Magic activity of β-lactams against intracellular Methicillin-Resistant *S. aureus*: role of acidic pH

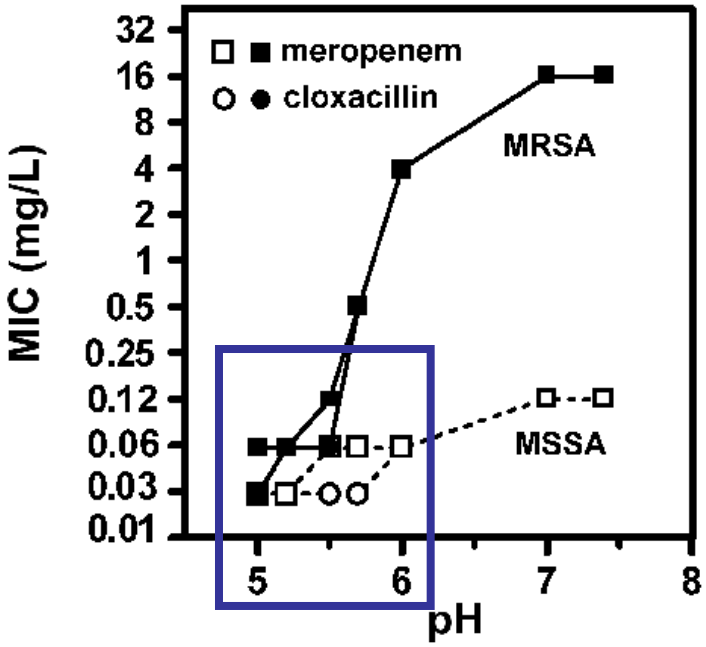
Sandrine Lemaire, Françoise Van Bambeke and Paul M. Tulkens

Unité de Pharmacologie cellulaire et moléculaire
Université Catholique de Louvain,
Brussels, BELGIUM

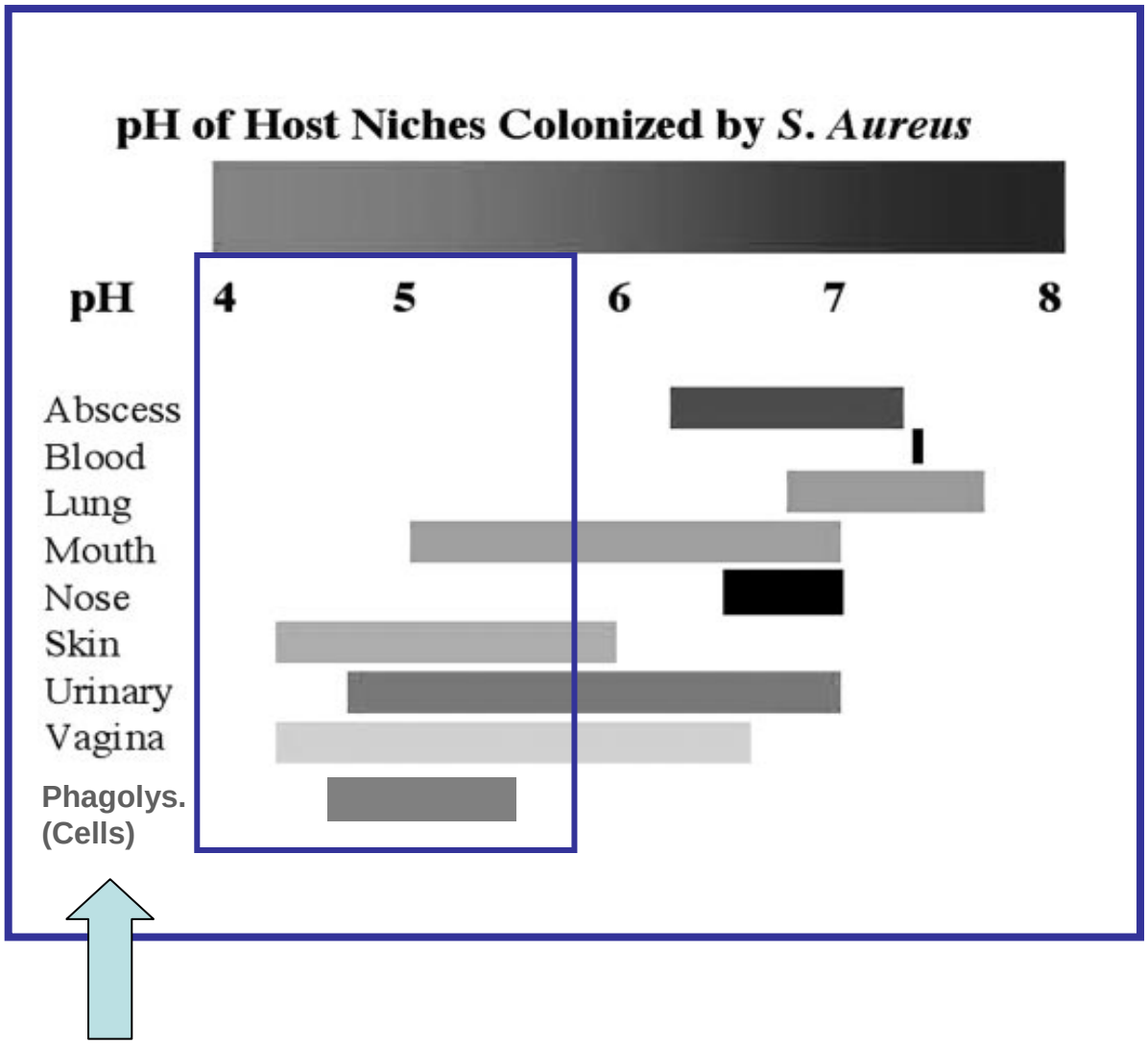
Methicillin Resistant *S. aureus* (MRSA)

- Reduced susceptibility to β-lactams
- Acquisition of a low-affinity Penicillin Binding Protein 2a (transpeptidase activity)
- Exposition to a low pH (5.0 to 5.5) has been shown to significantly reduce methicillin resistance

  (Sabath et al, AAC, 1972; Lemaire et al, AAC, 2007)



S. aureus sojourns in various acidic environments



- *S. aureus* can grow and divide normally in a broad pH range (5 to 9)

- S. aureus survives in various acidic environments, such as the mouth, the skin, the urinary tract, the vagina, and various types of eukaryotic cells

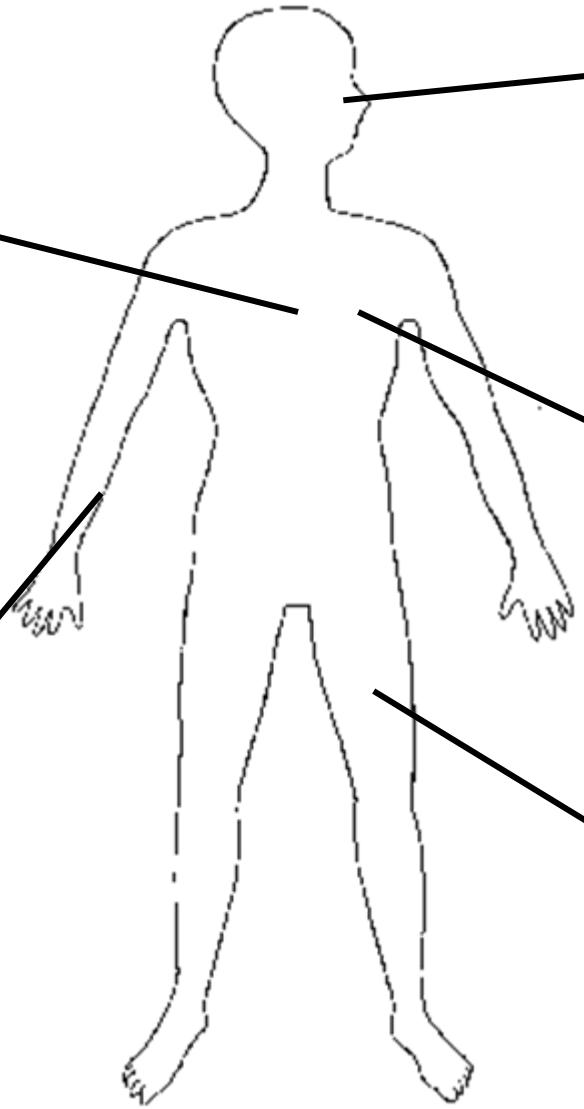
Weinrick et al, J. Bacteriol., 2004

Part I. Role for intracellular infections

Intracellular *S. aureus*

PULMONARY INFECTIONS ASSOCIATED WITH CYSTIC FIBROSIS
Jarry and Cheung, Infect Immun, 2006

RECURRENT RHINOSINUSITIS
Clement et al, J Infect Dis, 2005

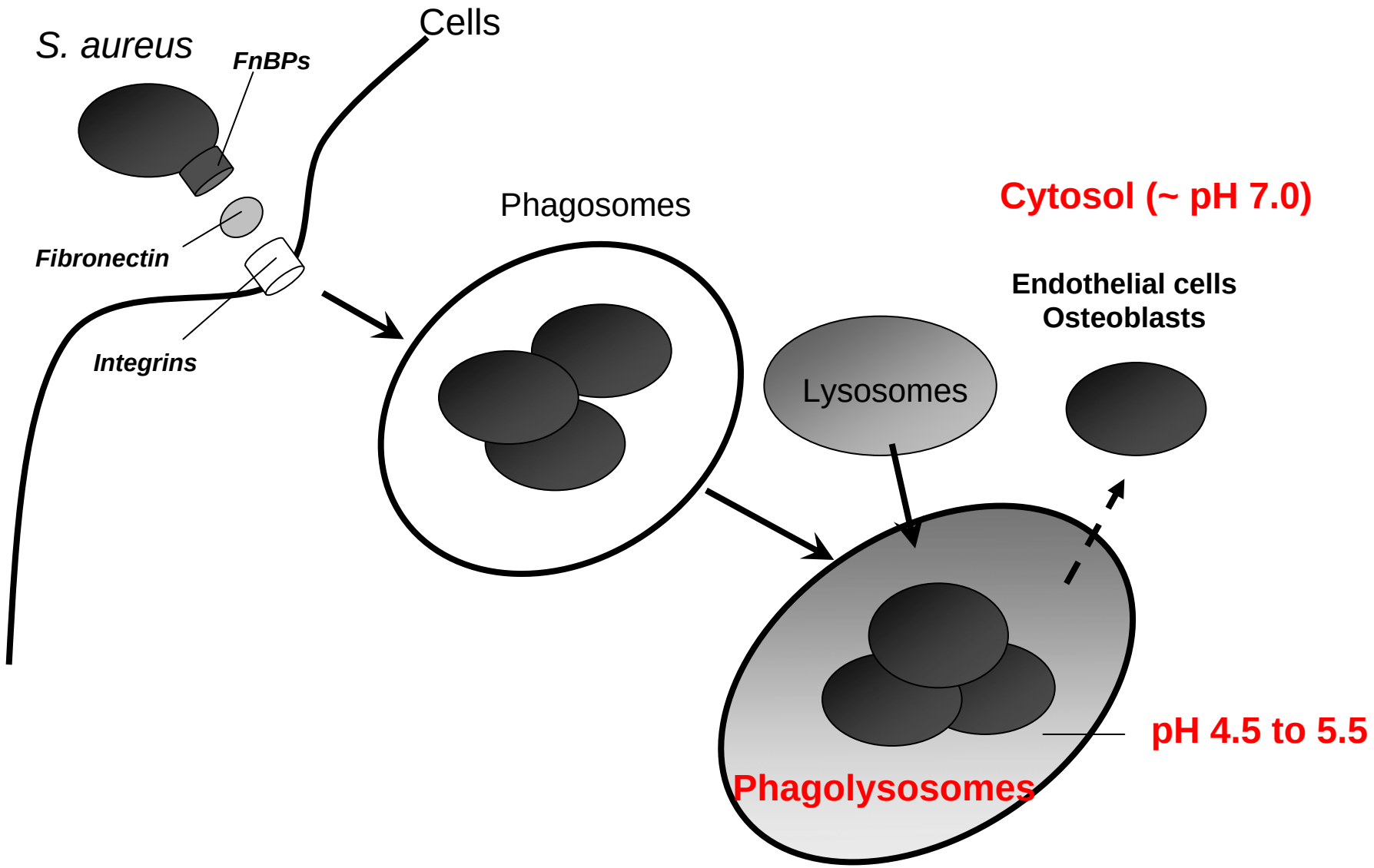


ENDOCARDITIS
Sinha and Herrmann, Thromb Haemost, 2005

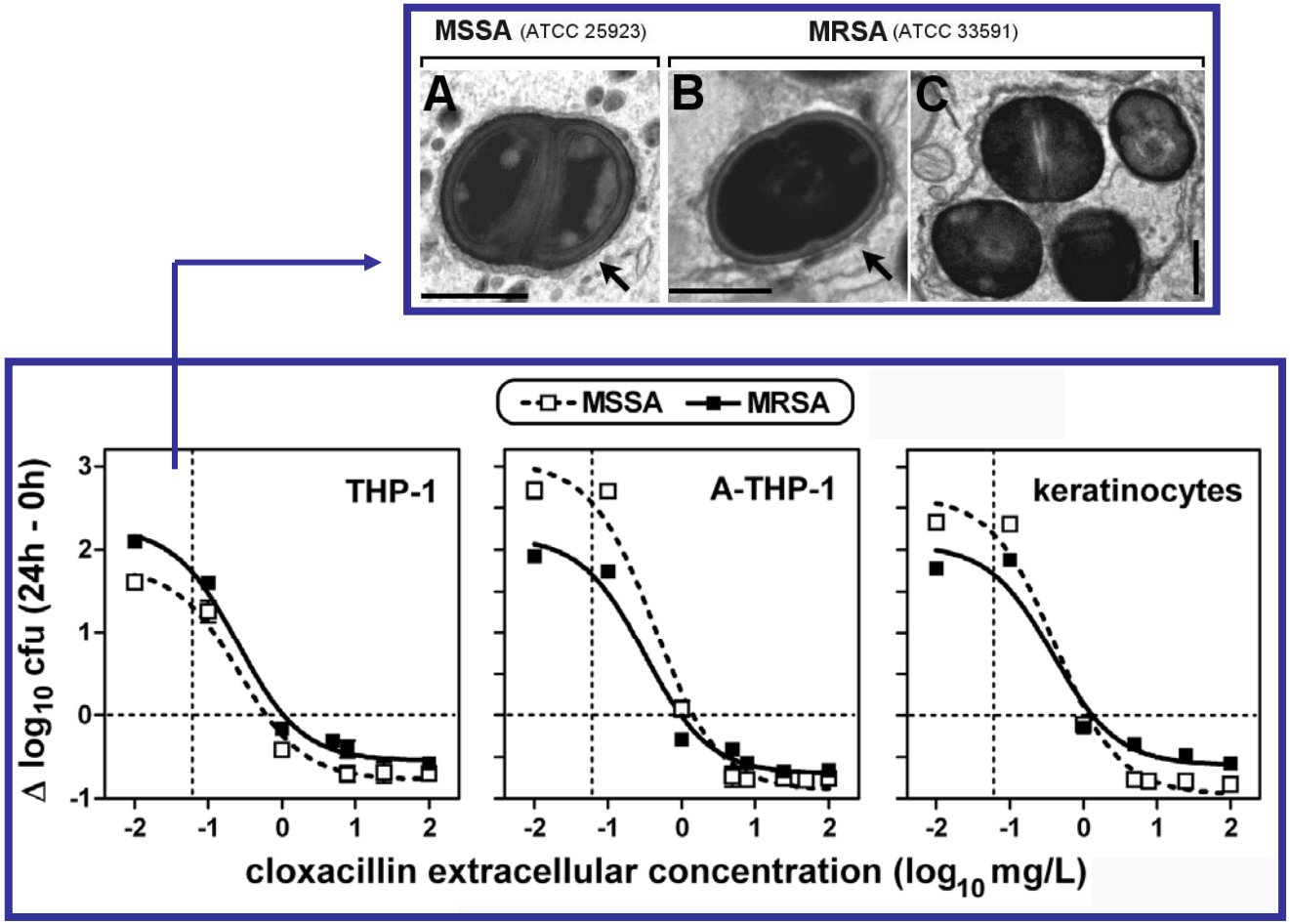
COMPLICATED SKIN INFECTIONS
Mempel et al, Br J Dermatol., 2002

OSTEOMYELITIS
Ellington et al, J Bone Joint Surg Br., 2003

Intracellular lifestyle of *S. aureus*



Restored activity of cloxacillin against intr. MRSA

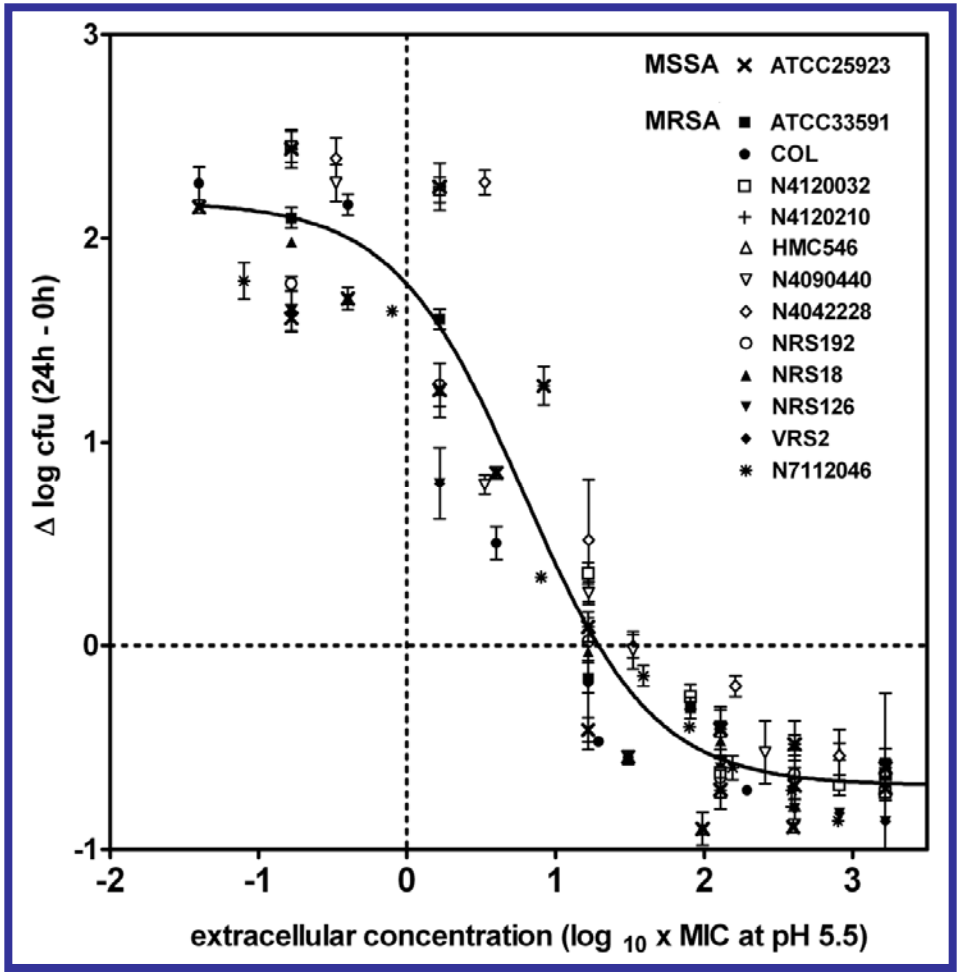


In phagolysosomial model of infection, cloxacillin shows similar activity against both MSSA and MRSA

* MSSA, ATCC 25923 ; MRSA, ATCC 33591

Lemaire et al, AAC, 2008

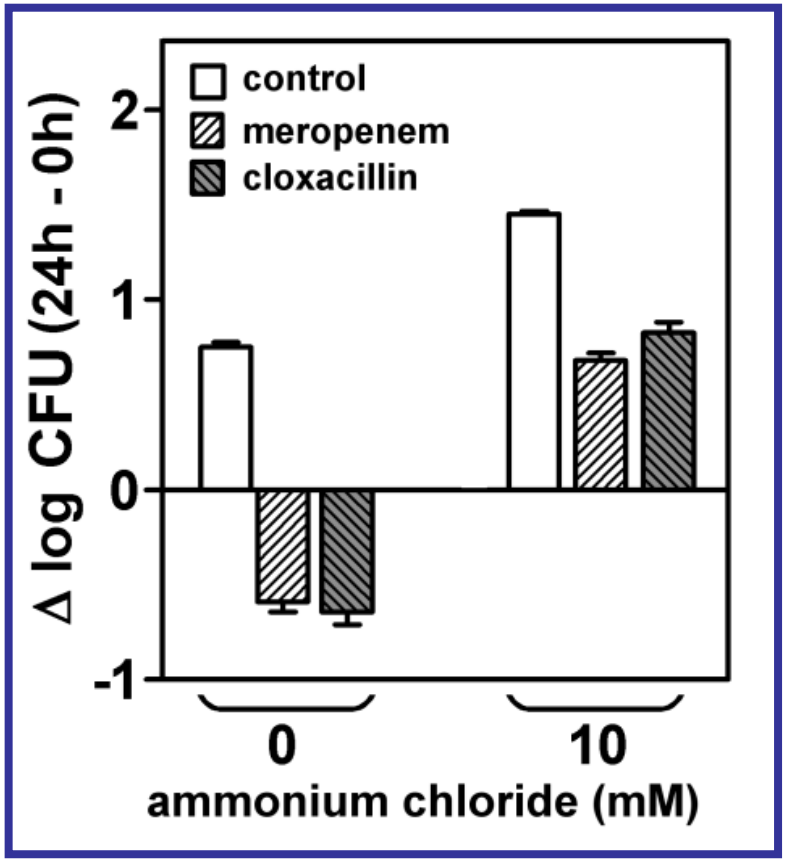
Restored activity of cloxacillin against intr. MRSA



Restoration of β-lactam activity can be extended
to MRSA isolates of current clinical and epidemiological interest

Lemaire et al, AAC, 2008

Role of acidic pH (i)

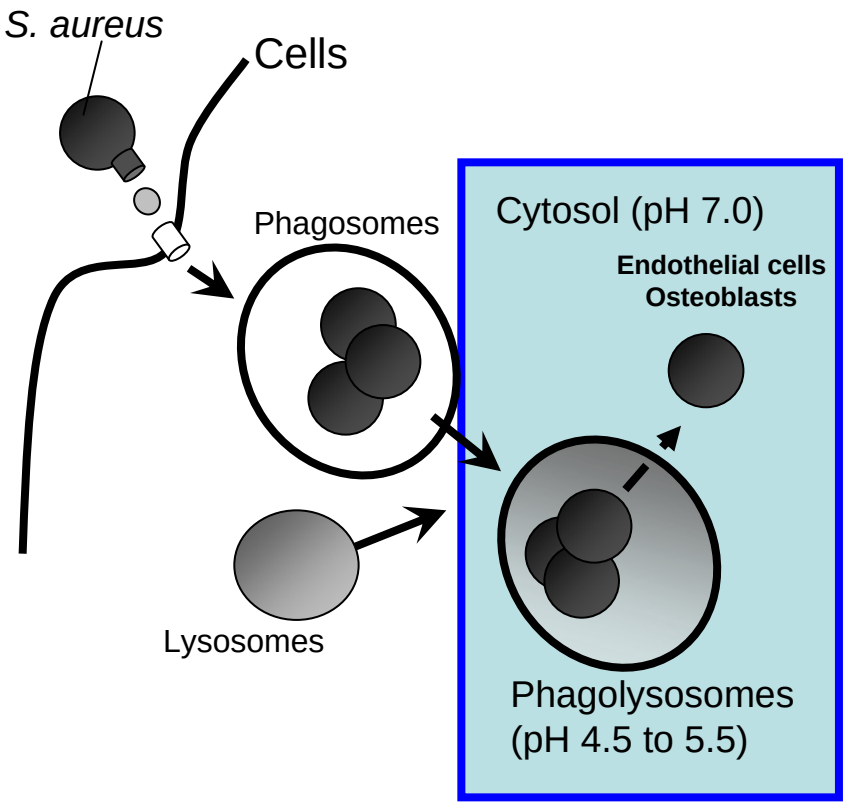


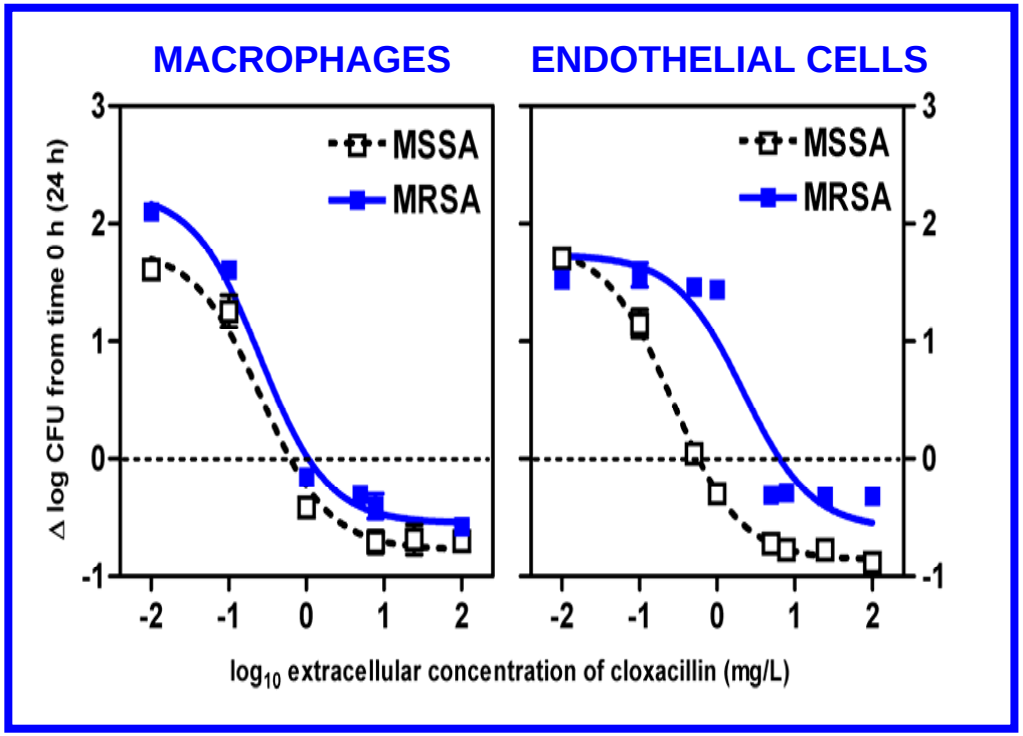
- **Ammonium chloride, a lysosomotropic agent, neutralizes the pH of lysosomal organelles**

- **Neutralization of the lysosomal pH makes MRSA insensitive to the action of both cloxacillin and meropenem**

Lemaire et al, AAC, 2007

Role of acidic pH (ii)



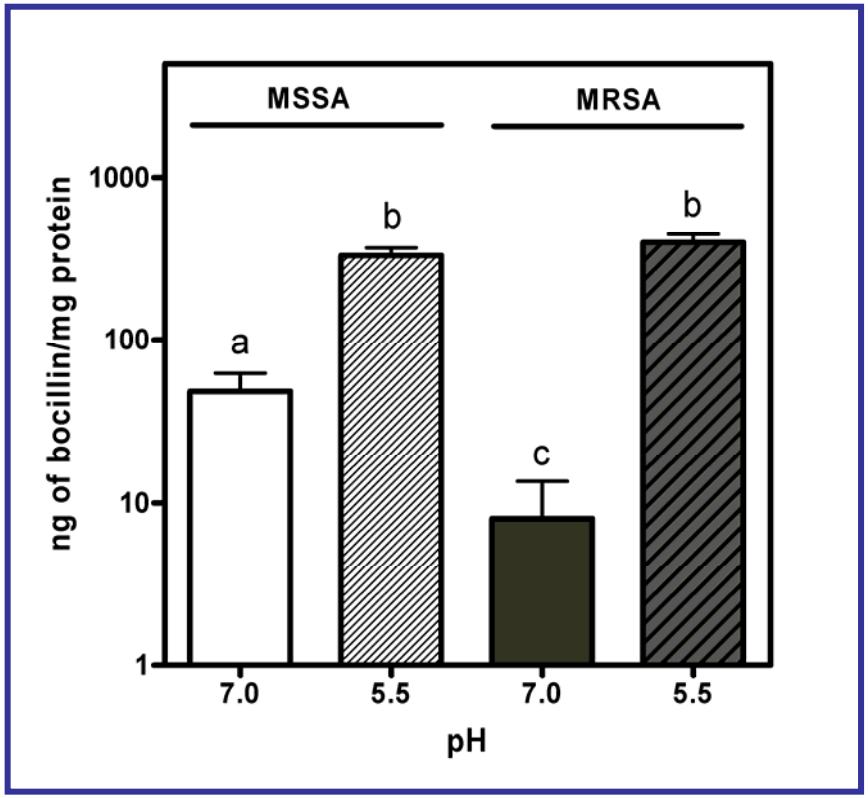


In endothelial cells (HUVEC cell line)

- *S. aureus* gains access to the cytosol (where pH is not in the acid)
- Cloxacillin shows less activity towards MRSA compared to MSSA

Part II. Role for the bacteria

A. Binding to PBPs



- The influence of pH on total penicillin-binding properties was measured with intact bacteria using BOCILLIN FL, a fluorescent derivative of penicillin V.

- MRSA grown in acidic conditions shows a larger binding of BOCILLIN FL compared to bacteria grown in neutral conditions

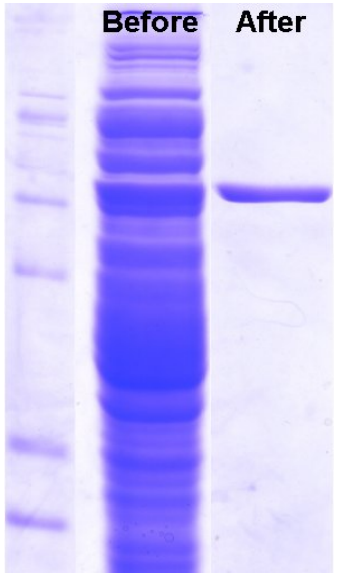
- No significant difference is noted between MSSA and MRSA for bacteria grown at pH 5.5

Lemaire et al, JBC, 2008

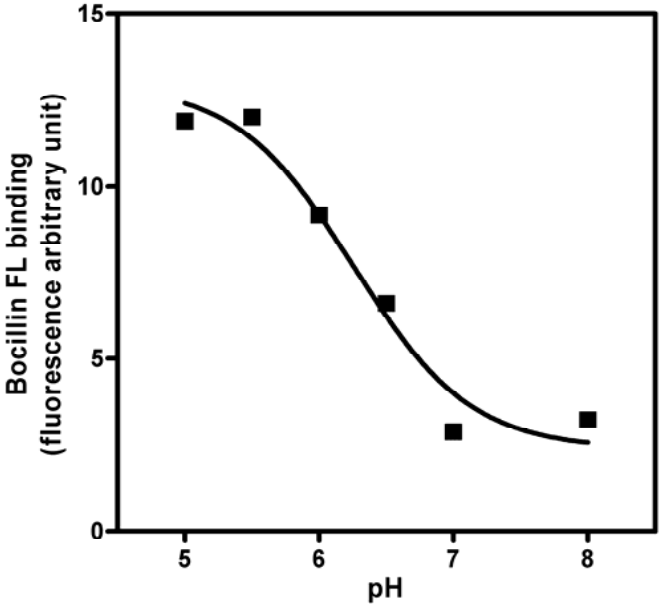
B. Studies with PBP2a (i)

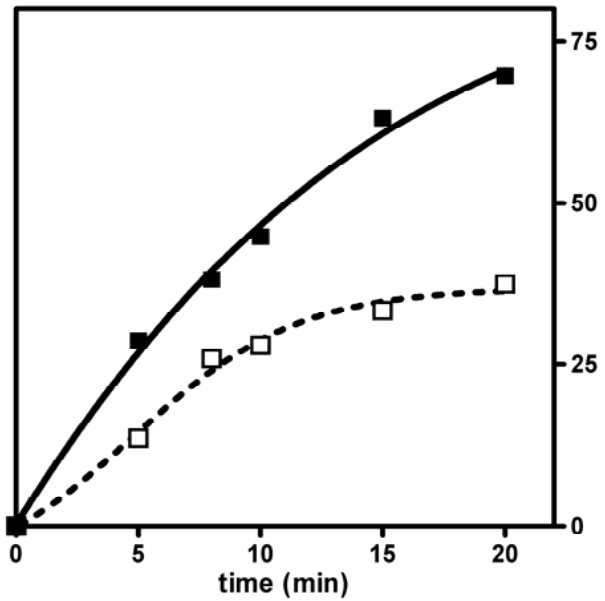
INFLUENCE OF PH ON THE BINDING TO PBP2a

Purified PBP2a



BOCILLIN FL Binding



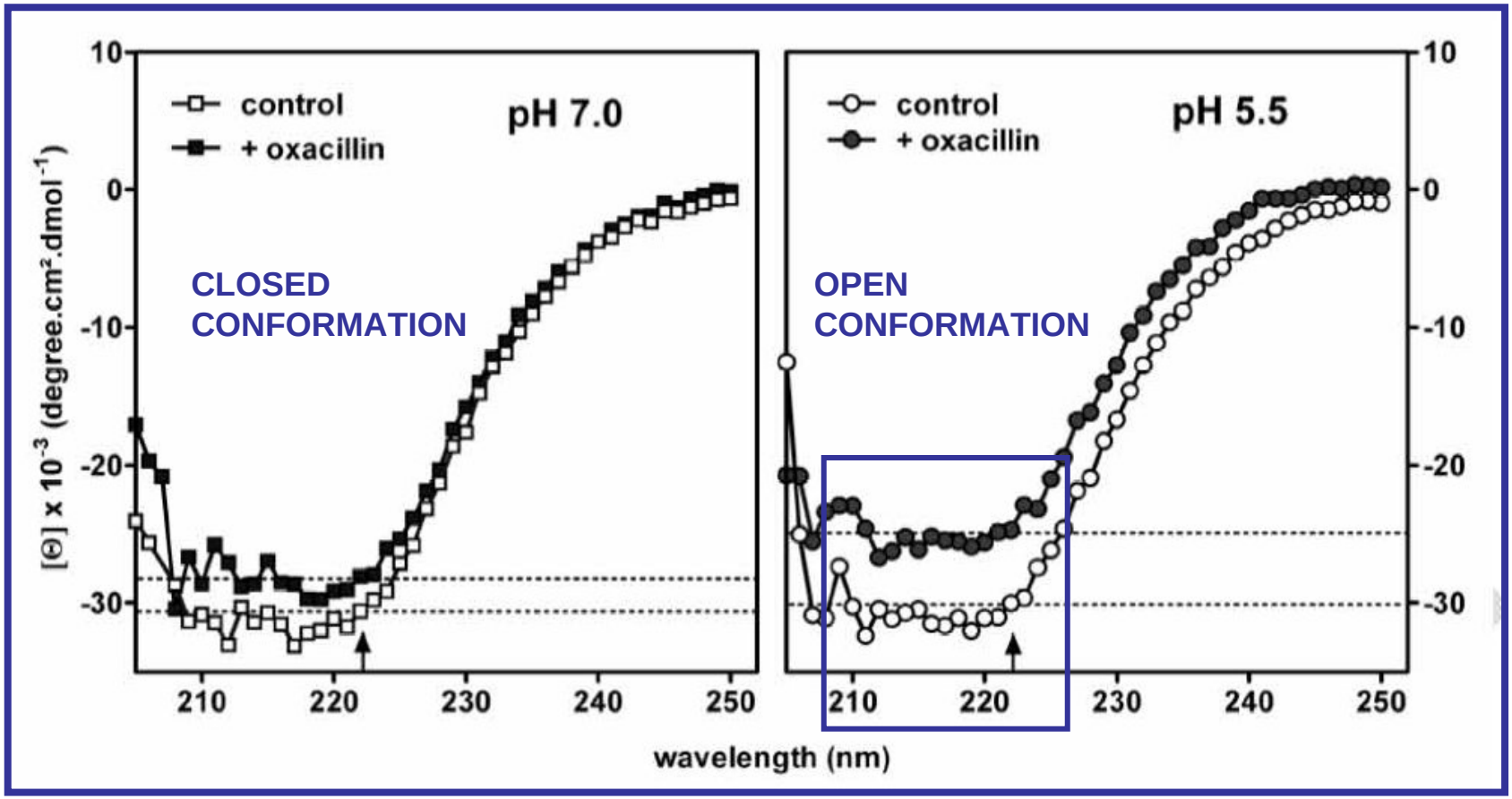


At a lower pH, PBP2a binds β-lactams more avidly and more rapidly

Lemaire et al, JBC, 2008

B. Studies with PBP2a (iii)

CIRCULAR DICHROISM SPECTRA



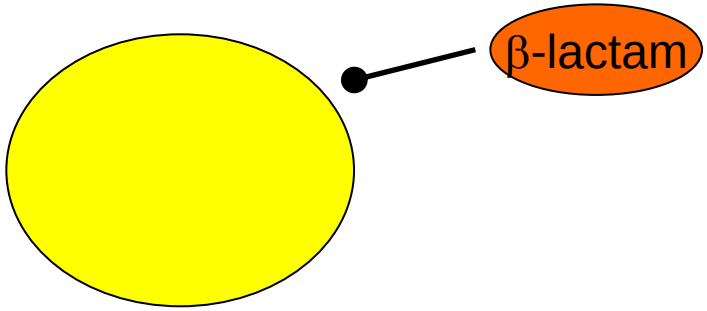
At pH 5.5 : PBP2a undergoes a conformational change in the presence of oxacillin, consistent with the opening of the active site

Lemaire et al, JBC, 2008

Conclusions: PBP2a

pH 7.0

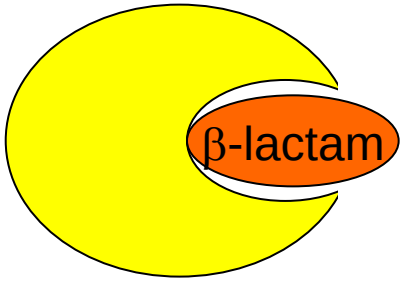
PBP2a : closed conformation of the active site



- Poor access by the antibiotic to the active site of the protein
- Poor inhibition of PBP2a by β-lactam, conferring resistance to these agents

pH 5.5

PBP2a : open conformation of the active site



- Improved access by the antibiotic to the active site of the protein
- Increased inhibition of PBP2a by β-lactam

Acknowledgments

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all the old and new FACMists …

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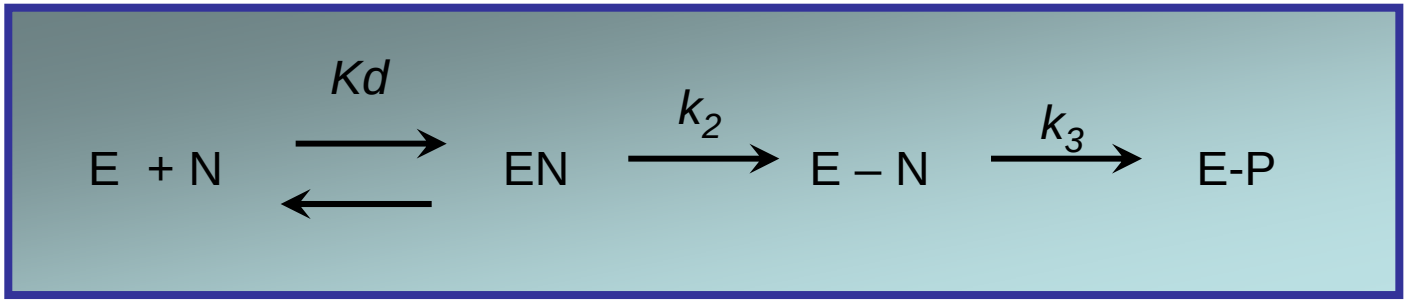
B. Studies with PBP2a (ii)

INFLUENCE OF PH ON KINETIC PARAMETERS OF INTERACTIONS BETWEEN NITROCEFIN AND PBP2A

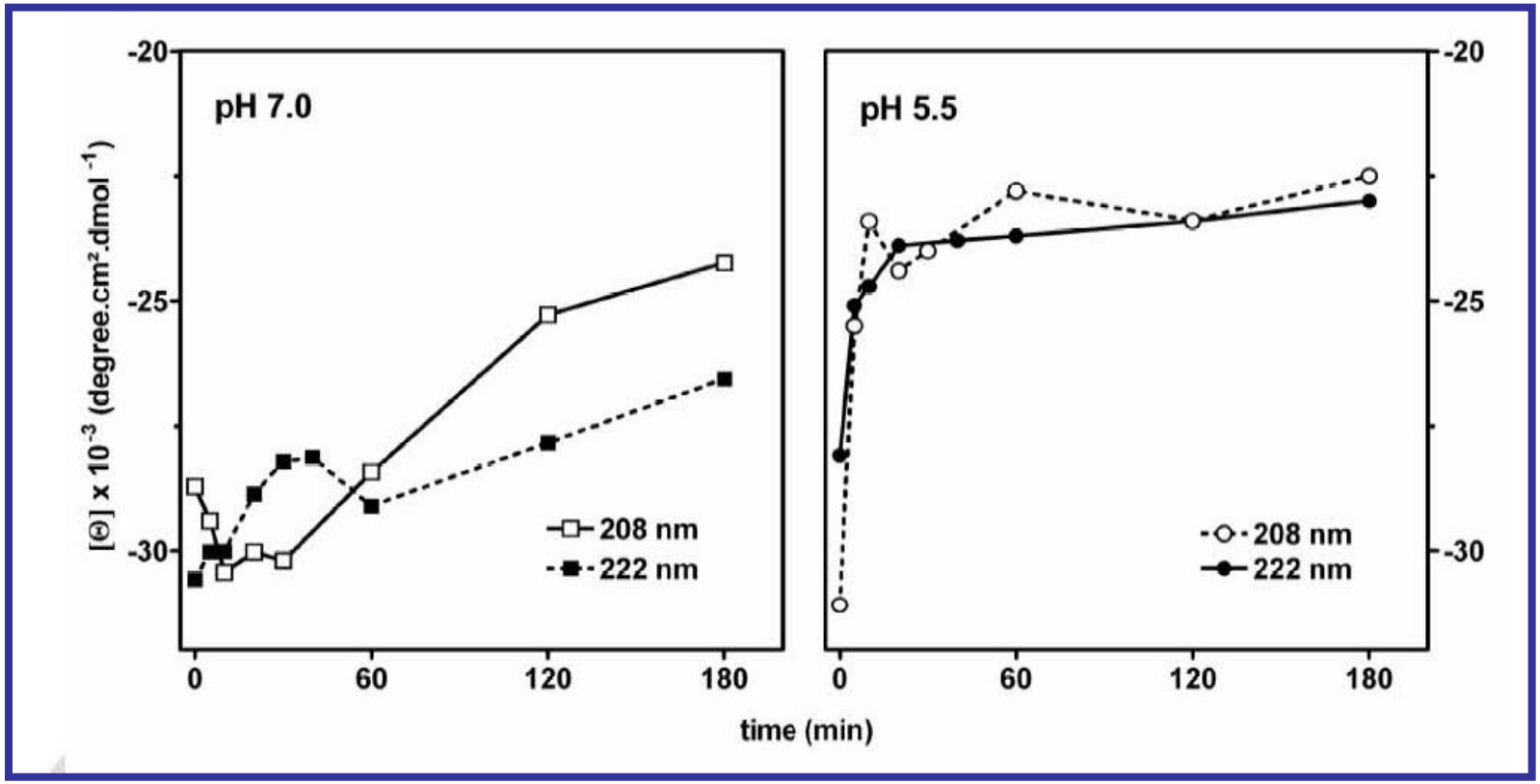


Parameters	pH	
	7.0	5.5
Kd (µM)	195 ± 28	100 ± 30
k_2 (s^{-1} x 10^3)	6.0 ± 0.6	15.2 ± 1.5
k_3 (s^{-1} x 10^6)	2.5 ± 0.2	2.8 ± 0.2

Lemaire et al, JBC, 2008

B. Studies with PBP2a (iv)

CIRCULAR DICHROISM SPECTRA



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Lemaire et al, JBC, 2008