



Angiotensin II-mediated ATP release and calcium signaling in the kidney

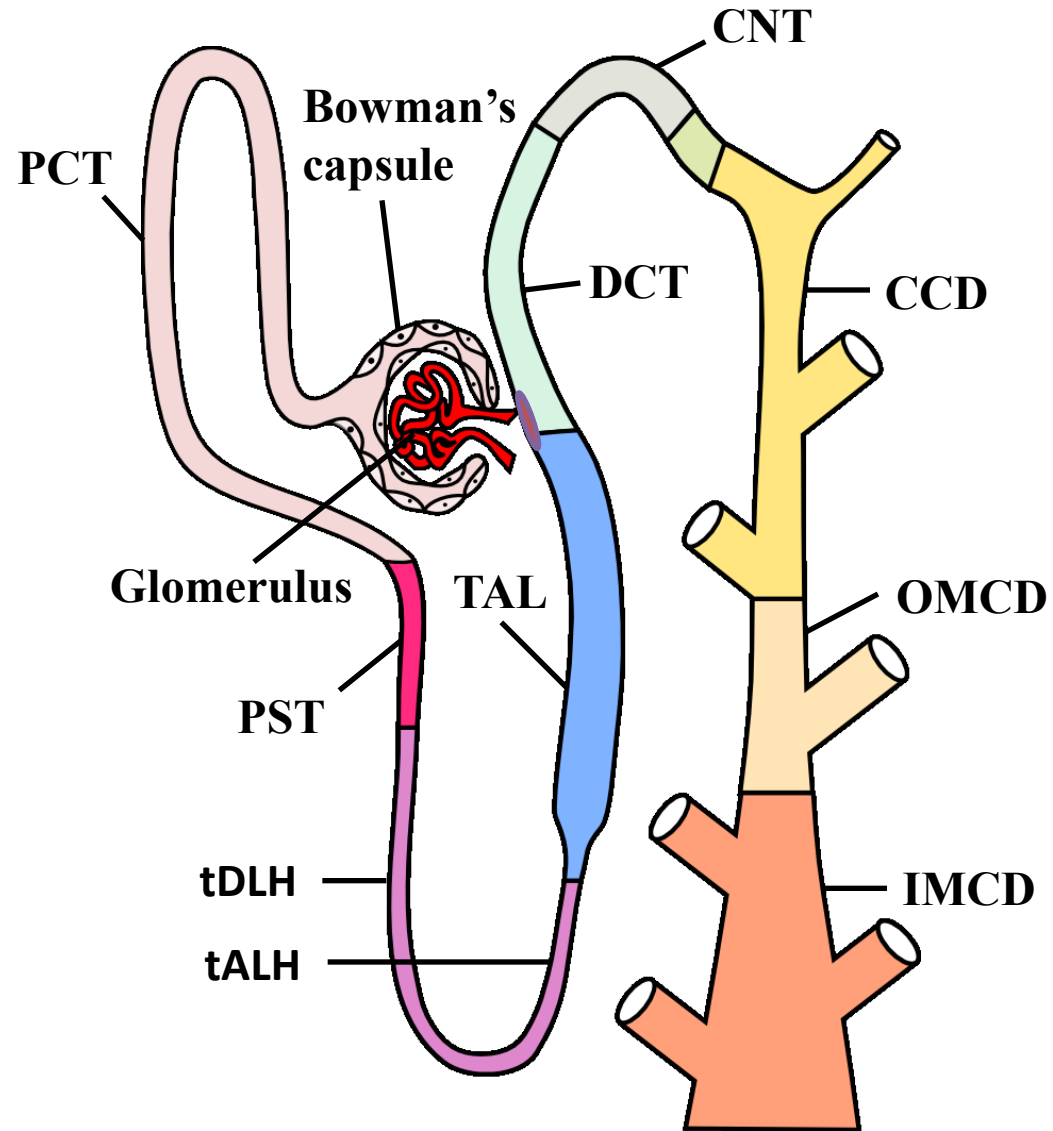
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Role of the epithelial Na⁺ channel (ENaC) in salt-sensitive hypertension and mechanisms of its regulation by EGF

Институт Эволюционной Физиологии РАН

12 ч. 26 декабря 2012 г.
Малый конференц зал

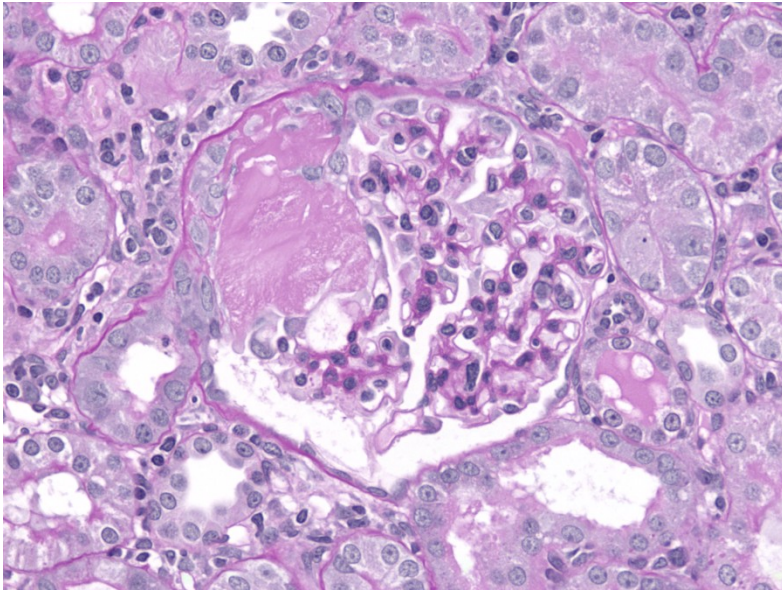




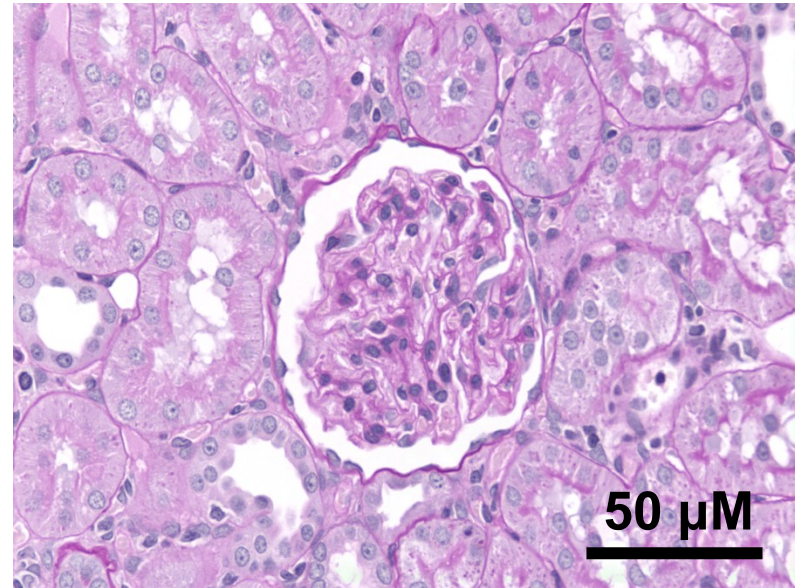


Focal Segmental Glomerulosclerosis

FSGS



Normal glomerulus

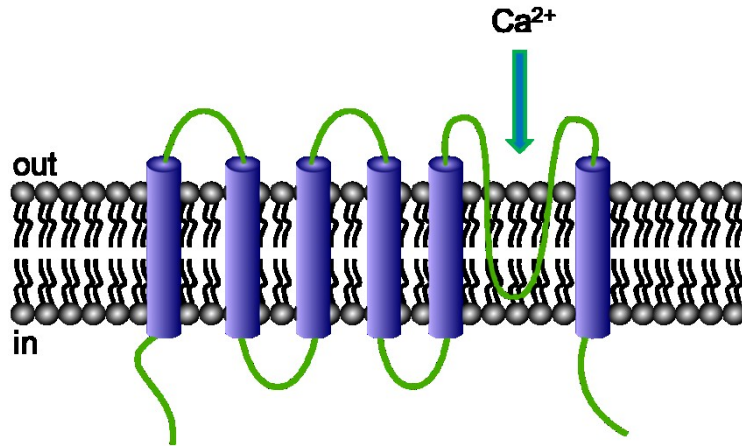


- ☐ Albuminuria
- ☐ Hypertension
- ☐ Hyperlipidemia
- ☐ Proteinuria
- ☐ edema

OUTLINE

TRPC (transient receptor potential canonical cation channels) are permeable to cations, with a selectivity of Ca^{2+} over Na^{+} .

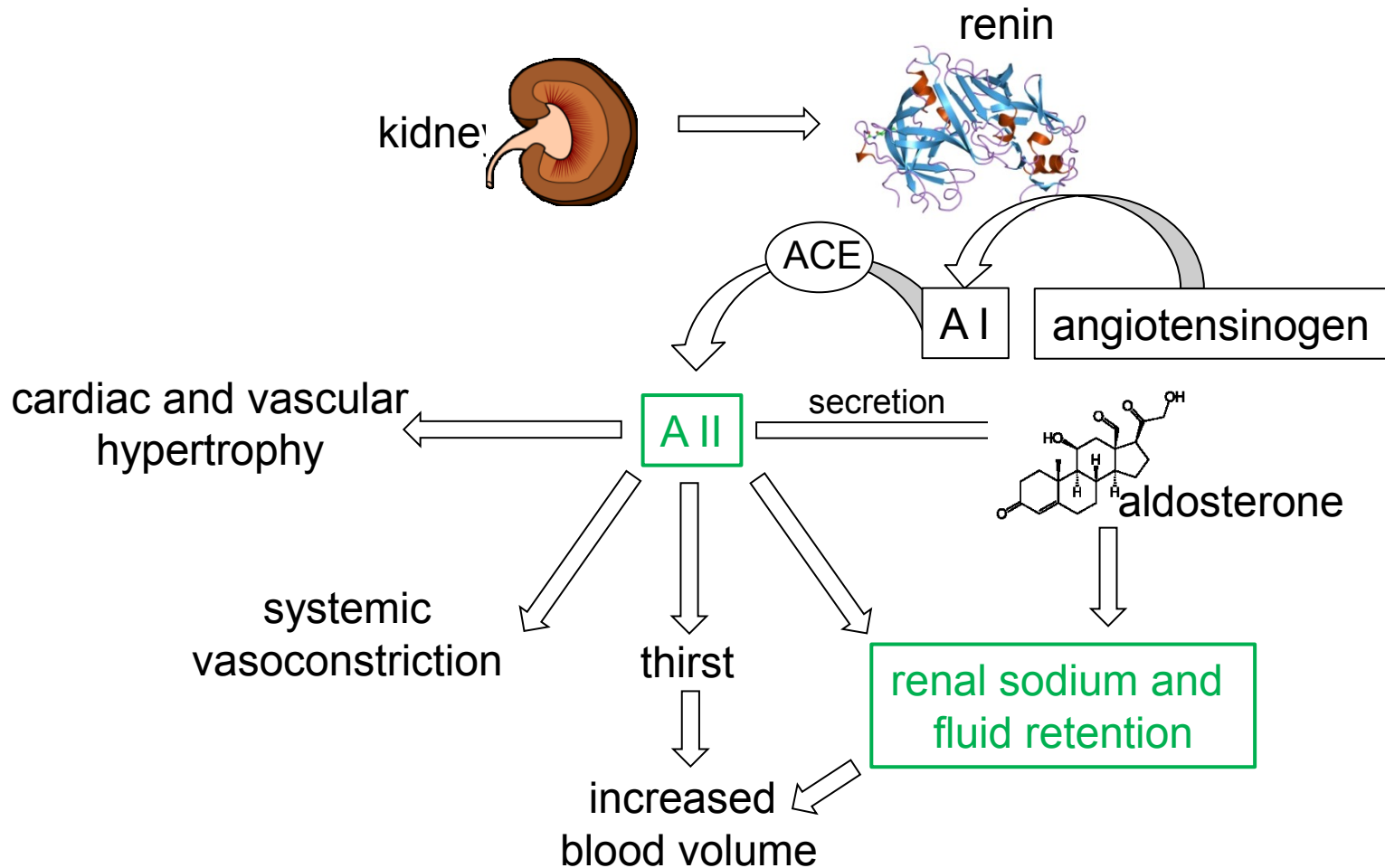
These channels are involved in a variety of cell functions due to their ability to regulate intracellular calcium signaling.



- Reiser et al., *Nat Genet*, 2005
- Winn et al., *Science*, 2005

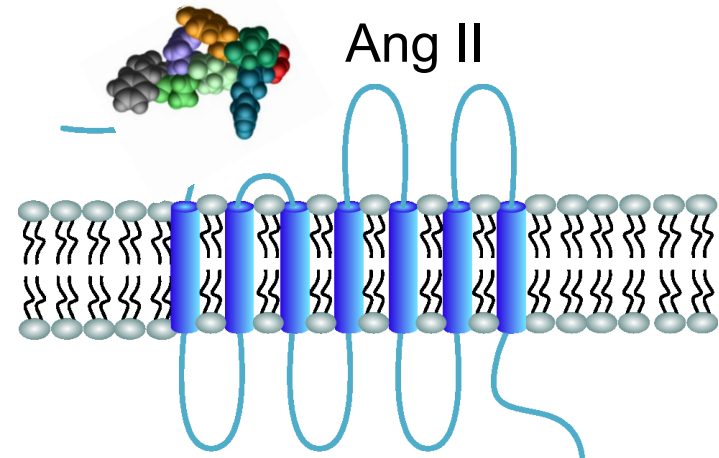
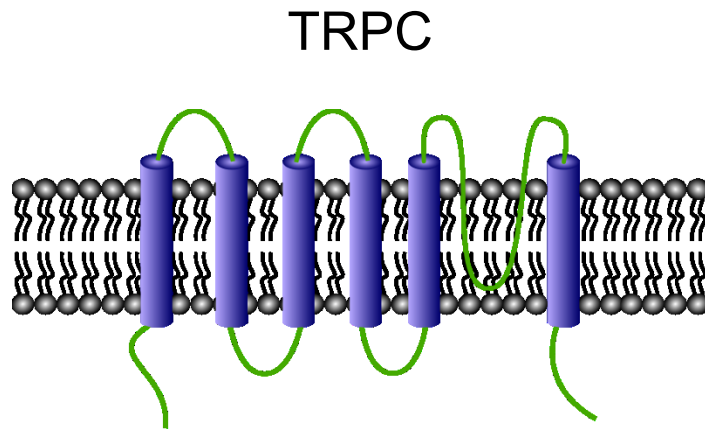
TRPC6 protein is the genetic basis for an autosomal dominant form of *focal segmental glomerulosclerosis* (FSGS), that is an important cause of nephrotic syndrome and kidney failure in adults.

Interstitial angiotensin II (Ang II) is a peptide hormone that is a part of the RAAS and is a major target for drugs that lower blood pressure



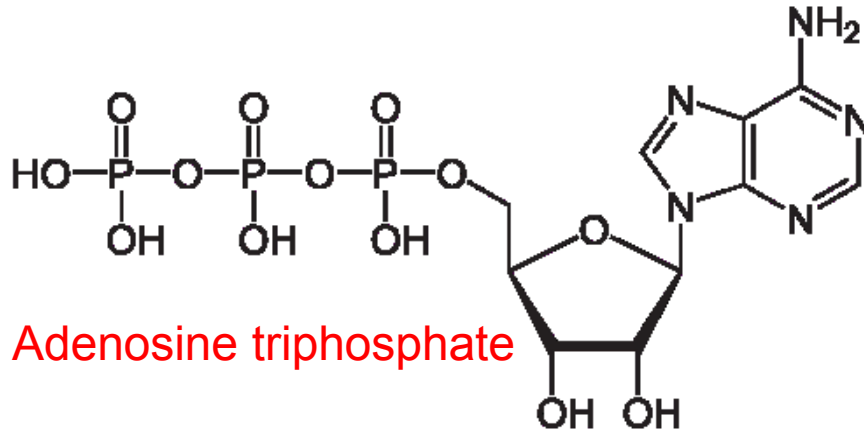
Angiotensin II and TRPC channels

- ❑ Ang II is a key mediator of renal inflammation and fibrosis in progressive chronic nephropathies
- ❑ expression of Ang II is increased in patients with glomerulopathies

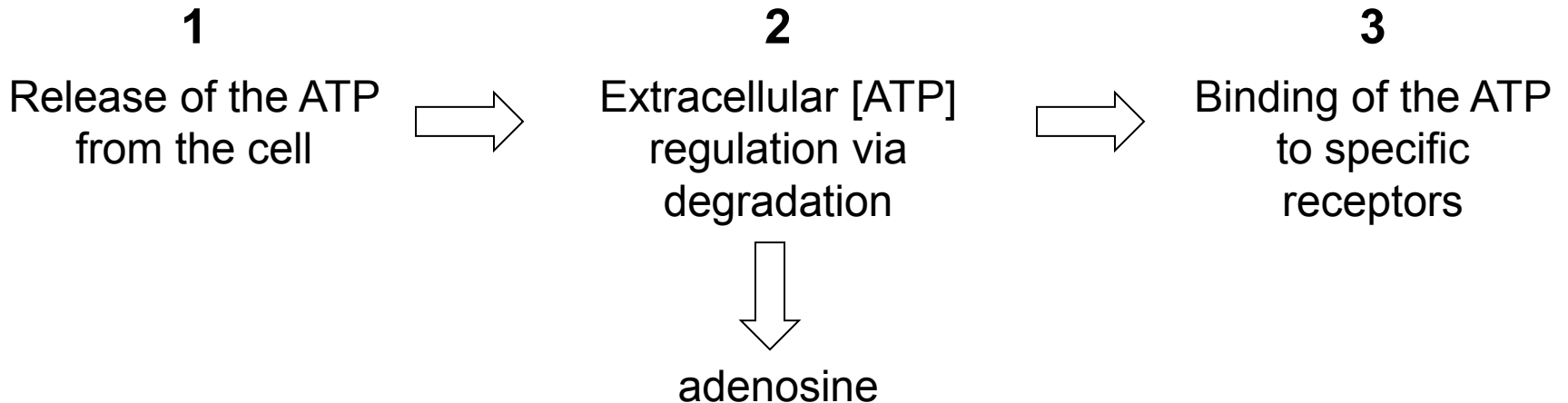


- ❑ **TRPC6** channels participate in the formation of Ang II – induced albuminuria
- ❑ Alteration of **TRPC6** expression and the Ca^{2+} influx is involved in Ang II induced apoptosis
- ❑ Deleterious effects of Ang II on podocytes and its pathogenic role in glomerular diseases involve enhanced **TRPC6** expression

ATP signaling



Extracellular signaling molecule
reported more than 75 years ago



P2 receptors

❑ P2X receptors

- Ionotropic
- Nonselective cation channel permeable to Ca^{2+}

❑ P2Y receptors

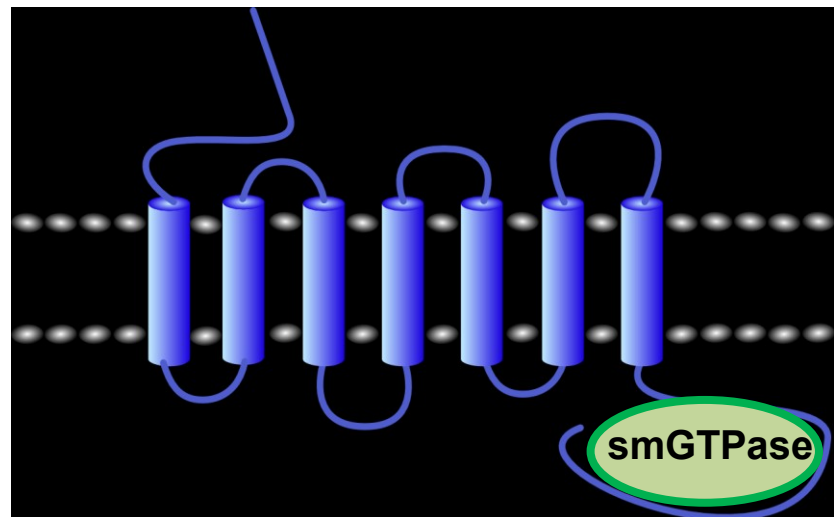
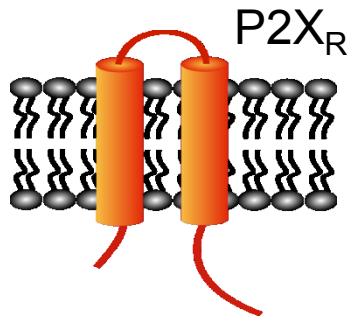
- G-protein coupled
- Evokes IP_3 -mediated Ca^{2+} mobilization

various isoforms

$\text{P2X}_{1\cdots 7}$

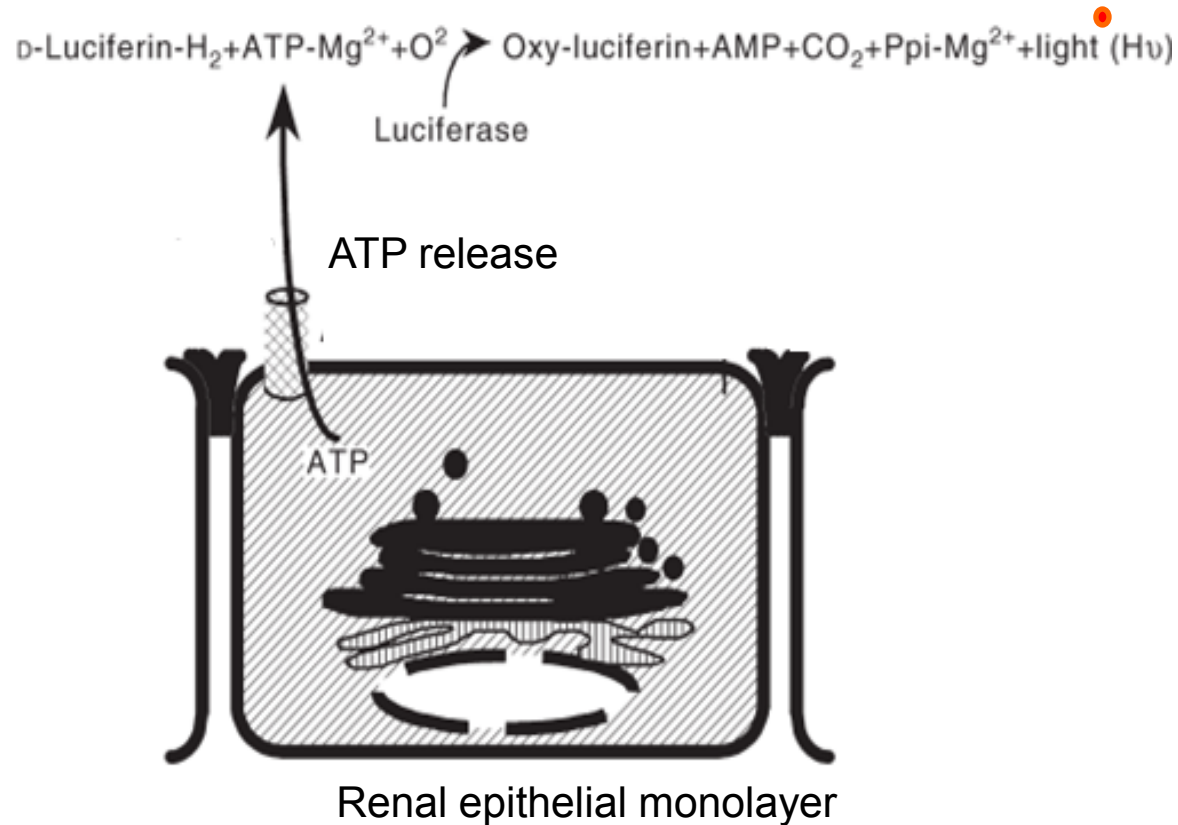
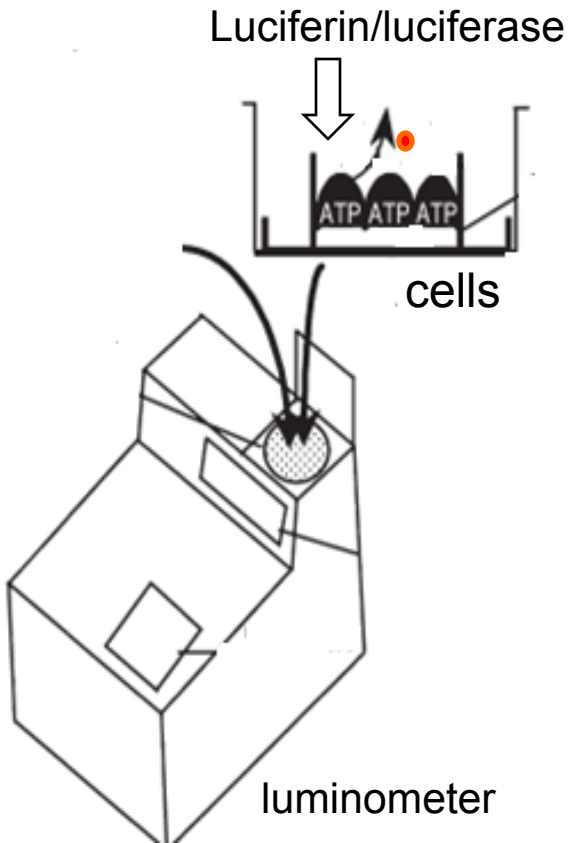
$\text{P2Y}_{1\cdots 14}$

- ✓ Isoforms differ in tissue distribution, affinity for nucleotides, antagonist/agonist specificity, may form multimeres
- ✓ Are expressed at both apical and basolateral membranes of renal epithelial cells, in the renal vasculature, glomerular mesangial cells and podocytes



Assays used for ATP release detection

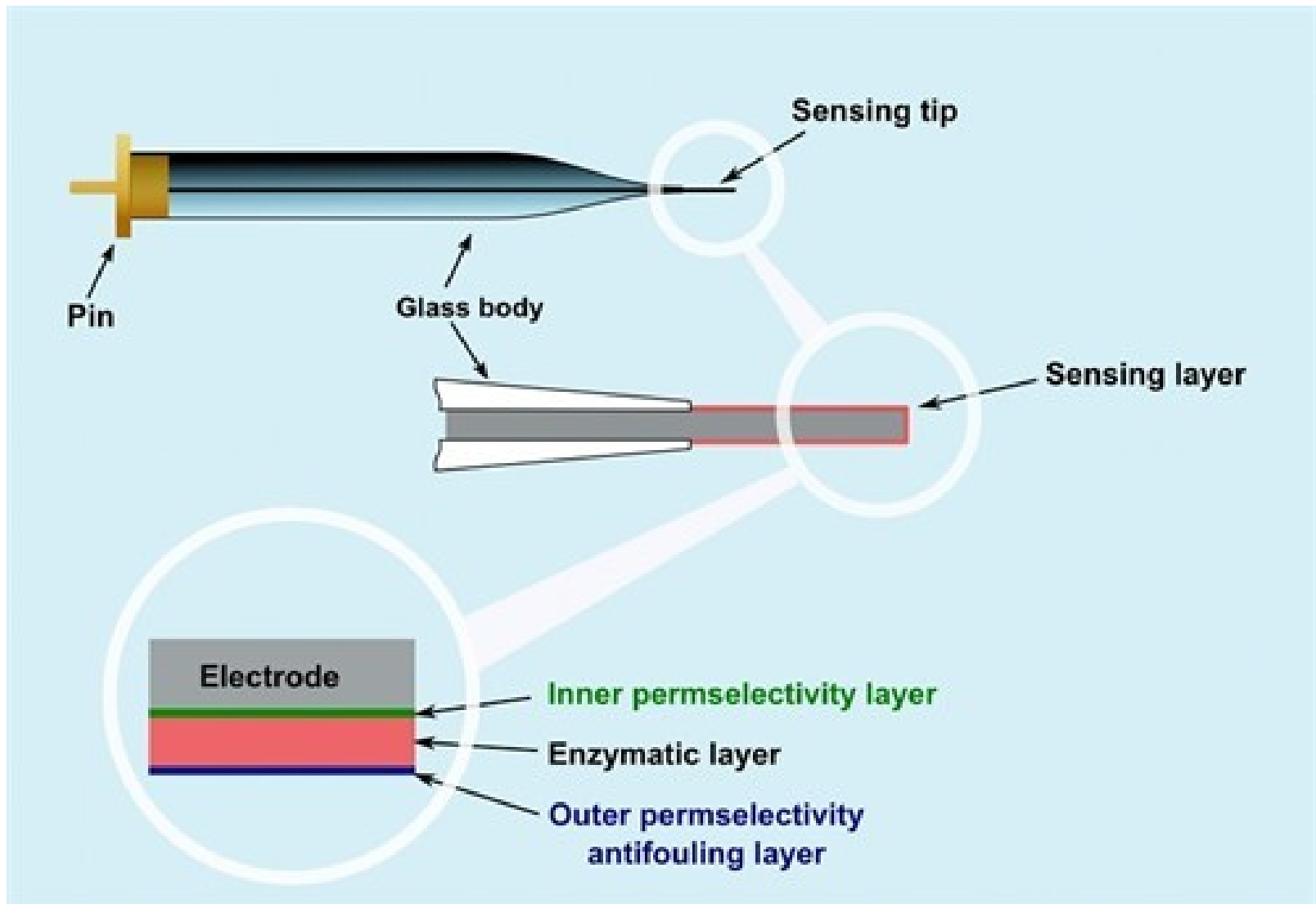
Bioluminescence



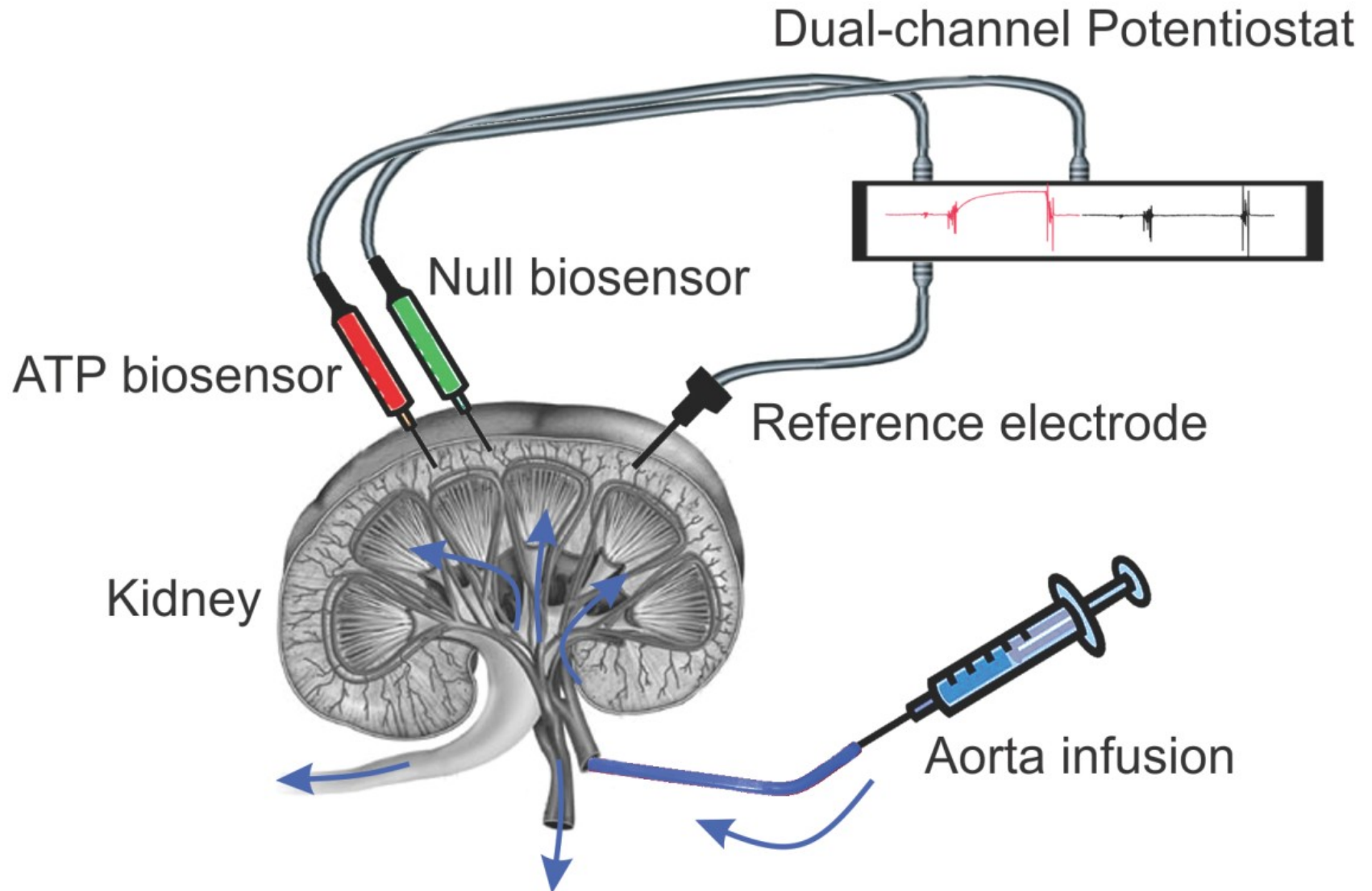
In the kidney: interstitial fluid collection and kit measurements

Assays used for ATP and hydrogen peroxide detection

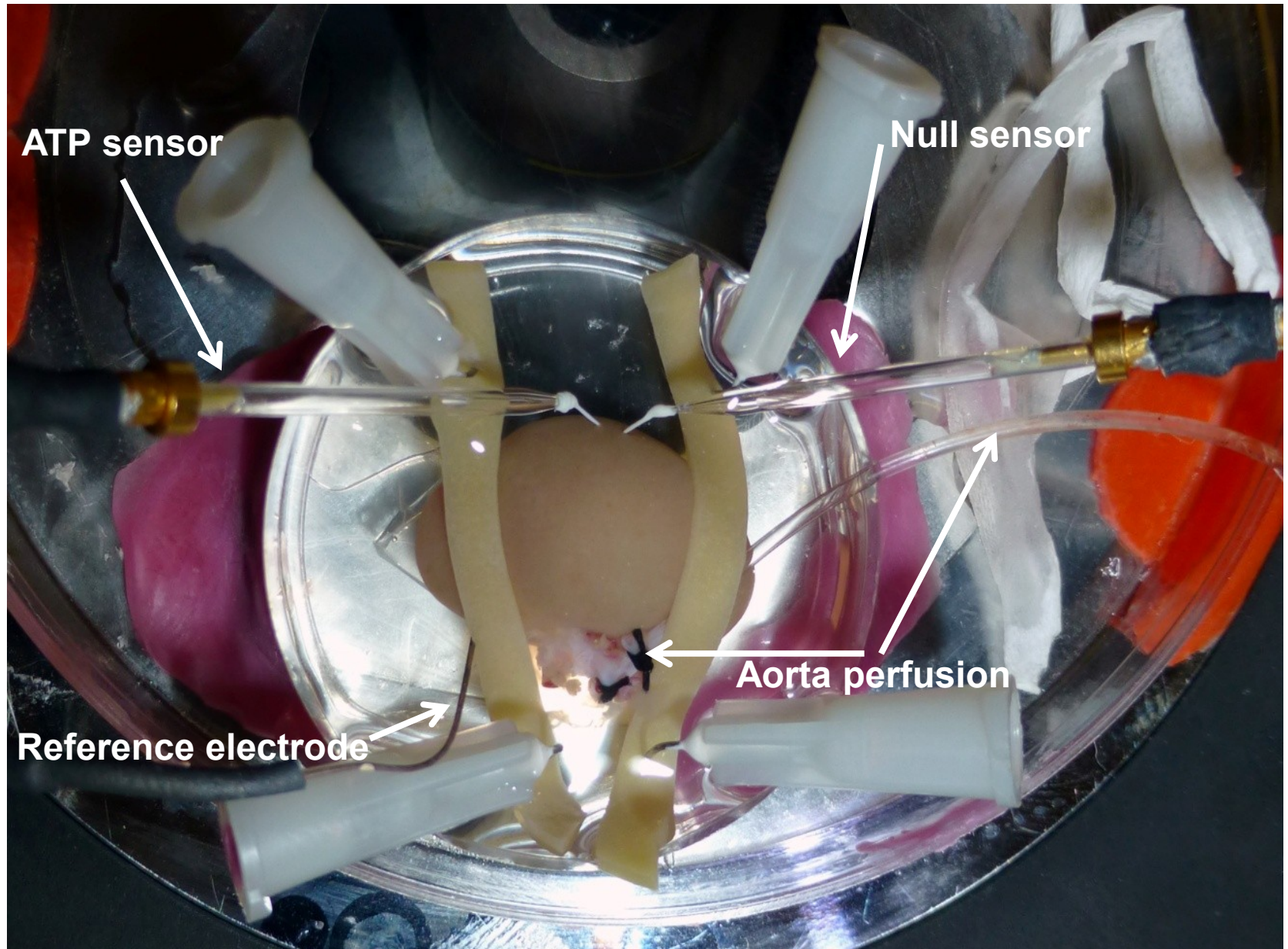
Biosensors



ATP and H₂O₂ detection setup scheme

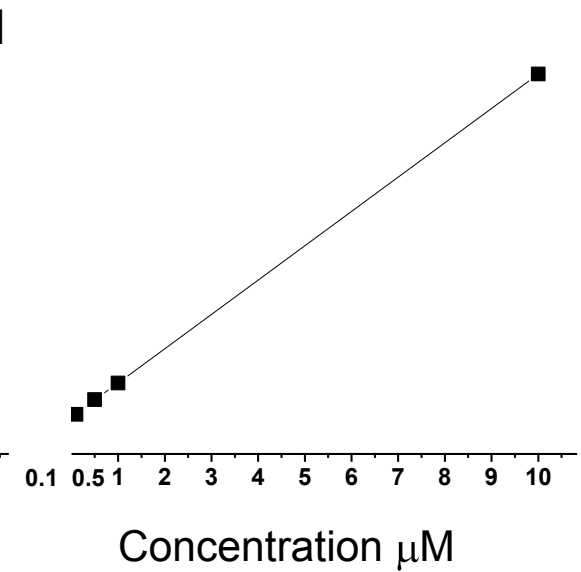
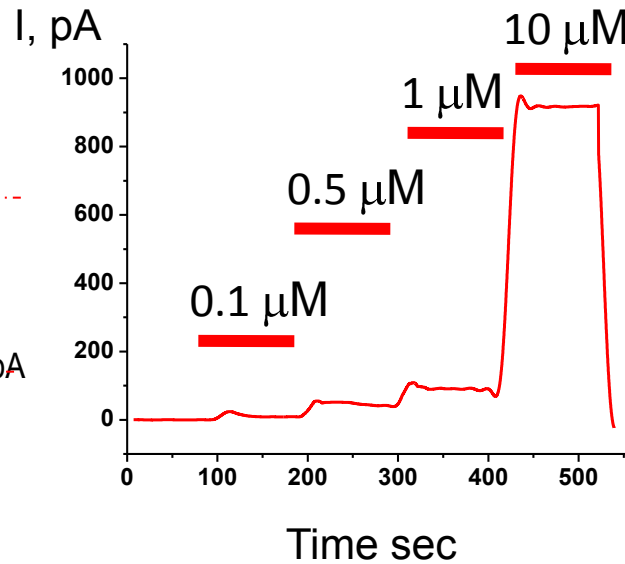
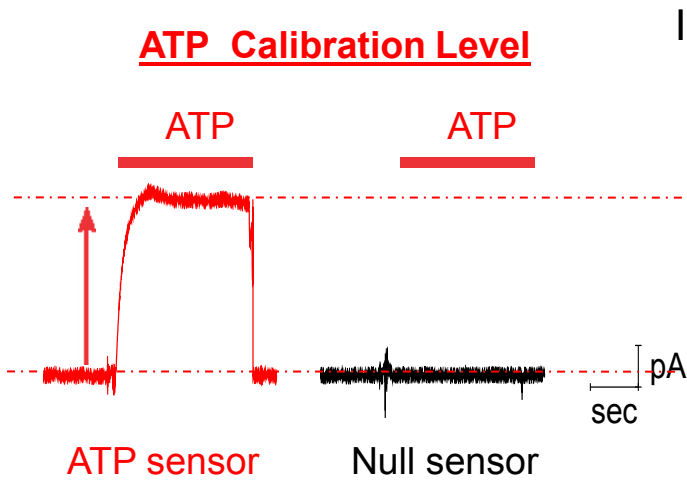


Real ATP and H₂O₂ detection setup

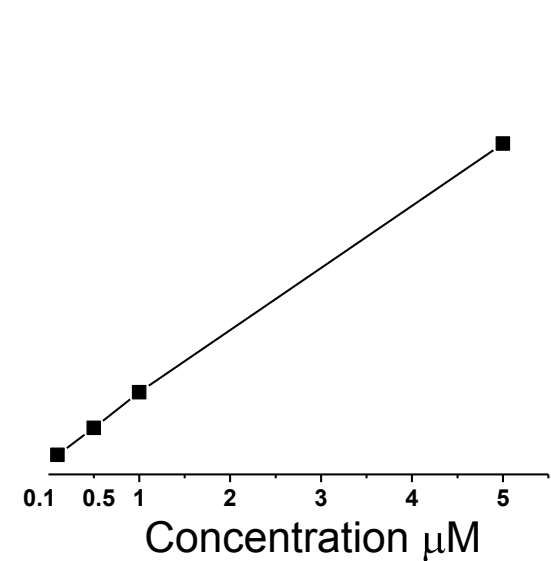
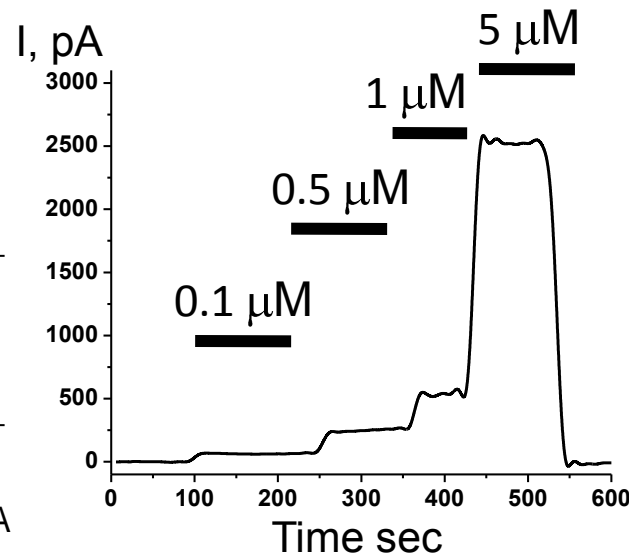
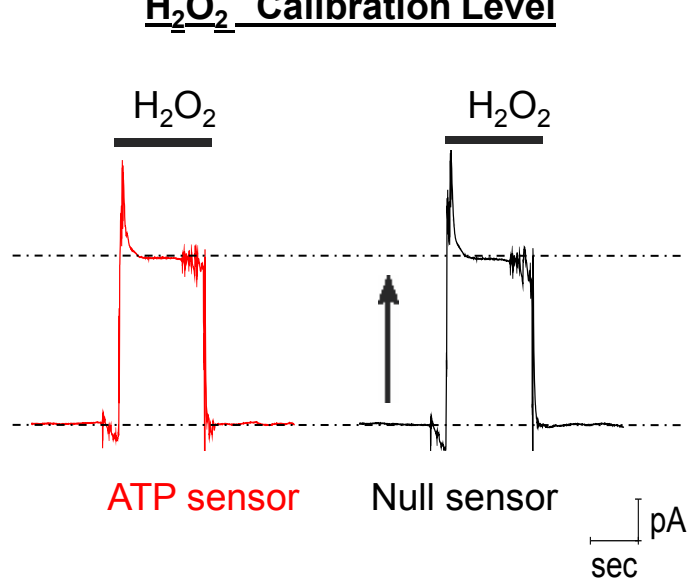


Calibration of the sensors

ATP Calibration Level



H₂O₂ Calibration Level



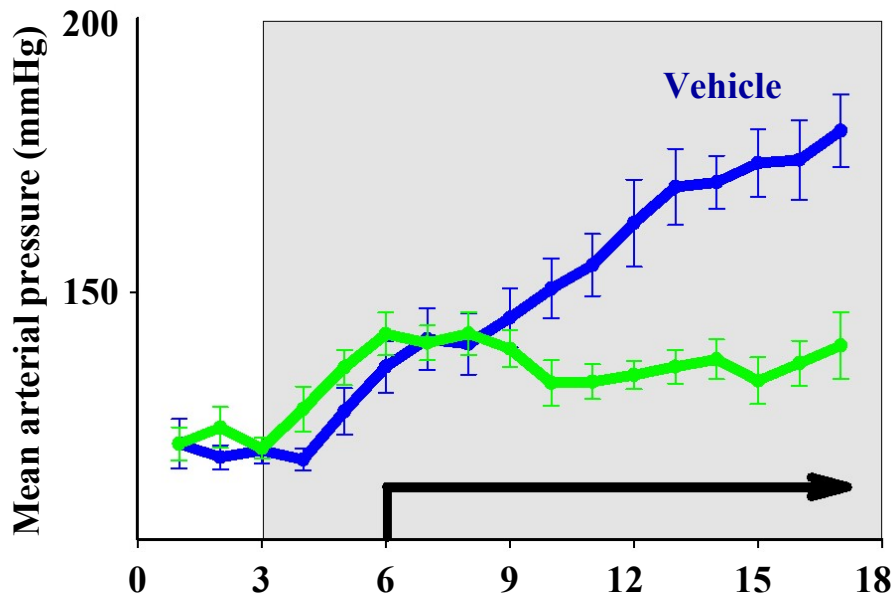
Sprague Dawley rats

Dahl Salt-Sensitive (SS) rats

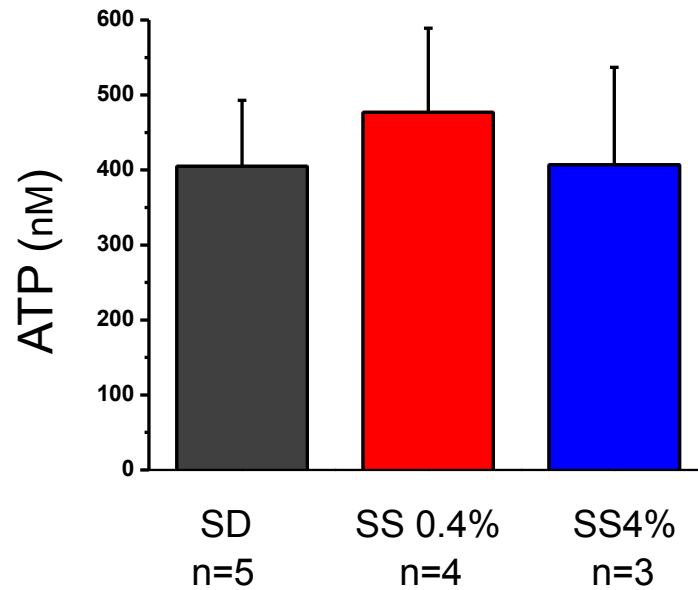
- Blood pressure salt-sensitivity
- Chronic kidney injury
- Reduced renal function
- Low renin hypertension



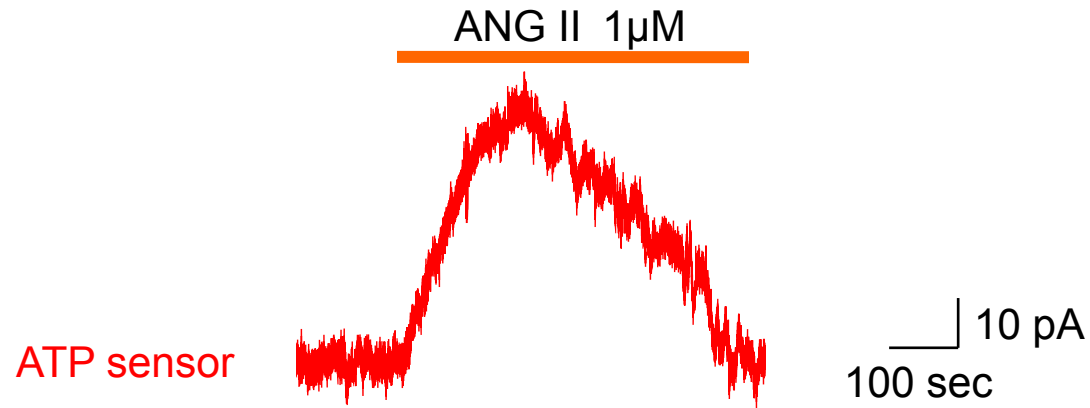
Arterial pressure



Basal levels of ATP in the kidney cortex



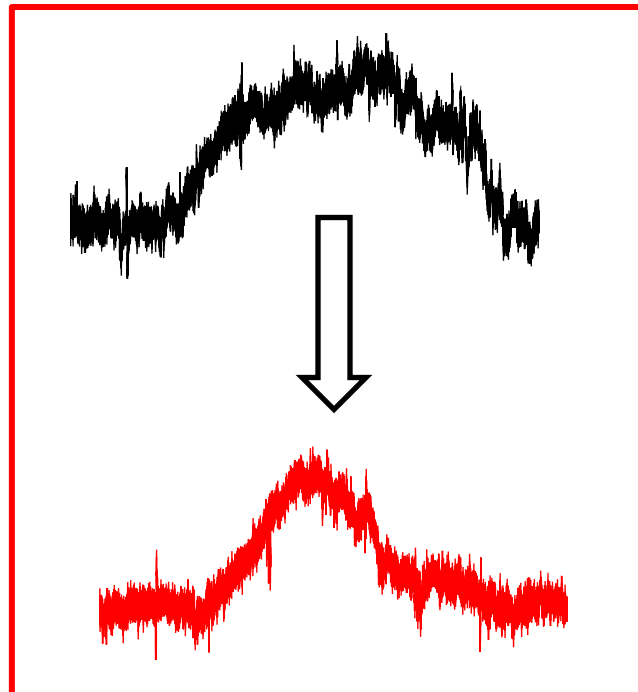
Angiotensin II effect on ATP production in the kidney



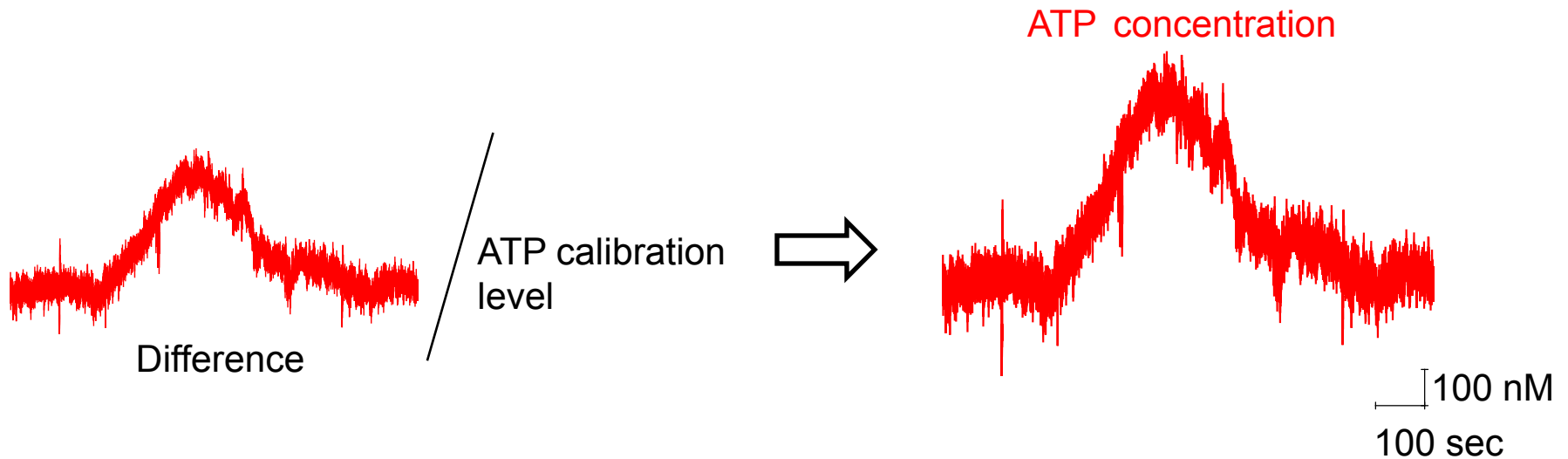
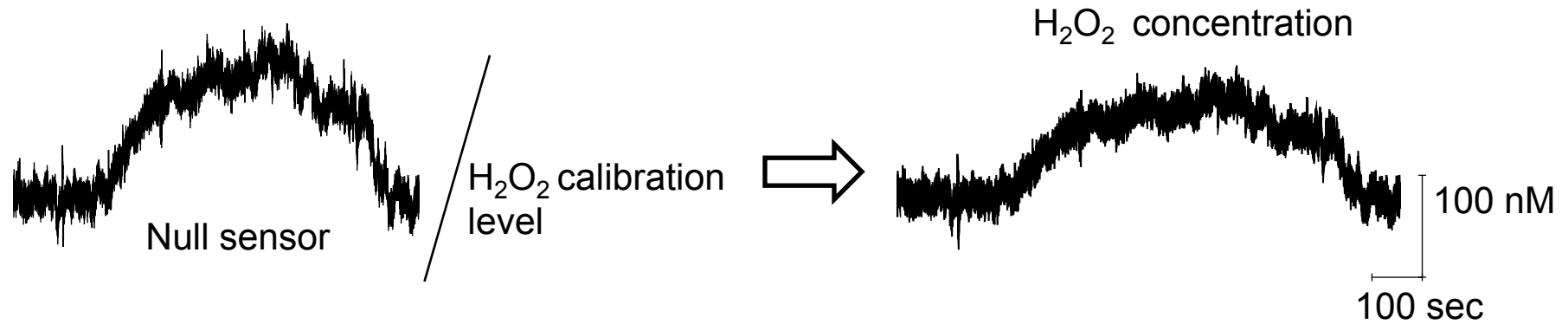
Subtraction

Null sensor

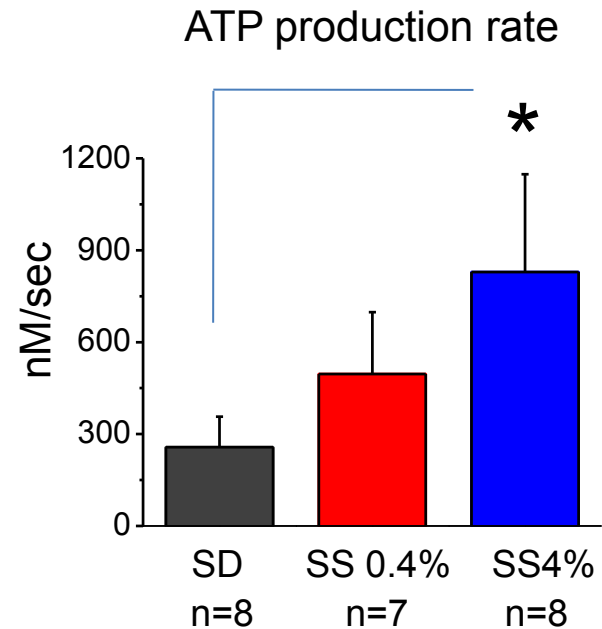
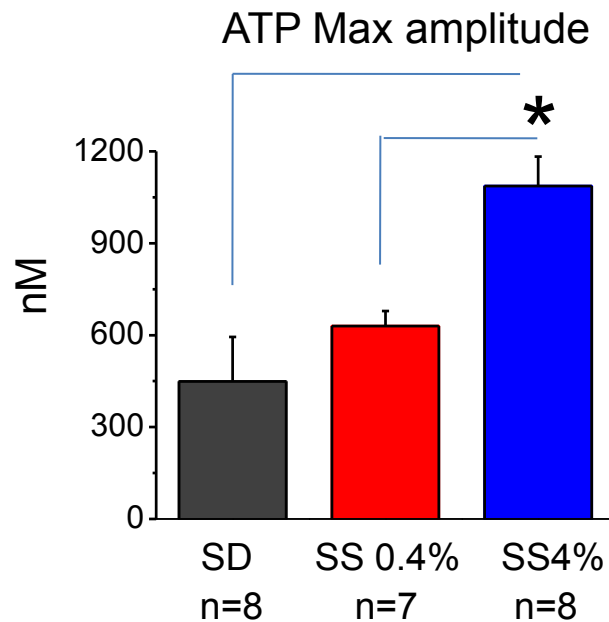
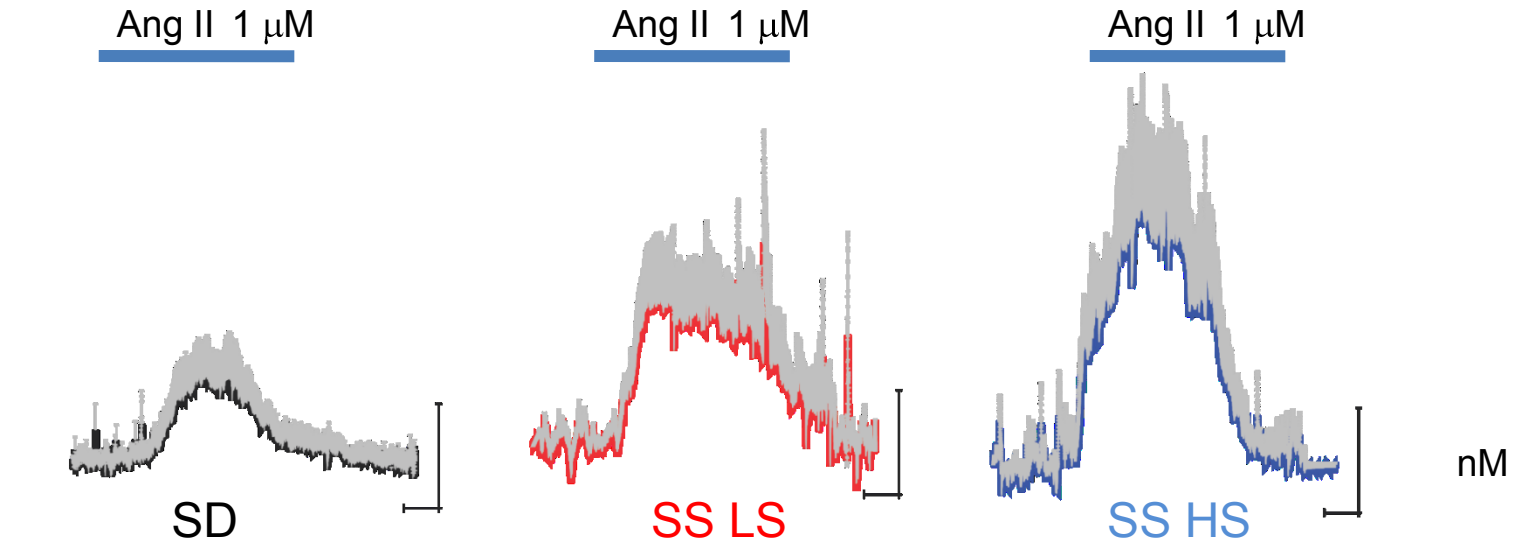
Difference



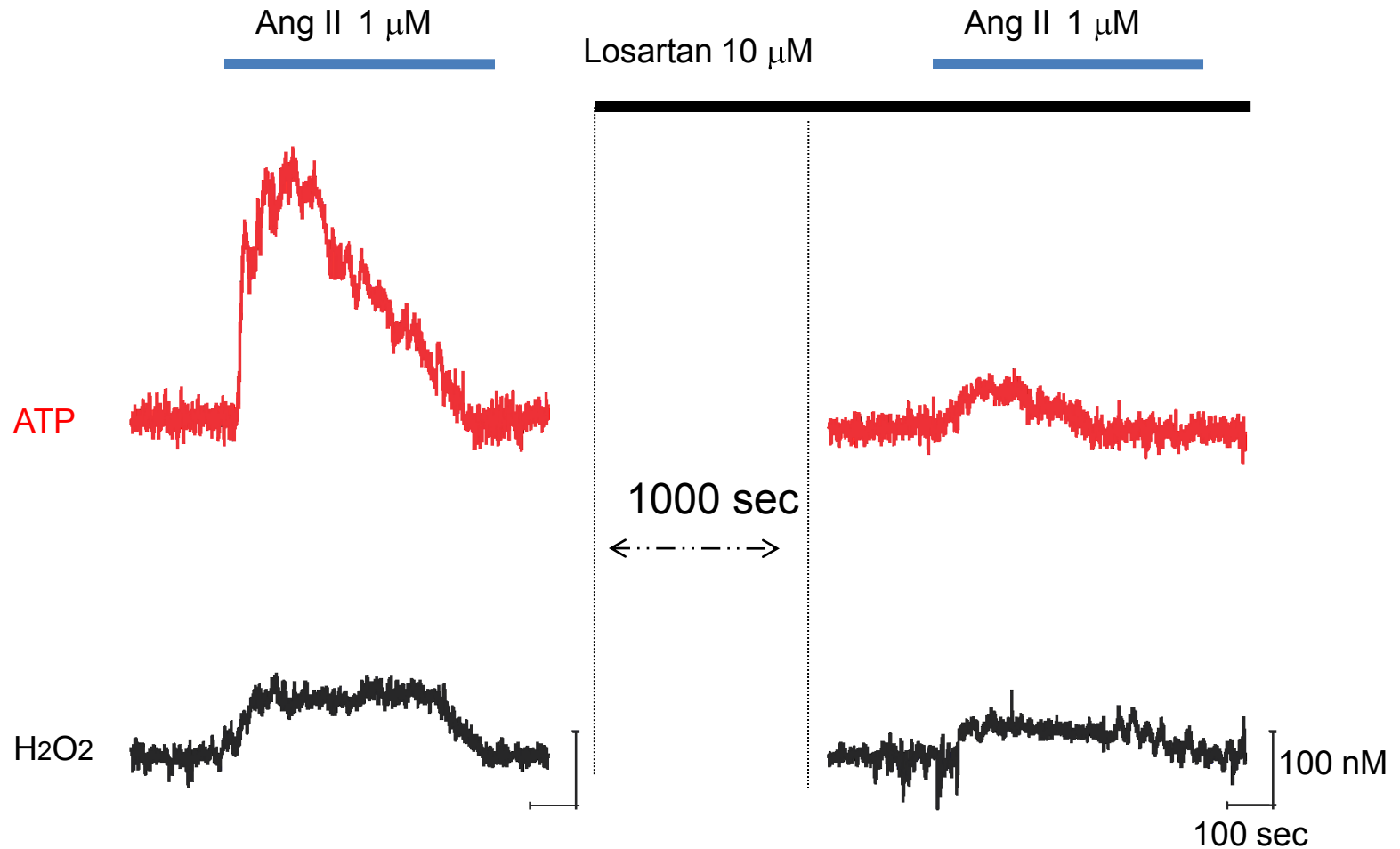
Representative concentration calculations



Angiotensin II effect on ATP production in the kidney

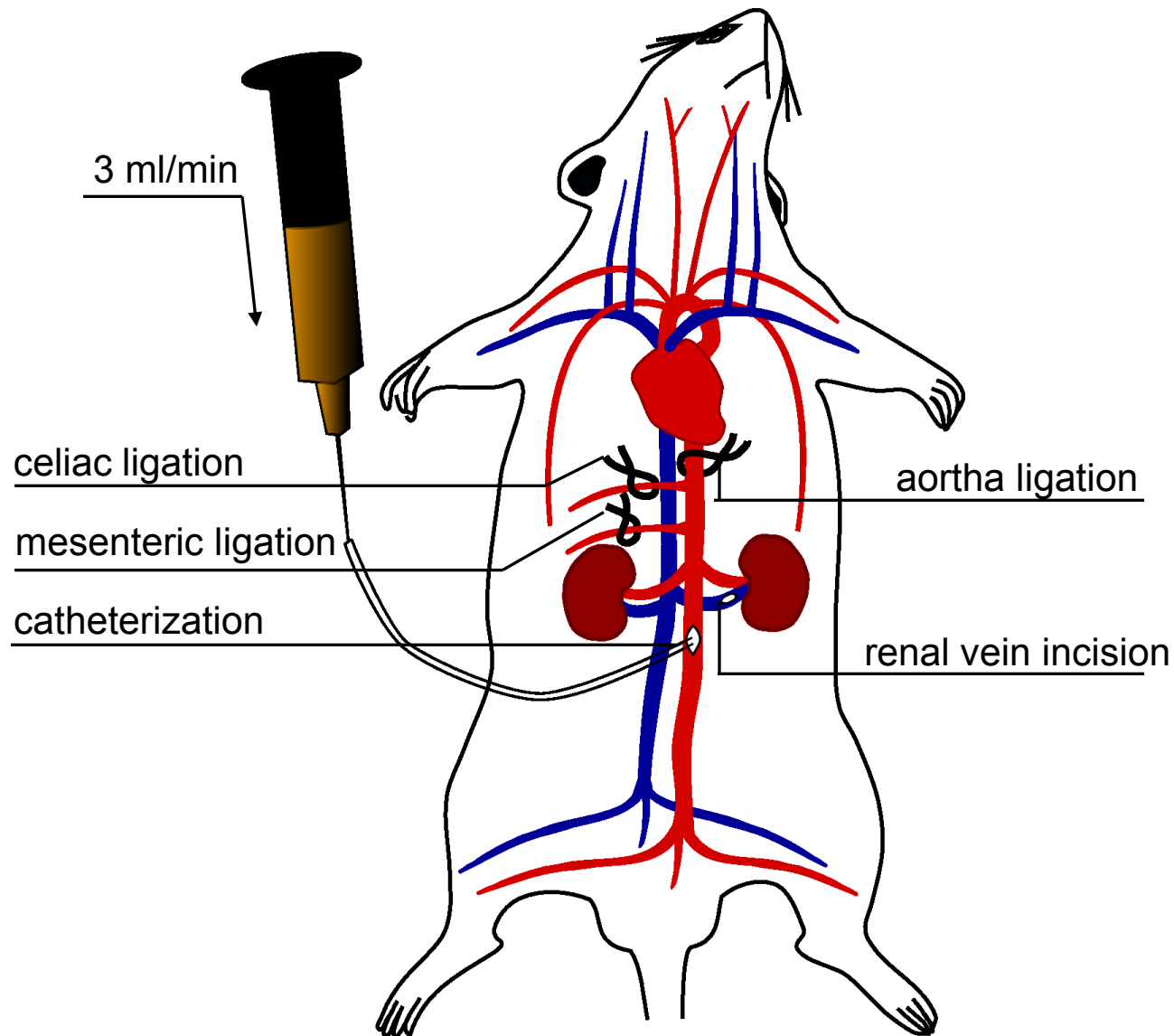


Inhibition of AT_1 receptor blocks effects of Ang II on ATP and H_2O_2 production in the kidney

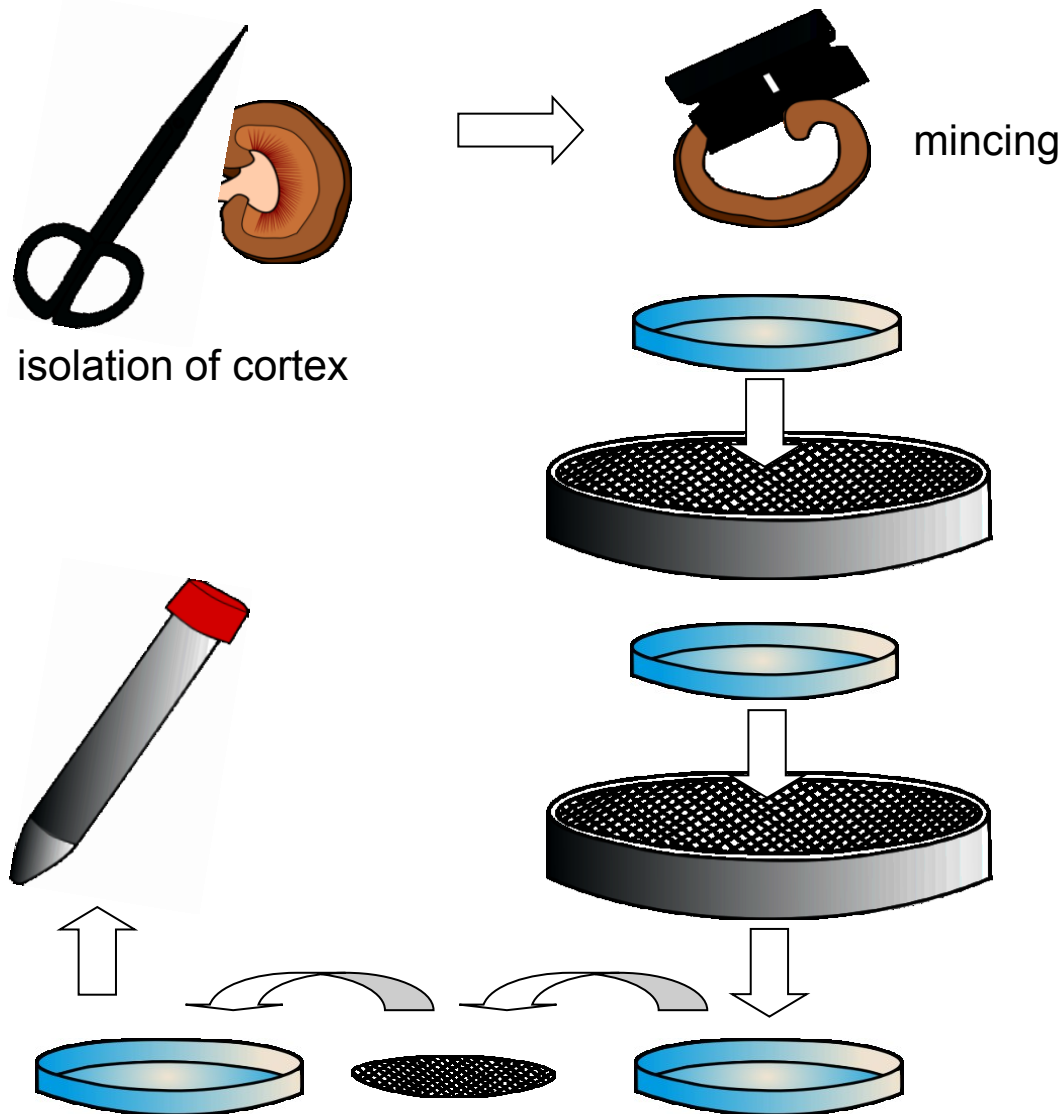


- ❑ We established a new approach to acutely measure endogenous substances (ATP, hydrogen peroxide etc) release in the whole perfused kidney
- ❑ Ang II causes ATP release in the Sprague Dawley and SS rats kidney cortex and this effect is elevated in SS rats
- ❑ Effect of Ang II is mediated through AT₁ receptor

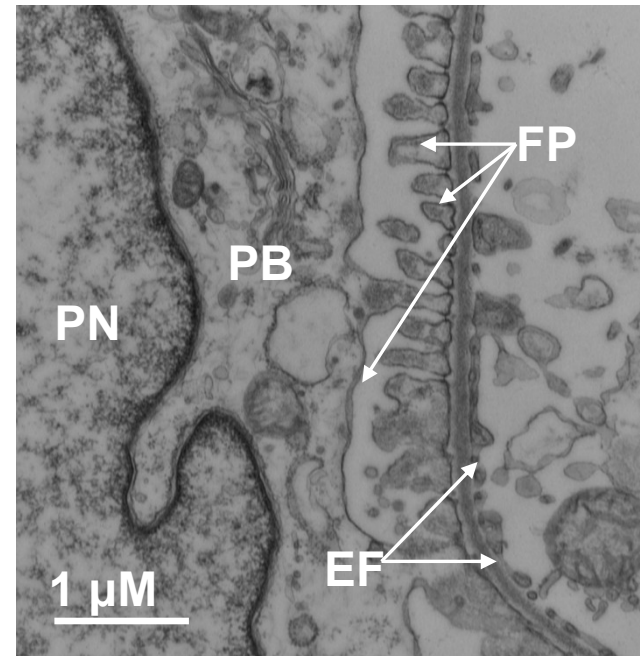
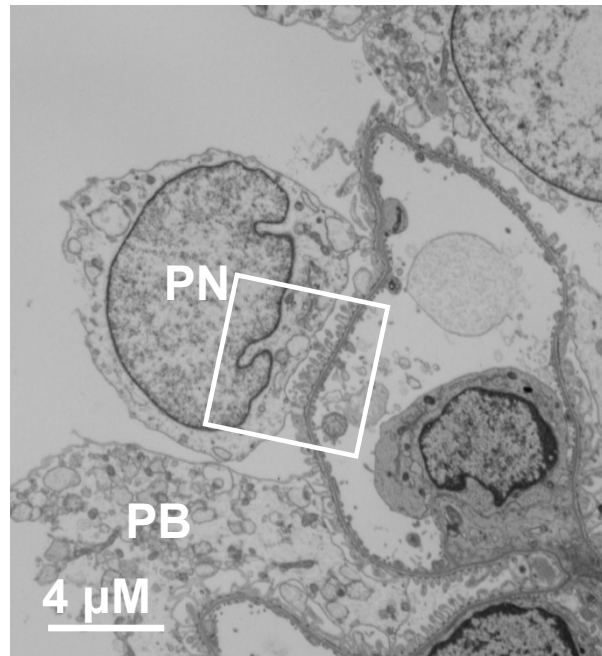
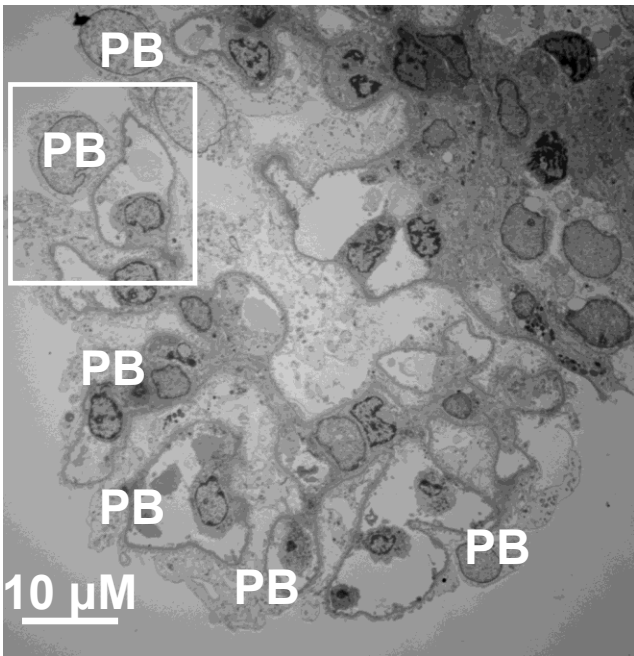
Rat preparation for isolation of the kidney



Isolation of the rat glomeruli

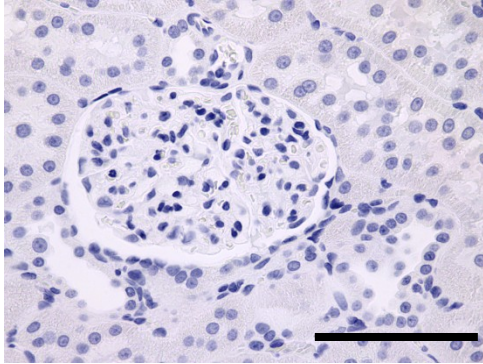


Electron microscopy of freshly isolated decapsulated glomeruli

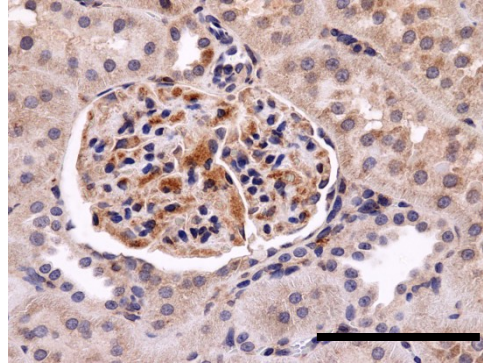


Expression profile of TRPC6 channels in the glomeruli

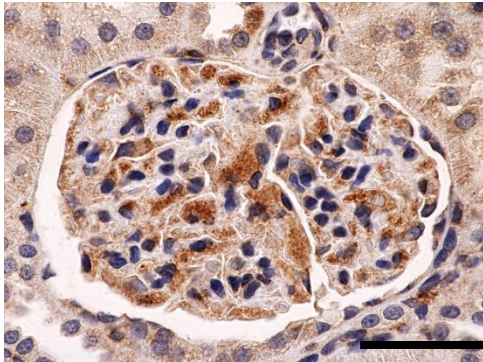
control (40X)



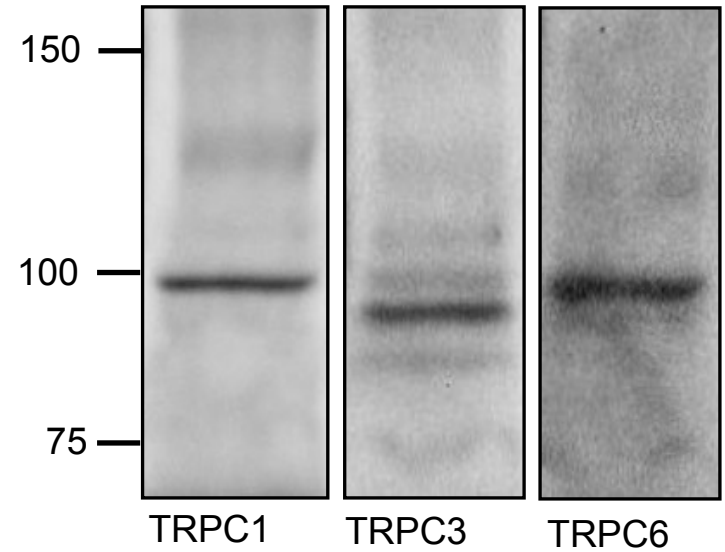
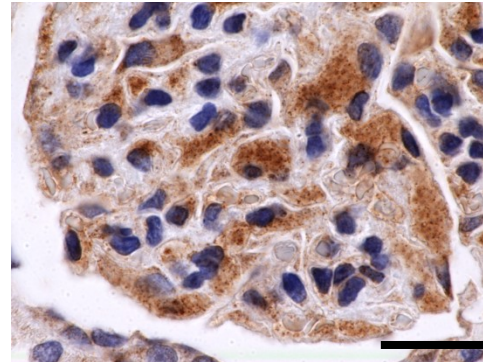
TRPC6 (40X)



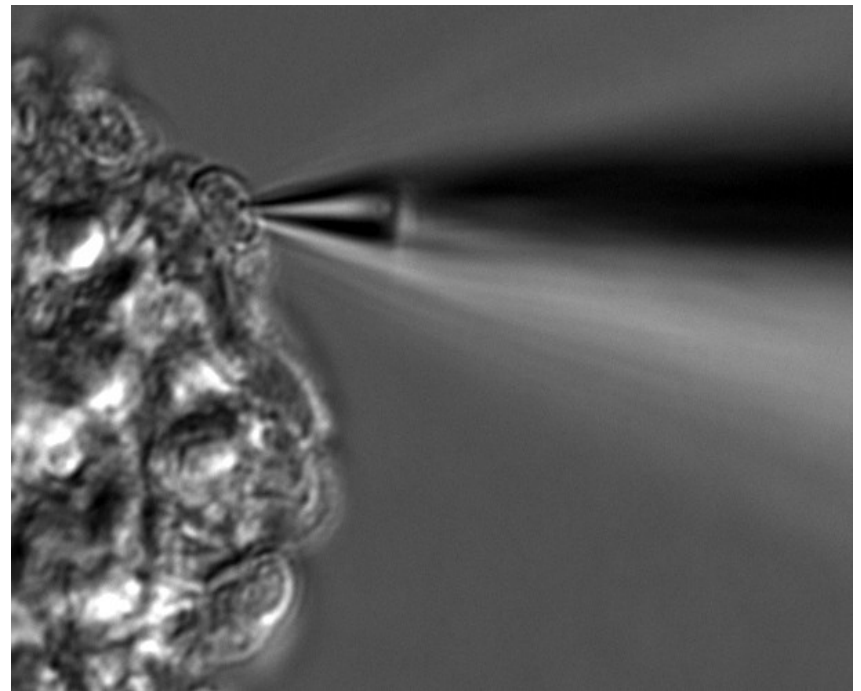
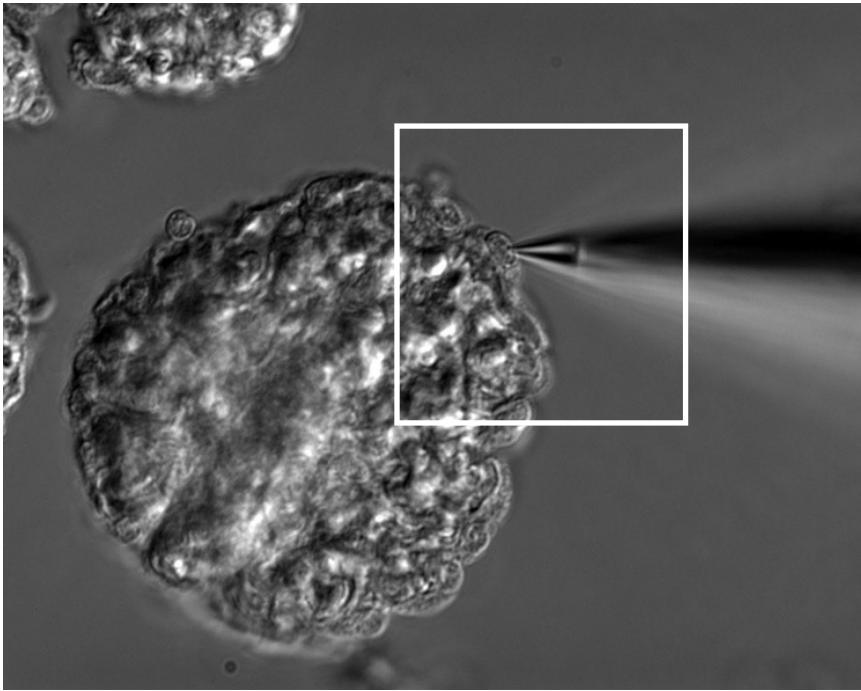
60X



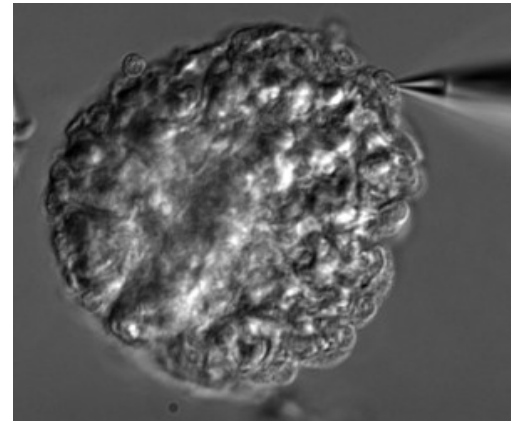
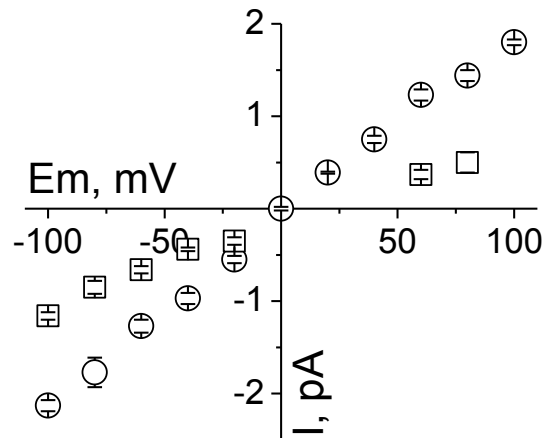
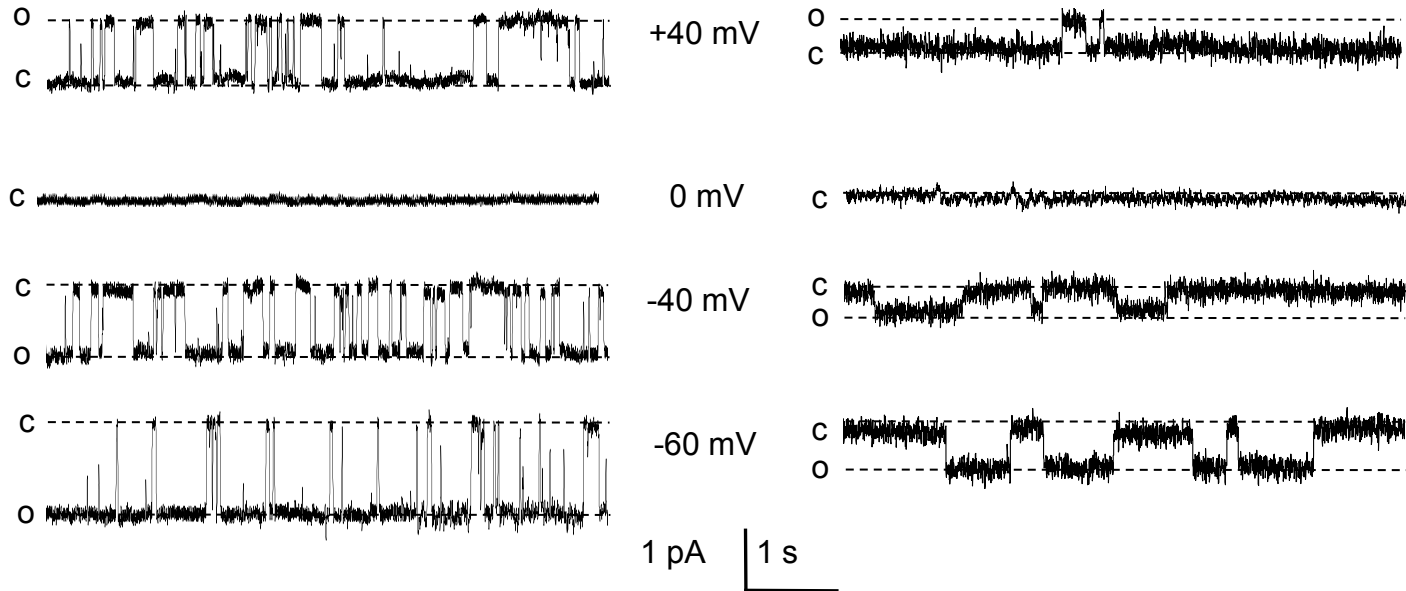
100X



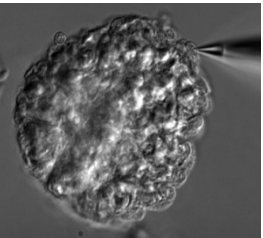
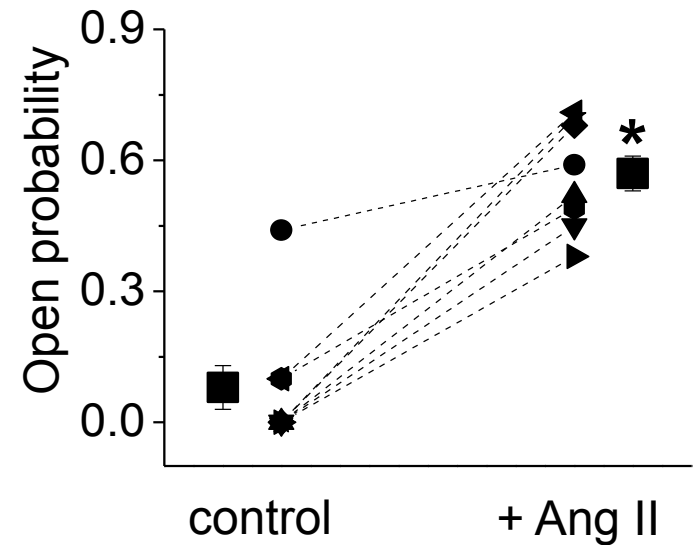
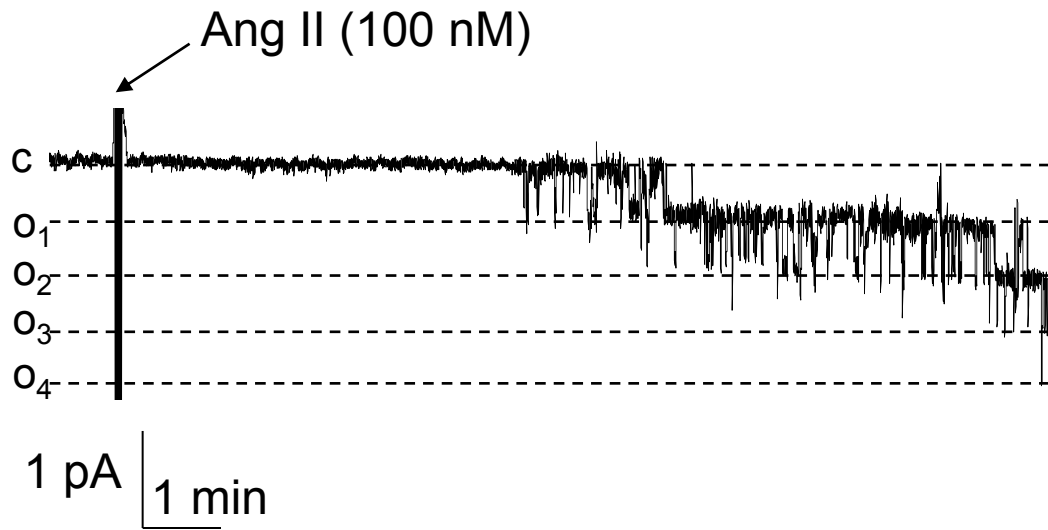
Decapsulated rat glomerulus in the patch-clamp setup



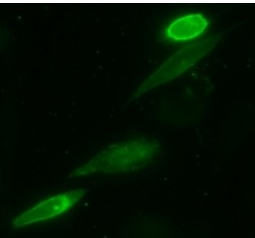
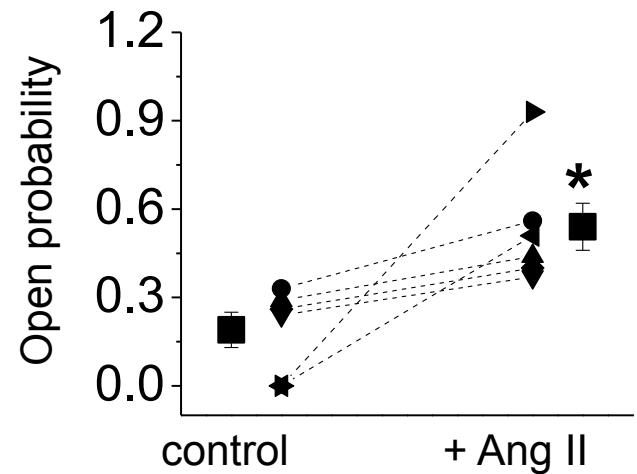
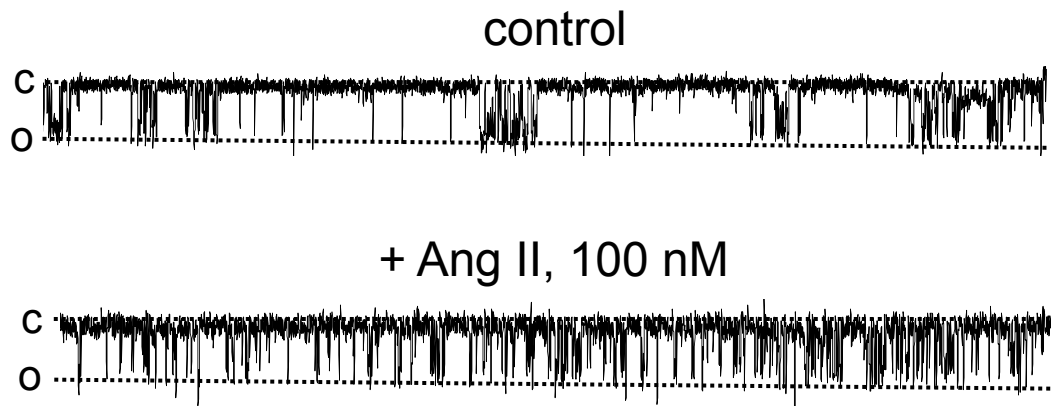
Single-channel recordings in podocytes



Ang II activates TRPC channels in the freshly isolated glomeruli

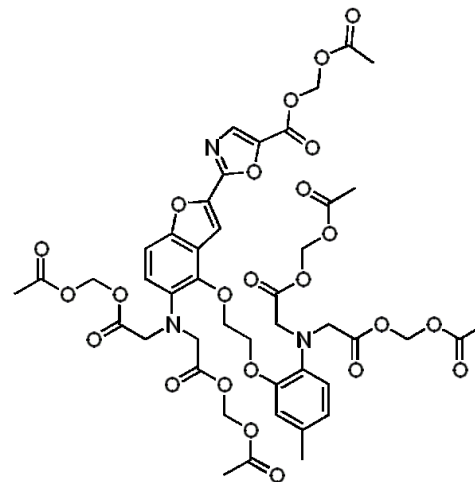
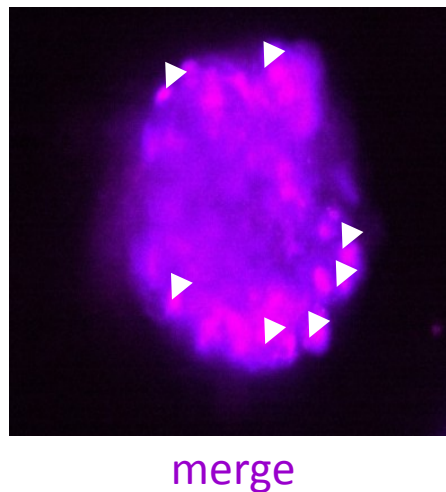
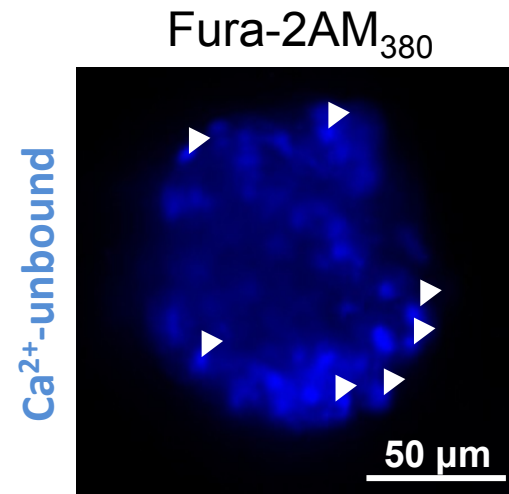
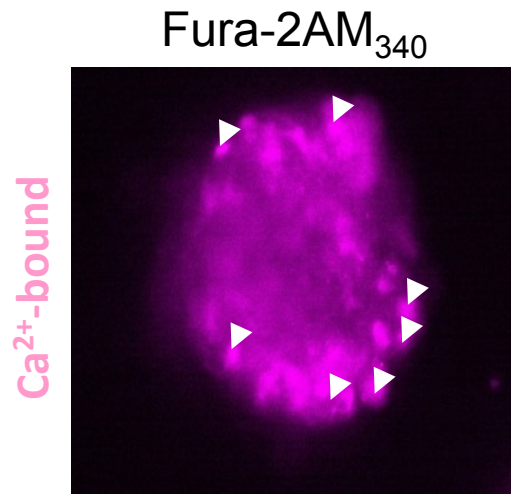


Ang II activates TRPC6 channel in transfected CHO cells

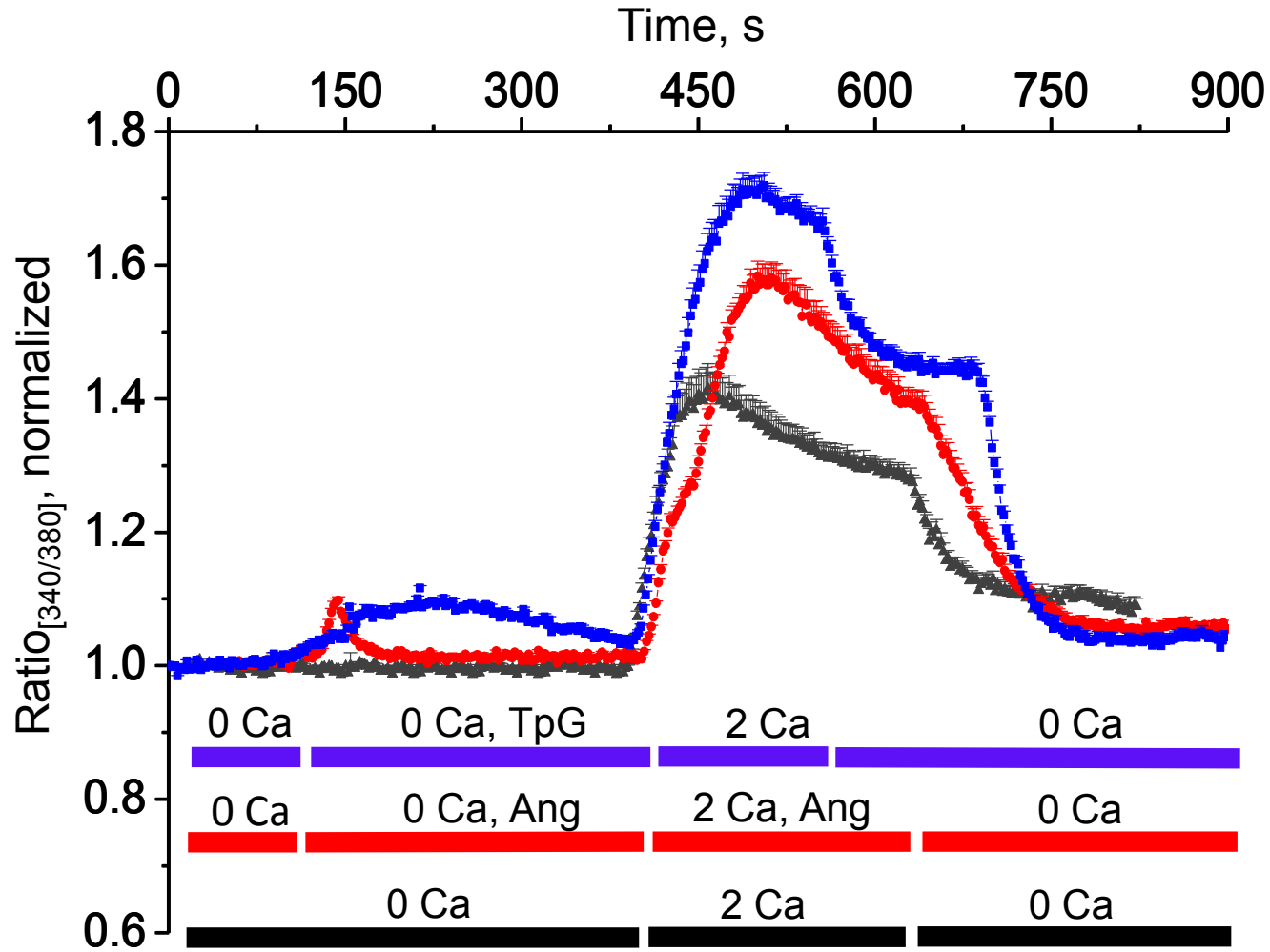


- ❑ We established an unique approach allowing us to do single channel analysis of endogenous channels in podocytes of freshly isolated glomeruli
- ❑ Ang II acutely activated TRPC channels in rat podocytes
- ❑ Ang II acutely activated TRPC6 channel in transfected CHO cells

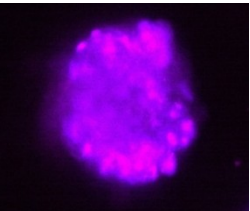
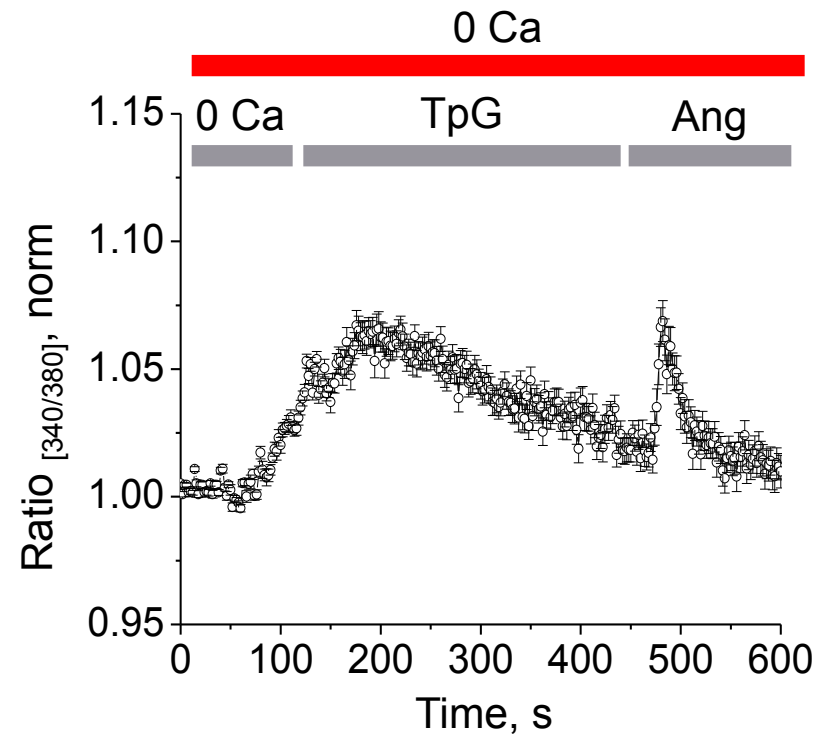
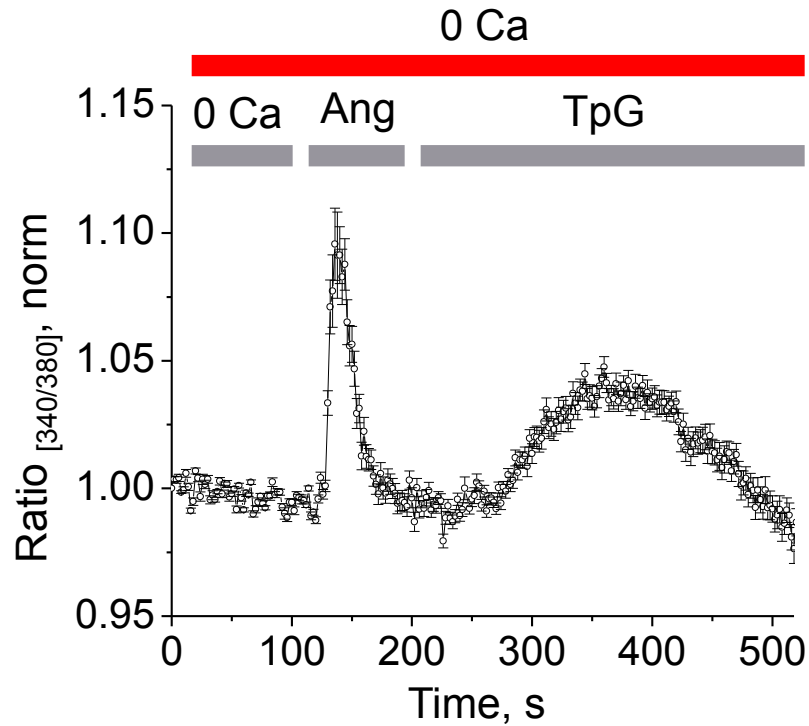
Fura-2AM calcium concentration measurements in the rat glomeruli



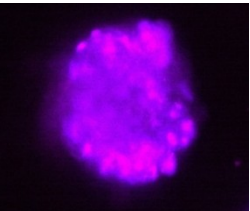
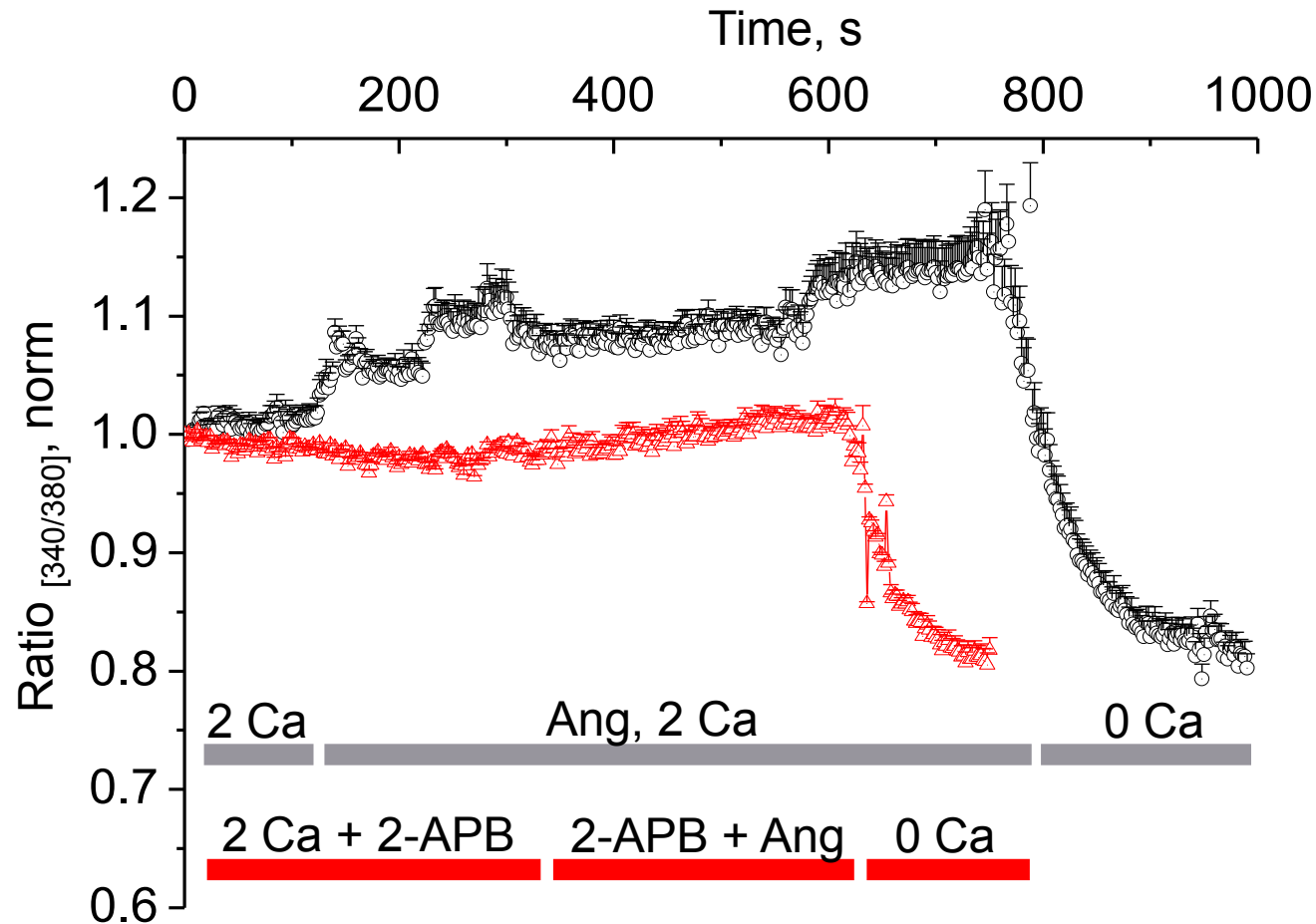
Ang II evokes $[Ca^{2+}]_i$ elevation in the freshly isolated glomeruli



Ang II-stimulated $[Ca^{2+}]_i$ peak lasts after calcium depot depletion with thapsigargin

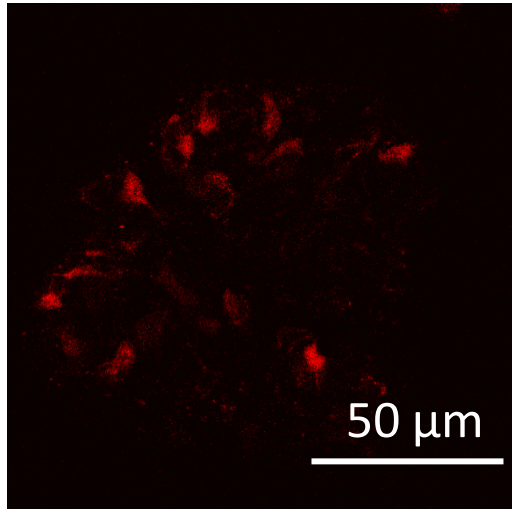


2-APB blocks Ang II – mediated elevation in $[Ca^{2+}]_i$

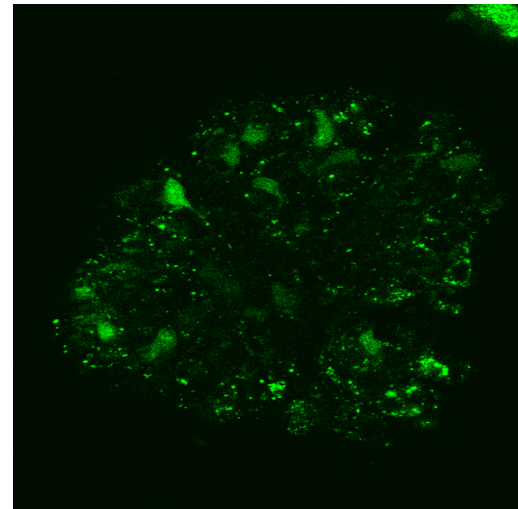


Fluo4/FuraRed Ca^{2+} concentration measurements in the rat glomeruli

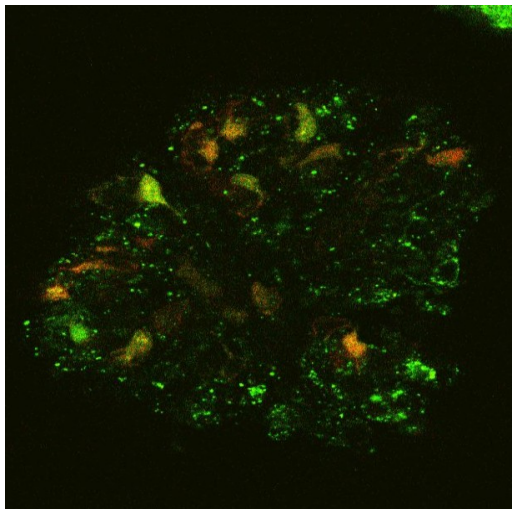
Fluo4



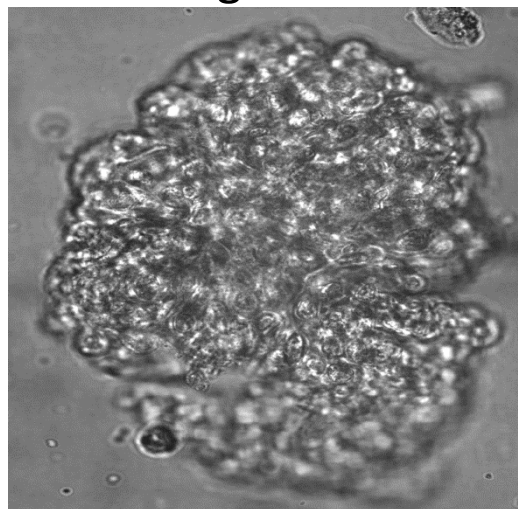
FuraRed



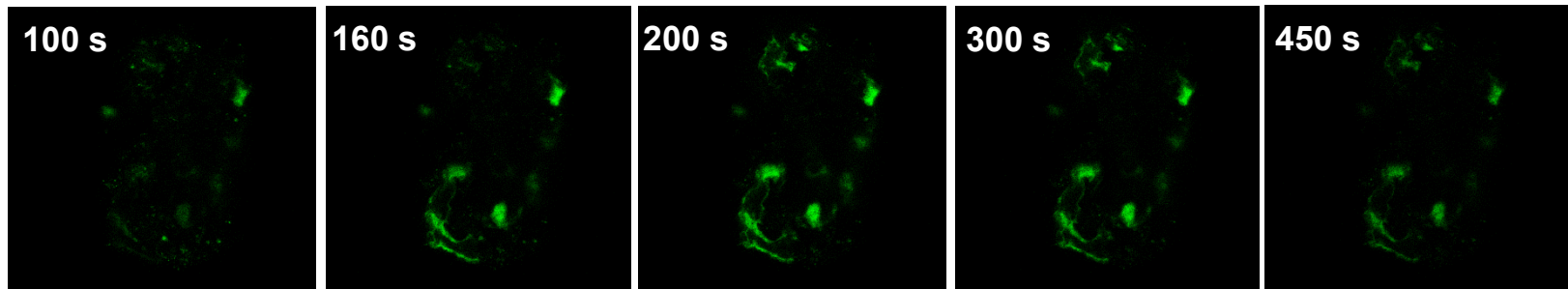
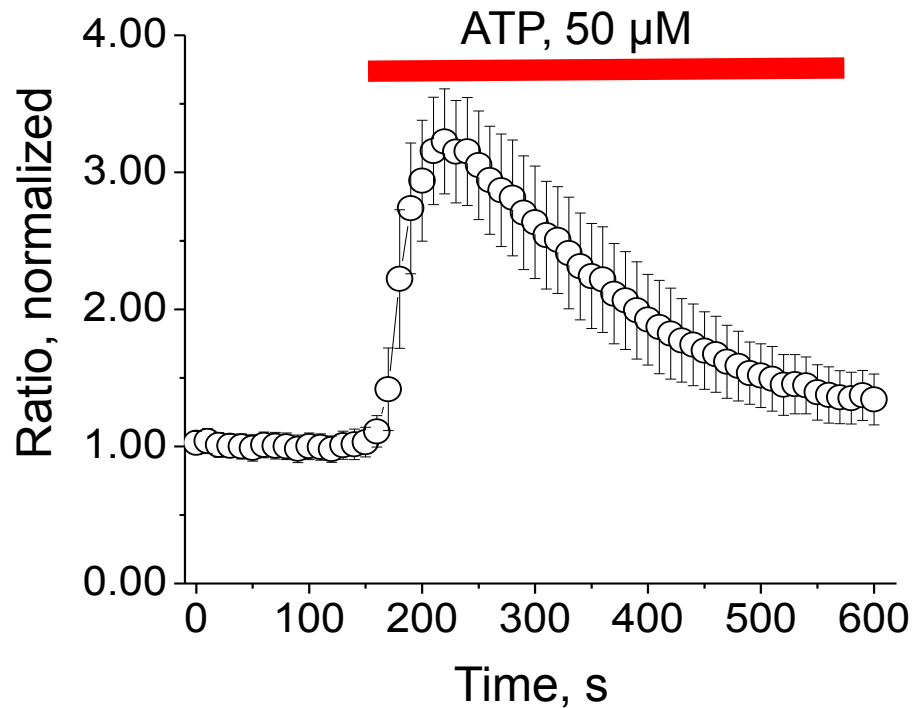
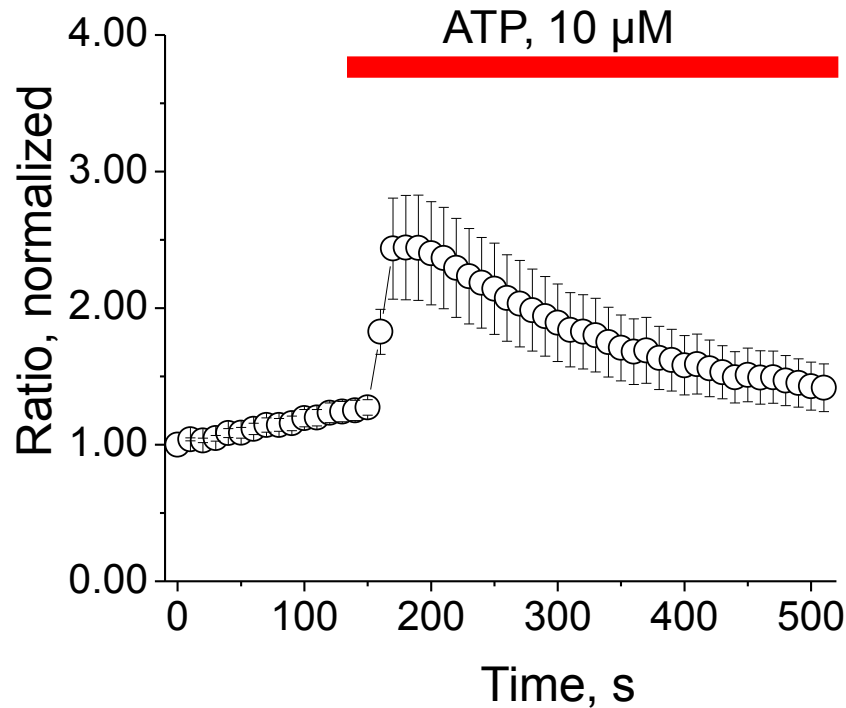
merge



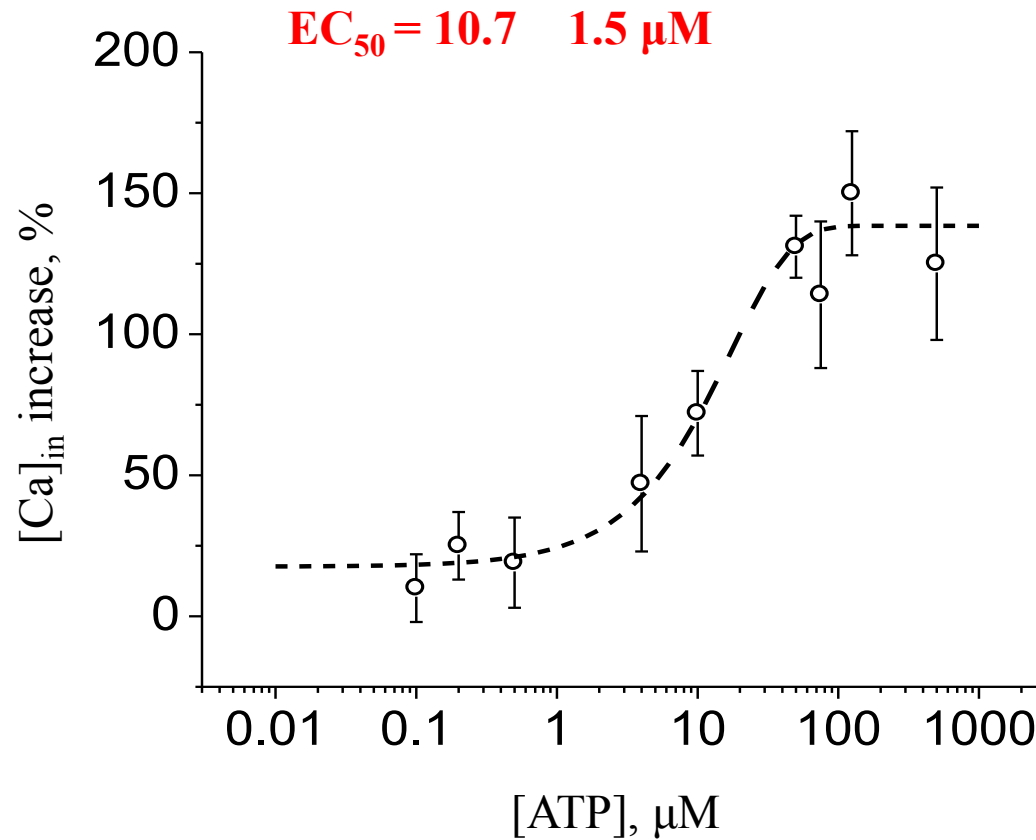
brightfield



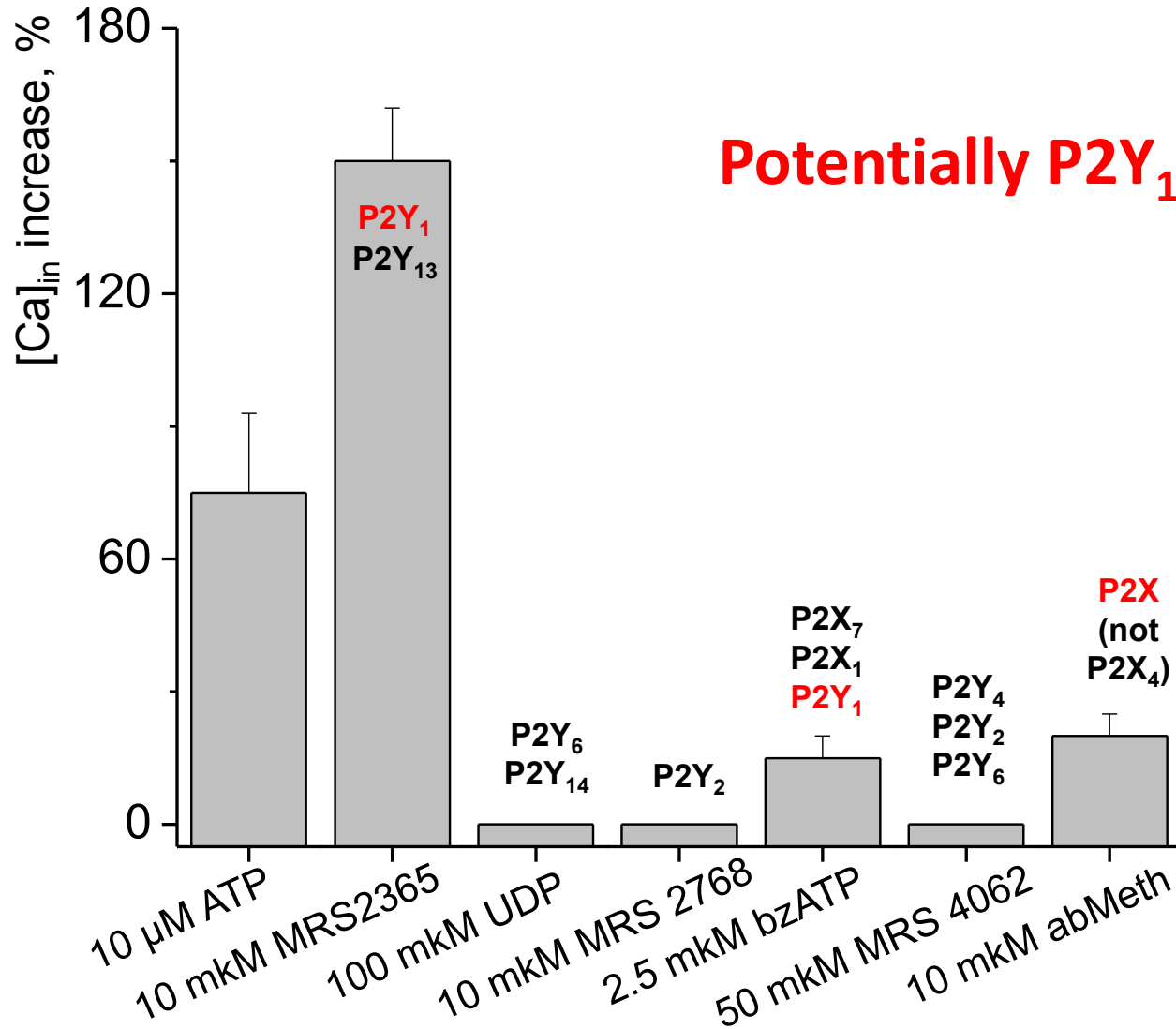
ATP evokes rapid increase of $[Ca^{2+}]_i$ in podocytes of the Sprague Dawley rat glomeruli



ATP concentration EC_{50} in Sprague Dawley rat glomeruli

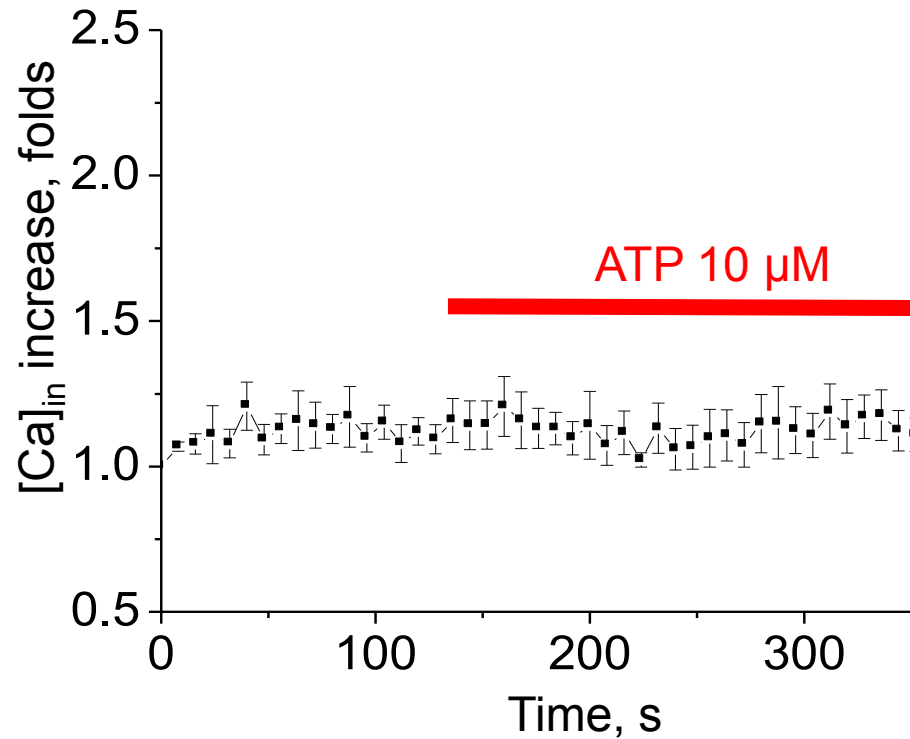


P2Y₁ receptor is the main responsible for calcium influx in the podocytes

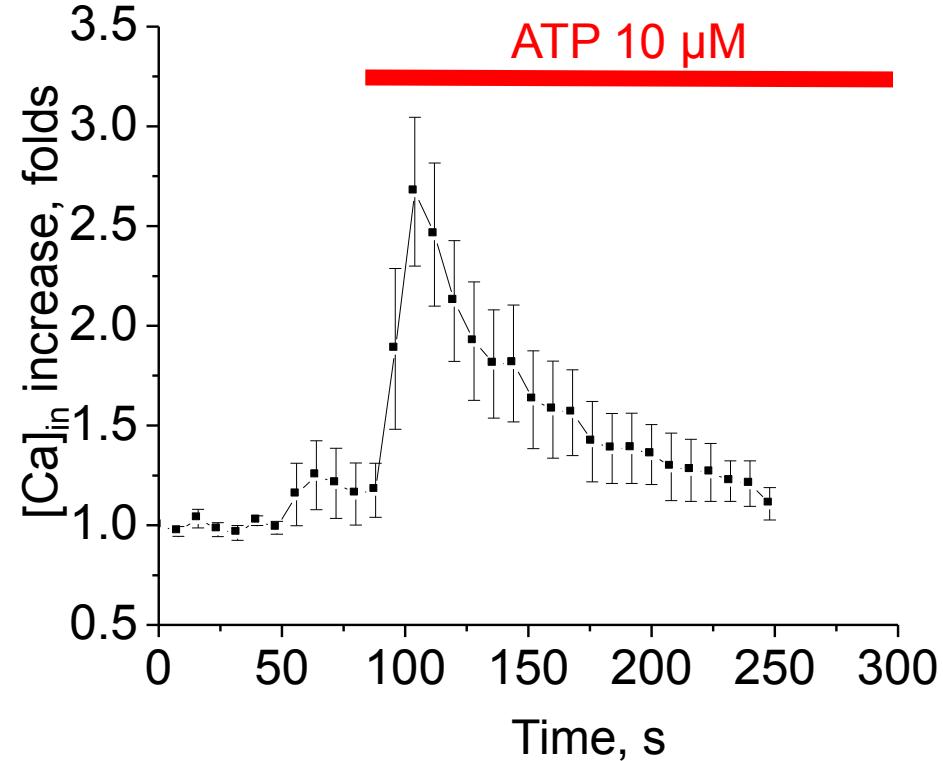


P2Y₁ receptor is the main responsible for calcium influx in the podocytes

MRS2500, 1 nM

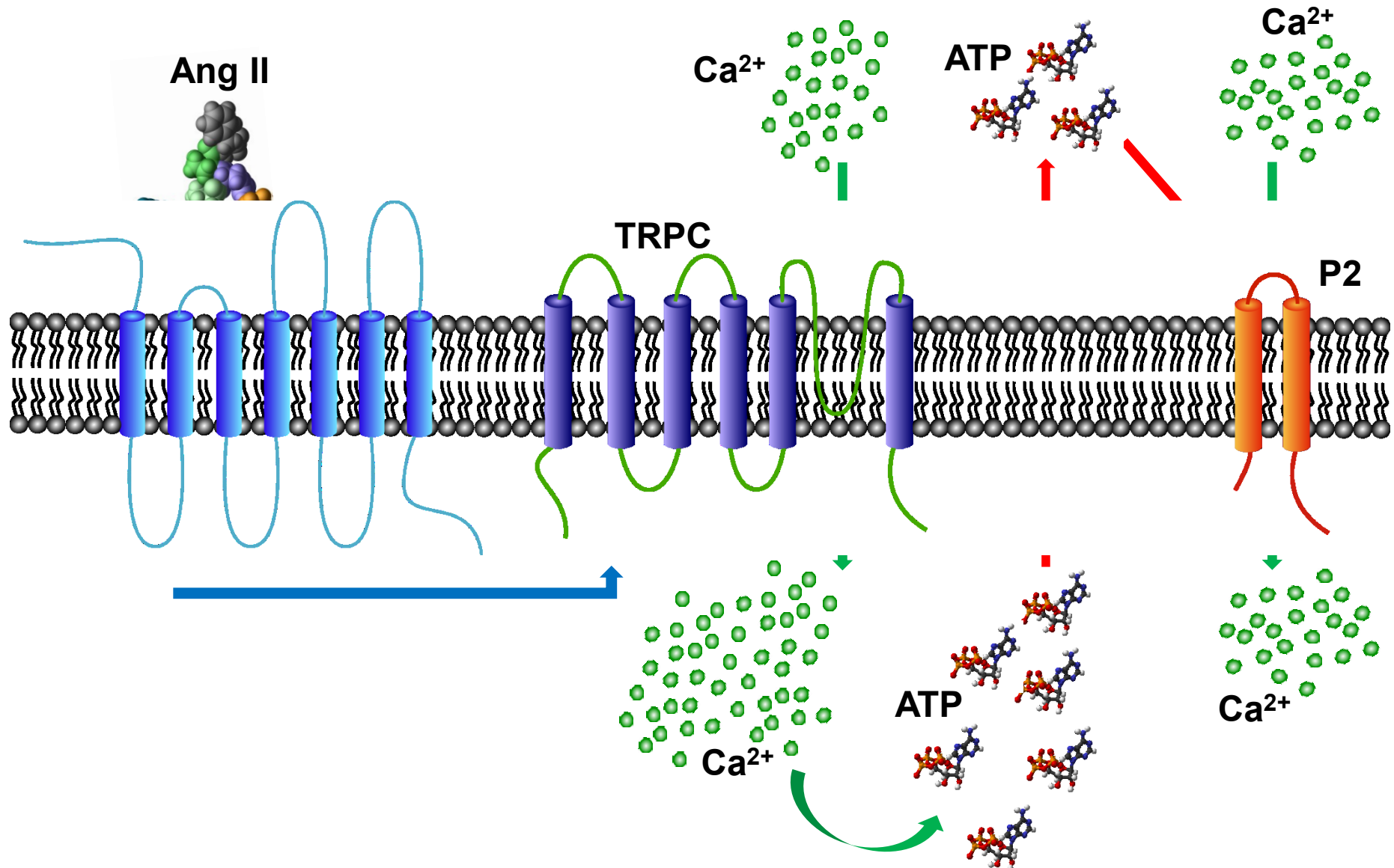


Control ATP application



- ❑ Ang II application results in calcium influx and Ca^{2+} depot release in the podocytes of the glomeruli
- ❑ Ang II causes ATP release in the Sprague Dawley rat kidney cortex and P2Y_1 receptor mediates this effects

Proposed scheme of regulation



Thanks to



Allen W. Cowley, Jr., PhD
Robert Ryan, BS } (MCW)

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Yuri Negulyaev, PhD

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