

# X-ray Crystallography of Concanavalin-A and IF7

Zaphron Richardson, Kathleen Dwyer; Maplewood Richmond Heights High School, MO 63143  
Norma Duke, SBC-CAT

## Abstract

The development of vascular (blood) supply is an essential step in the growth and metastasis of malignant tumors. Annexin 1, involved in human anti-inflammatory processes, is of use as a potential anticancer therapeutic; it is capable of highly specific tumor vasculature recognition. Recent articles have identified the carbohydrate ligand-mimicking 7-mer peptide, IFLWLQR (IF7) as capable of targeting annexin A1 in mouse tumors. IF7 can exhibit “unprecedented tumor targeting activity”, and has been detected in mouse tumors within a few minutes of intravenous injection of the peptide [PNAS, 108(49), 19587-19592 (2011)]. Thus, IF7 may have the potential to act as a delivery vehicle of anticancer drugs to the location of the tumor. Concanavalin-A is a carbohydrate binding protein, originally extracted from Jack Bean *Canavalia ensiformis*. It binds to various sugars, glycoproteins and glycolipids, by recognition of a  $\alpha$ -D-mannosyl or a  $\alpha$ -D-glucosyl group. We have grown crystals of Concanavalin-A, cross-linked them with glutaraldehyde, then soaked them in a solution of IF7, in an attempt to identify the peptide’s biologically active conformation.

## Procedure

1. Order concanavalin-A from Sigma-Aldrich (C2010). Remove nascently binding metals by dissolving protein in 30% (w/v) NaCl solution, adding 1.0M HCl until  $\sim$ pH 1.2. Stir at room temperature for 30 minutes, then dialyze 3x against Milli-Q water; pH of solution returns to  $\sim$ pH 6.
2. Dialyze protein solution 3x against 0.40M NaCl, 0.05M NaOAc, pH 5.2.
3. Centrifuge to clarify solution; filter over 0.22 $\mu$ m filter.
4. FIRST, Bring to 1mM MnCl<sub>2</sub>; allow to incubate at room temperature for 1 hour. THEN, bring to 1mM CaCl<sub>2</sub>; allow to incubate at room temperature for additional 1 hour.
5. Dialyze protein solution (23 mg/ml) against 0.10N NaNO<sub>3</sub>, 0.050M tri-acetate, 0.20% NaN<sub>3</sub>, 1mM MnCl<sub>2</sub>, 1mM CaCl<sub>2</sub>, pH 5.2. Allow solution to sit quietly at room temperature for approximately 1-2 weeks.
6. Cross-link crystals for 40 minutes in dialysis solution containing 2.5% glutaraldehyde; place crystals in dialysis solution containing peptide and 25% DMSO and soak for 3 hours.
7. Obtain crystal, transfer to same solution plus 30% MPD. Freeze crystal in liquid nitrogen.
8. Examine samples using APS beamline 19-BM, and analyze the diffraction patterns to obtain results.



Fig 1. APS SBC-CAT 19-BM Beamline.

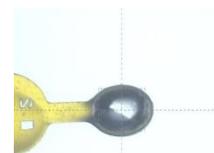


Fig 2. Native crystal of concanavalin-A.

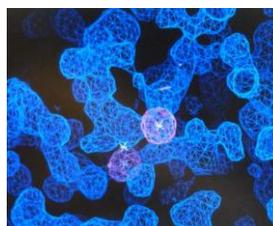


Fig 3. Mn and Ca cation binding sites



Fig. 4 Maplewood Richmond Heights students

## Results

Two sets of crystals were examined with x-ray diffraction. The first set was of native concanavalin-A, prior to cross-linking with glutaraldehyde; this allowed us to obtain the structure of “protein alone”, and judge the quality of the unaltered crystals. The second set was of concanavalin-A, cross-linked with glutaraldehyde, then soaked in IF7 peptide solution for 3 hours.

We identified the protein in both electron density maps, and compared the results. Unfortunately, the peptide had not bound to the concanavalin-A during the 3 hour soak. A similar study, using a 6-mer peptide, had soaked the crystals for 20 days; this experiment could be repeated, using a longer soak time.

The diffraction data sets were collected at a wavelength of 0.97911 Å; the merged data for the native concanavalin-A was, on average, 45x redundant. The data allowed us to determine, ab initio, the phasing of the concanavalin-A protein (using the bound manganese and calcium cations), and to verify the binding sites of each of the cations.

## Conclusion

In this experiment we were able to test for the binding of IF7 peptide to the sugar-binding domain of Jack Bean concanavalin-A. Despite soaking the crystal for three hours, we did not see any evidence of the peptide binding; a longer soak time may be required for the peptide to be observed in the electron density map, or the peptide may not bind to concanavalin-A.

We were able to determine the three-dimensional structure of concanavalin-A, ab initio, from the observed data, and verify the binding sites of the manganese and calcium cations within the protein. The Advanced Photon Source’s source of x-rays was invaluable to the determination of this structure.

## References

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