

	Experiment title: Preliminary characterization of crystals of the Spc24/Spc25 subcomplex of the Ndc80 complex	Experiment number: MX-394
Beamline: ID14-2 ID14-2	Date of experiment: from: 07/05/2005 to: 09/05/2005 from: 21/07/2005 to: 22/07/2005	Date of report: 24/07/2005
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Report:

The Ndc80 complex is a constituent of the outer plate of the kinetochore and plays a critical role in establishing stable kinetochore-microtubule interactions required for chromosome segregation in mitosis. The Ndc80 complex is evolutionarily conserved and contains the four subunits Spc24, Spc25, Nuf2 and Ndc80 (whose human homologue is called Hec1). All four subunits are predicted to contain globular domains and extensive coiled-coils regions. To gain an insight into the organization of the human Ndc80 complex, we have reconstituted it using recombinant methods and studied a number of biophysical parameters, including the hydrodynamic properties. This analysis showed that the recombinant Ndc80 complex is very similar or identical to the endogenous HeLa cell complex and consists of a 1:1:1:1 stoichiometry of the four subunits and a very elongated shape. Two tight Hec1/Nuf2 and Spc24/Spc25 sub-complexes, each stabilized by a parallel heterodimeric coiled-coil, maintain this organization. These sub-complexes tetramerize via an interaction of the C- and N-terminal portions of the Hec1/Nuf2 and Spc24/Spc25 coiled-coils, respectively. We have been able to raise crystals of a segment of the Spc24/Spc25 complex containing part of the coiled-coil region and the two globular domains. The crystals diffract X-ray to a maximal resolution of 3.8 Å. They belong to a tetragonal space group with unit cell dimensions $a, b = 194.97, c = 173.74, \alpha, \beta, \gamma = 90.00$. Selenomethione and heavy atom derivatives are being generated.