

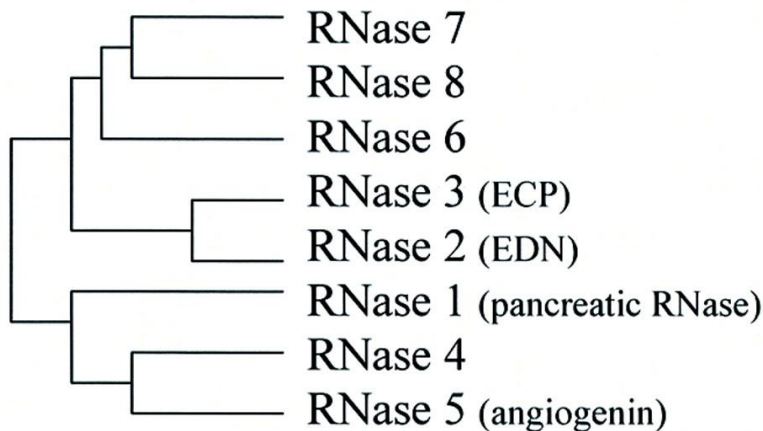
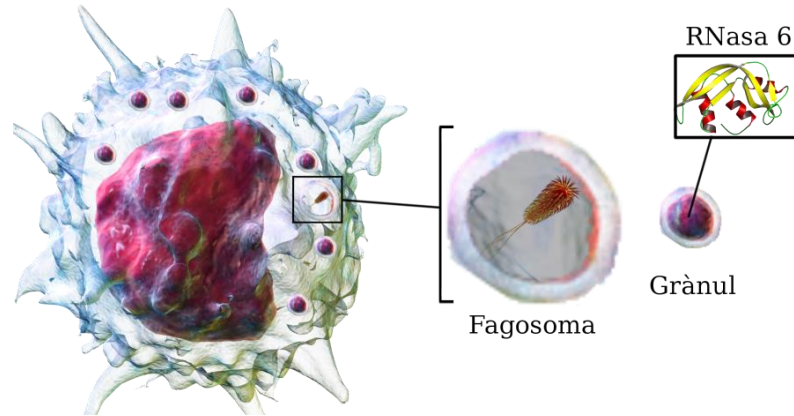
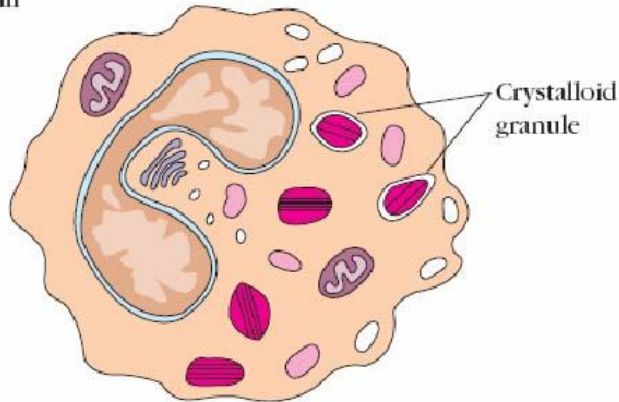
Human secretory RNases as multifaceted antimicrobial proteins

Ester Boix



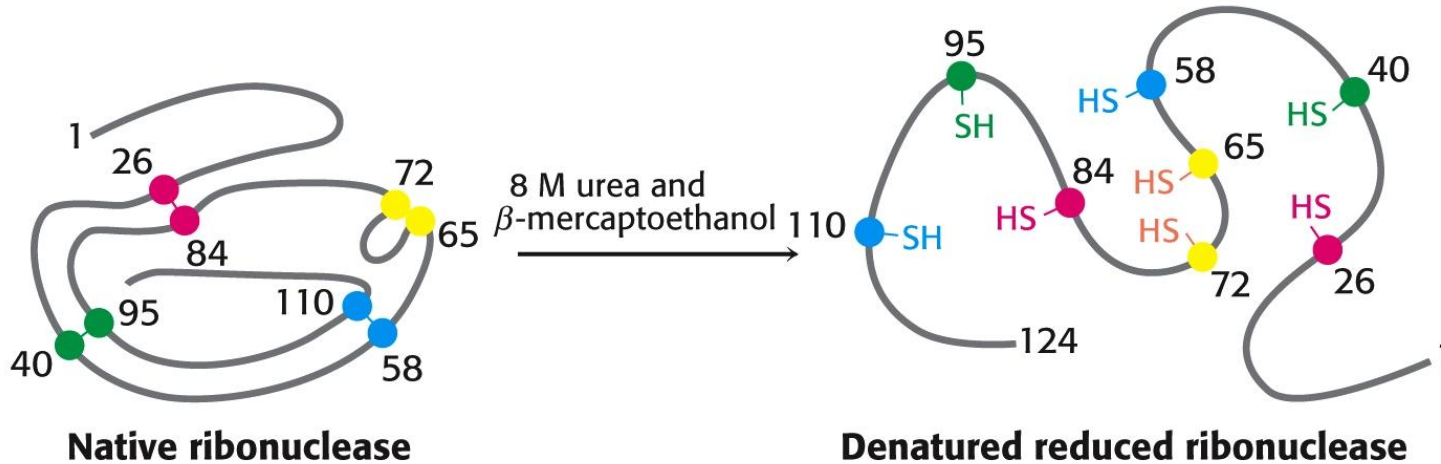
Human secretory RNases

Eosinophil

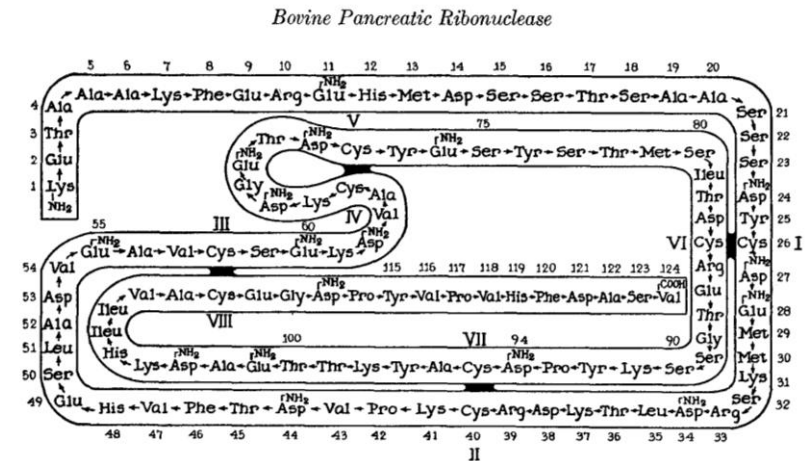


| Substrate | k_{cat} s^{-1} | K_m mM | k_{cat}/K_m $M^{-1} s^{-1}$ |
|-----------------------|--------------------------------|-----------------|----------------------------------|
| C>p | $(1.4 \pm 0.2) \times 10^{-2}$ | 1.5 ± 0.3 | 11 |
| U>p | $(4.3 \pm 0.4) \times 10^{-3}$ | 1.0 ± 0.2 | 4 |
| CpA | 4.2 ± 0.3 | 2.4 ± 0.3 | 1750 |
| UpA | 6.2 ± 0.4 | 5.4 ± 0.5 | 1150 |
| UpG | | | ND ^a |
| UpU>p | | | 38 ^b |
| (Up) ₂ U>p | 0.56 ± 0.03 | 1.4 ± 0.1 | 400 |
| (Up) ₃ U>p | 1.2 ± 0.2 | 0.7 ± 0.18 | 1714 |
| (Up) ₄ U>p | 1.4 ± 0.1 | 0.17 ± 0.02 | 8235 |

RNase A, the best studied enzyme

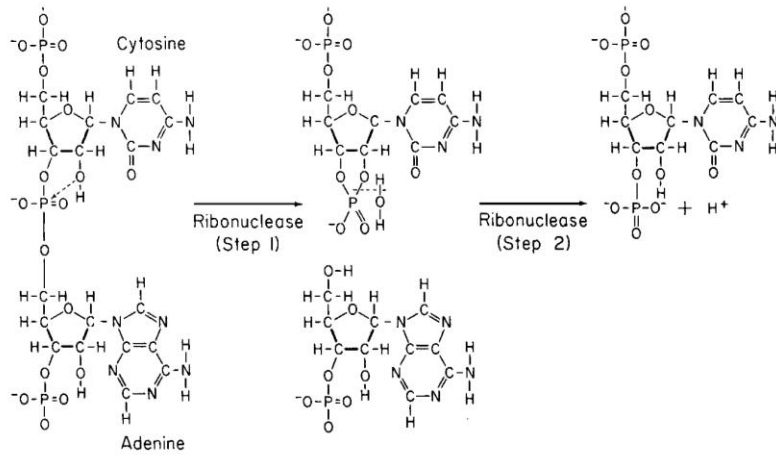


Christian Anfinsen. Nobel Lecture (1972).

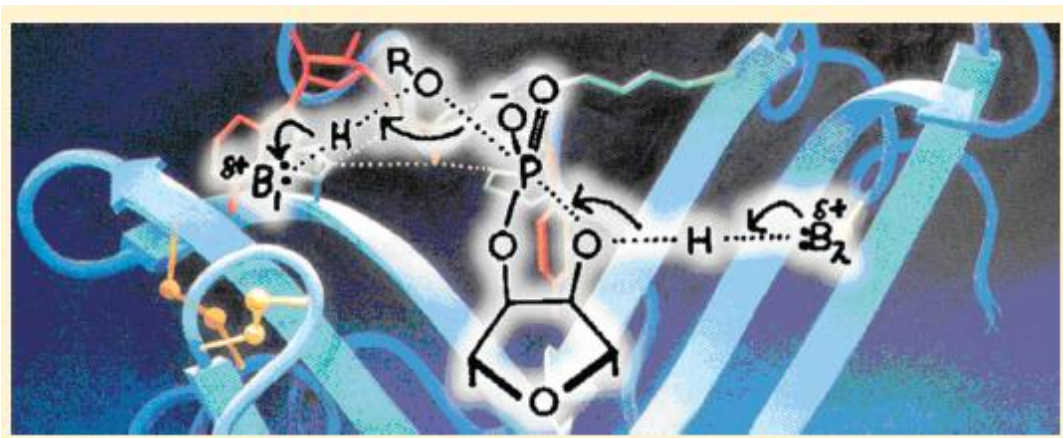


Bruce Merrifield. Nobel Lecture (1984).

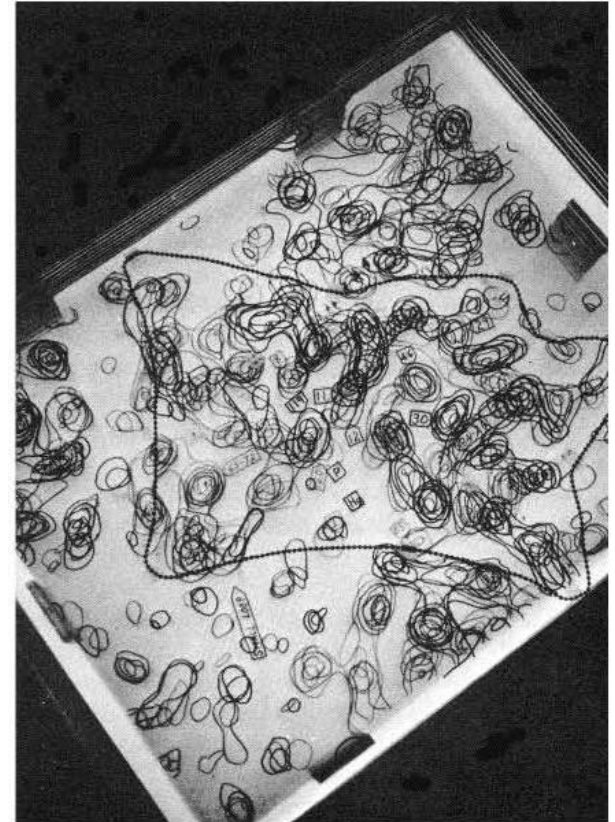
RNase A, the first catalytic mechanism



Standord Moore and William Stein. Nobel Lecture (1972).

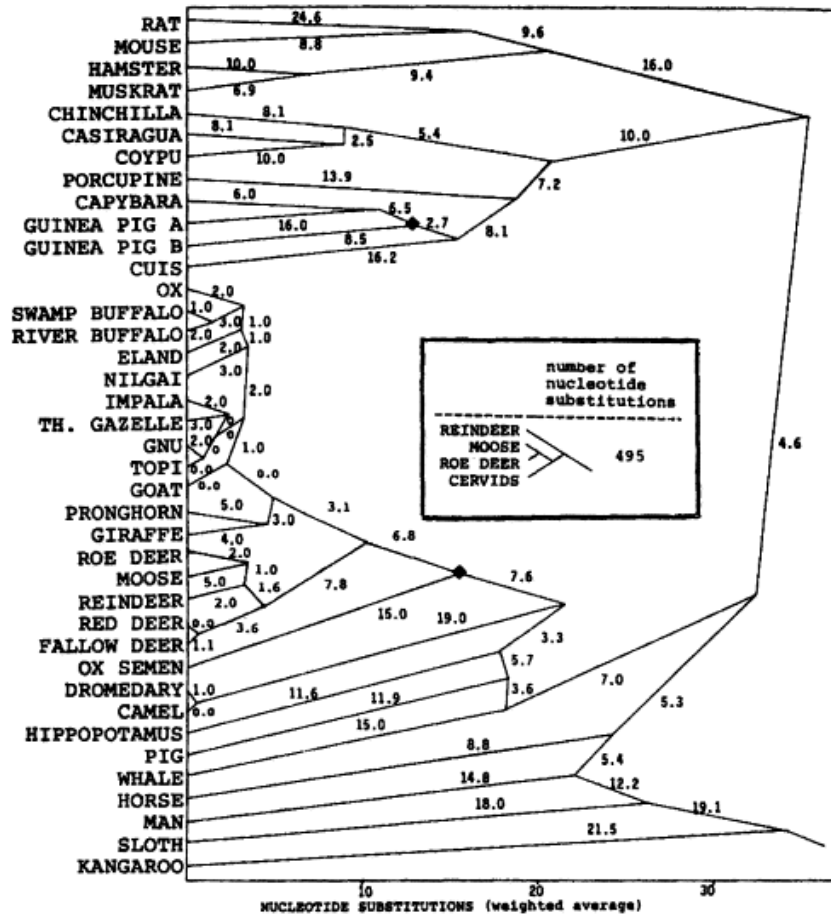


Cuchillo et al. Biochemistry (2011).

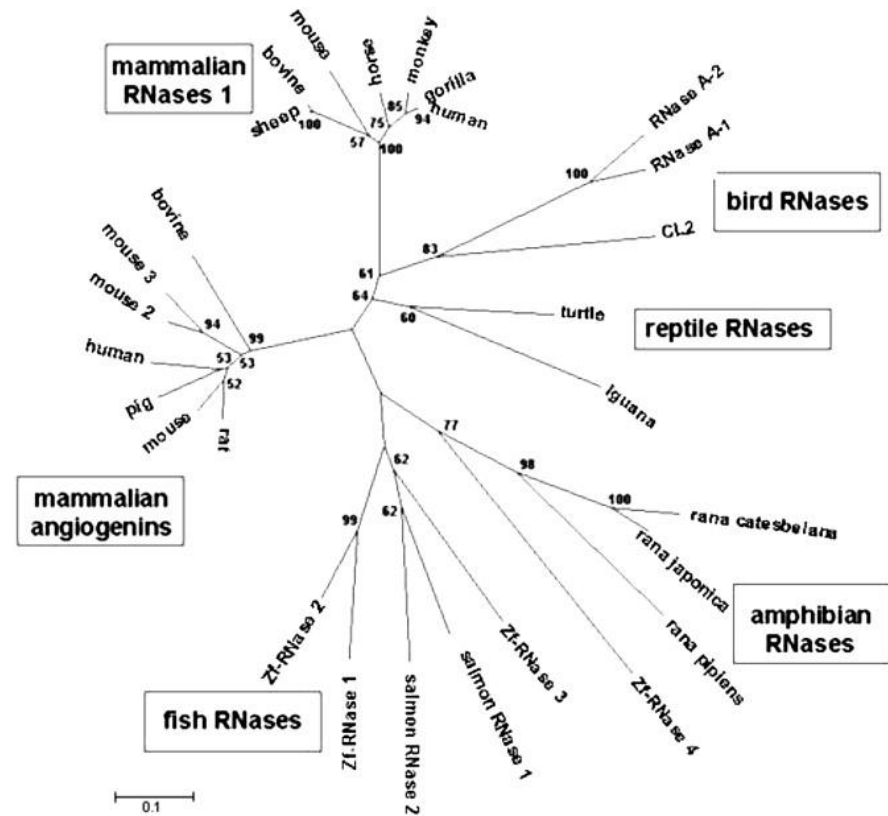


Wyckoff et al. *J. Biol. Chem.* (1967)

Pioneering evolution studies of mammalian RNases



Beintema et al. (1986) *Mol. Biol. Evol.* 3, 262



Pizzo and D'Alessio (2007)

An ancestral host defense role?

Zebrafish Ribonucleases Are Bactericidal: Implications for the Origin of the Vertebrate RNase A Superfamily

Soochin Cho and Jianzhi Zhang

Department of Ecology and Evolutionary Biology, University of Michigan



NIH Public Access Author Manuscript

J Leukoc Biol. Author manuscript; available in PMC 2009 June 8.

Published in final edited form as:

J Leukoc Biol. 2008 May ; 83(5): 1079–1087. doi:10.1189/jlb.1107725.

RNase A Ribonucleases and Host Defense: an Evolving Story

Helene F. Rosenberg*

Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health. Bethesda. Maryland 20892

NIH-PA Author Man

REVIEW

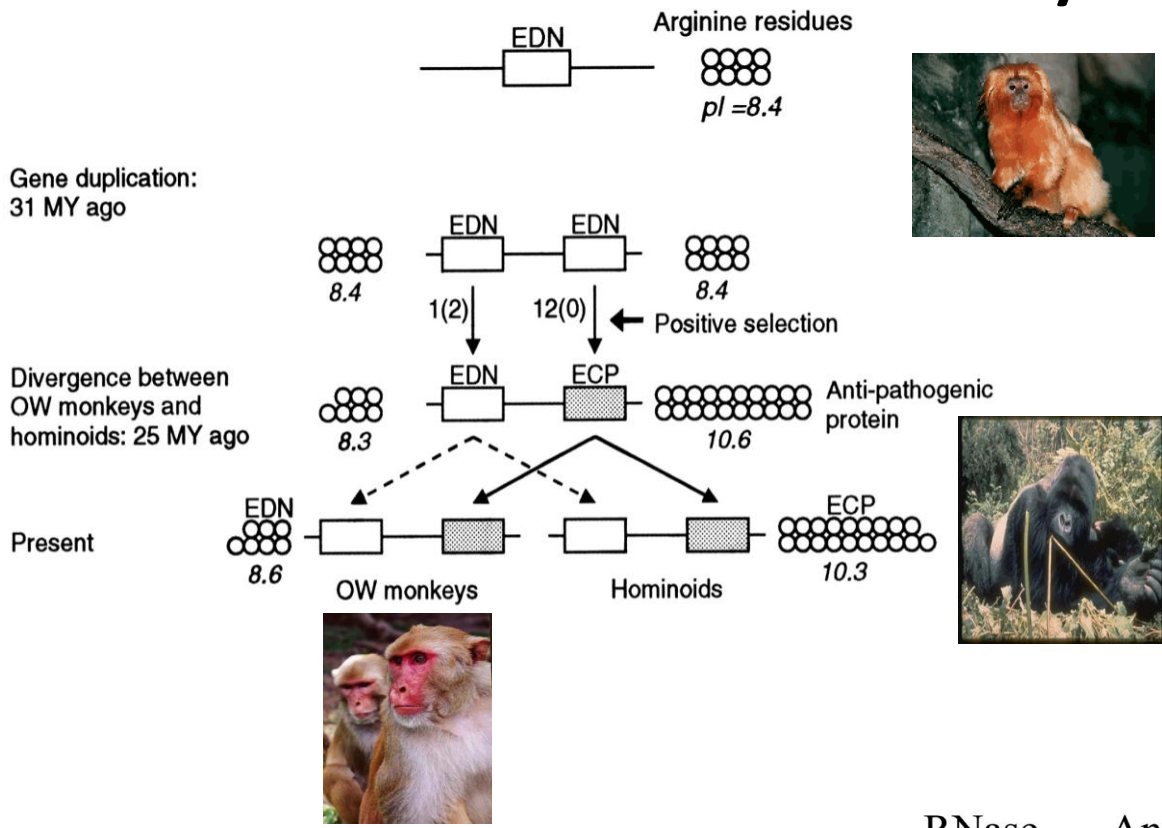
www.rsc.org/molecularbiosystems | Molecular BioSystems

Mammalian antimicrobial proteins and peptides: overview on the RNase A superfamily members involved in innate host defence

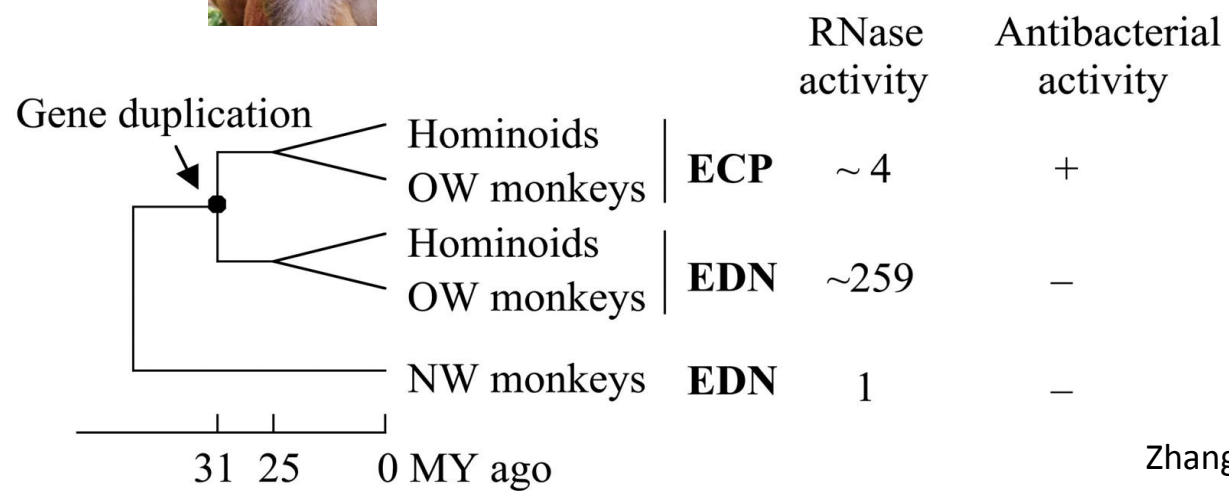
Ester Boix* and M. Victòria Nogués



Cationization versus catalytic efficiency

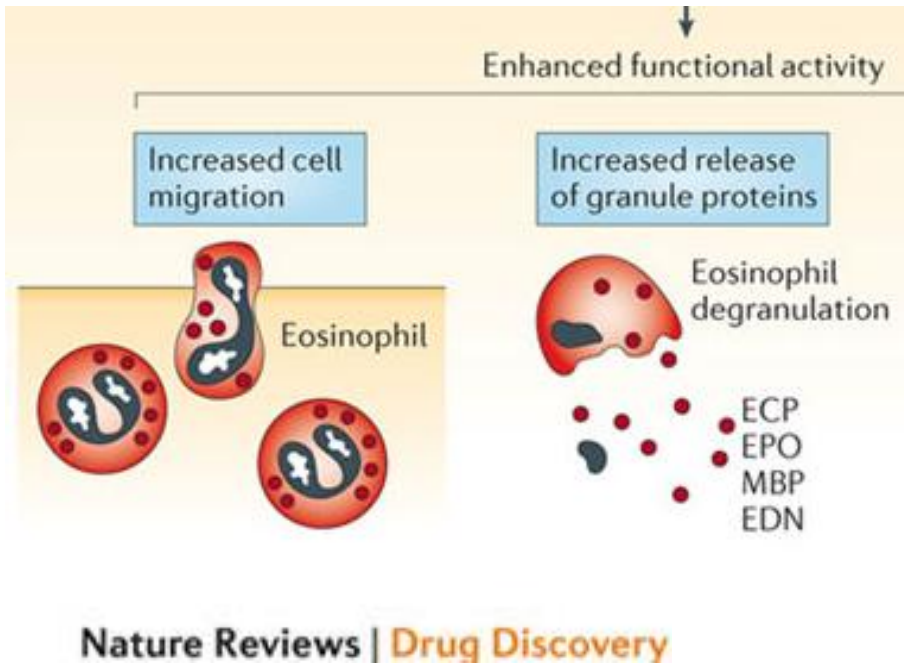


Zhang et al., *PNAS* (1998)

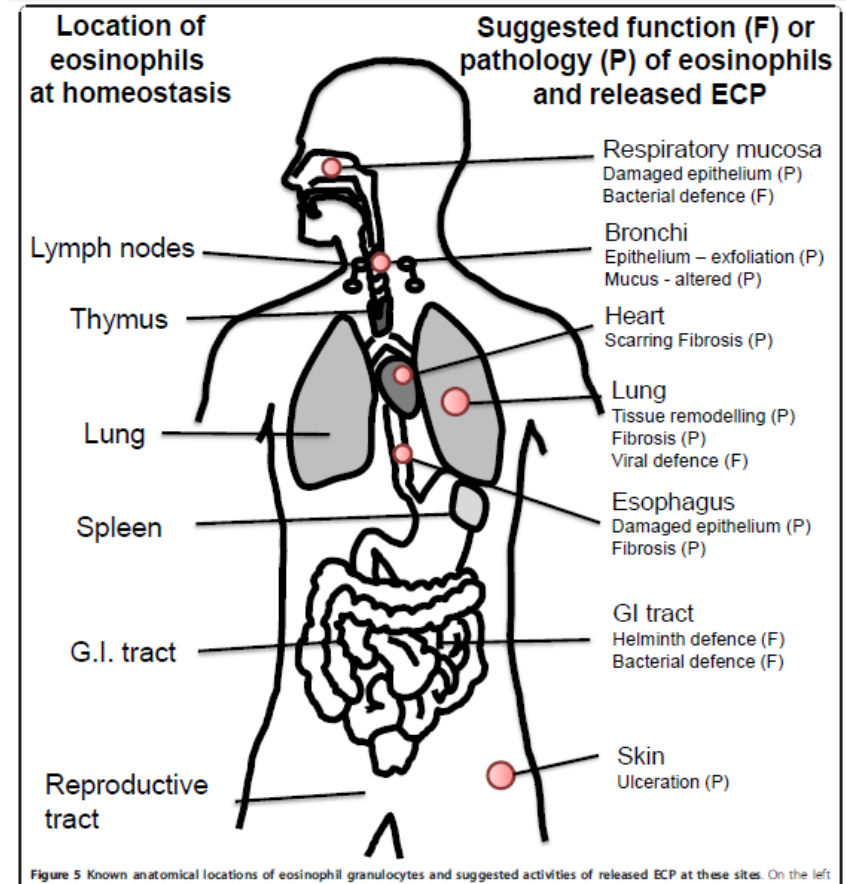


Zhang et al. *PNAS* (2002)

hRNases in host defense

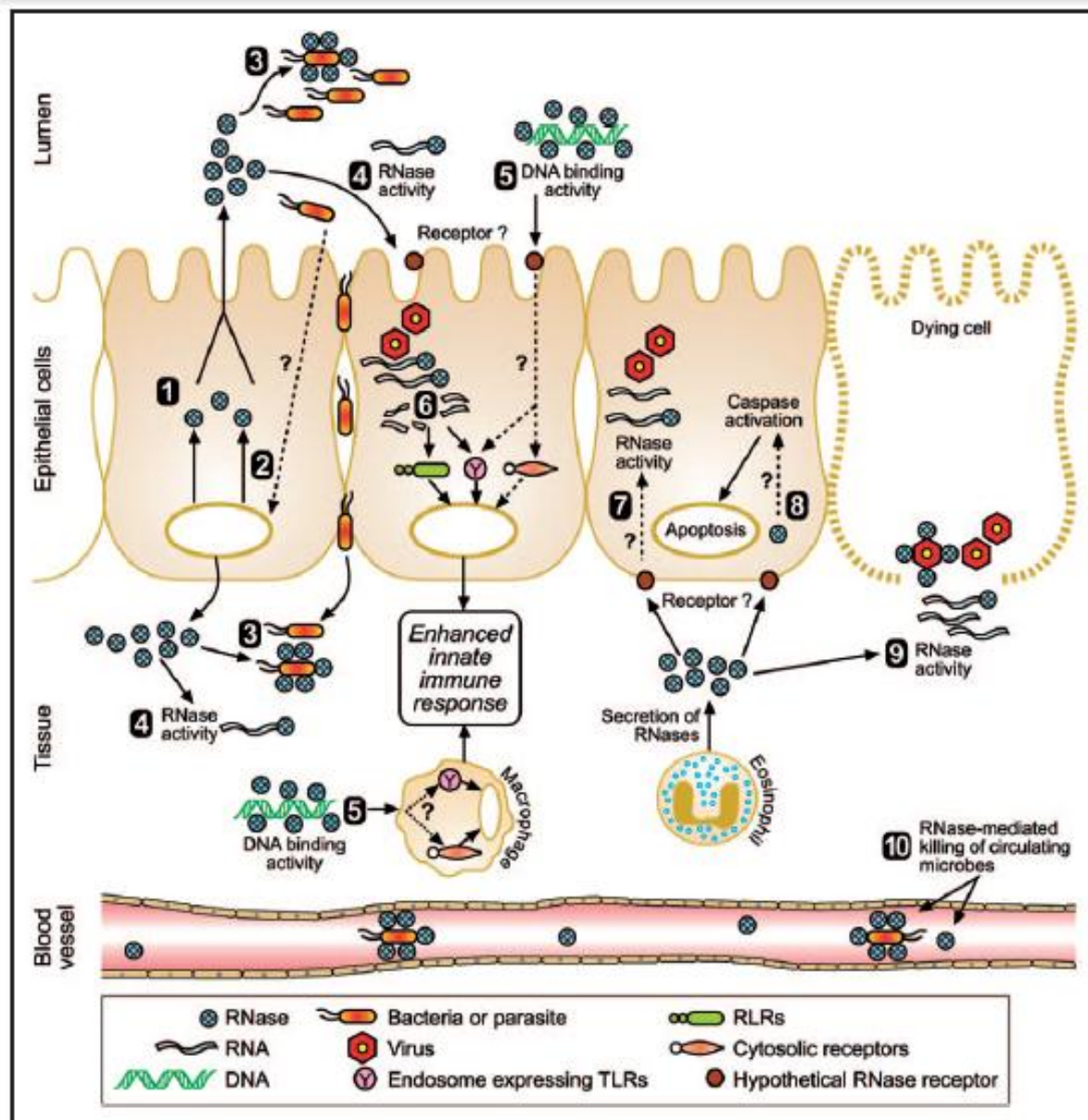


Fulkerson and Rothenberg *Nature Reviews* 2013



Bystrom et al. 2011

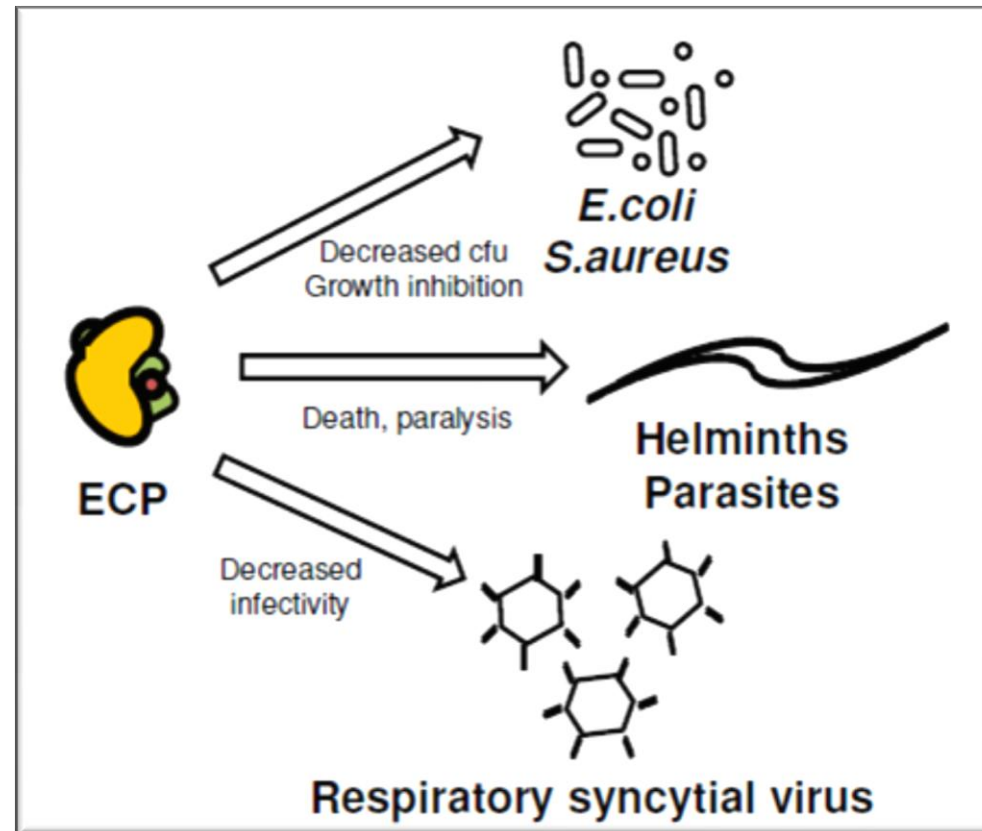
Proposed mechanisms for secretory RNases



Gupta and Wheeler, 2013

hRNases antipathogen activities

- Helminths:
 - *Schistosoma mansoni*, *Brugia malayi*, ...
- Protozoa:
 - *Leishmania*, *Trypanosoma cruzi*, *T. brucei*, and *Plasmodium*
- Bacteria:
 - *E. coli*, *S. aureus*, *Mycobacterium*,...
- Viruses:
 - RSV,..

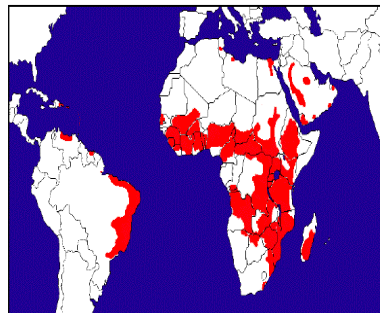


hRNases antiparasite activities

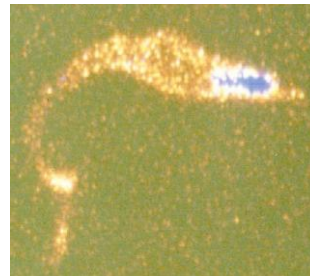
Schistosomiasis



Schistosoma mansoni



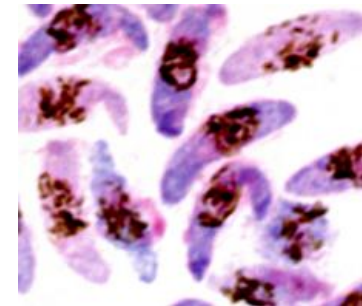
Chagas disease



Trypanosoma



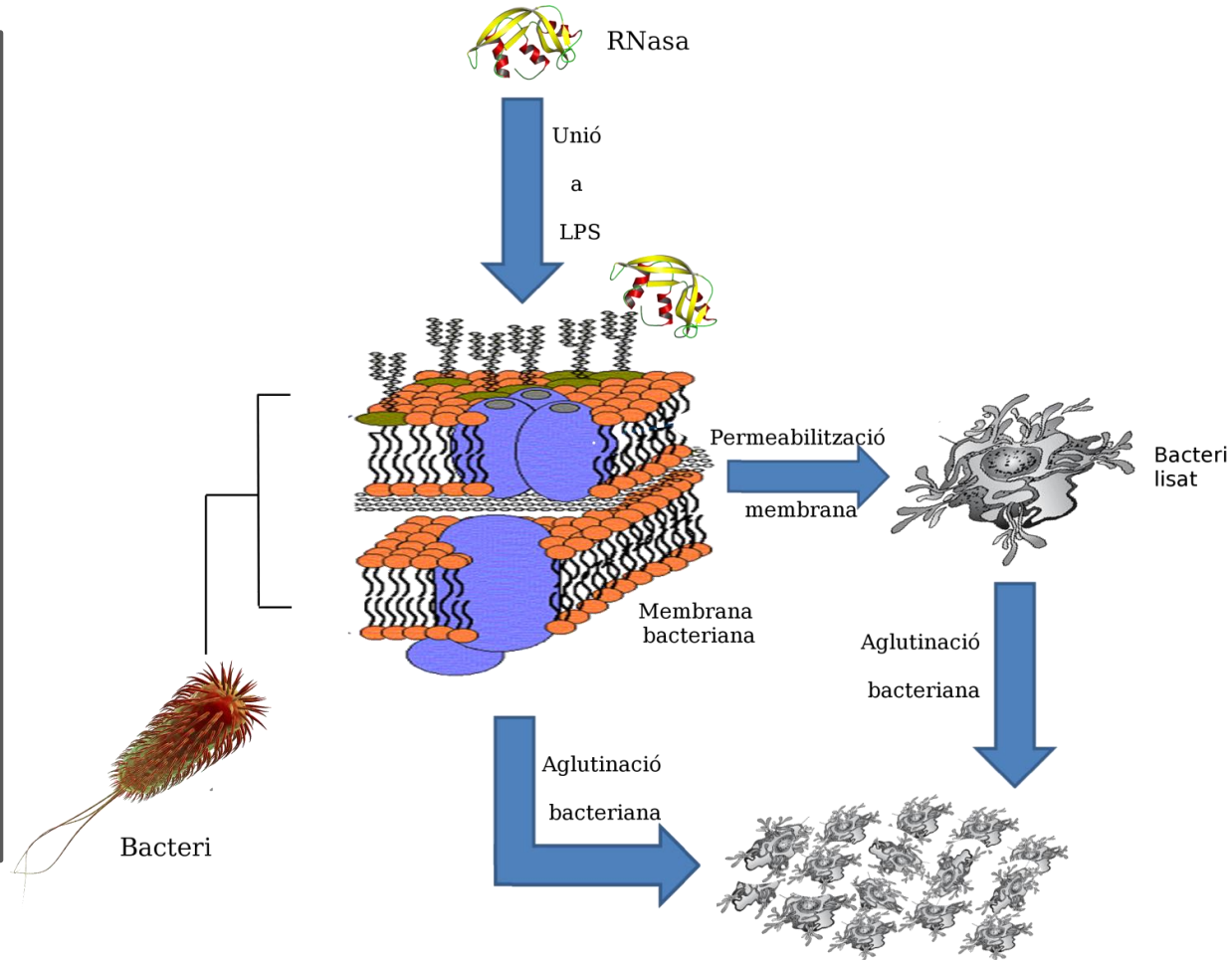
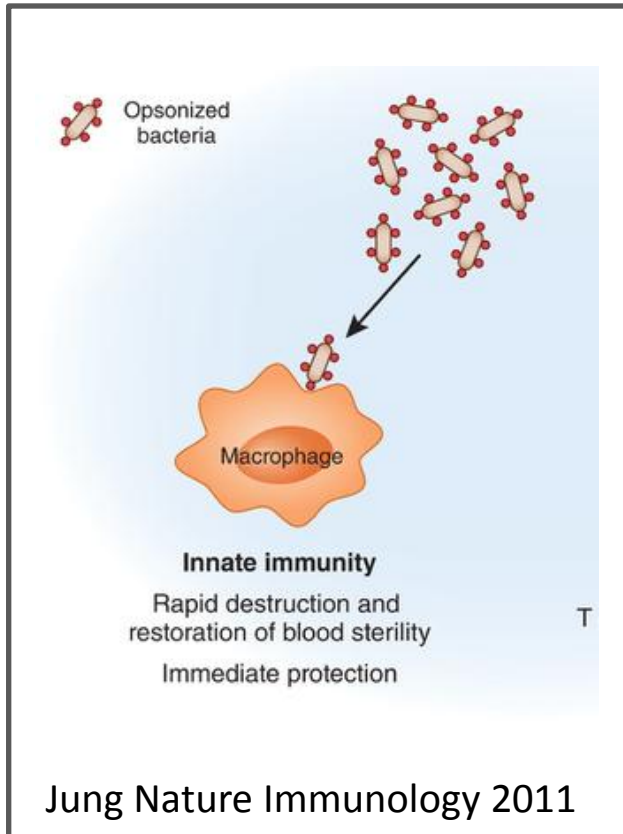
Malaria



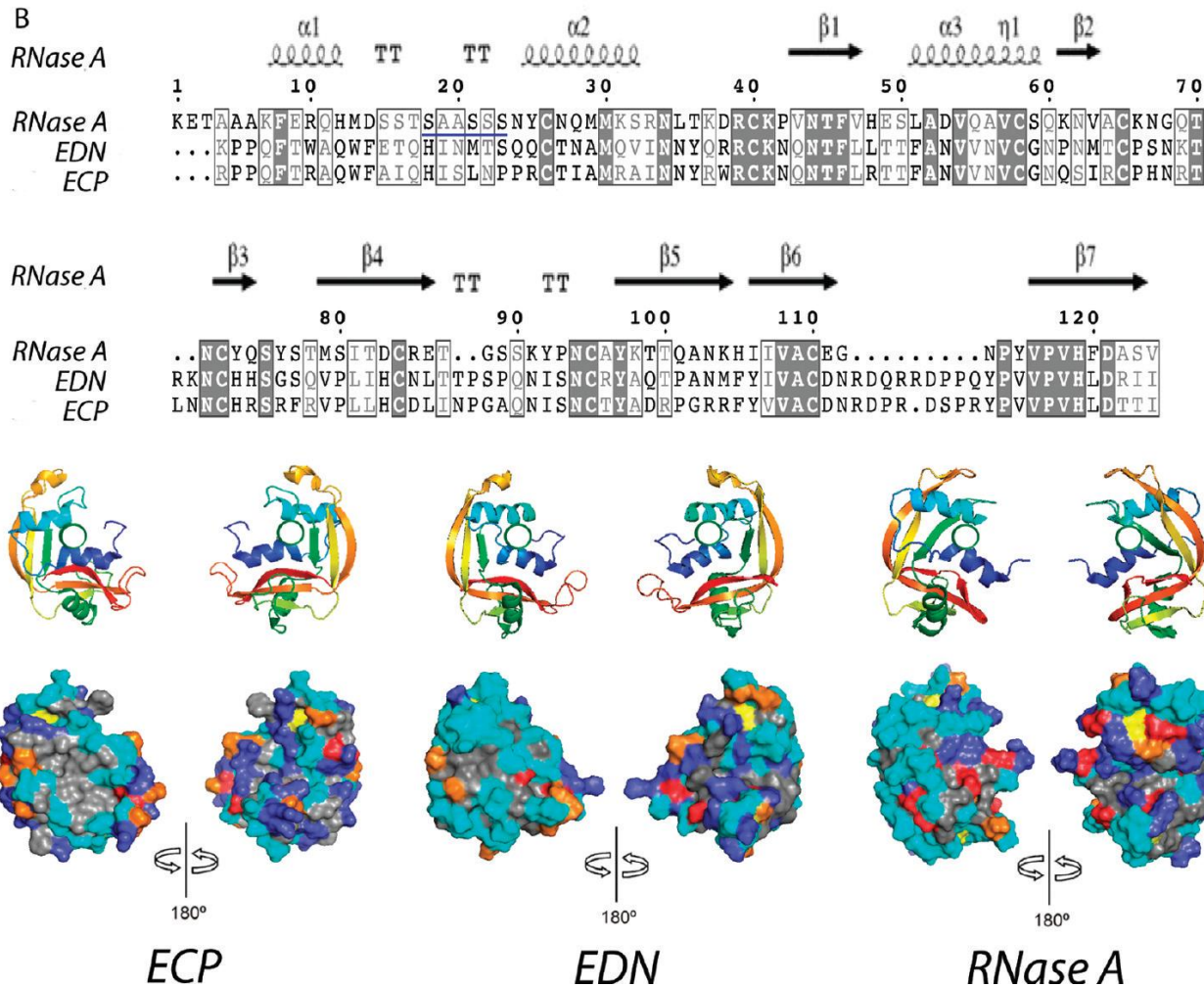
Plasmodium falciparum



hRNases bactericidal activity

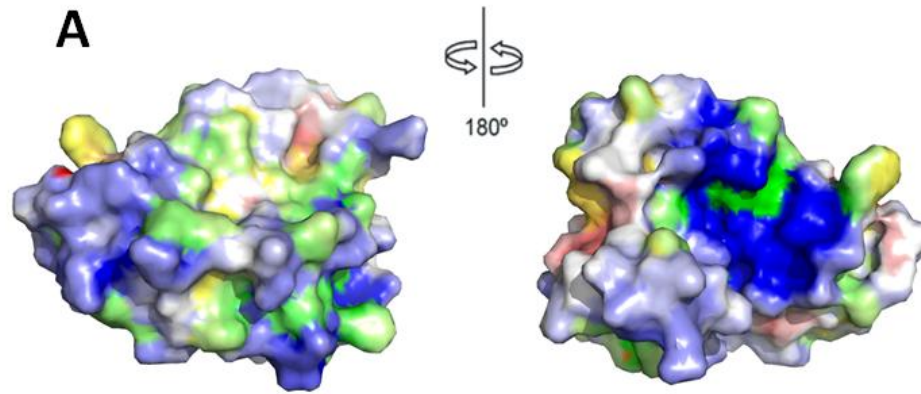


Structural determinants for antimicrobial proteins

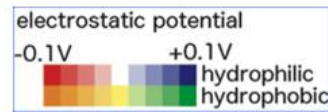


Dissecting hRNases properties

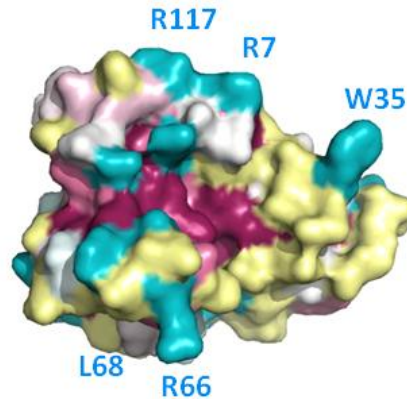
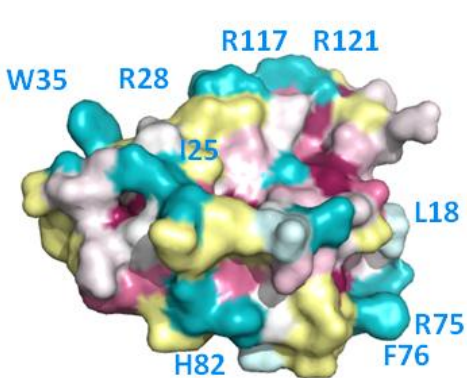
hRNase 3



pI ~ 11



B

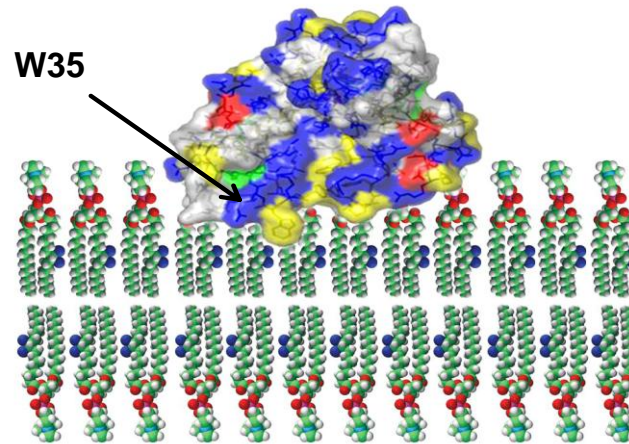


| | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| : | I | A | M | R | A | I | N | N | Y | R | W | R | C | K | N | Q | N | T | F | L | R | T | T | F |
| : | I | A | M | R | V | I | N | N | Y | Q | R | R | C | K | N | Q | N | T | F | L | R | T | T | F |
| : | T | A | M | R | V | I | N | N | Y | Q | R | R | C | K | D | Q | N | T | F | L | R | T | T | F |
| I | N | A | M | Q | V | I | N | N | F | Q | R | R | C | K | N | Q | N | T | F | L | R | T | T | F |
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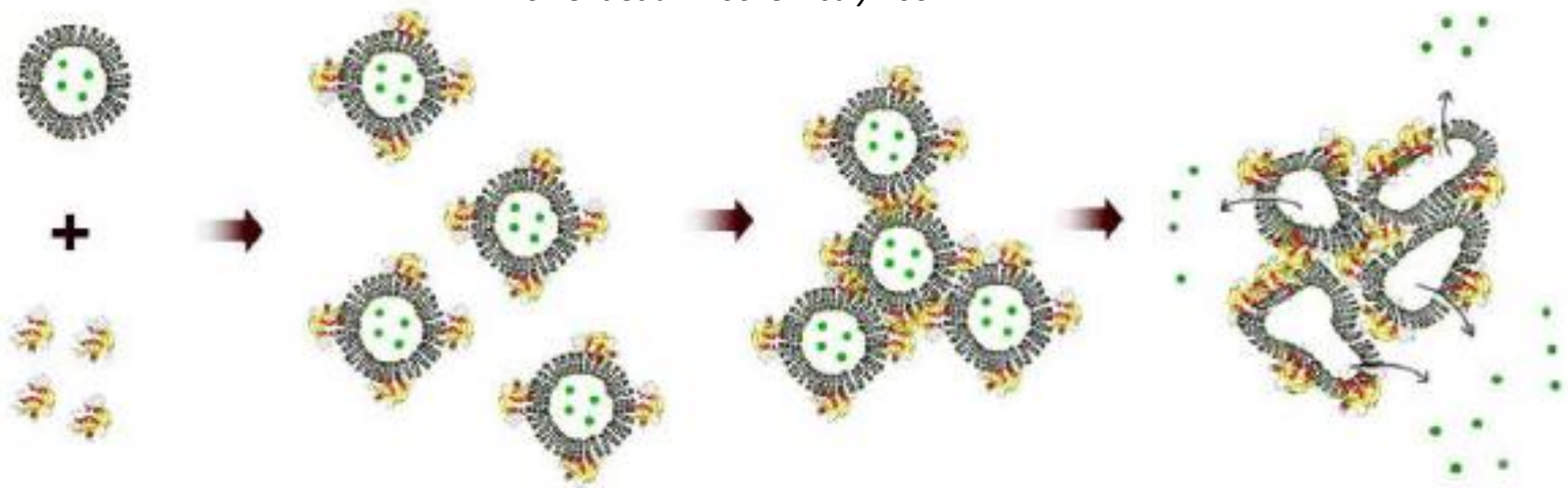


Conservation score

hRNases action on model membranes

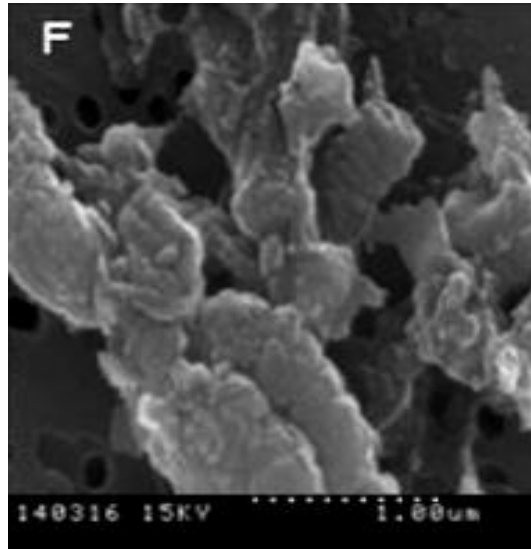


Torrent et al. *Biochemistry* 2007

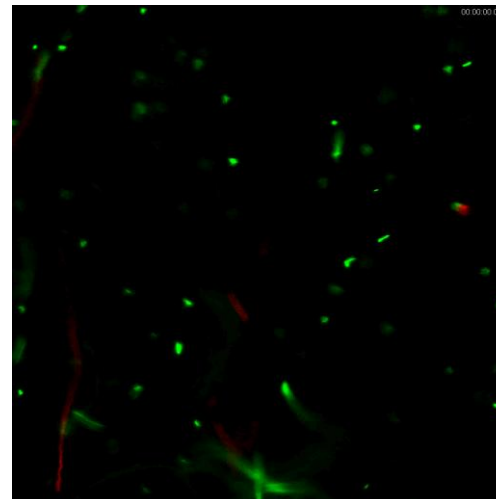
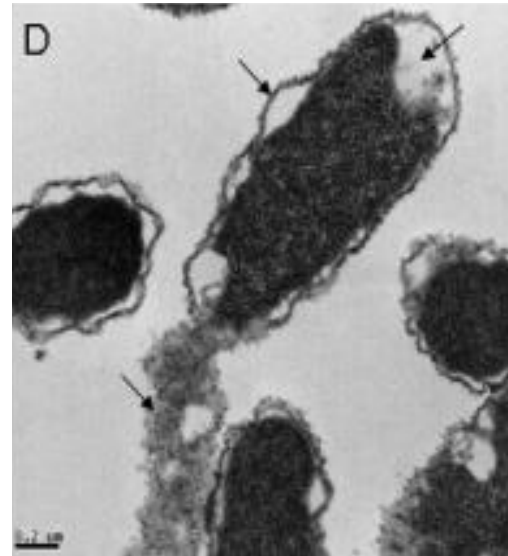


Torrent et al. *Biochim Biophys Acta*, 2009

hRNase 3 action on bacteria envelope

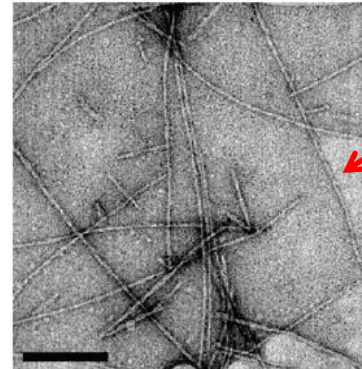
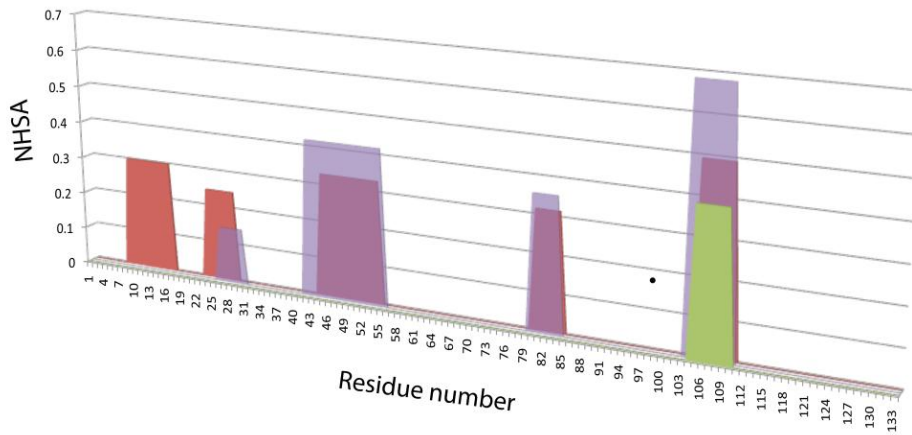
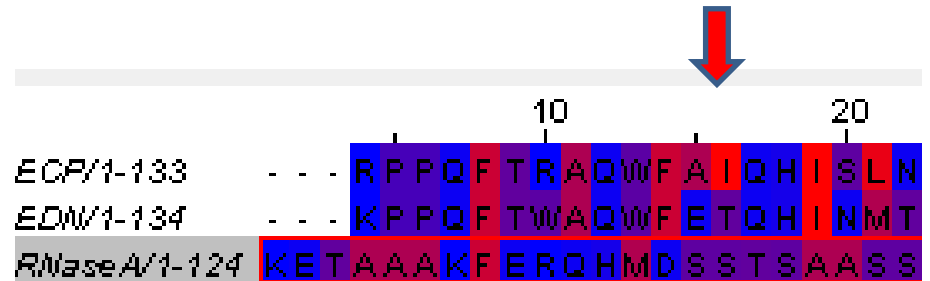
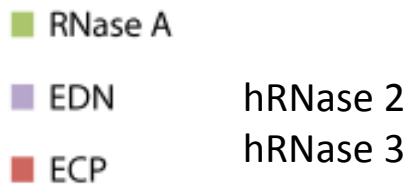


Torrent et al., *Biochemistry* 2008



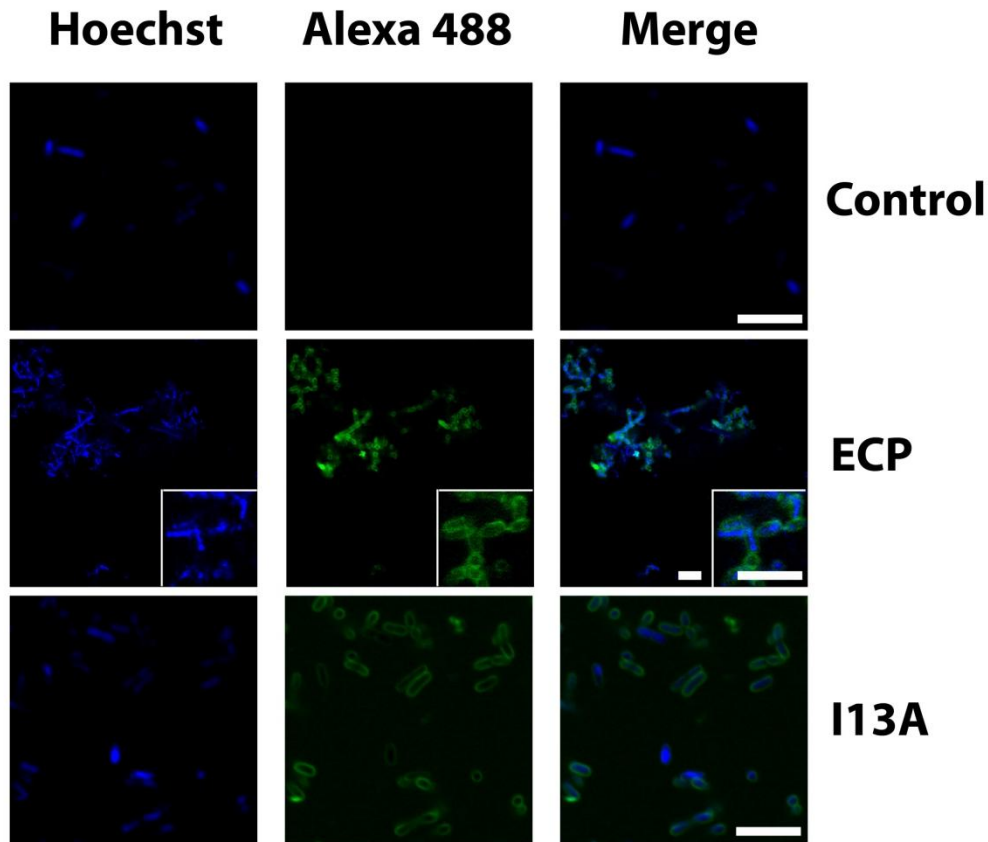
Torrent et al., *FEBSJ* 2010

An hydrophobic patch promotes hRNase 3 self-aggregation



ECP(1-19) peptide

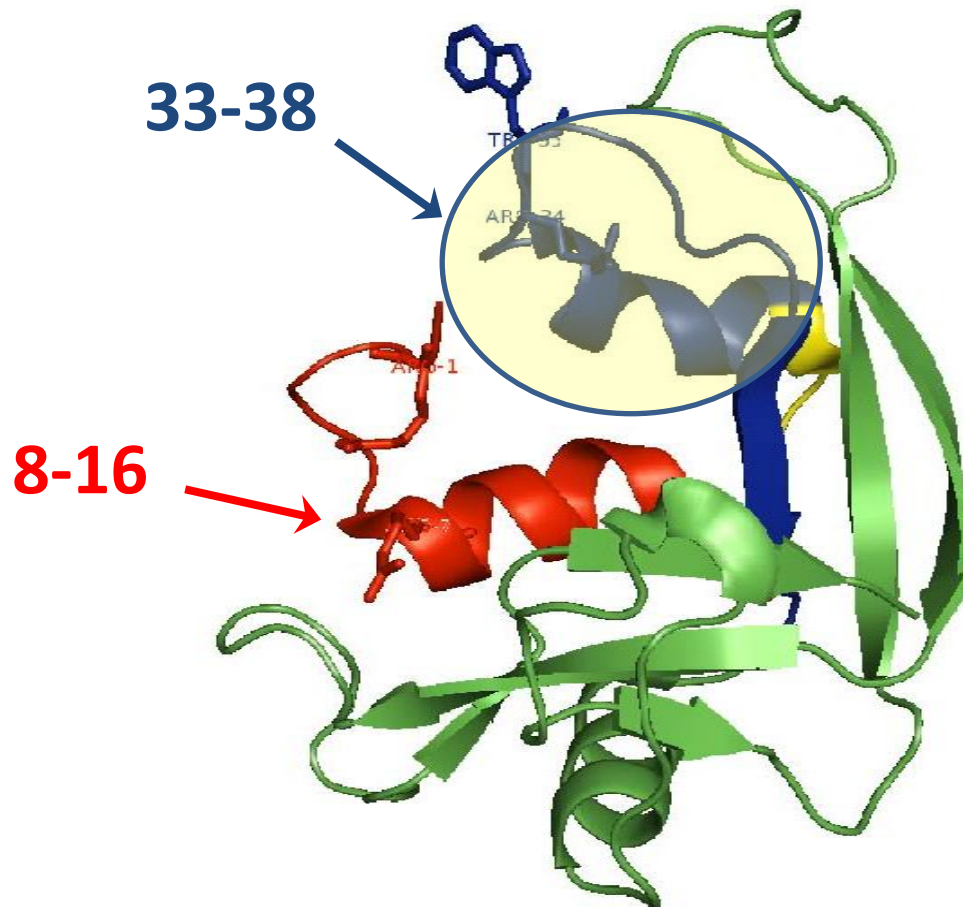
Protein self- aggregation triggers bacteria agglutination



| | MIC ₁₀₀ (μM) | |
|----------------------|-------------------------------|------|
| | Phosphate buffer ^a | |
| | ECP | I13A |
| <i>E. coli</i> | 0.40±0.10 | >5 |
| <i>P. aeruginosa</i> | 0.60±0.15 | >5 |
| <i>A. baumannii</i> | 0.75±0.15 | >5 |

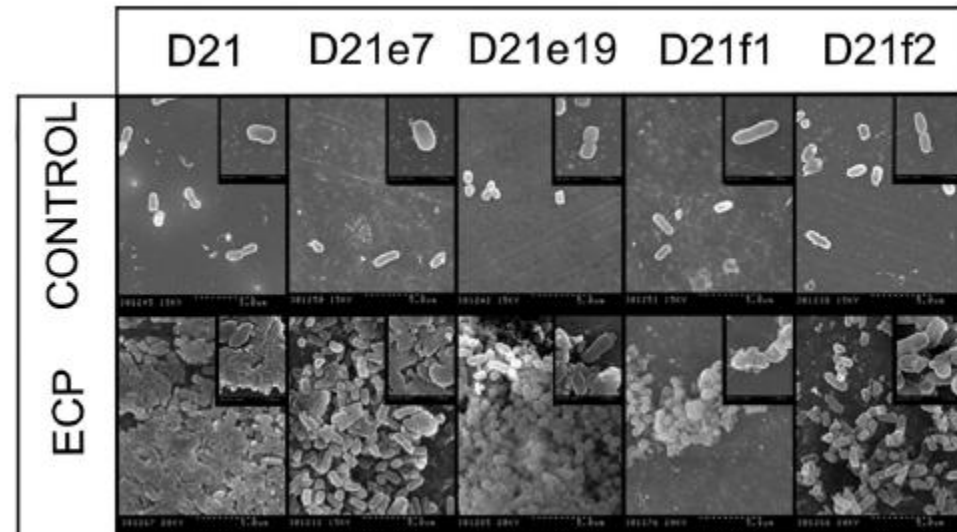
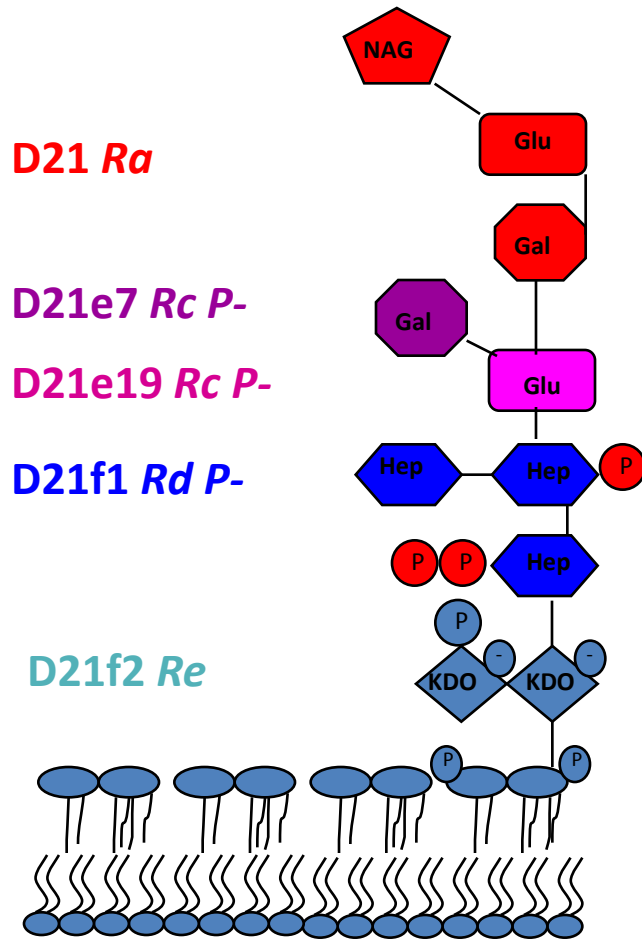
| | MAC (μM) | |
|----------------------|-------------------------------|------|
| | Phosphate buffer ^a | |
| | ECP | I13A |
| <i>E. coli</i> | 0.25±0.1 | >5 |
| <i>P. aeruginosa</i> | 0.5±0.1 | >5 |
| <i>A. baumannii</i> | 1.0±0.2 | >5 |

A domain involved in hRNase 3 binding to heterosaccharides



RPPQFTR**AQWFAIQHI**SLNPPRSTIAMRAINNY**RWR**SKNQNTFLR

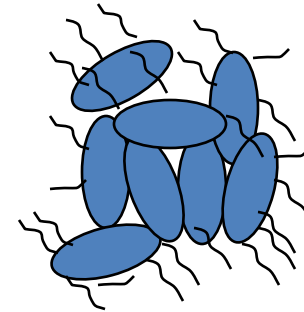
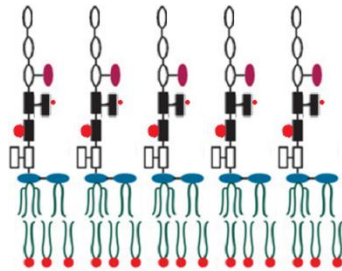
Structural determinants for hRNase 3 binding to lipopolysaccharides



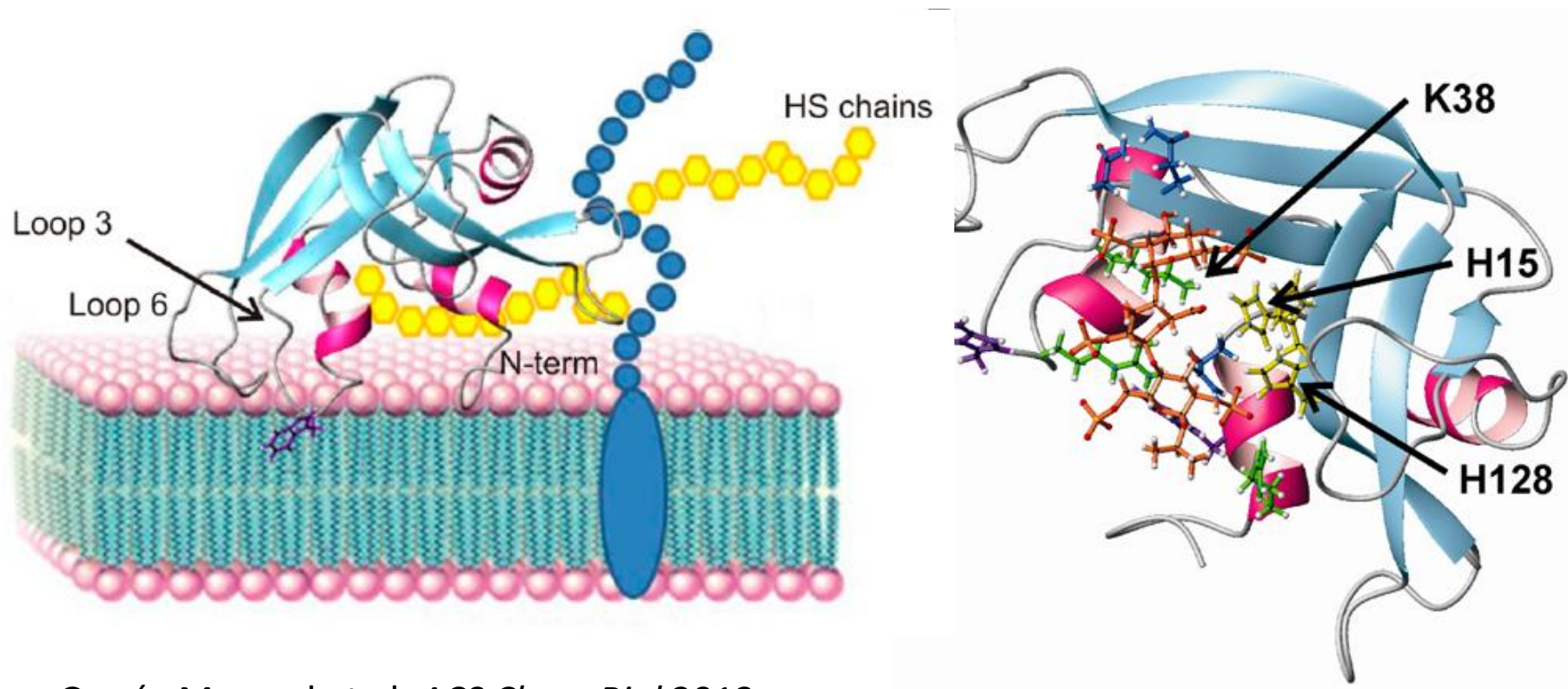
E. coli k12 mutant strains

Pulido et al. AAC 2012

Binding to lipopolysaccharides triggers bacteria agglutination

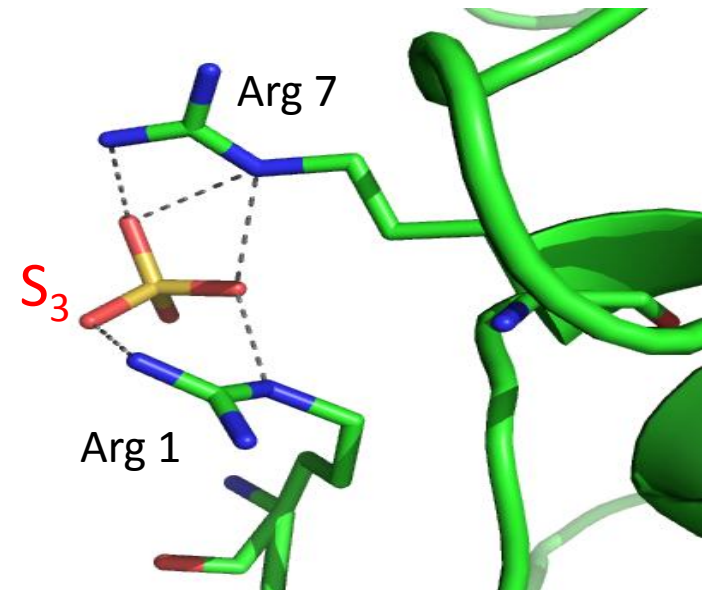
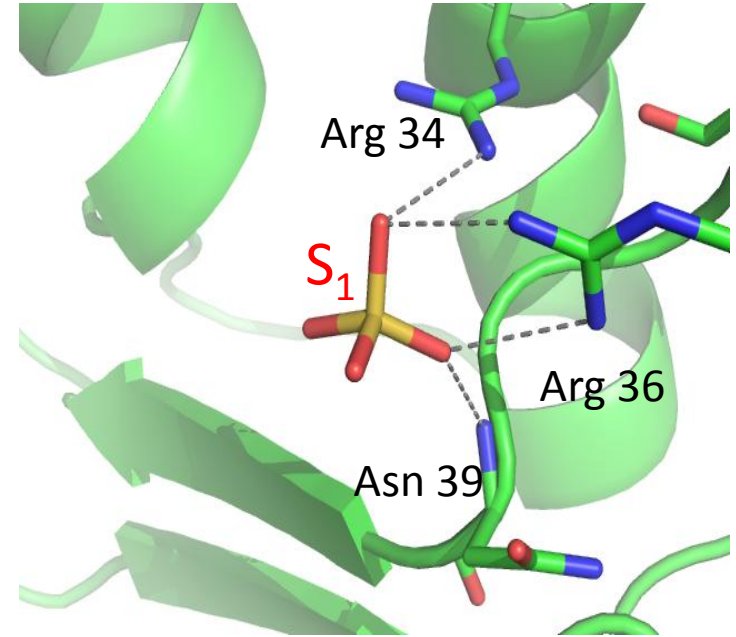
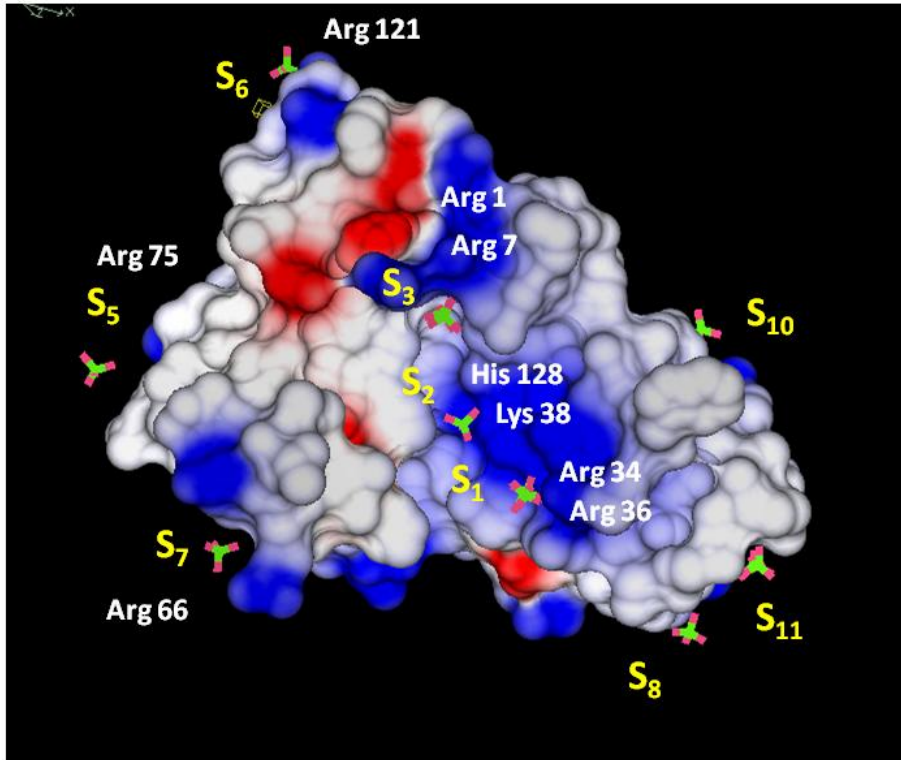


Binding to glycosaminoglycans



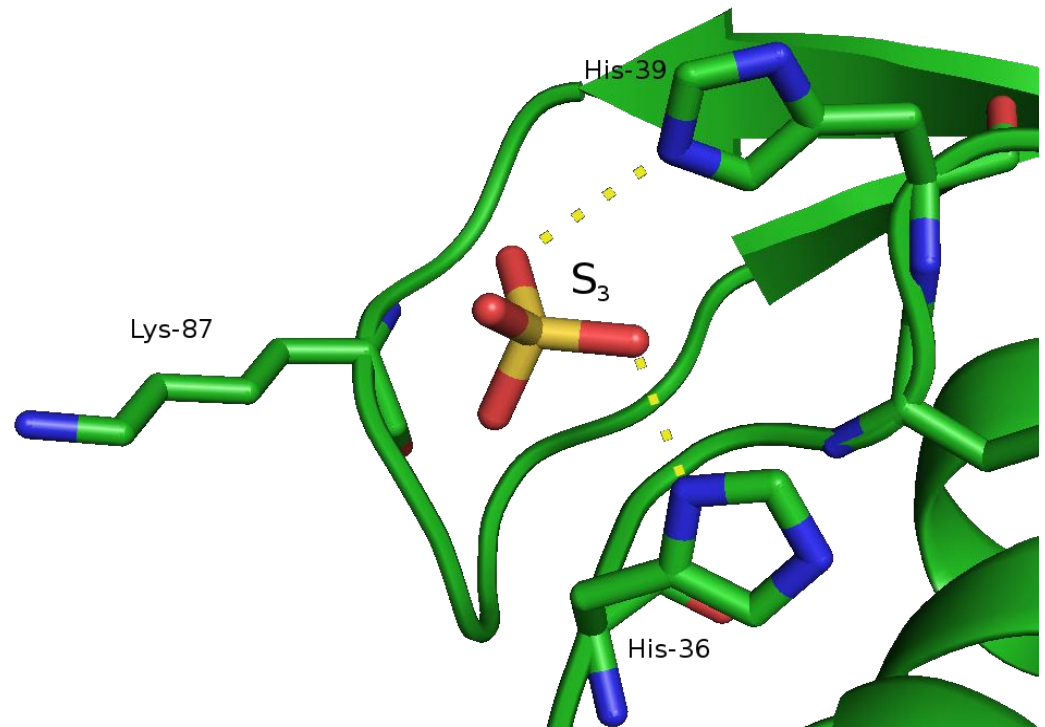
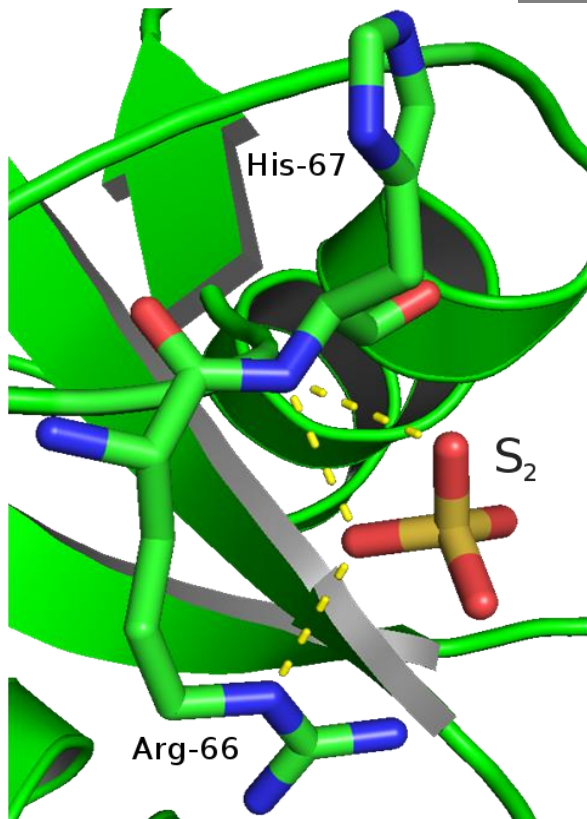
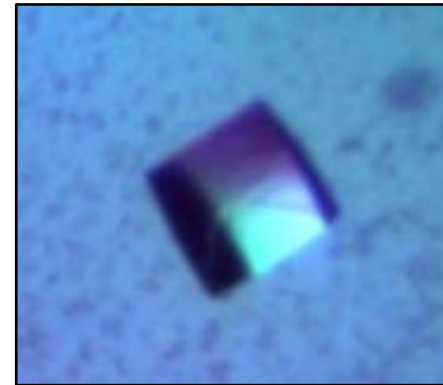
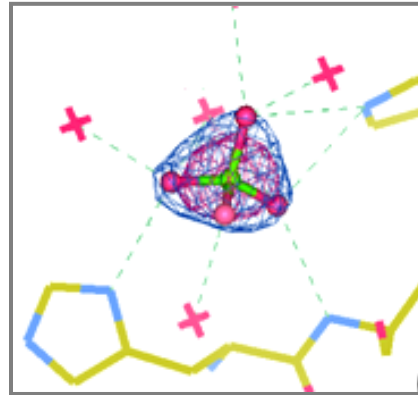
García-Mayoral et al. *ACS Chem Biol* 2013

hRNase 3 – SO₄ complex structure

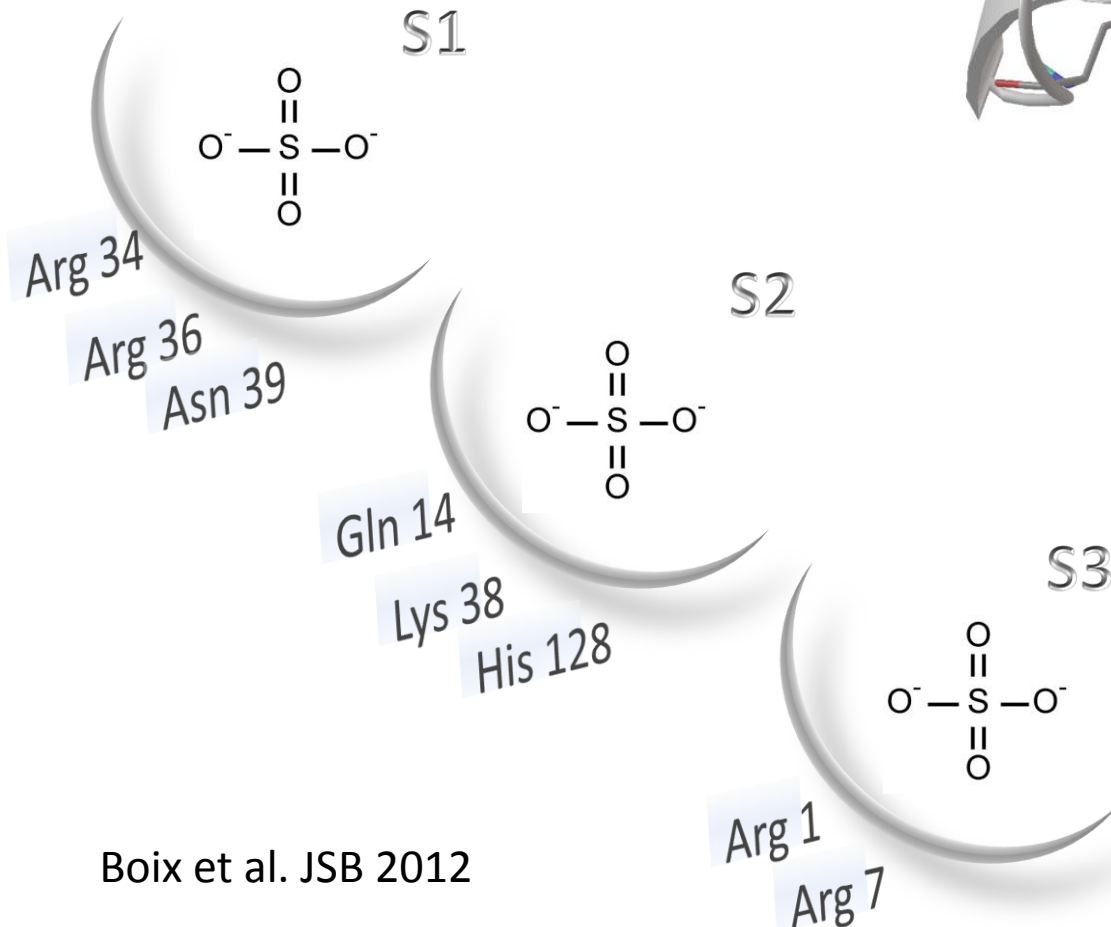


Boix et al. JSB 2012

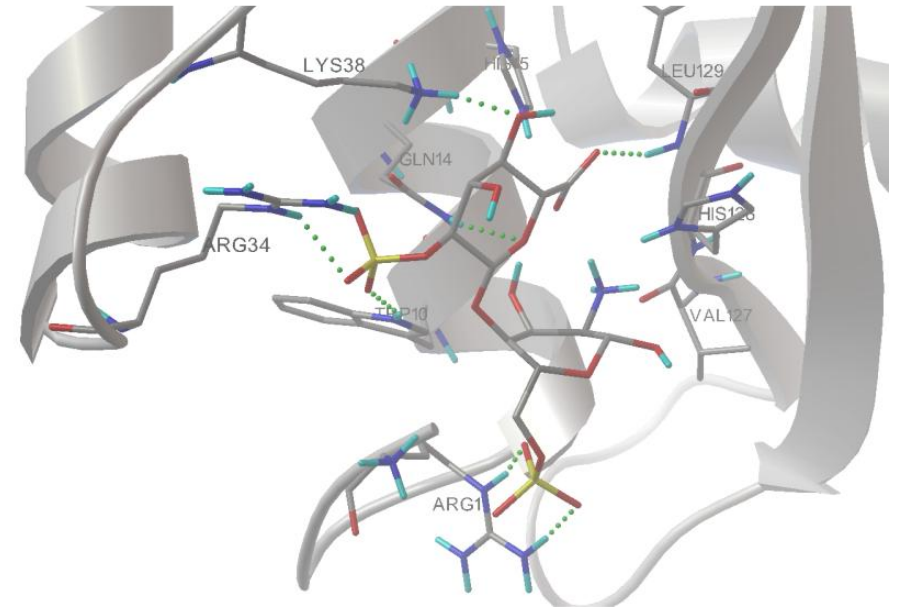
hRNase 6 – SO₄ complex structure



hRNase 3 binding to sulfated GAGs

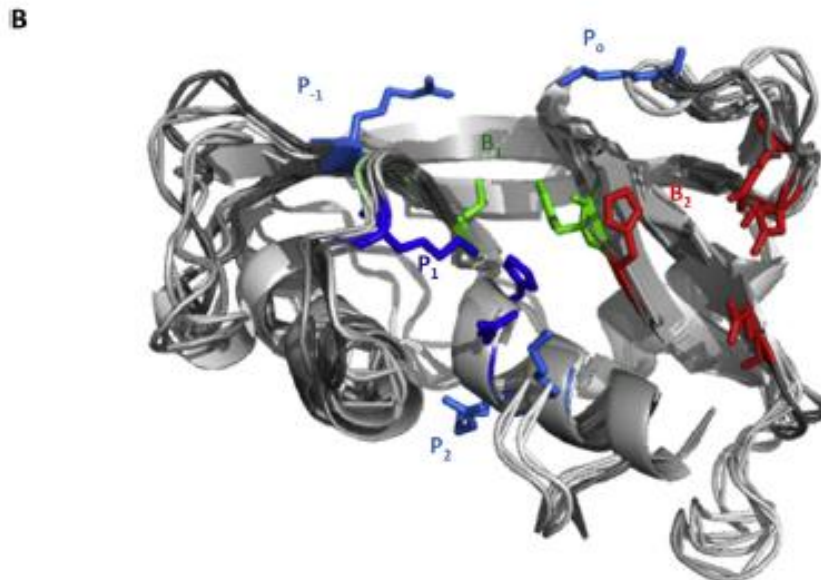
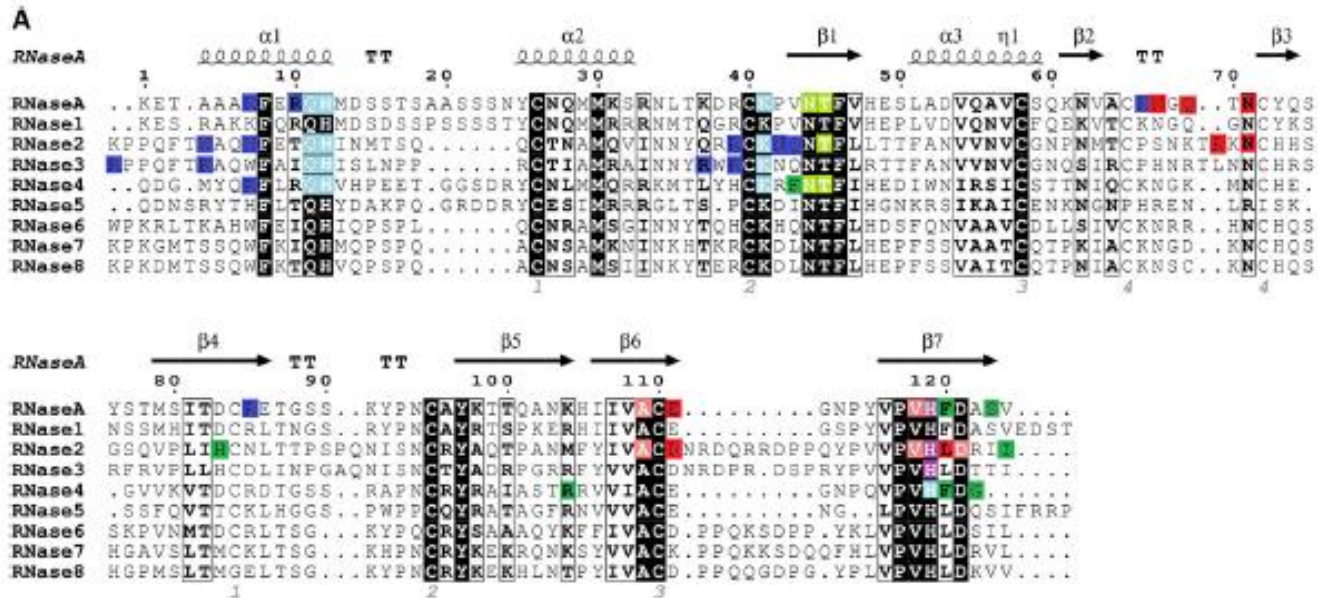


Boix et al. JSB 2012

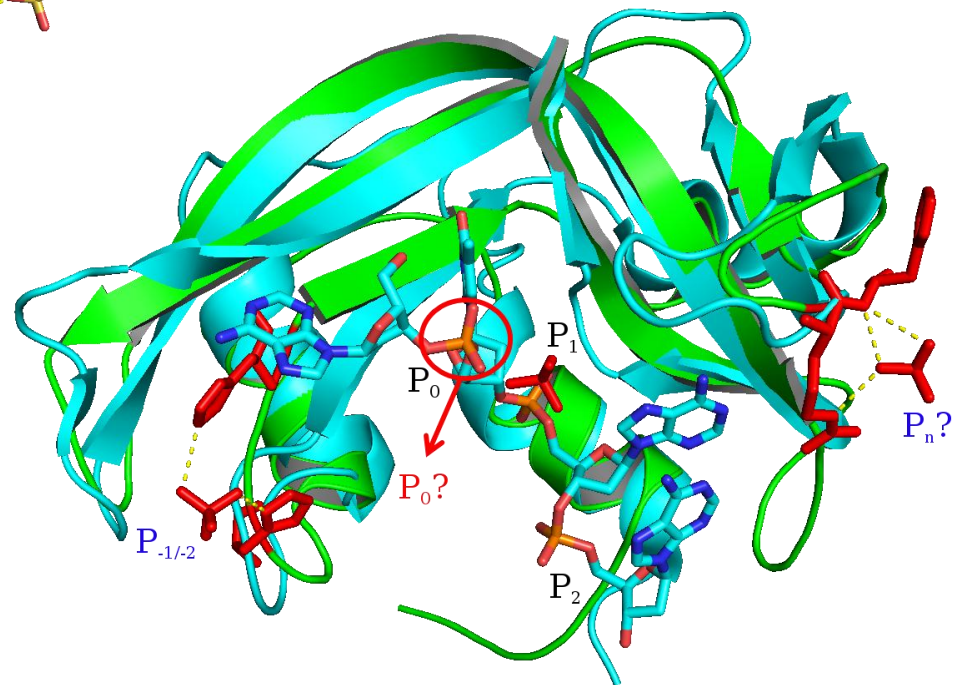
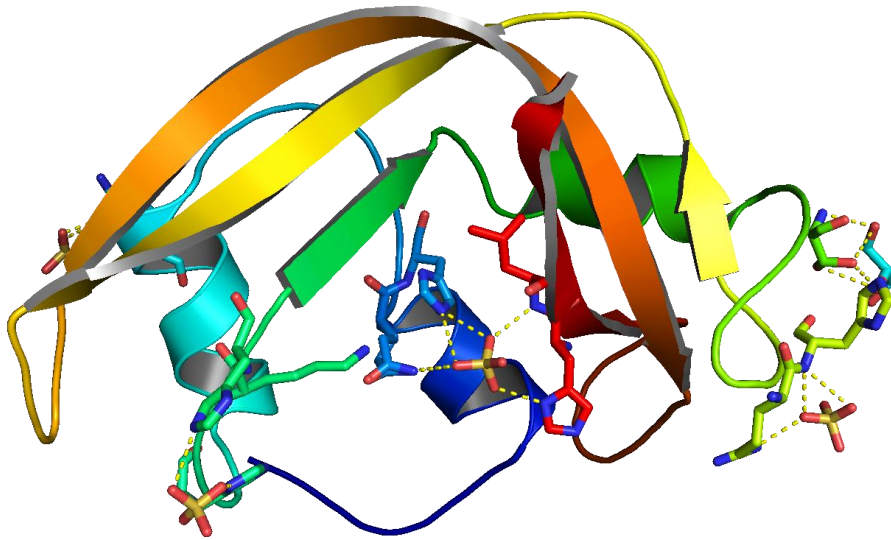


Torrent et al. JMR 2010

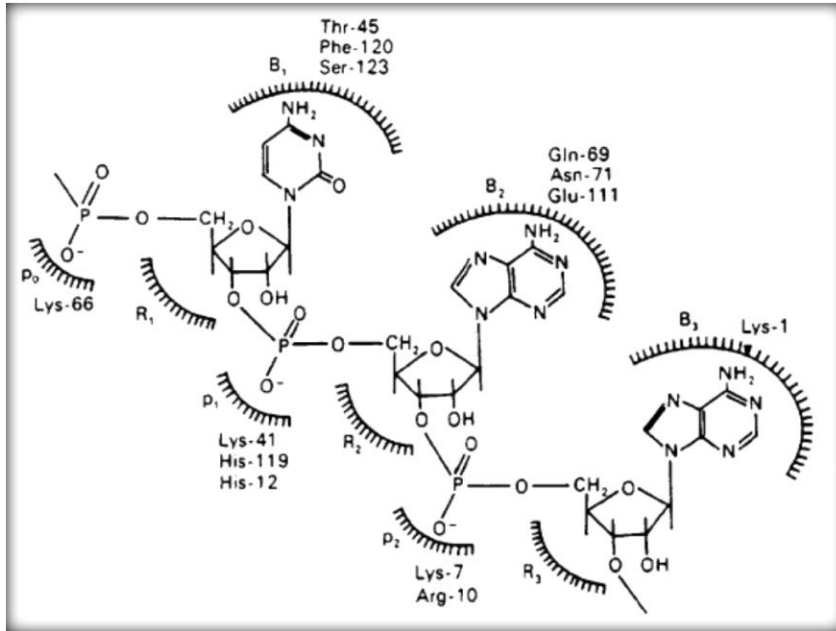
Further exploring hRNases binding sites



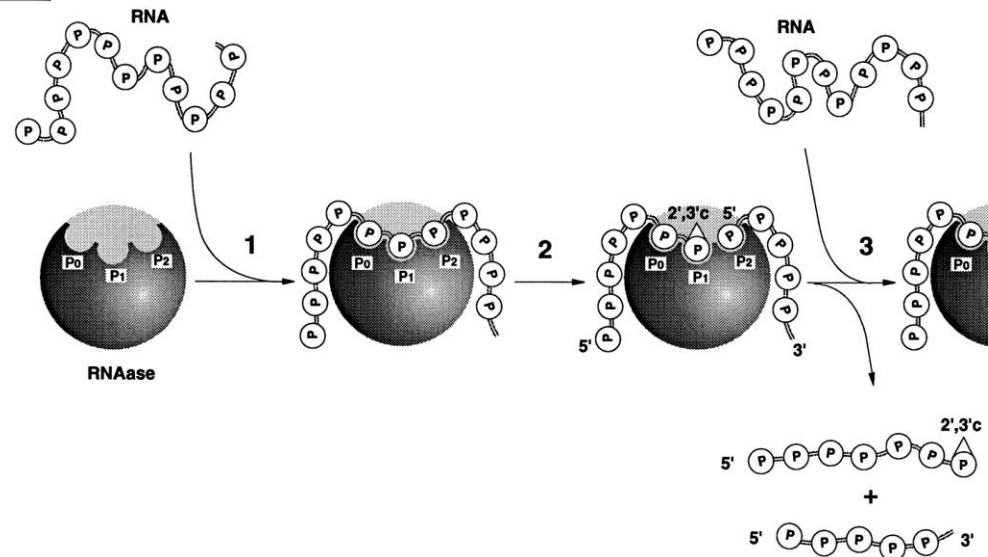
hRNase 6 nucleotide binding pattern



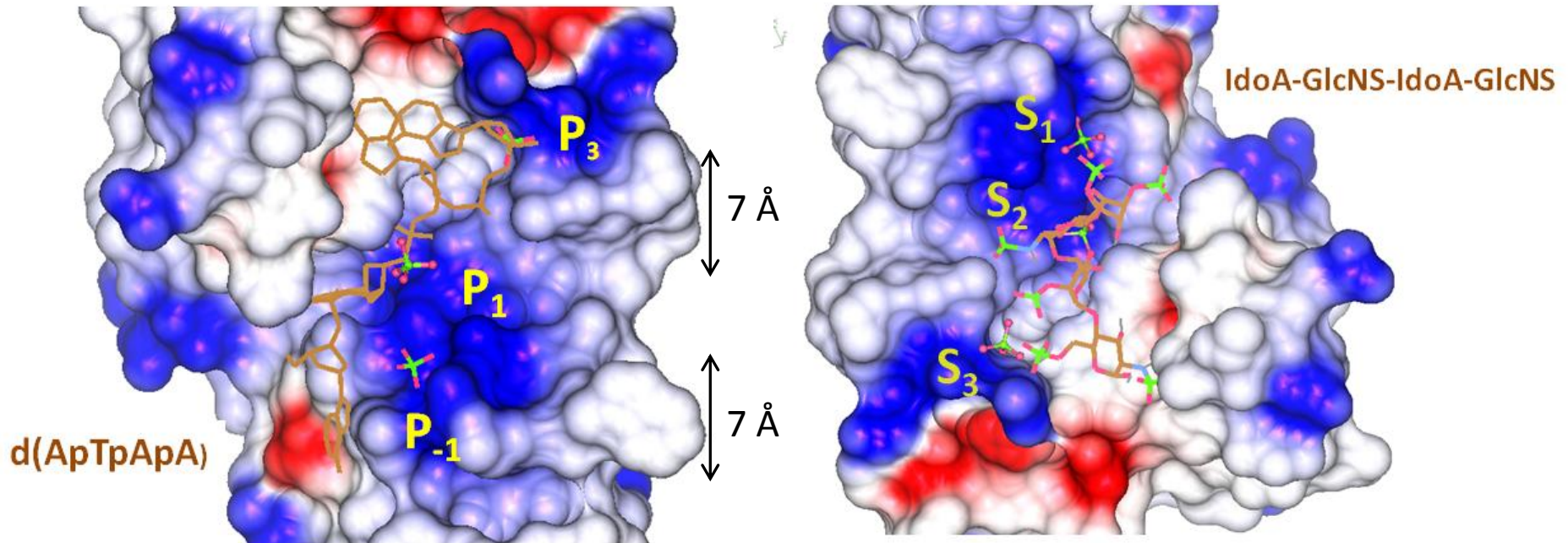
Binding sites for a polymeric substrate



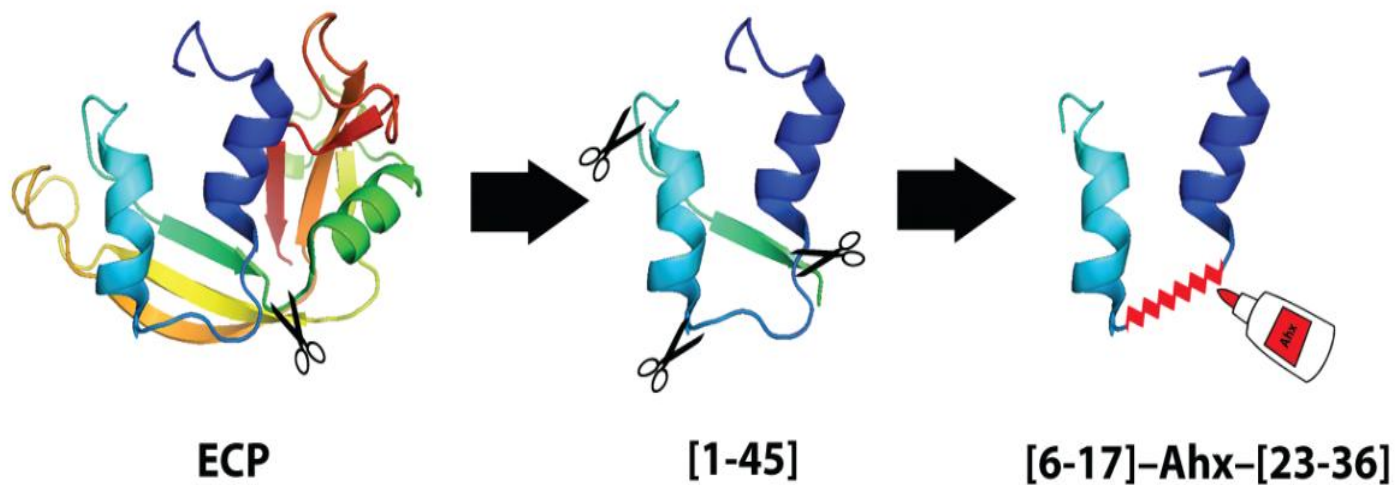
RNase A substrate subsites



A common binding pattern for nucleotides and saccharides



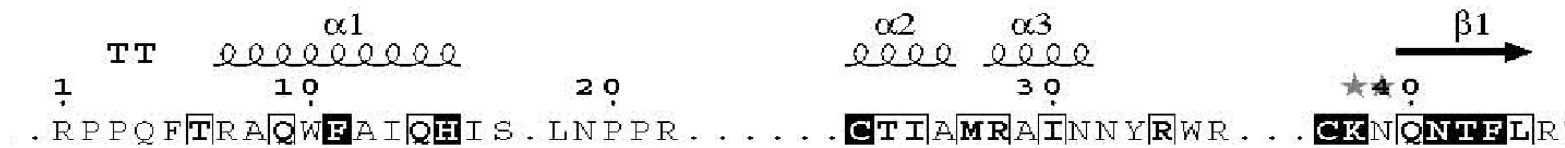
A protein scaffold- a source of antimicrobial peptides



Torrent et al. *J Med Chem.* 2011

RPPQFTR**AQWFAIQH****ISLNPPRSTIAMRAINNYRWR****SKNQNTFLR**

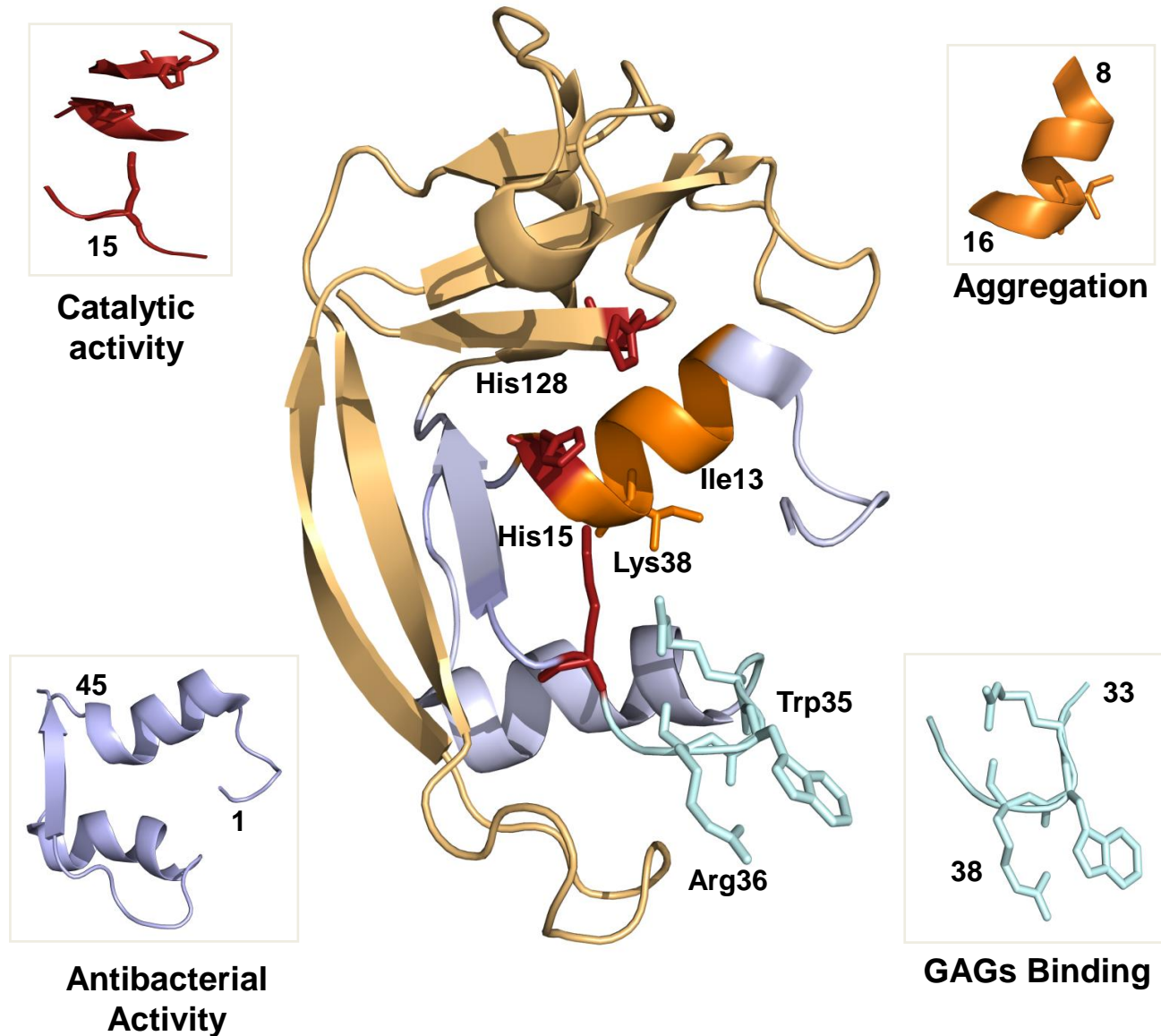
Design of N-terminus antimicrobial peptides



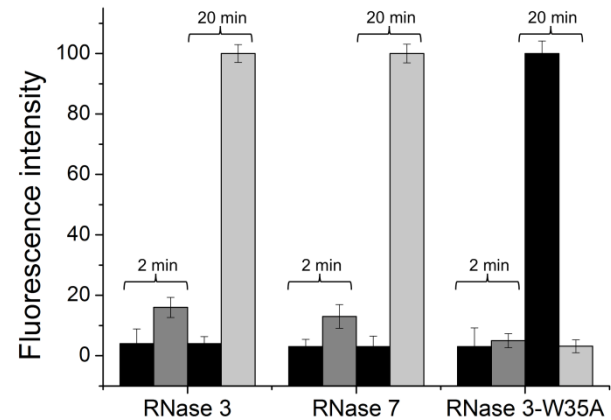
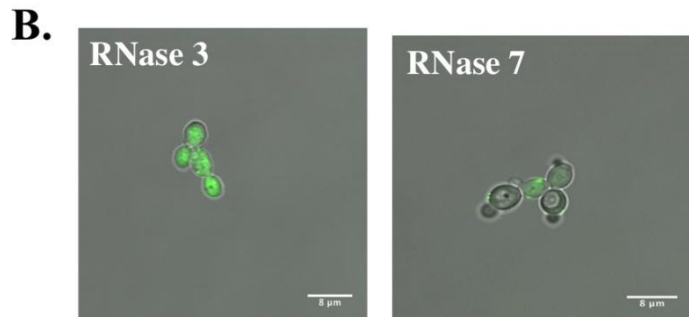
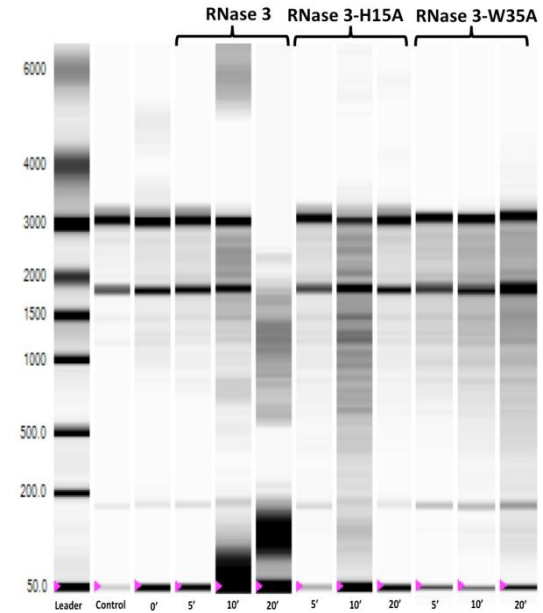
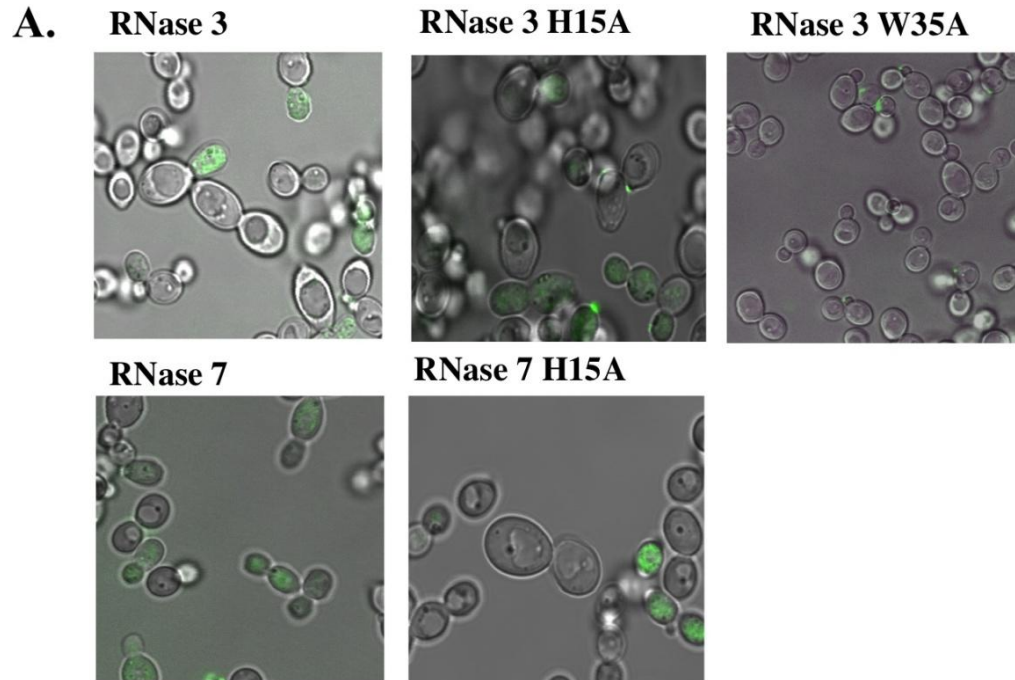
ECP

- | | | |
|----------|------------------|---|
| 1 | 1-45: | RPPQFTRAQWF AIQH ISLNP PR STIAMRAINNYRWR SKNQNTFLR |
| 2 | 24-45: | TIAMRAINNYRWR SKNQNTFLR |
| 3 | 1-19: | RPPQFTRAQWF AIQH ISLN |
| 4 | 8-45: | AQWF AIQH ISLNP PR STIAMRAINNYRWR SKNQNTFLR |
| 5 | 8-36: | AQWF AIQH ISLNP PR STIAMRAINNYRWR |
| 6 | 16-45: | ISLNP PR STIAMRAINNYRWR SKNQNTFLR |
| 7 | [6-17]Ahx[23-36] | TRAQWF AIQH IS-Ahx-STIAMRAINNYRWR |
| 8 | [8-17]Ahx[23-36] | AQWF AIQH IS-Ahx-STIAMRAINNYRWR |
| 9 | [8-15]Ahx[23-31] | AQWF AIQH -Ahx---STIAMRAIN |

The multiple faces of hRNase 3



hRNases antifungal activity



hRNases as multifunctional proteins

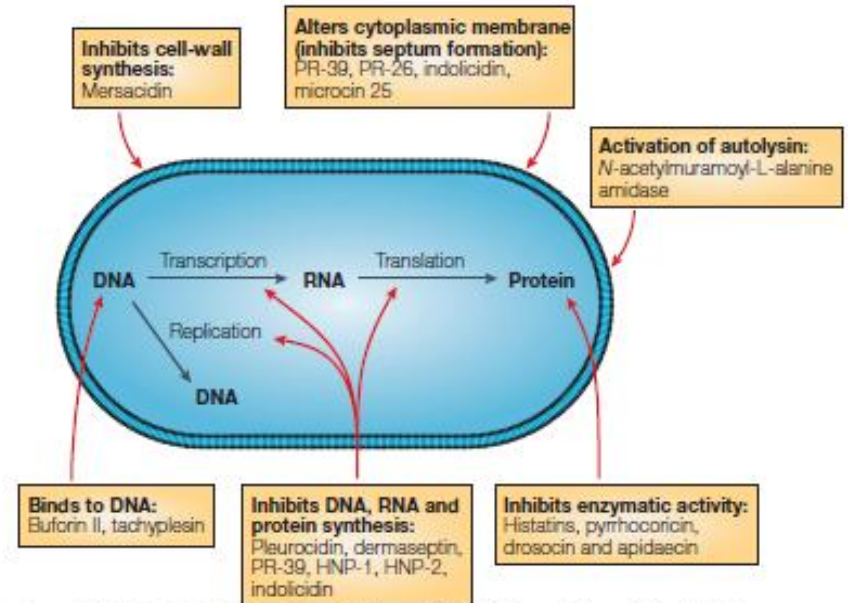
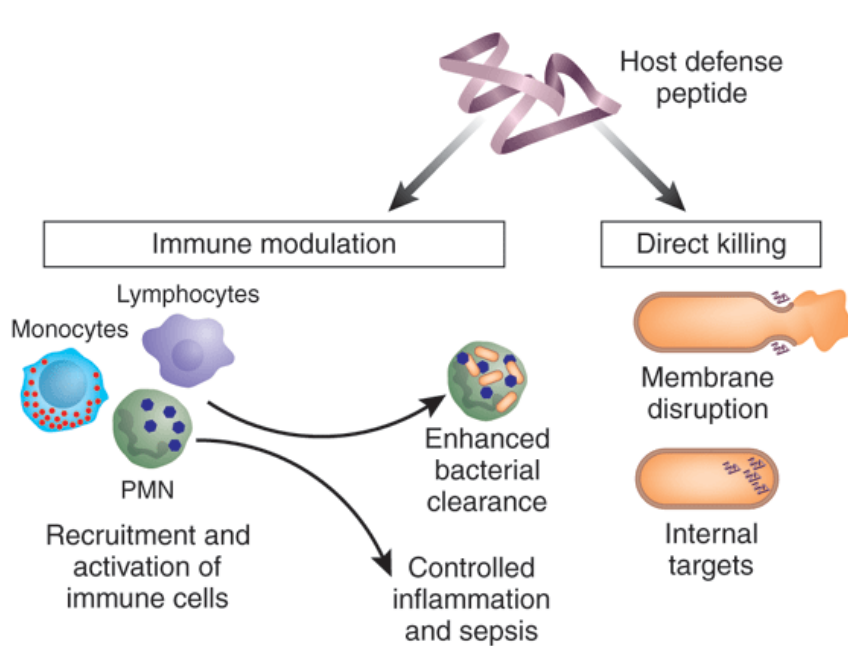


Figure 6 | Mode of action for intracellular antimicrobial peptide activity. In this figure *Escherichia coli* is shown as the target microorganism

Hancock and Sahl, *Nature Biotech.* (2006)

Brodgen *Nat Rev Microbiol* 2005



- **, Vivian Angelica Salazar, Javi Arranz, Guillem Prats, Marc Torrent, David Pulido, Mohammed Moussaoui, José Antonio Blanco, Claudi Cuchillo and Victòria Nogués.**

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