroni, M. Calligaris, W. M. Attia, Synthesis and characterization of 2 new classes of ruthenium(III)-sulfoxide complexes with nitrogen donor ligands (L)sNa[trans-$RuCl_4(R_2SO)(L)$] and mer,cis-$RuCl_3(R_2SO)$- $(R_2SO)(L)$ the crystal-structure of Na[trans-$RuCl_4$(DMSO)- $(NH_3)$],2DMSO, Na[trans-$RuCl_4$(DMSO)(Im)],$H_2O$,$Me_2CO$ (Im) imidazole) and mer,cis-$RuCl_3$(DMSO)(DMSO)$(NH_3)$, *Inorg. Chim. Acta*, **1993**, *203*, 205-217.