# Stoichiometry Distribution of the Nephrin/Nck/WASP Signalsome

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***Short Abstract* – Nephrin is a transmembrane protein essential for the formation of actin-based foot processes in glomerular podocytes. Its cytosolic domain contains three tyrosine motifs which recruit Nck, a SH3-SH3-SH3-SH2 protein, upon phosphorylation. SH3 domains of Nck recruit WASP to form the Nephrin/Nck/WASP signalsome by binding the poly-proline motifs in WASP [1]. The multi-valences of the components may provide the signalsome multiple copies of each player and varying stoichiometry. The presence of multiple nephrin in the signalsome may promote nephrin clustering and the oligomerization of WASP may be responsible for the integrity of the actin cytoskeleton. Here, we are investigating the hypothesis.**

## I. Purpose

In the foot processes of the glomerular podocytes, nephrin clusters to form the cell-cell junctions, the slit diaphragm [2]. Over sixty nephrin mutations have been discovered in congenital nephritic syndrome of the Finnish type, a disease whose hallmark is the absence of the slit diaphragm [2]. However, the molecular basis of nephrin clustering is currently unknown.

Proper control of the actin cytoskeleton by the nephrin/Nck/WASP signalsome in the foot process is essential for glomerular filtration [3]. The molecular mechanism by which the singalsome regulates actin polymerization is also unclear.

Every component of the Nephrin/Nck/WASP signalsome has multiple binding sites to recruit several copies of other components to form supramolecular complexes. Pawson and coworkers show that the multi-valences of at least nephrin or Nck are required to maintain integrity of the actin cytoskeleton [3]. An interesting hypothesis is that the multi-valences of the signalsome drive nephrin clustering by non-covalently cross-linking multiple nephrin and controls the activity towards Arp2/3 complex, an actin nucleator, by oligomerizing WASP. The ongoing project will test the hypothesis by studying the population distributions of nephrin and WASP in the stoichiometry-undefined signalsome. The main goal is to characterize the stoichiometry distribution of Nephrin/Nck/WASP signalsome in a reconstituted membrane tethered system by single molecule analyses.

## II. Results and Experimental Designs

### *A. Dimerization increases WASP activity*

Dimerizing agents of WASP, including dimeric Nck SH3, substantially increases WASP activity towards Arp2/3-mediated actin polymerization [4].

### *B. Biochemical characterization*

We will generate membrane-tethered, phosphorylated, and fluorophore-labeled C-terminal domain of nephrin. In addition, we will engineer fluorophore-labeled WASP proteins containing varying numbers of SH3 binding motifs.

We will characterize the binding affinities of these proteins using ITC and fluorescence anisotropy. We will also perform pyrene actin polymerization assays to assess how the multi-valences of the signalsome affect the activity of Arp2/3 complex.

### *C. Single molecular measurements*

We will carry out single molecular measurement of the membrane tethered nephrin/Nck/WASP singalsome, in which nephrin and WASP are labeled with different fluorophores. In these measurements, we will determine stoichiometry distribution of nephrin and WASP in the signalsomes as a function of the concentrations of the players. The distribution of WASP in the singalsomes will show the correlation of the oligomeric states of WASP with its activity towards Apr2/3 activation. The distribution of nephrin will reveal the role of multi-valences of the singalsome in forming the slit diaphragm.

### *D. Modeling and computer simulations*

Ordinary differential equations with deterministic variables are formulated for high concentration scenarios, and models including stochasticity for low concentration scenarios will be formulated. Computer modeling will be carried out. Predictions from the modeling will be compared with experimental observations in bulk biochemical assays and single molecule studies.

## III. Future Directions

Over the longer term, we plan to investigate the dynamic behavior of the signalsome.

## References

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