Serendipitous SAD Phasing

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We describe here the solution of the structure of a bisintercalator-DNA complex with the help of an anomalous scatterer fortuitously present in the structure.

The DNA bisintercalator Echinomycin is characteristic of a family of anticancer drugs that interfere with both replication and transcription. The crystal structures of Triostin A[1] and Echinomycin[1] in complex with (CGTACG)2 showed for the first time Hoogsteen base pairing in a double stranded DNA.

In our laboratory we grew crystals of the complex of Echinomycin with two other sequences: (GCGTACGC)2 and (ACGTACGT)2, which show also the canonical Hoogsteen base pairing outside the intercalation site. Over time, a different crystal form grew in the the same drops whose structure could not be solved by molecular replacement. A native high resolution dataset was collected at BL14.1 at BESSY on this crystal form and showed a weak but significant anomalous signal although no heavy atom was included in the crystallization conditions. SAD phasing showed one single heavy atom site from which an interpretable electron density map could be obtained. The heavy atom proved to be a mixture of metals of unknown origin.

The structure shows a mixture of the canonical Hoogsteen base pairing together with the Watson-Crick mode that was suggested by NMR data, showing great flexibility of the DNA duplex in accommodating the bisintercalation and that Hoogsteen base pairing is not a necessary condition for Echinomycin binding. It offers a detailed three-dimensional model for molecular dynamics simulations of binding for both Hoogsteen and Watson-Crick base pairing around the intercalation site.

[1]Ughetto G., Wang A.H.J., Quigley G.J., van der Marel G.A., van Boom J.H., Rich A., *Nucl. Acids Res*., 1985, **13**, 2305.

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