

Video Article

In vivo Quantification of G Protein Coupled Receptor Interactions using Spectrally Resolved Two-photon Microscopy

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Abstract

The study of protein interactions in living cells is an important area of research because the information accumulated both benefits industrial applications as well as increases basic fundamental biological knowledge. Förster (Fluorescence) Resonance Energy Transfer (FRET) between a donor molecule in an electronically excited state and a nearby acceptor molecule has been frequently utilized for studies of protein-protein interactions in living cells. The proteins of interest are tagged with two different types of fluorescent probes and expressed in biological cells. The fluorescent probes are then excited, typically using laser light, and the spectral properties of the fluorescence emission emanating from the fluorescent probes is collected and analyzed. Information regarding the degree of the protein interactions is embedded in the spectral emission data. Typically, the cell must be scanned a number of times in order to accumulate enough spectral information to accurately quantify the extent of the protein interactions for each region of interest within the cell. However, the molecular composition of these regions may change during the course of the acquisition process, limiting the spatial determination of the quantitative values of the apparent FRET efficiencies to an average over entire cells. By means of a spectrally resolved two-photon microscope, we are able to obtain a full set of spectrally resolved images after only one complete excitation scan of the sample of interest. From this pixel-level spectral data, a map of FRET efficiencies throughout the cell is calculated. By applying a simple theory of FRET in oligomeric complexes to the experimentally obtained distribution of FRET efficiencies throughout the cell, a single spectrally resolved scan reveals stoichiometric and structural information about the oligomer complex under study. Here we describe the procedure of preparing biological cells (the yeast *Saccharomyces cerevisiae*) expressing membrane receptors (sterile 2 α -factor receptors) tagged with two different types of fluorescent probes. Furthermore, we illustrate critical factors involved in collecting fluorescence data using the spectrally resolved two-photon microscopy imaging system. The use of this protocol may be extended to study any type of protein which can be expressed in a living cell with a fluorescent marker attached to it.

Video Link

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Protocol

1. Plasmid design

The proteins of interest are fused to one of two different fluorescent labels, as described next. The fluorescent labels not only provide information about the location of the proteins within the cell, but also quantitative information regarding the homo-oligomerization of the protein¹. The technique of Fluorescence Resonance Energy Transfer (FRET)²⁻⁴ is used to accumulate the information about the protein interactions⁵⁻¹⁰. FRET utilizes the principle that a transfer of energy can occur from an optically excited molecule (typically referred to as the donor, D) to an unexcited molecule (typically referred to as acceptor, A) via dipole-dipole interactions¹¹, if the two molecules come in close proximity to one another. In designing the plasmids encoding the fusion proteins, two key features should be borne in mind, as follows.

1. Selecting a compatible pair of fluorescent tags is of the utmost importance in FRET studies. Ideally, the fluorescent tag combination must meet two criteria: an appreciable overlap in wavelengths between the emission spectrum of the donor tag and the excitation spectrum of the acceptor tag, and a large separation between the center wavelength of the laser pulse spectrum and the excitation spectrum of the acceptor (i.e., large Stokes shift). Two variants of the green fluorescent protein^{12, 13} are particularly well suited to meet these criteria: the GFP₂ as a donor¹⁴ and the yellow fluorescent protein (YFP)¹² or a variant of it called Venus¹⁵ as the acceptor. Cerulean¹⁶ could also be used as a donor, though with somewhat more modest results.
2. In quantitative FRET imaging it is best if only one donor in a molecular complex is excited at a time (on average), so that no competition occurs between donors for transferring energy to the same acceptor in complexes that contain more than one donor and one acceptor. This leads to simplifications in the theory and data analysis process¹⁷. Because of that, the signal level is usually low. To compensate for this, the

expression level of the proteins of interest should be relatively high. High expression levels are achieved by using a high-copy plasmid to carry the protein gene into the cell.

2. Yeast transformation

It is advantageous to maximize the percentage of the cell population expressing the protein constructs of interest. For this purpose, we transform an auxotrophic strain of yeast cells (*Saccharomyces cerevisiae*), unable to produce tryptophan or uracil, with two different plasmids, one containing the selectable marker tryptophan and one the marker uracil. The plasmids also contain the genes which carry instructions for the cell to express the protein of interest tagged with one of the two fluorescent probes. The transformed cells are grown on solid medium plates which also lack tryptophan and uracil. At least three types of cells should be transformed: cells expressing the proteins of interest tagged with both types of fluorescent probes, cells expressing only proteins tagged with the donor fluorescent probes and cells expressing only proteins tagged with the acceptor fluorescent probes.

1. Prepare solid medium plates (1.7 g/L of yeast nitrogen base w/o amino acids, 1.6 g/L yeast synthetic dropout medium w/o uracil and tryptophan, 2% [w/v] glucose, 2% [w/v] agar) to be used as the growth medium for the transformed cells at least one day prior to the cell transformation. The solid medium plates can be used for up to two months after preparation if they remain free of any contaminant.
2. For each plasmid pair to be transformed, add 10 mL of YPD (10 g/L Bacto Yeast Extract, 20 g/L Bacto Peptone, and 2% [w/v] glucose) to an erlenmeyer flask. Inoculate the YPD with a colony of the auxotrophic strain of yeast cells to be transformed.
3. Place the erlenmeyer flask containing the yeast culture in a laboratory shaker, which provides an orbital swirling action on the samples, and incubate overnight at 30° C. The following morning, start monitoring the growth of the cell culture by periodically removing a small volume of the culture and testing its optical density (OD). The growth of the cell culture should be in mid-logarithmic phase, i.e. an OD of 0.5-1.0, at the time of the transformation.
4. Deposit 5 μ L of both types of plasmids into a sterile microcentrifuge tube for each plasmid pair to be transformed.
5. For each plasmid pair to be transformed, add 5 μ L of salmon sperm DNA (5 mg/ml) to an empty microcentrifuge tube. Submerge the bottom half of the microcentrifuge tube containing the salmon sperm DNA in boiling water for five minutes. To prevent the cap of the microcentrifuge tube from popping open, only submerge the bottom half of the microcentrifuge tubes in the water. After removing from the boiling water, place the microcentrifuge tube containing salmon sperm DNA in ice for at least two minutes.
6. Add 5 μ L of salmon sperm DNA to each microcentrifuge tube containing plasmids.
7. Measure the OD of the growing yeast culture to confirm that it has reached mid-logarithmic phase (i.e. OD reading between 0.5 and 1.0).
8. Centrifuge the yeast suspension at 1000xg for two minutes.
9. Pour off the supernatant and resuspend the yeast pellet in sterilized deionized water using a vortex mixer.
10. Centrifuge the suspension once again at 1000xg for two minutes.
11. Discard the supernatant and resuspend the pellet in a solution of 0.1M LiOAc in T.E (1.02 g LiAc, 10 mL of 1M Tris at pH 7.4, and 10 mL 0.1 M EDTA at pH 8.0). The volume of 0.1M LiOAc in T.E should be equal to 1/100th of the original yeast culture volume in YPD.
12. Distribute 100 μ L of cells to each of the microcentrifuge tubes containing the plasmids. Flick each tube gently in order to mix the cells with the plasmids.
13. Add 400 μ L of 44% [w/v] polyethylene glycol (PEG₄₀₀₀) solution to each of the microcentrifuge tubes.
14. Gently mix the PEG₄₀₀₀ with the cells and plasmids. In order to avoid breaking the cells, do not use a vortex mixer at this point. To gently mix, hold the microcentrifuge cap and the bottom of the microcentrifuge tube between forefinger and thumb, slowly invert the tube, and then slowly bring the tube back to the upright position. Rotate the tube 90° about its cylindrical axis and slowly invert again. Repeat this process a number of times so that the cells slide down the entire surface area of the walls of the microcentrifuge tube.
15. Place the microcentrifuge tubes in a 30° incubator for 45 minutes.
16. After 45 minutes incubation, remove the cells from the incubator and repeat the gentle mixing procedure described in 14.
17. Place the microcentrifuge tubes in a 42° water bath and incubate for 15 minutes. Once again, only submerge the bottom half of the microcentrifuge tubes in the water.
18. After the 42° incubation, add 1 mL of sterilized deionized water to the microcentrifuge tubes.
19. Centrifuge the tubes in a tabletop microfuge for 5 seconds. Remove the supernatant from the tubes using a micropipetter.
20. Add 100 μ L of sterilized deionized water to each of the microcentrifuge tubes and mix the sterilized deionized water with the cells using the inversion process.
21. Add the contents of a single microcentrifuge tube to a growth medium plate which selects for the particular plasmids added to that microcentrifuge tube. Add 4-6 sterilized glass beads (1 mm diameter). Shake the plate containing the droplet of transformed yeast cells and glass beads in order that the cells are spread over the surface of the agar growth medium. Repeat this process for each set of transformed cells.
22. Place the plates in a 30° incubator for 3- 5 days. After 3-5 days, multiple yeast colonies of 1-3 mm diameter will be visible on the surface of the growth medium. At this time, the cells are ready to be imaged in the spectrally resolved two-photon microscope.

3. Calibrating the spectrally resolved two-photon microscope

The spectrally resolved two-photon microscope used in these studies has been described in detail elsewhere^{18, 19}. Briefly, a high-power solid-state continuous wave laser is used to pump a mode-locked Ti: Sapphire laser which generates femtosecond pulses of wavelengths ranging from ~750 to 830 nm. The laser beam is focused by a high NA microscope objective to a diffraction-limited spot on the sample. Excitation only occurs in the near focal region of the beam due to the low probability of two-photon excitation. A pair of orthogonal computer-controlled scanning mirrors are used to translate the focus of the beam within the plane of the sample in two different directions (referred to as x and y directions throughout protocol). The back-propagating fluorescence emission from an illuminated voxel of the sample is dispersed into spectral components by a transmission grating placed in the path of the emission before the light falls incident onto the pixels of an EM-CCD array. Thus, the full spectral profile of any fluorescent molecule/molecules within the focal region of the beam is obtained along one dimension (y direction) of the CCD array. Using the movement of one of the two scanning mirrors, the location of the laser focus can be scanned within the sample along a line perpendicular to the y direction. By keeping the shutter of the CCD camera open during this single line scan, the spectral profiles of the fluorescent molecules residing along the entire line of the scan are collected in one single sweep of the laser beam across the sample.

Accumulating multiple line scans of this nature at different y locations within the cell gives the spectral profiles of a large number of fluorescent complexes residing within the sample. The images obtained from the line scans are reconstructed to give multiple x - y fluorescence intensity spatial maps of the sample at different wavelengths^{18,19}. In order to accurately reconstruct and calculate the actual wavelength corresponding to the x - y fluorescent intensity spatial maps, the line scanning protocol must be calibrated using a sample with a well-characterized fluorescence emission spectrum.

1. The spectrally resolved two-photon microscope accumulates spectral information about the oligomer complexes in the imaged cell by scanning the focus of the excitation beam across the sample of interest in a number of locations.
2. A pair of orthogonal scanning mirrors is used to translate the focus of the laser beam across the sample in two different directions. The movement of the scanning mirrors is computer controlled and synchronized with the extraction of fluorescence intensity data from the CCD camera.
3. From the line scans, fluorescent intensity spatial maps corresponding to a particular wavelength of light can be reconstructed. In order to accurately reconstruct x - y fluorescent intensity spatial maps and calculate the actual wavelength corresponding to each of these maps, the line scanning protocol must be calibrated using fluorescein solution.
4. To begin the calibration procedure, center the excitation spectrum at a wavelength of 800 nm, which is 2X the maximum excitation wavelength of the donor tag, GFP₂. To center the excitation spectrum, translate the prism located within the laser cavity to modify the group velocity dispersion. The prism is mounted on a computer controlled linear motorized stage.
5. Using the computer program the camera is interfaced to, send a command to the CCD chip to lower the temperature of the CCD chip to its lowest attainable temperature in order to reduce dark noise.
6. Pipette 10 μ L of fluorescein solution onto a microscope slide. Cover with a coverslip so that a thin layer of the sample is uniformly dispersed in the region between the coverslip and the microscope slide.
7. Place a small drop of immersion oil on the surface of the coverslip
8. Now fasten the microscope slide to the x - y - z translation stage and translate the slide/stage in the optical axis direction by manually adjusting the linear actuator which controls the stage movement in the optical axis direction. Translate the slide/stage until the microscope objective comes into contact with the drop of immersion oil.
9. Using the computer program which controls the camera, switch to a video mode of data acquisition so the emitted light striking the CCD array is displayed on the computer screen in real time. Slowly, adjust the micrometer controlling the translation stage in the optical axis direction to bring the sample into the focal spot of the laser beam.
10. When the fluorescein sample is in focus, the emission will appear as a sharp line on the CCD array. Download the readout of the pixel intensities from the CCD array to the computer using the computer program controlling the camera. Measure the pixel intensity as a function of position on the CCD array by opening the downloaded matrix of intensity values in Image J and drawing a line through the fluorescent region. Use the Image J command Analyze→Plot Profile to create a plot illustrating the emission spectrum of the fluorescein sample.
11. Adjust the incremental parameter of the mirror which controls the movement of the laser focus in the y direction, such that adjacent y positions within the sample result in the movement of the peak of the fluorescence spectra by exactly one pixel along the spectral dimension on the CCD array. Monitor this by downloading the fluorescein spectra intensity value image for two different y positions in the sample, opening the intensity images with Image J, and finding the peak pixel position for each of the fluorescence spectra using the Image J cursor.
12. Leaving the shutter of the CCD open, scan the laser focus across the sample in the x direction. The light emitted from each voxel along the line of the scan should fall incident upon the CCD array.
13. Store the data from the CCD array obtained for this line scan and then clear the pixels of the CCD array.
14. Move the position of the laser focus in the y direction by the amount determined in step 11 of this section.
15. Scan the laser beam in the x direction across the sample, once again leaving open the shutter of the CCD array and store the data.
16. Repeat this line scanning procedure until a physical area ~50% larger than the dimensions of a single biological cell has been illuminated by laser light.
17. The relationship between row number of a particular line scan image and wavelength should be used to reconstruct the images to obtain multiple x - y fluorescent intensity spatial maps at different wavelengths. To obtain the x - y fluorescence emission image for a particular wavelength (λ_j), find the row number on the image obtained from the first line scan that corresponds to this wavelength. Then the adjacent row of the subsequent line scan image corresponds to the next row of the fluorescence emission image for that particular wavelength. Stack all the image rows that correspond to this wavelength to obtain the x - y fluorescent intensity spatial maps of the sample at that wavelength. Repeat this procedure for all other obtainable wavelengths.
18. To calculate the wavelength associated with each x - y fluorescent intensity spatial map, determine the background corrected normalized fluorescence intensity of each reconstructed image as a function of image index (j). The relationship between camera pixel position in the spectral dimension and the wavelength of the emitted photons is approximately linear; therefore the relationship between the reconstructed x - y fluorescent intensity spatial map images and corresponding wavelength is also linear and calculated as follows:

$$\lambda_j = m(j - j_{\max}) + \lambda_{\max} \text{ where the value of } m \text{ is represented by: } m = \frac{(\lambda_{\max} - \lambda_{1/2})}{(j_{\max} - j_{1/2})}$$

In the above formalism, the symbol λ_j represents the wavelength of the j^{th} reconstructed image. The values of $\lambda_{\max}$ and $\lambda_{1/2}$ are extracted from the emission spectrum of the fluorescein sample and correspond to the wavelengths at which the fluorescence intensity of the fluorescing sample is a maximum and half of maximum, respectively. The image indices $j_{\max}$ and $j_{1/2}$ correspond to the reconstructed images possessing the maximum fluorescence intensity and one half of the maximum, respectively.

4. Collecting data on biological samples of interest

1. To collect data on your samples, first remove from the incubator the plates with transformed yeast colonies. There should be at least three types of transformed cells full-width half-maximum (FWHM):

1. cells expressing the proteins of interest tagged with both types of fluorescent probes
 2. cells expressing only proteins tagged with the donor fluorescent probes, and
 3. cells expressing only proteins tagged with the acceptor fluorescent probes.
2. Add 100 μ L of 100 mM KCl to a microcentrifuge tube. Using a micropipette tip, scrape 3-5 yeast colonies off of the plate of cells expressing proteins tagged with both donor and acceptor fluorescent probes, and inoculate the 100 mM KCl with these cells.
 3. Remove 10 μ L of the cell suspension and dispense it on a fresh microscope slide, cover the droplet with a coverslip, and place a droplet of oil on the surface of the coverslip.
 4. Manually close the shutter in the path of the laser beam to block the laser light from reaching the microscope objective.
 5. Fasten the microscope slide to the x-y-z translation stage and translate the slide/stage in the optical axis direction until the microscope objective comes into contact with the drop of immersion oil.
 6. The wide field image of the sample will be severely blurred because of the presence of the transmission grating in the emission path. Therefore place a bandpass filter with a small FWHM in the optical path preceding the transmission grating. Turn on the wide field light illumination, and go to the camera video mode of data collection. Slowly translate the translation stage in the optical axis direction while viewing the image of the cells on the camera screen until the cells are brought into focus
 7. Translate the stage in either the x or the y direction in order to bring a single cell to the location of the laser beam focus.
 8. Turn off the wide field illumination source. Remove the bandpass filter from the emission path.
 9. Unblock the laser beam for a short time (< 1 second), while simultaneously viewing the signal received at the CCD array. If a fluorescent signal is detected on the CCD array during the time the laser beam is incident upon the cell, then perform a full fluorescence data acquisition scan of this cell. It is vital to use the same scanning parameters, specifically number of lines and y increment, as determined in the calibration procedure demonstrated earlier.
 10. Repeat the cell location and fluorescence data acquisition process for a large number of cells expressing proteins attached to both donor tags and acceptor tags.
 11. After accumulating fluorescence images of cells expressing proteins tagged with both receptors, repeat the entire process for both cells expressing only proteins tagged with the donor fluorescent probes and cells expressing only proteins tagged with the acceptor fluorescent probes.
 12. Using the procedure described in section 3, reconstruct all scans to obtain spectrally resolved x-y fluorescent intensity spatial maps for each set of data.

5. Image analysis

Each pixel of the x-y fluorescent intensity spatial maps, which result from a single scan of a fluorescing biological cell, contains the full spectral profile of the molecular complex residing in the excitation voxel corresponding to that particular pixel. If the proteins of interest are interacting with one another, this spectral profile will contain a mixture of signals from both the donor and acceptor fluorescent probes. In order to determine the nature of the interactions between the proteins of interest, the spectral profiles from a large number of these protein complexes in a cell must be sampled and the apparent FRET efficiency (E_{app}), defined as the proportion of the excited states of the donor fluorescent probe that become transferred to the acceptor fluorescent probe via FRET, of each pixel must be calculated.

1. Determine the background-corrected fluorescence intensity for each of the reconstructed images described in section 4 for cells expressing proteins tagged with only the donor fluorescent probes and normalize to the maximum value. Because each of these reconstructed images corresponds to a separate wavelength (λ_j) this collection of normalized fluorescence intensity values corresponds to the measured emission spectrum of the donor probes, $I^D(\lambda_j)$.
2. Repeat step 1 for cells expressing proteins tagged with only the acceptor fluorescent tags to obtain the measured emission spectrum of the acceptor probes, $I^A(\lambda_j)$. Since the acceptor probe is chosen such that it is rarely directly excited using the laser source, the signal emanating from acceptor only samples will be low and possibly on the same order of magnitude as the cells inherent autofluorescence signal. Therefore, the measured acceptor fluorescence spectrum calculated in this manner may need to be corrected to remove the contribution of autofluorescence before proceeding.
3. Using the normalized spectrum obtained in step 1 and in step 2, spectrally decompose each pixel of the fluorescence emission spectrum of cells expressing both proteins tagged with donor fluorescent probes and proteins tagged with acceptor fluorescent probes using the relation $I(\lambda_j) = k^{DA} I^D(\lambda_j) + k^{AD} I^A(\lambda_j)$ where $I(\lambda_j)$ represents each particular pixels measured fluorescence spectrum. The values of k^{DA} and k^{AD} are proportional to the fluorescence emission from donors in the presence of acceptors and from acceptors in the presence of donors, respectively⁵.
4. Calculate the apparent FRET efficiency which maps the interaction of proteins in the cell at each pixel using the formula,

$$E_{app} = \frac{1}{1 + \frac{Q^A k^{DA} w^D}{Q^D k^{AD} w^A}} \quad \text{Here, } Q^D \text{ and } Q^A \text{ are the quantum yields of donor fluorescent probe and acceptor fluorescent probe}$$

respectively, and w^D and w^A are the integrals of the normalized donor and acceptor emission spectrum, respectively^{19, 20}.

5. Finally, create a histogram using the calculated values of E_{app} for each pixel, where the number of times a specific E_{app} value occurs is plotted against that particular value of E_{app} .

6. Histogram Analysis

In order to extract quantitative information from the E_{app} histograms, each histogram is theoretically fit with a sum of Gaussian functions, according to the following formula:

$$\sum_{i=1}^n A_i \exp \left[\frac{-(E_{app} - E_{0i})^2}{2\sigma_i^2} \right],$$

where A_i is the amplitude, σ_i the standard deviation, and E_{0i} the most probable FRET efficiency of the i^{th} Gaussian function. Different oligomer sizes and configurations lead to different numbers (n) of E_{0i} and different correlations between them^{1, 17}. For instance, for a rhombus-shaped tetramer, five peaks are predicted, given by the expressions listed in Table 1.

Table 1. Relationship between each of the five Gaussian peak center values and the pairwise FRET efficiency predicted for a rhombus shaped tetramer.

E_{01}	E_{02}	E_{03}	E_{04}	E_{05}
$\frac{2}{3}E_p$	E_p	$\frac{E_p(3+E_p)}{2(1+E_p)}$	$\frac{2E_p}{1+E_p}$	$\frac{3E_p}{1+2E_p}$

That means only one E_{0i} value needs to be adjusted in the process of data fitting - the one corresponding to E_p - while the other four are computed from the value of E_p .

1. Assume an oligomer model and identify the number of Gaussians to be used in fitting the E_{app} histograms¹. Fit the histogram by adjusting E_p , A_i , and σ_i . Note that the relative disposition of the histograms is fixed by the model. Minimize the chi square of the fit or some other goodness-of-fit functional.
2. Assume another likely oligomer model and repeat step number 1.
3. Choose the model that best fit the data for the number of parameters used. That may require using a statistical test (such as the chi square per number of degrees of freedom).

Representative Results

Illustrated in Figure 1 are the resultant k^{DA} , k^{AD} , and E_{app} spatial maps computed from spectrally resolved two-photon microscopy measurements performed on a yeast cell expressing the Sterile 2 α -factor (Ste2p)^{21, 22}.

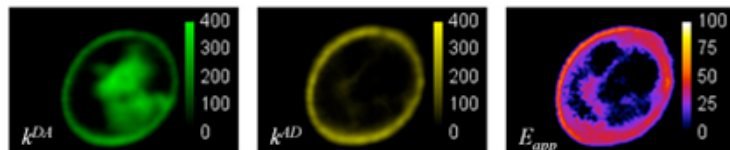


Figure 1. Yeast cell expressing the sterile 2 α -factor receptor. Two-dimensional maps of donor (k^{DA}) and acceptor (k^{AD}) signals were obtained from the spectral deconvolution procedure applied to each pixel location of images taken using the spectrally resolved two-photon microscope of a yeast cell expressing the sterile 2 α -factor receptor (Ste2p). Fluorescence intensities are assigned false colors according to their values and the scale is shown as an inset. A two-dimensional map of the apparent FRET efficiencies were computed using the k^{DA} and k^{AD} images.

A histogram plot was prepared using the values extracted from the E_{app} map of the cell shown in Figure 1. Pictured in Figure 2 is the fitted histogram plot corresponding to the measured E_{app} values (circles) of the cell pictured in Figure 1. The red solid line represents the best fit to the measured data using the sum of the individual Gaussian functions (solid green lines).

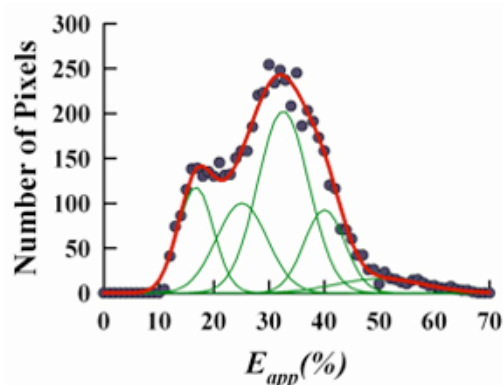


Figure 2. Histogram plot of E_{app} values for a yeast cell expressing the Sterile 2 α -factor receptor. The histogram plot which corresponds to the measured values (circles) of E_{app} for the cell pictured in Figure 1 is shown. Individual Gaussian functions (solid green lines) are summed

to simulate (solid red line) the measured values. Gaussian function parameters are: $E_{01}=16.7$; $A_1=118.9$; $\sigma_1=3.2$; $E_{02}=25.1$; $A_2=100.0$; $\sigma_2=4.6$; $E_{03}=32.6$; $A_3=202.6$; $\sigma_3=4.6$; $E_{04}=40.1$; $A_4=92.6$; $\sigma_4=3.7$; $E_{05}=50.1$; $A_5=16.0$; $\sigma_5=7.9$.

The relationships between Gaussian means needed to fit the data presented in the histogram of Figure 2 are given in Table 1. The number and correlation between Gaussian functions corresponds to the number and correlations between expected E_{app} values for rhombus shaped tetramer oligomer complexes. Therefore, it is apparent from the spectrally resolved two-photon measurements that the Ste2p oligomer complex assumes the form of a rhombus shaped tetramer *in vivo*¹⁹.

Discussion

In this presentation, we illustrated how to determine the size and structural information about a protein oligomer complex *in vivo*. While the data presented was obtained from a specific membrane receptor (i.e. Ste2p) expressed in yeast cells, the method is all encompassing in that it can be applied to any type of protein expressed in any type of cell, the only stipulation being that the proteins are tagged with the appropriate fluorescent markers. Future modifications to this protocol will involve enhancing the instrument to achieve higher scanning speeds in an attempt to monitor time-dependent protein oligomer size and structure changes.

Disclosures

No conflicts of interest declared.

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