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NMR studies on oligonucleotide – Methylene blue conjugates targeting double-helical nucleic acids

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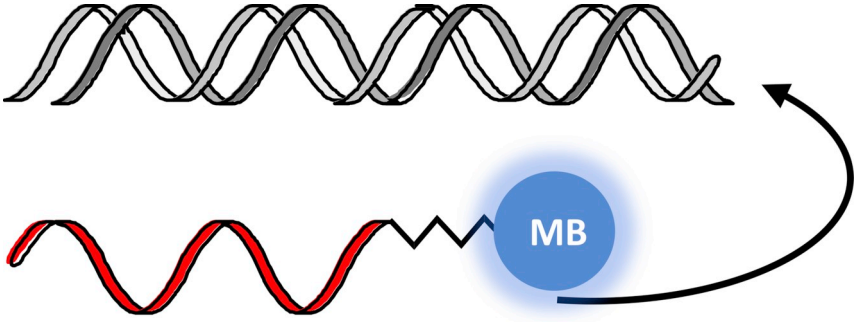
HIGHLIGHTS

- Oligonucleotide – methylene blue conjugates allow for triplex formation.
- The MB dye shows no distinct long-lived interaction with the triplex.
- MB preferentially interacts at the triplex-duplex junction.
- MB stacking on an external TAT triad yields significant triplex stabilization.

GRAPHICAL ABSTRACT



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ABSTRACT

Methylene blue (MB) – nucleic acid interactions are of considerable interest due to the photosensitizing activity of the dye with potential applications in medicine and biotechnology. Covalent attachment of the MB to an oligonucleotide through a flexible heptamethylene linker enabled a positioning of the dye moiety to specific sites through triplex formation with a target duplex. NMR studies demonstrated interactions of MB with the nucleic acids. In sequences with the MB moiety facing the triplex-duplex junction with an alternating CG duplex overhang next to a T·A·T triple-helical tract, proton resonances experienced severe linebroadening upon MB binding and point to kinetically labile complexes with exchange among different binding modes. For sequences with the MB moiety facing a terminal T·A·T base triad of the triplex tract, structural heterogeneity decreased when compared to a triplex without MB attached to the third strand. Also, the thermal stability of the latter construct increased significantly in the presence of MB, indicating external end stacking as predominant binding mode. Without any obvious disruptions of sequential imino-imino NOE contacts within the triplex and duplex tracts, a most favorable intercalation between T·A·T base triples or CG base pairs is not supported by the present data under our experimental conditions.

1. Introduction

Methylene blue (MB), a phenothiazinium dye, has attracted much attention as a DNA binding ligand due to its use as an electrochemical biosensor [1,2] and as a photosensitizer, causing strand cleavage after photoactivation by generating singlet oxygen [3,4]. This photosensitization process on MB might find its application as a noninvasive therapeutic tool in modern photomedicine. The generation of singlet

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oxygen will occur with an efficiency that depends on the structure and sequence of the MB-DNA complex involved in the photolysis process [5]. Notably, studies on the interaction between the dye and double-helical nucleic acids have been rather controversial. Computational and experimental studies using optical methods have been devoted to reveal the strength and DNA binding mode of the dye [6–10]. MB intercalation was suggested as the predominant binding mode in alternating GC sequences at a low ionic strength and at a high DNA-to-dye molar ratio, while binding in the major or minor groove of DNA was found under other experimental conditions [11–14]. MB has also been reported to interact preferentially with guanine, thus exhibiting DNA base specificity [15]. Although a large body of information about MB-DNA interactions has been provided so far, a high-resolution structure of the complex from X-ray or NMR data has not become available to date.

DNA triple helix formation through binding of a third strand oligonucleotide into the major groove of a DNA duplex constitutes a powerful strategy to target specific DNA sequences. Either a pyrimidine third strand or a purine third strand can recognize a homopurine tract of the duplex by forming pH-dependent parallel or antiparallel triplexes, respectively [16,17]. Using such a triplex approach, small DNA binding molecules can be tethered to the third strand oligonucleotide and guided to its target site following triplex formation [18,19]. As an added advantage, such a duplex targeting conjugate will also reduce problems due to various stoichiometries and will limit potential ligand aggregation to yield a better defined complex. In the present paper, we have prepared an MB-oligonucleotide conjugate with the dye covalently coupled to a 3′-aminoalkyl-modified DNA oligonucleotide third strand via a flexible hydrocarbon linker. The ability of the oligonucleotide-dye conjugate for triplex formation and the binding of MB to various competing binding sites in the formed construct, namely triplex stem, triplex-duplex junction, and duplex overhang, was studied by 1D and 2D ^{1}H NMR measurements in 100 mM NaCl solution. Still lacking details on MB binding to DNA structures, information on the preferred binding site and binding mode in such triple-helical constructs will be important for the potential use of MB, in particular for the site-directed damaging of DNA targets.

2. Materials and methods

2.1. Materials

Starting reagents of p.a. quality were purchased from Sigma Aldrich GmbH, Germany, and used without further purification. Solvents for syntheses were dried prior to use when necessary. Deuterated solvents were purchased from Deutero GmbH, Germany, and used after passing through dry molecular sieves. MB-oligonucleotide conjugates were synthesized as described previously [20,21]. Briefly, a carboxybutyl-functionalized methylene blue derivative was prepared from *N*-methylamine, 5-bromovaleric acid ethyl ester, and *N*,*N*-dimethylphenylenediamine in four steps. Activation by N-hydroxysuccinimide followed by amide coupling with a 3′-aminoalkyl-derivatized oligonucleotide yielded the MB-oligonucleotide conjugate. The latter was purified by HPLC prior to use. Specific 4-^{15} N labeled 2′-deoxycytidine was prepared in five steps as described starting with 2′-deoxyuridine and amination with $^{15}NH_3$ [22,23]. The labeled nucleoside was subsequently converted to its corresponding phosphoramidite for solid-phase oligonucleotide synthesis.

2.2. NMR spectroscopy

Samples were prepared by dissolving the oligonucleotide in 0.5 mL 90% H_2O/10% D_2O containing 100 mM NaCl. The pH was adjusted to pH 5 with concentrated HCl solution. For measurements in D_2O, the H_2O/D_2O samples were lyophilized several times from D_2O and finally redissolved in 0.5 mL of 99.996% D_2O. NMR spectra were recorded on a Bruker AVANCE 600 MHz spectrometer equipped with 5 mm probes. 1D and 2D NOESY spectra were recorded in either D_2O or 90% H_2O/10% D_2O. The carrier frequency was placed on the water resonance and a delay of 20 μs was used within the binomial pulses in the WATERGATE sequence for maximum excitation of the imino proton region of the spectrum. Phase-sensitive NOESY spectra were acquired with mixing times of 120 to 250 ms and with a repetition delay of 2 s in the time proportional phase incrementation (TPPI) mode with 2 K complex data points in t_2 and 512 real data points in t_1. FID's of one-dimensional ^{1}H NMR experiments were multiplied by an exponential window function with 2 Hz linebroadening. ^{1}H chemical shifts were referenced relative to the H_2O signal (δ_H = 4.96 and 4.84 ppm at 10 °C and 20 °C, respectively).

3. Results and discussion

For the studies on the interaction of methylene blue within a triple-helical DNA, three different triplexes with a pyrimidine third strand were employed. These involved a trimolecular triplex and two bimolecular triplexes with a T4-loop linking complementary strand of the duplex target to possibly increase stabilities of the triple-helical constructs. The MB moiety was tethered to the triplex-forming third strand oligonucleotide by a moderately long flexible linker as described in detail previously [20,21]. This allows for unrestrained interactions in the vicinity of the dye attachment at the triplex 3′-end. All sequences with residue numbers are listed in Table 1.

3.1. Trimolecular triplex

Triple-helices were assigned using established methods for right-handed helical DNA [24]. Initially, oligonucleotide triplexes were assigned without attached methylene blue. The AAA·TTT tract in the sequences together with the characteristic downfield shifted C^+ imino protons were convenient starting points to assign exchangeable and non-exchangeable protons. NOE connectivities anticipated for a triple-helical oligonucleotide were observed for **Tt1** (Figs. 1 and S1). As expected for the trimolecular triplex, C21, G1, and G10 iminos noticeably suffer from fraying effects at the 5′- and 3′-termini and were therefore not observed. Also, assignment of the C23 imino proton was only possible through a 1D NMR spectrum. Although some additional cross-peaks appear in the imino region that do not correspond to the expected sequential connectivities within the triplex, they do not prevent the assignment of the major triplex resonances. Starting from imino-imino connectivities, proton chemical shifts were also determined for amino, aromatic and some of the T methyl resonances (Table S1).

In the corresponding triple-helical MB-oligonucleotide conjugate **Tt1-MB** significant signal linebroadening was observed (Fig. 2). This can be attributed to i) various complexes that might coexist in solution giving rise to structural heterogeneity with heterogenous linebroadening in the spectra; and ii) dynamic processes between species of different conformation or different binding sites in an intermediate exchange regime, resulting in homogenous linebroadening.

The broadened resonances severely hampered proton resonance assignments but still allowed for an almost full assignment of the imino proton resonances in the dye-oligonucleotide conjugate. Imino-imino connectivities in the triple-helical conjugate **Tt1-MB** are shown in Fig. 3. Obviously, imino signals at or close to the triplex-duplex junction with the MB attachment site, e.g. T_{15}, T_{26}, T_{14}, T_{27}, G_8, and G_{12} have notably experienced the largest linebroadening among all imino resonances (see also Fig. 2). However, since T_{16} can be recognized from its crosspeak to G_4, the T_{15} imino resonance could accordingly be identified. Likewise, T_{27} could be ascribed to T_{26} through its weak crosspeak which has previously been assigned by following C_{24} imino contacts. Finally, T_{14} and G_8 imino resonances were identified by their weak mutual crosspeak, whereas G_{12} and G_{10} imino signals were hard to observe. The T_{22} imino proton shows a crosspeak to C_{23} amino protons. Also, the C_{24} imino resonance exhibits crosspeaks to G_4 H8 and

Table 1
Sequence and residue numbers for tri- and bimolecular triple-helical oligonucleotides.

name	sequence
Tt1	3′- C_{20} T_{19} C_{18} C_{17} T_{16} T_{15} T_{14} C_{13} G_{12} C_{11} 5′- G_1 A_2 G_3 G_4 A_5 A_6 A_7 G_8 C_9 G_{10} 5′- C_{21} T_{22} C_{23} C_{24} T_{25} T_{26} T_{27} -3′
Tt1-MB	3′- C_{20} T_{19} C_{18} C_{17} T_{16} T_{15} T_{14} C_{13} G_{12} C_{11} 5′- G_1 A_2 G_3 G_4 A_5 A_6 A_7 G_8 $^{15}C_9$ G_{10} 5′- C_{21} T_{22} C_{23} C_{24} T_{25} T_{26} T_{27} ----MB
Tb1	C_{20} T_{19} C_{18} C_{17} T_{16} T_{15} T_{14} C_{13} G_{12} C_{11} 5′- G_1 A_2 G_3 G_4 A_5 A_6 A_7 G_8 C_9 G_{10} C_{21} T_{22} C_{23} C_{24} T_{25} T_{26} T_{27} -3′
Tb1-MB	C_{20} T_{19} C_{18} C_{17} T_{16} T_{15} T_{14} C_{13} G_{12} C_{11} 5′- G_1 A_2 G_3 G_4 A_5 A_6 A_7 G_8 C_9 G_{10} C_{21} T_{22} C_{23} C_{24} T_{25} T_{26} T_{27} ----MB
Tb2	C_{13} G_{12} C_{11} C_{20} T_{19} C_{18} C_{17} T_{16} T_{15} T_{14} - 5′ G_8 C_9 G_{10} G_1 A_2 G_3 G_4 A_5 A_6 A_7 -3′ C_{21} T_{22} C_{23} C_{24} T_{25} T_{26} T_{27} -3′
Tb2-MB	C_{13} G_{12} C_{11} C_{20} T_{19} C_{18} C_{17} T_{16} T_{15} T_{14} - 5′ G_8 C_9 G_{10} G_1 A_2 G_3 G_4 A_5 A_6 A_7 -3′ C_{21} T_{22} C_{23} C_{24} T_{25} T_{26} T_{27} ----MB

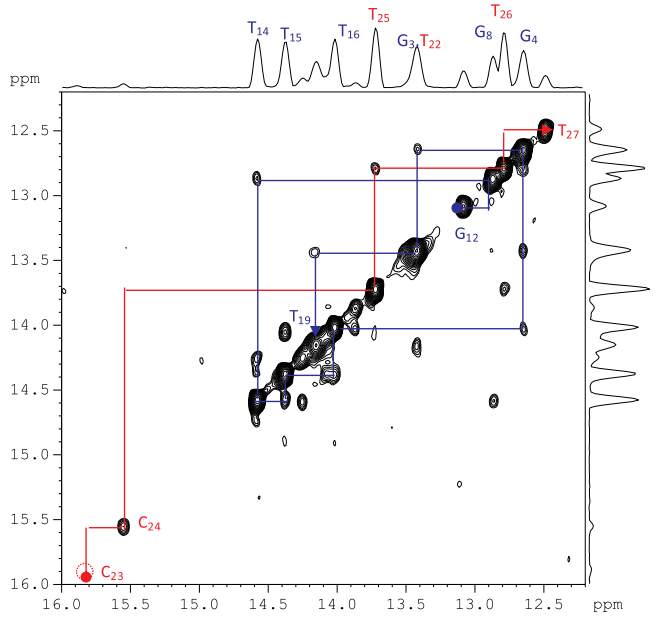

Fig. 1. Imino-imino region of a 2D NOE spectrum of **Tt1** with 100 mM NaCl (pH 5) at 15 °C in 90% H_2O/10% D_2O and a mixing time of 250 ms. Blue and red lines correspond to Watson-Crick and Hoogsteen imino-imino sequential contacts. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

its own H5 resonance (Fig. S2). Based on the crosspeak pattern, formation of a triple helix in the presence of MB is evident despite the fact that the duplex overhang as well as the triplex-duplex junction seem to be structurally perturbed. It is also important to note, that no significant disruption of imino-imino connectivities was observed in the NOESY spectra, giving no indication of intercalation as the predominant binding mode of MB within the triplex-duplex construct.

In an attempt to better follow and localize MB interactions, cytosine C9 within the duplex target was specifically labeled with the ^{15}N isotope at its amino group. Employing an inverse 2D $^{1}H-^{15}N$ HMQC experiment for spectral editing effectively filters out all proton resonances of the sample except for the cytosine amino protons scalar coupled to the ^{15}N isotope. Whereas a single crosspeak of the two C9 amino protons in fast exchange was observed for the ^{15}N labeled single strand, addition of the complementary strand resulted in the appearence of two separate signals of slowly exchanging Watson-Crick hydrogen- and non-hydrogen-bonded amino protons of the duplex (Fig. S3). Finally, the third strand was added in a 1:1 stoichiometric ratio to allow for triplex formation. In the absence of methylene blue two slightly shifted amino signals of the duplex overhang were observed, whereas upon addition of the third strand conjugate the sample displayed no crosspeak even within a range of temperatures. These results again suggest significant MB-induced structural perturbations of the duplex overhang as indicated by the NOESY experiments. However, information on specific interactions remains vague as a result of heterogenous and/or homogenous linebroadening effects.

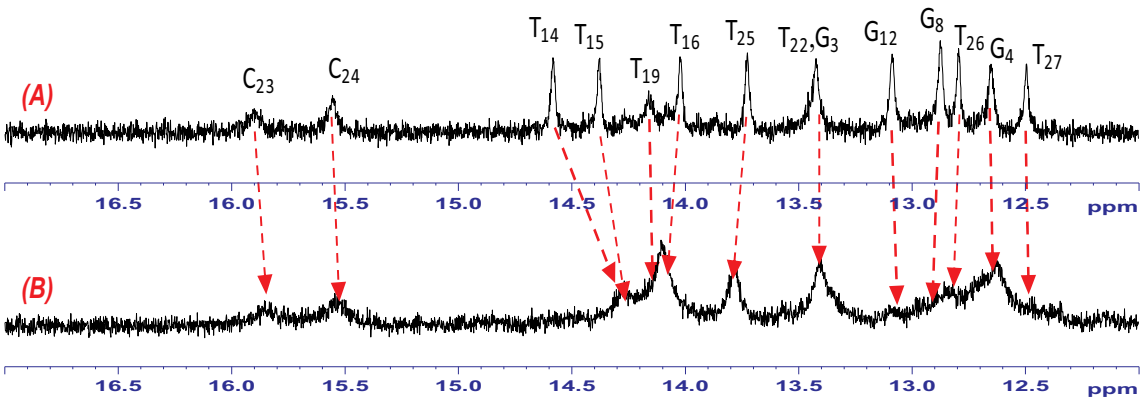

Fig. 2. Imino spectral region of ^{1}H NMR spectra of **Tt1** (A) and the MB conjugate **Tt1-MB** (B) in 90% H_2O/10% D_2O (100 mM NaCl, pH 5).

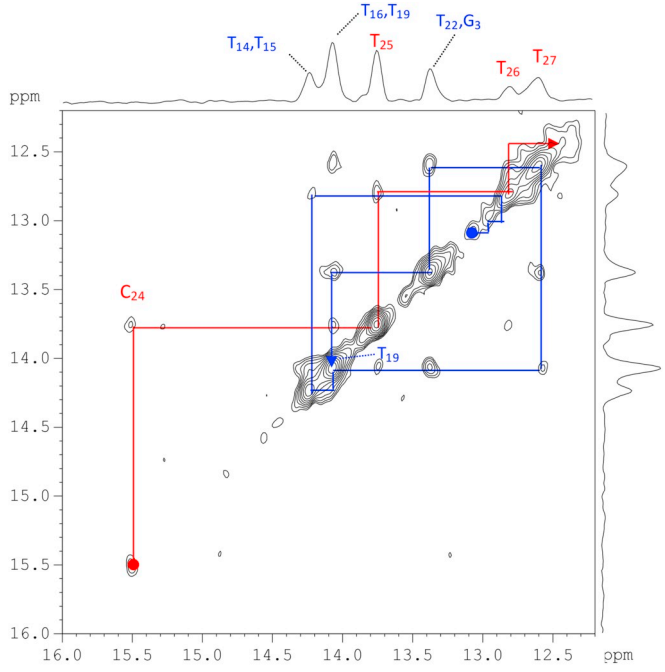

Fig. 3. Portion of a NOESY spectrum with imino-imino contacts of **Tt1-MB** in 90% H_2O/10% D_2O (100 mM NaCl, pH 5) at 15 °C and a mixing time of 250 ms. Sequential connectivities within the Watson-Crick duplex and the Hoogsteen third strand are indicated by blue and red lines, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Bimolecular triplex

For additional duplex stabilization, we also performed NMR experiments with a bimolecular triplex **Tb1-MB** employing a hairpin duplex target of the same sequence as **Tt1**. A very similar crosspeak pattern was observed and most imino protons were assigned in analogy to **Tt1-MB** (Fig. S4). As with the trimolecular construct, imino resonances at the triplex-duplex junction exhibit the most significant linebroadening effects among all iminos. Thus, linebroadening of all three guanine iminos of the duplex overhang, which hampered their unambiguous assignment, indicates that the loop-promoted stabilization did not help in sharpening the duplex resonances in the presence of MB. However, again no noticeable disruption of sequential imino-imino connectivities was observed in **Tb1-MB**, excluding intercalation into the DNA as the predominant binding mode of MB.

To exclude any potential MB-induced DNA strand breaks, the sample of the triplex conjugate was adjusted to pH 7, resulting in the dissociation of the third strand in case of the pH sensitive parallel triplex. 1D ^{1}H NMR spectra on the neutral solution showed all expected imino resonances of an intact duplex, excluding any photo-induced strand breaks as the origin of structural heterogeneity.

3.3. Chemical shift footprints

More information on potential interaction sites of MB with the triplex can be gained by following chemical shift changes of imino protons upon triplex formation (Fig. 4). As expected, no significant shift was observed for the imino resonances in C·G·C$^+$ triplets. This site should be unfavorable for MB interaction as a result of the repulsion between the positively charged MB and the protonated cytosine in the third strand. Accordingly, the negligible shielding or deshielding effect of the T_{22}·A_2·T_{19} imino protons located between two C·G·C$^+$ triplets can easily be rationalized. Although some assignments at the duplex overhang are missing mainly due to terminal fraying effects, there is no clear indication of dye intercalation between CG base pairs of the overhang as may be anticipated from previous studies on CG duplexes at low ionic strength [12,14]. Likewise, MB intercalation between consecutive T·A·T triples of the triplex stem as suggested for poly(dT·dA·dT) [25] cannot be excluded but corresponding complexes are short-lived and hardly constitute major species. In contrast, the chemical shift footprints clearly point to the triplex-duplex junction as major site that experiences most significant imino proton shielding effects. A most favorable intercalation site at triplex-duplex junctions has often been observed for DNA binding ligands [26–28], however, the present shielding effect with a maximum of 0.3 ppm does not corroborate a strong intercalative binding of the MB dye. Rather, it indicates partial intercalation of modest strength in line with only moderate affinities found for MB towards DNA [29,30]. Weaker binding is also expected in solutions with a relatively high ionic strength which additionally decreases electrostatic contributions of binding the cationic dye [31,32]. On the other hand, the chemical shift footprints with their localized major shifts of imino protons at the triplex-duplex junction mostly exclude predominant binding in the major or minor groove.

3.4. Bimolecular triplex with shifted duplex overhang

To further evaluate structural elements favored for MB binding, a construct **Tb2** has been designed for additional studies (see Table 1). Here, the same duplex overhang was placed at the opposite side of the triplex stem with the methylene blue now positioned at the blunt triplex terminus with its three contiguous T·A·T base triplets. Such a construct is expected to give more conclusive information about the binding preference of MB towards a T·A·T-rich triple-helical region without the additional presence of a duplex overhang. Using the same experimental conditions, this triple-helical construct was measured with and without

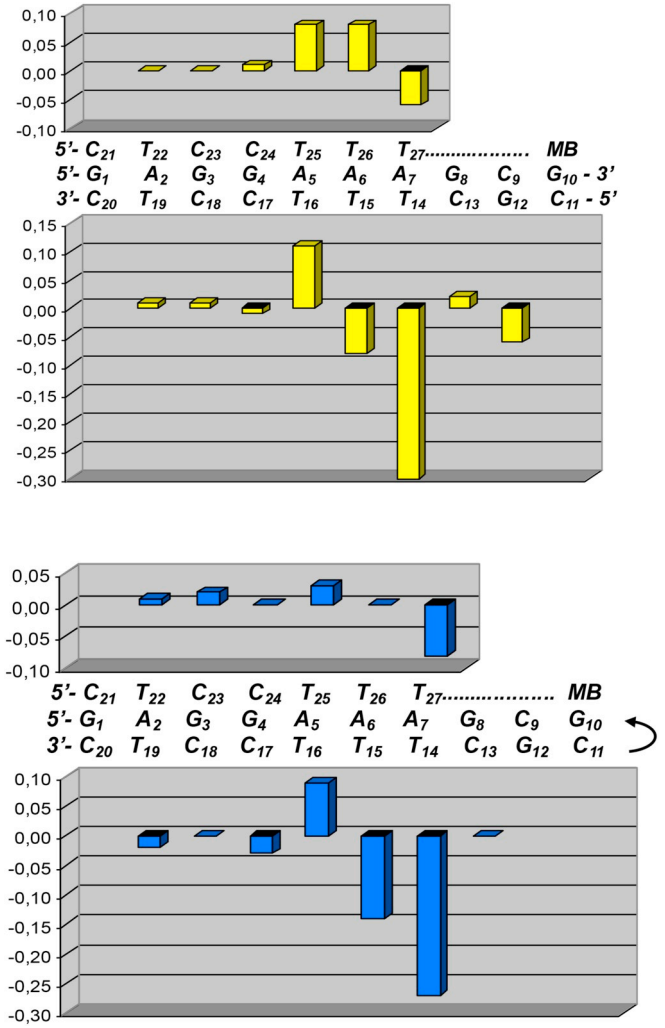

Fig. 4. Chemical shift differences (in ppm) of Hoogsteen iminos (top) and Watson-Crick iminos (bottom) between (A) the **Tt1-MB** conjugate and **Tt1** as well as between (B) the **Tb1-MB** conjugate and **Tb1.**

MB in the third strand. Corresponding 1D ^{1}H NMR spectra are presented in Fig. 5. The pattern of imino proton resonances of **Tb2** without MB shows the characteristic features of a triplex but with considerable structural heterogeneity and the formation of various species in contrast to **Tt1** and **Tb1**. As a consequence, proton assignments with more detailed structural information of coexisting species were not possible.

Notably, in contrast to previous observations the imino resonances of the MB-DNA conjugate **Tb2-MB** were better resolved compared to the reference construct without MB and allowed for an almost complete resonance assignment of the imino protons (Fig. 5B). Apparently, MB enables selective stabilization of a **Tb2** triplex structure. Portions of a NOESY spectrum of **Tb2-MB** are shown in Fig. 6. Surpringly, the exchange crosspeaks of iminos due to some minor conformations are still evident. Resonances of **Tb2-MB** were assigned in a similar way as performed for the other conjugates. Again, the typical sequential imino connectivity path T_{16}-G_4-G_3-T_{19} was easily identified. Also, the T_{22} imino proton was unambiguously ascribed from its typical crosspeak to C_{23} amino protons. Three typical downfield shifted imino resonances around and above 15 ppm were assigned to C_{21}, C_{23}, and C_{24}, respectively. The C_{21} imino signal appeared somewhat sharper than observed in the other constructs. The C_{24} imino resonance shows a strong crosspeak to G_4 H8 and a weak contact to its own H5 proton in close analogy to the other sequences. Although very weak, T_{25}-T_{26} and T_{26}-T_{27} Hoogsteen imino-imino contacts can be observed, again indicating no or only partial MB intercalation within the triple-helical T·A·T tract. On the other hand, unobservable T_{14}-T_{15} as well as T_{15}-T_{16} Watson-Crick imino-imino crosspeaks may result from their similar chemical shifts and corresponding crosspeaks close to the diagonal.

No chemical shift footprints could be constructed for the **Tb2** triplexes due to various coexisting species of the MB-free structure. Interestingly, UV thermal denaturation studies showed a noticeable thermal stabilization for the triplex of ΔT_m = 6.1 °C upon MB attachment to **Tb2** (Table S3). On the other hand, only a small triplex stabilization of 2.5 °C and 1.4 °C in line with rather weak interactions was observed when binding methylene blue to the third strand in the **Tt1** and **Tb1** constructs, respectively. Thus, given the same T·A·T tract located next to the tethered MB in all triplex conjugates, itis strongly suggested that methylene blue preferentially binds through outer stacking onto the T·A·T base triple at the triplex blunt end, a unique structural feature of **Tb2-MB**. Such a favored outer stacking of MB was previously also found for an MB-oligonucleotide conjugate upon formation of a duplex with a complementary single strand [20]. As expected, the high-temperature duplex-single strand transition is not influenced by covalent attachment of MB to the third strand, thus again indicating that no irreversible dye-induced degradation at the duplex overhang has occurred.

Unfortunately, MB-DNA crosspeaks were not observable in any of the 2D NOE spectra due to the significant linebroadening of resonances upon triplex formation, preventing a direct identification of specific

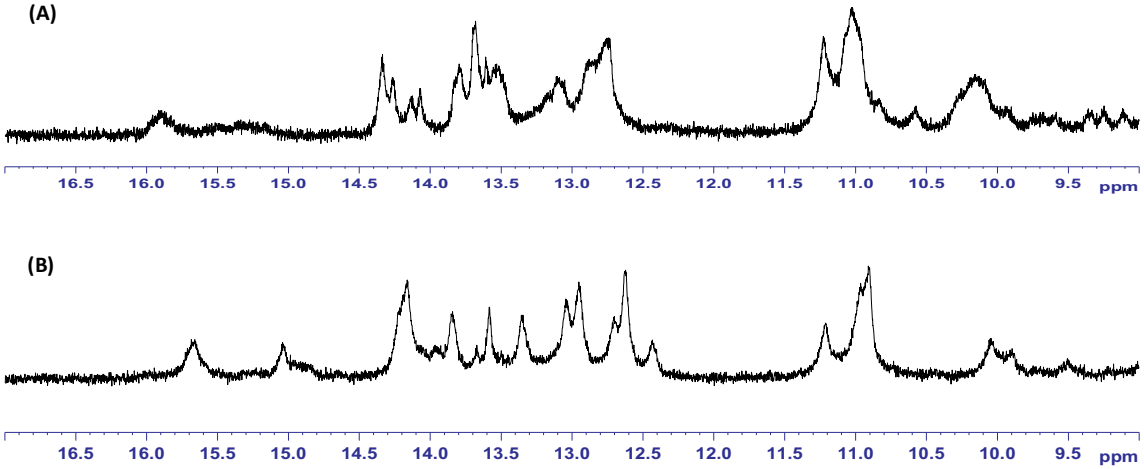

Fig. 5. Imino region of 1D ^{1}H NMR spectra of (A) **Tb2** and (B) **Tb2-MB** in H_2O (100 mM NaCl, pH 5).

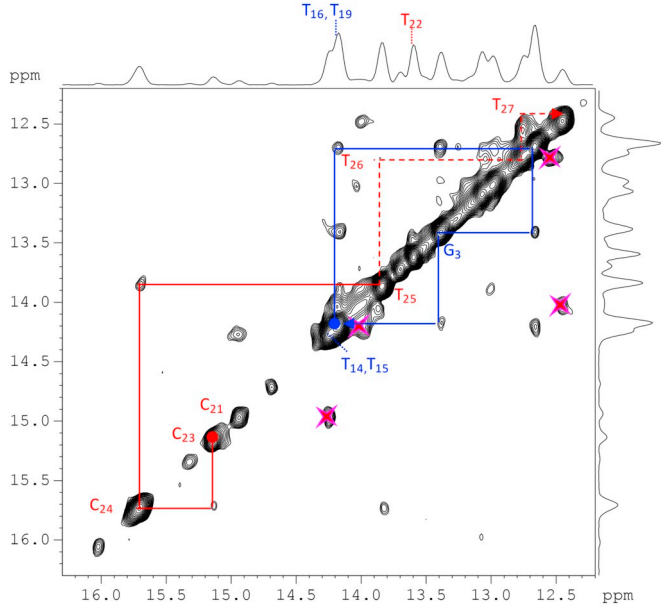

Fig. 6. Imino-imino 2D NOE spectral region of ***Tb2-MB*** in 90% H_2O/10% D_2O (100 mM NaCl, pH 5) at 10 °C (mixing time 120 ms). Strong exchange cross-peaks are marked by a cross. Sequential connectivities within the Watson-Crick duplex and the Hoogsteen third strand are indicated by blue and red lines, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

long-lived binding modes of the dye. However, based on scalar and dipolar couplings, proton resonances of the dye can be assigned in a straightforward manner in the free oligonucleotide-dye conjugate. Corresponding characteristic patterns of scalar and dipolar couplings also discerned in the spectra of triplexes allowed for partial proton assignments of the dye in case of **Tb2-MB** and full assignments for **Tb1-MB** (Fig. S6 and Table S2). Notably, chemical shift differences of dye resonances in the triple-helical complexes and the single-stranded conjugate are < 0.1 ppm in all cases, again pointing to only weak and short-lived interactions between the dye and DNA.

4. Conclusion

Methylene blue tethered to a third strand oligonucleotide supports triplex formation with a duplex and thus allows for targeted MB-induced photochemical or redox reactions at localized sites within double-helical DNA based on a triplex approach. However, dye-DNA interactions are only moderately strong and short-lived, precluding the determination of a high-resolution complex structure with a single defined binding mode. While partial intercalation into base pairs and base triples, as suggested by previous optical measurements with the free dye, cannot be excluded based on the experimental data, the NMR data point to the presence of different binding geometries and the formation of kinetically labile complexes. Under the experimental conditions employing a 100 mM NaCl containing buffer, binding is favored at duplex-triplex junctions and outer T·A·T base triplets, indicating that the formation of an intercalation cavity within the triplex or duplex stem is energetically unfavorable and hardly compensated by stacking interactions with the dye. Such a behavior has to be considered for the future design of artificial DNA-based photonucleases that employ methylene blue as photoactivating agent. Clearly, the requirement of an acidic pH for parallel triplexes as used in this study will restrict applications in vivo, yet the use of base analogs or purine third strands to form antiparallel triplexes is anticipated to alleviate such limitations.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bpc.2019.106314.

References

[1] S.O. Kelley, J.K. Barton, N.M. Jackson, M.G. Hill, Bioconjug. Chem. 8 (1997) 31.
[2] L. Cui, M. Lu, Y. Li, B. Tang, C. Zhang, Biosens. Bioelectron. 102 (2018) 87.
[3] C. OhUigin, D.J. McConnell, J.M. Kelly, W.J. van der Putten, Nucleic Acids Res. 15 (1987) 7411.
[4] R.A. Floyd, J.E. Schneider, D.P. Dittmer, Antivir. Res. 61 (2004) 141.
[5] T. Liu, F. Zhang, P. Chen, G.-Q. Tang, L. Lin, Chin. Phys. Lett. 28 (2011) 014208.
[6] J.J. Nogueira, L. González, Biochemistry 53 (2014) 2391.
[7] M. Hossain, G. Suresh Kumar, Mol. BioSyst. 5 (2009) 1311.
[8] P.O. Vardevanyan, A.P. Antonyan, M.A. Parsadanyan, M.A. Shahinyan, L.A. Hambardzumyan, J. Appl. Spectrosc. 80 (2013) 595.
[9] R. Rohs, H. Sklenar, J. Biomol. Struct. Dyn. 21 (2004) 699.
[10] W.-Y. Li, J.-G. Xu, X.-W. He, Anal. Lett. 33 (2000) 2453.
[11] E.M. Tuite, B. Nordén, Spectrochim. Acta A 189 (2018) 86–92.
[12] R. Rohs, H. Sklenar, R. Lavery, B. Röder, J. Am. Chem. Soc. 122 (2000) 2860.
[13] B. Nordén, F. Tjerneld, Biopolymers 21 (1982) 1713.
[14] E. Tuite, B. Norden, J. Am. Chem. Soc. 116 (1994) 7548.
[15] T. Friedmann, D.M. Brown, Nucleic Acids Res. 5 (1978) 615.
[16] P. Beal, P. Dervan, Science 251 (1991) 1360.
[17] J.-C. François, T. Saison-Behmoaras, C. Hélène, Nucleic Acids Res. 16 (1988) 11431.
[18] D. Ossipov, J. Chattopadhyaya, Tetrahedron 54 (1998) 5667.
[19] H. Moser, P. Dervan, Science 238 (1987) 645.
[20] J. Jähnchen, M.G.M. Purwanto, K. Weisz, Biopolymers 79 (2005) 335.
[21] M.G.M. Purwanto, Int. J. Appl. Chem. 11 (2015) 475.
[22] G. Kupferschmitt, J. Schmidt, T. Schmidt, B. Fera, F. Buck, H. Rüterjans, Nucleic Acids Res. 15 (1987) 6225.
[23] D. Leitner, W. Schröder, K. Weisz, Biochemistry 39 (2000) 5886.
[24] J. Feigon, K.M. Koshlap, F.W. Smith, Methods Enzymol. 261 (1995) 225.
[25] E. Tuite, B. Nordén, J. Chem. Soc. Chem. Commun. (1995) 53.
[26] D.A. Collier, J.-L. Mergny, N.T. Thuong, C. Helene, Nucleic Acids Res. 19 (1991) 4219.
[27] A. Eick, F. Riechert-Krause, K. Weisz, Bioconjug. Chem. 23 (2012) 1127.
[28] J. Dickerhoff, F. Riechert-Krause, J. Seifert, K. Weisz, Biochimie 107 (2014) 327.
[29] M. Hossain, P. Giri, G.S. Kumar, DNA Cell Biol. 27 (2008) 81.
[30] L.Z. Zhang, G.-Q. Tang, J. Photochem. Photobiol. B Biol. 74 (2004) 119.
[31] I. Saha, G.S. Kumar, Dyes Pigments 96 (2013) 81.
[32] E.T. Mudasir, D.H. Wahyuni, N. Tjahjono, H.I. Yoshioka, Spectrochim. Acta A Mol. Biomol. Spectrosc. 77 (2010) 528.

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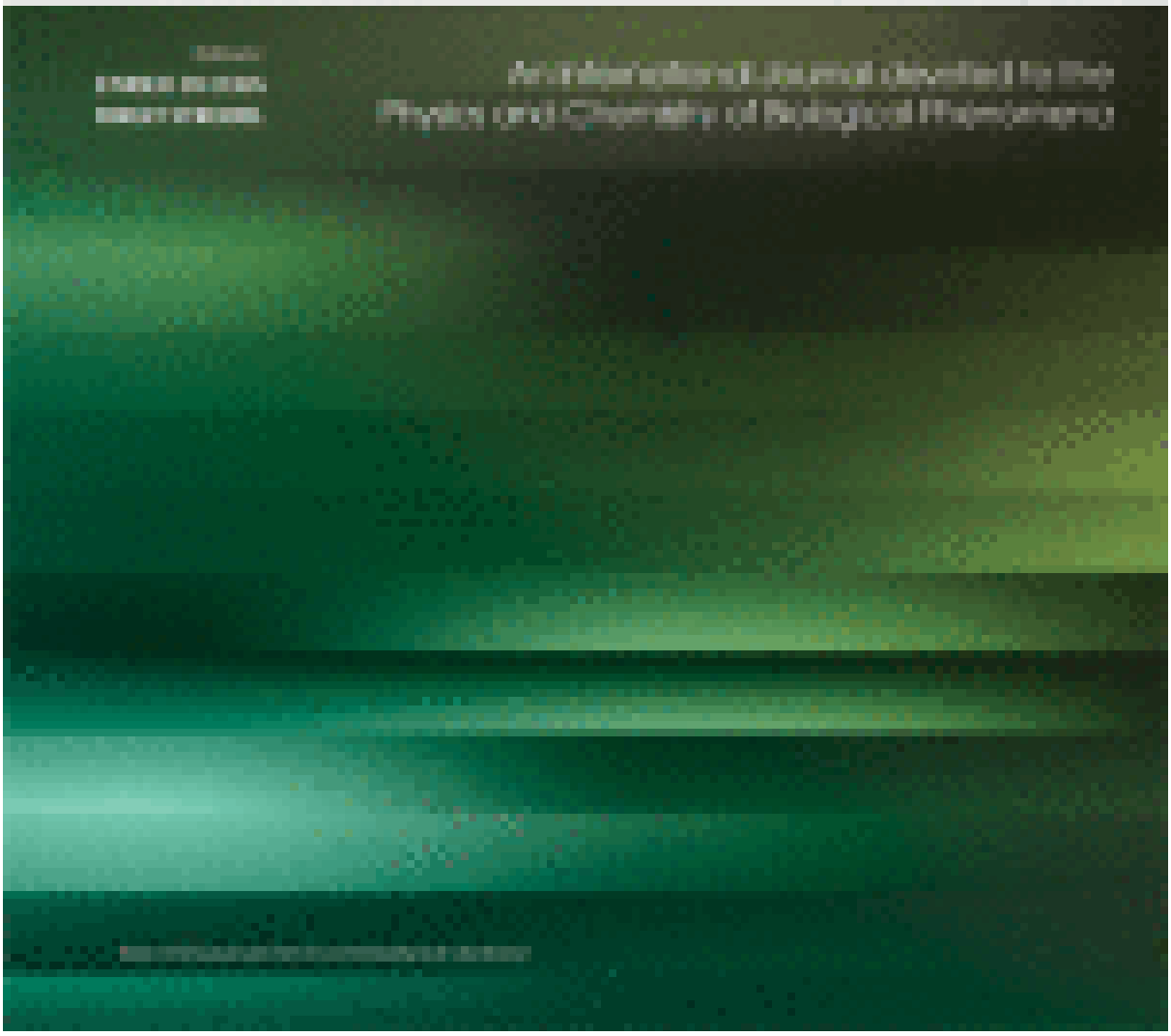

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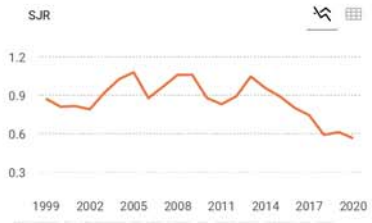

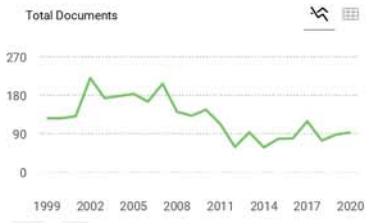

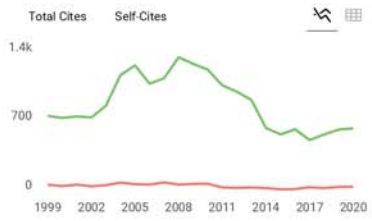

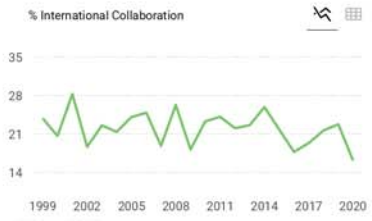

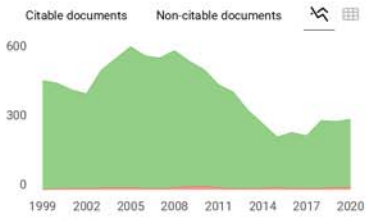

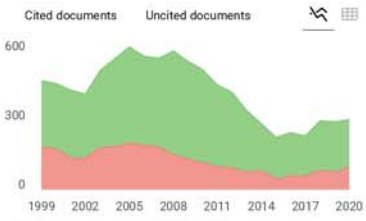

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National Institutes of Health, National Heart, Lung, and Blood Institute, Biochemistry and Biophysics Center, Bethesda, Maryland, United States of America

Protein folding, Protein aggregation, Amyloid formation, Protein-lipid interactions, Fluorescence spectroscopy

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Tata Institute of Fundamental Research Department of Chemical Sciences, Mumbai, India

Fluorescence spectroscopy, protein aggregation, single molecule, optical microscopy, protein folding

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Molecular Biophysics (in particular Biophysics of Proteins), UV-VIS-IR Spectroscopy, Structure and Dynamics of Proteins, Proteins Aggregation and Intermolecular Interactions, Proteins as Biomaterials.

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Nucleosomes, chromatin regulation, NMR spectroscopy, protein conformation and dynamics

Martin Muschol

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Institute of Biophysics National Research Council Unit of Trento, Trento, Italy

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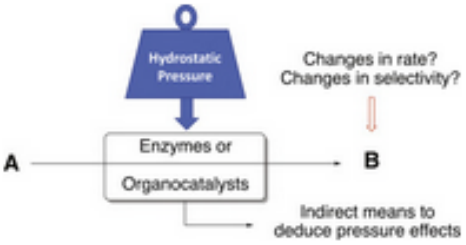

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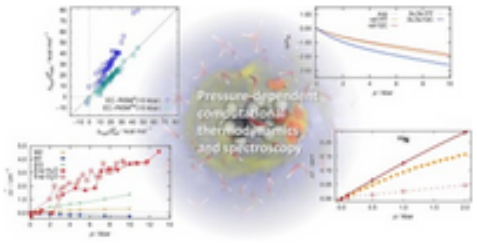

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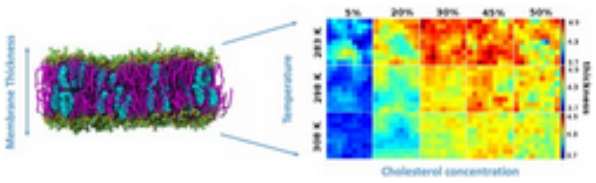

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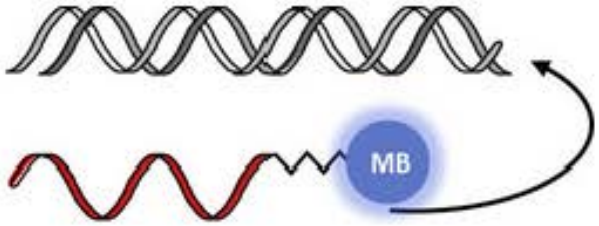

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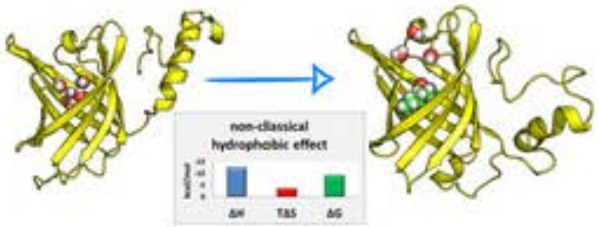

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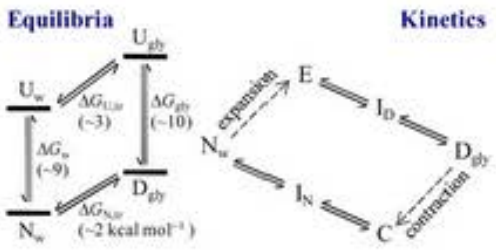

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Refutation of the cation-centric torsional ATP synthesis model and advocating murburn scheme for mitochondrial oxidative phosphorylation

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The acclaimed explanation for mitochondrial oxidative phosphorylation (mOxPhos) is a proton or cation centric scheme. Such ideas were recently disclaimed and *in lieu*, an evidence-based oxygen-centric explanation, murburn concept, was proposed. The new understanding vouches for catalytic roles of diffusible reactive oxygen species (DROS). The involvement of DROS explains the "non-discoverability of an enzyme-linked high-energy phosphorylating intermediate", a historical predicament, which had fueled several trans-membrane potential (TMP) based mechano-electrical explanations like the Nath model. This communication aims to briefly apprise the readers some lacunae and inadmissible aspects of the Nath model and project the appeal of murburn scheme of mOxPhos.

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Consolidation of Nath's torsional mechanism of ATP synthesis and two-ion theory of energy coupling in oxidative phosphorylation and photophosphorylation

Sunil Nath

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Abstract

In a recent publication, Manoj raises criticisms against consensus views on the ATP synthase. The radical statements and assertions are shown to contradict a vast body of available knowledge that includes i) pioneering single-molecule biochemical and biophysical studies from the respected experimental groups of Kinosita, Yoshida, Noji, Börsch, Dunn, Gräber, Frasch, and Dimroth etc., ii) state-of-the-art X-ray and EM/cryo-EM structural information garnered over the decades by the expert groups of Leslie-Walker, Kühlbrandt, Mueller, Meier, Rubinstein, Sazanov, Duncan, and Pedersen on ATP synthase, iii) the pioneering energy-based computer simulations of Warshel, and iv) the novel theoretical and experimental works of Nath. Valid objections against Mitchell's chemiosmotic theory and Boyer's binding change mechanism put forth by Manoj have been addressed satisfactorily by Nath's torsional mechanism of ATP synthesis and two-ion theory of energy coupling and published 10 to 20 years ago, but these papers are not cited by him. This communication shows conclusively and in great detail that none of his