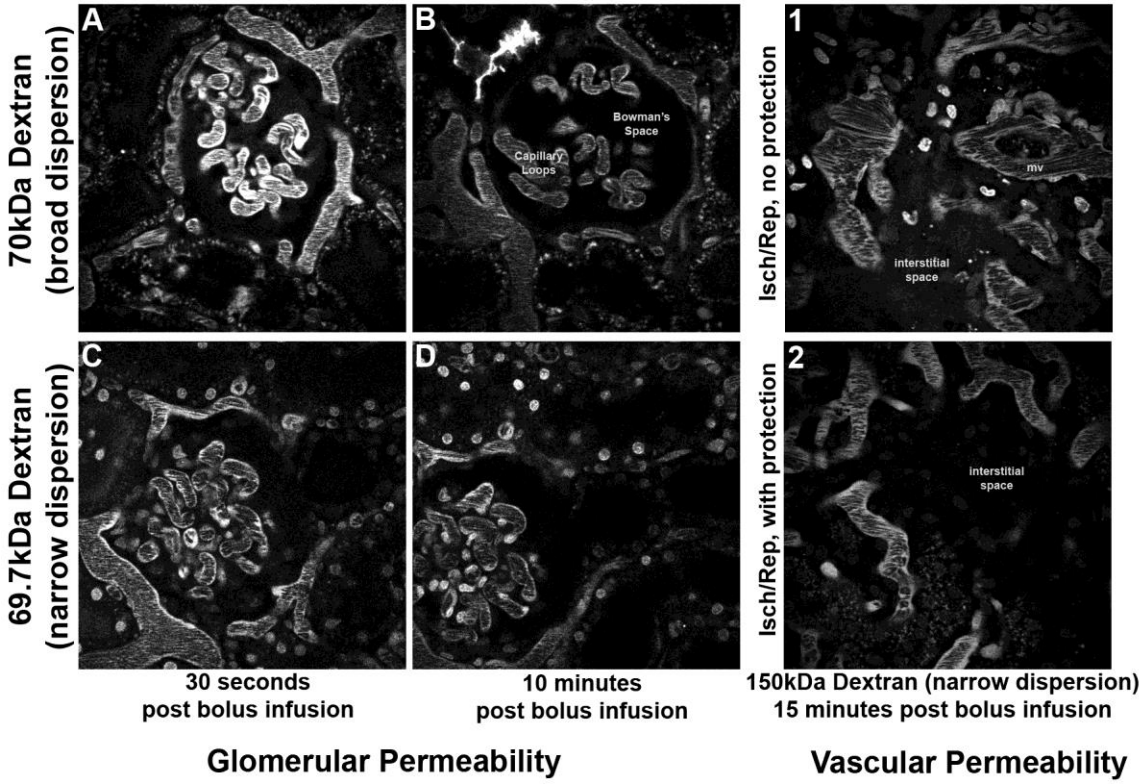
EXAMPLES OF INTRAVITAL STUDIES USING FLUORESCENT-DEXTRANS FROM DIFFERENT SOURCES (contribution by Ruben M Sandoval)

Note; The 69.7kDa FITC-dextran and the 150kD FITC-dextran were supplied by TdB Consultancy AB, SWEDEN



Glomerular Permeability

To better understand the data from the GSC experiments, images from those studies are shown in panels A-D. The capillary loops that show where the plasma intensities are taken, and the Bowman's Space that shows the urinary volume are labeled in panel B. Panels A and C show an image taken 30 seconds after the bolus infusion of the 70kDa and 69.7 kDa dextran, respectively. Note the filtration of large quantities of the small molecular weight dextran components into the Bowman's space in panel A, while panel C shows less material within the same space for the 69.7kDa dexran. Ten minutes after the initial bolus, the Bowman's Space for the 70kDa dextran (panel B) and the 69.7kDa dextran (panel D) appears clear, leading to the decrease in GSC seen for both dextrans.

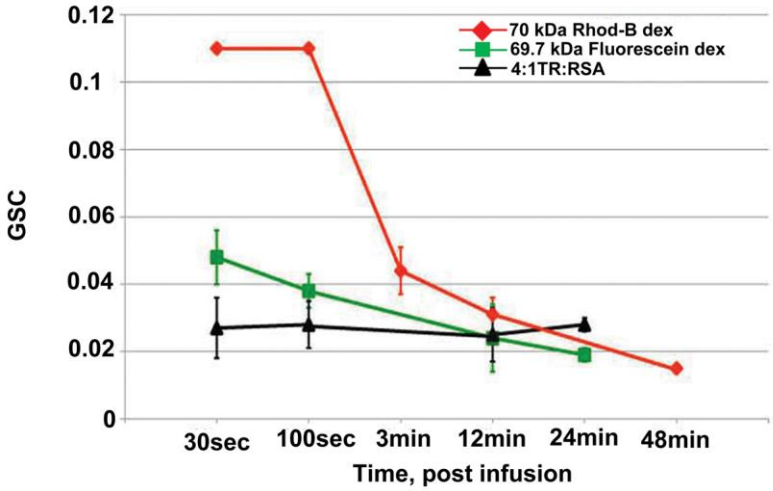
Vascular Permeability

The same principles that govern the behavior of dextran clearance by the glomerulus into the urinary space also apply the microvasculature and interstitial space (mv, interstitial space, labeled in panel 1). Normally, a fluorescent dextran

with a molecular weight of 150kDa remains tightly retained within the microvasculature and will not leak into the surrounding interstitial space. In a study looking at protection of vascular integrity after ischemia and reperfusion (panels 1 and 2), a clear difference between the conditions was seen 15 minutes after the bolus infusion. Panel 2 shows an image with a prominent section of interstitial space lacking any of the 150kDa dextran within; in sharp contrast to panel 1 showing a large amount of the compound within the defined space. Had the 150kDa dextran contained small molecular weight contaminants, no such difference in protection would have been determined, as the small contaminants would have freely crossed into the interstitial space despite the retention of microvascular integrity.

A portion of the glomerular permeability data was taken from **RM Sandoval *et al*. "Multiple factors influence glomerular albumin permeability in rats." *J Am Soc Nephrol*, 2012 Mar, 23(3)447-57**.

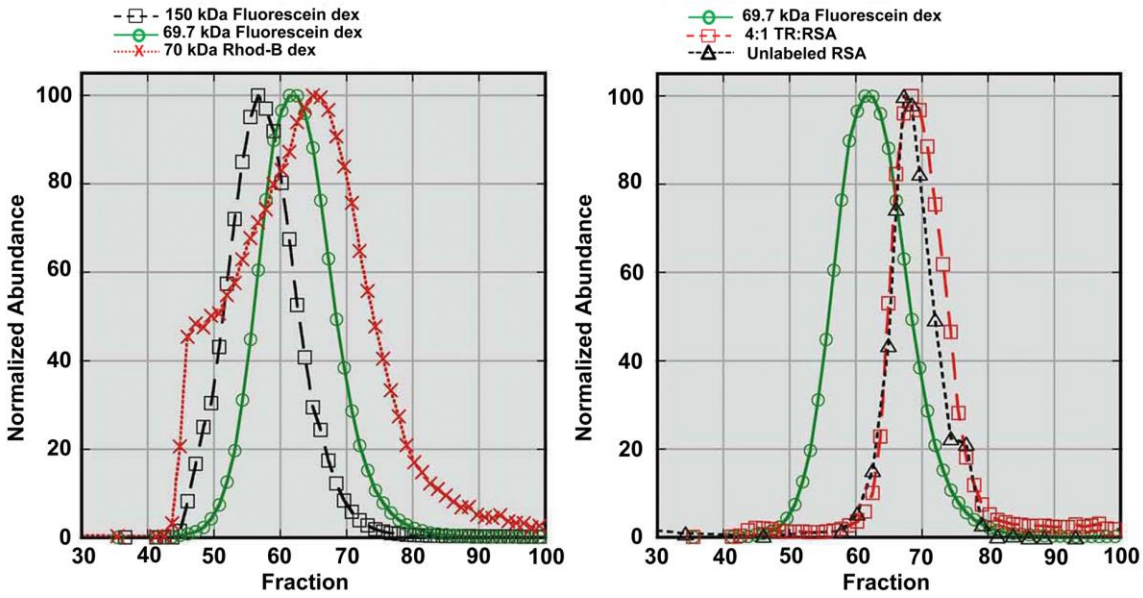
Timed Glomerular Sieving Coefficient (GSC):



Intravital 2-photon microscopy allows for the determination of the glomerular sieving coefficient of fluorescent compounds by taking the intensity within the Bowman's space (the urinary space) and dividing it by the intensity within the plasma; after respectively subtracting background. This value should remain constant (time independent) for a compound of uniform molecular weight. For compounds such as dextrans that have a range of sizes, this value will decrease over time as the smaller components are cleared first (leading to higher GSC values initially). The retention of the larger components within the vasculature will produce a decrease in GSC. In the graph, the GSC values for the 69.7kDa fluorescein dextran, Texas Red labeled rat serum albumin and the 70kDa Rhodmaine B dextran are shown. For each compound, a 100 second time series during the bolus infusion was taken followed by 3D image volumes at prescribed time points to determine the GSC. The value for the fluorescent albumin

remained constant throughout the study, reflecting the uniformity in size. The 69.7kDa fluorescein dextran had a slight but noticeable decrease in GSC over the span of the study due to its narrow size distribution of its constituent dextran components. In contrast, the 70kDa Rhodamine B dextran because of the very broad size distribution of its constituent components had a GSC value that spanned an order of magnitude for the duration of the study.

Gel Chromatography Studies



The two panels show the analysis of a series of compounds by gel filtration chromatography to determine the poly-dispersity of several fluorescent dextran conjugates. In both panels the fraction number at the bottom corresponds to decreasing molecular weights with increasing fraction number. Here, smaller molecules will interact with the matrix longer and take longer to elute from the column. All values from each fraction were normalized to the highest fluorescent reading.

The left panel shows a graph for a broadly dispersed 70kDa Rhodamine B Dextran, a narrowly dispersed 69.7kDa fluorescein dextran and a narrowly dispersed 150kDa fluorescein dextran from TdB. Fractions from both the 69.7 and 150 kDa dextrans produce narrow bell shaped curves. In contrast the competitors 70kDa dextran produces an irregular shaped curve that contain constituent dextrans that span a broad range of molecular weights, some larger than the 150kDa dextran and some that are freely filtered by the kidney.

The right panel shows a graph for the 69.7kDa dextran and unlabeled and Texas Red labeled rat serum albumin. The labeled and un-labeled rat serum albumin produce narrower curves than the dextrans analyzed because albumin is a globular protein having a singular molecular weight of 65kDa. Note the similar

shape of the curve between the rat serum albumins and the 69.7 and 150 kDa dextrans.

This data is taken from **RM Sandoval *et al*. ”Multiple factors influence glomerular albumin permeability in rats.” *J Am Soc Nephrol*, 2012 Mar, 23(3)447-57**.