High Resolution Crystallographic Analysis of the Competitive Binding of a Novel Nucleotide Analog GS-9148-Diphosphate to HIV-1 Reverse Transcriptase

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16th Conference on Retroviruses and Opportunistic Infections
February 8-11, 2009
Montréal, Canada

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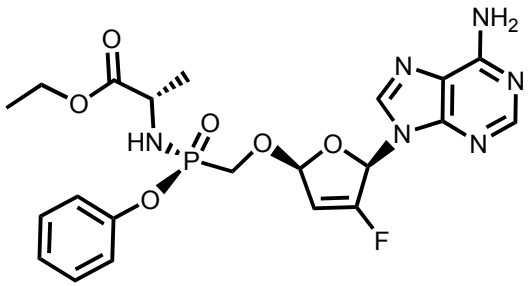
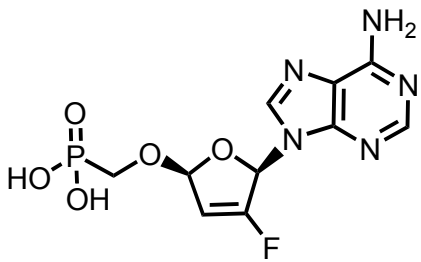
Introduction

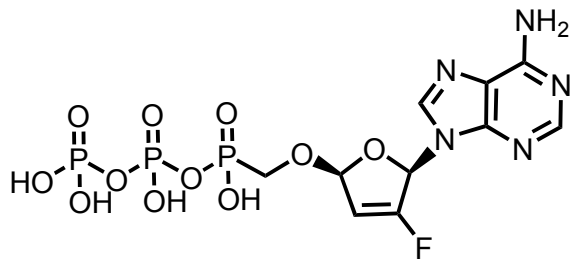
- GS-9148 is a phosphonate analog of dAMP
- Orally administered as its lymphoid–targeted amidate prodrug GS-9131



GS-9131
(Prodrug)



GS-9148
(Parent nucleotide)



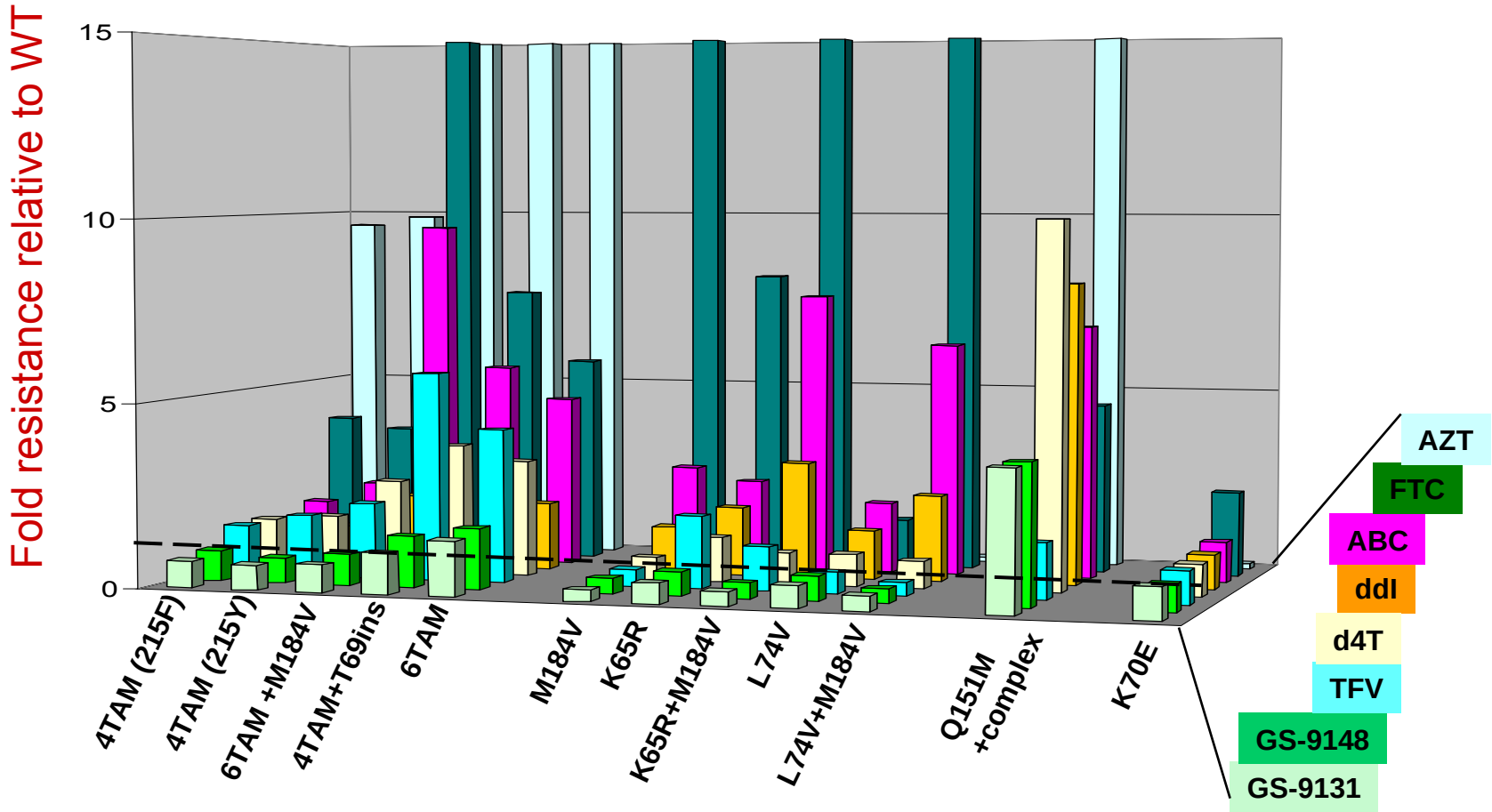
GS-9148-DP
(Active metabolite)

GS-9148/GS-9131 Profile

- Unique resistance profile
  - Maintains activity against M184V, K65R, L74V, and multiple TAM's
- Low potential for class-specific toxicities
  - Based on preclinical and *in vitro* studies
- Synergistic *in vitro* with other HIV therapies, no evidence for antagonism with tenofovir
- *In vivo* pharmacokinetic profile supporting QD oral dosing
  - High intracellular levels and prolonged retention (> 24h) of the active metabolite in PBMCs

Ray *et al.,* (2008) Antimicrobial Agents and Chemotherapy, 52 (2) 648-654
Cihlar *et al.,* (2008) Antimicrobial Agents and Chemotherapy, 52 (2) 655-665

Favorable Resistance Profile of GS-9148/GS-9131



- **PhenoSense assay** (Monogram Biosciences)

White *et al.*, (2008) 9th Annual Symposium on Antiviral Drug Resistance

Goal of this Structure Study

- Objective:
  - Understand the unique resistance profile of GS-9148 from a structural perspective

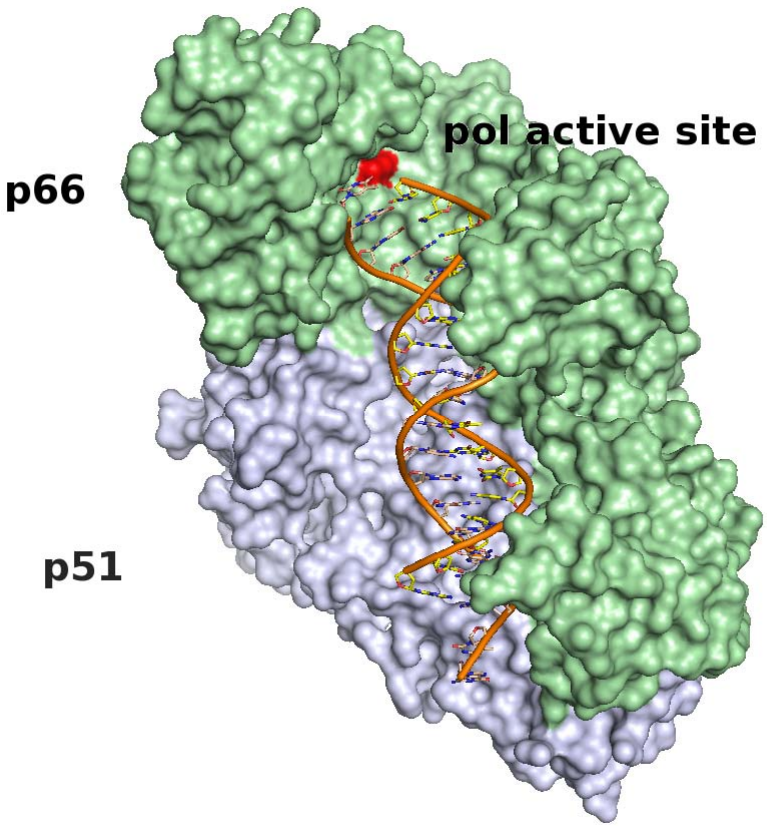
- Methods:
  - RT:DNA complex was formed by covalently tethering
  - Primer strand is dideoxy-terminated and positioned in priming site allowing a dNTP to occupy nucleotide site
  - Novel crystal form was identified ($C222_1$ space group)
  - Structures with GS-9148-DP and dATP were determined by soaking compound into RT:DNA crystals

Huang *et al.,* (1998) Science, 282(5394):1669
Sarafianos *et al.,* (2003) JBC, 278(18):16280

Crystal Structures Discussed

- RT:DNA complex structures
  - Binary fingers-open conformation
  - GS-9148-DP ternary complex
  - dATP ternary complex

RT:DNA Crystal Structure



- **Primer strand is terminated with a dideoxy nucleotide**
- **Primer is covalently tethered to Q258C mutation in p66**

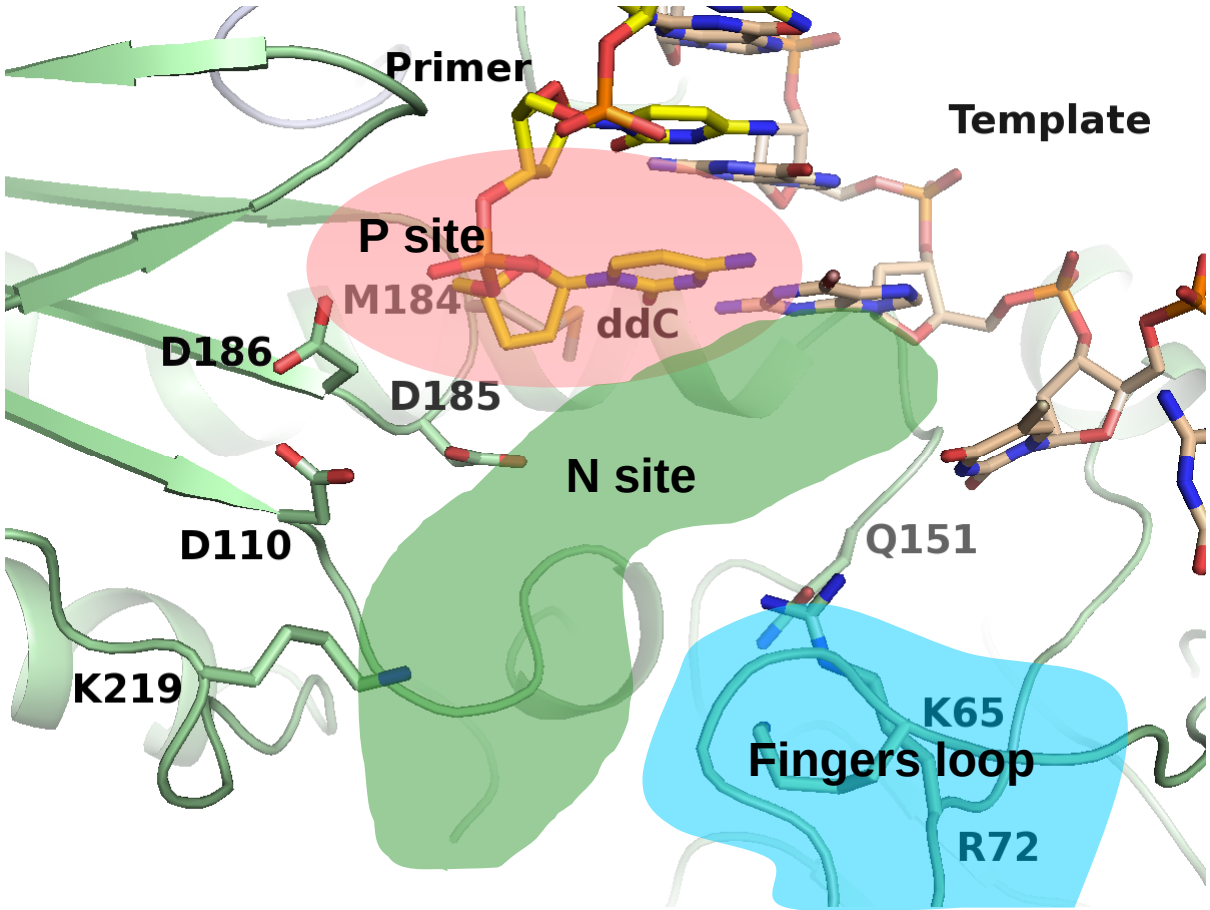
5’-ACAGTCCCTGTTCGGGCGCCC$_{dd}$-3’

Site of tethering to Q258C

Resolution =3.1Å

R/R$_{free}$=23.9/33.9

HIV-1 RT Polymerase Active Site



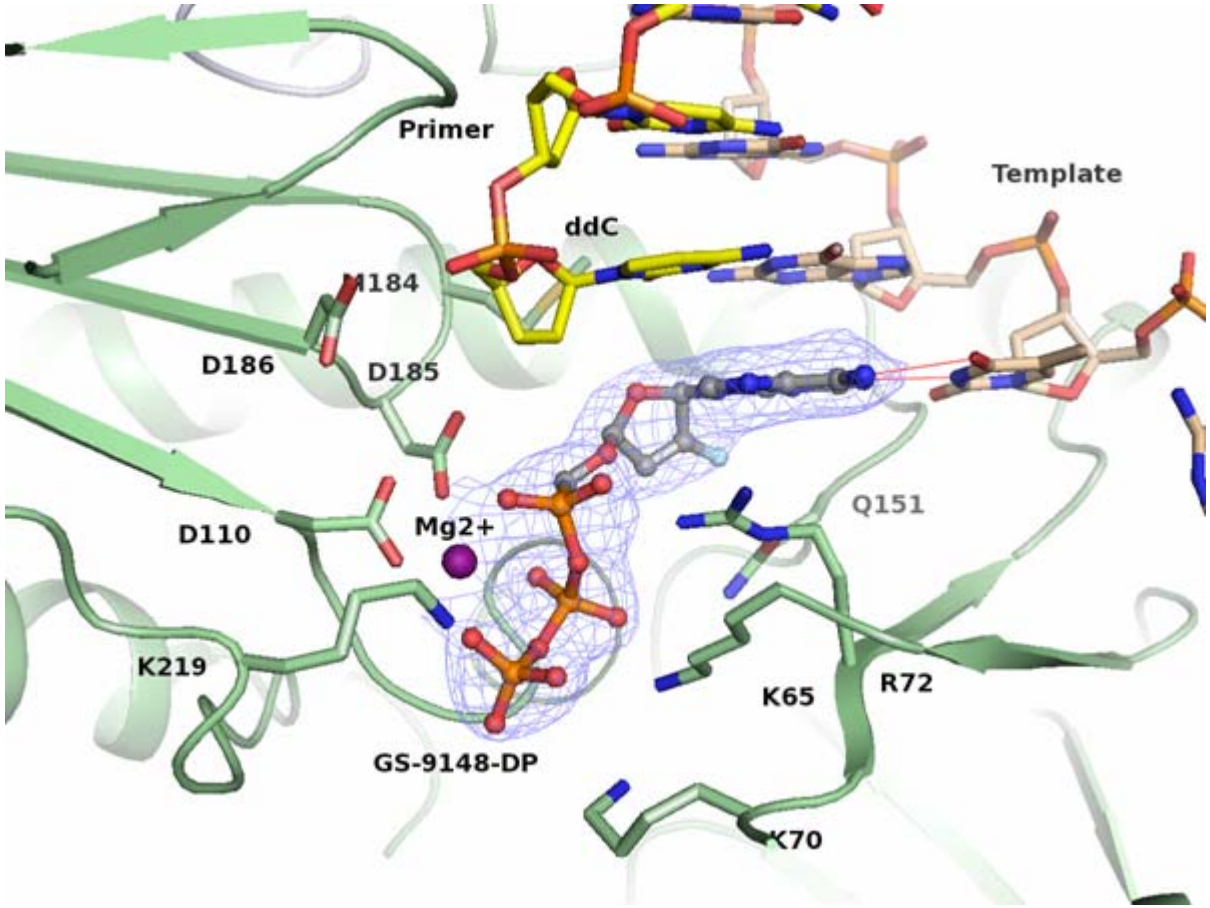
P site: priming site

- **Dideoxy termination prevents reaction with incoming dNTP**
- **Allows visualization of GS-9148-DP in N site**

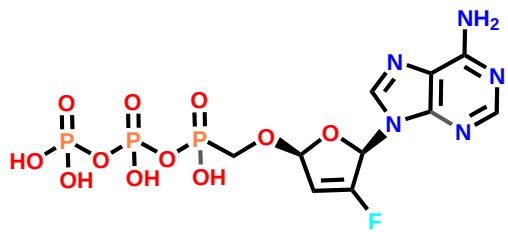
N site: nucleotide binding site

Fingers loop: moves to bind incoming dNTP

GS-9148-DP Electron Density in N site



- Electron density clearly describes all atoms of GS-9148-DP
- Normal Watson-Crick base pair with templating base



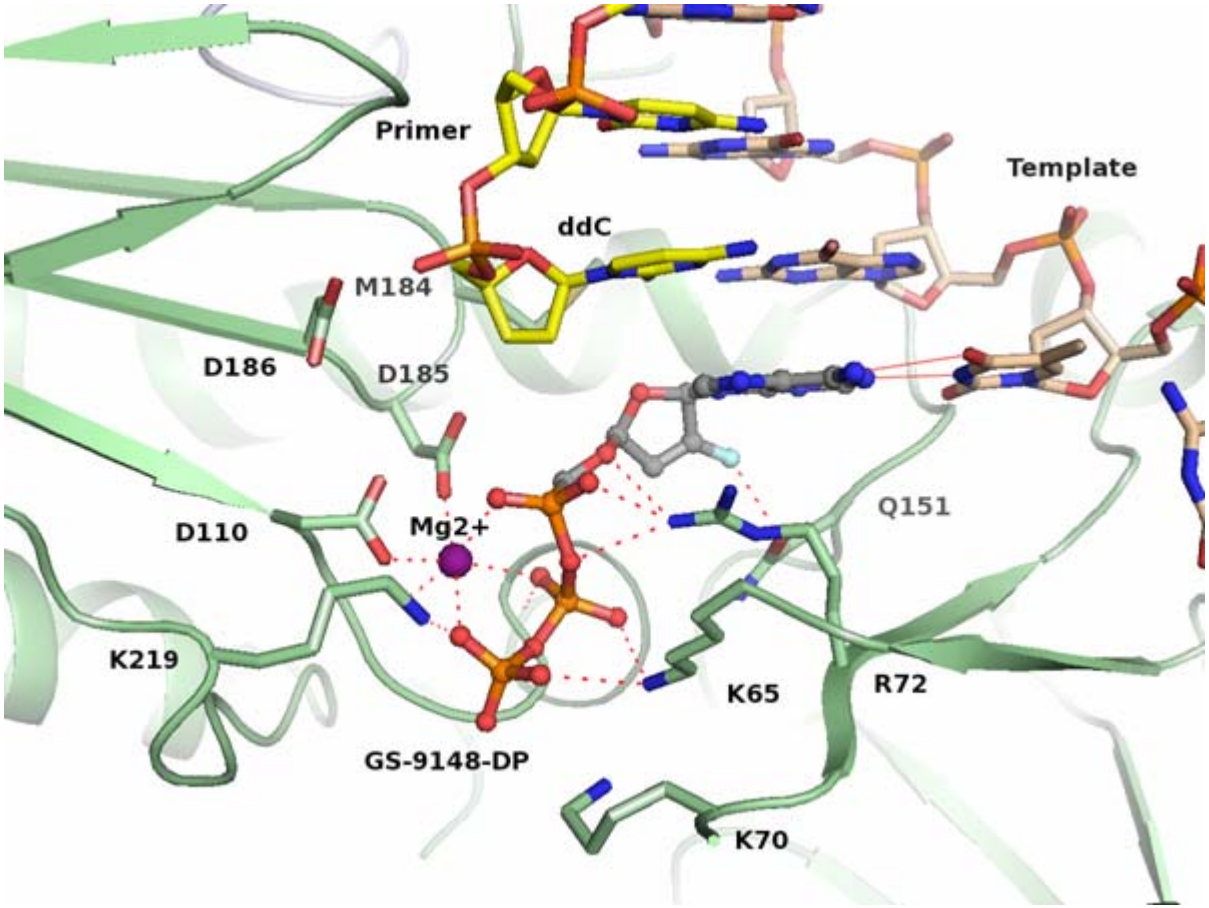
GS-9148-DP

Resolution =2.7Å

R/R_{free}=21.2/27.5

omit map drawn @1.0σ

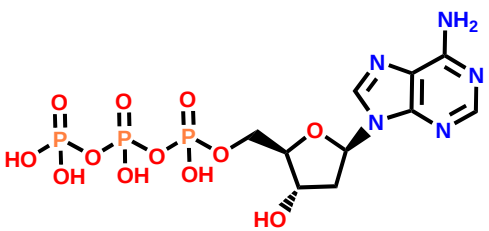
Recognition between RT and GS-9148-DP



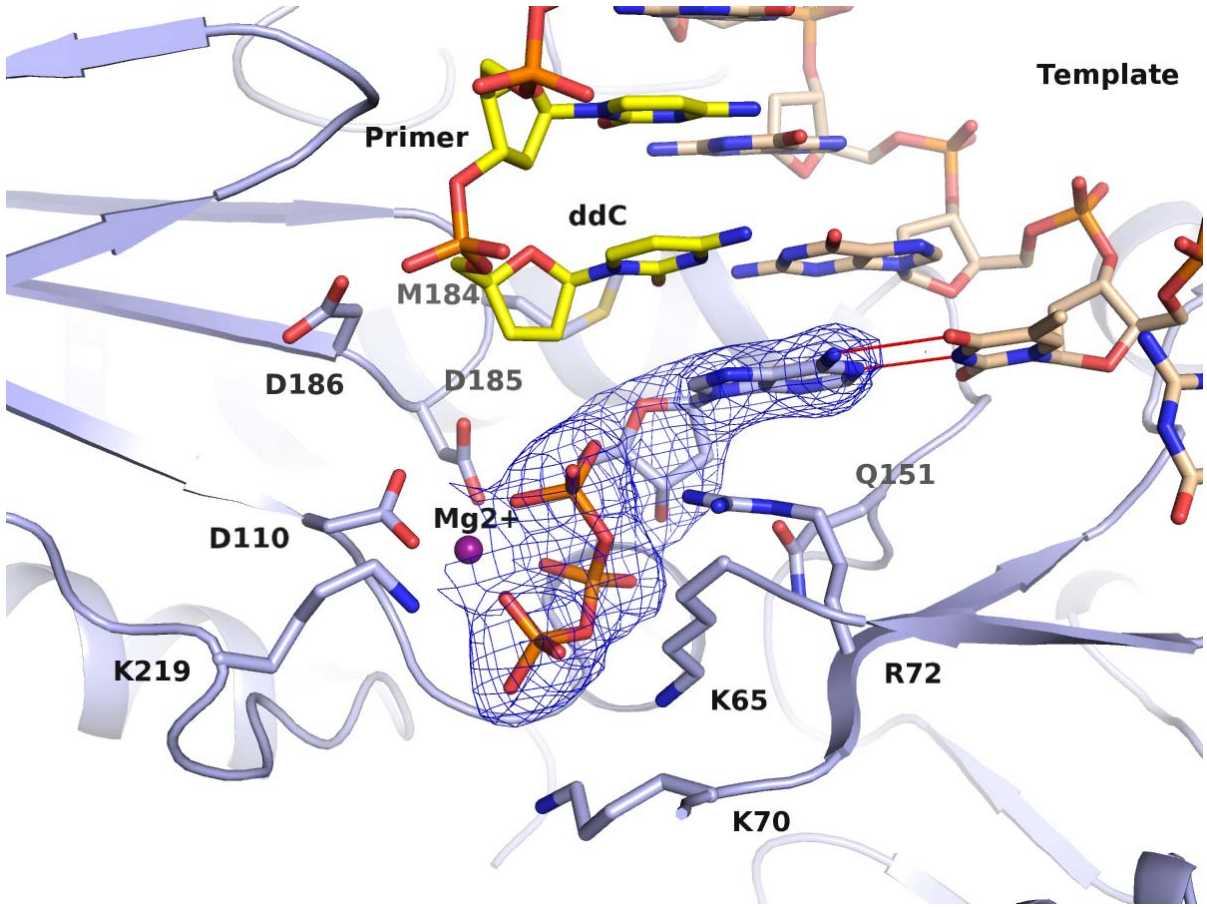
- Mg^{2+} has octahedral coordination to D110, D185, and phosphates
- K65, R72, and K219 help coordinate phosphates
- Q151 in close proximity to 2'-fluoro
- K70 in vicinity of γ-phosphate, but no interaction

Electron Density for dATP Binding in N site

- Clear electron density for all atoms of dATP
- Normal Watson-Crick base pairing



dATP



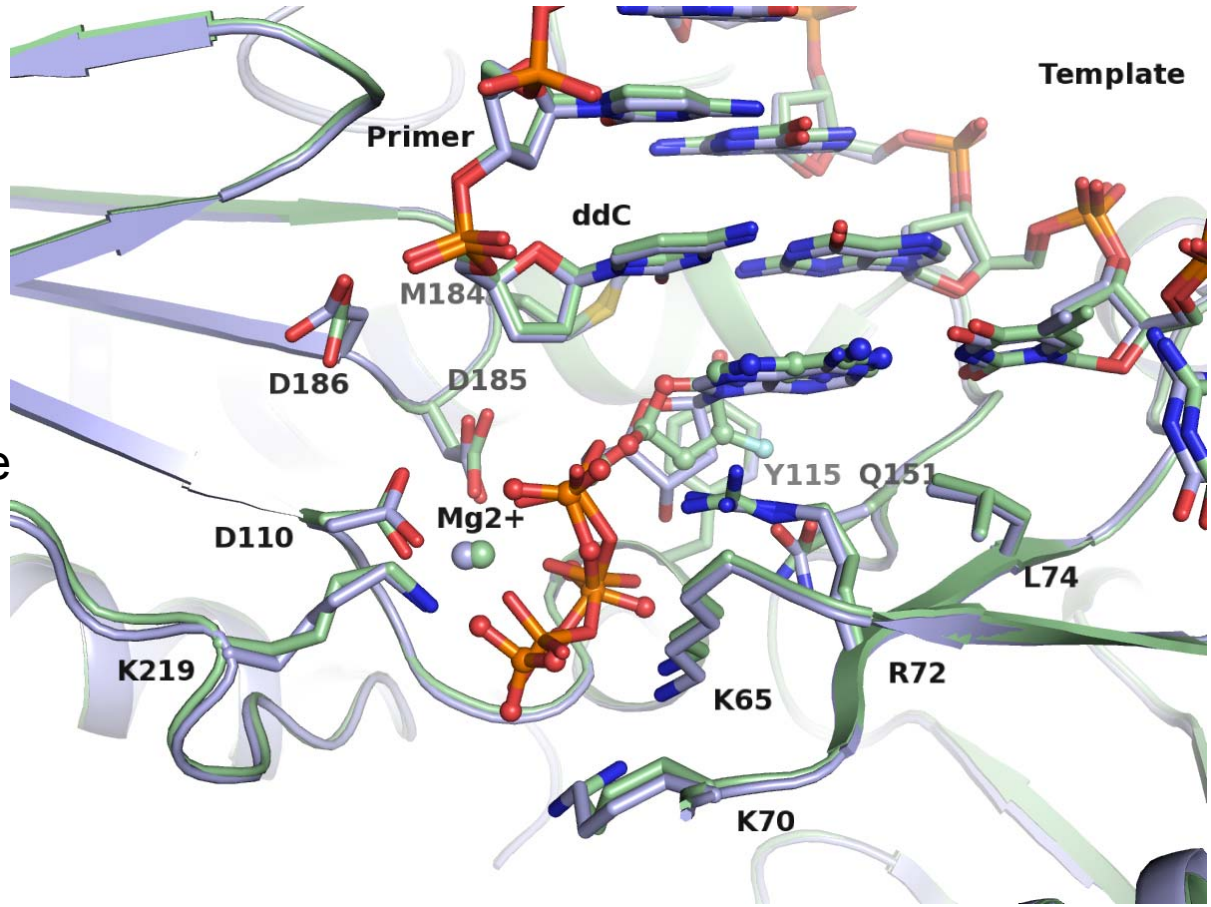
Resolution =2.9Å

R/R_{free}=20.7/26.9

omit map drawn @1.0σ

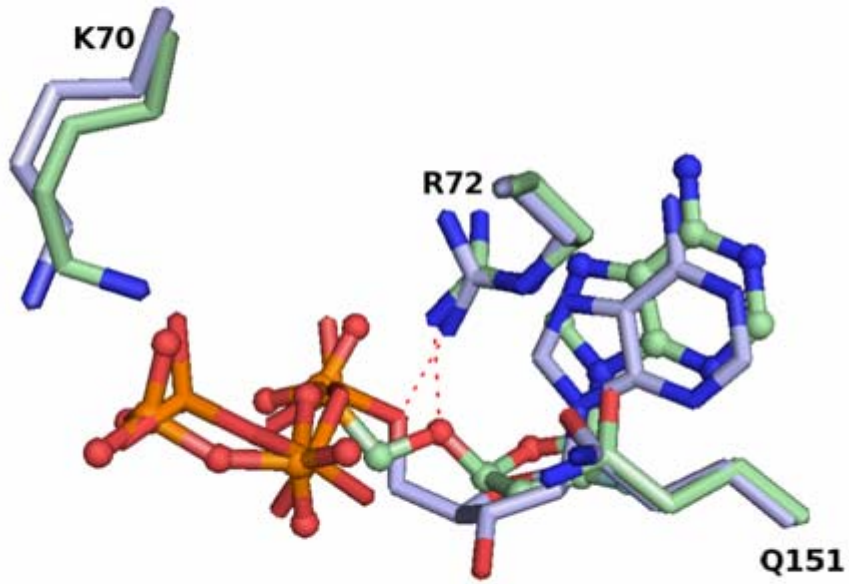
Overall Comparison between GS-9148-DP and dATP Structures

- GS-9148-DP and dATP bind in a similar fashion
  - Same H-bonds between phosphates and R72, K65, K219
  - Same Mg^{2+} coordination
- Small shift in position of the dihydrofuran ring of GS-9148-DP compared to ribose of dATP
- M184, L74, and Y115 side chains show no shift between structures



GS-9148-DP *vs* dATP

- R72 interacts with phosphonate oxygen of GS-9148-DP and phosphate oxygen of dATP
- No interaction of K70 to either GS-9148-DP or dATP
- 2'-Fluoro of GS-9148-DP extends closer Q151 than 2'H of dATP



RT: GS-9148-DP

RT: dATP

Conclusions

- GS-9148-DP is a good mimic of dATP
  - Similarity in binding modes is consistent with potency against a wide range of mutations
- GS-9148-DP 2'-fluoro is in close proximity to Q151
  - Consistent with reduced susceptibility to Q151M complex in patient isolates
- Goals of future structure studies:
  - GS-9148 incorporated in the P site
  - Exploring the mechanism of retained activity against TAMs
    - Potential resistance to excision

Acknowledgements

Protein Chemistry
Xiaohong Liu
Nilima Kutty
Debi Jin
Magdeleine Hung
Kathy Brendza
Martin McDermott
Roman Sakowicz

Structural Chemistry
Todd Appleby
John Somoza
Robert Anderson
Jarmila Jancarik

Clinical Virology
Michael Miller
Nicolas Margot

Monogram Biosciences

TriLink BioTechnologies

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