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DePaul University Honors Program Senior Thesis

# Protein Music: Solo for Bovine Rhodopsin

February 9th, 2014

This project realizes protein structure as an instrument. I wrote a brief composition for such an instrument derived from the structure of bovine rhodopsin. The sounds generated by this instrument are to be projected around the listeners by eight speakers arranged in a cube. An accompanying video shows the protein being played. I also include a score and the Pure Data patch used to realize the score. My intention is to musically celebrate refinement of the human knowledge of life at the molecular scale.

## Science as Artistic Inspiration

Since the beginning of human culture, artists' communications required an understanding of the human sensory pathways. Historically, this understanding was subconsciously imbedded in the artist's creative practice handed down through generations as theory, technique, and experience. Technologies like the Camera Obscura allowed artists to directly explore semi-realistic models of sensory organs. Objective awareness of the mechanisms of perception increase the potency of artist's communications.

Modern research and technology further refine our understanding of the senses. We have begun to understand the biological molecules enabling our perception of light, sound, and many other channels of perception. Artists are now able to explore the very small structures enabling their communications. In this modern understanding, proteins are the main actors behind life processes. Proteins contain both genetic information in their sequence of amino acids and structural information in their specific folded forms and movements. Among their many functions they facilitate communication within cells, between cells, within organisms, and between organisms.

Before this project I wanted to focus on the protein mechanism of hearing. Hearing has been extensively studied at the cellular scale; however, the proteins involved are not yet fully understood. Tiny hairs on top of specialized cells in the cochlea give rise to auditory synaptic activity. A study in 2011[1] implicates two proteins from the connexin family in the hearing pathway. These proteins form doors between cells known as gap junctions. Another study maps the distribution of these proteins in the cochlea.[2] This sense is more complicated than a single protein signaling sequence and probably involves many specialized cells and the movement of ionic fluids around and between them.[3]

For this project my faculty advisor Justin Maresh suggested I focus on vision as it is a well documented, relatively simple sensory pathway. Vision is realized as the polarization of specialized cells coating the back of the retina. These cells are divided into an inner segment and a protruding rod or cone segment filled with disk-shaped sub-membranes (see Figure 1). A protein called rhodopsin imbedded in these disk-membranes changes shape when it is hit with a single photon of light. The change in shape activates nearby transducin molecules which in turn remove inhibitory caps from phosphodiesterase enzymes. The uncapped phosphodiesterase enzymes reduce the concentration of cyclic-guanosine-monophosphate converting it into 5'-guanosine-monophosphate. The former molecule holds open the gates of ion channels in membrane of the rod or cone segment. A drop in its concentration causes the channels to close, leading to electrical polarization as ions continue to be pumped out from the inner segment. A series of proteins reset the system. The chain of events leading to cell polarization amplifies the

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[1] Yan Qu, Wenxue Tang, Binfei Zhou, Shoeb Ahmad, Qing Chang, Xiaoming Li, Xi Lin, "Early developmental expression of connexin26 in the cochlea contributes to its dominate functional role in the cochlear gap junctions," *Biochemical and Biophysical Research Communications* vol. 417 (2012) pages 245-250 <www.elsevier.com/locate/ybbrc>. This study analyses the function of connexin26 and connexin30 in the mouse cochlea.

[2] Wei Liu, Marja Boström, Anders Kinnefors, Helge Rask-Anderson, "Unique Expression of Connexins in the Human Cochlea," *Hearing Research* vol. 250 (2009) pages 55-62 <www.elsevier.com/locate/heares>. This study uses protein-specific chemical staining techniques to map the distribution of connexin30 and connexin26 in five surgically removed human cochleae.

[3] Robles, Luis and Mario A. Ruggero, "Mechanics of the Mammalian Cochlea." *Physiological Reviews.* vol. 81 no. 3 july 2001, pg. 1305 to 1352. A review of the cellular mechanics of hearing offering a description of the cochlea as an electro-chemical transducer.

visual signal: a single rhodopsin molecule activates many transducin molecules and a single transducin molecule activates many phosphodiesterases.[4]

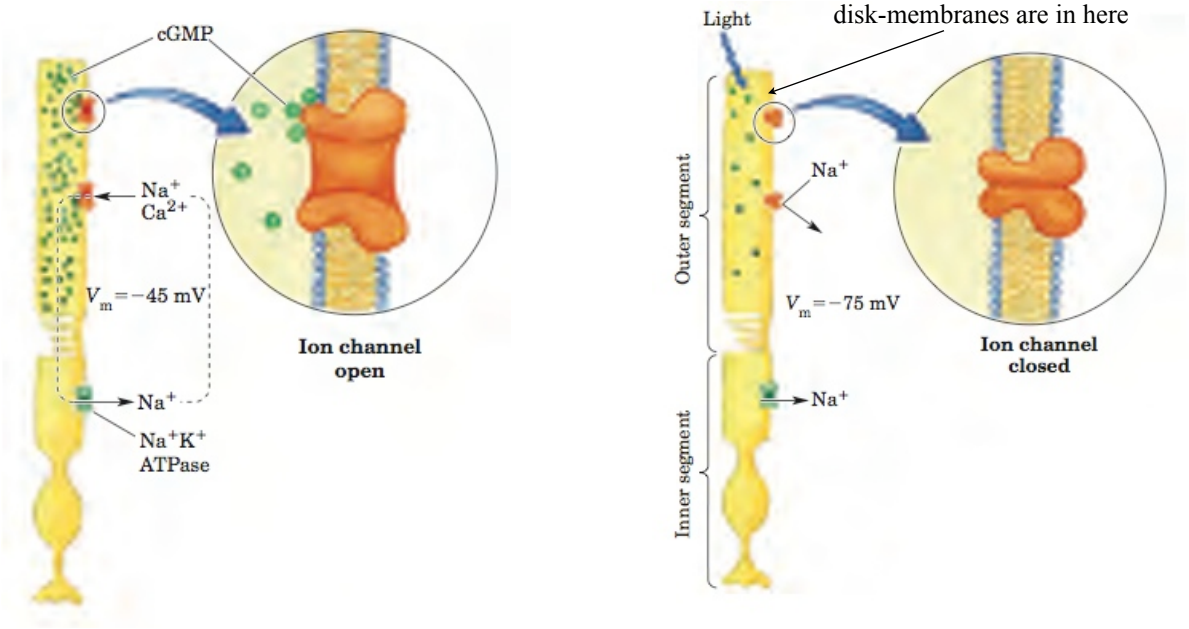


Figure 1: Cross-section of a rod cell showing dark (left) and light (right) conditions. (adapted from *Lehninger Principles of Biochemistry* page 457. See footnote 4 for citation)

As the project developed I decided to use only rhodopsin since it is the first signaling protein or "primary event" of vision.[5] Rhodopsin is a trans-membrane protein with seven helices bundled around a central pocket. The carbon structure of retinol, a form of vitamin A1, inhabits this pocket (see Figure 2). Before exposure to light, retinol exists in a bent configuration. Upon absorbing a single photon the retinol molecule flips into a straight configuration causing the protein's shape to shift slightly. This slight shift opens a hole on the outside of the disk-membrane and raises a small flag, both of which work to bind and activate transducin. Though essential to the function of the protein, this shifting is far too subtle to create a musical effect. I

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[4] Nelson, David L., Michael M. Cox, *Lehninger Principles of Biochemistry,* (W. H. Freeman, Fourth Edition, 2004). The visual sense is discusses as an example of G-protein coupled signaling pathways on page 456. For a deeper treatment of this sense see Rodieck, Robert W, *The First Steps in Seeing,* (Sunderland, Mass: Sinauer Associates, 1998).

[5] Wang et. al. "Vibrationally Coherent Photochemistry in the Femtosecond Primary Event of Vision," *Science,* New Series, Vol. 266, No. 5184 (1994), pgs. 422-424. Femtosecond pump-probe experiments on the transition states between dark and light states of rhodopsin cited here for the term "primary event."

chose to use only the active form of rhodopsin because its published structure is believed to be the most physiologically relevant.[6]

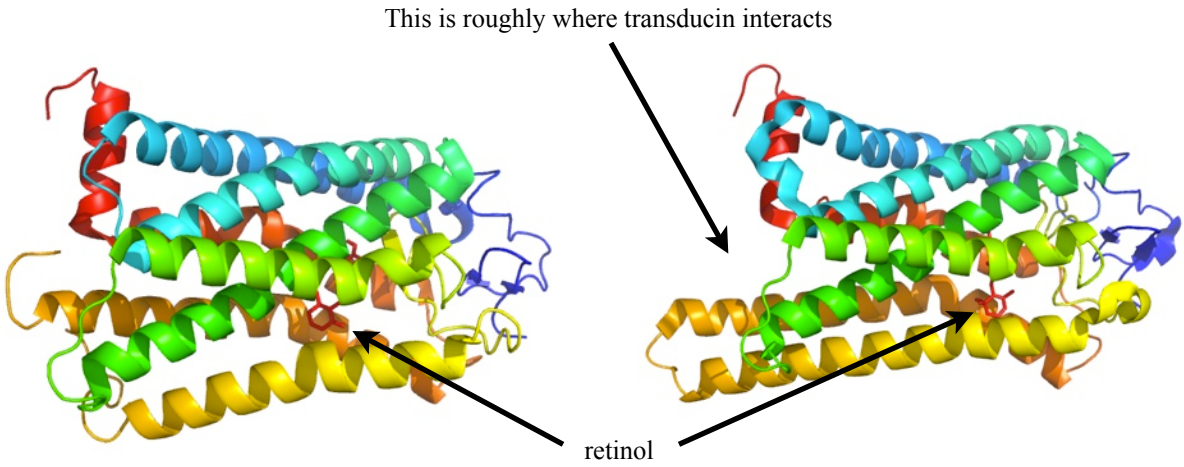


Figure 2: dark (left) and light (right) states of rhodopsin. For both: the right side points into the sub-membrane, the left side points out into the rod or cone cytosol. Blue indicates the amino terminus, red the carboxylic terminus. Note how the retinol flips. Rendered from protein data bank entries 1f88 (left) and 3pqr (right) in PyMOL.[7]

## Protein Music

Douglas Hofstadter was perhaps the first to compare proteins to musical compositions. In his book *Gödel, Escher, Bach* he describes the translation of genetic information into amino acid sequences with the metaphor of an analog tape machine: "When a 'tape' of mRNA passes through the 'playing head' of a ribosome, the 'notes' which are produced are *amino acids,* and the 'pieces of music' which they make up are *proteins."*[8] He connects the layers of protein structure to musical phrases, sections, movements, and pieces.

The first to actually transcribe genes into musical notes was Susumu Ohno, a geneticist known for work on gender determination and junk DNA.[9] Ohno used an "inviolable rule" to

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[6] 3pqr (active rhodopsin) uses only ammonium sulfate, 1f88 (inactive rhodopsin) uses a variety of other chemicals and includes unnatural metal ions in the model.

[7] <http://www.rcsb.org/pdb/home/home.do> accessed throughout the course of this project.

[8] Hofstadter, Douglas R., *Gödel, Escher, Bach: an Eternal Golden Braid,* (New York: Basic Books Inc. 1979), pg. 519, italics his.

[9] Oliver, Myrna, "Susumu Ohno; Geneticist Wrote Music Based on DNA," *LA Times* (January 19th, 2000), Obituary.

map the four DNA bases to diatonic pitches.[10] Rhythm, meter, and phrasing distinguish Ohno's music from modern computer generated protein musics. He seems to have understood the need for creative interpretation of raw scientific data. (see Figure 3)

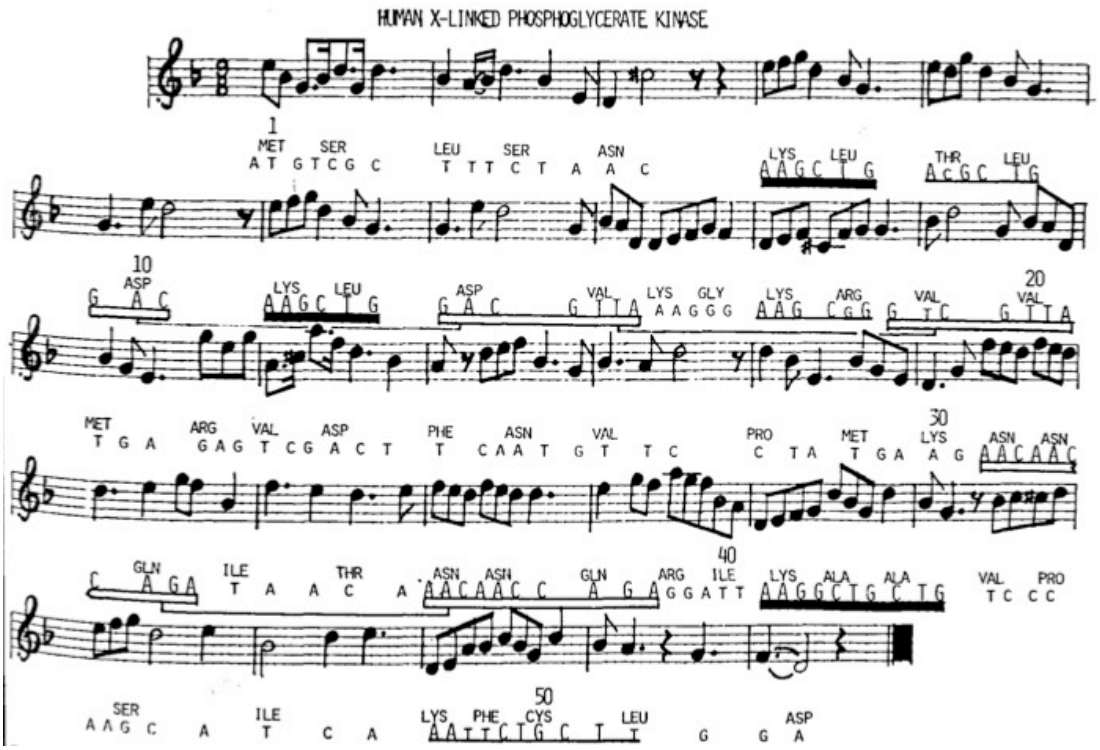


Figure 3: Ohno's realization of human x-linked phosphoglycerate kinase as a short piece for violin. (See footnote 10 for citation.)

Advancement of computer technology made protein music extremely easy to produce. Seeking new musical structure John Dunn began creating protein music in the early 1990s.[11] Dunn's music incorporates varying timbres and textures but retains the element of sequential recitation. A group at MIT describes an aesthetically flexible method with the intention of making these sequences musically accessible to a wider audience.[12] The general approach is

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[10] Ohno, Susumu and Midori Ohno, "The All Pervasive Principle of Repetitious Recurrence Governs Not Only Coding Sequence Construction But Also Human Endeavor in Musical Composition," *Immunogenetics*, Volume 24, Issue 2: 1986, pages 71-78.

[11] Dunn, John and Mary Anne Clark, "Life Music: The Sonification of Proteins," *Leonardo*, Vol. 32, no. 1 (1999), pg. 30. Dunn's music and software is available online at <http://www.algoart.com/music.htm>

[12] Shi, X. J., Y. Y. Cai, and C. W. Chan, "Electronic Music for Bio-Molecules Using Short Music Phrases," *Leonardo,* vol. 40, no. 2 (2007), pgs. 137-141. Includes links to musical samples.

neatly summarized by Wikipedia: “protein music is a musical genre or form, composed by converting protein sequences, such as genes, to musical notes.”[13]

The results of these approaches bore me. The ratio between number of possible elements (4 DNA bases or 20 amino acids) and number of elements combined in a typical protein (hundreds of amino acids or thousands of atoms) suggests that regardless of specific conversion choices the results will be monotonous. Some may be interested in this effect but for me it does not resonate with the intricate sheets and spirals of three-dimensional protein structure.

## Rhodopsin as Instrument

One of this project’s goals was to sonically communicate three-dimensional protein structure. I treat this structure as an imaginary instrument. Each amino acid of the backbone is represented by a simple sound positioned as determined by the protein model within a cube of speakers. Playing the instrument reveals the structure by activating spatially-defined groups of amino acids.

The pitch of each sound is determined by the position of that amino acid in the protein sequence: the highest pitch is at the protein’s amino terminus, the lowest pitch at the carboxyl terminus. A frequency range from 93Hz to 10,303Hz is divided into 326 eighth-steps, 48 steps per octave. In the piece I variably compress this dissonance into a major scale. The intention is to form a connection between the complexity of the model and commonly heard pitch structures. The dissonance/consonance relationship also defines formal sections creating a sense of tension and release.

The pitches are realized as sine waves: up to four per amino acid at integer multiples of the given frequency. The fundamental represents the alpha carbon of each amino acid. Integer multiples represent polar atoms (nitrogen and oxygen) in side chains. They are variably faded in and out so that at times we only hear the protein’s backbone and at times we hear each polar atom.

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[13]<http://en.wikipedia.org/wiki/Protein_music> accessed January 20th, 2014

Amplitude is variably pulsed in patterns determined by the molecular structure of amino acid. The same parameter which controls amplitude of the harmonics controls the pulse depth. Discrete pulses represent the atoms of each molecule (see Figure 4). At times the pulses are unified around 256 BPM; at times their speed is proportional to their position in the sequence. The retinol component is represented by random pulses and slight frequency variation reflecting its dynamic shape-changing role.

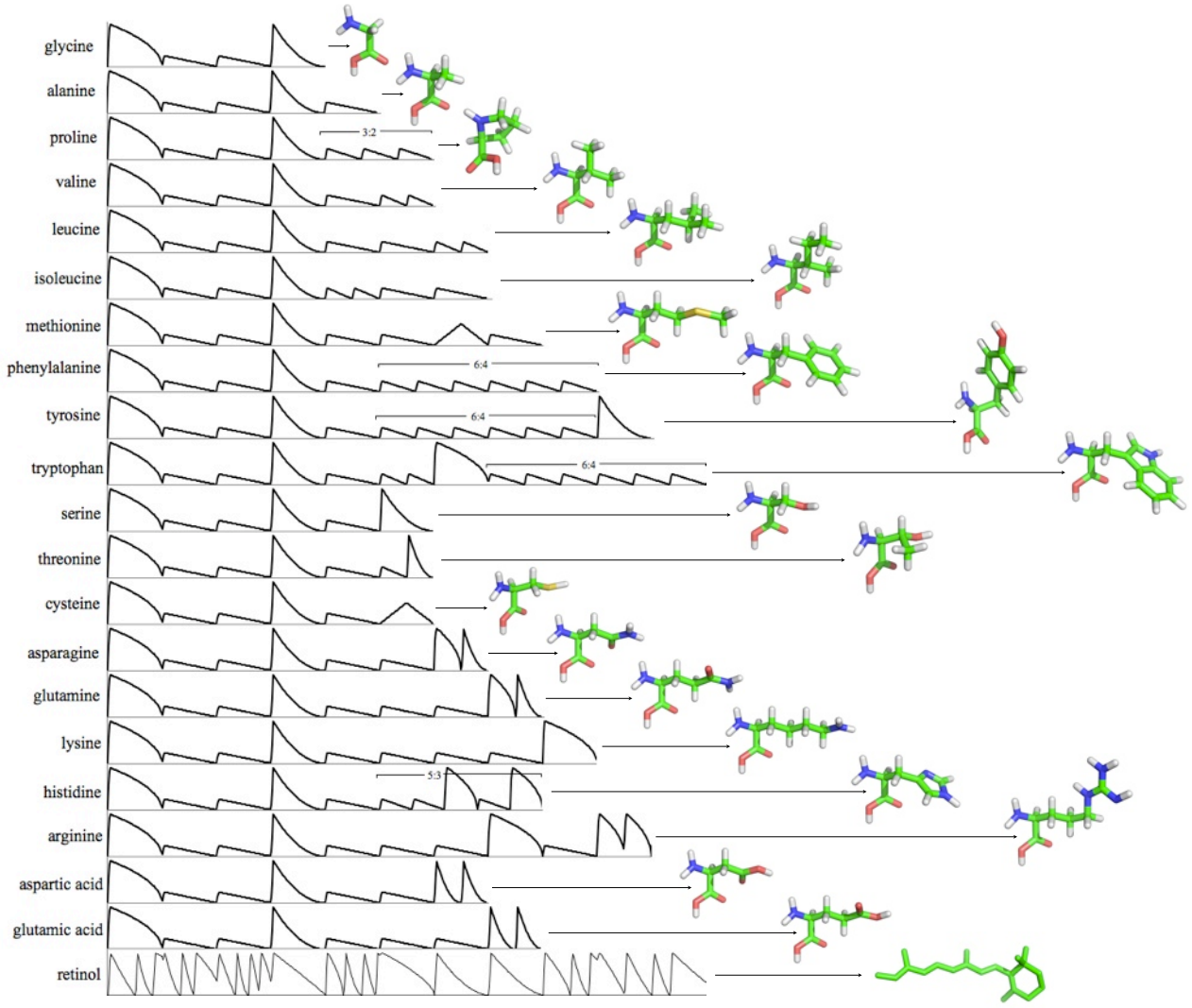


Figure 4: amplitude traces of pulse patterns used to represent amino acids. Each pattern is cycled from left to right. Stick models on right show the three-dimensional structures of the amino acids with carbon in green, nitrogen in blue, and oxygen in red (from Maresh's "Genes and Proteins" class, rendered in PyMOL). Note how the elements correspond to curvature with carbon as flat, oxygen as negative curvature, and nitrogen as positive curvature. The trace for retinol is only a sample: in the piece it is continuously varied by Pure Data's random number generator.

To play the instrument I filtered the three-dimensional protein model so that only specific regions of space are activated at a given point in time. I used mathematically-defined spheres and planes to determine the amplitude of each amino acid. For spheres I calculate the distance from each amino acid to a center point; for planes I calculate the distance from each amino acid to the nearest point on that plane. Distance was transformed to amplitude by:

$$\text{amplitude} = \sqrt{\text{radius}^2 - \text{distance}^2}$$

This circular cutoff provides smooth transitions between sounds as the activated regions move around the protein model. I was inspired by the technique of sweeping multiple keys on a keyboard with the palm or fist.

The sounds generated by this instrument are to be projected around the listeners by eight speakers positioned at the vertices of a large cube. Each speaker's signal is determined by the distance from that speaker to each amino acid. Loudness is proportional to the inverse square of distance. Phase is proportional to distance so that sounds are realized at a slightly earlier phase in the speakers they are closer to. The phase scaling factor is based on the speed of sound at 20 degrees Celsius (343.21 meters per second) and the ratio relating virtual distance in the model to physical distance between the speakers. The .wav file included here was calculated for a 1.2 meter cube I built by hanging small speakers from microphone stands. Should the piece be played on a larger speaker cube or under different atmospheric conditions, a different phase scaling factor would be desirable. The intention is to create a semi-realistic three-dimensional sonic "image" of the protein-model instrument.

To help the listener understand what they are hearing I accompany the music with a video. We see the instrument played: the gain of each amino acid, the rotation, and the scaling over time. A dark gray cube superimposed around the protein model shows the virtual locations of the speakers. Carbon atoms are colored to show the protein's sequence; oxygen and nitrogen atoms are red and blue respectively. The color scheme is borrowed from PyMOL's "spectrum" option used in Figure 2 and also connects to the use of pitch with higher frequencies corresponding to blue and lower frequencies to red.

If the efficiency of computation were such that these sounds could be realized in realtime, the model could be played live using a controller such as a camera or iPad. My current program renders the model roughly ten times slower than realtime. I must score the movements of parameters, then wait for the computer to realize the audio and video.

## First Repertoire for Rhodopsin

I used the sequence of rhodopsin to determine the large-scale structure of this piece though unlike other protein musics (see page 4) the pulse of amino acids is not present on the musical surface. Each amino acid gets one 40th of a minute yielding a total of 8 minutes 9 seconds. Material is segregated by secondary structural divisions: helical sections correspond to freely composed spheres, sheet sections to horizontal cross-sections, and loops to silence (see Figure 5). In the freely composed sphere sections the number of voices reflects the number of previous helices that section seems to touch determined by looking at the model in PyMOL.

The sphere sections were composed in two phases. First, I used Pure Data's built-in "data structure" objects to determine the sphere's radius and trajectory through the sequence. The spheres trace the helix represented in the duration scheme though speed and direction vary.

Second, I determined the model's rotation,

| Time (min:sec) | Sequence # (40 BPM) | Material |
|---|---|---|
| | 1-3 | silence |
| 0:4.5 | 4-6 | cross section |
| | 7-8 | silence |
| 0:12 | 9-11 | cross section |
| | 12-32 | silence |
| 0:48 | 33-65 | one moving sphere |
| | 66-70 | silence |
| 1:45 | 71-101 | one moving sphere |
| | 102-106 | silence |
| 2:39 | 107-141 | one moving sphere |
| | 142-149 | silence |
| 3:43.5 | 150-173 | two moving spheres |
| | 174-178 | silence |
| 4:27 | 179-181 | cross section |
| | 182-185 | silence |
| 4:37.5 | 186-189 | cross section |
| | 190-198 | silence |
| 4:57 | 199-236 | two moving spheres |
| | 237-239 | silence |
| 5:58.5 | 240-278 | one moving sphere |
| | 279-285 | silence |
| 7:7.5 | 286-310 | three moving spheres |
| 7:45 | 311-326 | one moving sphere |

Figure 5: duration scheme

and scale between the speakers using the accelerometer on my iPod touch. Viewing a three-dimensional model requires the viewer to constantly rotate, translate, and scale the two dimensional projection on screen; however, the use of a cursor creates jerky, discontinuous motions. By employing the physical sensors on my iPod I was able to literally hold the model in my hand as the piece played, making continuous alterations to these parameters.

For each cross section I determined an angle on the XZ plane. A perpendicular amplitude plane (as described on page 8) was then moved across the model at this angle over the amount of time indicated in the duration scheme. The first two cross sections show only the back bone; the second two include pulses and harmonics showing the types of amino acids.

The score includes the data structure objects read by the program, a background showing how the objects map onto the sequence, and scales for pitch and time. Colored lines show the structural divisions both vertically and horizontally using the same color scheme as the images in Figure 2 and the video (blue at the beginning, red at the end). Each sphere is shown as three stacked grey traces. The vertical position of the top trace determines the position in the sequence used as center. The width of each trace controls a specific sonic parameter: the top (dark gray) shows radius, the middle (medium gray) shows dynamic, the bottom (light gray) shows the extent to which the type of amino acid is shown in pulses and timbre. The area covered by the top trace does not intuitively correspond to what is heard because of how the protein is folded. In the process of composing I often adjusted the radius based on visual feedback to make sure it included the right amino acids.

Three additional lines are constructed beneath the colored sequence. The red line determines the degree to which eighth steps are compressed into the major scale: the bottom indicates that only the major scale is heard, the top indicates eight steps. I associate the major scale with the inside of the disk membrane and eighth steps with the outside. Going through the sequence, the helices go back and forth between the inside and outside as shown by this line. The blue line shows the degree to which the speed of pulse patterns depends on the position in the protein's sequence: at the bottom all amino acids are at the same speed, at the top their speed is proportional to their position in the sequence. I chose to unify the pulses at two points in the

sequence corresponding to two amino acids participating in a disulfide bond. This bond connects the third helix with a central sheet stabilizing the bundle of helices. I mark these moments with rhythmic unity though the effect is subtle. The black line shows the scale (or zoom) factor. This trace was not determined with other score objects but using my iPod as described at the top of page 10. The recorded scale data was redrawn onto this part of the score to help connect with the video and sonic realizations.

In addition to the score I include the Pure Data patch used to write and realize this music. Though far from perfected, it represents a substantial portion of the work I have done for this project. A record of the objects used for the score is included and the reader is encouraged to view, modify, re-realize, or design a completely different piece. An understanding of the Pure Data language is not necessary since I have annotated basic operation of the patch in the main window. An iPod is also not necessary as the model can be rotated with the cursor on screen or using a different physical controller. Though it would be desirable for the patch to read any protein model a couple details prevent this: I have not developed a universal method to deal with ligands and the pitch-scaling, score-background-generating, and visualizing algorithms all depend on specifics of the rhodopsin model. Still, the experienced Pure Data user should be able to adapt what is offered to many different protein models.

For a “universal protein instrument” it would be better to begin in a lower level language. While Pure Data is a sophisticated and accessible tool for audio programming, its structure requires bulky realizations of logic which could be distilled to a single line of C or Java. Further, all of Pure Data’s processes are currently resolved on a single processor thread wasting much of the power of my dual-core MacBook Pro. These factors contributed to the long processing times I experienced working on this project. Though I used Pure Data’s “data structure” objects it also seems desirable to develop a specific system of notation for protein instruments. I would like to show many parameters in a single visual object and avoid the awkward stacked traces used in this project.

# Project History

The ideas presented here developed over a seven month period. On its first and second submissions this project received an incomplete. I developed and tested many different schemes for converting the model data into sound. I am indebted to my faculty advisors for allowing me the time to find and realize suitable ideas.

There are two previous schemes worth mentioning. The first split the three spatial dimensions of the model into time, pitch, and panorama between two speakers. I realized several of the proteins in the visual signaling pathway using this scheme and included a video showing how the models were sectioned through time. These realizations are interesting sonic experiences though musically primitive and somewhat monotonous.

The second form used four speakers to create a two dimensional panoramic space and inherited the first form's use of pitch. I freed time from its spatial mapping and used it instead to sketch the protein's sequence in branching glissandi. Static sinusoids were turned on as the glissandi passed the corresponding atoms culminating in a complete sonic image of the model. The result exhibited similar unnatural pitch material and was monotonous as my speculation at the top of page 6 suggests. While working on this scheme the idea of relating amplitude to distance occurred to me, and I began using spheres and other shapes to selectively filter the structure. Without Christopher Jones' comment about using eight speakers, this scheme might have developed into a similar piece as the one presented here with a different use of pitch.

Previous presentations of the project fell short of an essential goal described in my initial proposal. They were not in themselves music. Music is sound intentionally structured as experience. The structures are not themselves experiences: they are static approximations of living molecules. Protein structure is truly experienced when properly connected with an understanding of the underlying chemical dynamics.[14] I realized that for this project I needed to

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[14] Myers, Natasha, "Molecular Embodiments and the Body-work of Modeling in Protein Crystallography," *Social Studies of Science,* Vol. 38, No. 2 (2008), pg. 163-199. Myers' article changed my appreciation for the models in the protein data bank.

actively shape an experience of the model rather than rely on the model to create experience for me.

The current scheme uses four more speakers to free pitch from its spacial mapping. As described on page 6 the model is transformed into an imaginary instrument. I freely composed music for this instrument within certain time-boundaries. A different composer could compose a completely different piece exposing the same structure.

The ideas developed in this project have many further possibilities. I would like to package a virtual meta-instrument to interpret and play any protein model. I have begun experiments using an iPad to control the amplitude shapes though it would also be possible to incorporate three-dimensional camera technology to directly interact with the instrument in the sound space. I envision an interactive installation: participants could reach into the sound space while hearing and seeing regions of the protein surrounding their arms. I would also like to develop my voice as composer creating more repertoire for protein instruments, perhaps even ensembles. The original goals for this project have been fulfilled, and I must carefully decide how to further share these ideas with the world.