

## NRP Endocrine Disruptors

### Final Summary

Original project title <b>Development of chemical sensors and affinity-directed fractionation techniques</b>
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Project number <b>4050-66586</b>

### Development of chemical sensors for endocrine disrupting compounds

*New, fast and robust analytical tools are required to measure endocrine disrupting effects of chemicals. This project demonstrated the potential of chemical sensors to suit this need.*

### Research questions

Quick and robust analytical tools are urgently needed for high throughput screening of the multitude of daily-used chemicals for estrogenic effects. A chemical sensor approach based on the affinity between a nuclear receptor and ligands and determination by mass spectrometry provides a rapid alternative to in vitro tests, has the potential for automatization and hence for processing large numbers of samples.

### Results

The ligand-binding domain (LBD) of the estrogen receptor was over-expressed in *E. coli*, producing a fusion protein that could be stored over months and was compatible with the mass spectrometric methods used (MALDI and ESI). Ligand-binding experiments showed a higher receptor affinity for E2 than for the environmental hormone nonylphenol. This agrees with relative estrogenicities determined with in vitro test. That the interaction was specific, i.e. involving the LBD could be shown with competitive ligand-binding experiments with radioactive E2.

Having provided a proof of principle for the chemical sensor approach, the interaction of cadmium with the LBD was investigated, since also Cadmium had been shown to interact with the endocrine system, its mode of action is not well understood. Again, E2 showed an order of magnitude higher affinity for the LBD. ICP-MS measurements of the Cd concentration then showed that despite its higher affinity, increasing E2 concentrations were unable to replace Cd in the binding cavity. On the other hand, increasing Cd concentration did affect E2-LBD binding, which

is either caused by direct competition for the active site, or conformational changes induced by interactions of Cd with the nuclear receptor. Additional proof of the specificity of Cd interaction with the LBD is provided by the activation of the estrogenic response in the yeast estrogen screen.

### **Perspectives**

Automation promises to provide the short cycle times needed for high throughput screening. Furthermore, in order to better mimic the natural system, the full length estrogen receptor should be evaluated in future work.