

Characterization of the thermostable nature of the alpha and beta tubulin  
proteins in *Cyanidium caldarium* and *Cyanidioschyzon merolae*

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(ABSTRACT)

Microtubules are critically important cytoskeletal elements. Together with microtubule associated proteins (MAPs), they form the latticework on which eukaryotic life exists. Simply put, microtubules are polymers of tubulin heterodimers, which are composed of the globular proteins alpha and beta tubulin. In vivo, these monomers associate with one another to form heterodimers, which then polymerize to form microtubules.

In mammals, microtubule polymerization is a temperature-dependent process with an optimum of 37 °C (Detrich et al., 2000). If temperatures exceed this optimal temperature by even a few degrees, the microtubule will begin to dissemble due to denaturation of the tubulin subunit and permanent loss of both shape and function will occur. This thermal barrier seems to be consistent in most eukaryotic organisms.

Two exceptions are the thermophilic red algae, *Cyanidium caldarium* and *Cyanidioschyzon merolae*. These thermophilic acidophiles have been discovered in volcanic vents around the globe from Yellow Stone Park to Italy and grow at optimal temperatures of around 55°C. These organisms have been primarily studied in the

context of evolutionary biology because of their primitive characteristics. Very little is known about the molecular biology of these organisms, and certainly nothing is known about how the biochemistry of these organisms brings about the ability to survive the harsh conditions of their environment. Currently, my hypothesis concerning the thermostable tubulin expressed within these organisms is that there may be key amino acid differences in the tubulin's primary structure that confer enhanced thermostability. I am testing this hypothesis by sequencing the alpha and beta tubulin genes of *Cyanidium caldarium* and *Cyanidioschyzon merolae*, generating homology models of the tubulin dimers, and comparing these models to a known mesophilic tubulin heterodimer structure in order to identify potential structural differences.

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## **Table of Contents**

|                                                 |    |
|-------------------------------------------------|----|
| Title Page .....                                | i  |
| Abstract .....                                  | ii |
| Acknowledgments .....                           | iv |
| Dedication .....                                | v  |
| Table of Contents .....                         | vi |
|                                                 |    |
| Introduction .....                              | 1  |
| Microtubules and Tubulin .....                  | 1  |
| Tubulin Structure .....                         | 4  |
| Thermophilic Algae.....                         | 7  |
| Protein Modeling .....                          | 10 |
|                                                 |    |
| Materials and Methods .....                     | 12 |
| DNA extraction from the red algae .....         | 12 |
| Sequence Analysis and Homology Modeling .....   | 18 |
| Computational Thermostability Experiments ..... | 19 |

|                                                                 |     |
|-----------------------------------------------------------------|-----|
| Results .....                                                   | 21  |
| Sequencing the alpha and beta tubulin genes .....               | 22  |
| Sequence alignments and analysis .....                          | 27  |
| Analysis of the tertiary structure of the tubulin monomer ..... | 34  |
| Computational analysis of tubulin monomers .....                | 63  |
| <br>                                                            |     |
| Discussion .....                                                | 69  |
| Conclusion .....                                                | 76  |
| <br>                                                            |     |
| Literature Cited .....                                          | 80  |
| Appendix A .....                                                | 86  |
| Appendix B .....                                                | 95  |
| Curriculum Vitae .....                                          | 104 |

## **List of Figures**

|                                                                                                                                           |    |
|-------------------------------------------------------------------------------------------------------------------------------------------|----|
| <b>Figure 1</b> Microtubule Assemble.....                                                                                                 | 2  |
| <b>Figure 2</b> The structure of the bovine brain tubulin heterodimer.....                                                                | 6  |
| <b>Figure 3</b> Rapid Amplification of Genomic Ends (RAGE PCR).....                                                                       | 17 |
| <b>Figure 4</b> Amplification of internal portion of alpha and beta tubulin genes from<br><i>C. caldarium</i> and <i>C. merolae</i> ..... | 23 |
| <b>Figure 5</b> RAGE PCR amplification of N-terminus regions of tubulin genes from<br><i>C. caldarium</i> .....                           | 24 |
| <b>Figure 6</b> Alpha tubulin genomic DNA and predicted amino acid sequences.....                                                         | 25 |
| <b>Figure 7</b> Beta tubulin genomic DNA and predicted amino acid sequences.....                                                          | 26 |
| <b>Figure 8</b> Alignment of alpha tubulin genes from <i>C. caldarium</i> and <i>C. merolae</i> .....                                     | 28 |
| <b>Figure 9</b> Alignment of alpha tubulin amino sequences from <i>C. caldarium</i><br>and <i>C. merloae</i> .....                        | 29 |

|                                                                                                                                                    |    |
|----------------------------------------------------------------------------------------------------------------------------------------------------|----|
| <b>Figure 10</b> Alignment of beta tubulin amino sequences from <i>C. caldarium</i><br>and <i>C. merolae</i> .....                                 | 30 |
| <b>Figure 11</b> Alignment of thermophilic alpha tubulin consensus sequences.....                                                                  | 31 |
| <b>Figure 12</b> Alignment thermophilic of beta tubulin consensus sequences.....                                                                   | 32 |
| <b>Figure 13:</b> A Ramachandran Plot of the solved structure of pig alpha and beta<br>tubulin heterodimer.....                                    | 37 |
| <b>Figure 14</b> Secondary structure map of thermophilic alpha tubulin.....                                                                        | 38 |
| <b>Figure 15</b> Secondary structure map of thermophilic beta tubulin.....                                                                         | 39 |
| <b>Figure 16</b> The six faces of the thermophilic tubulin heterodimer.....                                                                        | 41 |
| <b>Figure 17</b> In <i>C. caldarium</i> and <i>C. merolae</i> a hydrophobic leucine <sup>31</sup> is present<br>in thermophilic alpha tubulin..... | 42 |
| <b>Figure 18</b> In <i>C. caldarium</i> and <i>C. merolae</i> a basic arginine <sup>39</sup> is an addition<br>to thermophilic alpha tubulin.....  | 43 |

|                                                                                                                                                  |    |
|--------------------------------------------------------------------------------------------------------------------------------------------------|----|
| <b>Figure 19</b> In <i>C. caldarium</i> and <i>C. merolae</i> a polar serine <sup>50</sup> is present in thermophilic alpha tubulin.....         | 44 |
| <b>Figure 20</b> In <i>C. caldarium</i> and <i>C. merolae</i> a hydrophobic proline <sup>84</sup> is present in thermophilic alpha tubulin.....  | 45 |
| <b>Figure 21</b> In <i>C. caldarium</i> and <i>C. merolae</i> a basic arginine <sup>98</sup> is present in thermophilic alpha tubulin.....       | 46 |
| <b>Figure 22</b> In <i>C. caldarium</i> and <i>C. merolae</i> a hydrophobic glycine <sup>288</sup> is present in thermophilic alpha tubulin..... | 47 |
| <b>Figure 23</b> In <i>C. caldarium</i> and <i>C. merolae</i> a polar glutamine <sup>326</sup> is present in thermophilic alpha tubulin.....     | 48 |
| <b>Figure 24</b> In <i>C. caldarium</i> and <i>C. merolae</i> a basic lysine <sup>335</sup> is present in thermophilic alpha tubulin.....        | 49 |
| <b>Figure 25</b> In <i>C. caldarium</i> and <i>C. merolae</i> a polar serine <sup>359</sup> is present in thermophilic alpha tubulin.....        | 50 |
| <b>Figure 26</b> In <i>C. caldarium</i> and <i>C. merolae</i> a polar glutamine <sup>42</sup> is present in thermophilic alpha tubulin.....      | 51 |

|                                                                                                                                                 |    |
|-------------------------------------------------------------------------------------------------------------------------------------------------|----|
| <b>Figure 27</b> In <i>C. caldarium</i> and <i>C. merolae</i> a polar threonine <sup>56</sup> is present<br>in thermophilic alpha tubulin.....  | 52 |
| <b>Figure 28</b> In <i>C. caldarium</i> and <i>C. merolae</i> a polar glutamine <sup>212</sup> is present<br>in thermophilic alpha tubulin..... | 53 |
| <b>Figure 29</b> In <i>C. caldarium</i> and <i>C. merolae</i> a polar serine <sup>267</sup> is present<br>in thermophilic alpha tubulin.....    | 54 |
| <b>Figure 30</b> In <i>C. caldarium</i> and <i>C. merolae</i> a polar serine <sup>269</sup> is present<br>in thermophilic alpha tubulin.....    | 55 |
| <b>Figure 31</b> In <i>C. caldarium</i> and <i>C. merolae</i> a polar threonine <sup>276</sup> is present<br>in thermophilic alpha tubulin..... | 56 |
| <b>Figure 32</b> In <i>C. caldarium</i> and <i>C. merolae</i> a polar threonine <sup>279</sup> is present<br>in thermophilic alpha tubulin..... | 57 |
| <b>Figure 33</b> In <i>C. caldarium</i> and <i>C. merolae</i> a basic histidine <sup>283</sup> is present<br>in thermophilic alpha tubulin..... | 58 |
| <b>Figure 34</b> In <i>C. caldarium</i> and <i>C. merolae</i> a polar serine <sup>320</sup> is present<br>in thermophilic alpha tubulin.....    | 59 |

|                                                                                                                                                       |    |
|-------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| <b>Figure 35</b> In <i>C. caldarium</i> and <i>C. merolae</i> a hydrophobic glycine <sup>356</sup> is present<br>in thermophilic alpha tubulin.....   | 60 |
| <b>Figure 36</b> In <i>C. caldarium</i> and <i>C. merolae</i> an acidic aspartic acid <sup>363</sup> is present<br>in thermophilic alpha tubulin..... | 61 |
| <b>Figure 37</b> In <i>C. caldarium</i> and <i>C. merolae</i> a hydrophobic cysteine <sup>366</sup> is present<br>in thermophilic alpha tubulin.....  | 62 |
| <b>Figure 38</b> RMS and atomic fluctuation graphs of mesophilic and thermophilic<br>alpha tubulin.....                                               | 65 |
| <b>Figure 39</b> RMS and atomic fluctuation graphs of mesophilic and thermophilic<br>beta tubulin.....                                                | 67 |

**List of Tables**

|                                                                                                                                              |    |
|----------------------------------------------------------------------------------------------------------------------------------------------|----|
| <b>Table 1</b> Primers used to generate tubulin sequence.....                                                                                | 15 |
| <b>Table 2</b> Non-conserved amino acid substitutions and additions between <i>C. caldarium</i><br>and <i>C. merolae</i> and mesophiles..... | 33 |
| <b>Table 3</b> All non-conserved changes, their location, and effect.....                                                                    | 79 |

## **Introduction**

### **Microtubules and Tubulin**

Microtubules are vital cytoskeletal elements that are involved in cellular activities ranging from cell division to axonal transport. These cytoskeletal elements are polymers of alpha-beta tubulin heterodimers that are composed of two globular proteins. The amino acid sequences of both alpha and beta tubulin are very highly conserved throughout all eukaryotic organisms (Dutcher, 2001). For example, alpha tubulin from *Trichomonas vaginalis* has a 94.4% identity with alpha tubulin from pig brain (Noel et al., 2001). In addition, the three-dimensional structures of alpha and beta subunits are so similar that they are almost superimposable (Nogales et al., 1998). The subunits interact with one another in the presence of the nucleotide GTP and five specialized chaperone proteins to form the heterodimer; heterodimers are thought only rarely to disassociate from one another in the cell (Mandelkow and Mandelkow, 1995). Given the appropriate temperature and concentration, the heterodimers will polymerize without the participation of any other molecule, a process known as self-assembly (Figure 1).

While both tubulin subunits bind GTP, they do so in very different ways. Alpha tubulin binds GTP irreversibly using a binding motif called the non-exchangeable site; beta tubulin binds GTP reversibly and hydrolyzes it using a binding motif called the exchangeable site, because the GDP can be exchanged for GTP (Nogales et al., 1999). Both sites must be associated with GTP in order for tubulin dimers to polymerize into microtubules.

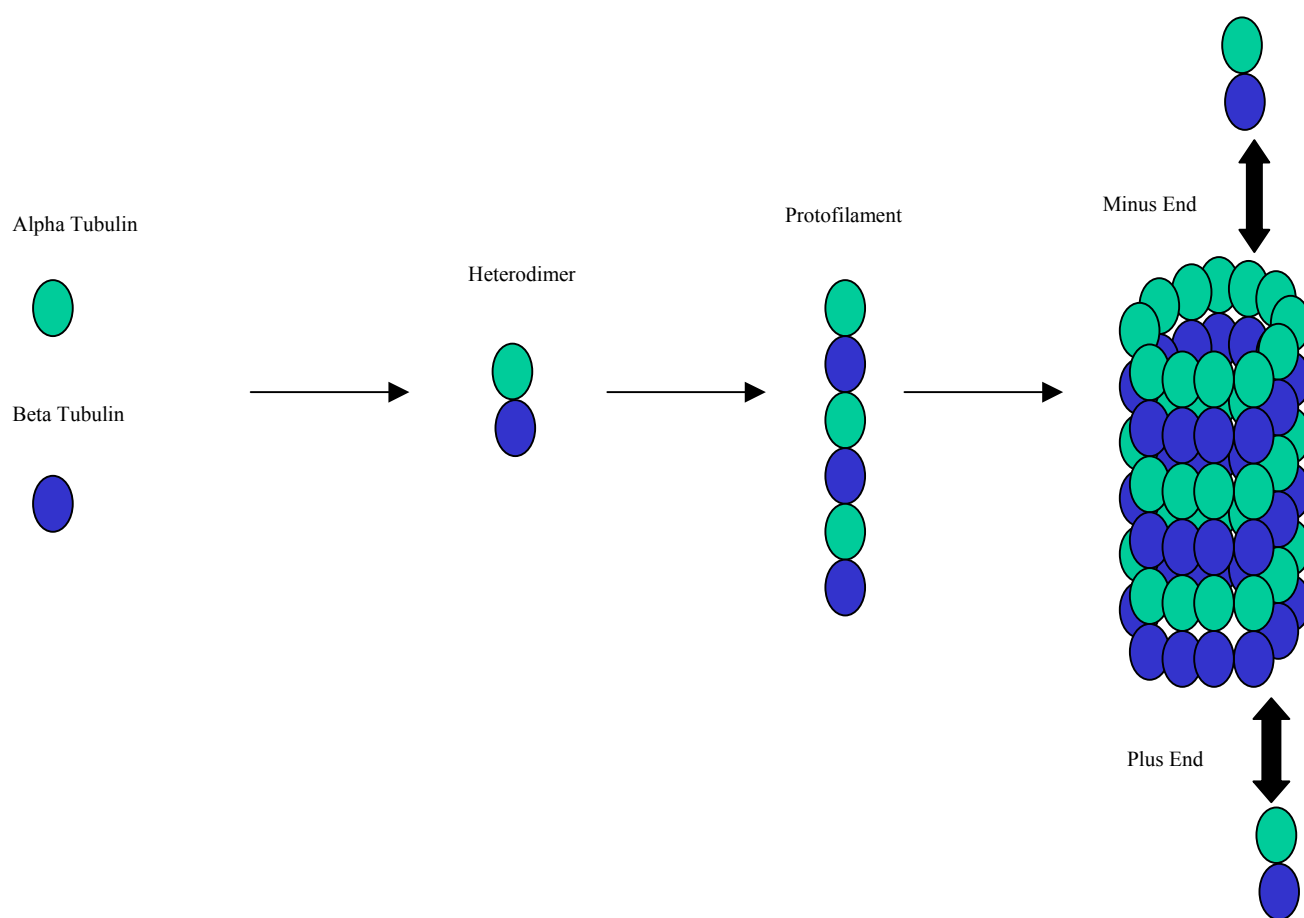


Figure 1: Microtubule Assembly. Alpha and beta tubulin assemble in the cell to form heterodimers. These heterodimers then attach longitudinally to form protofilaments. Thirteen of these protofilaments line up laterally and form a ring of protofilaments called a microtubule. Once the microtubule is completely assembled, heterodimers assembled and disassemble at each end; this type of interaction where populations of microtubules are constantly being partially disassembled and reassembled is called steady state.

To be effective as cytoskeletal elements, microtubules must be highly organized within the cell. To accomplish this, most cells have employed a structure called the microtubule-organizing center (MTOC) (Vaughn and Harper, 1998). This, in most cases, is the structure from which the microtubules radiate outward into the cell. These MTOCs allow the microtubules to become organized in such a way as to help perform the correct role in the cell (Baas, 1996). Using the MTOC as a reference point, one can clearly identify two different ends for any given microtubule with one end at the MTOC (the minus end) and the other projecting out into the cell (the plus end) (Dammermann et al., 2003). However, the polarity of the microtubule does not come from the MTOC, rather, it is a function of how microtubules assemble. Microtubules assemble in a regular pattern with the alpha subunit always interacting with the beta subunit of the longitudinally adjacent heterodimer, and vice versa (Figure 1). This head-to-tail assembly gives the polymer a regular pattern and two distinct ends (Mayer, Jurgens 2002). Since microtubules have two distinct ends, they are classified as polar filaments. These ends represent the regular pattern made by head-to-tail assembly, with the alpha tubulin monomer always exposed at the minus end and the beta tubulin always at the plus end (Dutcher 2001).

The basic structure of a microtubule is that of a hollow cylinder and most of the microtubules found in the cell will possess this sort of structure. There are, however, variations on this theme. Doublet and triplet microtubules have been observed in cells, but these are found only in specialized structures such as axonemes and basal bodies (Mayer U., 2002)

In vitro, polymerization of purified tubulin is very much a function of

concentration and temperature. Heterodimers will start to polymerize only when a threshold concentration exists. The critical concentration, denoted  $C_c$ , is the threshold concentration at which heterodimers will polymerize into microtubules in vitro. If concentrations were to dip below this, the microtubules would depolymerize. Critical concentration is only a potential problem in vitro because the cell must always maintain a high enough concentration of alpha-beta heterodimers to form microtubules; this is a necessity to the survival of the cell. The other major factor in microtubule assembly is temperature. The optimal temperature for mammalian microtubule polymerization is around 35-39°C. If the temperature fluctuates too far from this range in either direction, then the heterodimers disassemble from one another and the microtubule falls apart (Wallin and Stromberg, 1995). Whether or not the microtubule can reassemble later depends on whether the subunits simply disassembled due to cold or whether they are denatured due to heat. There are, however, exceptions to these thermal effects (Gupta et al., 2001). It has been shown that microtubules from some cold water fish are intrinsically cold stable (Detrich et al., 2000), while tubulin from certain fungi and algae are heat stable.

### **Tubulin Structure**

Alpha and beta tubulin monomers are very similar in tertiary structure (Li et al., 2002a). Each monomer is composed of two beta sheets composed of six and four strands each connected by intervening loops to twelve surrounding alpha helices (Figure 2) (Nogales et al., 1998). The amino acid sequence or primary structure of the tubulin

monomer structure has been divided into three functional domains, an N-terminal domain consisting of 205 residues, which forms a Rossmann fold typical of nucleotide binding proteins (Rossmann et al., 1974), an intermediate domain consisting of residues 206-381, and a C-terminal domain consisting of residues 382-451 (Downing and Nogales, 1998).

It is currently thought that the loop between the alpha helix H1 and the second beta strand S2, also known as the N-loop, is the most variable in primary structure and may help determine functional differences between the alpha and beta monomers (Fygenson et al., 2004) such as nucleotide binding and hydrolysis. Contacts between heterodimers forming a protofilament (Figure 1) are known as longitudinal contacts while contacts between adjacent protofilaments to form a microtubule are known as lateral contacts (Figure 1). Only lateral contacts have been well defined, longitudinal contacts between tubulin monomers are large and not well defined (Lowe et al., 2001). The N-loop and the M-loop play significant roles in lateral contacts between protofilaments in both alpha and beta tubulin (Nogales, Wolf et al 1998). These loops on the adjacent protofilaments interact with each other to form the microtubule.

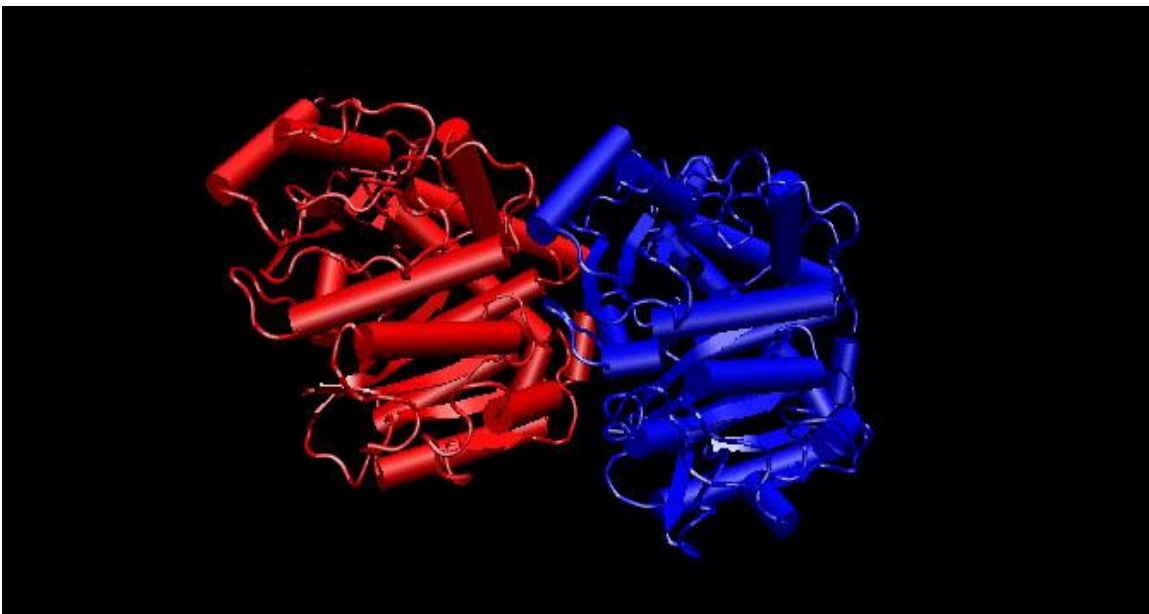


Figure 2: The structure of the bovine brain tubulin heterodimer. The alpha tubulin is colored red and the beta tubulin is colored blue. VMD rendering is based upon 1TUB.pdb file (Nogales, Wolf et al 1998). Cylinders represent alpha helices, arrows represent beta strands while intervening strands represent loops.

## Thermophilic Algae

This study focuses on the primitive red algae *C. merolae* and *C. caldarium*. Both organisms are thermophiles and acidophiles found in volcanic springs in a variety of locations (Pinto G., 1994). These organisms are obligate autotrophes, which means they rely on photosynthesis exclusively as a food source. *C. merolae* and *C. caldarium* have nuclear genomes around 8 Mbp and 18 Mbp respectively, consistent with those of other single celled eukaryotes (Toda et al., 1995).

The environment in which these algae live would almost seem to be better suited for archaea than eukaryotes (Roberts, 1998). However, although these organisms live at a pH range of 2-4, their cytosol is very near neutral (Seckbach, 1994). This implies two things, that there is a mechanism for keeping the cytosol near neutrality, and that any surface proteins present are stable at a low pH (Rothschild and Mancinelli, 2001). Also both organisms can exist at temperature ranges from 40 - 55 C° (Donachie et al., 2002). It should therefore be no surprise that the genomic DNA from both organisms shows a G-C content of over 55% (Takahashi, Takano et al. 1995).

It would stand to reason that the functional differences reported thus far must have underlying structural differences within the molecular machinery of the algae. It has been the structural differences and the evolutionary relationships between these algae and other plants that has consumed most of the molecular and biochemical research done on these organisms to date. For example, through microscopy and a variety of molecular techniques it has been discovered that *C. merolae* has no observable contractile ring, and the actin gene has substitutions present in otherwise normally well-conserved regions.

(Takahashi et al., 1995.)

### **Protein stability**

As seen in the case of mammalian tubulin, many eukaryotic molecules only function in a narrow temperature range. But this does not mean that these organisms do not have to contend with occasional temperatures slightly outside of this range. Recent studies have suggested that most organisms take care of this problem by employing one of two strategies. One strategy (used by eukaryotes to counteract temperature outside their normal temperature range) is to produce a chaperone protein to bind to the target enzyme and keep it from denaturing, or to refold denatured proteins (Pace, 2000). These types of proteins are generally called heat or cold shock proteins. Another strategy is to have multiple forms of a given enzyme with different optimal temperatures. An example of this is found in fish for the enzyme acetylcholinesterase (Hickman et al., 1998).

However, even molecules designed to function at inhospitable temperatures, such as the heat and cold shock proteins, have to employ certain structural strategies in order to survive the temperature ranges in which they function. Therefore, inherent thermostability is more relevant to this project because *C. merolae* and *C. caldarium* exist at temperatures that are prohibitively high for other eukaryotes, and the possibility that heat shock proteins are constantly engaged seems extremely unlikely.

From the literature, there seem to be four general strategies for thermo-stabilizing a protein. The use of disulfide bonds, salt bridges, hydrogen bonds, and changes in the overall electrostatic environment of the protein's surface (Flores and Ellington, 2002).

The first, disulfide bonds, are bonds formed between two cysteine side chains. These side chains are usually on different polypeptides, or if on the same polypeptide, can be quite far apart in terms of the primary structure. These disulfide bonds work to stabilize the protein by “locking” the polypeptide into a given conformation. It has been shown experimentally that proteins in which disulfide bonds were removed through point mutation have severely curtailed activity at elevated temperatures when compared to the wild type (Li et al., 2002b).

Salt bridges are accomplished by the interaction of oppositely charged side chains with one another to produce ionic bonds (Pace, 2000). These salt bridges work very well by “locking down” particular segments of the protein, thus limiting the movement and possible loss of conformation of a protein exposed to high temperatures. For example, after a comprehensive genome study, it was found that the genomes of hyperthermophiles such as *Pyrococcus horikoshii* have proteins that contain more salt bridges than comparative mesophiles (Das and Gerstein, 2000).

The third strategy used to stabilize proteins is the formation of hydrogen bonds. Hydrogen bonds form between two partially charged atoms. Though they may be weaker than a covalent bond individually, they are more common in proteins than disulfide bonds or salt bridges and thus can have more of an affect on the tertiary structure. These types of interactions have been suggested as the most common method of protein stabilization (Pucciarelli, 2002). For example, the protein SP1 from *Populus tremula* which has been shown to be thermostable, relies on hydrogen bonds to stabilize external loops (Rathore 2004).

Lastly, some proteins are stabilized by a change in the overall electrostatic

potential. For example, the cold shock protein Bs-Csp was converted to a thermophilic protein by adding a leucine at position 66 and thus dramatically changing the electrostatic potential (Perl et al., 2000). These changes which include hydrophobic-hydrophobic interactions are precipitated by a few “keystone” side chains which, while not having a specific local effect, are responsible for changing the overall electrostatic potential of the surface of the protein. (Torrez et al., 2003). This change is thought to affect the flexibility of the protein (Grottesi et al., 2002).

### **Protein Modeling**

A significant amount of work in the field of molecular biology is devoted to the sequencing of genes. However, once this task is completed, the next step in understanding the true nature of the protein encoded by the gene is the analysis of the tertiary structure of that protein; this is the first step in understanding how the protein actually interacts in its environment (Gigant et al., 2000). There are two basic ways to discern the tertiary structure of a protein, X-ray crystallography or nuclear magnetic resonance (NMR) (Sali and Kuriyan, 1999).

Given the large variety of proteins in existence, and because of the inability of modern science to determine the tertiary structure of a great many proteins, it is impractical to attempt to solve protein structures by X-ray crystallography or nuclear magnetic resonance (NMR) (Baker and Sali, 2001). This is where protein modeling using computer algorithms can help. There are two different types of computer-assisted protein modeling, de novo and homology modeling. De novo modeling assumes that

every individual atom in the protein will find its lowest energy conformation. The algorithms involved in these types of programs are specifically designed to find this conformation level. This type of modeling is rife with problems due to its many assumptions (Baker and Sali, 2001). For example, the underlying assumption that an atom will always assume its absolute lowest energy state is sound, but tends to break down when conflicts arise involving alternate conformations of equal thermal stability. The de novo modeling system also has problems taking into account chaperone-assisted folding, because of de novo modeling inability to predict the effect the chaperon will have on the protein. The second type of computer-assisted modeling works using an existing protein structure as template. This type of approach, called homology modeling, is a powerful tool because it does not make nearly as many assumptions.

With this in mind, there are some issues that must be addressed when using homology modeling. One of those issues is that the model created using homology modeling is only as good as the original template provided. The second issue to be addressed is the overall sequence similarity between the template and target protein. There must be at least 30% sequence identity between the template and target protein before the homology model can approach accuracy (Sanchez et al., 2000). Homology modeling seems to be the best choice for the current project because the tubulin structure has been solved for bovine brain tubulin, and tubulin is known for its high conservation throughout the eukaryotic genome. Therefore the tubulin proteins will have more than enough identity to produce a useful homology model.

## **Materials and Methods**

### **DNA extraction from the red algae, *C. merolae* and *C. caldarium***

Algal cultures were grown in Allen's Medium (Takahashi et al., 1995.) at 45°C to cell saturation levels ( $>10^7$  for *C. caldarium* and  $>10^8$  for *C. merolae*), and 45 ml of saturated culture were collected and centrifuged for 10 minutes at 3300xg. The pellet was resuspended in 5 ml stasis buffer (50 mM Tris HCl pH 8.0 and 20 mM EDTA), and was centrifuged as described above. This washing process was repeated two more times and then 1 ml of stasis buffer was added to the pellet and the solution was transferred to a 2 ml tube containing 1.4 ml of 0.5 mm silicon beads. This tube was agitated at 4200 rpm for 190 seconds three times per sample in a mini-beadbeater (Biospec Products). SDS (1% final concentration) and Proteinase K (1mg/ml final concentration) were also added to the cell lysate and the solution was incubated for 16 hours at 60°C.

After the incubation, the solution was collected in a 15ml conical tube and an equal volume of Tris-saturated phenol was added. This mixture was separated into two distinct phases, an aqueous phase and organic phase, by centrifugation at 2000xg for 3 minutes. The aqueous phase was then collected and the process was repeated with phenol and chloroform, the aqueous phase was collected from each of those extractions. After the final extraction, 3M sodium acetate (pH 5.0) was added to the aqueous phase followed by 2.5 volumes of ice cold 100% ethanol. This solution was incubated at -80°C for one hour, centrifuged for five minutes at 10,000g, and the DNA-containing pellet was washed with 70% ethanol, dried overnight, and resuspended in ddH<sub>2</sub>O.

### Sequencing of the *C. merolae* and *C. caldarium* alpha and beta tubulin

The internal portions of the alpha and beta tubulin genes were amplified from *C. merolae* and *C. caldarium* genomic DNA using polymerase chain reaction (PCR). PCR reactions used Taq polymerase for 34 cycles with 95°C as a denaturing temperature, 50°C as an annealing temperature, and 72°C as an extension temperature. Alpha tubulin sequences were amplified using 1F and ATBB primers, while beta tubulin sequences were amplified using BTBA and ATBB primers (Table 1). ATBB and BTBA were degenerate primers designed based upon highly conserved portions of the eukaryotic alpha and beta tubulin genes. Amplified products were run on a 1% agarose gel and bands of the correct size were cut from the gel and purified using a Qiaquick gel extraction kit (Qiagen), ligated into the pT7Blue-3 (Novagen) plasmid and transformed into Nova Blue *E. coli*. After clones containing the tubulin insert were identified by blue white screening and colony PCR, plasmids were purified (Clonetechn minprep kit), and the insert was sequenced at the Virginia Bioinformatics Institute (VBI) core laboratory facility. Both strands of each insert were sequenced at least twice to insure accuracy and a Blast search was done against Genbank plant DNA sequences to confirm that each insert was in fact alpha or beta tubulin.

Once the internal tubulin sequences were determined, the N-terminal regions of the alpha and beta tubulin genes from *C. caldarium* were obtained using rapid amplification of genomic ends (RAGE) PCR (Liu and Baird, 2001) as illustrated in Figure 3. This was accomplished by partially digesting 10 µg of genomic DNA with the

restriction enzyme NlaIII. After digestion, terminal transferase (New England Biolabs) was used to add a poly cytosine tail to each DNA fragment. A selective anchor primer (SAP) containing a poly guanine region was used in combination with a gene specific primer (GSP1) to perform the first round of PCR. Then a second round of PCR using a second gene specific primer (GSP2) was used in combination with a universal amplification primer (UAP) that corresponds to regions inside SAP, this second round was done to further refine the product from round one PCR. Products amplified from the second round of RAGE PCR were run on a 1% agarose gel and bands > 500 base pairs were cut, cloned and sequenced as described above. The gene specific primers 1R and 2R were used in the case of N-terminus RAGE PCR. RAGE PCR was also done in an attempt to obtain the C-terminal regions of the tubulin genes from *C. caldarium*, but failed.

After the sequences of the N-terminal regions of the *C. caldarium* alpha and beta tubulin genes were determined, a primer was designed that annealed to upstream regions of the tubulin genes. This primer (AUP) or (BUP) was coupled with the downstream primer ATBB in a standard PCR reaction using PFU polymerase, to confirm the N-terminal sequence of *C. caldarium*. This PCR product was cloned and sequenced as described above.

**Table 1: Primers used to generate tubulin sequence**

**ATBB: CCY TCY TCC ATD CCY TCD CCR ACR TAC CAR TG**

**BTBA: GGT CAR TRY GGH AAY CAR RTY GG**

**A1F: GCC CTC GGT GGT GGA TGA AGT GC**

**A2F: TAT TTG TCG GCG GTC ACT CG**

**B1F: TCA GCA AGA TTC GCG AGG AGT A**

**B2F: AGA ACT CGG CGT ACT TTG TCG**

**BUP: GCG TGC GCA ATC GTT ACA A**

**B3F: CGA TGG AAT TTA CGG AAG CAG A**

**AUP: CCC TTG GCG TTG GTT CTG TT**

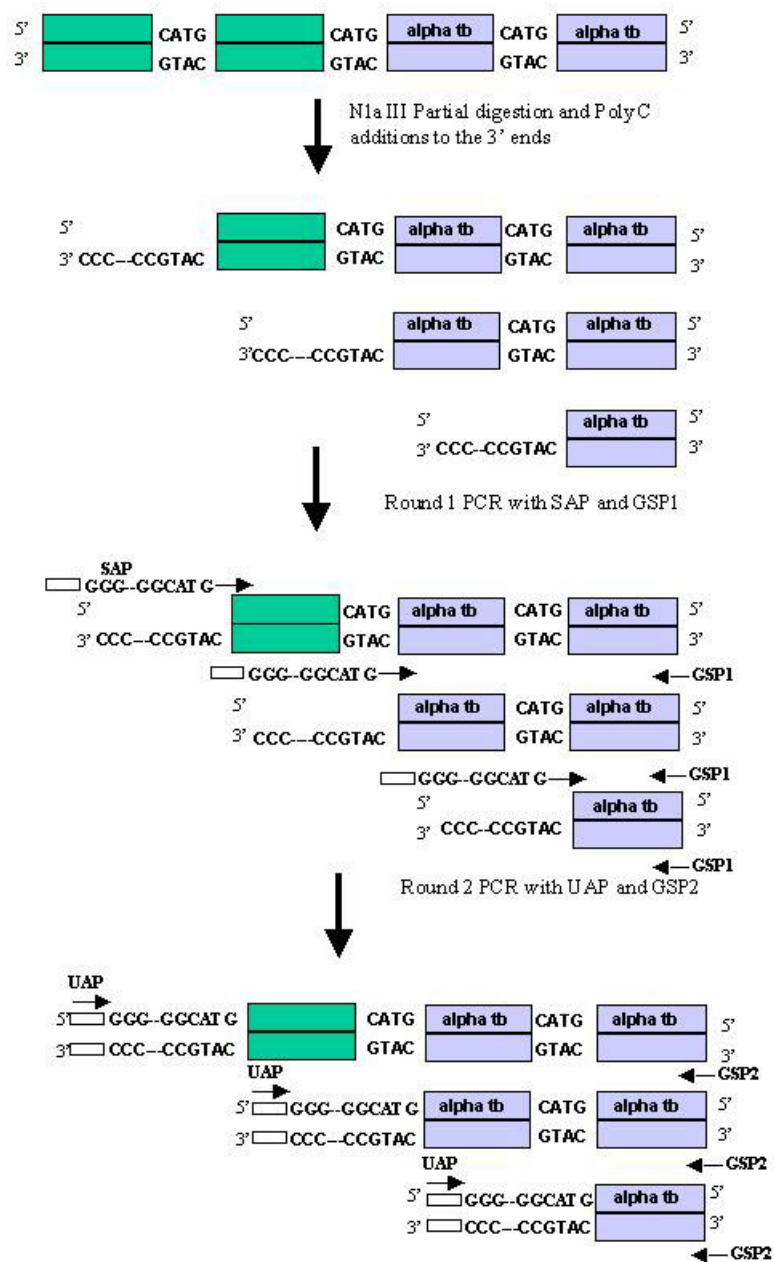
**SAP: GGA GAC TGA CAT GGA CTG AAG GAG TAA AGGHHGGHHGGGCAT**

**UAP: CCT CTG ACT GTA CCT GAC TTC CTC ATT**

**Key: D = A or T or G, H = A or C or T, R = A or G, Y = C or T**

**Underlined regions indicate complementary portions between primers UAP and SAP**

Figure 3: Rapid Amplification of Genomic Ends (RAGE PCR). Nla III partial digestion generates DNA fragments of varying sizes with a 3' overhang. Terminal transferase then adds poly-C to each 3' end. Tailed fragments of interest were then amplified by a Selective Anchor Primer (SAP) and gene specific primers (GSP1). A second round of PCR uses a second gene specific primer (GSP2) with Universal Amplification Primer (UAP), which anneals to SAP (Liu and Baird, 2001).



## Sequence Analysis and Homology Modeling

Once the nucleotide sequence for the *C. caldarium* tubulin genes were obtained in the current study and the complete sequence for *C. merolae* was determined independently (Nozaki 2003), the amino acid sequences were predicted using the program Sixframe (Biology Workbench). The predicted alpha and beta amino acid sequences were aligned with 250 mesophilic counterparts in order to identify amino acid differences that may contribute to the inherent stability observed in these organisms. These alignments were generated by Clustal Alignment (Biology Workbench). Differences in the amino acid sequences between the thermophiles and the mesophiles that did not change the charge or significantly alter the size of the amino acid side chain were considered conserved substitutions and not analyzed in this study.

The tertiary structures of the algal alpha and beta tubulin proteins were modeled using the homology modeling software Modeller version 6.00 (Baker and Sali, 2001). This was accomplished by placing the amino acids from the algal tubulin monomer in the same three-dimensional orientation as a previously solved structure of bovine brain tubulin, 1TUB.pdb (Nogales, 1998). Modeller produced five approximate models of the same structure each based on different energetic conformations. A PERL script file was used to average the five models into one. The energy minimization software Assisted Model Building with Energy Refinement (AMBER) (Gogonea et al., 1999) was then used to place all atoms in their most favorable energy conformation.

Once the models were produced and optimized, the visualization software Visual Molecular Dynamics (VMD) was used to identify the positions of the non-conserved

differences in the tertiary structure of the algal models. Amino acids residing within 5, 3.5, and 2 Å of the non-conserved differences were identified using the program visual molecular dynamics (VMD) (Kumar et al., 2000). Each non-conserved difference was analyzed along with its tertiary neighbors for their contributions to the overall thermostability of the heterodimer.

To illustrate possible contributions to thermostability, two renderings were taken of each non-conserved difference, one to illustrate the location of non-conserved differences in the tertiary structure of the monomer and the second to illustrate which amino acid side chains interact with the non-conserved differences. All renderings were taken showing every non-conserved difference and all amino acids within 3.5 angstroms to illustrate all possible interactions. The distance of 3.5 angstroms was chosen to reduce the confusion generated by having a large number of amino acids in one rendering possibly obscuring each other. Also, with a resolution of 3.7 angstroms associated with the solved structure, 3.5 angstroms is the closest one can get when determining at which three dimensional orientation the side chain is likely to be with a large degree of confidence. The non-conserved differences and all amino acids with 3.5 angstroms were then analyzed to find possible mechanisms for the improved thermostability apparent in the algal tubulin.

### **Computational Thermostability Experiments:**

Protein data bank (PDB) files of alpha and beta tubulin monomers from *C. merolae* and *C. caldarium* generated using Modeller, and the PDB file of the alpha and beta tubulin heterodimer (1TUB.PDB) (Nogales 1999), were loaded into the Leap

module of AMBER. A box of water molecules was built around each tubulin monomer so that the solvation box was constructed so that no water molecule came within nine angstroms of the edge of the box. Sodium ions were added to the solvation box to neutralize any charges present in the tubulin monomers. Molecular dynamics was performed for 100 ps with the Sander module of AMBER, to equilibrate the water molecules and sodium ions. Then each system was minimized and heated to 45°C over a period of 45 ps. Each system was then equilibrated for an additional 35 ps under constant volume conditions after which constant pressure conditions were imposed for the remainder of the simulation (total simulation time was 1 ns). The tubulin monomers were then analyzed for total atomic root mean squared (RMS) deviation in angstroms relative to the starting structure and each amino acid side chain was analyzed for atomic positional fluctuation in angstroms. These data were graphed and the tubulin monomers from the thermophiles were compared to the tubulin monomers of the mesophile for differences in RMS and atomic fluctuation values.

## **Results**

This project was accomplished in three distinct stages. The goal for the first stage was to sequence the entire alpha and beta tubulin genes from two species of red algae, *C. merolae* and *C. caldarium*. After the internal portions of the *C. merolae* and *C. caldarium* tubulin genes were sequenced, RAGE PCR was used to amplify the N-terminal alpha and beta tubulin sequences from *C. caldarium*. After the nucleotide sequences were determined, the project then proceeded to the second stage, analysis of the predicted amino acids sequences. To accomplish this, 250 mesophilic alpha tubulin sequences were compared to the alpha tubulin amino acid sequences from *C. caldarium* and *C. merolae* in order to find unique amino acid positions shared in both thermophiles but absent in mesophiles. This process was repeated with beta tubulin sequences. Once these unique amino acid positions were found, the side chains were analyzed to determine if the amino acid differences altered the chemistry of that residue. If so, it was logged as a non-conserved difference and analyzed in the third stage.

In stage three, a structural analysis was performed on the algal tubulin monomers. This was accomplished by building a homology model of the tertiary structure of each thermophilic tubulin monomer and mapping the non-conserved substitutions onto these models. These non-conserved differences were analyzed in conjunction with neighboring amino acids to assess what, if any, contributions these could make to thermo-stabilizing the tubulin monomer, protofilament, and microtubule.

### Sequencing the alpha and beta tubulin genes of *C. merolae* and *C. caldarium*

PCR was used to amplify the internal portions of both alpha and beta tubulin genes from *C. merolae* and *C. caldarium*. Gel electrophoresis of the amplified products revealed bands of the expected size of 1030 bp for alpha tubulin and 1100 bp for beta tubulin (Figure 4), which were then cloned and sequenced. After the internal portions of the tubulin genes were sequenced, (Nozaki et al., 2003) published the complete sequences of alpha and beta tubulin from *C. merolae*, which led me to focus on sequencing the complete alpha and beta tubulin genes *C. caldarium*. RAGE PCR was used to determine the N-terminal sequences of the alpha and beta tubulin monomers of *C. caldarium*. RAGE PCR was also used in an effort to complete the C-terminal regions of the alpha and beta tubulin sequences from *C. caldarium* although this was ultimately unsuccessful, leaving an estimated 90 to 100 nucleotides from the completed sequences from *C. caldarium*.

Genomic DNA was digested with *Nla*III (Figure 5a) and fractionated on a 1% agarose gel, which produced a smearing pattern with an intense band at approximately 250 bp. This DNA was used in RAGE PCR to amplify N-terminal ends for both alpha and beta tubulin from *C. caldarium*. After two rounds of RAGE PCR, a diffuse band was seen at around 500 bp for both alpha and beta tubulin genes (Figure 5b). After the internal and N-terminal sequences for both alpha and beta tubulin from *C. caldarium* were determined (Figure 6), the project entered the next phase.

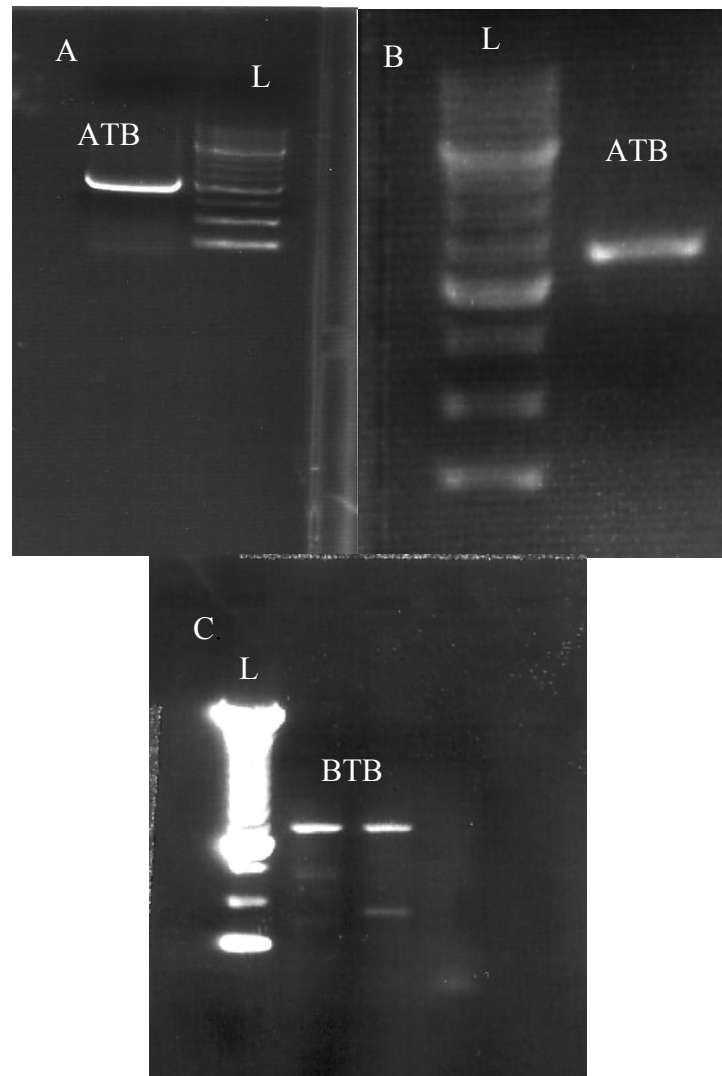


Figure 4: Amplification of internal portion of alpha and beta tubulin genes from *C. caldarium* and *C. merolae*. PCR amplification of 1030 bp internal portion of alpha tubulin from *C. caldarium* (A) and *C. merolae* (B). This PCR was done with A1F as a forward primer and ATBB as a degenerate reverse primer. PCR amplification of 1100 bp internal portion of beta tubulin from *C. caldarium* and *C. merolae* (C). This PCR was done with BTBA as a forward primer and ATBB as the reverse primer. Molecular weight standards (L) used were 250 bp ladders (Novagen).

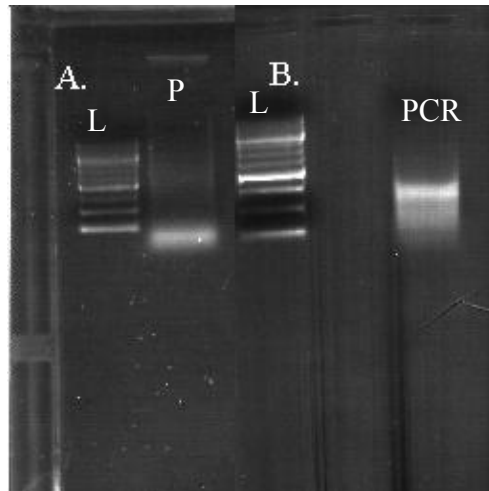


Figure 5: RAGE PCR amplification of N-terminus regions of tubulin genes from *C. caldarium*. (A) *C. caldarium* genomic DNA was digested with *Nla*III and fractionated for one hour on an agarose gel (P), then all DNA > 500 bp was extracted and prepared for RAGE PCR. (B) Diffuse band ~ 500bp was produced by the second round of RAGE PCR. These results are characteristic of both alpha and beta N-terminal reactions. A 250 base pair ladder (L) was used on both gels.

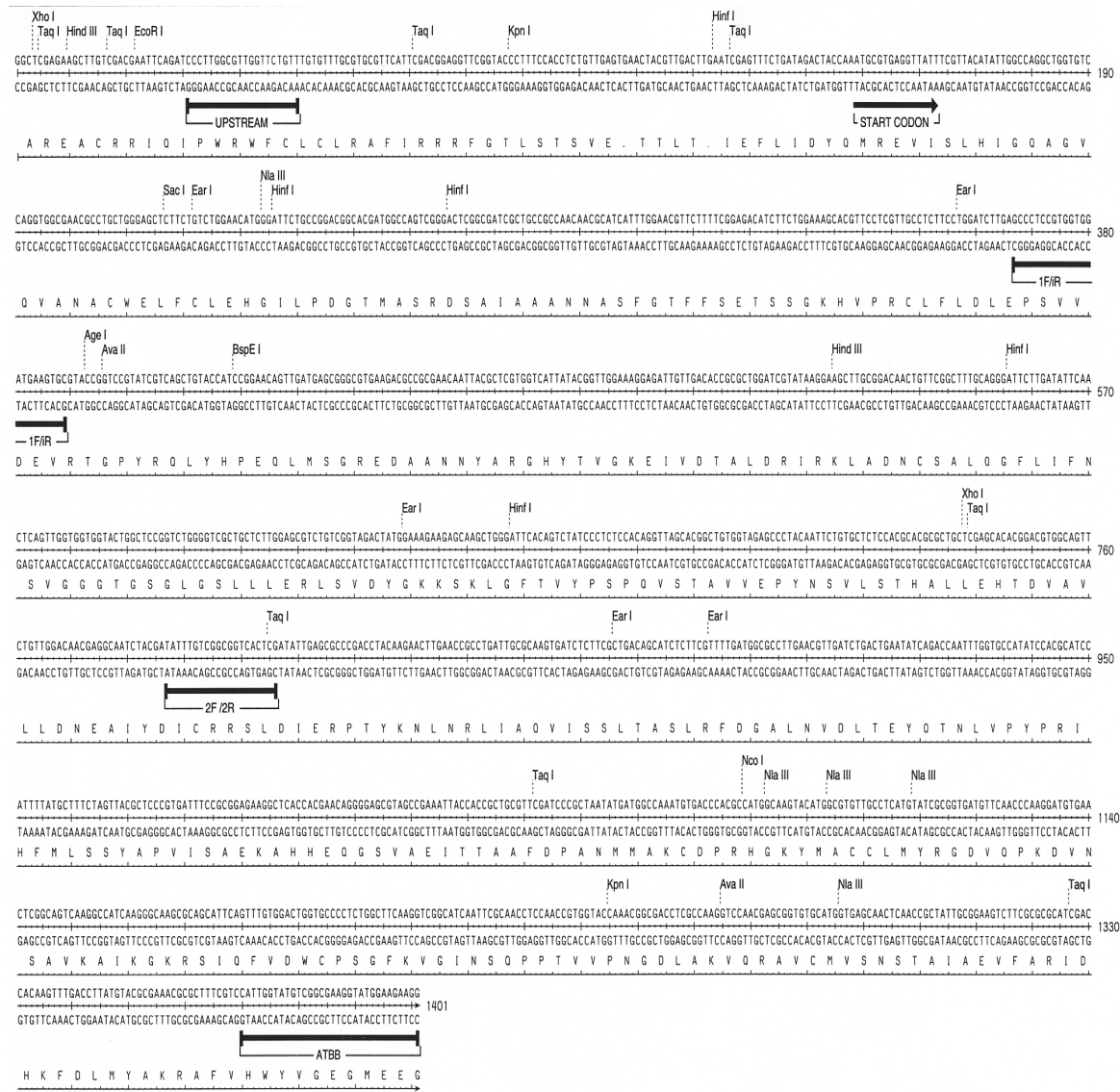


Figure 6: Alpha tubulin genomic DNA and predicted amino acid sequences (*C. caldarium*). The locations of the primers used to generate this 1401bp sequence are indicated.

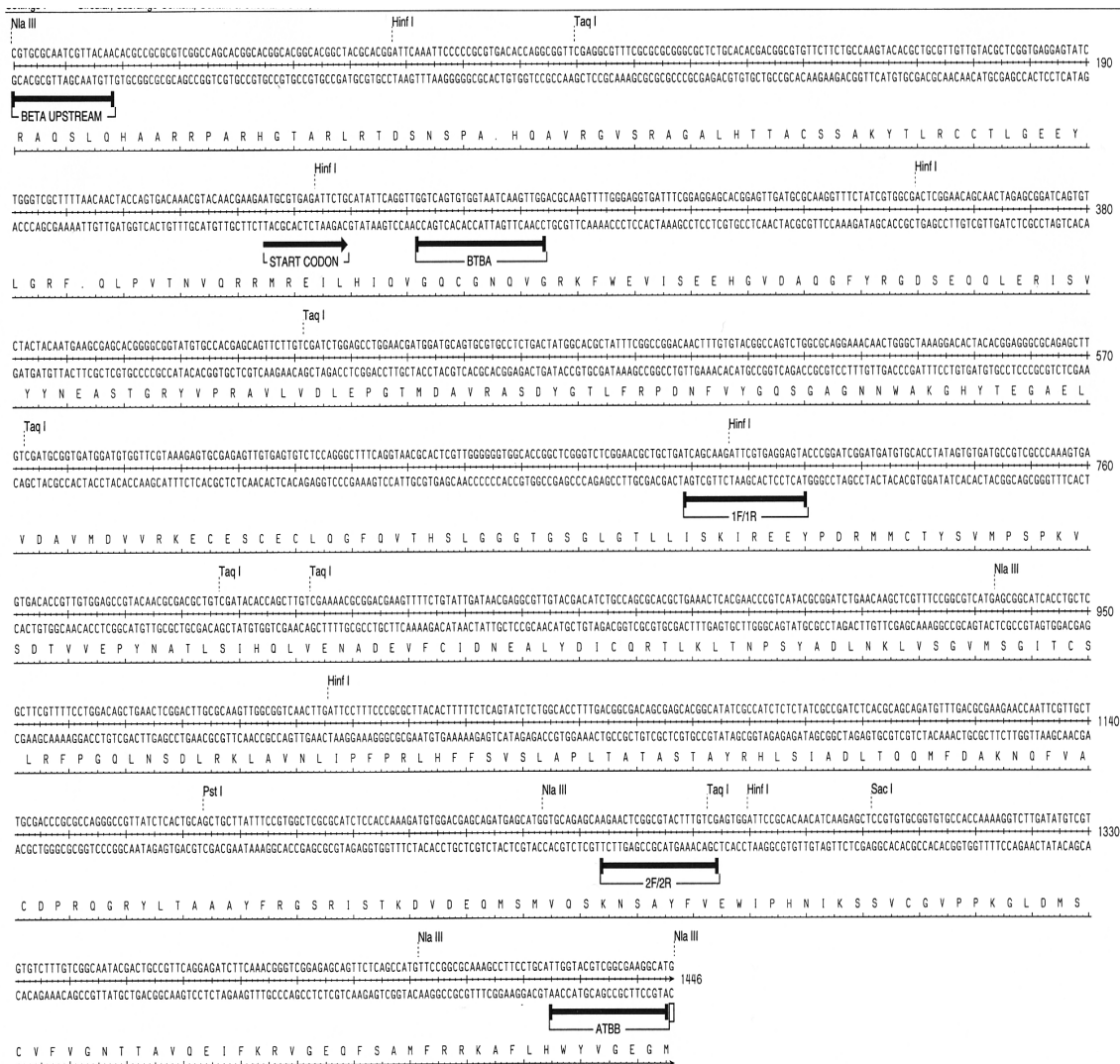


Figure 7: Beta tubulin genomic DNA and predicted amino acid sequences (*C. caldarium*).

The locations of the primers used to generate this 1446bp sequence are indicated.

## Sequence alignment and analysis

Both alpha and beta algal tubulin genes are highly conserved, with over 75% identity at the nucleotide level (Figure 8) and over 90% identity at the amino acid level (Figures 9 and 10). This is consistent with the high conservation seen between many other closely related tubulin monomers. For example the amino acid sequences of human and porcine alpha tubulin are 95% identical.

When amino acid sequences for the alpha and beta tubulin monomers from the thermophiles were compared to their mesophilic counterparts, 19 total differences were identified in the alpha tubulin monomer (Figure 11), 9 of which changed the charge or significantly changed the size of the side chain and were termed non-conserved (Table 2). Comparisons done with beta tubulin identified 25 total differences (Figure 12), 12 of which were non-conserved (Table 2). Five of the 19 changes in the alpha tubulin monomer were between residues 30 to 60 and 5 substitutions were located between residues 325 to 370. In the beta tubulin monomer 8 of the 25 changes were located between residues 269 and 310 and 6 were located between residues 355 and 380. Approximately 71% of the non-conserved changes were either ionic or polar amino acids substituted for hydrophobic amino acids in the mesophiles.

This alignment illustrates the 76.4% identity between the alpha tubulin genes from the thermophiles. Dashes at the end of sequence indicate lack of sequence data at the C-terminus.

```

      10      20      30      40      50      60
MREVISLHIGQAGVQVANACWELFCLEHGILPDGTMASRDSAIAAANNASFGTFFSETSS
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
MREVISLHIGQAGVQVANACWELFCLEHGILPDGTMASRDSAIAAANNASFGTFFSETSS
      10      20      30      40      50      60

      70      80      90     100     110     120
GKHVPRCLFLDLEPSVVDEVRTGPYRQLYHPEQLMSGREDAANNYARGHYTVGKEIVDTA
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
GKHVPRCLFLDLEPSVVDEVRTGPYRQLYHPEQLMSGREDAANNYARGHYTVGKEIVDTA
      70      80      90     100     110     120

     130     140     150     160     170     180
LDRIRKLADNCSALQGFLIFNSVGGGTGSGLSLLERLSVDYGKSKLGFVYPSPQVS
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
LDRIRKLADNCSALQGFLIFNSVGGGTGSGLSLLERLSVDYGKSKLGFVYPSPQVS
     130     140     150     160     170     180

     190     200     210     220     230     240
TAVVEPYNSVLSTHALLEHTDVAVLDDNEAIYDICRRSLDIERPTYKNLNRLIAQVISSL
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
TAVVEPYNSVLSTHALLEHTDVAVLDDNEAIYDICRRSLDIERPTYKNLNRLIAQVISSL
     190     200     210     220     230     240

     250     260     270     280     290     300
TASLRFDGALNVDLLEYQTNLVYPRIHFMLSSYAPVISA EKAHHEQGSVAEITTAADF
P
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
TASLRFDGALNVDLLEYQTNLVYPRIHFMLSSYAPVISA EKAHHEQGSVAEITTAADF
P
     250     260     270     280     290     300

     310     320     330     340     350     360
ANMMAKCDPRHGKYMCCCLMYRGDVQPKDVNSAVKAIKSKRSIQFVDWCPSGFKVGINSQ
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
ANMMAKCDPRHGKYMCCCLMYRGDVQPKDVNSAVKAIKSKRSIQFVDWCPSGFKVGINSQ
     310     320     330     340     350     360

     370     380     390     400     410
PPTVVPNGDLAKVQRAVCMVSNSTAIAEVFARIDHKFDLMYAKRAVHWYVGEEMEE---
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
PPTVVPNGDLAKVQRAVCMVSNSTAIAEVFARIDHKFDLMYAKRAVHWYVGEEMEEGEF
     370     380     390     400     410     420

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SEAREDLAALEKDYIEVAADTNEEEEEY
      430      440

```

Figure 9: Alignment of alpha tubulin amino sequences from *C. caldarium* (top) and *C. merolae* (bottom). This alignment illustrates the 93% identity between the alpha tubulin amino acid sequences of the thermophiles. There are three differences, all of which are conserved (K227R, T295S, G339S). Dashes indicate lack of sequence data.

```

      10      20      30      40      50      60
MREILHIQVGQCGNQVGRKFEVISEEHGVDAQGFYRGDSEQQLERISVYYNEASTGRYV
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
MREILHIQVGQCGNQIGRKFEVISEEHGVDAQGFYHGDSEQQLERISVYYNEASTGRYV
      10      20      30      40      50      60

      70      80      90     100     110     120
PRAVLVDLEPGTMDAVRASDYGTIFRPDNFVYGQSGAGNNWAKGHYTEGAELVDAVMDVV
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
PRAVLVDLEPGTMDAVRASEYGTIFRPDNFVYGQSGAGNNWAKGHYTEGAELVDAVMDVV
      70      80      90     100     110     120

     130     140     150     160     170     180
RKECESCECLQGQFQVTHSLGGGTGSGGLGTLISKIREEYPDRMMCTYSVMPSPKVS DTVV
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
RKECESCECLQGQFQVTHSLGGGTGSGGLGTLISKIREEYPDRMMCTYSVMPSPKVS DTVV
     130     140     150     160     170     180

     190     200     210     220     230     240
EPYNATLSIHQLVNADEVFCIDNEALYDICQRTLKLTNPSYADLNKLVSGVMSGITCSL
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
EPYNATLSIHQLVNADEVFCIDNEALYDICQRTLKLTNPSYADLNKLVSGVMSGITCSL
     190     200     210     220     230     240

     250     260     270     280     290     300
RFPGQLNSDLRKLA VNLIPF PRLHFFSVSLAPLTATASTAYRHLSIADLTQQMFDAKNQF
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
RFPGQLNSDLRKLA VNLIPF PRLHFFSVSLAPLTATASTAYRHLSIADLTQQMFDAKNQF
     250     260     270     280     290     300

     310     320     330     340     350     360
VACDPRQG RYLTA AAYFRGSRISTKDVDEQMSMVQSKNSAYFVEWIPHNKSSVCGVPPK
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
VACDPRQG RYLTA AAYFRGSRISTKDVDEQMSMVQSKNSAYFVEWIPHNKSSVCGVPPK
     310     320     330     340     350     360

     370     380     390     400     410
GLDMSCVFVGNTTAVQEIFKRVGEQFSAMFRRKAFLHWYVGE GMDPMEFTEAESNM----
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
GLDMSCVFVGNTTAVQEIFKRVGEQFSAMFRRKAFLHWYTGE GMDPMEFTEAESNMNDLV
     370     380     390     400     410     420

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SEYQQYEQAVA EDEDEDVEQEFGAYRTGEEDRTAIESSIMS
      430      440      450      460

```

Figure 10: Alignment of beta tubulin amino sequences from *C. caldarium* (top) and *C. merolae* (bottom). This alignment illustrates the 90% identity between the beta tubulin amino acid sequences of the thermophiles. There are three differences, all of which are conserved (V16I, R37H, D79E). Dashes indicate lack of sequence data.

Figure 11: Alignment of thermophilic alpha tubulin consensus sequence (top) and a mesophilic consensus sequence determined from 250 known sequences (bottom). This alignment illustrates the 19 differences present in the thermophiles not found in any mesophilic sequence analyzed. Dashes indicate lack of sequence data.

```

      10      20      30      40      50      60
MREILHIQVGQCGNQVGRKFWEVISEEHGVDAQGFYRGDSEQQLERISVYYNEASTGRYV
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
MREILHIQVGQCGNQIGRKFWFVISEEHGVDA SGFYRGDSELQLERISVYYNEASGGRYV
      10      20      30      40      50      60

      70      80      90     100     110     120
PRAVLVDLEPGTMDAVRASDYGTLFRPDNFVYGQSGAGNNWAKGHYTEGAELVDAVMDVV
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
PRAVLVDLEPGTMDSVRASDYGTLFRPDNFVYGQSGAGNNWAKGHYTEGAELVDAVMDVV
      70      80      90     100     110     120

     130     140     150     160     170     180
RKECESCECLQGFQVTHSLGGGTGSGLGTLISKIREEYPDRMMCTYSVMPSPKVSDTVV
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
RKECESCECLQGFQVTHSLGGGTGSGLGTLISKIREEYPDRMMCTYSVMPSPKVSDTVV
     130     140     150     160     170     180

     190     200     210     220     230     240
EPYNATLSIHLVENADEVFCDNEALYDICQRTLKLTNP SYADLNKLVSGVM SGITCSL
::::::::::::::::::::::::::::::::::::::::::::: ::::::::::::::::::::
EPYNATLSIHLVENADEVFCDNEALYDICFRTLKLTNP SYGDLNHLVSGVM SGITCSL
     190     200     210     220     230     240

     250     260     270     280     290     300
RFPGQLNSDLRKLAVNLIPFPR LHFFSVSLAPLTATASTAYRHL SIADLTQQMFDAKNQF
:::::::::::::::::::::::::::: . :::::::::: :: :: ::::::::::::::::::::
RFPGQLNSDLRKLAVNLIPFPR LHFFLILLAPLTARASQAYRALSVADLTQQMFDAKNMM
     250     260     270     280     290     300

     310     320     330     340     350     360
VACDPRQGRYLTAAYFRGSRISTKD VDEQMSMVQSKNSAYFVEWIPHN IKSSVCGVPPK
:::::::::::::::::::::::::: ::::::::::::::::::::::::::::::::::::
AACDPRHGRYLTAAYFRG-RISTKD VDEQMSMVQSKNSSYFVEWIPHN IKSSVCDVPPK
     310     320     330     340     350

     370     380     390     400     410
GLDMSCV FVGNTTAVQE I FKR VGEQFSAMFRRKAF LHWYV GEGMDPMEFTEAESNM----
:: :: ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
GLKMSAAFVGNTTAIQELFKRVGEQFSAMFRRKAF LHWYT GEGMDPMEFTEAESNMNDLV
60      370     380     390     400     410

-----
SEYQQYEQAVA EDEDEDVEQEFGAYRTGEEDRTAIESSIMS
20      430     440     450     460

```

Figure 12: Alignment thermophilic of beta tubulin consensus sequence (top) and a mesophilic consensus sequence derived from 250 known sequences (bottom). This alignment illustrates the 25 differences present in the thermophiles not found in any mesophilic sequence analyzed. Dashes indicate lack of sequence data.

Table 2: Non-conserved amino acid substitutions and additions between *C. caldarium* and *C. merolae* and mesophiles. The number indicates the position of the amino acid in the algal sequence, the left abbreviation represents the algal tubulin amino acid, while the right abbreviation represents the mesophilic amino acid.

| Alpha tubulin                 | Beta Tubulin                      |
|-------------------------------|-----------------------------------|
| 31 Leu for Gln (N-Loop)       | 42 Gln for Leu (N-Loop)           |
| 39 Addition of Arg (N-Loop)   | 56 Thr for Gly (N-Loop)           |
| 50 Addition of Ser (N-Loop)   | 212 Gln for Phe (H6 Loop)         |
| 84 Pro for Thr (H2-S3 Loop)   | 267 Ser for Leu (H8-S7 Loop)      |
| 98 Arg for Ile S3-(H3' Loop)  | 269 Ser for Leu (S7 Loop)         |
| 288 Gly for Leu (M-Loop)      | 276 Thr for Arg (M-Loop)          |
| 326 Gly for Val (H10)         | 279 Thr for Gln (M-Loop)          |
| 335 Lys for Gly (H10-S9 Loop) | 283 His for Ala (M-Loop)          |
| 359 Ser for Tyr (S9-S10 Loop) | 320 Addition of Ser (S8-H10 Loop) |
|                               | 356 Gly for Asp (S9-S10 Loop)     |
|                               | 363 Asp for Lys (S9-S10 Loop)     |
|                               | 366 Cys for Ala (S9-S10 Loop)     |

### **Analysis of the tertiary structure of the tubulin monomer**

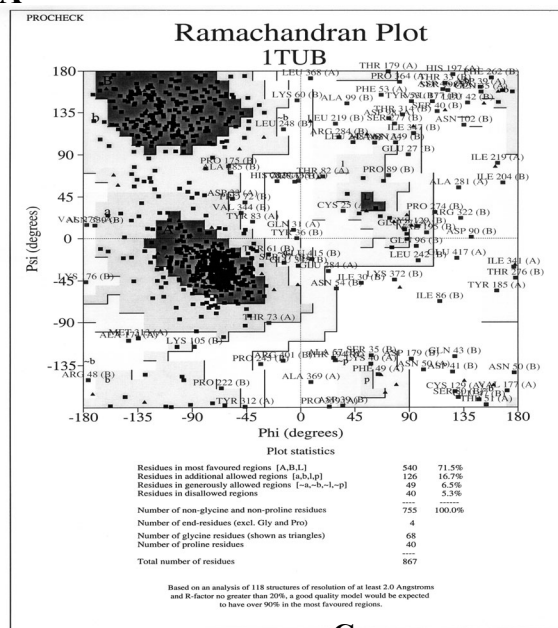
When the sequence of either thermophilic tubulin is compared to that of the mesophilic porcine tubulin, the tubulin sequence used to solve the tertiary structure, total conservation dropped to ~55% identity in the nucleotide sequences and ~75% identity in the amino acid sequences. This drop may seem substantial, but it is well over the threshold needed to make an adequate homology model (Sali and Kuriyan, 1999). Homology models were generated by Modeller by taking the primary structure of the thermophilic tubulin and arranging it into the same tertiary structure as its mesophilic tubulin template. After modeling each of the four thermophilic tubulin monomers and comparing them to their mesophilic counterparts, several analyses were made. First, a Ramachandran plot was used to evaluate each of the models and the solved mesophilic structure (Figure 13). It was found that all models had ~72-75% of their phi and psi angles in the most thermodynamically-favorable regions with an additional ~15-20% in favorable regions. While it is recommended that models have 90% of their phi and psi angles in most favorable regions (Baker and Sali, 2001), the results from the thermophile are the same as the solved structure.

When non-conserved substitutions found in the primary structure of the thermophilic tubulin monomers were mapped to the tertiary structure (Figures 16-37), it was found that they mostly were located in loop regions of the proteins (Figures 14,15). There are three main categories of secondary structure present in the tubulin monomers, alpha helices, beta strands, and loops (some of which connect beta strands to beta strands or alpha helices to beta strands and so on). Some loops also serve as interfaces for lateral

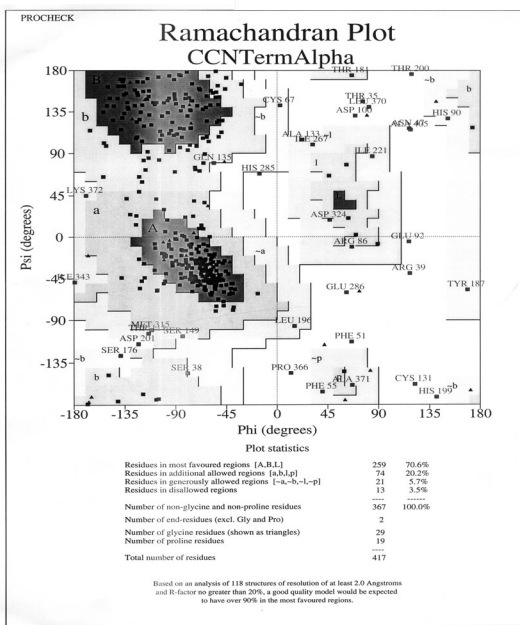
and longitudinal interactions between monomers (Li et al, 2002a). Several non-conserved differences, in particular, map to the N-loop and M-loop, which are responsible for lateral interactions between protofilaments forming a microtubule. There are also several non-conserved changes in the S9-S10 loop, which lies near the M-loop on the inside surface of the microtubule (Lowe et al., 2001). This loop is thought to play a substantial role in stabilizing the M-loop. In fact, two thirds of all non-conserved changes identified are clustered around the N-loop, M-loop, or S9-S10 loop, all dealing with lateral interaction in some respect.

Figure 13: A Ramachandran Plot of the solved structure of pig alpha and beta tubulin heterodimer (A), having ~72% of their phi and psi angles in the most thermodynamically favorable regions with an additional ~16% in favorable regions. A Ramachandran Plot of the modeled structure of algal alpha (B) and beta tubulin (C), having ~74% of their phi and psi angles in the most thermodynamically favorable regions with an additional ~22% in favorable regions.

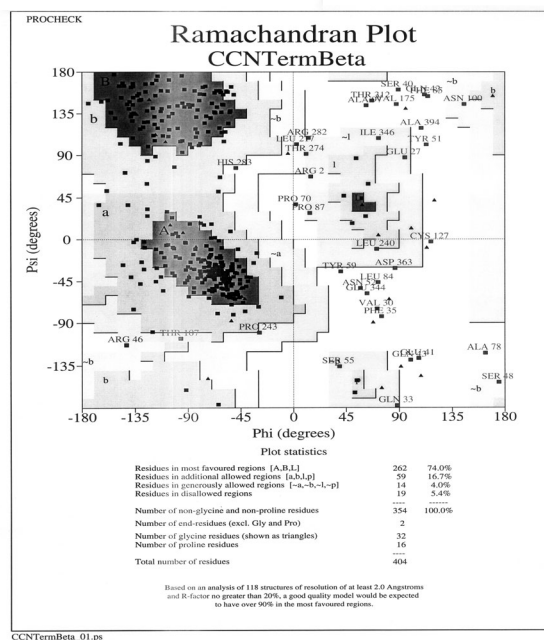
A



B



C



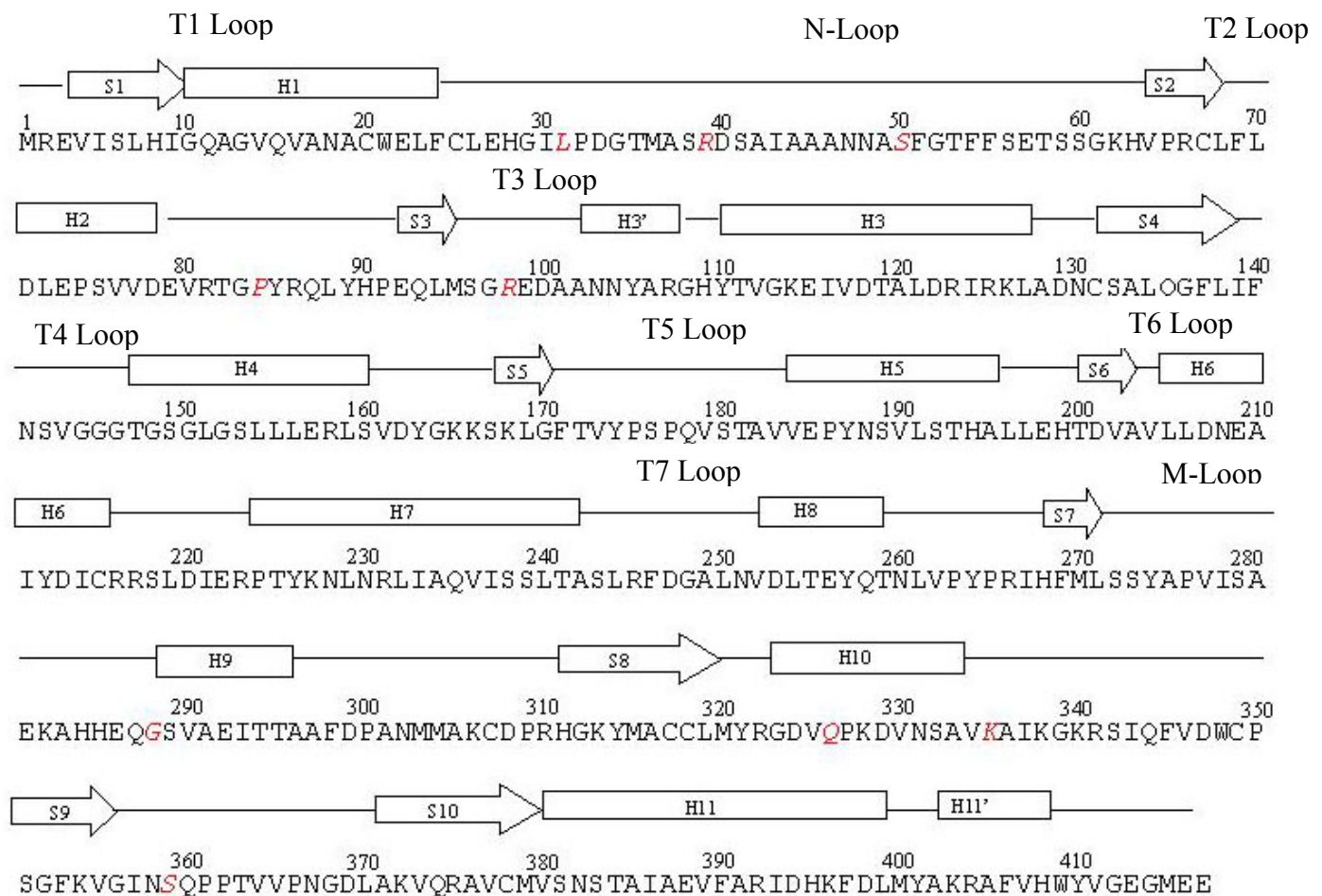


Figure 14: Secondary structure map of thermophilic alpha tubulin. Rectangles indicate alpha helices, arrows indicate beta strands, and lines indicate loops. All secondary structures are numbered in order of appearance in the primary structure. Black letters indicate amino acids found in mesophiles and thermophiles, while red letters indicate amino acids unique to the *C. caldarium* and *C. merolae* in that position.

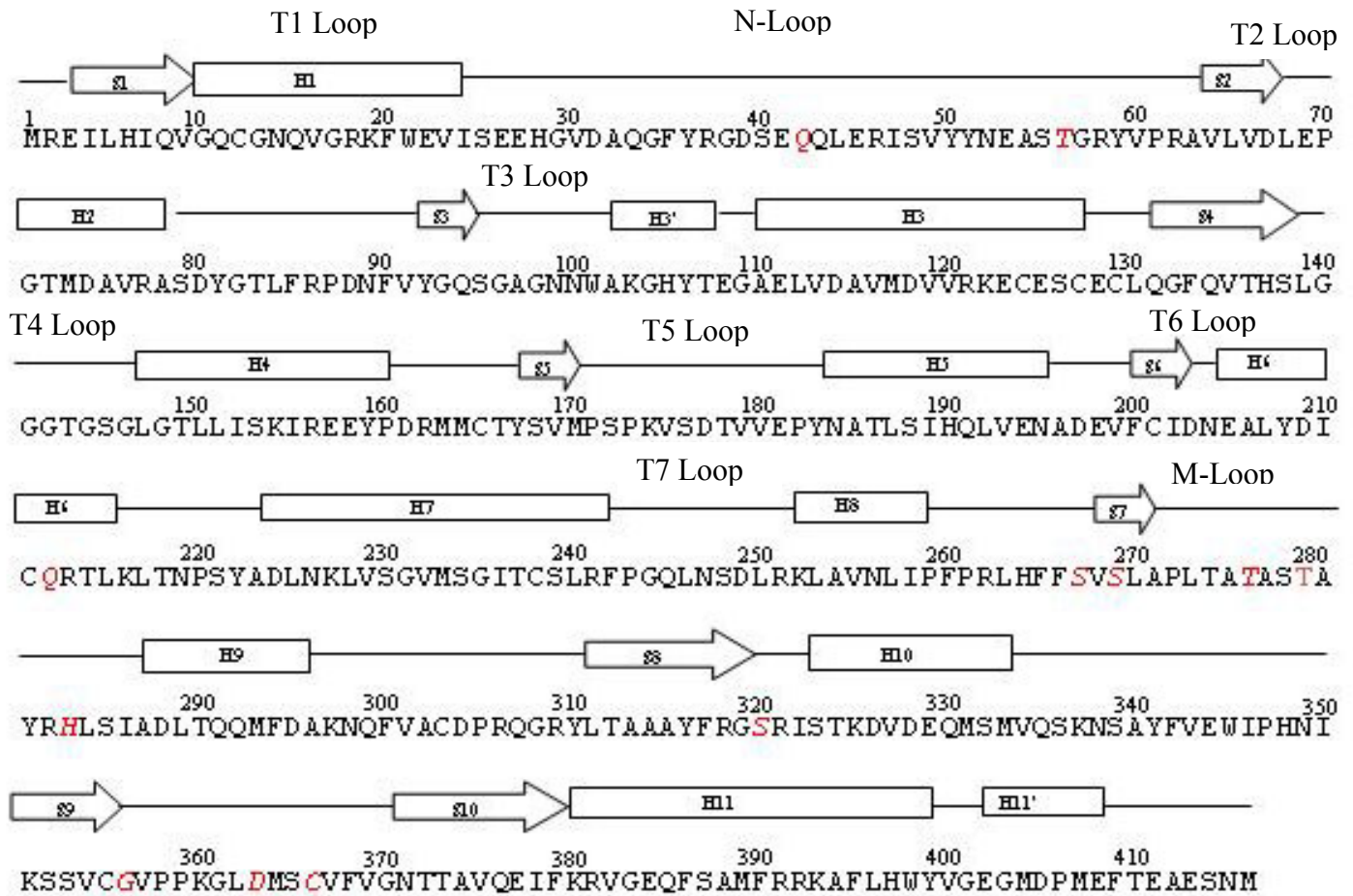
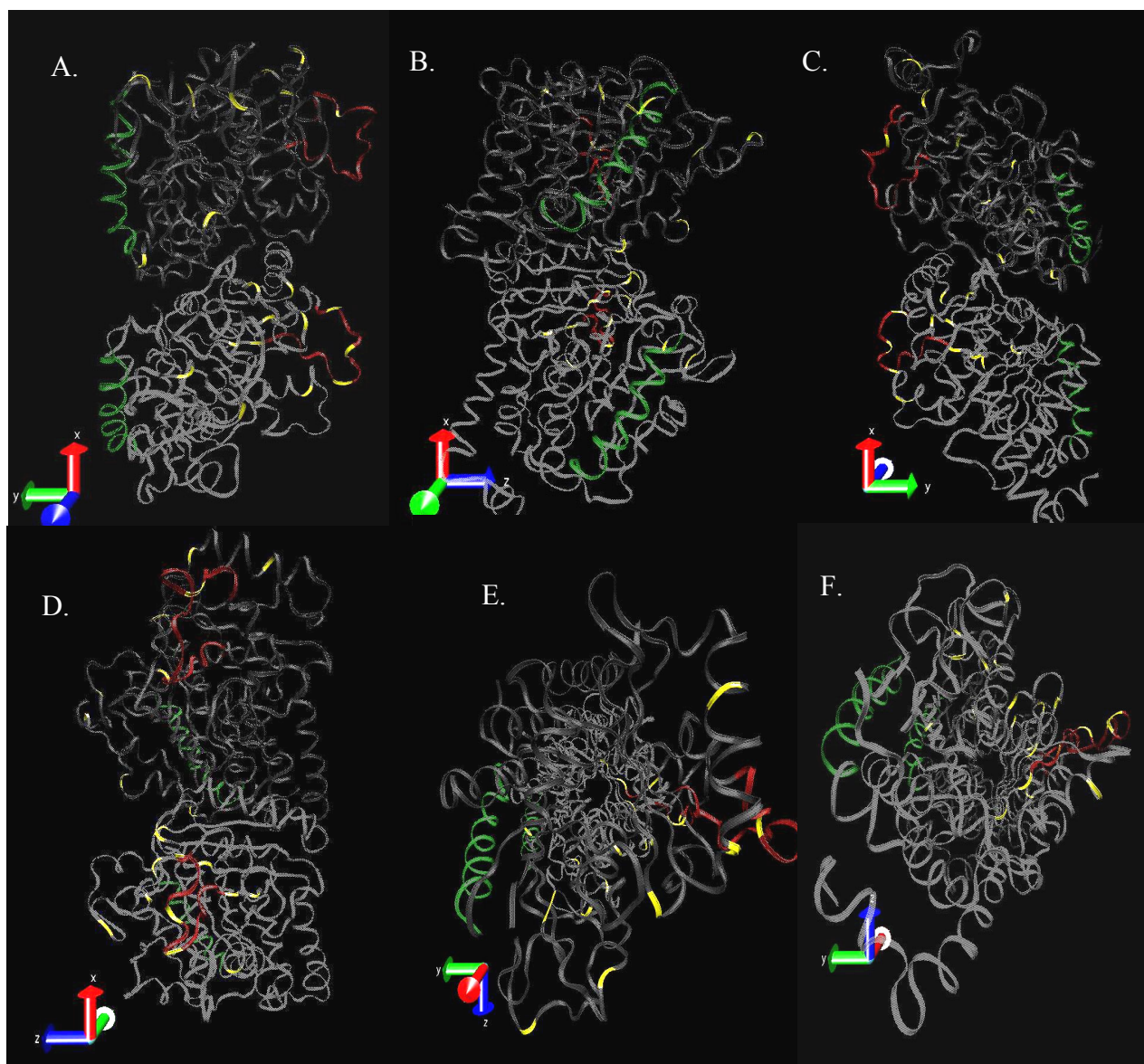
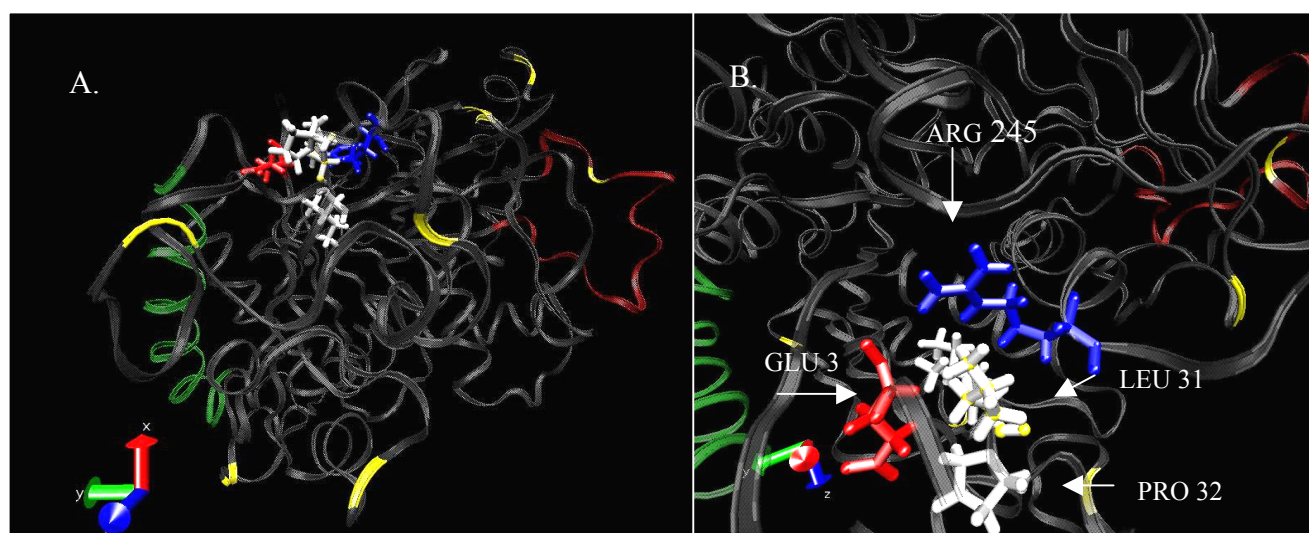


Figure 15: Secondary structure map of thermophilic beta tubulin. Rectangles indicate alpha helices, arrows indicate beta strands, and lines indicate loops. All secondary structures are numbered in order of appearance in the primary structure. Black letters indicate amino acids found in mesophiles and thermophiles, while red letters indicate amino acids unique to the *C. caldarium* and *C. merolae* in that position.

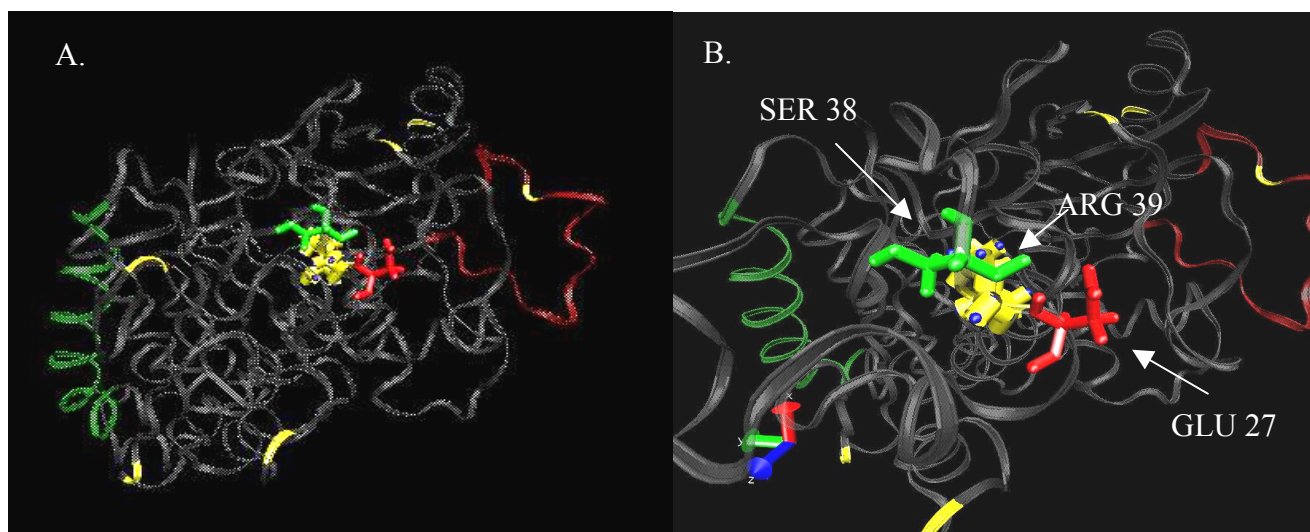
Figure 16: The six faces of the thermophilic tubulin heterodimer. Image A depicts the heterodimer as seen from outside the microtubule with the H3 helix (green) on the left and the M-loop (red) to the right. The top monomer (gray) is alpha tubulin, the bottom (white) is beta tubulin. B has been rotated to the right by 90 degrees with the H3 helix facing outward. C is a view of the inside of the microtubule with the H3 helix on the right and the M-loop on the left. D is a view is the M-loop in the front of the heterodimer. E is a view of the heterodimer from the alpha (-) end of the heterodimer. F is a view from the beta (+) end of the heterodimer.





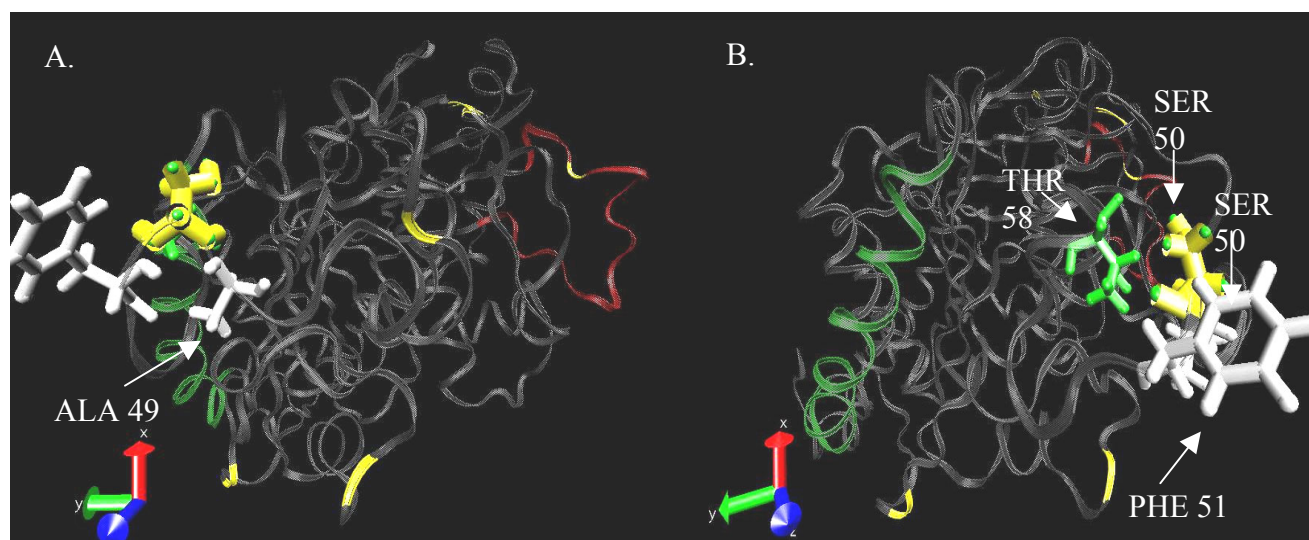
| Non-conserved substitution     | Within 5 angstroms                                                                                                                                                                                                                                                                                                                                                                     | Within 2 angstroms                                                                                                            |
|--------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|
| LEU <sup>31</sup> (H1-S2 Loop) | MET <sup>1</sup> (N-Term)<br>ARG <sup>2</sup> (N-Term)<br>GLU <sup>3</sup> (N-term)<br>VAL <sup>4</sup> (S1)<br>HIS <sup>28</sup> (H1-S2 Loop)<br>ILE <sup>30</sup> (H1-S2 Loop)<br>PRO <sup>32</sup> (H1-S2 Loop)<br>ASP <sup>33</sup> (H1-S2 Loop)<br>GLY <sup>34</sup> (H1-S2 Loop)<br>PHE <sup>246</sup> (T7 loop)<br>ARG <sup>245</sup> (T7 Loop)<br>LEU <sup>254</sup> (T7 Loop) | GLU <sup>3</sup> (N-Term)<br>LEU <sup>30</sup> (H1-S2 Loop)<br>PRO <sup>32</sup> (H1-S2 Loop)<br>ARG <sup>245</sup> (T7 Loop) |

Figure 17: In *C. caldarium* and *C. merolae* a hydrophobic leucine<sup>31</sup> (yellow/white) replaces the polar glutamine normally found in mesophilic alpha tubulin(A). Arginine<sup>245</sup> (blue/basic), glutamic acid<sup>3</sup> (red/acidic), proline<sup>32</sup> (white/hydrophobic), leucine<sup>30</sup> (white/hydrophobic), and leucine<sup>254</sup> (white/hydrophobic) are also pictured as they lie within 3.5 angstroms of leucine<sup>31</sup>. A closer view (B) of the amino acids to show orientation and possible hydrophobic-hydrophobic interaction between side chains leucine<sup>31</sup> and proline<sup>32</sup>. Parenthetical references in the table refer to locations of amino acids in secondary structure.



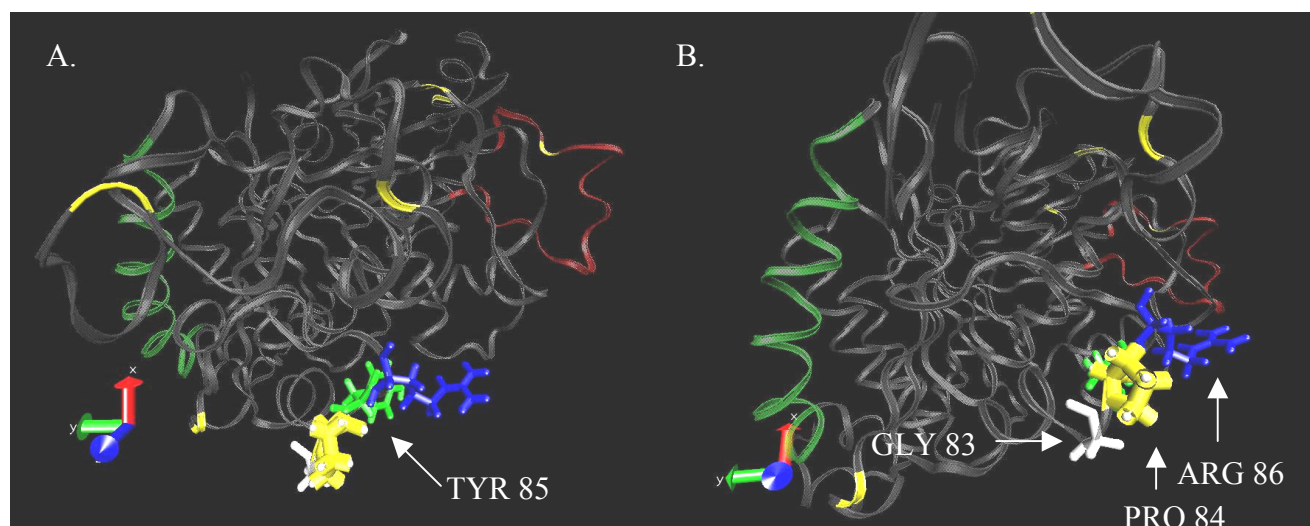
| Non-conserved substitution     | Within 5 angstroms                                                                                                                                                                                                                                                                                             | Within 2 angstroms                                               |
|--------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------|
| ARG <sup>39</sup> (H1-S2 Loop) | LEU <sup>26</sup> (H1-S2 Loop)<br>GLU <sup>27</sup> (H1-S2 Loop)<br>MET <sup>36</sup> (H1-S2 Loop)<br>ALA <sup>37</sup> (H1-S2 Loop)<br>SER <sup>38</sup> (H1-S2 Loop)<br>ASP <sup>40</sup> (H1-S2 Loop)<br>SER <sup>41</sup> (H1-S2 Loop)<br>ILE <sup>43</sup> (H1-S2 Loop)<br>ALA <sup>46</sup> (H1-S2 Loop) | SER <sup>38</sup> (H1-S2 Loop)<br>GLU <sup>27</sup> (H1-S2 Loop) |

Figure 18: In *C. caldarium* and *C. merolae* a basic arginine <sup>39</sup> (yellow/blue) is an addition found only in thermophilic alpha tubulin (A). Serine <sup>38</sup> (green/polar) and glutamic acid <sup>27</sup> (red/acidic) are also pictured as they are within 3.5 angstroms of arginine<sup>39</sup>. This image is a closer view (B) of the amino acids to show orientation and possible interaction of side chains. This also shows a possible salt bridge between arginine <sup>39</sup> and glutamic acid <sup>27</sup>. Parenthetical references in the table refer to locations of amino acids in secondary structure.



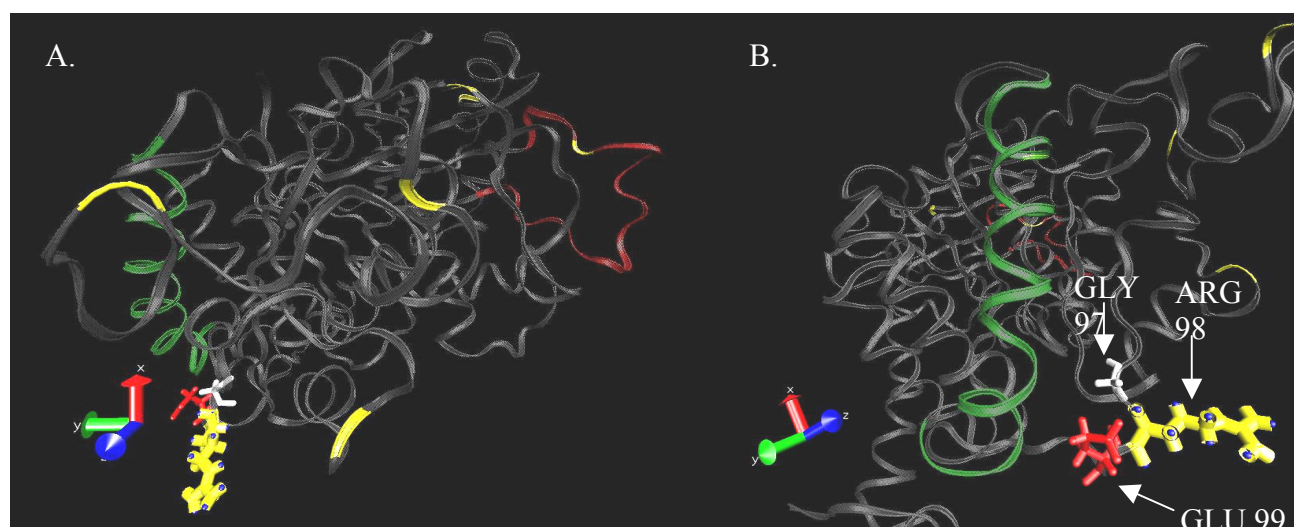
| Non-conserved substitution     | Within 5 angstroms                                                                                                                                                     | Within 2 angstroms                                               |
|--------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------|
| SER <sup>50</sup> (H1-S2 Loop) | ASN <sup>47</sup> (H1-S2 Loop)<br>ASN <sup>48</sup> (H1-S2 Loop)<br>ALA <sup>49</sup> (H1-S2 Loop)<br>PHE <sup>51</sup> (H1-S2 Loop)<br>THR <sup>58</sup> (H1-S2 Loop) | ALA <sup>49</sup> (H1-S2 Loop)<br>PHE <sup>51</sup> (H1-S2 Loop) |

Figure 19: In *C. caldarium* and *C. merolae* a polar serine<sup>50</sup> (yellow/green) is an addition found only in the thermophilic alpha tubulin in alpha tubulin (A). Alanine<sup>49</sup> (white/hydrophobic), phenylalanine<sup>51</sup> (white/hydrophobic), and threonine<sup>58</sup> (green/polar) are also pictured as they within 3.5 angstroms of serine<sup>50</sup>. This image is a closer view (B) of the amino acids to show orientation and possible interaction of side chains. The interaction between serine<sup>50</sup> and threonine<sup>58</sup> forms a possible hydrogen bond not seen in the mesophilic alpha tubulin monomer. Parenthetical references in the table refer to locations of amino acids in secondary structure.



| Non-conserved substitution<br>PRO <sup>84</sup> (H2-S3 Loop) | Within 5 angstroms             | Within 2 angstroms             |
|--------------------------------------------------------------|--------------------------------|--------------------------------|
|                                                              | VAL <sup>80</sup> (H2-S3 Loop) | GLY <sup>83</sup> (H2-S3 Loop) |
|                                                              | ARG <sup>81</sup> (H2-S3 Loop) | TYR <sup>85</sup> (H2-S3 Loop) |
|                                                              | GLY <sup>83</sup> (H2-S3 Loop) | ARG <sup>86</sup> (H2-S3 Loop) |
|                                                              | TYR <sup>85</sup> (H2-S3 Loop) |                                |
|                                                              | ARG <sup>86</sup> (H2-S3 Loop) |                                |
|                                                              | GLN <sup>87</sup> (H2-S3 Loop) |                                |
|                                                              | LEU <sup>88</sup> (H2-S3 Loop) |                                |

Figure 20: In *C. caldarium* and *C. merolae* a hydrophobic proline<sup>84</sup> (yellow/white) replaces polar threonine in alpha tubulin (A). Arginine<sup>86</sup> (basic/blue), glycine<sup>83</sup> (white/hydrophobic), and tyrosine<sup>85</sup> (green/polar) are also pictured as they within 3.5 angstroms of a proline<sup>84</sup>. This image is a closer view (B) of the amino acids to show orientation and possible interaction of side chains. This amino acid is the start of the H2-S3 loop. This substitution may help elongate the loop not seen in the mesophilic alpha tubulin monomer. Parenthetical references in the table refer to locations of amino acids in secondary structure.



| Non-conserved substitution  | Within 5 angstroms                                                                                                                                                                                 | Within 2 angstroms                                         |
|-----------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------|
| ARG <sup>98</sup> (T3 Loop) | LEU <sup>72</sup> (H2)<br>GLU <sup>73</sup> (H2)<br>PRO <sup>74</sup> (H2)<br>SER <sup>96</sup> (T3 Loop)<br>GLY <sup>97</sup> (T3 Loop)<br>GLU <sup>99</sup> (T3 Loop)<br>ILE <sup>116</sup> (H3) | GLY <sup>97</sup> (T3 Loop)<br>GLU <sup>99</sup> (T3 Loop) |

Figure 21: In *C. caldarium* and *C. merolae* a basic amino acid arginine<sup>98</sup> (yellow/blue) replaces a hydrophobic isoleucine in alpha tubulin (A). Glutamic acid<sup>99</sup> (acidic/red) and glycine<sup>97</sup> (hydrophobic /white) are also pictured as they are within 3.5 angstroms of arginine<sup>98</sup>. This image is a closer view (B) of the amino acids to show orientation and possible interaction of side chains not seen in the mesophilic alpha tubulin monomer. Parenthetical references in above table refer to location of amino acid in secondary structure.

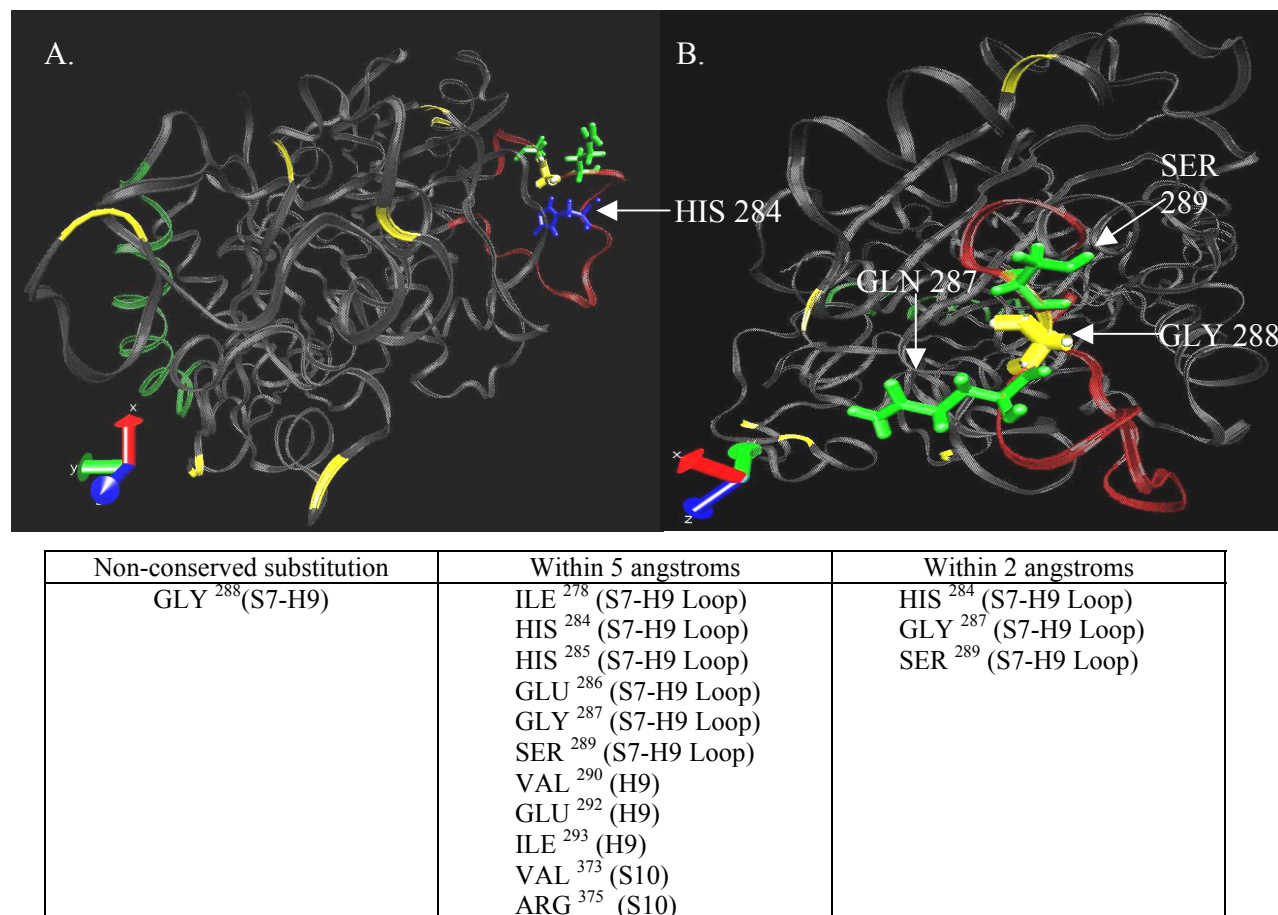
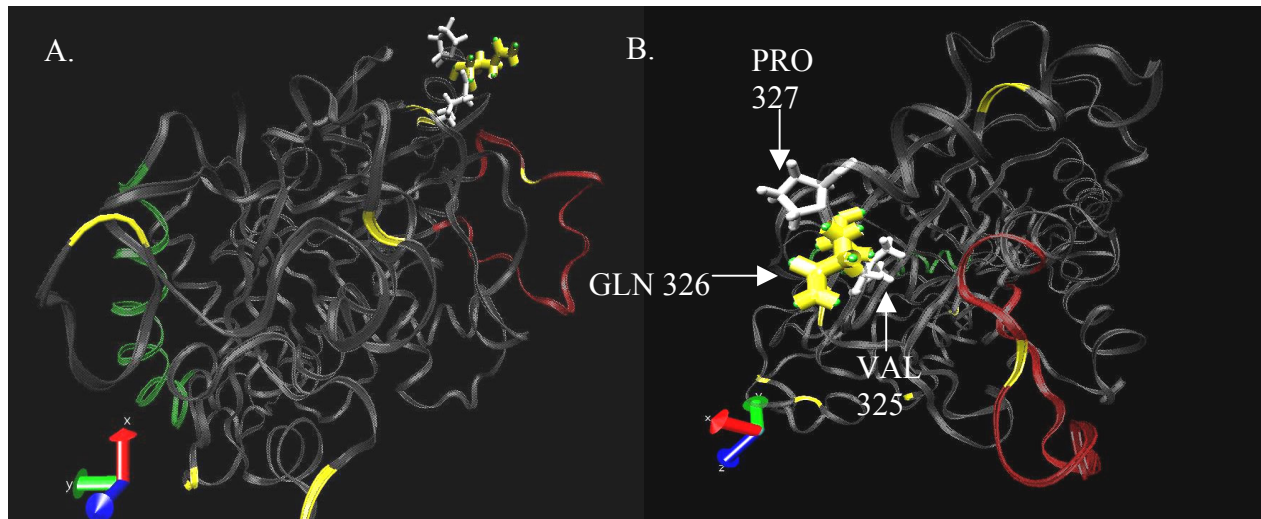
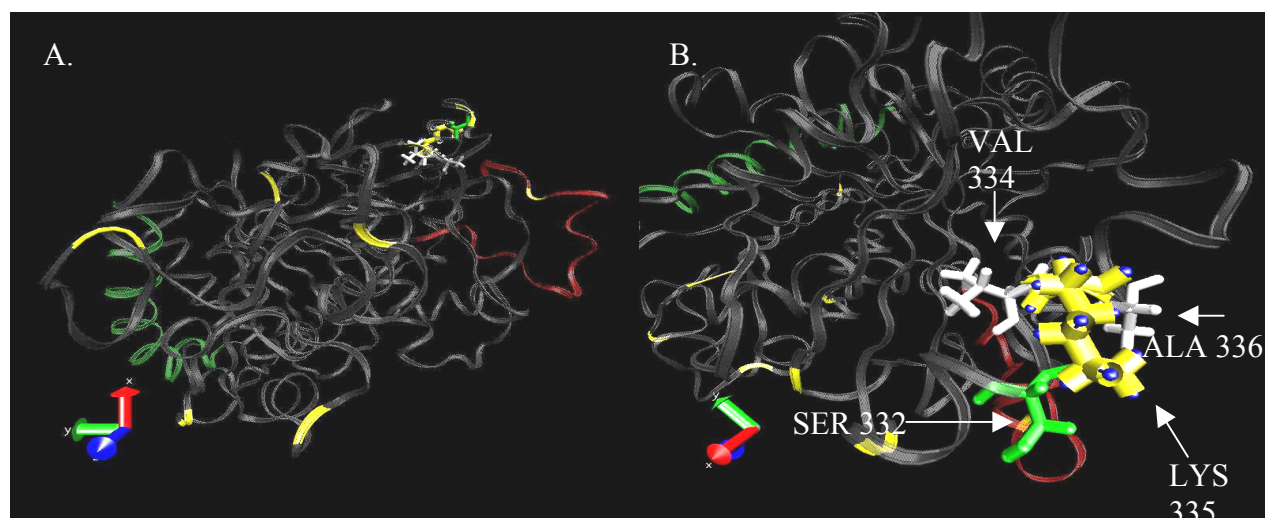


Figure 22: In *C. caldarium* and *C. merolae* a hydrophobic glycine<sup>288</sup> (yellow/white) replaces a larger hydrophobic valine in alpha tubulin (A). Histidine<sup>284</sup> (basic/blue), glutamine<sup>287</sup> (polar/green), and serine<sup>289</sup> (polar/green) are also pictured as they are within 3.5 angstroms of glycine<sup>288</sup>. This image is a closer view (B) of the amino acids to show orientation and possible interaction of side chains. The smaller size of the glycine may help facilitate a hydrogen bond between glutamine<sup>287</sup> and serine<sup>289</sup> not seen in the mesophilic alpha tubulin monomer. Parenthetical references in the table refer to locations of amino acids in secondary structure.



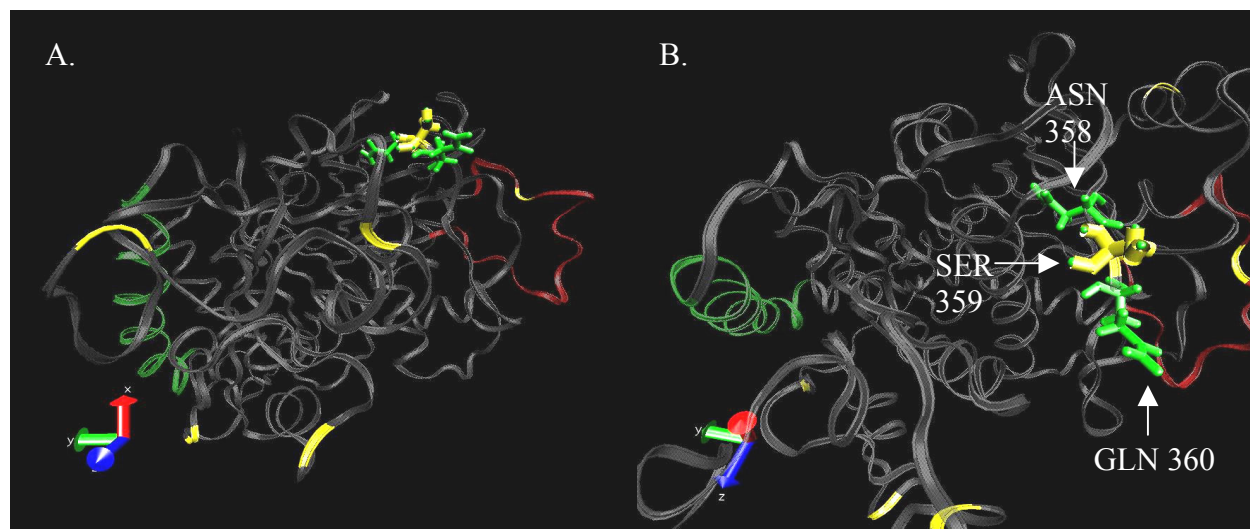
| Non-conserved substitution | Within 5 angstroms                                                                                                                                                                                                                                                      | Within 2 angstroms                                   |
|----------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|
| GLN <sup>326</sup> (H10)   | VAL <sup>290</sup> (H9)<br>ASP <sup>324</sup> (S8-H10 Loop)<br>VAL <sup>325</sup> (H10)<br>PRO <sup>327</sup> (H10)<br>LYS <sup>328</sup> (H10)<br>ASP <sup>329</sup> (H10)<br>VAL <sup>330</sup> (H10)<br>ASN <sup>331</sup> (H10)<br>ILE <sup>357</sup> (S9-S10 Loop) | VAL <sup>325</sup> (H10)<br>PRO <sup>327</sup> (H10) |

Figure 23: In *C. caldarium* and *C. merolae* a polar glutamine<sup>326</sup> (yellow/green) replaces a hydrophobic valine in alpha tubulin (A). Valine<sup>325</sup> (hydrophobic/white and proline<sup>327</sup> (hydrophobic/white) are also pictured as they are within 3.5 angstroms of glutamine<sup>326</sup>. This image is a closer view (B) of the amino acids to show orientation and possible interaction of side chains. Parenthetical references in above table refer to location of amino acid in secondary structure.



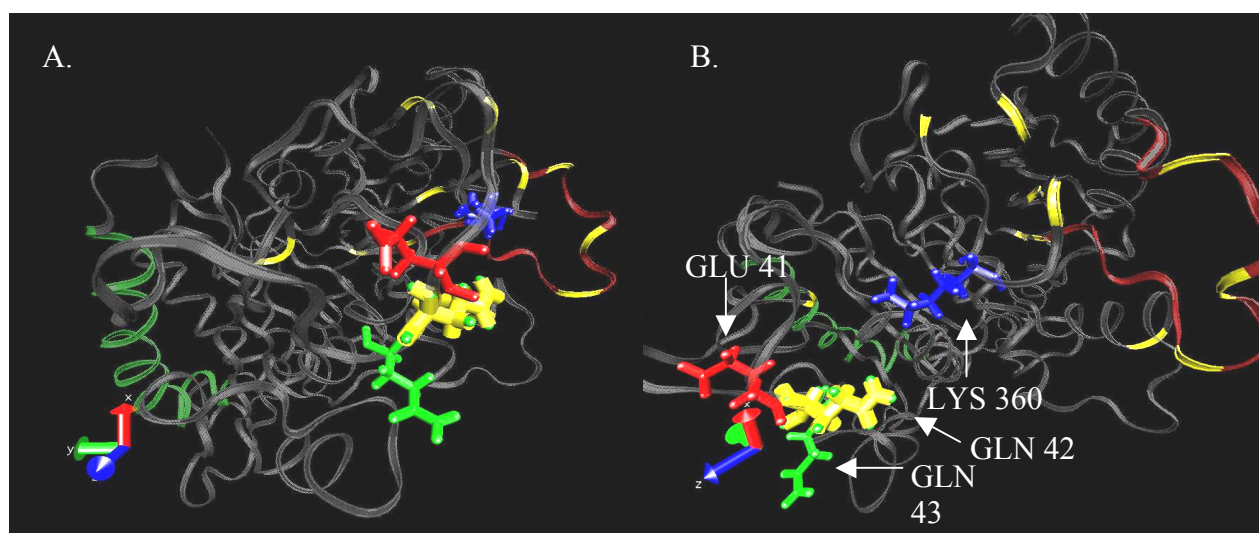
| Non-conserved substitution       | Within 5 angstroms                                                                                                                                                                                                                                                                                                  | Within 2 angstroms                                           |
|----------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------|
| LYS <sup>335</sup> (H10-S9 Loop) | VAL <sup>330</sup> (H10)<br>ASN <sup>331</sup> (H10)<br>SER <sup>332</sup> (H10)<br>ALA <sup>333</sup> (H10)<br>VAL <sup>334</sup> (H10)<br>ALA <sup>336</sup> (H10-S9 Loop)<br>ILE <sup>337</sup> (H10-S9 Loop)<br>LYS <sup>338</sup> (H10-S9 Loop)<br>SER <sup>339</sup> (H10-S9 Loop)<br>PHE <sup>353</sup> (S9) | VAL <sup>334</sup> (H10)<br>ALA <sup>336</sup> (H10-S9 Loop) |

Figure 24: In *C. caldarium* and *C. merolae* (yellow/blue) a basic lysine <sup>335</sup> replaces small hydrophobic glycine in alpha tubulin (A). Serine <sup>332</sup> (polar/green), valine <sup>334</sup> (hydrophobic/white), and alanine <sup>336</sup> (hydrophobic/white) are also pictured as they are within 3.5 angstroms of glutamine <sup>326</sup>. This image is a closer view (B) of the amino acids to show orientation and possible interaction of side chains. The lysine <sup>335</sup> may interact with glutamine <sup>326</sup> to form a hydrogen bond not seen in the mesophilic alpha tubulin monomer. Parenthetical references in the table refer to locations of amino acids in secondary structure.



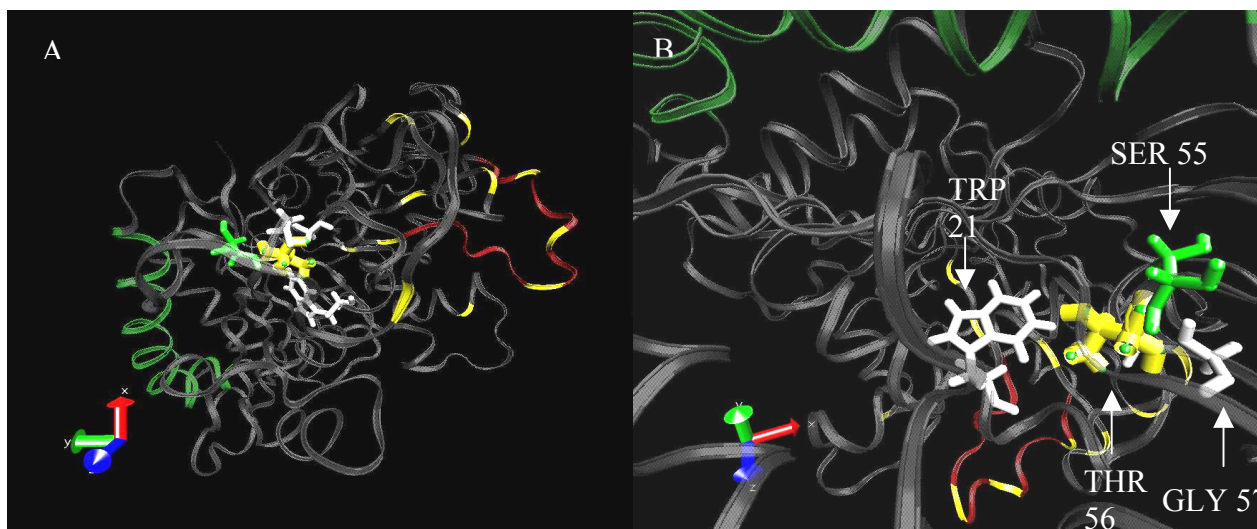
| Non-conserved substitution       | Within 5 angstroms                                                                                                                                                                                                                                                                                                                                                                                              | Within 2 angstroms                                                   |
|----------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------|
| SER <sup>359</sup> (S9-S10 Loop) | ALA <sup>242</sup> (T7 Loop)<br>SER <sup>243</sup> (T7 Loop)<br>PHE <sup>246</sup> (T7 Loop)<br>ASP <sup>247</sup> (T7 Loop)<br>GLY <sup>248</sup> (T7 Loop)<br>ASN <sup>258</sup> (H8)<br>ARG <sup>322</sup> (S8-H10 Loop)<br>ASP <sup>324</sup> (S8-H10 Loop)<br>ILE <sup>357</sup> (S9-S10 Loop)<br>ASN <sup>358</sup> (S9-S10 Loop)<br>GLN <sup>360</sup> (S9-S10 Loop)<br>PRO <sup>361</sup> (S9-S10 Loop) | ASN <sup>358</sup> (S9-S10 Loop)<br>GLN <sup>360</sup> (S9-S10 Loop) |

Figure 25: In *C. caldarium* and *C. merolae* a polar serine<sup>359</sup> (yellow/green) replaces a more hydrophobic tyrosine in alpha tubulin (A). Asparagine<sup>358</sup> (polar/green) and glutamine<sup>360</sup> (polar/green) are also pictured as they are within 3.5 angstroms of serine<sup>359</sup>. This image is a closer view (B) of the amino acids to show orientation and possible interaction of side chains. Parenthetical references in the table refer to locations of amino acids in secondary structure.



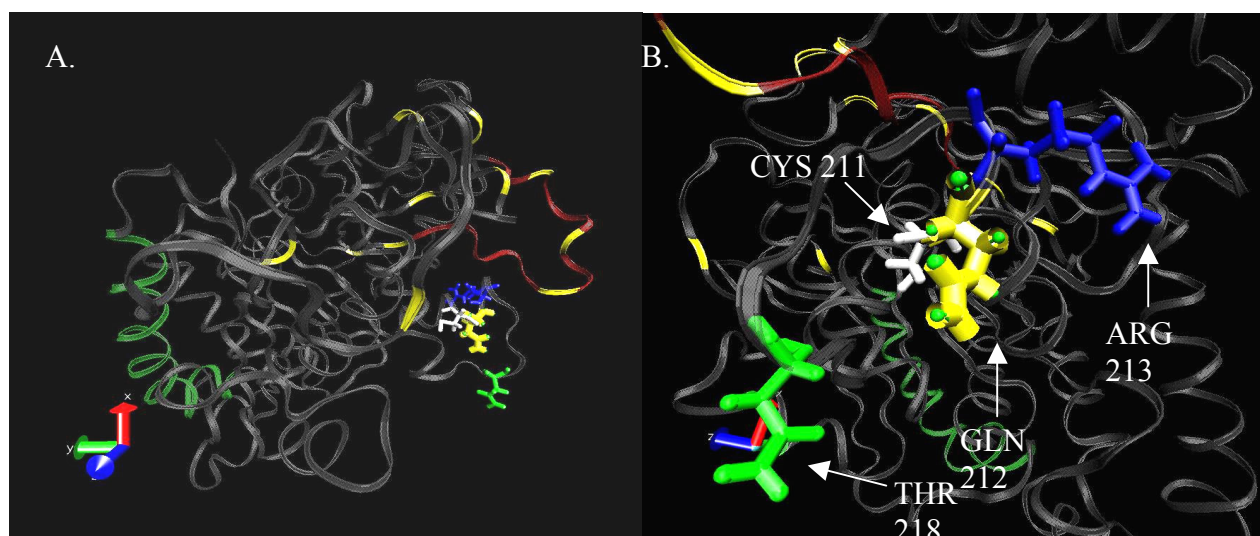
| Non-conserved substitution     | Within 5 angstroms                                                                                                                                                                                                                                                        | Within 2 angstroms                                               |
|--------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------|
| GLN <sup>42</sup> (H1-S2 Loop) | SER <sup>25</sup> (H1-S2 Loop)<br>SER <sup>40</sup> (H1-S2 Loop)<br>GLU <sup>41</sup> (H1-S2 Loop)<br>GLN <sup>43</sup> (H1-S2 Loop)<br>LEU <sup>44</sup> (H1-S2 Loop)<br>GLU <sup>45</sup> (H1-S2 Loop)<br>ARG <sup>46</sup> (H1-S2 Loop)<br>LYS <sup>360</sup> (S9-S10) | GLU <sup>41</sup> (H1-S2 Loop)<br>GLN <sup>43</sup> (H1-S2 Loop) |

Figure 26: In *C. caldarium* and *C. merolae* a polar glutamine<sup>42</sup> (yellow/green) replaces a hydrophobic leucine in beta tubulin (A). Lysine<sup>360</sup> (basic/blue), glutamic acid<sup>41</sup> (acidic/red), and glutamine<sup>43</sup> (polar/green) are also pictured as they are within 3.5 angstroms of glutamine<sup>42</sup>. This image is a closer view (B) of the amino acids to show orientation and possible interaction of side chains. One such possible interaction involves an interaction between glutamine<sup>42</sup> and lysine<sup>360</sup>. Parenthetical references in the table refer to locations of amino acids in secondary structure.



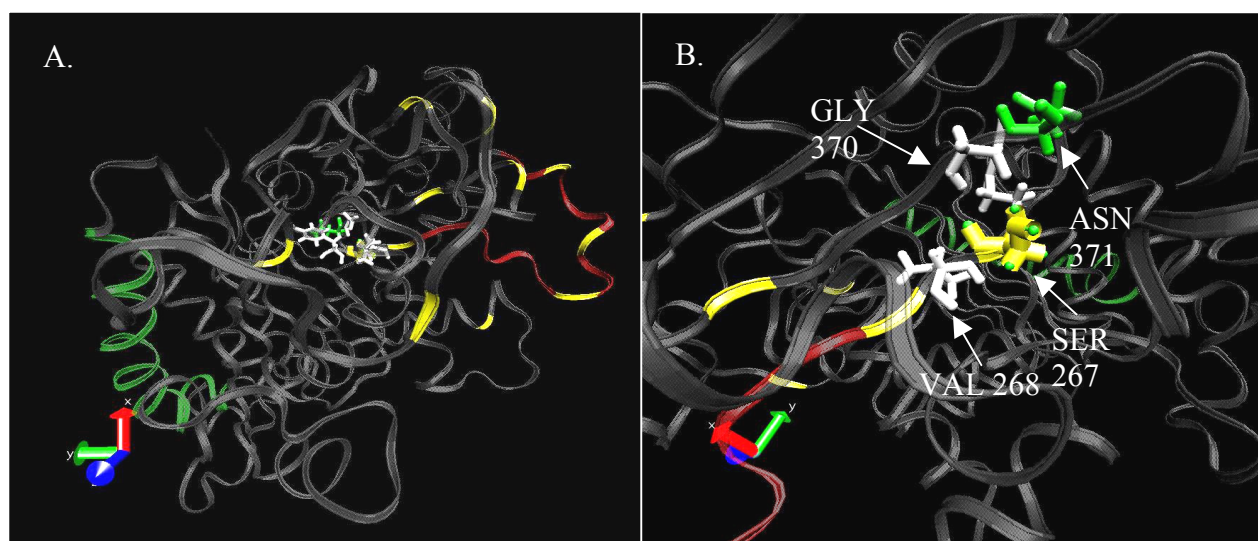
| Non-conserved substitution     | Within 5 angstroms                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Within 2 angstroms                                                                         |
|--------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|
| THR <sup>56</sup> (H1-S2 Loop) | HIS <sup>6</sup> (S1)<br>TRP <sup>21</sup> (H1)<br>ILE <sup>24</sup> (H1-S2 Loop)<br>GLU <sup>27</sup> (H1-S2 Loop)<br>HIS <sup>28</sup> (H1-S2 Loop)<br>GLY <sup>29</sup> (H1-S2 Loop)<br>VAL <sup>30</sup> (H1-S2 Loop)<br>ASP <sup>31</sup> (H1-S2 Loop)<br>ALA <sup>32</sup> (H1-S2 Loop)<br>ARG <sup>46</sup> (H1-S2 Loop)<br>SER <sup>55</sup> (H1-S2 Loop)<br>GLY <sup>57</sup> (H1-S2 Loop)<br>ARG <sup>58</sup> (H1-S2 Loop)<br>ARG <sup>62</sup> (H1-S2 Loop)<br>ALA <sup>63</sup> (H1-S2 Loop) | TRP <sup>21</sup> (H1)<br>SER <sup>55</sup> (H1-S2 Loop)<br>GLY <sup>57</sup> (H1-S2 Loop) |

Figure 27: In *C. caldarium* and *C. merolae* a polar threonine <sup>56</sup> (yellow/green) replaces a hydrophobic glycine in beta tubulin (A). Tryptophan <sup>21</sup> (hydrophobic/white), serine <sup>55</sup> (polar/green), and glycine <sup>57</sup> (hydrophobic/white) are also pictured as they are within 3.5 angstroms of threonine <sup>56</sup>. This image is a closer view (B) of the amino acids to show orientation and possible interaction of side chains. Parenthetical references in the table refer to locations of amino acids in secondary structure.



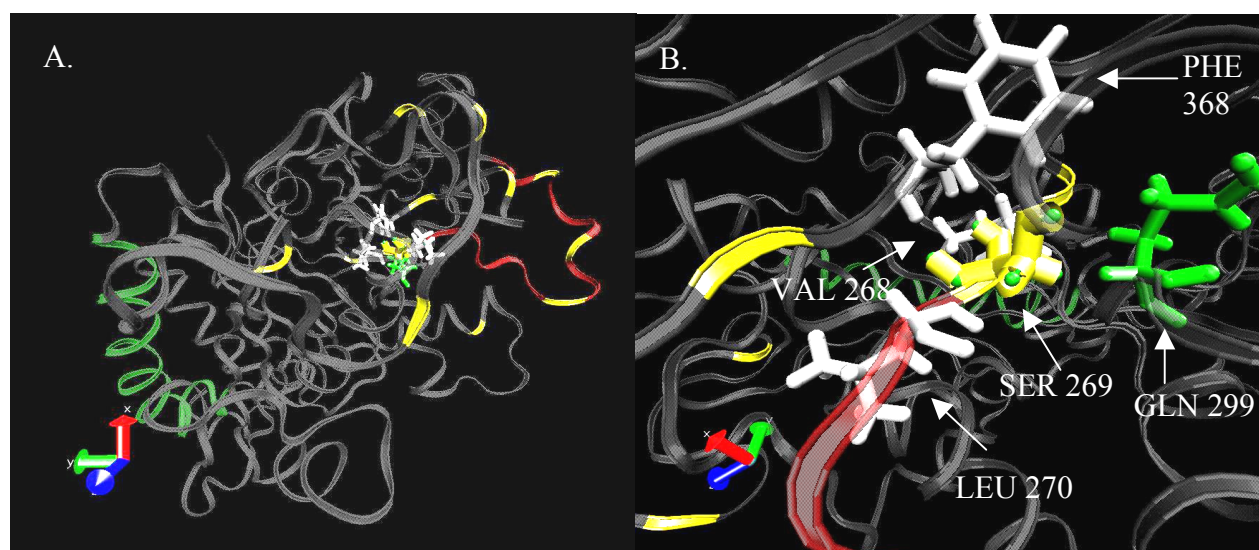
| Non-conserved substitution | Within 5 angstroms                                                                                                                                                                                                                                                                                                                                                                                                  | Within 2 angstroms                                 |
|----------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------|
| GLN <sup>212</sup> (H6)    | LEU <sup>207</sup> (H6)<br>TYR <sup>208</sup> (H6)<br>ASP <sup>209</sup> (H6)<br>ILE <sup>210</sup> (H6)<br>CYS <sup>211</sup> (H6)<br>ARG <sup>213</sup> (H6)<br>THR <sup>214</sup> (H6-H7 Loop)<br>LEU <sup>215</sup> (H6-H7 Loop)<br>LYS <sup>216</sup> (H6-H7 Loop)<br>LEU <sup>217</sup> (H6-H7 Loop)<br>THR <sup>218</sup> (H6-H7 Loop)<br>ASN <sup>219</sup> (H6-H7 Loop)<br>PRO <sup>220</sup> (H6-H7 Loop) | CYS <sup>211</sup> (H6)<br>ARG <sup>213</sup> (H6) |

Figure 28: In *C. caldarium* and *C. merolae* a polar glutamine<sup>212</sup> (yellow/green) replaces a hydrophobic phenylalanine in beta tubulin (A). Cysteine<sup>211</sup> (hydrophobic/white), arginine<sup>213</sup> (basic/blue), and threonine<sup>218</sup> (polar/green) are also pictured as they are within 3.5 angstroms of glutamine<sup>212</sup>. This image is a closer view (B) of the amino acids to show orientation and possible interaction of side chains. One such interaction includes a possible attractive electrostatic interaction between glutamine<sup>212</sup> and threonine<sup>218</sup>. Parenthetical references in the table refer to locations of amino acids in secondary structure.



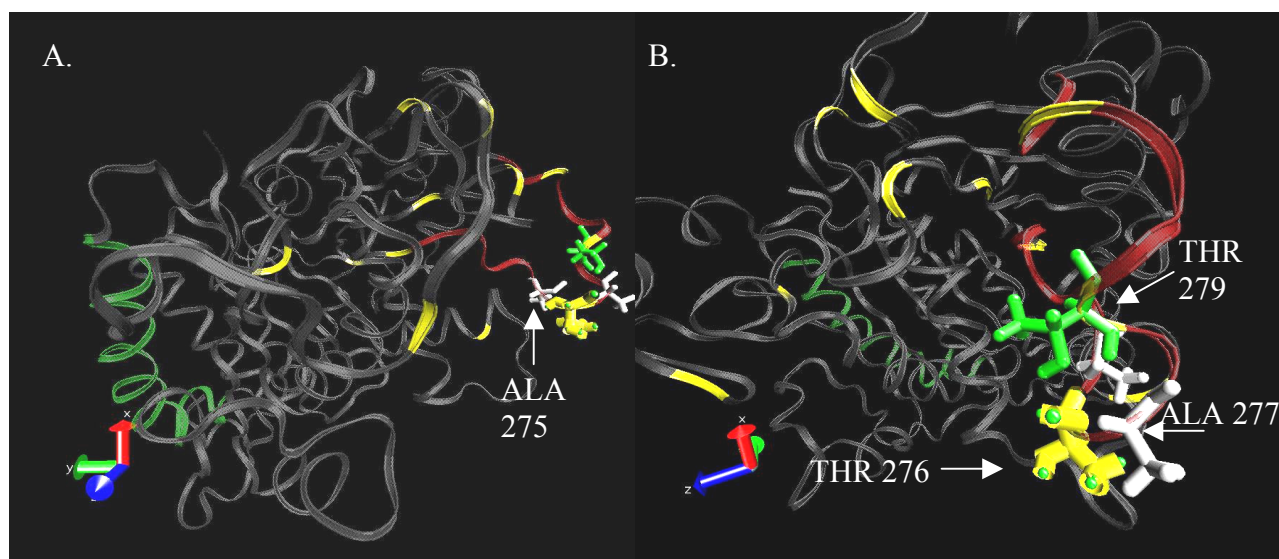
| Non-conserved substitution      | Within 5 angstroms                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Within 2 angstroms                                         |
|---------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------|
| SER <sup>267</sup> (H8-S7 Loop) | PHE <sup>200</sup> (S6)<br>CYS <sup>201</sup> (S6)<br>PHE <sup>265</sup> (H8-S7 Loop)<br>PHE <sup>266</sup> (H8-S7 Loop)<br>VAL <sup>268</sup> (H8-S7 Loop)<br>SER <sup>269</sup> (H8-S7 Loop)<br>GLN <sup>299</sup> (H9-S8 Loop)<br>PHE <sup>300</sup> (H9-S8 Loop)<br>VAL <sup>301</sup> (H9-S8 Loop)<br>CYS <sup>303</sup> (H9-S8 Loop)<br>PRO <sup>305</sup> (H9-S8 Loop)<br>TYR <sup>310</sup> (H9-S8 Loop)<br>VAL <sup>369</sup> (S9-S10 Loop)<br>GLY <sup>370</sup> (S9-S10 Loop)<br>ASN <sup>371</sup> (S10)<br>THR <sup>372</sup> (S10)<br>VAL <sup>375</sup> (S10) | VAL <sup>268</sup> (S7)<br>GLY <sup>370</sup> (H9-S8 Loop) |

Figure 29: In *C. caldarium* and *C. merolae* a polar serine<sup>267</sup> (yellow/green) replaces a hydrophobic leucine in beta tubulin (A). Valine<sup>268</sup> (polar/green), asparagine<sup>371</sup> (polar/green), and glycine<sup>370</sup> (hydrophobic/white) are also pictured as they are within 3.5 angstroms of serine<sup>267</sup>. This image is a closer view (B) of the amino acids to show orientation and possible interaction of side chains. One possible interaction between serine<sup>267</sup> and asparagine<sup>371</sup> may form a hydrogen bond. Parenthetical references in the table refer to locations of amino acids in secondary structure.



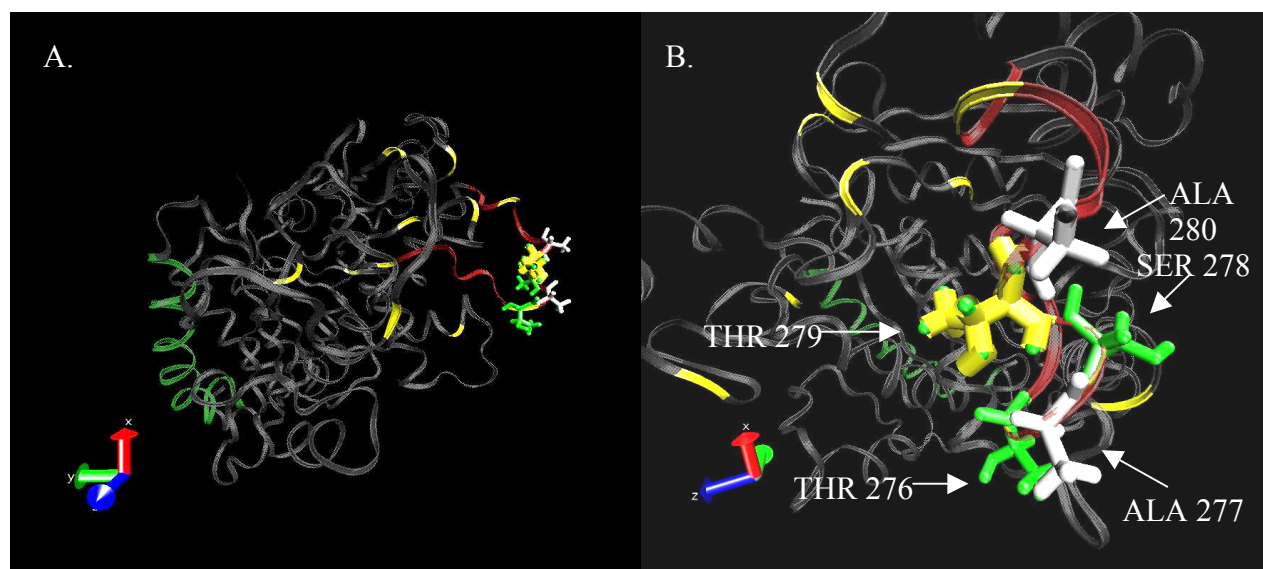
| Non-conserved substitution | Within 5 angstroms                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Within 2 angstroms                                                                            |
|----------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|
| SER <sup>269</sup> (S7)    | VAL <sup>232</sup> (H7)<br>SER <sup>267</sup> (S7)<br>VAL <sup>268</sup> (S7)<br>LEU <sup>270</sup> (S7-H9 Loop)<br>ALA <sup>271</sup> (S7-H9 Loop)<br>LEU <sup>273</sup> (S7-H9 Loop)<br>GLN <sup>292</sup> (H9)<br>MET <sup>293</sup> (H9)<br>PHE <sup>294</sup> (H9)<br>ASN <sup>298</sup> (H9-S8 Loop)<br>GLN <sup>299</sup> (H9-S8 Loop)<br>PHE <sup>300</sup> (H9-S8 Loop)<br>CYS <sup>366</sup> (S9-S10 Loop)<br>VAL <sup>367</sup> (S9-S10 Loop)<br>PHE <sup>368</sup> (S9-S10 Loop)<br>VAL <sup>369</sup> (S9-S10 Loop) | LEU <sup>270</sup> (S7-H9 Loop)<br>GLN <sup>299</sup> (H9-S8 Loop)<br>VAL <sup>268</sup> (S7) |

Figure 30: In *C. caldarium* and *C. merolae* a polar serine<sup>269</sup> (yellow/green) replaces a hydrophobic leucine in beta tubulin (A). Phenylalanine<sup>368</sup> (hydrophobic/white), glutamine<sup>299</sup> (polar/green), valine<sup>268</sup> (hydrophobic/white), and leucine<sup>270</sup> (hydrophobic/white) are also pictured as they are within 3.5 angstroms of serine<sup>267</sup>. This image is a closer view (B) of the amino acids to show orientation and possible interaction of side chains. One such possible interaction between serine<sup>269</sup> and glutamine<sup>299</sup> may form a hydrogen bond. Parenthetical references in the table refer to locations of amino acids in secondary structure.



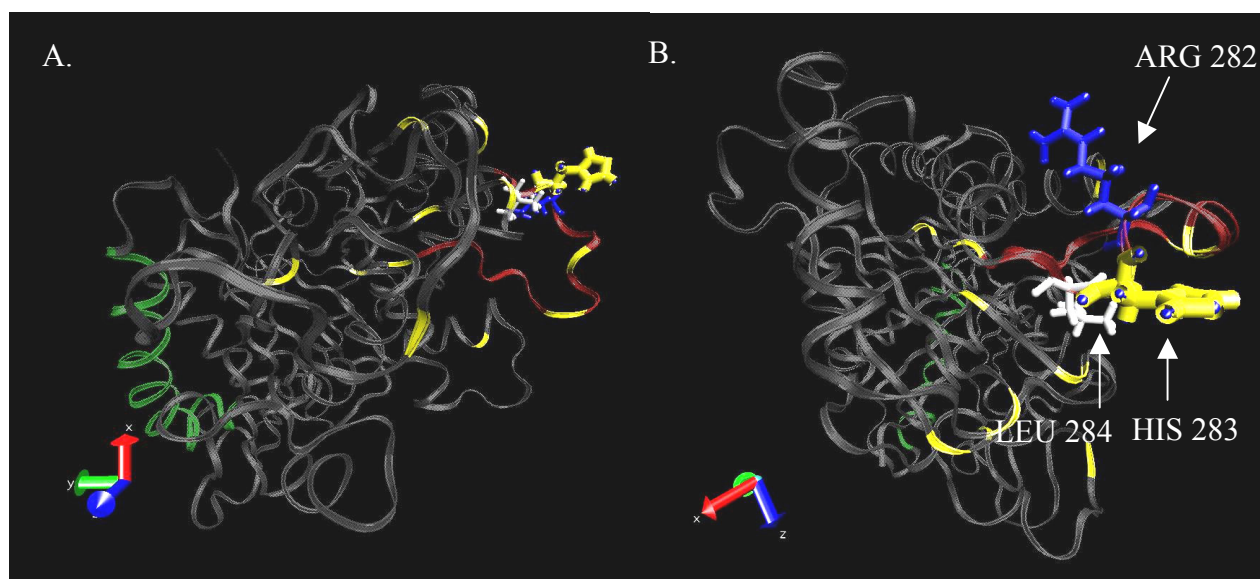
| Non-conserved substitution      | Within 5 angstroms                                                                                                                                                                                                                                                                   | Within 2 angstroms                                                 |
|---------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------|
| THR <sup>276</sup> (S7-H9 Loop) | LEU <sup>215</sup> (H6-H7 Loop)<br>LYS <sup>216</sup> (H6-H7 Loop)<br>LEU <sup>217</sup> (H6-H7 Loop)<br>LEU <sup>273</sup> (S7-H9 Loop)<br>ALA <sup>275</sup> (S7-H9 Loop)<br>ALA <sup>277</sup> (S7-H9 Loop)<br>SER <sup>278</sup> (S7-H9 Loop)<br>THR <sup>279</sup> (S7-H9 Loop) | ALA <sup>275</sup> (S7-H9 Loop)<br>ALA <sup>277</sup> (S7-H9 Loop) |

Figure 31: In *C. caldarium* and *C. merolae* a polar threonine <sup>276</sup> (yellow/green) replaces a basic arginine in beta tubulin (A). Alanine <sup>275</sup> (hydrophobic/white), threonine <sup>279</sup> (polar/green), and alanine <sup>277</sup> (hydrophobic/white) are also pictured as they are within 3.5 angstroms of threonine <sup>276</sup>. This image is a closer view (B) of the amino acids to show orientation and possible interaction of side chains. Parenthetical references in the table refer to locations of amino acids in secondary structure.



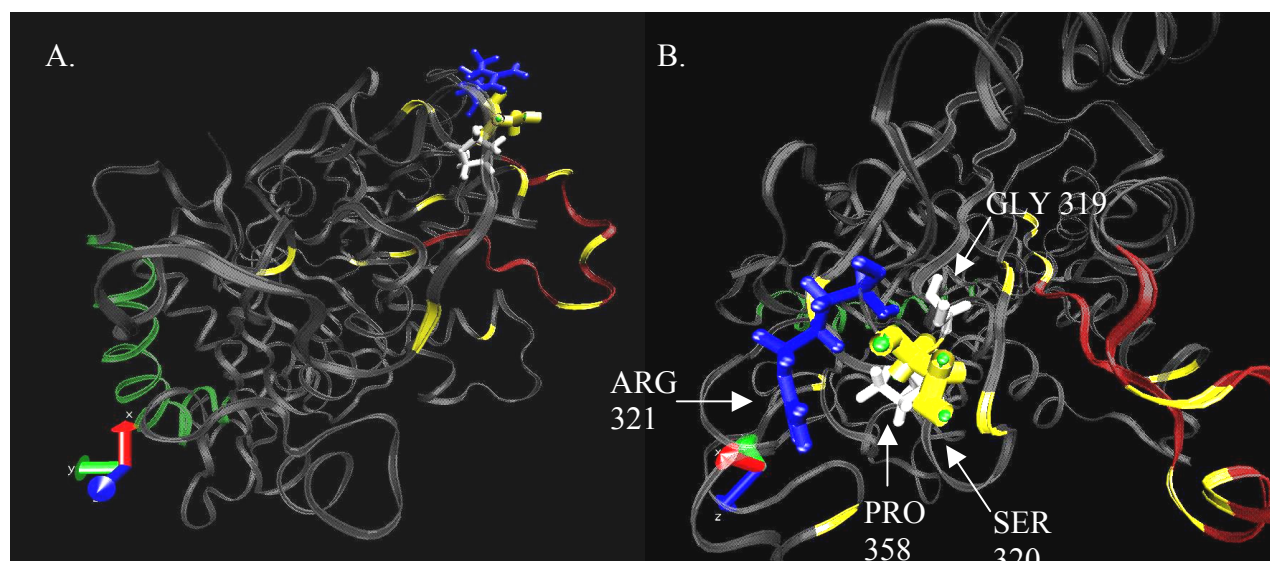
| Non-conserved substitution      | Within 5 angstroms                                                                                                                                                                                                                                | Within 2 angstroms                                                 |
|---------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------|
| THR <sup>279</sup> (S7-H9 Loop) | THR <sup>274</sup> (S7-H9 Loop)<br>ALA <sup>275</sup> (S7-H9 Loop)<br>THR <sup>276</sup> (S7-H9 Loop)<br>ALA <sup>277</sup> (S7-H9 Loop)<br>SER <sup>278</sup> (S7-H9 Loop)<br>ALA <sup>280</sup> (S7-H9 Loop)<br>TYR <sup>281</sup> (S7-H9 Loop) | SER <sup>278</sup> (S7-H9 Loop)<br>ALA <sup>280</sup> (S7-H9 Loop) |

Figure 32: In *C. caldarium* and *C. merolae* a polar threonine <sup>279</sup> (yellow/green) replaces a larger glutamine in beta tubulin (A). Serine <sup>278</sup> (polar/green), alanine <sup>280</sup> (hydrophobic/white), threonine <sup>276</sup> (polar/green), and alanine <sup>277</sup> (hydrophobic/white) are also pictured as they are within 3.5 angstroms of threonine <sup>279</sup>. This image demonstrates the location of the substitution on the beta monomer. This image is a closer view (B) of the amino acids to show orientation and possible interaction of side chains. Parenthetical references in the table refer to locations of amino acids in secondary structure.



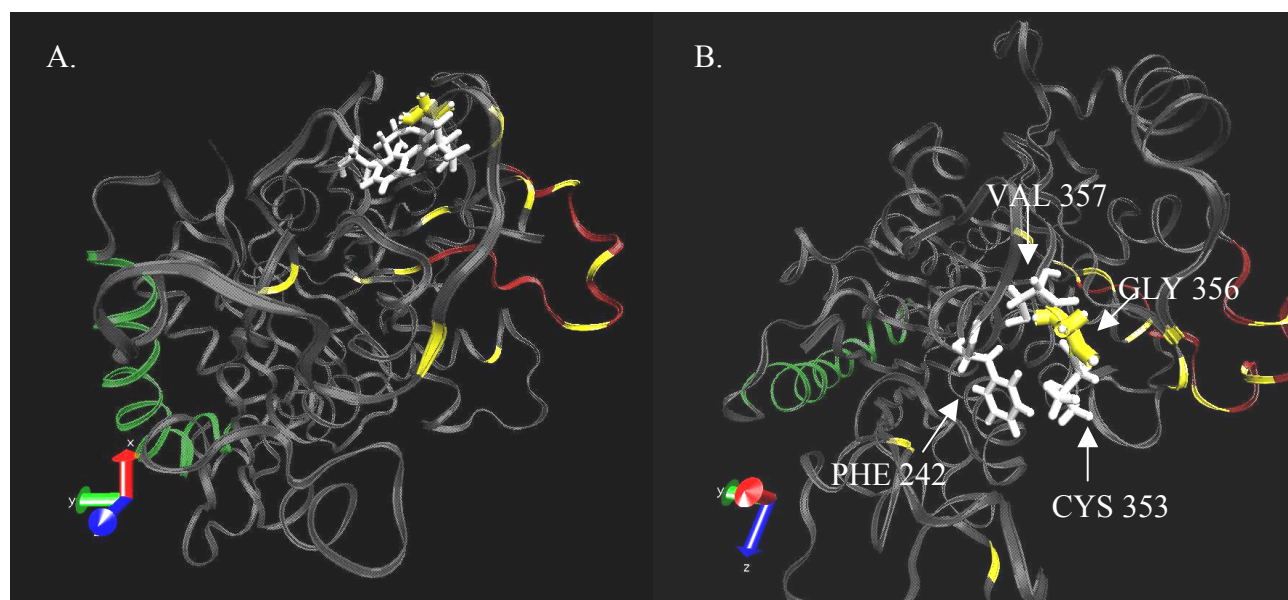
| Non-conserved substitution      | Within 5 angstroms                                                                                                                                                   | Within 2 angstroms                                                 |
|---------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------|
| HIS <sup>283</sup> (S7-H9 Loop) | ALA <sup>280</sup> (S7-H9 Loop)<br>TYR <sup>281</sup> (S7-H9 Loop)<br>ARG <sup>282</sup> (S7-H9 Loop)<br>LEU <sup>284</sup> (S7-H9 Loop)<br>MET <sup>384</sup> (H11) | ARG <sup>282</sup> (S7-H9 Loop)<br>LEU <sup>284</sup> (S7-H9 Loop) |

Figure 33: In *C. caldarium* and *C. merolae* a basic histidine <sup>283</sup> (yellow/blue) replaces a hydrophobic glycine in beta tubulin (A). Arginine <sup>282</sup> (basic/blue), alanine <sup>280</sup> (hydrophobic/white), and alanine <sup>284</sup> (hydrophobic/white) are also pictured as they are within 3.5 angstroms of histidine <sup>283</sup>. This image is a closer view (B) of the amino acids to show orientation and possible interaction of side chains. Parenthetical references in the table refer to locations of amino acids in secondary structure.



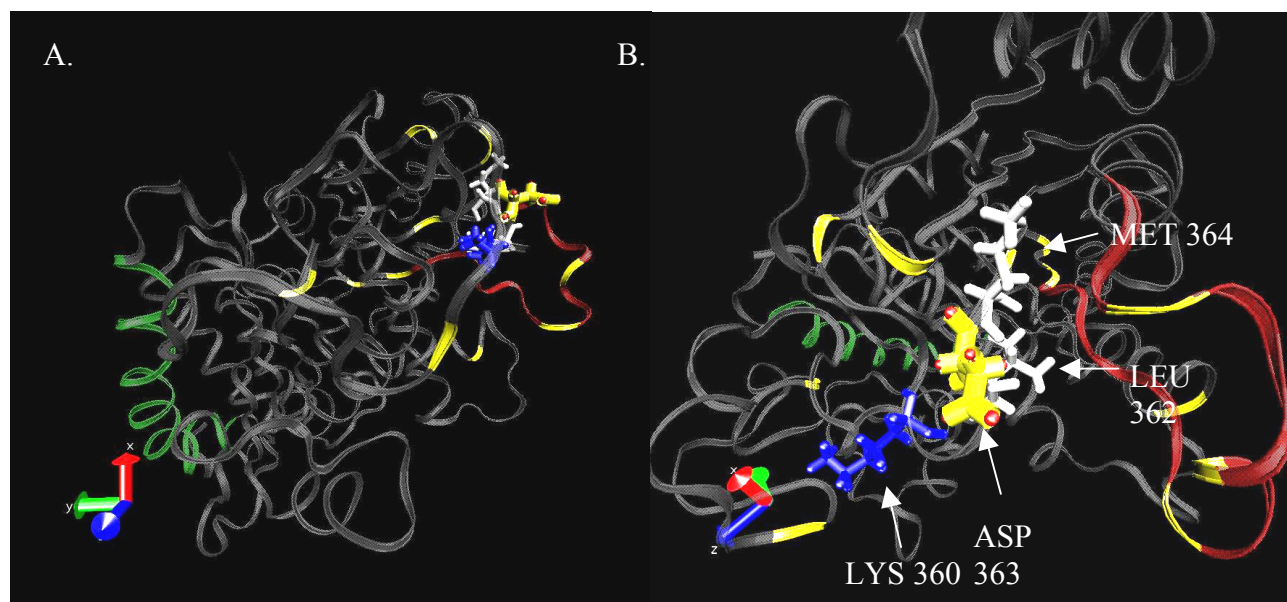
| Non-conserved substitution | Within 5 angstroms                                                                                                                                                                                                                                                                                                                                 | Within 2 angstroms                                                                   |
|----------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| SER <sup>320</sup> (S8)    | ARG <sup>318</sup> (S8)<br>GLY <sup>319</sup> (S8)<br>ARG <sup>321</sup> (S8-H10 Loop)<br>ILE <sup>322</sup> (S8-H10 Loop)<br>VAL <sup>357</sup> (S9-S10 Loop)<br>PRO <sup>358</sup> (S9-S10 Loop)<br>PRO <sup>359</sup> (S9-S10 Loop)<br>ASP <sup>363</sup> (S9-S10 Loop)<br>MET <sup>364</sup> (S9-S10 Loop)<br>SER <sup>365</sup> (S9-S10 Loop) | GLY 319 (S8)<br>ARG <sup>321</sup> (S8-H10 Loop)<br>PRO <sup>358</sup> (S9-S10 Loop) |

Figure 34: In *C. caldarium* and *C. merolae* a polar serine<sup>320</sup> (yellow/green) is an addition found only in the thermophilic beta tubulin (A). Arginine<sup>321</sup> (basic/blue), glycine<sup>319</sup> (hydrophobic/white), and proline<sup>358</sup> (hydrophobic/white) are also pictured as they are within 3.5 angstroms of serine<sup>320</sup>. This image is a closer view (B) of the amino acids to show orientation and possible interaction of side chains. Parenthetical references in the table refer to locations of amino acids in secondary structure.



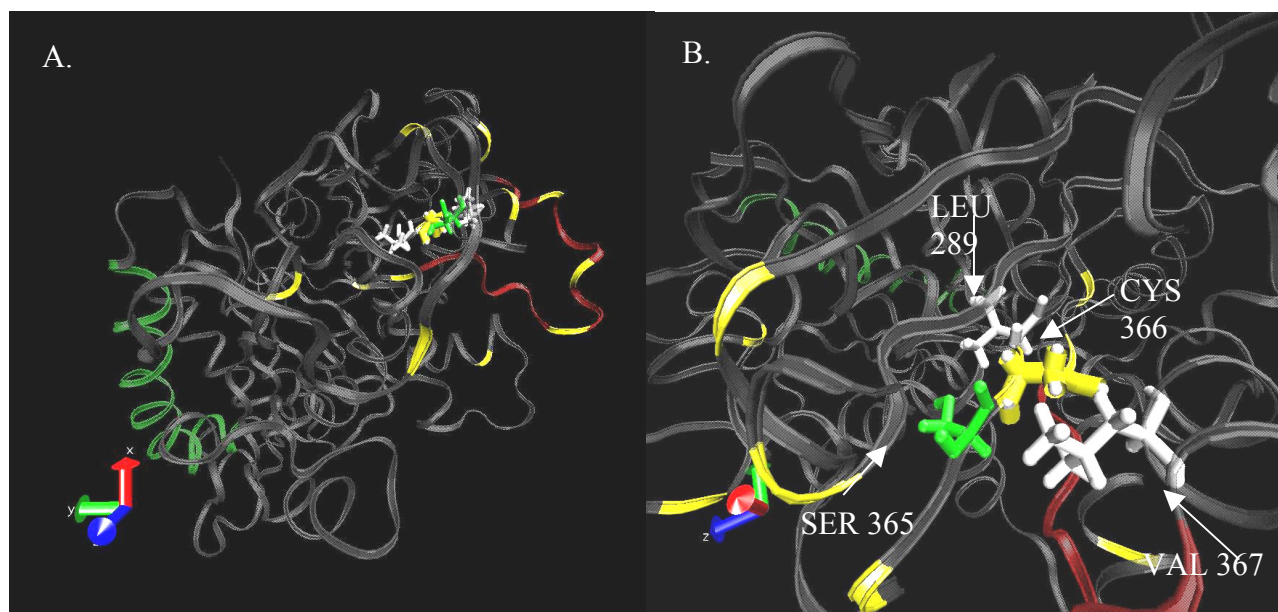
| Non-conserved substitution      | Within 5 angstroms                                                                                                                                                                                                                                                                                                                    | Within 2 angstroms                                          |
|---------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------|
| GLY <sup>356</sup> (S9-S10Loop) | PHE <sup>242</sup> (H7-H8 Loop)<br>PRO <sup>243</sup> (H7-H8 Loop)<br>GLY <sup>244</sup> (H7-H8 Loop)<br>GLN <sup>245</sup> (H7-H8 Loop)<br>ARG <sup>318</sup> (S8)<br>ARG <sup>321</sup> (S8-H10 Loop)<br>VAL <sup>354</sup> (S9)<br>CYS <sup>355</sup> (S9)<br>VAL <sup>357</sup> (S9-S10 Loop)<br>PRO <sup>358</sup> (S9-S10 Loop) | CYS <sup>353</sup> (S9)<br>VAL <sup>357</sup> (S9-S10 Loop) |

Figure 35: In *C. caldarium* and *C. merolae* a hydrophobic glycine <sup>356</sup> replaces a polar asparagine in beta tubulin (A). Valine <sup>357</sup> (hydrophobic/white), cysteine <sup>353</sup> (hydrophobic/white), and phenylalanine <sup>242</sup> (hydrophobic/white) are also pictured as they are within 3.5 angstroms of glycine <sup>356</sup>. This image is a closer view (B) of the amino acids to show orientation and possible interaction of side chains. One possible such interaction may be a hydrophobic-hydrophobic interaction between glycine <sup>356</sup> and phenylalanine <sup>242</sup>. Parenthetical references in the table refer to locations of amino acids in secondary structure.



| Non-conserved substitution       | Within 5 angstroms                                                                                                                                                                                                                                                                                            | Within 2 angstroms                                                   |
|----------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------|
| ASP <sup>363</sup> (S9-S10 Loop) | LEU <sup>284</sup> (S7-H9 Loop)<br>LYS <sup>319</sup> (S8)<br>SER <sup>320</sup> (S8)<br>PRO <sup>358</sup> (S9-S10 Loop)<br>PRO <sup>359</sup> (S9-S10 Loop)<br>LYS <sup>360</sup> (S9-S10 Loop)<br>GLY <sup>361</sup> (S9-S10 Loop)<br>LEU <sup>362</sup> (S9-S10 Loop)<br>MET <sup>364</sup> (S9-S10 Loop) | LEU <sup>362</sup> (S9-S10 Loop)<br>MET <sup>364</sup> (S9-S10 Loop) |

Figure 36: In *C. caldarium* and *C. merolae* an acidic aspartic acid<sup>363</sup> (yellow/red) replaces a basic lysine in beta tubulin (A). Lysine<sup>360</sup> (basic/blue), leucine<sup>362</sup> (hydrophobic/white), and methionine<sup>364</sup> (hydrophobic/white) are also pictured as they are within 3.5 angstroms of asparagine<sup>363</sup>. This image is a closer view (B) of the amino acids to show orientation and possible interaction of side chains. The substitution may reduce like-like repulsion between lysine<sup>360</sup> and the lysine<sup>363</sup> usually found in mesophilic tubulin. Parenthetical references in the table refer to locations of amino acids in secondary structure.



| Non-conserved substitution       | Within 5 angstroms                                                                                                                                                                                                                                                                                                                                                                                                                                    | Within 2 angstroms                                                                              |
|----------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|
| CYS <sup>366</sup> (S9-S10 Loop) | SER <sup>269</sup> (S7)<br>LEU <sup>270</sup> (S7)<br>ASP <sup>288</sup> (H9)<br>LEU <sup>289</sup> (H9)<br>THR <sup>290</sup> (H9)<br>GLN <sup>292</sup> (H9)<br>MET <sup>293</sup> (H9)<br>ALA <sup>315</sup> (S8)<br>TYR <sup>316</sup> (S8)<br>PHE <sup>317</sup> (S8)<br>ARG <sup>318</sup> (S8)<br>LEU <sup>362</sup> (S9-S10 Loop)<br>SER <sup>365</sup> (S9-S10 Loop)<br>VAL <sup>367</sup> (S9-S10 Loop)<br>PHE <sup>368</sup> (S9-S10 Loop) | LEU <sup>289</sup> (H9)<br>SER <sup>365</sup> (S9-S10 Loop)<br>VAL <sup>367</sup> (S9-S10 Loop) |

Figure 37: In *C. caldarium* and *C. merolae* a hydrophobic cysteine<sup>366</sup> (yellow/white) replaces a smaller alanine in beta tubulin (A). Valine<sup>367</sup> (hydrophobic/white), leucine<sup>289</sup> (hydrophobic/white), and serine<sup>365</sup> (polar/green) are also pictured as they are within 3.5 angstroms of cysteine<sup>366</sup>. This image is a closer view (B) of the amino acids to show orientation and possible interaction of side chains. One of these interactions may be a hydrophobic-hydrophobic interaction between cysteine<sup>366</sup> and leucine<sup>289</sup>. Parenthetical references in the table refer to locations of amino acids in secondary structure

## Computational analysis of tubulin monomers

In order to test whether the non-conserved differences identified in the algal tubulin monomers could affect thermostability, it was important to determine whether the algal tubulin behaved differently from the mesophilic tubulin at temperatures approaching 55°C, the temperature commonly found in the algal habitat. Alpha and beta tubulin monomers from both *C. caldarium* and *C. merolae* were analyzed in silico using molecular dynamics simulations to find differences between the thermophilic and the mesophilic tubulin in regard to RMS and atomic fluctuation values. For example, if there were major differences between the RMS values of the thermophiles and the mesophiles, then this would suggest that there was a difference between overall atomic motion between the thermophilic and mesophilic monomers, while atomic fluctuations would represent a more local change within the monomer. It is assumed that to maintain conformation and molecular integrity the thermophile would have to have less atomic motion at 55°C than would the mesophile.

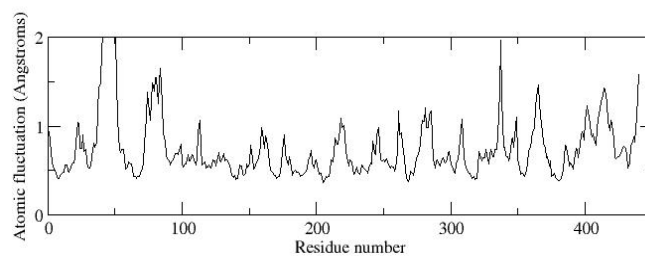
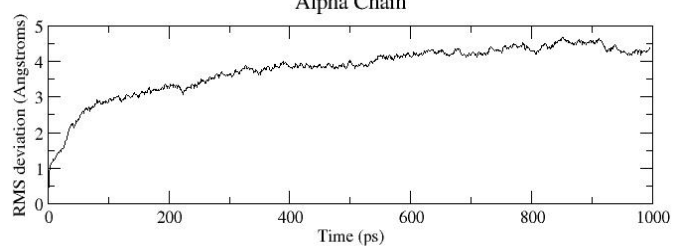
Atomic fluctuation and RMS values for alpha and beta tubulin monomers were compared to those from the porcine tubulin structure after simulated heating to 55°C. In alpha tubulin, the RMS deviation was an average of one angstrom higher for the mesophile with a RMS deviation of 4.5 Å, indicating more movement away from the starting structure for the mesophilic alpha monomer than for either of the thermophilic monomers (Figure 38). The N-loop, amino acids 35 to 70, exhibited atomic fluctuations of more than 2.5 Å in the pig tubulin alpha chain, compared to less than 2 Å atomic fluctuation in the N-loops of either thermophilic alpha tubulin monomer. The only other

major difference between the atomic fluctuation of the mesophilic and thermophilic alpha tubulin occurred in the region of residues ~340-350. This region had an atomic fluctuation of 2 Å in the mesophile compared to 1.5 in the thermophile. The M-loop does not show any significant differences between the mesophilic and thermophilic alpha tubulin, which was expected since there was only one non-conserved difference within that region as compared to three in the N-loop.

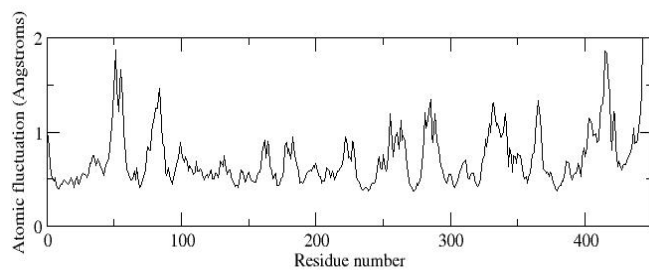
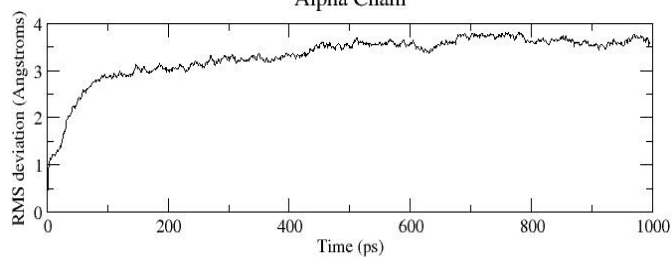
In beta tubulin, there was no difference in the RMS values between mesophilic and thermophilic tubulin monomers (Figure 39). In addition, there were no clear differences in the atomic fluctuations between mesophilic and either of the thermophilic beta tubulin monomers. In some cases there were large-scale differences between the thermophiles, which was unexpected due to the high conservation of amino acid sequence between thermophiles. For example, there was a difference of 0.5 Å of atomic fluctuation in the N-loop between the beta monomers.

Figure 38: RMS and atomic fluctuation graphs of mesophilic (Pig) and thermophilic (*C. caldarium* and *C. merolae*) alpha tubulin over 1ns at 55°C. Pig alpha tubulin had a maximum RMS value of 4.5 Å as compared to a maximum RMS of 3.5 in both alpha thermophilic tubulins. Major differences in atomic fluctuation were found in regions of the primary structure at ~ amino acids 30-50 and 340-350, with the mesophile having atomic fluctuation values of 2.5 and 2 Å, respectively, as compared to thermophilic values of 2 and 1.5 Å, respectively.

Pig Tubulin  
Alpha Chain



*C. merolae* Tubulin  
Alpha Chain



*C. caldarium* Tubulin  
Alpha Chain

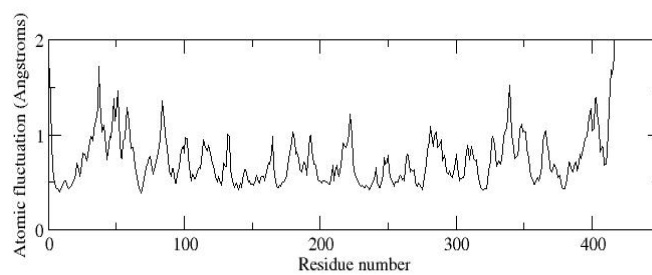
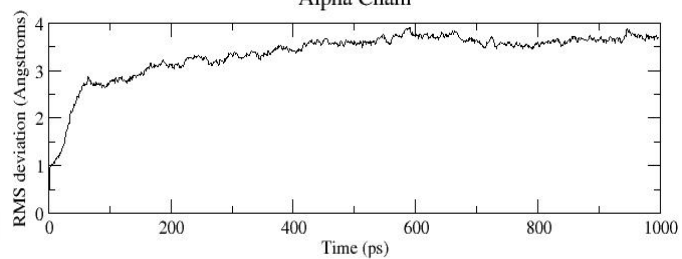
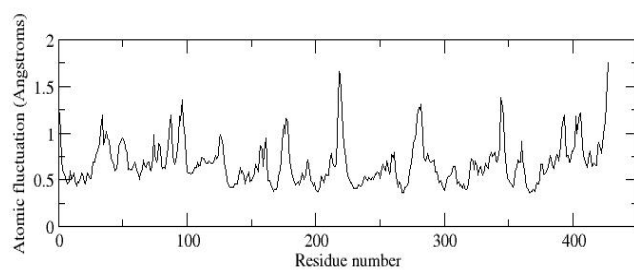
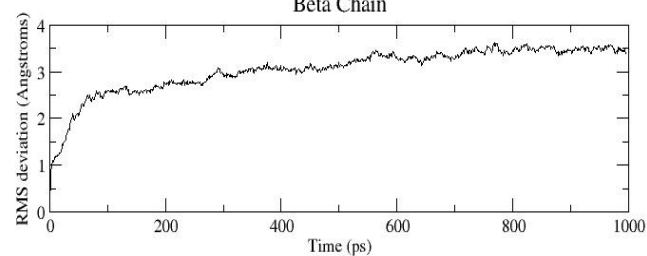
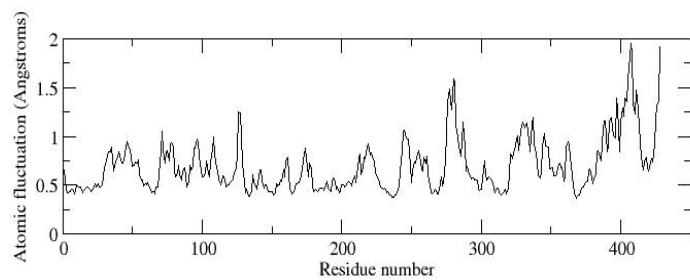
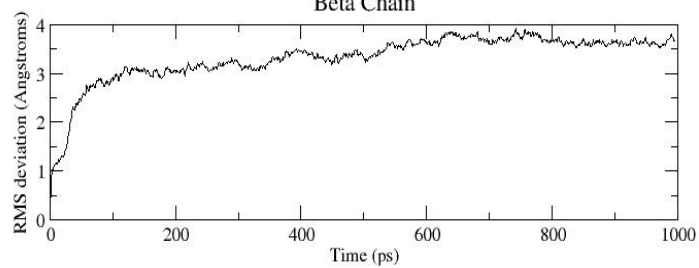


Figure 39: RMS and atomic fluctuation graphs of mesophilic (Pig) and thermophilic (*C. caldarium* and *C. merolae*) beta tubulin monomers in angstroms over 1ns at 55°C. Pig tubulin had RMS values comparable to both of the beta thermophilic tubulins. Major differences in atomic fluctuation were not found in regions of the primary structure between the mesophile and thermophilic beta tubulin, although there were unexpected differences in atomic fluctuations between the thermophilic tubulin in the N-terminal regions.

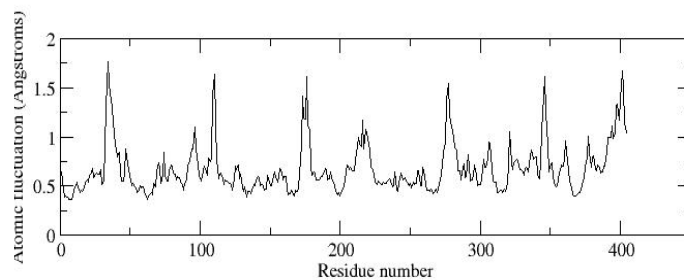
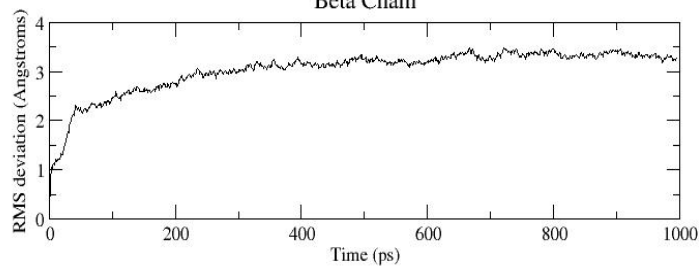
Pig Tubulin  
Beta Chain



*C. merolae* Tubulin  
Beta Chain



*C. caldarium* Tubulin  
Beta Chain



## **Discussion**

When the predicted amino acid sequences for the algal thermophilic tubulin monomers were compared to 250 mesophilic tubulin amino acid sequences, nine non-conserved differences were found in alpha tubulin and 12 in beta tubulin. These differences were characterized and mapped to the tertiary structure of algal thermophilic tubulin monomers produced by homology modeling. Based on the analysis of amino acids within 2, 3.5, and 5 of the non-conserved differences, in many cases it is possible to make predictions as to how such differences may affect the overall thermostability of the algal tubulin monomers. I will discuss each non-conserved difference and its potential local effects, then I will draw conclusions about how these non-conserved differences and their local effects contribute to the overall thermostability of the microtubule.

### **Non-conserved differences and their local effects**

#### **Alpha Tubulin**

In the algal thermophile, a hydrophobic leucine<sup>31</sup> is substituted for polar glutamine normally found in a mesophilic alpha tubulin. This leucine is surrounded by two hydrophobic molecules, phenylalanine<sup>246</sup> and leucine<sup>254</sup>, which have the potential for hydrophobic-hydrophobic interactions (Lesk, 2003). A hydrophobic-hydrophobic interaction between leucine<sup>31</sup> located in the N-loop and phenylalanine<sup>246</sup> or leucine<sup>254</sup>, both located in the T7 loop, could potentially anchor the N-loop to the T7 loop restricting

the movement of the N-loop at higher temperatures and thus allowing the loop to maintain its shape. This anchoring effect may help account for the lower atomic fluctuations seen in N-loop in the alpha algal tubulin when compared to the mesophilic tubulin (Figure 36). The ability to maintain the structure of the N-loop is critical to maintaining a microtubule because it is thought to be one of the major sites of protofilament lateral interaction as discussed earlier (Li et al., 2002a).

Also in the N-loop basic arginine<sup>39</sup> is a addition found only in the algal thermophiles. This arginine is within 2 Å of glutamic acid<sup>27</sup>, an acidic amino acid found in a different part of the N-loop. Since these oppositely charged residues are in such close proximity to one another, there arises a possibility of a salt bridge forming between them. Salt bridges are rarely found within the same secondary structure (Das and Gerstein, 2000) but the N-loop is one of the largest and least well-defined areas in the heterodimer structure (Gogonea et al., 1999). As such, a salt bridge may help this large loop retain its shape by locking two parts of the loop together.

The last non-conserved difference in the N-loop of the alpha tubulin monomer in both thermophiles is the addition of serine<sup>50</sup>. This amino acid is found within 3.5 Å of another polar amino acid threonine<sup>58</sup>. As with arginine<sup>39</sup> and glutamic acid<sup>27</sup>, serine<sup>50</sup> and threonine<sup>58</sup> are both within the N- loop. Although neither of these polar amino acids can form a salt bridge, each may be able to form a hydrogen bond with each other. As with salt bridges, hydrogen bonds between amino acid side chains in the same secondary structure are rare, but may serve to stabilize a specific orientation in the N-loop (Kumar S., 2000). Outside the N-loop, non-conserved differences are not found clustered in any one part of the secondary structure of the algal alpha tubulin monomer.

At position 84 in the algal thermophile a hydrophobic praline<sup>84</sup> replaces a polar threonine in alpha tubulin. This position in mesophilic tubulin is often at the very end of the second helix (H2'), before the H2-S3 loop. However, in the thermophile it appears that the proline in that position facilitates the start of the loop due to its shape, resulting in an elongated H2-S3 loop. The low density maps from this region make it hard to predict the function of this loop (Downing and Nogales, 1998). However, this elongated loop may provide more area for possible heterodimer-heterodimer interactions unique to these thermophilic tubulin. Also a basic arginine<sup>98</sup> replaces a hydrophobic isoleucine<sup>98</sup>. Arginine<sup>98</sup> is in the T3 loop, which is a loop between S3 and H3. The H3 helix had previously been implicated as the main lateral attachment point of the tubulin monomer (Nogales et al., 1999), and although data has recently called this argument into question, the H3 helix is still thought to be vitally important to the overall function of the heterodimer. Arginine<sup>98</sup> is within 3.5 angstroms of the residue glutamic acid<sup>73</sup> residing in the H2 helix suggesting a possible salt bridge between the two of them. An ionic bond between the T3 loop and H2 helix may help stabilize the H3 helix at higher temperatures and help the T3 loop maintain its conformation.

The M-loop also is thought to be a main point of lateral attachment between the protofilaments (Nogales et al., 1998). In the thermophile a small hydrophobic glycine<sup>288</sup> replaces a larger hydrophobic valine<sup>288</sup> in the alpha tubulin. This substitution comes at the very end of the M-loop and the smaller amino acid that may lessen any steric interference to glutamine<sup>287</sup> and serine<sup>289</sup> forming a hydrogen bond. The hydrogen bond may help the M-loop keep its conformation at high temperatures.

All portions of the tubulin monomer following the M-loop are considered C-

terminal. C-terminal region has a higher variability than any other part of the tubulin monomer when comparing tubulin monomers to one another. Therefore, one may expect to find more non-conserved differences in this area, even though their contribution to thermostability may be questionable.

In the H10 helix of the thermophilic alpha tubulin, a polar glutamine replaces a hydrophobic valine. This polar amino acid is within 4 angstroms of an aspartic acid <sup>326</sup> within the S8-H10 loop. The electrostatic interaction between the two amino acids may help anchor the S8-H10 loop to the H10 helix. This interaction may reduce the movement of the loop at higher temperatures and help to hold the C-terminal region together.

Another non-conserved difference in the C-terminal region of the thermophilic tubulin is lysine <sup>335</sup>. The lysine replaces a hydrophobic glycine in the H10-S9 loop and is within 5 Å of phenylalanine <sup>353</sup> located in the ninth beta strand S9. This substitution may help anchor the H10-S9 loop to the internal S9 strand via a cation pi interaction. This interaction is formed when a positively charged amino acid interacts with the pi electrons in an aromatic ring.

The last substitution in the thermophile is serine <sup>359</sup>. This polar amino acid replaces a tyrosine located in the S9-S10 loop. The more external T7 loop is within four angstroms of serine <sup>359</sup>. The T7 loop connects the H7 and H8 helices together. A possible attractive electrostatic interaction between serine <sup>359</sup> and asparagine <sup>258</sup> located at the beginning of the H8 helix may help anchor that portion of the molecule, thus helping the H8 helix and the T7 loop keep their shape at higher temperatures. This interaction may not be possible in the mesophilic tubulin due to the steric hindrance caused by the

aromatic ring attached to tyrosine.

## **Beta Tubulin**

In thermophilic beta tubulin a polar glutamine<sup>42</sup> replaces a hydrophobic leucine located in the N-loop. This substitution adds another polar amino acid into an already highly polar region of the N-loop. Although there is a possibility of a hydrogen bond between lysine<sup>360</sup> from the S9-S10 loop and glutamine<sup>42</sup>, it is possible that amino acid side chains within the N-loop may also interact with glutamine<sup>42</sup>. This intra-loop interaction may be important to the stability of the N-loop by replacing hydrophobic amino acids with polar or charged amino acids in this region.

Another N-loop substitution present in the beta thermophile is threonine<sup>56</sup>. This polar amino acid replaces a hydrophobic glycine. The substitution takes place in a part of the N-loop that is within four angstroms of beta strand S1. It is possible that in addition to the above mentioned hypothesis for polar residues replacing hydrophobic residues in the N loop, this region of the N loop could also be anchored to the internal portion of the molecule by virtue of an attractive electrostatic interaction between the polar threonine<sup>56</sup> located in the N loop and the basic histidine<sup>6</sup> located in beta strand S1.

The first substitution outside of the N-loop is glutamine<sup>212</sup>. This polar amino acid replaces a hydrophobic phenylalanine in the helix H6. A non-conserved substitution inside a helix may seem to have little effect on the thermostability of the beta tubulin monomer, however part of the loop that connects the H6 and H7 helices folds back toward the H6 helix and thus glutamine<sup>212</sup> is within 3.5Å of threonine<sup>218</sup> located in the

H6-H7 loop. These two polar residues may form a hydrogen bond and thus help the H6-H7 loop maintain its shape at higher temperatures.

The next amino acid takes place near the M-loop region of the beta monomer. It is the polar amino acid serine <sup>267</sup>, and it replaces a hydrophobic leucine located near the end of the H8-S7 loop. The non-conserved substitution inside the H8-S7 loop is within 3.5 angstroms of the beta strand S10. There is a possible attractive electrostatic interaction between serine <sup>267</sup> inside the H8-S7 loop and asparagine <sup>371</sup> located in the S10 beta strand, which may help stabilize the H8-S7 loop at higher temperatures.

Another non-conserved substitution taking place only two positions downstream of serine <sup>267</sup> is serine <sup>269</sup>. The polar amino acid replaces a hydrophobic leucine located in the seventh beta strand S7 and the substitution is within 3.5 angstroms of many different parts of the molecule (3 loops and 2 helices). Therefore, it is possible that this region “ties” together many of these different parts of the molecule. There is a possible hydrogen bond between serine <sup>269</sup> located in the beta strand S7 and glutamine <sup>299</sup> located in the H9-S8 loop. This hydrogen bond may help the H9-S8 loop maintain conformation at higher temperatures by anchoring it to the S7 beta strand located in the internal portion of the molecule.

The third non-conserved difference taking place in the region of the M-loop is threonine <sup>276</sup>. The polar amino acid replaces a basic arginine. This substitution of a polar amino acid allows a possible attractive electrostatic interaction between threonine <sup>276</sup> and lysine <sup>216</sup> located in the H6-H7 loop, where before there was a like charge repulsion. A bond between these two amino acids in these two loops may help the M-loop maintain the critically important conformation needed for lateral interactions.

One particularly interesting non-conserved substitution occurs at position 279 in the beta monomer. In the thermophile a threonine replaces a glutamine located in the M loop. This substitution is located in the most external portion of the M-loop and is not close enough to any of the other secondary structures to interact. Thus, this substitution does not seem to contribute to the thermostability of the heterodimer.

The last non-conserved difference in the M-loop of the beta monomer is histidine<sup>283</sup>. In the thermophile this basic amino acid replaces a hydrophobic glycine. This difference is within four angstroms of the H11 helix located near the outside of the molecule. There is a possible hydrogen bond between histidine<sup>283</sup> located in the M-loop and methionine<sup>384</sup> located in the H11 helix. The hydrogen bond may help the M-loop maintain its conformation by anchoring the M-loop to the H11 helix. The conformation of the M-loop is critical for lateral interaction between heterodimers.

The only addition in the thermophilic beta tubulin is a polar serine. The addition occurs in the beta strand S8 buried in the core of the molecule. There doesn't seem to be any oppositely charged amino acids within bonding range, and thus there seems to be no obvious beneficial effect of this addition on the beta tubulin.

The next region of algal beta tubulin to have non-conserved differences is the S9-S10 loop and it has three. The first of these three substitutions is a small hydrophobic amino acid glycine<sup>356</sup> replacing a polar asparagine and is within 3.5 angstroms of phenylalanine<sup>242</sup> located in the more external H7-H8 loop. This implies a possible hydrophobic-hydrophobic interaction between the residues. The interaction may help anchor the H7-H8 loop at higher temperatures and help the H8 helix from moving around.

Secondly, there is a replacement of lysine for aspartic acid at position 363 and may help reduce the charge repulsion present in the mesophilic protein between lysine<sup>363</sup> and lysine<sup>360</sup>. This may help the loop maintain its conformation.

Finally, a larger hydrophobic cysteine replaces a smaller leucine located in the S9-S10 loop at position 366 and comes within 3.5 Å of helix H9, and located at the C-terminus the M-loop. The close proximity of the two structures allows for a hydrophobic-hydrophobic interaction between cysteine<sup>366</sup> and leucine<sup>289</sup> located in the H9 helix, which may help stabilize the H9 helix and thus help keep the M-loop in its native conformation.

## Conclusions

After identifying and characterizing the non-conserved differences unique to the algal tubulin, my thinking has been refined about the mechanism governing the ability of the tubulin heterodimer to maintain its conformation at higher temperatures. Originally it was hypothesized that the tubulin suffered a loss of tertiary structure at a global level, this is not the current thinking, however.

After mapping the substitutions to the tertiary structure it was found that over 75% percent of the non-conserved differences map to loops and 33% of these non-conserved substitutions mapped to the H1-S2 loop (Table 3). As stated above, the N-loop is thought to be a major player in lateral interactions between protofilaments, interfacing with the M-loop of the adjacent protofilament. Not to be forgotten is the S9-S10 loop

that is thought to stabilize the M-loops in the heterodimer. The 5 non-conserved differences in the N-loop, 5 non-conserved differences in the M loop, and 4 non-conserved differences in the S9-S10 loop of the alpha and beta thermophilic tubulin show that two thirds of all non-conserved changes occur in these areas, suggesting that these regions must be greatly changed to keep the shape of those loops stable and the lateral interactions open. Several thermophilic studies have shown that loop regions are the first part of the molecule to lose their conformation at elevated temperatures (Daggett and Levitt, 1993). This clustering pattern of the non-conserved differences suggests that the regions responsible for maintaining lateral interactions between protofilaments are much more likely to lose their shape than others, or at least that the ability to form microtubules is dependent on the M, N, and S9-S10 loops keeping their conformations. With these data in mind, I suggest that when one heats tubulin to 55°C, global losses of conformation are not likely to take place given that 55°C is far from the hyperthermophilic environment in which other thermophilic proteins exist. It is more likely that local loss of conformation take place, and from the atomic fluctuation data showing large amounts of movement in the N-loop of the mesophilic tubulin and the clustering of non-conserved substitutions it seems likely that these key areas are at least in part the N, M, and S9-S10 loops. The overall strategy that seems to be employed here is to save the conformation of these loops at higher temperatures and thus keep the lateral interactions key in forming microtubules intact.

In the future, the predictions may in my analysis of the mechanism governing the thermostability of the alpha and beta tubulin monomer from *C. caldarium* and *C. merolae* can be tested by mutating each of the non-conserved differences found in the thermophile

and expressing the tubulin. This would allow for the testing the role each non-conserved difference plays in thermo-stabilizing the microtubule.

Several technical problems must be resolved. First need to obtain the C-terminal regions of the *C. caldarium* tubulin genes by developing a different strategy than any described previously. Also, a way must be found to express this algal tubulin so that it folds correctly, to date this has not been accomplished by any investigator known. Although it is theoretically possible to place algal DNA into another organism of expression purposes, it has never been done. The organism would need to have the necessary array of chaperone proteins to properly fold tubulin. Several possibilities exist, first the use of a cell free extract such a rabbit reticulocyte lysate or the use of insect cells could also be tested to see if they carry the proteins necessary to fold tubulin. Also each non-conserved difference should be analyzed by point mutation in order to determine which of these non-conserved differences actually plays a role in thermostability.

**Table 3: All non-conserved changes, their location, and effect**

| Name amino acid of change | Number of amino acid in thermophilic tubulin | Location of non-conserved change | Name of amino acid counterpart in the mesophile | Possible overall effect              |
|---------------------------|----------------------------------------------|----------------------------------|-------------------------------------------------|--------------------------------------|
| Leucine                   | 31 (Alpha)                                   | H1-S2 (N) Loop                   | Glutamine                                       | Hydrophobic-hydrophobic bond         |
| Arginine                  | 39 (Alpha)                                   | H1-S2 (N) Loop                   | Serine                                          | Salt bridge                          |
| Serine                    | 50 (Alpha)                                   | H1-S2 (N) Loop                   | Alanine                                         | Hydrogen bond                        |
| Proline                   | 84 (Alpha)                                   | H2-S3 Loop                       | Threonine                                       | Elongation of H2-S3 Loop             |
| Arginine                  | 98 (Alpha)                                   | T3 Loop                          | Isoleucine                                      | Salt bridge                          |
| Glycine                   | 288 (Alpha)                                  | S7-H9 (M) Loop                   | Valine                                          | Lessen steric interference           |
| Glutamine                 | 326 (Alpha)                                  | H10                              | Valine                                          | Attractive electrostatic interaction |
| Lysine                    | 335 (Alpha)                                  | H10-S9 Loop                      | Glycine                                         | Cation-pi interaction                |
| Serine                    | 359 (Alpha)                                  | S9-S10 Loop                      | Tyrosine                                        | Attractive electrostatic interaction |
| Glutamine                 | 42 (Beta)                                    | H1-S2 (N) Loop                   | Leucine                                         | Increase charge in region            |
| Threonine                 | 56 (Beta)                                    | H1-S2 (N) Loop                   | Glycine                                         | Attractive electrostatic interaction |
| Glutamine                 | 212 (Beta)                                   | H6                               | Phenylalanine                                   | Hydrogen bond                        |
| Serine                    | 267 (Beta)                                   | H8-S7 Loop                       | Leucine                                         | Hydrogen bond                        |
| Serine                    | 269 (Beta)                                   | S7                               | Leucine                                         | Hydrogen bond                        |
| Threonine                 | 276 (Beta)                                   | S7-H9 (M) Loop                   | Arginine                                        | Attractive electrostatic interaction |
| Threonine                 | 279 (Beta)                                   | S7-H9 (M) Loop                   | Glutamine                                       | N/A                                  |
| Histidine                 | 283 (Beta)                                   | S7-H9 (M) Loop                   | Glycine                                         | Hydrogen bond                        |
| Serine                    | 320 (Beta)                                   | S8                               | Addition                                        | N/A                                  |
| Glycine                   | 356 (Beta)                                   | S9-S10 Loop                      | Asparagine                                      | Hydrophobic-hydrophobic interaction  |
| Aspartic Acid             | 363 (Beta)                                   | S9-S10 Loop                      | Lysine                                          | Reduce like-like charge repulsion    |
| Cysteine                  | 366 (Beta)                                   | S9-S10 Loop                      | Alanine                                         | Hydrophobic-hydrophobic interaction  |

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## **Appendix A**

Mesophilic Sequences used to identify non-conserved differences

|                |                                                                                                                      |
|----------------|----------------------------------------------------------------------------------------------------------------------|
| SDSCNR:221803  | <b>alpha-tubulin 1 [Eleusine indica] CAA06619.1 [AJ005599], 451 AA</b>                                               |
| SDSCNR:180464  | <b>alpha-1 tubulin [Hirudo medicinalis] AAB07890.1 [U67677], 451 AA</b>                                              |
| SDSCNR:2381953 | <b>ENSANGP00000002667 [Anopheles gambiae str. PEST] XP_309723.1 [XM_309723], 462 AA</b>                              |
| SDSCNR:2662167 | <b>similar to Tubulin alpha-3/alpha-7 chain [Homo sapiens] XP_054284.4 [XM_054284], 459 AA</b>                       |
| SDSCNR:2440596 | <b>(AAQ94598) Tubulin alpha 6 [Brachydanio rerio (Zebrafish) (Danio rerio)], 450 AA</b>                              |
| SDSCNR:153458  | <b>(P18258) Tubulin alpha-1 chain [Paracentrotus lividus (Common sea urchin)], 452 AA</b>                            |
| SDSCNR:301827  | <b>alpha-tubulin [Homo sapiens] CAA25855.1 [X01703], 451 AA</b>                                                      |
| SDSCNR:670601  | <b>(Q98TS1) Alpha tubulin subunit [Oncorhynchus nerka (Sockeye salmon)], 451 AA</b>                                  |
| SDSCNR:1767080 | <b>(Q8T6A5) Alpha tubulin 1 [Aplysia californica (California sea hare)], 451 AA</b>                                  |
| SDSCNR:316611  | <b>bA408E5.3 (novel protein (orthologue of mouse [Homo sapiens] CAC12714.1 [AL139327], 449 AA</b>                    |
| SDSCNR:2663781 | <b>similar to tubulin alpha-1 chain - Chinese [Rattus norvegicus] XP_217040.1 [XM_217040], 453 AA</b>                |
| SDSCNR:153782  | <b>(P41383) Tubulin alpha-2/alpha-4 chain [Patella vulgata (Common limpet)], 452 AA</b>                              |
| SDSCNR:2436700 | <b>neural alfa2 tubulin [Paracentrotus lividus] AAQ90468.1 [AY388462], 452 AA</b>                                    |
| SDSCNR:2682284 | <b>similar to tubulin, alpha 6; tubulin alpha 6 [Rattus norvegicus] XP_237718.2 [XM_237718], 566 AA</b>              |
| SDSCNR:306934  | <b>tubulin, alpha, ubiquitous [Homo sapiens] NP_006073.1 [NM_006082], 451 AA</b>                                     |
| SDSCNR:1751548 | <b>(Q8WQ47) Alpha tubulin [Lepidoglyphus destructor (Storage mite)], 450 AA</b>                                      |
| SDSCNR:578655  | <b>alpha tubulin [Chionodraco rastrospinosus] AAG15365.1 [AF263276], 450 AA</b>                                      |
| SDSCNR:2086631 | <b>tuba6-prov protein [Xenopus laevis] AAH46841.1 [BC046841], 449 AA</b>                                             |
| SDSCNR:260573  | <b>(Q40831) Tubulin alpha-1 chain [Pelvetia fastigiata], 453 AA</b>                                                  |
| SDSCNR:128177  | <b>(P52273) Tubulin alpha chain [Bombyx mori (Silk moth)], 450 AA</b>                                                |
| SDSCNR:460593  | <b>(P18288) Tubulin alpha chain, testis-specific [Oncorhynchus mykiss (Rainbow trout) (Salmo gairdneri)], 450 AA</b> |
| SDSCNR:2268878 | <b>(Q7YZZ4) Putative alpha-tubulin [Oikopleura dioica], 450 AA</b>                                                   |
| SDSCNR:148257  | <b>alpha-Tubulin at 85E CG9476-PA [Drosophila melanogaster]</b>                                                      |

NP\_524297.1 [NM\_079573], 449 AA  
SDSCNR:446969 alpha tubulin [Notothenia coriiceps] AAC97928.1 [AF082027], 451 AA  
SDSCNR:1443172 (Q95UU2) Alpha-tubulin [Monosiga brevicollis], 451 AA  
SDSCNR:148258 alpha-Tubulin at 84D CG2512-PB [Drosophila melanogaster] NP\_731169.1 [NM\_169189], 450 AA  
SDSCNR:578620 (Q9DFT3) Alpha tubulin [Notothenia coriiceps (black rockcod)], 451 AA  
SDSCNR:496509 (P02550) Tubulin alpha chain [Sus scrofa (Pig)], 451 AA  
SDSCNR:2414579 TUBA2 protein [Homo sapiens] AAH57810.1 [BC057810], 450 AA  
SDSCNR:1786803 TRANSPORT PROTEIN[Mutant, 440 AA  
SDSCNR:578654 alpha tubulin [Chionodraco rastrispinosus] AAG15363.1 [AF263274], 451 AA  
SDSCNR:2465140 Unknown (protein for MGC:73108) [Danio rerio] AAH60904.1 [BC060904], 451 AA  
SDSCNR:180465 alpha-2 tubulin [Hirudo medicinalis] AAB07728.1 [U67676], 451 AA  
SDSCNR:2538149 alpha 1-tubulin [Laodelphax striatellus] AAR92032.1 [AY508717], 449 AA  
SDSCNR:1450937 STRUCTURAL PROTEIN|tubulin beta chain, 451 AA  
SDSCNR:484249 tubulin, chain A - pig, 440 AA  
SDSCNR:446970 (Q9YHW1) Alpha tubulin [Notothenia coriiceps (black rockcod)], 451 AA  
SDSCNR:149509 (Q94572) Tubulin alpha-3 chain (Alpha-III tubulin) [Homarus americanus (American lobster)], 451 AA  
SDSCNR:2081760 similar to tubulin, alpha 1 [Danio rerio] AAH45847.1 [BC045847], 450 AA  
SDSCNR:2518453 Unknown (protein for MGC:77601) [Danio rerio] AAH62826.1 [BC062826], 449 AA  
SDSCNR:2268881 putative alpha-tubulin [Oikopleura dioica] AAP80598.1 [AF465696], 450 AA  
SDSCNR:578656 (Q9DFR7) Alpha tubulin [Chionodraco rastrispinosus (Ocellated icefish)], 449 AA  
SDSCNR:1795614 RH71862p [Drosophila melanogaster] AAM29636.1 [AY113631], 449 AA  
SDSCNR:2440821 (AAH59428) Zgc:73046 protein [Brachydanio rerio (Zebrafish) (Danio rerio)], 449 AA  
SDSCNR:2437133 tubulin, alpha 4 [Danio rerio] AAQ91285.1 [AY391473], 449 AA  
SDSCNR:2437132 tubulin, alpha 2 [Danio rerio] AAQ91280.1 [AY391468], 449 AA  
SDSCNR:257412 tubulin alpha-1 chain [Guillardia theta] NP\_113413.1 [NC\_002753], 448 AA  
SDSCNR:167136 alpha-tubulin [Blepharisma japonicum] AAB61232.1 [AF003250], 395 AA  
SDSCNR:2268879 (Q7YZZ3) Putative alpha-tubulin [Oikopleura dioica], 450 AA  
SDSCNR:174282 alpha-2 tubulin [Giardia intestinalis] AAK35049.1 [AF331826],

454 AA  
 SDSCNR:2061772 MGC53359 protein [*Xenopus laevis*] AAH44001.1 [BC044001],  
 450 AA  
 SDSCNR:615733 tubulin alpha chain, brain-specific isotype (clone pTUB5) -  
 chum salmon, 444 AA  
 SDSCNR:152027 (Q06331) Tubulin alpha chain [*Octopus dofleini* (Giant  
 octopus)], 451 AA  
 SDSCNR:2414616 Unknown (protein for MGC:71743) [*Homo sapiens*]  
 AAH57811.1 [BC057811], 450 AA  
 SDSCNR:1830670 similar to Tubulin alpha-3/alpha-7 chain [*Homo sapiens*]  
 XP\_053177.4 [XM\_053177], 450 AA  
 SDSCNR:458575 (P30436) Tubulin alpha chain [*Oncorhynchus keta* (Chum  
 salmon)], 444 AA  
 SDSCNR:2015769 alpha-tubulin [*Giardia intestinalis*] AAN78305.1 [AY161326],  
 439 AA  
 SDSCNR:2015765 alpha-tubulin [*Encephalitozoon intestinalis*] AAN78301.1  
 [AY161322], 453 AA  
 SDSCNR:592619 (Q9GLW6) Alpha-tubulin (Fragment) [*Macaca mulatta*  
 (Rhesus macaque)], 442 AA  
 SDSCNR:180369 (Q25008) Tubulin alpha-1 chain (Alpha-I tubulin) [*Homarus*  
 americanus (American lobster)], 451 AA  
 SDSCNR:578626 alpha tubulin [*Notothenia coriiceps*] AAG15326.1 [AF255564],  
 450 AA  
 SDSCNR:2062420 (Q86GG3) Alpha-tubulin (Fragment) [*Lehmannia valentiana*],  
 413 AA  
 SDSCNR:180355 alpha-2-tubulin [*Gecarcinus lateralis*] AAC47523.1 [U92646],  
 451 AA  
 SDSCNR:2377995 (P09644) Tubulin alpha-5 chain [*Gallus gallus* (Chicken)], 448  
 AA  
 SDSCNR:609281 tubulin alpha-5 chain - chicken, 448 AA  
 SDSCNR:731454 alpha-tubulin [*Reclinomonas americana*] AAK37433.1  
 [AF267182], 384 AA  
 SDSCNR:347783 tubulin, alpha 8 [*Mus musculus*] NP\_059075.1 [NM\_017379],  
 449 AA  
 SDSCNR:312294 tubulin, alpha 8 [*Homo sapiens*] NP\_061816.1 [NM\_018943],  
 449 AA  
 SDSCNR:792782 alpha-tubulin [*Diophrys* sp. PRP2001] AAK72393.1  
 [AY037857], 394 AA  
 SDSCNR:2446719 Unknown (protein for MGC:76934) [*Danio rerio*] AAH66482.1  
 [BC066482], 449 AA  
 SDSCNR:2086639 Unknown (protein for MGC:55727) [*Danio rerio*] AAH46889.1  
 [BC046889], 449 AA  
 SDSCNR:456626 alpha tubulin [*Gallus gallus*] CAA30852.1 [X08061], 447 AA  
 SDSCNR:149456 (P50719) Tubulin alpha chain [*Haemonchus contortus* (Barber  
 pole worm)], 450 AA  
 SDSCNR:180354 alpha-1-tubulin [*Gecarcinus lateralis*] AAC47522.1 [U92645],

451 AA  
SDSCNR:140889 MEChanosensory abnormality MEC-12, TuBulin,  
[Caenorhabditis elegans] NP\_497663.1 [NM\_065262], 450 AA  
SDSCNR:1785213 STRUCTURAL PROTEIN|(rb3), 423 AA  
SDSCNR:2257012 hypothetical protein [Homo sapiens] CAD97815.1 [BX537702],  
479 AA  
SDSCNR:135189 hypothetical protein C44B11.3 - Caenorhabditis elegans, 457  
AA  
SDSCNR:144015 alpha-3 tubulin [Caenorhabditis elegans] BAA22203.1  
[D21134], 449 AA  
SDSCNR:168468 alpha-tubulin [Cercomonas ATCC50319] AAC67375.1  
[AF043927], 384 AA  
SDSCNR:130258 TuBulin, Alpha (49.9 kD) (tba-2) [Caenorhabditis elegans]  
NP\_493270.1 [NM\_060869], 448 AA  
SDSCNR:1823053 alpha-tubulin [Opisthonecta matiensis] AAM50063.1  
[AY101351], 395 AA  
SDSCNR:615719 tubulin alpha chain - slime mold (Physarum polycephalum), 450  
AA  
SDSCNR:242594 alpha-tubulin-1 [Bigelowiella natans] AAC68503.1 [AF043928],  
384 AA  
SDSCNR:242595 alpha-tubulin-2 [Bigelowiella natans] AAC68504.1 [AF043929],  
384 AA  
SDSCNR:2729947 (Q7XYS1) Alpha-tubulin (Fragment) [Cyanidium caldarium],  
326 AA  
SDSCNR:2524922 alpha-tubulin [Trifolium repens] AAP32191.1 [AY192359], 384  
AA  
SDSCNR:770706 TuBulin, Alpha (tba-2) [Caenorhabditis elegans] NP\_740926.1  
[NM\_171845], 448 AA  
SDSCNR:131691 TuBulin, Alpha (50.0 kD) (tba-1) [Caenorhabditis elegans]  
NP\_492600.1 [NM\_060199], 449 AA  
SDSCNR:132431 TuBulin, Alpha (50.0 kD) (tba-4) [Caenorhabditis elegans]  
NP\_496351.1 [NM\_063950], 448 AA  
SDSCNR:1740160 alpha-tubulin [Hymenolepis diminuta] AAL84895.1  
[AF482688], 450 AA  
SDSCNR:213691 (Q9MB28) Alpha-tubulin (Fragment) [Chlorella ellipsoidea],  
380 AA  
SDSCNR:615727 tubulin alpha chain - nematode (Haemonchus contortus), 449  
AA  
SDSCNR:133947 tubulin alpha-1 chain - Caenorhabditis elegans, 449 AA  
SDSCNR:1823052 (Q8MTH9) Alpha-tubulin (Fragment) [Opisthonecta  
henneguyi], 395 AA  
SDSCNR:275555 alpha-tubulin [Goniomonas truncata] AAD02566.1 [AF050087],  
391 AA  
SDSCNR:1853797 (Q8MVU2) Alpha-tubulin 1 (Fragment) [Homarus gammarus  
(European lobster) (Homarus vulgaris)], 396 AA  
SDSCNR:462495 tubulin alpha chain - African clawed frog, 450 AA

- SDSCNR:2268876 putative alpha-tubulin [*Oikopleura dioica*] AAP80593.1  
[AF465691], 451 AA
- SDSCNR:180356 (O01943) Alpha-3-tubulin (Fragment) [*Gecarcinus lateralis*  
(Blackback land crab)], 443 AA
- SDSCNR:1525603 TuBulin, Alpha (tba-9) [*Caenorhabditis elegans*] NP\_508555.2  
[NM\_076154], 456 AA
- SDSCNR:1823051 alpha-tubulin [*Vorticella microstoma*] AAM50062.1  
[AY101350], 395 AA
- SDSCNR:2113679 alpha-tubulin [*Streblomastix strix*] AAO46112.1 [AY188855],  
384 AA
- SDSCNR:1853800 alpha-tubulin 2 [*Strongylocentrotus droebachiensis*]  
AAM73989.1 [AF465682], 396 AA
- SDSCNR:149541 (P90656) Alpha-tubulin (Fragment) [*Histriculus cavicola*], 380  
AA
- SDSCNR:149507 (Q94570) Tubulin alpha-2 chain (Alpha-II tubulin) [*Homarus  
americanus* (American lobster)], 451 AA
- SDSCNR:2113677 alpha-tubulin [*Streblomastix strix*] AAO46110.1 [AY188853],  
384 AA
- SDSCNR:2202921 tubulin alpha-6 chain (TUA6) [*Arabidopsis thaliana*]  
NP\_849388.1 [NM\_179057], 427 AA
- SDSCNR:143809 alpha-tubulin [*Colpoda* sp.] CAA64074.1 [X94348], 380 AA
- SDSCNR:149179 alpha-tubulin [*Frontonia* sp.] CAA12201.1 [AJ224897], 382 AA
- SDSCNR:1853804 alpha-tubulin 3 [*Ciona intestinalis*] AAM73993.1 [AF465686],  
396 AA
- SDSCNR:1853802 alpha-tubulin 1 [*Ciona intestinalis*] AAM73991.1 [AF465684],  
396 AA
- SDSCNR:721532 alpha-tubulin [*Malawimonas jakobiformis*] AAK27846.1  
[AF267181], 384 AA
- SDSCNR:154294 alpha-tubulin [*Stentor coeruleus*] CAA90014.1 [Z49853], 382  
AA
- SDSCNR:167137 alpha-tubulin [*Spirostomum* sp.] AAB61233.1 [AF003251], 394  
AA
- SDSCNR:1886582 tubulin alpha chain - *Stentor coeruleus* (fragment), 384 AA
- SDSCNR:2440649 alpha-tubulin [*Cyanidium caldarium*] AAP75758.2  
[AY302129], 418 AA
- SDSCNR:2147075 (Q84KQ3) Alpha-tubulin [*Cyanidioschyzon merolae* (Red  
alga)], 448 AA
- SDSCNR:237077 (P11481) Tubulin alpha-1/alpha-2 chain [*Volvox carteri*], 451  
AA
- SDSCNR:220054 (P09204) Tubulin alpha-1 chain [*Chlamydomonas  
reinhardtii*], 451 AA
- SDSCNR:219080 (Q9ZRJ4) Tubulin alpha chain [*Chlorella vulgaris*], 451 AA
- SDSCNR:152076 (P28287) Tubulin alpha chain [*Oxytricha granulifera*], 450  
AA
- SDSCNR:220056 (P09205) Tubulin alpha-2 chain [*Chlamydomonas*

reinhardtii], 451 AA  
SDSCNR:731710 alpha-tubulin [*Euglena gracilis*] AAK37833.1 [AF182557], 451 AA  
SDSCNR:221728 (P33625) Tubulin alpha chain [*Euglena gracilis*], 451 AA  
SDSCNR:2449187 alpha-tubulin [*Miscanthus floridulus*] CAD26891.1 [AJ437642], 450 AA  
SDSCNR:2129458 (Q84TK6) Alpha-tubulin 1 [*Populus tremuloides* (Quaking aspen)], 451 AA  
SDSCNR:238919 (P33627) Tubulin alpha-6 chain (Alpha-6 tubulin) [*Zea mays* (Maize)], 450 AA  
SDSCNR:240714 (O22349) Tubulin alpha-3 chain (Alpha-3 tubulin) [*Eleusine indica* (Goosegrass)], 450 AA  
SDSCNR:155562 (P41351) Tubulin alpha chain [*Tetrahymena thermophila*], 449 AA  
SDSCNR:151946 (P11237) Tubulin alpha-1/2/3 chain [*Naegleria gruberi*], 453 AA  
SDSCNR:801414 (Q93XB1) Alpha tubulin subunit [*Rosa hybrid cultivar*], 451 AA  
SDSCNR:153915 alpha-tubulin [*Reticulomyxa filosa*] CAA65329.1 [X96475], 451 AA  
SDSCNR:226623 (P28752) Tubulin alpha-1 chain [*Oryza sativa* (Rice)], 450 AA  
SDSCNR:2440719 alpha-tubulin 2 [*Gossypium hirsutum*] AAQ92662.1 [AY345604], 450 AA  
SDSCNR:241807 alpha-tubulin [*Chloromonas* sp. ANT1] AAB86649.1 [AF032876], 451 AA  
SDSCNR:2449183 (CAD26887) Alpha-tubulin [*Miscanthus sinensis* (Japanese silver grass)], 450 AA  
SDSCNR:2449189 alpha-tubulin [*Miscanthus floridulus*] CAD26893.1 [AJ437644], 450 AA  
SDSCNR:1996317 alpha tubulin [*Physcomitrella patens*] BAC24800.1 [AB096719], 451 AA  
SDSCNR:1996316 (Q8H933) Alpha tubulin [*Physcomitrella patens* (Moss)], 449 AA  
SDSCNR:151936 (Q25563) Tubulin alpha-13 chain [*Naegleria gruberi*], 453 AA  
SDSCNR:228367 (P46259) Tubulin alpha-1 chain [*Pisum sativum* (Garden pea)], 452 AA  
SDSCNR:2449182 (CAD26886) Alpha-tubulin [*Miscanthus sinensis* (Japanese silver grass)], 450 AA  
SDSCNR:226701 (P33629) Tubulin alpha chain [*Prunus dulcis* (Almond) (*Prunus amygdalus*)], 450 AA  
SDSCNR:2440720 alpha-tubulin 4 [*Gossypium hirsutum*] AAQ92663.1 [AY345605], 450 AA  
SDSCNR:225353 (Q02245) Tubulin alpha-5 chain (Alpha-5 tubulin) [*Zea mays* (Maize)], 450 AA

SDSCNR:615718 tubulin alpha chain - *Euglena gracilis*, 451 AA  
 SDSCNR:254592 (Q9M3T2) Alpha-tubulin [*Betula verrucosa* (White birch) (*Betula pendula*)], 451 AA  
 SDSCNR:155856 (P10872) Tubulin alpha chain [*Tetrahymena pyriformis*], 449 AA  
 SDSCNR:153916 alpha-tubulin [*Reticulomyxa filosa*] CAA65330.1 [X96476], 451 AA  
 SDSCNR:216664 tubulin alpha-1 chain (TUA1) [*Arabidopsis thaliana*] NP\_176654.1 [NM\_105148], 450 AA  
 SDSCNR:257566 (Q43473) Tubulin alpha-1 chain [*Hordeum vulgare* (Barley)], 450 AA  
 SDSCNR:216671 tubulin alpha-3/alpha-5 chain (TUA5) [*Arabidopsis thaliana*] NP\_197479.1 [NM\_121983], 450 AA  
 SDSCNR:241808 (O22661) Alpha-tubulin [*Chloromonas* sp. ANT3], 451 AA  
 SDSCNR:2449179 (CAD24766) Alpha-tubulin [*Miscanthus sinensis* (Japanese silver grass)], 450 AA  
 SDSCNR:2449186 alpha-tubulin [*Miscanthus sinensis*] CAD26890.1 [AJ437641], 450 AA  
 SDSCNR:2449178 (CAD24765) Alpha-tubulin [*Miscanthus sinensis* (Japanese silver grass)], 450 AA  
 SDSCNR:155825 (P10873) Tubulin alpha chain [*Toxoplasma gondii*], 453 AA  
 SDSCNR:216672 tubulin alpha-2/alpha-4 chain (TUA4) [*Arabidopsis thaliana*] NP\_171974.1 [NM\_100360], 450 AA  
 SDSCNR:153683 (Q94712) Alpha-tubulin [*Paramecium tetraurelia*], 449 AA  
 SDSCNR:126253 (Q94713) Alpha-tubulin [*Paramecium tetraurelia*, and *Paramecium caudatum*], 449 AA  
 SDSCNR:215583 (P33623) Tubulin alpha-1 chain [*Anemia phyllitidis* (Fern)], 451 AA  
 SDSCNR:216673 tubulin alpha-6 chain (TUA6) [*Arabidopsis thaliana*] NP\_193232.1 [NM\_117582], 450 AA  
 SDSCNR:2440718 alpha-tubulin 1 [*Gossypium hirsutum*] AAQ92661.1 [AY345603], 451 AA  
 SDSCNR:1426304 AT4g14960/dl3520c [*Arabidopsis thaliana*] AAL16174.1 [AF428406], 450 AA  
 SDSCNR:2145589 (Q84UZ5) Alpha-tubulin [*Ceratopteris richardii* (Triangle waterfern)], 450 AA  
 SDSCNR:1468317 (Q8VXD0) Alpha-tubulin [*Nicotiana tabacum* (Common tobacco)], 451 AA  
 SDSCNR:257600 (Q9ZRR5) Tubulin alpha-3 chain [*Hordeum vulgare* (Barley)], 451 AA  
 SDSCNR:153327 alpha tubulin [*Plasmodium falciparum* 3D7] NP\_704579.1 [NC\_004330], 453 AA  
 SDSCNR:2449180 alpha-tubulin [*Miscanthus floridulus*] CAD24767.1 [AJ431264], 450 AA  
 SDSCNR:1468318 (Q8VXC9) Alpha-tubulin [*Nicotiana tabacum* (Common tobacco)], 451 AA

|                |                                                                                                 |
|----------------|-------------------------------------------------------------------------------------------------|
| SDSCNR:154650  | (P09243) Tubulin alpha-2 chain [ <i>Stylonychia lemnae</i> ], 449 AA                            |
| SDSCNR:1468316 | alpha-tubulin [ <i>Nicotiana tabacum</i> ] CAD13176.1 [AJ421411], 450 AA                        |
| SDSCNR:152560  | alpha-tubulin ii [ <i>Plasmodium falciparum</i> 3D7] NP_702868.1 [NC_004318], 450 AA            |
| SDSCNR:221802  | (O22347) Tubulin alpha-1 chain (Alpha-1 tubulin) [ <i>Eleusine indica</i> (Goosegrass)], 451 AA |
| SDSCNR:2449181 | alpha-tubulin [ <i>Miscanthus floridulus</i> ] CAD24768.1 [AJ431265], 450 AA                    |
| SDSCNR:2731012 | (Q7RHC6) Tubulin/FtsZ family, putative [ <i>Plasmodium yoelii yoelii</i> ], 445 AA              |
| SDSCNR:238916  | (P14640) Tubulin alpha-1 chain (Alpha-1 tubulin) [ <i>Zea mays</i> (Maize)], 451 AA             |
| SDSCNR:238915  | (P14641) Tubulin alpha-2 chain (Alpha-2 tubulin) [ <i>Zea mays</i> (Maize)], 451 AA             |
| SDSCNR:2415692 | (AAQ81585) Putative tubulin alpha-2/alpha-4 chain [ <i>Brassica napus</i> (Rape)], 450 AA       |
| SDSCNR:257567  | (Q96460) Tubulin alpha-2 chain [ <i>Hordeum vulgare</i> (Barley)], 451 AA                       |
| SDSCNR:2065304 | (Q84XV0) Alpha tubulin [ <i>Populus tremuloides</i> (Quaking aspen)], 451 AA                    |
| SDSCNR:226621  | alpha-tubulin [ <i>Oryza sativa</i> (japonica cultivar-group)] CAA62916.1 [X91806], 451 AA      |
| SDSCNR:2449188 | (CAD26892) Alpha-tubulin [ <i>Miscanthus floridulus</i> ], 450 AA                               |
| SDSCNR:582140  | (AAS07163) Alpha tubulin [ <i>Oryza sativa</i> (japonica cultivar-group)], 451 AA               |
| SDSCNR:2730782 | (Q7RQE4) Tubulin alpha chain [ <i>Plasmodium yoelii yoelii</i> ], 453 AA                        |
| SDSCNR:221803  | alpha-tubulin 1 [ <i>Eleusine indica</i> ] CAA06619.1 [AJ005599], 451 AA                        |
| SDSCNR:268389  | (Q9ZRB7) Tubulin alpha chain [ <i>Triticum aestivum</i> (Wheat)], 451 AA                        |
| SDSCNR:258641  | (Q9M4L4) Alpha-tubulin [ <i>Miscanthus sinensis</i> (Japanese silver grass)], 450 AA            |
| SDSCNR:628721  | (Q9FRU1) Alpha tubulin [ <i>Nicotiana tabacum</i> (Common tobacco)], 450 AA                     |
| SDSCNR:2449184 | (CAD26888) Alpha-tubulin [ <i>Miscanthus sinensis</i> (Japanese silver grass)], 450 AA          |
| SDSCNR:215654  | (Q38771) Tubulin alpha chain [ <i>Avena sativa</i> (Oat)], 447 AA                               |
| SDSCNR:148616  | alpha tubulin [ <i>Eimeria acervulina</i> ] CAA61255.1 [X88776], 453 AA                         |
| SDSCNR:148993  | (Q08114) Tubulin alpha chain [ <i>Euplotes octocarinatus</i> ], 450 AA                          |
| SDSCNR:258802  | (Q9M4L3) Alpha-tubulin [ <i>Miscanthus sinensis</i> (Japanese silver grass)], 450 AA            |

|                      |                                                                                                             |
|----------------------|-------------------------------------------------------------------------------------------------------------|
| SDSCNR:572008        | <b>alpha-tubulin [Daucus carota] AAG02564.1 [AY007250], 451 AA</b>                                          |
| SDSCNR:1703912       | <b>(Q8WRT6) Alpha-tubulin [Euplotes focardii], 449 AA</b>                                                   |
| SDSCNR:154619        | <b>(P50258) Tubulin alpha-1A chain [Physarum polycephalum (Slime mold)], 450 AA</b>                         |
| SDSCNR:240713        | <b>(O22348) Tubulin alpha-2 chain (Alpha-2 tubulin) [Eleusine indica (Goosegrass)], 447 AA</b>              |
| SDSCNR:596103        | <b>(Q9BHW8) Probable tubulin alpha chain (Alpha tubulin, copy 1) [Leishmania major], 451 AA</b>             |
| SDSCNR:150167        | <b>(Q25262) Alpha tubulin [Leishmania donovani], 451 AA</b>                                                 |
| SDSCNR:226622        | <b>alfa-tubulin [Oryza sativa (japonica cultivar-group)] CAA62917.1 [X91807], 451 AA</b>                    |
| SDSCNR:153609        | <b>(P11480) Tubulin alpha-2B chain (Tubulin alpha-E chain) [Physarum polycephalum (Slime mold)], 449 AA</b> |
| SDSCNR:156094        | <b>(P04106) Tubulin alpha chain [Trypanosoma brucei rhodesiense], 451 AA</b>                                |
| SDSCNR:1708465       | <b>alpha tubulin [Trypanosoma cruzi] AAL75955.1 [AF455115], 451 AA</b>                                      |
| SDSCNR:223397        | <b>alpha tubulin [Hordeum vulgare subsp. vulgare] AAB08791.1 [U40042], 451 AA</b>                           |
| SDSCNR:2377996       | <b>(P28268) Tubulin alpha chain [Euplotes vannus], 450 AA</b>                                               |
| SDSCNR:149103        | <b>tubulin alpha chain - Euplotes vannus, 449 AA</b>                                                        |
| SDSCNR:155919        | <b>(Q27352) Tubulin alpha chain [Trypanosoma cruzi], 451 AA</b>                                             |
| SDSCNR:156101        | <b>alpha-tubulin [Trypanosoma cruzi] AAA99441.1 [L37345], 425 AA</b>                                        |
| SDSCNR:615729        | <b>tubulin alpha-1 chain - Stylonychia lemnae, 445 AA</b>                                                   |
| SDSCNR:241693        | <b>(O22635) Tubulin [Oryza sativa (Rice)], 451 AA</b>                                                       |
| SDSCNR:225531        | <b>(P22275) Tubulin alpha-3 chain (Alpha-3 tubulin) [Zea mays (Maize)], 450 AA</b>                          |
| SDSCNR:170564        | <b>alpha tubulin [Cryptosporidium parvum] AAM69358.1 [U65379], 454 AA</b>                                   |
| SDSCNR:154597        | <b>(P07304) Tubulin alpha-1 chain [Stylonychia lemnae], 445 AA</b>                                          |
| SDSCNR:153606        | <b>(P04105) Tubulin alpha-1B chain (Tubulin alpha-N chain) [Physarum polycephalum (Slime mold)], 449 AA</b> |
| User Entered         | <b>test</b>                                                                                                 |
| User Entered, copied | <b>CM ALpha tubulin Pubulished</b>                                                                          |
| User Entered         | <b>Pig Alpha solved structure</b>                                                                           |

## **Appendix B**

Mesophilic beta tubulin sequences used to identify non-conserved differences

|                |                                                                                                    |
|----------------|----------------------------------------------------------------------------------------------------|
| SDSCNR:652065  | <b>Sequence 9 from Patent WO0104281. CAC28487.1 [AX076229], 454 AA</b>                             |
| SDSCNR:216668  | <b>tubulin beta-7 chain (TUB7) [Arabidopsis thaliana] NP_180515.1 [NM_128508], 449 AA</b>          |
| SDSCNR:149457  | <b>beta-tubulin - nematode (Haemonchus contortus), 448 AA</b>                                      |
| SDSCNR:577128  | <b>(Q9GT32) Beta-tubulin isoform 1-3 [Cylicocyclus nassatus], 448 AA</b>                           |
| SDSCNR:226440  | <b>tubulin beta chain - rice, 447 AA</b>                                                           |
| SDSCNR:228834  | <b>tubulin beta chain [Oryza sativa (japonica cultivar-group)] NP_915874.1 [NM_190985], 447 AA</b> |
| SDSCNR:615763  | <b>beta-tubulin R1623 - rice, 446 AA</b>                                                           |
| SDSCNR:454271  | <b>(P09653) Tubulin beta-5 chain (Beta-tubulin class-V) [Gallus gallus (Chicken)], 446 AA</b>      |
| SDSCNR:225356  | <b>(Q41783) Tubulin beta-6 chain (Beta-6 tubulin) [Zea mays (Maize)], 446 AA</b>                   |
| SDSCNR:2042991 | <b>MGC52834 protein [Xenopus laevis] AAH41265.1 [BC041265], 446 AA</b>                             |
| SDSCNR:155918  | <b>(P08562) Tubulin beta chain [Trypanosoma cruzi], 442 AA</b>                                     |
| SDSCNR:174455  | <b>(Q9N611) Beta-tubulin [Cylicocyclus nassatus], 448 AA</b>                                       |
| SDSCNR:124719  | <b>Silkworm mRNA for beta-tubulin, complete cds. BAA19845.1 [AB003287], 447 AA</b>                 |
| SDSCNR:2663074 | <b>similar to RIKEN cDNA 2310057H16 [Rattus norvegicus] XP_225867.2 [XM_225867], 447 AA</b>        |
| SDSCNR:760023  | <b>RIKEN cDNA 2310057H16 [Mus musculus] NP_080749.2 [NM_026473], 447 AA</b>                        |
| SDSCNR:1749422 | <b>tubulin beta-1 [Gossypium hirsutum] AAL92026.1 [AF484959], 445 AA</b>                           |
| SDSCNR:238938  | <b>(Q43695) Tubulin beta-3 chain (Beta-3 tubulin) [Zea mays (Maize)], 445 AA</b>                   |
| SDSCNR:2397056 | <b>beta-tubulin [Oryza sativa (japonica cultivar-group)] BAC82429.1 [AB104732], 444 AA</b>         |
| SDSCNR:154135  | <b>(Q9N2N6) Tubulin beta chain (Beta-tubulin) [Euplotes focardii], 444 AA</b>                      |
| SDSCNR:2436196 | <b>(AAQ88114) Beta-tubulin 1 [Physcomitrella patens (Moss)], 443 AA</b>                            |
| SDSCNR:1913872 | <b>beta-tubulin Cce-1a [Cylicocyclus elongatus] AAM95350.1 [AF487844], 448 AA</b>                  |
| SDSCNR:615766  | <b>tubulin beta chain GRU-1 - nematode (Haemonchus</b>                                             |

|                |                                                                                                                                                      |
|----------------|------------------------------------------------------------------------------------------------------------------------------------------------------|
| SDSCNR:243339  | <b>contortus) (isolate resistant Utrecht), 448 AA</b>                                                                                                |
| SDSCNR:149459  | <b>(Q9ZPN9) Tubulin beta-2 chain (Beta-2 tubulin)</b><br><b>[Eleusine indica (Goosegrass)], 448 AA</b>                                               |
| SDSCNR:2436195 | <b>beta-tubulin [Haemonchus contortus] AAA29170.1</b><br><b>[M76493], 448 AA</b>                                                                     |
| SDSCNR:1913870 | <b>(AAQ88113) Beta-tubulin 6 [Physcomitrella patens</b><br><b>(Moss)], 443 AA</b>                                                                    |
| SDSCNR:257885  | <b>beta-tubulin Cyca-2a [Cyathostomum catinatum]</b><br><b>AAM95345.1 [AF487839], 448 AA</b>                                                         |
| SDSCNR:2030482 | <b>(P37392) Tubulin beta-1 chain [Lupinus albus</b><br><b>(White lupine)], 447 AA</b>                                                                |
| SDSCNR:578618  | <b>class II beta tubulin isotype [Homo sapiens]</b><br><b>AAN85571.1 [AY159127], 445 AA</b>                                                          |
| SDSCNR:261080  | <b>beta tubulin [Notothenia coriiceps] AAG15317.1</b><br><b>[AF255555], 456 AA</b>                                                                   |
| SDSCNR:698474  | <b>(P29500) Tubulin beta-1 chain [Pisum sativum</b><br><b>(Garden pea)], 450 AA</b>                                                                  |
| SDSCNR:257570  | <b>tubulin beta-4 chain [Oryza sativa (japonica cultivar-</b><br><b>group)] NP_912596.1 [NM_187707], 447 AA</b>                                      |
| SDSCNR:580377  | <b>(P93176) Tubulin beta chain (Beta tubulin)</b><br><b>[Hordeum vulgare (Barley)], 447 AA</b>                                                       |
| SDSCNR:243341  | <b>Homo sapiens cDNA FLJ12278 fis, clone</b><br><b>MAMMA1001735, highly similar to TUBULIN</b><br><b>BETA-5 CHAIN. BAB14016.1 [AK022340], 446 AA</b> |
| SDSCNR:238940  | <b>(Q9ZPN7) Tubulin beta-4 chain (Beta-4 tubulin)</b><br><b>[Eleusine indica (Goosegrass)], 446 AA</b>                                               |
| SDSCNR:1849809 | <b>(Q43697) Tubulin beta-5 chain (Beta-5 tubulin) [Zea</b><br><b>mays (Maize)], 445 AA</b>                                                           |
| SDSCNR:149128  | <b>(Q8MPB3) Beta tubulin (Tubulin beta chain,</b><br><b>probable) [Cryptosporidium parvum], 444 AA</b>                                               |
| SDSCNR:2440721 | <b>beta-tubulin [Encephalitozoon hellem] AAB12034.1</b><br><b>[L47271], 439 AA</b>                                                                   |
| SDSCNR:229010  | <b>(AAQ92664) Beta-tubulin 3 [Gossypium hirsutum</b><br><b>(Upland cotton)], 430 AA</b>                                                              |
| SDSCNR:676354  | <b>beta-tubulin [Oryza sativa] AAA66495.1 [L19598],</b><br><b>447 AA</b>                                                                             |
| SDSCNR:155920  | <b>tubulin beta MGC4083 [Homo sapiens] NP_115914.1</b><br><b>[NM_032525], 446 AA</b>                                                                 |
| SDSCNR:615776  | <b>beta tubulin [Trypanosoma cruzi] AAA91958.1</b><br><b>[M96849], 441 AA</b>                                                                        |
| SDSCNR:152028  | <b>Tubulin beta-2 chain - soybean, 449 AA</b><br><b>(P35394) Tubulin beta chain [Octopus dofleini (Giant</b><br><b>octopus)], 447 AA</b>             |
| SDSCNR:243340  | <b>(Q9ZPN8) Tubulin beta-3 chain (Beta-3 tubulin)</b><br><b>[Eleusine indica (Goosegrass)], 446 AA</b>                                               |
| SDSCNR:268392  | <b>(Q9ZRB0) Tubulin beta-3 chain (Beta-3 tubulin)</b>                                                                                                |

SDSCNR:1945859 [Triticum aestivum (Wheat)], 445 AA  
 class I beta tubulin [Danio rerio] AAN33030.1  
 [AF528096], 444 AA  
 SDSCNR:1741096 TUBULIN BETA CHAIN [Encephalitozoon cuniculi]  
 NP\_597591.1 [NC\_003230], 439 AA  
 SDSCNR:216667 tubulin beta-6 chain (TUB6) [Arabidopsis thaliana]  
 NP\_196786.1 [NM\_121263], 449 AA  
 SDSCNR:570079 beta tubulin [Arabidopsis thaliana] NP\_568437.1  
 [NM\_122291], 449 AA  
 SDSCNR:615771 tubulin beta-1 chain - rice, 447 AA  
 SDSCNR:268391 (Q9ZRB1) Tubulin beta-2 chain (Beta-2 tubulin)  
 [Triticum aestivum (Wheat)], 447 AA  
 SDSCNR:268390 (Q9ZRB2) Tubulin beta-1 chain (Beta-1 tubulin)  
 [Triticum aestivum (Wheat)], 445 AA  
 SDSCNR:150350 (Q25315) Beta-tubulin [Leishmania major], 443 AA  
 SDSCNR:1102319 (Q93Y15) Beta tubulin [Arabidopsis thaliana  
 (Mouse-ear cress)], 449 AA  
 SDSCNR:1913868 (Q8MV66) Beta-tubulin Cyp-1a [Cyathostomum  
 pateratum], 448 AA  
 SDSCNR:2440725 beta-tubulin 9 [Gossypium hirsutum] AAQ92668.1  
 [AY345610], 445 AA  
 SDSCNR:260767 (P50261) Tubulin beta-3 chain [Porphyra purpurea],  
 445 AA  
 SDSCNR:341198 tubulin beta chain (clone 16T) - Chinese hamster,  
 444 AA  
 SDSCNR:128615 (Q17299) Tubulin beta chain [Caenorhabditis  
 briggsae], 441 AA  
 SDSCNR:228835 (P46265) Tubulin beta chain [Oryza sativa (Rice)],  
 447 AA  
 SDSCNR:148263 beta-Tubulin at 85D CG9359-PA [Drosophila  
 melanogaster] NP\_524290.2 [NM\_079566], 446 AA  
 SDSCNR:148261 (P83130) Tubulin beta-2 chain [Drosophila erecta  
 (Fruit fly)], 446 AA  
 SDSCNR:615762 beta-tubulin R2242 - rice, 444 AA  
 SDSCNR:268393 (Q9ZRA9) Tubulin beta-4 chain (Beta-4 tubulin)  
 [Triticum aestivum (Wheat)], 445 AA  
 SDSCNR:216665 tubulin beta-2/beta-3 chain (TUB3) [Arabidopsis  
 thaliana] NP\_568960.1 [NM\_125665], 450 AA  
 SDSCNR:235301 (P12460) Tubulin beta-2 chain [Glycine max  
 (Soybean)], 449 AA  
 SDSCNR:2022497 putative tubulin beta-6 chain [Arabidopsis thaliana]  
 BAC42563.1 [AK117925], 449 AA  
 SDSCNR:223775 (Q40106) Tubulin beta-2 chain (Beta-2 tubulin)  
 [Lupinus albus (White lupine)], 448 AA  
 SDSCNR:615754 tubulin beta chain - Neurospora crassa, 447 AA  
 SDSCNR:225358 (Q41785) Tubulin beta-8 chain (Beta-8 tubulin) [Zea

|                |                                                                                                          |
|----------------|----------------------------------------------------------------------------------------------------------|
| SDSCNR:225357  | mays (Maize)], 445 AA<br>(Q41784) Tubulin beta-7 chain (Beta-7 tubulin) [Zea mays (Maize)], 445 AA       |
| SDSCNR:226945  | (P24637) Tubulin beta chain [Pneumocystis carinii], 442 AA                                               |
| SDSCNR:149230  | tubulin [Geodia cydonium] CAA76576.1 [Y17002], 449 AA                                                    |
| SDSCNR:148262  | D.melanogaster beta-2 tubulin mRNA, complete cds; beta-2 tubulin. AAA28991.1 [M20420], 446 AA            |
| SDSCNR:1760077 | beta-tubulin [Crypthecodinium cohnii] AAM02970.1 [AF421537], 445 AA                                      |
| SDSCNR:243338  | (Q9ZPP0) Tubulin beta-1 chain (Beta-1 tubulin) [Eleusine indica (Goosegrass)], 445 AA                    |
| SDSCNR:138818  | MEChanosensory abnormality MEC-7, tubulin (49.3 [Caenorhabditis elegans] NP_509313.1 [NM_076912], 441 AA |
| SDSCNR:216669  | tubulin beta-8 chain - Arabidopsis thaliana, 449 AA                                                      |
| SDSCNR:216674  | tubulin beta-1 chain (TUB1) [Arabidopsis thaliana] NP_177706.1 [NM_106228], 447 AA                       |
| SDSCNR:615757  | tubulin beta-5 chain - chicken, 446 AA                                                                   |
| SDSCNR:1945855 | (Q8H6N0) Beta-tubulin 1 [Gossypium hirsutum (Upland cotton)], 444 AA                                     |
| SDSCNR:275562  | (Q9ZTL3) Nucleomorph beta-tubulin (Fragment) [Guillardia theta (Cryptomonas phi)], 387 AA                |
| SDSCNR:652064  | Sequence 7 from Patent WO0104281; unnamed protein product. CAC28486.1 [AX076227], 454 AA                 |
| SDSCNR:220678  | Beta-tubulin [Zinnia elegans] BAA82637.1 [D63136], 448 AA                                                |
| SDSCNR:577127  | beta-tubulin isoform 1-2 [Cyllococyclus nassatus] AAG13960.1 [AF283766], 448 AA                          |
| SDSCNR:225813  | N.crassa (benomyl-resistant mutant) beta-tubulin gene, complete cds. AAA33617.1 [M13630], 447 AA         |
| SDSCNR:268394  | (Q9ZRA8) Tubulin beta-5 chain (Beta-5 tubulin) [Triticum aestivum (Wheat)], 447 AA                       |
| SDSCNR:238933  | (P18025) Tubulin beta-1 chain (Beta-1 tubulin) [Zea mays (Maize)], 446 AA                                |
| SDSCNR:156093  | (P04107) Tubulin beta chain [Trypanosoma brucei rhodesiense], 442 AA                                     |
| SDSCNR:216666  | tubulin beta-5 chain (TUB5) [Arabidopsis thaliana] NP_564101.1 [NM_101856], 449 AA                       |
| SDSCNR:1888690 | tubulin beta-2 chain - garden pea (fragment), 449 AA                                                     |
| SDSCNR:615737  | tubulin beta chain - human, 445 AA                                                                       |
| SDSCNR:2729948 | (Q7XYS0) Beta-tubulin (Fragment) [Cyanidium caldarium], 379 AA                                           |
| SDSCNR:744475  | tubulin beta subunit [Guillardia theta] NP_113213.1 [NC_002751], 441 AA                                  |

|                |                                                                                                 |
|----------------|-------------------------------------------------------------------------------------------------|
| SDSCNR:1808507 | <b>(Q8MUW6) Beta-tubulin [Oxytricha granulifera], 442 AA</b>                                    |
| SDSCNR:731711  | <b>beta-tubulin [Euglena gracilis] AAK37837.1 [AF182555], 445 AA</b>                            |
| SDSCNR:154622  | <b>(P07436) Tubulin beta-1 chain [Physarum polycephalum (Slime mold)], 467 AA</b>               |
| SDSCNR:505182  | <b>beta 1A tubulin - slime mold (Physarum polycephalum), 445 AA</b>                             |
| SDSCNR:245098  | <b>b-tubulin [Entosiphon sulcatum] AAD49555.1 [AF095840], 445 AA</b>                            |
| SDSCNR:244992  | <b>(Q9ZSW1) Tubulin beta-1 chain (Beta-1 tubulin) [Cyanophora paradoxa], 447 AA</b>             |
| SDSCNR:128008  | <b>(Q04709) Tubulin beta chain [Babesia bovis], 441 AA</b>                                      |
| SDSCNR:151938  | <b>(P34108) Tubulin beta chain [Naegleria gruberi], 451 AA</b>                                  |
| SDSCNR:1808506 | <b>beta-tubulin [Gastrostyla steinii] AAM43913.1 [AF510204], 442 AA</b>                         |
| SDSCNR:154651  | <b>(P11857) Tubulin beta chain (Beta-1 and beta-2) [Stylonychia lemnae], 442 AA</b>             |
| SDSCNR:1808509 | <b>beta-tubulin [Uroleptus gallina] AAM43918.1 [AF510209], 442 AA</b>                           |
| SDSCNR:1808508 | <b>beta-tubulin [Sterkiella histriomuscorum] AAM43916.1 [AF510207], 442 AA</b>                  |
| SDSCNR:2731134 | <b>(Q7RCR6) Tubulin beta chain [Plasmodium yoelii yoelii], 445 AA</b>                           |
| SDSCNR:151937  | <b>beta-tubulin [Naegleria gruberi] CAA56940.1 [X81050], 451 AA</b>                             |
| SDSCNR:174768  | <b>beta tubulin [Stylonychia mytilus] AAF00924.1 [AF188162], 442 AA</b>                         |
| SDSCNR:276489  | <b>beta-tubulin [Pythium ultimum] AAF22515.1 [AF115397], 446 AA</b>                             |
| SDSCNR:148905  | <b>(Q9NFZ6) Tubulin beta-2 chain (Beta-tubulin 2) [Echinococcus multilocularis], 445 AA</b>     |
| SDSCNR:168153  | <b>beta tubulin [Trichuris trichiura] AAB99949.1 [AF034219], 444 AA</b>                         |
| SDSCNR:153328  | <b>tubulin beta chain, putative [Plasmodium falciparum 3D7] NP_700558.1 [NC_004314], 445 AA</b> |
| SDSCNR:1443173 | <b>(Q95UU1) Beta-tubulin [Monosiga brevicollis], 444 AA</b>                                     |
| SDSCNR:148264  | <b>(P08841) Tubulin beta-3 chain [Drosophila melanogaster (Fruit fly)], 454 AA</b>              |
| SDSCNR:1756544 | <b>beta-tubulin [Bombyx mori] BAB86855.1 [AB072310], 446 AA</b>                                 |
| SDSCNR:155563  | <b>(P41352) Tubulin beta chain [Tetrahymena thermophila], 443 AA</b>                            |

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| SDSCNR:153684  | (P33188) Tubulin beta-1 chain [Paramecium tetraurelia], 442 AA                                   |
| SDSCNR:183385  | beta-Tubulin at 56D CG9277-PB [Drosophila melanogaster] NP_523795.2 [NM_079071], 447 AA          |
| SDSCNR:172029  | (Q9U7Q1) Beta tubulin [Trichuris trichiura (Whipworm)], 444 AA                                   |
| SDSCNR:1808510 | beta-tubulin [Hypotrichida sp. AL] AAM43919.1 [AF510210], 442 AA                                 |
| SDSCNR:148260  | D.melanogaster beta-1 tubulin mRNA, complete cds. AAA28989.1 [M20419], 447 AA                    |
| SDSCNR:2731341 | (Q7R3R8) GLP_82_78422_77079 [Giardia lamblia ATCC 50803], 447 AA                                 |
| SDSCNR:2415336 | (AAH58304) Hypothetical protein wu:fj33a08 [Brachydanio rerio (Zebrafish) (Danio rerio)], 444 AA |
| SDSCNR:1846945 | beta-Tubulin at 60D CG3401-PA [Drosophila melanogaster] NP_523842.2 [NM_079118], 454 AA          |
| SDSCNR:615741  | tubulin beta-2 chain - Tetrahymena pyriformis, 443 AA                                            |
| SDSCNR:274973  | (O04386) Tubulin beta chain [Chlamydomonas incerta], 443 AA                                      |
| SDSCNR:148904  | (Q9NFZ7) Tubulin beta-1 chain (Beta-tubulin 1) [Echinococcus multilocularis], 448 AA             |
| SDSCNR:2378000 | (P05304) Tubulin beta chain [Giardia lamblia (Giardia intestinalis)], 446 AA                     |
| SDSCNR:149313  | tubulin beta chain - Giardia lamblia, 445 AA                                                     |
| SDSCNR:260458  | (O59837) Tubulin beta chain [Phytophthora cinnamomi], 444 AA                                     |
| SDSCNR:155857  | (P10876) Tubulin beta chain [Tetrahymena pyriformis], 443 AA                                     |
| SDSCNR:237078  | (P11482) Tubulin beta-1/beta-2 chain [Volvox carteri], 443 AA                                    |
| SDSCNR:220058  | (P04690) Tubulin beta-1/beta-2 chain [Chlamydomonas reinhardtii], 443 AA                         |
| SDSCNR:1805649 | (Q8N6N5) Tubulin, beta, 2 [Homo sapiens (Human)], 445 AA                                         |
| SDSCNR:578617  | beta tubulin [Notothenia coriiceps] AAG15316.1 [AF255554], 445 AA                                |
| SDSCNR:2452192 | Unknown (protein for MGC:73008) [Rattus norvegicus] NP_954525.1 [NM_199094], 445 AA              |
| SDSCNR:2061738 | (Q7ZY50) Hypothetical protein [Xenopus laevis (African clawed frog)], 445 AA                     |
| SDSCNR:1960229 | tubulin, beta, 2 [Homo sapiens] AAH24038.1 [BC024038], 445 AA                                    |
| SDSCNR:301828  | tubulin, beta, 2 [Homo sapiens] NP_006079.1 [NM_006088], 445 AA                                  |

SDSCNR:461909 (P30883) Tubulin beta-4 chain [*Xenopus laevis* (African clawed frog)], 445 AA

SDSCNR:459030 (Q91240) Tubulin beta- chain [*Pseudopleuronectes americanus* (Winter flounder) (*Pleuronectes americanus*)], 445 AA

SDSCNR:128897 tubulin, Beta (49.8 kD) (tbb-4) [*Caenorhabditis elegans*] NP\_509585.1 [NM\_077184], 444 AA

SDSCNR:615743 beta-tubulin - *Plasmodium berghei*, 444 AA

SDSCNR:152562 beta-tubulin [*Plasmodium berghei*] AAA29500.1 [M58750], 443 AA

SDSCNR:2015766 beta-tubulin [*Encephalitozoon intestinalis*] AAN78302.1 [AY161323], 438 AA

SDSCNR:676504 (Q9BTZ0) Hypothetical protein (Fragment) [*Homo sapiens* (Human)], 466 AA

SDSCNR:154628 (P12458) Tubulin beta-2 chain (Tubulin beta-major chain) [*Physarum polycephalum* (Slime mold)], 454 AA

SDSCNR:594068 tubulin, beta 3 [*Mus musculus*] NP\_075768.1 [NM\_023279], 450 AA

SDSCNR:675614 (Q13509) Tubulin beta-4 chain (Tubulin beta-III) [*Homo sapiens* (Human)], 450 AA

SDSCNR:153498 (P11833) Tubulin beta chain [*Paracentrotus lividus* (Common sea urchin)], 447 AA

SDSCNR:363782 beta-tubulin [synthetic construct] AAF01152.1 [AF162281], 446 AA

SDSCNR:1524138 *Oryzias latipes* DNA, MHC Class I Region, complete cds, section 2/2. BAB83857.1 [AB073377], 444 AA

SDSCNR:148994 (Q08115) Tubulin beta chain (Beta-tubulin) [*Euplotes octocarinatus*], 444 AA

SDSCNR:2015770 beta-tubulin [*Giardia intestinalis*] AAN78306.1 [AY161327], 438 AA

SDSCNR:324085 tubulin, beta, 4 [*Homo sapiens*] NP\_006077.1 [NM\_006086], 450 AA

SDSCNR:454265 (P09652) Tubulin beta-4 chain (Beta-tubulin class-III) [*Gallus gallus* (Chicken)], 449 AA

SDSCNR:185081 (O17449) Tubulin beta-1 chain (Beta-1 tubulin) [*Manduca sexta* (Tobacco hawkmoth) (Tobacco hornworm)], 447 AA

SDSCNR:578616 beta tubulin [*Chionodraco rastrospinosus*] AAG15328.1 [AF255954], 445 AA

SDSCNR:664339 *Mus musculus* adult male hippocampus cDNA, RIKEN full-length enriched library, clone:2900057E16 product:tubulin beta-4 chain homolog [*Mus musculus*], full insert sequence. BAB28967.1 [AK013717], 444 AA

SDSCNR:2377999 (Q9D6F9) Tubulin beta-4 chain [*Mus musculus*

(Mouse)], 444 AA  
 SDSCNR:615764 tubulin, beta 4 [Mus musculus] NP\_033477.2  
 [NM\_009451], 444 AA  
 SDSCNR:155858 (Q7Z2D1) Beta-tubulin [Tetrahymena pyriformis],  
 443 AA  
 SDSCNR:149083 (P20365) Tubulin beta chain (Beta-tubulin)  
 [Euplotes crassus], 446 AA  
 SDSCNR:2087029 MGC53205 protein [Xenopus laevis] AAH46853.1  
 [BC046853], 444 AA  
 SDSCNR:149075 (Q27380) Tubulin beta chain [Eimeria tenella], 449  
 AA  
 SDSCNR:155826 (P10878) Tubulin beta chain [Toxoplasma gondii],  
 449 AA  
 SDSCNR:2530288 MGC76202 protein [Xenopus tropicalis]  
 AAH64873.1 [BC064873], 449 AA  
 SDSCNR:152900 (P14140) Tubulin beta chain [Plasmodium  
 falciparum], 445 AA  
 SDSCNR:2207369 (Q7Z1I4) Beta-tubulin [Schistosoma japonicum  
 (Blood fluke)], 443 AA  
 SDSCNR:1756542 beta-tubulin [Bombyx mori] BAB86853.1  
 [AB072308], 447 AA  
 SDSCNR:448112 (Q9YHC3) Tubulin beta-1 chain [Gadus morhua  
 (Atlantic cod)], 445 AA  
 SDSCNR:615755 tubulin beta chain 15 - rat, 445 AA  
 SDSCNR:454269 (P32882) Tubulin beta-2 chain (Beta-tubulin class-  
 II) [Gallus gallus (Chicken)], 445 AA  
 SDSCNR:662750 tubulin, beta [Mus musculus] NP\_076205.1  
 [NM\_023716], 445 AA  
 SDSCNR:300872 (P04350) Tubulin beta-5 chain (Tubulin 5 beta)  
 [Homo sapiens (Human)], 444 AA  
 SDSCNR:128507 (P18241) Tubulin beta-1 chain [Brugia pahangi  
 (Filarial nematode worm)], 448 AA  
 SDSCNR:167786 beta-tubulin [Onchocerca volvulus] AAC13548.1  
 [AF019885], 448 AA  
 SDSCNR:167785 beta-tubulin [Dirofilaria immitis] AAC13547.1  
 [AF019884], 448 AA  
 SDSCNR:2446727 tubulin, beta, 2 [Danio rerio] NP\_942104.1  
 [NM\_198809], 445 AA  
 SDSCNR:2333527 tubulin, beta, 2 [Danio rerio] AAH62827.1  
 [BC062827], 445 AA  
 SDSCNR:496513 (P02554) Tubulin beta chain [Sus scrofa (Pig)], 445  
 AA  
 SDSCNR:360500 (P04691) Tubulin beta chain (T beta-15) [Rattus  
 norvegicus (Rat)], 445 AA  
 SDSCNR:144372 (O18342) Beta-tubulin [Halocynthia roretzi (Sea  
 squirt)], 445 AA

SDSCNR:484250     **tubulin, chain B - pig, 427 AA**  
SDSCNR:2182340     **beta tubulin [Aplysia californica] AAP13560.1**  
                         **[AY256661], 449 AA**  
SDSCNR:725838     **tubulin, beta, 2 [Mus musculus] AAH05547.1**  
                         **[BC005547], 445 AA**  
SDSCNR:462644     **tubb5-prov protein [Xenopus laevis] AAH49004.1**  
                         **[BC049004], 444 AA**  
SDSCNR:310945     **tubulin, beta 5 [Mus musculus] NP\_035785.1**  
                         **[NM\_011655], 444 AA**  
SDSCNR:1756541     **(Q8T8B3) Beta-tubulin [Bombyx mori (Silk moth)],**  
                         **447 AA**

## **CURRICULUM VITA OF MATTHEW S. ARNOLD**

**Update: 5 March, 2004**

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**DATE AND PLACE OF BIRTH:** 23, May 1979, Abingdon, VA.

### **ACADEMIC DEGEERS:**

B.S. Biology, 2001, Radford University, Radford, VA 24142.  
M.S. Biology, 2004, Virginia Tech, Blacksburg, VA 24061

### **PROFESSIONAL EXPERIENCE:**

Teaching Assistant, Virginia Polytechnic Institute and University- Blacksburg, VA, 2001-2002.

Biol 1015: General Biology Laboratory I

Biol 1015 General Biology Laboratory II

Biol 1116 Principles of Biology Laboratory II

Biol 1216 Principles of Biology Laboratory II for Majors

Biol 1205H Honors Principles of Biology Laboratory

Averaged 3.8 using a 4.0 scale

### **GRANTS:**

#### **Received:**

2001 Sigma Xi: Title: Sequencing and Modeling alpha and beta tubulin from the thermophilic algae *C. caldarium* and *C. merolae*. Amount (\$150)

2002 GRDP Title Sequencing and Modeling alpha and beta tubulin from the thermophilic algae *C. caldarium* and *C. merolae*. Amount (300)

**PRESENTATIONS OF ORIGINAL RESEARCH**

2001 Radford University Undergraduate Forum. Title: Startle Response in Zebra Fish in Response to Adverse Stimuli.

2003 Poster Presentation Biology Graduate Research Forum Title: Characterization of Thermophilic Tubulin Genes

**GENE BANK SUBMISSIONS**

Cyanidium caldarium alpha-tubulin gene AY302129

Cyanidium caldarium beta-tubulin gene AY302130