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Novel Cosic resonance (standing wave) solutions for components of the JAK–STAT cellular signaling pathway: A convergence of spectral density profiles

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ABSTRACT

Cosic discovered that spectral analyses of a protein sequence after each constituent amino acid had been transformed into an appropriate pseudopotential predicted a resonant energy between interacting molecules. Several experimental studies have verified the predicted peak wavelength of photons within the visible or near-visible light band for specific molecules. Here, this concept has been applied to a classic signaling pathway, JAK–STAT, traditionally composed of nine sequential protein interactions. The weighted linear average of the spectral power density (SPD) profiles of each of the eight "precursor" proteins displayed remarkable congruence with the SPD profile of the terminal molecule (CASP-9) in the pathway. These results suggest that classic and complex signaling pathways in cells can also be expressed as combinations of resonance energies.

In contemporary cellular chemistry the mechanisms by which changes within an outer boundary (the plasma cell membrane) and an inner boundary (the nucleus) intercalate have been based upon a serial causality [1]. The operational model is that in order to satisfy the conditions for locality successive activations of different proteins mediate the changes along the surface of the plasma cell membrane to the nucleus that then initiates and effectively controls the ultimate structures and functions of the cell. The fundamental presumption is that the structural or spatial patterns of the molecule determine its interactions and its functions. This approach is a classic example of the particulate or matter-based description of cell function. However there may be an equally valid energy-based process [2] involving the correlative spatial resonance [3] and resulting spectral power density (SPD) of the specific properties of the units of molecules. The approach allows the potential inclusion of energy-based processes that are dependent upon the sequential units of molecular structure. The comparison would be analogous to de Broglie's matter waves, which assumed that a sequence of particles could also be expressed as a pattern of waves. Here we present evidence that the JAK (Janus Kinase)–STAT (Signal Transducer and Activator of Transcription) pathway, one of the classic signaling pathways within the cell whose final component affects the nucleus, can be described as a resonance pattern that is composed of the spectral characteristics of the pathway that converge at the nuclear interface as CASP-9. The protein interactions can be considered a transfer of resonant energy between interacting molecules through an oscillating physical field that could be expressed within the domain of classic photons.

While investigating a persistent anomaly that some complex molecular structures with quite different geometries displayed similar functional characteristics Cosic [3,4] developed the Resonance Recognition Model (RRM). Spectral analyses (Fast Fourier Transform) are usually applied to temporal phenomena in order to discern the power densities of specific intervals of frequencies or periodicities. Cosic applied the concept to spatial sequences. She equated each amino acid with a value that was employed as an inference of the pseudopotential of the de-localized electrons. They are electrons whose orbits exceed distances of the individual atom (over several adjacent atoms) or molecule that define the unit entity of a composite structure. De-localized electrons from a particular amino acid were assumed to determine

Abbreviations: BCLxl, B-cell lymphoma-extra large; CASP-9, caspase 9; cSrc, cellular Src kinase; EIIP, pseudopotential of electron–ion interaction; Il, interleukin; JAK, Janus Kinase; PBS, phosphate buffered saline; RRM, Resonance Recognition Model; SPD, spectral power density; STAT, Signal Transducer and Activator of Transcription; Tyk2, tyrosine kinase 2

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the major component of the electronic distribution of the entire protein. The energy of the delocalized electrons had been calculated as the pseudopotential of electron–ion interaction (EIIP) of each amino acid residue. These serial numerical values for each amino acid represented the distribution of free electron energies along the protein molecule. When spectral analyzed the intrinsic spatial frequency with the most power was employed to predict the molecule's primary photon emissions. Cosic's concept is that "each specific biological function within the protein or DNA is characterized by one frequency".

Several of our experiments and quantifications [5–8] have supported Cosic's concept. We had demonstrated that during the hours after melanoma cells were removed from standard incubation temperatures and remained at room temperature reliable and conspicuous shifts in the dominant frequency of the visible photon emissions were measured by digital photomultiplier units. The power density shifts between 400 and 800 nm were discerned by employing appropriate filters. The potential coupling between Cosic's predictions of photon frequencies based upon the spectral analysis of the delocalized electron values for the serial amino acids composing a protein were verified to a resolution of 10 nm (the limits of a filter). Compounds which are known to inhibit components of cell signaling and proliferation for a specific photon emission frequency as predicted by Cosic diminished the radiant flux density photon emissions while compounds that facilitate the specific components increased the radiant flux density of photon emissions.

The validity of the Cosic model can also be seen with molecules defined by simpler structures. For example bovine albumin is a protein that contains 607 amino acids. The calculated wavelength from the Cosic Resonant Recognition Model for this molecule is ~380 nm. The average of a triplicate of experiments where 4 g of albumin had been placed in 50 mL of PBS (phosphate buffered saline) and measured for photon emissions confirmed this prediction. This was completed by placing 1.5 cm^3 of this solution in a quartz cuvette and then measuring fluorescent counts by a RSM-OLIS spectrofluorometer. Fig. 1 shows the fluorescent counts for the solvent only and the counts for the same solvent containing the albumin. The major peak of spectral power displayed by the albumin (red arrow) was about 385 nm. This is well within measurement error of the peak predicted by the RRM. The Cosic procedure assumes a linear sequence of amino acids. One would expect smaller modulating contribution from higher order, non-linear spatial configurations. The sources of the smaller peaks have not been established clearly. However our preliminary experiments and quantitative analyses suggested they could indeed reflect the second and higher order conformational features of the molecule's geometry.

The question we then addressed is whether or not the RRM would reveal new relationships that are represented as

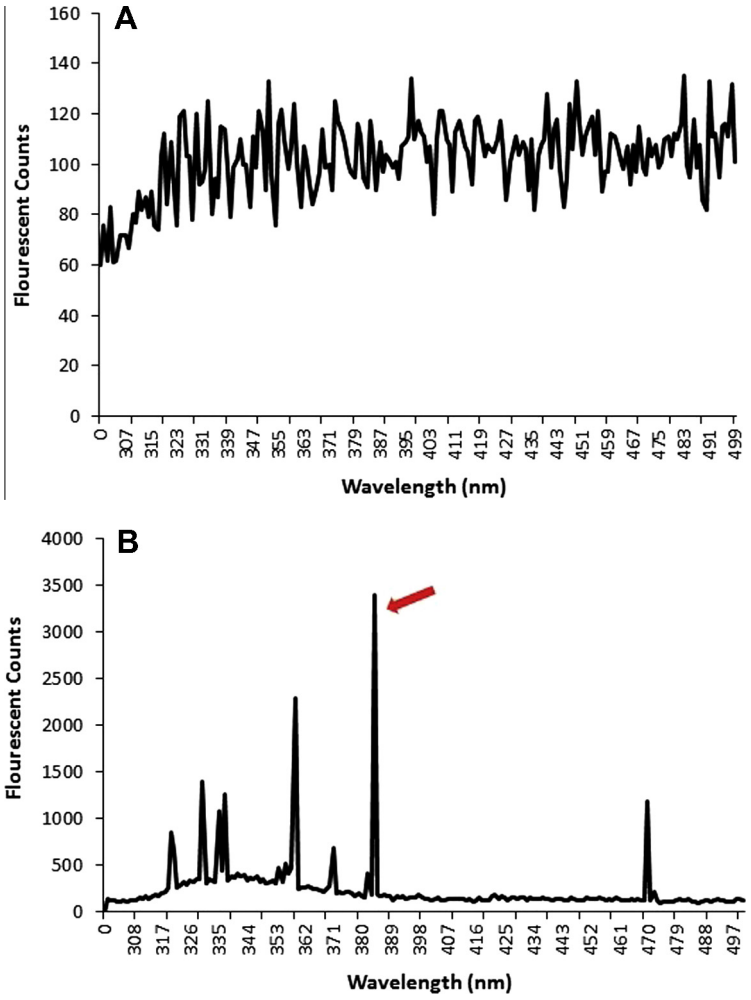

Fig. 1. Fluorescence (photon) counts for the medium (left) and the medium containing bovine albumin (right). The red arrow refers to the peak wavelength in nm predicted by Cosic's Recognition Resonance Model.

periodicities of spatial power densities in different molecular structures that have been shown to be intercalated by serial interactions that define a "pathway". In other words could an entire "signaling" pathway be characterized by a "single spectral pattern" in a manner similar to Cosic's concept that *each biological function of a protein is characterized by one frequency*. To date the RRM has been applied to single unit phenomenon such as a specific molecule. Although a traditional particulate approach to bioorganic chemistry would first invoke the successive transmission of minute structural changes mediated by a particular quantity of energy in different molecules, the operation of resonance predicts that the spatial spectral densities of the de-localized electrons of the components of a pathway should converge at the final "step".

The JAK (Janus kinase) STAT (Signal Transducer and Activator of Transcription) signaling pathway has been considered a primary source of "information" from chemical stimuli external to the cell boundary to the promoters of gene expression from the DNA within the nucleus. The sequential steps presently understood for this pathway include: Il2, Il6, JAK1, Tyk2, cSrc, STAT3, STAT5, BCLxl and CASP-9 [1]. The JAK–STAT pathway has remained relatively stable as inferred by evolutionary analysis from the original persistent life forms (green algae) that first emerged about 3 billion years ago to present day mammals. Disruption of this pathway has been correlated with various malignant cell disorders and disrupted immune functions.

To test the hypothesis that the combined Spectral Power Densities of the delocalized electron values for each of the mediating proteins between the cell surface and nuclear interface in the JAK–STAT pathway would converge to reflect the SPD profile of the molecule (CASP-9) directly influencing the nucleus, the Cosic procedure was applied to each molecule. This was completed by substituting each amino acid in the molecule by the appropriate pseudopotential calculated by Cosic. Because simultaneous spectral analysis for the program we employed (Spectra, SPSS-16) required equal length of cases, the pseudopotential values for each protein that was shorter than the longest protein were continued so that all molecules displayed equal cases.

To discern an optimal range for the numerical frequency associated with the spectral analyses the estimated width of amino acid was assumed to be 0.35 nm. It was divided by the numerical

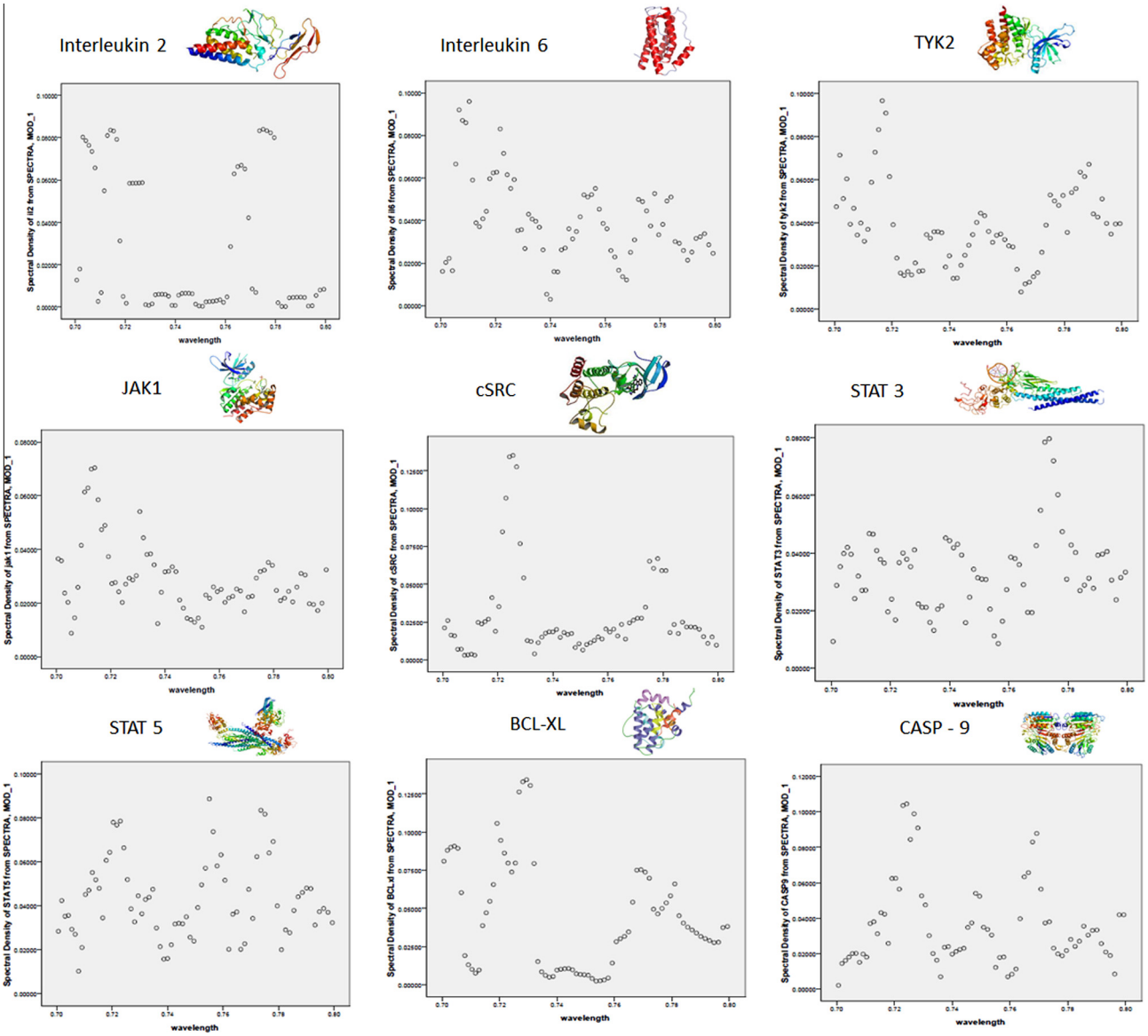

Fig. 2. Stylized shapes and descriptions of the proteins composing the JAK–STAT pathway (on top) and the distribution of Spectral Power Densities predicted by the Cosic Method for each protein between interleukin 2 (on the receptor) and CASP-9 at the end of the pathway.

frequency. We selected ⩽0.80 nm for this "functional" wavelength for convenience and for aesthetic reasons. It would be approximately πd of the average of width (d) of the median width of critical bond distances such as covalent and ionic bonds that constitute hydrogen bonds. Although perhaps spurious, this cutoff value demonstrated the most robust effect. It included the actual range (numerical frequency) from the spectral analysis of between 0.438 and 0.500. If one assumed Cosic's value for a residual amino acid width of 0.38 nm, the numerical frequency would not change.

The results are shown in Fig. 2. The spectral power density values (n = 73, Δf = 0.001) between 0.70 and 0.80 nm (numerical frequency 0.500–0.438) for each of the 9 components of the JAK–STAT pathway reflect the differences of the resonance characteristics of these molecules. Expanding inclusion of numerical frequencies up to and including the entire range of SPDs (n = 584) did not demonstrate this powerful relationship. In fact the effect size diminished progressively. The degree of convergence between the first molecule, Interleukin 2, within the outer boundary (the plasma cell membrane) and the nucleus (CASP-9) is not obvious. Even serially adjacent proteins in the pathway do not appear to share any similarity of Spectral Power Densities.

However when the spectral profiles of the 8 precursor proteins before CASP-9 were averaged, the results were more compelling. Multiple regression analyses, which removed redundant variance indicated that 50% of the variance in the spectral profile of CASP-9 [$F(3,69) = 23.71$, $p < .001$] could be predicted by the linear combination of the spectral densities (unstandardized partial regression coefficients or slopes in parentheses) of cSrc (0.347), tyk2 (−0.350), and BCLxl (0.18) with a constant of 0.3. Fig.3 shows the distribution of the predicted values over the base frequency units that were employed by Cosic for single molecules. These units, which are required for the calculation of potential photon wavelength, are the raw spectral frequency values from the Fast-Fourier Transform without correction for absolute values of the units.

The conspicuous congruence between the values predicted by the equation from the weighted linear combination of the spectral densities of the three precursors and the raw spectral density for CASP-9 is shown in Fig. 4. The closed circles refer to the raw CASP-9 values and the open circles refer to the predicted values. Lag/lead analyses of the 73 cases (Δfs) for ⩽0.8 nm (or numerical frequency ⩾0.438) for the SPDs for each molecule, that is, including the unit shifts in SPD in the regression equation for the 8 precursor variables to a maximum of ±3 spectral units (Δfs) with maximum steps = 7 (to accommodate sample size), increased the strength of the association to 0.90 [$F(7,65) = 39.58$, $p < .001$]. All precursors except Interleukin 6 and Tyk2 entered the equation. Only cSrc entered the equation twice (no lag/lead and a lead by 3 units). The overlaps between the actual SPD profile for CASP-9 and the predicted SPD profile based upon the weighted averages of the spectral densities approached an identity (Fig. 5) if one assumes the classic statistical measurement error of ∼10%.

The results indicated that the linear combination (or "weighted" average) of the SPD profiles based upon the serial values of the pseudopotential of each amino acid in each protein in the pathway reflect the SPD profile of the final molecule in the pathway. In other words the combination of the constituents along the pathway "averages" or "combines" to produce the final pattern that presumably would affect the nucleus. These results suggest that a specific signaling pathway could be described by their combined spectral patterns such that an aggregate pattern would reflect the entire pathway.

In the present analyses SPD calculations were completed for the undimerized form of STAT 3 and STAT 5. Although there would be a difference in SPDs for the two proteins in the dimerized state, the focus for these analyses was to discern the inherent SPD signatures of these proteins in their essential form (i.e., no accommodation for phosphorylation or ubiquitination states) and how *each* protein sequentially contributes to the whole pathway. JAK–STAT converges with other pathways such as ERK and mTOR. Consequently, other pathways converging on JAK–STAT could modify the calculation. The convoluted networks of molecular pathways that exist within a cell will still display SPD resonance between molecules. One would expect these other "contributors" to be congruent with the spectral properties of the pathway.

This particular application of the Cosic procedure suggests that "information" transferred within molecular pathways may be mediated through energies within proteins that display "similar" resonances. Malignant and normal cells can display different primary "pathways". Understanding the functionality of the molecular resonance "pathway" might reveal new strategies by which cells can be activated or inhibited. The resonant energies within

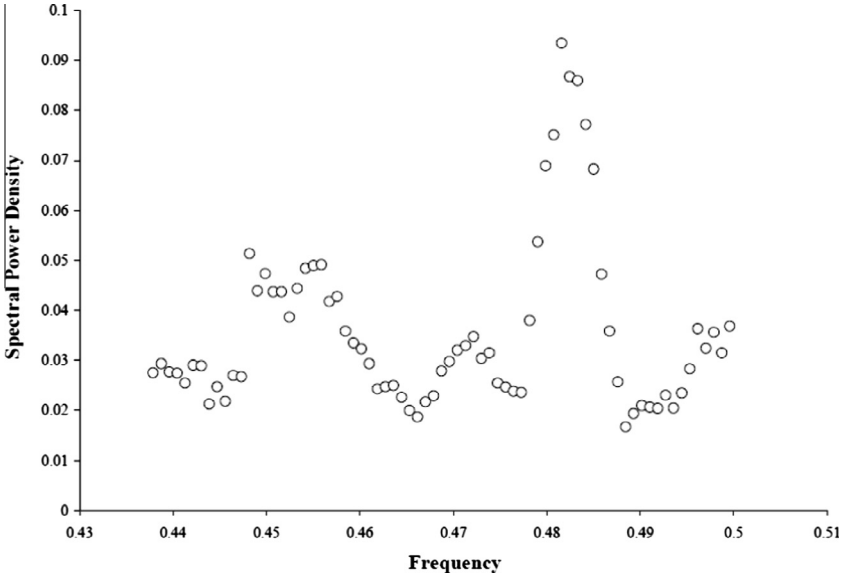

Fig. 3. Predicted Spectral Density Profile for CASP-9 based upon the optimal combination of SPDs as a function of numerical frequency according to multiple regression analyses of the antecedent proteins in the JAK–STAT pathway.

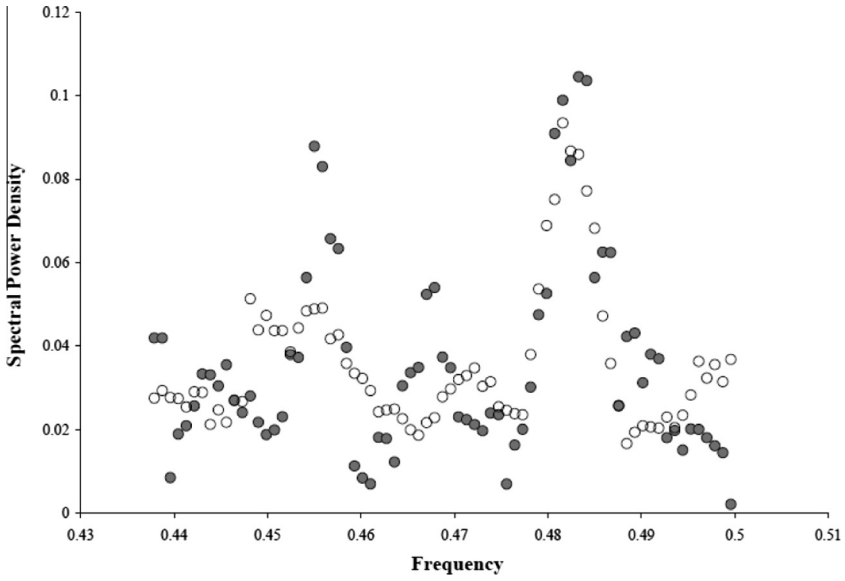

Fig. 4. Congruence of distribution of Spectral Power Densities profiles for pseudopotentials for composite amino acids as a function of numerical frequency for CASP-9 (closed circles) and that predicted (open circles) by the composite SPDs of precursor proteins in the pathway.

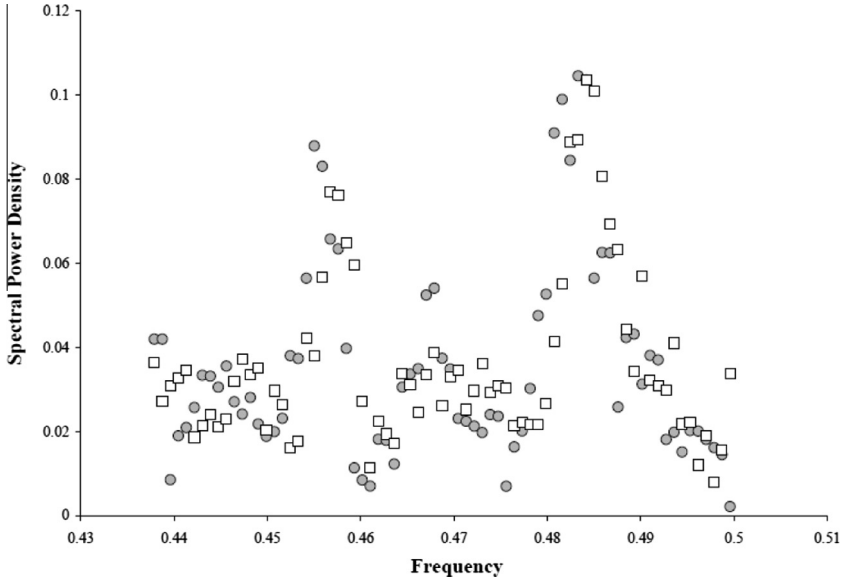

Fig. 5. Maximum congruence of SPD profiles for CASP-9 (closed circles) and that predicted (open squares) by the most optimal combination of ±3 Δf shifts in numerical frequency of SPDs for precursor proteins in the JAK–STAT pathway.

each protein may explain their role and relationship with antecedent and subsequent proteins. If we know the resonance pattern of the initial or terminal molecule in pathway displayed by malignant cells, applying a pattern that is optimally congruent could be specifically disruptive without affecting other pathways. This would occur through a temporally dynamic physical process rather than a frank shift in molecular structure.

There are several potentially important implications of these results. First, what may be equally as important as molecular structure in the mediation of information from location A (the external boundary) to location B (the nucleus, an inner boundary) is the resonant pattern of the molecule. Second, the importance of the resonant pattern of SPD of the amino acid sequence of the protein indicates that there could be potential substitution of "carrier" molecules. From this perspective the persistence of the "same" and reliable protein in a pathway might only reflect the *prominence* (molarity or activity) of that molecular species in the typical cell rather than the uniqueness of a particular molecule. The critical feature may be the correlative quantity of energy [2]. This approach would mean that in other cell systems, such as malignant lines and cancers where different molecules might be dominant because of a different metabolism, other proteins with similar resonance patterns could mediate the serial reactions. Such "stimulus substitution" would be relevant to pharmacological or chemical strategies that target the molecular component of these pathways. This traditional approach attempts to simulate structural similarities at the interface of molecular interactions rather than the similarities of the system's resonance.

Perhaps the most significant result of these analyses is the suggestion of three peaks (two major) of SPDs that could define

or at least alternatively represent the resonance pattern of the pathway. According to Cosic's solution, which divides the constant 201 by the peak numerical frequencies in Fig. 5, the maxima for photon flux densities should cluster around the wavelengths of 441, 430 and 416 nm. When the frequencies for these wavelengths were calculated and multiplied by Planck's constant (6.626×10^{-34} J s) the differences between the first two was 1×10^{-20} J and between the second and third wavelength was 1.6×10^{-20} J. This is within the range of the energies associated with hydrogen bonds (0.04–0.3 eV, 0.7–2.2×10^{-20} J). It may not be spurious that the equivalent wavelength for this increment of energy is 10 μm, the typical width of the prototypical cell which is also the median solution for the wavelength at physiological temperatures according to Wein's law (0.29 cm deg/K) [2].

This particular quantity of energy may be a fundamental "unit" [2] for a variety of cellular mechanisms that include the action potential of axons, the binding of ligands to receptors and the energy per molecule for intracellular processes. It is also the energy produced by electric forces over the average distance between potassium ions forming the single layer of ions that have been attributed historically to the source of the resting membrane potential for the classical cell plasma membrane [2]. The importance of this increment of energy in cell processes has been shown by direct experimentation with cells [9] and human brain activity [10].

This value is also within the range of the protons in the second shell hydrogen bonds, according to Decoursey [11], and is consistent with empirical measurement of the mobility of protons. The role of protons within the hydronium ions of water, which determines pH and hence the rate and even occurrence of many enzymatic and proteinaceous reactions, may have been underestimated in many conceptions of signaling pathways. Proton channels are copiously represented within plasma membranes and may be essential for accommodating the proton accumulation associated with normal metabolism.

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