Molecular Recognition of the Complement Component C3 by Compstatin

Georgios Archontis

Department of Physics, University of Cyprus

TAGp meeting, LAPTH, Annecy-le-Vieux

October 20-22, 2010

Acknowledgements

- Dr. Phanourios Tamamis, Panagiota Pierrou (U. of Cyprus).
- Prof. Dimitrios Morikis (Dept. of Bioengineering, UC Riverside).
- Prof. Christodoulos Floudas, Megan Bellows (Dept. of Chemical Engineering, Princeton University).
- Prof. John D. Lambris, Department of Pathology, University of Pennsylvania.

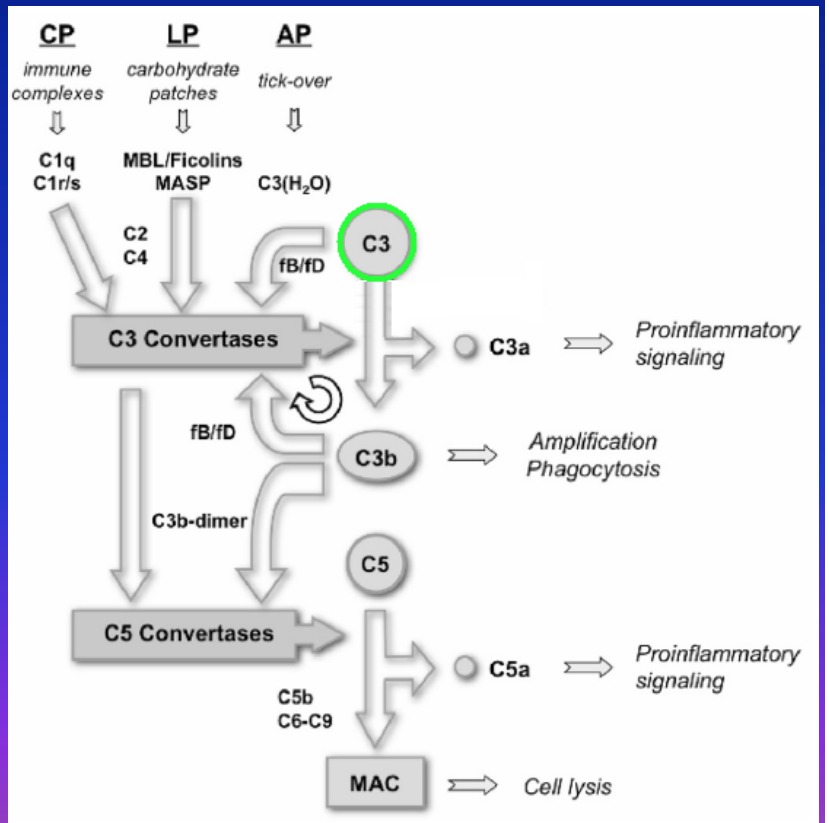
Complement: An Immune Surveillance System

- Set of plasma proteins that activates a cascade of proteolytic reactions on microbial surfaces, coating them with fragments recognized by phagocytic receptors on macrophages.
- Also involved in processes such as synapse maturation, tissue regeneration, angiogenesis and lipid metabolism.

Reviewed in Ricklin et al. Nature Immunology 11:785 (2010)

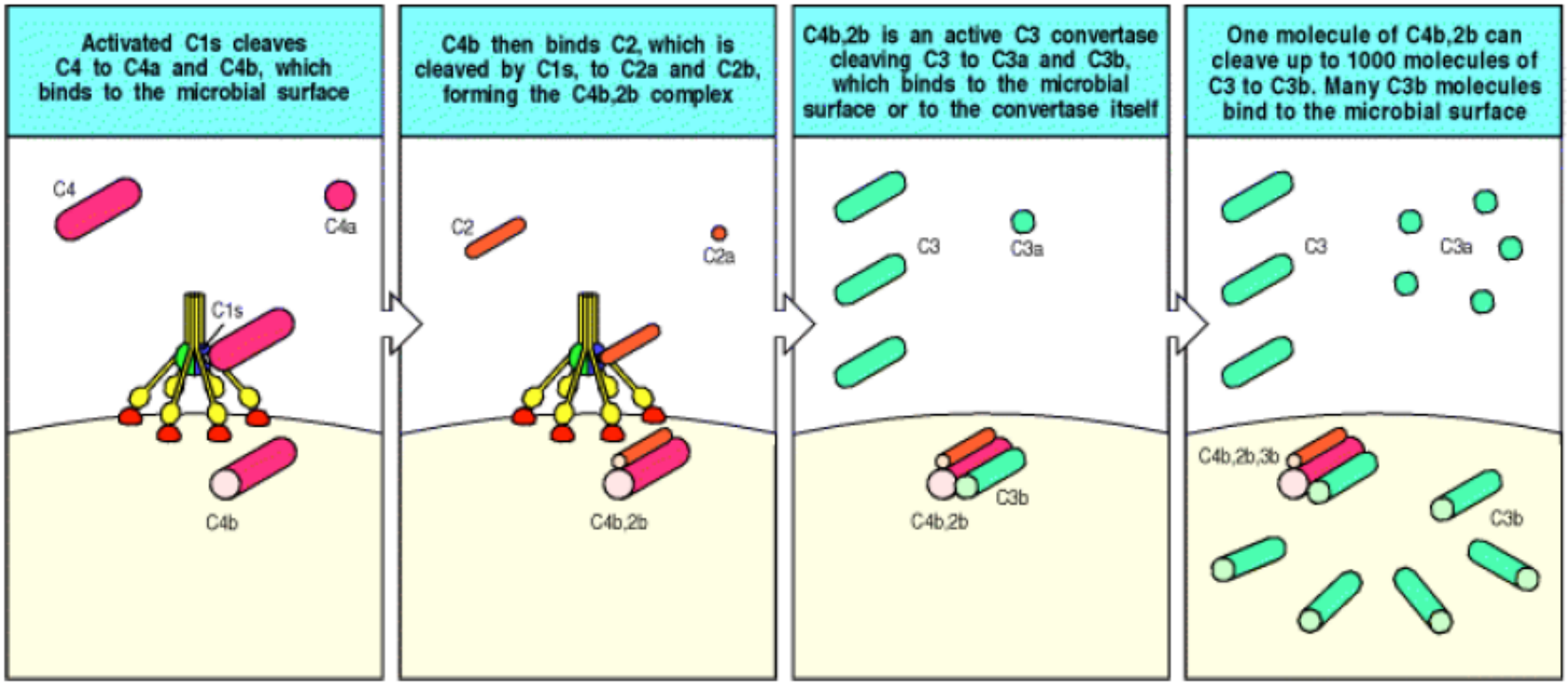
Proceeds through three pathways (classical, lectin or alternative), all converging to the cleavage of protein C3 into C3b and C3a.

- C3b contains an active, solvent-exposed thioester group, which forms a covalent bond at the surface of infecting pathogen, signaling its destruction by phagocytes.



Ricklin et al. Current topics in Complement II, doi:10:1007/978-0-387-78952-1_20)

Schematic depiction of the amplified production of the C3b protein fragment in the classical pathway. The C3b fragments bind to the pathogen surface, labeling it for destruction.



Charles Janeway et al. Immunobiology 6th Ed, Garland Science

Inappropriate complement activation is responsible for several pathological conditions and complement related diseases.

Complement involvement in disease

Acute disorders
- Adult respiratory distress syndrome
- **Asthma**
- Burns, wound healing
- Hyperacute rejection (organ transplant)
- Guillain-Barré syndrome
- Ischemia-reperfusion injury
  - **Heart attack**
  - Skeletal muscle
  - Stroke
  - Lung inflammation
- Multiple organ dysfunction syndrome
- Septic shock
- Trauma, hemorrhagic shock
- Xenotransplantation

Reaction to Biomaterials / Implants
- Angioplasty
- Cardiopulmonary bypass
- Hemodialysis
- Platelet storage

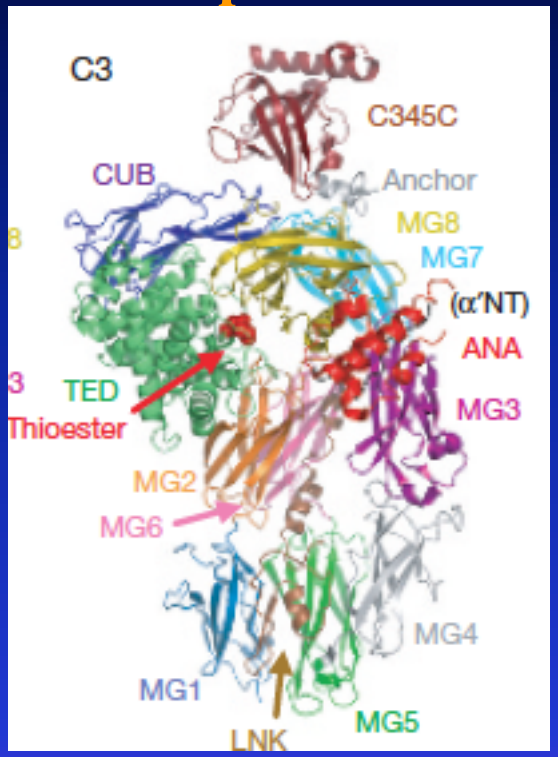
Chronic disorders
- Age-related macular degeneration
- **Alzheimer's disease**
- Autoimmune diseases
  - Ancylosing spondylitis
  - Angiodema
  - Crohn's disease
  - Glomerulonephritis
  - Hemolytic-uremic syndrome
  - **Rheumatoid arthritis**
  - Multiple sclerosis
  - Myasthenia gravis
  - Neisserial infection
  - Paroxysmal nocturnal hemoglobinuria
  - Psoriasis
  - Pyogenic bacterial infections
  - Systemic lupus erythematosus
  - Ulcerative colitis
- Hemolysis
- Infertility
- Obesity
- Organ rejection (transplantation)
- Thrombosis
- Type I diabetes Mellitus

The development of drugs for the regulation of complement is of significant medical interest.

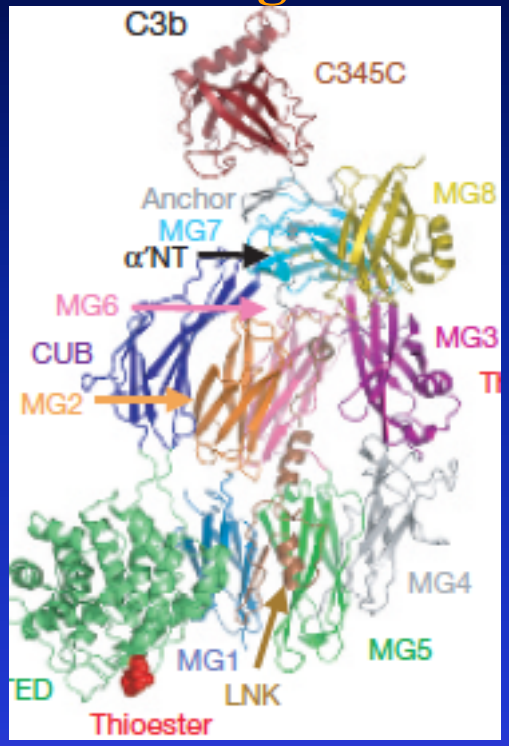
Reviewed in Ricklin and Lambris Adv. Exp. Med. Biol. 632 (2008)

C3: Good target for complement-directed drug discovery, due to its core role in all pathways.

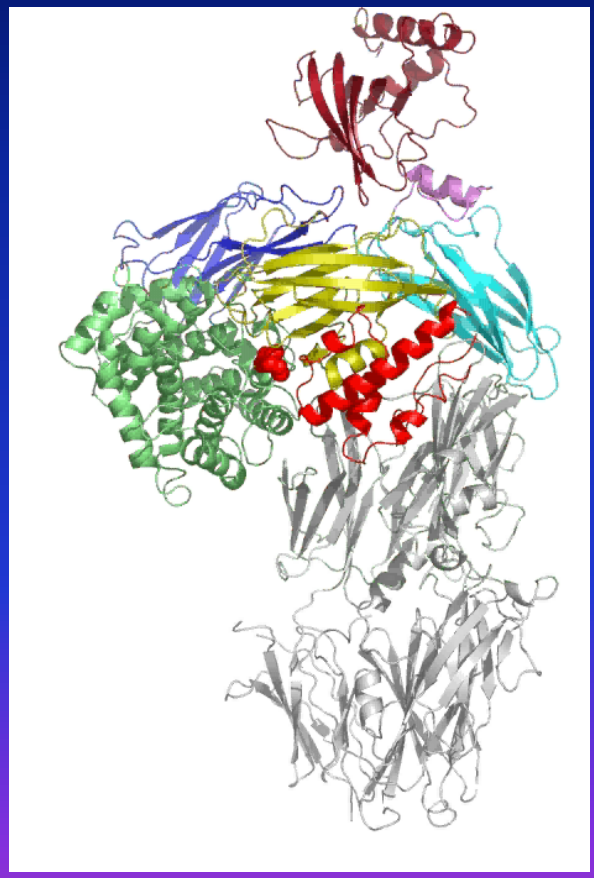
C3 protein



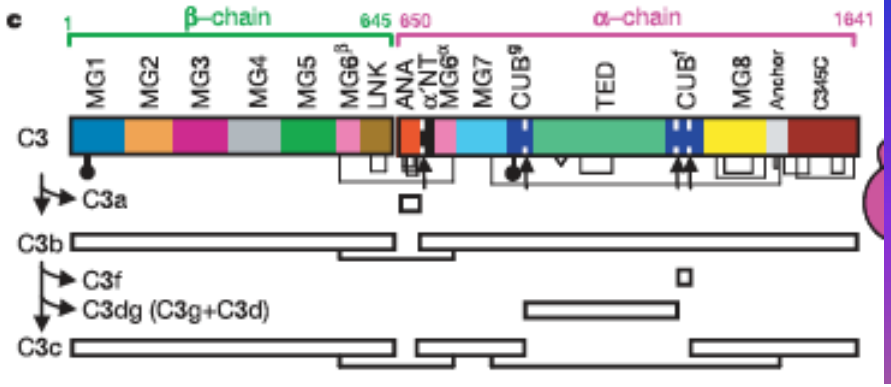
C3b fragment



Schematic depiction of the conformational change C3 → C3b



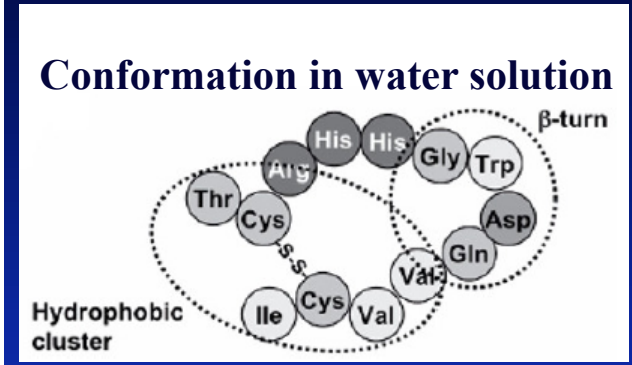
Janssen et al., Nature 444:214 (2006)

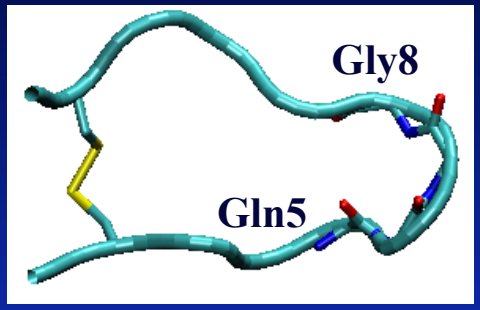


1641 residues
13 domains

Compstatin: A promising C3 inhibitor

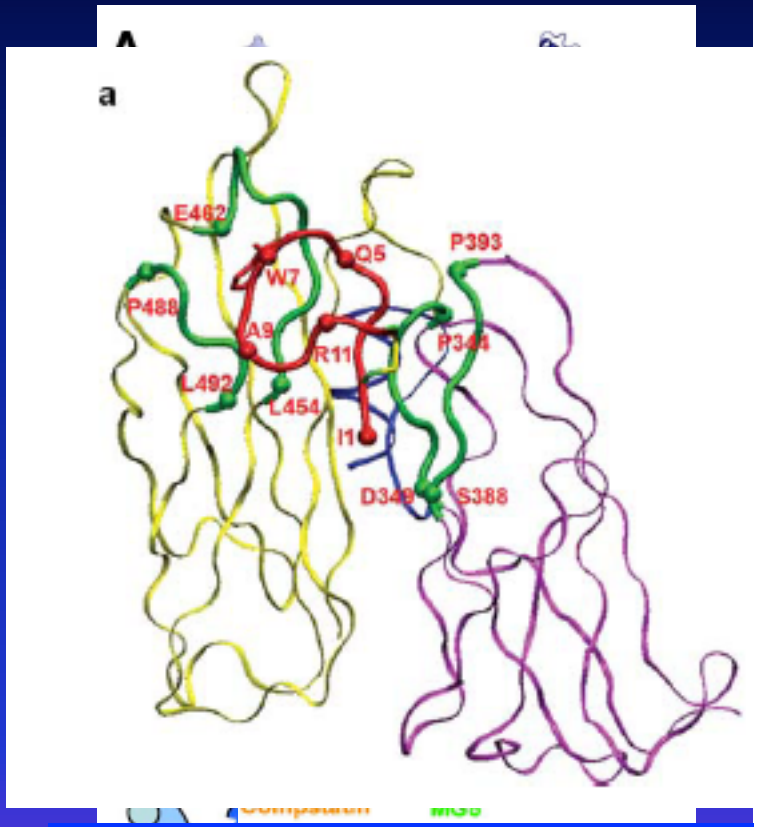
- 13 residue peptide: ICVVQDWGHHRCT.
- Maintained in a cyclic conformation via disulfide bridge **Cys2-Cys12**.



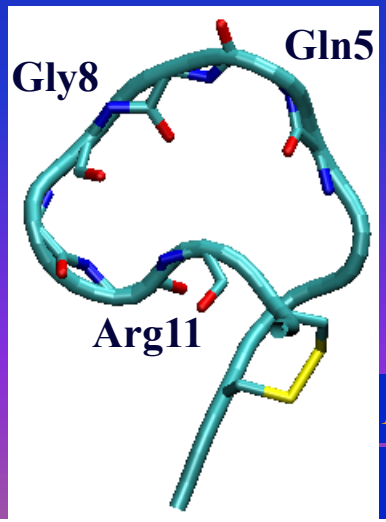


Segment 5-8 in β-turn & terminal ends in hydrophobic cluster.

Morikis et al. Prot. Sci 7:619 (1998)
Mallik et al. Proteins 52:130 (2003)
Tamamis et al. J Mol Graph. Model. 26:571 (2007)



Binding conformation, in complex with C3.



The binding conformation is different! (RMSD = 3.7 Å).

The turn 5-8 is no longer present. Instead, a new turn 8-11 is stabilized, allowing compstatin to interact with four protein sectors.

Ricklin and Lambris Adv. Exp. Med. Biol. (2008)

Janssen et al, J. Biol. Chem. 282 (2007)

Compstatin binds between domains MG4/MG5

C3:C3bBb complex formation is probably inhibited due to steric interference from compstatin.

Janssen et al. J. Biol. Chem. 282:29241 (2007)

Early mutation studies on compstatin

analogue name	sequence[a]	IC_{50} (μM)[b]	RIA[d]
compstatin[f]	I[CVVQDWGHHRC]T-NH_2	53.6	1
Ac-compstatin[f]	Ac-I[CVVQDWGHHRC]T-NH_2	18.1	3
Ac-H9A-NH_2	Ac-I[CVVQDWGAHRC]T-NH_2	12.4	4
Ac-V4T-NH_2	Ac-I[CVTQDWGHHRC]T-NH_2	68.3	1
Ac-V4S-NH_2	Ac-I[CVSQDWGHHRC]T-NH_2	50.9	1
Ac-V4H-NH_2	Ac-I[CVHQDWGHHRC]T-NH_2	10.5	5
Ac-V4F-NH_2	Ac-I[CVFQDWGHHRC]T-NH_2	10.2	5
Ac-V4Y/H9A-NH_2	Ac-I[CVYQDWGAHRC]T-NH_2	3.8	14
Ac-V4W/H9W-NH_2	Ac-I[CVWQDWGWHRC]T-NH_2	3.1	17
Ac-V4W-NH_2	Ac-I[CVWQDWGHHRC]T-NH_2	2.2	24
Ac-V4W/H9A	Ac-I[CVWQDWGAHRC]T	2.0	27
Ac-V4W/H9A-NH_2	Ac-I[CVWQDWGAHRC]T-NH_2	1.2	45
	CVVADWGHHRC	910	
	CVVQAWGHHRC	257	
	CVVQDAGHHRC	182	
	CVVQDWAHHRC	>1,200	

Alanine mutations show that residues 5-8 are critical for activity.

Insertion of an aromatic residue at position 4 increases activity, with Trp4 the best substitution.

Alanine insertion at position 9 increases activity slightly.

Double mutant Val4Trp/His9Ala yields the best inhibitor with natural aminoacids to-date.

Cys2-Cys12 disulfide bridge is necessary for activity.

Morikis et al. Protein Science 7:619-627 (1998)

Mallik et al. J. Med. Chem. 48:274 (2005)

Human C3c – Compstatin Complex:
Questions and Goals

- Identify important interactions in the human complex.
- Suggest changes that may yield improved human C3c inhibitors.

Non-primate mammal – Compstatin Complexes:
Questions and Goals

Compstatin is inactive against C3c of non-primate mammals.

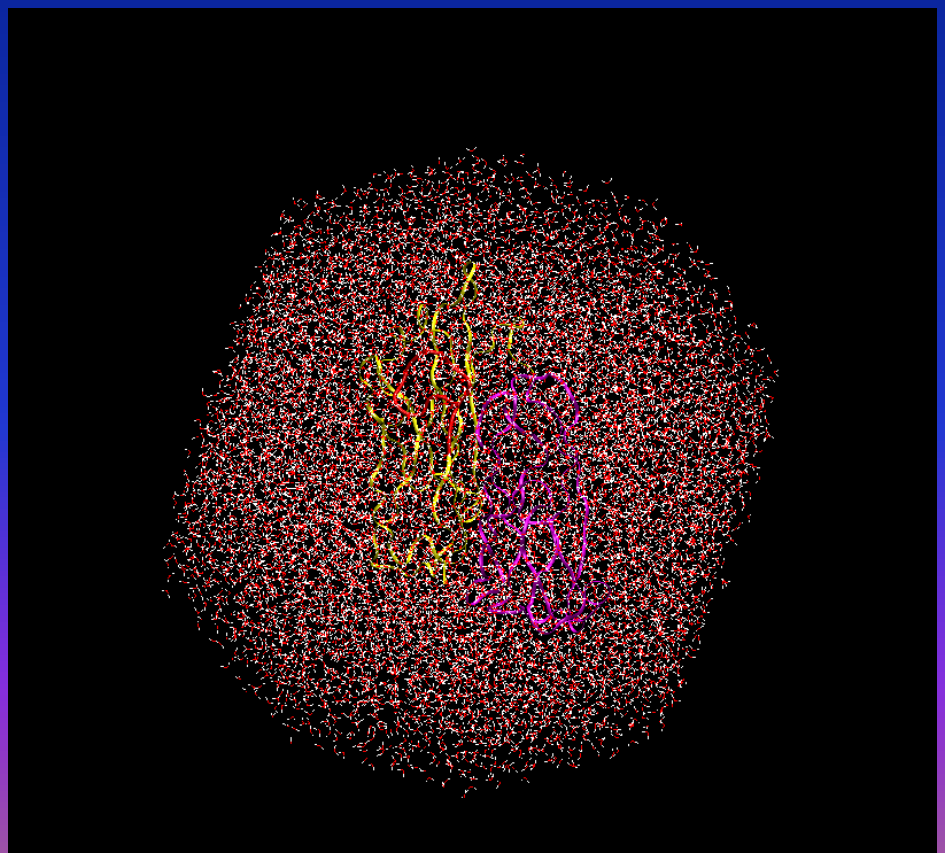
- Explain this compstatin species specificity by MD simulations.
- Possibly develop effective inhibitors against non-primate species to test disease models in non-primate animals.

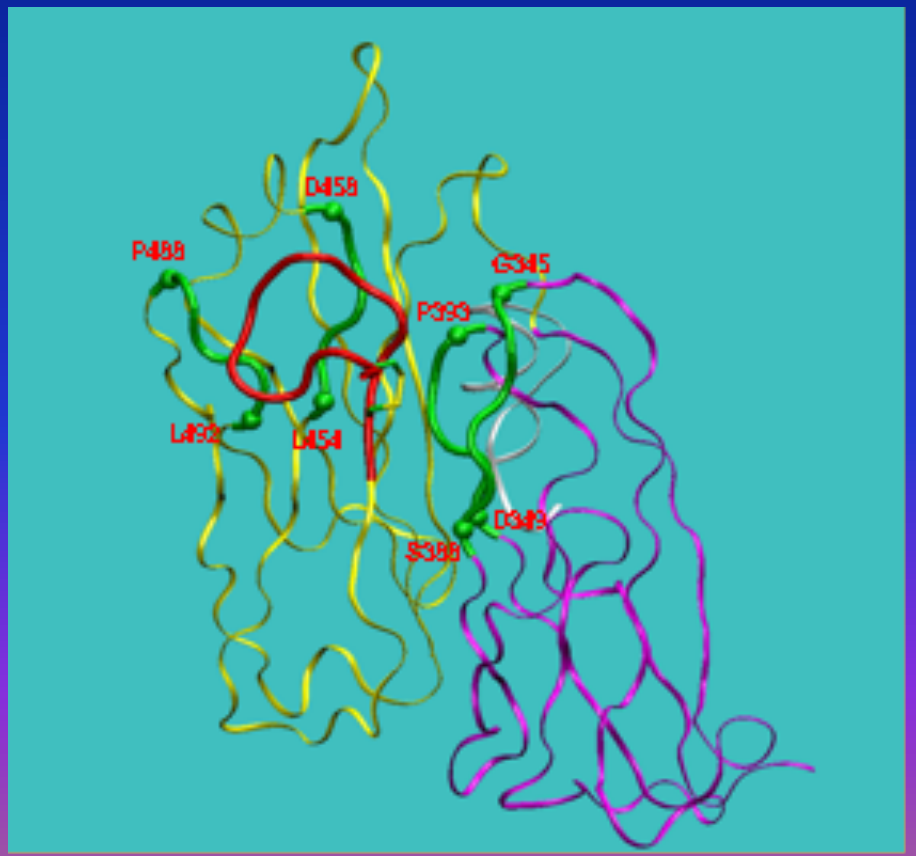
Tamamis et al, Proteins (2010)

Complexes of Compstatin – Human/Rat C3c: Methods

Tamamis et al, Proteins (2010)

- Multiple simulations of human / rat C3c, in complex with compstatin or free. Length of production runs: 7 ns.
- The complex / free proteins were immersed in a 89-Å octahedral water box.
- Main-chain heavy atoms of an external protein shell, 20 Å beyond compstatin, were harmonically restrained to their initial crystallographic positions.

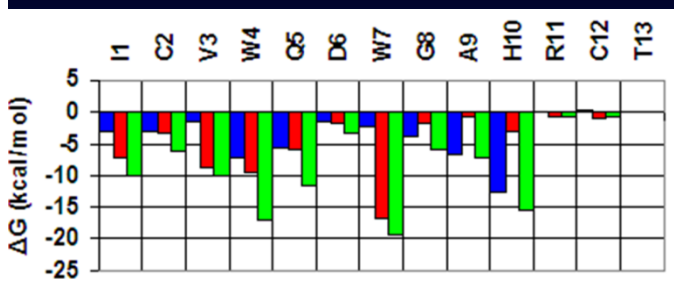


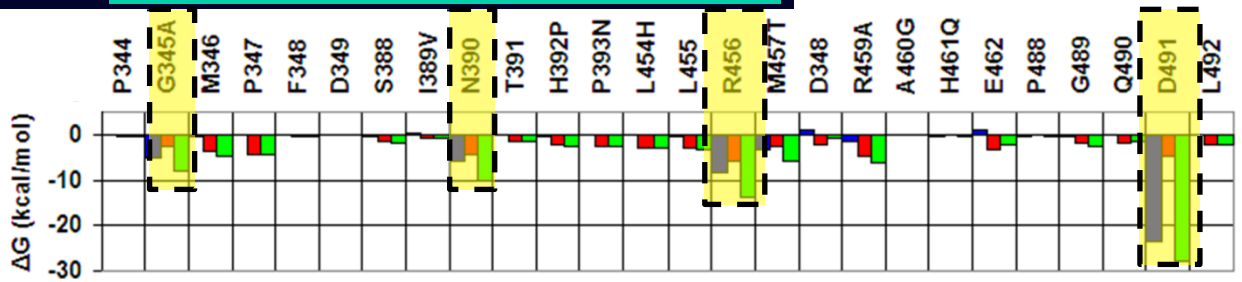


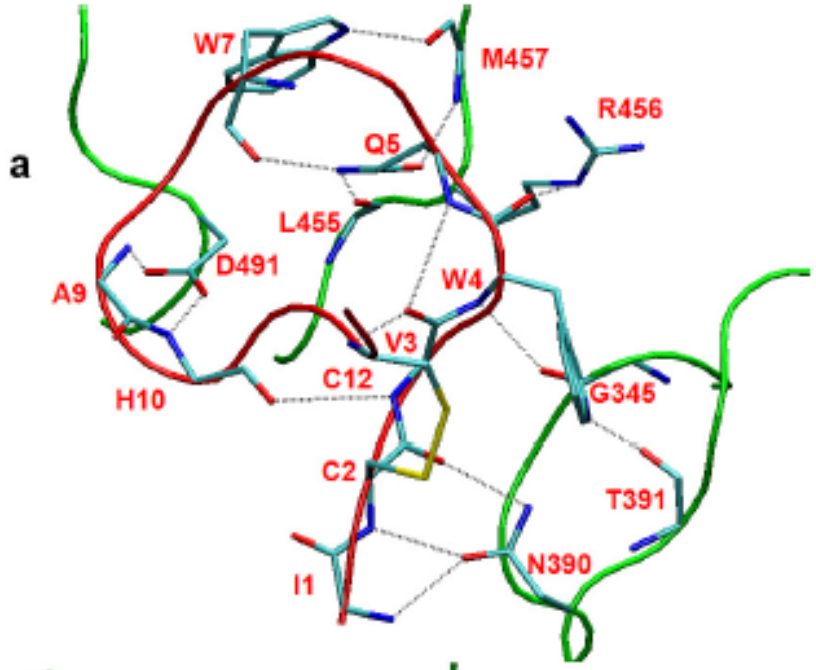
Human Complex

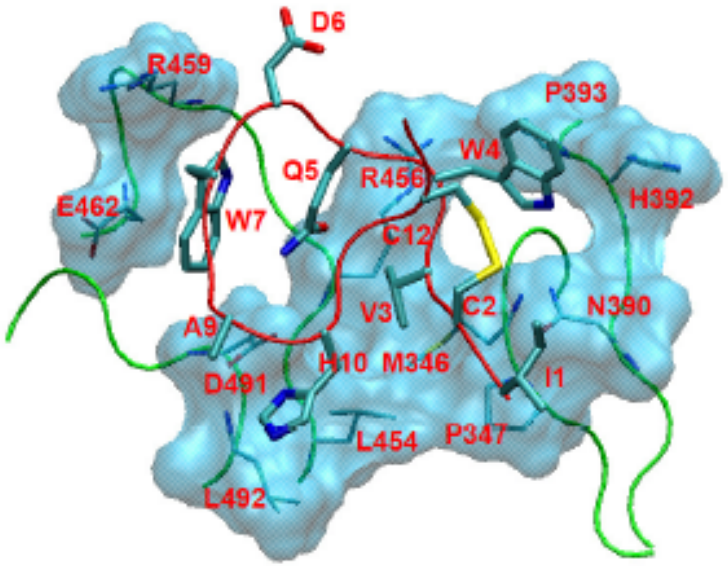
Residue Interaction Energies

- Polar
- Non-polar
- Total









Tamamis et al, Proteins (2010)

- Side-chain (non-polar) contacts are also accurately retained:
  - Val3 stable hydrophobic cluster with Met346, Pro347 and Leu454.
  - Trp4 packs between the Cys2 - Cys12 disulfide-bridge and Pro393.
  - Trp7 makes contacts with the non-polar parts of Gln5, Arg459 and Glu462.
  - His10 near a hydrophobic nucleus formed by Leu454 and Leu492.

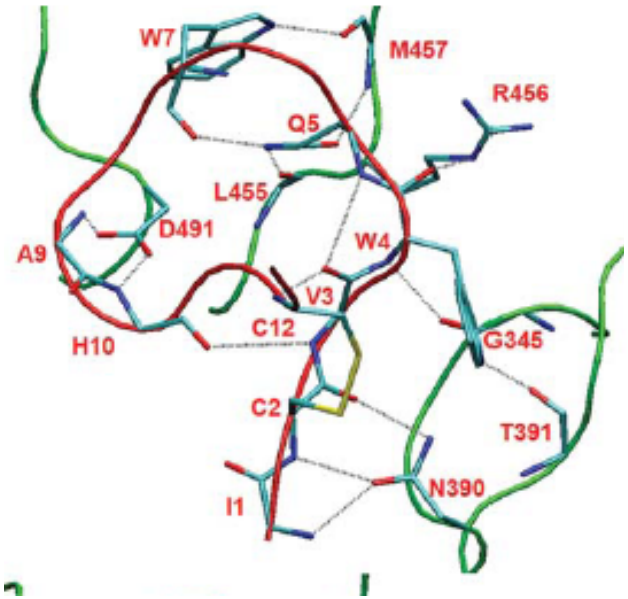
- X-tal hb reproduced:
  - Ile1 / Cys2 - Asn390.
  - Trp4 - Gly345, Arg456.
  - Gln5 - Leu455, Met457.
  - Trp7 - Met457.
  - Ala9 / His10 - Asp491.

Alignment of primate and non-primate C3

Boxes mark regions contacting compstatin.

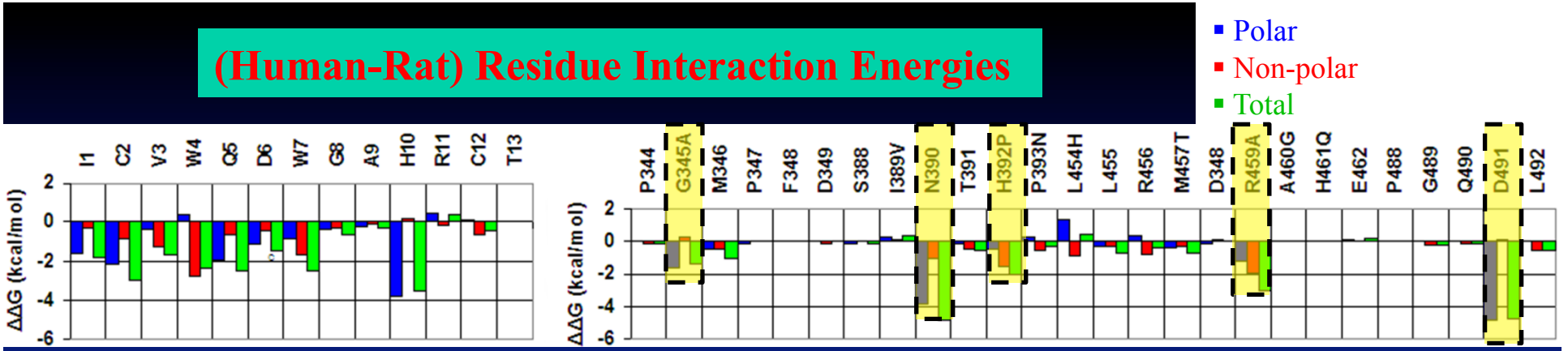
Key mutations from human to non-primate C3:

H392P
P393N/D
R459A/P
D491A/V

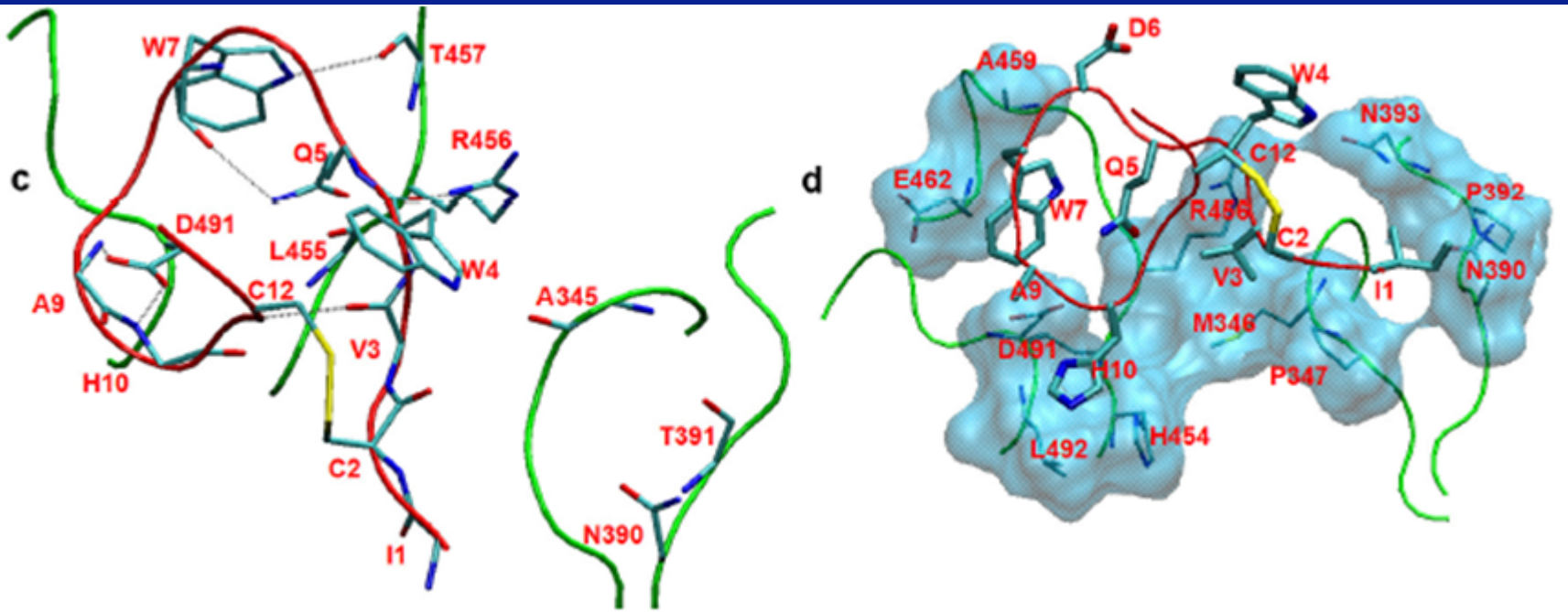



```
                330        340        350        360        370        380
Homo sapiens    SPYQIHFTKTPKYFKPGMPFDLMVFVTNPDGSPAYRVPVAVQGEDTVQSLTQGDGVAKLS
Pan troglodytes SPYQIHFTKTPKYFKPGMPFDLMVFVTNPDGSPAYRVPVAVQGEDTVQSLTQGDGVAKLS
Macaca mulatta  SPYQIHFTKTPKYFKPGMPFDLMVFVTNPDGSPAYRVPVAVQGEDAVQSLTQGDGVAKLS
Rattus norvegicus SPYQIHFTKTPKFFKPAMPFDLMVFVTNPDGSPARRVPVVTQG-SDAQALTQDDGVAKLS
Bos taurus      SPYQIHFTKTPKFFKPAMPFDLMVYVTNPDGSPARHIPVVTQG-SNVQSLTQDDGVAKLS
Mus musculus    SPYQIHFTKTPKFFKPAMPFDLMVFVTNPDGSPASKVLVVTQG-SNAKALTQDDGVAKLS
Canis familiaris SPYQIHFTKTPKFFKPAMPFDLMVFVTNPDGSPAPHVPVGIQN-YRVQALTQKDGVAKLT
Guinea pig      SPYQIHFTKTPKYFKPAMPFEIMVLVTNPDGSPAPHVPVVTQG-SNVQSLTQADGVARLS
Sus scrofa      SPYQIHFTKTPKFFKPAMPFDLMVYVTNPDGSPARHIPVVTED-FKVRSLTQEDGVAKLS
                ************:***.***::** ********* :: *  :.   .::*** ****:*:

                390        400        410        420        430        440
Homo sapiens    INTHPSQKPLSITVRTKKQELSEAEQATRTMQALPYSTVGNSNNYLHLSVLRTELRPGET
Pan troglodytes INTHPSQKPLSITVRTKKQELSEAEQATSTMQALPYSTVGNSNNYLHLSVPRTELRPGET
Macaca mulatta  INTHPSQKPLSITVRTKKRELSEAEQATRTMEAQPYSTVGNSNNYLHLSVPRAELRPGET
Rattus norvegicus VNTPNNRQPLTITVSTKKEGIPDARQATRTMQAQPYSTMHNSNNYLHLSVSRVELKPGDN
Bos taurus      INTQNKRDPLTITVRTKKDNIPEGRQATRTMQALPYNTQGNSNNYLHLSVPRVELKPGET
Mus musculus    INTPNSRQPLTITVRTKKDTLPESRQATKTMEAHPYSTMHNSNNYLHLSVSRMELKPGDN
Canis familiaris INTPDSKKPLHITVSTKKEGILESRQATRTMEVQPYNTIGNSRNYLHLSVPRMELKPGET
Guinea pig      INTPNTRQPLSVTVQTKKGGIPDARQAINTMQALPYTTMYNSNNYLHLSMPRTELKPGET
Sus scrofa      INTPDNRNSLPITVRTEKDGIPAARQASKTMHVLPYNTQGNSKNYLHLSLPRVELKPGEN
                :**  .:..* :** *:*  :  ..**  **.. **.*  **.******: * **:**:.

                450        460        470        480        490        500
Homo sapiens    LNVNFLLRMDRAHEAKIRYYTYLIMNKGRLLKAGRQVREPGQDLVVLPLSITTDFIPSFR
Pan troglodytes LNVNFLLRMDRAHEAKIRYYTYLIMNKGRLLKAGRQVREPGQDLVVLPLSITTDFIPSFR
Macaca mulatta  LNVNFLLRMDRTQEAKIRYYTYLIMNKGKLLKVGRQVREPGQDLVVLPLSITTDFIPSFR
Rattus norvegicus LNVNFHLRTDAGQEAKIRYYTYLVMNKGKLLKAGRQVREPGQDLVVLSLPITPEFIPSFR
Bos taurus      LNVNFHLRTDPGEQAKIRYYTYMIMNKGKLLKVGRQYREPGQDLVVLPLTITSDFIPSFR
Mus musculus    LNVNFHLRTDPGHEAKIRYYTYLVMNKGKLLKAGRQVREPGQDLVVLSLPITPEFIPSFR
Canis familiaris LNVNFHLRTDPSKEAQIRYYTYLIMNKGKILKVGRQERESGQDLVVLPLTITSDFIPSFR
Guinea pig      INVNFHLRSDPNQEAKIRYYTYLIMNKGKLLKVGRQPREPGQALVVLPMPITKELIPSFR
Sus scrofa      LNVNFHLRTDPGYQDKIRYFTYLIMNKGKLLKVGRQPRESGQVVVVLPLTITTDFIPSFR
                :**** ** *   : :***:**::****::**.*** **.** :***.:.** ::*****
```

(Human-Rat) Residue Interaction Energies



Weaker interactions in the rat complex



- The displacement destabilizes the compstatin binding position.

- Interactions are weaker in the rat complex.

Tamamis et al, Proteins (2010)

Weaker interactions in the rat complex

•**Missing** hydrogen bonds **:**

➢**The displacement of protein segment 388-393** disrupts the hydrogen-bonding interactions Ile1/ Cys2 main-chain - Asn390 side-chain and Trp4 side-chain - Thr391.

➢The Gln5 side-chain makes weaker polar interactions with rat C3c (partial loss of hydrogen bonds).

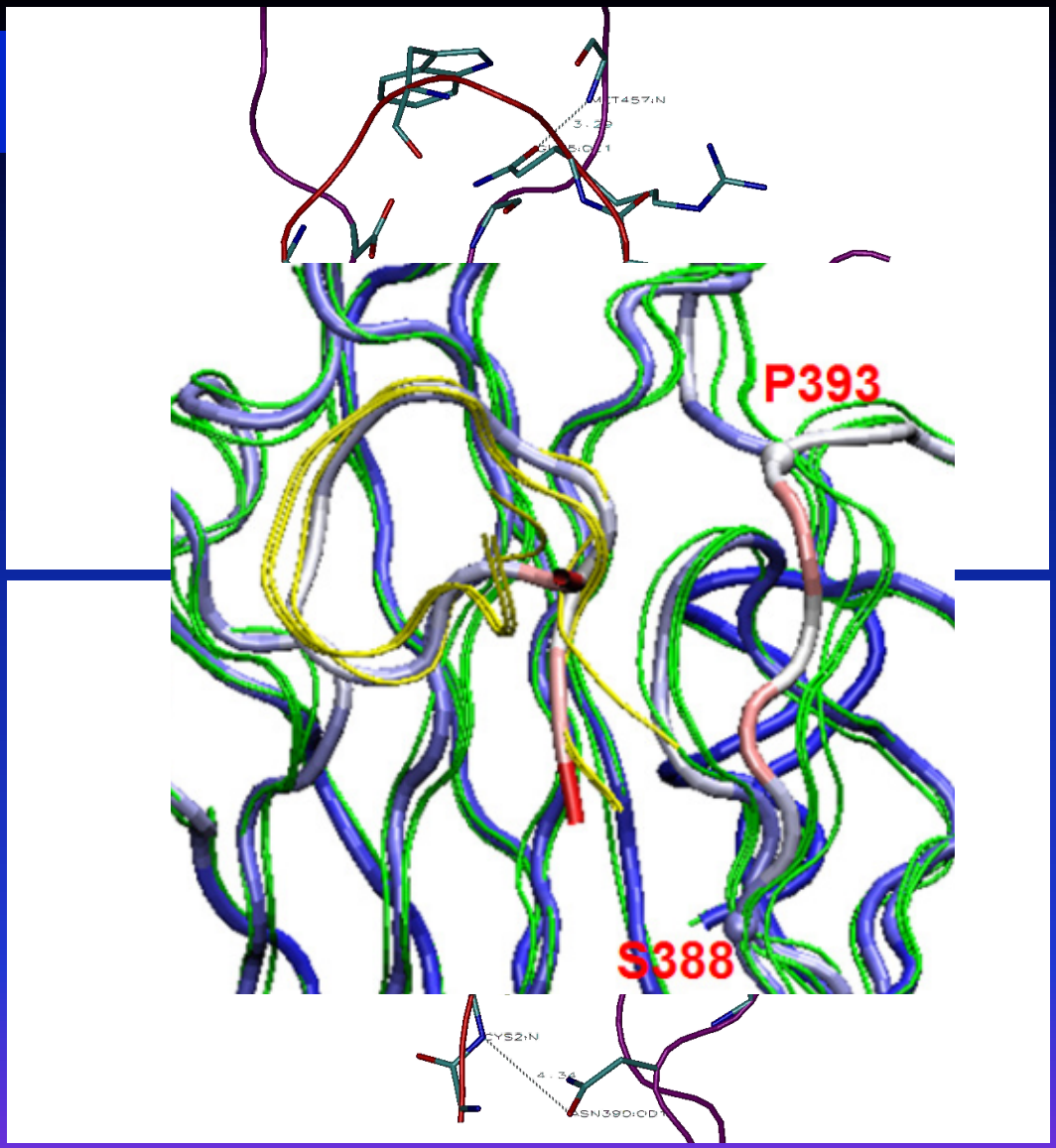
➢**His10: largest reduction in interaction energy** (loss of hydrogen bonds with the Asp491 side-chain)

•Side-chain (non-polar) contacts **missing:**

➢As the sector 388-393 moves away, the Trp4 side-chain explores alternative conformations, and the hydrophobic cluster of residues Val3, Met346, Pro347 and Leu454 is destroyed.

➢The R459A mutation results in reduced non-polar interactions with Trp7.

Tamamis et al, Proteins (2010)



TYPICAL HUMAN C3c RUN

TYPICAL RAT C3c RUN

- Displacement of sector 388-393 is most prominent, and observed in multiple runs.
- It eliminates interactions between C3 and the compstatin N-terminal end.
- As a result, the compstatin binding position is destabilized and the interactions
- with the 488-492 sector are also weakened.

Human and Rat Complexes: Association and Interaction Energies (MM-GBSA estimate)

P + L ⟶ PL

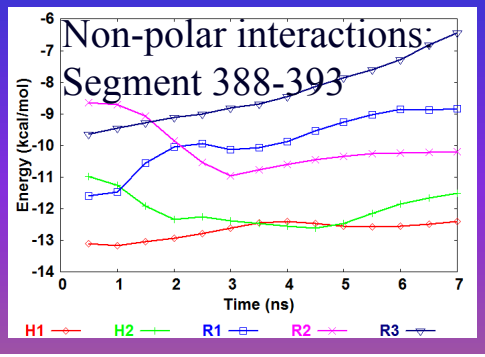
Human: **-55 kcal/mol** (np/p: -61/+6 kcal/mol)
Rat: **-46 kcal/mol** (np/p: -52/+6 kcal/mol)

- **The non-polar free-energy component is more negative in the human complex, reflecting better shape complementarity.**
- **The polar free-energy component is positive (opposing complex formation).**

Polar Interactions: Reflect the formation of specific interactions Involving polar/charged groups in the complex.

Human complex: -47 kcal/mol.
Rat complex: -36 kcal/mol.

Stronger in human complex, consistent with higher hydrogen-bond occupancies.



Tamamis et al, Proteins (2010)

Conclusions

- The simulations of the human complex reproduce the recently determined crystallographic structure and interactions and provide quantitative estimates of the interactions between the ligand and the protein.
- The simulations of the rat C3c complex provide an explanation for the lack of compstatin activity against non-primate C3: The rat C3 protein undergoes local conformational changes, which disrupt specific polar and non-polar interactions with compstatin and reduce the stability of the complex.
- Many of the rat C3c mutations are also observed in other non-primate mammals. Presumably, these mutations also contribute to the loss of compstatin affinity for C3c in these non-primate mammals.

Tamamis et al, Proteins (2010)