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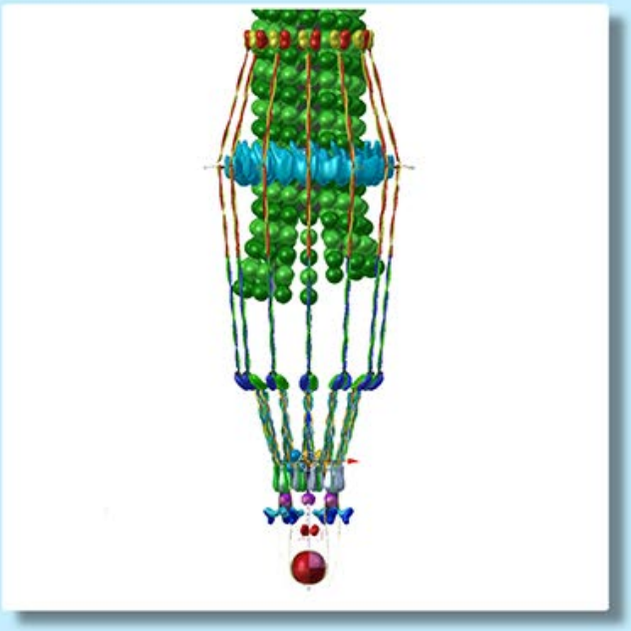

Working yeast kinetochore model
(by Alyona Fulp, Bloom Lab)

Probing the 3D Architecture of the Inner Kinetochore

The fidelity of chromosome segregation is necessary to maintain genome stability and prevent aneuploid disorders. During segregation, the kinetochore is assembled onto centromeric DNA and achieves the attachment of microtubule plus ends, which provide the force to physically segregate chromosomes to the two poles of the cell. The kinetochore is a macromolecular protein machine composed of eight different protein multi-component complexes, the DNA-binding components of which define the inner kinetochore, and the microtubule-binding components of which define the outer kinetochore. The 3D protein architecture of the kinetochore in living cells remains poorly understood due to the resolution limits of live-cell imaging techniques. Here, we used two-color, *in vivo* fluorescence microscopy to determine the positions of three inner kinetochore proteins, Ame1, Mtw1, and Nnf1, along the budding yeast kinetochore axis with nanometer resolution. Rather than remaining in a defined linear order along the kinetochore axis, inner kinetochore proteins localized to both the microtubule- and DNA-facing side of the centromeric nucleosome in dividing cells. Furthermore, the frequency with which they are found on the DNA-facing side increases with their distance from the nucleosome. We attribute this behavior to the flexibility of the DNA substrate, which demonstrates the importance of incorporating substrate dynamics into our understanding of kinetochore structure and function.