THE BOWERS GROUP

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Structural Examination of the Peptide-Zinc interaction in the Divalent Oxytocin Complex

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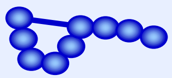
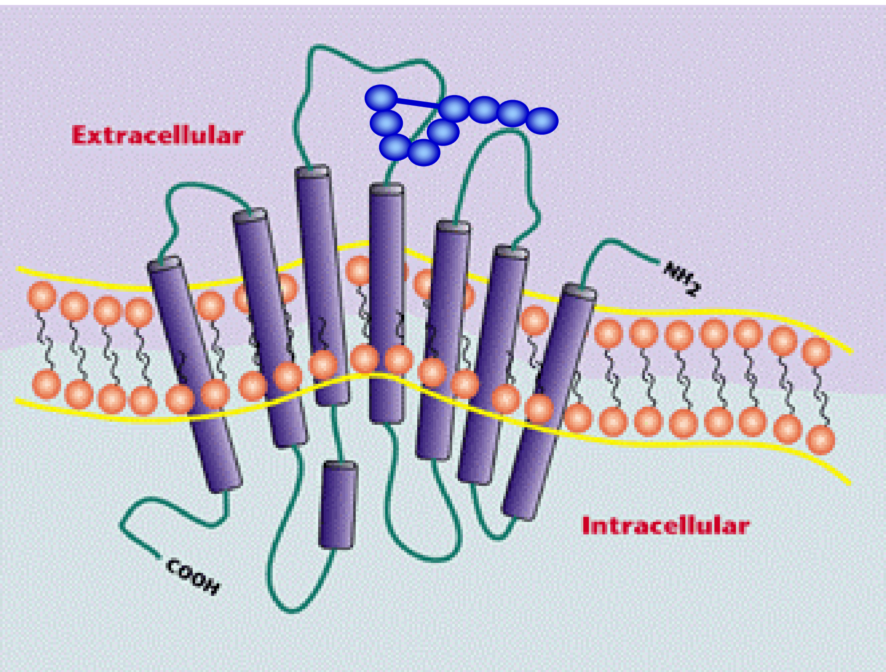
Oxytocin (OT) Structure

OT

Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly(NH_2)

S – S





- Virtually all vertebrate species have an OT-like hormone:
  - Disulfide bridge b/t residues 1 and 6
  - Cyclic portion, 3 residue amidated tail

- Synthesized in posterior pituitary and released into circulation.

- Receptor is G-protein found in smooth muscle cells.

Conformation of OT ligand dramatically affects binding to receptor.

Oxytocin (OT) Function

- OT is found in equivalent concentration in both sexes.

- OT has been linked to several physiological activities:

  1) Uterine contractions during birth
  2) Lactation

Also responsible for "Affiliation" in mammals.

Establishment of complex social and bonding behaviors related to reproduction and the care of offspring.

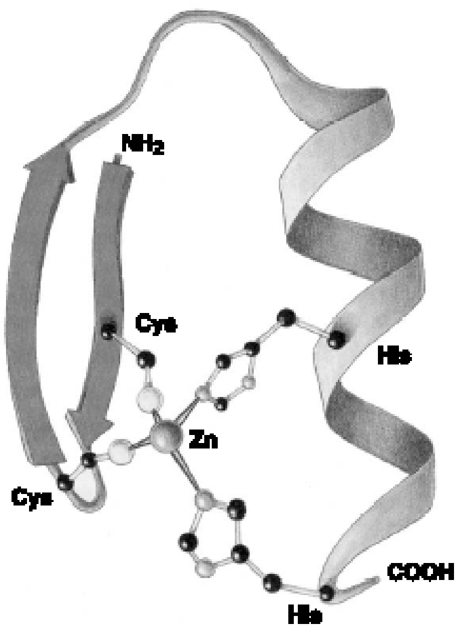
i.e: maternal behavior, infant separation distress, mate formation

- Linked to autism.

Metal-OT Complexes

Why Zinc?

Zinc Fingers: Protein motif that binds DNA
Transcription Factors necessary for DNA replication.



~ 30 residues
OT = 9 residues

OT and Divalent Metal Cations

- Essential elements and other metals have been found to form complexes with OT.
  i.e.: Cu^{2+}, Zn^{2+}, Co^{2+}, Mg^{2+}, Ca^{2+}, Ni^{2+}

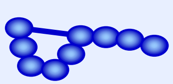
- The presence of divalent cation is essential for specific binding of OT to receptor.
  (Pearlmutter and Soloff, *J Biol Chem*, **1979**)

Research Objectives

Characterize the OT-Zinc complex.

Observation:	Question:
Divalent metal cations required for OT-Receptor binding.	Does Zinc bind to receptor or ligand?
OT has high affinity for divalent metal.	What are the binding properties of the OT-metal complex?
Lock and key model: Receptor binding is conformation-dependent.	Does Zinc cause a conformational change which will enhance binding?

OT



Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly(NH_2)

S – S

Experimental methods

- Mass spectrometry

Cross section
(collisions with helium)

- Hydration
(equilibrium with water vapor)

Theoretical methods

- Molecular mechanics
- Density functional theory

Protons/Metal ions

Molecule size/shape

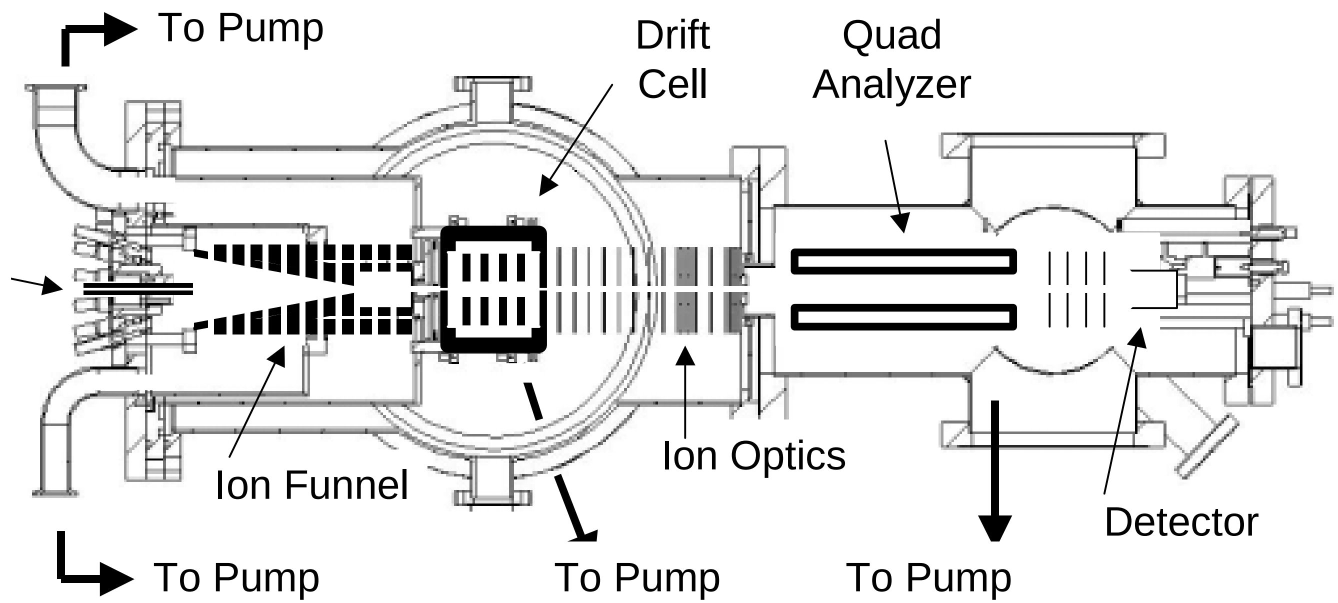
Molecule surface

Molecule structure

Cross section

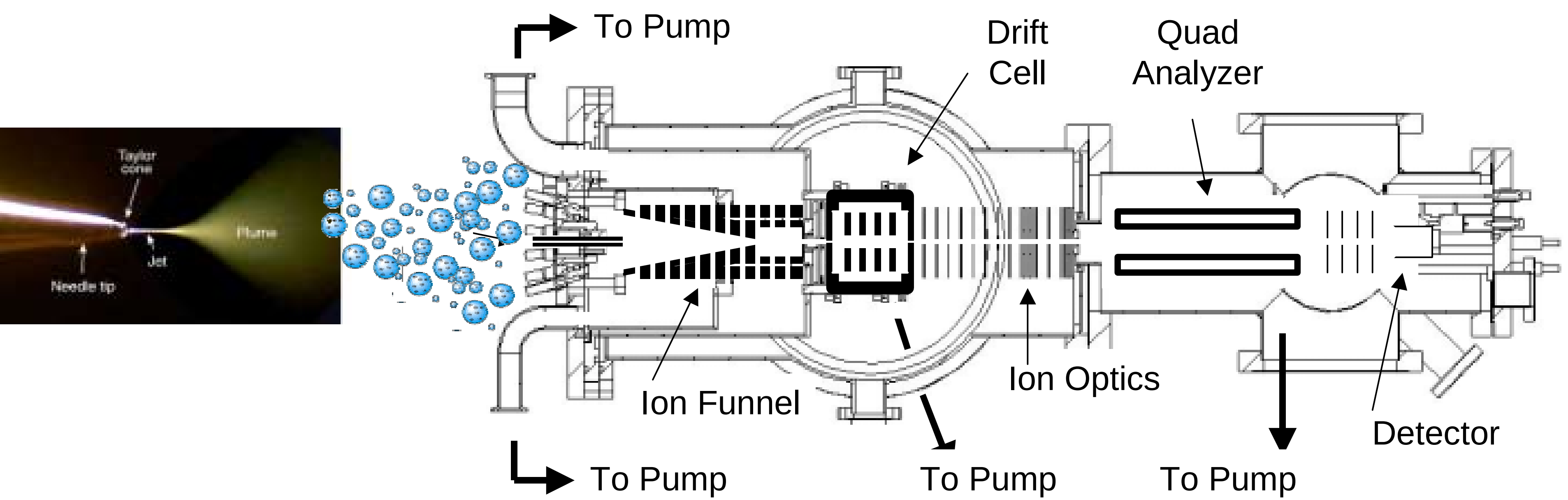
Instrumentation

ESI Ion Source → Ion Funnel → Drift Cell → MS → Detector



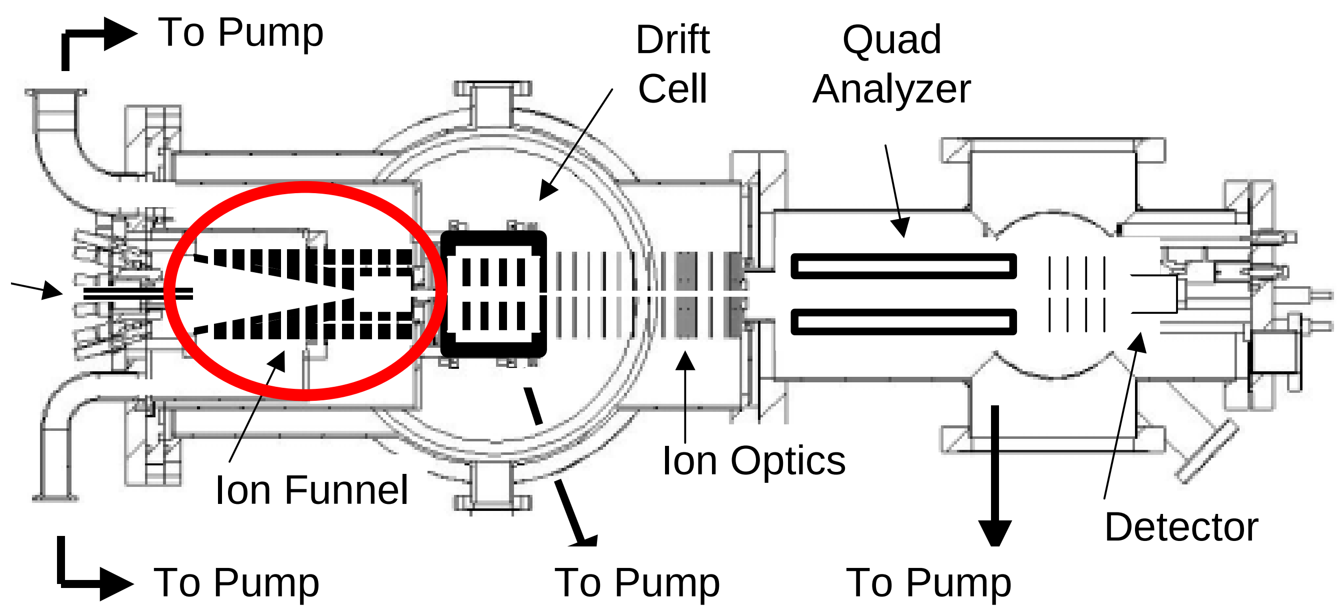
Instrumentation

ESI Ion Source → Ion Funnel → Drift Cell → MS → Detector



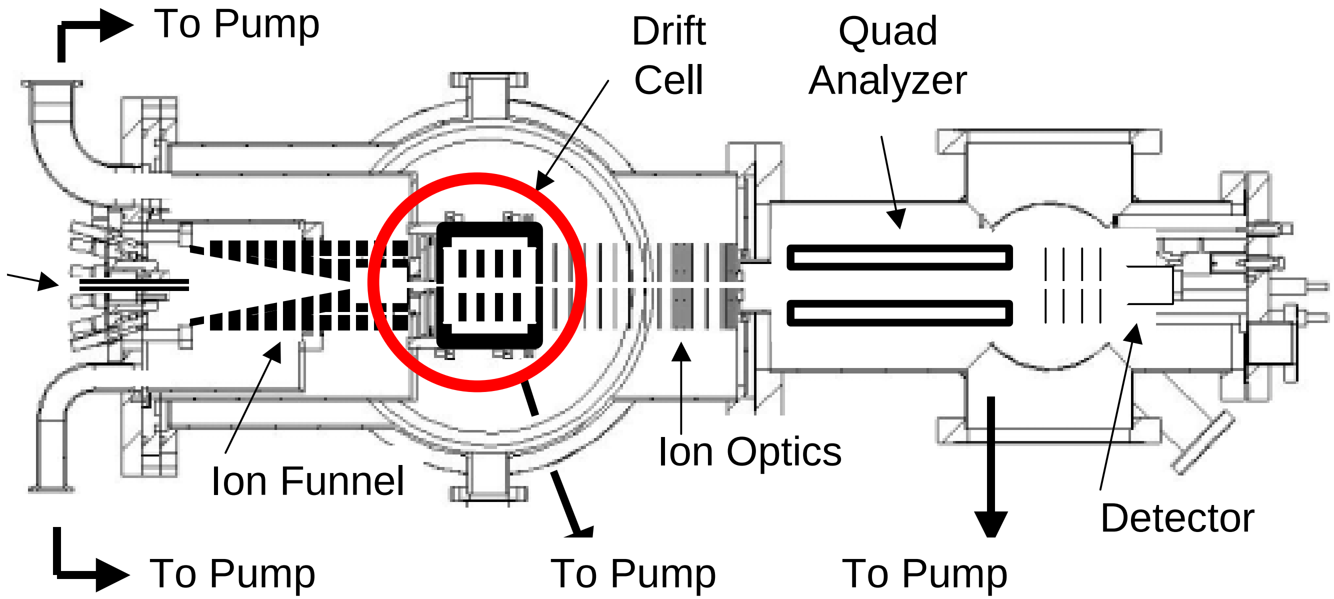
Instrumentation

ESI Ion Source → **Ion Funnel** → Drift Cell → MS → Detector

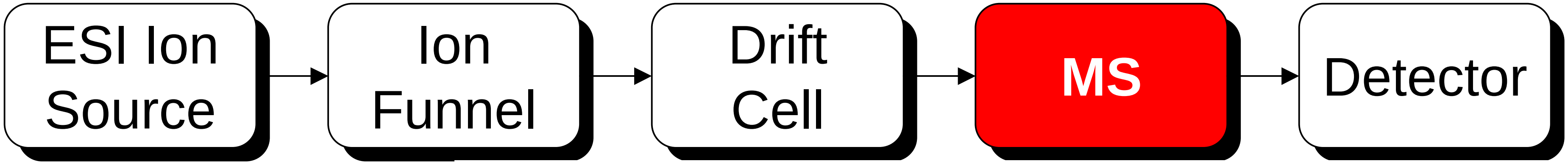


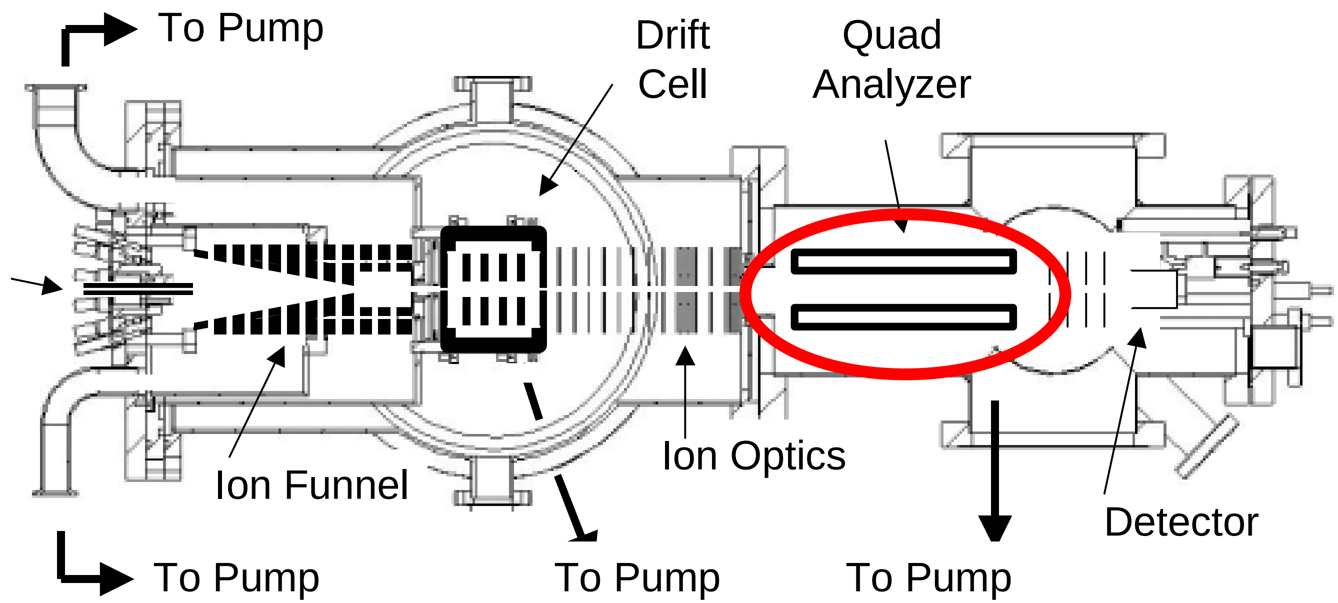
Instrumentation

ESI Ion Source → Ion Funnel → **Drift Cell** → MS → Detector



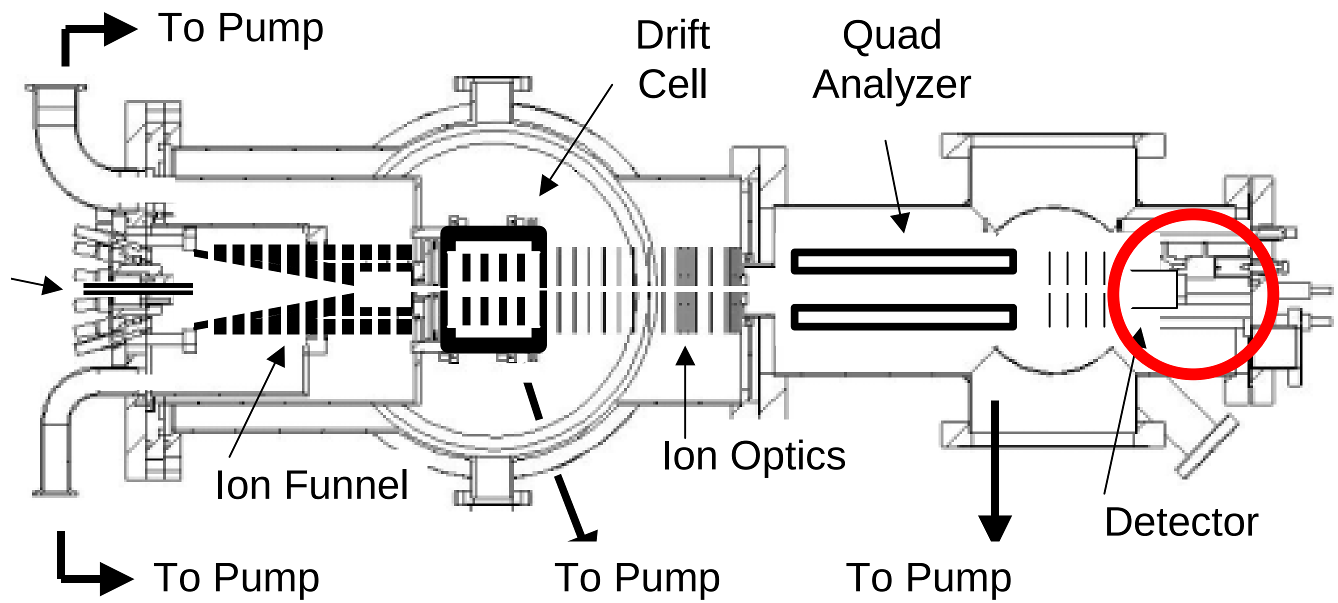
Instrumentation



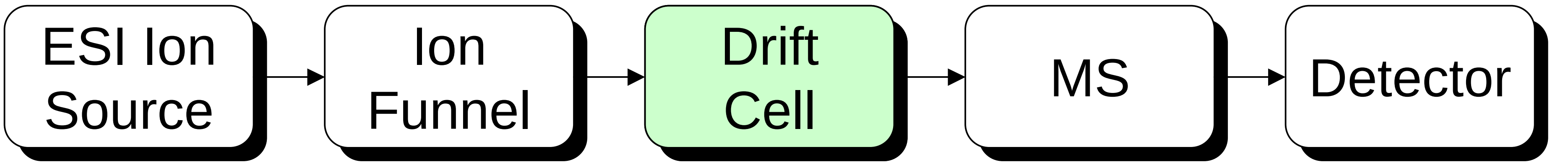


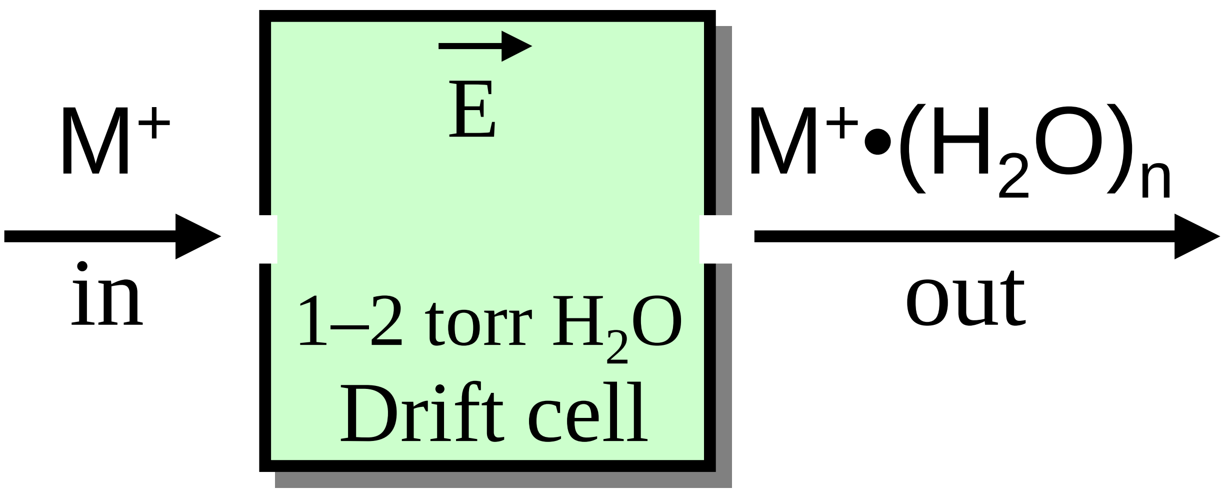
Instrumentation

ESI Ion Source → Ion Funnel → Drift Cell → MS → **Detector**

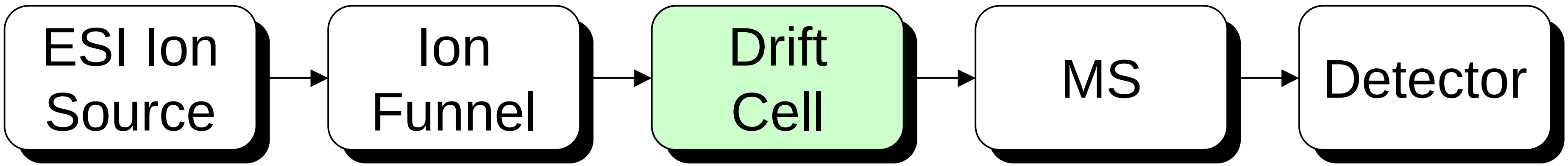


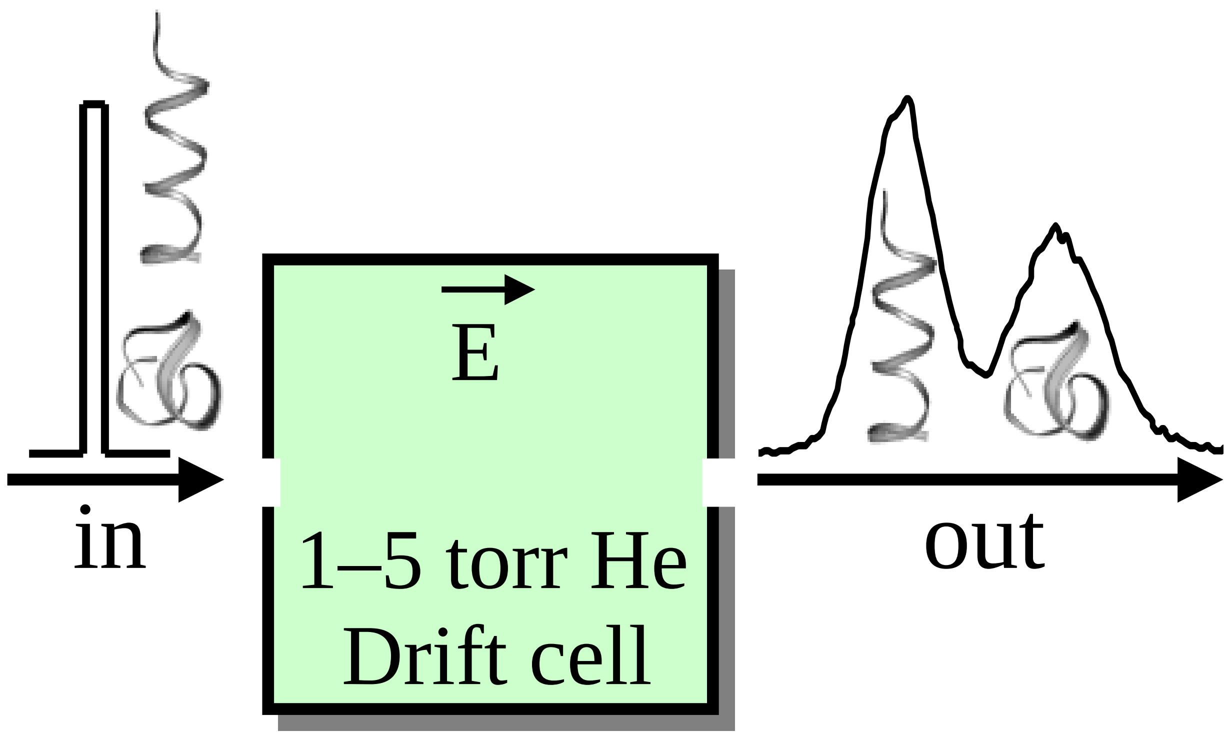
Hydration under equilibrium conditions





Cross section measurements





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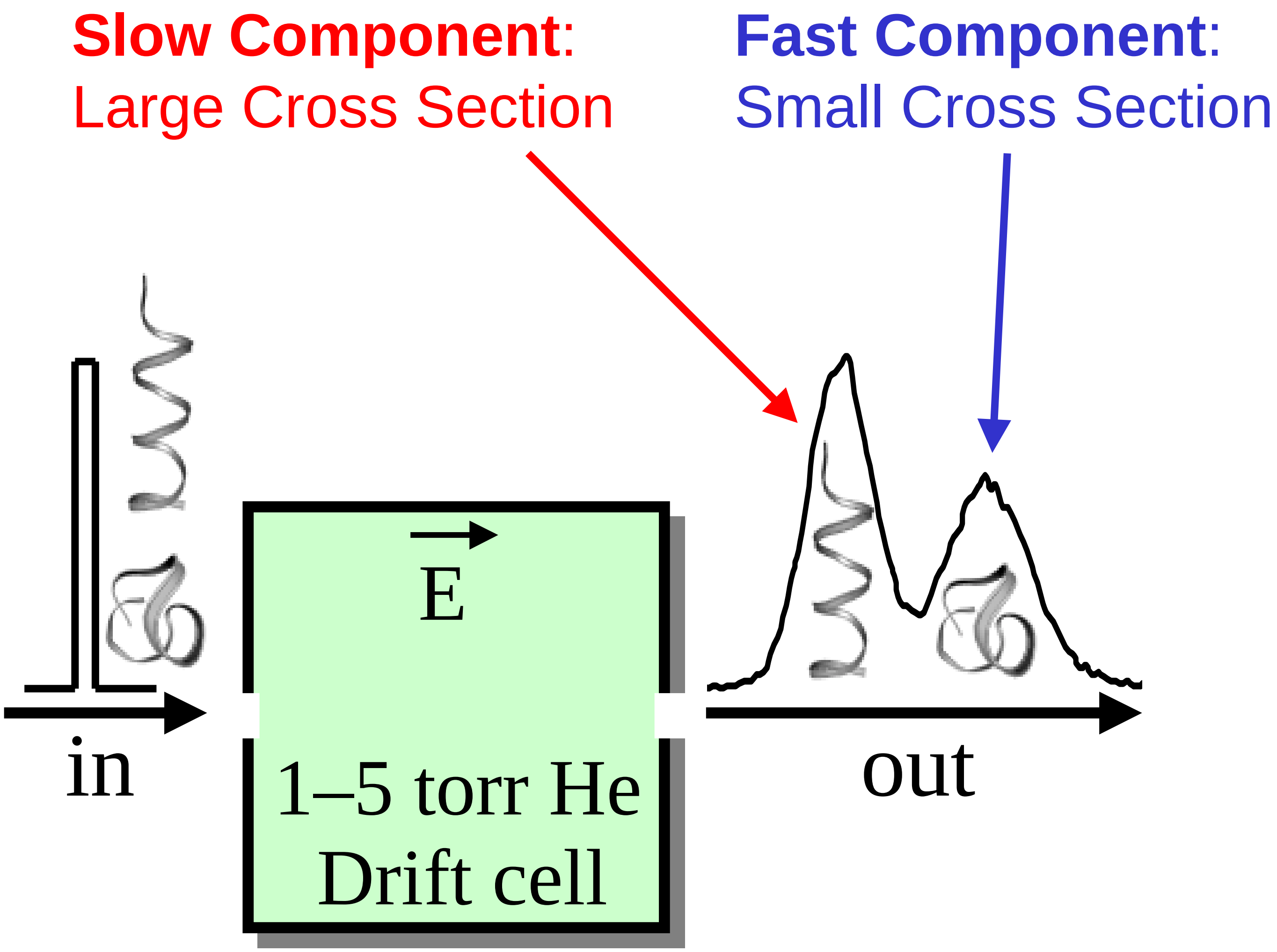
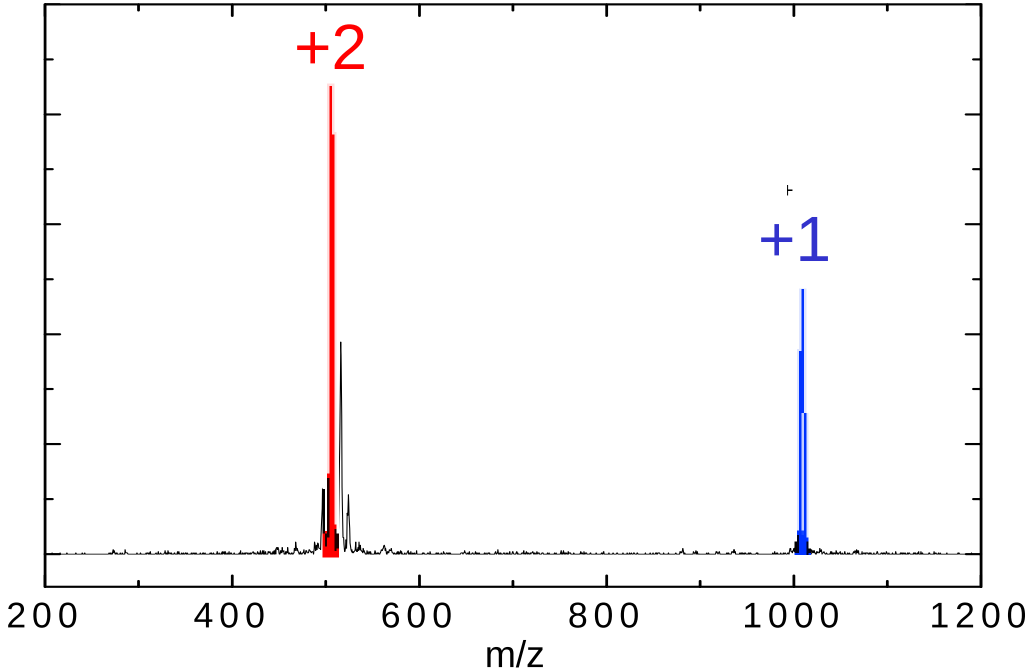
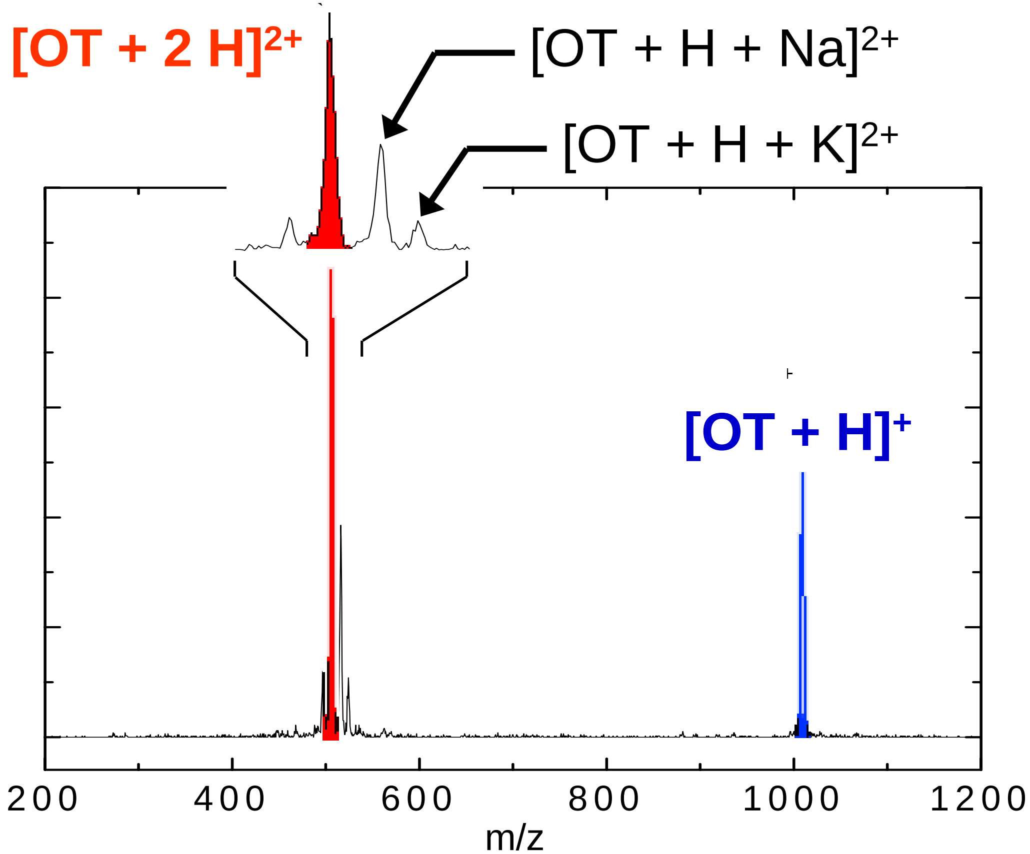


Experimental Results

Mass Spectrum Oxytocin



Mass Spectrum Oxytocin



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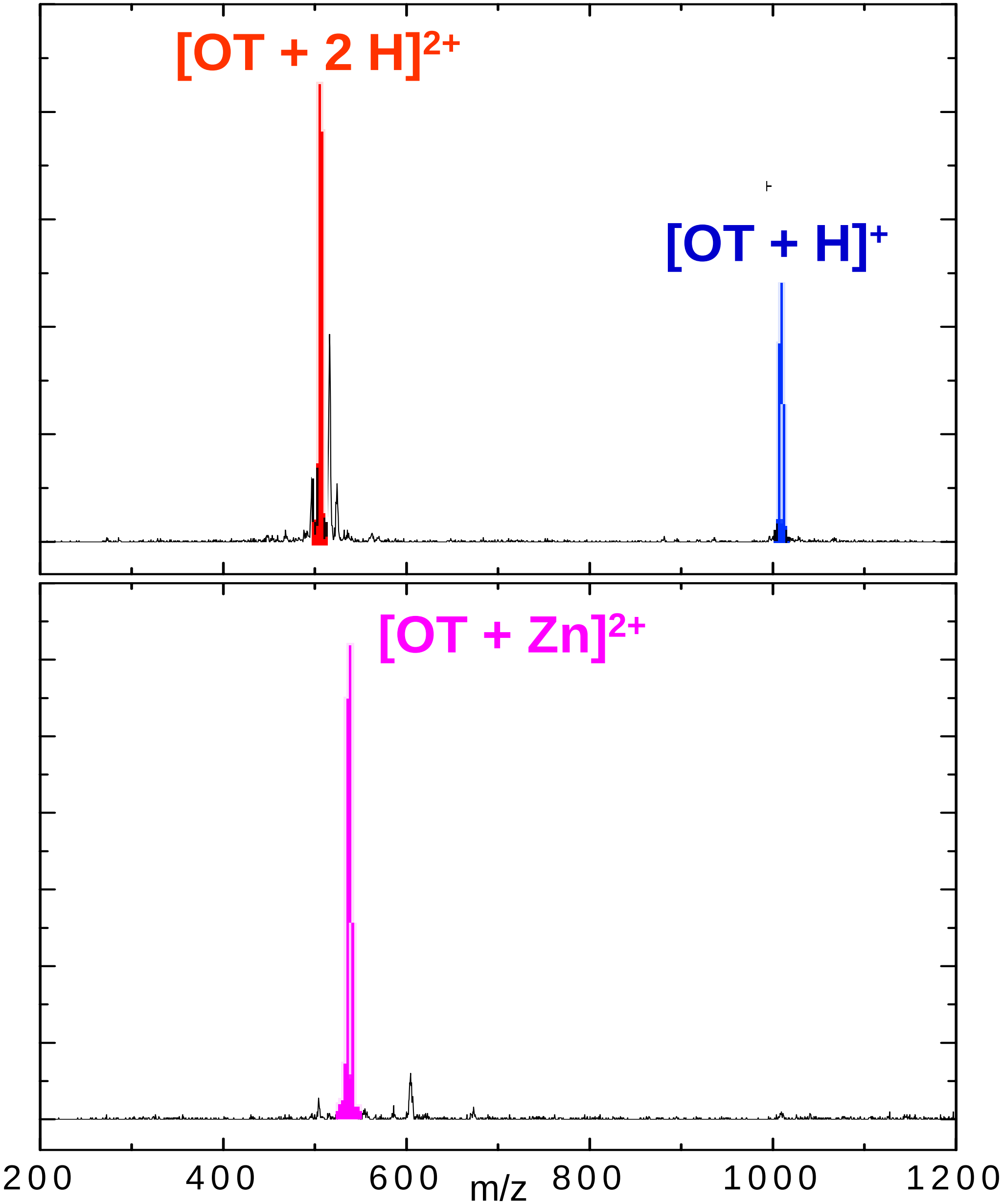
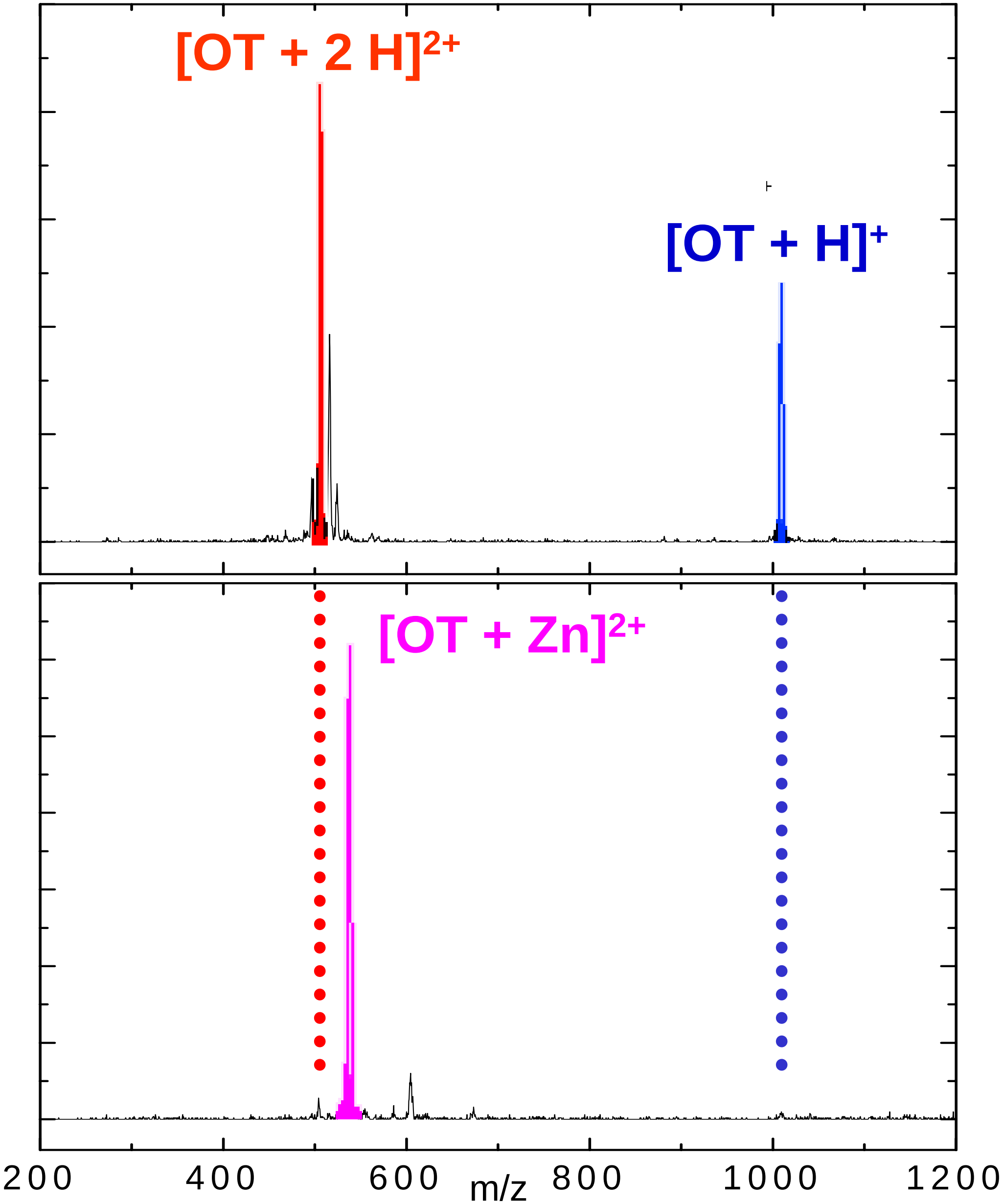


with $ZnCl_2$

Mass Spectrum Oxytocin



with $ZnCl_2$

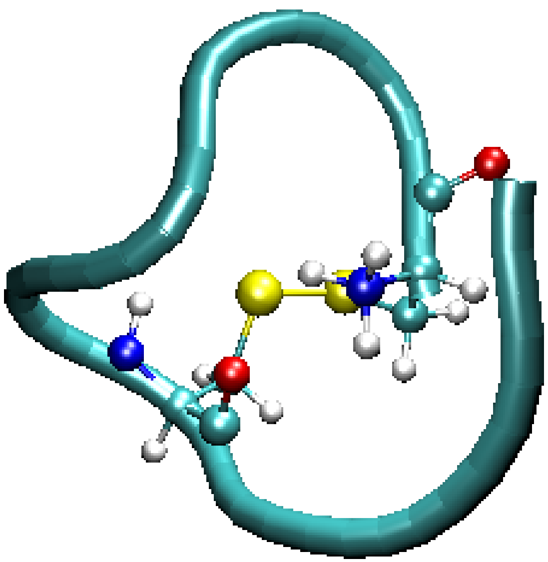
How does zinc interact with OT?

Theory Results: OT-H$^+$

Bare OT

Theory: MM - CHARMM force field
DFT - SVP basis set; BP86 functional

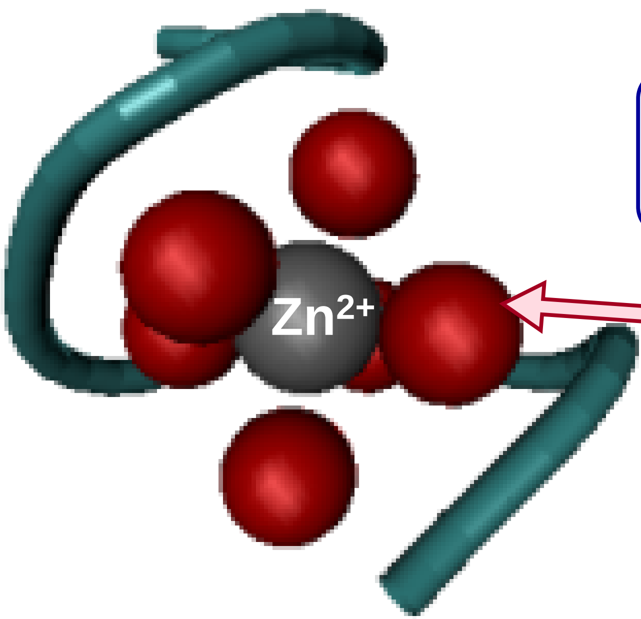
OT Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly(NH_2)
S – S



228 Å^2 (calc)
230 Å^2 (exp)

Theory Results: OT-Zn^{2+} Complex

Theory: MM - CHARMM force field
DFT - SVP basis set; BP86 functional



OT Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly(NH_2)
S – S

donating backbone carbonyl oxygen

octahedral coordination sphere

Zn–O distance = 204-215 pm
Zn^{2+} + O ionic radaii = 214 pm

236 Å^2 (calc)
236 Å^2 (exp)

Isotocin (IT)

OT Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly(NH_2)
S – S

IT Cys-Tyr-Ile-Ser-Asn-Cys-Pro-Ile-Gly(NH_2)
S – S

Osteichthyes – Bony Fish

Isotocin (IT)

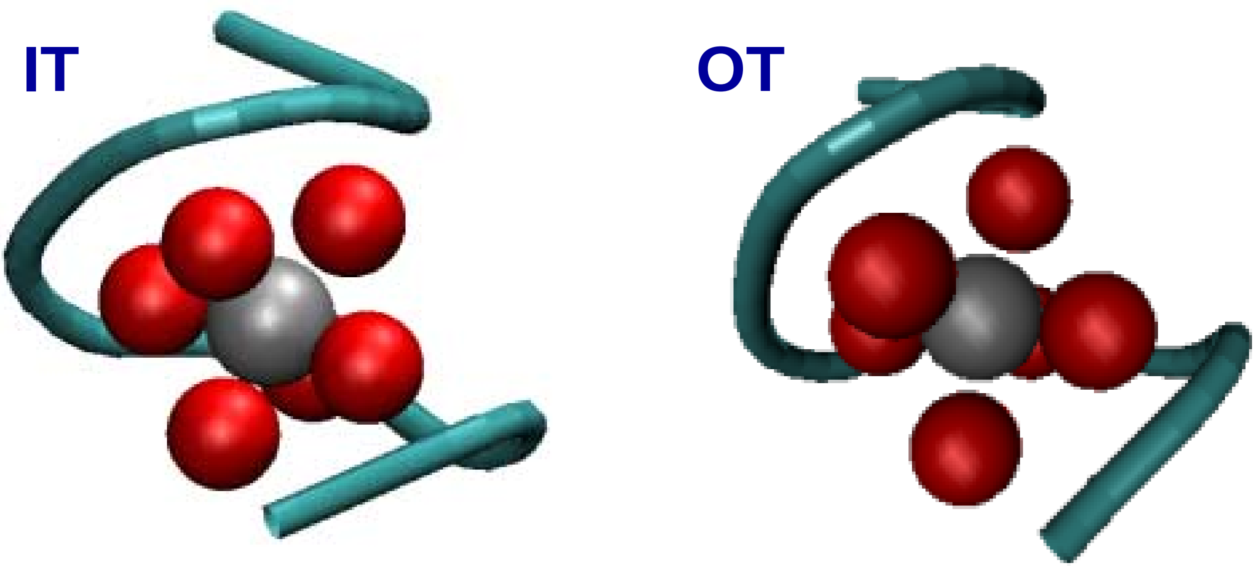
OT Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly(NH_2)

S – S

IT Cys-Tyr-Ile-Ser-Asn-Cys-Pro-Ile-Gly(NH_2)

S – S

Osteichthyes – Bony Fish



donating backbone oxygen

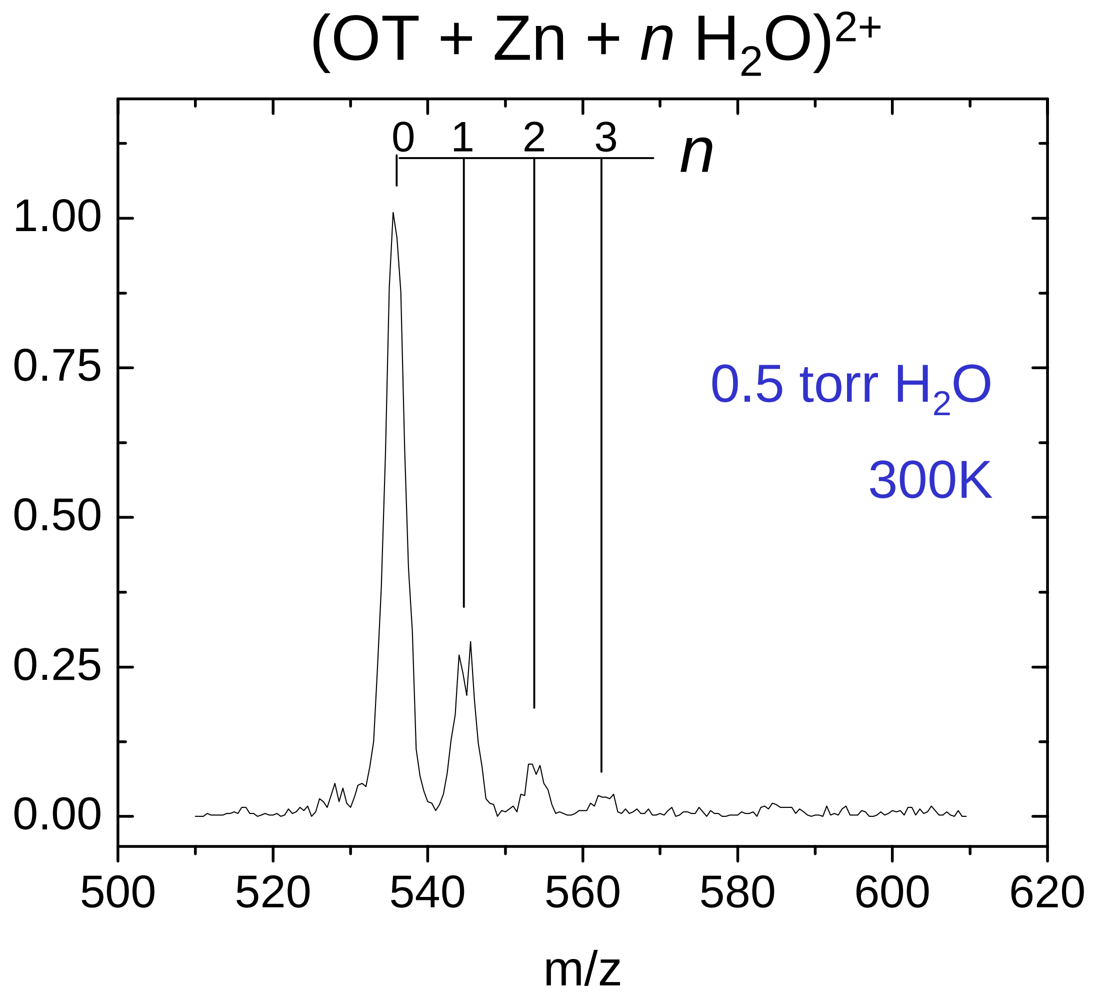
Comparison With Experimental Results

1.) Cross Section

σ [Å^2]	*Exp.*	*MD**
(OT+H)$^+$	230	228
(OT+Zn)$^{2+}$	236	236
(IT+Zn)$^{2+}$	222	225

2.) Hydration

Hydration of (Oxytocin+Zn)$^{2+}$



Hydration data

$(OT+H)^{+}$

n (H_2O)	*1*	*2*	*3*
$\Delta \mathbf{H}_{hydr}$ [kcal/mol]	-7.4	-8.3	-7.4
$\Delta \mathbf{S}_{hydr}$ [cal/mol·K]	-14.9	-18.6	-15.8

$(OT+Zn)^{2+}$

n (H_2O)	*1*	*2*
$\Delta \mathbf{H}_{hydr}$ [kcal/mol]	-9.6	-8.6
$\Delta \mathbf{S}_{hydr}$ [cal/mol·K]	-18.9	-15.9

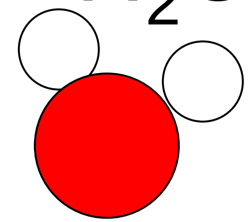
Water binding energies

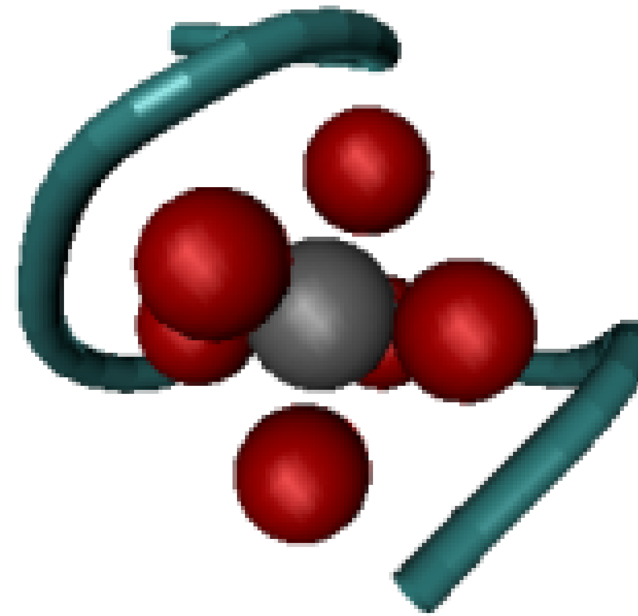
$Zn^{2+} \cdots H_2O$ 96 kcal/mol [1]
Peptide $\cdots H_2O$ 10 kcal/mol [2]
$(OT+H)^{+} \cdots H_2O$ 7 kcal/mol

$(OT+Zn)^{2+} \cdots H_2O$ 10 kcal/mol

Consistent with OT-water interaction rather than zinc-water interaction

H_2O





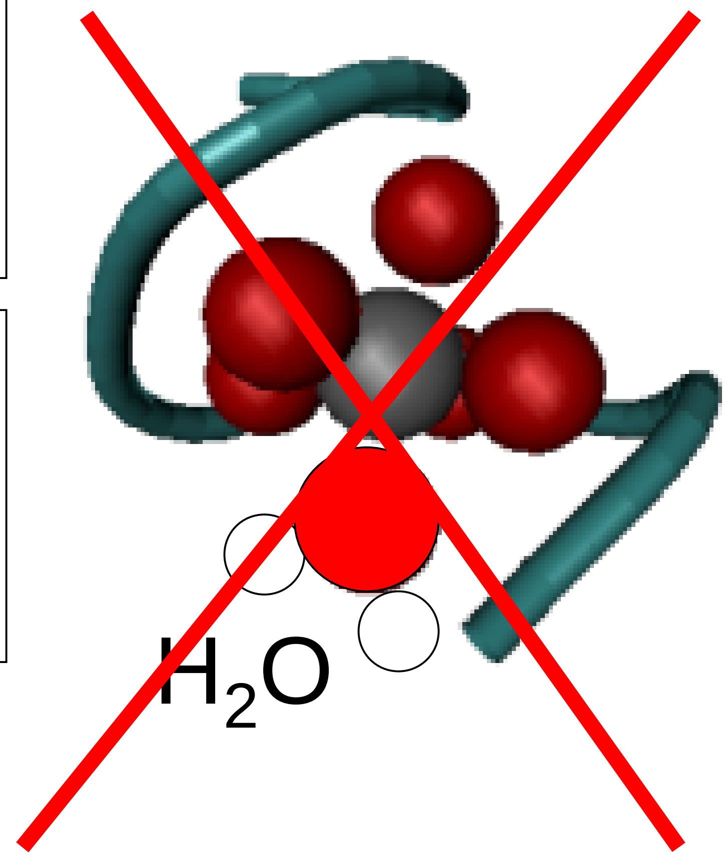
[1] Bock et al. JACS 1995, 117, 3754
[2] Lui et al. JACS 2003, 125, 8458

Water binding energies

$Zn^{2+} \cdots H_2O$	96 kcal/mol [1]
Peptide $\cdots H_2O$	10 kcal/mol [2]
$(OT+H)^{+} \cdots H_2O$	7 kcal/mol

$(OT+Zn)^{2+} \cdots H_2O$	10 kcal/mol

Consistent with OT-water interaction rather than zinc-water interaction



[1] Bock et al. JACS 1995, 117, 3754
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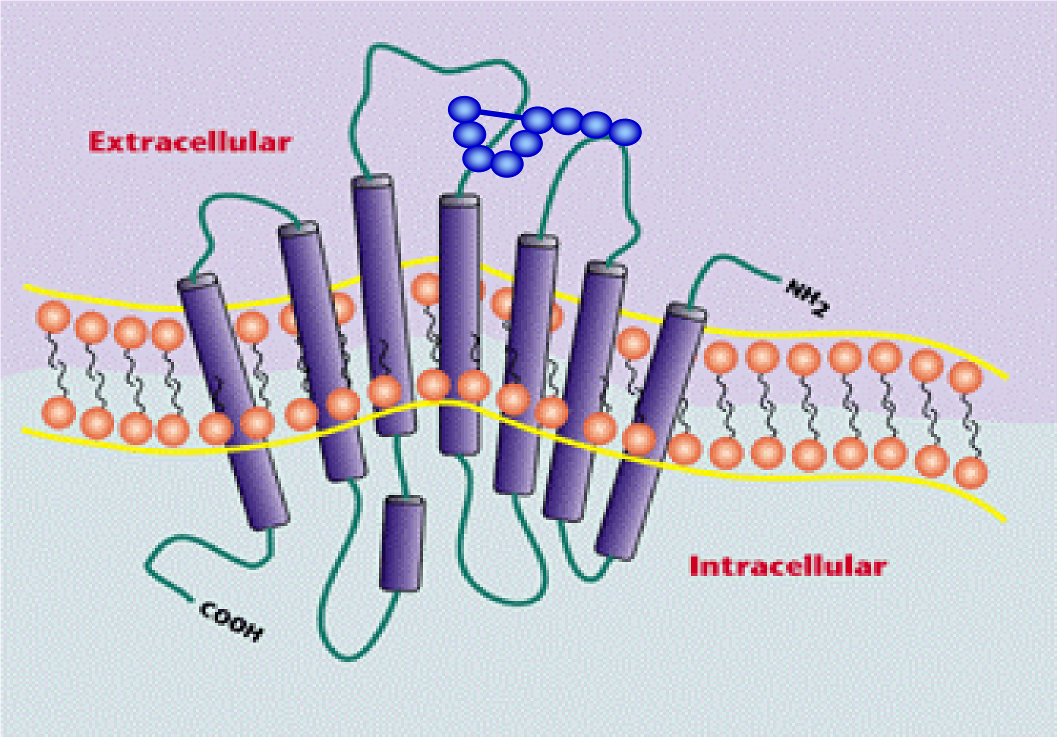
Hydration and Cross Section Measurements confirm that Zinc is buried in the structure.

Oxytocin (OT) Receptor Interaction

- OT receptor sequence is known, but ligand binding is not.

- The cyclic portion of OT binds to extracellular loop.
  The linear portion binds to another extracellular loop.

- Isoleucine in third position has been found to be crucial to receptor binding.



Oxytocin (OT) Receptor Interaction

Why do divalent metals increase OT binding?

OT

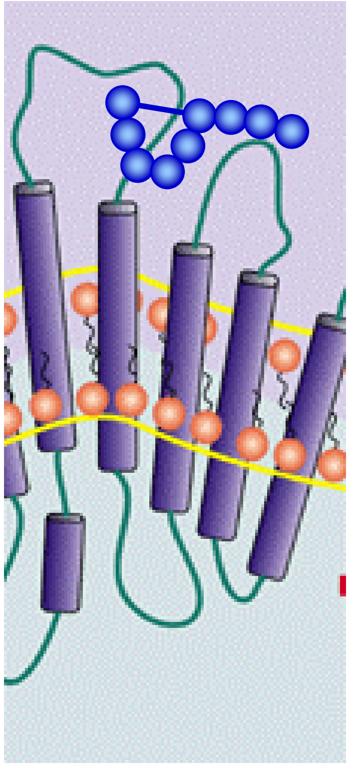
Cys-Tyr-**Ile-Gln-Asn**-Cys-Pro-Leu-Gly(NH_2)

S - S

Vasopressin

Cys-Tyr-**<u>Phe</u>-Gln-Asn**-Cys-Pro-<u>Arg</u>-Gly(NH_2)

S - S

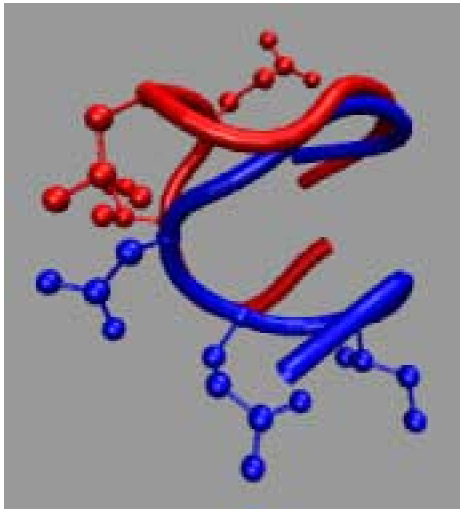


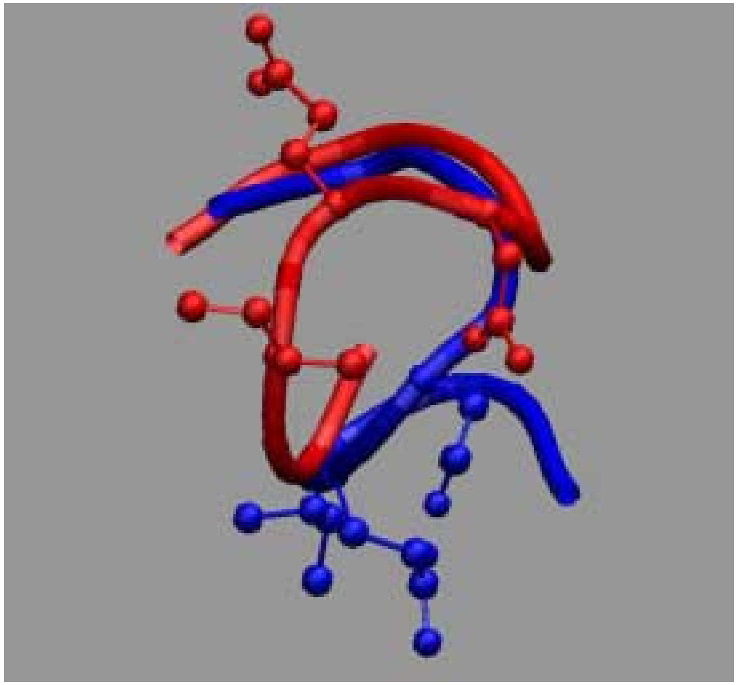
Residue 3-5 are found to be crucial for cyclic hormone-receptor interaction.

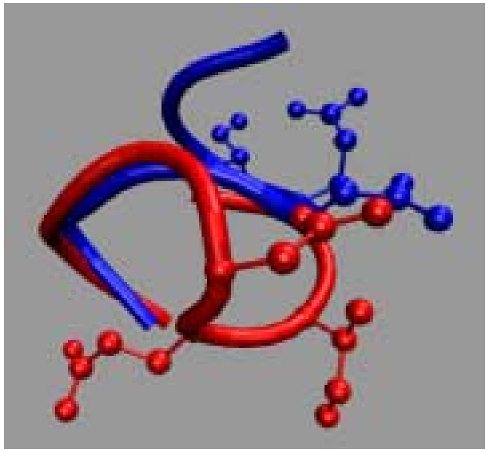
Hydrophobic residue Ile/Phe-3 forms hydrophobic pocket interaction

Conserved Gln-4 and Asn-5 interact with conserved residues in the receptor.

Side Chain Conformation



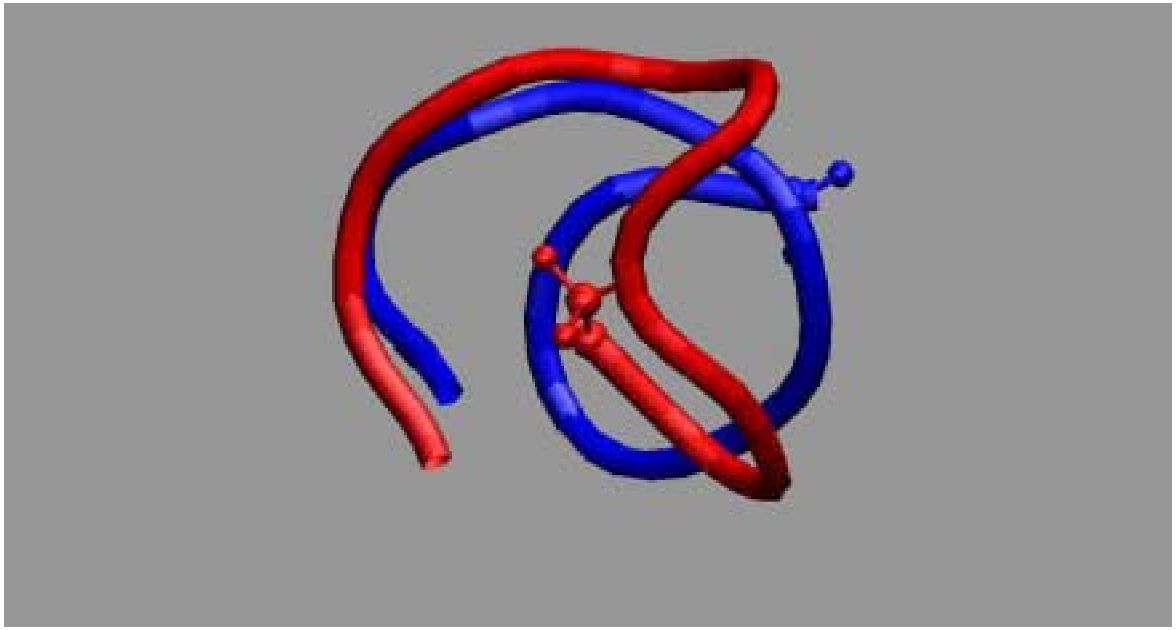




Bare OT

OT- Zinc Complex

N-terminus Conformation



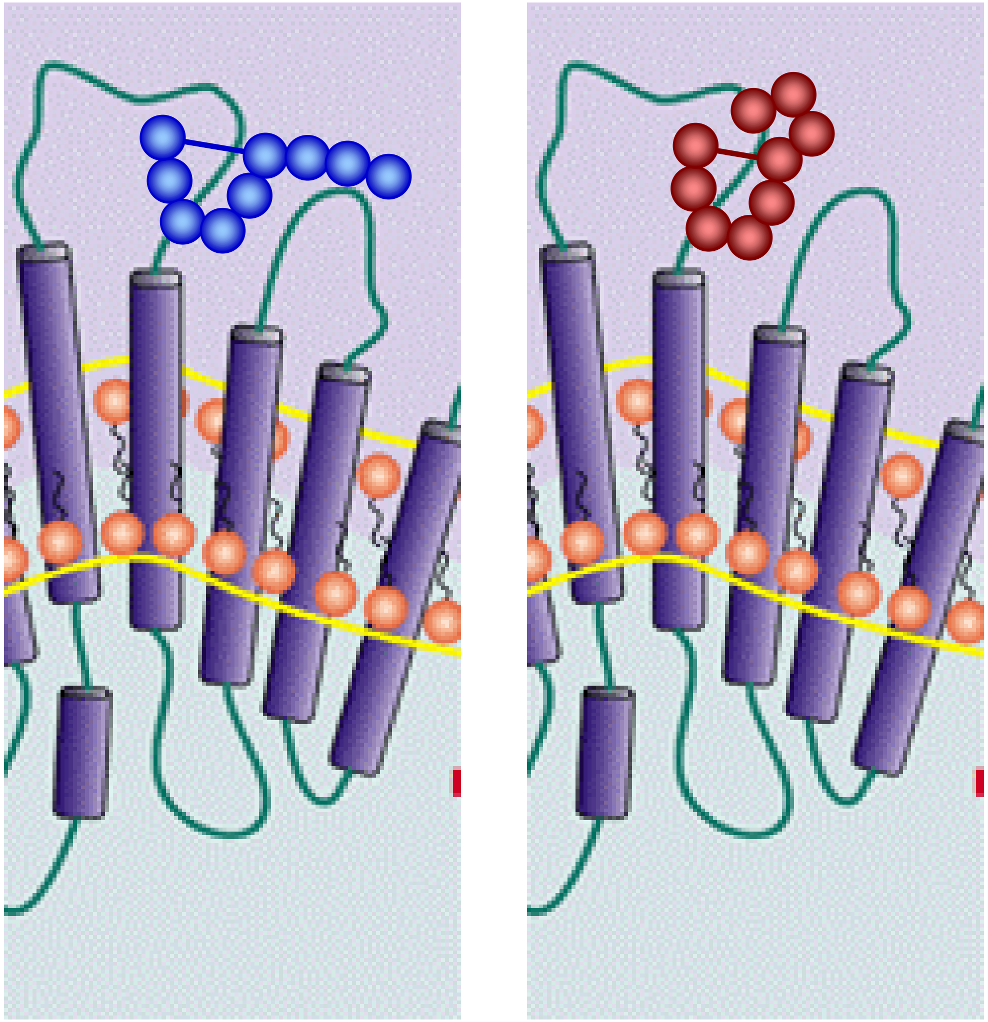
Interaction of the cyclic portion is stabilized by salt bridge interaction
Between NH3+ and Glutamic Acid in receptor.

Zinc displaces N-terminus from the interior of peptide.

Bare OT

OT- Zinc Complex

Compact vs. Extended

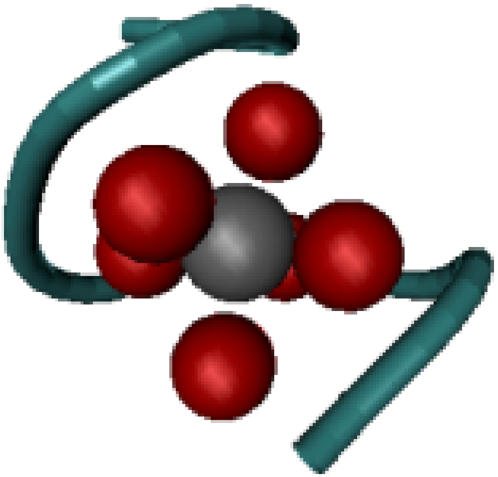


Bare OT

OT- Zinc Complex

Conclusions

Experiments confirm that Zinc is buried in peptide structure.



•A dramatic conformational change occurs as OT binds to zinc.

Similar change is seen in IT.

• Conformational change appears more favorable for receptor binding.

Acknowledgements

- **Dengfeng Liu**
  Oli Th. Ehrler
  Xiaohua Zhang

- **Professor Bowers**

- **Bowers Group**
  Dr. Thomas Wyttenbach
  Dr. Catherine Carpenter