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RUTINOSIDASE

LABYRINTHINE JOURNEY FOR X-RAY STRUCTURE

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Abstract

Obtaining well diffracting crystals and solving protein structure can be tedious work and successful process may include various crystallization techniques and tricks. Here we present one didactic story of crystallization α-L-Rhamnosyl-β-D-glucosidase (Rutionsidase) from *Aspergilus niger*. During the crystallization process, we performed screening using vapour diffusion method, optimization by counter diffusion technique, and final crystals soaking of heavy atoms in micro batch experiments, which allowed structure solution by SIRAS. However, to repeat the crystal growth, we had to deglycosylate the enzyme and perform new screening followed by Matrix Microseed Screening. Moreover, as final reproducible procedure, for growing the protein crystals, we used under oil micro batch experiments. With this optimised method, we were able to grow crystal that diffracted upto 1.27 Å resolution and see structural details that shall be used in the future.

I

Rutinosidase from *Aspergillus niger*

Recombinant production in *Pichia pastoris*

Purified and concentrated to 35 g/l

Screening

II

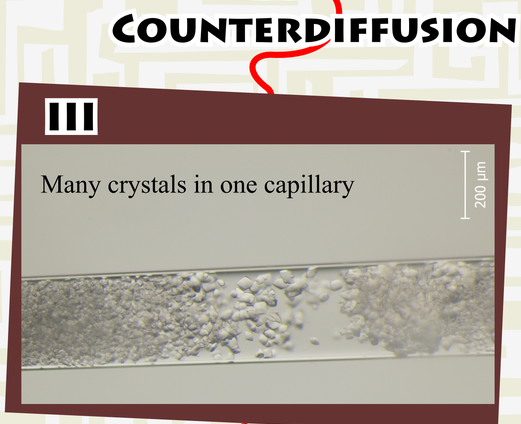
One hit in 192 vapor diffusion experiments

0.2 M Zinc Acetate
0.1 M Imidazole pH 8.0
20% PEG 3000

Counterdiffusion

III

Many crystals in one capillary



Under oil microbatch

IV

Crystals from capillary were extracted by breaking the capillary into the precipitating solution under oil to prevent evaporation.

Some crystals were transferred into precipitant solution containing 100 mM NaI.

Data collection

V

Data collected at BESSY, Berlin, Germany

$P4_12_12$

Structure solved by SIRAS upto 1.9 Å

New crystal growing

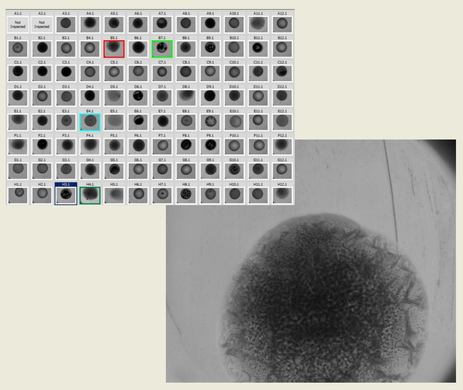
VI

NO CRYSTALS AT ALL

Deglycosylation and new screening

VII

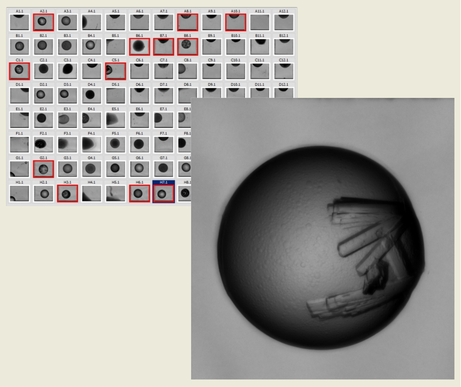
One hit in 192 conditions



Random Microseed Matrix Screening

VIII

Twelve crystal hits in 96 conditions



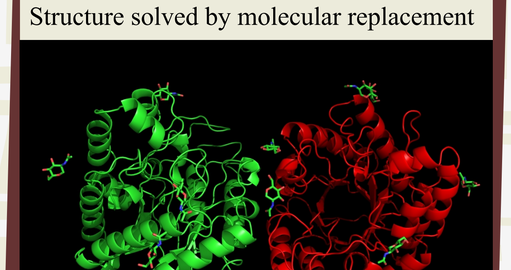
Data collection

IX

Data collected at homesource upto 1.8 Å

$P2_12_12_1$

Structure solved by molecular replacement



Optimization in microbatch

X

Precipitation solution derived from newly found condition

20 mM Amonium Sulfate
0.1 M Bis-Tris pH 5.5
25% PEG 3350

PEG concentration from 20% to 30%

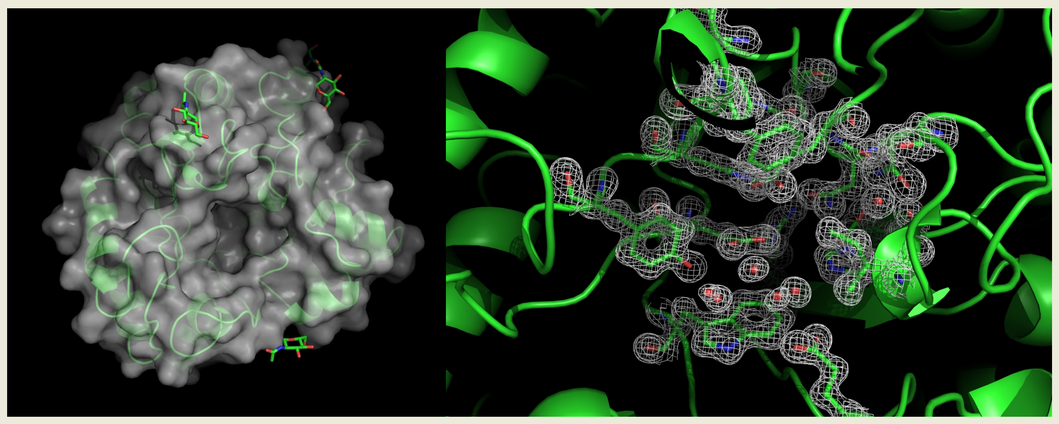
Two concentrations of seed stock

Quest for glory
Find the structure

TO THE LABYRINTH

XI

Overall structure of rutinosidase and detail of active site with electron density map contoured at 1.5σ



Data collected at BESSY, Berlin, Germany

Crystal diffracted upto 1.27 Å

R/R_{free} = 0.16 / 0.18

Structure contains 358 AA and 5 N-acetylglucosamine molecules

Overall fold is TIM barrel consisting of 8 α-helices and 8 parallel β-strands

Similar deposited structure is Glucan 1,3-β-glucosidase with RMSD of 226 Cα atoms 2.2 Å

Conclusion

Finding crystal structure and reproducible crystal preparation process is not easy task and in many cases using several techniques is crucial. Therefore diversity of methods is in every crystallization process beneficial and combining advantageous attributes of each technique can lead to the best results.

In this case information from future crystal structures of rutinosidase with substrates and products will serve for protein engineering to affect it's substrate specificity for synthesis purposes.