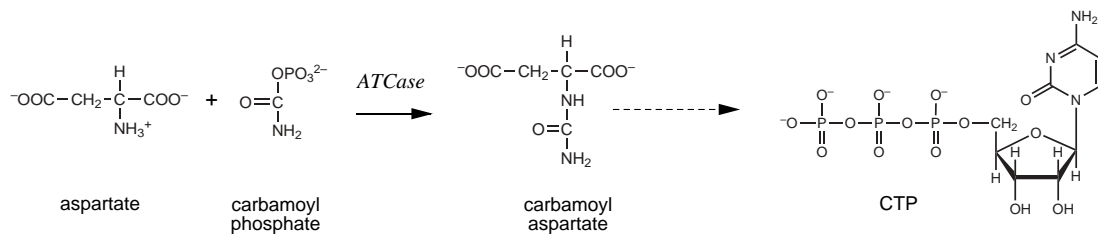


SYSTEMS BIOLOGY: TUTORIAL 2 CONFORMATION CHANGES IN PROTEINS

ATCase

ATCase (Aspartate transcarbamoylase or, more correctly, aspartate carbamoyl transferase) of bacteria is a regulatory enzyme, being the first committed step in the synthesis of the pyrimidine nucleotide triphosphate, CTP:



Like the threonine deaminase enzyme of the isoleucine synthesis pathway mentioned in your lectures, it is subject to allosteric inhibition by the 'end-product' of the pathway, CTP.

As with our simple model of an allosteric enzyme, ATCase is a multi-subunit protein which can exist in either a tense (T) or relaxed (R) state. However, there are two differences. First, ATCase is a hexamer, rather than a dimer. Second, each of the six basic units of ATCase is itself made up of two dissimilar subunits — a catalytic subunit (with a zinc atom, which is involved in the catalysis) and a regulatory subunit. The regulatory subunit contains a binding site for CTP, which is only available (makes a good fit) in the T-state.

ATCase has been crystallized in both T-state (by binding CTP) and R-state (by binding a non-reactive analogue of the two substrates, phosphonacetylaspartate) and the two structures determined. It transpires that the basic unit of symmetry is not the hexamer, but a dimer of the catalytic–regulatory subunit combination.

Objective

The objective is to compare the structures of the R-state and T-state of ATCase using *RasMol* and demonstrate that the overall three-dimensional structure is different in the two states. This will be done by measuring inter-atomic distances.

Instructions

- Download PDB files 5AT1 and 8ATC from the Protein Data Bank.
 - 5AT1 contains the structure co-ordinates of T-state complexed with CTP.
 - 8ATC contains the structure co-ordinates of R-state.
- Start with 8ATC (it will have the extension .pdb) and load it into *RasMol*.

We need to distinguish the four chains.

- Select *Chain* from the Colours menu.

The two catalytic subunits (A and C) are coloured green and blue, respectively, whereas the two regulatory subunits (B and D) are coloured brown and cyan, respectively. (When you click on an atom or residue in a chain, the chain designation is given on the command line.)

The catalytic subunits each contain a substrate analogue (PAL) and the regulatory subunits each contain a zinc atom. To visualize them:

- Type *select ligands* on the command line.
- Select *Spacefill* from the Display menu.

The PAL will be seen as a large molecule, whereas the zinc atoms will appear as single spheres.

It should be evident that catalytic subunit A is associated with regulatory subunit B, and catalytic subunit C with regulatory subunit D. The difference in structure of the R- and T-states is manifest in a difference in the relative positions of the AB and CD pairs. To show this we will make measurements between suitable landmarks on them, starting with the zinc atom.

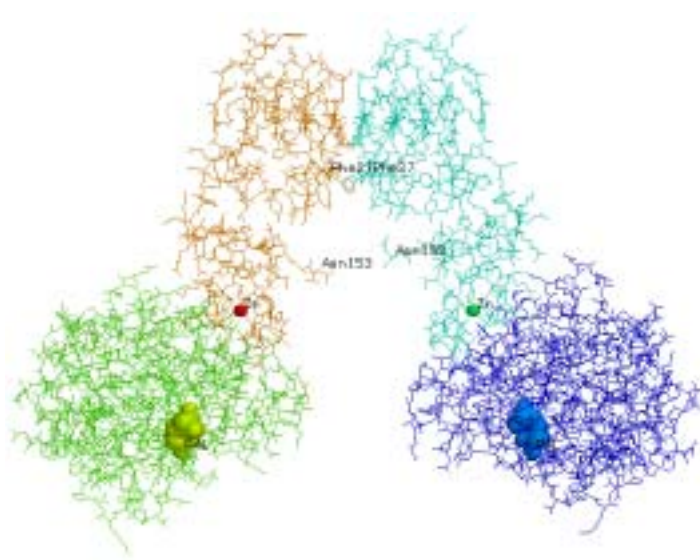
- Type *set picking distance*.
- Zoom in first, if necessary, and click first on one zinc atom and then on the other.

The distance between the atoms appears on the command line as illustrated. Note this down.

```
RasMol> set picking distance
RasMol>
Atom #1: ZN154B.ZN (3993)
RasMol>
Atom #2: ZN154D.ZN (8060)
Distance ZN154B.ZN-ZN154D.ZN: 38.170
```

- Find some other suitable 'landmarks' and repeat the measurement, making sure that you have clicked on the corresponding atoms.

A couple of suggested examples are shown below (with a white background).



- Close 8ATC (perhaps 'export' an image first — .bmp format for Windows).
- Load 5AT1, proceeding as for 8ATC.
- Type *select CTP* on the command line to visualize the CTP:

Be careful making your measurements that you compare exactly the same atoms as in 8ATC,

Ras

It is implicit in the description of the function of G-proteins in signal transduction that the functional subunit, although a monomer, can exist in two distinct conformations. One conformation is active and binds GTP, the other is inactive and binds GDP, the change in conformation accompanying the hydrolysis of GTP.

Because of the great interest in the G-protein, *ras*, this has been crystalized in the two different conformations, one bound to GDP and the other bound to a non-hydrolysable analogue of GTP (GNP — guanosyl-imido-triphosphate).

Objective

The objective is to compare the GTP-bound and GDP-bound *ras* structures. In this case the bound nucleotides make it easy to see the large structural change that occurs in one part of the chain.

Instructions

- Download PDB files 4Q21 and 5P21 from the Protein Data Bank.
 - 4Q21 contains the structure co-ordinates of *ras* bound to GDP.
 - 5P21 contains the structure co-ordinates of *ras* bound to the GTP analogue.

- Start by loading 4Q21.
- Type *background white* on the command line.

It will be easiest to orient the protein if we can see its characteristic helices.

- Select *Ribbons* from Display menu.

Now visualize the GDP and display it.

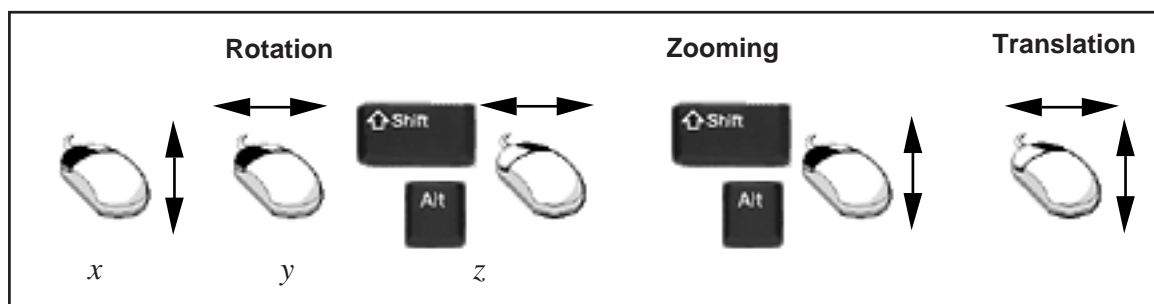
- Type *select GDP* on the command line.
- Select *Ball & Stick* from Display menu.

The part of the protein that shows the clearest difference in the two structures is a region of helix from amino acid residues 67–74 approx.

- Type *select 67-74* on the command line.
- Type *colour red* on the command line.
- Orient the protein to the position shown in the illustration and export an image of it.
- You may also make measurements (e.g. to the guanine base) if you wish.
- Close 4Q21, Load 5P21 and repeat, trying to match as well as possible the position of the helices (other than the one you have coloured) and the nucleotide.



RasMol Reference

**Display**

There are several different menu options for displaying molecules, but it is important to realize that they only apply to those molecules that have been selected. At start-up this is all molecules, but subsequently a subset may be selected.

Display	
Wireframe	Shows bonds between atoms
Backbone	For viewing 'winding' of chain
Sticks	Like Wireframe, but more solid
Spacefill	How it 'really' is
Ball & Stick	Atoms as balls, bonds as sticks
Ribbons	Like Backbone but with 2 ^o structure
Strands	Variant on Ribbons
Cartoons	Variant on Ribbons

Colouring

To colour selected atoms use a command such as:
colour red (colours selected atoms red etc.)

Recognized colours are *red, redorange, orange, yellow, green, cyan, blue, purple* and *white*.

The most useful *general* colour schemes are:

Colours	
Monochrome	
CPK	N = blue, O = red, S = yellow, etc.
Shapely	
Group	N-terminus = blue Æ C-terminus = red
Chain	Chains coloured differently
Temperature	
Structure	Helix = magenta, Sheet = yellow
User	
Model	
Alt	

The background colour can also be changed, e.g.
set background white

Help

help commands (lists commands for which help is available)

help select (gives help for 'select')

Atom labels

Clicking on an atom gives information on the command line, but the atom can also be labelled:

label on (selected atoms are labelled)

label off (selected atoms are unlabelled)

However, to label a residue the trick is to select one atom and then specify the label you want-, e.g.

select lys95.CA (lysine-95 alpha carbon)

label 'Lys 95'

Measuring intermolecular distances

These can be measured by first typing:

set picking distance

and then clicking on the two relevant atoms.

Selecting and Restricting

'Select' selects subsections of a molecule for subsequent operation, leaving the rest of the molecule visible. 'Restrict' is similar but deletes the rest of the molecule from view. Commands are illustrated for 'select', but all also work with 'restrict'.

select all (everything selected)

*select ** (everything selected)

select 0 (nothing selected)

select protein

select ligand

select nucleic

select hydrophobic (hydrophobics selected)

select polar (hydrophilic residues selected)

select Mg (etc.)

select sheet (only b-sheet residues selected)

*select *a* (only chain 'a' selected)

select backbone (selects C, N, Ca backbone)

*select *.ca* (selects α-carbons)

select 2-10, 22 (selects residues 2-10 and 22)

select lys95 (selects lys-95)

select within(4.0, ligand) (only atoms within 4.0 Å of ligand selected)

select !water (etc. *deselects* water)

Remember that you may need to select a display option after using these commands with *restrict*.

You can add to restricted views using *select*.

Centring

Determines the point of rotation.

centre (centres on midpoint of molecule)

centre 26 (centres on residue 26)

Introduction to *RasMol* from Basic Bioinformatics Module

- Open *RasMol* (*raswin.exe* from *Programs* sub-menu in the Windows *Start* menu).
- Type *set background white* in the command window.
- Load the file, *5ICD.pdb*, after selecting *Open* in the *File* menu.

The protein will appear in the wire-frame representation shown (i). This is useful for a biologist to study individual amino acids, but we cannot relate it easily to our linear string.

- Type *restrict protein*
 - Select *Backbone* from the *Display* menu.
- The protein appears as in (ii), with the start visible at the bottom. However the end is difficult to locate.

- Select *Group* from the *Colours* menu.
- You can now trace the chain along the colour gradient from blue to red, rotating the molecule as necessary.

- Select *Spacefill* from the *Display* menu.
- You can now see how the protein is a 'solid' object, rather than full of space (iii). (This view makes it difficult to see 'inside' the protein, though.) Now let us look at isocitrate, the reacting molecule ('substrate') bound to the protein.

- Select *Wireframe* from the *Display* menu.
- Select *CPK* from the *Colours* menu.
- Type *select ligand*.
- Select *Spacefill* from the *Display* menu.

You can now see the isocitrate, apparently inside the protein (iv).

- Type *colour blue*.
- Type *select protein*.
- Select *Spacefill* from the *Display* menu.

By rotating the molecule you should be able to see that the (blue) isocitrate, is sitting in a pocket in the protein (v) where the catalysis occurs.

