Nucleation of large scale of furin crystals from nanoparticle seeds

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Furin proteases are important in developing therapeutics for a range of drugs including antivirals for COVID -19 (Figure 1a). However, in a general, methods for crystallizing furin – drug complexes are not well- developed. In particular, the growth of crystals suitable for characterization by synchrotron XRD is currently a limitation for development of therapeutics from other proteases. Our focus was to developing robust crystallization conditions for growth of good quality protease crystals using nanoparticles to aid the crystallization process. Particularly, this method illustrates protein crystallization without the use of ligands, His-tags, or other modifications [1].

According to the *Modified Hanging drop method* (Figure 1b) is typically used for growing crystals of furin [2]. In this method, equal volumes of the protein solution (comprising of Buffer + Nanoparticle + Protein) and the crystallization solution are used. We have developed these protocols further and tested specific experimental protocols for growing crystals of furin. The recommended sample concentration is 5-25 mg/ml in dilute (25 mM or less) buffer. We typically aim to target a protein solution concentration of 9 mg/mL. The crystallization solution we use in our lab involves the following reagents: 0.1 M tris, 0.1 M sodium potassium phosphate/2.8 M sodium chloride (pH 6.1 – 7) / 16% (w/vol) and *gold nanoparticles (AuNPs)* [3]. We also use the Using this in-house protocol for the crystallization of furin, we were able to grow excellent quality crystals of furin (Figure 1c). The furin crystals were grown in our laboratory at room temperature over a period of 11 hours screened under the Single crystal x ray diffraction and Cryo EM.

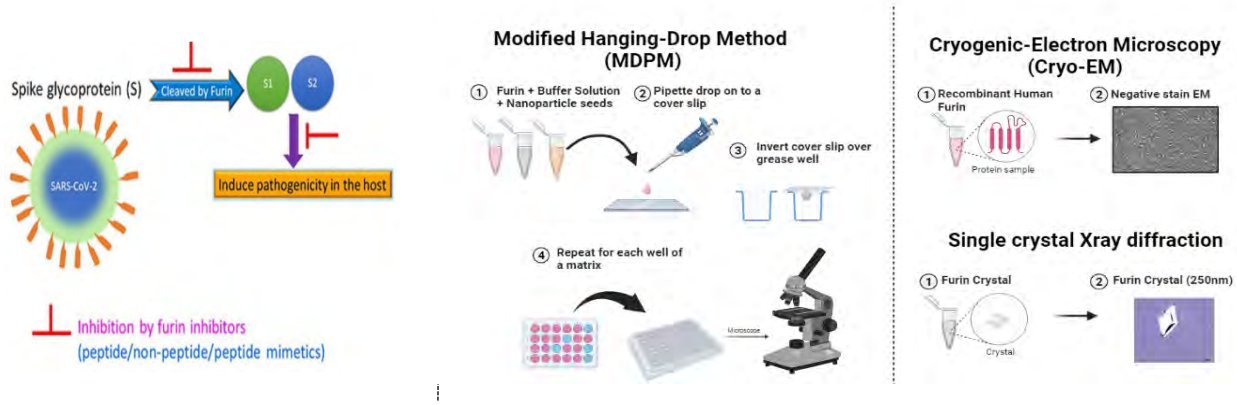

Figure 1. (a) Recombinant Human Furin, (b) Modified Hanging Drop Method, (c) Cryo-EM & (d)Single Crystal Xrd for furin crystallization.

Reference

[1] Dessau MA, Modis Y. *Protein crystallization for X-ray crystallography*. J Vis Exp. 2011 Jan 16;(47):2285. doi: 10.3791/2285. PMID: 21304455; PMCID: PMC3182643.

[2] David Sheehan, Carolyn O'Mahony, Miquel Coll, *A modification of the hanging drop method of protein crystallisation suitable for an undergraduate class practical*, Biochemical Education, Volume 26, Issue 2,1998, Pages 173-175.

[3] Matthew N. Martin, James I. Basham, Paul Chando, and Sang-Kee Eah, *Charged Gold Nanoparticles in Non-Polar Solvents: 10-min Synthesis and 2D Self-Assembly,* Langmuir 2010 26 (10), 7410-7417.