ESRF	Experiment title: STRUCTURE DETERMINATION OF POLYAMINE OXIDASE	Experiment number: LS - 943
Beamline: ID14 3	**Date of Experiment:** from: 19/4/98 to: 21/4/98	**Date of Report:** 11/ 8/98
Shifts: 6	**Local contact(s):** WIM BURMAISTER	*Received at ESRF :*

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A total of 8 data sets were collected from crystals of two FAD-dependent enzymes.

Polyamine oxidase catalyses the oxidation of spermine, a central reaction in the polyamine metabolism. Data from four crystals soaked in different competitive inhibitors have been collected. The main results deriving from the experiment are: (1) *structure determination* of *the enzyme* (by MIR and threefold averaging) *has been completed* (Rfactor=19.5% for 216570 reflections). Polyamine oxidase represents the first flavin-dependent amine oxidase for which the 3D structure is available; (2) cancer cells strictly require polyamines for their growth. The structural analysis of 4 *enzyme-inhibitors* has provided information on the *catalytic and substrate specificity properties.* This knowledge will provide the starting point for the design of compounds with anti-cancer activity.

L-aspartate oxidase catalyses the first step in the bacterial de novo biosynthesis of NAD. The enzyme shares a number of functional and structural (i.e. 30% sequence identity) properties with the flavin subunit of succinate dehydrogenase. In our laboratory, we have been working on this enzyme for four years, encountering a number of difficulties with reproducibility of crystals and identification of good heavy atom derivatives. Data collection at ESRF has eventually led to the solution of the three-dimensional structure. A native and three heavy atom derivatives have been collected. These data were used for phasing by the program SHARP. The resulting map was of first class quality. L-asparrate oxidase displays a partially new fold and its structure will be of general interest for gaining insight into the class of succinate dehydrogenase/fumarate reductase oxidoreductases.

Experiment	Resol. (Å)	Measured reflections	Independ. reflections	Overall compl. (%)	Compl. highest resol. shell	Overall Rfactor (%)	Rfactor highest resol. shell	Comment
Polyamine oxidase (P6522, a=b=185 Å, c=280 Å)								
Saoking in:								
-Guazatine	1.9	677712	208.000	94.3	81.6	10.1	38.0	Complex with an herbicide
-Diamino octane	1.9	774835	215873	97.9	87.9	8.1	32.8	Competitive inhibitor
-Diamino dodecane	1.9	785004	216570	98.1	88.4	8.2	33.5	Binding only in one subunit
-MDL72527*	1.9	704940	211025	96.6	95.2	8.9	17.3	Substrate analogue, insightful for catalytic mechanism
L-aspartate oxidase (P3221, a=b=85 Å, c=160 Å)								
-Native	2.4	73298	25520	97.6	94.3	8.7	39.0	
-0.1 mM PCMBS	2.8	57124	16323	98.6	97.0	9.1	19.9	Good derivative
-1.0 mM PCMBS	2.3	101590	29355	98.4	93.7	7.9	28.3	Poorly isomorphous
-4 mM OsO_4	1.9	156169	46528	89.0	70.5	5.1	41.7	Data set used for refinement

* N,N'-bis(2,3- butadienil)-1,4-butanediamine