# New insights into the effect of azithromycin on Pseudomonas aeruginosa


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## Aantibiotics, *Aeruginosa*, Attachment

- Antibiotics resistance is a global threat of increasing significance (1)
- Established methods focus on killing bacteria, leading to emergence of resistance (Antibacterials)
- Targeting diseases factors instead may reduce resistance development (Antivirulants)
- One of the most significant factors is biofilm formation developing trough bacterial attachment
- Presented here is a new method for measuring attachment kinetics in high throughput – Here *Pseudomonas aeruginosa* PAO1 exposed to several concentrations of Azithromycin
- Infections of pseudomonas aeruginosa, are often treated using Azithromycin, causing reduction of biofilm formation.

## High-throughput, quantitative measurement of bacterial attachment kinetics on seconds scale

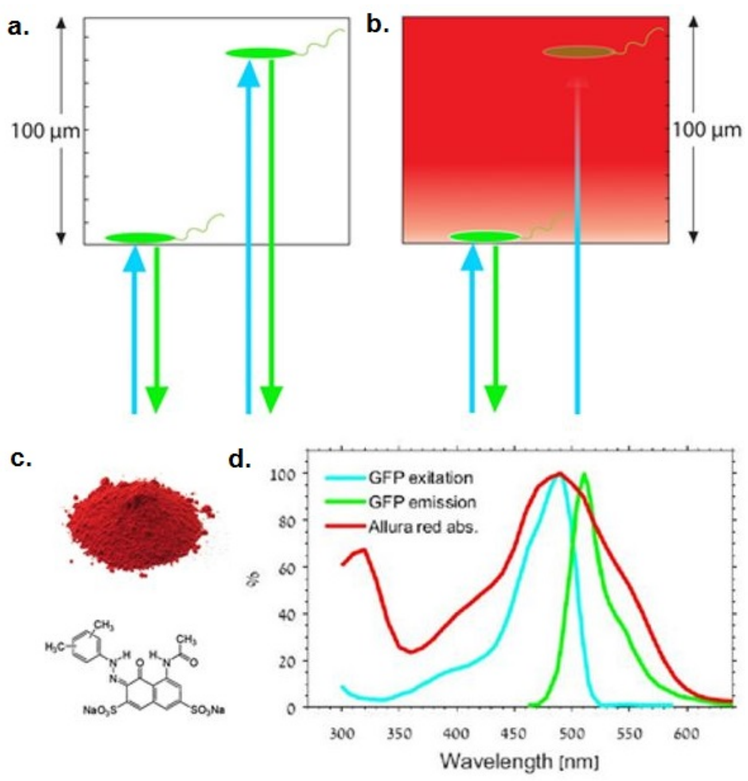


**Figure 1** experimental setting bacterial fluoresce from different depth within the microtiter well in absence (**a**) and presence (**b**) of dye **c.** Allura red AC powder and structure **d.** Allura red AC absorption spectrum vs. $GFP_{mut2}$ excitation and emission spectra.

To follow bacterial attachment a fluorescently tagged culture is read from the bottom of a standard microtiter plate. Supplementing the culture with dye allows focusing on the signal emitted by attached bacteria (Figure 1). This setting allow repeated measurement in high frequency.

## Attachment kinetics in several Azithromycin concentrations

GFP tagged bacteria were cultivated and diluted in M9 medium to $OD_{600nm}$=0.01 in several azithromycin concentrations and supplemented with dye in a microtiter plate. Bottom fluorescence was read every 2 minutes for a period of 24 hours to follow bacteria attachment

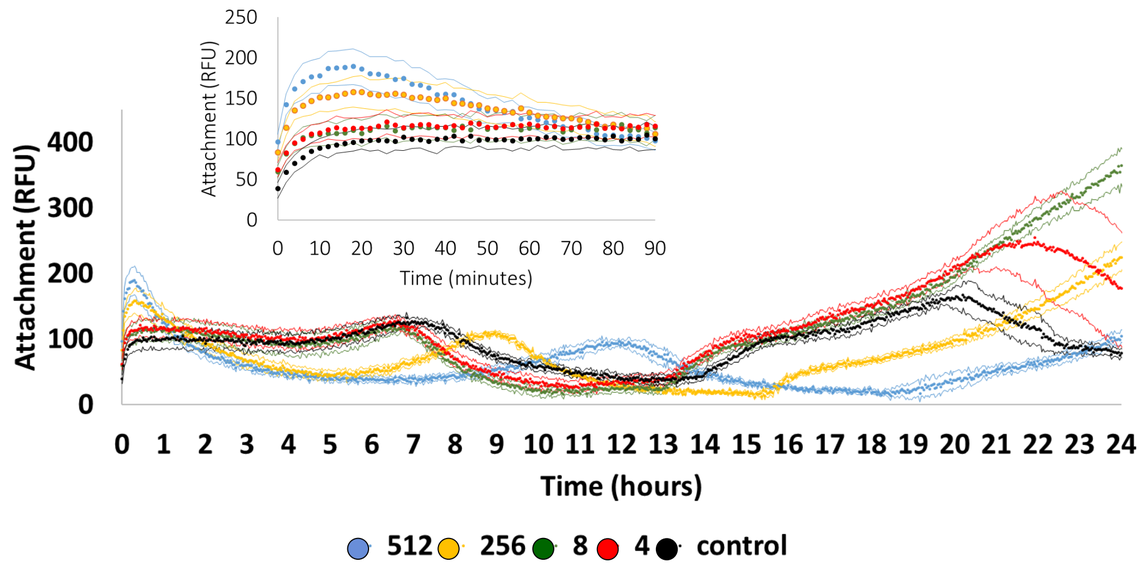


**Figure 2** Pseudomonas aeruginosa attachment in various Azithromycin concentrations, n=7 (per treatment), symbols represent sampling times, flanking curves stand for ±1SD. Blowout of the first 90 minutes shows the sampling rate enabled by the system.

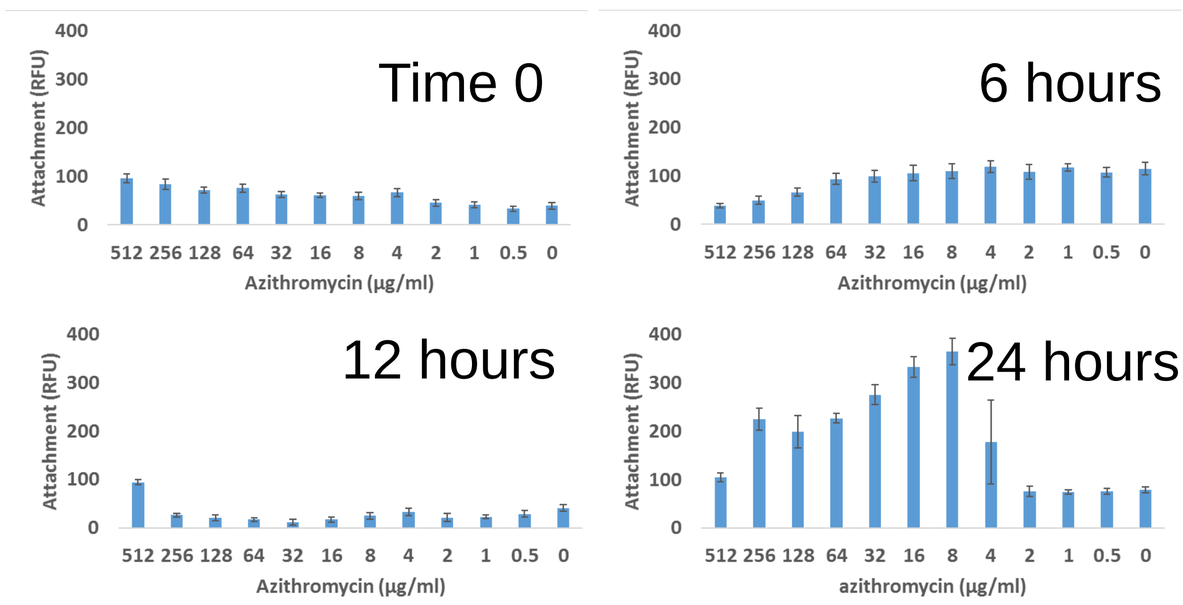


**Figure 3** Pseudomonas aeruginosa attachment in various Azithromycin concentrations at various time points along the 24 hours kinetics. n=7, Error bars stand for ±1SD.

- Bacterial attachment and biofilm formation are highly dynamic, and modulation of attachment in reaction to different azithromycin concentrations is complex
- Measured values are highly dependent on sampling time
- A kinetic approach allows a more complete picture and may assist in the development of effective treatments

## Bibliography


1. Ventola, C. L. (2015). The antibiotic resistance crisis: part 1: causes and threats. Pharmacy and Therapeutics, 40(4), 277.

2. Kimura, S., Mori, N., Kai, T., Ishii, Y., Yamaguchi, K., & Tateda, K. (2017). Azithromycin Modulates 3′, 5′-cyclic Diguanylic Acid Signaling in Pseudomonas aeruginosa. Journal of infection and chemotherapy, 23(8), 550-555.

3. Shteindel, N., Yankelev. D., Gerchman. Y. (coming soon) High-throughput, quantitative measurement of bacterial attachment kinetics on seconds scale.