MS5-P49 Crystal structures of RSEGFP and RSGREEN0.7 reveal photoswitching in EGFP-derived fluorescent proteins

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Reversible photoswitchable fluorescent proteins (PS-FPs) play a key role in superresolution fluorescence microscopy such as PALM *(1)* and pcSOFI *(2)*. Although several examples of these PS-FPs are well known, we developed the rsGreen series, a palette of PS-FPs, based on the frequently used Enhanced Green Fluorescent Protein (EGFP, *3,4*). In comparison to rsEGFP *(5)*, another EGFP-based PS-FP, the rsGreens show an enhanced maturation efficiency at 37°C and faster photoswitching behavior, which makes them suitable for subcellular and superresolution visualization studies.

One of these new FPs, rsGreen0.7 has been crystallized, both in its green-emitting (green-on) and dim (green-off) state. They have the typical β-barrel fold as seen in other FPs. These crystal structures give a first glance at the photoswitching mechanism of EGFP-based PS-FPs. Comparison of the green-on and green-off state shows that photoswitching is accompanied by a *cis-trans* isomerization of the chromophore, which is situated in the center of the barrel. Surprisingly, this isomerization is different when compared with Dronpa *(6,7)* and other well-known PS-FPs. Both in Dronpa and rsGreen0.7, the *p*-hydroxyphenyl moiety of the green-off state chromophore is out of plane compared with the green-on state. However, in rsGreen0.7, this effect is more pronounced. Moreover, the orientation of this moiety is completely different between both proteins.

During the design of rsGreen0.7, residues that could influence the photoswitching behavior and/or the barrel-stability were mutated. As we also determined the crystal structure of its parent, rsEGFP, we were able to elucidate the effect of these mutations and to get a better understanding of the relationship between structure and function in this family of PS-FPs.

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MS5-P50 The structural basis for an essential subunit interaction in influenza virus RNA polymerase

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Influenza A virus is a major human and animal pathogen with the potential to cause catastrophic loss of life. Influenza virus reproduces rapidly, mutates frequently, and occasionally crosses species barriers. The recent emergence of swine-origin influenza H1N1 and avian influenza related to highly pathogenic forms of the human virus has highlighted the urgent need for new effective treatments. Here we demonstrate the importance to viral replication of a subunit interface in the viral RNA polymerase which presents a new set of potential drug binding sites entirely independent of surface antigen type. No current medication targets the heterotrimeric RNA polymerase complex. All three subunits, PB1, PB2, and PA are required for both transcription and replication. PB1 carries the polymerase active site, PB2 includes the capped-RNA recognition domain, and PA is involved in assembly of the functional complex, but so far very little structural information has been reported for any of them. We describe two crystal structures of complexes made by fragments of PA and PB1, and PB1 and PB2[1, 2]. These novel interfaces are surprisingly small, yet they play a crucial role in regulating the 250 kDa. polymerase complex, and are completely conserved among swine, avian and human influenza viruses. Given their importance to viral replication and strict conservation, the PA/PB1 and PB1/PB2 interfaces appear to be promising targets for novel anti-influenza drugs of use against all strains of influenza A virus. It is hoped that the structures presented here will assist the search for such compounds.

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