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### Modeling Mammalian Carboxypeptidase O Expression Patterns with the Thirteen-lined Ground Squirrel (*Ictidomys tridecemlineatus*)

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# Modeling mammalian carboxypeptidase O expression patterns with the thirteen-lined ground squirrel (*Ictidomys tridecemlineatus*)

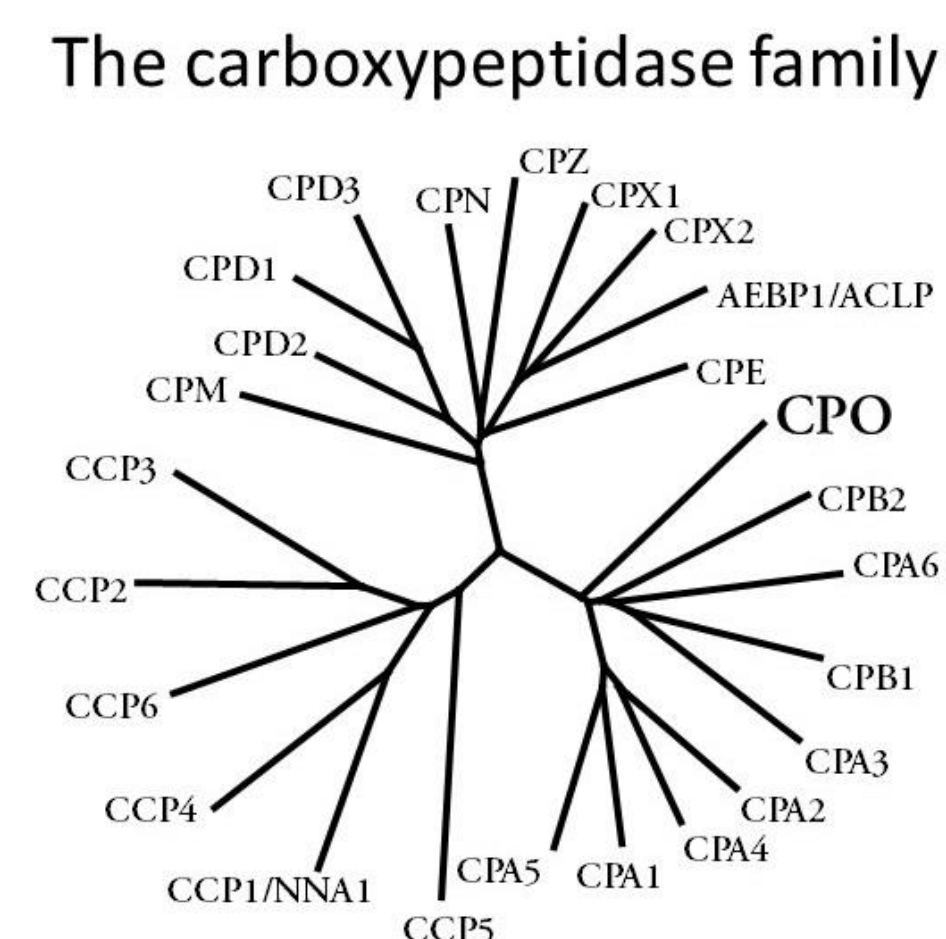
## Abstract

Carboxypeptidase O (CPO) is a protease that cuts acidic amino acids from the carboxyl terminus of a substrate protein. Besides its potential digestive application, not much else is known about its other activities in the body. This study aims to describe the expression pattern of CPO using the thirteen-lined ground squirrel as a model mammalian system. Thus far we have sampled tissue from one ground squirrel and analyzed those samples via Western blot. Immunoreactive bands likely to be CPO (molecular weight approximately 42 kDa) were seen in kidney, liver, and small intestine tissue samples. Further investigation will include immunohistochemical analysis.

## Introduction

### Why is it important to understand CPO?

- Metalloproteases play key roles in digestion and in the modification of other proteins (Arolas et al. 2007).
- CPO is part of the CPA subfamily and is thought to play a complementary role to that of CPA1 and CPB in the small intestine.
- Not much is known about its expression in the body.



### Why the thirteen-lined ground squirrel?

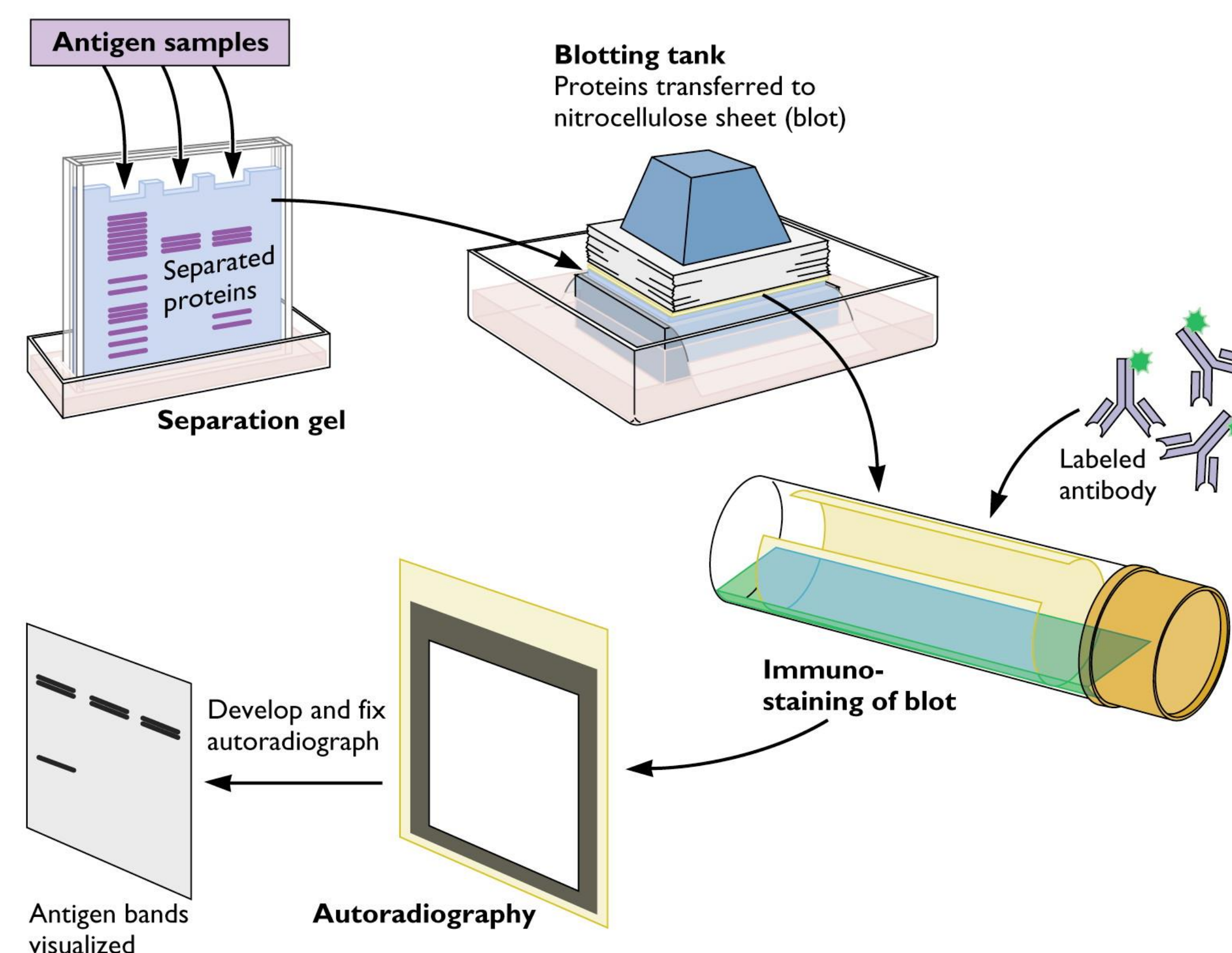
- The mouse, the preferred mammalian model system, does not express CPO. (Marques et al. 2012)
- A human CPO antibody is able to recognize this organism's CPO.
- A population of thirteen-lined ground squirrels lives on the Andrews University campus and has already been a subject of study in the Department of Biology.
- The genome of the thirteen-lined ground squirrel has been sequenced and its CPO sequence can be compared to human CPO.



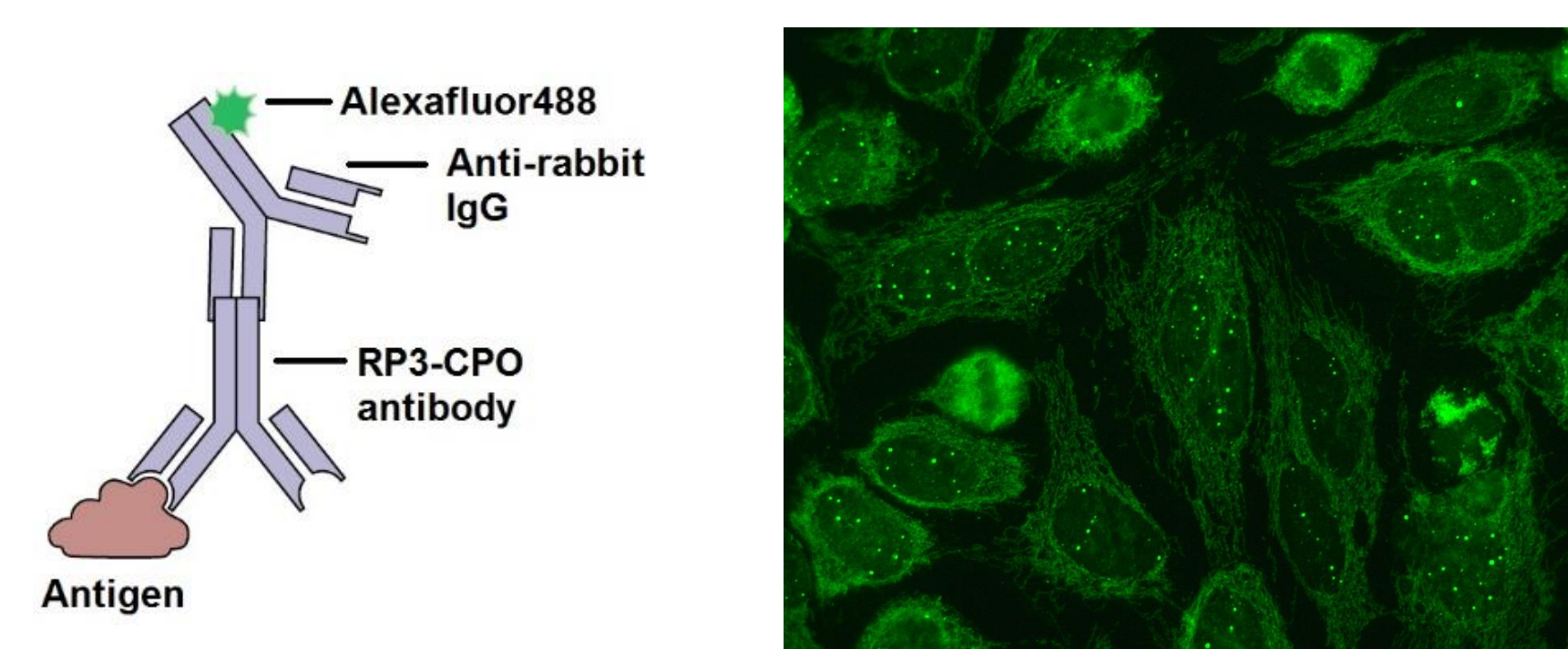
## Methods

### 1 Capture, euthanasia, and dissection of ground squirrels to collect tissue samples

### 2 Western blot analysis to see which tissues produce CPO and how much CPO is expressed



### 3 Immunohistochemistry to analyze expression patterns



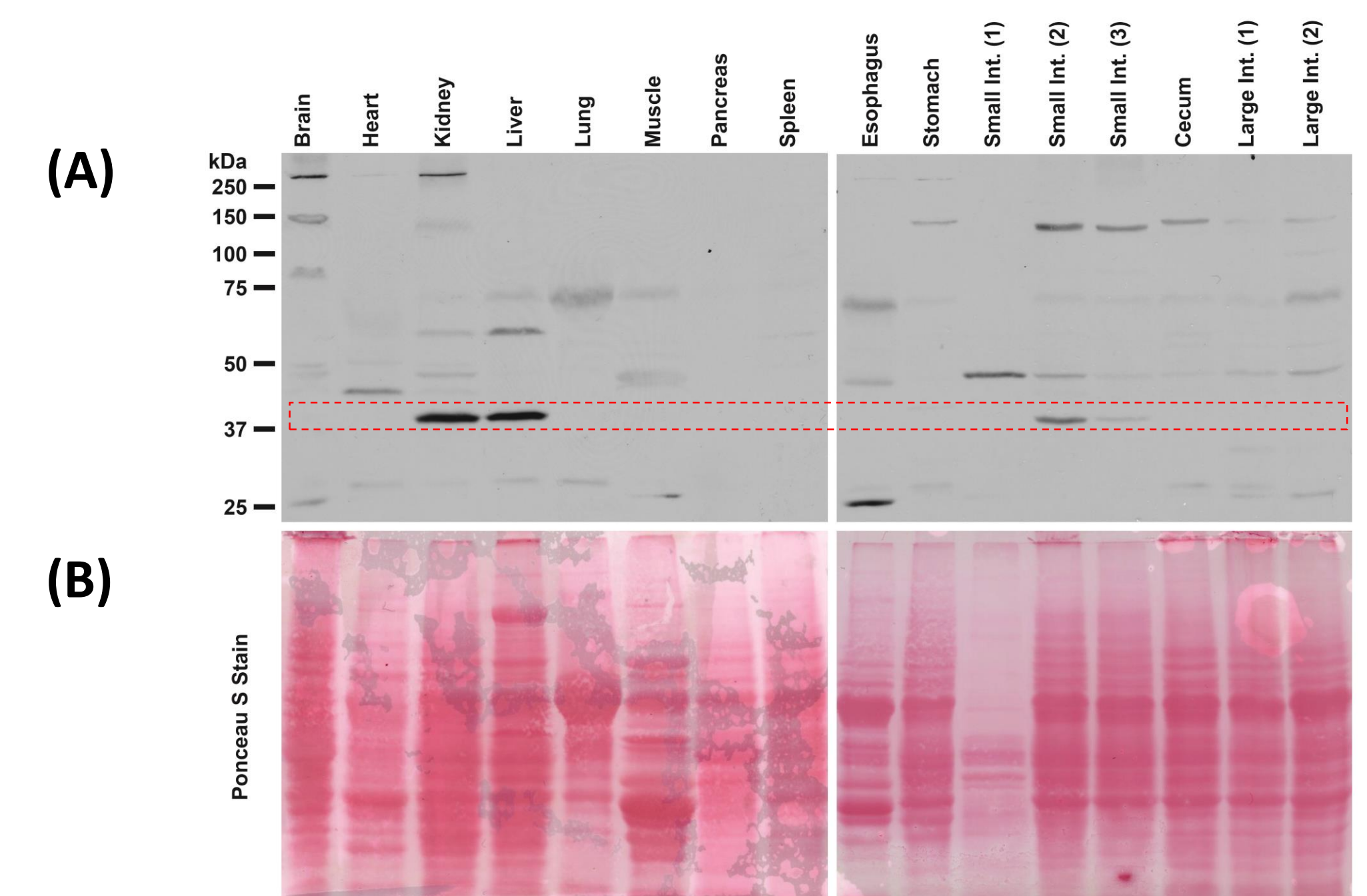
## Selected Bibliography

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- Lyons PJ and Fricker LD, (2011) Carboxypeptidase O is a glycosylphosphatidylinositol-anchored intestinal peptidase with acidic amino acid specificity. *J Biol Chem*, 286:39023-32.
- Marques AC et al., (2012) Evidence for conserved post-transcriptional roles of unitary pseudogenes and for frequent bifunctionality of mRNAs. *Genome Biol*, 13:R102.

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## Results

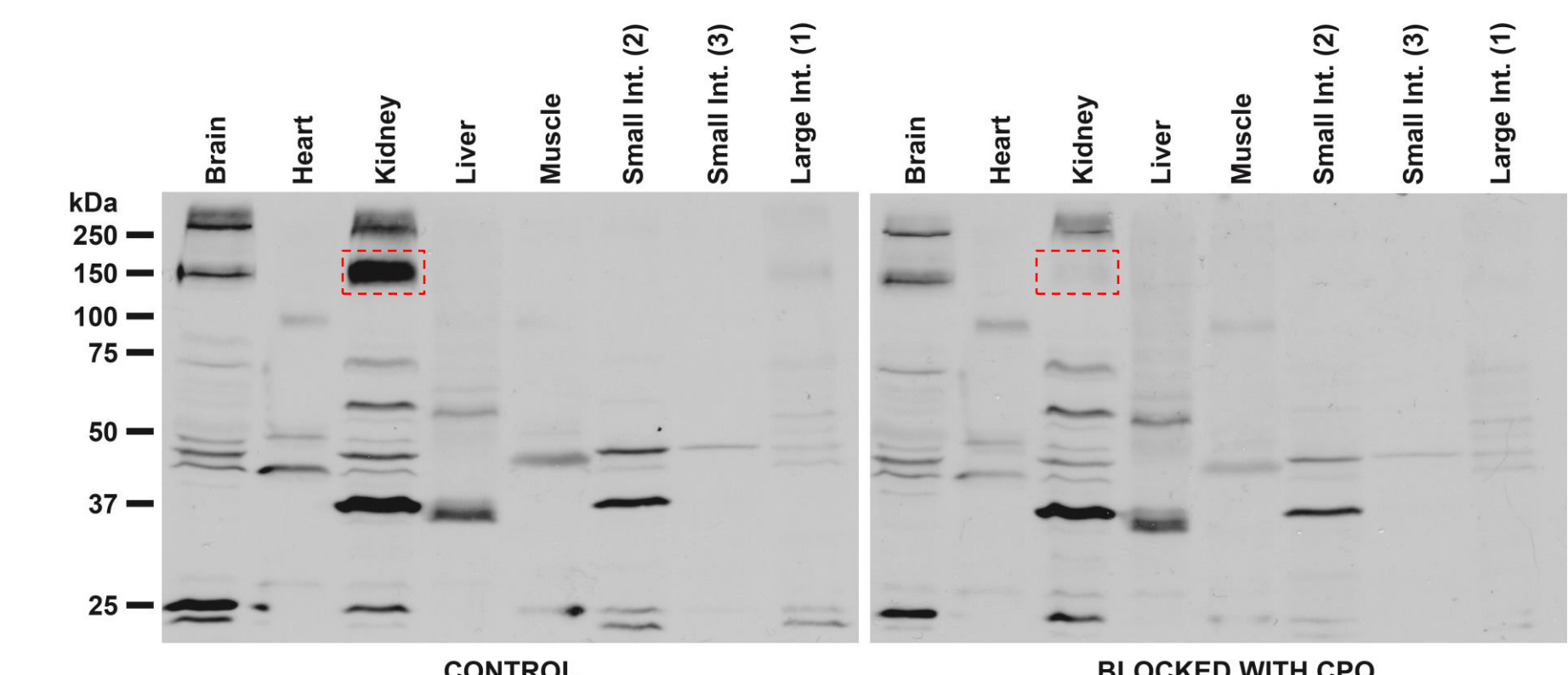
### 1 Western blot of thirteen-lined ground squirrel tissues



(A) Protein samples from the tissues of a thirteen-lined ground squirrel were separated by SDS-PAGE, transferred onto a nitrocellulose membrane, and stained with RP3 CPO antibody. The radiograph was produced by chemiluminescence of a horseradish peroxidase (HRP) reaction. The area at ~42 kDa, the molecular weight of CPO, is highlighted in red.

(B) Ponceau S staining of the nitrocellulose membrane visualized the protein contents of the tissue extractions.

### 2 Blocking antibody with antigen modifies specific binding



Two identical protein sample groups were analyzed for nonspecific binding of the RP3 CPO antibody. One group was stained with the RP3 CPO antibody alone. The second group was stained with RP3 CPO antibody blocked with human CPO. A missing band at ~150 kDa is highlighted in red.

## Conclusions

- Immunoreactive bands present at 42 kDa, the approximate weight of CPO, indicate that CPO is present in kidney, liver, and small intestine tissues.
- An immunoreactive band at ~150 kDa in kidney disappears when stained with antigen-blocked antibody suggesting that it is CPO.