Staphylococcal quorum sensing and enabling technologies for synthetic biology

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Staphylococcus aureus

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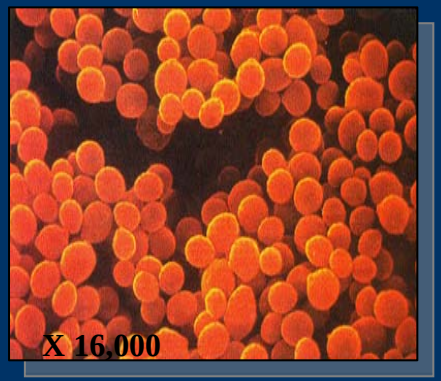
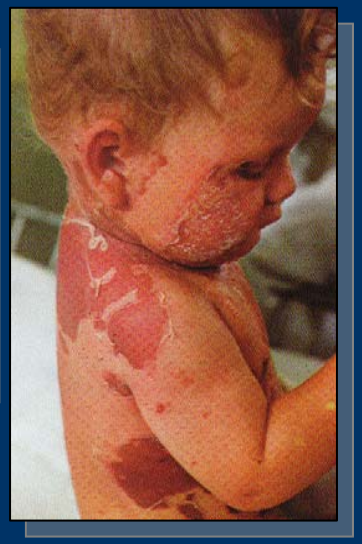
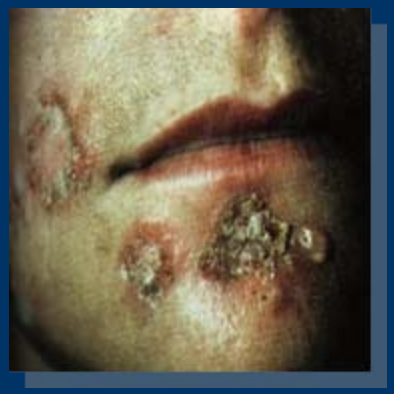


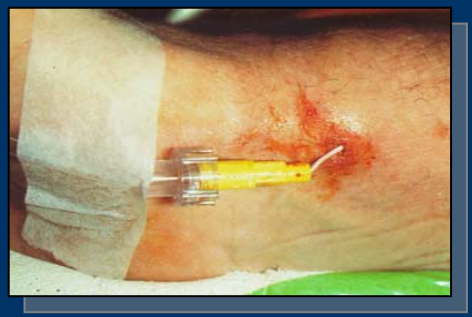
S. aureus



Scalded Skin Syndrome



Impetigo



Catheter site infection

Staphylococci cause a wide range of infections

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S. aureus

- a major pathogen in human infectious diseases
- emergence of multi-antibiotic-resistant strains
- capacity to synthesise extracellular and cell-wall associated proteins (virulence factors)
- coordinated expression of virulence factors is under the control of global regulatory systems (i.e. quorum sensing mediated)
- *agr* locus is the best characterised system

agr-mediated virulence in *S. aureus*

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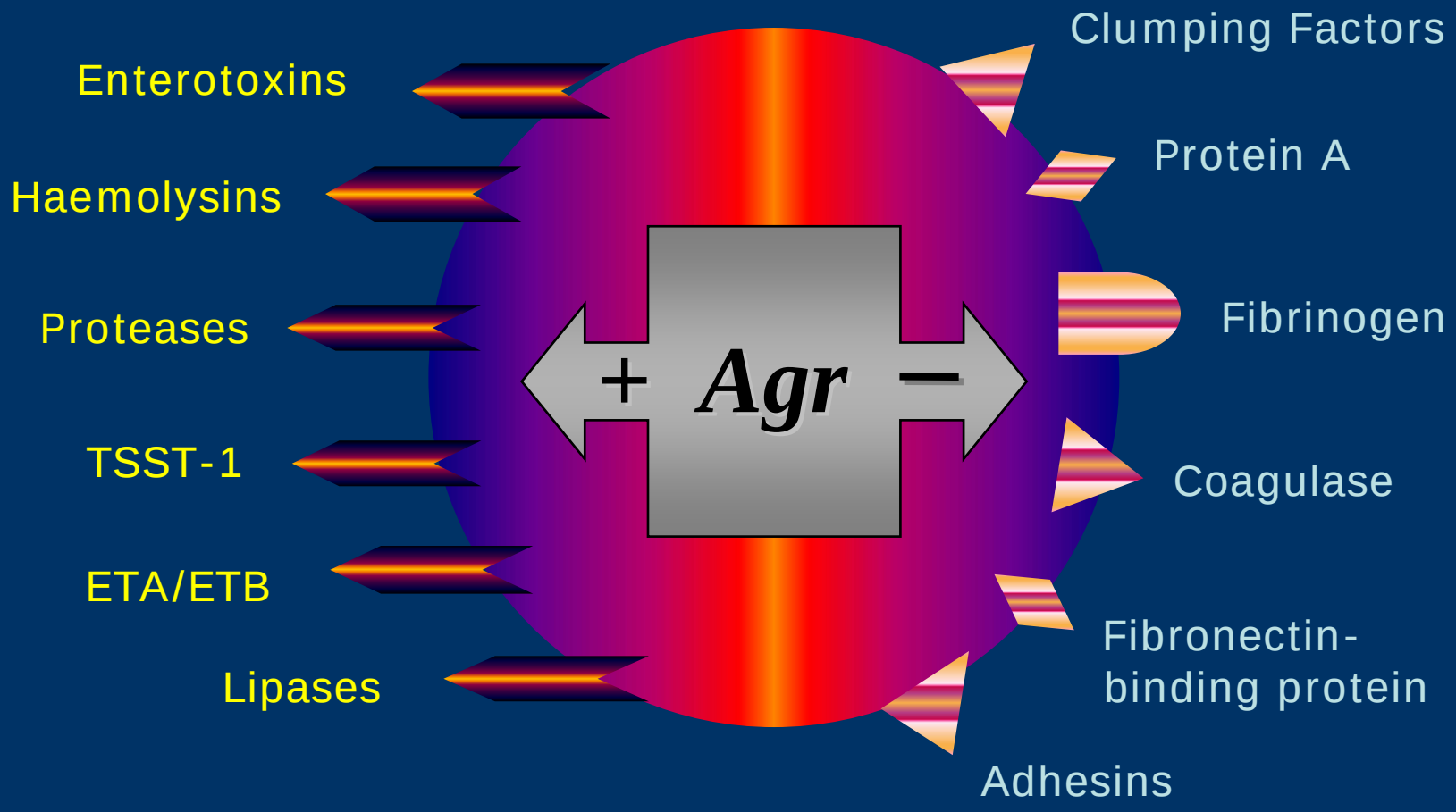

Agr = Accessory Gene Locus

agr locus in *S. aureus* - a two-component signal transduction system

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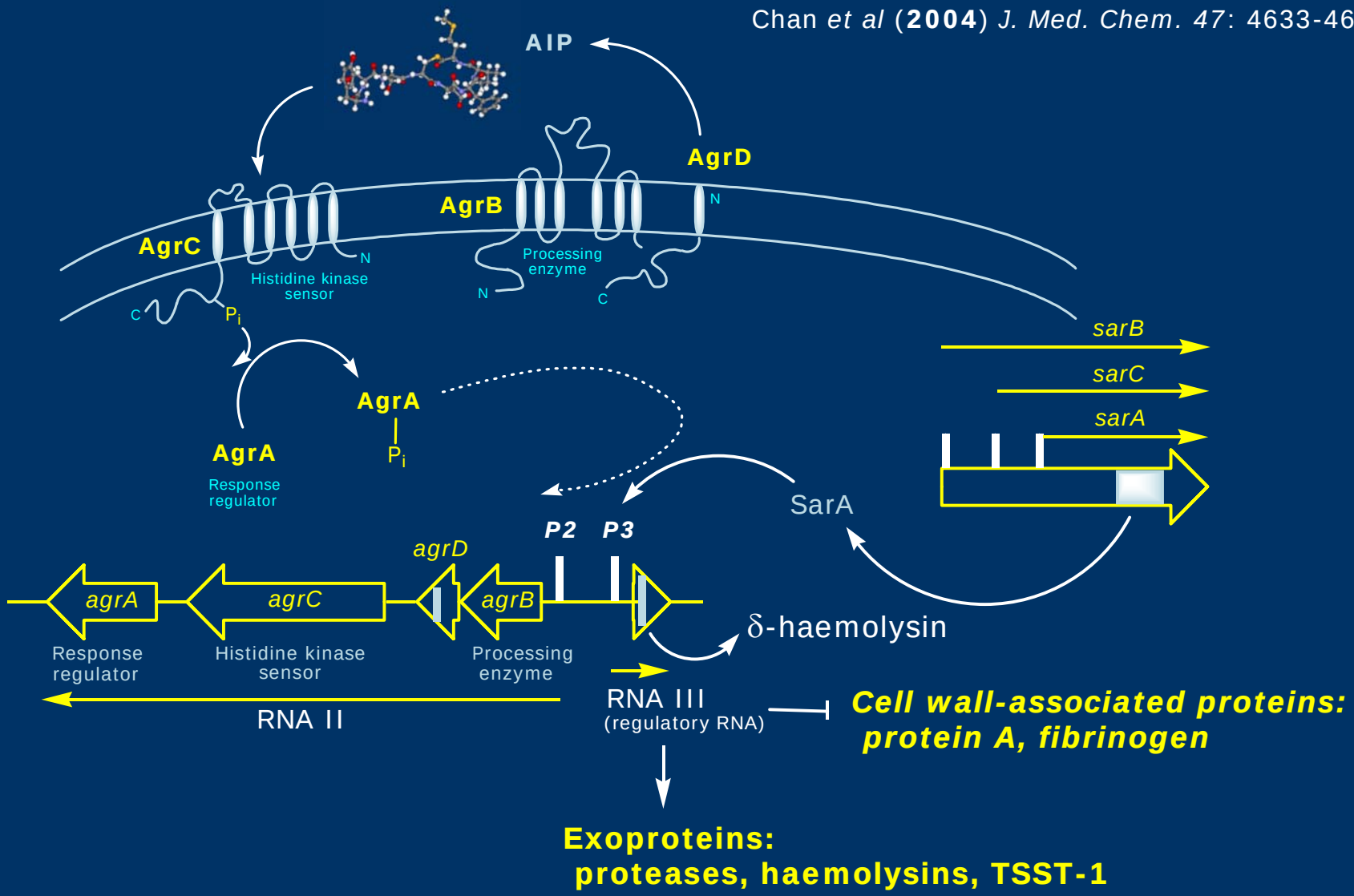
Chan *et al* (**2004**) *J. Med. Chem.* *47*: 4633-4641



AIP1 in *S. aureus*

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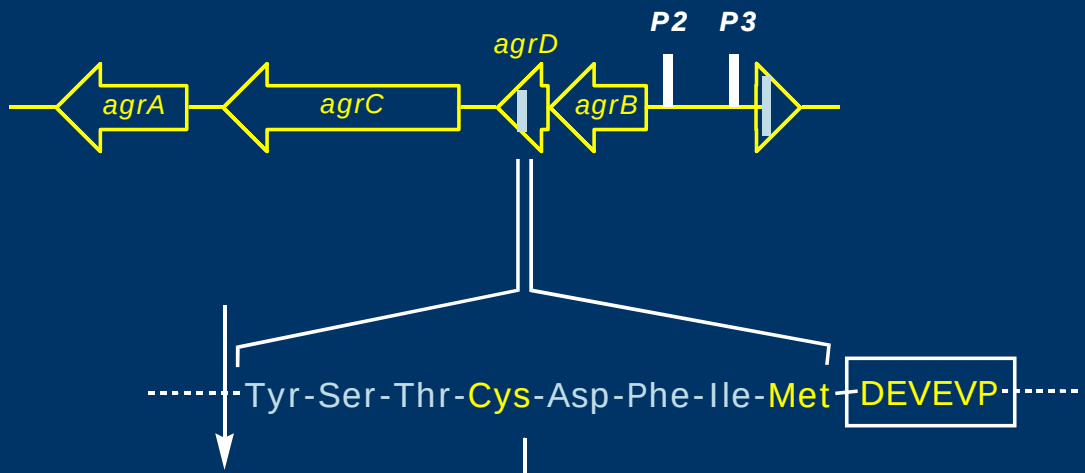
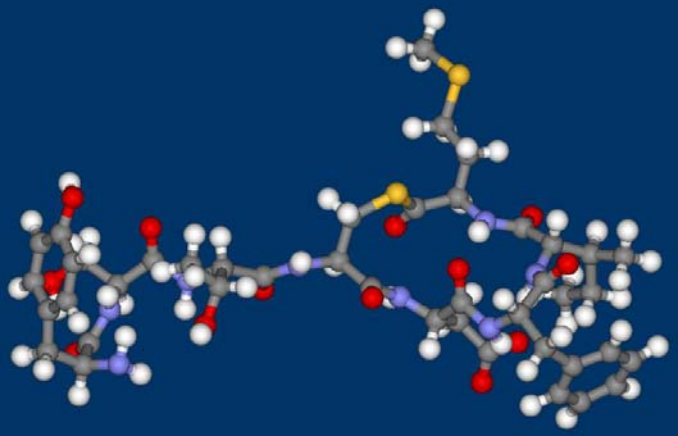
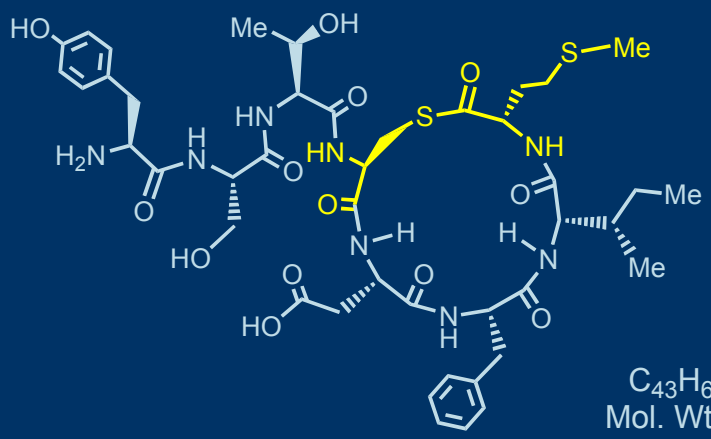


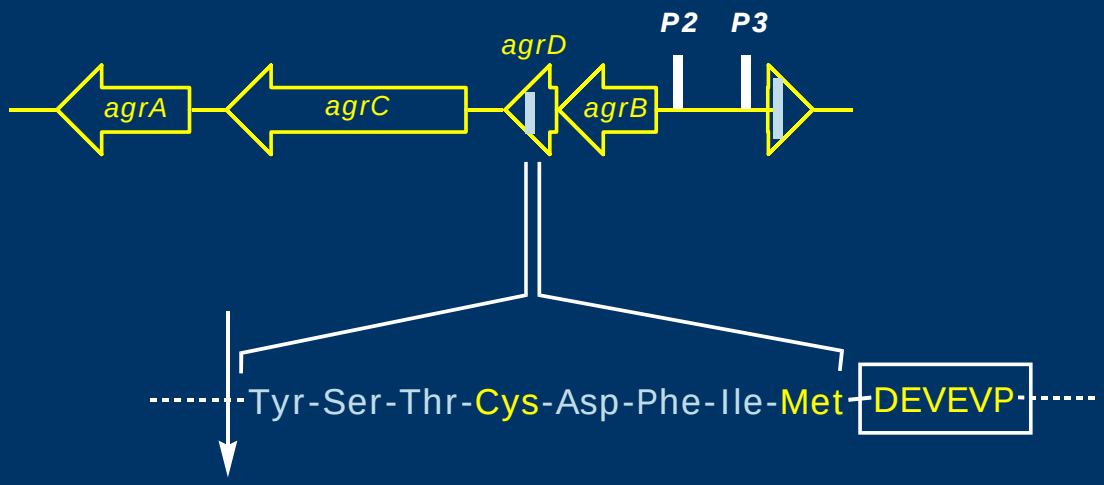




$C_{43}H_{60}N_8O_{13}S_2$
Mol. Wt.: 961.1146

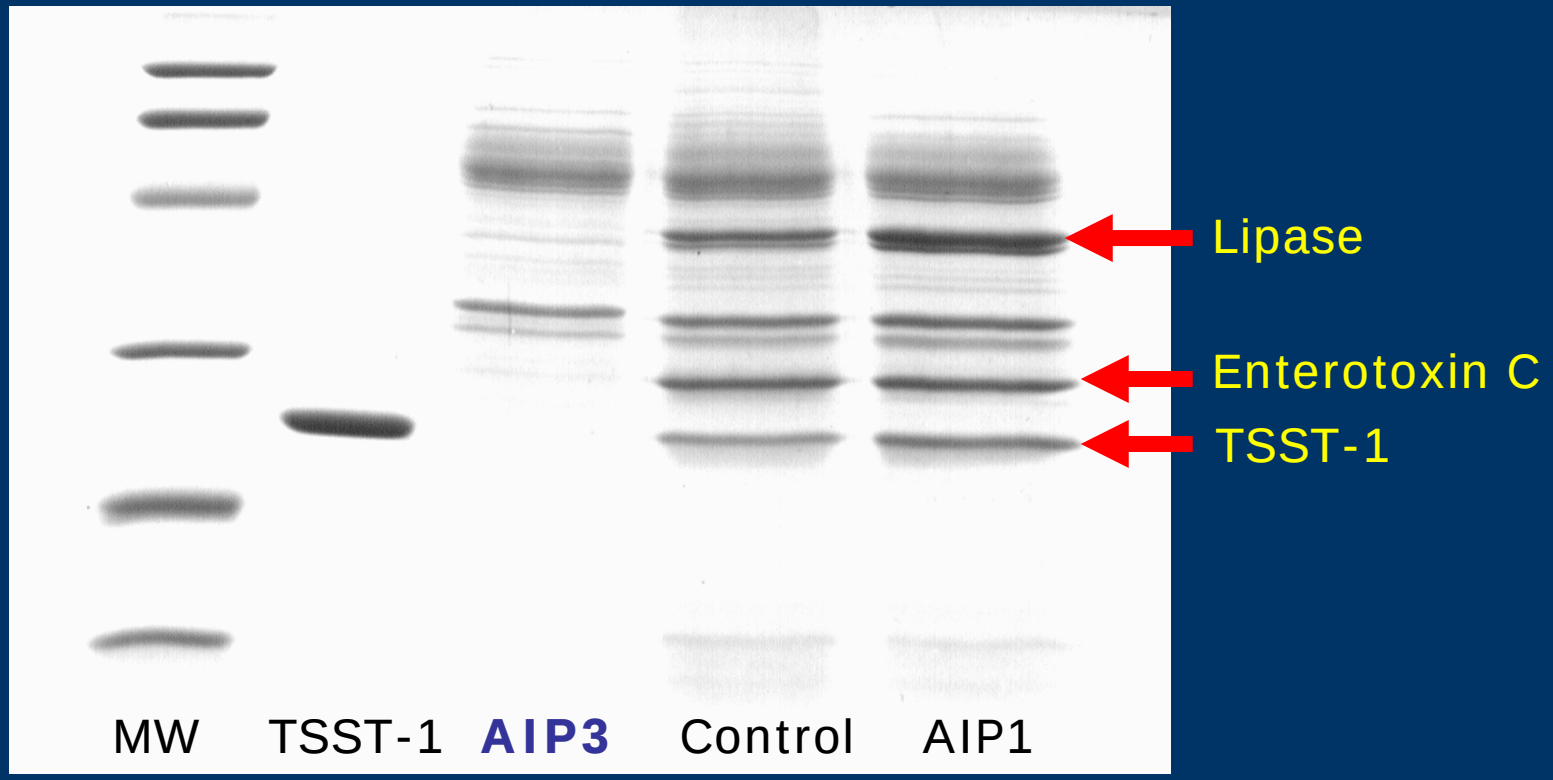
- 16-membered macrocyclic structure (thiolactone)

Staphylococcal AIPs



Gly-Val-Asn-Ala-**Cys**-Ser-Ser-Leu-Phe	*S. aureus* Group-2
Ile-Asn-**Cys**-Asp-Phe-Leu-Leu	*S. aureus* Group-3
Tyr-Ser-Thr-**Cys**-Tyr-Phe-Ile-Met	*S. aureus* Group-4
Asp-Ile-**Cys**-Asn-Ala-Tyr-Phe	*S. lugdunensis*
Asp-Ser-Val-**Cys**-Ala-Ser-Tyr-Phe	*S. epidermidis*
Arg-Ile-Pro-Thr-**Ser**-Thr-Gly-Phe-Phe	*S. intermedius*

Cross-inhibition: Inhibition of exotoxins production in a *S. aureus* Group-1 by AIP3



McDowell *et al* (**2001**) *Mol. Microbiol. 41*: 503-512

Inhibitors of two-component signal transduction system: AgrC antagonists

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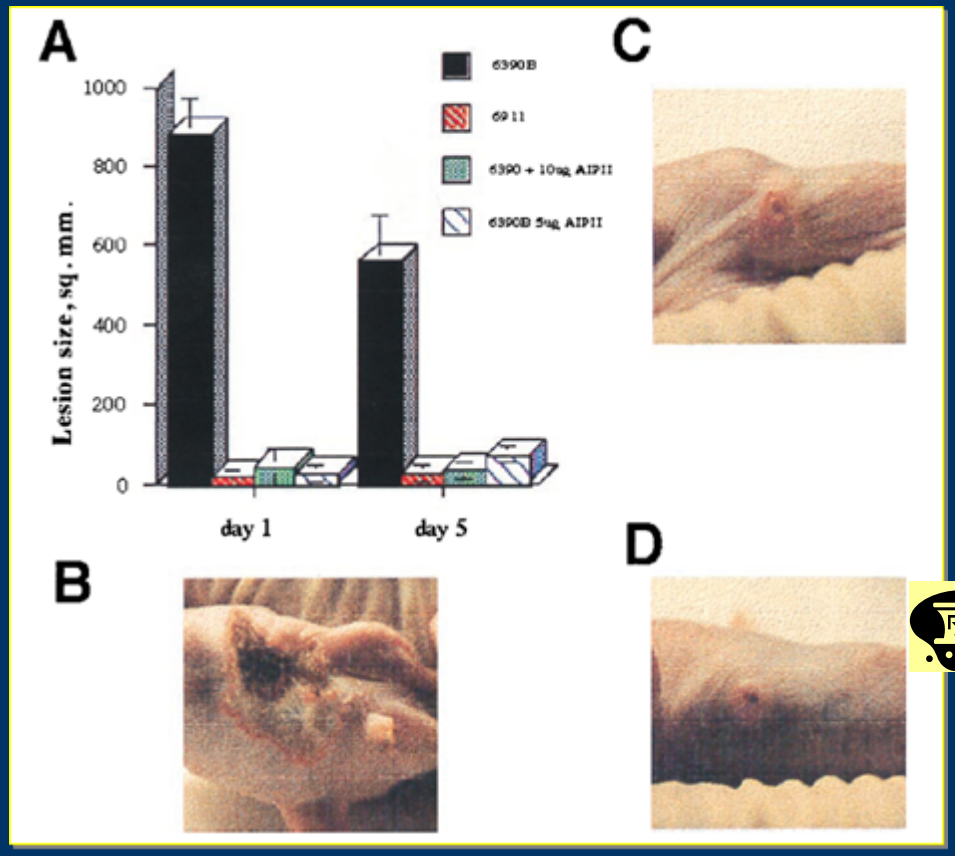
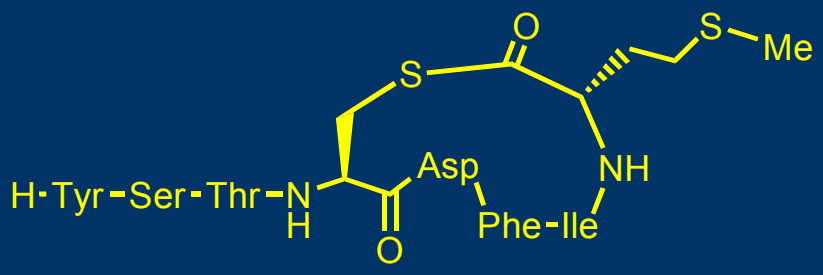


Attenuation of staphylococcal skin abscess (induced by s.c. 10^8 c.f.u. of *S. aureus* group-1) in 6-8 weeks old hairless mice using co-administered AIP2 at *ca.* 10 nM g^{-1}
Typical lesion sizes at day 5 (**b**) without treatment and (**d**) with AIP2 treatment.

Mayville *et al* (**1999**) *Proc. Natl. Acad. Sci. 96*: 1218-1223

Structure-activity studies of AIP1 using *S. aureus* Group-1



- Ala scan
- D-Amino acid scan

Synthesized peptides and their activity on the *S. aureus* Group-1 *agr* system (AgrC-1)

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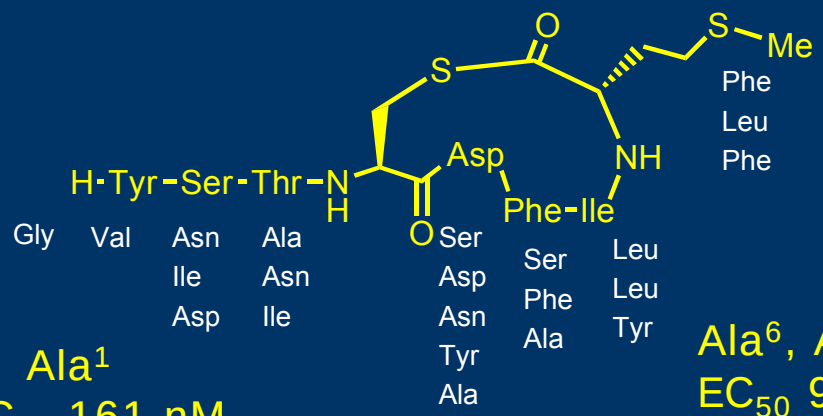
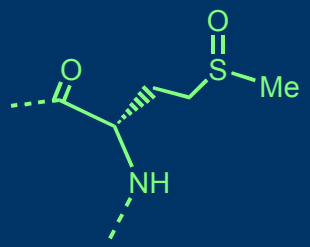
AIP1: EC_{50} 19 nM

- Ala scan



- Nle, Pro, Ser, Glu, Lys: *no activity*



Ala[6], Ala[7] , Ala[8]
EC_{50} 9-11 μM (*no activity*)

Ala[1]
EC_{50} 161 nM

Ala[2]
EC_{50} 9 nM

Ala[3]
EC_{50} 248 nM

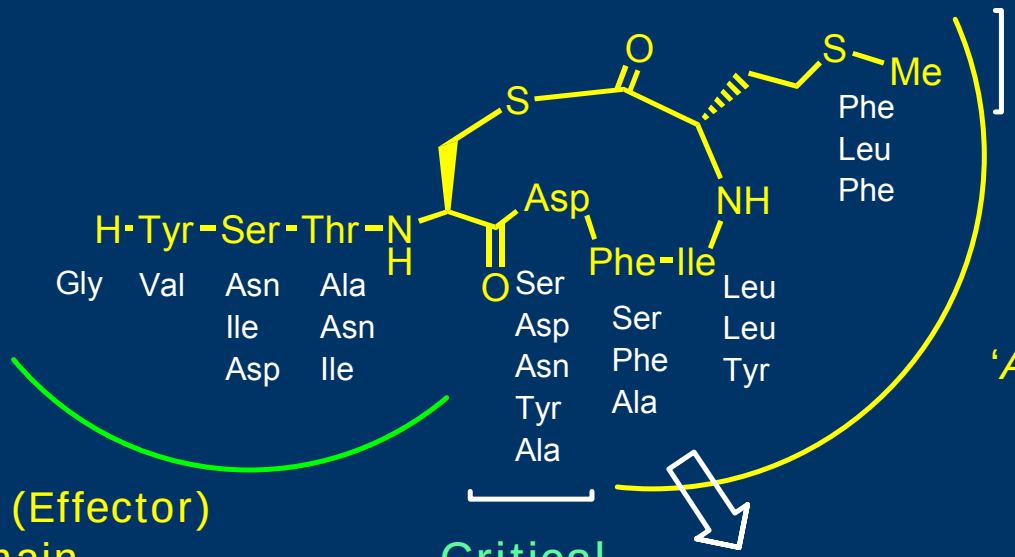
Ala[5]
IC_{50} 21 ± 9 nM

Gly[5] IC_{50} 1.4 μM; Abu[5] IC_{50} 137 ± 65 nM

S. aureus Group-2: AgrC-2
Ala[5] IC_{50} 4 ± 4 nM; Abu[5] IC_{50} 2.8 ± 0.4 nM

Summary of structure-activity relationship (SAR)

- 16-membered macrocyclic structure essential for binding to AgrC-1
- Competitive inhibitors based on the cyclic structure

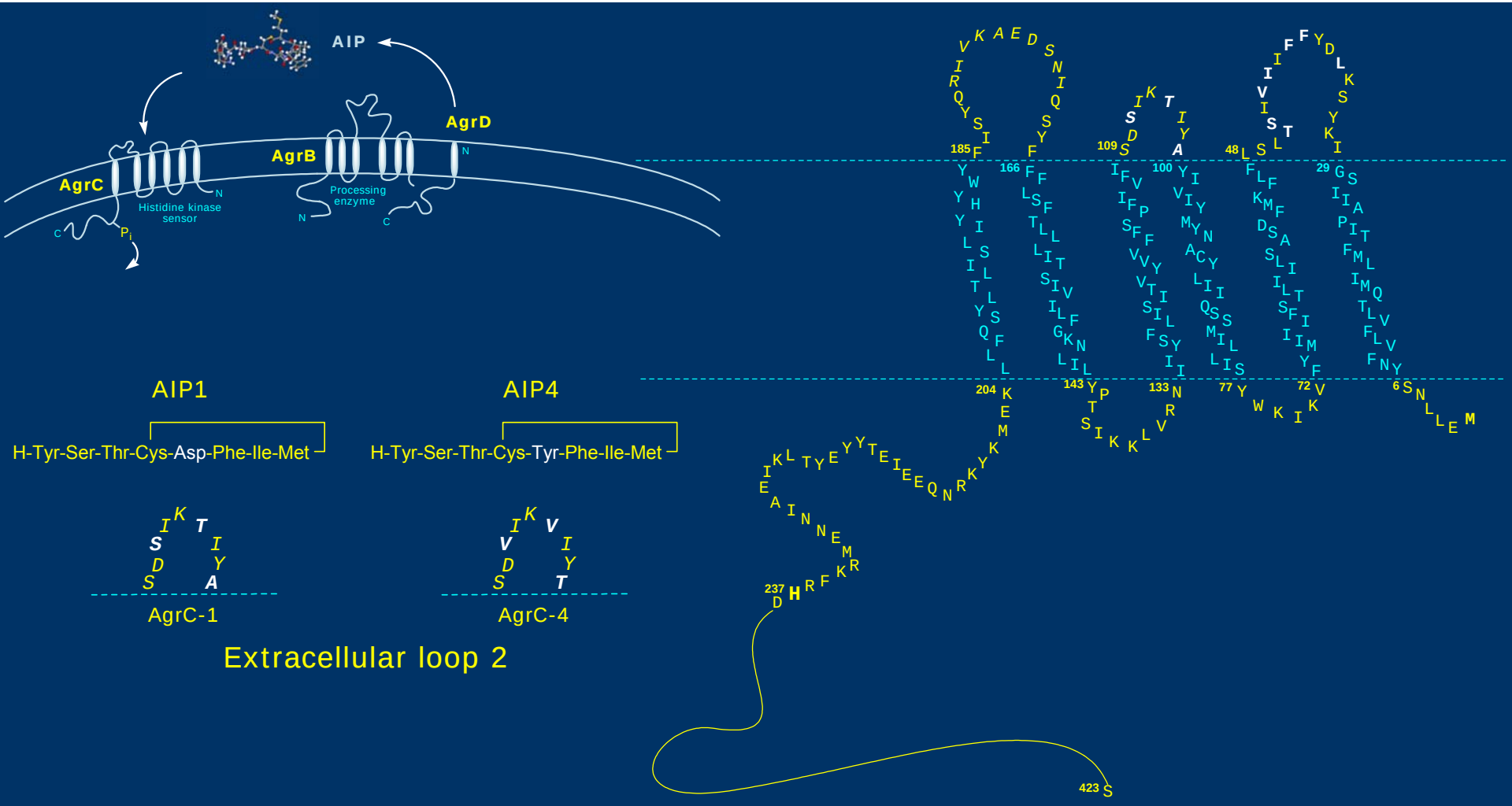


'*Message*' (Effector) domain
Also contributes to enhanced binding

Critical residue
Activation/Inhibition

'*Address*' domain:
Hydrophobic binding domain

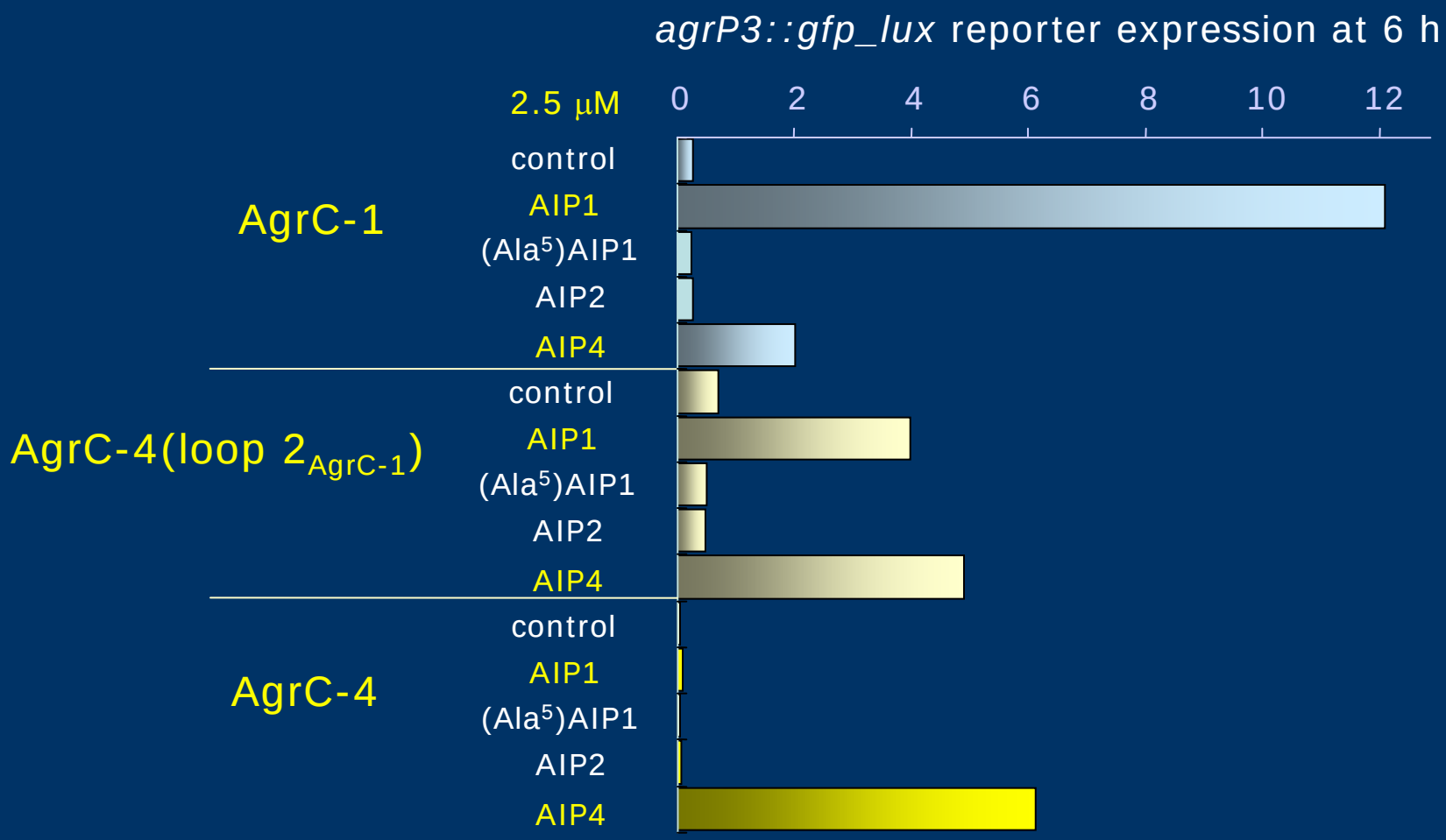
The AgrC kinase sensor



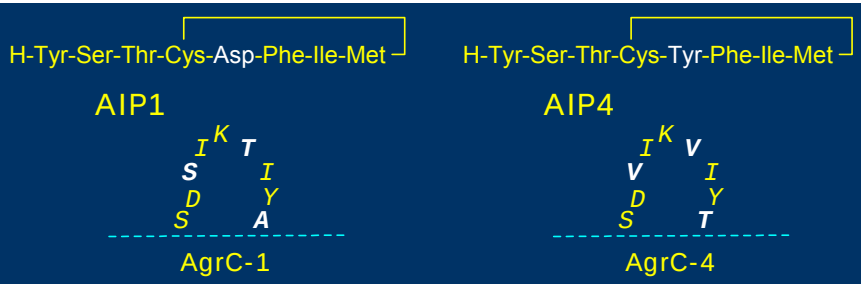
Transmembrane topology of AgrC-1 predicted using TMHMM

(http://www.cbs.dtu.dk/services/TMHMM-2.0/)

AIP is recognised by AgrC-1/4 extracellular loop 2



The AgrC kinase sensor: contribution of extracellular loop 2 to AIP recognition



Jensen *et al* (**2008**) *JMB 381*: 300-309

Native and mutant AgrC4 proteins	EC_{50} (nM)	
	AIP-1	AIP-4
AgrC4 (wild type)	3542 ± 997	9 ± 1
T101A,V104T,V107S (Group I loop 2)	33 ± 2	170 ± 12
T101A	754 ± 47	29 ± 1
V104T	6357 ± 349	512 ± 57
V107S	451 ± 30	9 ± 3
T101A, V104T	12064 ± 1654	587 ± 10
T101A, V107S	407 ± 45	22 ± 3
V104T, V107S	4335 ± 457	551 ± 48
Native and mutant AgrC1		
AgrC1 (wild type)	6 ± 1	107 ± 20
A101T	30 ± 2	22 ± 5
T104V	235 ± 38	269 ± 49
S107V	150 ± 15	ND
A101T, T104V	47 ± 2	15 ± 2
A101T, S107V	99 ± 10	19 ± 3

The AgrC kinase sensor: activity of (Ala^5)AIP-1 against mutant AgrC

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H-Tyr-Ser-Thr-Cys-Asp-Phe-Ile-Met

AIP1

I K T
S I
D Y
S A

AgrC-1

H-Tyr-Ser-Thr-Cys-Tyr-Phe-Ile-Met

AIP4

I K V
V I
D Y
S T

AgrC-4

Jensen *et al* (**2008**) *JMB 381*: 300-309

H-Tyr-Ser-Thr-Cys-**Ala**-Phe-Ile-Met

Ala → Thr
GCA → *ACA*

Native and mutant AgrC4 and AgrC1 proteins	Activity (nM)	
	Activation, EC_{50}	Inhibition, IC_{50}
AgrC4		11 ± 2
T101A,V104T,V107S (Group I loop 2)	389 ± 12	
T101A		73 ± 13
V104T		16 ± 2
V107S		4718 ± 318
T101A, V104T		28 ± 5
T101A, V107S		255 ± 15
V104T, V107S		10 ± 3
AgrC1 (wild type)		7 ± 1
A101T	120 ± 24	
T104V	556 ± 38	
S107V	4053 ± 785	
A101T, T104V	26 ± 1	
A101T, S107V	16 ± 1	
A101T, T104V,S107V (Group IV loop 2)	1235 ± 303	

Conclusions I

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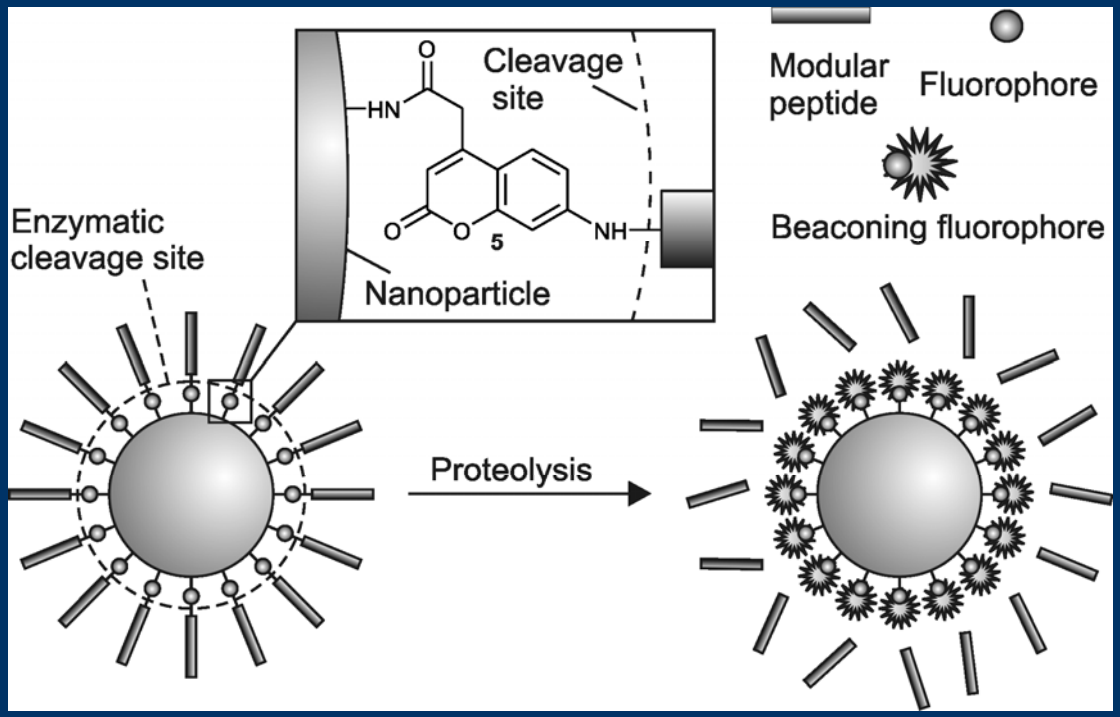
- The staphylococcal quorum sensing *agr* system offers unique targets for the development of novel anti-infective agents
- SAR studies using AIP1 has yielded potent AgrC antagonists
- The extracellular loop 2 is one of the domains involved in recognition of AIPs.
- A new vocabulary for staphylococcal communication was discovered

The protease responsive nanoprobe based on peptidyl coumarin substrate



Synthesis of bifunctional 3-arylcoumarins and their fluorogenic properties

Welser *et al* (**2009**) *Chem Commun*: 671-673

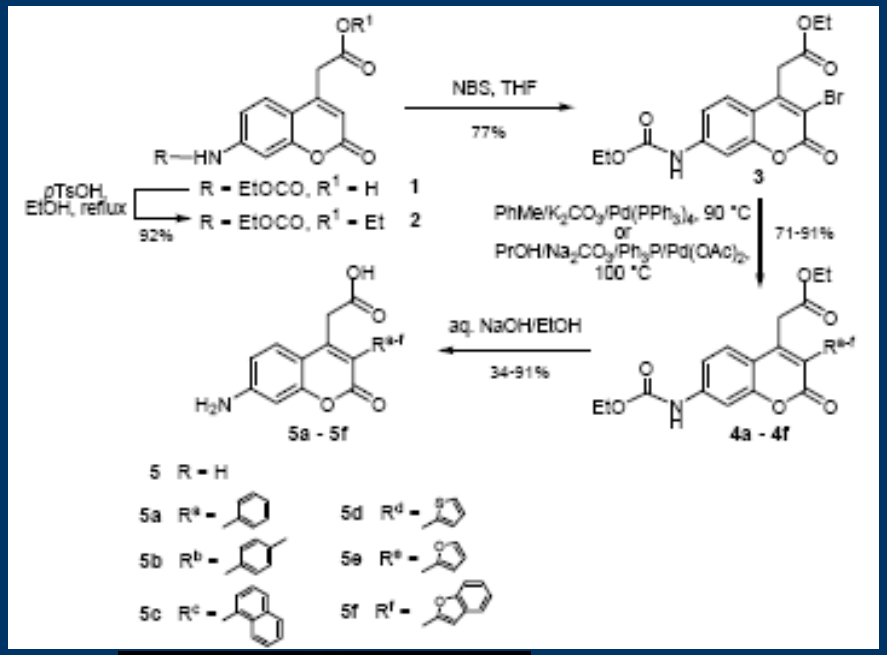
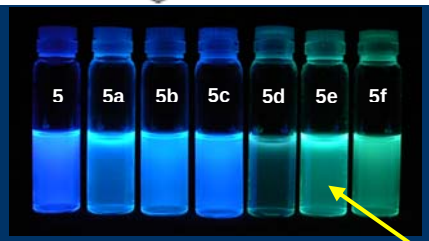
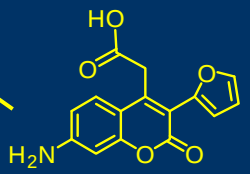


Table 1 Photophysical properties of **5** and its derivatives **5a–5f**.

Reagent	$\lambda_{max/ex}$ [a]	λ_{em} [a]	log ε [b]	Φ_f [a,c]	τ [a,d] (ns)
5	345	450	4.11	0.99	5.0
5a	355	460	4.33	0.83	3.7
5b	350	460	4.18	0.68	3.5
5c	355	457	4.41	0.75	3.6
5d	360	484	4.47	0.11	0.6
5e	370	490	4.21	0.26	2.9 (0.7)/0.4 (0.3)
5f	380	496	4.32	0.30	3.2 (0.6)/0.4 (0.4)

[a] Measurements carried out in PBS/10% DMSO. [b] Molar extinction coefficient at λ_{max} (L mol^{-1} cm^{-1}; EtOH/5%DMSO). [c] Quantum yields. [d] Fluorescence lifetime; the numbers in parenthesis represent relative amplitudes.





5e

Proteolysis of subtilisin responsive nanoprobes

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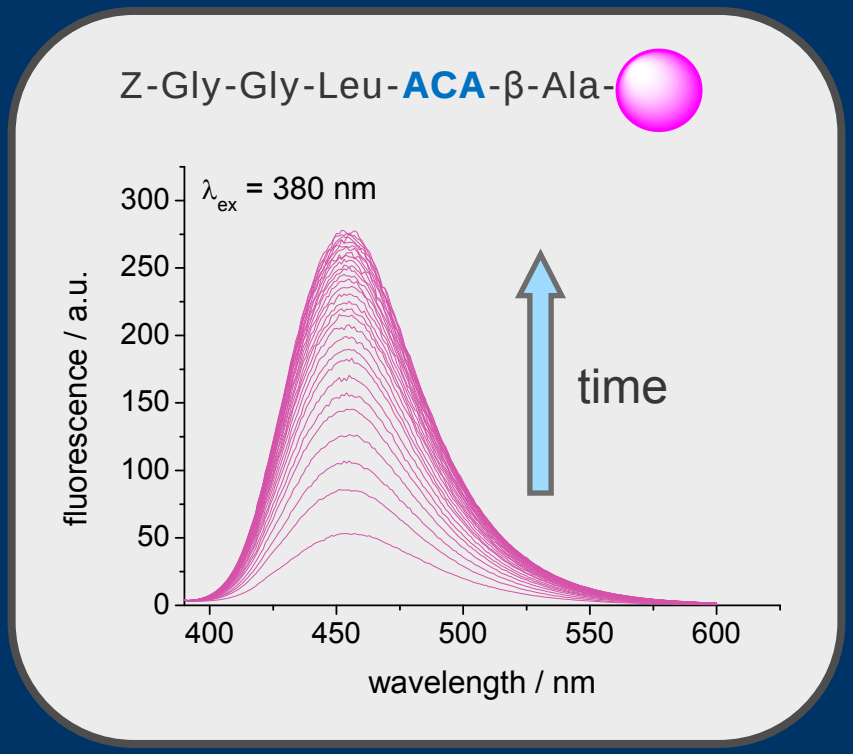

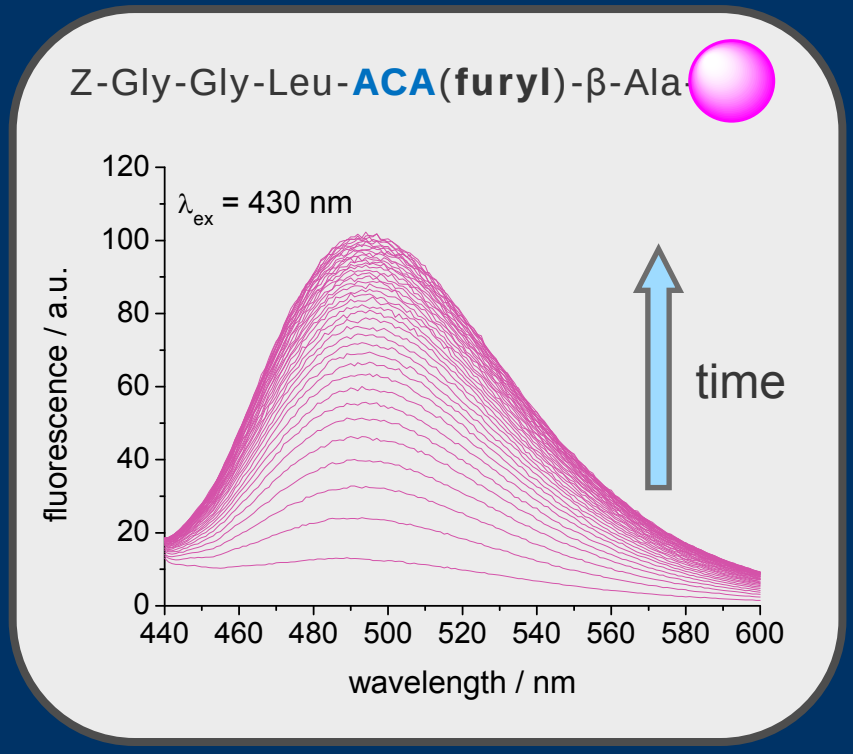

Conclusions II

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- A robust green fluorescent bifunctional 3-furylcoumarin dye was developed

- Protease response nanosensors were obtained by the conjugation of peptidyl 7-amino-4-carboxymethyl-3-furylcoumarin to poly(acrylamide)-based nanoparticles

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Dr Huw Williams

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