

## Investigating the mechanism of Cd-binding by the rainbow trout estrogen receptor using ICP/MS and MALDI-TOF

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Recent studies have shown that certain heavy metals can mimic the effect of the endogenous estrogen receptor (ER) agonist 17 $\beta$ -estradiol (E2) and lead to estrogen receptor activation. Possible interaction sites of the human ER have been identified using molecular biology tools (Stoica et al. *Endocrinology*, 2003, 144, 2425). We have used a combination of ICP/MS and MALDI to evaluate interactions of the ER with Cd on the macro- and micro levels.

MALDI analysis of the peptide mass profiles following proteolytic digestion was used to identify the sites of the rainbow trout ligand-binding domain involved in cadmium-binding. The results from these experiments indicated that Cd preferentially shields Cys groups against various chemical modifications, underlining their involvement in Cd-binding. This agrees well with known strong metal-binding properties of thiol groups in e.g. phytochelatins.

Competitive binding experiments with radio-labelled E2 indicated a ten times higher affinity of E2. However, ICP/MS showed that despite this higher affinity, increasing E2 concentrations were unable to release pre-equilibrated Cd from the ligand-binding domain. Increasing Cd concentrations on the other hand resulted in a release of pre-equilibrated E2 or prevented binding of E2 possibly due to conformational changes induced by Cd-binding. Additional investigation of this phenomenon using MALDI in combination with hydrogen/deuterium exchange experiments is currently underway.