new dimension
for drug discovery

*Enamine Computational Services Report*

# **Customer** – Customer
# **Project** – SETDB1

September 15th, 2025

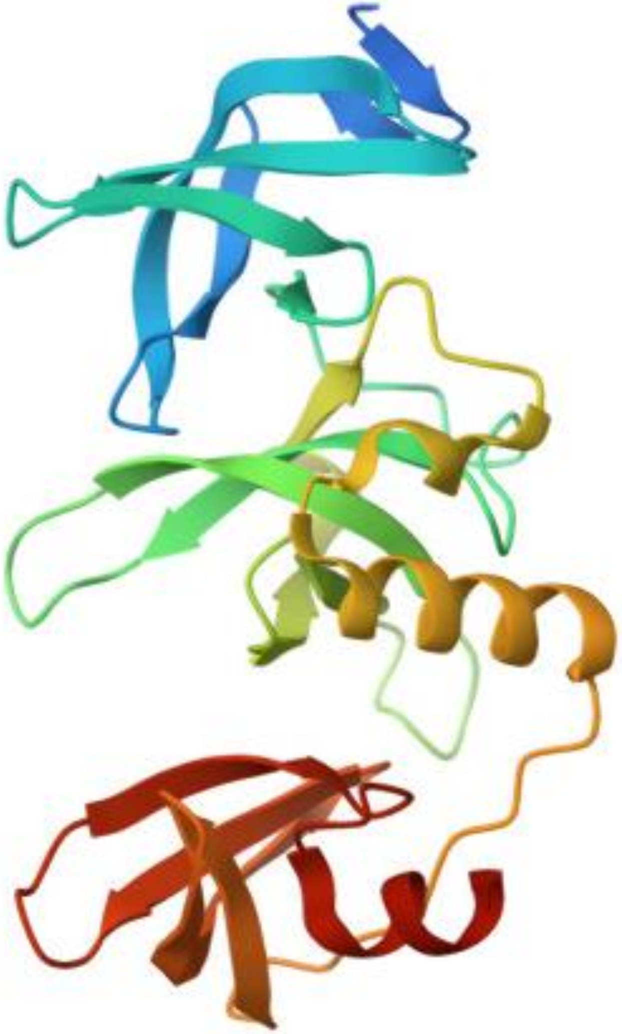
*Demo Material – For Illustration Purposes*

# Our evaluation and proposal

# SETDB1

*Demo Material – For Illustration Purposes*

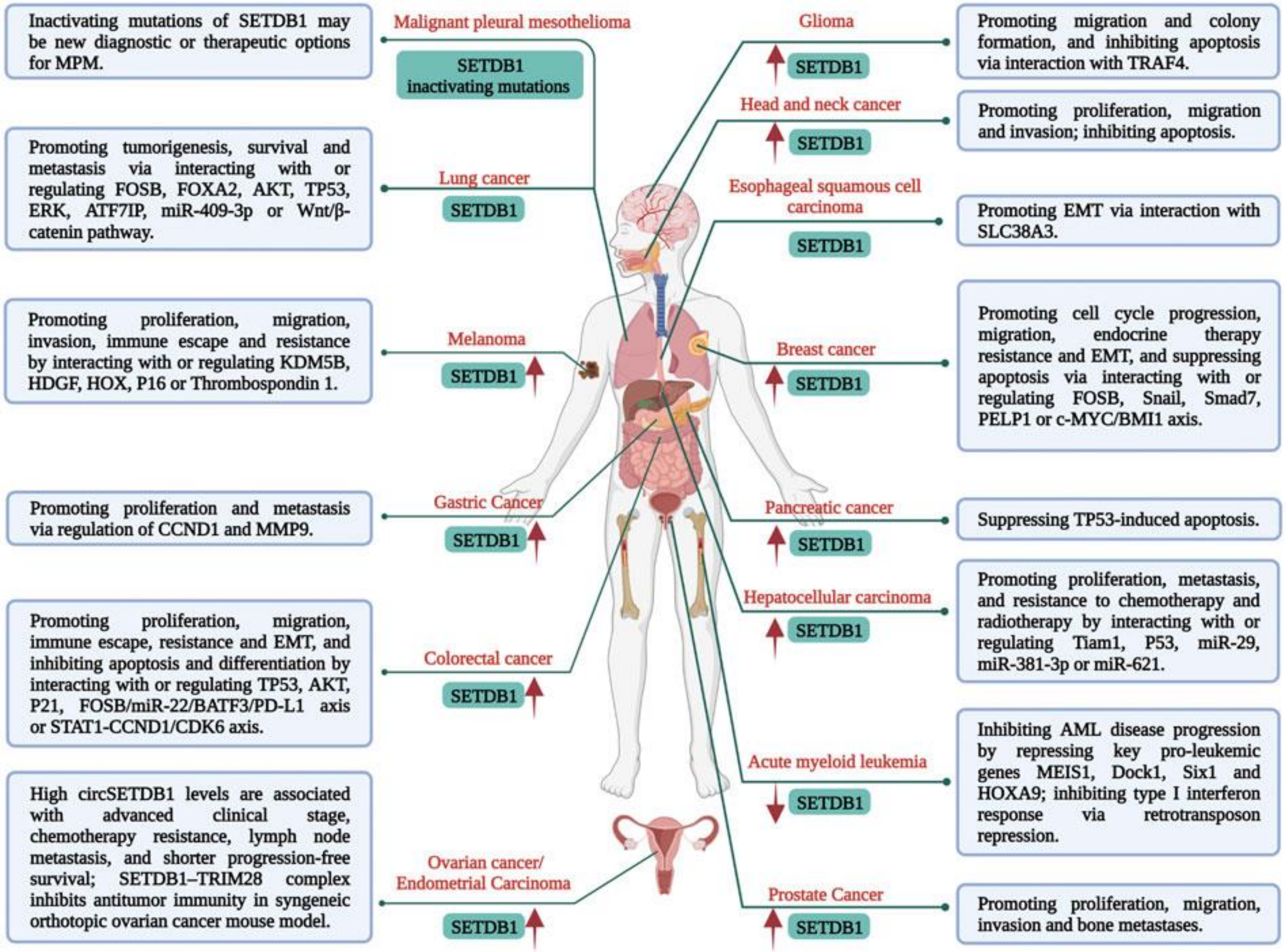
The SET Domain Bifurcated Histone Lysine Methyltransferase 1 (SETDB1) is a prominent member of the Suppressor of Variegation 3-9 (SUV39)-related protein lysine methyltransferases (PKMTs), comprising three isoforms that differ in length and domain composition. SETDB1 is widely expressed in human tissues, methylating Histone 3 lysine 9 (H3K9) residues, promoting chromatin compaction and exerting negative regulation on gene expression.



https://doi.org/10.3390/life11080817

# Multiple functions and mechanisms of SETDB1 regulation in malignancies

*Demo Material – For Illustration Purposes*



**Malignant pleural mesothelioma** — SETDB1 inactivating mutations

Inactivating mutations of SETDB1 may be new diagnostic or therapeutic options for MPM.

**Lung cancer** — SETDB1

Promoting tumorigenesis, survival and metastasis via interacting with or regulating FOSB, FOXA2, AKT, TP53, ERK, ATF7IP, miR-409-3p or Wnt/β-catenin pathway.

**Melanoma** — SETDB1 ↑

Promoting proliferation, migration, invasion, immune escape and resistance by interacting with or regulating KDM5B, HDGF, HOX, P16 or Thrombospondin 1.

**Gastric Cancer** — SETDB1 ↑

Promoting proliferation and metastasis via regulation of CCND1 and MMP9.

**Colorectal cancer** — SETDB1 ↑

Promoting proliferation, migration, immune escape, resistance and EMT, and inhibiting apoptosis and differentiation by interacting with or regulating TP53, AKT, P21, FOSB/miR-22/BATF3/PD-L1 axis or STAT1-CCND1/CDK6 axis.

**Ovarian cancer/ Endometrial Carcinoma** — SETDB1 ↑

High circSETDB1 levels are associated with advanced clinical stage, chemotherapy resistance, lymph node metastasis, and shorter progression-free survival; SETDB1–TRIM28 complex inhibits antitumor immunity in syngeneic orthotopic ovarian cancer mouse model.

**Glioma** — ↑ SETDB1

Promoting migration and colony formation, and inhibiting apoptosis via interaction with TRAF4.

**Head and neck cancer** — ↑ SETDB1

Promoting proliferation, migration and invasion; inhibiting apoptosis.

**Esophageal squamous cell carcinoma** — SETDB1

Promoting EMT via interaction with SLC38A3.

**Breast cancer** — ↑ SETDB1

Promoting cell cycle progression, migration, endocrine therapy resistance and EMT, and suppressing apoptosis via interacting with or regulating FOSB, Snail, Smad7, PELP1 or c-MYC/BMI1 axis.

**Pancreatic cancer** — ↑ SETDB1

Suppressing TP53-induced apoptosis.

**Hepatocellular carcinoma** — ↑ SETDB1

Promoting proliferation, metastasis, and resistance to chemotherapy and radiotherapy by interacting with or regulating Tiam1, P53, miR-29, miR-381-3p or miR-621.

**Acute myeloid leukemia** — ↓ SETDB1

Inhibiting AML disease progression by repressing key pro-leukemic genes MEIS1, Dock1, Six1 and HOXA9; inhibiting type I interferon response via retrotransposon repression.

**Prostate Cancer** — ↑ SETDB1

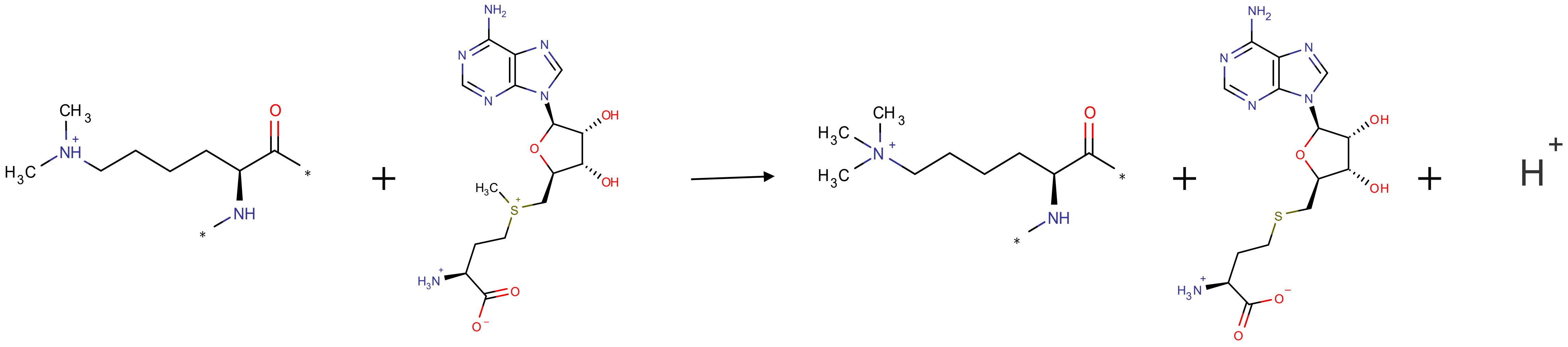
Promoting proliferation, migration, invasion and bone metastases.

SETDB1 amplification plays pivotal roles in tumourigenesis and progression, such as promoting cell proliferation, migration, invasion, epithelialmesenchymal transition (EMT), metastasis, resistance, and immune evasion.

doi: 10.3389/fphar.2022.1073713

# Catalytic activity

SETDB1 regulates gene expression by di-/trimethylating lysine 9 (K9) of H3 protein across various chromatin regions. As a chromatin regulator, it mediates H3K9 methylation, a repressive modification that compacts chromatin by recruiting Heterochromatin protein 1 (HP1), reducing accessibility and impacting gene expression. This compaction inhibits transcription factor binding, thus repressing transcription.



*N*$^6$,*N*$^6$-dimethyl-L-lysyl$^9$-[histone H3]

S-adenosyl-L-methionine

N6,N6,N6-trimethyl-L-lysyl9-[histone H3]

S-adenosyl-L-homocysteine

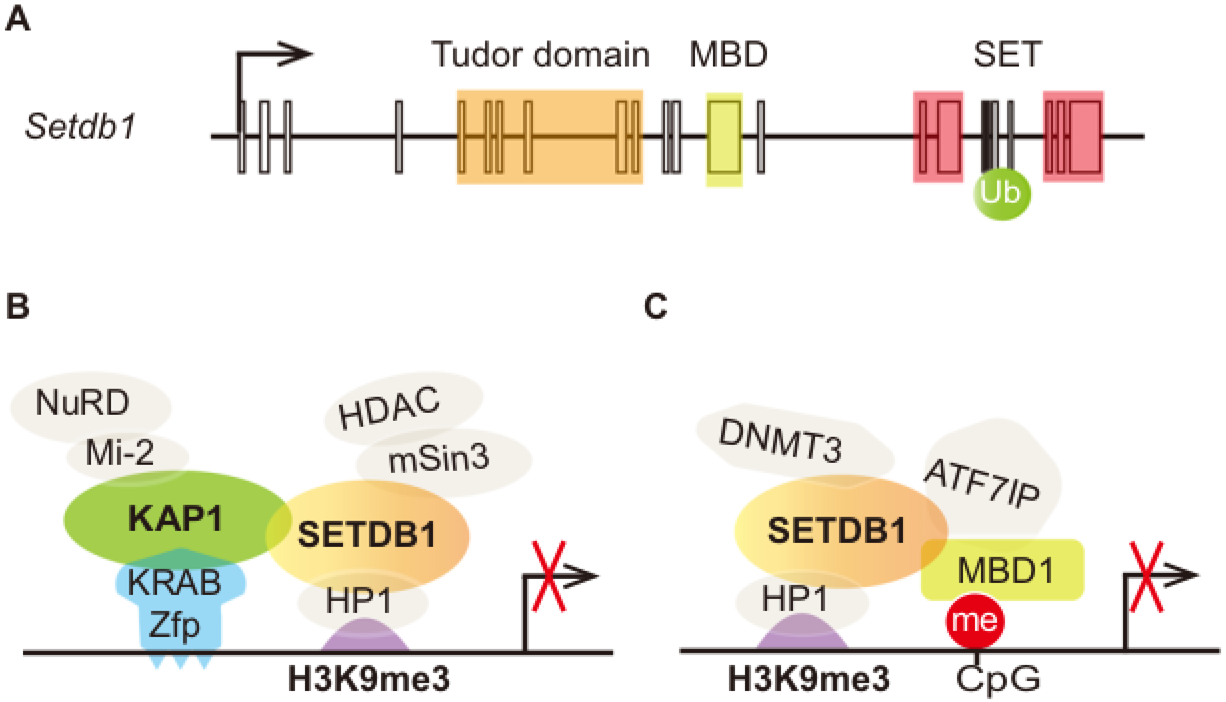
# SETDB1-associated chromatin repressive complex

*Demo Material – For Illustration Purposes*

*a* Setdb1 gene structure containing a Tudor domain, encoding protein MBD binding domain. The SET domain contains a ubiquitination (Ub) site on lysine-867.

*b* SETDB1/KAP1/ KRAB-Zfp complex. SETDB1 interacts with KAP1 and is recruited by KRAB-Zfp in a sequence specific manner. The H3K9me3 signal is established and recognized by HP-1; together with other repressive signals from the SIN3A/HDAC1/2 corepressor complex and the Mi-2/ NuRD (nucleosome remodeling deacetylase) local transcriptional repression is established.

*c* SETDB1/MBD-1/ATF7IP complex mediates the interaction between H3K9me3 and DNA methylation. DNA methyltransferases (DNMTs) also interacts with SETDB1.

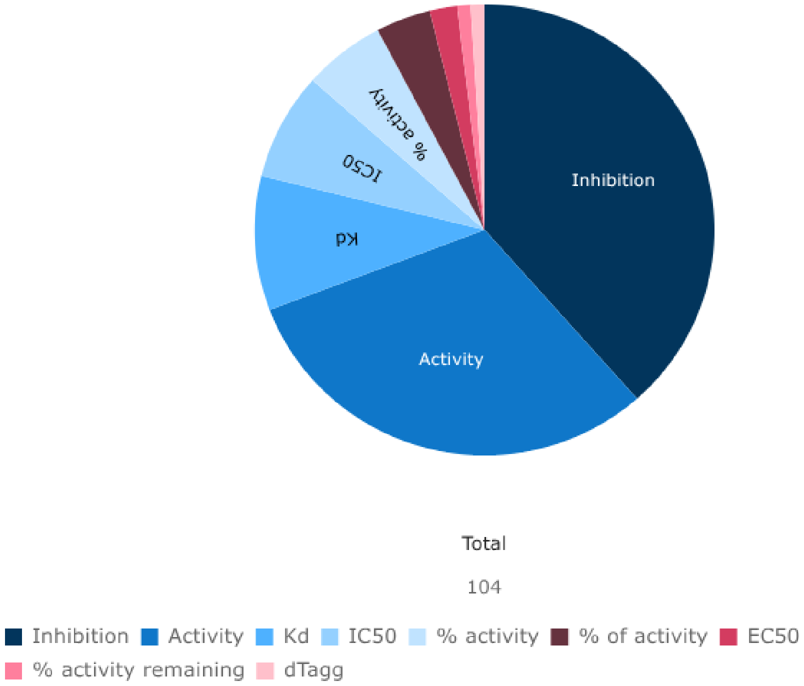


https://doi.org/10.1038/s41398-020-0797-7

# SETDB1

*Uniprot:* Q15047
*Protein:* Histone-lysine N-methyltransferase SETDB1
*Gene:* SETDB1

*ChEMBL:* CHEMBL2321646



# Triple Tudor Domain

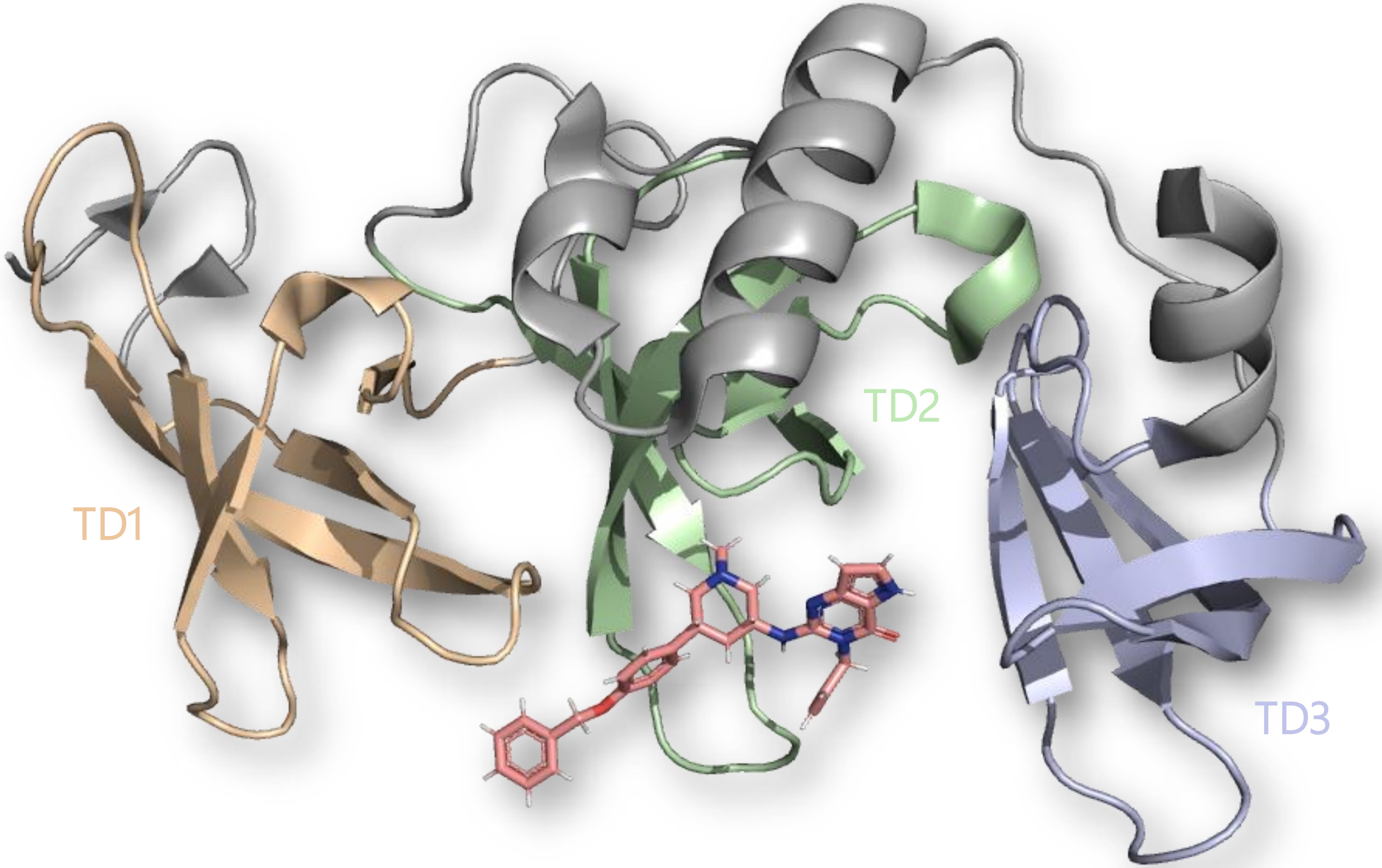
*Demo Material – For Illustration Purposes*

Tudor Domain 1: 201-251
Tudor Domain 2: 261-314
Tudor Domain 3: 350-395

PDB – 7CJT

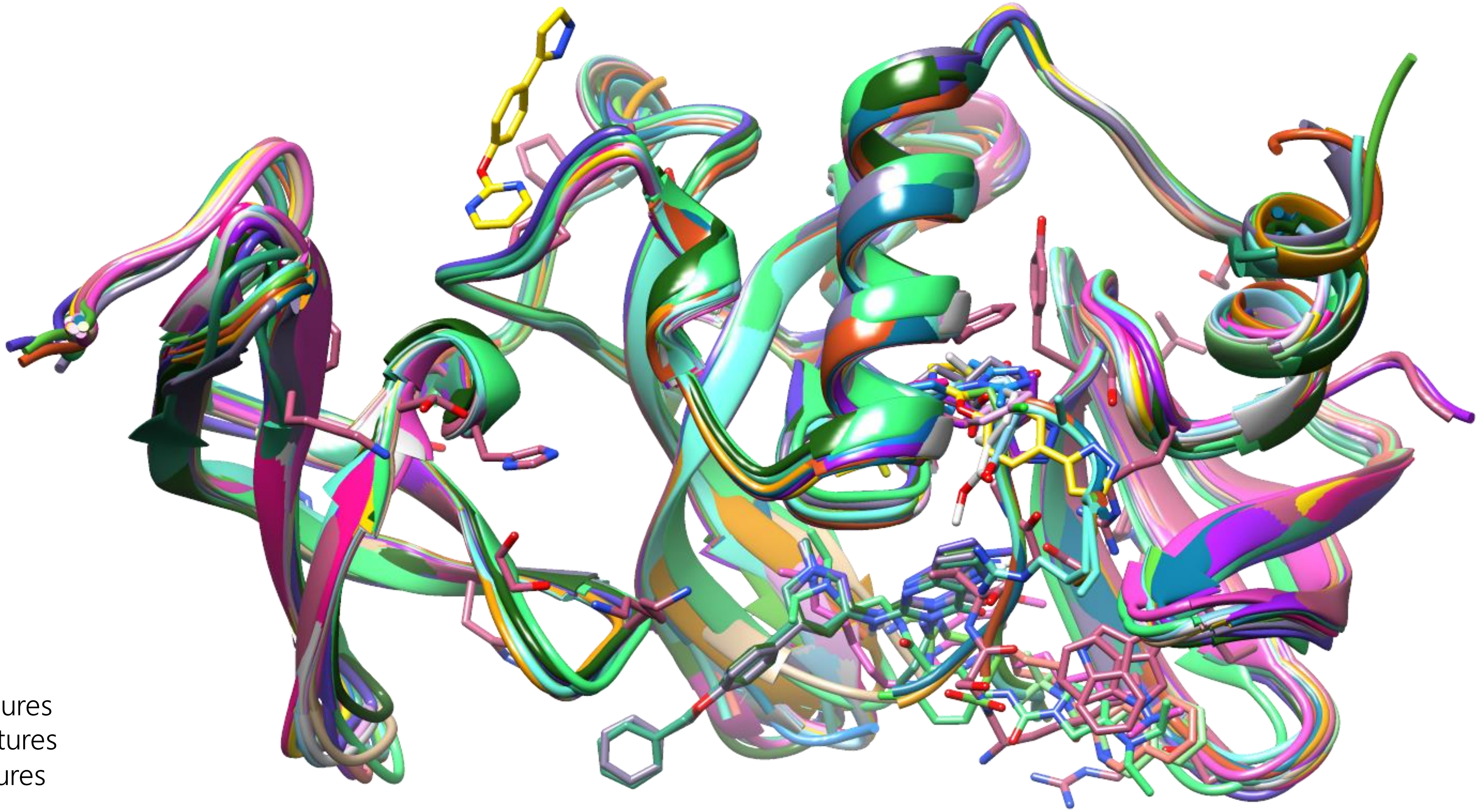
Interacts with already methylated histones, stabilizing them and ensuring proper positioning for further methylation.

Inhibition of Tudor-domain-mediated interactions is a promising approach for the treatment of diseases associated with epigenetic disorders.



# PDB SETDB1

*Demo Material – For Illustration Purposes*

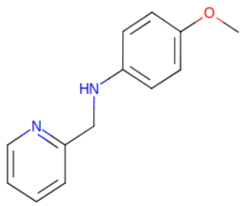


*UniProt:* Q15047
Structures – 23

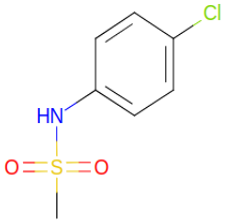
Ligand-contained:
Pocket 1 – 7 structures
Pocket 2 – 8 structures
Peptide – 6 structures

# Pocket 1

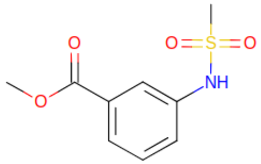
*Demo Material – For Illustration Purposes*



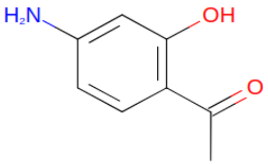
5KCH



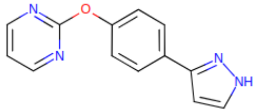
5KCO



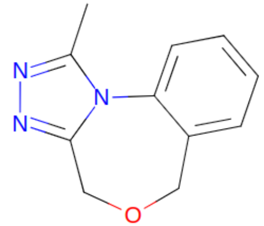
5KH6



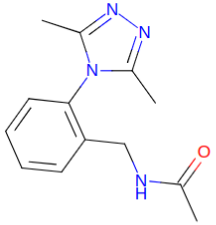
5QT1



5QT2

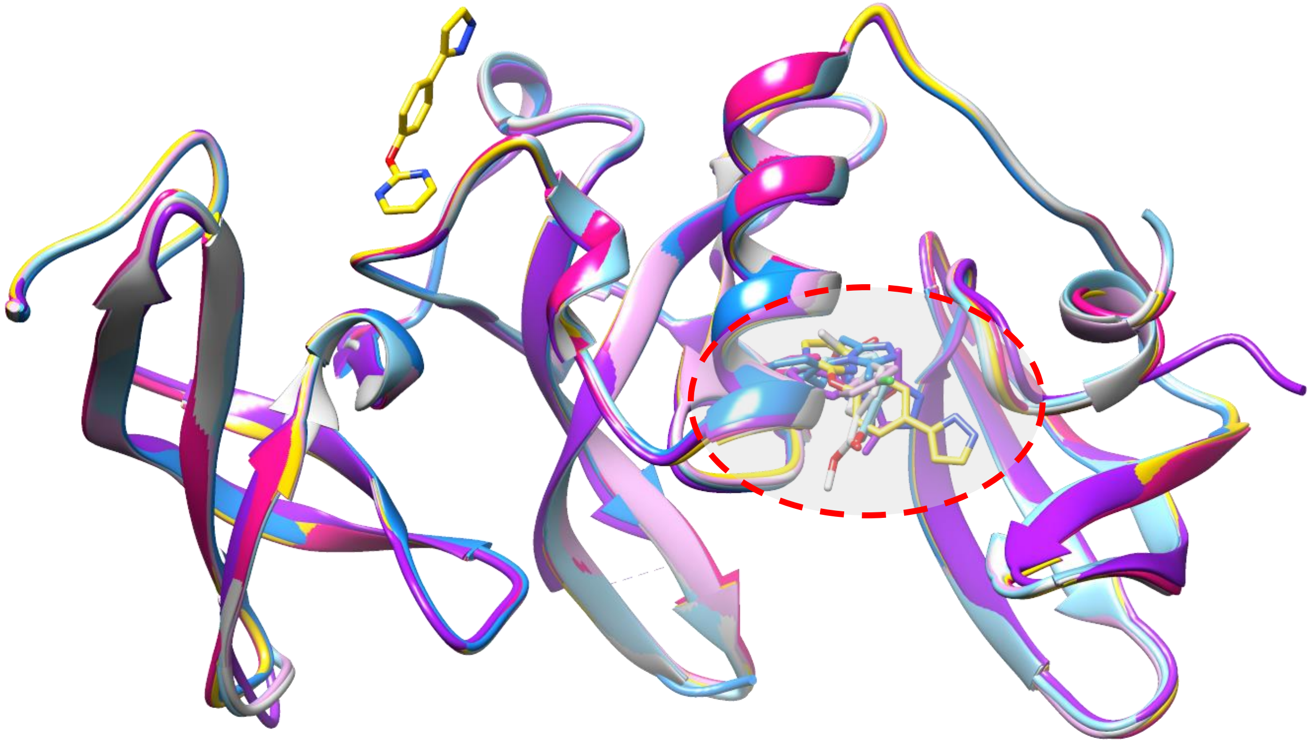


6AU2



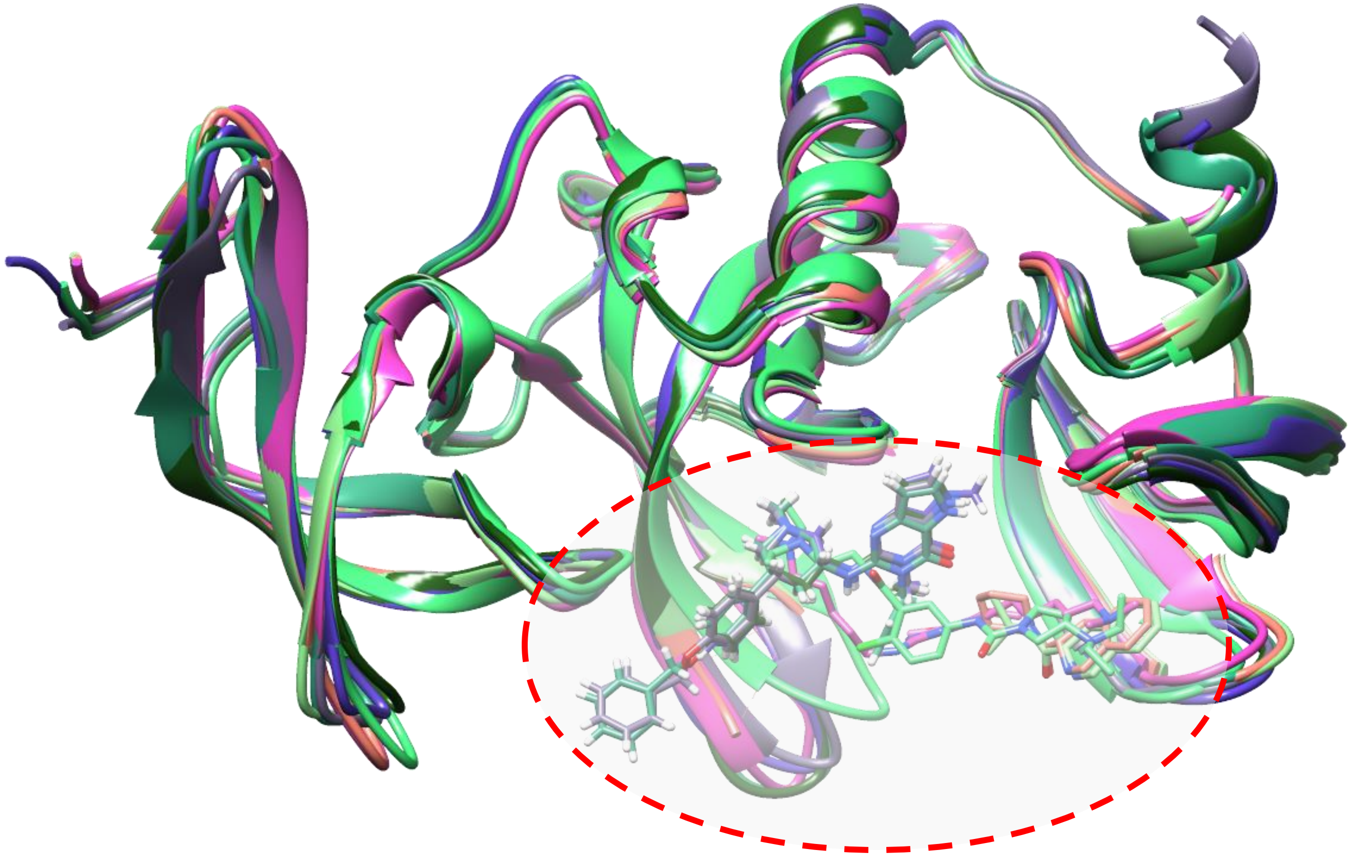
6AU3

| Identifier | Color | Resolution | Chain | Ligand |
|---|---|---|---|---|
| 5KCH | 🟦 | 1.70 Å | A | Fragment candidate |
| 5KCO | 🟪 | 1.47 Å | A | Fragment candidate |
| 5KH6 | ⬜ | 2.05 Å | A | fragment candidate |
| 5QT1 | 🟥 | 1.58 Å | A | FMOMB000017a |
| 5QT2 | 🟨 | 1.59 Å | A | FMOPL000074a |
| 6AU2 | 🟦 | 1.63 Å | A | Triazole fragments |
| 6AU3 | 🟪 | 1.80 Å | A | Aryl triazole fragments |



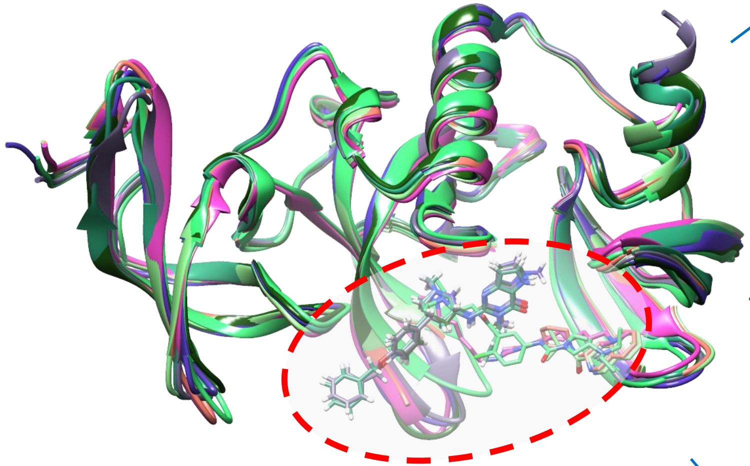
# Pocket 2

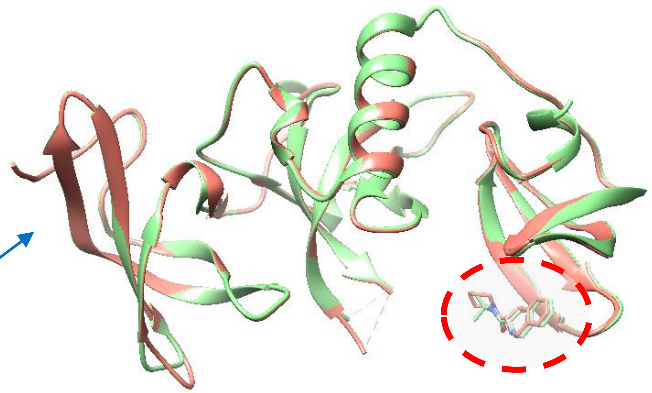
| Identifier | Color | Resolution | Chain | Ligand |
|---|---|---|---|---|
| 5KE2 | 🟩 | 1.56 Å | A | inhibitor XST06472A |
| 5KE3 | 🟧 | 1.70 Å | A | fragment MRT0181a |
| 7C9N | 🟦 | 2.47 Å | A/B | Compound 1 |
| 7CAJ | 🟩 | 2.20 Å | A/D | Compound 2 |
| 7CD9 | 🟪 | 1.60 Å | A/B | Compound 6 |
| 7CJT | 🟩 | 2.47 Å | A/B/C/D | (R,R)-59 |
| 8G5E | 🟪 | 1.98 Å | A | UNC6535 |
| 8UWP | 🟩 | 1.77 Å | A/B | MR46747 |

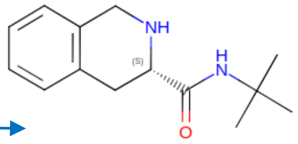


# Pocket 2

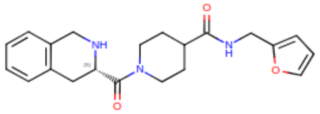
*Demo Material – For Illustration Purposes*



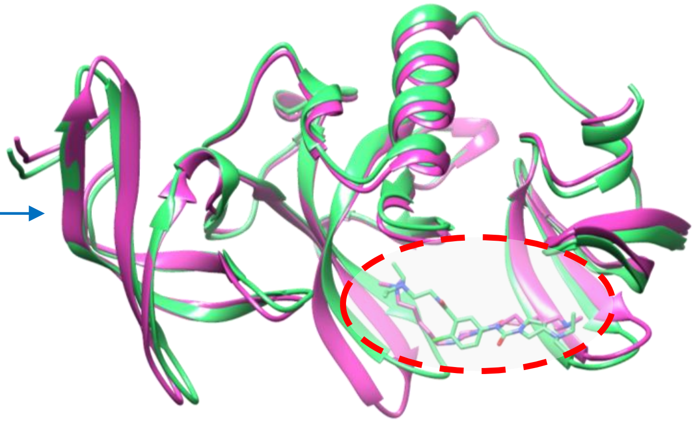


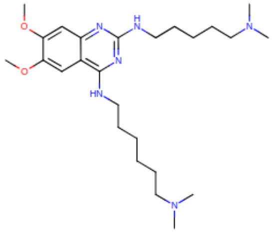


5KE2

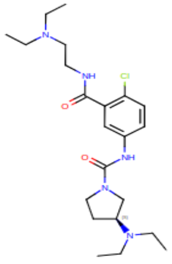


5KE3

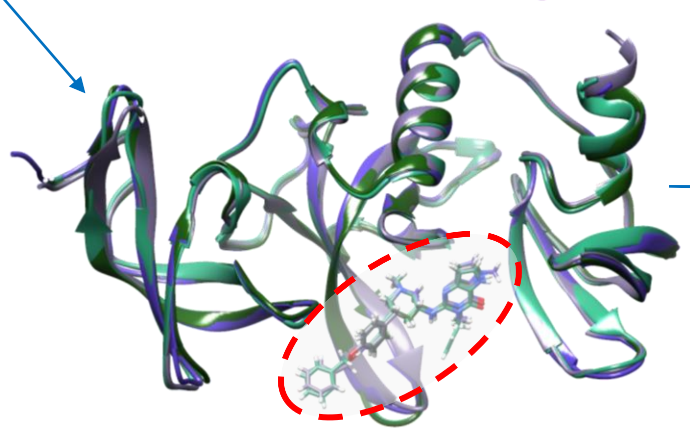


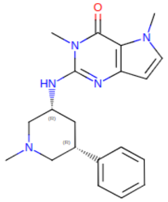


8G5E

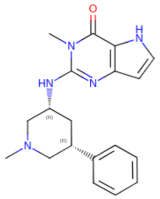


8UWP

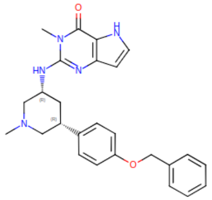




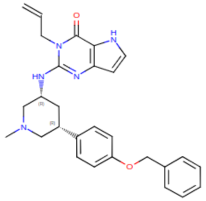
7C9N



7CAJ

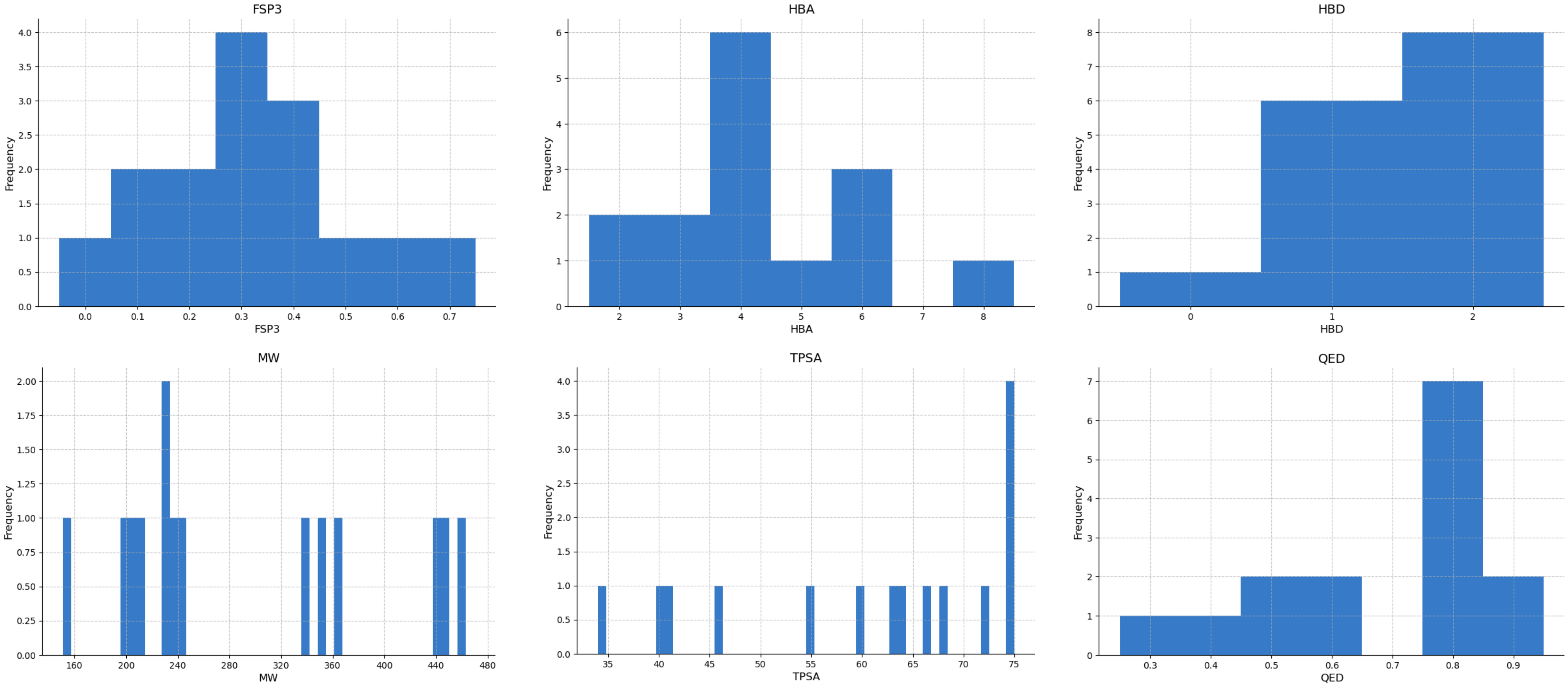


7CD9



7CJT

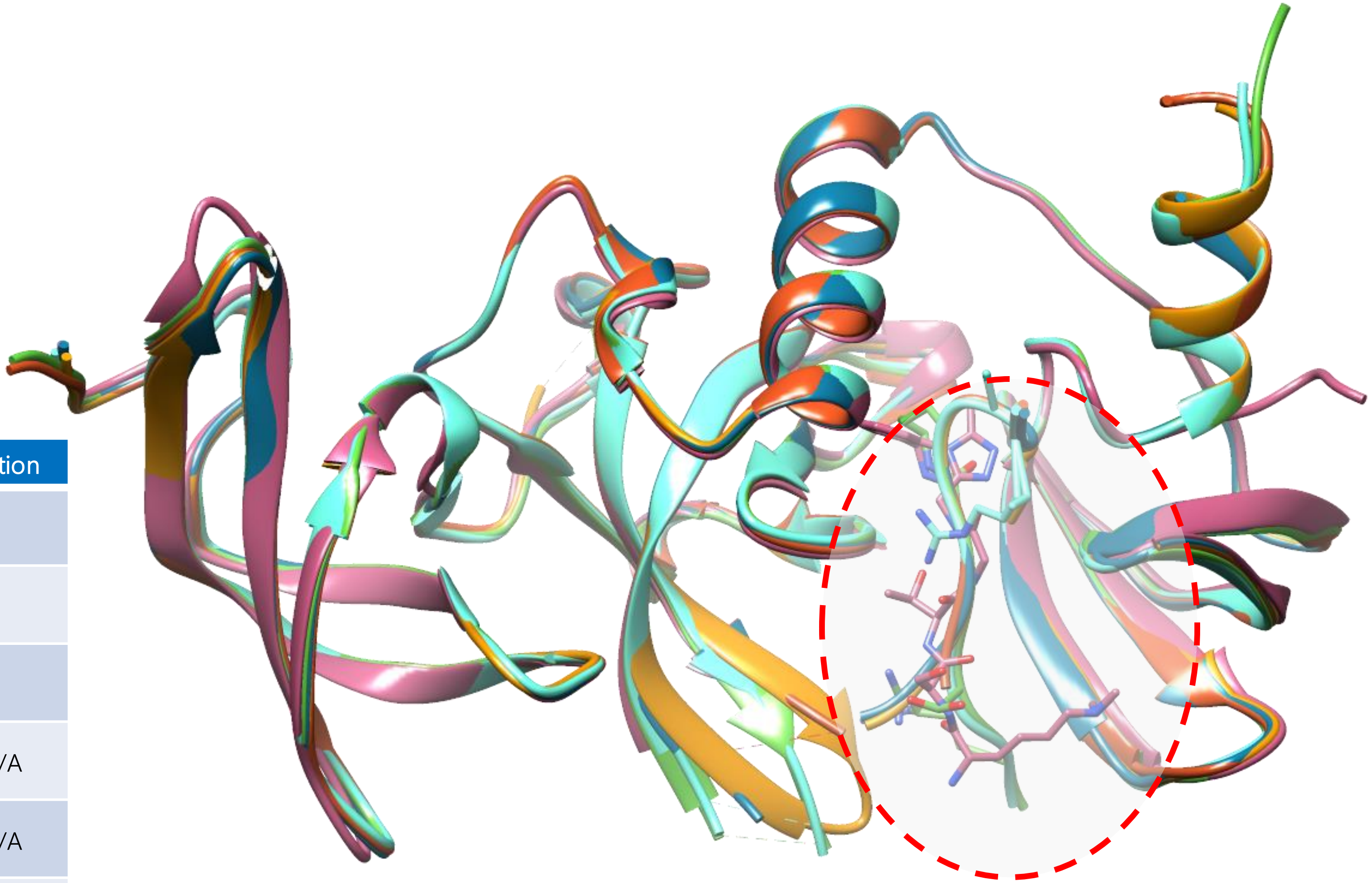
# Physicochemical properties of ligand



# Peptide

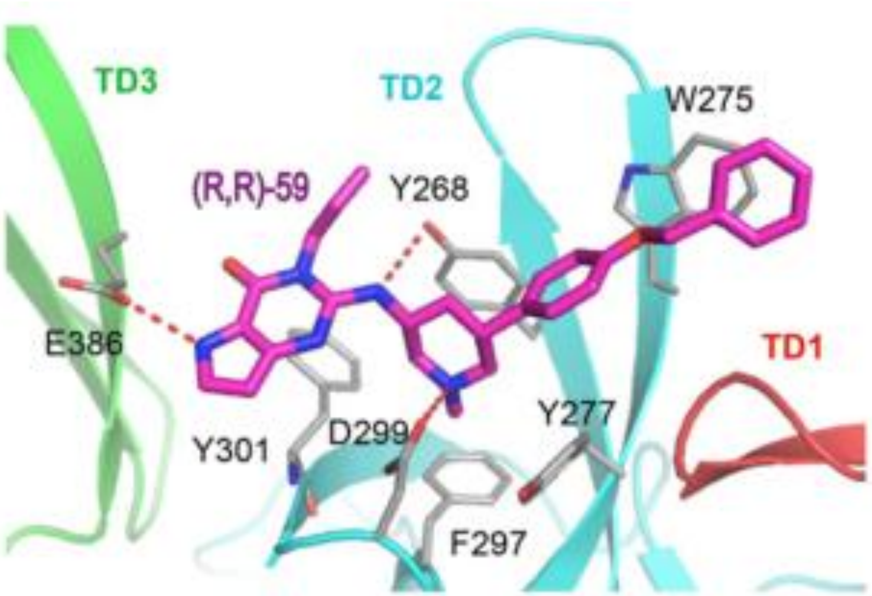
*Demo Material – For Illustration Purposes*

| Identifier | Color | Resolution | Chain | Ligand | Mutation |
|---|---|---|---|---|---|
| 6BHD | 🟩 | 1.25 Å | A | modified H3 peptide | |
| 6BHE | 🟦 | 1.35 Å | A | modified H3 peptide | |
| 6BHG | 🟥 | 1.45 Å | A | modified H3 peptide | |
| 6BHH | 🟦 | 1.85 Å | A | modified H3 peptide | 1 W/A |
| 6BHI | 🟧 | 1.40 Å | A | modified H3 peptide | 1 W/A |
| 6BPI | 🟪 | 1.64 Å | A | aryl triazole fragment peptide conjugates | |



# Binding modes of (R,R)-59 with SETDB1-TTD

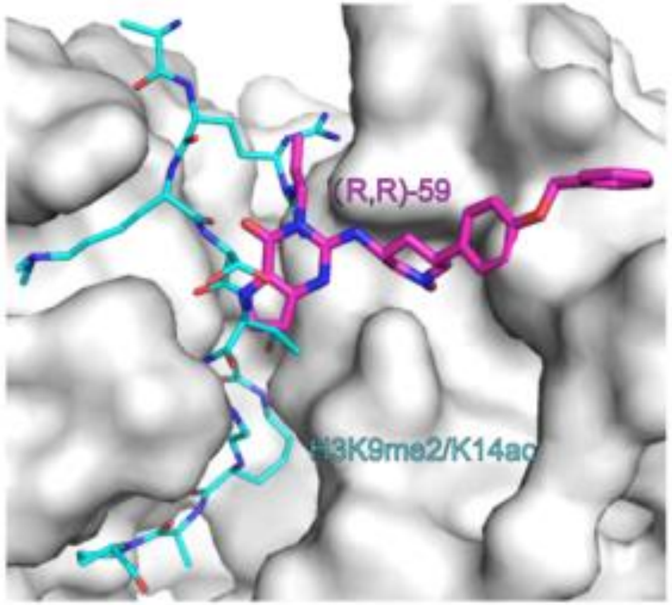
*Demo Material – For Illustration Purposes*



The (R,R)-59 molecule is located in the binding site between the TD2 and TD3 domains.

Main interactions:

- Location in the aromatic "pocket" formed by the amino acid residues Y301, Y268, W275, Y277 and F297.
- Hydrogen bond with the phenolic oxygen of Y268.

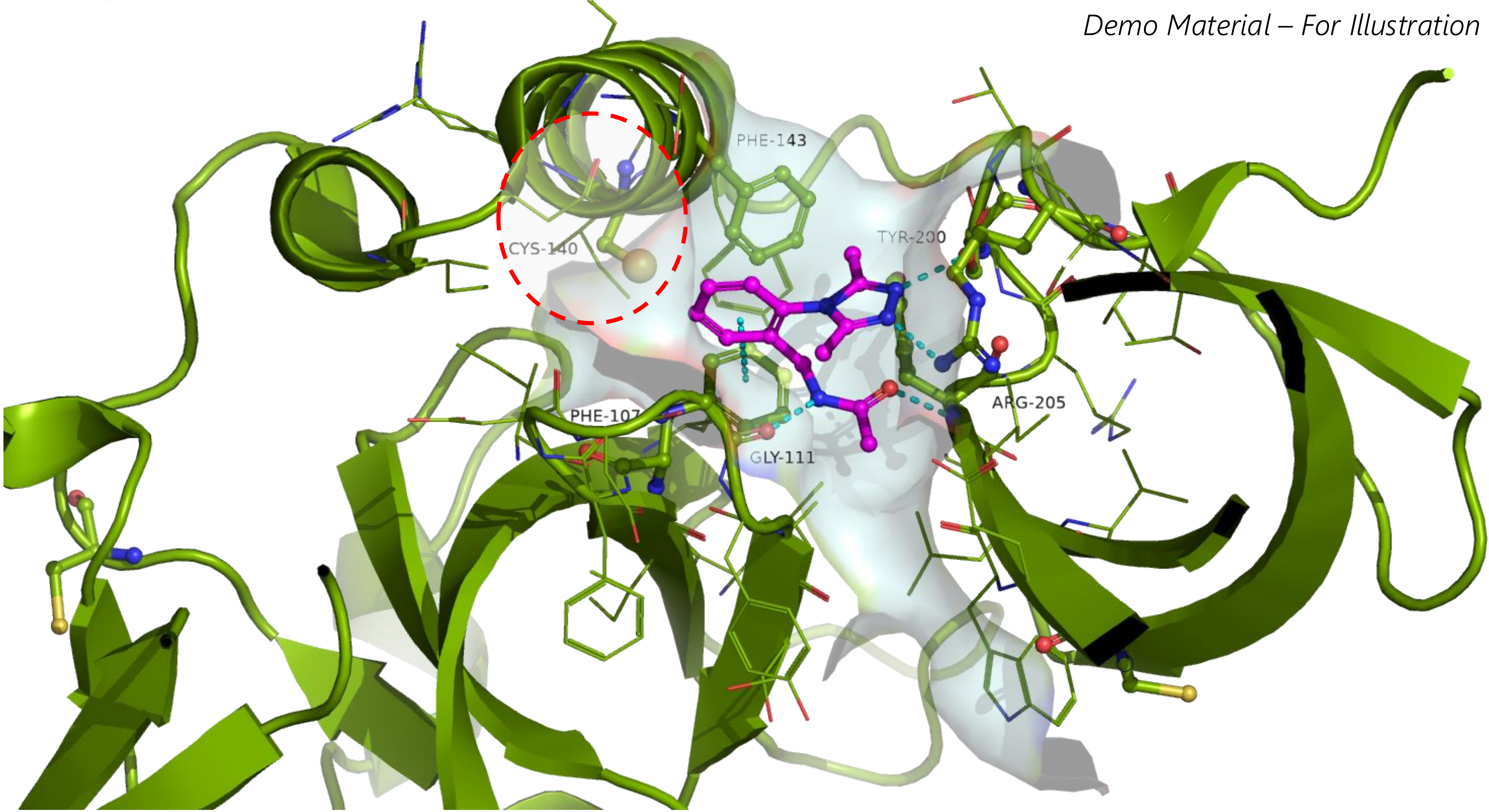


A superposition of crystal structures of the TTD-(R,R)-59 complex (PDB entry: 7CJT) and TTD-H3 peptide (PDB ID: 6BHD). Structural superposition demonstrates that (R,R)-59 and the H3 peptide occupy the same binding site on the protein surface.

https://doi.org/10.1002%2Fanie.202017200

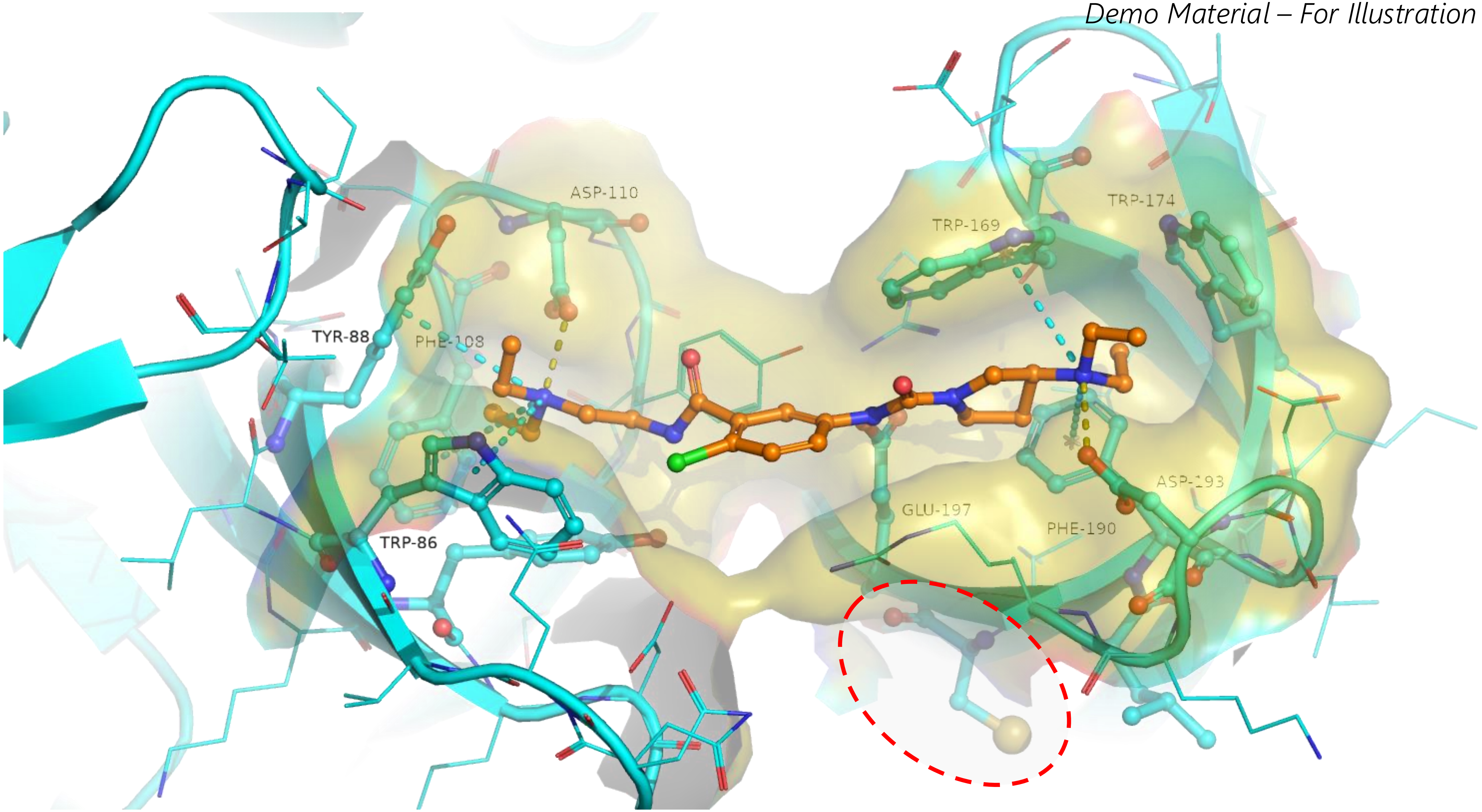
# Binding mode 6AU3

*Demo Material – For Illustration Purposes*



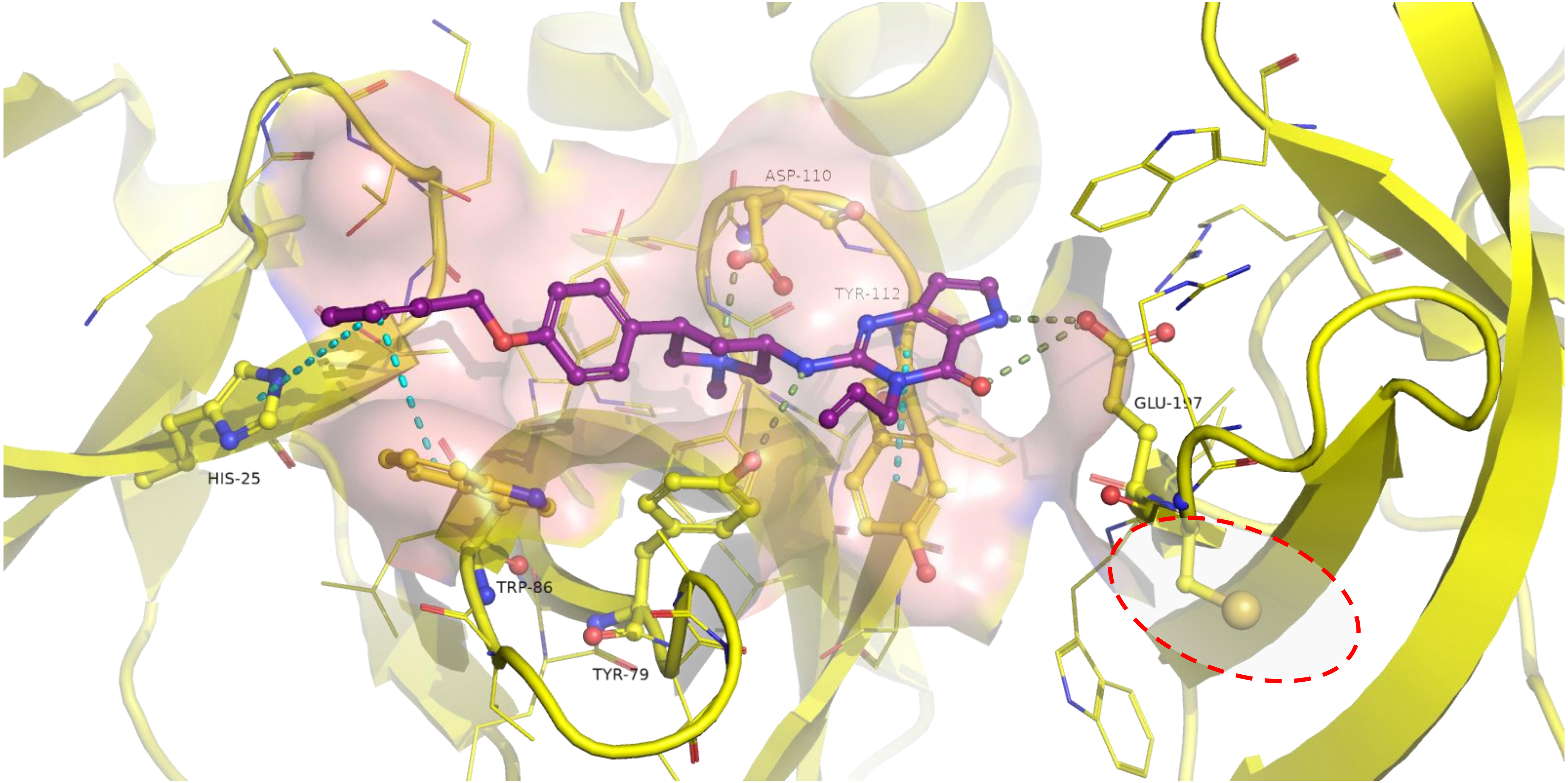
# Binding mode 8UWP

*Demo Material – For Illustration Purposes*

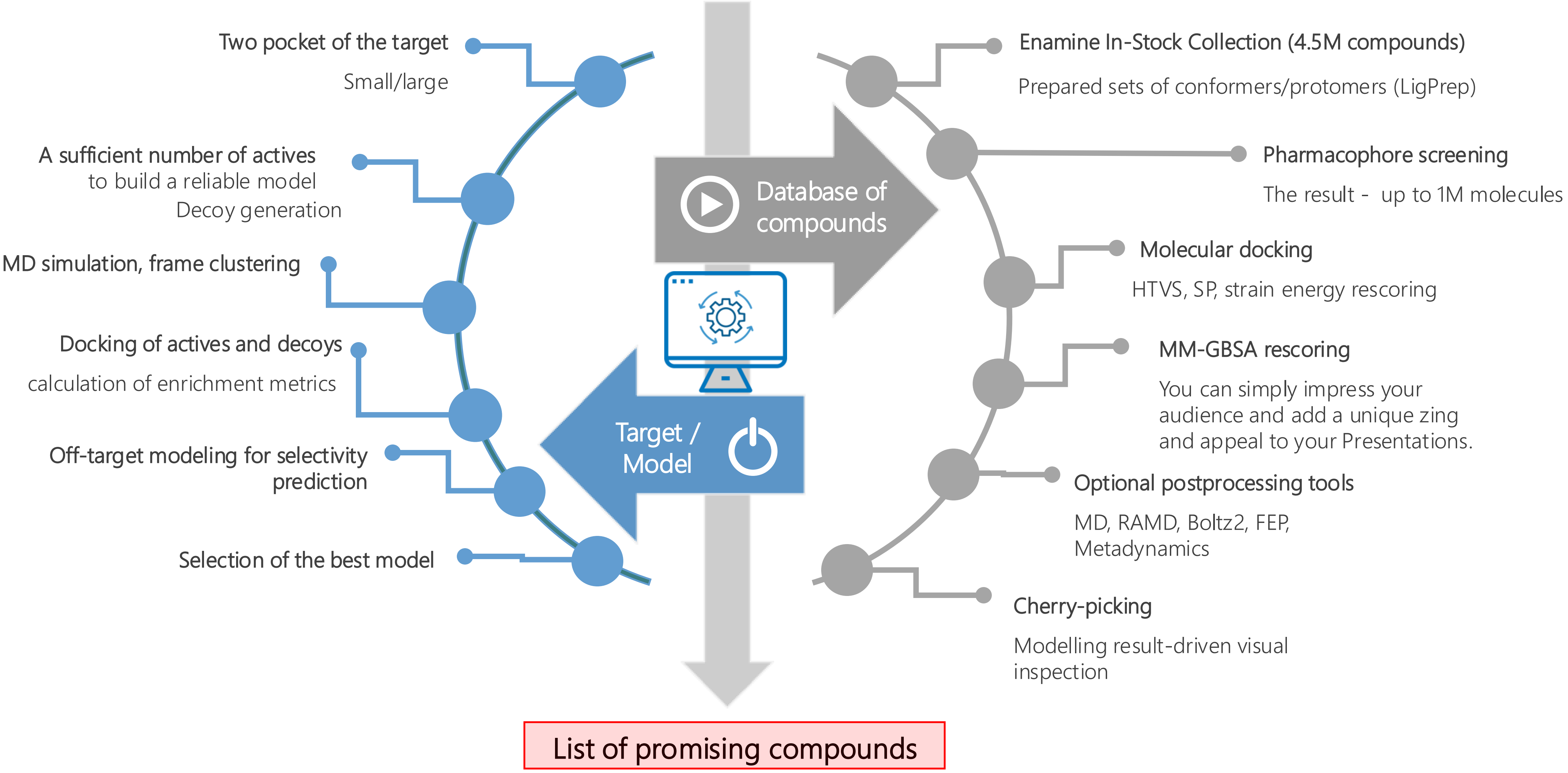


# Binding mode 7CJT

*Demo Material – For Illustration Purposes*



# Our proposed workflow



Two pocket of the target
Small/large

A sufficient number of actives
to build a reliable model
Decoy generation

MD simulation, frame clustering

Docking of actives and decoys
calculation of enrichment metrics

Off-target modeling for selectivity prediction

Selection of the best model

Enamine In-Stock Collection (4.5M compounds)
Prepared sets of conformers/protomers (LigPrep)

Pharmacophore screening
The result - up to 1M molecules

Molecular docking
HTVS, SP, strain energy rescoring

MM-GBSA rescoring
You can simply impress your audience and add a unique zing and appeal to your Presentations.

Optional postprocessing tools
MD, RAMD, Boltz2, FEP, Metadynamics

Cherry-picking
Modelling result-driven visual inspection

List of promising compounds

new dimension
for drug discovery

Enamine

*Demo Material – For Illustration Purposes*

# Thank you!

**CADD project was performed by the CADD team at Enamine**