Biofilm Dispersing NO-Donor Cephalosporins Activated by β-Lactamase

A/Prof Mike Kelso

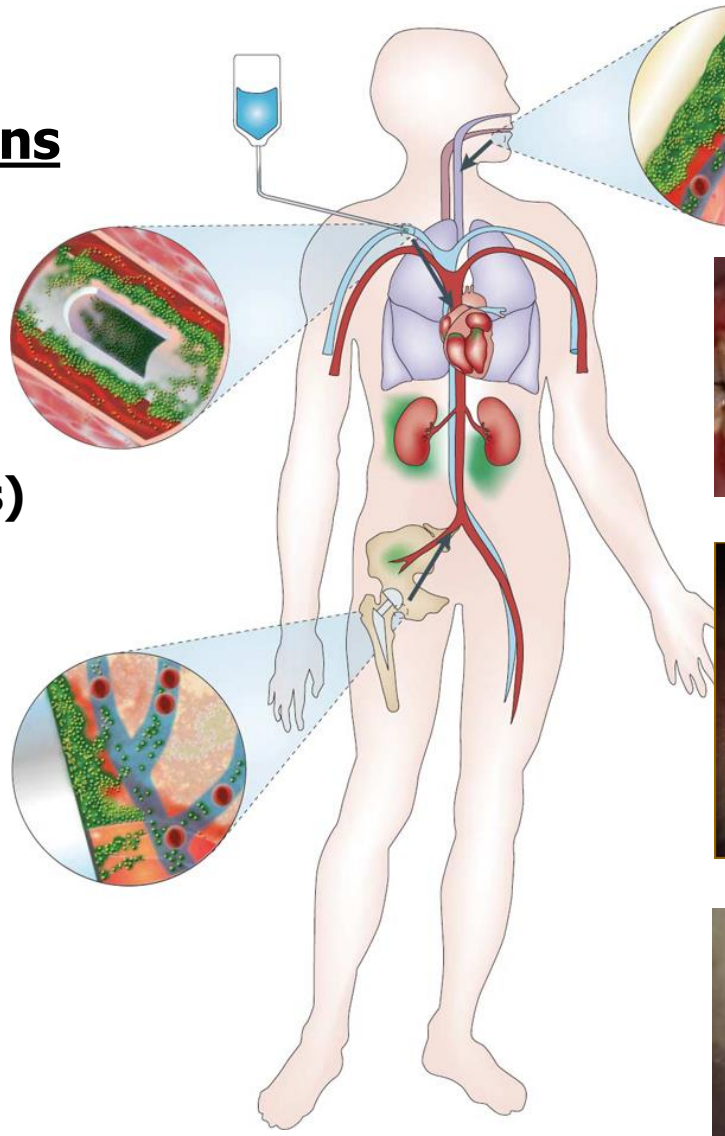
School of Chemistry
University of Wollongong
Australia

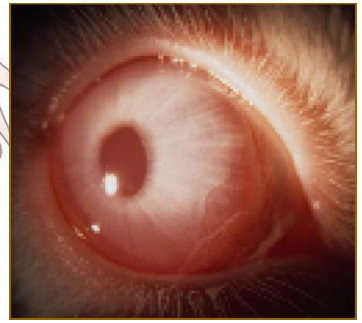
World Congress and Exhibition on Antibiotics, Las Vegas, USA Sept 2015

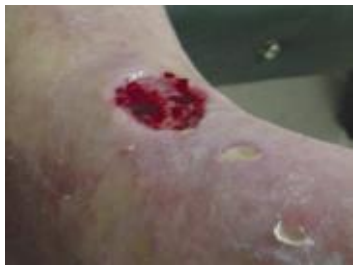
Bacterial biofilms cause chronic disease

Medical device contaminations
- Dialysis catheters
- Prosthetic implants
- Contact lenses

Tissue infections
- Oral cavity (plaque, gingivitis)
- Lungs (*P. aeruginosa* in CF)
- Urinary tract
- Cervix
- Ears (otitis media)
- Eyes
- Diabetic wounds
- Heart (endocarditis)







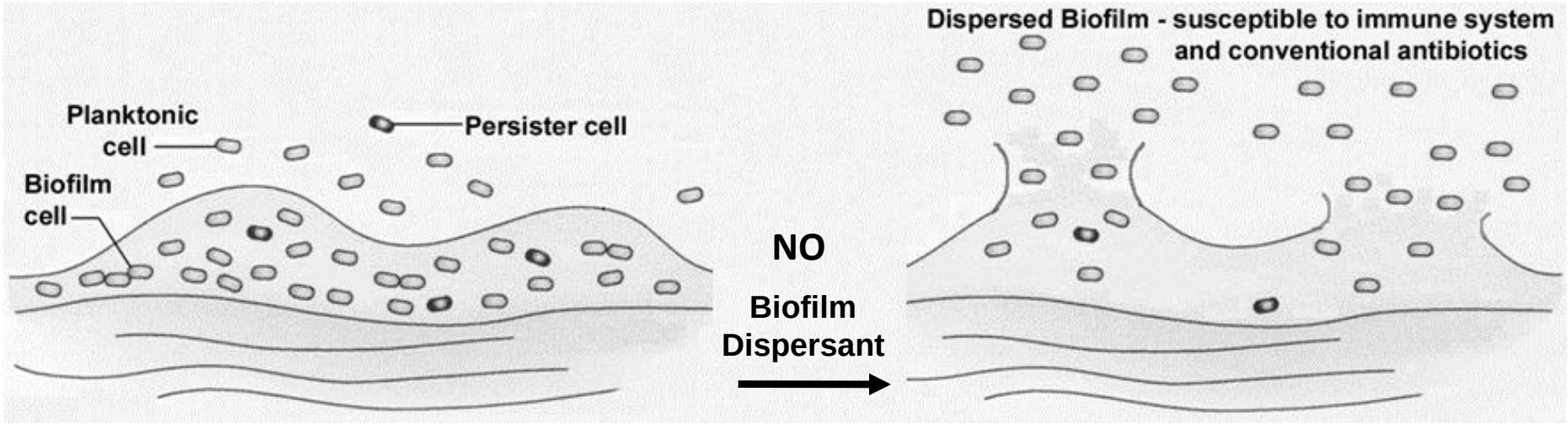
Biofilms: A Major Mechanism of Bacterial Resistance

Planktonic bacteria up to 1000x more susceptible to antibiotics

Bacterial biofilm life-cycle

- **Biofilms = chronic infections**
- **Poor penetration of antimicrobials into biofilms**
- **Physical protection from immune cells**
- **Persister cells**

<u>Hypothesis:</u> If bacteria can be induced to disperse from biofilms they will become more susceptible (up to 1000 x) to host immune defences and conventional antibiotics.



JOURNAL OF BACTERIOLOGY, Nov. 2006, p. 7344–7353
0021-9193/06/$08.00+0 doi:10.1128/JB.00779-06

Vol. 188, No. 21

Involvement of Nitric Oxide in Biofilm Dispersal of *Pseudomonas aeruginosa*

Nicolas Barraud,[1] Daniel J. Hassett,[2] Sung-Hei Hwang,[2] Scott A. Rice,[1] Staffan Kjelleberg,[1]* and Jeremy S. Webb[1,3]

School of Biotechnology and Biomolecular Sciences, Centre for Marine Biofouling and Bio-innovation, and Environmental Biotechnology Cooperative Research Centre, University of New South Wales, Sydney, NSW 2052, Australia[1]; Department of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267[2]; and School of Biological Sciences, University of Southampton, Bassett Crescent East, Southampton SO16 7PX, United Kingdom[3]

Nitric Oxide: A Key Mediator of Biofilm Dispersal with Applications in Infectious Diseases

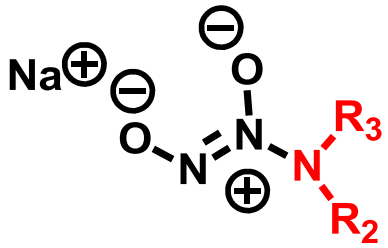
Barraud, N.; Kelso, M. J.; Rice, S.; Kjelleberg, S.

***Curr. Pharm. Des.* 2015, 21, 31-42.**

- **<u>Problem:</u>** **Systemic NO exposure may cause unacceptable side effects due to the multitude of biological functions mediated by NO in humans.**

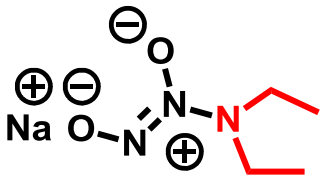
- **<u>Solution:</u>** **Localise NO release from an NO donor to the immediate vicinity of biofilm infections through the use of a biofilm-activated prodrug**

Diazeniumdiolates: Versatile NO Donors

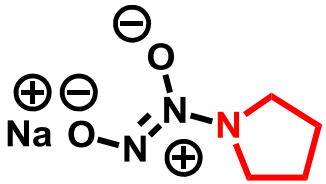


- **Stable solids that spontaneously fragment to release NO when dissolved in neutral aqueous solutions (*p*H 7)**

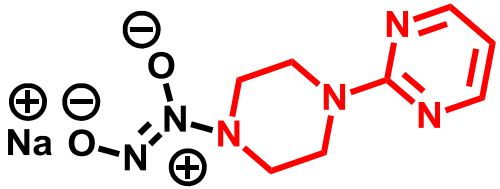
- **Half-life ($t_{1/2}$) of NO release can be tuned by varying R_2 and R_3**



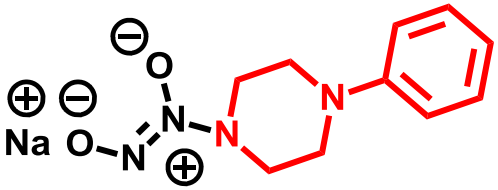
$t_{1/2}$ = 2 min



$t_{1/2}$ = 2.8 s



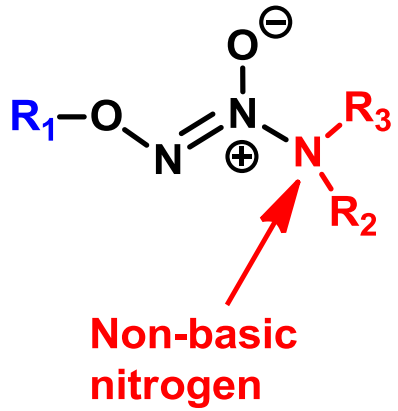
$t_{1/2}$ = 1.9 min



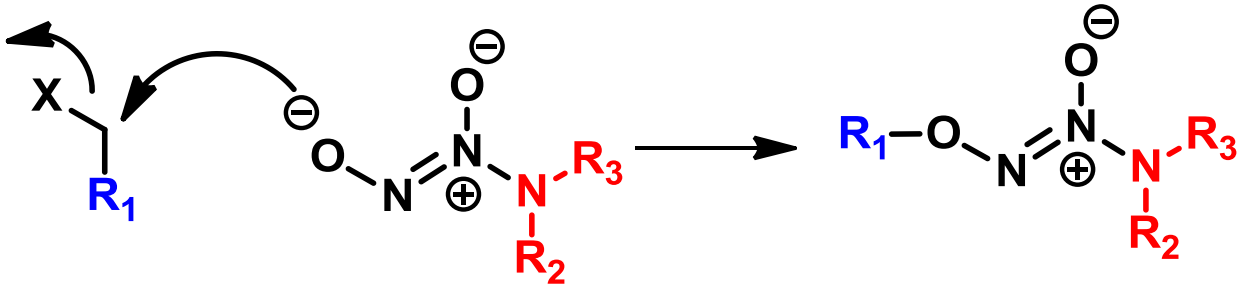
$t_{1/2}$ = 2.0 min

Diazeniumdiolates: Versatile NO Donors

- O^2-alkylated diazeniumdiolates (R_1 = alkyl group) are stable compounds

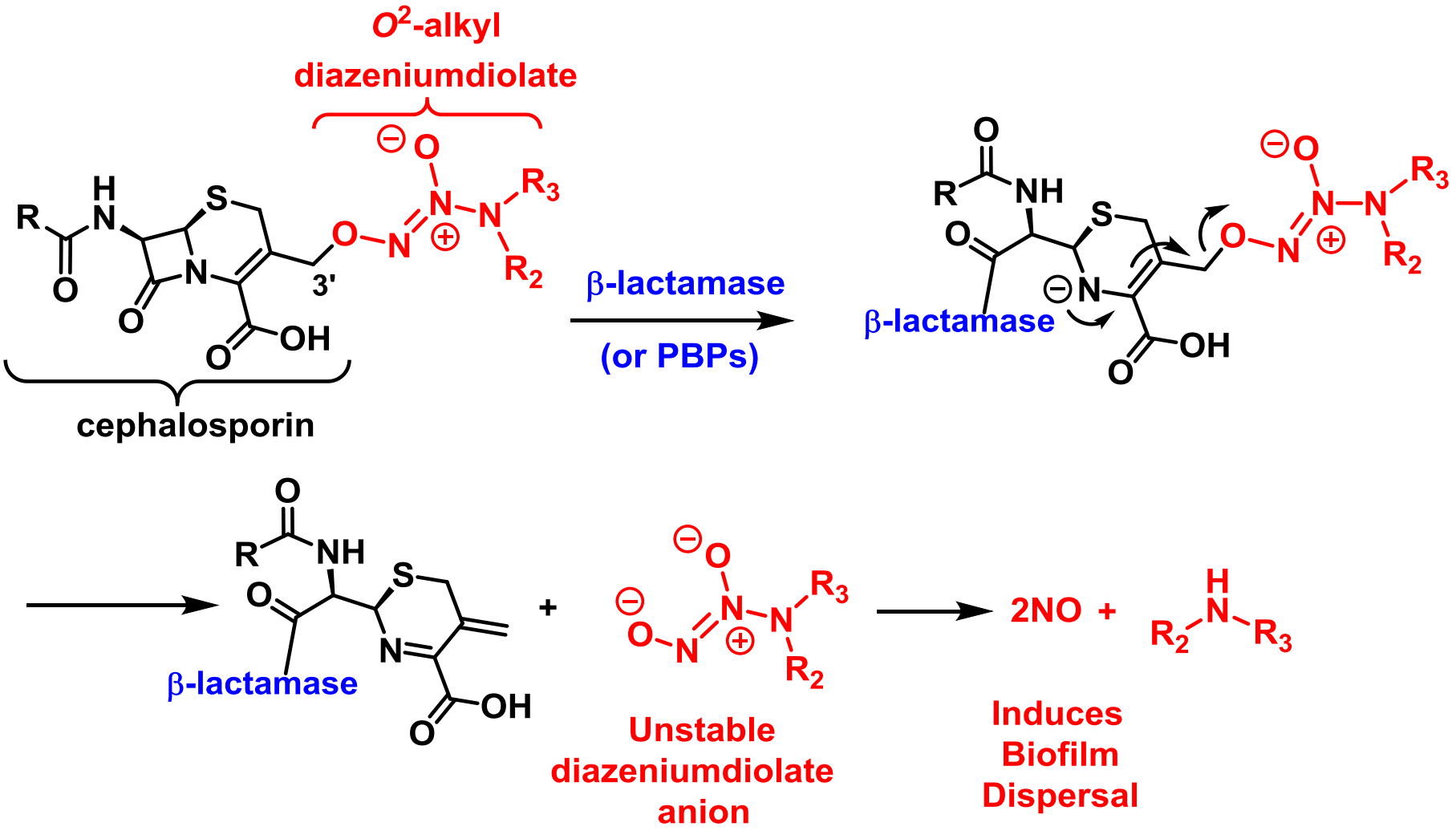


- Easily synthesised by selective O^2-alkylation with alkyl halides

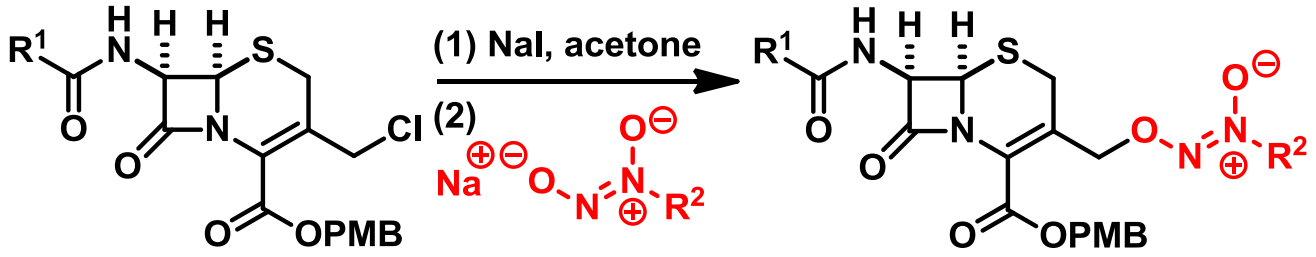


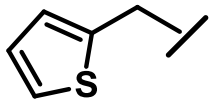
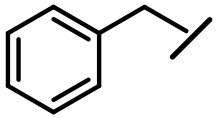
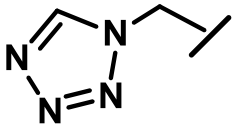
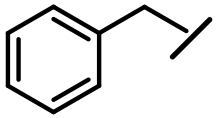
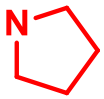
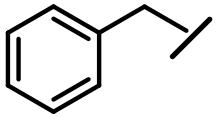
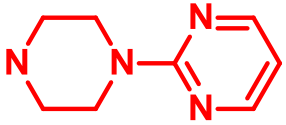
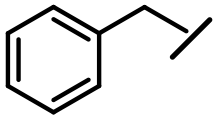
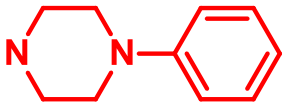
Cephalosporin-3′-Diazeniumdiolates as NO Donor Prodrugs

- Cephalosporins bearing O^2-alkyldiazeniumdiolates at the 3′position should release diazeniumdiolate anions following reaction with β-lactamases

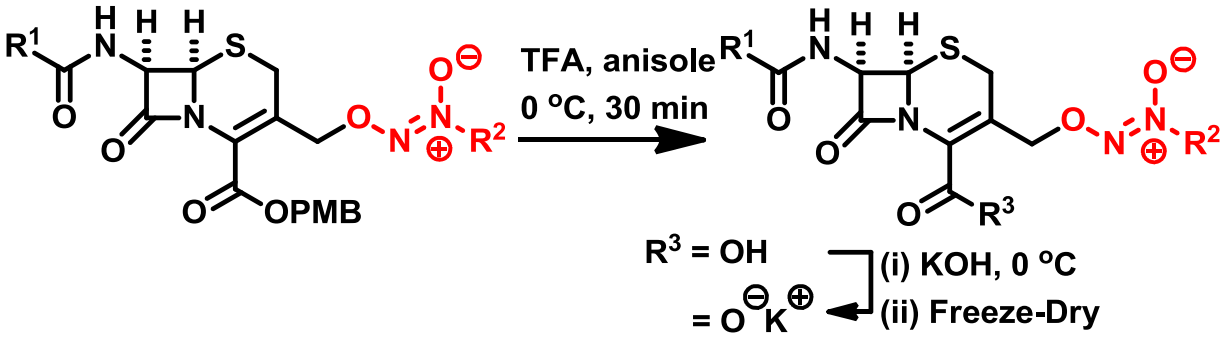


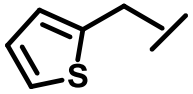
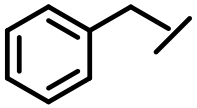
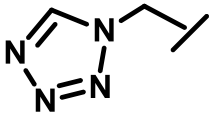
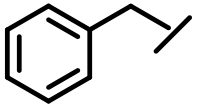
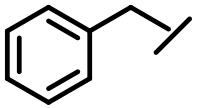
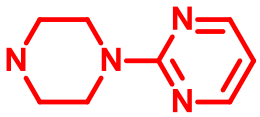
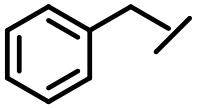
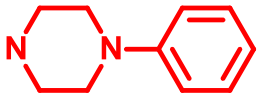
Alkylation of Diazeniumdiolates with Cephalosporins



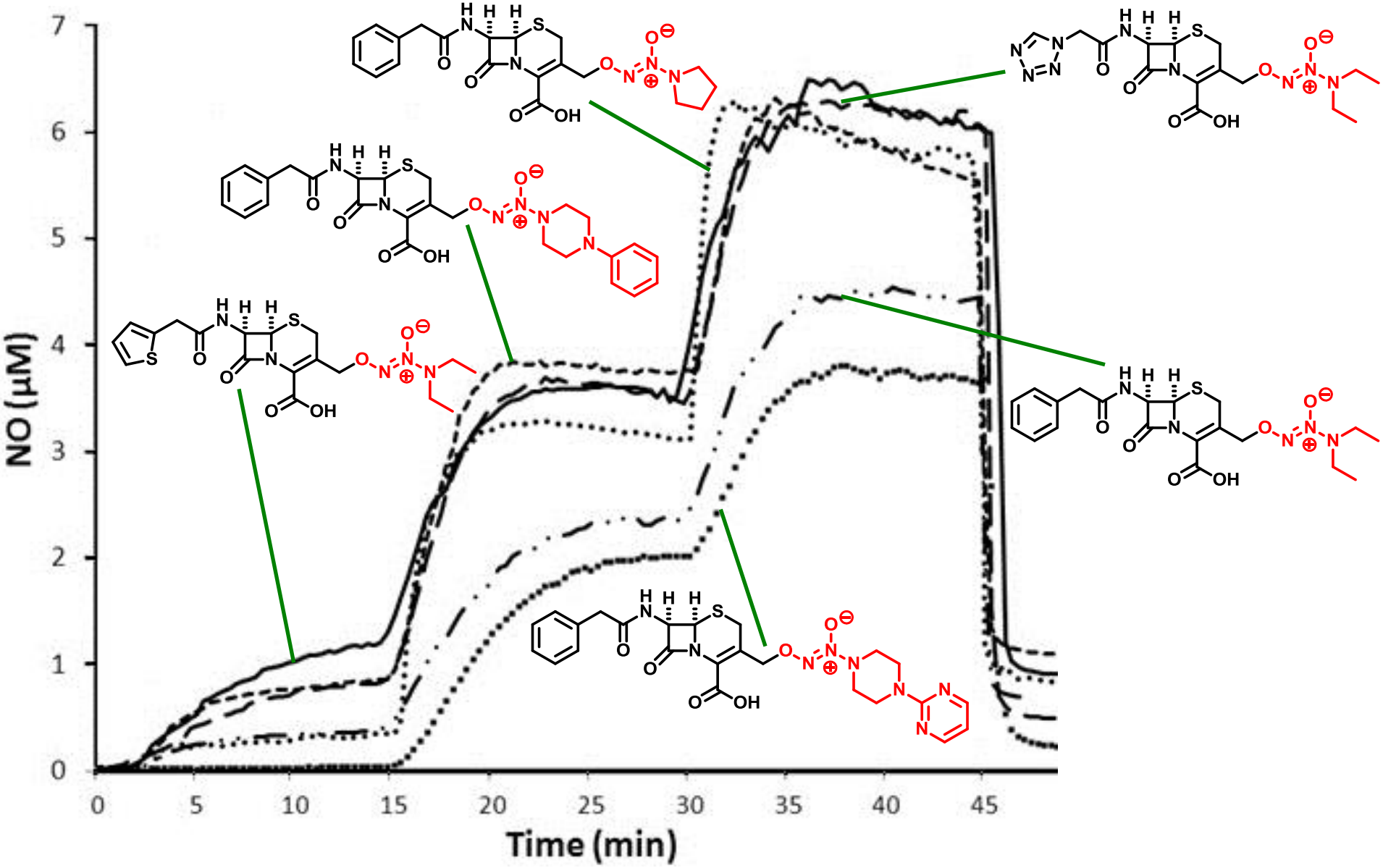
R^1	R^2	Yield %
	NEt_2	85
	NEt_2	75
	NEt_2	14
		66
		39
		80

PMB Ester Deprotection

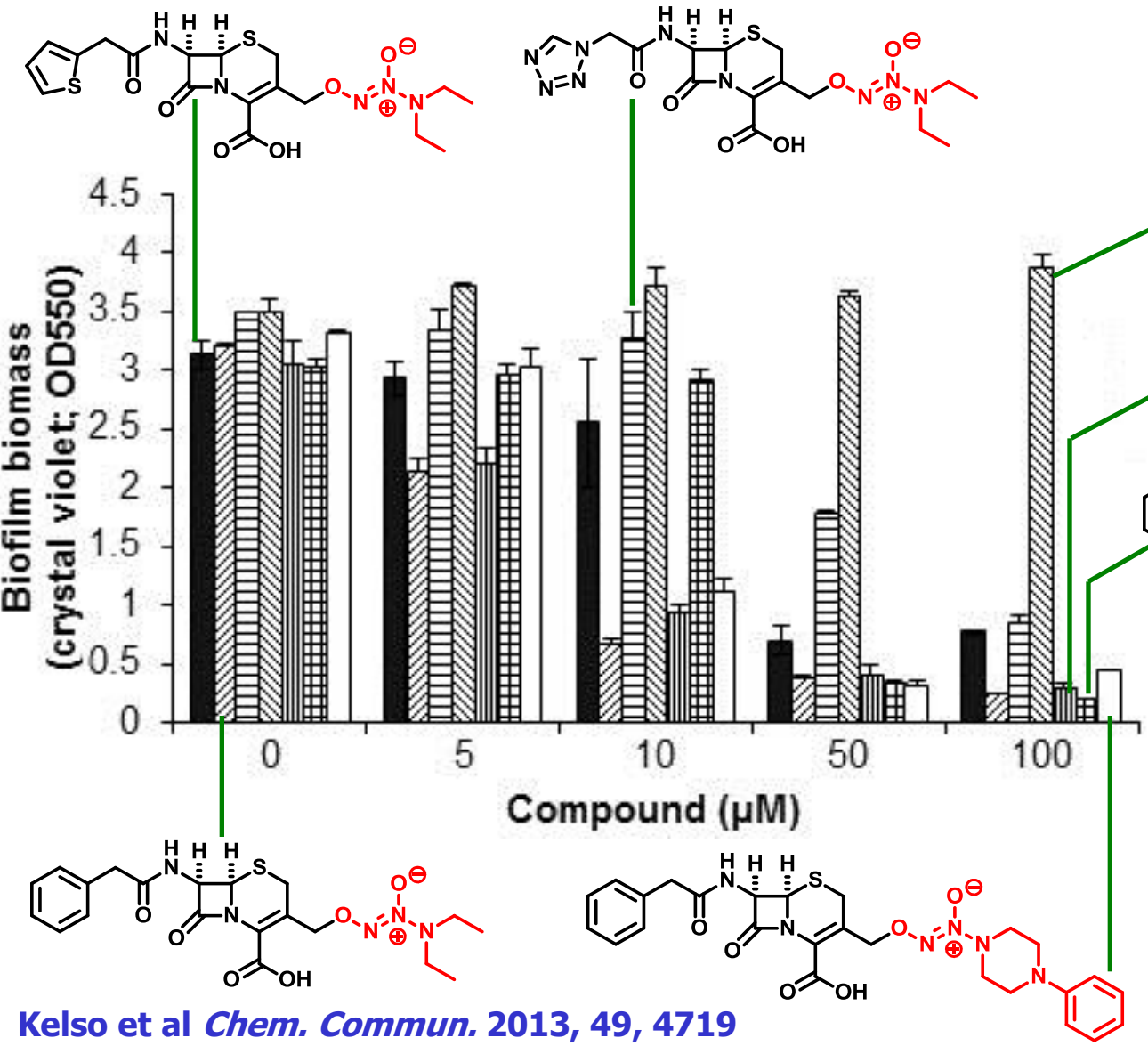


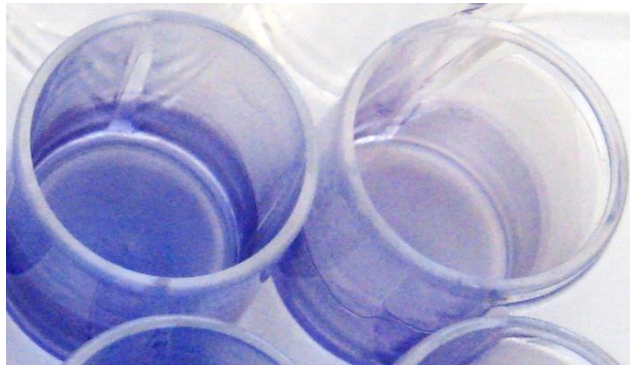
R1	R2	R3 = OH Yield %	R3 = O⊖K⊕ Yield %
	NEt_2	36	99
	NEt_2	80	99
	NEt_2	81	ND
		83	98
		93	94
		67	ND

β-Lactamase Triggered Release of NO



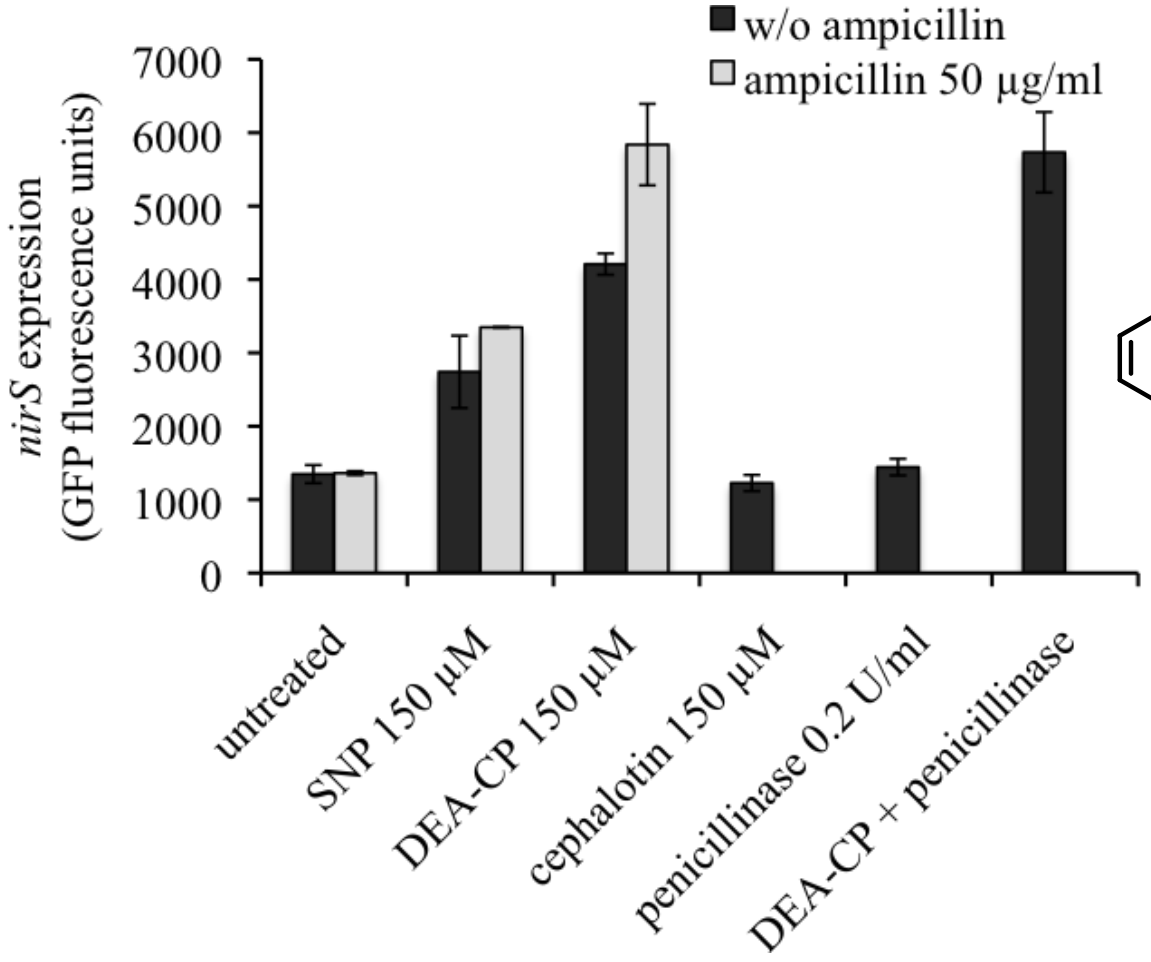
In Vitro Dispersion of *Pseudomonas aeruginosa* Biofilms

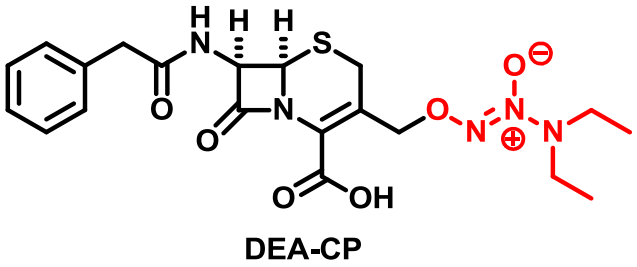




Kelso et al *Chem. Commun.* 2013, 49, 4719

DEA-CP Triggers NO-Induced Gene Expression in *P. aeruginosa*



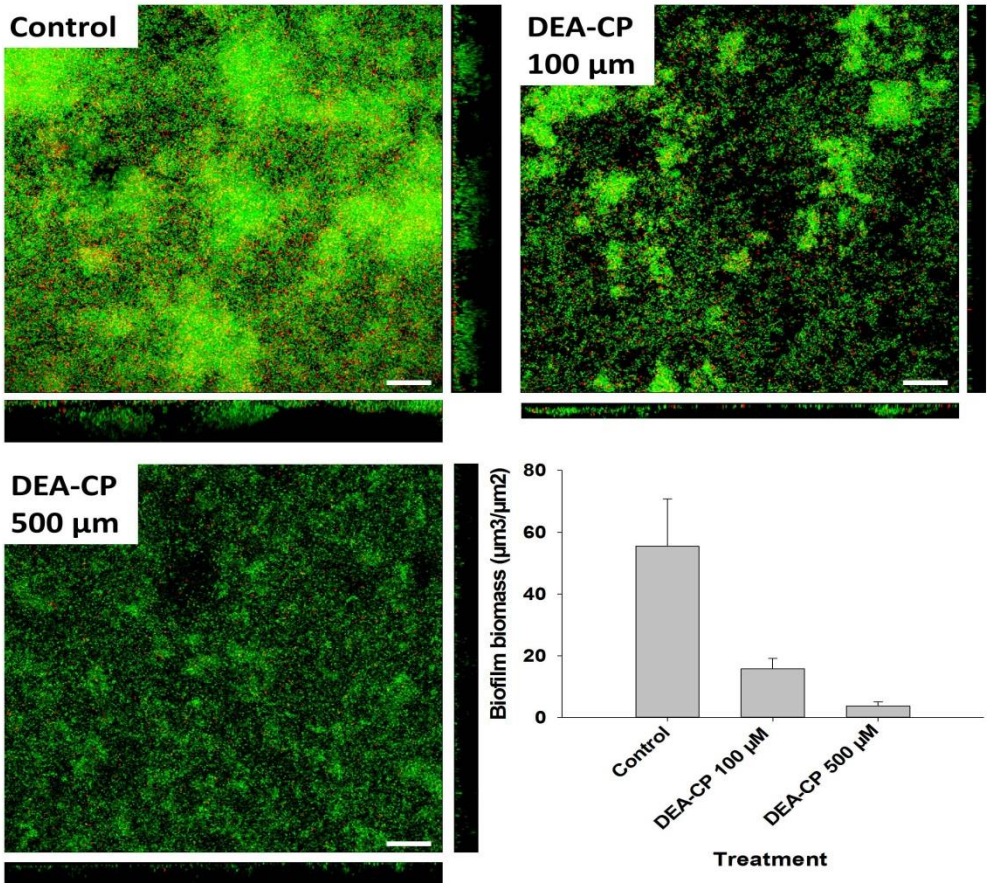


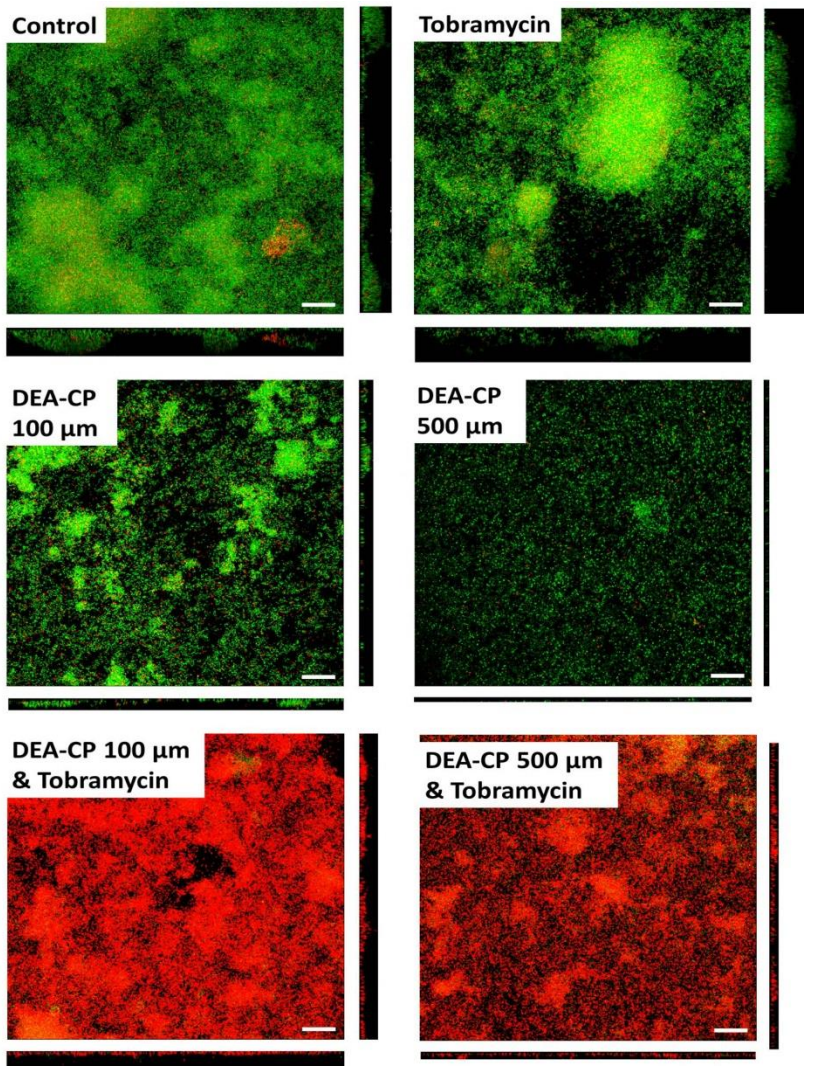
DEA-CP

P. aeruginosa **NSGFP reporter strain**

Kelso et al *Angew. Chem. Int. Ed.* 2012, 51, 9057 – 9060

Combination Approach: Activity Against *P. aeruginosa* Biofilms from CF Sputum Clinical Isolates





Dr Jeremy Webb and Prof Saul Faust
University of Southampton (unpublished)

Holy Grail: Bactericidal Anti-Biofilm NO-Donor Cephalosporins

PBP-activation would give:

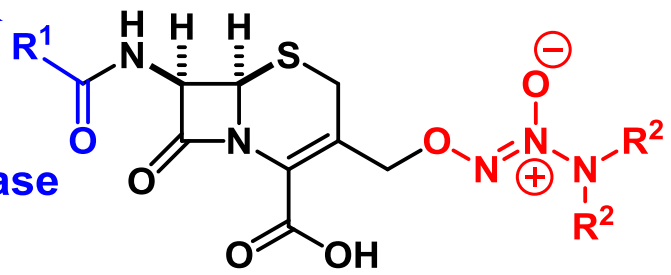
(1) release of NO and biofilm dispersion (same mechanism as β-lactamase activation)
(2) kill biofilm and released planktonic cells like a classic cephalosporin

Requires full-blown med-chem program….Seeking licensing partner

Patent coverage

R^1 = any R^1 group from any clinically used cephalosporin, past or present

R^2 = any diazeniumdiolate (> 100 reported)



****Modulate PBP vs β-lactamase sensitivity****

****Modulate NO release $t_{1/2}$ and physicochemical properties****

Is it Possible to find a Holy Grail Molecule?

Non-β-lactamase producing *Streptococcus pneumoniae* (planktonics)

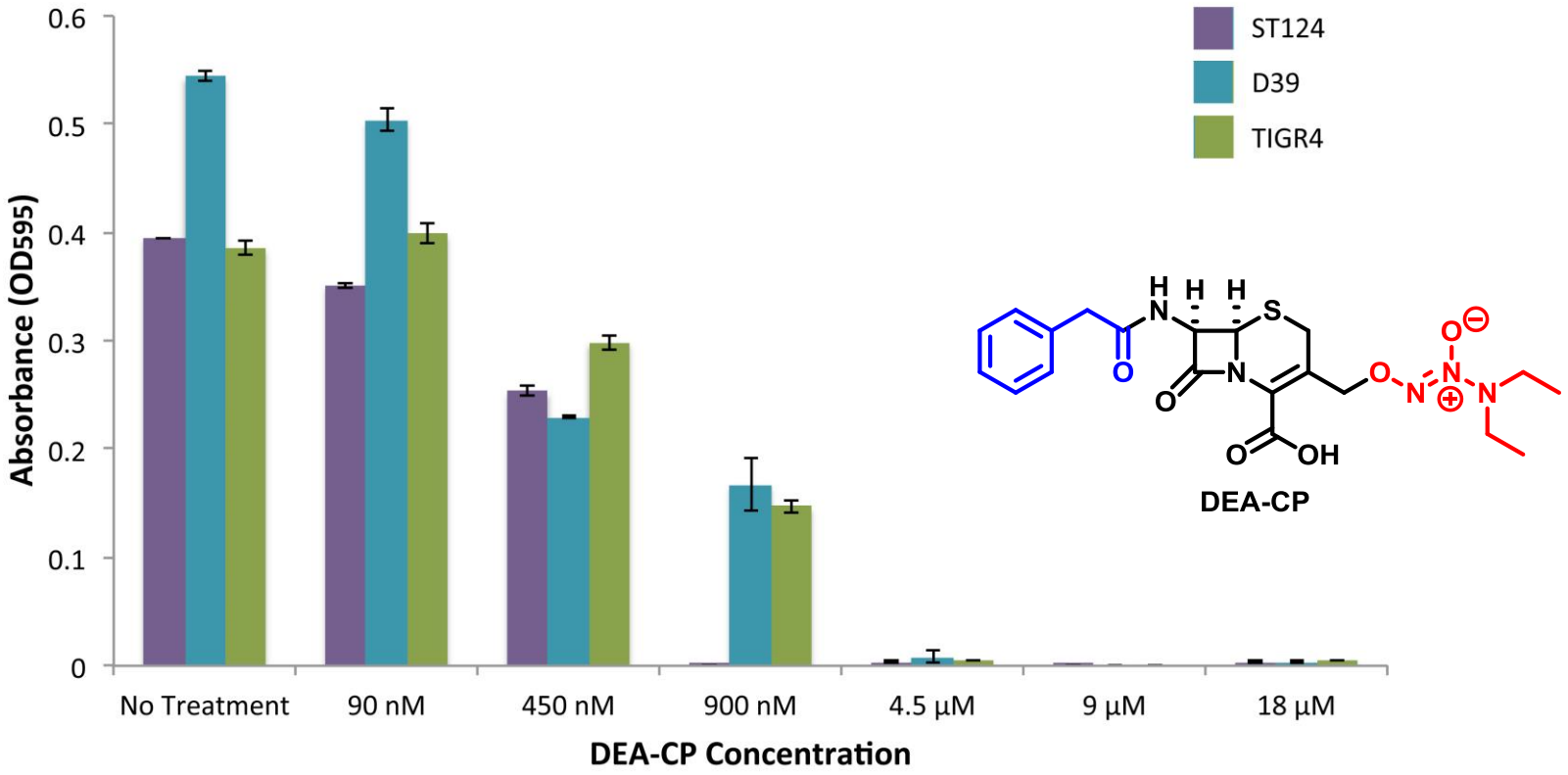


Figure 1: The effect of DEA-CP treatment on planktonic *S. pneumoniae*. Strains ST124, D39 and TIGR4 were treated with DEA-CP for 18 hours and absorbance (OD595) measured to determine the minimum inhibitory concentration.

Is it Possible to find a Holy Grail Molecule?

Established *S. pneumoniae* biofilms

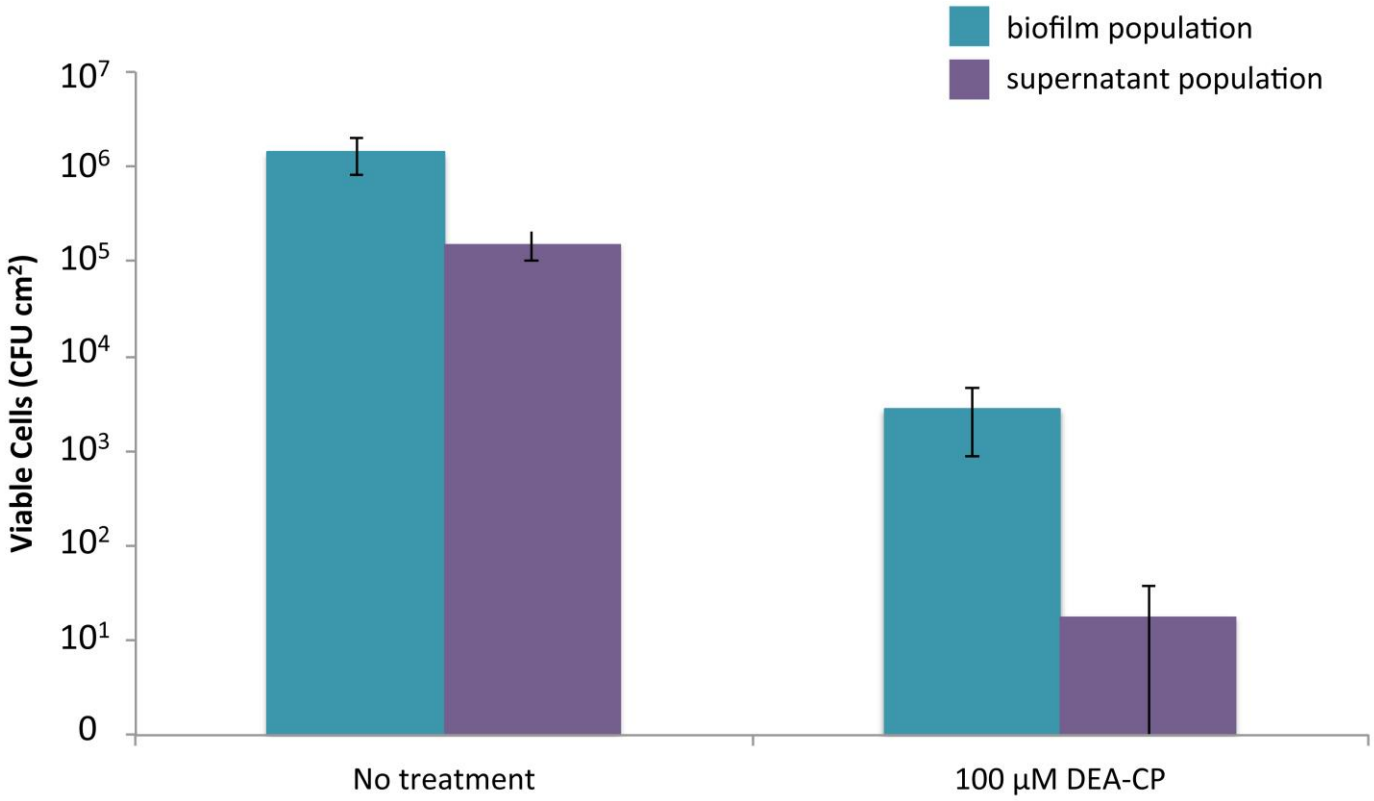
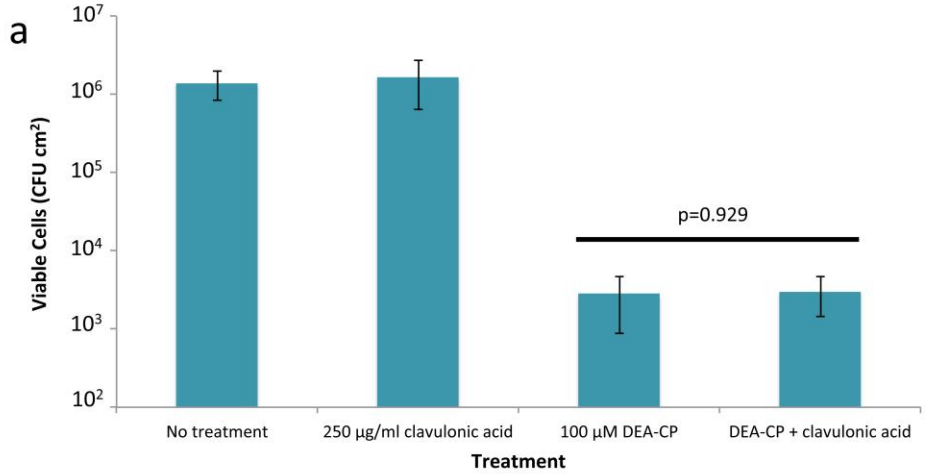


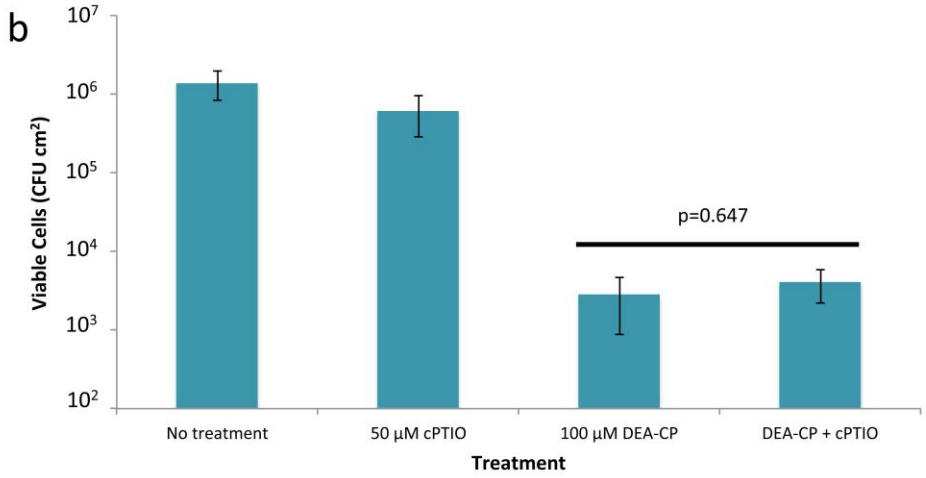
Figure 3: The effect of DEA-CP treatment on viability of *S. pneumoniae* ST124 *in vitro* biofilm supernatant population. 48 h ST124 biofilms were treated with DEA-CP for 2 hours and viability of pneumococci in the biofilm and surrounding media measured by CFU enumeration. *p≤0.05.

Is it Possible to find a Holy Grail Molecule? YES!!!

Established *S. pneumoniae* biofilms



Confirmed: Activity not due to β-lactamase triggered NO release

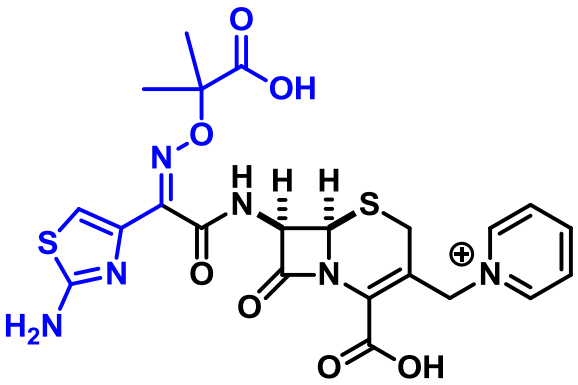


Confirmed: Activity not due to bactericidal NO activity.

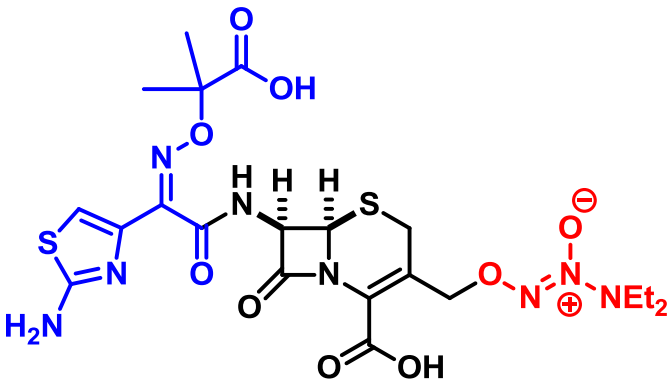
Conclusion: Compound acting via reaction with PBP like classic cephalosporin

Figure 4: The response of *S. pneumoniae* ST124 *in vitro* biofilms to DEA-CP treatment in the presence of clavulonic acid and cPTIO. 48 h ST124 biofilms were treated with **a)** clavulonic acid and **b)** cPTIO in the absence and presence of 100 µM DEA-CP, and biofilm viability measured by CFU enumeration.

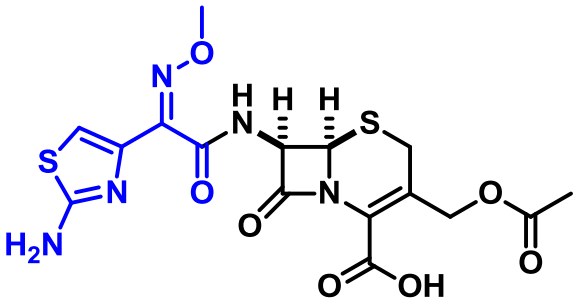
Current Synthetic Targets



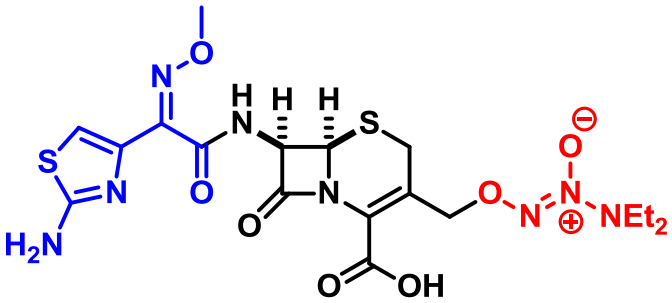
Ceftazidime



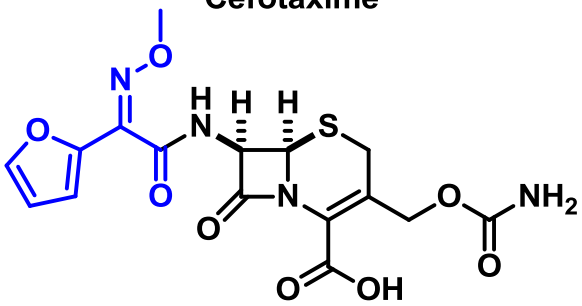
DEA-Ceftazidime



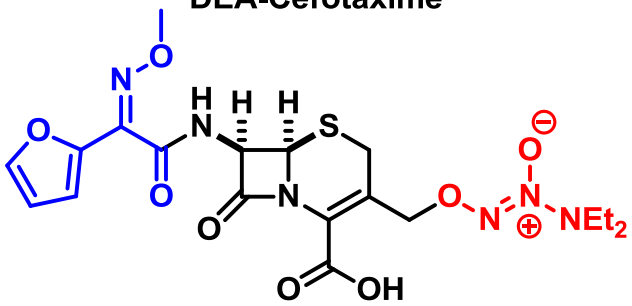
Cefotaxime



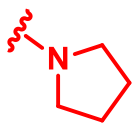
DEA-Cefotaxime

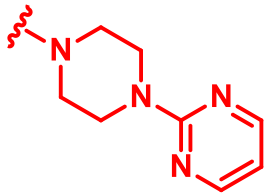


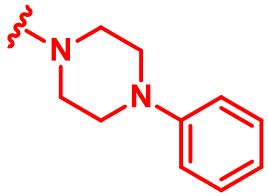
Cefuroxime



DEA-Cefuroxime







Acknowledgements

UOW
Dr Bharat Kardak
Dr Rao Yepuri

UNSW
Dr Nicolas Barraud
A/Prof Scott Rice
Prof Staffan Kjelleberg

Funding: NHMRC Project Grant (568841)

University of Southampton
Jeremy Webb
Saul Faust
Ray Allan
Funding: SPARKS (UK)

NHMRC
Australian Government
National Health and Medical Research Council

CYSTIC FIBROSIS Australia

NSi NewSouth Innovations