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**Hypothesis** 

### In Silico mutagenesis and docking studies of active site residues suggest altered substrate specificity and possible physiological role of Cinnamoyl CoA Reductase 1 (LI-CCRH1)

### Prashant Sonawane, Krunal Patel, Rishi Kishore Vishwakarma, Somesh Singh & Bashir Mohammad Khan\*

Plant Tissue Culture and Biochemical Sciences Division, CSIR-National Chemical Laboratory, Pune 411008, India; Bashir Mohammad Khan - Email: bm.khan@ncl.res.in; Phone: 912025902220; Fax: 912025902645; \*Corresponding author

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#### Abstract:

Cinnamoyl CoA reductase (CCR) carries out the first committed step in monolignol biosynthesis and acts as a first regulatory point in lignin formation. CCR shows multiple substrate specificity towards various cinnamoyl CoA esters. Here, *in silico* mutagenesis studies of active site residues of LI-CCRH1 were carried out. Homology modeling based modeled 3D structure of LI-CCRH1 was used as template for *in silico* mutant preparations. Docking simulations of LI-CCRH1 mutants with CoA esters by AutoDock Vina tools showed altered substrate specificity as compared to wild type. The study evidences that conformational changes, and change in geometry or architecture of active site pocket occurred following mutations. The altered substrate specificity for active site mutants suggests the possible physiological role of CCR either in lignin formation or in defense system in plants.

Keywords: Cinnamoyl CoA reductase 1, Mutagenesis, Homology Modeling, Docking Simulations, Substrate Specificity.

**Abbreviations:** LI-CCRH1, *Leucaena leucocephala* cinnamoyl CoA reductase 1, OPLS, Optimized Potentials for Liquid Simulations, RMSD, Root Mean Square Deviation.

#### Background:

Lignin, an integral cell wall component of plants, is a phenolic heteropolymer of monolignols namely, *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol **[1]**. Among several genes involved in lignin biosynthesis, Cinnamoyl CoA Reductase (CCR, EC 1.2.1.44) plays a key regulatory role in lignin formation **[2]**. Hydroxycinnamoyl CoA esters of general phenylpropanoid pathway become destined to form respective monolignols after action of CCR. Being entry point enzyme in monolignol biosynthesis, CCR diverts phenylpropanoid derived metabolites towards lignin.

specificity CCR exhibits substrate for different hydroxycinnamoyl CoA esters (p-coumaroyl CoA, caffeoyl CoA, feruloyl CoA, 5-hydroxyferuloyl CoA and sinapoyl CoA); and reduce them to corresponding aldehydes. Cinnamoyl CoA esters are the common precursors of wide range of phenolic compounds and flavonoids. For example, coumaroyl CoA esters are the substrates for chalcone synthase enzyme, the first catalytic step towards secondary metabolites synthesis. Secondary metabolites are considered as the first line of defense against pathogens and diseases. Differential substrate specificity of CCR has been correlated to its exclusive or

redundant function inside the cell either in lignification (feruloyl CoA/ sinapoyl CoA as most preferred substrate) during plant development or in defense mechanism (Coumaroyl CoA as favored substrate) [3-5].

The major limitation in understanding structure-function relationship of CCR is lack of its three dimensional structure till date by any experimental means. Homology modeling provides an alternative approach for constructing three dimensional structures if X-ray crystal structure data is not available. We constructed 3D model of LI-CCRH1 using Dihydroflavanol Reductase from *Vitis vinifera* as template (PDB ID: 2c29). Putative active site residues involved in substrate binding, stabilization and catalysis were identified based on amino acid sequence analysis and docking simulations. These residues

were further investigated and confirmed by site directed mutagenesis and chemical modification studies (data unpublished).

Here, the present study was aimed to investigate the effects of various substitution mutations (*in silico*) of active site residues on substrate specificity of LI-CCRH1. Five different *in silico* mutants were prepared for each amino acid residue and subjected to docking simulations with different hydroxycinnamoyl CoA esters. Based on docking energies obtained, substrate preferences for each mutant were determined. These substrate specificities were used to predict the possible role of *in silico* LI-CCRH1 mutants either in lignin formation or in defense mechanisms.



**Figure 1:** Cinnamoyl CoA esters binding energy change for *in silico* active site mutants. X-axis represents LI-CCRH1 amino acid mutants generated by homology modeling, using TRITON/MODELLER software, and Y-axis is respective binding energies for CoA esters docked with each individual mutant. The '\*' shows best docked substrate with most negative binding energy among particular mutant.

### Methodology:

#### Starting molecule

Three dimensional model of LI-CCRH1, generated using MODELLER 9v9, was used as template for *in silico* mutagenesis studies (Protein Model Database ID: PM0078699).

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#### Preparation of the in silico mutants

Active site of LI-CCRH1 is made up of ten residues, Phe30, Ile31, Arg51, Asp77, Ser136, Tyr170, Lys174, Val200, Ser200 and His215. Five one-point substitution mutants were prepared for each active site residue. The three dimensional structures of *in* 

*silico* mutants were constructed by homology modeling, using the program TRITON interfaced with MODELLER **[6-8]**. Each mutant homology model was evaluated for its stereo chemical quality using PROCHECK **[9]** and also checked for environmental profile using ERRAT (Structure analysis and verification servers).

#### Substrate docking

Docking studies were carried out with five different hydroxycinnamoyl CoA esters; 4-coumaroyl CoA, caffeoyl CoA, feruloyl CoA, 5- hydroxyferuloyl CoA, sinapoyl CoA. Substrate molecules were downloaded from Pubchem database on NCBI, and converted to 3D molecules using LigPrep module in the schrodinger suite (LigPrep, version 2.4; Schrodinger: New York, 2010). Protein-ligand complexes were minimized within an RMSD of 0.30 Å with force field OPLS2005 using MacroModel package (MacroModel, version 9.8; Schrodinger: New York, 2010). Protein- ligand docking simulations were conducted using AutoDock Vina tool to prepare the systems for calculations [10]. For each ligand, around 100 docking runs with default parameters were performed treating protein as rigid and the ligand as flexible. The results were visualized using PyMoI (The PyMOL Molecular Graphics System, Version 1.5.0.4 Schrodinger, LLC.), wherein all the conformations for each of the ligand were found to be within the cavity of protein indicating that the docking run was free from errors. The conformational clusters with lowest binding energy (Ea) for each ligand were considered for further studies.



**Figure 2**: Surface representation of the catalytic active site in LI-CCRH1 model (close up view) and docking of cinnamoyl CoA esters in substrate binding pocket of LI-CCRH1; caffeoyl CoA (tv green), feruloyl CoA (yellow), hydroxyferuloyl CoA (blue), coumaroyl CoA (orange) and sinapoyl CoA (cyan). Active site residues are shown in white color.

#### Results & Discussion: Wild type LI-CCRH1

Docking simulations of different hydroxycinnamoyl CoA esters with LI-CCRH1 showed feruloyl CoA (-9.9 Kcal/mole) as most favored substrate over others (-8.8 to -9.7 Kcal/mole) (data unpublished). Better affinity of LI-CCRH1 towards feruloyl CoA indicates its possible role in lignification during growth and development of plants (Figure 2).

#### Phenylalanine30

Phe30 residue interacts with CoA esters via its main chain function and takes part in substrate binding or stabilization. Five mutants, namely F30C, F30L, F30S, F30V and F30Y were

ISSN 0973-2063 (online) 0973-8894 (print) Bioinformation 9(5): 224-232 (2013) generated by homology modeling using TRITON software. **Table 1 (see supplementary material)** shows a comparison of the MODELLER produced mutants with respect to RMSD. Docking studies showed that F30S and F30V showed feruloyl CoA as favored substrate; while mutants F30L, F30S and F30Y showed preference for coumaroyl CoA **Table 2 (see supplementary material)**. The main chain function of all mutants was expected to remain same even after mutation. Thus, there should be conformational alterations or change in geometry of active site following mutations resulting in differential substrate specificity (**Figure 1 & 3 A,B**). The RMSD values for phe30 mutants lay between 0.107-0.125 Å. Mutants F30C and F30V may play a role in lignin biosynthesis; while F30S, F30Y and F30L could be important for defense cascades **I3-5I**.

#### Isoleucine31

Ile31 residue also plays role in substrate binding through its main chain function. Mutant I31F showed more negative binding energy for hydroxyferuloyl CoA; while I31M exhibited equal affinity for coumaroyl and hydroxyferulol CoA. Mutant I31N demonstrated better affinity for sinapoyl CoA over others. Substitution with serine and threonine (I31S and I31T) resulted in feruloyl CoA as preferred substrate with equal binding energy (-10 Kcal/mole) (Figure 1, Table 2). In silico mutations of Ile31 lead to overall change in architecture of active site pocket (Figure 3 C,D). Slightly higher RMSD value was observed for mutant I31M compared to other Ile31 mutants (Table 1).

#### Arginine51

In wild type LI-CCRH1, Arg51 interacts with CoA esters via its main chain as well as side chain and plays important role in substrate binding or stabilization. In mutant R51G, coumaroyl CoA showed most favorable binding energy (-9.7 Kcal/mole). Substitution of Arg51 by glycine altered side chain from polar charged to small compact neutral residue, resulting in marked decrease in accessible surface area and leads to loss of interactions with substrate (Table 2). Similar decrease in surface area was observed with mutants R51I, R51S and R51T except for R51K, which showed similar area as that of wild type. Except R51K, all three remaining mutants have shown better preference for coumaroyl CoA; and thus could play role in defense. Mutant R51K showed affinity towards caffeoyl CoA (Table 2) (Figure 1 & 3 E, F). Thus, all mutants R51G, R51I, R51S, R51K and R51T may function in defense [3, 4].

#### Aspartate77

No Asp77 interactions were observed during docking of LI-CCRH1 with different CoA esters; but site directed mutagenesis and chemical modification confirmed its role in CCR catalyzed reaction (data not shown). D77 is present partially on surface and in proximity of R51. Substitution with alanine and glycine resulted in slight decrease in surface areas, but substrate affinities differ for both these mutants; D77A showed specificity towards coumaroyl CoA while D77G displayed equal affinity for Caffeoyl CoA and sinapoyl CoA (Figure 1, Table 2). This may be due to change in structure of binding pocket and allowing better conformations to interact with other amino acids. Mutant D77H showed more negative binding energy for sinapoyl CoA (-9.7 Kcal/mol). D77N has same substrate affinity (feruloyl CoA) as that of wild type. In mutation D77Y, change

from D to Y altered small polar side chain to bulky hydrophobic aromatic ring, induced a significant increase in accessibility for

surface area **(Figure 3 G,H).** RMSD values for all mutants are very comparable to each other (0.104-0.122 Å) **(Table 1).** 



Figure 3: The best docked structures of cinnamoyl CoA esters into the active site of *in silico* LI-CCRH1 mutants at various positions. Mutated LI-CCRH1 molecule (red) is represented as solid surface, whereas docked structure is shown as sticks. Mutated amino acid is displayed in blue color and remaining active site residues are shown in white. (A) F30C mutant, feruloyl CoA (B) F30Y mutant, coumaroyl CoA (C) I31F mutant, coumaroyl CoA (D) I31N mutant, sinapoyl CoA (E) R51I mutant, coumaroyl CoA (F) R51K mutant, caffeoyl CoA (G) D77H mutant, sinapoyl CoA (H) D77Y mutant, coumaroyl CoA.

#### Serine136

In wild type LI-CCRH1, Ser136 plays a key role in catalysis and is a part of reaction centre. Mutations in ser136 caused change in the geometry of active site and non specific interactions with substrates have been increased. Ser136 is buried in the substrate binding pocket and mutations resulted in partial or complete exposure of mutant residue. Coumaroyl CoA was found to be better substrate for mutant S136A and S136C. S136Y showed preference for caffeoyl CoA and, S136P along with S136T showed favored specificity for feruloyl CoA (Table 2) (Figure 1 and 4 A,B). Mutants S136P and S136T could have functional role in lignin formation; while mutants S136Y, S136A and S136C might be involved in defense [3-5].

#### Tyrosine170

Tyr170 acts as catalytic base and accepts hydride from NADPH and transfers to Serine residues in catalysis reaction. Tyr170 is completely buried in active site pocket and is surrounded by Lys174, Ser212, His215 and Ser136 catalytic residues. Mutant Y170C displayed less number of interactions compared to wild type; still sufficient for displaying affinity towards coumaroyl CoA. Mutant Y170D exhibited better binding energy for feruloyI CoA (-9.7 Kcal/mol) (Figure 1). In case of mutant Y170D, increase in number of hydrogen bond interactions with Arg51 was observed. Arg51 is distantly present from Y170D. This indicates the drastic change in architecture of binding pocket and significant changes in pKa values of pocket. Y170F and Y170N showed preference for coumaroyI CoA; while Y170H demonstrated affinity for feruloyI CoA (Table 2 & Figure 4 C, D).

#### Lysine174

Lys174 residue promotes hydride transfer in CCR mediated reduction reactions. Docking simulations of K174E and K174T mutants showed coumaroyl CoA as preferred substrate (-10 Kcal/mole) (Figure 1 & Table 2). On the other hand, K174M mutant is specific for feruloyl CoA; while K174N and K174R have favorable binding energy for hydroxyferuloyl CoA (Figure 1). Thus, mutant K174R may play role in both, that is, either in lignin biosynthesis or defense system [3, 4]. Lys174 is deeply buried in active site pocket and same conformational profile was also observed in all mutants (Figure 4 E,F). Mutant K174R

shows the lowest RMSD (0.097 Å) among the all mutants generated (Table 1).

#### Valine200

Replacement of Val200 with glycine and alanine displayed substrate specificity towards coumaroyl CoA. These substitutions have not altered aliphatic side chain profile of mutant residues. On the other hand, substitution of Valine by positively charged Aspargine resulted in charge redistribution along the active site pocket and allowed favorable conformational changes for substrate binding (feruloyI CoA). V200M residue exhibited increased affinity for coumaroyI CoA (Table 2 & Figure 4 G,H). V200E mutant could possibly be involved in monolignol biosynthesis. Mutants V200G, V200M, V200A may take part in secondary metabolite synthesis [3, 4] (Supplementary Figure S1).

#### Serine212

Ser212 residue is involved in proton shuttle mechanism and thus participates in CCR catalysis. Mutations in Ser residue (deeply buried) by Gly, Ile, Glu and Arg showed partial or complete exposure of mutated residues. These exposed mutated residues altered substrate binding conformation and favored coumaroyl CoA as substrate (Figure 1 & Supplementary Figure S2 A, B). Thus, these structural changes altered shape of active site pocket and assisted more number of interacting residues with maximum interactions. S212T has shown feruloyI CoA as promising substrate (Figure 1 & Supplementary Figure S2 C) (Table 2). All mutants of ser212 except S212T may function in secondary metabolism, ultimately in providing defense [3-5].

#### Histidine215

His215 also takes part in CCR mediated reduction reactions either in substrate binding or catalysis. H215R and H215Y mutants showed significant increase in surface area due to bulky and long side chains. Both mutants showed same binding energy (-10.5 Kcal/mol), but for different substrates; that is, coumaroyl CoA is specific for H215R and hydroxyferuloyl CoA for H215Y (Figure 1 & Supplementary Figure S2 E, F). Replacement of His215 by Asp and Gln demonstrated affinity for coumaroyl CoA (Figure 1). Lastly, replacement of His with Leu displayed preference for sinapoyl CoA (Table 2) (Figure 1 & Supplementary Figure S2 D). Thus, only H215L mutant may possibly take part in lignification; while remaining mutants might prefer secondary metabolite pathway [3, 4].



Figure 4: Cinnamoyl CoA esters conformations (sticks) in the active site of mutants LI-CCRH1 (surface, red). Color code for mutated and remaining active site residue is same as mentioned in Figure 3 (A) S136C mutant, coumaroyl CoA (B) S136Y mutant, caffeoyl CoA (C) Y170F mutant, coumaroyl CoA (D) Y170H mutant, coumaroyl CoA (E) K174E mutant, coumaroyl CoA (F) K174N mutant, hydroxyferuloyl CoA (G) V200L mutant, hydroxyferuloyl CoA (H) V200M mutant, coumaroyl CoA. Lysine174 residue is deeply buried inside the binding pocket, thus it is not visible in E, F surface diagrams.

#### Conclusion:

In conclusion, in silico mutation analysis of active site residues of LI-CCRH1 displayed differential substrate specificity. This in ISSN 0973-2063 (online) 0973-8894 (print) Bioinformation 9(5): 224-232 (2013)

differential substrate specificity mainly was due to conformational changes in substrate binding pocket or change geometry/architecture/shape of active site or

increase/decrease in number of interactions following mutations or charge redistribution along active site and physiochemical properties of mutated residues. On the basis of substrate specificity, possible physiological role of mutant CCRs could be predicted.

#### Acknowledgement:

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#### **Reference:**

- [1] Boerjan J et al. Annu Rev Plant Biol. 2003 54: 519 [PMID: 14503002]
- [2] Lacombe E et al. Plant J. 1997 11: 429 [PMID: 9107033]

- [3] Zhou R et al. Proc Natl Acad Sci U S A. 2010 1071: 17803 [PMID: 20876124]
- [4] Escamilla-Trevino L *et al. New phytol.* 2010 **185:** 143 [PMID: 19761442]
- [5] Li L et al. Plant Cell Physiol. 2005 46: 1073 [PMID: 15870094]
- [6] Eswar N et al. Curr Protoc Bioinformatics. 2006 15: 5.6.1 [PMID: 18428767]
- [7] Sali A & Blundell TL, J Mol Biol. 1993 234: 779 [PMID: 8254673]
- [8] Prokop M et al. Bioinformatics. 2008 24: 1955 [PMID: 18603567]
- [9] Laskowski RA et al. J Biomol NMR. 1996 8: 477 [PMID: 9008363]
- [10] Trott O & Olson A, J Comput Chem. 2010 31: 455 [PMID: 19499576]

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### Supplementary material:



Figure S1: Cinnamoyl CoA esters binding energy for in silico Val200 mutants. X-axis represents V200 mutants generated by homology modeling, using TRITON/MODELLER software, and Y-axis is respective binding energies for CoA esters docked with each individual mutant. The '\*' shows best docked substrate with most negative binding energy among particular mutant.



Figure S2: Docking of cinnamoyl CoA esters in active site of CCRH1 mutants. A) S212I mutant, coumaroyl CoA (B) S212R mutant, coumaroyl CoA (C) S212T mutant, feruloyl CoA (D) H215L mutant, sinapoyl CoA (E) H215R mutant, coumaroyl CoA (F) H215Y mutant, hydroxyferuloyl CoA.

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Table 1	DeatMaan	Course Doudations			mendulos disativa site multante af LLCCDL1
Table 1	Root iviean 3	square Deviations	RIVISD	) OF TRITUN/IVIODELLER	produced active site mutants of LI-CCRHT.

$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Residues	Mutants	RMSD (Å)
F30L         0.119           F30S         0.107           F30Y         0.122           F30Y         0.120           131F         0.107           131M         0.144           131N         0.115           Ile31         131S           131S         0.110           131T         0.104           R51G         0.153           R51I         0.116           R51T         0.099           D77A         0.122           D77A         0.122           D77A         0.122           D77H         0.112           D77G         0.122           D77H         0.104           S136A         0.144           S136A         0.144           S136A         0.134           Y170C         0.134           Y170C         0.134           Y170D         0.124           Y170D         0.124           Y170F         0.114           Y170F         0.114           Y170F         0.114           Y170F         0.114           Y170F         0.114           Y170F         0.11		F30C	0.125
F30S         0.107           F30V         0.122           F30Y         0.120           F30Y         0.120           F30Y         0.120           F30Y         0.120           F30Y         0.120           F30Y         0.120           F31M         0.144           J31N         0.115           III         0.107           F31S         0.110           R51G         0.153           R51H         0.115           R51K         0.138           Arg51         R51S         0.112           D77A         0.112         0.104           Ser136         0.134         0.144           S136A         0.144         1.11           Asp77         D77N         0.104           S136A         0.144         1.11           Asp77         D77N         0.104           S136A         0.134         1.11           Asp77         D71N         0.114           Tyr170         1.131         S136P           V170C         0.134         1.11           K174N         0.115         1.11           Y170P		F30L	0.119
Phe30         F30V         0.122           F30Y         0.120           I31F         0.107           I31M         0.114           I31N         0.115           IB31         131S         0.110           I31T         0.104           R51G         0.153           R51I         0.115           Arg51         R51S         0.116           R51S         0.116           R51T         0.099           D77A         0.122           D77G         0.122           D77H         0.111           Asp77         D77N         0.104           S136A         0.144           S136A         0.144           S136A         0.144           S136C         0.120           S136P         0.134           Y170C         0.134           Y170C         0.134           Y170F         0.114           Tyr170         Y170H         0.114           Y170F         0.114           Y170F         0.114           Y170F         0.114           Y170F         0.114           Y170F         0.114     <		F30S	0.107
F30Y         0.120           131F         0.107           131M         0.144           131N         0.115           131S         0.110           131T         0.104           R51G         0.153           R51I         0.115           R51K         0.138           Arg51         R51S         0.116           R51T         0.099           D77A         0.112           D77G         0.122           D77H         0.111           Asp77         D77N         0.104           S136A         0.144           S136C         0.122           D77H         0.111           Asp77         D77N         0.104           S136A         0.144           S136P         0.134           Y170C         0.134           Y170C         0.134           Y170D         0.124           Y170F         0.114           Tyr170         Y170H         0.115           Y170N         0.140           K174R         0.097           Y170H         0.123           V200E         0.128           <	Phe30	F30V	0.122
I31F         0.107           I31M         0.144           I31N         0.115           I31S         0.110           I31T         0.104           R51G         0.153           R51I         0.115           R51K         0.138           Arg51         R51S         0.116           R51T         0.099           D77A         0.112           D77G         0.122           D77H         0.111           Asp77         D77N         0.104           Si36C         0.120           Si36C         0.120           Si36C         0.134           Y170D         0.124           Y170F         0.114           Y200E         0.128           V200E         0.128           V200E         0.128 <td></td> <td>F30Y</td> <td>0.120</td>		F30Y	0.120
131M         0.144           131N         0.115           131S         0.110           131T         0.104           R51G         0.153           R51I         0.115           R51K         0.138           Arg51         R51S         0.116           R51T         0.099           D77A         0.112           D77G         0.122           D77H         0.104           Saf6C         0.120           D77H         0.104           Saf6C         0.120           Saf6C         0.120           Saf6C         0.120           Saf6C         0.120           Saf6C         0.120           Saf6C         0.134           Saf6C         0.134           Y170C         0.134           Y170D         0.124           Y170F         0.114           Tyr170         Y170H           K174K         0.097           K174K         0.017           Lys174         K174K           K174K         0.017           V200E         0.132           V200C         0.132		131F	0.107
IIB31       I31N       0.115         IIB31       I31S       0.110         IIB31       I31S       0.104         IIB31       R51G       0.153         R511       0.115       R51         R511       0.099       0.112         D77G       0.122       0.111         Arg51       D77H       0.112         D77H       0.114       111         Asp77       D77N       0.104         S136C       0.120       134         Ser136       S136T       0.134         Y170C       0.134       134         Y170C       0.134       134         Y170F       0.114       115         Y170F       0.114       119         Y170N       0.108       128         Y200E       0.128       129         Y200E       0.128       120         Y200F       0.116       121         Y200F       0.128       120         Y200F<		I31M	0.144
IIe31       I31S       0.110         I31T       0.104         R51G       0.153         R51I       0.115         R51K       0.138         Arg51       R51S       0.116         R51T       0.099         D77A       0.112         D77G       0.122         D77H       0.104         Si36A       0.144         S136C       0.120         Si36A       0.144         S136C       0.120         Si36P       0.134         Y170C       0.139         Y170C       0.139         Y170F       0.114         Tyr170       Y170H       0.115         Y170F       0.114         Tyr170       Y170F       0.114         Y170F       0.114         Y170N       0.140         K174N       0.118         V200E       0.128         V200E       0.128         V200E       0.128         V200E       0.116         V200E       0.128         V200C       0.116         V200E       0.128         V200A       0.116 <t< td=""><td></td><td>I31N</td><td>0.115</td></t<>		I31N	0.115
I31T         0.104           R51G         0.153           R51I         0.115           R51K         0.138           Arg51         R51S         0.116           R51T         0.099           D77A         0.112           D77G         0.122           D77H         0.111           Asp77         D77N         0.104           Si36A         0.144           S136C         0.120           S136P         0.134           Ser136         S136T         0.134           Y170C         0.139           Y170C         0.139           Y170F         0.114           Tyr170         Y170F         0.114           Y170F         0.114           Y170F         0.114           Y170N         0.140           K174E         0.153           K174N         0.108           Lys174         K174R         0.097           K174N         0.108           Lys174         K174R         0.097           K174N         0.108           V200E         0.132           V200E         0.132           V200A<	lle31	I31S	0.110
R51G         0.153           R511         0.115           R51K         0.138           R51S         0.116           R51T         0.099           D77A         0.112           D77G         0.122           D77H         0.114           Asp77         D77N         0.104           S136A         0.144           S136C         0.120           S136P         0.134           Ser136         S135T         0.134           Y170C         0.134           Y170C         0.134           Y170C         0.134           Y170C         0.134           Y170D         0.124           Y170F         0.114           Tyr170         Y170H         0.140           K174M         0.115           K174M         0.118           V200E         0.128           V200G         0.116           V200E         0.128           V200C         0.132           V200A         0.116           V2120         V200A         0.116           V2120         V200A         0.116           V2120         V2120 </td <td></td> <td>I31T</td> <td>0.104</td>		I31T	0.104
R511         0.115           R51K         0.138           R51S         0.116           R51T         0.099           D77A         0.112           D77G         0.122           D77H         0.111           Asp77         D77N         0.104           S136A         0.144           S136A         0.144           S136P         0.134           Ser136         S136T         0.131           Ser136         S136T         0.134           Y170C         0.124         Y170C           Y170D         0.124           Y170F         0.114           Tyr170         Y170F         0.114           K174E         0.153           K174M         0.108           Lys174         K174R         0.097           K174N         0.108           V200E         0.132           V200E         0.132           V200C         0.132           V200C         0.132           V200A         0.116           V200A         0.116           V2120         V200A         0.116           V2120         V200A		R51G	0.153
Arg51         R51K         0.138           R51S         0.116           R51T         0.099           D77A         0.112           D77G         0.122           D77H         0.111           Asp77         D77N         0.104           S136A         0.144           S136C         0.120           S136P         0.134           Ser136         S136T         0.134           Y170C         0.124           Y170D         0.124           Y170D         0.124           Y170D         0.124           Y170D         0.140           K174R         0.097           K174R         0.197           Y170N         0.140           K174R         0.097           K174R         0.132           V200E         0.132           V200A         0.116           S212Q		R51I	0.115
Arg51       R51S       0.116         R51T       0.099         D77A       0.112         D77G       0.122         D77H       0.104         Sap77       D77N         Asp77       D77N         S136A       0.144         S136C       0.120         Saf6P       0.134         Ser136       S136T       0.131         Y170C       0.124         Y170D       0.124         Y170F       0.114         Tyr170       Y170H       0.115         Y170N       0.140         K174R       0.097         K174N       0.108         Lys174       K174R       0.097         K174R       0.097         K174R       0.097         K174R       0.097         K174R       0.097         K174R       0.097         V200E       0.132         V200E       0.132         V200E       0.132         V200E       0.132         V200E       0.132         V200A       0.116         S212I       0.114         S212Q       0.114		R51K	0.138
R51T         0.099           D77A         0.112           D77G         0.122           D77H         0.111           Asp77         D77N         0.104           S136A         0.144           S136C         0.120           S136P         0.134           Ser136         S136T         0.134           Y170C         0.139         Y170C           Y170D         0.124         Y170C           Y170F         0.114         Y170F           Tyr170         Y170H         0.115           Y170N         0.140         K174N           K174N         0.119         K174N           Lys174         K174R         0.097           K174N         0.108         V200E           V200E         0.128           V200G         0.116           V200G         0.116           V200A         0.116           V200A         0.116           V2120         V200A           V200A         0.116           V2120         0.139           Ser212         S212R         0.130           S212V         0.139           Ser212	Arg51	R51S	0.116
D77A         0.112           D77G         0.122           D77H         0.111           Asp77         D77N         0.104           S136A         0.144           S136C         0.120           S136P         0.134           S136P         0.134           Y170C         0.134           Y170C         0.134           Y170D         0.124           Y170F         0.114           Tyr170         Y170F           Y170N         0.140           K174M         0.115           Y170N         0.140           K174M         0.119           K174M         0.108           Lys174         K174R         0.097           K174M         0.123           V200E         0.128           V200G         0.116           V200G         0.116           V200A         0.116           S212G         0.169           S212I         0.114           S212Q         0.130           S212I         0.114           S212Q         0.139           Ser12         S212R         0.130           S212T<	0	R51T	0.099
Asp77         D77G         0.122           D77H         0.111           D77N         0.104           D77Y         0.104           S136A         0.144           S136C         0.120           S136F         0.134           Ser136         S136F         0.134           Y170C         0.134           Y170C         0.134           Y170D         0.124           Y170F         0.114           Tyr170         Y170H         0.153           K174M         0.119         K174N           K174N         0.108         V200E           Lys174         K174R         0.097           K174N         0.132         V200E           V200C         0.132         V200L           V200C         0.132         V200G           V200A         0.116         S212G           V200A         0.116         S212G           Ser212         S212R         0.130           S212F         0.121         H14           S212R         0.130         S212F           S212F         0.121         H215D           H215D         0.117         H215D <td></td> <td>D77A</td> <td>0.112</td>		D77A	0.112
Asp77       D77H       0.111         D77N       0.104         D77Y       0.104         \$136A       0.144         \$136C       0.120         \$136F       0.134         Ser136       \$136F       0.134         \$136P       0.134         \$136P       0.134         \$136P       0.134         \$136Y       0.134         \$170D       0.124         \$170D       0.124         \$170D       0.140         \$170D       0.140         \$170D       0.140         \$170D       0.140         \$170D       0.140         \$170N       0.140         \$17170       \$170H       0.115         \$170N       0.140         \$174M       0.119         \$174M       0.108         \$200       0.128         \$200E       0.128         \$200E       0.132         \$212G       0.160         \$212G       0.169         \$212I       0.114         \$212Q       0.132         \$212Q       0.132         \$212Q       0.139         \$2		D77G	0.122
Asp77       D77N       0.104         D77Y       0.104         S136A       0.144         S136C       0.120         S136P       0.134         Ser136       S136T       0.131         S136P       0.134         Y170C       0.139         Y170D       0.124         Y170F       0.114         Tyr170       Y170F         Y170N       0.140         K174E       0.153         K174N       0.108         Lys174       K174R       0.097         K174N       0.116         V200E       0.128         V200E       0.132         V200G       0.116         V200A       0.116         V200A       0.116         S212G       0.169         S212I       0.114         S212N       0.139         Ser212       S212R       0.130         S212F       0.121         H215D       0.117         H215D       0.117         H215Q       0.139		D77H	0.111
D77Y         0.104           \$136A         0.144           \$136C         0.120           \$136P         0.134           \$er136         \$136T         0.131           \$136Y         0.134           Y170C         0.139           Y170D         0.124           Y170F         0.114           Tyr170         Y170F           Y170F         0.114           Tyr170         Y170H           Y170N         0.140           K174K         0.153           K174N         0.108           Lys174         K174R           V200E         0.128           V200G         0.116           V200C         0.132           V200A         0.116           V200A         0.116           S212G         0.169           S212I         0.114           S212N         0.139           Ser212         S212R         0.130           S212T         0.121           H215D         0.117           H215D         0.117           H215D         0.139           H15215         H215R         0.125	Asp77	D77N	0.104
Similar         0.144           Similar         0.120           Similar         0.134           Ser136         Similar           Similar         0.131           Similar         0.134           Y170C         0.134           Y170D         0.124           Y170F         0.114           Tyr170         Y170H         0.115           Y170N         0.140           K174E         0.153           K174M         0.119           K174N         0.108           Lys174         K174R         0.097           K174T         0.123           V200E         0.128           V200G         0.116           V200G         0.116           V200L         0.132           Val200         V200A         0.116           S212I         0.114           S212I         0.114           S212I         0.114           S212I         0.116           S212I         0.117           S212I         0.139           S212I         0.139           S212I         0.121           H215D         0.117		D77Y	0.104
Ser136         \$136C         0.120           Sindep         0.134           Ser136         \$136T         0.131           Sindep         0.134           Y170C         0.139           Y170D         0.124           Y170F         0.114           Tyr170         Y170H         0.115           Y170N         0.140           K174E         0.153           K174M         0.119           K174N         0.108           Lys174         K174R         0.097           K174T         0.123           V200E         0.128           V200G         0.116           V200G         0.116           V200L         0.132           Val200         V200A         0.116           S212I         0.114           S212G         0.169           S212I         0.116           S212I         0.1139           Ser212         S212R         0.139           Ser212         S212R         0.139           S212T         0.121         14215D           H215D         0.117         14215L           H215Q         0.139         142		S136A	0.144
Ser136       \$136P       0.134         Ser136       \$136T       0.131         \$136Y       0.134         Y170C       0.139         Y170D       0.124         Y170F       0.114         Tyr170       Y170H       0.153         K174E       0.153         K174M       0.119         K174N       0.108         Lys174       K174R       0.097         K174T       0.123         V200E       0.128         V200G       0.116         V200L       0.132         Val200       V200M       0.111         V2020A       0.116         S212G       0.169         S212I       0.114         S212Q       0.130         S212I       0.114         S212Q       0.130         S212I       0.116         S212I       0.117         H215D       0.117         H215D       0.117         H215Q       0.139         His215       H215R       0.125		S136C	0.120
Ser136       \$136T       0.131         \$136Y       0.134         Y170C       0.139         Y170D       0.124         Y170F       0.114         Tyr170       Y170H       0.153         K174E       0.153         K174M       0.119         K174N       0.108         Lys174       K174R       0.097         K174T       0.123         V200E       0.128         V200G       0.116         V200L       0.132         Val200       V200M       0.111         V2020A       0.116         S212G       0.169         S212I       0.114         S212Q       0.130         S212I       0.114         S212I       0.116         S212I       0.116         S212I       0.116         S212I       0.116         S212I       0.116         S212I       0.117         H215D       0.117         H215D       0.117         H215D       0.108         H215Q       0.139		S136P	0.134
S136Y       0.134         Y170C       0.139         Y170D       0.124         Y170F       0.114         Tyr170       Y170H       0.115         Y170N       0.140         K174E       0.153         K174M       0.119         K174N       0.108         Lys174       K174R       0.097         K174T       0.123         V200E       0.128         V200G       0.116         V200L       0.132         Val200       V200M       0.111         V200A       0.116         S212G       0.169         S212I       0.114         S212N       0.139         Ser212       S212R       0.130         S212T       0.121         H215D       0.117         H215D       0.117         H215D       0.117         H215Q       0.139	Ser136	S136T	0.131
Y170C       0.139         Y170D       0.124         Y170F       0.114         Tyr170       Y170H       0.115         Y170N       0.140         K174E       0.153         K174M       0.119         K174N       0.108         Lys174       K174R       0.097         K174T       0.123         V200E       0.128         V200G       0.116         V200L       0.132         Val200       V200M       0.111         V200A       0.116         S212G       0.169         S212I       0.114         S212R       0.130         S212I       0.114         S212N       0.139         Ser212       S212R       0.130         S212T       0.121         H215D       0.117         H215D       0.117         H215Q       0.139         His215       H215R       0.125		S136Y	0.134
Y170D       0.124         Y170F       0.114         Tyr170       Y170H       0.115         Y170N       0.140         K174E       0.153         K174M       0.119         K174N       0.108         Lys174       K174R       0.097         K174T       0.123         V200E       0.132         V200G       0.116         V200L       0.132         Val200       V200A       0.116         Ser212       S212G       0.169         S212I       0.114         S212N       0.139         Ser212       S212R       0.130         S212T       0.121         H215D       0.117         H215D       0.117         H215D       0.117         H215Q       0.139		Y170C	0.139
Y170F       0.114         Tyr170       Y170H       0.115         Y170N       0.140         K174E       0.153         K174M       0.119         K174N       0.108         Lys174       K174R       0.097         K174T       0.123       V200E       0.132         V200G       0.116       V200L       0.132         Val200       V200M       0.111         V200A       0.116         S212G       0.169         S212I       0.114         S212N       0.139         Ser212       S212R       0.130         S212T       0.121         H215D       0.117         H215D       0.117         H215Q       0.139		Y170D	0.124
$\begin{array}{cccccccc} Tyr170 & Y170H & 0.115 \\ Y170N & 0.140 \\ K174E & 0.153 \\ K174M & 0.119 \\ K174N & 0.108 \\ Lys174 & K174R & 0.097 \\ K174T & 0.123 \\ V200E & 0.128 \\ V200G & 0.116 \\ V200L & 0.132 \\ Val200 & V200M & 0.111 \\ V200A & 0.116 \\ S212G & 0.169 \\ S212I & 0.114 \\ S212N & 0.139 \\ Ser212 & S212R & 0.130 \\ S212T & 0.121 \\ H215D & 0.117 \\ H215L & 0.108 \\ H215Q & 0.139 \\ His215 & H215R & 0.125 \\ \end{array}$		Y170F	0.114
Y170N       0.140         K174E       0.153         K174M       0.119         K174N       0.108         Lys174       K174R       0.097         K174T       0.123         V200E       0.128         V200G       0.116         V200L       0.132         Val200       V200M       0.111         V200A       0.116         S212G       0.169         S212I       0.114         S212N       0.130         S212T       0.130         S212T       0.121         H215D       0.117         H215D       0.117         H215D       0.117         H215Q       0.139	Tyr170	Y170H	0.115
K174E       0.153         K174M       0.119         K174N       0.108         Lys174       K174R       0.097         K174T       0.123         V200E       0.128         V200L       0.132         Val200       V200M       0.111         V200A       0.116         S212G       0.169         S212I       0.114         S212V       0.139         Ser212       S212R       0.130         S212T       0.121         H215D       0.117         H215D       0.117         H215D       0.1139         H1s215       H215R       0.125		Y170N	0.140
K174M0.119K174N0.108Lys174K174RK174T0.123V200E0.128V200G0.116V200L0.132Val200V200MV200A0.111V200A0.116S212G0.169S212I0.114S212N0.139Ser212S212RL1200.121H215D0.117H215D0.117H215D0.139His215H215R0.125		K174E	0.153
K174N       0.108         Lys174       K174R       0.097         K174T       0.123         V200E       0.128         V200G       0.116         V200L       0.132         Val200       V200M         V200A       0.116         S212G       0.169         S212I       0.114         S212N       0.139         Ser212       S212R       0.130         S212T       0.121         H215D       0.117         H215D       0.117         H215Q       0.139         His215       H215R       0.125		K174M	0.119
Lys174       K174R       0.097         K174T       0.123         V200E       0.128         V200G       0.116         V200L       0.132         Val200       V200M         V200A       0.116         S212G       0.169         S212I       0.114         S212N       0.139         Ser212       S212R       0.130         S212T       0.121         H215D       0.117         H215D       0.117         H215Q       0.139         His215       H215R       0.125		K174N	0.108
K174T       0.123         V200E       0.128         V200G       0.116         V200L       0.132         Val200       V200M         V200A       0.116         S212G       0.169         S212I       0.114         S212N       0.139         Ser212       S212R       0.130         S212T       0.121         H215D       0.117         H215D       0.117         H215Q       0.139         His215       H215R       0.125	Lys174	K174R	0.097
V200E         0.128           V200G         0.116           V200L         0.132           Val200         V200M         0.111           V200A         0.116           S212G         0.169           S212I         0.114           S212N         0.139           Ser212         S212R         0.130           S212T         0.121           H215D         0.117           H215D         0.108           H215Q         0.139           His215         H215R         0.125		K174T	0.123
V200G       0.116         V200L       0.132         Val200       V200M       0.111         V200A       0.116         S212G       0.169         S212I       0.114         S212N       0.130         Ser212       S212R       0.130         S212T       0.121         H215D       0.117         H215D       0.108         H215Q       0.139		V200E	0.128
V200L       0.132         Val200       V200M       0.111         V200A       0.116         S212G       0.169         S212I       0.114         S212N       0.139         Ser212       S212R       0.130         S212T       0.121         H215D       0.117         H215D       0.108         H215Q       0.139		V200G	0.116
Val200       V200M       0.111         V200A       0.116         S212G       0.169         S212I       0.114         S212N       0.139         Ser212       S212R       0.130         S212T       0.121         H215D       0.117         H215L       0.108         H215Q       0.139		V200L	0.132
V200A       0.116         S212G       0.169         S212I       0.114         S212N       0.139         Ser212       S212R       0.130         S212T       0.121         H215D       0.117         H215L       0.108         H215Q       0.139	Val200	V200M	0.111
S212G       0.169         S212I       0.114         S212N       0.139         Ser212       S212R       0.130         S212T       0.121         H215D       0.117         H215L       0.108         H215Q       0.139         His215       H215R       0.125		V200A	0.116
S212I       0.114         S212N       0.139         Ser212       S212R       0.130         S212T       0.121         H215D       0.117         H215L       0.108         H215Q       0.139         His215       H215R       0.125		S212G	0.169
Ser212       S212N       0.139         Ser212       S212R       0.130         S212T       0.121         H215D       0.117         H215L       0.108         H215Q       0.139         His215       H215R		S212I	0.114
Ser212 S212R 0.130 S212T 0.121 H215D 0.117 H215L 0.108 H215Q 0.139 His215 H215R 0.125	0 010	S212N	0.139
H215D 0.121 H215D 0.117 H215L 0.108 H215Q 0.139 His215 H215R 0.125	Ser212	S212R	0.130
H215D 0.117 H215L 0.108 H215Q 0.139 His215 H215R 0.125		52121	0.121
H215L 0.108 H215Q 0.139 His215 H215R 0.125		H215D	0.117
H215Q 0.139 His215 H215R 0.125		H215L	0.108
HISZ10 HZ15K 0.125		H215Q	0.139
H215V 0.110	LI212	П213K H215V	0.120

 Table 2:
 Summary from docking simulations of LI-CCRH1 mutants with five different cinnamoyl CoA esters. For each mutant, more negative binding energy (preferred substrate) is indicated in red color.

Active site residues	Mutants	Binding Energies (Kcal/mole)					
in LI-CCRH1	LI-CCRH1	Caffeoyl	Feruloyl CoA	Hydroxy-	Coumaroyl	Sinapoyl	
		CoA	5	Feuloyl CoA	CoA	CoA	
Phe30	F30C	-9.4	-9.8	-8.7	-9.4	-8.9	
	F30L	-9.4	-10.3	-9.7	-10.5	-9.4	
	F30S	-9.1	-9.3	-9.7	-10.2	-9.1	
	F30V	-9.3	-9.4	-9.1	-9.0	-9.1	
	F30Y	-9.3	-9.1	-8.9	-9.6	-9.5	
lle31	I31F	-9.2	-8.9	-9.8	-9.5	-9.2	
	I31M	-9.2	-9.4	-9.7	-9.7	-9.2	
	131N	-9.4	-9.7	-9.3	-10.0	-10.1	
	I31S	-9.3	-10.0	-8.8	-9.0	-9.3	
	I31T	-9.0	-10.0	-9.2	-9.8	-9.3	
Arg51	R51G	-8.9	-9.1	-8.5	-9.7	-9.0	
0	R51I	-9.1	-9.1	-8.9	-9.6	-9.5	
	R51K	-10.0	-9.4	-9.9	-9.5	-9.5	
	R51S	-8.7	-9.6	-9.1	-9.8	-9.2	
	R51T	-9.4	-9.0	-9.1	-9.8	-9.4	
Asp77	D77A	-9.4	-9.8	-9.2	-10.0	-9.1	
•	D77G	-9.7	-9.6	-9.4	-9.5	-9.7	
	D77H	-8.9	-9.2	-9.7	-9.7	-9.8	
	D77N	-9.4	-10.2	-9.4	-8.9	-9.2	
	D77Y	-9.2	-8.9	-9.1	-10.1	-9.4	
Ser136	S136A	-9.0	-9.1	-9.5	-9.7	-9.4	
	S136C	-9.3	-9.3	-9.4	-9.6	-9.2	
	S136P	-10.2	-10.3	-9.8	-9.6	-9.3	
	S136T	-9.1	-9.6	-9.4	-9.5	-8.7	
	S136Y	-9.2	-8.7	-8.1	-8.8	-9.0	
Tyr170	Y170C	-8.8	-9.2	-8.7	-9.3	-9.1	
•	Y170D	-8.7	-9.7	-9.2	-9.0	-8.4	
	Y170F	-9.2	-9.1	-9.1	-9.3	-8.9	
	Y170H	-9.0	-9.5	-8.8	-8.9	-9.2	
	Y170N	-9.8	-8.8	-8.8	-9.9	-9.1	
Lys174	K174E	-9.1	-9.5	-9.8	-10.0	-9.9	
•	K174M	-9.6	-9.8	-9.6	-9.8	-9.6	
	K174N	-9.9	-9.3	-10.5	-10.2	-10.3	
	K174R	-9.3	-9.1	-9.9	-9.9	-9.3	
	K174T	-9.5	-9.2	-9.3	-9.9	-9.7	
Val200	V200E	-9.7	-10.2	-9.6	-9.8	-9.8	
	V200G	-9.4	-9.6	-9.2	-10.1	-9.4	
	V200L	-9.3	-9.3	-9.7	-9.4	-9.6	
	V200M	-9.0	-9.7	-9.8	-9.9	-9.2	
	V200A	-9.4	-9.6	-10.1	-10.5	-10.2	
Ser212	S212G	-9.1	-9.0	-9.3	-9.8	-9.6	
	S212I	-9.4	-9.7	-9.6	-10.3	-10.2	
	S212N	-9.8	-10.0	-8.9	-10.3	-9.6	
	S212R	-9.9	-10.0	-10.1	-10.5	-9.8	
	S212T	-9.7	-10.0	-9.8	-9.5	-9.6	
His215	H215D	-10.3	-9.8	-9.6	-10.5	-9.9	
	H215L	-9.5	-9.6	-9.2	-9.5	-9.7	
	H215Q	-9.2	-10.0	-9.0	-10.1	-9.5	
	H215R	-9.5	-9.7	-9.4	-10.5	-9.7	
	H215Y	-10.4	-10.1	-10.5	-9.9	-10.1	