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Origin of Different Color Hues in Fluorescent Proteins with the Same Chromophore¹ ALEKSANDER REBANE, MIKHAIL DROBIZHEV, SHANE TILLO, NIKOLAY MAKAROV, THOMAS HUGHES, Montana State University — Fluorescent proteins (FPs) exhibit broad variety of absorption and emission colors even though some mutants share the same chromophore structure. We demonstrate that in red FPs including DsRed, mRFP, and mFruits (absorption peak 540 to 590 nm), as well as in green FPs including EGFP, TagGFP, mWasabi, GX variants, and mTFP variants (absorption peak 450 to 500 nm) the colors are caused by internal Stark effect. We use quantitative two-photon absorption spectroscopy to show that the colors changes can be explained by quadratic Stark shifts due to variations of the strong local electric field within the beta barrel. This allows us, for the first time tour knowledge, to directly measure the internal electric field inside a protein. The obtained maximum values up to 10 to 100 MV/cm in the mFruits series are rather large, however, these field strengths are still 1 -2 orders less than those required to ionize the chromophore. These measured values also correspond well with previous theoretical estimates for different proteins. Our finding suggests a new way to sense electrical fields in biological systems, while it also bring order to a bewildering diversity of FP properties

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