# Supporting Information

## Rational Design of a Peptide Ligase from HRV-3C Protease

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## 1. Plasmid map

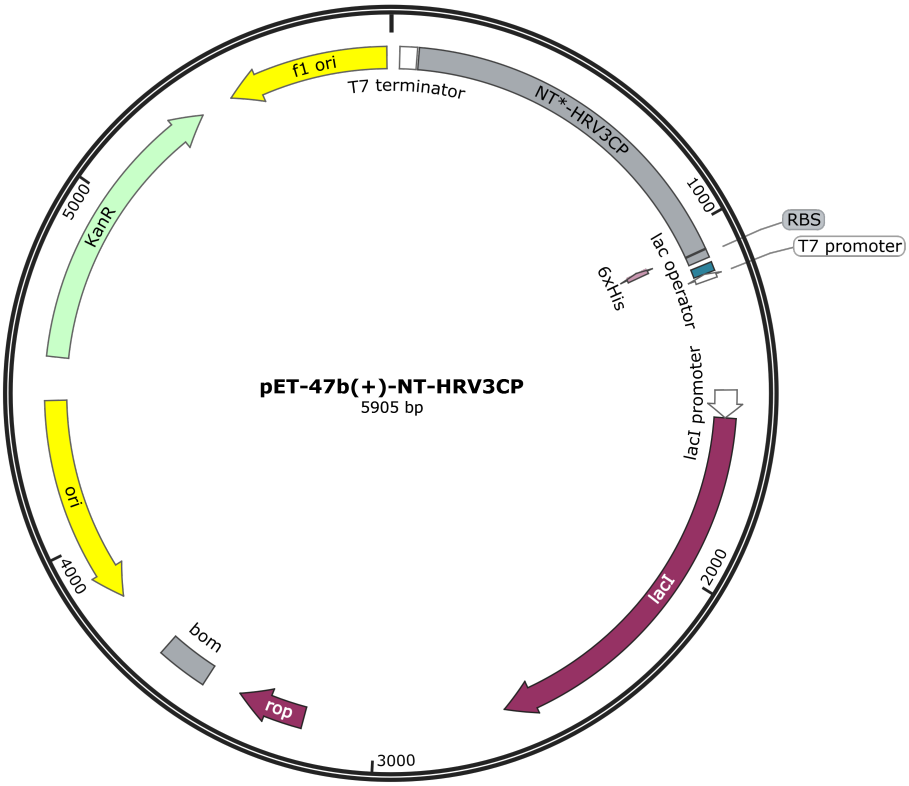


Figure 1: Plasmid map of pET-47b(+)-NT-HRV3CP used for protein expression. Key features are indicated, including the T7 promoter, the N-terminal His-tag, the coding sequence for NT*-HRV3CP, and the kanamycin resistance gene (KanR) for selection. The total size of the plasmid is 5.9kb

## 2. Primer Table

| Primer Name | Sequence (5′–3′) | Target Mutant |
|---|---|---|
| **Design 1** | | |
| 19-24-F | AACCATTTTAAACAGACGAATGGTCATAATGTTTTTACGCAGC | Mutagenesis of residues 19-24 |
| 19-24-R | CGTCTGTTTAAAATGGTTTTTACCGGTCTGGGTATTCATGA | |
| 105-107-F | GTAAATTTACCTGCATGAACAACCAGGGTTGCATCA | Mutagenesis of residues 105-107 |
| 105-107-R | CATGCAGGTAAATTTACCAACACCATCCTGGAAGT | |
| **Design 2** | | |
| 19-26-F | AAGCCATAATCGGTCAGAATGGTCATAATGTTTTTACGCAGC | Mutagenesis of residues 19-26 |
| 19-26-R | CTGACCGATTATGGCTTTTATAGCGGTCTGGGTATTCATGATCG | |
| 106-107-F | GGTAAACAGGCTGCTATGAACAACCAGGGTTGCA | Mutagenesis of residues 106-107 |
| 106-107-R | CATAGCAGCCTGTTTACCAACACCATCCTGGAAGT | |
| 143-145-F | CACAATTACCCGGTTTGGTTGCATAATCATAACGAATC | Mutagenesis of residues 143-145 |
| 143-145-R | CAAACCGGGTAATTGTGGTGGTGTTCTGTGTGCA | |

Table 1: Primers used for site-directed mutagenesis in this study.

## 3. Force Field Parameters

All quantum chemical calculations were performed using the ORCA 6.1 program package. The additional bonding interactions mediated by the ester intermediate are detailed below. The complete enzyme-substrate ester intermediate structures were optimized at the GFN1-xTB[1] level of theory with the ALPB(water) solvation model. Based on the optimized geometry, the ester intermediate moiety was extracted. The N-terminal and C-terminal of the intermediate were capped with methyl-acetyl (ACE) and N-methyl (NME), then subjected to electronic structure calculations. RESP2 (0.5)[2] charges were fitted using Multiwfn at the wB97X-2-D4/def2-TZVPP level using the RIJCOSX def2-TZVPP/C auxiliary basis set and the SMD solvation model for water and the gas phase. Vibrational frequency analysis were calculated at the wB97X-D4/def2-TZVP level using the RIJCOSX auxiliary basis set and the CPCM solvation model for water.

Bonded parameters were parametrized in Sobtop, with all atoms assigned AMBER force field atom types. Bond and angle parameters were derived via the mSeminario method using the Hessian matrix obtained from vibrational frequency analysis. For dihedral parameters, prebuilt bonded parameters were prioritized where available, then missing ones were supplemented by mSeminario method. The derived RESP charges are provided in Chapter 3.6.

The bonded model from Marina et al. was used to describe zinc coordination in $Zn^{2+}$-binding proteins[3]. For $Ca^{2+}$-binding proteins, the 12-6 non-bonded model from Merz et al. was applied.

### 3.1 SER&PHE : Subtilisin/Aqualigase

#### Bonds

| i | j | func | c0 | c1 |
|---|---|---|---|---|
| SER-OG | PHE-C | 1 | 0.133242 | 3.000599E+05 |

#### Pairs

| i | j | func |
|---|---|---|
| SER-CA | PHE-C | 1 |
| SER-HB1 | PHE-C | 1 |
| SER-HB2 | PHE-C | 1 |
| SER-CB | PHE-CA | 1 |
| SER-CB | PHE-O | 1 |
| SER-OG | PHE-CB | 1 |
| SER-OG | PHE-N | 1 |
| SER-OG | PHE-HA | 1 |

#### Angles

| i | j | k | func | c0 | c1 |
|---|---|---|---|---|---|
| SER-CB | SER-OG | PHE-C | 1 | 129.029 | 1.404181E+03 |
| SER-OG | PHE-C | PHE-CA | 1 | 122.974 | 5.531702E+02 |
| SER-OG | PHE-C | PHE-O | 1 | 119.052 | 5.002754E+02 |

#### Dihedrials

| i | j | k | l | func | c0 | c1 | c2 |
|---|---|---|---|---|---|---|---|
| SER-CA | SER-CB | SER-OG | PHE-C | 9 | 0.000 | 1.60247 | 3 |
| SER-CA | SER-CB | SER-OG | PHE-C | 9 | 180.000 | 3.34720 | 1 |
| SER-CB | SER-OG | PHE-C | PHE-CA | 9 | 180.000 | 11.29680 | 2 |
| SER-CB | SER-OG | PHE-C | PHE-O | 9 | 180.000 | 11.29680 | 2 |
| SER-CB | SER-OG | PHE-C | PHE-O | 9 | 180.000 | 5.85760 | 1 |
| SER-OG | PHE-C | PHE-CA | PHE-N | 9 | 0.000 | 0.00000 | 2 |
| SER-OG | PHE-C | PHE-CA | PHE-CB | 9 | 0.000 | 0.00000 | 2 |
| SER-OG | PHE-C | PHE-CA | PHE-HA | 9 | 0.000 | 0.00000 | 2 |
| SER-OG | PHE-CA | PHE-C | PHE-O | 4 | 180.000 | 43.93200 | 2 |

### 3.2 CYM&PHE : Subtiligase

#### Bonds

| i | j | func | c0 | c1 |
|---|---|---|---|---|
| CYM-SG | PHE-C | 1 | 0.174644 | 1.377031E+05 |

#### Pairs

| i | j | func |
|---|---|---|
| CYM-CA | PHE-C | 1 |
| CYM-HB1 | PHE-C | 1 |
| CYM-HB2 | PHE-C | 1 |
| CYM-CB | PHE-CA | 1 |
| CYM-CB | PHE-O | 1 |
| CYM-SG | PHE-CB | 1 |
| CYM-SG | PHE-N | 1 |
| CYM-SG | PHE-HA | 1 |

#### Angles

| i | j | k | func | c0 | c1 |
|---|---|---|---|---|---|
| CYM-CB | CYM-SG | PHE-C | 1 | 101.781 | 1.299887E+03 |
| CYM-SG | PHE-C | PHE-CA | 1 | 114.088 | 6.376070E+02 |
| CYM-SG | PHE-C | PHE-O | 1 | 123.254 | 4.820048E+02 |

#### Dihedrals

| i | j | k | l | func | c0 | c1 | c2 |
|---|---|---|---|---|---|---|---|
| CYM-CA | CYM-CB | CYM-SG | PHE-C | 9 | 0.000 | 1.39467 | 3 |
| CYM-SG | PHE-C | PHE-CA | PHE-N | 9 | 0.000 | 0.00000 | 2 |
| CYM-SG | PHE-C | PHE-CA | PHE-CB | 9 | 0.000 | 0.00000 | 2 |
| CYM-SG | PHE-C | PHE-CA | PHE-HA | 9 | 0.000 | 0.00000 | 2 |
| CYM-SG | PHE-CA | PHE-C | PHE-O | 4 | 180.000 | 43.93200 | 2 |
| CYM-CB | CYM-SG | PHE-C | PHE-CA | 2 | -162.148 | 6.430506E+02 | |
| CYM-CB | CYM-SG | PHE-C | PHE-O | 2 | 12.828 | 5.975791E+02 | |

### 3.3 CYM&GLN : HRV-3C

#### Bonds

| i | j | func | c0 | c1 |
|---|---|---|---|---|
| GLN-C | CYS-SG | 1 | 0.178541 | 1.302229E+05 |

#### Pairs

| i | j | func |
|---|---|---|
| CYM-CA | GLN-C | 1 |
| CYM-HB1 | GLN-C | 1 |
| CYM-HB2 | GLN-C | 1 |
| CYM-CB | GLN-CA | 1 |
| CYM-CB | GLN-O | 1 |
| CYM-SG | GLN-CB | 1 |
| CYM-SG | GLN-N | 1 |
| CYM-SG | GLN-HA | 1 |

#### Angles

| i | j | k | func | c0 | c1 |
|---|---|---|---|---|---|
| GLN-CA | GLN-C | CYM-SG | 1 | 122.720 | 4.691712E+02 |
| GLN-O | GLN-C | CYM-SG | 1 | 117.165 | 3.303963E+02 |
| GLN-C | CYM-SG | CYM-CB | 1 | 105.765 | 1.446423E+03 |

#### Dihedrials

| i | j | k | l | func | c0 | c1 | c2 |
|---|---|---|---|---|---|---|---|
| GLN-N | GLN-CA | GLN-C | CYM-SG | 9 | 0.000 | 0.00000 | 2 |
| GLN-CB | GLN-CA | GLN-C | CYM-SG | 9 | 0.000 | 0.00000 | 2 |
| GLN-HA | GLN-CB | GLN-C | CYM-SG | 9 | 0.000 | 0.00000 | 2 |
| GLN-CA | GLN-O | GLN-C | CYM-SG | 4 | 180.000 | 43.93200 | 2 |
| GLN-CA | GLN-C | CYM-SG | CYM-CB | 2 | 74.560 | 5.047826E+02 | |
| GLN-O | GLN-C | CYM-SG | CYM-CB | 2 | -111.419 | 3.908476E+02 | |

### 3.4 CYS&ASN : Butelase 2/Bu2g(V/GA)

#### Bonds

| i | j | func | c0 | c1 |
|---|---|---|---|---|
| ASN-C | CYM-SG | 1 | 0.174440 | 1.909970E+05 |

#### Pairs

| i | j | func |
|---|---|---|
| CYM-CA | ASN-C | 1 |
| CYM-HB1 | ASN-C | 1 |
| CYM-HB2 | ASN-C | 1 |
| CYM-CB | ASN-CA | 1 |
| CYM-CB | ASN-O | 1 |
| CYM-SG | ASN-CB | 1 |
| CYM-SG | ASN-N | 1 |
| CYM-SG | ASN-HA | 1 |

#### Angles

| i | j | k | func | c0 | c1 |
|---|---|---|---|---|---|
| CYM-CB | CYM-SG | ASN-C | 1 | 104.365 | 1.477785E+03 |
| CYM-SG | ASN-C | ASN-CA | 1 | 126.659 | 3.730087E+02 |
| CYM-SG | ASN-C | ASN-O | 1 | 120.447 | 4.222326E+02 |

#### Dihedrals

| i | j | k | l | func | c0 | c1 | c2 |
|---|---|---|---|---|---|---|---|
| CYM-CA | CYM-CB | CYM-SG | ASN-C | 9 | 0.000 | 1.39467 | 3 |
| CYM-SG | ASN-C | ASN-CA | ASN-N | 9 | 0.000 | 0.00000 | 2 |
| CYM-SG | ASN-C | ASN-CA | ASN-HA | 9 | 0.000 | 0.00000 | 2 |
| CYM-SG | ASN-C | ASN-CA | ASN-CB | 9 | 0.000 | 0.00000 | 2 |
| CYM-SG | ASN-CA | ASN-C | ASN-O | 4 | 180.000 | 43.93200 | 2 |
| CYM-CB | CYM-SG | ASN-C | ASN-CA | 2 | -63.583 | 7.735360E+02 | |
| CYM-CB | CYM-SG | ASN-C | ASN-O | 2 | 114.594 | 7.366940E+02 | |

### 3.5 SER&TYR : Trypsin/Trypsiligase

#### Bonds

| i | j | func | c0 | c1 |
|---|---|---|---|---|
| SER-OG | TYR-C | 1 | 0.133800 | 2.829375E+05 |

#### Pairs

| i | j | func |
|---|---|---|
| SER-CA | TYR-C | 1 |
| SER-HB1 | TYR-C | 1 |
| SER-HB2 | TYR-C | 1 |
| SER-CB | TYR-CA | 1 |
| SER-CB | TYR-O | 1 |
| SER-OG | TYR-CB | 1 |
| SER-OG | TYR-N | 1 |
| SER-OG | TYR-HA | 1 |

#### Angles

| i | j | k | func | c0 | c1 |
|---|---|---|---|---|---|
| SER-CB | SER-OG | TYR-C | 1 | 123.588 | 1.146986E+03 |
| SER-OG | TYR-C | TYR-CA | 1 | 121.247 | 5.311808E+02 |
| SER-OG | TYR-C | TYR-O | 1 | 118.972 | 4.967151E+02 |

#### Dihedrals

| i | j | k | l | func | c0 | c1 | c2 |
|---|---|---|---|---|---|---|---|
| SER-CA | SER-CB | SER-OG | TYR-C | 9 | 0.000 | 1.60247 | 3 |
| SER-CA | SER-CB | SER-OG | TYR-C | 9 | 180.000 | 3.34720 | 1 |
| SER-CB | SER-OG | TYR-C | TYR-CA | 9 | 180.000 | 11.29680 | 2 |
| SER-CB | SER-OG | TYR-C | TYR-O | 9 | 180.000 | 11.29680 | 2 |
| SER-CB | SER-OG | TYR-C | TYR-O | 9 | 180.000 | 5.85760 | 1 |
| SER-OG | TYR-C | TYR-CA | TYR-N | 9 | 0.000 | 0.00000 | 2 |
| SER-OG | TYR-C | TYR-CA | TYR-CB | 9 | 0.000 | 0.00000 | 2 |
| SER-OG | TYR-C | TYR-CA | TYR-HA | 9 | 0.000 | 0.00000 | 2 |
| SER-OG | TYR-CA | TYR-C | TYR-O | 4 | 180.000 | 43.93200 | 2 |

### 3.6 Ester Intermediate's Atom Charge

| SER&PHE : Subtilisin/Aqualigase | | | | | | |
|---|---|---|---|---|---|---|
| **SER** | | | | **PHE** | | |
| Atom name | Atom Type | RESP Charge | \| | Atom name | Atom Type | RESP Charge |
| N | N | -0.099980 | \| | N | N | -0.639300 |
| H | H | 0.209561 | \| | H | H | 0.298096 |
| CA | XC | -0.428951 | \| | CA | CX | 0.453849 |
| HA | H1 | 0.194192 | \| | HA | H1 | 0.021223 |
| CB | 2C | 0.065268 | \| | CB | CT | -0.574547 |
| HB1 | H1 | 0.142267 | \| | HB1 | HC | 0.136210 |
| HB2 | H1 | 0.142267 | \| | HB2 | HC | 0.136210 |
| OG | OH | -0.411480 | \| | CG | CA | 0.414377 |
| C | C | 0.865695 | \| | CD1 | CA | -0.314557 |
| O | O | -0.700476 | \| | HD1 | HA | 0.175905 |
| | | | \| | CE1 | CA | -0.103927 |
| | | | \| | HE1 | HA | 0.135433 |
| | | | \| | CZ | CA | -0.204169 |
| | | | \| | HZ | HA | 0.145660 |
| | | | \| | CE2 | CA | -0.032029 |
| | | | \| | HE2 | HA | 0.125047 |
| | | | \| | CD2 | CA | -0.385551 |
| | | | \| | HD2 | HA | 0.209969 |
| | | | \| | C | C | 0.619919 |
| | | | \| | O | O2 | -0.596185 |
| **CYM & PHE : Subtiligase** | | | | | | |
| **CYM** | | | | **PHE** | | |
| Atom name | Atom Type | RESP Charge | \| | Atom name | Atom Type | RESP Charge |
| N | N | -0.399439 | \| | N | N | -0.484043 |
| H | H | 0.234766 | \| | H | H | 0.271790 |
| CA | XC | 0.008866 | \| | CA | CX | 0.153457 |
| HA | H1 | 0.072497 | \| | HA | H1 | 0.120438 |
| CB | 2C | 0.108853 | \| | CB | CT | -0.260217 |
| HB1 | H1 | 0.108853 | \| | HB1 | HC | 0.099320 |
| HB2 | H1 | -0.067044 | \| | HB2 | HC | 0.099320 |
| SG | SH | -0.108677 | \| | CG | CA | 0.249433 |
| C | C | 0.752511 | \| | CD1 | CA | -0.274204 |
| O | O | -0.686425 | \| | HD1 | HA | 0.162808 |
| | | | \| | CE1 | CA | -0.111861 |
| | | | \| | HE1 | HA | 0.142556 |
| | | | \| | CZ | CA | -0.184474 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | \| | HZ | HA | 0.144202 |
| | | | \| | CE2 | CA | -0.108849 |
| | | | \| | HE2 | HA | 0.139198 |
| | | | \| | CD2 | CA | -0.266143 |
| | | | \| | HD2 | HA | 0.187509 |
| | | | \| | C | C | 0.405373 |
| | | | \| | O | O2 | -0.510373 |
| **CYM & GLN : HRV-3C** | | | | | | |
| **CYM** | | | | **GLN** | | |
| Atom name | Atom Type | RESP Charge | \| | Atom name | Atom Type | RESP Charge |
| N | N | -0.584409 | \| | N | N | -0.433087 |
| H | H | 0.343441 | \| | H | H | 0.210571 |
| CA | XC4 | -0.232359 | \| | CA | CX | 0.194266 |
| HA | H1 | 0.194115 | \| | HA | H1 | 0.054944 |
| CB | CT | -0.263114 | \| | CB | 2C | -0.031403 |
| HB3 | H1 | 0.174374 | \| | HB2 | HC | 0.039304 |
| HB2 | H1 | 0.174374 | \| | HB3 | HC | 0.039304 |
| SG | SH | -0.050250 | \| | CG | 2C | -0.237631 |
| C | C | 0.932639 | \| | HG2 | HC | 0.102703 |
| O | O | -0.649238 | \| | HG3 | HC | 0.102703 |
| | | | \| | CD | C | 0.843780 |
| | | | \| | OE1 | O | -0.681942 |
| | | | \| | NE2 | N | -0.982784 |
| | | | \| | HE21 | H | 0.434188 |
| | | | \| | HE22 | H | 0.403924 |
| | | | \| | C | C | 0.338698 |
| | | | \| | O | O2 | -0.437110 |
| **CYM & ASN : Butelase 2/Bu2g(V/GA)** | | | | | | |
| **CYM** | | | | **ASN** | | |
| Atom name | Atom Type | RESP Charge | \| | Atom name | Atom Type | RESP Charge |
| N | N | 0.196967 | \| | N | N | -0.959374 |
| H | H | 0.083926 | \| | H | H | 0.407511 |
| CA | XC | -0.653926 | \| | CA | CX | 0.567236 |
| HA | H1 | 0.186371 | \| | HA | H1 | 0.068667 |
| CB | CT | -0.258915 | \| | CB | 2C | -0.811920 |
| HB1 | H1 | 0.215073 | \| | HB1 | HC | 0.166786 |
| HB2 | H1 | 0.215073 | \| | HB2 | HC | 0.166786 |
| SG | SH | -0.163123 | \| | CG | C | 1.075060 |
| C | C | 1.108068 | \| | OD1 | O | -0.723788 |
| O | O | -0.698781 | \| | ND2 | N | -0.994454 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | \| | HD21 | H | 0.416590 |
| | | | \| | HD22 | H | 0.423083 |
| | | | \| | C | C | 0.519970 |
| | | | \| | O | O2 | -0.552887 |
| **SER & TYR : Trypsin/Trypsiligase** | | | | | | |
| **SER** | | | | **TYR** | | |
| Atom name | Atom Type | RESP Charge | \| | Atom name | Atom Type | RESP Charge |
| N | N | -0.572133 | \| | N | N | -0.542960 |
| H | H | 0.327313 | \| | H | H | 0.314216 |
| CA | XC | -0.118844 | \| | CA | CX | 0.239564 |
| HA | H1 | 0.110435 | \| | HA | H1 | 0.088779 |
| CB | 2C | -0.199754 | \| | CB | CT | -0.522172 |
| HB1 | H1 | 0.139968 | \| | HB1 | HC | 0.213591 |
| HB2 | H1 | 0.139968 | \| | HB2 | HC | 0.213591 |
| OG | OH | -0.246829 | \| | CG | CA | 0.088667 |
| C | C | 1.004785 | \| | CD1 | CA | -0.090570 |
| O | O | -0.714530 | \| | HD1 | HA | 0.157691 |
| | | | \| | CE1 | CA | -0.434305 |
| | | | \| | HE1 | HA | 0.208851 |
| | | | \| | CZ | C | 0.490648 |
| | | | \| | OH | OH | -0.640941 |
| | | | \| | HH | HO | 0.445892 |
| | | | \| | CE2 | CA | -0.346052 |
| | | | \| | HE2 | HA | 0.208206 |
| | | | \| | CD2 | CA | -0.153404 |
| | | | \| | HD2 | HA | 0.150136 |
| | | | \| | C | C | 0.633235 |
| | | | \| | O | O2 | -0.593045 |

## 4. Docking Simulations

Based on the established Ester Intermediate structure, a grid box was defined surrounding the amide carbonyl group. The center of the grid was set at the centroid of the amide carbonyl group, with the grid dimensions set to 50 × 50 × 50 points. Affinity maps were then generated using AutoGrid4. The docking process was performed using AutoDock Vina with the AD4 scoring function, and the exhaustiveness parameter was set to 50. After 5,000 docking runs, the conformation with the substrate N-terminus positioned closest to the electrophilic carbon atom of the amide carbonyl group was selected as the initial structure for subsequent LiGaMD3 simulations.

## 5. MM/PBSA calculations

Molecular Mechanics/Poisson-Boltzmann Surface Area (MM/PBSA) is a powerful computational approach used to estimate the binding affinity(ΔG) of a ligand to a receptor.The binding energy for each trajectory frame is evaluated using the following equations:

The binding energy ($\Delta G_{\text{bind}}$) can be decomposed into contributions of different interactions and expressed as the sum of enthalpic and entropic contributions:

$$\Delta G_{\text{bind}} = \Delta H - T\Delta S = \Delta E_{\text{mm}} + \Delta G_{\text{solv}} - T\Delta S \tag{1}$$

where the molecular mechanics energy ($\Delta E_{\text{mm}}$) is further decomposed into bonded and non-bonded terms:

$$\begin{aligned} \Delta E_{\text{mm}} &= \Delta E_{\text{bonded}} + \Delta E_{\text{non-bonded}} \\ &= \left(\Delta E_{\text{bonds}} + \Delta E_{\text{angles}} + \Delta E_{\text{dihedrals}}\right) + \left(\Delta E_{\text{ele}} + \Delta E_{\text{vdw}}\right) \end{aligned} \tag{2}$$

The solvation free energy ($\Delta G_{\text{solv}}$) is composed of polar and non-polar contributions:

$$\Delta G_{\text{solv}} = \Delta G_{\text{polar}} + \Delta G_{\text{non-polar}} = \Delta G_{\text{PB}} + (\gamma \cdot \text{SASA} + b) \tag{3}$$

where $\Delta E_{\text{mm}}$, $\Delta G_{\text{solv}}$ and $T\Delta S$ are the changes in the gas-phase molecular mechanics energy, solvation free energy, and conformational entropy upon ligand binding. $\Delta G_{\text{polar}}$ is the electrostatic or polar component of the solvation free energy evaluated by the Poisson-Boltzmann (PB) model, and $\Delta G_{\text{non-polar}}$ is the hydrophobic or nonpolar component proportional to the molecular solvent accessible surface area (SASA).Because of only the relative binding free energies of similar ligands are needed and heavy computational cost, changes in the conformational entropy are neglected. Final binding energy terms are averaged over multiple configurations or several MD snapshots. To calculate the binding free energy and interaction between the Mutants of HRV-3C and its substrates. We utilized the gmx_MMPBSA program to perform calculations. Using the first frame of the trajectory as the reference for alignment with the Mutants of HRV-3C protein backbone, trajectory segments exhibiting plateaued RMSD profiles with no substantial global changes are selected. These segments are evenly divided into 3*1000 snapshots.

# Bibliography

[1] S. Grimme, C. Bannwarth, and P. Shushkov, "A Robust and Accurate Tight-Binding Quantum Chemical Method for Structures, Vibrational Frequencies, and Noncovalent Interactions of Large Molecular Systems Parametrized for All spd-Block Elements (Z = 1–86)," *Journal of Chemical Theory and Computation*, vol. 13, no. 5, pp. 1989–2009, 2017, doi: 10.1021/acs.jctc.7b00118.

[2] F.-Y. Dupradeau *et al.*, "The R.E.D. tools: advances in RESP and ESP charge derivation and force field library building," *Phys. Chem. Chem. Phys.*, vol. 12, no. 28, pp. 7821–7839, 2010, doi: 10.1039/C0CP00111B.

[3] M. Macchiagodena, M. Pagliai, C. Andreini, A. Rosato, and P. Procacci, "Upgrading and Validation of the AMBER Force Field for Histidine and Cysteine Zinc(II)-Binding Residues in Sites with Four Protein Ligands," *Journal of Chemical Information and Modeling*, vol. 59, no. 9, pp. 3803–3816, 2019, doi: 10.1021/acs.jcim.9b00407.