The Rossmann fold

Relationship between sequence, structure and function.

Anna Casas, Júlia Gasull and Nerea Vega
1. **Introduction**: Adenine nucleotides
   1. The most commonly used organic cofactors

2. **The Rossman fold**: Nucleotide-binding proteins
   1. The fold: a/b
   2. Topological switch diagram
   3. The Rossmann Hypothesis
   4. Aligning to find a consensus sequence

3. **The NAD-binding pocket**
   1. UDP-galactose 4-epimerase
   2. Amino acid residues interactions
   3. The role of glycine
   4. The role of water
   5. Stereospecific transference of the H

4. **What about ATP?** The Walker motif
INTRODUCTION

Adenine-nucleotides

All organisms possess small molecular weight co-factors with a crucial role in several metabolic and regulatory pathways.

Co-factor: non-protein chemical compound that is bound to a protein and is required for the protein’s biological activity.

- Organic
  - Coenzymes
  - Prosthetic group

- Inorganic (Mg)

*NAD and FAD serve as cofactors in many essential biologic processes, such as glycolysis (NAD) and the citric acid cycle (FAD and NAD)*

Schulz, G.E., Schirmer, R.H. Principles of protein structure
# Index

1. **Introduction**: Adenine-dinucleotides  
   1. The most commonly used organic cofactors

2. **The Rossman fold**: Nucleotide-binding proteins  
   1. The fold: a/b  
   2. Topological switch diagram  
   3. The Rossmann Hypothesis  
   4. Aligning to find a consensus sequence

3. **The NAD-binding pocket**  
   1. UDP-galactose 4-epimerase  
   2. Amino acid residues interactions  
   3. The role of glycine  
   4. The role of water  
   5. Stereospecific transference of the H

4. **FAD vs. NAD –binding proteins**

5. **What about ATP/GTP?** The Walker motif
The Rossmann Fold

Introduction to Rossmann fold

*Rossmann et. al. (1974)* described two $\beta$-$\alpha$-$\beta$-$\alpha$-$\beta$ units forming a *six-stranded parallel $\beta$-sheet* flanked by *four $\alpha$-helices* in the structure of some dinucleotide-binding proteins.

ONE ROSSMANN FOLD UNIT = $\beta$-$\alpha$-$\beta$-$\alpha$-$\beta$.

Schulz, G.E., Schirmer, R.H. Principles of protein structure
The Rossmann Fold

β-α-β motif

Two adjacent β-strands in the aminoacid sequence are joined by an α-helix at opposite edges to form a parallel β-strand in the structure.

The loop connecting C-end of the β-strand with N-end of the α-helix often have conserved amino acid sequence in homologous proteins and is involved in forming the active site.

Right-handed.

Schulz, G.E., Schirmer, R.H. Principles of protein structure
The Rossmann Fold

Dinucleotide-binding fold

Rossmann fold is a super-secondary structural open sheet domain composed of alternating α-helices and β-strands along the backbone. The β-strands are therefore mostly parallel.

Carl Branden, John Tooze. Introduction to protein structure
The Rossmann Fold

NAD-binding Rossmann fold

Fig. 4 Rossmann fold (1EK5)

Carl Branden, John Tooze. Introduction to protein structure
The Rossmann Fold

SCOP classification

**Fold: NAD(P)-binding Rossmann-fold domains**

- **core:** 3 layers, a/b/a; parallel beta-sheet of 6 strands, order 321456
- The nucleotide-binding modes of this and the next two folds/superfamilies are similar

**Lineage:**

1. **Root:** scop
2. **Class:** Alpha and beta proteins (a/b) [51349]
   - Mainly parallel beta sheets (beta-alpha-beta units)
3. **Fold:** NAD(P)-binding Rossmann-fold domains [51734]
   - core: 3 layers, a/b/a; parallel beta-sheet of 6 strands, order 321456
   - The nucleotide-binding modes of this and the next two folds/superfamilies are similar

**Superfamilies:**

1. NAD(P)-binding Rossmann-fold domains [51735] (12)
   - Superfamily
     1. Alcohol dehydrogenase-like, C-terminal domain [51736] (28) 
     - N-terminal all-beta domain defines family
2. Tyrosine-dependent oxidoreductases [51751] (108) 
   - also known as short-chain dehydrogenases and SDR family
   - parallel beta sheet is extended by 7's strand, order 3214567, left-handedmony connection between strands 6 and 7
The Rossmann Fold

Rossmann fold

Although dinucleotide-binding domains show very low overall sequence homology, large portions of their proteins backbones superimpose very well due to Rossmann fold.
The Rossmann Fold

**Structure is more conserved than sequence**

*The degree of conservation of the three-dimensional structure is much higher than the degree of conservation of the amino acid sequence.*
The Rossmann Fold

Structure is more conserved than sequence

The degree of conservation of the three-dimensional structure is much higher than the degree of conservation of the amino acid sequence.
The Rossmann Fold

The Rossmann hypothesis

A large number of proteins had incorporate the Rossmann fold as a consequence of a **gene fusion** process. These proteins have acquired a new function.
UDP-galactose-4-epimerase (GALE)

A nucleotide-binding protein

**Nterminal** (Met1-Thr189) $\rightarrow$ NAD binding

- 7-stranded parallel $\beta$-sheet
- 5-$\alpha$-helix

**Cterminal** (Gly190-Ala348) $\rightarrow$ UDP sugar binding

Conserved features among these proteins:

- GXGXXG motif
- Side chains interactions with the dinucleotide

Fig. 5 Single Nterminal and Cterminal GALE domains

NAD-binding proteins

Nicotinamide adenine dinucleotide (NAD)

The main role of NAD+ cofactor is the redox reactions.

Reactions of this type are catalyzed by a large group of enzymes called oxidoreductases.

Binding domain for NAD consists of two paired Rossmann fold.

Fig. 2 Nicotinamide adenine dinucleotide

Fig. 6 Single Rossman fold: A β-α-β-α-β unit.
NAD-binding proteins

Aminoacid residue interactions

<table>
<thead>
<tr>
<th>SIDE CHAIN</th>
<th>RESIDUES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polar</td>
<td>Tyr13, Tyr37, Asn157</td>
</tr>
<tr>
<td>Apolar</td>
<td>Ile14</td>
</tr>
<tr>
<td>+ charge (basic)</td>
<td>Lys161</td>
</tr>
<tr>
<td>- charge (acidic)</td>
<td>Asp33, Asp66</td>
</tr>
</tbody>
</table>

These amino acid residues are not conserved, but so do their properties.

Fig.2 Main amino acid interactions in the NAD+ binding pocket.

NAD-binding proteins

The role of water in the binding pocket

Water molecules mediate about 30% of hydrogen bonds.

Concentrated around the pyrophosphate group.

Important parameter for the dinucleotide recognition.


Fig.2 Water molecules interacting with NAD+.
NAD-binding proteins

How does NAD cofactor stabilize itself into the protein?

- GLYCINES → no side chains
- A-helix → overall positive dipole moment

Fig.2 Gly conserved sequence in Rossmann fold.

Fig.2 α-helix peptide dipole are parallel to the helix axis.

Bottoms CA,, et al. 2002.
but... is it enough?

NO
NAD-binding proteins

The role of water is crucial

- Structurally conserved water molecule
- Conserved hydrogen-bonding pattern

Invariant hydrogen bonds
- Last conserved Gly (αA)
- Dinucleotide pyrophosphate

Variant hydrogen bonds
- 2nd/3rd conserved Gly
- C-terminal residue of 4β

Fig. 7 A single water conserved molecule.
1EK5: STRUCTURE OF HUMAN UDP-GALACTOSE 4-EPIMERASE

<table>
<thead>
<tr>
<th>Identifier</th>
<th>Formula</th>
<th>Name</th>
<th>Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAD</td>
<td>(C_{21}H_{27}N_{7}O_{14}P_{2} )</td>
<td>NICOTINAMIDE-ADENINE-DINUCLEO ...</td>
<td>Ligand Explorer</td>
</tr>
</tbody>
</table>

1EK6: STRUCTURE OF HUMAN UDP-GALACTOSE 4-EPIMERASE

<table>
<thead>
<tr>
<th>Identifier</th>
<th>Formula</th>
<th>Name</th>
<th>Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>MG</td>
<td>Mg</td>
<td>MAGNESIUM ION</td>
<td>Ligand Explorer</td>
</tr>
<tr>
<td>NAI</td>
<td>(C_{21}H_{29}N_{7}O_{14}P_{2} )</td>
<td>1,4-DIHYDRONICOTINAMIDE ...</td>
<td>Ligand Explorer</td>
</tr>
<tr>
<td>TMA</td>
<td>(C_{4}H_{12}N )</td>
<td>TETRAMETHYLAMMONIUM ION</td>
<td>Ligand Explorer</td>
</tr>
<tr>
<td>UPG</td>
<td>(C_{15}H_{24}N_{2}O_{17}P_{2} )</td>
<td>URIDINE-5'-DIPHOSPHATE-GLUCOSE</td>
<td>Ligand Explorer</td>
</tr>
</tbody>
</table>
NAD-binding proteins

Stereospecific transference of the H

UDP-galactose-4-epimerase

NAD-binding proteins

Stereospecific transference of the H

\[ \text{NAD}^+ \text{ OXIDIZING AGENT} \]

accepts electrons from other molecules and becomes reduced

\[ \text{NADH REDUCING AGENT} \]
donates electrons to other molecules and becomes oxidized

P-loop

Phosphate-binding motif

The phosphate binding loop (P-loop) is the common motif in mononucleotide binding proteins.

The three-dimensional structure of the P-loop, *preceded by a β-sheet and followed by an α-helix*, is similar in different protein families.

Fig.9 Phosphate-binding loop (P-loop)

Fig.10 Superimposition of different protein families with the P-loop.

C. Ramakrishnan, et al. 2001
P-loop

Walker A motif

The basic structural requirement of a P-loop in most ATP-binding enzymes is its consensus sequence GXXXXGK T/S.

This pattern is mostly present as a structurally and energetically favorable loop that appears to provide more room to surround and manipulate the nucleotide phosphate.

Apha and beta proteins:

**Fold**: P-loop containing nucleoside triphosphate hydrolases

**Superfamily**: P-loop containing nucleoside triphosphate hydrolases

6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase, kinase

Tymidylate kinase

Deoxyctydine kinase

Adenylate kinase

UMP/CMP kinase

Uridine-cytidine kinase 2

Adenosine-5-phosphosulfate kinase

CFTR

---

C. Ramakrishnan, et al. 2001
P-loop

Walker A motif

What is important in the Walker A motif?

Variable quartet (XXXX), however G (12.3 %), A (11.9 %), S (9.8 %), V (8.4 %) and T (5.9 %) occur more commonly than other amino-acids.

A glycine-rich loop.

Lysine (K) and Threonine (T) residue.

---

Fig. 12 T-coffee alignment among different P-loop containing nucleoside triphosphate hydrolases.

C. Ramakrishnan, et al. 2001
P-loop

Walker A motif

The glycine-rich residues in the loop clearly play an important conformational role in maintaining the structure of the loop.

Fig.13 Gycine rich-loop

Fig.14 Glycine-rich loop.

Priva et al, 2011.
P-loop

**Walker A motif**

The *lysine residue* in the consensus sequence GXXXXGKT/S is crucial for the direct interaction with the phosphates of ATP.

The eight residue is usually a hydroxyl-containing residue: a *threonine* or *serine*.

Fig.2 Walker A motif.
P-loop

P-loop and ATP

Fig. 12 Interaction between ADP and an a.

Fig. 13 Interaction between ATP and an adenylate kinase.

C. Ramakrishnan, et al. 2001
The Walker B motif is another integral part of the ATP-binding site.

The aspartate residue is required for ATP hydrolysis and is preceded by four hydrophobic residues: hhhhD
P-loop

Walker B motif

Fig. 16 Walker B motif (1RKB)

C. Ramakrishnan, et al. 2001
Thank you for your attention!
References


Wierenga RK, De Mayer CH, Hol WG