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Effects of surface biotin density on lipid monolayer-assisted 2D crystallization of streptavidin at the aqueous solution-vapor interface MASAFUMI FUKUTO, MATTHEW LOHR, SUNTAO WANG, SUMIT KEWALRAMANI, LIN YANG, Brookhaven National Laboratory — Adsorption and two-dimensional (2D) crystallization of soluble protein streptavidin on a biotinylated lipid monolayer at an aqueous solution-vapor interface have been studied extensively since the 1990s. These previous studies, largely based on fluorescence microscopy and *ex-situ* electron microscopy measurements, revealed the effects of protein modifications and aqueous buffer conditions, such as pH and ionic strength. We have examined the dependence of 2D streptavidin crystallization on the areal biotin density in the lipid monolayer template, using Brewster-angle microscopy (BAM) and *in-situ* x-ray reflectivity and grazing-incidence x-ray diffraction (GID). The lipid monolayer consisted of a binary mixture of DMPC and DPPE-x-biotin, and the biotin density was controlled by varying the lipid composition while keeping the area per lipid fixed. Both BAM and GID results demonstrate that in order for 2D crystallization of streptavidin to occur, the surface biotin density must exceed a threshold, corresponding to approximately two biotins per protein. The results highlight the importance of well-defined molecular orientations to the 2D crystallization of proteins.

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