Biophysical Chemistry 319 (2025) 107392

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: www.elsevier.com/locate/biophyschem

SPR is a fast and straightforward method to estimate the binding constants of cyclic dinucleotides to their binding partners, such as STING or poxin

Hagen Sülzen, Martin Klima, Vojtech Duchoslav, Evzen Boura *

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v.v.i, Flemingovo nám. 2, 166 10 Prague 6, Czech Republic

ARTICLE INFO

Keywords:
SPR
ITC
STING
Poxin
Cyclic dinucleotide

ABSTRACT

The development of small molecule drugs that target protein binders is the central goal in medicinal chemistry. During the lead compound development process, hundreds or even thousands of compounds are synthesized, with the primary focus on their binding affinity to protein targets. Typically, IC_{50} or EC_{50} values are used to rank these compounds. While thermodynamic values, such as the dissociation constant (KD), would be more informative, they are experimentally less accessible. In this study, we compare isothermal calorimetry (ITC) with surface plasmon resonance (SPR) using human STING, a key protein of innate immunity, and several cyclic dinucleotides (CDNs) that serve as its ligands. We demonstrate that SPR, with recent technological advancements, provides KDs that are sufficiently accurate for drug development purposes. To illustrate the versatility of our approach, we also used SPR to estimate the KD of poxin binding to cyclic GMP-AMP (cGAMP) that serves as a second messenger in the innate immune system. In conclusion, SPR offers a high benefit-to-cost ratio, making it an effective tool in the drug design process.

1. Introduction

Development of small molecules that are protein binders and can be used as drugs in human medicine is the primary goal of the medicinal chemistry field. During the development of a lead compound, often hundreds or even thousands of compounds are synthesized and have to be individually characterized. All properties, such as stability, solubility, bioavailability and toxicity, are important. Yet, (almost) always, the first property measured of any new compound is its ability to bind to its target protein. Therefore, drug discovery usually begins with a library screen based on a single parameter (e.g., binding or inhibition above a certain threshold). This can be done experimentally, virtually, or increasingly through a combination of both methods [1,2]. Later IC_{50} or EC_{50} values are obtained and used as a scoring function to identify compounds for further characterization. IC_{50} or EC_{50} values are used mostly because they are relatively easier to obtain than dissociation constants (KDs) and often suffice to rank the compounds. In addition, both in vitro reactions and cell-based assays are now commonly performed in a high-throughput format. However, neither IC_{50} nor EC_{50} are thermodynamic quantities and their values depend on the specific conditions of each assay where they are determined. Consequently, more direct methods are often preferred. A simple, time-saving method is the determination of the melting point (T_m), which, although not entirely precise, is based on the principle that when a protein binds a strong ligand, its T_m typically increases (though exceptions do exist). Of course, ideally, one would aim to obtain a thermodynamic quantity, such as the KD of the protein-ligand complex, to serve as the most effective scoring function in facilitating medicinal chemistry efforts.

However, obtaining KDs might be experimentally demanding. Recently, we and others aimed to develop agonists of STING (stimulator of interferon genes) [3–7] which is a crucial molecule of innate immunity [8,9]. STING is a transmembrane protein and a key member of the cGAS-STING pathway, responsible for detecting cytosolic DNA, which is absent in healthy eukaryotic cells. Consequently, STING plays a crucial role in both cancer and antiviral responses [10]. It is not surprising that the cGAS-STING pathway was also identified as a key contributor to abnormal type I interferon responses in COVID-19 [11].

The natural STING ligands are cyclic dinucleotides (CDNs), primarily the canonical cyclic 2′-3′-GMP-AMP (2′3′-cGAMP), which is found in metazoans and is produced by the cyclic GMP-AMP synthase (cGAS) [12]. STING also binds other naturally occurring CDNs bearing two of the primary purine nucleobases (guanine, adenine, hypoxanthine). These mostly bacterial CDNs can be linked via phosphate linkage in three different ways (2′,2′-, 2′,3′-, and 3′,3′-CDNs). Besides these natural

* Corresponding author.
E-mail address: boura@uochb.cas.cz (E. Boura).

https://doi.org/10.1016/j.bpc.2025.107392
Received 22 November 2024; Accepted 19 January 2025
Available online 20 January 2025
0301-4622/© 2025 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

phosphate-linked CDNs, many artificial compounds with different linkers have been prepared linked with phosphorothioate-, phosphoroamidate-, amide-, urea-, 1,2,3-triazole/, or N-acylsulfonamide moieties [13–17]. The ribose moiety is also tolerant to alteration, such as the carba-modification, isonucleoside-modification, removal of the hydroxyl group(s) or their replacement with fluorine [4,5,7,18,19]. Many of these changes may lead to a higher affinity of the modified CDNs to STING [20–22].

Previously, we used ITC (isothermal calorimetry) to determine the precise values of KDs of CDNs to STING [19]. ITC is considered as the 'gold standard' for measuring KDs, as it allows both the protein and ligand to be unlabeled and in solution, closely resembling biological conditions [23,24]. However, experiments are usually very time consuming as the technique necessitates large quantities of protein that must be recombinantly produced and purified [19]. In this study, we compare binding affinities obtained by ITC to a quick alternative, surface plasmon resonance (SPR). SPR was used to measure binding of small molecules already 20 years ago [25]. However, here we utilize the recent advancement in SPR technology that greatly improved signal to noise ratio in SPR measurements and allows to quickly and reliably measure binding of small molecules to relatively large proteins [26,27]. Unfortunately, it is still impossible to measure very fast kinetics, unless some special method such as rapid sample switching is used [28].

By comparison to KDs previously determined by ITC, we find that in this case the SPR derived KDs are sufficiently precise for most applications.

2. Materials and methods

2.1. Protein expression and labeling

The ligand binding domain of human STING and the Mpox poxin were expressed and purified as described previously [29,30]. Both proteins were biotinylated using the EZ-Link NHS-PEG4-Biotinylation kit (Thermo Scientific) according to the manufacturer's instructions. Briefly, the purified protein was transferred to phosphate-buffered saline (PBS, pH 7.4) using size exclusion chromatography on a pre-equilibrated Superdex75 Increase 10/300 GL column (Cytiva). After mixing the buffer-exchanged protein with a 20 mM solution of NHS-PEG4-Biotin to achieve a final molar ratio of protein to biotin of 1:3, the reaction was incubated for 2 h on ice before quenching with 100 mM hydroxylamine (pH 8.5) and incubation for another 1 h on ice. The STING-biotin conjugates were separated from excess biotin using size exclusion chromatography on a Superdex75 Increase 10/300 GL column (Cytiva), pre-equilibrated with PBS. The purified, biotinylated proteins were concentrated to 2 mg/ml, aliquoted and stored at −80 °C until further use.

2.2. SPR measurements

All SPR measurements were performed at 25 °C using a Series S sensor chip CAP (Cytiva) on a Biacore 1S+ system (Cytiva). All binding analyses and dilution series were performed in SPR running buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 3 mM EDTA, 0.005 % (*v*/v) TWEEN-20). Flow paths 3 and 4 were saturated with undiluted CAPture reagent before coupling the biotinylated ligands to flow path 4 at 2 μl min^{-1} for 240 s. Subsequently, a continuous flow of buffer was applied until a stable baseline was reached. Poxin was immobilized to 1695 RU whereas STING was immobilized to a final level of 1050 RU (F2cGAMP, cGAMP), 798 RU (cdiGMP) and 786 RU (F2cdiGMP), respectively.

All compounds were purchased from InvivoGen and their dilution series were applied to flow paths 3 and 4 at 30 μl min^{-1} for 60 s (poxin) or 120 s (STING) with sufficient time in between injections for the analyte to completely dissociate (300–600 s). The binding data was double reference subtracted using the Biacore Insight evaluation software (Cytiva). First, the response observed on the blank reference surface (flow path 3) caused by analyte injection was subtracted from the binding response of the analyte to the ligand on the active flow cell (flow path 4) to account for unspecific binding of the analyte to the sensor chip surface. Second, a buffer blank injection response, previously corrected by 4–3 subtraction, was subtracted from each analyte injection response to mitigate bulk refractive index changes and injection artifacts across all analyte concentrations.

Steady state affinity was subsequently evaluated using the Biacore Insight evaluation software (Cytiva). Following double reference subtraction, the steady state response (R_{eq}) (median response calculated for a 5 s window, 5 s before the end of the injection) for each analyte concentration is plotted against the respective analyte concentration. Based on the curvature of the plot, the Evaluation Software estimates the maximum response (R_{max}) that can be achieved by occupying all analyte binding sites of the ligand, and subsequently derives the estimated KD. The resulting plots were exported and redrawn in Graphpad Prism 10 for enhanced clarity.

3. Results

3.1. Binding affinities as determined by SPR

To determine whether surface plasmon resonance may serve as a timesaving and cost-effective alternative to ITC for characterization of potential drug candidates targeting STING, we assessed the binding affinities of four, well characterized cyclic dinucleotides to STING, immobilized via biotin capture on a CAP chip (Fig. 1).

We performed all the experiments on the latest generation of the Biacore 1S+ SPR system with a 40 Hz data collection frequency, however, we were still unable to reliably determine the binding kinetics due to the high association/dissociation rates of the CDNs tested (Fig. 1). Consequently, all binding affinities were determined via steady state affinity. In good agreement with prior studies characterizing STING interactions via ITC [19], significant differences in the binding affinity of the analytes were observed.

To determine the KD for the vertebrate cGAMP (2′3′-cGAMP), we used a 2-fold dilution series of the analyte, with concentrations spanning from 80 μM to 156 nM. A good fit to the experimental data was obtained, as illustrated by the low χ^2 value (Fig. 1, Table 1) and the KD was estimated to be 7.66 ± 3.7 μM, the lowest binding affinity for STING among the CDNs analyzed.

When performing the experiment with an identical dilution series of the bacterial 3′-3′-cyclic-di-GMP (cdiGMP), a slight increase in binding affinity over the vertebrate CDN was observed (KD = 5.45 ± 2.6 μM) (Fig. 1). A χ^2 of 8.44 × 10^{-3} indicates a reliable fit of the measured binding data (Table 1).

A much greater improvement in binding affinity to STING was observed for F2cdiGMP, a cdiGMP analogue with fluorinations in the 2′ position of both ribose rings. Using a 4-fold dilution series ranging from 10 μM to 39 nM, the binding affinity was determined to be 916 ± 68 nM, representing a 5- to 7-fold increase in affinity when compared to the two previous CDNs (Fig. 1). Despite using a 4-fold dilution series, thereby effectively reducing the number of analyte concentrations by half, the resulting χ^2 of 3.63 × 10^{-3} indicates that the quality of the fit was not impaired (Table 1).

The lowest KD among the CDNs tested here was observed for the fluorinated cGAMP analogue, F2cGAMP. Fluorination in the 2′ position of both ribose rings increased the binding affinity over the naturally occurring cGAMP by more than one order of magnitude, resulting in a KD of 177 ± 66 nM (Fig. 1). While the use of a 4-fold dilution series, spanning analyte concentrations from 5 μM to 4.9 nM, resulted in a still reasonable fit of the steady-state model (χ^2 of 7.27 × 10^{-2}, Table 1).

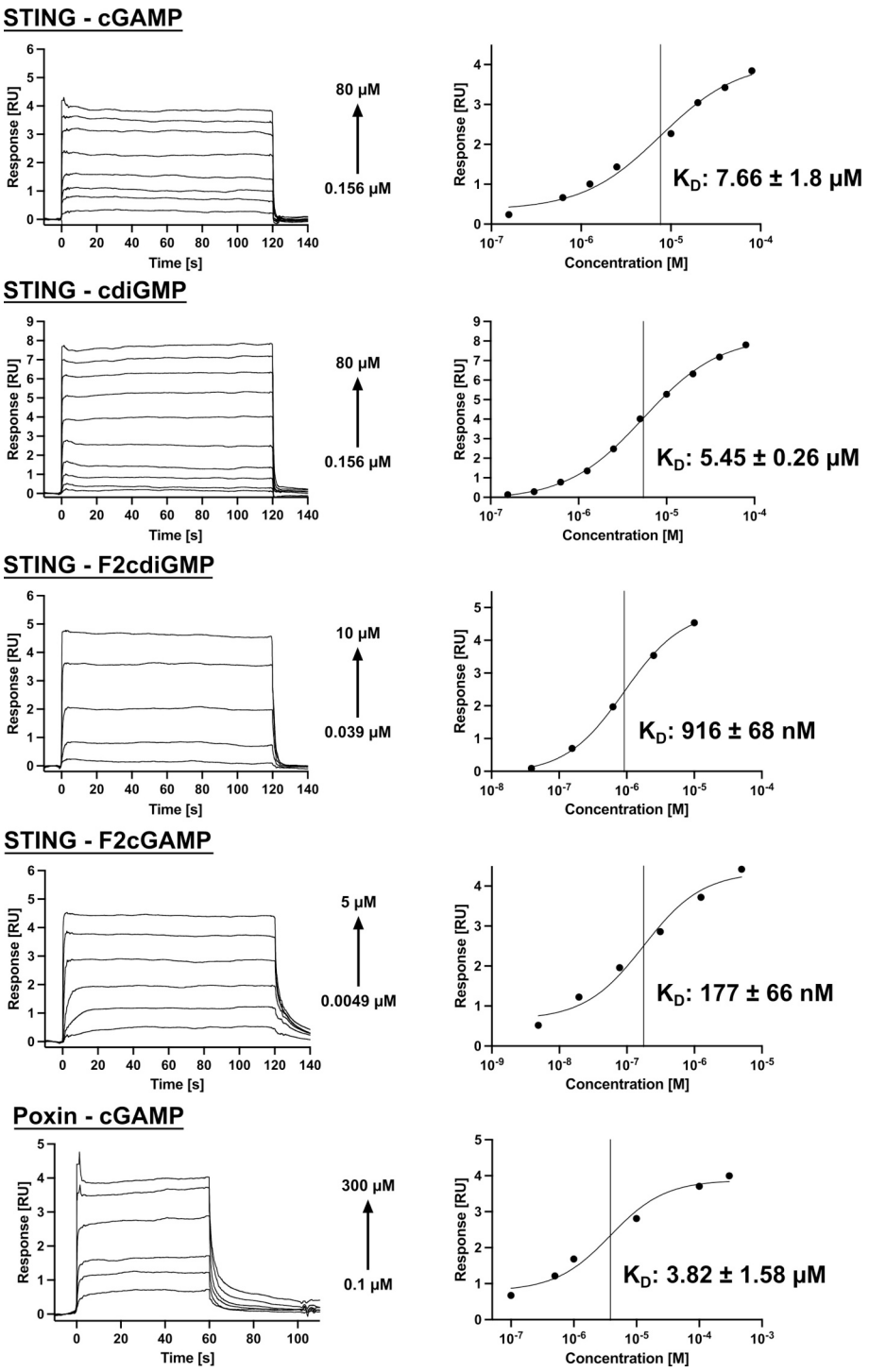

Fig. 1. Surface plasmon resonance allows for comparative determination of binding affinities of various cyclic dinucleotides to STING and poxin. Reference subtracted sensorgrams for all ligand:analyte interactions are depicted alongside the analyte concentration range used (left). Req vs analyte concentration plots and the binding affinities (± standard error (SE)) derived therefrom, as determined using the Biacore Insight Evaluation software (Cytiva), are shown (right).

Table 1
Binding affinities of STING and poxin to various cyclic dinucleotides as determined by surface plasmon resonance. All interaction parameters were determined using the Biacore Insight Evaluation software.

Analyte	Ligand	Immobilization level (RU)	Included concentrations [µM]	KD [µM]	SE_{KD} [µM]	R_{max} [RU]	Affinity $\chi 2$ [RU^2]
cGAMP	Poxin	1695	0.1–300	3.82	1.58	3.1	6.07×10^{-2}
	STING	1050	0.156–80	7.66	1.8	3.7	2.60×10^{-2}
cdiGMP	STING	789	0.156–80	5.45	2.6	8.4	8.44×10^{-3}
F2cdiGMP	STING	786	0.039–10	0.916	0.068	5	3.63×10^{-3}
F2cGAMP	STING	1050	0.0049–5	0.177	0.066	3.7	7.27×10^{-2}

3.2. The binding affinity of cGAMP to the poxin nuclease as determined by SPR

To determine whether SPR can be used for other CDN-binding proteins, we used it to measure the binding of cGAMP to Mpox poxin [30], an unusual nuclease that certain viruses use to evade the cGAS-STING pathway [31,32]. We performed the experiment in the same manner; poxin was biotinylated and immobilized as STING. We used the analyte in the concentration range from 0.1 μM to 300 μM and we observed a KD of 3.82 μM with a reasonable χ^2 of 6.07 × 10^{-2}, implicating that the binding affinity is in the same range as observed for STING binding to CDNs (Fig. 1). However, it is important to point out that this observed KD is actually virtual. It represents a combination of KDs for cGAMP (with the K_{on} for cGAMP) and the cleavage product Gp[2′–5′]Ap[3′] (with the K_{off} being a combination of cGAMP and its cleavage product, Gp[2′–5′]Ap[3′]). It is presented solely to illustrate that our approach can be extended beyond STING. We do not claim any accuracy for its value, although it is most likely correct, at least in terms of the order of magnitude.

3.3. Comparison to ITC

In a previous study, we determined the precise binding affinity of the same four cyclic dinucleotides to STING via ITC [19]. Similarly, to the data presented here, the fluorinated analogues F2cGAMP and F2cdiGMP exhibited the highest affinity to STING, with KDs of 25 ± 14 nM and 370 ± 50 nM, respectively. The KD of the naturally occurring cGAMP was previously determined to be 1.9 μM whereas cdiGMP exhibited the weakest binding affinity of 2.5 μM (all values are compared in Table 2).

4. Discussion

Overall, the binding affinities determined by SPR align well with the observations made using ITC. The SPR measurements lead to the correct identification of the two strongest binders, and the KD values of the two weaker binders were of the same magnitude as the values derived from ITC, although their ranking in terms of binding affinity was reversed but within the experimental error (Table 2).

The most likely cause of the observed differences in the absolute value of the KD between SPR and ITC measurements is the inaccurate determination of the maximum response (R_{max}). During the evaluation of the steady state affinity using the Biacore Insight Evaluation software, the default fitting process begins with initial values for the parameters in the equation of the model which are subsequently automatically refined to minimize the sum of the squared residuals. By default, the initial value for R_{max} is the maximum response observed during injection of the analyte. However, in cases where the steady-state response (R_{eq}) vs. analyte concentration plot does not adequately approach the curve's asymptote (representing the maximum response, R_{max}), the observed curvature of the plot becomes critical. With sufficient curvature, the asymptote can still be reliably extrapolated, even with fewer data points. Conversely, if the curvature of the plot is insufficient, this extrapolation becomes prone to error. Consequently, the refined R_{max} value may deviate significantly from the maximum response observed during the experiment, as exemplified by the interaction between STING and F2cGAMP (Fig. 1, Table 1). These inaccuracies are not necessarily reflected by the standard error (SE) of the R_{max}, as the SE_{Rmax} is below 10 % of R_{max} for all CDNs analyzed and thus in an acceptable range (Table 1).

While full saturation of the immobilized ligand typically requires analyte concentrations above 100-fold of the KD, it should be noted that the highest concentration used for all analytes tested here exceeds the estimated KD by at least 10-fold (Fig. 1), a concentration typically regarded as sufficient to reliably approximate the maximum response [33]. As evident by the insufficient curvature of the R_{eq} vs analyte concentration plot, this was not the case for the CDNs investigated here.

Table 2
Binding affinities of STING to various cyclic dinucleotides determined by surface plasmon resonance compared to previously derived ITC values.

Ligand	KD by SPR [μM]	KD by ITC [μM]
cGAMP	7.66 ± 1.8	1.90 ± 0.40
cdiGMP	5.45 ± 2.6	2.50 ± 0.60
F2cdiGMP	0.916 ± 0.068	0.37 ± 0.05
F2cGAMP	0.177 0.066	0.025 ± 0.014

It is noteworthy that the four cyclic dinucleotides tested exhibited large variations in their ability to fully saturate the immobilized ligand. While the highest concentrations of cGAMP and F2cGAMP elicited only 11 % of the theoretical R_{max}, injection of F2cdiGMP and cdiGMP resulted in 20 and 35 %, respectively.

When assessing the binding affinity via steady state analysis, the R_{eq} at an analyte concentration equal to the KD should be less than half of the maximum response (R_{max}) for the data to be considered reliable. As a consequence of the aforementioned inaccuracy in the estimation of R_{max}, this was only the case for the determination of binding affinity of cdiGMP and F2cdiGMP to STING (Table 1). Noticeably, the KD values of these analytes, as measured by SPR, most closely resemble the affinities determined by ITC (Table 2).

5. Conclusion

From the data presented here, we conclude that SPR can serve as an effective alternative to ITC to rapidly determine binding affinities across a broad range of potential drug candidates. This is illustrated by the accurate identification of the two strongest binders among the four cyclic dinucleotides tested, as well as the correct assessment of magnitude of binding affinity for the remaining analytes.

While all experiments presented in this study were performed with the same, basic experimental setup and analyzed with default settings during the evaluation of the binding affinity, the reliable determination of relative binding affinities among drug candidates with KD values in the same order of magnitude requires a more accurate estimation of R_{max}. While this could theoretically be achieved by manually estimating the maximum response using a well-characterized control analyte with high affinity, most medicinal chemistry projects do not require such a high level of accuracy. Instead, the focus is on achieving the highest possible price-to-performance ratio, which the SPR method in our setup fulfills.

Consequently, SPR may serve as a good technique to quickly select strong binders in a large pool of novel drug candidates, while complementary, more accurate methods like ITC will be necessary if exact KD values are required.

CRediT authorship contribution statement

Hagen Sülzen: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Martin Klima:** Formal analysis, Investigation. **Vojtech Duchoslav:** Investigation. **Evzen Boura:** Conceptualization, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare no competing interests.

Acknowledgment

This research was funded by the project the National Institute Virology and Bacteriology (Programme EXCELES, Project No. LX22NPO5103) - Funded by the European Union - Next Generation EU. RVO: 61388963 is also acknowledged.

References

[1] J. Bajorath, Integration of virtual and high-throughput screening, Nat. Rev. Drug Discov. 1 (11) (2002) 882–894.

[2] T. Polgar, G.M. Keseru, Integration of virtual and high throughput screening in lead discovery settings, Comb. Chem. High Throughput Screen. 14 (10) (2011) 889–897.

[3] Z. Vavrina, P. Perlikova, N. Milisavljevic, F. Chevrier, M. Smola, J. Smith, M. Dejmek, V. Havlicek, M. Budesinsky, R. Liboska, L. Vanekova, J. Brynda, E. Boura, P. Rezacova, M. Hocek, G. Birkus, Design, synthesis, and biochemical and biological evaluation of novel 7-Deazapurine cyclic dinucleotide analogues as STING receptor agonists, J. Med. Chem. 65 (20) (2022) 14082–14103.

[4] M. Dejmek, M. Sala, A. Brazdova, L. Vanekova, M. Smola, M. Klima, P. Brehova, M. Budesinsky, M. Dracinsky, E. Prochazkova, M. Zavrel, O. Simak, O. Pav, E. Boura, G. Birkus, R. Nencka, Discovery of isonucleotidic CDNs as potent STING agonists with immunomodulatory potential, Structure 30 (8) (2022) 1146–1156 e11.

[5] M. Dejmek, A. Brazdova, T. Otava, M.P. Polidarova, M. Klima, M. Smola, Z. Vavrina, M. Budesinsky, M. Dracinsky, R. Liboska, E. Boura, G. Birkus, R. Nencka, Vinylphosphonate-based cyclic dinucleotides enhance STING-mediated cancer immunotherapy, Eur. J. Med. Chem. 259 (2023) 115685.

[6] S. Basu, S. Middya, M. Banerjee, R. Ghosh, D.C. Pryde, D.B. Yadav, R. Shrivastava, A. Surya, The discovery of potent small molecule cyclic urea activators of STING, Eur. J. Med. Chem. 229 (2022) 114087.

[7] S. Stazzoni, D.F.R. Bohmer, F. Hernichel, D. Ozdemir, A. Pappa, D. Drexler, S. Bauernfried, G. Witte, M. Wagner, S. Veth, K.P. Hopfner, V. Hornung, L. M. Konig, T. Carell, Novel poxin stable cGAMP-derivatives are remarkable STING agonists, Angew. Chem. Int. Ed. Eng. 61 (40) (2022) e202207175.

[8] K. Mosallanejad, J.C. Kagan, Control of innate immunity by the cGAS-STING pathway, Immunol. Cell Biol. 100 (6) (2022) 409–423.

[9] C. Ritchie, J.A. Carozza, L.Y. Li, Biochemistry, cell biology, and pathophysiology of the innate immune cGAS-cGAMP-STING pathway, Annu. Rev. Biochem. 91 (2022) 599–628.

[10] A. Ablasser, Z.J.J. Chen, cGAS in action: expanding roles in immunity and inflammation, Science 363 (6431) (2019) 1055.

[11] J. Di Domizio, M.F. Gulen, F. Saidoune, V.V. Thacker, A. Yatim, K. Sharma, T. Nass, E. Guenova, M. Schaller, C. Conrad, C. Goepfert, L. de Leval, C. von Garnier, S. Berezowska, A. Dubois, M. Gilliet, A. Ablasser, The cGAS-STING pathway drives type I IFN immunopathology in COVID-19, Nature 603 (7899) (2022) 145.

[12] K.P. Hopfner, V. Hornung, Molecular mechanisms and cellular functions of cGAS-STING signalling, Nat. Rev. Mol. Cell Biol. 21 (9) (2020) 501–521.

[13] W. Chang, M.D. Altman, C.A. Lesburg, S.A. Perera, J.A. Piesvaux, G.K. Schroeder, D.F. Wyss, S. Cemerski, Y. Chen, E. DiNunzio, A.M. Haidle, T. Ho, I. Kariv, I. Knemeyer, J.E. Kopinja, B.M. Lacey, J. Laskey, J. Lim, B.J. Long, Y. Ma, M. L. Maddess, B.S. Pan, J.P. Presland, E. Spooner, D. Steinhuebel, Q. Truong, Z. Zhang, J. Fu, G.H. Addona, A.B. Northrup, E. Parmee, J.R. Tata, D.J. Bennett, J. N. Cumming, T. Siu, B.W. Trotter, Discovery of MK-1454: a potent cyclic dinucleotide stimulator of interferon genes agonist for the treatment of cancer, J. Med. Chem. 65 (7) (2022) 5675–5689.

[14] C. Pal, T.K. Chakraborty, Synthesis of amide-linked cyclic dinucleotide analogues with pyrimidine bases, Asian J. Org. Chem. 6 (10) (2017) 1421–1427.

[15] B.L. Gaffney, R.A. Jones, Synthesis of c-di-GMP analogs with Thiourea, urea, carbodiimide, and Guanidinium linkages, Org. Lett. 16 (1) (2014) 158–161.

[16] R. Amador, J.J. Vasseur, G. Birkus, E. Bignon, A. Monari, G. Clave, M. Smietana, Synthesis of original cyclic dinucleotide analogues using the Sulfo-click reaction, Org. Lett. 26 (4) (2024) 819–823.

[17] C.R. Dialer, S. Stazzoni, D.J. Drexler, F.M. Muller, S. Veth, A. Pichler, H. Okamura, G. Witte, K.P. Hopfner, T. Carell, A click-chemistry linked 2′3′-cGAMP analogue, Chemistry 25 (8) (2019) 2089–2095.

[18] M. Klima, M. Dejmek, V. Duchoslav, A. Eisenreichova, M. Sala, K. Chalupsky, D. Chalupska, B. Novotna, G. Birkus, R. Nencka, E. Boura, Fluorinated cGAMP analogs, which act as STING agonists and are not cleavable by poxins: structural basis of their function, Structure 32 (4) (2024), p. 433–439 e4.

[19] M. Smola, O. Gutten, M. Dejmek, M. Kozisek, T. Evangelidis, Z.A. Tehrani, B. Novotna, R. Nencka, G. Birkus, L. Rulisek, E. Boura, Ligand strain and its conformational complexity is a major factor in the binding of cyclic dinucleotides to STING protein, Angew. Chem. Int. Ed. Eng. 60 (18) (2021) 10172–10178.

[20] B. Novotna, L. Hola, M. Stas, O. Gutten, M. Smola, M. Zavrel, Z. Vavrina, M. Budesinsky, R. Liboska, F. Chevrier, J. Dobias, E. Boura, L. Rulisek, G. Birkus, Enzymatic synthesis of 3′-5′, 3′-5’ cyclic dinucleotides, their binding properties to the stimulator of interferon genes adaptor protein, and structure/activity correlations, Biochemistry 60 (48) (2021) 3714–3727.

[21] M. Pimkova Polidarova, P. Brehova, M.M. Kaiser, M. Smola, M. Dracinsky, J. Smith, A. Marek, M. Dejmek, M. Sala, O. Gutten, L. Rulisek, B. Novotna, A. Brazdova, Z. Janeba, R. Nencka, E. Boura, O. Pav, G. Birkus, Synthesis and biological evaluation of phosphoester and phosphorothioate prodrugs of STING agonist 3′,3′-c-Di(2’F,2’dAMP), J. Med. Chem. 64 (11) (2021) 7596–7616.

[22] B. Novotna, L. Vanekova, M. Zavrel, M. Budesinsky, M. Dejmek, M. Smola, O. Gutten, Z.A. Tehrani, M. Pimkova Polidarova, A. Brazdova, R. Liboska, I. Stepanek, Z. Vavrina, T. Jandusik, R. Nencka, L. Rulisek, E. Boura, J. Brynda, O. Pav, G. Birkus, Enzymatic preparation of 2′-5′,3′-5’-cyclic dinucleotides, their binding properties to stimulator of interferon genes adaptor protein, and structure/activity correlations, J. Med. Chem. 62 (23) (2019) 10676–10690.

[23] R. Perozzo, G. Folkers, L. Scapozza, Thermodynamics of protein-ligand interactions: history, presence, and future aspects, J. Recept. Signal Transduct. Res. 24 (1–2) (2004) 1–52.

[24] S.G. Krimmer, G. Klebe, Thermodynamics of protein-ligand interactions as a reference for computational analysis: how to assess accuracy, reliability and relevance of experimental data, J. Comput. Aided Mol. Des. 29 (9) (2015) 867–883.

[25] D.G. Myszka, Analysis of small-molecule interactions using Biacore S51 technology, Anal. Biochem. 329 (2) (2004) 316–323.

[26] D. Capelli, V. Scognamiglio, R. Montanari, Surface plasmon resonance technology: recent advances, applications and experimental cases, Trac-Trends Anal. Chem. (2023) 163.

[27] N. O'Connell, Protein ligand interactions using surface plasmon resonance, Methods Mol. Biol. 2365 (2021) 3–20.

[28] C.A. MacGriff, S.P. Wang, N.J. Tao, Four-port microfluidic flow-cell with instant sample switching, Rev. Sci. Instrum. 84 (10) (2013).

[29] M. Smola, G. Birkus, E. Boura, No magnesium is needed for binding of the stimulator of interferon genes to cyclic dinucleotides, Acta Crystallogr. F Struct. Biol. Commun. 75 (Pt 9) (2019) 593–598.

[30] V. Duchoslav, E. Boura, Structure of monkeypox virus poxin: implications for drug design, Arch. Virol. 168 (7) (2023) 192.

[31] J.B. Eaglesham, K.L. McCarty, P.J. Kranzusch, Structures of diverse poxin cGAMP nucleases reveal a widespread role for cGAS-STING evasion in host-pathogen conflict, Elife (2020) 9.

[32] J.B. Eaglesham, Y.D. Pan, T.S. Kupper, P.J. Kranzusch, Viral and metazoan poxins are cGAMP-specific nucleases that restrict cGAS-STING signalling, Nature 566 (7743) (2019) 259.

[33] J.A. Marquart, SPRpages - Getting a feeling for the curves, in: Handbook of Surface Plasmon Resonance, 2nd edition, 2017, pp. 106–148.