|  |  |  |
| --- | --- | --- |
| Applicant:  Seketoulie Keretsu | Title: Discovery of small molecules and dinucleotide analogs as STING agonist for cancer immunotherapy | |
| **Key Personnel:** | **Organization:** | **Role Category:** |
| Seketoulie Keretsu | Chosun University | Prospective Post-Doctoral Candidate |
| Professor Dr. Andrea Bender | Cambridge University | Principle Investigator |

**Abstract**

Immune evasion via T cell exhaustion, secretion of immunosuppressive mediators and expression of proteins that modulate immune checkpoint, is a well-established hallmark for cancer ([1](#_ENREF_1)). T cell infiltration to the tumor microenvironment has been considered to be a prerequisite for an efficient response to immunotherapeutic treatment ([2](#_ENREF_2),[3](#_ENREF_3)). Hence, redirecting the immune response to the tumor has been considered an important therapeutic route for cancer treatment.

The enzyme cyclic-GMP-AMP synthase (cGAS) detects cytoplasmic self DNA or tumor-derived DNA and generates the secondary messenger cyclic Guanosine Monophosphate–Adenosine Monophosphate (cGAMP) ([4](#_ENREF_4)). cGAMP binds to the stimulator of interferon genes (STING) and activates a cascade of downstream signaling events responsible for activation and infiltration of T cells into the tumor microenvironment and promotes antitumor responses. Hence, STING agonists with the potential to induce immune response are considered promising drug candidates for cancer. To this end, several nucleotidic and non-nucleotidic STING agonists have been developed, some of which showed antitumor activity in animal models and are in the early stages of clinical trials ([2](#_ENREF_2),[5](#_ENREF_5),[6](#_ENREF_6)).

The objective of this proposal is to determine the mechanism of STING activation by it endogenous ligand cGAMP at the molecular level and to develop novel STING agonists using state-of-the-art computational drug design techniques such as molecular docking, pharmacophore modeling, virtual screening, classical molecular dynamics (MD) and binding energy calculation.

**Specific Aims**

**Aim 1: To determine the mechanism of activation of STING by the endogenous cyclic dinucleotide cGAMP.**

The cryo-electron microscopy (cryo-EM) structure of the apo state STING (human) and the cGAMP-STING (human) complex by Shang et al., (2019) will be used for the study ([7](#_ENREF_7),[8](#_ENREF_8)). All-atom classical MD simulation of the apo-STING and STING-cGAMP complex will be performed to study the dynamic interactions between the STING protein and cGAMP at the binding interface and to study the cGAMP induced conformational changes in STING ([9](#_ENREF_9)). This will be followed by Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) based binding free energy calculations of the STING-cGAMP complexes with the objective to study the binding affinities between STING and cGAMP and to identify residues important for binding with STING ([10](#_ENREF_10)).

**Aim 2: To develop novel nucleotidic STING agonists based on the structure of cGAMP**.

Taking the endogenous cyclic dinucleotide (CDN) cGAMP as an initial structure ([11](#_ENREF_11)), dinucleotide analogs of cGAMP will be designed to mimic the interactions of cGAMP with STING. The observations made in Aim 1 will be used in guiding the process of optimizing the ligands. Molecular docking will be performed to study the binding interactions. The binding affinity of the designed compounds will be tested iteratively using all-atom classical MD simulations and binding energy calculations ([9](#_ENREF_9)).

**Aim 3: To discover small molecules from publicly available databases with virtual screening.**

Based on the study from Aim 1, interactions that were significant for the activation of STING will be identified. Pharmacophore models will be developed to search for small molecules that can mimic the interactions of cGAMP with STING. This will be followed by molecular docking of the compounds with STING to evaluate the binding score. The compounds that showed high binding scores and interactions with STING will be further studied using classical MD simulations and free energy calculations ([9](#_ENREF_9)). The compounds collected from publicly available databases such as ZINC database ([12](#_ENREF_12)) or ChEMBL database ([13](#_ENREF_13)) will be used for the study. The compounds that has high binding affinity with binding site residues of STING will be selected as potential STING agonists. These compounds maybe experimentally studied to test its antitumor activity.

**Research Strategy**

**Significance**

Immunotherapy has emerged as a remarkable pharmaceutical approach to mitigate previously untreatable tumors and metastatic cancers. A major of this was achieved via the immune checkpoint blockade by triggering antitumor T cell response. However, a major drawback of the use of immune checkpoint blockade was in the case of non-immunogenic tumors in which cancer fails to elicit T cell response ([2](#_ENREF_2)). Earlier studies have shown that the infiltration of T cells into tumor microenvironment could be an important indicator of effective response to immunotherapic treatment ([3](#_ENREF_3)). Hence, efforts to invoke the infiltration of T-cell into the tumor microenvironment could be an effective approach to improve the antitumor activity.

**C:\Users\Keretsu\Documents\sting.tif**

**Figure 1. (a)**  The cGAS-STING-IRF3 immune signaling pathway (adapted from Ref. ([14](#_ENREF_14))) **(b)** The apo state of the STING homodimer from the cryo-EM (PDB code **6NT5**) is shown. The two monomers are shown in cyan and magenta color. The cytoplasmic domain of the cGAMP-STING complex (PDB code **4KSY**), shown in gray color, is aligned with the apo-STING structure.

The cGAS-STING-IRF3 pathway constitutes an important signaling pathway in the innate immune system, protecting the body against various pathogen invasions (Figure **1a**) ([14](#_ENREF_14)). The presence of microbial DNA or self DNA in the cytoplasm represents a danger signal and consequently triggers the innate immune response. The cytoplasmic DNA is detected by the enzyme cGAS which generates the secondary messenger cGAMP. The cGAMP binds to STING, inducing conformational changes in the STING cytoplasmic domain, which leads to its activation. This is followed by production of antiviral and pro-inflammatory cytokines such as type 1 interferons (INFs) leading to the infiltration of T-cells into the tumor micro-environment, invoking immune response. Hence, STING is considered an important target for antitumor drugs and vaccine adjuvants ([5](#_ENREF_5),[11](#_ENREF_11)).

Great strides have been made in understanding the structural and functional of aspects STING and components of the innate immune system. However, several aspects of the cGAS-STING-IRF3 signaling mechanism are yet to be uncovered, particularly in understanding how the cGAMP binding leads to activation of the STING. Several nucleotidic and non-nucleotidic STING agonists have been developed over the last few years, some of which have entered clinical trials. The 2’, 3’-cGAMP, which is the endogenous ligand of STING, and its isomers 3’, 3’-cGAMP, 3’, 2’-cGAMP, 2’, 2’-cGAMP were comprehensively studied and tested in animal models for pharmaceutical utility ([11](#_ENREF_11),[15](#_ENREF_15),[16](#_ENREF_16)). In addition, various nucleotidic analogs based on c-di-GMP ([17](#_ENREF_17),[18](#_ENREF_18)), c-di-AMP ([17](#_ENREF_17),[18](#_ENREF_18)), etc., were developed, some of which showed high potency for anti-tumor activity. However, the therapeutic utility of dinucleotide analogs has been limited as these compounds have low stability and were susceptible to hydrolysis by various nucleases and phosphodiesterase ([19](#_ENREF_19)). Hence, finding nucleotide-based STING agonists with high stability and resistance against hydrolysis poses a critical challenge for therapeutic intervention in cancer treatment.

The STING agonist 5,6-dimethylxanthenone-4-acetic acid (DMXAA) is a non-nucleotidic compound and showed potent anti-tumor activity in mouse models. However, DMXAA failed to clear clinical trial as it showed no potency for human STING activation ([20](#_ENREF_20)). Recently, Zhang et al. reported three small-molecule screened from the ZINC database as STING agonist ([21](#_ENREF_21)). These compounds showed potential STING activity and had antitumor activity in animal experiments. Amidobenzimidazole (ABZL) was reported by Ramanjulu et al., as STING agonist with anti-tumor activity in mouse ([22](#_ENREF_22)). However, activity data of the compound on human-related experiments were not reported. Though significant progress has been made in developing small molecule STING agonists, currently, there are no non-nucleotidic STING agonists in clinical trials.

**Innovation**

Computer-Aided Drug Design (CADD) methodologies have emerged as powerful tools in the arena of drug design and have been frequently used alongside experimental methods. The use of computational techniques could play a vital role in the discovery of STING agonists. To this end, Tsuchiya et al., (2016) studied the conformational changes at the c-terminal tail (CTT) of STING to understand the mechanism of activation of STING. However, the study was done in water medium without considering the lipid membrane environment of STING. In another study, Zhang at al., (2019) have performed docking based virtual screening of the ZINC database for STING agonists. The study was performed based on the static crystal structure of Amidobenzimidazole-STING complex and did not consider the pharmacophore information or the dynamic conformational changes of STING. With the literature regarding the structure and function of STING collected over the last decade and the availability of full-length cryo-EM structure of Apo-STING and cGAMP-STING, the computational study of STING presents a unique opportunity to target the cGAS-STING-IRF3 signaling pathway.

In this spirit, this project has been designed to study the binding interactions of STING and its endogenous ligand cGAMP and to design novel STING agonists. In this study, a comparative study of the apo-STING and cGAMP-STING will be performed using molecular dynamics simulation to identify the important residues for the activation of STING. Based on the structure of cGAMP, novel analogs will be designed by substituting the phosphodiester linkers in cGAMP to improve resistance against hydrolysis. Pharmacophore models will be developed based on the cGAMP-STING structure to screen publicly available databases such as ZINC ([12](#_ENREF_12)) and the Chemical database maintained by European Molecular Biology Laboratory (ChEMBL) ([13](#_ENREF_13)).

**Approach**

This section will sequentially give a detail description of the methods and procedures to be used to address the research problems.

**Aim 1: To understand the mechanism of STING activation and to identify the structural factors that are important for the activation.**

1a. **Preparation of protein structures.**

Recently, the cryo-EM structures of human apo state STING (PDB code **6NT5**) and the human cGAMP-STING (PDB code **4KSY**) were reported by Shang et al. (2019). The structures are shown in **Figure 1b**. These cryo-EM structures of the full-length apo state STING structure and cGAMP-STING structures will be used for computational studies.

The structure of the apo STING (PDB code **6NT5**) has several missing residues at the transmembrane region (GLY 49- SER 80, TYR 107- PRO 116) and at the cGAMP binding site (GLN 228- VAL 239 and GLU 249- ASN 250). These missing residues will be modeled by homology modeling methods with modeling tools such as Modeller or SWISS-MODEL ([23-25](#_ENREF_23)). The cGAMP-STING structure has a high resolution of 1.8 Å. However, the transmembrane regions of the protein were not included. This structure will be used as a template for modeling the binding site residues. The final model will be energy minimized to relax the structure with molecular dynamics simulations. Following this, the cGAMP will be placed at the binding site of STING by alignment of the modeled structure with the structure of cGAMP-STING in pymol.

1b. **Molecular Dynamics (MD) simulation of apo state STING.**

To study the global conformational changes and the conformational changes adopted by binding site residues, MD simulations of the STING in apo state will be performed ([9](#_ENREF_9)). The input parameters for the simulation will be generated using CHAMM-GUI and the MD production run and analyses will be done using the GROMACS package ([26](#_ENREF_26),[27](#_ENREF_27)). Visualization of the results will be done with VMD and pymol ([28-30](#_ENREF_28)).

STING is a transmembrane protein located in the endoplasmic reticulum (ER). The ER membrane mostly consists of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) lipids, with small amount of cholesterol and other lipids. Following best practices recommended for MD simulations in membrane environment, we will develop a mixed lipid bilayer comprising of palmitoyloleoylphosphatidylcholine (POPC) lipids, palmitoyloleoylphosphatidylethanolamine (POPE) lipids, and cholesterol in the ratio (4:4:1) ([31-33](#_ENREF_31)). Lipids present in small amounts will not be considered in the simulation as the lipid mixing is a computationally expensive task and can lead to artifacts in the simulation if not adequately simulated ( usually in terms of several microseconds).

The protein will be parameterized using the CHARMM22 force field which was derived specifically for all-atom protein simulations. The STING structure will be inserted into the lipid bilayer using CHARMM-GUI. The orientation of the protein with respect to the hydrocarbon core of the lipid bilayer will be done using Orientations of Proteins in Membranes (OPM) ([34](#_ENREF_34),[35](#_ENREF_35)). OPM is a web-based server commonly used to calculate the rotational and translational positions of transmembrane proteins in membrane. The output of OPM can be used alongside GHARMM-GUI to orient the protein-ligand in lipid membrane system. The system will be solvated with explicit solvent (TIP3P water model), followed by addition of NaCl ions to neutralize the charge of the system. Restrained constant Number of particles, Volume, and Temperature (NVT) ensembles and Number of particles, Pressure, and Temperature (NPT) ensembles will be performed to remove steric clashes between the water molecules and the protein-ligand system and to establish the system for production run at physiological condition of 300K temperature and 1 bar pressure. Finally, the production run of the system will be performed without restraining for 1μs. The choice to perform the simulation for 1μs was based on observations made in earlier studies of membrane simulations. Typically, membrane protein requires several microseconds to milliseconds time scale simulations for adequate sampling. However, unlike other membrane proteins such as G-protein coupled receptors (GPCRs) ([36](#_ENREF_36)), which are fully immersed in the lipid bilayer, the STING protein has a transmembrane domain and a cytoplasmic domain. The binding site of cGAMP was formed at the interface of the cytoplasmic domain of the two STING monomers. Earlier MD simulations of STING with the agonist cGAMP and cGMP in water medium have shown the equilibration of the system near 400 ns ([37](#_ENREF_37)). Hence, optimistically, a simulation time of 1 microsecond was chosen for the simulation in this study. It may be noted that the simulation time maybe extended based on visualization and based on the statistical analyses of the thermodynamic properties of the system.

1c. **MD simulation of the cGAMP-STING complex.**

The binding of the endogenous ligand 2’, 3’-cGAMP at the binding site of the STING dimer leads to conformational changes and its activation. Hence, to study the ligand-induced structural changes in STING, all-atom MD simulations of the 2’, 3’-cGAMP in complex with STING will be performed and the simulation results will be analyzed.

The CHARMM force fields CHRMM19 (united atom) and CHARMM22 (all-atom) were developed for parameterizing proteins and the CHARMM27 is developed for parameterizing DNA, RNA, and lipids. In addition, the CHARMM general force field (CGenFF) was developed for various drug-like molecules including large heterocyclic scaffolds ([38](#_ENREF_38)). These force fields and their combinations are available for parameterizing protein-ligand systems in GHARMM-GUI. Hence, the parameter for the cGAMP-STING complex in lipid membrane will be prepared using CHARMM-GUI. Here also, the procedure used in Aim 1b will be used to generate the parameters.

1d. **Binding free energy calculation**

Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) based binding free energy of cGAMP-STING will be calculated using the g\_mmpbsa program by Baker et al., (2001) and Kumari et al., (2014) ([10](#_ENREF_10),[39](#_ENREF_39)). The objective of binding energy calculation is to determine the binding affinity of cGAMP to STING and to elucidate the contribution of the energy terms such as electrostatic, hydrophobic, polar and non-polar solvation energy, etc., to the total binding energy. The role and significance of the individual residues to the total binding energy will also be determined by energy decomposition.

The binding energy consists of vacuum molecular mechanics (MM) potential energy of the bonded and non-bonded interactions, polar and non-polar solvation energy. Free energy for each individual entity (protein, ligand, protein-ligand complex) was represented by the general expression given below,

where is the protein or ligand or protein-ligand complex, is the average molecular mechanic potential energy in vacuum. and are the polar and non-polar solvation energies respectively.

The vacuum potential energy will be calculated based on molecular mechanics (MM) force-field parameters. Polar solvation energy will be calculated by solving the Poisson-Boltzmann (PB) equation. Non-polar solvation energy will be calculated based on the Solvent Accessible Surface Area (SASA) model. The energy components () will be collected from equilibrated regions of the MD production trajectory. Snapshots will be extracted from the trajectory every 0.5 ns. These steps are standard practice in molecular dynamics simulations of protein-ligand systems. Binding contribution of the individual protein residues to the total binding energy will be calculated using the MmPbSaDecomp.py program from the g\_mmpbsa package ([10](#_ENREF_10)).

**Aim 2: Developing hydrolysis resistant dinucleotide analogs with STING agonist activity**

C:\Users\Keretsu\Documents\strategy.tif

**Figure 2. (a)** The structure of the endogenous ligand (2’,3’-cGAMP) of STING **(b)** The strategy to be used to design the cyclic dinucleotide analogs by substitutions of the phosphodiester bonds in cGAMP with linker moieties. An example of linkers to be used as substitutions are shown in the table.

Natural cyclic dinucleotide (CDN) and their derivatives have demonstrated promising antitumor activity and immunostimulating effects both in vitro and in vivo. However, natural CDN and their derivatives are poor pharmaceutical drug candidates due to their instability and high polarity ([19](#_ENREF_19)). CDN based compounds consist of two nucleotides that share two phosphodiester bonds and the phosphorus atoms at the phosphodiester links/bonds were negatively charged and were susceptible to hydrolysis by enzymes such as phosphodiesterase. The endogenous second messenger compound 2’, 3’-cGAMP is a heterodimer linked by one 3’-5’-phosphodiester and one 2’-5’-phosphodiester and is the most potent STING agonist in human (**Figure 2a**). The CDN compound 2’, 3’-cGAMP has a high affinity for human STING with a dissociation constant of 4.59 nM ([11](#_ENREF_11)).

Taking advantage of the structure of cGAMP, various substitutions will be made for the phosphodiester bond with linker moieties as shown in **Figure 2b**.

2a. **Designing analogs**

The proposed designed compounds will be sketched and energy minimized using modeling tools such as SYBYL ([40](#_ENREF_40)), Open Eye or Discovery Studio ([41-43](#_ENREF_41)). This will be followed by molecular docking of the designed compounds into the binding site of STING. Molecular docking of the compounds in STING will be done using Autodock Vina program.

2b. **MD simulation of the designed compounds**

Based on the observations made in Aim 1c, the MD simulation of the designed compounds with STING will be performed to attain stable binding. The protein-ligand from the docking study will be used as the initial structure for the MD study. The parameterization of the proteins and ligands in the membrane environment will be performed iteratively for all protein-ligand complexes. The parameterization procedure used in Aim 1b and 1c will be used for the parameter preparation of the systems.

2c. **Binding energy calculation**

Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) based free energy calculations of the protein-ligand complexes will be performed to access the binding affinities of the designed compounds. The procedure given in Aim 1d will be used for the binding energy evaluations. Compounds showing strong binding interactions with STING will be selected as potential STING agonists.

**AIM 3: To perform pharmacophore-based virtual screening on publicly available databases for potential STING agonists.**

3a. **Pharmacophore model development:**

Pharmacophore models will be developed based on the interactions observed in cGAMP-STING (PDB code **4KSY**) to identify small molecules that can mimic the cGAMP-STING interactions. Identification of the pharmacophore and the development of the models can be done using GALAHAD in Tripos SYBYL. Alternatively, pharmacophore models can be developed using publicly available tools such as ZINCPhramer ([12](#_ENREF_12)).

3b. **Constructing the database of small molecules:**

Small molecules will be downloaded from databases like ZINC or ChEMBL. The compounds will be preprocessed to remove charged ions and compounds that do not possess drug-like properties. This can be done based on Lipinski’s rule of five using packages available in SYBYL, Open Eye or Discovery Studio. The 3-Dimensional conformations of the compounds will be generated using Concord package in SYBYL or other publicly available packages such as Avogadro ([44](#_ENREF_44)).

3c. **Screening compounds.**

Based on the pharmacophore model, a query will be initiated to search the database of small molecules. The hit compounds from the virtual screening will be further accessed by docking it into the binding site of STING and then scoring the binging interactions. The rigid receptor docking will be performed by in-house scripting that runs the Autodock Vina program for multiple dockings. The compounds that showed strong binding interactions will be selected as potential STING agonists.

4. **MD simulation and free energy calculation.**

The selected compounds from the virtual screening will be further studied using MD simulation. Binding affinities of the selected hit compounds will be evaluated using the procedure given in Aim 1c and Aim 1d respectively.

**Project Timeline:**

The project was designed to ensure that the objectives of the study can be completed within a tentative period of 2 years or less.

**References:**

1. Hanahan, D. and Weinberg, R.A. (2011) Hallmarks of cancer: the next generation. *cell*, **144**, 646-674.

2. Marloye, M., Lawler, S.E. and Berger, G. (2019). Future Science.

3. Gajewski, T.F., Schreiber, H. and Fu, Y.-X. (2013) Innate and adaptive immune cells in the tumor microenvironment. *Nature immunology*, **14**, 1014.

4. Wu, J., Sun, L., Chen, X., Du, F., Shi, H., Chen, C. and Chen, Z.J. (2013) Cyclic GMP-AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA. *Science*, **339**, 826-830.

5. Ablasser, A. (2019) Inflammation clues in STING protein structure. *Nature*, **567**.

6. Barber, G.N. (2015) STING: infection, inflammation and cancer. *Nature Reviews Immunology*, **15**, 760-770.

7. Shang, G., Zhang, C., Chen, Z.J., Bai, X.-c. and Zhang, X. (2019) Cryo-EM structures of STING reveal its mechanism of activation by cyclic GMP–AMP. *Nature*, **567**, 389.

8. Zhang, C., Shang, G., Gui, X., Zhang, X., Bai, X.-c. and Chen, Z.J. (2019) Structural basis of STING binding with and phosphorylation by TBK1. *Nature*, **567**, 394.

9. Pronk, S., Páll, S., Schulz, R., Larsson, P., Bjelkmar, P., Apostolov, R., Shirts, M.R., Smith, J.C., Kasson, P.M. and Van Der Spoel, D. (2013) GROMACS 4.5: a high-throughput and highly parallel open source molecular simulation toolkit. *Bioinformatics*, **29**, 845-854.

10. Kumari, R., Kumar, R., Consortium, O.S.D.D. and Lynn, A. (2014) g\_mmpbsa A GROMACS tool for high-throughput MM-PBSA calculations. *Journal of chemical information and modeling*, **54**, 1951-1962.

11. Shi, H., Wu, J., Chen, Z.J. and Chen, C. (2015) Molecular basis for the specific recognition of the metazoan cyclic GMP-AMP by the innate immune adaptor protein STING. *Proceedings of the National Academy of Sciences*, **112**, 8947-8952.

12. Koes, D.R. and Camacho, C.J. (2012) ZINCPharmer: pharmacophore search of the ZINC database. *Nucleic acids research*, **40**, W409-W414.

13. Gaulton, A., Bellis, L.J., Bento, A.P., Chambers, J., Davies, M., Hersey, A., Light, Y., McGlinchey, S., Michalovich, D. and Al-Lazikani, B. (2011) ChEMBL: a large-scale bioactivity database for drug discovery. *Nucleic acids research*, **40**, D1100-D1107.

14. Ablasser, A. and Gulen, M.F. (2016) The role of cGAS in innate immunity and beyond. *Journal of molecular medicine*, **94**, 1085-1093.

15. Zhang, X., Shi, H., Wu, J., Zhang, X., Sun, L., Chen, C. and Chen, Z.J. (2013) Cyclic GMP-AMP containing mixed phosphodiester linkages is an endogenous high-affinity ligand for STING. *Molecular cell*, **51**, 226-235.

16. Gao, P., Ascano, M., Wu, Y., Barchet, W., Gaffney, B.L., Zillinger, T., Serganov, A.A., Liu, Y., Jones, R.A. and Hartmann, G. (2013) Cyclic [G (2′, 5′) pA (3′, 5′) p] is the metazoan second messenger produced by DNA-activated cyclic GMP-AMP synthase. *Cell*, **153**, 1094-1107.

17. Jin, L., Hill, K.K., Filak, H., Mogan, J., Knowles, H., Zhang, B., Perraud, A.-L., Cambier, J.C. and Lenz, L.L. (2011) MPYS is required for IFN response factor 3 activation and type I IFN production in the response of cultured phagocytes to bacterial second messengers cyclic-di-AMP and cyclic-di-GMP. *The Journal of Immunology*, **187**, 2595-2601.

18. Liu, P., Sharon, A. and Chu, C.K. (2008) Fluorinated nucleosides: synthesis and biological implication. *Journal of fluorine chemistry*, **129**, 743-766.

19. Li, L., Yin, Q., Kuss, P., Maliga, Z., Millán, J.L., Wu, H. and Mitchison, T.J. (2014) Hydrolysis of 2′ 3′-cGAMP by ENPP1 and design of nonhydrolyzable analogs. *Nature chemical biology*, **10**, 1043.

20. Bibby, M., Phillips, R.M., Double, J. and Pratesi, G. (1991) Anti-tumour activity of flavone acetic acid (NSC 347512) in mice–influence of immune status. *British journal of cancer*, **63**, 57.

21. Zhong, S., Li, W., Bai, Y., Wu, B., Wang, X., Jiang, S., Zhao, Y., Ren, J., Li, H. and Jin, R. (2019) Computational study on new natural compound agonists of stimulator of interferon genes (STING). *PloS one*, **14**, e0216678.

22. Ramanjulu, J.M., Pesiridis, G.S., Yang, J., Concha, N., Singhaus, R., Zhang, S.-Y., Tran, J.-L., Moore, P., Lehmann, S. and Eberl, H.C. (2018) Design of amidobenzimidazole STING receptor agonists with systemic activity. *Nature*, **564**, 439.

23. Eswar, N., Eramian, D., Webb, B., Shen, M.-Y. and Sali, A. (2008), *Structural proteomics*. Springer, pp. 145-159.

24. Guex, N. and Peitsch, M.C. (1997) SWISS‐MODEL and the Swiss‐Pdb Viewer: an environment for comparative protein modeling. *electrophoresis*, **18**, 2714-2723.

25. Schwede, T., Kopp, J., Guex, N. and Peitsch, M.C. (2003) SWISS-MODEL: an automated protein homology-modeling server. *Nucleic acids research*, **31**, 3381-3385.

26. Brooks, B.R., Brooks III, C.L., Mackerell Jr, A.D., Nilsson, L., Petrella, R.J., Roux, B., Won, Y., Archontis, G., Bartels, C. and Boresch, S. (2009) CHARMM: the biomolecular simulation program. *Journal of computational chemistry*, **30**, 1545-1614.

27. Jo, S., Kim, T., Iyer, V.G. and Im, W. (2008) CHARMM‐GUI: a web‐based graphical user interface for CHARMM. *Journal of computational chemistry*, **29**, 1859-1865.

28. Humphrey, W., Dalke, A. and Schulten, K. (1996) VMD: visual molecular dynamics. *Journal of molecular graphics*, **14**, 33-38.

29. Hsin, J., Arkhipov, A., Yin, Y., Stone, J.E. and Schulten, K. (2008) Using VMD: an introductory tutorial. *Current protocols in bioinformatics*, **24**, 5.7. 1-5.7. 48.

30. DeLano, W.L. (2002).

31. Yeagle, P.L. (2016) *The membranes of cells*. Academic Press.

32. Smith, D.J., Klauda, J.B. and Sodt, A.J. (2018) Simulation Best Practices for Lipid Membranes [Article v1. 0]. *Living Journal of Computational Molecular Science*, **1**, 5966.

33. Feller, S.E. (2000) Molecular dynamics simulations of lipid bilayers. *Current opinion in colloid & interface science*, **5**, 217-223.

34. Lomize, M.A., Lomize, A.L., Pogozheva, I.D. and Mosberg, H.I. (2006) OPM: orientations of proteins in membranes database. *Bioinformatics*, **22**, 623-625.

35. Lomize, M.A., Pogozheva, I.D., Joo, H., Mosberg, H.I. and Lomize, A.L. (2011) OPM database and PPM web server: resources for positioning of proteins in membranes. *Nucleic acids research*, **40**, D370-D376.

36. Miao, Y. and McCammon, J.A. (2018) Mechanism of the G-protein mimetic nanobody binding to a muscarinic G-protein-coupled receptor. *Proceedings of the National Academy of Sciences*, **115**, 3036-3041.

37. Tsuchiya, Y., Jounai, N., Takeshita, F., Ishii, K.J. and Mizuguchi, K. (2016) Ligand-induced ordering of the C-terminal tail primes STING for phosphorylation by TBK1. *EBioMedicine*, **9**, 87-96.

38. Vanommeslaeghe, K. and MacKerell Jr, A.D. (2012) Automation of the CHARMM General Force Field (CGenFF) I: bond perception and atom typing. *Journal of chemical information and modeling*, **52**, 3144-3154.

39. Baker, N.A., Sept, D., Joseph, S., Holst, M.J. and McCammon, J.A. (2001) Electrostatics of nanosystems: application to microtubules and the ribosome. *Proceedings of the National Academy of Sciences*, **98**, 10037-10041.

40. Ash, S., Cline, M.A., Homer, R.W., Hurst, T. and Smith, G.B. (1997) SYBYL line notation (SLN): A versatile language for chemical structure representation. *Journal of chemical information and computer sciences*, **37**, 71-79.

41. Bolton, E.E., Chen, J., Kim, S., Han, L., He, S., Shi, W., Simonyan, V., Sun, Y., Thiessen, P.A. and Wang, J. (2011) PubChem3D: a new resource for scientists. *Journal of cheminformatics*, **3**, 32.

42. Toolkit, O. (2013) OpenEye Scientific Software. *Santa Fe, NM*.

43. Biovia, D.S. (2017). Release.

44. Hanwell, M.D., Curtis, D.E., Lonie, D.C., Vandermeersch, T., Zurek, E. and Hutchison, G.R. (2012) Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. *Journal of cheminformatics*, **4**, 17.