Interactions between graphyne and biological constituents.

Susan Carvalho Mathias[a].

[a] Campus Prof. Pedro C. Fornari, Padre Anchieta University Center, Jundiaí, Brazil, susancarvalhomathias@gmail.com.

Abstract. As biomedical nanomaterial applications advance, the concomitant escalation of unanticipated interactions between two-dimensional nanomaterials and biological or pharmaceutical constituents poses a growing concern. The meticulous examination of potential graphyne interactions serves not only to mitigate the occurrence of unforeseen biochemical complications but also to catalyse the creation of related medical treatments. Graphyne, a nanomaterial of substantial interest, has garnered attention for its multifaceted utility, including drug delivery mechanisms, DNA interaction, radiation protection, enzyme substitute, and cholesterol removal, among other applications. A comprehensive analysis of scholarly literature about this subject increases the popularisation of varieties of this nanomaterial. To ascertain potential interactions, an array of methodologies was further explained, including Density-functional theory (DFT), Density of States (DOS and PDOS), Charge Decomposition Analysis (CDA), and Molecular dynamics (MD) simulations. Furthermore, this extensive review will include a nuanced examination of structural variations of graphyne, regarding its acetylene linkages. Despite the extensive diversity of graphyne variants beyond the commonly known alpha, beta, and gamma forms, the alternative manifestations might be omitted in the scope of biomedical simulations. To systematically embrace the extensive range of variations, we shall employ the nomenclature "GnYf". The primary focus is to elucidate the biochemical implications of such structural configurations.

Keywords. Graphyne, Molecular Dynamics, Biochemistry, Drug Delivery, Radiation, Nanozyme, Antibacterial, Photothermal therapy, Biosensing.

1. Introduction

In recent years, the evolution of nanomaterials has significantly changed biomedical research. Among these materials, graphyne, a two-dimensional carbon allotrope, has emerged as a promising candidate with unique structural and physicochemical properties [1] This interest in graphyne is primarily attributed to its diacetylene linkages and Dirac cones, which generate interesting functionalities.

The structural diversity of graphyne presents an extensive area for exploration. These variations, distinguished by the number of acetylene linkages and their geometric arrangements influence the electronic properties of the material.[2] This difference leads to a myriad of applications in drug delivery [3], bioimaging [4], and biosensing [5], among other implementations. Furthermore, the exceptional properties of graphyne, coupled with its biocompatibility, elevate its potential for interfacing with biological systems at the nanoscale. One of the attributes of graphyne is its role in drug delivery. Simulations and experimental studies have unveiled its proficiency in adsorbing a spectrum of pharmaceutical compounds, including various anticancer agents. [3,6,7] This adsorption facilitated through hydrogen bonding and chemisorption, has triggered interest in its potential utilisation for targeted drug delivery, offering a glimpse into a promising era of medicine. The interactions between graphyne and biomolecules extend beyond drug delivery, as evidenced by its ability to effectively detect nucleobases and DNA strands. [8,5] This unique capacity has implications for the development of biosensors and diagnostics, offering insights into molecular interactions and biological processes.

2. Graphyne

An acetylene chain connects multiple (n) acetylenes with a single bond. Within the domain of graphyne

(GY) structures, their distinctive features are fundamentally characterised by two key parameters: firstly, the numerical value denoted as "n," signifying the number of acetylene units within the structure, and secondly, the geometric configuration they assume.[2] Among the myriad geometric configurations, the most prevalent classifications encompass the alpha (G1Y7), beta (G1Y4), and gamma (G1Y1) variants, as shown in **Fig. 1**.

Nevertheless, it is noteworthy that the theoretical underpinnings of graphyne extend to seven distinct geometric configurations, collectively denoted as GnYf, where the variable "f" can take on values ranging from one to seven. An alternate nomenclature convention, widely adopted within the scientific discourse, integrates the parameter "n" directly into the nomenclature, resulting in designations such as graphdiyne, graphtriynes, and so forth.

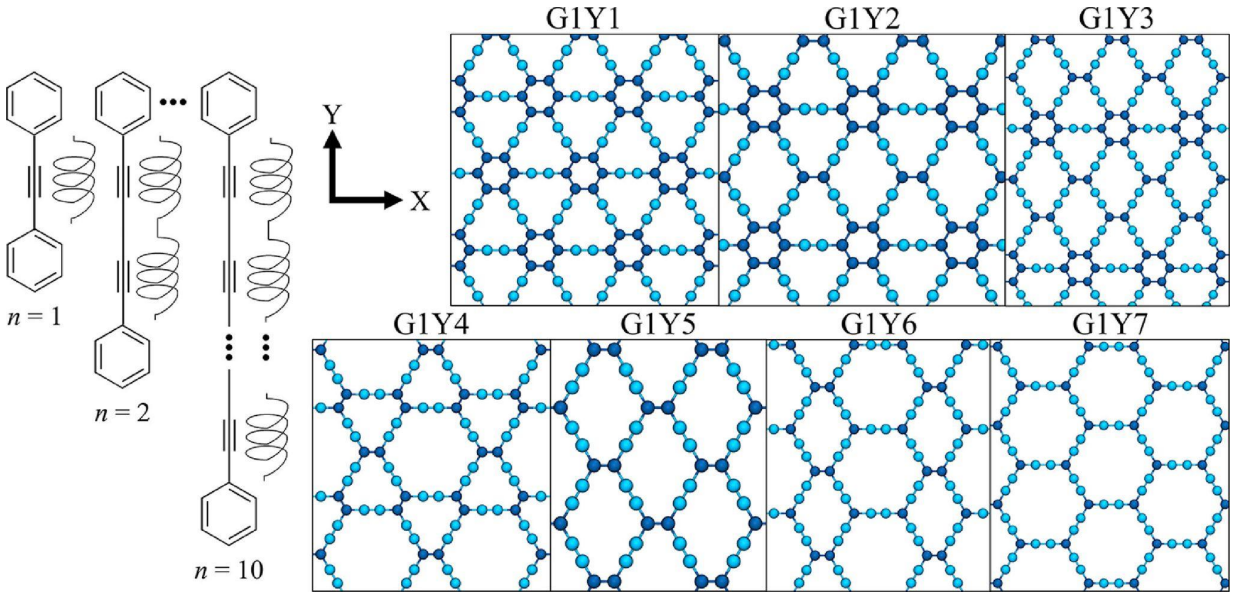

Fig. 1 - Geometric configurations and acetylene lengths. [2] Under a Creative Commons licence (CC BY-NC-ND 4.0).

In the context of graphyne nanotubes, the nomenclature "GyNT" is conventionally adopted. [9] This nomenclature practice is driven by extensive research predominantly centred around alpha, beta, and gamma graphynes, where the geometric descriptor typically assumes a prefix position. The representation of the acetylene chain within this nomenclature is written in an alphabetical format. For instance, the designation αGDyNT illustrates a graphyne nanotube characterised by alpha geometry (f=7) with two acetylene units composing the chain (n=2).

Another pivotal concept of graphyne geometry concerns the specific cut pattern leading to the formation of nanoribbons and nanosheets. When the division results in a zigzag pattern, it is denoted by the nomenclature 'ZNR,' whereas if it originates depressions and elevations resemble an armchair-like structure, the designation "ANR" is applied. [10]

3. Biomedical applications

The following section delves into select contemporary biomedical and biochemical applications. It is conceivable that further explorations have already been approached, with the possibility of additional investigations emerging in the future.

3.1 Cholesterol removal

A robust linkage is established between cholesterol and graphyne, with the strength of these bonds amplifying in tandem with the augmentation of acetylene linkages (n), once it incites an out-of-plane deformation. The presence of the cholesterol molecule within the cavities of graphyne enhances its propensity to aggregate similar molecules. An assessment of the effectiveness of alpha, beta, and gamma graphyne variants revealed a superior performance by gamma graphyne.[11]

3.2 Biosensing

A Density Functional Theory (DFT) study was performed, elucidating the propensity of both G1Y1 graphyne and its boron-substituted nanosheets to adsorb the nucleobases cytosine and guanine. The physisorption phenomenon originated from the influence of weak van der Waals forces, facilitating subsequent nucleobase desorption. The consequential adsorption-induced modifications in the energy of graphyne precipitate discernible alterations in the conductivity of the nanosheet. This discovery facilitates the development of techniques designed for cytosine and guanine detection.[8]

In addition, GDY (or G2YF) exhibited the capability to detect single and double-stranded DNA. This proficiency lends itself to the development of real-time fluorescent DNA biosensors, wherein DNA

is initially endowed with coloration, subsequently witnessing a shift in its wavelength frequency upon interaction. Such an attribute has been substantiated through both computational simulations and practical experimentation, where a measurable alteration in wavelength (with sensitivity as low as 25pM) was discerned. Furthermore, it is noteworthy that the formation of double-stranded DNA weakens the interactions between the single strands and graphdiyne, causing the single strands to detach from the material. In comparison with graphene, GDY nanosheets have demonstrated superior efficacy in this context.[5]

3.3 Drug delivery

Analogous to graphene, G1Y1 has exhibited notable efficacy in drug delivery. Employing charge distribution analysis (CDA) and density of states (DOS) analysis, Munir et al. substantiated the formation of a stable molecular complex between graphyne and Daunorubicin. The emergence of a dipole moment, in conjunction with the prevalent occurrence of weak interactions, guarantees mobility within biochemical systems.Consequently, this dynamic allows for the effortless detachment of the drug from the graphyne carrier at the specified target site.[3]

The nanosheet corresponding to G1Y1 has exhibited efficacy in the transport of pharmaceuticals, specifically sorafenib and regorafenib, which find application in cancer therapy. Following the protonation of these drugs, a tenuous hydrogen bonding interaction ensued among the molecules, thereby facilitating their targeted release to the desired cell.[6]

The G1Y1 nanosheet has also been forecasted to engage with quercetin and 5-fluorouracil via drug protonation, as evidenced by DOS analysis. This substantiates its capacity to effectively deliver these compounds. [7]

3.4 Membrane Insertion

In molecular dynamics simulations, the potential interactions of G1Y1 with cellular membranes have raised hypotheses regarding graphyne-linked cytotoxicity. Regardless, it demonstrated the capability to permeate a phosphatidylcholine membrane and effectively extract a substantial quantity of phospholipids, hinting at promising antibacterial applications. [12]

3.5 Lung cancer detection

According to DFT simulations (Generalised Gradient Approximation with additional DOS, PDOS, and PBE), gamma graphyne (G1Y1) has exhibited a noteworthy propensity for adsorbing aniline and ortho-toluidine, thereby instigating discernible perturbations in its electric dipole moment. This observed dissimilarity bears substantial potential for the detection of these constituents within the human respiratory system, leveraging the analysis of exhaled breath. However, it is noteworthy that the magnitude of this alteration was notably more pronounced in twin graphene, thereby designating it as a more suitable candidate for the proposed analytical methodology. The study also conducted simulations for styrene and benzene, but the energetic variation within graphyne was found to be negligible. [13]

3.6 Nanofilter for cigarette smoke

Acrolein, acrylamide, and nicotine are common chemicals in cigarettes, contributing to concerns of toxicity and carcinogenicity. A pioneering investigation introduces the utilization of graphyne nanotube γ-GNT within cigarettes to sequester these toxic compounds before they enter the human organism. Robust affinities were anticipated between tobacco smoke constituents and graphyne nanotubes, substantiating the viability of such chemisorption. The deployment of Density of States (DOS) maps corroborated the presence of the envisaged chemical binding, thereby augmenting the prospects for potential nanofiltration applications in the future. [14]

3.7 Radiation protection

Motivated by its distinctive molecular arrangement, characterised by a potent conjugated π-system and highly reactive diacetylene linkages, graphdiyne presents intriguing prospects as a radioprotective agent. In vitro investigations have unveiled that bovine serum albumin-modified graphdiyne nanoparticles efficiently quench free radicals, attenuate radiation-induced DNA harm within cellular structures, and enhance cell viability amidst ionizing radiation exposure. In vivo assessments have further attested that graphdiyne-BSA can safeguard murine bone marrow DNA against radiation-induced damage, thereby restoring superoxide dismutase and malondialdehyde levels, two pivotal indicators of radiation-induced injury, to their baseline levels. Furthermore, the biocompatibility profile and the lack of discernible systemic toxic responses to graphdiyne-BSA nanoparticles have been duly substantiated. [15]

A hydrogel composed of nanosized graphdiyne-loaded sodium hyaluronate was designed for radiation protection, in an effort to decrease skin damage caused by radiotherapy. The astonishing hydrogel is biologically safe and able to decrease ulcers and edema caused by X-ray contact. It also reduces the time of skin damage, promoting its recovery. [16]

3.8 Photothermal therapy

Employing a light-absorptive agent, photothermal therapy uses light irradiation to induce hyperthermia within the tumour region, thereby facilitating the infliction of irreversible harm upon tumour cells. The remarkable capacity of the photothermal agent to effectively transmute light irradiation into thermal energy, when subjected to low-power-density laser sources, mitigates the potential for collateral damage to adjacent healthy

tissues. Due to its substantial photothermal conversion efficacy, graphdiyne has been recently employed as both a photothermal agent and a contrast-enhancing component for photothermal therapy and in vivo tumour imaging. [4]

3.9 Antibacterial activity

It has been observed that the death of bacteria occurs when their membrane is exposed to wrapping, insertion, disruption, and oxidative stress caused by G2Y1 and G2Y1-O. Gram-positive bacteria are more susceptible to this than Gram-negative bacteria, and G2Y1 is more effective than G2Y1-O. However, both graphdiyne and its oxygen-substituted version showed mild toxicity towards human embryonic cells.[17]

3.10 Nanozyme

Nanozymes are nanomaterials that have enzyme-like properties and are considered to be one of the most promising enzyme substitutes. Due to its high specific surface area, abundant surface chemistry, and easily functionalized structure, GDY is a potential candidate for enzyme replacement. GDYO, which is obtained from the oxidation of GDY, was used as a peroxidase mimic. Hence, a colorimetric sensing platform was developed based on the peroxidase-like activity of GDYO to detect hydrogen peroxide. [18]

4. Negative biochemical effects

As the utilisation of nanomaterials advances in innovative biochemical applications, the possibility of encountering adverse consequences becomes evident.

4.1 Correlation with Alzheimer's disease

Experimental evidence suggests that Alzheimer's disease (AD) is closely related to β-amyloid (Aβ) accumulation. The Aβ peptide was spontaneously assembled in the G1Y1 surface as a monatomic adsorption layer, where the Aβ peptides performed sequential straightening, nucleation, and assembly. The direction of the peptide aggregation was strongly affected by the acetylene bonds of graphyne. The Aβ chains preferred to be arranged in the direction of the “recliner”, while the self-assembly extended in the direction of the “zigzag”. The aromatic rings of Aβ peptide in recliner arrangement interacted more with C≡C bonds, compared to the zigzag direction, enhancing the adsorption. [19]

4.2 Cytotoxicity

G2Yf oxide appears to exhibit enhanced biocompatibility and reduced susceptibility to cytotoxic effects when compared to graphene. Nonetheless, it retains the potential to serve as a viable ligand for binding biomolecules and aromatic compounds, facilitated by surface hydrophobic interactions and π-π arrangements between the aromatic domains and the unsaturated bonds. [20]

Additionally, the structure of Calmodulin is significantly disrupted by Graphyne (G1Y1) nanosheets, leading to the suppression of its Calcium-regulating function and a potential disruption of the calcium signal transduction pathway. [21]

5. Predominant methodologies

5.1 Density Functional Theory

The electronic density (ρ), within a system containing n electrons, signifies the number of electrons per unit volume at a specified point (r). Valence electrons typically occupy regions characterised by lower electron density, while those closer to the nucleus exhibit greater electron density. The fundamental objective of Density Functional Theory (DFT) simulations is to employ electronic density as the primary variable for elucidating attributes of multi-electron systems.[22] This approach enables the description and characterization of solids in terms of their constituent atoms.

The most prevalent Density Functional Theory (DFT) categories include Generalised Gradient Approximation (GGA), Local Density Approximation (LDA), and hybrid models. The LDA model decomposes the total energy into multiple components, each characterised by uniform electronic density and distinct values. The energies associated with these local elements are then summed. This approach performs admirably in scenarios featuring gradual charge density variations, such as covalent systems and simple metals. However, it yields substantial errors when dealing with van der Waals interactions, hydrogen bonds, metal oxides, adsorption energy calculations, or band gaps.

The GGA model addresses the system non-homogeneously, incorporating local and semi-local terms in the electronic density and its gradient. However, this model exhibits inaccuracies in electronic band structure calculations, despite its precision in the majority of systems.

Hybrids and derivatives of those previous categories enable a more rigorous mathematical framework, amenable to adaptation for diverse scenarios contingent upon bond types and material properties.

5.2 Density of states

The Density of States (DOS) represents the quantification of available quantum mechanical energy levels per unit of energy of the system. In this framework, a “state” means a permissible energy quantum level wherein an electron can reside within the specified system.[23] Partial Density of States (PDOS) follows the same principle, focusing on specific atomic orbitals.

5.3 Charge Decomposition Analysis

The methodology involves the fragmentation of molecular orbitals and entails an examination of the electronic interplay between constituent fragments X and Y. This examination includes an assessment of donation phenomena and the antagonistic polarisation interactions between them. Through the amalgamation of unoccupied orbitals from the fragments, this approach enables the determination of whether the linkage between them exhibits characteristics akin to a donor-acceptor bond.[24]

5.4 Molecular Dynamics

Molecular dynamics simulation, a computational approach, predicts the temporal evolution of each constituent atom within a molecular entity or protein, demonstrating the atomic interplay. These intricate simulations aptly encapsulate a diverse spectrum of pivotal biomolecular phenomena, including conformational alterations, ligand binding, and the intricate folds assumed by proteins. Some methods might offer insights into atomic coordinates at the astonishingly temporal precision of femtoseconds. Such techniques further enable anticipations concerning the atomic-level reactions of biomolecules when subjected to external perturbations, whether as a result of a ligand introduction or removal, protonation, phosphorylation, or mutational events. [25]

6. Frequency of appearance

As some configurations are popularised, others are still unusual within the field of biomedicine and biochemistry. **Tab. 1** conveys such heterogeneous distribution. The use of "f" implies the geometry was unclear or absent. The content of the table is limited to data within the cited scholarly literature.

Tab. 1 - Applications and their correlated graphynes.

Application	Graphyne	Methods	Ref.
Cholesterol removal	G1Y1, G1Y4, G1Y7	Molecular Dynamics	[11]
Nucleobase detection	G1Y1	DFT	[8]
DNA detection	G2YF	DFT, electron microscopy	[5]
Drug delivery	G1Y1	CDA, DFT DOS	[3,6,7]
Membrane insertion	G1Y1	MD	[12]
Lung cancer detection	G1Y1	DFT	[13]
Cigarette nano filter	γ-GNT	DOS	[14]
Radiation protection	G2YF-BSA, G2YF-SH	In vivo	[15,16]
Cholesterol removal	G1Y1, G1Y4, G1Y7	Molecular Dynamics	[11]
Nucleobase detection	G1Y1	DFT	[8]
DNA detection	G2YF	DFT, electron microscopy	[5]
Photothermal therapy	G2YF	In vivo	[4]
Antibacterial agent	G2Y1, G2Y1-O	Plate Counting	[17]
Nanozyme	G2Y1-O	Absorbance change analysis	[18]

7. Conclusion

It is worth noting that a substantial portion of biochemical research has predominantly focused on G1Y1 and G2Y1 graphyne, thus revealing an academic knowledge gap to be explored. This situation underscores the considerable prospects for future investigations, which could include a broader spectrum of graphyne variations. A plethora of unexplored biomedical applications remains fertile ground for scholarly inquiry, demanding studies to elucidate potential adverse factors such as cytotoxicity and unforeseen interactions within biological systems.

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9. References

[1] Kang J, Wei Z, Li J. Graphyne and Its Family: Recent Theoretical Advances. *ACS applied materials & interfaces.* 2019; 11(3):2692–706.

[2] Kanegae GB, Fonseca AF. Effective acetylene length dependence of the elastic properties of different kinds of graphynes. *Carbon Trends*. Campinas; 2022; 3 p.

[3] Munir I, Perveen M, Nazir S, Khera RA, Ayub AR, Ayub K, et al. Therapeutic potential of graphyne as a new drug-delivery system for daunorubicin to treat cancer: A DFT study. *Journal of Molecular Liquids.* 2021;336:116327.

[4] Liu JM, Chen C, Zhao Y. Progress and Prospects of Graphdiyne-Based Materials in Biomedical Applications. *Advanced Materials.* 2019;31(42):1804386–6.

[5] Parvin N, Jin Q, Wei Y, Yu R, Zheng B, Huang L, et al. Few-Layer Graphdiyne Nanosheets Applied for Multiplexed Real-Time DNA Detection. *Advanced Materials*. 2017 ;29(18):1606755–5.

[6] Srimathi U, Nagarajan V, Chandiramouli R. Investigation on graphdiyne nanosheet in adsorption of sorafenib and regorafenib drugs: A DFT approach. *Journal of Molecular Liquids.* 2019;277:776–85.

[7] Nagarajan V, Chandiramouli R. A study on quercetin and 5-fluorouracil drug interaction on graphyne nanosheets and solvent effects — A first-principles study. *Journal of Molecular Liquids.* 2019;275:713–22.

[8] Madhumitha S, Nagarajan V, Chandiramouli R. Adsorption behavior of cytosine and guanine nucleobases on graphyne nanosheets: A DFT study. *Computational and Theoretical Chemistry*. 2019; 1163:112514.

[9] Kang B, Lee JY. Electronic properties of α-graphyne nanotubes. *Carbon.* Suwon; 2015; 84:246–53.

[10] Ivanovskii AL. Graphynes and graphdyines. *Progress in Solid State Chemistry.* 2013; 41(1–2):1–19.

[11] Zhang L, Wang X. Mechanisms of graphyne-enabled cholesterol extraction from protein clusters. *RSC Advances*. 2015; (16):11776–85.

[12] Gu Z, Yang Z, Luan B, Zhou X, Hong L, Zhou H, et al. Membrane Insertion and Phospholipids Extraction by Graphyne Nanosheets. The *Journal of Physical Chemistry C.* 2017; 121(4):2444–50.

[13] Majidi R, Nadafan M. Detection of exhaled gas by γ-graphyne and twin-graphene for early diagnosis of lung cancer: A density functional theory study. *Physics Letters A* 2020; 384(1):126036.

[14] Nagarajan V, R. Chandiramouli. γ-Graphyne nanotube as nano filter for cigarette smoke based on chemisorption properties – A first-principles study. D*iamond and Related Materials.* 2019;97:107436–6.

[15] Xie J, Wang N, Dong X, Wang C, Du Z, Mei L, et al. Graphdiyne Nanoparticles with High Free Radical Scavenging Activity for Radiation Protection. *ACS Applied Materials & Interfaces*. 2018 Mar 6;11(3):2579–90.

[16] Xie J, Zhao M, Wang C, Zhu S, Niu W, Yuan Y, et al. External use of Nano-graphdiyne hydrogel for skin radioprotection via both physically shielding of Low-energy X-ray and chemically scavenging of Broad-spectrum free radicals. *Chemical Engineering Journal.* 2022; 430:132866–6.

[17] Zhu Z, Bai Q, Li S, Li S, Liu M, Du F, et al. Antibacterial Activity of Graphdiyne and Graphdiyne Oxide. *Advanced Materials*. 2020; 16(34):2001440–0.

[18] Ma W, Xue Y, Guo S, Jiang Y, Wu F, Yu P. Graphdiyne oxide: a new carbon nanozyme. Chemical Communications. 2020; 56(38):5115–8.

[19] Wang X, Hu Y, Li L, Ju R, Han X, Tan H, et al. Acetylenic Bond Layout Drives the Directional Assembly of Amyloid-like Peptides on Graphyne: Implications for Biomedical Safety Applications of Graphyne. *ACS applied nanomaterials.* 2023.

[20] Zheng T, Gao Y, Deng X, Liu H, Liu J, Liu R, et al. Comparisons between Graphene Oxide and Graphdiyne Oxide in Physicochemistry Biology and Cytotoxicity. *ACS Applied Materials & Interfaces*. 2018 ;10(39):32946–54.

[21] Feng M, Bell DR, Luo J, Zhou R. Impact of graphyne on structural and dynamical properties of calmodulin. *Physical Chemistry Chemical Physics.* 2017;19(15):10187–95.

[22] Kohn W, Sham LJ. Density functional theory. Conference Proceedings-Italian Physical Society 1996 (Vol. 49, pp. 561-572). Editrice Compositori.

[23] Nicholas. Introduction. Elsevier eBooks. 2003;1–53.

[24] Minkin V. Charge Decomposition Analysis (CDA). *IUPAC Standards Online.* 2016;

[25] Hollingsworth SA, Dror RO. Molecular Dynamics Simulation for All. *Neuron*. 2018; 99(6):1129–43.