



**University of Dundee**

## **Interaction and Signalling Networks**

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*Published in:*  
Microbiology

*DOI:*  
[10.1099/mic.0.000401](https://doi.org/10.1099/mic.0.000401)

*Publication date:*  
2017

*Document Version*  
Peer reviewed version

[Link to publication in Discovery Research Portal](#)

### *Citation for published version (APA):*

Kirkpatrick, C. L., Lesouhaitier, O., Malone, J. G., An, S-Q., & Caly, D. L. (2017). Interaction and Signalling Networks: a report from the fourth 'Young Microbiologists Symposium on Microbe Signalling, Organisation and Pathogenesis'. *Microbiology*, 163(1), 4-8. <https://doi.org/10.1099/mic.0.000401>

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# Microbiology

## Interaction and Signalling Networks: a report from the fourth 'Young Microbiologists Symposium on Microbe Signalling, Organisation and Pathogenesis' --Manuscript Draft--

<b>Manuscript Number:</b>	MIC-D-16-00311R1
<b>Full Title:</b>	Interaction and Signalling Networks: a report from the fourth 'Young Microbiologists Symposium on Microbe Signalling, Organisation and Pathogenesis'
<b>Short Title:</b>	A report from the fourth Young Microbiologists Symposium
<b>Article Type:</b>	Meeting Report
<b>Section/Category:</b>	Host-microbe interaction
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1 **Interaction and Signalling Networks: a report from the fourth ‘Young Microbiologists**  
2 **Symposium on Microbe Signalling, Organisation and Pathogenesis’**

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13

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16 **Running title:** A report from the fourth Young Microbiologists Symposium

17

18 **Keywords:** gene regulation, signalling, secretion, host-pathogen interactions, microbe-  
19 microbe interactions.

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## 25 **Abstract**

26 At the end of June, over 120 microbiologists from 18 countries gathered in Dundee, Scotland  
27 for the fourth edition of the Young Microbiologists Symposium on “Microbe Signalling,  
28 Organisation and Pathogenesis”. The aim of the symposium was to give early career  
29 microbiologists the opportunity to present their work in a convivial environment and to interact  
30 with senior world-renowned scientists in exciting fields of microbiology research. The meeting  
31 was supported by the Microbiology Society, the Society of Applied Microbiology, the  
32 American Society for Microbiology with further sponsorship from the European Molecular  
33 Biology Organisation and The Royal Society of Edinburgh. In this report, we highlight some  
34 themes that emerged from the many interesting talks and poster presentations, and some of the  
35 other activities that were on offer at this energetic meeting.

36

## 37 **Introduction**

38 The fourth Young Microbiologists Symposium (YMS2016) took place at the Apex City Quay  
39 Hotel in Dundee, Scotland on the 29<sup>th</sup> and 30<sup>th</sup> June 2016. The conference gathered 126  
40 scientists coming from 18 countries and was organized by **Helge Dorfmueller** and **Robert**  
41 **Ryan**, from University of Dundee, and **Delphine Caly** from University of Lille in France. The  
42 main objective of the YMS2016 was to bring together early career microbiologists. The  
43 symposium programme covered several hot topics in microbiology and touched on current  
44 areas of interest to microbiologists including intracellular signalling, antibiotic resistance,  
45 bacterial secretion and host-microbe interactions. Renowned experts, who led sessions, and the  
46 many junior microbiologists who attended provided insight and new findings into these  
47 exciting areas. A novelty to this year’s meeting was that participants were given the opportunity  
48 to attend a PLOS Pathogens writing and publishing workshop, chaired by **Neil Mabbott** from

49 the Roslin Institute and University of Edinburgh in Scotland, which provided valuable advice  
50 for PhD students and junior post-docs on how to write scientific papers and achieve successful  
51 publication.

52

### 53 **Sensing, transduction and intracellular signalling**

54 The YMS2016 kicked off with the FEMS keynote lecture from **Ute Römling** (Karolinska  
55 Institutet, Sweden), who described the identification of the *Pseudomonas aeruginosa* clone C  
56 strain cluster prevalent in patients, clinics and the environment worldwide. As part of this  
57 research, Ute discussed how her group identified the PACGI-1 genomic island in this cluster,  
58 and showed that it contributes to heat-shock resistance by encoding protein quality-control  
59 systems (Lee *et al.*, 2015). Next, Ute described her group's work on the ubiquitous bacterial  
60 second messenger signal cyclic-di-GMP in *Salmonella enterica* serovar Typhimurium, which  
61 controls rdar (red dry and rough) biofilm formation and virulence as part of a complex  
62 regulatory network involving the transcriptional regulator CsgD. Ute explained how her lab  
63 have identified and characterised several key players in this network, including the diguanylate  
64 cyclase AdrA, the cellulose synthase cyclic di-nucleotide-binding protein BcsE, and the  
65 degenerate phosphodiesterase STM1697, which controls flagellar gene transcription through  
66 binding to the master regulator FlhDC (Ahmad *et al.*, 2013; Le Guyon *et al.*, 2015) and gave  
67 perspectives on novel regulatory pathways.

68 These themes were built upon in the first session, which was opened by **Max Dow** (University  
69 College Cork, Ireland). Max discussed the structure-function relationship of HD-GYP domains  
70 which degrade the second messenger cyclic-di-GMP. Max began with a summary of his lab's  
71 work on the protein RpfG, which contains a HD-GYP domain, and controls virulence and  
72 motility in the plant pathogen *Xanthomonas campestris* (Ryan *et al.*, 2010). Recently, Max and

73 collaborators have determined the structures of PmGH, an enzymatically active HD-GYP  
74 protein from *Persephonella marina* (Bellini et al., 2014) and PA2572, an enzymatically-  
75 inactive YN-GYP variant from *P. aeruginosa* (Bellini et al., unpublished). The work on  
76 PmGH suggested that active HD-GYP domains could be sub-divided into those with two or  
77 three metal-ion cofactors. In contrast, PA2572 carried no metals but was able to interact with  
78 other proteins via the GYP 'loop'.

79 **Lisa Bowman** (Imperial College London, UK) described a second, equally interesting  
80 dinucleotide second messenger; cyclic-di-AMP. Pioneering work from the Gründling lab has  
81 shown that cyclic-di-AMP regulates potassium and osmolyte uptake in *Staphylococcus aureus*,  
82 and is produced by the membrane bound cyclase DacA (Corrigan et al., 2011). Lisa discussed  
83 her work to expand on the existing model for cyclic-di-AMP signalling by explaining her  
84 inventive use of a BioLog phenotypic microarray to determine the function of YbbR, an  
85 uncharacterised component of the DacA membrane protein complex. Based on this screen and  
86 suppressor mutagenesis, Lisa proposed that YbbR acts as a localisation determinant for DacA  
87 at the membrane, controlling local pools of c-di-AMP especially under stress conditions.

88 In the final talk in this session, **Francesca D'Angelo** (University Roma Tre, IT) attracted  
89 significant interest and many audience questions with her talk on the generation of synthetic  
90 cells. These synthetic cells consist of liposomes containing biological molecules, and represent  
91 an ambitious new approach to drug delivery (Stano et al., 2012). After demonstrating that the  
92 HSL signal could be produced *in vitro*, Francesca built on this by encapsulating the functional  
93 HSL production system in her synthetic cells, protecting the HSL pathway from externally  
94 added inhibitors. The next step for this project will be to generate synthetic cells that can sense  
95 signals as well as produce an output.

96

97

98 **Symbiosis, pathogenesis and mechanisms of host interaction**

99 The ASM keynote lecture was presented by **Scott Hultgren** (Washington University, USA).

100 Scott gave a fantastic and informative overview of his research into urinary tract infections

101 (UTIs) by *E. coli*, which are mediated by the activities of type I pili. Building on structural

102 models of pili, Scott first showed that high and low-affinity mannose-binding forms of the

103 terminal FimH adhesin exist in equilibrium, with both states required for effective infection.

104 He then moved on to a discussion of the clinical aspects of UTI, showing that bladder cells are

105 remodelled by sensitisation to UTI, and thereafter are significantly more likely to become re-

106 infected. Scott's talk finished with a description of several promising lines of research into UTI

107 treatment, including an anti-pilus vaccines, and drugs targeting both pili and the FimH adhesin.

108 The host-microbe interactions session covered a large spectrum of topics introduced in the

109 ASM lecture including polymicrobial infection, the use of new tools for studying host-microbe

110 interactions in real time and the impact of both host communication signals and small metabolic

111 compounds.

112 **Marvin Whiteley** (University of Texas, USA) showed that microbe-microbe interactions

113 increase bacterial resistance to host defences (Ramsey & Whiteley, 2009) and allow synergistic

114 effect for some pathogenic bacteria (Turner *et al.*, 2015), using various examples of

115 interactions, such as *P. aeruginosa* and *S. aureus* in the cystic fibrosis lungs or *Aggregatibacter*

116 *actinomycetemcomitans* and *Streptococcus gordonii* that form biofilms in the oral cavity. The

117 highly organised wound communities and the precise spacing between bacteria during

118 polymicrobial infection are required for infectious success (Stacy *et al.*, 2015), and Marvin

119 explained why understanding this process could help in improving therapeutic strategies. The

120 following talk was given by **Andrew Roe** (University of Glasgow, UK) who presented a new

121 tool for studying protein interactions specifically dedicated to the host-pathogen interaction

122 research field. This tool, named LOV for light-oxygen-voltage sensing domain, enables the  
123 visualisation of bacterial cells attached to host cells. In parallel, Andrew showed how the LOV  
124 tool could be very suitable to study the direct translocation of bacterial type III effectors into  
125 host cells. Andrew's talk was illustrated by amazing images obtained by the fusion of a LOV-  
126 based reporter with the *Shigella flexneri* effector IpaB, demonstrating the interaction with the  
127 host cell actin network (Gawthorne *et al.*, 2016).

128 The use of mass spectrometry imaging in microbiology was discussed by **Heather Hulme**  
129 (University of Glasgow, UK), who showed that it could be a valuable tool for identifying  
130 biomarkers during an infection process. Using the example of mesenteric lymph node infection  
131 by *Salmonella*, Heather showed that palmitoylcarnitine (PalC), which is localised and  
132 accumulates in the damaged infected tissue, could be measured and used as a potential  
133 biomarker of infection.

134 The host environment encountered by bacteria plays a role in the success of infections. In this  
135 context, **Tuuli Ahlstrand** (University of Turku, Finland) showed that biofilms formed by the  
136 opportunistic pathogen *A. actinomycetemcomitans* could disrupt the host inflammation  
137 response by binding and internalising the proinflammatory cytokine interleukin-1 $\beta$  (Paino *et al.*,  
138 2012), which is enhanced by a specific bacterial sensor named bacterial interleukin receptor I  
139 (BilRI) (Ahlstrand *et al.*, 2016; Paino *et al.*, 2013). In the same vein, **James Connolly**  
140 (University of Glasgow, UK) demonstrated how pathogenic *E. coli* integrates host signals in  
141 order to regulate its ability to colonize the urinary tract. More precisely, James demonstrated  
142 how D-serine influences both gene content and virulence factor expression in pathogenic *E.*  
143 *coli* (Connolly *et al.*, 2015) and how bacteria use a D-serine sensing system to adapt to their  
144 environment (Connolly *et al.*, 2016). Another way to prevent bacterial infection, using  
145 inhibitors of multivalent adhesion molecule 7 (MAM7), was described by **Daniel Stones**  
146 (University of Birmingham, UK) who described a bead-coupled recombinant MAM7 that not



147 only prevented bacterial adhesion and infection in rats, but also did not affect cytokines release  
148 and the wound healing process, suggesting a promising drug to counteract infection (Krachler  
149 *et al.*, 2011).

150

## 151 **Bacterial shape, secretion and development**

152 This session began and ended with a review of new developments in our understanding of the  
153 operation of the bacterial type VI secretion system (T6SS). This multi-protein complex is a  
154 delivery system for protein-based toxins targeted at other bacteria or at eukaryotic cells, while  
155 the bacteria that are the source of the toxins also express specific immunity proteins to protect  
156 themselves. **Alain Filloux** (Imperial College London, UK) presented a recently published  
157 structural study (Planamente *et al.*, 2016), focused on a previously uncharacterised component  
158 of the complex, the TssA baseplate. The Filloux group showed that TssA forms a circular  
159 baseplate-like structure that assembles onto the membrane-facing end of the TssBC sheath,  
160 sharing structural and functional homology with the gp6 baseplate of T4 bacteriophage, and is  
161 essential for T6SS activity.

162 Bacterial lifestyle changes often require remodelling of the cell envelope, whether to permit  
163 the entry of extracellular DNA during competence or to generate a spore that will be more  
164 resistant to the external environment than the mother cell from which it develops. **Emma**  
165 **Denham** (University of Warwick, UK) presented her group's ongoing work on the role of  
166 small RNAs in bacterial growth heterogeneity using *Bacillus subtilis* as their model system.  
167 This talk focused on one notable sRNA-controlled process, the AbrB-dependent transition from  
168 exponential to stationary phase (Mars *et al.*, 2015), where AbrB expression is regulated by the  
169 small RNA S1022. Modified AbrB levels lead to phenotypic heterogeneity, suggesting a novel  
170 sRNA-regulated bet-hedging strategy.

171 **Tessa Quax** (University of Freiburg, Germany) provided the conference's only talk on  
172 Archaea, specifically on archaellum-mediated motility in these organisms. Named  
173 "archaellum" due to its extreme structural difference to the bacterial flagellum, this  
174 substructure resembles the type IV pili seen in bacteria in terms of its components and assembly  
175 mechanism. Surprisingly, Tessa showed it can also interact with a CheY-like component of a  
176 chemotaxis system as the bacterial flagellum does, despite the extreme evolutionary divergence  
177 between these two kingdoms of life and the completely different composition of their  
178 respective motility organelles. Finally, **Francesca Cianfanelli** from the Coulthurst group  
179 (University of Dundee, UK) presented her work on the T6SS of *Serratia marcescens* and the  
180 specific interactions of VgrG and PAAR proteins at the tip of the T6SS "spike". This showed  
181 that PAAR proteins are essential for T6SS function and that particular VgrG-PAAR  
182 combinations are required for full T6SS-dependent antibacterial activity, including activity  
183 mediated by cargo adaptors that are not normally considered dependent on specific VgrG  
184 proteins (Cianfanelli *et al.*, 2016).

185

## 186 **Bacterial inter-species and inter-kingdom interactions**

187 The final session covered the topic of inter-species and inter-kingdom interactions, which  
188 included talks regarding interactions within complex communities, between microbes, and the  
189 various host signals/triggers that shape the interactions within these communities. A  
190 captivating example of the former was presented by **Christoph Tang** (University of Oxford,  
191 UK) who delivered the EMBO lecture. Christoph described that temperature is one of the most  
192 important environmental cues that act on regulatory networks of pathogenic microbes. His  
193 group discovered and characterised the RNA thermometer CsaA from *Neisseria meningitidis*,  
194 an elegant mechanism that this microbe uses to adapt to different temperature changes.

195 Christoph explained how using NMR spectroscopy and SHAPE (Selective 2'-OH acylation  
196 analysed by primer extension) assays, the group discovered that at low temperature (30°C), all  
197 base pair regions of C<sub>ss</sub>A are stably formed, and the ribosome cannot access the RBS which is  
198 fully occluded (Barnwal *et al.*, 2016). As the temperature is raised, the RNA structure starts to  
199 unfold and by 42°C, the thermometer structure is fully open, leading to efficient translation.  
200 Taken together, it suggests that C<sub>ss</sub>A acts as a rheostat, whose stability is optimized to respond  
201 in a small temperature range such as occurs within the upper airways during infection.

202 Continuing with the theme of environmental cues altering the response of the microbial  
203 community during infection, **Vanessa Sperandio** (UT Southwestern Medical Center, USA)  
204 showed that enterohaemorrhagic *E. coli* (EHEC) senses fucose cleaved from the mucus layer  
205 in the colon by *Bacteroides thetaiotaomicron* through the histidine kinase FusK. It then rewires  
206 its transcription, repressing the expression of the LEE and fucose utilisation genes (Pacheco *et*  
207 *al.*, 2012). However, without mucus as a carbon source, *B. thetaiotaomicron* starts to secrete  
208 succinate, which upon being taken up by EHEC is sensed by the Cra transcription factor as a  
209 clue to a gluconeogenic environment. Cra binds to another transcription factor, KdpE, which  
210 is a response regulator (RR) phosphorylated by the QseC adrenergic sensor, to integrate  
211 adrenergic and sugar sensing to activate virulence gene expression at the interface with the  
212 intestinal epithelium. Through the interaction with another RR; QseB, QseC also represses the  
213 expression of the *fusKR* genes, further derepressing the virulence regulon. These data suggest  
214 a new layer of complexity in the inter kingdom signalling that underlies EHEC pathogenicity.

215 Given what is now known regarding the contribution of the host microbiota to health there is  
216 an urgent need for relevant animal models. **Beckie Ingram** (Queens College Belfast, UK) gave  
217 an inspiring talk about her group's work on developing appropriate murine models for  
218 understanding the pathophysiology of lung inflammation and the pathogenesis of lung disease  
219 in cystic fibrosis. These approaches will become crucial in improving our understanding of

220 microbial community interactions in the field of infectious diseases. Finally, **Clare**  
221 **Kirkpatrick** (University of Geneva, Switzerland) discussed the role of toxin-antitoxin (TA)  
222 systems in bacterial interactions and how they can shape the community. Clare discussed her  
223 recent work on the HigBA system from *Caulobacter crescentus* and revealed that this TA  
224 system acts as a switch to regulate bacterial growth and induce cell death upon antibiotic-  
225 induced DNA damage (Kirkpatrick *et al.*, 2016). This novel regulatory mechanism could  
226 potentially be used to develop new treatments to clear bacterial infections.

227

## 228 **Conclusions**

229 This symposium, like previous meetings (Caly *et al.*, 2012, 2014; Ryan *et al.*, 2009), covered  
230 many fascinating areas of microbiology. As always the forum allowed the attendees to gain  
231 many insights into up and coming areas and techniques in bacteriology, and provided junior  
232 microbiologists the opportunity to present and discuss their work. This was successfully  
233 achieved judging the numerous interactions between junior and senior scientists observed  
234 during and between scientific sessions.

235 After the final session, a number of awards were distributed. These included the Frontiers in  
236 Microbiology short talk prize that went to **Fang-Fang Wang** (Chinese Academy of Sciences  
237 Beijing, China) for her excellent presentation entitled, “Receptor histidine kinase directly binds  
238 plant chemical to promote bacterial adaptation in host plant”. The Nature Reviews in  
239 Microbiology, Trends in Microbiology, Biochemical Journal and Molecular Microbiology  
240 poster prizes went to several PhD students working on outstanding projects. The meeting  
241 finished on relaxed note with a Ceilidh organised in the Apex hotel following the conference  
242 dinner.

243 Overall, the feedback from attendees was very positive; participants appreciated the quality of  
244 the scientific programme and the intimate atmosphere of the small conference. A post-meeting  
245 survey reported that 71% of the survey participants (n = 68) found the scientific programme  
246 ‘very good’ and 83% were interested in attending a future YMS conference (n = 65). One of  
247 the participants, who gave a talk as a junior post-doc at the YMS2012 and is now setting up  
248 her laboratory, used this opportunity to advertise for positions and made several promising  
249 contacts. This bodes well for further iterations of the meeting in the future.

250

## 251 **Acknowledgements**

252 We are deeply grateful to the participants who agreed for their work to be described in this  
253 report and we apologise to those whose work could not be mentioned due to space constraints.  
254 We also would like to thank all the speakers and participants for contributing to the success of  
255 this meeting. The organisers are extremely grateful to Erin Stanbridge, Kushal Rugjee, Birte  
256 Hollmann, Anne Six, the members of the Division of Molecular Microbiology in University of  
257 Dundee and Debbie Ree from the Dundee and Angus Convention Bureau for their help and  
258 support with the organisation of the meeting. We also thank the American Society for  
259 Microbiology, the European Molecular Biology Organization, the Federation of European  
260 Microbiological Societies, the Microbiology Society, the Society for Applied Microbiology  
261 and the Royal Society of Edinburgh and all our other sponsors for their financial support.

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