Protein Structure and Function

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1 Principles of X-ray crystallography

1.1 Scattering

- When light hits matter...
  - Vibration→scattering (all directions)
  - Energy level transitions→absorption and emission (fluorescence)
  - Photochemical reactions (e.g. photosynthesis)
- scattering can give rise to refraction and diffraction
- experiments on scattering
  - turbidity (reduction in intensity)
  - angular dependence
  - changes in λ

1.2 Wnt3-Fz8 Complex (Hirai et al. 2019)

- crystals of lysine-methylated and deglycosylated human Wnt3 (hWnt3)-mFz8 CRD complex were obtained by X-ray crystal structure was solved by molecular replacement and refined to a resolution of 2.8Å.

1.2.1 Difficulties in crystallisation and their solutions

- Strong hydrophobic property of Wnt proteins caused by a covalent lipid modification

Optimisation and chemical modifications conducted to ensure high expression yields, enhanced solubility and sample homogeneity

1.2.1.1 Solubility

- Failed attempts on making crystallization constructs
  - afamin can solubilize Wnt proteins; when coexpressed and complexed, Wnt3 and 3a can be purified to homogeneity. However, diffraction-quality crystals could not be obtained after repeated trials.
  - coexpression with mFz8 CRD after Janda's success; but found purified Wnt3a-Fz8 CRD complex still remained hydrophobic and required detergents during concentration, which hampered crystallisation
- Successful: N-terminal truncation of Wnts to mimic the cleavage of the N-terminal peptide by a metalloprotease Tiki, which has been reported to reduce the overall hydrophobicity
  - N-terminal 20 residues were removed from hWnt3/mWnt3a constructs
  - PA-hWnt3(ΔN)/PA-mWnt3a(ΔN) coexpressed with mFz8 CRD C-terminally fused with modified human Fc.
  - confirmed that complexes were fully soluble in aqueous buffer and could be concentrated to > 5 mg/ml without detergents

1.2.1.2 Optimisation

- Initially (following Janda) attached normal (with hinge region) human IgG1 Fc to the C-terminal of mFz8 CRD, intervened by a TEV protease cleavage sequence for the later Fc removal
  - although complex formed with high yield, it could not be cleaved at all; different linker lengths showed no improvements
- decided to use IdeS protease to remove Fc.

Hirai, Hidenori, Kyoko Matoba, Emiko Mihara, Takao Arimori, and Junichi Takagi. 2019. "Crystal Structure of a Mammalian Wnt–Frizzled Complex." *Nature Structural & Molecular Biology* 26 (5): 372–79. https://doi.org/10.1038/s41594-019-0216-z.