

RESEARCH INTERESTS

My group has diverse research interests in the broad area of bacterial signal transduction and bioengineering. We use several bacterial systems and experimental approaches to address the questions of interest. The approaches used in the lab involve bioinformatics, transcriptomics, genetics, protein-ligand biochemistry, photochemistry, protein and metabolic engineering. The three areas described below define our core interests.

(i) **Cyclic dimeric GMP signaling in bacteria** (see references 24, 26, 27, 32, 33, 36, 37, 40–43 below)

Several years ago, we have realized that cyclic dimeric GMP, c-di-GMP, must be a universal signaling molecule in Bacteria whose role had been grossly underappreciated (reviews 26, 41, 42). We carried out some pioneering work on the enzymology c-di-GMP synthesis and degradation (24, 27, 33, 40) and identified the first type of c-di-GMP receptors (32). By now, it is well established that c-di-GMP plays a central role in bacterial transition from the single-cellular lifestyle to the surface-attached multicellular lifestyle. Surface-attached bacteria can form biofilms, communities of cells growing in the self-produced extracellular matrices. The majority of chronic infections involve bacterial pathogens growing in biofilms, where cells are much less susceptible to antibiotics. Therefore, understanding how biofilms are formed and destroyed has not only both basic science but also medical importance. The current focus of the lab is on elucidating molecular mechanisms through which c-di-GMP controls bacterial motility and biofilms (43). We also study (in collaboration with several groups) mechanisms of c-di-GMP signaling in bacterial pathogens (36, 37).

43. Fang, X. and **M. Gomelsky**. 2010. A c-di-GMP-dependent mechanism regulating bacterial flagellar motility. *Mol Microbiol* 76:1295-1305. (Faculty of 1000 Biology: "Recommend")

42. **Gomelsky, M.** 2010. The core pathway: diguanylate cyclases, phosphodiesterases, and c-di-GMP-binding proteins. Chapter 4, pp. 37-56. *In* The second messenger cyclic di-GMP. (A. Wolfe and K. Visick, eds.) ASM Press, Washington, DC.

41. **Gomelsky, M.** 2009. C-di-GMP-binding CRP-like protein: a spectacular new role for a veteran signal transduction actor. Commentary. *J Bacteriol* 191: 6785–6787.

40. Barends, T.R.M., E. Hartmann, J. Griesse, T. Beitlich, N.V. Kirienko, D.A. Ryjenkov, J. Reinstein, R.L. Shoeman, **M. Gomelsky**[#] and I. Schlichting[#]. 2009. Structure and mechanism of a bacterial light-regulated cyclic nucleotide phosphodiesterase. *Nature* 459:1015-1018 ([#], *corresponding authors*). (Faculty of 1000 Biology: "Must read")

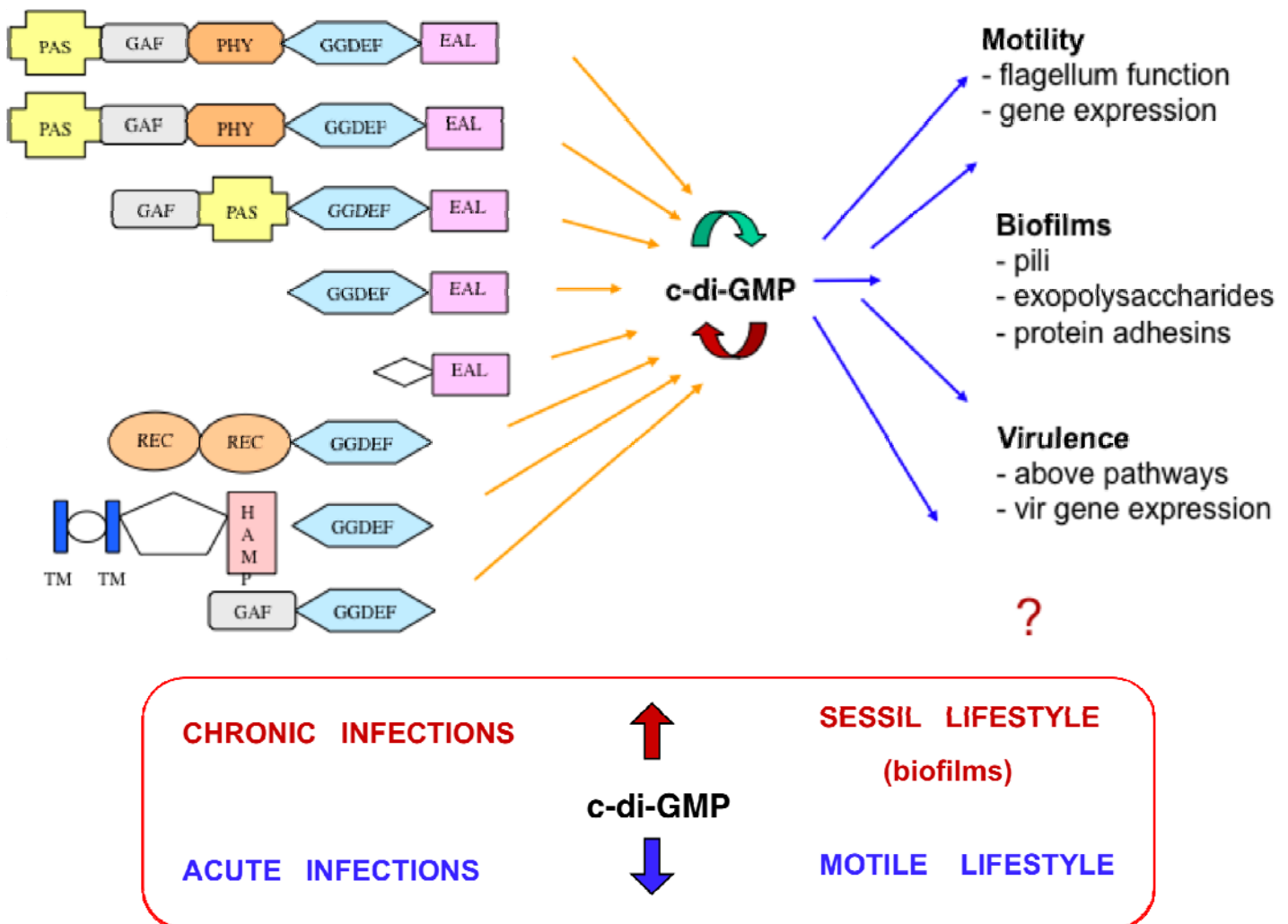
37. Holland, L.M., S.T. O'Donnell, D.A. Ryjenkov, L. Gomelsky, S.R. Slater, P.D. Fey, **M. Gomelsky** and J.P. O'Gara. 2008. A staphylococcal GGDEF domain protein regulates biofilm formation independently of c-di-GMP. *J Bacteriol* 190:5178–5189.

36. Claret, L., S. Miquel, N. Vieille, D.A. Ryjenkov, **M. Gomelsky** and A. Darfeuille-Michaud. 2007. The flagellar sigma factor FliA regulates adherence and invasion of Crohn disease-associated *Escherichia coli* via a c-di-GMP-dependent pathway. *J Biol Chem* 282:33275-33283. (Faculty of 1000 Biology: "Recommend")

33. Tarutina, M., D.A. Ryjenkov and **M. Gomelsky**. 2006. An unorthodox bacteriophytochrome from *Rhodobacter sphaeroides* involved in turnover of the second messenger c-di-GMP. *J Biol Chem* 281:34751–34758.

32. Ryjenkov, D.A., R. Simm, U. Römling, and **M. Gomelsky**. 2006. The PilZ domain is a receptor for the second messenger c-di-GMP. The PilZ domain protein YcgR controls motility in enterobacteria. *J Biol Chem* 281:30310-30314. (Accelerated publication)
27. Schmidt, A.J., D.A. Ryjenkov and **M. Gomelsky**. 2005. The ubiquitous protein domain EAL is a c-di-GMP-specific phosphodiesterase: Enzymatically active and inactive EAL domains. *J Bacteriol* 187:4774-4781.
26. Römling, U., **M. Gomelsky** and M.Y. Galperin. 2005. C-di-GMP: the dawning of a novel bacterial signalling system. MicroReview. *Mol Microbiol* 57:629-639.
24. Ryjenkov, D.A., M. Tarutina, O.V. Moskvina and **M. Gomelsky**. 2005. Cyclic diguanylate is a ubiquitous signaling molecule in bacteria: Insights into the biochemistry of the GGDEF protein domain. *J Bacteriol* 187:1792-1798.

Fig. 1. C-di-GMP signaling pathways affect various physiological processes in bacteria. GGDEF domain proteins are diguanylyl cyclases (DGC) involved in c-di-GMP synthesis; EAL domain proteins are phosphodiesterases (PDE) involved in c-di-GMP degradation.



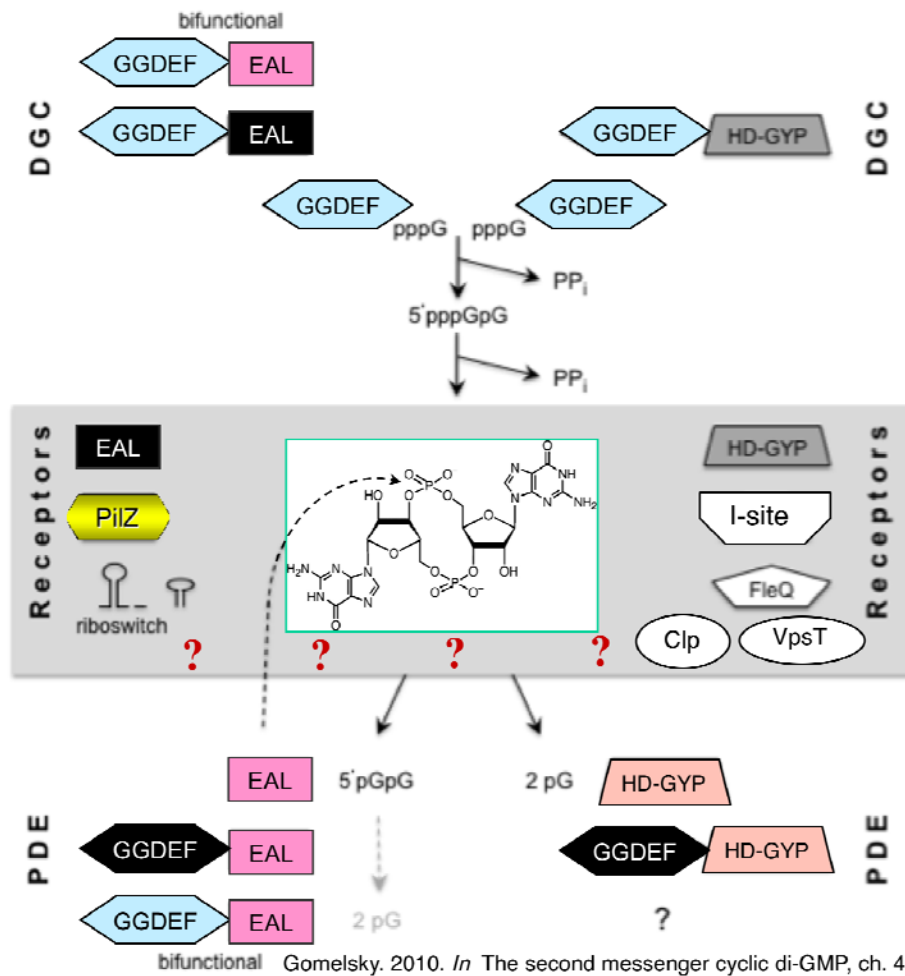
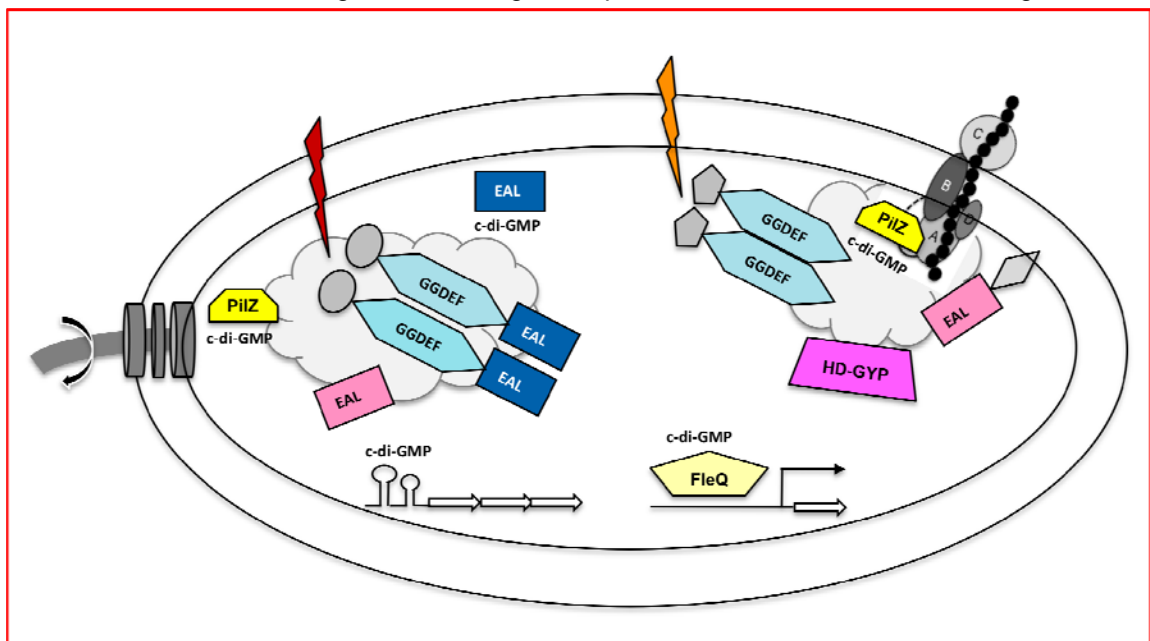


Fig. 2. Overview of c-di-GMP metabolic enzymes and receptors. (Adapted from ref. 42)

Fig. 3. c-di-GMP-dependent signaling pathways in a hypothetical cell affecting flagellum rotation, exopolysaccharide synthesis, and gene expression (via a c-di-GMP-dependent transcription factor and a riboswitch). Clouds surrounding c-di-GMP targets emphasize the idea of local c-di-GMP gradients.



(ii) **Gene regulation, transcriptomics and metabolic engineering of
*Rhodobacter sphaeroides***
(ref. 17, 18, 20, 22, 23, 25, 30, 34, 35, 38, 44, 45)

We have been interested in oxygen- and light-sensing regulators that control photosynthesis genes in the facultative phototroph, *Rhodobacter sphaeroides*. We have identified and characterized several such regulators (18, 20, 25, 35, 38, 44). In early 2000s, we have designed the *R. sphaeroides* GeneChip (22) and performed a number of transcriptomics studies to understand responses of *R. sphaeroides* to light, oxygen, oxidative stress and other environmental stimuli (23, 30, 34). Based on the extensive transcriptomics data, we have constructed (in collaboration with P. Ivanov, Moscow State Univ.) a transcription regulation database (<http://rhodobase.org>) that allows us to interrogate gene regulatory networks in this bacterium (Moskvin et al., submitted). A new and exciting direction that grew out of our familiarity with *R. sphaeroides* genomics and physiology involves metabolic modeling (45) and engineering of this bacterium for biofuel production.

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35. Moskvin, O.V., S. Kaplan, M-A. Gilles-Gonzalez and **M. Gomelsky**. 2007. Novel heme-based oxygen sensor with a revealing evolutionary history. *J Biol Chem* 282:28740-28748. (Faculty of 1000 Biology: "Recommend")
34. Zeller, T., M.A. Mraheil, O.V. Moskvin, K. Li, **M. Gomelsky** and G. Klug. 2007. Regulation of hydrogen peroxide-dependent gene expression in *Rhodobacter sphaeroides*: Regulatory functions of OxyR. *J Bacteriol* 189:3784-3792.
30. Zeller, T., O.V. Moskvin, K. Li, G. Klug and **M. Gomelsky**. 2005. Transcriptome and physiological responses to hydrogen peroxide in the facultatively phototrophic bacterium *Rhodobacter sphaeroides*. *J Bacteriol* 187:7232-7242.
25. Moskvin, O.V., L. Gomelsky and **M. Gomelsky**. 2005. Transcriptome analysis of the *Rhodobacter sphaeroides* PpsR regulon: PpsR as a master regulator of photosystem development. *J Bacteriol* 187:2148-2156.
23. Braatsch, S., O.V. Moskvin, G. Klug and **M. Gomelsky**. 2004. Responses of the *Rhodobacter sphaeroides* transcriptome to blue light under semiaerobic conditions. *J Bacteriol* 186:7726-7735.
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18. Braatsch, S., **M. Gomelsky**, S. Kuphal, and G. Klug. 2002. A single flavoprotein, AppA, from *Rhodobacter sphaeroides* integrates both redox and light signals. *Mol Microbiol* 45:827-836.

17. **Gomelsky, M.**, I.M. Horne, H.-J. Lee, J.M. Pemberton, A.G. McEwan and S. Kaplan. 2000. Domain structure, oligomeric state, and mutation analysis of PpsR, the *Rhodobacter sphaeroides* repressor of photosystem gene expression. *J Bacteriol* 182:2253-2261.

Fig. 4. Oxygen- and light-dependent formation of the photosynthetic apparatus in *R. sphaeroides*.

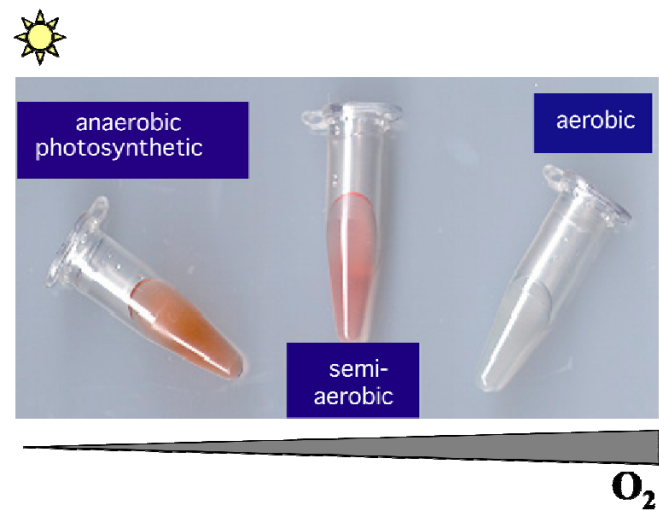
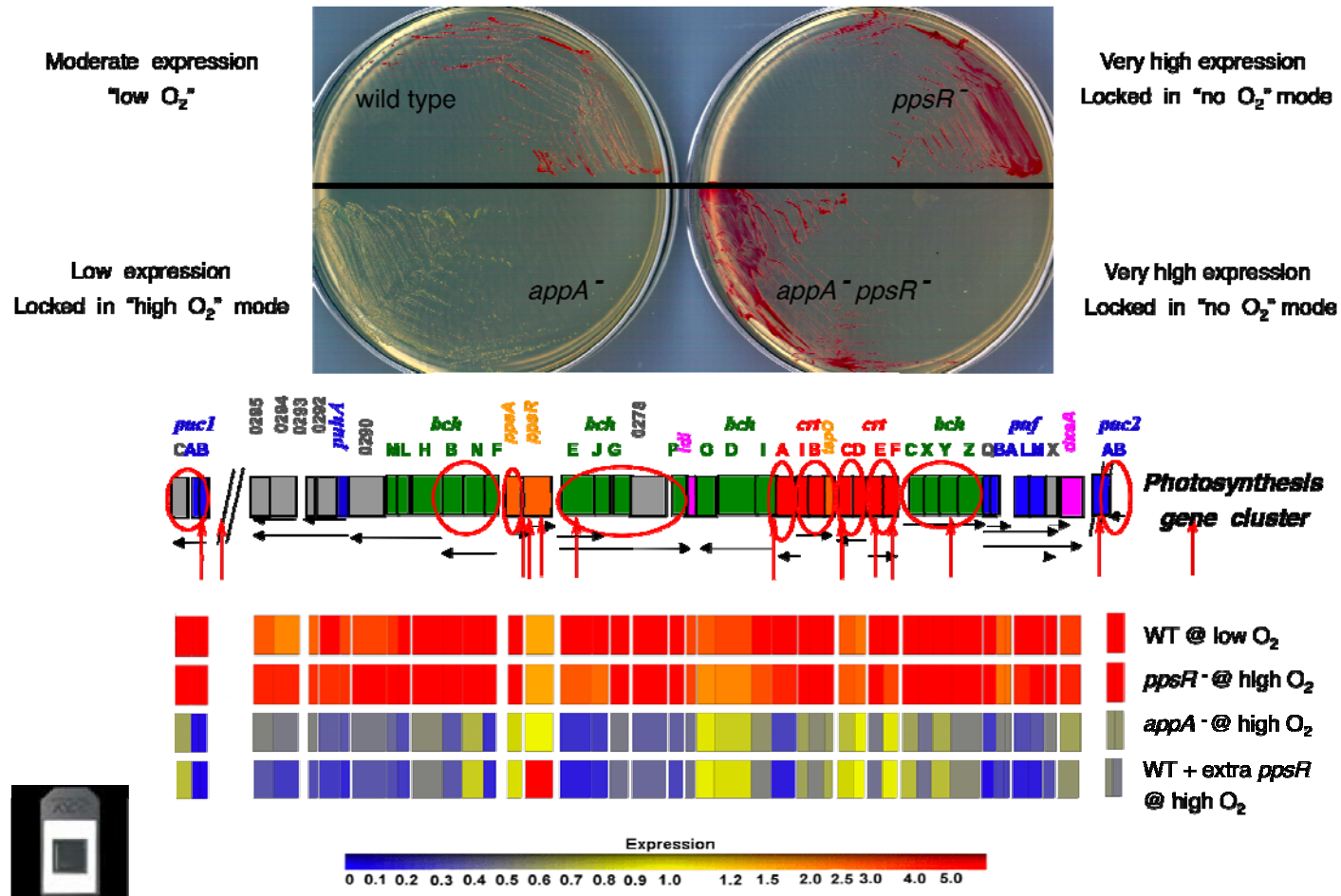


Fig. 5. Genetic and transcripto-mics description of the AppA -PpsR regulatory system. AppA a dual, oxygen and light, sensor , acts as an antirepressor of the transcriptional repressor PpsR.



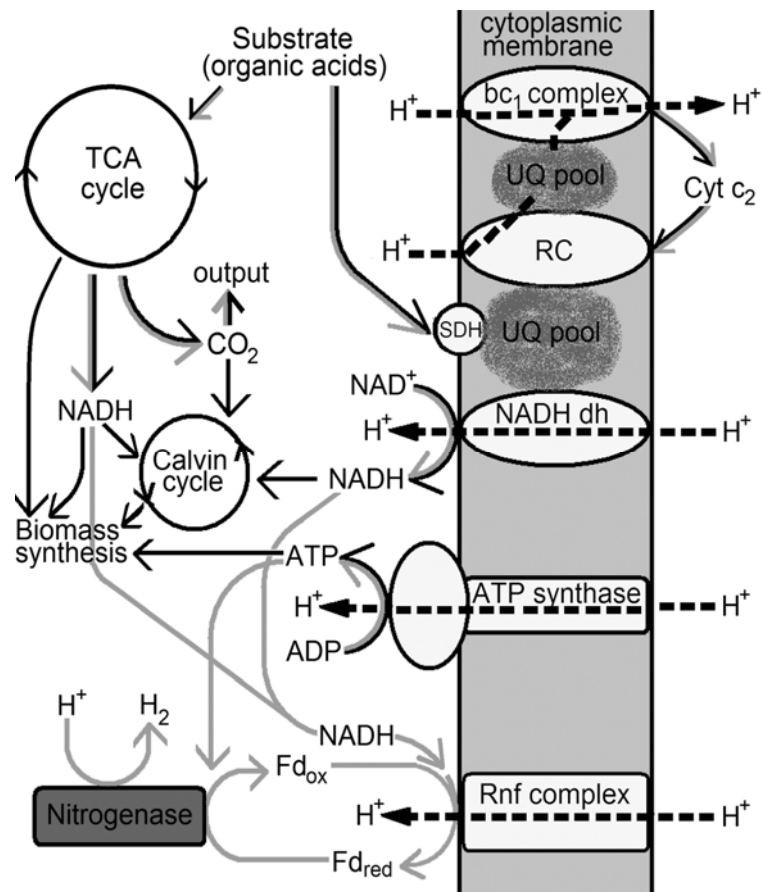
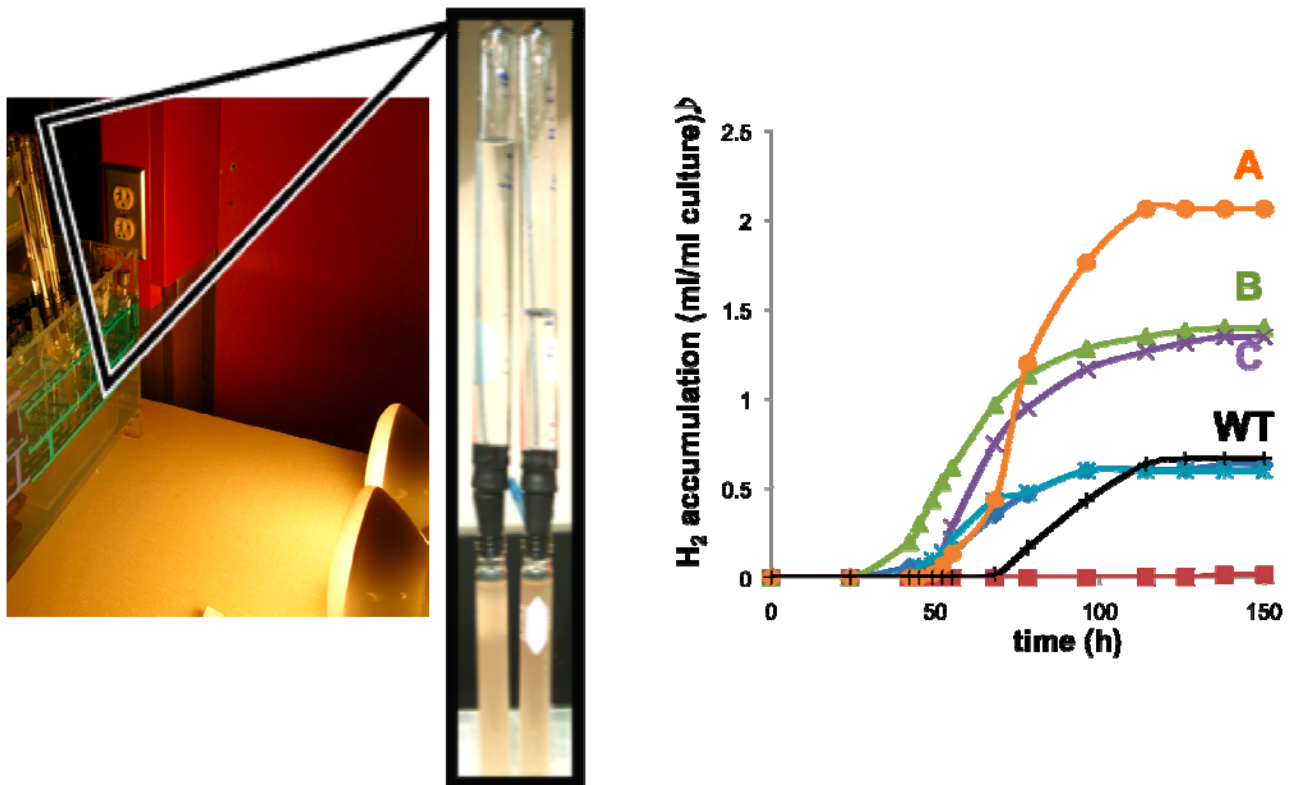


Fig. 6. Metabolic reconstruction of the central pathways in *R. sphaeroides* (45).

Fig. 7. Phototrophic production of hydrogen gas by metabolically engineered in *R. sphaeroides*. Left panel, experimental setup; hydrogen pushes out water and is accumulated in the inverted tubes. Right panel, hydrogen accumulation in various constructed mutants (A-C) and the wild-type strain (WT).



(iii) Characterization of photoreceptor proteins and engineering synthetic light-activated signaling pathways

(ref. 18, 19, 29, 33, 39, 40)

Light-activated proteins have the potential to revolutionize biomedical research. Such proteins can be delivered into model organisms (optogenetics) to control various activities in vivo with the spatiotemporal precision that supersedes that of chemicals (drugs). One of the photosynthesis gene regulators that we identified early on, AppA (6, 10, 13), turned out to function as a dual sensor of oxygen and light (18, 19). Amazingly, AppA senses each of these signals via novel mechanisms that we have uncovered in collaboration with G. Klug (Univ. of Giessen) and M.A. Gilles-Gonzalez (Univ. of Texas Southwestern Medical Center) (19, 35, 44). We are performing structure-function analysis of proteins containing the photoreceptor domain BLUF identified in AppA – in collaboration with I. Schlichting's group (Max Planck Institute for Biomedical Research - Heidelberg) (29, 39, 40). We have identified several light-activated enzymes that control synthesis and degradation of nucleotide second messenger (e.g., c-di-GMP, cAMP). We are characterizing these proteins and building synthetic signaling pathways that can be manipulated by light.

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39. Tyagi, A., A. Penzkofer, J. Griese, I. Schlichting, N.V. Kirienko, **M. Gomelsky**. 2008. Photodynamics of blue-light-regulated phosphodiesterase BlrP1 protein from *Klebsiella pneumoniae* and its photoreceptor BLUF domain. *Chem Physics* 354:130-141.

33. Tarutina, M., D.A. Ryjenkov and **M. Gomelsky**. 2006. An unorthodox bacteriophytochrome from *Rhodobacter sphaeroides* involved in turnover of the second messenger c-di-GMP. *J Biol Chem* 281:34751–34758.

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18. Braatsch, S., **M. Gomelsky**, S. Kuphal, and G. Klug. 2002. A single flavoprotein, AppA, from *Rhodobacter sphaeroides* integrates both redox and light signals. *Mol Microbiol* 45:827-836.

Fig. 8. Engineered light-dependent *E. coli* cells expressing blue-light activated adenylyl cyclase BlaC (growth seen only in the irradiated bucking bronco image [Ryu et al., submitted]).



Fig. 9. X-ray structure of the blue-light activated c-di-GMP phosphodiesterase, BlrP1 (40).

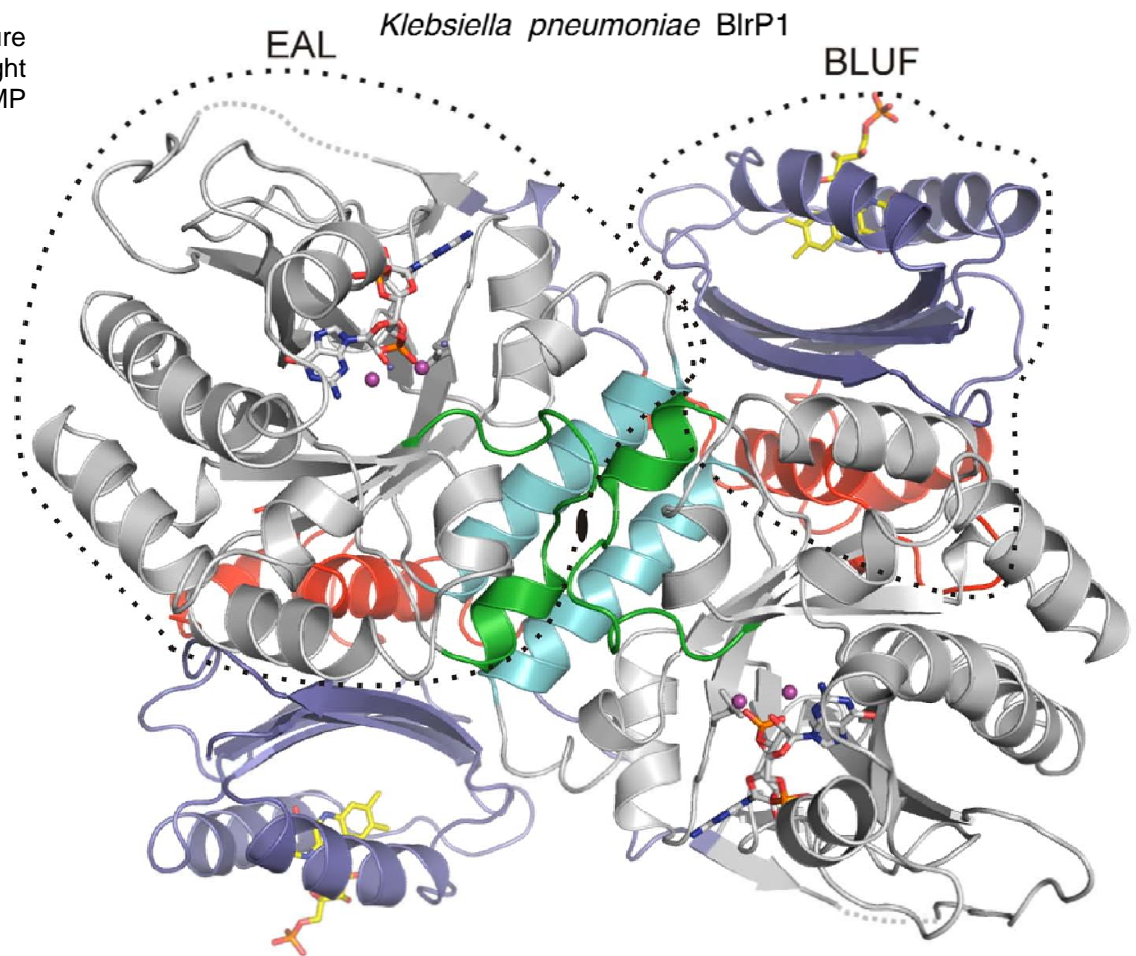


Fig. 10. Engineered light-controlled behavior (biofilm formation ; motility) in *E. coli*.

