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STRUCTURE, DYNAMICS AND SOLVATION OF HIV-1 PROTEASE
WILDTYPE COMPLEXED WITH INHIBITORS BY MOLECULAR DYNAMIC
SIMULATIONS

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A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Chemistry

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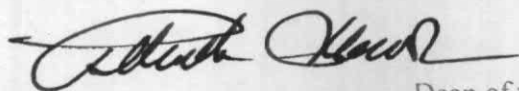
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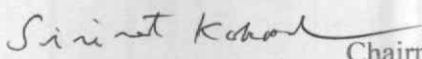
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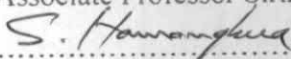
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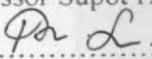


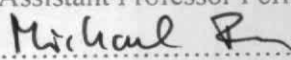
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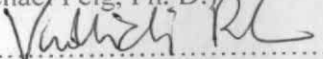
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กิตติยาพร วิทยานรากุล: โครงสร้าง พลศาสตร์และชอลเวชันของเอชไอวี-1 โปรทีเอสไวด์ไทป์กับสารยับยั้ง โดยการใช้การจำลองพลวัตเชิงโมเลกุล (STRUCTURE, DYNAMICS AND SOLVATION OF HIV-1 PROTEASE WILDTYPE COMPLEXED WITH INHIBITORS BY MOLECULAR DYNAMIC SIMULATIONS) อ.ที่ปรึกษา: ศ. ดร. สุพจน์ หารหนองบัว, อ.ที่ปรึกษาร่วม: ผศ. ดร. พรเทพ สมพรพิสุทธิ์, ดร. Michael Feig, 108 หน้า.

สารยับยั้งไวรัส human immunodeficiency ชนิด 1 โปรทีเอส (HIV-1 PR) เกือบทั้งหมดประกอบด้วย ไฮดรอกซิล-เอทิลลีน ซึ่งมีบทบาทสำคัญในการจำเรสซิดิวส์แอสปาร์ติคบริเวณเร่งของเอนไซม์ เป็นที่แน่ชัดว่าสัมพรรคภาพ ของการยึดเหนี่ยวมีส่วนสัมพันธ์กับสถานะการแตกตัวเป็นไอออนที่เหมาะสมของโซ่ข้างของสารยับยั้งที่ยึดกับเรสซิดิวส์ สถานะโปรโตเนชันของเรสซิดิวส์บริเวณเร่งยังไม่สามารถทราบได้แน่ชัด เราจึงตัดสินใจทำการจำลองพลวัตเชิงโมเลกุลและการคำนวณพลังงานอิสระแบบดั้งเดิมและแบบดัดแปลง เพื่อที่จะปรับปรุงการทำนายสถานะโปรโตเนชันของสารประกอบเชิงซ้อนกับยาทั้งหกชนิดซึ่งประกอบด้วย โลพินาเวียร์ (LPV), ริโทนาเวียร์ (RTV), ซาควินาเวียร์ (SQV), อินดินาเวียร์ (IDV), แอมพรินาเวียร์ (APV) และ เนลฟินาเวียร์ (NFV) จึงได้ทำการจำลองระบบของของทุกสถานะโปรโตเนชันที่เป็นไปได้ของเรสซิดิวส์บริเวณเร่ง ซึ่งประกอบด้วยโมโนโปรโตเนตที่แอสปาร์ติกที่ตำแหน่ง 25 (D25) และที่ โปรโตเนตที่แอสปาร์ติกที่ตำแหน่ง 25' (D25'), ไดโปรโตเนชัน (D25,25') และ อันโปรโตเนชัน (D-) ของระบบสารประกอบเชิงซ้อนสารยับยั้งกับเอนไซม์ การคำนวณพลังงานเสรี ($\Delta G_{binding}$) โดยใช้วิธีแบบมาตรฐานและแบบลูกผสมของ กลศาสตร์โมเลกุลปัวร์ซองโบลทซ์แมน หรือ เจอเนอร์ลไลซ์ และ โซลเวนท์แอสเซสลิเบิล เซอเฟสเอเรีย (MMPB(GB)/SA) การศึกษานี้ ได้พิจารณา $\Delta G_{binding}$ เนื่องจากการเปลี่ยนแปลงโปรโตเนชันที่ $pH = 7$ ผลการศึกษาพบว่า วิธี MMPB/SA แบบลูกผสมให้ประโยชน์เพียงเล็กน้อยในการให้ค่าสัมบูรณ์พลังงานอิสระ ขณะที่การใช้การประมาณค่าเงินเนอร์ลไลซ์ บอร์น กระทบความถูกต้องของการคำนวณสัมพรรคภาพการยึดจับอย่างมีนัยสำคัญ บนพื้นฐานของ $\Delta G_{binding}$ และ การวิเคราะห์วิธีการคำนวณพบว่า โมโนโปรโตเนชันเป็นสถานะที่เหมาะสมสำหรับ สารยับยั้งทั้ง 6 ชนิดกล่าวคือ D25 สำหรับ LPV, SQV และ IDV และ D25' สำหรับ RTV, APV และ NFV การศึกษานี้ยังได้ขยายขอบเขตเพื่อทำนายการกลายพันธุ์ระดับโมเลกุลที่เนื่องมาจากการใช้ยับยั้ง เอชไอวี-1 โปรทีเอสทั้ง 6 ชนิด และการใช้ยาโอเซลทามิเวียร์เพื่อยับยั้งไข้หวัดที่ทำงานที่เอนไซม์นิวรามินิเดส โดยใช้พลังงานอิสระยึดจับดีคอมโพสิชันเป็นเกณฑ์ ผลการทำนายเรสซิดิวส์ที่คาดว่าจะเกิดการกลายพันธุ์สำหรับทั้งสองกรณีสอดคล้องเป็นอย่างดีกับข้อมูลทางการแพทย์สำหรับการดื้อยาในระดับสูงและปานกลาง

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KITIYAPORN WITTAYANARAKUL: STRUCTURE, DYNAMICS AND SOLVATION OF HIV-1 PROTEASE WILDTYPE COMPLEXED WITH INHIBITORS BY MOLECULAR DYNAMIC SIMULATIONS. THESIS ADVISOR: PROF. SUPOT HANNONGBUA, Ph.D., THESIS CO-ADVISOR: ASST. PROF. PORNTHEP SOMPORNPIST, Ph.D., MICHAEL FEIG, Ph.D., 108 pp.

Almost all of the human immunodeficiency virus type I protease (HIV-1 PR) inhibitors contain a hydroxyl-ethylene moiety, which plays an essential role in recognition of the enzyme through the aspartic active site residues. Apparently, the affinity of the binding is associated with a proper ionization state of the sidechain of the inhibitor-bound residues. The protonation state of the active site residues is not clearly understood. We decided to carry out molecular dynamics simulations and conventional and modified free energy calculations to improve a prediction of the protonation state of the HIV-1 PR in complex with six HIV-1 PR drugs including Lopinavir (LPV), Ritonavir (RTV), Saquinavir (SQV), Indinavir (IDV), Amprenavir (APV), and Nelfinavir (NFV). All possible protonation states of the active site residues including monoprotonated at Asp25 (D25), monoprotonated at Asp25' (D25'), diprotonation (D25,25'), and unprotonation (D-), were used to set up the system of HIV-1 PR-drug complexes for the simulations. The binding free energy ($\Delta G_{binding}$) was computed using a standard and hybrid methods of molecular mechanic Poisson Boltzmann or Generalized Born, and solvent accessible surface area (MMPB (GB)/SA). In this study, the $\Delta G_{binding}$ due to the protonation change at $pH = 7$ was also taken into account. Comparison among the method used, the hybrid MMPB/SA approach offers a slightly advantage in reproducing absolute binding free energies whereas the use of Generalized Born approximation significantly affects the accuracy of the computed binding affinities. Based on the $\Delta G_{binding}$ and the computational analysis, monoprotonation is the optimal state for the 6 drugs, D25 for LPV, SQV, and IDV and D25' for RTV, APV, and NFV. The study was extended to predict molecular mutation due to the HIV-1 PR complexed with the 6 inhibitors and the influenza neuraminidase complexed with oseltamivir using the decomposition binding free energy as a criteria. The predicted mutation residues for both cases are in good agreement with the high and intermediate level of resistant reported clinically.

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LIST OF ABBREVIATIONS

CHAPTER 1		Section
AIDS	Acquired immune deficiency syndrome	1.1
HIV	Human immunodeficiency virus	1.1
Asp	Aspartic	1.1
ORF	Open reading frames	1.2
RT	Reverse transcriptase	1.2
PR	Protease	1.2
RNA	Ribonucleic acid	1.3
DNA	Deoxyribonucleic acid	1.3
UNAIDS	Joint United Nations Programme on HIV/AIDS	1.3
WHO	World health Organization	1.3
Gp	Glycoprotein	1.3.1
CD4	Cluster of differentiation	1.3.1
ENV	Envelope	1.3.1
Gag	group-specific antigen	1.3.1
Pol	Polymerase	1.3.1
Tat	Transactivator	1.3.1
Rev	Regulator of viral express	1.3.1
Vif	Viral infectivity	1.3.1
Vpr	Viral protein	1.3.1
Vpu	Viral protein U	1.3.1
Nef	Negative-regulation factor	1.3.1
FDA	Food and Drug Administration	1.5
LPV	Lopinavir	1.5
RTV	Ritonavir	1.5
SQV	Saquinavir	1.5
IDV	Indinavir	1.5
APV	Amprenavir	1.5
NFV	Nelfinavir	1.5
TPV	Fosamprenavir	1.5
FPV	Fosamprenavir	1.5

		Section
DRV	Darunavir	1.5
ATV	Atazanavir	1.5
HAART	Highly active antiretroviral therapy	1.6
MMPB/SA	Molecular mechanic Poisson Boltzmann/solvent accessible surface area	1.7
MMGB/SA	Molecular mechanic Generalized Born/solvent accessible surface area	1.7
MMGBMV/SA	Molecular mechanic Generalized Born molecular volume/solvent accessible surface area	1.7
 CHAPTER 2		
MD	Molecular dynamics simulations	2.1
BPTI	Bovine pancreatic trypsin inhibitor	2.1
F	Force	2.1.1
m	Mass	2.1.1
a	Acceleration	2.1.1
r	distance	2.1.1
E	Potential function	2.1.1
v	Velocity	2.1.1
E_{bonded}	Bonded term	2.1.2
$E_{non-bonded}$	Non-Bonded term	2.1.2
k	Force constant	2.1.3.1
ΔG_{bind}	Binding free energy	2.2
ΔH	Enthalpic term	2.2
ΔS	Entropic term	2.2
K_{eq}	Equilibrium constant	2.2
q	Charge	2.2.1.2
SASA	Solvent accessible surface area	2.2.1.3
I	Moment of inertia	2.2.1.4

CHAPTER 3		Section
FEP	Free energy perturbation	3.1
D25	monoprotonated at aspartic25	3.1
D25'	monoprotonated at aspartic25'	3.1
D25,25'	monoprotonated at both of aspartic 25 and aspartic 25'	3.1
D-	Unprotonation	3.1
TI	Thermodynamic integration	3.1
PDB	Protein Data Bank	3.2.1
$\Delta G_{\text{association}}$	Gibbs energy of protein-ligand association	3.1
$\Delta G_{\text{solvation}}$	Solvation binding free energy	3.1
$\Delta G_{\text{free}(D25 \rightarrow D25,25')}$	Free energy change from D25 to D25,25'	3.3.2
$\Delta G_{(\text{EnzH} \rightarrow \text{Enz}')}$	Free energy change from protonated aspartate enzyme to deprotonated aspartate enzyme	3.3.2
$\Delta G_{(\text{AspH} \rightarrow \text{Enz}')}$	Free energy change from protonated free aspartate to deprotonated free aspartate	3.3.2
CHAPTER 4		
DC	Decomposition energy	4.1
N	Neuraminidase	4.1
ΔG_{res}	Binding free energy change between individual residue and inhibitor	4.2
ΔG_{res}	Binding free energy change between individual residue and inhibitor	4.2
$\langle \Delta G^{\text{res+inhibitor}} \rangle$	average binding free energy between individual residue located on the protein and inhibitor	4.2
$\langle \Delta G^{\text{res}} \rangle$	average binding free energy of individual residue	4.2
$\langle \Delta G^{\text{inhibitor}} \rangle$	average binding free energy of inhibitor	4.2
OTV	Oseltamivir	4.3.2
RMSD	Root mean square displacement	4.3.3