J Biol Inorg Chem (2014) 19 (Suppl 2):S815–S832
DOI 10.1007/s00775-014-1163-0

POSTER PRESENTATION

Metal–nucleic acid interactions

P 110

Metal-mediated base pairs with 6-substituted purines: a combined experimental and computational approach

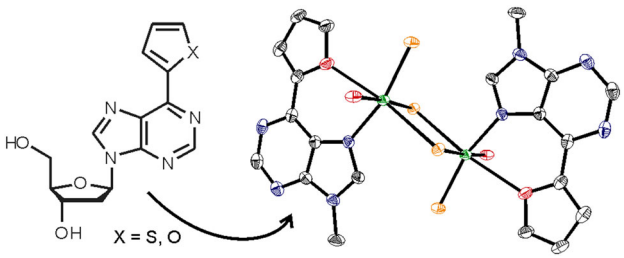
Indranil Sinha[1,2], Celia Fonseca Guerra[3], F. Matthias Bickelhaupt[3,4], Jens Müller[1,2]

[1]Westfälische Wilhelms-Universität Münster and
[2]NRW Graduate School of Chemistry, Corrensstraße 28/30, 48149, Münster, Germany;
[3]Department of Theoretical Chemistry and Amsterdam Center for Multiscale Modeling (ACMM), Scheikundig Laboratorium der Vrije Universiteit, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands;
[4]Institute of Molecules and Materials, Radboud University Nijmegen, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands

The use of DNA as sequence-specific, self-assembling building blocks has become a promising promenade for the construction of bio-inspired nanoarchitectures. In this context, metal-mediated base pairs have evolved as a convenient and versatile method for the site-specific functionalization of nucleic acids with metal ions. In metal-mediated base pairs, the hydrogen bonds present in natural base pairs are formally replaced by coordinative bonds to metal ions. Hence, these transition metal ions can be site-specifically introduced into the oligonucleotide sequence and are located inside the duplex along the helical axis [1].

Purine derivatives are of great interest for metal-mediated base pairs because of their structural similarity to the natural nucleobases. We performed a systematic study of a family of purine-based artificial nucleosides with an additional donor moiety attached to the C6 position. Incorporation of these nucleosides into DNA led to a variety of new metal-mediated base pairs [2]. In principle, different coordination modes are feasible (Watson–Crick edge *vs.* Hoogsteen edge, resulting in antiparallel *vs.* parallel-stranded DNA). The experimental conditions (ionic strength, pH) can be used to pre-select the conformation of the DNA. Computational studies were used to elucidate the most stable and preferred conformation as well as the kinetics of the formation of the metal-mediated base pairs.

Financial support by GSC-MS, NWO-CW, NWO-EW, COST CM1105 is acknowledged.



References

1. Scharf P, Müller J (2013) ChemPlusChem 78:20–34; Johannsen S, Megger N, Böhme D, Sigel RKO, Müller J (2010) Nat Chem 2:229–234; Megger DA, Fonseca Guerra C, Hoffmann J, Brutschy B, Bickelhaupt FM, Müller J (2011) Chem Eur J 17:6533–6544
2. Sinha I, Kösters J, Hepp A, Müller J (2013) Dalton Trans 42:16080–16089

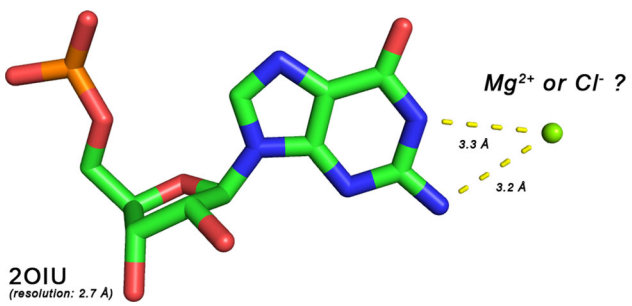
P 111

Electron density misattributions in RNA crystallographic structures: Mg^{2+} or anions in ribozymes?

Luigi D'Ascenzo[1], Pascal Auffinger[1]

[1]Architecture et Réactivité de l'ARN, Université de Strasbourg, Institut de Biologie Moléculaire et Cellulaire du CNRS, 15 rue René Descartes, 67084 Strasbourg, France

Magnesium cations are considered to be essential for structure and function of RNA systems and were supposed to govern the catalytic mechanism of all ribozyme types. This paradigm has evolved in the last decade. Divalent metal ions were confirmed as catalysts in self-splicing ribozymes but displaced by nucleobases in self-cleaving ones. In order to understand the role of Mg^{2+} cations in their catalytic mechanism, reliable crystal structures are required. Yet, the attribution of electron densities to ionic species is not always straightforward and can lead to quite disturbing misattributions [1]. We present a case of misattribution that was shown to take place at high concentrations of either Cl^- or SO_4^{2-} ions and that has been associated with several ribozyme crystal structures. These systematic errors are associated with electron densities located at ≈3.2 Å (Cl^-) or ≈3.8 Å (SO_4^{2-}) from electropositive sites such as $-NH_2$ groups (see figure). These results question further the participation of divalent metals in self-splicing ribozymes. Such misattribution errors might be avoided through a better understanding of anion-binding properties to nucleic acids [1,2].



References
1. Auffinger P, Bielecki L, Westhof E (2004) Structure 12:379–388
2. D'Ascenzo L, Auffinger P (2014) in: Methods in Molecular Biology. Nucleic acids crystallography: Methods and Protocols Ennifar E (ed.) Humana Press in press.

P 112

Condensation properties of antitumor dinuclear Ru(II) arene complexes with aliphatic linkers: relation to DNA binding and cytotoxicity

Olga Novakova[1], Martina Stankova[1], Jaroslav Malina[1], Bernhard K. Keppler[2], Viktor Brabec[1]
[1]Institute of Biophysics, Academy of Sciences of the Czech Republic, CZ-61265 Brno, Czech Republic. olga@ibp.cz;
[2]University of Vienna, Institute of Inorganic Chemistry, Austria

Ruthenium(II) organometallic complexes have been gaining popular interest as potential anticancer agents. Biological effects of a number of these compounds are connected with their binding to nuclear DNA. The resulting DNA damage triggers downstream effects including inhibition of replication and transcription, cell cycle arrest, and apoptosis or necrosis. Structures of new, water-soluble dinuclear Ru(II) arene compounds with long flexible linkers make it possible to predict that they can also condense DNA. These complexes, in which two {(η^6-p-isopropyltoluene)RuCl[3-(oxo-κO)-2-methyl-4-pyridinonato-κO_4]} units are linked by aliphatic chains of different length [$(CH_2)_n$ (n = 4, 6, 8, 12)] were found to exert promising cytotoxic effects in human cancer cells. A pronounced influence of the spacer length on the in vitro anticancer activity was found.

DNA condensation and cross-linking by these dinuclear Ru(II) arene compounds were examined by various methods of biophysics, biochemistry and molecular biology. The complexes bind DNA forming intrastrand and interstrand cross-links in one DNA molecule. These dinuclear complexes also form specific DNA lesions which can efficiently cross-link proteins to DNA. Very interesting phenomenon specific for the DNA binding of these dinuclear Ru(II) arene complexes is that they form interduplex cross-links that are tethered by ruthenium–DNA bonds. In accordance with the ability of dinuclear Ru(II) arene complexes to cross-link two DNA duplexes, the results of the present work demonstrate that these dinuclear complexes also condense DNA. The concept for the design of agents based on dinuclear Ru(II) arene complexes with sufficiently long linkers between two Ru centers may result in new compounds which exhibit a variety of biological effects and can be also useful in nucleic acids research.

Financial support by the Czech Science Foundation (13-08273S) is gratefully acknowledged.

References
1. Novakova O, Nazarov AA, Hartinger ChG, Keppler BK, Brabec V (2009) Biochem Pharmacol 77:364–374
2. Mendoza-Ferri MG, Hartinger ChG, Mendoza MA, Groessl M, Egger AE, Eichinger RE, Mangrum JB, Farrell NP, Maruszak M, Bednarski PJ, Klein F, Jakupec MA, Nazarov AA, Severin K, Keppler BK (2009) J Med Chem 52:916–925

P 113

X-ray crystal structure of an octameric RNA duplex and different divalent and trivalent metal ions

Michelle F. Schaffer[1], Joachim Schnabl[1], Bernhard Spingler[1], Guanya Peng[2], Vincent Olieric[2], Roland K.O. Sigel[1]
[1]Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland;
[2]Swiss Light Source at Paul Scherrer Institute, 5232 Villigen, Switzerland

The principle of charge neutralization and electrostatic condensation require cations to overwhelm the repulsive forces of the negatively charged backbone of RNA to adopt its three-dimensional structure [1,2]. A precise structural knowledge of RNA-metal ion interaction is crucial to understand the role of metal ions in the catalytic or regulatory activity of RNA [1,3]. In our study we use an octameric RNA duplex as a model system to investigate the coordination of various metal ions to specific binding sites and to understand the interactions between metal ions and RNA.

We were able to solve the crystal structure of the octameric RNA duplex in presence of six different di- and trivalent metal ions and could extend the knowledge of the influence of metal ions for conformational changes in RNA structure

The results reveal the strong influence of cations for a more compact RNA structure, although the kind of metal ions employed has structurally no particular influence. We considered different parameters to carefully assign the positions of the metal ions and suggest two prevalent positions in the investigated octameric RNA structures. One is located at the phosphate backbone, the second cation is in the centre of the RNA, interacting by a particular inner-sphere binding to O4 of uracil in presence of calcium, cobalt and copper. In addition our study reveals for the first time a RNA structure associated with copper.

Financial support by the ERC, the Swiss National Science Foundation and the University of Zurich is gratefully acknowledged.

References
1. Sigel RKO (2005) Eur J Inorg Chem 12:2281–2292
2. Freisinger E, Sigel RKO (2007) Coord Chem Rev 251:1834–1851
3. Pyle AM (2002) J Biol Inorg Chem 7:679–690

P 114

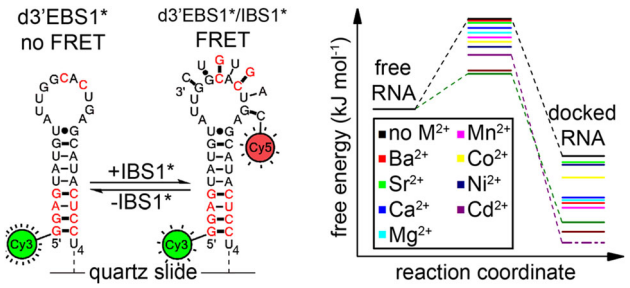
Dissecting the role of cations in RNA tertiary structure formation by single-molecule fluorescence

Sebastian L. B. König, Danny Kowerko, Mokrane Khier, Roland K. O. Sigel
Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland. sebastian.koenig@uzh.ch

RNA folding and function are largely dependent on the presence of cations. Using single-molecule Förster Resonance Energy Transfer (smFRET) and intron–exon recognition sequences of the self-splicing group II intron Sc.ai5γ as a model system (IBS1*/d3′EBS1*, see Figure), we have dissected the influence of eleven M^{2+} ions along the extended Irving-Williams series on RNA–RNA interaction [1,2]. Rigorous analysis of the thermodynamics and the kinetics reveals that the metal ion acts as a cofactor in both the docking and the undocking reaction. Excellent agreement with the characteristic metal ion complex stabilities along the extended Irving-Williams series shows that RNA/RNA docking depends on ionic strength and relies on nitrogen or carbonyl coordination, while undocking chiefly depends on the disruption of specific cation-phosphate bonds (see Figure) [2].

This study shows for the first time that RNA/RNA interaction correlates with the intrinsic coordination chemistry of the metal ion involved. It is further the first application of smFRET in a systematic characterisation of cation-dependent nucleic acid structure formation.

Financial support by the European Research Council (MIRNA N° 259092), the Swiss National Science Foundation and the University of Zurich (FK-57010302 and FK-13-108) is gratefully acknowledged.



References

1. Kruschel D, Skilandat M, Sigel RKO (2014) RNA 20:295–307
2. Sigel H, Griesser R (2005) Chem Soc Rev 34:875–900

P 115

Structure and stability of the human BCL2 RNA G-quadruplex

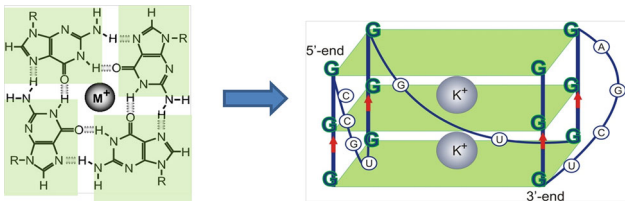
Alicia Domínguez-Martín, Roland K. O. Sigel

Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland. alicia.dominguez@chem.uzh.ch

Guanine-quadruplexes (G4) are structures formed within guanine-rich DNA or RNA sequences when four guanine bases are associated through cyclic Hoogsteen hydrogen bonds forming planar G-quartets. These quartets stack onto each other resulting in a right-handed helical conformation stabilized by the presence of metal ions [1]. Recently, the *in cellulo* existence of RNA G-quadruplex structures in mRNAs has been evidenced [2]. For example, RNA G4 present in the 5′-unstranslated region of the mRNA of the BCL2 (B Cell Lymphoma/leukemia-2) proto-oncogene was shown to down-regulate the expression of BCL2 proteins over expressed in several types of human cancers [3,4]. In this context, the study of this RNA G4 sequence is of great relevance for future novel anticancer therapy.

We evaluated the thermal stability of the BCL2 RNA G4 through a series of UV melting experiments. CD spectroscopy has also been used to probe the G-quadruplex folding topology based on well-known patterns in CD spectra. G-quadruplex formation and structure was observed by monitoring the imino proton region of ^{1}H NMR spectra. CD and NMR titrations clearly show the stabilization of the structure upon addition of KCl. However, time evolution experiments suggest the existence of more than one G-quadruplex stable conformation.

Financial support by the Fundacion Ramon Areces (A.D.M.), the Swiss National Science Foundation (R.K.O.S.), within the COST Action CM1105 (Swiss State Secr. Res. Innov. to R.K.O.S.) and by the University of Zurich is gratefully acknowledged.



References

1. Halder K, Hartig JS (2011) Met Ions Life Sci 9:125–139
2. Biffi G, Di Antonio M, Tannahill D, Balasubramanian S (2014) Nat Chem 6:75–80
3. Bugaut A, Balasubramanian S (2012) Nucleic Acids Res 40:4727–4741
4. Adams JM, Cory S (1998) Science 281:1322–1326

P 116

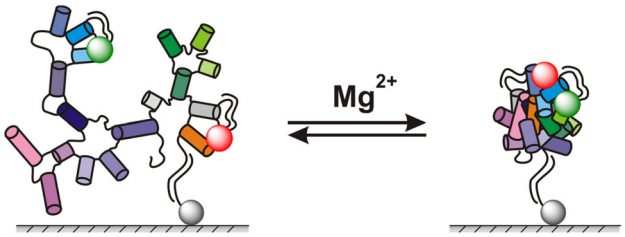
Peptide nucleic acids: superior FRET-labels for single molecule studies of RNA structure and folding

Anita G. Schmitz, Susann Zelger-Paulus, Philipp Anstaett, Gilles Gasser, Roland K. O. Sigel

Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland. anita.schmitz@chem.uzh.ch

We are interested in studying the dynamic folding behaviour of large RNAs via single molecule Förster Resonance Energy Transfer (smFRET). One of the systems that we are investigating is the group II intron of *Saccharomyces cerevisiae*, a catalytically active ribozyme [1]. A very challenging and crucial step is the labelling of such large RNA constructs with fluorophores. To this end, the current state-of-art technique is to add short complementary DNA-sequences that carry the fluorophores. However, the binding strength of these labels is generally fairly weak and heavily dependent on the conditions. More efficient labelling techniques are therefore of great interest. Peptide nucleic acid (PNA) is a non-natural analogue of DNA. It has been used extensively due to its superior binding strength towards DNA and RNA. So far, the influence of PNA on the folding behaviour of flexible RNA constructs has not been sufficiently investigated and it remains unclear if it can be used as an unbiased probe in smFRET studies. Herein, we present first experimental evidence, that indeed the binding strength of PNA based labels is superior to DNA based ones. Furthermore, the catalytic activity of the group II intron is similar with both labels, as well as with unlabelled RNA. smFRET studies show comparable general trends, but also differences between DNA- and PNA-labelled constructs. These differences have implications for the interpretation of the RNA structure and its flexibility [2].

Financial support by an ERC starting Grant 2010 (259092-MIRNA to R. K. O. S.), within the COST Action CM1105, and the University of Zurich is gratefully acknowledged.



References

1. Steiner M, Karunatilaka KS, Sigel RKO, Rueda D (2008) Proc Natl Acad Sci USA 105:13853–13858
2. Schmitz AG, Zelger-Paulus S, Gasser G, Sigel RKO (2014) submitted

P 117

Reversible stabilization of transition metal-binding DNA G-quadruplexes

David M. Engelhard[1], Roberta Pievo[2], Guido H. Clever[1]

[1]Institute for Inorganic Chemistry, Georg-August-University of Göttingen, Tammannstraße 4, 37077 Göttingen, Germany. dengelh2@uni-goettingen.de

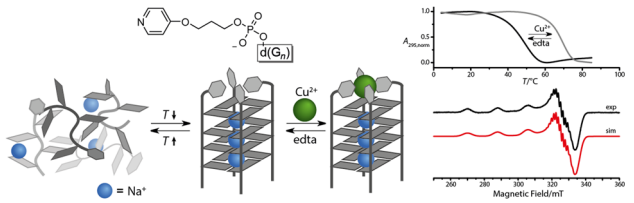
[2]Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Göttingen, Germany

Among the secondary structure motives of DNA, G-quadruplexes are now intensely studied as research indicates that

G-quadruplex formation is preventing human telomere elongation, and therefore shortening cancer cell lifetimes, as well as participating in gene expression of oncogenes [1]. G-quadruplexes self-assemble from guanine-rich oligonucleotides by Hoogsteen base pairing. Differences in strand orientation and connecting loops of the oligonucleotides give rise to a high diversity of topologies, one of many remarkable aspects which render G-quadruplexes valuable as tools in the fields of diagnostics and DNA nanotechnology [2]. In this respect it is interesting to gain control over topology formation and the stability of G-quadruplex assemblies, e.g. by regulation of de- and renaturation, as well as to incorporate non-biogenic functionalities (e.g. fluorescence or magnetism).

We synthesized tetramolecular G-quadruplexes with a terminal "metal base-tetrad", which self-assemble from Guanine-rich oligonucleotide strands. Each strand is modified with a covalently bound pyridine unit, together capable of transition metal coordination. The formation of the G-quadruplexes in buffer solution, together with their ability to reversibly coordinate transition metal ions, was monitored by UV–VIS and CD spectroscopy, as well as gel electrophoresis and EPR studies [3].

Currently we are extending this strategy to more complex G-quadruplex metal base-tetrad constructs with a focus on metal interactions and quadruplex topology.



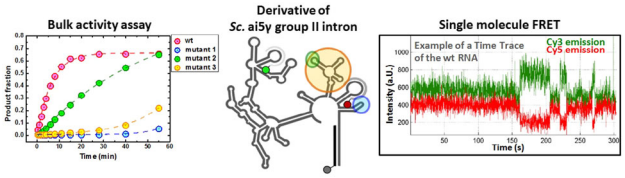
References
1. Collie GW, Parkinson GN (2011) Chem Soc Rev 40:5867–5892
2. Davis JT (2004) Angew Chem Int Ed 43:668–698
3. Engelhard DM, Pievo R, Clever GH (2013) Angew Chem Int Ed 52:12843–12847

P 118

From bulk to single molecule RNA studies: Point mutations reveal specific intra domain interactions essential for group II intron ribozyme folding pathway

Erica Fiorini, Danny Kowerko, Richard Börner and Roland K. O. Sigel
Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland. erica.fiorini@chem.uzh.ch

Group II introns belong to the class of self-splicing ribozymes and are genetic elements found in the genome of bacteria, plants and lower eukaryotes [1]. These RNAs are active upon formation of specific long-range tertiary interactions that define a precise conformation influenced by co-factors such as Mg^{2+} [2]. We study the folding pathway of the *Sc.* ai5γ group II intron through point mutations in the RNA sequence in positions responsible for inter-domain docking. Combining bulk activity assays and single molecule Fluorescent Resonance Energy Transfer (smFRET) experiments we test the effect of these mutations on the catalytic activity and folding pathway of this ribozyme. In both, bulk and single molecule experiments, different mutations have distinct kinetic effects on the activity and the folding. In particular smFRET allowed us to quantify the differences in the population for each conformational state depending on the mutated nucleobases [3]. Although essential for the folding process, Mg^{2+}, even at high concentration, cannot compensate the presence of the mutations. From the information obtained by the different mutations we understand the importance of different tertiary contacts. We can interpret them and obtain the timeline from unfolded, via intermediates, to the folded form of the ribozyme.



Financial support by the European Research Council (to R.K.O.S.) and the University of Zurich is gratefully acknowledged.

References
1. Pyle AM (2010) Crit Rev Biochem Mol Biol 45:215–232
2. Steiner M, Rueda D, Sigel RKO (2009) Angew Chem Int Ed 48: 9739–9742
3. König SLB, Hadzic M, Fiorini E, Börner R, Kowerko D, Blanckenhorn WU, Sigel RKO (2013) PLoS ONE 8:e84157

P 119

Aromatic-ring stacking between indole and nucleobase residues. The effect of bridge formation

Astrid Sigel, Helmut Sigel
Department of Chemistry, Inorganic Chemistry, University of Basel, Spitalstrasse 51, CH-4056 Basel, Switzerland.
Helmut.Sigel@unibas.ch

Aromatic-ring stacking is prominent among the noncovalent interactions occurring in biosystems; they are important, e.g., for the formation of protein-nucleic acid adducts and they are crucial for selectivity [1]. We consider here the interactions between the amino acid tryptophan, $H(Trp)^{\pm}$, and the nucleotides adenosine 5′-monophosphate, AMP^{2-}, or cytidine 5′-monophosphate, CMP^{2-}. The corresponding stability constants are listed in the Table (^1H NMR; D_2O; 27 °C; $I = 0.1$–0.15 M, KNO_3 [1]). Clearly, adduct formation

Entry	(T)(N)	$K^{(N)}_{(T)(N)}$ (M^{-1})
1	$(Trp)(AMP)^{3-}$	2.24 ± 0.58
2	$[H(Trp)](AMP)^{2-}$	6.83 ± 1.62
3	$(Trp)(CMP)^{3-}$	0.14 ± 0.05
4	$[H(Trp)](CMP)^{2-}$	0.77 ± 0.42

is more pronounced between $H(Trp)^{\pm}$ and AMP^{2-} than between Trp^- and AMP^{2-}. The repulsion between the $-COO^-$ $(Trp)^-$ and the $-PO_3^{2-}$ (AMP^{2-}) groups is expected to be small due to their distance. Hence, the main reason for the enhanced stability of the $[H(Trp)](AMP)^{2-}$ adduct is that in $H(Trp)^{\pm}$ the amino group carries a proton and thus an interaction between the $-NH_3^+$ group and the $-PO_3^{2-}$ unit of AMP^{2-} occurs, giving rise to an ionic bridge (or hydrogen bond) between the indole and adenine residues forming the stack. This bridge enhances the adduct stability

(entry 2) by a factor of ca. 3 and thus facilitates the indole-adenine recognition. The analogous observation is made with the CMP^{2-} systems (entries 3, 4) though these adducts are considerably less stable than those formed with AMP^{2-} due to the smaller size of the pyrimidine compared with the adenine residue. With the AMP^{2-} systems, three different isomers may form with $H(Trp)^{\pm}$, which are in equilibrium with each other [1]: the stacked form $[H(Trp)\cdot AMP]^{2-}_{st}$, the adduct with a sole ionic interaction $[H(Trp)\cdot AMP]^{2-}_{ii}$, and the "closed" isomer in which the stack is bridged by the intramolecular NH_3^+/PO_3^{2-} interaction, $[H(Trp)\cdot AMP]^{2-}_{cl}$; their formation degrees are 33, 15, and 53 %, respectively [1]. These calculations are based on the values in the Table and on the stability constant of the ionic adduct, $K^{AMP}_{[H(Trp)]AMP/ii} = 1.0$ [1]. Evidently, the ionic bridge (or hydrogen bond) can be replaced by a bridging metal ion that coordinates glycinate-like to Trp^- and also to the phosphate group of AMP^{2-}. With Ni^{2+} as bridging metal ion (with Zn^{2+} or Cu^{2+} precipitation occurred), the "closed" isomer in the intramolecular equilibrium, open $\rightleftharpoons$ closed, amounts to about 57 %; with CMP^{2-} the closed ternary complex forms only to about 11 % (possibly not at all) [2]. Overall it is no surprise to find that protein-nucleic acid interactions occur in Nature. among others, on the basis of Trp/indole-AMP/adenine stacks (see, e.g., [1]).

Supported by the Department of Chemistry of the University of Basel.

References

1. Sigel A, Operschall BP, Sigel H (2014) J Biol Inorg Chem 19: I Bertini memorial issue
2. Yamauchi O, Odani A, Masuda H, Sigel H (1996) Met Ions Biol Syst 32: 207–270

P 120

Chemical modifications of coenzyme B_{12} allow to investigate the interaction mechanism of the B_{12}-*btuB* riboswitch system

Anastasia Musiari, Sofia Gallo, Roland K.O. Sigel
Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland, anastasia.musiari@chem.uzh.ch

The *btuB* riboswitch is a bacterial RNA sequence able to control gene expression of a protein involved in the B_{12} transport by specifically binding coenzyme B_{12}, its natural metabolite [1]. Coenzyme B_{12} can interact with this large RNA through different moieties. Our research focuses on the understanding of this binding mechanism, investigating the influence of B_{12} derivatives differently modified. Previous works already demonstrated the importance of the adenosyl moiety for the affinity of the metabolite to the RNA, meanwhile the corrin ring is determinant for the correct structural rearrangement of the riboswitch [2,3]. Moreover, recent X-ray structures of B_{12}-riboswitches highlighted the presence of a specific binding pocket for the adenosyl moiety, meanwhile the great hydrogen-bonding potential of coenzyme B_{12} is not fully exploited [4,5].

To continue this structural study and to elucidate the role of a correct H-bonding and electrostatic network for the B_{12}-RNA interaction, we synthesized new B_{12} derivatives modified on the corrin ring sidechains *b* and *e*. In both coenzyme B_{12} and vitamin B_{12}, many primary amide sidechains are protruding from the corrin ring. We modified these sidechains to study how the presence of a carboxylic group or secondary/tertiary amidic groups influences the structural rearrangement of the *btuB* riboswitch. Both vitamin B_{12} and coenzyme B_{12} derivatives have been synthesized and tested. To investigate the impact of these chemical modifications, we exploited in-line probing assays.

The experiments performed in this work confirmed the importance of the adenosyl moiety to get a high binding affinity to the riboswitch. Indeed coenzyme B_{12} derivatives show an affinity in the nanomolar range, meanwhile vitamin B_{12} derivatives have an affinity in the micromolar range. Chemical modifications on the sidechain *e* seem to affect the structural rearrangement of the RNA but not the affinity. Meanwhile the presence of a secondary amide on the sidechain *b* increases the affinity to the riboswitch and leads to differences in the cleavage pattern of the RNA. The presence of a negative charge in the corrin ring sidechains usually decreases the binding affinity and leads to differences in the RNA structural rearrangement.

Financial support by the European Research Council (ERC starting grant 2010 259092-MIRNA to R.K.O.S.), the Swiss State Secretariat for Education Research and Innovation (COST Action CM1105) and the University of Zurich is gratefully acknowledged.

References

1. Nahvi A, Sudarsan N, Ebert MS, Zou X, et al. (2002) Chem Biol 9:1043–1049
2. Gallo S, Oberhuber M, Sigel RKO, Kräutler B (2008) ChemBioChem 9:1408–1414
3. Gallo S, Mundwiler S, Alberto R, Sigel RKO (2011) Chem Commun 47:403–405
4. Peselis A, Serganov A (2012) Nat Struct Mol Biol. 19:1182–1184
5. Johnson JE Jr, Reyes FE, Polaski JT, Batey RT (2012) Nature 492:133–137

P 121

Gene regulation on the RNA level. The B_{12} dependent *btuB* riboswitch studied with single molecule FRET

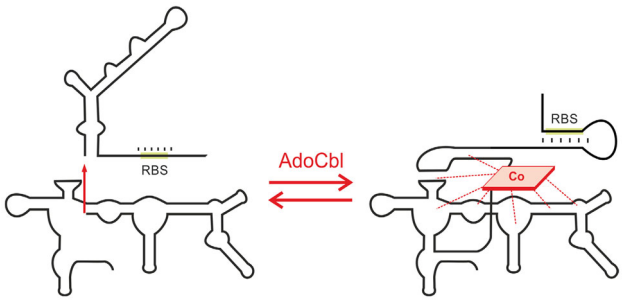
Richard Börner, Michelle Schaffer, Sofia Gallo, Roland K.O. Sigel
Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland. richard.boerner@chem.uzh.ch

The *btuB* riboswitch is a promising candidate for understanding gene regulation at the RNA level [1,2]. This B_{12} specific RNA is encoded in the 5′-untranslated region (UTR) of the *btuB gene* encoding a coenzyme B_{12} (AdoCbl) transporter found among other bacteria, especially in *E. coli*. Upon binding of B_{12}, a conformational *switch* of the *btuB* aptamer occurs, thus inhibiting the expression of the cellular B_{12} transporter (compare Figure). This controls the uptake of AdoCbl and ensures its constant level in the cytosol. RNA as polyanionic polymer is known to form secondary structures as well as tertiary contacts upon binding of mono and divalent metal ions. The folding of the *btuB* is therefore strongly dependent on the metal ion concentration [3]. In particular, the presence of Mg^{2+} is essential for the formation of different conformational states and thus for the functional *switch* of the aptamer upon cofactor binding.

Herein, we use Förster resonance energy transfer on a single molecule level (smFRET) to characterize the conformational states and the folding kinetics of the aptamer region of the *btuB* riboswitch. We developed a modified sequence of the *btuB* riboswitch labelled with the Cy3–Cy5 fluorophore pair and biotin for surface immobilization. Preliminary folding studies on the bulk level ensure for best in vitro folding conditions. Inline probing experiments test the switching behaviour and control for the activity of the FRET construct. Both will allow for thorough comparisons with former bulk studies [1–3]. The investigation of the *btuB* riboswitch on the single molecule level will complement our experiments. Thereby, we will especially focus on the

influence of AdoCbl and the function of Mg^{2+} for folding and switching to propose a first kinetic model for the *btuB* riboswitch.

Financial support by the University of Zurich, the European Research Council (to R.K.O.S.), the Swiss National Science Foundation and the Swiss State Secretariat for Education and Research (COST Action CM1105) is gratefully acknowledged.



References

1. Winkler WC, Breaker RR (2003) ChemBioChem 10:1024–1032
2. Perdrizet GA, Artsimovitch I, Furman R, Sosnick TR, Pan T (2012) Proc Natl Acad Sci USA 109:3323–3328
3. Choudhary PK, Sigel RKO (2014) RNA 20:36–45

P 122

Influence of Hg^{II} on the intercalation of $[Ru(bpy)_2(dppz)]^{2+}$ to DNA

Bhaumik S. Dave, Silke Johannsen, Roland K. O. Sigel

Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland. bhaumik.dave@chem.uzh.ch

DNA, due to its robust structural features and unique self-assembly properties, is a very attractive molecule for application in nanotechnology and medicinal technology. Replacing the hydrogen bonds between the complementary nucleobases by coordinative bonds to transition-metal ions generates the so called metal-mediated base pairs [1]. One of the most interesting applications is the usage of these metal-modified biomolecules as building block for nanowires. Metal-modified nucleic acids are expected to possess better conductive properties than their natural counterparts due to the functionalization with metal ions along the helical axis [2]. The best studied metal mediated base-pair is the Thymine-Hg^{II}-Thymine base pair. In this case the rather unstable thymine–thymine mismatch is strongly stabilised by the coordination of one Hg^{II} ion between the two opposite N3 nitrogens of the thymine–thymine base pair. In the absence of Hg^{II} ions, the mismatch region adopts an unusual non-helical fold that upon addition of Hg^{II} converts into a stable B-helical conformation [3].

In this study we use the metallointercalator $[Ru(bpy)_2(dppz)]^{2+}$ to observe small structural deviations between a natural, T–T mismatched and a Hg^{II} modified 17 bp long DNA. The emission properties of the $[Ru(bpy)_2(dppz)]^{2+}$ complex, a well-known DNA Light-switch, are known to be strongly dependent on the intercalation site [4]. We characterised the binding interaction of the racemic $[Ru(bpy)_2(dppz)]^{2+}$ complex and the separated Δ and Λ enantiomers using spectroscopic techniques like UV–VIS, CD, Fluorescence and NMR. Our first results show that the emission is significantly influenced by the presence of Hg^{II} ions within the DNA helix. The much lower emission observed for the Hg^{II}-modified DNA compared to the natural and T–T mismatched sequences can be twofold. On the one hand, the intercalation can be affected due to small distortions of the overall structure, or due to a direct interaction of the Hg^{II} ions with the metal complex resulting in altered electronic properties of the metal complex. Furthermore, we observed a strong difference in the intercalative properties to the different DNA models between the Λ and Δ enantiomers.

Financial support by the University of Zurich and within the COST Action CM1105 is gratefully acknowledged.

References

1. Takezawa Y, Shionoya M (2012) Acc Chem Res 45:2066–2076
2. Scharf P, Müller J (2013) ChemPlusChem 78:20–34
3. Kondo J, Yamada T, Hirose C, Okamoto I, Tanaka Y, Ono A (2014) Angew Chem 126: 2417–2420
4. Lim M H, Song H, Olmon E D, Dervan E E, Barton J K (2009) Inorg Chem 48:5392–5397

P 123

Structural investigation of the D1κζext region of group II intron Sc.ai5γ

Serranda Gashi, Maria Pechlaner, Roland K. O. Sigel

Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland. serranda.gashi@chem.uzh.ch

Group II introns are self-splicing RNAs and belong to the class of large ribozymes [1]. They share a common ancestry with the eukaryotic spliceosome and have possible applications in biotechnology and gene therapy raising the interest in their structure and mechanism. Group II introns have a highly conserved secondary structure comprising six domains around a central wheel that folds into a complex three-dimensional structure in the presence of Mg^{2+} ions [2]. Domain 1 (D1) and domain 5 (D5) of this large ribozyme serve as the minimal catalytically active structure [3]. Both, folding and catalytic activity is only achieved in the presence of Mg^{2+} ions. The first folding step is carried out by a small region within D1, a three-way junction containing the so called κζ element (D1κζ). This κζ element provides also the platform for D5 docking, forming the catalytic core of the group II intron. We have recently solved the NMR solution structure of the D1κζ region from the yeast mitochondrial group II intron Sc.ai5γ in the presence of Mg^{2+} ions [4]. However, under our experimental conditions we could not achieve D5 docking probably due to false intradomain interactions [4]. In this project we are interested to investigate the extended D1κζ region (D1κζext) including the neighbouring coordination loop. The presence of the coordination loop is expected to disrupt the intradomain interaction and thus favouring the D1κζ/D5 docking. To reach our final goal we first focus on the structural characterization of the D1κζext region in solution by NMR spectroscopy. We started using a 57 nt long construct containing both, the κ region and the coordination loop. Preliminary results confirmed that the three-way junction is stabilized by Mg^{2+} ions [4], while interestingly the coordination loop seems to adopt a defined structure already in the absence of Mg^{2+} ions.

Financial support by the Swiss National Science Foundation (RKOS) and the University of Zurich is gratefully acknowledged.

References

1. Sigel RKO (2005) Eur J Inorg Chem 2005: 2281–2292
2. Steiner M, Karunatilaka KS, Sigel RKO Rueda D (2008) Proc Natl Acad Sci USA 105:13853–13858
3. Michels WJJ, Pyle AM (1995) Biochemistry 34: 2965–2977
4. Donghi D, Pechlaner M, Finazzo C, Knobloch B, Sigel RKO (2013) Nucl Acids Res 41:2489–2504

P 124

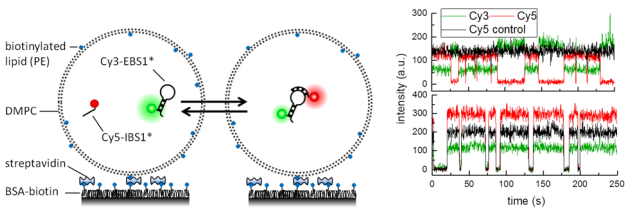
Following inter- and intramolecular dynamics of single encapsulated RNA molecules by FRET spectroscopy

Mélodie C.A.S. Hadzic, Sebastian L.B. König, Danny Kowerko, Roland K.O. Sigel

Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland. email: melodie.hadzic@uzh.ch

Single molecule FRET (Förster resonance energy transfer) is a state-of-the-art technique to investigate molecular dynamics revealing sparse spatial configurations and kinetic heterogeneities hidden in bulk experiments [1]. In bimolecular reactions, direct surface immobilisation of one reactive element allows long observation time but might bias the authenticity of the response. Co-encapsulation in surface-tethered vesicles ensures both free diffusion and constant proximity of the reactants. After experimental procedure optimisation, we co-encapsulated in a 1:1 ratio a previously studied RNA duplex (fluorophore-labelled EBS1* and IBS1*) and followed the successive association/dissociation kinetics over time by 2-colour sm-FRET depending on the Mg^{2+} concentration. As encapsulation permits to keep both RNAs in focal position—regardless if the duplex is formed or dissociated—we could alternatively collect the constant Cy5 emission from direct excitation by alternating laser pulses. Hereby, we detected a new type of event unknown from previous surface tethered studies, in which all fluorescence is quenched simultaneously. Significance tests based on the evaluation of the cross-sample variability [2], reveal a new conformation displaying Cy3–Cy5 distance lower than 2 nm.

Financial support from the ERC Starting Grant (to R.K.O. Sigel), and the Forschungskredit of the University of Zürich (to M.C.A.S Hadzic) is gratefully acknowledged.



References

1. König SLB, Kowerko D., Sigel RKO (2013) CHIMIA 67: 240–243
2. König SLB, Hadzic MCAS, Fiorini E, Börner R, Kowerko D, Sigel RKO (2013) PLoS ONE 8: e84157

P 125

Biologically relevant RNA G-quadruplex structure studied at the single-molecule level

Helena Guiset Miserachs, Roland K. O. Sigel

Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland. helena.guisetmiserachs@chem.uzh.ch

Guanine-rich nucleic acid sequences have the tendency to aggregate into non-canonical, K^+-sensitive helical structures, known as G-quadruplexes [1]. These sequences are highly enriched in regulatory regions of DNA and RNA, which suggests that they play a role in vivo [2, 3]. RNA G-quadruplexes, more stable than their DNA counterparts and easier to form (as RNA is more often single stranded in the cell), offer new possibilities as novel antitumor targets. Such structures are often located in regulatory, non-coding regions of the messenger RNAs (mRNAs) of oncogenes. We chose NRAS (Neuroblastoma RAS viral oncogene homolog), an oncogene that is overexpressed in some types of leukemias and melanomas. It contains a 18-nt G-quadruplex sequence in the 5′ untranslated region of its mRNA, which has been shown to be stable and inhibit translation in vitro [4]:

5′-GGGAGGGGCGGGUCUGGG-3′

We are working in setting up a system to visualize the NRAS RNA molecules individually, via single-molecule Förster Resonance Energy Transfer (smFRET). We are interested in elucidating the dynamics and kinetics of the RNA G-quadruplex formation and dissociation, as well as observing possible folding or unfolding intermediates.

Financial support within the COST Action CM1105, by an ERC Starting Grant 2010 (R.K.O.S.), a UZH Forschungskredit Grant 2013 (H.G.M.), by the Swiss National Science Foundation (R.K.O.S.) and by the University of Zurich is gratefully acknowledged.

References

1. Williamson JR (1994) Annu Rev Biophys Biol Struct 23:703–730
2. Maizels N (2006) Nature Struct Mol Biol 13:1055–1059
3. Bugaut A, Balasubramanian S (2012) Nucleic Acids Res 40:4727–4741
4. Kumari S, Bugaut A, Huppert JL Balasubramanian S (2007) Nat Chem Biol 3:218–221

P 126

The effect of metal ions on nucleic acids interactions, seen at the single molecule level

Mokrane Khier, Sebastian L.B. König, Danny Kowerko[1], Roland K.O. Sigel

Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland. mokrane.khier@chem.uzh

The coined role of RNA in the "modern dogma" and the increasing appreciation of RNA for biotechnological and medical applications demands more than ever a clear picture and fundamental understanding of how RNA folds and acts.

In our study, single molecule Förster Resonance Energy Transfer (smFRET) has been applied to characterize the effect of metal ions on an RNA tertiary contact: an RNA hairpin, known as EBS1* (Exon Binding Site 1), interacting with its cognate (Intron Binding Site 1). The cognate was either an RNA (IBS1*) or DNA (dIBS1*) fragment [1]. Our results revealed that the two interactions differ slightly in the conformation of their bound state, in a qualitative agreement with the NMR results performed on the same system [2]. In parallel, the thermodynamic and kinetic analysis shows that the affinity of EBS1* toward the RNA cognate is almost two orders of magnitude higher than its affinity toward the DNA one, in an independent manner from the nature of metal ions in the solution. However, in the case of EBS1*–IBS1* interaction, the presence of Mg^{2+} leads to a pronounced heterogeneity in the kinetic of this interaction due to the presence of at least two kinetic rates describing the dissociation process, a finding that was not observed for the same interaction in the presence of high amount of K^+ nor in the case of EBS1*–dIBS1* in the presence of both Mg^{2+} and high amount of K^+.

Based on our results and previous work that had pointed out at least three potential binding site for metal ions in EBS1*–IBS1* structure [3], we propose a model for the interaction where the kinetic heterogeneity seen in the presence of Mg^{2+} results from the different combination of occupying the three binding pockets within EBS1*–IBS1* structure.

Financial support by the European Research Council (MIRNA N° 259092), the Swiss National Science Foundation to Roland. K. O. Sigel and the University of Zurich (FK-570110302 to Sebastian L.

B. König and FK-13-108 to Danny Kowerko) are gratefully acknowledged.

References
1. Su LJ, Qin PZ, Michels WJ, Pyle AM (2001) J Mol Biol 306: 655–668
2. Skilandat M, Sigel RKO submitted
3. Kruschel D, Skilandat M, Sigel RKO (2014) RNA 20:295–307

P 127

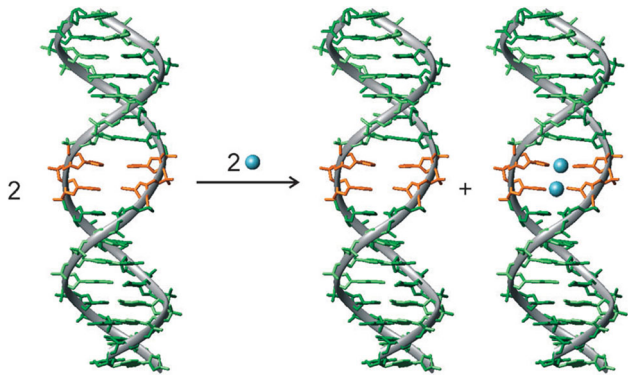
Thermodynamics of the formation of metal-mediated base pairs

Kristina Schweizer[1], Bart Jan Ravoo[2], Jens Müller[1]
[1]Institut für Anorganische und Analytische Chemie, Westfälische Wilhelms-Universität Münster, Corrensstrasse 28/30, 48149 Münster, Germany. kristina@muellerlab.org; [2]Organisch-Chemisches Institut, Westfälische Wilhelms-Universität Münster, Corrensstrasse 40, 48149 Münster, Germany

The introduction of metal-mediated base pairs is a convenient method for the site-specific functionalization of nucleic acids [1]. Hence, the base pair within a nucleic acid double helix is no longer mediated by hydrogen bonds but rather by coordinative bonds to a central metal ion.

Artificial nucleosides, in particular azole-based nucleosides (azole = imidazole, triazole, tetrazole) have proven to be excellent building blocks for Ag(I)-modified DNA [2]. The association constants for the formation of azole complexes outside the DNA context are known [2a]. Hence, the constants for the formation of Ag(I)-mediated azole base pairs within a DNA double helix were determined with isothermal titration calorimetry. Through a combination of CD- and UV-spectroscopy and isothermal titration calorimetry we demonstrated that DNA double helices comprising two neighbouring artificial imidazole-Ag(I)-imidazole base pairs incorporate the Ag(I) ions in a cooperative fashion (see illustration) [3]. Other systems, e.g. oligonucleotides with two non-consecutive artificial imidazole-Ag(I)-imidazole base pairs, were studied as well.

Financial support by the DFG (SFB 858) is gratefully acknowledged.



References
1. a) Scharf P, Müller J (2013) ChemPlusChem 78:20–34, b) Megger DA, Megger N, Müller J (2012) Met Ions Life Sci 10:295–317
2. a) Müller J, Böhme D, Lax P, Morell Cerdà M, Roitzsch M (2005) Chem Eur J 11:6246–6253, b) Böhme D, Düpre N, Megger DA, Müller J (2007) Inorg Chem 46:10114–10119, c) Johannsen S, Megger N, Böhme D, Sigel RKO, Müller J (2010) Nat Chem 2: 229–234
3. Petrovec K, Ravoo BJ, Müller J (2012) Chem Commun 48:11844–11846

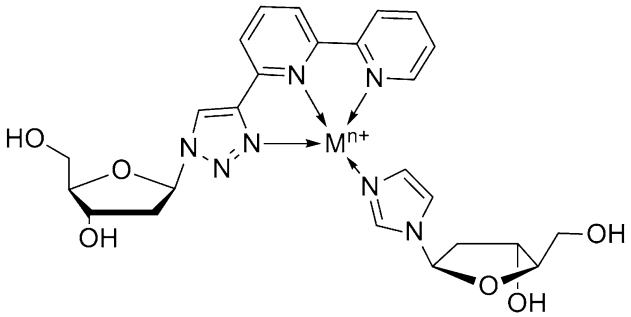
P 128

A metal-mediated base pair with a triazole-based tridentate ligand

Stefanie Litau, Jens Müller
Institut für Anorganische und Analytische Chemie, Westfälische Wilhelms-Universität Münster, Corrensstr. 28/30, 48149 Münster, Germany. steffi@muellerlab.org

The incorporation of artificial base pairs into nucleic acid double helices is a convenient method for the site-specific functionalization of these self-assembling biomolecules with metal ions [1]. We have established various nucleic acid systems with metal-mediated base pairs derived from azole ligands [2, 3]. Moreover, we recently developed a metal-mediated base pair with a trigonal planar coordination geometry of the central transition metal ion. This geometry was achieved by combining one bidentate and one monodentate ligand [4]. The triazole-based ligand used in that context was the first of an entire family of "click" nucleosides [5]. The latest addition to this family is a metal-mediated base pair with a triazole-based *tridentate* ligand and a complementary monodentate nucleoside. The synthesis of the new artificial nucleoside and the metal-binding properties of oligonucleotides containing the respective base pair will be reported.

Financial support by the Deutsche Forschungsgemeinschaft (TRR61) is gratefully acknowledged.



References
1. Scharf P, Müller J (2013) ChemPlusChem 78:20–34
2. Johannsen S, Megger N, Böhme D, Sigel RKO, Müller J (2010) Nat Chem 2:229–234
3. Kumbhar S, Johannsen S, Sigel RKO, Waller MP, Müller J (2013) J Inorg Biochem 127:203–210
4. Richters T, Müller J (2014) Eur J Inorg Chem 2014:437–441
5. Richters T, Krug O, Kösters J, Hepp A, Müller J (2014) Chem Eur J (doi: 10.1002/chem.201402221)

P 129

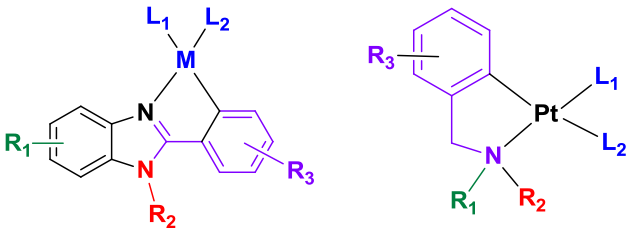
Hit to lead: SAR studies of cyclometalated benzimidazole and dimethylbenzylamine platinum group metals anticancer compounds

Jose Ruiz[1], Gorakh Yellol[1], Ana Zamora[1], Jyoti Yellol[1], Sergio Perez[1], Antonio Donaire[1], Venancio Rodríguez[1], Alicia Buceta[1], Natalia Cutillas[1], Patrick J. Bednarski[2], Christoph Janiak[3], Vera Vasylyeva[3]

[1]Department of Inorganic Chemistry and Regional Campus of International Excellence (Campus Mare Nostrum), University of Murcia and Instituto Murciano de Investigación Biosanitaria, Campus de Espinardo, E-30071 Murcia, Spain.
[2]Pharmaceutical & Medicinal Chemistry, Institute of Pharmacy, Ernst-Moritz-Arndt University Greifswald, 17487 Greifswald, Germany.
[3]Institut für Anorganische Chemie und Strukturchemie Universität Düsseldorf, D-40204 Düsseldorf, Germany

Organometallic compounds with properties somewhat intermediate between classical inorganic and organic drugs have recently been considered as promising alternatives in medicinal chemistry. These are relatively lipophilic and can be endowed with a huge variety of functionalized organic ligands with very specific reactivities. Due to the exciting results from previous studies of dimethylbenzylamine (DMBA) Pt complexes and some smart phenyl-benzimidazole Ru(II) and Ir(III) derivatives [1], various sets of small libraries have been synthesized focusing on different aspects of the core molecules benzimidazole and DMBA to modulate anticancer activity: (i) to modulate the lipophilicity by the *N*-substitution (R2) with different alkyl and aryl groups; (ii) for SAR studies- different function group substitutions (R1) such as EDG, EWG, alkyl, aryl groups etc.; (iii) exploring the aromaticity of the ligand- substituting one phenyl group by two to four aromatic ones; (iv) linking to heterocyclic moieties resulting bis-heterocyclic compounds; and, (v) producing different combinations of secondary ligands attached to the metal (L1 and L2) like chloro, *p*-cymene, *penta*-methylcyclopentadiene, phosphines and small heterocycles.

Financial support by the European Union Seventh Framework Programme—Marie Curie COFUND (FP7/2007–2013) under U-IMPACT Grant Agreement 267143, the Spanish Ministerio de Economía y Competitividad and FEDER (Project SAF2011-26611), by Fundación Séneca (Project 15354/PI/10) and COST ACTION CM1105.



Reference

1. Yellol GS, Donaire A, Yellol JG, Vasylyeva V, Janiak C, Ruiz J (2013) Chem Commun 49:11533–11535

P 130

Purification and isolation of platinated RNA

Marianthi Zampakou, Daniela Donghi
Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland. marianthi.zampakou@chem.uzh.ch

Nucleic acids are an important potential drug target and we are currently investigating the way in which platinum anticancer drugs interact with RNA. These drugs are normally thought to exert their activity upon covalent binding to DNA purine nitrogens [1]. However in recent years potential alternative binding partners have been explored, including RNA [2]. The latter has many important functions in vivo and the disruption of these processes can have serious consequences [3]. It has already been reported that some RNA dependent activities are inhibited upon administration of platinum drugs, but still little is known on the effects of platinum drugs on RNA structure and biology [2–4].

In order to understand how platinum drugs affect RNA structure we use as a model RNA a 27 nucleotide long construct derived from the mitochondrial group II intron ribozyme *Sc*.ai5γ [5]. As platination agent we use oxaliplatin which is FDA approved anticancer drug that is used in clinics worldwide [6]. Gel electrophoresis mobility shift assays were used to identify the best experimental conditions to obtain monoplatinated RNA in high yield and separate it from the unreacted RNA. The platinated adducts collected from the gels were then further purified and isolated by HPLC. The characterization of the pure monoplatinated adducts is now in progress using a combination of different techniques including mass spectrometry, UV–Vis, CD and NMR spectroscopy.

Financial support by the Swiss National Science Foundation (*Ambizione* fellowship PZ00P2_136726 to DD), by the University of Zurich and within the COST Action CM1105 is gratefully acknowledged.

References

1. Alderden RA, Hall MD, Hambley TW (2006) J Chem Educ 83:728–734
2. Chapman EG, Hostetter AA, Osborn MF, Miller AL, DeRose VJ (2011) Met Ions Life Sci 9:347–377
3. Chapman EG, De Rose VJ (2010) J Am Chem Soc 132:1946–1952
4. Hostetter AA, Chapman EG, De Rose VJ (2009) J Am Chem Soc 131:9250–9257
5. Donghi D, Pechlaner M, Finazzo C, Knobloch B, Sigel RKO (2013) Nucleic Acids Res 41:2489–2504
6. Ibrahim A, Hirschfeld S, Cohen MH, Griebel DJ, Williams GA, Pazdur R (2004) The Oncologist 9:8–12

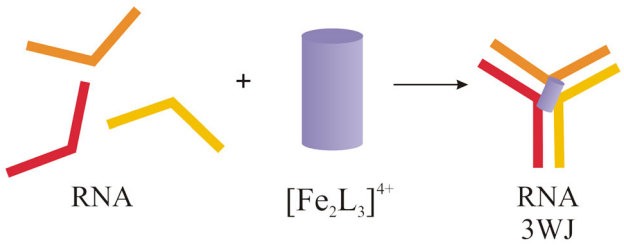
P 131

Recognition of an RNA three-way junction by a *de novo* designed drug

Susann Zelger-Paulus[1], Siriporn Phongtongpasuk[2], Michael J. Hannon[2], Roland K. O. Sigel[1]
[1]Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland. supaulu@aci.uzh.ch.
[2]School of Chemistry, University of Birmingham, Edgbaston, Birmingham B152TT, UK

RNA is a very exciting biomedical target because of its high structural diversity and the ability to regulate essential processes during RNA maturation. Ribosomal RNA, the hammerhead or introns of pre-mRNA are only few examples of catalytically active RNAs called ribozymes. Structure and function of such ribozymes are inextricably linked with each other. The complex architecture of RNA is represented by countless bulges, loops, helices or junctions. One special structural element is the Y-shaped three way junction (3WJ) which can take form of a perfectly shaped or a bulged junction. The detection and intercalation of such 3WJs by small molecules is of high importance since various RNA processes could be controlled this way. We demonstrate the recognition and stabilization of an perfect RNA 3WJ by a designed cylinder-shaped drug ($[Fe_2L_3]^{4+}$) through native gel studies [1]. In addition, we can illustrate that even bulged 3WJs are stabilized by the iron cylinder. A competition assay with analogous RNA and DNA sequences and the composition of RNA/DNAs constructs showed that the 3WJ formation induced by the cylinder is sequence dependent. In summary, we demonstrate that the recognition and formation of an RNA 3WJ by the iron cylinder is a very good approach of how drug design should be faced and offers high potential towards medical applications.

Financial support within the COST Action CM1105, from the Swiss State Secretariat for Education and Research, the University of Zurich, and the University of Birmingham are gratefully acknowledged.



Reference

1. Phongtongpasuk S, Paulus S, Schnabl J, Sigel RKO, Spingler B, Hannon MJ, Freisinger E (2013) Angew Chem Int Ed 52:11513–11516

P 132

Rhenium(I)-dppz complexes as potential RNA binding agents

Elena Alberti[1], Michael P. Coogan[2], Daniela Donghi[1]

[1]Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland. elena.alberti@chem.uzh.ch;
[2]Department of Chemistry, Faraday Building, Lancaster University, Bailrigg, Lancaster, LA1 4YB, United Kingdom

There is an increasing interest in the development of small molecules as structure-selective binding agents for bioimaging and along this line luminescent metal complexes have been extensively studied due to their suitable photophysical, spectroscopic and electrochemical properties [1, 2]. Interestingly, while their interaction with DNA is widely studied, a small amount of information is available to date on their RNA binding properties.

Re(I)-dppz complexes belong to the large class of d-block lumophores which includes also Ru(II) and Ir(III) and their interaction with DNA has been already studied some years ago [3]. Besides their binding properties to CT-DNA, it was also shown that their cellular uptake varies upon changing their axial ligand. It has been proved that these changes do not affect their optical properties but result in a different cell localization including RNA-rich regions [3]. Even if it is well known that Re(I)-dppz complexes are capable of binding nucleic acids via metallo-intercalation, the field is still rather unexplored, especially on their RNA interaction. For these reasons we decided to investigate the interaction of different mononuclear rhenium complexes with several RNA models containing the most common secondary structural features. Both the effects of varying the RNA model and the axial ligand of the Re(I)-dppz complexes are evaluated to rationalize the structural origin of interaction preferences.

We are currently investigating the changes of the optical properties of one of these complexes upon interaction with a 27 nucleotide construct by means of UV/Visible and fluorescence spectroscopy. This study enables us to evaluate the effect of the structural features contained in the model, e.g. internal and terminal loops on the binding properties. Moreover, as the NMR structure in solution of this RNA sequence is available [4], preliminary NMR studies have been performed to determine the site of interaction. Further NMR studies are in progress to investigate RNA structural changes upon rhenium complex binding.

Financial support by the Swiss National Science Foundation (*Ambizione* fellowship PZ00P2_136726 to DD), by the University of Zurich (including the *Forschungskredit* FK-13-107 to DD) and within the COST Action CM1105 is gratefully acknowledged.

References

1. Coogan MP, Fernández-Moreira V (2014) Chem Commun 50:384–399
2. Fernández-Moreira V, Thorp-Greenwood FL, Coogan MP (2010) Chem Commun 46:186–202
3. Thorp-Greenwood FL, Coogan MP, Mishra L, Kumari N, Rai G, Saripella S (2012) New J Chem 36:64–72
4. Donghi D, Pechlaner M, Sigel RKO unpublished

P 133

The CPEB3 ribozyme pseudoknot is tied up by magnesium(II)

Miriam Skilandat, Magdalena-Rowińska-Żyrek, Roland K. O. Sigel

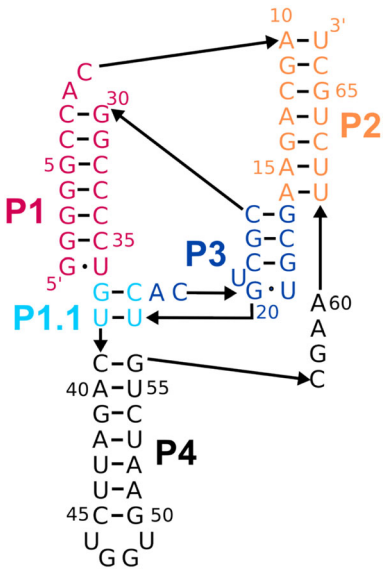
Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland. miriam.skilandat@chem.uzh.ch

The CPEB3 ribozyme is the only small self-cleaving ribozyme that has been shown to be highly conserved in mammalian genomes [1]. It is hypothesized to be evolutionary related to the HDV (hepatitis delta virus) ribozyme since both have the nested double-pseudoknot fold and the catalytic chemistry in common [1, 2]. However, there is to date neither an explanation for this relation nor any knowledge about the role of the CPEB3 ribozyme in the cell.

In this study we focus on the structural effects of metal-ion binding to CPEB3, which is known to use Mg(II) for its catalytic mechanism. We present the first results in elucidating the structure of CPEB3 in solution, obtained by NMR spectroscopy. We demonstrate that, in the absence of multivalent metal ions, the three major helices P1, P2 and P4 of CPEB3 and thus also the P1-P2 pseudoknot are formed according to the proposed secondary structure [1]. There is evidence for several Mg(II) binding sites within CPEB3. Binding of Mg(II) leads to a compaction of the ribozyme and to the formation of additional base pairs and stacking interactions in the ribozyme core, thereby indicating the formation of the internal loop P3 and possibly the second pseudoknot.

These results are prove of the remarkable similarity of the CPEB3- and HDV fold and shed light on the important role of Mg(II) for formation of the nested double-pseudoknot motif.

Financial support by the Swiss National Science Foundation (RKOS), the University of Zurich and a Marie Curie fellowship (No. PIEF-GA-2012-329700; MRZ) is gratefully acknowledged.



References
1. Salehi-Ashtiani K, Lupták A, Litovchick A, Szostak JW (2006) Science 313:1788–1792
2. Webb C-HT, Lupták A (2011) RNA Biology 8:719–727

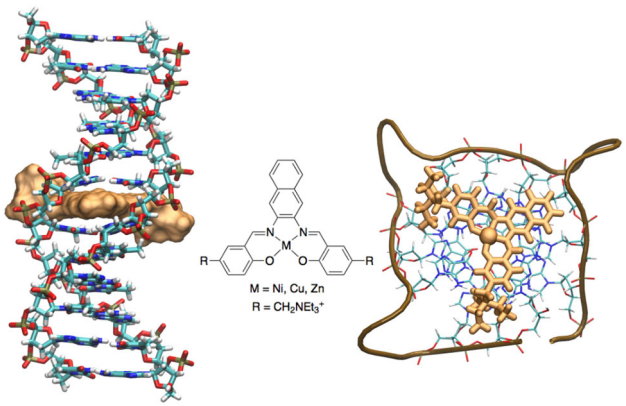
P 134

G4-DNA *vs.* B-DNA binding of Schiff base transition metal complexes

Giampaolo Barone, Alessio Terenzi, Riccardo Bonsignore, Angelo Spinello, Anna Maria Almerico, Antonino Lauria
Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche, Università di Palermo, Viale delle Scienze, Ed. 17, 90128 Palermo, Italy. giampaolo.barone@unipa.it

The competitive binding of nickel(II), copper(II) and zinc(II) complexes toward B- and G4-DNA was addressed through spectroscopic titrations and rationalized by computational investigations, consisting of molecular dynamics simulations followed by density functional theory/molecular mechanics (DFT/MM) calculations [1]. The experimental DNA binding studies clearly highlight the selectivity of the compounds, in particular the nickel(II) complex, toward G4-DNA from both *h-Telo* and *c-myc*. Moreover, the compounds show biological activity against HeLa and MCF-7 cancer cell lines. Remarkably, the experimental DNA-binding affinity trend of the three metal complexes, obtained from the DNA-binding constants as $\Delta G^\circ = -RT\ ln(K_b)$, is reproduced by the Gibbs formation free energy calculated by DFT/MM for the DNA-binding complexes, in the implicit water solution.

Financial support by the University of Palermo is gratefully acknowledged.



Reference
1. Lauria A, Bonsignore R, Terenzi A, Spinello A, Giannici F, Longo A, Almerico AM, Barone G (2014) Dalton Trans 43:6108–6119

P 135

NMR localization of Mg^{2+} ions in the mammalian CPEB3 ribozyme

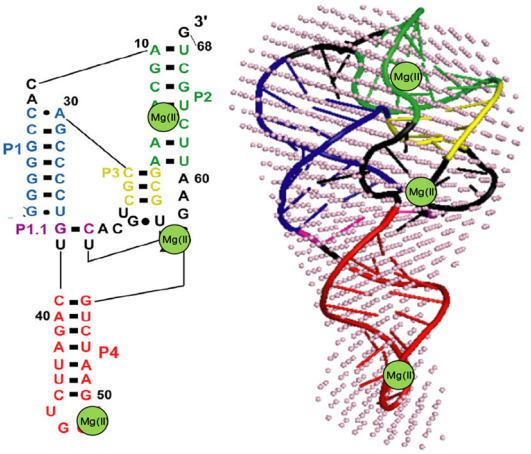
Magdalena Rowińska-Żyrek, Miriam Skilandat, Roland K. O. Sigel
Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland. magdalena.zyrek@chem.uzh.ch

The CPEB3 ribozyme is a small, highly conserved, mammalian, self cleaving, non-coding RNA located in the second intron of the *cpeb3* gene [1]. Most of the available knowledge about this ribozyme is based on comparative studies with the HDV (Hepatitis Delta Virus) ribozyme; both are predicted to fold into a similar pseudoknot structure and use a self-cleavage mechanism which employs a catalytic site cytosine [1, 2].

In this study, we focus on understanding the impact of Mg^{2+} ions on the structure of the CPEB3 ribozyme. Several direct metal ion counting techniques show that there are at least three specific binding sites for this metal and NMR spectroscopy precisely shows their localisation. In order to distinguish an outer- from an innersphere Mg^{2+} binding, $[Co(NH_3)_6]^{3+}$ is used as a spectroscopic probe for $[Mg(H_2O)_6]^{2+}$ [3].

The importance of these metal binding sites is underlined by co-transcriptional cleavage studies, which show the impact of single mutations of the predicted metal binding sites on the catalytical activity of the ribozyme.

Financial support by the Marie Curie IEF (no. PIEF-GA-2012-329700 to MRZ) and the Swiss National Science Foundation (to RKOS) is gratefully acknowledged.



References
1. Salehi-Ashtiani K, Luptak A, Litovchick A, Szostak JW (2006) Science 313:1788–1792.
2. Webb C, Riccitelli NJ, Ruminski DJ, Luptak A (2009) Science 326: 953–932
3. Skilandat M, Rowinska-Zyrek M, Sigel RKO (2014) J Biol Inorg Chem DOI:10.1007/s00775-014-1125-6.

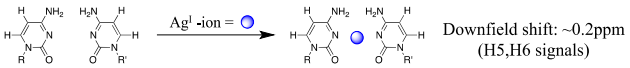
P 136

Proton and nitrogen-15 NMR spectroscopic studies of Ag^I-mediated C–C base-pairs

Takenori Dairaku[1], Itaru Okamoto[2], Kyoko Furuita[3], Shuji Oda[1], Daichi Yamanaka[1], Hidetaka Torigoe[4], Tetsuo Kozasa[4] Yoshinori Kondo,[1] Akira Ono[2], Chojiro Kojima[3], Vladimír Sychrovský[5], Yoshiyuki Tanaka[1]
[1]Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai 980-8578, Japan;
[2]Faculty of Engineering, Kanagawa University, Yokohama, Kanagawa 221-8686 Japan;
[3]Institute for Protein Research, Osaka University, Suita, Osaka 565-0871, Japan;

[4]Faculty of Science, Tokyo University of Science; Shinjuku-ku, Tokyo 162-8601, Japan;
[5]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, 16610, Praha 6, Czech Republic

The structure of Ag^I-mediated Cytosine–Cytosine base-pairs (C–Ag^I–C) in DNA duplex was not clearly understood. Our previous ^{1}H NMR spectroscopic studies of DNA duplex with a single C–C mismatch revealed that the stoichiometry between C–C mismatch and Ag^I-ion was 1:1 [1, 2]. In ^{1}H NMR spectra of a self-complementary DNA duplex1: d(5′-ATAATAATAAACTTTATTATTAT-3′)$_2$ [2] and non-self-complementary DNA duplex2: d(5′-TTAATAATATACT TAATTATAAT-3′)/d(5′-TTAATAATATACTTAATTATAAT-3′), the H5 and H6 signals of the cytosine residues were down-field shifted by ~0.2 ppm upon the formation of the C–Ag^I–C base-pair. Also, this characteristic down-field shift was found in ^{1}H NMR spectra of cytosine mononucleoside [2]. Furthermore, in ^{15}N NMR spectra of ^{15}N-labeled cytosine mononucleoside, the N3 signal of the cytosine was drastically up-field shifted by ~22 ppm upon the binding with Ag^I-ion in conjunction with ~0.2 ppm down-field shifts for H5 and H6 resonances [2]. These data indicated that the binding site of Ag^I-ion is N3 of cytosine. In addition, degree of down-field shifts for H5 and H6 resonances in the cytosine nucleoside (~0.2 ppm) were the same as those of DNA duplexes. This similarity in chemical shift perturbations between cytosine and cytosine residues in the DNA duplexes suggested that Ag^I-binding site in DNA duplexes were also N3 of cytosine residues. Because ^{15}N NMR spectroscopy was employed as a powerful tool for determining chemical structures of metal-mediated base-pairs [3, 4], we will also discuss ^{15}N NMR spectroscopic data for DNA duplexes.



References
1. Ono A, Cao S, Togashi H, Tashiro M, Fujimoto T, Machinami T, Oda S, Miyake Y, Okamoto I, Tanaka Y (2008) Chem Commun 4825–4827
2. Torigoe H, Okamoto I, Dairaku T, Tanaka Y, Ono A, Kozasa T (2012) Biochimie 94:2431–2440
3. Tanaka Y, Oda S, Yamaguchi H, Kondo Y, Kojima C, Ono A (2007) J Am Chem Soc 129:244–245
4. Johannsen S, Megger N, Böhme D, Sigel RKO, Müller J (2010) Nat Chem 2:229–234

P 137

A luminescent purine-based ligand for metal-mediated base pairs

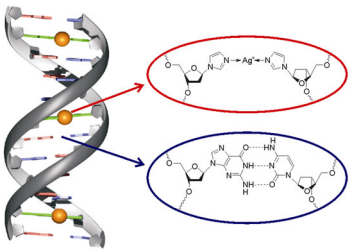
Soham Mandal, Jens Müller
Institut für Anorganische und Analytische Chemie & The Graduate School of Chemistry, Westfälische Wilhelms-Universität Münster, Corrensstr. 28/30, 48149, Münster, Germany. soham@muellerlab.org

DNA, the self assembling supramolecule, plays a pivotal role in chemical evolution using its capacity to store and transfer genetic information, its optimized hybridization properties and highly specific molecular recognition, resulting in diversified applications.

Introducing transition metal ions in DNA by exploiting metal-mediated base pairs offers a promising and versatile bottom-up strategy for its site-specific functionalization [1, 2]. Applicability is furthermore extended by replacing natural nucleobases by metal-mediated non-natural base pairs aiming to expand the genetic four letter code. This allows formation of a duplex with one or more metal-mediated base pairs interspersed between natural ones, helices with a long stretch of metalated base pairs and duplexes with metal arrays in a sequence-controlled pre-defined order [3]. Hence, metal-based properties can be introduced into modified nucleic acids [2]. This broadens the potential application of DNA as a functionalized nano-scale object.

We report for the first time the use of a luminescent bidentate purine-based moiety for an application in metal-mediated base pairs. Its synthesis and detailed characterization, including the formation of model complexes, will be presented. Moreover, first attempts towards its incorporation into different DNA sequences and the metal-binding properties of the resulting nucleic acids will be reported.

Financial support by the Graduate School of Chemistry (GSC MS) is gratefully acknowledged.



Representation of B-DNA with metal-mediated base pairs (top) and regular base pairs (bottom). (adapted from reference 1).

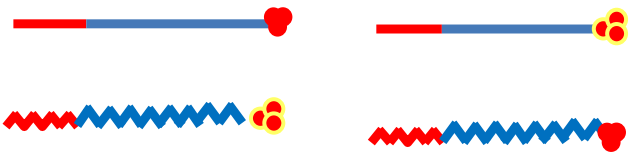
References
1. Müller J (2008) Eur J Inorg Chem 3749–3763
2. Scharf P, Müller J (2013) ChemPlusChem 78:20–34
3. Johannsen S, Megger N, Böhme D, Sigel RKO, Müller J (2010) Nat Chem 2:229–234

P 138

Insights into the structural determinants for the formation of fluorescent silver nanoclusters on DNA, RNA and DNA-RNA chimeras

Peter W. Thulstrup[1], Pratik Shah[2], Seok Keun Cho[2], Morten J. Bjerrum[1], Seong Wook Yang[2]
[1]Department of Chemistry, University of Copenhagen, Universitetsparken 5, 2100 Copenhagen, Denmark. pwt@chem.ku.dk;
[2]UNIK Center for Synthetic Biology, Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederikberg, Denmark

Recently, silver nanoclusters (AgNCs) bound to nucleic acids have become a highly promising group of fluorescent probes, displaying an intense response in the visible and near-infrared spectral regions. Through variation of the nucleic acid composition numerous different properties can be achieved, for instance to allow for the detection of micro RNA in solution [1]. It is known that probe design is complex, as not only base sequence but nucleic acid conformation is important [2]. RNA has been shown to host fluorescent AgNCs [3], and here we find that the emission properties are strongly dependent on the



backbone composition in a comparison of two probe systems using both DNA, RNA and DNA-RNA chimera sequences as the scaffold for AgNC formation. One probe sequence shows strongly enhanced

fluorescence with RNA and is subdued with DNA, and another probe acts in the opposite way. In addition to the fluorescence properties, the systems are characterized through capillary electrophoresis and circular dichroism spectroscopy. This is one of the first studies of RNA-based probes and it seems clear that the choice of backbone will be an important parameter in the design of new fluorescent probes based on AgNCs.

Financial support by the "Centre for Synthetic Biology" (Grant 09-065274) is acknowledged, and we thank Thomas Günter-Promosky for use his fluorimeter.

References

1. Yang SW, Vosch T (2001) Anal Chem 83:6935–6939
2. Shah P, Rorvig-Lund A, Chaabane SB, Thulstrup PW, Kjaergaard HG, Fron E, Hofkens J, Yang SW, Vosch T (2012) ACS Nano 6:8803–8814
3. Schultz D, Gwinn E (2001) Chem Commun 47:4715–4717

P 139

Stabilisation and nucleic acids binding of a +3 ion: the aluminium/cacodylate/RNA system

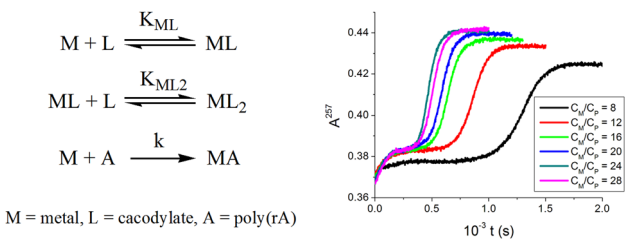
Tarita Biver[1], Natalia Busto[2], Begoña García[2], Matteo Lari[1], José-Maria Leal[2], Hector Lozano[2], Fernando Secco[1], Marcella Venturini[1]

[1]Department of Chemistry and Industrial Chemistry, University of Pisa, Via Risorgimento 35, 56126 Pisa, Italy. tarita.biver@unipi.it;
[2]Department of Chemistry, University of Burgos, Plaza Misael Bañuelos s.n., 09001 Burgos, Spain

Metal ions influence the three-dimensional architecture and function of nucleic acids, can induce folding of nucleic acids strands, or even can aid catalytic mechanism in ribozymes. Therefore, investigations of metal ion binding to specific sites, and of the ability to stabilize local motifs or particular non-canonical structures are of primary interest [1].

We have recently found that Mg(II) and Ni(II) cations are able to induce quadruplex and triplexes formation starting from duplex poly(rA)poly(rU) under still unexplored high ions concentrations. We have now extended our research to the study of the less explored class of tervalent metal ions. The interaction between aluminium(III) and poly(rA) nucleic acid, both in the form of single or double strand, has been analysed. The Al^{3+}/poly(rU) system has been also investigated for comparison purposes. At the pH range needed for these experiments (pH = 5–7) the stability of Al^{3+} in solution is guaranteed by the cacodylate buffer that also complexes the metal ion. It is shown that the binding occurs indeed, with features that differ on passing from poly(rA) to poly(rA)poly(rA). The fast process of the metal ion binding to RNA is studied by means of the initial rate analysis in different reactants conditions and a binding mechanism is proposed (figure, left). This enables the main binding species to be individuated. In the case of poly(rA) a slow cooperative aggregation of the single strands is found to occur (figure, right), that is favoured by the presence of the aluminium ion. The different binding features will be discussed.

Financial support by Obra Social "La Caixa" is gratefully acknowledged.



Reference

1. Sigel RKO, Sigel H (2010) Acc Chem Res 43:974–984

P 140

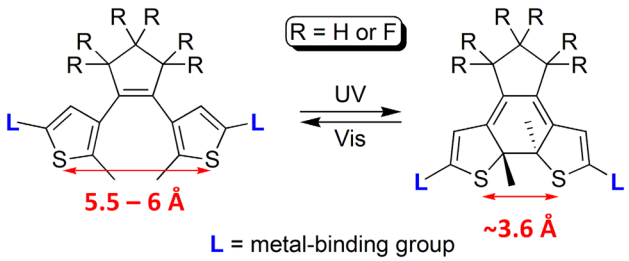
DNA-interacting photoswitchable metal complexes based on dithienyl-cyclopentene ligands

Andreu Presa[1], Guillem Vázquez[1], Ivana Borilovic[1], Olivier Roubeau[2], Patrick Gamez[1,3]

[1]Departament de Química Inorgànica, Facultat de Química, Universitat de Barcelona, Martí i Franquès 1-11, 08028 Barcelona, Spain. andreu.presa@ub.edu;
[2]Instituto de Ciencia de Materiales de Aragón (ICMA), CSIC and Universidad de Zaragoza, Plaza San Francisco s/n, 50009 Zaragoza, Spain;
[3]Institució Catalana de Recerca i Estudis Avançats (ICREA), Passeig Lluís Companys 23, 08010 Barcelona, Spain

Dithienylethene-based compounds of the type illustrated in the figure below are well known for their potential use as molecular switches, as they undergo reversible ring closure upon irradiation with UV or visible light [1–3], giving rise to their contraction or expansion, respectively. Surprisingly, their use for biological applications has not yet been extensively studied. Here we present a new series of photoswitchable coordination compounds obtained from both symmetrical and unsymmetrical dithienylcyclopentene ligands. The open and closed forms of these metal complexes not only exhibit interesting optical properties, but also show clearly distinct DNA-interacting behaviours.

Financial support by the Ministerio de Economía y Competitividad (MINECO) of Spain (Project CTQ2011-27929-C02-01). COST Action CM1105 is kindly acknowledged.



References

1. Feringa BL (ed) Molecular Switches Wiley–VCH Weinheim 2001
2. Irie M (2000) Chem Rev 100:1685–1716
3. Hanazawa M, Sumiya R, Horikawa Y, Irie M (1992) J Chem Soc Chem Commun 206–207

P 141

Targeting DNA structures using metallosupramolecular complexes

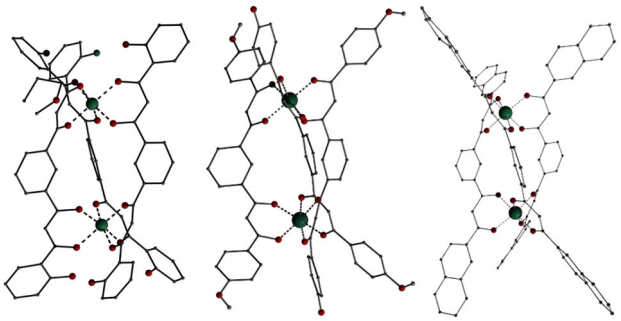
Rosa F. Brissos[1], David Aguilà[1], Mohanad Darawsheh[1], Leoni Barrios[1], Olivier Roubeau[2], Guillem Aromí[1], Patrick Gamez[1,3]

[1]Departament de Química Inorgànica, Facultat de Química, Universitat de Barcelona, Martí i Franquès 1-11, 08028 Barcelona, Spain. rosa.brissos@qi.ub.es;
[2]Instituto de Ciencia de Materiales de Aragón (ICMA), CSIC and Universidad de Zaragoza, Plaza San Francisco s/n, 50009 Zaragoza, Spain;

[3]Institució Catalana de Recerca i Estudis Avançats (ICREA), Passeig Lluís Companys 23, 08010 Barcelona, Spain

The molecular recognition of DNA duplex in the major groove is a topical strategy to develop therapeutic agents (antigene compounds), taking into account that the major groove is the binding site of proteins playing a key role in replication, transcription or recombination. These recognition processes occur via specific hydrogen-bonding (donor/acceptor) contacts with the edges of the base pairs [1]. Here, we report on the design and preparation of metallosupramolecular complexes, *i.e.* metallohelicates obtained from β-diketone ligands (see figure below), which are able to target specific DNA locations and/or conformations. The biological activity, namely the potential interaction of these new compounds with the DNA major groove, has been examined using UV–Vis spectroscopy, fluorescence dye-displacement techniques, circular dichroism (CD), gel electrophoresis and Atomic-Force Microscopy (AFM).

Financial support by the Ministerio de Economía y Competitividad (MINECO) of Spain (Project CTQ2011-27929-C02-01). COST Action CM1105 is kindly acknowledged.



Reference

1. Lusby PJ (2010) Annu Rep Prog Chem, Sect A Inorg Chem 106:319–339

P 142

Anion-π interactions in biological molecules derived from purine and pyrimidine bases

Angel Terrón, Miquel Barceló-Oliver, Juan J. Fiol, Angel García-Raso, Antonio Frontera

Department of Chemistry, Universitat de les Illes Balears, Campus UIB, 07122 Palma de Mallorca, Spain

Long chain N-alkyl substituted purines and pyrimidines have been used as biological models. We summarize the results from the characterization of the bases and their coordination chemistry focusing the attention on the anion-π interactions. The results are obtained from X-ray diffraction studies of well-characterized molecules, and theoretical studies based on the experimental data [1]. Some of the selected examples are indicated in Table 1.

Financial support by the Universitat de les Illes Balears is gratefully acknowledged. A.F. thanks the DGICYT of Spain (projects CTQ2011-27512/BQU and CONSOLIDER INGENIO 2010 CSD2010-00065, FEDER funds) and the Direcció General de Recerca i Innovació del Govern Balear (project 23/2011, FEDER funds) for financial support.

Table 1. Energetic (interaction energies in kcal/mol) and geometric parameters (anion to ring centroid distances in Å) computed for several anion-π complexes. 6R and 5R stands for six- and five-membered rings, respectively

Anion-π interaction		Distance	Energy
Bromide/(N^1CytH)C_6	[2]	3.58	−21.7
Chloride/(N^6AdeH)C_{10}	[3]	3.48	−15.6
Chloride/(N^6AdeHC_{10})···(N^6AdeHC_{10})	[3]	3.45	−25.2
($ZnCl_3$)Cl/(AdeH)$_2C_3$, N^1 protonated, 6R	[4]	3.58	−77.8
($ZnCl_3$)Cl/(AdeH)$_2C_3$, N^1 protonated, 5R	[4]	3.32	−83.7
($ZnCl_3$)Cl/(HypH)$_2C_3$, N^7 protonated, 6R	[4]	3.20	−82.4
($HgCl_3$)Cl/(AdeH)$_2C_3$, N^1 protonated, 6R	[4]	3.29	−83.3

References

1. Fiol JJ, Barceló-Oliver M, Tasada A, Frontera A, Terrón A, García-Raso A (2013) Coord Chem Rev 257:2705–2715
2. Barceló-Oliver M, Baquero BA, Bauzá A, García-Raso A, Terrón A, Mata I, Molins E, Frontera A (2012) CrystEngComm 14:5777–5784
3. García-Raso A, Albertí FM, Fiol JJ, Lagos Y, Torres M, Molins E, Mata I, Estarellas C, Frontera A, Quiñonero D, Deyà PM (2010) Eur J Org Chem 2010:5171–5180
4. García-Raso A, Albertí FM, Fiol JJ, Tasada A, Barceló-Oliver M, Molins E, Escudero D, Frontera A, Quiñonero D, Deyà PM (2007) Inorg Chem 46:10724–10735

P 143

Synthesis, characterization and DNA interaction study of some copper(II) complexes with substituted terpyridine ligands

Amparo Caubet[1], Jordi Grau[1], Patrick Gámez[1,2], Olivier Roubeau[3]

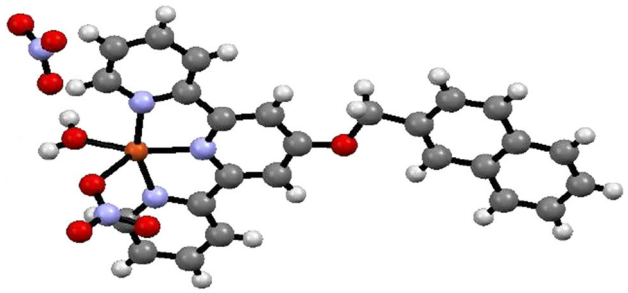
[1]Departament de Química Inorgànica, Universitat de Barcelona, Martí i Franquès 1-11 08028 Barcelona, Spain. amparo.caubet@qi.ub.es; [2]Institució Catalana de Recerca i Estudis Avançats (ICREA), Passeig Lluís Companys 23, 08010 Barcelona, Spain; [3]Instituto de Ciencia de Materiales de Aragón (ICMA), CSIC y Universidad de Zaragoza, Plaza San Francisco s/n, 50009 Zaragoza Spain

Cisplatin and related platinum based antitumor drugs are widely used to treat cancer. However, their administration is limited due to toxic side effects, such as neurotoxicity, emetogenesis and nephrotoxicity, and many tumors display intrinsic or acquired resistance against these drugs [1]. Therefore, it is of great interest to develop new anticancer drugs based on other transition-metal complexes.

Copper complexes provide a potential alternative. Among the essential metal ions in the human body, Cu^{2+} is third in abundance after Fe^{3+} and Zn^{2+}, and it plays very important roles in several biological processes. Also many copper(II) complexes have been reported to efficiently cleave DNA by an oxidative or hydrolytic mechanism [2].

In this communication we report the synthesis and characterization of some copper(II) compounds with substituted 2,2′:6′,2″-terpyridine ligands. The interaction between the ligands and their metal complexes with DNA has been studied by UV–Vis spectroscopy titrations, fluorescent indicator displacement and electrophoretic mobility.

Financial support by the Ministerio de Economía y Competitividad of Spain (Project CTQ2011-27929-C02-01) is gratefully acknowledged.



References

1. Galanski M, Jakupec MA, Keppler BK (2005) Curr Med Chem 12:185–211
2. Li GY, Du KJ, Wang JQ, Liang JW, Kou JF, Hou XJ, Ji LN, Chao H (2013) J Inorg Biochem 119:43–53

P 144

New mixed-ligand Cu(II) complexes acting as "self-activating" chemical nucleases

Inês Rodrigues[1], Filipa Mendes[1], Elisa Palma[1], Isabel Correia[2], Fernanda Carvalho[2], Isabel C. Santos[1], Fernanda Marques[1], Isabel Santos[1], António Paulo[1], Sofia Gama[1]

[1]Centro de Ciências e Tecnologias Nucleares-C[2]TN, Instituto Superior Técnico, Universidade de Lisboa, Campus Tecnológico e Nuclear, Estrada Nacional 10, km 139.7, 2695-066 Bobadela, LRS-Portugal;
[2]Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Avenida Rovisco Pais 1, 1049-001 Lisboa, Portugal

There has been considerable interest in the recent years for the development of DNA cleaving reagents aiming at their application in biotechnology and medicine [1]. Transition metal ions show diverse structural features, variable oxidation and spin states and redox properties in different complexes, offering plenty of opportunities to discover novel artificial nucleases.

Among the first row transition elements, copper has got a special interest in this regard since the discovery of the first chemical nuclease by Sigman et al. [2]. Copper has high affinity for the nucleobases and copper complexes possess biologically accessible redox properties [1]. Several copper complexes have been proposed as potential anticancer substances and cancer inhibiting agents, as they demonstrate remarkable anticancer activity and show general toxicity lower than platinum compounds [3]. Very recently, mixed ligand copper(II) complexes were found to exhibit prominent anticancer activity by inducing apoptosis, binding strongly and cleaving DNA [3].

In order to develop novel artificial nucleases, several mixed bipyridine-terpyridine Cu(II) complexes were synthesized. The ability of the ligands and Cu(II) complexes to cleave DNA was evaluated by monitoring the conversion of supercoiled ΦX174 plasmid DNA to nicked circular and linear DNA. All the Cu(II) complexes tested present a high artificial nuclease activity. Their cytotoxic properties were also tested on A2780 and A2780cisR cell lines. All the new mixed-ligand Cu(II) complexes presented a higher cytotoxicity when compared with cisplatin, and were able to significantly overcome cisplatin resistance.

The authors would like to acknowledge FCT (PTDC/QUI–QUI/114139/2009, SFRH/BPD/29564/2006 grant to SGama and FCT Investigator Grant to FMendes) and COST CM1105 Action for financial support.

References

1. Ghosh K, Kumar P, Tyagi N, Singh UP, Goel N (2011) Inorg Chem Commun 14:489–492
2. Sigman DS, Graham DR, Daurora V, Stern AM (1979) J Biol Chem 254:12269-12272
3. Loganathan R, Ramakrishnan S, Suresh E, Riyasdeen A, Akbarsha MA, Palaniandavar M (2012) Inorg Chem 51:5512–5532

P 145

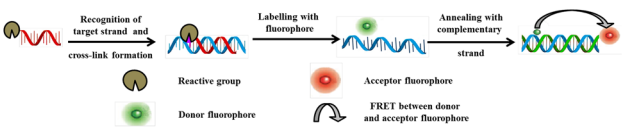
Site-specific covalent post-transcriptional labeling of oligonucleotides for the study of single molecules

Igor Oleinich, David Egloff, Eva Freisinger

Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland. igor.oleinich@chem.uzh.ch

Single molecule spectroscopy has been used intensively to investigate mechanisms in living cells [1]. This technique requires the site-specific functionalization of native biomolecules while retaining their structures and functions. Currently, our research focuses on a new labeling strategy for the site-specific functionalization of long oligonucleotides [2, 3]. The newly developed strategy is based on a modular system consisting of three parts: a short oligonucleotide recognition sequence, a reactive group that is specifically designed to generate the respective functional group, as well as a linker between recognition sequence and reactive group.

The basic principle of this approach relies on the annealing of the reactive strand with the target oligonucleotide to position the reactive group close in space to the target nucleotide. Following, if need be, the activation of the reactive group, generally first a cross-linked duplex is formed, which upon cleavage of the reactive group or the linker results in the new functionality that can be used to attach, e.g., a fluorescent dye. Among the new functional groups that we were able to introduce so far are alkine, aldehyde, and thiol groups.



Financial support by the University of Zurich, the Swiss National Science Foundation (EF), and the COST CM1105 Action is gratefully acknowledged.

References

1. Wang Z, Zhang K, Shen Y, Smith J, Bloch S, Achilefu S, Wooley KL, Taylor J-S (2013) Org Biomol Chem 11:3159–3167
2. Oleinich IA, Freisinger E (2014) to be submitted
3. Egloff D, Freisinger E (2014) submitted

P 146

Interaction of Pd^{2+} complexes of 2,6-disubstituted pyridines with nucleoside 5′-monophosphates

Oleg Golubev, Tuomas Lönnberg, Harri Lönnberg
University of Turku, Department of Chemistry, FIN-20014 Turku, Finland. oleg.golubev@utu.fi

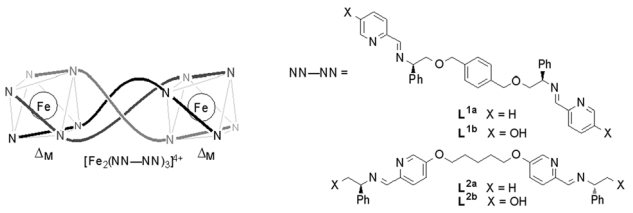
To learn more about the underlying principles of metal-ion-mediated recognition of nucleic acid bases, $PdCl^+$ complexes of six 2,6-disubstituted pyridines, viz. pyridine-2,6-dicarboxamide, its N^2,N^6-dimethyl and N^2,N^6-diisopropyl derivatives, 6-carbamoylpyridine-2-carboxylic acid, 6-aminomethylpyridine-2-carboxamide and its N^2-methyl derivative, were prepared and their interaction with nucleoside 5′-monophosphate (NMP) was studied by ^{1}H NMR spectroscopy in D_2O at pH 7.2. The binding sites within the nucleobases were assigned on the basis of Pd^{2+} induced changes in chemical shifts of the base moiety proton resonances. The mole fractions of NMPs engaged in mono- or dinuclear Pd^{2+} complexes were determined at various concentrations by comparing the intensities of the aromatic and anomeric protons of the complexed and uncomplexed NMPs. Some of the pyridine complexes showed moderate discrimination between the NMPs.

P 147

Recognition of alternative DNA and RNA structures by bimetallic triple-stranded ferro-helicates

Jaroslav Malina[1], Peter Scott[2], Viktor Brabec[1]
[1]Institute of Biophysics, Academy of Sciences of the Czech Republic, v.v.i., Kralovopolska 135, CZ-61265 Brno, Czech Republic;
[2]Department of Chemistry, University of Warwick, Gibbet Hill Road, Coventry CV4 7AL, UK. malina@ibp.cz

Metal-templated helicates commonly comprise three bis(bidentate) NN—NN ligands wrapped in a helical array around two metal ions [often iron(II)]. They are of a similar size and shape to natural biomolecule recognition units, such as α-helices or zinc fingers that recognize the major groove of the DNA. There is a hope that they can be developed into useful molecules in medicinal chemistry as a non-peptide mimetics. Indeed, promising anticancer and antimicrobial activity has been reported for some helicates [1,2] and it has been hypothesized that binding to DNA is responsible for this behavior [1]. Unfortunately, the helicates have remained difficult to use in the medicinal arena because they contain mixtures of isomers, are insoluble, or are too difficult to synthesize. Recently, it has been reported a new strategy to prepare optically pure, water-stable, functionalized metallo-helical assemblies that have been called 'flexicates' [3, 4]. The experiments revealed that in addition to specific interactions with DNA, flexicates exhibit very promising antimicrobial activity against MRSA (methicillin-resistant *S. aureus*) and *E. coli*, alongside low toxicity towards the nematode worm *C. elegans* [4]. Flexicates have been also shown to have comparable activity to cisplatin against the cell lines MCF7 and A2780 and ~5-fold higher activity against the cisplatin-resistant A2780cis [5].



We have been investigating interactions of the two classes of iron(II) flexicates with alternative DNA and RNA structures regarded as potential drug targets via a range of biophysical techniques. The results show that L^{1a} flexicates have significantly enhanced binding and stabilizing properties towards DNA three-way and four-way junctions and also towards DNA and RNA bulges.

References

1. Richards AD, Rodger A, et al. (2009) Int J Antimicrob Agents 33:469–472
2. Pascu GI, Hotze ACG, et al. (2007) Angew Chem Int Ed 46: 4374–4378
3. Howson SE, Scott P, et al. (2011) Dalton Trans 40:10268–10277
4. Howson SE, Bolhuis A, et al. (2012) Nat Chem 4:31–36
5. Brabec V, Howson SE, et al. (2013) Chem Sci 4:4407–4416

P 148

NMR study of metal ion interactions with the D1 κ-ζ region of a group II intron

Simona Bartova, Maria Pechlaner, Daniela Donghi, Roland K. O. Sigel
Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland. bartovai@vscht.cz

Self-splicing group II introns are highly structured RNA molecules, containing a characteristic secondary and catalytically active tertiary structure, which is formed only in the presence of Mg^{2+} [1, 2]. This divalent metal ion initiates the first folding step governed by the κ-ζ element of domain 1 (D1), which also provides the platform for other domain docking [3, 4]. Mg^{2+} can coordinate to RNA via inner or outer-sphere interactions [5]. In order to better characterize these interactions, other metal ions can be used to mimic Mg^{2+}, namely $[Co(NH_3)_6]^{3+}$ for outer-sphere interaction and Cd^{2+} for inner- and outer-sphere interactions, having also a higher affinity.

We performed a detailed multinuclear NMR study of Cd^{2+} and $[Co(NH_3)_6]^{3+}$ interactions with the κ-ζ region of the group II intron ribozyme *Sc*.ai5γ from baker's yeast [4]. The aim was to confirm the stabilization of the κ-ζ region induced by these metal ions, as well as to characterize their binding sites. Accordingly, several 1D (^{1}H, ^{31}P, ^{113}Cd) and 2D ([^{1}H, ^{13}C]-HSQC, ^2J-[^{1}H, ^{15}N]-HSQC) NMR experiments were recorded to map the spectral changes upon addition of different amounts of the metal ions. Our NMR data reveal a strong interaction of Cd^{2+} with G1N7 and proved the macrochelate formation of the corresponding phosphate group and G1N7. Other interactions of Cd^{2+} were detected in the GAAA-tetraloop, the κ region and around the three-way junction. $[Co(NH_3)_6]^{3+}$ interaction was also confirmed by multinuclear NMR spectroscopy. Together with our recently published data on Mg^{2+} interaction [4] we now present a much better understanding of Mg^{2+} binding to D1 κ-ζ.

Financial support by the Swiss National Science Foundation (to DD and RKOS) and the University of Zurich is gratefully acknowledged.

References

1. Pyle AM (2002) J Biol Inorg Chem 7:679–690
2. Sigel RKO (2005) Eur J Inorg Chem 2281–2292
3. Waldsich C, Pyle AM (2007) Nat Struct Mol Biol 14:37–44
4. Donghi D, Pechlaner M, Finazzo C, Knobloch B, Sigel RKO (2013) Nucleic Acids Res 41:2489–2504
5. Donghi D, Sigel RKO (2012) Methods Mol Biol 848:253–273

P 149

Anti-cancer effects of Pt(IV) complex in cisplatin-resistant ovarian cancer cells

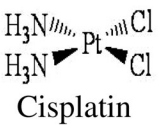
Takao Tobe, Karin Shimizu, Miki Motoyama, Koji Ueda, Yoshinori Okamoto, Nakao Kojima

Faculty of Pharmacy, Meijo University, 150 Yagotoyama, Tempaku-ku, Nagoya 468-8503, Japan

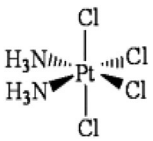
Platinum(IV) [Pt(IV)] complexes are an anti-cancer prodrug to be activated via reduction by which they exert Pt(II)-based drug activities in cells including the target cancer cells. Pt(IV) complexes show less adverse effects such as renal toxicity because of their low reactivity with biological molecules compared to a typical anti-cancer drug cisplatin. This different reactivity prompted us to investigate the effectiveness of Pt(IV) on cisplatin-resistant cancer cells with its mechanisms. This study aims to elucidate the anti-cancer effects of Pt(IV) complex.

In this study we have investigated *cis*-diammine-tetrachloro-Pt(IV) [*cis*-Pt(IV)] and cisplatin of the cytotoxicity against human ovarian cancer cell lines (A2780), and its cisplatin-resistant subline (A2780cis), with their influx/efflux patterns using ICP-MS. Pt–DNA-crosslink formation was also determined by agarose gel electrophoresis, in which the crosslink was quantified by decrease of ethidium bromide staining of DNA.

Cisplatin completely failed to decrease the survival of A2780cis cells at a concentration that killed A2780, whereas *cis*-Pt(IV) exerted cytotoxicity in both cell lines. Pt–DNA-crosslink formation assay showed *cis*-Pt(IV) bound to DNA only in the presence of reductant such as glutathione (GSH) or ascorbic acid, indicating cisplatin production. However, it is also known excess amount of GSH suppress DNA binding of cisplatin. Indeed, intracellular GSH level in A2780cis cells was about 3 times higher as compared with A2780 cells; therefore cisplatin would be inactivated in A2780cis cells. These findings suggest that *cis*-Pt(IV) is actually reduced to cisplatin in GSH-rich environment and then induce cytotoxicity by somehow escaping from GSH-dependent inactivation. Pt(IV)-specific subcellular distribution might contribute this paradoxical advantageous effect of GSH on *cis*-Pt(IV). Intracellular accumulation of Pt was reduced by competing a related transporter CTR1 by copper in *cis*-Pt(IV) treatment, whereas cytotoxicity was not suppressed. Therefore CTR1 is involved in the intake of Pt complexes including *cis*-Pt(IV), but the alternative pathway(s) could be responsible for anti-cancer effect of Pt(IV) complexes.



Cisplatin



cis-Pt(IV)

P 150

Stability and halide selectivity of the fluoride riboswitch explained

Mohit Chawla[1], Romina Oliva[2], Luigi Cavallo[1]

[1]King Abdullah University of Science and Technology, KAUST Catalysis Center, Thuwal, 23955-6900, Saudi Arabia;
[2]University "Parthenope" of Naples, Department of Sciences and Technologies Centro Direzionale Isola C4, 80143 Naples, Italy

Riboswitches are short mRNA segments deputed to control gene expression in response to the selective binding of a metabolite. Currently, over 20 classes of riboswitches are known, targeting metabolites that are either neutral or positively charged molecules, since this reduces or even favors the interaction of the metabolite with the negatively charged RNA backbone. This simple consideration has been recently questioned by the discovery of a riboswitch that targets a negatively charged fluoride anion. This fluoride sensing riboswitch was found to activate the expression of genes that encode fluoride transporters. Besides targeting the small fluoride anion with good efficiency, this riboswitch has also remarkable halide selectivity, since no chloride binding was observed in presence of KCl. The X-ray structure of the fluoride riboswitch evidenced that the fluoride is the central unit keeping together a small cluster of three Mg^{2+} cations, whose coordination sphere is completed by either oxygen atoms of phosphate groups, and by water molecules, one of them acting as a bridge between two of the Mg^{2+} ions.

The peculiar halide selectivity and the structure of the fluoride riboswitch provoke a series of questions: (i) Is the small Mg^{2+}/F^{-}/phosphate/water cluster at the center of the riboswitch a stable entity on its own? (ii) Considering that water molecules bridging metal cations are known to be quite acidic, which is the acidity of the bridging water of the central cluster of the fluoride riboswitch? (iii) Which is the origin of the halide selectivity? In this contribution we provide a clear explanation to the above three fundamental questions, based on static and dynamic density functional theory calculations.

P 151

Nano-arrays of DNA-binding cylinders

Jenifer C. White, Michael J. Hannon

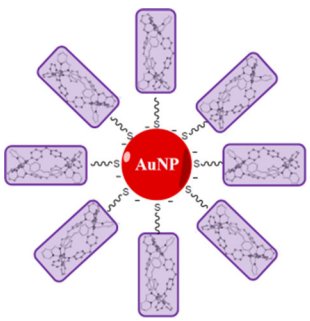
Department of Chemistry, University of Birmingham, Birmingham, B15 2TT, England. jcw129@bham.ac.uk

Metallo-supramolecular helicates are able to bind to DNA via a number of interactions. The di-nuclear supramolecular cylinders; $[Fe_2L_3]^{4+}$ and $[Ru_2L_3]^{4+}$ ($L = C_{25}H_{20}N_4$), have a size and shape comparable to those of zinc fingers [1]. Consequently, it is able to fit into the major groove of B-DNA and bind at the heart of DNA fork structures to induce structural change. These interactions mean the cylinder has the potential to act as a potent apoptotic and cytostatic agent [2, 3].

It is aimed to combine this supramolecular chemistry with nanotechnology, by attaching the helicate to the surface of gold nanoparticles, AuNPs. In doing so, it is hoped to enhance the interactions currently observed with DNA, ultimately with the aim of causing hindrance to DNA replication as a result of enhanced coiling, resulting in a more potent anticancer therapy.

In an attempt to achieve this, triple stranded helicates were synthesised with varying functionality in an attempt to attachment the cylinders to the AuNP surface. The use of surfactant coated nanoparticles and the electrostatic interactions between cationic cylinders and anionic AuNPs have also being investigated.

Supramolecular helicates have been functionalised using the novel use of click chemistry. These new structures are shown to bind to DNA, inducing a structural change, hence the use of click chemistry will be investigated to add functionality to the surface of AuNPs [4].



References

1. Hannon MJ, Moreno V, Prieto MJ, Moldrheim E, Sletten E, Meistermann I, Isaac CJ, Sanders KJ, Rodger A (2001) Angew Chem Int Ed 40:879–884
2. Hotze ACG, Hodges NJ, Hayden RE, Sanchez-Cano C, Paines C, Male N, Tse M-T, Bunce CM, Chipman JK, Hannon MJ (2008) Chem Biol 15:1258–1267
3. Khalid S, Hannon MJ, Rodger A, Rodger PM (2006) Chem Eur J 12:3493–3506
4. Zhou Y, Wang S, Zhang K, Jiang X (2008) Angew Chem Int Ed 47:7454–7456