Immunoelectron microscopy

1) **Sectioning**: Ultrathin sections of osmicated renal tissue were mounted directly onto 200 mesh nickel grids (alternatively on formvar-coated 200 mesh nickel grids that are enhanced with carbon on each side).

2) **Antigen retrieval**: The sections were incubated in 3% NaI04 (Sodium periodate) for 1 h, and subsequently heated in citrate buffer (0.01 M, pH 6.0) at 144 °C for 15 min in sealed PCR-tubes in a wet autoclave (Certoclav, Sterilizer GmbH, Austria).

3) **Blocking**: An incubation step with 10% BSA (diluted in PBS, pH 7.2) for 4h at room temperature was introduced prior to immunolabeling to block non-specific labeling.

4) **Primary antibody**: The sections were incubated with anti-X in 10% BSA in PBS for 1h at 56°C, followed by rinsing 3 x 5 min in 3% BSA.

5) **Secondary antibody**: The sections were incubated with the secondary immunoreagent (antibodies coupled to 15 nm colloidal gold particles) in 3% BSA for 75 minutes at room temperature, followed by rinsing for 4 x 5 min in distilled water.

6) **Counterstaining**: Finally, the sections were routinely stained with uranyl acetate and lead citrate.

References:


Kindly provided by Dr Sverre-Henning Brorson (Oslo, Norway)