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Vibrational Spectroscopy of Nucleic Acids

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ABSTRACT

During 1950s, X-Ray fiber diffraction technique was widely used in numerous researches about the structure of nucleic acids and later, single crystal diffraction has been the most implemented technique for this purpose [1-4]. Such investigations were led to identification of the structural details of some right- and left–handed DNA and RNA helices at the atomic resolution level. While nucleic acid crystals are necessary for reliable X-Ray Diffraction (XRD) data acquisition, the triple helical structures have not been successfully crystalized yet. Further, the biological importance of the nucleic acid structure can be understood if the geometry of DNA and RNA in aqueous solutions is clearly known. In this regard, it has been confirmed that vibrational spectroscopy can be effectively used to investigate the structure of nucleic acid {the limitation of NMR (Nuclear Magnetic Resonance) is the size of the entities that can be studied}.

INTRODUCTION

Using vibrational spectroscopy, both infrared (IR) spectroscopy [5,6] and Raman spectroscopy [7-9], for structural identification of nucleic acid backs to 1950s. Some advantages of this technique are listed:

(a) Samples can be investigated in both polycrystalline and crystalline states as well as in various conditions such as solution, hydrated fibers or films.

(b) The size of the considered structure is not a limitation and hence, it allows studying numerous and various structures including, but not limited to, native DNA or RNA, polynucleotides, DNA fragments obtained by enzymatic or chemical cleavage, or short synthetic oligonucleotides.

(c) This is a non-destructive technique and a little amount of sample is enough for investigation (typically 100 gr).

(d) It allows to simply correlating the structures identified in the solid state using X-Ray crystal diffraction and in solution using NMR. Therefore, the structural results in aqueous solutions can be estimated from single-crystal investigation through comparing the corresponding IR and/or Raman spectra.

(e) It easily allows varying numerous external parameters such as ionic strength, nature of counter ions, pH, temperature or hydration.

A set of marker IR bands and Raman lines can be obtained by combining X-Ray, IR and Raman techniques using the same DNA or RNA crystals as well as characteristic of different geometries in solid state and more detected in amorphous (hydrated fibers and films) and solution states [10-20]. It is possible to simply follow various conformational transitions such as helix → coil, B → A, B → Z, antiparallel → parallel and double helical → triple helical [21-24]. Vibrational spectroscopy can be effectively used to determine the base pairing schemes (Watson–Crick, reverse Watson-Crick, Hoogsteen, etc.), the sugar conformations (C2'-endo, S-type or C3'-endo, N-type) and the glycosidic torsion in some nucleosides (anti or syn) [25-39]. In addition, the stability of the obtained structures (melting of double and triple helices) can be analyzed using this technique [40-45].

The aim of the current book chapter is discussing the IR bands and Raman lines as structure indicators of various DNA and RNA double helices. Then, multistranded structures (triple helices and quadruplexes) and their characterization are investigated and finally, the effectiveness of vibrational spectroscopy for understanding the hydration of nucleic acids is discussed. In the text, RNA (r) and DNA (d) sequences are written using the usual notation (i.e. A, G, C, T, and U designate nucleotides containing, respectively, adenine, guanine, cytosine, thymine and uracil bases) [46,47].

Vibration spectroscopy is of many advantages, among them providing structural data for nucleic acids in various physical states, are very important [48-50]. The IR spectra of nucleic acids can be seen in a crystalline form, as a hydrated film at high (98%) relative humidity and in solution. It should be pointed out that the latter spectrum was recorded using a multiple internal reflection device in which the DNA concentration expressed as phosphates was 20mM, which is lower than the typical concentrations used in NMR experiments [51-59].

As going from the crystalline state to the dilute solution, the ratio of the relative intensities of the absorptions characteristic of the nucleic acids (principally observed in the 1800-600 cm^{-1} region) and of the solvent (in the present case around 3400 cm^{-1} for H_2O) is clearly decreased [60-63]. Although the amount of interfering solvent absorptions is very high in low-concentration solution spectra, it is possible to extract structural data to analyze them [64,65]. It can be evidently shown that the recorded spectrum of rAn-rUn in H_2O solution [66,67]. It can be obviously seen that the nucleic acid bands in some informative regions is hidden by the solvent absorptions [68-70]. The spectrum of the same sample after H_2O-D_2O exchange shows that these water contributions are shifted toward lower wavenumbers due to the use of D_2O as the solvent [71-77]. It can be seen that the difficulty of observation of the nucleic acid absorptions is reduced as the important H_2O contributions shift from their original location, at around 1600 cm^{-1} and below 1000 cm^{-1}, toward around 1200 cm^{-1} and below 600 cm^{-1} [78, 84]. It is possible to obtain valuable data for vibrations of different parts of the nucleic acid molecules using a parallel study in both solvents. As can be seen, the region ranges between 1800-600 cm^{-1} is divided into four domains, numbered I to IV [85-95].

The range between 1800 and 1500 cm^{-1} is named as domain I. In this domain, the peaks are principally corresponding to in-plane double-bond stretching vibrations of the bases which are characteristic of various base pairing schemes in double and multiple stranded DNA and RNA structures. This spectral domain is basically investigated in D_2O solutions.

The range between 1500 and 1250 cm^{-1} is a base-sugar fingerprint region named as domain II in which there are some absorptions that are particularly sensitive to the anti or syn conformation of purine nucleosides.

The range between 1250 and 1000 cm^{-1} named as domain III in which there are two high peaks related to the IR spectra of nucleic acids. The first peak is located around 1230 cm^{-1} which belongs to the anti-symmetric stretching vibrations of the phosphate groups and its position is a function of the secondary structure of the nucleic acid. However, the position of the second peak is approximately unique, at around 1089 cm^{-1}, for almost all DNA or RNA spectra and belongs to the symmetric stretching vibration of the phosphate groups. As a result of this uniqueness, this band can be considered as an internal standard for spectral normalization.

The region below 1000 cm^{-1} is named as domain IV in which there are various absorptions such as vibrations of the phosphodiester chain, vibrations of the sugars sensitive to the pucker

(C_{20}-endo, S-type or C_{30}-endo, N-type) and out-of-plane double bond vibrations of the bases. It is easier to investigate this domain in D_2O solutions.

A series of issues in using H_2O-D_2O exchange including non–specific deuteration of labile Hydrogens in NH groups of thymines, NH_2 groups of adenines, guanines and cytosines, and of OH groups in RNA sugars may cause band shifts and hence, should be kept in mind. Moreover, at high temperature, Hydrogens attached to Carbon atoms, such as C(8) of purine bases, can be exchanged very slowly.

It should be pointed out that comparing all the informative spectral data on a single figure is not practically possible due to the presence of numerous peaks of these IR spectra with various and therefore, they are divided into domains I-IV.

DOUBLE HELICAL STRUCTURES

It is well known that double helical DNA is highly polymorphic in contrast to RNA. As base sequence effects, curvatures, kinks, and groove width variations were appeared, the modulation of the initial regular double helix of Watson and Crick were detected. In addition, new double helical structures (parallel duplexes) also were presented. The helical parameters have been standardized to describe all these macromolecules.

Canonical Antiparallel Duplexes

Right-handed B and A families

Aqueous solutions with low-ionic-strength and fibers or films exposed to high relative humidity are suitable media to observe B-DNA which is basically considered as the main structural form in vivo. However, low-water-activity conditions, e.g., water-ethanol solutions or fibers or films exposed to below 76% RH, are essential for stabilizing A-DNA. The double stranded right handed RNA has always been observed in the A family conformation in a solution. Among the differences between both structures going from B to A, the most important ones are: (a) Producing a typical central void as the x displacement of the base pairs displace in x direction and lead to moving the helix axis within the major groove when the A family structure is viewed from the top; and (b) The variation of the sugar pucker from C2'-endo (S-type) to C3'-endo (N-type).

The first observation of Raman spectra for A and B genus forms were related to fibers of native DNAs and then in oligomer crystals. IR studies concerning these conformations have been developed.

It can be seen that the spectra for the d(CCCCCGCGGGGG)2 crystal. The crystal was also studied by X-Ray Diffraction (XRD) and the results were shown that a complete turn of A DNA is formed. As can be seen, the spectra for rAn-rUn in solution are illustrated which can be considered as the characteristic of the A family form of double helical RNA. Also, it can be seen that the spectra for dAn-dTn in solution which are the characteristic of the B family form of double helical DNA. It

should be noted that the average values of wavenumbers are listed which introduce the families of geometries. However, the exact values may be differing by several wavenumbers as they are strongly dependent on the sequence of the nucleic acid. The results corresponded to each described structure show the exact positions of them.

It can be seen that the sharp band resulted from the base pairing in double stranded helices in the B geometry and the A geometry are at around 1715 cm^{-1} and 1705 cm^{-1}, respectively, while the antisymmetric phosphate stretching vibration are located at 1224 cm^{-1} and 1243 cm^{-1}, respectively. A sugar band can be seen around 1188 cm^{-1} for A DNA. The absorption around 835 cm-1 indicates the S-type sugar pucker of the B family form double helix. However, the presence of N-type sugars in the A family double helix is indicated by absorptions around 807 and 864 cm^{-1}.

Here, we show that how B and A geometries for nucleic acids containing A-T or A-U base pairs can be characterized. For this purpose, a series of double stranded polynucleotides are investigated and their corresponding IR spectra within domain II are shown for those were formed by homobase strands and (e)-(h) for those were formed by regularly alternating purine-pyrimidine strands. Adenine and thymine (or uracyl) are the bases while the sugars deoxyribose or ribose. As a result, the characteristic of B and A geometries has been determined by a set of IR absorptions. It was found that the bands of adenosine and thymidine in the B geometry which were at 1344 cm^{-1}, 1328 and 1281 cm^{-1}, respectively, can be observed in the A geometry at 1335 cm^{-1} and 1275 cm^{-1} as the overlap of adenosine and thymidine and thymidine, respectively. The IR spectra from within domain IV are shown. It can be seen that for the spectra of d(A-T)n-d(A-T)n in high hydration conditions while domain IV is the same but in low hydration conditions. However, it can be seen that the spectra of r(A-U)8-r(A-U)8 in solution. It is possible to distinguish between both geometries through characteristic absorptions of C2'-endo, S-type sugars at 841 cm^{-1} and of C3'-endo, N-type sugars around 810, 864 and 880 cm^{-1}.

DNA-RNA hybrid structures

There are some conditions in which DNA-RNA hybrids can be formed in vivo; e.g., during transcription of DNA into RNA or reverse transcription of viral RNA into DNA. In addition, the antisense gene expression control strategy is strongly dependent on the formation of hybrids between generally modified DNA oligonucleotides (future potential pharmaceuticals) and the targeted messenger RNA or viral RNA. For designing the distinguish between both geometries, hence, it is very important to have a good knowledge about the ability of an oligomer to form a hybrid and the resulted structure.

One of the best methods for adopting the heteronomous geometries of hybrids with one complete RNA strand and one DNA strand such as rAn-dTn, dAn-rUn or rCn-dIn (I = Inosine) is fiber X-Ray Diffraction (XRD). This issue is confirmed by the Raman study in solution of the rA8-dT8 hybrid. The IR spectra of rAn-dTn and dAn-rUn are investigated, respectively. The above mentioned characteristic marker bands of adenosines with sugars in N- or S-type conformations

(respectively 1344 and 1335 cm-1) and thymidines (respectively 1328, 1281 cm-1 and 1335, 1275 cm-1) demonstrate the adoption of an N-type geometry and the deoxyribose strand sugars an S-type geometry in both hybrids of the ribose strand sugars. It should be pointed out that the previously performed investigations have been shown that sugar puckers of hybrids with sequences containing all four bases cannot be clearly identified by NMR and they are not uniform.

In order to evaluate the potential use of modified DNA oligomers in gene therapy, they are tested in a way that rapid enzymatic degradation is not allowed *in vivo*. IR spectroscopy has been confirm that it is possible to form DNA-RNA hybrids with DNA containing (methylene)-methylimino instead of native phosphodiester linkages. All sugars adopt the N-type conformation in such hybrids.

Left-handed Z family

The investigation of the d(CGCGCG)2 crystal by X-Ray Diffraction (XRD) was led to proposing the left-handed double helical Z conformation. The results obtained from the Raman spectra of the crystal of the high-ionic-strength solution of the oligomer and of the d(G-C)n-d(G-C)n polymer has emerged the interesting investigations about the presence of such left-handed structures (Z family form) in biological systems. The Raman spectra of Z conformation have been previously described and there is an in-depth discussion about the IR Z-form bands in the literature.

The Z-form spectra, i.e. the Raman spectra of solutions and IR spectra of hydrated films, are related to the three regularly alternating purine-pyrimidine polymers. The same IR spectra are studied while presents the scale of the base fingerprint region (part of domain II) is expanded. Through a specific interactions of divalent transition metal ions on the N(7) sites of the purines, the left-handed structures incorporating A-T base pairs in hydrated films has been stabilized. It has been confirmed through the IR spectroscopy implemented on polynucleotides containing guanines and/or adenines deuterated on the C(8) site that the interaction is localized. In solutions, however, it has been shown that the Z form also can be determined by interaction with transition metal ions in low-water-activity conditions and it can be characterized by Raman spectroscopy. The level of the purine nucleosides is one of the major differences between the right- and left-handed helices. In right-handed geometries, guanosines and/or adenosines are in the anti-conformation while in left-handed geometry they are in the syn conformation. This difference can be obviously recognized on the IR spectra as the anti dG and/or anti dA bands located around 1420 and 1374 cm^{-1} are shifted to around 1410 and 1354 cm^{-1} for syn dG and/or syn dA. This is also happened on the Raman spectra as the anti dG and/or anti dA bands located around 682 and 727 cm^{-1} are shifted to around 625 and 622 cm^{-1} for syn dG and/or syn dA.

The characteristic marker peaks of the B, A and Z geometries can be easily used to determine the antiparallel double helical conformations. The markers often used for this purpose are investigated. Only the spectra that concern sequences containing G-C base pairs are illustrated for simplicity. The Raman spectra of the B form of d(G-C)n-d(G-C)n, the A form of d(CCCCCGCGGGGG)2

and the Z form of d(G-C)n-d(G-C)n are studied, respectively. However, the IR spectra of (d-f) the B, A and Z forms of d(CCCGCGGG)2 are investigated, respectively. It should be noted that the above mentioned marker bands of B, A and Z geometries are of similar hatches on the Raman and IR spectra.

Conformational transitions

The transition from helix to coil in nucleic acids as well as the transitions between different double helical geometries resulted from the change in the temperature or the hydration of the DNA samples can be easily determined by the vibrational spectroscopy. Several examples are given below.

Helix to Coil Transition. In the early 1970s, the transition from helix to coil, known as melting transition, was firstly characterized by Raman spectroscopy for the native DNAs and polynucleotides. However, the IR spectroscopy also was used for this purpose. An example of transition from helix to coil for a DNA is studied. The relative intensity of the adenine absorption, which is very sensitive to base pairing, located at 1622 cm^{-1} can be measured to identify the melting of the d(A-G)7-d(T-C)7 duplex structure on the IR spectra recorded in D_2O. As can be seen, the melting occurs at around 70°C in these experimental conditions. As discussed in Section "Purine Motif Triplexes", 3.2., the multiphasic melting in triple helices containing T*A-T or A*A-T base triplets can be easily identified by this adenine band. It can be observed that melting causes a decrease in intensity of the symmetric stretching vibration band of the phosphates (located at 1089 cm^{-1}) and shifts it to lower wavenumbers (1064 cm^{-1}).

The involvement of guanine or cytosine in base pairing is dependent on the DNA sequence and it can be identified by measuring the evolution of the relative intensities of absorptions assigned to guanine (1576 cm^{-1}) or cytosine (1524 cm^{-1}) vibrations.

B to A Transition. The Raman experiments performed on the fibers exposed to various ethanol/water mixtures and the IR experiments using polarized radiation on oriented hydrated films can be used to identify the conformational transition from B to A for native DNAs. During the transition from B to A, a reorientation occurs for the phosphate groups which can be determined by linear dichroism measurement. In the A form, the perpendicular polarization of the non-dichroic B-form antisymmetric phosphate stretching vibration which locates around 1224 cm^{-1} occurs at around 1243 cm^{-1}. Vice versa, the symmetric phosphate stretching vibration around 1089 cm^{-1} becomes parallel during the same transition. The conformational transition can be identified by using the orientations of the corresponding transition moments as probes.

The modification of sugar pucker can be considered to identify the same B to A transition using the characteristic S-type and N-type sugar bands. It can be seen that for a transition from B to A for d(A-T)n-d(A-T)n in film, which resulted from a decrease in the RH to which the sample was exposed. While the only thing that can be observed at 98% RH is the marker band of S-type sugars at 841 cm^{-1}, it is possible to detect a contribution around 866 cm^{-1} at 81% RH which confirms the

beginning of the S-type to N-type sugar repuckering. By more decreasing down to 71% RH and below it, it can be seen that all markers of N-type geometry (808, 866, 880 cm^{-1}) are emerged while no absorption can be seen around 841 cm^{-1} which indicates that the transition from B to A is complete.

B to Z Transition. As the ionic strength of d(G-C)n-d(G-C)n increases, a conformational transition would occur from B to Z. Previously, the Raman spectra of d(G-C)n-d(G-C)n had been obtained in low- and high ionic-strength conditions. However, it was not possible to certainly assign the high salt spectrum to the left-handed structure as there was not enough crystallographic data at that time.

During the crystallization process, it is possible to see a conformational transition in oligonucleotide duplexes. However, the structural results from X-Ray crystal diffraction and NMR solution studies will be different in this condition. It can be seen that such apparent discrepancy can be easily explained by vibrational spectroscopy for two duplexes which have been investigated in solution (a,c) and crystalline states (b,d). The Raman data are studied while the IR data are illustrated. It can be seen that in both cases, crystallization leads to a transition from a right-handed B form to a left-handed Z form. The Raman peaks characteristic of the B form for d(CACGTG)2 occurs in solution at around 682 and 830 cm^{-1}, while those are found in the crystal for the Z form at around 621 and 807 cm^{-1}. Similar results are obtained by IR spectroscopy as the IR bands characteristic of the B form for d(CCGCGG)2 are observed in solution at around 1087, 1372 and 1420 cm^{-1} while those are found in the crystal for the Z form at around 924, 1320, 1360 and 1413 cm^{-1}.

Parallel DNA Duplexes

The newly discovered parallel structures for DNAs have been firstly observed in hairpin structures and some particular linear duplexes. It seems that they have a key functional role in gene expression and recombination. There are various types of parallel DNA helices which are dependent on the DNA sequence, the pH, and the counter ions and some types of them can be stabilized by the addition of drugs. Various researchers have been proposed some models for base pairing in parallel duplexes. The spectra of the parallel structures recorded in D_2O (spectral domain I) are illustrated while the corresponding spectra of classical antiparallel duplexes with identical base sequences and that of a single stranded structure are investigated, respectively.

The Raman and IR spectroscopy have been used to investigate the secondary structure of parallel DNAs containing only A-T base pairs. The spectrum of a parallel duplex 50-d(AAA AAA AAA ATA ATT TTA AAT ATT T)-30-50-d(TTT TTT TTT TAT TAA AAT TTA TAA A)-30 is studied. New results show that the characteristic marker bands of the C(2)DO(2) and C(4)DO(4) carbonyl stretching vibrations of thymines and for the antiparallel stranded reference spectrum are emerged at 1685, 1668 cm^{-1} and 1696, 1663 cm^{-1}, respectively. This difference is due to the reverse Watson-Crick base pairing of A-T with Hydrogen bonds which is established between N(6)H (A)Đ Đ ĐO(2) (T) and N(1) (A)Đ Đ ĐHN(3) (T).

It can be seen that another example of reverse Watson–Crick base pairing for sequences with mixed A-T/G-C composition. A 50-50 linker is introduced in the sequence during DNA synthesis 30-d(TTA TAG GGA T)-50-linker-50-d(A TCC CTA TAA)-30 to force the parallel character of the duplex in which the linker is -PO-(CH_2CH_2O)3P-. The parallel structure can be stabilized by Lithium counter ions. It can be seen that the spectrum of the corresponding antiparallel duplex 50-d(AAT ATC CCT A)-30-linker-50-d(T AGG GAT ATT)-30 with regular Watson-Crick base pairing which recorded in the same conditions. The absorptions of the thymine carbonyl can be found at around 1683 and 1668 cm^{-1} which confirms the presence of reverse Watson-Crick A-T base pairing.

It is also possible to form a parallel duplex structure in acidic pH conditions, for the IR characterization of such duplexes. A non-perfectly matched antiparallel duplex (10 out of 14 A-T or G-C Watson-Crick base pairs) is studied as the oligonucleotide 50-d(AGA AAA AAG AAG AA)-30 can form with 30-d(TTC TTC TTT TTT CT)-50 at neutral pH. By decreasing the pH to 4, cytosines are protonized. Hence, a new structure is formed in the presence of Mg_2C counter ions. As a result of this, the C(4)DO(4) thymine carbonyl band shifts to lower wavenumbers (1657 cm^{-1}) while the thymine band at 1641 cm^{-1} completely disappears. It confirms that a non-perfectly matched antiparallel Watson-Crick duplex is reorganized into a perfectly matched parallel Hoogsteen paired duplex with isomorphous A-T and G-CC base pairs.

It is well known that the oligonucleotides formed by regularly alternating A and G bases are self-associate and have the ability to form parallel duplexes. A parallel duplex which formed by a non-regularly alternating AG-rich sequence d(GAA GAG AAA GAA GG) in acidic pH conditions is investigated. The considerable IR spectrum of the single strand at pH 7 decrease of the guanine contribution at 1565 cm-1 confirms that the double stranded parallel DNA at pH 4 is formed.

TRIPLE HELICAL STRUCTURES

During the 1950s, triple helical structures were firstly developed for RNA homopolymers, rAn and rUn45 and then, the literature were continuously enriched by various researchers about the nucleic acid triplexes, with more emphasize on the fundamental potential of them in pharmaceutical applications in gene therapy. NMR has been used to obtain structural data in solution. It is very interesting that triple helices could not crystallize yet. There are rare researches about X-Ray crystal diffraction concerning the formation of base triplets using interactions between dangling ends of incompletely base paired duplexes or with duplex junctions.

It is well known that the main types of base triplets are the pyrimidine motif pyr*pur-pyr and the purine motif pur*pur-pyr (In this notation, the "*" indicates the Hydrogen bonds between the third strand written on the left and the purine strand of the original duplex while the " - " indicates the Watson-Crick Hydrogen bonds of the duplex.).

The base triplets of pyrimidine motif are T*A-T (or U*A-U for ribonucleotides) and CC*G-C (it should be noted that the formation of an H bond with the guanine N(7) is only possible if the third strand cytosines on the N(3) site is protonized). The Hoogsteen paired to the purine bases of the duplex are the third strand bases and the base triplets are isomorphous. The orientation of the third strand is parallel to the purine strand of the Watson-Crick duplex. If G-C base pairs are targeted, an acidic pH is necessary to form such triplexes.

The base triplets of the purine motif are A*A-T, G*G-C and T*A-T but its three canonical base triplets are not isomorphous and hence, there is a possibility of backbone distortions when the base triplets are mixed. It is considered that the third strand is oriented antiparallel to the purine strand of the duplex. However, its orientation may be changed from antiparallel to parallel when the third strand is composed of both guanines and thymines. This change is dependent on the number of GpT and TpG steps in the sequence. The third strand polarity is parallel when only G*G-C base triplets are present. It is possible to observe purine motif triplexes in neutral pH. Generally, it is necessary to have divalent ions such as Mg_2C or Mn_2C for stabilizing purine motif triplexes.

Vibrational spectroscopy is a powerful technique to detect the formation of the triple helices, to identify the Hydrogen bonding scheme between the third strand and the duplex purine strand bases, and to characterize the sugar conformations of the different strands. The third strand orientation can be determined in combination with molecular modeling investigations. The best method to detect triple helical formation is investigating the base vibrations involved in the structure. Valuable data can be obtained by IR spectroscopy as shown in this section. The Raman spectra of triple helices are also fully described.

Pyrimidine Motif Triplexes

In this section, various IR spectra obtained from D_2O solutions (spectral domain I) of DNA and RNA homopolymer triple helices formed by various canonical pyrimidine base triplets, are shown (for dTn*dAn-dTn; for rUn*rAn-rUn; for rCn C*rGn-rCn; for dCn C*dGn-dCn; and for a 29-mer RNA intramolecular triple helix formed at acidic pH, which contains both CC*G-C and U*A-U base triplets, r(GAGAGAACCCC-UUCUCUC-UUUU-CCUCCUCCUU). The spectra of the corresponding duplexes are shown for dAn-dTn; for rAn-rUn; for rGn-rCn; for dGn-dCn; and for the 29mer RNA at neutral pH.

The absence of the low-wavenumber adenine band at 1622 cm^{-1} (1632 cm^{-1}) which confirms the involvement of the purine in Hoogsteen Hydrogen bonds with the third strand thymine (uracyl) demonstrates that T*A-T (U*A-U) base triplets is formed. If any of the four duplexes dAn-dTn, dAn-rUn, rAn-dTn or rAn-rUn combine with either a rUn or a dTn third strand, eight different triple helices will form leading to very similar IR spectra in the base double bond in-plane stretching vibration region. The first conclusion of this issue is that triple helices can be formed for any possible initial geometry of the duplex in solution (B form for dAn-dTn, heteronomous forms

for dAn-rUn and rAn-dTn, A form for rAn-rUn). The second one is that it is possible to identify the third strand base pairing (Hoogsteen) and its orientation (parallel to the purine duplex strand) in all these triplexes.

If triple helices contain T*A-T base triplets, another third-strand base pairing can exist. For instance, the orientation of the third strand of triple helices formed with a third alpha anomeric dTn strand remains parallel to the polypurine duplex strand. However, the difference is that the third strand binding scheme changes from Hoogsteen to reverse Hoogsteen. For intramolecular triple helices containing T*A-T base triplets, this is the similar case but the orientation of these helices is forced to antiparallel. It should be pointed out that the adenine absorption band at 1622 cm^{-1} is not presented in the spectra of these two last triplexes which confirms the T*A-T base triplets are formed. The presence of two different C(2)DO(2) carbonyl stretching vibrations near 1712 and 1695 cm^{-1} shows that a third strand reverse Hoogsteen Hydrogen binding.

A high-wavenumber band around 1710 cm^{-1} on the IR spectrum shows that the CC*G-C base triplets is obtained after protonation of the N(3) of cytosine in acidic pH conditions. Using alpha anomeric third strand deoxycytidines, triple helices containing CC*G-C base triplets can be formed. In this case, the type of third strand pairing is still Hoogsteen but its orientation is switched from parallel to antiparallel.

Both T*A-T (U*A-U) and CC*G-C triplets will simultaneously form when the targeted sequence contains both purine bases while the corresponding absorptions overlap on the IR spectrum. It is possible that the IR spectra of a 29-mer RNA fold backs twice on itself to form a duplex with a dangling unpaired tail in neutral or basic pH conditions and in acidic pH conditions, after protonation of the unpaired cytosines which is an intramolecular triple helix. An interesting note is that the adenosine band at 1633 cm^{-1}, which is the characteristic of the formation of the U*A-U base triplets, is not presented on the spectrum recorded at acidic pH, while a cytosine band at 1705 cm^{-1} is found, which is the characteristic of the CC*G-C base triplet formation. It should be pointed out that similar trends have also been found for an intramolecular triple helix formed by a chimeric DNA-RNA oligonucleotide d(GAGAGAA-CCCC-TTCTCTC-TTTT)-r(CUCUCUU) containing rCC*dG-dC and rU*dA-dT nucleotide motives at acidic pH.

The sugar geometries in the triplexes also can be detected by vibrational spectroscopy in addition to their formation and the base pairing. The Raman and IR spectra of three pyrimidine motif triple helices, i.e. rUn*rAn-rUn, dTn*dAn-dTn and dCnC*dGn-dCn are investigated, respectively. For rUn*rAn-rUn, an N-type geometry is adopted by all sugars at 815 and 868 cm^{-1} IR bands and at 815 and 863 cm^{-1} Raman lines. For dTn*dAn-dTn, however, an S-type geometry is adopted by all sugars with a peak at around 841 cm-1 for both IR and Raman spectra. Further, both types of sugar geometries are identified for dCn C*dGn-dCn by IR with bands at 867 and 829 cm^{-1}. However, the sugars of the deoxyguanosine strand are detected in the Raman spectrum in an S-type conformation (line at 684 cm^{-1}).

Purine Motif Triplexes

It can be seen that the IR spectra of purine motif triplexes for spectral domain I. The double stranded dGn.dCn has been modified by adding either a third dGn or rGn strand. The presence of an absorption around 1715 cm^{-1} assigned to a C(6)DO(6) guanine carbonyl stretching vibration is a sign of formation of the triple helix. It has been shown by molecular modeling investigations using the IR data that the Hoogsteen-type base pairing with the dGn strand of the duplex involves the third strand and it is oriented parallel to this strand.

The A*A-T is the second possible base triplet in purine motif triplexes. The spectrum of double stranded dAn.dTn and that of the antiparallel triple helix containing A*A-T base triplets induced by folding back twice on itself of the dA10-linker-dA10-linkerdT10 oligonucleotide are studied. As indicated in Section "Conformational Transitions", 2.1.4., the variations of the relative intensity of the adenine absorption located at 1625 cm^{-1} against temperature show a biphasic melting of the structure. The molecular modeling data along with the IR results are in good accordance with a reverse Hoogsteen-like third strand base pairing.

It can be seen that how the sugar conformations in purine motif triplexes is determined. It can be observed in the IR spectra of dGn*dGn-dCn and rGn*dGn-dGn in spectral domain IV that there are both N- and S-type sugar conformations in these triplexes. When the third dGn strand is replaced by an rGn strand, the intensity ratio of the characteristic bands of both geometries at 860 and 835 cm-1, respectively, is inverted. It can be obviously seen in the corresponding Raman spectra for dGn*dGn-dCn that there are two different backbone geometries (lines at 808 and 841 cm^{-1}). In addition, it is clear that the guanosine sugars are equally divided into the S-type conformation (line at 689 cm^{-1}) and the N-type conformation (line at 662 cm^{-1}). However, all guanosine sugars are in the N-type conformation for rGn*dGn-dGn. As a result, it can be concluded that the dCn strand and dGn strand of the Watson-Crick duplex are of S- and N-type sugars, respectively, while the third strand has either N-type sugars (rGn strand) or S-type sugars (dGn strand).

Recombinant Three-Stranded Structures

It has been proposed that the formation of three-stranded complexes with a third strand parallel to the identical strand of the Watson-Crick duplex which are able to accommodate all four bases is a putative intermediate in the homologous recombination process mediated via the RecA protein. It has been demonstrated by electron microscopy technique that RecA protein leads to unwinding of the nucleic acid which in turn leads to an extended form of it. New results illustrate the IR spectrum of an intramolecular triplex with parallel homologous strands which induced by forced folding back on itself of the oligonucleotide d(CATGCTAACT)-linker-d(AGTTAGCATG)-linker-d(CATGCTAACT). Considering the characteristic S–type sugar absorption at 838 cm^{-1}, the spectrum of the mixture of the hairpin duplex with a third strand previously coated by RecA protein is investigated. It should be pointed out that similar oligonucleotide sequences were used in both experiments. The band at 875 cm^{-1} shows the existence of N-type sugars which in turn,

confirms that the three stranded structure formed in the vicinity of the protein are undergoing unwinding.

QUADRUPLEXES

There are many repeating sequences in the telomeric DNAs existed at chromosome extremities in which guanine and cytosine clusters are located on different strands. The possibility of formation of G quadruplexes and intercalated C-CC parallel duplexes of opposite polarity (i-motif) have been demonstrated by both NMR and X-Ray crystal diffraction investigations. If counter ions or pH conditions change to some extent, an important conformational reorganization may be occurred in these multistranded nucleic acids. For example, variation of KC and NaC concentrations can lead to change in geometry of guanine quadruplexes in telomeres. The Raman spectroscopy has been used to investigate G quadruplexes and C-CC quadruplexes (i-motif).

It can be seen that the recorded IR spectra in D_2O and H_2O solutions of d(TCCCCC) at neutral pH (a,c) and acidic pH (b,d) as an example of IR evidence for cytosine–rich quadruplex formation (i-motif). It has been shown that this sequence can form the i-motif quadruplex structure at acidic pH. A base pairing consisting of hemiprotonated C-CC base pairs with three Hydrogen bonds can be formed when cytosine is protonated at Nitrogen N(3). There is a N(3)HCĐ Đ ĐN(3) Hydrogen bond at the center of the cytosine pairs while there are two Hydrogen bonds that contain the N(4) H_2 amino group and the C(2)DO(2) carbonyl group. Both parallel-stranded base-paired duplexes of the i-motif which stabilized by C-CC base pairs are intercalated within each other in opposite polarity.

It can be seen that the single C(2)DO(2) stretching vibration of the neutral, non-associated dC residues of d(TCCCCC) is located at 1652 cm^{-1}. According to the proposed base pairing scheme, p electrons can be better localized on the carbonyl groups due to protonation and Hydrogen bonding. The presence of two high frequency shifted carbonyl bands at 1697 cm^{-1} and 1666 cm^{-1} confirm that at room temperature and acidic pH, the hemiprotonated C-CC base pairs in the d(TCCCCC) self-association is formed. Considering self-complexation, the intensity of motions of the N(3) atom decreased considerably and the bands characteristic of the unassociated unprotonated dC residues shifts to 1511 cm^{-1} and 1528 cm^{-1} from 1505 cm^{-1} and 1524 cm^{-1}. However, the mode at 1618 cm^{-1} mode which involves the ND_2 scissoring of the neutral cytosines is shifted to 1610 cm^{-1}.

It can be observed that at acidic pH and room temperature, the bands observed at 868 cm^{-1} and 801 cm^{-1} are the characteristic of N-type sugars and the band at 838 cm^{-1} is the characteristic of S-type sugars in region IV. The interesting issues are the unusual large intensity and broad bandwidth of the absorption at 868 cm^{-1} while there is not any contribution at 896 cm^{-1}.

Moreover, the tetraplex structure of d(TCCCCC) at acidic pH is also formed with a low-frequency shift of the band assigned to the symmetric stretching vibration of the phosphate groups from 1088-1090 cm^{-1} to 1080 cm^{-1} for all double stranded (both parallel and antiparallel) DNA helices.

HYDRATION OF NUCLEIC ACIDS

The adopted conformation by a nucleic acid is mainly dependent on the presence of ions and water. It was previously mentioned that the B to A interconversion in double helical DNA can be easily detected by the hydration of the nucleic acid. It has been well known that water is a key parameter for recognizing macromolecular activities. A direct way for measuring the hydration is vibrational spectroscopy using the solvent absorptions. One of the methods for measuring the hydration number of DNA is using Raman scattering of the O-H stretching modes in DNA films. Falk *et al*. proposed a correlation between the IR data and gravimetric measurements performed on native DNAs. Moreover, the hydration in double stranded rAn-rUn and dAn-dTn can also be estimated. An example of this measurement for the dTn*dAn-dTn triple helix can be obtained by measuring the water absorption at 3400 cm^{-1} from the spectra of triplex films recorded at decreasing relative humidities. As can be seen, the plots are normalized for the nucleotide content of the samples. It should be noted that the water content per nucleotide is highly decreased as the triple helix is formed through binding of the third strand in the major groove of the duplex. It can be estimated that how many triple helix of around 17 water molecules are solved using gravimetric measurements performed on the same samples compared to 21 for the duplex. This process is not performed on the case of the formation of the rUn*rAn-rUn triplex by addition of a third rUn strand to the rAn-rUn duplex. In this case, the average hydration is found to be around 15 water molecules per nucleotide for the triplex, similar to the duplex.

CONCLUSION AND FUTURE STUDIES

The use of vibrational spectroscopy as an effective technique to characterize the structure of nucleic acids, to obtain exact local conformational parameters and to demonstrate that the resulted macromolecules are stable has been widely accepted since the early 1950s. However, it can be hopefully used in future studies about the nucleic acids in complex biological systems.

References

1. Heidari A. “An Analytical and Computational Infrared Spectroscopic Review of Vibrational Modes in Nucleic Acids”, Austin J Anal Pharm Chem. 3: 1058, 2016.
2. Samanta A, Merrett K, Gerasimov M and Griffith M. 21 - Ocular applications of bioresorbable polymers-from basic research to clinical trials, In Bioresorbable Polymers for Biomedical Applications, Woodhead Publishing. 2017; 497-523.
3. Hausmann O. Chapter 9 - Drug Allergy, In Middleton’s Allergy Essentials, edited by Robyn E. O’Hehir, Stephen T. Holgate and Aziz Sheikh, Elsevier. 2017; 225-247.
4. Bernstein PS, Li B, Vachali PP, Gorusupudi A, Shyam R, et al. The basic and clinical science underlying carotenoid-based nutritional interventions against ocular disease, Progress in Retinal and Eye Research. 2016: 34-66.
5. Ali A, Gittelman M. Research paradigms and useful inventions in medicine: Patents and licensing by teams of clinical and basic scientists in Academic Medical Centers, Research Policy. 2016; 1499-1511.
6. Giri TK. 5 - Nanoarchitectured Polysaccharide-Based Drug Carrier for Ocular Therapeutics, In Nanoarchitectonics for Smart Delivery and Drug Targeting, edited by Alina Maria Holban and Alexandru Mihai Grumezescu. William Andrew Publishing. 2016; 119-141.

7. Kador PF, Wyman M, Peter J. Oates, Aldose reductase, ocular diabetic complications and the development of topical Kinostat®, Progress in Retinal and Eye Research. 2016; 54: 1-29.

8. Forrester JV, Dick AD, Paul G. McMenamin, Fiona Roberts, et al. Chapter 4 - Biochemistry and cell biology, In The Eye (Fourth Edition). W.B. Saunders. 2016; 157-268.

9. Gittelman M. The revolution re-visited: Clinical and genetics research paradigms and the productivity paradox in drug discovery, Research Policy. 2016; 1570-1585.

10. Quimby JM. Update on Medical Management of Clinical Manifestations of Chronic Kidney Disease, Veterinary Clinics of North America: Small Animal Practice. 2016; 6: 1163-1181.

11. Chia J, Goh G, Bard F. Short O-GalNAc glycans: regulation and role in tumor development and clinical perspectives, Biochimica et Biophysica Acta (BBA) - General Subjects. 2016; 8: 1623-1639.

12. Ole Andersen. Chapter 4 - Chelation Treatment During Acute and Chronic Metal Overexposures-Experimental and Clinical Studies, In Chelation Therapy in the Treatment of Metal Intoxication, edited by Jan Aaseth, Guido Crisponi and Ole Andersen, Academic Press. 2016; 85-252.

13. Dex S, Lin D, Shukunami C, Docheva D. TENOgenic MODULating INsider factor: systematic assessment on the functions of tenomodulin gene, Gene. 2016; 587: 1-17.

14. Yuan L, Xu H, Yuan J, Deng X, Xiong W, et al. A novel FN1 variant associated with familial hematuria: TBMN?. Clinical Biochemistry. 2016; 10-11:816-820.

15. Straub V, Bertoli M. Where do we stand in trial readiness for autosomal recessive limb girdle muscular dystrophies? Neuromuscular Disorders. 2016; 2: 111-125.

16. Babizhayev MA. Generation of reactive oxygen species in the anterior eye segment. Synergistic codrugs of N-acetylcarnosine lubricant eye drops and mitochondria-targeted antioxidant act as a powerful therapeutic platform for the treatment of cataracts and primary open-angle glaucoma, BBA Clinical. 2016; 49-68.

17. Gouveia RM and Connon CJ. 7 - Collagen scaffolds for corneal regeneration, In Woodhead Publishing Series in Biomaterials, Woodhead Publishing. 2016; 151-177.

18. Mishra A, Krishnan B, Raman R, Sharma Y. Ca2 + and ß?-crystallins: An affair that did not last?, Biochimica et Biophysica Acta (BBA) - General Subjects. 2016; 299-303.

19. Anbarasu K, Sivakumar J. Multidimensional significance of crystallin protein–protein interactions and their implications in various human diseases, Biochimica et Biophysica Acta (BBA) - General Subjects. 2016; 222-233.

20. Kiss M, Kiss AA, Radics M, Popovics N, Hermesz E, et al. Drosophila type IV collagen mutation associates with immune system activation and intestinal dysfunction, Matrix Biology. 2016; 120-131.

21. Chen Y, Young Y. Bilateral simultaneous sudden sensorineural hearing loss, Journal of the Neurological Sciences. 2016; 139-143.

22. Cooper ML, Crish SD, Inman DM, Horner PJ, Calkins DJ. Early astrocyte redistribution in the optic nerve precedes axonopathy in the DBA/2J mouse model of glaucoma, Experimental Eye Research. 2016; 22-33.

23. Legendre L. Anatomy and Disorders of the Oral Cavity of Guinea Pigs, Veterinary Clinics of North America: Exotic Animal Practice. 2016; 825-842.

24. Chang J, Huang Y, Cunningham CM, Han K, Chang M, et al. Matrix metalloproteinase 14 modulates signal transduction and angiogenesis in the cornea, Survey of Ophthalmology. 2016; 4: 478-497.

25. Brody T. Chapter 25 - Drug Safety, In Clinical Trials (Second Edition), Academic Press, Boston, 2016, Pages 483-568.

26. Xu X, Dickey DD, Chen S, Giangrande PH. Structural computational modeling of RNA aptamers, Methods, Volume 103, 1 July 2016, Pages 175-179.

27. Tonin AA, Calado AMC, Bottari NB, Dalenogare D, Gustavo R, et al. Novel markers of inflammatory response and hepatic dysfunction in canine leishmaniasis, Comparative Immunology, Microbiology and Infectious Diseases. 2016; 61-64.

28. Bersano A, Morbin M, Ciceri E, Bedini G, Berlit P, et al. The diagnostic challenge of Divry van Bogaert and Sneddon Syndrome: Report of three cases and literature review, Journal of the Neurological Sciences. 2016; 77-83.

29. Vardanyan R and Hruby V. Chapter 31 - Antibacterial Drugs, In Synthesis of Best-Seller Drugs, Academic Press. 2016; 645-667.

30. Wang G, Nie Q, Zang C, Zhang B, Zhu Q, et al. Self-Assembled Thermoresponsive Nanogels Prepared by Reverse Micelle ? Positive Micelle Method for Ophthalmic Delivery of Muscone, a Poorly Water-Soluble Drug, Journal of Pharmaceutical Sciences. 2016; 9: 2752-2759.

31. Piperigkou Z, Karamanou K, Engin AB, Gialeli C, Docea AO, et al. Emerging aspects of nanotoxicology in health and disease: From agriculture and food sector to cancer therapeutics, Food and Chemical Toxicology. 2016: 42-57.

32. Panel of experts from the National AIDS Plan (PNS) AIDS Study Group (GeSIDA) Spanish Society of Occupational Health Safety at Work (SEMST) Spanish Society of Preventive Medicine Public Health Hygiene (SEMPSPH) Spanish Association of Specialists in Occupational Medicine (AEEMT) Spanish Society of Occupational Health in Public Administration (SESLAP) National Association of Occupational Medicine in Health (ANMTAS) Spanish Society of Paediatric Infectology (SEIP) Spanish Society for Emergency Medicine (SEMES) Study Group for Viral Hepatitis-SEIMC (GEHEP) Spanish Federation of Occupational Health Nursing (FEDEET), Executive summary of the consensus document on post-exposure prophylaxis against HIV, HBV and HCV in adults and children, Enfermedades Infecciosas y Microbiología Clínica. 2016; 2: 122-131.

33. Chen F, Liu X. Advancing biomaterials of human origin for tissue engineering, Progress in Polymer Science. 2016; 86-168.

34. Wen R, Hu L, Qu G, Zhou Q, Jiang G. Exposure, tissue biodistribution, and biotransformation of nanosilver, NanoImpact. 2016; 18-28.

35. Meltzer EO. Allergic Rhinitis: Burden of Illness, Quality of Life, Comorbidities, and Control, Immunology and Allergy Clinics of North America, Volume 36, Issue 2, May 2016, Pages 235-248.

36. Bozkurt MF, Salanci BV, Ugur Ö. Intra-Arterial Radionuclide Therapies for Liver Tumors, Seminars in Nuclear Medicine. 2016; 4: 324-339.

37. Yakubu MT, Olutoye AF. Aphrodisiac activity of aqueous extract of Anthonotha macrophylla P. Beauv. leaves in female Wistar rats, Journal of Integrative Medicine.2016; 5: 400-408.

38. Manivasagan P, Junghwan Oh. Marine polysaccharide-based nanomaterials as a novel source of nanobiotechnological applications, International Journal of Biological Macromolecules. 2016; 315-327.

39. Hildreth CD. Preparing the Small Animal Hospital for Avian and Exotic Animal Emergencies, Veterinary Clinics of North America: Exotic Animal Practice. 2016; 2: 325-345.

40. Forrester JV, Dick AD, McMenamin PG, Roberts F, Pearlman E. Chapter 7 - Immunology, In The Eye (Fourth Edition) W.B. Saunders. 2016; 370-461.

41. Lalwani G, D'Agati M, Khan AM, Sitharaman B. Toxicology of graphene-based nanomaterials, Advanced Drug Delivery Reviews. 2016; 109-144.

42. Brownlee M, Aiello LP, Cooper ME, Vinik AI, Plutzky J, et al. Chapter 33 - Complications of Diabetes Mellitus, In Williams Textbook of Endocrinology (Thirteenth Edition), edited by Shlomo Melmed, Kenneth S. Polonsky, P. Reed Larsen and Henry M. Kronenberg, Content Repository Only! 2016; 1484-1581.

43. Nishida T, Inui M, Nomizu M. Peptide therapies for ocular surface disturbances based on fibronectin–integrin interactions, Progress in Retinal and Eye Research. 2015; 38-63.

44. Bron AJ, Argüeso P, Irkec M, Bright FV. Clinical staining of the ocular surface: Mechanisms and interpretations, Progress in Retinal and Eye Research. 2015; 36-61.

45. Bolton ID. Chapter 5 - Basic Physiology of Macaca fascicularis, In The Nonhuman Primate in Nonclinical Drug Development and Safety Assessment, edited by Joerg Bluemel, Sven Korte, Emanuel Schenck and Gerhard F. Weinbauer, Academic Press. 2015; 67-86.

46. Priyanka Arya, Mark A. Rainey, Sohinee Bhattacharyya, Bhopal C. Mohapatra, Manju George, Murali R. Kuracha, Matthew D. Storck, Vimla Band, Venkatesh Govindarajan, Hamid Band, The endocytic recycling regulatory protein EHD1 Is required for ocular lens development, Developmental Biology, Volume 408, Issue 1, 1 December 2015, Pages 41-55.

47. Rahman EZ, Lam PK, Chu C, Moore Q, Pflugfelder SC. Corneal Sensitivity in Tear Dysfunction and its Correlation With Clinical Parameters and Blink Rate, American Journal of Ophthalmology. 2015; 5: 858-866.

48. Lee JS, Kim Y, Kim D, Shin G, Lee JH, et al. Acute and subchronic (28 days) oral toxicity studies of Codonopsis lanceolata extract in Sprague–Dawley rats, Regulatory Toxicology and Pharmacology. 2015; 3: 491-497.

49. Lubrano E, Cantini F, Costanzo A, Girolomoni G, Prignano F, et al. Measuring psoriatic disease in clinical practice. An expert opinion position paper, Autoimmunity Reviews. 2015; 10: 864-874.

50. Ahmed S, Jelani M, Alrayes N, Mohamoud HSA, Almramhi MM, et al. Exome analysis identified a novel missense mutation in the CLPP gene in a consanguineous Saudi family expanding the clinical spectrum of Perrault Syndrome type-3, Journal of the Neurological Sciences. 2015; 1-2: 149-154.

51. Zhang SX, Ma JH, Bhatta M, Fliesler SJ, Wang JJ. The unfolded protein response in retinal vascular diseases: Implications and therapeutic potential beyond protein folding, Progress in Retinal and Eye Research. 2015; 111-131.

52. Smith DM, Fisher D, Blier P, Ilivitsky V, Knott V. The separate and combined effects of monoamine oxidase A inhibition and nicotine on the mismatch negativity event related potential, Pharmacology Biochemistry and Behavior. 2015; 137: 44-52.

53. Vivo DCD, Darras BT, Ryan MM, Jones HR. Chapter 1 - Introduction: Historical Perspectives, In Neuromuscular Disorders of Infancy, Childhood, and Adolescence (Second Edition), Academic Press. 2015; 3-16.

54. Underwood WJ, Blauwiekel R, Delano ML, Gillesby R, Mischler SA, et al. Chapter 15 - Biology and Diseases of Ruminants (Sheep, Goats, and Cattle), In Laboratory Animal Medicine (Third Edition), edited by James G. Fox, Lynn C. Anderson, Glen M. Otto, Kathleen R. Pritchett-Corning and Mark T. Whary, Academic Press. 2015; 623-694.

55. Yang Y, Kwon J, Shim I, Kim H, Kim P, et al. Evaluation of toxicity to triclosan in rats following 28 days of exposure to aerosol inhalation, Regulatory Toxicology and Pharmacology. 2015; 2: 259-268.

56. White CA, Ghazan-Shahi S, Adams MA. ß-Trace Protein: A Marker of GFR and Other Biological Pathways, American Journal of Kidney Diseases. 2015; 1: 131-146.

57. Ferreira S, Ortiz A, Germain DP, Viana-Baptista M, Caldeira-Gomes A, et al. The alpha-galactosidase A p.Arg118Cys variant does not cause a Fabry disease phenotype: Data from individual patients and family studies, Molecular Genetics and Metabolism. 2015; 2: 248-258.

58. De Vivo DC, Paradas C, DiMauro S. Chapter 41 - Mitochondrial Encephalomyopathies, In Neuromuscular Disorders of Infancy, Childhood, and Adolescence (Second Edition), edited by Basil T. Darras, H. Royden Jones, Monique M. Ryan and Darryl C. De Vivo, Academic Press. 2015; 796-833.

59. Gyang PV, Akinwale OP, Lee Y, Chuang T, Orok AB, et al. Seroprevalence, disease awareness, and risk factors for Toxocara canis infection among primary schoolchildren in Makoko, an urban slum community in Nigeria, Acta Tropica. 2015; 135-140.

60. O'Rourke DP, Lertpiriyapong K. Chapter 19 - Biology and Diseases of Reptiles, In Laboratory Animal Medicine (Third Edition), edited by James G. Fox, Lynn C. Anderson, Glen M. Otto, Kathleen R. Pritchett-Corning and Mark T. Whary, Academic Press. 2015; 967-1013.

61. Flores JP, Blanco GE, Rivas MP, Montero MA, Breysse MM, et al. Central retinal artery occlusion and infective endocarditis: Rigor does matter, Archivos de la Sociedad Española de Oftalmología. 2015; 11: 546-548.

62. Dubail J, Apte SS. Insights on ADAMTS proteases and ADAMTS-like proteins from mammalian genetics. Matrix Biol. 2015; 44-46: 24-37.

63. Fox DA, Chapter 18 - Retinal and visual system: occupational and environmental toxicology, In: Marcello Lotti and Margit L. Bleecker, Editor(s), Handbook of Clinical Neurology. 2015; 325-340.

64. Bentea E, Demuyser T, Liefferinge JV, Albertini G, Deneyer L, et al. Absence of system xc- in mice decreases anxiety and depressive-like behavior without affecting sensorimotor function or spatial vision, Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2015; 3: 49-58.

65. Uchiyama M, Lockley SW. Non-24-Hour Sleep-Wake Rhythm Disorder in Sighted and Blind Patients, Sleep Medicine Clinics. 2015; 4: 495-516.

66. Oohashi T, Edamatsu M, Bekku Y, Carulli D. The hyaluronan and proteoglycan link proteins: Organizers of the brain extracellular matrix and key molecules for neuronal function and plasticity, Experimental Neurology. 2015; 134-144.

67. Hunt C. Neurological Examination and Diagnostic Testing in Birds and Reptiles, Journal of Exotic Pet Medicine. 2015; 1: 34-51.

68. Hierro C, Rodon J, Tabernero J. Fibroblast Growth Factor (FGF) Receptor/FGF Inhibitors: Novel Targets and Strategies for Optimization of Response of Solid Tumors, Seminars in Oncology. 2015; 801-819.

69. Scomparin A, Polyak D, Krivitsky A, Satchi-Fainaro R. Achieving successful delivery of oligonucleotides - From physico-chemical characterization to in vivo evaluation, Biotechnology Advances. 2015; 6: 1294-1309.

70. Agnifili L, Pieragostino D, Mastropasqua A, Fasanella V, Brescia L, et al. Chapter 1 - Molecular biomarkers in primary open-angle glaucoma: from noninvasive to invasive, In: Giacinto Bagetta and Carlo Nucci, Editor(s), Progress in Brain Research. 2015; 1-32.

71. Matalon R, Matalon KM, Radhakrishnan GL. Chapter 31 - The Mucopolysaccharidoses, In Rosenberg's Molecular and Genetic Basis of Neurological and Psychiatric Disease (Fifth Edition), edited by Roger N. Rosenberg and Juan M. Pascual, Academic Press. 2015; 347-363.

72. Mora-Rillo M, Arsuaga M, Ramírez-Olivencia G, de la Calle F, Borobia AM, et al. Acute respiratory distress syndrome after convalescent plasma use: treatment of a patient with Ebola virus disease contracted in Madrid, Spain, The Lancet Respiratory Medicine. 2015; 7: 554-562.

73. Aleksandrov AP, Mirkov I, Demenesku J, Ninkov M, Zolotarevski L, et al. Strain differences in contact hypersensitivity reaction to dinitrochlorobenzene (DNCB) in rats, Food and Chemical Toxicology. 2015; 94-103.

74. King AM, Aaron CK. Organophosphate and Carbamate Poisoning, Emergency Medicine Clinics of North America. 2015; 1: 133-151.

75. Gong X, Rubin LP. Role of macular xanthophylls in prevention of common neovascular retinopathies: Retinopathy of prematurity and diabetic retinopathy, Archives of Biochemistry and Biophysics. 2015; 40-48.

76. Laidlaw TM, Boyce JA. Platelets in patients with aspirin-exacerbated respiratory disease, Journal of Allergy and Clinical Immunology. 2015; 6: 1407-1414.

77. Jin S, Daniell H. The Engineered Chloroplast Genome Just Got Smarter, Trends in Plant Science. 2015; 10: 622-640.

78. Nemzek JA, Lester PA, Wolfe AM, Dysko RC, Myers DD. Chapter 12 - Biology and Diseases of Dogs, In Laboratory Animal Medicine (Third Edition), edited by James G. Fox, Lynn C. Anderson, Glen M. Otto, Kathleen R. Pritchett-Corning and Mark T. Whary, Academic Press. 2015; 511-554.

79. Whitmore SS, Sohn EH, Chirco KR, Drack AV, Stone EM, et al. Complement activation and choriocapillaris loss in early AMD: Implications for pathophysiology and therapy, Progress in Retinal and Eye Research. 2015; 1-29.

80. Casarrubea M, Jonsson GK, Faulisi F, Sorbera F, Di Giovanni G, et al. T-pattern analysis for the study of temporal structure of animal and human behavior: A comprehensive review, Journal of Neuroscience Methods. 2015; 34-46.

81. Li Y, Zhao J, Qu Y, Gao Y, Guo Z, et al. Synchrotron radiation techniques for nanotoxicology, Nanomedicine: Nanotechnology, Biology and Medicine. 2015; 6: 1531-1549.

82. Avendaño C, Menéndez JC. Chapter 2 - Antimetabolites That Interfere with Nucleic Acid Biosynthesis, In Medicinal Chemistry of Anticancer Drugs. 2015; 23-79.

83. Anthony W. Norman and Helen L. Henry, Chapter 16 - The Pineal Gland, In Hormones (Third Edition), Academic Press. 2015; 351-361.

84. Seghatchian J. The multifaceted aspects of blood cell-derived microparticles: An intriguing aspect of my past subject of studies, Transfusion and Apheresis Science. 2015; 2: 103-105.

85. Romano GL, Platania CBM, Forte S, Salomone S, Drago F, et al. P.1.a.012 Common microRNAs in Alzheimer's disease and retinal degenerative diseases, European Neuropsychopharmacology; 2015: S165.

86. Mason CA. Chapter 208 - Protecting the Abused or Neglected Horse, In Robinson's Current Therapy in Equine Medicine (Seventh Edition), edited by Kim A. Sprayberry and N. Edward Robinson, W.B. Saunders, St. Louis. 2015; 904-907.

87. McLaughlin PJ, Zagon IS. Duration of opioid receptor blockade determines biotherapeutic response, Biochemical Pharmacology. 2015; 3: 236-246.

88. Tocidlowski ME. Chapter 25 - Musophagiformes, In Fowler's Zoo and Wild Animal Medicine, Volume 8, edited by R. Eric Miller and Murray E. Fowler, W.B. Saunders, St. Louis. 2015; 206-209.

89. Kim JJ, Yi JS, Kim SH, Pae CU. P1.a.011 Association of AHI1 polymorphisms with the diagnosis and treatment outcome of schizophrenia, European Neuropsychopharmacology. 2015; S164-S165.

90. Knottenbelt DC, Patterson-Kane JC, Snalune KL. 27 - Approach to the suspected or confirmed cancer case, In Clinical Equine Oncology. 2015; 415-428.

91. Takekita Y, Fabbri C, Kato M, Nonen S, Sakai S, et al. P1.a.013 Effect of HTR7 gene variants on the efficacy of second-generation antipsychotics with 5-HT7R antagonism in Japanese schizophrenia patients, European Neuropsychopharmacology. 2015; S165-S166.

92. Caussa JE, Vila EH. Epidermal growth factor, innovation and safety, Medicina Clínica (English Edition). 2015; 7: 305-312.

93. Palmer H, Fisher V. Chapter 33 - Considerations for Special Administration Techniques, In The Nonhuman Primate in Nonclinical Drug Development and Safety Assessment, edited by Joerg Bluemel, Sven Korte, Emanuel Schenck and Gerhard F. Weinbauer, Academic Press. 2015; 627-645.

94. Mancinelli E. Neurologic Examination and Diagnostic Testing in Rabbits, Ferrets, and Rodents, Journal of Exotic Pet Medicine. 2015; 1: 52-64.

95. Perfect JR, 264 - Cryptococcosis (Cryptococcus neoformans and Cryptococcus gattii), In Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases (Eighth Edition), Content Repository Only! 2015; 2934-2948.e5.