A structural overview of Pontin, Reptin and their complex(es)

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Two proteins with many names…

PONTIN

RuvBL1 [RuvB-like 1 (*E. coli*)]

NMP238
ECP54
INO80H
RVB1
Pontin52
Rvb1
TAP54-α
TIH1
TIP49
TIP49A

456 aa, 50.2 kDa

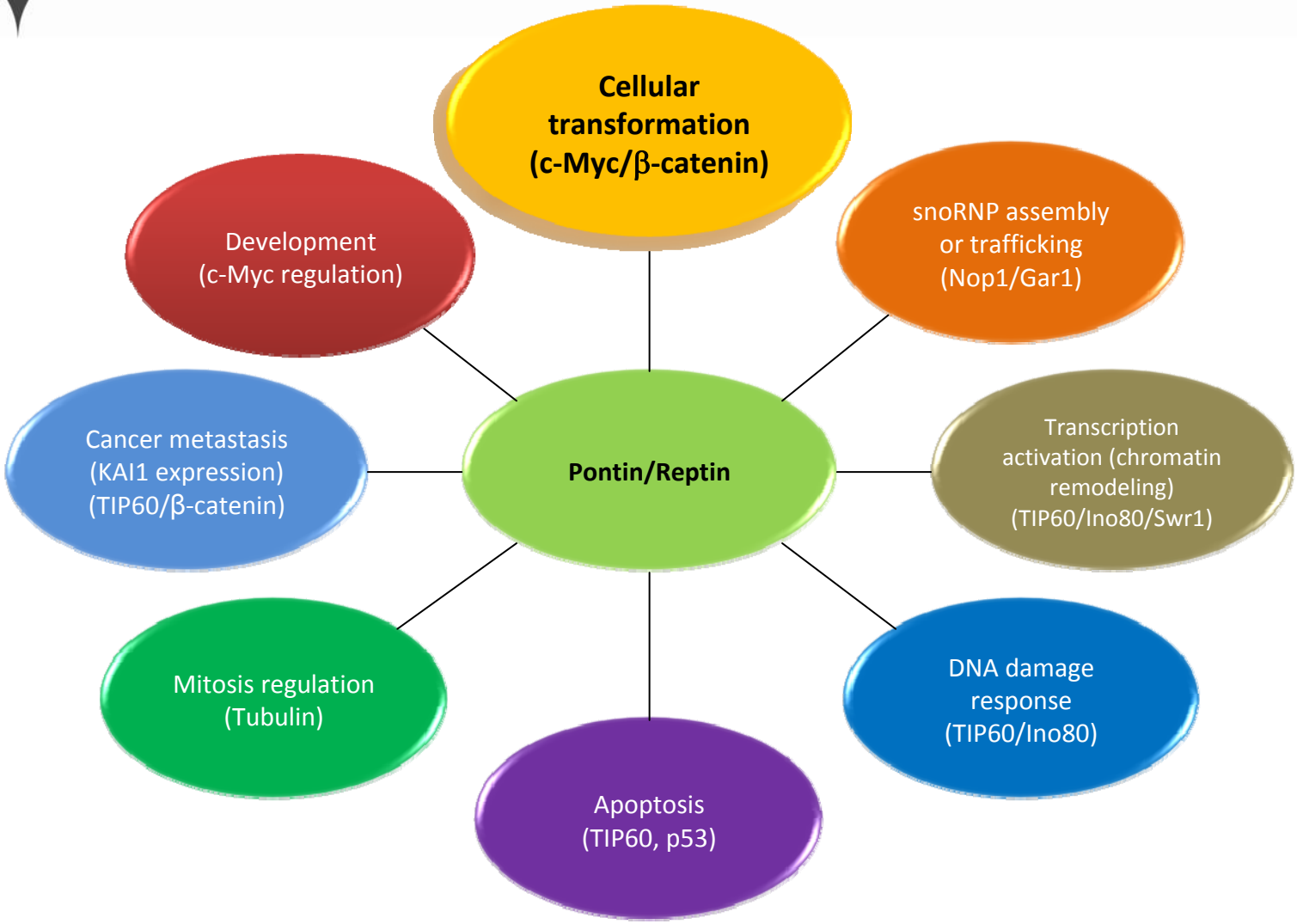
REPTIN

RuvBL2 [RuvB-like 2 (*E. coli*)]

CGI-46
ECP51
INO80J
RVB2
Reptin52
Rvb2
TAP54-β
TIH2
TIP48
TIP49B

463 aa, 52 kDa

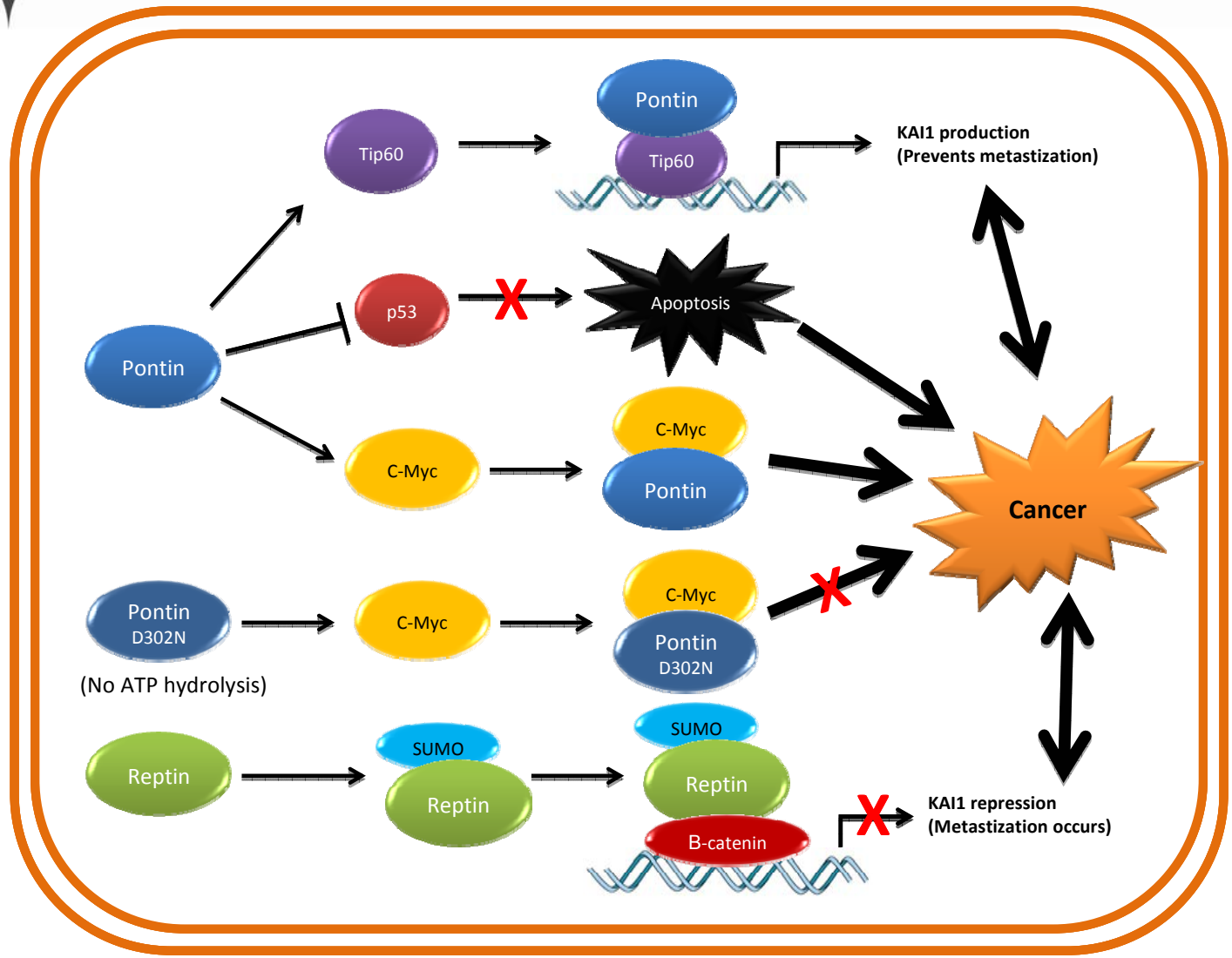
…and functions



Adapted from Jha and Dutta (2009) Mol. Cell 34:521-533

…and functions

Pontin, Reptin and Prostate Cancer



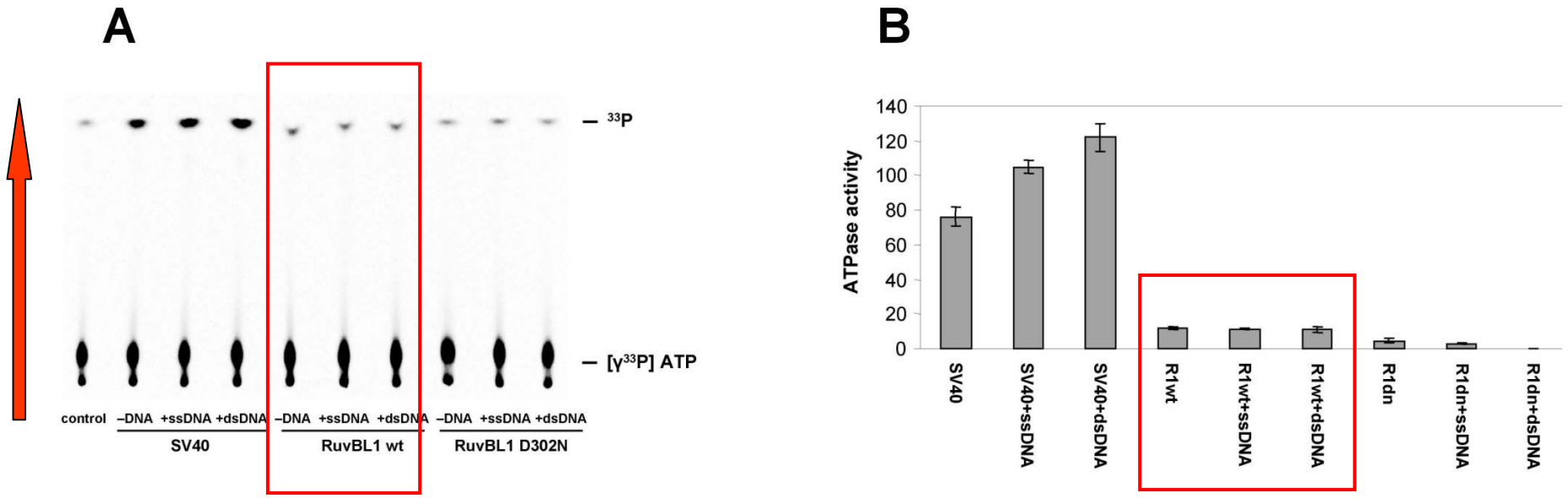
Pontin and Reptin are AAA+ proteins…

Human Pontin and Reptin:

- Show high **evolutionary conservation**; distinct orthologs exist in all eukaryotes as well as in archeabacteria;
- Belong to **AAA+ family of ATPases** (associated with diverse cellular activities);
- **AAA+ proteins:** share a common topology, generally form hexameric ring structures and contain conserved motifs for ATP binding and/or hydrolysis (**Walker A** and **B**, **sensors 1** and **2**, **arginine finger**) as well as oligomerization (**arginine finger**);
- AAA+ proteins can transform the **chemical energy** from the chemical reaction ATP $\rightarrow$ ADP + P_i into **mechanical forces**; function requires **ATPase activity**;

…with low ATPase activity…

Human Pontin – ATPase assay



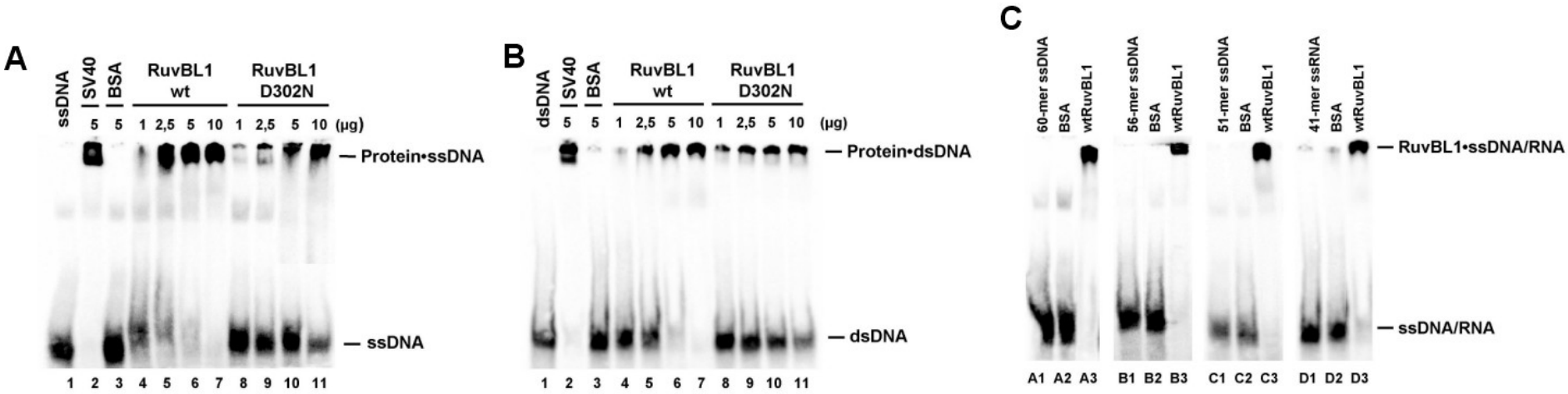
A - Free ^{33}P phosphate produced by hydrolysis of ATP was separated from [$\gamma^{33}P$] ATP by thin-layer chromatography. Free phosphate and ATP were visualized by autoradiography.

B - quantification of ATPase activity (moles of ATP hydrolyzed per mole of protein).

Pontin has low ATPase activity.

…that can bind ssDNA/RNA and dsDNA…

Human Pontin –Nucleic Acid binding assay

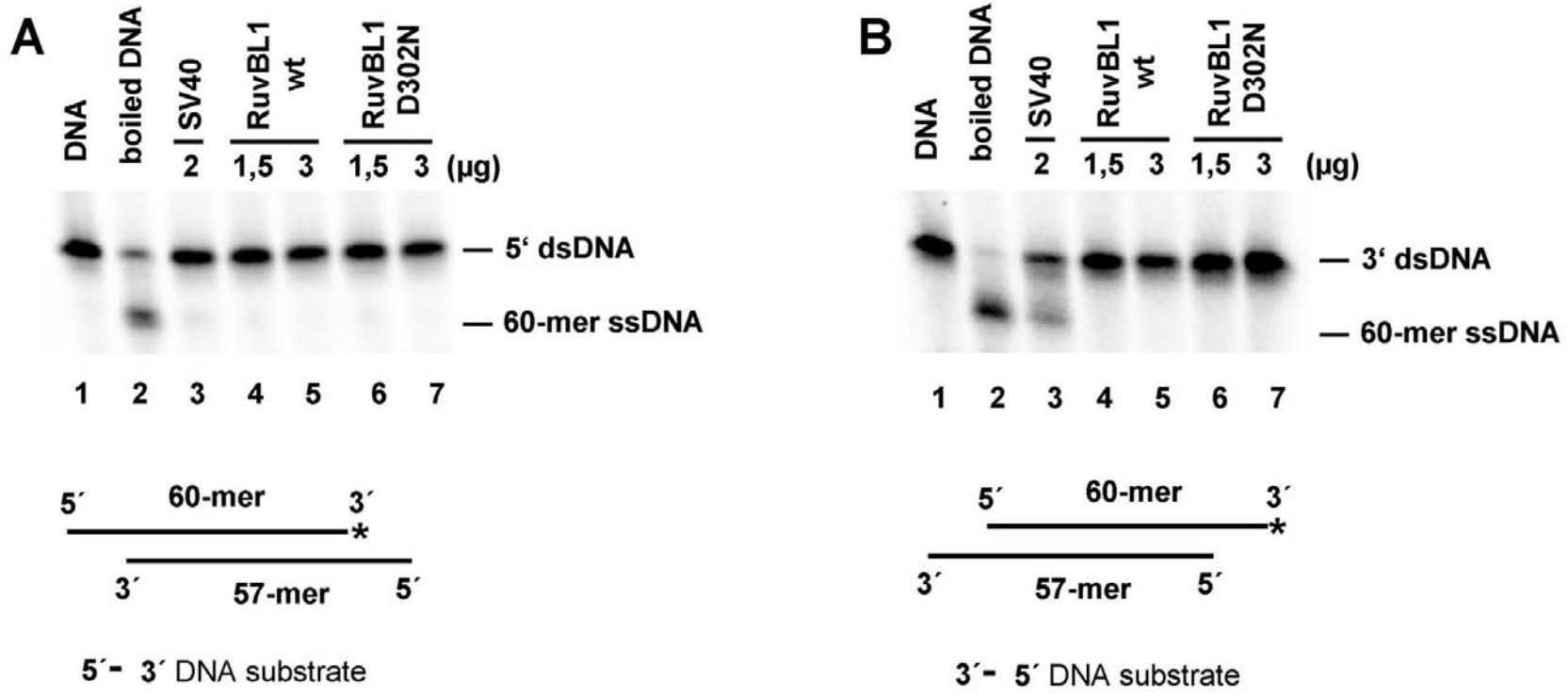


A - ssDNA and **B -** dsDNA binding of human Pontin protein by electrophoretic mobility shift assay (EMSA); **C -** further EMSA tests using three different ssDNA substrates with diverse sequences and a ssRNA substrate, to confirm nucleic acid binding to RuvBL1 in a sequence-independent fashion. The samples were analyzed on a 6% nondenaturing polyacrylamide gel and visualized by autoradiography.

Pontin can bind ssRNA/DNA as well as dsDNA.

…but have no DNA helicase activity

Human Pontin – Helicase activity assay

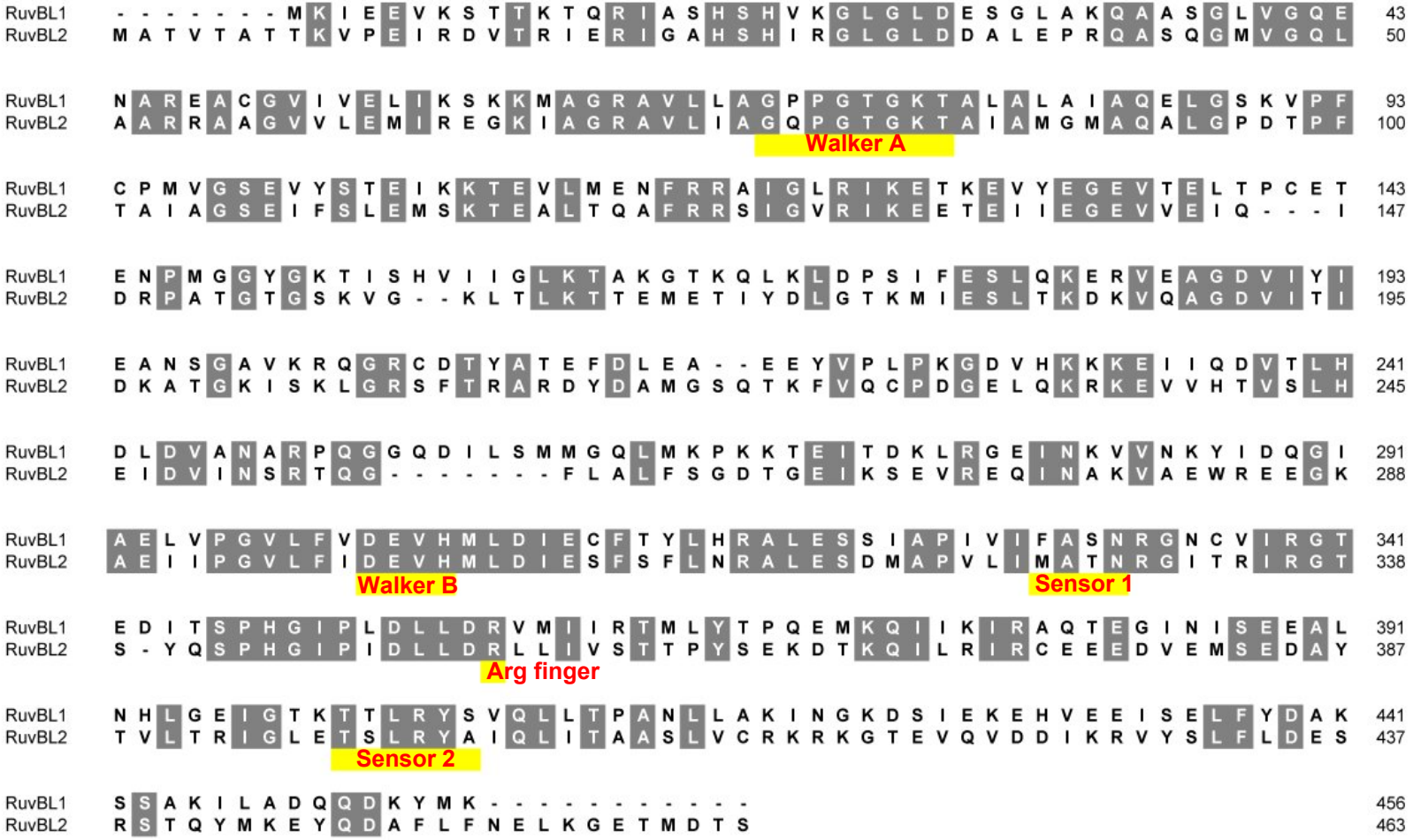


Helicase activity assay of human RuvBL1 using a 5' to 3' DNA substrate (**A**) and a 3' to 5' substrate (**B**). An asterisk denotes the ^{33}P label.

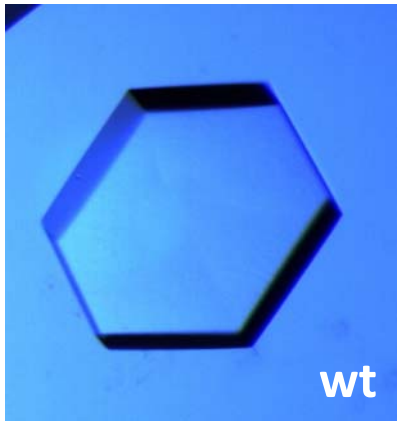
Purified Pontin has no measurable DNA helicase activity.

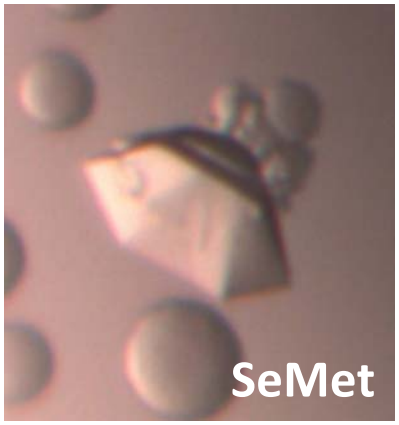
Human Pontin and Reptin are homologs

41% identity and 64% similarity



Crystallization of human Pontin





Crystals grown using as precipitant Sodium Malonate 1.6 M at pH 6.0

Cryoprotecting solution: Sodium Malonate 2 M at pH 6.0

Problems:

- Polymorphism induced by cryocooling
- Radiation damage

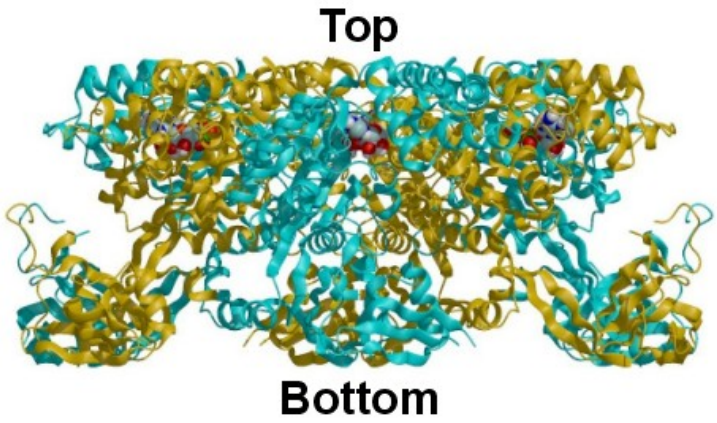
Diffraction data collected at the ESRF

3D structure determined by the SAD method from a SeMet derivative crystal

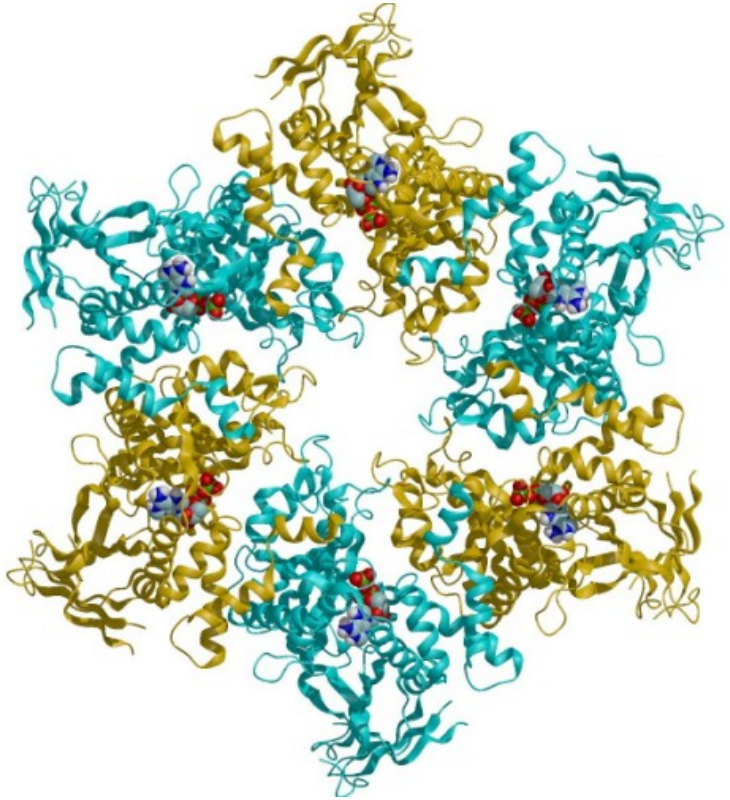
Gorynia et al., (2006) *Acta Crystallogr. F* 62:61-66.

The 3D structure of human Pontin

An hexameric ring

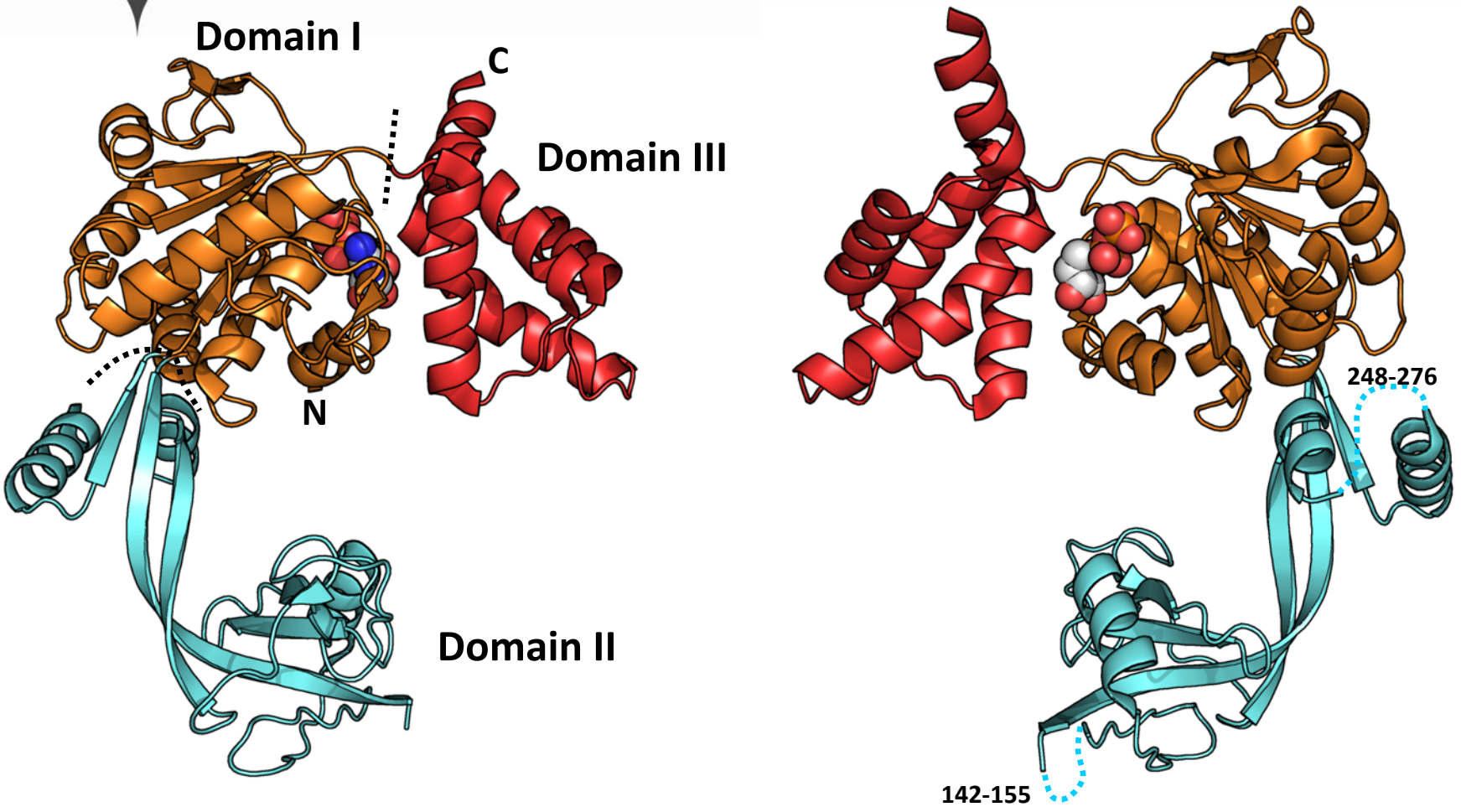


Resolution: 2.2 Å



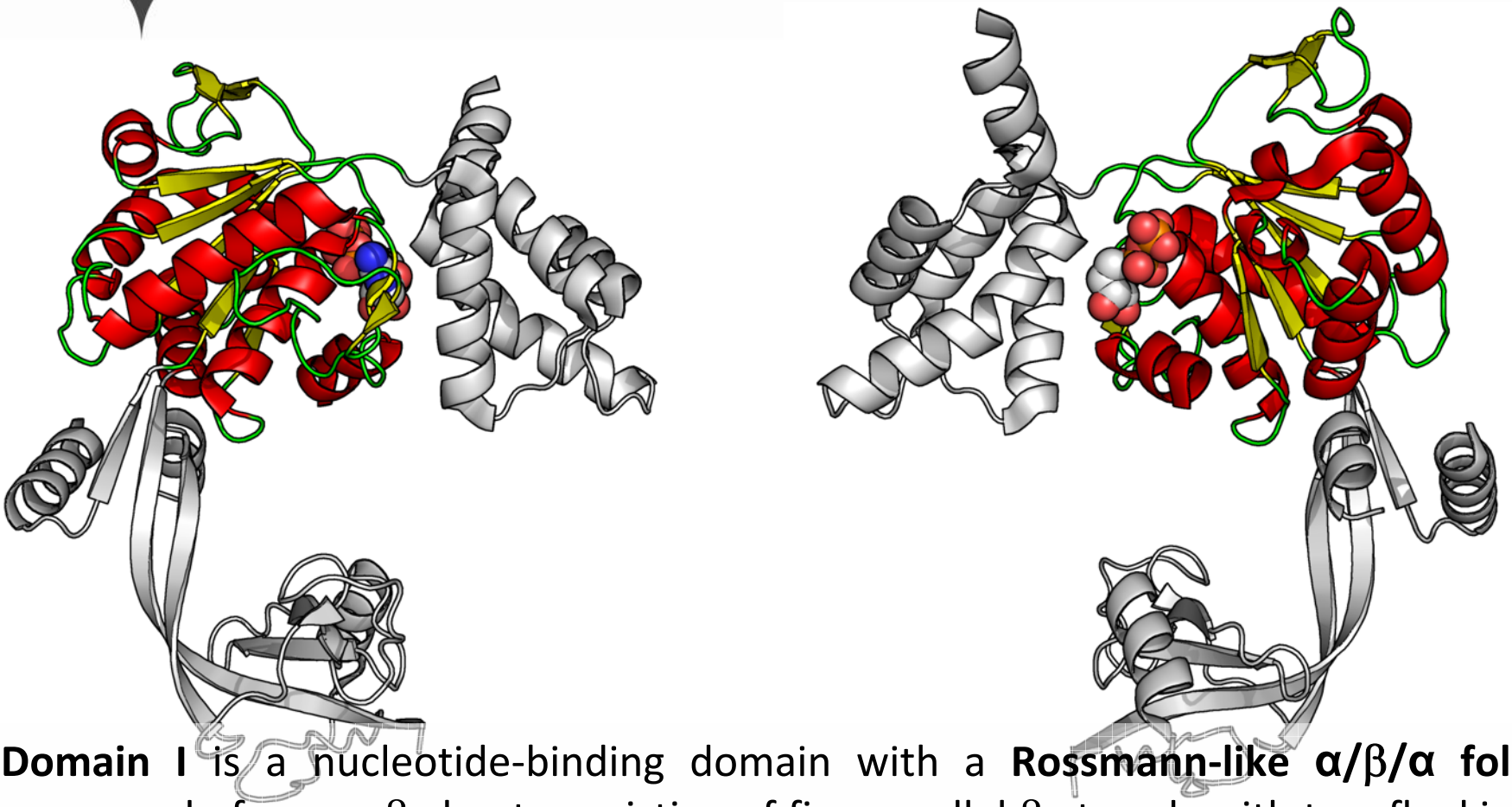
The external diameter of the hexameric ring ranges between **94** and **117 Å** and the central channel has an approximate diameter of **18 Å**. Its top surface appears to be remarkably **flat**.

The 3D structure of the human pontin monomer



Consists of three domains, of which the first and the third are involved in **ATP binding** and **hydrolysis**.

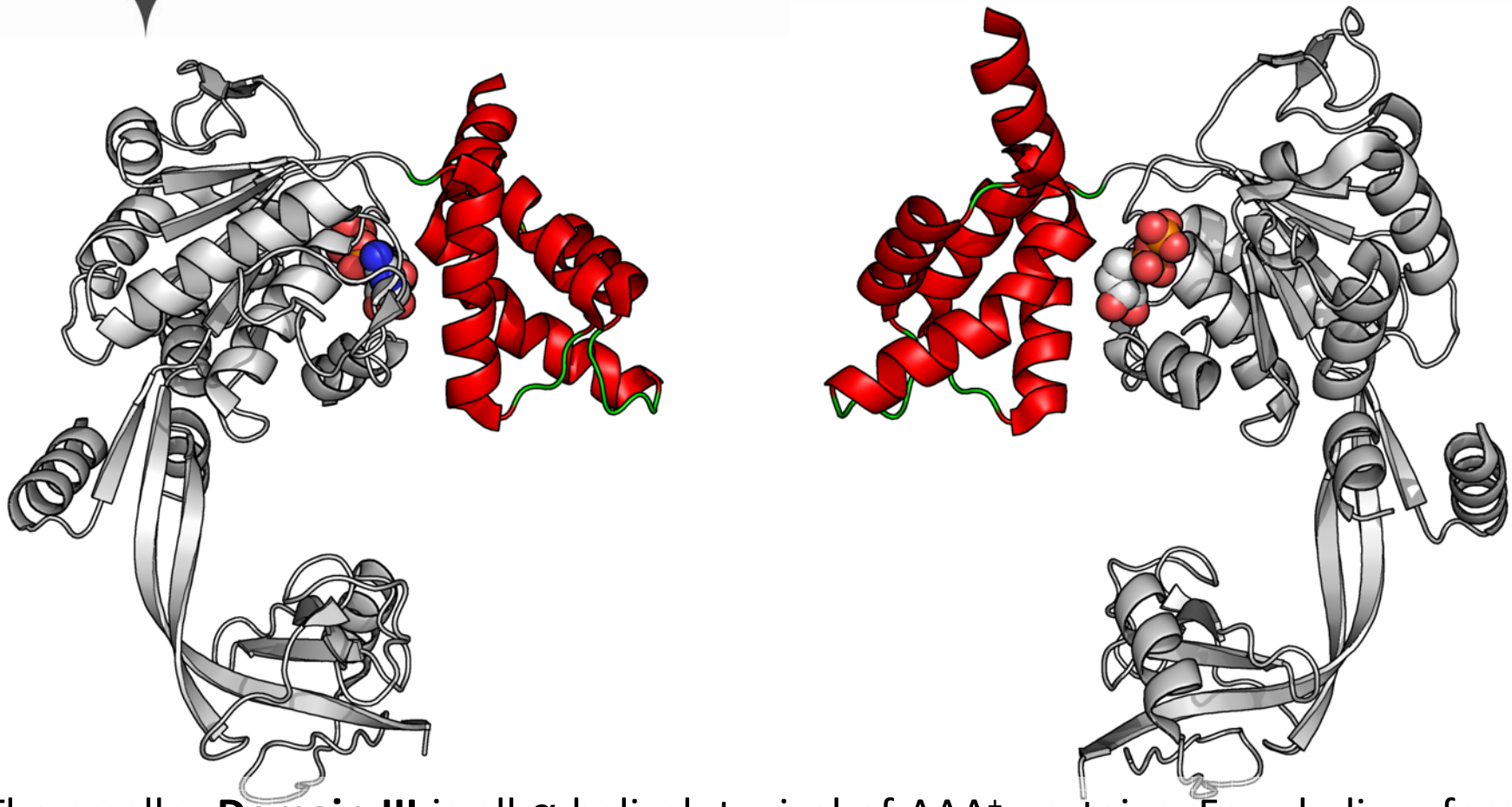
The 3D structure of the human pontin monomer



Domain I is a nucleotide-binding domain with a **Rossmann-like α/β/α fold** composed of a core β-sheet consisting of five parallel β-strands with two flanking α-helices on each side.

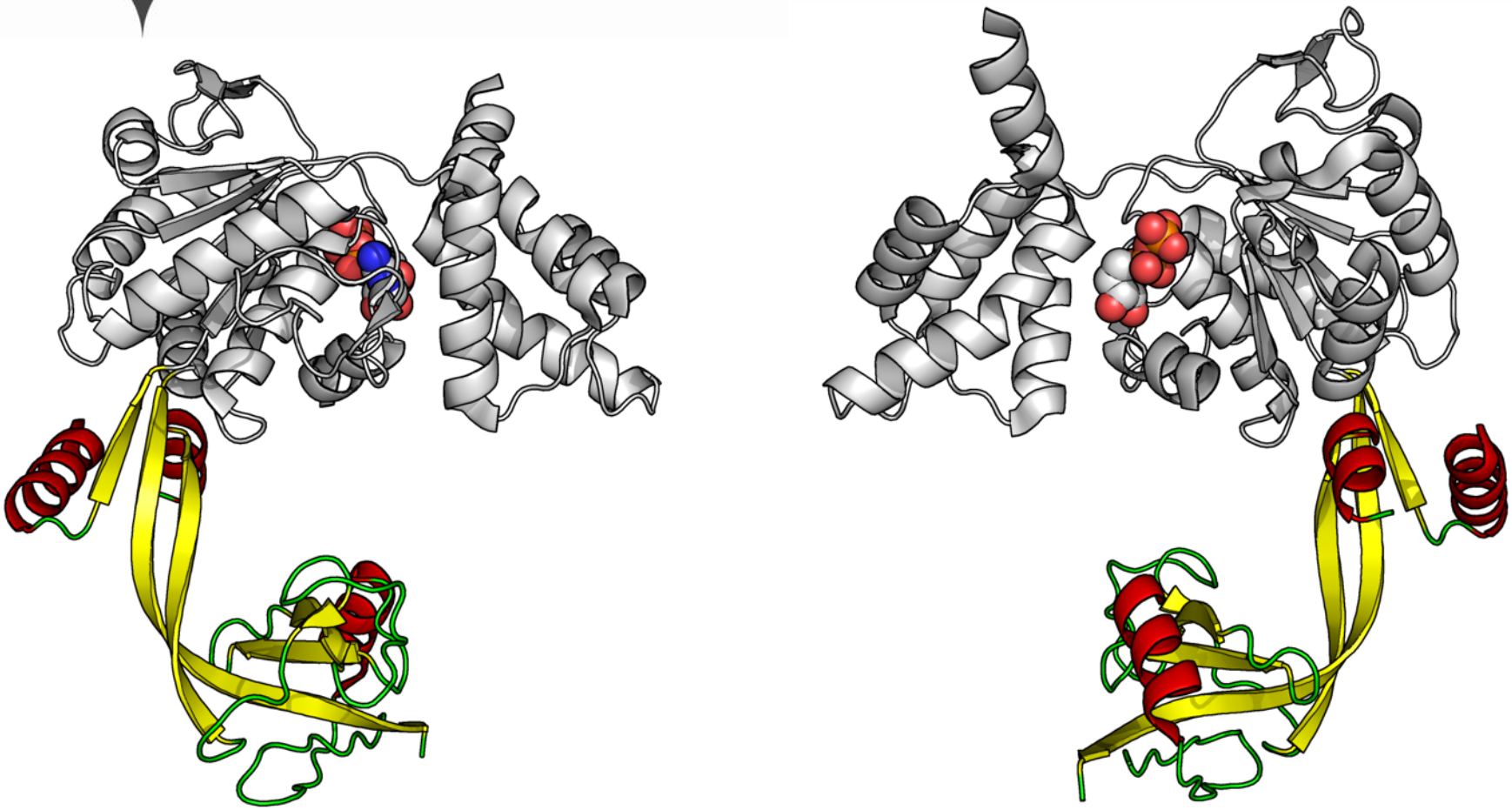
The core β-sheet is similar to the AAA^+ module of other AAA^+ family members.

The 3D structure of the human pontin monomer



The smaller **Domain III** is all α-helical, typical of AAA^+ proteins. Four helices form a bundle located near the 'P-loop', important for ATP-binding, which covers the nucleotide-binding pocket at the interface of **Domain I** and **Domain III**.

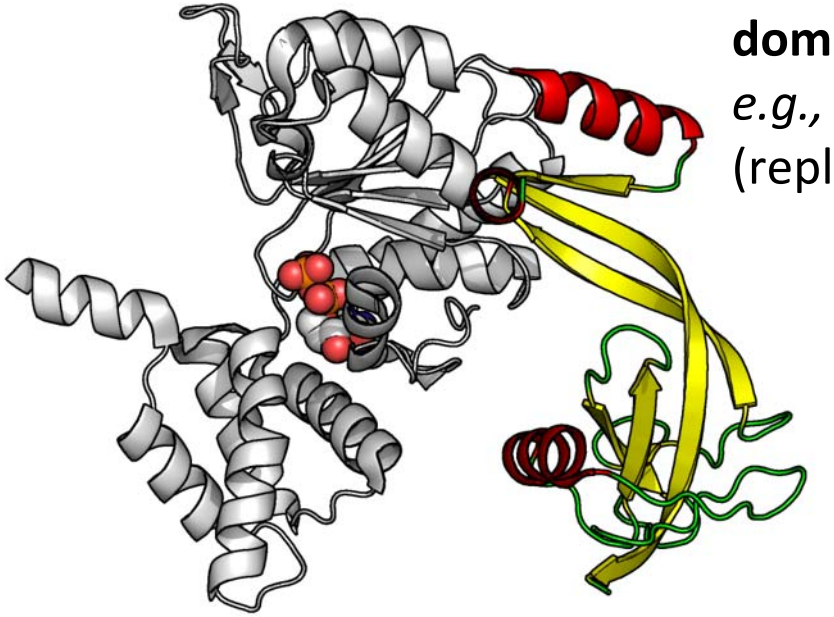
The 3D structure of the human pontin monomer



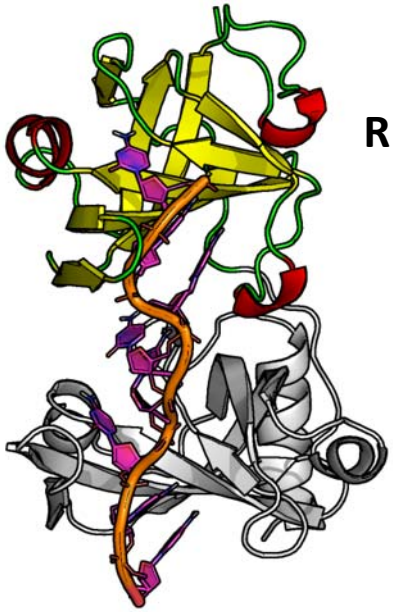
Domain II is as a **~170 residue** insertion between the Walker A and Walker B motifs in Domain I and is unique to Pontin and Reptin

A possible role for Domain II in Pontin/Reptin

Domain II is structurally similar to **DNA-binding domains** of proteins involved in DNA metabolism, *e.g.,* the highly conserved eukaryotic protein RPA (replication protein A)



Pontin Domain II



RPA Domain I

RPA
PDB 1JMC
(Bokharev et al., 1997)

Domain II may represent a new functional domain of eukaryotic AAA^{+} motor proteins important for DNA/RNA binding

AAA+ proteins are ATP-driven molecular machines

All AAA+ proteins use **ATP binding and/or hydrolysis** to exert **mechanical forces**.

Some examples:

- **NSF-D2** (membrane fusion) (Lenzen et al, 1998)

- **bacteriophage T7 gene 4 ring helicase** (Singleton *et al.*, 2000)

- **RuvB** (branch migration) (Putnam et al, 2001)

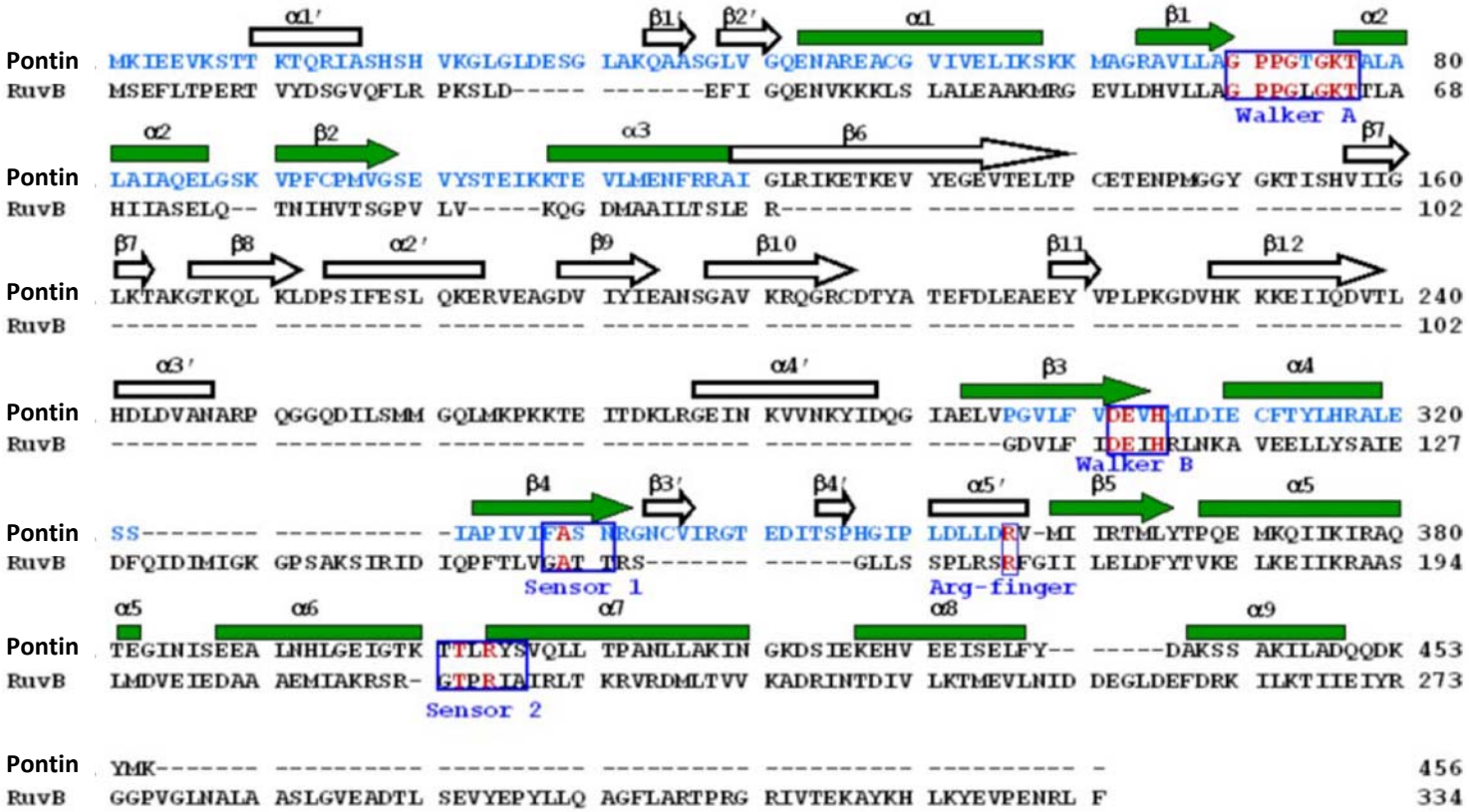
- **SV40 large tumor antigen helicase** (replication of viral DNA) (Li *et al.*, 2003, Gai *et al.*, 2004)

- **hexameric ATPase P4 of dsRNA bacteriophage ϕ12** (RNA packaging inside the virus capsid) (Mancini *et al.*, 2004)

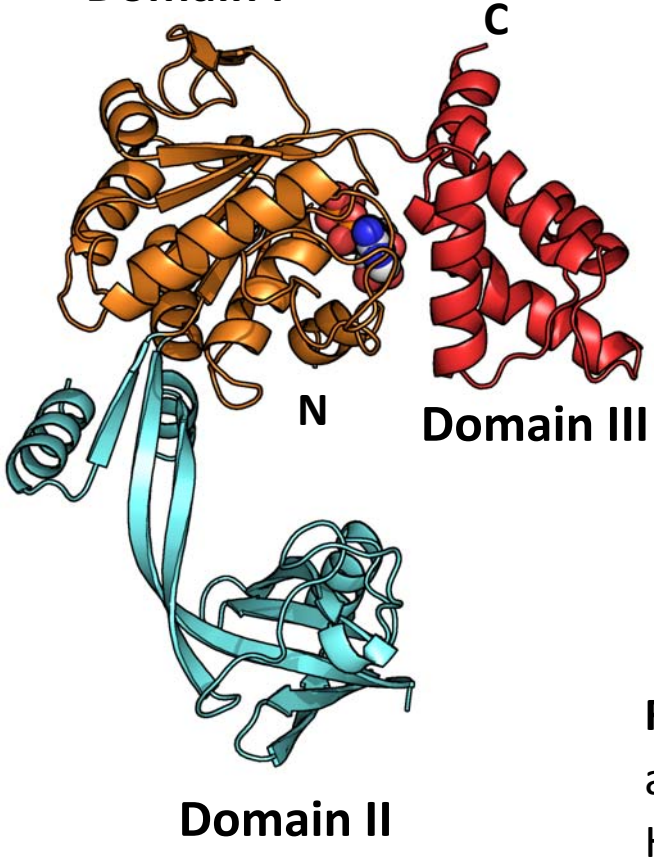
- **AAA+ domain of PspF** (transcription activation) (Rappas *et al.*, 2006)

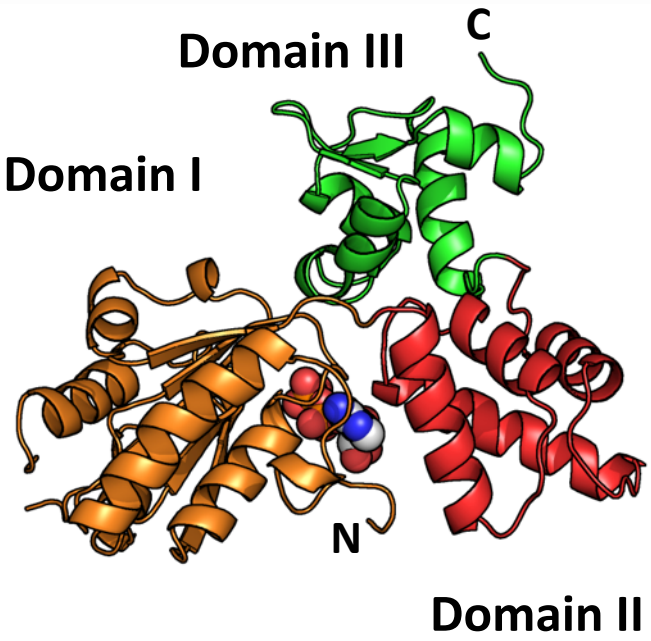
AAA+ proteins are ATP-driven molecular machines

Pontin is the **eukaryotic homolog** of the bacterial DNA-dependent ATPase and helicase **RuvB**.



AAA+ proteins are ATP-driven molecular machines





Thermotoga maritima **RuvB**
PDB 1IN7 (Puttnam et al., 2001)

RuvB assembles into functional homohexameric rings and is the motor that drives branch migration of the Holliday junction in the presence of **RuvA** and **RuvC** during homologous recombination.

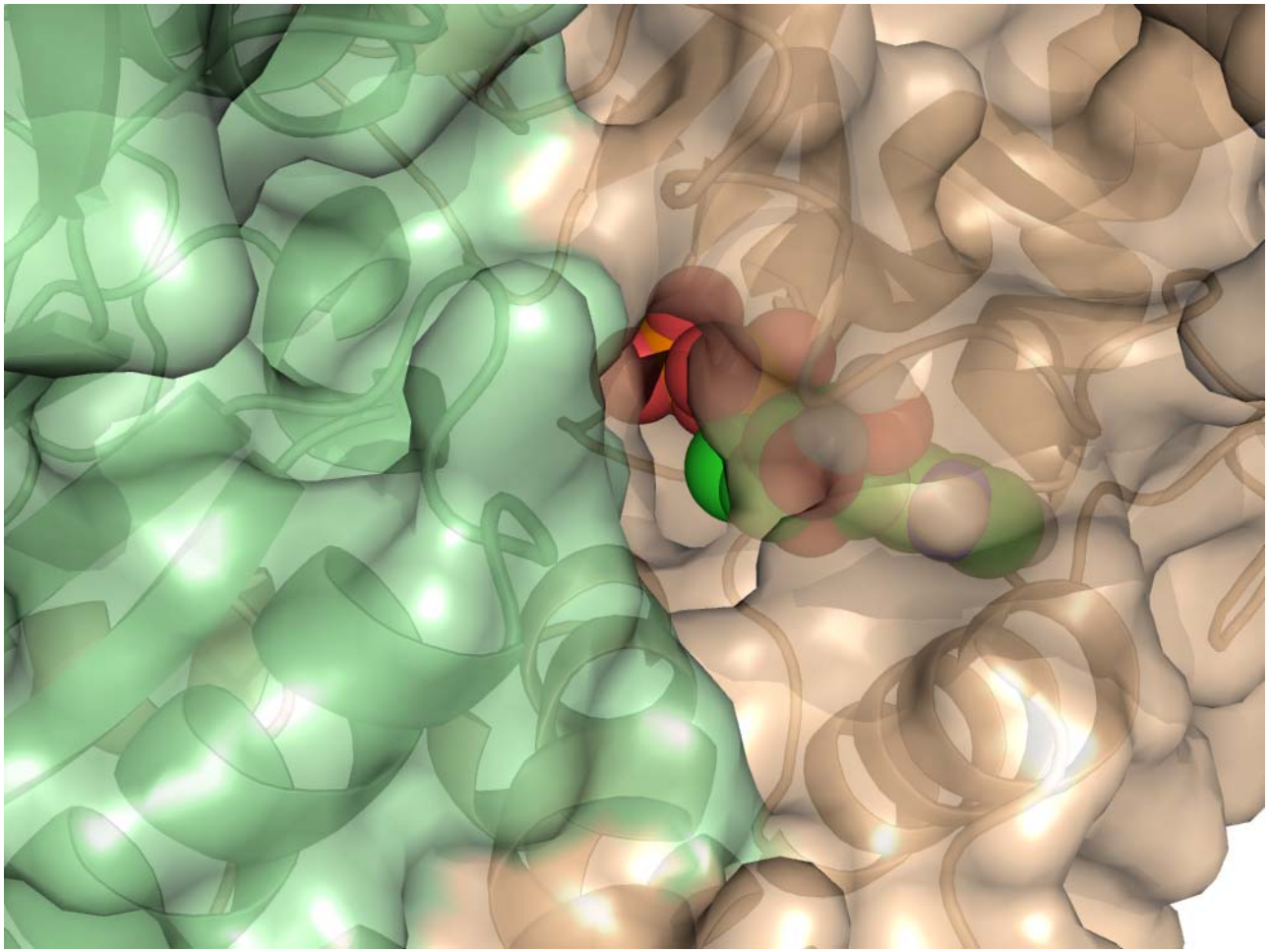
AAA+ proteins are ATP-driven molecular machines

The ability to hydrolyze ATP is essential for the biological function of Pontin.

However, purified heterologously expressed Pontin has only low ATPase activity.

Why?

The Pontin nucleotide-binding pocket



1. The nucleotide-binding pocket is blocked by hexamer formation:
ADP ↔ ATP exchange is hindered.

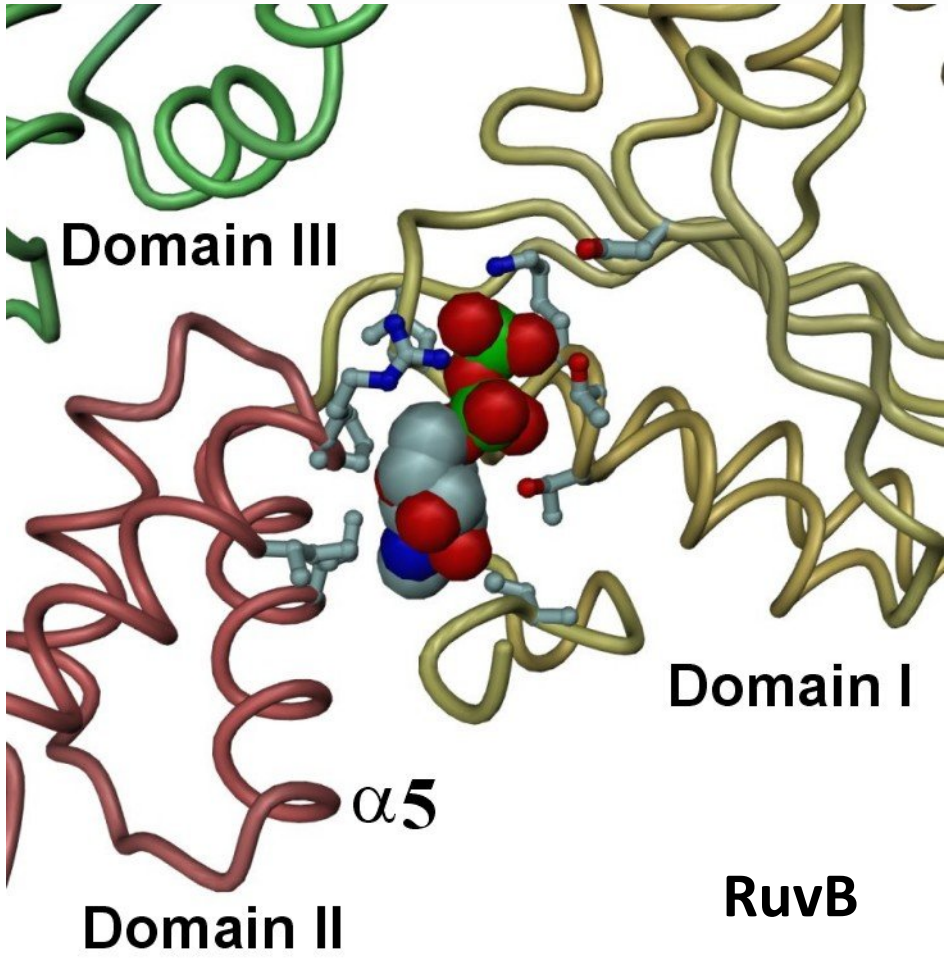
The Pontin nucleotide-binding pocket

Molecule	PDB code	Location of nucleotide binding pocket	Ligand	Accessible area (Å^2)	Ligand hydrogen bonds with [Ligand nr. atoms with hydrophobic contacts to] protein/water atoms				
					Adenine	Sugar	P_α	P_β	P_γ
Pontin	2C9O	DI/DIII interface	ADP	13.5	5 [4]	1 [1]	5	6	--
AAA+ Domain PspF	2C98	DI/DII interface	ADP	114.5	4 [3]	3 [1]	3	7	--
RuvB	1IN7	DI/DII interface	ADP	39.4	3 [5]	0 [1]	3	7	--
NSF-D2	1D2N	DI/DII interface	AMPPNP, Mg^{2+}	55.7	3 [4]	3 [0]	3	3	5
SV40 LTag Helicase	1SVL	M/M interface	ADP, Mg^{2+}	37.4	2 [3]	1 [1]	3	10	--
Bϕ12 ATPase P4	1W44	M/M interface	ADP	90.1	3 [5]	3 [2]	5	3	--
BT7 G4 Ring Helicase	1E0J	M/M interface	AMPPNP, Mg^{2+}	44.1	0 [4]	1 [1]	2	4	3

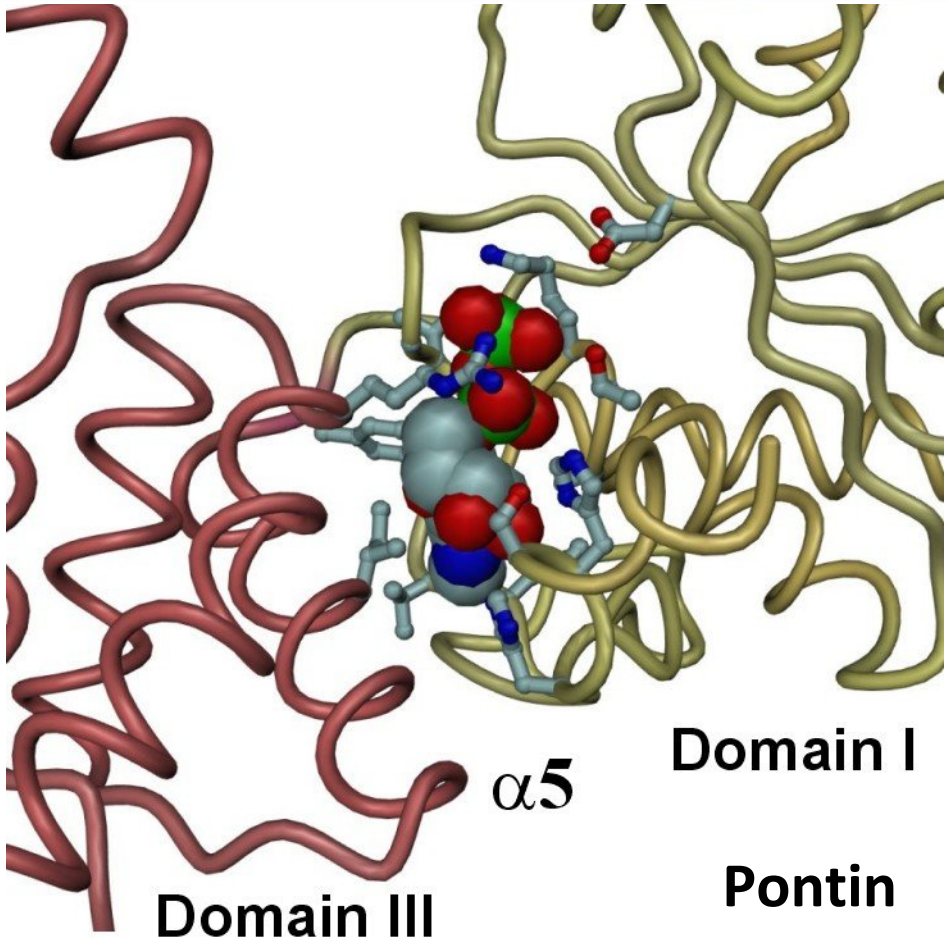
The nucleotide binding pocket is located either at the interface between two domains within a monomer (Dm/Dn interface) or at the interface between two adjacent monomers in the hexamer (M/M interface).

2. The NBP of Pontin has a low solvent accessibility and a high number of interactions: the ADP is tightly bound. Exchange with ATP, a pre-requisite for ATPase activity, is hindered.

Human Pontin vs. *T. maritima* RuvB – ADP tight binding



Human Pontin vs. *T. maritima* RuvB – ADP tight binding



Human Pontin – Conclusions

❖ The crystal structure of the **Pontin/ADP hexamer** reveals that human Pontin consists of **three domains**, of which the first and the third are involved in ATP binding and hydrolysis.

❖ Structural homology suggests that the second domain, which is **unique in AAA$^+$ proteins** and not present in RuvB, is a **DNA/RNA binding domain**.

❖ The biochemical assays show that the Pontin hexamer has a **marginal ATPase activity**, **binds nucleic acids** (ssRNA/DNA and dsDNA) and has **no significant DNA helicase activity.**

❖ The hexameric structure of the Pontin/ADP complex, combined with our biochemical results, suggest that, while Pontin has all the structural characteristics of an **AAA$^+$ molecular motor**, even of an ATP-driven helicase, its activation requires **conformational changes** to allow ADP exchange with ATP.

Matias et al., (2006) *JBC* 281:38918-38929.

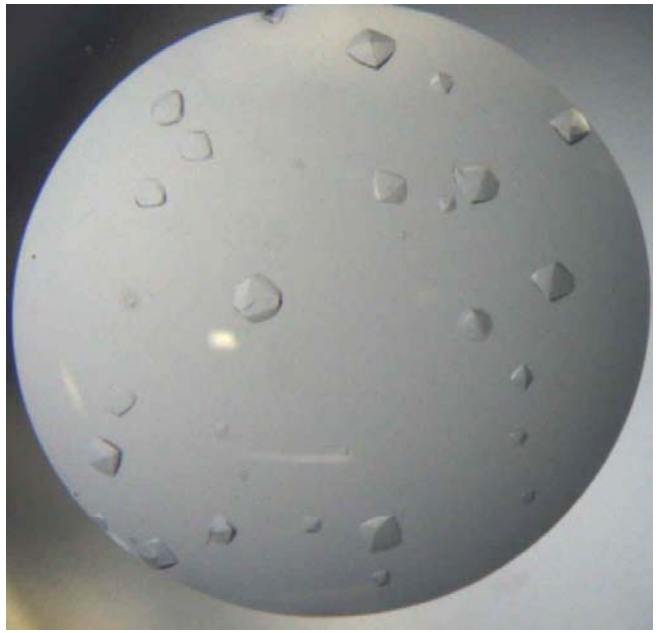
Human Reptin – A Parenthesis

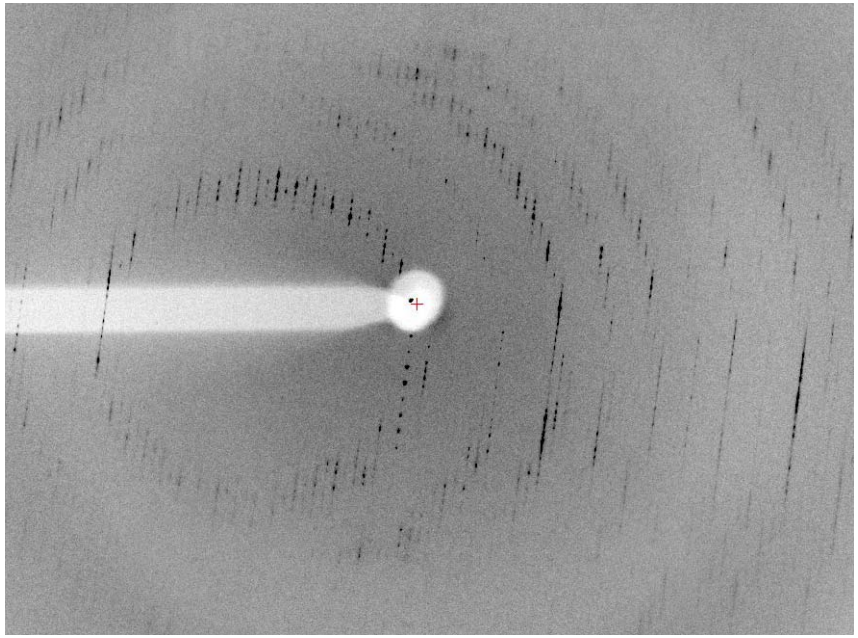
– Human Reptin has been produced and purified as for Pontin

– Crystals of poor quality were obtained

– Measured diffraction data showed crystals to be multiple

– No 3D structure of **full-length** human Reptin is known to date



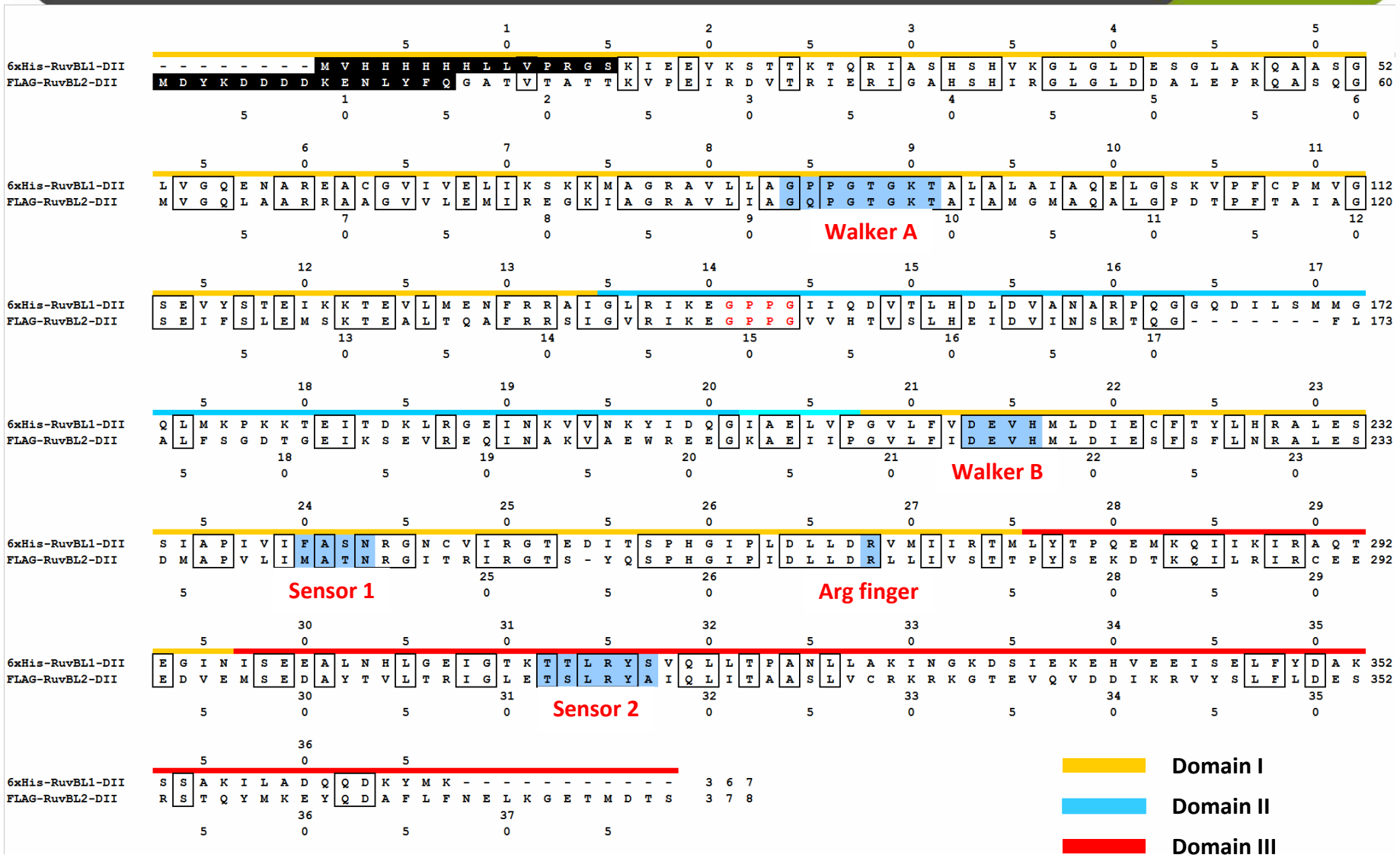


But see Petukhov et al., (2012) Structure 20:1321-1331

Human Pontin/Reptin complex - expression

- All our crystallization trials with co-expressed full-length **His_6-tagged Pontin** and **FLAG-tagged Reptin** failed.
- For crystallization purposes, Domain II of both Pontin and Reptin was truncated (Pontin-ΔDII and Reptin-ΔDII).
- Residues **T127-E233** in Pontin and **E134-E237** in Reptin were replaced by a **GPPG** linker.
- **His_6-tagged Pontin-ΔDII** and **FLAG-tagged Reptin-ΔDII** were co-expressed in *E. coli* using the pETDuet vector (Novagen) (pETDuet-His_6-Pontin-ΔDII_FLAG-Reptin-ΔDII).

Human Pontin/Reptin complex - expression



Human Pontin/Reptin complex - purification

Three purification steps were necessary to obtain a clean and uniform Pontin/Reptin complex using two affinity purifications and a gel filtration:

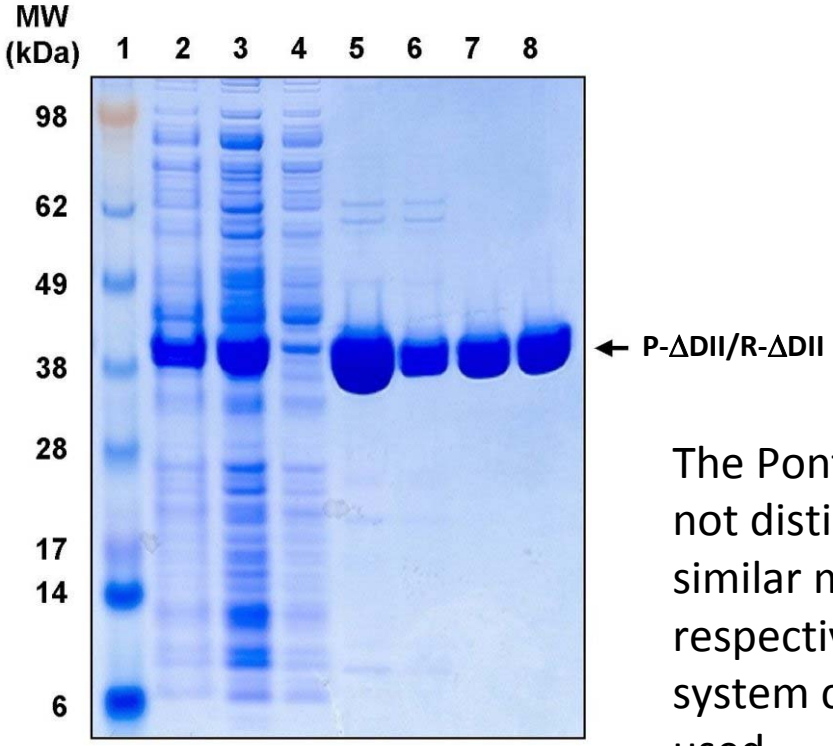
1st step – Ni-NTA
Pontin-ΔDII/Reptin-ΔDII complex binds to column via His_6-Pontin-ΔDII; free Reptin-ΔDII and impurities are removed.

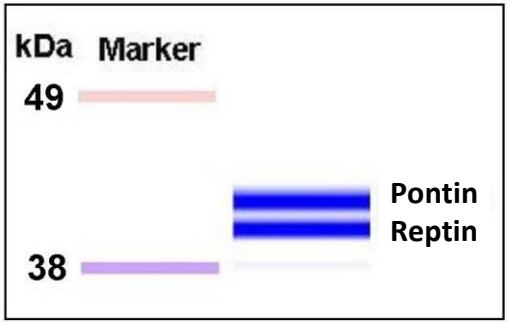
2nd step – ANTI-FLAG affinity column
Pontin-ΔDII/Reptin-ΔDII complex binds to column via FLAG-Reptin-ΔDII; free Pontin-ΔDII and impurities are removed.

3rd step – Gel filtration, polishing (16/60 Superdex 200)
Pontin-ΔDII/Reptin-ΔDII complex elutes **as a dodecamer**, and is separated from FLAG peptides and any remaining Pontin-ΔDII and Reptin-ΔDII monomers.

Human Pontin/Reptin complex - purification

SDS-PAGE of Pontin-ΔDII/Reptin-ΔDII complex purification:
1 – MW markers; 2 – after cell disruption; 3 – soluble proteins; 4 – Ni-NTA flowthrough;
5 – Ni-NTA pool; 6 – Anti-FLAG affinity flowthrough; 7 – Anti-FLAG affinity pool;
8 – Gel filtration pool.

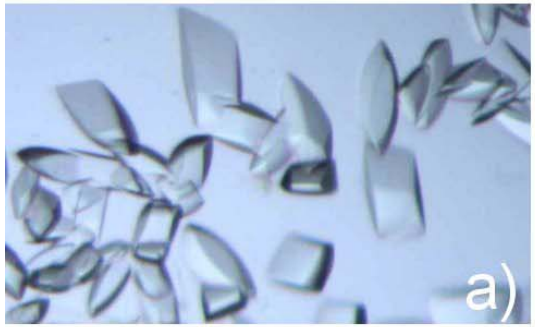


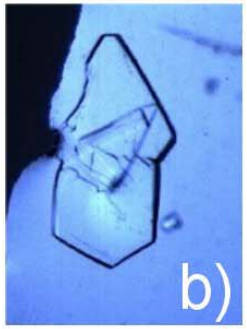


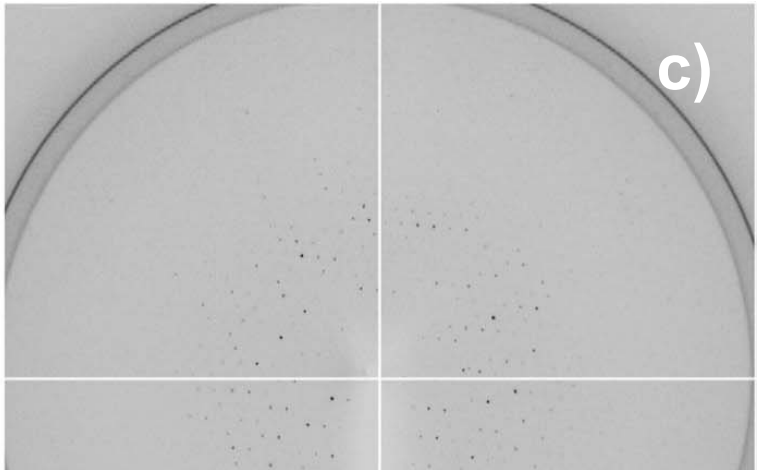
The Pontin-ΔDII and Reptin-ΔDII monomers were not distinguishable in the SDS-PAGE gel due to the similar molecular weights of 40.5 and 42.4 kDa, respectively; an automated electrophoresis system capable of separating the two bands was used.

Human Pontin/Reptin complex - crystallization

After screening and optimization, the best diffracting crystals were obtained with a reservoir solution of 0.8 M LiCl, 10 % PEG 6000 and 0.1 M Tris pH 7.5. Cryocooling was not very effective and usually degraded the diffraction quality.







a) Crystals of the Pontin-ΔDII/Reptin-ΔDII complex; **b)** optimized hexagonal-shaped plates used for preliminary structure determination; **c)** One crystal diffracted to 4 Å resolution and was used to measure diffraction data at ESRF ID14-2 leading to a preliminary structure determination. The crystal was a fragment of a thin (*ca.* 20 μm) hexagonal-shaped plate.

Human Pontin/Reptin complex – structure determination

✓ The 4 Å resolution diffraction data could be processed with similar statistics in two different but related space groups: $C222_1$ and $P2_1$.

✓ The 3D structure of the Pontin-ΔDII/ReptinΔDII complex was solved by the Molecular Replacement method in both space groups – search model: the Pontin monomer, truncated to reflect the shortened domain II region.

✓ Solution obtained: a **dodecamer** formed by **two hexamers**.

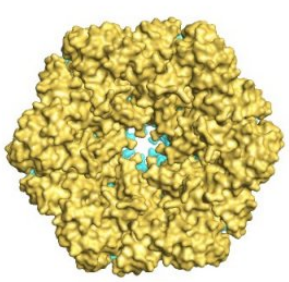
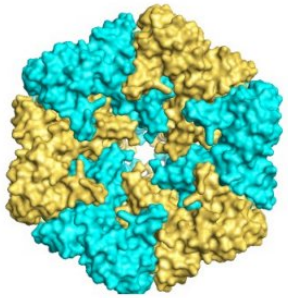
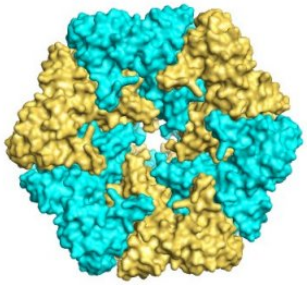
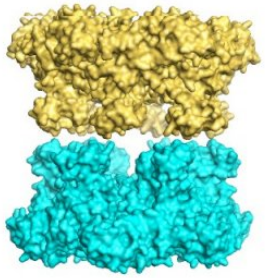
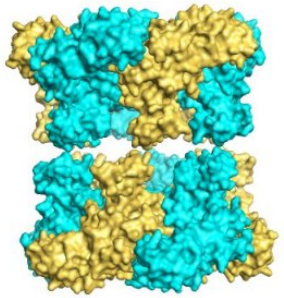
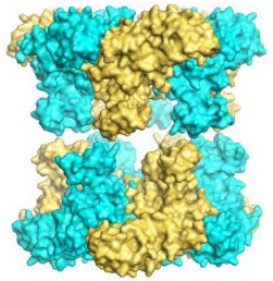
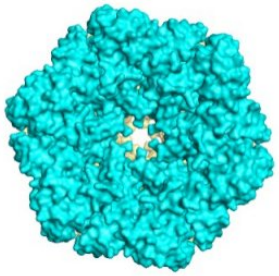
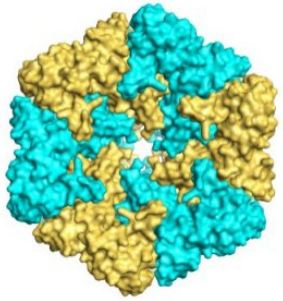
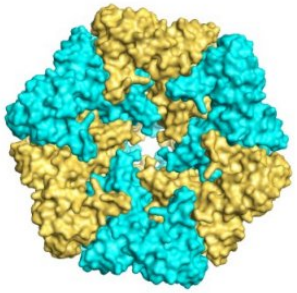
✓ In $P2_1$ a **full dodecamer** constitutes the asymmetric unit; in $C222_1$ only **one hexamer** is contained in the asymmetric unit.

✓ The high similarity between the 3D structures of Pontin-ΔDII and Reptin-ΔDII combined with the low data resolution, made rather difficult the distinction between Pontin and Reptin monomers, as well as between space groups $C222_1$ and $P2_1$.

✓ The precise determination of the space group has **significant implications** to the **dodecamer structure**.

Human Pontin/Reptin complex – structure determination

Point-group symmetry of the dodecamer

	6	32	32
Top			
Side			
Bottom			

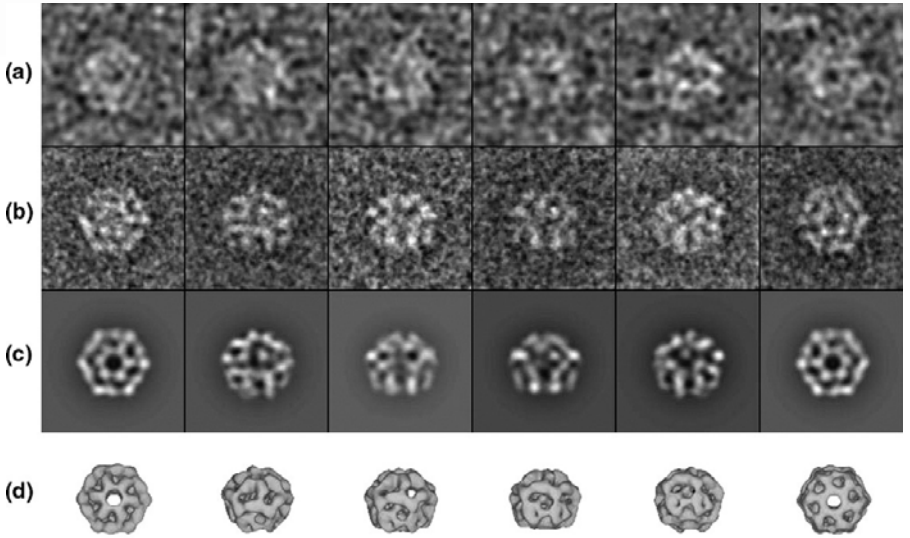
Space-group symmetry of the crystal structure

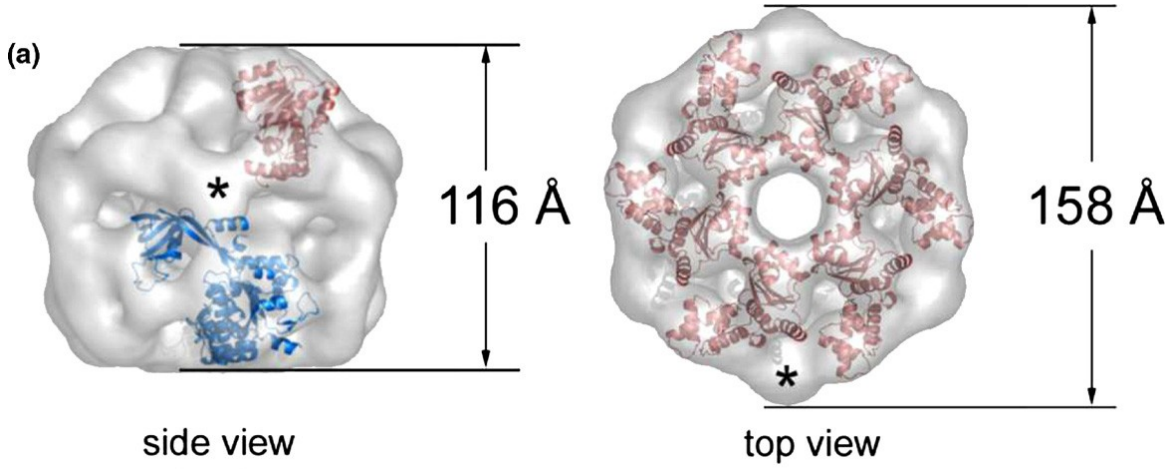
$P2_1$	$P2_1$ or $C222_1$	$P2_1$

Previous structural work on Pontin/Reptin complexes

Electron microscopy of the Human Pontin/Reptin complex

Puri *et al.* (2007) – 20 Å resolution, asymmetric **dodecamer**, possibly two homohexamers facing each other.

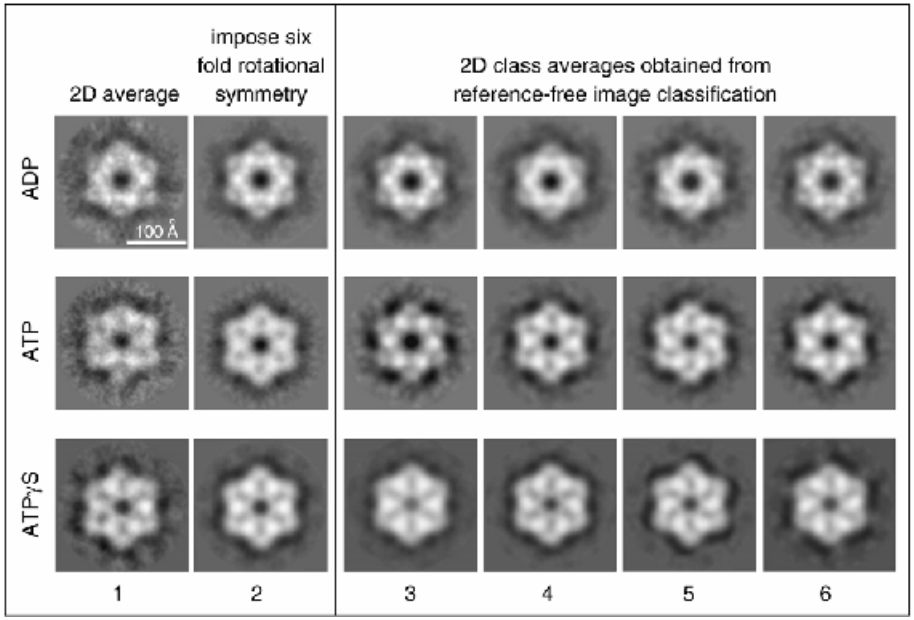




But see López-Perrote *et al.*, (2012) Nucl. Acids Res. doi:10.093/nar/gks871

Previous structural work on Pontin/Reptin complexes

Electron microscopy of Yeast Pontin/Reptin complex



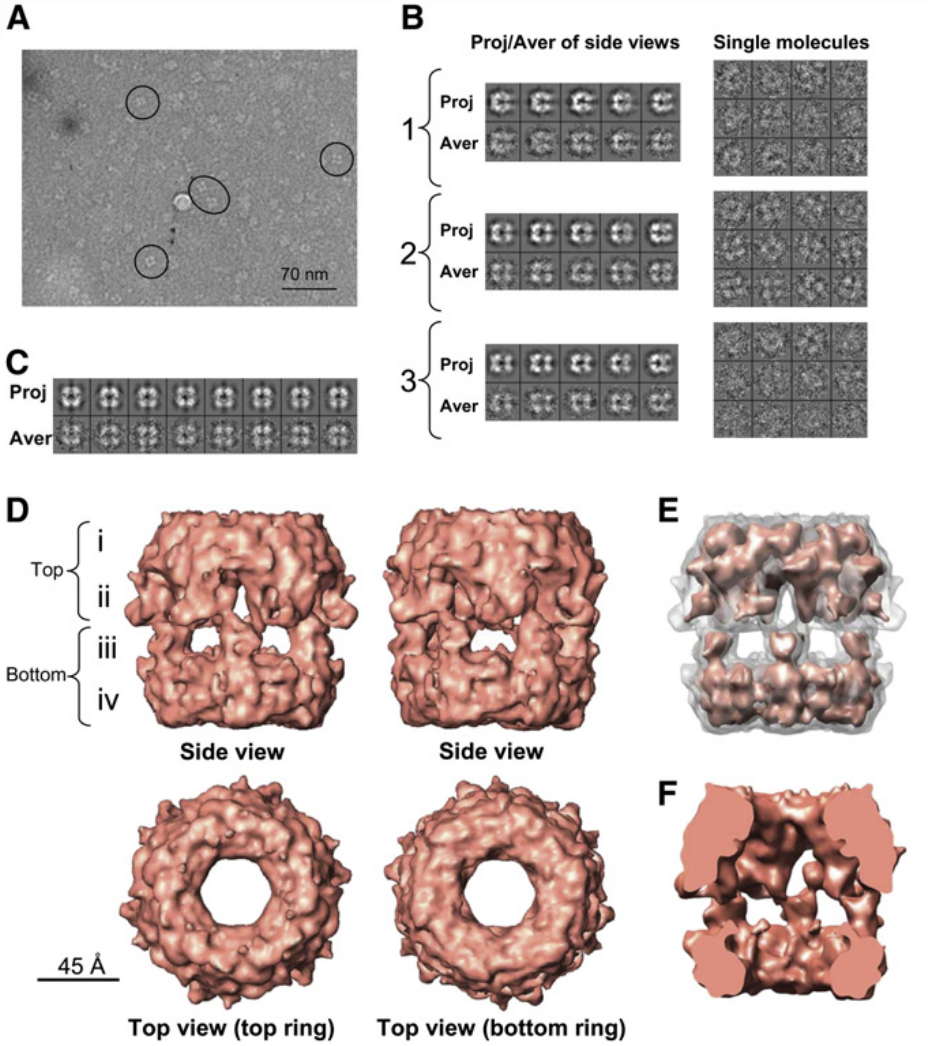
Gribun *et al.* (2008) – **heterohexamers**, probably made of alternating Pontin and Reptin monomers.

Previous structural work on Pontin/Reptin complexes

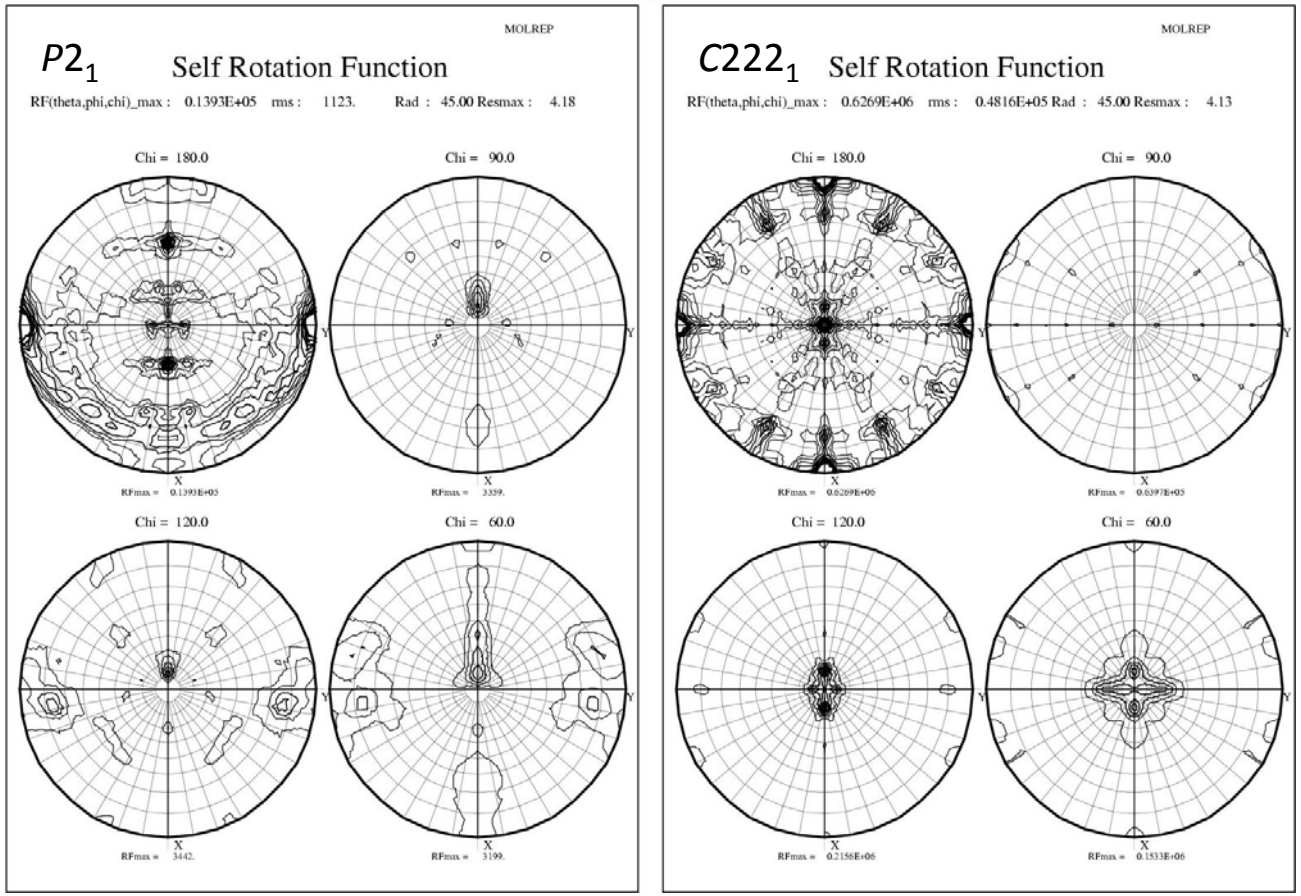
Electron microscopy of the Yeast Pontin/Reptin complex

Torreira *et al.* (2008) – 13 Å resolution, asymmetric **dodecamer**, possibly two homohexamers facing each other.

See also Cheung *et al.* (2010).



Human Pontin/Reptin complex – homo vs. heterohexamers

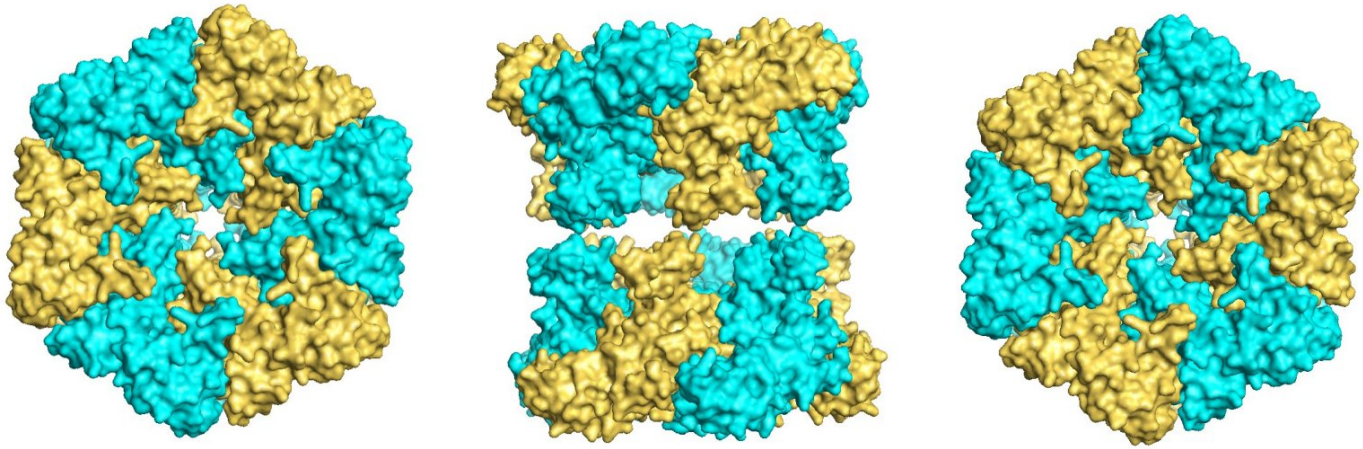


Self-rotation calculations support the **double heterohexamer** in $P2_1$ or $C222_1$: the peaks in the κ=120° section are stronger than those in the κ=60° section.

Human Pontin/Reptin complex – homo vs. heterohexamers

Density modification calculations with DM for each of the 4 different possibilities (3 in $P2_1$, 1 in $C222_1$) gave best results for **a dodecamer made of two heterohexamers in $C222_1$**.

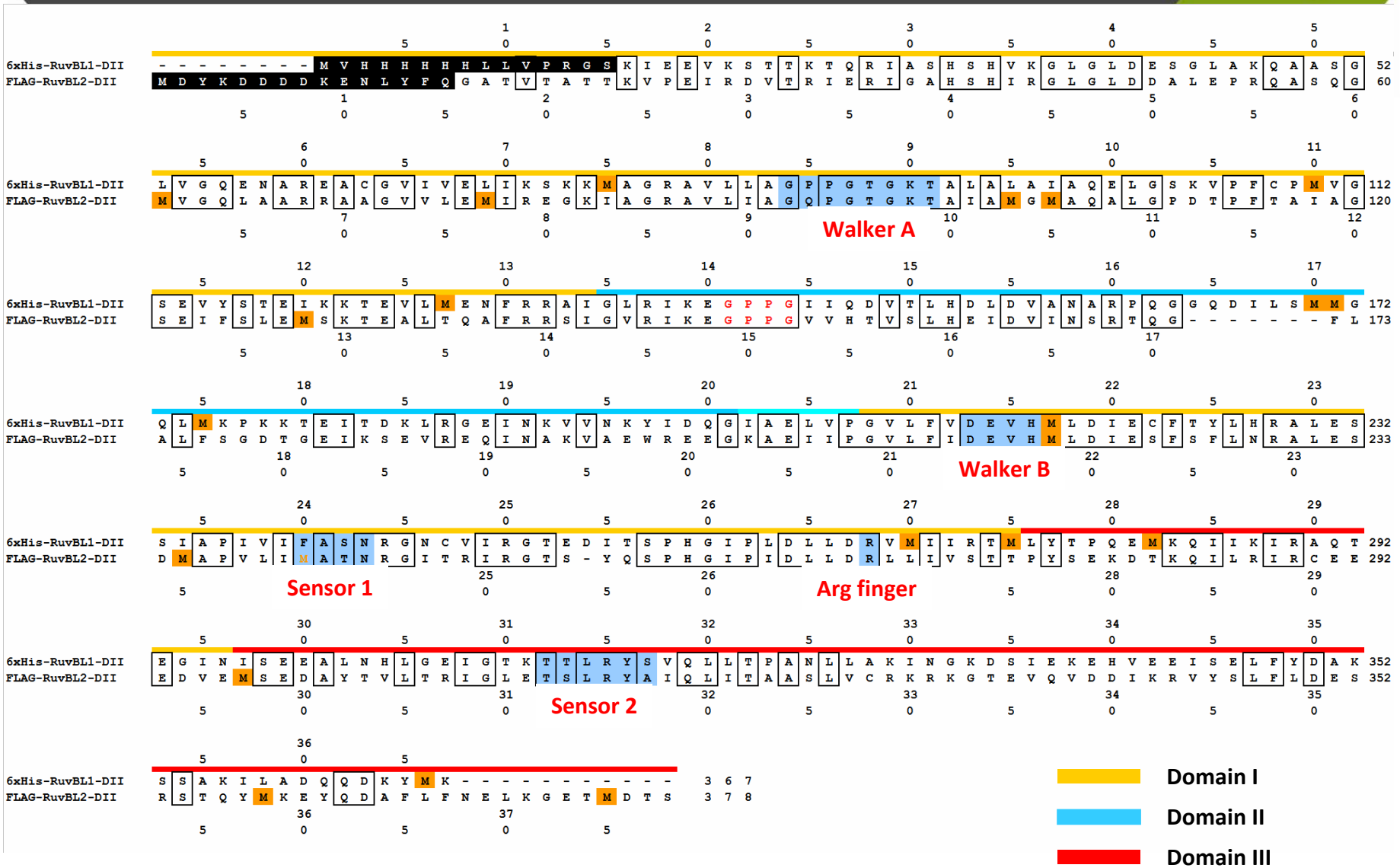
Still, no model for Reptin-ΔDII chains could be built.



Top Side Bottom

This interpretation of the results was not accepted by reviewers and this work could not be published.

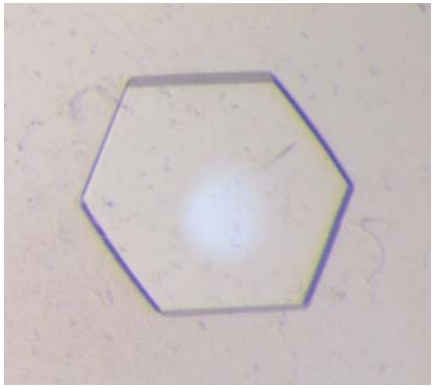
Human Pontin/Reptin complex – homo vs. heterohexamers

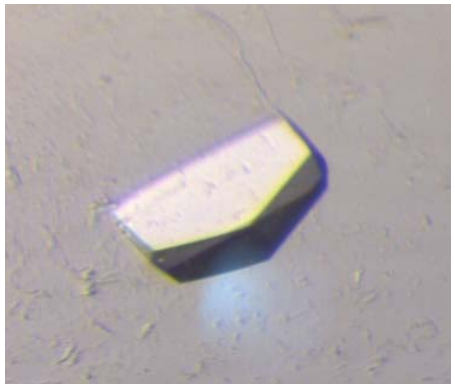


Human Pontin/Reptin complex – SeMet derivative

Pontin-ΔDII and Reptin-ΔDII each contain 11 methionine residues, and with one exception they occupy **different locations** in the sequence.

To elucidate the dodecamer composition by X-ray crystallography, the expression, purification and crystallization of a Se-Met derivative was undertaken.

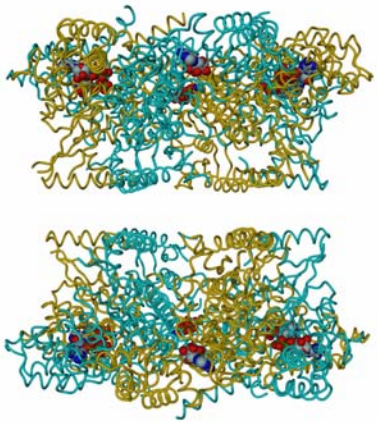


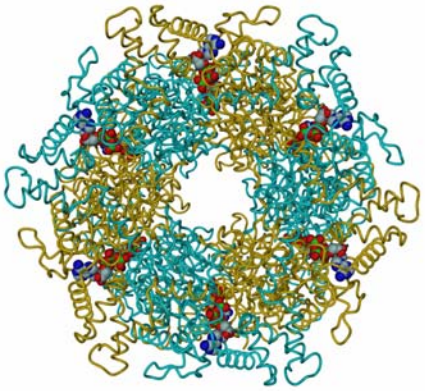


The best crystals of the Se-Met Pontin-ΔDII/Reptin-ΔDII complex were obtained at 4°C within one week by the sitting drop vapor diffusion technique, using a protein concentration of 12 mg/mL and 20 mM Tris-HCl pH 8.0, 200 mM NaCl, 10 % glycerol, 4 mM MgCl2, 4 mM ADP, 0.5 mM TCEP as the precipitating solution.

Human SeMet Pontin/Reptin complex – 3D structure

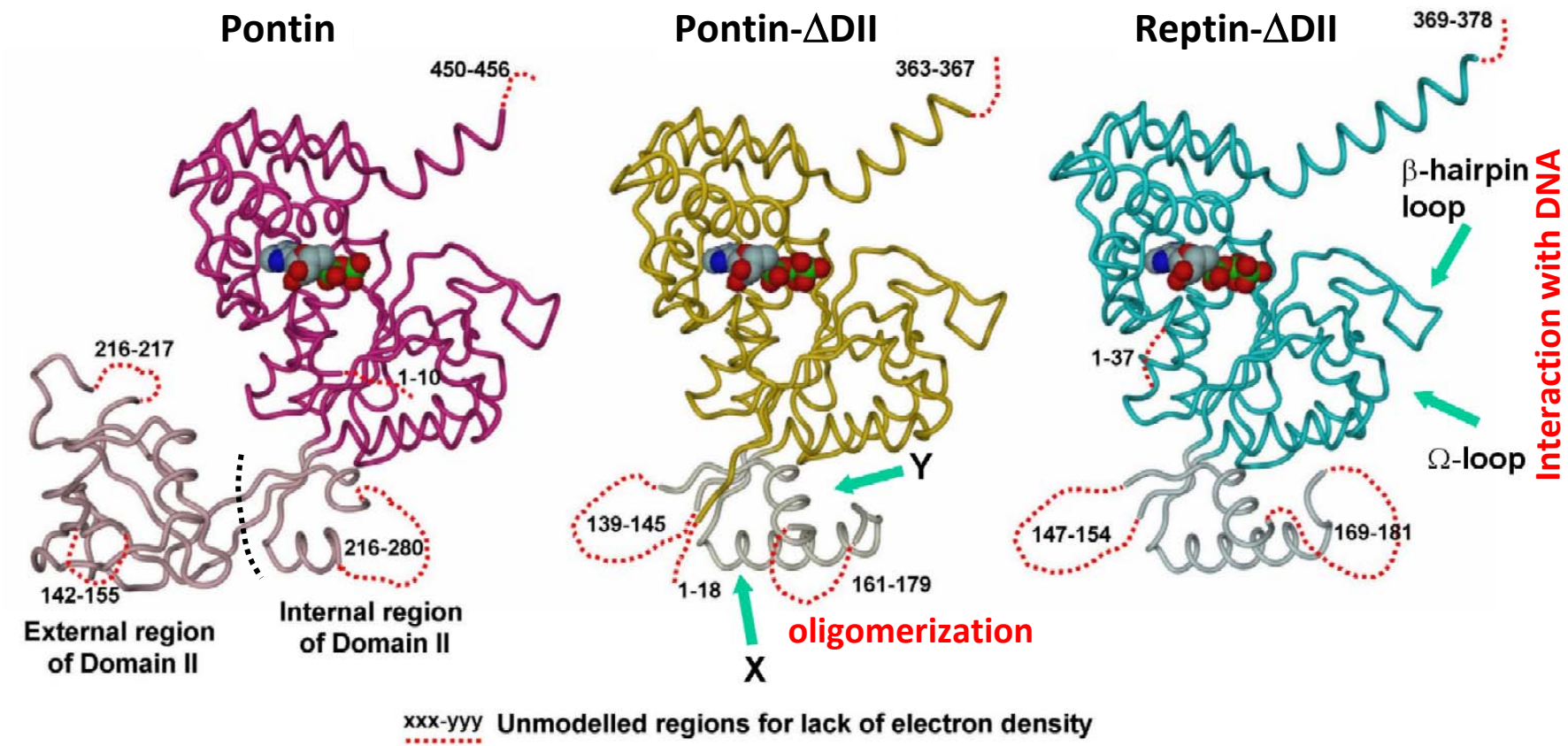
- The 3D structure was determined from a 3-wavelength MAD data set collected at ESRF ID-29 to a maximum resolution of 3 Å. Space group was unambiguously determined as $C222_1$.
- The **Pontin-ΔDII** and **Reptin-ΔDII** monomers could be distinguished. The structure was refined with BUSTER at 3 Å resolution to final R and R-free values of 0.178 and 0.205. No water molecules were added.





The new results confirmed those previously obtained at 4 Å – The complex crystallizes as a dodecamer with alternating **Pontin-ΔDII** and **Reptin-ΔDII** monomers. One heterohexamer is present in the asymmetric unit of space group $C222_1$, the second being generated by a crystallographic 2-fold rotation axis.

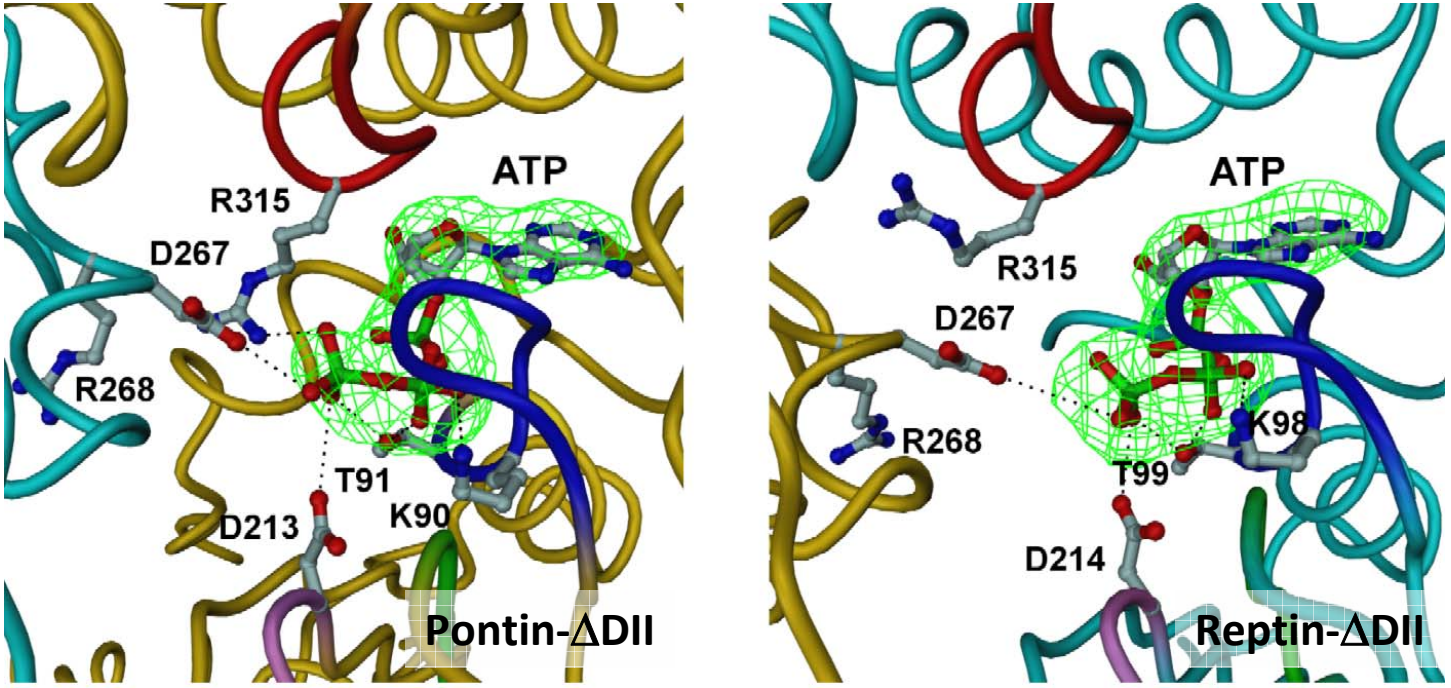
Human SeMet Pontin/Reptin complex – the monomers



Human SeMet Pontin/Reptin complex – the NBP

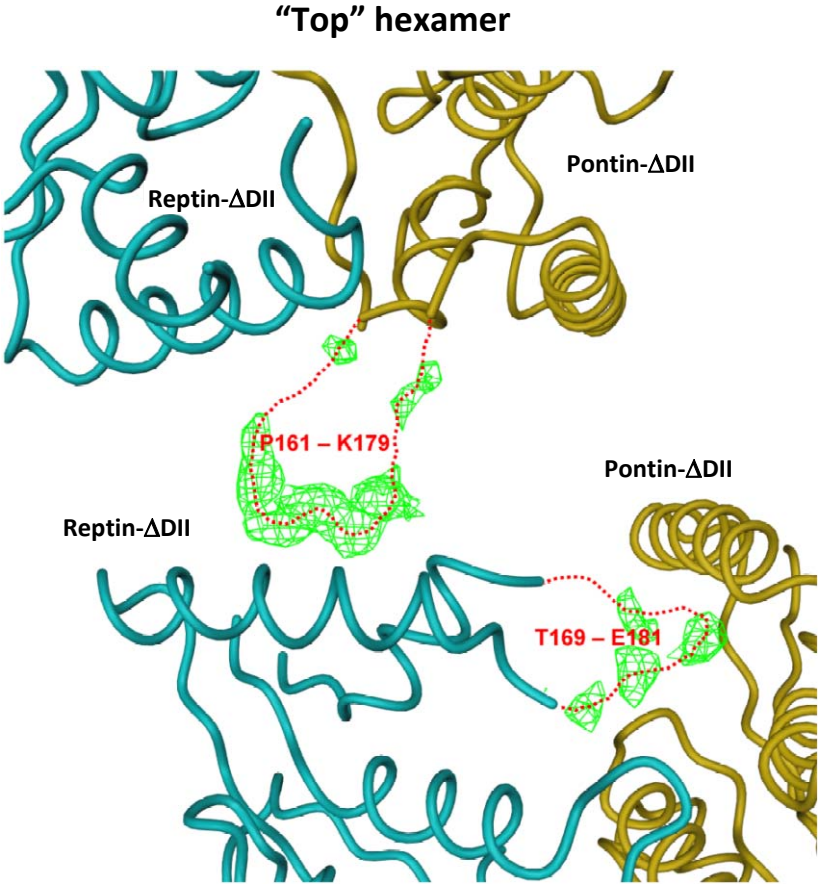
❖ No ATP was added at any stage during purification or crystallization.

❖ However, the nucleotide-binding pockets of every Pontin-ΔDII and Reptin-ΔDII monomer in the complex clearly showed electron density that could be interpreted as a mixture of ADP and ATP.



|Fo|-|Fc|: 3.0 σ (after initial refinement without ATP in the model).

Human SeMet Pontin/Reptin complex – dodecamerization



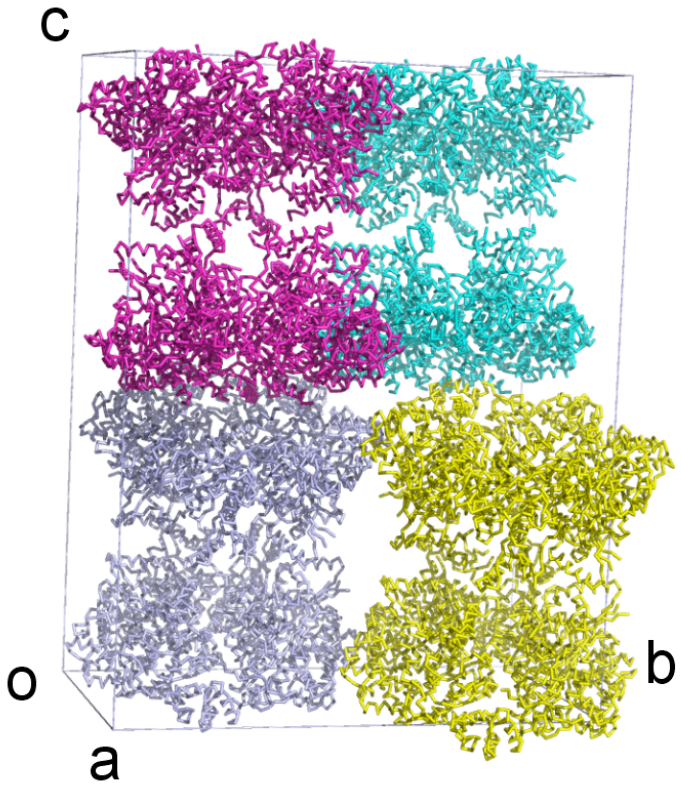
Interactions between hexamers in the dodecamer are **ill-defined** – poor electron density – probably resulting from mixed conformations

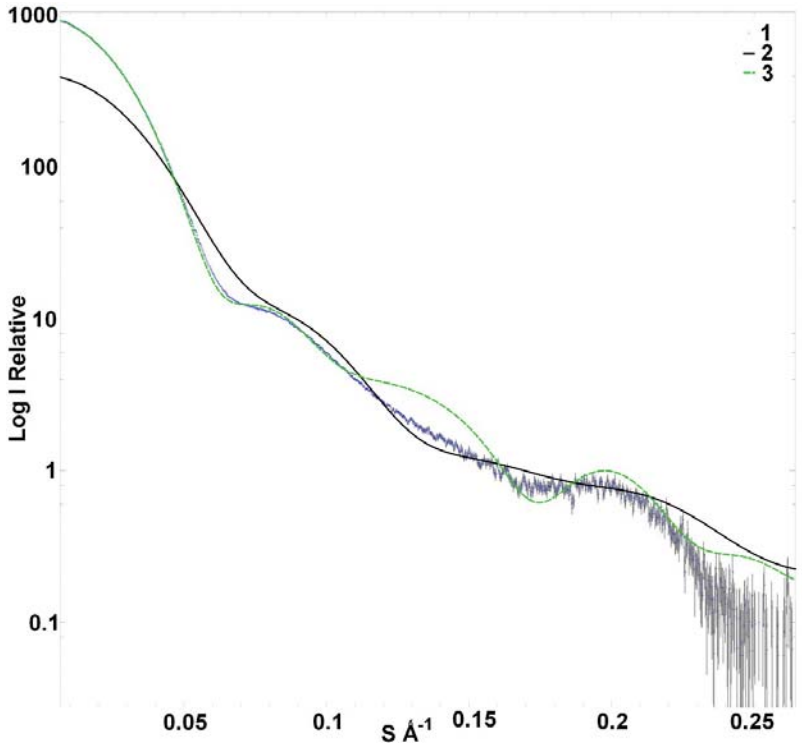
Is the complex really a dodecamer ?

There is no "direct" structural evidence, but...

Human SeMet Pontin/Reptin complex – dodecamerization

Crystal packing and SAXS data support the existence of a dodecameric complex.





(1) raw SAXS data; (2) fit by the crystallographic hexamer;
(3) fit by the crystallographic dodecamer after modelling of missing loops.

Human SeMet Pontin/Reptin complex – dodecamerization

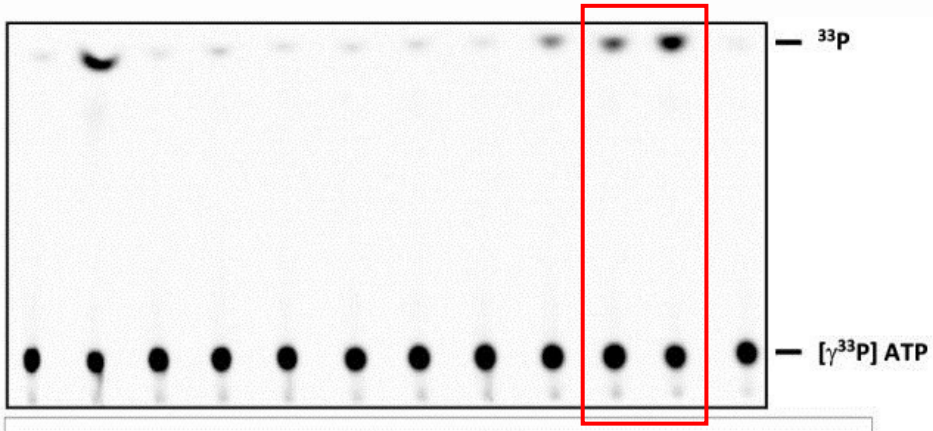
Dodecamer formation is favoured by Domain II truncation

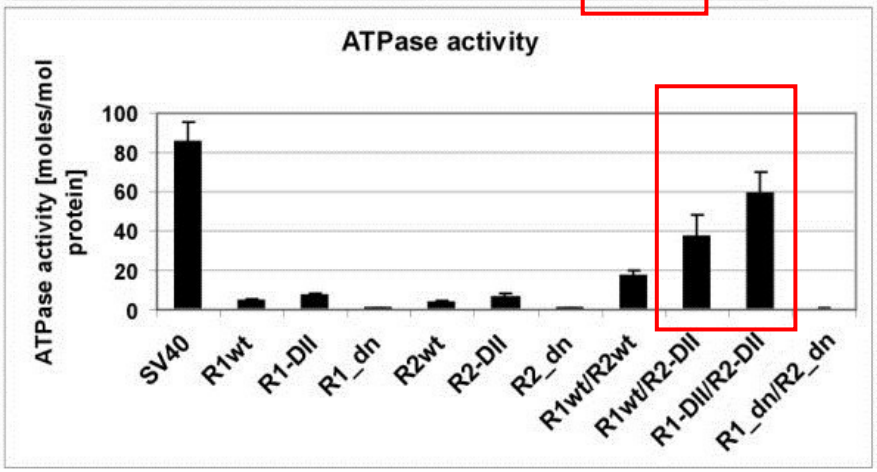
Table 2
Volume fractions of monomers, hexamers and dodecamers in solutions of RuvBL1, RuvBL2 and their complexes.

Sample	Monomer (%)	Hexamer (%)	Dodecamer (%)	χ
RuvBL1wt (< 6 mg/mL)	97	3	0	2.9
RuvBL1wt (> 6 mg/mL)	0	100	0	1.58
RuvBL2wt	0	82	18	5.35
RuvBL2ΔDII	0	77	23	1.4
RuvBL1wt/RuvBL2wt	0	54	46	2.92
RuvBL1wt/RuvBL2ΔDII	0	0	100	1.5
RuvBL1ΔDII/RuvBL2ΔDII	0	0	100	1.5

The accuracy of the volume fractions calculated with OLIGOMER (Konarev et al., 2003) is about 2 % for all constructs.

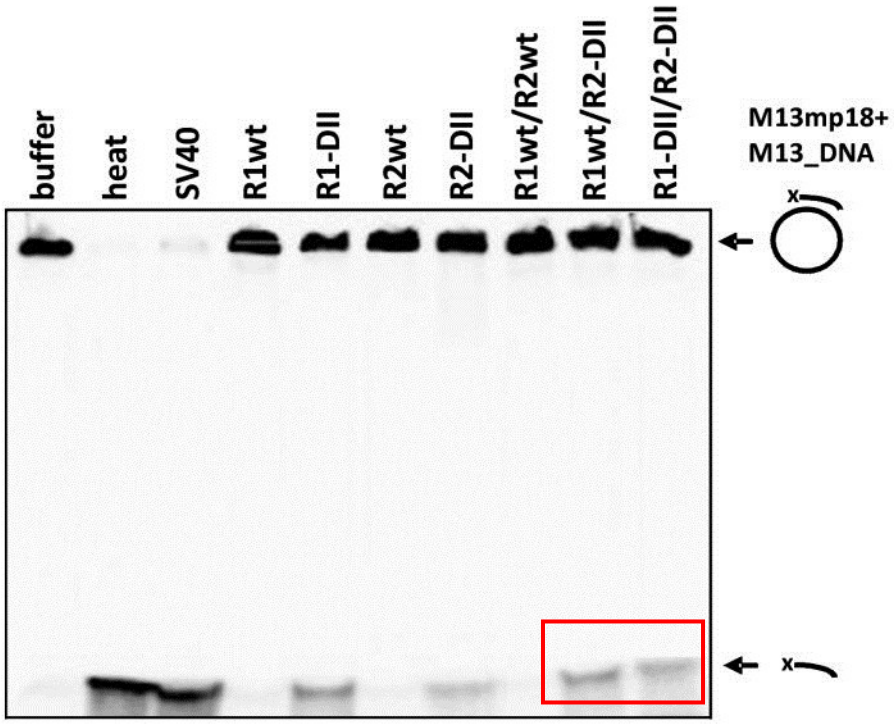
Human Pontin/Reptin complex – ATPase assay





The complexes with a truncated Domain II have a significant increase in ATPase activity

Human Pontin/Reptin complex – helicase assay



The complexes with a truncated Domain II have a significant increase in helicase activity

Human Pontin/Reptin complex – conclusions

The complex is a dodecamer formed by a double hexamer

Although the interacting regions have poor electron density, the crystal packing and the oligomerization studies in solution support this conclusion.

The hexamers are heterohexamers

The 3D structure of the Se-Met derivative has provided definitive proof.

Domain II is involved in regulation of ATP hydrolysis and helicase activity

The truncated complex exhibits a marked increase in ATPase and helicase activities over the wild-type complex and the isolated proteins. Truncation of domain II may mimic *in vivo* activation induced by cofactors, allowing a more efficient ADP/ATP exchange and helicase activity.

Gorynia et al., (2011) *J Struct Biol* 176:279-291

Human Pontin/Reptin complex – open questions

What are the details of hexamer-hexamer interaction in the dodecamer?

The electron density is poorly defined. Better crystals and/or mutants are needed.

What are the details of the ATP hydrolysis?

The present results suggest an “all-or-none” mechanism but more data is needed.

What are the details of the interaction with DNA?

The 3D structure of a complex with ssDNA or dsDNA is needed.

MAJOR hurdle to be overcome

The diffraction quality of the crystals: more than 150 crystals of the native complex were screened and only one crystal diffracted to about 3.5 Å.

Human Pontin/Reptin complex – open questions

Is this the only type of RuvBL1/RuvBL2 complex ?

Different complex types may exist, depending on the function exerted. Also, influence of tags in oligomerization must be considered [Cheung *et al.* (2010)].

Table 2
Volume fractions of monomers, hexamers and dodecamers in solutions of RuvBL1, RuvBL2 and their complexes.

Sample	Monomer (%)	Hexamer (%)	Dodecamer (%)	χ
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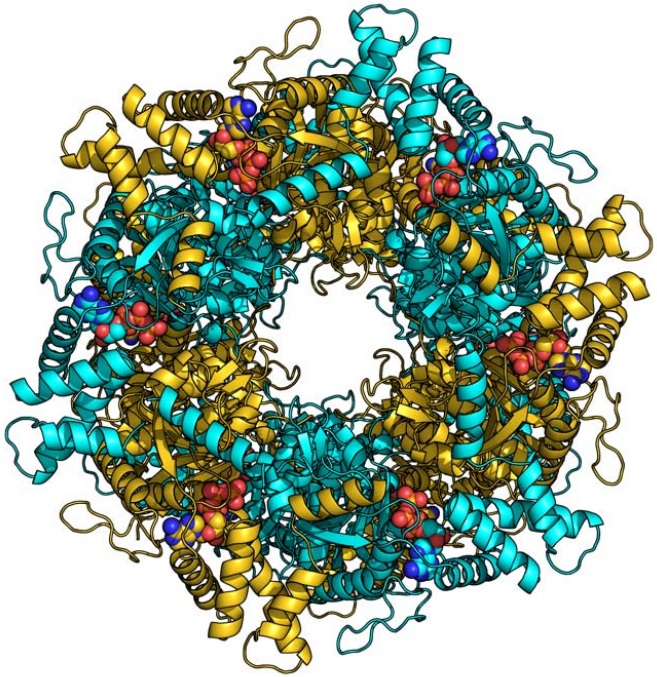
RuvBL1ΔDII/RuvBL2ΔDII	0	0	100	1.5

The accuracy of the volume fractions calculated with OLIGOMER (Konarev et al., 2003) is about 2 % for all constructs.

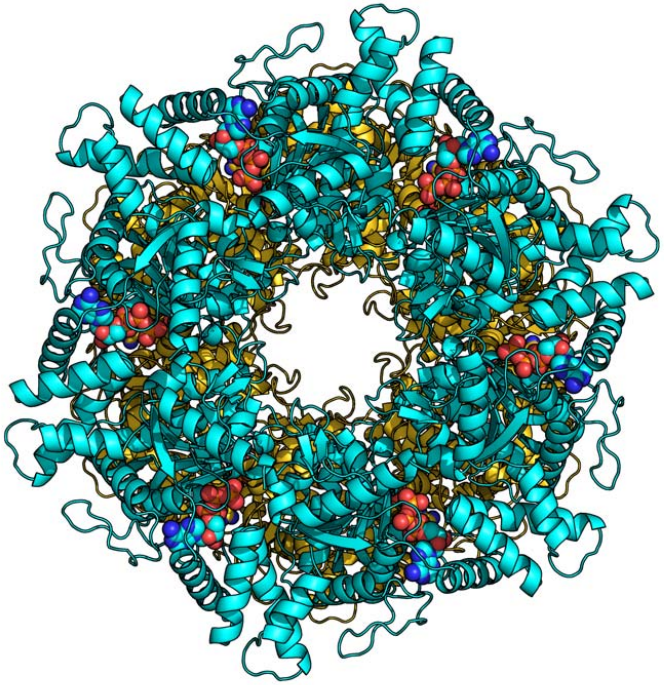
Coexpression of Pontin and Reptin = heterohexameric complex ?

Separate expression of Pontin and Reptin = homohexameric complex ?

Human Pontin/Reptin complex – open questions

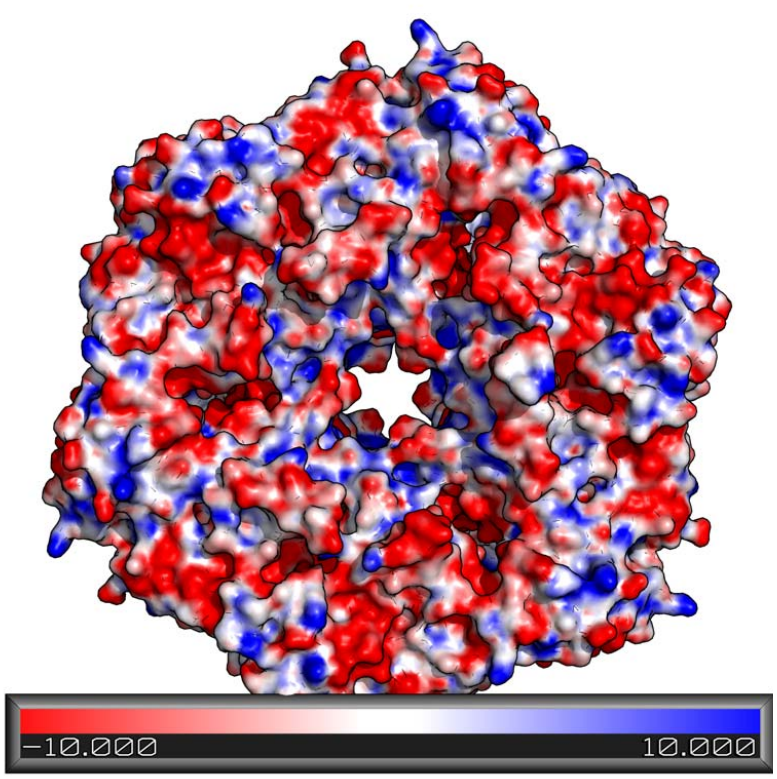


Heterohexameric complex (crystal structure)

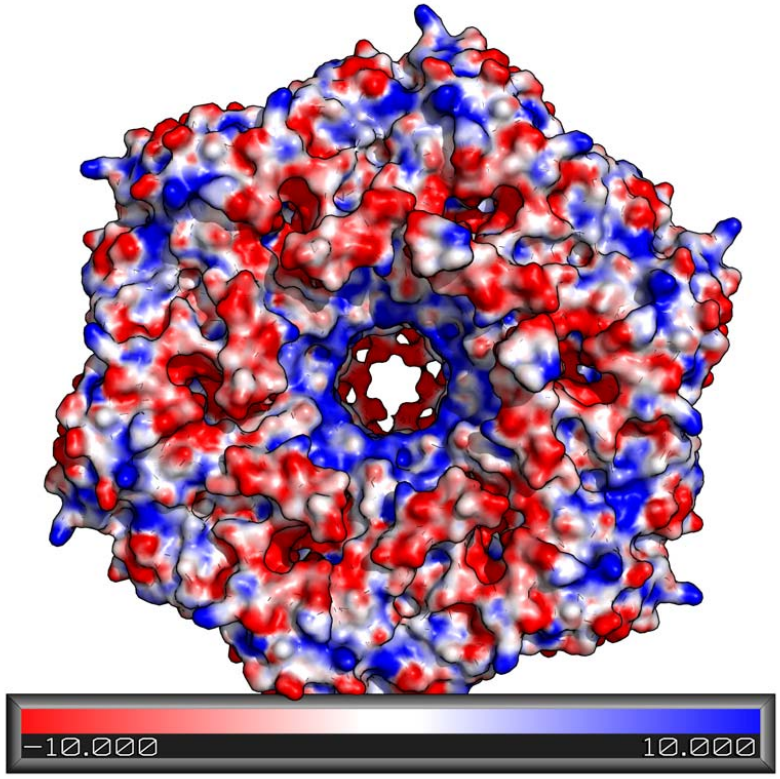


Homohexameric complex (model)

Human RuvBL1/RuvBL2 complex – open questions

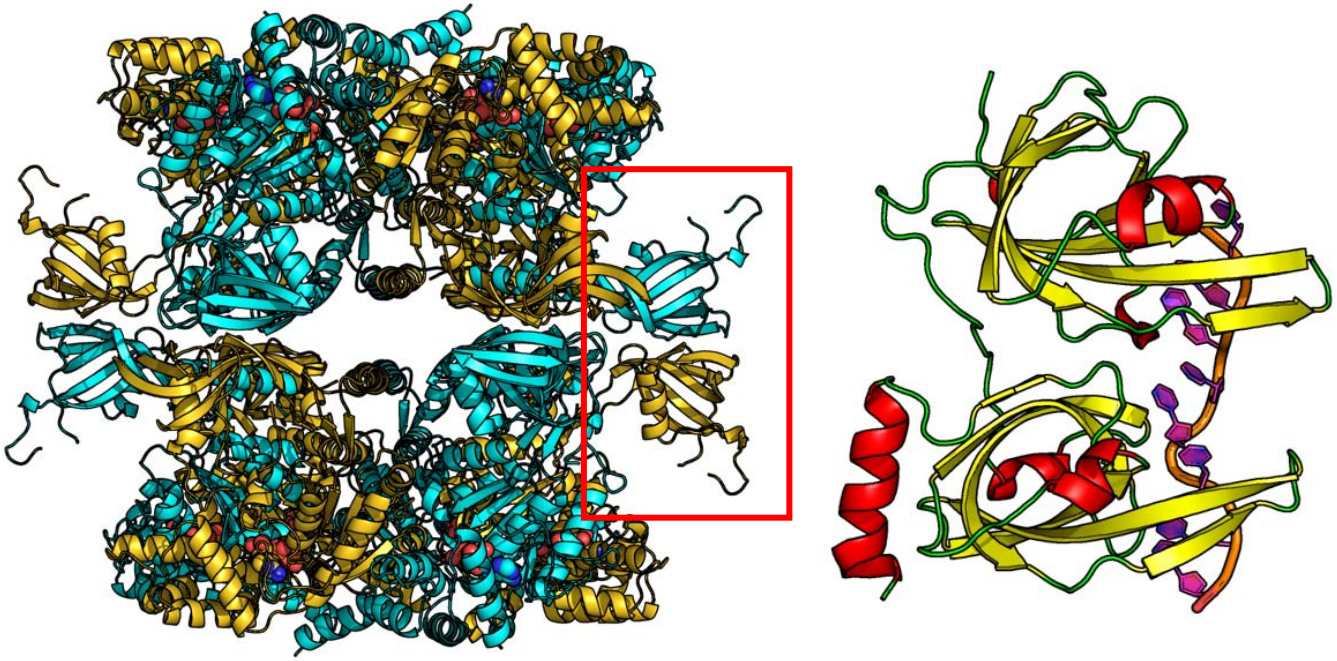


Heterohexameric complex
ssDNA/RNA as substrate ?



Homohexameric complex
dsDNA as substrate ?

Human Pontin/Reptin complex – open questions



RPA
PDB 1JMC
(Bokharev et al., 1997)

Model of the full-length heterohexameric complex
Domain II from Pontin interacts with Domain II from Reptin

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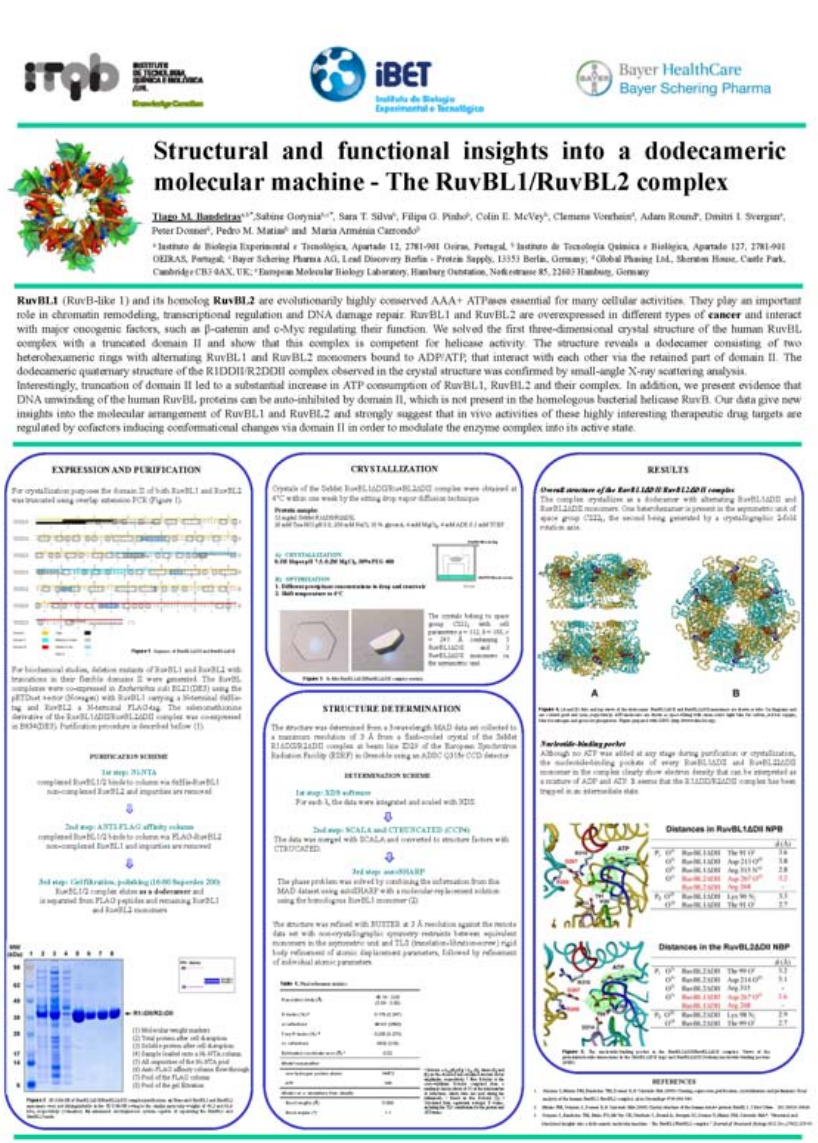
Clemens Vonrhein

EMBL-Hamburg
Adam Round
Dmitri Svergun

Human Pontin/Reptin complex – poster



Structural and functional insights into a dodecameric molecular machine - The RuvBL1/RuvBL2 complex

Tiago M. Bandeiras[a,b*], Sabine Gorynia[b,c*], Sara T. Silva[b], Filipa G. Pinho[b], Colin E. McVey[b], Clemens Vonrhein[d], Adam Round[e], Dmitri I. Svergun[e], Peter Donner[f], Pedro M. Matias[b] and Maria Arménia Carrondo[b]

[a] Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2781-901 Oeiras, Portugal, [b] Instituto de Tecnologia Química e Biológica, Apartado 127, 2781-901 OEIRAS, Portugal, [c] Bayer Schering Pharma AG, Lead Discovery Berlin - Protein Supply, 13353 Berlin, Germany, [d] Global Phasing Ltd., Sheraton House, Castle Park, Cambridge CB3 0AX, UK, [e] European Molecular Biology Laboratory, Hamburg Outstation, Notkestrasse 85, 22603 Hamburg, Germany

RuvBL1 (RuvB-like 1) and its homolog **RuvBL2** are evolutionarily highly conserved AAA+ ATPases essential for many cellular activities. They play an important role in chromatin remodeling, transcriptional regulation and DNA damage repair. RuvBL1 and RuvBL2 are overexpressed in different types of **cancer** and interact with major oncogenic factors, such as β-catenin and c-Myc regulating their function. We solved the first three-dimensional crystal structure of the human RuvBL complex with a truncated domain II and show that this complex is competent for helicase activity. The structure reveals a dodecamer consisting of two heterohexameric rings with alternating RuvBL1 and RuvBL2 monomers bound to ADP/ATP, that interact with each other via the retained part of domain II. The dodecameric quaternary structure of the R1DDII/R2DDII complex observed in the crystal structure was confirmed by small-angle X-ray scattering analysis.
Interestingly, truncation of domain II led to a substantial increase in ATP consumption of RuvBL1, RuvBL2 and their complex. In addition, we present evidence that DNA unwinding of the human RuvBL proteins can be auto-inhibited by domain II, which is not present in the homologous bacterial helicase RuvB. Our data give new insights into the molecular arrangement of RuvBL1 and RuvBL2 and strongly suggest that in vivo activities of these highly interesting therapeutic drug targets are regulated by cofactors inducing conformational changes via domain II in order to modulate the enzyme complex into its active state.

EXPRESSION AND PURIFICATION

CRYSTALLIZATION

STRUCTURE DETERMINATION

RESULTS

REFERENCES

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Diamond Light Source, Didcot, UK (XRC).

Deutsches Elektronen-Synchrotron, Hamburg, Germany (SAXS).