Modeling Family 2 Cystatins and their interaction with Papain

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Supplementary Material

Table SI

Result of Secondary structure analysis

		Alpha helix	Extended strand	Random coil	Beta Turn	Ambiguous states
Cystatin M/E	DSC	33.88%	10.74%	55.37%		
	GOR4	32.23%	26.45%	41.32%		
	HNN	28.93%	15.70%	55.37%		
	PHD	35.54%	16.53%	47.93%		
	Predator	34.71%	19.83%	45.45%		
	SOPMA	42.98%	12.40%	39.67%		
	Sec.Cons.	32.23%	9.09%	48.76%		9.92%
Cystatin S	DSC	22.31%	17.36%	60.33%		
	GOR4	32.23%	23.14%	44.63%		
	HNN	35.54%	16.53%	47.93%		
	PHD	24.79%	22.31%	52.89%		
	Predator	34.71%	10.74%	54.55%		
	SOPMA	32.23%	14.05%	49.59%		
	Sec.Cons.	27.27%	14.88%	52.89%		4.96%
Cystatin SA	DSC	23.97%	20.66%	55.37%		
	GOR4	36.36%	19.01%	44.63%		
	HNN	38.84%	16.53%	44.63%		
	PHD	27.27%	18.18%	54.55%		
	Predator	35.54%	10.74%	53.72%		
	SOPMA	32.23%	15.70%	47.11%	4.96%	

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	Sec.Cons.	28.93%	14.05%	49.59%	7.44%
Cystatin SN	DSC	20.66%	17.36%	61.98%	
	GOR4	28.93%	19.83%	51.24%	
	HNN	33.88%	15.70%	50.41%	
	PHD	27.27%	19.01%	53.72%	
	Predator	33.06%	10.74%	56.20%	
	SOPMA	29.75%	16.53%	49.59%	4.13%
	Sec.Cons.	29.75%	13.22%	51.24%	5.79%
Cystatin C	DSC	30.14%	10.27%	59.59%	
	GOR4	34.25%	15.07%	50.68%	
	HNN	38.36%	10.96%	50.68%	
	PHD	39.73%	16.44%	43.84%	
	Predator	30.82%	4.11%	65.07%	
	SOPMA	33.56%	15.75%	44.52%	6.16%
	Sec.Cons.	31.51%	10.96%	52.74%	4.79%

Figure S1: Ramachandran Plot of all the final four models by PROCHECK program.

Figure S2: Verify3D validation report of the refined models.

Figure S3: 3D profile scores of final structures using ERRAT Server.

Figure S4: ANOLEA plot, negative values (black) designate residues in a favorable environment and positive values (red) indicate residues in an unfavorable environment.

Table SII

Interaction Energy of (IE>5 kcal/mol) interface residues of Papain-Cystatin Complexes

Residue	Papain-Cystatin M/E			Papain-Cystatin S			Papain-Cystatin SA			Papain-Cystatin SN		
	IE	VDW	E_IE	IE	VDW	E_IE	IE	VDW	E_IE	IE	VDW	E_IE
A:GLN19	<u>-8.85</u>	-3.34	-5.51									
A:GLY20	<u>-9.12</u>	-2.58	-6.59									
A:SER21	-7.22	-5.46	-1.76	-7.56	-2.48	-5.07	<u>-14.47</u>	-1.26	-13.20	<u>-17.29</u>	-2.14	-15.15
A:CYS22				<u>-6.18</u>	-2.95	-3.23						
A:TYR61				-6.44	-6.03	-0.41	-10.05	-8.07	-1.98	<u>-12.88</u>	-2.76	-10.13
A:CYS63										<u>-5.88</u>	-1.18	-4.69
A:ASN64	<u>-15.99</u>	-3.74	-12.25	<u>-17.36</u>	-7.06	-10.30	<u>-23.78</u>	-7.46	-16.32	<u>-23.19</u>	-5.87	-17.32
A:TYR67							<u>-13.50</u>	-1.71	-11.79	<u>-21.10</u>	-5.74	-15.35
A:TRP69										<u>-8.40</u>	-2.14	-6.26
A:GLN112										<u>-16.19</u>	2.31	-18.50
A:GLU135	<u>-43.55</u>	4.40	-47.95									
A:LYS139	<u>-38.69</u>	1.60	-40.30	<u>-47.80</u>	5.83	-53.63	<u>-54.78</u>	6.42	-61.20			
A:GLN142				<u>-16.77</u>	0.81	-17.59	<u>-12.93</u>	-3.44	-9.49			
A:LYS156	<u>-62.63</u>	6.63	-69.26	<u>-57.25</u>	0.27	-57.53	<u>-66.91</u>	0.27	-67.17	<u>-51.02</u>	-0.81	-50.22
A:VAL157	<u>-14.88</u>	0.86	-15.74	<u>-16.46</u>	-3.67	-12.79				<u>-10.93</u>	-1.47	-9.46
A:ASP158	<u>-52.37</u>	-4.24	-48.12	<u>-19.39</u>	-5.44	-13.95	<u>-15.64</u>	-6.12	-9.52	<u>-10.42</u>	-4.61	-5.81
A:HIS159	<u>-10.36</u>	-3.96	-6.40	-5.07	-4.20	-0.87	-8.35	-5.86	-2.48	<u>-9.90</u>	-5.57	-4.32
A:TRP177	-8.84	-8.09	-0.75				-5.49	-4.35	-1.14			
A:SER205										<u>-15.40</u>	-0.06	-15.34
I:GLU1	<u>-90.15</u>	9.05	-99.20									
I:GLY1										<u>-32.45</u>	2.06	-34.50
I:ARG3	<u>-69.48</u>	1.82	-71.30									
I:ASP6	-38.80	2.71	-41.51				<u>-38.80</u>	2.71	-41.51			
I:ASP9	<u>-43.95</u>	3.72	-47.67							<u>-44.67</u>	4.85	-49.52
I:GLU43				<u>-40.53</u>	2.82	-43.35	<u>-37.88</u>	1.61	-39.50			
I:GLN44							<u>-12.19</u>	-0.04	-12.15	<u>-9.31</u>	-3.38	-5.93
I:LEU45	-8.24	-6.96	-1.28									
I:THR45				<u>-15.60</u>	0.95	-16.55				<u>-16.72</u>	-4.11	-12.61
I:ILE45							-5.87	-4.21	-1.66			
I:VAL46	<u>-14.64</u>	-6.31	-8.34				<u>-9.66</u>	-8.31	-1.35	<u>-11.01</u>	-8.54	-2.47
I:PHE46				<u>-12.30</u>	-10.30	-2.00						
I:ALA47	<u>-11.08</u>	-5.28	-5.80									
I:GLY47				<u>-9.73</u>	-1.24	-8.48	<u>-10.75</u>	-4.84	-5.91	<u>-11.32</u>	-4.72	-6.59
I:GLY48				<u>-7.52</u>	-2.67	-4.86						
I:LYS50	<u>-17.72</u>	-0.48	-17.24									
I:ASN50							<u>-6.31</u>	-2.85	-3.45	-5.66	-3.20	-2.46
I:GLU89										<u>-12.63</u>	-0.55	-12.09
I:TYR91				<u>-15.64</u>	-6.13	-9.51	-10.43	-8.59	-1.83	<u>-10.18</u>	-8.50	-1.68
I:PRO94				<u>-13.30</u>	-4.13	-9.17	<u>-10.79</u>	-4.84	-5.95	-8.09	-5.33	-2.76
I:TRP95							<u>-22.83</u>	-15.07	-7.76	<u>-8.74</u>	-10.63	1.89
I:GLU96							-8.66	-2.30	-6.36			
I:ASP97							<u>-53.47</u>	5.91	-59.38			
I:ARG98							<u>-8.44</u>	-0.87	-7.57	<u>-32.46</u>	-1.15	-31.31
I:TRP99	<u>-21.30</u>	-13.13	-8.17									
I:GLN100	-6.83	-3.40	-3.43									
I:SER100							<u>-10.58</u>	-0.08	-10.50			
I:LYS103										<u>-13.70</u>	0.12	-13.82

Note: Residues involved in Hydrogen bond (HB) are underlined, and in solvent accessible surface area (SASA) are italicized in IE column. Residues common in all four or three of the four complexes are in **bold**. Chain A represents Papain and I stand for cystatin.

Table SIII

Relative solvent accessible surface areas (%) of interface residues of Papain–Cystatin Complexes [Δ SAS>30]

Residue	Cystatin M/E	Cystatin S	Cystatin SA	Cystatin SN
	Δ SASA (%)	Δ SASA (%)	Δ SASA (%)	Δ SASA (%)
A:GLY20	<u>42.1</u>			
A:SER21	76.1		42.9	<u>47.2</u>
A:GLY23	40.3	<u>37.1</u>	36.2	40
A:TYR61				<u>30.1</u>
A:ASN64	<u>48.1</u>	<u>55.5</u>	<u>62</u>	<u>50.5</u>
A:ALA137	39.7			
A:LYS156	<u>37.5</u>		<u>37.1</u>	<u>51.7</u>
A:VAL157		<u>31.38</u>	30.3	<u>30.6</u>
A:ASP158	<u>43.9</u>	<u>50.51</u>	<u>52.9</u>	<u>49.2</u>
I:GLU1	<u>46.2</u>			
I:ARG3	<u>39.9</u>			
I:LEU45	59.7			
I:THR45		<u>54.5</u>		<u>57.6</u>
I:VAL46	<u>45.1</u>		59.6	58.2
I:PHE46		46.7		
I:ALA47	<u>71.3</u>			
I:GLY47		<u>36.7</u>	<u>52.6</u>	<u>52.7</u>
I:TYR91				33.3
I:PRO94		<u>46.2</u>	32.7	
I:TRP95		<u>56.7</u>	<u>59</u>	39.3
I:TRP99	<u>44.4</u>			
I:VAL102		45.6		31.7

Note: Residues present in both, HB & SASA analysis *are underlined & italicized*. Residues common in all four or three of the four complexes are in **bold**. Chain A corresponds to Papain and I denotes cystatin.

Table SIV

Hydrogen Bonds (Å) among interface residues of Papain-Cystatin Complexes

Papain-Cystatin M/E		Papain-Cystatin S		Papain-Cystatin SA		Papain-Cystatin SN	
A:GLN19:NE2 - <i>I:ALA47:O</i>	2.94	A:LYS139:NZ - I:ASP97:OD1	2.86	A:CYS25:SG - <i>I:GLY47:O</i>	3.16	A:TYR67:OH - I:GLU89:OE2	2.70
A:CYS25:SG - <i>I:VAL46:O</i>	3.04	<u>A:LYS139:NZ -</u> <u>I:ASP97:OD2</u>	<u>2.59</u>	<u>A:TYR67:OH -</u> <u>I:GLN44:OE1</u>	<u>2.66</u>	A:TRP69:NE1 - I:GLN44:OE1	2.78
A:LYS139:NZ - I:ASP9:OD1	3.14	A:GLN142:NE2 - <i>I:PRO94:O</i>	2.71	<u>A:LYS139:NZ -</u> <u>I:ASP97:OD1</u>	<u>2.64</u>	<i>A:LYS156:NZ -</i> I:ASP9:OD1	2.82
<u>A:LYS139:NZ -</u> <u>I:ASP9:OD2</u>	<u>2.62</u>	A:GLN142:NE2 - I:ASP97:OD1	2.78	<u>A:LYS139:NZ -</u> <u>I:ASP97:OD2</u>	<u>2.64</u>	<u>A:LYS156:NZ -</u> <u>I:ASP9:OD2</u>	<u>2.62</u>
<u>A:LYS156:NZ -</u> <u>I:GLU1:OE1</u>	<u>2.64</u>	<u>A:LYS156:NZ -</u> <u>I:GLU43:OE1</u>	<u>2.64</u>	A:GLN142:NE2 - <i>I:TRP95:O</i>	2.88	A:HIS159:ND1 - <i>I:GLY47:O</i>	2.87
<u>A:LYS156:NZ -</u> <u>I:GLU1:OE2</u>	<u>2.65</u>	A:LYS156:NZ - <i>I:THR45:OG1</i>	2.72	A:GLN142:NE2 - I:ASP97:OD2	3.18	<u>I:GLY1:N -</u> <u>A:GLN112:OE1</u>	<u>2.66</u>
A:HIS159:ND1 - <i>I:VAL46:O</i>	2.82	<i>I:GLY47:N -</i> A:VAL157:O	2.74	<u>A:LYS156:NZ -</u> <u>I:ASP6:OD1</u>	<u>2.65</u>	I:GLY1:N - A:SER205:OG	2.77
<u>I:GLU1:N -</u> <u>A:LYS156:O</u>	<u>2.66</u>	I:GLY48:N - A:VAL157:O	2.97	<u>A:LYS156:NZ -</u> <u>I:GLU43:OE1</u>	<u>2.66</u>	<i>I:THR45:OG1 -</i> A:VAL157:O	2.72
<u>I:GLU1:N -</u> <u>A:VAL157:O</u>	<u>2.67</u>	I:TYR91:OH - A:ASP158:O	2.78	I:GLN44:NE2 - A:TYR67:OH	3.12	I:GLY48:N - A:ASP158:O	3.20
<i>I:ARG3:NH1 -</i> A:GLU135:OE2	2.81	<i>I:TRP95:NE1 -</i> A:CYS22:O	2.96	<i>I:GLY47:N -</i> A:ASP158:O	3.03	I:ARG98:NH1 - A:SER21:O	2.76
<u>I:ARG3:NH1 -</u> <u>A:ASP158:OD2</u>	2.72	I:LEU101:N - A:ASN64:OD1	2.90	I:GLY48:N - A:ASP158:O	3.09	I:ARG98:NH2 - A:CYS63:O	3.02
<u>I:ARG3:NH2 -</u> <u>A:GLU135:OE2</u>	<u>2.59</u>			I:ASN50:ND2 - A:ASN64:O	3.02	I:ARG98:NH2 - A:ASN64:OD1	2.76
I:LYS50:NZ - A:ASN64:OD1	2.76			I:ARG98:NH2 - A:SER21:O	2.78	I:LYS103:NZ - A:TYR61:OH	2.83
<i>I:TRP99:NE1 -</i> A:GLY20:O	2.86			I:SER100:OG - A:ASN64:OD1	2.74	I:LYS103:NZ - A:TYR67:OH	3.14

Note: Residues present in SASA analysis are *italicized* and residues involved in SSHB (<2.7 Å) are underlined. Chain A symbolizes Papain whereas I indicate cystatin.

Table SV

Triadic Hydrogen Bonds

Papain-Cystatin M/E	Papain-Cystatin S	Papain-Cystatin SA	Papain-Cystatin SN
I:ASP9: <u>A:LYS139:I:ASP9</u>	I:ASP97: <u>A:LYS139:I:ASP97</u>	A:CYS25: <i>I:GLY47:A:ASP158</i>	I:GLU89:A:TYR67:I:LYS103
<u>I:GLU1:A:LYS156:I:GLU1</u>	<i>I:PRO94:A:GLN142:I:ASP97</i>	<u>I:GLN44:A:TYR67:I:GLN44</u>	<u>I:ASP9:A:LYS156:I:ASP9</u>
<u>A:VAL157:I:GLU1:A:LYS156</u>	<u>I:GLU43:A:LYS156:I:THR45</u>	<u>I:ASP97:A:LYS139:I:ASP97</u>	<u>A:GLN112:I:GLY1:A:SER205</u>
A:GLU135: <i>I:ARG3:A:ASP158</i>	<i>I:GLY47:A:VAL157:I:GLY48</i>	<i>I:ASP97:A:GLN142:I:TRP95</i>	<i>A:SER21:I:ARG98:A:CYS63</i>
A:CYS25: <i>I:VAL46:A:HIS159</i>		<u>I:ASP6:A:LYS156:I:GLU43</u>	A:TYR67:I:LYS103:A: <i>TYR61</i>
<u>I:ARG3:A:GLU135:I:ARG3</u>		<i>I:GLY47:A:ASP158:I:GLY48</i>	A:CYS63:I:ARG98:A:ASN64
		I:ASN50:A:ASN64:I:SER100	

Note: Residues present in *SASA analysis* are italicized and residues involved in SSHB are underlined.
Chain A & I signify Papain and Cystatin respectively.