Thermostability investigation of Fatty Acid Binding Protein from *Cataglyphis fortis* by fluorescence spectroscopy using genetically introduced tryptophan residues

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Värmestabilitetsmätningar av ett fettsyrabindande protein från *Cataglyphis fortis* med fluorescensspektroskopi med hjälp av genetisk introducerade tryptofaner

**Författare**
Elin Röjdeby

**Sammanfattning**

The desert ant *Cataglyphis fortis* is one of the hyperthermophilic species of *Cataglyphis*. It lives in the Sahara desert and forages during the hottest hours of the day when it can get up to 70°C in the sand. The body temperature of the ant during the foraging runs can reach a maximum of 55°C. Since *C.fortis* is one of few eukaryotic hyperthermophilic species, its proteins probably have a high thermostability. Investigating the thermostability can give valuable information about the principles of protein folding and stability in hyperthermophiles.

Fatty acid binding proteins (FABPs) have an important role in the cell taking up and transporting fatty acids and regulating metabolic and inflammatory pathways. FABPs have been extensively studied and structures from several species have been determined. The determined structures of all FABPs are very similar why thermostability studies of FABP from *C.fortis* are highly relevant.

Fluorescence spectroscopy is an easy and fast method to measure intrinsic protein fluorescence. Tryptophans were genetically introduced into three different positions in FABP to be used as environmental sensitive probes. Complementing the measurement results with a model of the 3D structure of FABP from *C.fortis* gave additional information about the ligand binding.

The (local) thermostability of the mutants can be detected by shift in wavelength maximum during temperature ramping experiments. All mutants are stabilised in the presence of fatty acids. The mutant with tryptophan positioned closest to the supposed ligand binding residues (Y11W) is most affected. The mutant with tryptophan situated farthest from the supposed binding residues (Y52W) shows a stabilisation of $T_m$ less evident than for Y11W. Thus, the structural changes following fatty acid binding are more obvious in the environment close to the binding site.

However, the third mutant C87W shows no significant stabilisation although positioned closer to the fatty acid binding site than Y52. This is probably due to the size difference between the original and introduced amino acid in the mutation. Since the high value of the starting $\lambda_{max}$ for C87W implies that C87W is quite exposed to the aqueous solvent, the residue is likely to not be subsumed in the protein tertiary structure.

Further, the myristic acid stabilise the melting temperature of all the mutants while octanoic acid only has a local effect of Y11W increasing the cooperativity. This implies different binding properties and that myristic acid stabilise the entire protein while octanoic acid only has a local stabilisation effect around the ligand binding site.

**Nyckelord**
Cataglyphis fortis, FABP, fluorescence spectroscopy, tryptophan, mutants
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1 Abstract

The desert ant *Cataglyphis fortis* is one of the hyperthermophilic species of *Cataglyphis*. It lives in the Sahara desert and forages during the hottest hours of the day when it can get up to 70°C in the sand. The body temperature of the ant during the foraging runs can reach a maximum of 55°C. Since *C.fortis* is one of few eukaryotic hyperthermophilic species, its proteins probably have a high thermostability. Investigating the thermostability can give valuable information about the principles of protein folding and stability in hyperthermophiles.

Fatty acid binding proteins (FABPs) have an important role in the cell taking up and transporting fatty acids and regulating metabolic and inflammatory pathways. FABPs have been extensively studied and structures from several species have been determined. The determined structures of all FABPs are very similar why thermostability studies of FABP from *C.fortis* are highly relevant.

Fluorescence spectroscopy is an easy and fast method to measure intrinsic protein fluorescence. Tryptophans were genetically introduced into three different positions in FABP to be used as environmental sensitive probes. Complementing the measurement results with a model of the 3D structure of FABP from *C.fortis* gave additional information about the ligand binding.

The (local) thermostability of the mutants can be detected by shift in wavelength maximum during temperature ramping experiments. All mutants are stabilised in the presence of fatty acids. The mutant with tryptophan positioned closest to the supposed ligand binding residues (Y11W) is most affected. The mutant with tryptophan situated farthest from the supposed binding residues (Y52W) shows a stabilisation of $T_m$ less evident than for Y11W. Thus, the structural changes following fatty acid binding are more obvious in the environment close to the binding site.

However, the third mutant C87W shows no significant stabilisation although positioned closer to the fatty acid binding site than Y52. This is probably due to the size difference between the original and introduced amino acid in the mutation. Since the high value of the starting $\lambda_{max}$ for C87W implies that C87W is quite exposed to the aqueous solvent, the residue is likely to not have subsumed in the protein tertiary structure.

Further, the myristic acid stabilise the melting temperature of all the mutants while octanoic acid only has a local effect of Y11W increasing the cooperativity. This implies different binding properties and that myristic acid stabilise the entire protein while octanoic acid only has a local stabilisation effect around the ligand binding site.
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Abbreviations

FABP Fatty Acid Binding Protein
*C.*fortis Cataglyphis fortis
*M.*musculus Mus musculus
*L.*migratoria Locust migratoria
*A.*mellifera Apis mellifera
Y11W Tyrosine substituted for tryptophan at position 11
Y52W Tyrosine substituted for tryptophan at position 52
C87W Cysteine substituted for tryptophan at position 87
Tm Melting temperature
B-value Temperature factor of the Ca atom
PyMol 3D molecular visualisation system
Jmol Java viewer for chemical structures in 3D
2 Introduction

The desert ant *Cataglyphis fortis* is one of the hyperthermophilic species of *Cataglyphis*. It lives in the Sahara desert and forages during the hottest hours of the day when it can get up to 70°C in the sand. To manage these extreme temperatures the *Cataglyphis* species uses different methods to protect themselves from the heat. They run very fast, the hotter the faster they run, they have long legs (4mm) that elevate them above the hot surface of the sand and they take rests at less hot areas during their foraging. The body temperature of the ant during the foraging runs is above 50°C and can reach a maximum of 53-55°C. *Cataglyphis* ants can survive a body temperature of 50°C for at least ten minutes, but would not survive the maximal temperature for very long. The thermal resistance of *C.fortis* is not yet examined, but for the ants to survive high body temperatures, their biomolecules need to be heat stable [1,2,3,4]. Examining thermostable proteins can give valuable information about the mechanisms of protein stability.

Fatty acid binding protein (FABP) is the common name for a family of intracellular proteins that reversibly bind fatty acids and other lipophilic substances with high affinity. The physiological functions of these lipid chaperones are not entirely understood, but probably they take up and transport fatty acids in the cell and regulate metabolic and inflammatory pathways. The low specificity of the FABPs make them able to bind fatty acids with different length [5,6,7].

FABPs have a common tertiary structure although not similar in primary sequences. Conserved sequences can be found in all FABPs, even from distantly related species. The structures of the proteins are similar and they all form a twisted β-barrel around a hydrophobic interior. The barrel consists of ten anti-parallel β-sheets capped by a helix-turn-helix motif in one end [5,8]. To investigate the ligand binding site and its relative position to the mutated residues, the 3D structure of FABP was modelled. The known structure of FABP from *mus musculus* was used as a template inserting the primary sequence of FABP from *C.fortis*.

The purpose of the thesis was to investigate the thermostability and ligand binding properties of FABP found in *C. fortis*. *C.fortis* is a hyperthermophilic species and its proteins are probable to have a high thermostability due to the sometimes high body temperature of the ant. Investigating the thermostability can give valuable information about the principles of protein folding and stability in hyperthermophiles. FABPs have an important role in the cell transporting fatty acids why studies of fatty acid binding are of interest.

Fluorescence spectroscopy is an easy and fast method to measure intrinsic protein fluorescence. Tryptophans were genetically introduced into three different positions in FABP to be used as environmental sensitive probes. The (local) thermostability of the mutants was detected by shift in wavelength maximum during temperature ramping experiments. Complementing the measurement results with a model of the 3D structure of FABP from *C.fortis* gave additional information about the ligand binding.
3 Theoretical background

3.1 Fluorescence spectroscopy
A molecule can absorb energy which excites the molecule from the ground electronic state to a vibrationally and rotationally excited level of the excited electronic state. When in this excited state, different relaxation transitions can occur. The most common way for excitation energy to be dissipated is the non-radiative transition, fluorescence is rather rare and most fluorescent probes contain aromatic rings or ring systems. Fluorescence means that the molecule relaxes to the ground electronic state by emitting a photon. The emitted photon has less energy than the absorbed radiation meaning that the emission has a longer wavelength than the absorption.

The energy of the photons emitted from a solution when excited with light at a specific wavelength is measured orthogonally in a fluorescence spectrometer, with a dark background. Fluorescence is therefore more sensitive than absorption spectroscopy (which is measured with the light source as background) and small differences in fluorescence intensity can be detected [1,9,10].

3.2 Intrinsic protein fluorescence
In a protein, there are three intrinsic fluorophores: tryptophan, tyrosine and phenylalanine. Tryptophan has the highest quantum yield (emission efficiency).

Tryptophan, as well as tyrosine and phenylalanine, is absorbing light at 280 nm, why the resulting spectra contains contributions from all three amino acid residues. To selectively excite the tryptophan residues an excitation wavelength of 295 nm can be used [9].

Using intrinsic fluorescence gives information about the tertiary structure of the protein. This is due to the influence of environmental factors such as solvent, pH, quenchers, neighbouring groups, etc. in the local environment of the probe(s).

Quenching is the shortening of the lifetime of the excited state. The quenchers, such as water molecules, decrease the quantum yield of the fluorophores by non-radiating processes. The absorbed energy is transferred to the quenching molecule and the resulting total emission energy decreases. The two types of quenching is dynamic quenching, where a quencher collides with the fluorophores in the excited state, and static quenching, in which the quencher forms a complex with the fluorophore [9,11].

- When a fluorophore moves into a less polar environment, its $\lambda_{\text{max}}$ shifts to shorter wavelengths (blue shift) and the $\lambda_{\text{max}}$ intensity increases. This is due to the decrease in quenching.
- In a polar environment, increasing the temperature causes the quantum yield of the fluorophore to decrease with the increasing temperature. Little change in quantum yield is observed in a nonpolar environment when increasing the temperature.
- Tryptophan fluorescence is quenched by neighbouring protonated acidic groups [9].

Globular proteins in aqueous solution prefer to keep its hydrophobic residues together inside the protein where they are less quenched. This builds a hydrophobic interior of the protein when in its native state. A tryptophan hidden in the protein structure is in an environment more nonpolar than when the protein is denatured. When denatured, the environment consists of the solvent, which is more polar than inside the protein [12].

If the tryptophan is situated in a hydrophobic environment in the native protein, the emission spectra are often shifted towards longer wavelengths (red shift) when the native structure is disrupted (denatures). This is due to the change of polarity in the environment of the tryptophan. Measuring the red shift of the tryptophan gives information about the proportion of denaturation of the local structure where the tryptophan is situated.
3.3 Protein stability

3.3.1 Protein structure
The primary structure is the linear order of the amino acid residues in the protein that are covalently linked. Depending on the primary structure, the polypeptide chain forms a secondary structure consisting of regular local structures. The most usual secondary structures in globular proteins are α helices, β strands and turns. The tertiary structure is the specific three-dimensional shape of the folded polypeptide chain. It is stabilised by interactions between amino acid residues close or far apart in the primary sequence. The quaternary structure is the interactions between two or more separate polypeptide chains, or subunits [13,14].

3.3.2 Stability of the protein structure
A protein is usually only biologically active when in its folded, native state. Its activity is determined by the interactions between the native protein and its environment. Stabilisation of the native fold is due to intramolecular forces in the protein. Cysteine residues can form strong covalent bonds in pairs called disulfide bridges or cystines when in a favourable position to each other. Most important to the stability of proteins are though the noncovalent bonds. The interactions of atoms in liquid solution are not well understood because of the complexity of the liquid state. Molecules dissolved in aqueous solutions are highly affected by the interactions between water molecules and ions, dipoles and hydrogen bond donors and acceptors in the molecule.

The noncovalent bonds are van der Waals interactions, hydrogen bonds and charged groups (electrostatic) interactions. Hydrophobic interactions include the positive effect of van der Waals interactions between nonpolar groups and the negative effect of the hydration of these groups. Hydration includes all complex interactions emerged between non-interacting nonpolar molecules and water.

Van der Waals interactions occur between adjacent, uncharged, non-bonded atoms because of their fluctuating dipoles. Although the van der Waals interactions are weak, they are also many due to the close distance between the atoms in the protein. This makes their contribution to the overall stability of the protein considerable. Because of their weakness, van der Waals interactions work at short distances while the hydration effect works at long distances (exceeding the size of a water molecule). The hydrophobic interaction is therefore attractive at short distances and repulsive at long distances.

Hydrogen bonds are formed intramolecularly between polar amino acid residues in the protein. When the protein is folded the hydrogen bonds stabilise the α helices and β sheets in the secondary structure, which helps form the overall structure of the folded protein.

Interactions between charged groups occur between oppositely charged side chains and the NH₃⁺ and COO⁻ groups in the ends of the polypeptide chains. The positively charged amino acids are lysine, arginine and histidine, and the negatively charged amino acids are aspartate and glutamate. To some extent also tyrosine and cysteine take part in the interaction with positively charged residues. When facing the solvent the forces of charged residues are weakened due to quenching. The low frequency of these residues together with their solvated status makes their contribution to the overall protein stability low.

The most important contributions to the stability of a native protein are the van der Waals interactions between the nonpolar groups and hydrogen bonds between the polar groups in the protein molecule. The nonpolar groups are often clustered together forming a nonpolar interior in globular proteins due to the hydrophobic interactions [13,15].

3.3.3 Denaturation stability
When folded, the protein has some stability against losing its native state when exposed to changes in the environment. But at some critical threshold of pH, temperature, pressure or denaturant concentration, the protein cooperatively loses its native state and denatures. The stability of a small, globular, two state protein like FABP is measured as the Gibbs energy difference between the native and denatured state. This is a combination of the entropy and enthalpy differences of the two states. When the temperature increases the protein absorbs the heat energy and as a result the enthalpy and entropy increases. The change in Gibbs energy difference then urges the denaturation process [15,16].
Denaturation is the loss of the three-dimensional structure and function of a protein. The denaturation does not necessarily mean that the protein is totally unfolded, but partially folded with no function.

Heat denaturation affects the weak intramolecular interactions. They are many, but weak, meaning that if some interactions are broken, interactions in their close proximity are likely to brake too. This is called cooperativity. If the temperature is slowly increased, the protein usually loses its conformation in a narrow temperature range. This points to that the loss of structure is a cooperative process in that the loss of structure in one part of the protein destabilises other parts of the protein. Conditions that lead to the disruption of one moiety of a protein structure are therefore likely to denature the protein completely [17,18].

The melting temperature ($T_m$) of a protein can be detected as the temperature at half of the wavelength increase in a graph with $\lambda_{max}$ on the y-axis and temperature on the x-axis. It is used as a measurement of thermostability and a higher $T_m$ indicates a more thermostable protein.

### 3.4 Ligand binding

Specific molecular recognition mechanisms such as a ligand binding are based on a structural complementarity. The ligand fits into the binding pocket of the protein where it interacts with residues inside the protein. More interactions tend to stabilise the protein structure [13,19].

### 3.5 Flexibility

Depending on each residues interactions with other residues in a protein their flexibility can differ considerable. The flexibility of residues in a protein sequence, expressed as B-values, can be obtained from high resolution X-ray crystal structures. The B-value is a measurement of the uncertainty in the atom position and usually comprises the thermal vibration and static disorder effects. The normalised B-value ($B'$) for all $C_\alpha$ was calculated as follows:

$$B' = (B - \langle B \rangle)/\sigma_B$$

Where the $\langle B \rangle$ is the mean B value of all $C_\alpha$ B-values in a protein and $\sigma_B$ is their standard deviation [20].
4 Materials and methods

4.1 Selection with His-tag

The protein mutants were cloned in expression vector pET-28a which contains a His tag. A His tag is a polyhistidine moiety containing six histidine residues that is fused with the protein sequence. The His-tag binds strongly to a nickel-chelate-nitrilotriacetate (Ni-NTA) chromatography column and is used to purify the protein from other proteins in the sample. The His tag has not been cleaved off the protein [9].

4.2 Protein concentration

There are more or less exact methods of determining the protein concentration. Usually there is no need for determining the concentration to decimal place accuracy why faster and easier methods can be used. A very easy and useful method with sufficiently high accuracy is ultraviolet absorption of tryptophan, tyrosine and cystines in the protein at 280nm and Beer-Lamberts law. The extinction coefficient $\varepsilon$ is calculated from the number of tryptophans, tyrosines and cysteines forming disulphide bridges (cystines) in the protein. When the cysteines are reduced, they do not contribute to the extinction coefficient $\varepsilon$ [9]. The cysteines of FABP do not form a disulphide bridge. The absorbance at 280nm is measured and the concentration calculated from Beer-Lamberts law.

$$\varepsilon = \#Trp \cdot \varepsilon_{Trp} + \#Tyr \cdot \varepsilon_{Tyr} + \#Cystine \cdot \varepsilon_{Cystine}$$

$$\varepsilon_{Y11W} = \varepsilon_{Y52W} = 1 \cdot 5500 + 2 \cdot 1490 = 8480 \text{ M}^{-1}\text{cm}^{-1}$$

$$\varepsilon_{C87W} = 1 \cdot 5500 + 3 \cdot 1490 = 9970 \text{ M}^{-1}\text{cm}^{-1}$$

Beer-Lamberts law: $A = \varepsilon \cdot l \cdot c$

Where $A$ is the absorbance at 280nm, $l$ is the pathlength of the cuvet used to measure the absorbance and $c$ is the protein concentration. For the primary sequence of FABP from *C.fortis*, see Appendix 1.

4.3 Intrinsic protein fluorescence

FABP from *C.fortis* does not have any tryptophan residue, why tryptophan has been genetically introduced in three mutants, replacing a tyrosine or cysteine residue. The three mutants are Y11W, Y52W and C87W and are situated in different $\beta$-strands in the “bottom” of the protein binding pocket (Figure 1). Y11W has a tyrosine substituted with a tryptophan at position 11 from the N-terminal in the primary sequence. Y52W has similarly a tyrosine substituted with a tryptophan at position 52 and C87W has a cysteine substituted with a tryptophan at position 87.

The fluorescence measurements were performed with a FluoroMax-4 spectrofluorometer (Horiba Jobin Yvon, S/N 0148-1107). The excitation wavelength was held constant at 295nm and the emission wavelength was scanned in the 310-380nm wavelength area. The slits for incoming and outgoing light were set to 4mm.

Protein stocks were originally suspended and frozen in Tris-HCl buffer (20mM Tris-HCl, 150mM NaCl, 250-500mM Imidazole, pH 8). The buffer of the proteins were changed to K$_2$HPO$_4$ buffer (20mM K$_2$HPO$_4$, pH 7.66, 2% glycerol) to minimise pH change effects when heating the samples.
4.4 Thermostability of the mutants
Batches from protein stocks were diluted with K$_2$HPO$_4$ buffer (20mM K$_2$HPO$_4$, pH 7.66, 2% glycerol) to an approximate concentration of 4μM. The excitation wavelength was 295 nm and the emission spectrum was registered in the wavelength area 310-380nm. Temperature ramping was done with an equilibration time of 3 minutes in 2˚C steps from 22 to 80˚C.

4.5 Ligand binding
FABP binds fatty acids and three different fatty acids were used as ligands; octanoic acid (C$_8$H$_{16}$O$_2$), sebacic acid (HOOC(CH$_2$)$_8$COOH) and myristic acid (C$_{14}$H$_{28}$O$_2$). All are straight carbon chains with a carboxylic acid in one end, sebacic acid has one carboxylic acid in each end (Figure 2). To a protein sample, ligands were added and the shift in wavelength maximum ($\lambda_{max}$) detected. Ligands were added in 30 times excess (120μL) to a final protein concentration of 4μM in K$_2$HPO$_4$ buffer (20mM K$_2$HPO$_4$ pH 7.66, 2% glycerol) from a 1mM stock solution to a total volume of 1mL.
The ligands a) sebacic acid (HO₂C(CH₂)₆CO₂H), b) octanoic acid (C₈H₁₆O₂) and c) myristic acid (C₁₄H₂₈O₂). Images are from Wikimedia commons.

4.6 Temperature ramping with ligands
The same conditions as for earlier described thermostability measurements were used. Temperature ramping measurements of the three mutants without and with octanoic acid and myristic acid were carried out.

4.7 55 degree measurements
The mutant Y52W without and with myristic acid ligand was kept in 55°C to investigate the heat stability over time. Fluorescence emission spectra were registered continuously.

4.8 Managing the sample data
The sample data from the fluorescence spectrometer was corrected with the buffer data. A sliding window of five values was used to detect the fluorescence intensity peak and avoid local highs. Then the wavelength of the fluorescence peak ($\lambda_{\text{max}}$) was accessed.
5 Results

5.1 Protein concentration

From Beer-Lamberts law, the protein concentrations of the mutants were calculated after measuring the absorbance of the stock solutions at 280nm with a HITACHI U-2000 spectrophotometer.

\[ A_{Y11W} = 0.192 \text{ a.u.} \quad c_{Y11W} = \frac{0.192}{1.8480} = 22.6\mu M \]

\[ A_{Y52W} = 0.638 \text{ a.u.} \quad c_{Y52W} = \frac{0.638}{1.8480} = 73.5\mu M \]

\[ A_{C87W} = 0.299 \text{ a.u.} \quad c_{C87W} = \frac{0.299}{1.9970} = 30.0\mu M \]

5.2 Thermostability of the mutants

Initially, the thermostability of the three mutants was investigated by fluorescence spectroscopy using the intrinsic tryptophan. By exiting the tryptophan residue at 295nm and taking up an emission wavelength scan, the wavelength with the maximum fluorescence intensity was detected. The maximum fluorescence intensity was then registered for each temperature scan in the temperature ramping program, that is, every second degree from 22 to 80˚C.

The shift in wavelength maximum gives information about the thermostability of the mutants (Figure 3). Since the measurements of each mutant only reflect the local stability of the introduced tryptophan, the expected results will differ unless the denaturation process is extremely cooperative. The melting temperature (T_m) of the protein mutants can be detected in Figure 3 as the temperature at half of the wavelength increase. The approximate T_m of Y52W is 60˚C, of Y11W 50˚C and of C87W at least 60˚C (the levelling out of the curve is not entirely in the measurement range).

The slope of the curves is an indication of how fast (small temperature span) the local environment of each mutant changes. For Y52W the temperature span is most narrow, for Y11W the temperature span is wider and C87W shows the widest temperature span for the change in \( \lambda_{\text{max}} \). The starting wavelength differs between the three mutants due to the distinct local environment of each tryptophan.
5.3 Ligand binding
An investigation of the shift in maximum wavelength when adding different fatty acid ligands to respective mutant was carried out. As seen in Table 1, the shift in $\lambda_{\text{max}}$ when adding ligands differs between the mutants. For Y11W and C87W a shift of 2nm respectively 1nm were detected, whereas for Y52W no spectral shift at all could be detected.

Table 1 The shift in $\lambda_{\text{max}}$ of the different mutants when adding a 30 times excess of different ligands.

<table>
<thead>
<tr>
<th></th>
<th>$\lambda_{\text{max}}$ Y11W (nm)</th>
<th>$\lambda_{\text{max}}$ Y52W (nm)</th>
<th>$\lambda_{\text{max}}$ C87W (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No ligand</td>
<td>331</td>
<td>335</td>
<td>340</td>
</tr>
<tr>
<td>Octanoic acid</td>
<td>330</td>
<td>335</td>
<td>341</td>
</tr>
<tr>
<td>Sebacic acid</td>
<td>332</td>
<td>335</td>
<td>339</td>
</tr>
<tr>
<td>Myristic acid</td>
<td>333</td>
<td>335</td>
<td>339</td>
</tr>
</tbody>
</table>

5.4 Temperature ramping with ligands
From the heat ramping results of the Y11W mutant, a clear increase of $T_m$ of approximately 8 degrees and a change in sigmoidality could be observed when adding myristic acid (Figure 4). When adding octanoic acid to the Y11W mutant, a change in the slope and the sigmoidal shape of the curve was detected.

The heat denaturation measurements of the mutant Y52W without ligand, with octanoic acid or myristic acid showed that the protein was approximately 3 degrees more heat stable with the myristic acid present, as seen in Figure 5. With the addition of octanoic acid, a nonsignificant change of sigmoidal shape was observed. The slopes of the curves did not change to any great extent with the addition of ligands.

For the C87W mutant a slight but not significant stabilisation could be detected in presence of octanoic acid and myristic acid (Figure 6). The slopes of the curves were the same, but the sigmoidality increased with the addition of the two ligands. Also, a slight increase in $T_m$ was detected upon adding myristic acid.
Heat stability of Y11W

Figure 4 $\lambda_{\text{max}}$ shift during heat denaturation of Y11W without and with octanoic acid and myristic acid ligands respectively. Lines are best-fit Boltzmann sigmoidal least squares.

Heat stability of Y52W

Figure 5 $\lambda_{\text{max}}$ shift during heat denaturation of Y52W without and with octanoic acid and myristic acid ligands respectively. Lines are best-fit Boltzmann sigmoidal, least squares.
Heat stability of C87W

![Graph showing heat stability with wavelength vs temperature for C87W and its ligands]

Figure 6: $\lambda_{\text{max}}$ shift during heat denaturation of C87W without and with octanoic acid and myristic acid ligands respectively. Lines are best-fit Boltzmann sigmoidal least squares.
5.5 Modelling of FABP
To investigate the ligand binding site and its relative position to the mutated residues, the 3D structure of FABP was modelled. The tertiary structure of FABP from C.fortis has not yet been solved, why a predicted structure was modelled in PyMol. All determined FABP structures are very similar and they all form a twisted β-barrel around a hydrophobic interior. The amino acid sequence of FABP from C.fortis was inserted in the known tertiary structure of adipocyte FABP from mus musculus (Figure 1).

5.6 55 degree measurements
Because of the sometimes high body temperature of the ant, its proteins must be heat stable enough for the ant to survive at high temperatures. Investigating the stability of the protein at 55˚C over time gives information about how long time the protein is functionally active in this temperature.

According to the results from the temperature ramping of Y52W, adding myristic acid would make the mutant more stable (Figure 5). At 55˚C the \( \lambda_{\text{max}} \) of Y52W was just above 340nm and with added myristic acid, the \( \lambda_{\text{max}} \) was 338nm. The expected result was therefore a slower denaturation of Y52W with added myristic acid than without at 55˚C.

The protein slowly denatured with approximately 2nm \( \lambda_{\text{max}} \) shift per half hour up to a \( \lambda_{\text{max}} \) of 342nm (Figure 7). Y52W without ligand had a slightly faster denaturation than with myristic acid during the first three and a half hours. The Y52W mutant with myristic acid was stable at a \( \lambda_{\text{max}} \) of 342nm during several more hours. After more than 120 hours the proteins were almost fully denatured with a \( \lambda_{\text{max}} \) of 411nm (Y52W) and 353nm (Y52W + myristic acid) (not shown in figure).

Figure 7 Heat denaturation of Y52W without and with myristic acid ligand over time. After more than 120 hours Y52W had a \( \lambda_{\text{max}} \) at 411nm and Y52W with myristic acid ligand had a \( \lambda_{\text{max}} \) at 353nm. Lines are 2nd order smoothened with 8 neighbours.
6 Discussion

6.1 Protein concentration

Some of the original protein samples were only about 90% pure from the SDS-PAGE which influenced the accuracy of the concentration calculations. This can be a problem when fluorescence spectroscopy is used since the intensity is dependent of the actual concentration. Thus, to bypass this problem only the shifts in $\lambda_{max}$ were analysed as they are independent of the protein concentration and therefore allow a comparison between the mutants.

6.2 Thermostability of the mutants

The mutated positions in FABP from *C.fortis* were all situated in the bottom part of the binding pocket as modelled in Figure 1. To get information from different local environments they were situated in separate β-strands. Figure 3 shows the results of the temperature ramping measurements of the three mutants. The distinct appearances of the curves in Figure 3 are due to the different thermostability and cooperativity of the areas where the mutants are positioned.

The different slopes and sigmoidality of the curves in Figure 3 indicated different cooperativity of the local structures of the mutants. Cooperativity of denaturation is when one disrupted area causes other parts in the folded protein to become destabilised. A flexible area in a protein is less inclined to become destabilised by cooperativity since it is not as dependent on fixed interactions as a more stable area is. A flexible moiety can probably position itself in several ways, still being in an energetically favourable position. A fixed structure is more influenced by a change of interactions, and thereby destabilised.

By comparing the normalised temperature factors, or B’-values, between the mutated positions in the FABP from *M.musculus*, information about the flexibility of the positions were gained. The B’-value of residues in *M.musculus* corresponding to the mutated positions in *C.fortis* showed that Y11 was most flexible (B’-value: 0.41), Y52 less flexible (B’-value: -0.57) and C87 least flexible (B’-value: -0.89) [20,21]. However, the amino acid residues of *M.musculus* at corresponding positions were not the same as in *C.fortis* why these values were just an indication of the flexibilities. Still, the B’-values gave an estimation of the difference in flexibility between the mutated positions. The substitution in the mutant C87W replaced a cysteine with a tryptophan which has a much larger side chain. This is likely to destabilise the moiety and thereby increase the flexibility beyond the B’-value of corresponding residue in *M.musculus*.

The local melting temperature for the Y11W mutant of approximately 50°C was the lowest melting temperature of the three mutants (Figure 3). This indicated a low thermostability of this moiety. Furthermore, as detected in Figure 3, the local environment of Y11W changed over a wide temperature range which indicated a less cooperative behaviour. The low slope and low $T_m$ could be explained by flexibility of this moiety, as was confirmed by its comparatively high B’-value. The position of Y11W was in the first β-strand from the N-terminal (Figure 1). A residue in the end of a protein primary or secondary sequence is more inclined to be flexible than a residue in the middle of the sequence due to fewer interactions.

Figure 3 showed that the melting temperature of Y52W was quite high (~60°C) and that its local environment changed in a narrow temperature range. The sigmoidality and high slope of the curve indicated that the conformation of this moiety denatured cooperatively. This result, together with the comparatively low B’-value and high melting temperature, suggested that this residue was situated in a rigid environment. Y52W was situated in the third β-strand from the N-terminal (Figure 1), which also indicated that the residue was stable. The more interactions a β-strand has, the more stable it is, why strands in the middle of a β-sheet is more inclined to be stable than one in the end.

The $T_m$ for C87W was quite high (~60°C) which indicated that it was stable. C87W also had the highest starting $\lambda_{max}$-values and was therefore likely to have the most exposed tryptophan from the start (Figure 3). This was probably due to that tryptophan is a much larger amino acid than the substituted cysteine which means it does not fit inside the hydrophobic interior of the protein. Thus, the tryptophan in C87W was then exposed to the polar solvent to a higher extent than the tryptophans in the other two mutants. Since
tryptophan in Y52W and Y11W replaced the larger amino acid tyrosine, they were more likely to fit inside the hydrophobic interior of the protein and therefore emit photons with a lower $\lambda_{\text{max}}$, as is seen in Figure 3. In Figure 3, the slope of the C87W mutant curve was low, meaning that this area had low cooperativity. C87W was placed at the end of the sixth $\beta$-strand from the N-terminal (Figure 1), where it unmutated is likely to be stable. This, together with the high melting temperature, indicated that the area was stable. At the same time the low slope and sigmoidality of the curve suggested it had a low cooperativity and was flexible. The mutation of cysteine 87 to tryptophan could have affected the local structure of the protein, as the high starting value of $\lambda_{\text{max}}$ indicated, but the differing results of the measurements of this mutant did not give a clear indication of what had happened in the protein.

6.3 Ligand binding

FABP binds fatty acids in vivo making the investigation of fatty acid ligand binding interesting to study. When binding a ligand, the protein increases its number of interactions which has a stabilising effect of the native structure. The ligands used for the ligand binding measurements were octanoic acid ($\text{C}_8\text{H}_{16}\text{O}_2$), myristic acid ($\text{C}_{14}\text{H}_{28}\text{O}_2$) and sebacic acid ($\text{HO}_2\text{C}(\text{CH}_2)_8\text{CO}_2\text{H}$) (Figure 2).

Ligand binding experiments were carried out to investigate the ligands’ stabilising effects of the mutants. The study aimed at investigating whether the binding of the ligands could be detected by the tryptophans and what effects the change in environment due to ligand binding had on the tryptophans. This was performed by adding an excess of different ligands to the mutants and register the fluorescence spectra. The binding of the ligands can cause a shift in $\lambda_{\text{max}}$. Table 1 shows that Y11W was the mutant with the greatest shift in $\lambda_{\text{max}}$ when adding an excess of ligands. C87W showed a small shift in $\lambda_{\text{max}}$ while Y52W showed no shift.

The sebacic acid was used to see how the ligand positioned itself in the binding pocket of FABP. If the ligand had its tail towards the bottom of the protein, the carboxyl group of sebacic acid was likely to have a quenching effect of the tryptophans. This would cause a shift in $\lambda_{\text{max}}$ greater than the ones in Table 1, and sebacic acid was therefore likely to instead curl up into a U-shape as in Figure 9.

The octanoic acid is six carbons shorter than myristic acid why it possibly would affect the tryptophans differently than myristic acid. Due to Table 1, the differences in binding octanoic acid and myristic acid were small. To investigate the ligand binding further, temperature ramping measurements were performed with octanoic acid and myristic acid.

6.4 Temperature ramping with ligands

Further studies with temperature ramping from 22 to 80°C were performed with octanoic acid and myristic acid (Figure 2). Octanoic acid consists of a carboxyl group and a hydrophobic chain of seven carbons (COOH(CH$_2$)$_7$). The longer myristic acid has a carboxyl group and 13 carbons in its hydrophobic chain (COOH(CH$_2$)$_{13}$).

As seen in Figure 4, Y11W clearly had a higher melting temperature when binding myristic acid than without ligand (approximately 4°C higher). Moreover, the tendency for Y11W binding any of the two ligands, but most distinct for the octanoic acid ligand, was an increase in cooperativity. This could be detected as a narrowing of the temperature range for the shift in $\lambda_{\text{max}}$ and a more sigmoidal shape of the curve.

The increase in melting temperature (of more than 3°C) when myristic acid was added to Y52W suggested a stabilisation of the protein structure (Figure 5). Adding octanoic acid gave no significant stabilisation. Octanoic acid had eight carbons while myristic acid had 14. The difference in length of their hydrophobic chains probably caused a difference in binding properties which could be the reason to their distinct results. The narrow temperature range of the denaturation implied that the cooperativity for Y52W was quite high both with and without ligand binding.

The curves of C87W with and without ligands in Figure 6 were very similar. A tendency of increased melting temperature and cooperativity could be detected for the mutant with myristic acid added. This mutant had least cooperativity (widest temperature span for denaturation) and it only showed a slight change with ligand present (Figure 3). This suggested that the structure surrounding C87W was not much affected by the ligand binding due to its flexibility or long distance to the ligand binding site.
Myristic acid had the tendency to stabilise the protein structure, detected as a higher melting temperature, of all the mutants. This suggested that it increased the total stability of the protein. Octanoic acid increased the cooperativity of Y11W and hardly affected the other two mutants at all, indicating a more local stabilisation.

The most pronounced increase in $T_m$ and cooperativity upon adding ligands were for the mutant Y11W. For Y52W smaller changes were detected and for C87W the detected effects were the least. The difference in influence for the mutants upon ligand binding could possibly be a result of where the ligands bind with respect to the mutated amino acids. To examine this further, a modelling of the tertiary structure was carried out.

6.5 Modelling of FABP

Modelling of FABP from *C.fortis* in the tertiary structure of FABP from *M.musculus* was carried out using PyMol. The program was used to investigate where specific residues were likely to be situated in the tertiary structure of the protein.

One tyrosine and two arginines in FABPs are involved in binding the carboxyl group of the fatty acid ligands. Compared to the conserved regions of FABPs, the most likely candidates were R108, R128 and Y130 in the FABP sequence of *C.fortis* [5,8,22]. This was also supported by the suggested positions from the modelling of FABP from *C.fortis*. The model showed that the only two basic amino acids that pointed inwards to the interior of the protein were R108 and R128. All other basic amino acids were directed towards the solvent in the model and could therefore not bind a ligand in the binding pocket. Also Y130 pointed inwards in the model and could thereby be active in the binding of a ligand.

Two of the suggested ligand binding amino acids, the R128 and Y130 residues, were situated adjacent to the Y11 residue and quite far from the Y52 and C87 residues (Figure 8). The third residue, R108, was situated between Y11 and C87, but still far from Y52. If this model was correct, Y11 was the mutated position situated closest to the supposed binding residues and Y52 farthest away.

The ligand only fills up to half of the binding pocket, leaving the other half water-filled. Ligands can also curl to a U-shape in the top of the binding pocket (Figure 9) [5,8,22]. When curling to a U-shape, the ligand itself would not affect the tryptophans in the bottom of the binding pocket to any great extent (Figure 9). However, a conformational change due to the ligand binding could affect the environment of the tryptophans.

The temperature ramping investigation of mutants with ligands showed that Y11W was the most affected mutant by ligand binding (Figure 4). Y11W was the mutant with the highest increase of $T_m$ in presence of myristic acid (approximately 4°C) and the mutant with the greatest change in cooperativity when binding octanoic acid. The high influence of ligand binding on Y11W coincided with the modelled position of Y11 close to the ligand binding residues (Figure 8). These results implied that the position Y11 was affected by ligand binding due to its close proximity to the amino acids binding the ligand. Either the ligand itself affected the local environment of the tryptophan, or a conformational change due to ligand binding.

In the temperature ramping results, Y52W showed an increase in $T_m$ when binding myristic acid (more than 3°C increase) and almost no change when binding octanoic acid (Figure 5). These results implied that Y52W was more affected by the longer myristic acid than by the shorter octanoic acid (Figure 2). From the suggested position of the modelling, Y52 was situated farthest from, and Y11 closest to, the ligand binding residues (Figure 8). Myristic acid influenced both Y11W and Y52W with an increase in $T_m$, which suggested a higher stability of the entire protein structure. Comparing the effects of octanoic acid binding between Y11W and Y52W, Y52W was not as affected as Y11W (Figure 4, Figure 5). This implied that octanoic acid had a local effect due to the close proximity to the Y11 position. In Table 1, the influence of sebacic acid and myristic acid was most similar, while the effect of octanoic acid differed. These results could depend on that myristic acid, just like sebacic acid, curled up to a U-shape while octanoic acid only reached the tryptophan of Y11 and affected it directly.

From the results of the temperature ramping with ligands, C87W showed just a slight increase of cooperativity and $T_m$ upon binding myristic acid ligand and just a tendency to higher cooperativity when binding octanoic acid (Figure 6). However small, the influence of the ligands confirmed the earlier theory that myristic acid stabilised the total structure of the protein, while octanoic acid only had a local effect.
Its modelled position implied that C87 was situated somewhat close to the R108 residue, but farther away from the R128 and Y130 residues. Compared to the effects of Y11W and Y52W relative their positions, the ligand binding did not affect C87W as much as expected in the temperature ramping results (Figure 4, Figure 5, Figure 6). A possible reason to why C87W was not much affected by the ligand binding was the local change of conformation due to the big difference in size between the original and introduced amino acid. This theory, in combination with the high value of the starting $\lambda_{\text{max}}$ for C87W (Figure 6), suggested that C87W was quite exposed to the aqueous solvent and had not subsumed in the protein tertiary structure. A residue in the protein interior would be more shielded from the quenching effect of the water molecules in the aqueous solvent. This would yield a lower starting $\lambda_{\text{max}}$ such as for the other two mutants (Figure 4, Figure 5).

How the ligand positions itself in the binding pocket is not entirely clear. The ligand binding results for sebacic acid in Table 1 suggested that sebacic acid was curled up to a U-shape (Figure 9). The similarity between the myristic acid and sebacic acid results further indicated that also myristic acid curled up to a U-shape (Table 1). In Table 1, the octanoic acid affected the mutants Y11W and C87W differently than sebacic acid and myristic acid did which could be a result of other binding properties. Myristic acid increased the melting temperature for all mutants while octanoic acid increased the cooperativity for Y11W, but not the other two mutants to any great extent. These differences could depend on their different binding properties which caused myristic acid to stabilise the total protein structure while octanoic acid only affected the local environment of the ligand binding residues (Y11).

6.6 55 degree measurements
The last measurements were of the thermostability over time at a fixed temperature of 55°C. Only the Y52W mutant without ligand and with myristic acid were measured.

Y52W was somewhat more stable with myristic acid than without as could be detected as a slower denaturation (shift in $\lambda_{\text{max}}$) of Y52W with myristic acid in Figure 7. This was expected from the higher $T_m$ of Y52W with myristic acid than without in the temperature ramping results (Figure 5). The higher $T_m$ means that the mutant has a higher thermostability with myristic acid than without.

Within five minutes of heating both Y52W with and without myristic acid to 55°C, the $\lambda_{\text{max}}$ started to shift towards longer wavelengths, indicating denaturation (Figure 7). For Y52W with added myristic acid, the curve then levelled out at 341nm and after more than 24 hours it was still stable at a $\lambda_{\text{max}}$ of approximately 341nm. When more than six days had passed, the Y52W with myristic acid had denatured almost completely to a $\lambda_{\text{max}}$ of 353nm (not shown in figure). No measurement for Y52W without ligand after 24 hours was available, but after more than five days its $\lambda_{\text{max}}$ was approximately 411nm (not shown in figure).

Some kind of structural change, at least in the local area, occurred during the first three and a half hours for both Y52W with and without myristic acid. The structure of Y52W with myristic acid was then stabilised for a period of time before further conformational changes took place. The structural changes seemed not to affect the ability to bind a ligand, as the stabilisation of the structure with myristic acid was still apparent after more than three hours in 55°C.
Figure 8 Modelling of fatty acid binding residues in FABPs from different species (modelled in PyMol). a) shows the position of the three mutation sites C87 (the leftmost), Y52 (the middle) and Y11 (the rightmost) in FABP (dark blue stick models) from C.fortis in respect to arginine 108, 128 and tyrosine 130 (magenta stick model), b) shows the conserved arginine 108, 128 and tyrosine 130 in FABP from L.migratoria (magenta stick model) (PDB code 2FLJ), c) shows the conserved arginine 106, 126 and tyrosine 128 in FABP from M.musculus (magenta stick model) (PDB code 3HK1)
6.7 Comparison to other FABPs

The closest match to FABP from *C.fortis* when making a blast search in Swissprot was FABP from *Nylanderia*, an ant called Caribbean crazy ant (89.6% similarity). When matched to other species, the most similar sequence was FABP from *Apis mellifera*, the honeybee (81.3% similarity). These were not available as 3D-structures in the PDB-database. A sequence available as 3D structures in the PDB database and somewhat close to the FABP of *C.fortis* was FABP from *L.migratoria*, a grasshopper (51.5% similarity). Another 3D structure available in the PDB database was adipocyte FABP from *M.musculus*, the house mouse, which was used as a tertiary structure template for modelling the tertiary structure of FABP from *C.fortis* (46.7% similarity). All similarity calculations have been performed using Genestream [23] and are available in Appendix 1.

The tyrosine (Y130) and arginine (R108 and R128) residues that supposedly are involved in ligand binding were conserved between the FABPs from *Nylanderia*, *A.mellifera*, *M.musculus*, *L.migratoria* and *C.fortis*. The conservation supported the supposition that these three residues bind ligand. Comparisons of the primary sequences showed that FABP from *C.fortis* was more equal to the FABP from *L.migratoria* than *M.musculus* (Appendix 1). This is probably due to that both *C.fortis* and *L.migratoria* are insects, unlike *M.musculus*. Consequently the tertiary structure of FABP from *L.migratoria* was probably more alike the FABP from *C.fortis* and would be better to use as tertiary structure template in the modelling.

Figure 8 shows the supposed ligand binding sites in the crystal structures of FABP from *L.migratoria* (b), *M.musculus* (c) and the modelled primary structure of *C.fortis* in the tertiary structure of *M.musculus* (a).

![Figure 9 Binding of ligand. Epidermal FABP from *H.sapiens* binding a palmitic acid (C_{16}H_{32}O_{2}) (PDB code: 1b56). Modelled in Jmol.](image-url)
7 Concluding remarks

Fluorescence spectroscopy is a fast and easy method for making measurements of proteins compared to the more time consuming and complex NMR (nuclear magnetic resonance) and X-ray crystallography methods. The results from the fluorescence measurements in this report indicate that fluorescence methods can complement NMR and X-ray crystallography when specific information about ligand binding is desired.

From X-ray crystallography studies of FABP, the most likely binding site for ligands in FABP involves the conserved residues corresponding to R108, R128 and Y130 in FABP from C.fortis [22]. Y11W is positioned closest to the presumed ligand binding site and is also most affected by ligand binding in the thermostability measurements. Y52W is less affected by ligand binding and is also situated a longer distance from the presumed ligand binding site. This implies that the influence of the tryptophan upon ligand binding is affected by the distance to the ligand binding site. The results for C87W do not agree with this theory, but this mutant is likely to have a changed structure due to the mutation and its results are therefore a lot harder to interpret.

Further, the myristic acid stabilise the melting temperature of all the mutants while octanoic acid only has a local effect of Y11W increasing the cooperativity. This implies different binding properties and that myristic acid stabilise the entire protein while octanoic acid only has a local stabilisation effect around the ligand binding site. The thermostability measurements and 3D modelling results suggest that the ligand binding is similar between FABP from C.fortis and FABP from other organisms.

8 Future prospects

It would be interesting to complement these local stability investigations with global thermostability measurement of the secondary and tertiary structure using circular dichroism (CD). Measuring the mutants and the wild type of FABP could give valuable information of the $T_m$ and if it differs between the mutants and the wild type. Since CD can give global information about the tertiary structure, a combination with the local fluorescence data would be valuable.

Introducing tryptophans in other positions would also be interesting. The function of the binding pocket of FABP could be investigated closer. F60 has been suggested to be an important residue for the swinging doorway that contracts the binding pocket (F57 in the reference) [5]. To introduce a tryptophan in this position or in a position closer to the top of the binding pocket can give further information about the swinging doorway function and/or the functions of ligand binding.

An X-ray crystallography is more complex and time consuming, but could give valuable information about the mutants and if they resemble the wild type or if the mutations have had structural consequences.
9 Acknowledgements

First and foremost I would like to thank Magdalena Svensson, my supervisor during the thesis. You have given me valuable inspiration concerning the approach of the thesis, interpreting the results and setting the framework of the report. Thank you for your help, your time and your enthusiastic way that has elevated me. I would also like to direct a thank you to my examiner Lars-Göran Mårtensson for rewarding discussions about methods and tolerance with my questions. Lisa Henriksson, Linnéa Nilebäck, Maria Pennsäter, Henric Enstedt, Torbjörn Sveds, Marie Roth, Alexander Sandberg and Susanna Ekeblad, thank you for rewarding discussions about methods of measurement, laboratory conditions and results over very nice lunches. Thank you Gabriel Gustafsson and Tommy Larsson for helping me getting started and answering my questions. Raul Campos Melo, thank you for taking your time to answer my questions and show me the fluorescence spectrophotometers and other practicalities. I would also like to thank Pontus Askbrink for discussing and reading my report giving me suggestions of improvement. Last, but not least, I would like to thank Jenny Ornefalk and Gustav Gahm for your constant support, it has been crucial to me during this turbulent time.
10 References


11 Appendix 1

Primary sequences

FABP – C.fortis
MSINEILGKR YKLSSSENFD DFMKALGVGMVTRKMATVSPV VYTLKTTSTF KNEIEFKFLG
EEFDEDTVDG RKVKSVCNTLE GNKLIQVQKG DKNTTIREFE TPTEMEAIMK VDDIVCTRXYQ KIQE

FABP – Nylanderia
MSSEVLGKR YKLSSFNFDY MKALGVGMVTRKMATVSPVV VELTEKDFGE YTLKTTSTFK SAIEIKFLGGE
EFDEETVDGR KVSCTLDGNNKLVQVQKGEK QTTIEREFSS TEMKAIMKVD DIICTRVYK IQE

FABP – Apis mellifera
MPDFLGKRYL YSSENFDDFD MKAIGDDATR KFKLKTSQTK KINEEFKLGE
EEFDEETVDGR KSCTLDGE GNKLQVQKGEK QTTIEREFSS TEMKAIMKVD DIICTRVYK IQE

Muscle-FABP – L.migratoria
MKVEFAGIKY KLDSTQFPFF YMKAGLALSP VIELEVLQDG KFKLKTSQTK KINEEFKLGE
EEFDEETVDGR KVSCTLDGE GNKLQVQKGEK QTTIEREFSS TEMKAIMKVD DIICTRVYK IQE

Adipocyte-FABP – M.musculus
CDAFVWTWLVSSENFDDDF KVEHMAGKPNM IISVEVDQIY KFKEFKEDGE
EEFDEETVDGR KSCTLDGE GNKLQVQKGEK QTTIEREFSS TEMKAIMKVD DIICTRVYK IQE

Sequence alignments

FABP Nylanderia and FABP C.fortis
>_ Nylanderia 133 aa vs. _> C.fortis 134 aa
scoring matrix: , gap penalties: -12/-2
89.6% identity; Global alignment score: 770

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81.3% identity; Global alignment score: 700

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FABP L.migratoria and FABP M.musculus
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:......................:
_ ECVMKGVSTRYERA

29
FABP C.fortis and A-FABP M.musculus

>_ FABP M.musculus 131 aa vs.  
>_ FABP C.fortis 134 aa

scoring matrix: , gap penalties: -12/-2  
46.7% identity;  
Global alignment score: 331

10 20 30 40 50
700834 C--DAFVG-T-WKLVSSENFDDYMKEVGVGFATRKVAGMAKPNMIISVNGDLVVTIRSESTF  
- MSINEILGKRYKLSSSENFFDMKALGVMVTRKMGAVTSPVVELTEKDGVYTLKTTSTF  
- 10 20 30 40 50
60 70 80 90 100 110
700834 KNTETISFKLVEFDEITADDRKVKSIITLDGGALVQVQKWGDKGSTTIIKXRKRKGDKLTVVEC  
- KNTETIKFLGEFEDTDGRKVSCTLEGNKLIQVQKGAMNKTIEREFTPTEM--EA  
- 70 80 90 100 110

FABP C.fortis and M-FABP L.migratoria

>_ FABP C.fortis 134 aa vs.  
>_ fabp L.migratoria 134 aa

scoring matrix: , gap penalties: -12/-2  
51.5% identity;  
Global alignment score: 418

10 20 30 40 50
845269 MSINEILGKRKYKLSSSENFDDMFKALGVMVTRKMGAVTSPVVELTEKDGVYTLKTTST  
- M-VKEFAGIKYKLDSQTNFEEMKAGIERKAGLALSPIVELEVLGDKFKLTSKTA  
- 10 20 30 40 50
60 70 80 90 100 110
845269 KNTETIKFLGEFEDTDGRKVSCTLEG-NKLIQVQKGDKNMTIEREFTPTMEAI  
- KNTETIKLFGEFEDTDGRKVSCTLEGNKLIQVQKGDKNMTIEREFTPTMEAI  
- 60 70 80 90 100 110

845269 MKVDDIVCTRIVYKIQE  
- 120 130

845269 IKLGDVLATRIYKAQ--