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Uncovering Conceptual Biases in DNA Stabilization: A Student-Led Investigation

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Abstract:

Deoxyribonucleic acid (DNA) stands as one of the most foundational concepts in life sciences, essential for students to master. However, when surveyed about the forces that stabilize the double-stranded DNA structure, many students exhibited a conceptual bias—favoring base pairing as the primary stabilizing force, while overlooking the equally critical role of base stacking interactions. To investigate the origins of this misconception, students conducted a comprehensive analysis of 35 widely used textbooks. Their findings revealed that one-third of these texts explicitly emphasized base pairing as the sole stabilizing force in their written content. Furthermore, two-thirds of the textbook contained illustrations that reinforced this bias, visually highlighting base pairing while neglecting base stacking. Recognizing this bias, students embarked on a literature review to gain a more accurate and nuanced understanding of DNA stabilization. Through this research, we identified three concept areas—DNA structure and function, environmental effects on DNA, and DNA-protein interactions—to illustrate how base pairing and base stacking work in concert to stabilize the antiparallel double helical structure of DNA. This interplay between base pairing and base stacking is crucial not only for the structural integrity of DNA, but also for its biological functionality. By addressing this conceptual bias, we aim to promote a more balanced and scientifically accurate representation of DNA stabilization in educational materials.

Introduction

Double stranded deoxyribonucleic acid (dsDNA) is a fundamental biomolecule, providing the basis for life on earth. It was first isolated from white blood cells in the late 19th century by Friedrich Miescher, although at the time its implications for genetic inheritance were not realized (Dahm, 2003). In 1945, Oswald Avery, Colin MacLeod, and Maclyn McCarty confirmed DNA as the carrier of genetic information, along with the observations of Fredrick Griffith that highlighted the possibility for the exchange of genetic information between bacterium (McCarty & Avery, 1945). Soon after, structures for DNA were proposed, such as Linus Pauling's triple helix (Pauling, 1953). Pauling used cryogenic electron microscopy images of DNA published by Robley Williams and X-ray diffraction patterns by William Astbury to theorize that one structural unit of DNA consisted of three polynucleotides (Figure 1a; Astbury, 1938). The structure described in his manuscript consists of a triple helix with the phosphate groups closely packed in the core and the nitrogenous bases adenine, thymine, guanine, and cytosine positioned outwards, exposed to solvent (Pauling, 1953).

In retrospect, one might immediately question the role of the hydrophobic effect in Pauling's structure, as its existence is contingent on the association of polar phosphate groups within a hydrophobic pocket, which would be disfavoured by repulsive forces (Tanford, 1978). Further, in this structure the hydrophilic phosphates are unable to interact with the water molecules present in an aqueous cellular environment—an oversight that limits the overall stability of the structure. Having worked on protein secondary structure, it is likely that Pauling's DNA triple helix was influenced by the α-helix, which positions the variable side chains of amino acids outwards (Pauling, 1951). In addition to the

irreconcilable conflict of inter-molecular forces, the triple helix did not provide any mechanism to explain the biological function of DNA as the heritable material.

In the same year, James Watson and Francis Crick published an alternative proposal for DNA structure, now known as the double helix model (Watson & Crick, 1953; Figure 1b). Their proposed structure consists of two antiparallel sugar-phosphate chains connected by phosphodiester linkages; both intertwined in right-handed helices. Backbone sugars are bound to heterocyclic nucleobases, composed of single and double-ringed carbon backbones (pyrimidines and purines, respectively), via an N-glycosidic linkage. These nucleobases are oriented parallel, pairing selectively such that purines bind pyrimidines, allowing for the transmission of genetic information. In canonical base pairing, adenine pairs with thymine (AT) through two hydrogen bonds (H-bonds), while guanine pairs with cytosine (GC) through three H-bonds. Watson and Crick implemented X-ray diffraction images from Rosalind Franklin, providing the biophysical parameters to construct their model of the DNA double helix (Franklin & Gosling, 1953). Unlike in Pauling's triple helix, the double helix is stabilized through the hydrophobic effect, whereby the hydrophilic phosphate groups interact with the polar solvent, and the hydrophobic nucleobase are buried. Currently, the DNA double helix model proposed by Watson and Crick is accepted as the correct structure for B-form DNA.

The Watson and Crick (WC) model for DNA utilizes four distinct stabilizing forces. For instance, the phosphate groups within the backbone of the helix have been found to establish stable electrostatic interactions with common intracellular cations such as sodium and magnesium when deprotonated at physiological condition (Huguet et al., 2017; Feughelman et al., 1955). Additionally, these phosphate groups form phosphodiester

linkages with deoxyribose sugars through dehydration reactions, providing stability to the structure through strong covalent bonds (Elliott & Elliott, 2005). Nitrogenous bases influence the stability of dsDNA through base pairing (facilitated by H-bonds) and base stacking (facilitated by orbital overlap and van der Waals forces between stacked heterocyclic bases) (Krueger et al., 2006; Poater et al., 2014; Li et al., 2009). While the four forces mentioned above (phosphodiester bonds, ionic interactions, base pairing, and base stacking) work synergistically to stabilize DNA, the amount each force contributes to total stability remains a subject of ongoing debate and investigation.

Base pairing, mediated by H-bonds between complementary bases, AT and GC, reveals its critical role in DNA replication, transcription, and in the fidelity of genetic information flow (Petersheim & Turner, 1983). The H-bonds between base pairs help anchor the DNA strands, particularly at the ends, where base stacking interactions are weaker (Matray & Kool, 1998). This function is essential for maintaining the structural integrity of linear DNA and preventing genomic degradation (Matray & Kool, 1998). In contrast, base stacking interactions, driven by van der Waals forces and the hydrophobic effect, contribute significantly to the enthalpic stability of dsDNA (Krueger et al., 2006). These stacking interactions are enhanced by the overlap of π-orbitals between neighboring bases and the additive contributions of van der Waals forces, providing a strong stabilizing effect, and thus crucial for the structural integrity and overall stability of the DNA duplex (Friedman & Honig, 1992).

Student perspectives on the stabilizing forces of dsDNA offer another lens to assess the broader impact of the base pairing versus base stacking discourse. Survey data collected from biochemistry and molecular biology undergraduates at an Atlantic Canadian

university revealed that many second-year students identified base pairing as the primary stabilizing force (Figure 2a). A smaller proportion recognized both base pairing and base stacking as contributing to stability, while very few considered base stacking alone to be significant. Notably, this trend persisted into their third year of study, with little to no improvement in their understanding (Figure 2b). Despite increased academic exposure, students continued to place disproportionate emphasis on base pairing; these results prompted our inquiry into the following research questions:

1. How might textbook representations both in writing and in figure contribute to students' oversimplified view on DNA stability?
2. How does the current literature represent the intermolecular forces involved in DNA stability?

This study investigates textbooks and literature to answer these two research questions. Further, the paper presents three key areas that exemplify a holistic depiction of dsDNA—DNA structure and function, environmental effects on DNA, and DNA-protein interaction— with the aim that their acknowledgement addresses this gap in student understanding and prevents the perpetuation of conceptual bias.

Methods

Textbook Analysis

The textbooks selected for analysis spanned a range of disciplines within the natural sciences, including biochemistry, molecular biology, genetics, biology, and biological chemistry. (Ahern et al., 2018; Alberts et al., 2022; Anthony-Cahill et al., 2019; Armstrong, 1983; Berg et al., 2002; Berg et al., 2023; Boyer, 1999; Campbell & Farrell, 2003; Campbell & Reece, 2002; Cox et al., 2025; Cox et al., 2015; Devlin, 2002; Dow et al., 1996; Elliott & Elliott, 1997; Elliott & Elliott, 2005; Ferrier, 2014; Garrett & Grisham, 2005; Horton et al., 2012; Jakubowski & Flatt, 2018; Klostermeier & Rudolph, 2018; McKee & McKee, 2016; McMurry et al., 2013; McMurry et al., 2017; Miesfeld & McEvoy, 2017; Miesfeld & McEvoy, 2021; Nelson & Cox, 2008; Nelson & Cox, 2021; Panini, 2013; Pierce, 2019; Pratt & Cornely, 2021; Tansey, 2020; Timberlake, 2010; Tymoczko et al., 2019; Voet & Voet, 2011; Voet et al., 2016). The objective of the analysis was to identify potential biases in how base stacking and base pairing interactions are presented as stabilizing forces in B-form DNA. Subsequent text and figure analysis considered only textbook chapters devoted to DNA structure. To assess textual bias, a decision tree was developed, employing a series of binary (yes/no) questions (Figure 3). Textbooks were categorized based on whether they presented an explicit preference for base stacking or base pairing as the primary stabilizing interaction. If the text explicitly stated that one interaction contributed more significantly to DNA stability than the other, it was classified as biased towards that force. In cases where no such explicit statement was provided, the relative volume of discussion dedicated to each interaction was evaluated. Textbooks that devoted substantially more content to one force were considered biased towards that force. If there was no explicit statement, and the

volumes of discussion for both forces were equivalent, textbook content was classified as unbiased.

In the second part of the analysis, figures depicting B-form DNA were evaluated for visual bias. A figure was deemed unbiased if it clearly illustrated both base pairing and base stacking, using visual indicators that allow students to recognize its presence and significance without prior knowledge of the stabilizing forces of DNA. Base pairing was typically identified by dashed lines between two nucleobases representing H-bonds; base stacking was expected to meet an equivalent standard of clarity, indicated using lines, shading, electron clouds or labels (Figure 4). Figure captions were excluded from this analysis, as it was found that there was a poor correlation between the mention of base stacking in the figure caption and its representation within the figure itself.

Literature Review

Literature research on the topic of forces that stabilize B-DNA was conducted by interested students who were recruited voluntarily through alternative assessment as part of a year-long laboratory experience at a third-year level. Students who were part of the alternative assessment group used their research and literature review to replace the credits that would be normally evaluated through writing lab reports. Since both the alternative and regular assessments were aimed to help students develop scientific writing skills, students from either group were unlikely to be disadvantaged throughout the laboratory learning process. During literature research and review, students focused on gaining a holistic and unbiased view on the forces that stabilize B-DNA from the current literature.

In total, 14 students chose to pursue the alternative assessment guided by a series of scaffolded formative assessments including bibliography, article summary, argument development, multiple drafts at different stages, and eventually the final report, which was used as the basis for developing the current manuscript in submission (Hati & Bhattacharyya, 2024). Students were encouraged to work in pairs at the early stages of research and article summary and then were promoted to work in a slightly larger groups, up to 6 people, at the later stage upon incorporating community data contributed by all 14 members. The scaffolded formative assessments were aimed to keep students on track of the year-long project while receiving timely feedback informing the next stage of writing. Two students were recruited over the summer of 2025 in a special topics course to take charge on compiling and finalizing the manuscript. The compiled document was read, edited, and revised by all students repeatedly, and the final editing was done by the instructor before submitting the manuscript for peer review.

Results & Discussion:

1. How might textbook representations both in writing and in figure contribute to students' oversimplified view on DNA stability?

Through a systematic analysis of both textbook content and figures, 35 textbooks were allocated into five out of the nine categories in Figure 3 (Table 1). This initial analysis revealed that the largest proportion of textbooks explicitly favoured base pairing both in text and in figure as the dominant force that stabilizes the B-form of DNA, which might have influenced instructors and thus predisposed students to favour base paring as the dominant force that stabilizes B-DNA during teaching and learning respectively.

We further analyzed the texts and figures separately to reveal additional trends that might be masked from the previous overall analysis. When looking only at the texts in the 35 textbooks analyzed, we found a relatively equal distribution for books favouring pairing, stacking, and unbiased (Figure 5). This distribution represented that in two thirds of the textbooks, the authors showed a clear preference of one force over the other. Notably, there are striking contradictions across textbooks which could cause confusion among students.

For instance, Elliott & Elliott (2005) assert unequivocally, "DNA almost always exists as a double strand … What holds them together? The answer is complementary base pairing." In stark contrast, Pratt & Cornely (2021) state, "Instead, stability depends mostly on stacking interactions, which are a form of van der Waals interactions, between adjacent base pairs."

A closer look at textbooks reveals a clear divide. Voet & Voet (2011) emphasize the contribution of H-bonds, noting that the melting point for double stranded DNA "…increases linearly with the mole fraction of G-C base pairs, which indicates that triply hydrogen bonded G-C base pairs are more stable than A-T base pairs." Conversely, Miesfeld & McEvoy (2021) challenges this view by stating "Sequences rich in G-C base pairs have more stability than do sequences rich in A-T base pairs primarily due to more favourable base stacking interactions (not because of differences in the number of hydrogen bonds)." Similarly, Devlin (2002) acknowledges the historical neglect of stacking, stating "The relative importance of base stacking versus hydrogen bonding in stabilizing the double helix was not always appreciated … experiments with reagents that reduce the stability of the double helix illustrate the greater importance of stacking interactions." When these claims are presented across different sources without reconciliation, they contribute to a conflicting educational narrative.

When analysing only the figures from the 35 textbooks, we found two thirds of the textbooks' figures favoured base pairing, while the remaining third showed a more unbiased representation of both base pairing and stacking (Figure 6). Taken together with the text-only analysis, we found that even though 23 textbooks in writing favoured either base stacking or neutrality—for which we would expect their figures to support either base stacking or an unbiased illustration, respectively—in practice only 12 of those textbooks showed unbiased figure representation, while no book showed figures that favoured base stacking, a nearly 50% reduction in highlighting the importance of base stacking in stabilizing B-form DNA. This discrepancy illuminates a critical issue—visual aids disproportionately favor base pairing, thereby potentially creating a disconnect between textual explanations and visual illustrations during learning.

It can be concluded that the disparity between textbook content and figures correlates with the students' preference for base pairing. This suggests that the treatment of dsDNA stability in textbooks might influence student understanding (Figure 2). Textbook figures, often powerful instructional tools for reinforcing concepts, overwhelmingly represent base pairing as the main DNA stabilizing force. As visual learning strongly influences cognitive retention, students and instructors may be unintentionally guided towards emphasizing base pairing, despite the texts recognizing stacking forces as an equivalent if not more stabilizing force. Thus, the prevalence of base pair focused figures appears to play a critical role in shaping student understanding and their subsequent misconceptions about DNA stabilization. Addressing this imbalance through promoting clearer, more balanced visual representations in educational materials could help rectify

student misunderstandings, ensuring a more complete comprehension of the true nature of DNA stability.

2. How does the current literature represent the intermolecular forces involved in DNA stability?

To help students and teachers to approach the concept of DNA stability from a more holistic standpoint, we have chosen three commonly discussed topics in life science programs that illustrate the interplay of the intermolecular interactions involved in stabilizing the DNA double helical structure, emphasizing base pairing and stacking.

Example 1: The Structure-Function Relationship of B-DNA

The overall literature review suggests there is a strong interplay between the forces, highlighting that both base pairing and stacking are necessary for the stability and functionality of DNA (Zhou et al., 1999; Guckian et al., 2000). Matray et al. utilized non-H-bonding analogs *in vitro* and observed that base stacking interactions alone could only stabilize for the loss of 40% of H-bonding, after which the DNA adopted a hairpin structure (1998). In fact, it has been found that base stacking can enhance H-bond strength, suggesting that the two forces are mutually beneficial (Mignon et al., 2005).

However, it should be acknowledged that the previous claims do not reflect B-DNA in biological systems, and this may be the source of discrepancy amongst findings. It is not unlikely that in controlling experimental conditions, contributions of one force have been overlooked or influenced by experimental parameters. This can be addressed by considering the function of B-DNA as genetic material and how this is influenced by structure, sequence,

and environmental conditions. Evidently, many characteristic functions of B-DNA utilize both base pairing and base stacking uniquely; not as energetically efficiently as an *in vitro* experiment, but in a way that provides enormous benefit *in vivo*.

In a cellular environment, DNA has been observed in three structural formations: A, Z, and B. Unlike A- and Z-DNA, B-DNA is favoured in aqueous environments. This is attributed to the lack of slide, which can be thought of as a parameter describing the relative deformation of neighbouring base pairs from a completely overlapped orientation (Hunter, 1997). Thus, the lack of slide accommodates hydrophobic interactions between stacked bases and solvation of the major and minor grooves (Hunter, 1993). Despite this, studies have shown that the absence of slide in B-DNA creates electrostatic repulsion in a GC base step. This repulsion is not observed in A-DNA, which contains a negative slide, yet there is only a minimal amount of A-DNA observed in the cell (Calladine & Drew, 1984). The abundance of B-DNA proves that the enhanced stacking and solvation effects of B-DNA are more favourable within the cell. However, preference for B-DNA *in vivo* does not only arise from base stacking. A study by Kool and colleagues highlights that when replacing up to 40% of the nucleobases in B-DNA with nonpolar analogs incapable of H-bonding, similar stability is achieved, but the degree of solubility is lost (Hirao et al., 2012; Kool et al., 2000). This is because in the absence of H-bonds, non-polar base analogues can achieve a larger extent of overlap, resulting in a lower solvent-accessible surface area (Hirao et al., 2012; Kool et al., 2000; Loakes et al., 1997). Therefore, despite stacking interactions contributing extensively to the stabilization of B-DNA, base pairing is essential for achieving a structure with optimal solubility in a biological context (Kool, 2001; Kool et al.; 2000, Guckian et al., 2000).

The fate of a given stretch of DNA is also contingent on nucleobase composition and hence, relative stability. Stabilization by base stacking and base pairing differs amongst base pairs, resulting in DNA sequences with segregated nucleotide motifs of distinct properties (Jabbari et al., 2019). Specifically, GC-rich sequences are more stable than AT-rich sequences. This is in part due to the additional H-bond in GC pairs, and due to the reinforcement of stacking forces by the overlap of electropositive amide nitrogen and electronegative carbonyl oxygen between diagonal bases in a GC rich sequence (Nieuwland et al., 2022). Furthermore, the presence of both stacking and pairing in B-DNA is crucial for optimizing cellular processes. Considering DNA breathing events that take place during replication and transcription, an AT-rich consensus sequence upstream of the gene has relatively weak stability, enabling the actions of helicase (Gutiérrez-Flores et al., 2017; Krueger et al., 2006). Conversely, sequences rich in GC have enhanced stability, and an extreme example of this is the G rich region in telomere, which forms G-quadraplexes to stabilize and protect the chromosomal ends via enhanced base paring and stacking.

Example 2: Environmental Effect on DNA Stability

Base pairing and base stacking interactions can be influenced by environmental factors, with solvation emerging as a significant area of interest. The impacts of solvation on base pairing have been extensively studied using both computational and experimental methodologies.

Quantum chemical computational studies have allowed researchers to delve into the behaviour of WC base pairs while considering structural and environmental factors. Density Functional Theory (DFT) calculations indicate that solvation has a destabilizing effect on the formation of DNA duplexes, due to a slight decrease in cavitation energy, which reflects the

energy required to create a void in the solvent for the solute. The solvent concentration and types significantly influence base pairing by not only reducing H-bonding but also equilibrating the interactions between H-bonding and π-π stacking for both AT and GC base pairs (Poater et al., 2014; Nieuwland et al., 2022).

Experimental investigations utilizing calorimetry and spectroscopy techniques have supported computational predictions regarding the stability of base pairing. Differential scanning calorimetry and isothermal titration calorimetry experiments have shown that the hydration of DNA minor grooves plays a crucial role in stabilizing H-bonds between base pairs (Privalov & Crane-Robinson, 2018). The release of structured water molecules during DNA melting significantly contributes to the enthalpic and entropic aspects of dsDNA stability, underscoring the importance of solvent mediated interactions in base pairing (Privalov & Crane-Robinson, 2018). Additionally, studies employing Atomic Force Microscopy (AFM) have provided direct measurements of the strength of DNA H-bonds, confirming that variations in ionic strength within solvent conditions directly affect base pairing (Zhang et al., 2015). Under low-salt conditions, DNA H-bonds demonstrate increased instability, while higher concentrations of NaCl enhance the force required to dissociate complementary strands.

Molecular orbital calculations utilizing DFT with dispersion corrections have revealed that solvation effects significantly influence stacking interactions in B-DNA (Poater et al., 2014; Tran et al., 2022). The findings indicate that base stacking interactions are more robust in hydrophobic environments, whereas they weaken in aqueous solvation conditions due to enhanced hydration-induced electrostatic shielding. Free energy calculations of stacked DNA duplexes under varying solvent conditions have demonstrated that water molecules

disrupt base stacking by altering the electrostatic potential of π–π interactions (Punnoose et al., 2025).

Single-molecule force spectroscopy experiments, including optical tweezers and AFM measurements, provide direct mechanical insights into base-stacking interactions (Zhang et al., 2015). These studies indicate that the forces required to disrupt base stacking in individual DNA molecules depend on solvent conditions and ionic strength. Notably, in high-salt environments, stacking interactions become increasingly strong, while in low-salt conditions, reduced electrostatic shielding results in weakened π–π interactions, leading to enhanced DNA flexibility and instability (Yakovchuk, 2006).

Interestingly, the identity of the salt in the solvent also influences the relative strength of base pairing and stacking in stabilizing B-DNA. Huguet et al. conclude that the presence of the monovalent cation sodium, increases the contribution of stacking interactions towards duplex stability, and the presence of the divalent cation magnesium increased the contribution of base pairing (Bizarro et al., 2012; Huguet et al., 2017; McFail-Isom et al., 1998; Punnoose et al., 2025; Viader-Godoy et al., 2024;). These results suggest that the ionic strength and stability of the duplex's environment influences the relative strength of its pairing and stacking interactions. This phenomenon is caused by the inhibition of base stacking in the presence of magnesium rather than the activation of base pairing in sodium (Punnoose et al., 2025; Bizarro et al., 2012; Viader-Godoy et al., 2024; McFail-Isom et al., 1998). Magnesium ions in the major groove of B-DNA have been demonstrated to be able to pull nucleobases partially out of the duplex, causing unstacking, which likely plays a key mechanistic role in DNA bending, strand separation, and DNA-protein recognition and

complexation (Punnoose et al., 2025; Bizarro et al., 2012; Viader-Godoy et al., 2024; McFail-Isom et al., 1998; Naskar et al., 2019).

Example 3: DNA Stability Through the Lens of Protein Stability and its Interaction

Protein folding is taught often repeatedly through biochemistry classes, where students understand the fundamental roles of inter and intramolecular forces stabilizing proteins. Applying the understanding of protein stability to DNA stability may be a conducive channel to help students develop a holistic view on how B-DNA is stabilized.

Protein primary structure is linked through peptide (amide) bonds via dehydration reactions. Localized amino acids on the linear chain begin to interact with each other to form secondary structures, α-helices and β-strands, through H-bonds. Eventually these distal folded motifs and domains coalesce to form a three-dimensional structure driven by the hydrophobic effect, whereby the polar and charged amino acids are exposed to the solvent, maximizing the interactions with water, while hydrophobic amino acids are buried inside, maximizing van der Waals interactions. This is the tertiary structure and often the final structure of a protein. If a protein requires multiple subunits to function, protomers could associate through H-bonds, ionic interactions, disulfide bonds, and van der Waals interactions, forming a quaternary structure. Likewise, in DNA, each polynucleotide is linked through a series of dehydration reactions, forming phosphodiester bonds. The two polynucleotides form the well-known double stranded helix due to hydrophobic effect, reminiscent of protein folding process.

These complementary interactions in stabilizing protein and DNA also dictate how the two molecules interact with each other. For example, DNA helicase directly binds to DNA through conserved aromatic (Trp or Phe) and electropositive (Arg) residues, stacking with the

bases of single stranded DNA and gripping the negatively charged phosphodiester backbone, respectively (Bhattacharyya & Keck, 2014). In fact, how helicase functions to unwind DNA also reflects the importance of both base pairing and stacking in DNA stability. In processive helicases, the unwinding process involves the breaking of multiple base pairs with each binding event. Helicases break the H-bonds between complementary base pairs in an ATP-dependent manner, with separation occurring at the junction of single-stranded and double-stranded regions (Patel & Donmez, 2006). This separation is coupled to the translocation of the helicase.

While helicases primarily disrupt the H-bonds between base pairs, their activity also impacts the base stacking interactions within double-stranded DNA. The unwinding process generates DNA supercoiling and torsional strain ahead of the helicase, which can cause adjacent base pairs to distort from their optimal stacking conformation, thereby weakening the stacking interactions in stressed regions (Duguet, 1997). Additionally, helicases create local single-stranded DNA regions, where base stacking interactions are disrupted due to the absence of base pairing (Brosh, 2015). In summary, helicase activity underscores the interdependence of base pairing and base stacking in maintaining the structural integrity of DNA.

Conclusion

Through our investigation of student conceptual bias on the role of base pairing in stabilizing dsDNA, we identified that a major contributor to the bias may be the oversimplified illustration of textbook figures, where base stacking is often neglected. Further, we presented three commonly taught topics in life sciences, reinforcing the important role of base stacking in DNA stability by adopting a holistic approach in

explaining DNA structure-function relationships, environmental effects on DNA, and Helicase-DNA interactions. During our investigation, we observed related misconceptions involving the explanation for the higher melting temperature (T_m) of GC-rich sequences, that are concerning enough to merit further investigation. Some textbooks continue to attribute this solely to the presence of three H-bonds in GC base pairs, compared to two in AT pairs. For instance, one textbook states, "Because there are three hydrogen bonds between G and C but only two between A and T, DNA that contains high concentrations of A and T denatures at a lower temperature than G-and C-rich DNA." (Harvey & Ferrier, 2013). Even in cases where this is not explicitly stated, textbook organization may inadvertently reinforce this oversimplified view. For example, we found several textbooks introduce base pairing immediately before posing questions about T_m comparison among different GC rich sequences, potentially leading students to infer that H-bonding is the primary determinant of T_m, and thus DNA stability. Another outdated claim appears in *Lehninger Principles of Biochemistry* (2008) concerning the sequence dependence of base stacking interactions, claiming they are "...largely non-specific with respect to the identity of the bases...". This statement may further entrench the misconception that base stacking does not contribute meaningfully to DNA stability.

Encouragingly, some textbooks have adopted a more accurate and research-aligned perspective. For example, Miesfeld and McEvoy's *Biochemistry* (2021) correctly notes, "Each unique double-stranded DNA sequence has a unique T_m. Sequences rich in G-C base pairs have more stability than do sequences rich in A-T base pairs primarily due to favourable base stacking interactions (not because of differences in number of hydrogen bonds)." This view aligns with current thermodynamic analyses, which indicate that base

stacking interactions contribute approximately 60% of the Gibbs free energy stabilizing double-stranded DNA, while base pairing accounts for the remaining 40% (Privalov & Crane-Robinson, 2020). These interactions are particularly strong in GC-rich sequences, where enhanced π-π stacking and greater overlap lead to significantly higher stability. In contrast, AT-rich sequences exhibit weaker stacking interactions, resulting in lower overall stability and reinforcing the sequence-dependent nature of base stacking (Yakovchuk, 2006).

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Figures & Tables

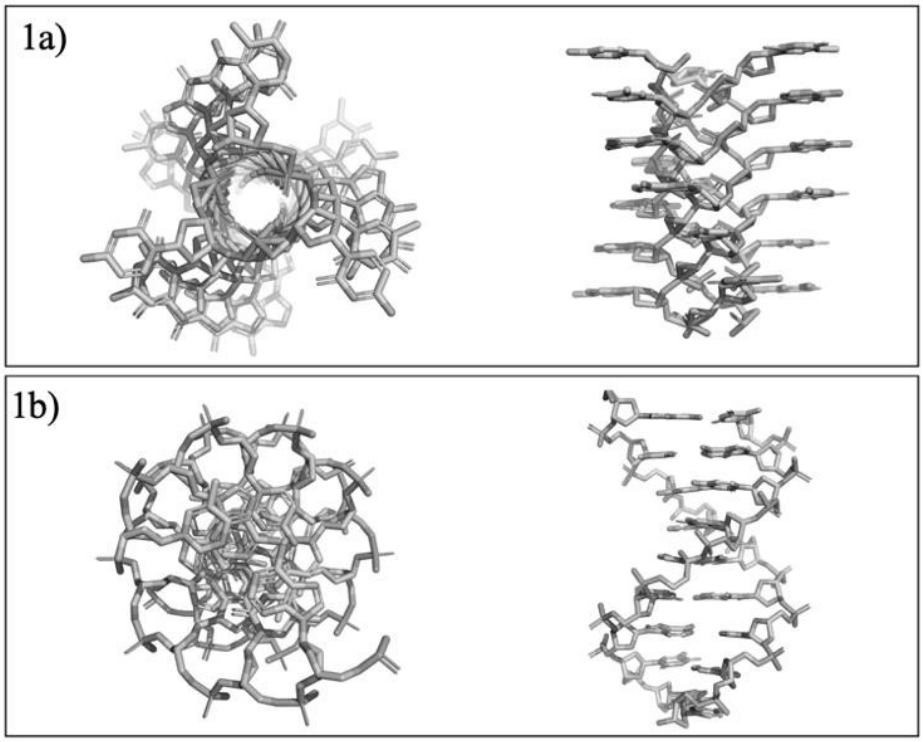

Figure 1. Molecular models of triple helix DNA (1a) and double helix DNA (1b). a) Birds'-eye and side-on views of Pauling's' triple helix adapted in PyMOL from the model published by Thomas Shafee, under the Creative Commons Attribution 4.0 International licence, with no changes made to the model aside from colouring (Shafee, 2019). 1b) Bird's-eye and side-on views of the Watson-Crick model for double helix DNA, generated from a random sequence using the "fnab" command in PyMOL.

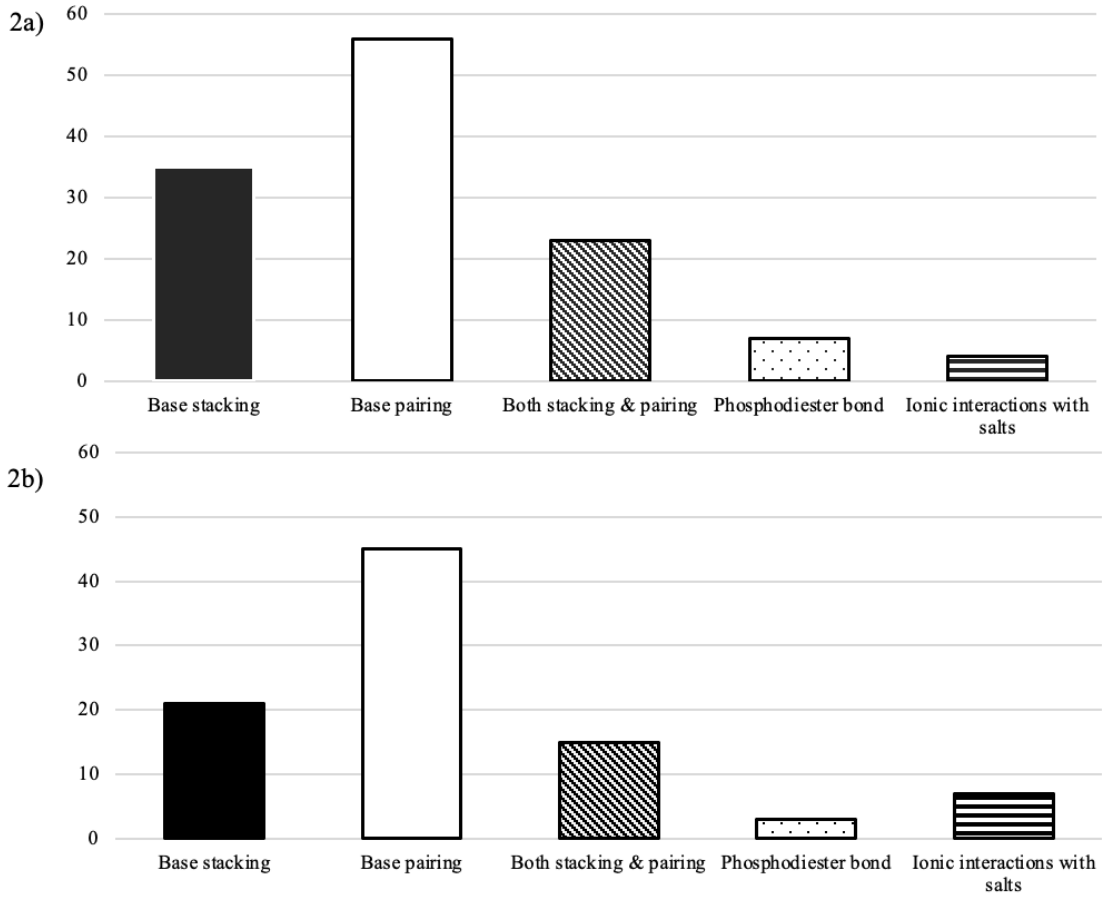

Figure. 2 Students' perception of the intermolecular forces that stabilize double stranded DNA. a) Survey of Atlantic Canadian university biochemistry and molecular biology undergraduate students in a second-year biochemistry course regarding what they believed to be the stabilizing force(s) of double stranded DNA. b) Survey of the same cohort of undergraduate students in their third year on what they believed to be the stabilizing force(s) of double stranded DNA.

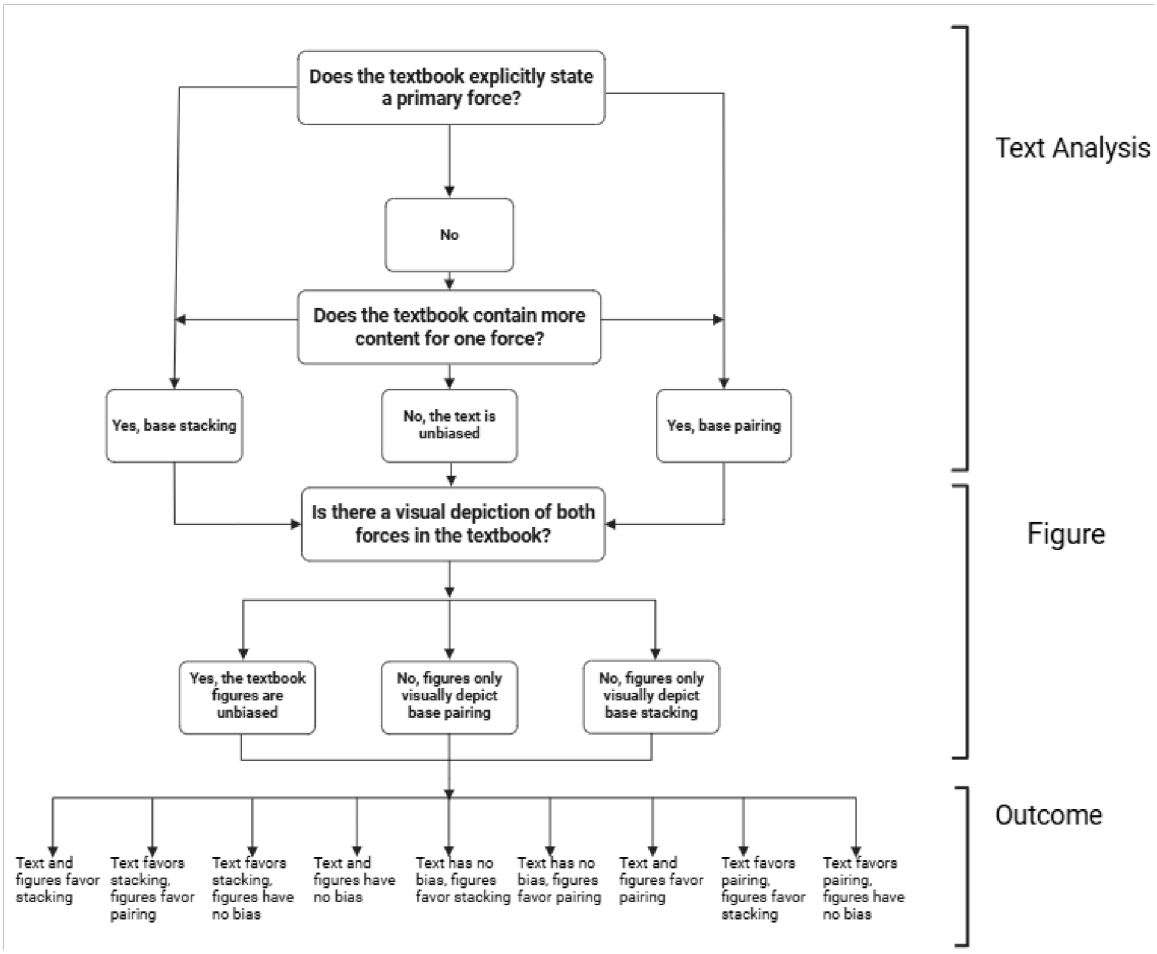

Figure 3. Decision tree used to assess base pairing or base stacking biases in textbooks.

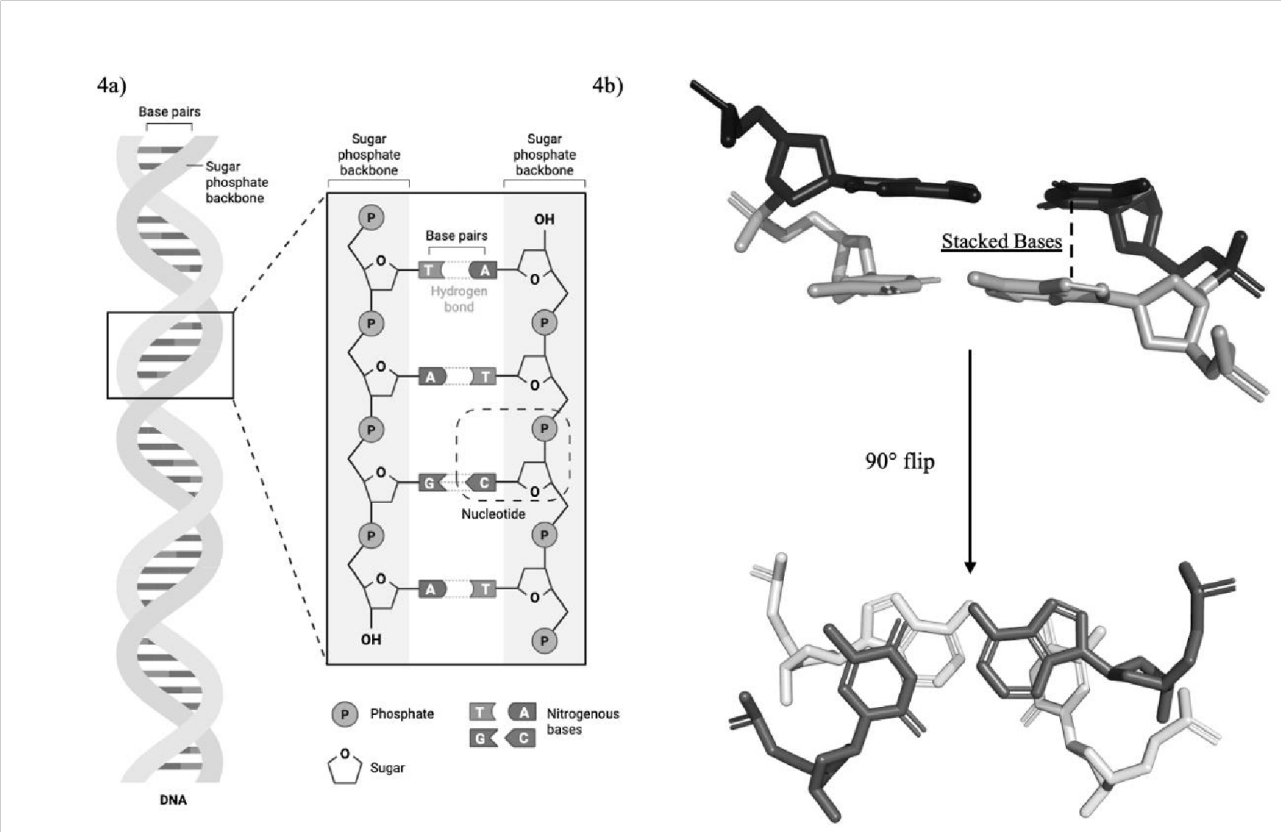

Figure 4. Representation of figure biases in illustrating the forces that stabilize double stranded DNA. a) Representation of a figure clearly indicating base pairing, taken from BioRender. 4b) Representation of a figure clearly indicating base stacking from a side-on and birds'-eye view, generated in PyMOL using the "fnab" command.

Table 1. Textbooks sorted into categories based on the criteria outlined in the Methods.

Category	Number of Textbooks
Texts favour base stacking; Figures favour base pairing	5
Texts favour base stacking; Figures unbiased	5
Texts unbiased; Figures favour base pairing	6
Text unbiased; Figures unbiased	7
Text favour base pairing; Figures favour base pairing	12

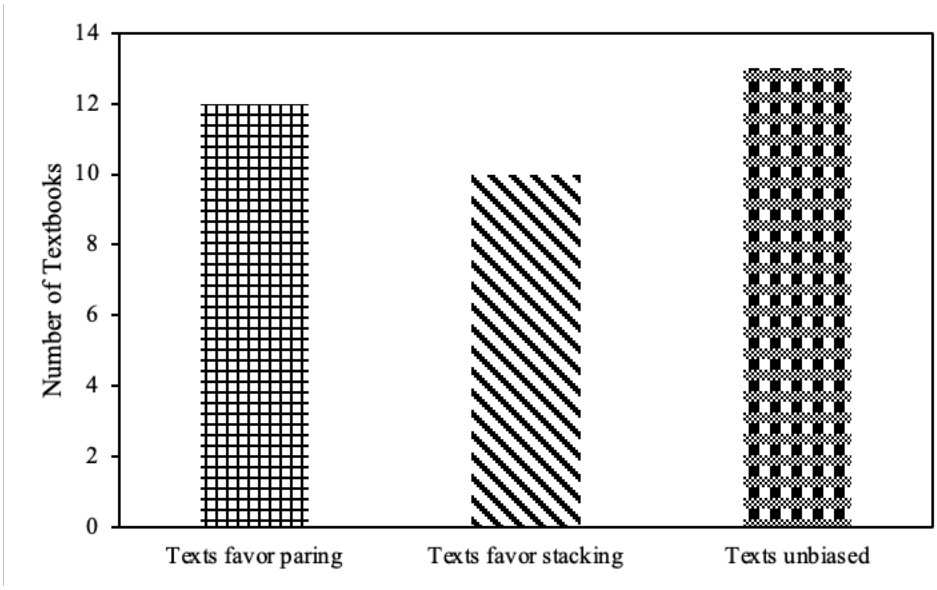

Figure 5. Number of textbooks that were assigned as having written texts biased towards pairing, stacking, or having no bias.

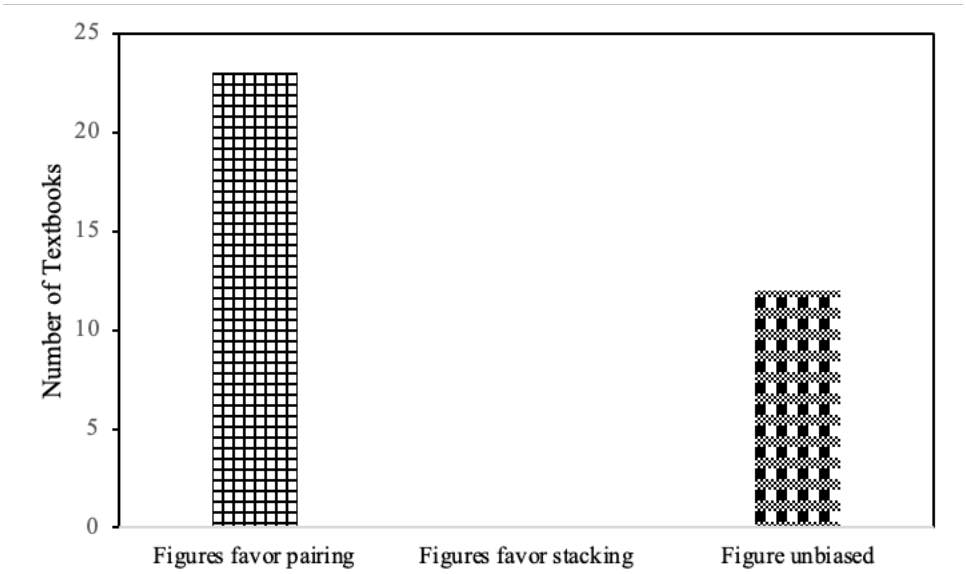

Figure 6. Number of textbooks that were assigned as having figures biased towards pairing, stacking, or having no bias.