Review

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A GIBAC-based selectivity strategy for the design of PDE5 inhibitors to minimize visual disturbances

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Article

A GIBAC-Based Selectivity Strategy for the Design of PDE5 Inhibitors to Minimize Visual Disturbances

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Abstract: Phosphodiesterase type 5 (PDE5) inhibitors are widely used in the treatment of various conditions, including erectile dysfunction and pulmonary hypertension. Despite their clinical efficacy, these drugs quite often cause visual disturbances due to off-target effects on Phosphodiesterase type 5 (PDE6) in the retina. This review explores the application of a general intermolecular binding affinity calculator (GIBAC)-based binding selectivity strategy in the design of PDE5 inhibitors, aiming to enhance binding selectivity and minimize visual side effects. This GIBAC strategy integrates computational structural biological approaches to iteratively refine drug-target binding affinities, thereby improving target specificity and the structural biophysical limit for the efficacy-safety balance of PDE5 inhibitors. Through detailed analysis of PDE5's and PDE6's biological role and the molecular mechanisms underlying visual disturbances, this article underscores the necessity of target binding selectivity in PDE5 inhibitor design in future. Additionally, this article discusses the practical applications of GIBAC in computational drug discovery and design, along with the future directions and the potential for GIBAC to transform PDE5 inhibitor development, ultimately enhancing therapeutic outcomes and patient safety. Finally, this article calls for a GIBAC-based paradigm shift in computational drug discovery and design towards the continued development of the pharmaceutical industry.

Keywords: sildenafil; Phosphodiesterase type 5 (PDE5); Phosphodiesterase type 6 (PDE6); efficacy-safety balance; GIBAC

An Introduction to Phosphodiesterase Type 5 (PDE5) and Phosphodiesterase Type 6 (PDE6)

Phosphodiesterase type 5 (PDE5) and phosphodiesterase type 6 (PDE6) are crucial enzymes involved in cellular signaling pathways with significant implications in human health and disease [1–3]. PDE5, predominantly found in smooth muscle cells of the vasculature and corpus cavernosum, plays a pivotal role in the regulation of cyclic guanosine monophosphate (cGMP) levels [4]. PDE5 inhibitors cause vasodilation in the penis and lung by blocking the breakdown of cyclic guanosine monophosphate (cGMP) which results in prolongation of the action of mediators of vasodilation including nitric oxide (NO) [5]. Clinically, inhibitors of PDE5, such as sildenafil, tadalafil, and vardenafil, are widely prescribed for the treatment of erectile dysfunction, pulmonary hypertension, and other cardiovascular conditions [6]. However, despite their therapeutic efficacy, PDE5 inhibitors are associated with a notable side effect profile, notably visual disturbances, attributed to off-target effects on PDE6 [7]. PDE6, primarily expressed in rod and cone photoreceptor cells of the retina, serves as a key mediator in the visual transduction cascade [8].

In general, the essential biological function of phosphodiesterase (PDE) type enzymes is to regulate the cytoplasmic levels of intracellular second messengers, 3′,5′-cyclic guanosine monophosphate (cGMP) and/or 3′,5′-cyclic adenosine monophosphate (cAMP) [7]. PDE targets have 11 isoenzymes. Of these enzymes [7], PDE5 (Figure 1) has attracted a special attention over the years after its recognition as being the target enzyme in treating erectile dysfunction. Due to the amino acid sequence and the secondary structural similarity of PDE6 and PDE11 with the catalytic domain of PDE5, first-generation PDE5 inhibitors (i.e. sildenafil and vardenafil) are also competitive inhibitors of PDE6 and PDE11 [9]. Thus, inhibition of PDE6 by PDE5 inhibitors can disrupt phototransduction processes, leading to transient visual changes, including altered color perception and sensitivity to light [9]. Understanding the distinct roles of PDE5 and PDE6 in cellular signaling pathways is essential for elucidating the

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mechanisms underlying both the therapeutic effects and adverse reactions associated with PDE5 inhibitors [8]. Moreover, it underscores the importance of developing strategies to enhance binding selectivity towards PDE5 while minimizing off-target interactions with PDE6, thereby mitigating visual disturbances and improving the overall safety profile of these drugs [8].

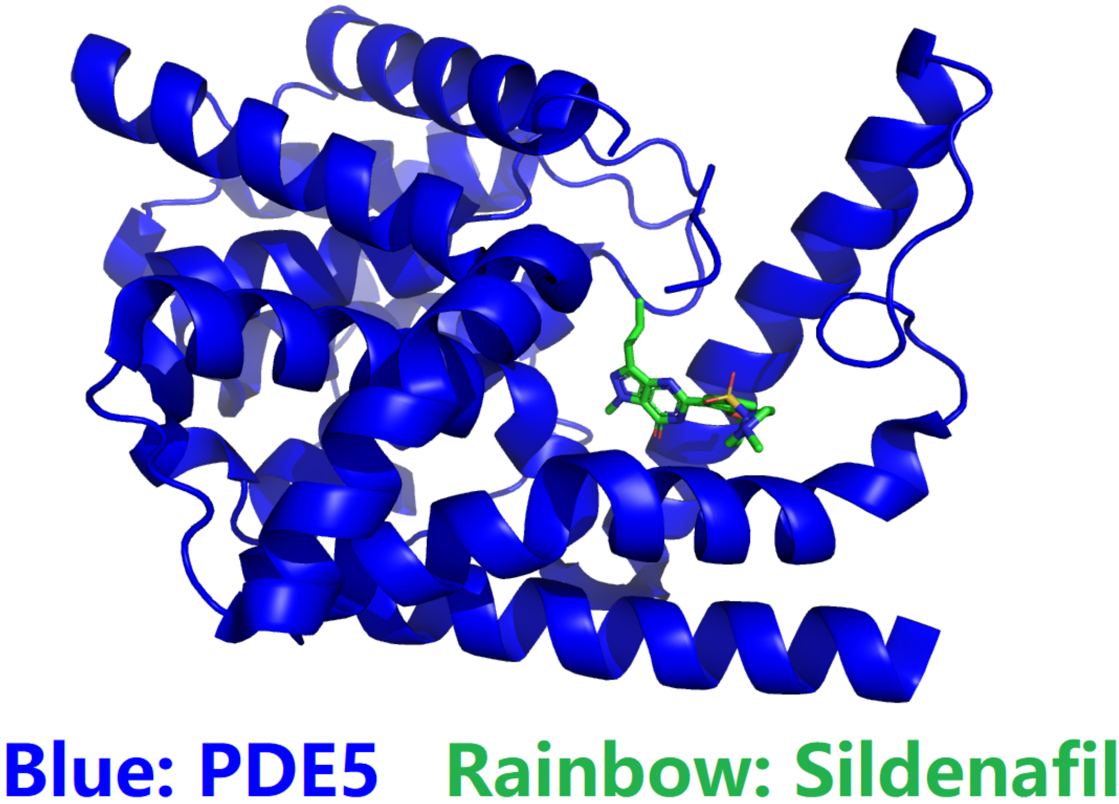

Figure 1. Crystal structure of human phosphodiesterase 5 (PDE5) complexed with sildenafil (PDB ID: 1UDT) [10,11]. In this figure, the blue cartoon represents the catalytic domain of PDE5, while the small molecule in rainbow sticks represents sildenafil. This figure was prepared with PyMol [12]

Specifically, the amino acid sequence of the catalytic domain of PDE5 is listed in italics in fasta format as below,

>3SHY_1 | Chain A | cGMP-specific 3′,5′-cyclic phosphodiesterase | Homo sapiens (9606)

MGSSHHHHHHSSGLVPRGSHMEETRELQSLAAAVVPSAQTLKITDFSFSDFELSDLETALCTIRMFTDLNLVQNFQMKHEVLCRWILSVKKNYRKNVAYHNWRHAFNTAQCMFAALKAGKIQNKLTDLEILALLIAALSHDLDHRGVNNSYIQRSEHPLAQLYCHSIMEHHHFDQCLMILNSPGNQILSGLSIEEYKTTLKIIKQAILATDLALYIKRRGEFFELIRKNQFNLEDPHQKELFLAMLMTACDLSAITKPWPIQQRIAELVATEFFDQGDRERKELNIEPTDLMNREKKNKIPSMQVGFIDAICLQLYEALTHVSEDCFPLLDGCRKNRQKWQALAEQQ

and the amino acid sequence of the entire PDE5 is listed in italics in fasta format as below,

>PDE5A_(UniProtKB ID: O76074)_cGMP-specific 3′,5′-cyclic phosphodiesterase

MERAGPSFGQQRQQQQPQQQKQQQRDQDSVEAWLDDHWDFTFSYFVRKATREMVNAWFAERVHTIPVCKEGIRGHTESCSCPLQQSPRADNSAPGTPTRKISASEFDRPLRPIVVKDSEGTVSFLSDSEKKEQMPLTPPRFDHDEGDQCSRLLELVKDISSHLDVTALCHKIFLHIHGLISADRYSLFLVCEDSSNDKFLISRLFDVAEGSTLEEVSNNCIRLEWNKGIVGHVAALGEPLNIKDAYEDPRFNAEVDQITGYKTQSILCMPIKNHREEVVGVAQAINKKSGNGGTFTEKDEKDFAAYLAFCGIVLHNAQLYETSLLENKRNQVLLDLASLIFEEQQSLEVILKKIAATIISFMQVQKCTIFIVDEDCSDSFSSVFHMECEELEKSSDTLTREHDANKINYMYAQYVKNTMEPLNIPDVSKDKRFPWTTENTGNVNQQCIRSLLCTPIKNGKKNKVIGVCQLVNKMEENTGKVKPFNRNDEQFLEAFVIFCGLGIQNTQMYEAVERAMAKQMVTLEVLSYHASAAEE

ETRELQSLAAAVVPSAQTLKITDFSFSDFELSDLETALCTIRMFTDLNLVQNFQMKHEVLCRWILSVK
KNYRKNVAYHNWRHAFNTAQCMFAALKAGKIQNKLTDLEILALLIAALSHDLDHRGVNNSYIQRSE
HPLAQLYCHSIMEHHHFDQCLMILNSPGNQILSGLSIEEYKTTLKIIKQAILATDLALYIKRRGEFFELIR
KNQFNLEDPHQKELFLAMLMTACDLSAITKPWPIQQRIAELVATEFFDQGDRERKELNIEPTDLMNR
EKKNKIPSMQVGFIDAICLQLYEALTHVSEDCFPLLDGCRKNRQKWQALAEQQEKMLINGESGQAK
RN

and the amino acid sequences of four (α, β, γ and δ) subunits of PDE6 (Figure 2) are also listed in italics in fasta format as below:

>PDE6A_(UniProtKB ID: P16499)_Human Retinal Rod cGMP-specific 3′,5′-cyclic phosphodiesterase subunit alpha

MGEVTAEEVEKFLDSNIGFAKQYYNLHYRAKLISDLLGAKEAAVDFSNYHSPSSMEESEIIFDLL
RDFQENLQTEKCIFNVMKKLCFLLQADRMSLFMYRTRNGIAELATRLFNVHKDAVLEDCLVMPDQE
IVFPLDMGIVGHVAHSKKIANVPNTEEDEHFCDFVDILTEYKTKNILASPIMNGKDVVAIIMAVNKV
DGSHFTKRDEEILLKYLNFANLIMKVYHLSYLHNCETRRGQILLWSGSKVFEELTDIERQFHKALYTV
RAFLNCDRYSVGLLDMTKQKEFFDVWPVLMGEVPPYSGPRTPDGREINFYKVIDYILHGKEDIKVIP
NPPPDHWALVSGLPAYVAQNGLICNIMNAPAEDFFAFQKEPLDESGWMIKNVLSMPIVNKKEEIVG
VATFYNRKDGKPFDEMDETLMESLTQFLGWSVLNPDTYESMNKLENRKDIFQDIVKYHVKCDNEEI
QKILKTREVYGKEPWECEEEELAEILQAELPDADKYEINKFHFSDLPLTELELVKCGIQMYYELKVVDK
FHIPQEALVRFMYSLSKGYRKITYHNWRHGFNVGQTMFSLLVTGKLKRYFTDLEALAMVTAAFCHDI
DHRGTNNLYQMKSQNPLAKLHGSSILERHHLEFGKTLLRDESLNIFQNLNRRQHEHAIHMMDIAIIA
TDLALYFKKRTMFQKIVDQSKTYESEQEWTQYMMLEQTRKEIVMAMMMTACDLSAITKPWEVQSQ
VALLVAAEFWEQGDLERTVLQQNPIPMMDRNKADELPKLQVGFIDFVCTFVYKEFSRFHEEITPMLD
GITNNRKEWKALADEYDAKMKVQEEKKQKQQSAKSAAAGNQPGGNPSPGGATTSKSCCIQ

>PDE6B_(UniProtKB ID: P35913)_Human Retinal Rod cGMP-specific 3′,5′-cyclic phosphodiesterase subunit beta

MSLSEEQARSFLDQNPDFARQYFGKKLSPENVAAACEDGCPPDCDSLRDLCQVEESTALLELV
QDMQESINMERVVFKVLRRLCTLLQADRCSLFMYRQRNGVAELATRLFSVQPDSVLEDCLVPPDSEI
VFPLDIGVVGHVAQTKKMVNVEDVAECPHFSSFADELTDYKTKNMLATPIMNGKDVVAVIMAVNK
LNGPFFTSEDEDVFLKYLNFATLYLKIYHLSYLHNCETRRGQVLLWSANKVFEELTDIERQFHKAFYT
VRAYLNCERYSVGLLDMTKEKEFFDVWSVLMGESQPYSGPRTPDGREIVFYKVIDYVLHGKEEIKVIP
TPSADHWALASGLPSYVAESGFICNIMNASADEMFKFQEGALDDSGWLIKNVLSMPIVNKKEEIVGV
ATFYNRKDGKPFDEQDEVLMESLTQFLGWSVMNTDTYDKMNKLENRKDIAQDMVLYHVKCDRDE
IQLILPTRARLGKEPADCDEDELGEILKEELPGPTTFDIYEFHFSDLECTELDLVKCGIQMYYELGVVRK
FQIPQEVLVRFLFSISKGYRRITYHNWRHGFNVAQTMFTLLMTGKLKSYYTDLEAFAMVTAGLCHDI
DHRGTNNLYQMKSQNPLAKLHGSSILERHHLEFGKFLLSEETLNIYQNLNRRQHEHVIHLMDIAIIAT
DLALYFKKRAMFQKIVDESKNYQDKKSWVEYLSLETTRKEIVMAMMMTACDLSAITKPWEVQSKV
ALLVAAEFWEQGDLERTVLDQQPIPMMDRNKAAELPKLQVGFIDFVCTFVYKEFSRFHEEILPMFDR
LQNNRKEWKALADEYEAKVKALEEKEEEERVAAKKVGTEICNGGPAPKSSTCCIL

>PDE6G_(UniProtKB ID: P18545)_Human Retinal rod rhodopsin-sensitive cGMP 3′,5′-cyclic phosphodiesterase subunit gamma

MNLEPPKAEFRSATRVAGGPVTPRKGPPKFKQRQTRQFKSKPPKKGVQGFGDDIPGMEGLGTD
ITVICPWEAFNHLELHELAQYGII

>PDE6D_(UniProtKB ID: O43924)_Human Retinal rod rhodopsin-sensitive cGMP 3′,5′-cyclic phosphodiesterase subunit delta

MSAKDERAREILRGFKLNWMNLRDAETGKILWQGTEDLSVPGVEHEARVPKKILKCKAVSREL
NFSSTEQMEKFRLEQKVYFKGQCLEEWFFEFGFVIPNSTNTWQSLIEAAPESQMMPASVLTGNVIIET
KFFDDDLLVSTSRVRLFYV

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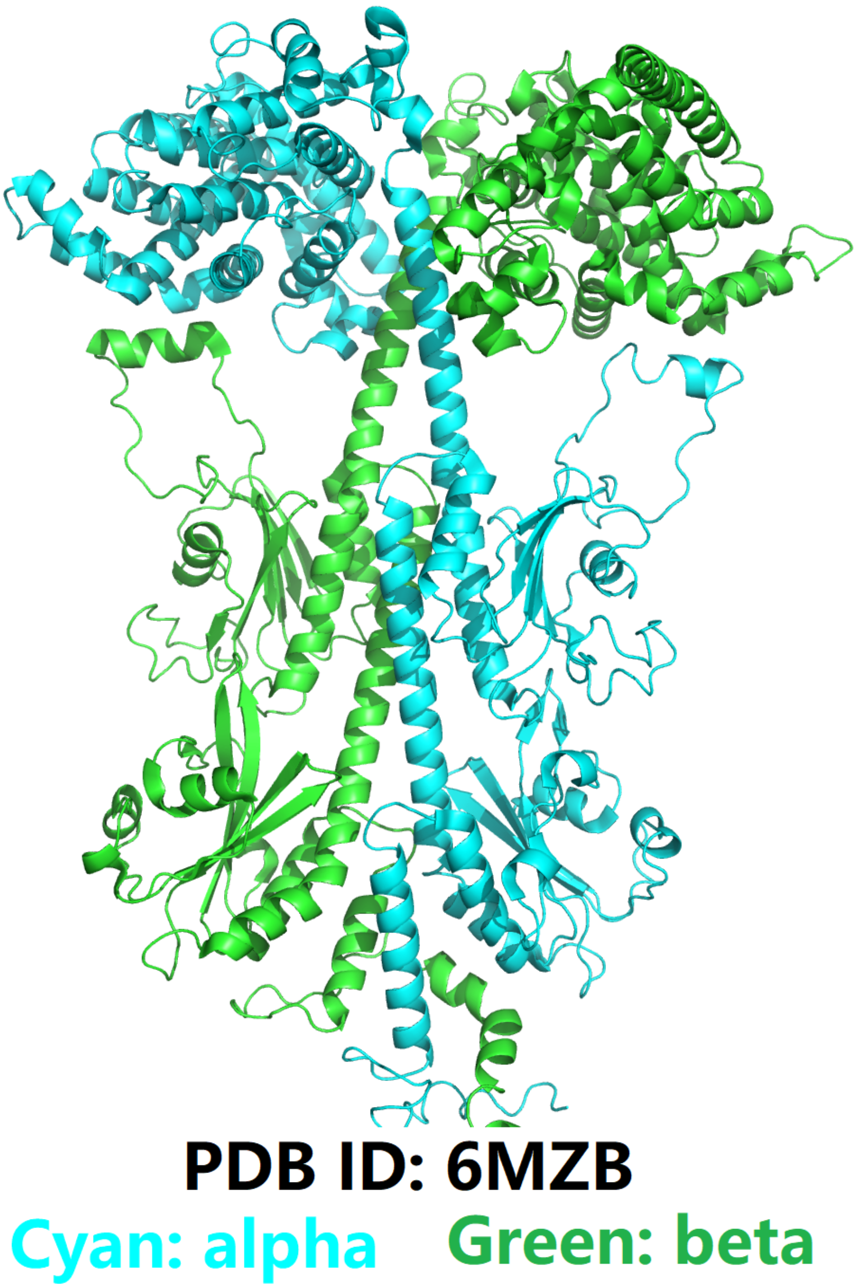

Figure 2. Cryo-EM structure of phosphodiesterase 6 (PDB ID: 6MZB) [13,14]. In this figure, cyan represents the α subunit of PDE6, while green represents the β subunit of PDE6. This figure was prepared with PyMol [12]

To further account for the molecular and structural characterizations of PDE6, the amino acid sequences of α, β and γ subunits of PDE6 are also listed in italics in fasta format as below:

>6MZB_1 | Chain A[auth B] | Rod cGMP-specific 3′,5′-cyclic phosphodiesterase subunit beta | Bos taurus (9913)

MSLSEGQVHRFLDQNPGFADQYFGRKLSPEDVANACEDGCPEGCTSFRELCQVEESAALFELVQDMQENVNMERVVFKILRRLCSILHADRCSLFMYRQRNGVAELATRLFSVQPDSVLEDCLVPPDSEIVFPLDIGVVGHVAQTKKMVNVQDVMECPHFSSFADELTDYVTRNILATPIMNGKDVVAVIMAVNKLDGPCFTSEDEDVFLKYLNFGTLNLKIYHLSYLHNCETRRGQVLLWSANKVFEELTDIERQFHKAFYTVRAYLNCDRYSVGLLDMTKEKEFFDVWPVLMGEAQAYSGPRTPDGREILFYKVIDYILHGKEDIKVIPSPPADHWALASGLPTYVAESGFICNIMNAPADEMFNFQEGPLDDSGWIVKNVLSMPIVNKKEEIVGVATFYNRKDGKPFDEQDEVLMESLTQFLGWSVLNTDTYDKMNKLENRKDIAQDMVLYHVRCDRE

EIQLILPTRERLGKEPADCEEDELGKILKEVLPGPAKFDIYEFHFSDLECTELELVKCGIQMYYELGVVR KFQIPQEVLVRFLFSVSKGYRRITYHNWRHGFNVAQTMFTLLMTGKLKSYYTDLEAFAMVTAGLCH DIDHRGTNNLYQMKSQNPLAKLHGSSILERHHLEFGKFLLSEETLNIYQNLNRRQHEHVIHLMDIAII ATDLALYFKKRTMFQKIVDESKNYEDRKSWVEYLSLETTRKEIVMAMMMTACDLSAITKPWEVQSK VALLVAAEFWEQGDLERTVLDQQPIPMMDRNKAAELPKLQVGFIDFVCTFVYKEFSRFHEEILPMFD RLQNNRKEWKALADEYEAKVKALEEDQKKETTAKKVGTEICNGGPAPRSSTCRIL

>6MZB_2 | Chain B[auth A] | Rod cGMP-specific 3′,5′-cyclic phosphodiesterase subunit alpha | Bos taurus (9913)

MGEVTAEEVEKFLDSNVSFAKQYYNLRYRAKVISDLLGPREAAVDFSNYHALNSVEESEIIFDLL RDFQDNLQAEKCVFNVMKKLCFLLQADRMSLFMYRARNGIAELATRLFNVHKDAVLEECLVAPDS EIVFPLDMGVVGHVALSKKIVNVPNTEEDEHFCDFVDTLTEYQTKNILASPIMNGKDVVAIIMVVNK VDGPHFTENDEEILLKYLNFANLIMKVFHLSYLHNCETRRGQILLWSGSKVFEELTDIERQFHKALYT VRAFLNCDRYSVGLLDMTKQKEFFDVWPVLMGEAPPYAGPRTPDGREINFYKVIDYILHGKEDIKVI PNPPPDHWALVSGLPTYVAQNGLICNIMNAPSEDFFAFQKEPLDESGWMIKNVLSMPIVNKKEEIVG VATFYNRKDGKPFDEMDETLMESLTQFLGWSVLNPDTYELMNKLENRKDIFQDMVKYHVKCDNEE IQTILKTREVYGKEPWECEEEELAEILQGELPDADKYEINKFHFSDLPLTELELVKCGIQMYYELKVVD KFHIPQEALVRFMYSLSKGYRRITYHNWRHGFNVGQTMFSLLVTGKLKRYFTDLEALAMVTAAFCH DIDHRGTNNLYQMKSQNPLAKLHGSSILERHHLEFGKTLLRDESLNIFQNLNRRQHEHAIHMMDIAI IATDLALYFKKRTMFQKIVDQSKTYETQQEWTQYMMLDQTRKEIVMAMMMTACDLSAITKPWEV QSKVALLVAAEFWEQGDLERTVLQQNPIPMMDRNKADELPKLQVGFIDFVCTFVYKEFSRFHEEITP MLDGITNNRKEWKALADEYETKMKGLEEEKQKQQAANQAAAGSQHGGKQPGGGPASKSCCVQ

>6MZB_3 | Chains C, D | Retinal rod rhodopsin-sensitive cGMP 3′,5′-cyclic phosphodiesterase subunit gamma | Bos taurus (9913)

MNLEPPKAEIRSATRVMGGPVTPRKGPPKFKQRQTRQFKSKPPKKGVQGFGDDIPGMEGLGTD ITVICPWEAFNHLELHELAQYGII

Background on Currently Available PDE5 Inhibitors and Their Action Mechanisms

Phosphodiesterase type 5 (PDE5) inhibitors represent a class of drugs with diverse clinical applications and profound therapeutic significance [1–3]. Primarily known for their efficacy in the management of erectile dysfunction (ED), PDE5 inhibitors, including sildenafil (Viagra, Figure 1), tadalafil (Cialis), and vardenafil (Levitra), exert their effects by selectively inhibiting the enzymatic activity of PDE5. By blocking the degradation of cyclic guanosine monophosphate (cGMP), PDE5 inhibitors prolong the vasodilatory effects initiated by nitric oxide (NO) release upon sexual stimulation [7]. This results in enhanced blood flow to the corpus cavernosum of the penis, facilitating erectile function [5]. Beyond their use in ED, PDE5 inhibitors have demonstrated efficacy in the treatment of pulmonary arterial hypertension (PAH) by promoting vasodilation and inhibiting pulmonary vascular remodeling [15]. Furthermore, emerging research suggests potential applications of PDE5 inhibitors in various other conditions, including benign prostatic hyperplasia (BPH) and Raynaud's phenomenon [16].

At the molecular level, PDE5 inhibitors bind directly to the catalytic site of PDE5 (Figure 1), preventing the hydrolysis of cGMP to guanosine monophosphate (GMP). From the amino acid sequence point of view, the binding between PDE5 inhibitor (e.g., Sildenafil) and PDE5's catalytic site is located between sequence position number 536 and 860 (Figure 3). This binding between PDE5 inhibitor (e.g., Sildenafil) leads to an accumulation of cGMP within smooth muscle cells, resulting in prolonged relaxation and vasodilation. Additionally, the inhibition of PDE5 in other tissues, such as the pulmonary vasculature, contributes to the therapeutic effects observed in PAH [7]. However, the non-selective nature of some PDE5 inhibitors can lead to off-target interactions with other phosphodiesterase isoforms, notably PDE6 in the retina, resulting in visual disturbances. Understanding the molecular mechanisms underlying PDE5 inhibitor action is crucial for optimizing their therapeutic benefits while minimizing adverse effects [19,20].

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3SHY   ............................................................   0
PDE5A  MERAGPSFGQQRQQQQPQQQKQQQRDQDSVEAWLDDHWDFTFSYFVRKATREMVNAWFAE  60

3SHY   ............................................................   0
PDE5A  RVHTIPVCKEGIRGHTESCSCPLQQSPRADNSAPGTPTRKISASEFDRPLRPIVVKDSEG 120

3SHY   ............................................................   0
PDE5A  TVSFLSDSEKKEQMPLTPPRFDHDEGDQCSRLLELVKDISSHLDVTALCHKIFLHIHGLI 180

3SHY   ............................................................   0
PDE5A  SADRYSLFLVCEDSSNDKFLISRLFDVAEGSTLEEVSNNCIRLEWNKGIVGHVAALGEPL 240

3SHY   ............................................................   0
PDE5A  NIKDAYEDPRFNAEVDQITGYKTQSILCMPIKNHREEVVGVAQAINKKSGNGGTFTEKDE 300

3SHY   ............................................................   0
PDE5A  KDFAAYLAFCGIVLHNAQLYETSLLENKRNQVLLDLASLIFEEQQSLEVILKKIAATIIS 360

3SHY   ............................................................   0
PDE5A  FMQVQKCTIFIVDEDCSDSFSSVFHMECEELEKSSDTLTREHDANKINYMYAQYVKNTME 420

3SHY   ............................................................   0
PDE5A  PLNIPDVSKDKRFPWTTENTGNVNQQCIRSLLCTPIKNGKKNKVIGVCQLVNKMEENTGK 480

3SHY   .................................MGSSHHHHHHSSGLVPRGSHMEETREL  27
PDE5A  VKPFNRNDEQFLEAFVIFCGLGIQNTQMYEAVERAMAKQMVTLEVLSYHASAAEEETREL 540

3SHY   QSLAAAVVPSAQTLKITDFSFSDFELSDLETALCTIRMFTDLNLVQNFQMKHEVLCRWIL  87
PDE5A  QSLAAAVVPSAQTLKITDFSFSDFELSDLETALCTIRMFTDLNLVQNFQMKHEVLCRWIL 600

3SHY   SVKKNYRKNVAYHNWRHAFNTAQCMFAALKAGKIQNKLTDLEILALLIAALSHDLDHRGV 147
PDE5A  SVKKNYRKNVAYHNWRHAFNTAQCMFAALKAGKIQNKLTDLEILALLIAALSHDLDHRGV 660

3SHY   NNSYIQRSEHPLAQLYCHSIMEHHHFDQCLMILNSPGNQILSGLSIEEYKTTLKIIKQAI 207
PDE5A  NNSYIQRSEHPLAQLYCHSIMEHHHFDQCLMILNSPGNQILSGLSIEEYKTTLKIIKQAI 720

3SHY   LATDLALYIKRRGEFFELIRKNQFNLEDPHQKELFLAMLMTACDLSAITKPWPIQQRIAE 267
PDE5A  LATDLALYIKRRGEFFELIRKNQFNLEDPHQKELFLAMLMTACDLSAITKPWPIQQRIAE 780

3SHY   LVATEFFDQGDRERKELNIEPTDLMNREKKNKIPSMQVGFIDAICLQLYEALTHVSEDCF 327
PDE5A  LVATEFFDQGDRERKELNIEPTDLMNREKKNKIPSMQVGFIDAICLQLYEALTHVSEDCF 840

3SHY   PLLDGCRKNRQKWQALAEQQ...............   347
PDE5A  PLLDGCRKNRQKWQALAEQQEKMLINGESGQAKRN   875

X  non-conserved
X  ≥ 50% conserved
```

Figure 3. Sequence alignment (https://www.genome.jp/tools-bin/clustalw) of human cGMP-specific 3′,5′-cyclic phosphodiesterase (PDE5, UniProtKB ID: O76074) and its catalytic domain whose structure is experimentally determined (PDB ID: 3SHY [17,18]).

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Challenges in the Design of PDE5 Inhibitors: Balancing Efficacy and Safety

Challenges in PDE5 Inhibitor Design encompass a spectrum of factors, notably the prevalence of common side effects, particularly visual disturbances, and the imperative for enhanced selectivity in drug design [5,20]. Among the various adverse reactions associated with PDE5 inhibitors, visual disturbances represent a significant concern. These disturbances primarily arise due to off-target effects on phosphodiesterase type 6 (PDE6) in the retina [21]. PDE6, a crucial enzyme involved in phototransduction, is responsible for the hydrolysis of cyclic guanosine monophosphate (cGMP) in photoreceptor cells. By inhibiting PDE6 activity, PDE5 inhibitors disrupt the delicate balance of cGMP levels in the retina, leading to alterations in visual perception [9]. The mechanism underlying visual disturbances involves perturbations in the phototransduction cascade, affecting processes such as light sensitivity and color discrimination. Moreover, the non-selective nature of some PDE5 inhibitors exacerbates this issue, as they may interact with other phosphodiesterase isoforms, further compromising visual function [22]. Thus, the challenge lies in designing PDE5 inhibitors with improved selectivity profiles, minimizing off-target interactions with PDE6 while maintaining therapeutic efficacy. Addressing these challenges is paramount for optimizing the safety and tolerability of PDE5 inhibitors, thereby enhancing their clinical utility in the treatment of erectile dysfunction and other related conditions [5].

Defining the Challenge in PDE5 Inhibitor Design with a Structural and Biophysical Perspective

As is known, ligand-receptor binding affinity is an essential parameter in computer-assisted drug discovery and structure-based drug design [23]. Thanks to the continued development of experimental structural biology and the half-a-century old Protein Data Bank (PDB) [24–28], a comprehensive structural biophysical analysis becomes possible [29,30] for specific ligand-receptor complex structures deposited in PDB, such that our understanding of the structural and biophysical basis of their interfacial stability is able to help us modify the binding affinity of certain drug target and its interacting partners [31–35]. Take PDE5 as an example here, where the structural key is the design of non-PDE6-binding PDE5 inhibitor (abbreviated below as molecule **X**), ensuring that:

1. molecule **X** does bind to PDE5, i.e., ΔG (kcal/mol) < 0; and
2. molecule **X** does not bind to PDE6, i.e., ΔG (kcal/mol) $= 0$ or ΔG (kcal/mol) > 0; and
3. in the absence of molecule **X**, cGMP does bind to PDE5 as usual (ΔG_{noelec} = -9.07 kcal/mol [10,11] as calculated by Prodigy [36]) and is able to be catalyzed by PDE5; and
4. in the presence of molecule **X**, cGMP does not bind to PDE5 (i.e., ΔG (kcal/mol) $= 0$ or ΔG (kcal/mol) > 0), such that PDE5 is unable, or at least not as able as in the absence of molecule **X**, to catalyze cGMP.

To put it simply, the structural key to the design of non-PDE6-binding PDE5 inhibitor (molecule **X**) here is also described in [37] as the question for a ChatGPT-like chatbot for drug discovery & design in future: can you generate a K_d-ranked list of therapeutic candidates which targets X (e.g., PDE5) but not Y (e.g., PDE6)?

A GIBAC-Based Selectivity Strategy for the Design of Non-PDE6-Binding PDE5 Inhibitors

On August 11, 2022, the concept of a general intermolecular binding affinity calculator (GIBAC) was for the first time proposed in an MDPI preprint [38] and defined as below:

$$K_d = f(molecules, envPara) \quad (1)$$

where <u>*molecules*</u> represents the molecular system described either in strings (e.g., amino acid sequences, strings of SMILES to represent small molecules [39,40]), or in graphs to describe PTMs (e.g., glycosylated proteins) and PEMs (e.g., insulin icodec of Novo Nordisk [41–43]).

On October 19, 2023, the concept of GIBAC (Equation 1) was for the first time updated, including its inception, definition (Equation 1), construction, practical applications, technical challenges and

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limitations, and future directions [37,44]. As defined in [37], a real GIBAC (Equation 1) is able to meet the criteria listed as below:

1. a real GIBAC needs to take genetic variations into account; and
2. a real GIBAC needs to work even without structural information; and
3. for a real GIBAC, a variety of factors need to be taken into account, such as temperature, pH [45,46], site-specific protonation states (e.g., side chain pK^a of protein) [47,48], post-translational modifications (PTMs) [49–51], post-expression modifications (PEMs) [52,53], buffer conditions [54], et cetera; and
4. a real GIBAC requires a general forcefield for all types of molecules [55]; and
5. a real GIBAC requires a universal notation system for accurate and flexible description of all molecular types and drug modalities [56,57]; and
6. a real GIBAC is able to be used the other way around, i.e., to be used as a search engine for therapeutic candidate(s). With such a GIBAC-based search engine, a list of therapeutic candidates can be retrieved and ranked according to drug-target K_d value(s), with input parameters including drug target(s) and a desired drug-target K_d value or a range of it.

As mentioned above, the binding between PDE5 inhibitor (e.g., sildenafil) and PDE5's catalytic domain is located between sequence position number 536 and 860 (Figure 3) of PDE5. From Figure 4, it can been seen that the three amino acid sequences (PDE5, PDE6A and PDE6B) do possess a certain degree of sequence homology. Yet, the three sequences are also different from one another, especially for the region which corresponds to the catalytic domain of PDE5, i.e., the region between sequence position number 536 and 860 (Figure 3) of PDE5. Thus, from a structural and biophysical point of view, it is conceivable to design non-PDE6-binding PDE5 inhibitors, which selectively targets PDE5, binds to its binding pocket as shown in Figure 1, and does not target PDE6 at all, i.e., ΔG (kcal/mol) $= 0$ or ΔG (kcal/mol) > 0 [37].

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PDE5A  MERAGPSFGQQRQQQQPQQQKQQQRDQDSVEAWLDDHWDFTFSYFVRKATREMVNAWFAE   60
PDE6A  .....................MGEVTAEEVEKFLDSNIGFAKQYYNLHYRAKLISDLLGA   39
PDE6B  ......................MSLSEEQARSFLDQNPDFARQYFGKKLSPENVAAAC.E   37

PDE5A  RVHTIPVCKEGIRGHTESCSCPLQQSPRADNSAPGTPTRKISASEFDRPLRPIVVKDSEG  120
PDE6A  KEAAVDFSNYHSPSSME...........................................   56
PDE6B  DGCPPDCDSLRDLCQVE...........................................   54

PDE5A  TVSFLSDSEKKEQMPLTPPRFDHDEGDQCSRLLELVKDISSHLDVTALCHKIFLHIHGLI  180
PDE6A  ...........................ESEIIFDLLRDFQENLQTEKCIFNVMKKLCFLL   89
PDE6B  ...........................ESTALLELVQDMQESINMERVVFKVLRRLCTLL   87

PDE5A  SADRYSLFLVCEDSSNDKFLISRLFDVAEGSTLEEVSN...NCIRLEWNKGIVGHVAALG  237
PDE6A  QADRMSLFMYRTRNG.IAELATRLFNVHKDAVLEDCLVMPDQEIVFPLDMGIVGHVAHSK  148
PDE6B  QADRCSLFMYRQRNG.VAELATRLFSVQPDSVLEDCLVPPDSEIVFPLDIGVVGHVAQTK  146

PDE5A  EPLNIKDAYEDPRFNAEVDQITGYKTQSILCMPIKNHREEVVGVAQAINKKSGNGGTFTE  297
PDE6A  KIANVPNTEEDEHFCDFVDILTEYKTKNILASPIMNG.KDVVAIIMAVNKVDG..SHFTK  205
PDE6B  KMVNVEDVAECPHFSSFADELTDYKTKNMLATPIMNG.KDVVAVIMAVNKLNG..PFFTS  203
```

Figure 4. *Cont.*

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PDE5A   KDEKDFAAYLAFCGIVLHNAQLYETSLLENKRNQVLLDLASLIFEEQQSLEVILKKIAAT   357
PDE6A   RDEEILLKYLNFANLIMKVYHLSYLHNCETRRGQILLWSGSKVFEELTDIERQFHKALYT   265
PDE6B   EDEDVFLKYLNFATLYLKIYHLSYLHNCETRRGQVLLWSANKVFEELTDIERQFHKAFYT   263

PDE5A   IISFMQVQKCTIFIVDEDCSDSFSSVFHMECEELEKSSDTLTREHDANKINYMYAQYVKN   417
PDE6A   VRAFLNCDRYSVGLLDMTKQKEFFDVWPVLMGEVPPYSGPRTPDGREINFYKVIDYILHG   325
PDE6B   VRAYLNCERYSVGLLDMTKEKEFFDVWSVLMGESQPYSGPRTPDGREIVFYKVIDYVLHG   323

PDE5A   TMEPLNIPDVSKDKRFPWTTENTGNVNQQCIRSLLCTPIKNGKKN............KVI   465
PDE6A   KEDIKVIPNPPPDHWALVSGLPAYVAQNGLICNIMNAPAEDFFAFQKEPLDESGWMIKNV   385
PDE6B   KEEIKVIPTPSADHWALASGLPSYVAESGFICNIMNASADEMFKFQEGALDDSGWLIKNV   383

PDE5A   GVCQLVNKMEENTGKVKPFNRND........EQFLEAFVIFCGLGIQNTQMYEAVERAMA   517
PDE6A   LSMPIVNKKEEIVGVATFYNRKDGKPFDEMDETLMESLTQFLGWSVLNPDTYESMNKLEN   445
PDE6B   LSMPIVNKKEEIVGVATFYNRKDGKPFDEQDEVLMESLTQFLGWSVMNTDTYDKMNKLEN   443

PDE5A   KQMVTLEVLSYHASAAEEETR...................ELQSLAAAVVPSAQTLKITD   558
PDE6A   RKDIFQDIVKYHVKCDNEEIQKILKTREVYGKEPWECEEEELAEILQAELPDADKYEINK   505
PDE6B   RKDIAQDMVLYHVKCDRDEIQLILPTRARLGKEPADCDEDELGEILKEELPGPTTFDIYE   503

PDE5A   FSFSDFELSDLETALCTIRMFTDLNLVQNFQMKHEVLCRWILSVKKNYRKNVAYHNWRHA   618
PDE6A   FHFSDLPLTELELVKCGIQMYYELKVVDKFHIPQEALVRFMYSLSKGYRK.ITYHNWRHG   564
PDE6B   FHFSDLECTELDLVKCGIQMYYELGVVRKFQIPQEVLVRFLFSISKGYRR.ITYHNWRHG   562

PDE5A   FNTAQCMFAALKAGKIQNKLTDLEILALLIAALSHDLDHRGVNNSYIQRSEHPLAQLYCH   678
PDE6A   FNVGQTMFSLLVTGKLKRYFTDLEALAMVTAAFCHDIDHRGTNNLYQMKSQNPLAKLHGS   624
PDE6B   FNVAQTMFTLLMTGKLKSYYTDLEAFAMVTAGLCHDIDHRGTNNLYQMKSQNPLAKLHGS   622

PDE5A   SIMEHHHFDQCLMILNSPGNQILSGLSIEEYKTTLKIIKQAILATDLALYIKRRGEFFEL   738
PDE6A   SILERHHLEFGKTLLRDESLNIFQNLNRRQHEHAIHMMDIAIIATDLALYFKKRTMFQKI   684
PDE6B   SILERHHLEFGKFLLSEETLNIYQNLNRRQHEHVIHLMDIAIIATDLALYFKKRAMFQKI   682

PDE5A   IRKN..........QFNLEDPHQKELFLAMLMTACDLSAITKPWPIQQRIAELVATEFFD   788
PDE6A   VDQSKTYESEQEWTQYMMLEQTRKEIVMAMMMTACDLSAITKPWEVQSQVALLVAAEFWE   744
PDE6B   VDESKNYQDKKSWVEYLSLETTRKEIVMAMMMTACDLSAITKPWEVQSKVALLVAAEFWE   742

PDE5A   QGDRERKELNIEPTDLMNREKKNKIPSMQVGFIDAICLQLYEALTHVSEDCFPLLDGCRK   848
PDE6A   QGDLERTVLQQNPIPMMDRNKADELPKLQVGFIDFVCTFVYKEFSRFHEEITPMLDGITN   804
PDE6B   QGDLERTVLDQQPIPMMDRNKAAELPKLQVGFIDFVCTFVYKEFSRFHEEILPMFDRLQN   802

PDE5A   NRQKWQALAEQQEKMLINGESGQAKRN.............................   875
PDE6A   NRKEWKALADEYDAKMKVQEEKKQKQQSAKSAAAGNQPGGNPSPGGATTSKSCCIQ   860
PDE6B   NRKEWKALADEYEAKVKALEEKEEEERVAAKKVGTEICNGGPAPKSSTCCIL....   854

        X  non-conserved
        X  ≥50% conserved
```

Figure 4. Sequence alignment (https://www.genome.jp/tools-bin/clustalw) of human cGMP-specific 3′,5′-cyclic phosphodiesterase (PDE5, UniProtKB ID: O76074), PDE6A_(UniProtKB ID: P16499)_Human Retinal Rod cGMP-specific 3′,5′-cyclic phosphodiesterase subunit alpha (UniProtKB ID: P16499) and PDE6B_(UniProtKB ID: P35913)_Human Retinal Rod cGMP-specific 3′,5′-cyclic phosphodiesterase subunit beta (UniProtKB ID: P35913).

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In practice, the design of non-PDE6-binding PDE5 inhibitors is equivalent to a matter of the construction of a mini GIBAC based on experimental structures of PDE5 and its inhibitor(s) using currently available AI algorithms as listed in [37]. Specifically, the construction of a mini GIBAC based on experimental structures of PDE5 and its inhibitor(s) needs at least four key ingredients: data, algorithm, knowledge and computational power, e.g.,

1. experimental structures of PDE5 and its inhibitor(s);
2. experimental structures of PDE6 and its inhibitor(s);
3. PDE5-related computational structural data from AlphaFold database [**?**];
4. PDE6-related computational structural data from AlphaFold database [**?**];
5. synthetic (both apo and complex) PDE5-related structural data generators [**?**];
6. synthetic (both apo and complex) PDE6-related structural data generators [**?**];
7. feature extraction using a comprehensive structural biophysical analysis [29,30], including structural biophysics and interfacial geometrics [25,58] underlying the complex structures of PDE5 (or PDE6) and its inhibitors.
8. molecular docking & dynamics simulation tools [**? ?**].
9. synthetic K_d data generators [36];
10. side chain placement and energy minimization algorithms [**?**] to incorporate structural information of PTMs [49–51] and PEMs [52,53,59] into structural models.

Future Direction of GIBAC's Practical Application in Drug Discovery and Design

While drug-target K_d is an essential parameter for drug discovery & design, it is but one of the many aspects of drug discovery & design. Usually, K_d and ΔG has been used to indicate the efficacy of a drug. However, it has been shown that residence time (*RT*) is yet another indicator of efficacy for some systems [60,61]. In biophysics, the relationship between K_d, K_{on} (association rate constant), and K_{off} (dissociation rate constant) can be described as $K_d = K_{off}/K_{on}$, with *RT* representing the average time a molecule spends bound to its target before dissociation.

Essentially, computational drug discovery and design is a matter of a package of parameters focusing on efficacy, safety, et cetera, of the candidates. To this end, a package of biophysical parameters (in addition to K_d) allows a further generalization of the GIBAC originally coined in [38], leading to the concept of a real GIBC (Table 1), which is to be one biophysics-based future direction of GIBAC. Moreover, this article further discusses the potential of GIBAC to act as a ChatGPT-like chatbot for drug discovery & design in future, which is able to accurately, precisely and efficiently handle questions such as:

1. can you generate a K_d-ranked list of therapeutic candidates which targets X of different species, e.g., X of human [35,63], X of cat [64], X of horse [65]?
2. can you generate a K_d-ranked list of therapeutic candidates which targets X, Y and Z? such as vorolanib [66] or retatrutide [67]?
3. can you generate a K_d-ranked list of therapeutic candidates which targets *X* but not *Y* or *Z*?
4. can you generate a list of insulin analogues does not bind to IDE [42,68], but still binds to IR to ensure downstream signal transduction?
5. can you generate a set of mutations into the S protein to stabilize its conformation in the prefusion state for the design of a subunit vaccine of Covid-19 [69–71]?

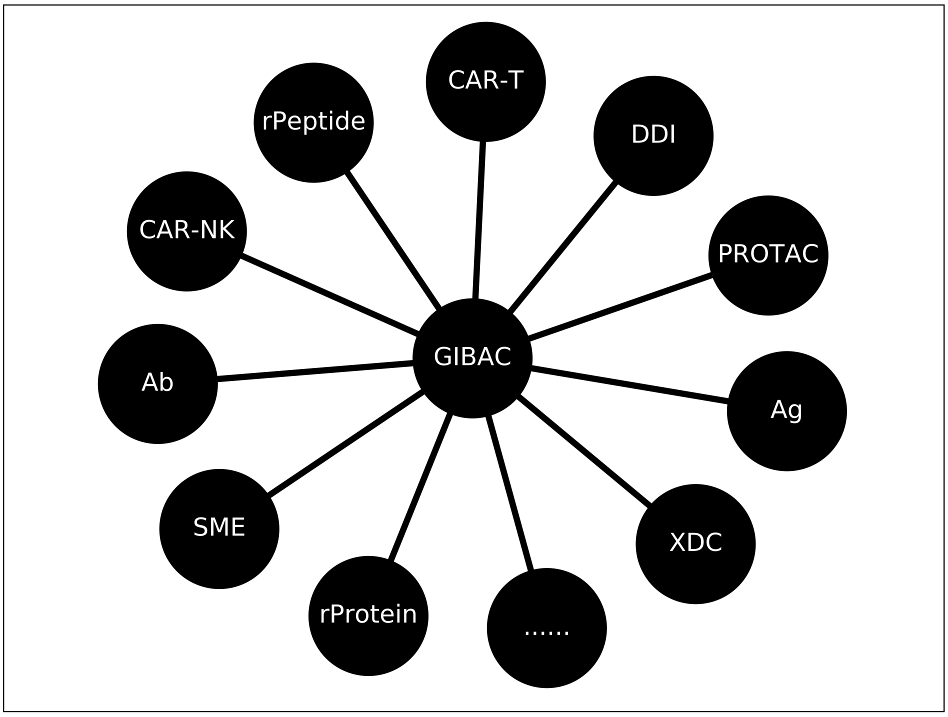

Figure 5. Areas where GIBAC are applicable and involved. SME, Antibody, Antigen, XDC, ApDC: Aptamer drug conjugate ADC: Ab drug conjugate, rPeptide, rProtein, PROTAC, DDI, Purify, Chimeric antigen receptor (CAR) T + CAR-NK [62] cell therapy relies on T cells that are guided by synthetic receptors to target and lyse cancer cells. CARs bind to cell surface antigens through an scFv (binder), the affinity of which is central to determining CAR T cell function and therapeutic success [37].

Table 1. A tabular description of a general intermolecular biophysics calculator (GIBC).

Input 1	Input 2	Output
$molAstring, molBstring, ...$	$envPara$	K_d, K_{on}, K_{off}, RT, pK_a
$molAgraph, molBgraph, ...$	$envPara$	K_d, K_{on}, K_{off}, RT, pK_a

Towards a GIBAC-Based Paradigm Shift in Computational Drug Discovery and Design

Finally, this article argues that the time (May 21, 2024) is now ripe for a GIBAC-based paradigm shift in computational drug discovery and design, and for the construction of such a GIBAC to be on the agenda of the drug discovery & design community, in light of

1. the crucial roles of K_d and ΔG in drug discovery & design; and
2. the availability of a substantial amount of structural and biophysical data, experimental and synthetic; and
3. the continued accumulation of our knowledge of the biophysics [72,73] underlying biological systems; and
4. the availability of a variety of AI algorithms [37] for drug discovery & design; and
5. the democratization of high performance computing since the beginning of this century, and its continued evolution towards scalable quantum computing [74], and perhaps computation beyond silicon [75,76] in future.

Ethical Statement

No ethical approval is required.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the author used OpenAI's ChatGPT in order to improve the readability of the manuscript, and to make it as concise and short as possible. After using this tool, the author reviewed and edited the content as needed and takes full responsibility for the content of the publication.

Author Contributions: Conceptualization, W.L.; methodology, W.L.; software, W.L.; validation, W.L.; formal analysis, W.L.; investigation, W.L.; resources, W.L.; data duration, W.L.; writing–original draft preparation, W.L.; writing–review and editing, W.L.; visualization, W.L.; supervision, W.L.; project administration, W.L.; funding acquisition, not applicable.

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