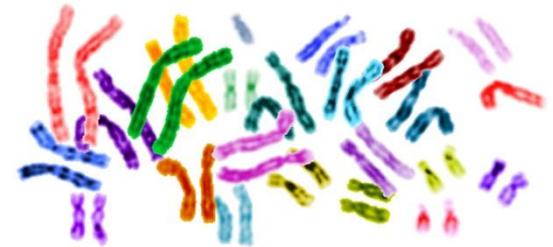
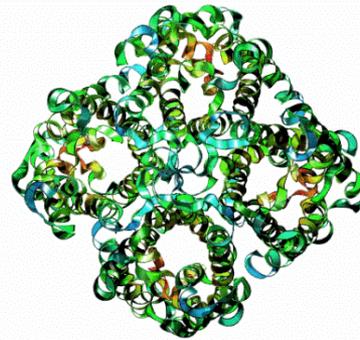
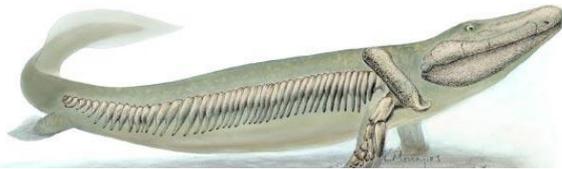


Water Transport: From Terrestrial Adaptation to Clinical Medicine



Prof. Dr. Med O. Devuyst

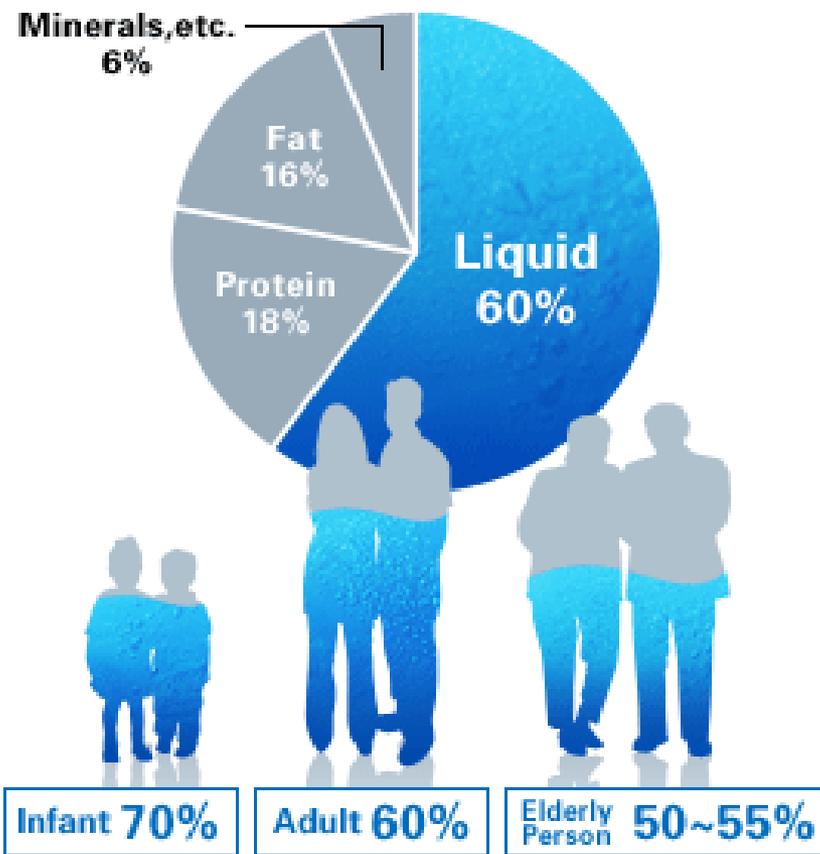
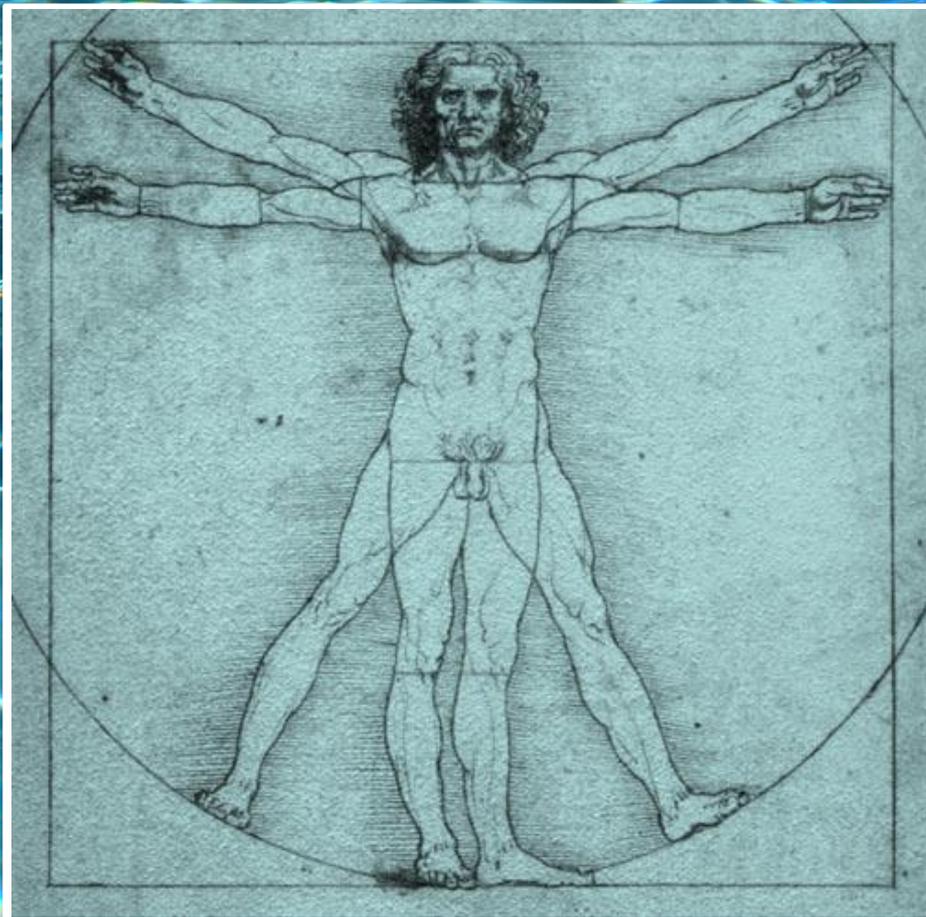
Brussels, May 23, 2014



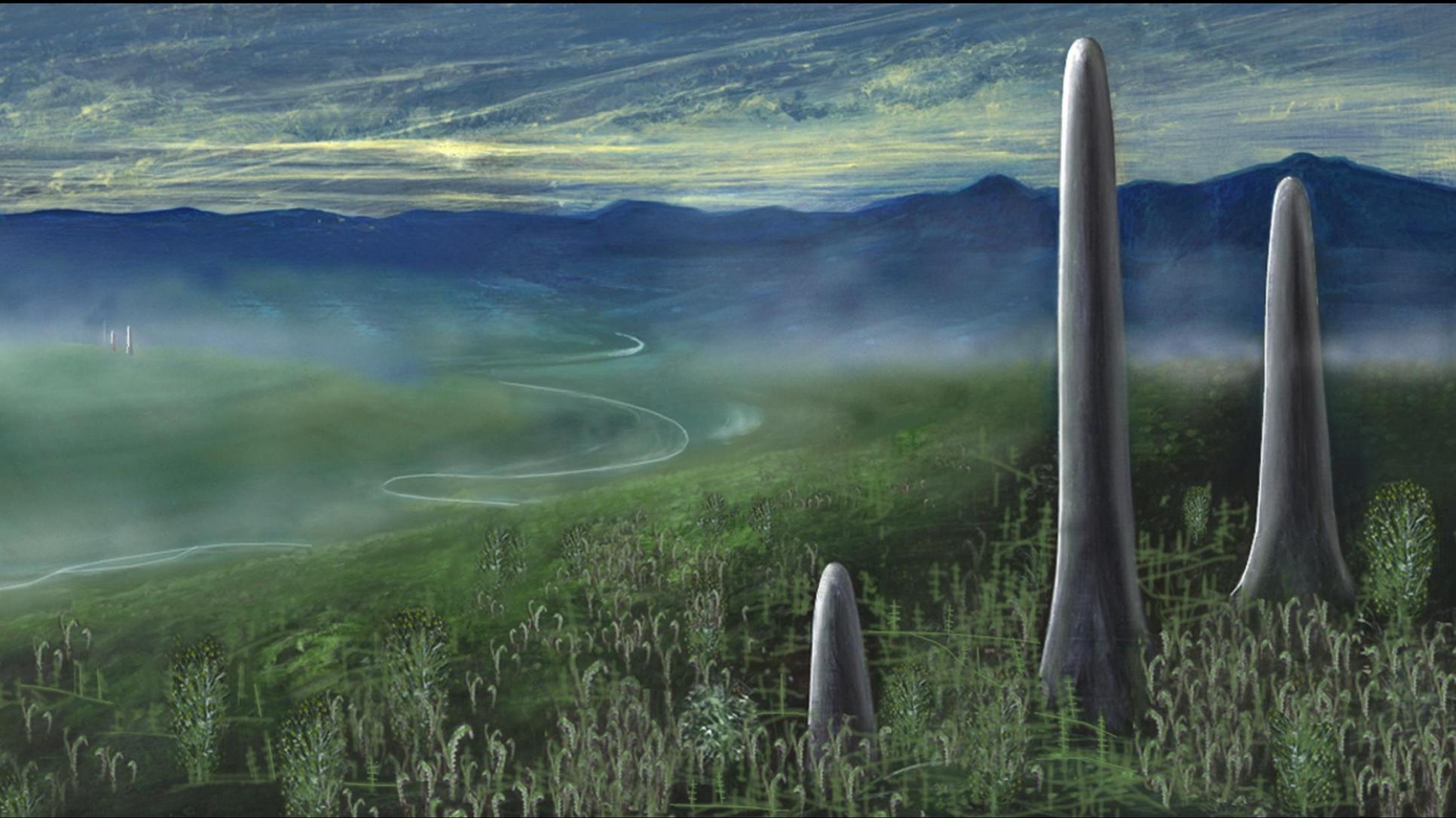
From the waves of
the North Sea

To the impetuous
mountain rivers



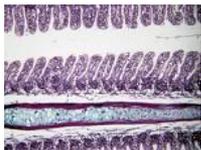


Devonian times, ~ 375 million years ago

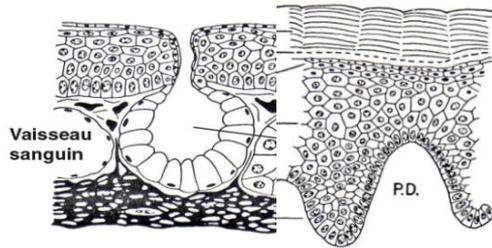




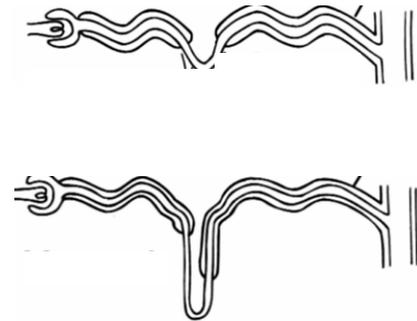
Need to decrease water losses:



Lung



Skin



Kidney

The evolution of man, from fish to college graduate.



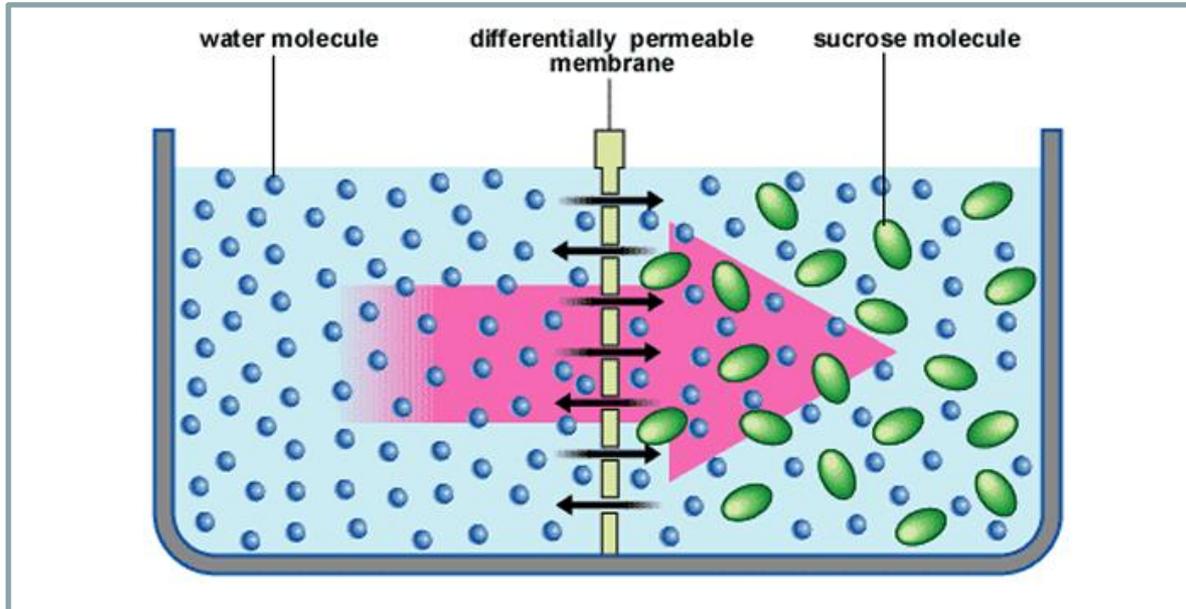
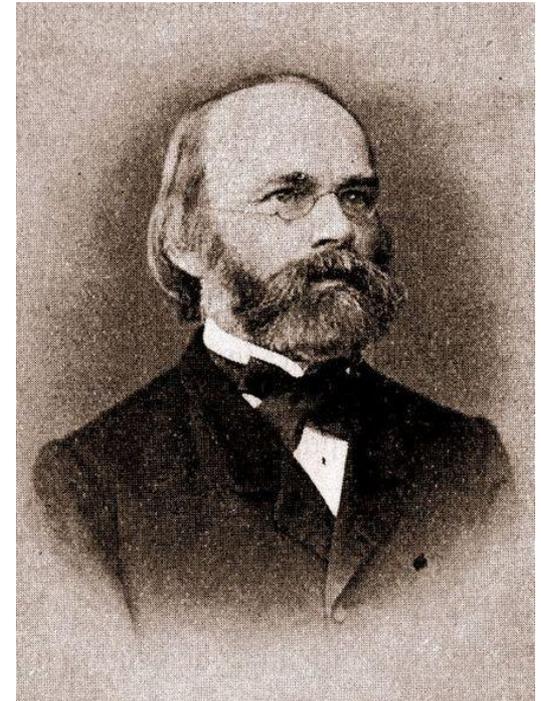
Zurich, Switzerland, around 1850



Karl Wilhelm von Nägeli (1817 – 1891)

- 1840 doctorate from the University of Zurich
- 1855 professor of botany at UZH

Naegeli, Carl Wilhelm von. 1856.
Die Individualität in der Natur mit vorzüglicher
Berücksichtigung des Pflanzenreiches. Zürich



Osmosis

Overton CE (1899)

On the general **osmotic properties of the cell**, their probable origin, and their significance for physiology.

Vierteljahrsschr Naturforsch Ges Zurich 44: 88–135

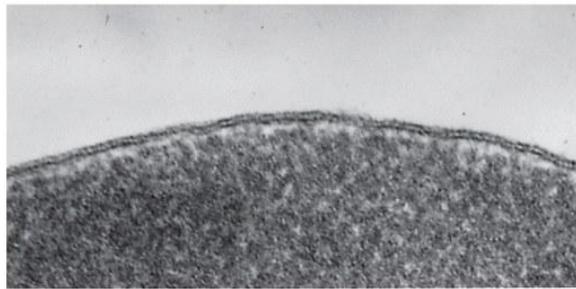


Charles Ernest Overton (1865–1933)

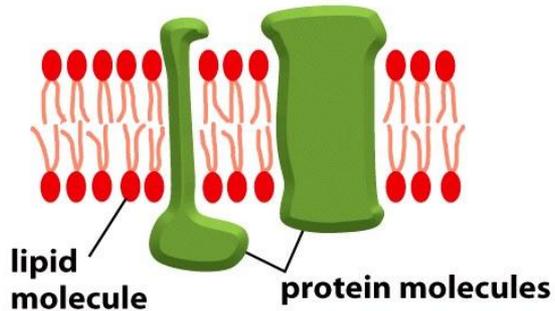
In 1890, Overton wurde er «Privatdocent der Biologie» an der Universität Zürich.

The Overton Rule

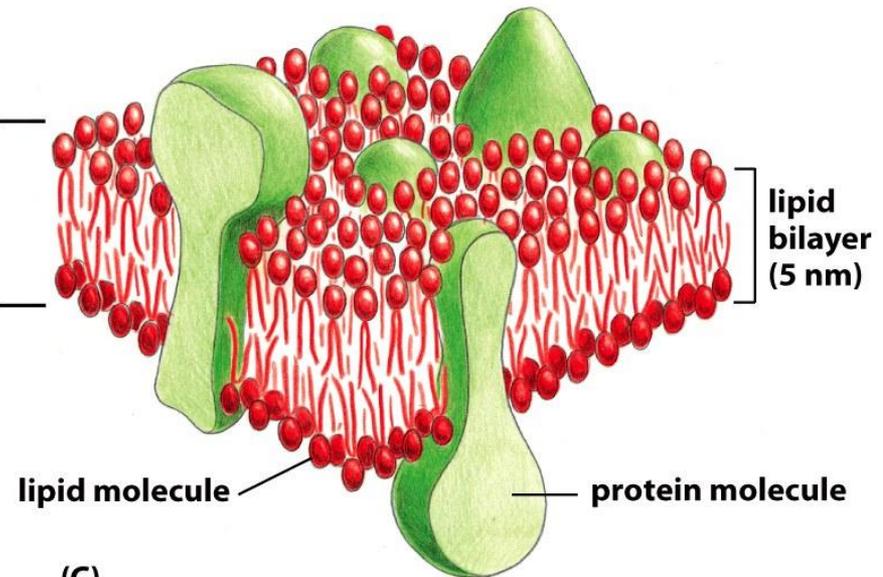
- The *boundary of cells* is mostly constituted of a *lipidic material*
- *Non-lipophilic substances* (incl. water) must use *specific pathways*



(A)

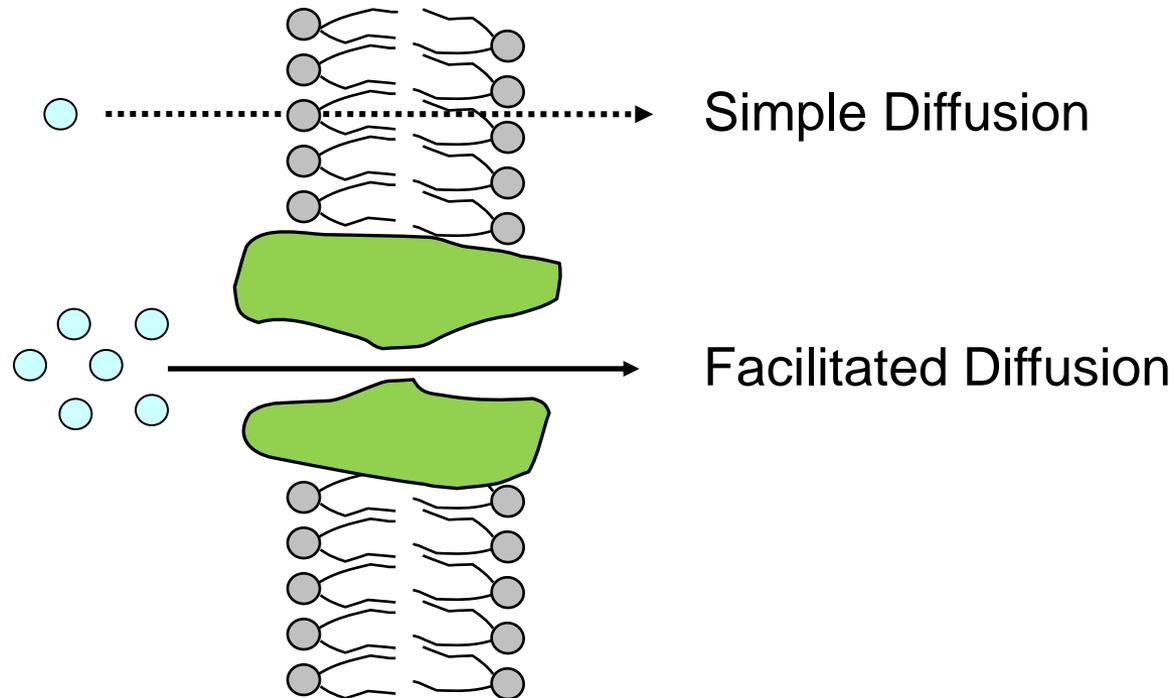


(B)



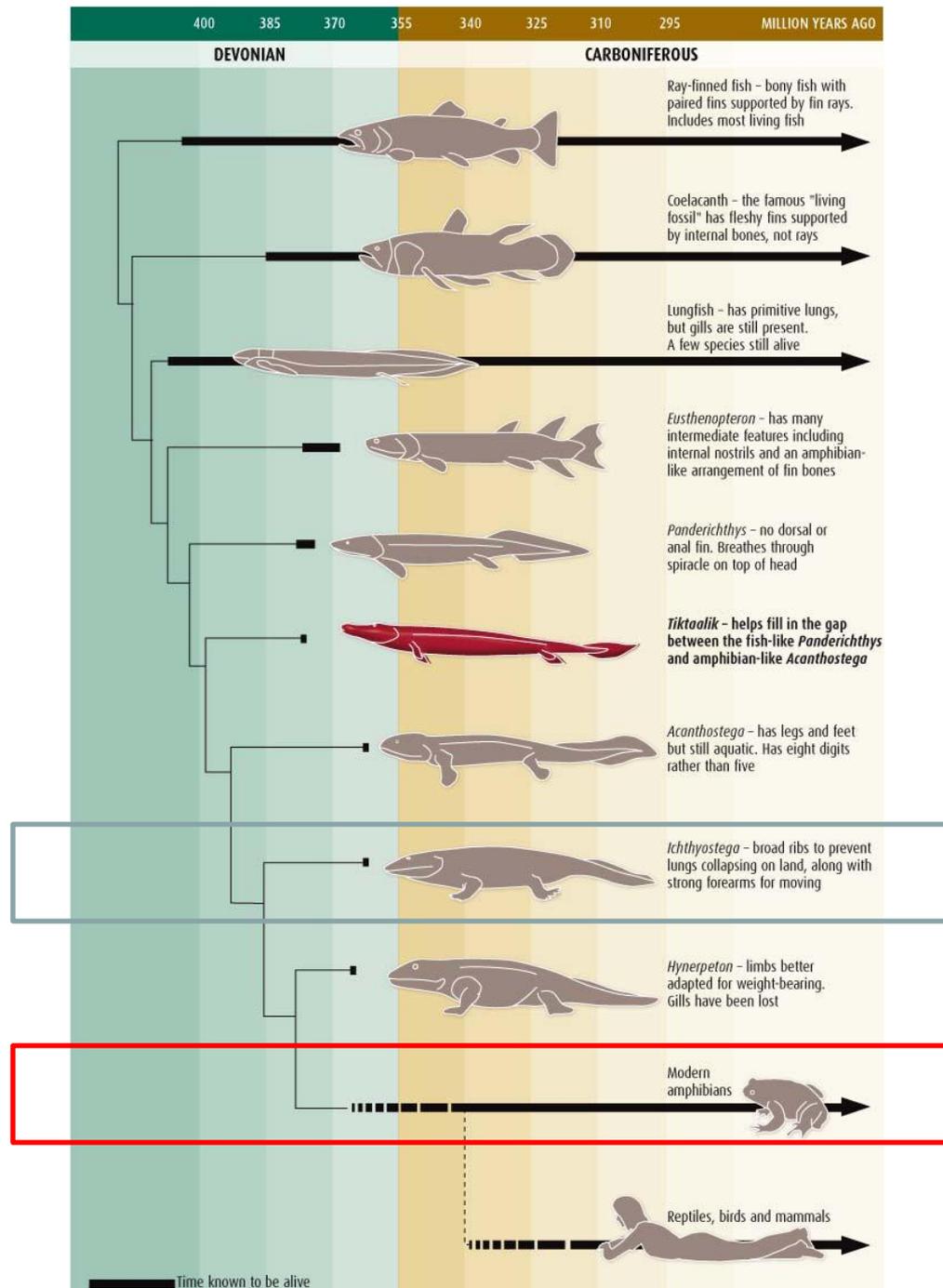
(C)

Debate: Water Permeability of Cell Membranes ?

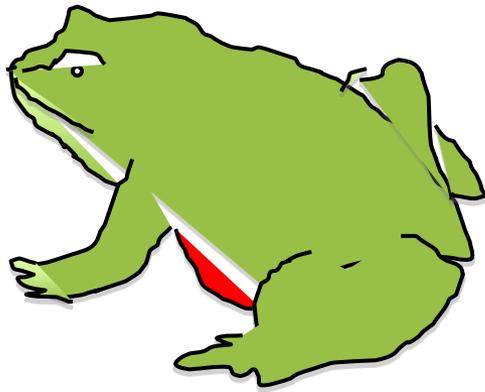


Osmotic
Gradient

Amphibians evolved from fishes in just a few millions years

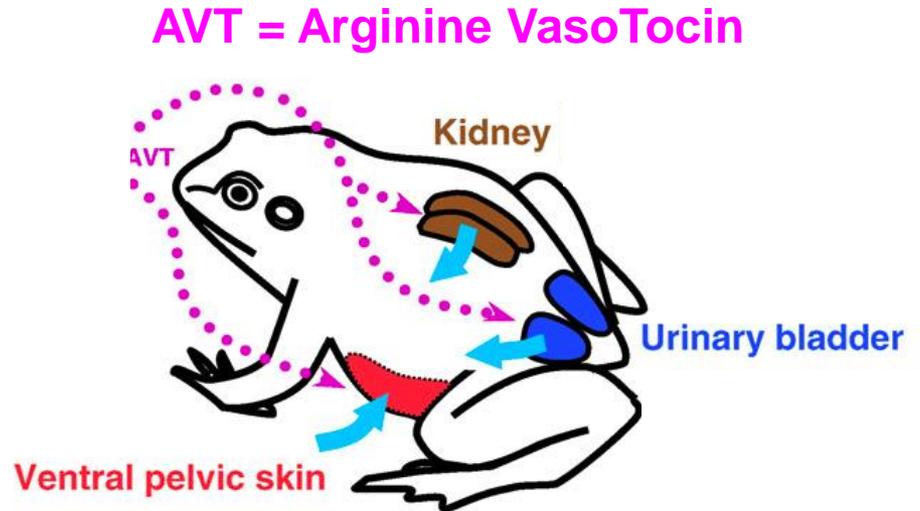


Adaptation to Terrestrial Life : Amphibians



Water losses +++

- Glandular skin
- Diluted urine

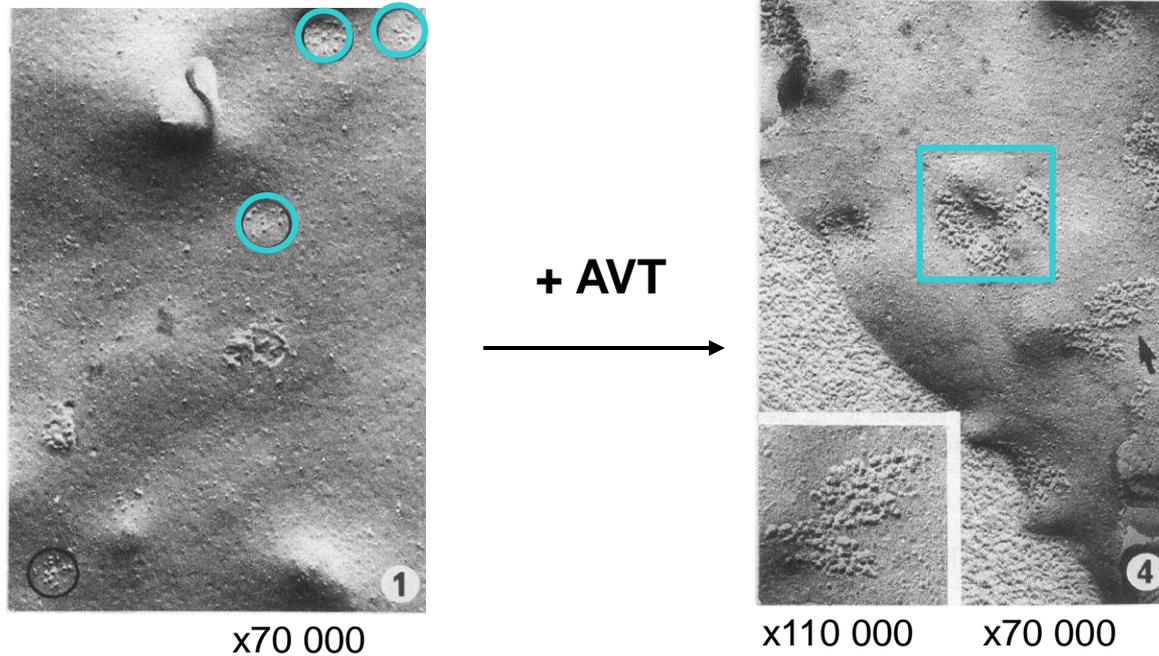


Transport systems

- Abdominal skin
- Urinary bladder

First Visualisation of Water Transport: Frog Bladder

Rana esculenta



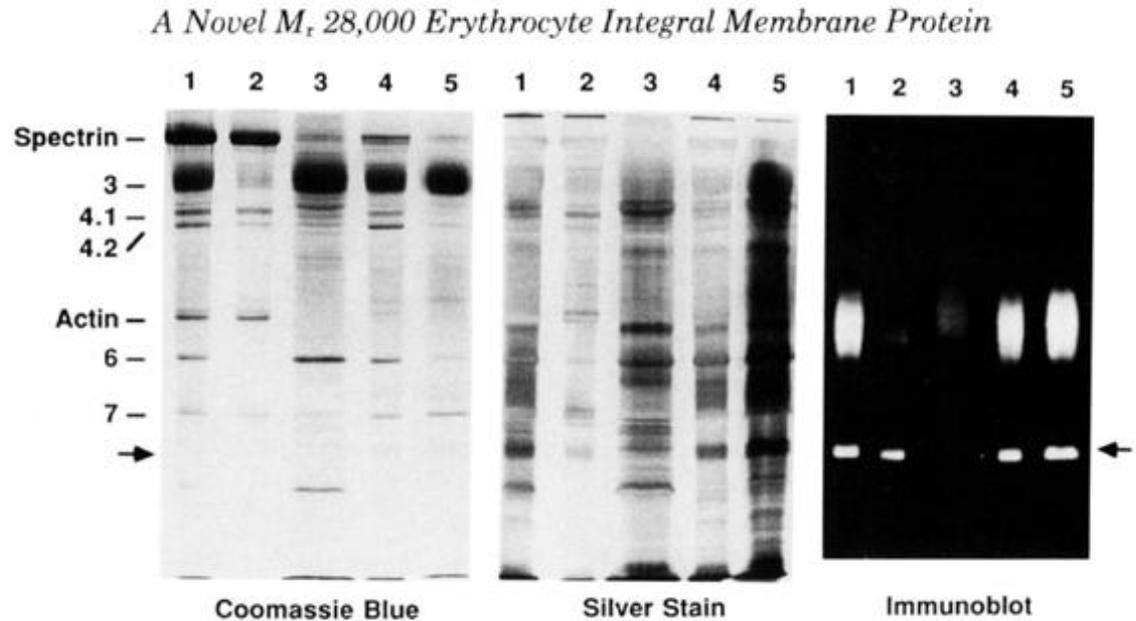
→ *Facilitated water diffusion across membranes*

The race to discover the Water channel gene



Identification, Purification, and Partial Characterization of a Novel Mr 28,00 Integral Membrane Protein from Erythrocytes and renal Tubules

Denker BM, Smith BL, Kuhajda FP, Agre P.



CHIP28 is not the Rhesus antigen – then what ?

CHIP 28 : Channel-like Integral Membrane Protein



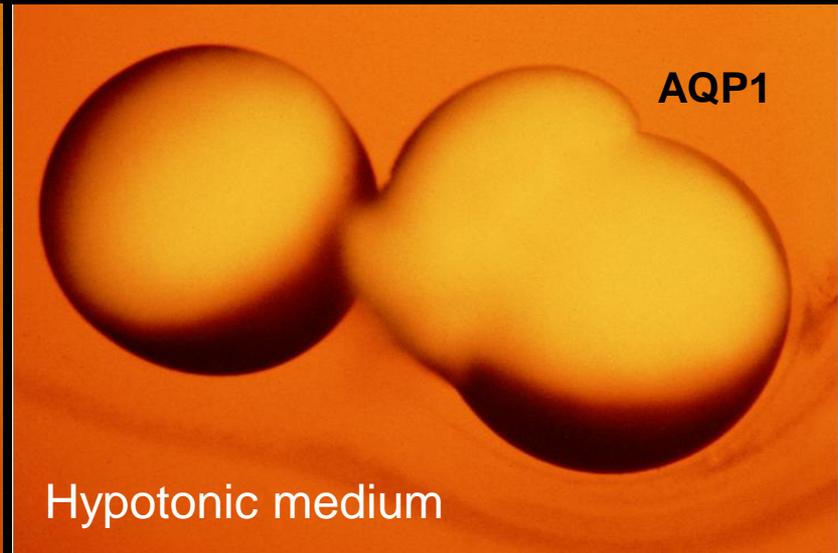
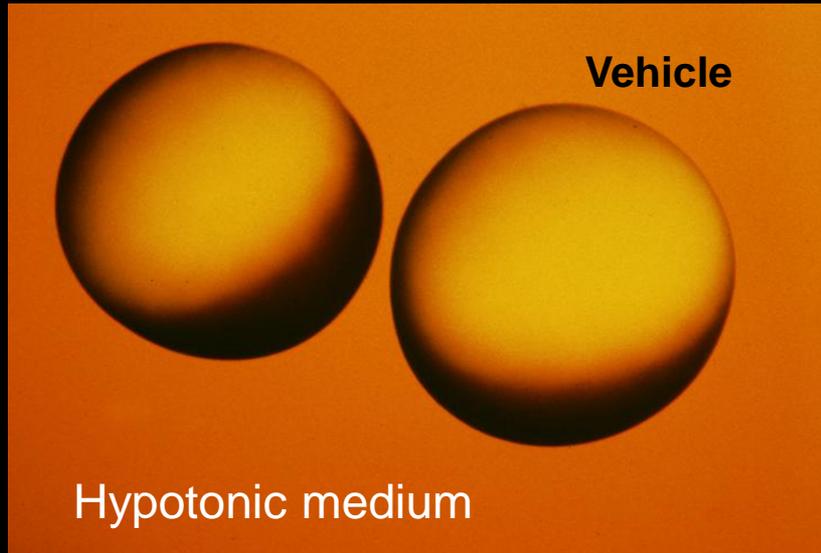
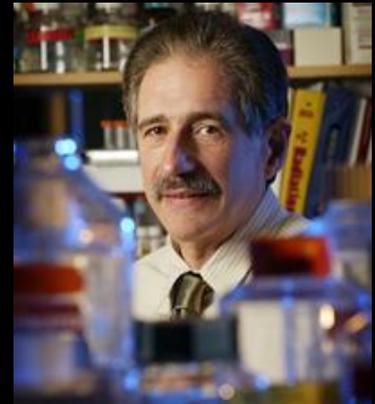
Is CHIP28 the long sought-after water channel ?

- channel-like integral membrane protein
- highly homologous with channels (MIP cornea)
- Mw 28 kDa
- 150,000 copies/RBC

→ Xenopus swelling assay: W.B. Guggino

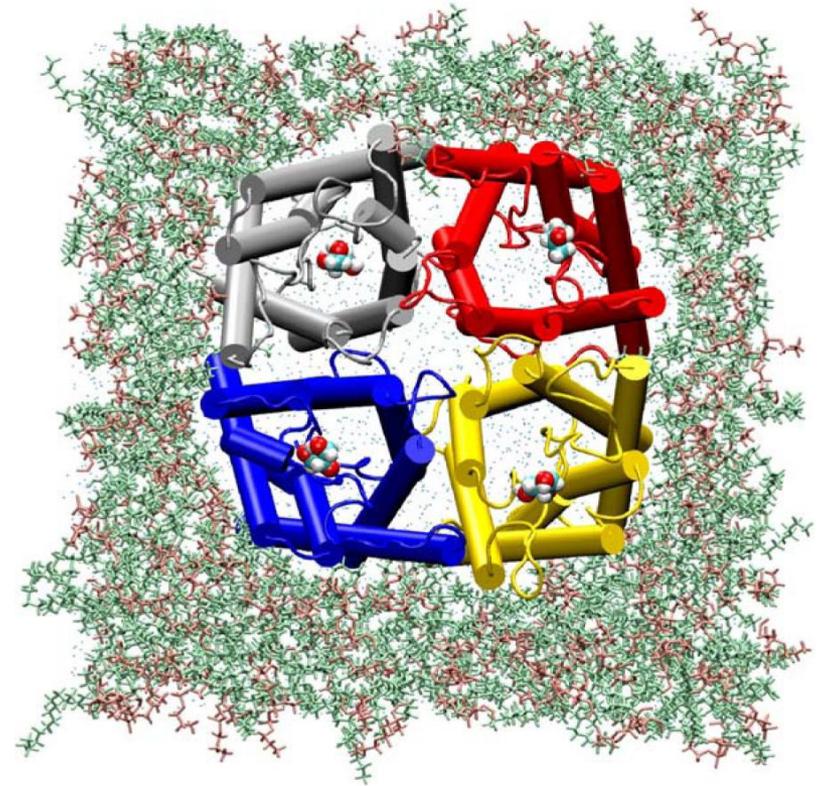
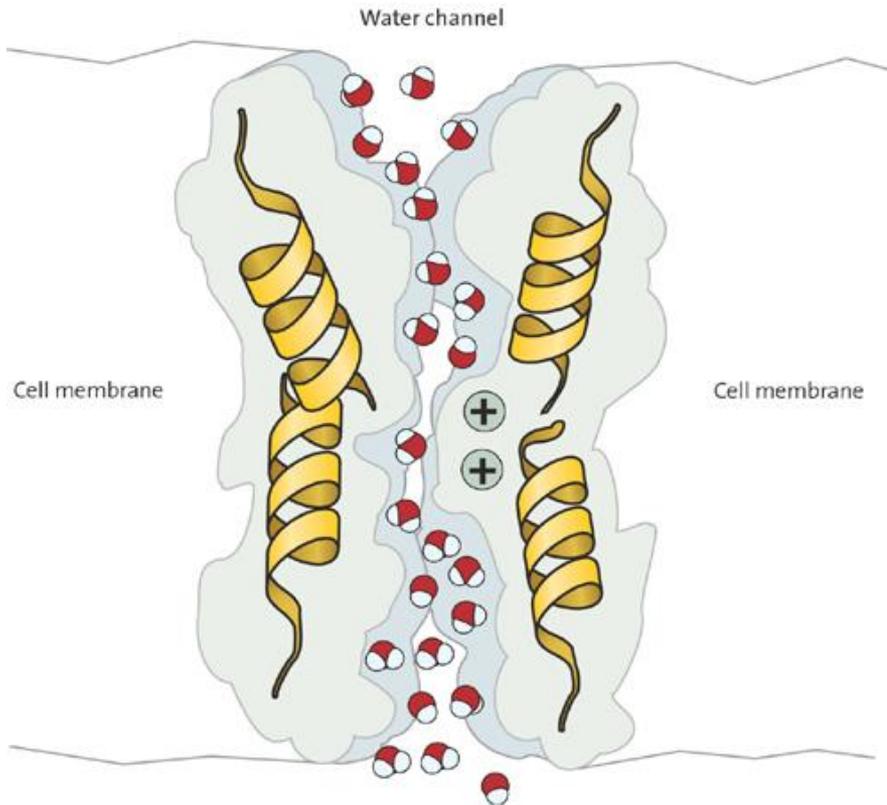
Appearance of Water Channels in *Xenopus* Oocytes Expressing Red Cell CHIP28 Protein

Gregory M. Preston, Tiziana Piazza Carroll,
William B. Guggino, Peter Agre*



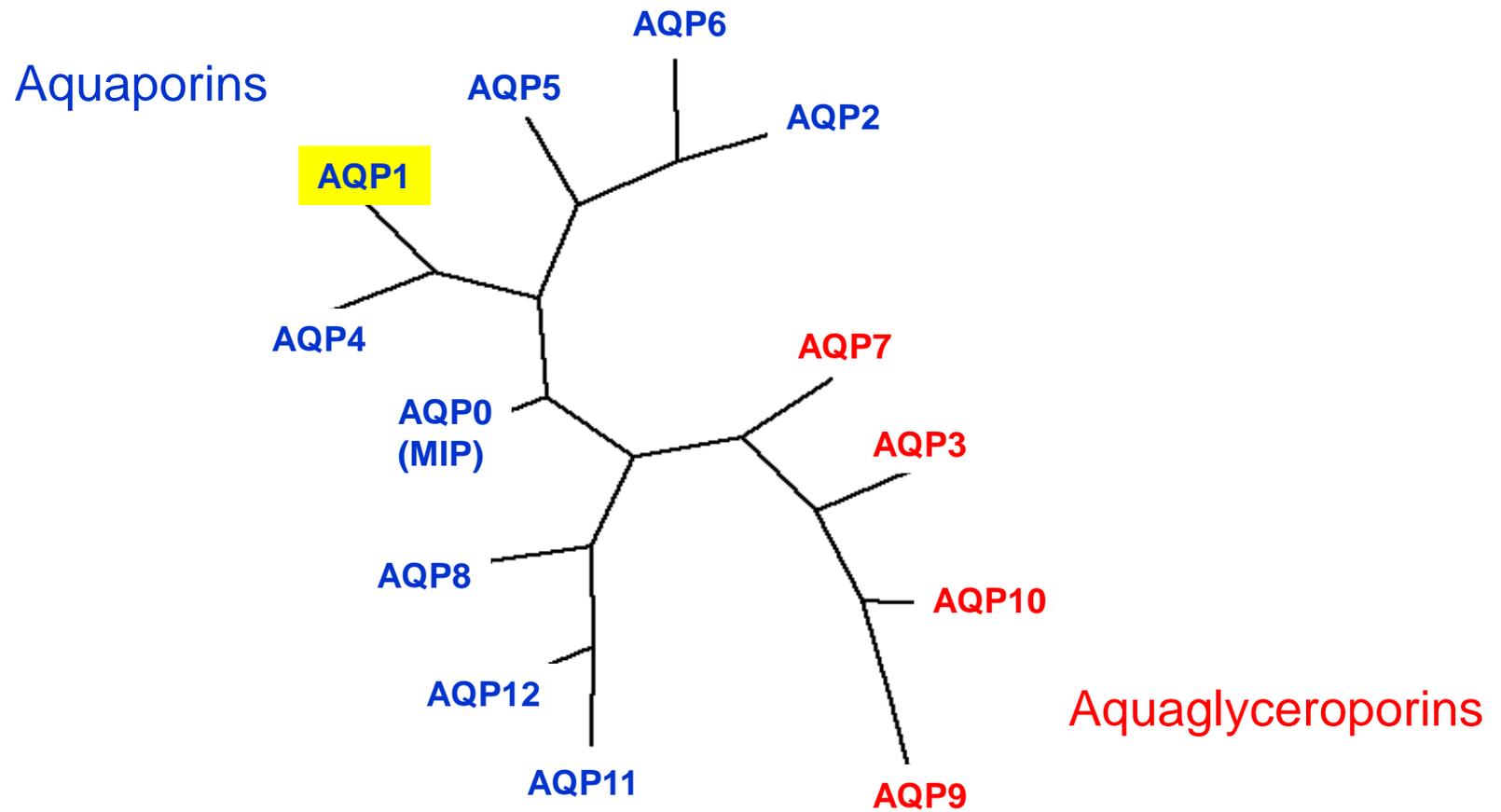
Cell swelling → facilitated water transport

Structure of Aquaporins



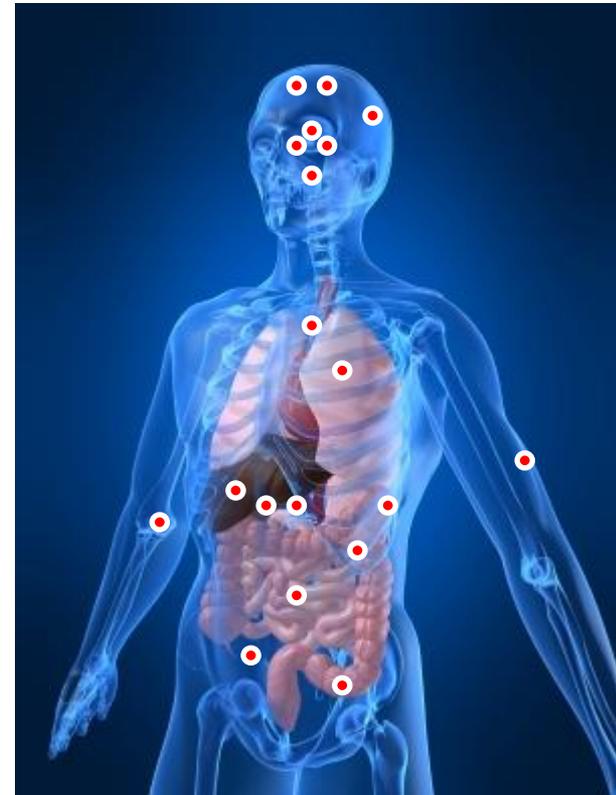
AQP1 tetramers: 3 billions of water molecules per second

The Family of Human Aquaporins

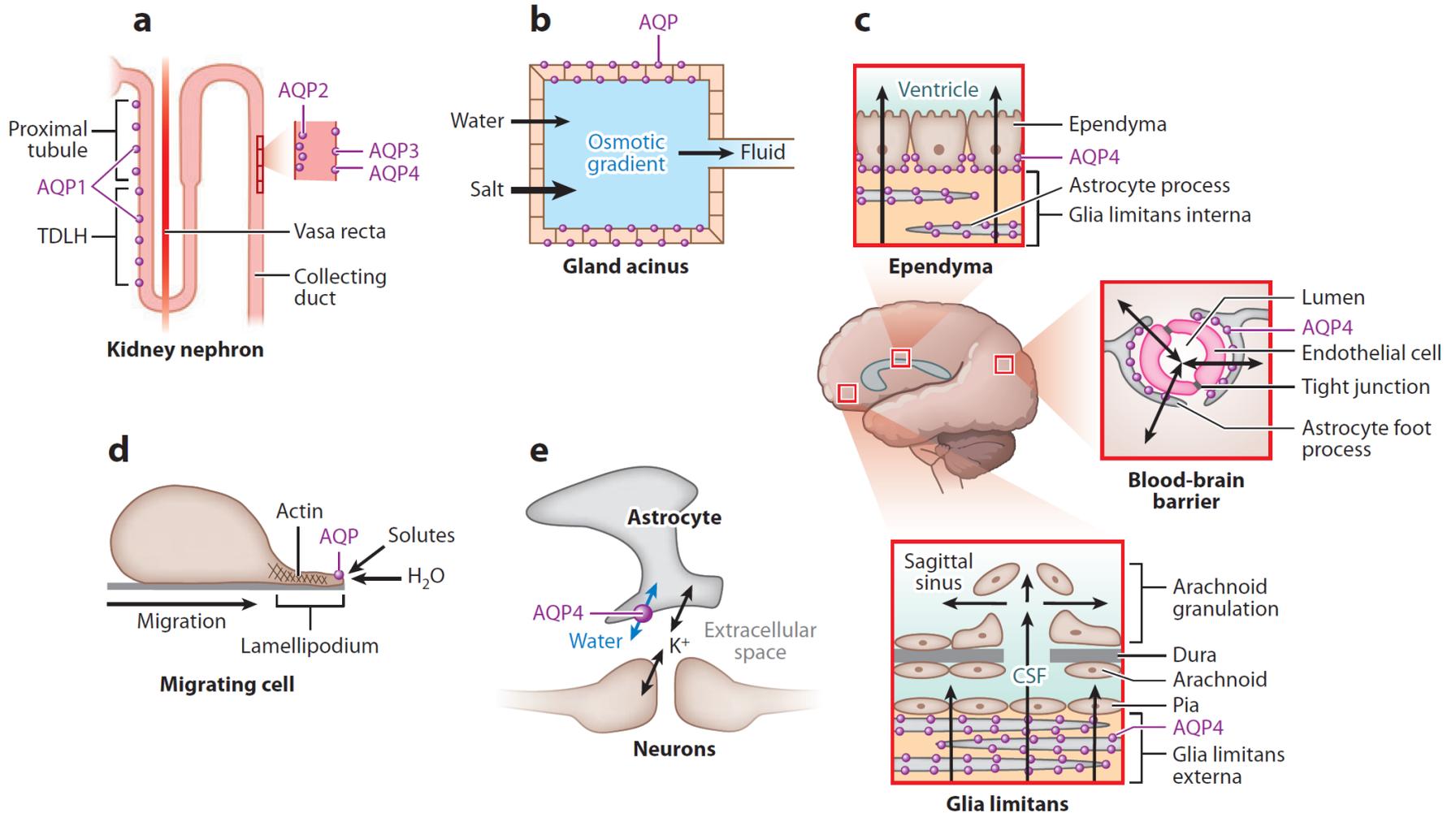


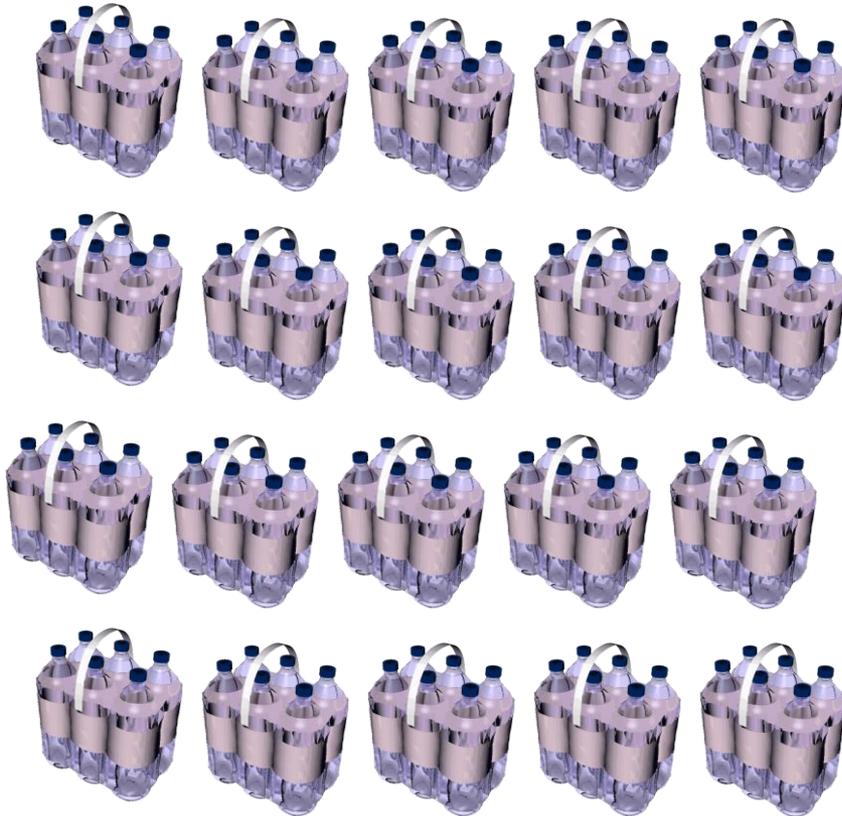
Distribution of Human Aquaporins

Aquaporin (AQP)	Tissue or Cell Type
AQP0	Eye (Lens)
AQP1	Choroid Plexus, Erythrocytes, Eye (Cornea), Gallbladder, Kidney, Lung, Liver, Pancreas
AQP2	Kidney
AQP3	Erythrocytes, Eye (Conjunctiva), Kidney, Skin, Upper Respiratory Tract
AQP4	Brain (Glial Cells), Eye (Retina), Kidney, Muscle
AQP5	Lacrimal Gland, Lung, Salivary Gland, Skin (Sweat Glands)
AQP6	Kidney
AQP7	Adipocytes, Kidney, Testis
AQP8	Colon, Kidney, Liver, Pancreas
AQP9	Brain, Leukocytes, Liver, Spleen
AQP10	Small Intestine

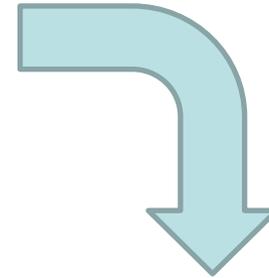


Aquaporins in Clinical Medicine





Filtration ~ 180L/day

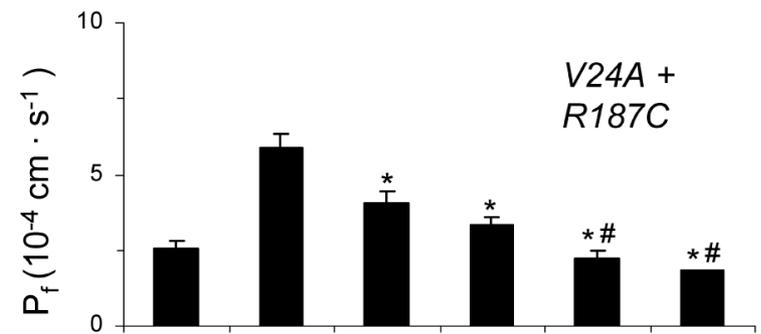
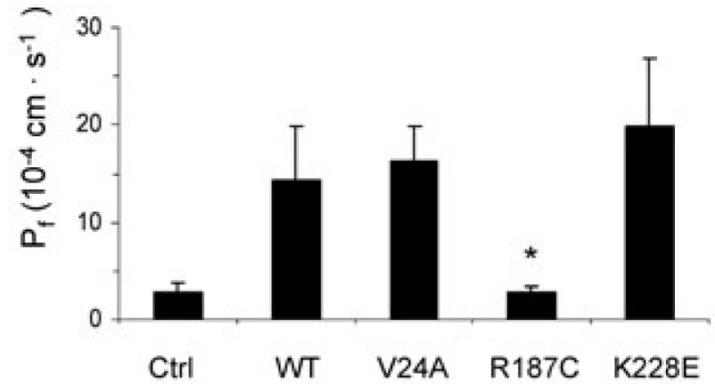
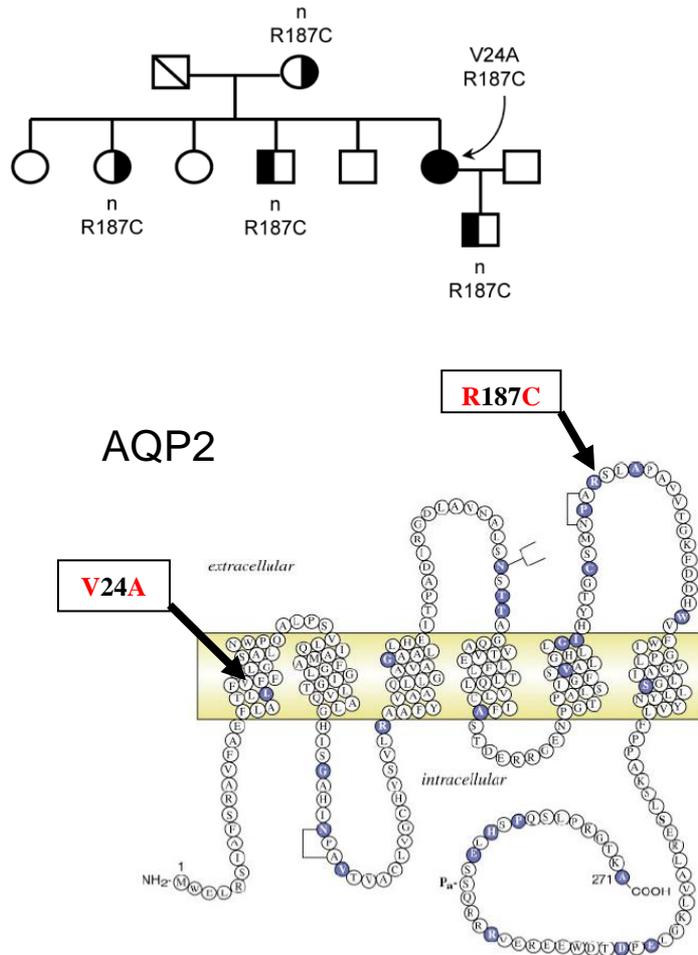


Urine ~ 1L/day

The kidneys reabsorb water massively

Sylvie, 39 year-old : Needs to drink 15-20 liters a day

- Urinates: **15** L/day
- Difficult scolarity, multiple hospitalizations (dehydration)



mutant	0	5	5	5	5	5
R187C	0	0	2.5	5	7.5	10



The Nobel Prize in Chemistry 2003

« for discoveries concerning channels in cell membranes »



Peter Agre & Roderick MacKinnon

“The Eucerin **Aquaporin-Active** provides a **maximum hydration** to the skin thanks to the **discovery of biologist Peter Agre...**”

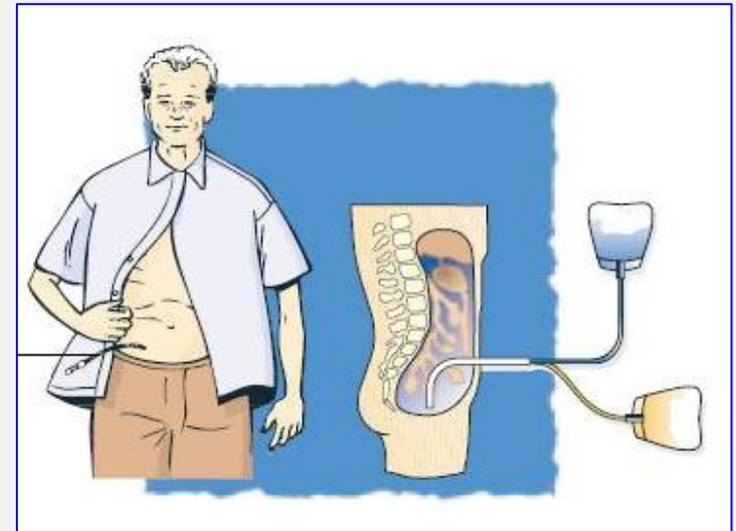


Aquaporins:

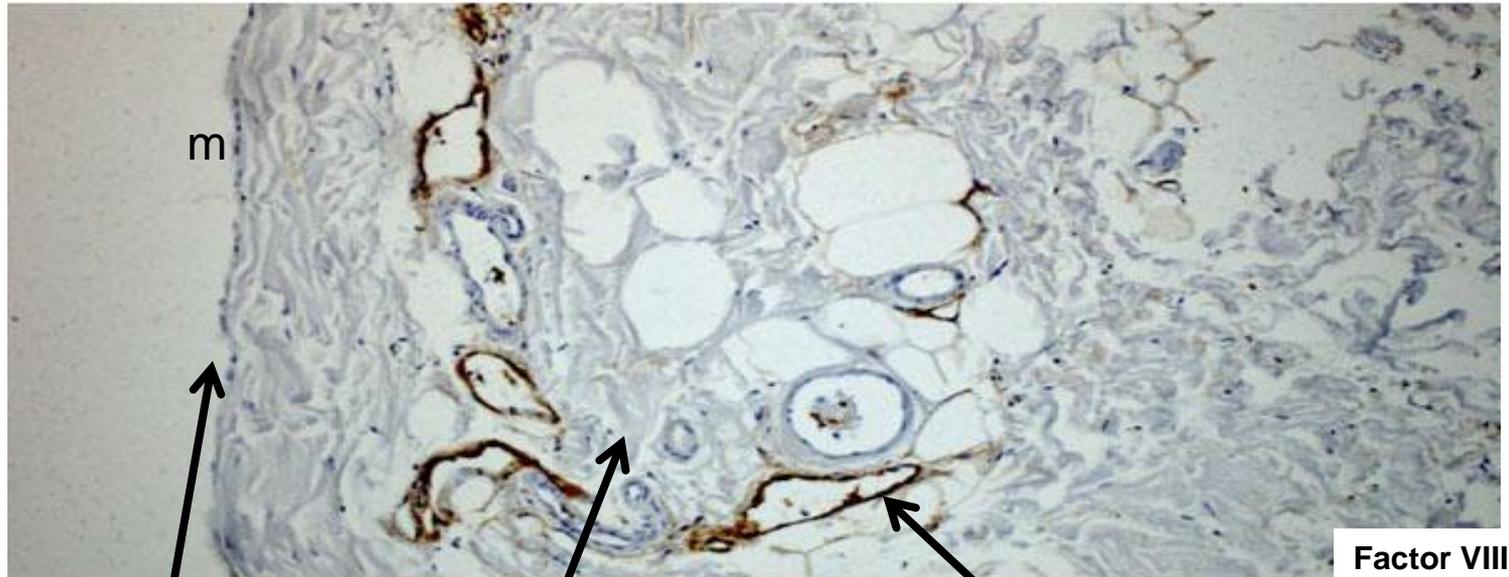
Plumbing system for peritoneal dialysis

Peritoneal Dialysis as Renal Replacement Therapy

- Peritoneal dialysis: a growing mode of RRT
- >200.000 patients treated worldwide
11% of all dialysis patients
- Advantages of PD:
 - *Relatively easy*
 - *Home-based therapy*
 - *Cheaper than in-center hemodialysis*
- Continuous dialysis
across natural membrane



The Peritoneal Membrane



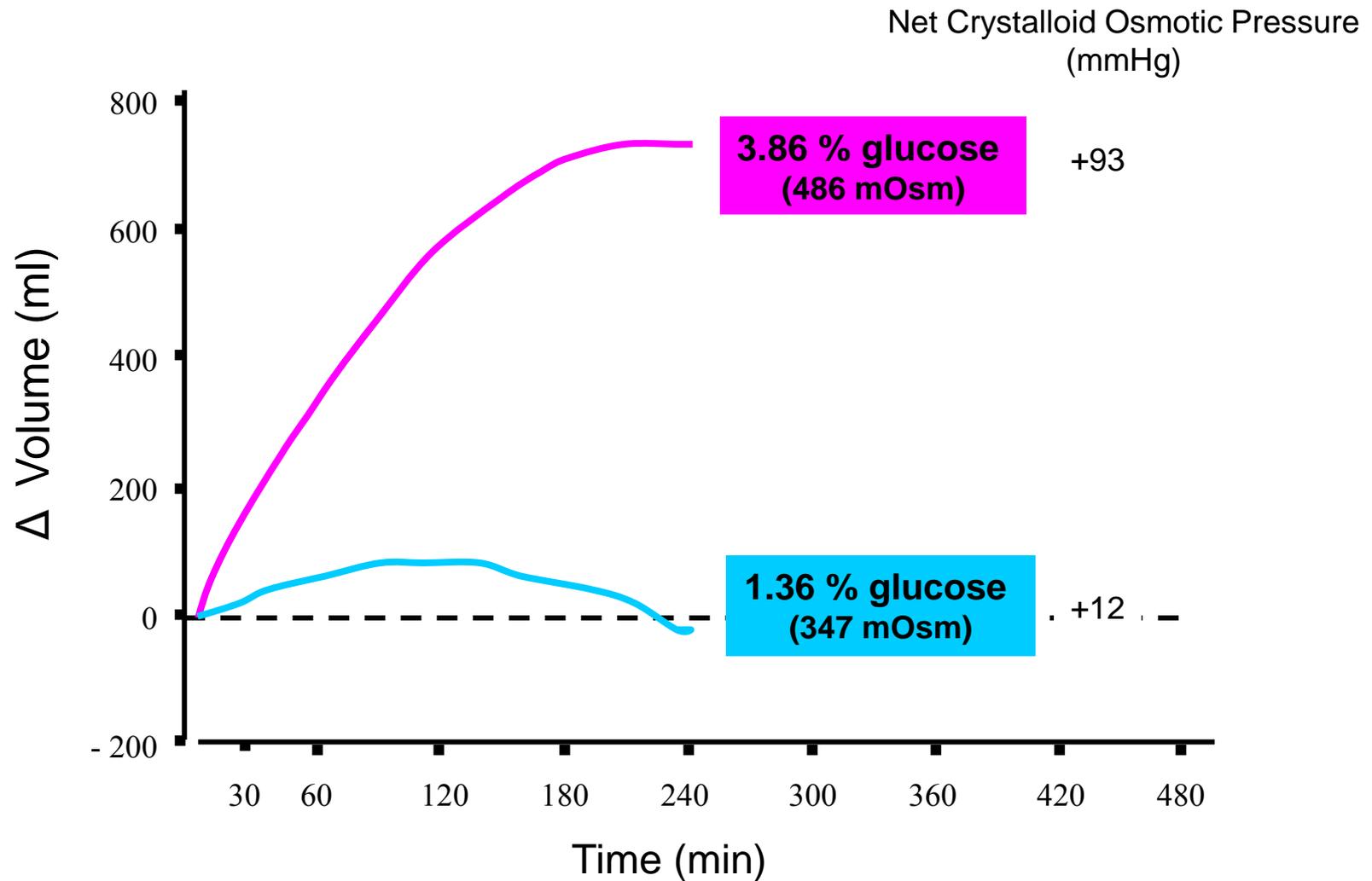
Mesothelium

Interstitium

Capillary endothelium

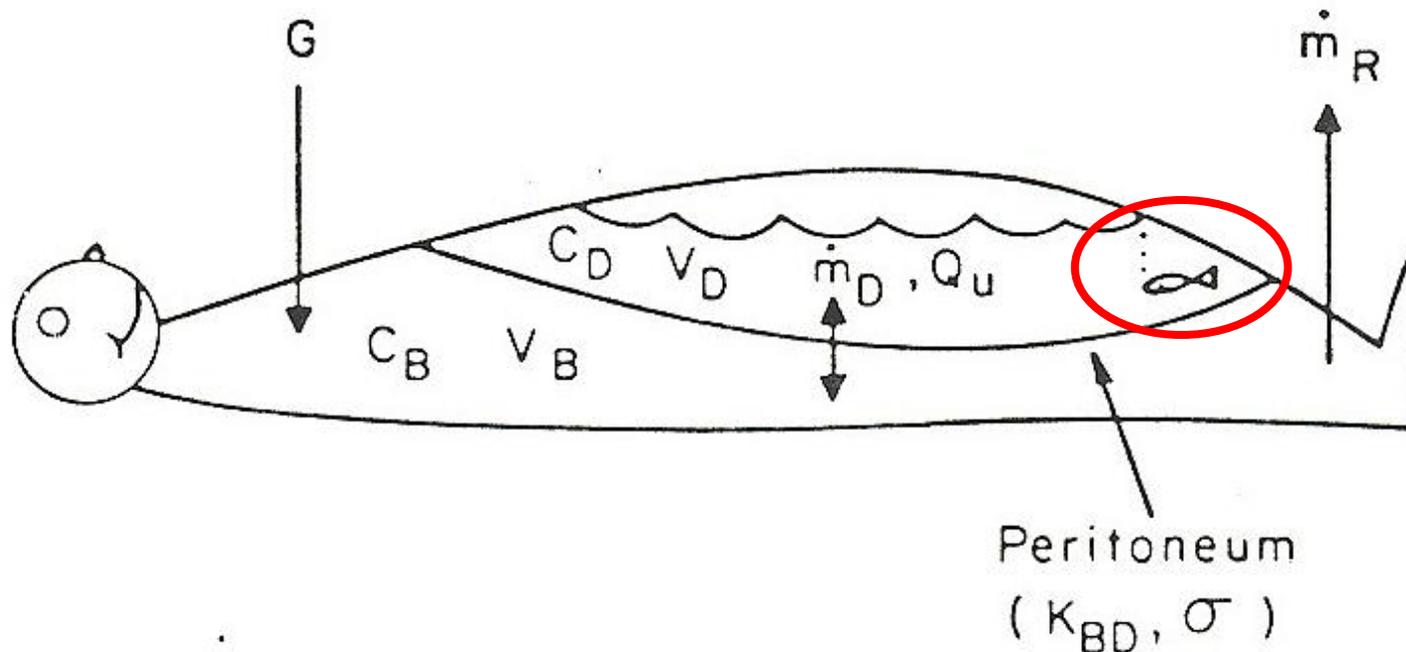
Main functional barrier to solute & fluid transport

Water Removal : Glucose as an Osmotic Agent



Peritoneal Transport: Models from the 1980's

Advanced mathematical modeling using **black-box-model**

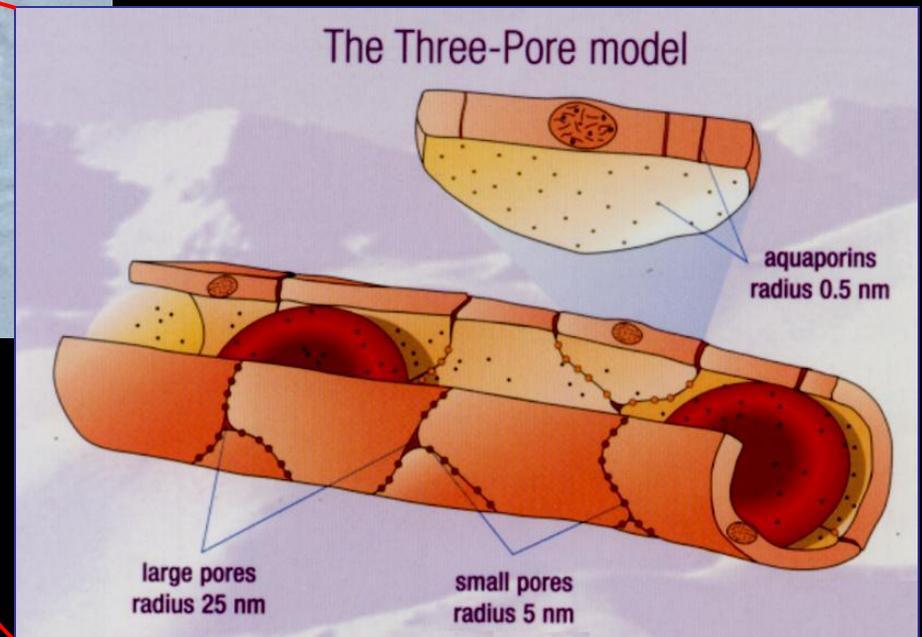


Transport Kinetics, In: Peritoneal Dialysis, 3rd Edition, Kluwer Academic Publ, Eds. Popovich, Moncrief and Pyle, Chap. 6, pp. 96-116, 1989

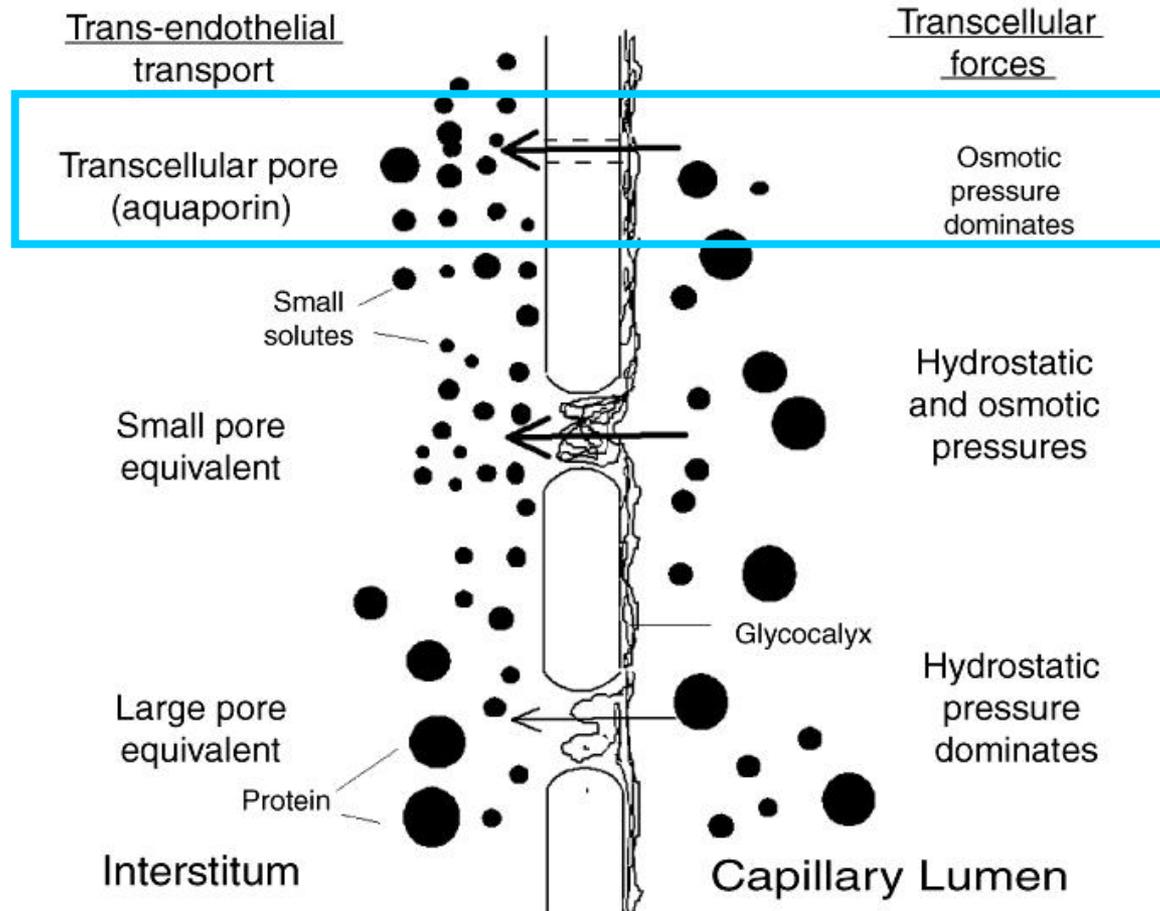
The Endothelium as a Functional Barrier in PD



Three-Pore model
B. Rippe

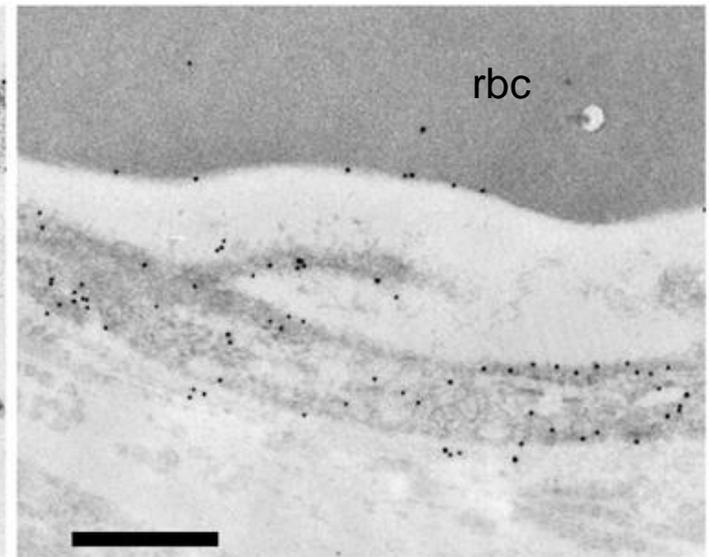
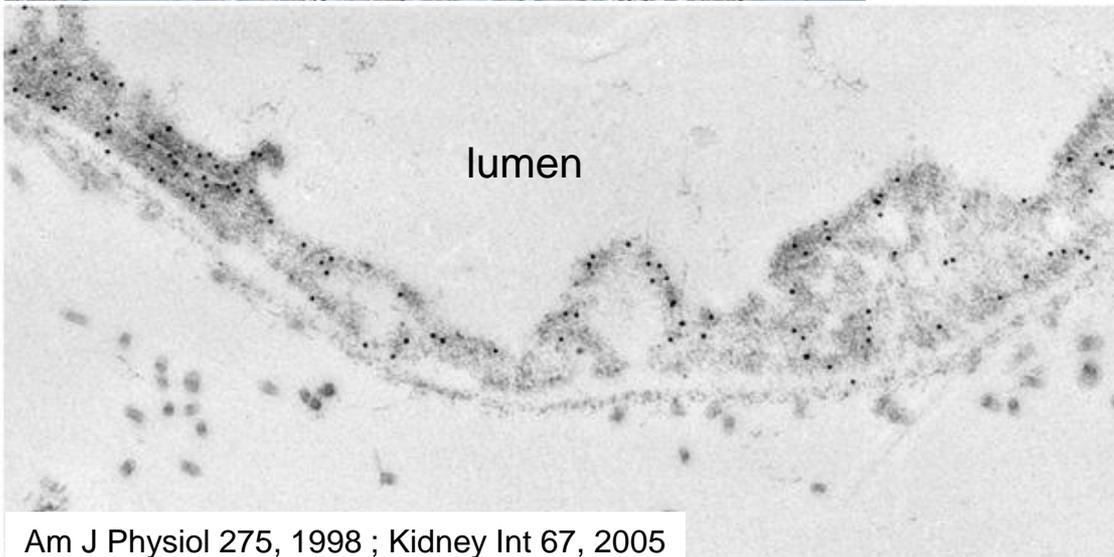
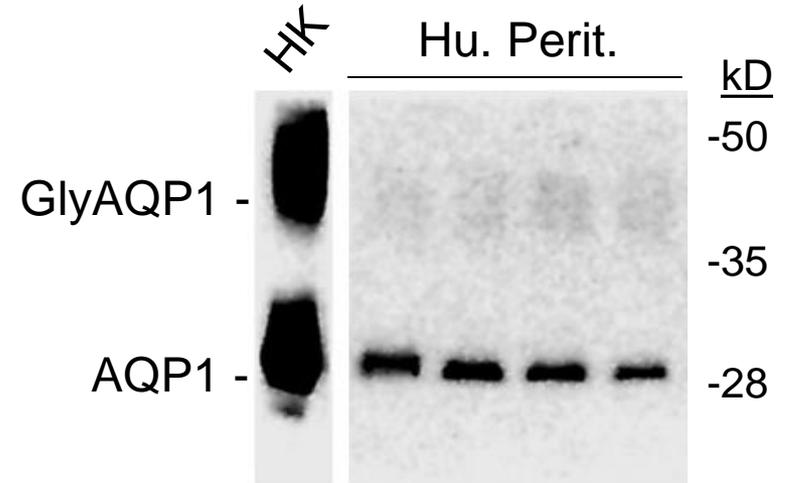


Ultra-small Pores across the Endothelium

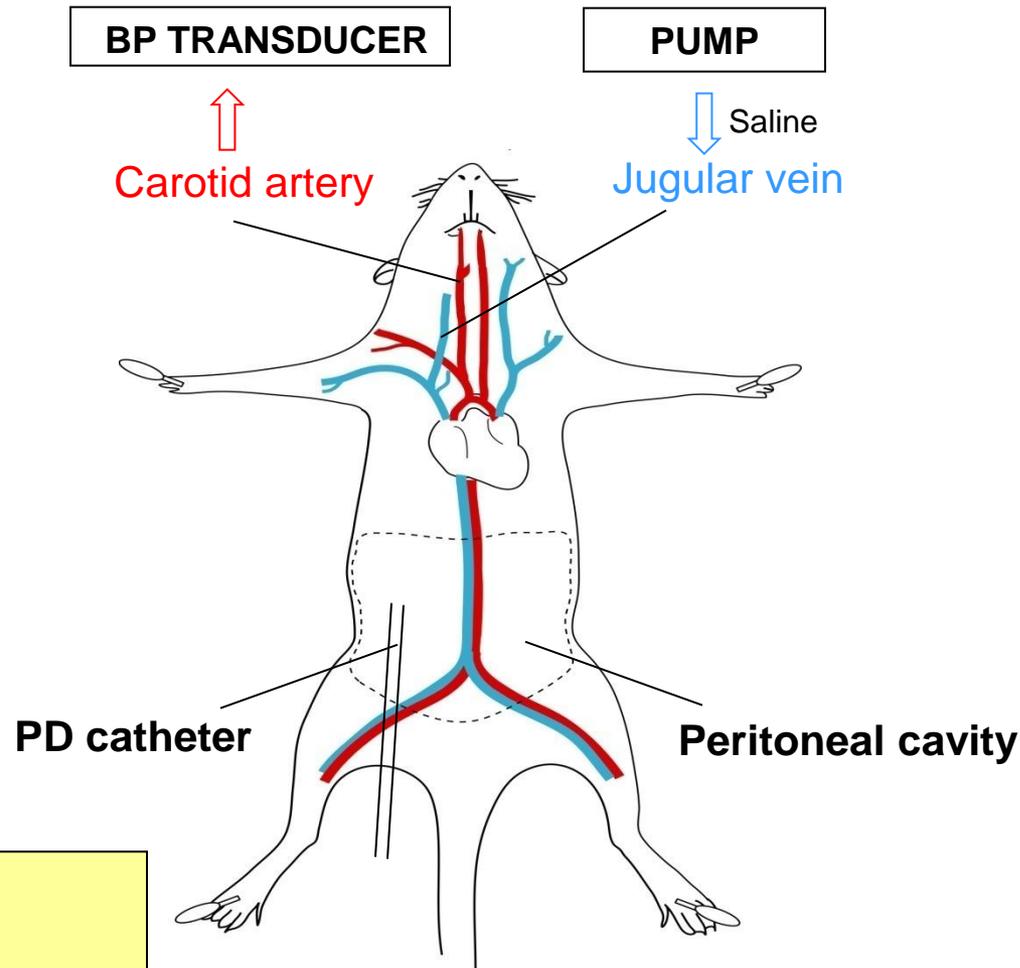


Ultra-small pores : predict to account for 50% of water removal (UF)

Distribution of Aquaporin-1 in the Endothelium Lining Peritoneal Capillaries

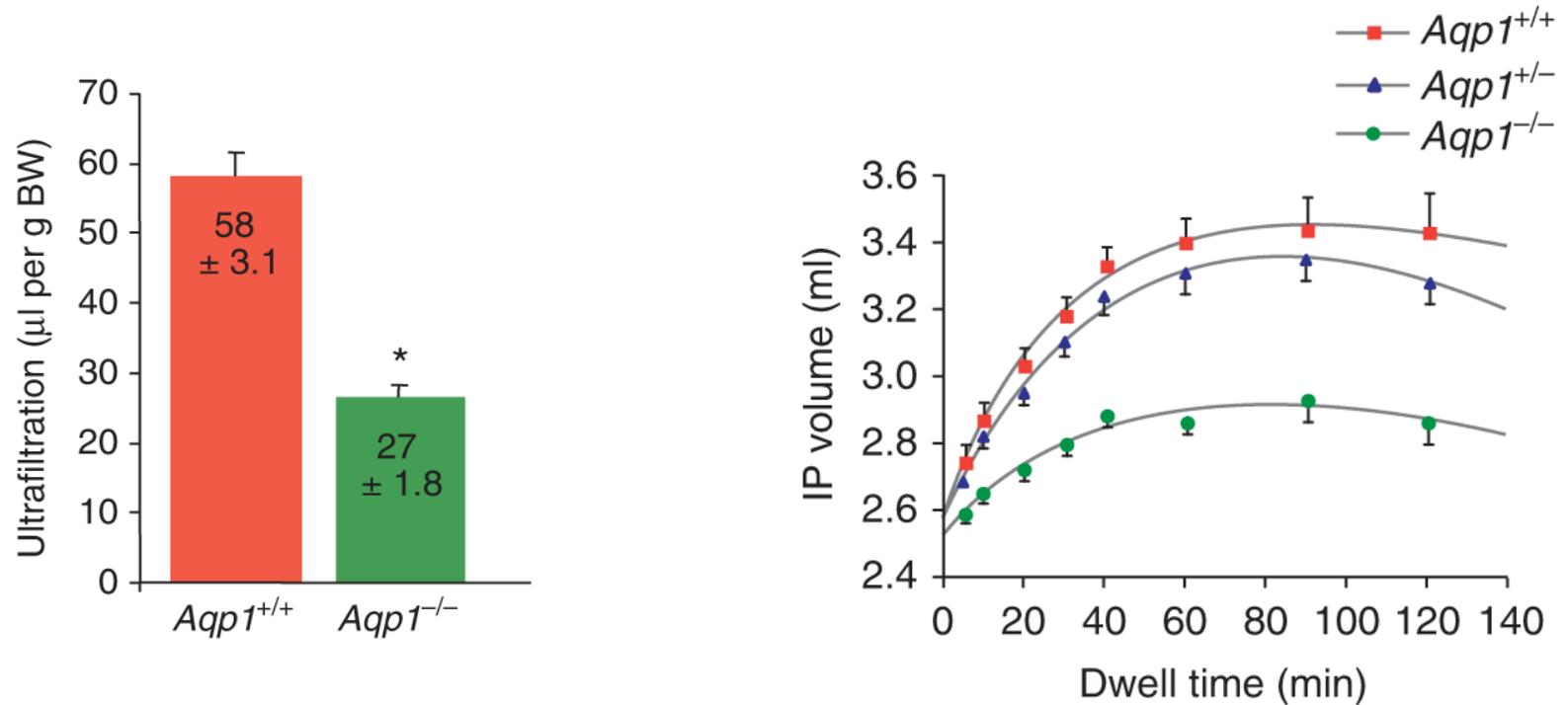


Peritoneal Dialysis in Mouse

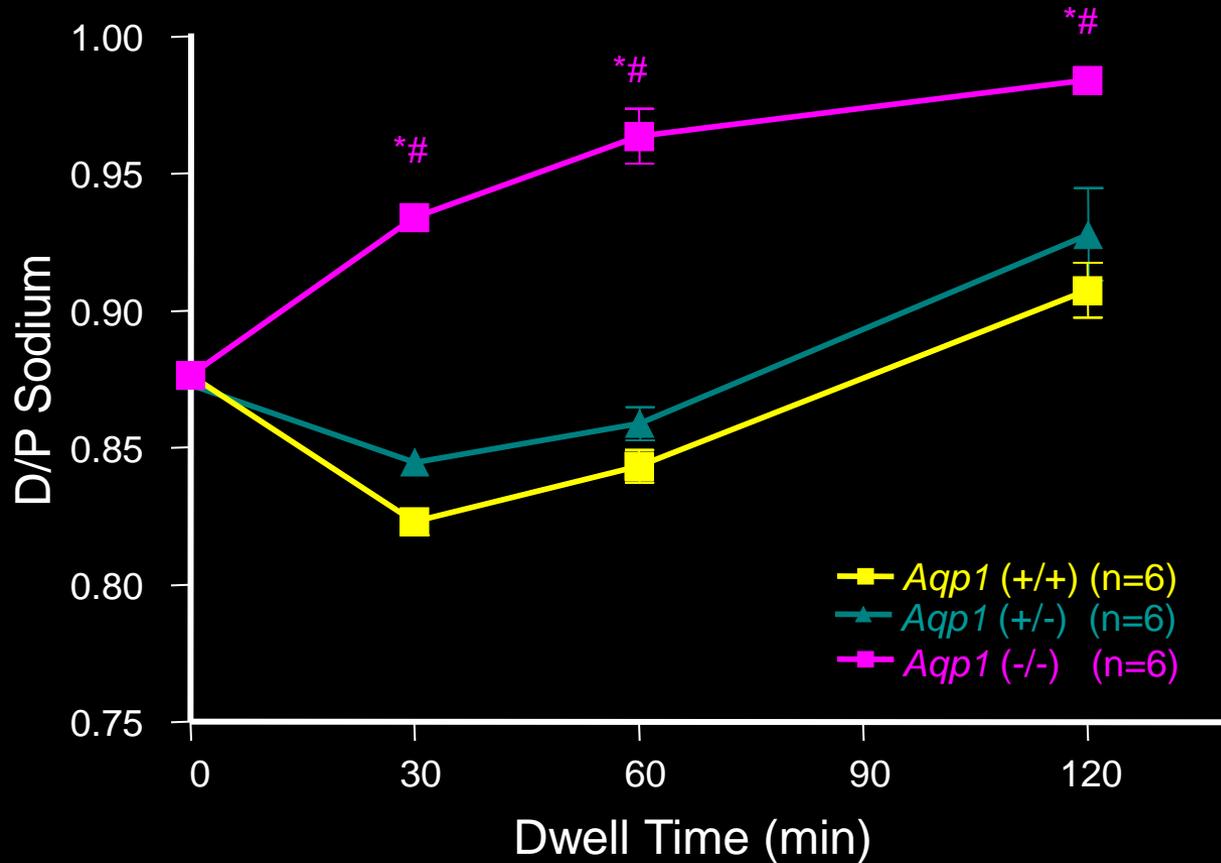


2-hour PET
2.5 ml dialysate
Sampling T0, T30, T60, T120
Synchron CX5 Beckman

Peritoneal Dilaysis in *Aqp1* Mice



Sodium Sieving in *Aqp1* KO Mice



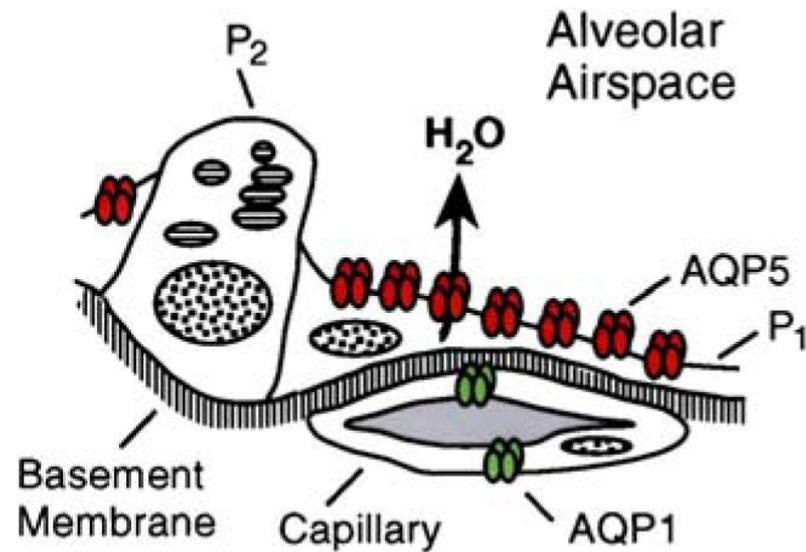
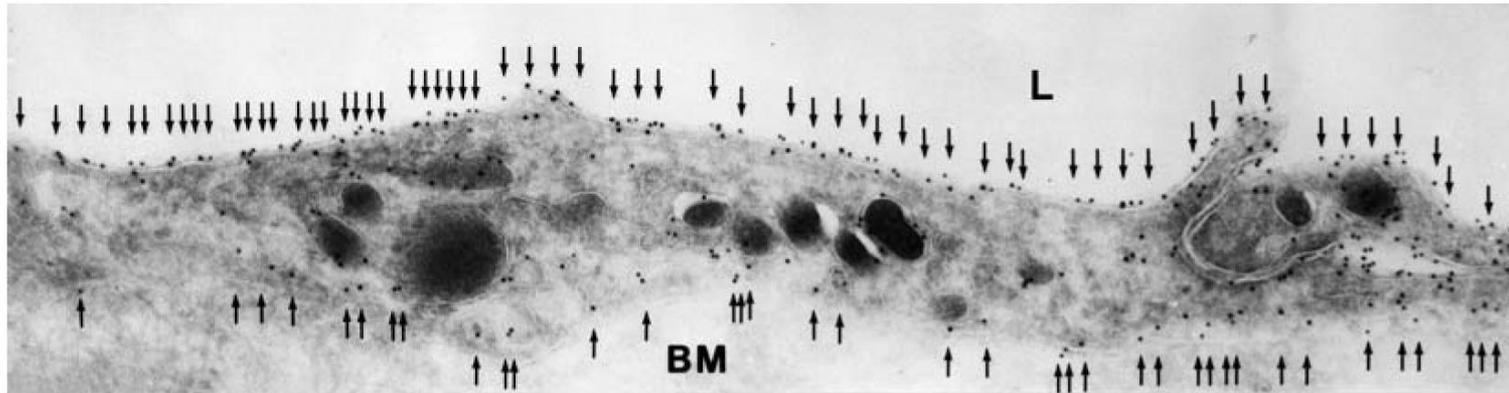
PET: 2ml, 7% glucose dialysate, 2 hours

Inducing aquaporins to improve dialysis ?

Dexamethasone accelerates fetal lung maturation



AQP1 in Capillary Endothelium : Lung

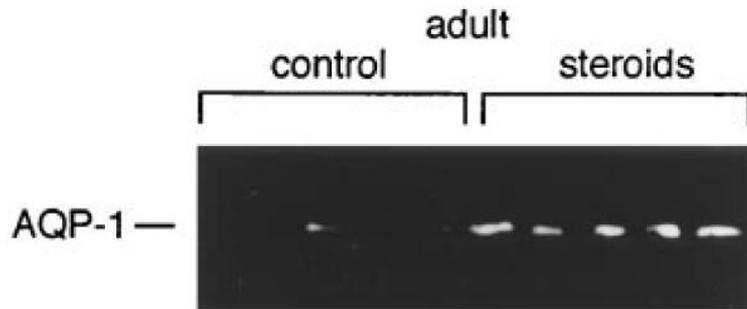
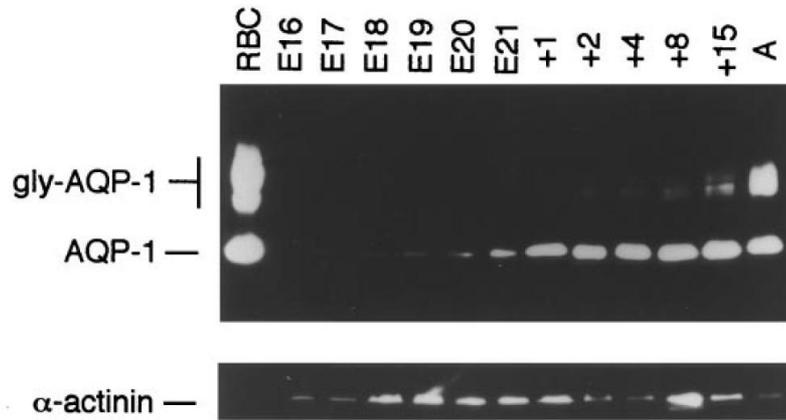


Aquaporin-1 Water Channel Protein in Lung

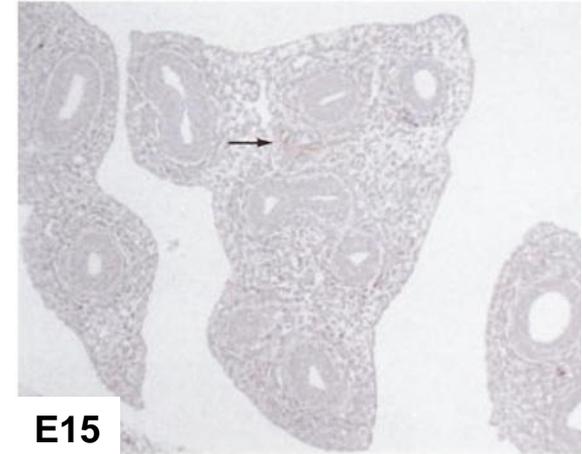
Ontogeny, Steroid-Induced Expression, and Distribution in Rat

Landon S. King,^{*‡} Søren Nielsen,[§] and Peter Agre^{*||}

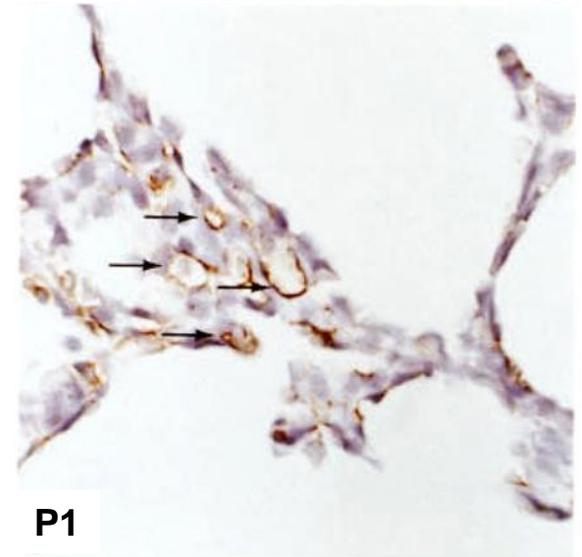
J Clin Invest 97, 1996



saline or betamethasone (0.35 mg/kg)

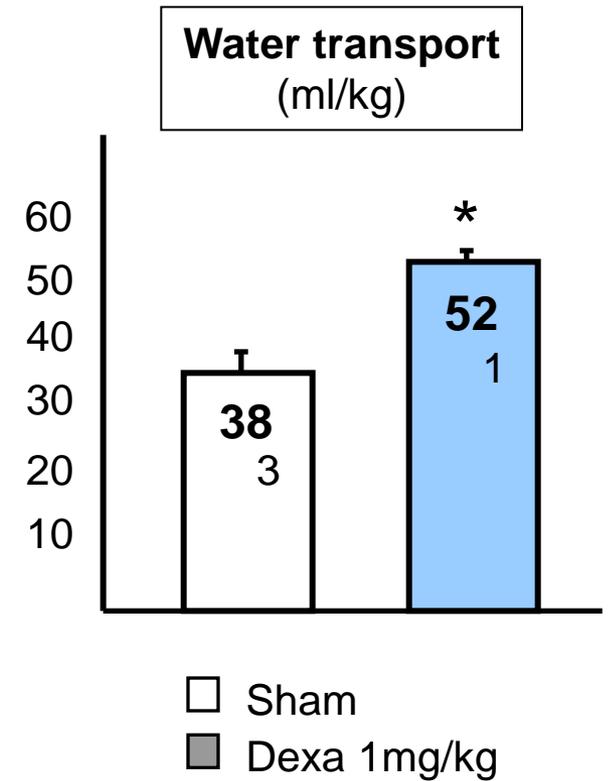
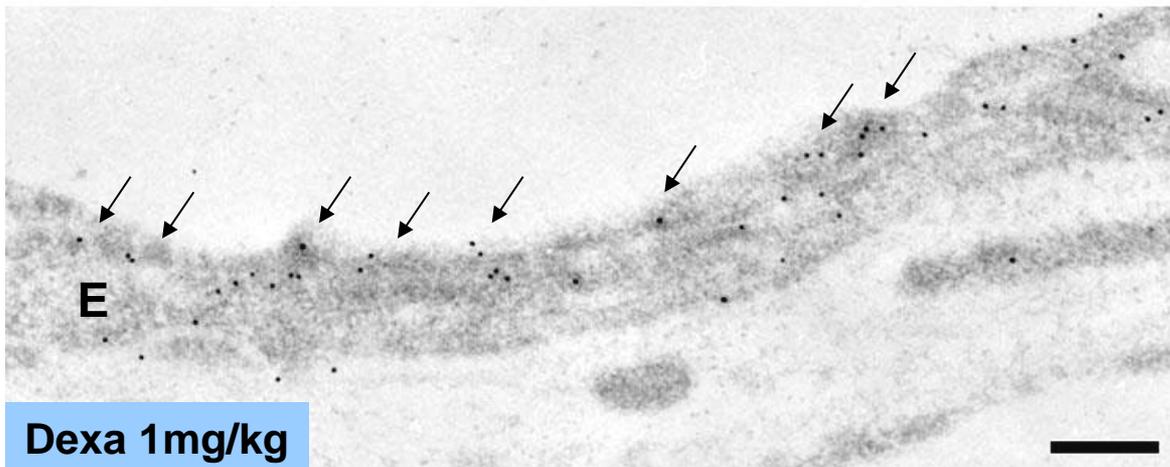
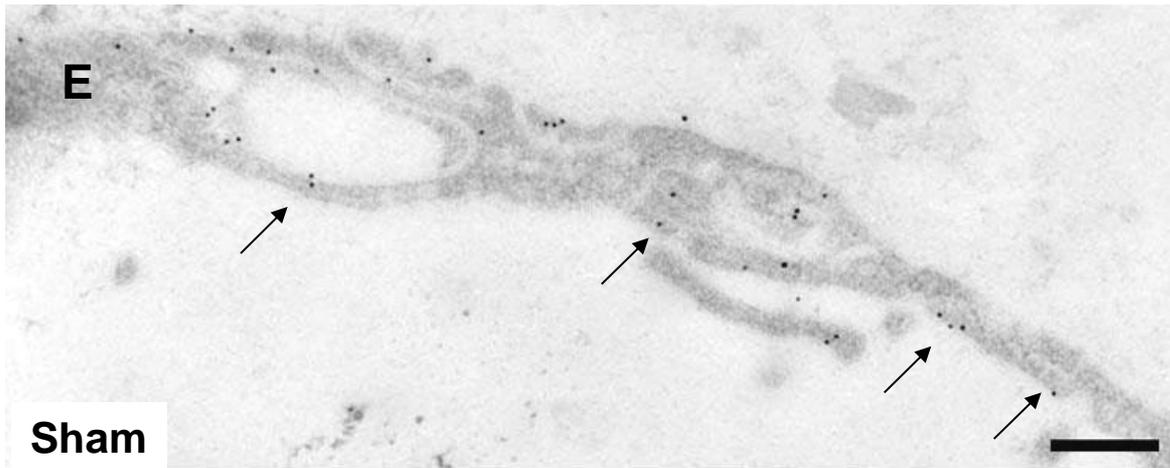


E15



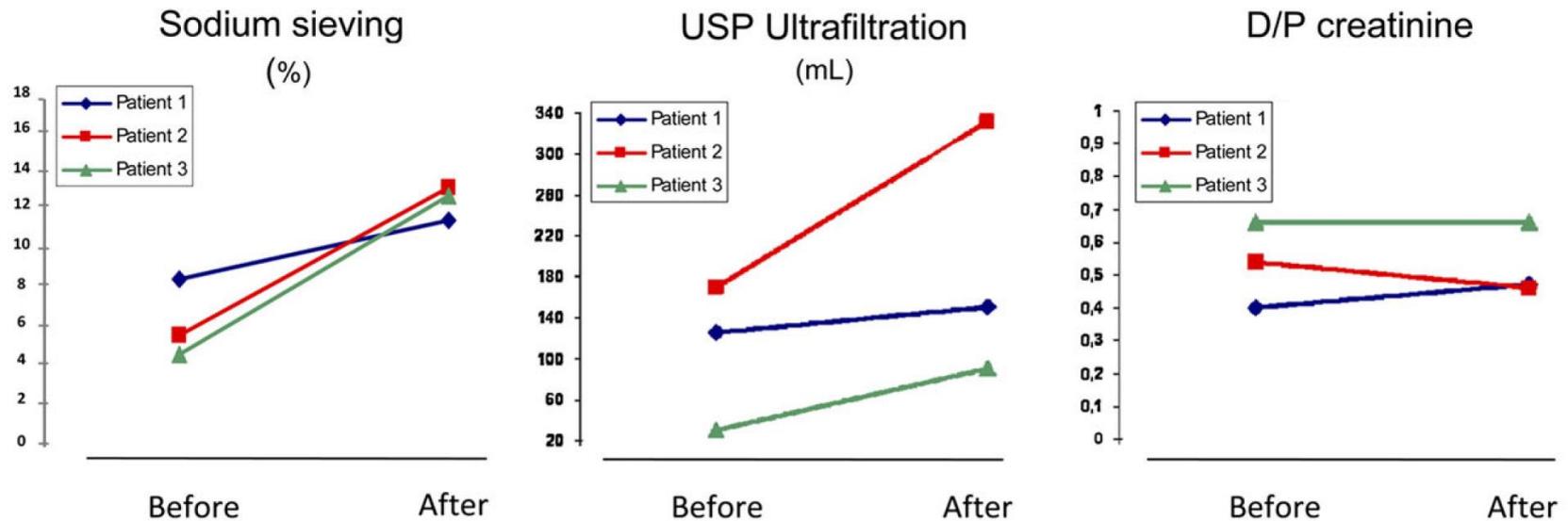
P1

Glucocorticoids upregulate AQP1 in peritoneal capillaries



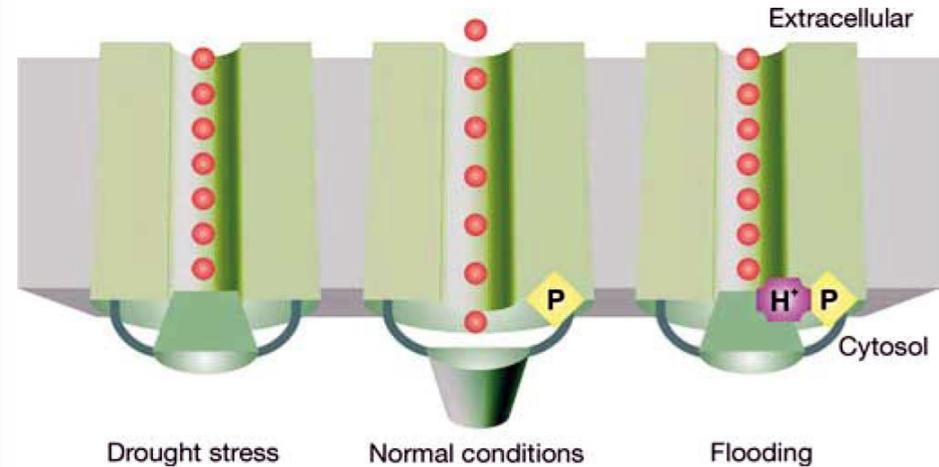
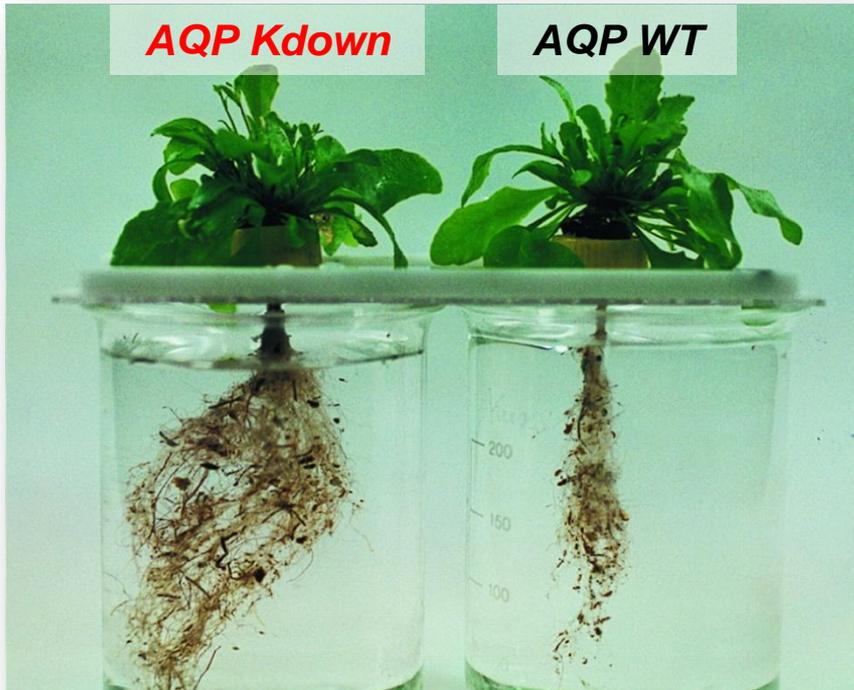
High-dose steroid treatment increases free water transport in peritoneal dialysis patients

Javier de Arteaga^{1,2}, Fabian Ledesma^{1,2}, Gabriela Garay^{1,2}, Carlos Chiurciu^{1,2},
Jorge de la Fuente^{1,2}, Walter Douthat^{1,2}, Pablo Massari^{1,2}, Sara Terryn³ and Olivier Devuyst^{3,4}



Gating AQP1 to improve water transport ?

Aquaporins Role in Plants: Gating Mechanisms

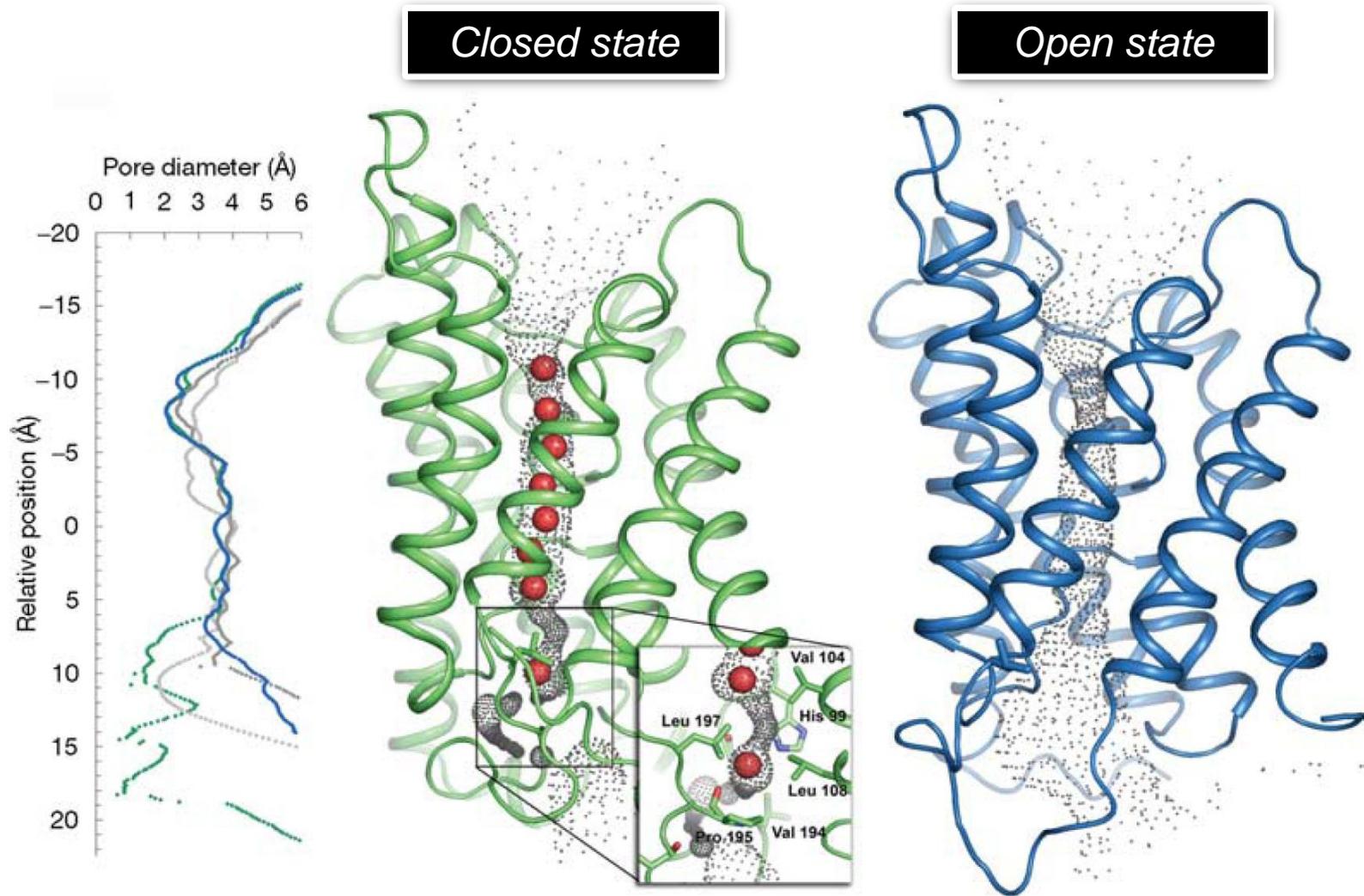


Fluctuations in water supply: regulation of AQPs in plants

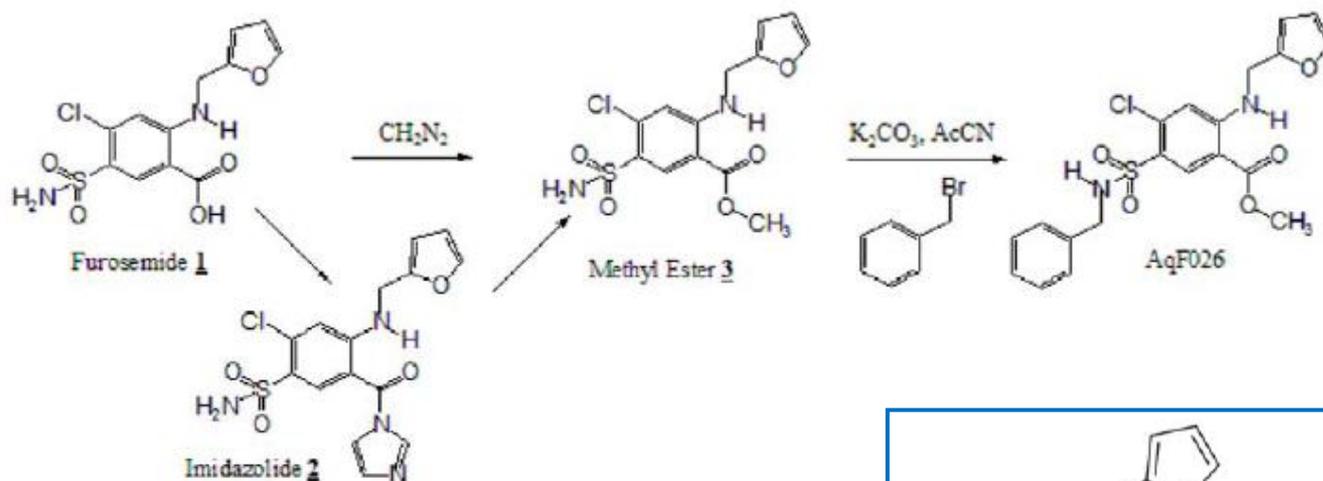
Drought stress → dephosphorylation of two conserved serine → channel closure

Flooding (acidification) → protonation of a conserved histidine → channel closure

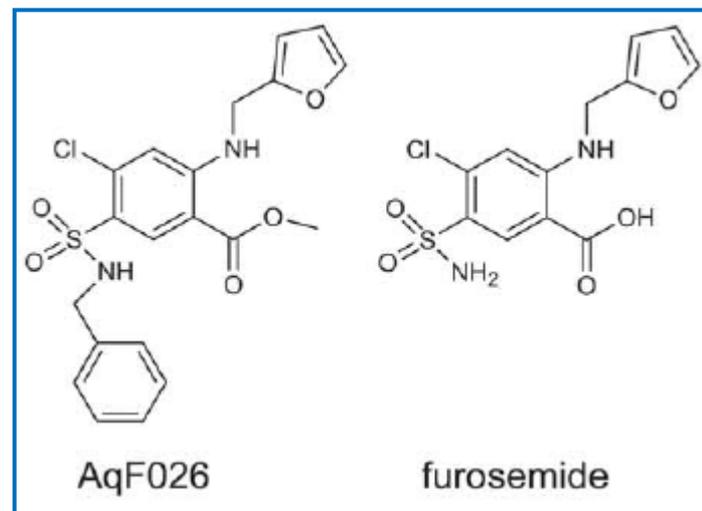
The gating of plant AQP involves loop D



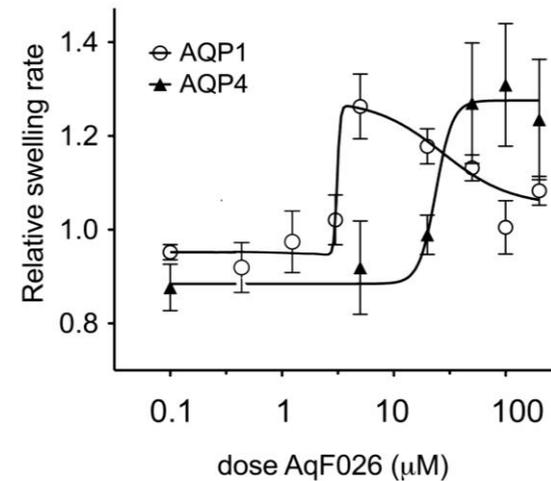
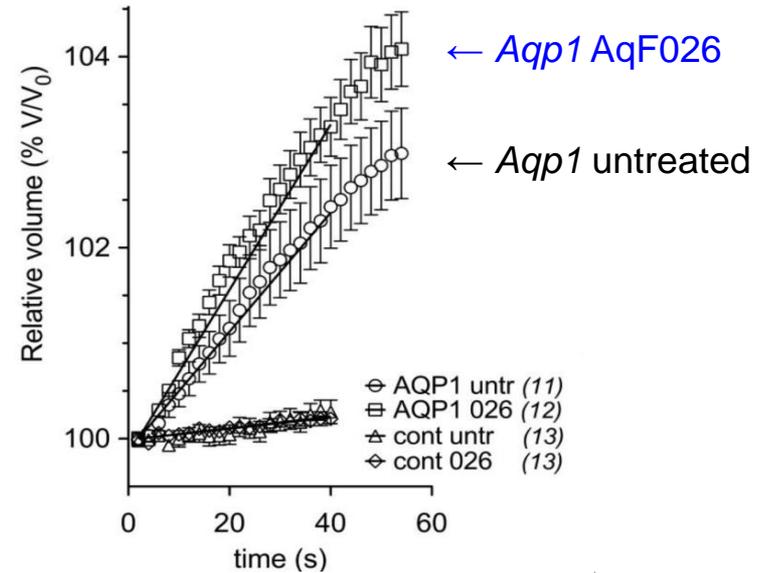
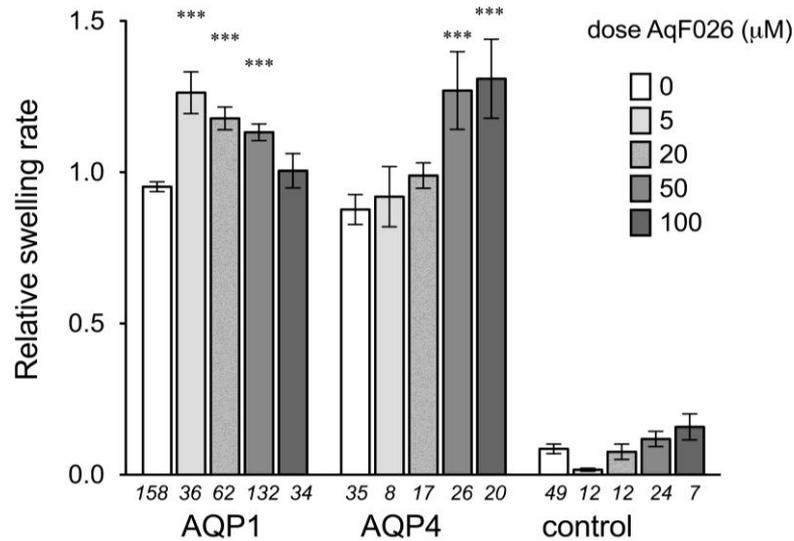
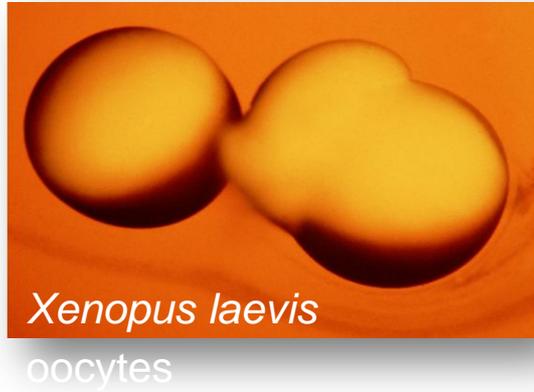
Chemical library screen: Aryl sulfonamides - AqF026



Distinctive sulfhydryl-linked
aromatic ring
of AqF026 vs. furosemide

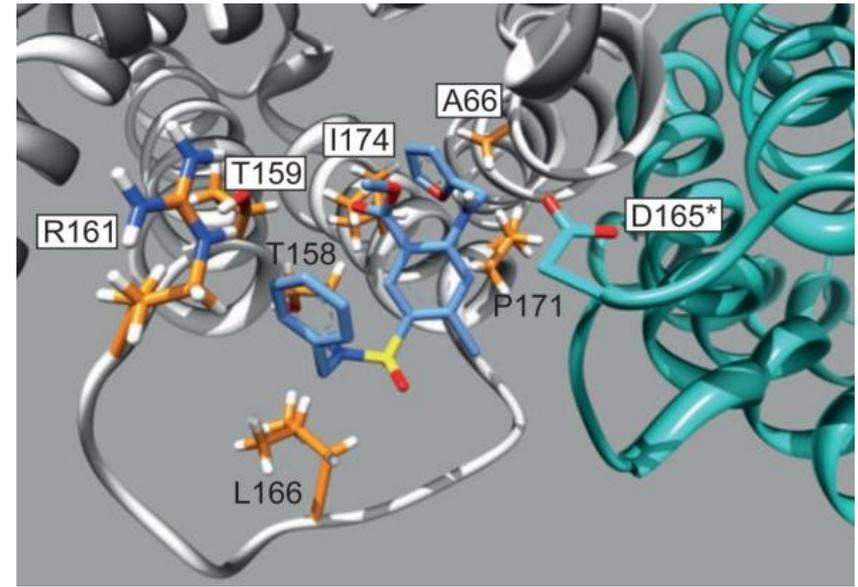
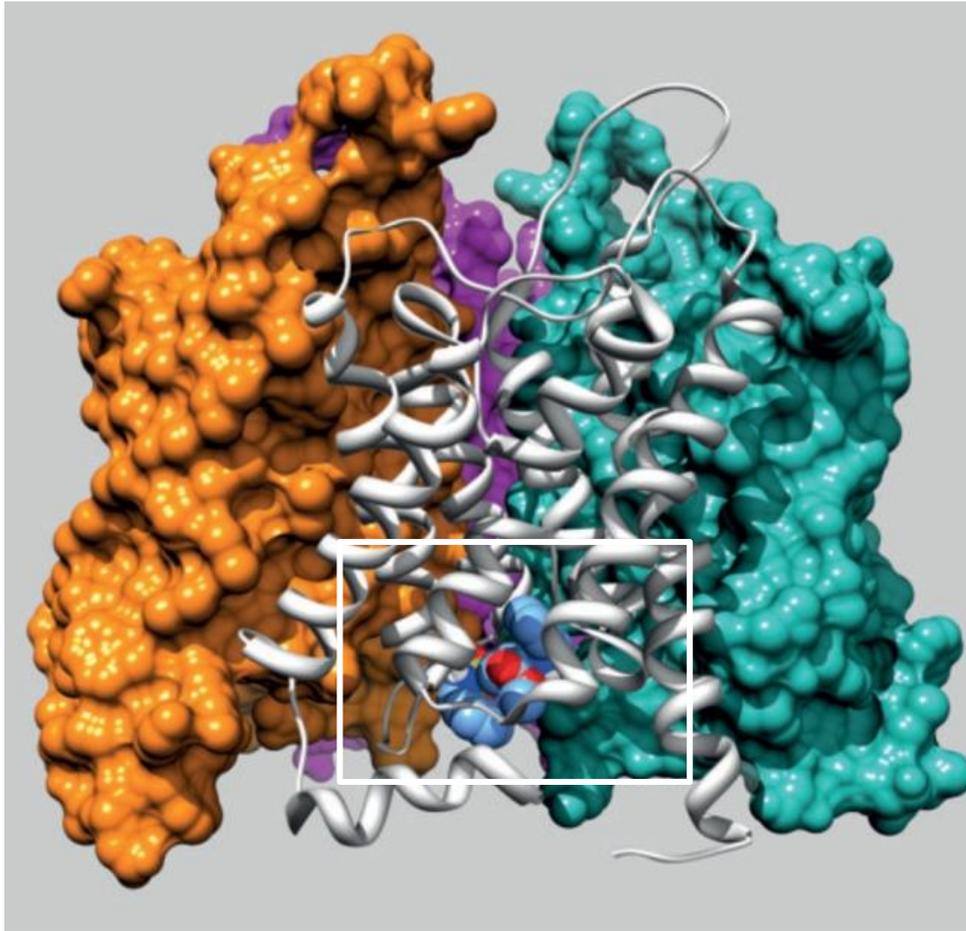


Effect of AqF026 on transport water *in vitro*



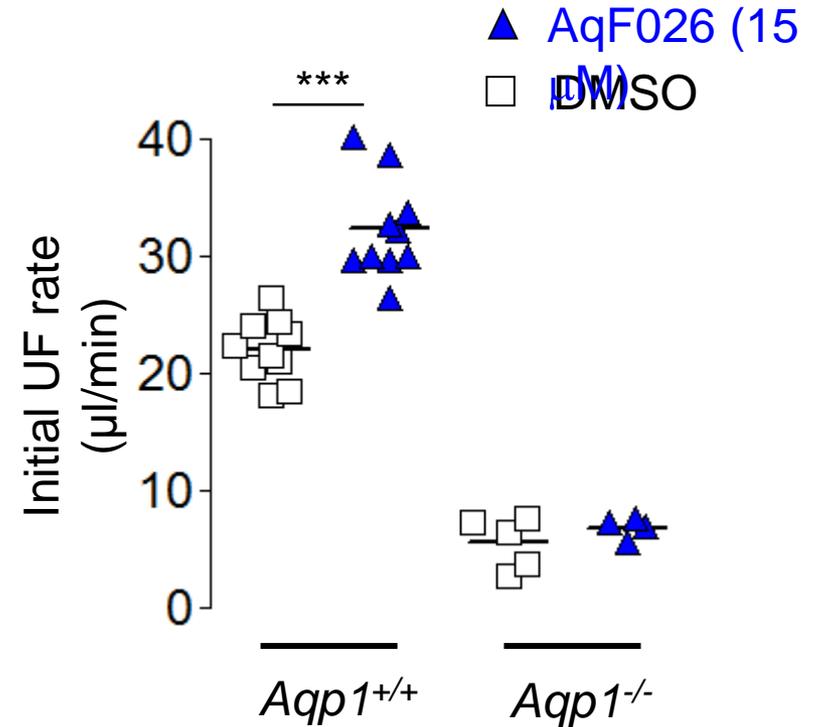
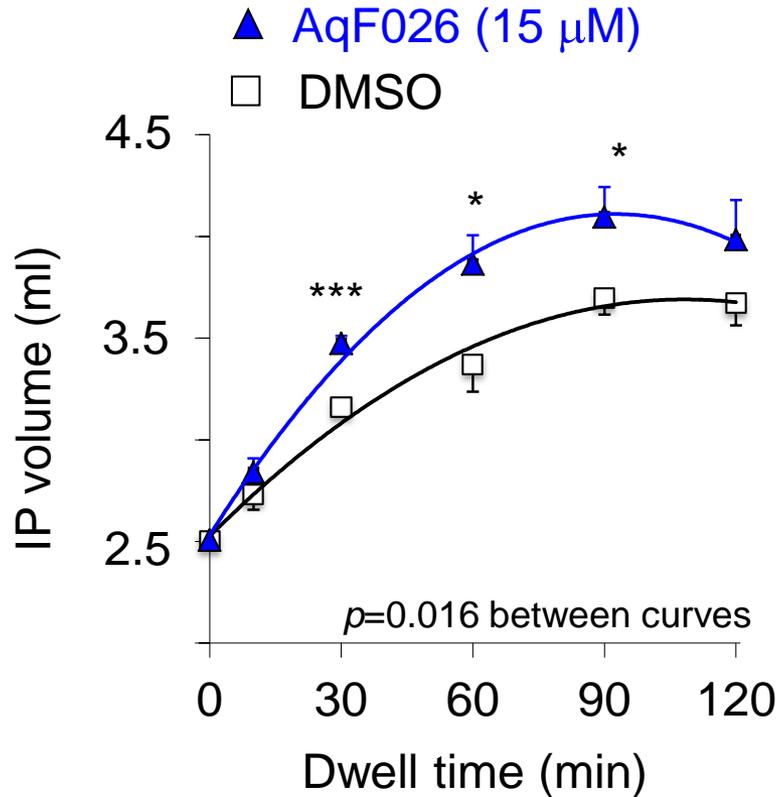
Potential of water transport : maximal at 5-20 μM for AQP1
(>50-100 μM for AQP4) with EC_{50} at 3.3 μM

Docking model suggests the binding of AqF026 to residues in the loop D domain



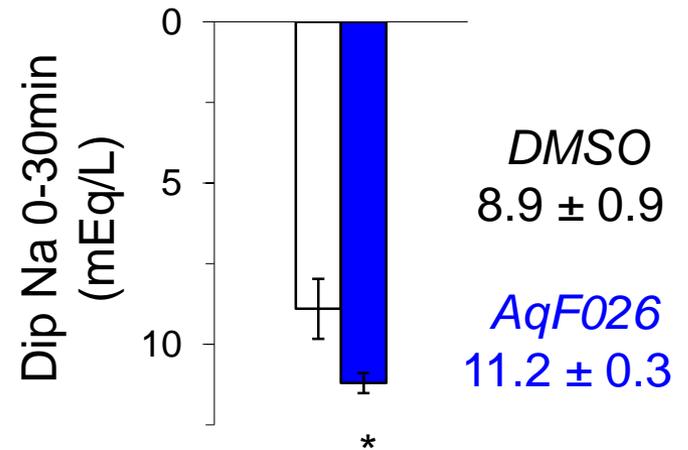
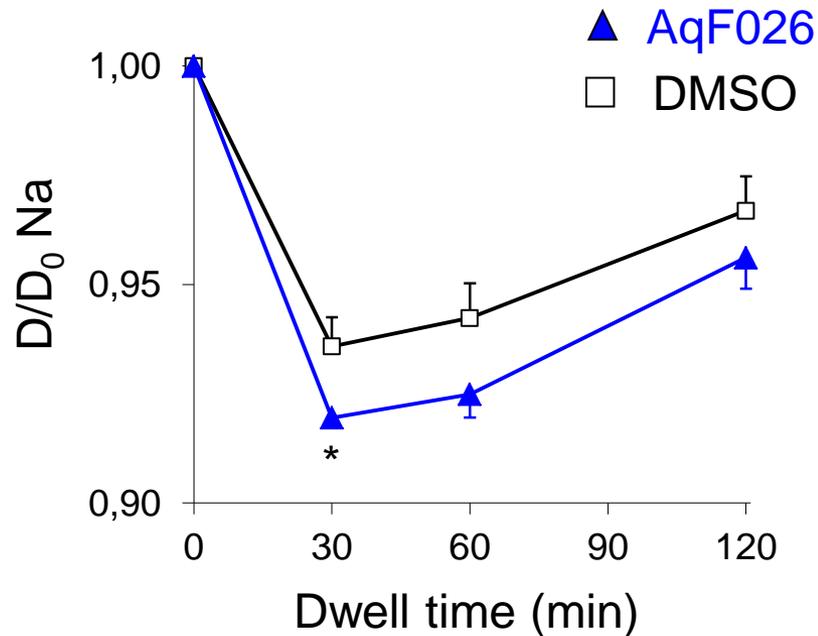
The binding site involves residues (Arg161, Thr159) located in the cytosolic D loop.

In vivo effect of AqF026: IPV curves and initial UF rate



1. AqF026 increases initial UF rate by ~50%
2. AqF026 has no effect on the initial UF rate in *Aqp1*-null mice

In vivo effect of AqF026 on the sodium sieving



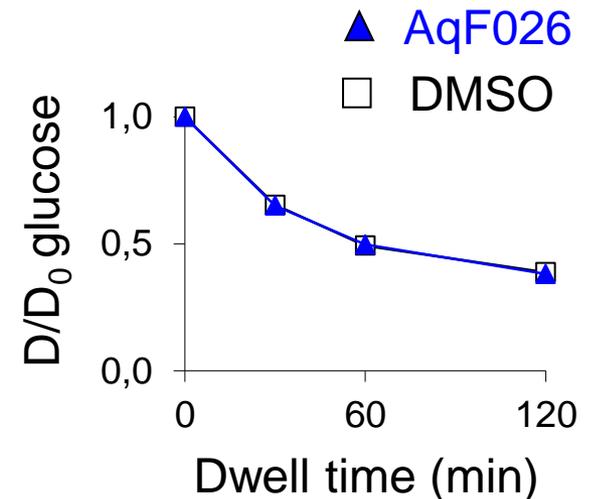
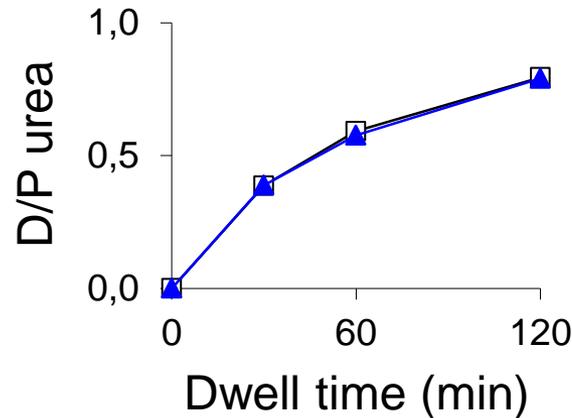
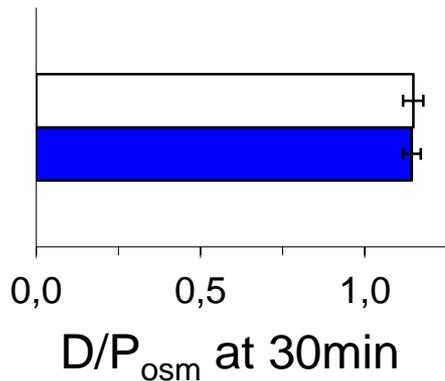
AqF026 increases the sodium sieving, reflecting the **enhanced free-water transport through AQP1**

AqF026: No Effect on Osmotic Gradient and Solute Removal

	Group (n)	Net UF ($\mu\text{l/g BW}$)	D/Posm at 30min	MTAC urea ($\mu\text{l/min}$)
<i>Aqp1</i> ^{+/+}	DMSO (16)	37.2 \pm 1.3	1.14 \pm 0.03	35.0 \pm 2.3
<i>Aqp1</i> ^{+/+}	026 (16)	42.8\pm1.4*	1.15 \pm 0.03	34.2 \pm 1.7
<i>Aqp1</i> ^{-/-}	DMSO (6)	15.7 \pm 1.9***	1.28 \pm 0.02	35.5 \pm 3.6
<i>Aqp1</i> ^{-/-}	026 (6)	15.6 \pm 1.6	1.27 \pm 0.03	36.4 \pm 7.0

+15%

*increase in net UF
after a 2-h dwell*



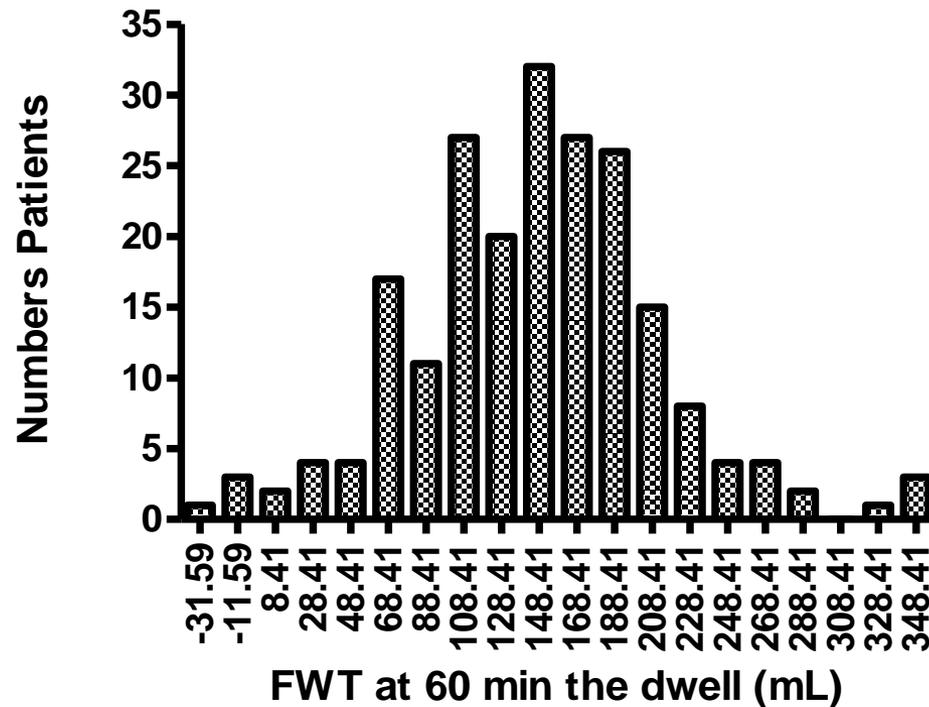
Tolerance of AqF026

- Well-established **safety index of loop diuretics** during acute or chronic administration
- **No overt tissue or organ toxicity** under the conditions tested
- **No diuretic effect**; biological screening:

	n/group	Normal values (SV129)	DMSO	AqF026
AST (UI/L)	6	71–201	180 ± 30	132 ± 9
ALT (UI/L)	6	45–84	12 ± 1	13 ± 1
LDH (UI/L)	9	2212-3150	978 ± 115	804 ± 89

Genetic influence on water transport ?

Distribution of Water Transport in PD Patients

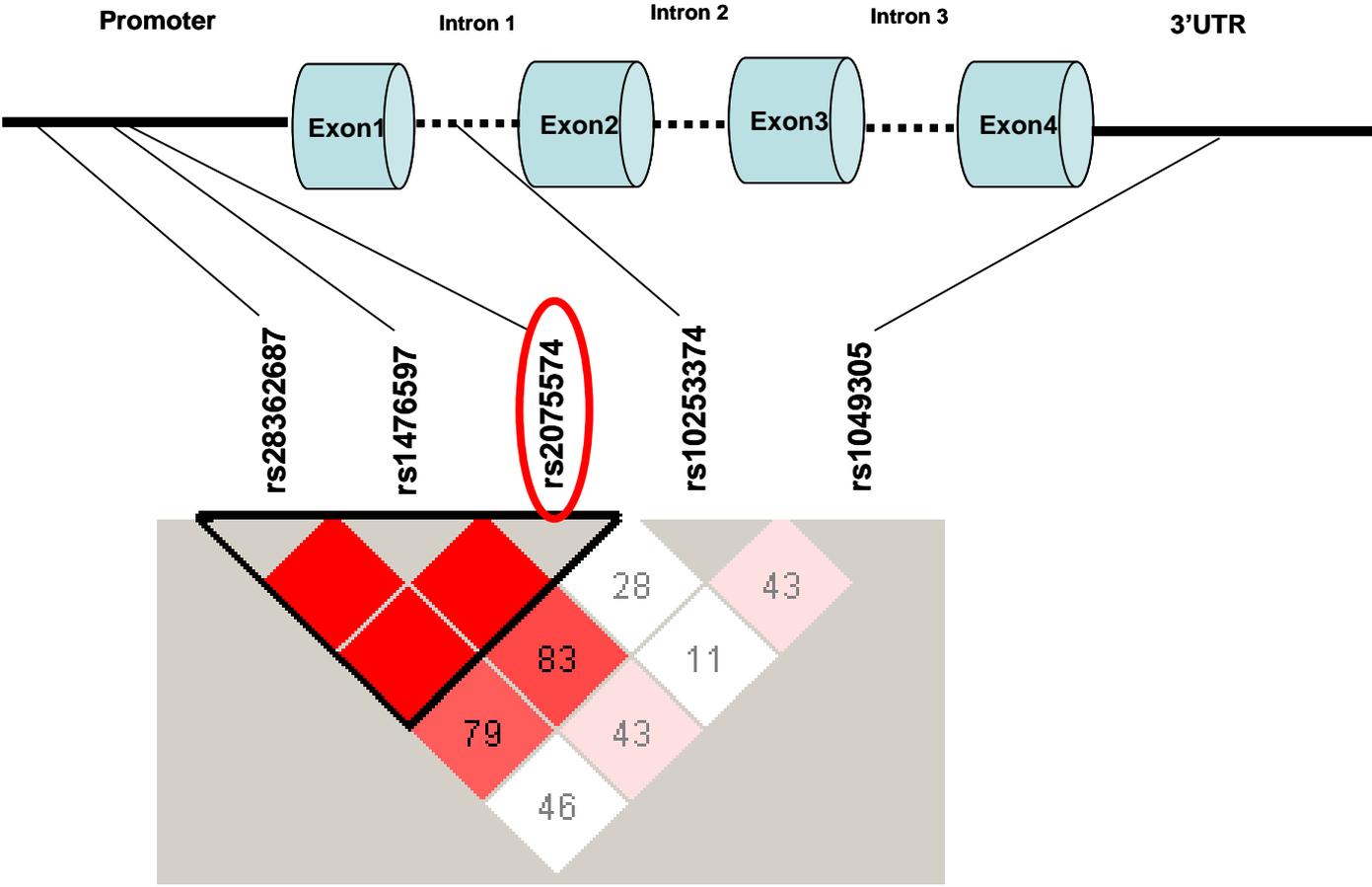


*Clinical variables account for only ~ 20% of the variability:
→ Variations in water channels ?*

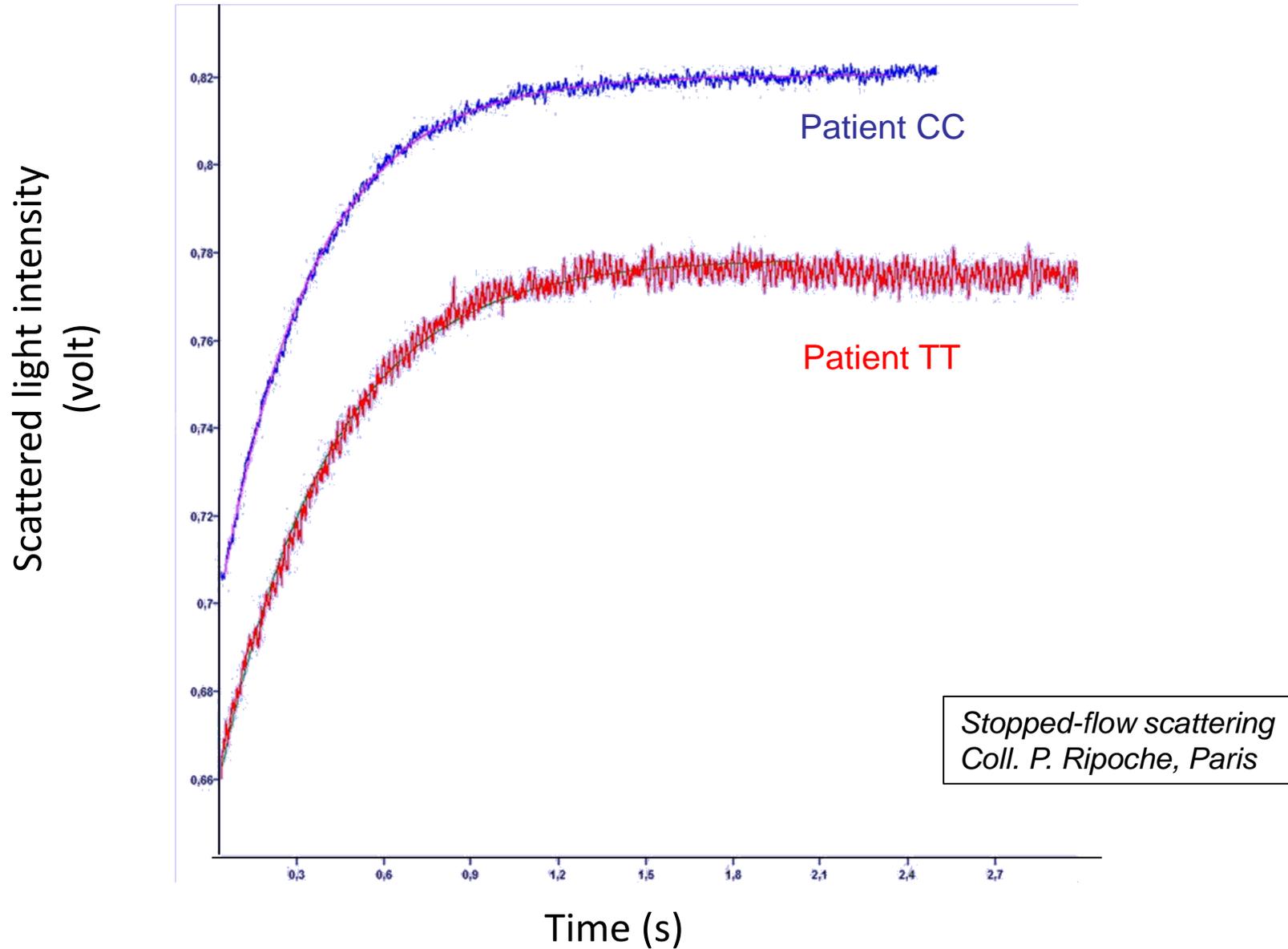
Influence of variants in *AQP1* on Water transport and PD Outcome

- Multicentric association study
 - UCL – KUL – CHC Liège: N=215
 - NECOSAD – AMC Amsterdam: N=500
 - Replication: UK and Spain cohorts
- Initial water flow, small solute transport
- Survival : technique and patient

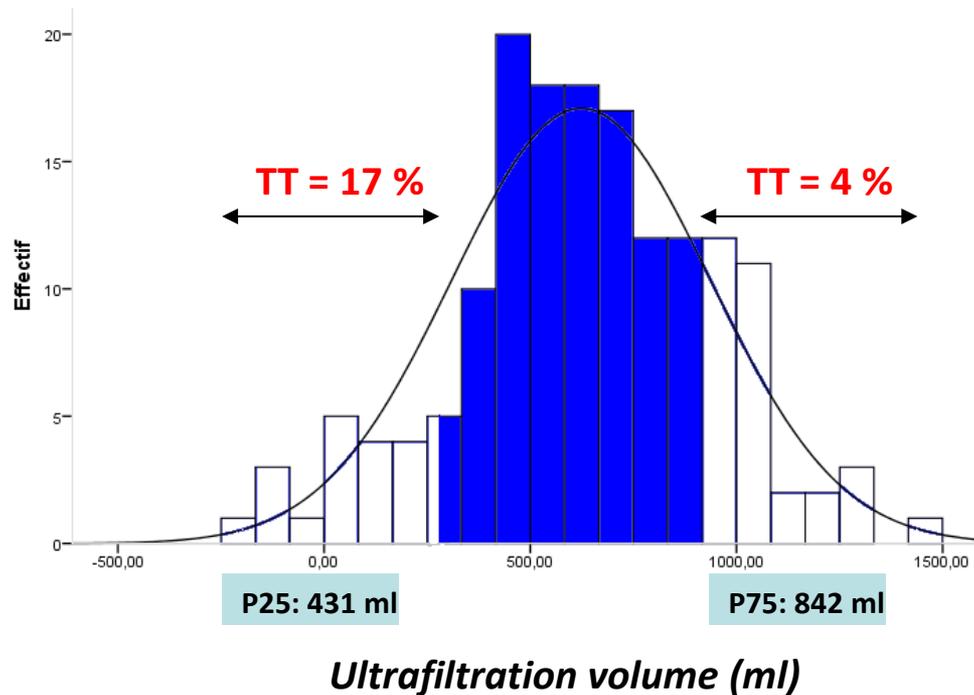
AQP1 Haplotype



AQP1 Genotype: Water transport in erythrocytes



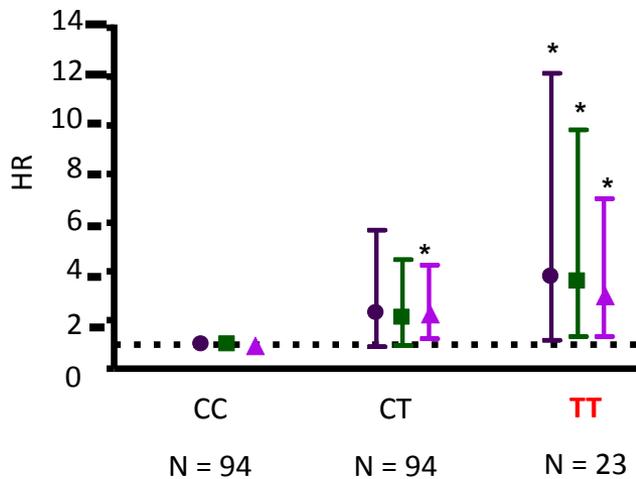
AQP1 Genotype and Transport



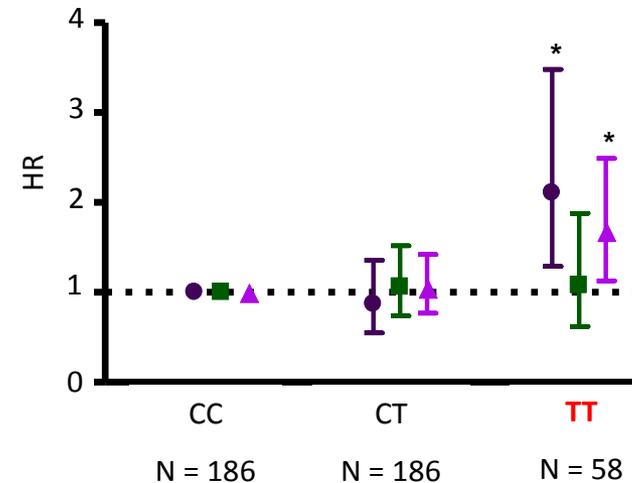
rs2075574	CC N = 94	CT N = 94	TT N = 23	P-value
Transport parameters				
UF Mean (Std)	588 (338)	673 (309)	483 (266)	0.02
Sieving Na Mean (Std) (n=155)	0.05 (0.03)	0.05 (0.02)	0.04 (0.03)	0.046
D/P creat 4 hours Mean (Std)	0.72 (0.12)	0.73 (0.11)	0.76 (0.12)	NS

AQP1 Genotype: Influence on Survival

Cohort UCL – KUL (N=211)



Cohort NECOSAD – AMC (N=430)



rs2075574	Death (33 events)*		Technique failure (41 events)**		Combined end point (65 events)***	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
CC (n = 94)	1		1		1	
CT (n = 94)	2.28 (0.91-5.70)	0.08	2.09 (0.97 – 4.49)	0.06	2.30 (1.24-4.26)	0.01
TT (n = 23)	3.78 (1.18 – 12.14)	0.03	3.59 (1.32-9.82)	0.01	3.04 (1.33-6.98)	0.01

* Adjusted for age, gender, CVE and diabetes
 ** Adjusted for age, gender, CVE, diabetes, UF, DPcreat and DDo gluc 4h
 *** Adjusted for age, gender, CVE, diabetes, UF, DPcreat and DDo gluc 4h

General Conclusions

- Water requires *specific carriers*, which have been acquired through evolution
 - *>1000 genes coding for carriers in mammalian genomes*
 - *Teological reason: control & regulation*
- Essential role of *AQP1 – archetype of water channel*
 - *kidney, lung, endothelium : peritoneal dialysis*
- Importance of *adequate animal models*
 - *Mechanistic studies & drug development - testing*
- *Advances in structure – regulation mechanisms*
 - *therapeutic implications & individual predictions*



Acknowledgements

UCL Brussels - Nephrology

J. Ni, J. Morelle
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University of Nagasaki

T. Nishino

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But man has in him the silence of the sea

R. Tagore

Thank you for your attention

End

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Modulating AQP1 (increasing water transport): Always beneficial ?

- AQP1 in endothelial cells: migration and angiogenesis
→ *Angiogenesis – peritonitis – long-term exposure*
- AQP1 in peritoneal macrophages: motility and phagocytosis
→ *Host defense – peritonitis*
- Interaction endothelial cells – circulating leukocytes

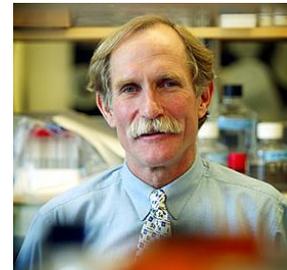
AqF026, a Novel Agonist of the Water Channel Aquaporin-1

- AqF026 increases water transport across AQP1 *in vitro*
- *In silico* modelling and site-directed mutagenesis support interaction between AqF026 and residues in the loop D domain of AQP1
- Application of AqF026 to the mouse model of PD demonstrates the efficacy of the AQP1 agonist to increase water transport *in vivo*
- AqF026 has potential therapeutic value for clinical situations based on osmotic water transport:
 - *Peritoneal dialysis, edema, angiogenesis, alterations of AQP1*

Acknowledgements



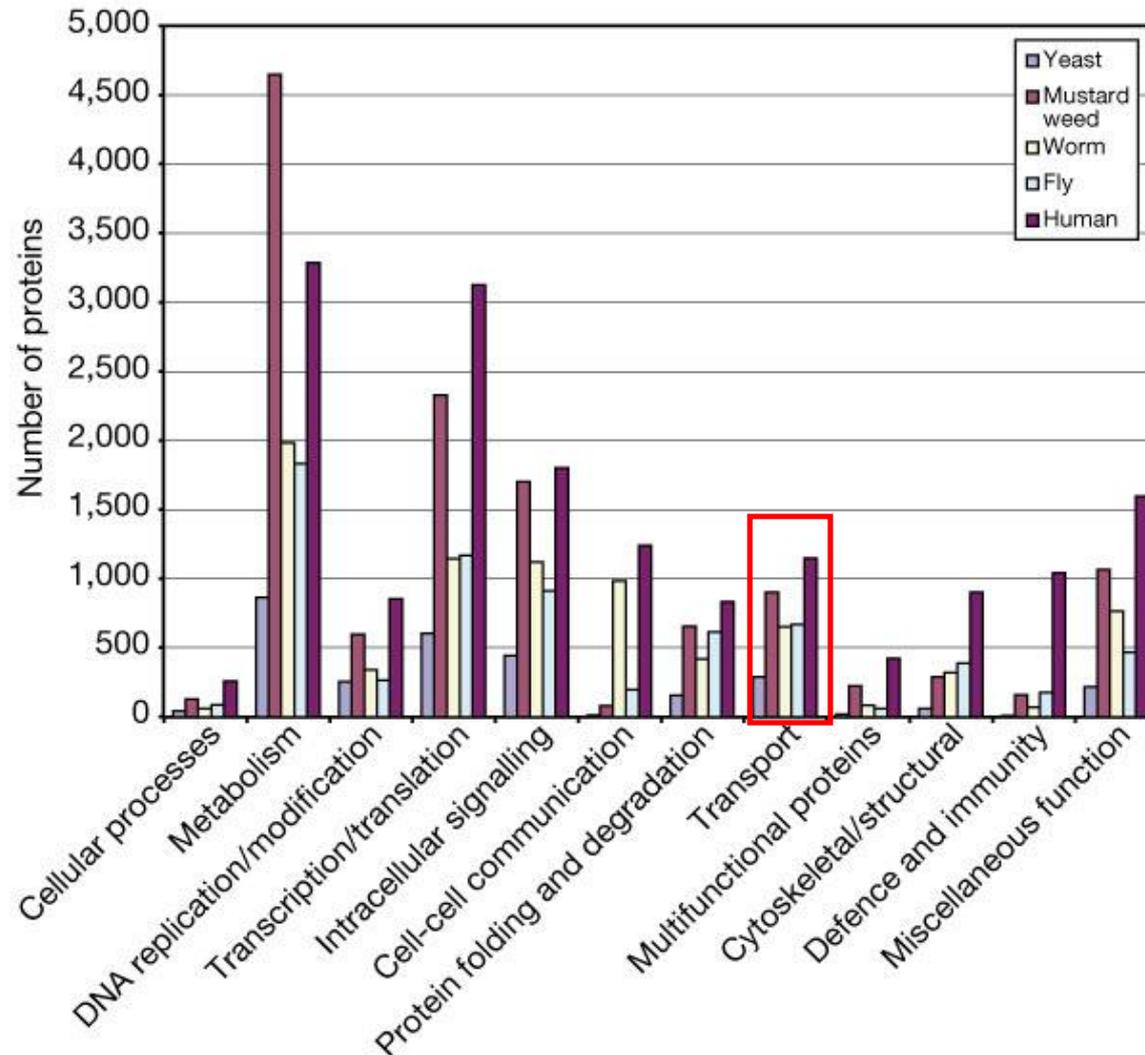
S. Shpun, J. Hoffmann, U. Katz



Universität
Zürich^{UZH}



The human genome contains > 1,000 genes for transport proteins



Quantitative visualization of passive transport across bilayer lipid membranes

John M. A. Grime*[†], Martin A. Edwards*[†], Nicola C. Rudd[†], and Patrick R. Unwin^{†*}

Significantly, the permeation coefficient decreased with acyl tail length contrary to previous work and to Overton's rule. The reasons for this difference are considered, and we suggest that the applicability of Overton's rule requires re-evaluation.

PNAS | September 23, 2008 | vol. 105 | no. 38 | 14277–14282

LETTER

Passive transport across bilayer lipid membranes: Overton continues to rule

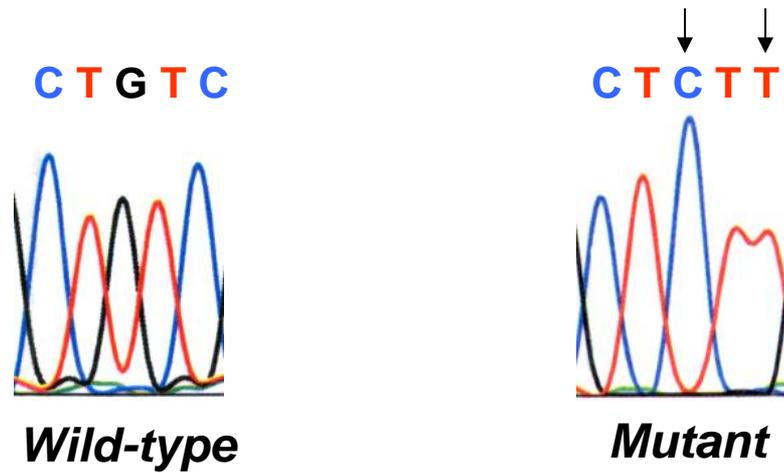
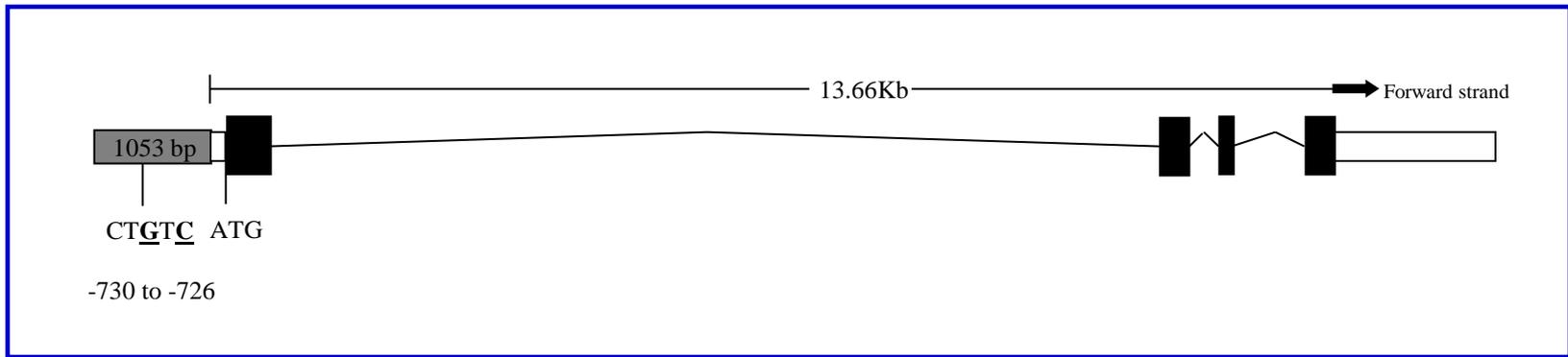
According to Overton's rule, membrane permeability (P_M) of a molecule increases with its hydrophobicity. Experiments with a series of carboxylic acids now suggest the opposite: the most hydrophilic acid exhibited the highest P_M (1).

The experiments, however, do not justify this conclusion

Andreas Missner^a, Philipp Kügler^{b,c}, Yuri N. Antonenko^d, and Peter Pohl^{a,1}

PNAS | December 30, 2008 | vol. 105 | no. 52 | E123

CTGTC Motif in the Promoter of *AQP1*



[Nephrol Dial Transplant.](#) 2011 Dec;26(12):4142-5.

High-dose steroid treatment increases free water transport in peritoneal dialysis patients.

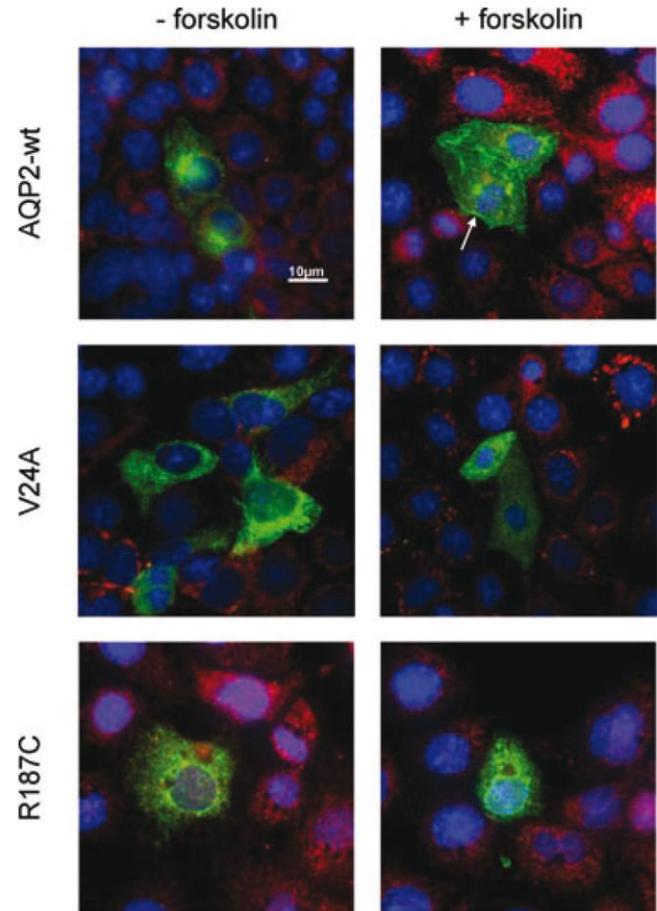
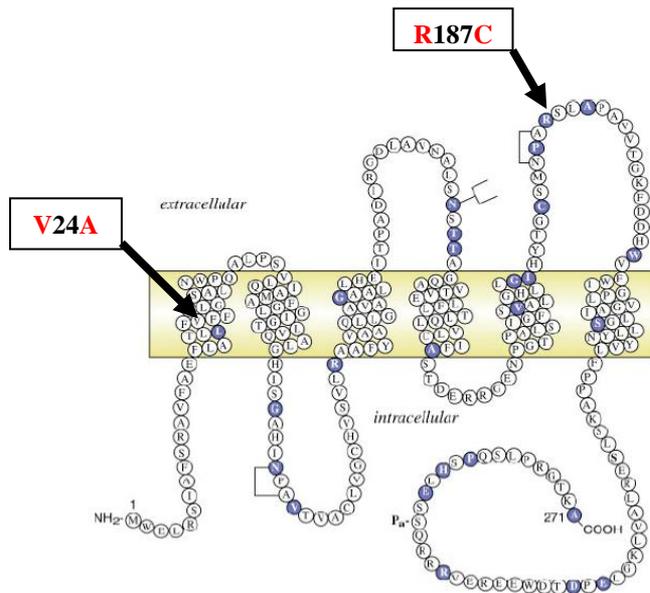
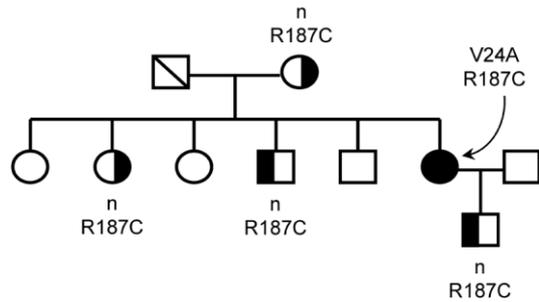
[de Arteaga J](#), [Ledesma F](#), [Garay G](#), [Chiurciu C](#), [de la Fuente J](#), [Douthat W](#), [Massari P](#), [Terry S](#), [Devuyst O](#).

Abstract

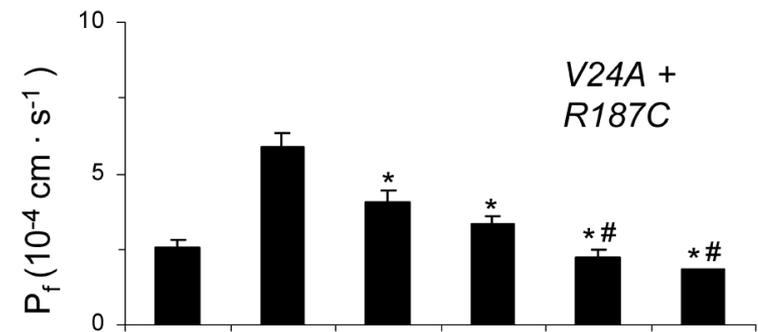
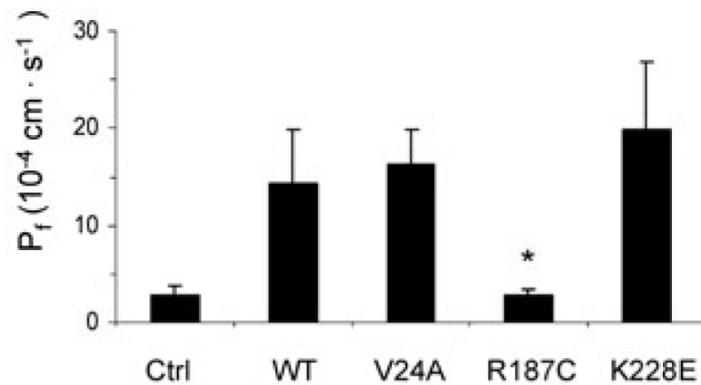
The water channel aquaporin-1 (AQP1) is the molecular counterpart of the ultrasmall pore that mediates free water transport during peritoneal dialysis (PD). Proof-of-principle studies performed in rats have shown that treatment with corticosteroids upregulates the expression of AQP1 in the peritoneal capillaries, causing a significant increase in free water transport. Whether such a beneficial effect could be observed in end-stage renal disease patients treated by PD remains unknown. Peritoneal transport parameters were evaluated in three patients on PD, shortly before and after living-donor renal transplantation and treatment with high-dose methylprednisolone (1.0-1.2 g/m²). As compared with pre-transplantation values, the post-transplantation test revealed an ~2-fold increase in the sodium sieving and ultrasmall pore ultrafiltration volume, suggesting an effect on AQP1 water channels. In contrast, there was no change in the parameters of small solute transport. The direct involvement of AQP1 in these changes is suggested by the expression of glucocorticoid receptors in the human peritoneum and the presence of conserved glucocorticoid response elements in the promoter of the human AQP1 gene.

Sylvie, 39 year-old

- Urinates **15 L/day**
 - Needs to drink : **15-20 L/day**
 - Difficult scolarity, multiple hospitalizations (dehydration)
- Asks for handicap recognition



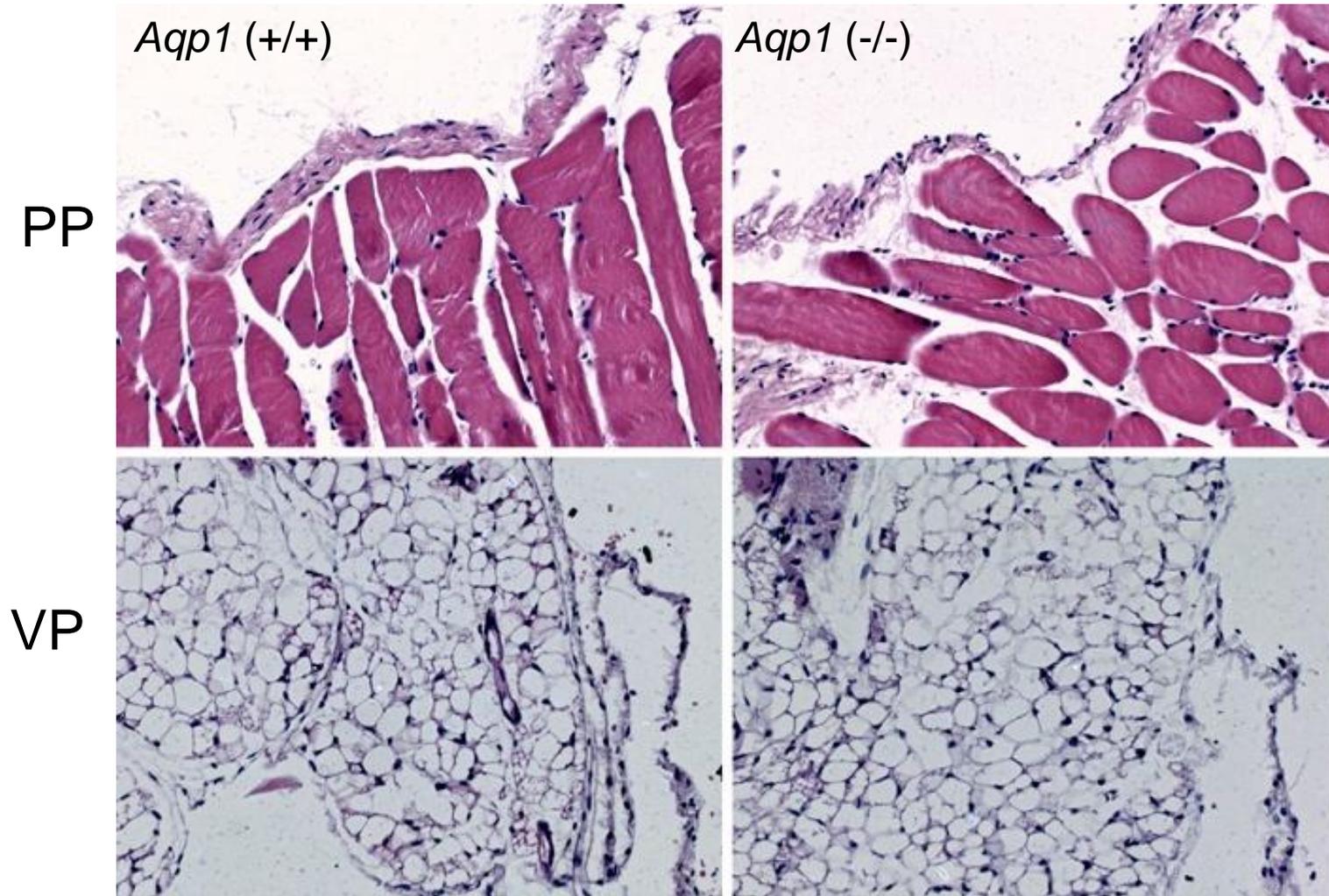
Compound heterozygous: combination of two mutations



mutant	0	5	5	5	5	5
R187C	0	0	2.5	5	7.5	10

→ Combined effect : decreased water transport *in vitro*

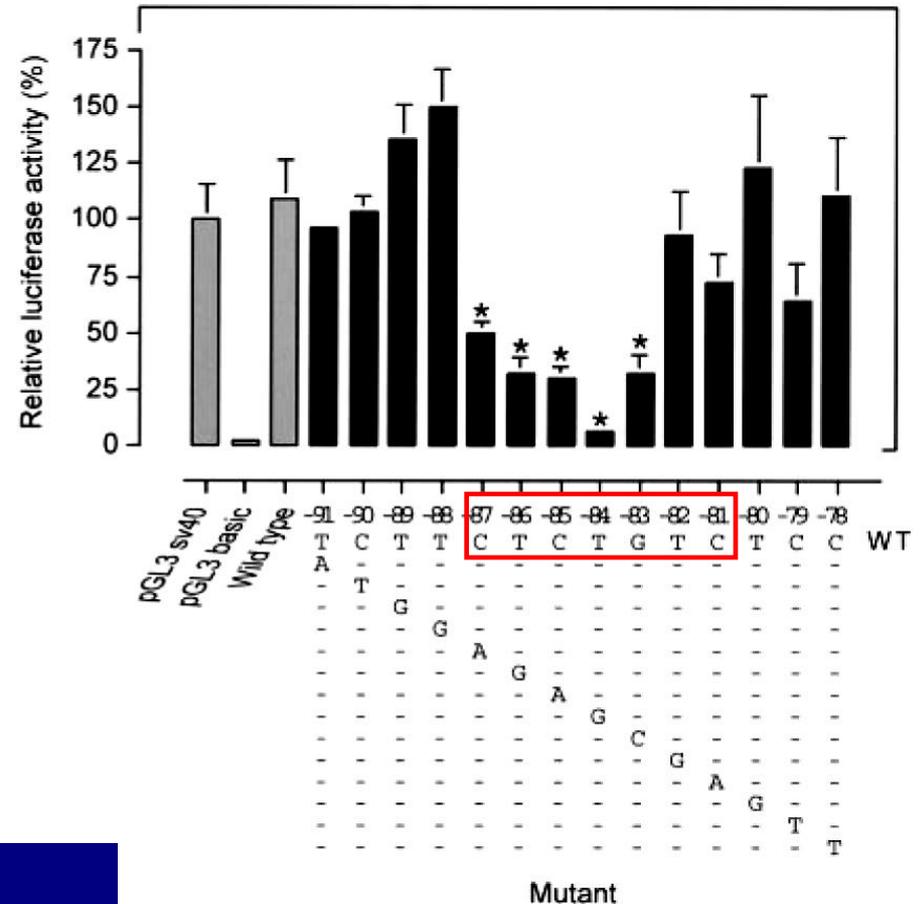
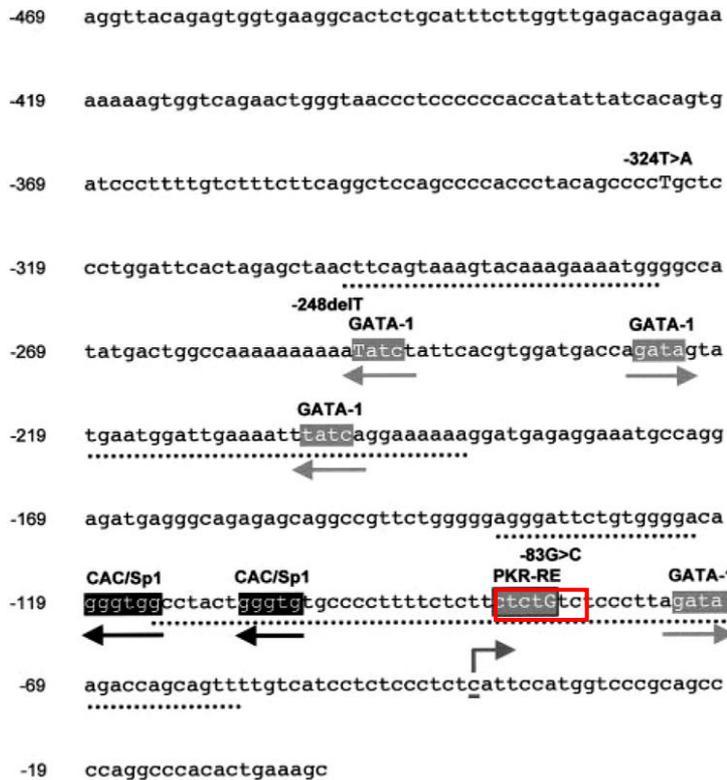
Peritoneal Structure in *Aqp1* Mice: Morphology



Disruption of a novel regulatory element in the erythroid-specific promoter of the human *PKLR* gene causes severe pyruvate kinase deficiency

Richard van Wijk, Wouter W. van Solinge, Claus Nerlov, Ernest Beutler, Terri Gelbart, Gert Rijksen, and Finn C. Nielsen

Blood 2003; 101: 1596-1602



CTCTG, core binding motif of a regulatory element for PKLR



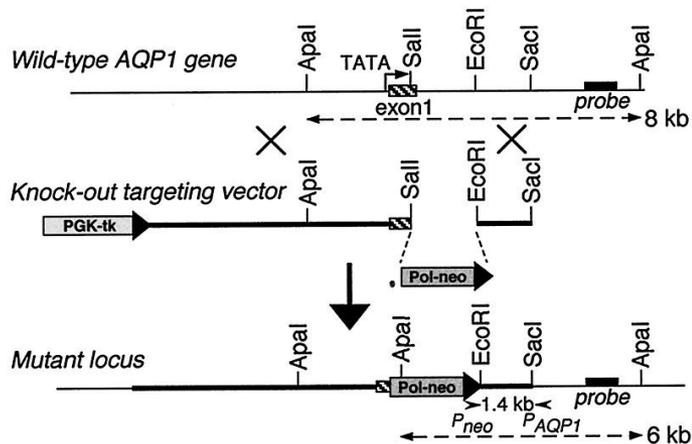




J Biol Chem. 1998 Feb 20;273(8):4296-9.

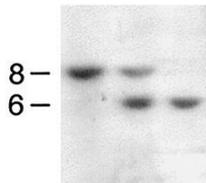
Severely impaired urinary concentrating ability in transgenic mice lacking aquaporin-1 water channels.

Ma T, Yang B, Gillespie A, Carlson EJ, Epstein CJ, Verkman AS.

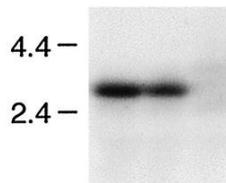


- Diabetes insipidus - nephrogenic
- ↓ water permeability across lung capillaries
- ↓ corneal water permeability
- ↓ cerebrospinal fluid production

kb +/+ +/- -/-

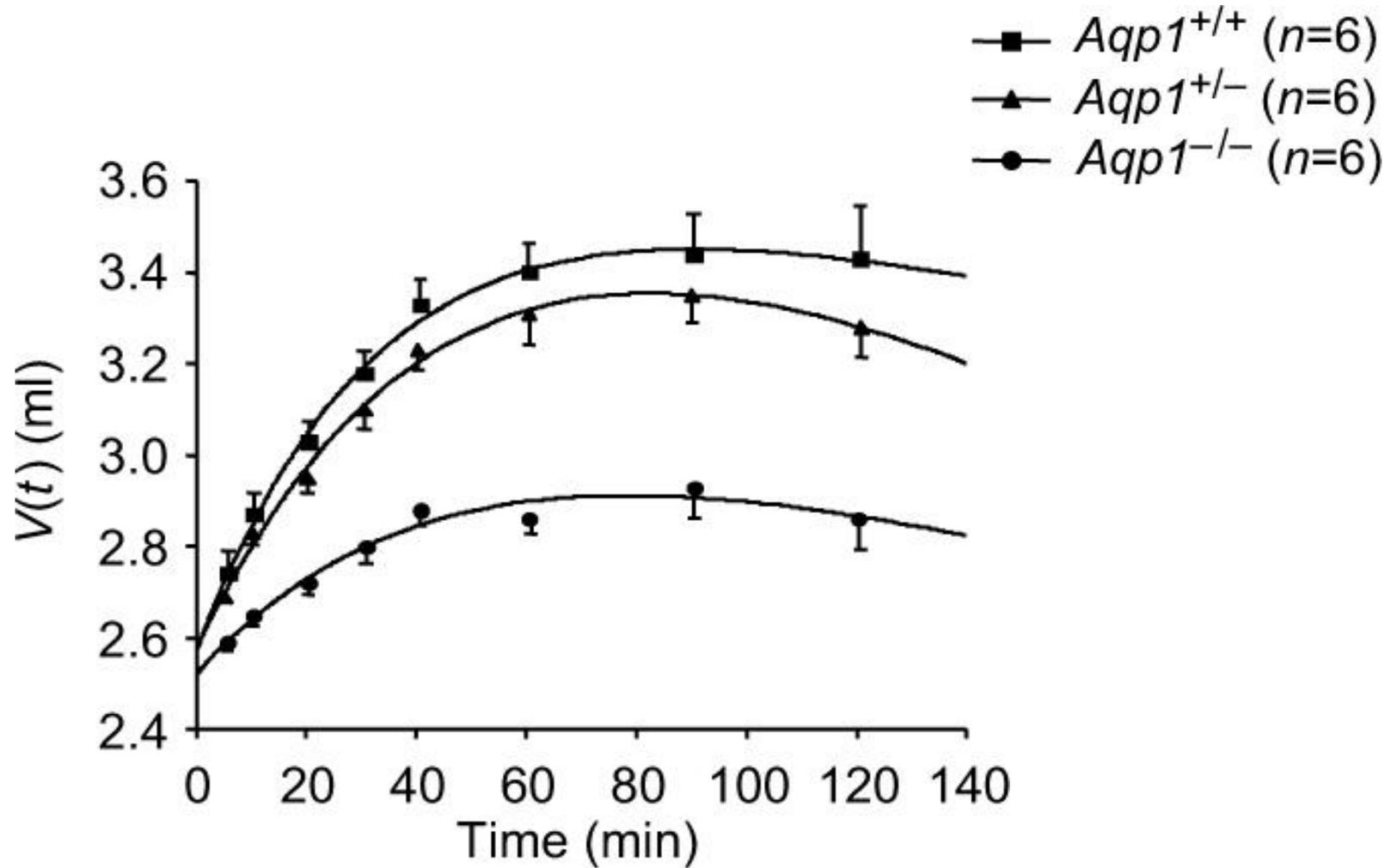


kb +/+ +/- -/-



→ Transport across the peritoneal membrane ?

Intraperitoneal volume during PD



Steroid Induction of AQP1: PET after Living Donor Transplantation

Parameter	Before → After
Sodium sieving (D/P sodium, T30-T0)	-8 → -16
Ultrafiltration USP (mL)	108 → 190
D/P creatinine	0.53 → 0.53

- N=3, living related kidney transplantation (F11y, M52y, M5y)
- Methylprednisolone, 1mg/m²
- MiniPET, before and 22 days after transplant

Early Formulations of Water Transport

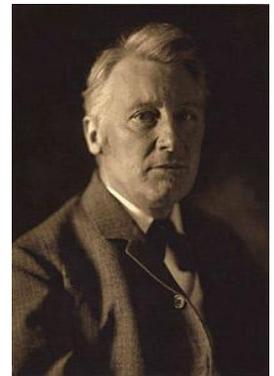
- **William Hewson (1773)** : lysis of red blood cells
→ Fluid-containing vesicles that shrink or swell depending on the nature of the bathing fluid

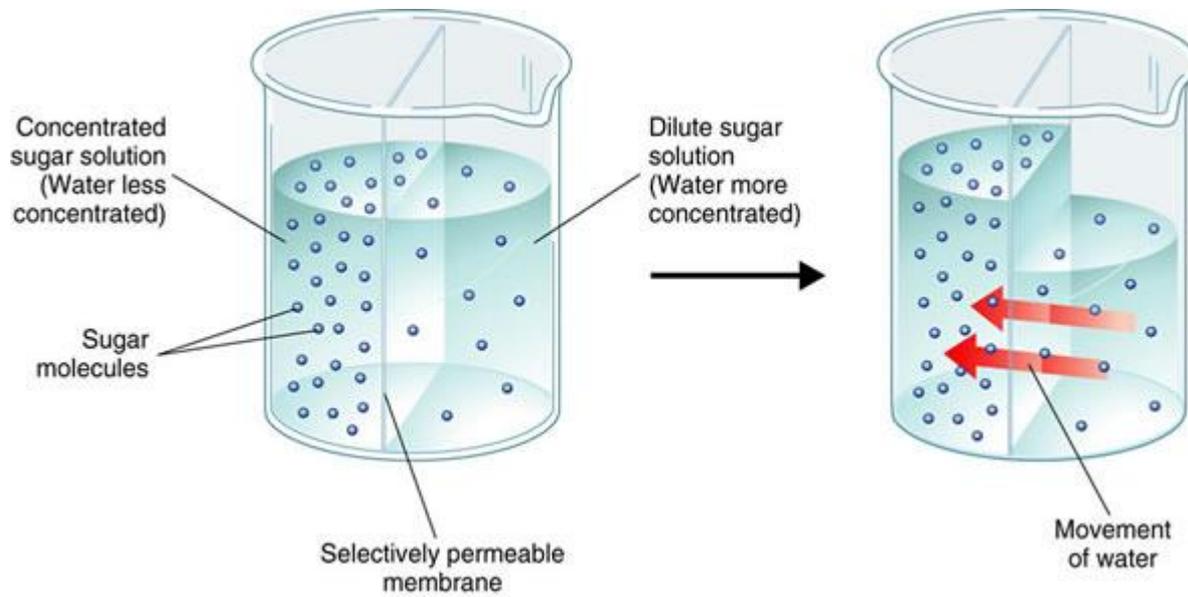


- **Karl von Nägeli (PD, University of Zurich 1855)**
→ osmosis in plant cells



- **Jakobus van't Hoff (1887)** : cells thought as osmometers
→ change in volume governed by bathing fluid composition
(Nobel Prize Chemistry 1901)





**Size
Restriction**

180H

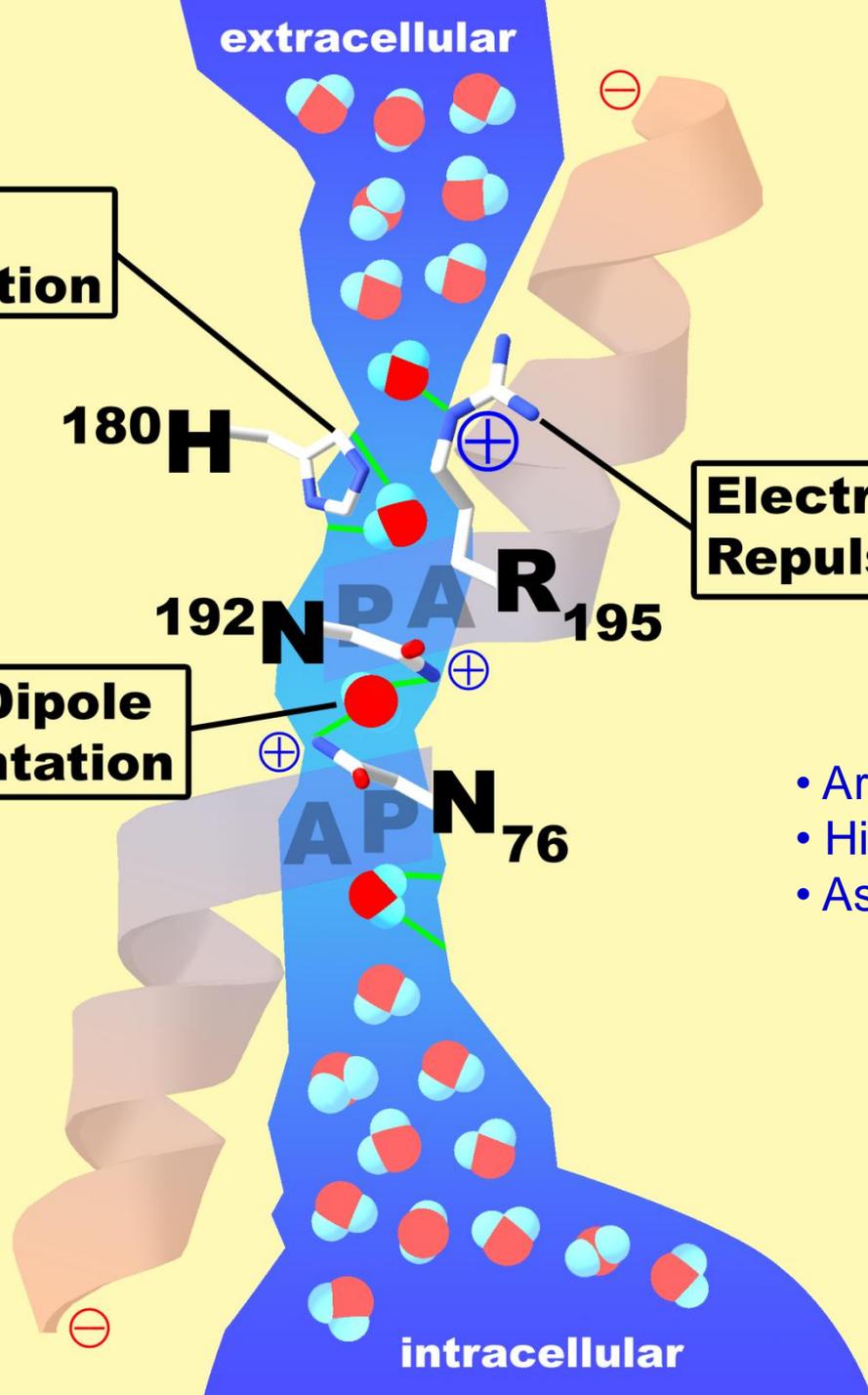
**Electrostatic
Repulsion**

**Water Dipole
Reorientation**

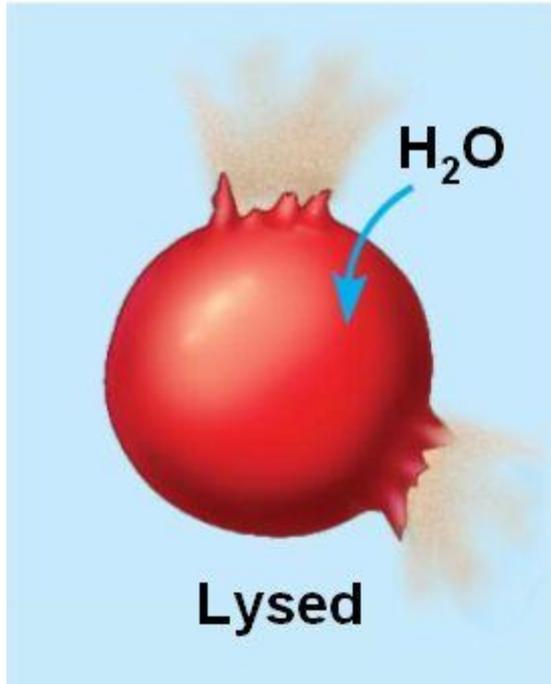
192N 195R

APN 76

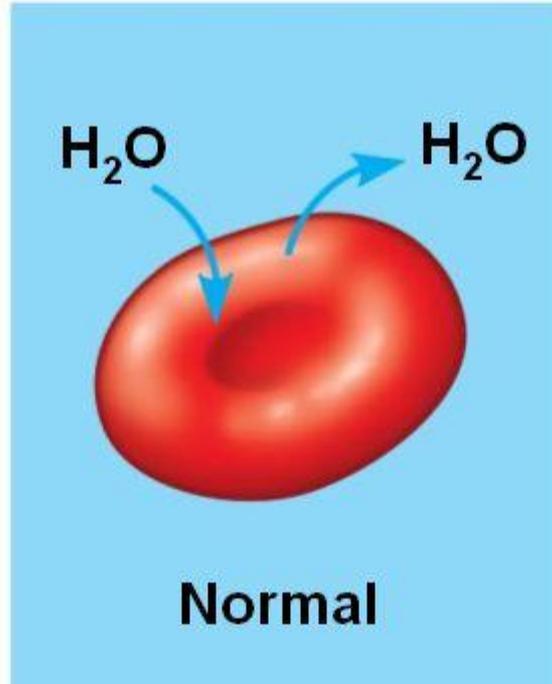
- Arg195
- His180
- Asn192 & 76



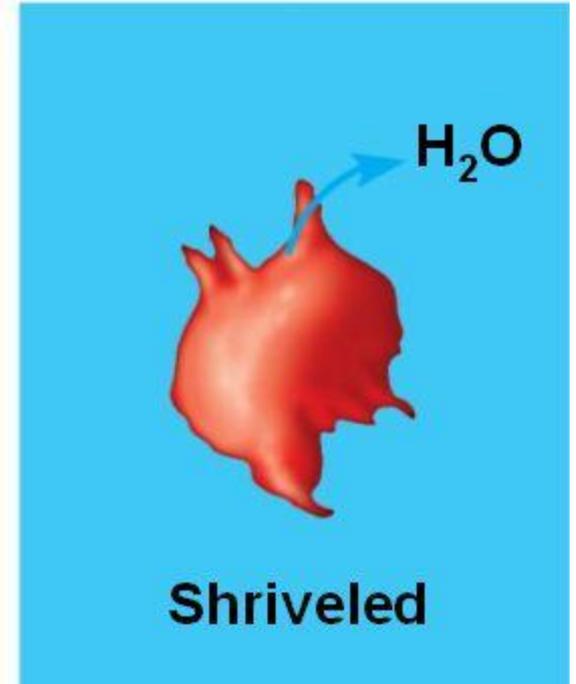
Hypotonic solution



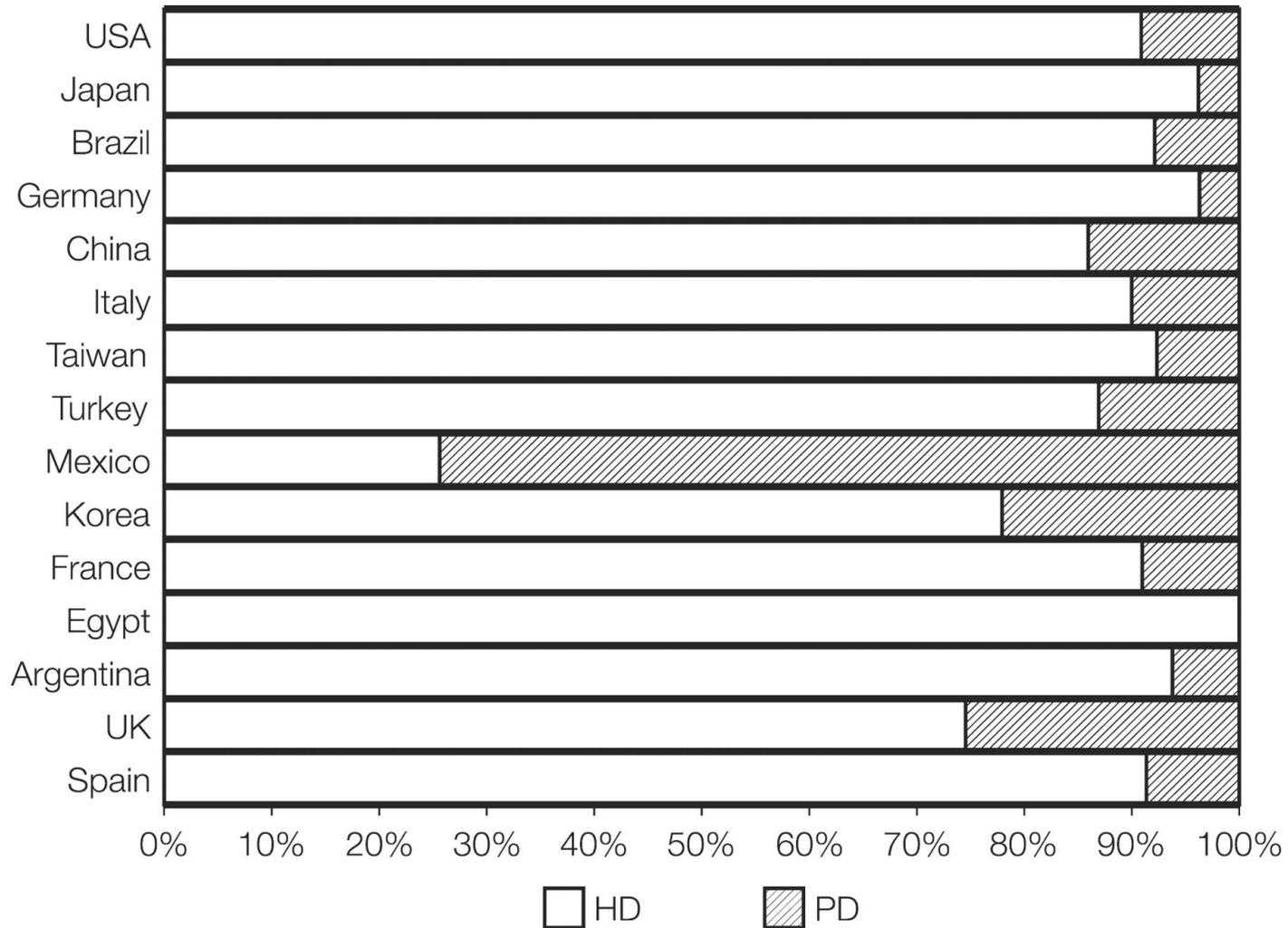
Isotonic solution



Hypertonic solution

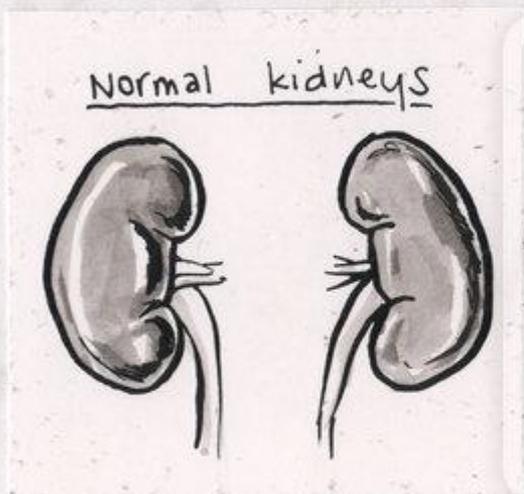


Comparison of HD and PD patient numbers in the 15 countries with the highest dialysis patient populations.



In 1995 I was diagnosed with POLYCYSTIC KIDNEY DISEASE.

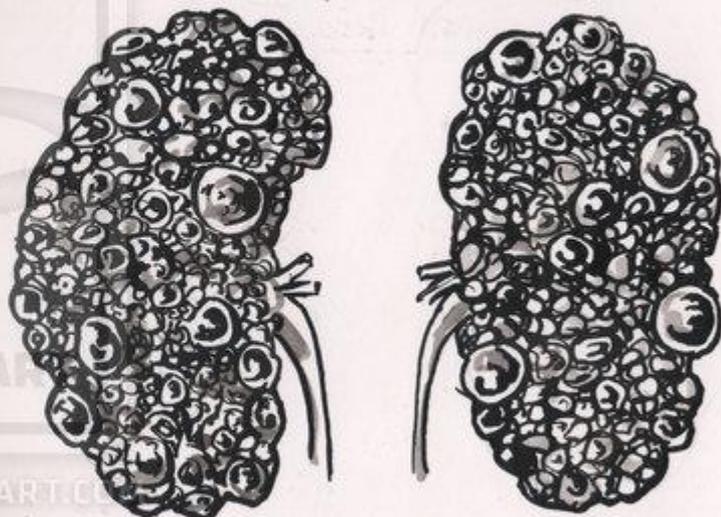
It was not a great surprise. It's a genetic condition, often hereditary. My Mum's father



had it, she and both her sisters had/have it, my brother (and I have it, three of my cousins have it. It usually begins to affect you as you get older. Over the years cysts grow on the

kidneys, causing them to grow in size and their function to gradually cease. High-blood pressure, infected cysts and urine infections are common side-effects.

POLYCYSTIC KIDNEYS



Eventually I will need dialysis or a transplant. I'll cross that bridge when I come to it.



Distribution of Human Aquaporins

Aquaporin	Permeability	Tissue distribution
AQP0	Water (low)	Lens
AQP1	Water (high)	Red blood cell, kidney, lung, vascular endothelium, brain, eye
AQP2	Water (high)	Kidney, vas deferens
AQP3	Water (high), glycerol (high), urea (moderate)	Kidney, skin, lung, eye, colon
AQP4	Water (high)	Brain, muscle, kidney, lung, stomach, small intestine
AQP5	Water (high)	Salivary gland, lacrimal gland, sweat gland, lung, cornea
AQP6	Water (low), anions ($\text{NO}_3^- > \text{Cl}^-$)	Kidney
AQP7	Water (high), glycerol (high), urea (high), arsenite	Adipose tissue, kidney, testis
AQP8 [†]	Water (high)	Testis, kidney, liver, pancreas, small intestine, colon
AQP9	Water (low), glycerol (high), urea (high), arsenite	Liver, leukocytes, brain, testis
AQP10	Water (low), glycerol (high), urea (high)	Small intestine

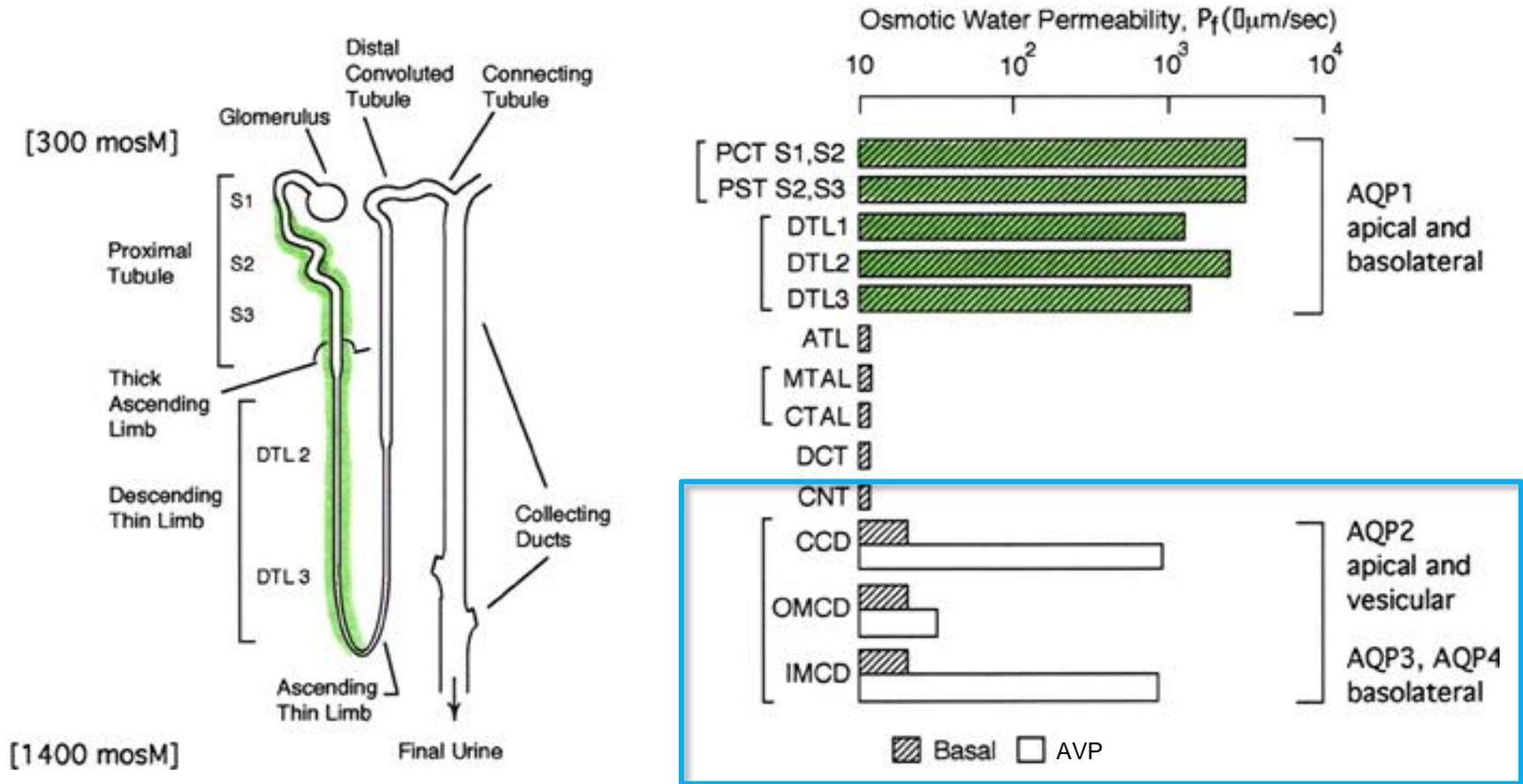
A quoi ça rime, l'Aquaporine ?

Théâtre

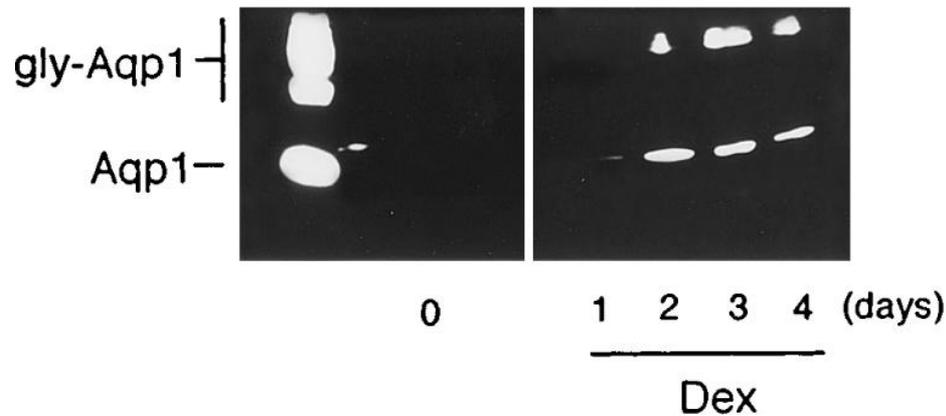
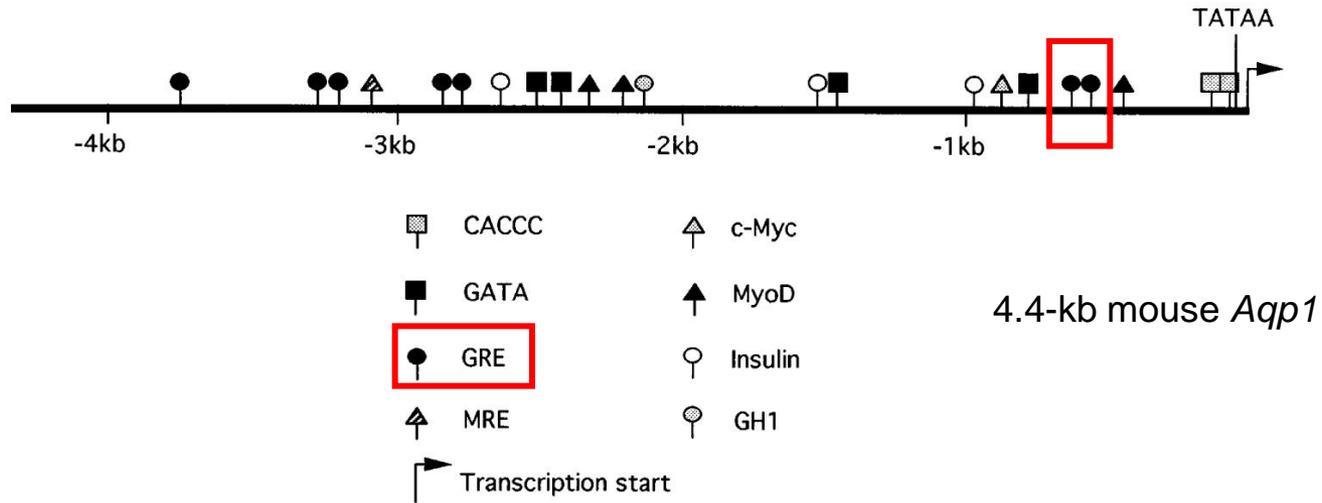


**Sylvie Déthiollaz
Vivienne Baillie Gerritsen**

Localization of Aquaporins & Water Permeability in Kidney

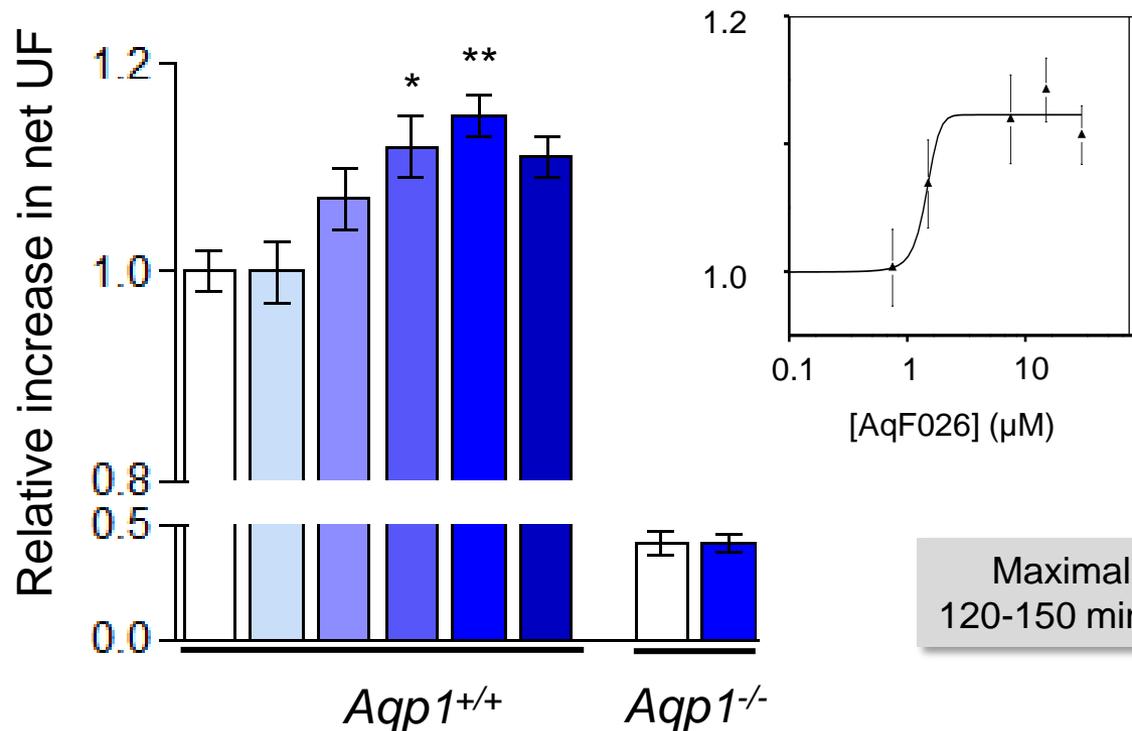


AQP1 Expression : Glucocorticoid Induction



In vivo Effect of AqF026: Dose-response

AqF026 (μM) \square 0 \square 1.5 \blacksquare 15
 \square 0.75 \square 7.5 \blacksquare 30



Maximal effect
120-150 min after i.v.

The effect of AqF026 is **dose-dependent**, with a maximal response observed for a concentration of **15 μM** and an **EC₅₀ value of 4.2 μM**