~12-ms interaction with the NPC, primarily follow the periphery on the nucleo-
oplasmic side and in the center of the NPC without occupying central axial
utilizing for passive diffusion of small molecules, and eventually dis-
sociating from the cytoplasmic side.

621-Pos Board B390
Charge Effects on Nucleocytoplasmic Transport Studied by Single-
Molecule Microscopy
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In eukaryotic cells, the transport of genetic material and proteins between
nucleus and cytoplasm is mediated by the nuclear pore complexes (NPCs) em-
bedded in the double-membrane nuclear envelope. A highly selective barrier
formed by phenylalanine-glycine (FG)-nucleoporin (Nup) with net positive
charge in the NPC allows for passive diffusion of signal-independent small
molecules (< 40 kDa) and transportin-facilitated translocation of signal-
dependent large molecules (up to 50 MDa). Previously it was suggested that
the positively charged FG-barrier would inhibit the transport of positively
charged transiting molecules. However, the fundamental inhibition mecha-
nism and the detailed pathways for both transport modes remain poorly under-
stood. Here, we employ an innovative single-molecule technique, single-point
edge-excitation subtraction (SPEED) microscopy, to track fluorescent sin-
gle molecules with different charges transiting through single native NPCs.
We have obtained the transport kinetics and the 3D transport pathways for
both passive diffusion of variously charged GFPs (27 kDa) and facilitated
translocation of transportin-cargo complexes with different charges. Our re-
sults indicate that (i): the positively charged selective barrier in the NPC pos-
sesses an axial central conduit for passive diffusion of small molecules with
different charges, and (ii) for transportin-facilitated translocation of differ-
tently charged cargos via the peripheral regions around the central conduit; and
(ii) the positively charged environment in the NPC significantly affects the
transport pathway, transport time and efficiency for both passive and facili-
tated transport.

Ryanodine & IP3 Receptors I

622-Pos Board B391
Exaggerated [Ca\(^{2+}\)]; Signaling and Alzheimer’s Disease-Like Phenotypes of
PS1M146V Mice are Attenuated by Decreasing Brain InsP, R1-Protein Levels
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Familial Alzheimer’s disease (FAD) is linked to mutations in the presenilin
(PS) homologs. FAD mutations have several cellular consequences, including
exaggerated intracellular Ca\(^{2+}\); ([Ca\(^{2+}\)]\(_i\)) signaling - enhanced sensitivity to ag-
onists of ER Ca\(^{2+}\); release and increased magnitudes of [Ca\(^{2+}\)]; signals. Single
channel recordings suggest exaggerated [Ca\(^{2+}\)]; signaling is due to a gain-of-
function interaction between FAD mutant PS and the inositol 1,4,5-trisphos-
phate receptor (InsP\(_3\);R, InsPR). However, the in vivo significance of the PS-InsP, R
interaction to exaggerated [Ca\(^{2+}\)]; signaling and the contribution of exagger-
ated [Ca\(^{2+}\)]; signaling to the development of AD are unknown. If exaggerated
[Ca\(^{2+}\)]; signaling is InsP\(_3\);R-dependent, we hypothesize that decreasing InsP\(_3\);R
protein expression will normalize [Ca\(^{2+}\)]; signaling. Therefore, we crossed the
opisthotonus (Opt) mouse, deficient in InsP\(_3\);R1 protein, to the PS1M146V
knock-in (PS1KIN) AD mouse model to investigate the contribution of
InsP\(_3\);R1-mediated [Ca\(^{2+}\)]; release to exaggerated [Ca\(^{2+}\)]; signaling, and the role
of exaggerated [Ca\(^{2+}\)]; signaling to the AD-like phenotypes of this mouse
model. We observed that the Opt allele normalizes exaggerated [Ca\(^{2+}\)];
release in vitro and ex vivo. Interestingly, in young (5wk) and mature
(3mth) PS1KIN mice we observed that the Opt allele normalized aberrantly
activated hippocampal biochemical pathways and the enhancement of CA3-
CA1 early long-term potentiation present in these mice - phenotypes that mirror
the early hippocampal hyperactivity in patients that later develop AD. These
findings indicate that FAD mutant PS-associated exaggerated [Ca\(^{2+}\)]; signaling
is InsP\(_3\);R1-dependent and a early event in Alzheimer’s disease.

623-Pos Board B392
Imaging the Motility of Inositol Trisphosphate Receptors in Intact
Mammalian Cells using Single Particle Tracking Photoactivated Localiza-
 tion Microscopy (Spptalm)
Ian Smith, Divya Swamithan, Ian Parker.
Neurobiology and Behavior, Irvine, CA, USA.
Inositol trisphosphate receptors (IP3\(_3\);Rs) are calcium-permeable channels in
the membrane of the endoplasmic reticulum (ER) that liberate calcium to generate
cytosolic calcium signals that control diverse cellular functions including gene
expression, secretion and synaptic plasticity. The spatial distribution of these
channels is crucial in determining the patterning of intracellular calcium sig-
nals. The mechanisms underlying the aggregation and maintenance of IP3\(_3\);Rs
into clusters are controversial. Local calcium puffs reflecting concerted open-
ings of clustered IP3\(_3\);Rs arise at just a few, fixed locations within a cell, suggest-
ing clusters are stable entities; and calcium blips generated by ‘lone’ IP3\(_3\);Rs are
also immotile. In contrast, GFP-tagged or immunostained IP3\(_3\);Rs show a dense
distribution throughout a cell. Moreover, the majority IP3\(_3\);Rs can diffuse freely
within the ER membrane, and aggregate into clusters following activation of
IP3 signaling and/or cytosolic calcium elevation. These apparently different
behaviors may be explained because calcium imaging detects only functional
IP3\(_3\);Rs, whereas immunostained or GFP-tagged IP3\(_3\);Rs report the entire popula-
tion of IP3\(_3\);R proteins. We therefore hypothesize that most IP3\(_3\);Rs are motile, but
functionally unresponsive. Local calcium signals arise, instead, from a small
subset of IP3\(_3\);Rs that are anchored, individually or in clusters, by association
with static cytoskeletal structures and possibly as a consequence of this anchor-
ing, display high sensitivity to IP3 to produce calcium signals. To test this
hypothesis we expressed type 1 IP3\(_3\);R tagged with a photoactivatable geneti-
cally encoded protein to track the motility of thousands of individual IP3\(_3\);R
molecules with nanoscale spatial and millisecond temporal resolution. We find
that IP3\(_3\);Rs can be distinguished into two groups with relatively high or
low motility, and that the apparently immotile IP3\(_3\);Rs are preferentially grouped
within tight clusters.

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624-Pos Board B393
Oligomerisation of the Inositol Trisphosphate Receptor Amino-Terminus
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The inositol 1,4,5-trisphosphate receptor (IP\(_3\);R) is a ubiquitously expressed
intracellular calcium release channel composed of four ~300kDa subunits.
Calcium signalling mediated via the IP\(_3\);R and the related rydanoide receptor (RYR)
is involved in numerous cellular processes including fertilisation, gene trans-
scription, neurotransmitter release and muscle contraction. Recently, we pre-
presented evidence that the RyR2 N-terminus self-associates into a tetrameric
form, which stabilises the closed conformation of the channel.

Given that the IP\(_3\);R and RyR amino-termini have a high degree of similarity in
both their protein sequence (~40%) and three-dimensional structure, we inves-
tigated the oligomerisation properties of the IP\(_3\);R N-terminus. A type 1 IP\(_3\);R
fragment (residues 1-667) was expressed in mammalian HEK293 cells and sub-
jected to chemical cross-linking. We found that the IP\(_3\);R N-terminal fragment
is capable of oligomerisation, forming predominantly tetramers. The presence
of a 15 amino acid splice insertion or of the cognate ligand, IP\(_3\), did not affect
tetramerisation. The IP\(_3\);R N-terminus self-interaction was further verified by
co-immunoprecipitation assays.

These studies indicate the putative involvement of the N-terminal region in
IP\(_3\);R oligomerisation. It is therefore possible that, in addition to its direct
role in ligand binding, the IP\(_3\);R N-terminus is involved in regulation of the
channel via inter-subunit interactions and that these two processes may be
directly linked.

625-Pos Board B394
Cytoplasmic [InsP\(_3\);] Drop Induces Transient High-Open-Probability
Gating Mode in Type 1 InsP\(_3\);R Channels
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Single-channel activities of homotetrameric recombinant rat type 1 InsP\(_3\);R
channels expressed in mutant DT40-3K0 cells with no endogenous InsP\(_3\);R
expression were studied in nuclear patch clamp experiments. In-nucleus ex-
periments with steady ligand conditions, the channel exhibits modal gating with
at least two modes: a high-\(P_o\) mode with \(P_o = 0.4-0.6\) and mean open duration
\((t_o) = 3\) ms; and a low-\(P_o\) mode with \(P_o < 0.05\) but \(t_o \text{ still} ~ 3\) ms. Cytoplasmic
free Ca\(^{2+}\); concentration ([Ca\(^{2+}\)]\(_o\)) regulates channel \(P_o\) biphasically by chang-
ing the likelihood of the channel being in the high-\(P_o\) mode and the mean
closed duration \((t_c)\) of the low-\(P_o\) mode. Even under optimal ligand conditions
([Ca\(^{2+}\)]\(_o\) = 2 \(\mu\)M and cytoplasmic InsP\(_3\); concentration \([\text{InsP}\(_3\);] = 10 \(\mu\)M), the
maximum \(P_o\) observed was only ~ 0.1, with the channel being in the low-
\(P_o\) mode the majority of time. InsP\(_3\) (33 nM to 10 \(\mu\)M) activates the channe-
by reducing its sensitivity to Ca\(^{2+}\); inhibition. In experiments with
cytoplasmic-side-out nuclear membrane patches, when initial [Ca\(^{2+}\)]\(_o\), was