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

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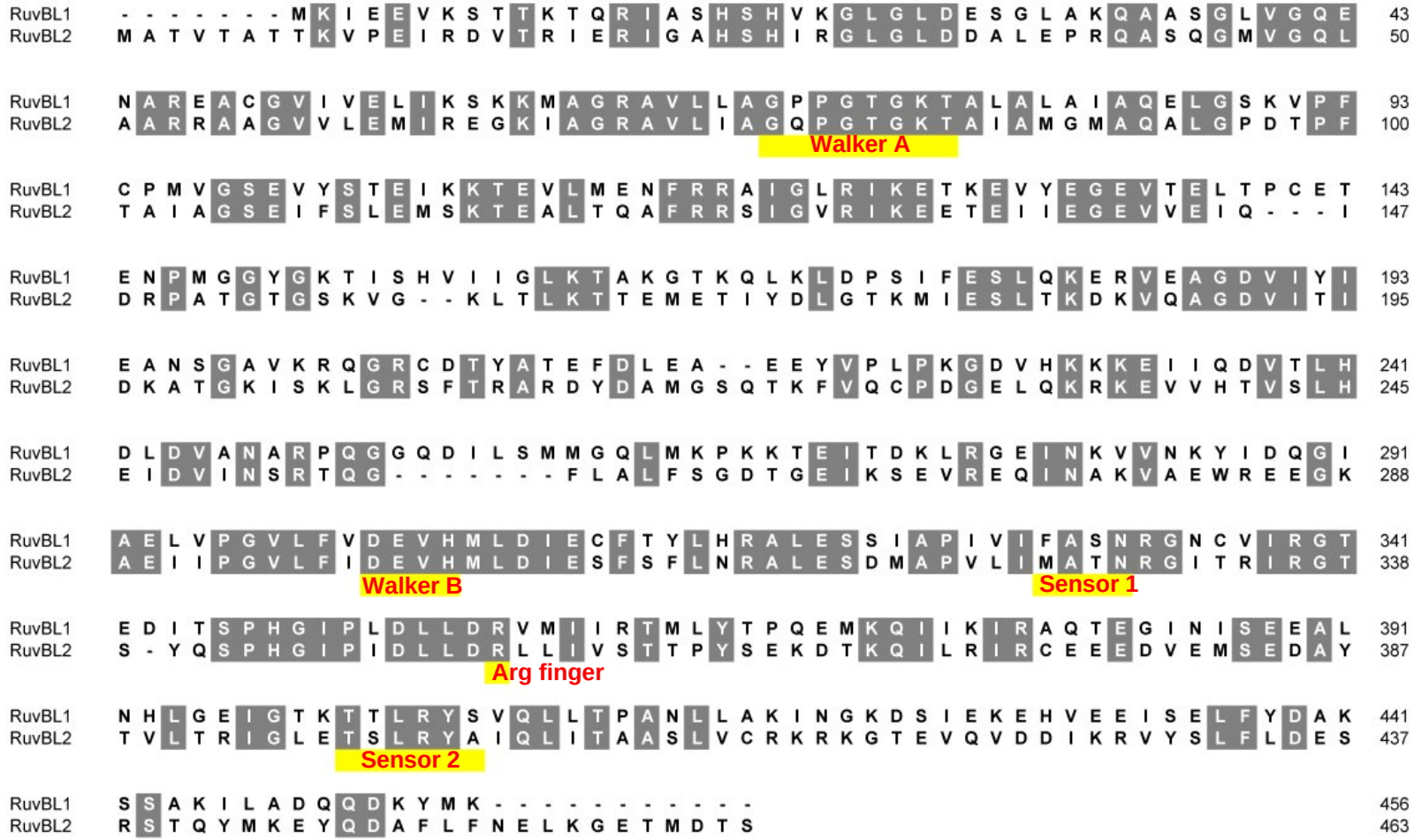
RuvBL1 [RuvB-like 1 (*E. coli*)]	**RuvBL2 [RuvB-like 2 (*E. coli*)]**
NMP238	CGI-46
ECP54	ECP51
INO80H	INO80J
PONTIN	REPTIN
RVB1	RVB2
Pontin52	Reptin52
Rvb1	Rvb2
TAP54-α	TAP54-β
TIH1	TIH2
TIP49	TIP48
TIP49A	TIP49B
456 aa, 50.2 kDa	463 aa, 52 kDa

Human RuvBL1 and RuvBL2:

- 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); this family includes nucleic acid processing enzymes, chaperones and proteases;
- **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**;

Human **RuvBL1** and **RuvBL2** are homologs,
sharing 41% identity and 64% similarity



Human RuvBL1 and RuvBL2:

- Are **ubiquitously expressed** proteins, especially abundant in heart, skeletal muscle and testis (RuvBL1) and in thymus and testis (RuvBL2)

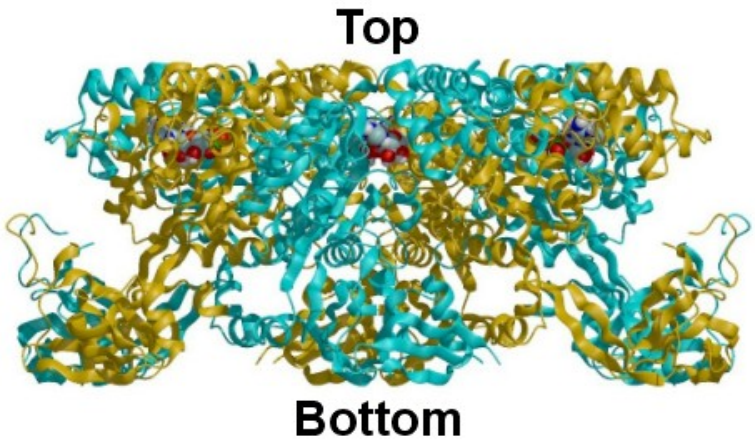
- Play roles in essential signaling pathways such as **c-Myc** and **β-catenin**

- RuvBL1 is required for the oncogenic transforming activity of **c-Myc**, **β-catenin** and the viral oncoprotein **E1A**

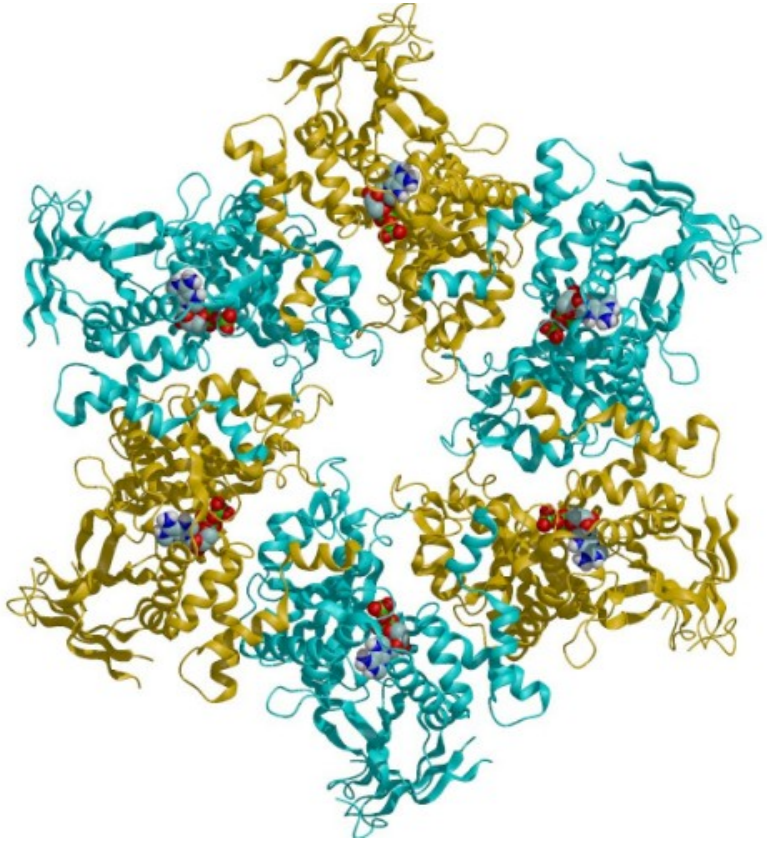
- Participate in chromatin remodelling as members of several complexes

- Are involved in transcriptional regulation, DNA repair, snoRNP biogenesis, and telomerase activity

The 3D structure of Human RuvBL1 – an hexameric ring

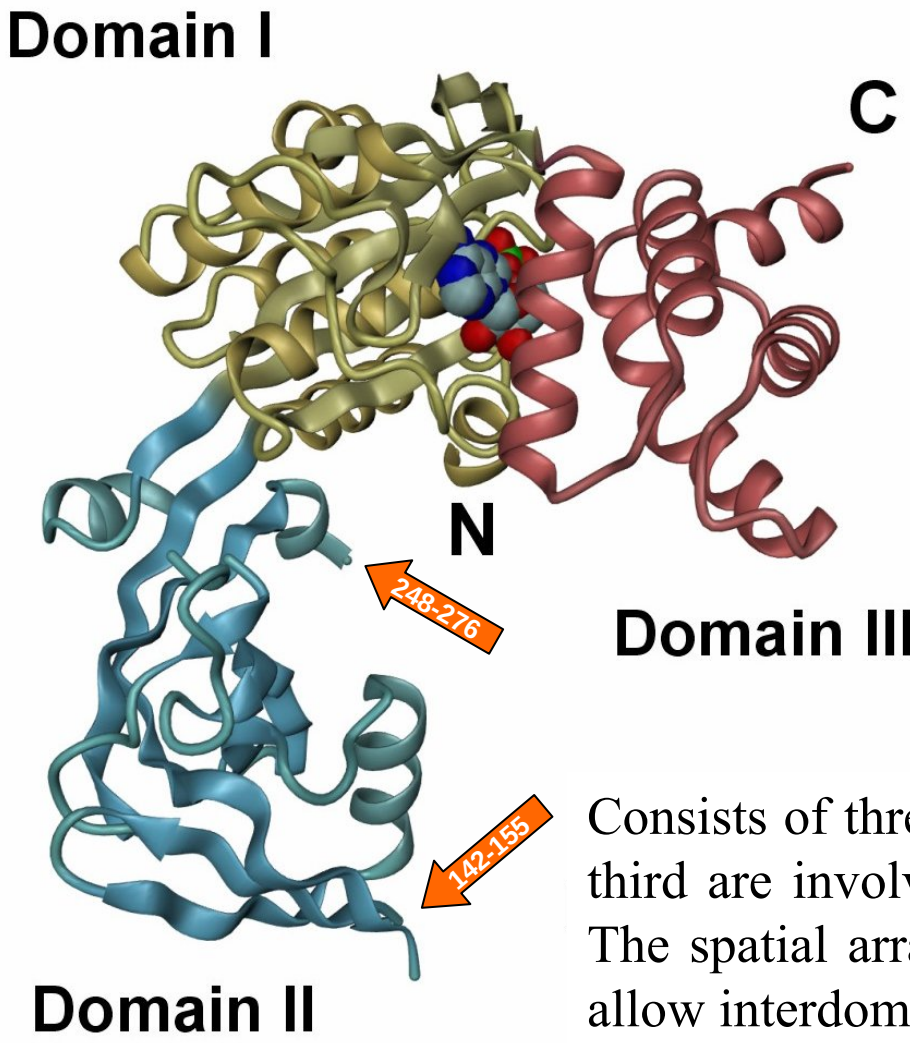


Resolution: 2.2 Å



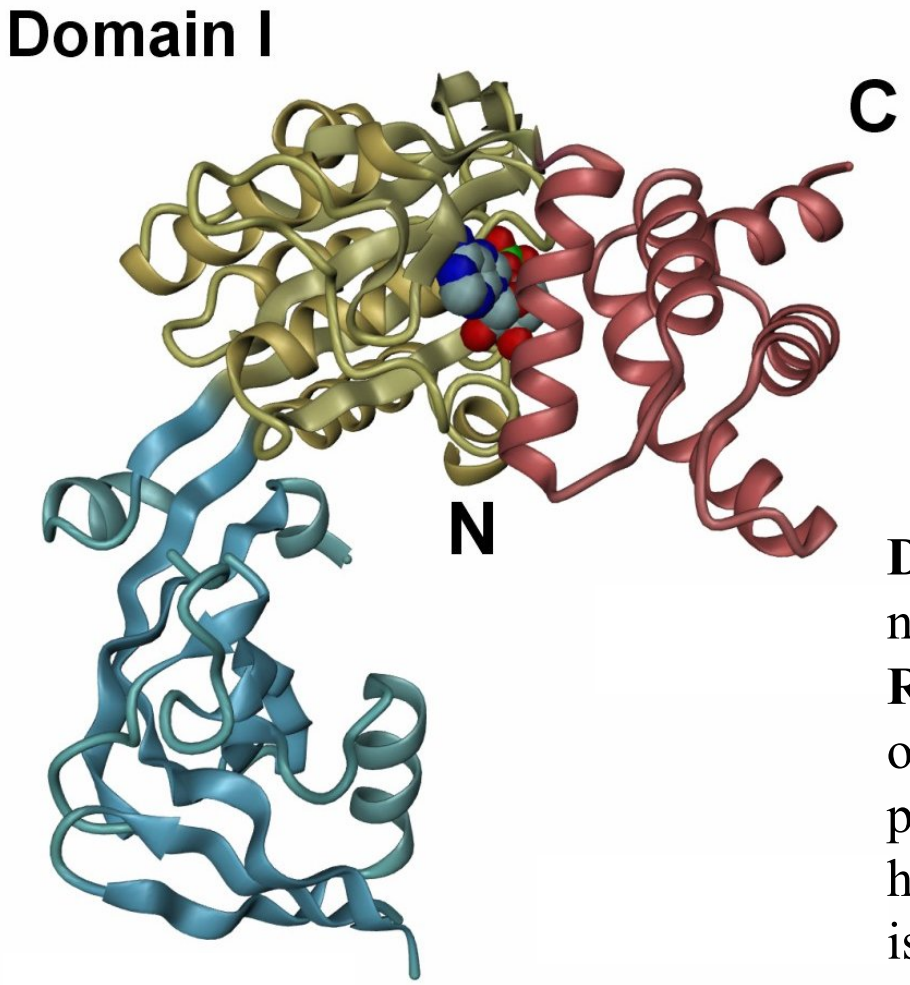
The external diameter of the hexameric ring ranges between **94** and **117 Å** and the central channel has an approximate diameter of **18 Å**. Its top surface appears to be remarkably **flat**.

Human RuvBL1 – the monomer 3D structure (I)



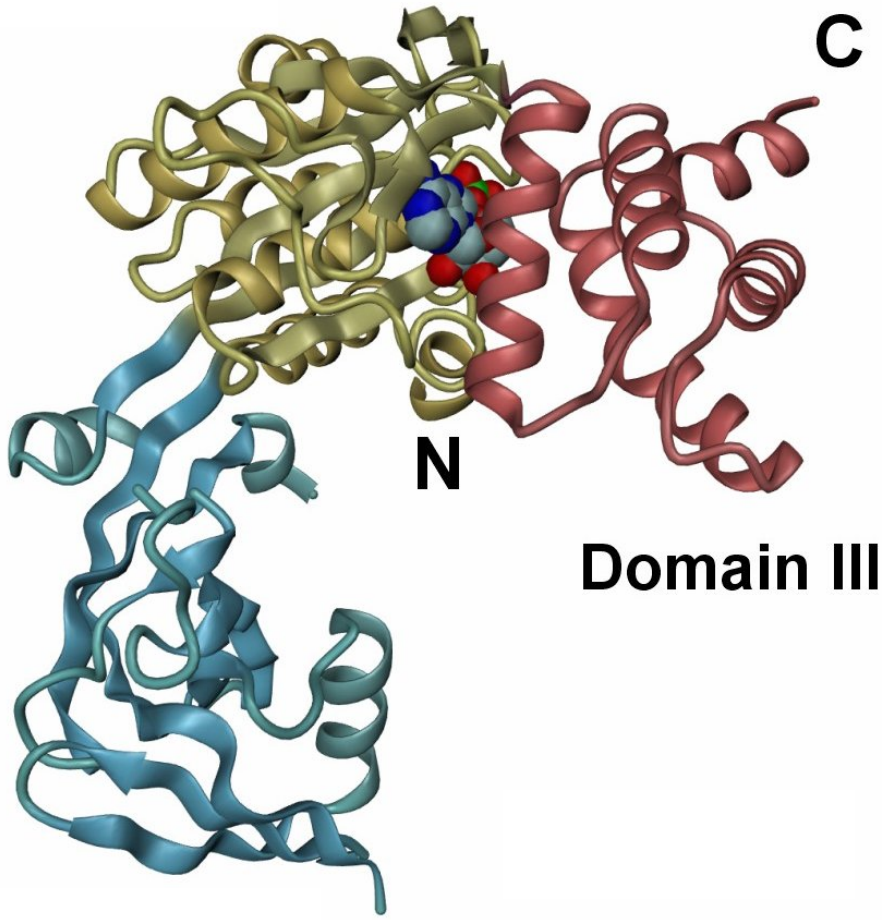
Consists of three domains, of which the first and the third are involved in **ATP binding** and **hydrolysis**. The spatial arrangement of the three domains could allow interdomain motions

Human RuvBL1 – the monomer 3D structure (II)



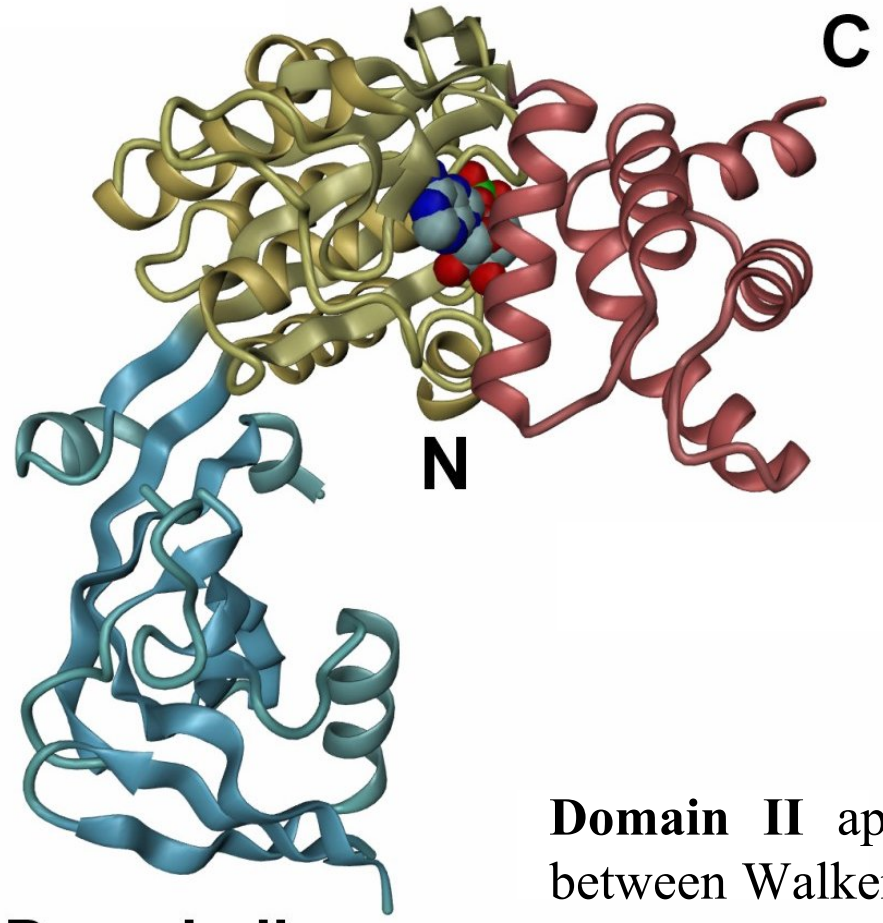
Domain I is a triangle-shaped nucleotide-binding domain with a **Rossmann-like α/β/α fold** composed of a core β-sheet consisting of five parallel β-strands with two flanking α-helices on each side. The core β-sheet is similar to the AAA^+ module of other AAA^+ family members.

Human RuvBL1 – the monomer 3D structure (III)



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**.

Human RuvBL1 – the monomer 3D structure (IV)



Domain II appears as a **~170 residue** insertion between Walker A and Walker B motifs in Domain I and is unique to RuvBL1 and RuvBL2

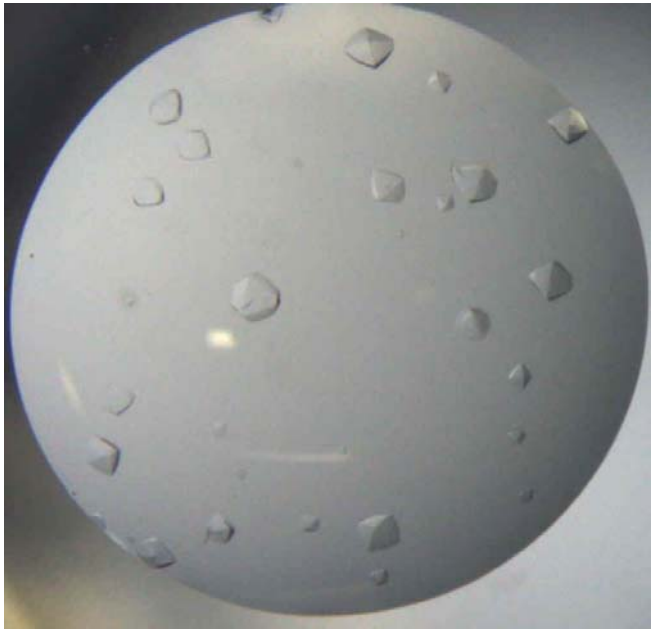
Human RuvBL1 – Biochemical Assays

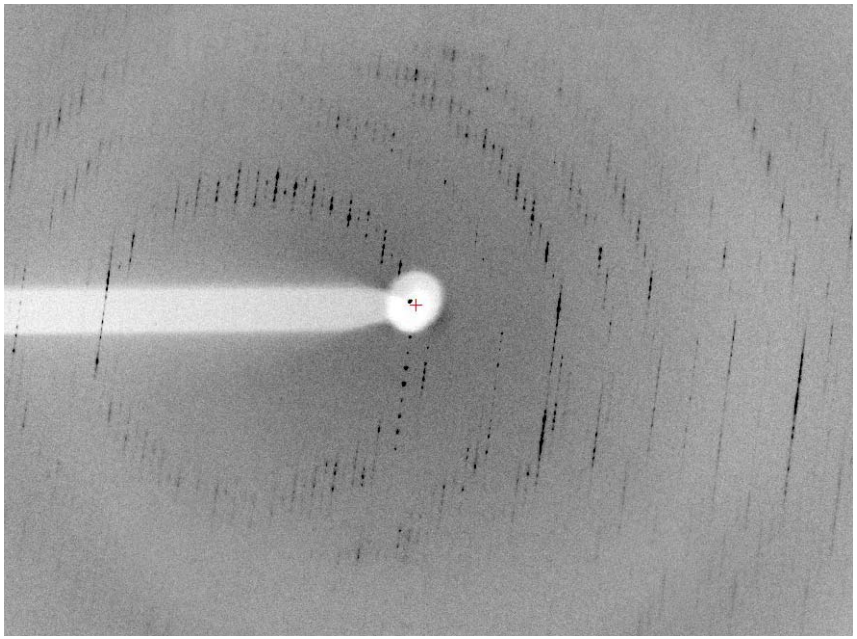
- RuvBL1 has low ATPase activity.
- RuvBL1 can bind ssRNA/DNA as well as dsDNA.
- Purified RuvBL1 has no measurable DNA helicase activity.

AAA$^+$ proteins are ATP-driven molecular machines –The ability to hydrolyze ATP is **essential** for the biological function of RuvBL1.

Human RuvBL2

- Human RuvBL2 was produced and purified as for RuvBL1
- Crystals of poor quality were obtained
- The measured diffraction data showed the crystals to be multiple
- No 3D structure of human RuvBL2 could be determined



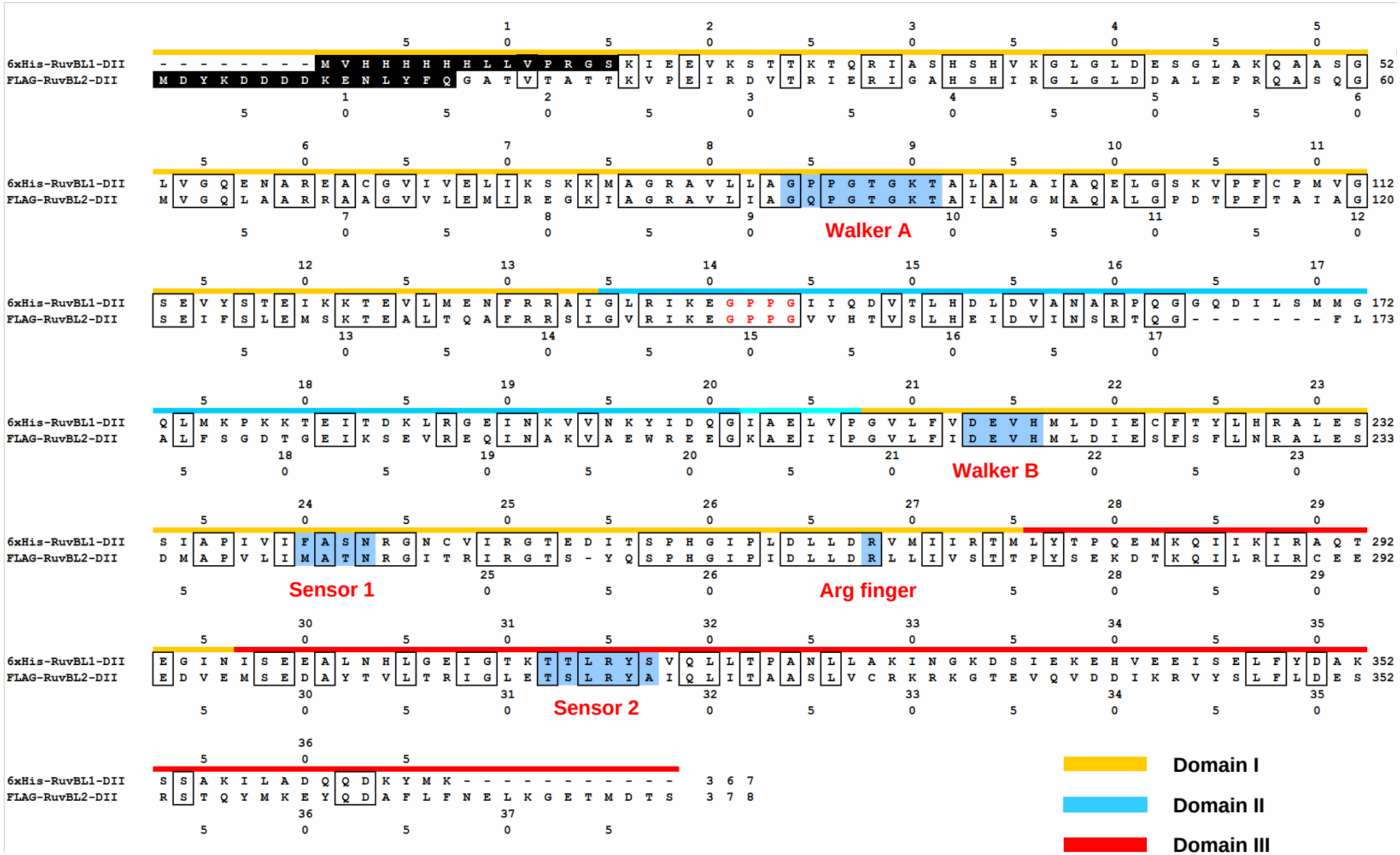


Human RuvBL1/RuvBL2 complex – expression

For crystallization purposes, Domain II of both RuvBL1 and RuvBL2 was truncated (RuvBL1ΔDII and RuvBL2ΔDII).

Residues **T127-E233** in RuvBL1 and **E134-E237** in RuvBL2 were replaced by a **GPPG** linker.

6xHis-tagged RuvBL1 and **FLAG-tagged** RuvBL2 were co-expressed in *E.coli* using the pETDuet vector (Novagen) (pETDuet-6xHis-RuvBL1ΔDII_FLAG-RuvBL2ΔDII).


6xHis-RuvBL1-DII
FLAG-RuvBL2-DII
Walker A
Walker B
Sensor 1
Arg finger
Sensor 2
Domain I
Domain II
Domain III

Human RuvBL1/RuvBL2 complex – purification and crystallization

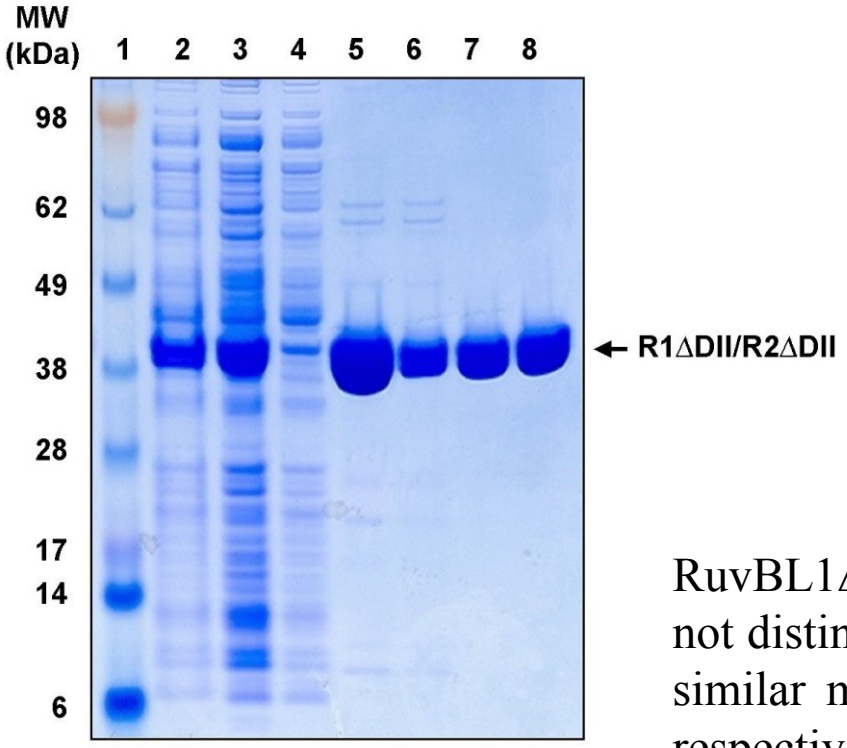
Three purification steps were necessary to obtain a clean and uniform complex of RuvBL1 and RuvBL2 using two affinity purifications and a gel filtration:

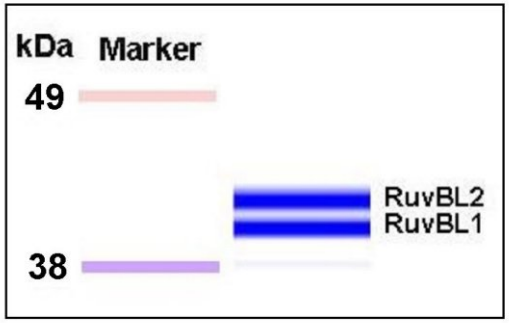
1st step – Ni-NTA
RuvBL1/RuvBL2 complex binds to column via 6xHis-RuvBL1;
free RuvBL2 and impurities are removed.

2nd step – ANTI-FLAG affinity column
RuvBL1/RuvBL2 complex binds to column via FLAG-RuvBL2;
free RuvBL1 and impurities are removed.

3rd step – Gel filtration, polishing (16/60 Superdex 200)
RuvBL1/RuvBL2 complex elutes **as a dodecamer**,
is separated from FLAG peptides and remaining RuvBL1 and RuvBL2 monomers.

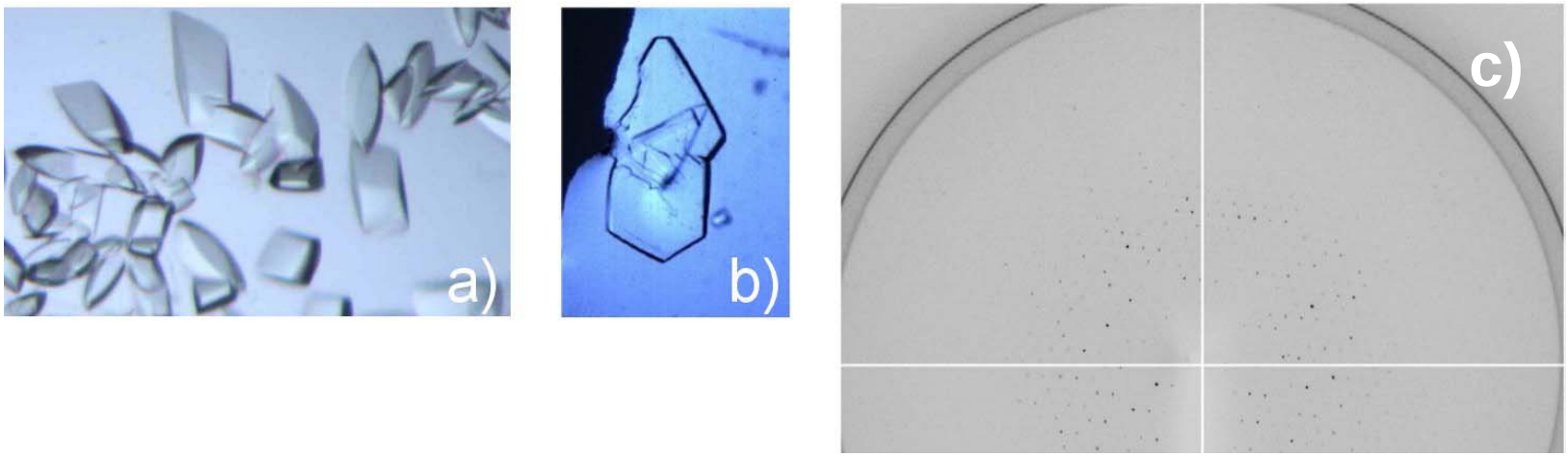
SDS-PAGE of RuvBL1ΔDII/RuvBL2ΔDII complex purification:
1 – MW markers; 2 – after cell disruption; 3 – soluble proteins; 4 – Ni-NTA flowthrough; 5 – Ni-NTA pool; 6 – Anti-FLAG affinity flowthrough; 7- Anti-FLAG affinity pool; 8 – Gel filtration pool.





RuvBL1ΔDII and RuvBL2ΔDII monomers were not distinguishable in the SDS-PAGE owing to the similar molecular weights of 40,5 and 42,4 kDa, respectively – an automated electrophoresis system capable of separating the RuvBL1 and RuvBL2 bands was used.

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) RuvBL1ΔDII/RuvBL2ΔDII crystals; **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. The ice rings surrounding the diffraction pattern may be due to accidental thawing and freezing of the crystal in the loop and may prevent seeing spots at a slightly higher resolution of about 3.5 Å.

Human RuvBL1/RuvBL2 complex – structure determination

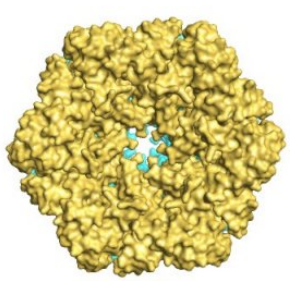
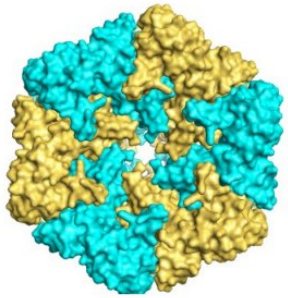
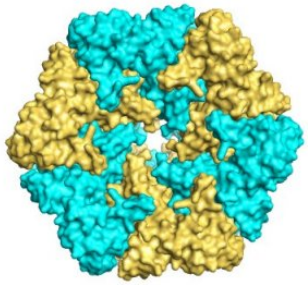
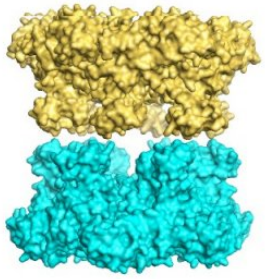
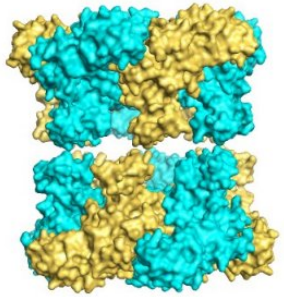
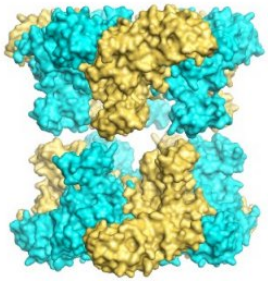
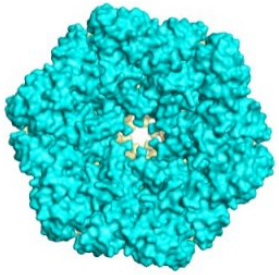
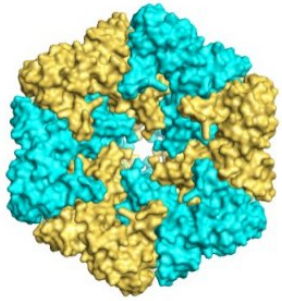
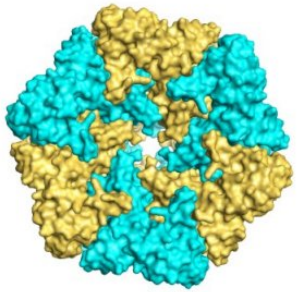
The diffraction data could be processed with similar statistics in two different but related space groups: $C222_1$ and $P2_1$.

The 3D structure of the RuvBL1ΔDII/RuvBL2ΔDII complex was solved by the Molecular Replacement method with PHASER in both space groups – search model: RuvBL1 monomer, truncated to reflect the shortened domain II region.

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

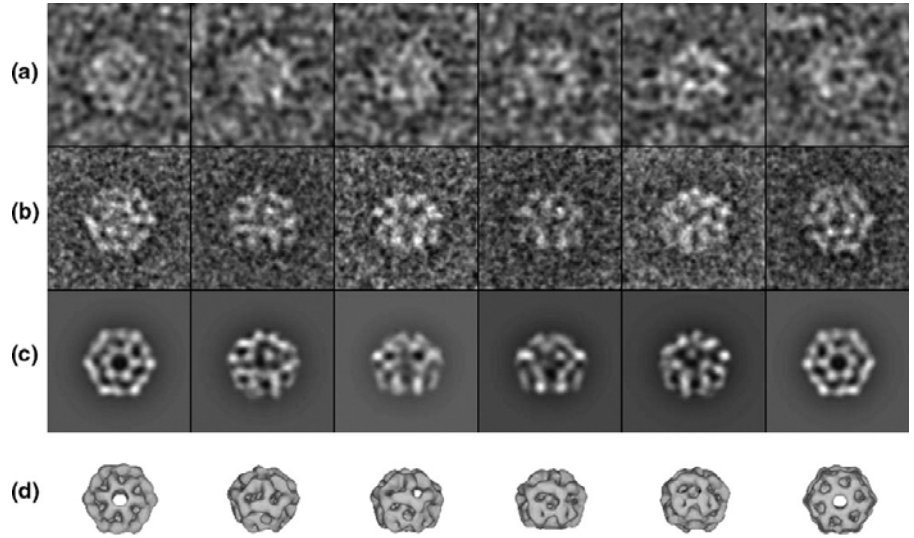
In $P2_1$ a full dodecamer constitutes the asymmetric unit; in $C222_1$ only one hexamer is contained in the asymmetric unit.

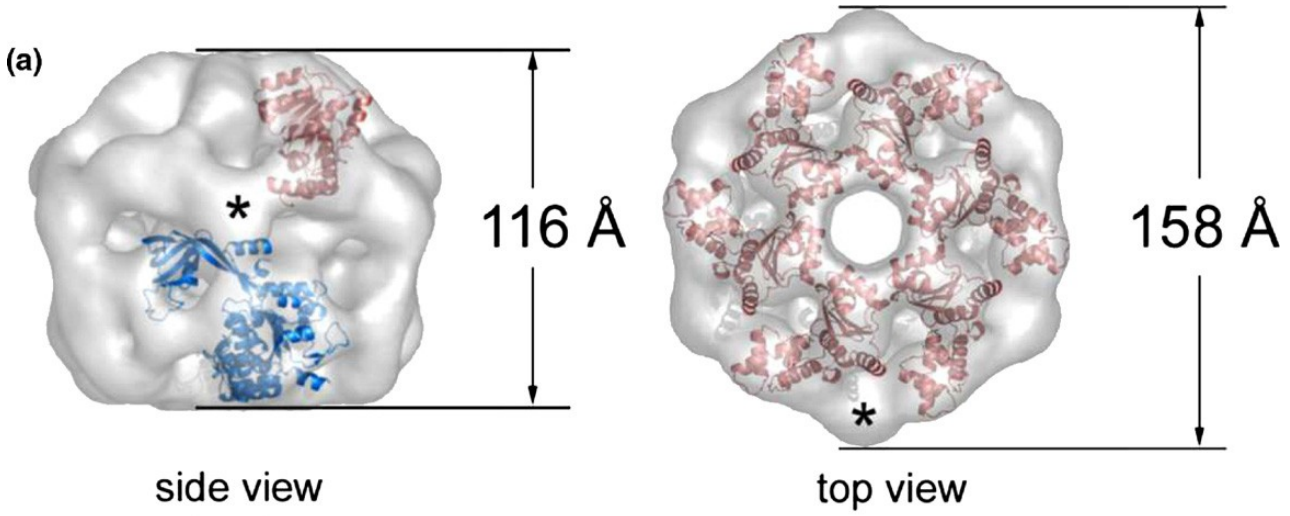
The high similarity between the 3D structures of the RuvBL1ΔDII and RuvBL2ΔDII combined with the low data resolution, made rather difficult the distinction between RuvBL1 and RuvBL2 monomers, as well as between space groups $C222_1$ and $P2_1$.

Point-group symmetry of the dodecamer	6	32	32
Top			
Side			
Bottom			
Space-group symmetry of the crystal structure	$P2_1$	$P2_1$ or $C222_1$	$P2_1$

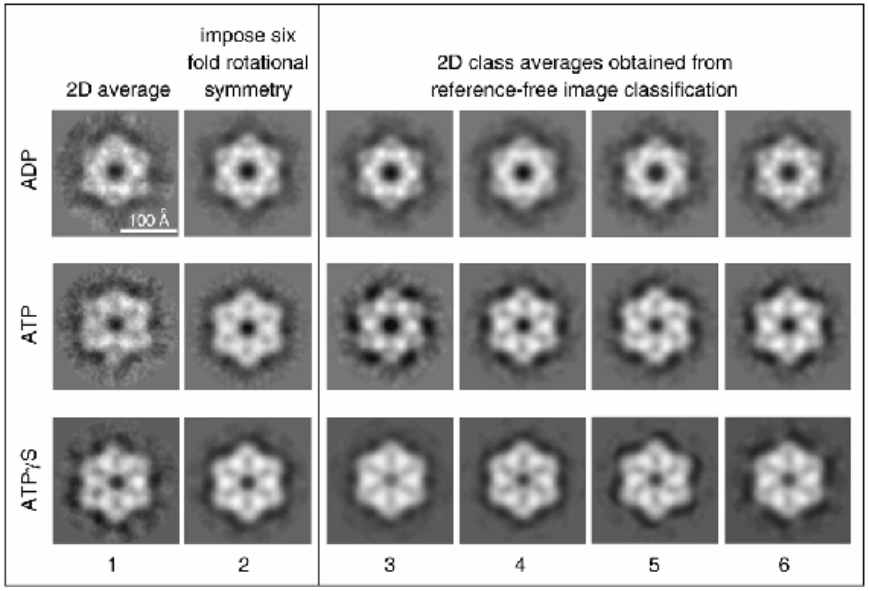
Previous structural work – electron microscopy of human RuvBL1/RuvBL2 complex

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

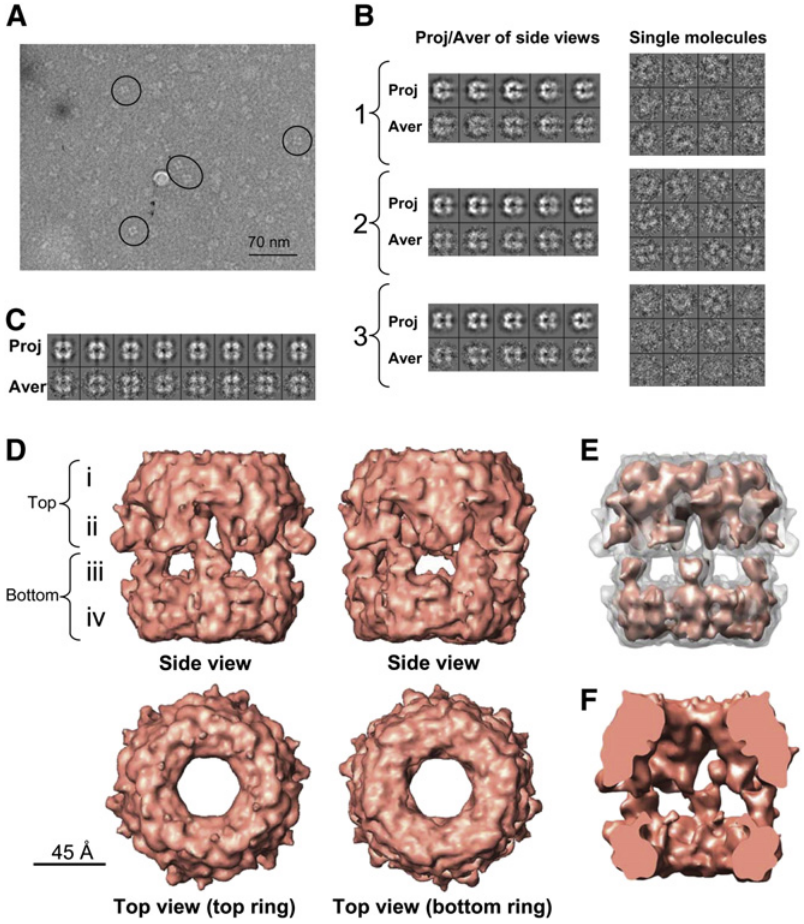




Previous structural work – electron microscopy of Yeast Rvb1/Rvb2 complex

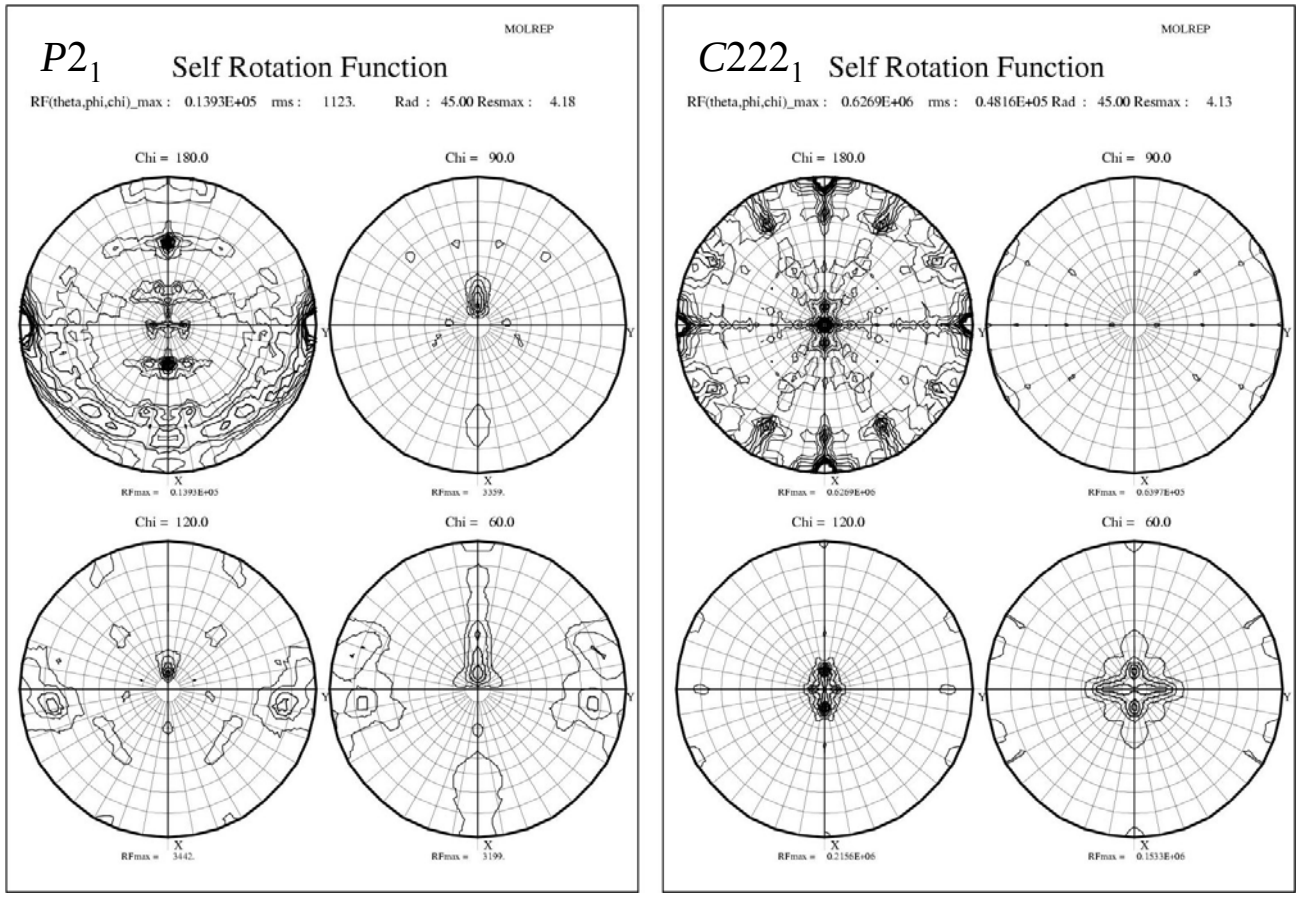


Gribun *et al.* (2008) – **heterohexamers**, probably made up of alternating RuvBL1 and RuvBL2 monomers.



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

Human RuvBL1/RuvBL2 complex – homo- or heterohexamers ?

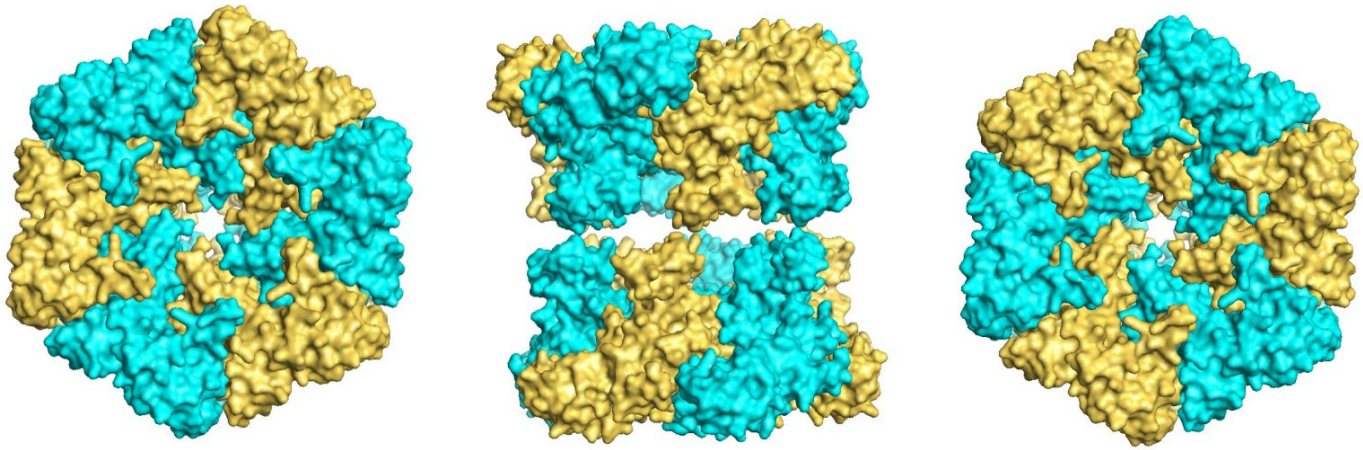


Self-rotation calculations with CCP4 MOLREP support the double heterohexamer in $P2_1$ or $C222_1$: the peaks in the κ=120º section are stronger than those in the κ=60º section.

Human RuvBL1/RuvBL2 complex – homo- or 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 RuvBL2ΔDII chains could be built.

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

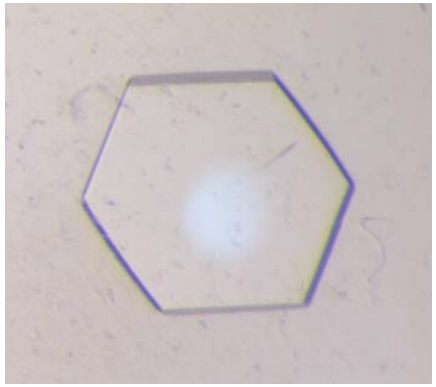


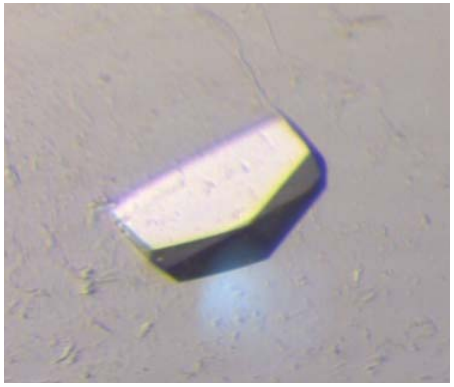
Human Se-Met RuvBL1/RuvBL2 complex

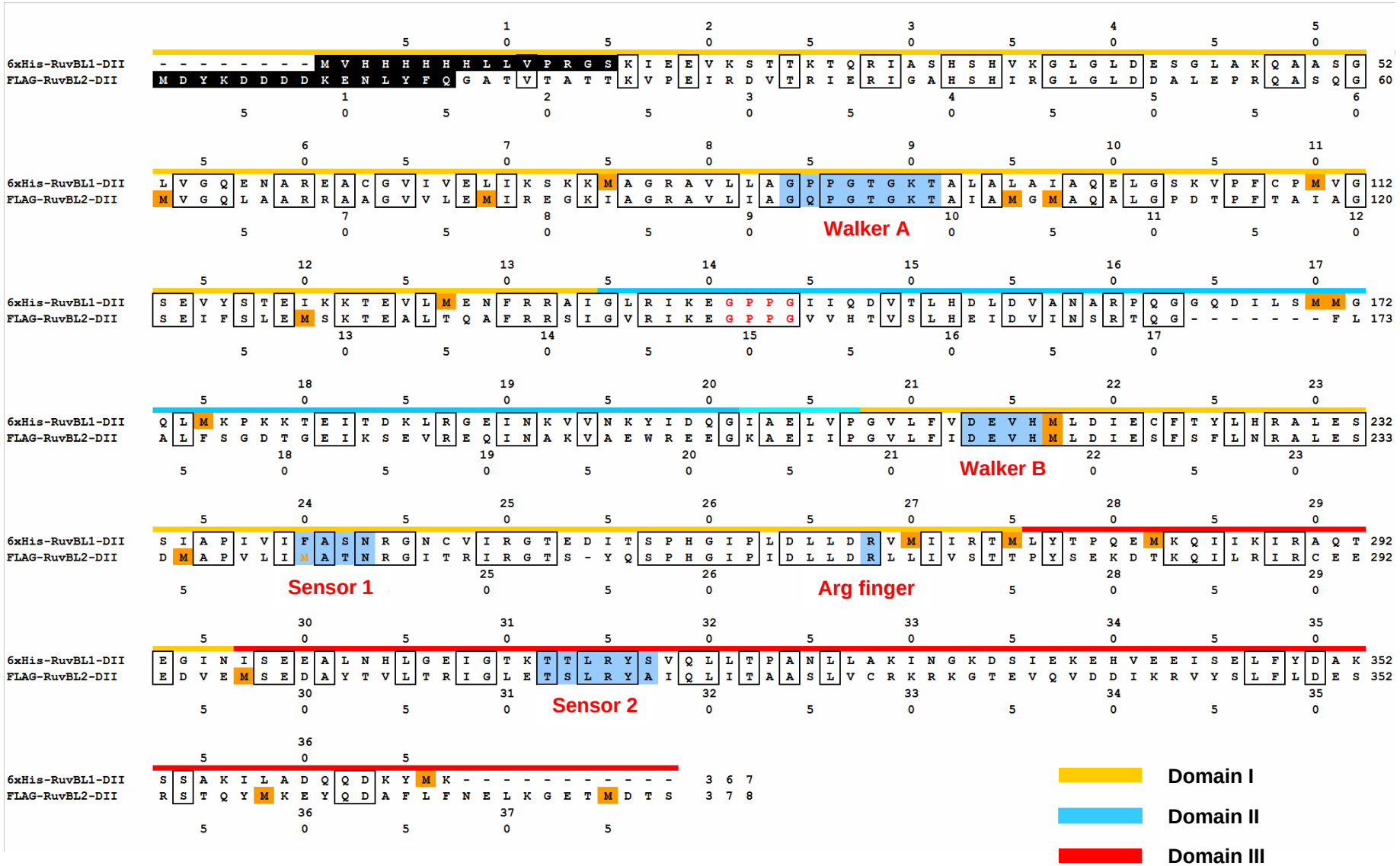
RuvBL1ΔDII and RuvBL2ΔDII each contain 11 methionine residues, and with one exception they occupy different locations in the sequence.

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 RuvBL1ΔDII/RuvBL2ΔDII 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.

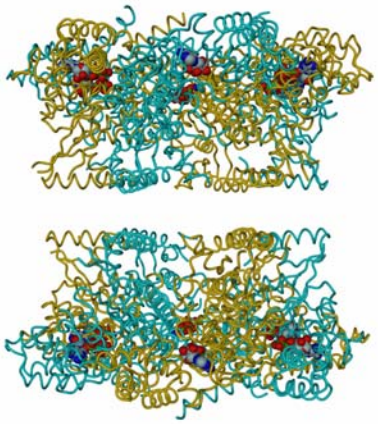


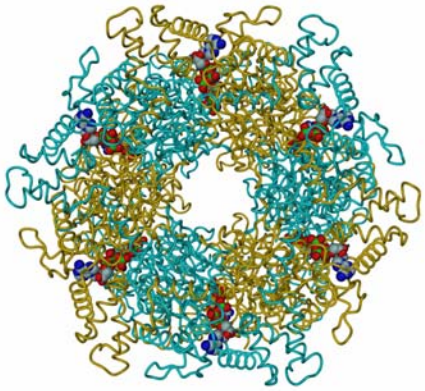



6xHis-RuvBL1-DII
FLAG-RuvBL2-DII
Walker A
Walker B
Sensor 1
Arg finger
Sensor 2
Domain I
Domain II
Domain III

Human Se-Met RuvBL1/RuvBL2 complex – structure determination

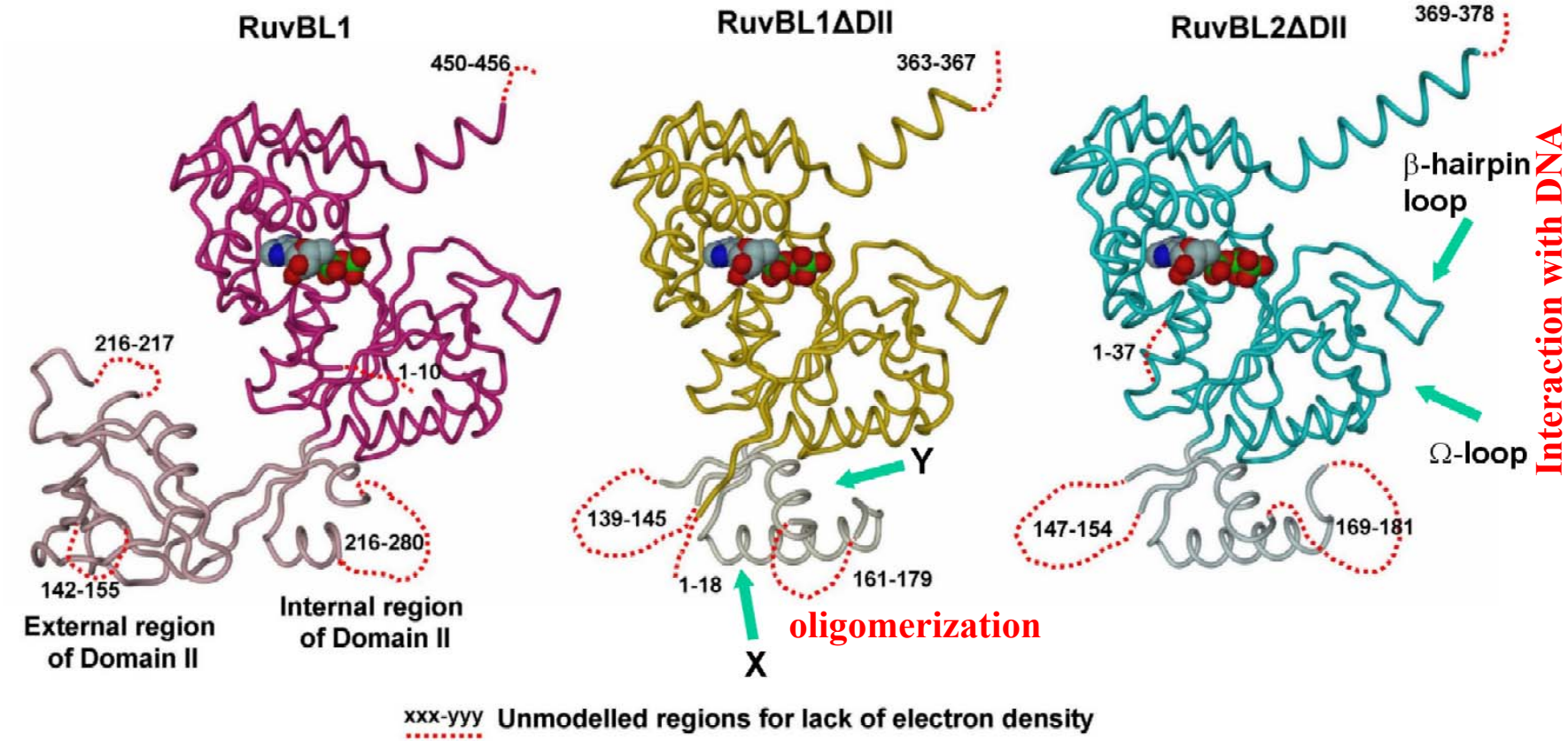
The structure was determined from a 3-wavelength MAD data set collected to a maximum resolution of 3 Å. Space group was unambiguously $C222_1$.
The phase problem was solved using autoSHARP by combining the information from this MAD dataset with a molecular replacement solution.
About 1800 residues of the expected 2235 could be built automatically with Buccaneer/REFMAC. The RuvBL1ΔDII and RuvBL2ΔDII 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.





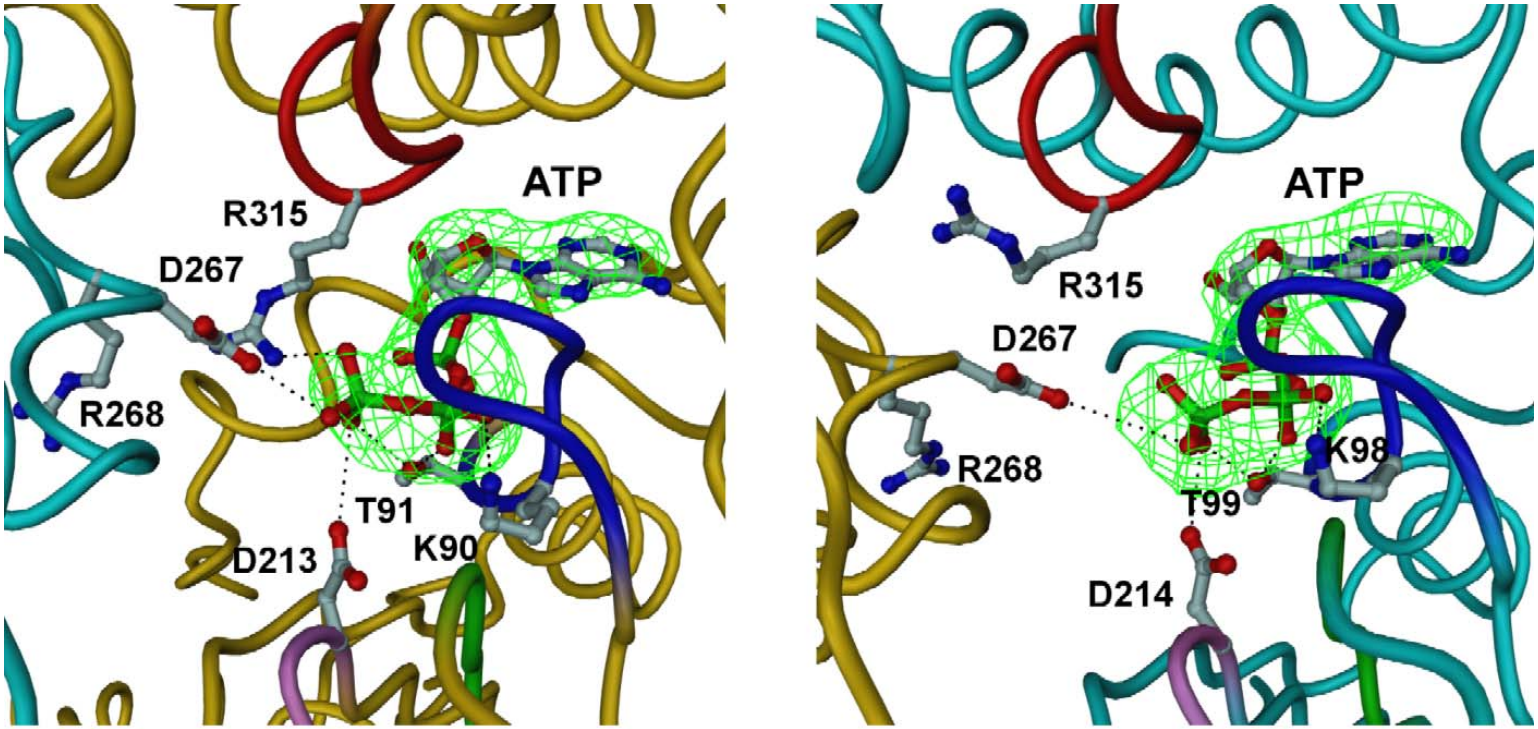
The new results confirmed those previously obtained at 4 Å – The complex crystallizes as a dodecamer with alternating RuvBL1ΔDII and RuvBL2ΔDII monomers. One heterohexamer is present in the asymmetric unit of space group $C222_1$, the second being generated by a crystallographic 2-fold rotation axis.

Human Se-Met RuvBL1/RuvBL2 complex – the monomer structures



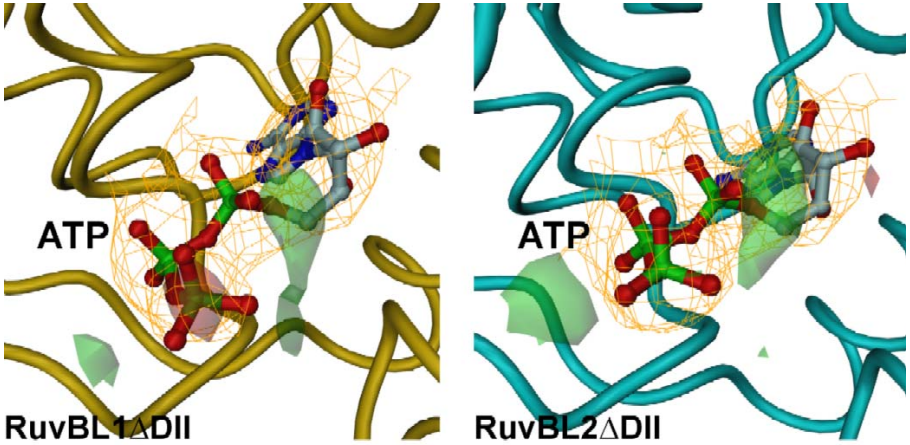
Human Se-Met RuvBL1/RuvBL2 complex – nucleotide binding pocket

No ATP was added at any stage during purification or crystallization.
However, the nucleotide-binding pockets of every RuvBL1ΔDII and RuvBL2ΔDII monomer in the complex clearly show electron density that can be interpreted as a mixture of ADP and ATP.



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

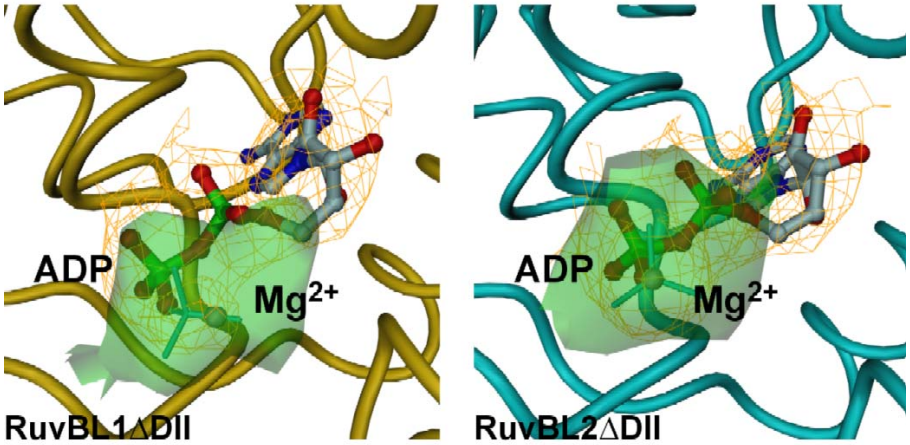
Human Se-Met RuvBL1/RuvBL2 complex – nucleotide binding pocket



Final electron density maps in the refined complex structure, assuming ATP is present in both RuvBL1ΔDII and RuvBL2ΔDII.

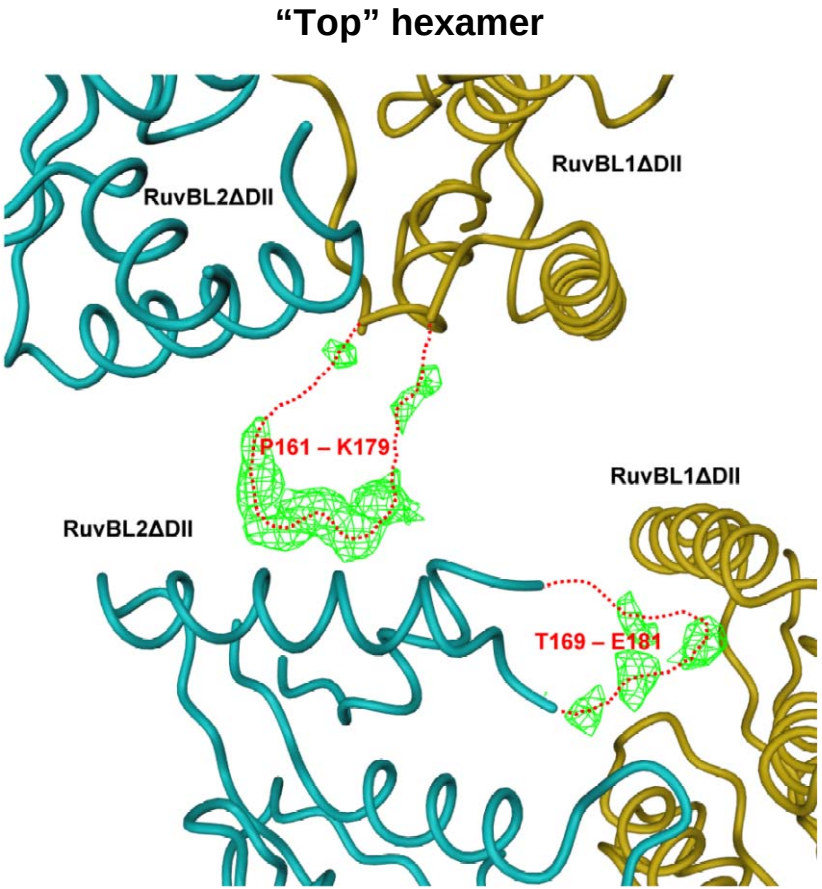
2|Fo|-|Fc|: 1.5 σ

|Fo|-|Fc|: -4.5 σ and 4.5 σ



Test refinements suggest that the ATP in RuvBL1ΔDII is partially hydrolyzed to ADP, whereas very little if any ATP is hydrolyzed in RuvBL2ΔDII.

Human Se-Met RuvBL1/RuvBL2 complex – dodecamerization



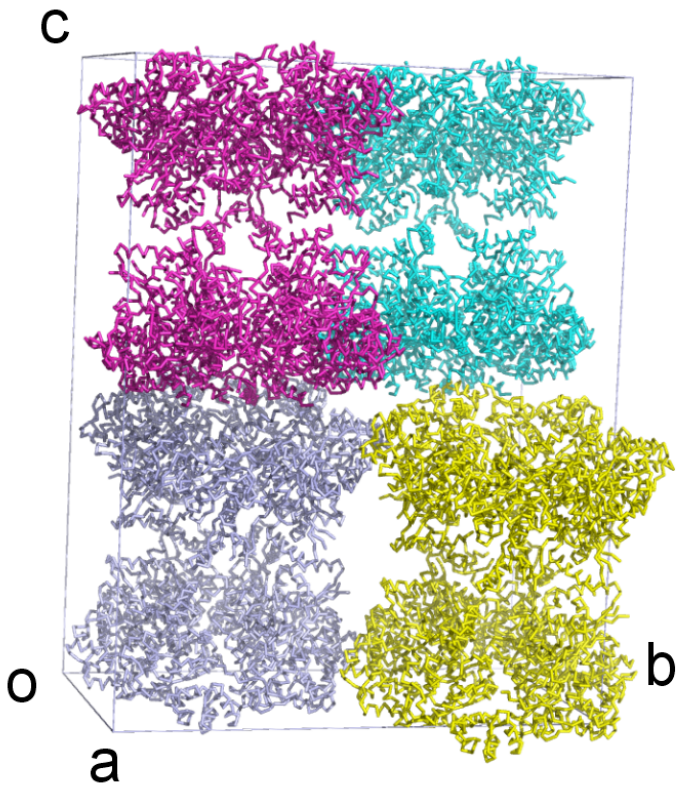
The interactions between hexamers in the dodecamer are **ill-defined** – poor electron density – probably resulting from mixed conformations

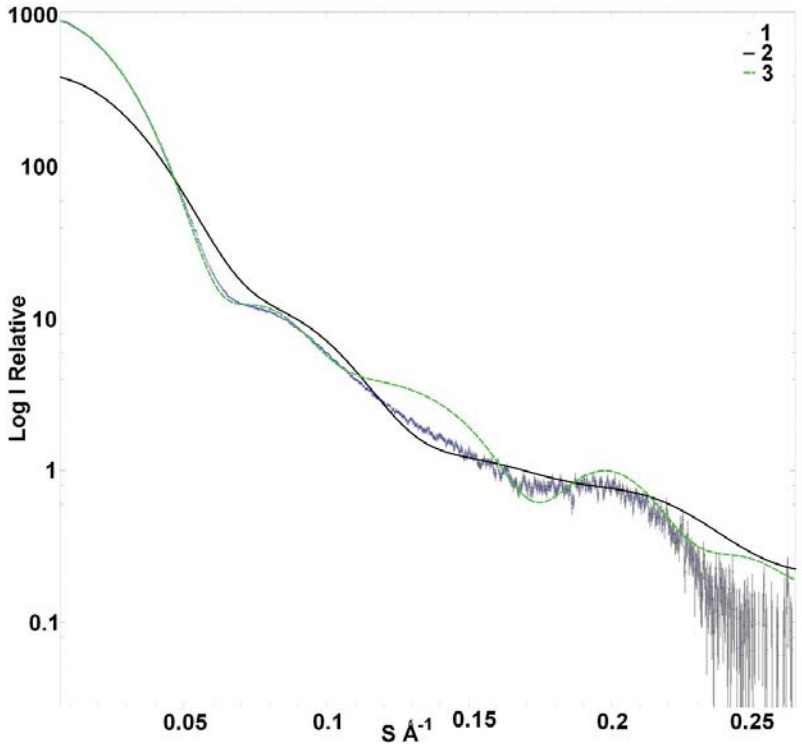
Is the complex really a dodecamer ?

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

Human Se-Met RuvBL1/RuvBL2 complex – dodecamerization

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





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

Human Se-Met RuvBL1/RuvBL2 complex – dodecamerization

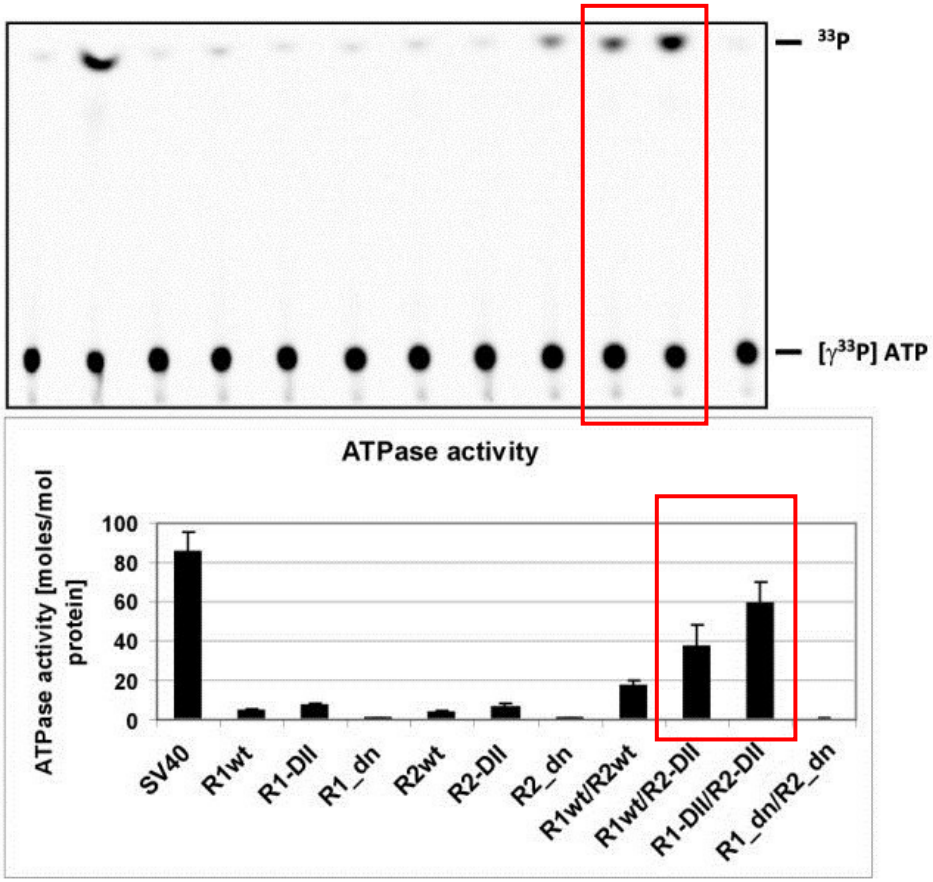
Dodecamer formation is favoured by Domain II truncation

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

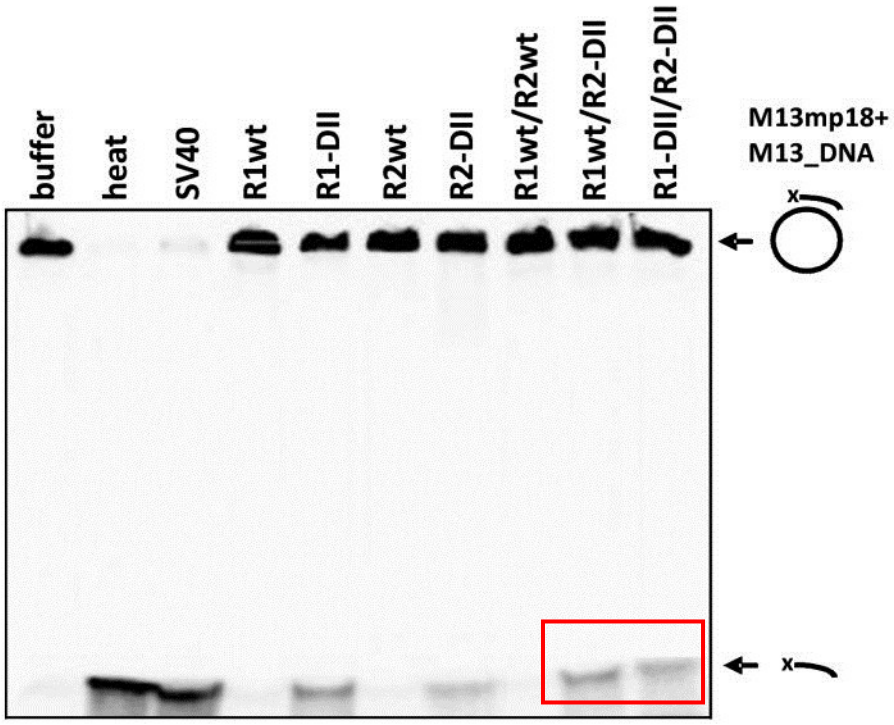
The accuracy of the volume fractions calculated with OLIGOMER (Konarev et al., 2003) is about 2 % for all constructs.

Human RuvBL1/RuvBL2 complex – biochemical assays



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

Human RuvBL1/RuvBL2 complex – biochemical assays



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

Human RuvBL1/RuvBL2 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.

In fact, cell cofactors bind to RuvBL1 and RuvBL2 within chromatin remodeling complexes, probably altering the conformation of domain II and allowing them to exert their helicase activity.

Human RuvBL1/RuvBL2 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.

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.

MAJOR hurdle to be overcome
The diffraction quality of the crystals: over 150 crystals of the native complex were screened and only one crystal diffracted to about 3.5 Å.

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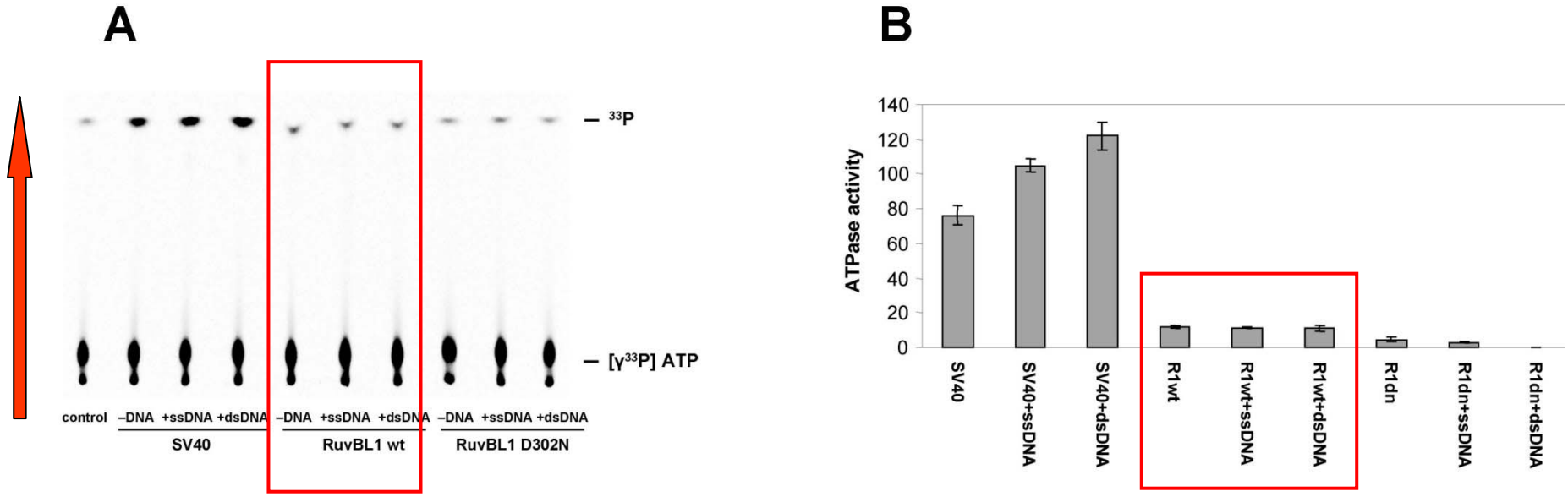
Diamond Light Source, Didcot, UK (XRC).

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

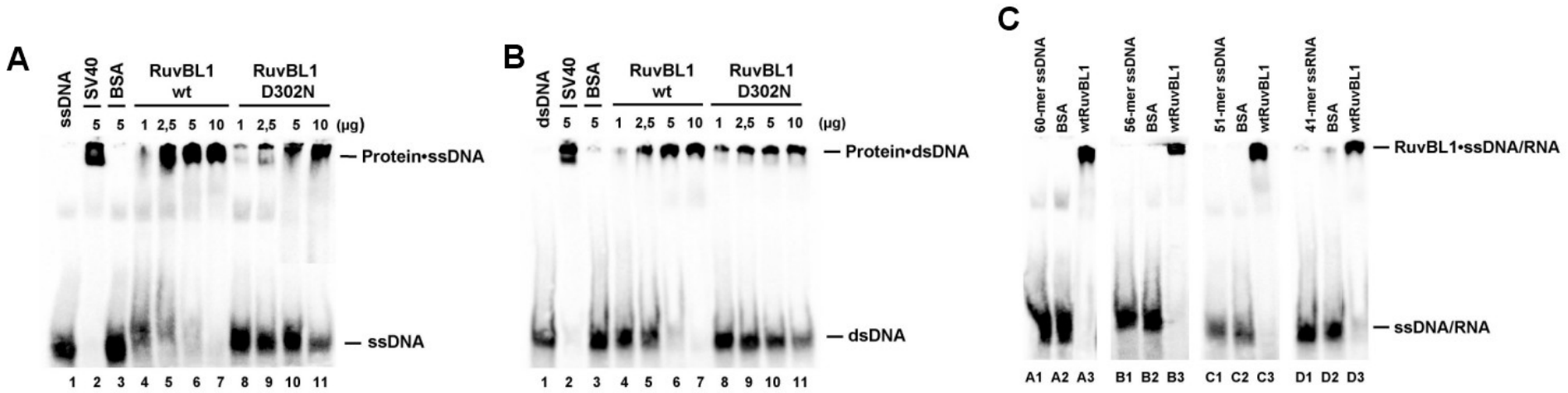
Human RuvBL1 – Biochemical Assays: ATPase



A - Free phosphate ^{33}P 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. Activity is expressed as moles of ATP hydrolyzed per mole of protein.

RuvBL1 has low ATPase activity.

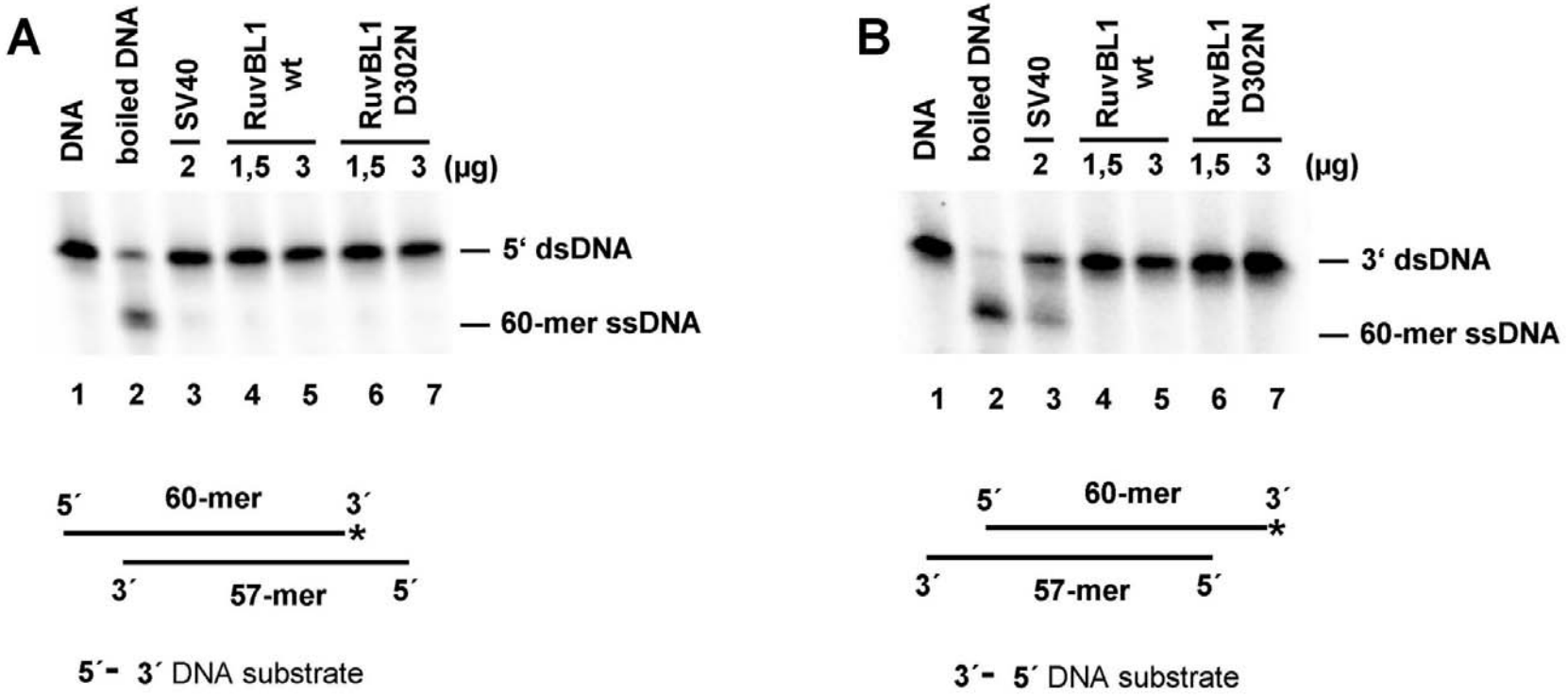
Human RuvBL1 – Biochemical Assays: Nucleic Acid binding



A - ssDNA and **B -** dsDNA binding of human RuvBL1 proteinby 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.

RuvBL1 can bind ssRNA/DNA as well as dsDNA.

Human RuvBL1 – Biochemical Assays: Helicase activity

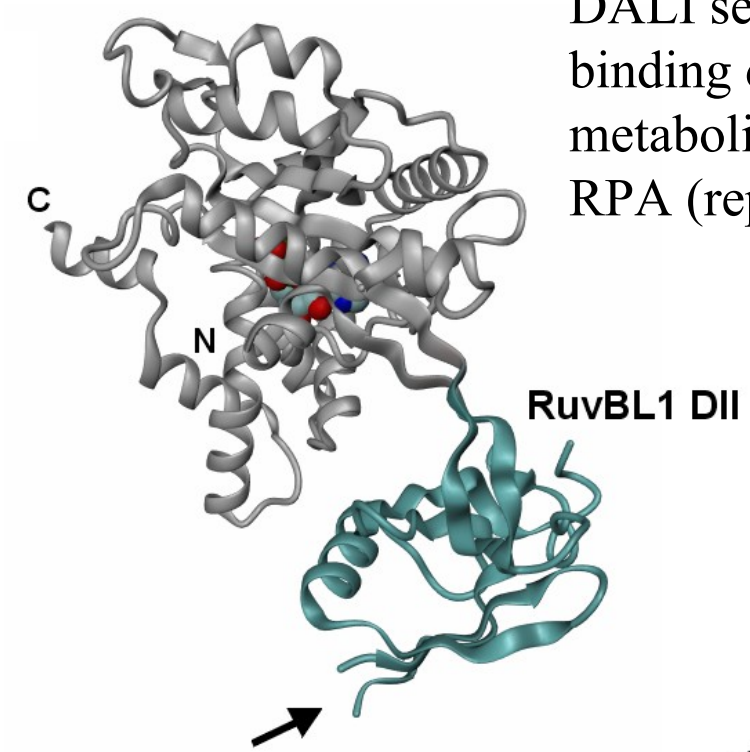


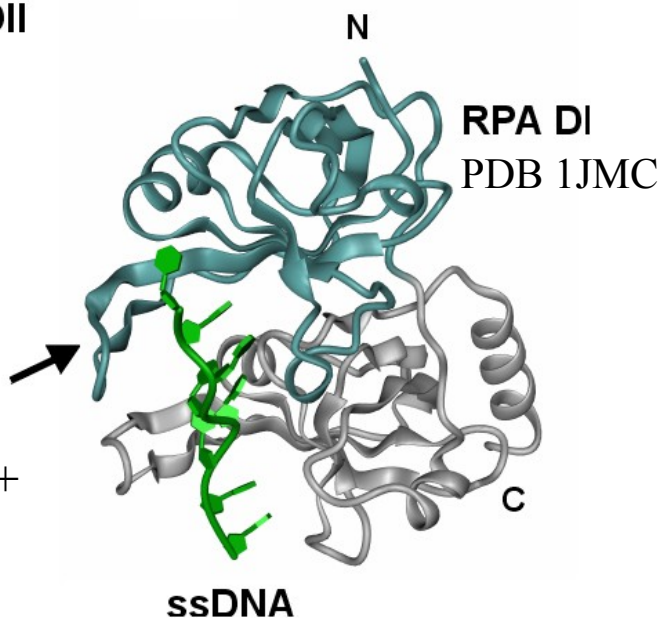
Helicase activity assay of human RuvBL1 using a 5' to 3' DNA substrate (**A**) and a 3' to 5' substrate (**B**). An asterisk denotes the ^{33}P label.

Purified RuvBL1 has no measurable DNA helicase activity.

Human RuvBL1 – the monomer 3D structure (V)

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

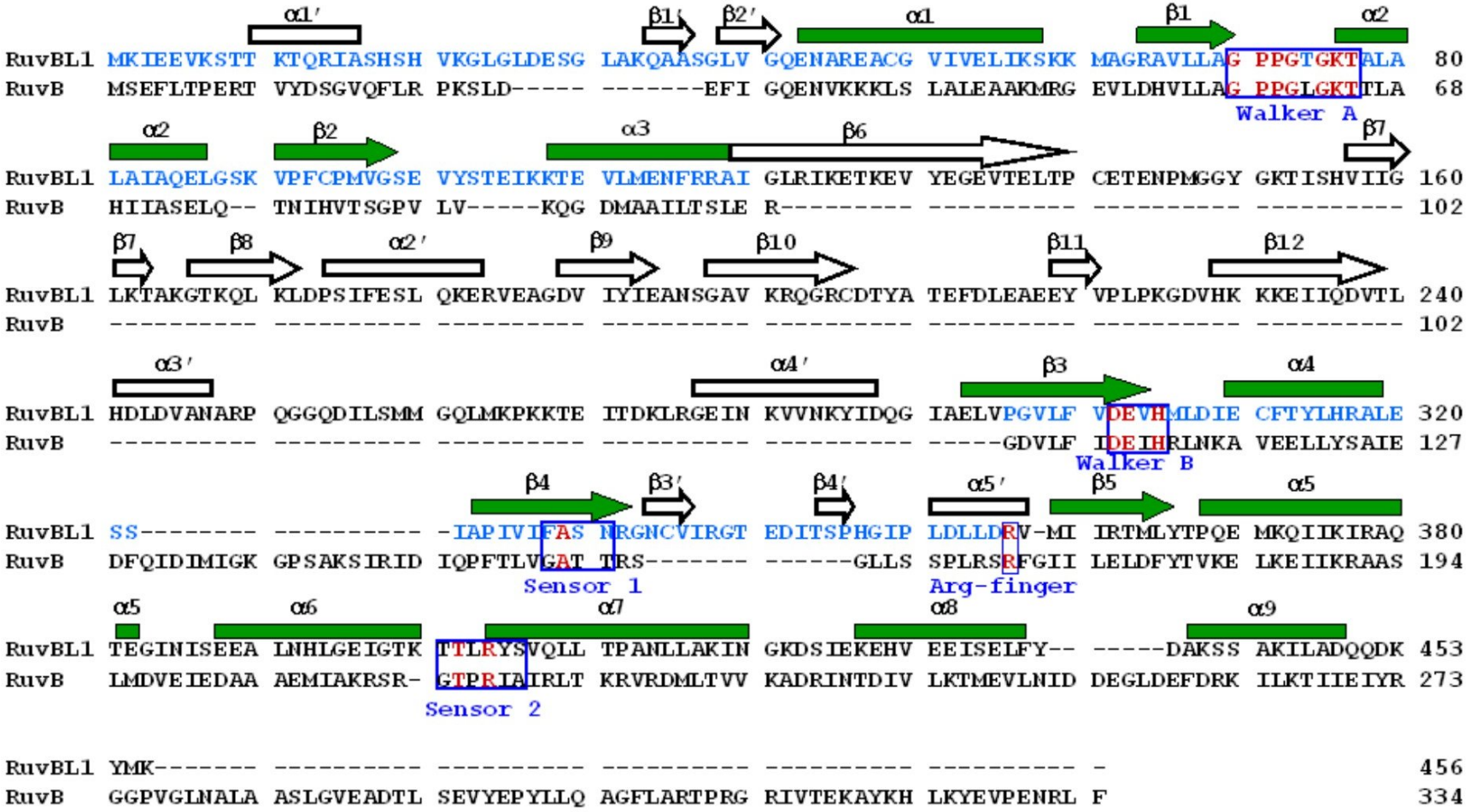




Domain II may represent a new functional domain of eukaryotic AAA+ motor proteins important for DNA/RNA binding

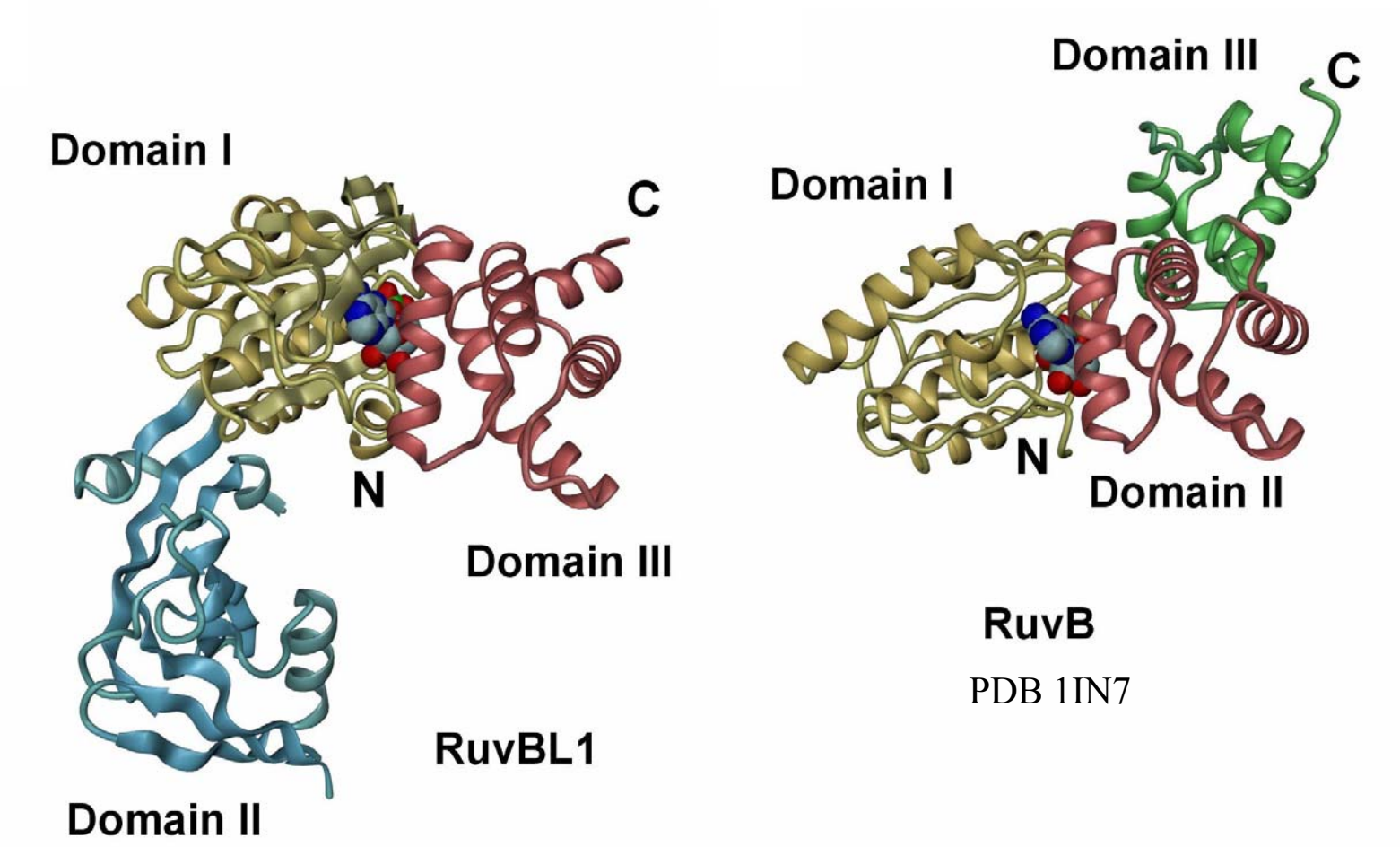
Human RuvBL1 – the monomer 3D structure (VI)

Closest structural homologue: *Thermotoga maritima* RuvB



Human RuvBL1 – the monomer 3D structure (VII)

Closest structural homologue: *Thermotoga maritima* RuvB



AAA+ proteins are ATP-driven molecular machines

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

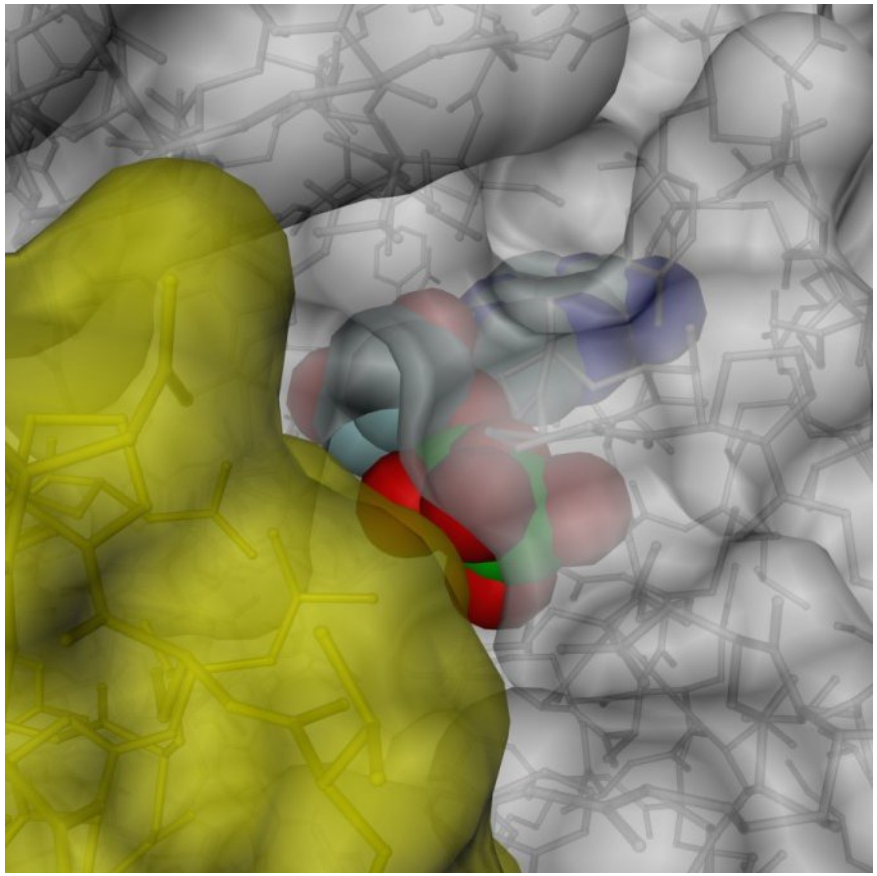
Some recent structures:

- **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

RuvBL1 is the **eukaryotic homolog** of the bacterial DNA-dependent ATPase and helicase RuvB.
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.

The ability to hydrolyze ATP is essential for the biological function of RuvBL1. However, purified exogenously expressed RuvBL1 has only low ATPase activity. Why?



1. Nucleotide-binding pocket is blocked by hexamer formation:
blocking greatly hinders ADP ↔ ATP exchange.

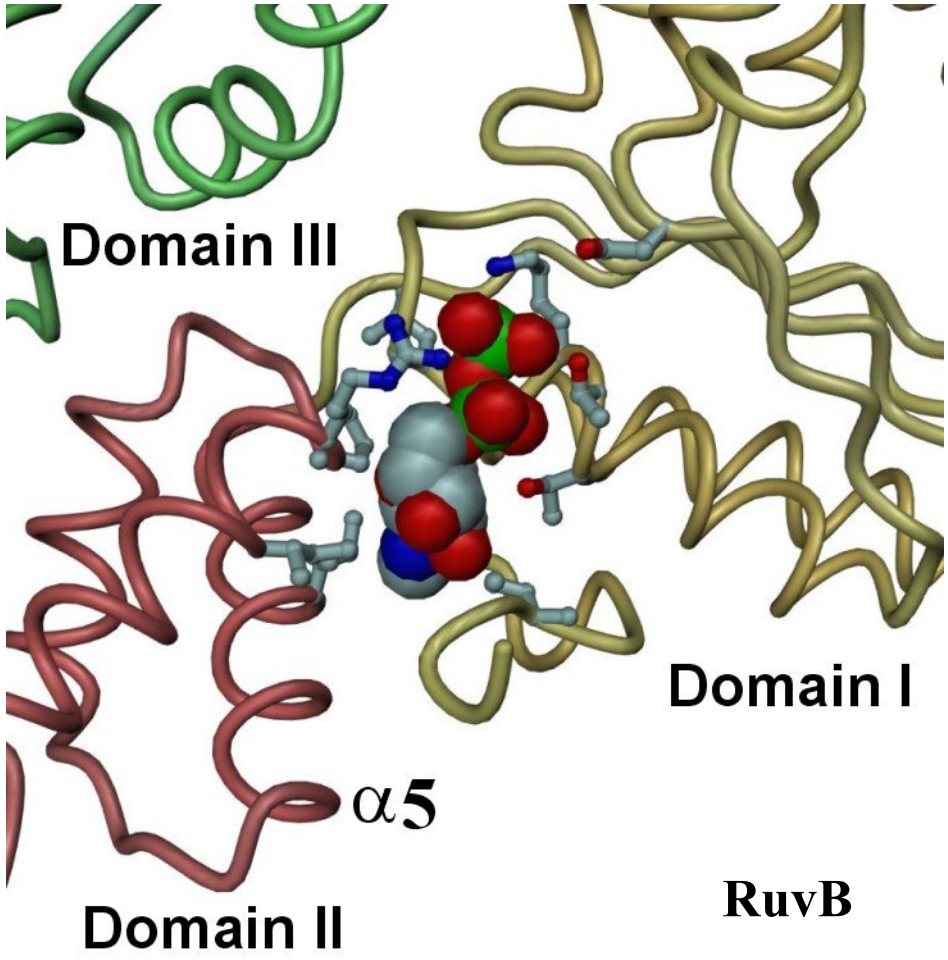
Human RuvBL1 – Characterisation of the nucleotide-binding pocket

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
RuvBL1	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

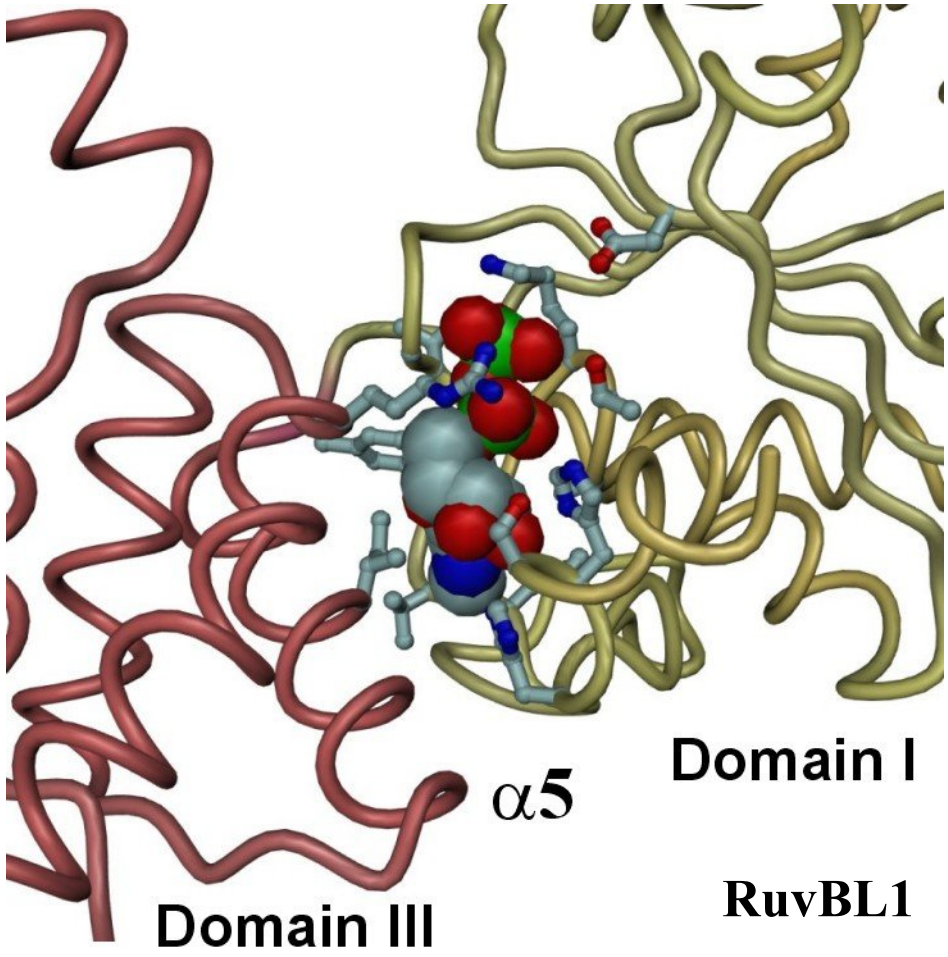
The nucleotide binding pocket is located either at the interface between two domains within a monomer (Dm/Dn interface) or at the interface between two adjacent monomers in the hexamer (M/M interface).

2. The NBP of RuvBL1 has the lowest 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 RuvBL1 vs. *T.maritima* RuvB – ADP tight binding



Human RuvBL1 vs. *T.maritima* RuvB – ADP tight binding



Human RuvBL1 – Conclusions

– The crystal structure of the **RuvBL1/ADP hexamer** reveals that human RuvBL1 consists of **three domains**, of which the first and the third are involved in ATP binding and hydrolysis.

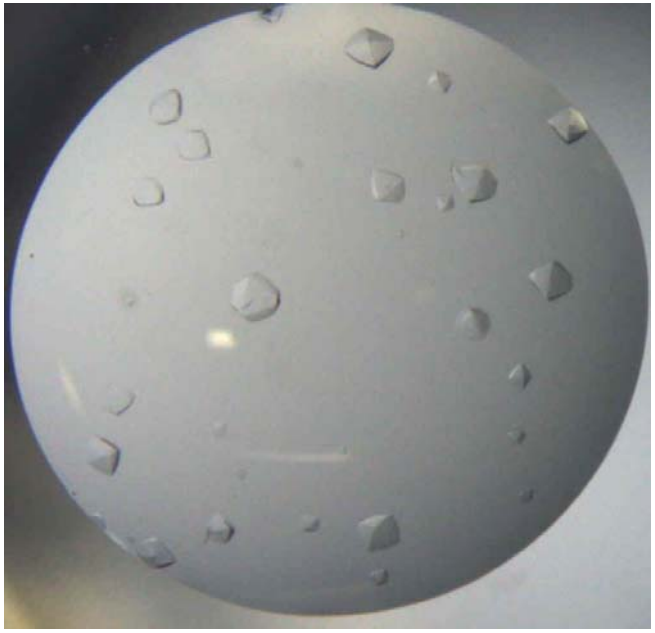
– Structural homology suggests that the second domain, which is **unique in AAA+ proteins** and not present in RuvB, is a **novel DNA/RNA binding domain**.

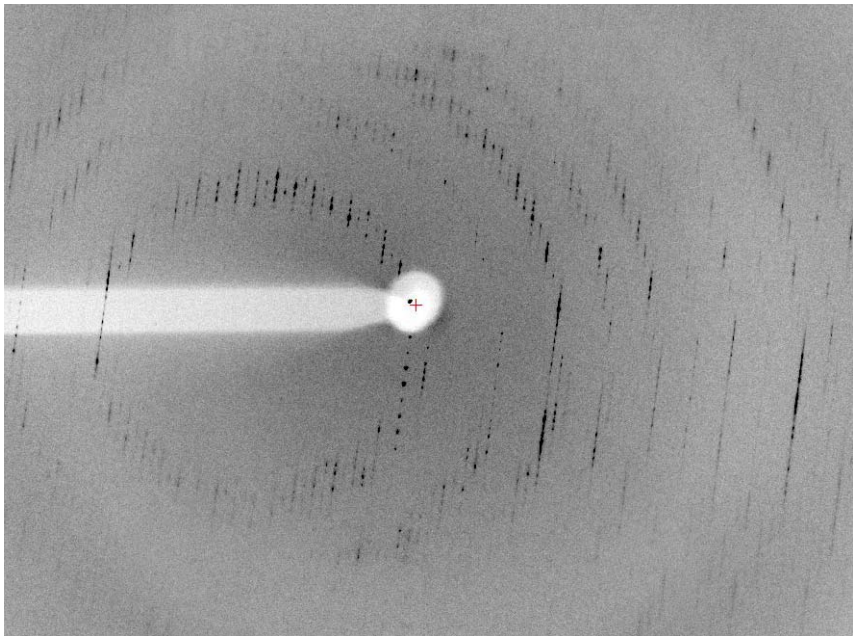
– The biochemical assays show that the RuvBL1 hexamer has a **marginal ATPase activity**, **binds nucleic acids** (ssRNA/DNA and dsDNA) and has **no significant DNA helicase activity.**

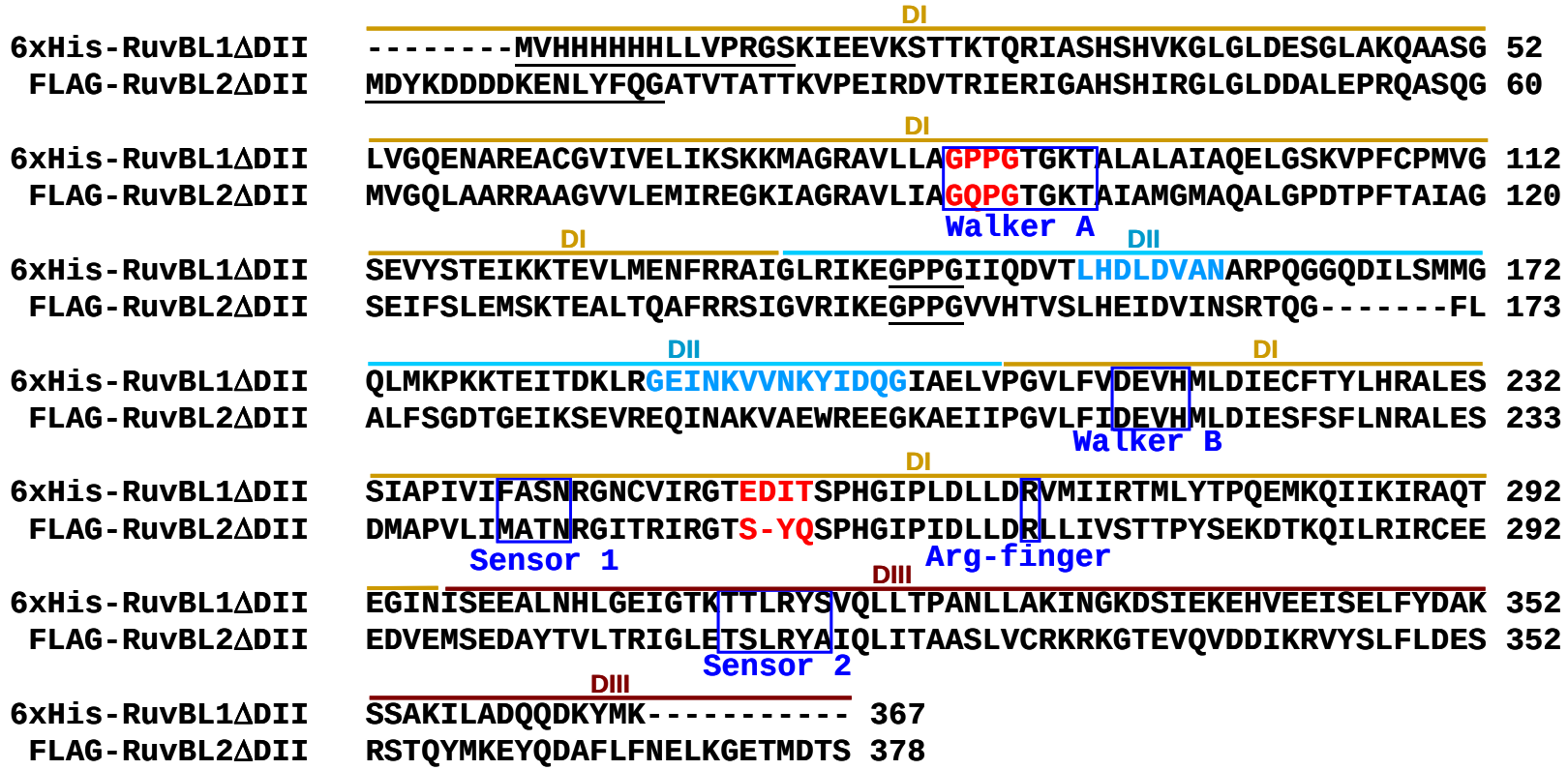
– The hexameric structure of the RuvBL1/ADP complex, combined with our biochemical results, suggest that, while RuvBL1 has all the structural characteristics of an **AAA+ molecular motor**, even of an ATP-driven helicase, **one or more as yet undetermined co-factors are essential to its activation**.

Human RuvBL2 – A Parenthesis

– Human RuvBL2 has been produced and purified as for RuvBL1
– Crystals of poor quality were obtained
– Measured diffraction data showed crystals to be multiple
– No 3D structure of human RuvBL2 is known to date






DI
6xHis-RuvBL1ΔDII --------MVHHHHHHLLVPRGSKIEEVKSTTKTQRIASHSHVKGLGLDESGLAKQAASG 52
FLAG-RuvBL2ΔDII MDYKDDDDKENLYFQGATVTATTKVPEIRDVTRIERIGAHSHIRGLGLDDALEPRQASQG 60
DI
6xHis-RuvBL1ΔDII LVGQENAREACGVIVELIKSKKMAGRAVLLAGPPGTGKTALALAIAQELGSKVPFCPMVG 112
FLAG-RuvBL2ΔDII MVGQLAARRAAGVVLEMIREGKIAGRAVLIAGQPGTGKTAIAMGMAQALGPDTPFTAIAG 120
Walker A
DI DII
6xHis-RuvBL1ΔDII SEVYSTEIKKTEVLMENFRRAIGLRIKEGPPGIIQDVTLHDLDVANARPQGGQDILSMMG 172
FLAG-RuvBL2ΔDII SEIFSLEMSKTEALTQAFRRSIGVRIKEGPPGVVHTVSLHEIDVINSRTQG------FL 173
DII DI
6xHis-RuvBL1ΔDII QLMKPKKTEITDKLRGEINKVVNKYIDQGIAELVPGVLFVDEVHMLDIECFTYLHRALES 232
FLAG-RuvBL2ΔDII ALFSGDTGEIKSEVREQINAKVAEWREEGKAEIIPGVLFIDEVHMLDIESFSFLNRALES 233
Walker B
DI
6xHis-RuvBL1ΔDII SIAPIVIFASNRGNCVIRGTEDITSPHGIPLDLLDRVMIIRTMLYTPQEMKQIIKIRAQT 292
FLAG-RuvBL2ΔDII DMAPVLIMATNRGITRIRGTS-YQSPHGIPIDLLDRLLIVSTTPYSEKDTKQILRIRCEE 292
Sensor 1
Arg-finger
DIII
6xHis-RuvBL1ΔDII EGINISEEALNHLGEIGTKTTLRYSVQLLTPANLLAKINGKDSIEKEHVEEISELFYDAK 352
FLAG-RuvBL2ΔDII EDVEMSEDAYTVLTRIGLETSLRYAIQLITAASLVCRKRKGTEVQVDDIKRVYSLFLDES 352
Sensor 2
DIII
6xHis-RuvBL1ΔDII SSAKILADQQDKYMK---------- 367
FLAG-RuvBL2ΔDII RSTQYMKEYQDAFLFNELKGETMDTS 378