



# Staphylococcal quorum sensing and enabling technologies for synthetic biology

Weng Chan

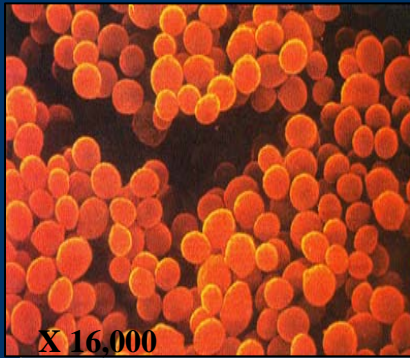
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# *Staphylococcus aureus*



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*S. aureus*



Scalded Skin  
Syndrome



Impetigo



Catheter site  
infection

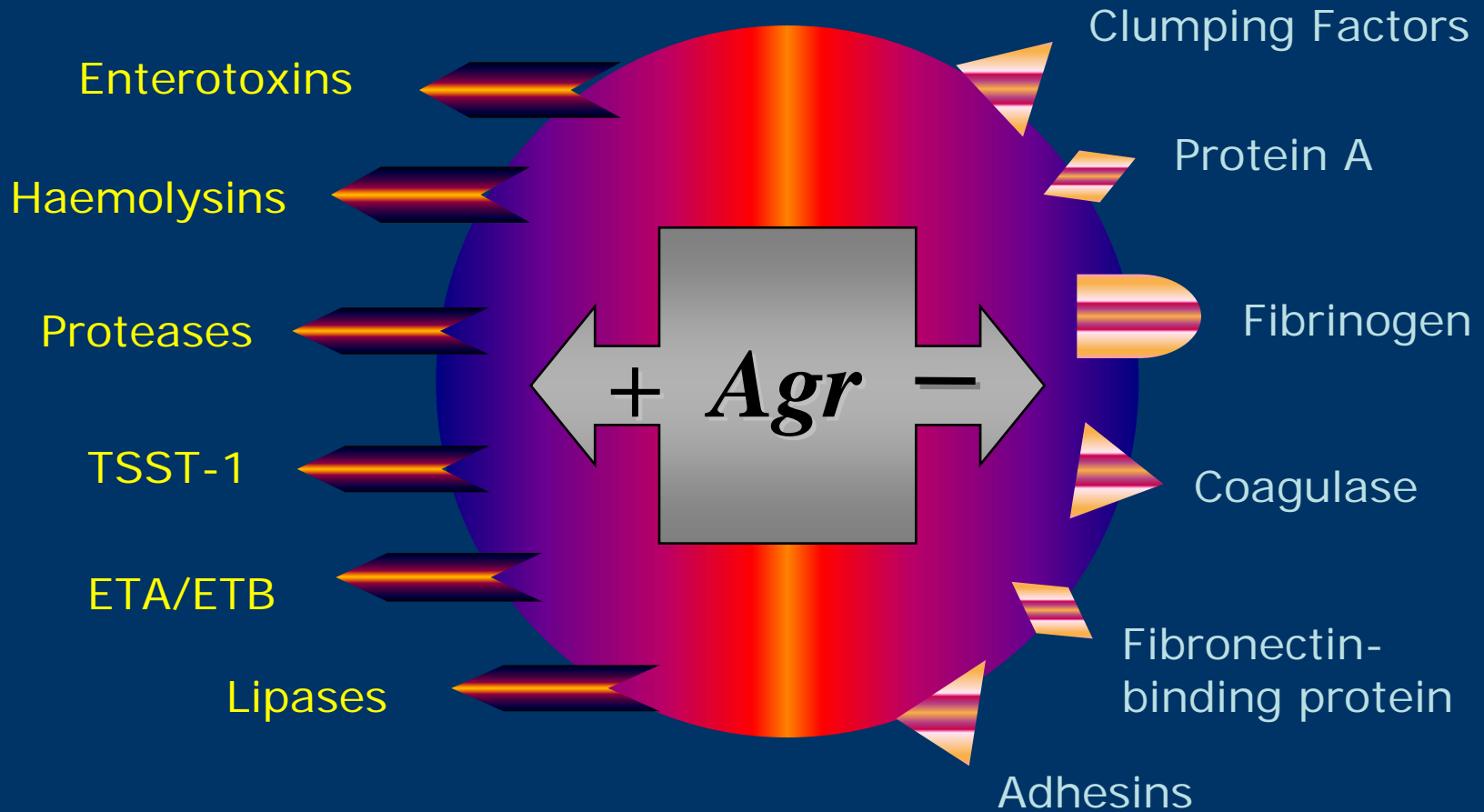
Staphylococci cause a wide range of infections



## *S. aureus*

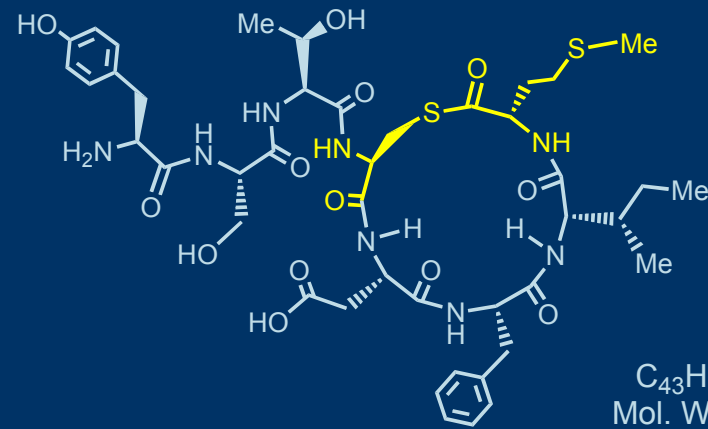
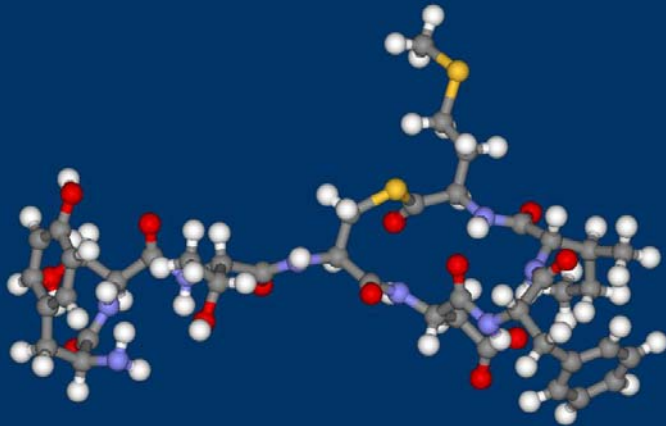
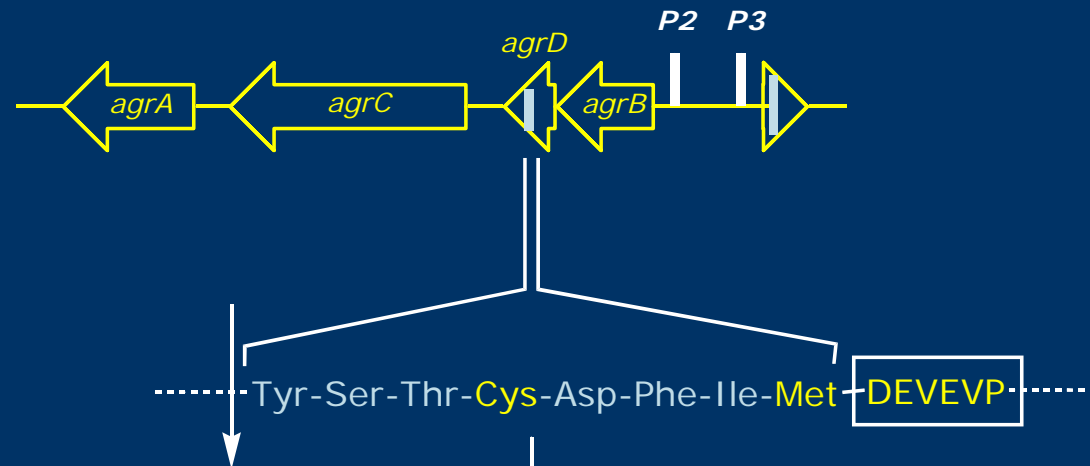
- a major pathogen in human infectious diseases
- emergence of multi-antibiotic-resistant strains
- capacity to synthesise extracellular and cell-wall associated proteins (virulence factors)
- coordinated expression of virulence factors is under the control of global regulatory systems (i.e. quorum sensing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

# *agr*-mediated virulence in *S. aureus*



*Agr* = Accessory Gene Locus

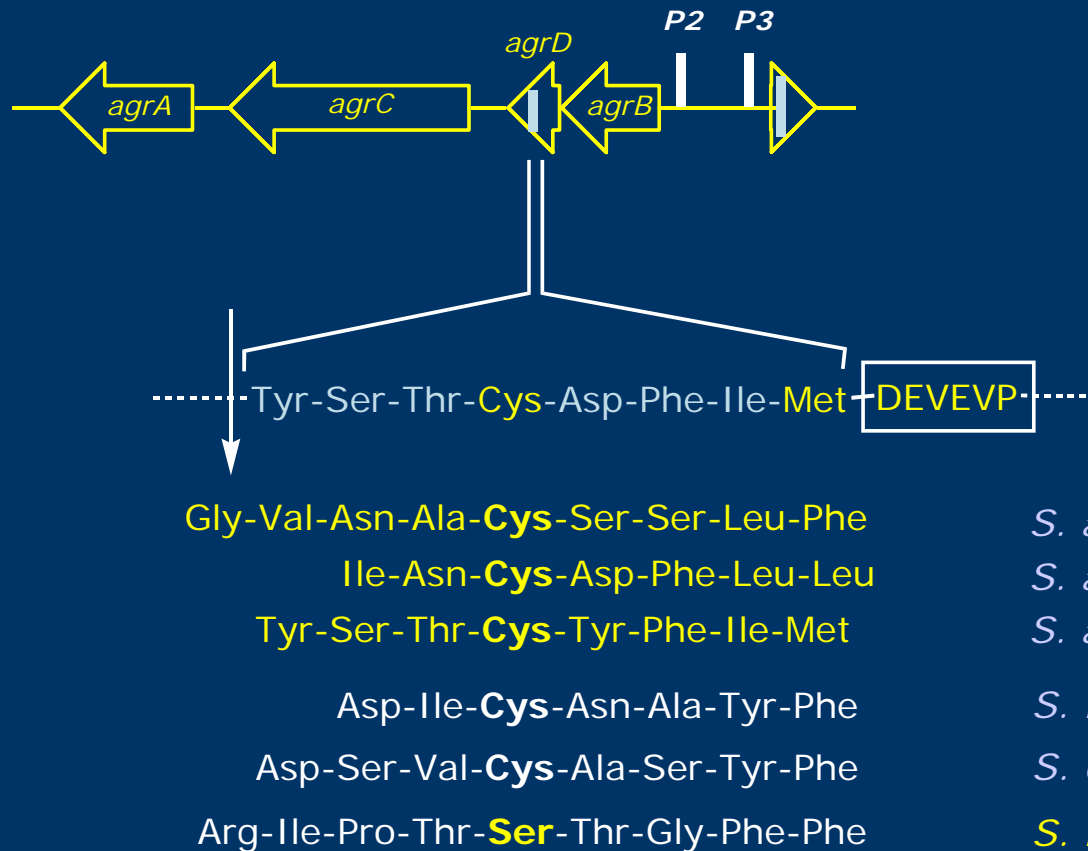




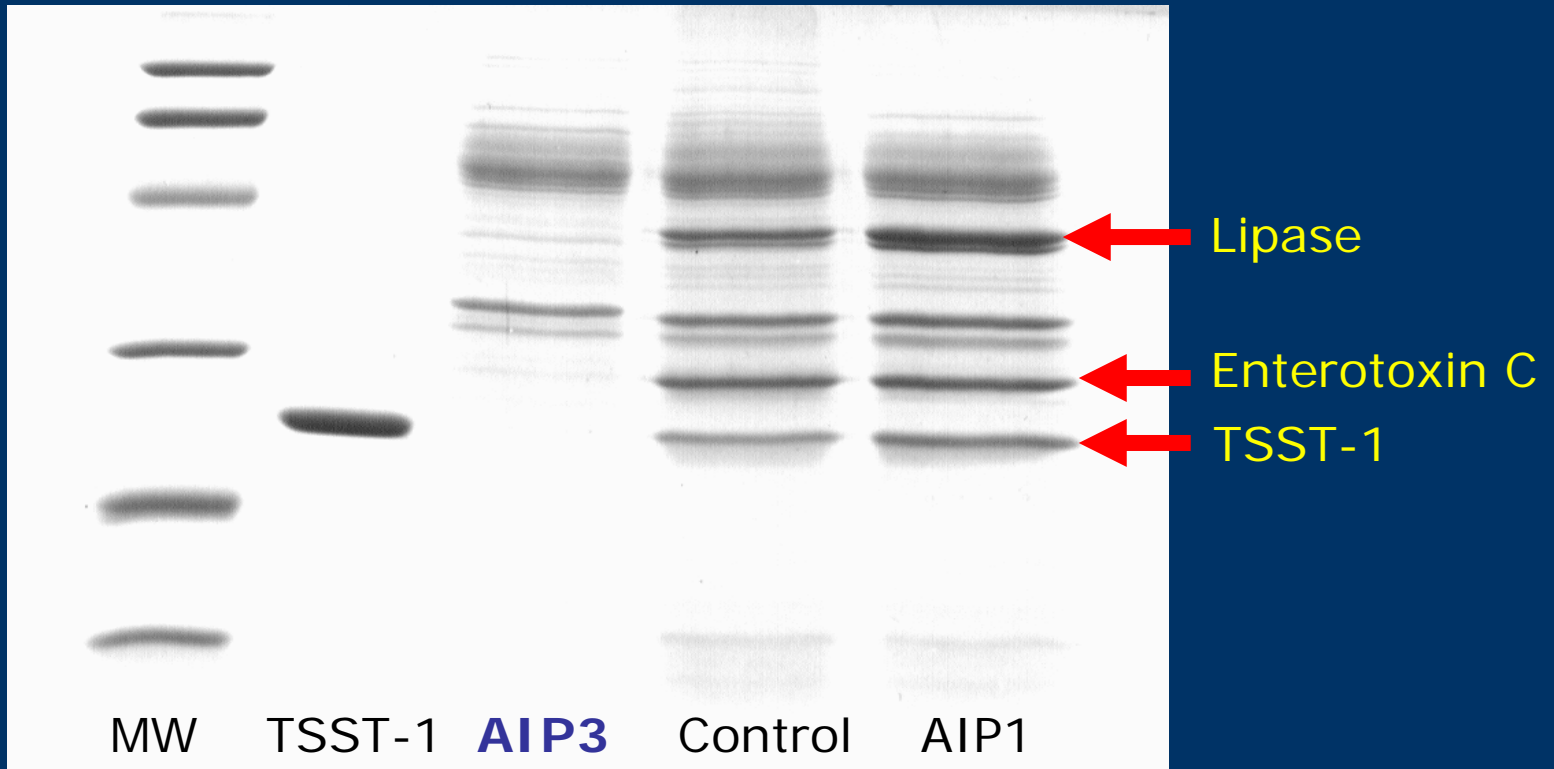
$C_{43}H_{60}N_8O_{13}S_2$   
Mol. Wt.: 961.1146

- 16-membered macrocyclic structure (thiolactone)

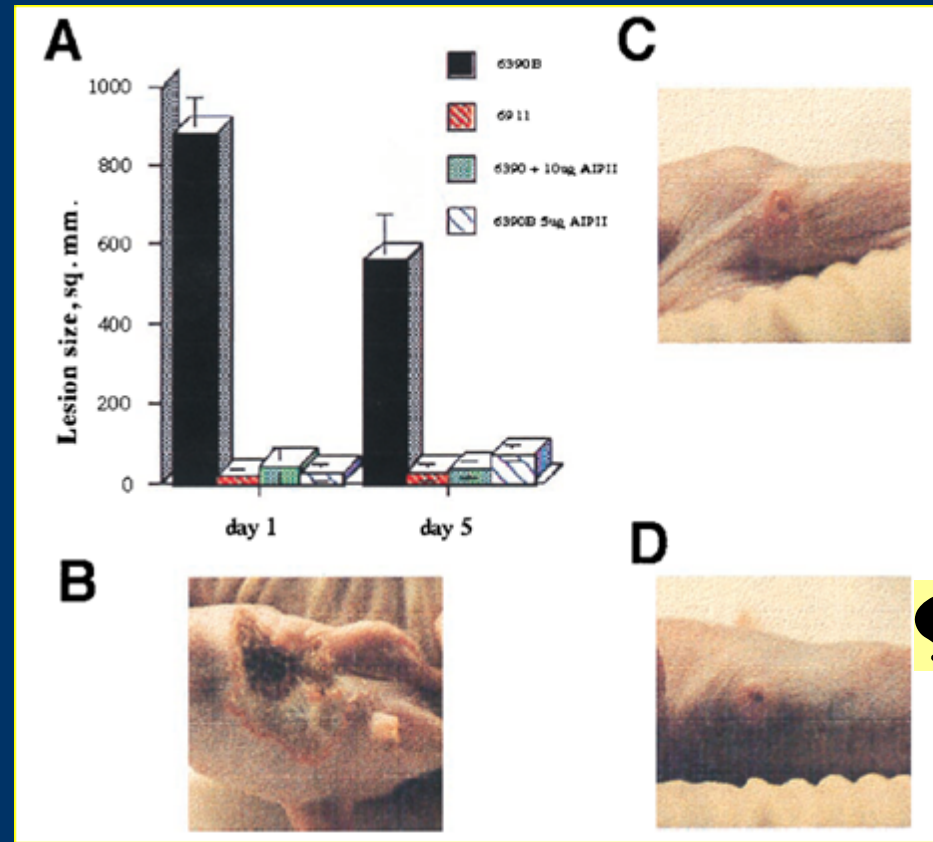
# Staphylococcal AIPs



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



# Inhibitors of two-component signal transduction system: AgrC antagonists

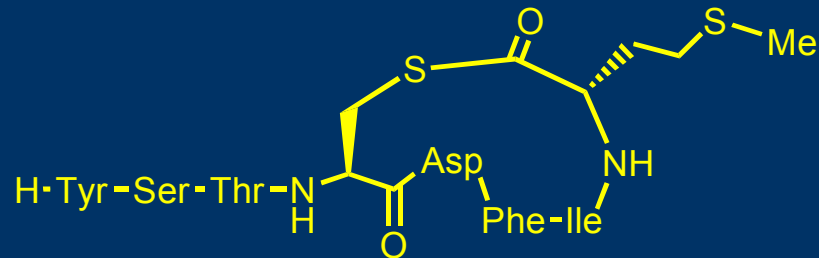


Attenuation of staphylococcal skin abscess (induced by s.c.  $10^8$  c.f.u. of *S. aureus* group-1) in 6-8 weeks old hairless mice using co-administered AIP2 at *ca.*  $10$  nM  $g^{-1}$   
Typical lesion sizes at day 5 (**b**) without treatment and (**d**) with AIP2 treatment.

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



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- Ala scan
- D-Amino acid scan

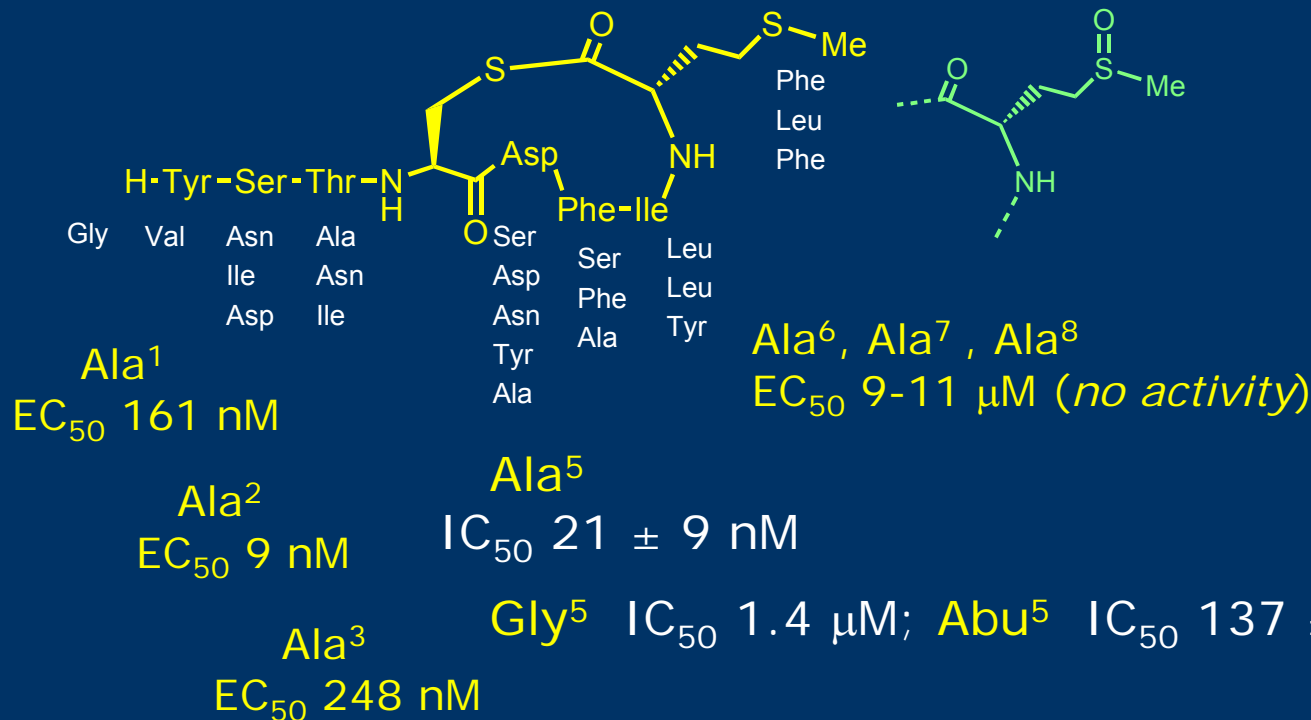
# Synthesized peptides and their activity on the *S. aureus* Group-1 *agr* system (AgrC-1)



AIP1: EC<sub>50</sub> 19 nM

- Ala scan

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



*S. aureus* Group-2: AgrC-2  
Ala<sup>5</sup> IC<sub>50</sub> 4 ± 4 nM; Abu<sup>5</sup> IC<sub>50</sub> 2.8 ± 0.4 nM





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



*agrP3::gfp\_lux* reporter expression at 6 h

2.5  $\mu$ M

0 2 4 6 8 10 12

AgrC-1

control

AIP1

(Ala<sup>5</sup>)AIP1

AIP2

AIP4

AgrC-4(loop 2<sub>AgrC-1</sub>)

control

AIP1

(Ala<sup>5</sup>)AIP1

AIP2

AIP4

AgrC-4

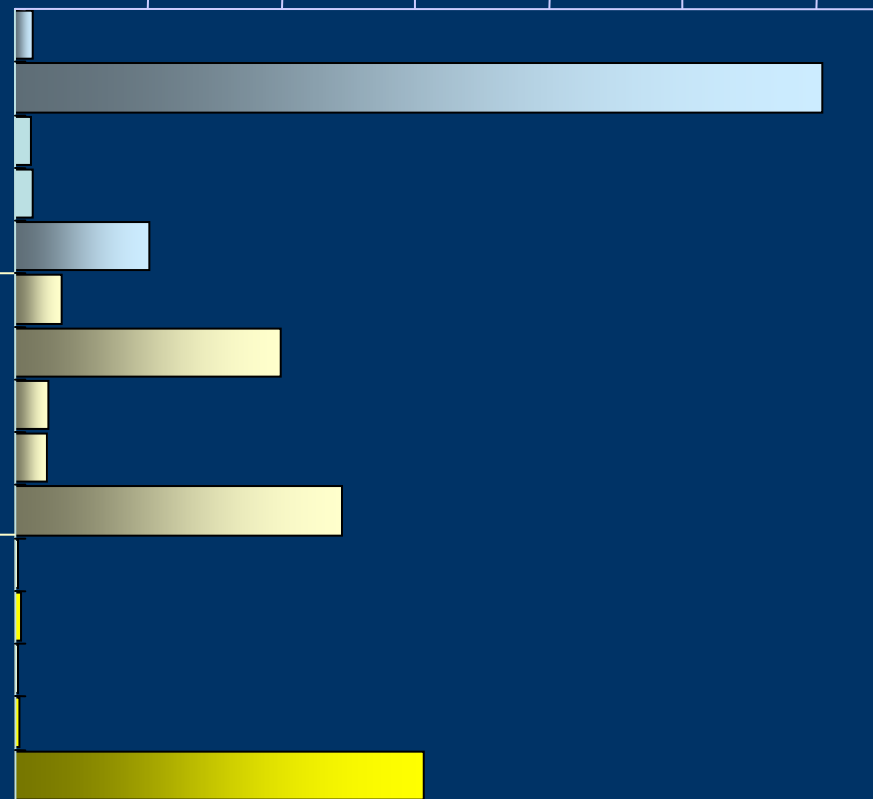
control

AIP1

(Ala<sup>5</sup>)AIP1

AIP2

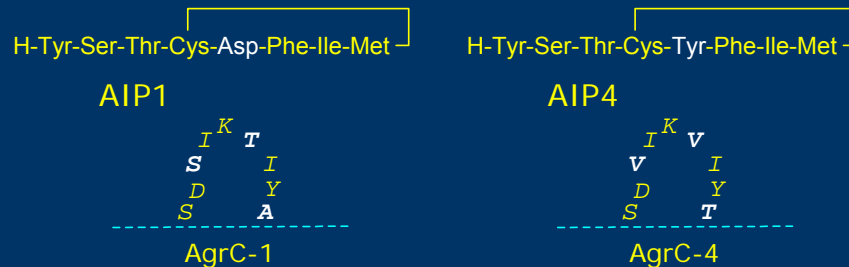
AIP4



# 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 <sub>50</sub> (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
<i>Native and mutant AgrC1</i>		
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<sup>5</sup>)AIP-1 against mutant AgrC

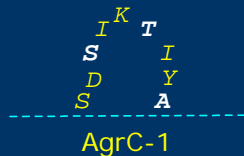


Jensen *et al* (2008) *JMB* 381: 300-309

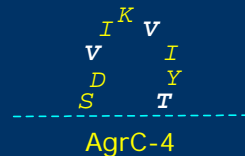
H-Tyr-Ser-Thr-Cys-Asp-Phe-Ile-Met

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

AIP1



AIP4



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

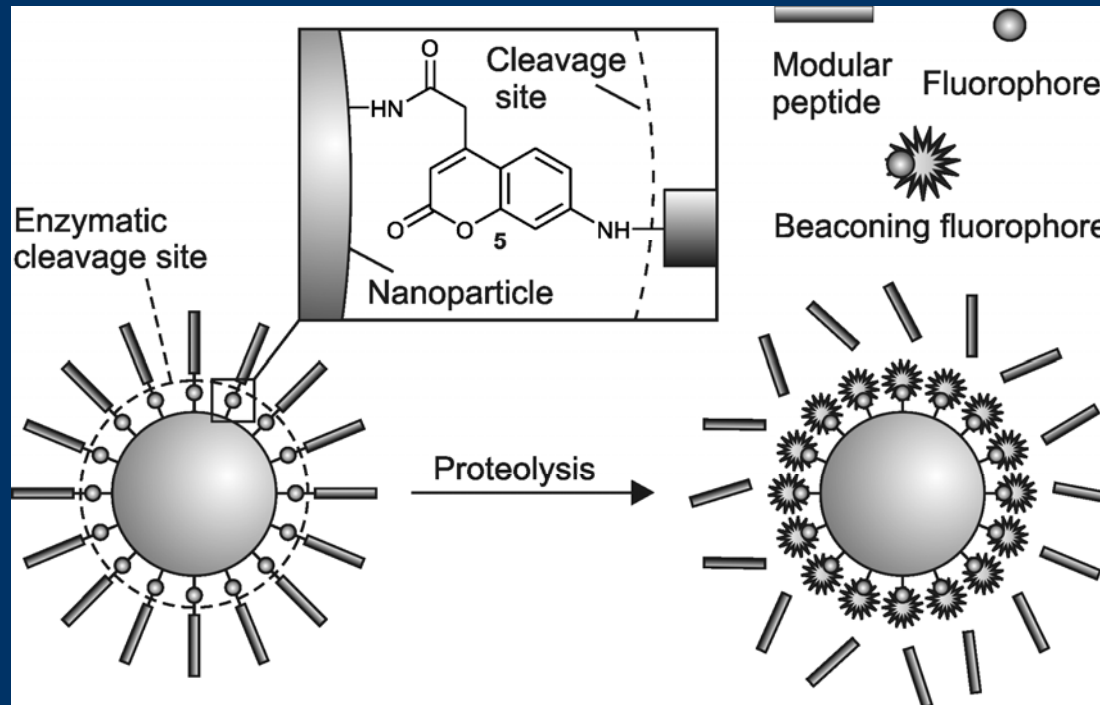
Ala → Thr  
GCA → ACA

Native and mutant AgrC4 and AgrC1 proteins	Activity (nM)	
	Activation, EC <sub>50</sub>	Inhibition, IC <sub>50</sub>
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	



- 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

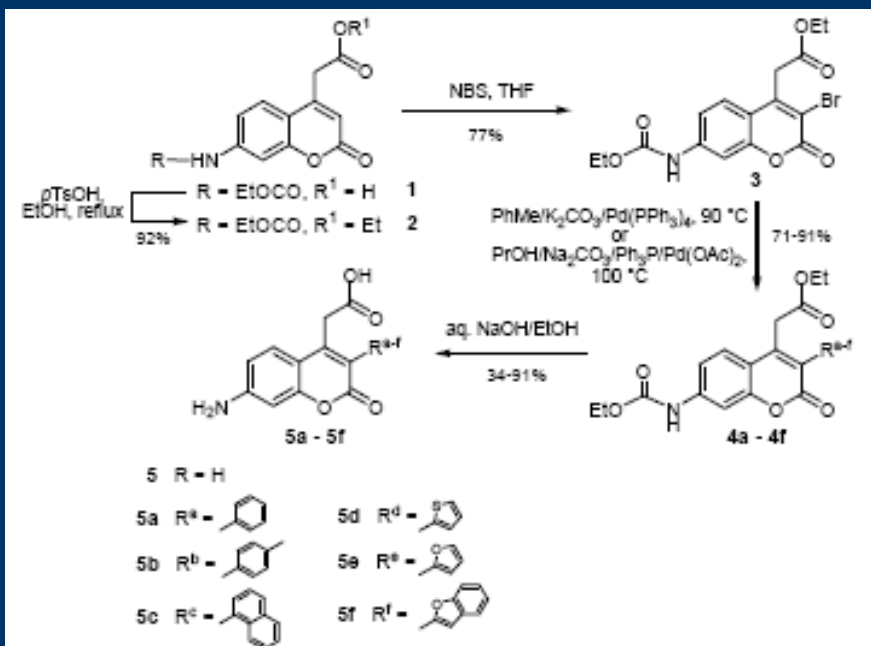


# Synthesis of bifunctional 3-arylcoumarins and their fluorogenic properties



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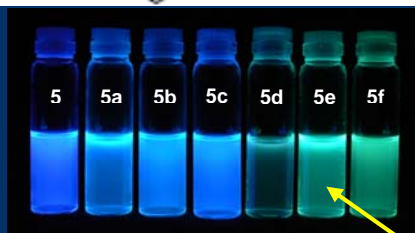
Welser *et al* (2009) *Chem Commun*: 671-673



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

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

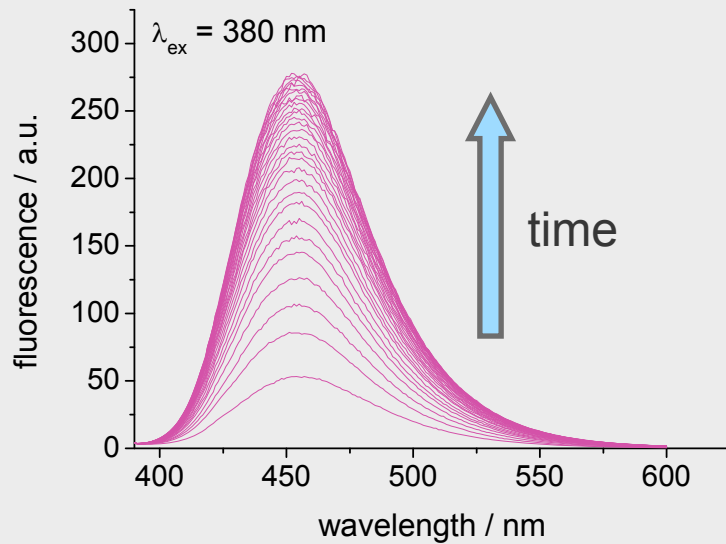
<sup>a</sup> Measurements carried out in PBS/10% DMSO. <sup>b</sup> Molar extinction coefficient at  $\lambda_{\text{max}}$  ( $\text{L mol}^{-1} \text{cm}^{-1}$ ; EtOH/5%DMSO). <sup>c</sup> Quantum yields. <sup>d</sup> Fluorescence lifetime; the numbers in parenthesis represent relative amplitudes.



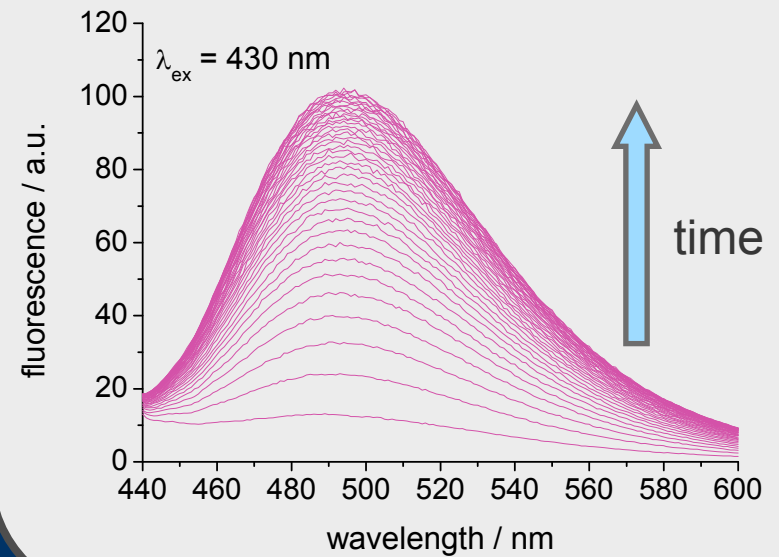
# Proteolysis of subtilisin responsive nanoprobes



Z-Gly-Gly-Leu-**ACA**- $\beta$ -Ala-



Z-Gly-Gly-Leu-**ACA(furyl)**- $\beta$ -Ala-





- 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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