Ph.D. Preliminary Examination

Single Molecule Fluorescent Measurements of Complex Systems

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Talk outline

- **Goal 1.** Critical exponents describing $1/f$ noise for Intermittent QDs.
- **Goal 2.** Distribution of $1/f$ noise for intermittent QDs.
- **Goal 3.** Compartmentalization of the surface of mammalian cells by cortical actin.
- **Goal 4.** Examination of Cav1.2 and beta 2 adrenergic receptor dynamics at the Kv2.1/ER/PM junction.
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Goal 1. Introduction
Power Spectral Density (PSD)

\[ S(f) = \lim_{T \to \infty} \frac{\left( \int_0^T I(t)e^{-2\pi ft} dt \right)^2}{T} \]
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\[ S_T(f) = \lim_{T \to \infty} \frac{\left( \int_{0}^{T} I(t) e^{-2\pi f t} dt \right)^2}{T} \]

\[ = \frac{(\Delta t)^2}{T} \left| \sum_{n=1}^{N} I(t_n) e^{-2\pi f n \Delta t} \right|^2 \]

\[ t_1 \quad t_2 \quad T = N\Delta T \]
Power Spectral Density (PSD)

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\[ = \frac{(\Delta t)^2}{T} \left| \sum_{n=1}^{N} I(t_n)e^{-2\pi fn} \right|^2 \]
$1/f$ noise (Pink noise)

$$S(f) \sim \frac{1}{f^\beta} \quad \text{with} \quad 0 < \beta < 2$$
1/$f$ noise (Pink noise)

- Occurs in many physical, biological and economic systems.
  - Evolution
  - Network traffic
  - Earthquakes
  - Semiconductor devices
  - Nanoscale electrodes
  - Organic fluorophores

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1/f noise (Pink noise)

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• No universal model.

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1/f noise (Pink noise)

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- No universal model.
- "Infrared catastrophe" ($\beta \geq 1$), violation of Parseval theorem.

$$S(f) \sim \frac{1}{f^\beta} \quad \text{with} \quad 0 < \beta < 2$$

$$\lim_{f_{\min} \to 0} \int_{f_{\min}}^{f_{\max}} S(f) df \to \infty$$
$1/f$ noise (Pink noise)

- Occurs in many physical, biological and economic systems.
- No universal model.
- “Infrared catastrophe” ($\beta \geq 1$), violation of Parseval theorem
  - Exists a transition frequency
  - Process is non-stationary

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Intermittent systems
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• Random switching between system microstates
• Power law (heavy tail) waiting times
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- Time averages remain random variable
Intermittent systems

- Random switching between system microstates
- Power law (heavy tail) waiting times
- No characteristic time
- Time averages remain random variable
- Non-stationary
An intermittent system: quantum dots

Core (CdSe)

Shell (Zns)

$2 - 10 \text{ nm}$

On state

$\psi(\tau) \sim t^{-(1+\alpha)}$

$S(f) \sim 1/f^\beta$

Off state

http://www.invitrogen.com
$1/f$ noise and intermittency
(Theoretical predictions)

On state $\psi(t) \sim t^{-(1+\alpha)}$  $S(f) \sim 1/f^\beta$

Off state

Theoretical prediction: $\langle S_T(f) \rangle \sim \frac{T^{\alpha-1}}{|f|^{2-\alpha}}$ as $\omega \to 0$ ($0 < \alpha < 1$)

Goal1. Results
Quantum Dot blinking

On state

Off state
Distribution of waiting times (1200 QDs)

\[ \psi(\tau) \sim \tau^{-(1+\alpha)} \]

\[ \alpha = 0.63 \]
Waiting times are power law distributed:

\[ S(f) \sim \frac{1}{f^\beta} \, , \quad \beta = 2 - \alpha = 1.37 \]

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\[ \text{Margolin and Barkai, J Stat Phys (2006)} \]

Truncation in on times:

\[ S(f) \sim \frac{1}{f^{\beta'}} \, , \quad \beta' = 2 - \alpha = 1.37 \]

\[ \beta_\text{<} = \alpha = 0.63 \]
Waiting times are power law distributed:

\[ S(f) \sim \frac{1}{f^{\beta}} , \quad \beta = 2 - \alpha = 1.37 \]

Truncation in on times:

\[ S(f) \sim \frac{1}{f^{\beta}} , \quad \beta_\leq = 2 - \alpha = 1.37 \]
\[ \beta_\geq = \alpha = 0.63 \]

Aging exponent

![Diagram showing aging exponent with logarithmic scales for PSD and frequency (f)].
Aging exponent

A

\[
\text{PSD (Hz}^{-1}) \quad f (\text{Hz})
\]

B

\[
\text{PSD} \times t^{0.12} \quad (\text{Hz}^{-11.2}) \quad f (\text{Hz})
\]
Aging exponent

Aging exponent = 0.12
Total power

If \( S(f) \sim \frac{1}{f^\beta} \) with \( \beta \geq 1 \), then,

\[
\lim_{f_{\min} \to 0} \int_{f_{\min}}^{f_{\max}} S(f) df \to \infty
\]

Infrared catastrophe
Total power

If \( S(f) \sim \frac{1}{f^\beta} \) with \( \beta \geq 1 \), then,

\[
\lim_{f_{\text{min}} \to 0} \int_{f_{\text{min}}}^{f_{\text{max}}} S(f)\,df \to \infty
\]

Transition frequency

Aging
Total power

- Existence of a transition frequency and aging of the PSD yields a finite total power.

Problem solved!
Timeline

1. Experiments (completed)
2. Analysis (completed)
3. Manuscript (published)
• Goal 1. Critical exponents describing $1/f$ noise for Intermittent QDs.

• **Goal 2.** Distribution of $1/f$ noise for intermittent QDs.

• Goal 3. Compartmentalization of the surface of mammalian cells by cortical actin.

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Theoretical predictions

• The PSD of two-state systems with power-low sojourn times remains a random variable in the limit of long time measurements.

Niemann et al., PRL. 2013.
Theoretical predictions

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• Large fluctuations should be observed between different power spectra of identical particles.

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Theoretical predictions

• The PSD of two-state systems with power-low sojourn times remains a random variable in the limit of long time measurements.
• Large fluctuations should be observed between different power spectra of identical particles.
• The distribution of PSD amplitudes converges to a Mittag-Leffler distribution.

Mittag-Leffler distribution

\[ ML(x, \alpha) = \frac{1}{\Gamma(1 + \alpha)} \left( \frac{1}{\alpha x^{1 + \frac{1}{\alpha}}} \right) l_\alpha \left( \frac{1}{\Gamma(1 + \alpha)} \right) \frac{1}{x^\alpha} \]

Gaol2. Results
Fluctuation of $1/f$ noise
Fluctuation of $1/f$ noise raw data

$$S(f) = \frac{A}{f^\beta}$$
Fluctuation of $1/f$ noise
raw data

$S(f) = \frac{A}{f^\beta}$

Closest ML distribution
With $\alpha = 0.82$
Fluctuation of $1/f$ noise
Thresholded data
Fluctuation of $1/f$ noise
Thresholded data
Fluctuation of $1/f$ noise
Thresholded data

Closest ML distribution
With $\alpha = 0.89$
Timeline

1. Experiments (completed)
2. Analysis (May 2015)
3. Manuscripts submission (May 2015)
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Goal 3. Introduction
Cell membrane and cortical actin

Engelke et al. *Physical biology* 2010

www.scs.illinois.edu
Molecules undergo hop diffusion in the plasma membrane

Transmembrane proteins anchored to the membrane skeleton
Phospholipid
Membrane skeleton

Molecules undergo hop diffusion in the plasma membrane

Transmembrane proteins anchored to the membrane skeleton

Membrane skeleton

Phospholipid


• Diffraction limit ~250 nm much larger than actin bundles (~40 nm)
Super resolution imaging of cortical actin

PhotoActivated Localization Microscopy (PALM)

Goal 3. Results

This project was started by a former student of our lab, Jenny Higgins.
TIRF vs. PALM
Watershed transformation
Characterization of compartments
live cells
Characterization of compartments fixed cells
Actin acts as a semipermeable fence for membrane proteins
Actin acts as a semipermeable fence for membrane proteins

Potassium channels (Kv2.1 and Kv1.4)
Actin acts as a semipermeable fence for membrane proteins

![Imagery](image.png)

**Bar Chart**

- **Kv2.1**: 0.2
- **Kv1.4**: 0.4
- **RW**: 0.6
Actin acts as a semipermeable fence for membrane proteins
Actin barriers play a significant role in membrane protein anomalous diffusion
Time line

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Goal 4. Introduction
Endoplasmic reticulum-plasma membrane (ER-PM) junction

Stefan CJ et al. *Current opinion in cell biology* 2013
Endoplasmic reticulum-plasma membrane (ER-PM) junction

Stefan CJ et al. *Current opinion in cell biology* 2013
ER–PM junction function in different cell types

ER-PM contact sites regulate:
- Ca^{2+} transport & dynamics
- phosphoinositide signaling
- tyrosine kinase signaling
- sterol lipid transfer
- membrane trafficking

Stefan CJ et al. Current opinion in cell biology 2013
ER-PM junctions in neurons

• First were observed in 1962 by electron microscopy.
ER-PM junctions in neurons

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• Their structure, components and function are not well understood.
ER-PM junctions in neurons

• First were observed in 1962 by electron microscopy.
• Their structure, components and function are not well understood.
• ER-PM junctions are regulated by stroke-related neuronal insults.
The ultimate goal

Fig. 1. Project overview

This Figure is courtesy of Dr. Tamkun
The ultimate goal

This Figure is courtesy of Dr. Tamkun
HEK cells as a model system for neurons

- Similar properties to immature neurons.
- Easy to culture and transfect.
- Possible to control the expression level of desired proteins.

www.celeromics.com
HEK cells as a model system for neurons

- Similar properties to immature neurons.
- Easy to culture and transfect.
- Possible to control the expression level of desired proteins.

Fox PD et al. *Molecular biology of the cell* 2013
Cav1.2 Channel can assemble with beta2 adrenergic receptors (β2ARs)

Davare et al., PRL. 2001.
Cav1.2 Channel can assemble with beta2 adrenergic receptors (β2ARs) 

Davare et al., PRL. 2001.

- The kinetics of this association and how these complexes relate to the Kv2.1/ER/PM junctions are unknown.
Goal 4. Progress up-to-date and research plan
Cav1.2 visualization in HEK cells
Cav1.2 channels are co-localized with Kv2.1 clusters

This Figure is courtesy of Dr. Tamkun
Research plan

1. Multi-color imaging of Cav1.2, β2AR, and ER-PM junctions
2. Labeling Cav1.2 and β2AR with QDs and image them
3. Track these channels individually and look at their interactions with each other and with ER-PM junctions in presence and absence of Kv2.1.
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  - Philip Fox

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- Dinah Loerke - DU (Tracking algorithms)
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