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Supporting information for article:

Conformational heterogeneity in apo and drug-bound structures of *Toxoplasma gondii* prolyl-tRNA synthetase

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Apol Apo2	397 GAMVTAKKDENFSEWYTQAIVRSEMIEYYDISGCYIMRPWAFHIWEKVQRFFDDEIKKMGVENSYFF GAMVTAKKDENFSEWYTQAIVRSEMIEYYD <mark>ISG</mark> CYIMRPWAFHIWEKVQRFFDDEIKKMGVENSYFF
Holl Hol2	GAMVTAKKDENFSEWYTQAIVRSEMIEYYDISGOYIMRPWAFHIWEKVQRFFDDEIKKMGVENSYFF GAMVTAKKDENFSEWYTQAIVRSEMIEYYDISGCYIMRPWAFHIWEKVQRFFDDEIKKMGVENSYFF
Apol Apo2	464 MFVS <mark>RHKLEKEKDHVEGFSP</mark> EVAWVTHYGDSPLPEKIAIRPTSETIMYPAYAKWIRSHRDLPLKLN(M <mark>FVSRHKLEKEKDHVEGFSPEVAWVTHYGDS</mark> PLPEKIAIRP <mark>TSETIMYPAYAKWIRSHRDLPLKLN(</mark>
Holl Hol2	MFVSRHKLEKEKDHVEG <mark>F</mark> SPEVAWVTHYGDS <mark>P</mark> LPEKIAIR <mark>PT</mark> SETIMYPAYAKWIRSHRDLPLKLN(MFVSRHKLEKEKDHVEGFSPEVAWVTHYGDSPLPEKIAIRPT <mark>SE</mark> TIMYPAYAKWIRSHRDLPLKLN(
Apol Apo2	465 531 WCSVVRWEFKQPTPFLRTREFLWQEGHTAHATEEEAWELVLDILELYRRWYEECLAVPVIKGEKSEG WCSVVRWEFKQPTPFLRTREFLWQEGHTAHATEEEAWELVLDILELYRRWYEECLAVPVIKGEKSEG
Holl Hol2	WCSVVRWEFKQPTPFIRTREFIWQEGHTAHATEEEAWELVLDILELYRRWYEECLAVPVIKGEKSEG WCSVVRWEFKQPTPFIRTREFIWQEGHTAHATEEEAWELVLDILELYRRWYEECLAVPVIKGEKSEG
Apol Apo2	532 EKFAGGKKTTTVEAFIPENGRGIQAATSHILGTNFAKMFEIEFEDEEGHKRLVHQTSWGCTTRSLGV EKFAGGKKTTTVEAFIPENGRGIQAATSHLLGTNFAKMFEIEFEDEEGHKRLVHQTSWGCTTRSLGV
Holl Hol2	EKFAGGKKTTTVEAFIPENGRGI <mark>O</mark> AATS <mark>H</mark> LLGTNFAKMFEIEFEDEEGHKRLVHQT <mark>S</mark> WGQTIRSLGV EKFAGGKKTTTVEAFIPENGRGI <mark>O</mark> AATSHLLGTNFAKMFEIEFEDEEGHKRLVHQTSWGQTIRSLGV
	599 665
Apol Apo2	MIMTHGDDKGLVIPPRVASVQVVIIPILFKDENTGEILGKCRELKTMLEKADIRVRIDDRSNYTPGW MIMTHGDDKGLVIPPRVASVQVVIIPILFKDENTGEILGKCRELKTMLEKADIRVRIDDRSNYTPG
Apol Apo2 Holl Hol2	MIMTHGDDKGLVIPPRVASVQVVIIPILFKDENTGEILGKCRELKTMLEKADIRVRIDDRSNYTPGW MIMTHGDDKGLVIPPRVASVQVVIIPILFKDENTGEILGKCRELKTMLEKADIRVRIDDRSNYTPGW MIMTHGDDKGLVIPPRVASVQVVIIPILFKDENTGEILGKCRELKTMLEKADIRVRIDDRSNYTPGW
Apo1 Apo2 Hol1 Hol2 Apo1 Apo2	MIMTHGDDKGLVIPPRVASVQVVIIPILFKDENTGEILGKCRELKTMLEKADIRVRIDDRSNYTPGW MIMTHGDDKGLVIPPRVASVQVVIIPILFKDENTGEILGKCRELKTMLEKADIRVRIDDRSNYTPGW MIMTHGDDKGLVIPPRVASVQVVIIPILFKDENTGEILGKCRELKTMLEKADIRVRIDDRSNYTPGW MIMTHGDDKGLVIPPRVASVQVVIIPILFKDENTGEILGKCRELKTMLEKADIRVRIDDRSNYTPGW 666 732 KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI
Apo1 Apo2 Hol1 Hol2 Apo1 Apo2 Hol1 Hol2	MIMTHGDDKGLVIPPRVASVQVVIIPILFKDENTGEILGKCRELKTMLEKADIRVRIDDRSNYTPGW MIMTHGDDKGLVIPPRVASVQVVIIPILFKDENTGEILGKCRELKTMLEKADIRVRIDDRSNYTPGW MIMTHGDDKGLVIPPRVASVQVVIIPILFKDENTGEILGKCRELKTMLEKADIRVRIDDRSNYTPGW MIMTHGDDKGLVIPPRVASVQVVIIPILFKDENTGEILGKCRELKTMLEKADIRVRIDDRSNYTPGW 666 732 KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI
Apol Apo2 Holl Hol2 Apo1 Hol1 Hol2 Apo1 Apo1 Apo1	MIMTHGDDKGLVIPPRVASVQVVIIPILFKDENTGEILGKCRELKTMLEKADIRVRIDDRSNYTPGW MIMTHGDDKGLVIPPRVASVQVVIIPILFKDENTGEILGKCRELKTMLEKADIRVRIDDRSNYTPGW MIMTHGDDKGLVIPPRVASVQVVIIPILFKDENTGEILGKCRELKTMLEKADIRVRIDDRSNYTPGW MIMTHGDDKGLVIPPRVASVQVVIIPILFKDENTGEILGKCRELKTMLEKADIRVRIDDRSNYTPGW 666 732 KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI
Apol Apo2 Holl Apo1 Apo2 Holl Apo2 Holl Apo2 Holl Hol2	MIMTHGDDKGLVIPPRVASVQVVIIPILFKDENTGEILGKCRELKTMLEKADIRVRIDDRSNYTPGW MIMTHGDDKGLVIPPRVASVQVVIIPILFKDENTGEILGKCRELKTMLEKADIRVRIDDRSNYTPGW MIMTHGDDKGLVIPPRVASVQVVIIPILFKDENTGEILGKCRELKTMLEKADIRVRIDDRSNYTPGW MIMTHGDDKGLVIPPRVASVQVVIIPILFKDENTGEILGKCRELKTMLEKADIRVRIDDRSNYTPGW 666 732 KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRTTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRTTGEAY
Apol Apo2 Holl Apo1 Apo2 Holl Hol2 Apo1 Hol1 Hol2 Apo1 Apo1 Apo1 Apo2	MIMTHGDDKGLVIPPRVASVQVVIIPILFKDENTGEILGKCRELKTMLEKADIRVRIDDRSNYTPGW MIMTHGDDKGLVIPPRVASVQVVIIPILFKDENTGEILGKCRELKTMLEKADIRVRIDDRSNYTPGW MIMTHGDDKGLVIPPRVASVQVVIIPILFKDENTGEILGKCRELKTMLEKADIRVRIDDRSNYTPGW 666 732 KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARI HEGIEKISTFDEVMPALNRKHLVLAPWCEDPESEEQIKKETQKLSEIQAIFAGDSEQVMTGAMKTLO HEGIEKISTFDEVMPALNRKHLVLAPWCEDPESEEQIKKETQKLSEIQAIFAGDSEQVMTGAMKTLO BOO 830 IPFDQPPMPEGTKCFYTGKPAKRWTLWGRSY IPFDQPPMPEGTKCFYTGKPAKRWTLWGRSY

Figure S1 Mapping of Structural Differences of Apo1, 2 and Holo1, 2 *Tg*PRS. Apo1 (PDB ID 5XIF) with Apo2 (PDB ID 6AA0) as well as Holo1 (PDB ID 5XIQ) and Holo2 (PDB ID 6AA8) sequences are shown. The 5 motifs noted are consistently disordered. Disordered in this sense here, is the electron density quality. These reveal the high structural malleability within apo-PRSs, and also drug-induced stability to regions that were otherwise disordered. These also reveal the multi-conformational nature of proteins with motifs having altered conformations. Disordered residues (red box), altered conformations (green box) and AMPPNP (ocean blue box), HF (cyan box) and FF interacting residues (magenta box) are shown.

Apo2 Holo2	331 GAMVTAKKDENFSEWYTQAIVRSEMIEYYD <mark>ISGCYIM</mark> RPWAFHIWEKVQRFFDDEIKKMGVENSYFP GAMVTAKKDENFSEWYTQAIVRSEMIEYYDISGCYIMRPWAFHIWEKVQRFFDDEIKKMGVENSYFP
Apo2 Holo2	398 M <mark>FVSRHKLEKEKDHVEGFSPEVAWVTHYGDS</mark> PLPEKIAIRPTSETIMYPAYAKWIRSHRDLPLKLNQ MFVSRHKLEKEKDHVEGFSPEVAWVTHYGDSPLPEKIAIRPTSETIMYPAYAKWIRSHRDLPLKLNQ
Apo2 Holo2	465 WCSVVRWEFKOPTPFLRTREFLWQEGHTAHATEEEAWELVLDILELYRRWYEECLAVPVIKGEKSEG WCSVVRWEFKOPTPFIRTREFLWQEGHTAHATEEEAWELVLDILELYRRWYEECLAVPVIKGEKSEG
Apo2 Holo2	532 EKFAGGKKTTTVEAFIPENGRGIQAATSHLLGTNFAKMFEIEFEDEEGHKRLVHQTSWGCTTRSLGV EKFAGGKKTTTVEAFIPENGRGIQAATSHLLGTNFAKMFEIEFEDEEGHKRLVHQTSWGCTIRSLGV
Apo2 Holo2	665 MIMTHGDDKGLVIPPRVASVQVVIIPILFKDENTGEILGKCRELKTMLEKADIRVRIDDRSNYTPGW MIMTHGDDKGLVIPPRVASVQVVIIPILFKDENTGEILGKCRELKTMLEKADIRVRIDDRSNYTPGW
Apo2 Holo2	732 KYNHWEVKGVPLRLELG <mark>PKDLAKGT</mark> ARVVRRDTGEAYQI <mark>SW</mark> ADLAPKLLELMEGIQRSLFEKAKARL KYNHWEVKGVPLRLELGPKDLAKGTARVVRRDTGEAYQISWADLAPKLLELMEGIQRSLFEKAKARL
Apo2 Holo2	733 HEGIEKISTFDEVMPALNRKHLVLAPWCEDPESEEQIKKETQK <mark>LSEIQAIEAGDSEQVMT</mark> GAMKTLC HEGIEKISTFDEVMPALNRKHLVLAPWCEDPESEEQIKKETQKLSEIQ <mark>AIEAGDSEOVM</mark> TGAM <mark>K</mark> TLC
Apo2 Holo2	800 830 IPFDQPPMPEGTKCFYTGKPAKRWTLWGRSY IPFDQPPMPEGTKCFYTGKPAKRWTLWGRSY

Figure S2 Mapping of Structural Differences of Apo2 vs. Holo2. Apo2 vs. Holo2 are shown. Disordered in this sense here, is the electron density quality. Disordered residues (red box), altered conformations (green box), AMPPNP interacting residues (ocean blue box) and FF-interacting residues (magenta box) are shown.



Figure S3 RMSD analyses of TgPRS Apo-Holo comparisons – related to Fig 6. RMSDs computed using GESAMT between A and B chains of Apo1, Apo2, Holo1 and Holo2 are shown color-coded in accordance with clusters of Apo-apo (blue), holo-holo (red) and apo-holo (green) comparisons. A1A – Apo1A, A1B – Apo1B, A2A – Apo2A, A2B – Apo2B, H1A – Holo1A, H1B – Holo1B, H2A – Holo2A, H2B – Holo2B. A shows the tabular data whilst B shows a graphical representation of the same.

Α		Set	RMSD (Å)
	1	3BJU_A vs 3BJU_B	0.453
	2	4YCU_A vs 4YCU_B	0.766
	3	3BJU A vs 4YCU A	0.994
	4	3BJU A vs 4YCU B	0.809
	5	3BJU_B vs 4YCU_A	0.757
	6	3BJU_B vs 4YCU_B	0.695



Figure S4 RMSD analyses of HsKRS ATP-Cladosporin bound comparisons – Related to Fig 7. RMSDs computed using GESAMT between A and B chains of 3BJU and 4YCU are shown colorcoded in accordance with clusters of ATP-ATP (blue), CLD-CLD (red) and ATP-CLD (green) comparisons. A shows the tabular data whilst B shows a graphical representation of the same.