Electronic Supporting Information (ESI)

Binding of Al(III) to synthetic RNA. Metal-mediated strand aggregation

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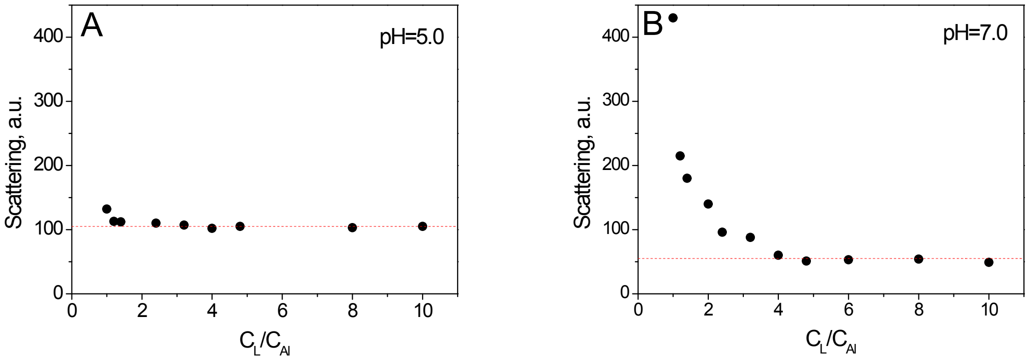

Fig. 1 ESI Scattering of the aluminum/cacodylate solutions for increasing C_L/C_{Al} ratios at (A) pH=5.0 and (B) pH=7.0. The dotted line is the solvent background. $C_{Al}=2.5\times10^{-3}$ M, I=0.1 M, $\lambda_{ex}=\lambda_{em}=250$ nm and T=25.0 °C.

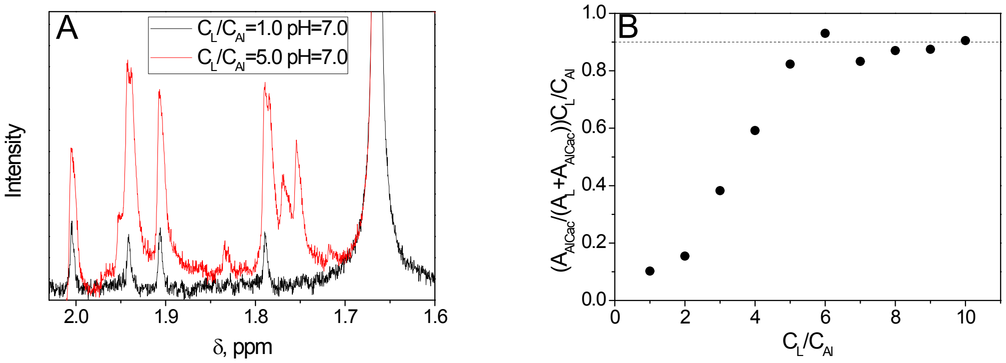

Fig. 2 ESI (A) ^{1}H-NMR spectra at C_L/C_{Al}=1.0 and C_L/C_{Al}=5.0 at pH=7.0. (B) Normalized area of AlCac peaks at pH=7.0 by the equation $A_{norm}=[A_{AlCac}/(A_L+A_{AlCac})]C_L/C_{Al}$, where A_{AlCac} is the area of the complex peaks and A_L the area of the free ligand. $[A_{AlCac}/(A_L+A_{AlCac})]$ is the relative area of the bound cacodylate; multiplication of the relative area by C_L yields the absolute concentration of the bound cacodylate and dividing by C_{Al} it yields the ratio between the ligand and the metal in the complex when the extent of the complex formation reaches the plateau. I=0.1 M ($NaClO_4$) and T=25.0 °C.

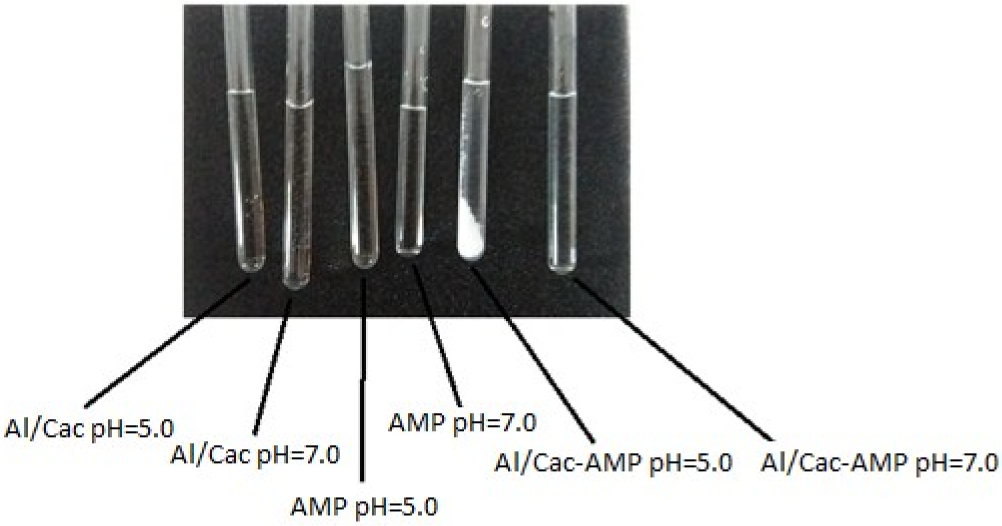

Fig. 3 ESI Photograph of the NMR solutions of the Al/Cac-AMP experiments. $C_{AlCac}=5.0\text{x}10^{-3}$ M, $C_p=5.0\text{x}10^{-3}$ M and I=0.1 M ($NaClO_4$).

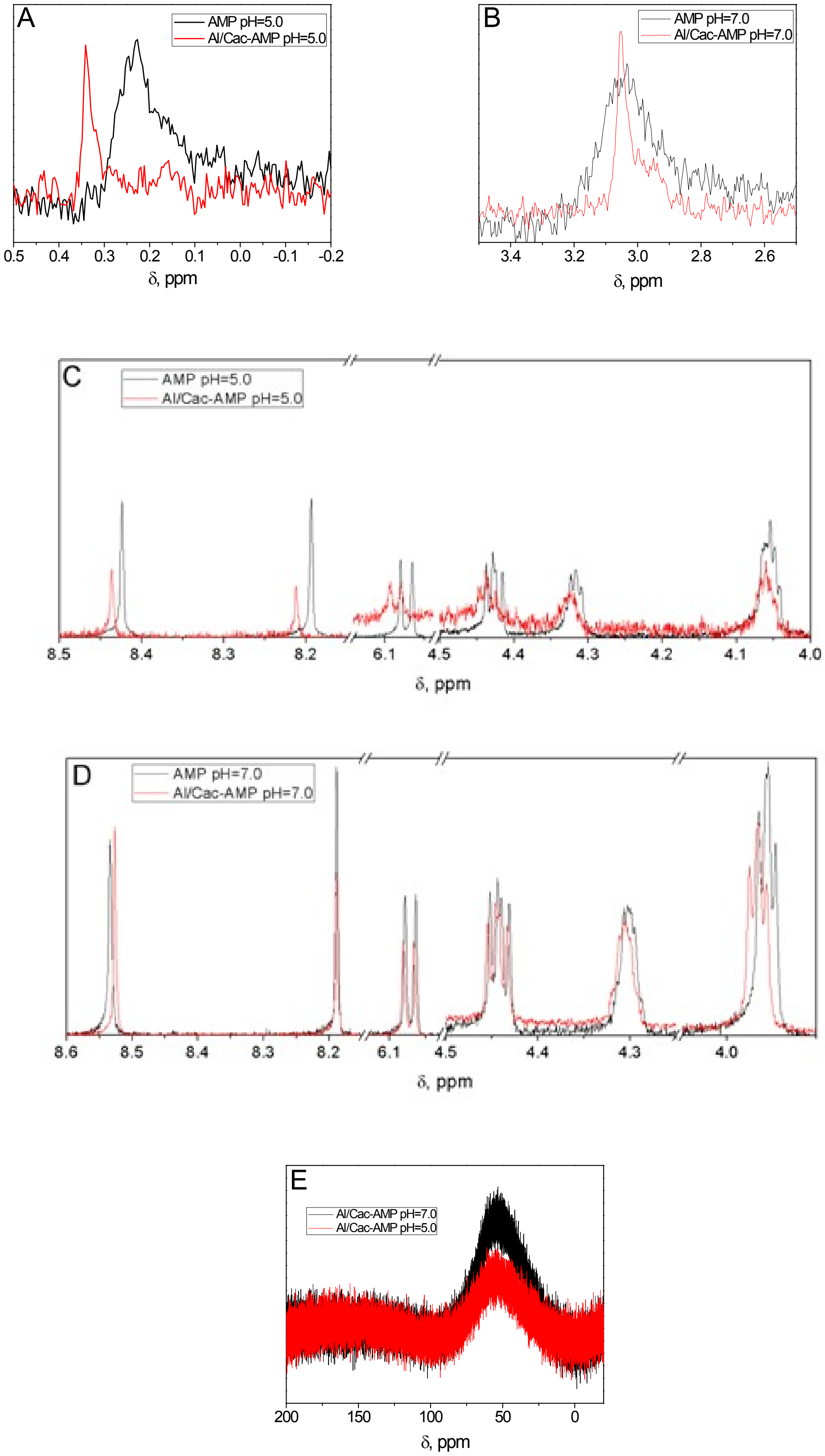

Fig. 4 ESI NMR spectra of Al/Cac and AMP solutions. (A) ^{31}P-NMR of AMP and Al/Cac-AMP at pH=5.0. (B) ^{31}P-NMR of AMP and Al/Cac-AMP at pH=7.0. (C) ^{1}H-NMR of AMP and Al/Cac-AMP at pH=5.0. (D) ^{1}H-NMR of AMP and Al/Cac-AMP at pH=7.0. (E) ^{27}Al-NMR of Al/Cac-AMP at pH=7.0 and 5.0. C_{AlCac}=5.0x10^{-3} M, C_P=5.0x10^{-3} M. I=0.1 M (NaClO$_4$) and T=25.0 °C.

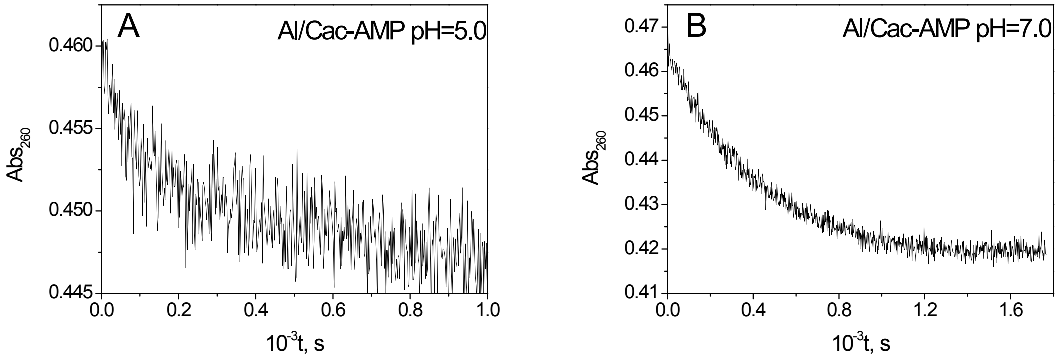

Fig. 5 ESI Kinetic curve of the Al/Cac-AMP system in excess of aluminium. (A) Al/Cac -AMP at pH=5.0 (B) Al/Cac -AMP at pH=7.0. $C_P=3.0\times10^{-5}$ M, $C_{AlCac}/C_P=10$, I=0.1 M ($NaClO_4$) and T=25.0 °C.

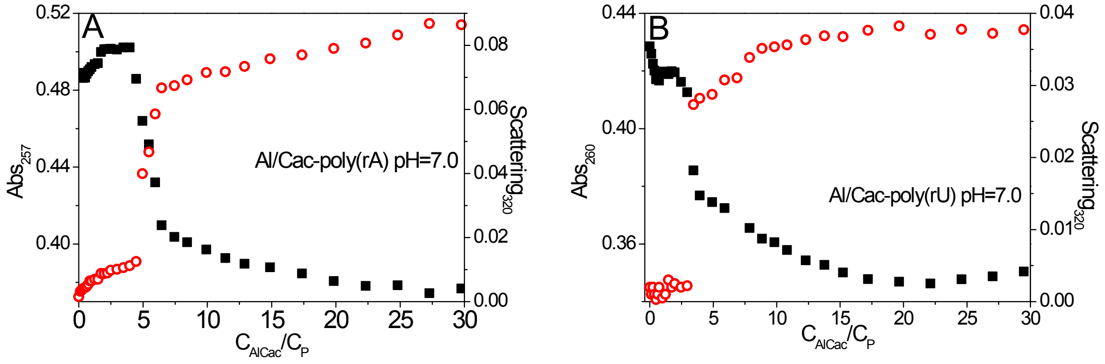

Fig. 6 ESI Comparison of the track at the absorbance maximum and 320 nm for (A) Al/Cac-poly(rA) and (B) Al/Cac-poly(rU). Absorbance maximum (full square) 257 nm for poly(rA) and 260 nm for poly(rU); Scattering at 320nm (open circle). The scattering value was subtracted from the absorbance maximum. $C_P=5.0\times10^{-5}$ M, I=0.1 M ($NaClO_4$), pH=7.0 and T=25.0 °C.

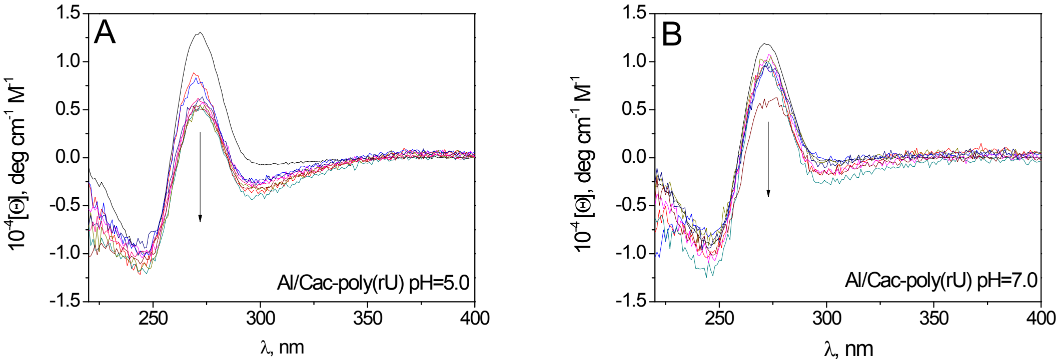

Fig. 7 ESI Circular dichroism of poly(rU) for increasing concentration of Al/Cac, at (A) pH=5.0 and (B) pH=7.0. $C_P=5.0\times10^{-5}$ M, $C_{AlCac}=0\text{-}5.0\times10^{-4}$ M, I=0.1 M ($NaClO_4$) and T=25.0 °C.

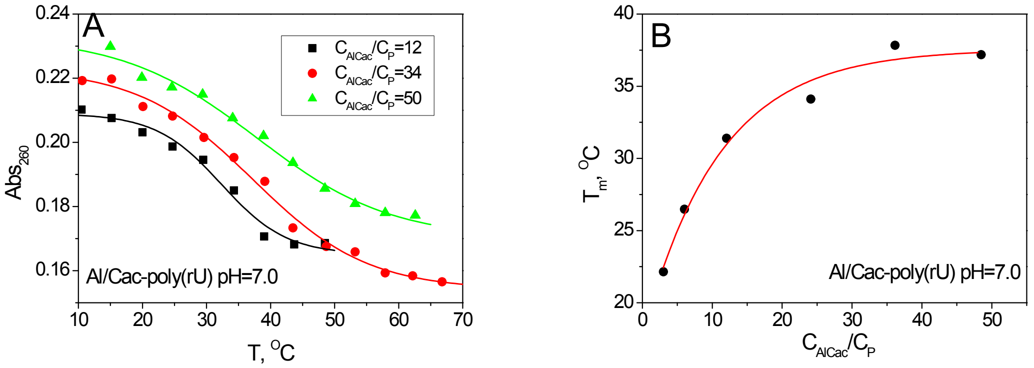

Fig. 8 ESI Denaturation proofs of the aggregated forms of Al/Cac-poly(rU). (A) Examples of melting curves. (B) Track of T_m for different C_{AlCac}/C_P ratios. $C_P=3.4x10^{-5}$ M, I=0.1 M ($NaClO_4$) and pH=7.0.

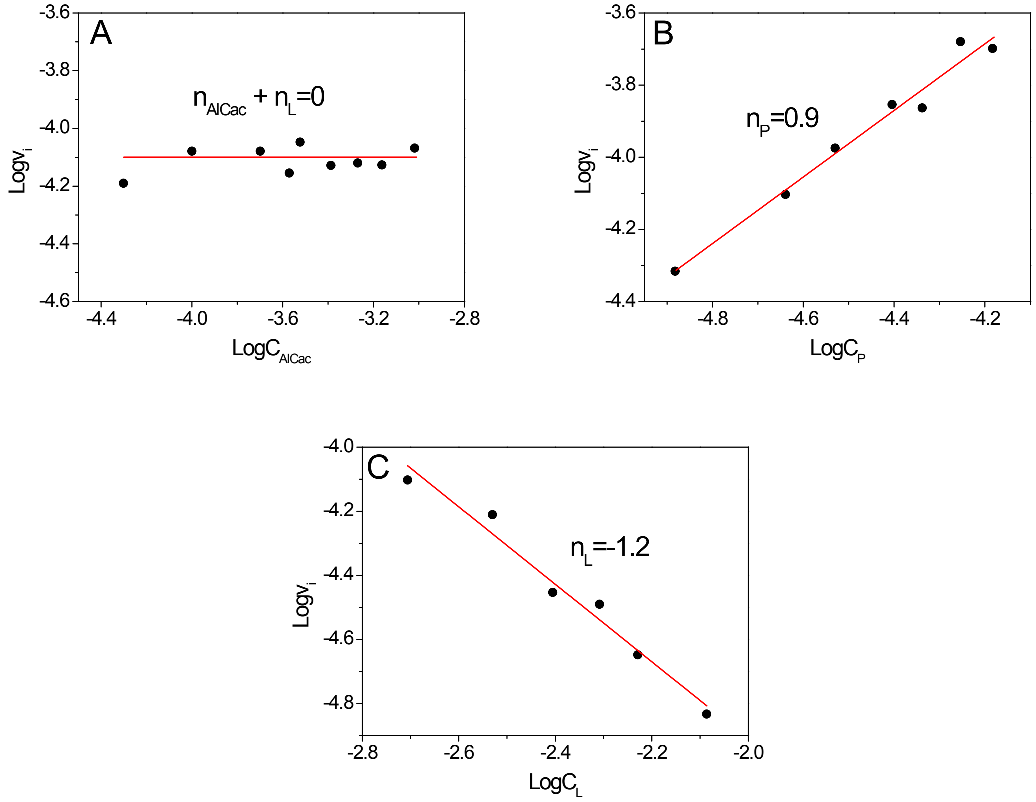

Fig. 9 ESI Dilogarithmic plots of initial rates *versus* (A) C_{AlCac}, (B) C_P and (C) C_L for Al/Cac -$[poly(rA)]_2$ at H=5.0. I=0.1 M ($NaClO_4$) and T=25.0 °C.

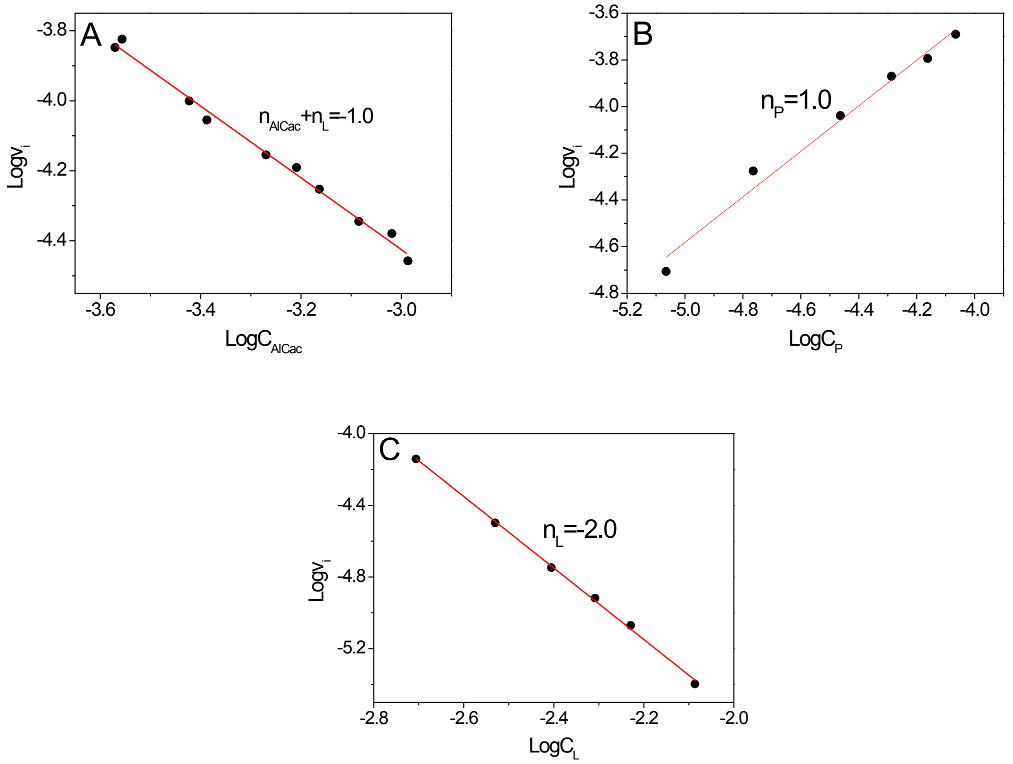

Fig. 10 ESI Dilogarithmic plots of initial rates *versus* (A) C_{AlCac}, (B) C_P and (C) C_L for Al/Cac-poly(rU) at pH=5.0. I=0.1 M ($NaClO_4$) and T=25.0 °C.

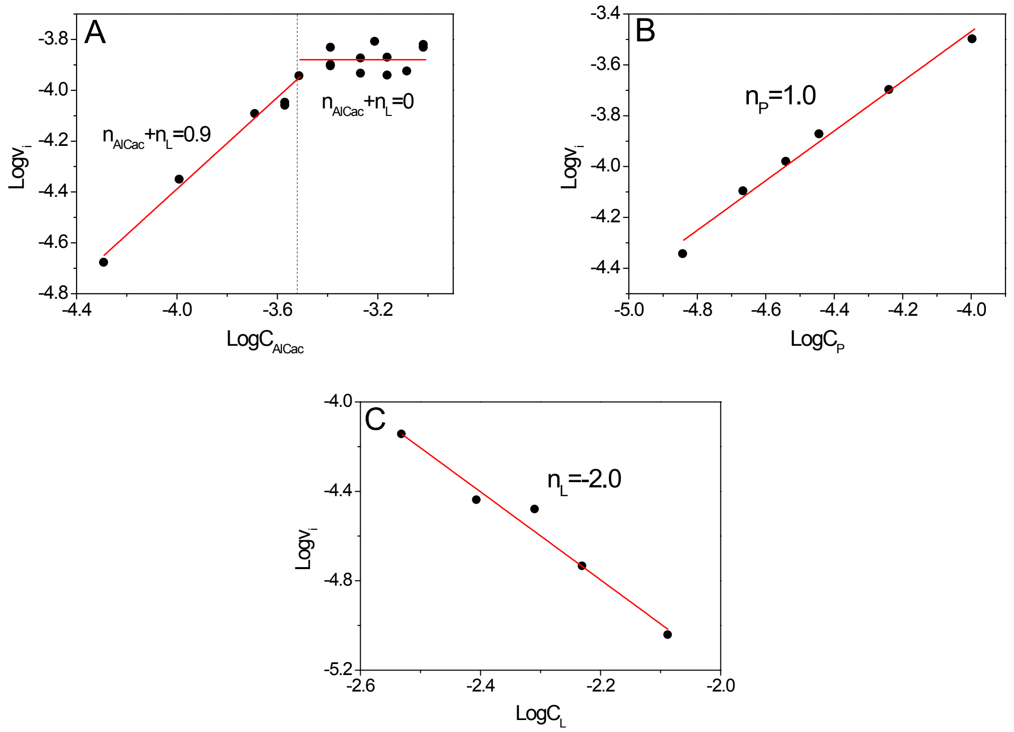

Fig. 11 ESI Dilogarithmic plots of initial rates versus (A) C_{AlCac}, (B) C_P and (C) C_L for Al/Cac-poly(rA) at pH=7.0. I=0.1 M ($NaClO_4$) and T=25.0 °C. The change in the reaction orders at the lower Al/Cac concentration was due to formation of noticeable amounts of M_2L.

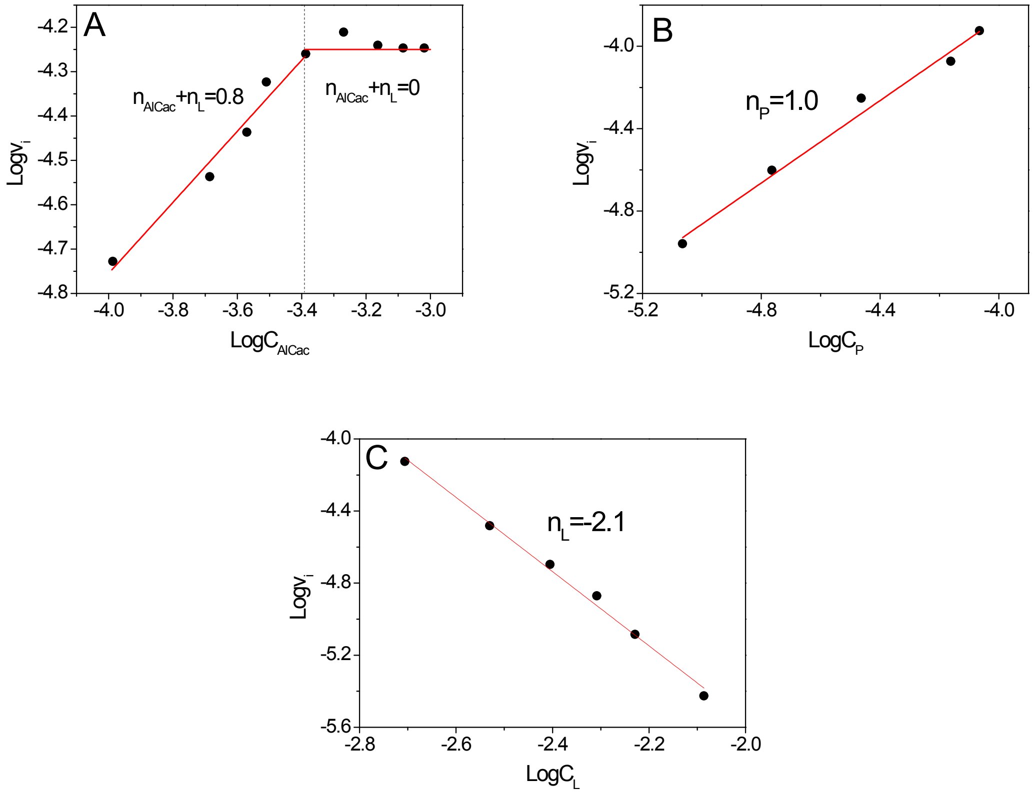

Fig. 12 ESI Dilogarithmic plots of initial rates *versus* (A) C_{AlCac}, (B) C_P and (C) C_L for Al/Cac-poly(rU) at pH=7.0. I=0.1 M ($NaClO_4$) and T=25.0 °C. The change in the reaction orders at the lower Al/Cac concentration was due to formation of noticeable amounts of M_2L.

Table 1 ESI Kinetic constants obtained from plots of the initial rate equations. I=0.1 M and T=25.0 °C.

	Al/Cac-[poly(rA)]$_2$ pH 5 ($s^{-1}M$)	Al/Cac-poly(rU) pH 5 ($s^{-1}M^2$)	Al/Cac-poly(rA) pH 7 ($s^{-1}M^2$)	Al/Cac-poly(rU) pH 7 ($s^{-1}M^2$)
Varying C_L	$k_3/(2K_2)=3.0\times10^{-2}$	$k_1/(2K_1K_2)= 7.8\times10^{-5}$	$k_2/(4K'^2_2)=0.34$	$k_{2'}/(4K'^2_2)=0.12$
Varying C_P	$k_3/(2K_2)= 7.4\times10^{-2}$	$k_1//(2K_1K_2)= 8.1\times10^{-5}$	$k_2/(4K'^2_2) =0.23$	$k_{2'}/(4K'^2_2)=0.12$
Varying C_{AlCac}	$k_3/(2K_2)= 5.0\times10^{-2}$	$k_1/(2K_1K_2)= 7.0\times10^{-5}$	$k_2/(4K'^2_2)=0.17$	$k_{2'}/(4K'^2_2)=0.10$
Mean Value (Confidence 95%)	$k_3/(2K_2)= 5\times10^{-2}$ (2.5×10^{-2})	$k_1/(2K_1K_2)= 7.6\times10^{-5}$ (6×10^{-6})	$k_2/(4K'^2_2)=0.24$ (0.10)	$k_{2'}/(4K'^2_2)=0.11$ (0.01)

Table 2 ESI Avrami parameters obtained for Al/Cac-poly(rA) aggregation. I=0.1 M, pH=7.0 and T=25.0 °C.

$C_P = 3.4\times10^{-5}$M (fixed)				$C_{AlCac} = 4.1\times10^{-4}$M (fixed)			
C_{AlCac} (10^4 M)	A	10^{-2} $t_{1/2}$, s	m	C_P (10^5M)	A	$10^{-2}t_{1/2}$, s	m
2.7	0.048±0.001	6.4±0.5	4.1±0.1	1.1	0.022±0.001	1.6±0.3	2.0±0.1
4.1	0.050±0.001	4.6±0.4	4.4±0.1	1.4	0.025±0.001	1.1±0.2	1.7±0.1
5.4	0.053±0.003	3.6±0.5	4.2±0.1	2.2	0.036±0.001	2.7±0.3	2.9±0.1
6.9	0.055±0.001	3.4±0.4	4.3±0.1	2.9	0.048±0.001	3.1±0.3	3.5±0.1
8.2	0.058±0.001	2.6±0.3	4.1±0.1	3.6	0.056±0.001	4.8±0.2	4.4±0.1
9.6	0.058±0.001	2.9±0.4	4.0±0.1	5.7	0.068±0.001	8.6±0.2	4.1±0.1
				7.2	0.078±0.001	13±2	3.8±0.1

Table 3 ESI Avrami parameters obtained for Al/Cac-poly(rU) aggregation. I=0.1 M, pH=7.0 and T=25.0 °C.

$C_P = 3.4\times10^{-5}$M (fixed)				$C_{AlCac} = 4.1\times10^{-4}$M (fixed)			
C_{AlCac} (10^4 M)	A	10^{-2} $t_{1/2}$, s	m	C_P (10^5M)	A	10^{-2} $t_{1/2}$, s	m
2.7	0.058±0.001	18±2	2.0±0.1	0.9	0.025±0.001	6.7±0.8	1.5±0.1
3.1	0.078±0.001	12±1	1.8±0.1	1.7	0.049±0.001	5.6±0.7	1.4±0.1
5.4	0.080±0.001	9±1	1.8±0.1	3.4	0.079±0.001	9±1	1.5±0.1
6.9	0.074±0.001	9±1	1.8±0.1				
8.2	0.079±0.001	8±1	1.5±0.1				
9.6	0.062±0.001	9±1	2.1±0.1				
10.3	0.075±0.001	9±1	1.4±0.1				

Appendix I. Determination of initial rate equation of Al/Cac-poly(rU) at pH=5.0.

For Al/Cac-poly(rU) at pH=5.0 the hypothesized model is

$$M_2L_2 \overset{1/K_2}{\rightleftarrows} M_2L + L \tag{I.1}$$

$$M_2L \overset{1/K_1}{\rightleftarrows} 2M + L \tag{I.2}$$

$$2M + P \xrightarrow{k_1} products \tag{I.3}$$

where M is the free metal, L the free ligand, P is the RNA monomeric unit, M_2L_2 and M_2L are the metal/ligand complexes. The apparent constants K_1 and K_2 of the metal/ligand complex at pH=5.0 are given by (I.4) and (I.5), with [M], [L], $[M_2L]$ and $[M_2L_2]$ the molar concentration of the free metal, the free ligand and the complexes at the equilibrium, respectively, whereas k_1 is the kinetic constant of the direct reaction.

$$K_1 = \frac{[M_2L]}{[M]^2[L]} \tag{I.4}$$

$$K_2 = \frac{[M_2L_2]}{[M_2L][L]} \tag{I.5}$$

The initial rate of the reaction is given by Equation (I.6)

$$v_i = k_1[M]^2[P] \tag{I.6}$$

Analytical concentration of the RNA (C_P) and metal (C_{AlCac}) are expressed by eqns (I.7) and (I.8)

$$C_P = [P] + [products] \tag{I.7}$$

$$C_{AlCac} = [M] + 2[M_2L_2] + 2[M_2L] + [products] \tag{I.8}$$

In the initial stage of the reaction, the concentration of the products is negligible with respect to the reactant concentrations. So, it can be excluded from (I.7) and (I.8). The reaction order with respect to L is n_L=-2, which indicates that large amounts of M_2L_2 are formed. Hence, $C_{AlCac} \approx 2[M_2L_2]$. In addition, as we work in excess of cacodylate, it follows that $[L] \approx C_L$.
From (I.4) and (I.5) one obtains (I.9)

$$[M]^2 = \frac{C_{AlCac}}{2K_1K_2C_L^2} \qquad (I.9)$$

and, then, equation (I.10) for the initial reaction rate, provided that reaction (I.1) and (I.2), are fast compared to reaction (I.3).

$$v_i = \frac{k_1}{2K_1K_2} \frac{C_{AlCac}C_P}{C_L^2} \qquad (I.10)$$

Appendix II. Determination of initial rate equation of Al/Cac -poly(rA) and Al/Cac-poly(rU) at pH=7.0.

For Al/Cac-poly(rU) and Al/Cac -poly(rA) at pH=7.0 the hypothesized model is

$$M_2L_2 \overset{1/K'_2}{\rightleftarrows} M_2L + L \qquad (II.1)$$

$$2M_2L + P \overset{k_2(k'_2)}{\rightarrow} products \qquad (II.2)$$

where L is the free ligand, P is the RNA monomeric unit, M_2L_2 and M_2L are the metal/ligand complexes. The apparent equilibrium constant K'_2 of the metal/ligand complex at pH=7.0 is given by (II.3) with [L], $[M_2L_2]$ and $[M_2L]$ the molar concentration of the free ligands and the complexes at the equilibrium, respectively, whereas k_2 and k'_2 are the kinetic constants of the forward reaction for the Al/Cac-poly(rA) and Al/Cac-poly(rU) systems, respectively.

$$K'_2 = \frac{[M_2L_2]}{[M_2L][L]} \qquad (II.3)$$

The initial rate of the reaction is:

$$v_i = k_2[M_2L]^2[P] \qquad (II.4)$$

Analytical concentration of the RNA (C_P) and metal (C_{AlCac}) are expressed by Equations (II.5) and (II.6)

$$C_P = [P] + [products] \qquad (II.5)$$

$$C_{AlCac} = 2[M_2L] + 2[M_2L_2] + [products] \qquad (II.6)$$

For the reasons given in Appendix I it turns out that for this system as well $C_{AlCac} \approx 2[M_2L_2]$ and $[L] \approx C_L$.
Rearranging (II.3) one obtains (II.7)

$$[M_2L]^2 = \frac{C_{AlCac}^{2}}{4K'^2_2 C_L^2} \qquad \text{(II.7)}$$

Which, introduced in (II.4), yields the expression (II.8) for the initial reaction rate.

$$v_i = \frac{k_2 \ C_{AlCac}^{2} C_P}{4K'^2_2 \quad C_L^2} \qquad \text{(II.8)}$$

Appendix III. Determination of initial rate equation Al/Cac-[poly(rA)]$_2$ at pH=5.0.

For Al/Cac-[poly(rA)]$_2$ at pH=5.0 the proposed model is

$$M_2L_2 \overset{1/K_2}{\rightleftarrows} M_2L + L \qquad \text{(III.1)}$$

$$M_2L + P \overset{k_3}{\rightarrow} products \qquad \text{(III.2)}$$

where L is the free ligand, P is the RNA monomeric unit, M_2L and M_2L_2 are the metal/ligand complexes. The apparent constant K_2 of the metal/ligand complex is given by (III.3), being [L], $[M_2L]$ and $[M_2L_2]$ the molar concentration of the free ligand and of the metal/ligand complexes at the equilibrium, respectively, whereas k_3 is the kinetic constant of the direct reaction.

$$K_2 = \frac{[M_2L_2]}{[M_2L][L]} \qquad \text{(III.3)}$$

The initial rate of the slow reaction is:

$$v_i = k_3[M_2L][P] \qquad \text{(III.4)}$$

Analytical concentration of the RNA (C_P) and metal (C_{AlCac}) are expressed by Equation (III.5) and (III.6)

$$C_P = [P] + [products] \tag{III.5}$$

$$C_{AlCac} = 2[M_2L] + 2[M_2L_2] + [products] \tag{III.6}$$

For the reasons given in Appendix I, it follows that $C_{AlCac} \approx 2[M_2L_2]$ and $[L] \approx C_L$.
From (III.3) one obtains (III.7)

$$[M_2L] = \frac{C_{AlCac}}{2K_2C_L} \tag{III.7}$$

which, introduced in (III.4), yields the initial reaction rate (III.8)

$$v_i = \frac{k_3\ C_{AlCac}C_P}{2K_2\quad C_L} \tag{III.8}$$