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Crystallization of ATG9 HDIR-ATG101:ATG13 complex

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DISCLAIMER

ABSTRACT

Crystallization protocol for the ATG9 HDIR-ATG101:ATG13 complex

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Crystallization

1 ATG9 HDIR (828-839) fused ATG101 (1-198):ATG13 (1-197) complex was concentrated to 6 mg/ml in 25 mM HEPES pH 7.5, 150mM NaCl, 1mM MgCl2, 1mM TCEP

2 Crystallization was carried out by sitting-drop vapor diffusion using an automated liquid-handling system (Mosquito, TTP LabTech, UK) at 288 K in 96-well plates

3 The protein solution was mixed with the reservoir buffer composed of 0.1 M HEPES pH 7.5, 0.2 M NaCl, 12% PEG8000 with a ratio of 1:1

4 Crystal trays were checked daily using a light microscopy for crystal growth

5 Large crystals were obtained in 2–4 days. Crystals were cryo-protectedin 28% glycerol/reservoir buffer and frozen in liquid N_2