JOSEPH F. FOSTER MEMORIAL
CHEMICAL BIOLOGY AND BIOCHEMISTRY SEMINAR

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"Regulation of membrane-localized signaling in native membrane environment."

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Abstract:

The oligomeric organization of membrane proteins in native cell membranes is a critical regulator of their function. High-resolution quantitative measurements of oligomeric assemblies directly from native membranes are indispensable to understanding membrane protein biology. However, this remains a challenging problem due to the inability to preserve the native environment while simultaneously attaining nanoscalespatial and precise molecular resolution coupled with insufficient sensitivity to analyze proteins at endogenous levels of expression. Addressing these challenges, we report a TIRF microscopy-based single-molecule photobleaching step analysis technique, Native-nanoBleach, to determine the oligomeric distribution of membrane proteins directly from native membranes at an effective spatial resolution of ~10 nm. We achieved this by capturing target membrane proteins in "native nanodiscs" that retain an annular ring of the proximal native environment using amphipathic copolymers. Counting subunits within single nanodiscs affords an effective lateral spatial resolution determined by the disc diameters (~10 nm). We applied Native-nanoBleach to quantify the oligomerization status (homo/heterooligomerization) of structurally and functionally diverse membrane proteins, including receptor tyrosine kinases (TrkA) and small GTPases (Ras) under growth-factor binding and oncogenic mutations, respectively. We demonstrate the presence of KRas dimers in native nanodiscs. Since previous data on supported lipid bilayers showed no intrinsic KRas dimerization properties, these results highlight the critical contribution of the native environment in aiding KRas dimerization. Native-nanoBleach is a compelling choice for studying membrane protein oligomerization – the spatial resolution is comparable to state-of-the-art technologies, has low sample requirements, can examine organellar-membrane localized proteins and applicable at endogenous expression levels, permitting easy adoption with access to commercial TIRF microscopes without requiring specialized hardware. This general experimental pipeline will usher in a new era of studying membrane protein organization *in their native-membrane environments* under various physiological and clinical conditions.