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The Application of Cryogenic TEM for Studying Protein–Metal–Organic Frameworks

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Protein–metal–organic frameworks (p-MOFs) are a prototypical example of how synthetic biological hybrid systems can be used to develop next-generation materials.(Doonan, et al., 2017; Liang, et al., 2015) Controlling p-MOF formation enables the design of hybrid materials with enhanced biological activity and high stability. However, such control is yet to be fully realized due to an insufficient understanding of the governing nucleation and growth mechanisms in p-MOF systems. TEM enables direct observation of morphological changes with atomic scale resolution to probe crystal growth. Although p-MOF crystals are stable under the high vacuum conditions of an electron microscope, the precursor phases to all crystalline structures synthesized in solution involve the formation of hydrated transient species.(De Yoreo, et al., 2015) These hydrated species are incompatible with standard TEM experiments, and we consequently utilized cryo-transmission electron microscopy to monitor ZIF-8 formation in the absence and presence of BSA.(Ogata, et al., 2020; Patterson, et al., 2017) The cryoTEM expeirments revealed the structural evolution of p-MOFs involves nonclassical pathways via dissolution–recrystallization of highly hydrated amorphous particles and solid-state transformation of a protein-rich amorphous phase.

The cryoTEM data is supported by x-ray diffraction, dynamic light scattering, dry state TEM and SEM, and correlative fluorescence microscopy and TEM experiments. These data demonstrate the importance of amorphous phases and particle–particle interactions in the formation of ZIF-8 and BSA-ZIF-8 composites. Aggregation of colloidal particles, such as the observed amorphous particles during ZIF-8 formation, is largely dependent on the electrostatic interactions between particles. Therefore, difference in nucleation and growth mechanisms can be explained by changes in interactions between the two types of amorphous particle: (1) the HmIm/Zn amorphous particles and (2) the protein/HmIm/Zn particles. To provide evidence of the generality of transient biomolecule/Zn/HmIm intermediates, we performed the same precursor studies on alternative proteins that enable formation of ZIF-8 biocomposites. On the basis of these data, we propose a general description of p-MOF crystallization which is best characterized by particle aggregation and colloidal theory for future synthetic strategies. Furthermore, we believe that controlling how proteins are encapsulated into p-MOFs may be most successful when approached from a colloidal organization perspective, emphasizing the electrostatic interactions that occur between precursor particles in solution.

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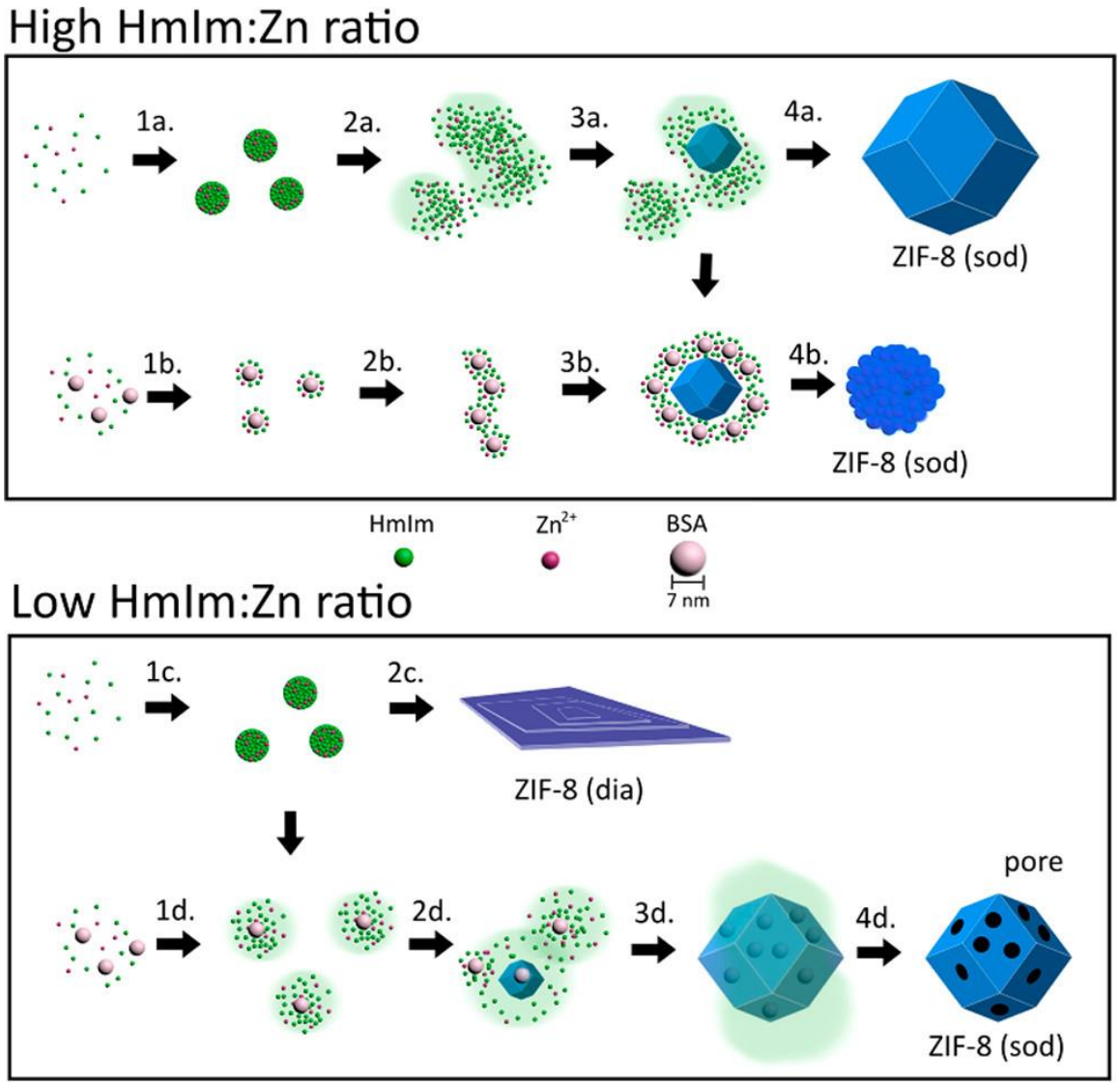

Figure 1. Proposed schematic of ZIF-8 formation with and without BSA. At high HmIm:Zn ratios, ZIF-8 crystal growth follows steps 1a–4a and reaches completion through 5a. In the presence of BSA, steps 1a–4a occur in parallel with a second process outlined in 1b–4b. At low HmIm:Zn ratios, ZIF-8 (dia) forms in the absence of BSA through the formation of amorphous particles outline in 1c–2c. Upon addition of BSA, HmIm and Zn ions complex at the protein surface and form a protein-induced amorphous phase that promotes ZIF-8 (sod) nucleation and growth outlined in 1d–4d.

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