Comments to the Editor

Response to Comment "Transient Complexes between Dark Rhodopsin and Transducin: Circumstantial Evidence or Physiological Necessity?" by D. Dell'Orco and K.-W. Koch

In retinal rod cells, absorption of a photon by the visual GPCR rhodopsin (R) initiates a cascade of biochemical reactions that amplifies the light signal and eventually generates an electrical response. Despite a vast number of experimental and simulation studies, the precise spatiotemporal mechanism by which rod cell phototransduction occurs on the supramolecular level is still elusive. As yet, the simultaneous observation of structure and dynamics in intact rod cells goes beyond experimental capabilities.

In Schöneberg et al. (1), we have combined kinetic data of G-protein activation reported in Heck and Hofmann (2) with explicit spatiotemporal simulations of receptor-G protein coupling in rod cell disk membranes, in order to investigate three different scenarios of the supramolecular arrangement of rhodopsin.

1. Rhodopsin does not form any supramolecular complexes and is freely diffusing.
2. Rhodopsin and G-protein form metastable but nonproductive dark complexes (RG) in addition to productive complexes (R*G).
3. Rhodopsin is arranged in immobile rows of dimers of different lengths, while the G-proteins are otherwise freely diffusing.

For each of the three scenarios (of which Scenarios 2 and 3 are not mutually exclusive), a consistent set of rate parameters could be identified that makes them consistent with the kinetic experimental data. Our results suggest additional experiments that may be conducted to further narrow down the microscopic mechanism of G-protein activation.

In Dell'Orco and Koch (3), the authors commented on our article (1). The authors focus on one aspect of our study, namely the dissociation rate (k_{off}) of the putative RG dark complexes and its relevance for the amplification mechanism. While we are in agreement that this rate must be sufficiently high to permit fresh supply of G protein to the active photoreceptors, they criticize our marginal note that the very slow rate of $k_{off} = 0.148\ s^{-1}$ reported in Table 1 of their article (4) is inconsistent with this requirement. They note to have emphasized in Dell'Orco and Koch (4) that only "relative values" of the surface plasmon resonance (SPR) kinetics should be used. They also point out that our simulations cannot be conclusive as to the role of the RG complexes in signal transduction. We respond to these comments in the following two sections.

Affinity and dissocation rate of the nonproductive precomplex between rhodopsin and G-protein

Although it is clear that active rhodopsin (R*) and G-protein (RG) transducin must interact in order to convey the activation signal, it was suggested that there is also an interaction between inactive Rhodopsin (R) and G-protein. The critical point is here the lifetime, or dissociation rate, of such a nonproductive precomplex.

Dell'Orco and Koch (4) have conducted SPR experiments of R-G interaction, and report binding and dissociation rates, as well as K_D values for R-G and R*-G in Table 1 in Dell'Orco and Koch (4). Although the aim of our article (1) was to produce an independent parameterization and not to criticize previous models, the comment (3) prompts us to respond to the parameters reported in Dell'Orco and Koch (4) in some detail.

1. In another work (3), Dell'Orco and Koch explain the fact that their off-rates are underestimated by the statement that "SPR has well known limitations in resolving fast dissociation processes". However, the apparent dissociation rate constants of $0.148 \pm 0.007\ s^{-1}$ for R-G dissociation and $0.00047 \pm 0.00015\ s^{-1}$ for R*-G reported in Dell'Orco and Koch (4) are very slow, and—as the authors admit in their response—much too slow. Both rates cannot apply to the in vivo situation, because that would essentially block phototransduction.
2. Although the authors of Dell'Orco and Koch (3) agree with this notion, they go further by applying the ratio of their off-rates of R-G versus R*-G of 315 to our R*-G dissociation rate of $200\ s^{-1}$ and conclude that then a physiologically realistic absolute off-rate would result. We believe that this calculation is not applicable, because our dissociation rate was obtained in the presence of GTP (2), while the dissociation rate of R*-G in the in vitro setup of Dell'Orco and Koch (4) is measured

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without GTP. Thus, the ratio of 315 is not applicable to a physiologically relevant case.

3. In Dell'Orco and Koch (4) it is stated that "The equilibrium dissociation constant K_D [...] was computed as the ratio between the k^{off} and the k^{on} values (Table 1)." As a result of the error in off-rates described in Point 1 above, these K_D values are associated with the same large relative error.
4. In their letter (3), the authors claim that despite the inaccuracies described above, the relationship of rates in the light and in the dark (1.6 for the on-rates and ≥ 315 for the off-rates) are reliable. We see no basis for this claim. The ratio of two systematically wrong numbers could only be correct if the error would be known to be a constant multiplicative factor, but this is not known.

In their response (3), Dell'Orco and Koch now confirm that the absolute dissociation rates reported in Table 1 of Dell'Orco and Koch (4) should indeed not be used (and thereby agree to our finding, that a much higher off-rate is applicable). We thank them for this clarification and would like to add that the dissociation constants and relative rates in Dell'Orco and Koch (4) cannot be considered reliable either.

Relevance of our simulation setup for the role of the RG complex

1. The authors state "Based on previously performed simulations by Dell'Orco and Schmidt (5) the analysis by Schöneberg et al. was significantly improved…"
   This statement is misleading. Our kinetic model is not at all based on Dell'Orco and Schmidt (5), but has been independently parameterized from a large set of real-time kinetic measurements of G* production as a function of GTP, GDP, G concentrations, and illumination intensities (2). Our general-purpose particle-based reaction-diffusion simulator READDY (6) is an original and independently developed software package. It has no relationship at all to the MATLAB scripts (The MathWorks, Natick, MA) used in Dell'Orco and Schmidt (5) and we have never seen or used any of their code. READDY was used to run high performance Brownian dynamics simulations, in continuous space, on the actual spherical geometry of the experiment (5), for particles that interact with particle-particle interaction potentials.
2. The statement of Dell'Orco and Koch (3) that, in Schöneberg et al. (1), we "raise doubts about the existence of such precomplexes, which would simply slow down the rate limiting steps in the cascade", is incorrect. On the contrary, we derive bounds for the dissociation rates of the precomplex under the assumption that the precomplex exists—and these bounds are consistent with kinetic experimental data.
3. Finally, in Dell'Orco and Koch (3) it is stated that our simulation setup (1) "cannot be conclusive as to the role of preformed RG complexes" and that the precomplexes should instead be considered in combination with supramolecular organization. We have chosen not to consider this situation in Schöneberg et al. (1) because it is arbitrarily complex and ill defined. Competing models with evidence for the supramolecular structure of rhodopsins have been published. The exact spatial organization, distribution of rhodopsin row lengths (if they exist), their distance, diffusion constants, the precise association/dissociation rates for R:G precomplexes, etc., will all affect the resulting kinetics. For reasons described in detail in Schöneberg et al. (1), we have chosen an analytic viewpoint in our study where we only look at the relevance of the two scenarios (precomplexes and racks) in separation. More realistic models will have to wait for additional experimental data, e.g., relating the supramolecular organization of rhodopsin to its functional state in situ.

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