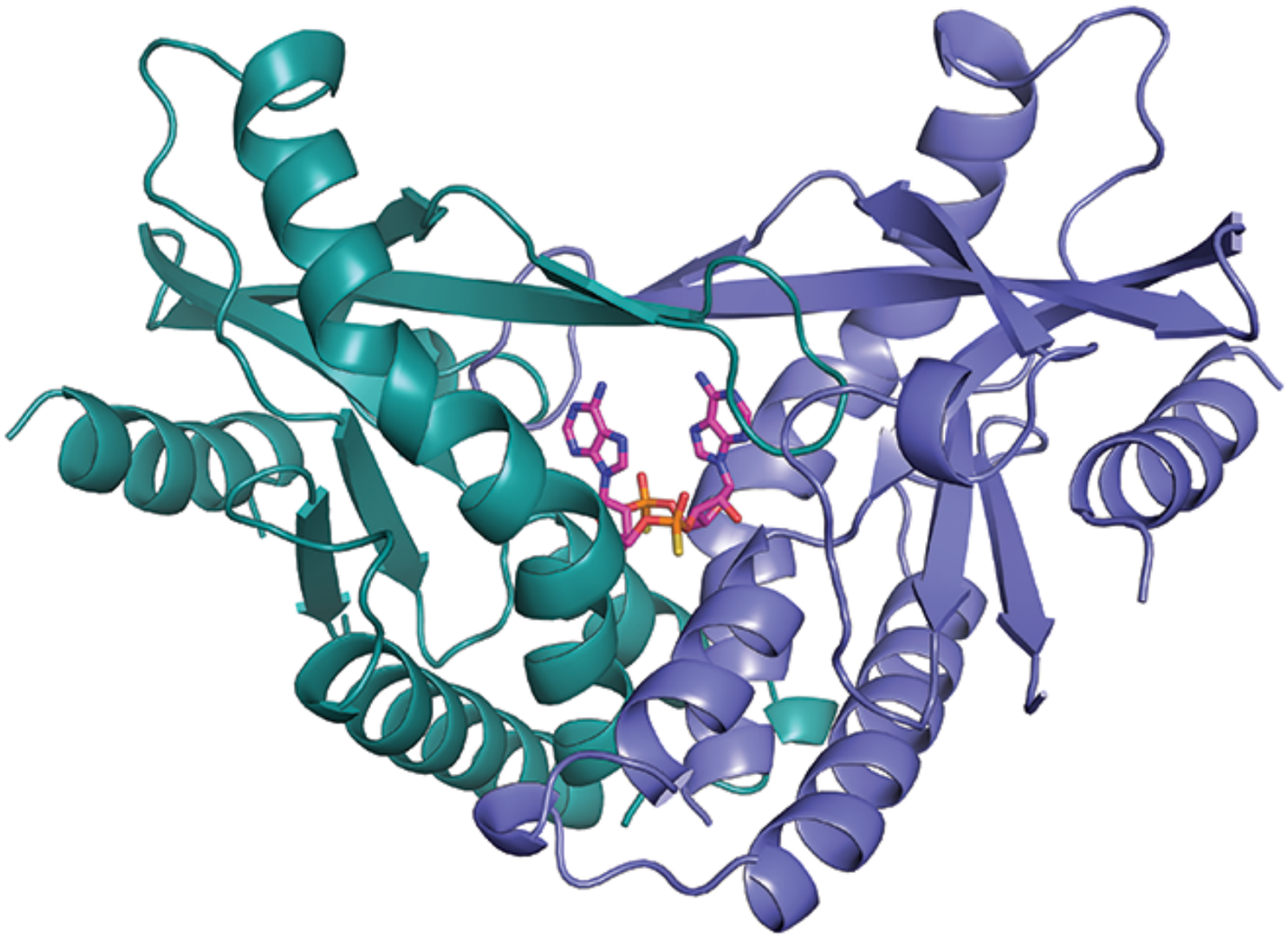
Immuno-Oncology: Targeting STING



Johannes Diesel

MacMillan Group Meeting

November 18, 2020

"STING Fever"

Company	Agent	Delivery	Program	Stage
Aduro/ Novartis	ADU-S100	IT	Small-molecule STING agonist	Ph1/2
Merck	MK-1454	IT	Small-molecule STING agonist	Ph1/2
Merck	MK-2118	IT/ SubQ	Small-molecule STING agonist	Ph1
Spring Bank	SB11285	IT/ IV	Small-molecule STING agonist	Ph1
GSK	GSK3745417	IV	Small-molecule STING agonist	Ph1
Bristol-Myers Squibb (IFM)	BMS-986301	IT	Small-molecule STING agonist	Ph1
Eisai	E7766	Unknown	Small-molecule STING agonist	Precl/ Disc
Takeda	TAK-676	Unknown	Small-molecule STING agonist	Precl/ Disc
Takeda/ Curadev	CRD5500	Unknown	Small-molecule STING agonist / "amendable to biconjugation as ADC"	Precl/ Disc
Abbvie (Mavupharma)	MAVU-104	Oral	ENPP1 inhibitor	Precl/ Disc
Synlogic	SYNB1891	IT	E. coli engineered to produce high levels of the STING agonist c-di-GMP	Precl/ Disc
Spring Bank	SB11325/ 11396	IV	Antibody conjugated STING agonists (Targets Unknown)	Precl/ Disc
Trillium Therapeutics	TTI-10001	Unknown	Small-molecule STING agonist	Precl/ Disc
Codiak Biosciences	exoSTING	Unknown	Engineered exosome	Precl/ Disc
Venn Therapeutics	VTX-001	IT	Adenovirus that produces the bacterial STING agonist c-di-GMP	Precl/ Disc
iTeos Therapeutics	Unnamed	IV	Small-molecule STING *pathway* activators	Precl/ Disc
Nimbus Therapeutics	Unnamed	Unknown	Small-molecule STING agonist	Precl/ Disc
Bicycle Therapeutics	Unnamed	Systemic	Bicycle conjugate	
Selvita	Unnamed	Unknown	Small-molecule to activate STING	Precl/ Disc
Stimunity	Unnamed	Unknown	Vectorized cGAMP – "virus like particle"	Precl/ Disc
StingInn	Unnamed	Unknown	Small-molecule STING agonists/ nucleic acid-based STING activators	Precl/ Disc
StingInn/ Vyriad	Unnamed	Unknown	Oncolytic viruses encoding STING *pathway* activators	Precl/ Disc
Venenum Biodesign	Unnamed	Unknown	Small-molecule STING agonist	Precl/ Disc

T. W. Dubensky, Tempest Therapeutics, *SITC 2019*, Conference Presentation.

Outline

- cGas-STING Pathway

- Structure of the STING Protein and the cGAMP-STING Complex

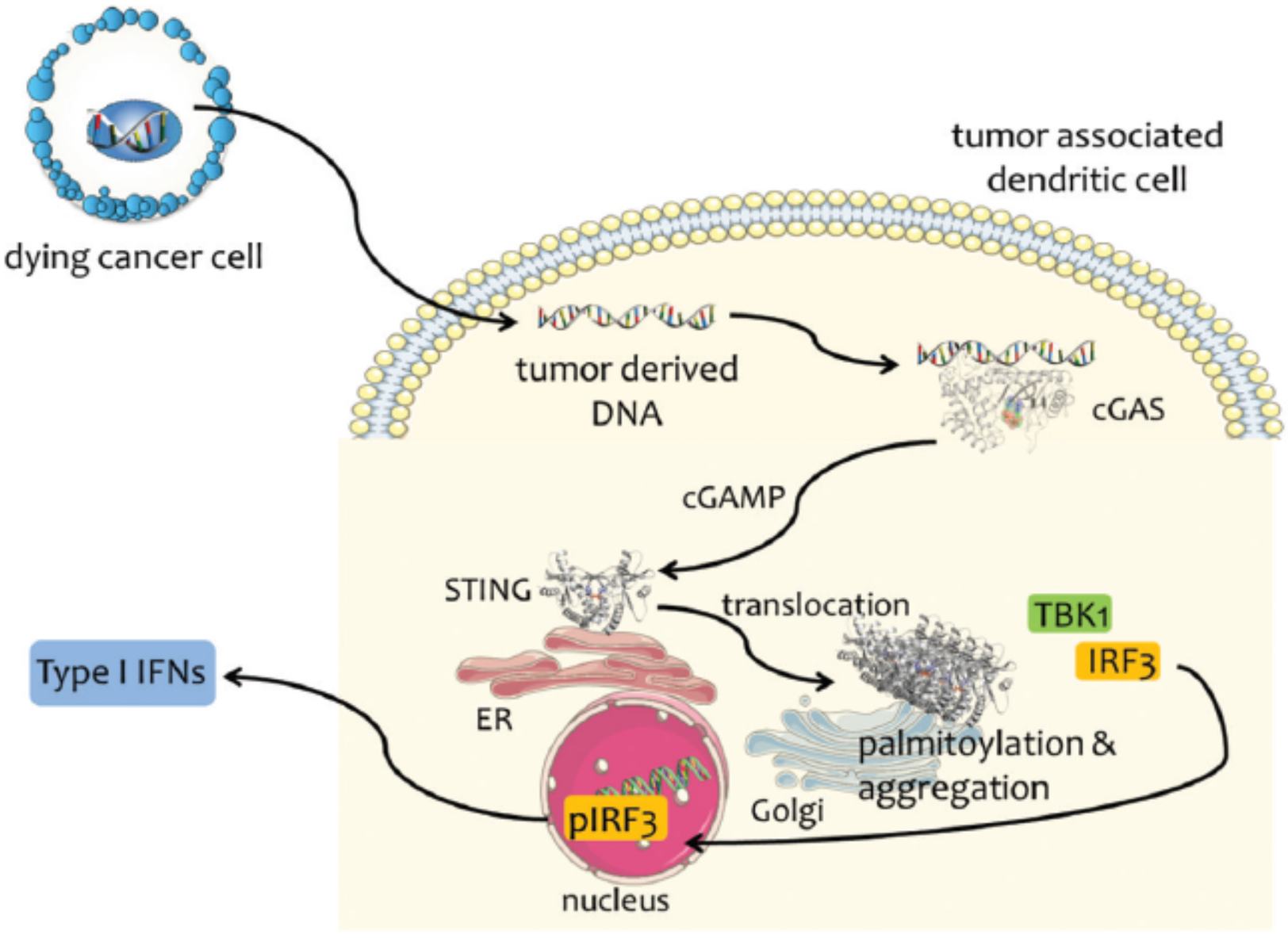
- Cyclic Dinucleotides (CDNs) as unique class of secondary messengers

- STING Agonists

  - CDN STING agonists

  - non-CDN STING agonists

cGas-STING pathway



cyclic GMP-AMP synthase (cGAS) is a DNA sensor activated by cytosolic DNA

cGAS generates the **second messenger 2',3'-cGAMP**, a cylic dinucleotide (CDN)

cGAMP is recognized by ER-bound adapter protein **stimulaor of interferon genes (STING)**

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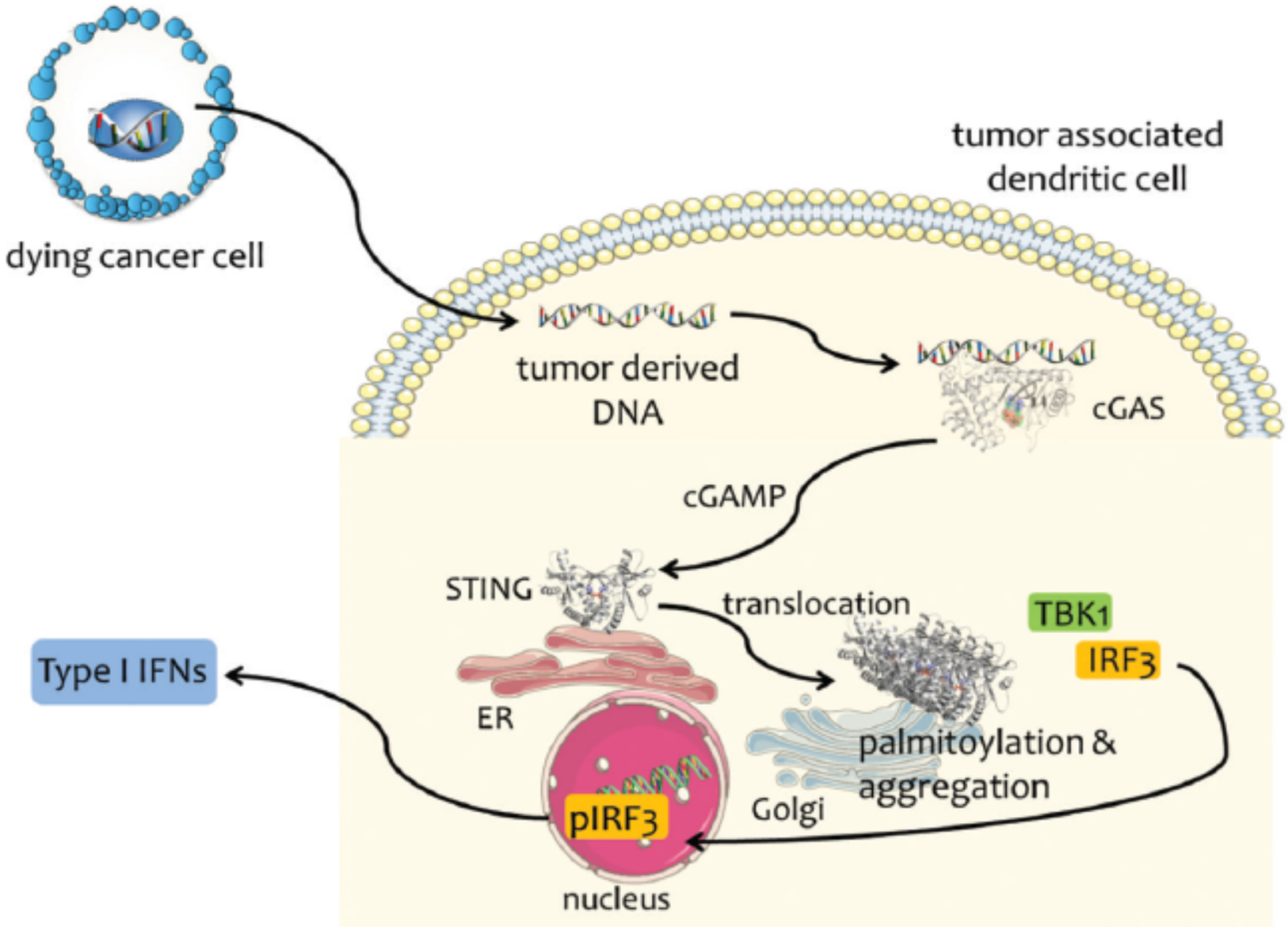
cGas-STING pathway



activated STING translocates to perinucluar Golgi compartments

palmitoylation dependent STING aggregation and recruiting of kinases TBK1 and IKK

phosphorylated STING binds IRF3, which dimerizes and translocates into the nucleus

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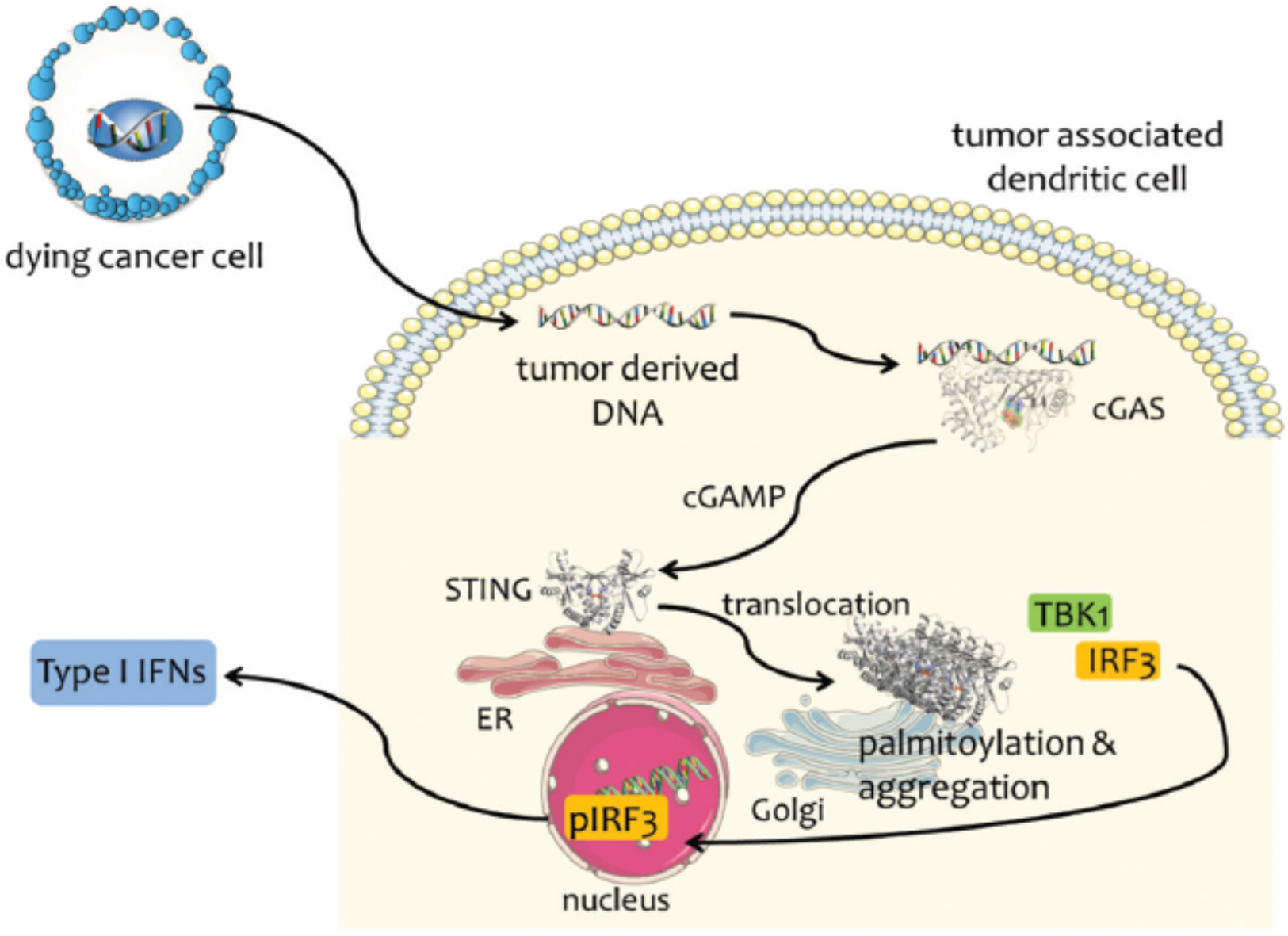
cGas-STING pathway



transcription of genes enconding **interferons (IFNs)** is initiated

IFNs exert cytotoxic effects on cancer cells and link innate and adaptive immune response

STING pathway is downregulated in various cancer cell lines (cancer immune evasion)

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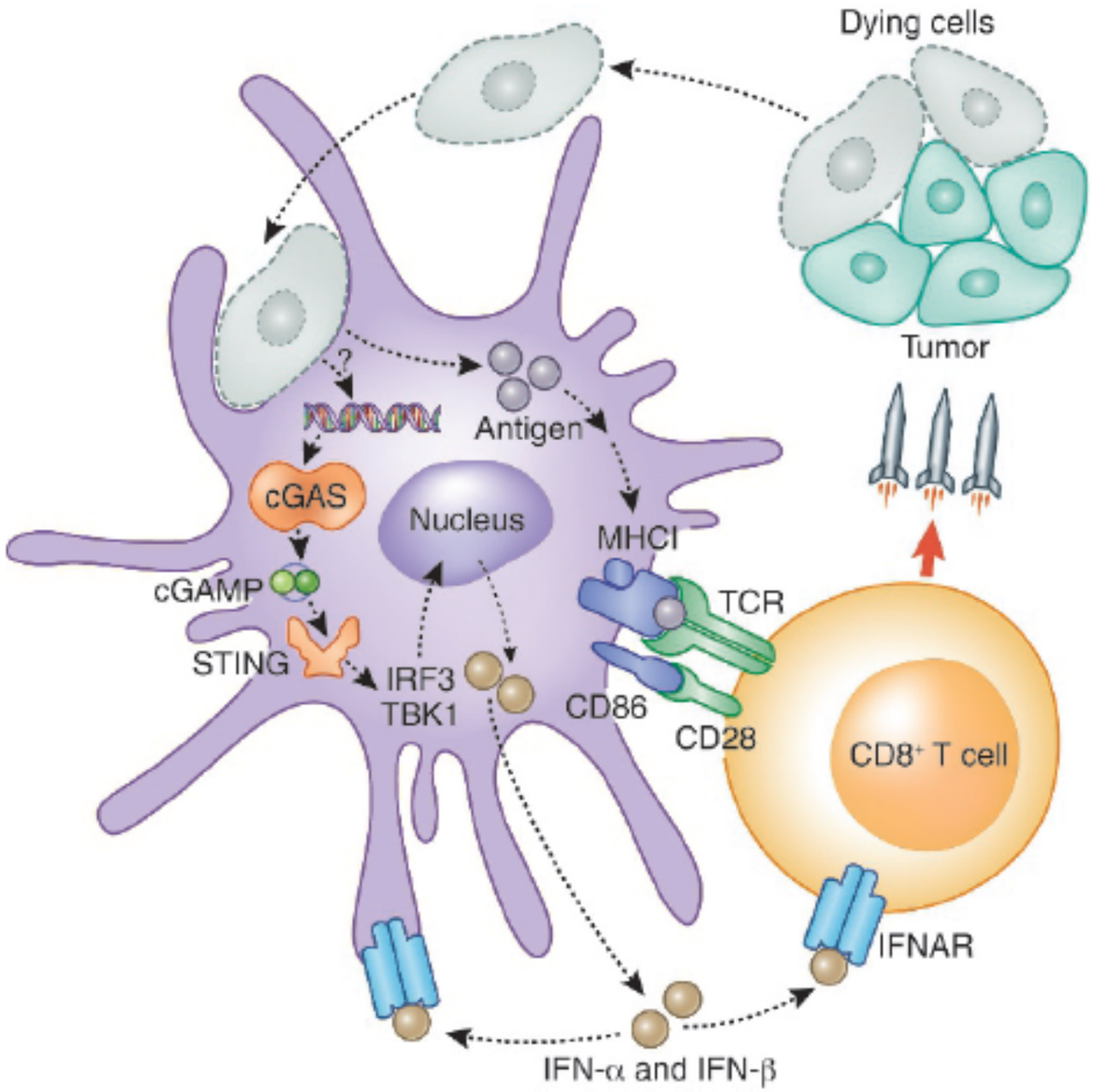
cGas-STING pathway



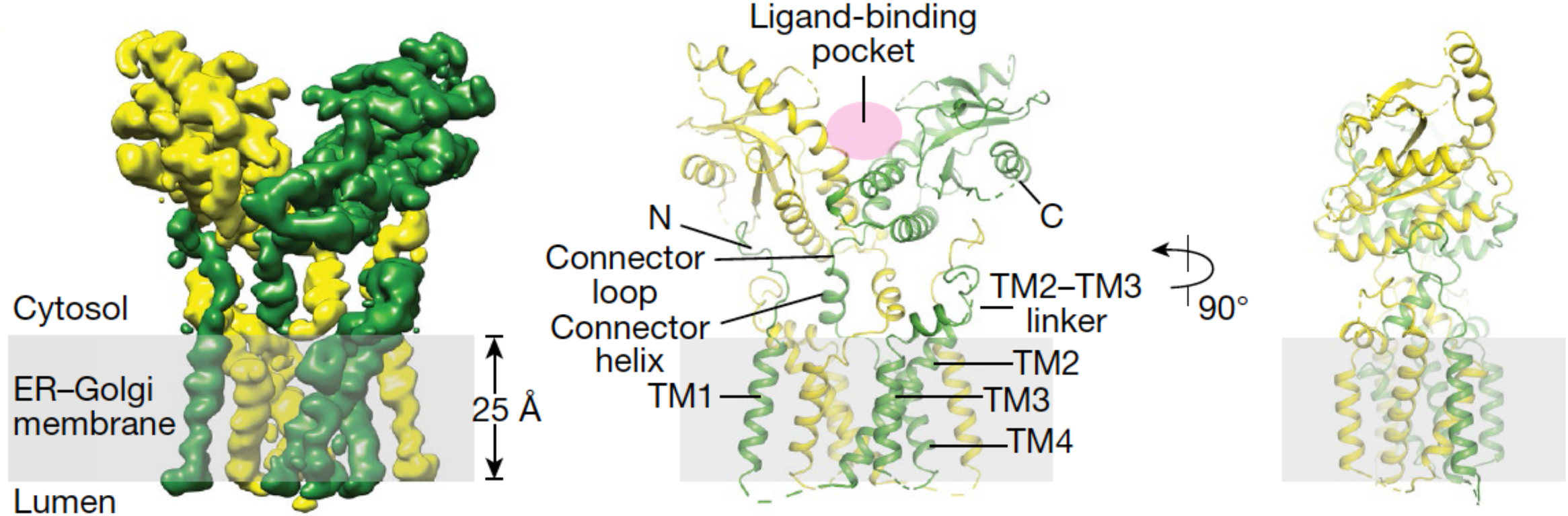
- STING agonists have large potential as effective anti/tumor agent
- most promising are combination therapies with checkpoint inhibitors

Q. Chen *et al. Nat. Immunol.* **2016,** *17*, 1142.

Structure of the STING Protein

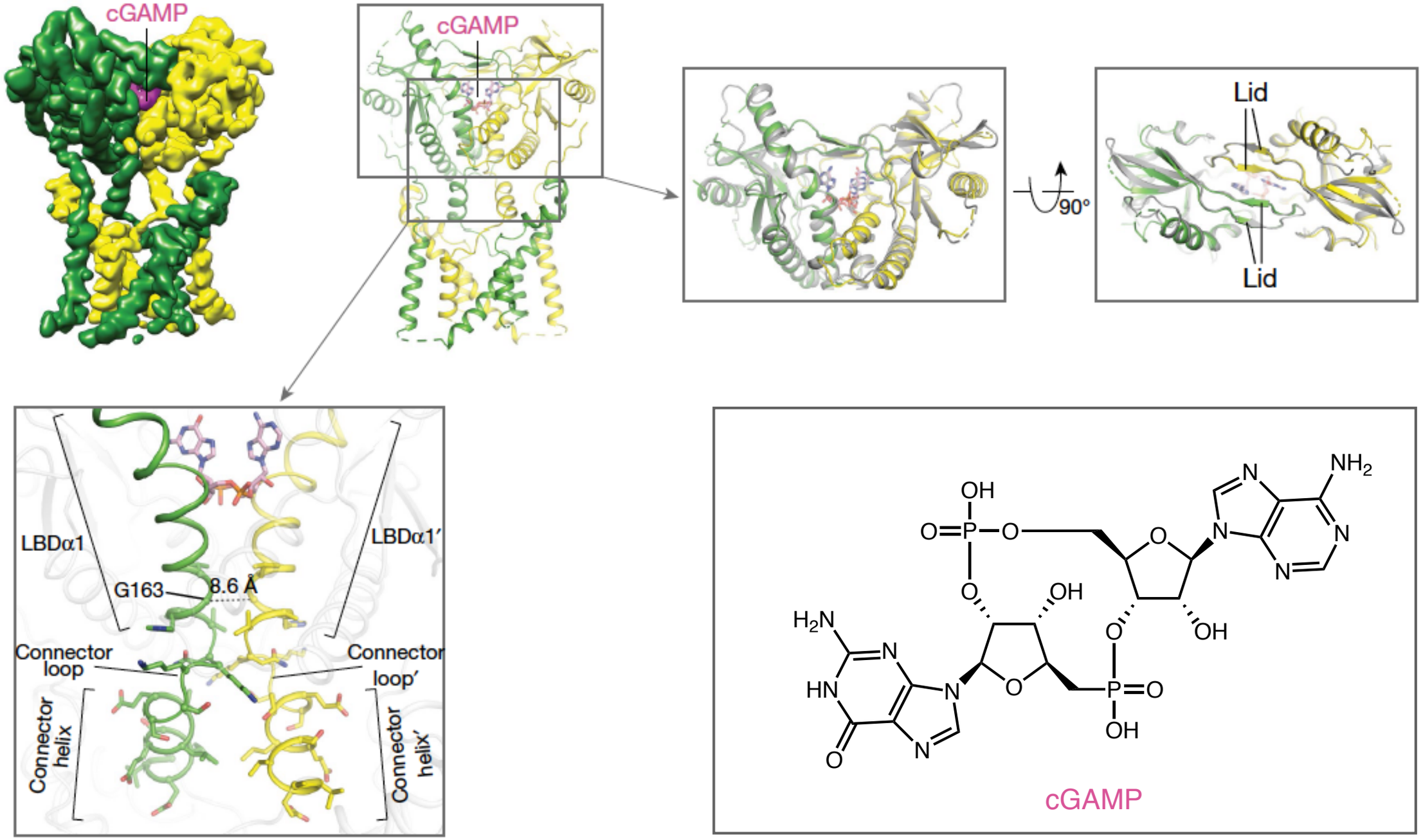
STING protein forms a V-shaped homodimer consisting of cytoplasmic *C*-terminal **ligand binding domain (LBD)** and *N*-teminal transmembrane domain.

Downstream signalling depends on *C*-terminal tail region. Ser366 is phosphorylated by TBK1 to form STING-IRF3 complex



X. Zhang et al. *Nature* **2019**, *567*, 389.

cGAMP-STING Complex

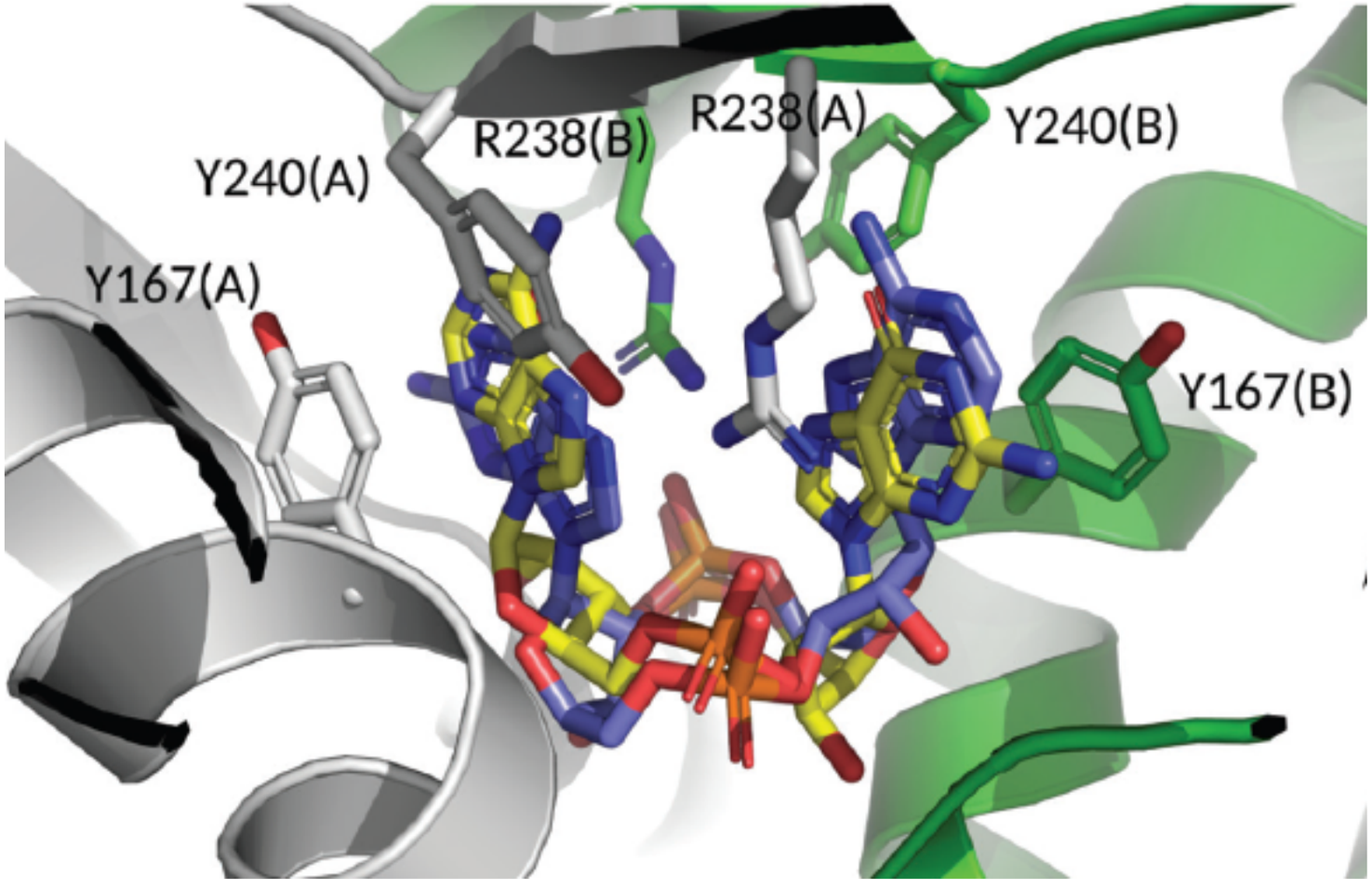


X. Zhang et al. *Nature* **2019**, *567*, 389.

cGAMP-STING Complex

Two distinct binding positions of the asymmetric ligand to the symmetric binding pocket

Main binding interactions are with Arg238 via charge reinforced hydrogen bonds to the phosphate groups and nucleobase π-stacking with Tyr167



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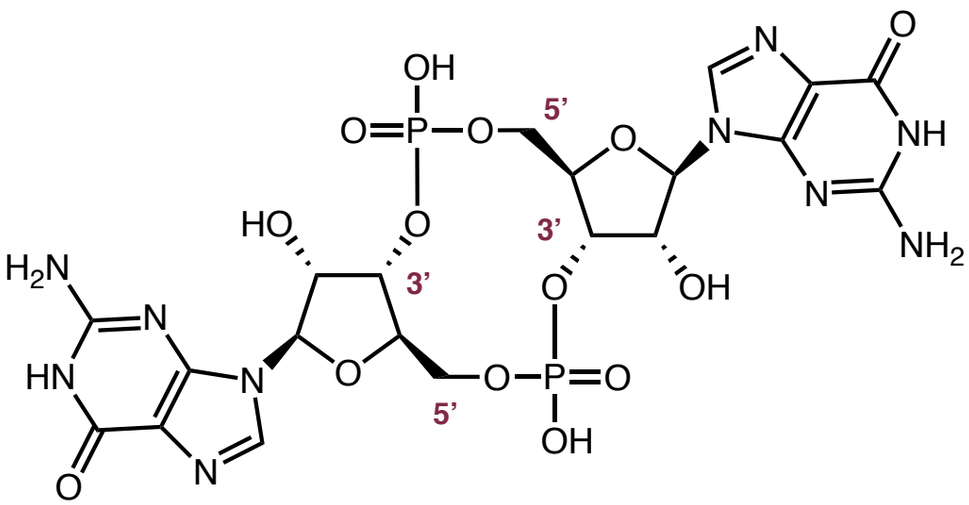
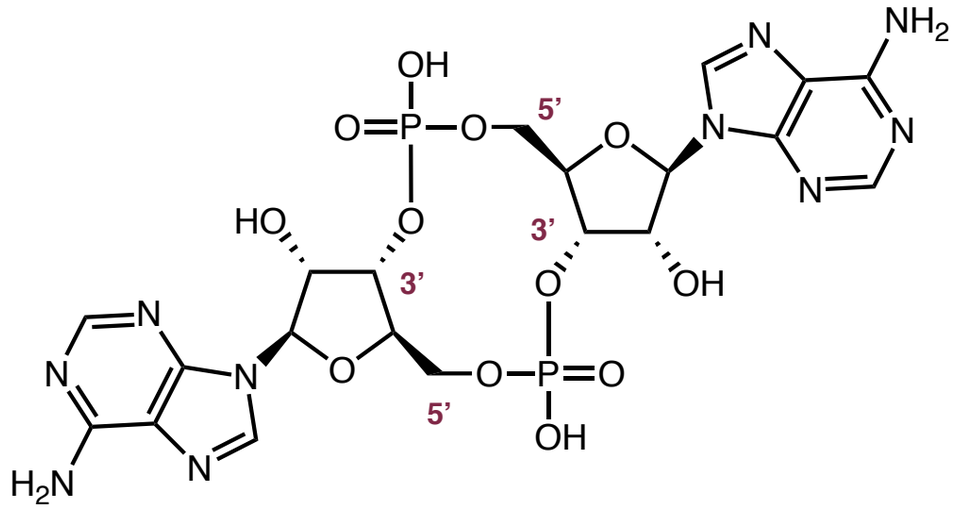
Cyclic Dinucleotides (CDNs)

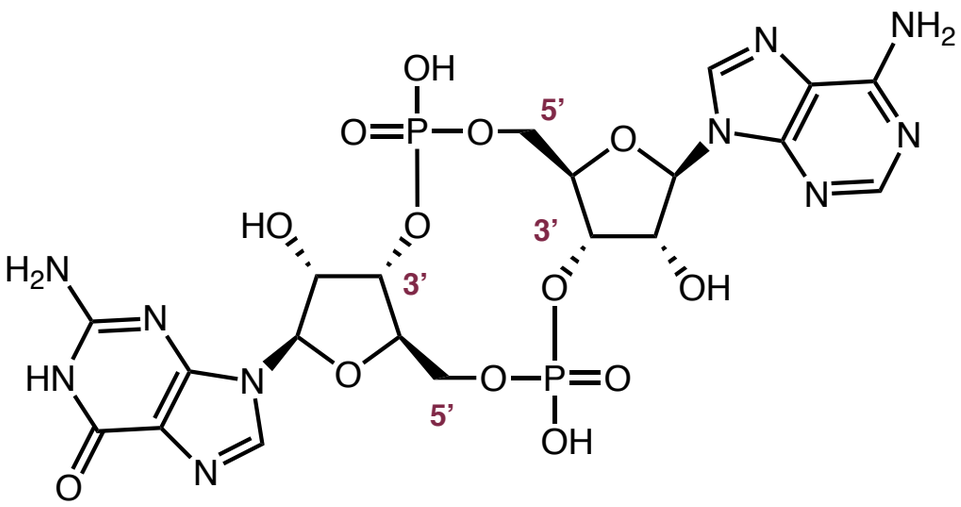


c-diGMP

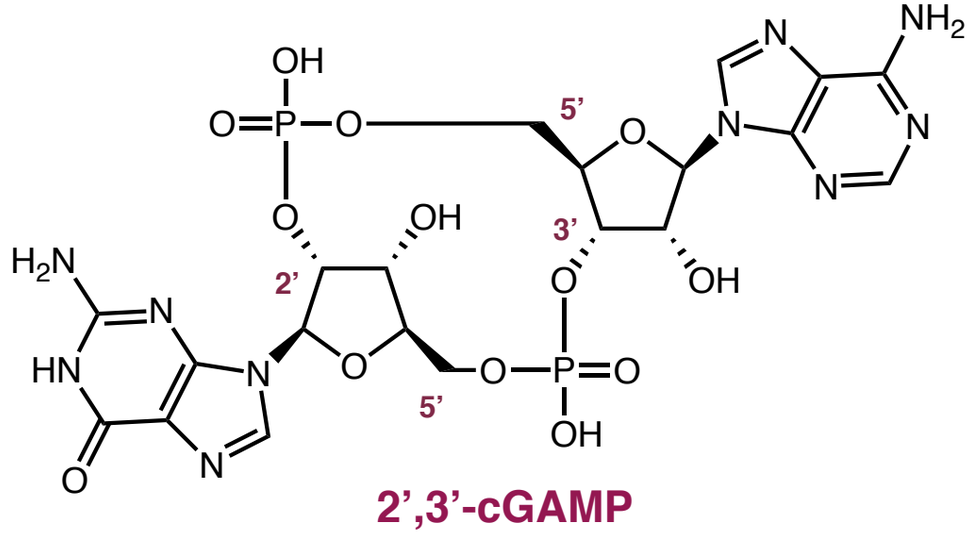


c-diAMP

CDNs are a structurally unique class of secondary messengers



3’,3’-cGAMP



2’,3’-cGAMP

2’,3’-cGAMP binds STING 100-fold stronger than 3’,3’-cGAMP

J. Oost, C. A. Kuttruff, H. Narr *2019 Medicinal Chemistry Reviews, 54*, 9.

Cyclic Dinucleotides (CDNs)

Rational for difference in total binding energies ΔG_{total} of 2',3'-cGAMP and 3',3'-cGAMP

Binding Energy STING/cGAMP

$$\Delta G_{total} = \Delta H_{total} - T\,\Delta S_{total}$$

$$\Delta S_{total} = \Delta S_{protein} + \Delta S_{ligand} + \Delta S_{water}$$

$$\Delta S_{ligand} = S_{ligand(bound)} - S_{ligand(free)}$$

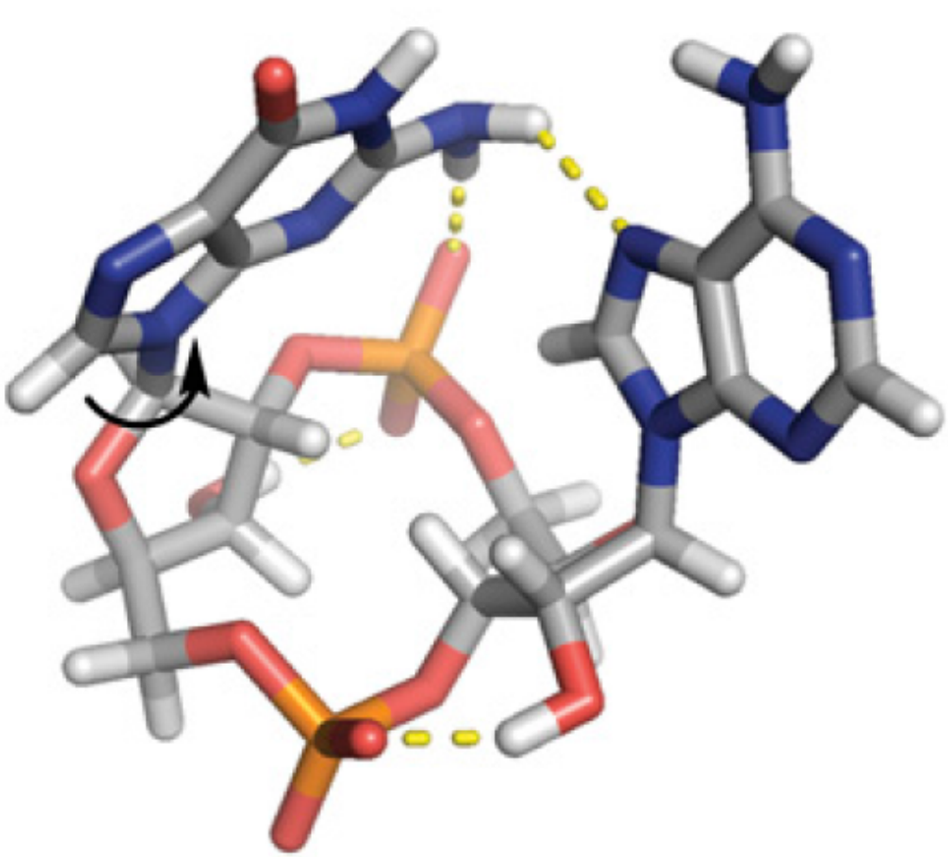
- $\Delta H_{STING/2',3'\text{-}cGAMP} \sim \Delta H_{STING/3',3'\text{-}cGAMP}$ (based on ITC data)
- $\Delta S_{protein}$ is comaparable as both 2',3'-cGAMP and 3',3'-cGAMP induce the same conformational change (based on X-ray structures)
- the ligands have the same volumes ($\sim$520 Å^3) hence ΔS_{water} is similar

different binding affinities originate from difference in ΔS_{ligand}

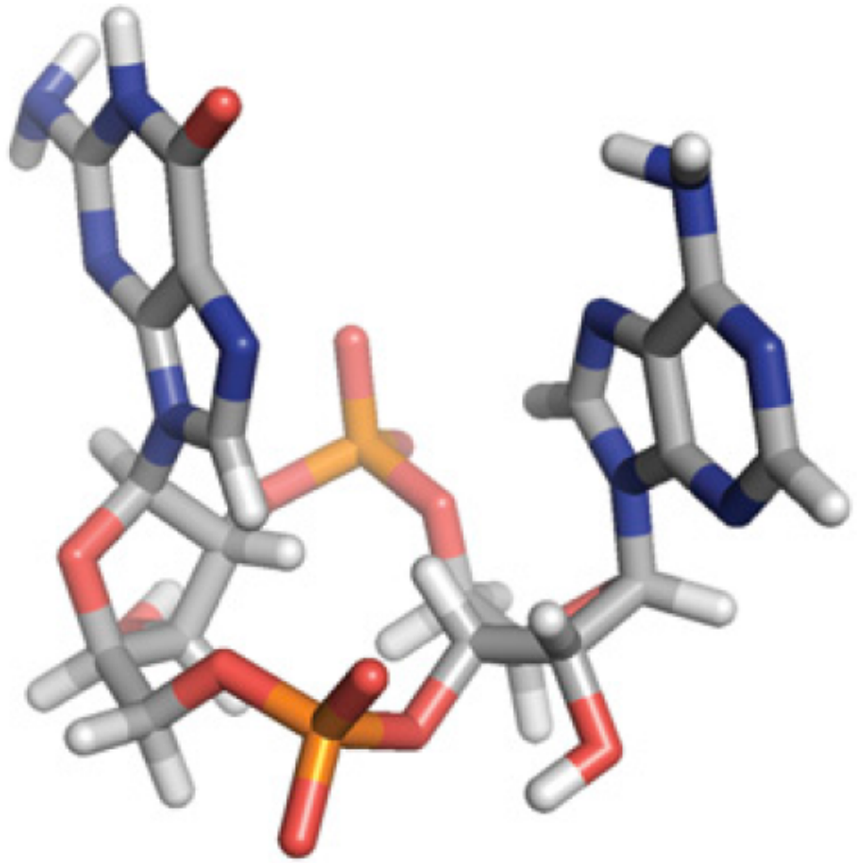
C. Chen *et al. PNAS,* **2015**, *112*, 8947.

Cyclic Dinucleotides (CDNs)

Rational for difference in total binding energies ΔG_{total} of 2',3'-cGAMP and 3',3'-cGAMP



closed equlibrium conformation of *2',3'-cGAMP*

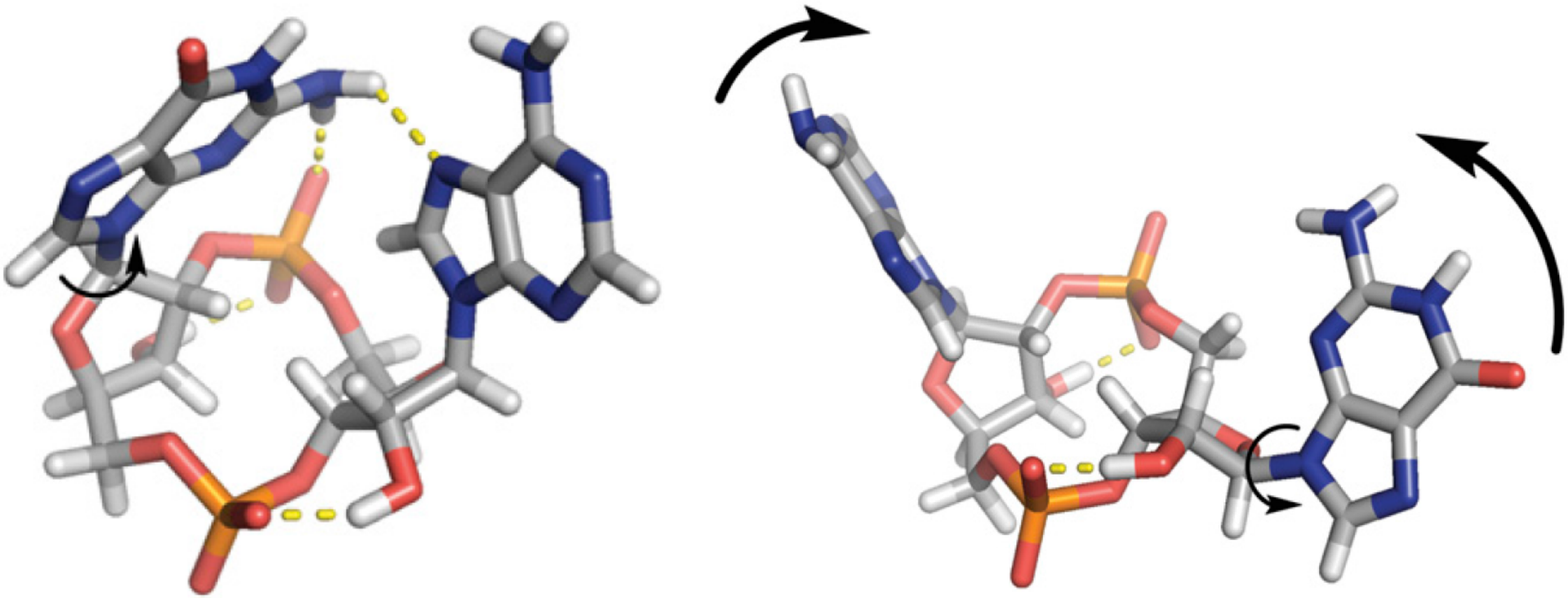


STING bound conformation

C. Chen *et al. PNAS,* **2015***, 112*, 8947.

Cyclic Dinucleotides (CDNs)

Rational for difference in total binding energies ΔG_{total} of 2',3'-cGAMP and 3',3'-cGAMP



closed equlibrium conformation of *2',3'-cGAMP*

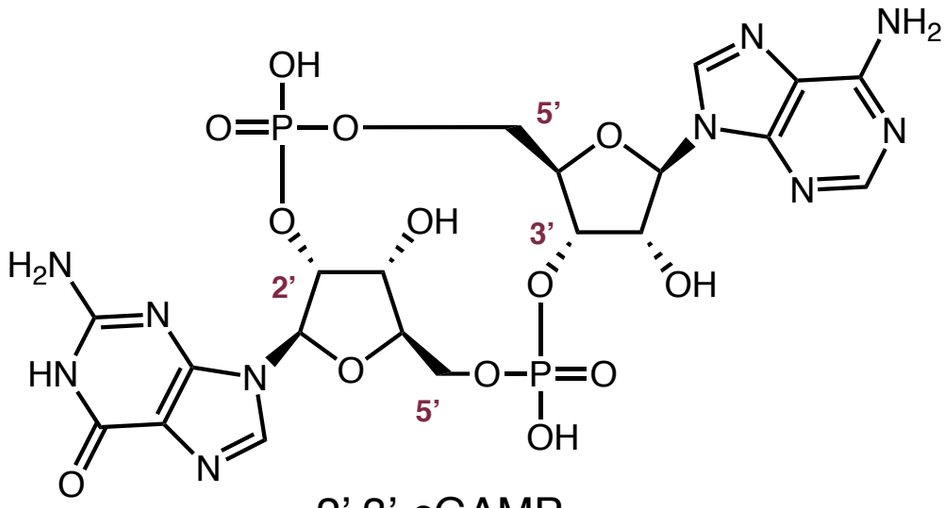
open equlibrium conformation of *3',3'-cGAMP*

2',3'-cGAMP binding to STING requires significantly less entropy cost

$S_{2',3'\text{-cGAMP(free)}} \ll S_{3',3'\text{-cGAMP(free)}}$

C. Chen *et al. PNAS,* **2015**, *112*, 8947.

CDN STING Agonists



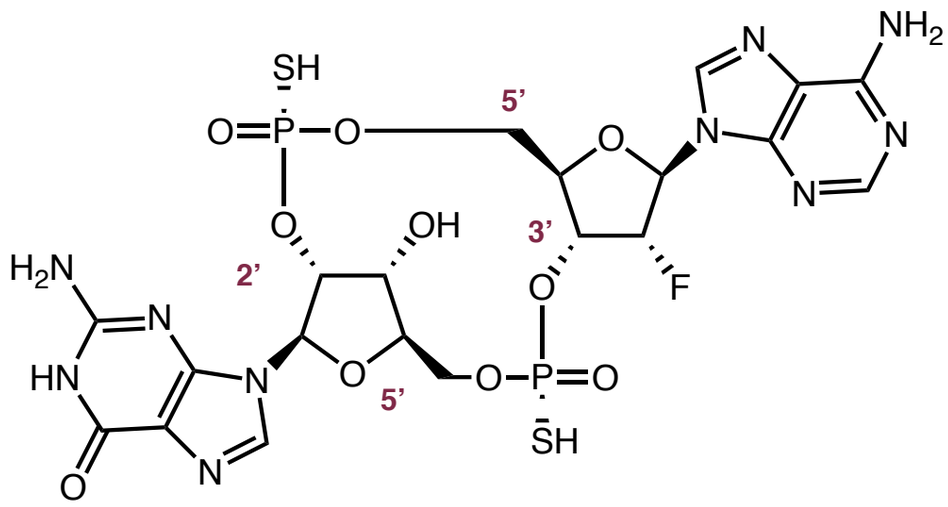
2',3'-cGAMP

STING WT DSF ΔT_M = 16.2 °C

Assay: Diffferential Scanning Fluorimetry DSF

- measures stabilzation of protein by ligand binding against thermal unfolding
- unfolding temperature is measured by increase of fluorescense of a dye binding to hydrophobic protein parts, which are exposed upon protein unfolding

- Aduro 2',3'-cGAMP analog



STING WT DSF ΔT_M = 27.3 °C

- increased binding affinity
- increased cellular uptake
- increased metabolic stability

F. H. Niesen *et al. Nat. Protoc.* **2007**, *2*, 2212.

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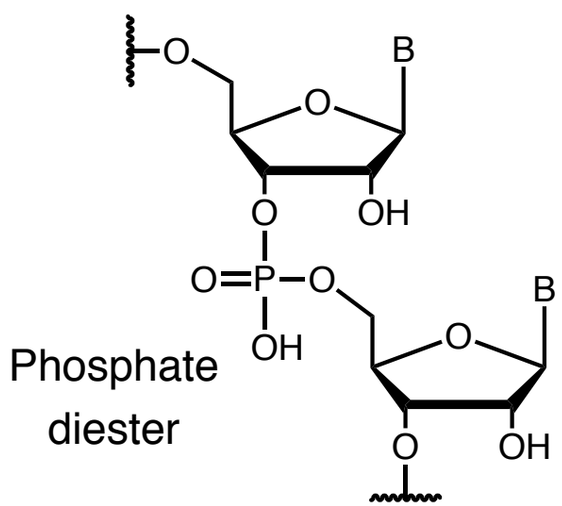
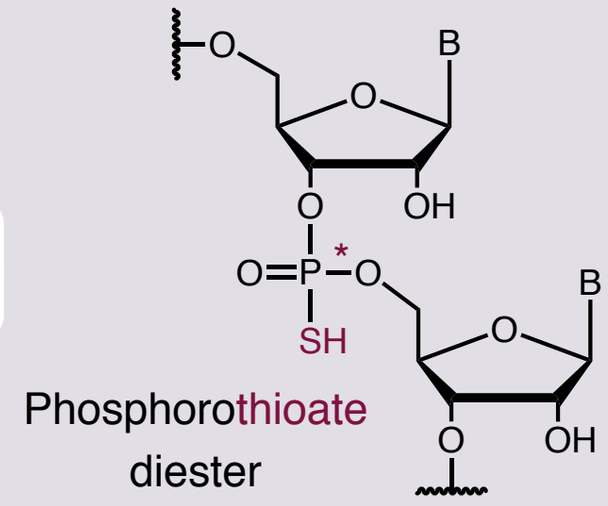
The Thio Effect





↑ increased celluar uptake

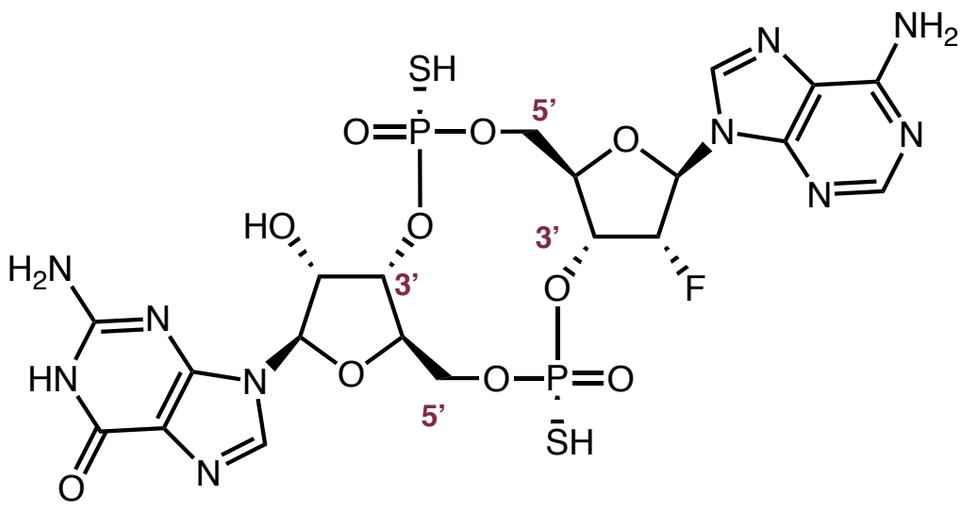
↑ higher metabolic stability

- decreased rate of hydrolysis caused by lower solvent stabilization of the pentavalent charged intermediate
- thio effect is widely applied in RNA-based drug discovery
- stereogenic phosphorus atom results in diastereomer formation

S. C. L. *et al.* Kamerlin *Org. Biomol. Chem.* **2015**, *13*, 5391.

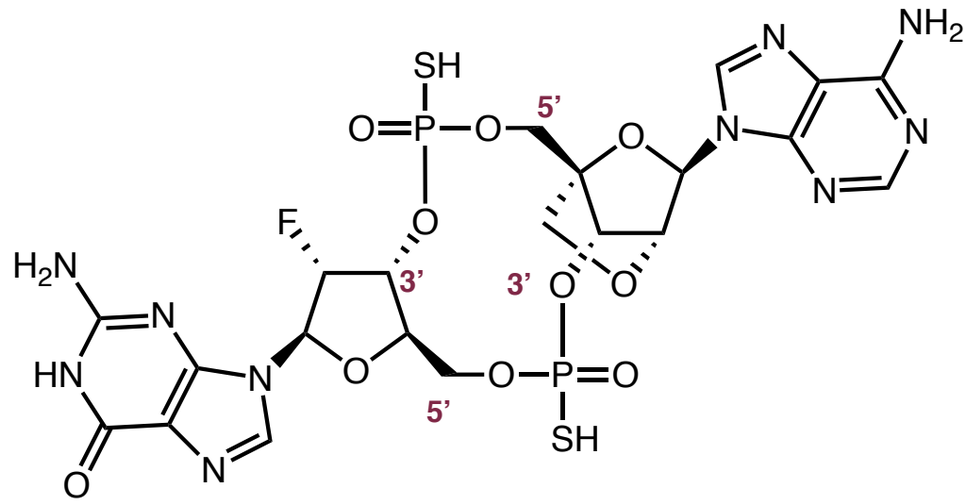
A. C. Hengge *et al. J. Org. Chem.* **2005**, *70*, 8437.

CDN STING Agonists



Aduro

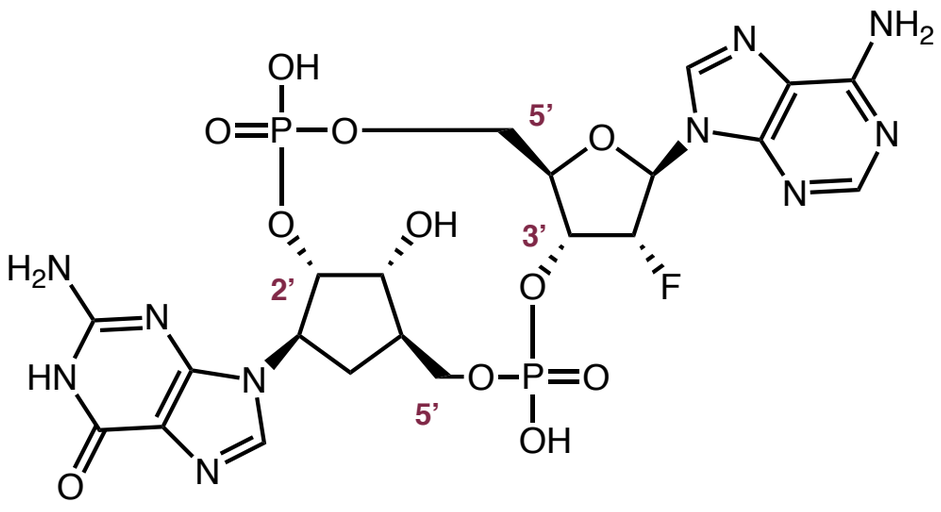
STING WT DSF ΔT_M = 19.2 °C



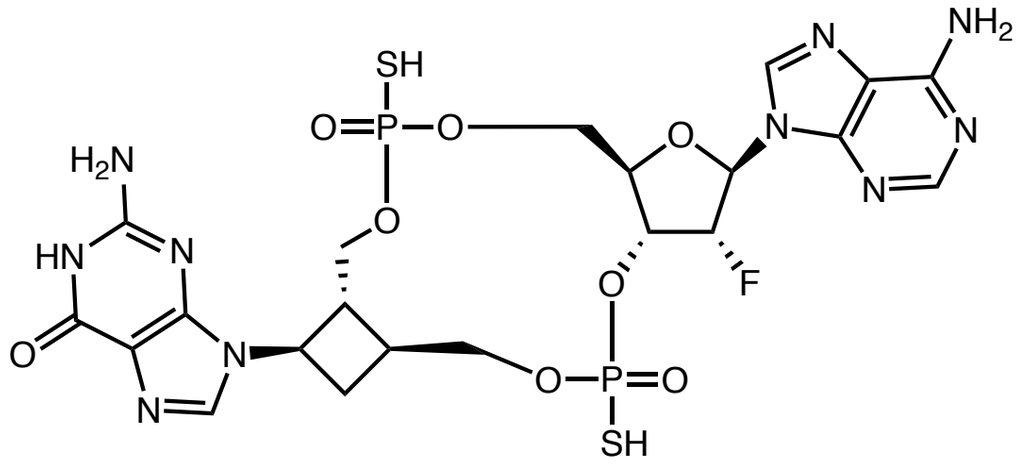
Boehringer Ingelheim (locked nucleic acid)

STING WT DSF ΔT_M = 30.3 °C

“late eluting” diastereomer



GSK



BMS

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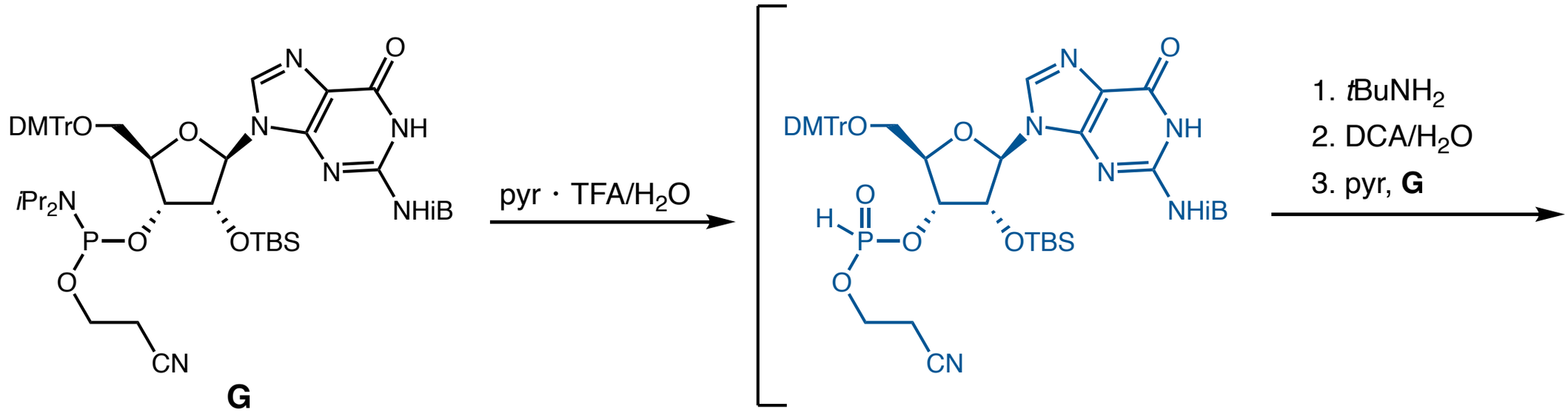
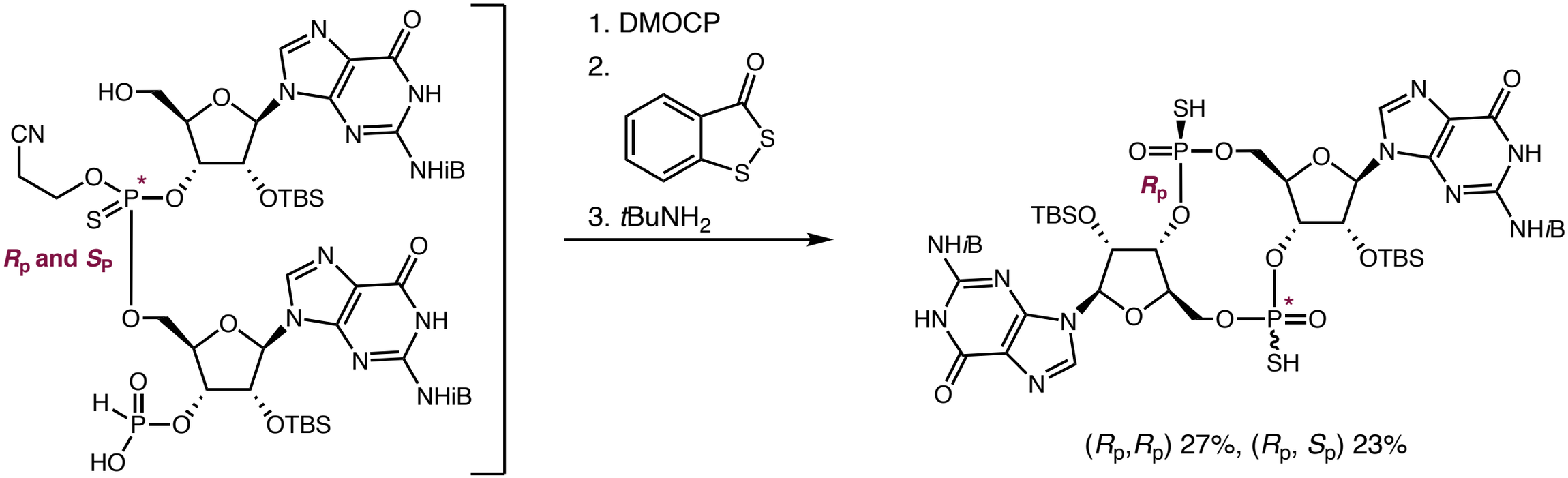
CDN Synthesis - Jones Protocol

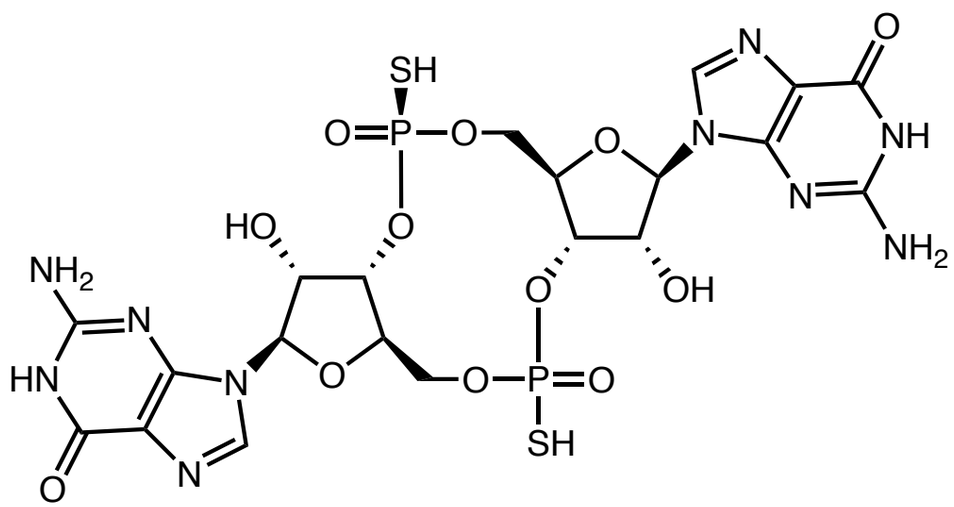


R. A. Jones *et al. Org. Lett.* **2010**, *12*, 3269.

CDN Synthesis - Jones Protocol



1. Diasteremer Seperation
2. $MeNH_2$
3. $Et_3N \cdot HF$



overall yield: (R_p,R_p) 19%, (R_p, S_p) 17%

c-diGMP phosphorothioate analog

R. A. Jones *et al. Org. Lett.* **2010**, *12*, 3269.

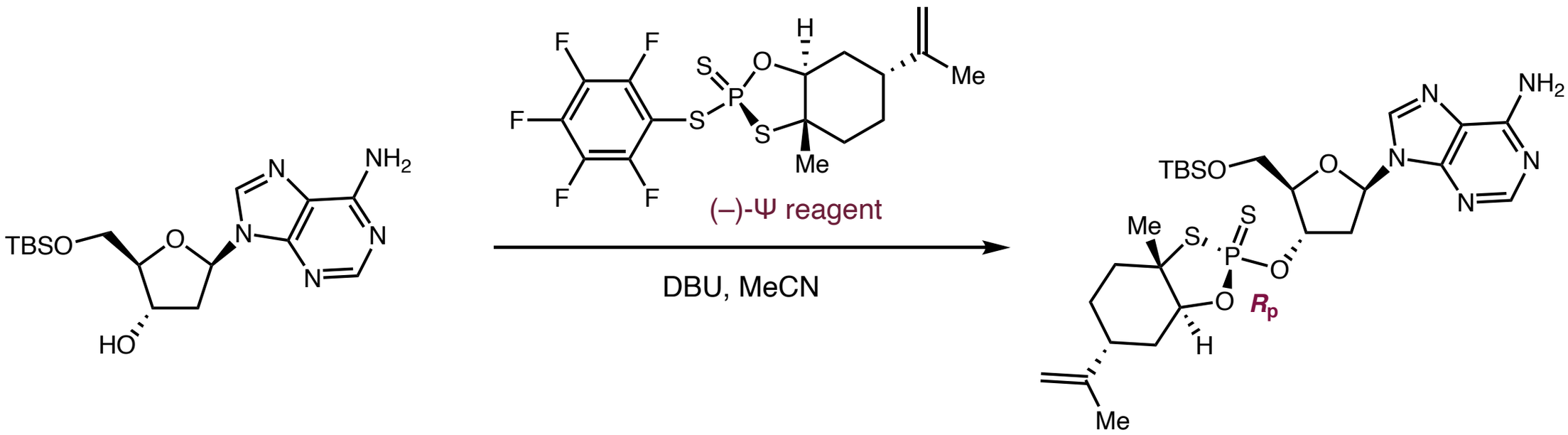
CDN Synthesis - Jones Protocol

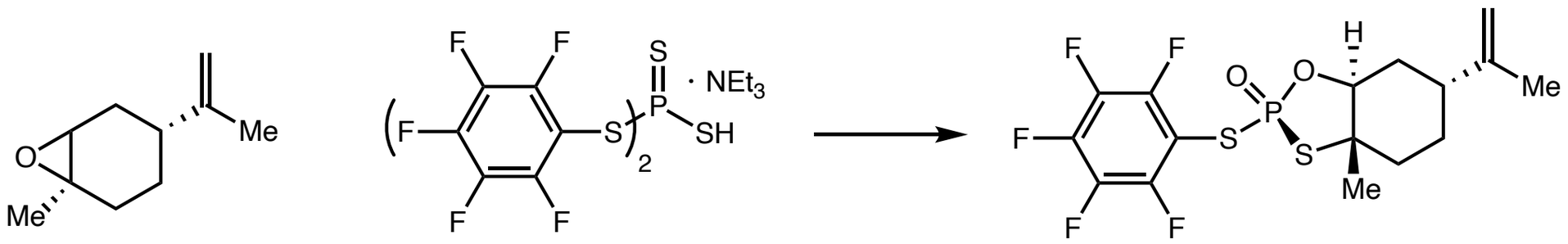
- efficient access to gram scale quantities of c-diGMP
- 8 steps are in one flask, <10h
- bis-phosphorothioate analogs of c-diGMP accessible

- mixture of up to 4 diastereomers, requires seperation
- protecting group manipulations
- functional group interconversions

R. A. Jones *et al. Org. Lett.* **2010**, *12*, 3269.

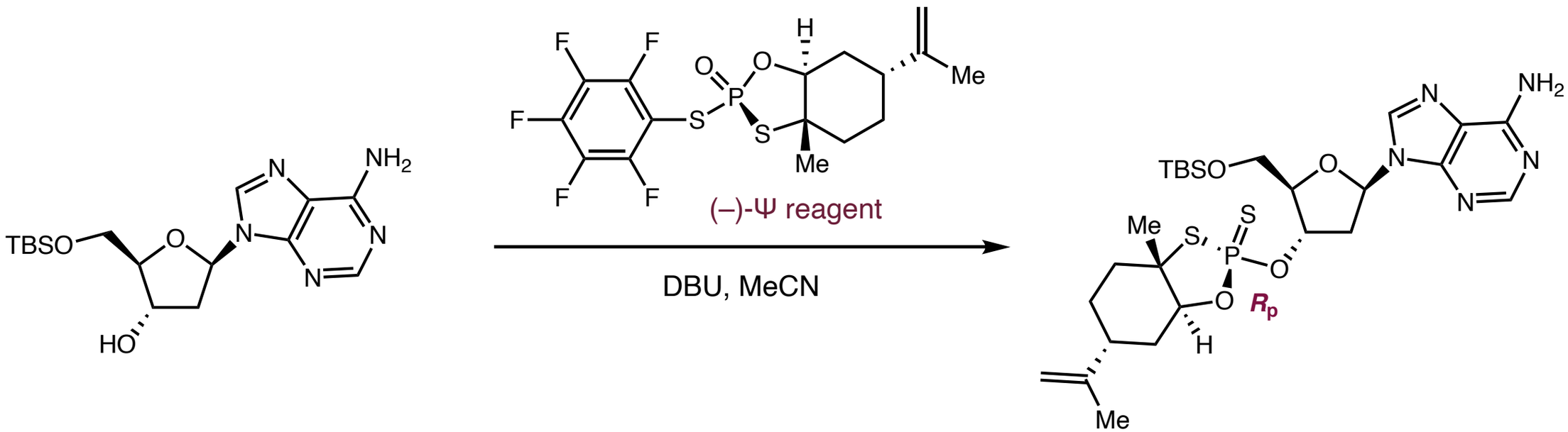
CDN Synthesis - Baran and BMS Protocol





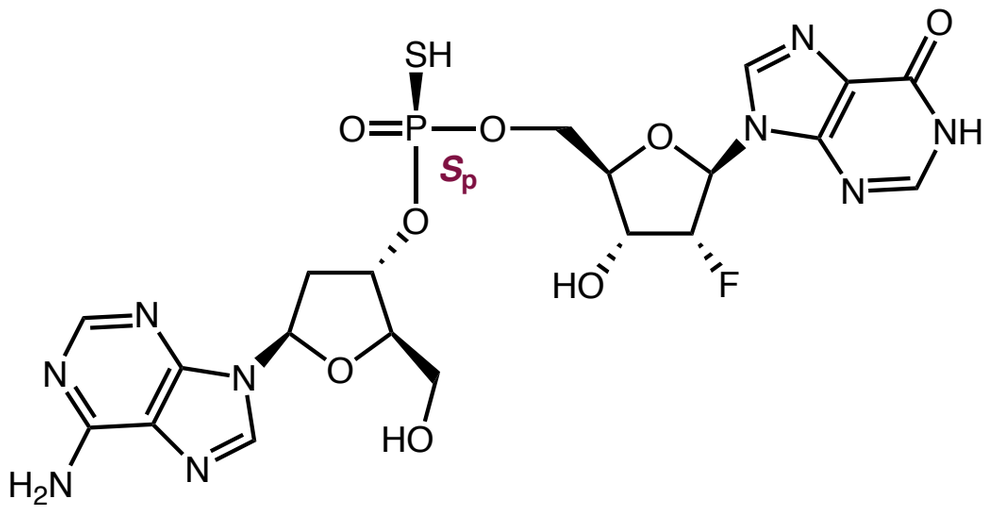
M. D. Eastgate, P. S. Baran *et al. Science* **2018**, *361*, 1234.

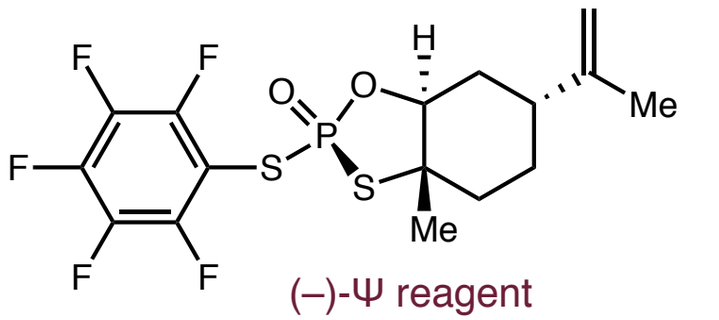
CDN Synthesis - Baran and BMS Protocol



M. D. Eastgate, P. S. Baran *et al. Science* **2018**, *361*, 1234.

CDN Synthesis - Baran and BMS Protocol

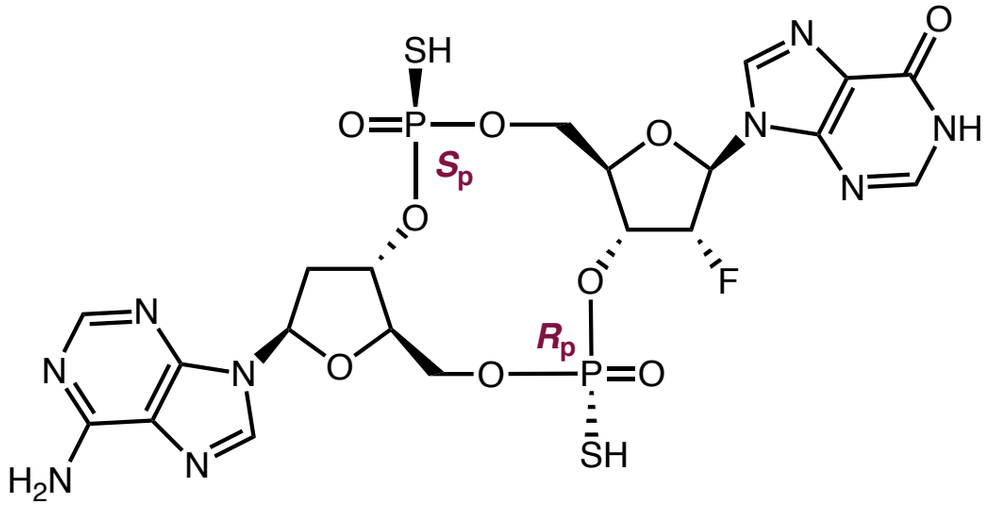




(–)-Ψ reagent

DBU, DMF

diol chemoselectivity controls diastereoselectivity



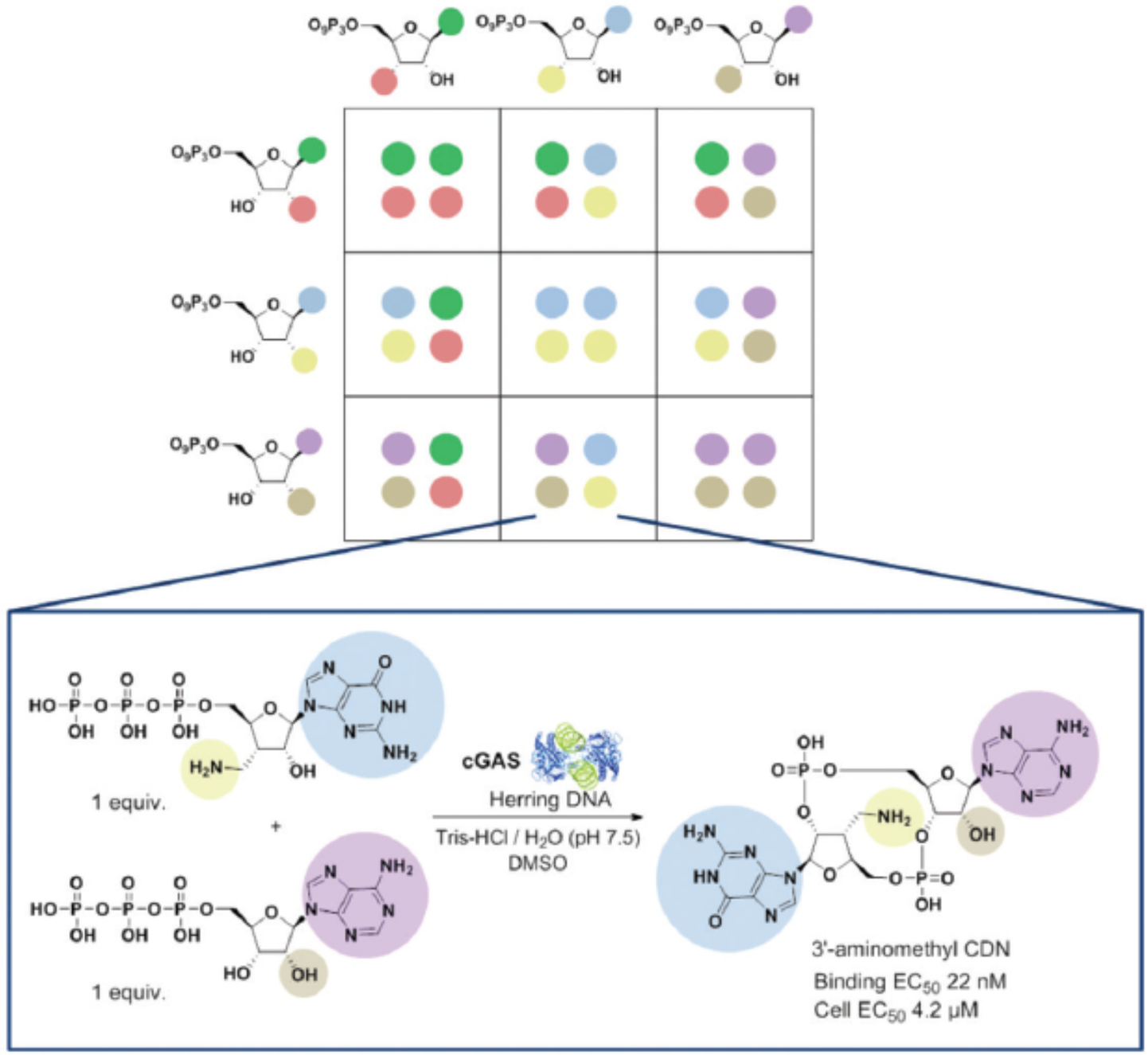
24%, single diastereomer

previously 4%, stereorandom

- avoids sensitive P(III)-reagents
- high step efficiency
- stereoselective

M. D. Eastgate, P. S. Baran *et al. Science* **2018**, *361*, 1234.

CDN Synthesis - Merck Biocatalytic Approach



M. D. Altmann *et al. Patent Application* WO2017/027646A1, **2017**.

J. Oost, C. A. Kuttruff, H. Narr *2019 Medicinal Chemistry Reviews, 54*, 9.

CDN STING Agonists

- variety of CDN STING agonists have advanced to clinical trials
- exclusively for solid tumors allowing intratumoral administration
- innovation needed to allow systematic administration to patients with multiple heterogenous tumors
- synthetic small molecules may be advantageous by providing improved permeability and easier synthetic acces

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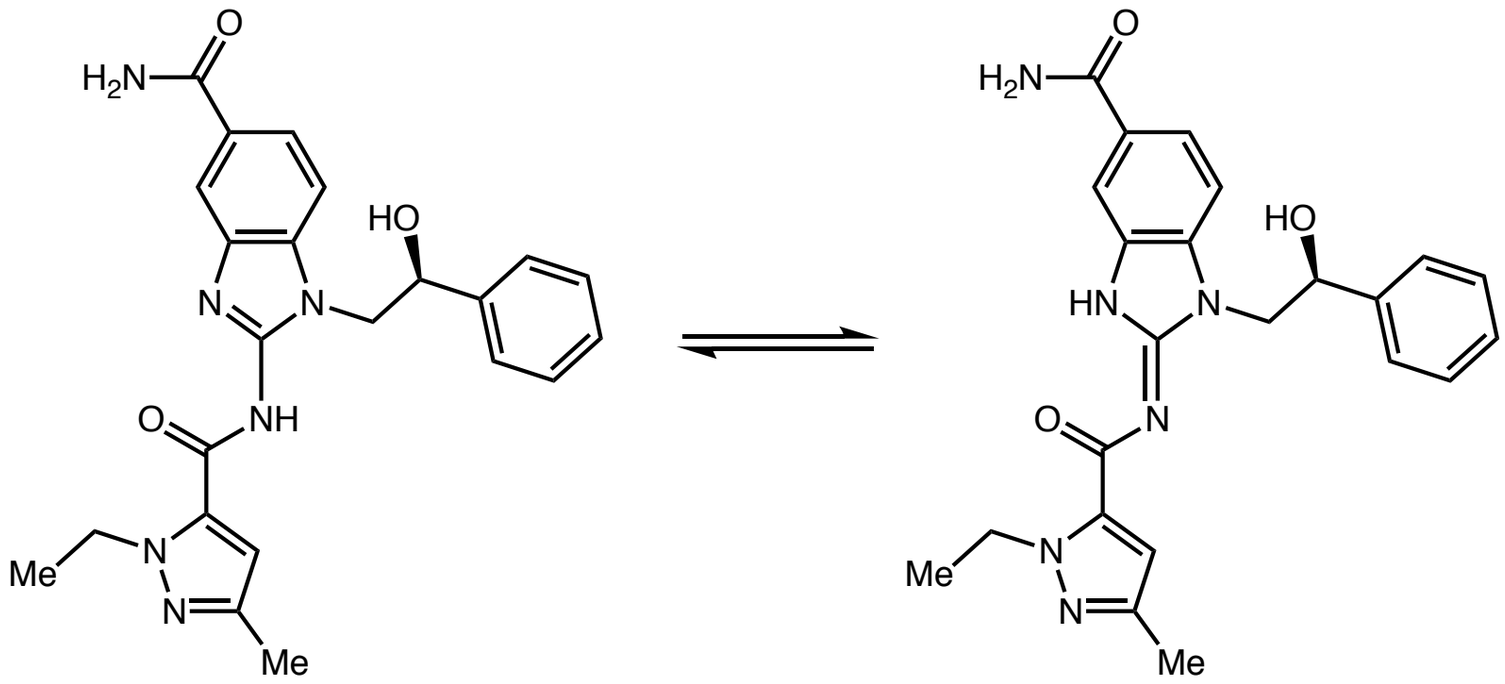
Non-Nucleotide STING Agonists

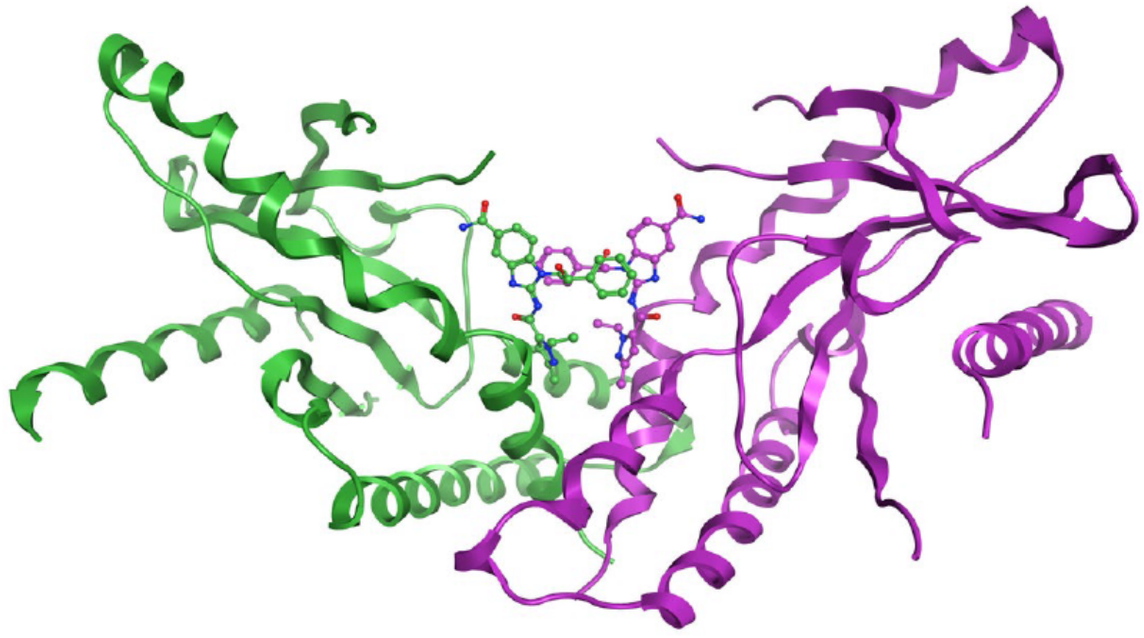


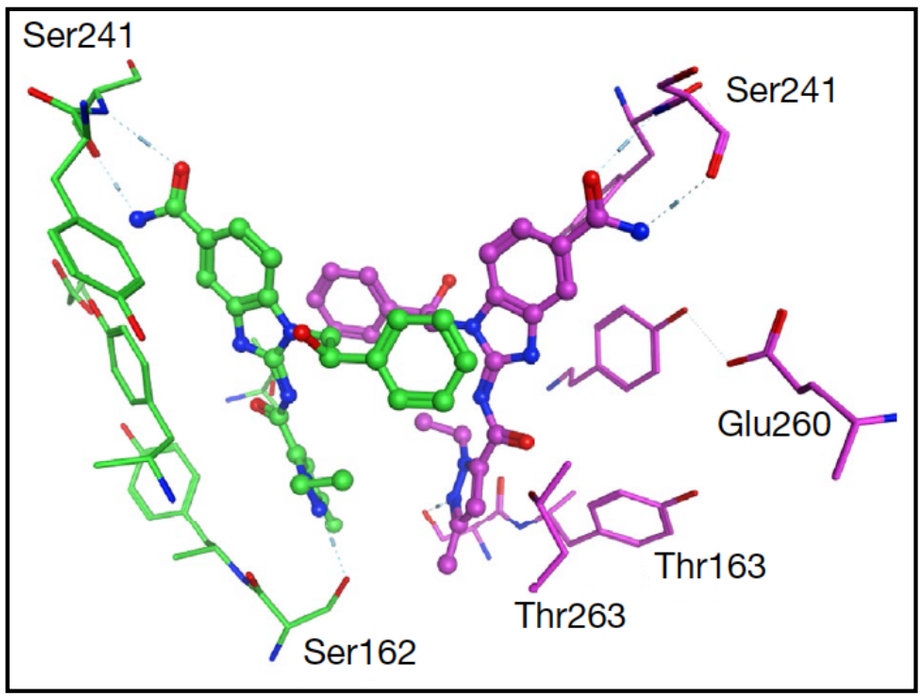
Amidobenzimidazol (ABZI)

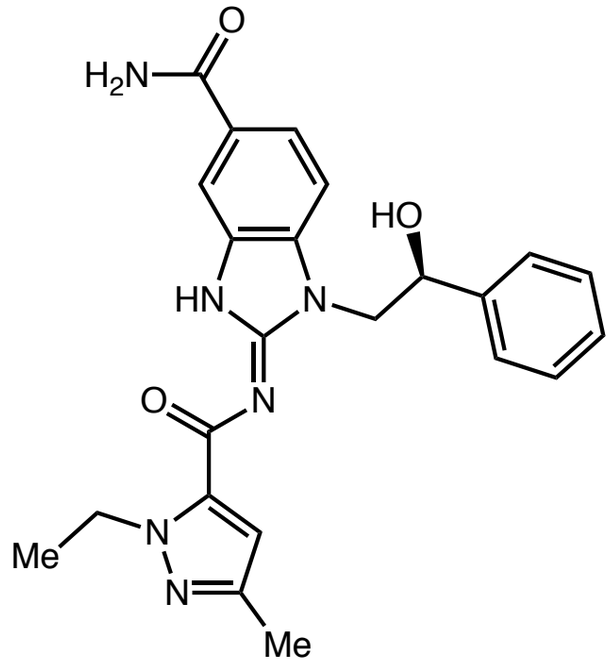
- HTS of small molecules that compete with binding of radiolabeled cGAMP
- ABZI identified with IC_{50} = 14 µmol
- two molecules ABZI bind to the STING cGAMP binding site

J. M. Ramanjulu *et al. Nature,* **2018**, *564*, 439.

Non-Nucleotide STING Agonists



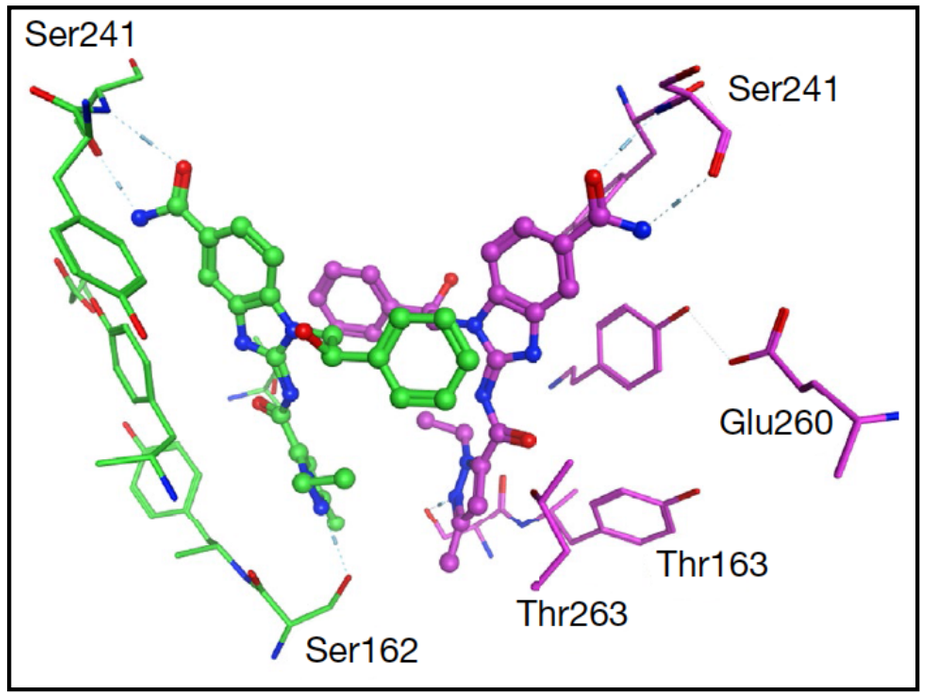


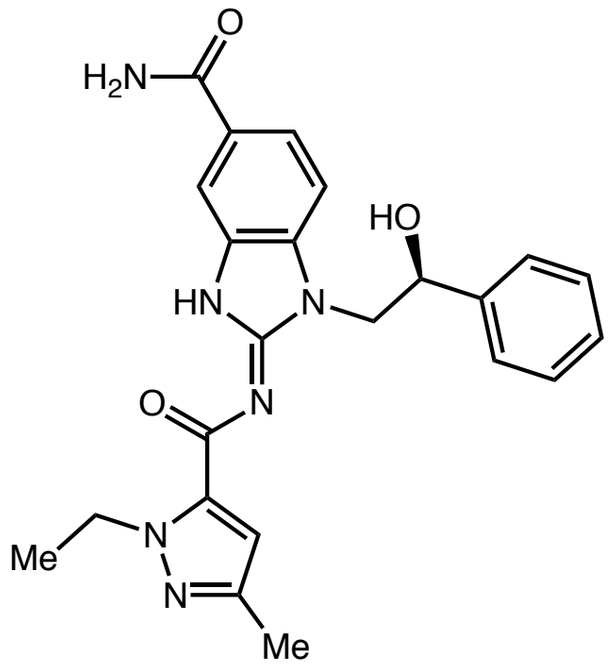


ABZI, IC_{50} = 14 µmol

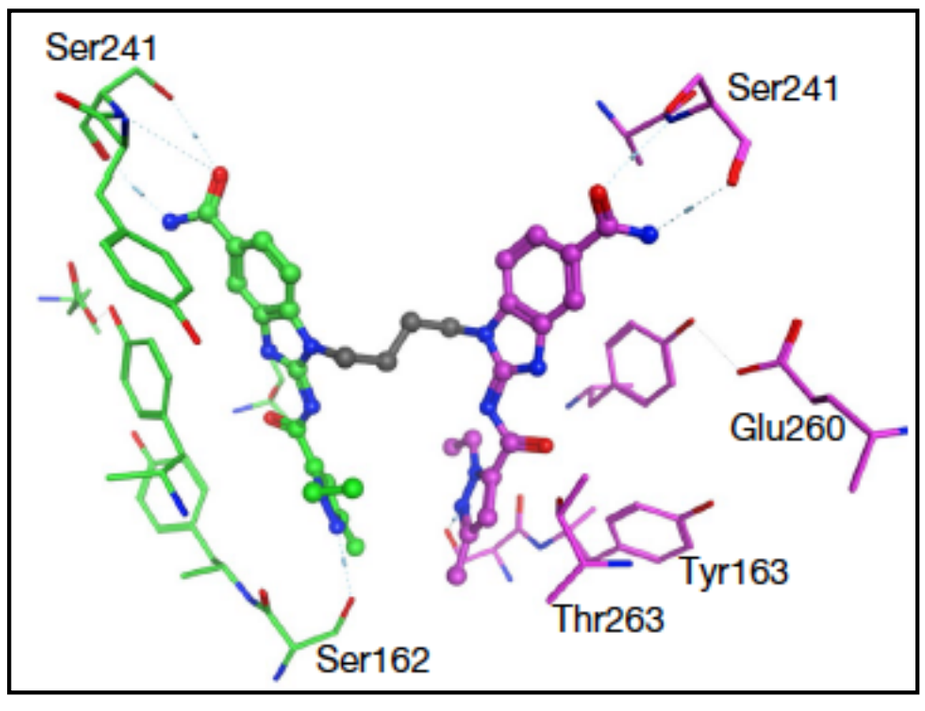
J. M. Ramanjulu *et al. Nature,* **2018***, 564*, 439.

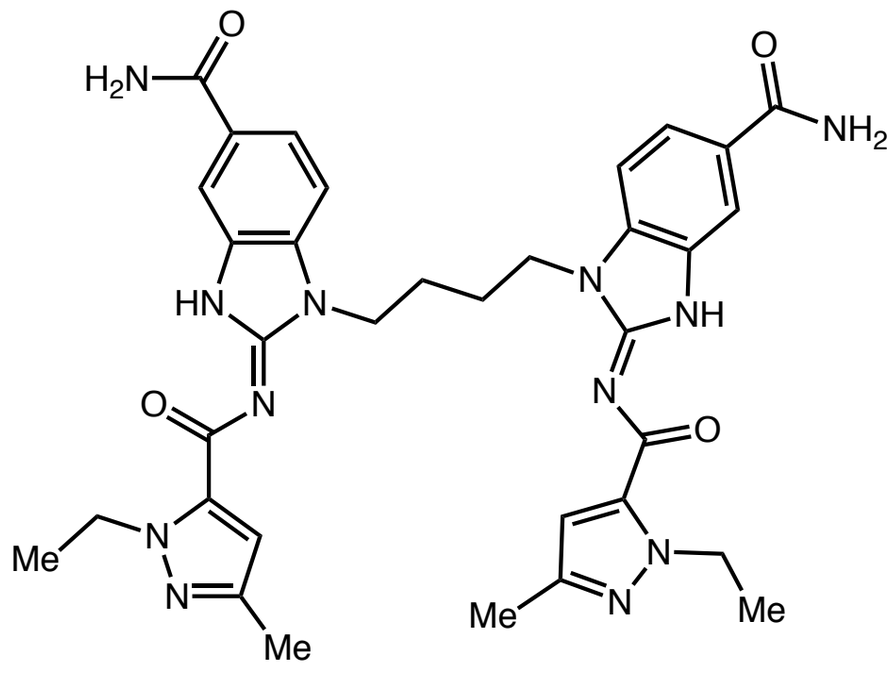
Non-Nucleotide STING Agonists





ABZI, IC_{50} = 14 µmol





diABZI, IC_{50} = 20 nmol

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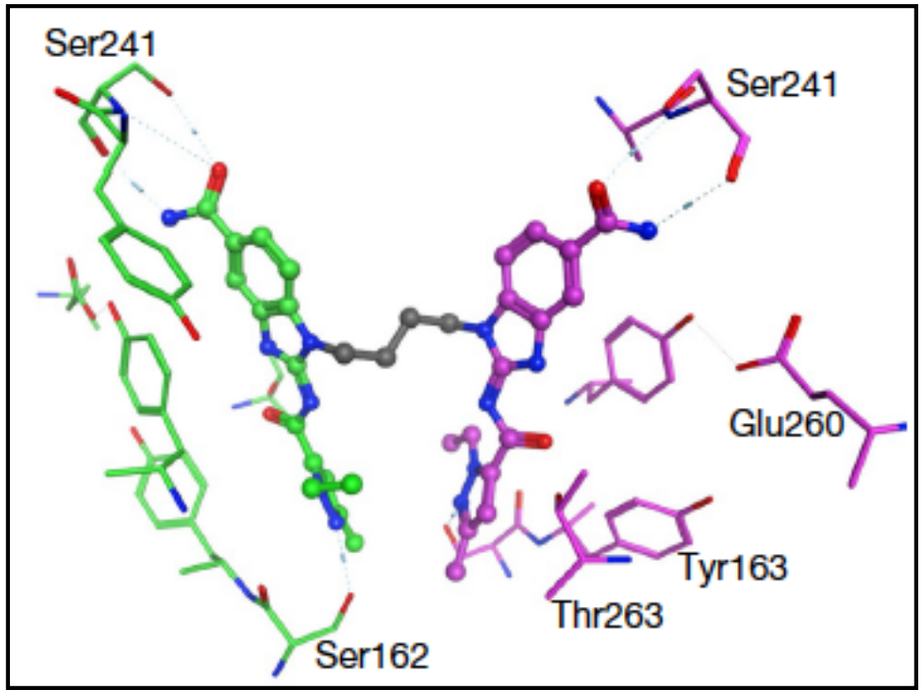
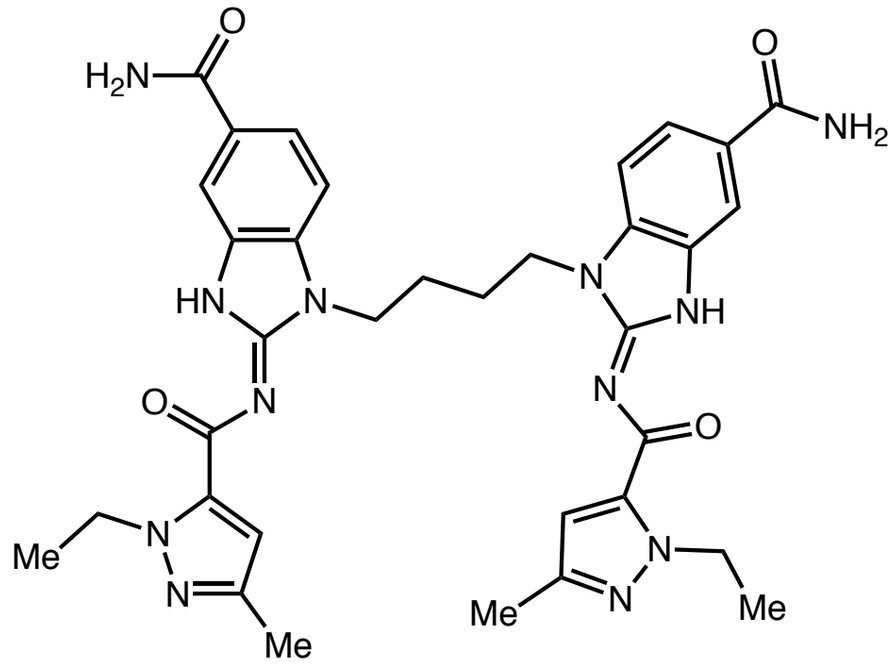
Non-Nucleotide STING Agonists





diABZI, IC_{50} = 20 nmol

Jencks' Principle

linked fragments reflect the **sum of binding energies of two unconnected fragments** if unfavorable interactions of the linker with the protein are avoided and the binding orientation is maintained

W. P. Jencks *Proc. Natl. Acad. Sci.* **1981**, *78*, 4046.

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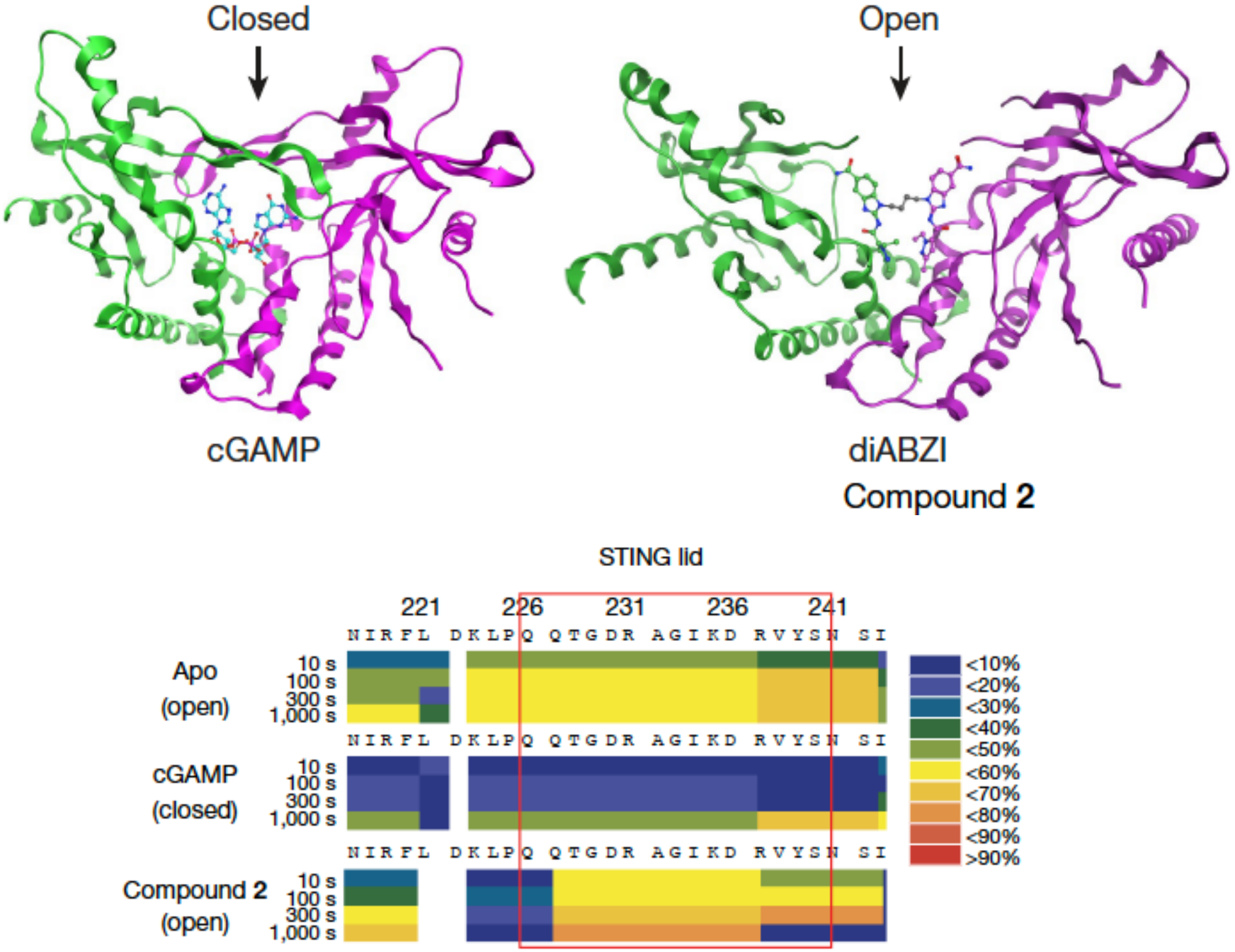
Non-Nucleotide STING Agonists



Conformational state of STING protein determined by hydrogen deuterium exchange (HDX) MS

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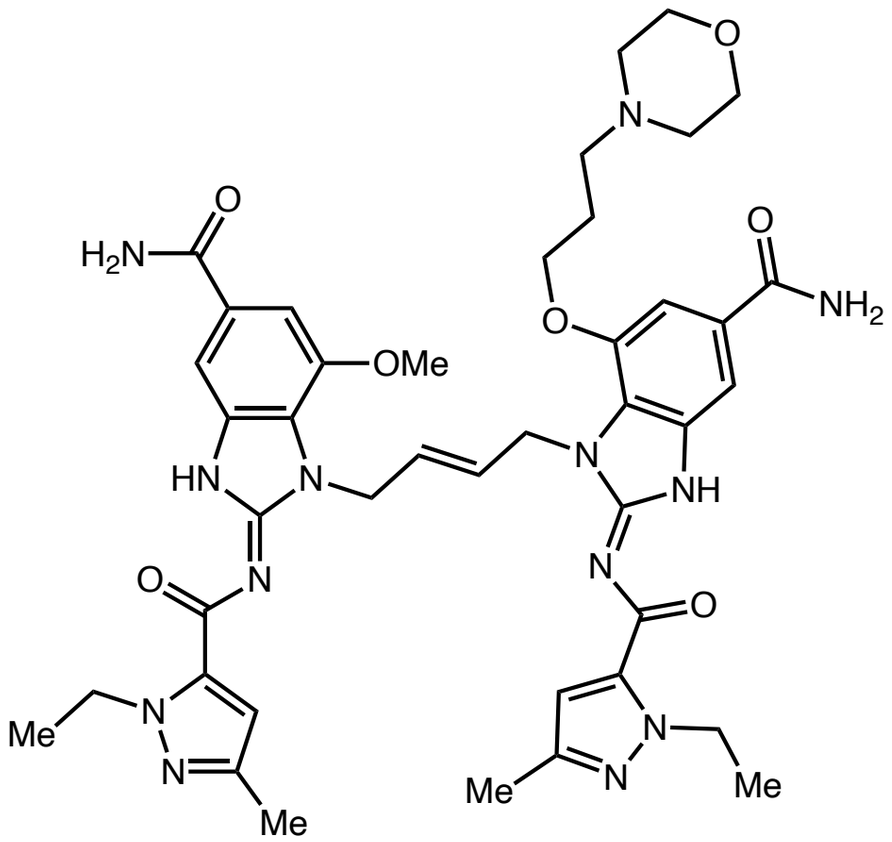
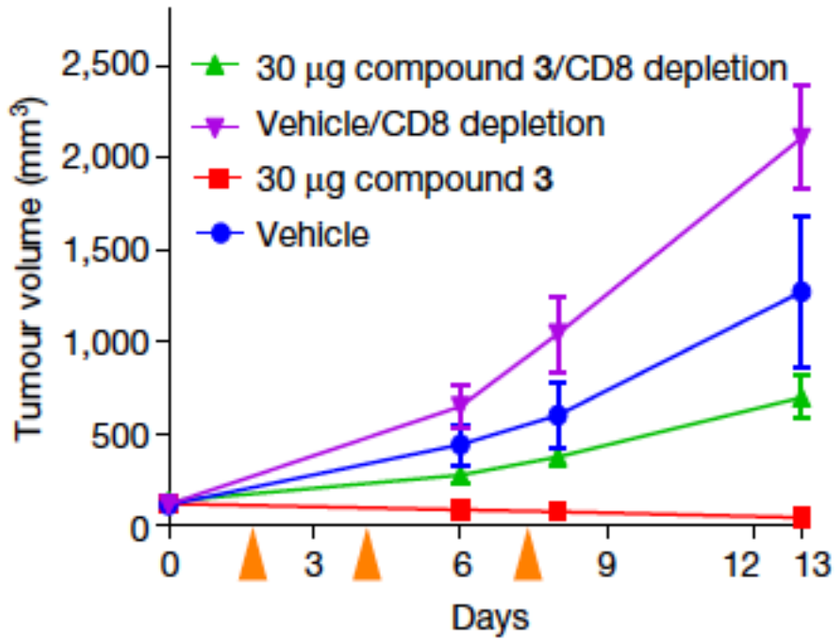
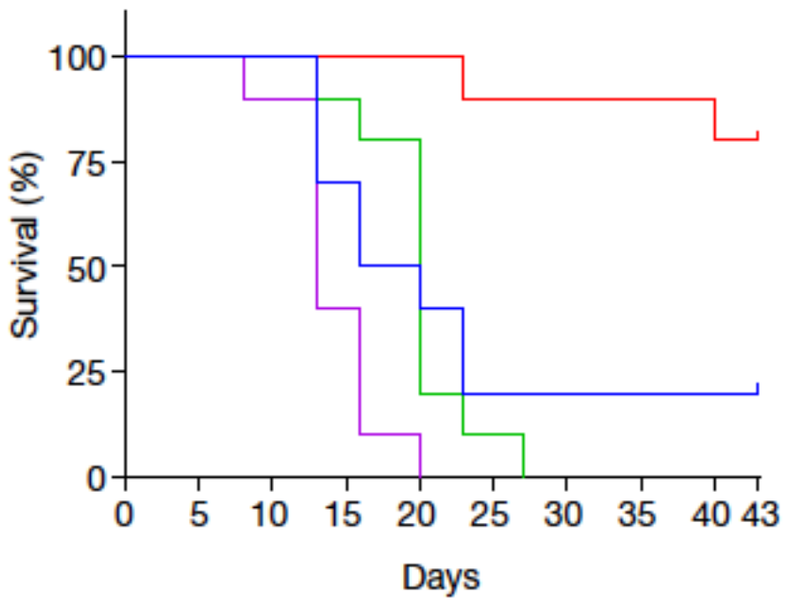
Non-Nucleotide STING Agonists

- intravenous administration of diABZI leads to adaptive $CD8^+$ T cell response in vivo



compound 3





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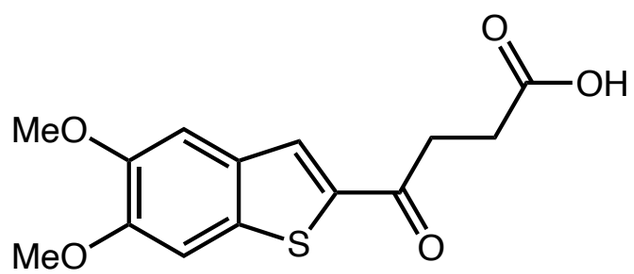
Non-Nucleotide STING Agonists

- Orally available non-nucleotide based STING Agonist



benzothiophene oxobutanoic acid (MSA-2)

- HTS of 2.4 million small molecules in phenotypic cell based assay
- MSA-2 induced IFN production only in STING containing THP-1 cells
- MSA-2 induced phosphorylation of STING pathway mediator proteins TBK-1 and IRF-3

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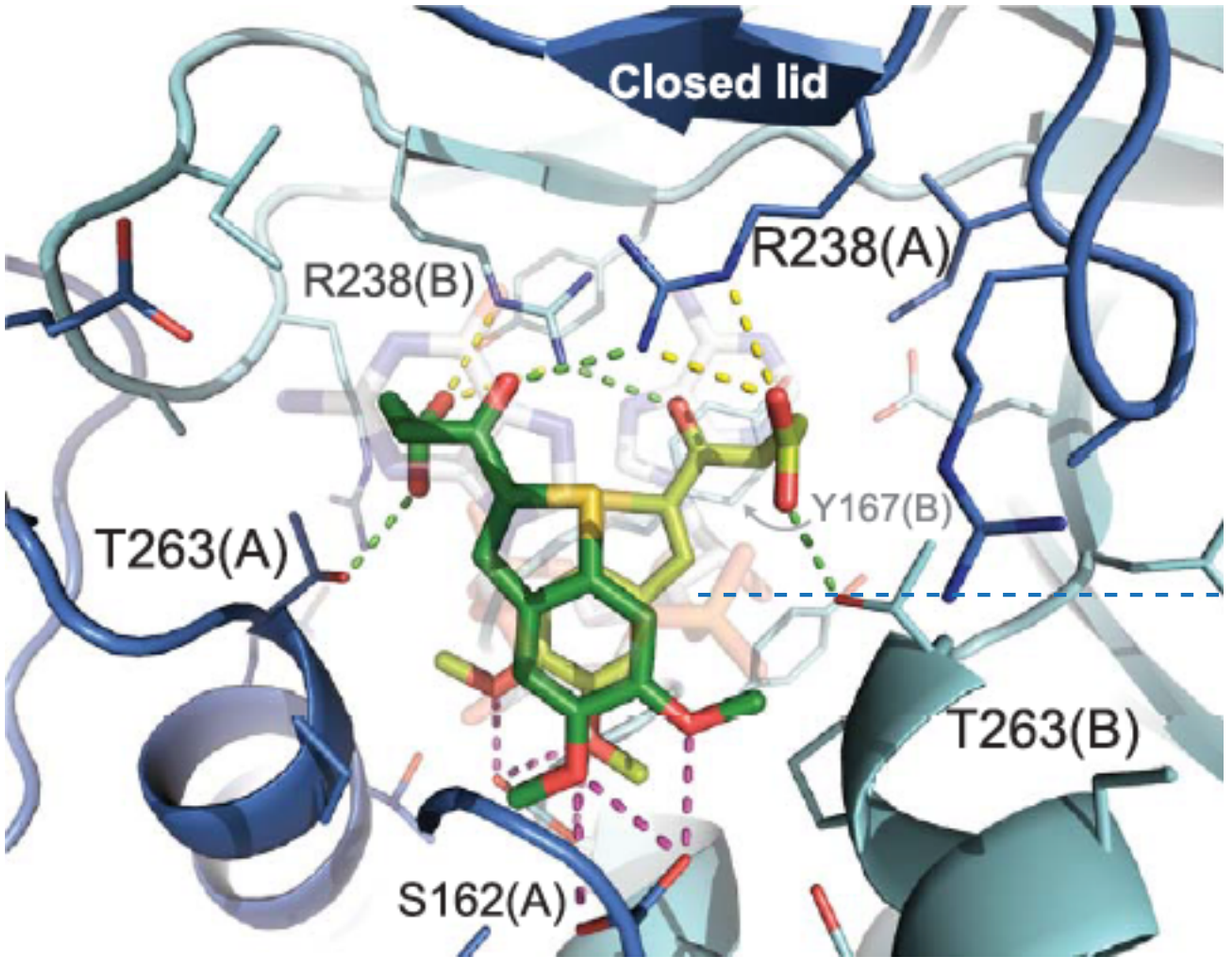
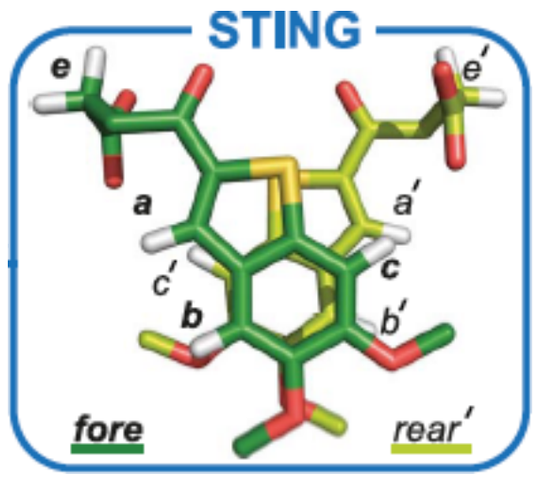
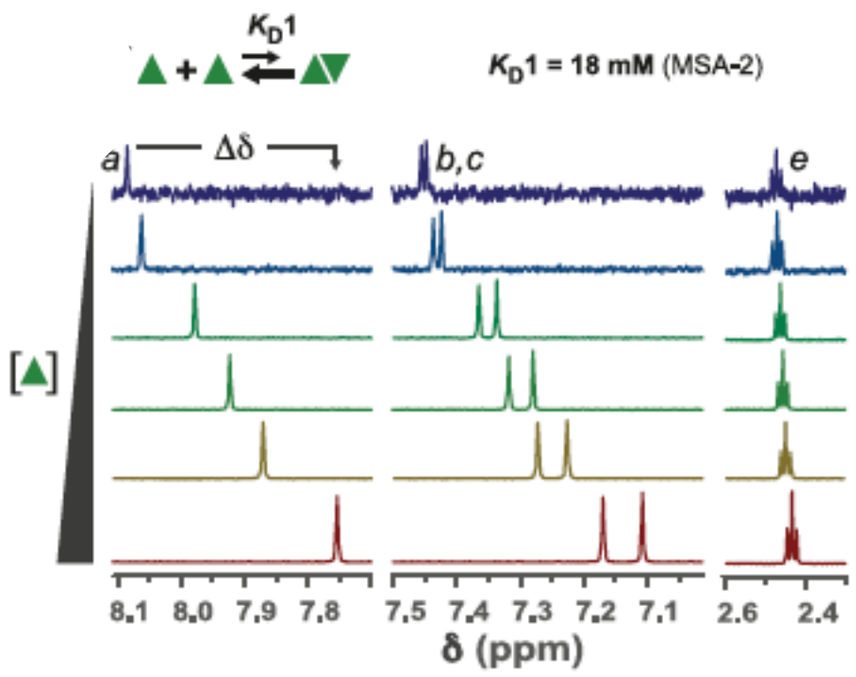
Non-Nucleotide STING Agonists



X-ray structure of MSA-2 bound to human STING

MSA-2 is bound as a non-covalent dimer to STING





Benzothiophene proton shifts change concentration dependend

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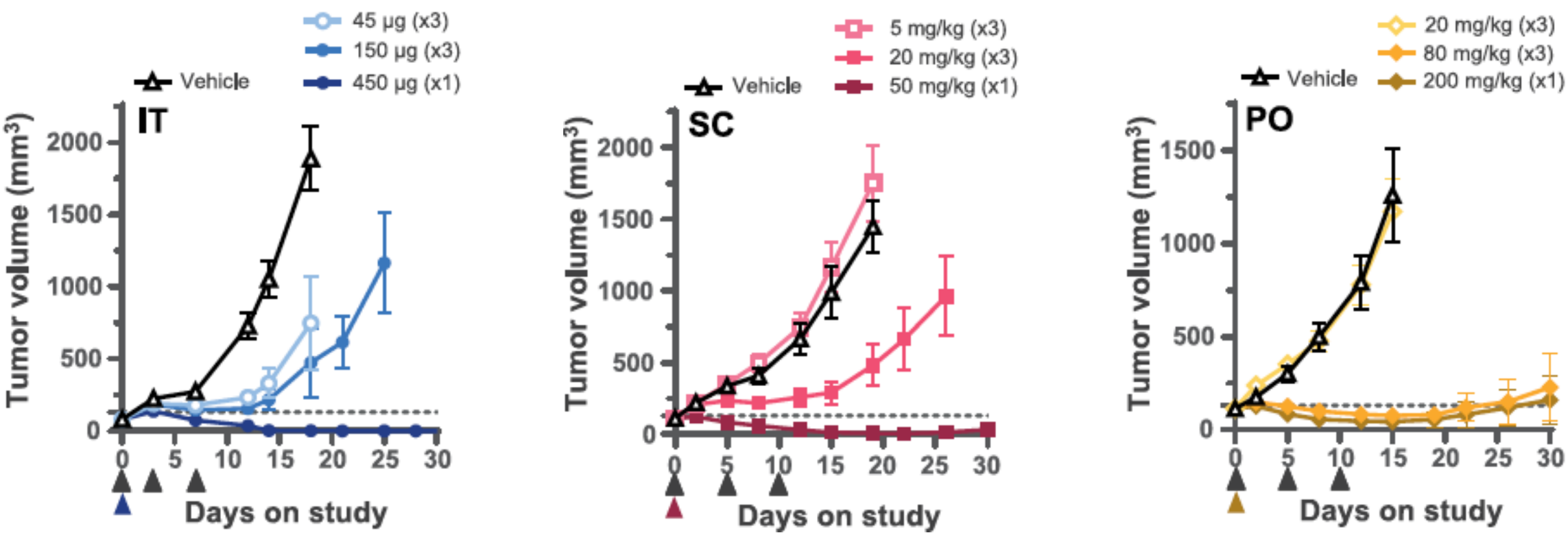
Non-Nucleotide STING Agonists

- Different administration routes of MSA-2 and effect on MC38 (colon carcinoma) tumor growth



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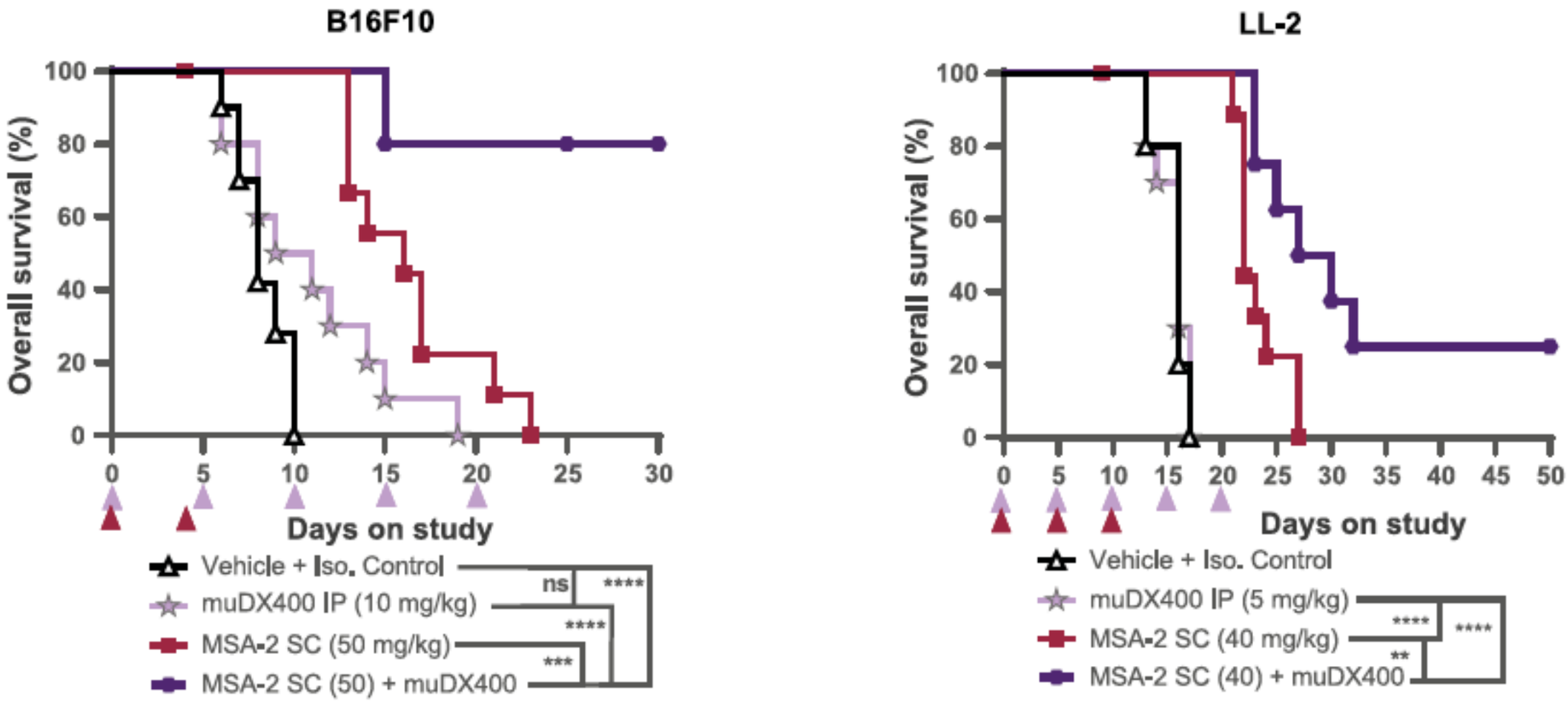
Non-Nucleotide STING Agonists

- MSA-2 enhances antitumor activity of anti-PD-1 immune checkpoint inhibitor in tumor models that are poorly responsive to PD-1 blockade

- MSA-2 and anti-PD-1 are synergistic in inhibiting tumor growth

→ both innate and adaptive immune function contribute to STING agonist-driven tumor regression



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