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THE CHALLENGE OF BACTERIAL RNA POLYMERASE BIOPHYSICAL CHARACTERIZATION

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Bacterial RNA polymerase (RNAP) is a multi-subunit enzyme that orchestrates all steps of transcription (synthesis of RNA from DNA templates) in bacteria and so has to integrate regulatory inputs from a wide array of transcription factors. In our studies of transcriptional regulation in Gram-positive bacteria, we routinely isolate RNAP complexes directly from their native hosts, such as *Mycobacterium smegmatis* and *Bacillus subtilis* [1, 2]. As expected, these preparations contain not only the core RNAP (bearing a His-tag on the ' subunit) but also numerous associated transcription factors and nucleic acids. Although heterologous overexpression in *E. coli* can partially alleviate this complexity, the resulting samples still typically contain multiple RNAP "species", including complexes formed with *E. coli*–derived proteins. This is largely due to the high degree of structural and functional conservation among bacterial RNAPs.

For rigorous structure–function analyses (including structural determination, mutational studies, and quantification of transcription factor affinities) it is essential to thoroughly characterize the composition of RNAP samples and to establish robust purification protocols. With the current development of biophysical techniques, we can employ new strategies for the characterization of such difficult samples (e.g. Mass Photometry [3]) and consequently improve our understanding of the complex biological systems. Here, we present our recent experience with the production and biophysical characterization of Gram-positive RNAP complexes, highlighting approaches that improve sample homogeneity and analytical reliability.

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2. Sudzinová P, Knežová Balgová T, Schwarz M, *et al.* Bacteria sense the antibiotic rifampicin through a widespread dual-promoter based alarm system. Nucleic Acids Res. (2026) **54**, gkaf1407. doi: 10.1093/nar/gkaf1407.
3. Wu D. & Piszczek G. Standard protocol for mass photometry experiments. *Eur Biophys J.* (2021) **50**, 403-409. doi: 10.1007/s00249-021-01513-9.

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HUMAN PAPILLOMAVIRUS VIRUS PARTICLES STUDIED BY HIGH-SPEED AFM

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Infection by human papillomavirus (HPV) is one of the most common viral infections and in the majority of cases is resolved asymptomatically, thus, posing little to no health threat. However, several HPV strains (e.g., HPV 16 and 18) are the leading cause of invasive cervical and oropharyngeal cancer worldwide and therefore, detailed understanding of HPV is highly desirable. Recently, differences in the mechanical properties of HPV in the absence and presence of the sulfated polysaccharide (heparin) were explained by Atomic Force Microscopy (AFM). Building up on these observations that illustrate the structural activation process of HPV with regular AFM, we explore various properties of pseudoviral HPV particles in close-to-physiological conditions using bio-high-speed (HS) AFM. Fast visualization of particle height (1-2 s per frame), real-time dynamics of HPV surface building blocks (250-500 ms per frame), and close to structural surface resolution of single HPV capsomeres is extracted using HS-AFM. Combined, these results are deepening our understanding of HPV, which could potentially lead to novel strategies for eliminating the virus or using it to our advantage as a potential nanocarrier.

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ANALOG COMPUTING IN BIOLOGICAL PHOTORECEPTORS: THE CASE OF A NEO-RHODOPSIN TANDEM

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Neorhodopsin (NeoR) is a newly discovered fungal photoreceptor that can reversibly switch between UV- and near-infrared-absorbing states within a single protein complex. Using ultrafast transient absorption and femtosecond stimulated Raman spectroscopy, we reveal an unexpected process: direct excitation-energy transfer between neighboring retinal chromophores inside a NeoR dimer. After UV excitation of the UV-absorbing form (NeoR367), the lowest excited state transfers energy to the near-IR form (NeoR690) on a ~200 ps timescale, competing with photochemistry. Structural data show that the two chromophores are separated by only ~29 Å, enabling efficient intradimer energy transfer despite originating from an optically forbidden excited state.

This finding demonstrates that biological photoreceptors can exchange excitation energy with each other, rather than acting as isolated light sensors. Such coupling creates a powerful evolutionary shortcut: spectral sensitivity can be extended or reshaped without redesigning the chromophore itself. In principle, energy transfer between coupled photoreceptors could allow UV sensitivity to be functionally "plugged" into a near-IR detector or vice versa. Our results therefore reveal a previously unrecognized design principle of photoreceptor evolution and suggest new strategies for engineering multiwavelength optogenetic and imaging tools. [1]

1. Ivo HM van Stokkum, Jakub Dostal, Thanh Nhut Do, Lifei Fu, Gregor Madej, Christine Ziegler, Peter Hegemann, Miroslav Kloz, Matthias Broser, John TM Kennis, Journal of the American Chemical Society 147 (17), 14468-14480

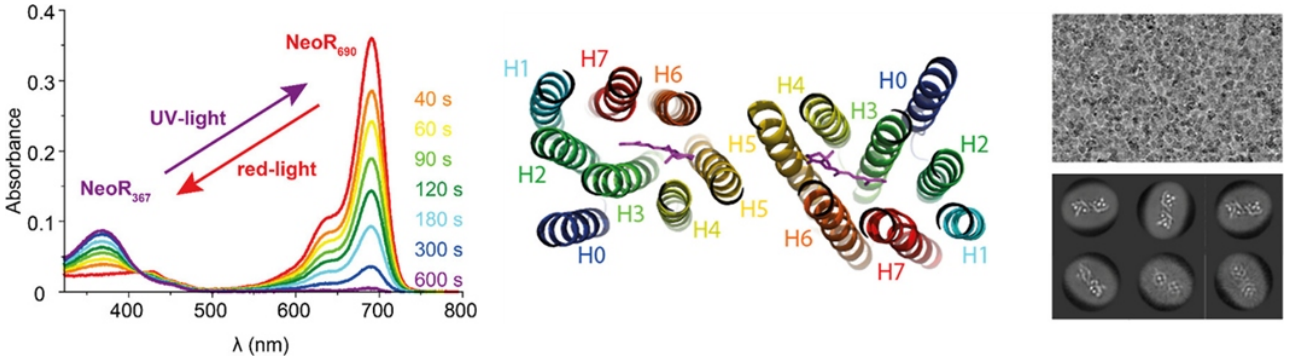

Figure 1. Structure and spectral dynamics of the Neo-Rhodopsin dimer. The tandem structure not only enhances sensitivity but also gives rise to qualitatively new functional dynamics.