

## ***In-silico* Approach Towards Identification of the Protein Responsible for Sjogren's Syndrome and Designing a Natural Anti-Inflammatory Remedy for the Same**

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### **Abstract**

Sjogren's Syndrome is an autoimmune disorder that attacks the body's moisture-producing glands, leading to progressively worsening dry mouth, eyes, vagina and skin, as well as fatigue. The syndrome boosts rheumatic disorders.

The mannose binding lectin (MBL) mutation protein is one of the cause for Sjogren's syndrome. A 3D structure of the MBL protein was modeled.

Pernin (glycosylated protein) extracted from green lipped mussel is found to have anti-inflammatory property. Its 3D structure was modeled.

The active component of ayurvedic herbs turmeric and licorice is found to have anti-inflammatory activity. Their structure was sketched using ACD/Chemsketch, combined and converted to \*.pdb. This combination is docked with pernin protein.

Again, the above combination is successfully docked with MBL protein which proves that the combination is effective in treating sjogren's syndrome.

**Keywords:** Sjogren's Syndrome; Autoimmune disorder; Rheumatic disorders; Bioinformatics; Mannose binding lectin; Ayurvedic herbs; Pernin; Homology modelling; Docking.

### **Introduction**

Sjögren's syndrome is an autoimmune disorder in which the body's immune system mistakenly reacts to the tissue in glands that produce moisture such as tear and

salivary glands. In 1933, Swedish physician Henrik Sjögren observed that a large number of his female patients were experiencing dry eyes and mouths, along with their arthritis symptoms. The condition became known as Sjögren's syndrome. It is a chronic, inflammatory disease that often progresses to a more complex, systemic disorder (generally affecting other organs in the body such as joints, kidneys, and intestinal tract). It is characterized by unusual infiltration of these glands that are responsible for fluid production by lymphocytes, thus causing decreased saliva (dry mouth), decreased tear production (dry eyes), and drying of other mucous membranes. Other features of this syndrome include swollen salivary glands, feeling of sand or grit in the eyes, difficulty swallowing, joint pain, and decreased sense of taste. Sjögren's syndrome can affect anyone at any age, but about 90% of those affected are women, the majority of whom are older than 40 [1, 2]. Sjogren's syndrome generally occurs in two ways: primary and secondary. Primary Sjogren's syndrome occurs by itself and is not associated with other diseases. Secondary Sjogren's syndrome occurs with rheumatic diseases such as rheumatoid arthritis, systemic lupus erythematosus ("lupus"), polymyositis, and some forms of scleredema. Rheumatic diseases are conditions that affect joints, bones, muscles, skin, and sometimes other organs. The cause of primary Sjogren's syndrome is complex and the factors initiating and driving autoimmunity in this disease are largely unknown. Certain disturbances of the immune system (i.e. B-cell hyperreactivity and enhanced levels of B-cell-activating factor/B-lymphocyte stimulator) play a central role in this entity. Whether this is a primary abnormality or the result of predisposing factors or infectious, e.g. viral, agents remains uncertain [3].

**The mannose binding lectin mutation protein (MBL)** is one of the cause for Sjogren's Syndrome. The gene coding for MBL is polymorphic, and variant alleles result in greatly reduced circulating MBL levels. The MBL gene (*mb12*) is located on chromosome 10 and is polymorphic at codons 52, 54 and 57. The presence of variant alleles at codon 54 has been associated with decreased functional levels of MBL in the circulation and in the vagina. The immune system lacks the usual controls. This causes white blood cells to invade glands in the body that produce moisture, such as the tear and salivary glands, and the Bartholin's glands in the vagina. They can destroy the glands and cause them to stop producing moisture [4, 5].

**The New Zealand green-lipped mussel**, also known as the New Zealand mussel or the greenshell mussel, *Perna canaliculus*, is a species of bivalve mollusc in the family Mytilidae. This species of mussel is endemic to New Zealand, and is also an introduced pest in Australian waters. A protein, designated pernin, found in the New Zealand green-lipped mussel, comprises almost all of the protein in cell-free haemolymph. It occurs as large, aggregate structures of several hundred units resembling small virus-like particles. Pernin is a non-pigmented, glycosylated protein, composed of 497 amino acids, which has an estimated molecular mass of 60 kDa. It is exceptionally rich in histidine (13.7%) and aspartic acid (12.3%), amino acids both known to participate in the binding of divalent metal cations. Studies have also found that *Perna canaliculus* inhibits the 5-lipoxygenase pathway, which leads to the formation of leukotrienes. Many of the products of these pathways have inflammation-supporting properties. The Green Lipped Mussel has been found to

have beneficial effects in relieving the pain, inflammation and symptoms associated with [6]:

- Osteoarthritis (degenerative joint disease)
- Rheumatoid Arthritis
- Bursitis
- Ankylosing Spondylitis
- Sports Related Injuries
- Lyme Disease
- Inflammatory Skin Conditions
- Connective Tissue Repair

Hence, is taken for this particular study.

**Ayurveda** is a system of traditional medicine native to the Indian Subcontinent and practiced in other parts of the world as a form of alternative medicine. In Sanskrit, the word Ayurveda consists of the words āyus, meaning 'life', and veda, meaning 'related to knowledge' or 'science'. Evolving throughout its history, Ayurveda remains an influential system of medicine in South Asia. The earliest literature of Ayurveda appeared during the Vedic period in India. The *Sushruta Samhita* and the *Charaka Samhita* were influential works on traditional medicine during this era. Ayurvedic practitioners also identified a number of medicinal preparations and surgical procedures for curing various ailments and diseases. It is one of the natural medical systems that run from 5000 of years. It is also called ancient medical system that was originated from India that believes in curing the diseases from the roots. It is one of the oldest medical system in the world.

Organic **Licorice** (*Glycyrrhiza uralensis*) is one of the most widely used herb from the ancient medical history of Ayurveda, both as a medicine and also as a flavoring herb to disguise the unpleasant flavor of other medications. It is a very sweet, moist, soothing herb that detoxifies and protects the liver and is also a powerful anti-inflammatory, being used in conditions as varied as arthritis and mouth ulcers. In India, it is cultivated in some parts of the hilly northern area, Delhi and South India. *Glycyrrhiza uralensis* has long been used throughout the world as a sweetener and in folk medicine because of its antioxidative, anti-inflammatory, antibacterial, antiulcerative properties. Its active component is **Glycirrhizin** [7, 8].

**Turmeric** (*Curcuma longa*) is a rhizomatous herbaceous perennial plant of the ginger family, Zingiberaceae. It is native to tropical South Asia and needs temperatures between 20°C and 30°C, and a considerable amount of annual rainfall to thrive. In medieval Europe, turmeric became known as Indian Saffron, since it was widely used as an alternative to the far more expensive saffron spice. Its active ingredient is **curcumin** and it has a distinctly earthy, slightly bitter, slightly hot peppery flavor and a mustardy smell. In Ayurvedic practices, turmeric has many medicinal properties and many in South Asia use it as a readily available antiseptic for cuts, burns and bruises. It is also used as an antibacterial agent. It is popular as a tea in Okinawa, Japan. Pakistanis also use it as an anti-inflammatory agent, and remedy for gastrointestinal discomfort associated with irritable bowel syndrome, and other digestive disorders. It is currently being investigated for possible benefits in

Alzheimer's disease, cancer, arthritis, and other biological disorders. Indians, in addition to its Ayurvedic properties, use turmeric in a wide variety of skin creams that are also exported to neighboring countries [9].

## Methodology

### Softwares/Web Servers used

- (1) modeller9v7  
MODELLER (copyright © 1989-2008 Andrej Sali)  
<http://salilab.org/index.html>
- (2) Swiss-PdbViewer v4.01 by Nicolas Guex , Alexandre Diemand , Manuel C. Peitsch , & Torsten Schwede (Swiss Institute of Bioinformatics)  
<http://spdbv.vital-it.ch/index.html>
- (3) ACD/ChemSketch Freeware, version 11.00, Advanced Chemistry Development, Inc., Toronto, ON, Canada, [www.acdlabs.com](http://www.acdlabs.com), 2008.
- (4) RAMPAGE: Assessment of the Ramachandran Plot  
MolProbity | Crystallography and Bioinformatics Group  
<http://mordred.bioc.cam.ac.uk/~rapper/rampage.php>  
S.C. Lovell, I.W. Davis, W.B. Arendall III, P.I.W. de Bakker, J.M. Word, M.G. Prisant, J.S. Richardson and D.C. Richardson (2002) Structure validation by Calpha geometry: phi,psi and Cbeta deviation. *Proteins: Structure, Function & Genetics*. 50: 437-450.
- (5) ArgusLab 4.0.1, Mark A. Thompson, Planaria Software LLC, Seattle, WA  
<http://www.arguslab.com>
- (6) HEX 5.1  
University of Aberdeen, Scotland, UK  
<http://www.csd.abdn.ac.uk/hex/>
- (7) Rasmol
- (8) LigandScout  
Structural Bioinformatics group at Tel -Aviv University  
<http://bioinfo3d.cs.tau.ac.il/PatchDock/index.html>
- (9) GRAMM-X: Protein Docking Web Server v.1.2.0  
Center for Bioinformatics: The University of Kansas  
<http://vakser.bioinformatics.ku.edu/resources/gramm/grammx/>

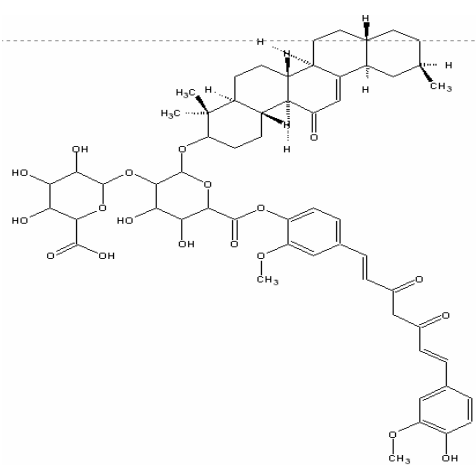
The target protein Mannose Binding Lectin (MBL) precursor (accession number AAK52907) was retrieved in fasta format from NCBI's Entrez protein database. To predict its 3D homology modelling is carried out using the software Modeller9v7 software. templates 1XPHA, 1TDQB, & 1G1SA and ten models were generated. The models generated by Modeller is analysed by Rampage Ramachandran Plot server and the best stable model is selected.

For proposed treatment principal alkaloids from Licorice herb (Glycirrizin) and Turmeric herb (Curcumin), both from Indian traditional Ayurvedic medication were selected. Again, protein Pernin found to have anti-inflammatory activity was selected.

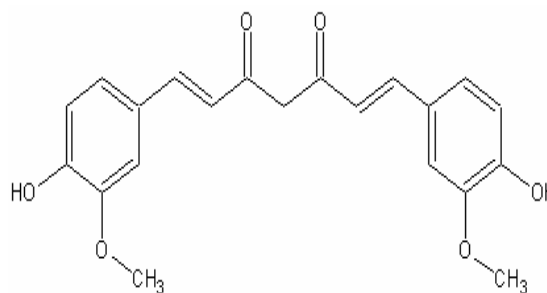
Authors take the combination of the three compounds as possible treatment for Sjogren's Syndrome.

The 3D structure of the protein Pernin (ncbi accession number AAK20952) was modeled using templates retrieved from BLAST search tool. The templates were 1FIGH, 1SDYA, & 1F18A. Ten models of Pernin protein was generated by modeller.

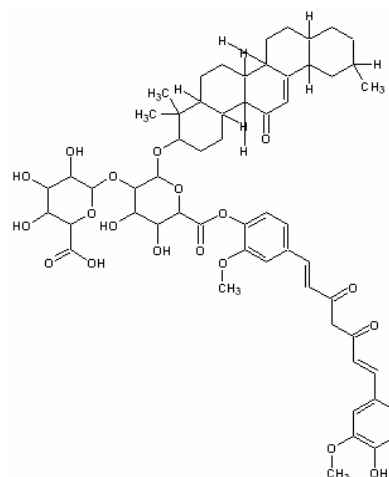
The structure of Glycirrizin and Curcumin was drawn using ACD/Chemsketch and converted to \*.pdb using argus lab software. This combination was docked with Pernin protein using HEX software. Again, the docked combination (Pernin, Glycirrizin and Curcumin combination) was docked with MBL protein using GRAMM-X software.



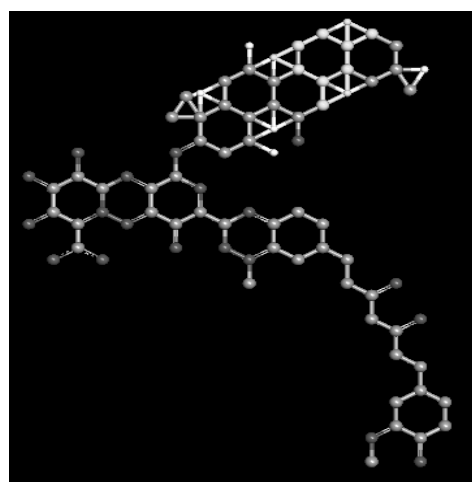
**Figure 1:** Chemical structure of glycirrizin drawn using ACD/Chemsketch software.



**Figure 2:** Chemical structure of curcumin drawn using ACD/Chemsketch software.



**Figure 3:** Combined Structure of glycirrizin & curcumin.



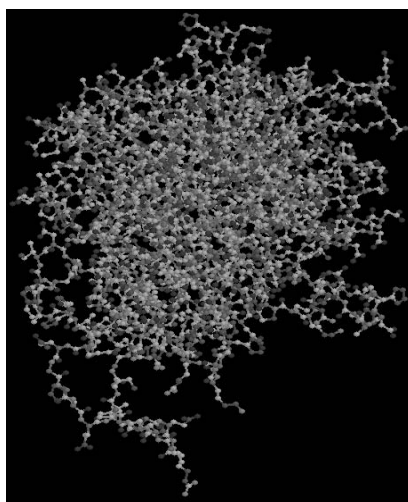
**Figure 4:** Combined Structure of glycirrizin & curcumin in Argus lab (\*.pdb).



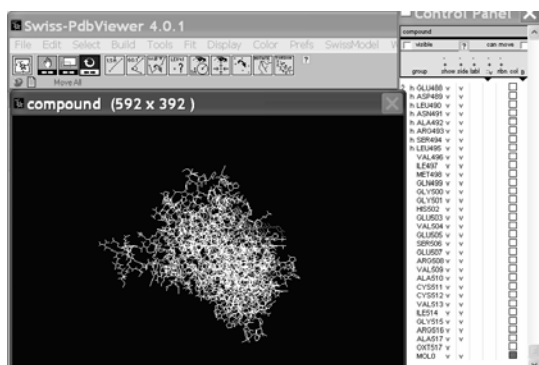
Number of residues in favoured region (~98.0% expected): 383 (74.4%)

Number of residues in allowed region (~2.0% expected): 76 (14.8%)

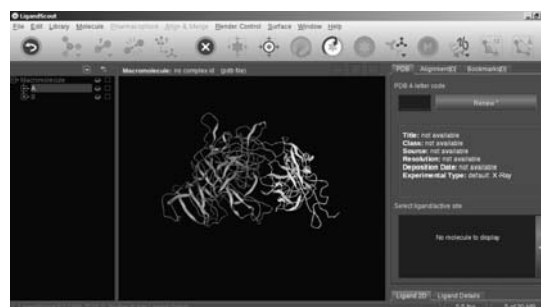
Number of residues in outlier region : 56 (10.9%)



**Figure 7:** PERNIN.pdb (visualization in Rasmol).



**Figure 8:** Docked structure PERNIN with glycirrizin & curcumin combination (visualization in SPDBV).



**Figure 9:** Docked structure MBL protein with PERNIN, glycirrizin & curcumin combination (visualization in LigandScout software).

## Conclusion

The successful docking of MBL.pdb protein with pernin, glycirrizin & curcumin combination proves that the combination can be effective in the treatment of Sjogren's Syndrome.

## Discussion

This *in-silico* work highlights Sjogren's Syndrome, an auto-immune disorder which has no treatment till present. The mutations in MBL protein (stated here) is one of the

cause of this disorder. It is found that Pernin (glycosylated protein) extracted from New Zealand green-lipped mussel has anti-inflammatory property and is used to treat Rheumatic arthritis, hence the authors use this particular protein as one of the possible remedy for Sjogren's Syndrome. (Sjogren's syndrome boosts Rheumatic arthritis).

Again, as per Indian Traditional Ayurvedic practice, we find the mention of herbs Licorice and Turmeric used to cure inflammatory and rheumatic disorders. Hence, the authors use this particular combination with pernin towards treatment/cure of this disorder.

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