Structural and Biochemical Characterization of the Human Cyclophilin Family of Peptidyl-Prolyl Isomerases

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Structural and Biochemical Characterization of the Human Cyclophilin Family of Peptidyl-Prolyl Isomerases

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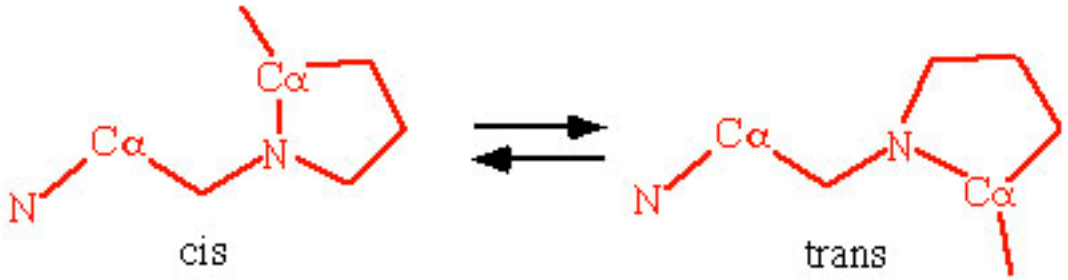
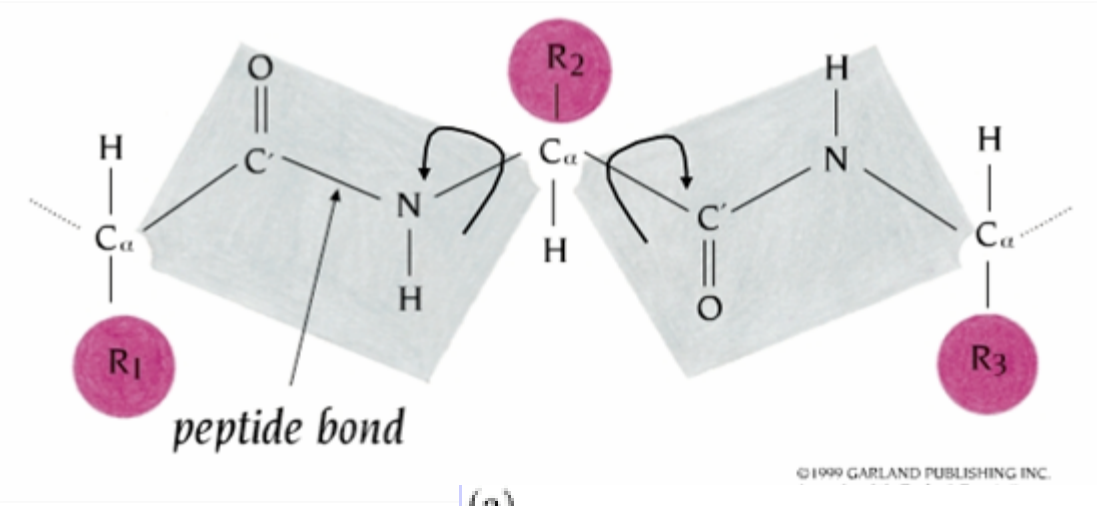
Abstract

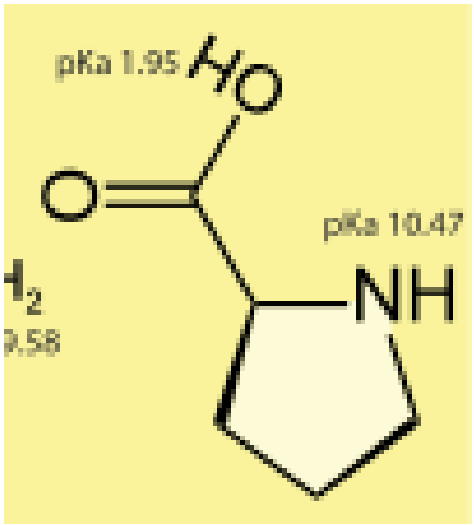
Peptidyl-prolyl isomerases catalyze the conversion between *cis* and *trans* isomers of proline. The cyclophilin family of peptidyl-prolyl isomerases is well known for being the target of the immunosuppressive drug cyclosporin, used to combat organ transplant rejection. There is great interest in both the substrate specificity of these enzymes and the design of isoform-selective ligands for them. However, the dearth of available data for individual family members inhibits attempts to design drug specificity; additionally, in order to define physiological functions for the cyclophilins, definitive isoform

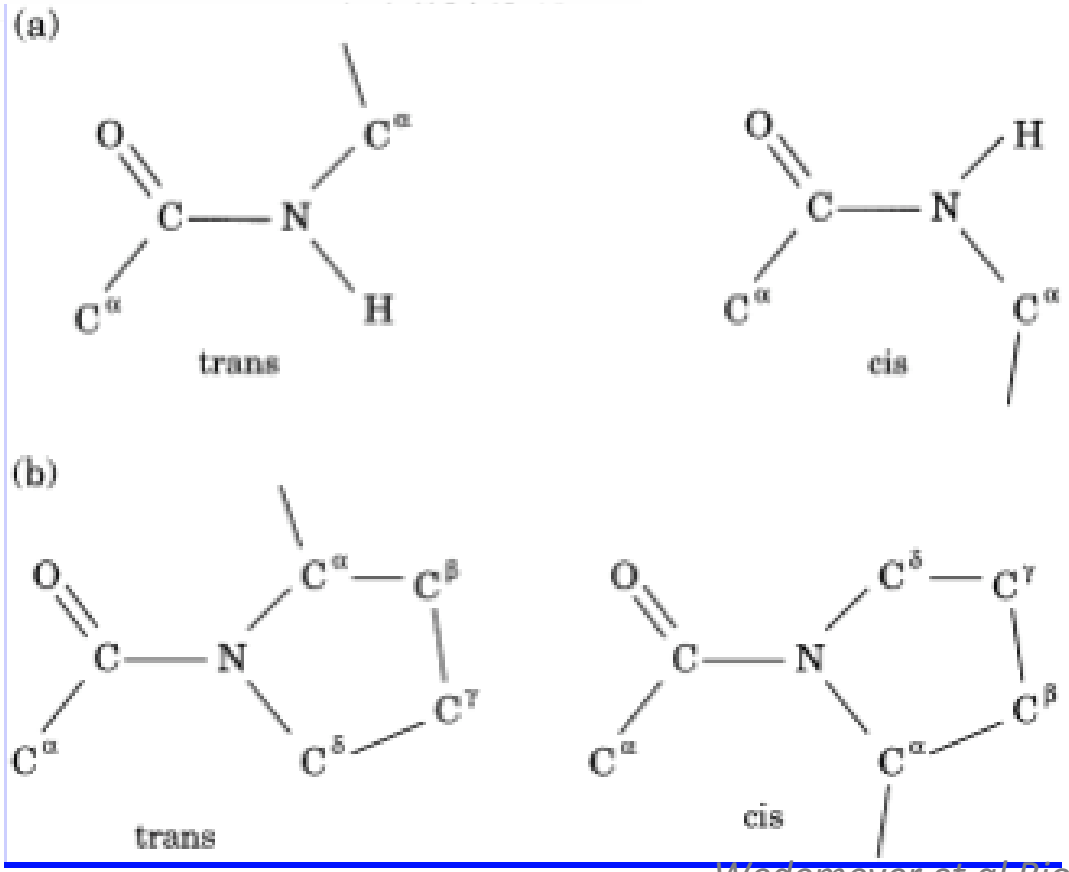
PPIases *Uurimisobjekt*

- Cyclophilin valkude perekonda kuuluvad **P**eptidyl-**P**rolyl **I**someraasid, mis katalüüsivad üleminekuid Proliini *cis* ja *trans* isovormide vahel.
- **ENZYME entry: EC 5.2.1.8**
  - **Rotamaas** Peptidylproline (omega=180) <=> peptidylproline (omega=0)









Wedemeyer et al Biochemistry 2002

Tähtsus

- Osalevad valgu õige struktuuri formeerumisel – chaperoni omadused
- On märklauaks immunisuppresandile ***cyclosporiinile***, mida kasutatakse immuunvastuse mahasurumisel organite siirdamisel
- **Pin1** isomeriseerib Pro juhul, kui eelnev amino-hape (S/T) on fosforüülitud
- **PPIA** moduleerib ka HIV infektsiooni läbi interaktsiooni kapsiidi (***gag***) valgu proliinide

this behaviour places Pin1 directly in the context of traditional signal transduction pathways

aga ka

- **PPIH** (SnuCyp20) osaleb splaisosoomides interageerudes 60K komponendiga tri-snRNP kompleksis – 3D struktuur tervest kompleksist näitab, et interaktsioonis osaleb piirkond mis on täpselt vastaspool "aktiiv saiti"
- **PPIE** on leitud RNA-äratundmis motiiv (RRM) ja detekteeritud RNA isomeraasne aktiivsus

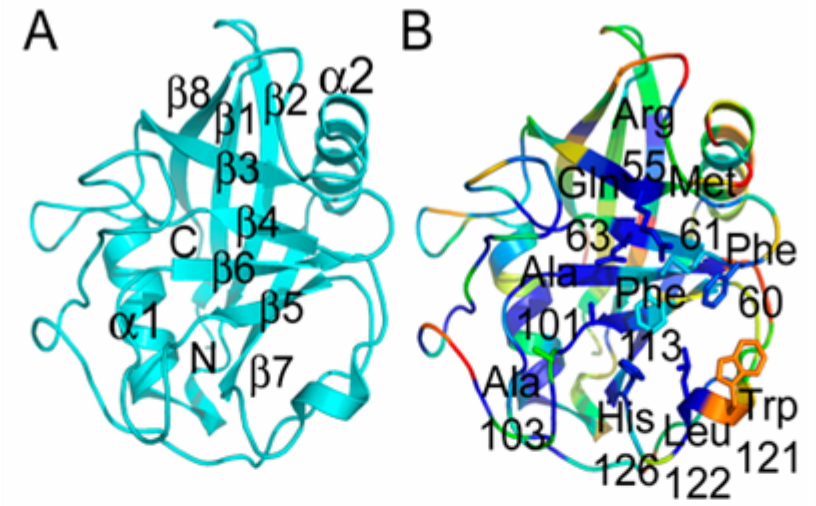
ja veel ....

- Inimeses on kirjeldatud 17 isovormi
- millest 7 on testitud isomeraasse aktiivsuse osasa
- Mille poolest erinevad isovormid erinevad ja milleks see hea on, on enam kui segane
- Isovormid pole eriti hästi kirjeldatud ei struktuurses ega fuktsionaalses mõttes

Selles töös

- Skreeniti proliini isomerisatsiooni võimet 15'l isovormil 17'st
- mõõdeti ka ***cyclosporiini*** seondumis affiinsust
- määrati struktuure (X-ray - 7 uut struktuuri lisaks 7'e teadaolevale)
- kasutati struktuurset infot katsetulemuste tõlgendamiseks

Structural elements of the cyclophilin fold and the definition of the active surface of PPIA



C

	55	60	61	63	101	113	121	122	126
PPIA	Arg	Phe	Met	Gln	Ala	Phe	Trp	Leu	His
PPIB	Arg	Phe	Met	Gln	Ala	Phe	Trp	Leu	His
PPIC	Arg	Phe	Met	Gln	Ala	Phe	Trp	Leu	His
PPID	Arg	Phe	Met	Gln	Ala	Phe	His	Leu	His
PPIE	Arg	Phe	Met	Gln	Ala	Phe	Trp	Leu	His
PPIF	Arg	Phe	Met	Gln	Ala	Phe	Trp	Leu	His
PPIG	Arg	Phe	Met	Gln	Ala	Phe	His	Leu	His
PPIH	Arg	Phe	Met	Gln	Ala	Phe	Trp	Leu	His
PPIL1	Arg	Phe	Met	Gln	Ala	Phe	Trp	Leu	His
PPIL2b	Arg	Phe	Val	Gln	Ala	Phe	Tyr	Leu	His
PPIL3	Arg	Phe	Met	Gln	Ala	Phe	His	Leu	Tyr
PPIL4	Asn	Phe	Ile	Gln	Val	Leu	Tyr	Leu	His
PPIL6	Arg	Gly	Met	Gln	Ala	Tyr	Tyr	Leu	Phe
PPWD1	Arg	Phe	Met	Gln	Ala	Phe	Trp	Leu	His
RANBP2	Arg	Phe	Val	Gln	Ala	Val	His	Leu	His
SDCCAG-10	Arg	Phe	Ile	Gln	Ala	Phe	Glu	Leu	His
NKTR	Arg	Phe	Met	Gln	Ala	Phe	His	Leu	His

Cyclophilin isoforms

Characterization of Cyclophilin Active Sites

- In order to elucidate the function of residues in the extended active site of the PPIase domain of the human cyclophilins, we probed the binding and catalytic function of these domains against either substrate or small-molecule inhibitors
- Three assays were utilized to explore these functions
  - **changes in thermal stability** (to assess cyclosporin binding)
  - **Cyclosporin binding**
  - **isomerisation activity** (Tetrapeptide Activity)

Lühendid: **CsA – Cyclosporin A**
CsC – Cyclosporin C
CsD – Cyclosporin D

A two-dimensional NMR experiment (1H/1H TOCSY) T^0 grad from RT --- > 80^0 C

Cyclophilin	Assay		Thermal Stability		ITC
	Tetrapeptide Activity	Cyclosporin Binding	Basal T_{agg} (°C)	ΔT_{agg}, CsC (°C)	K_d, CsA (nM)
PPIA	yes	yes	45.9	1.9	6.8
PPIB	yes	yes	60.0	3.4	8.4*
PPIC	yes	yes	50.6	7.0	7.7*
PPID	yes	yes	n/a	n/a	61
PPIE	yes	yes	60.4	6.8	6.9
PPIF	yes	yes	52.4	10.7	6.7*
PPIG	yes	yes	55.6	2	51
PPIH	yes	yes	54.4	7.3	160
PPIL1	yes	yes	49.1	1.7	9.8*
NKTR	yes	yes	45.4	3.0	488
PPWD1	yes	yes	50.6	5.2	168
PPIL2	no	no	50.9	n/a	n/d
PPIL6	no	no	60.0	n/a	n/d
RANBP2	no	no	n/a	n/a	n/d
SDCCAG-10	no	no	44.8	n/a	n/d
PPIL3	not tested	n/a	n/a	n/a	n/a
PPIL4	not tested	n/a	n/a	n/a	n/a

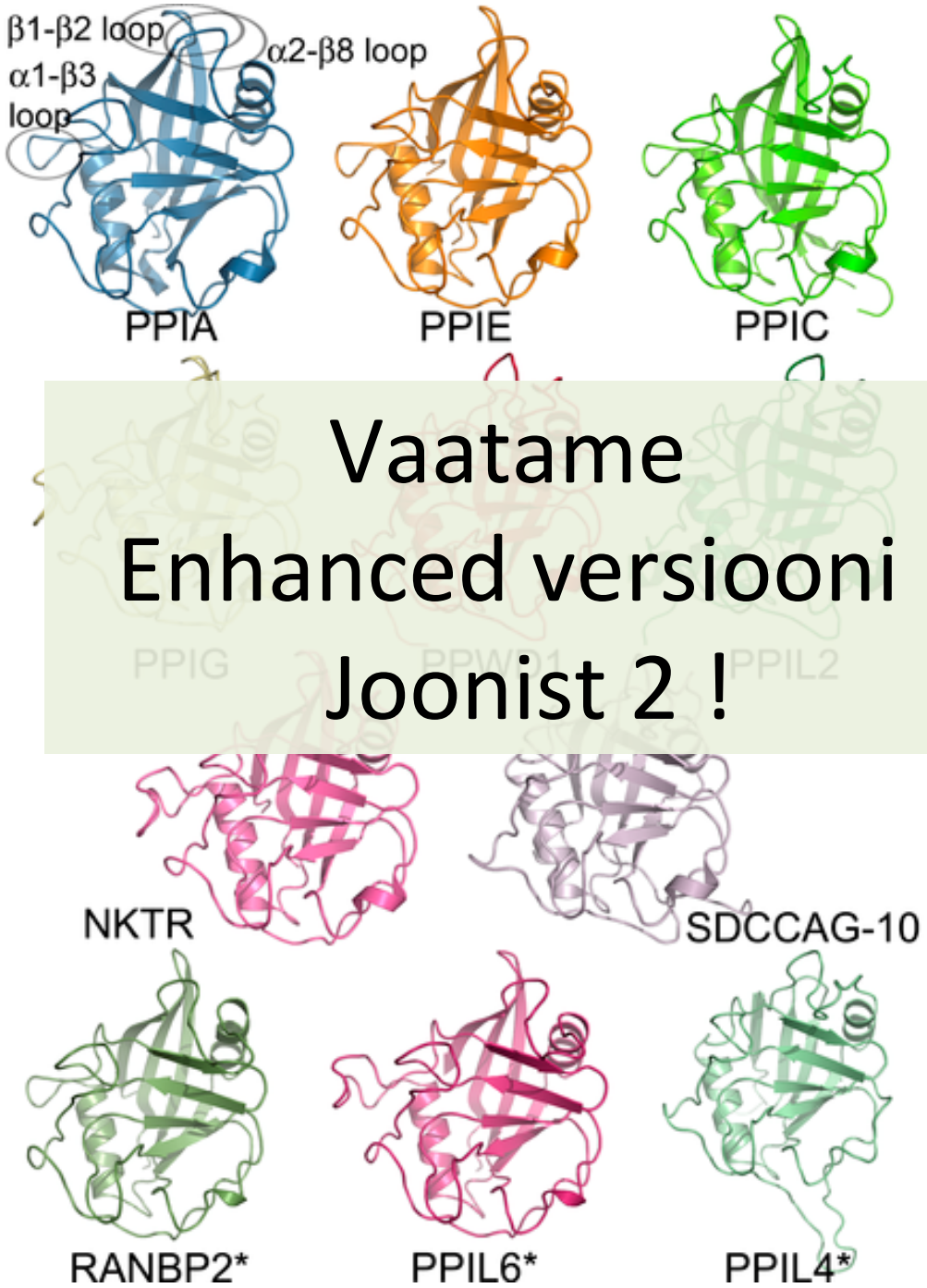
Tetrapeptide activity is defined as the collapse of substrate *cis/trans* peaks in the presence of highly purified PPIase protein, as previously described [30,40] and shown in Figure S1. Cyclosporin binding represents the combination of StarGazer and/or ITC data; briefly, any PPIase protein with a T_{agg} shift greater than 2°C in the presence of either cyclosporin A, C, or D is shown as a positive result. n/a indicates those cyclophilins that were either not tested or do not undergo a cooperative thermal transition [38,85]. The basal T_{agg} is shown for all family members for whom a cooperative thermal transition was observed, along with the observed T_{agg} shift for one cyclosporin compound (CsC). "n/d" indicates no binding isotherm was noted in ITC under the experimental conditions outlined in this study.
*For ITC data, asterisks indicate the K_i values obtained in a recent study of isoform-selective inhibitors for six cyclophilins [67].
doi:10.1371/journal.pbio.1000439.t001

Coclusions 1

- No binding was detected for PPIL2, PPIL6, or SDCCAG-10, making these, to our knowledge, the first set of human cyclophilins that have been found incompetent to ligate cyclosporin
- For all isoforms tested there was a strict correlation between the ability to bind cyclosporin and activity against the tetrapeptide substrates

In order to understand the molecular basis of these results,

- we sought structural coverage of the entire human cyclophilin enzymatic class.
- We determined crystal structures of seven human PPIase domains: PPIC, PPIE, PPIG, PPWD1, PPIL2, NKTR, and SDCCAG-10
- Combine these data with six previously determined structures (PPIA, PPIB, PPIF, PPIH, PPIL1, and PPIL3).



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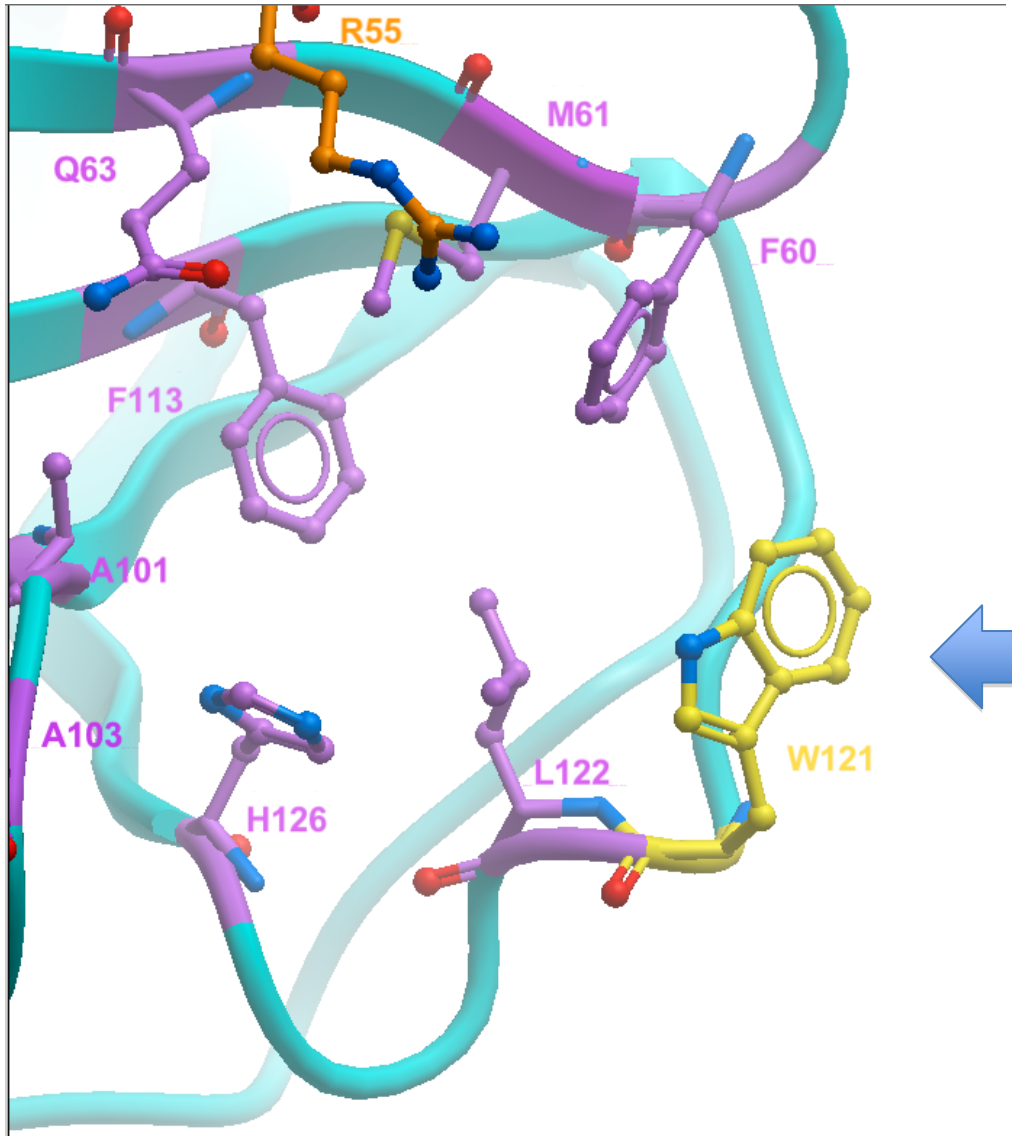
Joonist 2 !

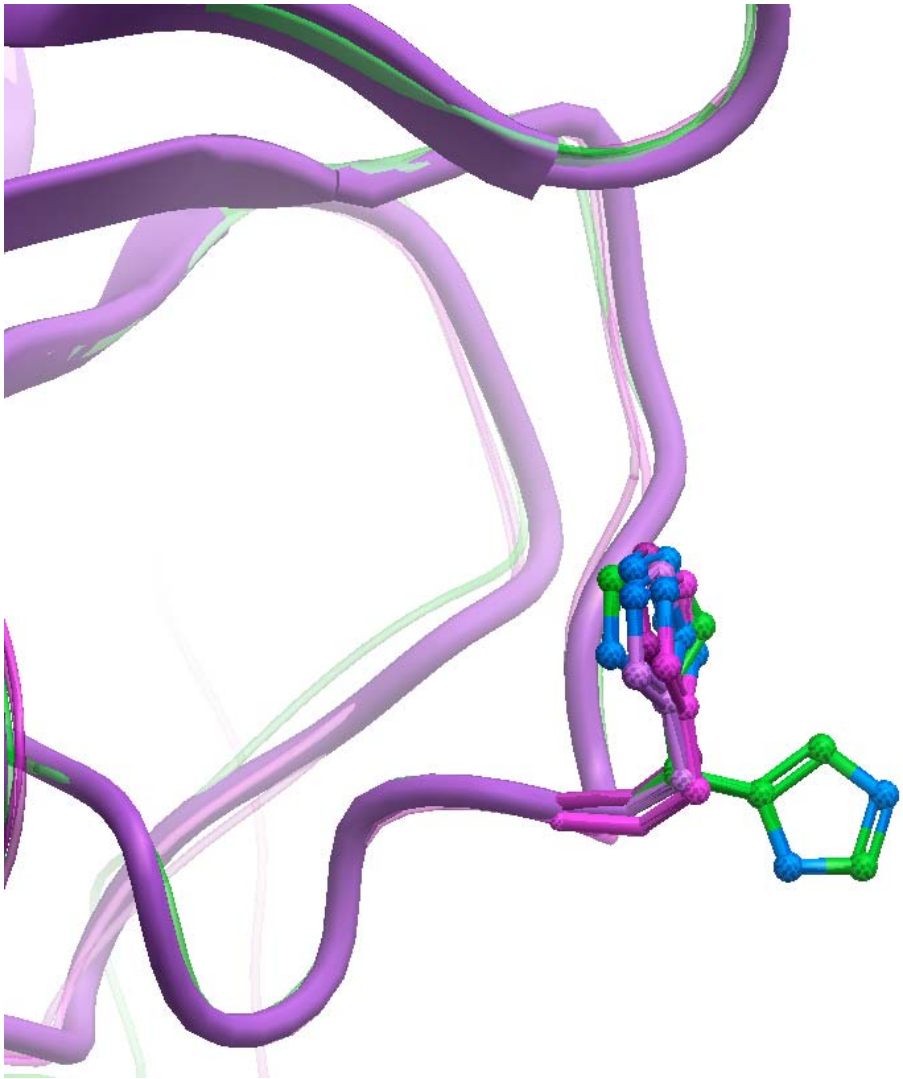
aktiivsait



the most striking correlation between cyclosporin binding, tetrapeptide identity, and active site residues is found at the **Trp121 position.**

Our results clearly show that a tryptophan ....

aktiivsait

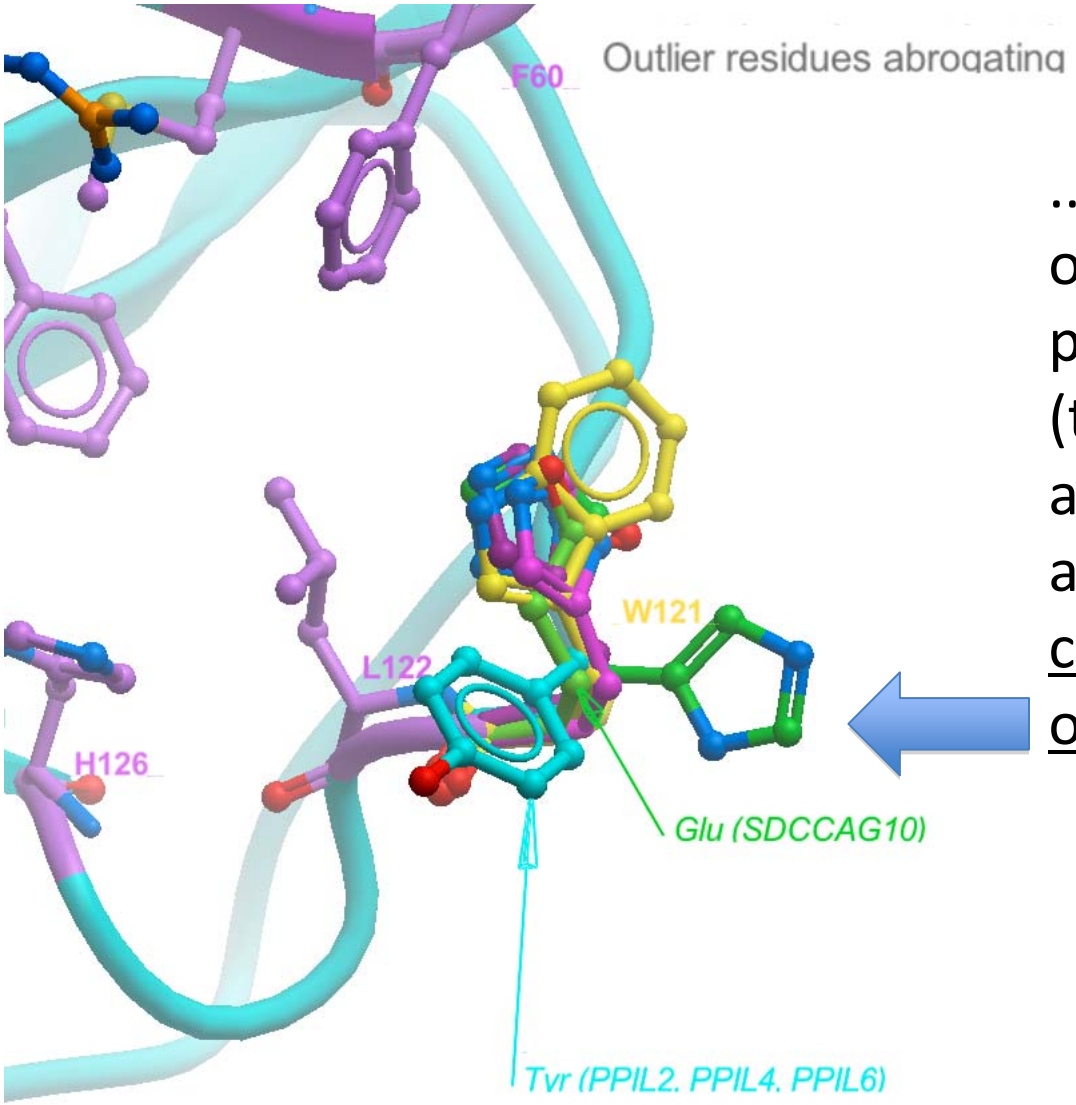


.... or histidine

at this position
is permissive for
cyclosporin binding

PPID, **PPIG**, **PPIL3, RANBP2**, and **NKTR**

aktiivsait



.... whilst other naturally occurring residues at this position
(tyrosine in PPIL2, PPIL4, and PPIL6, and glutamic acid in SDCCAG10) <u>abrogate cyclosporin binding under our experimental conditions</u>

The structural consequences of substitutions in the cyclophilin active site

SKIP !

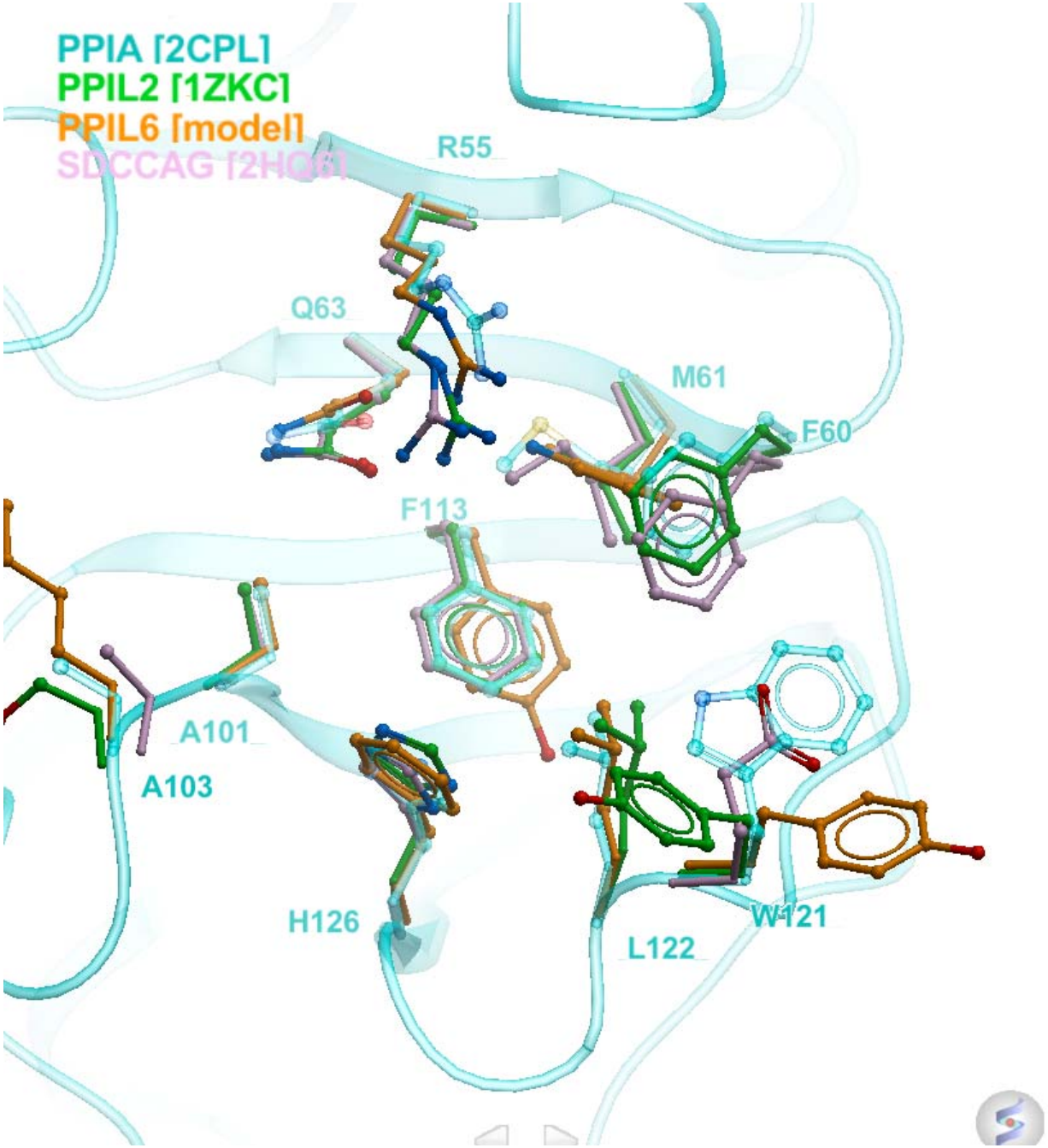
Three cyclophilins neither bound cyclosporin nor tetrapeptide

PPIL2, PPIL6, and SDCCAG-10

It is clear that these three proteins are quite divergent in the active site compared to PPIA
(vt joonis järgmisel slaidil)

they are the only isoforms that substitute the residue Trp121 with a non-histidine residue.

PPIL4 does not possess the otherwise strictly conserved Arg55


PPIA [2CPL]
PPIL2 [1ZKC]
PPIL6 [model]
SDCCAG [2HQ6]
R55
Q63
M61
F60
F113
A101
A103
H126
L122
W121

Expanding the Definition of the Cyclophilin Active Site: The S2 Pocket and Gatekeeper Hypothesis

Examination of the surface of the PPIase domains near the active site revealed two pockets that potentially contribute to substrate specificity, binding, and turnover.

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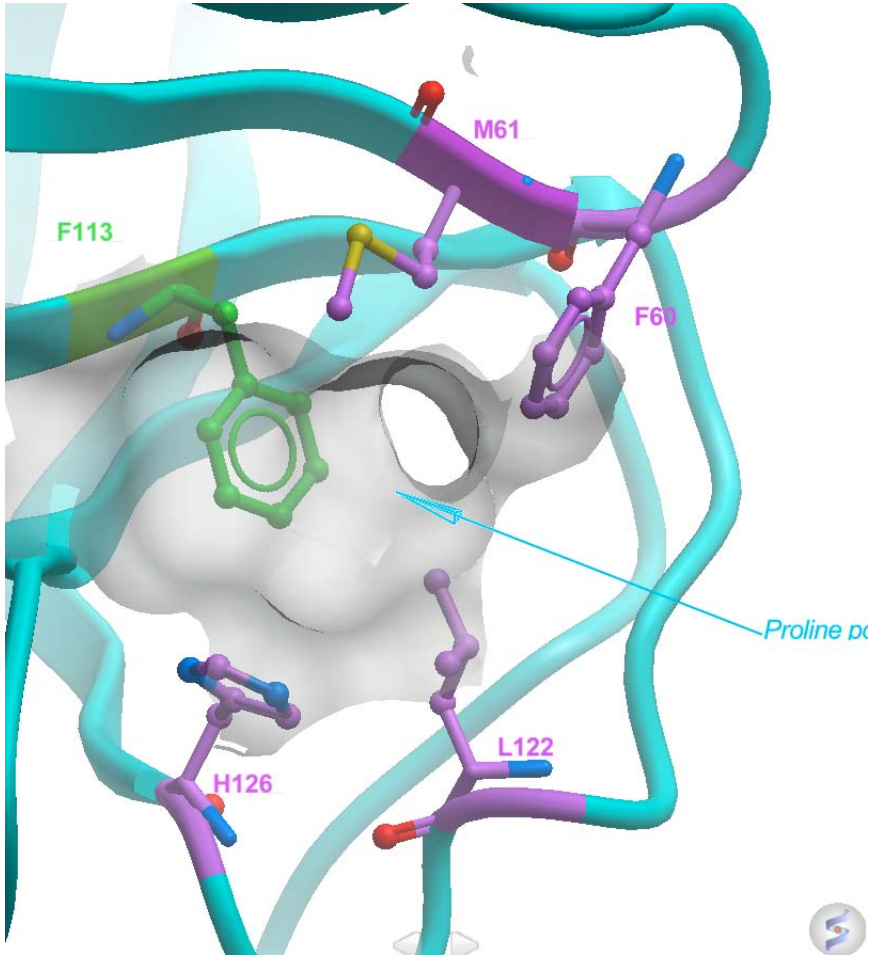
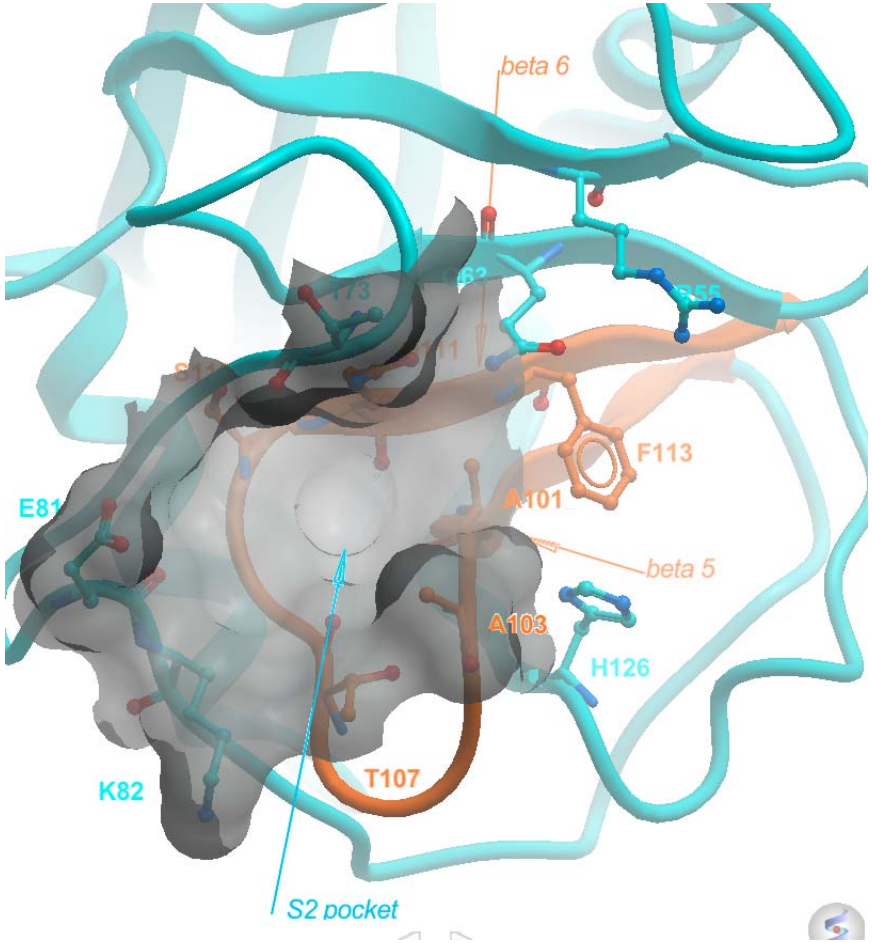
Enhanced versiooni
B Blokk!

The first pocket is the proline interaction surface

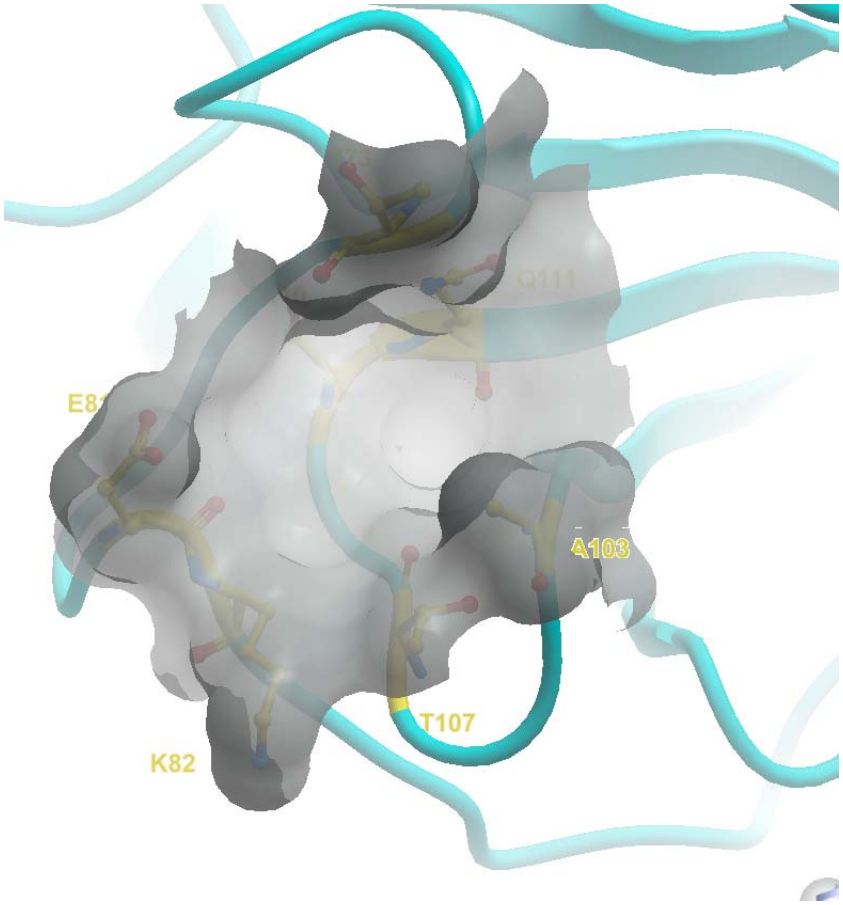


The second pocket forms a surface that likely interacts with substrate residue P2 or P3 relative to the substrate proline, and so will be named the S2 pocket hereafter



Indeed, the S2 pocket is extremely uniform across cyclophilins; it is deep and relatively nonspecific, so it can accommodate long, short, polar, or hydrophobic sidechains without penalty.

Gatekeepers



These gatekeeper residues at positions 81, 82, and 103 and the secondary gatekeeper at position 73 (so named because its position in most PPIase structures is pointed away from the S2 pocket) show major chemical and size variance.

Conclusion 2

- Gatkeeper positsioonid on isovormide spetsiifilised
- Gatkeeper positsioonid mõjutavad substraadi seondumist